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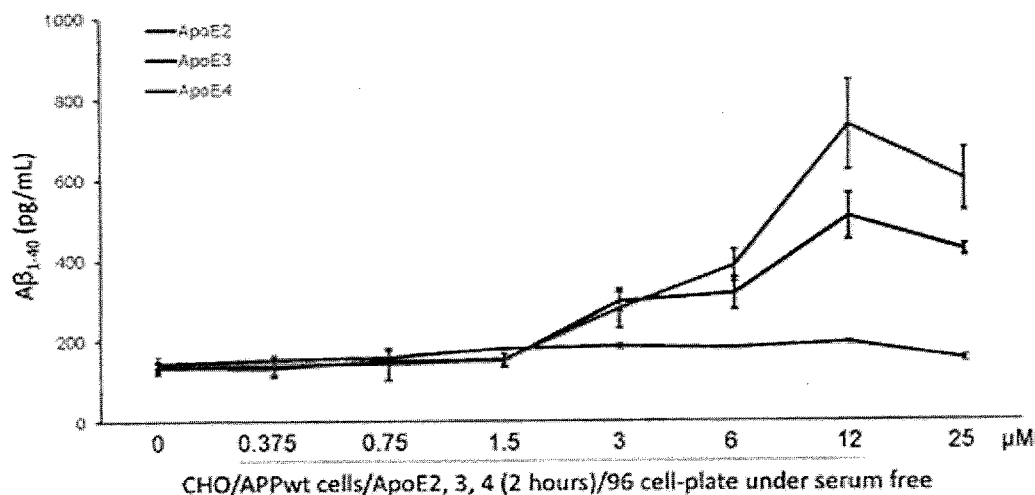


FIG. 1

(57) Abstract: Provided herein are compounds, compositions, formulations that can modulate binding of an ApoE protein to an ApoE receptor. Also provided herein are screening assays that can be capable of identifying compounds that can modulate binding of an ApoE protein to an ApoE receptor. Also provided herein are recombinant polypeptides that are capable of inhibiting binding of ApoE protein to an ApoE receptor. Also provided herein are transformed cells transgenic animals that can express a recombinant ApoE low-density lipoprotein receptor binding domain. Also provided herein are methods of administering a compound, compositions, or formulation thereof to a subject in need thereof, where the subject in need thereof suffers from or is at risk for a disease or disorder where abnormal ApoE binding to an ApoE receptor is at least part of the pathology of the disease or disorder.



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COMPOSITIONS AND METHODS OF MODULATING ABETA PROTEIN**CROSS-REFERENCE TO RELATED APPLICATIONS**

This application claims the benefit of and priority to co-pending U.S. Provisional Patent Application No. 62/331,055, filed on May 3, 2016, entitled "COMPOSITIONS AND METHODS OF MODULATING A β PROTEIN," and co-pending U.S. Provisional Patent Application No. 62/402,270, filed on September 30, 2016, entitled "COMPOSITIONS AND METHODS OF MODULATING A β PROTEIN," the contents of which are incorporated by reference herein in its entirety.

SEQUENCE LISTING

This application contains a sequence listing filed in electronic form as an ASCII.txt file entitled 292103-2710_ST25.txt, created on May 3, 2017. The content of the sequence listing is incorporated herein in its entirety.

BACKGROUND

Alzheimer's disease (AD) is an irreversible, progressive brain disorder that slowly destroys memory and thinking skills, and eventually the ability to carry out the simplest of tasks. AD is currently the sixth leading cause of death in the United States and ranks third in as the leading cause of death in the elderly. As such, there exists an urgent need for treatments for AD.

SUMMARY

Described herein are compositions that can include a compound configured to reduce binding of an apolipoprotein E (ApoE) protein to an ApoE receptor. In aspects the compound can specifically bind to a low-density lipoprotein receptor binding domain on the ApoE protein, wherein the LDL receptor binding domain has a polypeptide sequence according to SEQ ID NO: 1, or a polypeptide sequence that is about 90-100% identical to SEQ ID NO: 1, or a fragment thereof having at least 5 contiguous amino acids. The compound can be an antibody or fragment thereof. In embodiments, the compound can be a small molecule. The compound can be a competitive inhibitor of ApoE for the ApoE receptor. The compound can be a recombinant polypeptide, wherein the recombinant polypeptide can include or be a polypeptide sequence according to SEQ ID NO: 1 or a polypeptide sequence that is about 90-100% identical to SEQ ID NO: 1 and at least

sequence that is about 90-100% identical SEQ ID NO: 1. In some aspects, at least 3 or at least 6 lysine additional residues can be operatively coupled to the N-terminus of the polypeptide sequence according to SEQ ID NO: 1 or the polypeptide sequence that is about 90-100% identical SEQ ID NO: 1. In some aspects, the at least 3 additional lysine residues are operatively coupled to the C-terminus of the polypeptide sequence according to SEQ ID NO: 1 or the polypeptide sequence that is about 90-100% identical SEQ ID NO: 1. In some aspects, the at least 3 additional lysine residues are operatively coupled between the N-terminus and C-terminus of the polypeptide sequence according to SEQ ID NO: 1 or the polypeptide sequence that is about 90-100% identical SEQ ID NO: 1. The compositions described here can further include a pharmaceutical carrier.

Also provided herein are recombinant polypeptides that can include or be a polypeptide sequence according to SEQ ID NO: 1 or a polypeptide sequence that is about 90-100% identical to SEQ ID NO: 1. The recombinant polypeptide can further include at least 3 or at least 6 additional lysine residues operatively coupled to the polypeptide sequence according to SEQ ID NO: 1 or the polypeptide sequence that is about 90-100% identical SEQ ID NO: 1. In some aspects, the at least 3 or at least 6 lysine additional residues are operatively coupled to the N-terminus of the polypeptide sequence according to SEQ ID NO: 1 or the polypeptide sequence that is about 90-100% identical SEQ ID NO: 1. In some aspects, the at least 3 or at least 6 additional lysine residues are operatively coupled to the C-terminus of the polypeptide sequence according to SEQ ID NO: 1 or the polypeptide sequence that is about 90-100% identical SEQ ID NO: 1. In some aspects, the at least 3 or at least 6 additional lysine residues are operatively coupled between the N-terminus and C-terminus of the polypeptide sequence according to SEQ ID NO: 1 or the polypeptide sequence that is about 90-100% identical SEQ ID NO: 1.

Also described herein are polynucleotide sequences that can be configured to encode a recombinant polypeptide as described anywhere herein.

Also provided herein are vectors that can include vectors that can include a nucleotide sequence configured to encode a recombinant polypeptide as described herein. The vector can be an expression vector configured to express the recombinant polypeptide in a host cell.

Also provided herein are cells that can contain and/or express a recombinant polypeptide as described herein.

Also provided herein are cells that can include and/or express a polynucleotide sequence configured to encode a recombinant polypeptide as described herein.

Also provided herein are cells that can include a vector as described herein.

Also provided herein are transgenic animals that can include and/or express a recombinant polypeptide as described herein, a polynucleotide as described herein, a vector as described herein, and/or a cell as described herein.

Also provided herein are methods that can include the step of administering an amount of a composition as described herein, a recombinant polypeptide as herein, a polynucleotide as described herein, a vector as described herein, and/or as cell as described herein to a subject. The methods can further include the step of measuring binding of an apolipoprotein E (ApoE) to an ApoE receptor. The methods can further include the step of measuring A β protein formation.

The subject can have a neurologic disease or disorder. The subject can have Alzheimer's disease. The subject can have a cardiovascular disease or disorder.

Also provided herein are methods that can include the step of contacting a candidate compound with a recombinant polypeptide as described herein or an ApoE protein; and measuring binding of the recombinant polypeptide as described herein or the ApoE protein to an ApoE receptor present on the surface of a cell. In some aspects, the binding of the recombinant polypeptide as described herein or the ApoE protein to ApoE receptor can be measured by measuring ApoE receptor activity. In some aspects, the ApoE receptor can be a low-density lipoprotein receptor.

BRIEF DESCRIPTION OF THE DRAWINGS

Further aspects of the present disclosure will be readily appreciated upon review of the detailed description of its various embodiments, described below, when taken in conjunction with the accompanying drawings.

Fig. 1 shows a graph demonstrating *in vitro* amyloid β protein (A β) in CHO/APPwt cells in the presence and absence of different ApoE isoforms.

Fig. 2 shows a graph demonstrating *in vitro* amyloid β protein (A β) in CHO/APPwt cells in the presence and absence of ApoE LDL receptor binding domain peptide (SEQ ID NO.: 1).

Fig. 3 shows a graph demonstrating *in vitro* amyloid β protein (A β) in CHO/APPwt cells in the presence and absence of structurally modified ApoE LDL receptor binding domain peptide (ApoE LDLR).

Fig. 4 shows a graph demonstrating *in vitro* amyloid β protein (A β) in CHO/APPwt cells treated with Human plasma ApoE.

Fig. 5 shows a graph demonstrating *in vitro* amyloid β protein (A β) in CHO/APPwt cells treated with recombinant human ApoE3, 4 (rec hu ApoE3, 4) or Control (Ctl).

Fig. 6 shows a graph demonstrating *in vitro* amyloid β protein (A β) in CHO/APPwt cells treated with human ApoE LDL receptor binding domain (ApoE LDLR) (SEQ ID NO: 1).

Fig. 7 shows a graph demonstrating *in vitro* amyloid β protein ($A\beta$) in CHO/APPwt cells pre-treated with 3KApoE LDLR, human plasma, recombinant ApoE (rec ApoE), or FlagApoE LDLR.

Fig. 8 shows a graph demonstrating *in vitro* amyloid β protein ($A\beta$) in CHO/APPwt cells pre-treated with 3KApoE LDLR/ApoE LDLR, human plasma ApoE3 & 4, ApoB100 and LDL.

Figs. 9A-9F show data demonstrating human plasma LDL, ApoB100 and human recombinant ApoE3 & 4 proteins markedly promote amyloid β protein ($A\beta$) production. CHO cells overexpressing human wild-type APP (CHO/APPwt) cells were cultured in 96-well plate at 2×10^4 /well in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS), 1 mM sodium pyruvate and 100 U/mL of penicillin/streptomycin for 18 hours. These cells were further treated with human plasma LDL (hu LDL) (Figs. 9A and 9C), human plasma ApoB100 (hu ApoB100) (Figs. 9B and 9D), human recombinant ApoE3, 4 protein (hu rec ApoE3 or 4) or PBS (Ctrl) (Figs. 9E and 9F) at various concentrations in serum-free DMEM as indicated for 2 and 16 hours (h), followed by analysis of conditioned media and cell lysates by $A\beta$ 1-40, 42 ELISA, 82E1 western blotting (WB) analysis and protein assay, respectively. 82E1 WB analyses as shown below the $A\beta$ ELISA result histograms. The $A\beta$ ELISA results are representative of three independent experiments with each condition triplicated and presented as mean $\pm$ SD.

Figs. 10A-10C show data demonstrating ApoE LDL receptor binding domain peptide (ApoE-LBDP) treatment significantly increases $A\beta$ generation, which is structurally dependable. CHO/APPwt cells were cultured in 96-well plate at 2×10^4 /well in DMEM with 10% FBS for overnight and then serum-free DMEM in the presence or absence of ApoE LDL receptor binding domain peptide [ApoE-LBDP, LRVRLASHLRKLRKRLLRDA (residues 133-152)] as indicated for 2 and 16 hours (h). The conditioned media and cell lysates were analyzed by $A\beta$ 1-40, 42 ELISA (Fig. 10A), 82E1 WB analysis (Fig. 10B) and protein assay, respectively. In addition, (Fig. 10C) CHO/APPwt cells were cultured in 96-well plate (2×10^4 /well) in serum-free DMEM and treated with FlagApoE-LBDP, BiotinApoE-LBDP and 3 lysineApoE-LBDP (3KApoE-LBDP) in comparing to ApoE-LBDP at indicated doses for 2 hours (h). The conditioned media and cell lysates were prepared, and subjected to $A\beta$ 1-40, 42 ELISA ($**P < 0.01$; $***P < 0.001$ when compared to ApoE-LBDP) and protein assay. These results are representative of three independent experiments with each condition triplicated. Note: KKK-LRVRLASHLRKLRKRLLRDA; DYKDDDDK-LRVRLASHLRKLRKRLLRDA; Biotin-LRVRLASHLRKLRKRLLRDA.

Figs. 11A-11D show data demonstrating 6KApoE markedly inhibits $A\beta$ generation induced by human recombinant ApoE4 protein (hu rec ApoE4). CHO/APPwt cells were cultured in 96-well plate (2×10^4 /well) in serum-free medium and pre-treated with PBS (Ctrl), 3 lysine (3K), 6K lysine (6K), 3KApoE-LBDP (3KApoE), 6KApoE-LBDP (6KApoE), 7KApoE-

LBDP (7KApoE), 8KApoE-LBDP (8KApoE), 9KApoE-LBDP (9KApoE) peptide at 10 μ M for 15 minutes and went to treatment with or without human recombinant ApoE4 protein (hu rec ApoE4, 10 μ g/mL) for 2 hours. The conditioned media and cell lysates were prepared, and subjected to A β 1-40, 42 ELISA and protein assay. Further, CHO/APPwt cells were cultured in 96-well plate (2X10⁴/well) in serum-free DMEM and pre-treated with 6KApoE for 15 minutes at various doses as indicated and then went to treatment with human recombinant ApoE4 protein (hu rec ApoE4) for 2 and 16 hours. The conditioned media and cell lysates were prepared, and subjected to A β 1-40, 42 ELISA (Fig. 11C), 82E1 WB analysis (Fig. 11D) and protein assay, respectively. The A β 1-40, 42 ELISA results are representative of three independent experiments with each condition triplicated and presented as mean $\pm$ SD.

Figs. 12A-12F show data demonstrating LDL receptor is in part responsive for 6KApoE inhibition of A β generation induced by human recombinant ApoE4 protein. CHO/APPwt cells were cultured in 96-well plate (2X10⁴/well) in serum-free medium and pre-treated with 6KApoE at 1 μ M for 15 minutes and went treatment with an antagonist anti-LDLR antibody (anti-LDLR Ab) from 0 - 2.5 μ g/mL for 2 hours. The conditioned media and cell lysates were prepared, and subjected to A β 1-40, 42 ELISA (Fig. 12A), 82E1 WB analysis (Fig. 12B) and protein assay. Hamster LDLR siRNA and negative control were ordered from Thermo Fisher Scientific, transfection was performed following the protocol of lipofectamine RNAiMAX reagent provided by Thermo Fisher Scientific. Briefly, CHO APPwt cells were plated in 24 well plate 1X10⁵ cells/well for overnight, siRNAs were transfected at 10 nM as indicated, 24 hours after these cells were washed and treated with human recombinant ApoE4 protein (hu rec ApoE4) at 10 μ g/mL for 2 hours (h) in the presence or absence of 15-minute pre-treatment of 1 μ M 6KApoE. The conditioned media and cell lysates were prepared, and subjected to A β 1-40, 42 ELISA (Fig. 12C), anti-LDLR antibody WB evaluation (Fig. 12D) and protein assay. CHO/IdIA7 (IdIA7) and CHO wild-type (CHO) cells were provided by Dr. Monty Krieger (Massachusetts Institute of Technology, Cambridge, MA). The two cells were cultured in Ham's F-12 medium supplemented with 5% FBS, 2 mM L-glutamine. The cells were plated into 24-well plate at 1X10⁵ each well the day before transfection. PCMV6-APP695 (OriGene Technologies, Inc. Rockville, MD) was transfected to these cells using Lipofectamine® 3000 Transfection Reagent (Thermofisher Scientific) according to the instructions. Twenty-four hours after transfection, these cells were washed and treated with human recombinant ApoE4 protein (hu rec ApoE4) at 10 μ g/mL for 2 hours (h) in the presence or absence of 15-minute pre-treatment of 1 μ M 6KApoE. The conditioned media and cell lysates were prepared, and subjected to A β 1-40 ELISA (Fig. 12E), anti-LDLR antibody and APP processing WB evaluation (Fig. 12F), and protein assay. For A β ELISA, these results are representative of three independent experiments with each condition triplicated and presented as mean $\pm$ SD.

Figs. 13A-13B show graphs that can demonstrate amounts of A β ₁₋₄₀ (Fig. 13A) and A β ₁₋₄₂ (Fig. 13B) as measured by enzyme linked immunosorbent assay (ELISA) the brain of control (5XFAD) and treated (5XFAD/6KApoEp) mice after peripheral administration of 6KApoEp treatment.

5 Figs. 14A-14B show graphs that can demonstrate amounts of A β ₁₋₄₀ (Fig. 14A) and A β ₁₋₄₂ (Fig. 14B) as measured by ELISA in the blood of control (5XFAD) and treated (5XFAD/6KApoEp) mice.

Figs. 15A-15C show images of representative blots from a Western blot analysis for total A β (Fig. 15A), APP β -CFT (Fig. 15B) in control (5XFAD/Ctrl) and treated
10 (5XFAD/6KApoEp). Results were normalized to β -actin (Fig. 15C).

Figs. 16A-16G show images of representative blots from a Western blot analysis for Alzheimer-like acetylated (a-tau K²⁷⁴ (Fig. 16A) and a-tau K¹⁷⁴ (Fig. 16B)) and phosphorylated tau (p-tau Thr²³¹ (Fig. 16D), p-tau Thr⁴⁰⁴ (Fig. 16E), PHF (Fig. 16F), and total tau (Figs. 16C and 16G) in control (Ctrl) and treated (6KApoEp). Band density ratios of acetylated or
15 phosphorylated tau to total tau was also determined by densitometry analysis.

Figs. 17A-17B show graphs that demonstrate the results from the densitometry analysis of the Western blots demonstrated in Figs. 16A-16G. Band density ratios of acetylated or phosphorylated tau to total tau was also determined by densitometry analysis. (Ctrl (control), *P<0.05, **P<0.01, ***P<0.005)

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DETAILED DESCRIPTION

Before the present disclosure is described in greater detail, it is to be understood that this disclosure is not limited to particular embodiments described, and as such may, of course,
25 vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated
30 range, is encompassed within the disclosure. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges and are also encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure.

35 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 this disclosure

belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present disclosure, the preferred methods and materials are now described.

All publications and patents cited in this specification are cited to disclose and describe the methods and/or materials in connection with which the publications are cited. All such publications and patents are herein incorporated by references as if each individual publication or patent were specifically and individually indicated to be incorporated by reference. Such incorporation by reference is expressly limited to the methods and/or materials described in the cited publications and patents and does not extend to any lexicographical definitions from the cited publications and patents. Any lexicographical definition in the publications and patents cited that is not also expressly repeated in the instant application should not be treated as such and should not be read as defining any terms appearing in the accompanying claims. The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that the present disclosure is not entitled to antedate such publication by virtue of prior disclosure. Further, the dates of publication provided could be different from the actual publication dates that may need to be independently confirmed. As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present disclosure. Any recited method can be carried out in the order of events recited or in any other order that is logically possible.

Embodiments of the present disclosure will employ, unless otherwise indicated, techniques of molecular biology, microbiology, nanotechnology, organic chemistry, biochemistry, cell biology, and the like, which are within the skill of the art. Such techniques are explained fully in the literature.

Definitions

As used herein, "control" is an alternative subject or sample used in an experiment for comparison purpose and included to minimize or distinguish the effect of variables other than an independent variable.

As used herein, "biocompatible" or "biocompatibility" refers to the ability of a material to be used by a patient without eliciting an adverse or otherwise inappropriate host response in the patient to the material or a derivative thereof, such as a metabolite, as compared to the host response in a normal or control patient.

As used herein, "biodegradable" refers to the ability of a material or compound to be decomposed by bacteria or other living organisms or organic processes.

As used herein, "about," "approximately," and the like, when used in connection with a numerical variable, generally refers to the value of the variable and to all values of the variable that are within the experimental error (e.g., within the 95% confidence interval for the mean) or within $\pm 10\%$ of the indicated value, whichever is greater.

5 As used herein, "'effective amount" is an amount sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications, or dosages.

As used herein, "administering" can refer to an administration that is oral, topical, intravenous, subcutaneous, transcutaneous, transdermal, intramuscular, intra-joint, parenteral, intra-arteriole, intradermal, intraventricular, intracranial, intraperitoneal, 10 intralesional, intranasal, rectal, vaginal, by inhalation, by catheters, stents or via an implanted reservoir or other device that administers, either actively or passively (e.g. by diffusion) a composition the perivascular space and adventitia. For example a medical device such as a stent can contain a composition or formulation disposed on its surface, which can then dissolve or be otherwise distributed to the surrounding tissue and cells. The term "parenteral" 15 can include subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional, and intracranial injections or infusion techniques.

As used herein, "preventative" refers to hindering or stopping a disease or condition 20 before it occurs or while the disease or condition is still in the sub-clinical phase.

As used herein, "therapeutic" can refer to treating or curing a disease or condition.

As used interchangeably herein, "subject," "individual," or "patient," refers to a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, murines, simians, humans, farm animals, sport animals, and pets. The term "pet" 25 includes a dog, cat, guinea pig, mouse, rat, rabbit, ferret, and the like. The term farm animal includes a horse, sheep, goat, chicken, pig, cow, donkey, llama, alpaca, turkey, and the like.

The terms "operatively linked" or "operatively coupled" as used herein can refer to the association of nucleic acid sequences on a single nucleic acid fragment so that the function of one is regulated by the other. For example, a promoter is operatively linked with a coding 30 sequence when it is capable of regulating the expression of that coding sequence (i.e., the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operatively linked to regulatory sequences in a sense or antisense orientation. In one example, the complementary RNA regions can be operatively linked, either directly or indirectly, 5' to the target mRNA, or 3' to the target mRNA, or within the target mRNA, or a first 35 complementary region is 5' and its complement is 3' to the target mRNA. The term "operatively linked" as used herein can also refer to the direct or indirect linkage of any two nucleic acid sequences on a singly nucleic acid fragment such that they are indirectly or directly physically

connected on the same nucleic acid fragment. The term "operatively linked" as used herein can also refer to the insertion of a nucleic acid within the 5' and 3' end of another nucleic acid or the direct coupling of a nucleic acid to the 5' or 3' end of another nucleic acid.

As used herein, "specific binding," "specifically bind," and the like refer to binding which occurs between such paired species as enzyme/substrate, receptor/agonist, antibody/antigen, and lectin/carbohydrate which may be mediated by covalent or non-covalent interactions or a combination of covalent and non-covalent interactions. When the interaction of the two species produces a non-covalently bound complex, the binding which occurs is typically electrostatic, hydrogen-bonding, or the result of lipophilic interactions. Accordingly, "specific binding" occurs between a paired species where there is interaction between the two which produces a bound complex having the characteristics of an antibody/antigen or enzyme/substrate interaction. In particular, the specific binding is characterized by the binding of one member of a pair to a particular species and to no other species within the family of compounds to which the corresponding member of the binding member belongs. Thus, for example, an antibody preferably binds to a single epitope and to no other epitope within the family of proteins. As another non-limiting example, a miRNA can specifically bind preferably to a miRNA target and not to a non-specific nucleic acid sequence or if binding to a non-specific nucleic acid sequence occurs that no change in the expression or function of the non-specific nucleic acid can be observed or detected.

As used herein, "differentially expressed," refers to the differential production of RNA, including but not limited to mRNA, tRNA, miRNA, siRNA, snRNA, and piRNA transcribed from a gene or regulatory region of a genome or the protein product encoded by a gene as compared to the level of production of RNA or protein by the same gene or regulator region in a normal or a control cell. In another context, "differentially expressed," also refers to nucleotide sequences or proteins in a cell or tissue which have different temporal and/or spatial expression profiles as compared to a normal or control cell.

As used herein, "polypeptides" or "proteins" are amino acid residue sequences. Those sequences are written left to right in the direction from the amino to the carboxy terminus. In accordance with standard nomenclature, amino acid residue sequences are denominated by either a three letter or a single letter code as indicated as follows: Alanine (Ala, A), Arginine (Arg, R), Asparagine (Asn, N), Aspartic Acid (Asp, D), Cysteine (Cys, C), Glutamine (Gln, Q), Glutamic Acid (Glu, E), Glycine (Gly, G), Histidine (His, H), Isoleucine (Ile, I), Leucine (Leu, L), Lysine (Lys, K), Methionine (Met, M), Phenylalanine (Phe, F), Proline (Pro, P), Serine (Ser, S), Threonine (Thr, T), Tryptophan (Trp, W), Tyrosine (Tyr, Y), and Valine (Val, V).

As used herein, "gene" can refer to a hereditary unit corresponding to a sequence of DNA that occupies a specific location on a chromosome and that contains the genetic instruction for a characteristic(s) or trait(s) in an organism. "Gene" also refers to the specific

sequence of DNA that is transcribed into an RNA transcript that can be translated into a polypeptide or be a catalytic RNA molecule including but not limited to tRNA, siRNA, piRNA, miRNA, long-non-coding RNA and shRNA.

As used herein, "deoxyribonucleic acid (DNA)" and "ribonucleic acid (RNA)" generally refer to any polyribonucleotide or polydeoxribonucleotide, which can be unmodified RNA or DNA or modified RNA or DNA. RNA can be in the form of non-coding RNA such as tRNA (transfer RNA), snRNA (small nuclear RNA), rRNA (ribosomal RNA), anti-sense RNA, RNAi (RNA interference construct), siRNA (short interfering RNA), microRNA (miRNA), or ribozymes, aptamers or coding mRNA (messenger RNA).

As used herein, "nucleic acid sequence" and "oligonucleotide" also encompasses a nucleic acid and polynucleotide as defined elsewhere herein.

As used herein, "DNA molecule" includes nucleic acids/polynucleotides that are made of DNA.

As used herein, "nucleic acid" and "polynucleotide" generally refer to a string of at least two base-sugar-phosphate combinations and refers to, among others, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that can be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, polynucleotide as used herein refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The strands in such regions can be from the same molecule or from different molecules. The regions can include all of one or more of the molecules, but more typically involve only a region of some of the molecules. One of the molecules of a triple-helical region often is an oligonucleotide. "Polynucleotide" and "nucleic acids" also encompasses such chemically, enzymatically or metabolically modified forms of polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including simple and complex cells, inter alia. For instance, the term polynucleotide includes DNAs or RNAs as described above that contain one or more modified bases. Thus, DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritylated bases, to name just two examples, are polynucleotides as the term is used herein. "Polynucleotide" and "nucleic acids" also includes PNAs (peptide nucleic acids), phosphorothioates, and other variants of the phosphate backbone of native nucleic acids. Natural nucleic acids have a phosphate backbone, artificial nucleic acids can contain other types of backbones, but contain the same bases. Thus, DNAs or RNAs with backbones modified for stability or for other reasons are "nucleic acids" or "polynucleotide" as that term is intended herein.

As used herein, "microRNA" can refer to a small non-coding RNA molecule containing about 21 to about 23 nucleotides found in organisms, which functions in transcriptional and

post-transcriptional regulation of transcription and translation of RNA. "MicroRNA" can exist as part of a larger nucleic acid molecule such as a stem-loop structure that can be processed by a cell and yield a microRNA of about 21-23 nucleotides.

As used herein, "pharmaceutically acceptable carrier, diluent, binders, lubricants, glidants, preservative, flavoring agent, coloring agent, and excipient" refers to a carrier, diluent, binder, lubricant, glidant, preservative, flavoring agent, coloring agent, or excipient that is useful in preparing a pharmaceutical formulation that is generally safe, non-toxic, and is neither biologically or otherwise undesirable, and includes a carrier or excipient that is acceptable for veterinary use as well as human pharmaceutical use.

The term "treating", as used herein, can include inhibiting the disease, disorder or condition, e.g., impeding its progress; and relieving the disease, disorder, or condition, e.g., causing regression of the disease, disorder and/or condition. Treating the disease, disorder, or condition can include ameliorating at least one symptom of the particular disease, disorder, or condition, even if the underlying pathophysiology is not affected, such as treating the pain of a subject by administration of an analgesic agent even though such agent does not treat the cause of the pain.

As used herein, "overexpressed" or "overexpression" refers to an increased expression level of an RNA (coding or non-coding RNA) or protein product encoded by a gene as compared to the level of expression of the RNA or protein product in a normal or control cell.

As used herein, "underexpressed" or "underexpression" refers to decreased expression level of an RNA (coding or non-coding RNA) or protein product encoded by a gene as compared to the level of expression of the RNA or protein product in a normal or control cell.

As used herein, "expression" refers to the process by which polynucleotides are transcribed into RNA transcripts. In the context of mRNA and other translated RNA species, "expression" also refers to the process or processes by which the transcribed RNA is subsequently translated into peptides, polypeptides, or proteins.

As used herein with reference to the relationship between DNA, cDNA, cRNA, RNA, and protein/peptides, "corresponding to" or "encoding" can refer to the underlying biological relationship between these different molecules. As such, one of skill in the art would understand that operatively "corresponding to" can direct them to determine the possible underlying and/or resulting sequences of other molecules given the sequence of any other molecule which has a similar biological relationship with these molecules. For example, from a DNA sequence an RNA sequence can be determined and from an RNA sequence a cDNA sequence can be determined.

As used herein, "promoter" can include all sequences capable of driving transcription of a coding or a non-coding sequence. In particular, the term "promoter" as used herein refers

to a DNA sequence generally described as the 5' regulator region of a gene, located proximal to the start codon. The transcription of an adjacent coding sequence(s) is initiated at the promoter region. The term "promoter" also includes fragments of a promoter that are functional in initiating transcription of the gene.

5 As used herein, "selectable marker" or can refer to a gene whose expression allows one to identify cells that have been transformed with a nucleic acid (naked, contained in a vector, or a plasmid) containing the selectable marker gene. For instance, a recombinant nucleic acid may include a selectable marker operatively linked to a gene of interest (e.g. a gene encoding a recombinant polypeptide) and a promoter, such that expression of the
10 selectable marker indicates the successful transformation of the cell with the gene of interest. In some instances the selectable marker gene can be operative linked to a gene of interest such that when they are expressed as proteins the selectable marker allows for identification of expression of the gene of interest. "Selectable marker" can also be referred to herein as a reporter gene or polypeptide.

15 As used herein, "constitutive promoter" can refer to a promoter that allows for continual or ubiquitous transcription of its associated gene or polynucleotide. Constitutive promoters are generally are unregulated by cell or tissue type, time, or environment.

As used herein, "inducible promoter" can refer to a promoter that allows transcription of its associated gene or polynucleotide in response to a substance or compound (e.g. an
20 antibiotic, or metal), an environmental condition (e.g. temperature), developmental stage, or tissue type.

As used herein, "electroporation" is a transformation method in which a high concentration of plasmid DNA (containing exogenous DNA) or RNA is added to a suspension of host cell protoplasts, and the mixture shocked with an electrical field of about 200 to 600
25 V/cm.

As used herein, "plasmid" can refer to a non-chromosomal double-stranded DNA sequence including an intact "replicon" such that the plasmid is replicated in a host cell.

As used herein, the term "vector" can refer to a vehicle used to introduce an exogenous nucleic acid sequence into a cell. A vector can include a DNA molecule, linear or circular (e.g.
30 plasmids), which includes a segment encoding a polypeptide of interest operatively linked to additional segments that provide for its transcription and translation upon introduction into a host cell or host cell organelles. Such additional segments can include promoter and terminator sequences, internal ribosome entry site, and may also include one or more origins of replication, one or more selectable markers, an enhancer, a polyadenylation signal,
35 microRNA target sequences etc. Expression vectors are generally derived from yeast or bacterial genomic or plasmid DNA, or viral DNA, or can contain elements of both. The term "vector" can also include RNA or circular RNA vectors linked to additional segments that

provide for its translation upon introduction into a host cell or host cell organelles. Such additional segments can include 5'Cap, one or more selectable markers, an enhancer, a polyadenylation signal, polyA tail, microRNA target sequences etc. The term vector includes viral vectors and multi-vector viral vector systems (adeno, lentiviral, retroviral, and the like) that can be used to introduce a transgene into the genome of a cell. Such viral vectors and viral vector systems will be generally known by those skilled in the art.

As used herein, "identity," can refer to a relationship between two or more polypeptide sequences, as determined by comparing the sequences. In the art, "identity" also refers to the degree of sequence relatedness between polypeptide as determined by the match between strings of such sequences. "Identity" can be readily calculated by known methods, including, but not limited to, those described in (Computational Molecular Biology, *Lesk, A. M.*, Ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, *Smith, D. W.*, Ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, *Griffin, A. M., and Griffin, H. G.*, Eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, *von Heinje, G.*, Academic Press, 1987; and Sequence Analysis Primer, *Gribskov, M. and Devereux, J.*, Eds., M Stockton Press, New York, 1991; and *Carillo, H., and Lipman, D.*, SIAM J. Applied Math. 1988, 48: 1073. Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity are codified in publicly available computer programs. The percent identity between two sequences can be determined by using analysis software (e.g., Sequence Analysis Software Package of the Genetics Computer Group, Madison Wis.) that incorporates the *Needelman and Wunsch*, (J. Mol. Biol., 1970, 48: 443-453,) algorithm (e.g., NBLAST, and XBLAST). The default parameters are used to determine the identity for the polypeptides of the present disclosure.

As used herein, the term "transfection" refers to the introduction of an exogenous and/or recombinant nucleic acid sequence into the interior of a membrane enclosed space of a living cell, including introduction of the nucleic acid sequence into the cytosol of a cell as well as the interior space of a mitochondria, nucleus, or chloroplast. The nucleic acid may be in the form of naked DNA or RNA (unmodified or modified), it may be associated with various proteins or regulatory elements (e.g., a promoter and/or signal element, miRNA target sequences as described herein), or the nucleic acid may be incorporated into a vector or a chromosome.

As used herein, "transformation" or "transformed" refers to the introduction of a nucleic acid (e.g., DNA or RNA) into cells in such a way as to allow expression of the coding or non-coding portions of the introduced nucleic acid.

As used herein a "transformed cell" is a cell transformed with a nucleic acid sequence.

As used herein, a "transgene" refers to an artificial gene which is used to transform a cell of an organism, such as a bacterium or a plant.

As used herein, "transgenic" refers to a cell, tissue, or organism that contains a
5 transgene.

As used herein, the term "recombinant" generally refers to a non-naturally occurring nucleic acid, nucleic acid construct, or polypeptide. Such non-naturally occurring nucleic acids can include natural nucleic acids that have been modified, for example that have deletions, substitutions, inversions, insertions, *etc.*, and/or combinations of nucleic acid sequences of
10 different origin that are joined using molecular biology technologies (*e.g.*, a nucleic acid sequences encoding a "fusion protein" (*e.g.*, a protein or polypeptide formed from the combination of two different proteins or protein fragments), the combination of a nucleic acid encoding a polypeptide to a promoter sequence, where the coding sequence and promoter sequence are from different sources or otherwise do not typically occur together naturally (*e.g.*,
15 a nucleic acid and a constitutive promoter *etc.*). Recombinant also refers to the polypeptide encoded by the recombinant nucleic acid. Non-naturally occurring nucleic acids or polypeptides include nucleic acids and polypeptides modified by man, including but not limited to miRNA target sequences described herein.

As used herein, the term "exogenous DNA" or "exogenous RNA" or exogenous nucleic
20 acid sequence" or "exogenous polynucleotide" refers to a nucleic acid sequence that was introduced into a cell, organism, or organelle via transfection or transduction. Exogenous nucleic acids originate from an external source, for instance, the exogenous nucleic acid may be from another cell or organism and/or it may be synthetic and/or recombinant. While an exogenous nucleic acid sometimes originates from a different organism or species, it may also
25 originate from the same species (*e.g.*, an extra copy or recombinant form of a nucleic acid that is introduced into a cell or organism in addition to or as a replacement for the naturally occurring nucleic acid). Typically, the introduced exogenous sequence is a recombinant sequence.

Discussion

30 Alzheimer's disease (AD) is an irreversible, progressive brain disorder that slowly destroys memory and thinking skills, and eventually the ability to carry out the simplest of tasks. AD is currently the sixth leading cause of death in the United States and ranks third in as the leading cause of death in the elderly. Part of the pathology of AD is the abnormal formation of amyloid plaques and tau tangles in the brain and loss of connections between
35 neurons. Amyloid plaques are made up of amyloid beta peptides (A β), which are fragments of an amyloid precursor protein (APP).

Apolipoprotein E (ApoE) is a class of apolipoprotein found in the chylomicron and Intermediate-density lipoprotein (IDL) that is essential for the normal catabolism of triglyceride-rich lipoprotein constituents (Liu et al., 2013). In peripheral tissues, ApoE is primarily produced by the liver and macrophages, and mediates cholesterol metabolism in an isoform-dependent manner. In the central nervous system, ApoE is mainly produced by astrocytes, and transports cholesterol to neurons *via* APOE receptors, including LRP, which are members of the low density lipoprotein receptor (LDLR) gene family (Liu et al., 2013). Importantly, ApoE is involved in Alzheimer's disease and cardiovascular disease (Ian P. Stolermer, 2010).

ApoE is 299 amino acids long and contains multiple amphipathic α -helices. According to crystallography studies, a hinge region connects the N- and C-terminal regions of the protein. The N-terminal region (residues 1–167) forms an anti-parallel four-helix bundle such that the non-polar sides face inside the protein. Meanwhile, the C-terminal domain (residues 206–299) contains three α -helices which form a large exposed hydrophobic surface and interact with those in the N-terminal helix bundle domain through hydrogen bonds and salt-bridges. The N-terminal region also contains the low density lipoprotein receptor (LDLR)-binding site (residues 134–150) (Philips et al., 2014).

ApoE transports lipoproteins, fat-soluble vitamins, and cholesterol into the lymph system and then into the blood. It is synthesized principally in the liver, but has also been found in other tissues such as the brain, kidneys, and spleen (Baars et al., 2011). In the nervous system, non-neuronal cell types, most notably astroglia and microglia, are the primary producers of ApoE, while neurons preferentially express the receptors for ApoE (Zhang et al., 2013). There are seven currently identified mammalian receptors for ApoE, including LRP, which belong to the evolutionarily conserved LDLR family (Rogers et al., 2008).

APOE is polymorphic (Singh et al., 2006; Eisenberg et al., 2010) with three major alleles: *ApoE2* (cys112, cys158), *ApoE3* (cys112, arg158), and *ApoE4* (arg112, arg158) (Baars et al., 2011; Ghebranious et al., 2005). Although these allelic forms differ from each other by only one or two amino acids at positions 112 and 158, these differences alter ApoE structure and function, which have physiological consequences.

ApoE2 (rs7412), which has an allele frequency of approximately 7 percent, binds poorly to cell surface receptors compared with *ApoE3* and *E4* (Giveira et al., 1996). Most interestingly, *ApoE2*, which is severely defective in LDLR binding activity, with 2% LDLR binding activity compared with *ApoE3*, differs structurally from *ApoE3* and *ApoE4*, which bind avidly to LDLR. This reduced binding affinity of *ApoE2* is likely mediated by the presence of cysteine rather than arginine in position 158, thereby altering the conformation and size of the positive potential in the binding region (Mahley et al., 2009). *ApoE3* (rs429358), which has an allele frequency of approximately 79 percent (Alzheimer Research Forum), is considered the "neutral" Apo E genotype. In this isoform, the 134–150 binding region is largely solvent

exposed and forms a 20Å field of positive potential, likely available for receptor binding. ApoE4, which has an allele frequency of approximately 14 percent, has been implicated in atherosclerosis, Alzheimer's disease and impaired cognitive function (Corder et al., 1993; Deary et al., 2002

5 With that said, described herein are compounds, compositions, and formulations that can modulate the binding of an ApoE protein and a LDL receptor and/or an ApoE receptor. Also described herein are recombinant polypeptides that can contain an ApoE LDLR binding domain, and optionally, additional lysine residues. The compounds, compositions, and formulations described herein can reduce ApoE protein binding to an LDLR or ApoE receptor.
10 Also described herein are polynucleotides configured to encode the recombinant polynucleotides. Also described herein are cells and transgenic animals that can contain one or more recombinant polypeptides or polynucleotides described herein. Also provided herein are methods of administering the compounds, compositions, and formulations described herein to a subject. The subject can be suffering from a cardiovascular, vascular, or neurologic
15 disease or disorder. The subject can be suffering from A. The compounds, compositions, and formulations can be useful to treat cardiovascular, vascular, and/or neurologic diseases. Also provided herein are assays for detecting compounds, compositions, and formulations capable of modulating the binding of ApoE protein and a LDL receptor and/or an ApoE receptor.

Other compositions, compounds, methods, features, and advantages of the present
20 disclosure will be or become apparent to one having ordinary skill in the art upon examination of the following drawings, detailed description, and examples. It is intended that all such additional compositions, compounds, methods, features, and advantages be included within this description, and be within the scope of the present disclosure.

Compositions

25 Provided herein are compositions configured to modulate binding of a protein to an ApoE receptor. ApoE receptors are known in the art and include LDL receptor (LDLR), Very low-density lipoprotein receptors (VLDLRs), Apoer2, and lipoprotein receptor-related protein 1 (LRP1). The LDL receptor is generally known in the art and will be instantly appreciated by those of skill in the art. See e.g. Brown and Goldstein (1979) Proc. Natl. Acad. Sci. 76(7):3330-
30 3337; Hobbs et al., (1993) Hum. Mutat. 1(6): 445-466; May et al., (2003) Sci. STKE (175): PE12; and Braakman (2004) Cell. Mol. Life Sci. 61(19-20): 2461-2470. The compositions can be configured to reduce or decrease the amount of binding of an ApoE protein to an ApoE receptor as compared to a suitable control. Suitable controls will be instantly appreciated by one of ordinary skill in the art.

35 In embodiments, the composition can include a compound that can specifically bind to a LDLR binding domain of the ApoE protein. In embodiments, the LDLR binding domain of the ApoE protein can have a sequence according to SEQ ID NO.: 1. In other embodiments, the

LDLR binding domain of the ApoE protein can have a sequence that is about 90% to about 100% identical to SEQ ID NO.: 1. In further embodiments, the compound can specifically bind to a fragment of the LDLR binding domain wherein the fragment can contain at least 5 contiguous amino acids of SEQ ID NO.:1 (LRVRLASHLRKLRKRLLRDA) or a sequence that
5 is about 90% to about 100% identical to SEQ ID NO.: 1

In some embodiments, the compound that can modulate binding of an ApoE protein and an ApoE receptor can be an antibody or fragment thereof. The antibody can be configured to specifically bind to a LDLR binding domain of the ApoE protein. In embodiments, the LDLR binding domain of the ApoE protein can have a sequence according to SEQ ID NO.: 1. In other
10 embodiments, the LDLR binding domain of the ApoE protein can have a sequence that is about 90% to about 100% identical to SEQ ID NO.: 1. In further embodiments, the antibody or fragment thereof can specifically bind to a fragment of the LDLR binding domain wherein the fragment can contain at least 5 contiguous amino acids of SEQ ID NO.:1 (LRVRLASHLRKLRKRLLRDA) or a sequence that is about 90% to about 100% identical to
15 SEQ ID NO.: 1. The antibody can be a monoclonal antibody or a polyclonal antibody. Methods of producing antibodies and screening antibodies are generally known in the art and will be appreciated by one of ordinary skill in the art.

In other embodiments, the compound that can be configured to modulate binding of ApoE protein to an ApoE protein receptor can be a small molecule compound. As used in this
20 context, "small molecule" can refer to a low molecular weight organic or inorganic compound or composition (less than about 900 daltons) that can elicit a biologic response in a cell, tissue, organ, and/or organism and having a size on the order of about 10^{-9} m or less.

In some embodiments, the compound can be a competitive inhibitor of the ApoE for the ApoE receptor. In other words, in some embodiments, the compound can compete with
25 ApoE for binding to the ApoE receptor. While not being bound to theory, in this way the compound can reduce binding of the ApoE to the ApoE receptor.

In some embodiments, the compound can be a recombinant polypeptide. The recombinant polypeptide can contain a polypeptide sequence according to SEQ ID NO: 1 or a polypeptide sequence that is about 90-100% identical to SEQ ID NO: 1 and at least 3 or at
30 least 6 additional lysine residues operatively coupled to the polypeptide sequence according to SEQ ID NO: 1 or the polypeptide sequence that is about 90-100% identical SEQ ID NO: 1. In some embodiments, the number of additional lysine residues can range from 3 to 20. In other embodiments the number of additional lysine residues can range from 3-9. In some embodiments, the at least 3 lysine or at least 6 additional residues can be operatively coupled
35 to the N-terminus of the polypeptide sequence according to SEQ ID NO: 1 or the polypeptide sequence that is about 90-100% identical SEQ ID NO: 1. In other embodiments, the at least 3 or the at least 6 additional lysine residues are operatively coupled to the C-terminus of the

polypeptide sequence according to SEQ ID NO: 1 or the polypeptide sequence that is about 90-100% identical SEQ ID NO: 1. In further embodiments, the at least 3 or the at least 6 additional lysine residues are operatively coupled between the N-terminus and C-terminus of the polypeptide sequence according to SEQ ID NO: 1 or the polypeptide sequence that is about 90-100% identical SEQ ID NO: 1. In some embodiments, the recombinant polypeptide can be according to any one of SEQ ID NOs: 2-9. In some embodiments the number of additional lysine residues can be 6.

Recombinant Polypeptides, Polynucleotides, and Vectors

Provided herein are recombinant polypeptides that can contain a polypeptide sequence according to any one of SEQ ID NOs.: 1-9 or a polypeptide sequence that is about 90-100% identical to any one of SEQ ID NOs.: 1-9. The recombinant polypeptides can include one or more reporter proteins (also referred to as selectable markers) operatively linked to the polypeptide sequence according to any one of SEQ ID NOs.: 1-9 or a polypeptide sequence that is about 90-100% identical to any one of SEQ ID NOs.: 1-9. Exemplary reporter proteins include but are not limited to β -galactosidase, GUS; fluorescent proteins such as green fluorescent protein (GFP), cyan (CFP), yellow (YFP), red (RFP), luciferase, cell surface proteins and, epitope tags such as but not limited to, e.g. FLAG- and His-tags.

Modifications and changes can be made in the structure of the polypeptides of the present disclosure that result in a molecule having similar characteristics as the unmodified polypeptide (e.g., a conservative amino acid substitution). Modification techniques are generally known in the art. For example, certain amino acids can be substituted for other amino acids in a sequence without appreciable loss of activity. Because it is the interactive capacity and nature of a polypeptide that defines that polypeptide's biological functional activity, certain amino acid sequence substitutions can be made in a polypeptide sequence and nevertheless obtain a functional variant. Polypeptides with amino acid sequence substitutes that still retain properties substantially similar to polypeptides corresponding to any one of SEQ ID NOs.: 1-9 or a polypeptide sequence that is about 90-100% identical to any one of SEQ ID NOs.: 1-9 are within the scope of this disclosure.

Also provided herein are polynucleotides that can encode on or more of the recombinant polypeptides described herein. The polynucleotides can further include one or more selectable marker (or reporter) genes. Examples of selectable markers include, but are not limited to, DNA and/or RNA segments that contain restriction enzyme sites; DNA segments that encode products that provide resistance against otherwise toxic compounds including antibiotics, such as, spectinomycin, ampicillin, kanamycin, tetracycline, Basta, neomycin phosphotransferase II (NEO), hygromycin phosphotransferase (HPT)) and the like; DNA and/or RNA segments that encode products that are otherwise lacking in the recipient cell (e.g., tRNA genes, auxotrophic markers); DNA and/or RNA segments that encode products

which can be readily identified (e.g., phenotypic markers such as β -galactosidase, GUS; fluorescent proteins such as green fluorescent protein (GFP), cyan (CFP), yellow (YFP), red (RFP), luciferase, and cell surface proteins); the generation of new primer sites for PCR (e.g., the juxtaposition of two DNA sequence not previously juxtaposed), the inclusion of DNA sequences not acted upon or acted upon by a restriction endonuclease or other DNA modifying enzyme, chemical, etc.; epitope tags (e.g. FLAG- and His-tags), and, the inclusion of a DNA sequences required for a specific modification (e.g., methylation) that allows its identification. Other suitable markers will be appreciated by those of skill in the art.

In some embodiments, non-coding nucleotides can be placed at the 5' and/or 3' end of the polynucleotides encoding a polypeptide sequence according to any one of SEQ ID NOs.: 1-9 or a polypeptide sequence that is about 90-100% identical to any one of SEQ ID NOs.: 1-9 without affecting the functional properties of the molecule. A polyadenylation region at the 3'-end of the coding region of a polynucleotide can be included. The polyadenylation region can be derived from the endogenous gene, from a variety of other plant genes, from T-DNA, or through chemical synthesis. In further embodiments, the nucleotides encoding the polypeptide sequence according to any one of SEQ ID NOs.: 1-9 or a polypeptide sequence that is about 90-100% identical to any one of SEQ ID NOs.: 1-9 can be conjugated to a nucleic acid encoding a signal or transit (or leader) sequence at the N-terminal end (for example) of the polypeptide sequence according to any one of SEQ ID NOs.: 1-9 or a polypeptide sequence that is about 90-100% identical to any one of SEQ ID NOs.: 1-9 that co-translationally or post-translationally directs transfer of the polypeptide sequence according to any one of SEQ ID NOs.: 1-9 or a polypeptide sequence that is about 90-100% identical to any one of SEQ ID NOs.: 1-9. The polynucleotide sequence can also be altered so that the polypeptide sequence according to any one of SEQ ID NOs.: 1-9 or a polypeptide sequence that is about 90-100% identical to any one of SEQ ID NOs.: 1-9 is conjugated to a linker, selectable marker, or other sequence for ease of synthesis, purification, and/or identification of the protein. In one embodiment, the recombinant polynucleotide sequence includes at least one regulatory sequence operatively linked to the isolated nucleotide or cDNA sequences or fragments thereof.

To express a recombinant polynucleotide that encodes a polypeptide sequence according to any one of SEQ ID NOs.: 1-9 or a polypeptide sequence that is about 90-100% identical to any one of SEQ ID NOs.: 1-9 in a cell, the recombinant polynucleotide can be combined (e.g., in a vector) with transcriptional and/or translational initiation regulatory sequences, *i.e.* promoters, that direct the transcription of the gene and/or translation of the encoded protein in a cell. In some embodiments a constitutive promoter may be employed. Suitable constitutive promoters for mammalian cells are generally known in the art and include, but are not limited to SV40, CAG, CMV, EF-1 α , β -actin, RSV, and PGK. Suitable constitutive

promoters for bacterial cells, yeast cells, fungal cells are generally known in the art, such as a T-7 promoter for bacterial expression and an alcohol dehydrogenase promoter for expression in yeast.

In other embodiments, tissue-specific promoters or inducible promoters may be employed to direct expression of the exogenous nucleic acid in a specific cell type, under certain environmental conditions, and/or during a specific state of development. Suitable tissue-specific and inducible promoters are generally known in the art. In some embodiments, the tissue specific promoter is a brain, neuron, and/or neuron support cell specific promoter. For example, the calcium-calmodulin dependent protein kinase II α (CaM-KII α) promoter can be used, which can be specific for neurons of the forebrain. Other suitable brain, neuron, and neuron support cell specific promoters will be appreciated by those of ordinary skill in the art.

Also provided herein are vectors that can contain one or more of the polynucleotides or described herein. In embodiments, the vector can contain one or more polynucleotides that can encode a polypeptide according to any one of SEQ ID NOs.: 1-9 or a polypeptide sequence that is about 90-100% identical to any one of SEQ ID NOs.: 1-9. The vectors can be useful in producing transgenic bacterial, fungal, yeast, animal cells, and transgenic animals that can express a polypeptide according to any one of SEQ ID NOs.: 1-9 or a polypeptide sequence that is about 90-100% identical to any one of SEQ ID NOs.: 1-9. Within the scope of this disclosure are vectors containing one or more of the polynucleotide sequences described herein.

Cells and Transgenic Animals

Also provided herein are cells that are transformed with one or more polynucleotides (including vectors) described herein. The cells that are transformed with one or more polynucleotides described can express one or more recombinant polypeptides described herein. The cells can be bacterial, yeast, fungi, plant, or mammalian. Techniques for transforming cells are generally known in the art and can include, but are not limited to, transfection, electroporation, gene gun, and viral vector mediated transduction. The cells can be useful in the production of the recombinant polypeptides described herein. The cells can be useful in an assay to screen for candidate compounds that can bind or otherwise interact with and/or modulate the polypeptides described herein.

Also provided herein are transgenic animals, including but not limited to, mice, chickens, and pigs, that express one or more polypeptides described herein. Methods for producing transgenic animals that can express recombinant polypeptides are generally known in the art and will be appreciated by those of skill in the art.

Pharmaceutical formulations

Also provided herein are pharmaceutical formulations containing an amount of a cell-selective RNA molecule, corresponding DNA molecule (including vectors), and/or viron particle as

described herein. The amount can be an effective amount. Pharmaceutical formulations can be formulated for delivery via a variety of routes and can contain a pharmaceutically acceptable carrier. Techniques and formulations generally can be found in *Remington's Pharmaceutical Sciences*, Meade Publishing Co., Easton, Pa. (20th Ed., 2000), the entire disclosure of which is herein incorporated by reference. For systemic administration, an injection is useful, including intramuscular, intravenous, intraperitoneal, and subcutaneous. For injection, the therapeutic compositions of the invention can be formulated in liquid solutions, for example in physiologically compatible buffers such as Hank's solution or Ringer's solution. In addition, the therapeutic compositions can be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms are also included. Pharmaceutical compositions of the present invention are characterized as being at least sterile and pyrogen-free. These pharmaceutical formulations include formulations for human and veterinary use.

Suitable pharmaceutically acceptable carriers include, but are not limited to water, salt solutions, alcohols, gum arabic, vegetable oils, benzyl alcohols, polyethylene glycols, gelatin, carbohydrates such as lactose, amylose or starch, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid esters, hydroxyl methylcellulose, and polyvinyl pyrrolidone, which do not deleteriously react with the active composition.

The pharmaceutical formulations can be sterilized, and if desired, mixed with auxiliary agents, such as lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or aromatic substances, and the like which do not deleteriously react with the active composition.

The pharmaceutical formulations can be administered to a subject in need thereof. The subject in need thereof can have a disease, disorder, or a symptom thereof. Example disease or disorder can include, but are not limited to, a cardiovascular disease, a pulmonary disease, a brain disease, a renal disease, a liver disease, a blood disease, a nervous system disease, an intestinal disease, an ocular disease, and cancer. The pharmaceutical formulations can be disposed on or otherwise coupled to or integrated with a medical device, such as, but not limited to, catheters or stents, such that the pharmaceutical formulation is eluted from the medical device over a time period. The pharmaceutical formulation can therefore be delivered to a subject in need thereof during and/or after a procedure such as an angioplasty, vein graft or organ transplant. Other procedures where such a medical device would be useful will be appreciated by those of skill in the art.

A pharmaceutical formulation can be formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral,

intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerin, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. The construct, biologic molecules and pharmaceutical formulations thereof described herein can be disposed on or otherwise integrated with or coupled to a medical device such as, but not limited to, a catheter or stent, such that the construct, biological molecule can be released to the surrounding local area or systemically over a period of time after insertion or implantation into a subject in need thereof. These can also be referred to as drug eluting medical devices.

Pharmaceutical formulations suitable for injectable use can include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. For intravenous administration, suitable carriers can include physiological saline, bacteriostatic water, Cremophor EM™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). Injectable pharmaceutical formulations can be sterile and can be fluid to the extent that easy syringability exists. Injectable pharmaceutical formulations can be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, a pharmaceutically acceptable polyol like glycerol, propylene glycol, liquid polyethylene glycol, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it can be useful to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of injectable compositions can be brought about by incorporating an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating any of the compositions or recombinant polypeptides as described herein in an amount in an appropriate solvent with one or a combination of ingredients enumerated herein, as required, followed by filtered sterilization. Generally, dispersions can be prepared by incorporating the nucleic acid vectors

into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated herein. In the case of sterile powders for the preparation of sterile injectable solutions, examples of useful preparation methods are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated can be used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fluidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal the compositions or recombinant polypeptides described herein can be formulated into ointments, salves, gels, or creams as generally known in the art. In some embodiments, the compositions or recombinant polypeptides can be applied via transdermal delivery systems, which can slowly release the compositions or recombinant polypeptides for percutaneous absorption. Permeation enhancers can be used to facilitate transdermal penetration of the active factors in the conditioned media. Transdermal patches are described in for example, U.S. Pat. No. 5,407,713; U.S. Pat. No. 5,352,456; U.S. Pat. No. 5,332,213; U.S. Pat. No. 5,336,168; U.S. Pat. No. 5,290,561; U.S. Pat. No. 5,254,346; U.S. Pat. No. 5,164,189; U.S. Pat. No. 5,163,899; U.S. Pat. No. 5,088,977; U.S. Pat. No. 5,087,240; U.S. Pat. No. 5,008,110; and U.S. Pat. No. 4,921,475.

Administration of the compositions or recombinant polypeptides described herein is not restricted to a single route, but may encompass administration by multiple routes. For instance, exemplary administrations by multiple routes include, among others, a combination of intradermal and intramuscular administration, or intradermal and subcutaneous administration. Multiple administrations may be sequential or concurrent. Other modes of application by multiple routes will be apparent to the skilled artisan.

The pharmaceutical formulations can be administered to a subject by any suitable method that allows the agent to exert its effect on the subject in vivo. For example, the formulations or other compositions described herein can be administered to the subject by known procedures including, but not limited to, by oral administration, sublingual or buccal administration, parenteral administration, transdermal administration, via inhalation, via nasal delivery, vaginally, rectally, and intramuscularly. The formulations or other compositions described herein can be administered parenterally, by epifascial, intracapsular, intracutaneous, subcutaneous, intradermal, intrathecal, intramuscular, intraperitoneal, intrasternal, intravascular, intravenous, parenchymatous, and/or sublingual delivery. Delivery can be by injection, infusion, catheter delivery, or some other means, such as by tablet or spray.

For oral administration, a formulation as described herein can be presented as capsules, tablets, powders, granules, or as a suspension or solution. The formulation can contain conventional additives, such as lactose, mannitol, cornstarch or potato starch, binders, crystalline cellulose, cellulose derivatives, acacia, cornstarch, gelatins, disintegrators, potato starch, sodium carboxymethylcellulose, dibasic calcium phosphate, anhydrous or sodium starch glycolate, lubricants, and/or or magnesium stearate.

For parenteral administration (i.e., administration by through a route other than the alimentary canal), the formulations described herein can be combined with a sterile aqueous solution that is isotonic with the blood of the subject. Such a formulation can be prepared by dissolving the active ingredient in water containing physiologically-compatible substances, such as sodium chloride, glycine and the like, and having a buffered pH compatible with physiological conditions, so as to produce an aqueous solution, then rendering the solution sterile. The formulation can be presented in unit or multi-dose containers, such as sealed ampoules or vials. The formulation can be delivered by injection, infusion, or other means known in the art.

For transdermal administration, the formulation described herein can be combined with skin penetration enhancers, such as propylene glycol, polyethylene glycol, isopropanol, ethanol, oleic acid, N-methylpyrrolidone and the like, which increase the permeability of the skin to the nucleic acid vectors of the invention and permit the nucleic acid vectors to penetrate through the skin and into the bloodstream. The formulations and/or compositions described herein can be further combined with a polymeric substance, such as ethylcellulose, hydroxypropyl cellulose, ethylene/vinyl acetate, polyvinyl pyrrolidone, and the like, to provide the composition in gel form, which can be dissolved in a solvent, such as methylene chloride, evaporated to the desired viscosity and then applied to backing material to provide a patch.

Dosage forms

The pharmaceutical formulations or compositions described herein can be provided in unit dose form such as a tablet, capsule or single-dose injection or infusion vial. Where appropriate, the dosage forms described herein can be microencapsulated. The dosage form can also be prepared to prolong or sustain the release of any ingredient. In some embodiments, the complexed active agent can be the ingredient whose release is delayed. In other embodiments, the release of an auxiliary ingredient is delayed. Suitable methods for delaying the release of an ingredient include, but are not limited to, coating or embedding the ingredients in material in polymers, wax, gels, and the like. Delayed release dosage formulations can be prepared as described in standard references such as "Pharmaceutical dosage form tablets," eds. Liberman et. al. (New York, Marcel Dekker, Inc., 1989), "Remington – The science and practice of pharmacy", 20th ed., Lippincott Williams & Wilkins, Baltimore, MD, 2000, and "Pharmaceutical dosage forms and drug delivery systems", 6th Edition, Ansel

et al., (Media, PA: Williams and Wilkins, 1995). These references provide information on excipients, materials, equipment, and processes for preparing tablets and capsules and delayed release dosage forms of tablets and pellets, capsules, and granules. The delayed release can be anywhere from about an hour to about 3 months or more.

5 Coatings may be formed with a different ratio of water soluble polymer, water insoluble polymers, and/or pH dependent polymers, with or without water insoluble/water soluble non polymeric excipient, to produce the desired release profile. The coating is either performed on the dosage form (matrix or simple) which includes, but is not limited to, tablets (compressed with or without coated beads), capsules (with or without coated beads), beads, particle
10 compositions, "ingredient as is" formulated as, but not limited to, suspension form or as a sprinkle dosage form.

Examples of suitable coating materials include, but are not limited to, cellulose polymers such as cellulose acetate phthalate, hydroxypropyl cellulose, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose phthalate, and hydroxypropyl methylcellulose
15 acetate succinate; polyvinyl acetate phthalate, acrylic acid polymers and copolymers, and methacrylic resins that are commercially available under the trade name EUDRAGIT® (Roth Pharma, Westerstadt, Germany), zein, shellac, and polysaccharides.

In some embodiments, such as for treatments of plants, the topical formulation of a composition or pharmaceutical formulation described herein can be further formulated as a
20 spray and can include a suitable surfactant, wetting agent, adjuvants/surfactant (stickers, extender, plant penetrant, compatibility agents, buffers, drift control additives, and defoaming agents), or any combination thereof so as to be formulated as a spray. The compounds, any optional auxiliary active ingredient, suitable surfactant, wetting agent, adjuvants, or any combination thereof can be formulated as a solution, suspension, or emulsion. The spray
25 dosage form can be administered through a spraying device. In some embodiments, the spraying device can be configured to generate the sprayable formulation as a liquid solution is contacted with the complexed active agent compound or formulation thereof. In other embodiments, the sprayable dosage form is pre-made prior to spraying. As such, the spraying device can act solely as an applicator for these embodiments.

30 In further embodiments, such as for treatments of plants (e.g. such as a herbicide), the dosage form of composition or pharmaceutical formulation described herein thereof can be further formulated as a dust and can include a suitable dry inert carrier (e.g. talc chalk, clay, nut hull, volcanic ash, or any combination thereof so as to be formulated as a dust. The dust can contain dust particles of varying sizes. In some embodiments, the particle size can be
35 substantially homogenous. In other embodiments, the particle size can be heterogeneous. Dosage forms adapted as a dust can contain one or more adjuvants/surfactants (stickers,

extender, plant penetrant, compatibility agents, buffers, drift control additives, and defoaming agents).

In some embodiments, the dosage form can be formulated as a bait. In these embodiments, the complexed active agent compound or other formulation thereof can be further formulated to include a food or other attractive substance that can attract one or more insect or other pest. The bait dosage form can be formulated as a dust, paste, gel, or granule. Dosage forms adapted as baits can contain one or more adjuvants/surfactants (stickers, extender, plant penetrant, compatibility agents, buffers, drift control additives, and defoaming agents).

In additional embodiments, the dosage form can be formulated as granules or pellets that can be applied to the environment. These dosage formulations are similar to dust formulations, but the particles are larger and heavier. The granules can be applied to soil or other environmental area. Dosage forms adapted as granules or pellets can contain one or more adjuvants/surfactants (stickers, extender, plant penetrant, compatibility agents, buffers, drift control additives, and defoaming agents).

The dusts, granules, and pellets described herein can be formulated as wettable dusts, granules, and pellets, soluble dusts granules, and pellets, and/or water-dispersible granules, and/or dry flowables.

The dosage form can be adapted for impregnating (saturating) an object or device, which then can be carried by, worn, or otherwise coupled to an organism in need thereof. In some embodiments, the dosage form can be impregnated onto a collar, bracelet, patch, adhesive tape, livestock ear tags, clothing, blankets, plastics, nets, and paints. The composition or pharmaceutical formulation thereof can be formulated and impregnated in the object or device such that the composition or pharmaceutical formulation evaporates over time, which releases the composition and/or pharmaceutical formulation into the air and/or environment surrounding the organism and/or onto the organism.

The dosage form can be adapted as a fumigant, which is a formulation that forms a gas when utilized or applied. In some embodiments, the composition and/or pharmaceutical formulation thereof can be supplied as a liquid when packaged under pressure and change to a gas when they are released. In other embodiments, the composition and/or pharmaceutical formulation thereof can be supplied as a volatile liquid when enclosed in a container (not under pressure). Others can be formulated as solids that release gases when applied under conditions of high humidity or in the presence of high water vapor. Dosage forms adapted as fumigants can contain one or more adjuvants/surfactants (stickers, extender, plant penetrant, compatibility agents, buffers, drift control additives, and defoaming agents).

Effective Amounts

The pharmaceutical formulations can contain an effective amount of a composition described herein and/or an effective amount of an auxiliary agent. In some embodiments, the effective amount ranges from about 0.001 pg to about 1,000 g or more of a composition described herein. In some embodiments, the effective amount of the composition described herein can range from about 0.001 mg/kg body weight to about 1,000 mg/kg body weight. In yet other embodiments, the effective amount of the composition can range from about 1% w/w to about 99% or more w/w, w/v, or v/v of the total pharmaceutical formulation. In some embodiments, the effective concentration of a polypeptide according to any one of SEQ ID NOs.: 1-9 can be between about 3 to about 10 mM.

Combination Therapy

The pharmaceutical formulations or other compositions described herein can be administered to a subject either as a single agent, or in combination with one or more other agents. Additional agents include but are not limited to DNA, RNA, amino acids, peptides, polypeptides, antibodies, aptamers, ribozymes, guide sequences for ribozymes that inhibit translation or transcription of essential tumor proteins and genes, hormones, immunomodulators, antipyretics, anxiolytics, antipsychotics, analgesics, antispasmodics, anti-inflammatories, anti-histamines, anti-infectives, and chemotherapeutics.

Suitable antipyretics include, but are not limited to, non-steroidal anti-inflammants (e.g. ibuprofen, naproxen, ketoprofen, and nimesulide), aspirin and related salicylates (e.g. choline salicylate, magnesium salicylate, and sodium salicylate), paracetamol/acetaminophen, metamizole, nabumetone, phenazone, and quinine.

Suitable anxiolytics include, but are not limited to, benzodiazepines (e.g. alprazolam, bromazepam, chlordiazepoxide, clonazepam, clorazepate, diazepam, flurazepam, lorazepam, oxazepam, temazepam, triazolam, and tofisopam), serotonergic antidepressants (e.g. selective serotonin reuptake inhibitors, tricyclic antidepressants, and monoamine oxidase inhibitors), mebicar, afobazole, selank, bromantane, emoxypine, azapirones, barbituates, hydroxyzine, pregabalin, validol, and beta blockers.

Suitable antipsychotics include, but are not limited to, benperidol, bromoperidol, droperidol, haloperidol, moperone, pipaperone, timiperone, fluspirilene, penfluridol, pimozide, acepromazine, chlorpromazine, cyamemazine, dizyrazine, fluphenazine, levomepromazine, mesoridazine, perazine, pericyazine, perphenazine, pipotiazine, prochlorperazine, promazine, promethazine, prothipendyl, thioproperazine, thioridazine, trifluoperazine, triflupromazine, chlorprothixene, clopenthixol, flupentixol, tiotixene, zuclopenthixol, clotiapine, loxapine, prothipendyl, carpipramine, clozapramine, molindone, mosapramine, sulpiride, vernalipride, amisulpride, amoxapine, aripiprazole, asenapine, clozapine, blonanserin, iloperidone, lurasidone, melperone, nemonapride, olanzapine, paliperidone, perospirone, quetiapine, remoxipride, risperidone, sertindole, trimipramine, ziprasidone, zotepine, alstonie,

befeprunox, bitopertin, brexpiprazole, cannabidiol, cariprazine, pimavanserin, pomaglumetad methionil, vabicaserin, xanomeline, and ziconapine.

Suitable analgesics include, but are not limited to, paracetamol/acetaminophen, non-steroidal anti-inflammants (e.g. ibuprofen, naproxen, ketoprofen, and nimesulide), COX-2 inhibitors (e.g. rofecoxib, celecoxib, and etoricoxib), opioids (e.g. morphine, codeine, oxycodone, hydrocodone, dihydromorphine, pethidine, buprenorphine), tramadol, norepinephrine, flupiretine, nefopam, orphenadrine, pregabalin, gabapentin, cyclobenzaprine, scopolamine, methadone, ketobemidone, piritramide, and aspirin and related salicylates (e.g. choline salicylate, magnesium salicylate, and sodium salicylate).

Suitable antispasmodics include, but are not limited to, mebeverine, papverine, cyclobenzaprine, carisoprodol, orphenadrine, tizanidine, metaxalone, methocarbamol, chlorzoxazone, baclofen, dantrolene, baclofen, tizanidine, and dantrolene.

Suitable anti-inflammatories include, but are not limited to, prednisone, non-steroidal anti-inflammants (e.g. ibuprofen, naproxen, ketoprofen, and nimesulide), COX-2 inhibitors (e.g. rofecoxib, celecoxib, and etoricoxib), and immune selective anti-inflammatory derivatives (e.g. submandibular gland peptide-T and its derivatives).

Suitable anti-histamines include, but are not limited to, H₁-receptor antagonists (e.g. acrivastine, azelastine, bilastine, brompheniramine, buclizine, bromodiphenhydramine, carbinoxamine, cetirizine, chlorpromazine, cyclizine, chlorpheniramine, clemastine, cyproheptadine, desloratadine, dexbromapheniramine, dexchlorpheniramine, dimenhydrinate, dimetindene, diphenhydramine, doxylamine, ebasine, embramine, fexofenadine, hydroxyzine, levocetirizine, loratadine, meclozine, mirtazapine, olopatadine, orphenadrine, phenindamine, pheniramine, phenyltoloxamine, promethazine, pyrilamine, quetiapine, rupatadine, tripeleminamine, and triprolidine), H₂-receptor antagonists (e.g. cimetidine, famotidine, lafutidine, nizatidine, ranitidine, and roxatidine), tritoqualine, catechin, cromoglicate, nedocromil, and β 2-adrenergic agonists.

Suitable anti-infectives include, but are not limited to, amebicides (e.g. nitazoxanide, paromomycin, metronidazole, tinidazole, chloroquine, and iodoquinol), aminoglycosides (e.g. paromomycin, tobramycin, gentamicin, amikacin, kanamycin, and neomycin), anthelmintics (e.g. pyrantel, mebendazole, ivermectin, praziquantel, albendazole, miltefosine, thiabendazole, oxamniquine), antifungals (e.g. azole antifungals (e.g. itraconazole, fluconazole, posaconazole, ketoconazole, clotrimazole, miconazole, and voriconazole), echinocandins (e.g. caspofungin, anidulafungin, and micafungin), griseofulvin, terbinafine, flucytosine, and polyenes (e.g. nystatin, and amphotericin b), antimalarial agents (e.g. pyrimethamine/sulfadoxine, artemether/lumefantrine, atovaquone/proguanil, quinine, hydroxychloroquine, mefloquine, chloroquine, doxycycline, pyrimethamine, and halofantrine), antituberculosis agents (e.g. aminosaliclates (e.g. aminosalicylic acid), isoniazid/rifampin,

isoniazid/pyrazinamide/rifampin, bedaquiline, isoniazid, ethambutol, rifampin, rifabutin, rifapentine, capreomycin, and cycloserine), antivirals (e.g. amantadine, rimantadine, abacavir/lamivudine, emtricitabine/tenofovir, cobicistat/elvitegravir/emtricitabine/tenofovir, efavirenz/emtricitabine/tenofovir, avacavir/lamivudine/zidovudine, lamivudine/zidovudine, emtricitabine/tenofovir, emtricitabine/opinavir/ritonavir/tenofovir, interferon alfa-2v/ribavirin, peginterferon alfa-2b, maraviroc, raltegravir, dolutegravir, enfuvirtide, foscarnet, fomivirsen, oseltamivir, zanamivir, nevirapine, efavirenz, etravirine, rilpivirine, delaviridine, nevirapine, entecavir, lamivudine, adefovir, sofosbuvir, didanosine, tenofovir, avacavir, zidovudine, stavudine, emtricitabine, xalcitabine, telbivudine, simeprevir, boceprevir, telaprevir, lopinavir/ritonavir, fosamprenvir, dranuavir, ritonavir, tipranavir, atazanavir, nelfinavir, amprenavir, indinavir, sawuonavir, ribavirin, valcyclovir, acyclovir, famciclovir, ganciclovir, and valganciclovir), carbapenems (e.g. doripenem, meropenem, ertapenem, and cilastatin/imipenem), cephalosporins (e.g. cefadroxil, cephadrine, cefazolin, cephalexin, cefepime, ceflaroline, loracarbef, cefotetan, cefuroxime, cefprozil, loracarbef, cefoxitin, cefaclor, ceftibuten, ceftriaxone, cefotaxime, cefpodoxime, cefdinir, cefixime, cefditoren, cefizoxime, and ceftazidime), glycopeptide antibiotics (e.g. vancomycin, dalbavancin, oritavancin, and telvancin), glycyclines (e.g. tigecycline), leprostatics (e.g. clofazimine and thalidomide), lincomycin and derivatives thereof (e.g. clindamycin and lincomycin), macrolides and derivatives thereof (e.g. telithromycin, fidaxomicin, erythromycin, azithromycin, clarithromycin, dirithromycin, and troleandomycin), linezolid, sulfamethoxazole/trimethoprim, rifaximin, chloramphenicol, fosfomycin, metronidazole, aztreonam, bacitracin, penicillins (amoxicillin, ampicillin, bacampicillin, carbenicillin, piperacillin, ticarcillin, amoxicillin/clavulanate, ampicillin/sulbactam, piperacillin/tazobactam, clavulanate/ticarcillin, penicillin, procaine penicillin, oxacillin, dicloxacillin, and nafcillin), quinolones (e.g. lomefloxacin, norfloxacin, ofloxacin, gatifloxacin, moxifloxacin, ciprofloxacin, levofloxacin, gemifloxacin, moxifloxacin, cinoxacin, nalidixic acid, enoxacin, grepafloxacin, gatifloxacin, trovafloxacin, and sparfloxacin), sulfonamides (e.g. sulfamethoxazole/trimethoprim, sulfasalazine, and sulfasoxazole), tetracyclines (e.g. doxycycline, demeclocycline, minocycline, doxycycline/salicylic acid, doxycycline/omega-3 polyunsaturated fatty acids, and tetracycline), and urinary anti-infectives (e.g. nitrofurantoin, methenamine, fosfomycin, cinoxacin, nalidixic acid, trimethoprim, and methylene blue).

Suitable chemotherapeutics include, but are not limited to, paclitaxel, brentuximab vedotin, doxorubicin, 5-FU (fluorouracil), everolimus, pemetrexed, melphalan, pamidronate, anastrozole, exemestane, nelarabine, ofatumumab, bevacizumab, belinostat, tositumomab, carmustine, bleomycin, bosutinib, busulfan, alemtuzumab, irinotecan, vandetanib, bicalutamide, lomustine, daunorubicin, clofarabine, cabozantinib, dactinomycin, ramucirumab, cytarabine, cytoxan, cyclophosphamide, decitabine, dexamethasone, docetaxel, hydroxyurea,

decarbazine, leuprolide, epirubicin, oxaliplatin, asparaginase, estramustine, cetuximab, vismodegib, asparaginase erwinia chrysanthemi, amifostine, etoposide, flutamide, toremifene, fulvestrant, letrozole, degarelix, pralatrexate, methotrexate, floxuridine, obinutuzumab, gemcitabine, afatinib, imatinib mesylate, carmustine, eribulin, trastuzumab, altretamine, topotecan, ponatinib, idarubicin, ifosfamide, ibrutinib, axitinib, interferon alfa-2a, gefitinib, romidepsin, ixabepilone, ruxolitinib, cabazitaxel, ado-trastuzumab emtansine, carfilzomib, chlorambucil, sargramostim, cladribine, mitotane, vincristine, procarbazine, megestrol, trametinib, mesna, strontium-89 chloride, mechlorethamine, mitomycin, busulfan, gemtuzumab ozogamicin, vinorelbine, filgrastim, pegfilgrastim, sorafenib, nilutamide, pentostatin, tamoxifen, mitoxantrone, pegaspargase, denileukin diftitox, alitretinoin, carboplatin, pertuzumab, cisplatin, pomalidomide, prednisone, aldesleukin, mercaptopurine, zoledronic acid, lenalidomide, rituximab, octretide, dasatinib, regorafenib, histrelin, sunitinib, siltuximab, omacetaxine, thioguanine (tioguanine), dabrafenib, erlotinib, bexarotene, temozolomide, thiotepa, thalidomide, BCG, temsirolimus, bendamustine hydrochloride, triptorelin, arsenic trioxide, lapatinib, valrubicin, panitumumab, vinblastine, bortezomib, tretinoin, azacitidine, pazopanib, teniposide, leucovorin, crizotinib, capecitabine, enzalutamide, ipilimumab, goserelin, vorinostat, idelalisib, ceritinib, abiraterone, epothilone, taflopiside, azathioprine, doxifluridine, vindesine, and all-trans retinoic acid.

Screening Assays

The recombinant polypeptides and transformed cells expressing one or more of the polypeptides described herein can be used in an *in vitro* screening assay to screen candidate compounds to determine if they are capable of modulating binding of an ApoE protein to an ApoE receptor. As such, provided herein are methods including the steps of contacting a candidate compound with a recombinant polypeptide as described herein or an ApoE protein; and measuring binding of the recombinant polypeptide or the ApoE protein to an ApoE receptor present on the surface of a cell. In embodiments, the ApoE receptor is a LDL receptor. Measurement of the binding between the ApoE receptor and the ApoE protein or recombinant polypeptide as described herein can be conducted by measuring ApoE receptor activity.

In some embodiments, the amount and/or concentration of one or more A β peptides in cell cultured media can be measured. By measuring A β peptide production by the cell culture, candidate compounds including, but not limited to any small molecular compounds, small peptides, and antibodies, can be screened for their ability to specifically block ApoE binding to an ApoE receptor, such as LDLR. While not being bound to theory, measuring A β peptide production can be stimulated by ApoE binding to an ApoE receptor, such as LDLR. Therefore, if A β peptide production, as measured by the amount and/or concentration of one or more A β peptides in the cell culture media, is modulated in response to the presence of a candidate compound, it can be determined (in view of suitable controls) that the candidate

compound influenced the binding of the ApoE to the ApoE receptor. In some embodiments, the binding of ApoE or a polypeptide as described herein to the ApoE receptor can be compared to a suitable control. One of ordinary skill in the art will instantly appreciate what constitutes a suitable control.

5 *Methods of Treating a Cardiovascular, Vascular, and/or Neurologic Disease or Disorder*

 The compositions, recombinant polypeptides, and pharmaceutical formulations described herein can be useful for treating a disease or disorder where abnormal ApoE binding to an ApoE receptor is part of the pathology of the disease or disorder. As such, the
10 compositions, recombinant polypeptides, and pharmaceutical formulations described herein can be useful for treating a cardiovascular, vascular, and/or neurological disease or disorder in a subject. The compositions, recombinant polypeptides, and pharmaceutical formulations described herein can be useful for treating AD.

 In some embodiments, a compositions, recombinant polypeptide, or a pharmaceutical
15 formulation described herein can be administered to a subject in need thereof. In some embodiments the composition or pharmaceutical formulation that can be administered to a subject in need thereof can include a polypeptide sequence according to SEQ ID NO: 1 or a polypeptide sequence that is about 90-100% identical to SEQ ID NO: 1 and at least 3 or at least 6 additional lysine residues operatively coupled to the polypeptide sequence according
20 to SEQ ID NO: 1 or the polypeptide sequence that is about 90-100% identical SEQ ID NO: 1. In some embodiments the number of additional lysine residues can be 6. In some embodiments the recombinant polypeptide sequence can be any one of SEQ ID NOs: 1-9. The subject in need thereof can be suffering from or at risk for a disease or disorder where abnormal ApoE binding to an ApoE receptor is part of the pathology of the disease or disorder.
25 The subject in need thereof can be suffering from or at risk for a cardiovascular, vascular, and/or neurological disease or disorder. The subject in need thereof can be suffering from or at risk for AD. In embodiments, ApoE binding to an ApoE receptor can be measured in the subject in need thereof. Methods for measuring specific binding of Apoe to an ApoE receptor can be determine by methods generally known in the art and will be instantly appreciated by
30 those of skill in the art.

EXAMPLES

 Now having described the embodiments of the present disclosure, in general, the following Examples describe some additional embodiments of the present disclosure. While
35 embodiments of the present disclosure are described in connection with the following examples and the corresponding text and figures, there is no intent to limit embodiments of

the present disclosure to this description. On the contrary, the intent is to cover all alternatives, modifications, and equivalents included within the spirit and scope of embodiments of the present disclosure.

5 Example 1. Apo3 and ApoE4, but not ApoE2, can promote amyloid β ($A\beta$) production.

Chinese hamster ovary (CHO) cells overexpressing human wild-type amyloid precursor protein (APPwt) (CHO/APPwt cells) were cultured in 96-well plate at about 2×10^4 /well in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS), 1 mM sodium pyruvate and 100 U/mL of penicillin/streptomycin for 18 hours. These
 10 cells were then further cultured in serum-free DMEM in the presence or absence of ApoE2, ApoE3, or ApoE4 at various concentrations as indicated for about 2 hours, followed by analysis of conditioned media and cell lysates by $A\beta_{1-40}$ ELISA and protein assay, respectively. The results are shown in Fig. 1

As shown in Fig. 1, human LDL, ApoB100 or ApoE3 and 4 treatment can promote APP
 15 β -cleavage in human wild type (wt) APP expressing CHO cells (CHO/APPwt) as evidenced by (a) increased $A\beta_{1-40, 42}$ production and (b) decreased sAPP α secretion.

Example 2. ApoE LDLR binding domain peptide treatment can increase $A\beta$ generation.

CHO/APPwt cells were cultured in DMEM with 10% FBS for about 18 hours and then
 20 serum-free DMEM in the presence or absence of ApoE LDLR binding domain peptide (LRVR LASH LRKL RKRL LRDA, residues 133-152) as indicated for about 2 hours. The conditioned media and cell lysates were analyzed by $A\beta_{1-40}$ ELISA and protein assay, respectively. The results are shown in Fig. 2.

As shown in Fig. 2 peptide (LRVRLASHLRKLRKLLRDA), representing the ApoE
 25 receptor binding region, promotes APP β -cleavage in CHO/APPwt cells as evidenced by (a) increased $A\beta_{1-40, 42}$ production and (b) decreased sAPP α secretion.

Example 3. ApoE LDLR binding domain peptide induced $A\beta$ production is structurally dependable.

CHO/APPwt cells were cultured in 96-well plate (about 2×10^4 /well) in serum-free
 30 medium and treated with ApoE LDLR, FlagApoE LDLR (SEQ ID NO.: 1 with an N-terminal FLAG tag), BiotinApo LDLR (SEQ ID NO.: 1 with an N-terminal Biotin) and 3 lysinesApoE LDLR (3KApoE LDLR (SEQ ID NO.: 1)) at indicated doses for about 2 hours. The conditioned media and cell lysates were prepared, and subjected to $A\beta$ ELISA ($**P < 0.01$; $***P < 0.001$
 35 when compared to ApoE LDLR) and protein assay. The results are demonstrated in Fig. 3. These results are representative of three independent experiments with each condition triplicated. Addition of 3-9 additional lysine residues at the N-terminal of the LDLR binding

domain of ApoE (SEQ ID NO.: 1 LRVRLASHLRKLRKLLRDA) can result in ApoE LDLR domain acting as an LDLR competitive antagonist to ApoE.

CHO/APPwt cells were cultured in 96-well plate (about 2×10^4 /well) in serum-free medium and treated with human plasma ApoE, human recombinant ApoE3, 4 (ApoE3, ApoE4) and ApoE LDLR as indicated doses and PBS (Ctrl) for about 2 hours. The conditioned media and cell lysates were prepared, and subjected to A β ELISA and protein assay. The results are demonstrated in Figs. 4-6. These results are representative of three independent experiments with each condition triplicated and presented as mean $\pm$ SD.

Example 4. 3KApoE LDLR markedly inhibits A β generation induced by human plasma ApoE3, human recombinant ApoE3 and ApoE4, human plasma LDL, and ApoB100.

CHO/APPwt cells were cultured in 96-well plate (about 2×10^4 /well) in serum-free medium and pre-treated with 3KApoE LDLR (SEQ ID NO.: 2) at about 10 μ M and then went the treatment with ApoE LDLR, human recombinant ApoE3, ApoE4, or FlagApoE LDLR (SEQ ID NO.: 1 with an N-Terminal FLAG tag) (results demonstrated in Fig. 7), human plasma ApoE3, ApoB100, or LDL (results demonstrated in Fig. 8) at indicated doses for about 2 hours. The conditioned media and cell lysates were prepared, and subjected to A β ELISA (* P < 0.05, ** P < 0.01; *** P < 0.001 when compared to control) and protein assay. These results are representative of three independent experiments with each condition triplicated. These results are representative of three independent experiments with each condition triplicated and presented as mean $\pm$ SD.

Example 5. 6KApoE markedly inhibits A β generation induced by human recombinant ApoE4 protein.

Figs. 9A-9F show data demonstrating human plasma LDL, ApoB100 and human recombinant ApoE3 & 4 proteins markedly promote amyloid β protein (A β) production. CHO cells overexpressing human wild-type APP (CHO/APPwt) cells were cultured in 96-well plate at 2×10^4 /well in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS), 1 mM sodium pyruvate and 100 U/mL of penicillin/streptomycin for 18 hours. These cells were further treated with human plasma LDL (hu LDL) (Figs. 9A and 9C), human plasma ApoB100 (hu ApoB100) (Figs. 9B and 9D), human recombinant ApoE3, 4 protein (hu rec ApoE3 or 4) or PBS (Ctrl) (Figs. 9E and 9F) at various concentrations in serum-free DMEM as indicated for 2 and 16 hours (h), followed by analysis of conditioned media and cell lysates by A β 1-40, 42 ELISA, 82E1 western blotting (WB) analysis and protein assay, respectively. 82E1 WB analyses as shown below the A β ELISA result histograms. The A β ELISA results

are representative of three independent experiments with each condition triplicated and presented as mean $\pm$ SD.

Figs. 10A-10C show data demonstrating ApoE LDL receptor binding domain peptide treatment significantly increases A β generation, which is structurally dependable. CHO/APPwt cells were cultured in 96-well plate at 2X10⁴/well in DMEM with 10% FBS for overnight and then serum-free DMEM in the presence or absence of ApoE LDL receptor binding domain peptide [ApoE-LBDP, LRVRLASHLRKLRKRLLRDA (residues 133-152)] as indicated for 2 and 16 hours (h). The conditioned media and cell lysates were analyzed by A β 1-40, 42 ELISA (Fig. 10A), 82E1 WB analysis (Fig. 10B) and protein assay, respectively. In addition, (Fig. 10C) CHO/APPwt cells were cultured in 96-well plate (2X10⁴/well) in serum-free DMEM and treated with FlagApoE-LBDP, BiotinApoE-LBDP and 3 lysineApoE-LBDP (3KApoE-LBDP) in comparing to ApoE-LBDP at indicated doses for 2 hours (h). The conditioned media and cell lysates were prepared, and subjected to A β 1-40, 42 ELISA (***P* < 0.01; ****P* < 0.001 when compared to ApoE-LBDP) and protein assay. These results are representative of three independent experiments with each condition triplicated. Note: KKK-LRVRLASHLRKLRKRLLRDA; DYKDDDDK-LRVRLASHLRKLRKRLLRDA; Biotin-LRVRLASHLRKLRKRLLRDA.

Figs. 11A-11D show data demonstrating 6KApoE markedly inhibits A β generation induced by human recombinant ApoE4 protein. CHO/APPwt cells were cultured in 96-well plate (2X10⁴/well) in serum-free medium and pre-treated with PBS (Ctrl), 3 lysine (3K), 6K lysine (6K), 3KApoE-LBDP (3KApoE), 6KApoE-LBDP (6KApoE), 7KApoE-LBDP (7KApoE), 8KApoE-LBDP (8KApoE), 9KApoE-LBDP (9KApoE) peptide at 10 μ M for 15 minutes and went to treatment with or without human recombinant ApoE4 protein (hu rec ApoE4, 10 μ g/mL) for 2 hours. The conditioned media and cell lysates were prepared, and subjected to A β 1-40, 42 ELISA and protein assay. Further, CHO/APPwt cells were cultured in 96-well plate (2X10⁴/well) in serum-free DMEM and pre-treated with 6KApoE for 15 minutes at various doses as indicated and then went to treatment with human recombinant ApoE4 protein (hu rec ApoE4) for 2 and 16 hours. The conditioned media and cell lysates were prepared, and subjected to A β 1-40, 42 ELISA (Fig. 11C), 82E1 WB analysis (Fig. 11D) and protein assay, respectively. The A β 1-40, 42 ELISA results are representative of three independent experiments with each condition triplicated and presented as mean $\pm$ SD.

Figs. 12A-12F show data demonstrating LDL receptor is in part responsive for 6KApoE inhibition of A β generation induced by human recombinant ApoE4 protein. CHO/APPwt cells were cultured in 96-well plate (2X10⁴/well) in serum-free medium and pre-treated with 6KApoE at 1 μ M for 15 minutes and went treatment with an antagonist anti-LDLR antibody (anti-LDLR Ab) from 0 - 2.5 μ g/mL for 2 hours. The conditioned media and cell lysates were prepared, and subjected to A β 1-40, 42 ELISA (Fig. 12A), 82E1 WB analysis (Fig. 12B) and protein

assay. Hamster LDLR siRNA and negative control were ordered from Thermo Fisher Scientific, transfection was performed following the protocol of lipofectamine RNAiMAX reagent provided by Thermo Fisher Scientific. Briefly, CHO APPwt cells were plated in 24 well plate 1×10^5 cells/well for overnight, siRNAs were transfected at 10 nM as indicated, 24 hours after these cells were washed and treated with human recombinant ApoE4 protein (hu rec ApoE4) at 10 $\mu\text{g/mL}$ for 2 hours (h) in the presence or absence of 15-minute pre-treatment of 1 μM 6KApoE. The conditioned media and cell lysates were prepared, and subjected to A β 1-40, 42 ELISA (Fig. 12C), anti-LDLR antibody WB evaluation (Fig. 12D) and protein assay. CHO/IdIA7 (IdIA7) and CHO wild-type (CHO) cells were provided by Dr. Monty Krieger (Massachusetts Institute of Technology, Cambridge, MA). The two cells were cultured in Ham's F-12 medium supplemented with 5% FBS, 2 mM L-glutamine. The cells were plated into 24-well plate at 1×10^5 each well the day before transfection. PCMV6-APP695 (OriGene Technologies, Inc. Rockville, MD) was transfected to these cells using Lipofectamine® 3000 Transfection Reagent (ThermoFisher Scientific) according to the instructions. Twenty-four hours after transfection, these cells were washed and treated with human recombinant ApoE4 protein (hu rec ApoE4) at 10 $\mu\text{g/mL}$ for 2 hours (h) in the presence or absence of 15-minute pre-treatment of 1 μM 6KApoE. The conditioned media and cell lysates were prepared, and subjected to A β 1-40 ELISA (Fig. 12E), anti-LDLR antibody and APP processing WB evaluation (Fig. 12F), and protein assay. For A β ELISA, these results are representative of three independent experiments with each condition triplicated and presented as mean $\pm$ SD.

Example 6.

The effect of peripheral 6KApoEp treatment on A β levels and Alzheimer-like hyperphosphorylated and acetylated tau pathologies in an AD mouse model was examined. Briefly, 5XFADmice, with 3APP and 2PS1 AD mutations, at 6 weeks of age (n=10, 5female/5male) were intraperitoneally (i.p.) treated with 6KApoEp (250 $\mu\text{g/kg}$ in 50 μL PBS) or PBS (50 μL) daily for 10 weeks. Following sacrifice, blood plasma and brain tissue homogenates were analyzed by A β 1-40,42 ELISA (Figs. 13A-13B and Figs. 14A-14B) and Western blot for total A β , APP β -CTF (Figs. 15A-15C) and Alzheimer-like acetylated (a-tau K²⁷⁴ and a-tau K¹⁷⁴) and phosphorylated tau (p-tau Thr²³¹, p-tau Thr⁴⁰⁴ and PHF (Figs. 16A-16G). The ELISA results are represented as mean $\pm$ SD (μg of A β 1_{40,42} per total cerebral protein). Band density ratios of acetylated or phosphorylated tau to total tau as determined by densitometry analysis shown in Figs. 17A-17B. Statistical *t*-test analyses of Western blot data revealed a significant decrease in the ratios of p-tau (Thr²³¹), p-tau (Thr⁴⁰⁴), PHF, a-tau(K²⁷⁴) and a-tau(K¹⁷⁴) to total tau in 6KApoEp-treated compared with PBS injected 5XFAD mice (Ctrl, *P<0.05, **P<0.01, ***P<0.005).

We claim:

1. A composition comprising:
a compound configured to reduce binding of an apolipoprotein E (ApoE) protein to an ApoE receptor.
2. The composition of claim 1, wherein the compound specifically binds to a low-density lipoprotein receptor binding domain on the ApoE protein, wherein the LDL receptor binding domain has a polypeptide sequence according to SEQ ID NO: 1, or a polypeptide sequence that is about 90-100% identical to SEQ ID NO: 1, or a fragment thereof comprising at least 5 contiguous amino acids.
3. The composition of any one of claims 1-2, wherein the compound is an antibody or fragment thereof.
4. The composition of any one of claims 1-2, wherein the compound is a small molecule.
5. The composition of claim 1, wherein the compound is competitive inhibitor of ApoE for the ApoE receptor.
6. The composition of any one of claims 1 or 5, wherein the compound is a recombinant polypeptide, wherein the recombinant polypeptide comprises a polypeptide sequence according to SEQ ID NO: 1 or a polypeptide sequence that is about 90-100% identical to SEQ ID NO: 1 and at least 3 or at least 6 additional lysine residues operatively coupled to the polypeptide sequence according to SEQ ID NO: 1 or the polypeptide sequence that is about 90-100% identical SEQ ID NO: 1.
7. The composition of claim 6, wherein the at least 3 or at least 6 lysine additional residues are operatively coupled to the N-terminus of the polypeptide sequence according to SEQ ID NO: 1 or the polypeptide sequence that is about 90-100% identical SEQ ID NO: 1.

8. The composition of claim 6, wherein the at least 3 additional lysine residues are operatively coupled to the C-terminus of the polypeptide sequence according to SEQ ID NO: 1 or the polypeptide sequence that is about 90-100% identical SEQ ID NO: 1.

9. The composition of claim 6, wherein the at least 3 additional lysine residues are operatively coupled between the N-terminus and C-terminus of the polypeptide sequence according to SEQ ID NO: 1 or the polypeptide sequence that is about 90-100% identical SEQ ID NO: 1.

10. The composition of any one of claims 1-9, further comprising a pharmaceutical carrier.

11. A recombinant polypeptide comprising:
a polypeptide sequence according to SEQ ID NO: 1 or a polypeptide sequence that is about 90-100% identical to SEQ ID NO: 1.

12. The recombinant polypeptide of claim 11, further comprising at least 3 or at least 6 additional lysine residues operatively coupled to the polypeptide sequence according to SEQ ID NO: 1 or the polypeptide sequence that is about 90-100% identical SEQ ID NO: 1.

13. The recombinant polypeptide of claim 12, wherein the at least 3 or at least 6 lysine additional residues are operatively coupled to the N-terminus of the polypeptide sequence according to SEQ ID NO: 1 or the polypeptide sequence that is about 90-100% identical SEQ ID NO: 1.

14. The recombinant polypeptide of claim 12, wherein the at least 3 or at least 6 additional lysine residues are operatively coupled to the C-terminus of the polypeptide sequence according to SEQ ID NO: 1 or the polypeptide sequence that is about 90-100% identical SEQ ID NO: 1.

15. The recombinant polypeptide of claim 12, wherein the at least 3 or at least 6 additional lysine residues are operatively coupled between the N-terminus and C-terminus of the polypeptide sequence according to SEQ ID NO: 1 or the polypeptide sequence that is about 90-100% identical SEQ ID NO: 1.

16. A polynucleotide sequence, wherein the polynucleotide sequence is configured to encode a recombinant polypeptide according to any one of claims 11-15.

17. A vector comprising:
a nucleotide sequence configured to encode a recombinant polypeptide according to any one of claims 11-15.

18. The vector of claim 17, wherein the vector is an expression vector configured to express the recombinant polypeptide in a host cell.

19. A cell comprising:
a recombinant polypeptide according to any one of claims 11-15.

20. A cell comprising:
a polynucleotide sequence according to claim 16.

21. A cell comprising a vector according to any one of claims 17-18.

22. A transgenic animal comprising:
a recombinant polypeptide according to any one of claims 11-15, a polynucleotide according to claim 16, a vector according to any one of claims 17-18, or a cell according to any one of claims 19-21.

23. A method comprising:
administering an amount of a composition according to any one of claims 1-10, a recombinant polypeptide according to any one of claims 11-15, a polynucleotide according to claim 16, a vector according to any one of claims 17-18, or a cell according to any one of claims 19-21 to a subject.

24. The method of claim 23, further comprising the step of measuring binding of an apolipoprotein E (ApoE) to an ApoE receptor.

25. The method of any one of claims 23-24, further comprising measuring A β protein formation.

26. The method of any one of claims 23-25, wherein the subject has a neurologic disease or disorder.

27. The method of any one of claims 23-26, wherein the subject has Alzheimer's disease.

29. The method of any one of claims 23-25, wherein the subject has a cardiovascular disease or disorder.

30. A method comprising:
contacting a candidate compound with a recombinant polypeptide according to claim 11 or an ApoE protein; and
measuring binding of the recombinant polypeptide according to claim 11 or the ApoE protein to an ApoE receptor present on the surface of a cell.

31. The method of claim 30, wherein the binding of the recombinant polypeptide according to claim 11 or the ApoE protein to ApoE receptor is measured by measuring ApoE receptor activity.

32. The method of any one of claims 30-31, wherein the ApoE receptor is a low-density lipoprotein receptor.

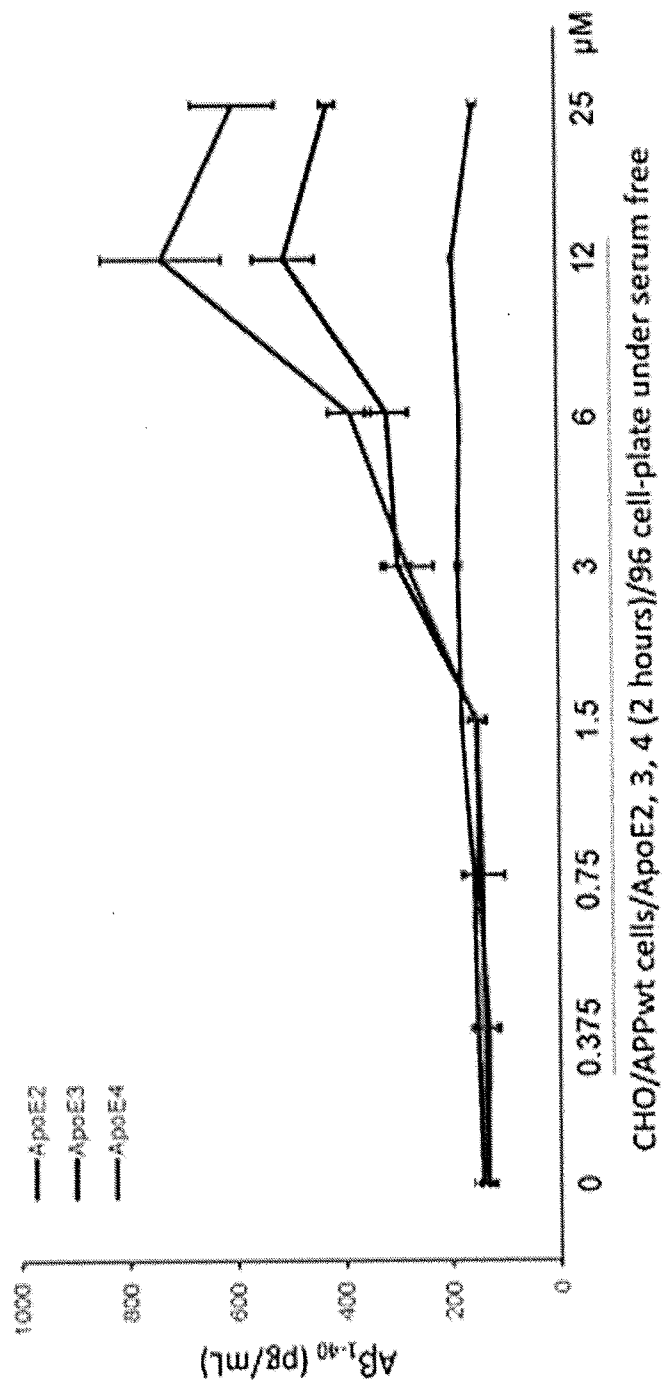


FIG. 1

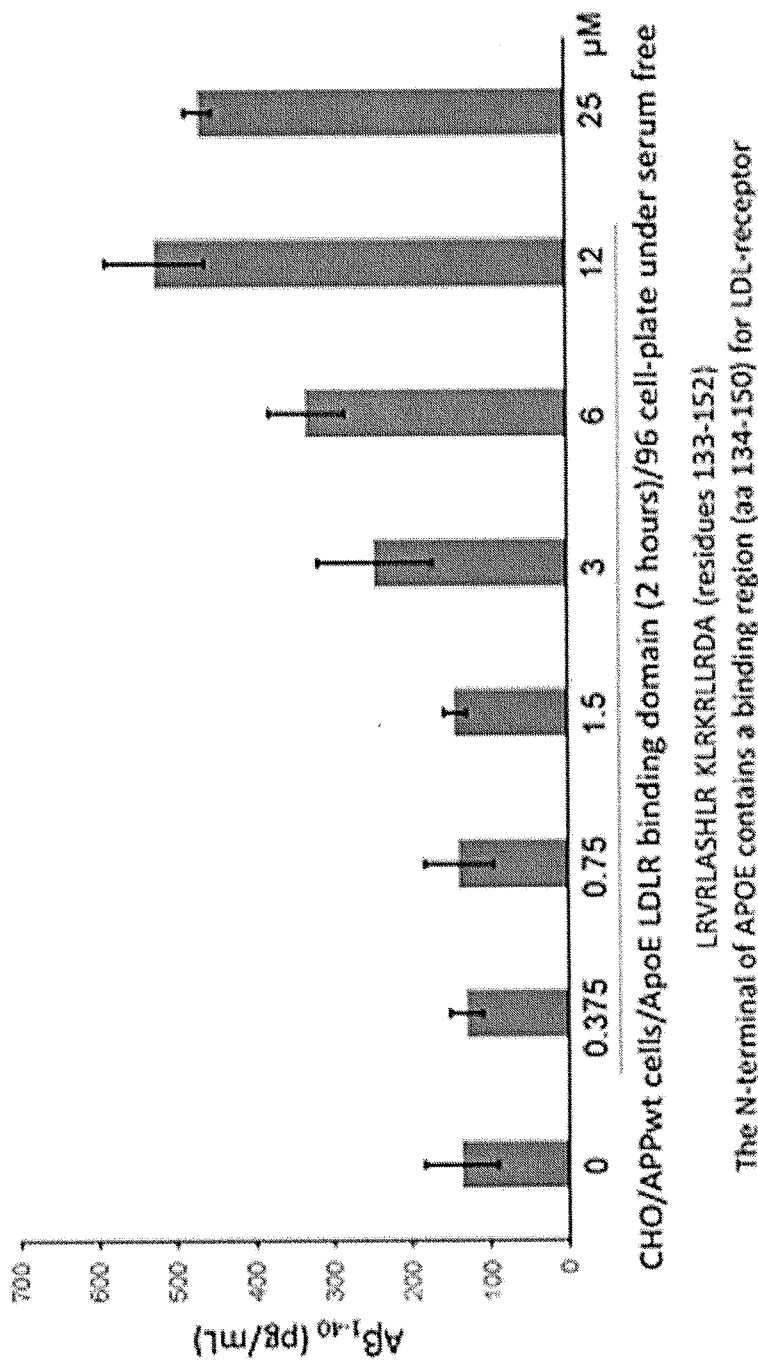


FIG. 2

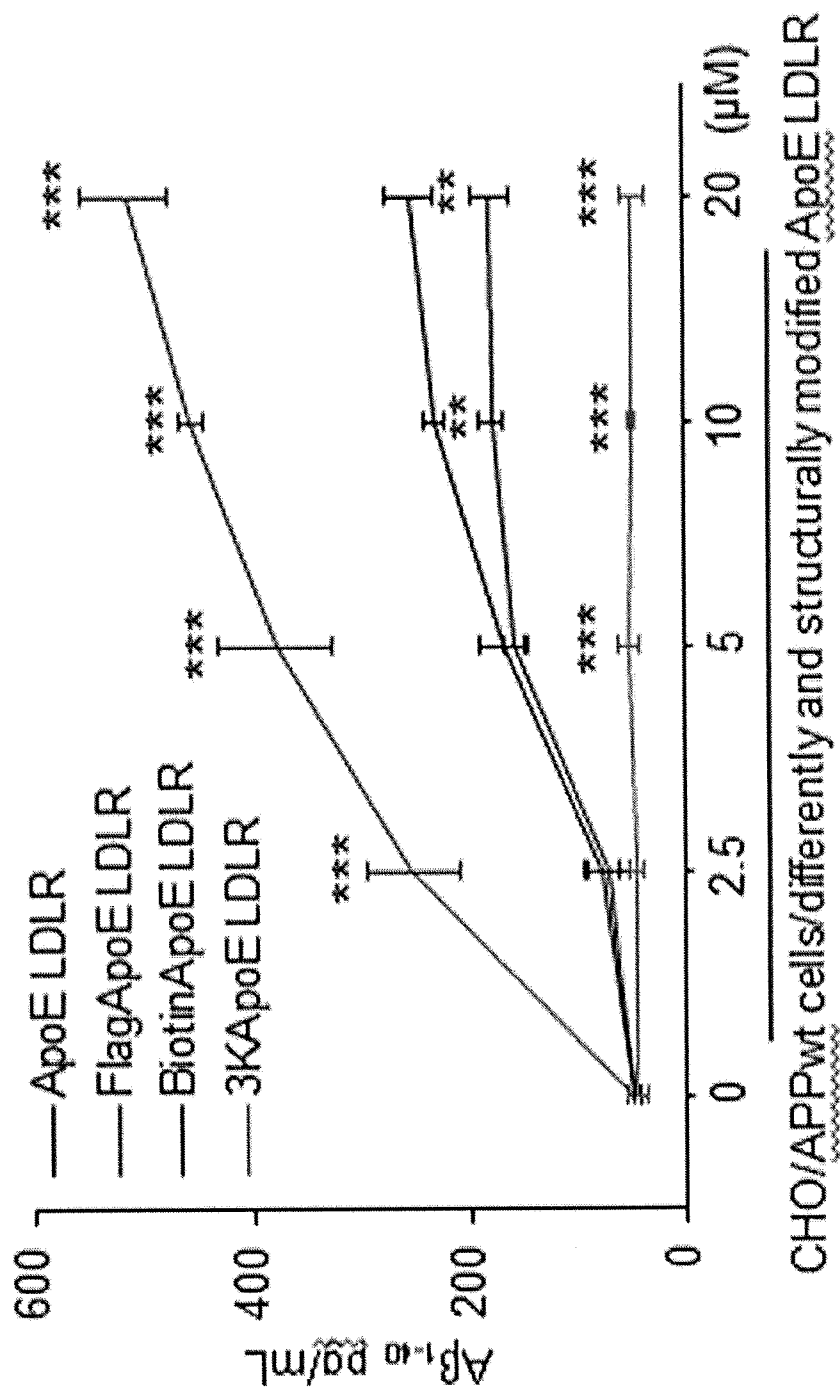


FIG. 3

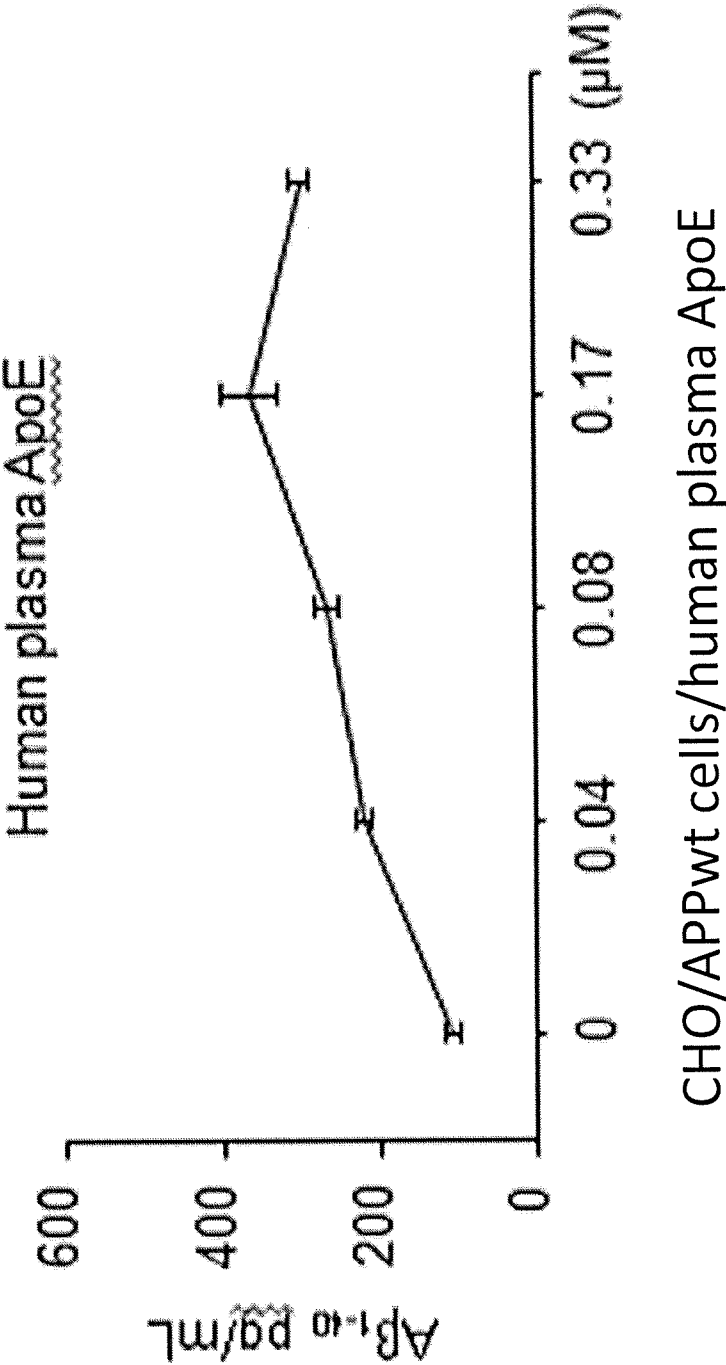
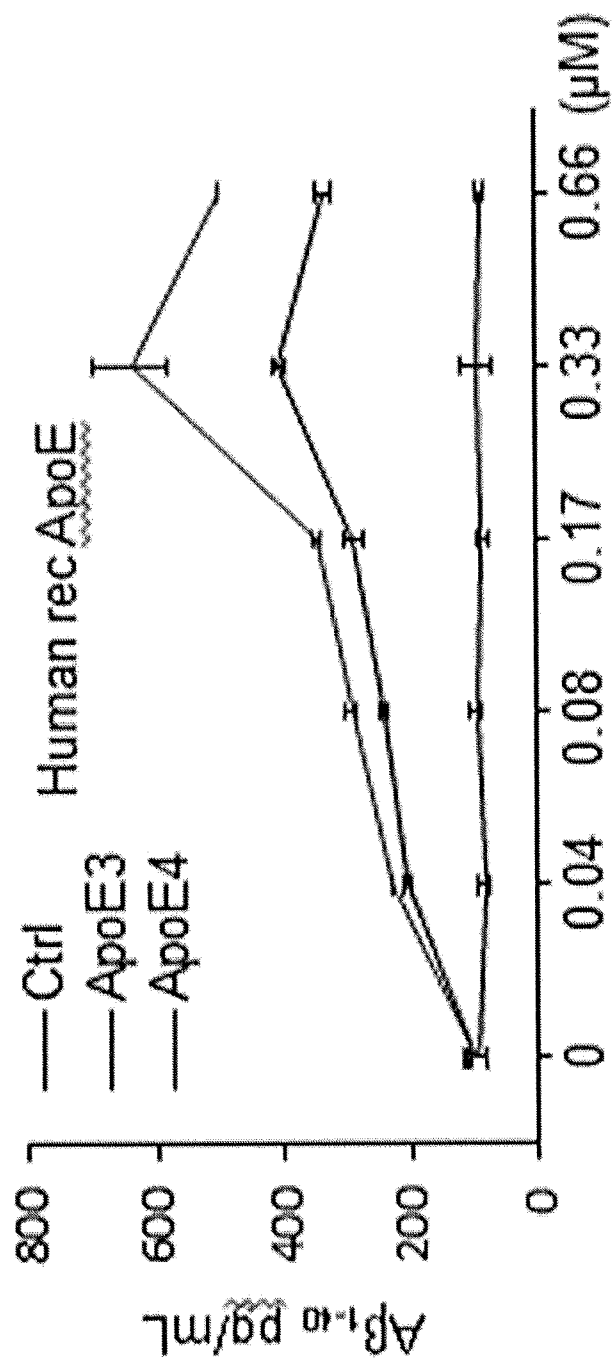


FIG. 4



CHO/APPwt cells/re hu ApoE3, 4 proteins

FIG. 5

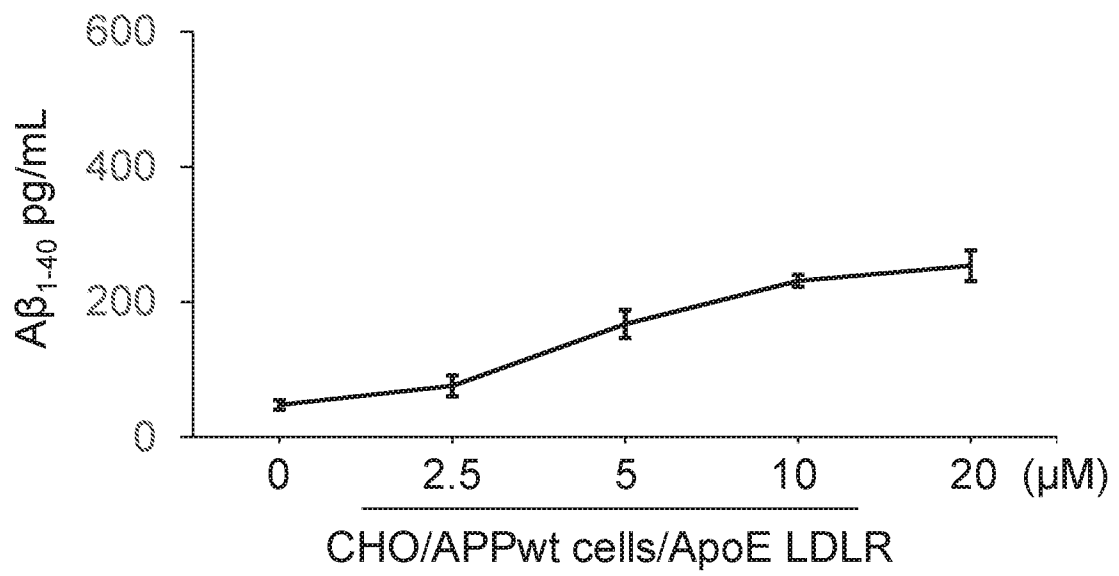


FIG. 6

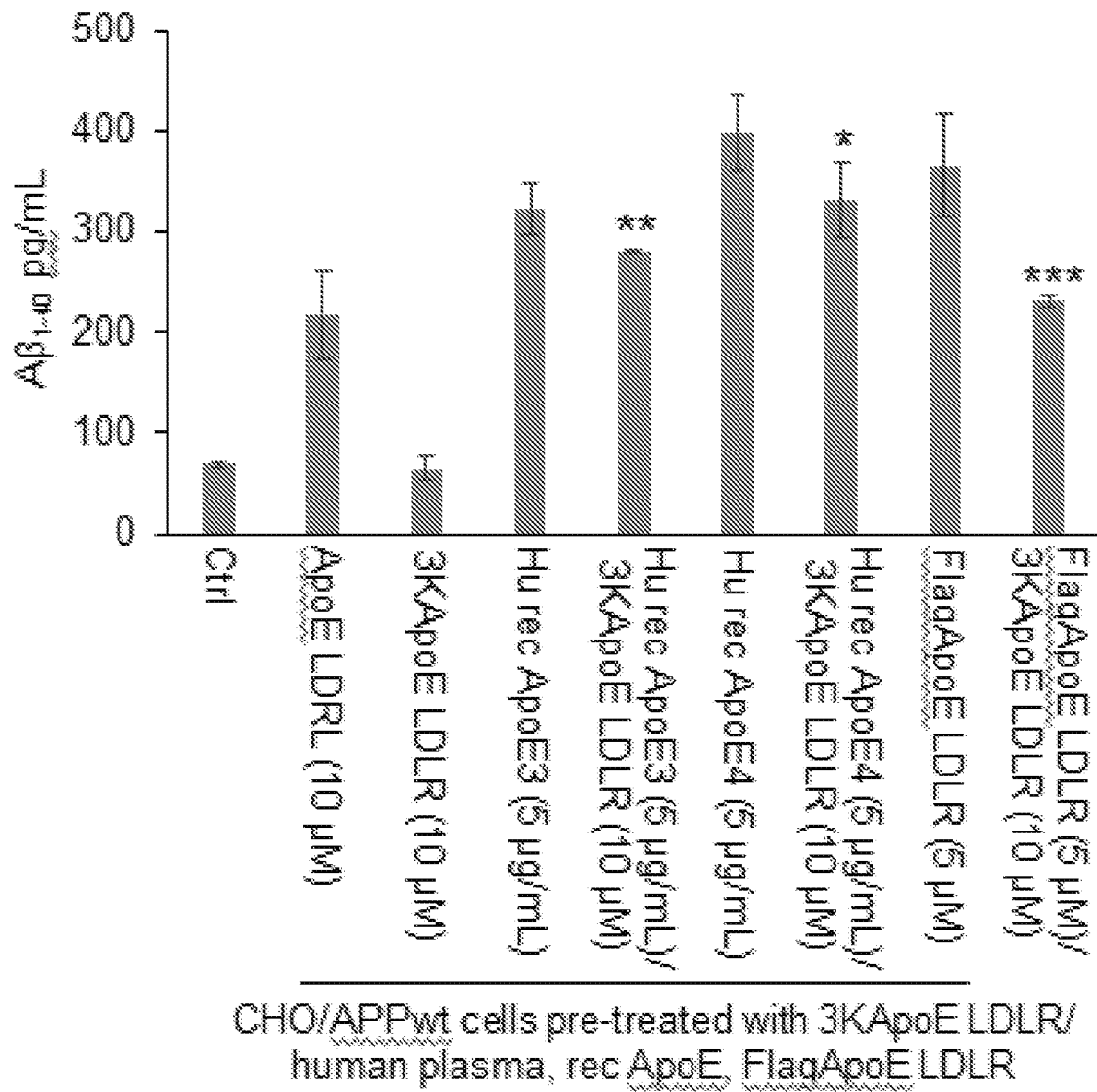


FIG. 7

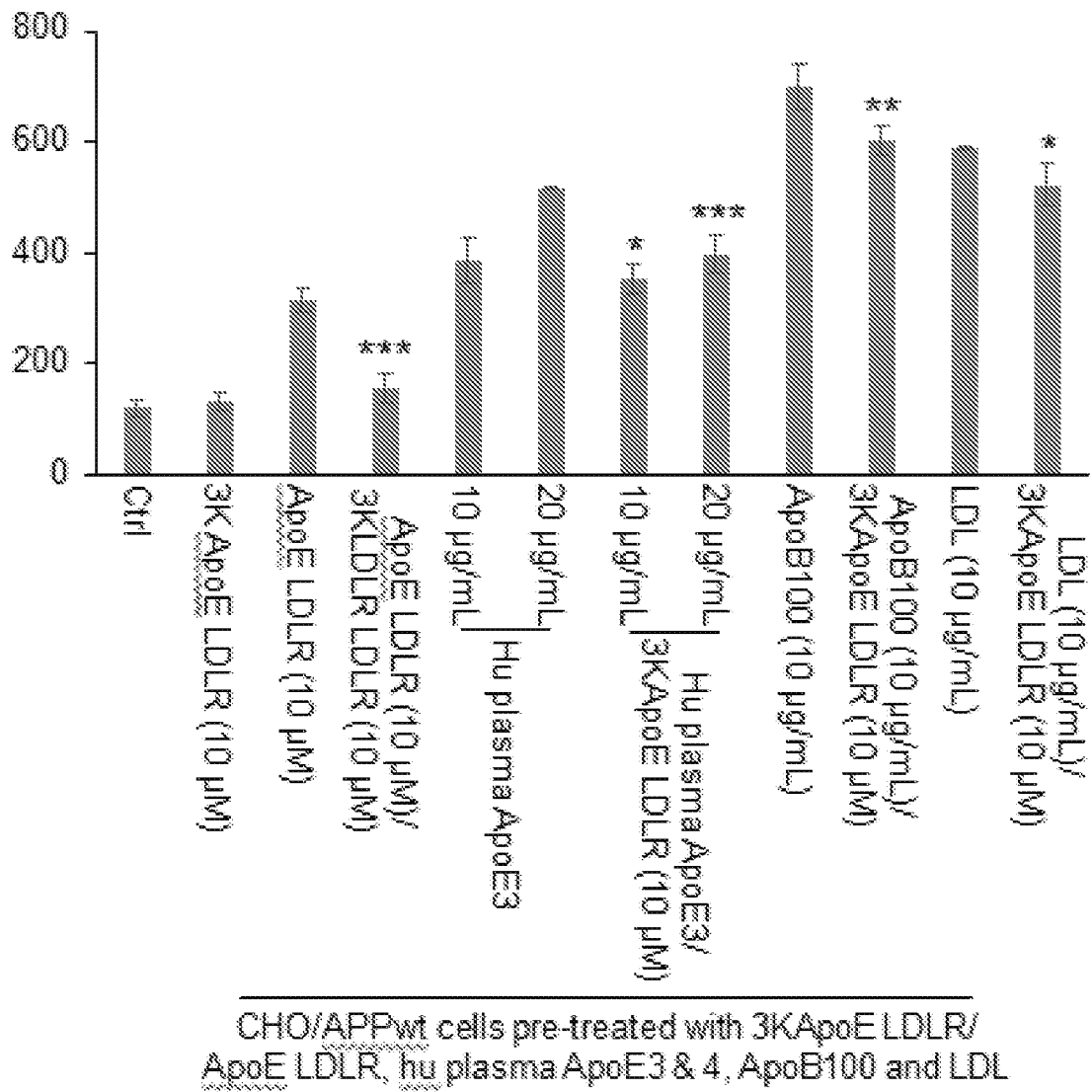


FIG. 8

FIG. 9A

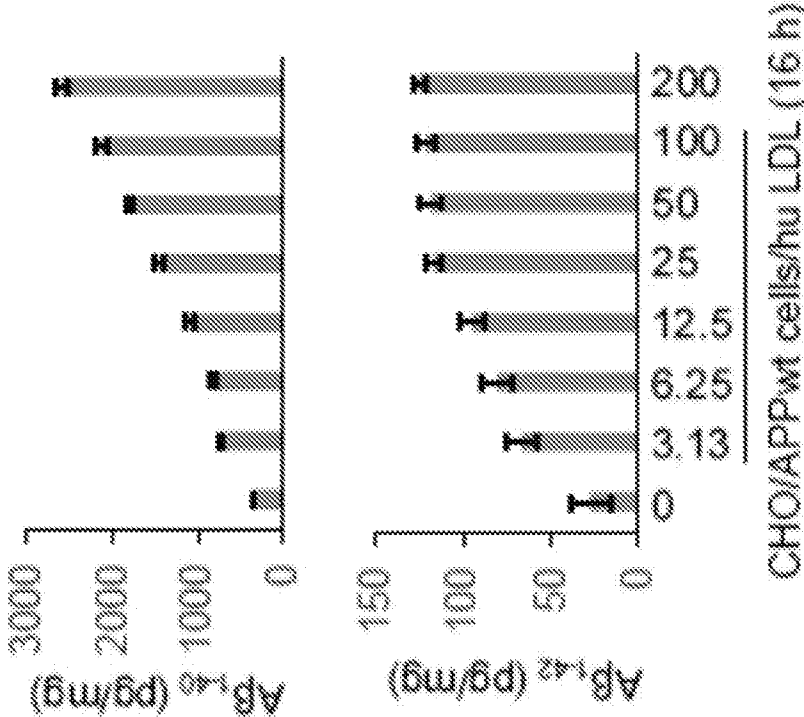
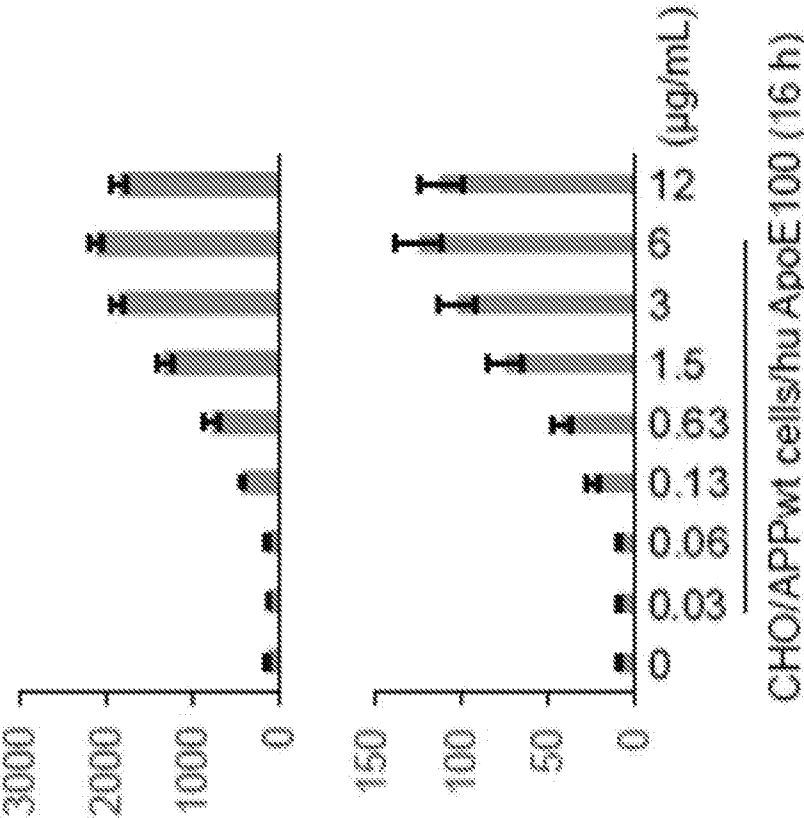


FIG. 9B



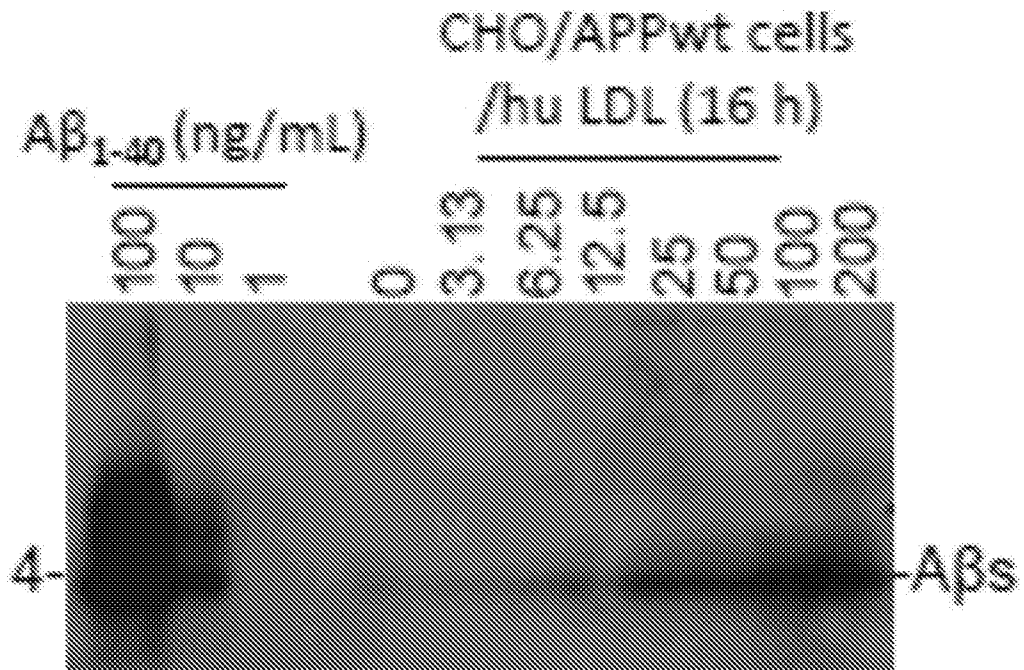


FIG. 9C

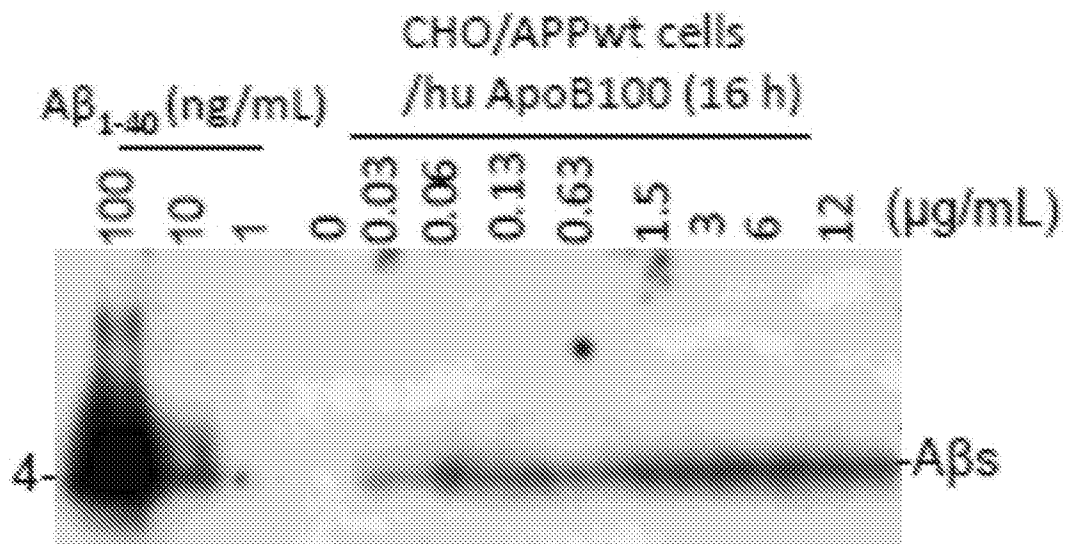


FIG. 9D

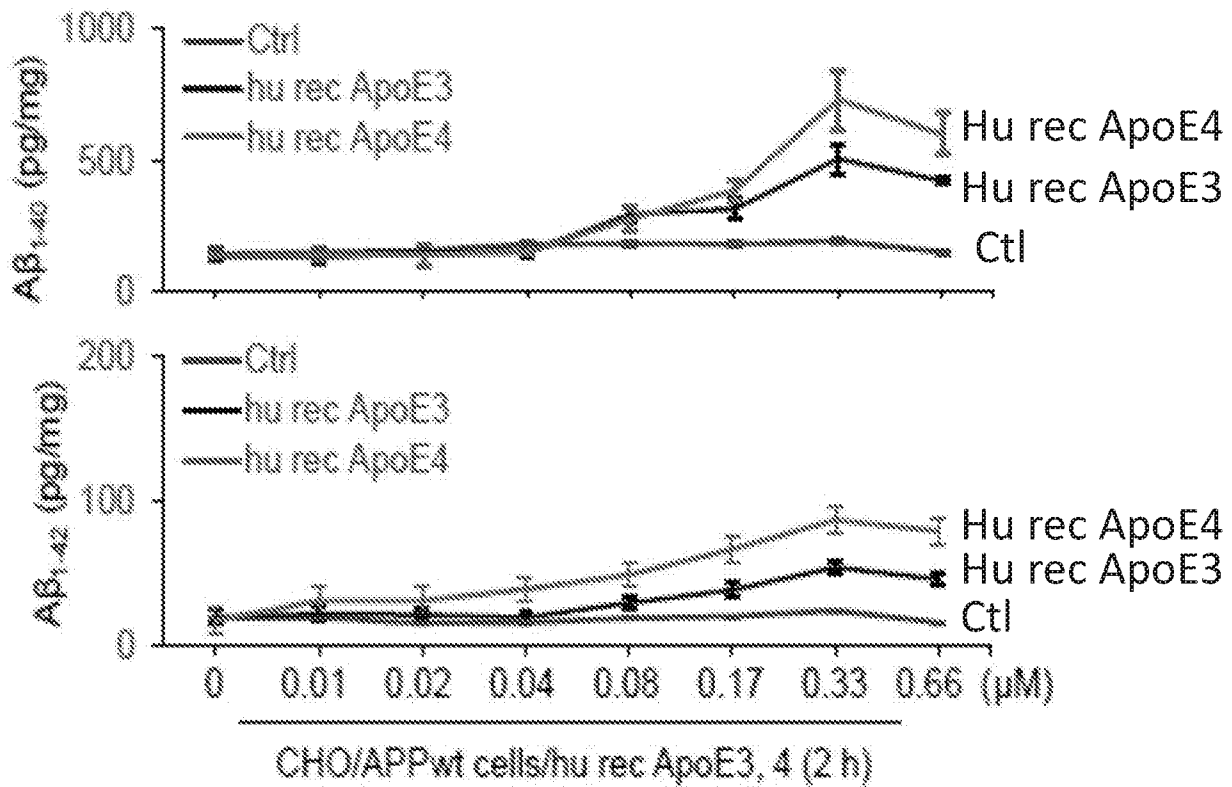


FIG. 9E

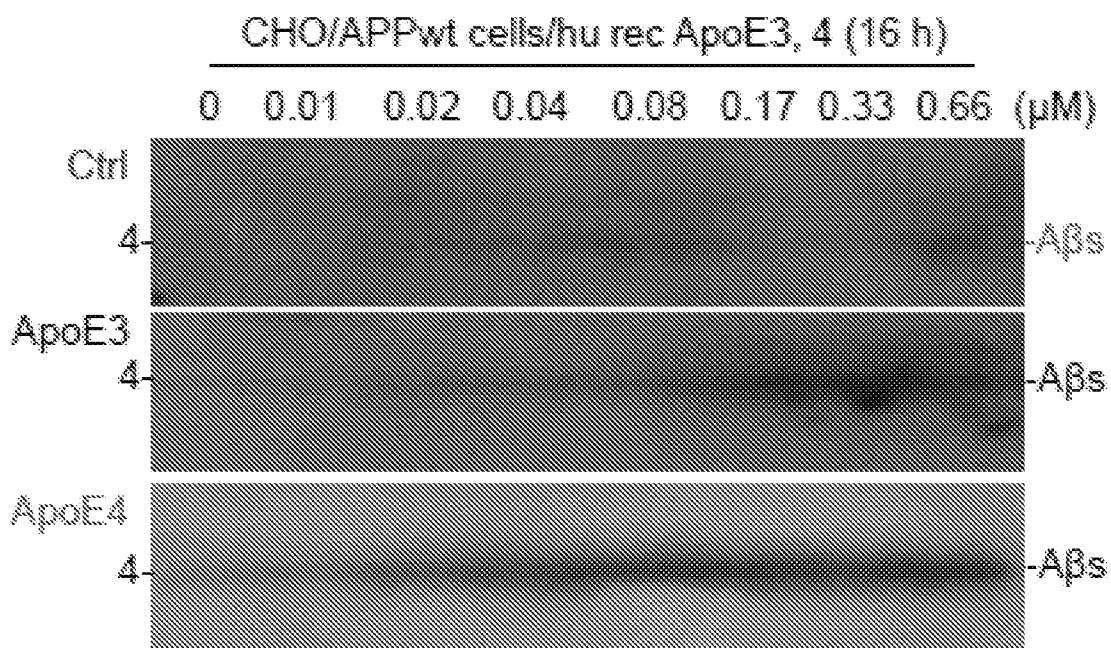


FIG. 9F

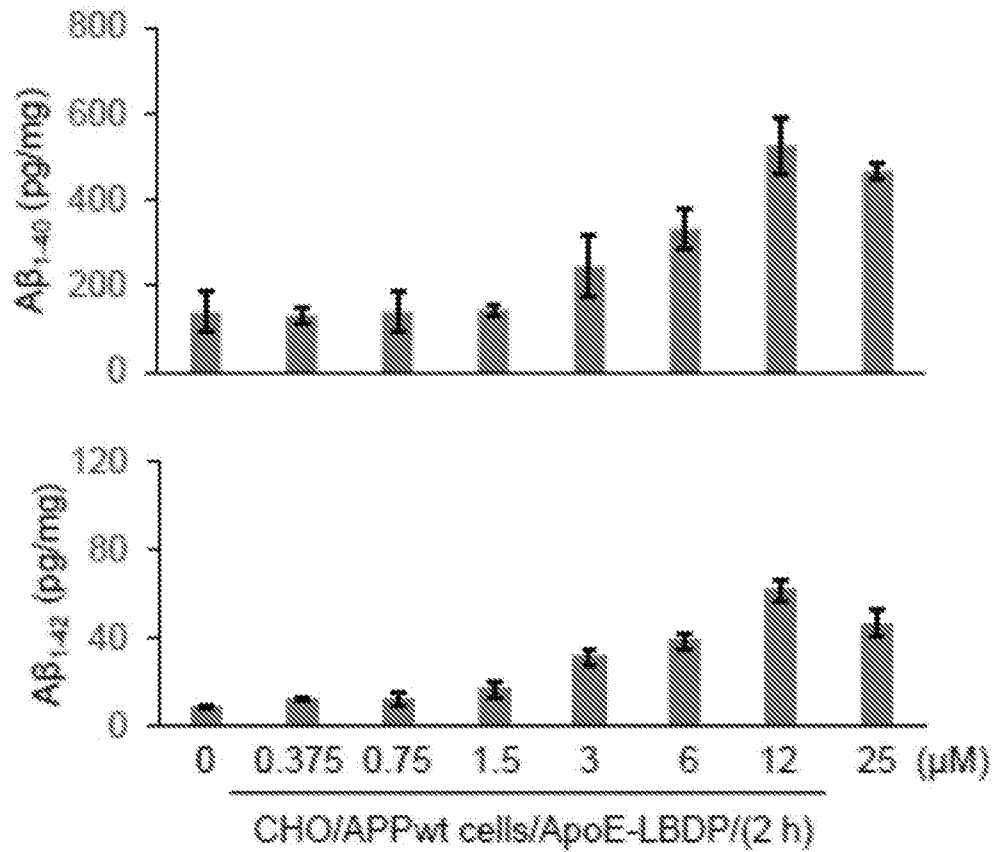


FIG. 10A

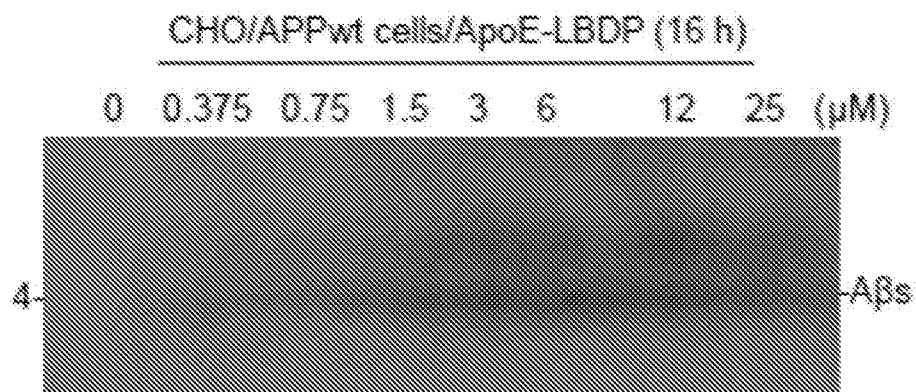


FIG. 10B

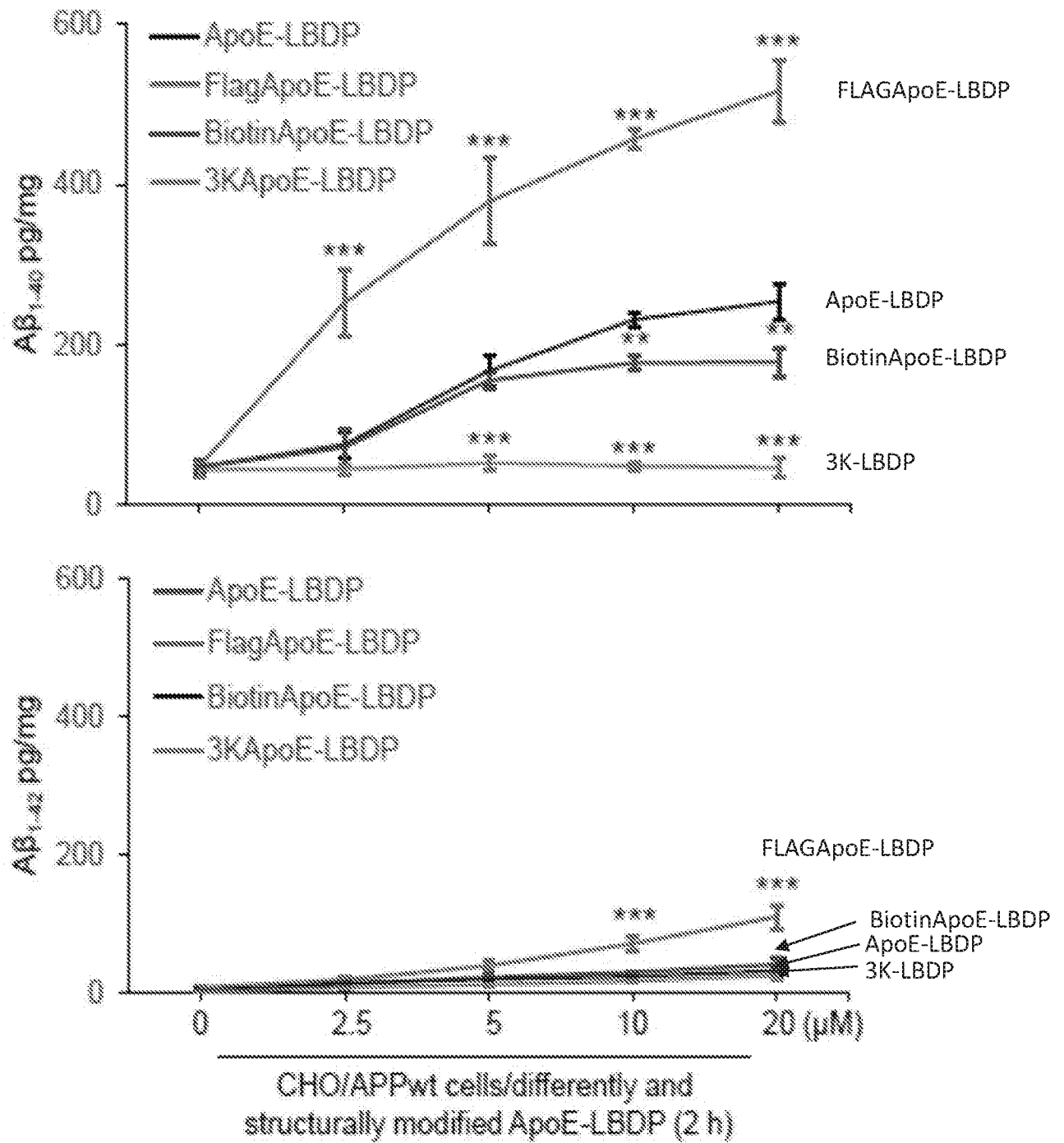
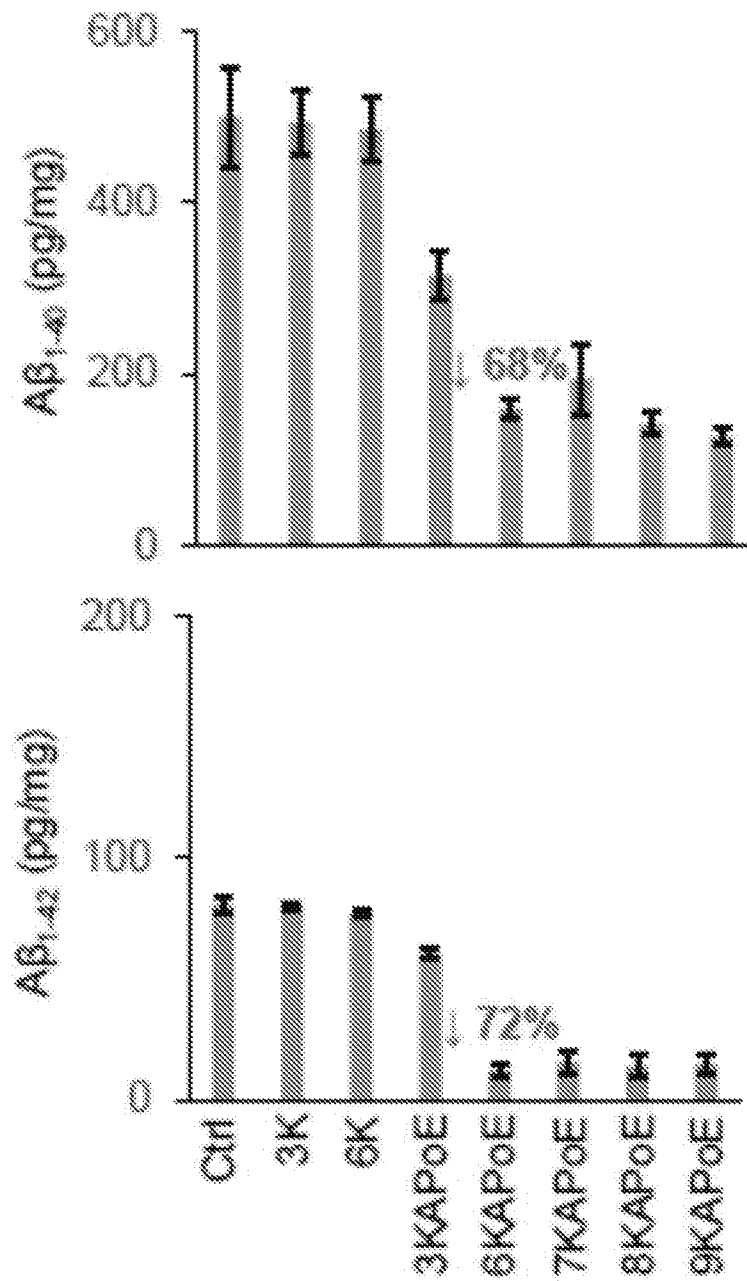


FIG. 10C



CHO/APPwt cells in the presence
of hu rec ApoE4 (10 µg/mL)

FIG. 11A

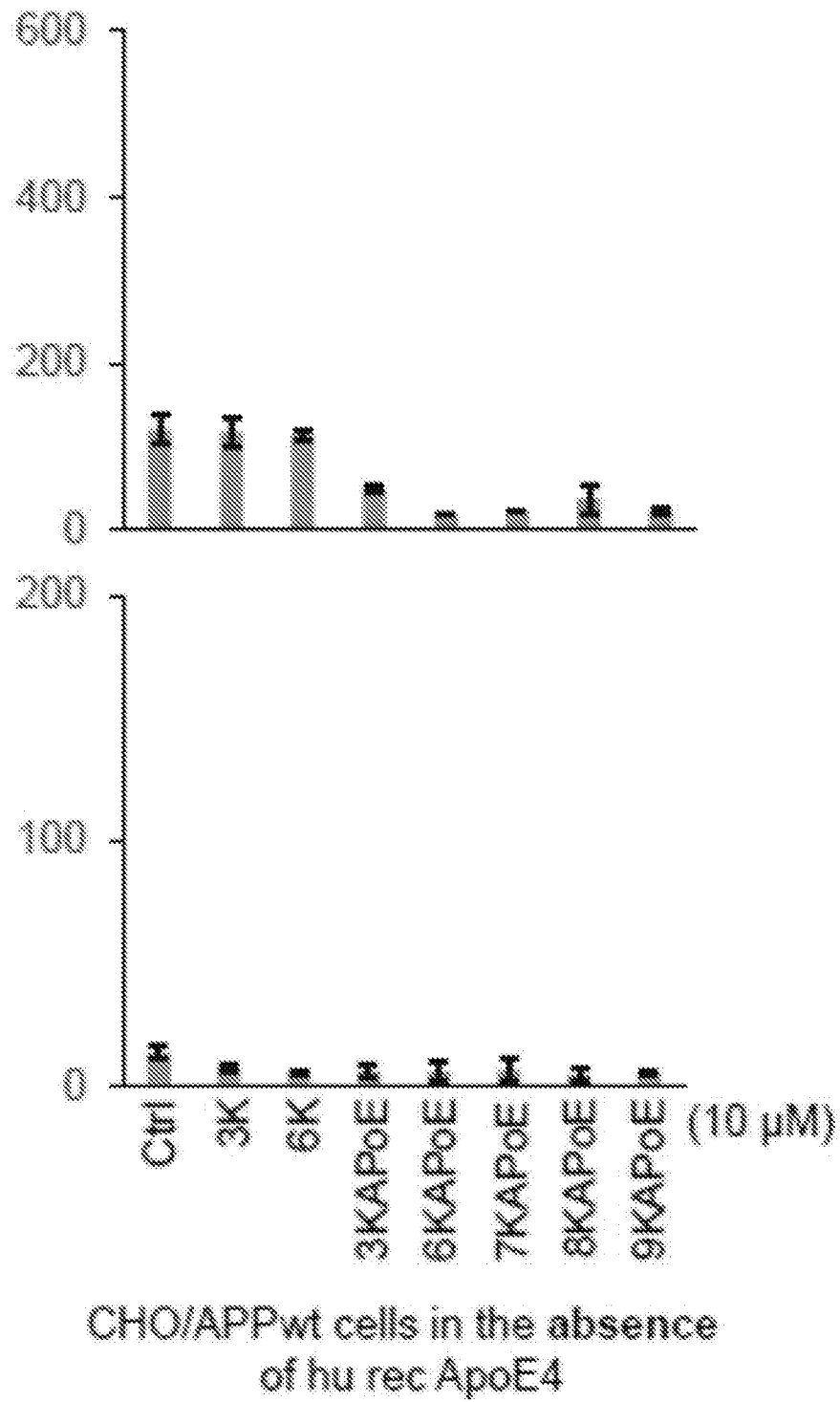


FIG. 11B

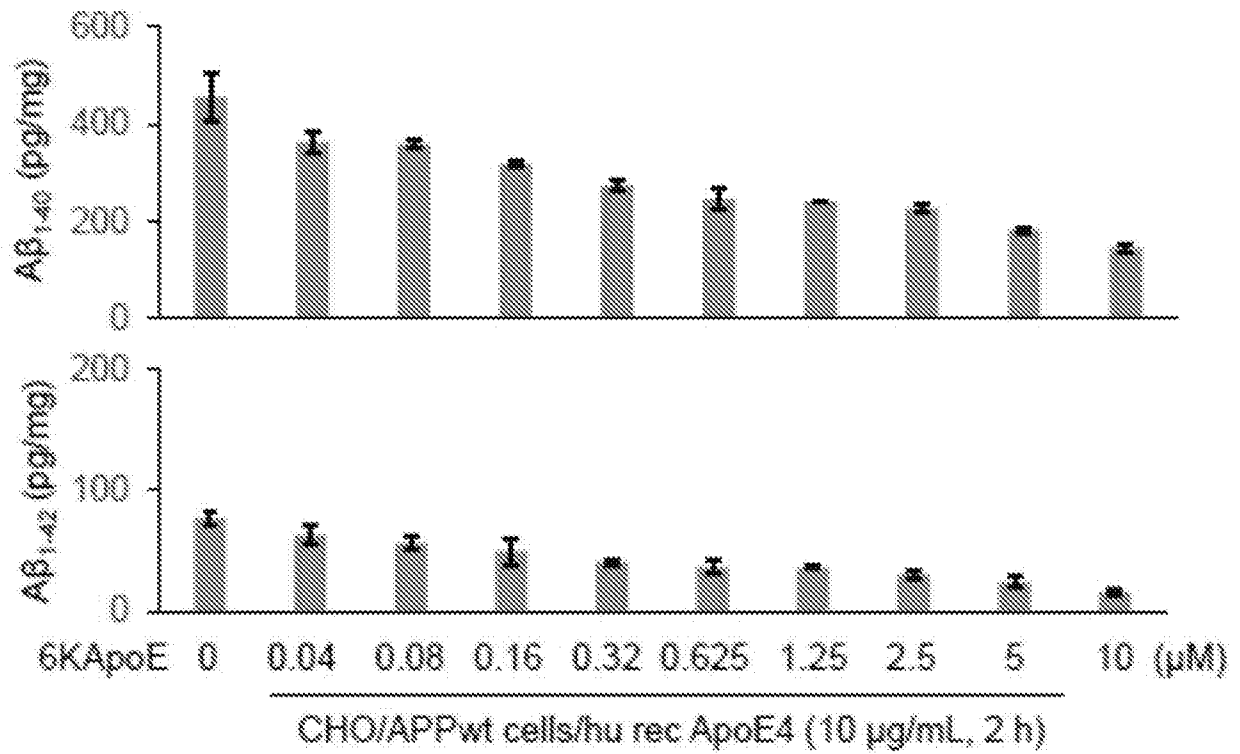


FIG. 11C

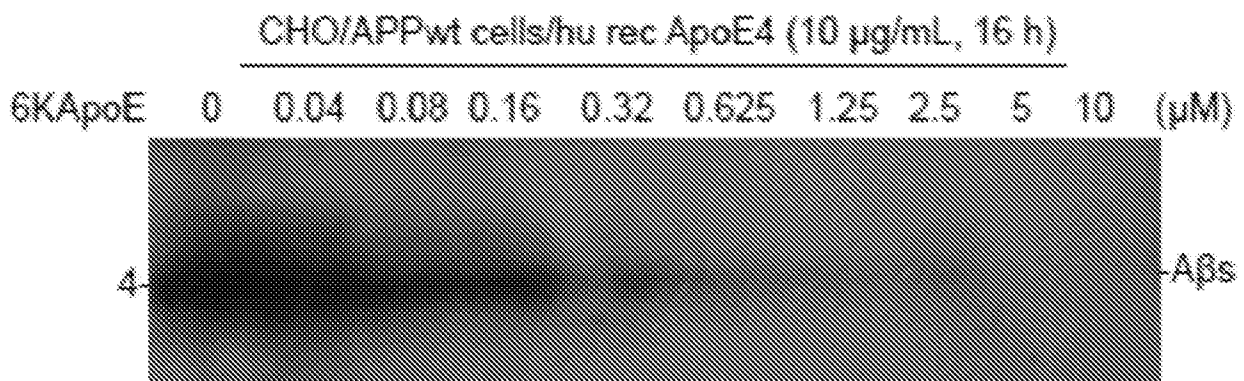


FIG. 11D

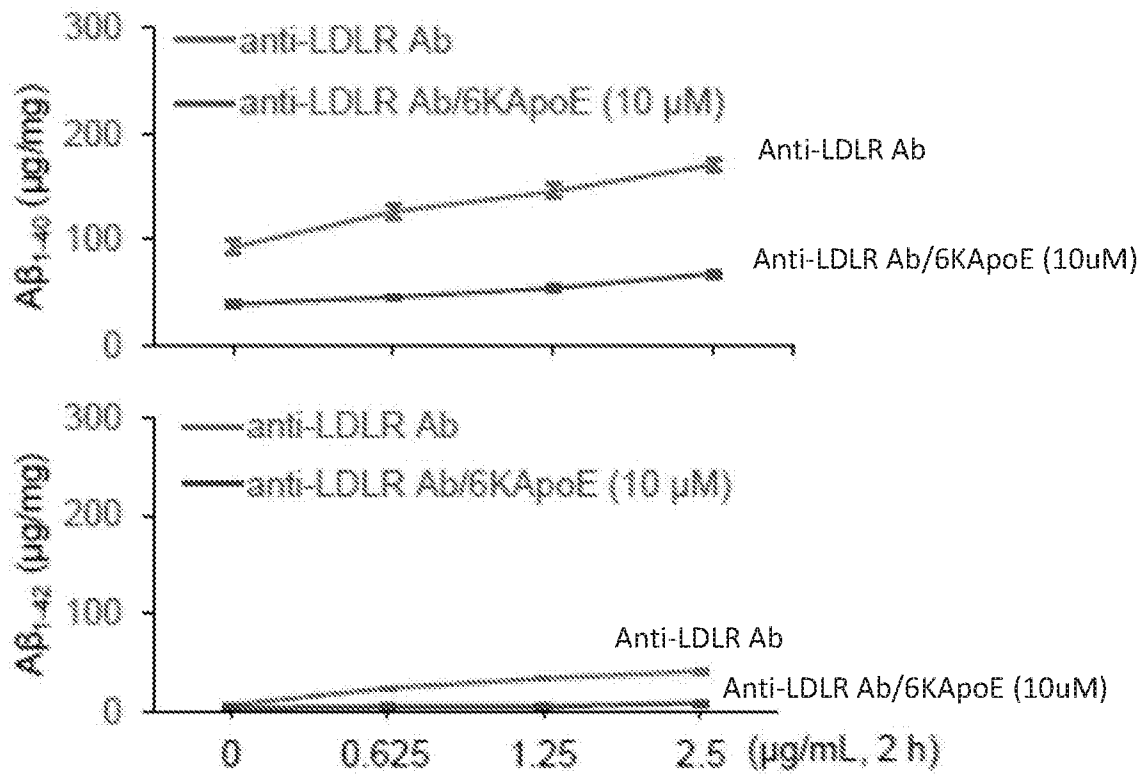


FIG. 12A

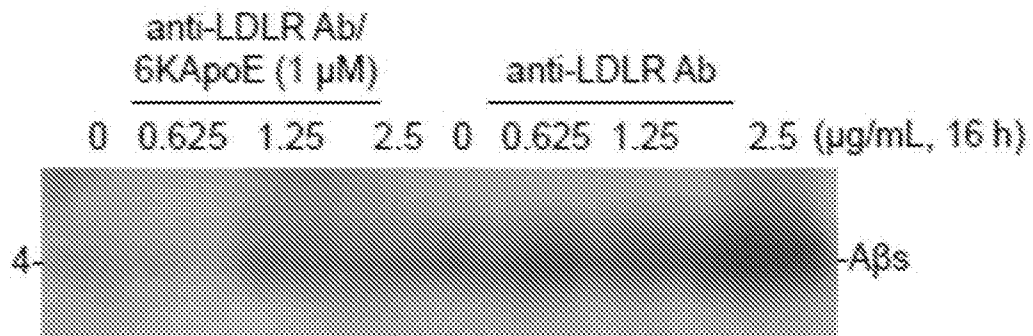


FIG. 12B

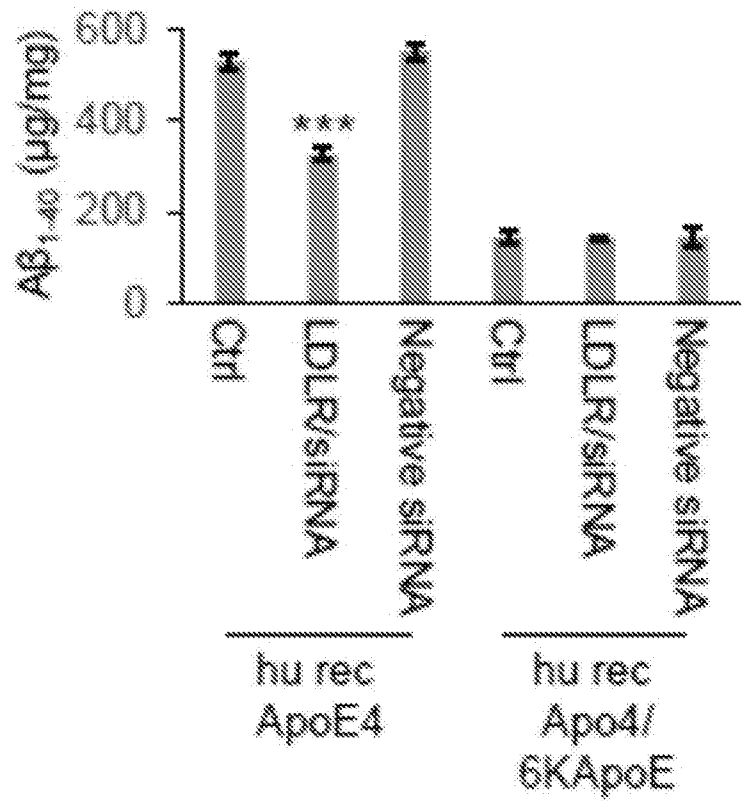


FIG. 12C

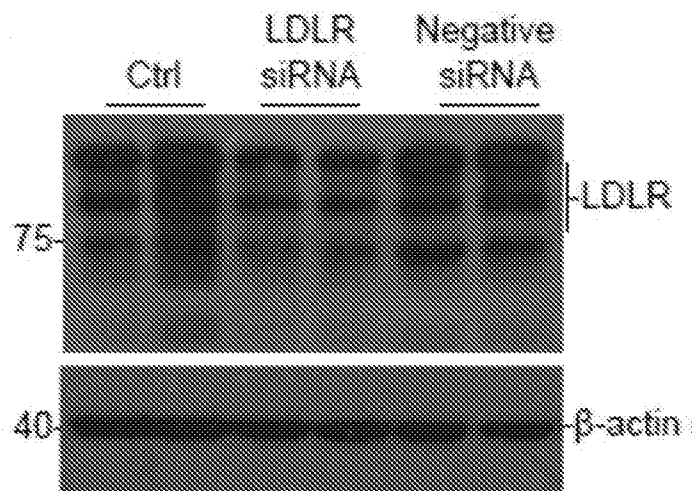


FIG. 12D

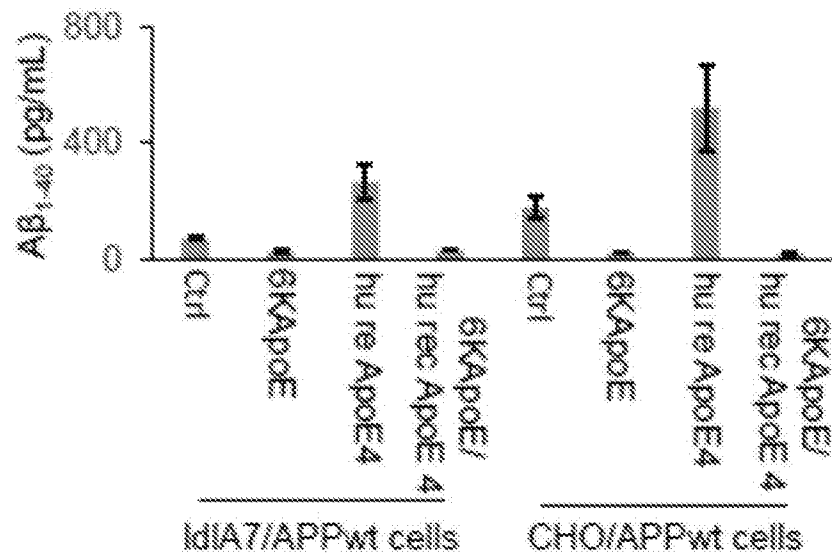


FIG. 12E

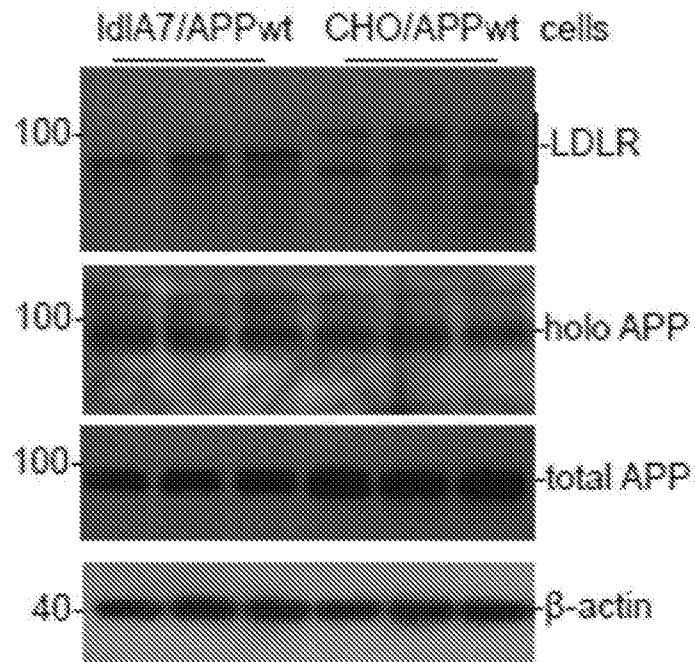


FIG. 12F

FIG. 13A

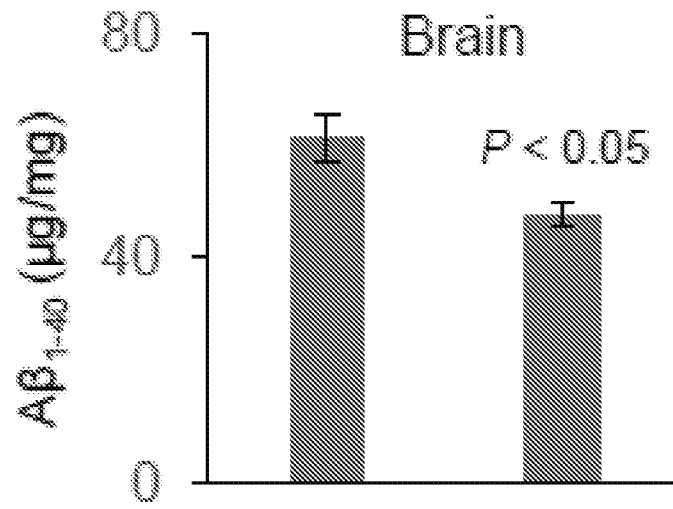


FIG. 13B

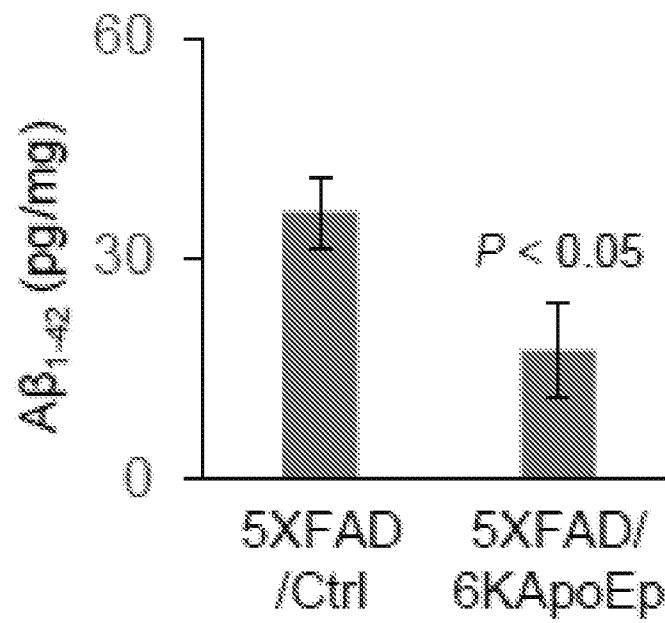


FIG. 14A

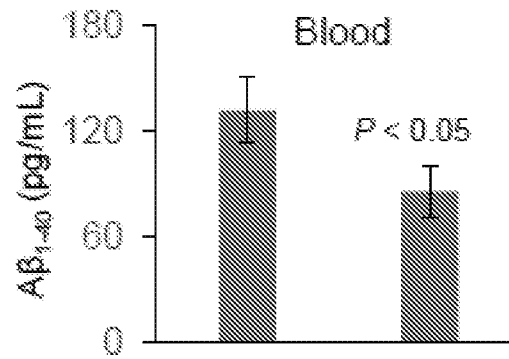
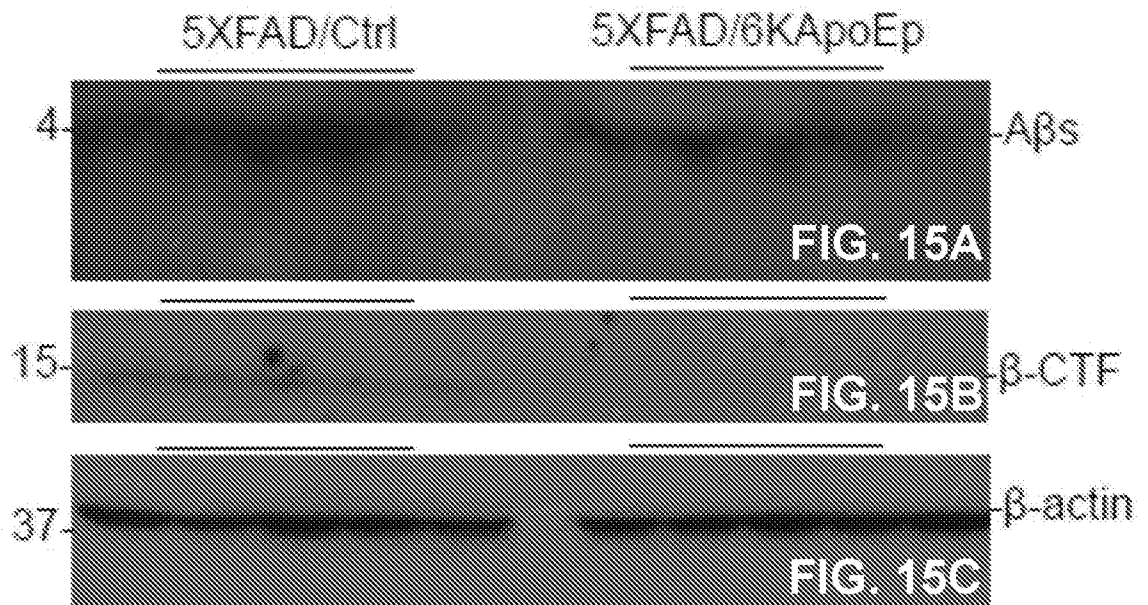
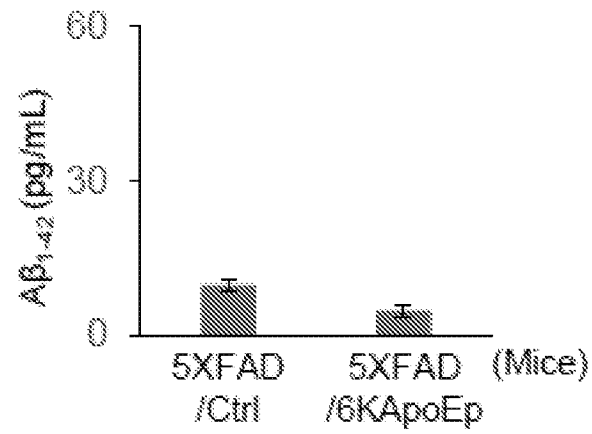
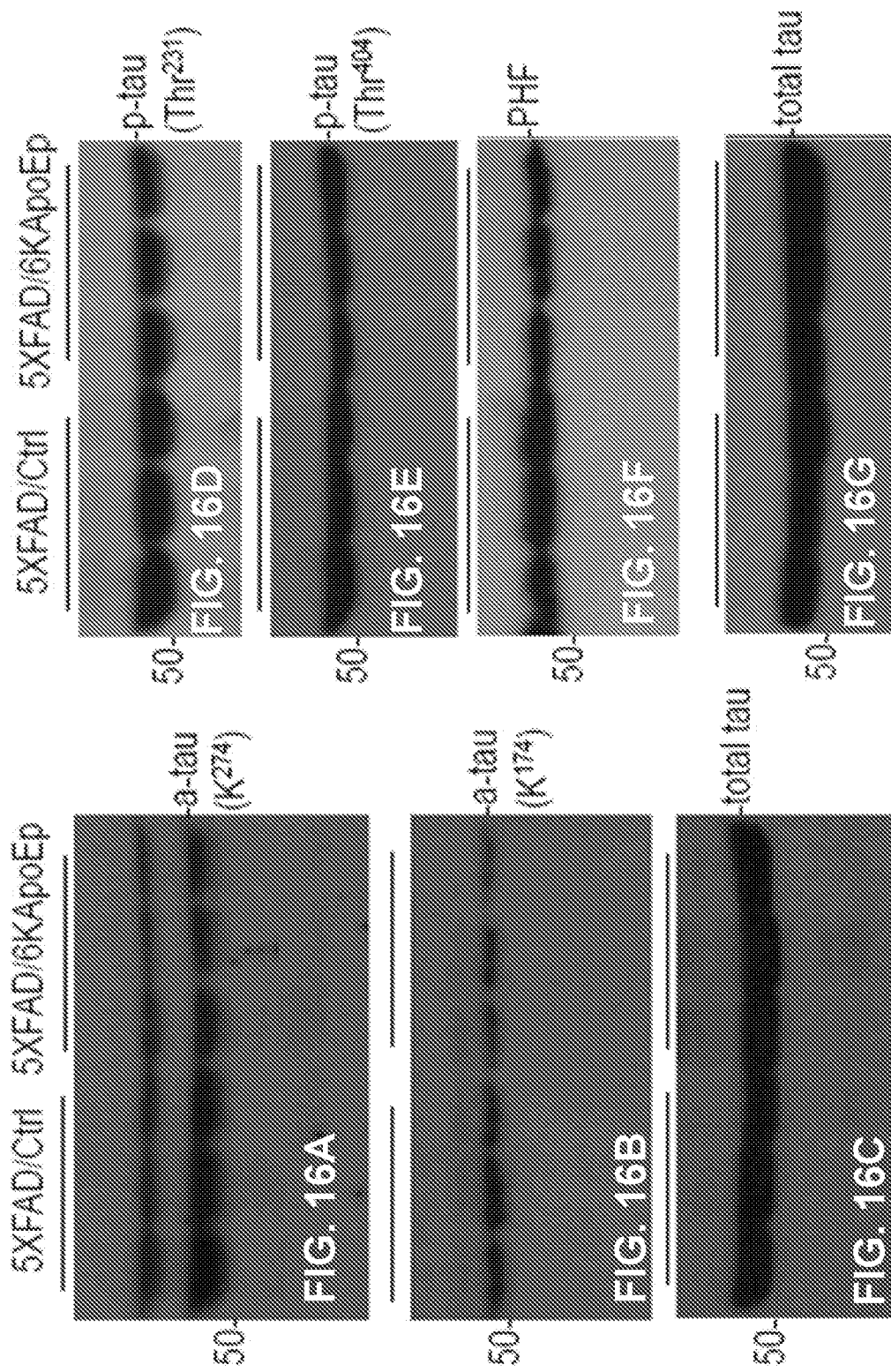


FIG. 14B





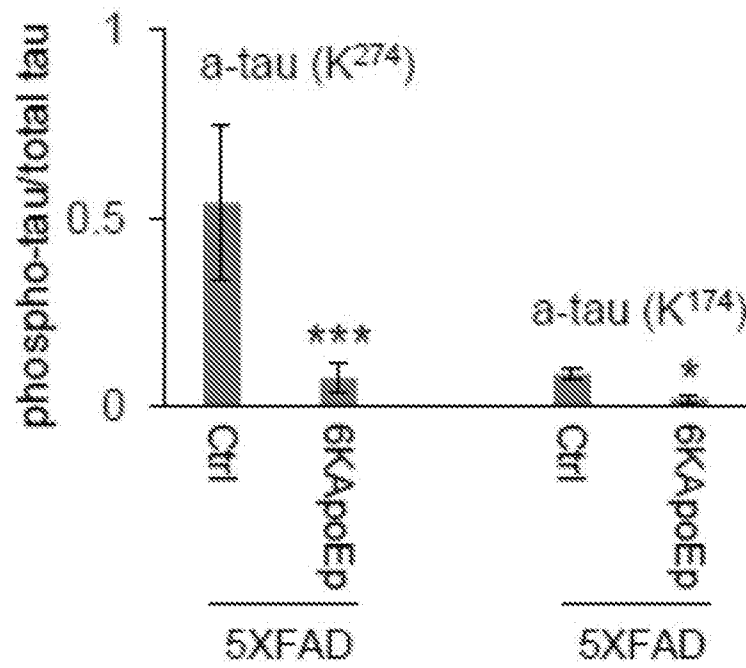


FIG. 17A

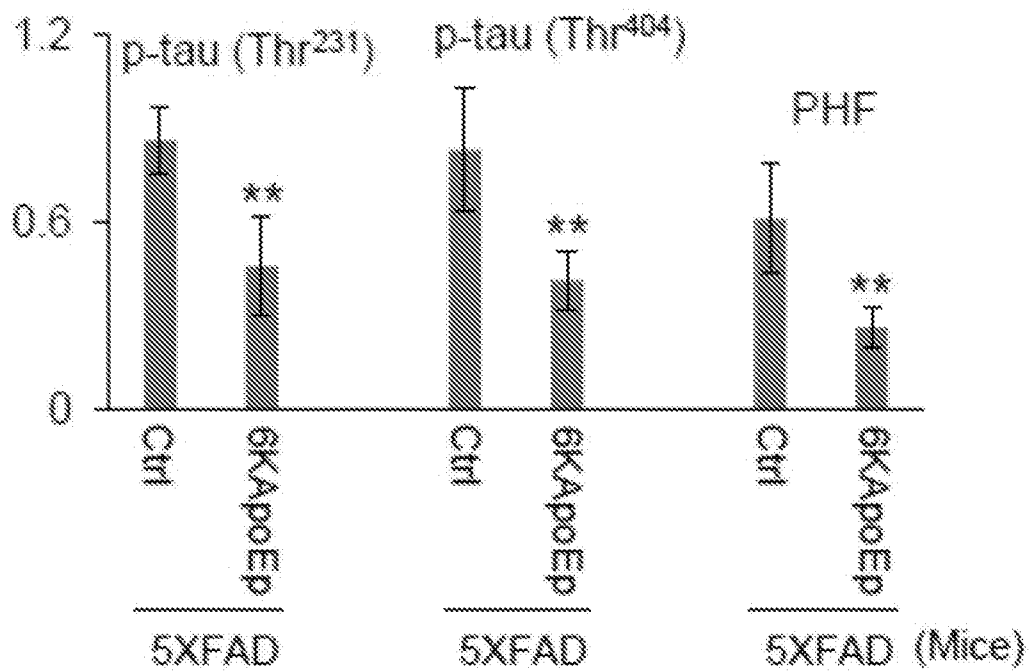


FIG. 17B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/030826

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 38/17; A61K 47/48; C07K 14/775; C07K 16/28 (2017.01)

CPC - A61K 47/48246; A61K 2039/505; A61K 2039/55516; C07K 14/775; C07K 16/2863; C07K 2317/24 (2017.02)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

USPC - 424/9.1; 424/130.1; 424/184.1; 435/7.1; 514/12; 514/7.4 (keyword delimited)

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/008823 A2 (MAYO FOUNDATION FOR MEDICAL EDUCATION AND RESEARCH et al) 20 January 2011 (20.01.2011) entire document	1, 2, 5-7, 11-13
X	US 7,608,246 B2 (VAN DEN ELZEN et al) 27 October 2009 (27.10.2009) entire document	1, 3, 5
X	WO 2016/064895 A1 (THE BRIGHAM AND WOMEN'S HOSPITAL, INC.) 28 April 2016 (28.04.2016) entire document	1, 4
X	US 2014/0179595 A1 (WANG et al) 26 June 2014 (26.06.2014) entire document	11, 16-20, 30
P, X	US 2017/0058017 A1 (MAYO FOUNDATION FOR MEDICAL EDUCATION AND RESEARCH) 02 March 2017 (02.03.2017) entire document	1, 2, 5-9, 11-20, 30
A	MAURICE et al. "A potential complication in the use of monoclonal antibodies: inhibition of apoB-mediated receptor binding by an anti-apoE antibody," Journal of Lipid Research, 01 April 1998 (01.04.1998), Vol. 30, Pgs. 587-596. entire document	1-9, 11-20, 30
A	WO 2012/075422 A2 (THE WASHINGTON UNIVERSITY et al) 07 June 2012 (07.06.2012) entire document	1-9, 11-20, 30

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

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

30 July 2017

Date of mailing of the international search report

16 AUG 2017

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Blaine R. Copenheaver

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

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/030826

Box No. 1 Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

- a. ☒ forming part of the international application as filed:
☒ in the form of an Annex C/ST.25 text file.
☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☒ furnished subsequent to the international filing date for the purposes of international search only:
☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).
2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs: 1-9 were searched.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/030826

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 10, 21-27, 29, 31, 32
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

Remark on Protest

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