

ANIMAL MODELS OF POSTNATAL CONDITIONS ASSOCIATED WITH REDUCED LEVELS OF PLASMALOGEN

Field of the Invention

[0001] The present invention generally relates to animal models for studying conditions associated with postnatal reduced levels of plasmalogens. More specifically, the present invention relates to inducible conditional knock out animal models in which levels of one or more of the proteins involved in the biosynthesis of plasmalogens is reduced. The inducible conditional knock out animal can be used to study the effect of reduced plasmalogen levels at any postnatal time during the life of the animal. In addition, the inducible conditional knock out animal can be used to study the effect of reduced plasmalogen levels specifically within a particular tissue or tissues. The inducible conditional knock out animal can also be used to test potential compounds in the treatment of conditions associated with reduced postnatal levels of plasmalogens.

Background of the Invention

[0002] Plasmalogens are membrane phospholipids, characterized by the presence of a vinyl-ether bond at the *sn*1 position of the glycerol backbone. They compose 15-20% of the total phospholipid content of a cell membrane, with neuronal tissues being particularly enriched¹. The vinyl-ether bond results in a conformation change which consequently has a major effect on the physical properties of a cellular membrane. A direct effect of decreased membrane plasmalogen is a reduced propensity for vesicular fusion²⁻⁴, a process critical for neurotransmission. Plasmalogens are also important for the structure of membrane microdomain regions required for the proper function of membrane-anchored proteins such as membrane-bound enzymes, receptors, and transporters⁵⁻⁷ as well as have potent antioxidant activity^{8,9}.

[0003] Numerous diseases have been linked to a plasmalogen deficiency including Alzheimer's disease (AD)^{10,11}, Parkinson's disease^{12,13}, Schizophrenia¹⁴, Down syndrome¹⁵, and Gaucher's disease¹⁶. Mechanistically, plasmalogens promote a specific cellular process called vesicular fusion, whereby small vesicles inside of cells merge with the plasma membrane. This is highly relevant in the brain because this fusion process is required for the release and re-uptake of neurotransmitters from neurons. A direct effect of decreased membrane plasmalogen is therefore reduced neurotransmission. Plasmalogens are also important for the structure of membrane

microdomain regions required for the proper function of membrane-anchored proteins such as membrane-bound enzymes, receptors, and transporters. The diverse consequences of plasmalogen deficiency, combined with the physiological importance of these functions, leads to the hypothesis that decreased plasmalogen levels could be underlying and driving the disease process of a number of diseases of aging. Confirming this direct relationship between disease state and plasmalogen levels has been prevented due to the absence of acceptable animal models of plasmalogen deficiency that occur only postnatally.

[0004] There are currently-available genetic models based on germline mutations in genes involved in plasmalogen biosynthesis, which mimic the human disease Rhizomelic chondrodysplasia punctata (RCDP). This disease has an extreme phenotype, with shortened proximal bones, stippled epiphyses around large joints, severe cognitive impairment, recurrent respiratory illness, cardiac malformations, and extremely shortened lifespan¹⁷⁻²⁰. The severity of the disorder highlights the criticality of proper plasmalogen levels in human development, function, and survival, however these animal models display numerous developmental abnormalities in the brain²¹⁻²³, as well as shortened lifespan. These models however are not useful in the context of understanding the role of plasmalogens in conditions associated with postnatal plasmalogen reductions, most notable neurodegenerative diseases. Transient knockdown of genes involved in plasmalogen biosynthesis using injected small hairpin RNA (sh-RNA) has been reported as an alternative to full knockout animals²⁴. While this approach did allow the researchers to control the timing and location of the knockdown, it has serious limitations in its utility of evaluating the disease process associated with chronic plasmalogen deficiency. The approach results in a knockdown of mRNA levels in only in a very limited region of the body, which in the case of the previously reported study, was the hippocampal region of the brain. While valuable in understanding the impact of plasmalogen reductions on a specific region of anatomy, it does not allow for the evaluation of the effect of systemic reductions in plasmalogen biosynthesis which occurs in human disease. Additionally, use of sh-RNA results in only a transient reduction of

mRNA levels and as such evaluations of chronic plasmalogen deficiency requires repeated injections.

[0005] The available animal models are therefore unsatisfactory and thus there is a need for animal models capable of mimicking adult onset of conditions associated with plasmalogen deficiency in humans.

Summary of the Invention

[0006] The shortcomings of the prior art are generally mitigated by the creation of the model as described herein for reducing postnatal levels of plasmalogens in a non-human animal or animal model. The model could be created in any number of ways, such as inducing recombination of a gene, or part thereof, involved in the biosynthesis of plasmalogens in an inducible conditional knock out non-human animal or a non-human animal model at a desired age or within a desired tissue.

[0007] In an embodiment of the invention, there's provided a transgenic, non-human animal or a non-human animal model comprising a genome of the transgenic, non-human animal or a non-human animal model that has a gene comprising at least one regulatory region, wherein the gene is a gene regulating a plasmalogen biosynthetic pathway. The regulatory region of the gene comprises at least one conditionally inducible gene editing site. The at least one conditionally inducible gene editing site includes either an exogenous nucleic acid sequence or an endogenous nucleic acid sequence. The genome further comprises at least one nucleic acid editing sequence that is integrated at a separate locus from the gene. The at least one nucleic acid editing sequence encodes a gene product that is capable of editing the conditionally inducible gene editing site thereby blocking or disrupting the expression of the gene that regulates the plasmalogen biosynthetic pathway. In another aspect of the invention, there could be more than one genes that are capable of regulating the plasmalogen biosynthetic pathway which are disrupted as noted above. In some aspect of the invention, the downregulation or disruption of the genes is conditionally triggered or induced by using an exogenous compound or an exogenous stimulus.

[0008] In another embodiment a transgenic non-human animal or animal model for post-natal, conditionally inducible, plasmalogen deficiency is provided. The animal or animal model has a

genome with: a gene with at least one regulatory region, wherein the gene is capable of regulating a plasmalogen biosynthetic pathway. The at least one regulatory region has at least one conditionally inducible gene editing site, wherein the gene editing site is capable of blocking expression of the gene when edited; and at least one nucleic acid editing sequence integrated at a separate locus from the gene, wherein the at least one nucleic acid editing sequence encodes a gene product capable of editing the at least one gene editing site when conditionally induced, thereby down-regulating or disrupting the plasmalogen biosynthetic pathway.

[0009] In another aspect of the invention, there's provided an animal model for post-natally and conditionally inducing plasmalogen deficiency. The animal model includes a transgenic, non-human animal with a genome that has a gene that is capable of regulating a plasmalogen biosynthetic pathway. The gene has at least one regulatory region. The regulatory region of the gene has at least one conditionally inducible gene editing site, wherein the conditionally inducible gene editing site is capable of blocking the expression of the gene responsible for plasmalogen biosynthetic pathway when edited. The genome further comprises at least one nucleic acid editing sequence that is integrated or incorporated at a separate locus from the gene. The at least one nucleic acid editing sequence is capable of encoding a gene product that edits the at least one conditionally inducible gene editing site when it's conditionally induced, thereby down-regulating or disrupting the plasmalogen biosynthetic pathway. The downregulation or disruption of the gene editing sequence is conditionally triggered or induced by using an exogenous compound or an exogenous stimulus.

[0010] In an aspect of the invention, the transgenic animal or the animal model comprising the transgenic animal is of any non-human animal. In some aspects of the invention, the non-human animal or the non-human animal model is a rodent.

[0011] In an aspect of the invention, the at least one conditionally inducible gene editing site further comprises a second, a third, a fourth, or a fifth conditionally inducible gene editing sites. The second, third, fourth or the fifth conditionally inducible gene editing sites, each has an exogenous nucleic acid sequence, or an endogenous nucleic acid sequence. The gene product

encoded by the nucleic acid editing sequence encodes is capable of editing the second, the third, the fourth, and the fifth conditionally inducible gene editing sites.

[0012] In some aspects of the invention, the one or more conditionally inducible gene editing sites, or in some instances the first, the second, the third, the fourth, and the fifth conditionally inducible gene editing sites, are LoxP sites, flippase recognition target (FRT) sites, attP and attB sites, sgRNA binding sites, FokI sites, zinc finger nuclease sites or any combination thereof.

[0013] In some aspects of the invention, the gene product encoded by the nucleic acid editing sequence is Cre, flippase, PhiC31, Cas9, an sgRNA, a crRNA, transcription activator-like effector nuclease (TALEN), zinc-finger nuclease (ZFN), any variant thereof, or any combination thereof.

[0014] In some aspects of the invention, the at least one conditionally inducible gene editing site or one or more conditionally inducible gene editing site are LoxP sites and the gene product is Cre or a variant thereof. The Cre gene product is a fusion protein which further includes a mutant estrogen ligand binding domain (ERT2). The Cre gene product is operatively linked to a promoter element with a tetracycline responsive element capable of inducing gene expression with the addition or removal of tetracycline from the transgenic animal.

[0015] In certain embodiments, the one or more conditionally inducible gene editing sites are FRT sites and the gene product is a flippase or a variant thereof. In certain embodiments, the one or more conditionally inducible gene editing sites are attP and attB sites and the gene product is PhiC31 or a variant thereof. In certain embodiments, the one or more conditionally inducible gene editing sites are FokI sites and the gene product is a TALEN or a variant thereof. In certain embodiments, the one or more conditionally inducible gene editing sites are zinc finger nuclease sites and the gene product is a zinc-finger nuclease. In certain embodiments, the one or more conditionally inducible gene editing sites are sgRNA binding sites and the gene product is a Cas9 or a variant thereof, and/or one or more of a sgRNA, a crRNA, or both, which are capable of binding the sgRNA binding sites. In certain embodiments, at least one conditionally inducible gene editing site is in proximity to the at least one regulatory region.

[0016] In certain embodiments, the at least one regulatory region is an exon, an element required for transcription of the gene, an element required for translation of the gene, an element required

for the function of the protein encoded by the genomic sequence comprising the gene, or any combination thereof. In certain specific embodiments, the at least one regulatory region is an exon.

[0017] In an aspect of the invention, the expression of the gene product is conditionally inducible. The expression of the gene product may be conditionally induced by the addition of an exogenous compound, an exogenous stimulus or both. In certain embodiments, the expression of the gene product may be conditionally induced by addition of tamoxifen, 4-hydroxytamoxifen (4-OHT), mifepristone, tetracycline, doxycycline, light, temperature or any combination thereof.

[0018] In some embodiments, the gene capable of regulating the plasmalogen biosynthetic pathway is fatty acid reductase 1 (FAR1), glyceronephosphate O-acyltransferase (GNPAT), alkylglycerone phosphate synthase (AGPS), acyl/alkyl-DHAP reductase, alkyl/acyl-GPA acyltransferase, phosphatidic acid phosphatase, ethanolamine phosphotransferase, plasmenylethanolamine desaturase, choline phosphotransferase or any combination thereof. In certain specific embodiments, the gene capable of regulating the plasmalogen biosynthetic pathway is glyceronephosphate O-acyltransferase (GNPAT). In the above embodiments, the at least one conditionally inducible gene editing site is not edited by the gene product at or before birth of the animal i.e. it's only edited post natally.

[0019] In certain embodiments, the transgenic animal or the animal model has one or more additional genes, each of the one or more additional genes comprising at least one regulatory region, wherein each of the one or more additional gene regulates a plasmalogen biosynthetic pathway. In these embodiments, the at least one additional conditionally inducible gene editing site is present within the at least one regulatory region. In these embodiments, the one or more additional genes may comprise a first additional conditionally inducible gene editing site and/or a second additional gene editing, wherein the first additional conditionally inducible gene editing site and/or the second additional conditionally inducible gene editing site of each of the at least one conditionally inducible gene editing sites comprise an exogenous nucleic acid sequence, or an endogenous nucleic acid sequence.

[0020] In some instances, the one of more additional genes can have a first, as second, a third, a fourth and a fifth conditionally inducible gene editing site. The second nucleic acid sequence may be integrated at a separate locus from the conditionally inducible gene editing sites, the first nucleic acid sequence, the first gene and the one or more additional genes, wherein the second nucleic acid

sequence encodes a second gene product capable of editing the one or more additional genes. The first i.e. the original gene product and the second gene product may be different.

[0021] In an aspect of the invention, the transgenic animal or animal model may be a rodent, and a genome of the rodent may include a glyceronephosphate O-acyltransferase (GNPAT) gene comprising loxP sites; and a gene encoding for a fusion protein of a Cre recombinase and a mutant estrogen ligand-binding domain (ERT2). In another alternate aspect of the invention, the transgenic animal or animal model may be a rodent, where a genome of the rodent comprises a glyceronephosphate O-acyltransferase (GNPAT) gene comprising loxP sites; and a Cre recombinase gene encoding Cre under the control of a tetracycline-responsive element. In another alternate aspect of the invention, the transgenic animal or animal model is a rodent, where a genome of the rodent has a glyceronephosphate O-acyltransferase (GNPAT) gene comprising one or more sites capable of binding an sgRNA and a promoter operatively connected to at least one sgRNA capable of binding the one or more sites capable of binding an sgRNA; and a gene encoding a Cas9 or variant thereof.

[0022] In another aspect of the invention, the transgenic animal or animal model may be a rodent, where a genome of the rodent has a first LoxP site upstream of exon 4 of the gene encoding glyceronephosphate O-acyltransferase (GNPAT) and a second LoxP site downstream of exon 4 of the gene glyceronephosphate O-acyltransferase (GNPAT); and a nucleic acid sequence encoding CAGGCre-ER^{T2}.

[0023] In another aspect of the invention, the transgenic animal or animal model may be a rodent, where the genome of the rodent has a first sgRNA site upstream of exon 4 of the gene encoding glyceronephosphate O-acyltransferase (GNPAT) capable of binding an sgRNA of GTGAGCTCCCCGGCCTCTC (SEQ ID NO:1) and a second sgRNA site downstream of exon 4 of the gene encoding glyceronephosphate O-acyltransferase (GNPAT) capable of binding an sgRNA of ATGGCAGACAGGGGCCCTTC (SEQ ID NO:2) and a first nucleic acid sequence operatively linked to a sequence encoding the sgRNA of GTGAGCTCCCCGGCCTCTC (SEQ ID NO:1), a second nucleic acid sequence operatively linked to a sequence encoding the sgRNA of GTGAGCTCCCCGGCCTCTC (SEQ ID NO:1), and a third nucleic acid sequence encoding

Cas9 or a variant thereof, wherein the Cas9 or variant thereof is operatively linked to an inducible regulatory element.

[0024] In another aspect of the invention, at least one genome of the transgenic animal or animal model comprises the genome. In the event, a cell of the transgenic animal or animal model is modified, only one genome of the transgenic animal or animal model will have the genome. In another aspect of the invention, all of the genomes of the transgenic animal or animal model comprises the genome.

[0025] In an aspect of the invention, a cell from the transgenic animal or animal model described hereinbefore is provided.

[0026] In another aspect of the invention, a method of reducing postnatal plasmalogen levels in a non-human animal or a non-human animal model is provided. The method includes providing the transgenic animal or the transgenic model as described hereinbefore, and inducing expression of the nucleic acid editing sequence encoding the gene product capable of editing the at least one conditionally inducible gene editing site, wherein the editing of the at least one conditionally inducible gene editing site decreases expression of the gene regulating the plasmalogen biosynthetic pathway.

[0027] In another aspect of the invention, use of the transgenic animal or animal model as described hereinbefore for reducing postnatal plasmalogen levels in a non-human animal or the non-human animal model, wherein the transgenic animal or animal model is for inducing expression of the nucleic acid editing sequence encoding the gene product capable of editing the at least one conditionally inducible gene editing site, wherein the editing of the at least one conditionally inducible gene editing site is for decreasing expression of the gene regulating the plasmalogen biosynthetic pathway.

[0028] In another aspect of the invention, the transgenic animal or animal model as described hereinbefore is provided for use in reducing postnatal plasmalogen levels in a non-human animal or animal model, wherein animal or animal model is used for inducing expression of the nucleic acid editing sequence encoding the gene product capable of editing the at least one conditionally inducible gene editing site, wherein the editing of the at least one conditionally inducible gene

editing site is for decreasing expression of the gene regulating the plasmalogen biosynthetic pathway.

[0029] In another aspect of the invention, a method of inducing a condition or disease in a non-human animal or animal model associated with reduced postnatal plasmalogen levels is provided. The method involves providing the transgenic animal or animal model as described hereinbefore. This is followed by a step of inducing expression of the nucleic acid editing sequence encoding the gene product capable of editing the at least one conditionally inducible gene editing site, wherein the editing of the at least one conditionally inducible gene editing site decreases expression of the gene regulating the plasmalogen biosynthetic pathway; and monitoring the transgenic animal or animal model for symptoms of the condition or disease.

[0030] In another aspect of the invention, use of the transgenic animal or the animal model as described herein before for inducing a condition or disease in a non-human animal or animal model associated with reduced postnatal plasmalogen levels, wherein the transgenic animal or animal model is for: inducing expression of the nucleic acid editing sequence encoding the gene product capable of editing the at least one conditionally inducible gene editing site, wherein the editing of the at least one conditionally inducible gene editing site is for decreasing expression of the gene regulating the plasmalogen biosynthetic pathway; and monitoring the transgenic animal or animal model for symptoms of the condition or disease.

[0031] In another aspect of the invention, the transgenic animal or the animal model described hereinbefore for use in inducing a condition or disease in a non-human animal or animal model associated with reduced postnatal plasmalogen levels is provided. The transgenic animal or animal model is used for inducing expression of the nucleic acid editing sequence encoding the gene product capable of editing the at least one conditionally inducible gene editing site, wherein the editing of the at least one conditionally inducible gene editing site is for decreasing expression of the gene regulating the plasmalogen biosynthetic pathway; and monitoring the transgenic animal or animal model for symptoms of the condition or disease.

[0032] In another aspect of the invention, a method for determining the efficacy of a compound or composition for treating a condition or disease associated with a reduced plasmalogen level is provided. The method involves providing the transgenic animal or the animal model described hereinbefore. This is followed by inducing expression of the nucleic acid editing sequence

encoding the gene product capable of editing the at least one conditionally inducible gene editing site, wherein the editing of the at least one conditionally inducible gene editing site is for decreasing expression of the gene regulating the plasmalogen biosynthetic pathway; administering the compound or the composition to the transgenic animal or animal model; and monitoring the transgenic animal or animal model for symptoms of the condition or disease. Alternatively, the method involves administering the compound or the composition to the transgenic animal or animal model; inducing expression of the nucleic acid editing sequence encoding the gene product capable of editing the at least one conditionally inducible gene editing site, wherein the editing of the at least one conditionally inducible gene editing site is for decreasing expression of the gene regulating the plasmalogen biosynthetic pathway; and monitoring the transgenic animal or animal model for symptoms of the condition or disease;. Alternatively, the method involves administering the compound or the composition to the transgenic animal or animal model and inducing expression of the nucleic acid editing sequence encoding the gene product capable of editing the at least one conditionally inducible gene editing site, wherein the editing of the at least one conditionally inducible gene editing site is for decreasing expression of the gene regulating the plasmalogen biosynthetic pathway; and monitoring the transgenic animal or animal model for symptoms of the condition or disease.

[0033] In some embodiments, use of the transgenic animal or the animal model for determination of the efficacy of a compound or composition for the treatment of a condition or disease associated with a reduced plasmalogen level is provided. In an alternate embodiment, the transgenic animal or the animal model as described hereinbefore is provided for use for in determination of the efficacy of a compound or composition for the treatment of a condition or disease associated with a reduced plasmalogen level.

[0034] In the above-recited aspects an embodiments of the invention, inducing expression of the nucleic acid editing sequence encoding the gene product capable of editing the at least one conditionally inducible gene editing site excises or modifies at least a portion of the gene. In these embodiments, the condition or disease associated with a reduced plasmalogen level is a neurodegenerative disease, presence of cataracts, respiratory disease, chronic inflammation, impaired myelination, metabolic syndrome, type II diabetes, or cardiovascular disease. In some specific embodiments, the neurodegenerative disease is Alzheimer's disease, Parkinson's disease, or Multiple sclerosis. In some further embodiments, the respiratory disease is bronchopulmonary

dysplasia (BPD) or chronic obstructive pulmonary disease (COPD). In these embodiments, the symptoms of the condition or disease comprise one or more of the following: a decreased plasmalogen level, and/or one or more symptoms associated with the diseases and/or conditions defined hereinbefore.

[0035] In another aspect of the invention, a method of generating the transgenic animal or the animal model is provided. The method involves, providing at least one non-human animal or animal model; introducing at least one gene editing sequence in a gene, part or regulatory element thereof, involved in the biosynthesis of plasmalogen to generate a first edited gene in the non-human animal or animal model; crossing the non-human animal or animal model with the first edited gene with a second non-human animal or animal model comprising a nucleic acid editing sequence at a separate locus encoding a gene product, to generate the transgenic animal or animal model; and inducing expression of the gene product in the transgenic animal or animal model to edit the first edited gene, wherein editing the first edited gene results in a decrease in the level of the gene product of the first edited gene, reduced activity of the first edited gene product of the first edited gene, reduced plasmalogen level in the transgenic animal or animal model or any combination thereof.

[0036] In any of the above-recited embodiments, the expression of the gene product is induced by providing the animal or animal model with an exogenous compound or an exogenous stimulus. The expression of the gene product may be induced by addition of tamoxifen, 4-hydroxytamoxifen (4-OHT), mifepristone, tetracycline, light, temperature or any combination thereof.

[0037] Other and further aspects and advantages of the present invention will be better understood upon the reading of the illustrative embodiments about to be described or will be indicated in the appended claims, and various advantages not referred to herein will occur to one skilled in the art upon employment of the invention in practice.

Brief Description of the Drawings

[0038] The above and other aspects, features and advantages of the invention will become more readily apparent from the following description, reference being made to the accompanying drawings in which:

[0039] Figure 1 shows a schematic of *Gnpat* gene region targeted for animal model creation including the relative locations of LoxP sites that results in the conditional deletion of exon 4

following administration of TMX and subsequent recombination between the LoxP sites, according to an embodiment.

[0040] Figure 2 shows boxplots of relative plasmalogen levels at one month post-TMX treatment in Homozygous *Gnpat* cKO Hemi CAGGCRE-ER mice. Total relative plasmalogen levels (based on the sum of all 8 measured individual species and normalized to the mean of vehicle) across various tissues. Asterisks denotes $p < 0.05$ versus the mean of the corresponding vehicle-treated plasmalogen.

[0041] Figure 3 shows boxplots of relative levels of specific plasmalogens at one month post-TMX treatment in *Gnpat* cKO mice across various tissues (A – Serum; B – Brain; C – Heart; D – Liver; E – Intestine; and F – Lung) based on the sum of all 8 measured individual species and normalized to the mean of vehicle in various tissues of vehicle- and TMX-treated animals. The first number in the x-axis labels represent the carbon length and saturation of the *sn1* sidechain; the second number represent the *sn2* sidechain. Asterisks indicate $p < 0.05$ versus wild-type. Asterisks denotes $p < 0.05$ versus the mean of the corresponding vehicle-treated plasmalogen.

[0042] Figure 4 shows boxplots of total relative plasmalogen levels at four months post-TMX treatment in Homozygous *Gnpat* cKO Hemi CAGGCRE-ER mice based on the sum of all 8 measured individual species and normalized to the mean of vehicle across various tissues. Asterisks indicate $p < 0.05$ versus wild-type. Asterisks denotes $p < 0.05$ versus the mean of the corresponding vehicle-treated plasmalogen.

[0043] Figure 5 shows boxplots of relative levels of specific plasmalogens at four month post-TMX treatment in *Gnpat* cKO mice across various tissues (A – Serum; B – Brain; C – Heart; D – Liver; E – Intestine; and F – Lung) based on the sum of all 8 measured individual species and normalized to the mean of vehicle in various tissues of vehicle- and TMX-treated animals. The first number in the x-axis labels represent the carbon length and saturation of the *sn1* sidechain; the second number represent the *sn2* sidechain. Asterisks indicate $p < 0.05$ versus wild-type. Asterisks denotes $p < 0.05$ versus the mean of the corresponding vehicle-treated plasmalogen.

[0044] Figure 6 shows the correlation between serum and brain plasmalogen levels among all 4 month animals, according to an embodiment.

[0045] Figure 7 shows open field test results. A). Mean distance travelled in the open field test by minute for animals following 1 month post-TMX treatment. B). Total mean distance travelled over 10 minutes following 1 month post-TMX treatment. C). Mean distance travelled by minute following 4 months post-TMX treatment. D). Total mean distance travelled over 10 minutes following 4 months post-TMX treatment.

[0046] Figure 8 shows the correlations between distance travelled and concentrations of individual plasmalogen species in the brain of 8 animals, according to an embodiment. Asterisks denote $p < 0.01$.

[0047] Figure 9 shows nerve conduction velocity (NCV) results. Boxplots of (A) latency, (B) NCV, and (C) amplitude across vehicle-treated, controls, and 4 month post-TMX treatment.

[0048] Figure 10 shows the correlation plots between serum plasmalogen levels and latency across all animals at 4 months post-TMX treatment, according to an embodiment.

[0049] Figure 11 shows the correlation plots between serum plasmalogen levels and NCV across all animals at 4 months post-TMX treatment, according to an embodiment.

[0050] Figure 12 shows boxplots of total relative plasmalogen levels (based on the sum of all 8 measured individual species) across various tissues at eight months post vehicle or TMX treatment in *Homozygous Gnpat^{del/flox} cKO Hemi CAGGCRE-ER mice*. Boxes represent the interquartile range with the horizontal line the median. Whiskers represent the min-max range of the data, with outliers shown as individual points. Asterisks denotes $p < 0.001$ versus the mean of the corresponding vehicle-treated plasmalogen.

[0051] Figure 13 shows boxplots of open field test results eight months post vehicle or TMX treatment in *Homozygous Gnpat^{del/flox} cKO Hemi CAGGCRE-ER mice* compared to wild-type controls. A). Mean total distance travelled, B). Mean speed travelled, C). Mean duration of activity. Boxes represent the interquartile range with the horizontal line the median. Whiskers represent the min-max range of the data, with outliers shown as individual points. Asterisks denote $p < 0.01$.

[0052] Figure 14 boxplots of percentage sleep eight months post vehicle or TMX treatment in *Homozygous Gnpat^{del/flox} cKO Hemi CAGGCRE-ER mice* compared to wild-type controls in light phase (A) or dark phase (B). Boxes represent the interquartile range with the horizontal line the

median. Whiskers represent the min-max range of the data, with outliers shown as individual points. Asterisks denote $p < 0.01$.

[0053] Figure 15 shows boxplots of the total brain volume for the left and right hemispheres at eight months post vehicle or TMX treatment in *Homozygous Gnp^{del/flox} cKO Hemi CAGGCRE-ER* mice compared to wild-type controls as determined by anatomical MRI. For each group the medians are represented by the horizontal line with the shaded box representing the interquartile range and the whiskers representing the min-max range. Outliers are represented by points outside the whiskers, Asterisks denote $p < 0.01$.

[0054] Figure 16 shows boxplots of the volume for the temporoparietal cortex at eight months post vehicle or TMX treatment in *Homozygous Gnp^{del/flox} cKO Hemi CAGGCRE-ER* mice compared to wild-type controls as determined by anatomical MRI. For each group the medians are represented by the horizontal line with the shaded box representing the interquartile range and the whiskers representing the min-max range. Outliers are represented by points outside the whiskers, Asterisks denote $p < 0.01$.

[0055] Figure 17 shows boxplots of the volume for the occipital cortex at eight months post vehicle or TMX treatment in *Homozygous Gnp^{del/flox} cKO Hemi CAGGCRE-ER* mice compared to wild-type controls as determined by anatomical MRI. For each group the medians are represented by the horizontal line with the shaded box representing the interquartile range and the whiskers representing the min-max range. Outliers are represented by points outside the whiskers, Asterisks denote $p < 0.01$.

[0056] Figure 18 shows boxplots of the volume for the hippocampus at eight months post vehicle or TMX treatment in *Homozygous Gnp^{del/flox} cKO Hemi CAGGCRE-ER* mice compared to wild-type controls as determined by anatomical MRI. For each group the medians are represented by the horizontal line with the shaded box representing the interquartile range and the whiskers representing the min-max range. Outliers are represented by points outside the whiskers, Asterisks denote $p < 0.01$.

[0057] Figure 19 shows boxplots of the volume for the olfactory areas at eight months post vehicle or TMX treatment in *Homozygous Gnp^{del/flox} cKO Hemi CAGGCRE-ER* mice compared to wild-type controls as determined by anatomical MRI. For each group the medians are represented by the horizontal line with the shaded box representing the interquartile range and the whiskers

representing the min-max range. Outliers are represented by points outside the whiskers, Asterisks denote $p < 0.01$.

[0058] Figure 20 shows boxplots of the volume for the striatum at eight months post vehicle or TMX treatment in *Homozygous Gnat^{del/flox} cKO Hemi CAGGCRE-ER* mice compared to wild-type controls as determined by anatomical MRI. For each group the medians are represented by the horizontal line with the shaded box representing the interquartile range and the whiskers representing the min-max range. Outliers are represented by points outside the whiskers, Asterisks denote $p < 0.01$.

[0059] Figure 21 shows boxplots of the volume for the thalamus at eight months post vehicle or TMX treatment in *Homozygous Gnat^{del/flox} cKO Hemi CAGGCRE-ER* mice compared to wild-type controls as determined by anatomical MRI. For each group the medians are represented by the horizontal line with the shaded box representing the interquartile range and the whiskers representing the min-max range. Outliers are represented by points outside the whiskers. Asterisks denote $p < 0.01$.

[0060] Figure 22 shows boxplots of the volume for the brainstem at eight months post vehicle or TMX treatment in *Homozygous Gnat^{del/flox} cKO Hemi CAGGCRE-ER* mice compared to wild-type controls as determined by anatomical MRI. For each group the medians are represented by the horizontal line with the shaded box representing the interquartile range and the whiskers representing the min-max range. Outliers are represented by points outside the whiskers. Asterisks denote $p < 0.01$.

[0061] Figure 23 shows boxplots of the white matter volume within the brain at eight months post vehicle or TMX treatment in *Homozygous Gnat^{del/flox} cKO Hemi CAGGCRE-ER* mice compared to wild-type controls as determined by anatomical MRI. For each group the medians are represented by the horizontal line with the shaded box representing the interquartile range and the whiskers representing the min-max range. Outliers are represented by points outside the whisker. Asterisks denote $p < 0.01$.

[0062] Figure 24 shows boxplots of Neurofilament light (NfL) concentrations in CSF (A) and plasma (B) at eight months post vehicle or TMX treatment in *Homozygous Gnat^{del/flox} cKO Hemi CAGGCRE-ER* mice compared to wild-type controls. For each group the medians are represented by the horizontal line with the shaded box representing the interquartile range and the whiskers

representing the min-max range. Outliers are represented by points outside the whiskers. Asterisks denote $p < 0.01$.

[0063] Figure 25 shows plasma levels of the 16:0/22:6 plasmalogen species in WT control, cKO control, cKO TMX Veh, and cKO TMX 1011 mice after four months of treatment with vehicle or PPI-1011.

[0064] Figure 26 shows the treatment with PPI-1011 increased brain volumes in plasmalogen-deficient mice as measured by anatomical MRI.

[0065] Figure 27 shows representative mass spectrometry image of plasmalogen species within the brain of plasmalogen deficient (TMX) and PPI-1011 treated mice compared to controls.

Detailed Description

[0066] The following description is of preferred embodiments by way of example only and without limitation to the combination of features necessary for carrying the invention into effect.

[0067] All terms are intended to be understood as they would be understood by a person skilled in the art. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the disclosure pertains. The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[0068] Although various features of the present disclosure can be described in the context of a single embodiment, the features can also be provided separately or in any suitable combination. Conversely, although the present disclosure can be described herein in the context of separate embodiments for clarity, the present disclosure can also be implemented in a single embodiment.

[0069] The following definitions supplement those in the art and are directed to the current application. Accordingly, the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

DEFINITIONS AND EMBODIMENTS

[0070] In this application, the use of the singular includes the plural unless specifically stated otherwise. It must be noted that, as used in the specification, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

[0071] In this application, the use of "or" means "and/or" unless stated otherwise. The terms "and/or" and "any combination thereof and their grammatical equivalents as used herein, can be used interchangeably. These terms can convey that any and all combinations are specifically contemplated. The term "or" can be used conjunctively or disjunctively, unless the context specifically refers to a disjunctive use.

[0072] Furthermore, use of the term "including" as well as other forms, such as "include", "includes," and "included," is not limiting.

[0073] Reference in the specification to "some embodiments," "an embodiment," "one embodiment" "alternate embodiment", or "other embodiments" means that a particular feature, structure, or characteristic described in connection with the embodiments is included in at least some embodiments, but not necessarily all embodiments, of the present disclosures.

[0074] As used in this specification and claim(s), the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps. It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method or composition of the present disclosure, and vice versa. Furthermore, compositions of the present disclosure can be used to achieve methods of the present disclosure.

[0075] The term "about" in relation to a reference numerical value and its grammatical equivalents as used herein can include the numerical value itself and a range of values plus or minus 10% from that numerical value. The term "about" or "approximately" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, i.e., the limitations of the measurement system. For example, "about" can mean within 1 or more than 1 standard deviation, per the practice in the art. Alternatively, "about" can mean a range of up to 20%, up to 10%, up to 5%, or up to 1% of a given value. In another example, the amount "about 10" includes 10 and any amounts from 9 to 11. In

yet another example, the term "about" in relation to a reference numerical value can also include a range of values plus or minus 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% from that value. Alternatively, particularly with respect to biological systems or processes, the term "about" can mean within an order of magnitude, preferably within 5-fold, and more preferably within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated the term "about" meaning within an acceptable error range for the particular value should be assumed.

[0076] The term "isolated" and its grammatical equivalents as used herein refer to the removal of a nucleic acid from its natural environment. Whether the nucleic acid is removed from nature (including genomic DNA and mRNA) or synthesized (including cDNA) and/or amplified under laboratory conditions, it is to be understood, however, that nucleic acids and proteins can be formulated with diluents or adjuvants and still for practical purposes be isolated. For example, nucleic acids typically are mixed with an acceptable carrier or diluent when used for introduction into cells.

[0077] As used herein, the term "gene" may refer to (a) a gene containing a DNA sequence encoding a protein, such as, for example, FAR1, GNPAT, or AGPS; (b) any DNA sequence that encodes a protein, such as, for example, FAR1, GNPAT, or AGPS, and/or; (c) any DNA sequence that hybridizes to the complement of the coding sequences of a protein or the coding sequence of a non-protein gene product, such as for example, an RNA. In certain embodiments, the term "gene" includes coding as well as noncoding regions, and preferably includes all sequences necessary for normal gene expression. As used herein, the term "gene" refers to a nucleic acid molecule capable of being used to produce mRNA, antisense RNA, siRNA, shRNA, miRNA, and the like. Genes may or may not be capable of being used to produce a functional protein. Genes can include both coding and non-coding regions (e.g., introns, regulatory elements including promoters, enhancers, termination sequences and 5' and 3' untranslated regions). A gene may be "isolated" by which is meant a nucleic acid molecule that is substantially or essentially free from components normally found in association with the nucleic acid molecule in its natural state. Such components include other cellular material, culture medium from recombinant production, and/or various chemicals used in chemically synthesizing the nucleic acid molecule.

[0078] Reference to a "gene" also includes within its scope reference to genes having a contiguous sequence, thus defining contiguous nucleic acid entities, as defined herein, or a non-contiguous sequence thus defining a non-contiguous nucleic acid entity as defined herein. In certain embodiments, the term "gene" includes within its scope the open reading frame encoding specific polypeptides, introns, and adjacent 5' and 3' non-coding nucleotide sequences involved in the regulation of expression. In this regard, the gene may further comprise control sequences such as promoters, enhancers, termination and/or polyadenylation signals that are naturally associated with a given gene, or heterologous control sequences. The gene sequences may be cDNA or genomic DNA or a fragment thereof. The gene may be introduced into an appropriate vector for extrachromosomal maintenance or for introduction into a host.

[0079] "Genome" as used herein refers to the entirety of an organism's hereditary information, represented by genes and non-coding sequences of DNA, either chromosomal or non-chromosomal genetic elements such as, linear polynucleotides, e.g., including the gene(s) to be assembled and/or recombined. Thus, the term "genome" is intended to include the entire DNA of an organism, including the nuclear DNA component, chromosomal or extrachromosomal DNA, as well as the cytoplasmic domain (e.g., mitochondrial DNA).

[0080] The terms "Nucleic Acid(s)", "Polynucleotide(s)", "oligonucleotide(s)", or "nucleotide(s)", or any grammatical equivalent as used herein refers to a polymeric form of nucleotides or nucleic acids of any length, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule. Thus, this term includes double and single stranded DNA, triplex DNA, as well as double and single stranded RNA. It also includes modified, for example, by methylation and/or by capping, and unmodified forms of the polynucleotide. The term is also meant to include molecules that include non-naturally occurring or synthetic nucleotides as well as nucleotide analogs. The nucleic acid sequences and vectors disclosed or contemplated herein can be introduced into a cell by, for example, transfection, transformation, or transduction.

[0081] The terms "identical" and its grammatical equivalents as used herein or "sequence identity" in the context of two nucleic acid sequences or amino acid sequences of polypeptides refers to the residues in the two sequences which are the same when aligned for maximum correspondence over a specified comparison window.

[0082] The term "substantially identical" and its grammatical equivalents as applied to nucleic acid sequences mean that a nucleic acid or amino acid sequence comprises a sequence that has at least 90% sequence identity or more, at least 95%, at least 98% and at least 99%), compared to a reference sequence using the programs described above, e.g., BLAST, using standard parameters. For example, the BLASTN program (for nucleotide sequences) uses as defaults a word length (W) of 11, an expectation (E) of 10, M=5, N=-4, and a comparison of both strands. Percentage of sequence identity is determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window can comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. In embodiments, the substantial identity exists over a region of the sequences that is at least about 25 bases in length, 50 bases in length, 100 bases in length, 125 bases in length, 150 bases in length and in embodiments, the sequences are substantially identical over at least about 180 bases. In embodiments, the sequences are substantially identical over the entire length of the coding regions.

[0083] A "donor plasmid", an "expression vector" or "vector" is any genetic element, e.g., a plasmid, chromosome, virus, transposon, behaving either as an autonomous unit of polynucleotide replication within a cell. (i.e. capable of replication under its own control) or being rendered capable of replication by insertion into a host cell chromosome, having attached to it another polynucleotide segment, so as to bring about the replication and/or expression of the attached segment. Suitable vectors include, but are not limited to, plasmids, transposons, bacteriophages and cosmids. Vectors can contain polynucleotide sequences which are necessary to effect ligation or insertion of the vector into a desired host cell and to effect the expression of the attached segment. Such sequences differ depending on the host organism; they include promoter sequences to effect transcription, enhancer sequences to increase transcription, ribosomal binding site sequences and transcription and translation termination sequences. Alternatively, expression vectors can be capable of directly expressing nucleic acid sequence products encoded therein without ligation or integration of the vector into host cell DNA sequences. In some embodiments,

the vector is an episomal expression vector, which is able to replicate in a host cell, and persists as an extrachromosomal segment of DNA within the host cell in the presence of appropriate selective pressure. Vector also can comprise a selectable marker gene. The term "selectable marker gene" as used herein refers to a nucleic acid sequence that allows cells expressing the nucleic acid sequence to be specifically selected for or against, in the presence of a corresponding selective agent.

[0084] The term "coding sequence" or "sequence encoding" as used herein refers to a segment of a polynucleotide that codes for protein. The region or sequence is bounded nearer the 5' end by a start codon and nearer the 3' end with a stop codon. Coding sequences can also be referred to as open reading frames. The present invention is further directed to a nucleotide construct comprising the nucleic acid as described above operatively linked to one or more regulatory elements. By "regulatory element", it is meant a portion of nucleic acid typically, but not always, upstream of a gene, and may be comprised of either DNA or RNA, or both DNA and RNA. Regulatory elements may include those which are capable of mediating organ specificity, or controlling developmental or temporal gene activation. Furthermore, "regulatory element" includes promoter elements, core promoter elements, elements that are inducible in response to an external stimulus, elements that are activated constitutively, or elements that decrease or increase promoter activity such as negative regulatory elements or transcriptional enhancers, respectively. By a nucleotide sequence exhibiting regulatory element activity it is meant that the nucleotide sequence when operatively linked with a coding sequence of interest functions as a promoter, a core promoter, a constitutive regulatory element, a negative element or silencer (i.e. elements that decrease promoter activity), or a transcriptional or translational enhancer.

[0085] As used herein, the term "transgenic non-human animal or animal model" or "transgenic non-human cell" may refer to an animal or animal model or cell that contains within its genome a specific sequence that has been disrupted or otherwise modified or mutated by the methods described herein or methods otherwise well known in the art. It is contemplated that the non-human animal or animal model or cell may be a mammal, bird, reptile, amphibian, fish, arthropod, vertebrate, or invertebrate. In certain embodiments, the non-human transgenic animal or animal model or cell is a mammal. In certain embodiments, the mammal or cell is a rodent such as a rat or mouse, a rabbit, a monkey, a guinea pig, a dog, a sheep, a horse, a cow, a cat, etc. In addition, a "transgenic animal or animal model" or "transgenic cell" may be a heterozygous animal

or cell (i.e., one modified allele and one wild-type allele) or a homozygous animal or cell (i.e., two modified alleles). An embryo is considered to fall within the definition of an animal. In certain embodiments, the transgenic animal or animal model or cell may be an embryo or a cell therefrom. The provision of an animal includes the provision of an embryo or fetus in utero, whether by mating or otherwise, and whether or not the embryo goes to term.

[0086] As contemplated herein, forms of administration may include, but are not limited to, injections, solutions, creams, gels, implants, pumps, ointments, emulsions, suspensions, microspheres, particles, microparticles, nanoparticles, liposomes, pastes, patches, tablets, capsules, transdermal delivery devices, sprays, aerosols, or other means familiar to one of ordinary skill in the art.

[0087] As used herein, the term “modification” or “modifying” or “modified”, in the context of a regulatory region or a conditionally inducible gene editing site, may comprise any modification to a conditionally inducible gene editing site, such as, for example, a deletion of one or more base pair, a substitution of one or more base pair, an addition of one or more base pair, wherein the gene comprising the editing site regulates a plasmalogen biosynthetic pathway and the modification changes the expression, level or activity of the gene comprising the conditionally inducible gene editing site.

[0088] As used herein, “a plasmalogen precursor” includes any and all known molecules that are transformed into a plasmalogen in the plasmalogen biosynthetic pathway. Preferably the plasmalogen precursor is an alkyl or alkenyl glycerol containing an ether or vinyl-ether at the sn1 position of the glycerol backbone.

[0089] As used herein, a reduction or reduced levels may comprise a decrease in levels of, for example, a plasmalogen, a plasmalogen precursor, or any biological molecule. In certain embodiments, a reduction or reduced levels may comprise a decrease in levels in the entire non-human animal, such as a systemic decrease, or a decrease in levels in a portion of the non-human animal, such as, for example, a cell, a fluid, a tissue, or any combination thereof.

[0090] As used herein, a “regulatory region” of a gene may be any coding and/or noncoding region of the gene that regulates the expression, activity and/or function of the gene and/or a product thereof. In certain embodiments, the regulatory region may be an exon, an element required for transcription of the gene, an element required for translation of the gene, an element required for

the function of the protein encoded by the genomic sequence comprising the gene, or any combination thereof. In certain embodiments, the regulatory region may be in proximity to the gene or the regulatory region may not be in proximity to the gene. In certain embodiments, a proximity to the gene may comprise a sequence within the gene, such as, for example, an exon or untranslated region, or may comprise a region outside of the gene, such as, for example, an intron, a promoter or enhancer. In certain embodiments, a proximity may comprise a regulatory region within 0 base pairs (bp) to 10000 bp upstream or downstream from a gene, such as 1bp, 2bp, 3bp, 4bp, 5bp, 6bp, 7bp, 8bp, 9bp, 10bp, 11bp, 12bp, 13bp, 14bp, 15bp, 16bp, 17bp, 18bp, 19bp, 20bp, 21bp, 22bp, 23bp, 24bp, 25bp, 26bp, 27bp, 28bp, 29bp, 30bp, 31bp, 32bp, 33bp, 34bp, 35bp, 36bp, 37bp, 38bp, 39bp, 40bp, 41bp, 42bp, 43bp, 44bp, 45bp, 46bp, 47bp, 48bp, 49bp, 50bp, 51bp, 52bp, 53bp, 54bp, 55bp, 56bp, 57bp, 58bp, 59bp, 60bp, 61bp, 62bp, 63bp, 64bp, 65bp, 66bp, 67bp, 68bp, 69bp, 70bp, 71bp, 72bp, 73bp, 74bp, 75bp, 76bp, 77bp, 78bp, 79bp, 80bp, 81bp, 82bp, 83bp, 84bp, 85bp, 86bp, 87bp, 88bp, 89bp, 90bp, 91bp, 92bp, 93bp, 94bp, 95bp, 96bp, 97bp, 98bp, 99bp, 100bp, 101bp, 102bp, 103bp, 104bp, 105bp, 106bp, 107bp, 108bp, 109bp, 110bp, 111bp, 112bp, 113bp, 114bp, 115bp, 116bp, 117bp, 118bp, 119bp, 120bp, 121bp, 122bp, 123bp, 124bp, 125bp, 126bp, 127bp, 128bp, 129bp, 130bp, 131bp, 132bp, 133bp, 134bp, 135bp, 136bp, 137bp, 138bp, 139bp, 140bp, 141bp, 142bp, 143bp, 144bp, 145bp, 146bp, 147bp, 148bp, 149bp, 150bp, 151bp, 152bp, 153bp, 154bp, 155bp, 156bp, 157bp, 158bp, 159bp, 160bp, 161bp, 162bp, 163bp, 164bp, 165bp, 166bp, 167bp, 168bp, 169bp, 170bp, 171bp, 172bp, 173bp, 174bp, 175bp, 176bp, 177bp, 178bp, 179bp, 180bp, 181bp, 182bp, 183bp, 184bp, 185bp, 186bp, 187bp, 188bp, 189bp, 190bp, 191bp, 192bp, 193bp, 194bp, 195bp, 196bp, 197bp, 198bp, 199bp, 200bp, 201bp, 202bp, 203bp, 204bp, 205bp, 206bp, 207bp, 208bp, 209bp, 210bp, 211bp, 212bp, 213bp, 214bp, 215bp, 216bp, 217bp, 218bp, 219bp, 220bp, 221bp, 222bp, 223bp, 224bp, 225bp, 226bp, 227bp, 228bp, 229bp, 230bp, 231bp, 232bp, 233bp, 234bp, 235bp, 236bp, 237bp, 238bp, 239bp, 240bp, 241bp, 242bp, 243bp, 244bp, 245bp, 246bp, 247bp, 248bp, 249bp, 250bp, 251bp, 252bp, 253bp, 254bp, 255bp, 256bp, 257bp, 258bp, 259bp, 260bp, 261bp, 262bp, 263bp, 264bp, 265bp, 266bp, 267bp, 268bp, 269bp, 270bp, 271bp, 272bp, 273bp, 274bp, 275bp, 276bp, 277bp, 278bp, 279bp, 280bp, 281bp, 282bp, 283bp, 284bp, 285bp, 286bp, 287bp, 288bp, 289bp, 290bp, 291bp, 292bp, 293bp, 294bp, 295bp, 296bp, 297bp, 298bp, 299bp, 300bp, 301bp, 302bp, 303bp, 304bp, 305bp, 306bp, 307bp, 308bp, 309bp, 310bp, 311bp, 312bp, 313bp, 314bp, 315bp, 316bp, 317bp, 318bp, 319bp, 320bp, 321bp, 322bp, 323bp, 324bp, 325bp, 326bp, 327bp, 328bp, 329bp, 330bp, 331bp, 332bp, 333bp,

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737bp, 738bp, 739bp, 740bp, 741bp, 742bp, 743bp, 744bp, 745bp, 746bp, 747bp, 748bp, 749bp, 750bp, 751bp, 752bp, 753bp, 754bp, 755bp, 756bp, 757bp, 758bp, 759bp, 760bp, 761bp, 762bp, 763bp, 764bp, 765bp, 766bp, 767bp, 768bp, 769bp, 770bp, 771bp, 772bp, 773bp, 774bp, 775bp, 776bp, 777bp, 778bp, 779bp, 780bp, 781bp, 782bp, 783bp, 784bp, 785bp, 786bp, 787bp, 788bp, 789bp, 790bp, 791bp, 792bp, 793bp, 794bp, 795bp, 796bp, 797bp, 798bp, 799bp, 800bp, 801bp, 802bp, 803bp, 804bp, 805bp, 806bp, 807bp, 808bp, 809bp, 810bp, 811bp, 812bp, 813bp, 814bp, 815bp, 816bp, 817bp, 818bp, 819bp, 820bp, 821bp, 822bp, 823bp, 824bp, 825bp, 826bp, 827bp, 828bp, 829bp, 830bp, 831bp, 832bp, 833bp, 834bp, 835bp, 836bp, 837bp, 838bp, 839bp, 840bp, 841bp, 842bp, 843bp, 844bp, 845bp, 846bp, 847bp, 848bp, 849bp, 850bp, 851bp, 852bp, 853bp, 854bp, 855bp, 856bp, 857bp, 858bp, 859bp, 860bp, 861bp, 862bp, 863bp, 864bp, 865bp, 866bp, 867bp, 868bp, 869bp, 870bp, 871bp, 872bp, 873bp, 874bp, 875bp, 876bp, 877bp, 878bp, 879bp, 880bp, 881bp, 882bp, 883bp, 884bp, 885bp, 886bp, 887bp, 888bp, 889bp, 890bp, 891bp, 892bp, 893bp, 894bp, 895bp, 896bp, 897bp, 898bp, 899bp, 900bp, 901bp, 902bp, 903bp, 904bp, 905bp, 906bp, 907bp, 908bp, 909bp, 910bp, 911bp, 912bp, 913bp, 914bp, 915bp, 916bp, 917bp, 918bp, 919bp, 920bp, 921bp, 922bp, 923bp, 924bp, 925bp, 926bp, 927bp, 928bp, 929bp, 930bp, 931bp, 932bp, 933bp, 934bp, 935bp, 936bp, 937bp, 938bp, 939bp, 940bp, 941bp, 942bp, 943bp, 944bp, 945bp, 946bp, 947bp, 948bp, 949bp, 950bp, 951bp, 952bp, 953bp, 954bp, 955bp, 956bp, 957bp, 958bp, 959bp, 960bp, 961bp, 962bp, 963bp, 964bp, 965bp, 966bp, 967bp, 968bp, 969bp, 970bp, 971bp, 972bp, 973bp, 974bp, 975bp, 976bp, 977bp, 978bp, 979bp, 980bp, 981bp, 982bp, 983bp, 984bp, 985bp, 986bp, 987bp, 988bp, 989bp, 990bp, 991bp, 992bp, 993bp, 994bp, 995bp, 996bp, 997bp, 998bp, 999bp, or 1000bp. In certain embodiments, a regulatory region may not be in proximity to a gene while still regulating expression, activity and/or function of said gene. In certain embodiments, a regulatory region not within proximity to a gene may be greater than 1000 bp, such as greater than 1000bp, greater than 1100bp, greater than 1200bp, greater than 1300bp, greater than 1400bp, greater than 1500bp, greater than 1600bp, greater than 1700bp, greater than 1800bp, greater than 1900bp, greater than 2000bp, greater than 2100bp, greater than 2200bp, greater than 2300bp, greater than 2400bp, greater than 2500bp, greater than 2600bp, greater than 2700bp, greater than 2800bp, greater than 2900bp, greater than 3000bp, greater than 3100bp, greater than 3200bp, greater than 3300bp, greater than 3400bp, greater than 3500bp, greater than 3600bp, greater than 3700bp, greater than 3800bp, greater than 3900bp, greater than 4000bp, greater than 4100bp, greater than 4200bp, greater than 4300bp, greater than 4400bp, greater than

4500bp, greater than 4600bp, greater than 4700bp, greater than 4800bp, greater than 4900bp, greater than 5000bp, greater than 5100bp, greater than 5200bp, greater than 5300bp, greater than 5400bp, greater than 5500bp, greater than 5600bp, greater than 5700bp, greater than 5800bp, greater than 5900bp, greater than 6000bp, greater than 6100bp, greater than 6200bp, greater than 6300bp, greater than 6400bp, greater than 6500bp, greater than 6600bp, greater than 6700bp, greater than 6800bp, greater than 6900bp, greater than 7000bp, greater than 7100bp, greater than 7200bp, greater than 7300bp, greater than 7400bp, greater than 7500bp, greater than 7600bp, greater than 7700bp, greater than 7800bp, greater than 7900bp, greater than 8000bp, greater than 8100bp, greater than 8200bp, greater than 8300bp, greater than 8400bp, greater than 8500bp, greater than 8600bp, greater than 8700bp, greater than 8800bp, greater than 8900bp, greater than 9000bp, greater than 9100bp, greater than 9200bp, greater than 9300bp, greater than 9400bp, greater than 9500bp, greater than 9600bp, greater than 9700bp, greater than 9800bp, greater than 9900bp, or greater than 10000bp from the gene. In certain embodiments, the regulatory region may comprise one or more conditionally inducible gene editing site. In certain embodiments, the regulatory region may comprise two conditionally inducible gene editing site.

[0091] As used herein, “a physiologically acceptable carrier” includes any and all solvents, dispersion media, coatings, adjuvants, stabilizing agents, diluents, preservatives, antibacterial and antifungal agents, isotonic agents, adsorption delaying agents, and the like.

[0092] The term "operably linked" as used herein refers to the physical and/or functional linkage of a DNA segment to another DNA segment in such a way as to allow the segments to function in their intended manners. A DNA sequence encoding a gene product is operably linked to a regulatory sequence when it is linked to the regulatory sequence, such as, for example, promoters, enhancers and/or silencers, in a manner which allows modulation of transcription of the DNA sequence, directly or indirectly. For example, a DNA sequence is operably linked to a promoter when it is ligated to the promoter downstream with respect to the transcription initiation site of the promoter, in the correct reading frame with respect to the transcription initiation site and allows transcription elongation to proceed through the DNA sequence. An enhancer or silencer is operably linked to a DNA sequence coding for a gene product when it is ligated to the DNA sequence in such a manner as to increase or decrease, respectively, the transcription of the DNA sequence. Enhancers and silencers can be located upstream, downstream or embedded within the coding regions of the DNA sequence. A DNA for a signal sequence is operably linked to DNA

coding for a polypeptide if the signal sequence is expressed as a pre-protein that participates in the secretion of the polypeptide. Linkage of DNA sequences to regulatory sequences is typically accomplished by ligation at suitable restriction sites or via adapters or linkers inserted in the sequence using restriction endonucleases known to one of skill in the art.

[0093] The term "induce", "induction" and its grammatical equivalents as used herein refer to an increase in nucleic acid sequence transcription, promoter activity and/or expression brought about by a transcriptional regulator, relative to some basal level of transcription or control systems used. Increase in nucleic acid sequence transcription, promoter activity and/or expression can also be brought about by a translation regulator such as a translation enhancing UTR sequence.

[0094] The term "promoter" refers to a region of a polynucleotide that initiates transcription of a coding sequence. Promoters are located near the transcription start sites of genes, on the same strand and upstream on the DNA (towards the 5' region of the sense strand). Some promoters are constitutive as they are active in all circumstances in the cell, while others are regulated becoming active in response to specific stimuli, e.g., an inducible promoter. The term "promoter activity" and its grammatical equivalents as used herein refer to the extent of expression of nucleotide sequence that is operably linked to the promoter whose activity is being measured. Promoter activity can be measured directly by determining the amount of RNA transcript produced, for example by Northern blot analysis or indirectly by determining the amount of product coded for by the linked nucleic acid sequence, such as a reporter nucleic acid sequence linked to the promoter.

[0095] A constitutive promoter directs the expression of a gene throughout the various parts of an organism and/or continuously throughout development of an organism. Any suitable constitutive promoter may be used to drive the expression of the proteins or fragments thereof as described herein.

[0096] The term "constitutive" as used herein does not necessarily indicate that a gene is expressed at the same level in all cell types, but that the gene is expressed in a wide range of cell types, although some variation in abundance is often observed.

[0097] "Inducible promoter" as used herein refers to a promoter which is induced into activity by the presence or absence of transcriptional regulators, e.g., biotic or abiotic factors. Inducible promoters are useful because the expression of genes operably linked to them can be turned on or

off with an inducer at certain stages of development of an organism or in a particular tissue. An inducible promoter is a promoter that is capable of directly or indirectly activating transcription of one or more DNA sequences or genes in response to an inducer. In the absence of an inducer the DNA sequences or genes will not be transcribed. Typically the protein factor that binds specifically to an inducible promoter to activate transcription is present in an inactive form which is then directly or indirectly converted to the active form by the inducer. The inducer can be a chemical agent such as a protein, metabolite, growth regulator, or a physiological stress imposed directly by heat, cold, or toxic elements or indirectly through the action of a pathogen or disease agent such as a virus. Non-limiting examples of inducible promoters include alcohol-regulated promoters, tetracycline-regulated promoters, steroid-regulated promoters, metal-regulated promoters, pathogenesis-regulated promoters, temperature-regulated promoters and light-regulated promoters, isopropyl- β -thiogalactopyranoside (IPTG) inducible promoter.

[0098] The term "transcriptional regulator" or "cis-acting regulatory elements" refers to a biochemical element that acts to prevent or inhibit the transcription of a promoter-driven DNA sequence under certain environmental conditions (e.g., a repressor or nuclear inhibitory protein), or to permit or stimulate the transcription of the promoter-driven DNA sequence under certain environmental conditions (e.g., an inducer or an enhancer).

[0099] The term "enhancer" or "translation enhancer" as used herein, refers to a DNA sequence that increases transcription of, for example, a nucleic acid sequence to which it is operably linked. Enhancers can be located many kilobases away from the coding region of the nucleic acid sequence and can mediate the binding of regulatory factors, patterns of DNA methylation, or changes in DNA structure. A large number of enhancers from a variety of different sources are well known in the art and are available as or within cloned polynucleotides (from, e.g., depositories such as the ATCC as well as other commercial or individual sources). A number of polynucleotides comprising promoters (such as the commonly-used CMV promoter) also comprise enhancer sequences. Enhancers can be located upstream, within, or downstream of coding sequences.

[00100] As used herein, the term "treatment", "treating", "amelioration" or its grammatical equivalents refers to obtaining a desired pharmacologic and/or physiologic effect. In embodiments, the effect is therapeutic, i.e., the effect partially or completely cures a disease and/or adverse symptom attributable to the disease.

[00101] In certain embodiments, a gene and/or one or more additional genes may comprise a gene regulating a plasmalogen biosynthetic pathway. As used herein, a plasmalogen biosynthetic pathway may refer to the series of steps, including enzymes and intermediate molecules for the synthesis of plasmalogens. Plasmalogens are a class of glycerophospholipids, characterized by a vinyl ether bond at the sn-1 position. Biosynthesis begins in the peroxisome by a series of non-redundant peroxisomal specific enzymes that create the ether bond which is reduced to the vinyl ether within the endoplasmic reticulum (ER). Plasmalogens are essential components of lipid membranes where they have been shown to play roles in vesicular transport, membrane protein activity and have antioxidant properties (reviewed [32]). Reductions in plasmalogen levels have also been reported in other neurodegenerative diseases including Alzheimer's disease [33-36], Parkinson's disease [37,38], Schizophrenia [39], Down syndrome [40] and Gaucher disease [41]. As used herein, a gene regulating a plasmalogen biosynthetic pathway may be a gene wherein modulating of said gene or product thereof, such as increasing or decreasing said gene or product thereof by gene editing, results in a change in a plasmalogen biosynthetic pathway. In certain embodiments, a gene regulating a plasmalogen biosynthetic pathway may be a gene wherein modulating said gene or product thereof, such as increasing or decreasing said gene or product thereof by gene editing, in combination with one or more additional gene modified by gene editing, results in a change in a plasmalogen biosynthetic pathway, wherein gene editing of said gene without editing of one or more additional gene may not result in changes in a plasmalogen biosynthetic pathway. In certain embodiments, a gene regulating a plasmalogen biosynthetic pathway may be a gene wherein modulating of said gene or product thereof, such as increasing or decreasing said gene or product thereof by gene editing, results in an enhancement of a modulation of a plasmalogen biosynthetic pathway produced by gene editing of an independent gene.

[00102] In certain embodiments, the regulatory region may regulate an expression, level, activity or any combination thereof of a gene regulatory a plasmalogen biosynthetic pathway. In certain embodiments, the gene regulating the plasmalogen biosynthetic pathway may be fatty acid reductase 1 (FAR1), glyceronephosphate O-acyltransferase (GNPAT), alkylglycerone phosphate synthase (AGPS), acyl/alkyl-DHAP reductase, alkyl/acyl-GPA acyltransferase, phosphatidic acid phosphatase, ethanolamine phosphotransferase, plasmenylethanolamine desaturase, choline phosphotransferase, any variant thereof, any orthologue, or any combination thereof. In certain embodiments, the transgenic non-human animal or animal model or cell may comprise one or more

conditionally inducible gene editing sites at a gene regulating a plasmalogen biosynthetic pathway, and one or more conditionally inducible gene editing sites at a one or more additional genes regulating a plasmalogen biosynthetic. In certain embodiments, the transgenic non-human animal or animal model or cell may comprise two editing sites in a GNPAT gene.

[00103] In certain embodiments, a genome of the transgenic, non-human animal or animal model may comprise at least one conditionally inducible gene editing site. As used herein, a “gene editing site” may be any site capable of directing a gene product capable of editing the editing site thereto, such as, for example, an enzyme or an enzyme:nucleic acid complex. In certain embodiments, the conditionally inducible gene editing site may be an endogenous sequence present in the genome or the non-human animal or animal model. In certain embodiments, the conditionally inducible gene editing site may be an exogenous sequence inserted into the genome of the non-human animal or animal model. In certain embodiments, the gene product capable of editing the editing site may bind to a consensus sequence therein. In certain embodiments, the gene product capable of editing the editing may be to and/or edit the editing site in the presence of an additional component, such as, for example an additional enzyme or nucleic acid, such as for example a CRISPR RNA or guide RNA. In certain embodiments, editing of the conditionally inducible gene editing site by the gene product may result in impaired expression, activity and/or function of the product of the edited gene.

[00104] In certain embodiments, a “conditionally inducible gene editing site” may comprise two editing sites, wherein the genomic region between said two editing sites may be excised, recombined, inverted, translocated or inactivated. In certain embodiments, the two editing sites may be edited by an enzyme or an enzyme in complex with one or more nucleic acids. In certain embodiments, the conditionally inducible gene editing site may comprise LoxP sites and the sites may be edited by a Cre. In certain embodiments, the conditionally inducible gene editing site may comprise guide RNA or equivalent binding site, and the sites may be edited by guide RNA or equivalents thereof capable of binding site guide RNA or equivalent binding sites, and Cas9 or a variant thereof.

[00105] In certain embodiments, at least one conditionally inducible gene editing site may be capable of modifying at least one regulatory region of a gene involved in the plasmalogen biosynthetic pathway. In certain embodiments, the at least one regulatory region may be critical

for a plasmalogen biosynthetic pathway. In certain embodiments, the modification of the at one regulatory region by the at least one conditionally inducible gene editing site may comprise a mutation, such as a base pair change, a deletion, such as a deletion of 1 or more base pairs, a recombination, or any combination thereof. In certain embodiments, modification of the at least one regulatory region comprising the at least one conditionally inducible gene editing site may modify an activity, function, expression and/or level of a gene product of the gene involved in the plasmalogen biosynthetic pathway.

[00106] In certain embodiments, the at least one conditionally inducible gene editing site may comprise an exogenous nucleic acid sequence. An exogenous nucleic acid sequence, as used herein, may be any sequence that is provided to a genome. The exogenous nucleic acid sequence may comprise sequences from a different species, the same species or any combination thereof. In certain embodiments, the exogenous sequence may be a sequence that directs gene editing. In certain embodiments, the exogenous sequence may comprise a consensus binding sequence, and said consensus binding sequence is capable of binding one or more gene editing products capable of editing the exogenous sequence or exogenous sequences. In certain embodiments, the exogenous sequence may comprise a consensus site for binding an endonuclease, recombinase, exonuclease, polymerase, ribonuclease, ligase, integrase, or any equivalent known to the skilled person.

[00107] In certain embodiments, the at least one conditionally inducible gene editing site may comprise an endogenous nucleic acid sequence. An endogenous nucleic acid sequence, as used herein, may be any sequence that is provided in a genome of the non-human animal or animal model or cell thereof. In certain embodiments, the endogenous sequence may be a sequence that directs gene editing. In certain embodiments, the endogenous sequence may comprise a consensus binding sequence, and said consensus binding sequence is capable of binding one or more gene editing products capable of editing the endogenous sequence or endogenous sequences. In certain embodiments, the endogenous sequence may comprise a consensus site for binding an endonuclease, recombinase, integrase, or any equivalent known to the skilled person. In certain embodiments, the endogenous sequence may comprise a sequence that is capable of binding an encoded nucleic acid, such as a ribonucleic acid including a CRISPR RNA or guide RNA, wherein said encoded nucleic acid may comprise 1-100bp complementary to said endogenous sequence, such as for example 1bp, 2bp, 3bp, 4bp, 5bp, 6bp, 7bp, 8bp, 9bp, 10bp, 11bp, 12bp,

13bp, 14bp, 15bp, 16bp, 17bp, 18bp, 19bp, 20bp, 21bp, 22bp, 23bp, 24bp, 25bp, 26bp, 27bp, 28bp, 29bp, 30bp, 31bp, 32bp, 33bp, 34bp, 35bp, 36bp, 37bp, 38bp, 39bp, 40bp, 41bp, 42bp, 43bp, 44bp, 45bp, 46bp, 47bp, 48bp, 49bp, 50bp, 51bp, 52bp, 53bp, 54bp, 55bp, 56bp, 57bp, 58bp, 59bp, 60bp, 61bp, 62bp, 63bp, 64bp, 65bp, 66bp, 67bp, 68bp, 69bp, 70bp, 71bp, 72bp, 73bp, 74bp, 75bp, 76bp, 77bp, 78bp, 79bp, 80bp, 81bp, 82bp, 83bp, 84bp, 85bp, 86bp, 87bp, 88bp, 89bp, 90bp, 91bp, 92bp, 93bp, 94bp, 95bp, 96bp, 97bp, 98bp, 99bp, or 100bp. In certain embodiments, the endogenous sequence may comprise a sequence that is capable of binding a nucleic acid, wherein the nucleic acid bound to the endogenous sequence may direct the activity of a gene product, such as an enzyme including Cas9 or a variant thereof, to said endogenous sequence to edit said endogenous sequence.

[00108] In certain embodiments, the at least one nucleic acid editing sequence may be integrated at a separate locus from the gene involved in the plasmalogen biosynthetic pathway and the at least one conditionally inducible gene editing site. As used herein, a separate locus may comprise any region of the genome of the non-human animal or animal model or cell that does not contain either the first gene or the at least one conditionally inducible gene editing site. In certain embodiments, the gene and the at least one conditionally inducible gene editing site are provided at regions of the genome such that Mendelian or near-Mendelian ratios of offspring may be observed when said non-human animals are mated with a non-human animal comprising the at least one nucleic acid editing sequence. In certain embodiments, the gene and the at least one conditionally inducible gene editing site are provided on a chromosome that is different than the chromosome providing the at least one nucleic acid editing sequence. In certain embodiments, the gene and the at least one conditionally inducible gene editing site are provided on a different arm of a same chromosome as the chromosome providing the at least one nucleic acid editing sequence.

[00109] In certain embodiments, the at least one nucleic acid editing sequence encodes a gene product capable of editing a conditionally inducible gene editing site. In certain embodiments, the expression of the at least one nucleic acid editing sequence may be regulated by constitutive regulatory systems, tissue-specific regulatory sequences or may be used with inducible systems wherein expression is regulated by introduction of an exogenous factor such as a small molecule, light or temperature. In certain embodiments, a gene product capable of editing a conditionally inducible gene editing site may be a nucleic acid, a protein or a combination thereof. In certain embodiments, the gene product capable of editing a conditionally inducible gene editing site may

comprise an enzyme. In certain embodiments, the gene product capable of editing a conditionally inducible gene editing site may comprise an enzyme capable of binding a nucleic acid and modifying said nucleic acid. In certain embodiments, the gene product capable of editing a conditionally inducible gene editing site may be an enzyme capable of binding a consensus nucleic acid sequence and/or a consensus binding motif or domain. In certain embodiments, the gene product capable of editing a conditionally inducible gene editing site may be an endonuclease, such as for example zinc-finger nuclease, TALEN, FokI, Cas9, or Cas9 variants, or a recombinase, such as for example Cre, PhiC31 or FRP.

[00110] In certain embodiments, the expression of a gene product capable of editing a conditionally inducible gene editing site may be regulated by a regulatory element from a gene, such as a promoter and/or enhancer that provides expression in a specific tissue, cell or combination thereof. Non-limiting examples of such regulatory elements from genes comprising:

Adipocytes Adiponectin (also known as Acrp30, AdipoQ, and GBP28), Adipoq, Adipsin, ALK7, ALBP/aP2 (adipocyte lipid-binding protein), C/EBP alpha/beta (CCAAT-enhancer binding protein), DOL54 (a pre-adipocyte marker), FABP (fatty acid binding protein), FABP4, GLUT4, GPDH (glycerol-3-phosphate dehydrogenase), Leptin, LPIN-1, LPL (lipoprotein lipase), Perilipin, PEPCK-C (Phosphoenolpyruvate carboxykinase), PPAR (peroxisome proliferator activated receptor), Pref-1 (Preadipocyte factor-1), Resistin, S-100, UCP-1/UCP-2 (Uncoupling protein), Mest/Pegl, aP2 Alveolar cells Alkaline phosphatase, Cytokeratin, HTI56, MEP-1, MPA (Maclura pomifera lectin), MPA binding glycoproteins (MPA-gp330), P2X7 and GABRP, pro-SPC, RAGE (receptor for advanced glycation endproducts), RTI(40), SBA (Soybean agglutinin), SPA (surfactant protein A, SP-A), SPB (surfactant protein B, SP-B), SPC (surfactant protein C, SP-C) Ameloblasts Ameloblastin, Amelogenin, Amelotin, AP-1 family proteins (c-Jun, JunB, JunD, c-Fos, FosB, Fra-1, and Fra-2), APC (adenomatous polyposis coli gene protein), Connexin43 (Cx43), Cytokeratin 14, Enamel matrix proteins (EMP), IGF-I receptor (Insulin-like growth factor-I receptor), TGF-beta 1, TSLC1 (Tumor suppressor in lung cancer-1) Apud cells Neuron-specific enolase (NSE) eBasal cells 34betaE1 2 (high molecular weight cytokeratin), Bcl-2, CD44, Keratin 14, p63, P-Cadherin, S100A6 (Calcyclin) Basophils BB1 (Basogranulin), Bsp-1, CCR3 (eotaxin-receptor), CD11a/CD11b/CD11c, CD13 (WS-80274, clone A8), CD44 and CD54, CD63 (gp53), CD69, CD107a (WS-80280, clone E63-880), CD164 (WS-80160, clone N6B6 and WS-80162, clone 67D2), CD203c (E-NPP3), CDwl 7 (lactosylceramide), IL-3, IL-4

Receptors, beta 1, beta 2, and beta 7 integrins, Interleukin-4 (IL-4), MBP (Major Basic Protein), MMCP-8, NCA, PSGL-1 (CD162), TLR4 (Toll-like receptor-4), B-cells B220, BLAST-2 (EBVCS), Bu-1, CD19, CD20 (L26), CD22, CD24, CD27, CD57, CD72, CD79a, CD79b, CD86, chB6, D8/17, Immunoglobulin Beta (B29), FMC7, L26, M17, MUM-1, Pax-5 (BSAP), PC47H, Cancer stem cells CD7, CD 10, CD18 (Integrin f32), CD19, CD20, CD24 (HSA), CD27, CD29 (Integrin CD31 (PECAM-1), CD33, CD34 (Mucosialin), CD38, CD44, CD49b (Integrin a2), CD49f (Integrin a6), CD74, CD90 (Thy-1), CD96 (Tactile), CD105 (Endoglin), CD117 (c-Kit), CD123 (IL-3Ra), CD133 (Prominin-1), CD138 (Syndecan-1), CD166 (ALCAM), CD184 (CXCR4), CD324 (E-cadherin), CD338 (ABCG2), D111, EpCAM (TROP-1), Jagged-2, Nestin, Notchl, Notch3, Notch4, Podoplanin, SSEA-1, SSEA-3, SSEA-4, TRA-1-60, TRA-1-80 Cardiomyocytes Adrenomedullin, ALCAM (CD166), alpha-Actinin, Annexin 5, CA 02756833 201 -0,3-27 Annexin 6, ANP (atrial natriuretic peptide), bFGF, BNP (brain natriuretic peptides), Cardiac troponin I (cTnI), Cardiac troponin-T (cTnT), CARP (cardiac adriamycin-responsive protein), Caveolin-2, Caveolin-3, CHAMP, CNP (C-type natriuretic peptide), Connexin-43, Desmin, dHAND, eHAND, GATA-4, GATA-6, H-FABP, InsUlin-like growth factor I (IGF-1), MEF2C, MHC (myosin heavy chain), MLC (myosin light chain), N-cadherin, Nlo(2.5 (cardiac homeobox protein), Oct-4, Pnmt (Phenylethanolamine N-methyltransferase), Sarcomeric alpha Actin/Actinin, Sarcomeric Myosin, Sarcomeric Tropomyosin, Skeletal alpha-Actin Chondrocytes AggreCAN, Annexin VI, betal Integrin (CD29), COMP (Cartilage oligomeric matrix protein), Cathepsin B, CD44, CD151, and CD49c, CEP-68 (Chondrocyte expressed protein-68), CMP (cartilage matrix protein, Matrilin-1), Collagen II, Collagen IX, Collagen X, IGF-I and IGF-II, MIA (Melanoma Inhibitory Activity), MMP13 (matrix metalloproteinase-13), Osteonectin (SPARC), PCNA, p21, Sox9, Syndecan-3, YKL39 and YKL40 Clara cells CC 10 (Clara cell secretory protein), CC16 (Clara cell secretory protein), CC26, CCSP (Clara cellspan> s secretory protein), CYP2F2/CYP2B4, Cytochrome P-450 (CYP450), NADPH reductase, SP-A, SP-B, SP-C, SP-D, Urinary protein 1, Uteroglobin, UGRP1 Dendritic cells ADAM19 (MADDAM), BDCA-2, CD1a, CD11c, CD21, CD83, CD86, CD208, CLIP-170/restin, Clusterin, DC-LAMP (CD208), DEC-205, Estrogen Receptor-alpha, Fascin, HLA-DR, NLDC-145, S-100 Endothelial cells ACE (angiotensin-converting enzyme), BNH9/BNF13, CD31 (PECAM-1), CD34, CD54 (ICAM-1), CD62P (p-Selectin GMP140), CD105 (Endoglin), CD146 (P1H12), D2-40, E-selectin, EN4, Endocan (ESM-1), Endoglin (CD105), Endoglyx-1, Endomuci, Endosialin (tumor endothelial

marker I, TEM-1, FB5), Eotaxin-3, EPAS1 (Endothelial PAS domain protein 1), Factor VIII related antigen, FB21, Flk-1 (VEGFR-2), Flt-1 (VEGFR-I), GBP-1 (guanylate-binding protein-1), GRO-alpha, Hex, ICAM-2 (intercellular adhesion molecule 2), LYVE-1, MRB (magic roundabout), Nucleolin, PAL-E (pathologische anatomie Leiden-endothelium), RPTPmu (Receptor protein tyrosine phosphatase mu), RTKs, sVCAM-1, TEM1 (Tumor endothelial marker 1), TEM5 (Tumor endothelial marker 5), TEM7 (Tumor endothelial marker 7), TEM8 (Tumor endothelial marker 8), Thrombomodulin (TM, CD141), VCAM-1 (vascular cell adhesion molecule-1) (CD106), VE-cadherin (CD144), VEGF (Vascular endothelial growth factor), vWF (von Willebrand factor) Enterocytes Amino-Peptidase N, Carbonic Anhydrase (CA), Carbamoylphosphate Synthase (CPS), CD10, Dipeptidyl Peptidase IV (DDP IV, CD26), E-Cadherin, Enterocytin, Glucose Transporter-5 (GLUTS), IAP (intestinal alkaline phosphatase), I-FABP (intestinal fatty acid-binding protein), L-FABP (liver fatty acid-binding protein), Lactase, Lectins, Neutral Endopeptidase (Endopeptidase 24.11; NEP; neprilysin), Sodium Glucose co-Transporter 1 (SGLT1), Sucrase Isomaltase (SI), Villin, Zonula Occludens (ZO1, ZO-1) Eosinophils BMK-13, CD9, CD44 and CD69, ECP (Eosinophil Cationic Protein, EGI/EG2), EDN (eosinophil derived neurotoxin), Eosinophil Peroxidase (EPO), Eosinophil Protein-X (EPX), IL-5, LA Antigen, MBP1/MBP2 (major basic protein), Epithelial cells A6 antigen, A33 antigen, Adenosine 5'-Triphosphatase (ecto-ATPase), Aminopeptidase N, APN/CD13, AUA1, BG8 (Lewis Y blood antigen), 20 02756833 201 -Crd-27 Bmi-1 oncoprotein, BRCA1, BTEB1, CA-125, Calcyclin, CAR-5, Carcinoembryonic Antigen (CEA), Cathepsin E (CaE), CCIO (Clara cell-specific protein), Cystatin C, Cytokeratins 8, 14, 18, and 19, Connexin-43 (Cx43), Desmin, EMA, Exo-1 (Pa-G14), EZH2, Ezrin, Foxal, GABRP, Galectin-3, GGT (gamma-glutamyl transpeptidase), Glutamine Synthetase, H4, HLA-DR, HME1, Keratin 5 (K5), Keratins 13 and 19, KL-6, Lactoferrin, LAMP-1 (lysosomal-associated membrane protein 1), Lectins, Leu-7, LhS28, Ly110, MI, MBEC, MEP-1, MEP7, MOC-31, NSE (neuron-specific enolase), Neutral Aminopeptidase, P2X7, p16, p16 (INK4A), p63, P-Cadherin, Prostate Derived Factor (PDF), PHM-5, PR1A3, Prominin-1 (CD133), Prostate Antigen (PA), Protein Gene Product 9.5 (PGP 9.5), Prostatic Binding Protein (PBP), PSCA (Prostate stem cell antigen), Rab13, RAGE, RLA (rat liver antigen), Rex-1 (zinc-finger protein-42, Zfp42), RTE 1, 2, 3, 7, 9 11, 12, 13, RTI40, Secretory Component (SC), SPA, SPB, SPC (surfactant proteins A, B, C), SPRR1B, SQM1 protein, Sucrase-isomaltase (SI), Thioesterase H, Transthyretin, VAT-1, Vimentin Erythrocytes BGP1, CD36,

CD47, CD71 (transferrin receptor), Globin, Glycophorin A (GPA), Glycophorin B, Hemoglobin, Rh Polypeptides and Rh Glycoprotein, N-Acetyl-9-0-Acetylneuraminic Acid, TER119, VLA4 Fibroblasts ER-TR7, FSP1, prolyl 4-hydroxylase (5B5) Germ cells 43-9F, AFP (alpha-fetoprotein), Aggrus, AP-2gamma, Axdazl, BMP15 (bone morphogenetic protein 15, CA-125, c-Kit (CD117), DAZ-like 1(DAZLI), Dppa3, EGFR (Epidermal growth factor receptor), GCNA1 (germ cell nuclear antigen 1, GCNA-1), GDF9 (growth and differentiation factor 9, Glypican 3, GP90-MC301, Keratin 7, Lactate Dehydrogenase (LD), Lactate Dehydrogenase Isoenzyme, LDH (lactate dehydrogenase isoenzyme 1), M2A, M-CSF, MAGE-44, MATER, OCT p53, PD-GFA, PLAP, Podoplanin, Proacrosin, RBMA (RNA-binding motif), telomerase, Tesmin, TEXI01, TRA-1-60, VASA, ZAR1, GCAP, sACE, Notch-1, c-kit, GFRalpha-1 Glial cells A2B5-antigen (A2B5), GD3, 04-antigen (04), RC1, Sox-1/Sox-2, Vimentin Goblet cells CDX-2, CK7, CK20, ITF, Keratin polypeptide 20 (K20), Lectins, Muc2, MUC5AC, MUC5B, PKD (PKCmu), Trefoil Factor (Tff3) Granulosa cells AMH (anti-mullerian hormone), Aromatase (CYP19A1), chZPC, Follicle regulatory protein (FRP), Inhibin, MCAM (Melanoma cell adhesion molecule, CDI46) Hematopoietic AC133, BAALC, CD31, CD34, CD43, CD44, CD45, CD84, Progenitors CD133/Prominin-1, CDCP1 (CUB-domain-containing protein I), C-Kit/CD117, Endomucin, Flk-2, Flk-2/F1t3, Flt-3L, LR-1, Ly-5, MYADM, Seal, SCGF, STK-1, TGF-beta2, Thy-1, Hepatoblasts alpha-Fetoprotein (AFP), C/EBP alpha, Cytokeratin 8, 14, and 18, Dfci/Pref-1, E-cadherin, Foxnlb, HNF4, Id3, Liv2 (liv-2), Proxl, SEK1, Interneurons Parvalbumin, Calretinin, Calbindin, CB1 (type 1 cannabinoid receptor, CCKpan (Cholecystokinin), ChAT (choline acetyltransferase), Chx10, DLX, EN1 (pan-Engrailed-1, EN-1), ER81, EVX1, GAD65, GABA(B) receptor I-like (GBRI-L1), GAD65, GAD67, GATA, GluR-8, ISL1, Lhx 1, Lhx5, Lhx3, Lhx6, mGluR1alpha, MOR, Nloc2-2 (NIcx2.2), NMDAR2D, NOS, Pax2, SDF-I, SPO, Substance P Receptor (SPR) Islet cells Beta-2/NeuroD, FoxA1, FoxA3, GAD (glutamic acid decarboxylase), GAD65/GAD67, Gdfl 1, GLUT1, GLUT3, GLUT2, GLUT4, 20 02756833 201 -Crd-27 IA2/ICA512, IAPP/amylin, IGRP, INGAP (islet neogenesis-associated protein), IPF1, Islet-1, MafB, Neurogenin 3 (Ngn3), NIOC6.1, Pax4, Pax6, PDX-1 (Pancreatic duodenal homeobox factor-1), PEK, STF-1 Keratinocytes Calmodulin, Calmodulin-like skin protein, CD24 (heat stable antigen, nectadrin), CD34, CD98, Epidermal calcium-binding protein (ECaBP), Filaggrin, GP37, gp80, hKPRP, ICAM-1, Involucrin, Keratinocyte transglutaminase, KL3, KPRP, Minoxidil Sulfotransferase, MTS24, p63, rSQ20 and hSQ16, SPR1 (small proline-rich protein-1), SPRR1,

SPRR1A, SPRR1B, SPRR2A, SQM I protein, Tob Kupfer Cells BGS-18, CD14, CD68, EDI, ED2, F4/80, Fucose Receptor, G6PD (glucose-6-phosphate dehydrogenase), Lectin, Lysozyme, TNF- α Langerhans Cells Acetylcholinesterase (AChE), ATPase, CD1a (Leu 6), E-Cadherin, Fascin, Fc gamma-receptor (FcR), HLA-DM, HLA-DR (Ia), KL-6, Langerin (CD207), MHC Class II, MT1, Neuron-Specific Enolase (NSE), OKT6, T6 (CD1) Leydig Cells 3 beta-HSD (3 - hydroxysteroid dehydrogenase, 3b-HSD), 7-dehydrocholesterol reductase (7-DHCR), 11 beta-hydroxysteroid dehydrogenase, Calretinin, Cyp17 and Cyp11a1, Esterase, Inhibin- α , IGF-1 (insulin like growth factor-1), INSL3 (Insulin-like factor 3), Ley I-L (Leydig insulin-like gene), LRH-1 (liver receptor homolog-1), Luteinizing Hormone (LH) receptor, Melan-A, Nestin, Neuron-Specific Enolase (NSE), P450arom (cytochrome P450 aromatase), PBR (Peripheral-type benzodiazepine receptor), Relaxin-like factor (RLF), SCC (P450 side-chain cleavage enzyme), STAR (steroidogenic acute regulatory protein), Steroidogenic Factor-1 (SF-1, Nr5a1, and Ad4bp), Thrombospondin 2 (TSP2), 301-1SD VI, PGD-synthetase, EST, 17 β HSD III, 3beta-hydroxysteroid dehydrogenase (3beta-HSD) VI, 17beta-hydroxysteroid dehydrogenase (17beta-HSD) III, vascular cell adhesion molecule 1, estrogen sulfotransferase, and prostaglandin D (PGD)-synthetase Leukocytes .. 8-OHdG (8-hydroxydeoxyguanosine), Beta2 Leukocyte Integrins (CD11/CD18), Cathepsin G, CD15 (leuM1), CD18 (MHM23), CD43 (leukosialin, leu-22), CD45, CD45RA/CD45RB/CD45RO, CD53 (Ox-44), CD68 (KPI, macrosialin), CD95 (fas), CD166, Diiodotyrosine (DIT), EFCC, Fecal Lactoferrin, Glucose-6-phosphatase (G-6-Pase), HLA (human leukocyte antigen), HLE (Human Leukocyte Elastase), ICAM-1, IL-8 (Interleukin-8), Li, Lactoferrin, LAM-1 (Leukocyte Adhesion Molecule-1), LAP (Leukocyte alkaline phosphatase), Lectins, L-selectin, LSP1 (Leukocyte-specific protein-1), Ly-9, M6 (leukocyte activation antigen), Mac-1, MPO (myeloperoxidase), VIP (Vasoactive Intestinal Polypeptide) Macrophage Carboxypeptidase M (CPM), Cathepsin K, Chitotriosidase, CD14, CD68 (Ki-M7, Y2/131, Y1/82A, EBM11), CD163, sCD163, CSF-1R (colony-stimulating factor-1 receptor), ED-1, ED-2, EMR1 (epidermal growth factor module-containing mucin-like receptor 1), Factor XIII-A, Ferritin, HAM-56, Ki-MIP, Lysozyme M, MAC-1/MAC-3, Myeloid-related protein (MRP) 14, RFD7/RFD9, RM3/1 Mast cells Carboxypeptidase A, Chymase, CD25, CD34, CD117 (c-Kit), Ki-MC1, Ki-MIP, LAMP-1/LAMP-2, Mast Cell Trypsin, PDG2 Melanocytes ETB (endothelin-B) receptor, HMB-45 (gp100), L-PGDS (lipocalin-type prostaglandin D synthase), MATP, Mell/Me12, Melan-A (A103), MeEM, Mitf (Microphthalmia-associated transcription factor),

PNL2, Tyrosinase (T4), Tyrosinase-related proteins (TRPs)/gp75 20 02756833 201 -Crd-27 Mesenchymal stem Msxl, TAX, Twistl cell Merkel Cells CD56, Chromogranin A (CGA), Cytokeratin 20, Fli-1 and CD99, Go alpha (alpha subunit of guanine nucleotide-binding protein Go), Keratin 20, NSE (neuron-specific enolase), TROMA-1, Villin Mesothelial Cells Calretinin, Cancer Antigen (CA)125, CD44, CD44H, Cytokeratin 5/6, Desmin, E-Cadherin, HBME-1, Keratin, Keratin7 (K7), MCp130, ME1/ME2, Mesothelin, N-Cadherin, Protein Phosphatase Inhibitor-1 (I-1), Thrombomodulin, Vimentin, WT1 (Wilms' tumour susceptibility gene 1) Monocytes Adipophilin, Angiotensin Converting Enzyme, CB12, CD11a (LFA-1 alpha), CD11b, CD14, CD15, CD54, CD62L (L-selectin), CD163, Cytidine Deaminase (CDD, EC 3.5.4.5), DH59B, Fe-receptors, Flt-1 (VEGFR-1), HLA-DR, hMGL, Ki-Mlp, Leucocyte tartrate-resistant acid phosphatase (FATRE), Leu-8, Lysozyme, Mannosyl Receptors, Peanut Agglutinin (PNA), Thromboplastin, Thymidine Phosphorylase (TP), TNF (Tumor necrosis factor), Urokinase (UK), VEP8 and VEP9, thiol-protein disulfide-oxidoreductase Motor Neurons ChAT (choline acetyltransferase), Choxl 0, En1, Even-skipped (Eve) transcription factor, Evx1/2, Fibroblast growth factor-1 (FGF1 or acidic FGF), HB9, Isl1 (Islet-1), Isl2, Islet1/2, Lim3, Nloc6, p75(NTR) (p75 neurotrophin receptor), REG2, Sim1, SMI32 (SMI-32), Zfh1 Myeloid cells Arginase-1, BM-1/BM-2/BM-3/BM-4 (Granulocyte), ClqR(P), CD11a/CD18, CD11b/CD11c, CD13, CD14, CD15, CD18 (beta(2) leukocyte integrin), CD31, CD33, CD34, CD38, CD43, CD123, CD138, CLL-1 (C-Type Lectin-Like Molecule-1), CSC-1, F4/80, Glut3, Elastase, GPIIb-IIIa, GR-1, Lactoferrin (LF), Ly498, Lysozyme, MAC-1, MC52, M01(CD11b), MPO (myeloperoxidase), MY3, MY4, MY7, MY7/MY9, MY8, MYADM, VIM-D5, Yml, Myoblasts Acetylcholinesterase (AChE), ADAM12, alpha- and beta-tropomyosin (pT), beta-Enolase, CD56, Desmin, Lactate Dehydrogenase (LDH), M-Cadherin (muscle cadherin), M-Cadherin (muscle cadherin), M-Calpain, M-CAM (melanoma cell adhesion molecule), MRF4 (myogenic/muscle regulating factor-4), Myf-5 (muscle regulatory factor-5), MyoD, Myogenin, Myosin, nls beta-Galactosidase, N-Cadherin (neural cadherin), p21, Phosphoprotein (pp(65;4.5)), Pax3, Pax7, PK-K (K-isozyme of pyruvate kinase), PK-M (M-isozyme of pyruvate kinase), Tbx3, Titn Myocytes ANP (Atrial natriuretic peptide), Arpp, BBF-1, BNP (B-type natriuretic peptide), Caveolin-3 (Cav-3), Connexin-43, Desmin, Dystrophin (Xp21), EGFP, Endothelin-1, FABP (Heart fatty-acid-binding protein), GATA-4, MEF-2 (MEF2), MLC2v, Myosin, N-cadherin, Nestin, Popdc2 (Popeye domain containing gene 2), Sarcomeric Actin, Troponin, Troponin 1 Myoepithelial 14-3-3sigma,

alpha-SMA, Caldesmon (CALD), Calponin, Carbonic Cells (MEC) Anhydrase III (CAIII), CD10, CD29 and 14-3-3sigma, CD109, Cytokeratin 14, Cytokeratin 17, EGFR, L2E3, Maspin, Neuropilin-1, Osteonectin (SPARC), p63, p75 neurotrophin receptor (p75NTR), P-cadherin, SMMHC (Smooth Muscle Myosin Heavy Chain), Thy-1 (thymocyte differentiation antigen), Vimentin Myofibroblasts Actin, Cadherin-11, Desmin, EDA (ED-A fibronectin), GB 42, Palladin 4Ig, SMA-alpha (smooth muscle actin-alpha), Transforming growth factor (TGF) beta 1, Thy-1, Tropomyosin-1 Natural Killer cells 2B4, CD2, CD3, CD7, CD16 (Leu 11b), CD33, CD45, CD56, CA 02756833 201 -0,3-27 CD57/1-[NK I, CD69, CD107a, CD161, CS I, HP (Helix pomatia) Receptors, LAT (linker for activation of T cells), Ly24 (Pgp-I), NKG2A and NKp80, NKH1 (N901), Protocadherin 15 (PCDH15) Neural stem cells CD15, CD24 (HSA), CD29 (Integrin CD49f (Integrin $\alpha 6$), (ICAM-1), CD81, CD95 (FAS/APO-1), CD133, CD140a (PDGFR α), CD146, CD184 (CXCR4), CD338 (ABCG2), Nestin, Notch1, SSEA-1 Neurons ABCA2 (ATP-binding cassette transporter-A2), Acetylcholinesterase, A1z-50, ATF3 (Activating transcription factor 3), Bcl-2, BM88, Calbindin D28, Bag 1, Beta-tubulin, c-Fos, Calbindin D28K, Calcineurin, Calretinin, Cerebellin, ChAT (choline acetyltransferase), Cytochrome oxidase, Cystathionine, DSS-3, ELF, HSV-1 (Herpes simplex virus type 1), importin alpha 5, MAG (myelin-associated glycoprotein), MAP2, MIT-23, NAA (N-Acetylaspartate), NADPH-diaphorase, Nestin, NeuN (neuronal nuclei), Neurofilament, Non-angiotensin II [(125)I] CGP42112, NSE (neuron specific enolase), NSP-C (Neuroendocrine-specific protein C), OMP (olfactory marker protein), Pax6, Pitx3, Tbr2, Tbr1, PGP9.5 (neuronal marker protein gene product 9.5), PKC (Protein kinase C), RC3/neurogranin, S199, SBDP120s, SSEA-1, Synapsin 1, TG-1, TGF-beta Neutrophils 8-hydroxydeoxyguanosine (8-OH-dGUA), B beta 30-43, CD11b, CD18, CD64, C-reactive protein (CRP), Gelatinase, Granulocyte Receptor-1 (Gr-1), HNE ANCAs, HNL (human neutrophil lipocalin), Human Neutrophil Peptides 1-3 (HNP-1-3), L-selectin, Lactoferrin, Lysozyme, Myeloperoxidase (MPO), Neutrophil Alkaline Phosphatase (NAP), Neutrophil Elastase (NE), NGAL (neutrophil gelatinase-associated lipocalin), Polymorphonuclear Neutrophil Elastase (PMN-E) Odontoblasts Alkaline Phosphatase (ALP), alpha 1 Type I Collagen (alpha I type I collagen), DMP 1 /DMP2 (dentin matrix protein), DPP (dentine phosphoprotein), DSP (dentin sialoprotein, dentinsialoprotein), DSPP (dentin sialophosphoprotein), Enamelysin, Mov13 allele, Nestin, OSAD (Osteoadherin), Osteopontin (OPN), Osteocalcin (OC), Phex (phosphate-regulating gene with homologies to endopeptidases on X-chromosome) Oocytes Bicaudal-D (Bic-D),

BMP15 (bone morphogenetic protein 15), c-kit, c-Mos, GDF9 (growth and differentiation factor 9), HBPP (heparin-binding placental protein), IGFBP-1, Kit Ligand (KL), Leptin, LH Receptor (LH-R), MATER (maternal antigen that embryos require), MSY2, NALP9, Orb, Oskar, p180, Pentraxin 3, VASA, ZP (zona pellucida, ZP1, ZP2, ZP3 or ZPA, ZPB, ZPC), ZAR1 (zygotic arrest 1) Osteoblasts Alkaline Phosphatase (ALP), alpha 1(1) procollagen, Bone Gla Protein (BGP), Bone Sialoprotein (BSP), Cbfa1/Osf2, Collagen Type I, Ell, Osteocalcin, Osteopontin, Phex, RP59 Osteoclasts acid ATPase, Calcitonin (CT) receptor (CTR), Carboxyterminal Telopeptide of Type I Collagen (1CTP), Cathepsin K, CKBB (creatine kinase BB), EDI, Kati-antigen (Katl-Ag), P1CP (procollagen carboxyterminal propeptide), RANK, Tartrate-resistant acid ATPase, TRAP (tartrate-resistant acid phosphatase), Vitronectin Receptor (VR, VNR) Paneth cells alpha-Defensins (cryptdins), Cryptdins, Cryptdin-1, Cryptdin-2, Cryptdin-3, Cryptdin-4, Defensins, Enhancing factor (EF), GM-CSF (granulocyte-macrophage colony-stimulating factor), HD-5 (Human Defensin 5), Lysozyme, Matrilysin, PLA2 (group II phospholipase A2), Trypsin Pericytes Alpha-smooth muscle actin (a-SMA), Angiopoietin-1, Angiopoietin-CA 02756833 201 -0,3-27 ' (Ang2), CD13, Desmin, Endosialin (CD248), NG2 Chondroitin sulfate proteoglycan, PDGFR-beta, RGS5, Thy-1 Phagocytes alpha-1-Antitrypsin, c-fms, CD11b/CD18 (beta 2 integrins), CD11c/CD18, CD14, CD36, CD64, CD68, CD204, CR3 (C3 receptor), CSF-1, ED1/ED2, F4/80, Mac-1, MARCO, M-CSF, MITE, MRP8/MRP14, Meloperoxidase (MPO), RFD7, S100 proteins, TacP (Tartrate-resistant acid phosphatase), TFEC, TPP-ase Platelet AK (adenylate kinase), Annexin V, BTG (beta-thromboglobulin), (thrombocyte) CD31, CD36, CD49b, CD62, CD62P (P-selectin), CD63 (glycoprotein-53), Glycocalicin (GC), GMP-140 (platelet alpha-granule membrane protein), GPV (Glycoprotein V), LAMP2 (lysosome-associated membrane protein-2), PAC-1, PDMP (platelet-derived microparticles), Platelet-Associated Factor XIIIa, Platelet Factor 4 (PF4), P-selectin (CD62P), Serotonin (5-HT), Thrombospondin (TSP), Thromboxane B2 Pneumocytes Alkaline Phosphatase, Aquaporin 5 (Aqp-5), Bauhinia purpurea lectin (BPL), Caveolins (Cav-1, -2, and -3), CD44v6, CD208 (DC-LAMP), CP4, Cx43, DC-LAMP (CD208), gp600, HTI56, ICAM-1, KL-6, MUC1, TI alpha, Thomsen-Friedenreich antigen, IF antigen, Thyroid Transcription Factor 1 (TTF-1) Podocytes alpha-actinin-4, B7-1, CD2AP, CD10, Cortactin, Desmin, Dystroglycan (DG), Ezrin, FAT, GLEPP1 (Glomerular epithelial protein 1), Lmx 1b, MAP-LC3 (Microtubule-associated protein 1 light chain 3), Myocilin, NEPH1, Nephrin, P-cadherin, PHM-5, (podocalyxin-like protein in humans), Podocin, Podoplanin, Podocalyxin

(PC), Synaptopodin, T-/H-cadherin (CDH13), VEGF, Vimentin, Wilms' tumor-1 protein (WT-1), ZO-1 (zonula occludens-1) Primordial germ Blimpl, Mili, Miwi, UTF1, AP-2, Eps8, GCNA1, OC13/4, PLAP, cell (gonocyte) VASA, Purkinje cells Aldolase C (Zebrin II), CaM-PDE (Calmodulin-dependent phosphodiesterase), Car8 CD3 (Leu-4), Calbindin (CaBP, 28-kDa calbindin-D, calcium binding protein Calbindin-D28K), Cerebellin, cGMP-dependent protein kinase, Clusterin, ELF, GABA-T (gamma-aminobutyric acid transaminase), GAD67 (67-kDa isoform of glutamic acid decarboxylase), Guanosine 3':5'-phosphate-dependent protein kinase, HDAC6, HFB-16 (KIAA0864 Protein), Inositol 1, 4, 5-triphosphate receptors (IP3R), L7, MAP2 (microtubule-associated protein 2), MAP-120 lCda, NMDA-NR1 (NMDA-R1 receptor subtype), OMP (olfactory marker protein), P400 protein, P450scc (P450 side-chain cleavage), PCA-1/PCA-2, PCPP-260 (Purkinje cell phosphoprotein of Mr 260,000), PDE5/PDE1B, PDE9A, PEP-19 (PEP19), PMCA (plasma membrane calcium pump), SERCA, Spot 35 protein (S-35), Zebrin I and Zebrin II Pyramid cells CaMK (calcium/calmodulin-dependent protein kinase II, CaMKII), Emxl, GluR2/3, MAP2 (microtubule-associated protein 2), MATH-2, mGluR1/mGluR5, Neurogranin/RC3, PSD-95/SAP90, RPTPalpha, RPTPgamma (receptor protein tyrosine phosphatase gamma), RPTPzeta/beta, SCIP, SMI-32, Tbrl, Zfp312, Pax6, Tbr2/Eomes, NeuroD, Reed-Stemberg CD15 (Leu-M1), CD30 (Ber-H2, Ki-1), CD74 (LN2), Fascin cells Sertoli cells ABP (androgen-binding protein), AMH (anti-Mullerian hormone), Calretinin, Cathepsin L, CK18, (Cytokeratin 18), Cytokeratin, Clusterin, Cyclic Potein-2 (CP-2), Dhh (Desert hedgehog), Desmin, Fas/FasL, GATA-1, GATA-4, Inhibin B, M2A, MIS (Mullerian CA 02756833 201 -0,3-27 inhibiting substance), Serotonin Receptor, SCF (stem cell factor), Sox9, Sulfated Glycoprotein-1 (SGP-1), Sulfated Glycoprotein-2 (SGP-2), Transferrin, Vimentin, WTI (Wilms' Tumor suppressor 1, WT-1) Spermatocytes 8D11, Acrosin Binding Protein (ACRBP), GCNA1, GP90-MC301, Lactate Dehydrogenase-X,(LDH-X), p73/5.7, Pgk-2, Proacrosin, SCP1/SCP2/SCP3 (Synaptonemal Complex Protein), SOX-17, SPTRX-3, TEX101, XMR, Spermatozoa Amidase, Aromatase, CD46, TEPA, Stellate cells alpha-SMA (smooth muscle actin, alpha), c-Myb, CRP2 (cysteine- and glycine-rich protein 2), Desmin, FAP (Fibroblast Activation Protein), GFAP, Reelin, S100, Synaptophysin, Vimentin, Vinculin Stromal cells Cadherin-11, Calretinin, CD10, CD117, Desmin, Endoglyx-1, Endosialin (TEM1, CD248), Fibroblast-Activation Protein (FAP), Neural Ganglioside GD2, Nucleostemin, Snep (stromal nidogen extracellular matrix protein), Tenascin, CD13, CD29, CD44, CD63, CD73, CD90, CD166, STRO-1, HOP-26 (CD63), CD49a,

SB-10 (CD166), Alpha and beta subunits of inhibin/activin, Alpha-smooth muscle actin Stem cells 4G10.3, AA4, AC133, Bcrp/ABCG2, c-Mpl, CD9, CD15, CD24, CD29, CD30, CD34, CD133 (Prominin-1), CDCP1, Connexin 43, Endoglin, ER-MP12, Fibroblast growth factor receptor-3, Flk-2, gpt, Human Rex-1 (hRex-1), importin alpha 1, Interleukin-2 receptors, Interleukin-3 receptor alpha chain, KDR, Keratin 19, c-kit, Lamin A/C, Macromolecular insoluble cold globulin (MICG), Musashi-1, Nanog, Nestin, N otchl, Nucleostemin, Oct4 (Oct-4), p63, Podocalyxin, R2/60, PSCA (Prostate stem cell antigen), Sox1, SOX2, SSEA-1, SSEA-3, Stem cell Antigen 1 and 2 (Sca-1 and Sca-2), Telomerase, Thy-1, Transcription factor Stat5, Synaptic cells Brain spectrin, Chromogranin A/Chromogranin C, Con A-binding glycoprotein, D2-protein, D3-protein, GAP-43 (Growth-Associated Protein-43), NCAM/N-CAM D2 (Neural cell adhesion molecule), p65, PSD95 (Post-Synaptic Density protein-95), Secretogranin II, Synapsin, Synaptin, Synaptobrevin, Synaptogyrin (p29), Synaptophysin, Synaptoporin, Synaptotagmin I, Syntaxin, SV2 (Synaptic vesicle protein 2), Vesicular glutamate transporters (VGLUT1 and VGLUT2) T cells ART2, CD1a, CD1d, CD2, CD3, CD4, CD5, CD7, CD8, CD11b (Mac-1), CD25 (interleukin 2 receptor alpha), CD38, CD45RO, CD72, CD134 (OX40), CD150, CRTAM, FOXP3, FT2, GPCA, HLA-DR, HML-1, HT23A, Leu-22, Ly-2, Ly-m22, MICG, MRC OX 8, MRC, OX-22, OX40, PD-1 (Programmed death-1), RT6, TCR (T cell receptor), Thy-1 (CD90), TSA-2 (Thymic shared Ag-2) Theca cells Alkaline phosphatase (AP), BMP-4, CYP17, NR5A1 (steroidogenic factor-1, SF-1) Thymocytes 20 alpha SDH, CD1, CD1a, CD2, CD4, CD5, CD8, CD25, CD26, CD45RA, CD53, CD69, CD71, CD150, CTX (cortical thymocyte-specific antigen of Xenopus), GIX, Granzymes, H-2, H-2D, HBA-71, ICT-1 antigen (thymocyte differentiation antigen), IL-7, Immature thymocyte antigen-1 (1MT-1), J11 d (heat-stable antigen), JL1, LFA-1 (lymphocyte function associated antigen-1) beta, Ly-1/Ly-2, Ly-2/3, Ly-24 and Ly6C, M241, MRC OX-2, Peanut agglutinin (PNA) receptor, Sca-1/Sca-2 (stem cell antigen), T3 (OKT 3), T6 (OKT 6), TAP (T cell-activating protein), THAM (thymocyte-activating molecule), Thy-1, Thy-1.1, Thy-2, Thymic shared antigen-1 (TSA-1), TL antigens (thymus leukaemia antigens), TL3, H-2, TL, Ly I and Ly2, Thy-1, Ly-1, Ly-2, T200, T1, T4, T5, 16, T8 Trophoblasts Cdkn1c, Cdx2, CHL1, Cytokeratin, Cytokeratin-7 (CK7), D1x3, FD0161G, Gcm1 (glial cells missing 1), H315, H316, Handl, HASH2, hCG (human chorionic gonadotropin), hCG-beta (Human chorionic gonadotrophin beta), HLA-A/HLA-B/HLA-CiFILA-G, hPL (human placental lactogen), Id-1, Id2, 1-mfa, Inhibin A, Integrins, Kip2, M30, Mash2, MNF116, NDOGI/NDOG2, OKT9, PAL-1

(plasminogen activator inhibitor-1), PHLDA2, Placental Lactogen (PL-1, PL-2), PLP-A/PLP-B/PLP-C/PLP-D/PLP-E/PLP-F/PLP-L/PLP-M/PLP-N, SBU-I, SP-1, TAI/TA2 (trophoblast antigens), and/or Tfeb. In certain embodiments, the gene product is Cre, Cas9 or any variants thereof, and the Cre, Cas9 or variants thereof are operably linked to a tissue-specific regulatory element.

[00111] In certain embodiments, the expression of a gene product capable of editing a conditionally inducible gene editing site may be regulated by a regulatory element, such as a promoter and/or enhancer that is capable of providing expression in every cell of the transgenic animal or animal model or substantially every cell of the transgenic animal or animal model. Non-limiting examples of such regulatory elements comprising:

ACTB (beta actin), CMV (cytomegalovirus), EF1 α (elongation factor 1 alpha), SV40 (simian vacuolating virus 40), TK (thymidine kinase), PGK (phosphoglycerate kinase), CAG (chicken beta actin) and/or UbC (ubiquitin c).

[00112] In certain embodiments, the at least one nucleic acid sequence encoding a gene product capable of editing the editing site may comprise a regulatory element comprising a tetracycline responsive element capable of inducing gene expression of the gene product with the addition or removal of tetracycline from the transgenic animal or animal model or the transgenic cell.

[00113] In certain embodiments, the conditionally inducible gene editing site, such as the at least one conditionally inducible gene editing site and/or one or more additional conditionally inducible gene editing site, comprises LoxP site and the at least one nucleic acid editing sequence encodes a Cre. In certain embodiments, the Cre may be operably linked to a regulatory element, such as a promoter, that restricts its expression to a specific tissue, cell, organ or combination thereof.

[00114] In certain embodiments, the activity, expression and/or function of Cre may be induced, wherein induction may comprise exposing the transgenic non-human animal or animal model or cell to an exogenous stimulus. In certain embodiments, the Cre may be fused to a peptide that regulates its activity, such that the Cre may only provide enzymatic activity in the presence of an exogenous compound, such as for example, tamoxifen or an analog or equivalent thereof. In

certain embodiments, the Cre may be fused to a mutant form of an estrogen receptor. In certain embodiments, the Cre may be Cre ERT2.

[00115] In certain embodiments, the at least one conditionally inducible gene editing site may further comprises a second conditionally inducible gene editing site and the second conditionally inducible gene editing site may comprise an exogenous nucleic acid sequence, or an endogenous nucleic acid sequence, and the nucleic acid editing sequence encodes the gene product capable of editing the second conditionally inducible gene editing site. In certain embodiments, the at least one conditionally inducible gene editing site may comprise two conditionally inducible gene editing sites, wherein each conditionally inducible gene editing site may flank a regulatory region. In certain embodiments, the at least one conditionally inducible gene editing site may comprises LoxP sites flanking a regulatory region. In certain embodiments, the at least one conditionally inducible gene editing site may comprise LoxP sites flanking an exon. In certain embodiments, the at least one conditionally inducible gene editing site may comprise LoxP site flanking a critical regulatory region in a Gnpat gene. In certain embodiments, the at least one conditionally inducible gene editing site may comprise LoxP sites flanking exon 2 in a Gnpat gene of a mouse. In certain embodiments, the at least one conditionally inducible gene editing site may comprise LoxP sites flanking a regulatory region, wherein the gene product capable of editing the conditionally inducible gene editing sites is a Cre.

[00116] In certain embodiments, the at least one nucleic acid editing sequence may comprise one or more sequences encoding a gene product capable of editing a conditionally inducible gene editing site, wherein each of the one or more sequence encoding the gene product capable of editing the conditionally inducible gene editing site may be provided in a same or different region of the genome. In certain embodiments, the at least one nucleic acid editing sequence comprises a sequence encoding one guide RNA or equivalent thereof, and a sequence encoding a Cas9 or variant thereof. In certain embodiments, the at least one nucleic acid editing sequence comprises two independent sequences each encoding one guide RNA or equivalent thereof, and a sequence encoding a Cas9 or variant thereof. In certain embodiments, the at least one nucleic acid editing sequence comprise two independent sequences each encoding one guide RNA or equivalent thereof, wherein each guide RNA is capable of binding conditionally inducible gene editing sites flanking a regulatory region. In certain embodiments, the at least one nucleic acid editing sequence comprise two independent sequences each encoding one guide RNA or

equivalent thereof, wherein each guide RNA is capable of binding conditionally inducible gene editing sites in a Gnpat gene. In certain embodiments, the at least one nucleic acid editing sequence comprise two independent sequences each encoding one guide RNA or equivalent thereof, wherein each guide RNA is capable of binding conditionally inducible gene editing sites flanking a regulatory region in a Gnpat gene. In certain embodiments, the at least one nucleic acid editing sequence comprise two independent sequences each encoding one guide RNA or equivalent thereof, wherein each guide RNA is capable of binding conditionally inducible gene editing sites flanking an exon of a Gnpat gene. In certain embodiments, the at least one nucleic acid editing sequence comprise two independent sequences each encoding one guide RNA or equivalent thereof, wherein each guide RNA is capable of binding conditionally inducible gene editing sites flanking exon 2 of a Gnpat gene.

[00117] In certain embodiments, the at least one conditionally inducible gene editing site may comprise guide RNA or equivalent binding sites flanking a regulatory region. In certain embodiments, the at least one conditionally inducible gene editing site may comprise guide RNA or equivalent binding sites flanking an exon. In certain embodiments, the at least one conditionally inducible gene editing site may comprise guide RNA or equivalent binding site flanking a critical regulatory region in a Gnpat gene. In certain embodiments, the at least one conditionally inducible gene editing site may comprise guide RNA or equivalent binding sites flanking exon 2 in a Gnpat gene of a mouse. In certain embodiments, the at least one conditionally inducible gene editing site may comprise guide RNA or equivalent binding sites flanking a regulatory region, wherein the gene product capable of editing the conditionally inducible gene editing sites is a Cas9 or variant thereof, and one or more guide RNA or equivalent thereof capable of binding the at least one editing site comprising the guide RNA or equivalent binding site.

[00118] In certain embodiments described herein, the conditionally inducible gene editing site(s) may comprise a sequence capable of binding a gene product or gene products encoded by at least one nucleic acid editing sequence. The skilled person, in light of the teachings herein, would be able to select an appropriate sequence for the conditionally inducible gene editing site, such that the gene product or products encoded by the at least one nucleic acid editing sequence are capable of modifying the conditionally inducible gene editing site. In certain embodiments, the conditionally inducible gene editing site may be one or more of the following: LoxP sites, flippase recognition target (FRT) sites, attP and attB sites, sgRNA binding sites, FokI sites, or zinc finger

nuclease sites. In certain embodiments, the conditionally inducible gene editing site or sites may be sequences that are capable of binding one or more of the following: Cre, FLP, PhiC31, Cas9, Cas9 variants, a guide RNA, a CRISPR RNA, FokI, zinger finer nuclease, a transcription activator-like effector nuclease, any variant thereof, or any combination thereof. In certain embodiments, the conditionally inducible gene editing site may be ATAACCTTCGTATANNNTANNNTATACGAAGTTAT (SEQ ID NO:3), wherein N may be any base pair, or any reverse complement or equivalent complementary sequence thereof. In certain embodiments, the conditionally inducible gene editing site may be one or more of the following, any reverse complement, or complementary sequence capable of binding a desired gene product or products:

ATAACCTTCGTATAATGTATGCTATACGAAGTTAT (SEQ ID NO:4),

ATAACCTTCGTATAATGTATaCTATACGAAGTTAT (SEQ ID NO:5),

ATAACCTTCGTATAATGTgTaCTATACGAAGTTAT (SEQ ID NO:6),

ATAACCTTCGTATAAAaGTATcCTATACGAAGTTAT (SEQ ID NO:7),

ATAACCTTCGTATAAgaAccaTATACGAAGTTAT (SEQ ID NO:8),

ATAACCTTCGTATAtaaTACCATATACGAAGTTAT (SEQ ID NO:9),

ATAACCTTCGTATAAgaTAGAATATACGAAGTTAT (SEQ ID NO:10),

ATAACCTTCGTATAcgaTAccaTATACGAAGTTAT (SEQ ID NO:11),

TACCGTTCGTATANNNTANNNTATACGAAGTTAT (SEQ ID NO:12),

ATAACCTTCGTATANNNTANNNTATACGAACGGTA (SEQ ID NO:13), or

ATAACCTTCGTATAatgtacatTATACGAAGTTAT (SEQ ID NO:14), wherein N may be any base pair.

[00119] In certain embodiments, the conditionally inducible gene editing site may be GAAGTTCCTATTCTctagaaaGTATAGGAACTTC (SEQ ID NO:15) or any reverse complement or equivalent complementary sequence thereof.

[00120] In certain embodiments, the conditionally inducible gene editing site may be GTGCCCCAACTGGGGTAACCTtGAGTTCTCTCAGTTGGGGG (SEQ ID NO:16) and/or

TGCGGGTGCCAGGGCGTGCCCTGGGCTCCCCGGGCGCGTACTCC (SEQ ID NO:17), or any reverse complement or equivalent complementary sequence thereof.

[00121] In certain embodiments, the at least one nucleic acid editing sequence encoding a gene product capable of editing the conditionally inducible gene editing site may be CAG-CREERT2.

[00122] In certain embodiments, the gene product may be Cre, flippase, PhiC31, Cas9, an sgRNA, a crRNA, transcription activator-like effector nuclease (TALEN), zinc-finger nuclease (ZFN), any variant thereof, or any combination thereof. In certain embodiments, the gene product is Cre and the conditionally inducible gene editing site or sites comprise LoxP sites. In certain embodiments, the gene product is one or more guide RNA or equivalent thereof, and a Cas9 or variant thereof, and the conditionally inducible gene editing sites are capable of binding said one or more RNA guide or equivalent thereof, wherein the Cas9 or variant thereof forms a ribonucleoprotein complex capable of editing the conditionally inducible gene editing site.

[00123] In certain embodiments, the expression of the gene product is for induction by the addition of an exogenous compound, an exogenous stimulus or both. In certain embodiments, an exogenous compound may refer to a compound that is not naturally occurring in the transgenic animal or animal model or transgenic cell, or the exogenous compound may refer to a naturally occurring compound that is for administration at a concentration that exceeds physiological levels to induce expression. In certain embodiments, the exogenous stimulus may be any stimulus that is not naturally occurring for the transgenic animal or transgenic model or transgenic cell. Non-limiting examples of exogenous stimulus may comprise light, such as a specific duration or wavelength, temperature or combination thereof. In certain embodiments, the exogenous compound may be tamoxifen, 4-hydroxytamoxifen, mifepristone, or any compound capable of inducing activity of a Cre fused to a mutant form of an estrogen receptor. In certain embodiments, the exogenous compound may be tetracycline or doxycycline.

[00124] In certain embodiments, in the event of more than one conditionally inducible gene editing sites are present, the first, the second, the third, the fourth, the fifth and other conditionally inducible gene editing sites are not edited by the gene product at or before birth of the animal. As used herein, “not edited by the gene product at or before birth of the animal” may refer to substantially all of the genomes of said transgenic animal or genomes of a transgenic cell in a non-

edited form, wherein the gene product or gene products encoded by the at least one nucleic acid editing sequence has not been expressed, has not edited a genome, or a combination thereof in the transgenic animal or animal model or cell. In certain embodiments, the conditionally inducible gene editing site or sites may be edited at or before birth of the animal when the exogenous compound or exogenous stimulus is provided to the transgenic animal at or before the birth of the transgenic animal, such as, for example, an embryo.

[00125] In certain embodiments, at least one genome of the transgenic animal or animal model or cell comprises the genome, wherein the genome may refer to a genome comprising at least one of: a conditionally inducible gene editing site in a regulatory region of a gene, a nucleic acid editing sequence encoding a gene product capable of editing the conditionally inducible gene editing site and may further comprise one or more additional editing sites in one or more additional regulatory regions of one or more additional genes, and one or more additional nucleic acid editing sequence encoding a gene product capable of editing the one or more additional conditionally inducible gene editing sites. In certain embodiments, the transgenic non-human animal or animal model or cell may be heterozygous for one or more of the elements. In certain embodiments, the transgenic non-human animal or animal model or cell may be homozygous for one or more of the elements. In certain embodiments, all of the genomes of the transgenic animal or animal model or cell may comprise the genome. In certain embodiments, genetic interactions between the one or more additional genes may occur that result in reduction of plasmalogen levels. For instance, a non-human transgenic animal or animal model or a cell having a first gene with normal plasmalogen expression/activity and a second gene with normal plasmalogen expression/activity, an interaction between the first and the second genes may result in decreased plasmalogen expression/activity. Alternatively, a non-human transgenic animal or animal model or a cell having a first gene with a reduced plasmalogen expression/activity and a second gene with normal plasmalogen expression/activity, an interaction between the first and the second genes may result in decreased plasmalogen expression/activity.

[00126] *Transgenic cells*

[00127] In an embodiment, there is provided herein a transgenic cell as described herein and/or a cell from a transgenic animal or animal model as described herein. In certain embodiments, the cell may be a cell derived or isolated from a transgenic animal or animal model

described herein, such that the cell comprises the genome of the transgenic animal or animal model. In certain embodiments, the cell may be an animal cell including a cell from a mammal, bird, reptile, amphibian, fish, arthropod, vertebrate, or invertebrate. In certain embodiments, the transgenic cell may be obtained by modifying a cell of a non-human animal to comprise a genome as described herein. The skilled person, in light of the teachings herein, would be able to select appropriate methods to obtain a transgenic cell as described herein. In certain embodiments, the transgenic cell may not be derived or isolated from a transgenic animal or animal model. In certain embodiments, the transgenic cell may be modified *ex vivo* and maintained *ex vivo*, such as, for example by culturing the cells under appropriate conditions. In certain embodiments, the transgenic cell may be immortalized or for immortalization.

[00128] In another embodiment, the transgenic cell may be obtained by modifying any known cell-line of a non-human animal or animal model to comprise a genome as described herein. The skilled person, in light of the teachings herein, would be able to select appropriate methods to obtain a transgenic cell-line as described herein. Some non-limiting examples would be tissue-specific Cre lines, inducible ubiquitous driver lines, or any suitable non-human animal cell line.

[00129] *Methods and uses of transgenic animals or animal models and cells as described herein*

[00130] In an embodiments herein, there is provided a method for reducing postnatal plasmalogen levels in a non-human animal or animal model comprising providing a transgenic non-human animal or animal model as described herein and inducing expression of the nucleic acid editing sequence encoding the gene product capable of editing the at least one conditionally inducible gene editing site, wherein the editing of the at least one conditionally inducible gene editing site may decrease expression of the gene regulating the plasmalogen biosynthetic pathway.

[00131] In an embodiment, there is provided a method for inducing a condition or disease in a non-human animal or animal model associated with reduced postnatal plasmalogen levels, the method may comprise providing a transgenic animal or animal model or cell as described herein; inducing expression of the nucleic acid editing sequence encoding the gene product capable of editing the at least one conditionally inducible gene editing site, wherein the editing of the at least one conditionally inducible gene editing site decreases expression of the gene regulating the

plasmalogen biosynthetic pathway; and monitoring the transgenic animal or animal model for symptoms of the condition or disease.

[00132] In an embodiment there is provided a method for determining the efficacy of a compound or composition for treating a condition or disease associated with a reduced plasmalogen level, the method may comprise providing a transgenic animal or animal model or cell as described herein; and one of the following:

1) inducing expression of the nucleic acid editing sequence encoding the gene product capable of editing the at least one conditionally inducible gene editing site, wherein the editing of the at least one conditionally inducible gene editing site is for decreasing expression of the gene regulating the plasmalogen biosynthetic pathway;

administering the compound or the composition to the transgenic animal or animal model;
and

monitoring the transgenic animal or animal model for symptoms of the condition or disease; or

2) administering the compound or the composition to the transgenic animal or animal model;

inducing expression of the nucleic acid editing sequence encoding the gene product capable of editing the at least one conditionally inducible gene editing site, wherein the editing of the at least one conditionally inducible gene editing site is for decreasing expression of the gene regulating the plasmalogen biosynthetic pathway; and

monitoring the transgenic animal or animal model for symptoms of the condition or disease; or

3) administering the compound or the composition to the transgenic animal or animal model and inducing expression of the nucleic acid editing sequence encoding the gene product capable of editing the at least one conditionally inducible gene editing site, wherein the editing of the at least one conditionally inducible gene editing site is for decreasing expression of the gene regulating the plasmalogen biosynthetic pathway; and

monitoring the transgenic animal or animal model for symptoms of the condition or disease.

[00133] In an embodiment, there is provided herein a method for generating a transgenic animal or animal model as described herein, the method may comprise: providing at least one non-human animal or animal model; introducing at least one gene editing sequence in a gene, part or regulatory element thereof, involved in the biosynthesis of plasmalogen to generate a first edited gene in the non-human animal or animal model; crossing the non-human animal or animal model with the first edited gene with a second non-human animal or animal model comprising a nucleic acid editing sequence at a separate locus encoding a gene product, to generate the transgenic animal or animal model; and inducing expression of the gene product in the transgenic animal or animal model to edit the first edited gene, wherein editing the first gene results in a decrease in the level of the gene product of the first edited gene, reduced activity of the first edited gene product of the first edited gene, reduced plasmalogen level in the transgenic animal or animal model or any combination thereof.

[00134] Certain embodiments of a method or use described herein may comprise inducing expression of a nucleic acid editing sequence encoding a gene product capable of editing the at least one conditionally inducible gene editing site. In certain embodiments of a method or use, the conditionally inducible gene editing site may comprise a plurality of conditionally inducible gene editing sites and the nucleic acid editing sequences encoding a gene product capable of editing the plurality of conditionally inducible gene editing sites is provided. In certain embodiments, wherein the conditionally inducible gene editing site comprises two or more conditionally inducible gene editing sites, the method may comprise one or more nucleic acid editing sequences capable of editing the plurality of conditionally inducible gene editing sites. In certain embodiments, the induction of expression may comprise administration of an exogenous compound to the transgenic animal or animal model or cell. In certain embodiments, the gene product capable of editing the gene may comprise a Cre ERT2 and the induction may comprise the addition of tamoxifen, 4-hydroxytamoxifen, mifepristone, or any compound capable of inducing activity of a Cre fused to a mutant form of an estrogen receptor. In certain embodiments, the nucleic acid sequence encoding a gene product capable of editing the at least one conditionally inducible gene editing site may be operably linked to an inducible regulatory element. In certain embodiments, the nucleic acid sequence encoding a gene product capable of editing the at least one conditionally inducible gene editing site may be operably linked to a tetracycline or a doxycycline responsive regulatory element. In certain embodiments, the nucleic acid sequence encoding a gene product capable of

editing the at least one conditionally inducible gene editing site may be regulated by the addition or removal of tetracycline and/or doxycycline from the transgenic animal or animal model or cell. In certain embodiments, the plurality of nucleic acid sequences encoding the gene products capable of editing the plurality of gene products comprises a gene product capable of editing each of the plurality of gene products, such as, 1 gene product, 2 gene products, 3 gene products, 4 gene products, 5 gene products, 6 gene products, 7 gene products, 8 gene products, 9 gene products or 10 or more gene products.

[00135] In certain embodiments, the editing of the at least one conditionally inducible gene editing site decreases expression of a gene regulating the plasmalogen biosynthetic pathway. In certain embodiments, the editing of one or more conditionally inducible gene editing sites decreases expression of the gene regulating the plasmalogen biosynthetic pathway. As used herein, a decrease in expression may refer to any reduction in expression of a gene regulating the plasmalogen biosynthetic pathway in comparison to a reference animal, value, tissue, sample or cell. In certain embodiments, the decrease in expression of a gene regulating the plasmalogen biosynthetic pathway may be a decrease in said gene, such that a symptom of a condition or disease is observed in the transgenic animal or animal model or cell when monitored. In certain embodiments, the decrease in expression of a gene regulating the plasmalogen biosynthetic pathway may not induce a symptom or condition in the transgenic animal or animal model or cell. In certain embodiments, the decrease in expression of a gene regulating the plasmalogen biosynthetic pathway is a decrease such that a symptom or condition is induced in the transgenic animal or animal model. In certain embodiments, the decrease in expression of a gene regulating the plasmalogen biosynthetic pathway may be a 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 1.1%, 1.2%, 1.3%, 1.4%, 1.5%, 1.6%, 1.7%, 1.8%, 1.9%, 2%, 2.1%, 2.2%, 2.3%, 2.4%, 2.5%, 2.6%, 2.7%, 2.8%, 2.9%, 3%, 3.1%, 3.2%, 3.3%, 3.4%, 3.5%, 3.6%, 3.7%, 3.8%, 3.9%, 4%, 4.1%, 4.2%, 4.3%, 4.4%, 4.5%, 4.6%, 4.7%, 4.8%, 4.9%, 5%, 5.1%, 5.2%, 5.3%, 5.4%, 5.5%, 5.6%, 5.7%, 5.8%, 5.9%, 6%, 6.1%, 6.2%, 6.3%, 6.4%, 6.5%, 6.6%, 6.7%, 6.8%, 6.9%, 7%, 7.1%, 7.2%, 7.3%, 7.4%, 7.5%, 7.6%, 7.7%, 7.8%, 7.9%, 8%, 8.1%, 8.2%, 8.3%, 8.4%, 8.5%, 8.6%, 8.7%, 8.8%, 8.9%, 9%, 9.1%, 9.2%, 9.3%, 9.4%, 9.5%, 9.6%, 9.7%, 9.8%, 9.9%, 10%, 10.1%, 10.2%, 10.3%, 10.4%, 10.5%, 10.6%, 10.7%, 10.8%, 10.9%, 11%, 11.1%, 11.2%, 11.3%, 11.4%, 11.5%, 11.6%, 11.7%, 11.8%, 11.9%, 12%, 12.1%, 12.2%, 12.3%, 12.4%, 12.5%, 12.6%, 12.7%, 12.8%, 12.9%, 13%, 13.1%, 13.2%, 13.3%, 13.4%, 13.5%, 13.6%, 13.7%, 13.8%, 13.9%, 14%,

14.1%, 14.2%, 14.3%, 14.4%, 14.5%, 14.6%, 14.7%, 14.8%, 14.9%, 15%, 15.1%, 15.2%, 15.3%, 15.4%, 15.5%, 15.6%, 15.7%, 15.8%, 15.9%, 16%, 16.1%, 16.2%, 16.3%, 16.4%, 16.5%, 16.6%, 16.7%, 16.8%, 16.9%, 17%, 17.1%, 17.2%, 17.3%, 17.4%, 17.5%, 17.6%, 17.7%, 17.8%, 17.9%, 18%, 18.1%, 18.2%, 18.3%, 18.4%, 18.5%, 18.6%, 18.7%, 18.8%, 18.9%, 19%, 19.1%, 19.2%, 19.3%, 19.4%, 19.5%, 19.6%, 19.7%, 19.8%, 19.9%, 20%, 20.1%, 20.2%, 20.3%, 20.4%, 20.5%, 20.6%, 20.7%, 20.8%, 20.9%, 21%, 21.1%, 21.2%, 21.3%, 21.4%, 21.5%, 21.6%, 21.7%, 21.8%, 21.9%, 22%, 22.1%, 22.2%, 22.3%, 22.4%, 22.5%, 22.6%, 22.7%, 22.8%, 22.9%, 23%, 23.1%, 23.2%, 23.3%, 23.4%, 23.5%, 23.6%, 23.7%, 23.8%, 23.9%, 24%, 24.1%, 24.2%, 24.3%, 24.4%, 24.5%, 24.6%, 24.7%, 24.8%, 24.9%, 25%, 25.1%, 25.2%, 25.3%, 25.4%, 25.5%, 25.6%, 25.7%, 25.8%, 25.9%, 26%, 26.1%, 26.2%, 26.3%, 26.4%, 26.5%, 26.6%, 26.7%, 26.8%, 26.9%, 27%, 27.1%, 27.2%, 27.3%, 27.4%, 27.5%, 27.6%, 27.7%, 27.8%, 27.9%, 28%, 28.1%, 28.2%, 28.3%, 28.4%, 28.5%, 28.6%, 28.7%, 28.8%, 28.9%, 29%, 29.1%, 29.2%, 29.3%, 29.4%, 29.5%, 29.6%, 29.7%, 29.8%, 29.9%, 30%, 30.1%, 30.2%, 30.3%, 30.4%, 30.5%, 30.6%, 30.7%, 30.8%, 30.9%, 31%, 31.1%, 31.2%, 31.3%, 31.4%, 31.5%, 31.6%, 31.7%, 31.8%, 31.9%, 32%, 32.1%, 32.2%, 32.3%, 32.4%, 32.5%, 32.6%, 32.7%, 32.8%, 32.9%, 33%, 33.1%, 33.2%, 33.3%, 33.4%, 33.5%, 33.6%, 33.7%, 33.8%, 33.9%, 34%, 34.1%, 34.2%, 34.3%, 34.4%, 34.5%, 34.6%, 34.7%, 34.8%, 34.9%, 35%, 35.1%, 35.2%, 35.3%, 35.4%, 35.5%, 35.6%, 35.7%, 35.8%, 35.9%, 36%, 36.1%, 36.2%, 36.3%, 36.4%, 36.5%, 36.6%, 36.7%, 36.8%, 36.9%, 37%, 37.1%, 37.2%, 37.3%, 37.4%, 37.5%, 37.6%, 37.7%, 37.8%, 37.9%, 38%, 38.1%, 38.2%, 38.3%, 38.4%, 38.5%, 38.6%, 38.7%, 38.8%, 38.9%, 39%, 39.1%, 39.2%, 39.3%, 39.4%, 39.5%, 39.6%, 39.7%, 39.8%, 39.9%, 40%, 40.1%, 40.2%, 40.3%, 40.4%, 40.5%, 40.6%, 40.7%, 40.8%, 40.9%, 41%, 41.1%, 41.2%, 41.3%, 41.4%, 41.5%, 41.6%, 41.7%, 41.8%, 41.9%, 42%, 42.1%, 42.2%, 42.3%, 42.4%, 42.5%, 42.6%, 42.7%, 42.8%, 42.9%, 43%, 43.1%, 43.2%, 43.3%, 43.4%, 43.5%, 43.6%, 43.7%, 43.8%, 43.9%, 44%, 44.1%, 44.2%, 44.3%, 44.4%, 44.5%, 44.6%, 44.7%, 44.8%, 44.9%, 45%, 45.1%, 45.2%, 45.3%, 45.4%, 45.5%, 45.6%, 45.7%, 45.8%, 45.9%, 46%, 46.1%, 46.2%, 46.3%, 46.4%, 46.5%, 46.6%, 46.7%, 46.8%, 46.9%, 47%, 47.1%, 47.2%, 47.3%, 47.4%, 47.5%, 47.6%, 47.7%, 47.8%, 47.9%, 48%, 48.1%, 48.2%, 48.3%, 48.4%, 48.5%, 48.6%, 48.7%, 48.8%, 48.9%, 49%, 49.1%, 49.2%, 49.3%, 49.4%, 49.5%, 49.6%, 49.7%, 49.8%, 49.9%, 50%, 50.1%, 50.2%, 50.3%, 50.4%, 50.5%, 50.6%, 50.7%, 50.8%, 50.9%, 51%, 51.1%, 51.2%, 51.3%, 51.4%, 51.5%, 51.6%, 51.7%, 51.8%, 51.9%, 52%, 52.1%, 52.2%, 52.3%, 52.4%, 52.5%, 52.6%, 52.7%, 52.8%, 52.9%, 53%, 53.1%, 53.2%, 53.3%, 53.4%, 53.5%, 53.6%, 53.7%, 53.8%, 53.9%, 54%, 54.1%, 54.2%, 54.3%,

54.4%, 54.5%, 54.6%, 54.7%, 54.8%, 54.9%, 55%, 55.1%, 55.2%, 55.3%, 55.4%, 55.5%, 55.6%,
55.7%, 55.8%, 55.9%, 56%, 56.1%, 56.2%, 56.3%, 56.4%, 56.5%, 56.6%, 56.7%, 56.8%, 56.9%,
57%, 57.1%, 57.2%, 57.3%, 57.4%, 57.5%, 57.6%, 57.7%, 57.8%, 57.9%, 58%, 58.1%, 58.2%,
58.3%, 58.4%, 58.5%, 58.6%, 58.7%, 58.8%, 58.9%, 59%, 59.1%, 59.2%, 59.3%, 59.4%, 59.5%,
59.6%, 59.7%, 59.8%, 59.9%, 60%, 60.1%, 60.2%, 60.3%, 60.4%, 60.5%, 60.6%, 60.7%, 60.8%,
60.9%, 61%, 61.1%, 61.2%, 61.3%, 61.4%, 61.5%, 61.6%, 61.7%, 61.8%, 61.9%, 62%, 62.1%,
62.2%, 62.3%, 62.4%, 62.5%, 62.6%, 62.7%, 62.8%, 62.9%, 63%, 63.1%, 63.2%, 63.3%, 63.4%,
63.5%, 63.6%, 63.7%, 63.8%, 63.9%, 64%, 64.1%, 64.2%, 64.3%, 64.4%, 64.5%, 64.6%, 64.7%,
64.8%, 64.9%, 65%, 65.1%, 65.2%, 65.3%, 65.4%, 65.5%, 65.6%, 65.7%, 65.8%, 65.9%, 66%,
66.1%, 66.2%, 66.3%, 66.4%, 66.5%, 66.6%, 66.7%, 66.8%, 66.9%, 67%, 67.1%, 67.2%, 67.3%,
67.4%, 67.5%, 67.6%, 67.7%, 67.8%, 67.9%, 68%, 68.1%, 68.2%, 68.3%, 68.4%, 68.5%, 68.6%,
68.7%, 68.8%, 68.9%, 69%, 69.1%, 69.2%, 69.3%, 69.4%, 69.5%, 69.6%, 69.7%, 69.8%, 69.9%,
70%, 70.1%, 70.2%, 70.3%, 70.4%, 70.5%, 70.6%, 70.7%, 70.8%, 70.9%, 71%, 71.1%, 71.2%,
71.3%, 71.4%, 71.5%, 71.6%, 71.7%, 71.8%, 71.9%, 72%, 72.1%, 72.2%, 72.3%, 72.4%, 72.5%,
72.6%, 72.7%, 72.8%, 72.9%, 73%, 73.1%, 73.2%, 73.3%, 73.4%, 73.5%, 73.6%, 73.7%, 73.8%,
73.9%, 74%, 74.1%, 74.2%, 74.3%, 74.4%, 74.5%, 74.6%, 74.7%, 74.8%, 74.9%, 75%, 75.1%,
75.2%, 75.3%, 75.4%, 75.5%, 75.6%, 75.7%, 75.8%, 75.9%, 76%, 76.1%, 76.2%, 76.3%, 76.4%,
76.5%, 76.6%, 76.7%, 76.8%, 76.9%, 77%, 77.1%, 77.2%, 77.3%, 77.4%, 77.5%, 77.6%, 77.7%,
77.8%, 77.9%, 78%, 78.1%, 78.2%, 78.3%, 78.4%, 78.5%, 78.6%, 78.7%, 78.8%, 78.9%, 79%,
79.1%, 79.2%, 79.3%, 79.4%, 79.5%, 79.6%, 79.7%, 79.8%, 79.9%, 80%, 80.1%, 80.2%, 80.3%,
80.4%, 80.5%, 80.6%, 80.7%, 80.8%, 80.9%, 81%, 81.1%, 81.2%, 81.3%, 81.4%, 81.5%, 81.6%,
81.7%, 81.8%, 81.9%, 82%, 82.1%, 82.2%, 82.3%, 82.4%, 82.5%, 82.6%, 82.7%, 82.8%, 82.9%,
83%, 83.1%, 83.2%, 83.3%, 83.4%, 83.5%, 83.6%, 83.7%, 83.8%, 83.9%, 84%, 84.1%, 84.2%,
84.3%, 84.4%, 84.5%, 84.6%, 84.7%, 84.8%, 84.9%, 85%, 85.1%, 85.2%, 85.3%, 85.4%, 85.5%,
85.6%, 85.7%, 85.8%, 85.9%, 86%, 86.1%, 86.2%, 86.3%, 86.4%, 86.5%, 86.6%, 86.7%, 86.8%,
86.9%, 87%, 87.1%, 87.2%, 87.3%, 87.4%, 87.5%, 87.6%, 87.7%, 87.8%, 87.9%, 88%, 88.1%,
88.2%, 88.3%, 88.4%, 88.5%, 88.6%, 88.7%, 88.8%, 88.9%, 89%, 89.1%, 89.2%, 89.3%, 89.4%,
89.5%, 89.6%, 89.7%, 89.8%, 89.9%, 90%, 90.1%, 90.2%, 90.3%, 90.4%, 90.5%, 90.6%, 90.7%,
90.8%, 90.9%, 91%, 91.1%, 91.2%, 91.3%, 91.4%, 91.5%, 91.6%, 91.7%, 91.8%, 91.9%, 92%,
92.1%, 92.2%, 92.3%, 92.4%, 92.5%, 92.6%, 92.7%, 92.8%, 92.9%, 93%, 93.1%, 93.2%, 93.3%,
93.4%, 93.5%, 93.6%, 93.7%, 93.8%, 93.9%, 94%, 94.1%, 94.2%, 94.3%, 94.4%, 94.5%, 94.6%,

94.7%, 94.8%, 94.9%, 95%, 95.1%, 95.2%, 95.3%, 95.4%, 95.5%, 95.6%, 95.7%, 95.8%, 95.9%, 96%, 96.1%, 96.2%, 96.3%, 96.4%, 96.5%, 96.6%, 96.7%, 96.8%, 96.9%, 97%, 97.1%, 97.2%, 97.3%, 97.4%, 97.5%, 97.6%, 97.7%, 97.8%, 97.9%, 98%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9%, or 100% decrease in expression in comparison with a reference. In certain embodiments, a reference may comprise a transgenic animal or animal model that does not comprise one or more of the conditionally inducible gene editing site or the at least one nucleic acid editing sequence, or the transgenic animal or animal model may not be treated with an exogenous compound or exogenous stimulus or both.

[00136] Non-limiting examples of conditions or diseases that may be associated with a reduced plasmalogen level are neurodegenerative disease, presence of cataracts, respiratory disease, chronic inflammation, impaired myelination, metabolic syndrome, type II diabetes, or cardiovascular disease. Non-limiting examples of neurodegenerative diseases that may be associated with a reduced plasmalogen level may comprise Alzheimer's disease, Parkinson's disease, or Multiple sclerosis. Non-limiting examples of respiratory diseases that may be associated with a reduced plasmalogen level may comprise bronchopulmonary dysplasia (BPD) or chronic obstructive pulmonary disease (COPD).

[00137] In certain embodiments, the transgenic animal or animal model and/or cell may be monitored or undergo monitoring for symptoms of a condition or disease. In certain embodiments, the condition or disease may be one or more associated with a reduced plasmalogen level. In certain embodiments, the symptoms to be monitored or for monitoring may comprise one or more symptom accompanying any condition or disease that may be associated with a reduced plasmalogen. Non-limiting examples of symptoms linked or associated with a decreased expression of a gene regulating the plasmalogen biosynthetic pathway may comprise: animal growth, joint development, joint movement, joint pain, facial development, cataracts, cardiac function, respiratory function, inflammation, liver size, liver function, cognitive impairment, neurological function, seizures, memory impairment, blood sugar level, neuronal cell loss, dopaminergic cell loss, cholinergic cell loss, muscle output, gait, bladder function, bowel function, coordination, vision, blood pressure, cholesterol level, insulin resistance, weight, and/or blood triglyceride level.

[00138] In certain embodiments, the expression of the gene product may be induced by providing the animal or animal model with an exogenous compound or an exogenous stimulus. The skilled person, in light of the teachings herein, would be able to select an appropriate method for inducing the gene product. In certain embodiments, the gene product or gene products may be induced by one or more of the following: tamoxifen, 4-hydroxytamoxifen (4-OHT), mifepristone, tetracycline, light, temperature or any combination thereof.

[00139] In certain embodiments of a method described herein, the step of providing at least one non-human animal or animal model may comprise providing an animal, such as a mammal, bird, reptile, amphibian, fish, arthropod, vertebrate, or invertebrate, wherein said non-human animal or animal model is for genome modification.

[00140] In certain embodiments of a method described herein, the step of introducing at least one gene editing sequence in a gene, part or regulatory element thereof, involved in the biosynthesis of plasmalogen to generate a first edited gene in the non-human animal or animal model may comprise any method or techniques known to the skilled person for transforming a genome of a non-human animal or animal model.

[00141] In certain embodiments of a method described herein, the step of crossing the non-human animal or animal model with the first edited gene with a second non-human animal or animal model comprising a nucleic acid editing sequence at a separate locus encoding a gene product, to generate the transgenic animal or animal model may comprise any method or technique known to the skilled person, in light of the teachings herein, required for the crossing (or mating) of organisms of said species.

[00142] *Nucleic acid*

[00143] In certain embodiments, there is provided herein an isolated nucleic acid comprising the sequence GTGAGCTCCCCGGCCTCTC (SEQ ID NO:1) and/or ATGGCAGACAGGGGCCCTTC (SEQ ID NO:2). In certain embodiment's, the isolated nucleic acid may comprise one or more additional elements operably linked to a sequence of GTGAGCTCCCCGGCCTCTC (SEQ ID NO:1) and/or ATGGCAGACAGGGGCCCTTC (SEQ ID NO:2). In certain embodiments, the isolated nucleic acid may further comprise one or both of a transcription termination sequence and a promoter operably linked to a sequence of

GTGAGCTCCCCGGCCTCTC (SEQ ID NO:1) and/or ATGGCAGACAGGGGCCCTTC (SEQ ID NO:2).

[00144] In certain embodiments, there is provided herein a recombinant DNA construct comprising a promoter operably linked to a sequence encoding a single guide RNA (sgRNA), wherein the sequence of the sgRNA is GTGAGCTCCCCGGCCTCTC (SEQ ID NO:1) and/or ATGGCAGACAGGGGCCCTTC (SEQ ID NO:2). In certain embodiments, the recombinant DNA construct may further comprise a transcription termination sequence.

[00145] As used herein, a nucleic acid may be “operably linked” when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, “operably linked” means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites or through other methods or techniques known to the skilled person, such as for example, a multi-enzyme assembly.

[00146] In certain embodiments, there is provided herein compositions comprising an isolated nucleic acid or isolated nucleic acids as described herein, or a recombinant DNA construct or constructs as described herein. In certain embodiments, the composition comprise at isolated nucleic acids or recombinant DNA constructs comprising sequences of GTGAGCTCCCCGGCCTCTC (SEQ ID NO:1) and/or ATGGCAGACAGGGGCCCTTC (SEQ ID NO:2). In certain embodiments, the composition may further comprise a Cas9 protein or variant thereof, or a recombinant nucleic acid encoding the Cas9 protein or variant thereof.

[00147] Therefore, the present disclosure is well adapted to attain the ends and advantages mentioned as well as those that are inherent therein. The particular embodiments disclosed above are illustrative only, as the present disclosure may be modified and practiced in different but equivalent manners apparent to those skilled in the art having the benefit of the teachings herein. Although individual embodiments are discussed, the disclosure covers all combinations of all those embodiments. Furthermore, no limitations are intended to the details of construction or design

herein shown, other than as described in the claims below. Also, the terms in the claims have their plain, ordinary meaning unless otherwise explicitly and clearly defined by the patentee. It is therefore evident that the particular illustrative embodiments disclosed above may be altered or modified and all such variations are considered within the scope and spirit of the present disclosure. If there is any conflict in the usages of a word or term in this specification and one or more patent(s) or other documents that may be referenced herein, the definitions that are consistent with this specification should be adopted.

[00148] Many obvious variations of the embodiments set out herein will suggest themselves to those skilled in the art in light of the present disclosure. Such obvious variations are within the full intended scope of the appended claims.

[00149] EXEMPLARY EMBODIMENT AND EXPERIMENTAL DATA

[00150] Current animal models for plasmalogen deficiency are based on germline mutations and display numerous developmental defects resulting in brain abnormalities and impaired function, consequently leading to early death and thereby precluding their use in studying the role of plasmalogen in adult-onset conditions associated with plasmalogen deficiencies. The inventors therefore sought to develop novel animal models in which a plasmalogen deficiency could be initiated only postnatally, rather than occurring during development. Preferably, triggering a plasmalogen deficiency could be temporally controlled in the animals so as to allow the identification of any age-related onset of conditions associated with plasmalogen deficiency, as well as characterization of the disease process resulting from the deficiency.

[00151] Described herein are examples of methods suitable for inducing a condition associated with reduced postnatal levels of plasmalogen in a non-human animal or animal model. Transgenic non-human animals in which plasmalogen deficiency can be conditionally, temporally or regionally induced are also described herein. It will be appreciated that embodiments and examples are provided for illustrative purposes intended for those skilled in the art and are not meant to be limiting in any way.

[00152] A novel method of inducing a condition associated with reduced levels of plasmalogens in a non-human animal or animal model will be described. A novel method of

reducing plasmalogen levels in a non-human animal or animal model will be described concurrently. A first non-human animal line in which a conditionally inducible gene editing site (e.g. loxP sites) have been introduced in a gene involved in the biosynthesis of plasmalogens is first generated. The conditionally inducible gene editing sites may be introduced anywhere in the gene (i.e. the floxed gene) in order to flank a region of the gene which is essential for generating a full length and fully functional protein. The resulting transgenic animal or animal model should show significant reduction in plasmalogen levels after the flanked region has been excised by recombinase. Various exons or combinations of introns and exons may be targeted in a gene or combination of genes, or parts thereof, involved in the biosynthesis of plasmalogens in order to generate transgenic animals showing significant systemic or tissue specific reductions (depending on the non-human animal model) in plasmalogen levels after the flanked region(s) has been excised by recombinase.

[00153] The method then entails generating an inducible conditional knock out non-human animal line by crossing the first non-human animal line with a second non-human animal line which carries the gene for the recombinase, which is linked to the genetic code for a ligand binding domain of a receptor that can be used to control the translocation of the recombinase enzyme into the nucleus. Any suitable means of inducing recombinase translocation into the nucleus is contemplated. For example, the second non-human animal line may comprise a *CreERT2* gene encoding for a fusion protein of Cre recombinase and mutant estrogen ligand-binding domain (ERT2) such that inducing Cre-mediated recombination of the gene is accomplished by administering tamoxifen to the inducible conditional knock out non-human animals. Alternatively, the second non-human animal line in which Cre-mediated recombination is inducible may comprise a Cre recombinase gene under the control of a tetracycline-responsive element (TRE) such that inducing Cre-mediated recombination of the targeted gene or genes is accomplished by administering tetracycline to the inducible conditional knock out non-human animals. Purified Cre recombinase or nucleic acids encoding for Cre recombinase may also be administered to the inducible conditional knock out non-human animal or animal models to induce Cre-mediated recombination of the targeted gene(s). Accordingly, the method entails inducing Cre-mediated recombination of the targeted gene(s) involved in the biosynthesis of plasmalogen in the inducible conditional knock out non-human animal or animal model, to obtain a conditional

knock out non-human animal or animal model in which plasmalogen levels are reduced and which exhibits symptoms of a condition associated with reduced levels of plasmalogens.

[00154] According to an embodiment, the gene involved in the biosynthesis of plasmalogen is fatty acid reductase 1 (*FARI*), glyceronephosphate O-acyltransferase (*GNPAT*), alkylglycerone phosphate synthase (*AGPS*), acyl/alkyl-DHAP reductase, alkyl/acyl-GPA acyltransferase, phosphatidic acid phosphatase, ethanolamine phosphotransferase, plasmenylethanolamine desaturase or choline phosphotransferase, *PEX7* and *PEX5*.

[00155] According to an embodiment, plasmalogen levels are reduced significantly, by at least 20% - 50%, and in some instances even higher reductions can be seen in the conditional knock out non-human animal or animal model compared to levels in an equivalent wild-type non-human animal.

[00156] According to an embodiment, the conditionally inducible gene editing site e.g. loxP sites have been introduced in a region flanking exon four of the *GNPAT* gene.

[00157] According to an embodiment, inducing Cre-mediated recombination of the *targeted* gene(s) in the inducible conditional knock out non-human animal line may be accomplished by administering about 50 mg of tamoxifen per kg of bodyweight for about 7 consecutive days in an inducible conditional knock out non-human animal comprising the floxed gene and the *CreERT2* gene. Other variations in the administration protocols and means of inducing Cre-mediated recombination of the targeted gene(s) are contemplated and remain within the teachings of the present invention.

[00158] According to an embodiment, the *GNPAT* conditional knock out in non-human animals exhibits symptoms of a condition associated with reduced levels of plasmalogens starting from about one month after inducing Cre-mediated recombination.

[00159] According to an embodiment, the condition associated with reduced levels of plasmalogens is a neurodegenerative disease, cataracts, respiratory disease, impaired myelination, chronic inflammation, metabolic syndrome, type II diabetes, or cardiovascular disease.

[00160] According to an embodiment, the neurodegenerative disease is Alzheimer's disease, Parkinson's disease, or Multiple sclerosis.

[00161] According to an embodiment, the respiratory disease is bronchopulmonary dysplasia (BPD) or chronic obstructive pulmonary disease (COPD).

[00162] Another aspect of the invention is a transgenic non-human animal or animal model whose genome comprises a gene involved in the biosynthesis of plasmalogens in which the conditionally inducible gene editing sites i.e. loxP sites have been introduced. The transgenic non-human animal or animal model further comprises a *CreERT2* gene encoding for a fusion protein of Cre recombinase and a mutant estrogen ligand-binding domain (ERT2) such that Cre-mediated recombination is accomplished by the CreERT2 protein following tamoxifen administration.

[00163] According to an embodiment, the genome of the transgenic non-human animal or animal model comprises loxP sites introduced in exon 4 of the glyceronephosphate O-acyltransferase (*GNPAT*) gene.

[00164] Another aspect of the invention is a transgenic non-human animal or animal model whose genome comprises a gene involved in the biosynthesis of plasmalogen in which loxP sites have been introduced. The transgenic non-human animal further comprises a Cre recombinase gene under the control of a tetracycline-responsive element such that Cre-mediated recombination is induced by the Cre recombinase protein following tetracycline administration. In some aspects of the invention, a tissue-specific induction of the Cre-recombination protein may also be possible.

[00165] According to an embodiment, the genome of the transgenic non-human animal or animal model may further comprise a ubiquitously expressed Cre recombinase such that the transgenic non-human animal is a full knock out of the targeted gene.

[00166] Another aspect of the present invention entails a method of determining the efficacy of a compound or pharmaceutical composition in treating a condition associated with reduced levels of plasmalogens in a non-human animal or animal model. The method involves administering the compound or pharmaceutical composition to be tested to a knock out non-human animal or animal model, in which the knockout of the enzyme has, or will be induced, and observing its impact on plasmalogen levels and the clinical condition. In an optional aspect of the

invention, the method may involve administering the compound or pharmaceutical composition to be tested to a knock out non-human animal or animal model, in which the knockout of the enzyme has, or will be induced, and administering a placebo to another knock out non-human animal or animal model. The impact on plasmalogen levels and the clinical condition in both animals are then compared to determine the efficacy of the compound or pharmaceutical composition.

[00167] In another aspect of the present invention, the method may be used to test the ability of a given plasmalogen-augmenting compound or pharmaceutical composition in increasing plasmalogen levels and/or in mitigating any clinical, behavioral and/or secondary biochemical or physiological effects of the plasmalogen deficiency..

Examples

[00168]

Example 1 - Homozygous *Gnpat* cKO Hemi CAGGCRE-ER mice

[00169] Mice were housed at Jackson Laboratories (Bar Harbor, ME) with 3-4 mice per cage in positively ventilated polysulfonate cages with HEPA-filtered air. The animal room was kept on a 12 h light/dark cycle (6:00 am to 6:00 pm light) using artificial fluorescent lighting. Room temperature was maintained at $22 \pm 4^{\circ}\text{C}$ and the relative humidity was $50 \pm 15\%$. The animal rooms had 15 air exchanges every hour and the animals were provided filtered tap water at a pH of 2.5 to 3.0 and normal rodent chow *ad libitum*. This work complied with the National Research Council's Guide for the Care and Use of Laboratory Animals. All animal handling was performed under approval from The Institutional Animal Care and Use Committee (IACUC) and Institutional Biosafety Committee (IBC) under Animal Use Summary (AUS) #: 21013. Veterinary care was available throughout the course of the study and animals were examined by veterinary staff when warranted by clinical signs and other changes.

*Generation of the Homozygous *Gnpat* cKO Hemi CAGGCRE-ER mouse line*

[00170] *Gnpat*^{flox/flox} mice were generated through the introduction of loxP sites using dsDNA in the regions flanking exon four of the *Gnpat* gene [Chr8:124863033-124890057 base pair (bp), positive strand] by microinjecting CRISPR RNA target guides into the embryo at the same time. Donor plasmid with homology arms of 2 kb and 1.6 kb flanking the loxP sites were used for homology driven repair. The upstream CRISPR target guide was: *Gnpat_in3_crRNA1*:

GTGAGCTCCCCGGCCTCTC (SEQ ID NO:1) while the downstream CRISPR target guide was: Gnpat_in4_crRNA1: ATGGCAGACAGGGGCCCTTC (SEQ ID NO:2). Six founder lines were produced however, only one contained the correct loxP sites without any additional deletions detected, and therefore this line was chosen to generate the *Gnpat*^{fl_{ox}/fl_{ox}} colony. Genotypes were confirmed using the following primers: Gnpat_genof2: TGCCAGCTTTCTACTTGCCA (SEQ ID NO:18 and Gnpat_genor2: GCAGTGACCTGACTGAGACC (SEQ ID NO:19).

[00171] *Heterozygous Gnpat cKO (Gnpat*^{fl_{ox}/WT}*) mice were first backcrossed to C57BL/6J (stock#00664) mice to produce another generation of Heterozygous Gnpat cKO (Gnpat*^{fl_{ox}/WT}*) mice, which were then crossed to produce Homozygous Gnpat cKO (Gnpat*^{fl_{ox}/fl_{ox}}*) animals. Heterozygous Gnpat cKO (Gnpat*^{fl_{ox}/WT}*) mice were also crossed with Hemizygous CAGGCre-ER*TM *mice*²⁵ *(C57BL/6J background; Jackson Laboratories Stock#004682) to produce mice that carried the hemizygous CAGGCre-ER*TM *transgene and were heterozygous for the floxed Gnpat allele. These mice were crossed Homozygous Gnpat cKO (Gnpat*^{fl_{ox}/fl_{ox}}*) animals, resulting in animals that were Gnpat*^{fl_{ox}/fl_{ox}} *and hemizygous for the CAGGCre-ER*TM *transgene, representing the new TMX-inducible Gnpat conditional knock out (Homozygous Gnpat cKO Hemi CAGGCRE-ER, Figure 1).*

Tamoxifen administration

[00172] Tamoxifen powder was dissolved in 100% ethanol (Sigma E7023) to 50 mg/mL at 55°C and was diluted 1:10 to 5 mg/mL in corn oil and stored at -20°C until the day of dosing. Vehicle preparation was identical with the exception of the addition of TMX powder. On the day of dosing, solutions were thawed at 37°C. Mice between 16 and 17 weeks of age were administered 50 mg/kg of TMX (or vehicle) using the standardized dosage volume of 10 mL/kg of body weight once daily for 7 consecutive days. Technicians performing all experiments were blinded to animal genotype and treatment group.

Homozygous Gnpat cKO Hemi CAGGCRE-ER characterization study design

[00173] A comparison of *Homozygous Gnpat cKO Hemi CAGGCRE-ER* animals was performed one month following treatment with either TMX or vehicle (3 animals per gender per group). All animals were assessed by both the open field test (day 30) and nerve conduction assay (day 33), before serum and tissue were harvested upon euthanasia on day 35.

[00174] A second comparison was performed four months following treatment with either TMX (6 females/8 males) or vehicle (6 females/7 males). This time point also included a third group of age-matched untreated C57BL/6J mice (8 females/8 males) to act as a wild-type (WT) control. All animals were assessed by both the open field test (day 95) and nerve conduction assay (day 125), before serum and tissue was harvested upon euthanasia on day 130.

Terminal Tissue Collection

[00175] Terminal blood collection was performed by cardiac puncture following euthanasia by carbon dioxide narcosis. The blood was collected in a serum separator tube and allowed to separate for 30-40 minutes at room temperature prior to centrifugation at 10 000 rpm. Serum was stored at -80°C until analysis.

[00176] Mice were subsequently transcardially perfused with 10 mL phosphate buffered saline (PBS). The brain, liver, heart, skeletal muscle (quadriceps and tibialis anterior), lung, and intestine were removed and placed in a screw top cap, flash frozen on dry ice and then stored at -80°C until analysis.

Lipid Quantification by FI-MS/MS

[00177] Serum extraction was performed on a 20 µL aliquot in 1.4 mL Thermo matrix tubes. Lipids were extracted into HPLC-grade water (20 µL) and 600 µL ethyl acetate with 2% water and 1% formic acid containing labelled internal standard [⁹D PlsEtn 18:0/18:1] at 0.05 µg/mL. Samples were hand mixed by inversion followed by vortexing for 30 minutes at 1500 rpm using a Mixmate. Next, samples were centrifuged for 5 minutes at 2000 rcf.

[00178] Tissue samples were extracted by placing approximately 50 mg of each tissue into a 2 mL safe-lock Eppendorf tube with all weights recorded for normalization. Two scoops (roughly 300 µL) of 0.5 mm zirconium oxide beads and 600 µL ethyl acetate with 2% water with 1% formic acid was added to each tube and then samples were homogenized using the Bullet Blender Storm Pro (Next Advantage) for 10 minutes. Each sample was mixed for 1 hour on a Mixmate at 1500 rpm to ensure complete lipid extraction, then centrifuged for 10 minutes at 3500 rcf. Based on the tissue weights, an aliquot of the eluant for all samples was removed from the tissue pellet and diluted to 50 mg/mL. Samples were further diluted in a tissue-specific manner in ethyl acetate with

2% water and 1% formic acid containing labelled internal standard (^3D PlsEtn 18:0/18:1 at 0.05 $\mu\text{g/mL}$) to bring the lipid levels within range for linear quantification.

[00179] A 100 μL aliquot of each extract was analyzed by flow injection tandem mass spectrometry (FI-MS/MS) on an Applied Biosystems Q-Trap mass spectrometer coupled with an Agilent 1200 HPLC system. Each transition was scanned for 50 milliseconds with a total acquisition time per sample of 1 minute. The mobile phase was an ethyl acetate:methanol:water solution at 80:15:5 at a flow rate of 450 $\mu\text{L/min}$. The standards and stable isotopes used were >99% purity by TLC and manufactured by Avanti Polar Lipids, Inc, and solvents were HPLC grade. The measured transitions are reported in Table 1. The results were processed using Analyst software (version 1.5.1) as μg lipid per mg of starting tissue ($\mu\text{g/mg}$) or, in the case of serum, μg lipid per mL of serum ($\mu\text{g/mL}$).

Table 1 – FI-MS/MS Transitions

Analyte	Molecular Formula	MRM transition
PlsEtn 16:0/18:1	$\text{C}_{39}\text{H}_{76}\text{NO}_7\text{P}$	700.5/281.2
PlsEtn 16:0/18:2	$\text{C}_{39}\text{H}_{74}\text{NO}_7\text{P}$	698.5/279.2
PlsEtn 16:0/20:4	$\text{C}_{41}\text{H}_{74}\text{NO}_7\text{P}$	722.5/303.2
PlsEtn 16:0/22:6	$\text{C}_{43}\text{H}_{74}\text{NO}_7\text{P}$	746.5/327.2
PlsEtn 18:0/18:1	$\text{C}_{41}\text{H}_{80}\text{NO}_7\text{P}$	728.5/281.2
PlsEtn 18:0/18:2	$\text{C}_{41}\text{H}_{78}\text{NO}_7\text{P}$	726.5/279.2
PlsEtn 18:0/20:4	$\text{C}_{43}\text{H}_{78}\text{NO}_7\text{P}$	750.6/303.2
PlsEtn 18:0/20:5	$\text{C}_{43}\text{H}_{76}\text{NO}_7\text{P}$	748.5/301.3
PlsEtn 18:0/22:6	$\text{C}_{45}\text{H}_{78}\text{NO}_7\text{P}$	774.5/327.2

Lipid Alterations in tamoxifen-treated Homozygous Gnpat cKO Hemi CAGGCRE-ER mice

[00180] The TMX-inducible *Homozygous Gnpat cKO Hemi CAGGCRE-ER mice* were allowed to develop normally for 16-17 weeks after birth, at which time they were treated with TMX by IP injection. Serum and tissue samples were collected at one and four months post-TMX and analyzed for plasmalogen levels. At one month post-TMX administration, total plasmalogen levels were significantly reduced compared to vehicle-treated levels, with the plasmalogen content in TMX-treated animals ranging between 50-75% of vehicle-treated levels, depending on the tissue (Figure 2). Within the serum, TMX-treated animals displayed total plasmalogen levels approximately 65% of vehicle levels ($p=0.028$). The most drastic reductions were seen in the heart ($p<0.001$), intestine ($p=0.0029$), and liver ($p=0.0009$), where levels were reduced by approximately 50% compared to vehicle treatment.

[00181] At the plasmalogen species-specific level (Figure 3) the effect of the knock out tended to broadly affect most plasmalogens, however, there were some exceptions and tissue-specific patterns that emerged at only one month post-TMX. In the serum, there was high variability in the individual plasmalogen levels in the vehicle- and TMX-treated plasmalogens with 18:1 and 18:2 sidechains at *sn2*. Interestingly, serum plasmalogens with unsaturated *sn2* sidechains (20:4 and 22:6) showed the most significant reductions (Figure 3, $p<0.05$ versus corresponding plasmalogen species in vehicle-treated animals). Within the brain, heart, and liver, all eight plasmalogen species were significantly reduced ($p<0.05$) in the TMX group (Figure 3). Intestine showed significant reductions in the four 18:0 plasmalogen species, as well as the 16:0/22:6 species (Figure 3). High variability was observed in the vehicle-treated lung tissues, which, although a general reduction of all plasmalogens was evident, only the 16:0/18:2 and 18:0/18:2 species were statistically significant ($p<0.05$; Figure 3).

[00182] At four months post-TMX, total plasmalogen levels within the serum and tissues were again significantly reduced (Figure 4), with levels ranging between 45-80% of vehicle-treated levels. Of particular interest was the further reduction in total plasmalogen levels between one and four months in the brain, with levels at four months of only 45% that of vehicle-treated levels.

[00183] Individual plasmalogen species for each tissue are shown in Figure 5. Within the serum, the majority of plasmalogen species are significantly reduced ($p<0.05$) with the exception

of 16:0/18:1 and 16:0/18:2. Brain and lung tissue displayed significant reductions in all eight plasmalogen species within the TMX-treated animals ($p < 0.05$). Intestine showed significant reductions in all plasmalogen species except 16:0/18:1 and 16:0/18:2, which were also the species which showed the largest variation in the vehicle-treated animals. The heart and liver showed similar plasmalogen species patterns, with both tissues having significant reductions in the 16:0/18:1, 16:0/20:4, 18:0/18:1, 18:0/20:4 and 18:0/22:6 species ($p < 0.05$; Figure 5).

[00184] As serum plasmalogen levels are commonly used as a surrogate for levels elsewhere in the body, particularly in the brain, plasmalogen levels between brain and serum were compared (Figure 6). All plasmalogens measured in the serum were significantly correlated with brain ($p < 0.0001$, F-stats between 20.6 and 51.8) for all plasmalogen species. Although there is variability evident among the animals, the results indicated that when a significant plasmalogen reduction occurs in the brain, it will translate into reduced serum levels.

[00185] Taken together, although the novel *Homozygous Gnpat cKO Hemi CAGGCRE-ER* animals were phenotypically indistinguishable following TMX treatment from either wild-type or vehicle-treated animals, reduced plasmalogen levels were detectable in the serum and tissues including the brain within one month of TMX treatment. Further reductions in the brain were particularly evident by four months post-TMX treatment. Overall, plasmalogen levels in *Homozygous Gnpat cKO Hemi CAGGCRE-ER* animals ranged between 45-75% of vehicle levels after 4 months, which is more analogous to what would be expected in individuals with neurodegenerative diseases of aging. As an example individuals with AD show that levels are in the range of a 50-90% of the levels reported in healthy controls^{11,26-29}, with greater reductions seen in individuals with more severe phenotypes and faster rate of progression^{26,30}. Similar levels of deficiency have also been reported in patients with Parkinson's disease^{12,31,32}. Therefore, the *Homozygous Gnpat cKO Hemi CAGGCRE-ER* animal model appears to be the first of its kind that is capable of mirroring the progressive plasmalogen decline observed in human neurodegenerative diseases. The usability of this model is increased by the strong correlation observed between serum and brain plasmalogen levels, which will allow future studies to monitor the status of PlsEtn levels within the living animal, as serum samples can easily be taken longitudinally.

Open Field

[00186] To evaluate the behavioral consequences of plasmalogen deficiency in adulthood, the adult animals were tested in the open field test at one and four month's post-TMX treatment. Hyperactivity in the open field is a phenomenon that has previously been reported in a variety of neurodegenerative mouse models³³⁻³⁵.

[00187] On the day of testing mice were placed in a square area (40 cm x 40 cm x 40 cm) made with clear Plexiglass and illuminated at $100,500 \pm 20$ lux. Animals were allowed to move freely for 10 minutes while activity was recorded using a sensitive infrared (IR) photobeam three-dimensional grid system that was invisible to the mice. Fusion software (Omnitech Electronics Inc) was used to determine the distance traveled in centimeters within each of the ten minutes of the trial.

[00188] At one month, animals trended towards increased distance travelled over the course of the test, however neither the individual minute distances (Figure 7A) nor the total distance travelled (Figure 7B) reached significance. By four months, the distance travelled in TMX-treated animals was significantly increased compared to wild-type and vehicle-treated animals across all minute intervals of the test as well as total distance travelled (Figures 7C and D). Indeed, by four months post-treatment, TMX-treated animals displayed significant hyperactivity by travelling approximately twice the distance as the vehicle-treated or wild-type animals within the ten minute test.

[00189] Since it is likely these behavioral changes are central nervous system (CNS)-mediated, the correlation between brain plasmalogens levels and total distance travelled was investigated (Figure 8). A robust and significant inverse correlation was observed between the levels of each PlsEtn measured and distance travelled ($p < 0.0001$, F-stats between 17.9-51.0).

[00190] A strong inverse correlation between the distance travelled and plasmalogen levels in the brain suggests that the reduced plasmalogens in the CNS is not only a contributing factor to the behavior, but that the observed effects are actually dose-dependent. That is, the lower the plasmalogen level, the more severe the behavioral phenotype. Furthermore, the most robust associations were with those plasmalogens containing polyunsaturated fatty acids at the *sn2*

position. Thus, these findings suggest that four months of progressive plasmalogen deficiency is sufficient to induce changes in the CNS that translate into behavioral deficits.

[00191] Interestingly, the magnitude of hyperactivity observed in the *Homozygous Gnpat cKO Hemi CAGGCRE-ER* animals after four months post-TMX is comparable to that seen within germline knock out animals^{4,36}. This suggests that the behavioral effects are related to the plasmalogen deficiency, and not due to developmental abnormalities. Further, the results demonstrate that severe plasmalogen reductions are not required to see significant behavioral changes. Germline knock out animals display negligible brain plasmalogen levels, but display similar behavioral changes as our cKO which shows only a 50% reduction in levels.

[00192] Historically, there have been a few different explanations presented for the hyperactivity phenotype in animal models. Impairments in both neurotransmitter levels and neurotransmitter release have been reported and suggested to underlie the increase in activity^{4,34}. Damage or neuronal death within the hippocampus as also been suggested to be the cause. Faizi *et al* suggested in their animal model, damage to the hippocampus prevented the mice from being able to generate a contextual map of the open field, resulting in the continuous exploration of the area³⁵. Considering the roles that plasmalogens have been shown to play within neuronal tissues, together with the strong correlation between plasmalogen species with a polyunsaturated fatty acid at *sn2*, it is reasonable to hypothesize that impaired neurotransmission could be causing the increased activity. There are two main phenomenon that would be expected to be impaired within a plasmalogen-deficient neuron. First, a change in membrane fluidity caused by reduced plasmalogen content would impact the membranes ability to form the inverse-hexagonal phase², leading to impaired vesicular fusion^{2,37}. This change would prevent efficient neurotransmitter release and reuptake. Additionally, plasmalogens are necessary for effective binding to neurotransmitter binding proteins³⁸, in which a reduction would impair neurotransmitter binding and signal transmission, resulting in cognitive effects. These impairments could lead to an inability for the TMX-treated animals to adequately map the open field, causing them to remain ambulatory longer than the wild-type or vehicle-treated animals. Future studies using this model may be performed to assess neurotransmitter levels, receptor binding, and vesicular fusion, focusing on the hippocampus.

Compound Muscle Action Potential and Nerve Conduction Velocity

[00193] Given the role of plasmalogens in neuronal structure and function, an investigation of whether plasmalogen deficiency would affect nerve conduction properties was performed. More specifically, characterization of the animal model outside of CNS function was performed using nerve tests to assess motor nerve function.

[00194] The mice were anesthetized for up to 5 minutes with 2-3% isoflurane in oxygen (O₂) while the body temperature was maintained constant. The hair of the right hind limb was trimmed with an electrical trimmer where needed. A thermostatically controlled heating pad was used to maintain normal body temperature and this was monitored with a rectal probe. The animal was stabilized by gently extending the body/limbs and taping the paws to the table surface. Three platinum or stainless-steel subdermal recording/stimulating needle electrodes (Grass F-E2, tip size 0.3 mm) were sterilized with vaporized hydrogen peroxide (VHP) prior to use and wiped between animals with 70% alcohol.

[00195] The stimulating electrodes were placed on each side of the sciatic nerve at the proximal thigh and stimulus intensity is increased until the compound muscle action potential (CMAP) was maximized. The stimulation range used was 0.7-2 mA. Motor responses were recorded using a PowerLab instrument and Labchart software (AD Instruments) from an intramuscular needle electrode in the tibialis anterior muscle.

[00196] The recording electrode was inserted into the plantar muscles and a reference electrode was placed in the skin between toes. To stimulate distally, a pair of subcutaneous stimulating electrodes was placed on both sides of the ankle to stimulate the lateral plantar nerve. For proximal stimulation, the electrodes were moved to the level of the sciatic notch, to the depth of the sciatic nerve.

[00197] Once all electrodes were in place, a current pulse was delivered at low frequency and the intensity was gradually increased from zero to 2 mA until a CMAP in the plantar muscles was evident on the oscilloscope. Three distally-produced CMAPs were recorded using a PowerLab instrument and Labchart software (AD Instruments). The procedure was repeated after moving the stimulation electrodes to the proximal stimulation point, and current was increased until a maximal CMAP was produced by proximal stimulation. Three proximally-produced CMAPs were

recorded. Amplitude and latency were calculated by averaging the three CMAP profiles, and the distance between the sites of stimulation was measured and used to calculate nerve conduction velocity (NCV).

[00198] A comparison of the CMAP, latency, amplitude and NCV following one month post-TMX showed no detectable differences between the TMX- and vehicle-treated controls (data not shown), as was observed in the open field study. The sample size of the one month study did not allow for an adequately powered assessment of the correlation between PlsEtn levels and nerve readings.

[00199] At four months post-TMX, however, there was a detectable increase in the latency relative to wild-type ($p=0.014$) and vehicle-treated ($p=0.021$) animals (Figure 9A). There was also a marginal decrease in NCV compared to wild-type ($p=0.025$) but not vehicle (Figure 9B), while amplitude was unchanged (Figure 9C). This suggests that plasmalogens play a role in mediating both the speed with which nerve impulses travel and the time required for the stimulus to evoke an action potential.

[00200] Interestingly, there was a strong inverse correlation between all serum plasmalogen levels and latency (Figure 10), with the exception of PlsEtn 16:0/18:1, where reduced plasmalogen levels were associated with increased latency. Less robust inverse correlations were observed between plasmalogen levels and NCV, although the trends were still evident and met statistical significance ($p<0.05$) for select plasmalogens (see Figure 11). Amplitude was shown to inversely correlate with three plasmalogen species 16:0/18:2 ($p=0.049$, F-stat 4.1), 16:0/22:6 ($p=0.028$, F-stat 5.2), and 18:0/22:6 ($p=0.035$, F-stat 4.8). This suggests that under conditions of plasmalogen deficiency, the latency period of the nerve stimulus is lengthened, resulting in an overall slowing of the velocity. Supporting this correlation, plasmalogen germline mutant mouse models have been previously reported to have reduced nerve conduction velocities^{5,39}. Moreover, a slight reduction in amplitude did correlate with PlsEtn species containing a DHA at the *sn2* position, although no overall difference in the amplitude in the TMX-treated group could be observed. Furthermore, no changes could be seen in the overall CMAP values, nor was there a correlation between these values and any of the serum PlsEtn levels (data not shown).

[00201] The standard clinical interpretation for increased latency and decreased NCV is linked to compromised myelin integrity. Given the importance of plasmalogens within the structure of myelin, and the reduction in myelin within the peripheral⁵ and central nervous system⁴⁰ of germline mouse models, it is plausible that plasmalogen deficiency in our model could be affecting myelin structure and/or function. The slight change in amplitude at four months could also suggest that chronic plasmalogen deficiency might lead to loss or damage of the synapse. Future studies are warranted to determine if amplitude is more substantially impacted following longer periods of deficiency along with detailed characterization of myelin structure.

[00202] In summary, a novel *Homozygous Gnpat cKO Hemi CAGGCRE-ER* animal model that is appropriate for evaluating adult-onset conditions associated with plasmalogen deficiency has been created. This novel *Homozygous Gnpat cKO Hemi CAGGCRE-ER* animal model is also appropriate for evaluating aging-related conditions associated with plasmalogen deficiency such as AD. It was shown that within one month of TMX treatments the impairment of GNPAT activity is sufficient to significantly reduce PlsEtn levels in serum and tissues, and by four months significant changes are evident in behavior and motor nerve function. While the magnitude of plasmalogen deficiency in this model was modest compared to previously described germline mutant models, the changes to behavior and nerve function are comparable. This confirms for the first time that the hyperactive phenotype and impaired nerve functions derive directly from the plasmalogen dysfunction and are not caused by developmental differences. Further, it was demonstrated that modest plasmalogen reductions in adulthood, comparable to those found in individuals with neurodegenerative diseases, are sufficient to impair function.

[00203] The *Homozygous Gnpat cKO Hemi CAGGCRE-ER* displays an indistinguishable phenotype from that of the wild-type prior to TMX administration, allowing for a variety of future assessments that would not have been possible using a full knock out model. Further, the *Homozygous Gnpat cKO Hemi CAGGCRE-ER* model allows for the ability to investigate neuroprotection and dose response studies using a plasmalogen precursor provided prior to deficiency, or early in the disease course. Future studies using this model will finally be able to address the questions remaining about the role of plasmalogens in the onset and progression of diseases associated with postnatal plasmalogen deficiency, as well as assess whether plasmalogen augmentation is a clinically viable treatment for these conditions.

[00204] Taken together, the novel inducible *Homozygous Gnpat cKO Hemi CAGGCRE-ER* mouse model represents the first animal model capable of evaluating the consequences of postnatal plasmalogen deficiency in relation to the disease onset and progression. In addition, this model provides an opportunity to evaluate the clinical potential of plasmalogen augmentation strategies in conditions associated with postnatal plasmalogen deficiency.

[00205] While illustrative and presently preferred embodiments of the invention have been described in detail hereinabove, it is to be understood that the inventive concepts may be otherwise variously embodied and employed and that the appended claims are intended to be construed to include such variations except insofar as limited by the prior art.

[00206] **Example 2 - *Gnpat^{del/flox} cKO Hemi CAGGCRE-ER* mice**

[00207] All mice were housed at Jackson Laboratories (Bar Harbor, ME) with 3-4 mice per cage in positively ventilated polysulfonate cages with HEPA-filtered air. The animal room was kept on a 12 h light/dark cycle (6:00 am to 6:00 pm light) using artificial fluorescent lighting. Room temperature was maintained at $22 \pm 4^{\circ}\text{C}$ and the relative humidity was $50 \pm 15\%$. The animal rooms had 15 air exchanges every hour and the animals were provided filtered tap water and normal rodent chow ad libitum. This work complied with the National Research Council's Guide for the Care and Use of Laboratory Animals. All animal handling was performed under approval from The Institutional Animal Care and Use Committee (IACUC) and Institutional Biosafety Committee (IBC) under Animal Use Summary (AUS) #: 21013. Veterinary care was available throughout the course of the study and animals were examined by veterinary staff when warranted by clinical signs and other changes. Later, animals were transferred to RI-MUHC ARD (Research Institute of the McGill University Health Centre, Animal Resource Division [subcontracted by Biospective, Montreal, QC]) with 1-3 mice per cage in positively ventilated standard cages (70 in²) with standard nesting and bio-huts attached to automate air filtering and water systems. The animal room was kept on a 12 h light/dark cycle (7:00 am to 7:00 pm light) using artificial fluorescent lighting. Room temperature was maintained at $21 \pm 3^{\circ}\text{C}$ and the relative humidity was $50 \pm 20\%$. The animals were provided filtered water through the attached water system and normal rodent chow ad libitum. The animals were monitored biweekly weighing for the duration of the study starting at the week of

baseline behavioral assessments and a Pain and Distress Assessment Score (PDAS) and a Body Condition Score (BCS) evaluated weekly. These studies were performed in accordance with Canadian Council on Animal Care (CCAC) guidelines and the internal Animal Use Protocol (AUP).

Generation of the $Gnpat^{del/flox}$ cKO Hemi CAGGCRE-ER mouse line

[00208] The *Gnpat* gene was targeted for the introduction of loxP sites using dsDNA in the regions flanking exon four of the *Gnpat* gene [Chr8:124863033-124890057 base pair (bp), positive strand] by microinjecting CRISPR RNA target guides into the embryo at the same time. Donor plasmid with homology arms of 2 kb and 1.6 kb flanking the loxP sites were used for homology driven repair. The upstream CRISPR target guide was: *Gnpat_in3_crRNA1*: GTGAGCTCCCCCGGCCTCTC (SEQ ID NO:1) while the downstream CRISPR target guide was: *Gnpat_in4_crRNA1*: ATGGCAGACAGGGGCCCTTC (SEQ ID NO:2). This resulted in one mouse line which contained the correct loxP sites without any additional deletions detected (*Gnpat^{flox/WT}*) and a second line which displayed a 381 bp deletion which contained exon four of the *Gnpat* gene, resulting in a full knockout animal line (*Gnpat^{del/WT}*). Genotypes were confirmed using the following primers: *Gnpat_genoF2*: TGCCAGCTTTCTACTTGCCA SEQ ID NO:18) and *Gnpat_genoR2*: GCAGTGACCTGACTGAGACC (SEQ ID NO:19).

[00209] *Heterozygous $Gnpat$ cKO ($Gnpat^{flox/WT}$)* mice were intercrossed to produce *Homozygous $Gnpat$ cKO ($Gnpat^{flox/flox}$)* animals. *Heterozygous $Gnpat$ KO ($Gnpat^{del/WT}$)* mice were crossed with hemizygous *CAGGCre-ERTM* mice²⁵ (C57BL/6J background; Jackson Laboratories Stock#004682) to produce mice that carried the hemizygous *CAGGCre-ER* transgene and were heterozygous for the deletion in the *Gnpat* allele (*Heterozygous $Gnpat$ KO Hemi CAGGCRE-ER*). Female *Heterozygous $Gnpat$ KO Hemi CAGGCRE-ER* mice were crossed to male *Homozygous $Gnpat$ cKO ($Gnpat^{flox/flox}$)* animals, resulting in animals that were *$Gnpat^{del/flox}$* and hemizygous for the *CAGGCre-ERTM* transgene, representing the new TMX-inducible *Gnpat* conditional knock out (*$Gnpat^{del/flox}$ cKO Hemi CAGGCRE-ER*).

[00210] *Tamoxifen administration*

[00211] Tamoxifen powder was dissolved in 100% ethanol (Sigma E7023) to 50 mg/mL at 55°C and was diluted 1:10 to 5 mg/mL in corn oil and stored at -20°C until the day of dosing. On

the day of dosing, solutions were thawed at 37°C. Mice between 4-7.5 months of age were administered 50 mg/kg of TMX (or vehicle) using the standardized dosage volume of 10 mL/kg of body weight once daily for 7 consecutive days. Technicians performing all experiments were blinded to animal genotype and treatment group. The animals were then aged for another 4 months. After 10 weeks they were transferred to RI-MUHC ARD (Research Institute of the McGill University Health Centre, Animal Resource Division [subcontracted by Biospective, Montreal, QC]) for the rest of the analyses were performed in accordance with Canadian Council on Animal Care (CCAC) guidelines.

Gnpat^{del/flox} cKO Hemi CAGGCRE-ER characterization

[00212] A characterization study was undertaken to evaluate lipidomic, behavioral and neuroimaging comparisons between wild-type controls (wild-type), *Gnpat^{del/flox} cKO Hemi CAGGCRE-ER* controls + vehicle (vehicle), *Gnpat^{del/flox}* and *cKO Hemi CAGGCRE-ER* + TMX (TMX). All animals were aged to eight months post-TMX treatment at which point behavioral and imaging studies (discussed below) were completed. Terminal blood samples were collected from each animal from the vena cava. The blood was transferred into an EDTA containing tube and placed on wet ice until processed into plasma. The tubes were centrifuged at 3000 x g for 15 minutes at 4°C within 30 minutes of the blood collection. The plasma was then placed into a clean Eppendorf tube and stored at -80°C until analysis.

[00213] Following euthanasia, animals were perfused with 20-40 mL of cold phosphate buffered saline. The liver, lungs, and intestines were dissected from the animals and collected into separate Eppendorf tubes and immediately snap frozen on dry ice. The tissues were stored at -80°C until analysis.

[00214]

Lipid Quantification by FI-MS/MS

[00215] Plasma extraction was performed on a 20 µL aliquot in 1.4 mL Thermo matrix tubes. Lipids were extracted into HPLC-grade water (20 µL) and 600 µL ethyl acetate with 2% water and 1% formic acid containing labelled internal standard [⁹D PlsEtn 18:0/18:1] at 0.05

µg/mL. Samples were hand mixed by inversion followed by vortexing for 30 minutes at 1500 rpm using a Mixmate. Next, samples were centrifuged for 5 minutes at 2000 rcf.

[00216] Tissue samples were extracted by placing approximately 50 mg of each tissue into a 2 mL safe-lock Eppendorf tube with all weights recorded for normalization. Two scoops (roughly 300 µL) of 0.5 mm zirconium oxide beads and 600 µL ethyl acetate with 2% water with 1% formic acid was added to each tube and then samples were homogenized using the Bullet Blender Storm Pro (Next Advantage) for 10 minutes. Each sample was mixed for 1 hour on a Mixmate at 1500 rpm to ensure complete lipid extraction, then centrifuged for 10 minutes at 3500 rcf. Based on the tissue weights, an aliquot of the eluant for all samples was removed from the tissue pellet and diluted to 50 mg/mL. Samples were further diluted in a tissue-specific manner in ethyl acetate with 2% water and 1% formic acid containing labelled internal standard (⁹D PlsEtn 18:0/18:1 at 0.05 µg/mL) to bring the lipid levels within range for linear quantification.

[00217] A 100 µL aliquot of each extract was analyzed by flow injection tandem mass spectrometry (FI-MS/MS) on an Applied Biosystems Q-Trap mass spectrometer coupled with an Agilent 1200 HPLC system. Each transition was scanned for 50 milliseconds with a total acquisition time per sample of 1 minute. The mobile phase was an ethyl acetate:methanol:water solution at 80:15:5 at a flow rate of 450 µL/min. The standards and stable isotopes used were >99% purity by TLC and manufactured by Avanti Polar Lipids, Inc, and solvents were HPLC grade. The measured transitions are reported in Table 1. The results were processed using Analyst software (version 1.5.1) as µg lipid per mg of starting tissue (µg/mg) or, in the case of serum, µg lipid per mL of serum (µg/mL).

Lipid Alterations in tamoxifen-treated Gnpat^{del/flox} cKO Hemi CAGGCRE-ER mice

[00218] Tissue and plasma samples were collected at the eight months post-TMX time points, and analyzed for plasmalogen levels. Total plasmalogen levels, as determined by summing the 8 measured individual plasmalogen species, were significantly reduced in the intestine, liver, lungs and plasma ($p < 0.001$; Figure 12) confirming the tamoxifen administration resulted in a chronic knockout of plasmalogen biosynthesis.

Open field behavioral assessment

[00219] Animals with germline mutations in the plasmalogen biosynthetic pathway have been shown to display a range of behavioral differences from healthy controls. Hyperactivity in the open field has been a consistent finding in animals with germline mutations in the plasmalogen biosynthetic pathway^{4,36}, but these studies were unable to differentiate if these behavioral changes are caused by differences in neurodevelopment or function. Using the *Gnpat^{del/flox} cKO Hemi CAGGCRE-ER* mice treated with TMX it was possible to assess the impact of plasmalogen deficiency in adulthood on activity levels in the open field.

[00220] Mice were placed in one corner of a beige 40 cm³ open field box with an overhead camera facing the corner. The recording began ~2 seconds after placing the animal to confirm camera detection thresholds and allow the experimenter to get out of the view of the mouse. The mice were allowed to move freely inside the box and were video recorded for 10 minutes. The recorded footage underwent manual quality control for accurate detection of the mouse and its rearing behavior. The footage was then analyzed by Bioseb (an automated tracking software) for distance traveled (meters), active time (seconds), rearing, and percentage of time in the center region of the field compared to the periphery.

[00221] The tracking data from the open field showed clear differences between the plasmalogen deficient TMX-treated mice compared with the wild-type and vehicle-treated control groups. The total distance travelled was comparable between the wild-type and vehicle-treated groups, while the TMX-treated animals travelled approximately twice the distances representing a significant increase ($p<0.01$; Figure 13A). Similarly, the TMX-treated animals had an increased mean speed ($p<0.01$; Figure 13B) and duration of activity ($p<0.01$; Figure 13C) in the open field compared with either wild-type or vehicle-treated animals.

Sleep behavioral assessment

[00222] Sleep disturbances have been shown to be an early symptom in neurodegenerative diseases, including Alzheimer's disease^{41,42} and Parkinson's disease^{43,44}. To assess whether chronic plasmalogen deficiency in adulthood has an impact on sleep quantity and quality, animals were housed in specialized PiezoSleep cages to monitor sleep and wake cycles during both the light and dark phases of the day.

[00223] The animals were individually housed in PiezoSleep cages for 3 consecutive days at approximately 8 months post-TMX administration, with light/dark cycles of 12h/12h and access to food and water ensured and closely monitored. The first 24 hours of data were excluded from analysis to allow for acclimatization to the cage, producing 48 hours total data for each assessment time. After 3 days the mice were returned to their home cages. The PiezoSleep Mouse Behavior Tracking System, a fully automated system, was used to quantify the sleep and wake cycles of the animals. For each animal, at each timepoint, the percentage of sleep versus wakefulness was calculated. Sleep bout data was also quantified and averaged over both the light and dark phase period. The sleep versus wake data was reviewed for quality control and animals with poor sleep versus wake delineation were excluded from the analysis.

[00224] Differences in sleep architecture were observed in the *Gnpat^{del/flox} cKO Hemi CAGGCRE-ER* mice, particularly those treated with TMX, when compared to wild-type control animals. While, there were no differences observed between the groups in the amount of time the animals spent sleeping during the light phase (Figure 14A), during the dark phase wild-type animals slept significantly more than any other group ($p < 0.001$), while the vehicle-treated mice slept an intermediate amount, and the TMX-treated group slept significantly less ($p < 0.001$; Figure 14B).

[00225] The overall shift in sleep duration and sleep bout length in the *Gnpat^{del/flox} cKO Hemi CAGGCRE-ER* mice TMX-treated animals compared with the wild-type and TMX untreated animals suggests that a chronic plasmalogen deficiency has a profound impact on sleep. This provides further support for the utility of *Gnpat^{del/flox} cKO Hemi CAGGCRE-ER* mice as a model to understand the impact of a postnatal plasmalogen deficiency. Further, sleep represents a meaningful intervenable endpoint that can be evaluated in the future to assess the clinical utility of treatment protocols designed to address chronic plasmalogen deficiency.

[00226] Anatomical MRI acquisition

[00227] Plasmalogens are critical components of both the myelin sheath and neuronal cell membranes. Numerous studies have shown that a reduction in plasmalogen levels can impact both the structure and function of brain cells, but with a lack of models that allow for induction of a plasmalogen deficiency in adulthood it has not been possible to assess the impact of this deficiency on the brains structure. Using our *Gnpat^{del/flox} cKO Hemi CAGGCRE-ER* model, we sought to

evaluate if a chronic plasmalogen deficiency in adult animals resulted in a measurable difference in the structure and size of the brain using anatomical MRIs using a 7T Bruker BioSpec 70/30 MRI system.

[00228] Each animal was anesthetized with an induction dose of 4-5% isoflurone and secured in an MRI-compatible bed. Animals were allowed to breathe spontaneously without medical ventilation. Respiration rate and body temperature were continuously monitored. The MRI acquisition included set-up, scout, and shimming. Following set up, anatomical images were acquired using a 3D balanced Steady-State Free Precision (b-SSFP) sequence. The phase-cycled images were combined using the sum-of-squares reconstruction method to minimize banding artifacts. Total scan time was approximately one hour per animal.

[00229] MRI scans underwent initial image QC at the time of scanning based on a quality control image containing multiple orthogonal views of the data. Scans with excessive motion or image artifacts were considered inadequate; failed scans were repeated. All images were processed using NIGHTWINGTM software package.

[00230] The b-SSFP images were utilized to generate an unbiased symmetric, anatomical template. Prior to template generation, each reconstructed image volume underwent image non-uniformity correction using the N3 algorithm, brain masking, and linear spatial normalization using a 12-parameter affine transformation to map individual images from native coordinate spaces to reference space. Briefly, the template generation process involved iterative (coarse-to-fine resolution) estimation of the nonlinear transformation to match each MRI scan to the evolving average of the population. The individual MRI scans were then linearly and non-linearly registered to this anatomical template^{45,46}.

[00231] The total brain volume was measured by the MRI for the left and right hemispheres. The volumes were comparable between the wild-type and vehicle control mice, while TMX-treated mice displayed a reduction in the whole brain volume ($p < 0.01$; Figure 15).

[00232] Regional brain volumes were calculated for all the major regions of interest within the brain. It was consistently observed that the wild-type and vehicle-treated control animals had comparable regional brain volumes, while the TMX-treated animals had reduced volumes. The differences between the control group and the TMX-treated groups were more

profound ($p < 0.01$) in the more posteriorly located regions of the brain, including the temporoparietal cortex (Figure 16), occipital cortex (Figure 17), hippocampus (Figure 18), olfactory area (Figure 19), striatum (Figure 20), thalamus (Figure 21), and brainstem (Figure 22).

[00233] In addition to regional difference in brain volume, the volume of the white matter, or myelin, was also compared. A similar pattern was seen in which the two control groups (wild-type and vehicle-treated) had similar white matter volumes, while the TMX-treated animals displayed significant reduction in white matter volume ($p < 0.01$; Figure 23). Given the high levels of plasmalogen in myelin it was expected that a loss of plasmalogen biosynthetic capacity in the TMX-treated *Gnpat^{del/flox} cKO Hemi CAGGCRE-ER* mice would result in a decreased volume. These data support the utility of the *Gnpat^{del/flox} cKO Hemi CAGGCRE-ER* model to study the neurodegenerative consequences of a chronic plasmalogen deficiency in adulthood and further support its utility in testing the clinical potential of new therapeutic strategies.

[00234] *Neurofilament light chain quantification*

[00235] Neurofilament light chain (NfL) is a protein component of the neuronal cytoskeleton. Neuronal damage leads NfL to be released into the blood and cerebrospinal fluid (CSF) and as such levels of NfL are considered to be a sensitive, although non-specific, marker of neuroinflammation. NfL is increasingly being used as a biomarker of neuroinflammation in the diagnosis and clinical monitoring of patients with Alzheimer's disease. The changes in brain structures seen in the TMX-treated animals were suggestive of neuroinflammation and therefore both plasma and CSF NfL levels were evaluated.

[00236] Plasma and CSF samples were collected from all mice at the time of euthanasia. For CSF extraction, animals were deeply anesthetized with Avertin (500 mg/kg) and placed in the prone position, with the head and body at a 120° angle. The skin and muscle at the nape of the neck were retracted. A 36 gauge Hamilton syringe was used to pierce the dura matter at the site of the cisterna magna. CSF was collected by capillary action and then transferred to a 0.2 mL low protein binding microcentrifuge tube, centrifuged to check for blood contamination, and frozen at -80°C until analysis. Blood collection and plasma preparation followed CSF collection, as described above. Quantification of NfL levels was performed by RayBiotech using the Simoa® (Quanterix™) single molecule protein detection platform.

[00237] NfL CSF levels were comparable between wild-type and vehicle-treated mice, while levels in the TMX-treated animals were approximately twice as high, representing a

statistically significant increase ($p < 0.001$; Figure 24A). Plasma levels of NfL showed the same trend with the TMX treatment resulting in a doubling of levels compared to either the wild-type or vehicle-treated group ($p < 0.001$ Figure 24B). Overall, these data suggest that a chronic plasmalogen deficiency in adulthood results in neuroinflammation, similar to that seen in individuals with Alzheimer's disease⁴⁷ or Parkinson's disease⁴⁸.

[00238] In summary, the *Gnpat*^{del/flox} *cKO Hemi CAGGCRE-ER* animal model represents a novel model for evaluating the behavioral and neurodegenerative consequences of adult-onset conditions associated with plasmalogen deficiency such as AD and PD. Eight months following TMX administration there was a significant changes in behavior as demonstrated by change in sleep architecture and activity in the open field, as well as in the structure of the brain, as demonstrated by reduced brain and white matter volumes. Additionally, NfL levels in the plasma and CSF suggest neuroinflammation occurs as a result of chronic plasmalogen deficiency. Together, this data clearly demonstrated that modest plasmalogen reductions in adulthood, comparable to those found in individuals with neurodegenerative diseases, are sufficient to impair behavior and function.

[00239] Example 3: Use of the *Gnpat*^{del/flox} *cKO Hemi CAGGCRE-ER* model of Alzheimer's disease to evaluate plasmalogen targeted therapeutics

[00240] All mice were housed at Jackson Laboratories (Bar Harbor, ME) with 3-4 mice per cage in positively ventilated polysulfonate cages with HEPA-filtered air. The animal room was kept on a 12 h light/dark cycle (6:00 am to 6:00 pm light) using artificial fluorescent lighting. Room temperature was maintained at $22 \pm 4^\circ\text{C}$ and the relative humidity was $50 \pm 15\%$. The animal rooms had 15 air exchanges every hour and the animals were provided filtered tap water and normal rodent chow ad libitum. This work complied with the National Research Council's Guide for the Care and Use of Laboratory Animals. All animal handling was performed under approval from The Institutional Animal Care and Use Committee (IACUC) and Institutional Biosafety Committee (IBC) under Animal Use Summary (AUS) #: 21013. Veterinary care was available throughout the course of the study and animals were examined by veterinary staff when warranted by clinical signs and other changes. Later, animals were transferred to RI-MUHC ARD (Research Institute of the McGill University Health Centre, Animal Resource Division [subcontracted by Biospective, Montreal, QC]) with 1-3 mice per

cage in positively ventilated standard cages (70 in²) with standard nesting and bio-huts attached to automate air filtering and water systems. The animal room was kept on a 12 h light/dark cycle (7:00 am to 7:00 pm light) using artificial fluorescent lighting. Room temperature was maintained at 21 ± 3°C and the relative humidity was 50 ± 20%. The animals were provided filtered water through the attached water system and normal rodent chow ad libitum. The animals were monitored biweekly weighing for the duration of the study starting at the week of baseline behavioral assessments and a Pain and Distress Assessment Score (PDAS) and a Body Condition Score (BCS) evaluated weekly. These studies were performed in accordance with Canadian Council on Animal Care (CCAC) guidelines and the internal Animal Use Protocol (AUP).

Generation of the $Gnpat^{del/flox}$ cKO Hemi CAGGCRE-ER mouse line

[00241] The *Gnpat* gene was targeted for the introduction of loxP sites using dsDNA in the regions flanking exon four of the *Gnpat* gene [Chr8:124863033-124890057 base pair (bp), positive strand] by microinjecting CRISPR RNA target guides into the embryo at the same time. Donor plasmid with homology arms of 2 kb and 1.6 kb flanking the loxP sites were used for homology driven repair. The upstream CRISPR target guide was: *Gnpat_in3_crRNA1*: GTGAGCTCCCCCGGCCTCTC (SEQ ID NO:1) while the downstream CRISPR target guide was: *Gnpat_in4_crRNA1*: ATGGCAGACAGGGGCCCTTC (SEQ ID NO:2). This resulted in one mouse line which contained the correct loxP sites without any additional deletions detected (*Gnpat^{flox/WT}*) and a second line which displayed a 381 bp deletion which contained exon four of the *Gnpat* gene, resulting in a full knockout animal line (*Gnpat^{del/WT}*). Genotypes were confirmed using the following primers: *Gnpat_genoF2*: TGCCAGCTTTCTACTTGCCA (SEQ ID NO:18) and *Gnpat_genoR2*: GCAGTGACCTGACTGAGACC (SEQ ID NO:19).

[00242] *Heterozygous Gnpat cKO ($Gnpat^{flox/WT}$)* mice were intercrossed to produce *Homozygous Gnpat cKO ($Gnpat^{flox/flox}$)* animals. *Heterozygous Gnpat KO ($Gnpat^{del/WT}$)* mice were crossed with hemizygous *CAGGCre-ERTM* mice²⁵ (C57BL/6J background; Jackson Laboratories Stock#004682) to produce mice that carried the hemizygous *CAGGCre-ER* transgene and were heterozygous for the deletion in the *Gnpat* allele (*Heterozygous Gnpat KO Hemi CAGGCRE-ER*). Female *Heterozygous Gnpat KO Hemi CAGGCRE-ER* mice were crossed to male *Homozygous Gnpat cKO ($Gnpat^{flox/flox}$)* animals, resulting in animals that were *Gnpat^{del/flox}* and hemizygous for

the *CAGGCre-ERTM* transgene, representing the new TMX-inducible *Gnpat* conditional knock out (*Gnpat^{del/flox} cKO Hemi CAGGCRE-ER*).

Tamoxifen and PPI-1011 Treatment

[00243] Tamoxifen (TMX) powder was dissolved in 100% ethanol (Sigma E7023) to 50 mg/mL at 55°C and was diluted 1:10 to 5 mg/mL in corn oil and stored at -20°C until the day of dosing. On the day of dosing (age 4 months), solutions were thawed at 37°C and were administered 50 mg/kg of TMX (or vehicle) using the standardized dosage volume of 10 mL/kg of body weight once daily for 7 consecutive days. PPI-1011, a synthetic 16:0/22:6/lipoic acid (sn1/sn2/sn3) ether precursor, or vehicle (liquid coconut oil with 0.1% thioglycerol) was administered by oral gavage for 4 months at a dose of 200 mg/kg every other day beginning 4 months post-TMX administration.

Table 2. Study Treatment Groups

Group	Strain	IP Treatment	Oral Treatment
Wild-type (WT)	C57Bl/6	NA	Vehicle
cKO Control	<i>Gnpat^{del/flox} cKO</i> <i>Hemi CAGGCRE-ER</i>	NA	Vehicle
cKO TMX	<i>Gnpat^{del/flox} cKO</i> <i>Hemi CAGGCRE-ER</i>	Tamoxifen	Vehicle
cKO 1011	<i>Gnpat^{del/flox} cKO</i> <i>Hemi CAGGCRE-ER</i>	Tamoxifen	PPI-1011

[00244] *Plasmalogen Analysis*

[00245] Plasma extraction was performed on a 20 µL aliquot in 1.4 mL Thermo matrix tubes. Lipids were extracted into HPLC-grade water (20 µL) and 600 µL ethyl acetate with 2% water and 1% formic acid containing labelled internal standard [⁹D PlsEtn 18:0/18:1] at 0.05 µg/mL. Samples were hand mixed by inversion followed by vortexing for 30 minutes at 1500 rpm using a Mixmate. Next, samples were centrifuged for 5 minutes at 2000 ref. A 100 µL aliquot of

each extract was analyzed by flow injection tandem mass spectrometry (FI-MS/MS) on an Applied Biosystems Q-Trap mass spectrometer coupled with an Agilent 1200 HPLC system. Results were processed using Analyst software (version 1.5.1) μg lipid per mL of plasma ($\mu\text{g}/\text{mL}$).

[00246] *PPI-1011 treatment normalizes plasmalogen levels in tamoxifen-treated $Gnpat^{\text{del/flox}}$ cKO Hemi CAGGCRE-ER mice*

[00247] Plasma samples were collected and analyzed for levels of the 16:0/22:6 plasmalogen, which is the primary metabolite of PPI-1011. Plasma levels of PlsEtn 16:0/22:6 were significantly reduced ($p < 0.001$; Figure 25) in the cKO TMX group relative to the wild-type and cKO vehicle groups, confirming a chronic knockout of plasmalogen biosynthesis. The cKO 1011 groups had significantly increased plasma PlsEtn 16:0/22:6 levels compared to cKO TMX animals ($p < 0.001$; Figure 25). In fact plasma concentrations in the cKO 1011 animals were comparable to those in the cKO control animals, confirming the ability of PPI-1011 to normalize plasma plasmalogen levels. Further, the ability to correct plasmalogen levels validates the utility of the $Gnpat^{\text{del/flox}}$ cKO Hemi CAGGCRE-ER mouse model to assess therapeutic treatments that target an underlying plasmalogen deficiency.

[00248] *Anatomical MRI acquisition*

[00249] Using our $Gnpat^{\text{del/flox}}$ cKO Hemi CAGGCRE-ER model, we sought to evaluate if treatment with PPI-1011 was able to rescue the reduction in brain volumes observed following induction of a chronic plasmalogen deficiency in adult animals using tamoxifen. Brain volumes were assessed using anatomical MRIs using a 7T Bruker BioSpec 70/30 MRI system.

[00250] Each animal was anesthetized with an induction dose of 4-5% isoflurone and secured in an MRI-compatible bed. Animals were allowed to breathe spontaneously without medical ventilation. Respiration rate and body temperature were continuously monitored. The MRI acquisition included set-up, scout, and shimming. Following set up, anatomical images were acquired using a 3D balanced Steady-State Free Precision (b-SSFP) sequence. The phase-cycled images were combined using the sum-of-squares reconstruction method to minimize banding artifacts. Total scan time was approximately one hour per animal.

[00251] MRI scans underwent initial image QC at the time of scanning based on a quality control image containing multiple orthogonal views of the data. Scans with excessive motion or image artifacts were considered inadequate; failed scans were repeated. All images were processed using NIGHTWINGTM software package.

[00252] The b-SSFP images were utilized to generate an unbiased symmetric, anatomical template. Prior to template generation, each reconstructed image volume underwent image non-uniformity correction using the N3 algorithm, brain masking, and linear spatial normalization using a 12-parameter affine transformation to map individual images from native coordinate spaces to reference space. Briefly, the template generation process involved iterative (coarse-to-fine resolution) estimation of the nonlinear transformation to match each MRI scan to the evolving average of the population. The individual MRI scans were then linearly and non-linearly registered to this anatomical template^{45,46}.

[00253] *Brain Volumes Increased with PPI-1011 Treatment*

[00254] Anatomical MRIs were performed on all animals and brain volumes were calculated for the left and right hemispheres. The cKO TMX group, known to have a chronic plasmalogen deficiency, showed significant reductions whole brain, white matter, and hippocampal volumes. This was anticipated due to the critical role plasmalogens play in maintaining the structure of the brain and in the composition of myelin. The cKO 1011 group showed increased volumes in the whole brain, white matter, and hippocampus, although only the whole brain and hippocampal volume increases reached significance ($p < 0.05$; Figure 26). These results confirm that increasing plasmalogen levels in a deficient animal results in clear physiological changes and further validates the utility of the *Gnpat*^{del/flox} cKO Hemi CAGGCRE-ER mouse model to evaluate the clinical utility of plasmalogen targeted treatment modalities.

[00255] *Mass Spectrometry Imaging*

[00256] Brains from study animals were flash frozen in liquid nitrogen and maintained at -80°C until analysis. Each brain was sliced sagittally, and sections were sublimed with 1,5-DAN matrix and analyzed by MALDI-MSI (negative ionization mode) and analyzed at a spatial resolution of 125 μ M (University of Montreal).

[00257] *PPP-1011 Increased Plasmalogen Levels as Shown by Mass Spectrometry Imaging*

[00258] Each brain slice was analyzed using mass spectrometry imaging (MSI) for levels of the 16:0/22:6 plasmalogen, known to be the primary metabolite of PPI-1011, as well as the 16:0/18:1 plasmalogen species which is the primary plasmalogen component in myelin. MSI clearly illustrated that different plasmalogen species appear to have different localizations, with DHA-containing species, for example, being localized preferentially to the cortical and non-myelin regions of the cerebellum, while plasmalogens containing more saturated fatty acids, for example 16:0/18:1, appear to be preferentially enriched in myelin tracts (arrows in Figure 27). cKO TMX animals showed reduced levels of both PlsEtn 16:0/22:6 and PlsEtn 16:0/18:1 in the brain compared to cKO control animals. This was expected given the plasmalogen deficiency noted in plasma, but confirmed the specificity and utility of the MSI method for measuring brain plasmalogen levels. Mice treated for four months with PPI-1011 showed increased levels of PlsEtn 16:0/22:6 and PlsEtn 16:0/18:1 in the brain (Figure 27). This data not only confirmed the ability of the plasmalogens that result from PPI-1011 treatment to cross the blood-brain barrier, but it also validated the utility of the *Gnpat^{del/flox} cKO Hemi CAGGCRE-ER* model to assess plasmalogen targeted therapeutic modalities as a treatment for the central nervous system-based consequences of chronic plasmalogen deficiency.

[00259] In summary, the *Gnpat^{del/flox} cKO Hemi CAGGCRE-ER* model represents not only a novel model for studying the consequences of chronic plasmalogen deficiency in adulthood, but also for evaluating therapies designed at correcting either the plasmalogen deficiency, or the biochemical or functional changes that result of the deficiency.

[00260] While illustrative and presently preferred embodiments of the invention have been described in detail hereinabove, it is to be understood that the inventive concepts may be otherwise variously embodied and employed and that the appended claims are intended to be construed to include such variations except insofar as limited by the prior art.

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Claims

1. A transgenic, non-human animal model for post-natal, conditionally inducible, plasmalogen deficiency comprising a genome with:
 - a) a gene with at least one regulatory region, wherein the gene is capable of regulating a plasmalogen biosynthetic pathway;
 - b) the at least one regulatory region comprising at least one conditionally inducible gene editing site, wherein the gene editing site is capable of blocking expression of the gene when edited; and
 - c) at least one nucleic acid editing sequence integrated at a separate locus from the gene, wherein the at least one nucleic acid editing sequence encodes a gene product capable of editing the at least one gene editing site when conditionally induced, thereby down-regulating or disrupting the plasmalogen biosynthetic pathway.
2. The transgenic, non-human animal model of claim 1, wherein the conditionally inducible gene editing site comprises an exogenous nucleic acid sequence and an endogenous nucleic acid sequence.
3. The transgenic, non-human animal model of claim 1 or 2, wherein the animal model is a rodent.
4. The transgenic, non-human animal model of any one of claims 1-3, wherein the at least one conditionally inducible gene editing site further comprises a second conditionally inducible gene editing site and the second gene editing site comprises an exogenous nucleic acid sequence, or an endogenous nucleic acid sequence, and the at least one nucleic acid editing sequence encodes a second gene product capable of editing the second gene editing site.
5. The transgenic, non-human animal model of any one of claims 1-4, wherein the at least one conditionally inducible gene editing site is LoxP site, flippase recognition target (FRT) site, attP and attB site, sgRNA binding site, FokI site, zinc finger nuclease site or any combination thereof.

6. The transgenic, non-human animal model of any one of claims 1-5, wherein the gene product is Cre, flippase, PhiC31, Cas9, an sgRNA, a crRNA, transcription activator-like effector nuclease (TALEN), zinc-finger nuclease (ZFN), any variant thereof, or any combination thereof.
7. The transgenic, non-human animal model of any one of claims 1-4, wherein the at least one conditionally inducible gene editing site is LoxP site and the gene product is Cre or a variant thereof.
8. The transgenic, non-human animal model of claim 7, wherein the Cre gene product is a fusion protein further comprising a mutant estrogen ligand binding domain (ERT2).
9. The transgenic, non-human animal model of claim 7, wherein the Cre gene product is operatively linked to a promoter element comprising a tetracycline responsive element capable of inducing gene expression with the addition or removal of tetracycline from the transgenic animal.
10. The transgenic, non-human animal model of any one of claims 1-4, wherein the at least one conditionally inducible gene editing site is FRT site and the gene product is a flippase or a variant thereof.
11. The transgenic, non-human animal model of any one of claims 1-4, wherein the at least one conditionally inducible gene editing site is attP and attB site and the gene product is PhiC31 or a variant thereof.
12. The transgenic, non-human animal model of any one of claims 1-4, wherein the at least one conditionally inducible gene editing site is FokI site and the gene product is a TALEN or a variant thereof.
13. The transgenic, non-human animal model of any one of claims 1-4, wherein the at least one conditionally inducible gene editing site is zinc finger nuclease site and the gene product is a zinc-finger nuclease.
14. The transgenic, non-human animal model of any one of claims 1-4, wherein the at least one conditionally inducible gene editing site is sgRNA binding site and the gene product is a Cas9 or a variant thereof, and/or a sgRNA, a crRNA, or both, capable of binding the sgRNA binding sites.

15. The transgenic, non-human animal model of any one of claims 1-14, wherein the at least one regulatory region is an exon, an element required for transcription of the gene, an element required for translation of the gene, an element required for the function of the protein encoded by the genomic sequence comprising the gene, or any combination thereof.
16. The transgenic, non-human animal model of any one of claims 1-15, wherein the at least one regulatory region is an exon.
17. The transgenic, non-human animal model of any one of claims 1-16, wherein expression of the gene product is conditionally inducible.
18. The transgenic, non-human animal model of claim 17, wherein expression of the gene product is conditionally induced by the addition of an exogenous compound, an exogenous stimulus or both.
19. The transgenic, non-human animal model of claim 17 or 18, wherein expression of the gene product is conditionally induced by addition of tamoxifen, 4-hydroxytamoxifen (4-OHT), mifepristone, tetracycline, doxycycline, light, temperature or any combination thereof.
20. The transgenic, non-human animal model of any one of claims 1-19, wherein the gene regulating the plasmalogen biosynthetic pathway is fatty acid reductase 1 (FAR1), glyceronephosphate O-acyltransferase (GNPAT), alkylglycerone phosphate synthase (AGPS), acyl/alkyl-DHAP reductase, alkyl/acyl-GPA acyltransferase, phosphatidic acid phosphatase, ethanolamine phosphotransferase, plasmenylethanolamine desaturase, choline phosphotransferase or any combination thereof.
21. The transgenic, non-human animal model of any one of claims 1-20, wherein the gene is glyceronephosphate O-acyltransferase (GNPAT).
22. The transgenic, non-human animal model of any one of claims 1-21, wherein the at least one conditionally inducible gene editing site is not edited by the gene product at or before birth of the animal.
23. The transgenic, non-human animal model of any one of claims 1-22, further comprising:

- a) one or more additional genes, each of the one or more additional genes comprising at least one regulatory region, wherein each of the one or more additional gene regulates a plasmalogen biosynthetic pathway; and
- b) the at least one regulatory region comprising an additional conditionally inducible gene editing site, wherein the additional gene editing site comprising a first additional conditionally inducible gene editing site and/or a second additional conditionally inducible gene editing site, wherein the first additional gene editing site and/or the second additional gene editing site of the additional gene editing sites comprise an exogenous nucleic acid sequence, or an endogenous nucleic acid sequence.
24. The transgenic, non-human animal model of claim 23, further comprising:
- c) a second conditionally inducible nucleic acid editing sequence integrated at a separate locus from the gene editing sites, the first nucleic acid editing sequence, the gene and the one or more additional genes, wherein the second conditionally inducible nucleic acid editing sequence encodes a second gene product capable of editing the one or more additional genes.
25. The transgenic, non-human animal model of claim 24, wherein the gene product and the second gene product are different.
26. The transgenic, non-human animal model of any one of claims 1-22, comprising a rodent, a genome of the rodent comprising:
- a glyceronephosphate O-acyltransferase (GNPAT) gene comprising loxP sites; and
- a gene encoding for a fusion protein of a Cre recombinase and a mutant estrogen ligand-binding domain (ERT2).
27. The transgenic, non-human animal model of any one of claims 1-22, comprising a rodent, a genome of the rodent comprising:
- a glyceronephosphate O-acyltransferase (GNPAT) gene comprising loxP sites; and

a Cre recombinase gene encoding Cre under the control of a tetracycline-responsive element.

28. The transgenic, non-human animal model of any one of claims 1-22, comprising a rodent, a genome of the rodent comprising:

a glyceronephosphate O-acyltransferase (GNPAT) gene comprising one or more sites capable of binding an sgRNA;

a promoter operatively connected to at least one sgRNA capable of binding the one or more sites capable of binding an sgRNA; and

a gene encoding a Cas9 or variant thereof.

29. The transgenic, non-human animal model of any one of claims 1-22, comprising a rodent, a genome of the rodent comprising:

a) a first LoxP site upstream of exon 4 of the gene encoding glyceronephosphate O-acyltransferase (GNPAT) and a second LoxP site downstream of exon 4 of the gene glyceronephosphate O-acyltransferase (GNPAT); and

b) a nucleic acid sequence encoding CAGGCre-ER^{T2}.

30. The transgenic, non-human animal model of any one of claims 1-22, comprising a rodent, a genome of the rodent comprising:

a) a first sgRNA site upstream of exon 4 of the gene encoding glyceronephosphate O-acyltransferase (GNPAT) capable of binding an sgRNA of GTGAGCTCCCCGGCCTCTC (SEQ ID NO:1) and a second sgRNA site downstream of exon 4 of the gene encoding glyceronephosphate O-acyltransferase (GNPAT) capable of binding an sgRNA of ATGGCAGACAGGGGCCCTTC (SEQ ID NO:2); and

b) a first nucleic acid sequence operatively linked to a sequence encoding the sgRNA of GTGAGCTCCCCGGCCTCTC (SEQ ID NO:1), a second nucleic acid sequence operatively linked to a sequence encoding the sgRNA of GTGAGCTCCCCGGCCTCTC

(SEQ ID NO:1), and a third nucleic acid sequence encoding Cas9 or a variant thereof, wherein the Cas9 or variant thereof is operatively linked to an inducible regulatory element.

31. The transgenic, non-human animal model of any one of claims 1-30, wherein at least one genome of the transgenic animal model comprises the genome.
32. The transgenic, non-human animal model of any one of claims 1-30, wherein all of the genomes of the transgenic animal model comprise the genome.
33. A cell from the non-human transgenic animal model of any one of claims 1-32.
34. A method of reducing postnatal plasmalogen levels in a non-human animal, the method comprising:
 - providing the non-human transgenic animal model of any one of claims 1-32; and
 - inducing expression of the nucleic acid editing sequence encoding the gene product capable of editing the at least one conditionally inducible gene editing site, wherein the editing of the at least one conditionally inducible gene editing site decreases expression of the gene regulating the plasmalogen biosynthetic pathway.
35. Use of the non-human transgenic animal model of any one of claims 1-32 for reducing postnatal plasmalogen levels in a non-human animal, wherein the non-human transgenic animal model is for inducing expression of the nucleic acid editing sequence encoding the gene product capable of editing the at least one conditionally inducible gene editing site, wherein the editing of the at least one conditionally inducible gene editing site is for decreasing expression of the gene regulating the plasmalogen biosynthetic pathway.
36. The non-human transgenic animal model of any one of claims 1-33 for use in reducing postnatal plasmalogen levels in a non-human animal, wherein the transgenic animal is for inducing expression of the nucleic acid editing sequence encoding the gene product capable of editing the at least one conditionally inducible gene editing site, wherein the editing of the at least one conditionally inducible gene editing site is for decreasing expression of the gene regulating the plasmalogen biosynthetic pathway.

37. A method of inducing a condition or disease in a non-human animal associated with reduced postnatal plasmalogen levels, the method comprising:

- providing the non-human transgenic animal model of any one of claims 1-32;
- inducing expression of the nucleic acid editing sequence encoding the gene product capable of editing the at least one conditionally inducible gene editing site, wherein the editing of the at least one conditionally inducible gene editing site decreases expression of the gene regulating the plasmalogen biosynthetic pathway; and
- monitoring the non-human transgenic animal model for symptoms of the condition or disease.

38. Use of the non-human transgenic animal model of any one of claims 1-32 for inducing a condition or disease in a non-human animal associated with reduced postnatal plasmalogen levels, wherein the non-human transgenic animal model is for:

- inducing expression of the nucleic acid editing sequence encoding the gene product capable of editing the at least one conditionally inducible gene editing site, wherein the editing of the at least one conditionally inducible gene editing site is for decreasing expression of the gene regulating the plasmalogen biosynthetic pathway; and
- monitoring the non-human transgenic animal model for symptoms of the condition or disease.

39. The non-human transgenic animal model of any one of claims 1-32 for use in inducing a condition or disease in a non-human animal associated with reduced postnatal plasmalogen levels, wherein the non-human transgenic animal model is for:

- inducing expression of the nucleic acid editing sequence encoding the gene product capable of editing the at least one conditionally inducible gene editing site, wherein the editing of the at least one conditionally inducible gene editing site is for decreasing expression of the gene regulating the plasmalogen biosynthetic pathway; and
- monitoring the transgenic animal for symptoms of the condition or disease.

40. A method for determining the efficacy of a compound or composition for treating a condition or disease associated with a reduced plasmalogen level, the method comprising:

- providing the non-human transgenic animal model of any one of claims 1-32;

- A1) inducing expression of the nucleic acid editing sequence encoding the gene product capable of editing the at least one conditionally inducible gene editing site, wherein the editing of the at least one conditionally inducible gene editing site is for decreasing expression of the gene regulating the plasmalogen biosynthetic pathway;

- B1) administering the compound or the composition to the non-human transgenic animal model; and

- C1) monitoring the non-human transgenic animal model for symptoms of the condition or disease; or

- A2) administering the compound or the composition to the non-human transgenic animal model;

- B2) inducing expression of the nucleic acid editing sequence encoding the gene product capable of editing the at least one conditionally inducible gene editing site, wherein the editing of the at least one conditionally inducible gene editing site is for decreasing expression of the gene regulating the plasmalogen biosynthetic pathway; and

- C2) monitoring the non-human transgenic animal model for symptoms of the condition or disease; or

- A3) administering the compound or the composition to the non-human transgenic animal model and inducing expression of the nucleic acid editing sequence encoding the gene product capable of editing the at least one conditionally inducible gene editing site, wherein the editing of the at least one conditionally inducible gene editing site is for decreasing expression of the gene regulating the plasmalogen biosynthetic pathway; and

- B3) monitoring the non-human transgenic animal model for symptoms of the condition or disease.

41. Use of the non-human transgenic animal model of any one of claims 1-32 for determination of the efficacy of a compound or composition for the treatment of a condition or disease associated with a reduced plasmalogen level.

42. The non-human transgenic animal model of any one of claims 1-32 for use for in determination of the efficacy of a compound or composition for the treatment of a condition or disease associated with a reduced plasmalogen level.

43. The method of any one of claims 34, 37 and 40, the use of any one of claims 35, 38 and 41, or the non-human transgenic animal model for use of any one of claims 36, 39 or 42, wherein inducing expression of the nucleic acid editing sequence encoding the gene product capable of editing the at least one conditionally inducible gene editing site excises or modifies at least a portion of the gene.

44. The method of claim 37 or 40, the use of claim 38 or 41, or the non-human transgenic animal model for use of claims 39 or 42, wherein the condition or disease associated with a reduced plasmalogen level is a neurodegenerative disease, presence of cataracts, respiratory disease, chronic inflammation, impaired myelination, metabolic syndrome, type II diabetes, or cardiovascular disease.

45. The method, use or transgenic animal for use of claim 44, wherein the neurodegenerative disease is Alzheimer's disease, Parkinson's disease, or Multiple sclerosis.

46. The method, use or transgenic animal for use of claim 44, wherein the respiratory disease is bronchopulmonary dysplasia (BPD) or chronic obstructive pulmonary disease (COPD).

47. The method of claim 40, the use of claim 41 or the non-human transgenic animal model for use of claim 42, wherein the symptoms of the condition or disease comprise one or more of the following: a decreased plasmalogen level, and/or one or more symptoms associated with the diseases and/or conditions defined in claim 44 or claim 45.

48. A method of generating the non-human transgenic animal model of any one of claims 1-32, the method comprising:

- providing at least one non-human animal;

- introducing at least one conditionally inducible gene editing sequence in a gene, part or regulatory element thereof, involved in the biosynthesis of plasmalogen to generate a first edited gene in the non-human animal;
- crossing the non-human animal with the first edited gene with a second non-human animal comprising a nucleic acid editing sequence at a separate locus encoding a gene product, to generate the non-human transgenic animal model; and
- inducing expression of the gene product in the non-human transgenic animal model to edit the first edited gene, wherein editing the first edited gene results in a decrease in the level of the gene product of the first edited gene, reduced activity of the first edited gene product of the first edited gene, reduced plasmalogen level in the transgenic animal or any combination thereof.

49. The method of any one of claims 34, 37, 40, and 48, the use of any one of claims 35, 38, 41, the non-human transgenic animal model for use of any one of claims 36, 39 and 42, or the method, use or non-human transgenic animal model for use of any one of claims 43-47, wherein the expression of the gene product is induced by providing the animal with an exogenous compound or an exogenous stimulus.

50. The method of any one of claims 34, 37, 40, and 48, the use of any one of claims 35, 38, 41, the non-human transgenic animal model for use of any one of claims 36, 39 and 42, or the method, use or non-human transgenic animal model for use of any one of claims 43-47 and 49, wherein the expression of the gene product is induced by addition of tamoxifen, 4-hydroxytamoxifen (4-OHT), mifepristone, tetracycline, light, temperature or any combination thereof.