



(51) International Patent Classification:

A61K 39/395 (2006.01) *A61K 31/135* (2006.01)
A01K 67/027 (2006.01) *A61P 9/10* (2006.01)

(21) International Application Number:

PCT/US2016/053351

(22) International Filing Date:

23 September 2016 (23.09.2016)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/233,290 25 September 2015 (25.09.2015)

US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: METHODS AND COMPOSITIONS FOR TREATMENT OF ATHEROSCLEROSIS

(57) Abstract: The present invention provides methods and compositions for treating atherosclerosis in a subject comprising the use of therapeutic compounds to reduce Reelin in the circulation of the subject, thereby reducing the adhesion of leukocytes to the vascular wall. The invention also provides methods and compositions for reducing leukocyte adhesion to the vascular wall in a subject.



TITLE**METHODS AND COMPOSITIONS FOR TREATMENT OF
ATHEROSCLEROSIS****CROSS REFERENCE TO RELATED APPLICATIONS**

5 This application claims the benefit of United States Provisional Application No. 62/233,290, filed September 25, 2015, herein incorporated by reference in its entirety.

INCORPORATION OF SEQUENCE LISTING

 The sequence listing that is contained in the file named
“UTSW010WO_ST25.txt,” which is 45,952 bytes as measured in Microsoft Windows
10 operating system and was created on September 22, 2016, is filed electronically herewith
and incorporated herein by reference.

FIELD OF THE INVENTION

 The present invention relates in general to the field of medicine. More
specifically, the invention relates to methods and compositions for treatment of
15 atherosclerosis.

BACKGROUND OF THE INVENTION

 Atherosclerosis is a chronic inflammatory process characterized by the
recruitment and transmigration of monocytes into the intima of large vessels and
subendothelial accumulation of lipids and lipid-laden foam cells, resulting in plaque
20 formation that can rupture and lead to infarction or death. Atherosclerosis is the leading
cause of morbidity and mortality in western countries and thus, effective therapies for
treatment and prevention of this disease are needed.

SUMMARY OF THE INVENTION

 In one aspect, the invention provides a method of treatment or prevention of
25 atherosclerosis in a subject comprising providing a therapeutic composition that
decreases the activity of Reelin protein in the blood stream of the subject, wherein the

treatment results in treatment or prevention of atherosclerosis in a subject or any symptoms thereof. In one embodiment, the reduction of disease symptoms comprises reduced leukocyte adhesion to the vascular wall in the subject. In another embodiment, targeting Reelin comprises reduction of Reelin protein expression or inhibition of Reelin
5 function in the circulation of a subject. In still another embodiment, the therapeutic composition comprises a protein, a peptide, a polypeptide, an RNA molecule, a peptidomimetic, an siRNA molecule, a decoy receptor, a carbohydrate, an antibody or antibody fragment, or a small molecule, wherein said therapeutic compound targets Reelin, resulting in a reduction of Reelin concentration and/or activity in the circulation
10 of the subject. In other embodiments, the antibody is a monoclonal antibody, or the decoy receptor is an Fc-coupled dimeric or monomeric decoy receptor. In another embodiment, the therapeutic composition performs a function comprising: (a) reducing or inhibiting the expression of a gene encoding Reelin; (b) reducing or inhibiting the expression of a Reelin protein; (c) reducing or inhibiting expression of a receptor for
15 Reelin; (d) reducing or inhibiting the function of a Reelin protein; or (e) binding to Reelin protein in such a way that the function is reduced or eliminated. In a further embodiment, the method further comprises administering an antithrombotic drug, an antibody targeting PCSK9, or an HMG-CoA reductase inhibitor or other therapeutic agent with the potential to reduce atherosclerosis in a subject.

20 In another aspect, the invention provides a method for reducing leukocyte adhesion to the vascular wall in a subject, comprising introducing to the subject a therapeutic compound targeting Reelin in the circulation of the subject, wherein said treatment results in reduced leukocyte adhesion to the vascular wall. In one embodiment, targeting Reelin comprises reduction of Reelin protein expression or inhibition of Reelin
25 function in the circulation of a subject. In another embodiment, the therapeutic compound comprises a protein, a peptide, a polypeptide, an RNA molecule, an siRNA molecule, a decoy receptor, a carbohydrate, an antibody or antibody fragment, or a small molecule, wherein said therapeutic compound targets Reelin, resulting in reduced leukocyte adhesion to the vascular wall of the subject. In another embodiment, the

antibody is a monoclonal antibody. In still another embodiment, the decoy receptor is an Fc-coupled dimeric or monomeric decoy receptor. In another embodiment, the therapeutic compound performs a function comprising: (a) reducing or inhibiting the expression of a gene encoding Reelin; (b) reducing or inhibiting the expression of a Reelin protein; (c) reducing or inhibiting expression of a receptor for Reelin; (d) reducing or inhibiting the function of a Reelin protein; or (e) binding to Reelin protein in such a way that the function is reduced or eliminated. In another embodiment, the method further comprises administering an antithrombotic drug, an antibody targeting PCSK9 or an HMG-CoA reductase inhibitor to the subject. In another embodiment, the method further comprises identifying a subject suspected of having atherosclerosis or being in need of atherosclerosis treatment.

In another aspect, the invention provides a therapeutic composition for treating atherosclerosis in a subject comprising a therapeutic compound capable of being delivered in an effective amount to reduce disease symptoms and a pharmaceutically acceptable carrier. In one embodiment, the therapeutic compound comprises a protein, a peptide, a polypeptide, an RNA molecule, a peptidomimetic, an siRNA molecule, a decoy receptor, a carbohydrate, an antibody or antibody fragment, or a small molecule, wherein said therapeutic compound targets Reelin, resulting in a reduction of Reelin in the circulation of the subject.

In another aspect, the invention provides an antibody, immunological fragment, or derivative thereof which immunologically binds Reelin, for the treatment of atherosclerosis, or diseases associated therewith or resulting therefrom.

In another aspect, the invention provides an siRNA complementary to all or a portion of a Reelin mRNA sequence provided as SEQ ID NO:3-5. In one embodiment, the siRNA functions to inhibit Reelin in a subject.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the invention. The invention may be better

understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein:

FIG. 1A – 1D – Shows induction of systemic Reelin-deficiency by tamoxifen injection and analysis of plasma lipids. (A) Representative western blots analyses of Reelin in plasma, brain, and liver extract prepared from previously tamoxifen-treated two month-old $Ldlr^{-/-}$ and DKO mice. Results for two representative mice are shown for each genotype. (B) Body weight, fasting plasma triglyceride, and cholesterol levels in male $Ldlr^{-/-}$ and DKO mice after 16 weeks of high cholesterol diet. $n=7-13$ for each group. (C) and (D) Lipoprotein profile analysis in $Ldlr^{-/-}$ and DKO mice after fed high cholesterol diet for 16 weeks. Pooled plasma (500 μ l) from 5 mice in each group was fractionated by Superose 6 chromatography and fractions were used for cholesterol (C) and triglyceride (D) determinations.

FIG. 2A and 2B – Shows generation of circulating Reelin deficiency using adenoviral delivery of Cre recombinase. (A) Western blot analysis for Reelin abundance in plasma and tissues obtained 2 weeks after jugular vein infusion of adenoviruses encoding either Cre recombinase or a β -Gal control. Samples for 3 mice per group are shown. (B) After fed high cholesterol diet for 16 weeks, body weight, plasma triglyceride, total cholesterol, and HDL-cholesterol levels were determined in fasted animals. $n=8$ animals per group, values are mean $\pm$ SEM, * $P<0.05$ versus Ad-Gal- $Reln^{fl/fl}$ $Ldlr^{-/-}$.

FIG. 3A and 3B – Shows global Reelin deficiency attenuates atherosclerotic lesion development in $Ldlr^{-/-}$ mice. (A) Representative en face photomicrographs and lesion analyses for whole aorta in male $Ldlr^{-/-}$ and DKO mice. (B) Representative photomicrographs of aortic root sections stained with Oil Red O and quantification of atheroma area in $Ldlr^{-/-}$ and DKO mice (Scale bar = 200 μ m). $n=10-16$ animals per group. In A and B, data represent mean $\pm$ SEM; ** $P<0.01$ vs $Ldlr^{-/-}$.

FIG. 4A and 4B – Shows knockdown of circulating Reelin decreases atherosclerotic lesion size in Ad-Cre- $Reln^{fl/fl}$ $Ldlr^{-/-}$ mice. (A) Representative en face

photomicrographs and lesion analyses for whole aorta in Ad-Cre-ReInfl/fl *Ldlr*^{-/-} and Ad-Gal-ReIn^{fl/fl} *Ldlr*^{-/-} mice. (B) Representative photomicrographs of aortic root sections stained with Oil Red O and quantification of atheroma area in Ad-Cre-ReIn^{fl/fl} *Ldlr*^{-/-} and Ad-Gal-ReIn^{fl/fl} *Ldlr*^{-/-} mice. Scale bar = 200 μ m, arrows indicate atherosclerotic lesions. n=6 animals per group. In A and B, data represent mean $\pm$ SEM; ** P<0.01 vs Ad-Gal-ReIn^{fl/fl} *Ldlr*^{-/-}.

FIG. 5A and 5B – Shows Global Reelin deficiency decreases monocyte recruitment to atherosclerotic lesions in *Ldlr*^{-/-} mice. (A) Representative aortic root cross-sections stained with anti-Mac-3 and anti- α -actin antibodies to detect macrophage (green) and smooth muscle cell accumulation (red). (B) Representative aortic sinus sections stained for VCAM1 and ICAM1. In (A) and (B), sections were counterstained with DAPI (blue) to stain nuclei, and size bars represent 50 μ m. Positive staining is indicated by the white arrows. In (A) and (B), bar graphs show summary data for area of positive staining normalized to lesion area, with at least 3 fields of view used to calculate an average for each section. N=7/group, data represent mean $\pm$ SEM; ** P<0.01 compared to *Ldlr*^{-/-}.

FIG. 6A, 6B, and 6C – Shows Global Reelin deficiency reduces leukocyte-endothelial cell adhesion, and it has no effect on circulating WBC number. (A) After peritoneal injection of tamoxifen daily for 5 d, intravital microscopy was performed in male *Ldlr*^{-/-} and DKO mice to evaluate leukocyte-endothelial cell adhesion in the mesenteric microcirculation. Representative still images are shown. (B) Summary data for leukocyte velocity and the number of adherent leukocytes. Values are mean $\pm$ SEM, n=10 for each group, * P<0.05, ** P<0.01 compared to *Ldlr*^{-/-} control group. (C) Total WBC count and numbers of leukocyte subtypes were determined in *Ldlr*^{-/-} and DKO mice (MO, monocyte; LY, lymphocyte; NE, neutrophils; EO, eosinophil; BA, basophil). n=6 for each group, data represent mean $\pm$ SEM.

FIG. 7A and 7B – Shows Reelin enhances monocyte-endothelial cell adhesion via Apoer2. (A) Monocyte adhesion to HAEC was evaluated under the following conditions: Vehicle, Mock and Reelin. Images show the endothelial cells (cobblestone shape) and adhering U937 monocytes (small, round cells). Scale bar = 200 μ m. (B)

Summary data for A. n=6, ** P<0.01. (C) and (D) HAEC transfected with control (control RNAi), double-stranded RNA targeting Vldlr (C) or Apoer2 (D) were exposed to Vehicle, Mock and Reelin, and monocyte adhesion was evaluated. In parallel, whole cell lysates were immunoblotted for Vldlr or Apoer2 and GAPDH (lower panels). n=3; **
 5 P<0.01 versus vehicle or mock control, †† P<0.01 versus control RNAi. (E) Reelin blunts VEGF-induced eNOS activation via Apoer2. HAEC were pretreated for 24h with Mock media or Reelin, and basal and VEGF-stimulated eNOS activity was measured. n=6, ** P<0.01 versus basal; ††, P<0.01 versus vehicle or Mock control. (F) HAEC transfected with control RNAi or double-stranded RNAi targeting Apoer2 were pretreated with
 10 Mock or Reelin, and basal and VEGF-stimulated eNOS activity was measured. n=6, ** P<0.01 versus VEGF alone; †† P<0.01 versus control RNAi. In B-F, values are mean ± SEM.

FIG. 8 – Shows sustained reduction of plasma Reelin abundance in mice after intraperitoneal injection of the anti-Reelin antibody CR50. Plasma samples were
 15 collected at the indicated time points from CR50 or mouse IgG injected mice. Reelin was measured by Western blot. Representative Western blots are shown.

FIG. 9 – Shows selective labeling of circulating monocytes in C57/B6 and CX₃CR1w^{GFP} mice. Representative still images are shown.

FIG. 10 – Shows complete removal of Reelin from mouse plasma by i.p. injection
 20 of the CR50 antibody.

FIG. 11 – Shows reduced monocyte adhesion to the vascular endothelium in mice treated with the anti-Reelin CR50 antibody in an *in vivo* monocyte adhesion assay in CX₃CR1w^{GFP} mice. Representative still images are shown.

FIG. 12 – Shows that treatment with anti-Reelin CR50 antibody blocks leukocyte
 25 adhesion to the vascular endothelium in CX₃CR1w^{GFP} mice.

FIG. 13 – Shows that Reelin-mediated IKBα activation is blocked by CR50 in HAECs. HAECs were treated with 20nM Reelin in the presence or absence of 200nM CR50 for the indicated time points (0, 1, 15, 30, 60 and 120 min). Protein was harvested

and subjected to 8% SDS-PAGE gels for pIKB α . IKB α and Calnexin were used as controls. Data were mean $\pm$ SEM from 3 independent experiments (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

FIG. 14 – Shows that Reelin activates NF- κ B in Neuro2A cells. Neuro2A cells were transfected with NF- κ B-Luc construct for 24 hrs, followed by treatment with Reelin at different concentrations (5, 10, 20 and 40nM) in the presence or absence of 10-fold CR50 for another 24 hrs. Cells were lysed for luciferase assay. TNF α (1ng/ml) was used as positive control. Data were mean $\pm$ SEM from 3 independent experiments (*, $p < 0.05$; **, $p < 0.01$).

FIG. 15 – Shows that Reelin activates NF- κ B in HAECs. HAECs were transfected with NF- κ B -Luc construct for 24 hrs, followed by treatment with 20nM Reelin in the presence or absence of 200nM CR50 for another 24 hrs. Cells were lysed for luciferase assay. TNF α (1ng/ml) was used as a positive control. Data were mean $\pm$ SEM from 3 independent experiments. *, $p < 0.05$.

FIG. 16 – Shows that endogenous Dab2 was successfully silenced in HAECs by siRNA targeting Dab2. HAECs were transfected with siRNA targeting human Dab2 at different concentrations (20 or 50nM) on Day 1 and 3. On Day 4, protein was harvested for Western blots.

FIG. 17 - Shows that Reelin activates the expression of mRNAs for endothelial adhesion molecules in HAECs. Summary plots are shown depicting the relative (Rel.) transcript abundance of VCAM and E-Selectin relative to HPRT1 in HAECs treated with mock or 20 nM Reelin after transfection with 20nM control (control RNAi) or 20nM double-stranded RNA targeting either Apoer2 or Dab2 (*, $P < 0.05$; **, $P < 0.01$).

BRIEF DESCRIPTION OF THE SEQUENCES

SEQ ID NO:1 – Sequence of double-stranded siRNA directed to Vldlr (s14811).

SEQ ID NO:2 – Sequence of double-stranded siRNA directed to Apoer2 (s15365).

SEQ ID NO:3 – Sequence of the complete coding sequence of human Reelin, provided as GenBank Accession No. U79716.1.

SEQ ID NO:4 – Sequence of transcript variant 1 of human Reelin, provided as GenBank Accession No. NM_005045.3.

5 **SEQ ID NO:5** – Sequence of transcript variant 2 of human Reelin, provided as GenBank Accession No. NM_173054.2.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides methods and compositions for treatment or prevention of atherosclerosis in a subject comprising introduction of a therapeutic compound targeting
10 Reelin in the circulation of the subject, wherein said treatment results in a reduction of disease symptoms in the subject. Also provided are methods and compositions for reducing leukocyte adhesion to the vascular wall in a subject, resulting in reduction of disease symptoms in the subject. In some embodiments, a therapeutic compound as described herein performs a function comprising: (a) reducing or inhibiting the
15 expression of a gene encoding Reelin; (b) reducing or inhibiting the expression of a Reelin protein; (c) reducing or inhibiting expression of a receptor for Reelin; (d) reducing or inhibiting the function of a Reelin protein; or (e) binding to Reelin protein in such a way that the function is reduced or eliminated.

Reelin is a large, multimodular secreted, extracellular matrix glycoprotein that
20 controls neuronal migration and positioning during brain development. It was originally found in the developing brain where it is secreted by Cajal-Retzius neurons in the marginal zone. After development, Reelin functions as a synaptic neuromodulator by binding to cognate receptors Apolipoprotein E receptor-2 (Apoer2) and very low-density lipoprotein receptor (Vldlr) in neurons. In the circulation, Reelin promotes thrombosis
25 and hemostasis. As described in detail in the Examples below, Reelin was inducibly deleted, either ubiquitously or restricted to the circulation, in atherosclerosis-prone low-density lipoprotein receptor-deficient (*Ldlr*^{-/-}) mice, and it was found that atherosclerosis progression was markedly attenuated, with mice showing reduced lesion macrophage

content and decreased expression of vascular cell adhesion molecule-1 (VCAM1) and intercellular adhesion molecule-1 (ICAM1) in the lesions. Intravital microscopy further revealed decreased leukocyte-endothelial cell adhesion in the absence of Reelin. In cultured human endothelial cells, Reelin enhanced monocyte adhesion and suppressed endothelial nitric oxide synthase (eNOS) activity in an Apoer2-dependent manner. The inventors thus discovered that circulating Reelin promotes atherosclerosis by increasing leukocyte-endothelial cell adhesion and facilitating the infiltration of inflammatory macrophages into the arterial wall via Apoer2. Targeting circulating Reelin thus presents a novel opportunity for the prevention of cardiovascular disease through prevention or reduction of leukocyte adhesion to the vascular wall.

The binding of Reelin to Apoer2 and Vldlr on the surface promotes tyrosine phosphorylation of the cytoplasmic adaptor protein disabled homolog 1 (Dab1). Phosphorylated Dab1 then activates a series of signal transduction mechanisms that control various cellular functions, including neuronal positioning during brain development, synaptic plasticity, and memory formation. Recent data from experimental and clinical studies indicate that increased Reelin expression is protective, while reduced Reelin expression is associated with several neurodegenerative disorders including Alzheimer's disease (AD).

In addition to the CNS, Reelin is found in the circulation, the liver, the pituitary pars intermedia, and in adrenal chromaffin cells. It promotes platelet spreading on fibrinogen and also plays a role in coagulation, by enhancing thrombin generation and the formation of fibrin clots. There is evidence that the two receptors for Reelin, Apoer2 (Lrp8) and Vldlr influence atherosclerosis severity. In macrophages, Apoer2 reduces stress-induced cell death and potentially retards the development of advanced atherosclerotic plaques. In contrast, macrophage Vldlr promotes atherosclerotic lesion development. Both Reelin receptors are also expressed in endothelial cells, where Apoer2 mediates the potential anti-atherogenic actions of Apolipoprotein E3.

Therapeutic Compounds or Compositions and Administration Thereof

In accordance with the invention, any therapeutic molecule that may be used to treat atherosclerosis by reducing Reelin protein expression or inhibiting its function in the circulation is within the scope of the present invention, including, but not limited to, a protein, a peptide, an RNA molecule, such as an siRNA, an antisense oligonucleotide, an antibody or antibody fragment, such as a monoclonal antibody or a polyclonal antibody, an Fc-coupled dimeric or monomeric decoy receptor, a peptidomimetic (*i.e.*, a small protein-like chain designed to mimic a peptide) that inhibits Reelin oligomerization or receptor binding, a carbohydrate, a small molecule, and the like. In some embodiments, such therapeutic compounds target Reelin mRNA or Reelin protein to reduce the activity of Reelin in a cell or individual subject. In another embodiment, cells that express Reelin into the circulation of the subject, including, but not limited to bone marrow-derived cells and Kupffer cells, can be targeted using a therapeutic compound described herein. In another embodiment, liposomes or viral vectors may be used to provide antibodies or other molecules of interest to a subject for targeting of Reelin. In further embodiments, a therapeutic compound described herein may be useful for targeting cells in the subject. It is envisioned that a therapeutic molecule of the invention may include, but is not limited to, an antibody or a humanized antibody targeting Reelin to reduce or eliminate its function, a siRNA or dsRNA to reduce expression of the Reelin transcript, or a decoy receptor to bind to Reelin and eliminate its function. In an embodiment, an antibody of the invention may be a humanized antibody, *i.e.* an antibody from a non-human species whose protein sequence has been modified to increase the similarity to antibody variants produced naturally in humans. In some embodiments, an siRNA in accordance with the invention may be complementary to all or a portion of the mRNA sequence provided as SEQ ID NO:3-5. In an embodiment, the siRNA functions to inhibit Reelin in a subject.

Any method of interfering with Reelin activity in a subject, such as inhibiting or eliminating the expression of Reelin protein or its gene in the circulation, or inhibiting or eliminating the function of Reelin protein in the circulation, known in the art may be useful in accordance with the invention. In other embodiments, a protein may be

combined with a non-naturally occurring pharmaceutically acceptable carrier such as one described herein. In some embodiments, treatment methods of the present invention involve direct delivery of such a therapeutic molecule or compound. In other embodiments, treatment methods of the present invention may involve direct delivery of, for example, a vector expressing a functional copy of a therapeutic compound or molecule, such as a protein, for example a vector expressing an antibody targeting Reelin or a decoy receptor to which Reelin may bind to reduce or eliminate its function. In further embodiments, treatment may comprise any combination of delivery of a therapeutic compound as described herein, delivery of a vector expressing a therapeutic compound or protein, or a combination of these with any known treatment for atherosclerosis, including but not limited to administering an antithrombotic drug, a drug that acts in cholesterol homeostasis, such as an antibody targeting proprotein convertase subtilisin/kexin type 9 (PCSK9) or an HMG-CoA reductase inhibitor (*i.e.*, a statin) to the subject.

Therapeutic compounds or compositions within the scope of the present invention may also contain other compounds, which may be biologically active or inactive. For example, one or more antibodies or decoy receptors described herein may be present, within a composition according to the invention. In some embodiments, antibodies or proteins useful with the present invention may be conjugated to other macromolecules. Therapeutic compounds or compositions may generally be used for prophylactic and/or therapeutic purposes. Embodiments of the invention provide therapeutic compounds or compositions for treatment of any inflammatory state involving the induction of endothelial inflammatory responses leading to or not leading to leukocyte extravasation as part of the pathogenic mechanism. In certain embodiments, therapeutic compounds or compositions provided by the invention are useful in methods of treating or preventing multiple sclerosis, where circulating monocytes aggravate or perpetuate the pathology. Other exemplary syndromes that can be treated using the compositions provided by the invention include arthritis or psoriasis, or any disorder in which excessive leukocyte/monocyte extravasation aggravates the disease process. For example, in

accordance with the invention, a composition as described herein may be provided to a subject, such as a human, before onset of symptoms in order to reduce atherosclerotic plaques or to prevent the symptoms caused by atherosclerosis. In other embodiments, such a composition may be provided to a subject, such as a human, after onset of
5 symptoms of atherosclerosis in order to provide treatment of atherosclerosis in the subject, such as by reducing or eliminating symptoms in the subject.

Therapeutic compounds or compositions may be provided to a subject in a single dose or multiple doses and as such provided in single-dose or multi-dose containers, such as sealed ampules or vials. Such containers may be sealed to preserve sterility of the
10 composition until use. In general, compositions as described herein may be stored as suspensions, solutions, or emulsions in oily or aqueous vehicles. Alternatively, such a composition may be stored in a freeze-dried condition requiring only the addition of a sterile liquid carrier immediately prior to use.

In one embodiment, the present invention provides a treatment method for
15 atherosclerosis or antiphospholipid syndrome, resulting from inhibition or elimination of the function of Reelin protein or the gene encoding Reelin protein, or a receptor to which Reelin binds. In certain embodiments, methods of the present invention may be used to treat or prevent atherosclerosis or antiphospholipid syndrome in a subject, or to treat any other diseases that may benefit from the use of decoy receptors for ApoER2 or Vldlr, for
20 example.

In one embodiment, the invention thus provides a method of treating a subject with atherosclerosis or antiphospholipid syndrome, the method comprising administering or delivering to the subject a therapeutic compound, for example an antibody targeting Reelin protein or a decoy receptor for Reelin, wherein the therapeutic compound is
25 introduced into the circulation of the subject.

In an embodiment, a nucleotide sequence encoding a therapeutic compound, for example an antibody targeting Reelin or a functional copy of a decoy receptor, may be introduced to the subject. For example, in accordance with the invention, a vector appropriate for use in a human or an animal, for example an adeno-associated viral

vector, may be used to insert or transduce a nucleotide sequence encoding antibody targeting Reelin or a functional copy of a decoy receptor to the subject as described herein.

In an embodiment, the invention provides a composition comprising a therapeutic compound that targets a Reelin protein or the gene encoding Reelin. In some 5 embodiments, an RNA molecule may be targeted through the use of an siRNA or a miRNA. In other embodiments, Reelin may be targeted through the use of a decoy receptor, such as an Fc-coupled dimeric or monomeric decoy receptor.

Accordingly, therapeutic compounds as described herein may be delivered to the 10 subject, for instance into the circulation, through a variety of routes. In other embodiments, a vector comprising a nucleic acid sequence encoding a therapeutic compound such as an antibody targeting Reelin or a decoy receptor. In general, any administration route that places the cells into the circulation of the subject may be used. In one embodiment of the invention, the cells may be injected intravenously.

As described herein, a therapeutic composition may be combined with a 15 pharmaceutically acceptable carrier. In some embodiments, a pharmaceutically acceptable carrier as described herein is non-naturally occurring. The selection of a suitable carrier may be determined in part by the particular composition being administered (*e.g.*, antibody, protein, modulatory compounds, or the like), as well as by 20 the particular method used to administer the composition. Accordingly, a wide variety of suitable formulations of therapeutic compositions are available that may of use in the present invention. Administration may be in any convenient manner, *e.g.*, by injection, oral administration, inhalation, transdermal application, or rectal administration.

Formulations suitable for parenteral administration, such as, for example, by 25 intraarticular (in the joints), intravenous, intramuscular, intradermal, intraperitoneal, and subcutaneous routes, include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended subject, and aqueous and nonaqueous sterile suspensions that can include suspending agents, solubilizers,

thickening agents, stabilizers, and preservatives. In the practice of this invention, compositions may be administered, for example, by intravenous infusion into the circulation, orally, topically, or intraperitoneally.

Such compositions may also comprise buffers (*e.g.*, neutral buffered saline or phosphate buffered saline), carbohydrates (*e.g.*, glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, bacteriostats, chelating agents such as EDTA or glutathione, adjuvants (*e.g.*, aluminum hydroxide), solutes that render the formulation isotonic, hypotonic, or weakly hypertonic with the blood of a subject, suspending agents, thickening agents, and/or preservatives. Alternatively, compositions of the present invention may be formulated as a lyophilizate. Compounds may also be encapsulated within liposomes using methods known in the art.

Injection solutions and suspensions may be prepared from sterile powders, granules, and tablets as described herein. An injection as described herein may involve a suspension of one or more of a purified or non-purified solution of an antibody, decoy receptor, protein, or other type of molecule as described herein. An injection solution may also contain a pharmaceutically acceptable carrier as described herein.

Formulations suitable for oral administration may consist of (a) liquid solutions, such as an effective amount of the packaged antibody, decoy receptor, protein, or other type of molecule suspended in diluents, such as water, saline or PEG 400; (b) capsules or tablets, each containing a predetermined amount of the active ingredient, as liquids, solids, granules or gelatin; (c) suspensions in an appropriate liquid; or (d) suitable emulsions. Tablet forms may include one or more of lactose, sucrose, mannitol, sorbitol, calcium phosphates, corn starch, potato starch, microcrystalline cellulose, gelatin, colloidal silicon dioxide, talc, magnesium stearate, stearic acid, and other excipients, colorants, fillers, binders, diluents, buffering agents, moistening agents, preservatives, flavoring agents, dyes, disintegrating agents, and pharmaceutically compatible carriers. Lozenge forms may comprise the active ingredient in a flavor, *e.g.*, sucrose, as well as pastilles comprising the active ingredient in an inert base, such as gelatin and glycerin or

sucrose and acacia emulsions, gels, and the like containing, in addition to the active ingredient, carriers known in the art.

The compound of choice, alone or in combination with other suitable components, may be made into aerosol formulations to be administered via inhalation.

5 Aerosol formulations may be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like.

The dose administered to a subject in the context of the present invention should be sufficient to effect a beneficial therapeutic response in the subject over time. The dose will be determined by the efficacy of the particular molecule employed and the condition
10 of the subject, as well as the body weight and/or surface area of the patient to be treated. The size of the dose also may be determined by the existence, nature, and extent of any adverse side-effects that accompany the administration of a particular molecule or therapeutic compound in a particular subject.

For administration, compounds and transduced cells of the present invention can
15 be administered at a rate determined by the LD-50 of the molecule or therapeutic compound, and the side-effects thereof at various concentrations, as applied to the mass and overall health of the subject. Administration may be accomplished via single, multiple, or divided doses.

Antibody Production

20 Methods that may be used to produce polyclonal and monoclonal antibodies that react specifically with a Reelin protein or fragment thereof are known in the art. Such techniques may include antibody preparation by selection of antibodies from recombinant antibody libraries in phage or other vectors, as well as preparation of polyclonal and monoclonal antibodies by immunizing rabbits or mice.

25 A number of antigens or antigenic regions comprising a Reelin protein or portions thereof may be used to produce antibodies specifically reactive to Reelin. For example, a Reelin protein or fragment thereof, may be isolated using any methods described herein or known in the art. Recombinant proteins may be expressed in prokaryotic or eukaryotic

cells and purified as described herein. Monoclonal and/or polyclonal antibodies may be produced using naturally occurring (in pure or impure form) or recombinant proteins using methods known in the art. Synthetic peptides derived from a Reelin sequence may also be used to generate antibodies and may be conjugated to a carrier protein and
5 injected into an animal capable of producing antibodies (*e.g.*, rabbit).

Methods of production of polyclonal antibodies are known to those of skill in the art. For example, an inbred strain of mice or rabbits may be immunized with a protein using a standard adjuvant, such as an adjuvant described herein, using a standard immunization protocol known in the art. When appropriately high titers of antibody to
10 the protein are obtained, antisera may be prepared and enrichment performed to obtain antibodies reactive to the protein.

Monoclonal antibodies may also be obtained by various methods known in the art. For example, spleen cells from an animal immunized with a desired antigen may be immortalized, commonly by fusion with a myeloma cell or through transformation with
15 Epstein Barr Virus (EBV), oncogenes, or retroviruses, or other methods well known in the art. The immortalized cells may then be screened for production of antibodies of the desired specificity and affinity for the antigen. Yield of the monoclonal antibodies produced by such cells may be enhanced by various techniques known in the art, for example by injection into the peritoneal cavity of a vertebrate host.

Monoclonal antibodies and polyclonal sera may be collected and titered against
20 the desired antigen or protein (*e.g.*, Reelin protein) in an immunoassay, for example, a solid phase immunoassay with the protein immobilized on a solid support. Antibodies specific only for a Reelin protein may also be made by subtracting out other cross-reacting proteins. In this manner, antibodies that bind only to the protein of choice may
25 be obtained.

Hybridomas may be employed to produce antibodies in accordance with the invention, in which a hybrid cell line may be used as the basis for the production of antibodies in large quantities for diagnostic or therapeutic use. A hybridoma may be

produced by injecting an antigen such Reelin protein into a mammal such as a mouse, collecting monoclonal antibody-producing cells produced thereby, and fusing the antibodies with a tumor cell to produce myelomas.

Once the specific antibodies against the desired antigen, such as a Reelin protein,
5 are available, the desired antigen may be detected using a variety of immunoassay methods. The antibody may also be a humanized antibody for therapeutic use with a non-natural, pharmaceutically acceptable carrier.

Protein either associated with or distinct from a Reelin protein may be detected
and/or quantified using any of a number of well recognized immunological binding
10 assays. Immunological assays may use an antibody that specifically binds to a protein or antigen of choice. The antibody may be produced by any of a number of methods well known to those of skill in the art. Immunoassays may also use a labeling agent to specifically bind to the complex formed by the antibody and antigen for detection
15 purposes. The labeling agent may itself be one of the moieties comprising the antibody/antigen complex. Thus, the labeling agent may be a labeled Reelin protein antibody. Alternatively, the labeling agent may be a third moiety, such as a secondary antibody, that specifically binds to the antibody/antigen complex. A secondary antibody may be specific to antibodies of the species from which the first antibody is derived. A
20 labeling agent can be modified with a detectable moiety, such as biotin, to which another molecule can specifically bind, such as streptavidin. A variety of detectable moieties are well known to those skilled in the art.

Immunoassays for detecting a protein such as Reelin in samples are well known
in the art. Such assays may be either competitive or noncompetitive, and may be either
quantitative or non-quantitative. Noncompetitive immunoassays are assays in which
25 antigen may be directly detected and, in some instances, the amount of antigen directly measured. In competitive assays, Reelin protein present in a sample is detected indirectly by a detectable signal associated with a known, added (exogenous) antigen displaced from an antibody by the antigen present in a sample. In this manner, such assays can also be adapted to provide for an indirect measurement of the amount of Reelin protein

present in the sample. Competitive binding immunoassays may also be used to determine cross-reactivity, in which any cross-reacting antibodies may be removed from pooled antisera. Additional assay types, including but not limited to western blot or liposome immunoassays may also be used in accordance with the present invention.

5 One of skill in the art will appreciate that it is often desirable to minimize nonspecific binding in immunoassays. Particularly, where the assay involves an antigen or antibody immobilized on a solid substrate it is desirable to minimize the amount of nonspecific binding to the substrate. Means of reducing such non-specific binding are well known to those of skill in the art.

10 An assay as described herein may include a label or detectable group that does not significantly interfere with the specific binding of the antibody used in the assay. A detectable group may be any material having a detectable physical or chemical property. Such detectable labels are known in the art and generally, any label useful in such methods may be applied to the present invention. Thus, a "label" as used herein may be
15 any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Useful labels in the present invention may include magnetic beads (*e.g.*, DYNABEADS™), fluorescent dyes (*e.g.*, fluorescein isothiocyanate, Texas red, rhodamine, and the like), radiolabels (*e.g.*, ^3H , ^{125}I , ^{35}S , ^{14}C , or ^{32}P), enzymes (*e.g.*, horse radish peroxidase, alkaline phosphatase, and/or any
20 others known in the art and used in ELISA), and colorimetric labels such as colloidal gold or colored glass or plastic beads (*e.g.*, polystyrene, polypropylene, latex, *etc.*).

 A label in accordance with the invention may be coupled directly or indirectly to the desired component of the assay according to methods well known in the art. As described above, a wide variety of labels may be used, with the choice of label depending
25 on sensitivity, ease of conjugation with the compound, stability requirements, or available instrumentation, among others.

 Non-radioactive labels may be attached by indirect means. Generally, a ligand molecule (*e.g.*, biotin) is covalently bound to the molecule. The ligand may then bind to

another molecule (*e.g.*, streptavidin), which may be either inherently detectable or covalently bound to a signal system, such as a detectable enzyme, a fluorescent compound, or a chemiluminescent compound. The ligands and their corresponding targets may be used in any suitable combination with antibodies that recognize a Reelin protein, or secondary antibodies that recognize a Reelin protein. The molecules may also be conjugated directly to signal generating compounds, *e.g.*, by conjugation to an enzyme or fluorophore. Enzymes of interest to be used as labels may be hydrolases, for example phosphatases, esterases and glycosidases, or oxidotases, such as peroxidases. Fluorescent compounds may include fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, and the like. Chemiluminescent compounds may include luciferin, 2,3-dihydrophthalazinediones, *e.g.*, luminol, or others known in the art.

Means of detecting labels are well known to those of skill in the art and will depend on the type of label used. For example, autoradiography may be used to detect a radioactive label, or fluorochromes may be used to detect a fluorescent label. Fluorescence may be detected visually, for example by electronic detectors such as charge coupled devices (CCDs) or photomultipliers, and the like. Similarly, enzymatic labels may be detected by providing the appropriate substrates for the enzyme and detecting the resulting reaction product. Colorimetric or chemiluminescent labels may be detected by observing a color associated with a particular label. In some embodiments, an assay formats may not require the use of a labeled component but rather may be detected by simple visual inspection.

Post-transcriptional Gene Suppression

As used herein the words “gene suppression,” is intended to refer to any of the well-known methods for reducing the levels of protein produced as a result of gene transcription to mRNA and subsequent translation of the mRNA. Gene suppression is also intended to mean the reduction of protein expression from a gene or a coding sequence including posttranscriptional gene suppression and transcriptional suppression. Post-transcriptional gene suppression is mediated by the homology between of all or a part of a mRNA transcribed from a gene or coding sequence targeted for suppression and

the corresponding double stranded RNA used for suppression, and refers to the substantial and measurable reduction of the amount of available mRNA available in the cell for binding by ribosomes. The transcribed RNA can be in the sense orientation to effect what is called co-suppression, in the anti-sense orientation to effect what is called anti-sense suppression, or in both orientations producing a double stranded RNA (dsRNA) to effect what is called RNA interference (RNAi). Transcriptional suppression is mediated by the presence in the cell of a dsRNA, a gene suppression agent, exhibiting substantial sequence identity to a promoter DNA sequence or the complement thereof to effect what is referred to as promoter trans suppression. Gene suppression may be effective against a native gene associated with a trait, *e.g.*, to suppress expression of a Reelin protein.

Post-transcriptional gene suppression may employ both sense-oriented and anti-sense-oriented, transcribed RNA which is stabilized, *e.g.*, as a hairpin and stem and loop structure. A preferred DNA construct for effecting post transcriptional gene suppression one in which a first segment encodes an RNA exhibiting an anti-sense orientation exhibiting substantial identity to a segment of a gene targeted for suppression, which is linked to a second segment encoding an RNA exhibiting substantial complementarity to the first segment. Such a construct would be expected to form a stem and loop structure by hybridization of the first segment with the second segment and a loop structure from the nucleotide sequences linking the two segments.

The present invention provides dsRNA or siRNA molecules for reduction or elimination of Reelin expression. siRNA technology is known in the art and used to study inhibition of, for example, apolipoprotein receptors such as ApoCIII (Gaudet *et al.*, *N Engl J Med* 373(5):438-47, 2015; Graham *et al.*, *Circulation Res* 112:1479-1490, 2013) ApoA (Tsimikas *et al.*, *Lancet* 2015), or chemicals useful in accordance with the invention, such as PCSK9 (Miao *et al.*, *Arterioscler Thromb Vasc Biol* 35(7):1589-96, 2015). The dsRNA or siRNA nucleotide sequences comprise double strands of polymerized ribonucleotide and may include modifications to either the phosphate-sugar backbone or the nucleoside. Modifications in RNA structure may be tailored to allow

specific genetic inhibition. In one embodiment, the dsRNA molecules may be modified through an enzymatic process so the siRNA molecules may be generated. The siRNA can efficiently mediate the down-regulation effect for some target genes. This enzymatic process may be accomplished by utilizing an RNase III enzyme or a DICER enzyme, present in the cells of an individual in the eukaryotic RNAi pathway. Both the DICER enzyme and RNase III, naturally occurring in an individual or made through recombinant DNA techniques, cleave larger dsRNA strands into smaller oligonucleotides. The DICER enzymes specifically cut the dsRNA molecules into siRNA pieces each of which is about 19-25 nucleotides in length while the RNase III enzymes normally cleave the dsRNA molecules into 12-15 base-pair siRNA. The siRNA molecules produced by the either of the enzymes have 2 to 3 nucleotide 3' overhangs, and 5' phosphate and 3' hydroxyl termini. The siRNA molecules generated by RNase III enzyme are the same as those produced by Dicer enzymes in the eukaryotic RNAi pathway and are hence then targeted and degraded by an inherent cellular RNA-degrading mechanism after they are subsequently unwound, separated into single-stranded RNA and hybridize with the RNA sequences transcribed by the target gene. This process results in the effective degradation or removal of the RNA sequence encoded by the nucleotide sequence of the target gene. The outcome is the silencing of a particularly targeted nucleotide sequence within the individual.

Inhibition of a target gene using the stabilized dsRNA technology of the present invention is sequence-specific in that nucleotide sequences corresponding to the duplex region of the RNA are targeted for genetic inhibition. RNA containing a nucleotide sequences identical to a portion of the target gene is preferred for inhibition. RNA sequences with insertions, deletions, and single point mutations relative to the target sequence have also been found to be effective for inhibition. In performance of the present invention, it is preferred that the inhibitory dsRNA and the portion of the target gene share at least from about 80% sequence identity, about 85% identity, about 90% sequence identity, about 91% identity, about 92% identity, about 93% identity, about 94% identity, or from about 95% sequence identity, about 96% identity, about 97%

identity, about 98% identity, about 99% sequence identity, or about 100% sequence identity. Alternatively, the duplex region of the RNA may be defined functionally as a nucleotide sequence that is capable of hybridizing with a portion of the target gene transcript. A less than full length sequence exhibiting a greater homology compensates
5 for a longer less homologous sequence. The length of the identical nucleotide sequences may be at least about 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, or at least about 1000 bases. Normally, a sequence of greater than 20-100 nucleotides may be used, or a sequence of greater than about 200-300 nucleotides, or a sequence of greater than about 500-1000 nucleotides, depending on the size of the target
10 gene. A dsRNA or siRNA as described herein may be able to tolerate sequence variations that might be expected due to genetic mutation, polymorphism, or evolutionary divergence. The introduced nucleic acid molecule may not need to be absolute homology, may not need to be full length, relative to either the primary transcription product or fully processed mRNA of the target gene. Therefore, those skilled in the art
15 will understand that 100% sequence identity between the RNA and the target gene is not required to practice the present invention.

The dsRNA or siRNA molecules may be synthesized either *in vivo* or *in vitro*. The dsRNA may be formed by a single self-complementary RNA strand or from two complementary RNA strands. Endogenous RNA polymerase of the cell may mediate
20 transcription *in vivo*, or cloned RNA polymerase can be used for transcription *in vivo* or *in vitro*. Inhibition may be targeted by specific transcription in an organ, tissue, or cell type; stimulation of an environmental condition (*e.g.*, infection, stress, temperature, chemical inducers); and/or engineering transcription at a developmental stage or age. The RNA strands may or may not be polyadenylated; the RNA strands may or may not
25 be capable of being translated into a polypeptide by a cell's translational apparatus.

The RNA, dsRNA, siRNA, or miRNA of the present invention may be produced chemically or enzymatically by one skilled in the art through manual or automated reactions *or in vivo* in another organism. RNA may also be produced by partial or total organic synthesis; any modified ribonucleotide can be introduced by *in vitro* enzymatic or

organic synthesis. The use and production of an expression construct are known in the art. If synthesized chemically or by *in vitro* enzymatic synthesis, the RNA may be purified prior to introduction into the cell. For example, RNA can be purified from a mixture by extraction with a solvent or resin, precipitation, electrophoresis, chromatography, or a combination thereof. Alternatively, the RNA may be used with no or a minimum of purification to avoid losses due to sample processing. The RNA may be dried for storage or dissolved in an aqueous solution. The solution may contain buffers or salts to promote annealing, and/or stabilization of the duplex strands.

As used herein, the term “nucleic acid” refers to a single or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5’ to the 3’ end. The “nucleic acid” may also optionally contain non-naturally occurring or altered nucleotide bases that permit correct read through by a polymerase and do not reduce expression of a polypeptide encoded by that nucleic acid. The term “nucleotide sequence” or “nucleic acid sequence” refers to both the sense and antisense strands of a nucleic acid as either individual single strands or in the duplex. The term “ribonucleic acid” (RNA) is inclusive of RNAi (inhibitory RNA), dsRNA (double stranded RNA), siRNA (small interfering RNA), mRNA (messenger RNA), miRNA (micro-RNA), tRNA (transfer RNA, whether charged or discharged with a corresponding acylated amino acid), and cRNA (complementary RNA) and the term “deoxyribonucleic acid” (DNA) is inclusive of cDNA and genomic DNA and DNA-RNA hybrids. The words “nucleic acid segment”, “nucleotide sequence segment”, or more generally “segment” will be understood by those in the art as a functional term that includes both genomic sequences, ribosomal RNA sequences, transfer RNA sequences, messenger RNA sequences, operon sequences and smaller engineered nucleotide sequences that express or may be adapted to express, proteins, polypeptides or peptides.

Kits

In still further embodiments, the present invention provides kits for use with the methods described herein for treatment of atherosclerosis in a subject, for instance comprising a composition or therapeutic compound as described herein for targeting

Reelin in a subject such as a canine, a feline, a mouse, or a human. Such a kit may include one or more antibodies targeting a Reelin protein or fragment thereof. A kit may further contain a decoy receptor such as an Fc-coupled dimeric or monomeric decoy receptor for administration to a subject. The kit may include one or more sterile
5 containers, for example a vial, a tube, a flask, or a syringe.

In further embodiments, the kit may include one or more tubes or wells of a culture or microtiter plate or other cell sterile cell culture plates or flasks into which components or reagents may be placed. The kit may allow for a single sample, or more than one sample. In some embodiments, the kit may include a plurality of plates or tubes,
10 which allow for numerous samples concurrently or consecutively.

The treatment reagents of the kit may take any one of a variety of forms. Such reagents may include, but are not limited to, media, diluents, or cell culture reagents known in the art and useful with the methods of the invention, for example, fetal bovine serum, antibiotics, or the like. Detection assays that are associated with and/or linked to a
15 given therapeutic agent or protein may be included in a kit according to the invention. Detectable labels that are associated with and/or attached to a secondary binding ligand are also contemplated. Exemplary secondary ligands are those secondary antibodies that have binding affinity for the first antibody. A number of exemplary assays are known in the art and/or all such assays may be employed in connection with the present invention.
20 Assays for some therapeutic compounds envisioned may be performed at certified analytical laboratories. When this is the case, the kits may include instructions and materials for submitting samples to the appropriate certified labs for analysis.

The kits may optionally include a suitably aliquoted composition of a therapeutic agent of the invention to serve as a control. The components of the kits may be packaged
25 either in aqueous media and/or in lyophilized form, and may be suitable for storage at any temperature. In another embodiment, kits of the present invention may comprise instructions or written directions for use of the kit.

Definitions

As used herein, an “antibody” refers to a polypeptide comprising a framework region from an immunoglobulin gene or fragments thereof that specifically binds and recognizes an antigen, such as a Reelin protein or fragment thereof. The recognized immunoglobulin genes may include the kappa, lambda, alpha, gamma, delta, epsilon, and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains may be classified as either kappa or lambda. Heavy chains may be classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD, and IgE, respectively.

An exemplary immunoglobulin (antibody) structural unit may comprise a tetramer, with each tetramer composed of two identical pairs of polypeptide chains, each pair having one “light” (about 25 kD) and one “heavy” chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain and variable heavy chain refer to these light and heavy chains.

Antibodies exist, *e.g.*, as intact immunoglobulins or as a number of well-characterized fragments produced by digestion with various peptidases. While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized *de novo* either chemically or by using recombinant DNA methodology. Thus, the term “antibody,” as used herein, also includes antibody fragments either produced by the modification of whole antibodies, or those synthesized *de novo* using recombinant DNA methodologies or those identified using other methods known in the art.

For preparation of antibodies, *e.g.*, recombinant, monoclonal, or polyclonal antibodies, many technique known in the art may be used. The genes encoding the heavy and light chains of an antibody of interest can be cloned from a cell and used to produce a recombinant monoclonal antibody. Gene libraries encoding heavy and light chains of monoclonal antibodies may also be used. Random combinations of the heavy and light chain gene products generate a large pool of antibodies with different antigenic

specificity. Techniques for the production of single chain antibodies or recombinant antibodies are found in the art and may be adapted to produce antibodies to polypeptides according to the invention. Phage display technology may also be used to identify antibodies and heteromeric fragments that specifically bind to selected antigens.

5 Antibodies may also be made bispecific, *i.e.*, able to recognize two different antigens, or heteroconjugates, *e.g.*, two covalently joined antibodies, or immunotoxins.

The phrase “specifically (or selectively) binds” to an antibody or “specifically (or selectively) immunoreactive with,” when referring to a protein or peptide, refers to a binding reaction that is determinative of the presence of the protein, often in a heterogeneous population of proteins. Thus, under particular immunoassay conditions, a specified antibody may bind to a particular protein at least two times the background and more typically more than 10 to 100 times background. Specific binding to an antibody under such conditions requires an antibody that is selected by virtue of its specificity for a particular protein. For example, polyclonal antibodies raised to a Reelin protein, polymorphic variants, alleles, orthologs, and variants thereof, or splice variants, or portions thereof, may be selected to obtain only those polyclonal antibodies that are specifically immunoreactive with Reelin and not with other proteins. This selection may be achieved by subtracting out antibodies that cross-react with other molecules. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein.

The phrase “functional effects” in the context of assays for testing compounds that modulate activity of Reelin includes the determination of a parameter that is indirectly or directly under the influence of Reelin, *e.g.*, a phenotypic or chemical effect. “Functional effects” may include *in vitro*, *in vivo*, and *ex vivo* activities and may be measured by any means known to those skilled in the art, such as changes in spectroscopic characteristics, shape, chromatographic, or solubility properties for a protein, measuring inducible markers or transcriptional activation of a protein; measuring

binding activity or binding assays, *e.g.* binding to antibodies; measuring changes in ligand or substrate binding activity, measuring cell surface marker expression, measurement of changes in protein levels, measurement of RNA stability, identification of downstream or reporter gene expression via, for example, chemiluminescence, fluorescence, colorimetric reactions, antibody binding, and/or inducible markers.

As used herein, “autologous” refers to a protein, cell, cell type, or the like, that is derived from the same individual who is to be treated. For example, in accordance with the invention, an autologous cell is a cell or a sample of cells that are isolated from an individual subject (*e.g.*, a canine or human), transduced with a construct comprising a polynucleotide sequence encoding a protein of interest, expanded in culture to create a population of cells all of which comprise the construct, and injected back into the same subject for treatment.

The terms “inhibitors,” “activators,” and “modulators” of Reelin nucleic acid or polypeptide sequences are used to refer to activating, inhibitory, or modulating molecules identified using *in vitro* and *in vivo* assays of the Reelin nucleic acid and polypeptide sequences. Inhibitors are compounds that may bind to, partially or totally block activity, decrease, prevent, delay activation, inactivate, desensitize, or down regulate the activity or expression of Reelin. Activators refer to compounds that increase, open, activate, facilitate, enhance activation, sensitize, agonize, or upregulate Reelin activity. Inhibitors, activators, or modulators also include genetically modified versions of Reelin, *e.g.*, versions with altered activity, as well as naturally occurring and synthetic ligands, substrates, antagonists, agonists, antibodies, peptides, cyclic peptides, nucleic acids, antisense molecules, ribozymes, small chemical molecules and the like. Assays for inhibitors and activators include, *e.g.*, expressing Reelin *in vitro*, in cells, or cell membranes, applying putative modulator compounds, and then determining the functional effects on activity, as described herein.

Test samples or assays comprising Reelin that are treated with a potential activator, inhibitor, or modulator may be compared to a control sample lacking the inhibitor, activator, or modulator in order to determine the extent of inhibition. Control

samples to which a test sample or assay is compared may be assigned a relative protein activity value of 100%. Inhibition of Reelin is achieved when the activity value of the test sample relative to the control sample is less than about 80%, including about 75%, about 70%, about 65%, about 60%, about 55%, about 50%, about 45%, about 40%, about 35%, about 30%, about 25%, about 20%, about 15%, about 10%, about 5%, and about 0%.

The terms “identical” or “percent identity,” in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (*i.e.*, about 60% identity, preferably 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher identity over a specified region, when compared and aligned for maximum correspondence over a comparison window or designated region) as measured using a BLAST or BLAST 2.0 sequence comparison algorithms with default parameters described below, or by manual alignment and visual inspection (*see, e.g.*, the NCBI web site found at ncbi.nlm.nih.gov/BLAST/ or the like). Such sequences are then referred to as “substantially identical.” This definition also refers to, or applies to, the complement of a particular sequence. The definition may also include sequences that have deletions, additions, and/or substitutions. Likewise, this definition also is intended to apply to complementarity between sequences.

For sequence comparison, one sequence typically serves as a reference sequence, to which other sequences are compared. When using a sequence comparison algorithm, reference and comparison sequences may be entered into a computer, and sequence algorithm program parameters are selected as desired. Percent sequence identities are then generated for the comparison sequences relative to the reference sequence, based on the parameters selected. An example of an algorithm that may be suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.*, (*Nuc Acids Res* 25:3389-3402, 1977) and Altschul *et al.*, (*J Mol Biol* 215:403-410, 1990), respectively. BLAST

and BLAST 2.0 are well known in the art and may be used to determine percent sequence identity for any nucleic acids or proteins, such as those described herein.

The present invention includes DNA molecules or siRNA molecules and proteins having at least about 80% (percent) sequence identity, about 81% sequence identity, about 82% sequence identity, about 83% sequence identity, about 84% sequence identity, about 85% sequence identity, about 86% sequence identity, about 87% sequence identity, about 88% sequence identity, about 89% sequence identity, about 90% sequence identity, about 91% sequence identity, about 92% sequence identity, about 93% sequence identity, about 94% sequence identity, about 95% sequence identity, about 96% sequence identity, about 97% sequence identity, about 98% sequence identity, about 99% sequence identity, or about 100% sequence identity to a Reelin coding sequence provided herein, for instance the Reelin sequences set forth as SEQ ID NOs:3-5. As used herein, the term “percent sequence identity” or “% sequence identity” refers to the percentage of identical nucleotides or amino acids in a linear polynucleotide or polypeptide sequence of a reference (“query”) sequence (or its complementary strand) as compared to a test (“subject”) sequence (or its complementary strand) when the two sequences are optimally aligned (with appropriate nucleotide or amino acid insertions, deletions, or gaps totaling less than 20 percent of the reference sequence over the window of comparison). Optimal alignment of sequences for aligning a comparison window are well known to those skilled in the art and may be conducted by tools such as the local homology algorithm of Smith and Waterman, the homology alignment algorithm of Needleman and Wunsch, the search for similarity method of Pearson and Lipman, and by computerized implementations of these algorithms such as GAP, BESTFIT, FASTA, and TFASTA available as part of the Sequence Analysis software package of the GCG® Wisconsin Package® (Accelrys Inc., San Diego, CA), MEGAlign (DNASar, Inc., 1228 S. Park St., Madison, Wis. 53715), and MUSCLE (version 3.6) (RC Edgar, Nucleic Acids Research (2004) 32(5):1792-1797) with default parameters. An “identity fraction” for aligned segments of a test sequence and a reference sequence is the number of identical components which are shared by the two aligned sequences divided by the total number

of components in the reference sequence segment, that is, the entire reference sequence or a smaller defined part of the reference sequence. Percent sequence identity is represented as the identity fraction multiplied by 100. The comparison of one or more sequences may be to a full-length sequence or a portion thereof, or to a longer sequence.

5 Proteins in accordance with the invention may be produced by changing (that is, modifying) a wild-type protein to produce a new protein with a novel combination of useful protein characteristics, such as altered V_{max}, K_m, substrate specificity, substrate selectivity, and protein stability. Modifications may be made at specific amino acid positions in a protein and may be a substitution of the amino acid found at that position in
10 nature (that is, in the wild-type protein) with a different amino acid. Proteins provided by the invention thus provide a new protein with one or more altered protein characteristics relative to the wild-type protein found in nature. In one embodiment of the invention, a protein may have altered protein characteristics such as improved or decreased activity against one or more herbicides or improved protein stability as compared to a similar
15 wild-type protein, or any combination of such characteristics. In one embodiment, the invention provides a Reelin antibody, and the DNA molecule or coding sequence encoding it, having at least about 80% sequence identity, about 81% sequence identity, about 82% sequence identity, about 83% sequence identity, about 84% sequence identity, about 85% sequence identity, about 86% sequence identity, about 87% sequence identity,
20 about 88% sequence identity, about 89% sequence identity, about 90% sequence identity, about 91% sequence identity, about 92% sequence identity, about 93% sequence identity, about 94% sequence identity, about 95% sequence identity, about 96% sequence identity, about 97% sequence identity, about 98% sequence identity, about 99% sequence identity, or about 100% sequence identity to a protein sequence such as set forth as SEQ ID NO:3-
25 5. Amino acid mutations may be made as a single amino acid substitution in the protein or in combination with one or more other mutation(s), such as one or more other amino acid substitution(s), deletions, or additions. Mutations may be made as described herein or by any other method known to those of skill in the art.

As used herein, the term “nucleic acid” refers to a single or double-stranded polymer of deoxyribonucleotide bases or ribonucleotide bases read from the 5’ to the 3’ end. A “nucleic acid” may also optionally contain non-naturally occurring or altered nucleotide bases that permit correct read through by a polymerase and do not reduce expression of a polypeptide encoded by that nucleic acid. The term “nucleotide sequence” or “nucleic acid sequence” refers to both the sense and antisense strands of a nucleic acid as either individual single strands or in the duplex. The term “ribonucleic acid” (RNA) is inclusive of RNAi (inhibitory RNA), dsRNA (double stranded RNA), siRNA (small interfering RNA), mRNA (messenger RNA), miRNA (micro-RNA), tRNA (transfer RNA, whether charged or discharged with a corresponding acylated amino acid), and cRNA (complementary RNA). The terms “nucleic acid segment,” “nucleotide sequence segment,” or more generally, “segment,” will be understood by those in the art as a functional term that includes genomic sequences, ribosomal RNA sequences, transfer RNA sequences, messenger RNA sequences, operon sequences, and smaller engineered nucleotide sequences that express or may be adapted to express, proteins, polypeptides or peptides. The nomenclature used herein is that required by Title 37 of the United States Code of Federal Regulations §1.822 and set forth in the tables in WIPO Standard ST.25 (1998), Appendix 2, Tables 1 and 3.

The term “gene” refers to components that comprise viral DNA or RNA, cDNA, viral intron and exon DNA, artificial viral DNA polynucleotide, or other DNA that encodes a peptide, polypeptide, protein, or RNA transcript molecule, and the genetic elements that may flank the coding sequence that are involved in the regulation of expression, such as, promoter regions, 5’ leader regions, 3’ untranslated region that may exist as native genes or transgenes in the genome. The gene or a fragment thereof can be subjected to polynucleotide sequencing methods that determines the order of the nucleotides that comprise the gene.

Polynucleotides as described herein may be complementary to all or a portion of a gene sequence, including a promoter, intron, coding sequence, exon, 5’ untranslated region, and 3’ untranslated region.

A particular nucleic acid sequence may also encompass “splice variants.” Similarly, a particular protein encoded by a nucleic acid implicitly encompasses any protein encoded by a splice variant of that nucleic acid. “Splice variants” are products of alternative splicing of a gene. After transcription, an initial nucleic acid transcript may be
5 spliced such that different (alternate) nucleic acid splice products encode different polypeptides. Mechanisms for the production of splice variants vary, but include alternate splicing of exons. Alternate polypeptides derived from the same nucleic acid by read-through transcription are also encompassed by this definition. Any products of a splicing reaction, including recombinant forms of the splice products, are included in this
10 definition.

The terms “polypeptide,” “peptide” and “protein” are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino
15 acid polymers and non-naturally occurring amino acid polymer.

The term “amino acid” refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, *e.g.*,
20 hydroxyproline, γ -carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, *i.e.*, an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, *e.g.*, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (*e.g.*, norleucine) or modified peptide
25 backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

5 Macromolecular structures such as polypeptide structures may be described in terms of various levels of organization. “Primary structure” refers to the amino acid sequence of a particular peptide. “Secondary structure” refers to locally ordered, three dimensional structures within a polypeptide. These structures are commonly known as domains, for example enzymatic domains, extracellular domains, transmembrane
10 domains, pore domains, or cytoplasmic tail domains. Domains are portions of a polypeptide that form a compact unit of the polypeptide. Exemplary domains include domains with enzymatic activity. A domains may be made up of sections of lesser organization such as stretches of β -sheet and α -helices. “Tertiary structure” refers to the complete three dimensional structure of a polypeptide monomer. “Quaternary structure”
15 refers to the three dimensional structure formed by the noncovalent association of independent tertiary units. Anisotropic terms are also known as energy terms.

A “label” or a “detectable moiety” is a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, chemical, or other physical means. For example, useful labels include ^{32}P , fluorescent dyes, electron-dense reagents, enzymes
20 (*e.g.*, as commonly used in an ELISA), biotin, digoxigenin, or haptens and proteins which can be made detectable, *e.g.*, by incorporating a radiolabel into the peptide or used to detect antibodies specifically reactive with the peptide.

The phrase “treating atherosclerosis” or “treatment of atherosclerosis” refers to ameliorating the effects of, or delaying, halting, or reversing the progress of, or delaying
25 or preventing the onset of, atherosclerosis as defined herein.

As used herein, a “therapeutic compound” or “therapeutic composition” refers to a molecule, such as an antibody or antibody fragment, such as a monoclonal antibody or a polyclonal antibody, RNA molecule, protein, peptide, polypeptide, an Fc-coupled

dimeric or monomeric decoy receptor, a peptidomimetic that inhibits Reelin oligomerization or receptor binding a carbohydrate, a small molecule, or the like, that has the activity of reducing or eliminating Reelin protein expression or inhibit its function. Such a compound or composition is meant to encompass a composition suitable for administration to a subject, such as a mammal, particularly a human subject. In general a “therapeutic composition” is sterile, and preferably free of contaminants that are capable of eliciting an undesirable response within the subject (*e.g.*, the compound(s) in the immunogenic composition is pharmaceutical grade). Therapeutic compositions may be designed for administration to subjects in need thereof via a number of different routes of administration including oral, intravenous, buccal, rectal, parenteral, intraperitoneal, intradermal, intratracheal, intramuscular, subcutaneous, inhalational, and the like.

As used herein, “subject” or “patient” refers to animals, including humans, who are treated with the therapeutic compounds or compositions or in accordance with the methods described herein. For diagnostic or research applications, a wide variety of mammals may be suitable subjects, including rodents (*e.g.*, mice, rats, hamsters), rabbits, primates, and swine, such as inbred pigs and the like.

As used herein, a “biological sample” or “sample” may include blood and blood parts including, but not limited to serum, plasma, platelets, or red blood cells; sputum, mucosa, tissue, cultured cells, including primary cultures, and transformed cells; biological fluids, stool, and urine. A biological sample may also include sections of tissues such as biopsy and autopsy samples, and frozen sections taken for histologic purposes. A biological sample may be obtained from a eukaryotic organism, such as a human. Any tissue appropriate for use in accordance with the invention may be used, for instance, skin, brain, spinal cord, adrenals, pectoral muscle, lung, heart, liver, duodenum, small intestine, large intestine, kidney, spleen, pancreas, adrenal gland, bone marrow, lumbosacral spinal cord, or blood.

As used herein, a “pharmaceutically acceptable carrier,” “pharmaceutically acceptable adjuvant,” or “adjuvant” refers to reagents, cells, compounds, materials, compositions, and/or dosage forms that are not only compatible with the

antibodies or other agents to be administered therapeutically, but also are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other complication commensurate with a reasonable benefit/risk ratio. Also included may be an agent that modifies the effect of other agents and is useful in preparing a therapeutic compound or composition that is generally safe, non-toxic, and neither biologically nor otherwise undesirable. Such an agent may be added to a therapeutic composition to modify the immune response of a subject by boosting the response such as to give a higher amount of antibodies and longer-lasting protection. Such an agent may include an excipient, diluent, carrier, or adjuvant that is acceptable for pharmaceutical use. Such an agent may be non-naturally occurring, or may be naturally occurring, but not naturally found in combination with other agents in the immunogenic composition.

The term “isolated compound” means a compound which has been substantially separated from, or enriched relative to, other compounds with which it occurs in nature. Isolated compounds are usually at least about 80%, at least 90% pure, at least 98% pure, or at least about 99% pure, by weight.

The term “unit dosage form,” as used herein, refers to physically discrete units suitable as unitary dosages for animal subjects, each unit containing a predetermined quantity of a compound (*e.g.*, a Reelin antibody as described herein) calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier, or vehicle. The specifications for unit dosage forms depend on the particular compound employed, the route and frequency of administration, the effect to be achieved, and the pharmacodynamics associated with each compound in the host.

As used herein, “cell culture,” “in culture,” or “cultured” refers generally to cells taken from a living organism and grown under controlled conditions. A “primary cell culture” is a culture of cells, tissues, or organs taken directly from an organism before the first subculture. Cells are expanded in culture when they are placed in a growth medium under conditions that facilitate growth and/or division, resulting in a larger population of the cells. When cells are expanded in culture, the rate of cell proliferation is sometimes

measured by the amount of time needed for the cells to double in number, referred to as “doubling time.”

As used herein, “introducing,” “delivering,” and “administering” refer to the therapeutic introduction of a therapeutic compound or composition as described herein to a subject. Administration may take place by any route that provides the therapeutic compound to the circulation of the subject in accordance with the invention.

The phrase “effective amount” refers to a concentration or amount of a therapeutic compound or composition as described herein, reagent, or other agent, that is effective for producing an intended result, including treatment of atherosclerosis as described herein. With respect to the administration of a therapeutic compound as disclosed herein, an effective amount may be any effective range or concentration. The exact dose will depend on the purpose of the treatment, and one of skill in the art will be able to determine such a dose using techniques known in the art.

As used herein, “expression” refers to the combination of intracellular processes, including transcription and translation undergone by a coding DNA molecule such as a structural gene to produce a polypeptide or functional nucleic acid (*e.g.*, an RNAi, antisense molecule, ribozyme, aptamer, etc.).

As used herein, “genetic Transformation” refers to a process of introducing a DNA sequence or construct (*e.g.*, a vector or expression cassette) into a cell or protoplast in which that exogenous DNA is incorporated into a chromosome or is capable of autonomous replication.

The term “about” is used herein to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value. The use of the term “or” in the claims is used to mean “and/or” unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and to “and/or.” When not used in conjunction closed wording in the claims or specifically noted otherwise, the words “a” and “an” denote “one or more.”

The terms “comprise,” “have,” and “include” are open-ended linking verbs. Any forms or tenses of one or more of these verbs, such as “comprises,” “comprising,” “has,” “having,” “includes” and “including,” are also open-ended. For example, any method that “comprises,” “has” or “includes” one or more steps is not limited to possessing only those one or more steps and also covers other unlisted steps. Similarly, any cell that “comprises,” “has” or “includes” one or more traits is not limited to possessing only those one or more traits and covers other unlisted traits.

EXAMPLES

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventors to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

Example 1

Animal models

Mice carrying the loxP-targeted *Reln* gene (encoding Reelin protein) were generated by gene targeting murine SV129J ES cells as described previously (Lane-Donovan, *et al. Science Signaling* 8, 2015) and mated with *Ldlr*^{-/-} (Ishibashi, *et al. The Journal of Clinical Investigation* 92:883-893, 1993) mice to yield double homozygotes (*Reln*^{fl/fl};*Ldlr*^{-/-}). *Reln*^{fl/fl};*Ldlr*^{-/-} mice were then crossbred with CAG-CreETR2 mice

(Jackson strain # 004682) to obtain CAG-Cre⁺ Reelin^{fl/fl};Ldlr^{-/-} mice and their Cre-negative littermates. To induce Cre-mediated DNA recombination, 6 week-old Reelin^{fl/fl};Ldlr^{-/-} mice with or without the CAG-Cre transgene were intraperitoneally injected with 0.135 mg/g body weight tamoxifen dissolved in sunflower oil for 5 consecutive days to yield global Reelin and Ldlr double knockout (DKO) mice, and Ldlr^{-/-} (control) mice. To elucidate the role of circulating Reelin in atherogenesis, 6 week-old Reelin^{fl/fl};Ldlr^{-/-} mice were intravenously injected with either adenovirus vector encoding Cre recombinase (Ad-Cre) to generate mice lacking circulating Reelin (Ad-Cre Reelin^{fl/fl};Ldlr^{-/-}), or with adenovirus vector encoding β -Galactosidase (Ad-Gal) to generate control mice (Ad-Gal Reelin^{fl/fl};Ldlr^{-/-}). The Ad-Cre and Ad-Gal were produced as described previously (Rohmann, *et al. Nature Biotechnology* 14:1562, 1996). Two weeks following injection with either tamoxifen or adenovirus, Reelin levels in the plasma, brain and liver were determined by western blotting. To evaluate atherosclerosis severity, eight-week-old male mice of each genotype, i.e. lacking or expressing Reelin, were placed on an atherogenic high cholesterol diet containing 21% (w/w) milk fat, 1.25% (w/w) cholesterol and 0.5% (w/w) cholic acid (TD 02028, Harlan Laboratories, Indianapolis, IN). In select experiments, white blood cell (WBC) counts and differentials were performed by collecting fresh blood obtained via retro-orbital bleeding in EDTA-containing tubes. A ProCyt Dx Hematology Analyzer (IDEXX Laboratories) was used to quantify the five major WBC subpopulations: neutrophils, lymphocytes, monocytes, eosinophils and basophils.

Example 2

Cell culture and transfection

Human aortic endothelial cells (HAEC, Cambrex Corp.) were cultured in EBM2 medium (Lonza) containing 10% FBS and used within 3-6 passages. The monocyte cell line U937 (human histiocytic lymphoma, ATCC) was grown in RPMI 1640 medium (Sigma-Aldrich) containing 10% FBS. In siRNA experiments, HAEC were transfected using siPORT amine transfection reagent (Life Technologies). Double-stranded siRNA directed to Vldlr (s14811) with the functional sequence

GCAGUGUAAUGGUAUCCGAtt (SEQ ID NO:1) or directed to Apoer2 (s15365) with the functional sequence CAUCCCUGAAUCUUCACCAAtt (SEQ ID NO:2) were purchased from Life Technologies, and control siRNA was from GE HEALTHCARE DHARMACON INC (D0018100250). The siRNA-mediated silencing of Apoer2 or Vldlr expression was evaluated by immunoblot analysis.

Example 3

Immunoblot analysis

Protein samples from plasma, tissues or HAEC whole cell lysates were prepared in RIPA buffer and separated by SDS-PAGE. After transfer onto nitrocellulose membranes (Bio-Rad), blots were probed separately with monoclonal mouse anti-Reelin (G10; Bergeyck, *et al. Journal of Neuroscience Methods* 82:17, 1998), rabbit anti-mouse Apoer2 and monoclonal mouse anti-Vldlr antibodies as indicated in the Figures. The Vldlr antibody was purchased from Millipore Corporation (Cat #MABS25) and the Apoer2 antibody was generated as described previously (Beffert, *et al. Journal of Neuroscience* 26:2041, 2006). The secondary antibody used was HRP-linked anti-mouse IgG or anti-rabbit IgG (GE Healthcare), and membranes were visualized with SuperSignal West Pico Chemiluminescence reagents and X-ray film. Band intensity was quantified using scanning densitometry of non-saturating autoradiograms with ImageJ software (NIH) within linear exposure range.

Example 4

Plasma lipids and lipoprotein profiles

At termination, blood was collected from mice via tail bleeding after an overnight fasting period, and plasma was separated by centrifugation. Plasma lipids (total cholesterol and triglyceride) were determined using kits from Thermo Scientific Company. HDL-cholesterol (HDL-C) was quantified after precipitation of apolipoprotein B-containing lipoproteins with an equal volume of a 20% polyethylene glycol solution. Lipoprotein profiles were determined by fractionation of pooled 500 μ l plasma from 5 mice in each group using a Superose 6 column (Amersham Pharmacia).

Example 5

Atherosclerotic lesion analysis

Mice fed a high cholesterol diet for 16 weeks were euthanized by anesthetic overdose. Hearts were perfused with PBS and 4% paraformaldehyde, and hearts and entire aorta were collected. For en face analysis, entire aortas from the heart extending 5-10 mm beyond the bifurcation of the iliac arteries were removed and dissected free of adjoining tissues, opened, and stained with Oil red O. Lesion extent was evaluated by morphometry of scanned images using ImageJ software. For the analysis of lesions in the aortic sinus, serial cryosections of 10 μ m thickness were taken from the region of the proximal aorta through the aortic sinuses and stained with Oil red O or hematoxylin. Quantitative immunostaining was performed using primary antibodies against Mac-3 (1:200; BD PharmingenTM), α -smooth muscle actin (1:200; Abcam), CD106 (1:40; BD PharmingenTM) or CD54 (1:40; R&D systems), and fluorescently-labeled secondary antibodies goat anti-rat Alexa Fluor 488 (ThermoFisher Scientific, A11006), donkey anti-goat Alexa Fluor 594 (ThermoFisher Scientific, A11058), or goat anti-rabbit Alexa Fluor 594 (ThermoFisher Scientific, A11012). Nuclei were counterstained with DAPI (ThermoFisher Scientific, P36935). Images were obtained with a Zeiss Axiophot microscope and the percentage of lesion area that was positively stained was determined using ImageJ software (NIH).

Example 6

Intravital microscopy for quantification of leukocyte-endothelial cell adhesion

Leukocyte-endothelial adhesion was evaluated as described previously (Umetani, *et al. Cell Metab.*, 2014). Briefly, 3-week-old male mice were intraperitoneally injected with vehicle or tamoxifen daily for 5 days. After tamoxifen-mediated knockdown of circulating Reelin was confirmed by immunoblotting, the mice were prepared for intravital microscopy. Endogenous leukocytes were fluorescence labeled by injection with Rhodamine-6G (100 μ l of 0.05% solution given via optic vascular plexus), and the mesentery was exposed for the observation and recording of images of leukocyte

adhesion and rolling using a Regita digital camera (QImaging). The velocity and quantity of leukocyte rolling was measured by Image-Pro v.6.2 (MediaCybernetics). In preliminary studies the effects of tamoxifen on adhesion were evaluated in Reln^{fl/fl};Ldlr^{-/-} mice treated daily for 5 days with tamoxifen or vehicle. WBC velocity was similar in tamoxifen-treated and vehicle-treated mice.

Example 7

Monocyte adhesion assay

The adhesion of U937 monocytes to monolayers of HAEC was evaluated as previously described (Ramesh, *et al. The Journal of Clinical Investigation* 121:120, 2011). Recombinant Reelin and mock conditioned media were collected and purified by column chromatography and size exclusion filtration from the supernatant of stably-transfected 293 cells and nontransfected 293 cells, respectively. Confluent HAEC were treated with vehicle, mock media (20µl per 1 ml) or Reelin (20µl per 1 ml) for 16h. Subsequently HAEC were washed with PBS and rinsed with RPMI 1640 medium, U937 monocytes (1×10⁶ cells/well) were added and incubated with HAEC under rotating conditions (benchtop incubator at 70 rpm) at 37°C for 20min, nonadhered monocytes were removed by gentle washing with PBS, cells were fixed with 1% paraformaldehyde for 10 min at room temperature, and the number of adherent cells was determined in triplicate per ×40 magnification field by Image J software. In select studies HAEC were transfected with control, Apoer2 or Vldlr siRNA prior to the treatment with mock media or Reelin.

Example 8

Endothelial nitric oxide synthase activation assay

Endothelial nitric oxide synthase (eNOS) activation was determined in intact endothelial cells by measuring the conversion of [14C]L-arginine to [14C]l-citrulline. Briefly, HAEC were pretreated with vehicle, mock media or Reelin for 30 min, and eNOS activity was then assessed over 15 min in the continued presence of vehicle, mock media or Reelin, in the absence (basal) or presence of VEGF (100 ng/ml). Findings were

replicated in 3 or more independent experiments.

Example 9

Statistical Analyses

All data are expressed as mean $\pm$ SEM. Two-tailed Student's t test or one-way ANOVA was used to assess differences between two groups or among more than two groups, respectively, with Newman-Keuls post-hoc testing following one-way ANOVA. P values < 0.05 (*) were considered significant, or highly significant ($p < 0.01$, **).

Example 10

Generation and characterization of conditional *Reln*^{-/-};*Ldlr*^{-/-} Mice

To explore the potential role of Reelin in the pathogenesis of atherosclerosis, *Ldlr*^{-/-} mice were crossed with floxed *Reln* mice expressing a tamoxifen-inducible Cre recombinase under the control of the ubiquitously active CAG promoter. Cre-negative littermates were used as controls. After intraperitoneal injection of tamoxifen for two weeks, immunoblot analysis confirmed the deletion of Reelin protein in the plasma, brains and livers of the double knockout (DKO) mice (FIG. 1A). To segregate the potential effects of circulating Reelin on atherosclerosis from those of systemic Reelin, mice with selective deletion of Reelin only in plasma were generated by injecting *Reln*^{fl/fl};*Ldlr*^{-/-} mice through the tail vein with adenovirus expressing Cre recombinase (Ad-Cre). Adenovirus expressing β -galactosidase (Ad-Gal) served as control. Immunoblot analysis demonstrated efficient and specific ablation of Reelin from the liver and from plasma, but not from the brain in the Ad-Cre-injected *Reln*^{fl/fl};*Ldlr*^{-/-} mice (FIG. 2A).

Plasma lipid parameters were determined in all mice after feeding with a high cholesterol diet for 16 weeks. As shown in FIG. 1B, no differences in body weight, plasma cholesterol or triglyceride levels were observed between DKO mice and *Ldlr*^{-/-} mice, and the atherogenic diet caused similarly severe hypercholesterolemia with comparable lipid profiles for cholesterol and triglyceride in the two groups of mice

(FIG. 1C, D). In the adenovirus treated groups, total plasma cholesterol was modestly increased in Ad-Gal *Reln^{fl/fl};Ldlr^{-/-}* mice compared to Ad-Cre *Reln^{fl/fl};Ldlr^{-/-}* mice, while plasma triglyceride and HDL-cholesterol (HDL-C) levels were similar in the two groups (FIG. 2B).

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Example 11

Reelin deficiency reduces atherosclerosis in *Ldlr^{-/-}* mice

Following high cholesterol feeding for 16 weeks beginning at eight weeks of age, aortas were excised and stained with Oil Red-O to visualize atherosclerotic plaques. En face analyses revealed a 52% decrease in atherosclerotic lesion area in aortas from DKO mice with global Reelin deletion compared to the aortas of control *Ldlr^{-/-}* mice (FIG. 3A). As shown in FIG. 3B, quantitative analysis of lesion areas in cross-sections of the aortic sinus showed a similar 47% reduction in lesion size in DKO mice compared to controls. Similarly, Ad-Cre *Reln^{fl/fl};Ldlr^{-/-}* mice displayed a 46% decrease of atherosclerotic lesion area in the aorta and a 73% reduction of aortic root lesions compared with Ad-Gal *Reln^{fl/fl};Ldlr^{-/-}* mice (FIG. 4A and 4B). Thus, both global and plasma-selective Reelin deficiency greatly diminished atherosclerotic lesion formation.

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Example 12

Reelin deficiency reduces adhesion molecule expression and macrophage infiltration

The presence of macrophages and smooth muscle cells in atherosclerotic plaques from DKO and control *Ldlr^{-/-}* mice was investigated by immunostaining with anti-Mac-3 and anti- α -actin antibodies, respectively. As shown in FIG. 5A, morphometric quantification of the lesions revealed a 32% reduction of Mac-3 positive macrophages in DKO versus *Ldlr^{-/-}* control mice. No difference in α -actin-positive (smooth muscle) area was observed between the two groups.

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The migration of monocytes into the subendothelium of arteries in atherosclerosis results from the activation of endothelial cells, which leads to the increased expression of leukocyte adhesion molecules. To determine the basis for the impact of Reelin on lesion macrophage accumulation, VCAM-1 and ICAM-1 expression in plaques was investigated

by immunostaining with anti-CD106 and anti-CD54 antibodies, respectively. VCAM-1 was reduced by 65% and ICAM-1 by 56% in plaques from DKO mice compared to *Ldlr*^{-/-} mice (FIG. 5B). At the same time, Mac-3, CD106 and CD54 immunoreactivity was similarly reduced in the lesions of Ad-Cre *Reln*^{fl/fl}; *Ldlr*^{-/-} compared to Ad-Gal *Reln*^{fl/fl}; *Ldlr*^{-/-} mice (data not shown). These results suggest that Reelin promotes macrophage foam cell accumulation in atherosclerotic lesions, and that this is driven by increased adhesion molecule expression.

Example 13

Reelin increases leukocyte-endothelial cell adhesion

To better understand the mechanisms by which Reelin promotes macrophage accumulation in atherosclerosis, intravital microscopy was performed to quantify leukocyte-endothelial adhesion *in vivo* in the setting of low versus high Reelin abundance. Male DKO and *Ldlr*^{-/-} mice were intraperitoneally injected for 5 days with tamoxifen, and the tamoxifen-induced reduction of circulating Reelin was confirmed by western blotting. Endogenous leukocytes were fluorescently labeled by injection of Rhodamine-6G, and intravital video microscopy was performed to visualize leukocyte adhesion to the endothelium in the mesenteric microvasculature. As shown in representative still images in FIG. 6A and in the quantitation provided in FIG. 6B, leukocyte velocity was increased by 25% and the number of adherent leukocytes was reduced by 78% in DKO mice compared with *Ldlr*^{-/-} mice. Total circulating WBC and leukocyte subsets were also quantified in DKO and *Ldlr*^{-/-} mice, and no differences were observed (FIG. 6C). Therefore, the intravital microscopy findings are not the result of an effect of Reelin on leukocyte abundance, but they instead they indicate that Reelin increases leukocyte-endothelial cell adhesion *in vivo*.

To determine if direct actions of Reelin on endothelial cells underlie the apparent increase in adhesion, and if these processes are operative in human endothelium, monocyte adhesion to cultured primary human aortic endothelial cells (HAEC) was investigated. Compared with vehicle and Mock treatment of HAEC, Reelin caused a >2-

fold increase in adhesion (FIG. 7A and 7B). To determine which Reelin receptor mediates the increase in endothelial cell-monocyte adhesion, siRNA knockdown of Vldlr or Apoer2 was performed. Whereas loss of Vldlr did not alter the effect of Reelin (FIG. 7C), Apoer2 knockdown effectively prevented the Reelin mediated increase of endothelial cell-monocyte adhesion (FIG. 7D). These results indicate that Reelin enhances adhesion via Apoer2 and not Vldlr in endothelium.

Example 14

Reelin antagonizes endothelial NOS through Apoer2

NO generated by endothelial eNOS is a key modulator of leukocyte-endothelial cell adhesion. Having previously shown that Apoer2 mediates antiphospholipid antibody-induced suppression of eNOS, which increases endothelial cell-leukocyte adhesion, it was next investigated whether Reelin alters eNOS activity in HAEC and whether Apoer2 participates. eNOS activation by VEGF was quantified by measuring ¹⁴C-arginine conversion to ¹⁴C-citrulline in intact HAEC. Whereas VEGF increased eNOS activity in non-treated and Mock-treated cells, Reelin potently attenuated eNOS activation (FIG. 7E). As shown in FIG. 7F, siRNA knockdown of Apoer2 fully prevented Reelin-mediated inhibition of eNOS activation. Thus, Reelin reduces eNOS activity via Apoer2.

Example 15

Treatment with Anti-Reelin Antibody Reduces Monocyte and Leukocyte Adhesion in the Vascular Endothelium

The effect of a reduction in plasma Reelin abundance on monocyte and leukocyte adhesion was investigated using the anti-Reelin antibody CR50 (RIKEN). Intraperitoneal injection of CR50 resulted in sustained reduction of plasma Reelin in mice (FIG. 8).

An *in vivo* leukocyte adhesion assay was developed in which monocytes were selectively labeled in CX₃CR1^{w^{GFP}} mice (FIG. 9).

It was demonstrated that Reelin can be completely removed from plasma by i.p. injection of the anti-Reelin CR50 antibody (FIG. 10). CX₃CR1^{w^{GFP}} mice treated with the anti-Reelin CR50 antibody exhibited reduced *in vivo* monocyte adhesion to the vascular endothelium (FIG. 11). FIG. 12 demonstrates reduced *in vivo* leukocyte adhesion to the vascular endothelium in CX₃CR1^{w^{GFP}} mice treated with CR50 antibody.

Example 16

Role of Reelin in the Signaling

Further assays were developed using the anti-Reelin CR50 antibody to investigate Reelin-mediated IKB α activation. HAEC's were treated with 20nM Reelin in the presence or absence of 200nM CR50. Reelin-mediated IKB α activation was blocked by treatment of HAECs with CR50 (FIG. 13).

Neuro2A cells were transfected with NF- κ B-Luc construct followed by treatment with Reelin at different concentrations in the presence or absence of 10-fold CR50. These experiments demonstrated that Reelin activates NF- κ B in Neuro2A cells, and that activation is attenuated by treatment with CR50 antibody (FIG. 14).

HAECs were transfected with NF- κ B-Luc construct followed by treatment with 20nM Reelin in the presence or absence of 200nM CR50. Reelin was shown to activate NF- κ B in HAECs, and that effect was blocked by treatment with CR50 antibody (FIG. 15).

In order to identify the role of Dab2 in Reelin activation, an assay was developed in which endogenous Dab2 was successfully deleted from HAECs using siRNA targeting human Dab2 (FIG. 16). The levels of endothelial adhesion molecules VCAM and E-Selectin relative to HRPT1 were evaluated in HAECs in the presence or absence of Reelin. Reelin was shown to activate the expression of mRNAs for endothelial adhesion molecules in HAECs (FIG. 17). Treatment of HAECs with double-stranded RNA targeting either Apoer2 or Dab2 significantly reduced VCAM1 and E-Selectin levels compared with cells treated with Reelin and control RNA. This demonstrates that the

effect of Reelin is mediated by a signaling cascade that requires Apoer2 and its intracellular adapter protein Dab2 (FIG. 17).

Example 17

Discussion

5 Besides the nervous system, Reelin is expressed by the liver and present at substantial concentrations in the circulation. In the present work, two distinct types of Reelin conditional knockout mice were employed to determine how Reelin impacts atherosclerosis. Consistent with earlier studies, the results indicate that the circulating pool of Reelin is peripherally-derived, primarily from the liver, and that it does not
10 originate from the CNS. Using these genetic loss-of-function strategies, it was demonstrated that Reelin promotes atherosclerosis in mice.

 Atherogenesis is a chronic inflammatory process characterized by the recruitment and transmigration of monocytes into the intima of large vessels and subendothelial accumulation of lipids and lipid-laden foam cells. The studies of Reelin deficiency
15 disclosed herein revealed that the adverse impact of Reelin on atherosclerosis severity entails the enhancement of macrophage accumulation in the atherosclerotic lesions. Prior studies of the Reelin receptors Apoer2 and Vldlr in the context of atherosclerosis investigated cell autonomous mechanisms of these receptors in macrophages, but did not address the role of their cognate ligand, Reelin. Functional analysis of macrophage
20 Apoer2 in *Ldlr*^{-/-} mice suggested that deficiency of the receptor enhances macrophage susceptibility to lipid accumulation and cell death to augment atherosclerotic plaque progression *in vivo*. In contrast, the transplantation of Vldlr-expressing macrophages into *Vldlr*^{-/-} mice markedly accelerated the development of atherosclerotic lesions, suggesting a proatherogenic role of macrophage Vldlr. A study limited to macrophages in
25 culture showed that the activation of Vldlr and Apoer2 by Reelin or ApoE3 induces ABCA1 expression to promote macrophage cholesterol efflux, suggesting a potential antiatherogenic role for Reelin. In another series of experiments the treatment of Vldlr- and Apoer2-overexpressing macrophages with ApoE promoted macrophage conversion

from the proinflammatory M1 to the antiinflammatory M2 phenotype, potentially indicating anti-inflammatory actions of the receptors. In contrast to these prior efforts, the present investigation entailing discrete manipulation of Reelin in mice comprehensively addresses how this neurodevelopmentally essential ligand influences atherosclerosis *in vivo*, and reveals how it aggravates vascular inflammation by enhancing macrophage adhesion and transmigration.

During atherosclerotic lesion development, endothelial dysfunction is an early event, preceding clinical manifestations and complications. After activation by oxidized lipids and proinflammatory cytokines, endothelial cells of the arterial wall express chemokines and adhesion molecules, which provoke leukocyte-endothelial cell adhesion, and in turn the recruitment of inflammatory cells into the lesions. Interestingly, in a prior study neither Vldlr deficiency nor endothelial overexpression of the Vldlr affected atherosclerotic lesion development in Ldlr knockout mice, suggesting that there is modest, if any role for Vldlr in endothelial cells in atherogenesis. On the contrary, it has been previously reported that Apoer2 in endothelial cells reduces NO synthesis and leukocyte-endothelial cell adhesion when Apoer2 is mediating the actions of antiphospholipid antibodies, but enhances NO signaling when ApoE3 is the ligand. The present disclosure demonstrates that hypercholesterolemic Ldlr^{-/-} mice lacking Reelin either systemically or only in their circulation display decreased expression of vascular adhesion markers resulting in attenuated leukocyte-endothelial cell adhesion and reduced atherosclerotic plaque size. Two important differences were observed in the studies in which recombinant adenoviruses were used to delete Reelin selectively in the circulation. First, the total lesion area was much greater in the mice that received recombinant adenovirus (FIG. 4A) than in the tamoxifen experiments (FIG. 3A). This was the case for both, the Ad-Gal control virus and the Ad-Cre virus, compared to the Cre⁺ and Cre⁻ groups that had received tamoxifen. Second, total plasma cholesterol levels were somewhat higher in the mice that had received Ad-Gal (FIG. 2B), while they were essentially identical in the mice that had received Ad-Cre (FIG. 2B) or tamoxifen (FIG. 1B). These differences likely reflect the contribution of adenovirus-induced inflammation

to the results and highlight a possible common complication of using virus-mediated gene transfer or other invasive manipulation (like bone marrow transplantation) in atherosclerosis experiments. Despite these caveats, the results of the adenovirus experiments are consistent with the results obtained in the tamoxifen-treated groups, further supporting the role of Reelin in atherogenesis.

Studies in primary human endothelial cells revealed that direct effects of Reelin on endothelium underlie its pro-inflammatory actions, and that these are likely related to Reelin inhibition of eNOS via Apoer2. Collectively these observations indicate that the negative impact of Reelin on atherosclerosis is at least partially related to its actions on the endothelium.

In summary, the conditional Reelin knockout mouse of the instant disclosure provides a new model to study the impact of Reelin on atherosclerosis and the underlying cellular processes. The cumulative results of the instant disclosure demonstrate for the first time that circulating Reelin promotes atherosclerosis development, and that this is likely attributed to a variety of synergistic molecular mechanisms. These include i) the Reelin-induced reduction of eNOS activity via Apoer2, resulting in ii) increased leukocyte-endothelial adhesion and iii) monocyte/macrophage accumulation in expanding atherosclerotic lesions. These cellular mechanisms, combined with a direct prothrombotic function of Reelin, represent peripheral proatherogenic effects of this essential regulator of brain development, synaptic plasticity, learning and memory. Reelin and its surprising modes of action in the context of atherosclerosis provide novel therapeutic targets to pursue in efforts to combat cardiovascular disease.

* * * * *

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and methods and in the steps or in the sequence of steps of the

method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and
5 modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

WHAT IS CLAIMED IS:

Claim 1. A method of treatment or prevention of atherosclerosis in a subject comprising providing to a subject an amount of a therapeutic composition effective to decrease the activity of Reelin in the circulation of the subject, wherein said treatment or prevention results in a reduction of disease symptoms in the subject.

Claim 2. The method of claim 1, wherein said reduction of disease symptoms comprises reduced leukocyte adhesion to the vascular wall in the subject.

Claim 3. The method of claim 1, wherein said decreased activity of Reelin comprises reduction of Reelin protein expression or inhibition of Reelin function in the circulation of a subject.

Claim 4. The method of claim 1, wherein said therapeutic composition comprises a protein, a peptide, a polypeptide, an RNA molecule, a peptidomimetic that inhibits Reelin oligomerization or receptor binding, an siRNA molecule, a decoy receptor, a carbohydrate, an antibody or antibody fragment, or a small molecule, wherein said therapeutic composition targets Reelin, resulting in a reduction of Reelin activity in the circulation of the subject.

Claim 5. The method of claim 4, wherein the antibody is a monoclonal antibody.

Claim 6. The method of claim 4, wherein the antibody is a humanized antibody.

Claim 7. The method of claim 4, wherein the decoy receptor is an Fc-coupled dimeric or monomeric decoy receptor.

Claim 8. The method of claim 1, wherein said therapeutic composition performs a function comprising:

- (a) reducing or inhibiting the expression of a gene encoding Reelin;
- (b) reducing or inhibiting the expression of a Reelin protein;
- (c) reducing or inhibiting expression of a receptor for Reelin;
- (d) reducing or inhibiting the function of a Reelin protein; or

(e) binding to Reelin protein in such a way that the function is reduced or eliminated.

Claim 9. The method of claim 1, further comprising administering an antithrombotic drug, an antibody targeting PCSK9, or an HMG-CoA reductase inhibitor to the subject.

Claim 10. A method for reducing leukocyte adhesion to the vascular wall in a subject, comprising administering to the subject a therapeutic compound targeting Reelin in the circulation of the subject, wherein said treatment results in reduced leukocyte adhesion to the vascular wall.

Claim 11. The method of claim 10, wherein said targeting Reelin comprises reduction of Reelin protein expression or inhibition of Reelin function in the circulation of a subject.

Claim 12. The method of claim 10, wherein said therapeutic compound comprises a protein, a peptide, a polypeptide, an RNA molecule, a peptidomimetic that inhibits Reelin oligomerization or receptor binding, an siRNA molecule, a decoy receptor, a carbohydrate, an antibody or antibody fragment, or a small molecule, wherein said therapeutic compound targets Reelin, resulting in reduced leukocyte adhesion to the vascular wall of the subject.

Claim 13. The method of claim 12, wherein the antibody is a monoclonal antibody.

Claim 14. The method of claim 12, wherein the decoy receptor is an Fc-coupled dimeric or monomeric decoy receptor.

Claim 15. The method of claim 10, wherein said therapeutic compound performs a function comprising:

- (a) reducing or inhibiting the expression of a gene encoding Reelin;
- (b) reducing or inhibiting the expression of a Reelin protein;
- (c) reducing or inhibiting expression of a receptor for Reelin;

(d) reducing or inhibiting the function of a Reelin protein; or

(e) binding to Reelin protein in such a way that the function is reduced or eliminated.

Claim 16. The method of claim 10, further comprising administering an antithrombotic drug, an antibody targeting PCSK9 or an HMG-CoA reductase inhibitor to the subject.

Claim 17. The method of claim 10, further comprising identifying a subject suspected of having atherosclerosis or being in need of atherosclerosis treatment.

Claim 18. A therapeutic composition for treating atherosclerosis in a subject comprising a therapeutic compound in an effective amount to reduce disease symptoms and a non-natural, pharmaceutically acceptable carrier.

Claim 19. The composition of claim 18, wherein the therapeutic compound comprises a protein, a peptide, a polypeptide, an RNA molecule, an siRNA molecule, a decoy receptor, a carbohydrate, an antibody or antibody fragment, or a small molecule, wherein said therapeutic compound targets Reelin, resulting in a reduction of Reelin in the circulation of the subject.

Claim 20. A human or humanized antibody, fragment, or derivative thereof which recognizes Reelin, for the treatment of atherosclerosis, or diseases associated therewith or resulting therefrom.

Claim 21. A pharmaceutical composition comprising an siRNA complementary to all or a portion of a Reelin mRNA sequence (SEQ ID NO:3-5).

Claim 22. The pharmaceutical composition of claim 21, wherein the siRNA functions to inhibit Reelin in a subject.

Claim 23. A method of treatment or prevention of multiple sclerosis, arthritis, or psoriasis in a subject comprising providing to a subject an amount of a therapeutic composition effective to decrease the activity of Reelin in the circulation of the subject,

wherein said treatment or prevention results in a reduction of disease symptoms in the subject.

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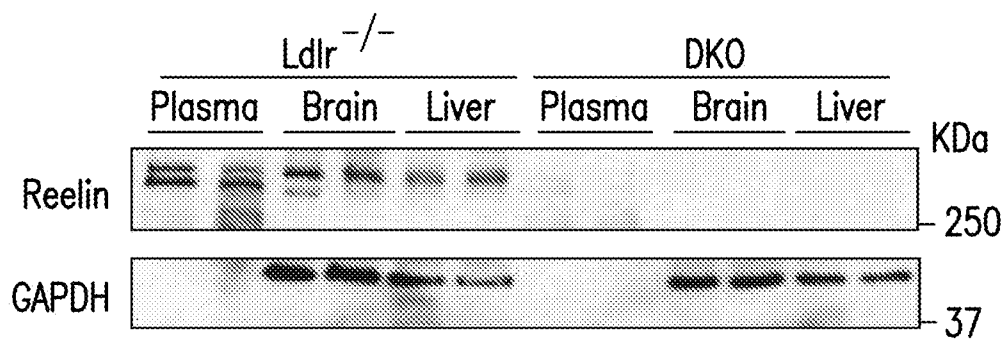


FIG.1A

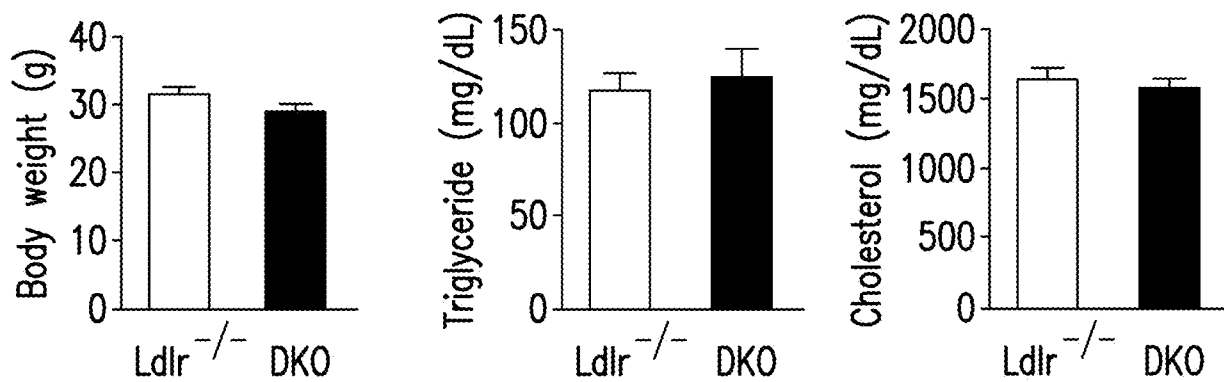


FIG.1B

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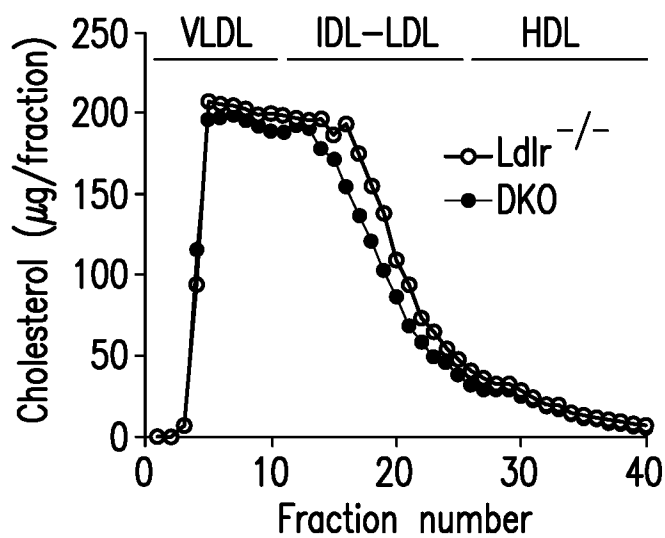


FIG.1C

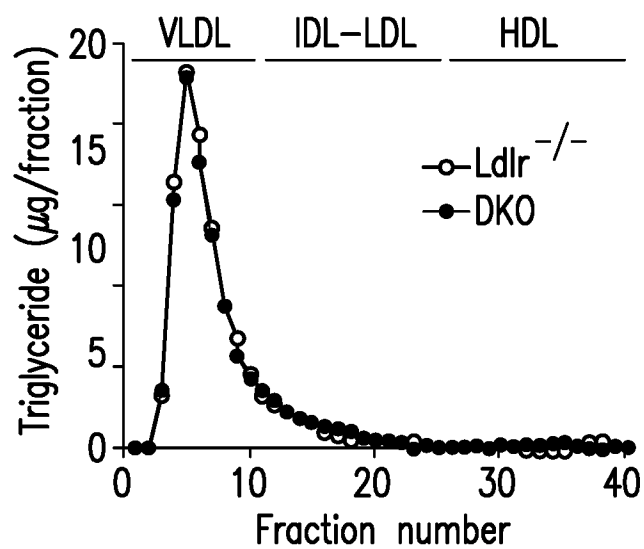


FIG.1D

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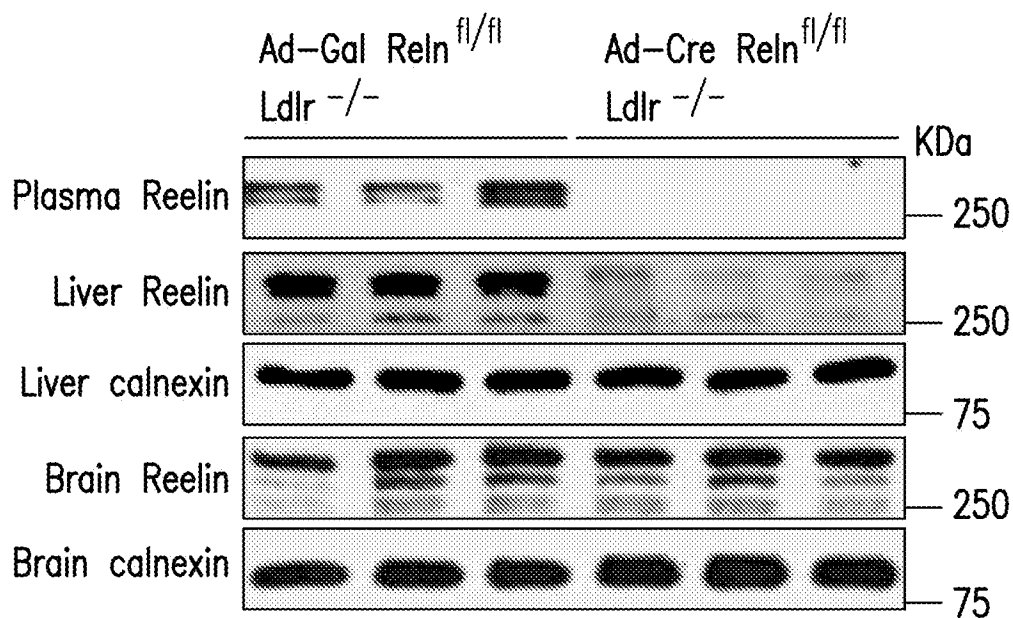


FIG.2A

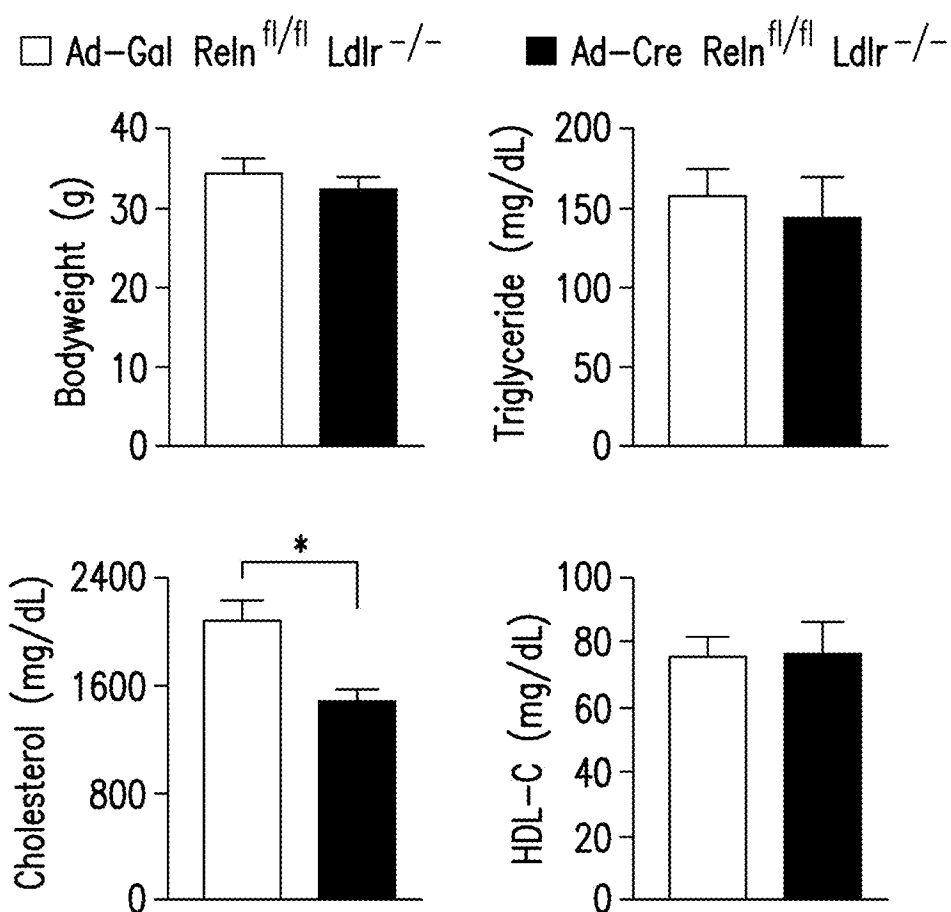


FIG.2B

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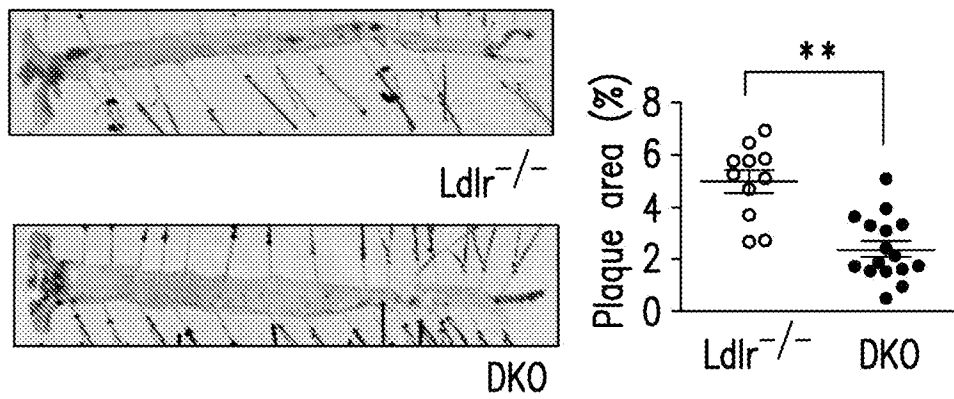


FIG.3A

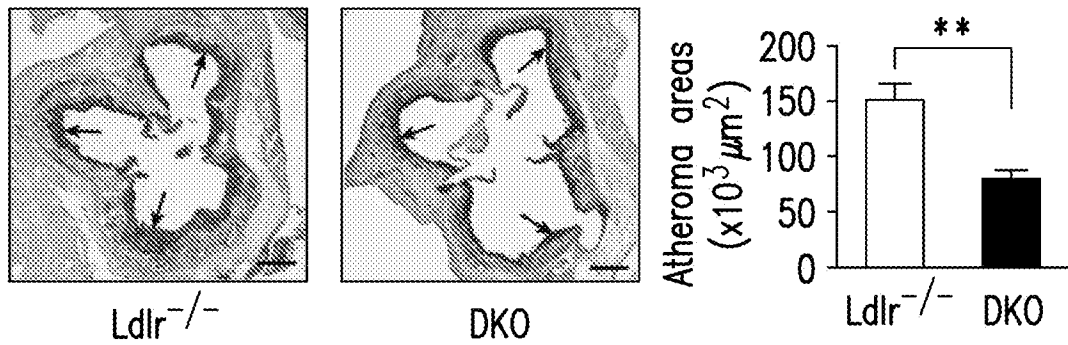


FIG.3B

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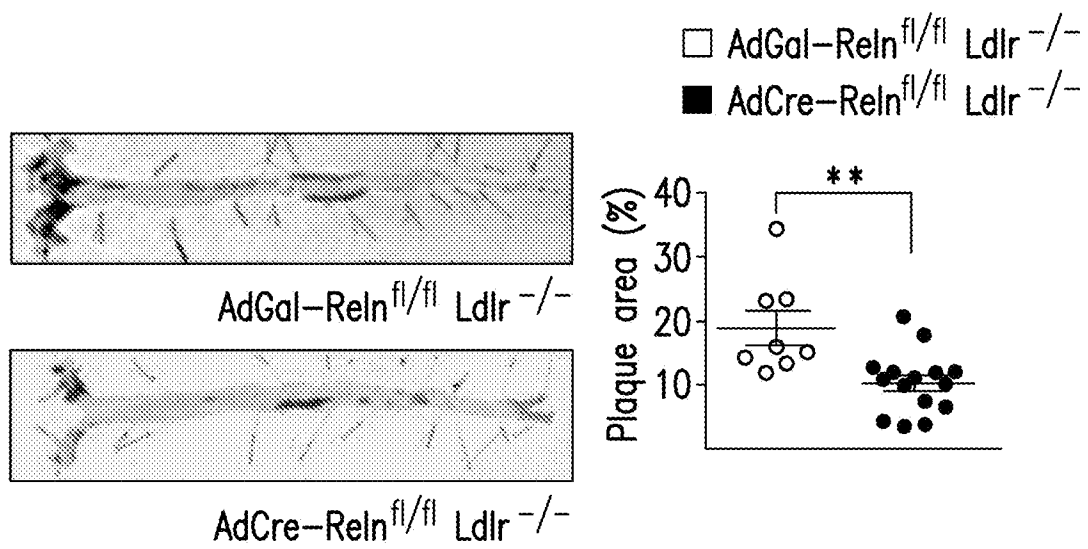


FIG.4A

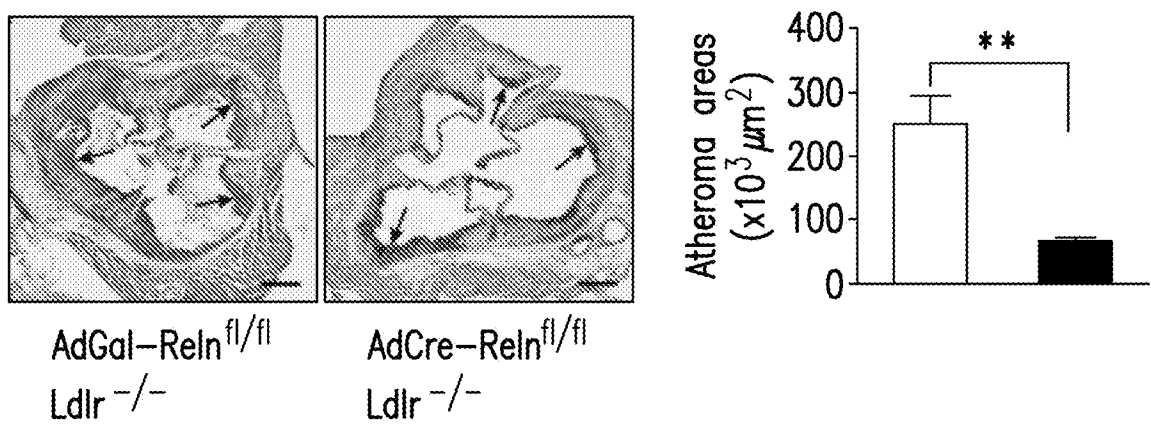


FIG.4B

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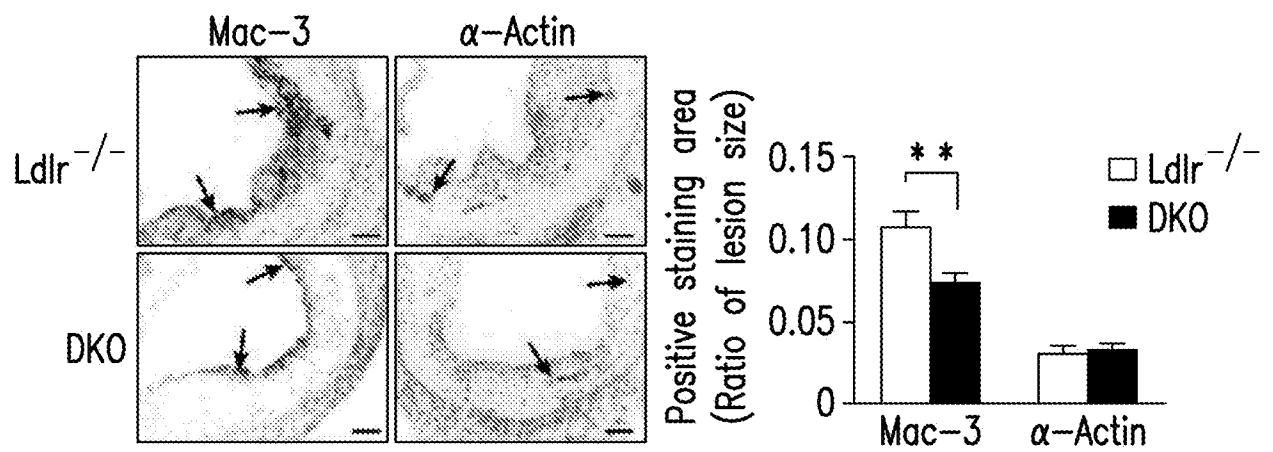


FIG.5A

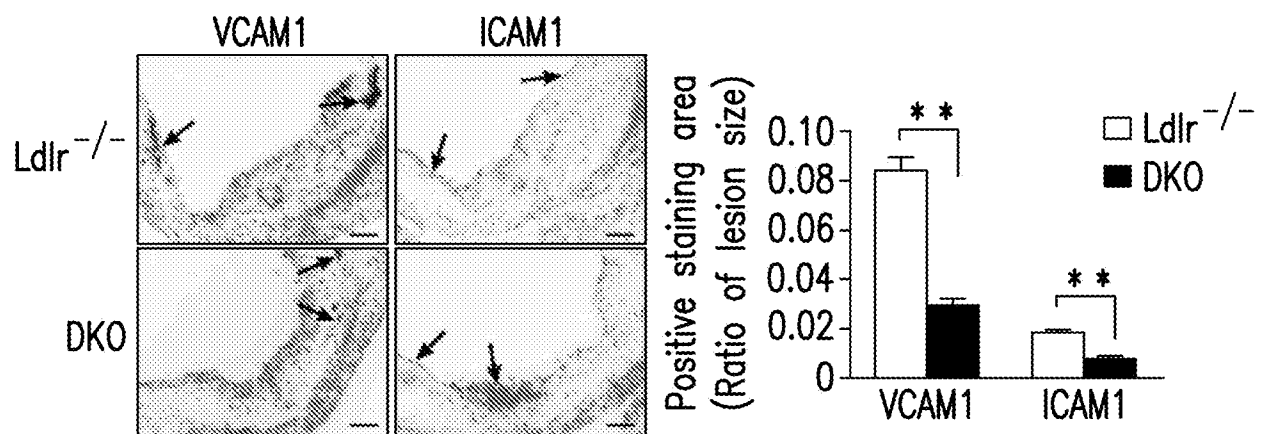


FIG.5B

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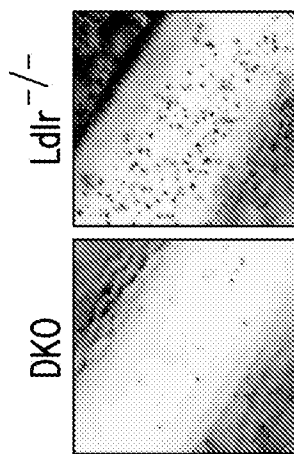


FIG.6A

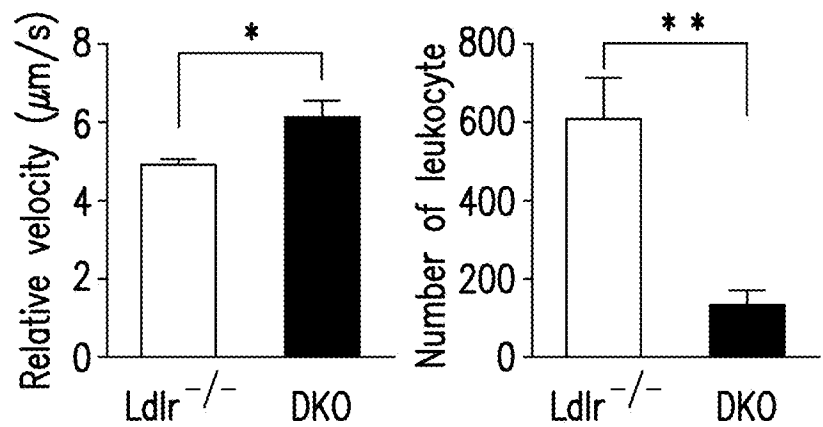


FIG.6B

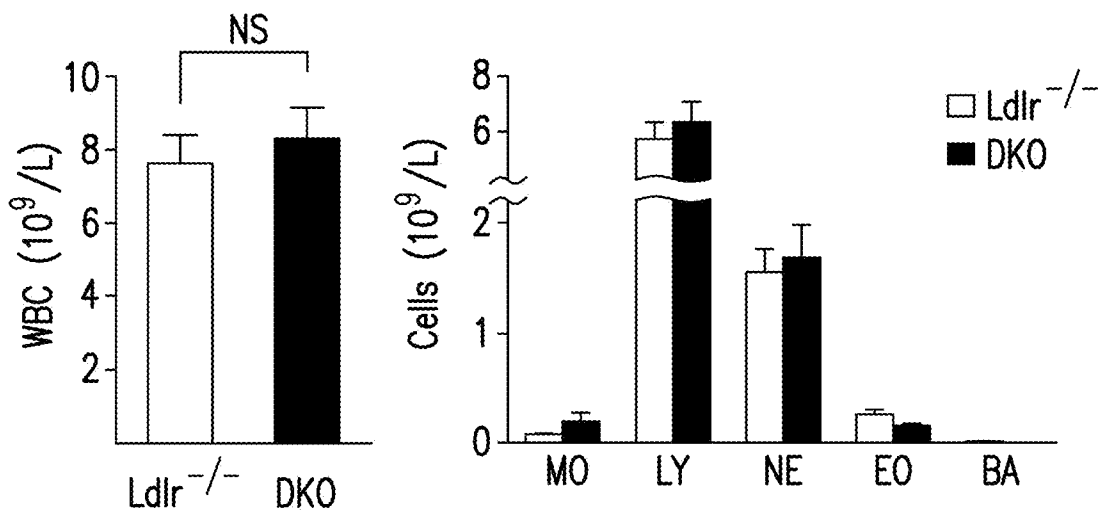


FIG.6C

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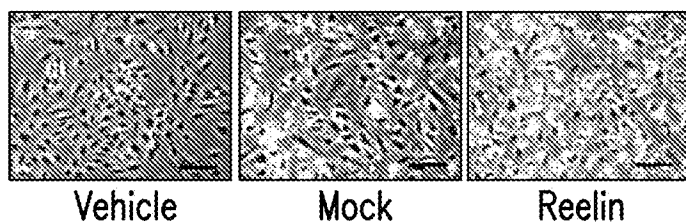


FIG. 7A

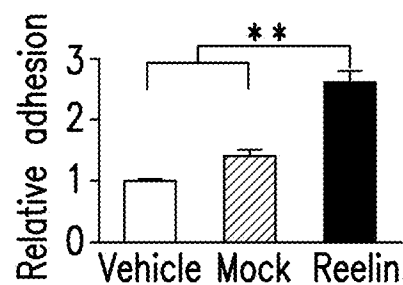


FIG. 7B

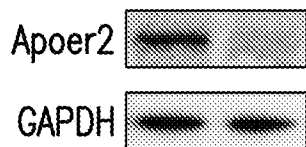
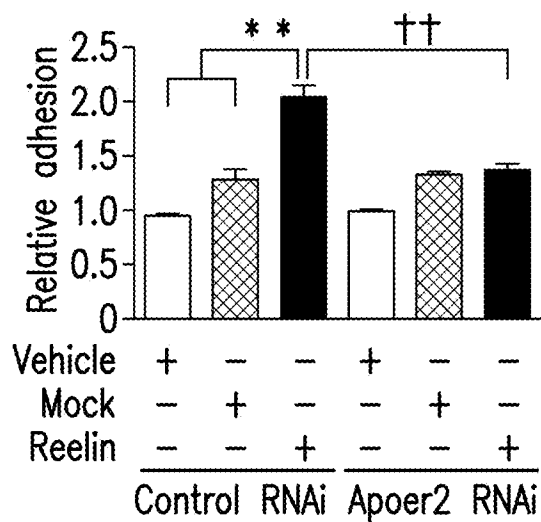


FIG. 7C

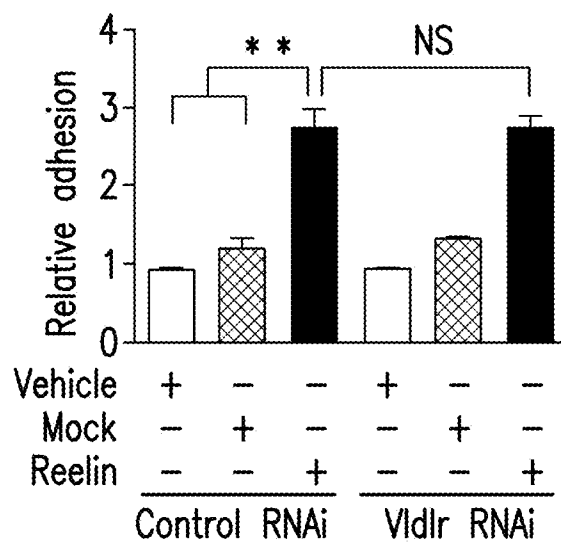


FIG. 7D

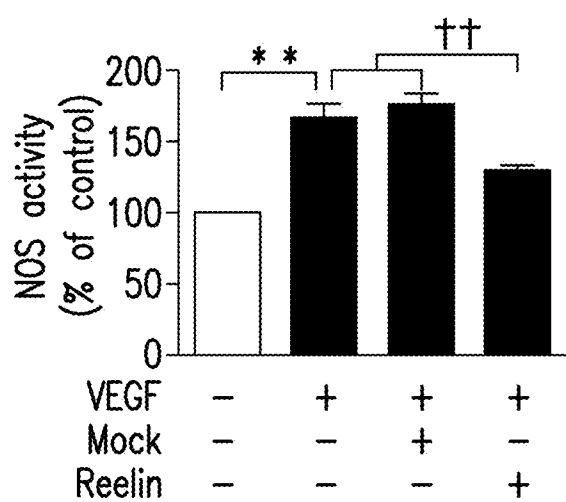


FIG. 7E

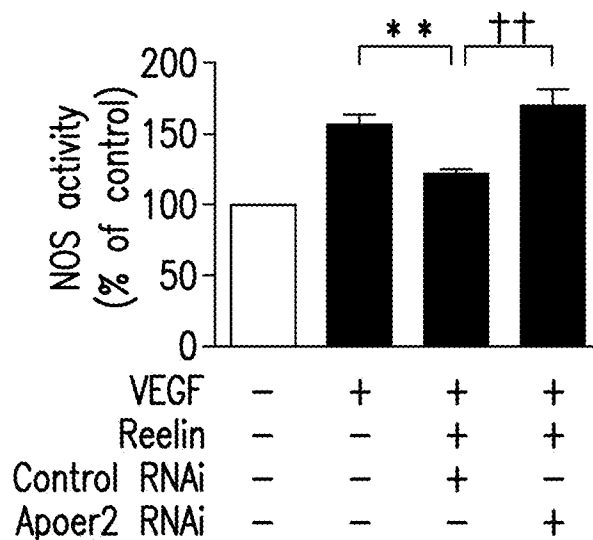


FIG. 7F

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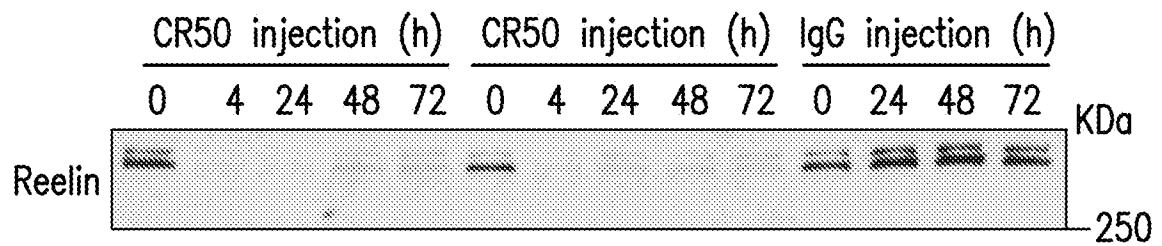


FIG.8

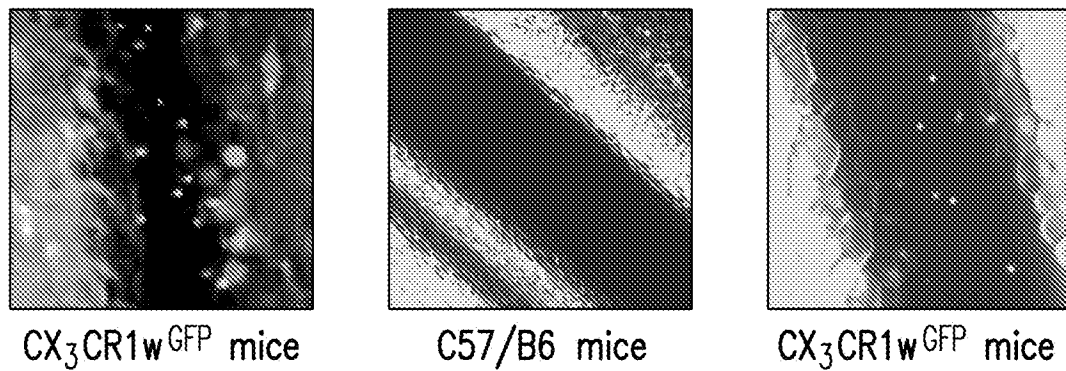


FIG.9

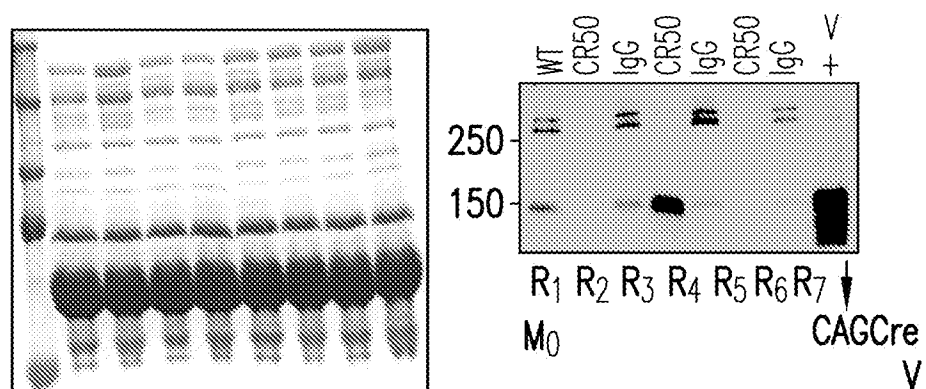


FIG.10

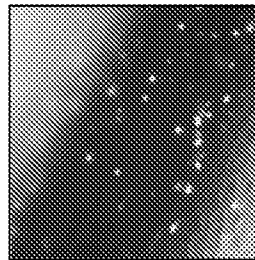
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In vivo monocyte adhesion in CX₃CR1^{GFP} mice

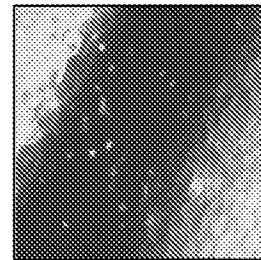
IgG group



Female mice

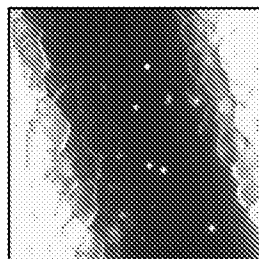


Male mice

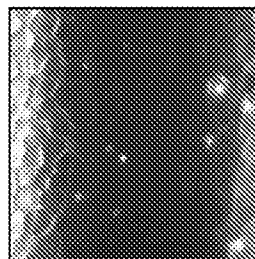


Male mice

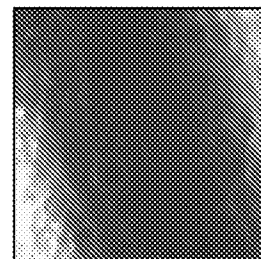
CR50 group



Male mice



Female mice



Male mice

FIG.11

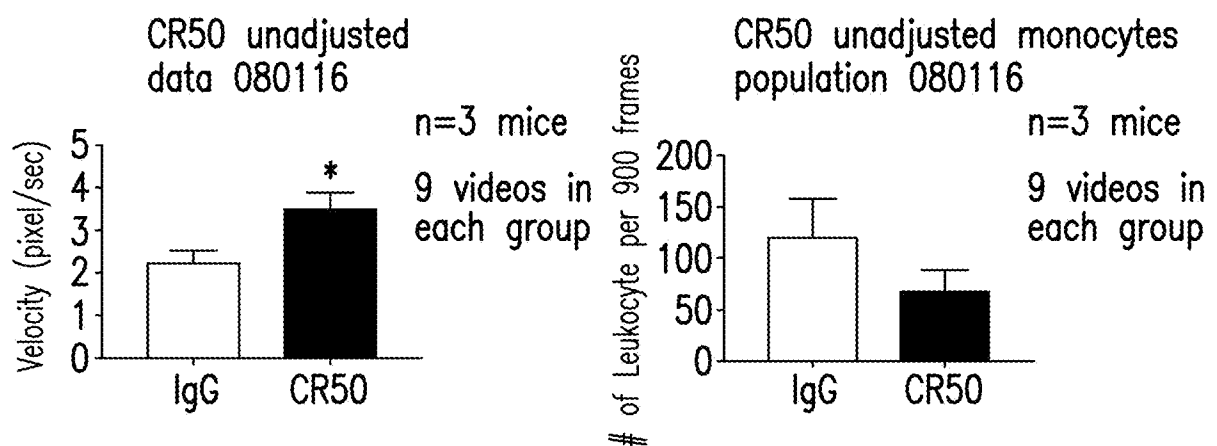
In vivo leukocyte adhesion in CX₃CR1^{GFP} mice

FIG.12

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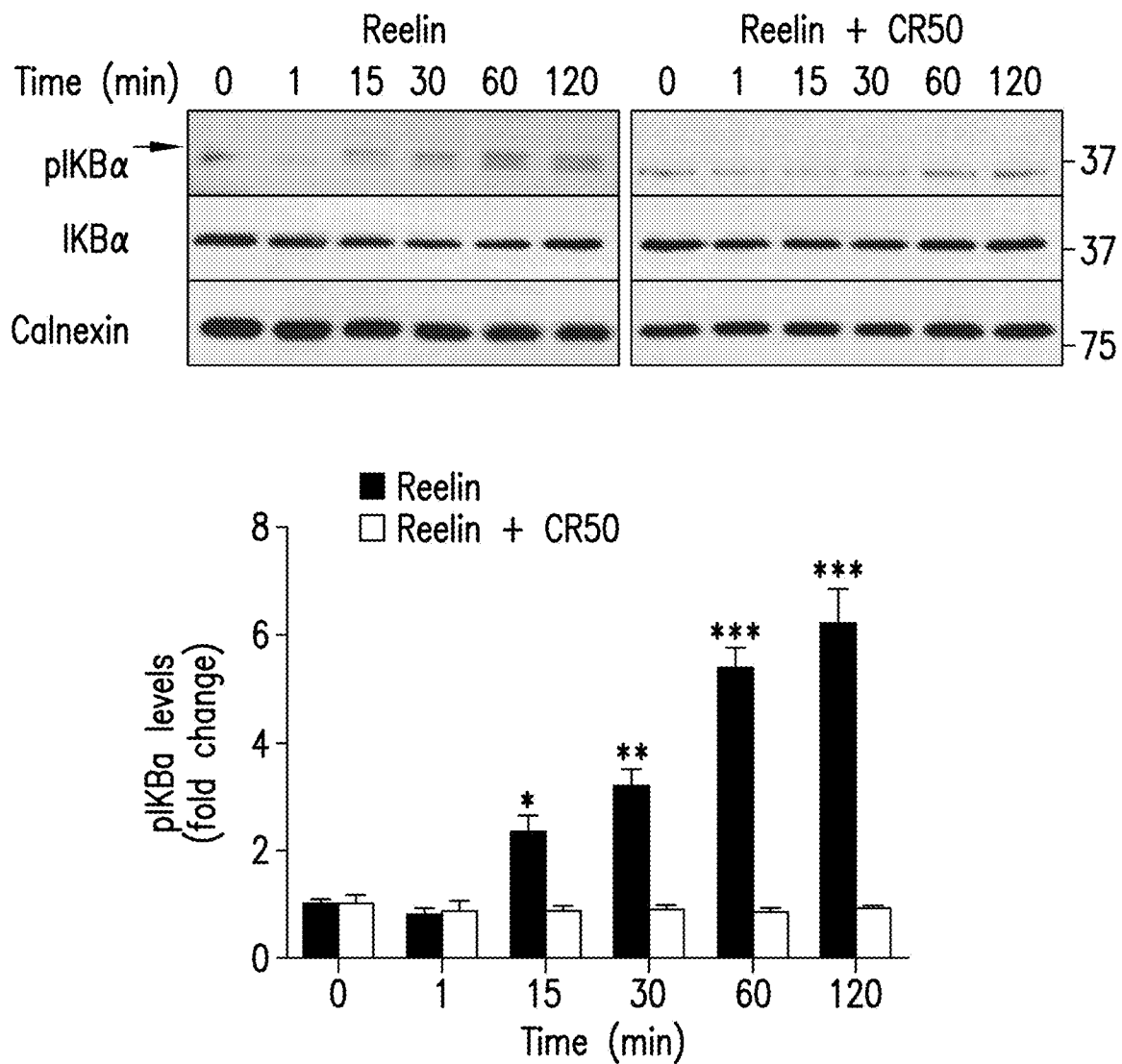


FIG.13

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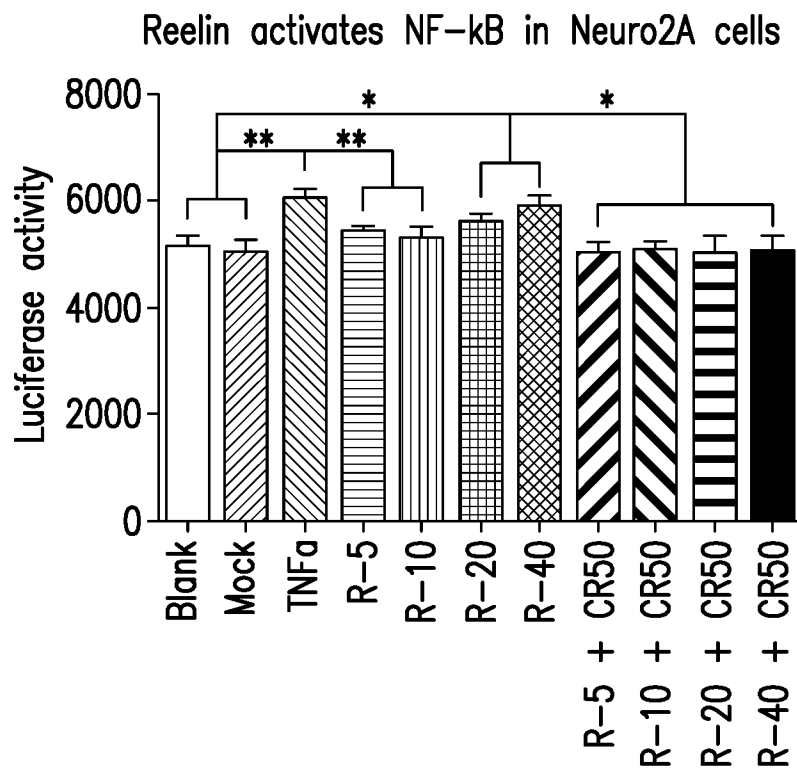


FIG. 14

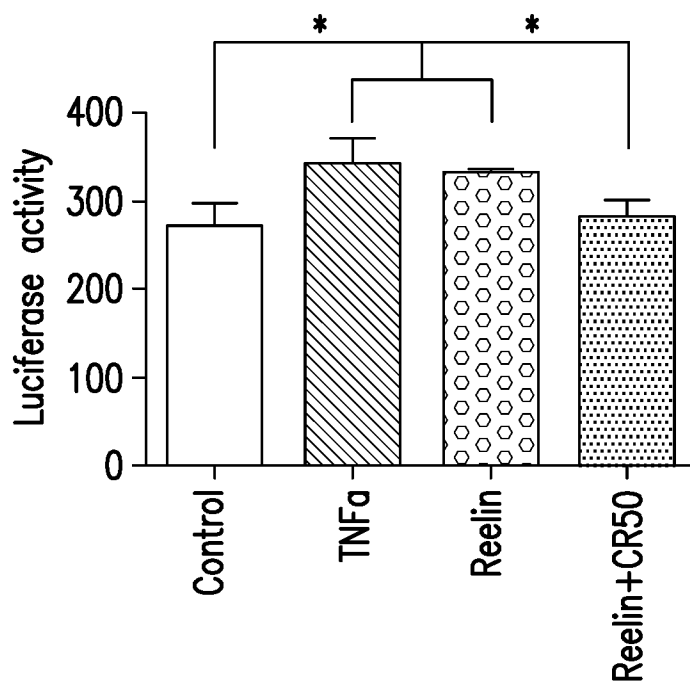


FIG. 15

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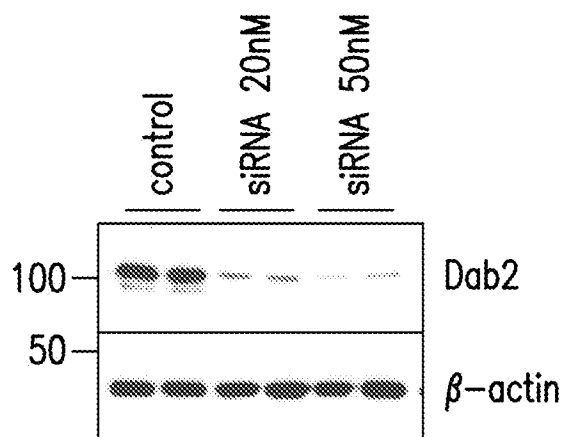


FIG. 16

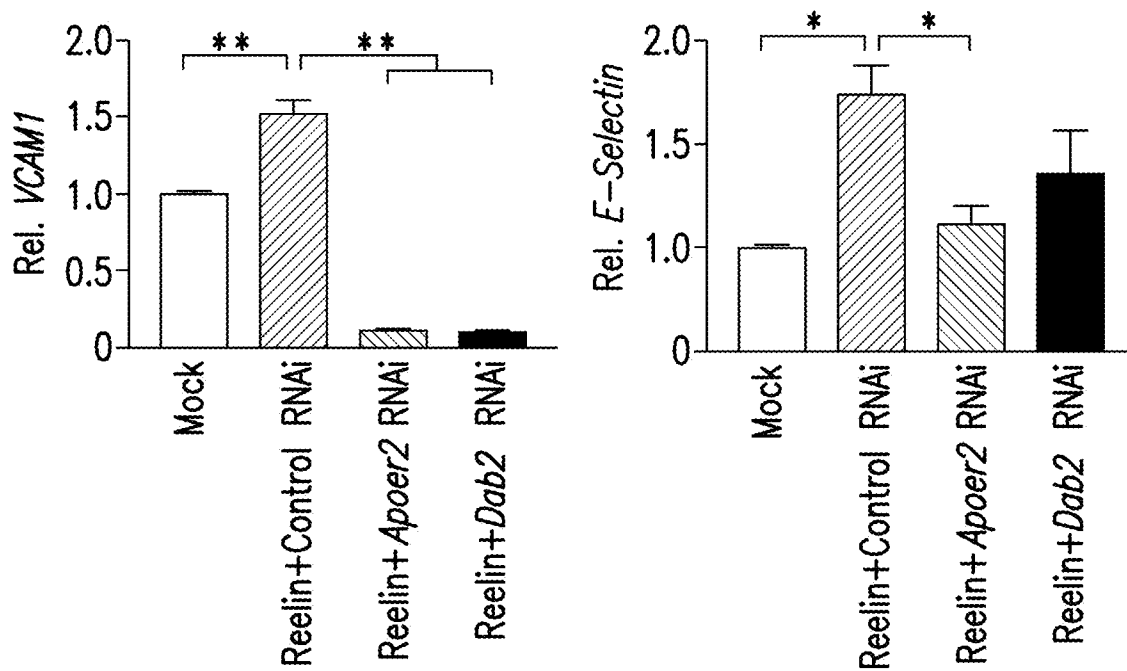


FIG. 17

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/053351

A. CLASSIFICATION OF SUBJECT MATTER INV. A61K39/395 A01K67/027 A61K31/135 A61P9/10 ADD.											
According to International Patent Classification (IPC) or to both national classification and IPC											
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) A61K C07K A01K											
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched											
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, BIOSIS, EMBASE, WPI Data											
C. DOCUMENTS CONSIDERED TO BE RELEVANT <table border="1"> <thead> <tr> <th>Category*</th> <th>Citation of document, with indication, where appropriate, of the relevant passages</th> <th>Relevant to claim No.</th> </tr> </thead> <tbody> <tr> <td>X</td> <td>US 6 323 177 B1 (CURRAN THOMAS [US] ET AL) 27 November 2001 (2001-11-27) column 6, paragraph 1 and 6; claims 1-17</td> <td>1-23</td> </tr> <tr> <td>A</td> <td> ----- MAY P ET AL: "Molecular mechanisms of lipoprotein receptor signalling", CMLS CELLULAR AND MOLECULAR LIFE SCIENCES, BIRKHÄUSER-VERLAG, BA, vol. 62, no. 19-20, 1 October 2005 (2005-10-01), pages 2325-2338, XP019200847, ISSN: 1420-9071, DOI: 10.1007/S00018-005-5231-Z page 2319, column 1, paragraph 3 ----- -/- </td> <td>1-23</td> </tr> </tbody> </table>			Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	X	US 6 323 177 B1 (CURRAN THOMAS [US] ET AL) 27 November 2001 (2001-11-27) column 6, paragraph 1 and 6; claims 1-17	1-23	A	----- MAY P ET AL: "Molecular mechanisms of lipoprotein receptor signalling", CMLS CELLULAR AND MOLECULAR LIFE SCIENCES, BIRKHÄUSER-VERLAG, BA, vol. 62, no. 19-20, 1 October 2005 (2005-10-01), pages 2325-2338, XP019200847, ISSN: 1420-9071, DOI: 10.1007/S00018-005-5231-Z page 2319, column 1, paragraph 3 ----- -/-	1-23
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.									
X	US 6 323 177 B1 (CURRAN THOMAS [US] ET AL) 27 November 2001 (2001-11-27) column 6, paragraph 1 and 6; claims 1-17	1-23									
A	----- MAY P ET AL: "Molecular mechanisms of lipoprotein receptor signalling", CMLS CELLULAR AND MOLECULAR LIFE SCIENCES, BIRKHÄUSER-VERLAG, BA, vol. 62, no. 19-20, 1 October 2005 (2005-10-01), pages 2325-2338, XP019200847, ISSN: 1420-9071, DOI: 10.1007/S00018-005-5231-Z page 2319, column 1, paragraph 3 ----- -/-	1-23									
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.											
* Special categories of cited documents : <table border="0"> <tr> <td> "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 </td> <td> "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 </td> </tr> </table>			"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							
"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		Date of mailing of the international search report									
20 December 2016		10/01/2017									
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Klee, Barbara									

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2016/053351

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	STRICKLAND D K ET AL: "Diverse roles for the LDL receptor family", TRENDS IN ENDOCRINOLOGY AND METABOLISM, ELSEVIER SCIENCE PUBLISHING, NEW YORK, NY, US, vol. 13, no. 2, 1 March 2002 (2002-03-01), pages 66-74, XP002376834, ISSN: 1043-2760, DOI: 10.1016/S1043-2760(01)00526-4 abstract page 71, column 2, paragraph 2 - page 72, column 1, paragraph 1; table 2 -----	1-23
A	KNUESEL ET AL: "Reelin-mediated signaling in neuropsychiatric and neurodegenerative diseases", PROGRESS IN NEUROBIOLOGY, ELSEVIER, AMSTERDAM, NL, vol. 91, no. 4, 1 August 2010 (2010-08-01), pages 257-274, XP027119040, ISSN: 0301-0082 [retrieved on 2010-04-22] page 258, column 2, paragraph 2 -----	1-23
A	WALTMANN MEAGHAN D ET AL: "Apolipoprotein E receptor-2 deficiency enhances macrophage susceptibility to lipid accumulation and cell death to augment atherosclerotic plaque progression and necrosis", BIOCHIMICA ET BIOPHYSICA ACTA. MOLECULAR BASIS OF DISEASE, AMSTERDAM, NL, vol. 1842, no. 9, 16 May 2014 (2014-05-16), pages 1395-1405, XP029014369, ISSN: 0925-4439, DOI: 10.1016/J.BBADIS.2014.05.009 page 1396, column 1, paragraph 3 page 1404, column 1, paragraph 3 - column 2, paragraph 1 -----	1-23
A	WO 2014/197752 A1 (REGENERON PHARMA [US]; SANOFI SA [FR]) 11 December 2014 (2014-12-11) claims 1-39 -----	1-23

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2016/053351

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6323177	B1	27-11-2001	NONE
WO 2014197752	A1	11-12-2014	
		AU 2014274844 A1	21-01-2016
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		KR 20160024906 A	07-03-2016
		US 2016115246 A1	28-04-2016
		WO 2014197752 A1	11-12-2014