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(71) Applicant: **REGENERON PHARMACEUTICALS, INC.** [US/US]; 777 Old Saw Mill River Road, Tarrytown, New York 10591-6707 (US).

(72) Inventors: **GUSAROVA, Viktoria**; c/o Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591-6707 (US). **MURPHY, Andrew J.**; c/o Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591-6707 (US). **GROMADA, Jesper**; c/o Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591-6707 (US). **GUO, Dayong**; 9612 W 116th Terrace, Overland Park, Kansas 66210 (US).

(74) Agent: **GROLZ, Edward W.**; Scully, Scott, Murphy & Presser, 400 Garden City Plaza, Suite 300, Garden City, New York 11530 (US).

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(54) Title: NON-HUMAN ANIMALS HAVING AN ENGINEERED *ANGPTL8* GENE

(57) Abstract: Non-human animals, and methods and compositions for making and using the same, are provided, wherein said non-human animals comprise a humanization of an Angiopoietin- like protein 8 (*ANGPTL8*) gene. Said non-human animals may be described, in some embodiments, as having a genetic modification to an endogenous *ANGPTL8* locus so that said non-human animals express a human ANGPTL8 polypeptide.

NON-HUMAN ANIMALS HAVING AN ENGINEERED *ANGPTL8* GENE

CROSS REFERENCE TO RELATED APPLICATION

[001] This application claims the benefit of priority from U.S. Provisional Application No. 62/291,446, filed February 4, 2016, the entire contents of which are incorporated herein by reference.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[002] The sequence listing in an ASCII text file, named as 34634_10232US01_SequenceListing of 38 kb, created on February 2, 2017, and submitted to the United States Patent and Trademark Office via EFS-Web, is incorporated herein by reference.

BACKGROUND

[003] According to the World Health Organization (WHO), cardiovascular diseases are the number one cause of death each year. In particular, it is estimated that 17.5 million people died from cardiovascular diseases in 2012, which accounts for about 31% of all global deaths. Cardiovascular diseases include disorders of the heart and blood and have several associated risk factors, which most notably include behavioral risk factors such as tobacco and/or alcohol use, unhealthy diet and obesity, and physical inactivity. Such behavioral risk factors include, for example, high blood pressure, high blood sugar and/or high blood lipid levels. Lipids (fat), which include both cholesterol and triglycerides, are not soluble in blood and are transported through the bloodstream via lipoproteins. Having high blood lipid levels can increase the risk for cardiovascular disease and require management via medicine, and, in some cases, surgery.

SUMMARY

[004] The present invention encompasses the recognition that it is desirable to engineer non-human animals to permit improved *in vivo* systems for identifying and developing new therapeutics and/or therapeutic regimens that can be used for the treatment of metabolic disorders that are, in some embodiments, characterized by lipid dysfunction. The present

invention also encompasses the recognition that it is desirable to engineer non-human animals to permit improved *in vivo* systems for identifying and developing new therapeutics that can be used to treat cardiovascular diseases, disorders or conditions. Further, the present invention also encompasses the recognition that non-human animals having an engineered Angiopoietin-like protein 8 (*Angptl8*) gene and/or otherwise expressing, containing (e.g., in the blood), or producing a human or humanized Angiopoietin-like protein 8 polypeptide are desirable, for example for use in identifying and developing therapeutics that can be used for the treatment of hypertriglyceridemia.

[005] In some embodiments, non-human animals having a genome comprising an engineered *Angptl8* gene are provided, which engineered *Angptl8* gene includes genetic material from two different species (e.g., a human and a non-human). In some embodiments, such an engineered *Angptl8* gene includes genetic material that encodes one or more coiled-coil domains of a human ANGPTL8 polypeptide. In some embodiments, such an engineered *Angptl8* gene includes genetic material that encodes an N-terminal region, in whole or in part, of a human ANGPTL8 polypeptide. Thus, in some embodiments, an engineered *Angptl8* gene of a non-human animal as described herein encodes an Angptl8 polypeptide that has a sequence that is all or substantially all human. In various embodiments, an Angptl8 polypeptide expressed by a non-human animal as described herein is expressed under the control of a non-human promoter (e.g., a non-human Angptl8 promoter).

[006] In some embodiments, a non-human animal is provided, whose genome comprises an *Angptl8* gene that comprises an endogenous portion and a human portion, wherein the endogenous and human portions are operably linked to non-human *Angptl8* regulatory elements.

[007] In some embodiments, a non-human animal is provided, that expresses a human ANGPTL8 polypeptide under the control of non-human *Angptl8* regulatory elements.

[008] In some embodiments, an endogenous portion of an *Angptl8* gene includes or comprises an endogenous non-human *Angptl8* promoter. In some embodiments, an endogenous portion of an *Angptl8* gene includes or comprises a 3' region or sequence immediately downstream of the 3' untranslated region of an endogenous non-human *Angptl8* gene at the endogenous non-human *Angptl8* locus.

[009] In some embodiments, an endogenous portion of an *Angptl8* gene includes or comprises 5' and/or 3' untranslated regions (UTRs). In some embodiments, an endogenous portion of an *Angptl8* gene includes 5' and/or 3' untranslated regions (UTRs) and further includes an endogenous *Angptl8* ATG start codon. In some embodiments, 5' and 3' UTRs of an endogenous *Angptl8* gene each have a sequence that is substantially identical or identical to the corresponding 5' and 3' UTRs that appear in a rodent *Angptl8* gene. In some certain embodiments, 5' and 3' UTRs of an endogenous *Angptl8* gene each have a sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% identical to the corresponding 5' and 3' UTRs that appear in SEQ ID NO:1 or SEQ ID NO:3.

[0010] In some embodiments, an *Angptl8* gene as described herein encodes a polypeptide having a sequence at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% identical to SEQ ID NO:6 or SEQ ID NO:8. In some embodiments, an *Angptl8* gene as described herein encodes a polypeptide having a sequence that is substantially identical or identical to SEQ ID NO:6 or SEQ ID NO:8.

[0011] In some embodiments, a human portion includes or comprises exons 1-4, in whole or in part, of a human *ANGPTL8* gene. In some embodiments, exons 1-4, in whole or in part, of a human *ANGPTL8* gene are at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% identical to the corresponding exons 1-4, in whole or in part, that appear in a human *ANGPTL8* mRNA sequence of SEQ ID NO:5. In some embodiments, exons 1-4, in whole or in part, of a human *ANGPTL8* gene are substantially identical or identical to the corresponding exons 1-4, in whole or in part, that appear in a human *ANGPTL8* mRNA sequence of SEQ ID NO:5. In some embodiments, a human portion further comprises the 3'UTR of a human *ANGPTL8* gene. In some embodiments, a human portion comprises a sequence that is codon-optimized for expression in a non-human animal.

[0012] In some embodiments, a human ANGPTL8 polypeptide includes or comprises an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98%

identical to amino acid residues 22-198 of SEQ ID NO:6 or SEQ ID NO:8. In some embodiments, a human ANGPTL8 polypeptide includes or comprises an amino acid sequence that is substantially identical or identical to amino acid residues 22-198 of SEQ ID NO:6 or SEQ ID NO:8.

[0013] In some embodiments, a human ANGPTL8 polypeptide is encoded by a sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% identical to SEQ ID NO:9. In some embodiments, a human ANGPTL8 polypeptide is encoded by a sequence that is substantially identical or identical to SEQ ID NO:9. In some embodiments, a human ANGPTL8 polypeptide is encoded by a sequence that is codon-optimized.

[0014] In some embodiments, a human ANGPTL8 polypeptide is a variant human ANGPTL8 polypeptide. In some embodiments, a variant human ANGPTL8 polypeptide is characterized by an R59W amino acid substitution. In some embodiments, a variant human ANGPTL8 polypeptide is characterized by a Q121X amino acid substitution. In some embodiments, a variant human ANGPTL8 polypeptide is characterized by or is associated with lower plasma low-density lipoprotein (LDL)-cholesterol and/or high-density lipoprotein (HDL)-cholesterol levels. In some embodiments, a variant human ANGPTL8 polypeptide is characterized by or is associated with augmented triglyceride levels. In some embodiments, a human ANGPTL8 polypeptide is encoded by a nucleic acid sequence placed at an endogenous non-human Angptl8 locus.

[0015] In some embodiments, an isolated non-human cell or tissue is provided, whose genome comprises an *Angptl8* gene as described herein. In some embodiments, a cell is a lymphocyte. In some embodiments, a cell is selected from a B cell, dendritic cell, macrophage, monocyte, and a T cell. In some embodiments, a tissue is selected from adipose, bladder, brain, breast, bone marrow, eye, heart, intestine, kidney, liver, lung, lymph node, muscle, pancreas, plasma, serum, skin, spleen, stomach, thymus, testis, ovum, and a combination thereof.

[0016] In some embodiments, an immortalized cell made, generated or produced from an isolated non-human cell as described herein is provided.

[0017] In some embodiments, a non-human embryonic stem (ES) cell is provided, whose genome comprises an *Angptl8* gene as described herein. In some embodiments, a non-human embryonic stem cell is a rodent embryonic stem cell. In some certain embodiments, a rodent embryonic stem cell is a mouse embryonic stem cell and is from a 129 strain, C57BL strain, or a mixture thereof. In some certain embodiments, a rodent embryonic stem cell is a mouse embryonic stem cell and is a mixture of 129 and C57BL strains.

[0018] In some embodiments, use of a non-human embryonic stem cell as described herein to make a non-human animal is provided. In some certain embodiments, a non-human embryonic stem cell is a mouse embryonic stem cell and is used to make a mouse comprising an *Angptl8* gene (or locus) as described herein. In some certain embodiments, a non-human embryonic stem cell is a rat embryonic stem cell and is used to make a rat comprising an *Angptl8* gene (or locus) as described herein.

[0019] In some embodiments, a non-human embryo comprising, made from, obtained from, or generated from a non-human embryonic stem cell as described herein is provided. In some certain embodiments, a non-human embryo is a rodent embryo; in some embodiments, a mouse embryo; in some embodiments, a rat embryo.

[0020] In some embodiments, use of a non-human embryo described herein to make a non-human animal is provided. In some certain embodiments, a non-human embryo is a mouse embryo and is used to make a mouse comprising an *Angptl8* gene (or locus) as described herein. In some certain embodiments, a non-human embryo is a rat embryo and is used to make a rat comprising an *Angptl8* gene (or locus) as described herein.

[0021] In some embodiments, a kit is provided, comprising an isolated non-human cell or tissue as described herein, an immortalized cell as described herein, non-human embryonic stem cell as described herein, a non-human embryo as described herein, or a non-human animal as described herein.

[0022] In some embodiments, a kit as described herein, for use in the manufacture and/or development of a drug (e.g., an antibody or antigen-binding fragment thereof) for therapy or diagnosis is provided.

[0023] In some embodiments, a kit as described herein, for use in the manufacture and/or development of a drug (e.g., an antibody or antigen-binding fragment thereof) for the treatment, prevention or amelioration of a disease, disorder or condition is provided.

[0024] In some embodiments, a transgene, nucleic acid construct, DNA construct, or targeting vector as described herein is provided. In some certain embodiments, a transgene, nucleic acid construct, DNA construct, or targeting vector comprises an *Angptl8* gene (or locus), in whole or in part, as described herein. In some certain embodiments, a transgene, nucleic acid construct, DNA construct, or targeting vector comprises a DNA fragment that includes an *Angptl8* gene (or locus), in whole or in part, as described herein. In some certain embodiments, a transgene, nucleic acid construct, DNA construct, or targeting vector comprises an *Angptl8* gene (or locus) that comprises any one of SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:15, SEQ ID NO:16 and SEQ ID NO:17. In some certain embodiments, a transgene, nucleic acid construct, DNA construct, or targeting vector comprises an *Angptl8* gene (or locus) that comprises SEQ ID NO:15, SEQ ID NO:16 and SEQ ID NO:17. In some certain embodiments, a transgene, nucleic acid construct, DNA construct, or targeting vector further comprises one or more selection markers. In some certain embodiments, a transgene, nucleic acid construct, DNA construct, or targeting vector further comprises one or more site-specific recombination sites (e.g., l*ox*P, *Frt*, or combinations thereof). In some certain embodiments, a transgene, nucleic acid construct, DNA construct, or targeting vector is depicted in Figure 3.

[0025] In some embodiments, use of a transgene, nucleic acid construct, DNA construct, or targeting vector as described herein to make a non-human embryonic stem cell, non-human cell, non-human embryo and/or non-human animal is provided.

[0026] In some embodiments, a method of making a non-human animal that expresses a human ANGPTL8 polypeptide from an endogenous *Angptl8* gene is provided, the method comprising (a) placing a genomic fragment into an endogenous *Angptl8* gene in a non-human embryonic stem cell, said genomic fragment comprising a nucleotide sequence that encodes a human ANGPTL8 polypeptide in whole or in part; (b) obtaining a non-human embryonic stem cell generated in (a); and, (c) creating a non-human animal using the non-human embryonic stem cell of (b).

[0027] In some embodiments, a nucleotide sequence comprises exons 1-4, in whole or in part, of a human *ANGPTL8* gene. In some embodiments, a nucleotide sequence further comprises a 3' UTR of a human *ANGPTL8* gene. In some embodiments, a nucleotide sequence encodes the mature form (i.e., without a signal peptide) of a human ANGPTL8 polypeptide. In some embodiments, a nucleotide sequence encodes amino acids 22-60, 77-134, 156-193 or 22-198 of a human ANGPTL8 polypeptide. In some embodiments, a nucleotide sequence comprises one or more selection markers. In some embodiments, a nucleotide sequence comprises one or more site-specific recombination sites. In some embodiments, a nucleotide sequence comprises a recombinase gene and a selection marker flanked by recombinase recognition sites, which recombinase recognition sites are oriented to direct an excision. In some embodiments, a recombinase gene is operably linked to a promoter that drives expression of the recombinase gene in differentiated cells and does not drive expression of the recombinase gene in undifferentiated cells. In some embodiments, a recombinase gene is operably linked to a promoter that is transcriptionally competent and developmentally regulated. In some embodiments, a promoter that is transcriptionally competent and developmentally regulated is or comprises SEQ ID NO:12, SEQ ID NO:13, or SEQ ID NO:14; in some certain embodiments, a promoter that is transcriptionally competent and developmentally regulated is or comprises SEQ ID NO:12. In some embodiments, a nucleotide sequence comprises one or more sequences that are codon-optimized for expression in a non-human animal. In some embodiments of a method of making a non-human animal that expresses a human ANGPTL8 polypeptide from an endogenous *Angptl8* gene, the method further comprises a step of breeding the rodent generated in (c) so that a rodent homozygous for expressing a human ANGPTL8 polypeptide from an endogenous *Angptl8* gene is created.

[0028] In some embodiments, a method of making a non-human animal whose genome comprises an *Angptl8* gene that encodes a human ANGPTL8 polypeptide is provided, the method comprising modifying the genome of a non-human animal so that it comprises an *Angptl8* gene that encodes a human ANGPTL8 polypeptide under the control of non-human animal *Angptl8* regulatory sequences, thereby making said non-human animal.

[0029] In some embodiments, an *Angptl8* gene is modified to include exons 1-4, in whole or in part, of a human *ANGPTL8* gene. In some embodiments, an *Angptl8* gene is modified to

include exons 1-4, in whole or in part, of a human *ANGPTL8* gene and modified to further include the 3' UTR of a human *ANGPTL8* gene.

[0030] In some embodiments, a non-human animal obtainable by (made from, obtained from, or generated from) any one of the methods as described herein is provided.

[0031] In some embodiments, a method of assessing triglyceride-lowering efficacy of a drug targeting human ANGPTL8 is provided, the method comprising the steps of administering the drug to a non-human animal as described herein, and performing an assay to determine one or more triglyceride-lowering properties of the drug targeting human ANGPTL8.

[0032] In some embodiments, a method of assessing the pharmacokinetic properties of a drug targeting human ANGPTL8 is provided, the method comprising the steps of administering the drug to a non-human animal as described herein, and performing an assay to determine one or more pharmacokinetic properties of the drug targeting human ANGPTL8.

[0033] In some embodiments, a drug targeting human ANGPTL8 is an ANGPTL8 antagonist. In some embodiments, a drug targeting human ANGPTL8 is an ANGPTL8 agonist. In some embodiments, a drug targeting human ANGPTL8 is an anti-ANGPTL8 antibody. In some embodiments, a drug targeting human ANGPTL8 is administered to the rodent intravenously, intraperitoneally or subcutaneously.

[0034] In some embodiments, a non-human animal is provided, whose genome comprises an engineered *Angptl8* gene that includes an endogenous portion that comprises the 5' UTR of an endogenous *Angptl8* gene, and a human portion that comprises exons 1-4, in whole or in part, and the 3' UTR of a human *ANGPTL8* gene, wherein the human portion is operably linked to an endogenous non-human *Angptl8* ATG start codon and operably linked to an endogenous non-human *Angptl8* promoter, and wherein the non-human animal expresses a human ANGPTL8 polypeptide in its serum. The engineered *Angptl8* gene may also include or is linked to or followed by the 3' UTR of an endogenous *Angptl8* gene, and/or a 3' sequence immediately downstream of the 3' UTR of an endogenous *Angptl8* gene at an endogenous *Angptl8* locus.

[0035] In some embodiments, a non-human animal model of hypertriglyceridemia is provided, which non-human animal expresses a human ANGPTL8 polypeptide as described herein.

[0036] In some embodiments, a non-human animal model of hypertriglyceridemia is provided, which non-human animal has a genome comprising an *Angptl8* gene as described herein.

[0037] In some embodiments, a non-human animal or cell as described herein is provided, for use in the manufacture and/or development of a drug for therapy or diagnosis.

[0038] In some embodiments, use of a non-human animal or cell as described herein in the manufacture and/or development of a drug or vaccine for use in medicine, such as use as a medicament is provided.

[0039] In some embodiments, a non-human animal or cell as described herein is provided, for use in the manufacture of a medicament for the treatment, prevention or amelioration of a disease, disorder or condition. In some embodiments, a disease, disorder or condition is hypertriglyceridemia. In some embodiments, a disease, disorder or condition is a cardiovascular disease, disorder or condition.

[0040] In some embodiments, use of a non-human animal or cell as described herein in the manufacture of a medicament for the treatment of a disease, disorder or condition characterized by lipid dysfunction is provided.

[0041] In some embodiments, use of a non-human animal or cell as described herein in the manufacture and/or development of an antibody that binds human ANGPTL8 is provided.

[0042] In various embodiments, non-human Angptl8 regulatory elements include a non-human Angptl8 promoter; in some certain embodiments, an endogenous non-human Angptl8 promoter.

[0043] In various embodiments, an *Angptl8* gene as described herein is a humanized *Angptl8* gene.

[0044] In various embodiments, a human portion of an *Angptl8* gene encodes an amino acid sequence that encodes, *inter alia*, an amino acid sequence of a human ANGPTL8 polypeptide that is responsible for lipid binding or binding ANGPTL3.

[0045] In various embodiments, a human portion of an Angptl8 polypeptide comprises an amino acid sequence of the coiled-coil domain(s) or the N-terminal region of a human ANGPTL8 polypeptide.

[0046] In various embodiments, non-human animals as described herein express a human ANGPTL8 polypeptide that is detectable in the serum of the non-human animal. In various embodiments, non-human animals as described herein do not detectably express an endogenous Angptl8 polypeptide in the serum of the non-human animal.

[0047] In various embodiments, non-human animals as described herein comprise an *Angptl8* gene (or locus) that includes SEQ ID NO:9 or SEQ ID NO:11. In various embodiments, non-human animals as described herein comprise an *Angptl8* gene (or locus) that includes SEQ ID NO:15 and SEQ ID NO:18.

[0048] In various embodiments, a non-human animal as described herein is a rodent; in some embodiments, a mouse; in some embodiments, a rat. In some embodiments, a mouse as described herein is selected from the group consisting of a 129 strain, a BALB/C strain, a C57BL/6 strain, and a mixed 129xC57BL/6 strain; in some certain embodiments, a C57BL/6 strain.

[0049] As used in this application, the terms "about" and "approximately" are used as equivalents. Any numerals used in this application with or without about/approximately are meant to cover any normal fluctuations appreciated by one of ordinary skill in the relevant art.

BRIEF DESCRIPTION OF THE DRAWING

[0050] The Drawing included herein, which is composed of the following Figures, is for illustration purposes only and not for limitation.

[0051] **Figure 1** shows a representative diagram, not to scale, of the genomic organization of non-human (e.g., mouse) and human Angiopoietin-like protein 8 (ANGPTL8) genes. Exons are numbered below each exon. Untranslated regions (open boxes) are also indicated for each gene. Relative portions of coding sequence that encode signal peptides are indicated above exon 1 for each gene.

[0052] **Figure 2** shows an alignment of representative amino acid sequences of human ANGPTL8 (hANGPTL8, SEQ ID NO:6), mouse Angptl8 (mAngptl8, SEQ ID NO:4), rat

Angptl8 (rAngptl8, SEQ ID NO:2) and engineered Angptl8 (engAngptl8; SEQ ID NO:8). Asterisk (*) indicates identical amino acids; colon (:) indicates conservative substitutions; period (.) indicates semiconservative substitutions; blank indicates non-conservative substitutions; boxed amino acid residues indicate signal peptide.

[0053] **Figure 3** shows a representative diagram, not to scale, of an exemplary method for humanization of a non-human *Angptl8* gene. **Top**: a targeting vector made according to Example 1 for insertion into a murine *Angptl8* locus via homologous recombination; **Bottom**: targeted murine *Angptl8* locus after insertion of the targeting vector via homologous recombination and recombinase-mediated deletion of a selection cassette. Selected nucleotide junction locations are marked with a line below each junction and each indicated by SEQ ID NO.

[0054] **Figure 4** shows a representative diagram, not to scale, of the genomic organization of mouse and human Angiopoietin-like protein 8 (*ANGPTL8*) genes indicating the approximate locations of probes employed in an assay described in Example 1. Lengths of an exemplary synthetic DNA fragment employed in humanization of an endogenous murine *Angptl8* gene and corresponding deletion are indicated below each respective gene and are described in Example 1.

[0055] **Figure 5** shows representative levels of triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HLD-C) in wild-type (WT) and mice homozygous for a humanized *Angptl8* gene ($ANGPTL8^{hum/hum}$) as described in Example 2. Lipid levels are presented as mg/dL in plasma separated from venous blood.

[0056] **Figure 6** shows representative tissue-specific (liver and adipose tissues) expression of human ANGPTL8 in mice homozygous for a humanized *Angptl8* gene as described in Example 3. The expression levels are shown as reads per kilo base of transcript per million mapped reads (RPKM)

[0057] **Figure 7** shows representative serum triglyceride levels in mice homozygous for a humanized *Angptl8* gene as described in Example 4 before and after administration of the anti-ANGPTL8 antibody or control (isotype-matched human IgG with irrelevant specificity).

Serum triglyceride levels are presented as mg/dL prior (prebleed) and after (post administration) treatment with antibody.

[0058] **Figures 8A-8K.** For mRNA sequences, bold font indicates coding sequence and consecutive exons, where indicated, are separated by alternating underlined text; for engineered mRNA sequences, human sequences are contained within parentheses. For amino acid sequences, signal sequences are indicated by underlined font. **8A.** *Rattus norvegicus Angptl8* mRNA (SEQ ID NO:1, NCBI Reference Sequence NM_001271710.1). **8B.** *Rattus norvegicus* Angptl8 amino acid (SEQ ID NO:2, NCBI Reference Sequence: NP_001258639.1). **8C.** *Mus musculus Angptl8* mRNA (SEQ ID NO: 3, NCBI Reference Sequence: NM_ 001080940.1). **8D.** *Mus musculus* Angptl8 amino acid (SEQ ID NO:4, NCBI Reference Sequence: NP_ 001074409.1). **8E.** *Homo sapiens ANGPTL8* mRNA (SEQ ID NO:5, NCBI Reference Sequence: NM_018687.6). **8F.** *Homo sapiens* ANGPTL8 amino acid (SEQ ID NO:6, NCBI Reference Sequence: NP_061157.3). **8G.** Exemplary Engineered Angptl8 mRNA (SEQ ID NO:7). **8H.** Exemplary Engineered Angptl8 amino acid (SEQ ID NO:8). **8I.** Exemplary synthetic DNA fragment for engineering a non-human *Angptl8* gene (SEQ ID NO:9; ~2,383bp including exons 1-4 and a 3' UTR of a human *ANGPTL8* gene). **8J.** Exemplary engineered Angptl8 allele including a selection cassette (SEQ ID NO:10; human sequence indicated in bold uppercase font, selection cassette sequence indicated in lowercase font, and mouse sequence indicated by regular uppercase font). **8K.** Exemplary engineered Angptl8 allele after recombinase-mediated excision of a selection cassette (SEQ ID NO:11; human sequence indicated in bold uppercase font, sequence remaining after recombinase-mediated deletion of a selection cassette indicated in lowercase font, and mouse sequence indicated by regular uppercase font).

[0059] **Figure 9A.** The nucleotide sequence across the upstream insertion point is shown which indicates endogenous mouse sequence (contained within the parentheses below with the ATG start codon in bold font) contiguous with human *ANGPTL8* genomic sequence at the insertion point.

[0060] **Figure 9B.** The nucleotide sequence across the 5' end of the self-deleting neomycin cassette is shown, which indicates human *ANGPTL8* genomic sequence contiguous

with cassette sequence (contained within the parentheses below with an XhoI site italicized and a *lox*P site in bold font) downstream of the insertion point.

[0061] **Figure 9C.** The nucleotide sequence across the downstream insertion point at the 3' end of the self-deleting neomycin cassette is shown, which indicates cassette sequence (contained within the parentheses below with a *lox*P site in bold font, an I-CeuI recognition site underlined and an NheI recognition site italicized) contiguous with mouse *Angptl8* genomic sequence.

[0062] **Figure 9D.** The nucleotide sequence across the downstream insertion point after deletion of the neomycin cassette (77bp remaining between a human *ANGPTL8* 3'UTR and a mouse *Angptl8* 3'UTR) i sshown, which indicates human and mouse genomic sequence juxtaposed with remaining cassette sequence (contained within the parentheses below with XhoI and NheI recognition sites italicized, a *lox*P site in bold, and an I-CeuI restriction site underlined).

DEFINITIONS

[0063] This invention is not limited to particular methods and experimental conditions described herein, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention is defined by the claims.

[0064] Unless defined otherwise, all terms and phrases used herein include the meanings that the terms and phrases have attained in the art, unless the contrary is clearly indicated or clearly apparent from the context in which the term or phrase is used. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, particular methods and materials are now described. All patent and non-patent publications mentioned herein are hereby incorporated by reference.

[0065] ***Approximately:*** as applied herein to one or more values of interest, refers to a value that is similar to a stated reference value. In certain embodiments, the term "*approximately*" or "*about*" refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise

stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0066] ***Biologically active***: as used herein, refers to a characteristic of any agent that has activity in a biological system, *in vitro* or *in vivo* (e.g., in an organism). For instance, an agent that, when present in an organism, has a biological effect within that organism, is considered to be biologically active. In particular embodiments, where a protein or polypeptide is biologically active, a portion of that protein or polypeptide that shares at least one biological activity of the protein or polypeptide is typically referred to as a "*biologically active*" portion.

[0067] ***Comparable***: as used herein, refers to two or more agents, entities, situations, sets of conditions, etc. that may not be identical to one another but that are sufficiently similar to permit comparison between them so that conclusions may reasonably be drawn based on differences or similarities observed. Those of ordinary skill in the art will understand, in context, what degree of identity is required in any given circumstance for two or more such agents, entities, situations, sets of conditions, etc. to be considered comparable.

[0068] ***Conservative:*** as used herein, refers to instances when describing a conservative amino acid substitution, including a substitution of an amino acid residue by another amino acid residue having a side chain R group with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of interest of a protein, for example, the ability of a receptor to bind to a ligand. Examples of groups of amino acids that have side chains with similar chemical properties include: aliphatic side chains such as glycine (Gly, G), alanine (Ala, A), valine (Val, V), leucine (Leu, L), and isoleucine (Ile, I); aliphatic-hydroxyl side chains such as serine (Ser, S) and threonine (Thr, T); amide-containing side chains such as asparagine (Asn, N) and glutamine (Gln, Q); aromatic side chains such as phenylalanine (Phe, F), tyrosine (Tyr, Y), and tryptophan (Trp, W); basic side chains such as lysine (Lys, K), arginine (Arg, R), and histidine (His, H); acidic side chains such as aspartic acid (Asp, D) and glutamic acid (Glu, E); and sulfur-containing side chains such as cysteine (Cys, C) and methionine (Met, M). Conservative amino acids substitution groups include, for example, valine/leucine/isoleucine (Val/Leu/Ile, V/L/I), phenylalanine/tyrosine (Phe/Tyr, F/Y), lysine/arginine (Lys/Arg, K/R), alanine/valine (Ala/Val, A/V), glutamate/aspartate (Glu/Asp,

E/D), and asparagine/glutamine (Asn/Gln, N/Q). In some embodiments, a conservative amino acid substitution can be a substitution of any native residue in a protein with alanine, as used in, for example, alanine scanning mutagenesis. In some embodiments, a conservative substitution is made that has a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet, G.H. et al., 1992, Science 256:1443-1445. In some embodiments, a substitution is a moderately conservative substitution wherein the substitution has a nonnegative value in the PAM250 log-likelihood matrix.

[0069] ***Control***: as used herein, refers to the art-understood meaning of a "*control*" being a standard against which results are compared. Typically, controls are used to augment integrity in experiments by isolating variables in order to make a conclusion about such variables. In some embodiments, a control is a reaction or assay that is performed simultaneously with a test reaction or assay to provide a comparator. As used herein, a "*control*" may refer to a "*control animal*". A "*control animal*" may have a modification as described herein, a modification that is different as described herein, or no modification (i.e., a wild-type animal). In one experiment, the "test" (i.e., the variable being tested) is applied. In the second experiment, the "control" (i.e., the variable being tested) is not applied. In some embodiments, a control is a historical control (i.e., of a test or assay performed previously, or an amount or result that is previously known). In some embodiments, a control is or comprises a printed or otherwise saved record. A control may be a positive control or a negative control.

[0070] ***Disruption***: as used herein, refers to the result of a homologous recombination event with a DNA molecule (e.g., with an endogenous homologous sequence such as a gene or gene locus). In some embodiments, a disruption may achieve or represent an insertion, deletion, substitution, replacement, missense mutation, or a frame-shift of a DNA sequence(s), or any combination thereof. Insertions may include the insertion of entire genes or fragments of genes, e.g., exons, which may be of an origin other than the endogenous sequence (e.g., a heterologous sequence). In some embodiments, a disruption may increase expression and/or activity of a gene or gene product (e.g., of a protein encoded by a gene). In some embodiments, a disruption may decrease expression and/or activity of a gene or gene product. In some embodiments, a disruption may alter sequence of a gene or an encoded gene product (e.g., an encoded protein). In some embodiments, a disruption may truncate or

fragment a gene or an encoded gene product (e.g., an encoded protein). In some embodiments, a disruption may extend a gene or an encoded gene product; in some such embodiments, a disruption may achieve assembly of a fusion protein. In some embodiments, a disruption may affect level but not activity of a gene or gene product. In some embodiments, a disruption may affect activity but not level of a gene or gene product. In some embodiments, a disruption may have no significant effect on level of a gene or gene product. In some embodiments, a disruption may have no significant effect on activity of a gene or gene product. In some embodiments, a disruption may have no significant effect on either level or activity of a gene or gene product.

[0071] ***Determining***, ***measuring***, ***evaluating***, ***assessing***, ***assaying*** and ***analyzing***: are used interchangeably herein to refer to any form of measurement, and include determining if an element is present or not. These terms include both quantitative and/or qualitative determinations. Assaying may be relative or absolute. "*Assaying for the presence of*" can be determining the amount of something present and/or determining whether or not it is present or absent.

[0072] ***Endogenous locus*** or ***endogenous gene***: as used herein, refers to a genetic locus found in a parent or reference organism prior to introduction of an alteration, disruption, deletion, insertion, modification, replacement, or substitution as described herein. In some embodiments, the endogenous locus has a sequence found in nature. In some embodiments, the endogenous locus is a wild-type locus. In some embodiments, the reference organism is a wild-type organism. In some embodiments, the reference organism is an engineered organism. In some embodiments, the reference organism is a laboratory-bred organism (whether wild-type or engineered).

[0073] ***Endogenous promoter***: as used herein, refers to a promoter that is naturally associated, e.g., in a wild-type organism, with an endogenous gene.

[0074] ***Engineered:*** as used herein refers, in general, to the aspect of having been manipulated by the hand of man. For example, in some embodiments, a polynucleotide may be considered to be "*engineered*" when two or more sequences that are not linked together in that order in nature are manipulated by the hand of man to be directly linked to one another in the engineered polynucleotide. In some particular such embodiments, an engineered

polynucleotide may comprise a regulatory sequence that is found in nature in operative association with a first coding sequence but not in operative association with a second coding sequence, is linked by the hand of man so that it is operatively associated with the second coding sequence. Alternatively or additionally, in some embodiments, first and second nucleic acid sequences that each encode polypeptide elements or domains that in nature are not linked to one another may be linked to one another in a single engineered polynucleotide. Comparably, in some embodiments, a cell or organism may be considered to be "*engineered*" if it has been manipulated so that its genetic information is altered (e.g., new genetic material not previously present has been introduced, or previously present genetic material has been altered or removed). As is common practice and is understood by those in the art, progeny of an engineered polynucleotide or cell are typically still referred to as "*engineered*" even though the actual manipulation was performed on a prior entity. Furthermore, as will be appreciated by those skilled in the art, a variety of methodologies are available through which "*engineering*" as described herein may be achieved. For example, in some embodiments, "*engineering*" may involve selection or design (e.g., of nucleic acid sequences, polypeptide sequences, cells, tissues, and/or organisms) through use of computer systems programmed to perform analysis or comparison, or otherwise to analyze, recommend, and/or select sequences, alterations, etc.). Alternatively or additionally, in some embodiments, "*engineering*" may involve use of *in vitro* chemical synthesis methodologies and/or recombinant nucleic acid technologies such as, for example, nucleic acid amplification (e.g., via the polymerase chain reaction) hybridization, mutation, transformation, transfection, etc., and/or any of a variety of controlled mating methodologies. As will be appreciated by those skilled in the art, a variety of established such techniques (e.g., for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation [e.g., electroporation, lipofection, etc.]) are well known in the art and described in various general and more specific references that are cited and/or discussed throughout the present specification. See e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989).

[0075] ***Gene***: as used herein, refers to a DNA sequence in a chromosome that codes for a product (e.g., an RNA product and/or a polypeptide product). In some embodiments, a gene includes coding sequence (i.e., sequence that encodes a particular product). In some

embodiments, a gene includes non-coding sequence. In some particular embodiments, a gene may include both coding (e.g., exonic) and non-coding (e.g., intronic) sequence. In some embodiments, a gene may include one or more regulatory sequences (*e.g.*, promoters, enhancers, *etc.*) and/or intron sequences that, for example, may control or impact one or more aspects of gene expression (e.g., cell-type-specific expression, inducible expression, etc.). For the purpose of clarity we note that, as used in the present application, the term "*gene*" generally refers to a portion of a nucleic acid that encodes a polypeptide; the term may optionally encompass regulatory sequences, as will be clear from context to those of ordinary skill in the art. This definition is not intended to exclude application of the term "*gene*" to non-protein-coding expression units but rather to clarify that, in most cases, the term as used in this document refers to a polypeptide-coding nucleic acid.

[0076] ***Heterologous***: as used herein, refers to an agent or entity from a different source. For example, when used in reference to a polypeptide, gene, or gene product present in a particular cell or organism, the term clarifies that the relevant polypeptide, gene, or gene product: 1) was engineered by the hand of man; 2) was introduced into the cell or organism (or a precursor thereof) through the hand of man (e.g., via genetic engineering); and/or 3) is not naturally produced by or present in the relevant cell or organism (e.g., the relevant cell type or organism type).

[0077] ***Host cell***: as used herein, refers to a cell into which a heterologous (e.g., exogenous) nucleic acid or protein has been introduced. Persons of skill upon reading this disclosure will understand that such terms refer not only to the particular subject cell, but also is used to refer to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "*host cell*" as used herein. In some embodiments, a host cell is or comprises a prokaryotic or eukaryotic cell. In general, a host cell is any cell that is suitable for receiving and/or producing a heterologous nucleic acid or polypeptide, regardless of the Kingdom of life to which the cell is designated. Exemplary cells include those of prokaryotes and eukaryotes (single-cell or multiple-cell), bacterial cells (e.g., strains of E. coli, Bacillus spp., Streptomyces spp., etc.), mycobacteria cells, fungal cells, yeast cells (e.g., S. cerevisiae, S. pombe, P. pastoris, P. methanolica, etc.), plant cells, insect cells (e.g., SF-9, SF-21,

baculovirus-infected insect cells, Trichoplusia ni, etc.), non-human animal cells, human cells, or cell fusions such as, for example, hybridomas or quadromas. In some embodiments, the cell is a human, monkey, ape, hamster, rat, or mouse cell. In some embodiments, the cell is eukaryotic and is selected from the following cells: CHO (e.g., CHO K1, DXB-11 CHO, Veggie-CHO), COS (e.g., COS-7), retinal cell, Vero, CV1, kidney (e.g., HEK293, 293 EBNA, MSR 293, MDCK, HaK, BHK), HeLa, HepG2, WI38, MRC 5, Colo205, HB 8065, HL-60, (e.g., BHK21), Jurkat, Daudi, A431 (epidermal), CV-1, U937, 3T3, L cell, C127 cell, SP2/0, NS-0, MMT 060562, Sertoli cell, BRL 3A cell, HT1080 cell, myeloma cell, tumor cell, and a cell line derived from an aforementioned cell. In some embodiments, the cell comprises one or more viral genes, e.g., a retinal cell that expresses a viral gene (e.g., a PER.C6™ cell). In some embodiments, a host cell is or comprises an isolated cell. In some embodiments, a host cell is part of a tissue. In some embodiments, a host cell is part of an organism.

[0078] ***Humanized***: is used herein in accordance with its art-understood meaning to refer to nucleic acids or polypeptides whose structures (i.e., nucleotide or amino acid sequences) include portions that correspond substantially or identically with structures of a particular gene or polypeptide found in nature in a non-human animal, and also include portions that differ from that found in the relevant particular non-human gene or protein and instead correspond more closely with comparable structures found in a corresponding human gene or polypeptide. In some embodiments, a "*humanized*" gene is one that encodes a polypeptide having substantially the amino acid sequence as that of a human polypeptide (e.g., a human protein or portion thereof – e.g., characteristic portion thereof). For example, in the case of a membrane receptor, a "*humanized*" gene may encode a polypeptide having an extracellular portion, in whole or in part, having an amino acid sequence as that of a human extracellular portion and the remaining sequence as that of a non-human (e.g., mouse) polypeptide. In the case of a secreted polypeptide, a "*humanized*" gene may encode a polypeptide having a mature peptide, in whole or in part, having a sequence as that of a human mature peptide and the signal sequence as that of a non-human (e.g., mouse) peptide. In some embodiments, a humanized gene comprises at least a portion of a DNA sequence of a human gene. In some embodiments, a humanized gene comprises an entire DNA sequence of a human gene or the DNA sequence of a human gene that encodes a mature peptide or polypeptide corresponding

to a mature human peptide or polypeptide. In some embodiments, a humanized polypeptide comprises a sequence having a portion that appears in a human polypeptide. In some embodiments, a humanized polypeptide comprises an entire sequence of a human polypeptide and is expressed from an endogenous locus of a non-human animal that corresponds to the homolog or ortholog of the human gene.

[0079] ***Identity***: as used herein in connection with a comparison of sequences, refers to identity as determined by a number of different algorithms known in the art that can be used to measure nucleotide and/or amino acid sequence identity. In some embodiments, identities as described herein are determined using a ClustalW v.1.83 (slow) alignment employing an open gap penalty of 10.0, an extend gap penalty of 0.1, and using a Gonnet similarity matrix (MACVECTOR™ 10.0.2, MacVector Inc., 2008).

[0080] ***In vitro***: as used herein refers to events that occur in an artificial environment, e.g., in a test tube or reaction vessel, in cell culture, etc., rather than within a multi-cellular organism.

[0081] ***In vivo***: as used herein refers to events that occur within a multi-cellular organism, such as a human and a non-human animal. In the context of cell-based systems, the term may be used to refer to events that occur within a living cell (as opposed to, for example, *in vitro* systems).

[0082] ***Isolated***: as used herein, refers to a substance and/or entity that has been (1) separated from at least some of the components with which it was associated when initially produced (whether in nature and/or in an experimental setting), and/or (2) designed, produced, prepared, and/or manufactured by the hand of man. Isolated substances and/or entities may be separated from about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% of the other components with which they were initially associated. In some embodiments, isolated agents are about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. As used herein, a substance is "*pure*" if it is substantially free of other components. In some embodiments, as will be understood by those skilled in the art, a substance may still be

considered "*isolated*" or even "*pure*", after having been combined with certain other components such as, for example, one or more carriers or excipients (e.g., buffer, solvent, water, etc.); in such embodiments, percent isolation or purity of the substance is calculated without including such carriers or excipients. To give but one example, in some embodiments, a biological polymer such as a polypeptide or polynucleotide that occurs in nature is considered to be "*isolated*" when: a) by virtue of its origin or source of derivation is not associated with some or all of the components that accompany it in its native state in nature; b) it is substantially free of other polypeptides or nucleic acids of the same species from the species that produces it in nature; or c) is expressed by or is otherwise in association with components from a cell or other expression system that is not of the species that produces it in nature. Thus, for instance, in some embodiments, a polypeptide that is chemically synthesized or is synthesized in a different cellular system from that which produces it in nature is considered to be an "*isolated*" polypeptide. Alternatively or additionally, in some embodiments, a polypeptide that has been subjected to one or more purification techniques may be considered to be an "*isolated*" polypeptide to the extent that it has been separated from other components: a) with which it is associated in nature; and/or b) with which it was associated when initially produced.

[0083] ***Locus*** or ***Loci***: as used herein, includes a specific location(s) of a gene (or significant sequence), DNA sequence, polypeptide-encoding sequence, or position on a chromosome of the genome of an organism. For example, an "*Angptl8 locus*" may refer to the specific location of an *Angptl8* gene, *Angptl8* DNA sequence, *Angptl8*-encoding sequence, or *Angptl8* position on a chromosome of the genome of an organism that has been identified as to where such a sequence resides. An "*Angptl8 locus*" may comprise a regulatory element of an *Angptl8* gene, including, but not limited to, an enhancer, a promoter, 5' and/or 3' UTR, or a combination thereof. Those of ordinary skill in the art will appreciate that chromosomes may, in some embodiments, contain hundreds or even thousands of genes and demonstrate physical co-localization of similar genetic loci when comparing between different species. Such genetic loci may be described as having shared synteny.

[0084] ***Non-human animal***: as used herein, refers to any vertebrate organism that is not a human. In some embodiments, a non-human animal is a cyclostome, a bony fish, a cartilaginous fish (e.g., a shark or a ray), an amphibian, a reptile, a mammal, and a bird. In

some embodiments, a non-human animal as described herein is a mammal. In some embodiments, a non-human mammal is a primate, a goat, a sheep, a pig, a dog, a cow, or a rodent. In some embodiments, a non-human animal as described herein is a small mammal, e.g., of the superfamily Dipodoidea or Muroidea. In some embodiments, a genetically modified animal as described herein is a rodent. In some embodiments, a rodent as described herein is selected from a mouse, a rat, and a hamster. In some embodiments, a rodent as described herein is selected from the superfamily Muroidea. In some embodiments, a genetically modified animal as described herein is from a family selected from Calomyscidae (e.g., mouse-like hamsters), Cricetidae (e.g., hamster, New World rats and mice, voles), Muridae (true mice and rats, gerbils, spiny mice, crested rats), Nesomyidae (climbing mice, rock mice, white-tailed rats, Malagasy rats and mice), Platacanthomyidae (e.g., spiny dormice), and Spalacidae (e.g., mole rates, bamboo rats, and zokors). In some certain embodiments, a genetically modified rodent as described herein is selected from a true mouse or rat (family Muridae), a gerbil, a spiny mouse, and a crested rat. In some certain embodiments, a genetically modified mouse as described herein is from a member of the family Muridae. In some embodiment, a non-human animal as described herein is a rodent. In some certain embodiments, a rodent as described herein is selected from a mouse and a rat. In some embodiments, a non-human animal as described herein is a mouse.

[0085] In some embodiments, a non-human animal as described herein is a rodent that is a mouse of a C57BL strain selected from C57BL/A, C57BL/An, C57BL/GrFa, C57BL/KaLwN, C57BL/6, C57BL/6J, C57BL/6ByJ, C57BL/6NJ, C57BL/10, C57BL/10ScSn, C57BL/10Cr, and C57BL/Ola. In some certain embodiments, a mouse as described herein is a 129 strain selected from the group consisting of a strain that is 129P1, 129P2, 129P3, 129X1, 129S1 (e.g., 129S1/SV, 129S1/SvIm), 129S2, 129S4, 129S5, 129S9/SvEvH, 129/SvJae, 129S6 (129/SvEvTac), 129S7, 129S8, 129T1, 129T2 (see, e.g., Festing et al., 1999, Mammalian Genome 10:836; Auerbach, W. et al., 2000, Biotech. 29(5):1024-1028, 1030, 1032). In some certain embodiments, a genetically modified mouse as described herein is a mix of an aforementioned 129 strain and an aforementioned C57BL/6 strain. In some certain embodiments, a mouse as described herein is a mix of aforementioned 129 strains, or a mix of aforementioned BL/6 strains. In some certain embodiments, a 129 strain of the mix as described herein is a 129S6 (129/SvEvTac) strain. In some embodiments,

a mouse as described herein is a BALB strain, e.g., BALB/c strain. In some embodiments, a mouse as described herein is a mix of a BALB strain and another aforementioned strain.

[0086] In some embodiments, a non-human animal as described herein is a rat. In some certain embodiments, a rat as described herein is selected from a Wistar rat, an LEA strain, a Sprague Dawley strain, a Fischer strain, F344, F6, and Dark Agouti. In some certain embodiments, a rat strain as described herein is a mix of two or more strains selected from the group consisting of Wistar, LEA, Sprague Dawley, Fischer, F344, F6, and Dark Agouti.

[0087] ***Nucleic acid***: as used herein, in its broadest sense, refers to any compound and/or substance that is or can be incorporated into an oligonucleotide chain. In some embodiments, a "*nucleic acid*" is a compound and/or substance that is or can be incorporated into an oligonucleotide chain via a phosphodiester linkage. As will be clear from context, in some embodiments, "*nucleic acid*" refers to individual nucleic acid residues (e.g., nucleotides and/or nucleosides); in some embodiments, "*nucleic acid*" refers to an oligonucleotide chain comprising individual nucleic acid residues. In some embodiments, a "*nucleic acid*" is or comprises RNA; in some embodiments, a "*nucleic acid*" is or comprises DNA. In some embodiments, a "*nucleic acid*" is, comprises, or consists of one or more natural nucleic acid residues. In some embodiments, a "*nucleic acid*" is, comprises, or consists of one or more nucleic acid analogs. In some embodiments, a nucleic acid analog differs from a "*nucleic acid*" in that it does not utilize a phosphodiester backbone. For example, in some embodiments, a "*nucleic acid*" is, comprises, or consists of one or more "*peptide nucleic acids*", which are known in the art and have peptide bonds instead of phosphodiester bonds in the backbone, are considered within the scope of the present invention. Alternatively or additionally, in some embodiments, a "*nucleic acid*" has one or more phosphorothioate and/or 5'-N-phosphoramidite linkages rather than phosphodiester bonds. In some embodiments, a "*nucleic acid*" is, comprises, or consists of one or more natural nucleosides (e.g., adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine, deoxythymidine, deoxyguanosine, and deoxycytidine). In some embodiments, a "*nucleic acid*" is, comprises, or consists of one or more nucleoside analogs (e.g., 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methylcytidine, C-5 propynyl-cytidine, C-5 propynyl-uridine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5-propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 7-

deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, O(6)-methylguanine, 2-thiocytidine, methylated bases, intercalated bases, and combinations thereof). In some embodiments, a "*nucleic acid*" comprises one or more modified sugars (e.g., 2'-fluororibose, ribose, 2'-deoxyribose, arabinose, and hexose) as compared with those in natural nucleic acids. In some embodiments, a "*nucleic acid*" has a nucleotide sequence that encodes a functional gene product such as an RNA or protein. In some embodiments, a "*nucleic acid*" includes one or more introns. In some embodiments, a "*nucleic acid*" is prepared by one or more of isolation from a natural source, enzymatic synthesis by polymerization based on a complementary template (*in vivo* or *in vitro*), reproduction in a recombinant cell or system, and chemical synthesis. In some embodiments, a "*nucleic acid*" is at least 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 20, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000 or more residues long. In some embodiments, a "*nucleic acid*" is single stranded; in some embodiments, a "*nucleic acid*" is double stranded. In some embodiments, a "*nucleic acid*" has a nucleotide sequence comprising at least one element that encodes, or is the complement of a sequence that encodes, a polypeptide. In some embodiments, a "*nucleic acid*" has enzymatic activity.

[0088] ***Operably linked***: as used herein, refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A control sequence "*operably linked*" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences. "*Operably linked*" sequences include both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in trans or at a distance to control the gene of interest. The term "*expression control sequence*", as used herein, refers to polynucleotide sequences, which are necessary to effect the expression and processing of coding sequences to which they are ligated. "*Expression control sequences*" include: appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance protein secretion. The nature of such control sequences differs depending upon

the host organism. For example, in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence, while in eukaryotes, typically, such control sequences include promoters and transcription termination sequence. The term "*control sequences*" is intended to include components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

[0089] ***Patient*** or ***subject***: as used herein, refers to any organism to which a provided composition is or may be administered, e.g., for experimental, diagnostic, prophylactic, cosmetic, and/or therapeutic purposes. Typical patients include animals (e.g., mammals such as mice, rats, rabbits, non-human primates, and/or humans). In some embodiments, a patient is a non-human animal. In some embodiments, a patient or subject (e.g., a non-human animal patient) may have a modification as described herein, a modification that is different as described herein or no modification (i.e., a wild-type non-human animal patient). In some embodiments, a non-human animal is suffering from or susceptible to one or more disorders or conditions. In some embodiments, a non-human animal displays one or more symptoms of a disease, disorder or condition. In some embodiments, a non-human animal has been diagnosed with one or more diseases, disorders or conditions.

[0090] ***Polypeptide***: as used herein, refers to any polymeric chain of amino acids. In some embodiments, a polypeptide has an amino acid sequence that occurs in nature. In some embodiments, a polypeptide has an amino acid sequence that does not occur in nature. In some embodiments, a polypeptide has an amino acid sequence that contains portions that occur in nature separately from one another (i.e., from two or more different organisms, for example, human and non-human portions). In some embodiments, a polypeptide has an amino acid sequence that is engineered in that it is designed and/or produced through action of the hand of man.

[0091] ***Promoter or Promoter sequence:*** as used herein, refers to a DNA regulatory region capable of being bound by an RNA polymerase in a cell (e.g., directly or through other promoter-bound polypeptides or substances) and initiating transcription of a coding sequence. A promoter sequence is, in general, bound at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements

necessary to initiate transcription at any level. The promoter may be operably associated with or operably linked to expression control sequences, including enhancer and repressor sequences or with a nucleic acid of interest that is to be expressed. In some embodiments, a promoter may be inducible. In some embodiments, an inducible promoter may be unidirectional or bi-directional. In some embodiments, a promoter may be a constitutive promoter. In some embodiments, a promoter can be a hybrid promoter, in which the sequence containing a transcriptional regulatory region is obtained from one source and the sequence containing a transcription initiation region is obtained from a second source. Systems for linking control elements to coding sequences within a transgene are well known in the art. For example, general molecular biological and recombinant DNA techniques are described in Principles of Gene Manipulation: An Introduction to Genetic Manipulation, 5th Ed., ed. By Old, R.W. and S.B. Primrose, Blackwell Science, Inc., 1994; Molecular Cloning: A Laboratory Manual, 2nd Ed., ed. by Sambrook, J. et al., Cold Spring Harbor Laboratory Press: 1989.

[0092] ***Recombinant***: as used herein, is intended to refer to polypeptides that are designed, engineered, prepared, expressed, created or isolated by recombinant means, such as polypeptides expressed using a recombinant expression vector transfected into a host cell, polypeptides isolated from a recombinant, combinatorial human polypeptide library (Hoogenboom H. R., 1997, TIB Tech. 15:62-70; Azzazy H., and Highsmith W. E., 2002, Clin. Biochem. 35:425-45; Gavilondo J. V., and Larrick J. W., 2002, BioTech. 29:128-45; Hoogenboom H., and Chames P., 2000, Immunology Today 21:371-8), antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (see e.g., Taylor, L. D. et al., 1992, Nucl. Acids Res. 20:6287-95; Kellermann S-A., and Green L. L., 2002, Curr. Opin. Biotechnol. 13:593-7; Little M. et al., 2000, Immunol. Today 21:364-370; Murphy, A.J. et al., 2014, Proc. Natl. Acad. Sci. U.S.A. 111(14):5153-8) or polypeptides prepared, expressed, created or isolated by any other means that involves splicing selected sequence elements to one another. In some embodiments, one or more of such selected sequence elements is found in nature. In some embodiments, one or more of such selected sequence elements is designed *in silico*. In some embodiments, one or more such selected sequence elements result from mutagenesis (e.g., *in vivo* or *in vitro*) of a known sequence element, e.g., from a natural or synthetic source. For example, in some embodiments, a

recombinant polypeptide is comprised of sequences found in the genome of a source organism of interest (e.g., human, mouse, etc.). In some embodiments, a recombinant polypeptide has an amino acid sequence that resulted from mutagenesis (e.g., *in vitro* or *in vivo*, for example in a non-human animal), so that the amino acid sequences of the recombinant polypeptides are sequences that, while originating from and related to polypeptides sequences, may not naturally exist within the genome of a non-human animal *in vivo*.

[0093] ***Replacement***: as used herein, refers to a process through which a "*replaced*" nucleic acid sequence (e.g., a gene) found in a host locus (e.g., in a genome) is removed from that locus, and a different, "*replacement*" nucleic acid is located in its place. In some embodiments, the replaced nucleic acid sequence and the replacement nucleic acid sequences are comparable to one another in that, for example, they are homologous to one another and/or contain corresponding elements (e.g., protein-coding elements, regulatory elements, etc.). In some embodiments, a replaced nucleic acid sequence includes one or more of a promoter, an enhancer, a splice donor site, a splice acceptor site, an intron, an exon, an untranslated region (UTR); in some embodiments, a replacement nucleic acid sequence includes one or more coding sequences. In some embodiments, a replacement nucleic acid sequence is a homolog of the replaced nucleic acid sequence. In some embodiments, a replacement nucleic acid sequence is an ortholog of the replaced sequence. In some embodiments, a replacement nucleic acid sequence is or comprises a human nucleic acid sequence. In some embodiments, a replacement nucleic acid sequence is or comprises an engineered nucleic acid sequence. In some embodiments, including where the replacement nucleic acid sequence is or comprises a human nucleic acid sequence, the replaced nucleic acid sequence is or comprises a rodent sequence (e.g., a mouse or rat sequence). The nucleic acid sequence so placed may include one or more regulatory sequences that are part of source nucleic acid sequence used to obtain the sequence so placed (e.g., promoters, enhancers, 5'- or 3'-untranslated regions, etc.). For example, in various embodiments, the replacement is a substitution of an endogenous sequence with a heterologous sequence that results in the production of a gene product from the nucleic acid sequence so placed (comprising the heterologous sequence), but not expression of the endogenous sequence; the replacement is of an endogenous genomic sequence with a nucleic acid sequence that encodes a polypeptide that has a similar function as a polypeptide encoded by the endogenous sequence (e.g., the

endogenous genomic sequence encodes a Angptl8 polypeptide, and the DNA fragment encodes one or more human ANGPTL8 polypeptides, in whole or in part). In various embodiments, an endogenous gene or fragment thereof is replaced with a corresponding human gene or fragment thereof. A corresponding human gene or fragment thereof is a human gene or fragment that is an ortholog of, or is substantially similar or the same in structure and/or function, as the endogenous gene or fragment thereof that is replaced.

[0094] ***Reference***: as used herein, describes a standard or control agent, cohort, individual, population, sample, sequence or value against which an agent, animal, cohort, individual, population, sample, sequence or value of interest is compared. In some embodiments, a reference agent, cohort, individual, population, sample, sequence or value is tested and/or determined substantially simultaneously with the testing or determination of the agent, cohort, individual, population, sample, sequence or value of interest. In some embodiments, a reference agent, cohort, individual, population, sample, sequence or value is a historical reference, optionally embodied in a tangible medium. In some embodiments, a reference may refer to a control. As used herein, a "*reference*" may refer to a "*reference animal*". A "*reference animal*" may have a modification as described herein, a modification that is different as described herein or no modification (i.e., a wild-type animal). Typically, as would be understood by those skilled in the art, a reference agent, animal, cohort, individual, population, sample, sequence or value is determined or characterized under conditions comparable to those utilized to determine or characterize the agent, animal (e.g., a mammal), cohort, individual, population, sample, sequence or value of interest.

[0095] ***Substantially***: as used herein, refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term "*substantially*" is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

[0096] ***Substantial homology***: as used herein, refers to a comparison between amino acid or nucleic acid sequences. As will be appreciated by those of ordinary skill in the art, two sequences are generally considered to be "*substantially homologous*" if they contain

homologous residues in corresponding positions. Homologous residues may be identical residues. Alternatively, homologous residues may be non-identical residues will appropriately similar structural and/or functional characteristics. For example, as is well known by those of ordinary skill in the art, certain amino acids are typically classified as "*hydrophobic*" or "*hydrophilic*" amino acids, and/or as having "*polar*" or "*non-polar*" side chains. Substitution of one amino acid for another of the same type may often be considered a "*homologous*" substitution. Typical amino acid categorizations are summarized below:

Alanine	Ala	A	Nonpolar	Neutral	1.8
Arginine	Arg	R	Polar	Positive	-4.5
Asparagine	Asn	N	Polar	Neutral	-3.5
Aspartic acid	Asp	D	Polar	Negative	-3.5
Cysteine	Cys	C	Nonpolar	Neutral	2.5
Glutamic acid	Glu	E	Polar	Negative	-3.5
Glutamine	Gln	Q	Polar	Neutral	-3.5
Glycine	Gly	G	Nonpolar	Neutral	-0.4
Histidine	His	H	Polar	Positive	-3.2
Isoleucine	Ile	I	Nonpolar	Neutral	4.5
Leucine	Leu	L	Nonpolar	Neutral	3.8
Lysine	Lys	K	Polar	Positive	-3.9
Methionine	Met	M	Nonpolar	Neutral	1.9
Phenylalanine	Phe	F	Nonpolar	Neutral	2.8
Proline	Pro	P	Nonpolar	Neutral	-1.6
Serine	Ser	S	Polar	Neutral	-0.8
Threonine	Thr	T	Polar	Neutral	-0.7
Tryptophan	Trp	W	Nonpolar	Neutral	-0.9
Tyrosine	Tyr	Y	Polar	Neutral	-1.3
Valine	Val	V	Nonpolar	Neutral	4.2

Ambiguous Amino Acids	3-Letter	1-Letter
Asparagine or aspartic acid	Asx	B
Glutamine or glutamic acid	Glx	Z
Leucine or Isoleucine	Xle	J
Unspecified or unknown amino acid	Xaa	X

[0097] As is well known in this art, amino acid or nucleic acid sequences may be compared using any of a variety of algorithms, including those available in commercial

computer programs such as BLASTN for nucleotide sequences and BLASTP, gapped BLAST, and PSI-BLAST for amino acid sequences. Exemplary such programs are described in Altschul et al., 1990, Basic local alignment search tool, J. Mol. Biol., 215(3): 403-410; Altschul et al., 1996, Methods Enzymol. 266:160-80; Altschul et al., 1997, Nucleic Acids Res. 25:3389-3402; Baxevanis et al., 1998 Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins, Wiley; and Misener et al. (eds.) (1999) Bioinformatics Methods and Protocols (Methods in Molecular Biology, Vol. 132), Humana Press. In addition to identifying homologous sequences, the programs mentioned above typically provide an indication of the degree of homology. In some embodiments, two sequences are considered to be substantially homologous if at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more of their corresponding residues are homologous over a relevant stretch of residues. In some embodiments, the relevant stretch is a complete sequence. In some embodiments, the relevant stretch is at least 9, 10, 11, 12, 13, 14, 15, 16, 17 or more residues. In some embodiments, the relevant stretch includes contiguous residues along a complete sequence. In some embodiments, the relevant stretch includes discontinuous residues along a complete sequence. In some embodiments, the relevant stretch is at least 10, 15, 20, 25, 30, 35, 40, 45, 50, or more residues.

[0098] ***Substantial identity***: as used herein, refers to a comparison between amino acid or nucleic acid sequences. As will be appreciated by those of ordinary skill in the art, two sequences are generally considered to be "*substantially identical*" if they contain identical residues in corresponding positions. As is well known in this art, amino acid or nucleic acid sequences may be compared using any of a variety of algorithms, including those available in commercial computer programs such as BLASTN for nucleotide sequences and BLASTP, gapped BLAST, and PSI-BLAST for amino acid sequences. Exemplary such programs are described in Altschul et al., 1990, Basic local alignment search tool, J. Mol. Biol., 215(3): 403-410; Altschul et al., 1996, Methods Enzymol. 266:160-80; Altschul et al., 1997, Nucleic Acids Res. 25:3389-3402; Baxevanis et al., 1998, Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins, Wiley; Misener et al., (eds.) (1999) Bioinformatics Methods and Protocols (Methods in Molecular Biology, Vol. 132), Humana Press. In addition to identifying identical sequences, the programs mentioned above typically provide an indication of the degree of identity. In some embodiments, two sequences are considered to be

substantially identical if at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more of their corresponding residues are identical over a relevant stretch of residues. In some embodiments, the relevant stretch is a complete sequence. In some embodiments, the relevant stretch is at least 10, 15, 20, 25, 30, 35, 40, 45, 50, or more residues.

[0099] ***Targeting vector*** or ***targeting construct***: as used herein, refers to a polynucleotide molecule that comprises a targeting region. A targeting region comprises a sequence that is identical or substantially identical to a sequence in a target cell, tissue or animal and provides for integration of the targeting construct into a position within the genome of the cell, tissue or animal via homologous recombination. Targeting regions that target using site-specific recombinase recognition sites (e.g., *lox*P and/or *Frt* sites) are also included. In some embodiments, a targeting construct further comprises a nucleic acid sequence or gene of particular interest, a selectable marker, control and or regulatory sequences, and other nucleic acid sequences that allow for recombination mediated through exogenous addition of proteins that aid in or facilitate recombination involving such sequences. In some embodiments, a targeting construct further comprises a gene of interest in whole or in part, wherein the gene of interest is a heterologous gene that encodes a polypeptide, in whole or in part, that has a similar function as a polypeptide encoded by an endogenous sequence. In some embodiments, a targeting construct further comprises a humanized gene of interest, in whole or in part, wherein the humanized gene of interest encodes a polypeptide, in whole or in part, that has a similar function as a polypeptide encoded by the endogenous sequence. In some embodiments, a targeting construct further comprises an engineered gene of interest, in whole or in part, wherein the engineered gene of interest encodes a polypeptide, in whole or in part, that has a similar function as a polypeptide encoded by an endogenous sequence.

[00100] ***Variant***: as used herein, refers to an entity that shows significant structural identity with a reference entity, but differs structurally from the reference entity in the presence or level of one or more chemical moieties as compared with the reference entity. In many embodiments, a "*variant*" also differs functionally from its reference entity. In general, whether a particular entity is properly considered to be a "*variant*" of a reference entity is based on its degree of structural identity with the reference entity. As will be appreciated by those skilled in the art, any biological or chemical reference entity has certain characteristic

structural elements. A "*variant*", by definition, is a distinct chemical entity that shares one or more such characteristic structural elements. To give but a few examples, a small molecule may have a characteristic core structural element (e.g., a macrocycle core) and/or one or more characteristic pendent moieties so that a variant of the small molecule is one that shares the core structural element and the characteristic pendent moieties but differs in other pendent moieties and/or in types of bonds present (single vs. double, E vs. Z, etc.) within the core, a polypeptide may have a characteristic sequence element comprised of a plurality of amino acids having designated positions relative to one another in linear or three-dimensional space and/or contributing to a particular biological function, a nucleic acid may have a characteristic sequence element comprised of a plurality of nucleotide residues having designated positions relative to on another in linear or three-dimensional space. For example, a "*variant polypeptide*" may differ from a reference polypeptide as a result of one or more differences in amino acid sequence and/or one or more differences in chemical moieties (e.g., carbohydrates, lipids, etc.) covalently attached to the polypeptide backbone. In some embodiments, a "*variant polypeptide*" shows an overall sequence identity with a reference polypeptide that is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99%. Alternatively or additionally, in some embodiments, a "*variant polypeptide*" does not share at least one characteristic sequence element with a reference polypeptide. In some embodiments, the reference polypeptide has one or more biological activities. In some embodiments, a "*variant polypeptide*" shares one or more of the biological activities of the reference polypeptide. In some embodiments, a "*variant polypeptide*" lacks one or more of the biological activities of the reference polypeptide. In some embodiments, a "*variant polypeptide*" shows a reduced level of one or more biological activities as compared with the reference polypeptide. In many embodiments, a polypeptide of interest is considered to be a "*variant*" of a parent or reference polypeptide if the polypeptide of interest has an amino acid sequence that is identical to that of the parent but for a small number of sequence alterations at particular positions. Typically, fewer than 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% of the residues in the variant are substituted as compared with the parent. In some embodiments, a "*variant*" has 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 substituted residue as compared with a parent. Often, a "*variant*" has a very small number (e.g., fewer than 5, 4, 3, 2, or 1) of substituted functional residues (i.e., residues that participate in a particular

biological activity). Furthermore, a "*variant*" typically has not more than 5, 4, 3, 2, or 1 additions or deletions, and often has no additions or deletions, as compared with the parent. Moreover, any additions or deletions are typically fewer than about 25, about 20, about 19, about 18, about 17, about 16, about 15, about 14, about 13, about 10, about 9, about 8, about 7, about 6, and commonly are fewer than about 5, about 4, about 3, or about 2 residues. In some embodiments, the parent or reference polypeptide is one found in nature. As will be understood by those of ordinary skill in the art, a plurality of variants of a particular polypeptide of interest may commonly be found in nature, particularly when the polypeptide of interest is an infectious agent polypeptide.

[00101] ***Vector***: as used herein, refers to a nucleic acid molecule capable of transporting another nucleic acid to which it is associated. In some embodiment, vectors are capable of extra-chromosomal replication and/or expression of nucleic acids to which they are linked in a host cell such as a eukaryotic and/or prokaryotic cell. Vectors capable of directing the expression of operatively linked genes are referred to herein as "*expression vectors*".

[00102] ***Wild-type***: as used herein, has its art-understood meaning that refers to an entity having a structure and/or activity as found in nature in a "*normal*" (as contrasted with mutant, diseased, altered, etc.) state or context. Those of ordinary skill in the art will appreciate that wild-type genes and polypeptides often exist in multiple different forms (e.g., alleles).

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

[00103] The present invention provides, among other things, improved and/or engineered non-human animals having heterologous genetic material encoding an Angiopoietin-like protein 8 (ANGPTL8) for determining the therapeutic efficacy of ANGPTL8 modulators (e.g., an anti-ANGPTL8 antibodies) for the treatment of metabolic disorders, and assays measuring lipid (e.g., triglyceride) metabolism, glucose homeostasis, various effects on body weight, composition and energy expenditure. It is contemplated that such non-human animals provide an improvement in determining the therapeutic efficacy of ANGPTL8 modulators and their potential for ANGPTL8 blockade. Therefore, the present invention is particularly useful for the development of anti-ANGPTL8 therapies for the treatment of diseases, disorders or conditions that result from or are characterized by various metabolic disorders, including triglyceride dysfunction, glucose intolerance and dyslipidemia (Zhang and Abou-Samra,

Cardiovascular Diabetology 2014, 13:133). In particular, the present invention encompasses the engineering of a non-human (e.g., murine) *Angptl8* gene resulting in expression of a human ANGPTL8 polypeptide in the serum of the non-human animal. Such non-human animals have the capacity to provide an *in vivo* animal model for determining the efficacy of anti-ANGPTL8 therapeutics in the treatment of metabolic disorders and/or cardiovascular diseases, disorders and/or conditions. In some embodiments, non-human animals as described herein demonstrate augmented triglyceride levels as compared to wild-type non-human animals. In some embodiments, non-human animals as described herein provide an *in vivo* animal model for lipoprotein metabolism. In some embodiments, non-human animals as described herein provide an *in vivo* animal model for hypertriglyceridemia.

[00104] In some embodiments, Angptl8 polypeptides expressed (or secreted) by a non-human animal as described herein comprise a sequence corresponding to amino acids 22-60, 77-134, 156-193 or 22-198 of a human ANGPTL8 polypeptide. In some embodiments, Angptl8 polypeptides encoded by genetic material within the genome of non-human animals described herein comprise a sequence corresponding to the signal peptide a murine Angptl8 polypeptide. In some embodiments, non-human animals as described herein comprise, at an endogenous *Angptl8* locus, an *Angptl8* gene that contains genetic material from the non-human animal and a heterologous species (e.g., a human). In some embodiments, non-human animals as described herein comprise an engineered *Angptl8* gene, wherein the engineered *Angptl8* gene comprises exons 1-4 of a human *ANGPTL8* gene, in whole or in part. In some embodiments, non-human animals as described herein comprise an engineered *Angptl8* gene, wherein the engineered *Angptl8* gene comprises the coding portion of exon 1 and exons 2-4 of a human *ANGPTL8* gene. In some embodiments, non-human animals as described herein comprise an engineered *Angptl8* gene, wherein the engineered *Angptl8* gene comprises the coding portion of exon 1 (or the coding portion of exon 1 excluding the start codon), exon 2, exon 3 and exon 4 (which includes the 3' UTR) of a human *ANGPTL8* gene. In some certain embodiments, non-human animals as described herein comprise an engineered *Angptl8* gene, wherein the engineered *Angptl8* gene comprises ~2,383bp of a human *ANGPTL8* gene corresponding to the coding portion of exon 1 beginning from immediately after the start codon through exon 4 including the 3' UTR (e.g., ~256 bp) of a human *ANGPTL8* gene. In some embodiments, non-human animals as described herein comprise an engineered *Angptl8*

gene at an endogenous *Angptl8* locus, wherein the engineered *Angptl8* gene comprises the 5' UTR of an endogenous *Angptl8* gene, the coding portion of exon 1, exon 2, exon 3 and exon 4 (which includes the 3' UTR) of a human *ANGPTL8* gene, operably linked to an endogenous *Angptl8* promoter; and in some embodiments, in creating such an engineered *Angptl8* gene, the coding portion of exon 1, exons 2-3, and the coding portion of exon 4 of the endogenous *Angptl8* gene at said endogenous *Angptl8* locus have been deleted. In various embodiments, non-human animals as described herein do not detectably express an endogenous Angptl8 polypeptide, in whole or in part.

[00105] Various aspects of the invention are described in detail in the following sections. The use of sections is not meant to limit the invention. Each section can apply to any aspect of the invention. In this application, the use of "or" means "and/or" unless stated otherwise.

Angiopoietin-like protein 8 (ANGPTL8)

[00106] ANGPTL8 (also referred to as TD26, RIFL, Lipasin, C19orf80 and Betatrophin) is a newly recognized ANGPTL family member that has been implicated in both triglyceride and glucose metabolism. Phylogenetic analysis has revealed that ANGPTL8 is closely related to ANGPTL3 and ANGPTL4 (Fu, Z. et. al., 2013, Biochem. Biophys. Res. Commun. 430:1126-31; Quagliarini F. et al; 2012, PNAS 109:19751-19756). ANGPTL8 is a secreted polypeptide expressed primarily in liver and adipose tissue, and, unlike related family members ANGPTL3 and ANGPTL4, lacks a C-terminal fibrinogen-like domain, but contains an N-terminal coiled-coil domain, much like other ANGPTL family members (Mattijssen F.,and Kersten S, Biochim Biophys Acta 1821, 2012:782-789).

[00107] Hepatic overexpression of ANGPTL8 is associated with hypertriglyceridemia, whereas inactivation of Angptl8 causes a reduction in plasma triglyceride levels (Quagliarini, F. et. al., 2012, Proc. Natl. Acad. Sci. USA 109(48):19751-6; Wang, Y. et. al., 2013, Proc. Natl. Acad. Sci. USA 110:16109-14). Despite reports that ANGPTL8 is involved in the regulation of lipids, the responsible mechanism is still under debate. To give but one example, one mechanism reasons that ANGPTL8 inhibits lipoprotein lipase activity, resulting in reduced triglyceride hydrolysis and clearance (Zhang, R. et.al., 2012, Biochem. Biophys. Res. Commun. 424:786-92). ANGPTL8 has also been reported to play a role in beta cell proliferation and beta cell mass in mice where insulin resistance was induced by insulin

receptor antagonist S961 (Yi, P. et. al. 2013, Cell 153:747-58). However, subsequent studies have revealed that ANGPTL8 is not required for beta cell function or the beta cell growth response to insulin resistance. Further, overexpression of ANGPTL8 does not increase beta cell area or improve glycemic control (Gusarova, V. et. al., 2014, Cell 159:691-6). Since hepatic overexpression of ANGPTL8 is associated with hypertriglyceridemia and inactivation of Angptl8 results in a reduction in plasma triglyceride levels, an inhibitor or antagonist of ANGPTL8 may prove effective in treating a disease characterized, in part, by elevated triglyceride levels, such as, but not limited to, hypertriglyceridemia. According to one report using wild-type mice, a monoclonal antibody to lipasin decreased serum triglyceride levels when injected intraperitoneally (Zhang, R., 2015, Endocrine Society's 97th Annual Meeting, Presentation No. OR13-6, March 5-8, San Diego, CA).

[00108] A more thorough and detailed understanding of ANGPTL8-mediated functions and the ANGPTL8 pathway in lipid metabolism, glucose homeostasis, effect on body weight, body composition, energy expenditure and cardiovascular function, is needed to develop practical targeted therapies for future treatment of human patients suffering from hypertriglyceridemia and other diseases, disorders or conditions characterized by elevated triglyceride and lipid levels.

ANGPTL8 sequences

[00109] ANGPTL8 (also referred to as TD26, RIFL, Lipasin, C19orf80 and Betatrophin) is a member of the Angiopoietin family of proteins. "ANGPTL8", as used herein, refers to a human ANGPTL8 polypeptide, and in some embodiments, a human ANGPTL8 polypeptide without a signal peptide (e.g., a polypeptide comprising the amino acid sequence as set forth in 22-198 of SEQ ID NO:6). Exemplary human ANGPTL8 amino acid (including the signal peptide) and mRNA sequences can be found in GenBank accession numbers NP_061157.3 (SEQ ID NO:6) and NM_018687.6 (SEQ ID NO:5), respectively (see Figures 8D and 8E). The N-terminal coiled-coil domains of human ANGPTL8 spans amino acid residues ~77-134 and 156-193 of SEQ ID NO:6.

[00110] Exemplary rodent (e.g., rat and mouse), human and engineered Angptl8 sequences are set forth in Figures 8A-8H. An exemplary synthetic DNA fragment for engineering a non-human *Angptl8* gene as described herein is also set forth in Figure 8I. For mRNA

sequences, bold font indicates coding sequence and consecutive exons, where indicated, are separated by alternating underlined text; for engineered mRNA sequences, human sequences are contained within parentheses. For amino acid sequences, signal sequences are indicated by underlined font.

DNA constructs and Production of Non-Human Animals Having A Humanized ANGPTL8 Gene

[00111] Typically, a polynucleotide molecule containing an *Angptl8 gene* (e.g., a heterologous or engineered *Angptl8* gene), in whole or in part, is inserted into a vector, preferably a DNA vector, in order to replicate the polynucleotide molecule in a suitable host cell.

[00112] Depending on size, an *Angptl8* gene or Angptl8-encoding sequence as can be cloned directly from cDNA sources available from commercial suppliers or designed *in silico* based on published sequences available from GenBank. Alternatively, bacterial artificial chromosome (BAC) libraries can provide heterologous *Angptl8* sequences from genes of interest (e.g., a heterologous *Angptl8* gene). BAC libraries contain an average insert size of 100-150kb and are capable of harboring inserts as large as 300kb (Shizuya, H. et al., 1992, Proc. Natl. Acad. Sci., U.S.A. 89:8794-7; Swiatek, P.J. and T. Gridley, 1993, Genes Dev. 7:2071-84; Kim, U.J. et al., 1996, Genomics 34:213-8; herein incorporated by reference). For example, human and mouse genomic BAC libraries have been constructed and are commercially available (e.g., Invitrogen, Carlsbad Calif.). Genomic BAC libraries can also serve as a source of heterologous *Angptl8* sequences as well as transcriptional control regions.

[00113] Alternatively, heterologous *Angptl8* sequences may be isolated, cloned and/or transferred from yeast artificial chromosomes (YACs). An entire heterologous gene or locus can be cloned and contained within one or a few YACs. If multiple YACs are employed and contain regions of overlapping homology, they can be recombined within yeast host strains to produce a single construct representing the entire locus. YAC arms can be additionally modified with mammalian selection cassettes by retrofitting to assist in introducing the constructs into embryonic stems cells or embryos by methods known in the art and/or described herein.

[00114] Exemplary mRNA and amino acid sequences for use in constructing a humanized *Angptl8* gene in a non-human animal are provided above. Other heterologous *Angptl8* sequences can also be found in the GenBank database or other sequence databases known in the art.

[00115] DNA constructs containing *Angptl8* sequences as described herein, in some embodiments, comprise human *ANGPTL8* genomic (or cDNA) sequences encoding at least about amino acids 22-60, 77-134, 156-193 or 22-198 of a human ANGPTL8 polypeptide operably linked to non-human regulatory sequences (e.g., a rodent promoter) for expression in a transgenic non-human animal. In some embodiments, DNA constructs containing *Angptl8* sequences as described herein comprise human *ANGPTL8* genomic (or cDNA) sequences encoding at least about amino acids 22-60,77-134, 156-193 or 22-198 of a human ANGPTL8 polypeptide operably linked to a non-human *Angptl8* promoter and one or more non-human *Angptl8* untranslated regions (e.g., 5' and/or 3' UTRs). Human and/or non-human *Angptl8* sequences included in DNA constructs described herein may be identical or substantially identical with human and/or non-human *Angptl8* sequences found in nature (e.g., genomic), artificial (e.g., synthetic) or may be engineered by the hand of man. In some embodiments, *Angptl8* sequences are synthetic in origin, and include a sequence or sequences that are found in a human *ANGPTL8* gene found in nature. For example, a DNA construct can include synthetic DNA that corresponds to exons 1-4 of a human *ANGPTL8* gene, and that encodes at least about amino acids 22-60, 77-134, 156-193 or 22-198 of a human ANGPTL8 polypeptide, operably linked to non-human *Angptl8* regulatory (e.g., promoter) and non-coding sequences (e.g., one or more non-human UTRs) so that a Angptl8 polypeptide having a sequence that is all or substantially all human is encoded by the resulting DNA construct. Alternatively, a DNA construct can include synthetic DNA that corresponds to the genetic material that encodes a functional portion of a human ANGPTL8 polypeptide (e.g., one or more coiled-coil domains, an N-terminal region) operably linked to non-human *Angptl8* regulatory (e.g., promoter) and coding sequences (e.g., one or more non-human exons) so that an Angptl8 polypeptide having human and non-human portions is encoded by the resulting DNA construct. In some embodiments, *Angptl8* sequences comprise a sequence naturally associated with a heterologous *Angptl8* gene (e.g., a human *ANGPTL8* gene). In some embodiments, *Angptl8* sequences comprise a sequence that is not naturally associated with a

heterologous *Angptl8* gene. In some embodiments, *Angptl8* sequences comprise a sequence that is optimized for expression in a non-human animal. In some embodiments, heterologous *Angptl8* sequences operably linked to non-human *Angptl8* sequences each encode a portion of an Angptl8 polypeptide that appears in separate polypeptides in nature. If additional sequences are useful in optimizing expression of heterologous *Angptl8* sequences, such sequences can be cloned using existing sequences as probes. Additional sequences necessary for maximizing expression of a heterologous *Angptl8* gene or heterologous *Angptl8*-encoding sequence can be obtained from genomic sequences or other sources depending on the desired outcome.

[00116] DNA constructs can be prepared using methods known in the art. For example, a DNA construct can be prepared as part of a larger plasmid. Such preparation allows the cloning and selection of the correct constructions in an efficient manner as is known in the art. DNA fragments containing one or more nucleotide coding sequences as described herein can be located between convenient restriction sites on the plasmid so that they can be easily isolated from the remaining plasmid sequences for incorporation into the desired animal.

[00117] Various methods employed in preparation of plasmids and host organisms containing them are known in the art. For other suitable expression systems for both prokaryotic and eukaryotic cells, as well as general recombinant procedures, see Principles of Gene Manipulation: An Introduction to Genetic Manipulation, 5th Ed., ed. By Old, R.W. and S.B. Primrose, Blackwell Science, Inc., 1994; Molecular Cloning: A Laboratory Manual, 2nd Ed., ed. by Sambrook, J. et al., Cold Spring Harbor Laboratory Press: 1989.

[00118] Non-human animals are provided that express human ANGPTL8 polypeptides, in whole or in part, in the serum of the non-human animals resulting from a genetic modification of an endogenous locus (e.g., an *Angptl8* locus) of the non-human animal that encodes an Angptl8 polypeptide. Suitable examples described herein include rodents, in particular, mice.

[00119] A humanized *Angptl8* gene, in some embodiments, comprises genetic material from a heterologous species (e.g., humans), wherein the humanized *Angptl8* gene encodes an Angptl8 polypeptide that comprises the encoded portion of the genetic material from the heterologous species. In some embodiments, a humanized *Angptl8* gene as described herein comprises genomic DNA of a heterologous species that encodes an Angptl8 polypeptide that

is expressed in the serum of the non-human animal, wherein the Angptl8 polypeptide has a sequence that is all or substantially all human. Non-human animals, embryos, cells and targeting constructs for making non-human animals, non-human embryos, and cells containing said humanized *Angptl8* gene are also provided.

[00120] In some embodiments, an endogenous *Angptl8* gene is deleted. In some embodiments, an endogenous *Angptl8* gene is altered, wherein a portion of the endogenous *Angptl8* gene is replaced with a heterologous sequence (e.g., a human *ANGPTL8* sequence, in whole or in part). In some embodiments, all or substantially all of an endogenous *Angptl8* gene is replaced with a heterologous gene (e.g., a human *ANGPTL8* gene, in whole or in part). In some embodiments, a portion of a heterologous *Angptl8* gene is inserted into an endogenous non-human *Angptl8* gene at an endogenous *Angptl8* locus. In some embodiments, the heterologous gene is a human gene. In some embodiments, the modification or humanization is made to one of the two copies of an endogenous *Angptl8* gene, giving rise to a non-human animal that is heterozygous with respect to the humanized *Angptl8* gene. In other embodiments, a non-human animal is provided that is homozygous for a humanized *Angptl8* gene.

[00121] In some embodiments, a non-human animal as described herein contains a human *ANGPTL8* gene, in whole or in part, at an endogenous non-human *Angptl8* locus. In some embodiments, a non-human animal as described herein contains a human *ANGPTL8* gene, in whole or in part, at a location other than an endogenous non-human *Angptl8* locus. Thus, non-human animals as described herein can be characterized as having a humanized or heterologous *Angptl8* gene. The replaced, inserted, modified or altered *Angptl8* gene at the endogenous *Angptl8* locus or a polypeptide expressed from such gene can be detected using a variety of methods including, for example, PCR, Western blot, Southern blot, restriction fragment length polymorphism (RFLP), or a gain or loss of allele assay. A humanized or heterologous *Angptl8* gene randomly inserted into the non-human animal genome may be detected by the same or similar means. In some embodiments, a non-human animal as described herein is heterozygous with respect to a humanized or heterologous *Angptl8* gene as described herein.

[00122] In various embodiments, a humanized *Angptl8* gene as described herein includes an *Angptl8* gene that has the coding portion of exon 1 (beginning from or immediately after the ATG start codon to the 3' end of exon 1), and exons 2-4, of a human *ANGPTL8* gene.

[00123] In various embodiments, a humanized *Angptl8* gene as described herein includes an *Angptl8* gene that has a first, second, third and fourth exon each having a sequence at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to a first, second, third and fourth exon that appear in SEQ ID NO:5 or SEQ ID NO:7.

[00124] In various embodiments, a humanized *Angptl8* gene as described herein includes a *Angptl8* gene that has a first, second, third and fourth exon each having a sequence that is substantially identical or identical to a first, second, third and fourth exon that appear in SEQ ID NO:5 or SEQ ID NO:7.

[00125] In various embodiments, a humanized *Angptl8* gene as described herein comprises a sequence at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to SEQ ID NO:10 or SEQ ID NO:11.

[00126] In various embodiments, a humanized *Angptl8* gene as described herein comprises a sequence that is substantially identical or identical to SEQ ID NO:10 or SEQ ID NO:11.

[00127] In various embodiments, a humanized *Angptl8* gene as described herein is or comprises SEQ ID NO:10 or SEQ ID NO:11.

[00128] In various embodiments, a humanized *Angptl8* gene as described herein comprises a 5' untranslated region having a sequence at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to a 5' untranslated region that appears in SEQ ID NO:1 or SEQ ID NO:3, and/or a 3' untranslated region having a sequence at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to a 3' untranslated region that appears in SEQ ID NO:1 or SEQ ID NO:3.

[00129] In various embodiments, a humanized *Angptl8* gene as described herein comprises a 5' untranslated region having a sequence that is substantially identical or identical to a 5'

untranslated region that appears in SEQ ID NO:1 or SEQ ID NO:3, and/or a 3' untranslated region having a sequence that is substantially identical or identical to a 3' untranslated region that appears in SEQ ID NO:1 or SEQ ID NO:3.

[00130] In various embodiments, a humanized *Angptl8* gene as described herein comprises a 5' untranslated region having a sequence that is substantially identical or identical to the 5' untranslated region of an endogenous non-human *Angptl8* gene, and/or a 3' untranslated region having a sequence that is substantially identical or identical to the 3' untranslated region of a human *ANGPTL8* gene. In particular embodiments, a humanized *Angptl8* gene as described herein comprises a 5' untranslated region having a sequence that is substantially identical or identical to a 5' untranslated region that appears in SEQ ID NO:1 or SEQ ID NO:3, and/or a 3' untranslated region having a sequence that is substantially identical or identical to the 3' untranslated region that appears in SEQ ID NO:5 or SEQ ID NO:7.

[00131] In specific embodiments, a humanized *Angptl8* gene as described herein comprises a 5' untranslated region of an endogenous non-human (e.g., mouse or rat) *Angptl8* gene, the coding portion of exon 1 of a human *ANGPTL8* gene, and exons 2-4 of a human *ANGPTL8* gene, which include the 3' UTR of the human *ANGPTL8* gene.

[00132] In various embodiments, a humanized *Angptl8* gene as described herein comprises a nucleotide coding sequence (e.g., a cDNA sequence) at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to a nucleotide coding sequence that appears in SEQ ID NO:5 or SEQ ID NO:7.

[00133] In various embodiments, a humanized *Angptl8* gene as described herein comprises a nucleotide coding sequence (e.g., a cDNA sequence) that is substantially identical or identical to a nucleotide coding sequence that appears in SEQ ID NO:5 or SEQ ID NO:7.

[00134] In various embodiments, a humanized *Angptl8* gene as described herein encodes an Angptl8 polypeptide that is identical or substantially identical to a human ANGPTL8 polypeptide. In various embodiments, a humanized *Angptl8* gene as described herein encodes an Angptl8 polypeptide that is identical or substantially identical to a full-length human ANGPTL8 protein translated from a human *ANGPTL8* gene (which includes a human

ANGPTL8 signal peptide, or the first 21 amino acids of a human ANGPTL8 full-length protein).

[00135] In various embodiments, a humanized *Angptl8* gene as described herein encodes an Angptl8 polypeptide having an amino acid sequence at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to an amino acid sequence that appears in SEQ ID NO:6 or SEQ ID NO:8.

[00136] In various embodiments, a humanized *Angptl8* gene as described herein encodes an Angptl8 polypeptide having an amino acid sequence that is substantially identical or identical to an amino acid sequence that appears in SEQ ID NO:6 or SEQ ID NO:8.

[00137] In various embodiments, an Angptl8 polypeptide produced by a non-human animal as described herein comprises an amino acid sequence at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to amino acid residues 22-198 of SEQ ID NO:6 or SEQ ID NO:8.

[00138] In various embodiments, an Angptl8 polypeptide produced by a non-human animal as described herein comprises an amino acid sequence that is substantially identical or identical to amino acid residues 22-198 of SEQ ID NO:6 or SEQ ID NO:8.

[00139] In various embodiments, an Angptl8 polypeptide produced by a non-human animal as described herein comprises an amino acid sequence at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to amino acid residues 77-134 of SEQ ID NO:6 or SEQ ID NO:8.

[00140] In various embodiments, an Angptl8 polypeptide produced by a non-human animal as described herein comprises an amino acid sequence that is substantially identical or identical to amino acid residues 77-134 of SEQ ID NO:6 or SEQ ID NO:8.

[00141] In various embodiments, an Angptl8 polypeptide produced by a non-human animal as described herein comprises an amino acid sequence at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to amino acid residues 156-193 of SEQ ID NO:6 or SEQ ID NO:8.

[00142] In various embodiments, an Angptl8 polypeptide produced by a non-human animal as described herein comprises an amino acid sequence that is substantially identical or identical to amino acid residues 156-193 of SEQ ID NO:6 or SEQ ID NO:8.

[00143] In various embodiments, an Angptl8 polypeptide produced by a non-human animal as described herein comprises an amino acid sequence at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to amino acid residues 22-60 of SEQ ID NO:6 or SEQ ID NO:8.

[00144] In various embodiments, an Angptl8 polypeptide produced by a non-human animal as described herein comprises an amino acid sequence that is substantially identical or identical to amino acid residues 22-60 of SEQ ID NO:6 or SEQ ID NO:8.

[00145] In various embodiments, an Angptl8 polypeptide produced by a non-human animal as described herein includes one or more coiled-coil domains, wherein said one or more coiled-coil domains comprise an amino acid sequence at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to one or more coiled-coil domains that appear in SEQ ID NO:6 or SEQ ID NO:8.

[00146] In various embodiments, an Angptl8 polypeptide produced by a non-human animal as described herein includes one or more coiled-coil domains, wherein said one or more coiled-coil domain comprise an amino acid sequence that is substantially identical or identical to one or more coiled-coil domains that appear in SEQ ID NO:6 or SEQ ID NO:8.

[00147] In various embodiments, an Angptl8 polypeptide produced by a non-human animal as described herein has an N-terminal region, which N-terminal region comprises an amino acid sequence at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to an N-terminal region that appears in SEQ ID NO:6 or SEQ ID NO:8.

[00148] In various embodiments, an Angptl8 polypeptide produced by a non-human animal as described herein has an N-terminal region, which N-terminal region comprises an amino acid sequence that is substantially identical or identical to an N-terminal region that appears in SEQ ID NO:6 or SEQ ID NO:8.

[00149] Compositions and methods for making non-human animals that express a human or humanized Angptl8 polypeptide, including specific polymorphic forms, allelic variants (e.g., single amino acid differences) or alternatively spliced isoforms, are provided, including compositions and methods for making non-human animals that express such polypeptides from a human promoter and a human regulatory sequence. In some embodiments, compositions and methods for making non-human animals that express such proteins from a non-human promoter and a non-human regulatory sequence are also provided. In some embodiments, compositions and methods for making non-human animals that express such proteins from an endogenous promoter and an endogenous regulatory sequence are also provided. In some certain embodiments, endogenous promoters and endogenous regulatory sequences are endogenous rodent promoters and endogenous rodent regulatory sequences. The methods include inserting the genetic material encoding a human ANGPTL8 polypeptide, in whole or in part, at a precise location in the genome of a non-human animal that corresponds to an endogenous *Angptl8* gene thereby creating a humanized *Angptl8* gene that expresses an Angptl8 polypeptide that is human in whole or in part. In some embodiments, methods include inserting genomic DNA corresponding to exons 1-4 of a human *ANGPTL8* gene, in whole or in part, into an endogenous Angptl8 gene of the non-human animal thereby creating a humanized gene that encodes an Angptl8 polypeptide that contains a human portion containing amino acids encoded by the inserted exons.

[00150] Where appropriate, the coding region of the genetic material or polynucleotide sequence(s) encoding a human (or humanized) ANGPTL8 polypeptide, in whole or in part, may be modified to include codons that are optimized for expression from cells in the non-human animal (e.g., see U.S. Patent No.'s 5,670,356 and 5,874,304). Codon optimized sequences are synthetic sequences, and preferably encode the identical polypeptide (or a biologically active fragment of a full length polypeptide which has substantially the same activity as the full length polypeptide) encoded by the non-codon optimized parent polynucleotide. In some embodiments, the coding region of the genetic material encoding a human (or humanized) ANGPTL8 polypeptide, in whole or in part, may include an altered sequence to optimize codon usage for a particular cell type (e.g., a rodent cell). For example, the codons of the genomic DNA corresponding to exons 1-4 of a human *ANGPTL8* gene, in whole or in part, to be inserted into an endogenous *Angptl8* gene of a non-human animal (e.g.,

a rodent) may be optimized for expression in a cell of the non-human animal. Such a sequence may be described as a codon-optimized sequence.

[00151] Methods for generating transgenic non-human animals, including knockouts and knock-ins, are well known in the art (see, e.g., Gene Targeting: A Practical Approach, Joyner, ed., Oxford University Press, Inc. (2000)). For example, generation of transgenic rodents may optionally involve disruption of the genetic loci of one or more endogenous rodent genes (or gene segments) and introduction of one or more heterologous genes (or *Angptl8*-encoding sequences) into the rodent genome, in some embodiments, at the same location as an endogenous rodent gene (or gene segments).

[00152] In some embodiments, heterologous (e.g., human or humanized) *Angptl8* genes or heterologous *Angptl8*-encoding sequences as described herein are randomly introduced in the genome of a rodent. In such embodiments, rodents comprising, containing or otherwise harboring randomly introduced heterologous (or humanized *Angptl8* genes or heterologous *Angptl8*-encoding sequences) can be characterized as having a heterologous *Angptl8* transgene or heterologous *Angptl8* transgene construct. Typically, a transgene and/or transgene construct includes, among other things, a nucleic acid sequence (encoding e.g., a polypeptide of interest, in whole or in part) that is introduced into a non-human cell (e.g., a rodent embryonic stem cell) by the hand of man using methods described herein or otherwise known in the art. Further, a transgene may be partly or entirely heterologous, i.e., foreign, to a non-human animal or cell into which it is introduced. A transgene can further include one or more transcriptional regulatory sequences and any other nucleic acid, such as introns or promoters (e.g., constitutive, tissue-specific, etc.), which may be necessary for expression of a selected nucleic acid sequence. In some embodiments, heterologous (or humanized) *Angptl8* genes or heterologous *Angptl8*-encoding sequences as described herein are introduced into an endogenous *Angptl8* gene in the genome of a rodent; in some certain embodiments, an endogenous *Angptl8* gene locus is altered, modified, or engineered to contain human *ANGPTL8* sequences (or gene fragments) operably linked to one or more non-human *Angptl8* sequences (or gene fragments).

[00153] As described herein, heterologous (or humanized) *Angptl8* genes or heterologous *Angptl8*-encoding sequences are operably linked to expression control sequences such as a

promoter to drive expression of the heterologous (or humanized) *Angptl8* in the non-human animals. In some embodiments, such promoters are non-human *Angptl8* promoters (e.g., a rodent *Angptl8* promoters). Persons of skill upon reading this disclosure will recognize that other non-human promoters may be operably linked to heterologous *Angptl8* sequences inserted into the genome of non-human animals as described herein regardless if such heterologous *Angptl8* sequences are placed at the same location as an endogenous non-human gene or randomly integrated in the genome of the non-human animal. In some embodiments, a non-human promoter is or comprises a constitutive promoter. In some embodiments, a non-human promoter is or comprises a viral promoter (e.g., simian virus promoter, herpes simplex virus promoter, papilloma virus promoter, adenovirus promoter, retrovirus promoter, etc.). In some embodiments, a non-human promoter is a mammalian promoter. Suitable examples of promoters that can be used in accordance with the present invention include, but are not limited to, SRα promoters, human or murine CMV promoters, EF1α promoters and SV40 early promoter regions. Other promoters that control expression of desired polypeptides in a tissue-specific manner are known in the art and can be employed in the methods described herein as desired. Further, promoters may be selected depending on a desired cell type for expression. Exemplary promoters can be found in, e.g., Villa-Komaroff et al., 1978, Proc. Natl. Acad. Sci. U.S.A. 75:3727-31; Benoist et al., 1981, Nature 290:304-10; Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-5; Brinster et al., 1982, Nature 296:39-42; DeBoer et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-5; Boshart et al., 1985, Cell 41:521-30; Foecking et al., 1986, Gene 45:101-5; Takebe et al., 1988, Mol. Cell. Bio. 8:466-72.

[00154] A humanized *Angptl8* gene approach employs a relatively minimal modification of the endogenous protein interactions and signaling and results in natural Angptl8-mediated functions and/or activity in the non-human animal, in various embodiments, because the genomic sequence of the *Angptl8* sequences are modified in a single fragment and therefore retain normal functionality by including necessary regulatory sequences. Further, in various embodiments, the modification does not affect the secretion of a functional Angptl8 polypeptide in the serum and maintains normal functions and/or interactions via binding to various lipids (e.g., triglycerides).

[00155] A schematic illustration (not to scale) of the genomic organization of an endogenous murine *Angptl8* gene and a human *ANGPTL8* gene is provided in Figure 1. An exemplary method for humanizing an endogenous murine *Angptl8* gene using a genomic fragment containing exons 1-4 and a 3' UTR of a human *ANGPTL8* gene is provided in Figure 3. As illustrated, a 2,383bp synthetic DNA fragment corresponding to exons 1-4 and a 3' UTR of a human *ANGPTL8* gene is inserted into the place of a 1,576bp sequence of an endogenous murine *Angptl8* gene locus via homologous recombination with a targeting construct. The 2,383bp synthetic DNA fragment may be cloned directly from human DNA or synthesized from a source sequence (e.g., GenBank accession no. NM_018687.6, SEQ ID NO:5). This genomic DNA includes the portion of the gene that encodes at least about amino acid residues 22-198 of a human ANGPTL8 polypeptide responsible for lipid binding.

[00156] A non-human animal (e.g., a mouse) having a humanized *Angptl8* gene at an endogenous Angptl8 locus can be made by any method known in the art. For example, a targeting vector can be made that introduces a human *ANGPTL8* gene, in whole or in part, with a selectable marker gene. Figure 3 illustrates a targeting vector that contains an endogenous *Angptl8* locus of a mouse genome comprising an insertion of a 2,383bp synthetic DNA fragment that corresponds to exons 1-4 (specifically, the coding portion of exon 1, exon 2, exon 3 and exon 4 which includes the 3' UTR) of a human *ANGPTL8* gene. As illustrated, the targeting construct contains a 5' homology arm containing sequence upstream of exon 1 (i.e., including the ATG start codon) of an endogenous murine *Angptl8* gene (~79kb), followed by the 2,383bp synthetic DNA fragment, a drug selection cassette (e.g., a neomycin resistance gene flanked on both sides by *lox*P sequences; ~5kb), and a 3' homology arm containing the 3' UTR of an endogenous murine *Angptl8* gene (~148kb). The targeting construct contains a self-deleting drug selection cassette (e.g., a neomycin resistance gene flanked by *lox*P sequences; see U.S. Patent Nos. 8,697,851, 8,518,392 and 8,354,389, all of which are incorporated herein by reference). Upon electroporation in embryonic stem cells, a modified endogenous *Angptl8* gene is created that includes 2,383bp of a human *ANGPTL8* gene (i.e., the coding portion of exon 1, exon 2, exon 3 and exon 4 which includes the 3' UTR) in the place of 1,576bp of an endogenous wild-type *Angptl8* gene, which is contained in the targeting vector. A humanized *Angptl8* gene is created resulting in a cell or non-human animal that expresses a humanized Angptl8 polypeptide that contains amino acids encoded by

the 2,383bp synthetic DNA fragment. The drug selection cassette is removed in a development-dependent manner, i.e., progeny derived from mice whose germ line cells containing the humanized *Angptl8* gene described above will shed the selectable marker from differentiated cells during development (see bottom of Figure 3).

[00157] Exemplary promoters than can be operably linked with drug selection cassettes and/or recombinase genes included in targeting vectors described herein are provided below. Additional suitable promoters that can be used in targeting vectors described herein include those described in U.S. Patent No.'s 8,697,851, 8,518,392 and 8,354,389; all of which are incorporated herein by reference). Exemplary promoter sequences include a Protamine 1 (Prm1) promoter (SEQ ID NO:12), a Blimp1 promoter 1kb (SEQ ID NO:13), and a Blimp1 promoter 2kb (SEQ ID NO:14).

[00158] In some embodiments, a non-human animal having a humanized *Angptl8* gene as described herein can be characterized as transgenic for the humanized *Angptl8* gene or a transgenic *Angptl8* non-human animal. Such descriptions are used interchangeably herein and refer to any non-naturally occurring non-human animal in which one or more of the cells of the non-human animal contain a heterologous *Angptl8* nucleic acid sequence and/or *Angptl8*-encoding sequence, in whole or in part, as described herein. In some embodiments, a heterologous *Angptl8* nucleic acid sequence and/or *Angptl8*-encoding sequence, in whole or in part, is introduced into a cell, directly or indirectly by introduction into a precursor cell, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus. In such embodiments, genetic manipulation does not include classic breeding techniques, but rather is directed to introduction of recombinant DNA molecule(s) that contain a heterologous *Angptl*8 nucleic acid sequence and/or *Angptl8*-encoding sequence, in whole or in part, as described herein. Such a molecule may be integrated within a chromosome, or it may be extrachromosomally replicating DNA. As described herein, transgenic non-human animals includes animals that are heterozygous or homozygous for a heterologous *Angptl8* nucleic acid sequence and/or *Angptl8*-encoding sequence, in whole or in part, and/or animals that have single or multiple copies of a heterologous *Angptl8* nucleic acid sequence and/or *Angptl8*-encoding sequence, in whole or in part, as described herein.

[00159] A transgenic founder non-human animal can be identified based upon the presence of a humanized *Angptl8* gene in its genome and/or expression of Angptl8 polypeptides containing amino acids encoded by the inserted genetic material in tissues or cells of the non-human animal. A transgenic founder non-human animal can then be used to breed additional non-human animals carrying the humanized *Angptl8* gene thereby creating a series of non-human animals each carrying one or more copies of a humanized *Angptl8* gene. Moreover, transgenic non-human animals carrying a humanized *Angptl8* gene can further be bred to other transgenic non-human animals carrying other transgenes (e.g., human immunoglobulin genes) as desired.

[00160] Transgenic non-human animals may also be produced to contain selected systems that allow for regulated or directed expression of the humanized *Angptl8* gene (or humanized *Angptl8* transgene). Exemplary systems include the Cre/*lox*P recombinase system of bacteriophage P1 (see, e.g., Lakso, M. et al., 1992, Proc. Natl. Acad. Sci. U.S.A. 89:6232-6) and the FLP/Frt recombinase system of S. cerevisiae (O'Gorman, S. et al, 1991, Science 251:1351-5). Such animals can be provided through the construction of "double" transgenic animals, e.g., by mating two transgenic animals, one containing a transgene comprising a selected modification (e.g., a humanized *Angptl8* gene or transgene) and the other containing a transgene encoding a recombinase (e.g., a Cre recombinase).

[00161] Although embodiments employing a humanized *Angptl8* gene in a mouse (i.e., a mouse with a *Angptl8* gene that encodes a Angptl8 polypeptide having a human sequence, in whole or in part) are extensively discussed herein, other non-human animals that comprise a humanized *Angptl8* gene are also provided. In some embodiments, such non-human animals comprise a humanized *Angptl8* gene operably linked to a rodent *Angptl8* promoter. In some embodiments, such non-human animals comprise a humanized *Angptl8* gene operably linked to an endogenous *Angptl8* promoter; in some embodiments, an endogenous rodent *Angptl8* promoter. In some embodiments, such non-human animals express a humanized Angptl8 polypeptide from an endogenous locus, wherein the humanized Angptl8 polypeptide comprises at least amino acid residues 22-60, 77-134, 156-193 or 22-198 of a human ANGPTL8 polypeptide. Such non-human animals include any of those which can be genetically modified to express a Angptl8 polypeptide as disclosed herein, including, e.g., mammals, e.g., mouse, rat, rabbit, pig, bovine (e.g., cow, bull, buffalo), deer, sheep, goat,

chicken, cat, dog, ferret, primate (e.g., marmoset, rhesus monkey), etc. For example, for those non-human animals for which suitable genetically modifiable ES cells are not readily available, other methods are employed to make a non-human animal comprising the genetic modification. Such methods include, e.g., modifying a non-ES cell genome (e.g., a fibroblast or an induced pluripotent cell) and employing somatic cell nuclear transfer (SCNT) to transfer the genetically modified genome to a suitable cell, e.g., an enucleated oocyte, and gestating the modified cell (e.g., the modified oocyte) in a non-human animal under suitable conditions to form an embryo.

[00162] For example, a rat pluripotent and/or totipotent cell can be from any rat strain, including, for example, an ACI rat strain, a Dark Agouti (DA) rat strain, a Wistar rat strain, a LEA rat strain, a Sprague Dawley (SD) rat strain, or a Fischer rat strain such as Fisher F344 or Fisher F6. Rat pluripotent and/or totipotent cells can also be obtained from a strain derived from a mix of two or more strains recited above. For example, a rat pluripotent and/or totipotent cell can be from a DA strain or an ACI strain. An ACI rat strain is characterized as having black agouti, with white belly and feet and an $RT1^{avl}$ haplotype. Such strains are available from a variety of sources including Harlan Laboratories. An example of a rat ES cell line from an ACI rat is an ACI.G1 rat ES cell. A Dark Agouti (DA) rat strain is characterized as having an agouti coat and an $RT1^{avl}$ haplotype. Such rats are available from a variety of sources including Charles River and Harlan Laboratories. Examples of a rat ES cell line from a DA rat are the DA.2B rat ES cell line and the DA.2C rat ES cell line. In some cases, rat pluripotent and/or totipotent cells are from an inbred rat strain. See, e.g., U.S. Patent Application Publication No. 2014-0235933 A1, incorporated herein by reference.

[00163] Methods for modifying a non-human animal genome (e.g., a pig, cow, rodent, chicken, etc. genome) include, e.g., employing a zinc finger nuclease (ZFN), a transcription activator-like effector nuclease (TALEN), or a Cas protein (i.e., a CRISPR/Cas system) to modify a genome to include a humanized *Angptl8* gene.

Methods Employing Non-Human Animals Having A Humanized ANGPTL8 Gene

[00164] The present invention is, among other things, based on the recognition that the creation of an *in vivo* system that exploits regulatory molecules of lipid metabolism can be made using a humanized *Angptl8* gene as described herein. Such an *in vivo* system allows for

the development of therapeutics and/or therapeutic regimens that focus on ameliorating the effects of lipid dysfunction in human patients. Further, such an *in vivo* system also provides for the development of therapeutics and/or therapeutic regimens that focus on altering Angiopoietin-associated regulation of lipid metabolism in hypertriglyceridemia and/or cardiovascular diseases, disorders or conditions.

[00165] Non-human animals as described herein provide an improved *in vivo* system and source of biological materials (e.g., cells) expressing human (or humanized) ANGPTL8 that are useful for a variety of assays. In various embodiments, non-human animals as described herein are used to develop therapeutics that target human ANGPTL8 and/or modulate human ANGPTL8 signaling (e.g., disrupting interactions with human ANGPTL8 binding partners, such as ANGPTL3). In various embodiments, non-human animals as described herein are used to screen and develop candidate therapeutics (e.g., antibodies) that block interaction of human ANGPTL8 with human ANGPTL3. In various embodiments, non-human animals as described herein are used to determine the binding profile of antagonists and/or agonists of human ANGPTL8 in a non-human animal as described herein; in some embodiments, non-human animals as described herein are used to determine the epitope or epitopes of one or more candidate therapeutic antibodies that bind human ANGPTL8.

[00166] In various embodiments, non-human animals as described herein are used to determine the pharmacokinetic profiles of anti-ANGPTL8 antibodies. In various embodiments, one or more non-human animals as described herein and one or more control or reference non-human animals are each exposed to one or more candidate therapeutic anti-ANGPTL8 antibodies at various doses (e.g., 0.1 mg/kg, 0.2 mg/kg, 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg, 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/mg, 7.5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 40 mg/kg, or 50 mg/kg or more). Candidate therapeutic antibodies may be dosed via any desired route of administration including parenteral and non-parenteral routes of administration. Parenteral routes include, e.g., intravenous, intraarterial, intraportal, intramuscular, subcutaneous, intraperitoneal, intraspinal, intrathecal, intracerebroventricular, intracranial, intrapleural or other routes of injection. Non-parenteral routes include, e.g., oral, nasal, transdermal, pulmonary, rectal, buccal, vaginal, ocular. Administration may also be by continuous infusion, local administration, sustained release from implants (gels, membranes or the like), and/or intravenous injection. Blood is isolated

from non-human animals (humanized and control) at various time points (e.g., 0 hr, 6 hr, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, or up to 30 or more days). Various assays may be performed to determine the pharmacokinetic profiles of administered candidate therapeutic antibodies using samples obtained from non-human animals as described herein including, but not limited to, total IgG, anti-therapeutic antibody response, agglutination, etc.

[00167] In various embodiments, non-human animals as described herein are used to measure the therapeutic effect of blocking or modulating human ANGPTL8 signaling and the effect on gene expression as a result of cellular changes. In various embodiments, a non-human animal as described herein or cells isolated therefrom are exposed to a candidate therapeutic that binds a human ANGPTL8 polypeptide (or a portion of a human ANGPTL8 polypeptide) in the non-human animal and, after a subsequent period of time, analyzed for effects on ANGPTL8-dependent processes, for example, triglyceride metabolism, lipoprotein lipase activity and uptake of various lipoproteins (e.g., low-density lipoprotein, LDL).

[00168] Non-human animals as described herein express human (or humanized) ANGPTL8 polypeptide, thus cells, cell lines, and cell cultures can be generated to serve as a source of human ANGPTL8 for use in binding and functional assays, e.g., to assay for binding or function of a ANGPTL8 antagonist or agonist, particularly where the antagonist or agonist is specific for a human ANGPTL8 sequence or epitope or, alternatively, specific for a human ANGPTL8 sequence or epitope that associates with ANGPTL3. In various embodiments, ANGPTL8 epitopes bound by candidate therapeutic antibodies can be determined using cells isolated from non-human animals as described herein. In various embodiments, a human (or humanized) ANGPTL8 polypeptide expressed by a non-human animal as described herein may comprise a variant amino acid sequence. In various embodiments, non-human animals as described herein express a human (or humanized) ANGPTL8 variant. In various embodiments, the variant is polymorphic at an amino acid position associated with ligand binding. In various embodiments, non-human animals as described herein are used to determine the effect of ligand binding through interaction with a polymorphic variant of human ANGPTL8. Exemplary variant human ANGPTL8 polypeptides include a variant characterized by an R59W (Quagliarini, F. et al., 2012, Proc. Nat. Acad. Sci. U.S.A. 109(48):19751-6) or Q121X (Clapham et al., BMC Endocr Disord.

2016, 16:7) amino acid substitution. In some embodiments, a variant human ANGPTL8 polypeptide is associated with lower plasma low-density lipoprotein (LDL)-cholesterol and/or high-density lipoprotein (HDL)-cholesterol levels. In some embodiments, a variant human ANGPTL8 polypeptide is associated with lower plasma triglycerides and/or HDL-cholesterol levels.

[00169] Cells from non-human animals as described herein can be isolated and used on an ad hoc basis, or can be maintained in culture for many generations. In various embodiments, cells from a non-human animal as described herein are immortalized (e.g., via use of a virus) and maintained in culture indefinitely (e.g., in serial cultures).

[00170] Non-human animals as described herein provide an *in vivo* system for assessing the pharmacokinetic properties of a drug (e.g., an ANGPTL8 modulator). In various embodiments, a drug may be delivered or administered to one or more non-human animals as described herein, followed by monitoring of, or performing one or more assays on, the non-human animals (or cells isolated therefrom) to determine the effect of the drug on the non-human animal. Pharmacokinetic properties include, but are not limited to, how an animal processes the drug into various metabolites (or detection of the presence or absence of one or more drug metabolites, including, toxic metabolites), drug half-life, circulating levels of drug after administration (e.g., serum concentration of drug), anti-drug response (e.g., anti-drug antibodies), drug absorption and distribution, route of administration, routes of excretion and/or clearance of the drug. In some embodiments, pharmacokinetic and pharmacodynamic properties of drugs are monitored in or through the use of non-human animals as described herein.

[00171] In some embodiments, performing an assay includes determining the effect on the phenotype and/or genotype of the non-human animal to which the drug is administered. In some embodiments, performing an assay includes determining lot-to-lot variability for a drug. In some embodiments, performing an assay includes determining the differences between the effects of a drug administered to a non-human animal as described herein and a reference non-human animal. In various embodiments, reference non-human animals may have a modification as described herein, a modification that is different from one as described herein or no modification (i.e., a wild-type non-human animal).

[00172] Exemplary parameters that may be measured in non-human animals (or in and/or using cells isolated therefrom) for assessing the pharmacokinetic properties of a drug include, but are not limited to, agglutination, autophagy, cell division, cell death, complement-mediated hemolysis, DNA integrity, drug-specific antibody titer, drug metabolism, gene expression arrays, metabolic activity, mitochondrial activity, oxidative stress, phagocytosis, protein biosynthesis, protein degradation, protein secretion, stress response, target tissue drug concentration, non-target tissue drug concentration, transcriptional activity and the like. In various embodiments, non-human animals as described herein are used to determine a pharmaceutically effective dose of a drug.

[00173] Non-human animals as described herein provide an *in vivo* system for the analysis and testing of a drug or vaccine. In various embodiments, a candidate drug or vaccine may be administered to one or more non-human animals as described herein, followed by monitoring of the non-human animals to determine one or more of the immune response to the drug or vaccine, the safety profile of the drug or vaccine, or the effect on a disease or condition. Exemplary methods used to determine the safety profile include measurements of toxicity, optimal dose concentration, efficacy of the drug or vaccine, and possible risk factors. Such drugs or vaccines may be improved and/or developed in such non-human animals.

[00174] Vaccine efficacy may be determined in a number of ways. Briefly, non-human animals described herein are vaccinated using methods known in the art and then challenged with a vaccine, or a vaccine is administered to already-infected non-human animals. The response of a non-human animal(s) to a vaccine may be measured by monitoring of, and/or performing one or more assays on, the non-human animal(s) (or cells isolated therefrom) to determine the efficacy of the vaccine. The response of a non-human animal(s) to the vaccine is then compared with control animals, using one or more measures known in the art and/or described herein.

[00175] Vaccine efficacy may further be determined by viral neutralization assays. Briefly, non-human animals described herein are immunized and serum is collected on various days post-immunization. Serial dilutions of serum are pre-incubated with a virus during which time antibodies in the serum that are specific for the virus will bind to it. The virus/serum mixture is then added to permissive cells to determine infectivity by a plaque

assay or microneutralization assay. If antibodies in the serum neutralize the virus, there are fewer plaques or lower relative luciferase units compared to a control group.

[00176] In various embodiments, non-human animals as described herein are used in efficacy studies to determine the *in vivo* effect of anti-ANGPTL8 therapeutics (e.g., anti-ANGPTL8 antibodies) on circulating triglyceride levels. For example, non-human animals as described herein are bled prior to administration of candidate therapeutics or controls and organized into various treatment groups as desired. Candidate therapeutics or controls are administered at a desired dosage and bled on consecutive days after administration. Plasma levels of triglycerides, glucose and/or insulin may be measured using collected serum. Levels of candidate therapeutics may also be measured as desired. Exemplary assays that can be used for detection of various molecules include ELISA assays and others as described in Wang, Y. et al., 2013, Proc. Nat. Acad. Sci. U.S.A. 110(40):16109-114; Quagliarini, F. et al., 2012, Proc. Nat. Acad. Sci. U.S.A. 109(48):19751-6.

[00177] In various embodiments, non-human animals as described herein are used to determine lipoprotein lipase (LPL) activity after treatment with anti-ANGPTL8 therapeutics (e.g., anti-ANGPTL8 antibodies). For example, non-human animals as described herein are bled prior to administration of candidate therapeutics or controls and put into various treatment groups as desired. Candidate therapeutics or controls are administered at a desired dosage and bled at consecutive days after administration. After sufficient time (e.g., several days), non-human animals are administered an anti-coagulant (e.g., heparin) so that LPL is released from vascular endothelial surfaces and blood is obtained from the non-human animals shortly thereafter. Post-heparin plasma is fractionated to separate LPL using heparin-Sepharose chromatography and LPL activities are assayed using a lipase substrate. For example, general methods and assays are described in Wang, Y. et al., 2013, Proc. Nat. Acad. Sci. U.S.A. 110(40):16109-114; Quagliarini, F. et al., 2012, Proc. Nat. Acad. Sci. U.S.A. 109(48):19751-6.

[00178] In various embodiments, non-human animals as described herein are used in lipid tolerance tests to determine triglyceride clearance by acute fat loading after treatment with anti-ANGPTL8 therapeutics (e.g., anti-ANGPTL8 antibodies). For example, non-human animals as described herein are bled prior to administration of candidate therapeutics or

controls and put into various treatment groups. Candidate therapeutics or controls are administered at a desired dosage. After several days, non-human animals are subjected to a fasting regimen following administration of a lipid emulsion (e.g., 20% concentration) according to body weight. Plasma triglyceride levels are determined in blood collected from non-human animals in each treatment group. For example, general methods and assays are described in Wang, Y. et al., 2013, Proc. Nat. Acad. Sci. U.S.A. 110(40):16109-114; Quagliarini, F. et al., 2012, Proc. Nat. Acad. Sci. U.S.A. 109(48):19751-6.

[00179] Non-human animals as described herein provide an improved *in vivo* system for the development and characterization of candidate therapeutics for use in hypertriglyceridemia. In various embodiments, non-human animals as described herein may be subjected to a specific feeding regimen (e.g. overfeeding or fasting), followed by administration of one or more candidate therapeutics. In some embodiments, candidate therapeutics may include a multi-specific antibody (e.g., a bi-specific antibody) or an antibody cocktail; in some embodiments, candidate therapeutics include combination therapy such as, for example, administration of mono-specific antibodies dosed sequentially or simultaneously. The non-human animals may be subjected to the feeding regimen for a sufficient time so that ANGPTL8 levels are at a high level in one or more locations (e.g., liver and/or adipose tissue) within the non-human animal. Plasma levels of triglyceride, glucose and/or insulin, and lipoprotein lipase activity, etc. may be measured both before and after administration of the candidate therapeutic(s). Cytoxicity of candidate therapeutics may also be measured in the non-human animal as desired.

[00180] Non-human animals as described herein may be used to develop one or more disease models to evaluate or assess candidate therapeutics and/or therapeutic regimens (e.g., monotherapy, combination therapy, dose range testing, etc.) to effectively treat diseases, disorders or conditions that affect humans. Various disease conditions may be established in non-human animals as described herein followed by administration of one or more candidate molecules (e.g., drugs targeting ANGPTL8) so that efficacy of the one or more candidate molecules in a disease condition can be determined. Non-human animals may be placed into different treatment groups according to dose so that an optimal dose or dose range that correlates with effective treatment of an established disease can be determined. In some embodiments, disease models include cardiovascular diseases, disorders or conditions.

[00181] Candidate molecules can be administered to non-human animal disease models using any method of administration including parenteral and non-parenteral routes of administration. Parenteral routes include, e.g., intravenous, intraarterial, intraportal, intramuscular, subcutaneous, intraperitoneal, intraspinal, intrathecal, intracerebroventricular, intracranial, intrapleural or other routes of injection. Non-parenteral routes include, e.g., oral, nasal, transdermal, pulmonary, rectal, buccal, vaginal, ocular. Administration may also be by continuous infusion, local administration, sustained release from implants (gels, membranes or the like), and/or intravenous injection. When a combination therapy is evaluated in non-human animals as described herein, candidate molecules can be administered via the same administration route or via different administration routes. When a dosing regimen is evaluated in non-human animals as described herein, candidate molecules may be administered at bimonthly, monthly, triweekly, biweekly, weekly, daily, at variable intervals and/or in escalating concentrations to determine a dosing regimen that demonstrates a desired therapeutic or prophylactic effect in a non-human animal in which one or more disease models has been established.

Kits

[00182] The present invention further provides a pack or kit comprising one or more containers filled with at least one non-human animal, non-human cell, DNA fragment (or construct), and/or targeting vector as described herein. Kits may be used in any applicable method (e.g., a research method). Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects (a) approval by the agency of manufacture, use or sale for human administration, (b) directions for use, or both, or a contract that governs the transfer of materials and/or biological products (e.g., a non-human animal or non-human cell as described herein) between two or more entities.

[00183] Other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments, which are given for illustration and are not intended to be limiting thereof.

EXAMPLES

[00184] The following examples are provided so as to describe to those of ordinary skill in the art how to make and use methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Unless indicated otherwise, temperature is indicated in Celsius, and pressure is at or near atmospheric.

Example 1. Modification of an endogenous Angiopoietin-like protein 8 gene

[00185] This example illustrates exemplary methods of modifying an endogenous *Angptl8* gene in a non-human mammal such as a rodent (e.g., a mouse) so that said endogenous *Angptl8* gene encodes a human ANGPTL8 polypeptide. The methods described in this example can be employed to modify an endogenous *Angptl8* gene of a non-human animal using any human sequence (e.g., a variant), or combination of human sequences (or sequence fragments) as desired. In this example, a 2,383bp synthetic DNA fragment containing exons 1-4 (excluding the ATG start codon) of a human *ANGPTL8* gene that appears in GenBank accession NM_018687.6 (SEQ ID NO: 5) was employed for modifying an endogenous *Angptl8* gene of a mouse. Alignment of mouse, human, and exemplary human ANGPTL8 polypeptide expressed by a rodent as described herein, with signal peptide indicated in boxes for each sequence, is depicted in Figure 2. Figure 3 shows a targeting vector for modifying an endogenous *Angptl8* gene of a rodent to encode a human ANGPTL8 polypeptide that was constructed using VELOCIGENE® technology (see, e.g., U.S. Patent No. 6,586,251 and Valenzuela et al., 2003, Nature Biotech. 21(6):652-659; herein incorporated by reference).

[00186] Briefly, mouse bacterial artificial chromosome (BAC) clone RP23-198h22 (Invitrogen) was modified to delete the sequence containing immediately downstream of the endogenous *Angptl8* ATG start codon to 9bp beyond the stop codon (i.e., exon 1 except the 5' 11 nucleotides, exons 2-3, and the 5' portion of exon 4 through 9bp beyond the stop codon) and insert just downstream of the human *ANGPTL8* ATG start codon to beyond the human *ANGPTL8* 3'UTR (i.e., the coding portion of exon 1 beginning just downstream of the ATG start codon through exon 4) using a 2,383bp synthetic DNA fragment, which encodes a human ANGPTL8 polypeptide. Endogenous DNA containing the 5' and 3' untranslated regions (UTRs) as well as the endogenous *Angptl8* ATG start codon were retained. Thus, exons 1-4 of a human *ANGPTL8* gene, without the human *ANGPTL8* start codon, was fused

in frame to the endogenous *Angptl8* ATG start codon. Sequence analysis of the 2,383bp synthetic DNA fragment (i.e., corresponding to exons 1-4 of a human *ANGPTL8* gene) confirmed all human *ANGPTL8* exons and splicing signals. Sequence analysis revealed that the sequence matched the reference genome and *ANGPTL8* transcript NM_018687.6.

[00187] The 2,383bp synthetic DNA fragment was synthesized by Genescript Inc. (Piscataway, NJ) and cloned into an ampicillin-resistant plasmid vector. Unique restriction enzyme recognition sites were employed to ligate a ~4,996bp self-deleting neomycin cassette flanked by recombinase recognition sites (*lox*P-hUb1-em7-Neo-pA-mPrm1-Crei-*lox*P; see U.S. Patent Nos. 8,697,851, 8,518,392 and 8,354,389, all of which are incorporated herein by reference). Subsequent selection in bacterial cells was performed via plating on agar medium containing neomycin. The targeting vector was linearized prior to homologous recombination with mouse BAC clone RP23-198H22. By design, the junction between the human *ANGPTL8* 2,383bp fragment and the mouse downstream sequence included a human *ANGPTL8* 3' UTR followed by a mouse *Angptl8* 3' UTR (Figure 3). The resulting targeting vector contained, from 5' to 3', a 5' homology arm containing ~79kb of mouse genomic DNA from BAC clone RP23-198h22, 2,383bp synthetic DNA fragment (corresponding to exons 1-4 of a human *ANGPTL8* gene), a self-deleting neomycin cassette flanked by *lox*P sites, and ~148kb of mouse genomic DNA from BAC clone RP23-198h22.

[00188] The modified bMQ-400O17 BAC clone described above was used to electroporate mouse embryonic stem (ES) cells to create modified ES cells comprising an endogenous *Angptl8* gene that is humanized from exon 1 (minus the ATG start codon) through exon 4 including a human *ANGPTL8* 3' UTR (i.e., deletion of 1,576bp of an endogenous *Angptl8* gene and insertion of 2.383bp of human *ANGPTL8*-encoding sequence). Positively targeted ES cells containing the modified *Angptl8* gene were identified by an assay (Valenzuela et al., supra) that detected the presence of the human *ANGPTL8* sequences (e.g., exons 1-4) and confirmed the loss and/or retention of mouse *Angptl8* sequences (e.g., exons 1-4 and/or 5' and 3' UTRs). Table 1 sets forth the primers and probes that were used to confirm modification of an endogenous *Angptl8* gene as described above (Figure 4).

[00189] The nucleotide sequence across the upstream insertion point included the following, which indicates endogenous mouse sequence (contained within the parentheses

below with the ATG start codon in bold font) contiguous with human *ANGPTL8* genomic sequence at the insertion point: (AAGGCAGCCG CAGCGGCCCG GGAACCACAC CCACGAAACT GTCAGCC**ATG**) CCAGTGCCTG CTCTGTGCCT GCTCTGGGCC CTGGCAATGG TGACCCGGCC (SEQ ID NO: 15) (Figure 9A). See, also, Figure 3.

[00190] The nucleotide sequence across the 5' end of the self-deleting neomycin cassette included the following, which indicates human *ANGPTL8* genomic sequence contiguous with cassette sequence (contained within the parentheses below with an XhoI site italicized and a *lox*P site in bold font) downstream of the insertion point: GGGAGACCCC ACCCAGCATG ATGTATGAAT ACCTCCCATT CAAGTGCCCA (*CTCGAG* **ATAACTTCG TATAATGTAT GCTATACGAA GTTAT** ATGCATGGCC TCCGCGCCGG GTTTTGGCGC CTCCCGCGGG CGCCCCCCTC CTCACGGCGA GCGCTGCCAC GTCAGACGAA GGGCGCAGCG AGCGTCCTGA) (SEQ ID NO:16) (Figure 9B). See, also, Figure 3.

[00191] The nucleotide sequence across the downstream insertion point at the 3' end of the self-deleting neomycin cassette included the following, which indicates cassette sequence (contained within the parentheses below with a *lox*P site in bold font, an I-CeuI recognition site underlined and an NheI recognition site italicized) contiguous with mouse *Angptl8* genomic sequence: (TTTCACTGCAT TCTAGTTGTG GTTTGTCCAA ACTCATCAAT GTATCTTATC ATGTCTGGA **ATAACTTCGTATAATGTATGCTATACGAAGTTAT** GCTAGTAACTATAACGGTCCTAAGGTAGCGA *GCTAGC*) GATGCCACCGA GGACCAGTTGT GCTGCAAGGAA CACTGAAGCG CTCCACC (SEQ ID NO:17) (Figure 9C). See, also, Figure 3.

[00192] The nucleotide sequence across the downstream insertion point after deletion of the neomycin cassette (77bp remaining between a human *ANGPTL8* 3'UTR and a mouse *Angptl8* 3'UTR) included the following, which indicates human and mouse genomic sequence juxtaposed with remaining cassette sequence (contained within the parentheses below with XhoI and NheI recognition sites italicized, a *lox*P site in bold, and an I-CeuI restriction site underlined): GGGAGACCCC ACCCAGCATG ATGTATGAAT ACCTCCCATT CAAGTGCCCA (*GTCGAG* **ATAACTTCGTATAATGTATGCTATACGAAGTTAT** GCTAGTAACTATAACGGTCCTAAGGTAGCGA *GCTAGC*) GATGCCACCG

AGGACCAGTT GTGCTGCAAG GAACACTGAA GCGCTCCACC (SEQ ID NO:18) (Figure 9D). See, also, Figure 3.

[00193] Positive ES cell clones were then used to implant female mice using the VELOCIMOUSE® method (see, e.g., U.S. Patent No. 7,294,754 and Poueymirou et al., 2007, Nature Biotech. 25(1):91-99) to generate a litter of pups containing an insertion of human *ANGPTL8* exons 1-4 (including human *ANGPTL8* 3' UTR) into an endogenous *Angptl8* locus of a mouse. Mice bearing the human *ANGPTL8* exons 1-4 (i.e., the 2,383bp synthetic DNA fragment) in place of endogenous *Angptl8* exons 1-4 were again confirmed and identified by genotyping of DNA isolated from tail snips using an assay as previously described (Valenzuela et al., supra) that detected the presence of the human *ANGPTL8* sequences (Figure 4). Pups are genotyped and cohorts of animals heterozygous for the human *ANGPTL8* sequences are selected for characterization.

TABLE 1

Name	Primer	Sequence (5'-3')	
7182mU	Forward	GGTGTTGGTGGCAGGTAAGAGT	(SEQ ID NO:19)
	Probe	TGAGGAAATGGTAAACCCAGAACAGA	(SEQ ID NO:20)
	Reverse	TGGTGTGTCATCAGGGTATGTTTC	(SEQ ID NO:21)
7182mD	Forward	TGAGCCTGGTGGGATTACTCT	(SEQ ID NO:22)
	Probe	TAGCAGTGGAAGTTGCCTAGGTCC	(SEQ ID NO:23)
	Reverse	CCGTCAAGGCCAGTGCTT	(SEQ ID NO:24)
7182hU	Forward	GCAAGCCTGTTGGAGACTCAG	(SEQ ID NO:25)
	Probe	CACCGTAGCTGCGACACTGTGG	(SEQ ID NO:26)
	Reverse	AGACACGAACTCCTCTTTGGA	(SEQ ID NO:27)
7182hD	Forward	TGGGCTGAGCCACATCTC	(SEQ ID NO:28)
	Probe	CAGACTCCACACAGCGGCGCT	(SEQ ID NO:29)
	Reverse	TCAGTTCCATCCAGGCAGATTC	(SEQ ID NO:30)

Example 2. Expression of human ANGPTL8 in non-human animals

[00194] This Example demonstrates that non-human animals (e.g., rodents) containing an engineered *Angptl8* gene according to Example 1 express (or secrete) human (or humanized) ANGPTL8 polypeptide that is detectable in the plasma of the non-human animal. In particular, as described below, non-human animals having an engineered *Angptl8* gene

demonstrate augmented triglyceride levels as compared to wild-type non-human animals (e.g., wild-type rodents) that contain a wild-type *Angptl8* gene.

[00195] Briefly, venous blood was collected at non-fasted conditions from wild-type (n=9) and mice homozygous for an engineered *Angptl8* gene (n=8) in EDTA tubes from the retroorbital plexus. Plasma was isolated by centrifugation of collected blood at 4,000 rpm for 10 minutes. Plasma was analyzed for expression of human ANGPTL8 by an ELISA assay using an anti-ANGPLT8 antibody.

[00196] The data demonstrated that human ANGPTL8 was secreted into the plasma of mice homozygous for a humanized *Angptl8* gene. In particular, protein expression of human ANGPTL8 averaged about 400 ng/mL for all humanized mice from which blood was collected. Thus, rodents containing an engineered *Angptl8* gene according to Example 1 detectably express (and secrete) human ANGPTL8 in the plasma. In particular, such expression (or secretion) of human ANGPTL8 is under the control of rodent Angptl8 regulatory elements (e.g., a rodent Angptl8 promoter) in these animals.

[00197] In another experiment, plasma from wild-type and mice homozygous for an engineered *Angptl8* gene (as described above) collected at non-fasted conditions was also used to determine plasma lipid levels.

[00198] Briefly, plasma lipids (triglycerides, total cholesterol, low-density lipoprotein cholesterol [LDL-C], high-density lipoprotein cholesterol [HDL-C]) were measured using serum chemistry analyzer ADVIA® 1800 (Siemens) according to manufacturer's specifications. Representative results are set forth in Figure 5.

[00199] As shown in Figure 5, rodents having an engineered *Angptl8* gene as described herein demonstrate augmented triglyceride levels as compared to wild-type rodents.

Example 3. Tissue Expression of human ANGPTL8 in non-human animals

[00200] This Example demonstrates that non-human animals (e.g., rodents) containing an engineered *Angptl8* gene according to Example 1 express (or secrete) human (or humanized) ANGPTL8 polypeptide that is detectable in various tissues of the non-human animal. In particular, as described below, non-human animals having an engineered *Angptl8* gene demonstrate expression of human ANGPTL8 in liver and adipose tissues.

[00201] Briefly, RNA preparation and RNAseq read mapping was performed as previously described (Mastaitis, J. et al., 2015, Proc. Natl. Acad. Sci. U.S.A. 112(6):1845-9) using tissues from mice homozygous for an engineered Angptl8 gene, as described in Example 1, collected at re-fed conditions. Representative results are set forth in Figure 6.

[00202] As shown in Figure 6, human ANGPTL8 expression was identified in liver and adipose tissues (e.g., white adipose, subcutaneous and brown fat) of humanized Anptl8 mice. This Example demonstrates that the engineering of a murine *Angptl8* gene as described herein results in expression of a human ANGPTL8 polypeptide in a tissue-specific manner and, therefore, provide an *in vivo* animal model for determining the efficacy of anti-ANGPTL8 therapeutics to lower triglyceride levels *in vivo*.

Example 4. In vivo efficacy of ANGPTL8 modulators

[00203] This Example demonstrates that non-human animals (e.g., rodents) modified to contain a humanized *Angptl8* gene according to Example 1 can be used in an *in vivo* assay to screen Angptl8 modulators (e.g., anti-ANGPTL8 antibodies) for their triglyceride-lowering efficacy. In this Example, representative anti-ANGPTL8 antibodies are screened in mice homozygous for a humanized *Angptl8* gene to determine the efficacy of monoclonal antibody therapy to lower elevated triglycerides.

[00204] Briefly, mice homozygous for an engineered *Angptl8* gene (as described above) were pre-bled 5 days before the experiment and sorted into treatment groups (n=5 per treatment group) based on their triglyceride levels so that the mean triglyceride level across each group was equal. Anti-ANGPTL8 antibodies or control (isotype-matched human IgG4 control with irrelevant specificity) were administered at 10mg/kg dose by subcutaneous injection on Day 0 of the study. Mice were bled at 4 days after injection and serum triglyceride levels were determined by ADVIA® 1800 Serum Chemistry Analyzer (Siemens). Results were expressed as Mean ± SEM for each group for all tested antibodies. Representative results are set forth in Figure 7.

[00205] As shown in Figure 7, anti-ANGPTL8 antibody therapy significantly reduced circulating triglyceride levels as compared to control antibody. Further, these data suggest that mice containing an engineered *Angptl8* gene as described in Example 1 express human ANGPTL8 and can be used in screening therapeutics for the treatment of elevated triglyceride

levels. Taken together, the present disclosure demonstrates that non-human animals provided herein offer an *in vivo* system for assessing the triglyceride-lowering efficacy of anti-ANGPTL8 antibodies and, in some embodiments, provide an *in vivo* animal model for hypertriglyceridemia.

EQUIVALENTS

[00206] Having thus described several aspects of at least one embodiment of this invention, it is to be appreciated that various alterations, modifications, and improvements will readily occur to those skilled in the art. Such alterations, modifications, and improvements are intended to be part of this disclosure, and are intended to be within the spirit and scope of the invention. Accordingly, the foregoing description and drawing are by way of example only and the invention is described in detail by the claims that follow.

[00207] Use of ordinal terms such as "first," "second," "third," etc., in the claims to modify a claim element does not by itself connote any priority, precedence, or order of one claim element over another or the temporal order in which acts of a method are performed, but are used merely as labels to distinguish one claim element having a certain name from another element having a same name (but for use of the ordinal term) to distinguish the claim elements.

[00208] The articles "a" and "an" as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to include the plural referents. Claims or descriptions that include "or" between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention also includes embodiments in which more than one, or the entire group members are present in, employed in, or otherwise relevant to a given product or process. Furthermore, it is to be understood that the invention encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, descriptive terms, etc., from one or more of the listed claims is introduced into another claim dependent on the same base claim (or, as relevant, any other claim) unless

otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise. Where elements are presented as lists, (e.g., in Markush group or similar format) it is to be understood that each subgroup of the elements is also disclosed, and any element(s) can be removed from the group. It should be understood that, in general, where the invention, or aspects of the invention, is/are referred to as comprising particular elements, features, etc., certain embodiments of the invention or aspects of the invention consist, or consist essentially of, such elements, features, etc. For purposes of simplicity those embodiments have not in every case been specifically set forth in so many words herein. It should also be understood that any embodiment or aspect of the invention can be explicitly excluded from the claims, regardless of whether the specific exclusion is recited in the specification.

[00209] Those skilled in the art will appreciate typical standards of deviation or error attributable to values obtained in assays or other processes described herein. The publications, websites and other reference materials referenced herein to describe the background of the invention and to provide additional detail regarding its practice are hereby incorporated by reference.

CLAIMS

1. A rodent whose genome comprises an integrated *Angptl8 gene* that comprises an endogenous portion and a human portion, wherein the endogenous and human portions are operably linked to rodent or human *Angptl8* regulatory elements.

2. The rodent of claim 1, wherein the rodent *Angptl8* regulatory elements include a rodent *Angptl8* promoter.

3. The rodent of claim 2, wherein the rodent *Angptl8* promoter is an endogenous rodent *Angptl8* promoter.

4. The rodent of any one of claims 1-3, wherein the endogenous portion of the *Angptl8* gene includes endogenous 5' and/or 3' untranslated regions (UTRs).

5. The rodent of claim 1, wherein the human *Angptl8* regulatory elements include a human *Angptl8* promoter.

6. The rodent of claim 4, wherein the 5' and 3' UTRs of the endogenous *Angptl8* gene each have a sequence that is substantially identical or identical to the corresponding 5' and 3' UTRs that appear in a rodent *Angptl8* gene.

7. The rodent of any one of claims 1-6, wherein the *Angptl8* gene encodes a polypeptide having a sequence that is at least 95% identical to SEQ ID NO:6.

8. The rodent of any one of claims 1-7, wherein the human portion includes exons 1-4, in whole or in part, of a human *ANGPTL8* gene.

9. The rodent of claim 8, wherein exons 1-4, in whole or in part, of a human *ANGPTL8* gene are at least 95% identical to the corresponding exons 1-4, in whole or in part, that appear in a human *ANGPTL8* mRNA sequence of SEQ ID NO:5.

10. The rodent of claim 8, wherein the human portion comprises a sequence that is codon-optimized for expression in the rodent.

11. A rodent that expresses a human ANGPTL8 polypeptide under the control of rodent *Angptl8* regulatory elements

12. The rodent of claim 11, wherein the rodent *Angptl8* regulatory elements include a rodent *Angptl8* promoter.

13. The rodent of claim 12, wherein the rodent *Angptl8* promoter is an endogenous rodent *Angptl8* promoter.

14. The rodent of any one of claims 11-13, wherein the human ANGPTL8 polypeptide includes an amino acid sequence that is at least 95% identical to amino acid residues 22-198 of SEQ ID NO:6.

15. The rodent of any one of claims 11-14, wherein the human ANGPTL8 polypeptide is encoded by a sequence that is at least 95% identical to SEQ ID NO:9.

16. The rodent of any one of claims 11-14, wherein the human ANGPTL8 polypeptide is encoded by a sequence that is codon-optimized.

17. The rodent of any one of claims 11-14, wherein the human ANGPTL8 polypeptide is a variant human ANGPTL8 polypeptide.

18. The rodent of claim 17, wherein the variant human ANGPTL8 polypeptide is characterized by an R59W amino acid substitution.

19. The rodent of claim 17, wherein the variant human ANGPTL8 polypeptide is characterized by a Q121X amino acid substitution.

20. The rodent of any one of claims 7-19, wherein the human ANGPTL8 polypeptide is encoded by a nucleic acid sequence placed at an endogenous *Angptl8* locus.

21. The rodent of any one of claims 1-20, wherein the rodent is a rat or mouse.

22. An isolated rodent cell or tissue whose genome comprises an *Angptl8* gene that comprises an endogenous portion and a human portion, wherein the endogenous and human portions are operably linked to rodent *Angptl8* regulatory elements.

23. A rodent embryonic stem cell whose genome comprises an *Angptl8* gene that comprises an endogenous portion and a human portion, wherein the endogenous and human portions are operably linked to rodent *Angptl8* regulatory elements.

24. A rodent embryo generated from the embryonic stem cell of claim 23.

25. A method of making a rodent that expresses a human ANGPTL8 polypeptide from an endogenous *Angptl8* gene, the method comprising

(a) placing a genomic fragment into an endogenous *Angptl8* gene in a rodent embryonic stem cell, said genomic fragment comprising a nucleotide sequence that encodes a human ANGPTL8 polypeptide in whole or in part;

(b) obtaining the rodent embryonic stem cell generated in (a); and,

(c) creating a rodent using the rodent embryonic stem cell of (b).

26. The method of claim 25, wherein the human ANGPTL8 polypeptide comprises a sequence that is at least 95% identical to amino acid residues 22-198 of SEQ ID NO:6.

27. The method of claim 25 or 26, wherein the nucleotide sequence comprises exons 1-4, in whole or in part, of a human *ANGPTL8* gene.

28. The method of claim 27, wherein the nucleotide sequence further comprises a 3' UTR of a human *ANGPTL8* gene.

29. The method of any one of claims 25-28, wherein the nucleotide sequence comprises one or more selection markers.

30. The method of any one of claims 25-29, wherein the nucleotide sequence comprises one or more site-specific recombination sites.

31. The method of claim 30, wherein the nucleotide sequence comprises a recombinase gene and a selection marker flanked by recombinase recognition sites, which recombinase recognition sites are oriented to direct an excision.

32. The method of claim 31, wherein the recombinase gene is operably linked to a promoter that drives expression of the recombinase gene in differentiated cells and does not drive expression of the recombinase gene in undifferentiated cells.

33. The method of claim 31, wherein the recombinase gene is operably linked to a promoter that is transcriptionally competent and developmentally regulated.

34. The method of claim 32 or 33, wherein the promoter is or comprises SEQ ID NO:12, SEQ ID NO:13, or SEQ ID NO:14.

35. The method of claim 34, wherein the promoter is or comprises SEQ ID NO:12.

36. The method of any one of claims 25-35, wherein the nucleotide sequence comprises one or more sequences that are codon-optimized for expression in a rodent.

37. The method of any one of claims 25-36, the method further comprises a step of breeding the rodent generated in (c) so that a rodent homozygous for expressing a human ANGPTL8 polypeptide from an endogenous *Angptl8* gene is created.

38. A method of making a rodent whose genome comprises an *Angptl8* gene that encodes a human ANGPTL8 polypeptide, the method comprising modifying the genome of a rodent so that it comprises an *Angptl8* gene that encodes a human ANGPTL8 polypeptide under the control of rodent *Angptl8* regulatory sequences, thereby making said rodent.

39. The rodent of claim 38, wherein the rodent *Angptl8* regulatory sequences include a rodent *Angptl8* promoter.

40. The method of claim 39, wherein the rodent *Angptl8* promoter is an endogenous rodent *Angptl8* promoter.

41. The method of any one of claims 38-40, wherein the human ANGPTL8 polypeptide comprises a sequence that is at least 95% identical to amino acid residues 22-198 of SEQ ID NO:6.

42. The method of any one of claims 38-40, wherein the human ANGPTL8 polypeptide is a variant human ANGPTL8 polypeptide.

43. The rodent of claim 42, wherein the variant human ANGPTL8 polypeptide is characterized by an R59W amino acid substitution.

44. The rodent of claim 42, wherein the variant human ANGPTL8 polypeptide is characterized by a Q121X amino acid substitution.

45. The method of any one of claims 38-44, wherein the *Angptl8* gene is modified to include exons 1-4, in whole or in part, of a human *ANGPTL8* gene.

46. The method of claim 45, wherein the *Angptl8* gene is modified to further include the 3' UTR of a human *ANGPTL8* gene.

47. The method of any one of claims 25-46, wherein the rodent is a mouse or rat.

48. A rodent obtainable from the method of any one of claims 25-47.

49. A method of assessing triglyceride-lowering efficacy of a drug targeting human ANGPTL8, the method comprising the steps of

administering the drug to a rodent whose genome comprises an *Angptl8* gene that comprises an endogenous portion and a human portion, wherein the endogenous and human portions are operably linked to rodent *Angptl8* regulatory elements; and

performing an assay to determine one or more triglyceride lowering properties of the drug targeting human ANGPTL8.

50. A method of assessing the pharmacokinetic properties of a drug targeting human ANGPTL8, the method comprising the steps of

administering the drug to a rodent whose genome comprises an *Angptl8* gene that comprises an endogenous portion and a human portion, wherein the endogenous and human portions are operably linked to rodent *Angptl8* regulatory elements; and

performing an assay to determine one or more pharmacokinetic properties of the drug targeting human ANGPTL8.

51. The method of claim 49 or 50, wherein the human portion comprises exons 1-4, in whole or in part, of a human *ANGPTL8* gene.

52. The method of claim 51, wherein the human portion further comprises the 3' UTR of a human *ANGPTL8* gene.

53. The method of any one of claims 49-52, wherein the drug targeting human ANGPTL8 is an ANGPTL8 antagonist.

54. The method of any one of claims 50-52, wherein the drug targeting human ANGPTL8 is an ANGPTL8 agonist.

55. The method of any one of claims 49-52, wherein the drug targeting human ANGPTL8 is an anti-ANGPTL8 antibody.

56. The method of any one of claims 49-55, wherein the drug targeting human ANGPTL8 is administered to the rodent intravenously.

57. The method of any one of claims 49-55, wherein the drug targeting human ANGPTL8 is administered to the rodent intraperitoneally.

58. The method of any one of claims 49-55, wherein the drug targeting human ANGPTL8 is administered to the rodent subcutaneously.

59. The method of any one of claims 49-58, wherein the rodent *Angptl8* regulatory elements include a rodent *Angptl8* promoter.

60. The method of claim 59, wherein the rodent *Angptl8* promoter is an endogenous rodent *Angptl8* promoter.

61. The method of any one of claims 49-60, wherein the rodent is a mouse or a rat.

62. A rodent whose genome comprises an *Angptl8* gene that includes

an endogenous portion that comprises the 5' and 3' UTRs of an endogenous *Angptl8* gene; and

a human portion that comprises exons 1-4, in whole or in part, of a human *ANGPTL8* gene;

wherein the human portion is operably linked to an endogenous rodent *Angptl8* promoter, and wherein the rodent expresses a human ANGPTL8 polypeptide in its serum.

63. The rodent of claim 62, wherein human ANGPTL8 polypeptide expressed by the rodent comprises an amino acid sequence that is at least 95% identical to amino acid residues 22-198 that appear in SEQ ID NO:6.

64. The method of claim 62 or 63, wherein the human ANGPTL8 polypeptide is a variant human ANGPTL8 polypeptide.

65. The rodent of claim 64, wherein the variant human ANGPTL8 polypeptide is characterized by an R59W amino acid substitution.

66. The rodent of claim 64, wherein the variant human ANGPTL8 polypeptide is characterized by a Q121X amino acid substitution.

67. The rodent of any one of claims 62-66, wherein the human portion comprises a sequence that is codon-optimized for expression in the rodent.

68. The rodent of any one of claims 62-67, wherein the rodent is a mouse or a rat.

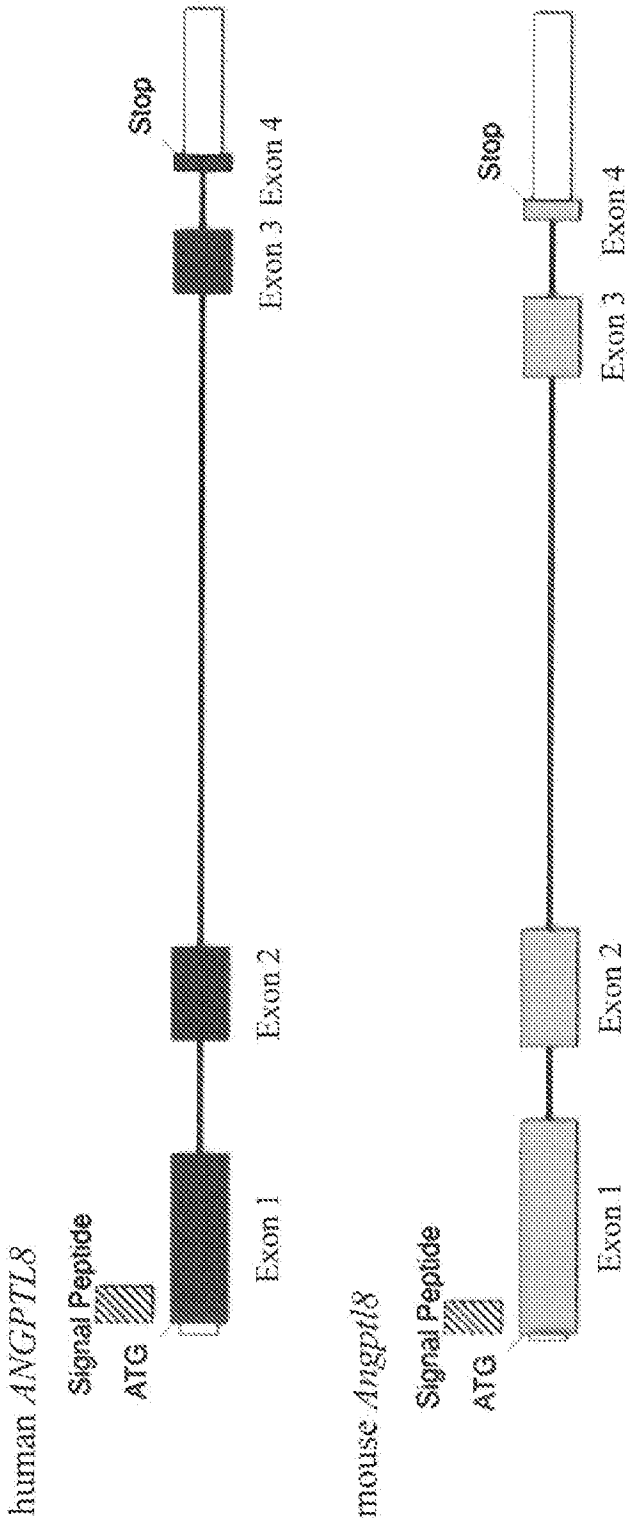
human ANGPTL8
Signal Peptide
ATG
Exon 1
Exon 2
Exon 3
Exon 4
Stop
mouse Angptl8
Signal Peptide
ATG
Exon 1
Exon 2
Exon 3
Exon 4
Stop

Figure 1

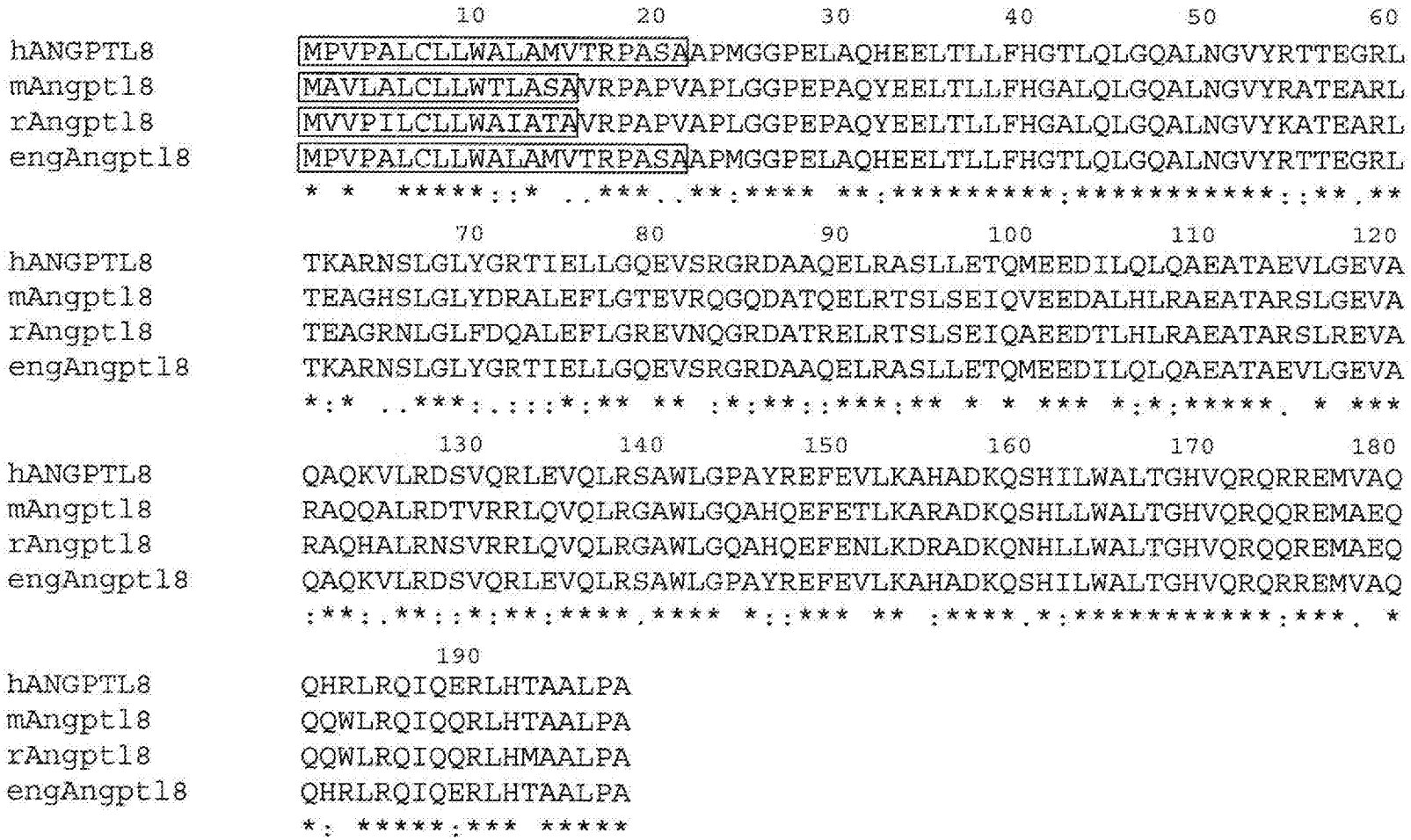
10 20 30 40 50 60
hANGPTL8 MPVPALCLLWALAMVTRPASAAPMGGPELAQHEELTLLFHGTLQLGQALNGVYRTTEGRL
mAngptl8 MAVLALCLLWTLASAVRPAPVAPLGGPEPAQYEELTLLFHGALQLGQALNGVYRATEARL
rAngptl8 MVVPILCLLWAIATAVRPAPVAPLGGPEPAQYEELTLLFHGALQLGQALNGVYKATEARL
engAngptl8 MPVPALCLLWALAMVTRPASAAPMGGPELAQHEELTLLFHGTLQLGQALNGVYRTTEGRL
70 80 90 100 110 120
hANGPTL8 TKARNSLGLYGRTIELLGQEVSRGRDAAQELRASLLETQMEEDILQLQAEATAEVLGEVA
mAngptl8 TEAGHSLGLYDRALEFLGTEVRQGQDATQELRTSLSEIQVEEDALHLRAEATARSLGEVA
rAngptl8 TEAGRNLGLFDQALEFLGREVNQGRDATRELRTSLSEIQAEEDTLHLRAEATARSLREVA
engAngptl8 TKARNSLGLYGRTIELLGQEVSRGRDAAQELRASLLETQMEEDILQLQAEATAEVLGEVA
130 140 150 160 170 180
hANGPTL8 QAQKVLRDSVQRLEVQLRSAWLGPAYREFEVLKAHADKQSHILWALTGHVQRQRREMVAQ
mAngptl8 RAQQALRDTVRRLQVQLRGAWLGQAHQEFETLKARADKQSHLLWALTGHVQRQQREMAEQ
rAngptl8 RAQHALRNSVRRLQVQLRGAWLGQAHQEFENLKDRADKQNHLLWALTGHVQRQQREMAEQ
engAngptl8 QAQKVLRDSVQRLEVQLRSAWLGPAYREFEVLKAHADKQSHILWALTGHVQRQRREMVAQ
190
hANGPTL8 QHRLRQIQERLHTAALPA
mAngptl8 QQWLRQIQQRLHTAALPA
rAngptl8 QQWLRQIQQRLHMAALPA
engAngptl8 QHRLRQIQERLHTAALPA

Figure 2

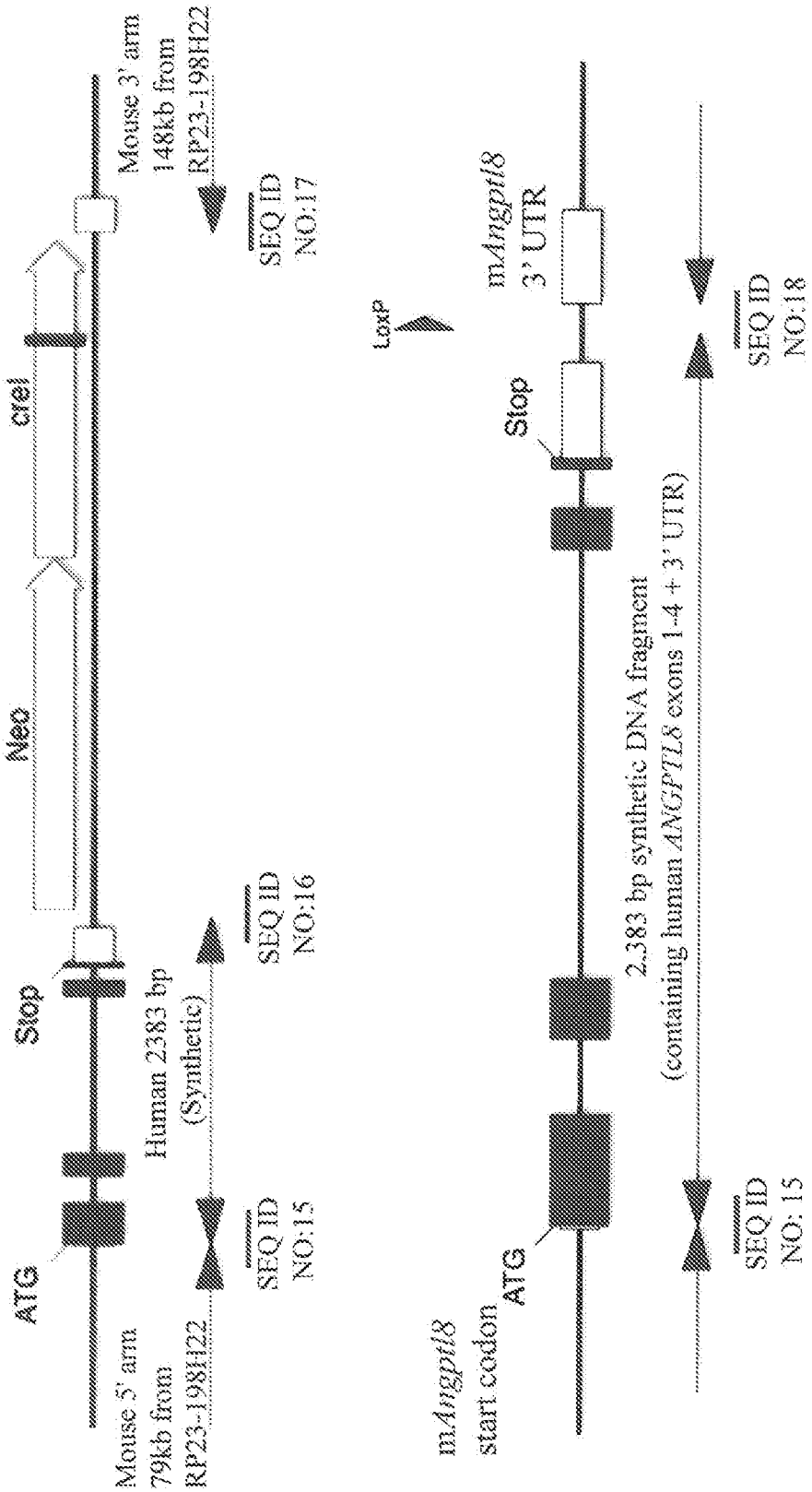
Mouse 5' arm
79kb from
RP23-198H22
ATG
Human 2383 bp
(Synthetic)
Stop
Neo
cre
Mouse 3' arm
148kb from
RP23-198H22
SEQ ID
NO:15
SEQ ID
NO:16
SEQ ID
NO:17
m*Angptl8*
start codon
ATG
2.383 bp synthetic DNA fragment
(containing human *ANGPTL8* exons 1-4 + 3' UTR)
LoxP
Stop
m*Angptl8*
3' UTR
SEQ ID
NO: 15
SEQ ID
NO:18

Figure 3

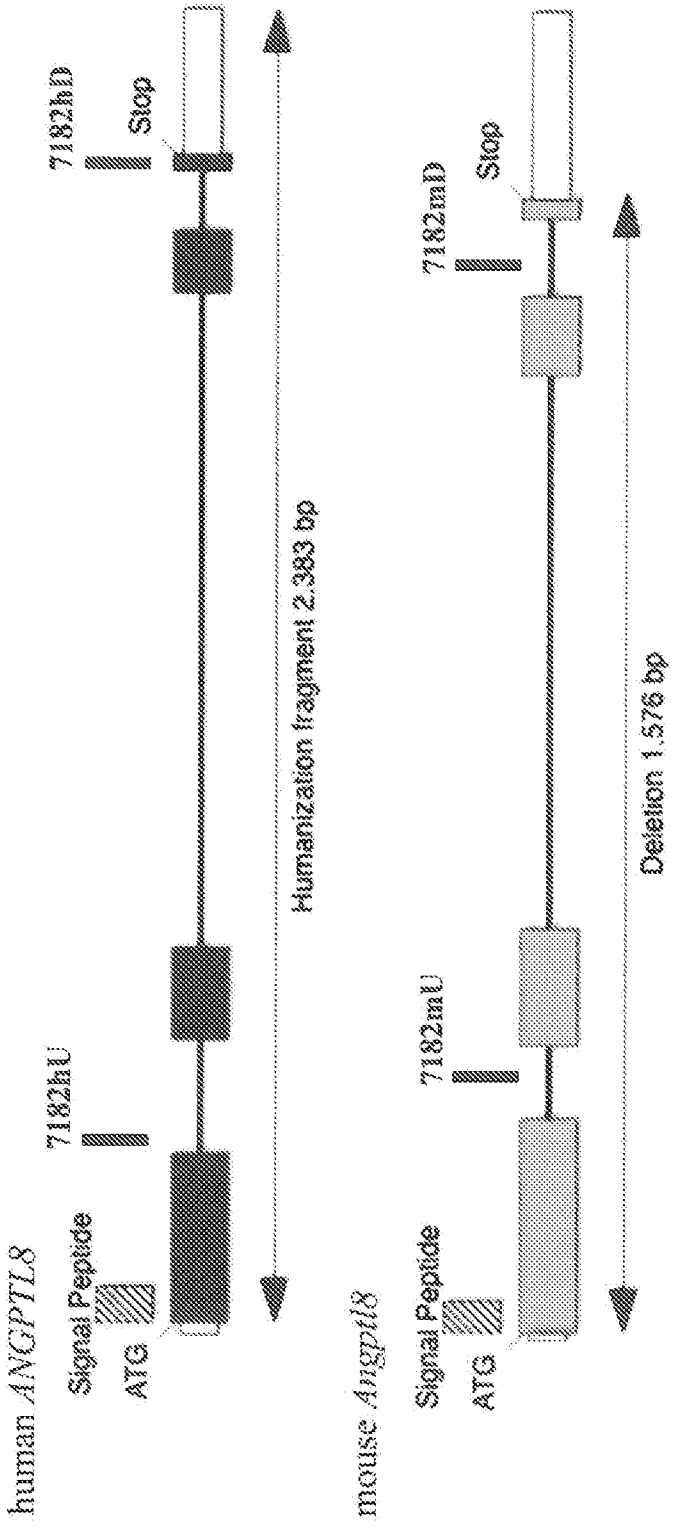
human ANGPTL8
7182hU
Signal Peptide
ATG
7182hD
Stop
Humanization fragment 2,383 bp
mouse Angptl8
7182mU
Signal Peptide
ATG
7182mD
Stop
Deletion 1,576 bp

Figure 4

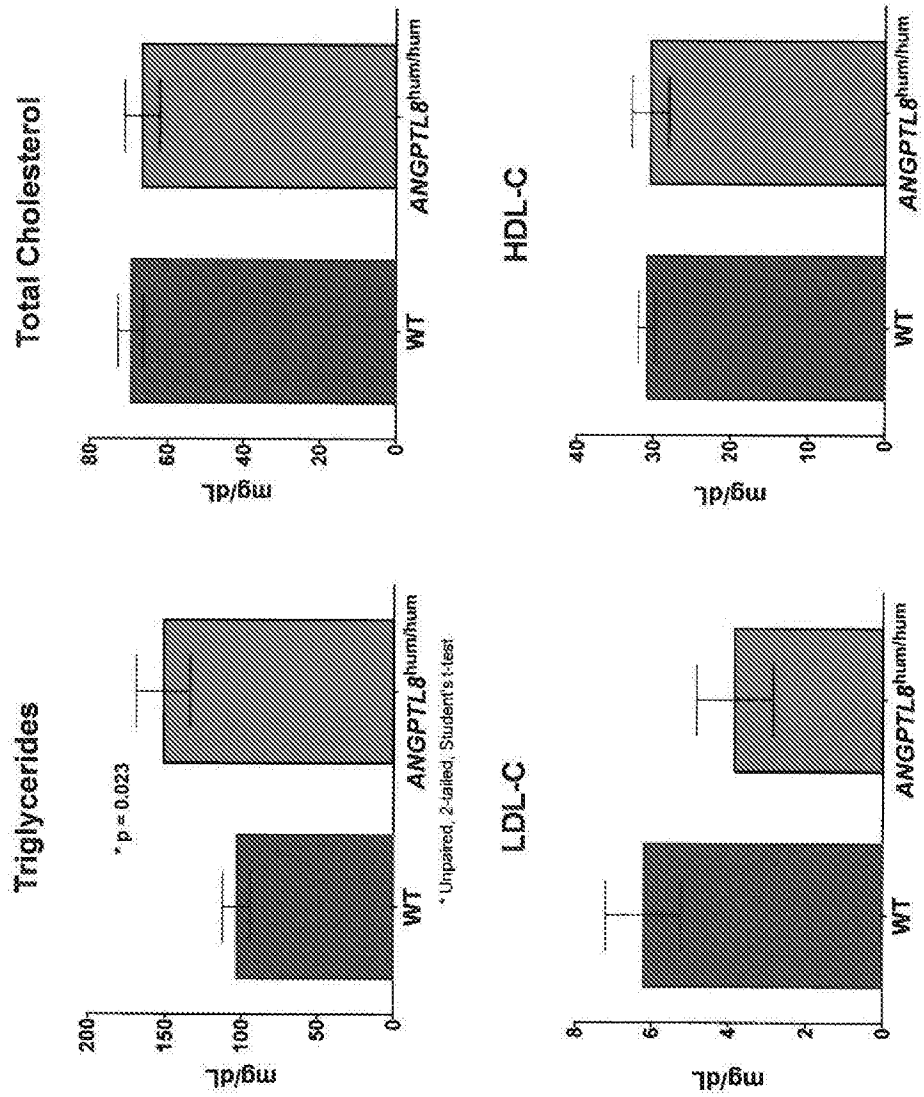
Total Cholesterol
mg/dL
0
20
40
60
80
WT
ANGPTL8hum/hum
HDL-C
mg/dL
0
10
20
30
40
WT
ANGPTL8hum/hum
Triglycerides
mg/dL
0
50
100
150
200
* p = 0.023
WT
ANGPTL8hum/hum
* Unpaired, 2-tailed, Student's t-test
LDL-C
mg/dL
0
2
4
6
8
WT
ANGPTL8hum/hum

Figure 5

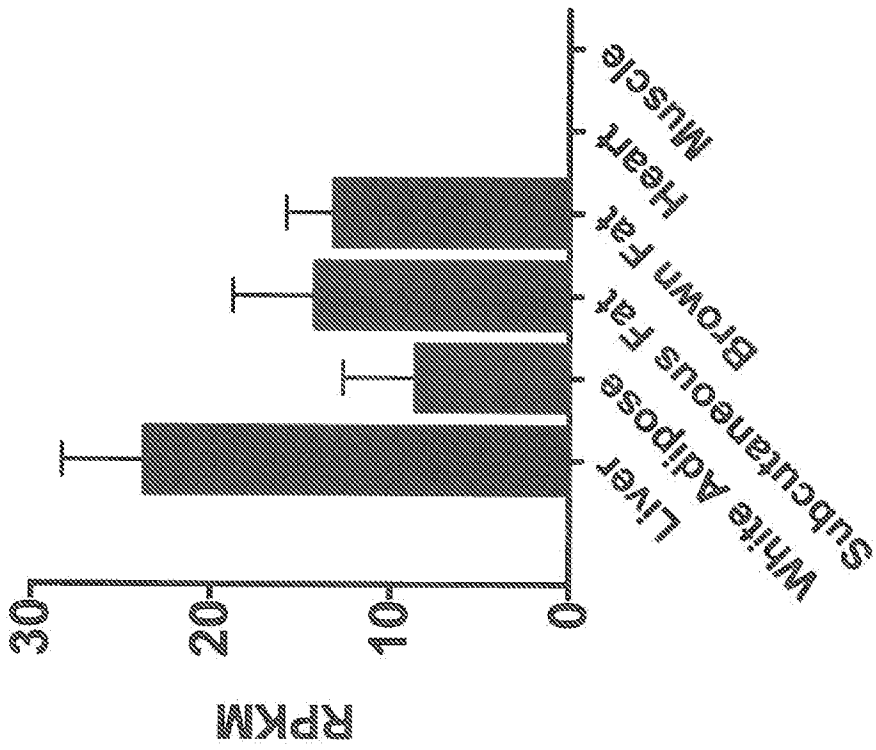
RPKM
30
20
10
0
Liver
White Adipose
Subcutaneous Fat
Brown Fat
Heart
Muscle

Figure 6

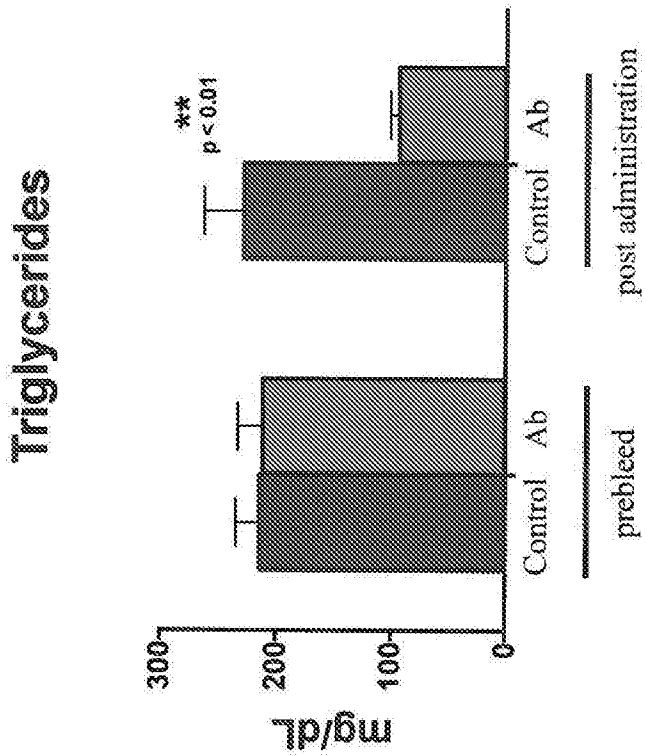
Triglycerides
mg/dL
300
200
100
0
** p < 0.01
Control Ab
Control Ab
prebleed
post administration

Figure 7

Figure 8A. *Rattus norvegicus Angptl8* mRNA (SEQ ID NO:1, NM_001271710.1):

ATACCCCCGAGACTGTCCACC**ATGGTTGTGCCTATTCTCTGCCTCCTATGGGC**
CATAGCAACAGCAGTGCGACCTGCCCCAGTGGCCCCTCTCGGTGGTCCAGA
GCCGGCCCAATATGAAGAGTTGACCCTGCTCTTTCACGGGGCCCTACAGCT
AGGTCAGGCCCTCAATGGTGTGTACAAAGCCACGGAAGCTCGCCTGACAG
AAGCTGGGCGCAACCTGGGCCTTTTTGACCAAGCACTGGAATTTCTGGGAA
GAGAGGTCAATCAGGGCCGGGATGCAACACGGGAGCTTCGCACCAGCTTG
TCGGAGATTCAGGCAGAAGAGGACACTTTACACCTTCGAGCAGAAGCCACA
GCCCGATCGCTGAGGGAAGTGGCCCGGGCCCAGCATGCTCTGCGGAACAG
TGTACGGAGACTACAAGTGCAGCTGAGAGGTGCCTGGCTAGGCCAAGCCC
ACCAAGAATTTGAGAATTTAAAGGATCGAGCCGATAAGCAGAACCACCTCT
TGTGGGCTCTCACTGGCCACGTGCAGCGACAGCAGCGTGAGATGGCAGAG
CAGCAACAGTGGCTGCGGCAGATCCAGCAGAGACTCCACATGGCAGCCCT
CCCAGCCTGAGACTACCTGGATGCCACTGAGGACCAGTTGTGCTGCAGGGAAC
ACTGAATGCGCTCCACCGGGCCTATCTATGAGCAGGGCCGACAGAGCTGGCTGC
CCATCAGCTAGACTTGGCCGGTGCACCCCGCTTCCTGGCAGAGCAGAGACAGAA
GCAAGCAGGCGGGATGGAAGGCAGAAGACAGCCCCGTGGAGAAGGCTGGAGA
AGGACATGAGCTCCCTTATGCCCCACACCCCACAATAAAAAAGAGGCAATCTAT
AAA

Figure 8B. *Rattus norvegicus* Angptl8 amino acid (SEQ ID NO:2, NP_001258639.1):

MVVPILCLLWAIATAVRPAPVAPLGGPEPAQYEELTLLFHGALQLGQALNGVYKAT
EARLTEAGRNLGLFDQALEFLGREVNQGRDATRELRTSLSEIQAEEDTLHLRAEATA
RSLREVARAQHALRNSVRRLQVQLRGAWLGQAHQEFENLKDRADKQNHLLWALT
GHVQRQQREMAEQQQWLRQIQQRLHMAALPA

Figure 8C. *Mus musculus Angptl8* mRNA (SEQ ID NO: 3, NM_ 001080940.1):

TGTCAGCCATGGCTGTGCTTGCTCTCTGCCTCCTGTGGACCTTAGCATCAGC
AGTGCGACCCGCTCCAGTGGCCCCTCTGGGTGGTCCAGAGCCAGCTCAATA
TGAAGAGCTGACCCTGCTCTTTCACGGGGCCCTGCAGCTAGGCCAGGCCCT
CAATGGCGTGTACAGAGCCACAGAGGCTCGCCTGACAGAAGCTGGGCACA
GCCTGGGCCTCTATGACAGAGCACTGGAATTCCTGGGGACAGAAGTCAGG
CAGGGCCAGGATGCCACACAGGAGCTTCGCACCAGCCTGTCGGAGATTCA
GGTGGAAGAGGACGCTTTACACCTTCGAGCTGAAGCCACAGCCCGATCACT
GGGGGAAGTGGCCCGGGCCCAGCAGGCTCTGCGGGACACTGTACGGAGAC
TACAAGTGCAGCTGAGAGGCGCCTGGCTCGGTCAAGCCCACCAAGAATTTG
AGACCTTAAAGGCTCGAGCTGATAAGCAGAGCCACCTCTTATGGGCTCTCA
CTGGCCACGTGCAGCGACAGCAGCGGGAGATGGCAGAGCAGCAACAGTGG
CTGCGACAGATCCAGCAGAGACTCCACACAGCAGCCCTCCCAGCCTGAGAC
TACCTGGATGCCACCGAGGACCAGTTGTGCTGCAAGGAACACTGAAGCGCTCCA
CCAGGCCCATGAACAGGGCTGACAGAGCCGGCTGCCCATCAGCTGGACCTGGC
CAGTGCACCCCGCTTCCTGGCAGAGCGGAGACAGAAGCAAGCAGGCGGGATGG
AAGGCAGAAGACAGAGCCCTGTGGAGGAGGGCTGGAAAAAGACACGAGCCCC
CTTATGCCCACACACCCCACAATAAAAGAGAACAGAGGCAATCTAAAAAAAAA
AAAAAAAAAAAAAA

Figure 8D. *Mus musculus* Angptl8 amino acid (SEQ ID NO:4, NP_ 001074409.1):

MAVLALCLLWTLASAVRPAPVAPLGGPEPAQYEELTLLFHGALQLGQALNGVYRA
TEARLTEAGHSLGLYDRALEFLGTEVRQGQDATQELRTSLSEIQVEEDALHLRAEAT
ARSLGEVARAQQALRDTVRRLQVQLRGAWLGQAHQEFETLKARADKQSHLLWAL
TGHVQRQQREMAEQQQWLRQIQQRLHTAALPA

Figure 8E. *Homo sapiens ANGPTL8* mRNA (SEQ ID NO:5, NM_018687.6):

ATACCTTAGACCCTCAGTCATGCCAGTGCCTGCTCTGTGCCTGCTCTGGGCC
CTGGCAATGGTGACCCGGCCTGCCTCAGCGGCCCCCATGGGCGGCCCAGA
ACTGGCACAGCATGAGGAGCTGACCCTGCTCTTCCATGGGACCCTGCAGCT
GGGCCAGGCCCTCAACGGTGTGTACAGGACCACGGAGGGACGGCTGACAA
AGGCCAGGAACAGCCTGGGTCTCTATGGCCGCACAATAGAACTCCTGGGG
CAGGAGGTCAGCCGGGGCCGGGATGCAGCCCAGGAACTTCGGGCAAGCCT
GTTGGAGACTCAGATGGAGGAGGATATTCTGCAGCTGCAGGCAGAGGCCA
CAGCTGAGGTGCTGGGGGAGGTGGCCCAGGCACAGAAGGTGCTACGGGAC
AGCGTGCAGCGGCTAGAAGTCCAGCTGAGGAGCGCCTGGCTGGGCCCTGC
CTACCGAGAATTTGAGGTCTTAAAGGCTCACGCTGACAAGCAGAGCCACAT
CCTATGGGCCCTCACAGGCCACGTGCAGCGGCAGAGGCGGGAGATGGTGG
CACAGCAGCATCGGCTGCGACAGATCCAGGAGAGACTCCACACAGCGGCG
CTCCCAGCCTGAATCTGCCTGGATGGAACTGAGGACCAATCATGCTGCAAGGA
ACACTTCCACGCCCCGTGAGGCCCCTGTGCAGGGAGGAGCTGCCTGTTCACTGG
GATCAGCCAGGGCGCCGGGCCCCACTTCTGAGCACAGAGCAGAGACAGACGCA
GGCGGGGACAAAGGCAGAGGATGTAGCCCCATTGGGGAGGGGTGGAGGAAGG
ACATGTACCCTTTCATGCCTACACACCCCTCATTAAAGCAGAGTCGTGGCATCTC
AAAAAAAAAAAAAAAAA

Figure 8F. *Homo sapiens* ANGPTL8 amino acid (SEQ ID NO:6, NP_061157.3):

MPVPALCLLWALAMVTRPASAAPMGGPELAQHEELTLLFHGTLQLGQALNGVYRT
TEGRLTKARNSLGLYGRTIELLGQEVSRGRDAAQELRASLLETQMEEDILQLQAEAT
AEVLGEVAQAQKVLRDSVQRLEVQLRSAWLGPAYREFEVLKAHADKQSHILWALT
GHVQRQRREMVAQQHRLRQIQERLHTAALPA

Figure 8G. Exemplary Engineered *Angptl8* mRNA (SEQ ID NO:7):

TGTCAGCCATG(CCAGTGCCTGCTCTGTGCCTGCTCTGGGCCCTGGCAATGG
TGACCCGGCCTGCCTCAGCGGCCCCCATGGGCGGCCCAGAACTGGCACAG
CATGAGGAGCTGACCCTGCTCTTCCATGGGACCCTGCAGCTGGGCCAGGC
CCTCAACGGTGTGTACAGGACCACGGAGGGACGGCTGACAAAGGCCAGGA
ACAGCCTGGGTCTCTATGGCCGCACAATAGAACTCCTGGGGCAGGAGGTC
AGCCGGGGCCGGGATGCAGCCCAGGAACTTCGGGCAAGCCTGTTGGAGAC
TCAGATGGAGGAGGATATTCTGCAGCTGCAGGCAGAGGCCACAGCTGAGG
TGCTGGGGGAGGTGGCCCAGGCACAGAAGGTGCTACGGGACAGCGTGCAG
CGGCTAGAAGTCCAGCTGAGGAGCGCCTGGCTGGGCCCTGCCTACCGAGA
ATTTGAGGTCTTAAAGGCTCACGCTGACAAGCAGAGCCACATCCTATGGGC
CCTCACAGGCCACGTGCAGCGGCAGAGGCGGGAGATGGTGGCACAGCAGC
ATCGGCTGCGACAGATCCAGGAGAGACTCCACACAGCGGCGCTCCCAGCC
TGAATCTGCCTGGATGGAACTGAGGACCAATCATGCTGCAAGGAACACTTCCAC
GCCCCGTGAGGCCCCTGTGCAGGGAGGAGCTGCCTGTTCACTGGGATCAGCCAG
GGCGCCGGGCCCCACTTCTGAGCACAGAGCAGAGACAGACGCAGGCGGGGACA
AAGGCAGAGGATGTAGCCCCATTGGGGAGGGGTGGAGGAAGGACATGTACCCT
TTCATGCCTACACACCCCTCATTAAAGCAGAGTCGTGGCATCTCAAAAAAAAAAA
AAAAAAA)

Figure 8H. Exemplary Engineered Angptl8 amino acid (SEQ ID NO:8):

MPVPALCLLWALAMVTRPASAAPMGGPELAQHEELTLLFHGTLQLGQALNGVYRT
TEGRLTKARNSLGLYGRTIELLGQEVSRGRDAAQELRASLLETQMEEDILQLQAEAT
AEVLGEVAQAQKVLRDSVQRLEVQLRSAWLGPAYREFEVLKAHADKQSHILWALT
GHVQRQRREMVAQQHRLRQIQERLHTAALPA

Figure 8I. Exemplary synthetic DNA fragment for engineering a non-human *Angptl8* gene (SEQ ID NO:9)

CCAGTGCCTGCTCTGTGCCTGCTCTGGGCCCTGGCAATGGTGACCCGGCCTGCCTCAGC
GGCCCCCATGGGCGGCCCAGAACTGGCACAGCATGAGGAGCTGACCCTGCTCTTCCATG
GGACCCTGCAGCTGGGCCAGGCCCTCAACGGTGTGTACAGGACCACGGAGGGACGGCT
GACAAAGGCCAGGAACAGCCTGGGTCTCTATGGCCGCACAATAGAACTCCTGGGGCAG
GAGGTCAGCCGGGGCCGGGATGCAGCCCAGGAACTTCGGGCAAGCCTGTTGGAGACTC
AGGTGGGCACCGTAGCTGCGACACTGTGGGGTGGCCAGGAGTCCAAAGAGGAGTTCGT
GTCTAGGGTAACCAACCATCCTGGTTTGCCCAGGACTGAAGGGATTCCTGGGATACAAG
ATTTTCAGCGATAAACTCAGGCAAGTCCTTAGGTACACAAAGATGAGTTGGACATCCTA
CTAGTGACCCACTGTTTATTAAGCAGATGGAGGAGGATATTCTGCAGCTGCAGGCAGAG
GCCACAGCTGAGGTGCTGGGGGAGGTGGCCCAGGCACAGAAGGTGCTACGGGACAGCG
TGCAGCGGCTAGAAGTCCAGCTGAGGAGCGCCTGGCTGGGCCCTGCCTACCGAGAATTT
GAGGTCTTAAAGGTAAGGAGCTCCCCCAACCCTAGTGGGCTGAGACCCTGATTTCCGGC
CAGAACTCGCTTCTGCACCTTGAGTCCCAAAGACCTCCCAGATCAGCCTCCCAGCTCTGT
GGCCTCTACCCTGCATGTCCCCAGACAAAACTCAAGTCCTTTTGTGTGCCTCAGTTTCCC
TTTTGTGTGCCTCAGTTGCAAATAAGGGCAACACCTGATATCTCACAGTAGGGCCAGGT
ACTCAATGCAGGTAAAATATTCAGCATGGGGCGGGCACACAGTTGGTGCTCAATAAATT
CTTTTTTTTTTTTTTTTTGAGACAGAGTCTCACTGTTGCCCAGGCTGGAGTGCAGTGGTGT
GATCTTGGCTCACTGCAACCTCCACCTCCTAGGTTCAAGTGATTCTCCTGCCTCAGCCTC
CTGAGTAGCTGGAATTACAGGTGCACCAGCTAATTTTTGTATTTTTTAGTAGAGATGGG
ATTTCACCATGTTGGCCAGGCTGGTCTCGAACTCCTGACCTCAAGGGATCTGCCTGCCTC
GGTTTCCCAAAGTGCTGGGATTACAGGTGTGAGCCACTACACCTGGCCAATAAATTCTT
ACTACTAGAGAAACTGGTAACATTTTGTGAGCACCCAGTAAGTACCCAGCACTGTTCTA
TGCCCTTTAATAATCCATATGATGGCCGGGCATGGTGGCTCATGCCTGTAATCCCAGCA
CTTTGGGTAGCTAAGGTGGGTGGAACACTTAAGGTCAGGAGTTCGAGACCACCCTGGCC
AACATGGTGAAACCCCGTCTCTACTAAAAATACAAAAAATTAGCTGGGCGTGGTGGCAC
ATGCCTGTAGTCCCAGCTACTCAGGAGGCTTAGGTAGGAGAATCGCTTGAACCTGGGAG
GTGGAGGTTGCAGTGAGCTGAGATCGTGTCATTGCACTCAGCCTGGGTGACAGAGAGA
GACTCAAAAAAAAAAAAAAATCCATAGGATGTTCATCACCTCCCCATGAAGTGAGTCCT
ATTTTATCCCCATTTTACAGATGGGGAAACTGAGGCCAAAGAGCATTGTTGACTTGCTG
GGTCACACAGATACAATGAGGGGCTGGGGCAGAGGGTCAGGGGATGGGAGGTGAGGT
GGCTGTCGGCTGAGGTTTCCATTCTGACCCCCACAGGCTCACGCTGACAAGCAGAGCCA
CATCCTATGGGCCCTCACAGGCCACGTGCAGCGGCAGAGGCGGGAGATGGTGGCACAG
CAGCATCGGCTGCGACAGATCCAGGAGAGGTGAGCCTGGCAGGGGTTTGGCAGGCAGG
GCAGTTGGATGGGGGGCGCACAGGGCAGCTGGAAAGGGGCCCCCTCACCTGGGCTGAG
CCACATCTCCCTCCCCAGACTCCACACAGCGGCGCTCCCAGCCTGAATCTGCCTGGATG
GAACTGAGGACCAATCATGCTGCAAGGAACACTTCCACGCCCCGTGAGGCCCCTGTGCA
GGGAGGAGCTGCCTGTTCACTGGGATCAGCCAGGGCGCCGGGCCCCACTTCTGAGCACA
GAGCAGAGACAGACGCAGGCGGGGACAAAGGCAGAGGATGTAGCCCCATTGGGGAGG
GGTGGAGGAAGGACATGTACCCTTTCATGCCTACACACCCCTCATTAAAGCAGAGTCGT
GGCATCTCACCCAGGGTGTCTGTGTGTGTCCTTGGCTTAGGGAGACCCCACCCAGCATG
ATGTATGAATACCTCCCATTCAAGTGCCCA

Figure 8J. Exemplary engineered *Angptl8* allele including a selection cassette (SEQ ID NO:10)

[7182allele]CACGAAACTGTCAGCCATGCCAGTGCCTGCTCTGTGCCTGCTCTGGGCCC
TGGCAATGGTGACCCGGCCTGCCTCAGCGGCCCCCATGGGCGGCCCAGAACTGGC
ACAGCATGAGGAGCTGACCCTGCTCTTCCATGGGACCCTGCAGCTGGGCCAGGCC
CTCAACGGTGTGTACAGGACCACGGAGGGACGGCTGACAAAGGCCAGGAACAGCC
TGGGTCTCTATGGCCGCACAATAGAACTCCTGGGGCAGGAGGTCAGCCGGGGCCG
GGATGCAGCCCAGGAACTTCGGGCAAGCCTGTTGGAGACTCAGGTGGGCACCGTA
GCTGCGACACTGTGGGGTGGCCAGGAGTCCAAAGAGGAGTTCGTGTCTAGGGTAA
CCAACCATCCTGGTTTGCCCAGGACTGAAGGGATTCCTGGGATACAAGATTTTCAG
CGATAAACTCAGGCAAGTCCTTAGGTACACAAAGATGAGTTGGACATCCTACTAGT
GACCCACTGTTTATTAAGCAGATGGAGGAGGATATTCTGCAGCTGCAGGCAGAGG
CCACAGCTGAGGTGCTGGGGGAGGTGGCCCAGGCACAGAAGGTGCTACGGGACA
GCGTGCAGCGGCTAGAAGTCCAGCTGAGGAGCGCCTGGCTGGGCCCTGCCTACCG
AGAATTTGAGGTCTTAAAGGTAAGGAGCTCCCCCAACCCTAGTGGGCTGAGACCC
TGATTTCCGGCCAGAACTCGCTTCTGCACCTTGAGTCCCAAAGACCTCCCAGATCA
GCCTCCCAGCTCTGTGGCCTCTACCCTGCATGTCCCCAGACAAAACTCAAGTCCTT
TTGTGTGCCTCAGTTTCCCTTTTGTGTGCCTCAGTTGCAAATAAGGGCAACACCTG
ATATCTCACAGTAGGGCCAGGTACTCAATGCAGGTAAAATATTCAGCATGGGGCG
GGCACACAGTTGGTGCTCAATAAATTCTTTTTTTTTTTTTTTTGAGACAGAGTCTC
ACTGTTGCCCAGGCTGGAGTGCAGTGGTGTGATCTTGGCTCACTGCAACCTCCACC
TCCTAGGTTCAAGTGATTCTCCTGCCTCAGCCTCCTGAGTAGCTGGAATTACAGGT
GCACCAGCTAATTTTTGTATTTTTTAGTAGAGATGGGATTTCACCATGTTGGCCAG
GCTGGTCTCGAACTCCTGACCTCAAGGGATCTGCCTGCCTCGGTTTCCCAAAGTGC
TGGGATTACAGGTGTGAGCCACTACACCTGGCCAATAAATTCTTACTACTAGAGAA
ACTGGTAACATTTTGTGAGCACCCAGTAAGTACCCAGCACTGTTCTATGCCCTTTA
ATAATCCATATGATGGCCGGGCATGGTGGCTCATGCCTGTAATCCCAGCACTTTGG
GTAGCTAAGGTGGGTGGAACACTTAAGGTCAGGAGTTCGAGACCACCCTGGCCAA
CATGGTGAAACCCCGTCTCTACTAAAAATACAAAAAATTAGCTGGGCGTGGTGGCA
CATGCCTGTAGTCCCAGCTACTCAGGAGGCTTAGGTAGGAGAATCGCTTGAACCT
GGGAGGTGGAGGTTGCAGTGAGCTGAGATCGTGTCATTGCACTCAGCCTGGGTGA
CAGAGAGAGACTCAAAAAAAAAAAAAAAATCCATAGGATGTTCATCACCTCCCCATG
AAGTGAGTCCTATTTTATCCCCATTTTACAGATGGGGAAACTGAGGCCAAAGAGCA
TTGTTGACTTGCTGGGTCACACAGATACAATGAGGGGCTGGGGCAGAGGGTCAGG
GGATGGGAGGTGAGGTGGCTGTCGGCTGAGGTTTCCATTCTGACCCCCACAGGCT
CACGCTGACAAGCAGAGCCACATCCTATGGGCCCTCACAGGCCACGTGCAGCGGC
AGAGGCGGGAGATGGTGGCACAGCAGCATCGGCTGCGACAGATCCAGGAGAGGT
GAGCCTGGCAGGGGTTTGGCAGGCAGGGCAGTTGGATGGGGGGCGCACAGGGCA
GCTGGAAAGGGGCCCCCTCACCTGGGCTGAGCCACATCTCCCTCCCCAGACTCCA
CACAGCGGCGCTCCCAGCCTGAATCTGCCTGGATGGAACTGAGGACCAATCATGC
TGCAAGGAACACTTCCACGCCCCGTGAGGCCCCTGTGCAGGGAGGAGCTGCCTGT
TCACTGGGATCAGCCAGGGCGCCGGGCCCCACTTCTGAGCACAGAGCAGAGACAG

Figure 8J (Continued)

ACGCAGGCGGGGACAAAGGCAGAGGATGTAGCCCCATTGGGGAGGGGTGGAGGA
AGGACATGTACCCTTTCATGCCTACACACCCCTCATTAAAGCAGAGTCGTGGCATC
TCACCCAGGGTGTCTGTGTGTCCTTGGCTTAGGGAGACCCCACCCAGCATGAT
GTATGAATACCTCCCATTCAAGTGCCCActcgagataacttcgtataatgtatgctatacgaagttatatgcatggcct
ccgcgccgggttttggcgcctcccgcgggcgcccccctcctcacggcgagcgctgccacgtcagacgaagggcgcagcgagcgtcctgatc
cttccgcccggacgctcaggacagcggcccgctgctcataagactcggccttagaaccccagtatcagcagaaggacattttaggacgggactt
gggtgactctagggcactggttttctttccagagagcggaacaggcgaggaaaagtagtcccttctcggcgattctgcggagggatctccgtggg
gcggtgaacgccgatgattatataaggacgcgccgggtgtggcacagctagttccgtcgcagccgggatttgggtcgcggttcttgtttgtggatc
gctgtgatcgtcacttggtgagtagcgggctgctgggctggccggggctttcgtggccgccgggccgctcggtgggacggaagcgtgtggaga
gaccgccaagggctgtagtctgggtccgcgagcaaggttgccctgaactgggggttgggggagcgcagcaaaatggcggctgttcccgagt
cttgaatggaagacgcttgtgaggcgggctgtgaggtcgttgaaacaaggtgggggggcatggtgggcggcaagaacccaaggtcttgaggcct
tcgctaatgcgggaaagctcttattcgggtgagatgggctggggcaccatctggggaccctgacgtgaagtttgtcactgactggagaactcggtt
tgtcgtctgttgcgggggcggcagttatggcggtgccgttgggcagtgcacccgtacctttgggagcgcgcgccctcgtcgtgtcgtgacgtcac
ccgttctgttggcttataatgcagggtggggccacctgccggtaggtgtgcggtaggcttttctccgtcgcaggacgcagggttcgggcctagggt
aggctctcctgaatcgacaggcgccggacctctggtgaggggagggataagtgaggcgtcagtttctttggtcggttttatgtacctatcttcttaag
tagctgaagctccggttttgaactatgcgctcggggttggcgagtgtgttttgtgaagttttttaggcacctttgaaatgtaatcatttgggtcaatatgt
aattttcagtgttagactagtaaattgtccgctaaattctggccgtttttggcttttttgttagacgtgttgacaattaatcatcggcatagtatatcggcata
gtataatacgacaaggtgaggaactaaaccatgggatcggccattgaacaagatggattgcacgcaggttctccggccgcttgggtggagaggc
tattcggctatgactgggcacaacagacaatcggctgctctgatgccgccgtgttccggctgtcagcgcaggggcgcccggttcttttttgtcaaga
ccgacctgtccggtgccctgaatgaactgcaggacgaggcagcgcggctatcgtggctggccacgacgggcgttccttgcgcagctgtgctcg
acgttgtcactgaagcgggaagggactggctgctattgggcgaagtgccggggcaggatctcctgtcatctcaccttgctcctgccgagaaagta
tccatcatggctgatgcaatgcggcggctgcatacgcttgatccggctacctgcccattcgaccaccaagcgaaacatcgcatcgagcgagcac
gtactcggatggaagccggtcttgtcgatcaggatgatctggacgaagagcatcaggggctcgcgccagccgaactgttcgccaggctcaagg
cgcgcatgcccgacggcgatgatctcgtcgtgacccatggcgatgcctgcttgccgaatatcatggtggaaaatggccgcttttctggattcatcg
actgtggccggctgggtgtggcggaccgctatcaggacatagcgttggctacccgtgatattgctgaagagcttggcggcgaatgggctgaccg
cttcctcgtgctttacggtatcgccgctcccgattcgcagcgcatcgccttctatcgccttcttgacgagttcttctgaggggatccgctgtaagtctg
cagaaattgatgatctattaaacaataaagatgtccactaaaatggaagtttttcctgtcatactttgttaagaagggtgagaacagagtacctacatttt
gaatggaaggattggagctacgggggtgggggtggggtgggattagataaatgcctgctctttactgaaggctctttactattgctttatgataatgtt
tcatagttggatatcataatttaaacaagcaaaaccaaattaagggccagctcattcctcccactcatgatctatagatctatagatctctcgtgggatc
attgtttttctcttgattcccactttgtggttctaagtactgtggtttccaaatgtgtcagtttcatagcctgaagaacgagatcagcagcctctgttccaca
tacacttcattctcagtattgttttgccaagttctaattccatcagacctcgacctgcagcccctagcccgggcgccagtagcagcacccacgtccac
cttctgtctagtaatgtccaacacctccctcagtccaaacactgctctgcatccatgtggctcccatttatacctgaagcacttgatggggcctcaatgt
tttactagagcccacccccctgcaactctgagaccctctggatttgtctgtcagtgcctcactggggcgttggataatttcttaaaaggtcaagttccc
tcagcagcattctctgagcagtctgaagatgtgtgcttttcacagttcaaatccatgtggctgtttcacccacctgcctggccttgggttatctatcagg
acctagcctagaagcaggtgtgtggcacttaacacctaagctgagtgactaactgaacactcaagtggatgccatctttgtcacttcttgactgtgac
acaagcaactcctgatgccaaagccctgcccacccctctcatgcccatatttggacatggtacaggtcctcactggccatggtctgtgaggtcctg
gtcctctttgacttcataattcctaggggccactagtatctataagaggaagagggtgctggctcccaggccacagcccacaaaattccacctgctc
acaggttggctggctcgacccaggtggtgtcccctgctctgagccagctcccggccaagccagcaccatgggtacccccaagaagaagagga
aggtgcgtaccgatttaaattccaatttactgaccgtacaccaaaatttgcctgcattaccggtcgatgcaacgagtgatgaggttcgcaagaacct
gatggacatgttcagggatcgccaggcgttttctgagcatacctggaaaatgcttctgtccgtttgccggtcgtgggcggcatggtgcaagttgaat
aaccggaaatggtttcccgcagaacctgaagatgttcgcgattatcttctatatcttcaggcgcgcggtctggcagtaaaaactatccagcaacattt
gggccagctaaacatgcttcatcgtcggtccgggctgccacgaccaagtgacagcaatgctgtttcactggttatgcggcggatccgaaaagaaa
acgttgatgccggtgaacgtgcaaaacaggctctagcgttcgaacgcactgatttcgaccaggttcgttcactcatggaaaatagtgatcgctgcc

Figure 8J (Continued)

aggatatacgtaatctggcatttctggggattgcttataacaccctgttacgtatagccgaaattgccaggatcagggttaaagatatctcacgtactg
acggtgggagaatgttaatccatattggcagaacgaaaacgctggttagcaccgcaggtgtagagaaggcacttagcctgggggtaactaaact
ggtcgagcgatggatttccgtctctggtgtagctgatgatccgaataactacctgttttgccgggtcagaaaaaatggtgttgccgcgccatctgcc
accagccagctatcaactcgcgccctggaagggatttttgaagcaactcatcgattgatttacggcgctaaggtaaatataaaatttttaagtgtataa
tgtgttaaactactgattctaattgtttgtgtatttaggatgactctggtcagagatacctggcctggtctggacacagtgcccgtgtcggagccgcg
cgagatatggcccgcgctggagtttcaataccggagatcatgcaagctggtggctggaccaatgtaaatattgtcatgaactatatccgtaacctgg
atagtgaaacaggggcaatggtgcgcctgctggaagatggcgattgatctagataagtaatgatcataatcagccatatcacatctgtagaggtttt
acttgctttaaaaaacctcccacacctcccctgaacctgaaacataaaatgaatgcaattgttgttgttaaacctgccctagttgcggccaattccag
ctgagcgtgcctccgcaccattaccagttggtctggtgtcaaaaataataataaccgggcaggggggatctaagctctagataagtaatgatcata
atcagccatatcacatctgtagaggttttacttgctttaaaaaacctcccacacctcccctgaacctgaaacataaaatgaatgcaattgttgttgttaa
cttgtttattgcagcttataatggttacaaataaagcaatagcatcacaaatttcacaaataaagcatttttttcactgcattctagttgtggtttgtccaaa
ctcatcaatgtatcttatcatgtctggaataacttcgtataatgtatgctatacgaagttatgctagtaactataacggtcctaaggtagcgagctagcG
ATGCCACCGAGGACCAGTTGTGCTGCAAGGAACACTGAAGCGCTCCACCAGGCCCATG
AACAGGGCTGACAGAG

Figure 8K. Exemplary engineered *Angptl8* allele after recombinase-mediated excision of a selection cassette (SEQ ID NO:11)

[7183allele]CACGAAACTGTCAGCCATGCCAGTGCCTGCTCTGTGCCTGCTCTGGGCCC
TGGCAATGGTGACCCGGCCTGCCTCAGCGGCCCCCATGGGCGGCCCAGAACTGGC
ACAGCATGAGGAGCTGACCCTGCTCTTCCATGGGACCCTGCAGCTGGGCCAGGCC
CTCAACGGTGTGTACAGGACCACGGAGGGACGGCTGACAAAGGCCAGGAACAGCC
TGGGTCTCTATGGCCGCACAATAGAACTCCTGGGGCAGGAGGTCAGCCGGGGCCG
GGATGCAGCCCAGGAACTTCGGGCAAGCCTGTTGGAGACTCAGGTGGGCACCGTA
GCTGCGACACTGTGGGGTGGCCAGGAGTCCAAAGAGGAGTTCGTGTCTAGGGTAA
CCAACCATCCTGGTTTGCCCAGGACTGAAGGGATTCCTGGGATACAAGATTTTCAG
CGATAAACTCAGGCAAGTCCTTAGGTACACAAAGATGAGTTGGACATCCTACTAGT
GACCCACTGTTTATTAAGCAGATGGAGGAGGATATTCTGCAGCTGCAGGCAGAGG
CCACAGCTGAGGTGCTGGGGGAGGTGGCCCAGGCACAGAAGGTGCTACGGGACA
GCGTGCAGCGGCTAGAAGTCCAGCTGAGGAGCGCCTGGCTGGGCCCTGCCTACCG
AGAATTTGAGGTCTTAAAGGTAAGGAGCTCCCCCAACCCTAGTGGGCTGAGACCC
TGATTTCCGGCCAGAACTCGCTTCTGCACCTTGAGTCCCAAAGACCTCCCAGATCA
GCCTCCCAGCTCTGTGGCCTCTACCCTGCATGTCCCCAGACAAAACTCAAGTCCTT
TTGTGTGCCTCAGTTTCCCTTTTGTGTGCCTCAGTTGCAAATAAGGGCAACACCTG
ATATCTCACAGTAGGGCCAGGTACTCAATGCAGGTAAAATATTCAGCATGGGGCG
GGCACACAGTTGGTGCTCAATAAATTCTTTTTTTTTTTTTTTTGAGACAGAGTCTC
ACTGTTGCCCAGGCTGGAGTGCAGTGGTGTGATCTTGGCTCACTGCAACCTCCACC
TCCTAGGTTCAAGTGATTCTCCTGCCTCAGCCTCCTGAGTAGCTGGAATTACAGGT
GCACCAGCTAATTTTTGTATTTTTTAGTAGAGATGGGATTTCACCATGTTGGCCAG
GCTGGTCTCGAACTCCTGACCTCAAGGGATCTGCCTGCCTCGGTTTCCCAAAGTGC
TGGGATTACAGGTGTGAGCCACTACACCTGGCCAATAAATTCTTACTACTAGAGAA
ACTGGTAACATTTTGTGAGCACCCAGTAAGTACCCAGCACTGTTCTATGCCCTTTA
ATAATCCATATGATGGCCGGGCATGGTGGCTCATGCCTGTAATCCCAGCACTTTGG
GTAGCTAAGGTGGGTGGAACACTTAAGGTCAGGAGTTCGAGACCACCCTGGCCAA
CATGGTGAAACCCCGTCTCTACTAAAAATACAAAAAATTAGCTGGGCGTGGTGGCA
CATGCCTGTAGTCCCAGCTACTCAGGAGGCTTAGGTAGGAGAATCGCTTGAACCT
GGGAGGTGGAGGTTGCAGTGAGCTGAGATCGTGTCATTGCACTCAGCCTGGGTGA
CAGAGAGAGACTCAAAAAAAAAAAAAAAATCCATAGGATGTTCATCACCTCCCCATG
AAGTGAGTCCTATTTTATCCCCATTTTACAGATGGGGAAACTGAGGCCAAAGAGCA
TTGTTGACTTGCTGGGTCACACAGATACAATGAGGGGCTGGGGCAGAGGGTCAGG
GGATGGGAGGTGAGGTGGCTGTCGGCTGAGGTTTCCATTCTGACCCCCACAGGCT
CACGCTGACAAGCAGAGCCACATCCTATGGGCCCTCACAGGCCACGTGCAGCGGC
AGAGGCGGGAGATGGTGGCACAGCAGCATCGGCTGCGACAGATCCAGGAGAGGT
GAGCCTGGCAGGGGTTTGGCAGGCAGGGCAGTTGGATGGGGGGGCGCACAGGGCA
GCTGGAAAGGGGCCCCCTCACCTGGGCTGAGCCACATCTCCCTCCCCAGACTCCA
CACAGCGGCGCTCCCAGCCTGAATCTGCCTGGATGGAACTGAGGACCAATCATGC
TGCAAGGAACACTTCCACGCCCCGTGAGGCCCCTGTGCAGGGAGGAGCTGCCTGT
TCACTGGGATCAGCCAGGGCGCCGGGCCCCACTTCTGAGCACAGAGCAGAGACAG
ACGCAGGCGGGGACAAAGGCAGAGGATGTAGCCCCATTGGGGAGGGGTGGAGGA
AGGACATGTACCCTTTCATGCCTACACACCCCTCATTAAAGCAGAGTCGTGGCATC
TCACCCAGGGTGTCTGTGTGTGTCCTTGGCTTAGGGAGACCCCACCCAGCATGAT
GTATGAATACCTCCCATTCAAGTGCCCActcgagataacttcgtataatgtatgctatacgaagttatgctagtaacta
taacggtcctaaggtagcgagctagcGATGCCACCGAGGACCAGTTGTGCTGCAAGGAACACTGAAGC
GCTCCACCAGGCCCATGAACAGGGCTGACAGAG

Figure 9A.

(AAGGCAGCCG CAGCGGCCCG GGAACCACAC CCACGAAACT GTCAGCC**ATG**) CCAGTGCCTG CTCTGTGCCT GCTCTGGGCC CTGGCAATGG TGACCCGGCC (SEQ ID NO: 15)

Figure 9B.

GGGAGACCCC ACCCAGCATG ATGTATGAAT ACCTCCCATT CAAGTGCCCA (*CTCGAG* **ATAACTTCG TATAATGTAT GCTATACGAA GTTAT** ATGCATGGCC TCCGCGCCGG GTTTTGGCGC CTCCCGCGGG CGCCCCCCTC CTCACGGCGA GCGCTGCCAC GTCAGACGAA GGGCGCAGCG AGCGTCCTGA) (SEQ ID NO:16)

Figure 9C.

(TTTCACTGCAT TCTAGTTGTG GTTTGTCCAA ACTCATCAAT GTATCTTATC ATGTCTGGA **ATAACTTCGTATAATGTATGCTATACGAAGTTAT** GCTAGTAACTATAACGGTCCTAAGGTAGCGA *GCTAGC*) GATGCCACCGA GGACCAGTTGT GCTGCAAGGAA CACTGAAGCG CTCCACC (SEQ ID NO:17)

Figure 9D.

GGGAGACCCC ACCCAGCATG ATGTATGAAT ACCTCCCATT CAAGTGCCCA (*GTCGAG* **ATAACTTCGTATAATGTATGCTATACGAAGTTAT** GCTAGTAACTATAACGGTCCTAAGGTAGCGA *GCTAGC*) GATGCCACCG AGGACCAGTT GTGCTGCAAG GAACACTGAA GCGCTCCACC (SEQ ID NO:18)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/016487

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/515 A01K67/027 A61K49/00 C07K16/22 A61K39/00
ADD.

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)
C07K A01K A61K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/098887 A2 (GENENTECH INC [US]; LEXICON GENETICS INC [US]; BYERS-HORNER ALLISON AN) 21 September 2006 (2006-09-21)	1-3,7-9, 11-15, 17, 20-27, 37-42, 45,47,48
Y	page 142, line 27 - line 37 page 202, line 9 - line 14 example 70.26; sequence 52	4-6,10, 16,18, 19, 28-36, 43,44, 46,49-68

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
3 April 2017	19/04/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Deleu, Laurent

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	ZHIYAO FU ET AL: "A lipasin/Angptl8 monoclonal antibody lowers mouse serum triglycerides involving increased postprandial activity of the cardiac lipoprotein lipase", SCIENTIFIC REPORTS, vol. 5, no. 1, 21 December 2015 (2015-12-21), XP055360939, DOI: 10.1038/srep18502 figure 1 -----	55

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