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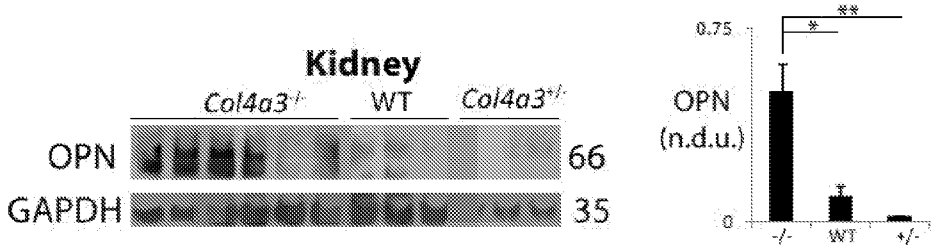
(54) Title: METHODS OF TREATING ALPORT SYNDROME

FIGURE 1A



(57) Abstract: Methods of using an osteopontin inhibitor in treating a type IV collagenopathy such as Alport Syndrome in a subject in need thereof are disclosed. The methods comprise administering an osteopontin inhibitor in an amount effective to improve cardiac function, kidney function, hearing, and/or vision in the subject.

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METHODS OF TREATING ALPORT SYNDROME

CROSS-REFERENCE TO RELATED APPLICATION AND INCORPORATION BY REFERENCE

[0001] This application claims the benefit of priority under 35 U.S.C. §119(e) of U.S. Provisional Patent Application No. 62/371,064, filed August 4, 2016, which is incorporated herein by reference.

[0002] This application contains, as a separate part of the disclosure, a sequence listing in computer-readable form (Filename: 50574A_Seqlisting.txt; Size: 4,166 bytes; Created: August 4, 2017 txt; Size: kilobytes; Created: August 4, 2017), which is incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0003] The present disclosure relates to methods of treating type IV collagenopathies such as Alport Syndrome.

BACKGROUND

[0004] Collagen type IV (Col4) is an essential component of basement membranes, which separate the epithelium from connective tissue. Alport Syndrome is an inherited disease caused by mutations in three collagen type IV genes, *COL4A3*, *COL4A4*, and *COL4A5*. The disease is characterized by progressive renal failure, hypertension, proteinuria, greatly increased risk of cardiovascular disease, and loss of hearing and vision. Current therapy for Alport Syndrome is aimed at ameliorating the symptoms and includes angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARB). Such treatments only slow the loss of kidney function or manage blood pressure and do not treat or prevent the other effects of the disease.

[0005] There remains a need for therapeutic options for Alport Syndrome and other Col4-related diseases to improve cardiac and kidney function, as well as hearing and vision.

SUMMARY

[0006] The present disclosure is directed to methods of treating a Col4-related disease such as Alport Syndrome in a patient in need thereof comprising administering a therapeutically effective amount of an osteopontin inhibitor to the subject. In one aspect, the present disclosure provides a method of improving cardiac systolic and diastolic function in a subject in need

thereof comprising administering an osteopontin inhibitor in an amount effective to lower blood pressure, increase the mean corpuscular hemoglobin concentration (MCHC) in the blood, decrease myocardial fibrosis, increase stroke volume, increase cardiac output, decrease isovolumetric relaxation time, decrease interventricular wall thickness, decrease myocyte hypertrophy, or a combination of any of the foregoing, in the subject. In another aspect, the present disclosure provides a method of improving kidney function in a subject in need thereof comprising administering an osteopontin inhibitor in an amount effective to decrease the urine albumin/creatinine (ALB/CRE) ratio, decrease the thickness of a glomerulus basement membrane, decrease fibrotic cell proliferation and/or fibrosis, decrease lipid accumulation in a renal tubule, decrease one or more of plasma kidney injury molecule-1 (KIM-1), plasma blood urea nitrogen (BUN), plasma creatinine (CRE), renal Dynamin3 (Dnm3), and plasma Galectin-3 (Gal-3), or a combination of any of the foregoing, in the subject. In one aspect, the present disclosure provides a method of treating hearing loss in a subject in need thereof, comprising administering an OPN inhibitor in an amount effective to improve or maintain hearing and/or decrease the thickness of the capillary basement membrane in the cochlea, in the subject. In another aspect, the disclosure provides a method of treating g loss of vision in a subject in need thereof, comprising administering an OPN inhibitor in an amount effective to improve or maintain vision, treat or prevent a lenticonus, increase the anterior chamber depth and/or the anterior chamber angle in the eye, decrease the thickness of the capillary basement membrane in the retina, or a combination of any of the foregoing, in the subject.

[0007] In one aspect, the OPN inhibitor is administered in an amount effective to alter gene expression, for example, to (a) decrease expression of one or more genes selected from *Snora75, LOC100302567, Dnm3*, *Mir24-1|Mir3074-1*, *B930095G15Rik*, *4933409K07Rik|Gm3893*, *4933409K07Rik|Gm3893*, *9530091C08Rik*, and *Gm20038* and/or (b) increase expression of one or more genes selected from *Syne1*, *Ogdhl*, *Myh11*, *Scd4*, *Aqp7*, *Slc17a7*, *Rps13*, *Hbb-b1|Hbb-b2|Beta-s*, *Il15*, *Cnn1*, and *Alas2|Apex2*.

[0008] In any of the methods of the disclosure, the osteopontin inhibitor is selected from an antibody, an aptamer, a small interfering RNA, an antisense oligonucleotide, a regulatory nucleic acid, a small molecule inhibitor, and combinations thereof. In one aspect, the osteopontin inhibitor is administered systemically, e.g., intravenously. In another aspect, the osteopontin inhibitor is administered locally, e.g., to the eye and/or ear.

[0009] In one aspect of any of the methods of the present disclosure, the subject has Alport Syndrome.

[0010] In various aspects, the disclosure provides:

[0011] 1. A method of treating a Col4-related disease in a patient in need thereof comprising administering a therapeutically effective amount of an osteopontin inhibitor to the subject.

[0012] 2. The method of paragraph 1, comprising administering the osteopontin inhibitor in an amount effective to decrease the blood pressure of the subject.

[0013] 3. The method of paragraph 1 or 2, comprising administering the osteopontin inhibitor in an amount effective to increase the mean corpuscular hemoglobin concentration (MCHC) in the blood of the subject.

[0014] 4. The method of any one of paragraphs 1-3, comprising administering the osteopontin inhibitor in an amount effective to improve cardiac systolic/or and diastolic function in the heart of the subject.

[0015] 5. The method of any one of paragraphs 1-4, comprising administering the osteopontin inhibitor in an amount effective to decrease a marker of renal dysfunction in the subject, optionally a marker selected from plasma kidney injury molecule-1 (KIM-1), plasma blood urea nitrogen (BUN), plasma creatinine (CRE), renal Dynamin3 (Dnm3), plasma Galectin-3 (Gal-3), and a combination of any of the foregoing.

[0016] 6. The method of any one of paragraphs 1-5, comprising administering the osteopontin inhibitor in an amount effective to decrease the urine albumin/creatinine ratio of the subject.

[0017] 7. The method of any one of paragraphs 1-6, comprising administering the osteopontin inhibitor in an amount effective to decrease the thickness of the basement membrane in the kidney, retina, and/or cochlea of the subject.

[0018] 8. The method of any one of paragraphs 1-7, comprising administering the osteopontin inhibitor in an amount effective to treat or prevent hearing loss in the subject.

[0019] 9. The method of any one of paragraphs 1-8, comprising administering the osteopontin inhibitor in an amount effective to treat or prevent loss of vision.

[0020] 10. The method of any one of paragraphs 1-9, comprising administering the osteopontin inhibitor in an amount effective to increase the anterior chamber depth and/or the anterior chamber angle in the eye of the subject.

[0021] 11. A method of improving cardiac function in a subject in need thereof comprising administering an osteopontin inhibitor in an amount effective to lower blood pressure, increase the mean corpuscular hemoglobin concentration (MCHC) in the blood, decrease myocardial fibrosis, increase stroke volume, increase cardiac output, decrease isovolumetric relaxation time, decrease interventricular wall thickness, decrease myocyte hypertrophy, or a combination of any of the foregoing, in the subject.

[0022] 12. A method of improving kidney function in a subject in need thereof comprising administering an osteopontin inhibitor in an amount effective to decrease the urine albumin/creatinine (ALB/CRE) ratio, decrease the thickness of a glomerulus basement membrane, decrease fibrotic cell proliferation and/or fibrosis, decrease renal lipid accumulation, decrease one or more of plasma kidney injury molecule-1 (KIM-1), plasma blood urea nitrogen (BUN), plasma creatinine (CRE), renal Dynamin3 (Dnm3), and plasma Galectin-3 (Gal-3), or a combination of any of the foregoing, in the subject.

[0023] 13. A method of treating hearing loss in a subject in need thereof, comprising administering an osteopontin inhibitor in an amount effective to improve or maintain hearing and/or decrease the thickness of the capillary basement membrane in the cochlea, in the subject.

[0024] 14. A method of treating loss of vision in a subject in need thereof, comprising administering an osteopontin inhibitor in an amount effective to improve or maintain vision, treat or prevent a lenticonus, increase the anterior chamber depth in the eye, increase the anterior chamber angle in the eye, decrease the thickness of the capillary basement membrane in the retina, or a combination of any of the foregoing, in the subject.

[0025] 15. The method of any one of paragraphs 1-14, wherein the subject has Alport Syndrome.

[0026] 16. The method of any one of paragraphs 1-15, wherein the osteopontin inhibitor is selected from an antibody, an aptamer, a small interfering RNA, an antisense oligonucleotide, a regulatory nucleic acid, a small molecule inhibitor, and combinations thereof.

[0027] 17. The method of any one of paragraphs 1-16, comprising administering the osteopontin inhibitor subcutaneously or intravenously.

[0028] 18. The method of any one of paragraphs 1-17, comprising administering the osteopontin inhibitor locally to the eye or ear of the subject.

[0029] 19. The method of paragraph 18, comprising administering the osteopontin inhibitor intravitreally and/or intratympanically.

[0030] 20. The method of any one of paragraphs 1-19, comprising administering the osteopontin inhibitor at least once a day.

[0031] 21. The method of any one of paragraphs 1-20, comprising administering the osteopontin inhibitor in a dosage of between about 0.1 mg and about 50 mg.

[0032] 22. The method of any one of paragraphs 1-21, comprising administering the osteopontin inhibitor in an amount effective to: (a) decrease expression of the Snora75 and/or LOC100302567 gene(s) in the heart; (b) increase expression of one or more genes in the heart selected from Syne1, Ogdhl, Myh11, Scd4, Aqp7, Slc17a7, Rps13, Hbb-b1|Hbb-b2|Beta-s, Il15, Cnn1, and Alas2|Apex2; (c) decrease expression of one or more genes in the kidney selected from Dnm3, Mir24-1|Mir3074-1, B930095G15Rik, 4933409K07Rik|Gm3893, 4933409K07Rik|Gm3893, 9530091C08Rik, and Gm20038; and/or (d) normalize the splicing events relative to wild-type in one or more genes selected from Dhx36, Zfp106, Dst, Fubp1, Elf2, Btbd1, Lrrc2, Abcd3, Ctsa, Fyco1, Abhd6, Serpinh1, Fgd4, and Ttn.

[0033] The foregoing summary is not intended to define every aspect of the invention, and other features and advantages of the present disclosure will become apparent from the following detailed description, including the drawings. The present disclosure is intended to be related as a unified document, and it should be understood that all combinations of features described herein are contemplated, even if the combination of features are not found together in the same sentence, paragraph, or section of this disclosure. In addition, the disclosure includes, as an additional aspect, all embodiments of the invention narrower in scope in any way than the

variations specifically mentioned above. With respect to aspects of the disclosure described or claimed with "a" or "an," it should be understood that these terms mean "one or more" unless context unambiguously requires a more restricted meaning. With respect to elements described as one or more within a set, it should be understood that all combinations within the set are contemplated. If aspects of the disclosure are described as "comprising" a feature, embodiments also are contemplated "consisting of" or "consisting essentially of" the feature. Additional features and variations of the disclosure will be apparent to those skilled in the art from the entirety of this application, and all such features are intended as aspects of the disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0034] **Figures 1A** and **1B** show OPN expression is increased in Alport mice. Western blots and corresponding densitometry shows elevated OPN expressions in *Col4a3*$^{-/-}$ versus WT or *Col4a3*$^{+/-}$ mouse kidneys (Figure 1A) and plasma (Figure 1B). N=3-6 mice per group. Quantification is based on three independent experiments. Data are mean±SEM. *P < 0.05; **P < 0.01. n.d.u = normalized densitometry units.

[0035] **Figures 2A** to **2G** show OPN deficiency increases life span and attenuates Alport pathology in *Col4a3*$^{-/-}$ mice. OPN deficiency in Alport mice causes an increase in lifespan by Kaplan Meier survival curve (Figure 2A), a reduction in body weight loss (Figure 2B), a reduction of albuminuria as measured by albumin and creatinine (ALB/CRE) ELISA (Figure 2C), a decrease in plasma Blood Urea Nitrogen (BUN) (Figure 2D) and creatinine (CRE) (Figure 2E) levels, an increase in blood Mean Corpuscular Hemoglobin Concentration (MCHC) (Figure 2F), and reductions in systolic blood pressure (SBP) and diastolic blood pressure (DBP) as recorded by tail-cuff blood pressure system (Figure 2G), all relative to Alport mice. Data were collected from animals at 8 to 9 weeks of age.

[0036] **Figures 3A** and **3B** show OPN deficiency improved cardiac structure and function in Alport mice. Echocardiography studies show significant improvement of systolic (stroke volume; Fig. 3A) and diastolic (isovolumetric relaxation time; Figure 3B) function in Alport hearts.

[0037] **Figures 4A** through **4C** show OPN deficiency prevents cardiac hypertrophy in *Col4a3*$^{-/-}$ mice. OPN deficiency rescues cardiac hypertrophy shown by decreased thickness of interventricular septum (IVS) (Figure 4A) and myocyte cross-sectional area (Figure 4B). H&E

images show rescue of IVS thickness (Figure 4C). Values are mean±SEM. P values: **p<0.01, ***p<0.001 using 1-way ANOVA with Tukey's post hoc test.

[0038] **Figures 5A** through **5C** show cardiac fibrosis is rescued by OPN deficiency in *Col4a3*$^{-/-}$ mice. OPN deficiency reduces cardiac fibrosis reflected by decreased interstitial activated fibroblasts shown by Electron Microscopy (Figure 5A), dividing/EdU-positive MLC2-negative interstitial cells (Figure 5B), and plasma levels of Galectin-3 (Figure 5C) in *Col4a3*$^{-/-}$ mice. Gal-3, Galectin-3. C, Collagen fibers, RER, Rough Endoplasmic Reticulum. Values are mean±SEM. P values: *p<0.05, **p<0.01, ***p<0.001 using one way ANOVA with Tukey's post hoc test.

[0039] **Figure 6A** to **6F** show OPN deficiency reduces fibrotic cell proliferation and prevents severe renal pathology in mouse Alport kidney. (Figure 6A) Fibrotic cell proliferation was visualized by EdU incorporation after 30 days of EdU injections and is elevated in Alport kidneys, but prevented by OPN deficiency. (Figure 6B) Kim-1 expression is elevated in renal tubules of Alport mice and rescued by OPN deficiency as shown by immunofluorescent staining and corresponding quantification. (Figure 6C) Dnm3 expression is elevated in Alport kidneys but reduced by OPN deficiency as shown by immunostaining. (Figure 6D) Oil red staining and corresponding quantification show extensive lipid accumulation in tubules of *Col4a3*$^{-/-}$ mice, that is reduced with OPN deficiency. (Figure 6E) Western blot and corresponding densitometry shows Dnm3 expression is significantly increased in Alport kidneys and normalized with OPN deficiency. N=3-4 mice per group. (Figure 6F) Representative electron microscopy images show podocytes effacements and disruption of basement membrane in *Col4a3*$^{-/-}$ mice (center) as compared to normal or restored morphology in wild-type (left) or *Col4a3*$^{-/-}$*OPN*$^{-/-}$ mice (right). Insets are shown at a higher magnification in the bottom row. In the zoomed-in images, scale bar = 20 μm. Data are mean±SEM. *P < 0.05; **P < 0.01***P < 0.001.

[0040] **Figures 7A** and **7B** show OPN deficiency improves high frequency hearing threshold in *Col4a3*$^{-/-}$ mice and reduces pathology of inner ear. (Figure 7A) Auditory brainstem response (ABR) thresholds across stimulus frequencies (4 to 16 kHz) and clicks are shown. OPN deficiency caused an improvement of hearing ability of Alport mice at the 16 KHZ stimuli. N=6-12 mice per group. (Figure 7B) Representative electron microscopy (EM) images show thickened basement membrane in Alport cochleas - a pathology that is reduced with OPN deficiency. Insets are shown at a higher magnification on the bottom.

[0041] **Figures 8A** and **8B** show OPN deficiency attenuates tendency of lenticonus in *Col4a3*$^{-/-}$ mice. (Figure 8A) Optical coherence tomography (OCT) studies show significant improvement in anterior chamber distance (ACD) and anterior chamber angle (ACA) dimension in Alport OPN deficient relative to Alport mice. N=2 mice per group. (Figure 8B) EM shows disrupted basement membrane of cornea in *Col4a3*$^{-/-}$ mice – a pathology that is reduced with OPN deficiency. Insets are shown at a higher magnification on the bottom.

DETAILED DESCRIPTION

[0042] The present disclosure provides methods of treating Alport Syndrome in a patient in need thereof comprising administering a therapeutically effective amount of an osteopontin inhibitor. Osteopontin (OPN), a secreted phosphoprotein, has not previously been studied in Alport Syndrome. Inhibition of OPN in Alport Syndrome subjects improves lifespan, decreases proteinuria and hypertension, improves renal and cochlear histology, and improves cardiac function, hearing ability, and eye abnormalities.

[0043] The following definitions may be useful in aiding the skilled practitioner in understanding the disclosure. Unless otherwise defined herein, scientific and technical terms used in the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art.

[0044] The term "osteopontin inhibitor" or "OPN inhibitor" refers to a small molecule and/or biologic inhibitor of the osteopontin (secreted phosphoprotein 1; SPP1) gene or protein. OPN inhibitors include antigen-binding peptides (e.g., antibodies), aptamers, and nucleic acids (e.g., microRNA, small interfering RNA, and antisense nucleotides). Examples of OPN inhibitors include, but are not limited to, the inhibitors described in U.S. Patent Application Nos. 20150366851, 20150004157, 20120264942, 20080262092, and International Patent Publication WO2009102438, incorporated herein by reference.

[0045] The terms "type IV collagen-related disease," "type IV collagenopathy" or "Col4-related disease" refers to a disease or disorder characterized by a defect in a Col4 gene or protein (e.g., COL4A1, COL4A2, COL4A3, COL4A4, COL4A5, and/or COL4A6). Examples include Alport Syndrome, Goodpasture's Syndrome, and Col4-related vascular and cardiovascular diseases, such as hypertension and heart failure with preserved ejection fraction, respectively.

[0046] The terms "therapeutically effective amount" and "effective amount" depend on the condition of a subject and dosing regimen. The terms refer to an amount of an OPN inhibitor effective to achieve a desired biological, e.g., clinical effect. A therapeutically effective amount varies with the nature of the disease being treated, the length of time that activity is desired, and the age and the condition of the subject. For example, a therapeutically effective amount of an OPN inhibitor according to the disclosure is an amount effective to decrease or improve one or more of the following symptoms: proteinuria, urinary albumin/creatinine levels, renal dysfunction/insufficiency, fibrotic cell proliferation, anemia, cardiac dysfunction, hearing deficits, eye/vision abnormalities (e.g., lenticonus), and hypertension. In one aspect, a therapeutically effective amount of an OPN inhibitor is an amount effective to increase the longevity of the subject relative to the expected lifespan in view of disease progression.

[0047] As used herein, the terms "patient" and "subject" may be used interchangeably and mean animals, such as dogs, cats, cows, horses, and sheep (*i.e.*, non-human animals) and humans.

[0048] As used herein, the term "pharmaceutically acceptable" means that the referenced substance, such as a compound of the present disclosure, or a composition containing the compound, or a particular excipient, are safe and suitable for administration to a patient. The term "pharmaceutically acceptable carrier" refers to a medium that does not interfere with the effectiveness of the biological activity of the active ingredient(s) and is not toxic to the host to which it is administered.

[0049] As used herein, the term "excipient" means any pharmaceutically acceptable additive, carrier, diluent, adjuvant, or other ingredient, other than the OPN inhibitor

[0050] As used herein the terms "treating", "treat" or "treatment" and the like include preventative (*e.g.,* prophylactic) and palliative treatment.

[0051] In jurisdictions that forbid the patenting of methods that are practiced on the human body, the meaning of "administering" of a composition to a human subject shall be restricted to prescribing a controlled substance that a human subject will self-administer by any technique (*e.g.*, orally, inhalation, topical application, injection, insertion, etc.). The broadest reasonable interpretation that is consistent with laws or regulations defining patentable subject matter is intended. In jurisdictions that do not forbid the patenting of methods that are practiced on the

human body, the "administering" of compositions includes both methods practiced on the human body and also the foregoing activities.

[0052] In one aspect, the present disclosure provides methods of treating a Col4-related disease such as Alport Syndrome in a subject in need thereof comprising administering a therapeutically effective amount of an OPN inhibitor to the subject. In one aspect, the OPN inhibitor is administered in an amount effective to treat a cardiac symptom of the disease in the subject. In another aspect, the present disclosure provides a method of improving cardiac function in a subject in need thereof, optionally a subject having a Col4-related disease such as Alport Syndrome, comprising administering an OPN inhibitor in an amount effective to lower blood pressure, increase the mean corpuscular hemoglobin concentration (MCHC) in the blood, decrease myocardial fibrosis, increase stroke volume, increase cardiac output, decrease isovolumetric relaxation time, decrease interventricular wall thickness, decrease myocyte hypertrophy, or a combination of any of the foregoing, in the subject. In one aspect, the OPN inhibitor is administered in an amount effective to decrease the blood pressure of the subject, e.g., the systolic blood pressure and/or the diastolic blood pressure, for example, by at least about 1 mm Hg, at least about 2 mm Hg, at least about 3 mm Hg, at least about 4 mm Hg, at least about 5 mm Hg, at least bout 6 mm Hg, at least about 7 mm Hg, at least about 8 mm Hg, at least about 9 mm Hg, at least about 10 mm Hg, at least about 20 mm Hg, at least about 30 mm Hg, at least about 40 mm Hg, at least about 50 mm Hg, at least about 60 mm Hg, at least about 70 mm Hg, at least about 80 mm Hg, at least about 90 mm Hg, or at least about 100 mm Hg. In another aspect, the OPN inhibitor is administered in an amount effective to increase the MCHC in the blood of the subject, for example, by at least about 1 g/dL, at least about 2 g/dL, at least about 3 g/dL, at least about 4 g/dL, at least about 5 g/dL, at least about 6 g/dL, at least about 7 g/dL, at least about 8 g/dL, at least about 9 g/dL, at least about 10 g/dL, at least about 15 g/dL, at least about 20 g/dL, at least about 25 g/dL, or at least about 30 g/dL. In one aspect, the OPN inhibitor is administered in an amount effective to decrease myocardial fibrosis in the subject. In one aspect, the OPN inhibitor is administered in an amount effective to increase cardiac output, e.g., ejection fraction and/or endocardial stroke volume, in the subject for example, by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, or at least about 30%, compared to pre-treatment.

[0053] In one aspect, the disclosure provides a method of treating a Col4-related disease such as Alport Syndrome in a subject in need thereof comprising administering a therapeutically effective amount of an OPN inhibitor to the subject to treat a renal symptom of the disease. In another aspect, the disclosure provides a method of improving kidney function in a subject in need thereof, optionally a subject having a Col4-related disease such as Alport Syndrome, comprises administering an OPN inhibitor in an amount effective to decrease the urine albumin/creatinine (ALB/CRE) ratio, decrease the thickness of a glomerulus basement membrane, decrease fibrotic cell proliferation and/or fibrosis, decrease lipid accumulation in a renal tubule, decrease one or more of plasma kidney injury molecule-1 (KIM-1), plasma blood urea nitrogen (BUN), plasma creatinine (CRE), renal Dynamin3 (Dnm3), and plasma Galectin-3 (Gal-3), or a combination of any of the foregoing in the subject. In one aspect, the OPN inhibitor is administered in an amount effective to decrease the urine ALB/CRE, for example, by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, or at least about 300%, in the subject, compared to pre-treatment. In another aspect, the OPN inhibitor is administered in an amount effective to decrease the thickness of a glomerulus basement membrane in the subject. In another aspect, the OPN inhibitor is administered in an amount effective to decrease fibrotic cell proliferation and/or fibrosis in the subject. In one aspect, the OPN inhibitor is administered in an amount effective to decrease lipid accumulation in a renal tubule in the subject. In aspect, the OPN inhibitor is administered in an amount effective to decrease a marker of renal dysfunction, for example, one or more of plasma KIM-1, plasma BUN, plasma CRE, renal Dnm3, and plasma Gal-3, optionally by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 125%, at least about 150%, at least about 175%, at least about 200%, at least about 250%, or at least about 300%.

[0054] In one aspect, the disclosure provides a method of treating Alport Syndrome in a subject in need thereof comprising administering a therapeutically effective amount of an OPN inhibitor to the subject to treat hearing loss. In another aspect, the disclosure provides a method of treating hearing loss in a subject in need thereof, optionally a subject having a Col4-related

disease such as Alport Syndrome, comprising administering an OPN inhibitor in an amount effective to improve or maintain hearing and/or decrease the thickness of the capillary basement membrane in a cochlea of the subject. In one aspect, the OPN inhibitor is administered in an amount effective to improve or maintain hearing, including, for example, high frequency (e.g., greater than 8 kHz) hearing, in the subject. In another aspect, the OPN inhibitor is administered in an amount effective the decrease the thickness of a basement membrane in the cochlea, e.g., in the stria vascularis, in the subject.

[0055] In one aspect, the disclosure provides a method of treating Alport Syndrome in a subject in need thereof comprising administering a therapeutically effective amount of an OPN inhibitor to the subject to treat loss of vision. In another aspect, the disclosure provides a method of treating loss of vision in a subject in need thereof, optionally a subject having a Col4-related disease such as Alport Syndrome, comprising administering an OPN inhibitor in an amount effective to improve or maintain vision, treat or prevent a lenticonus, increase the anterior chamber depth in the eye, increase the anterior chamber angle in the eye, decrease the thickness of the capillary basement membrane in the retina, or a combination of any of the foregoing, in the subject. In one aspect, the OPN inhibitor is administered in an amount effective to improve or maintain vision. In another aspect, the OPN inhibitor is administered in an amount effective to treat or prevent an eye deformity, for example, a lenticonus, e.g., a lenticonus anterior, increased anterior chamber depth and/or increased anterior chamber angle in the eye, and/or thickening of the capillaries in the retinal basement membrane. In another aspect, the OPN is administered in an amount effective to increase the anterior chamber depth in an eye of the subject, optionally by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, or at least about 30%. In another aspect, the OPN inhibitor is administered in an amount effective to increase the anterior chamber angle in an eye of the subject, optionally by at least about 1 degree, at least about 2 degrees, at least about 3 degrees, at least about 4 degrees, at least about 5 degrees, at least about 6 degrees, at least about 7 degrees, at least about 8 degrees, at least about 9 degrees, at least about 10 degrees, at least about 11 degrees, at least about 12 degrees, at least about 13 degrees, at least about 14 degrees, or at least about 15 degrees. In another aspect, the OPN inhibitor is administered in an amount effective to decrease the retinal basement membrane in the subject.

[0056] One of ordinary skill will appreciate that treating a disease or disorder such as Alport Syndrome does not require complete eradication of the disease or disorder. Any beneficial physiologic response is contemplated, such as improvement of kidney function, prevention or delay of kidney failure, improvement of cardiac function or prevention of cardiovascular disease, decrease in proteinuria and/or hematuria, reduction in blood pressure, improvement in or maintenance of hearing and/or vision, and any combination of the foregoing. Treating a disease or disorder such as Alport Syndrome also encompasses prevention of one or symptoms typically associated with the disease, which may entail a complete prevention of the symptom or a delay in onset or worsening of the symptom.

[0057] In some aspects, therapeutic efficacy can be measured using laboratory or clinical tests known in the art to evaluate symptoms associated with a type IV collagenopathy. Kidney function can be evaluated, for example, using imaging (e.g., ultrasound and CT scans), biopsies, and detection methods (e.g., ELISA) for quantitating biomarkers in patient samples, including blood and urine samples. Cardiac function and hypertension can be evaluated, for example, using electrocardiography, echocardiography, stress tests, positron emission tomography tests, imaging (e.g., magnetic resonance imaging and CT scans), and blood pressure cuffs. Hearing can be evaluated, for example, using audiometry exams, otoacoustic emissions tests, auditory brainstem response tests, and encephalograms. Eye abnormalities and vision can be evaluated, for example, using optical coherence tomography studies

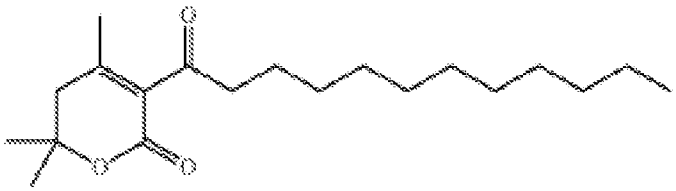
[0058] A particular administration regimen for a given subject will depend, in part, upon the compound or composition, the amount administered, the route of administration, and the cause and extent of any side effects. The amount administered to a subject (e.g., a mammal, such as a human) in accordance with the disclosure should be sufficient to effect the desired response over a reasonable time frame. Dosage typically depends upon the route, timing, and frequency of administration.

[0059] Purely by way of illustration, the methods of the present disclosure comprise administering, e.g., from about 0.1 mg/kg to about 15 mg/kg or more of an OPN inhibitor based on the body weight of the subject, depending on the factors mentioned above. In some aspects, the dosage ranges from about 0.1 mg/kg to about 0.5 mg/kg, about 1 mg/kg to about 3 mg/kg, about 0.5 mg/kg to about 5 mg/kg, about 0.2 mg/kg to about 0.8 mg/kg, about 5 mg/kg to about

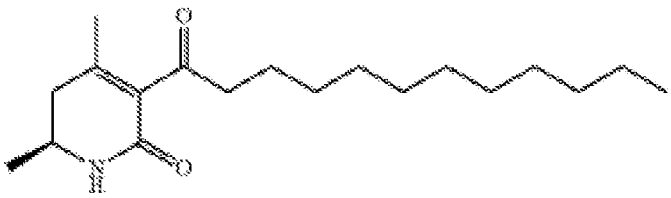
15 mg/kg, about 4 mg/kg to about 12 mg/kg, or about 0.1 mg/kg to about 2 mg/kg. For example, an OPN inhibitor may be administered to a human patient in an amount from between about 1 mg to about 50 mg, for example, about 1 mg, about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, or about 50 mg. The dosage is administered as needed, for example, one to three times daily, every other day, twice a week, weekly, every two weeks, monthly, or less frequently. The treatment period will depend on the particular condition and may last one day to several days, weeks, months, or years. The above dosages are exemplary of the average case, but there can be individual instances in which higher or lower dosages are merited, and such are within the scope of the present disclosure.

[0060] Suitable OPN inhibitors, and pharmaceutically acceptable compositions thereof, are known in the art. In various aspects, the OPN inhibitor is a small molecule and/or biologic inhibitor of OPN. OPN inhibitors include antigen-binding peptides (e.g., antibodies), aptamers, and nucleic acids (e.g., microRNA, small interfering RNA, and antisense nucleotides). Examples of OPN inhibitors include, but are not limited to, the inhibitors described in U.S. Patent Application Nos. 20150366851, 20150004157, 20120264942, 20080262092, International Patent Publication WO2009102438, Wang et al. *Journal of Immunology.* 2009;182(4):2485-91, Dai et al. *Cancer Immunol Immunother.* 2010;59(3):355-66, Zhao et al. *Am J Transl Res.* 2016;8(9):3645-3655, Xanthou et al. *Nature Medicine.* 2007,13(5):570-578, Mohamed et al. *PLOS One.* 2015; 10(4):e012331, Mason et al. *Mol Cancer Ther.* 2008;7(3):548-558 (*i.e.*, (-)-agelastatin A), Kiefer et al. *Diabetes.* 2010;59:935-946, Paliwal et al. *Aging.* 2012;4(8):553-566, Liaw et al. *Arteriosclerosis, Thrombosis, and Vascular Biology.* 1997;17:188-193, Kon et al. *J Cell Biochem.* 2002;84(2):420-432, and Jalvy et al. *Cardiovascular Research.* 2007;75:738-747, incorporated herein by reference. For example, the OPN inhibitor may be a dictyopyrone derivative or a dihydrodictyopyrone derivative represented by any of Chemical Formulas 1-16:

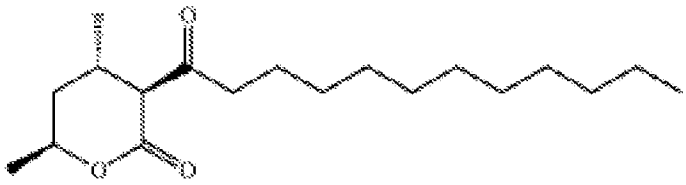
[Chemical Formula 1]



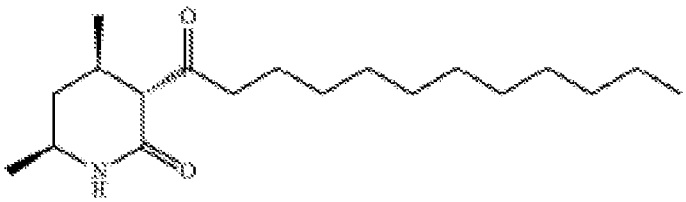
[Chemical Formula 2]



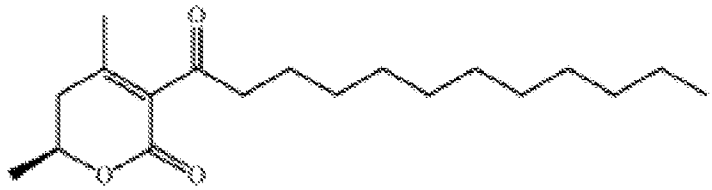
[Chemical Formula 3]



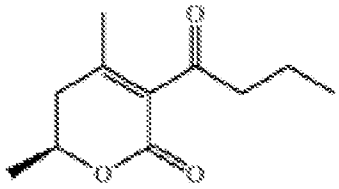
[Chemical Formula 4]



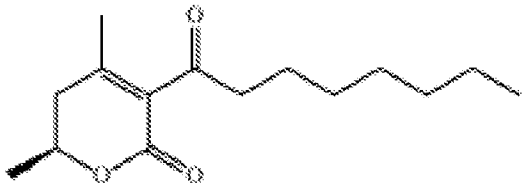
[Chemical Formula 5]



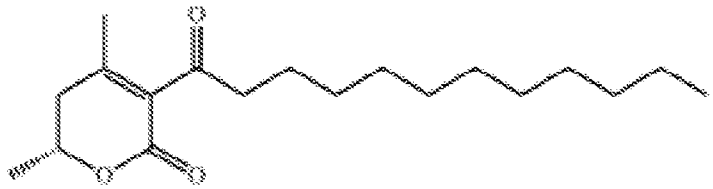
[Chemical Formula 6]



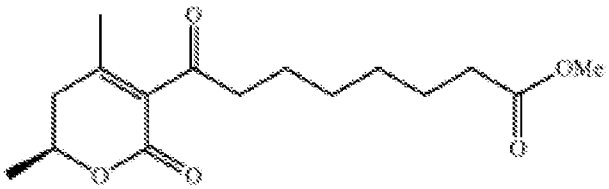
[Chemical Formula 7]



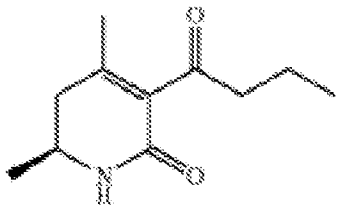
[Chemical Formula 8]



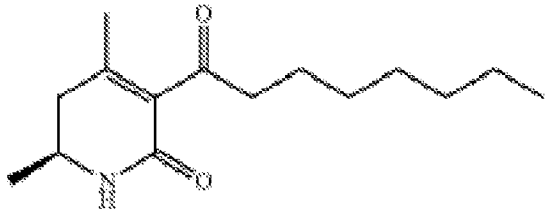
[Chemical Formula 9]



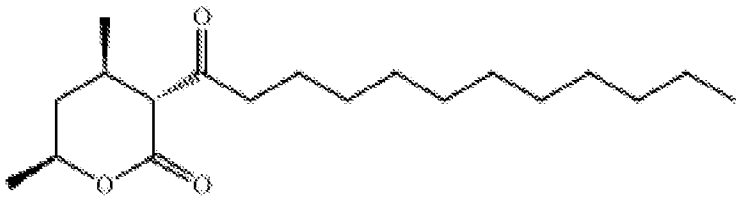
[Chemical Formula 10]



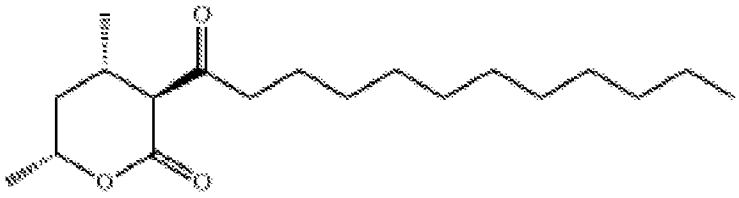
[Chemical Formula 11]



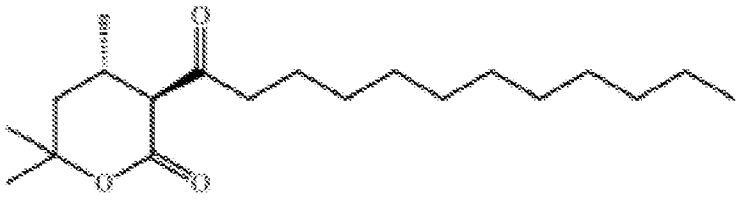
[Chemical Formula 12]



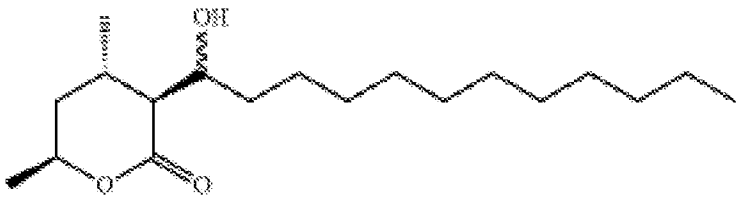
[Chemical Formula 13]



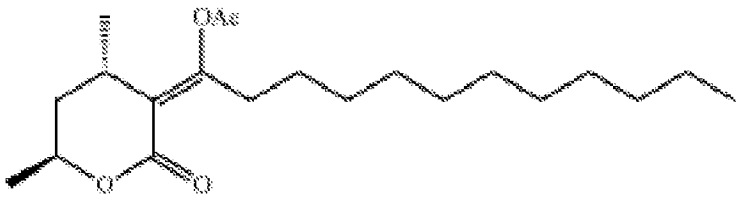
[Chemical Formula 14]



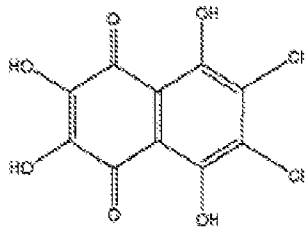
[Chemical Formula 15]



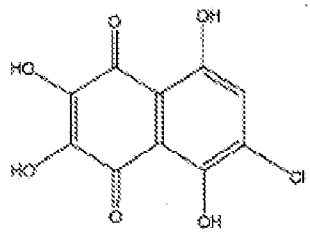
[Chemical Formula 16]



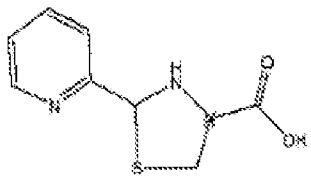
In still another example, the OPN inhibitor is a compound selected from compounds IPS-02001, IPS-02002, and IPS-02003:



(IPS-02001) :
6,7-Dichloro-2,3,5,8-tetrahydroxy-[1,4]naphthoquinone

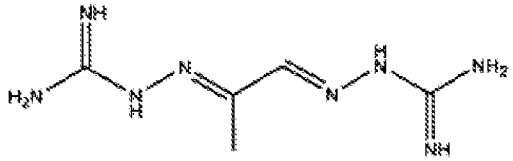


(IPS-02002) :
6-Chloro-2,3,5,8-tetrahydroxy-[1,4]naphthoquinon

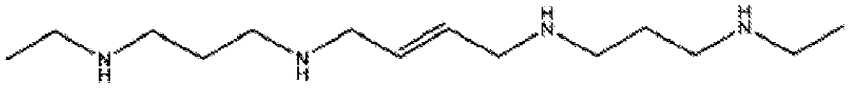


(IPS-02003) : 2-Pyridin-2-yl-thiazolidine-4-carboxylic acid

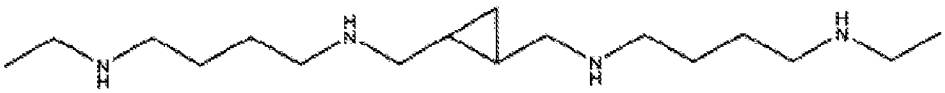
In another example, the OPN inhibitor is 1,1'[methylethanediylidene]dinitrilodiguanidine (MGBG), SL47, or SL93:



1,1'-[methylethanediylidenedinitrilo]diguanidine
Methylglyoxal bis(guanyl-hydrazone) or MGBG



N,N'-bis(3-ethylaminopropyl)-cis-but-2-ene-1,4-diamine
SL11047 or SL47



$N^1,N^{1'}$-(cyclopropane-1,2-diyl)bis(methylene))bis(N^4-ethylbutane-1,4-diamine)
SL11093 or SL93

In another example, the OPN inhibitor is polynucleotide aptamer that binds to and inhibits the function of osteopontin, such as an RNA aptamer that comprises a nucleotide sequence that is identical to any of SEQ ID NOS: 1-14 as shown in Table 4, or a nucleotide sequence that is at least 70% identical, e.g., at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to any of SEQ ID NOS: 1-14.

TABLE 4

Aptamer Name	Aptamer Sequence
OPN-R3	CGGCCACAGAAUGAAAAACCUCAUCGAUGUUGCAUAGUUG (SEQ ID NO: 1)
AP1	UCCCCAGCCCAUAGGAUCAAGCCAAACUCUCAUCCGCGAU (SEQ ID NO: 2)
AP2	UACAACUCCCGCUGGCCCCAACGCUCUACUCCGAGUAACG (SEQ ID NO: 3)
AP3	UACCCACCGGCCACGGGAACGAUCAGACGUCCCAUAAU (SEQ ID NO: 4)
AP4	UCAUUCGCCAAAUGGCGAACCAGCCGGUCGCAGCCAGGAU (SEQ ID NO: 5)
AP5	CUAACCCCGAGGACAUGUCACGCCGCGCAUAGAGAUUCUC (SEQ ID NO: 6)
AP6	AAAAUUGUGCGGUUUGCAGAUUAGAAGAGGUCCAUUUGUU (SEQ ID NO: 7)
AP7	CGGCUCUGAUAUGCUUCCUGGAAGCAGCGUUAUAGCCCAC (SEQ ID NO: 8)
AP8	AGACGUCAGAACCGGAAUAAACAGACGCUUAACUUUAGAA (SEQ ID NO: 9)
AP9	ACCCGCCGCAGAAAUUCCGCCCAUCCAGGACGCGGCGCAC (SEQ ID NO: 10)
AP10	AAUGCCCAUGCAGAAGCCAUCAAUCACAACUCGACCCCAA (SEQ ID NO: 11)
AP11	GCUUCAUGAAAGGCACGAAACCACCGCGCAUGGGA (SEQ ID NO: 12)
AP12	CGAGAAAUCGAAUUCCCGCGGCCGCCAUGGCGGCCGGGAG (SEQ ID NO: 13)
AP14	GGCCGCGGGAAUUCGAUUGGGGGAAUUCUAAUACGACUCA (SEQ ID NO: 14)

In another example, the OPN inhibitor is a siRNA comprising the sequence AAGAUGAUAGGUAUCUGAAAU (SEQ ID NO:15).

[0061] A composition comprising an OPN inhibitor may be in any suitable dosage form including, but not limited to, tablets, capsules, implants, depots, liquids, patches, lozenges, creams, gels, ointments, lotions, sprays, ear drops, and eye drops.

[0062] Compositions suitable for injection or instillation may comprise physiologically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions, or emulsions, and sterile powders for reconstitution into sterile injectable solutions or dispersions. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents, or vehicles include water, ethanol, polyols (propylene glycol, polyethylene glycol, glycerol, and the like), suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

[0063] The compositions may also contain adjuvants such as preserving, wetting, emulsifying, and dispersing agents. Microorganism contamination can be prevented by adding various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. The compositions may also include isotonic agents, for example, sugars, sodium chloride, and the like. Prolonged absorption of injectable pharmaceutical compositions can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0064] Suitable methods of administering a pharmaceutically acceptable composition comprising an OPN inhibitor are well-known in the art. Although more than one route can be used to administer a compound, a particular route can provide a more immediate and more effective reaction than another route. Depending on the circumstances, a compound or composition described herein is introduced into a surgical site, applied or instilled into a body cavity, absorbed through the skin or mucous membranes, inhaled, ingested and/or introduced into circulation. In one aspect, the compound or composition is administered orally. In another aspect, the compound or composition is injected intravenously and/or intraperitoneally. For example, in certain circumstances, it will be desirable to deliver the composition through injection, infusion, or deposition by intravenous, intraperitoneal, intracerebral (intra-parenchymal), intracerebroventricular, intracardiac, intraarterial, intraportal, intralesional, intramedullary, intrathecal, intraventricular, intranasal, subcutaneous, renal, otic, or ocular means; by controlled, delayed, sustained or otherwise modified release systems; or by implantation devices. In one aspect, a composition comprising an OPN inhibitor is administered locally to the eye and/or ear, e.g., ophthalmically, intraocularly, conjunctivally, intracorneally, intravitreally, retrobulbarly, auricularly, or intratympanically. Alternatively, an OPN inhibitor is administered via implantation of a matrix, membrane, sponge, or another appropriate material onto which the compound has been absorbed or encapsulated. Where an implantation device is used, the device is, in one aspect, implanted into or on the surface of any suitable tissue or organ, and delivery of an OPN inhibitor is, for example, via diffusion, timed-release bolus, or continuous administration. In one aspect, an OPN inhibitor comprises a targeting moiety specific for OPN, such as an antigen binding protein including, but not limited to, antibodies, antibody fragments, antibody derivatives, antibody analogs, and fusion proteins.

[0065] The present disclosure will be more readily understood by reference to the following example, which is provided by way of illustration and is not intended to be limiting.

Example

[0066] Materials and Methods

[0067] *Animals. Col4a3*$^{-/-}$ Alport mice on C57Bl6 or 129X1/SvJ background were purchased from Jackson Lab, crossed at least 10 times, and confirmed by genotyping (Transnetyx, TN).

[0068] *EdU Injections.* 5-ethynyl-2'-deoxyuridine (A10044, LifeTechnologies) was prepared at 1 mg/ml stock and filtered through 0.2 μm Nalgene syringe filter (190-2520, ThermoScientific). 100μl (100μg) EdU stock was administered daily to each mouse through intraperitoneal injections for up to 30 days to identify mitotic cells.

[0069] *Blood Pressure Recording.* Blood pressure measurements were carried out using CODA mouse tail-cuff system (Kent Scientific). Anesthesia was induced with 5% vaporized isoflurane at 0.8 L/min flow rate and maintained with 1% isoflurane during blood pressure recordings. Heart rate was monitored and body temperature was controlled within 37 ^{o}C to 39 ^{o}C. Two tail cuffs, occlusion and VPR, were equipped onto the tails. Occlusion tail cuff was inflated and deflated to control blood flow. VPR tail cuff was incorporated with specially designed differential pressure transducer that measured the systolic and diastolic blood pressure by determining the blood volume in the tail. Animals were trained three times to minimize stress-related blood pressure changes caused by the system prior to recording the final blood pressure values. Twenty systolic and diastolic blood pressure readings were recorded from each mouse and the fifteen most consistent readings were used for analysis.

[0070] *Hearing Test.* Auditory Brainstem Response (ABR) were performed on anesthetized mice at 8 to 9 weeks of age. ABR subdermal electrodes were placed on the vertex, both mastoids, and the left hind leg (ground). Using a commercial system (Intelligent Hearing Systems, Miami, FL), electroencephalograms (EEG) were amplified 100,000 times, band-pass filtered between 30 Hz and 1500 Hz and acquired using a sampling frequency of 10 Ksps in 102.4 ms long epochs. Acoustic stimuli using tone bursts at 4, 8, and 16 kHz were delivered with insert ear phones. For each frequency and epoch, eight, amplitude-modulated, tone bursts were presented to each ear at an average stimulation rate of 78.13 stimuli. The raw EEGs were averaged in blocks of 64 epochs to obtain the auditory evoked potential responses. Each epoch

lasted 102.4 ms and contained eight tone pips modulated by a 3 ms trapezoidal envelope (1 ms rise time, 1 ms plateau, and 1 ms falling time). ABR hearing threshold was defined as the minimum intensity required to produce an ABR response that was identifiable and consistent. Intensity level of stimuli were tested in 10 dB SPL intervals.

[0071] *Ocular Measurements.* Anterior-segment images were captured by optical coherence tomography (OCT) using a SD-OCT system (Bioptigen, NC). Central corneal thickness (CCC), anterior chamber depth (ACD) and anterior chamber angle (ACA) were determined using Image J software (ver. 2.0.0). Central corneal thickness was defined as the distance from the corneal epithelial surface to the corneal endothelium. Anterior chamber depth was defined as the distance from the corneal endothelium to the anterior surface of the lens capsule. Anterior chamber angle was defined as the angle formed between the anterior surface of the iris and the posterior surface of the cornea.

[0072] *Echocardiography.* Cardiac function was evaluated by a Vevo2100 echocardiography imaging system (Visual Sonics, Toronto, Canada) with a MS400 linear array transducer. Briefly, mice were anesthetized with 4% isoflurane at 0.8 L/min flow rate and maintained with 1% isoflurane. Following anesthesia, the mice were fixed in a supine position on a platform with an integrated temperature sensor, a heater, and ECG electrodes. The heart rate was monitored constantly and body temperature was maintained at 37 °C during measurements. Depilatory cream was used to remove fur from the region of procedure, and medical ultrasonographic acoustic gel was applied as a coupling fluid between the real-time microvisualization scan probe and the skin. Endocardial % Ejection Fraction (EF) was recorded in B-Mode and analyzed using a VEVO 770 software.

[0073] *Kidney Epithelial Cells Isolation and Culture.* Kidneys were harvested in DMEM+F12 media (10565-18, Gibco, Bartlesville, OK) supplemented with 1% Penicillin/Streptavidin (400-109, Gemini, Commack, NY). Medulla was removed, and the remaining kidney tissue was cut into pieces and digested in Collagenase type II (LS004176, Worthington, Columbus, OH) at 5mg/ml for 10min two times at 37 ^{o}C. Digested kidney pieces were then plated on a 2% gelatin (G2500-100g, Sigma, St. Louis, MO) coated 6-well plate. Tubule epithelial media (PCS400030, PCS400040, ATCC, Manassas, Virginia) was used.

[0074] *ELISA*. Albumin concentration was measured by ELISA (E90-134, Bethyl Laboratories, Montgomery, TX) using 1:500 to1:20,000 diluted urine samples collected from 8- to 9-week-old mice. Creatinine concentration was measured using 1:20 diluted urine samples by an ELISA assay (1012, Exocell, Philadelphia, PA). Albumin concentrations (mg/mL) were then normalized to creatinine (mg/mL) for analysis of albuminuria. Plasma Galectin-3 levels were measured by enzyme-linked immunosorbent assay (DY1197, R&D Systems, Minneapolis, Minnesota, USA) according to the manufacturer's instruction.

[0075] *Blood Pathology*. Blood samples were collected into Capiject tubes (T-MLHG, Terumo, Elkton, MD) by cardiac puncture and centrifuged to separate plasma from blood cells. Mean corpuscular hemoglobin concentration (MCHC), blood urea nitrogen (BUN) and creatinine levels were analyzed.

[0076] *Histochemistry*. De-paraffinized 4 μm kidney sections were processed for antigen retrieval using citrate buffer in a steamer for 45 minutes, permeabilized using 0.2% triton for 15 minutes, and blocked in 10% donkey serum in 1% TBST for 1 hour. EdU-555 was stained for 30 minutes as recommended by the manufacturer. Sections were then counter-stained for the glomeruli marker Synaptopodin (Synpo) (sc-21537, Santa Cruz Biotechnology, Santa Cruz, California) at 4 ^{o}C overnight, and staining was detected using anti-Goat Alexa Fluor-488 at RT for 30 minutes. Dnm3 was stained overnight and detected using biotinylated anti-Rabbit (BA-1000, Vector Laboratories, Burlingame, CA) at RT for 30 minutes followed by DAB peroxidase (HRP) amplification (SK-4100, Vector Laboratories, Burlingame, CA).

[0077] For OPN or KIM-1 staining, 10 μm frozen kidney sections were air dried, rinsed with PBS, permeabilized with 0.2% triton for 3 minutes and blocked with 10% donkey serum for 30 min at RT. OPN (ab3458, Abcam, Cambridge, MA) or Kim-1 (AF3689, R&D, Minneapolis, MN) antibodies were used at 4 ^{o}C overnight and detected using anti-Goat or anti-Rat Alexa Fluo-488 at RT for 30 minutes. For Oil red staining, frozen sections were processed as described above and incubated with 60% isopropanol for 5 min followed by oil red solution for 45 min. For immunostainings, primary and secondary antibody incubations were done in 10% donkey Serum in 1% TBST. Dnm3 and oil red staining images were captured on a Zeiss microscope using a 32x objective. OPN, Kim-1 and EdU fluorescent images were captured on a Zeiss LSM710 confocal

microscope using z-scanning at 40x magnification. Three to five kidneys/mice per group were used and six to ten images per kidney were acquired and quantified.

[0078] *Electron Microscopy*. Kidneys, cochlea and eyes were fixed for 24 hours (hrs) in 10% formalin, followed by 24 hrs in 2% glutaraldehyde, rinsed in wash buffer three times, and then postfixed in 2% osmium tetroxide in 0.1 M phosphate buffer overnight. After buffer rinses, they were dehydrated through a series of graded ethanols and embedded using Embed/Araldite (Electron Microscopy Sciences, Hatfield, PA) overnight in a 64 °C oven. Silver/gold sections were cut on a Leica Ultracut R (Leica) and stained in uranyl acetate and lead citrate. Images were captured by a Gatan Orius SC 200D CCD camera (Gatan, Pleasanton, CA, USA) in a JEM 1400 electron microscope (JEOL, Peabody, MA, USA).

[0079] *Microarrays and Bioinformatics*. Kidneys were lysed using Cell Disruption Buffer (Mirvana Paris Kit) and total RNA was extracted using Mirvana Paris Kit to yield a 260–280 nm absorbance ratio of 2.0. RNA concentration and integrity was determined by using an Agilent Bioanalyzer. A 1 μg aliquot of total RNA from each of the samples (3 samples per group; 2 groups per experiment) was processed using instructions and reagents supplied by the manufacturer (Affymetrix, Santa Clara, CA). Briefly, total RNA was reverse transcribed using a T7-Oligo (dT) Promoter Primer in the first-strand cDNA synthesis reaction. Following RNase H-mediated-DNA polymerase I-second-strand cDNA synthesis, the double-stranded cDNA was purified and served as a template for in vitro transcription in the presence of T7RNA polymerase and a biotinylated nucleotide analog/ribonucleotide mix, producing biotin-labeled complementary RNA (cRNA). cRNA probes were then purified, fragmented, and hybridized on Mouse ST2 gene expression arrays (45,101 probe sets). Background noise, housekeeping gene expression and 3'/5' ratio values of all chips were within quality control limits set by Affymetrix. Expression ratios were calculated as the power-2 exponential of the log2 differences. The acceptance criteria for gene array expression changes was a minimum 2-fold change in log2 (equivalent to 4-fold) and a one-way Analysis of Variance (ANOVA) t-test p-value of <0.05. All microarray raw files were submitted to the NCBI Gene Expression Omnibus (GEO) database (GSEXXX).

[0080] *Western Blots*. Protein concentration was measured using a Bradford assay. Samples were prepared and separated by a 4-12% Novex mini 15-well gradient gel (Bolt System, Life

Technologies), and probed with OPN (AF808, R&D, Minneapolis, MN), Dnm3 (ab3458, Abcam, Cambridge, MA), Kim-1 (AF3689, R&D, Minneapolis, MN) and GAPDH (sc-25778, Santa Cruz Biotechnology, Santa Cruz, California) antibodies. Signals were detected by chemiluminescence (Femto, Thomas Scientific) on photographic films. Digitized images were analyzed using Image J (NIH). Protein band densitometry was normalized to that of GAPDH, and the averaged results were plotted as normalized densitometry units (n.d.u.).

[0081] *Statistics*. For all experiments, N refers to the number of individual mice or individual culture plates. All data are expressed as mean ± S.E.M. P-values were calculated using Student's t-tests. Where indicated, p-values were calculated using ANOVA and corrected for multiple comparisons using Tukey Posthoc correction in GraphPad Prism. Repeated symbols represent P-values of different orders of magnitude, i.e. *P< 0.05, **P< 0.01.

Results

[0082] *OPN is substantially expressed in the tubules of Col4a3$^{-/-}$ mice*. The *Col4a3$^{-/-}$* knockout mouse phenocopies the symptom of Alport Syndrome, making it an ideal disease model (Cosgrove et al. *Genes Dev*. 1996;10:2981-2992). OPN expression was studied by immunostaining and western blots in kidney and/or plasma samples from WT and *Col4a3$^{-/-}$* mice. Immunostaining showed that OPN was substantially expressed in the tubules of *Col4a3$^{-/-}$* mice. Western blots revealed a dramatic increase of OPN expression in kidney (Fig. 1A) and plasma (Fig. 1B) of *Col4a3$^{-/-}$* mice.

[0083] *OPN deficiency increases life span and attenuates Alport pathology in Col4a3$^{-/-}$ mice*. Alport mice develop severe renal dysfunction and die approximately at 10 weeks of age. In order to test whether OPN deficiency increases lifespan in Alport mice, survivals of *Col4a3$^{-/-}$*, *Col4a3$^{-/-}$OPN$^{+/-}$* and *Col4a3$^{-/-}$OPN$^{-/-}$* animals and their body weights at 9 to 12 weeks of age were recorded. Kaplan Meier survival curves show that the *Col4a3$^{-/-}$OPN$^{-/-}$* and *Col4a3$^{-/-}$ OPN$^{+/-}$* mice significantly outlived the Alport mice (Fig. 2A), and that the body weights were also significantly increased in OPN deficient animals (Fig. 2B). Impressively, two *Col4a3$^{-/-}$ OPN$^{+/-}$* mice lived up to 20 weeks (Fig. 2A), doubling the typical lifespan of Alport animals. To study the functional effects of OPN deficiency on Alport pathology, urine and blood samples from animals at 8 to 9 weeks of age were analyzed. At 8 to 9 weeks of age, Alport mice develop severe proteinuria, renal dysfunction, hearing deficits and eye abnormalities. Using sandwich

Elisa, urinary albumin/creatinine levels were quantified, which substantially increased in Alport animals by 161 times compared to wild type (WT), and were significantly decreased by 50% in *Col4a3*$^{-/-}$*OPN*$^{+/-}$ and *Col4a3*$^{-/-}$*OPN*$^{-/-}$ mice as compared to Col4a3$^{-/-}$ mice (Fig. 2C). Other renal dysfunction parameters, plasma Blood Urea Nitrogen (BUN) and creatinine, were also measured, and very mild decrease were found in *Col4a3*$^{-/-}$*OPN*$^{+/-}$ and *Col4a3*$^{-/-}$*OPN*$^{-/-}$ mice as compared to *Col4a3*$^{-/-}$ mice (Fig. 2D and Fig. 2E). Interestingly, Galectin-3, whose expression correlates with end-stage renal dysfunction (ESRD), was found to be expressed two times higher in *Col4a3*$^{-/-}$ mice than WT and significantly decreased to control level in *Col4a3*$^{-/-}$*OPN*$^{+/-}$ and *Col4a3*$^{-/-}$*OPN*$^{-/-}$ mice (Fig. 2F). Blood MCHC levels were decreased in *Col4a3*$^{-/-}$ mice (Fig. 2G), suggesting anemia in the Alport mice, which was consistent with clinical reports that with onset of renal insufficiency, symptoms of chronic anemia may become evident as Alport disease progress. OPN deficiency significantly improved MCHC levels in Alport mice. Blood pressure was recorded using CODA mouse tail-cuff system (Kent Scientific). *Col4a3*$^{-/-}$ mice develop significantly higher blood pressures relative to WT mice (Kevan et al. 2016; submitted manuscript), but hypertension was markedly reduced in *Col4a3*$^{-/-}$*OPN*$^{-/-}$ and *Col4a3*$^{-/-}$ *OPN*$^{+/-}$ mice (Fig. 2H). Furthermore, since Alport patients have 1000x more risk of cardiovascular disease, cardiac function was analyzed by echocardiography, and OPN deficiency was found to reverse cardiac systolic and diastolic dysfunction (Fig. 3A and Fig. 3B).

[0084] *Cardiac hypertrophy and fibrosis are rescued by OPN deficiency in Col4a3*$^{-/-}$ *Mice.* Echocardiography data demonstrated cardiac hypertrophy in *Col4a3*$^{-/-}$ mice, as reflected by increased thickness of intraventricular septum diastole (Figure 4A). Furthermore, quantification of WGA images of cardiac myocyte cross-sectional area acquired showed significant increase in myocyte area in *Col4a3*$^{-/-}$ hearts (Figure 4B), supporting the hypertrophic phenotype (n=3 mice per group and 12 images per mouse). However, *Col4a3*$^{-/-}$ mice on hetero-or homozygote OPN KO background show normal myocardial wall thickness and cardiomyocyte area (Figure 4A-4C). In addition, OPN deficiency lowered fibrosis in *Col4a3*$^{-/-}$ mice as shown by decreasing interstitial "activated" fibroblasts as shown by electron microscopy (Figure 5A). Furthermore, microscopy and quantification data of EdU positive MLC2 negative interstitial cells showed reduction of fibrotic activity in the double knockout hearts (Figure 5B) (n=3 mice per group and 12 images per mouse). Additionally, elevated plasma levels of Galectin-3, a marker of diastolic dysfunction, in *Col4a3*$^{-/-}$ mice were also decreased by OPN deficiency (Figure 5C).

[0085] In order to study the mechanism by which OPN reduces cardiac systolic and diastolic function in Alport animals, global gene microarray studies were performed using heart samples from WT, *Col4a3*$^{-/-}$ and *Col4a3*$^{-/-}$*OPN*$^{-/-}$ mice. Two genes were found to be upregulated in Alport relative to WT kidneys, and then normalized by OPN deficiency (Table 1).

TABLE 1

Transcripts Cluster Id	FC (abs) ([Heart-Alport] vs [Heart-WT])	Regulation ([Heart-Alport] vs [Heart-WT])	FC (abs) ([Heart-Alport OPN Homo] vs [Heart-Alport])	Regulation ([Heart-Alport OPN Homo] vs [Heart-Alport])	Gene symbol	Gene description
17225169	2.4914064	up	2.501529	down	Snora75	smallnucleolarRNA,H/ACAbox75
17475818	2.0759642	up	2.2909088	down	LOC100302567	snoRNAMBI-142
17238920	2.1693795	down	3.1649513	up	Syne1	synapticnuclearenvelope1
17298556	3.2937572	down	6.9774137	up	Ogdhl	oxoglutaratedehydrogenase-like
17328368	2.095809	down	2.4237137	up	Myh11	myosin,heavypolypeptide11,smoothmuscle
17359698	2.3611708	down	2.583993	up	Scd4	stearoyl-coenzymeAdesaturase4
17424105	2.408188	down	2.2211387	up	Aqp7	aquaporin7
17477714	2.6463757	down	2.1116838	up	Slc17a7	solutecarrierfamily17(sodium-dependentinorganicphosphatecotransporter),member7
17493182	3.2724	down	10.451324	up	Rps13	ribosomalproteinS13
17494221	3.1911702	down	2.124569	up	Hbb-b1|Hbb-b2|Beta-s	hemoglobin,betaadultmajorchain|hemoglobin,betaadultminorchain|hemoglobinsubunitbeta-1-like
17510856	2.0189939	down	4.440632	up	Il15	interleukin15
17515402	2.2083213	down	2.0659814	up	Cnn1	calponin1
17538575	2.7673488	down	2.0064886	up	Alas2|Apex2	aminolevulinicacidsynthase2,erythroid|ap

						urinic/apyrimidinicendonuclease2

[0086] In one aspect, the OPN inhibitor is administered in an amount effective to alter gene/transcript expression (e.g., as measured in the heart), for example, to decrease expression of the *Snora75* and/or *LOC100302567* gene(s) and/or increase expression of one or more genes selected from *Syne1*, *Ogdhl*, *Myh11*, *Scd4*, *Aqp7*, *Slc17a7*, *Rps13*, *Hbb-b1|Hbb-b2|Beta-s*, *Il15*, *Cnn1*, and *Alas2|Apex2*.

[0087] It was also found that 14 genes showed significant difference in exonic expression (e.g., alternative splicing of exons) in the *Col4a3*$^{-/-}$ hearts, but the splicing events were normalized in the *Col4a3*$^{-/-}$ *OPN*$^{-/-}$ mice (Table 2). The data suggest that OPN deficiency may treat cardiac dysfunction via the identified gene/transcript targets listed in Tables 1 and 2.

TABLE 2

Transcripts Cluster Id	Splicing Index [Heart_WT] vs [Heart_Alport]	Gene symbol	Gene description
17405513	3.105732	Dhx36	DEAH(Asp-Glu-Ala-His)boxpolypeptide36
17390219	2.9435794	Zfp106	zincfingerprotein106
17211587	2.9246695	Dst	dystonin
17403625	2.7623181	Fubp1	farupstreamelement(FUSE)bindingprotein1
17405099	2.665932	Elf2	E74-likefactor2
17492847	2.6379364	Btbd1	BTB(POZ)domaincontaining1
17522536	2.4733872	Lrrc2	leucinerichrepeatcontaining2
17409920	2.4243236	Abcd3	ATP-bindingcassette,sub-familyD(ALD),member3
17379560	2.385108	Ctsa	cathepsinA
17532540	2.3834305	Fyco1	FYVEandcoiled-coildomaincontaining1
17297059	2.3299668	Abhd6	abhydrolasedomaincontaining6
17493658	2.0288756	Serpinh1	serine(orcysteine)peptidaseinhibitor,cladeH,member1
17328508	-2.114285	Fgd4	FYVE,RhoGEFandPHdomaincontaining4
17386778	-2.3689816	Ttn	titin

[0088] In one aspect, the OPN inhibitor is administered in an amount effective to normalize splicing events (e.g., as measured in the heart) relative to wild-type, for example, for one or more genes selected from *Dhx36*, *Zfp106*, *Dst*, *Fubp1*, *Elf2*, *Btbd1*, *Lrrc2*, *Abcd3*, *Ctsa*, *Fyco1*, *Abhd6*, *Serpinh1*, *Fgd4*, and *Ttn*.

[0089] *OPN deficiency reduces fibrotic cell proliferation and reduces kidney pathology in in Col4a3*$^{-/-}$ *kidney*. Kidney Injury Molecule-1 (KIM-1) is a type 1 transmembrane protein whose

expression is markedly up-regulated in the proximal tubules in acute tubular necrosis in rats and human patients (Han et al. *Kidney Int.* 2002;62:237-244) and Alport mice. KIM-1 was found dramatically increased in Alport mice compared to WT and significantly decreased to control level in *Col4a3*$^{-/-}$*OPN*$^{-/-}$ mice (Fig. 6B). Because Kim-1 is capable to promote renal fibrosis (Humphreys et al. *J Clin Invest.* 2013;123:4023-4035), decreased Kim-1 in *Col4a3*$^{-/-}$*OPN*$^{-/-}$ mice indicated an anti-fibrotic effect of OPN deficiency in Alport mice. To understand the impact of OPN knock-out on fibrotic cell proliferation, EdU, a thymidine analogue that incorporates into newly synthesized DNA, was injected intraperitoneally into WT, *Col4a3*$^{-/-}$ and *Col4a3*$^{-/-}$*OPN*$^{-/-}$ mice at a 100µg per 100uL concentration daily. Animals were sacrificed after a total of 30 injections. EdU incorporation was visualized by EdU fluorescent staining on paraffin kidney sections. To eliminate glomeruli, the kidney tissues were co-stained with Synaptopodin, a marker of glomeruli. The majority of EdU was present in tubules and interstitial space. In *Col4a3*$^{-/-}$ mice, extensive EdU incorporation was observed outside the glomeruli while WT and *Col4a3*$^{-/-}$ *OPN*$^{-/-}$ mice had almost undetectable level of EdU (Fig. 6A). Electron microscopy showed thickened basement membrane and podocytes effacement in the *Col4a3-/-* mice as other groups have reported (Gomez et al. *J Clin Invest.* 2015;125:141-156; Cosgrove et al. *Genes Dev.* 1996;10:2981-2992) (Fig. 6E). In *Col4a3*$^{-/-}$*OPN*$^{-/-}$ mice, structure of basement membrane and morphology of podocytes were much improved relative to the Alport mice (Fig. 6E). These data demonstrate that OPN expression in the injured Alport tubules correlated with high fibrotic cell proliferation. In order to study the mechanism by which OPN reduces fibrotic cell proliferation in Alport animals, global gene microarray studies were performed using kidney samples from WT, *Col4a3*$^{-/-}$ and *Col4a3*$^{-/-}$*OPN*$^{-/-}$ mice. Eleven genes were found to be upregulated in Alport relative to WT kidneys, and then normalized by OPN deficiency (Table 3). The data suggest that OPN deficiency may treat kidney dysfunction via the identified gene/transcript targets listed in Table 3.

TABLE 3

Transcripts Cluster Id	FC (abs) ([Kidney-Alport] vs [Kidney-WT])	Regulation ([Kidney-Alport] vs [Kidney-WT])	FC (abs) ([Kidney-Alport OPN Homo] vs [Kidney-Alport])	Regulation ([Kidney-Alport OPN Homo] vs [Kidney-Alport])	Gene symbol	Gene description
17213976	2.0559123	up	1.7654657	down		
17228906	2.0809784	up	1.5276362	down	Dnm3	dynamin3

17248406	2.1012573	up	1.6529104	down		
17288112	1.7395718	up	1.6874008	down	Mir24-1|Mir3074-1	microRNA24-1|microRNA3074-1
17302832	1.926296	up	1.5398612	down	B930095G15Rik	RIKENcDNA B930095G15 gene
17304145	2.4783666	up	1.5078232	down		
17305259	1.8952456	up	1.5298346	down		
17424412	3.3135536	up	1.5267851	down	4933409K07Rik|Gm3893	RIKENcDNA 4933409K07gene|predicted gene3893
17434248	3.3134856	up	1.540013	down	4933409K07Rik|Gm3893	RIKENcDNA 4933409K07gene|predicted gene3893
17518933	2.2847536	up	1.7172713	down	9530091C08Rik	RIKENcDNA 9530091C08gene
17548135	1.8419162	up	1.7043395	down	Gm20038	predictedgene,20038

[0090] In one aspect, the OPN inhibitor is administered in an amount effective to alter gene/transcript expression (e.g., as measured in the kidney), for example, to decrease expression of one or more genes selected from *Dnm3*, *Mir24-1|Mir3074-1*, *B930095G15Rik*, *4933409K07Rik|Gm3893*, *4933409K07Rik|Gm3893*, *9530091C08Rik*, and *Gm20038.*

[0091] Interestingly, Dynamin3 (Dnm3) was found to be significantly downregulated in *Col4a3*$^{-/-}$*OPN*$^{-/-}$ versus *Col4a3*$^{-/-}$ mice (Table 3). To validate the expression pattern of Dnm3, immunohistochemistry was performed on kidney sections and found that Dnm3 was highly expressed in the proximal tubules of *Col4a3*$^{-/-}$ mice and then normalized in the OPN deficient Alport mice (Fig. 6C). To further validate kidney expression of Dnm3, western blot on the kidneys was performed and found Dnm3 expression was significantly upregulated in *Col4a3*$^{-/-}$ mice compared to WT, and significantly downregulated in *Col4a3*$^{-/-}$*OPN*$^{-/-}$ mice.

[0092] Because DNM3 dysregulation in the tubules may have an effect on lipid accumulation in the tubules, Oil Red was used to stain for lipids in frozen kidney sections collected from the three genotypes. Impressively, extensive lipid deposition in the renal tubules was found in the *Col4a3*$^{-/-}$ mice relative to wild type mice, but was significantly reduced in the *Col4a3*$^{-/-}$*OPN*$^{-/-}$ mice (Fig. 6D).

[0093] *OPN deficiency improves high frequency hearing threshold and reduces cochlear pathology in Col4a3$^{-/-}$ mice.* Otopathology of Alport syndrome is characterized by high frequency sensorineural hearing loss. In order to examine the effects of OPN deficiency on hearing ability of Alport mice, animals were recorded responding to a series of clicks and pure tones stimuli through auditory brainstem response (ABR) test. No differences were observed across genotypes in response to pure tones stimuli and 4, 8, 16 KHZ clicks stimuli. However, *Col4a3$^{-/-}$* animals showed retarded responses to 16KHZ stimuli and *Col4a3$^{-/-}$OPN$^{-/+}$* and *Col4a3$^{-/-}$OPN$^{-/-}$* mice had significantly improved hearing ability to Alport animals (Fig. 7A). Previous studies have shown thickened capillary basement membrane in stria vascularies of *Col4a3$^{-/-}$* cochlea (Cosgrove et al. *Hear Res.* 1998;121:84-98). The EM studies showed that OPN deficiency reduced the thickening of capillary basement membrane of Alport cochleas (Fig. 7B).

[0094] *OPN deficiency reduces corneal pathology in Col4a3$^{-/-}$ mice.* Alport patients may develop the eye abnormality lenticonus. Optical coherence tomography (OCT) studies were performed to visualize cornea structures in the Alport mice with or without OPN. The central cornea thickness (CCC) was analyzed and a difference among groups was not observed (Fig. 8A), suggesting Alport animals do not have pathology changes in cornea thickness. The anterior chamber depth (ACD) – distance from cornea to lens, and anterior chamber angle -- angle between cornea and iris, was analyzed, and both were significantly reduced in *Col4a3$^{-/-}$* mice, but were rescued to control level in *Col4a3$^{-/-}$OPN$^{-/-}$* mice (Fig. 8A). In addition, EM revealed that the thickening of the capillaries in the Alport retinal basement membrane was reduced by OPN deficiency. The data supported a tendency of lenticonus in *Col4a3$^{-/-}$* mice that phenocopies symptoms of human Alport patients (Mahajan et al. *International Journal of Scientific Study.* 2013;01:4).

[0095] The foregoing Results are also observed in human kidney tubular cells and podocytes treated with an OPN inhibitor and Alport mice administered an OPN inhibitor, such as an OPN RNA aptamer, an OPN antibody, or an OPN small molecule inhibitor.

[0096] The foregoing Example demonstrates that reduction of OPN can improve lifespan, kidney function, cardiac function, renal and cochlear histology, eye deformities, and hearing ability in Alport subjects, demonstrating inhibition of OPN as a therapeutic target for Alport Syndrome.

[0097] All publications, patents and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this disclosure that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED:

1. A method of treating a Col4-related disease in a patient in need thereof comprising administering a therapeutically effective amount of an osteopontin inhibitor to the subject.

2. The method of claim 1, comprising administering the osteopontin inhibitor in an amount effective to decrease the blood pressure of the subject.

3. The method of claim 1 or 2, comprising administering the osteopontin inhibitor in an amount effective to increase the mean corpuscular hemoglobin concentration (MCHC) in the blood of the subject.

4. The method of claim 1 or 2, comprising administering the osteopontin inhibitor in an amount effective to improve cardiac systolic/or and diastolic function in the heart of the subject.

5. The method of claim 1 or 2, comprising administering the osteopontin inhibitor in an amount effective to decrease a marker of renal dysfunction in the subject, optionally a marker selected from plasma kidney injury molecule-1 (KIM-1), plasma blood urea nitrogen (BUN), plasma creatinine (CRE), renal Dynamin3 (Dnm3), plasma Galectin-3 (Gal-3), and a combination of any of the foregoing.

6. The method of claim 1 or 2, comprising administering the osteopontin inhibitor in an amount effective to decrease the urine albumin/creatinine ratio of the subject.

7. The method of claim 1 or 2, comprising administering the osteopontin inhibitor in an amount effective to decrease the thickness of the basement membrane in the kidney, retina, and/or cochlea of the subject.

8. The method of claim 1 or 2, comprising administering the osteopontin inhibitor in an amount effective to treat or prevent hearing loss in the subject.

9. The method of claim 1 or 2, comprising administering the osteopontin inhibitor in an amount effective to treat or prevent loss of vision.

10. The method of claim 1 or 2, comprising administering the osteopontin inhibitor in an amount effective to increase the anterior chamber depth and/or the anterior chamber angle in the eye of the subject.

11. A method of improving cardiac function in a subject in need thereof comprising administering an osteopontin inhibitor in an amount effective to lower blood pressure, increase the mean corpuscular hemoglobin concentration (MCHC) in the blood, decrease myocardial fibrosis, increase stroke volume, increase cardiac output, decrease isovolumetric relaxation time, decrease interventricular wall thickness, decrease myocyte hypertrophy, or a combination of any of the foregoing, in the subject.

12. A method of improving kidney function in a subject in need thereof comprising administering an osteopontin inhibitor in an amount effective to decrease the urine albumin/creatinine (ALB/CRE) ratio, decrease the thickness of a glomerulus basement membrane, decrease fibrotic cell proliferation and/or fibrosis, decrease renal lipid accumulation, decrease one or more of plasma kidney injury molecule-1 (KIM-1), plasma blood urea nitrogen (BUN), plasma creatinine (CRE), renal Dynamin3 (Dnm3), and plasma Galectin-3 (Gal-3), or a combination of any of the foregoing, in the subject.

13. A method of treating hearing loss in a subject in need thereof, comprising administering an osteopontin inhibitor in an amount effective to improve or maintain hearing and/or decrease the thickness of the capillary basement membrane in the cochlea, in the subject.

14. A method of treating loss of vision in a subject in need thereof, comprising administering an osteopontin inhibitor in an amount effective to improve or maintain vision, treat

or prevent a lenticonus, increase the anterior chamber depth in the eye, increase the anterior chamber angle in the eye, decrease the thickness of the capillary basement membrane in the retina, or a combination of any of the foregoing, in the subject.

15. The method of any one of claims 1 and 11-14, wherein the subject has Alport Syndrome.

16. The method of any one of claims 1 and 11-14, wherein the osteopontin inhibitor is selected from an antibody, an aptamer, a small interfering RNA, an antisense oligonucleotide, a regulatory nucleic acid, a small molecule inhibitor, and combinations thereof.

17. The method of any one of claims 1 and 11-14, comprising administering the osteopontin inhibitor subcutaneously or intravenously.

18. The method of any one of claims 1 and 11-14, comprising administering the osteopontin inhibitor locally to the eye or ear of the subject.

19. The method of claim 18, comprising administering the osteopontin inhibitor intravitreally and/or intratympanically.

20. The method of any one of claims 1 and 11-14, comprising administering the osteopontin inhibitor at least once a day.

21. The method of any one of claims 1 and 11-14, comprising administering the osteopontin inhibitor in a dosage of between about 0.1 mg and about 50 mg.

22. The method of any one of claims 1 and 11-14, comprising administering the osteopontin inhibitor in an amount effective to: (a) decrease expression of the *Snora75* and/or *LOC100302567* gene(s) in the heart; (b) increase expression of one or more genes in the heart selected from *Syne1*, *Ogdhl*, *Myh11*, *Scd4*, *Aqp7*, *Slc17a7*, *Rps13*, *Hbb-b1*\|*Hbb-b2*\|*Beta-s*, *Il15*, *Cnn1*, and *Alas2*\|*Apex2*; (c) decrease expression of one or more genes in the kidney selected

from *Dnm3*, *Mir24-1|Mir3074-1*, *B930095G15Rik*, *4933409K07Rik|Gm3893*, *4933409K07Rik|Gm3893*, *9530091C08Rik*, and *Gm20038*; and/or (d) normalize the splicing events relative to wild-type in one or more genes selected from *Dhx36*, *Zfp106*, *Dst*, *Fubp1*, *Elf2*, *Btbd1*, *Lrrc2*, *Abcd3*, *Ctsa*, *Fyco1*, *Abhd6*, *Serpinh1*, *Fgd4*, and *Ttn*.

22. Use of an osteopontin inhibitor in the manufacture of a medicament for treating a Col4-related disease in a patient in need thereof.

23. The use of claim 22, wherein the osteopontin inhibitor is in an amount effective to decrease the blood pressure of the subject.

24. The use of claim 22 or 23, wherein the osteopontin inhibitor is in an amount effective to increase the mean corpuscular hemoglobin concentration (MCHC) in the blood of the subject.

25. The use of claim 22 or 23, wherein the osteopontin inhibitor is in an amount effective to improve cardiac systolic/or and diastolic function in the heart of the subject.

26. The use of claim 22 or 23, wherein the osteopontin inhibitor is in an amount effective to decrease a marker of renal dysfunction in the subject, optionally a marker selected from plasma kidney injury molecule-1 (KIM-1), plasma blood urea nitrogen (BUN), plasma creatinine (CRE), renal Dynamin3 (Dnm3), plasma Galectin-3 (Gal-3), and a combination of any of the foregoing.

27. The use of claim 22 or 23, wherein the osteopontin inhibitor is in an amount effective to decrease the urine albumin/creatinine ratio of the subject.

28. The use of claim 22 or 23, wherein the osteopontin inhibitor is in an amount effective to decrease the thickness of the basement membrane in the kidney, retina, and/or cochlea of the subject.

29. The use of claim 22 or 23, wherein the osteopontin inhibitor is in an amount effective to treat or prevent hearing loss in the subject.

30. The use of claim 22 or 23, wherein the osteopontin inhibitor is in an amount effective to treat or prevent loss of vision.

31. The use of claim 22 or 23, wherein the osteopontin inhibitor is in an amount effective to increase the anterior chamber depth and/or the anterior chamber angle in the eye of the subject.

32. Use of an osteopontin inhibitor in the manufacture of a medicament for improving cardiac function in a subject in need thereof comprising the osteopontin inhibitor in an amount effective to lower blood pressure, increase the mean corpuscular hemoglobin concentration (MCHC) in the blood, decrease myocardial fibrosis, increase stroke volume, increase cardiac output, decrease isovolumetric relaxation time, decrease interventricular wall thickness, decrease myocyte hypertrophy, or a combination of any of the foregoing, in the subject.

33. Use of an osteopontin inhibitor in the manufacture of a medicament for improving kidney function in a subject in need thereof comprising the osteopontin inhibitor in an amount effective to decrease the urine albumin/creatinine (ALB/CRE) ratio, decrease the thickness of a glomerulus basement membrane, decrease fibrotic cell proliferation and/or fibrosis, decrease renal lipid accumulation, decrease one or more of plasma kidney injury molecule-1 (KIM-1), plasma blood urea nitrogen (BUN), plasma creatinine (CRE), renal Dynamin3 (Dnm3), and plasma Galectin-3 (Gal-3), or a combination of any of the foregoing, in the subject.

34. Use of an osteopontin inhibitor in the manufacture of a medicament for treating hearing loss in a subject in need thereof, comprising the osteopontin inhibitor in an amount effective to improve or maintain hearing and/or decrease the thickness of the capillary basement membrane in the cochlea, in the subject.

35. Use of an osteopontin inhibitor in the manufacture of a medicament for treating loss of vision in a subject in need thereof, comprising the osteopontin inhibitor in an amount effective to improve or maintain vision, treat or prevent a lenticonus, increase the anterior chamber depth in the eye, increase the anterior chamber angle in the eye, decrease the thickness of the capillary basement membrane in the retina, or a combination of any of the foregoing, in the subject.

36. The use of any one of claims 22 and 32-35, wherein the subject has Alport Syndrome.

37. The use of any one of claims 22 and 32-35, wherein the osteopontin inhibitor is selected from an antibody, an aptamer, a small interfering RNA, an antisense oligonucleotide, a regulatory nucleic acid, a small molecule inhibitor, and combinations thereof.

38. The use of any one of claims 22 and 32-35, wherein the osteopontin inhibitor is administered subcutaneously or intravenously.

39. The use of any one of claims 22 and 32-35, wherein the osteopontin inhibitor is administered locally to the eye or ear of the subject.

40. The use of any one of claims 22 and 32-35, wherein the osteopontin inhibitor is administered intravitreally and/or intratympanically.

41. The use of any one of claims 22 and 32-35, wherein the osteopontin inhibitor is administered at least once a day.

42. The use of any one of claims 22 and 32-35, wherein the osteopontin inhibitor is administered in a dosage of between about 0.1 mg and about 50 mg.

43. The use of any one of claims 22 and 32-35, wherein the osteopontin inhibitor is administered in an amount effective to: (a) decrease expression of the *Snora75* and/or

LOC100302567 gene(s) in the heart; (b) increase expression of one or more genes in the heart selected from *Syne1*, *Ogdhl*, *Myh11*, *Scd4*, *Aqp7*, *Slc17a7*, *Rps13*, *Hbb-b1|Hbb-b2|Beta-s*, *Il15*, *Cnn1*, and *Alas2|Apex2*; (c) decrease expression of one or more genes in the kidney selected from *Dnm3*, *Mir24-1|Mir3074-1*, *B930095G15Rik*, *4933409K07Rik|Gm3893*, *4933409K07Rik|Gm3893*, *9530091C08Rik*, and *Gm20038*; and/or (d) normalize the splicing events relative to wild-type in one or more genes selected from *Dhx36*, *Zfp106*, *Dst*, *Fubp1*, *Elf2*, *Btbd1*, *Lrrc2*, *Abcd3*, *Ctsa*, *Fyco1*, *Abhd6*, *Serpinh1*, *Fgd4*, and *Ttn*.

FIGURE 1A

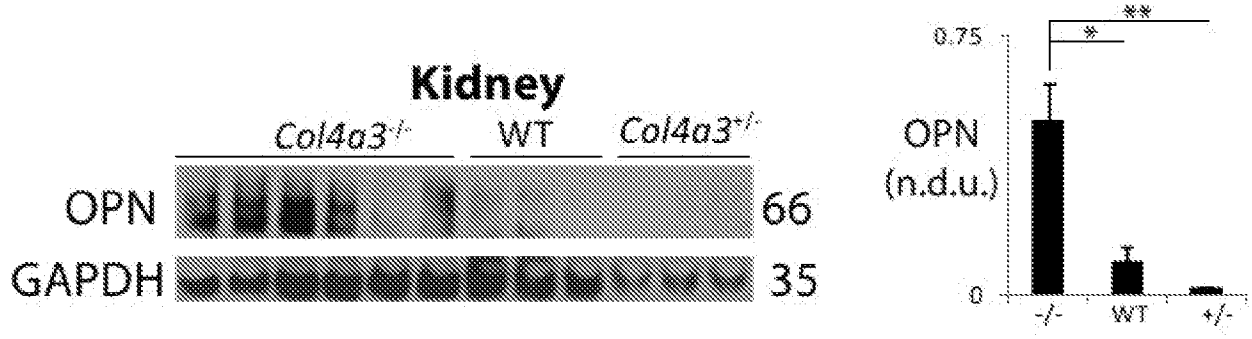


FIGURE 1B

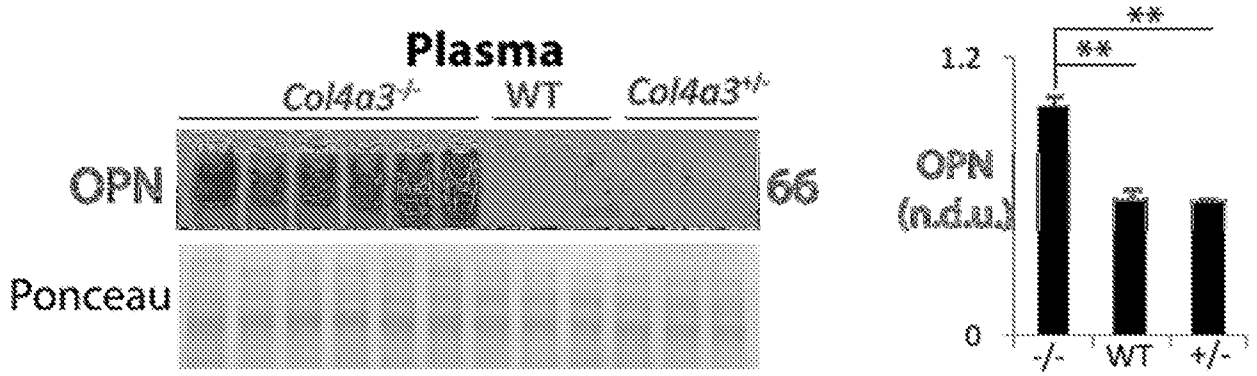


FIGURE 2A

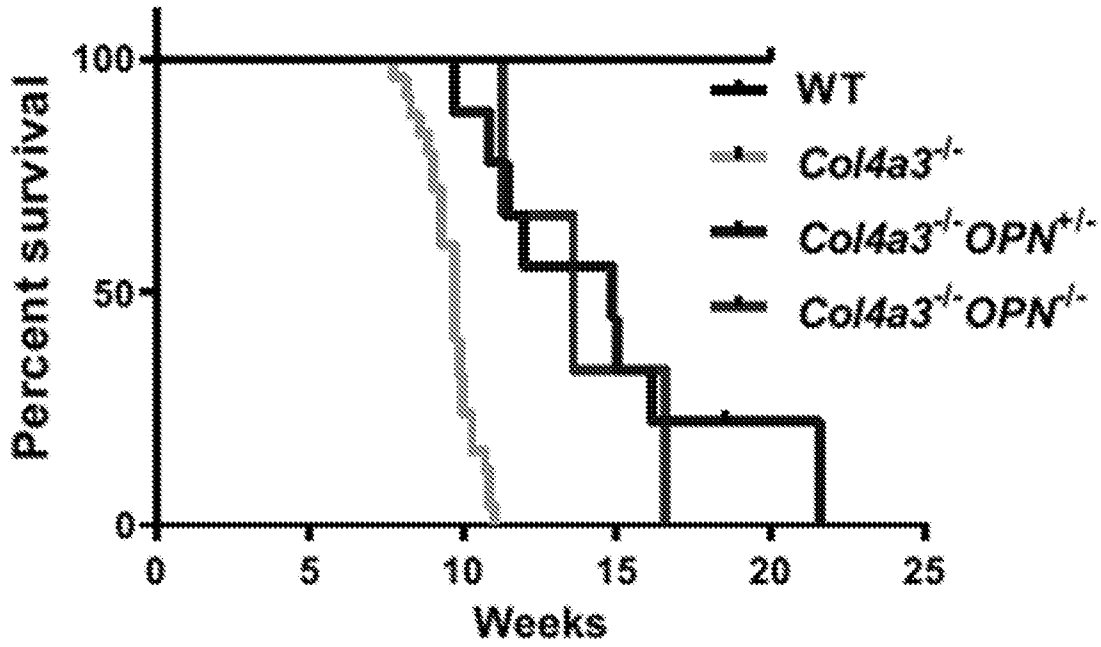


FIGURE 2B

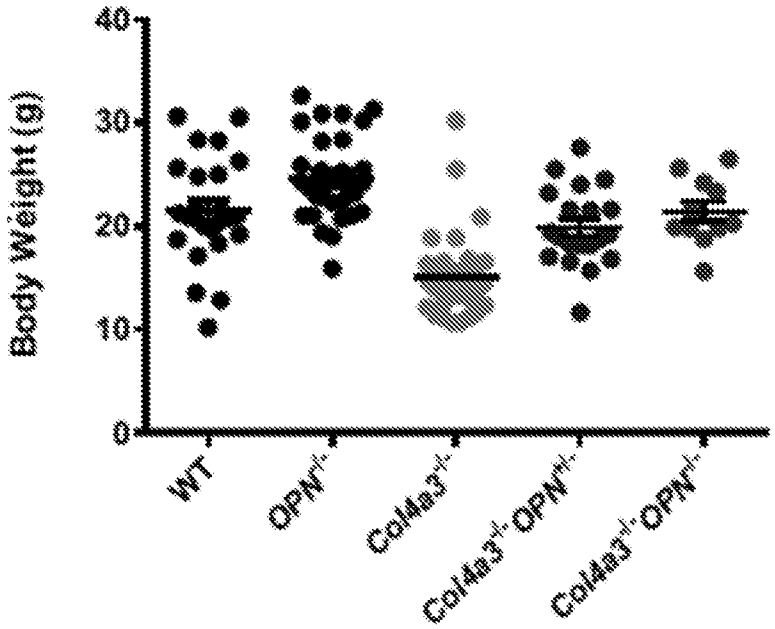


FIGURE 2C

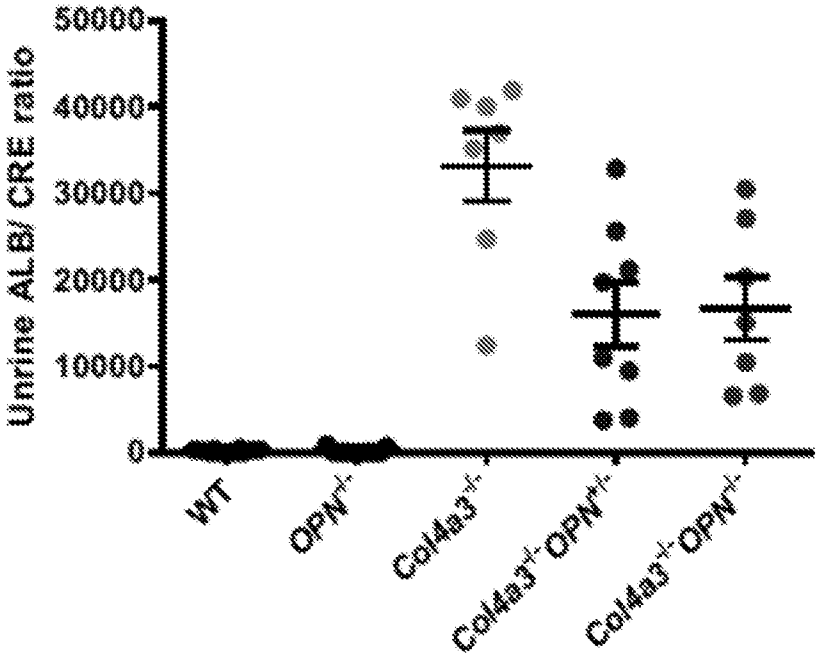


FIGURE 2D

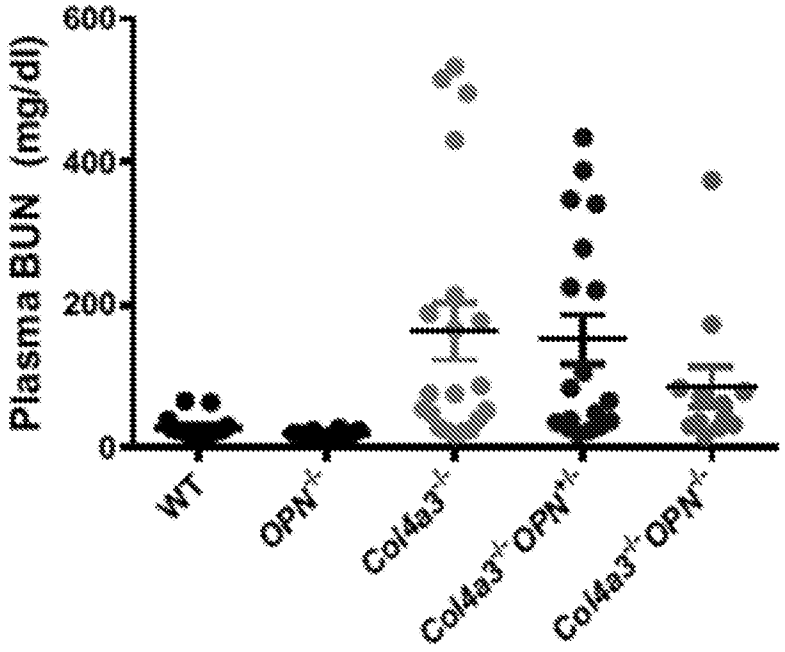


FIGURE 2E

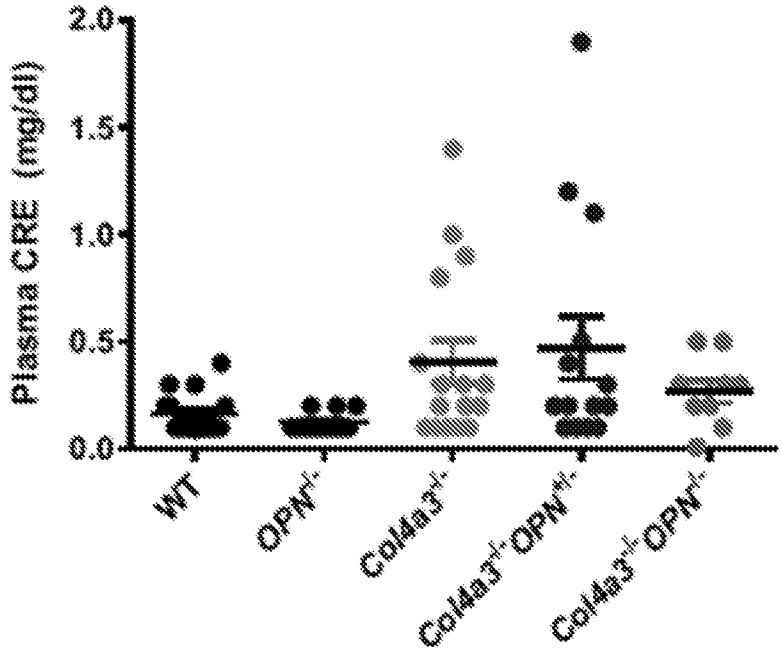

Plasma CRE (mg/dl)
2.0
1.5
1.0
0.5
0.0
WT
OPN-/-
Col4a3-/-
Col4a3-/-OPN-/-
Col4a3-/-OPN-/-

FIGURE 2F

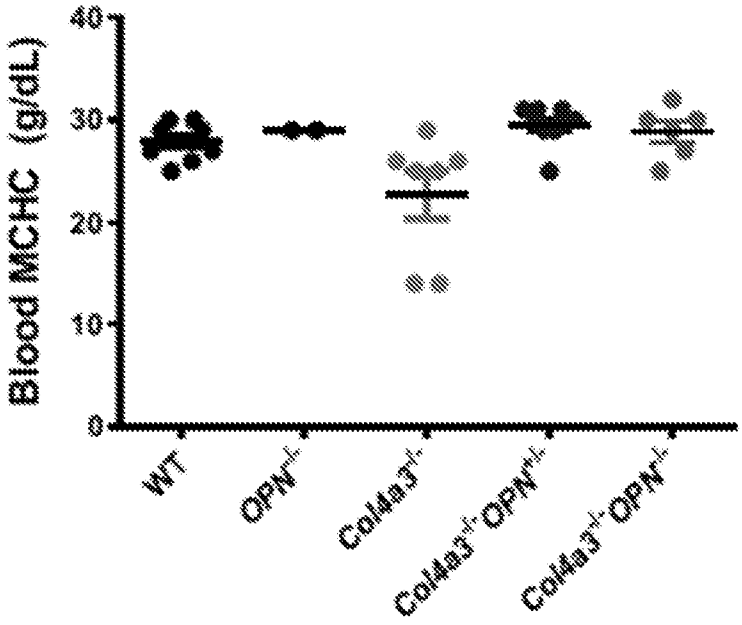


FIGURE 2G

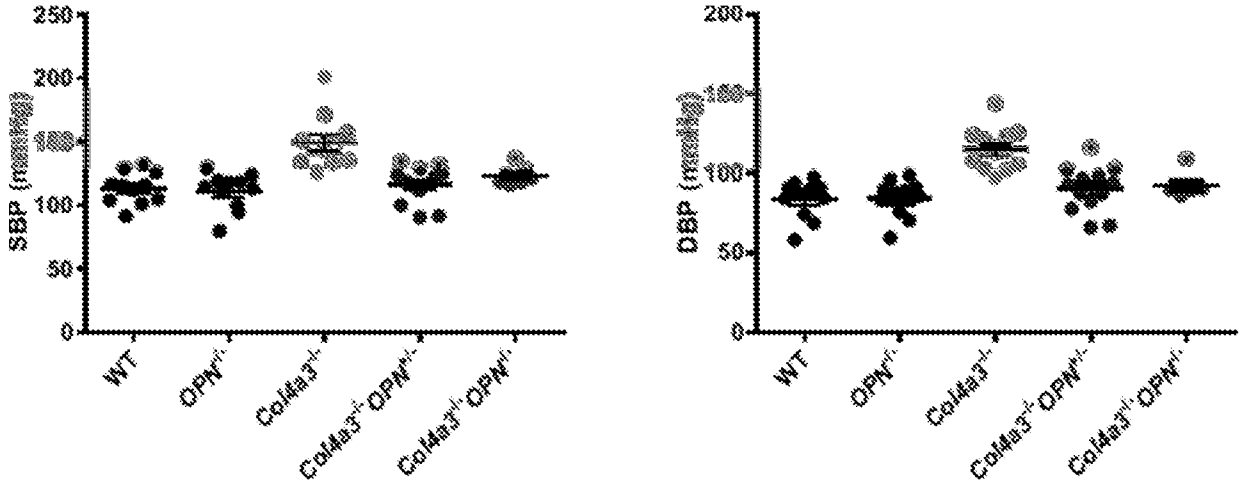


FIGURE 3A

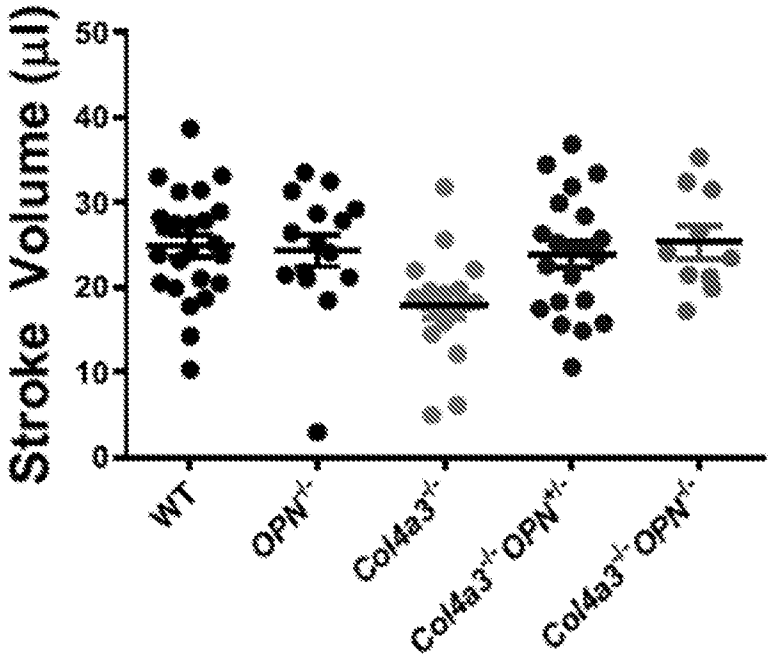


FIGURE 3B

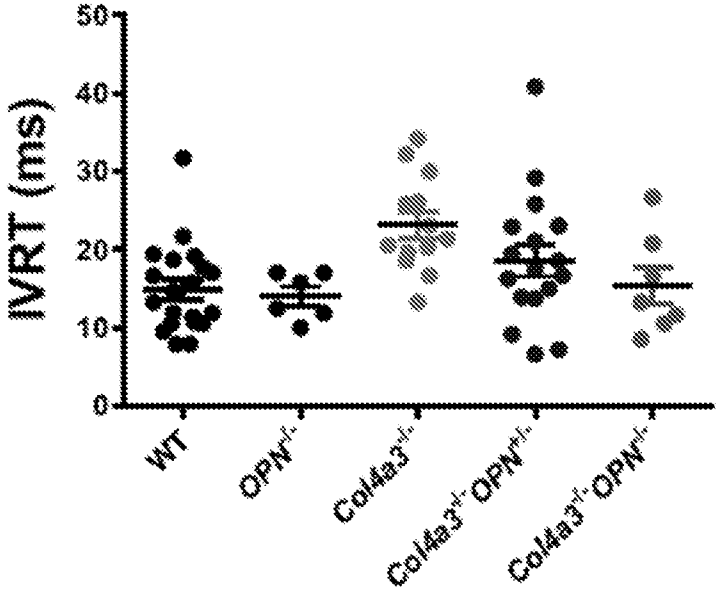


FIGURE 4A

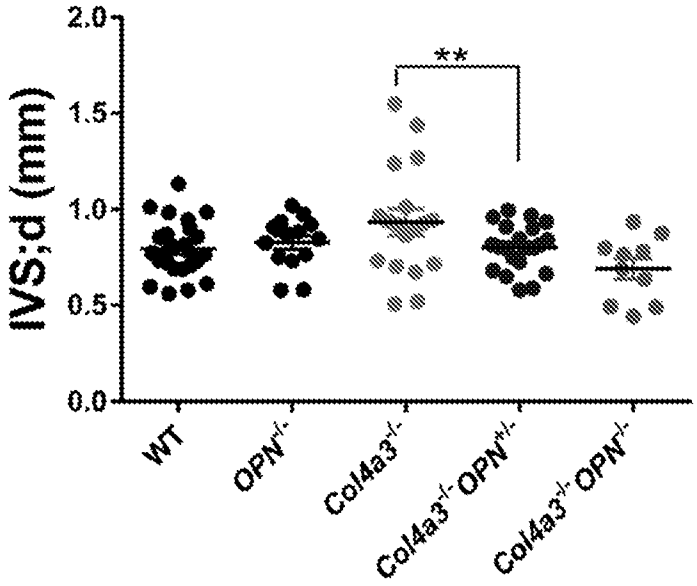


FIGURE 4B

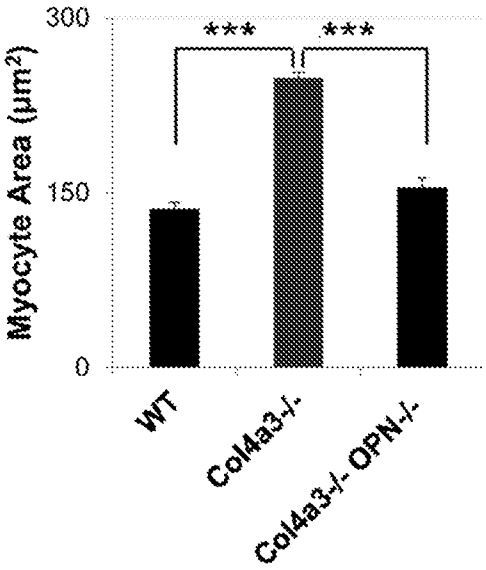


FIGURE 4C

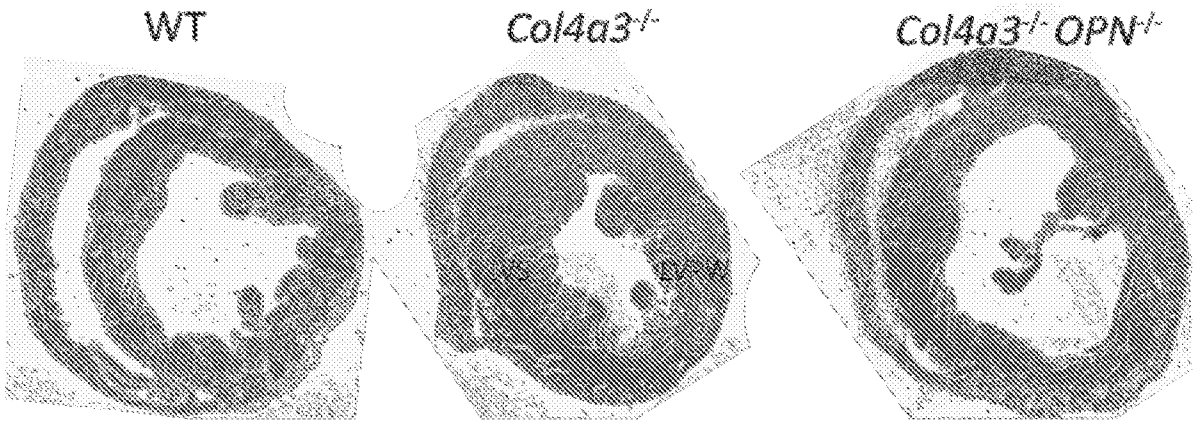


FIGURE 5A

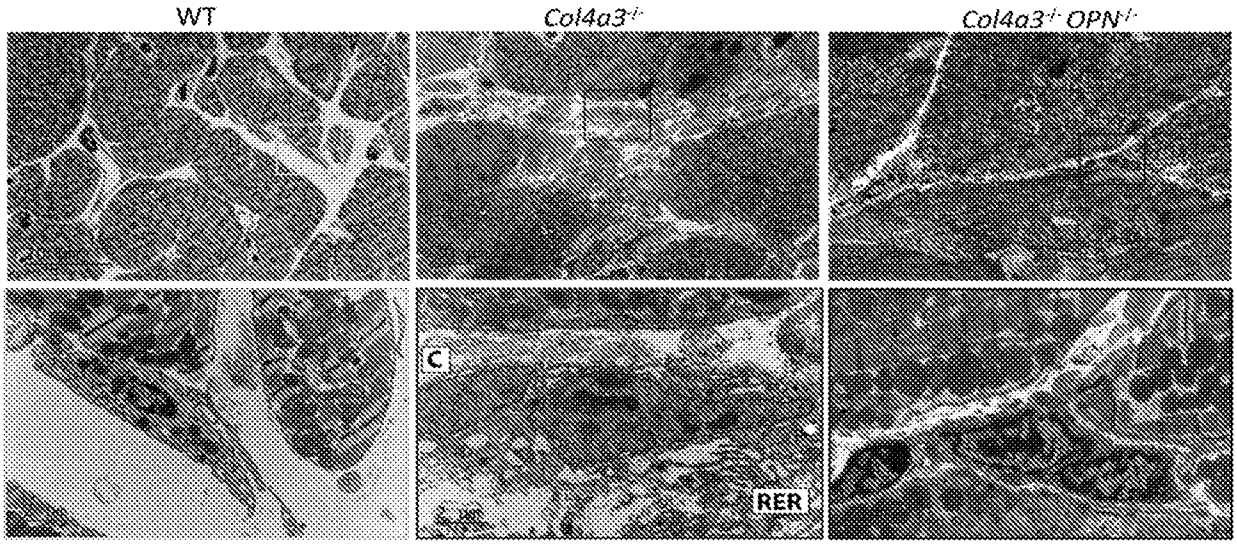


FIGURE 5B

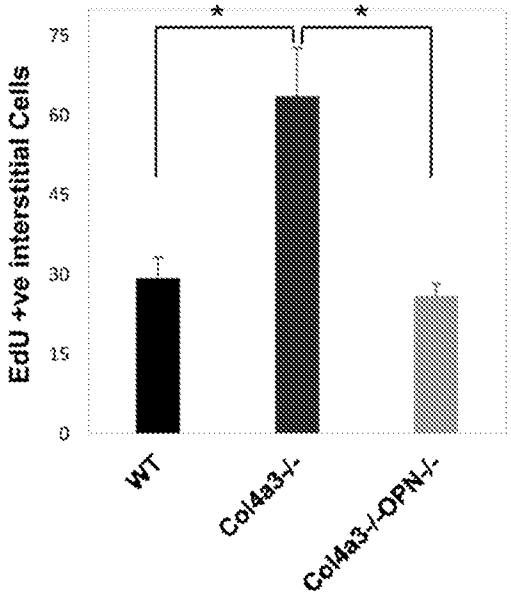


FIGURE 5C

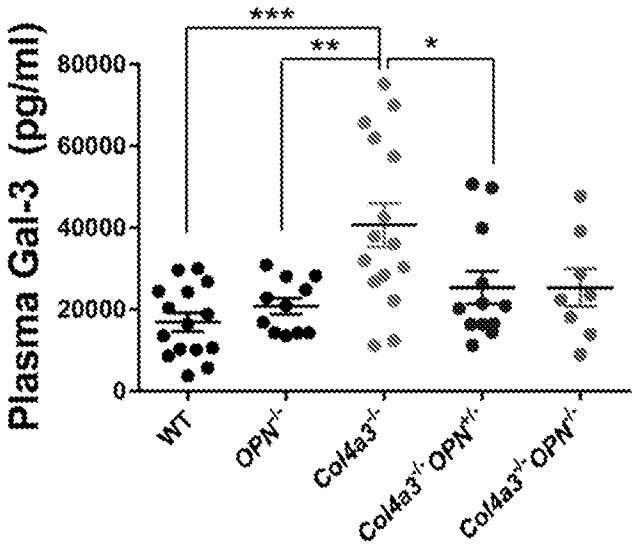


FIGURE 6A

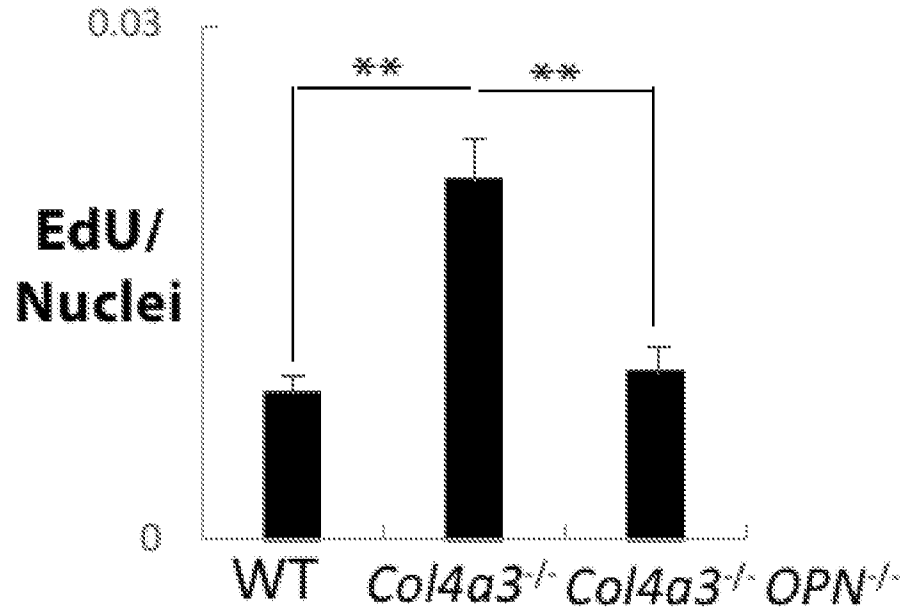


FIGURE 6B

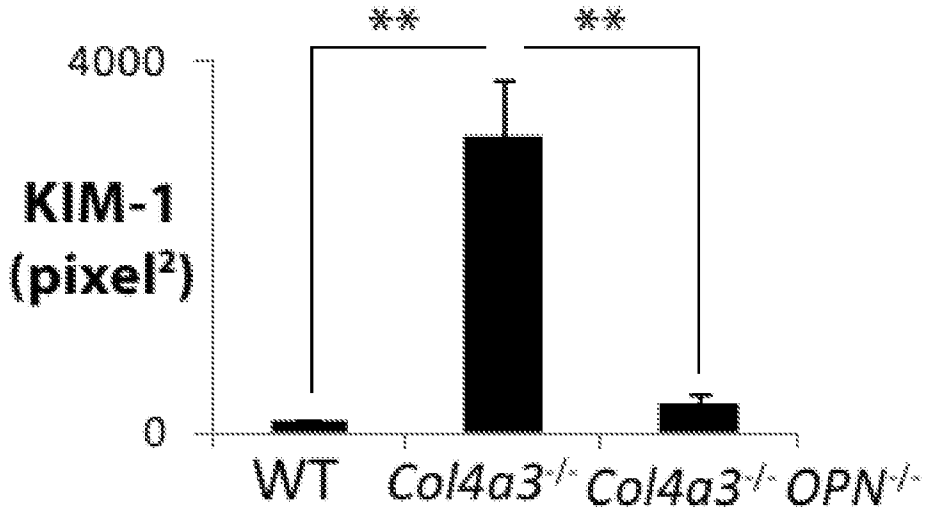


FIGURE 6C

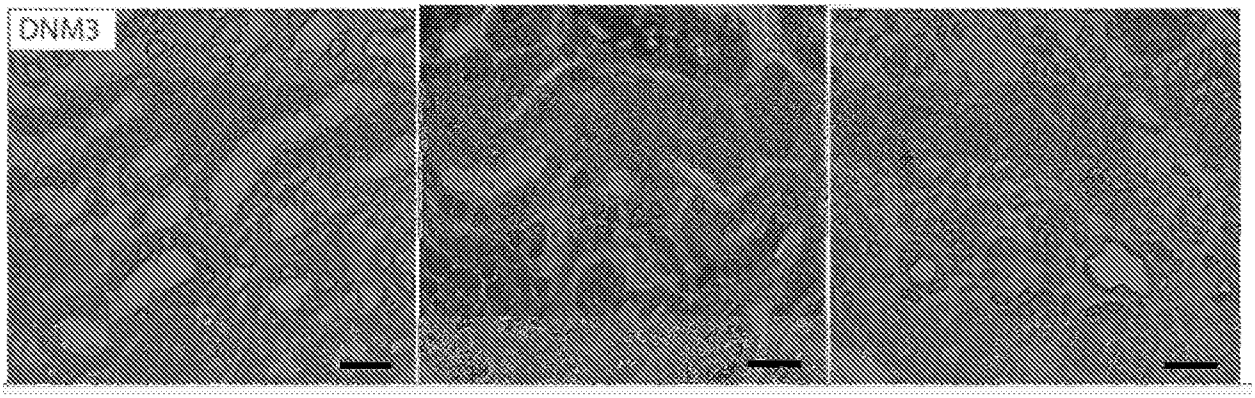
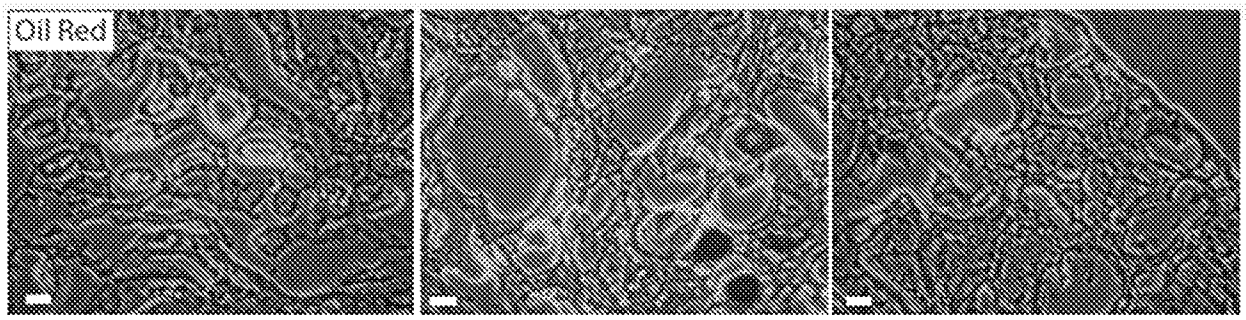


FIGURE 6D



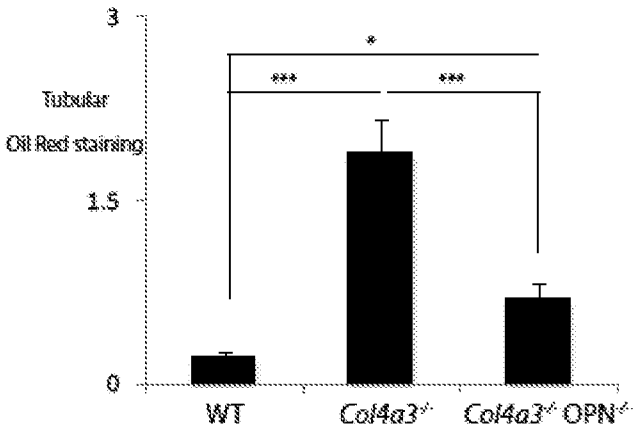


FIGURE 6E

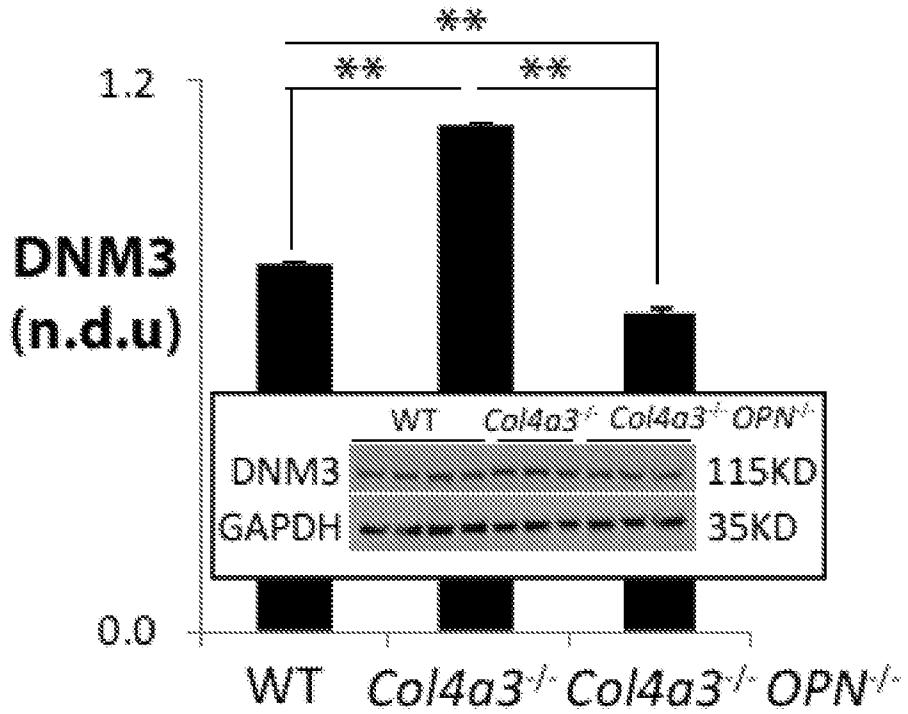


FIGURE 6F

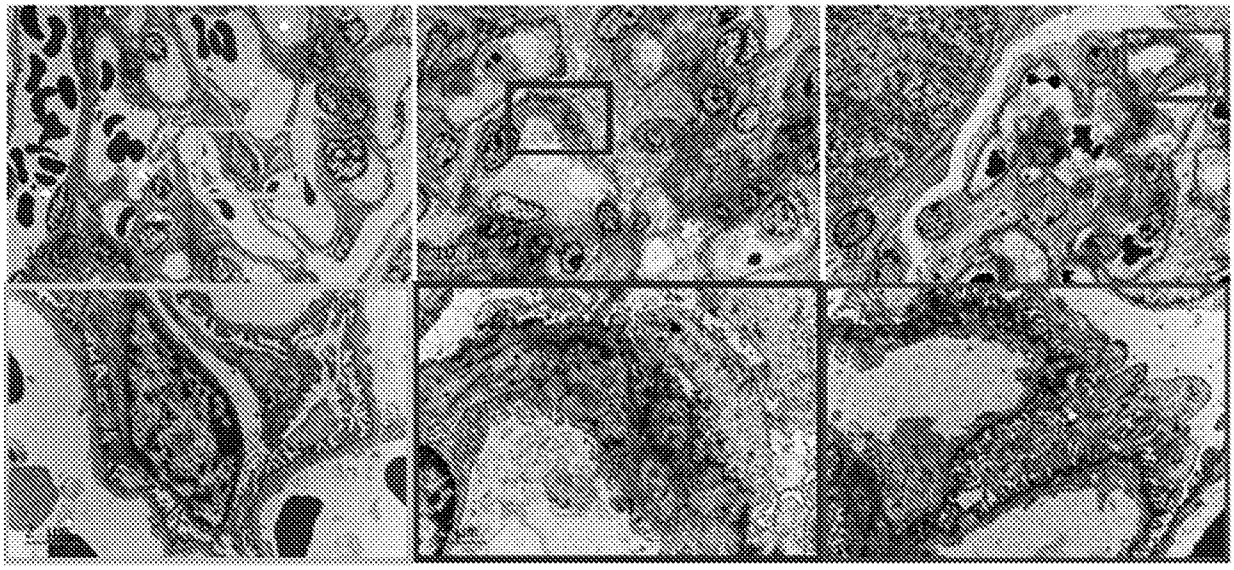


FIGURE 7A

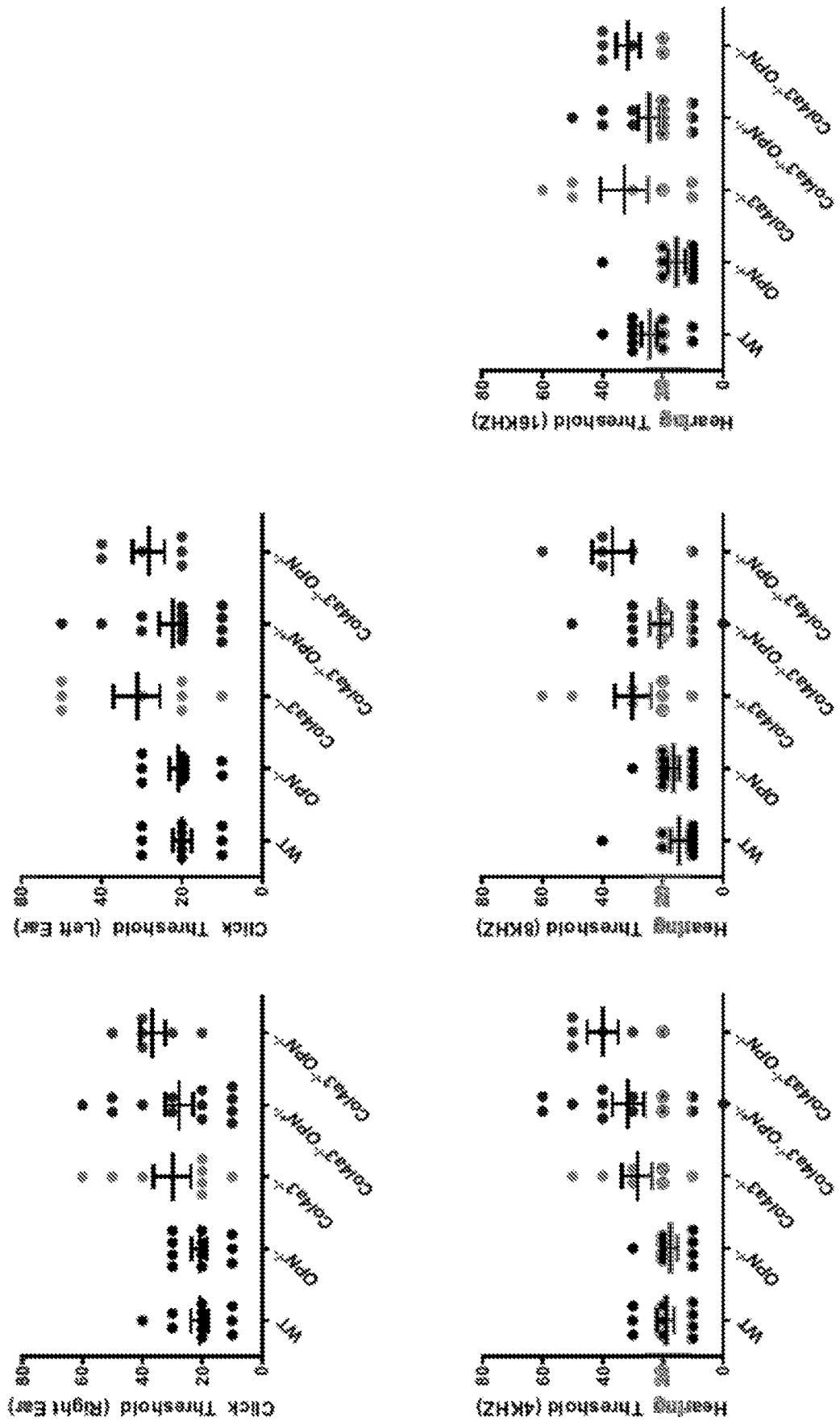

Hearing Threshold (16KHZ)
Hearing Threshold (8KHZ)
Hearing Threshold (4KHZ)
Click Threshold (Left Ear)
Click Threshold (Right Ear)
0
20
40
60
80
WT
OPN-/-
Col4a3
Col4a3-/-OPN-/-

FIGURE 7B

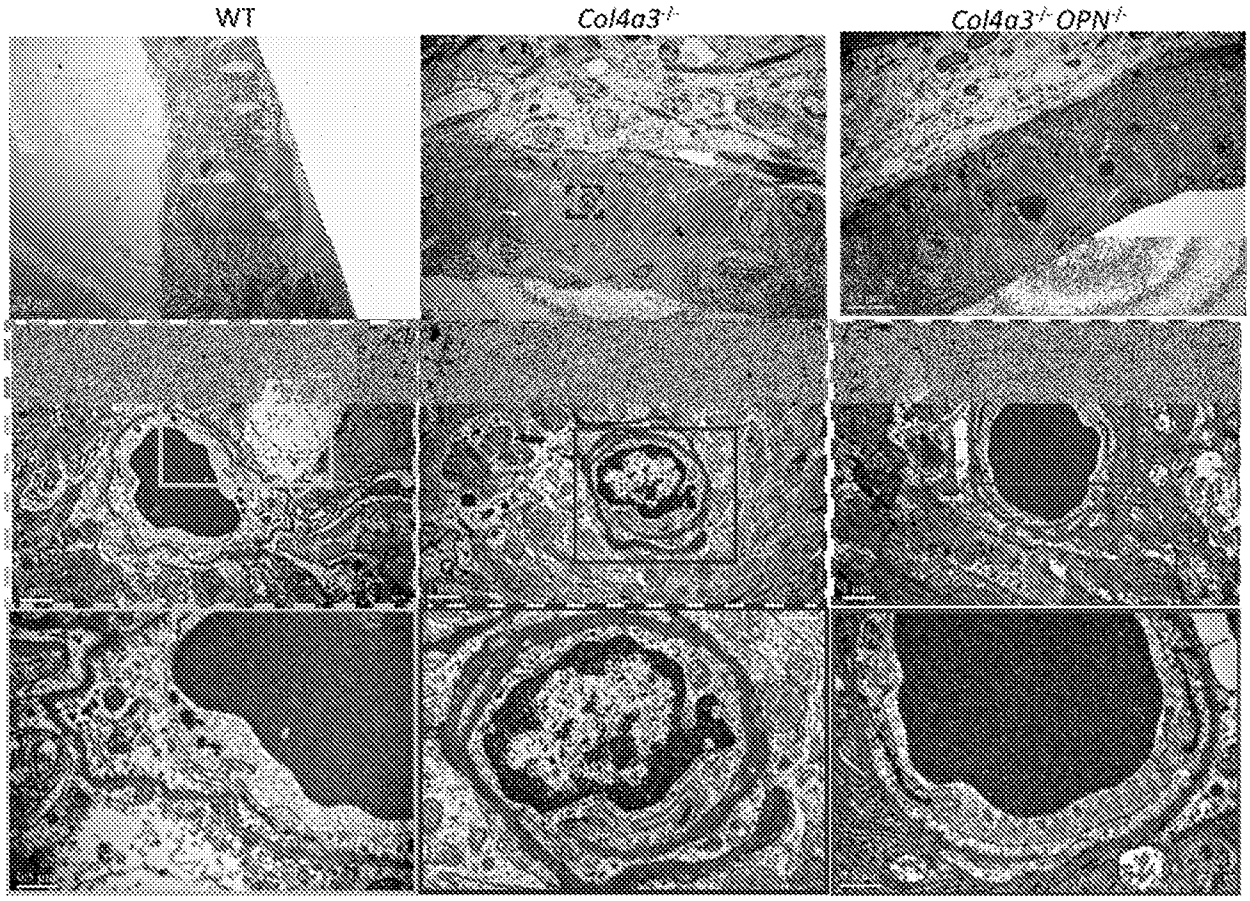


FIGURE 8A

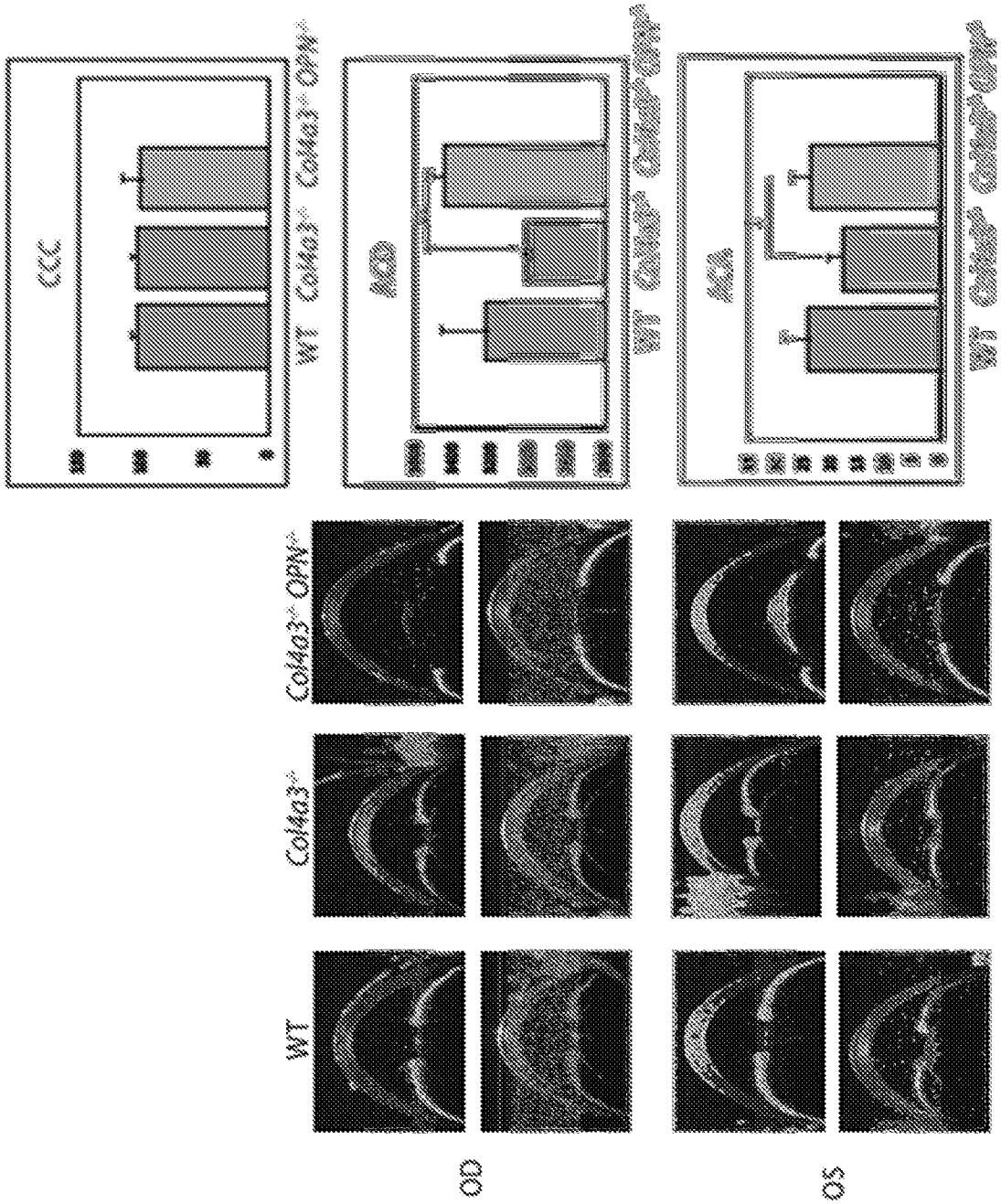
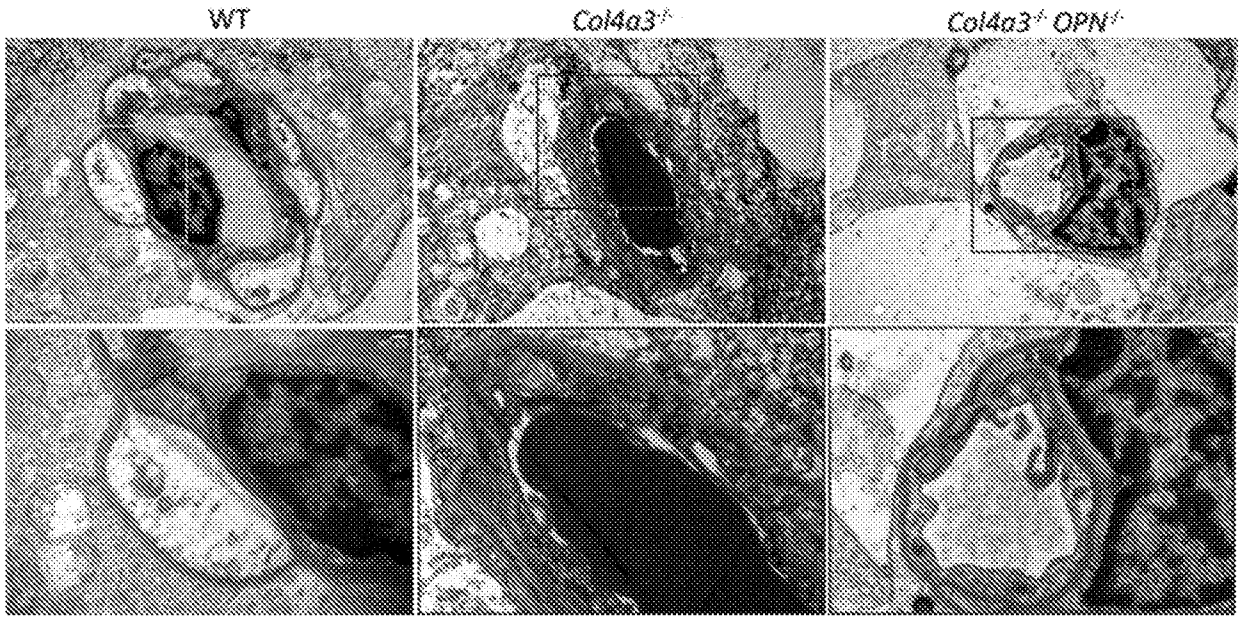


FIGURE 8B



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/045536

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

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

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/045536

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/165; A61K 31/7088; A61K 31/74; A61K 39/39; A61P 9/12 (2017.01)

CPC - A01K 2267/0306; A61K 38/00; A61K 39/00; A61K 48/00; C07K 14/78; C07K 16/2866; C12N 9/6491; C12N 15/113 (2017.08)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

USPC - 424/130.1; 435/375; 514/44A; 530/387.9; 530/387.1; 530/388.1; 530/388.26 (keyword delimited)

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2004/0037806 A1 (ROCHA et al) 26 February 2004 (26.02.2004) entire document	11 --- 2, 4, 21, 23, 25, 42
X -- Y	US 2012/0288507 A1 (QIAN et al) 15 November 2012 (15.11.2012) entire document	12 --- 5, 33
X -- Y	US 2015/0329866 A1 (QUARK PHARMACEUTICALS, INC.) 19 November 2015 (19.11.2015) entire document	13 --- 8, 29, 34
X -- Y	US 2012/0289604 A1 (MCKEARN et al) 15 November 2012 (15.11.2012) entire document	14, 35 --- 9, 30
X	US 6,458,590 B1 (MUKHERJEE et al) 01 October 2002 (01.10.2002) entire document	32
Y	US 2014/0140922 A1 (QUARK PHARMACEUTICALS, INC.) 22 May 2014 (22.05.2014) entire document	1, 2, 4-9, 16-21, 22b, 23, 25-30, 37-42
Y	XIE et al. "Expression, roles, receptors, and regulation of osteopontin in the kidney," Kidney Int. 01 November 2001 (01.11.2001), Vol. 60, Iss. 5, Pgs. 1645-1657. entire document	1, 2, 4-9, 16-21, 22b, 23, 25-30, 37-42

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

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
04 October 2017	06 NOV 2017

Name and mailing address of the ISA/US	Authorized officer
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/045536

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2009/023411 A1 (BAUSCH & LOMB INCORPORATED et al) 19 February 2009 (19.02.2009) entire document	19, 40
Y	PEI et al. "Osteopontin deficiency reduces kidney damage from hypercholesterolemia in Apolipoprotein E-deficient mice," Scientific Reports, 29 June 2016 (29.06.2016), Vol. 6, Iss. 28882, Pgs. 1-12. entire document	33
Y	KIM et al. "Patterns of Gene Expression Associated with Pten Deficiency in the Developing Inner Ear," PLoS One, 03 June 2014 (03.06.2014), Vol. 9, Iss. 6, Pgs. 1-8. entire document	34
Y	US 2005/0147602 A1 (LINDNER) 07 July 2005 (07.07.2005) entire document	39