



(51) International Patent Classification:

A61K 38/17 (2006.01) *A61K 48/00* (2006.01)
A61K 31/436 (2006.01) *A61K 45/06* (2006.01)
A61K 31/216 (2006.01)

(21) International Application Number:

PCT/US2017/041539

(22) International Filing Date:

11 July 2017 (11.07.2017)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/360,575 11 July 2016 (11.07.2016) US

(71) Applicant: **UNIVERSITY OF NORTH TEXAS
HEALTH SCIENCE CENTER AT FORT WORTH**
[US/US]; 3500 Camp Bowie Blvd., Fort Worth, TX
76109-2699 (US).

(72) Inventor: **ZODE, Gulab**; 3703 Byers Avenue, Fort Worth,
TX 76107 (US).

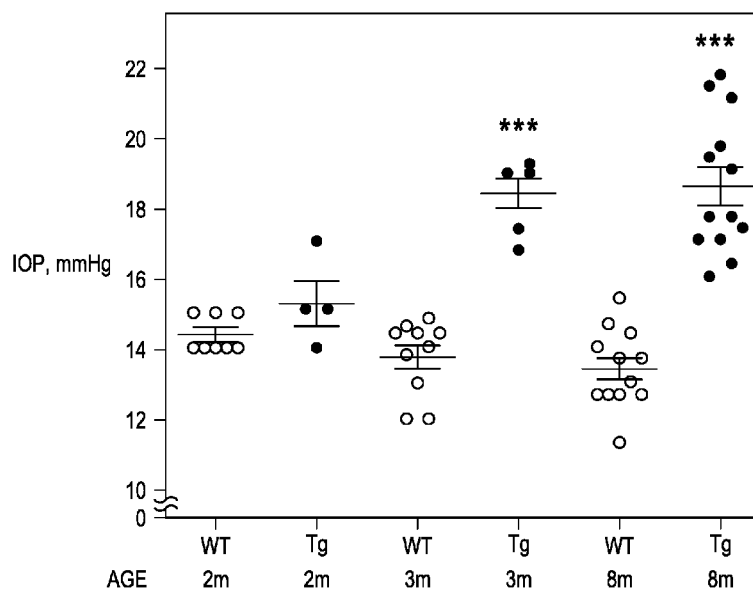
(74) Agent: **FLORES, Edwin, S.** et al.; Chalker Flores, LLP,
14951 North Dallas Parkway, Suite 400, Dallas, TX 75254
(US).

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

(84) Designated States (*unless otherwise indicated, for every
kind of regional protection available*): ARIPO (BW, GH,
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ,
UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,
TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,
EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,
MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,

(54) Title: TREATMENT FOR GLAUCOMA AND OTHER EYE DISEASES

FIG. 5A



(57) Abstract: The present invention includes a composition and method for the treatment of an eye disease comprising a therapeutically effective amount of an autophagy stimulator that treats or slows the progression of the eye disease by enhancing or stimulating autophagy or correcting an autophagy deficiency.

TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
KM, ML, MR, NE, SN, TD, TG).

Published:

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

TREATMENT FOR GLAUCOMA AND OTHER EYE DISEASES

TECHNICAL FIELD OF THE INVENTION

The present invention relates in general to the field of treatments for glaucoma and other eye diseases, and more particularly, to compositions and methods for enhancing autophagy to treat glaucoma and other eye diseases.

STATEMENT OF FEDERALLY FUNDED RESEARCH

This invention was made with government support under grant EY022077 and EYO26177 awarded by National Eye Institute. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Without limiting the scope of the invention, its background is described in connection with treatments for glaucoma.

Primary open angle glaucoma, a major cause of irreversible blindness, is often associated with elevated intraocular pressure (IOP) due to increased aqueous humor outflow resistance at the trabecular meshwork (TM). The pathological mechanisms leading to increased outflow resistance and IOP elevation are poorly understood.

Although the major pathology lies in the trabecular meshwork (increased outflow resistance elevates IOP in glaucoma), most current glaucoma treatments reduce IOP by reducing aqueous humor production by acting on ciliary body. In addition, these glaucoma treatments do not prevent blindness but merely halt progression of disease to some extent. Most glaucoma patients must undergo surgical procedures to reduce elevated IOP, which becomes expensive. Developing targeted treatments based on the understanding of TM pathology may improve clinical outcome and cure disease.

SUMMARY OF THE INVENTION

In one embodiment, the present invention includes a composition for the treatment of an eye disease comprising a therapeutically effective amount of an autophagy stimulator that treats or slows the progression of the eye disease by enhancing or stimulating autophagy or correcting an autophagy deficiency. In one aspect, the amount of the composition is sufficient to reduce at least one of protein misfolding, defective autophagy, or endoplasmic reticulum stress or increase autophagy. In another aspect, the autophagy stimulator is provided with a synergistic amount of a protein chaperone sufficient to treat or slow the progression of the eye disease. In another aspect, the eye disease comprises one or more symptoms selected from at least one of elevated intraocular pressure, increased aqueous humor outflow resistance at the trabecular meshwork, accumulation of misfolded proteins, or cell death. In another aspect, the

composition is adapted for subcutaneous, cutaneous, intravitreal, intraocular, or ocular administration. In another aspect, the eye disease is caused by misfolded proteins, the loss of clearance of misfolded proteins, the accumulation of misfolded proteins, or a decrease in lysosomal activity. In another aspect, the eye disease is glaucoma, endoplasmic reticulum (ER) stress, autophagy deficiency, age-related macular degeneration (AMD), or diabetic retinopathy. In another aspect, the composition further comprises one or more pharmaceutically acceptable excipients. In another aspect, the autophagy stimulator is selected from at least one of: beclin 1 protein, Tat-beclin1 peptide (SEQ ID NO:1), Tat-Beclin 1 D11 (SEQ ID NO:10), trehalose, ISRIB, or an mTOR inhibitor. In another aspect, the mTOR inhibitor is at least one of rapamycin, temsirolimus, everolimus, KU-0063794, WYE-354, AZD8055, metformin, or Torin-2. In another aspect, the protein chaperone is 4-phenylbutyrate or a salt thereof, 1-deoxygalactonojirimycin or a salt thereof, isofagomine or a salt thereof, fagomine isomers or a salt thereof, dimethylsulfoxide (DMSO) or a salt thereof, tauroursodeoxycholic acid (TUDCA) or a salt thereof, ursodeoxycholic acid (UDCA) or a salt thereof, glycine betaine (betaine) or a salt thereof, glycerolphosphocholine (GPC) or a salt thereof, methylamines or a salt thereof, or trimethylamine N-oxide (TMAO), or a Histone deacetylase (DHAC inhibitor selected from Vorinostat, Romidepsin, Chidamide, Panobinostat, Valproic acid (e.g., Mg valproate), Belinostat, Mocetinostat (MGCD0103), Abexinostat (PCI-24781), Entinostat (MS-275), SB939, Resminostat (4SC-201), Givinostat (ITF2357), Quisinostat, HBI-8000, Kevetrin, CUDC-101, AR-42, CHR-2845, CHR-3996, 4SC-202, CG200745, ACY-1215, lenalidomide, ME-344, sulforaphane, and/or alpha-crystallin protein or a salt thereof. In another aspect, the autophagy stimulator is an inhibitor of a CHOP gene, an ATF4 gene, or both, comprising a gene knockdown construct selected from at least one of an siRNA, a miRNA, an shRNA, an antisense RNA, or an sgRNA, a programmable gene editing nuclease (CRISPR-Cas) knockdown of ATF4 and/or CHOP, or a gene editing or therapy that reduces expression of ATF4 and/or CHOP. In another aspect, the sgRNA construct targets an exon, intron, or exon/intron junction of a human CHOP gene and/or an exon, intron, or exon/intron junction of a human ATF4 gene. In another aspect, the sgRNA construct comprises (GACCACTCTGTTTCCGTTTCC) (SEQ ID NO:4) and/or (AGGTCTCTTAGATGATTACC) (SEQ ID NO:5).

In another embodiment, the present invention includes a composition for the treatment of an eye disease comprising a therapeutically effective amount of autophagy stimulator or enhancer adapted for treatment of the eye. In one aspect, the amount of the composition is sufficient to

reduce at least one of protein misfolding, defective autophagy, or endoplasmic reticulum stress or increase autophagy. In another aspect, the eye disease comprises one or more symptoms selected from at least one of elevated intraocular pressure, increased aqueous humor outflow resistance at the trabecular meshwork, accumulation of misfolded proteins, or cell death. In another aspect, the composition is adapted for subcutaneous, cutaneous, intravitreal, or ocular administration. In another aspect, the eye disease is caused by misfolded proteins, the loss of clearance of misfolded proteins, the accumulation of misfolded proteins, or a decrease in lysosomal activity. In another aspect, the eye disease is glaucoma, endoplasmic reticulum (ER) stress, autophagy deficiency, age-related macular degeneration (AMD), or diabetic retinopathy. In another aspect, the composition further comprises one or more pharmaceutically acceptable excipients. In another aspect, the autophagy stimulator is selected from at least one of: beclin 1 protein, Tat-beclin1 peptide (SEQ ID NO:1), Tat-Beclin 1 D11 (SEQ ID NO:10), trehalose, ISRIB, or an mTOR inhibitor. In another aspect, the mTOR inhibitor is at least one of rapamycin, temsirolimus, everolimus, KU-0063794, WYE-354, AZD8055, metformin, or Torin-2. In another aspect, the amount is sufficient to treat or reduce the progression of the eye disease. In another aspect, the composition further comprises a chemical protein chaperone in an amount that is synergistic for treating the eye disease.

In yet another embodiment, the present invention includes a composition for the treatment of an eye disease comprising a therapeutically effective amount of an inhibitor of a CHOP gene, an ATF4 gene, or both, comprising a gene knockdown construct selected from at least one of an siRNA, a miRNA, an shRNA, an antisense RNA, or an sgRNA, a programmable gene editing nuclease (CRISPR-Cas) knockdown of ATF4 and/or CHOP, or a gene editing or therapy that reduces expression of ATF4 and/or CHOP.

In another embodiment, the present invention includes a method of treating eye disease comprising: identifying a subject in need of treatment for an eye disease; and providing the subject with an effective amount of an autophagy stimulator sufficient to reduce at least one of defective autophagy, endoplasmic reticulum stress, or elevated intraocular pressure or increase autophagy. In one aspect, the eye disease comprises one or more symptoms selected from at least one of increased aqueous humor outflow resistance at the trabecular meshwork, accumulation of misfolded proteins, or cell death. In another aspect, the autophagy stimulator is selected from at least one of: beclin 1 protein, Tat-beclin1 peptide (SEQ ID NO:1), Tat-Beclin 1 D11 (SEQ ID NO:10), trehalose, ISRIB, or an mTOR inhibitor. In another aspect, the mTOR inhibitor is at least one of rapamycin, temsirolimus, everolimus, KU-0063794, WYE-

354, AZD8055, metformin, or Torin-2. In another aspect, the method further comprises the step of providing the subject with a protein chaperone is selected from at least one of 4-phenylbutyrate or a salt thereof, 1-deoxygalactonojirimycin or a salt thereof, isofagomine or a salt thereof, fagomine isomers or a salt thereof, dimethylsulfoxide (DMSO) or a salt thereof, 5 tauroursodeoxycholic acid (TUDCA) or a salt thereof, ursodeoxycholic acid (UDCA) or a salt thereof, glycine betaine (betaine) or a salt thereof, glycerolphosphocholine (GPC) or a salt thereof, methylamines or a salt thereof, or trimethylamine N-oxide (TMAO), or a Histone deacetylase (DHAC inhibitor selected from Vorinostat, Romidepsin, Chidamide, Panobinostat, Valproic acid (e.g., Mg valproate), Belinostat, Mocetinostat (MGCD0103), Abexinostat (PCI-10 24781), Entinostat (MS-275), SB939, Resminostat (4SC-201), Givinostat (ITF2357), Quisinostat, HBI-8000, Kevetrin, CUDC-101, AR-42, CHR-2845, CHR-3996, 4SC-202, CG200745, ACY-1215, lenalidomide, ME-344, sulforaphane, and/or alpha-crystallin protein or a salt thereof, in a synergistic amount with the autophagy stimulator sufficient to treat the eye disease. In another aspect, the method further comprises the step of providing the subject with 15 a composition that comprises a synergistic amount of a protein chaperone and beclin1 protein, Tat-beclin1 peptide (SEQ ID NO:1), Tat-Becn1 D11 (SEQ ID NO:10), trehalose, ISRIB, rapamycin, temsirolimus, everolimus, KU-0063794, WYE-354, AZD8055, metformin, or Torin-2, or 4-phenylbutyrate or a salt thereof. In another aspect, the autophagy stimulator is adapted for subcutaneous, cutaneous, intravitreal, intraocular, or ocular administration. In 20 another aspect, the method further comprises the step of determining that the subject has a disease caused by misfolded proteins, the loss of clearance of misfolded proteins, the accumulation of misfolded proteins, or a decrease in lysosomal activity; and providing the subjected with the effective amount of a Tat-beclin1 peptide, Tat-beclin1 D11, trehalose, ISRIB, rapamycin, temsirolimus, everolimus, KU-0063794, WYE-354, AZD8055, metformin, 25 Torin-2, and sodium 4-phenylbutyrate to treat the eye disease. In another aspect, the eye disease is glaucoma, endoplasmic reticulum (ER) stress, autophagy deficiency, age-related macular degeneration (AMD), or diabetic retinopathy. In another aspect, the autophagy stimulator is an inhibitor of a CHOP gene, an ATF4 gene, or both, comprising a gene knockdown construct selected from at least one of an siRNA, a miRNA, an shRNA, an antisense RNA, an sgRNA, a programmable gene editing nuclease (CRISPR-Cas) knockdown 30 of ATF4 and/or CHOP, or a gene editing or therapy that reduces expression of ATF4 and/or CHOP. In another aspect, the sgRNA construct targets an exon, intron, or exon/intron junction of a human CHOP gene and/or an exon, intron, or exon/intron junction of a human ATF4 gene.

In another aspect, the sgRNA construct comprises (GACCACTCTGTTTCCGTTTCC) (SEQ ID NO:4) and/or (AGGTCTCTTAGATGATTACC) (SEQ ID NO:5).

In another embodiment, the present invention includes a method of lowering intraocular pressure in a mammal comprising administering to a subject in which such pressure lowering is desired, at least one of an effective amount of autophagy stimulator to achieve the desired lowering in pressure. In one aspect, the autophagy stimulator is selected from at least one of: beclin 1 protein, Tat-beclin1 peptide (SEQ ID NO:1), Tat-Beclin 1 D11 (SEQ ID NO:10), trehalose, ISRIB, or an mTOR inhibitor. In another aspect, the mTOR inhibitor is at least one of rapamycin, temsirolimus, everolimus, KU-0063794, WYE-354, AZD8055, metformin, or Torin-2. In another aspect, the method further comprises providing a synergistic amount of a protein chaperone for treating the eye disease. In another aspect, the protein chaperone is selected from at least one of 4-phenylbutyrate or a salt thereof, 1-deoxygalactonojirimycin or a salt thereof, isofagomine or a salt thereof, fagomine isomers or a salt thereof, dimethylsulfoxide (DMSO) or a salt thereof, tauroursodeoxycholic acid (TUDCA) or a salt thereof, ursodeoxycholic acid (UDCA) or a salt thereof, glycine betaine (betaine) or a salt thereof, glycerolphosphocholine (GPC) or a salt thereof, methylamines or a salt thereof, or trimethylamine N-oxide (TMAO), or a Histone deacetylase (DHAC) inhibitor selected from Vorinostat, Romidepsin, Chidamide, Panobinostat, Valproic acid (e.g., Mg valproate), Belinostat, Mocetinostat (MGCD0103), Abexinostat (PCI-24781), Entinostat (MS-275), SB939, Resminostat (4SC-201), Givinostat (ITF2357), Quisinostat, HBI-8000, Kevetrin, CUDC-101, AR-42, CHR-2845, CHR-3996, 4SC-202, CG200745, ACY-1215, lenalidomide, ME-344, sulforaphane, and/or alpha-crystallin protein or a salt thereof. In another aspect, the autophagy stimulator is an inhibitor of a CHOP gene, an ATF4 gene, or both, comprising a gene knockdown construct selected from at least one of an siRNA, a miRNA, an shRNA, an antisense RNA, an sgRNA, a programmable gene editing nuclease (CRISPR-Cas) knockdown of ATF4 and/or CHOP, or a gene editing or therapy that reduces expression of ATF4 and/or CHOP. In another aspect, the sgRNA construct targets an exon, intron, or exon/intron junction of a human CHOP gene and/or an exon, intron, or exon/intron junction of a human ATF4 gene. In another aspect, the sgRNA construct comprises (GACCACTCTGTTTCCGTTTCC) (SEQ ID NO:4) and/or (AGGTCTCTTAGATGATTACC) (SEQ ID NO:5).

In yet another embodiment, the present invention includes a method of evaluating a candidate drug believed to be useful in treating an eye disease, the method comprising: a) measuring at least one of protein misfolding, defective autophagy, or endoplasmic reticulum stress from

tissue suspected of having an eye disease from a set of patients; b) administering the candidate drug to a first subset of the patients, and a placebo to a second subset of the patients; c) repeating step a) after the administration of the candidate drug or the placebo; and d) determining if the candidate drug reduces the level of protein misfolding, defective autophagy, or endoplasmic reticulum stress that is statistically significant as compared to any reduction occurring in the second subset of patients, wherein a statistically significant reduction indicates that the candidate drug is useful in treating the eye disease.

BRIEF DESCRIPTION OF THE DRAWINGS

For a more complete understanding of the features and advantages of the present invention, reference is now made to the detailed description of the invention along with the accompanying figures and in which:

FIG. 1A shows the result from treating TM-3 cells expressing mutant myocilin with various concentrations of TS or TB for 24 hours. The cell lysates were subjected to Western blot analysis for MYOC, p62 and LC3B (n=3). FIG. 1B shows the co-localization of LC3B positive autophagic vesicles (green) with MYOC (red) upon TB treatment. FIG. 1C is a graph that shows ocular hypertensive Tg-MYOCY437H mice treated with topical TB (5mM) or TS (5mM) eye drops 3 times daily for 4 days and measurements of WT (n=2) or Tg-MYOCY437H mice (n=3) show that TB treatment significantly reduces elevated IOP.

FIG. 2A shows the results from treating TM-3 cells expressing mutant myocilin with or without trehalose (100mM) for 24 hours and cell lysates were subjected to Western blot analysis for myocilin and LC3B. FIG. 2B shows the results from treating TM-3 cells expressing mutant myocilin with or without rapamycin for 24 hours and cell lysates were subjected to Western blot analysis for myocilin and LC3B. Both trehalose and rapamycin reduced mutant myocilin and also increased LC3BII, a marker of stimulated autophagy.

FIG. 3 shows the results from treating TM-3 cells expressing mutant myocilin with PBA (1mM), TB or TB+PBA for 24 hours and cell lysates were subjected to Western blot analysis for MYOC.

FIG. 4 is a drawing that shows the ATF4 and CHOP signaling pathway.

FIGS. 5A to 5D show: FIG. 5A is a graph that shows IOP elevation in different aged group Tg-MYOC^{Y437H} mice compared to WT litter mates (n=8); FIG. 5B is a graph that shows reduced outflow facility in 7 months old Tg-MYOC^{Y437H} mice compared to WT littermates (n=8-10); FIG. 5C is a graph that shows IOP elevation in 0.1% topical ocular dex treated mice compared to vehicle treated mice; and FIG. 5D is a graph that shows reduced outflow facility in dex

treated mice compared to vehicle treated mice (** $p < 0.005$, *** $p < 0.0001$; vs veh-treated mice; n=8).

FIGS. 6A to 6C show Western blots of UPR markers, specifically: FIG. 6A is the anterior segment tissues of 6-month-old WT and *Tg-MYOC^{Y437H}* mice (n=3WT & 3 Tg); FIG. 6B is the anterior segment tissues of vehicle (n=4) and Dex (n=4)- treated mice; and FIG. 6C is a post-mortem human normal and glaucomatous TM tissues lysates (n=4).

FIG. 6D shows immunostaining for CHOP & KDEL in normal and glaucomatous human TM tissues.

FIGS. 7A to 7C show: FIG. 7A is a Western blot of MYOC in conditioned media (CM) and cell lysates (CL) of TM-5 cells expressing WT or mutant MYOC; FIG. 7B shows the MYOC accumulation and the presence of aggregates (shown by arrow); and FIG. 7C is a Western blot that shows the induction of apoptotic markers in mutant MYOC expressing cells.

FIG. 8 shows IOP measured in WT mice intravitreally injected with 107 p.f.u. of Ad5 expressing null, ATF4 and CHOP (10-days post injections).

FIGS. 9A and 9B show IOP levels were measured in 3 months old littermates of: FIG. 9A is a Wt mice, *Tg-MYOC^{Y437H}* mice, CHOP -/- mice & double mutant mice (*Tg-MYOC^{Y437H}/CHOP-/-*); FIG. 9B shows the effect of 0.1% topical ocular dex treated WT mice, Vehicle treated WT mice (0.1% EtOH), Dex treated CHOP-/- mice & Vehicle treated CHOP-/- mice.

FIGS. 10A and 10B show: FIG. 10A is a Western blot showing myocilin and ER stress markers in TM-5 cells stably expressing mutant MYOC were transiently transfected with plasmids expressing Cas9-CHOP or ATF4; and FIG. 10B shows the CRISPR-CAS9 mediated deletion of CHOP verified in CRISPR-CHOP transfected clones by surveyor nuclear digestion analysis.

FIGS. 11A and 11B show as follows: FIG. 11A is a Western blot of TM5 cells stably expressing mutant myocilin transfected with plasmid expressing CAS9-CHOP. Myocilin levels, autophagy marker (LC3B) and ER stress markers were analyzed; and FIG. 11B shows immunostaining showing Myocilin (Red), LC3B(Green) in TM5 cells stably expressing mutant myocilin transfected with plasmid expressing CAS9-CHOP.

FIGS. 12A and 12B show the effect of ISRIB on mutant myocilin accumulation. FIG. 12A shows a Western blot of MYOC, ATF4 and CHOP in TM cells expressing WT or mutant MYOC treated with vehicle or ISRIB for 48 hours. FIG. 12B is a graph that shows the IOP of the left eyes of *Tg-MYOC^{Y437H}* mice injected with ISRIB are compared with vehicle-injected contralateral right eyes. N=5; paired t-test.

FIGS. 13A and 13B show the effects of autophagy stimulator Tat-Beclin 1 D11, a shorter Tat-Beclin 1 peptide, on IOP. FIG. 13A shows WT and *Tg-MYOC^{Y437H}* mice were given topical eye drops of TS (left eyes) and TB (D11) in the contralateral right eyes for 2 weeks. IOP were measured weekly in the night. TB treatment reduced elevated IOP in *Tg-MYOC^{Y437H}* mice but
5 did not alter IOP in WT mice (n=6 each; one way ANOVA, *p<0.5). FIG. 13B shows IOP of the left eyes of *Tg-MYOC^{Y437H}* mice given Torin-2 eye drops topically for 1 week compared with vehicle eye drops given to the contralateral right eyes. N=8 ; P<0.001, paired t-test.

FIG. 14 is a graph that shows IOP measurements in mice treated with CR-ATF4.

FIG. 15 shows that knockdown of ATF4 or CHOP inhibits TGFβ2 signaling and prevents
10 abnormal Dex-induced ECM accumulation and ER stress in TM cells.

DETAILED DESCRIPTION OF THE INVENTION

While the making and using of various embodiments of the present invention are discussed in detail below, it should be appreciated that the present invention provides many applicable inventive concepts that can be embodied in a wide variety of specific contexts. The specific
15 embodiments discussed herein are merely illustrative of specific ways to make and use the invention and do not delimit the scope of the invention.

To facilitate the understanding of this invention, a number of terms are defined below. Terms defined herein have meanings as commonly understood by a person of ordinary skill in the areas relevant to the present invention. Terms such as “a”, “an” and “the” are not intended to
20 refer to only a singular entity, but include the general class of which a specific example may be used for illustration. The terminology herein is used to describe specific embodiments of the invention, but their usage does not delimit the invention, except as outlined in the claims. The present invention includes the treatment of glaucoma (and other ocular diseases) by enhancing or stimulating autophagy or correcting autophagy deficiency. Specifically, in addition to
25 glaucoma the present invention can be used for the treatment of other ocular diseases associated with, e.g., Endoplasmic Reticulum (ER) stress and/or autophagy deficiency, Age-related Macular Degeneration (AMD), and/or Diabetic Retinopathy.

The present invention also includes compositions and methods for gene therapy modulation (inhibition) of ATF4 and/or CHOP to enhance autophagy for treatment of glaucoma/ocular
30 diseases, including but not limited to, programmable gene editing nuclease (e.g., CRISPR-Cas) knockdown of ATF4 and/or CHOP, and/or other gene editing/therapy techniques to reduce expression of ATF4 and/or CHOP (including but not limited to siRNA, miRNA).

The present invention also includes methods for using, and compositions that include proteins/peptides (Beclin 1, Tat-Beclin, Tat-Beclin D11), or small molecules for use in making a medicament for treating, and methods of treating ocular diseases by enhancing autophagy to treat the glaucoma and other ocular diseases caused by decreased autophagy.

- 5 The present invention also includes methods and compositions that include one or more chemical enhancers of autophagy for treatment of glaucoma/ocular diseases (use of Trehalose, Rapamycin, Torin-2). The present invention also includes methods and compositions that include one or more mTOR inhibitors that induce autophagy, including but not limited to Rapamycin, Torin-2, everolimus, temsirolimus, KU-0063794, WYE-354, AZD8055,
10 metformin and derivatives, analogs or prodrugs thereof. Finally, the present invention also includes methods and compositions that use small molecule inhibitors of ATF4/CHOP pathway for treatment of glaucoma/ocular diseases (e.g., ISRIB).

- A dosage unit for use of the composition of the present invention, may be a single compound or mixtures thereof with other compounds. In one non-limiting example, the composition may
15 be a single dosed ampule or equivalent, or a dropper-bottle that includes the composition and can be provided to the subject in the form of drops. The compounds may be mixed together, may form ionic bonds, or covalent bonds. The composition of the present invention may be administered in cutaneous, subcutaneous, intraocular, or ocular form, all using dosage forms well known to those of ordinary skill in the pharmaceutical arts. Depending on the particular
20 location or method of drops, serums, elixirs, tinctures, suspensions, syrups, and emulsions may be used to provide the composition of the present invention to a patient in need of therapy for a medical condition or symptom. The composition may also be administered as any one of known salt forms of the compounds or molecules used in the composition.

- The composition of the present invention is typically administered in admixture with suitable
25 pharmaceutical salts, buffers, diluents, extenders, excipients and/or carriers (collectively referred to herein as a pharmaceutically acceptable carrier or carrier materials) selected based on the intended form of administration and as consistent with conventional pharmaceutical practices. Depending on the best location for administration, the composition may be formulated to provide, e.g., maximum and/or consistent dosing for the particular form for
30 ocular administration. While the composition may be administered alone, it will generally be provided in a stable salt form mixed with a pharmaceutically acceptable carrier. The carrier may be solid or liquid, depending on the type and/or location of administration selected.

Techniques and compositions for making useful dosage forms using the present invention are described in one or more of the following references: Anderson, Philip O.; Knoben, James E.; Troutman, William G, eds., Handbook of Clinical Drug Data, Tenth Edition, McGraw-Hill, 2002; Pratt and Taylor, eds., Principles of Drug Action, Third Edition, Churchill Livingstone, New York, 1990; Katzung, ed., Basic and Clinical Pharmacology, Ninth Edition, McGraw Hill, 2003; Goodman and Gilman, eds., The Pharmacological Basis of Therapeutics, Tenth Edition, McGraw Hill, 2001; Remington's Pharmaceutical Sciences, 20th Ed., Lippincott Williams & Wilkins., 2000; Martindale, The Extra Pharmacopoeia, Thirty-Second Edition (The Pharmaceutical Press, London, 1999); Remington: The Science and Practice of Pharmacy, Pharmaceutical Press; 22nd Edition (2012), relevant portions incorporated herein by reference.

The present inventors recently linked protein misfolding and endoplasmic reticulum (ER) stress to the development of glaucomatous trabecular meshwork (TM) damage and intraocular pressure (IOP) elevation. TM cells activate protective unfolded protein response (UPR) pathway to eliminate abnormal protein accumulation. When the levels of unfolded proteins overwhelm ER-associated proteosomal degradation, cells have the capacity to activate autophagy, a compensatory lysosomal degradation system. Autophagy can adapt to cellular stress and act as a cell survival mechanism via clearance of misfolded proteins and reduction of abnormal protein accumulation. Defective autophagy can lead to accumulation of misfolded and abnormal proteins and cell death. The role of autophagy in regulation of IOP was investigated herein and treatments for IOP demonstrated. The results herein demonstrate for the first time that autophagy is impaired in the TM of mouse models of glaucoma as well as in human post-mortem glaucomatous TM tissues. Therefore, drugs that correct misfolding as well as enhance autophagic degradation of misfolded protein may reduce ER stress and prevent IOP elevation. The present inventors have found that treating trabecular meshwork pathology by drugs that reduce protein misfolding and increase abnormal misfolded protein degradation by autophagy significantly improve clinical outcomes and prevent irreversible blindness.

In the past, most efforts have been focused on using strategies to correct protein misfolding using drugs that enhance protein secretion. The present inventors have shown that sodium 4-phenylbutyrate enhances secretion of misfolded mutant myocilin, thus reducing ER stress and protects from ocular hypertension. In addition, other groups have shown that this approach reduces mutant myocilin accumulation in cell culture (*Invest Ophthalmol Vis Sci.* 2007 Apr;48(4):1683-90). Until now, any role for autophagy in IOP elevation has not been explored. Cell culture studies suggest that knockdown of GRP94 reduces mutant myocilin and

enhances autophagic clearance of mutant myocilin. The present invention shows for the first time that autophagy is impaired in glaucoma models and treating mice or cells with one or more specific stimulator of autophagy enhances autophagic degradation of mutant myocilin, reducing elevated IOP. Further, when combined with a chemical chaperone, a surprising synergistic effect is shown herein for the combination of an autophagy stimulator (enhancer or agonist) and a chemical protein chaperon. Other chemical chaperones for use with the present invention include, e.g., Histone deacetylase inhibitors (HDAC inhibitors). Non-limiting examples of HDAC selective and non-selective inhibitors include, but are not limited to: Vorinostat, Romidepsin, Chidamide, Panobinostat, Valproic acid (e.g., Mg valproate), Belinostat, Mocetinostat (MGCD0103), Abexinostat (PCI-24781), Entinostat (MS-275), SB939, Resminostat (4SC-201), Givinostat (ITF2357), Quisinostat, HBI-8000, Kevetrin, CUDC-101, AR-42, CHR-2845, CHR-3996, 4SC-202, CG200745, ACY-1215, lenalidomide, ME-344, and/or sulforaphane. Another chaperone protein includes Alpha-crystallin and members of the Alpha-crystallin (such as Alpha-crystallin EMBL: PF00525 or IPR003090) that act as protein chaperones.

Beclin1. In alternative embodiments, the invention provides for use of a sequence comprising all or a subsequence of Beclin1 or a peptidomimetic or synthetic form thereof, or an equivalent thereof, that induces, stimulates or increases autophagy.

One example of a Beclin1 amino acid sequence for use with the present invention is as follows:

Length: 450 amino acids, Mass (Da): 51,896, Last modified: February 21, 2001 - v2: obtained from UniProtKB - Q14457-1 (BECN1_HUMAN).

	10	20	30	40	50
	MEGSKTSNNS	TMQVSFVCQR	CSQPLKLDTS	FKILDRVITI	Q ELTAPLLTTA
	60	70	80	90	100
25	QAKPGETQEE	ETNSGEEPFI	ETPRQDGVSR	RFIPPARMMS	TESANSFTLI
	110	120	130	140	150
	GEASDGGTME	NLSRRLKVTG	DLFDIMSGQT	DVDHPLCEEC	TDTLLEDQLDT
	160	170	180	190	200
	QLNVTENECQ	NYKRCLEILE	QMNEEDDSEQL	QMELKELALE	EERLIQEELED
30	210	220	230	240	250
	VEKNRKIVAE	NLEKVQAEAE	RLDQEEAQYQ	REYSEFKRQQ	LELDDELKSV
	260	270	280	290	300
	ENQMRYAQTQ	LDKLKKTNVF	NATFHIWHSG	QFGTINNFRL	GRLPSVPVEW
	310	320	330	340	350

NEINAAWGQT VLLLHALANK MGLKFQRYRL VPYGNHSTYLE SLTDKSKELP
 360 370 380 390 400
 LYCSGGLRFF WDNKFDHAMV AFLDCVQQFK EEVEKGETRF CLPYRMDVEK
 410 420 430 440 450
 5 GKIEDTGGS GSYSIKTQFN SEEQWTKALK FMLTNLKWGL AWWSSQFYNK

SEQ ID NO. 3, see www.uniprot.org/uniprot/Q14457#sequences.

Tat-beclin1 Peptide: Tat-beclin1 (TB) peptide was bought from EMD Millipore Corporation (MA, USA). TB was dissolved in sterile water and kept in -20°C. The peptide sequences for the Tat-beclin1 (TB) peptide is YGRKKRRQRRR-GG-TNVFNATFEIWHDGEFGT (SEQ ID NO:1). Tat-beclin 1 is cell permeable and induces autophagy. Tat-scrambled Peptide: Tat-scrambled peptide (TS) was bought from AnaSpec, Inc. (CA, USA). TS was dissolved in sterile water and kept in -20°C. The Tat-Beclin-1, scrambled peptide sequences is YGRKKRRQRRRGVGNDFFINHETTGFATEW (SEQ ID NO:2), and does not induce autophagy and was used as a negative control as described previously (1). Furthermore, a shorter amino acid sequences for Tat-Beclin 1, D11, having an amino acid sequence: RRRQRRKKRGYGGDHWIHFTANWV (SEQ ID NO:10), was also found to reduce IOP.

Treatment of trabecular meshwork (TM)-3 cells stably expressing DsRed tagged WT or Y437H mutant MYOC protein. The inventors recently generated TM-3 cells stably expressing DsRed tagged WT or Y437H mutant MYOC, which allows us to visualize myocilin in live cells and facilitates various therapeutic strategies. WT MYOC is secreted while mutant MYOC is not secreted and accumulates in the TM cells. Furthermore, mutant MYOC forms aggregates (B) and induces ER stress as described previously (2). TM-3 cells expressing mutant myocilin were grown on 24-well plate and incubated either with TS (control) or TB at various concentrations. 24 hours later, cell lysates were subjected for Western blot analysis of myocilin and ER stress as well as autophagy markers. Sodium 4-phenylbutyrate (PBA) was bought from Scandinavian Formulas, Inc. The PBA was dissolved in sterile water and cells were incubated at concentration of 1mM. Trehalose was bought from Sigma. TM cells were incubated at concentration of 100mM Rapamycin (bought from Sigma) of rapamycin for 24 hours.

Treatment of *Tg-MYOC*^{Y437H} mice with Tat-beclin1 peptide: A detailed characterization of *Tg-MYOC*^{Y437H} mice has been published previously (2). *Tg-MYOC*^{Y437H} mice on C57BL/6J background were crossed with pure strain AJ mice and F1 mice were intercrossed. Age-matched WT and *Tg-MYOC*^{Y437H} littermates were utilized for phenotype study and further biochemical analysis. Prior to treatment, IOP of WT and *Tg-MYOC*^{Y437H} mice was measured to

ensure IOP elevation in *Tg-MYOC^{Y437H}* mice. The left eyes of WT or *Tg-MYOC^{Y437H}* mice were given TB (5mM) while the contralateral right eyes were given TS eye drops (5mM) 3 times daily for 4 days. On fifth day, IOP were measured again.

Intraocular Pressure (IOP) Measurement.

5 Intraocular pressure (IOP) was determined in behaviorally trained conscious mice using a TonoLab rebound tonometer (Colonial Medical Supply), as described previously (3). Daytime IOP was measured between 9-11am. The first three individual IOP measurements made were discarded; the following 10 individual IOP measurements were recorded. The mean $\pm$ S.E.M. of the recorded readings was then calculated as the final IOP value for each eye at each IOP
10 measurement.

1. Tat-beclin 1 peptide induces autophagic degradation of mutant myocilin in TM-5 cells and topical ocular Tat-beclin1 reduces elevated IOP in *Tg-MYOC^{Y437H}* mice. The inventors determined whether improving autophagic flux via Tat-beclin (TB) 1 peptide reduces mutant MYOC. TM-5 cells stably expressing mutant MYOC were incubated with various doses of
15 Tat-beclin 1 (TB) or Tat-scrambled (TS) peptide (Fig.1A). Starting at 20uM, TB reduced mutant MYOC and also increased LC3B II form as well as reduced p62 levels. Increased LC3B II form and reduced p62 levels indicate that TB activates autophagy and also reduces accumulation of misfolded proteins. Immunostaining for LC3B revealed induction of autophagy and co-localization myocilin within autophagic vesicles upon TB treatment
20 (Fig.1B). Co-localization of myocilin with LC3B further suggests active autophagic degradation of mutant myocilin when treated with TB while TS treated cells did not exhibit autophagy. Next, the inventors treated ocular hypertensive *Tg-MYOC^{Y437H}* mice with topical TB or TS eye drops (Fig.1C). The left eye was treated with TB and the contralateral right eye was given TS peptide twice daily for 4 days. Topical ocular TB significantly reduced elevated
25 IOP compared to control contralateral eye treated with TS. TB treatment did not cause any toxicity or inflammation to the eyes.

2. General autophagy enhancers reduce mutant myocilin accumulation in TM cells. To test the inventors' findings that stimulation of autophagy reduces mutant myocilin accumulation in TM cells, the inventors tested the autophagy enhancers: Trehalose and rapamycin, on TM cells
30 expressing mutant myocilin. Several studies have shown that trehalose and rapamycin stimulates autophagy and reduce misfolded proteins (4-6). Western blot analysis for myocilin accumulation revealed that treatment with trehalose (FIG. 2A) or rapamycin (FIG. 2B) reduces mutant myocilin dramatically and also induced autophagy as evident from increased LC3BII form. These studies demonstrate that stimulation of autophagy via known autophagy

enhancers reduce mutant myocilin and further suggest that these autophagy enhancers can reduce elevated IOP in glaucoma. FIG. 2A shows the results from treating TM-3 cells expressing mutant myocilin with or without trehalose (100mM) for 24 hours and cell lysates were subjected to Western blot analysis for myocilin and LC3B. FIG. 2B shows the results from treating TM-3 cells expressing mutant myocilin with or without rapamycin for 24 hours and cell lysates were subjected to Western blot analysis for myocilin and LC3B. Both trehalose and rapamycin reduced mutant myocilin and also increased LC3B-II, a marker of stimulated autophagy. FIG. 3 shows the results from treating TM-3 cells expressing mutant myocilin with PBA (1mM), TB or TB+PBA for 24 hours and cell lysates were subjected to Western blot analysis for MYOC.

3. Combination of Tat-beclin and PBA synergistically reduces mutant myocilin accumulation in TM cells. The inventors have previously demonstrated that chemical chaperone; PBA reduces mutant myocilin and prevents glaucoma. The inventors demonstrated that PBA reduces elevated IOP via enhancing myocilin secretion and reducing endoplasmic reticulum stress in TM (2, 7). These data show that stimulation of autophagic degradation of mutant myocilin reduces elevated IOP. Therefore, it is possible that combination therapy of PBA to enhance secretion of mutant myocilin and autophagy stimulators to reduce mutant myocilin degradation will act synergistically to reduce protein misfolding and reduce elevated IOP more effectively. To test this hypothesis, the inventors treated TM cells expressing mutant myocilin with PBA alone, TB alone or combination of PBA with TB and examined mutant myocilin. Result show that PBA or TB alone reduces mutant myocilin. However, combination of PBA and TB reduce mutant myocilin more effectively than TB or PBA alone. These data demonstrate that a combination therapy of PBA and TB may reduce elevated IOP synergistically. Other chemical chaperones for use with the present invention include Histone deacetylase inhibitors (HDAC inhibitors) or an alpha-crystallin protein.

4. CRISPR for Glaucoma Treatment. Generation of plasmid expressing CRISPR-Cas9 targeting CHOP or ATF4. The human codon-optimized Cas9 plasmid pX459 (Addgene # 48139) was obtained from Addgene (10). sgRNAs were designed and constructed as described previously (10,11). Briefly, target 20 bp sequences starting with guanine and preceding the PAM motif (5'-NGG-3') were selected from mouse Chop exon 2 and human ATF4 exon 2. Potential off-target effects of sgRNA candidates were analyzed using the online tool CRISPR Design developed by Zhang's laboratory (<http://crispr.mit.edu/>).

One sgRNA in exon 2 of the mouse Chop gene (GACCACTCTGTTTCCGTTTCC) (SEQ ID NO:4) and exon 2 of the human ATF4 gene (AGGTCTCTTAGATGATTACC) (SEQ ID NO:5) was selected for transfection to create knockout cell lines. Clonal genomic DNA was extracted and used for PCR and Sanger sequencing. Primers for sequencing Chop were Chop-seqF (CACCACTCTTGACCCTGCTT) (SEQ ID NO:6) and Chop-seqR (GGGATTGAGGGTCACATCAT) (SEQ ID NO:7) and for ATF4 were ATF4-seqF (AACCGACAAAGACACCTTCG) (SEQ ID NO:8) and ATF4-seqR (ACCCTAGATCCCACCAGGAC) (SEQ ID NO:9).

Cell culture and Transfection. Human trabecular meshwork cells (TM-3) stably expressing human mutant myocilin were cultured in DMEM/F12 (Life Technologies) supplemented with 10% FCS. Cells were seeded in a 6-well plate at 1×10^6 cells/well. Transfection was performed with Lipofectamine 3000 using recommended conditions. Briefly, 2 μ g of plasmid containing a sgRNA targeting either Chop exon 2 or ATF4 exon 2 and 6 μ l Lipofectamine 2000 were diluted in 100 μ l Opti-MEM, mixed 1:1 and added to cells after 5 minute incubation. After 24 hours, TM cell lysate was utilized for Western blotting. Western blot analysis and immunostaining for myocilin and other ER stress markers was performed as described previously (8,9).

CRISPR mediated CHOP/ATF4 knockdown induces autophagy mediated degradation of mutant myocilin. Elevation of intraocular pressure (IOP) is the major and only treatable risk factor in Primary Open Angle Glaucoma (POAG). The trabecular meshwork (TM) tissue in the eye regulates outflow resistance and maintains normal IOP. The glaucomatous TM damage is known to increase outflow resistance, thus elevating IOP. However, the lack of understanding of the pathological pathways that cause TM dysfunction has limited drug development targeted at the TM pathology. The inventors have demonstrated that chronic endoplasmic reticulum (ER) stress plays a key role in the glaucomatous TM damage & IOP elevation in MYOC-associated glaucoma (2), glucocorticoid (GC)-induced glaucoma (8) and also in human POAG cases (9). Abnormal protein accumulation in the TM is associated with induction of ER stress and IOP elevation in both of these mouse models.

TM cells activate the protective unfolded protein response (UPR) pathway to eliminate unfolded protein load by sensing through the ER stress sensors PERK, ATF6 α , and IRE1, which then activate the downstream signaling to normalize ER homeostasis. Chronic and excessive ER stress can activate ATF4/CHOP signaling, which leads to apoptosis of TM cells and TM dysfunction. The inventors have also demonstrated that prodeath ER stress markers

ATF4 and CHOP are significantly increased in the TM of human POAG patients (3). The inventors determined if inhibition of prodeath ATF4 and CHOP signaling prevents myocilin misfolding and TM dysfunction.

To inhibit ATF4/CHOP signaling, the inventors designed CRISPR-Cas9 targeting ATF4 or CHOP. FIG. 4 is a drawing that shows the ATF4 and CHOP signaling pathway.

IOP elevation and reduced outflow facility in mouse models of mutant myocilin induced glaucoma (*Tg-Myoc^{Y437H}*) and Dex induced glaucoma.

FIGS. 5A to 5D show as follows: FIG. 5A is a graph that shows IOP elevation in different aged group *Tg-MYOC^{Y437H}* mice compared to wild-type (WT) litter mates (n=8); FIG. 5B is a graph that shows reduced outflow facility in 7 months old *Tg-MYOC^{Y437H}* mice compared to WT littermates (n=8-10); FIG. 5C is a graph that shows IOP elevation in 0.1% topical ocular dex treated mice compared to vehicle treated mice; and FIG. 5D is a graph that shows reduced outflow facility in dex treated mice compared to vehicle treated mice (** p< 0.005, *** p< 0.0001; vs veh-treated mice; n=8).

Induction of Prodeath ATF4 and CHOP in murine and human glaucoma. FIGS. 6A to 6C show Western blots of UPR markers, specifically: FIG. 6A is the anterior segment tissues of 6-month-old WT and *Tg-MYOC^{Y437H}* mice (n=3WT & 3 Tg); FIG. 6B is the anterior segment tissues of vehicle (n=4) and Dex (n=4)- treated mice; and FIG. 6C is a post-mortem human normal and glaucomatous TM tissues lysates (n=4). FIG. 6D show immunostaining for CHOP & KDEL in normal and glaucomatous human TM tissues.

Induction of Apoptotic markers in TM cells expressing mutant Myocilin. FIGS. 7A to 7C show: FIG. 7A is a Western blot of MYOC in conditioned media (CM) and cell lysates (CL) of TM-5 cells expressing WT or mutant MYOC; FIG. 7B shows the MYOC accumulation and the presence of aggregates (shown by arrow); and FIG. 7C is a Western blot that shows the induction of apoptotic markers in mutant MYOC expressing cells.

Overexpression of ATF4 in the TM elevates IOP in WT mice. FIG. 8 shows IOP measured in WT mice intravitreally injected with 107 p.f.u. of Ad5 expressing null, ATF4 and CHOP (10-days post injections).

Deletion of CHOP rescues IOP elevation in mouse models of glaucoma. FIGS. 9A and 9B show IOP levels were measured in 3 months old littermates of: FIG. 9A is a Wt mice, *Tg-MYOC^{Y437H}* mice, CHOP -/- mice and double mutant mice (*Tg-MYOC^{Y437H}/CHOP-/-*); FIG. 9B

shows the effect of 0.1% topical ocular dex treated WT mice, Vehicle treated WT mice (0.1% EtOH), Dex treated CHOP^{-/-} mice and vehicle treated CHOP^{-/-} mice.

CRISPR-mediated ATF4 or CHOP deficiency reduces mutant myocilin accumulation in TM cells. FIGS. 10A and 10B show: FIG. 10A is a Western blot showing myocilin and ER stress markers in TM-5 cells stably expressing mutant MYOC were transiently transfected with plasmids expressing Cas9-CHOP or ATF4; and FIG. 10B shows the CRISPR-CAS9 mediated deletion of CHOP verified in CRISPR-CHOP transfected clones by surveyor nuclear digestion analysis.

CRISPR mediated CHOP/ATF4 knockdown induces autophagy-mediated degradation of mutant myocilin. FIGS. 11A and 11B show: FIG. 11A is a Western blot of TM5 cells stably expressing mutant myocilin transfected with plasmid expressing CAS9-CHOP. Myocilin levels, autophagy marker (LC3B) and ER stress markers were analyzed; and FIG. 11B shows immunostaining showing Myocilin (Red), LC3B (Green) in TM5 cells stably expressing mutant myocilin transfected with plasmid expressing CAS9-CHOP.

FIGS. 12A and 12B show that Integrated Stress Response Inhibitor (ISRIB or *trans*-N,N'-(cyclohexane-1,4-diyl)bis(2-(4-chlorophenoxy)acetamide), ChemSpider No. 30773985) prevents mutant myocilin accumulation in TM-5 cells and reduces elevated IOP in *Tg-MYOC^{Y437H}* mice. The inventors examined whether ISRIB treatment reduces mutant myocilin accumulation in TM-5 cells stably expressing mutant myocilin. Compared to control TM-5, which shows accumulation of mutant myocilin, ISRIB (100 and 200nM) treatment dramatically reduced myocilin accumulation. ISRIB also inhibited ATF4 and CHOP as evident from reduced cellular protein levels. Furthermore, the inventors performed an intravitreal injection of vehicle (right eye) or ISRIB (2mM, 2ul, left eye) in ocular hypertensive *Tg-MYOC^{Y437H}* mice (4-months-old). IOP measurements after 1-week treatment revealed that ISRIB significantly reduced elevated IOP compared to the vehicle-treated contralateral eyes. In addition ISRIB treated mice did not show any signs of inflammation or other ocular toxicity in slit lamp analysis. FIG. 12A is a Western blot of MYOC, ATF4 and CHOP in TM cells expressing WT or mutant MYOC treated with vehicle or ISRIB for 48 hours. FIG. 12B is a graph that shows the IOP of the left eyes of *Tg-MYOC^{Y437H}* mice injected with ISRIB are compared with vehicle-injected contralateral right eyes. N=5; paired t-test.

Next, the inventors treated ocular hypertensive *Tg-MYOC^{Y437H}* mice with topical TB or TS eye drops (Fig. 13A). The left eye was treated with TB and the contralateral right eye was given TS peptide twice daily for 2 weeks. TB treatment did not alter IOP in WT mice. Topical ocular TB significantly reduced elevated IOP compared to control contralateral eye treated with TS.

TB treatment did not cause any toxicity or inflammation to the eyes. Similar to TB treatments, the inventors also used another stimulator of autophagy, Torin-2 ((9-(6-Amino-3-pyridinyl)-1-[3-(trifluoromethyl)phenyl]-benzo[h]-1,6-naphthyridin-2(1H)-one)). Torin-2 is a Mammalian target of rapamycin (mTOR) inhibitor. Treatment of ocular hypertensive *Tg-MYOC^{Y437H}* mice with Torin-2 dramatically reduced IOP within 1 week of treatment compared to the contralateral vehicle treated eyes (Fig. 13B). Fig. 13A compares WT and *Tg-MYOC^{Y437H}* mice were given topical eye drops of TS (left eyes) and TB (D11) in the contralateral right eyes for 2 weeks. IOP were measured weekly in the night. TB treatment reduced elevated IOP in *Tg-MYOC^{Y437H}* mice but did not alter IOP in WT mice (n=6 each; one way ANOVA, *p<0.5). 13B shows is a graph that shows the IOP of the left eyes of *Tg-MYOC^{Y437H}* mice given Torin-2 eye drops topically for 1 week compared with vehicle eye drops given to the contralateral right eyes. N=8 ; P<0.001, paired t-test. As such, the autophagy stimulators Tat-Beclin 1 D11 (shorter beclin peptide conjugated to Tat) and Torin-2 reduced elevated IOP in Tg mice.

Next, the inventors used CRISPR-Cas9 expressing guide RNA for ATF4 deletion rescues mouse model of glucocorticoid-induced glaucoma. The inventors have developed a mouse model of glucocorticoid-induced ocular hypertension. Weekly periocular injections of a potent glucocorticoid, dexamethasone acetate suspension (Dex) elevates IOP significantly starting from 1 week of treatment. To determine whether CRISPR-Cas9 targeted genetic deletion of ATF4 rescues mouse model of glucocorticoid-induced IOP elevation, C57 mice were injected with intravitreal injections of adenovirus expressing CRISPR-Cas9 ATF-4. One week after injections, mice were given periocular injections of Veh or Dex and IOP were monitored. IOP measurement revealed that control mice (without CR-ATF4) elevated IOP significantly while mice injected with CR-ATF4 did not elevate IOP significantly. These data demonstrate that targeting ATF4 knockdown via CRISPR-Cas9 rescues glucocorticoid-induced glaucoma.

FIG. 14 is a graph that shows IOP measurements in mice treated with CR-ATF4. C57 mice were injected with or without CR-ATF4. One week later, mice were treated with periocular injections of Veh or Dex and IOPs were monitored after 1 week. Compared to mice treated with Dex alone, mice treated with CR-ATF4 and Dex demonstrated significantly reduced IOP similar to vehicle treated mice.

ATF4 and CHOP knockdown prevents activation of TGF- β 2 signaling and also reduces intracellular ECM accumulation in the TM. The inventors have previously demonstrated that Dex induces expression of terminal ER stress markers such as ATF4 and CHOP. Moreover, CHOP knock out mice are protected from Dex-induced ocular hypertension. However, it is not

clear how loss of CHOP protects from Dex-induced IOP elevation. Here, the inventors examined whether loss of ATF4 or CHOP inhibits TGF β 2 signaling, thus preventing abnormal ECM accumulation and induction of ER stress (Figure 15). TM3 cells were transfected with CRISPR-Cas9 targeted to CHOP or ATF4 for 24 hours, and then treated with Veh or Dex for an additional 48 hours. Western blot analysis demonstrated that Dex increases precursor and active TGF β 2, fibronectin, and ER stress markers including GRP78, ATF4 and CHOP, which were reduced to normal levels in cells pretreated with CR-ATF4 or CR-CHOP. Western blot data further confirmed that CR-ATF4 or CR-CHOP treatment reduced protein levels of ATF4 and CHOP in TM cells transfected with these plasmids and treated with Dex. Furthermore, CHOP or ATF4 knockdown decreased Dex-induced expression of pro and active TGF β 2 levels (Figure 15).

FIG. 15 shows that knockdown of ATF4 or CHOP inhibits TGF β 2 signaling and prevents abnormal Dex-induced ECM accumulation and ER stress in TM cells. TM3 cells transiently transfected with CRISPR-Cas9-CHOP or CRISPR-Cas9-ATF4 and treated with Veh or Dex for 48 hours. Cell lysates were analyzed for expression levels of fibronectin, TGF β 2 and ER stress markers by Western blotting.

It was found that chronic or sustained ER stress either in mutant myocilin or Dexamethasone induced glaucoma trigger induction of ATF4/CHOP, which is associated with TM cell dysfunction/death and IOP elevation. Inhibition of ATF4/CHOP pathway by genetic knockdown (CAS9 mediated knockdown of ATF4 or CHOP) allows pro-survival UPR mechanisms to cope up with protein misfolding, thus preventing TM cell death and IOP elevation.

It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method, kit, reagent, or composition of the invention, and vice versa.

Furthermore, compositions of the invention can be used to achieve methods of the invention.

It will be understood that particular embodiments described herein are shown by way of illustration and not as limitations of the invention. The principal features of this invention can be employed in various embodiments without departing from the scope of the invention. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of this invention and are covered by the claims.

All publications and patent applications mentioned in the specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated
5 by reference.

The use of the word “a” or “an” when used in conjunction with the term “comprising” in the claims and/or the specification may mean “one,” but it is also consistent with the meaning of “one or more,” “at least one,” and “one or more than one.” The use of the term “or” in the claims is used to mean “and/or” unless explicitly indicated to refer to alternatives only or the
10 alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and “and/or.” Throughout this application, the term “about” is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

As used in this specification and claim(s), the words “comprising” (and any form of comprising, such as “comprise” and “comprises”), “having” (and any form of having, such as “have” and “has”), “including” (and any form of including, such as “includes” and “include”) or “containing” (and any form of containing, such as “contains” and “contain”) are inclusive or open-ended and do not exclude additional, unrecited elements or method steps. In
15 embodiments of any of the compositions and methods provided herein, “comprising” may be replaced with “consisting essentially of” or “consisting of”. As used herein, the phrase “consisting essentially of” requires the specified integer(s) or steps as well as those that do not materially affect the character or function of the claimed invention. As used herein, the term “consisting” is used to indicate the presence of the recited integer (e.g., a feature, an element, a characteristic, a property, a method/process step or a limitation) or group of integers (e.g.,
20 feature(s), element(s), characteristic(s), propertie(s), method/process steps or limitation(s)) only.

The term “or combinations thereof” as used herein refers to all permutations and combinations of the listed items preceding the term. For example, “A, B, C, or combinations thereof” is intended to include at least one of: A, B, C, AB, AC, BC, or ABC, and if order is important in
30 a particular context, also BA, CA, CB, CBA, BCA, ACB, BAC, or CAB. Continuing with this example, expressly included are combinations that contain repeats of one or more item or term, such as BB, AAA, AB, BBC, AAABCCCC, CBBAAA, CABABB, and so forth. The skilled artisan will understand that typically there is no limit on the number of items or terms in any combination, unless otherwise apparent from the context.

As used herein, words of approximation such as, without limitation, “about”, “substantial” or “substantially” refers to a condition that when so modified is understood to not necessarily be absolute or perfect but would be considered close enough to those of ordinary skill in the art to warrant designating the condition as being present. The extent to which the description may vary will depend on how great a change can be instituted and still have one of ordinary skilled in the art recognize the modified feature as still having the required characteristics and capabilities of the unmodified feature. In general, but subject to the preceding discussion, a numerical value herein that is modified by a word of approximation such as “about” may vary from the stated value by at least ± 1 , 2, 3, 4, 5, 6, 7, 10, 12 or 15%.

All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

REFERENCES

1. Shoji-Kawata S, Sumpter R, Leveno M, Campbell GR, Zou Z, Kinch L, Wilkins AD, Sun Q, Pallauf K, MacDuff D, et al. Identification of a candidate therapeutic autophagy-inducing peptide. *Nature*. 2013;494(7436):201-6.
2. Zode GS, Kuehn MH, Nishimura DY, Searby CC, Mohan K, Grozdanic SD, Bugge K, Anderson MG, Clark AF, Stone EM, et al. Reduction of ER stress via a chemical chaperone prevents disease phenotypes in a mouse model of primary open angle glaucoma. *J Clin Invest*. 2011;121(9):3542-53.
3. Wang WH, Millar JC, Pang IH, Wax MB, and Clark AF. Noninvasive measurement of rodent intraocular pressure with a rebound tonometer. *Invest Ophthalmol Vis Sci*. 2005;46(12):4617-21.
4. Sarkar S, Davies JE, Huang Z, Tunnacliffe A, and Rubinsztein DC. Trehalose, a novel mTOR-independent autophagy enhancer, accelerates the clearance of mutant huntingtin and alpha-synuclein. *J Biol Chem*. 2007;282(8):5641-52.
5. Sarkar S, Chigurupati S, Raymick J, Mann D, Bowyer JF, Schmitt T, Beger RD, Hanig JP, Schmued LC, and Paule MG. Neuroprotective effect of the chemical chaperone, trehalose

in a chronic MPTP-induced Parkinson's disease mouse model. *Neurotoxicology*. 2014;44(250-62).

6. Renna M, Jimenez-Sanchez M, Sarkar S, and Rubinsztein DC. Chemical inducers of autophagy that enhance the clearance of mutant proteins in neurodegenerative diseases. *J Biol Chem*. 2010;285(15):11061-7.
7. Zode GS, Bugge KE, Mohan K, Grozdanic SD, Peters JC, Koehn DR, Anderson MG, Kardon RH, Stone EM, and Sheffield VC. Topical ocular sodium 4-phenylbutyrate rescues glaucoma in a myocilin mouse model of primary open-angle glaucoma. *Invest Ophthalmol Vis Sci*. 2012;53(3):1557-65.
8. Zode GS et al. ER stress reduction rescues glaucoma in murine glucocorticoid-induced glaucoma. *J Clin Invest*. 2014;124(5):1956-65.
9. Peters JC et al. Increased Endoplasmic Reticulum Stress in Human Glaucomatous Trabecular Meshwork Cells and Tissues. *Invest Ophthalmol Vis Sci*. 2015;56(6):3860-8.

What is claimed is:

1. A composition for the treatment of an eye disease comprising a therapeutically effective amount of an autophagy stimulator that treats or slows the progression of the eye disease by enhancing or stimulating autophagy or correcting an autophagy deficiency.
- 5 2. The composition of claim 1, wherein the amount of the composition is sufficient to reduce at least one of protein misfolding, defective autophagy, or endoplasmic reticulum stress or increase autophagy.
3. The composition of claim 1, wherein the autophagy stimulator is provided with a synergistic amount of a protein chaperone sufficient to treat or slow the progression of the eye
10 disease.
4. The composition of claim 1, wherein the eye disease comprises one or more symptoms selected from at least one of elevated intraocular pressure, increased aqueous humor outflow resistance at the trabecular meshwork, accumulation of misfolded proteins, or cell death.
5. The composition of claim 1, wherein the composition is adapted for subcutaneous,
15 cutaneous, intravitreal, intraocular, or ocular administration.
6. The composition of claim 1, wherein the eye disease is caused by misfolded proteins, the loss of clearance of misfolded proteins, the accumulation of misfolded proteins, or a decrease in lysosomal activity.
7. The composition of claim 1, wherein the eye disease is glaucoma, endoplasmic
20 reticulum (ER) stress, autophagy deficiency, age-related macular degeneration (AMD), or diabetic retinopathy.
8. The composition of claim 1, wherein the composition further comprises one or more pharmaceutically acceptable excipients.
9. The composition of claim 1, wherein the autophagy stimulator is selected from at least
25 one of: beclin 1 protein, Tat-beclin1 peptide (SEQ ID NO:1), Tat-Beclin 1 D11 (SEQ ID NO:10), trehalose, ISRIB, or an mTOR inhibitor.
10. The composition of claim 9, wherein the mTOR inhibitor is at least one of rapamycin, temsirolimus, everolimus, KU-0063794, WYE-354, AZD8055, metformin, or Torin-2.
11. The composition of claim 3, wherein the protein chaperone is 4-phenylbutyrate or a salt
30 thereof, 1-deoxygalactonojirimycin or a salt thereof, isofagomine or a salt thereof, fagomine

- isomers or a salt thereof, dimethylsulfoxide (DMSO) or a salt thereof, tauroursodeoxycholic acid (TUDCA) or a salt thereof, ursodeoxycholic acid (UDCA) or a salt thereof, glycine betaine (betaine) or a salt thereof, glycerolphosphocholine (GPC) or a salt thereof, methylamines or a salt thereof, or trimethylamine N-oxide (TMAO), or a Histone deacetylase (DHAC inhibitor selected from Vorinostat, Romidepsin, Chidamide, Panobinostat, Valproic acid (e.g., Mg valproate), Belinostat, Mocetinostat (MGCD0103), Abexinostat (PCI-24781), Entinostat (MS-275), SB939, Resminostat (4SC-201), Givinostat (ITF2357), Quisinostat, HBI-8000, Kevetrin, CUDC-101, AR-42, CHR-2845, CHR-3996, 4SC-202, CG200745, ACY-1215, lenalidomide, ME-344, sulforaphane, and/or alpha-crystallin protein or a salt thereof.
12. The composition of claim 1, wherein the autophagy stimulator is an inhibitor of a CHOP gene, an ATF4 gene, or both, comprising a gene knockdown construct selected from at least one of an siRNA, a miRNA, an shRNA, an antisense RNA, or an sgRNA, a programmable gene editing nuclease (CRISPR-Cas) knockdown of ATF4 and/or CHOP, or a gene editing or therapy that reduces expression of ATF4 and/or CHOP.
13. The composition of claim 12, wherein the sgRNA construct targets an exon, intron, or exon/intron junction of a human CHOP gene and/or an exon, intron, or exon/intron junction of a human ATF4 gene.
14. The composition of claim 12, wherein the sgRNA construct comprises (GACCACTCTGTTTCCGTTTCC) (SEQ ID NO:4) and/or (AGGTCTCTTAGATGATTACC) (SEQ ID NO:5).
15. A composition for the treatment of an eye disease comprising a therapeutically effective amount of autophagy stimulator or enhancer adapted for treatment of the eye.
16. The composition of claim 15, wherein the amount of the composition is sufficient to reduce at least one of protein misfolding, defective autophagy, or endoplasmic reticulum stress or increase autophagy.
17. The composition of claim 15, wherein the eye disease comprises one or more symptoms selected from at least one of elevated intraocular pressure, increased aqueous humor outflow resistance at the trabecular meshwork, accumulation of misfolded proteins, or cell death.
18. The composition of claim 15, wherein the composition is adapted for subcutaneous, cutaneous, intravitreal, intraocular, or ocular administration.

19. The composition of claim 15, wherein the eye disease is caused by misfolded proteins, the loss of clearance of misfolded proteins, the accumulation of misfolded proteins, or a decrease in lysosomal activity.
20. The composition of claim 15, wherein the eye disease is glaucoma, endoplasmic
5 reticulum (ER) stress, autophagy deficiency, age-related macular degeneration (AMD), or diabetic retinopathy.
21. The composition of claim 15, wherein the composition further comprises one or more pharmaceutically acceptable excipients.
22. The composition of claim 15, wherein the autophagy stimulator is selected from at least
10 one of: beclin 1 protein, Tat-beclin1 peptide (SEQ ID NO:1), Tat-Beclin 1 D11 (SEQ ID NO:10), trehalose, ISRIB, or an mTOR inhibitor.
23. The composition of claim 22, wherein the mTOR inhibitor is at least one of rapamycin, temsirolimus, everolimus, KU-0063794, WYE-354, AZD8055, metformin, or Torin-2.
24. The composition of claim 15, wherein the amount is sufficient to treat or reduce the
15 progression of the eye disease.
25. The composition of claim 15, further comprising a chemical protein chaperone in an amount that is synergistic for treating the eye disease.
26. A composition for the treatment of an eye disease comprising a therapeutically effective amount of an inhibitor of a CHOP gene, an ATF4 gene, or both, comprising a gene knockdown
20 construct selected from at least one of an siRNA, a miRNA, an shRNA, an antisense RNA, or an sgRNA, a programmable gene editing nuclease (CRISPR-Cas) knockdown of ATF4 and/or CHOP, or a gene editing or therapy that reduces expression of ATF4 and/or CHOP.
27. A method of treating eye disease comprising:
identifying a subject in need of treatment for an eye disease; and
25 providing the subject with an effective amount of an autophagy stimulator sufficient to reduce at least one of defective autophagy, endoplasmic reticulum stress, or elevated intraocular pressure or increase autophagy.
28. The method of claim 27, wherein the eye disease comprises one or more symptoms selected from at least one of increased aqueous humor outflow resistance at the trabecular
30 meshwork, accumulation of misfolded proteins, or cell death.

29. The method of claim 27, wherein the autophagy stimulator is selected from at least one of: beclin 1 protein, Tat-beclin1 peptide (SEQ ID NO:1), Tat-Beclin 1 D11 (SEQ ID NO:10), trehalose, ISRIB, or an mTOR inhibitor.
30. The method of claim 29, wherein the mTOR inhibitor is at least one of rapamycin, temsirolimus, everolimus, KU-0063794, WYE-354, AZD8055, metformin, or Torin-2.
31. The method of claim 27, further comprising the step of providing the subject with a protein chaperone is selected from at least one of 4-phenylbutyrate or a salt thereof, 1-deoxygalactonojirimycin or a salt thereof, isofagomine or a salt thereof, fagomine isomers or a salt thereof, dimethylsulfoxide (DMSO) or a salt thereof, tauroursodeoxycholic acid (TUDCA) or a salt thereof, ursodeoxycholic acid (UDCA) or a salt thereof, glycine betaine (betaine) or a salt thereof, glycerolphosphocholine (GPC) or a salt thereof, methylamines or a salt thereof, or trimethylamine N-oxide (TMAO), or a Histone deacetylase (DHAC) inhibitor selected from Vorinostat, Romidepsin, Chidamide, Panobinostat, Valproic acid (e.g., Mg valproate), Belinostat, Mocetinostat (MGCD0103), Abexinostat (PCI-24781), Entinostat (MS-275), SB939, Resminostat (4SC-201), Givinostat (ITF2357), Quisinostat, HBI-8000, Kevetrin, CUDC-101, AR-42, CHR-2845, CHR-3996, 4SC-202, CG200745, ACY-1215, lenalidomide, ME-344, sulforaphane, and/or alpha-crystallin protein or a salt thereof, in a synergistic amount with the autophagy stimulator sufficient to treat the eye disease.
32. The method of claim 27, further comprising the step of providing the subject with a composition that comprises a synergistic amount of a protein chaperone and beclin1 protein, Tat-beclin1 peptide (SEQ ID NO:1), Tat-Beclin 1 D11 (SEQ ID NO:10), trehalose, ISRIB, rapamycin, temsirolimus, everolimus, KU-0063794, WYE-354, AZD8055, metformin, or Torin-2, or 4-phenylbutyrate or a salt thereof.
33. The method of claim 27, wherein the autophagy stimulator is adapted for subcutaneous, cutaneous, intravitreal, intraocular, or ocular administration.
34. The method of claim 27, further comprising the step of determining that the subject has a disease caused by misfolded proteins, the loss of clearance of misfolded proteins, the accumulation of misfolded proteins, or a decrease in lysosomal activity; and providing the subject with the effective amount of a Tat-beclin1 peptide, Tat-beclin1 D11, trehalose, ISRIB, rapamycin, temsirolimus, everolimus, KU-0063794, WYE-354, AZD8055, metformin, Torin-2, and sodium 4-phenylbutyrate to treat the eye disease.

35. The method of claim 27, wherein the eye disease is glaucoma, endoplasmic reticulum (ER) stress, autophagy deficiency, age-related macular degeneration (AMD), or diabetic retinopathy.
36. The method of claim 27, wherein the autophagy stimulator is an inhibitor of a CHOP gene, an ATF4 gene, or both, comprising a gene knockdown construct selected from at least one of an siRNA, a miRNA, an shRNA, an antisense RNA, an sgRNA, a programmable gene editing nuclease (CRISPR-Cas) knockdown of ATF4 and/or CHOP, or a gene editing or therapy that reduces expression of ATF4 and/or CHOP.
37. The method of claim 36, wherein the sgRNA construct targets an exon, intron, or exon/intron junction of a human CHOP gene and/or an exon, intron, or exon/intron junction of a human ATF4 gene.
38. The method of claim 36, wherein the sgRNA construct comprises (GACCACTCTGTTTCCGTTTCC) (SEQ ID NO:4) and/or (AGGTCTCTTAGATGATTACC) (SEQ ID NO:5).
39. A method of lowering intraocular pressure in a mammal comprising administering to a subject in which such pressure lowering is desired, at least one of an effective amount of autophagy stimulator to achieve the desired lowering in pressure.
40. The method of claim 39, wherein the autophagy stimulator is selected from at least one of: beclin 1 protein, Tat-beclin1 peptide (SEQ ID NO:1), Tat-Bec1 D11 (SEQ ID NO:10), trehalose, ISRIB, or an mTOR inhibitor.
41. The method of claim 40, wherein the mTOR inhibitor is at least one of rapamycin, temsirolimus, everolimus, KU-0063794, WYE-354, AZD8055, metformin, or Torin-2.
42. The method of claim 39, further comprising the step of providing a synergistic amount of a protein chaperone.
43. The method of claim 42, wherein the protein chaperone is selected from at least one of 4-phenylbutyrate or a salt thereof, 1-deoxygalactonojirimycin or a salt thereof, isofagomine or a salt thereof, fagomine isomers or a salt thereof, dimethylsulfoxide (DMSO) or a salt thereof, tauroursodeoxycholic acid (TUDCA) or a salt thereof, ursodeoxycholic acid (UDCA) or a salt thereof, glycine betaine (betaine) or a salt thereof, glycerolphosphocholine (GPC) or a salt thereof, methylamines or a salt thereof, or trimethylamine N-oxide (TMAO), or a Histone deacetylase (HDAC) inhibitor selected from Vorinostat, Romidepsin, Chidamide, Panobinostat,

Valproic acid (e.g., Mg valproate), Belinostat, Mocetinostat (MGCD0103), Abexinostat (PCI-24781), Entinostat (MS-275), SB939, Resminostat (4SC-201), Givinostat (ITF2357), Quisinostat, HBI-8000, Kevetrin, CUDC-101, AR-42, CHR-2845, CHR-3996, 4SC-202, CG200745, ACY-1215, lenalidomide, ME-344, sulforaphane, and/or alpha-crystallin protein or a salt thereof.

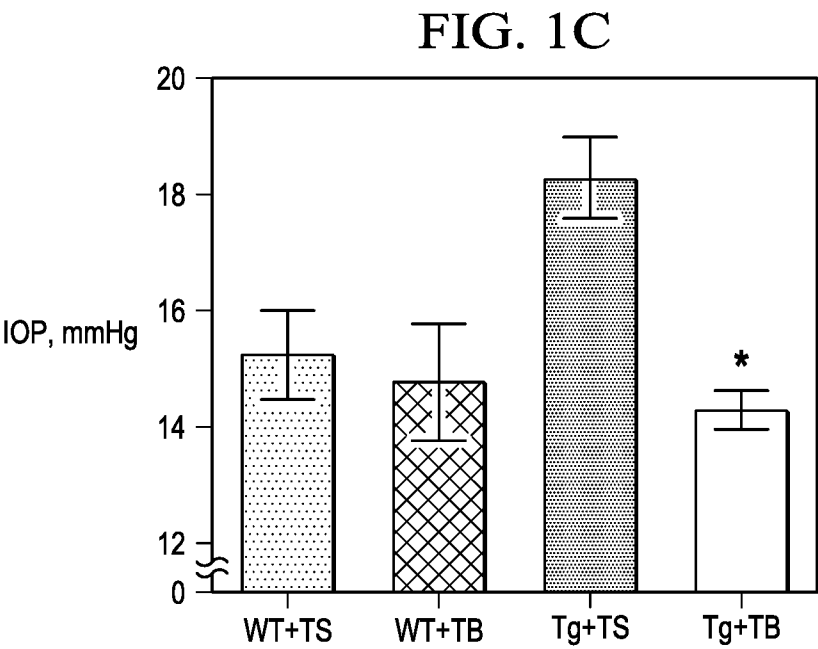
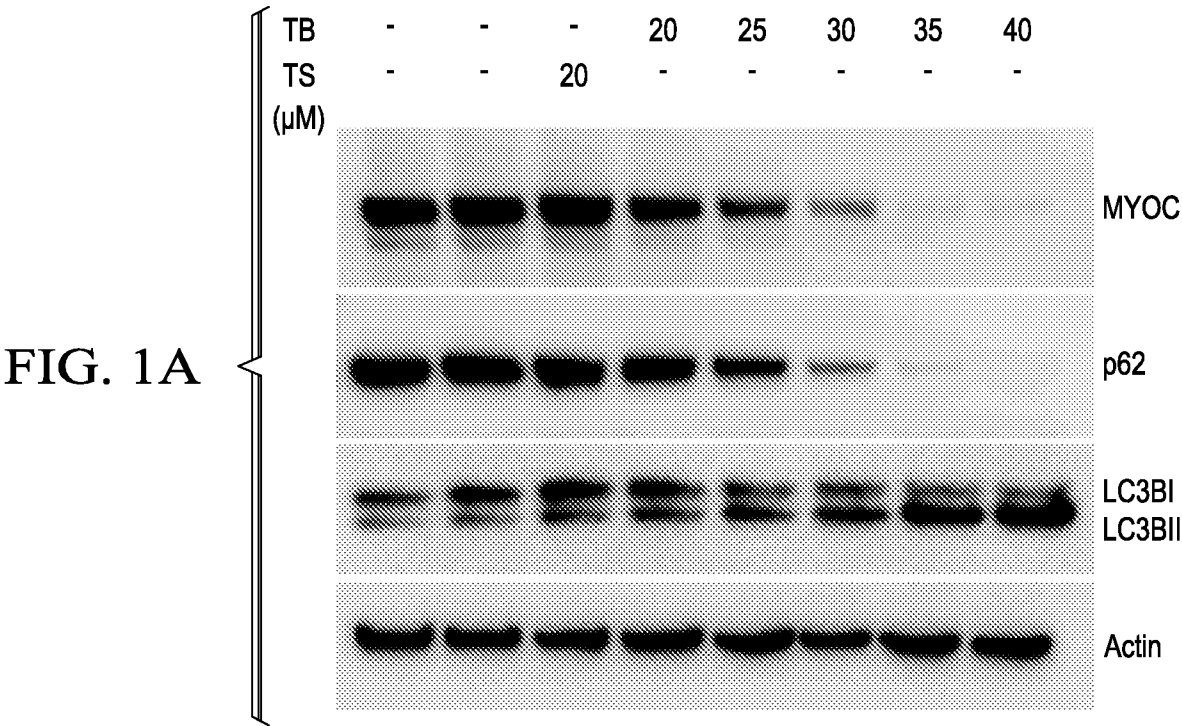
44. The method of claim 39, wherein the autophagy stimulator is an inhibitor of a CHOP gene, an ATF4 gene, or both, comprising a gene knockdown construct selected from at least one of an siRNA, a miRNA, an shRNA, an antisense RNA, an sgRNA, a programmable gene editing nuclease (CRISPR-Cas) knockdown of ATF4 and/or CHOP, or a gene editing or therapy that reduces expression of ATF4 and/or CHOP.

45. The method of claim 39, wherein the sgRNA construct targets an exon, intron, or exon/intron junction of a human CHOP gene and/or an exon, intron, or exon/intron junction of a human ATF4 gene.

46. The method of claim 39, wherein the sgRNA construct comprises (GACCACTCTGTTTCCGTTTCC) (SEQ ID NO:4) and/or (AGGTCTCTTAGATGATTACC) (SEQ ID NO:5).

47. A method of evaluating a candidate drug believed to be useful in treating an eye disease, the method comprising:

- a) measuring at least one of protein misfolding, defective autophagy, or endoplasmic reticulum stress from tissue suspected of having an eye disease from a set of patients;
- b) administering the candidate drug to a first subset of the patients, and a placebo to a second subset of the patients;
- c) repeating step a) after the administration of the candidate drug or the placebo; and
- d) determining if the candidate drug reduces the level of protein misfolding, defective autophagy, or endoplasmic reticulum stress that is statistically significant as compared to any reduction occurring in the second subset of patients, wherein a statistically significant reduction indicates that the candidate drug is useful in treating the eye disease.



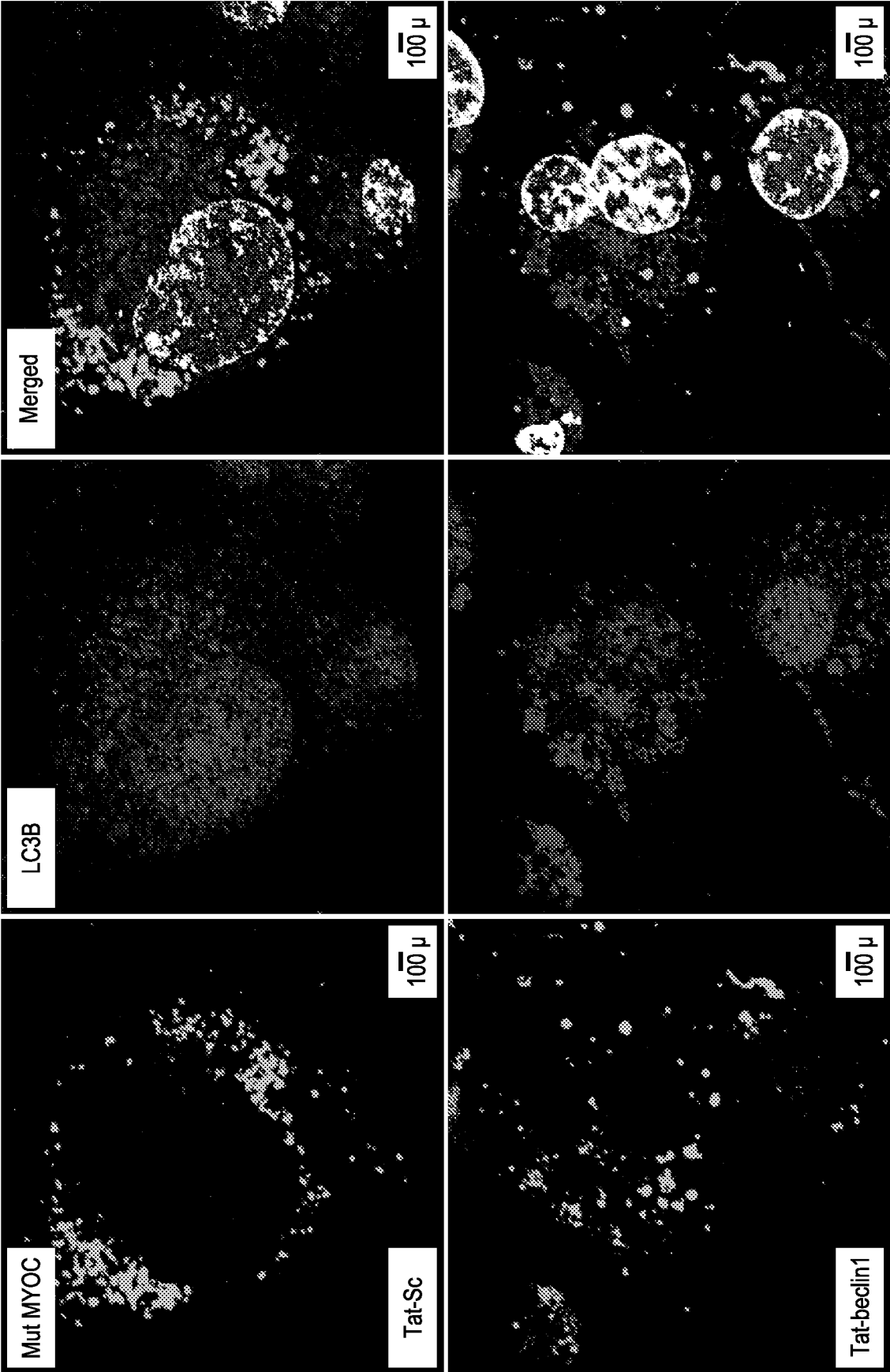


FIG. 1B

B I

B II

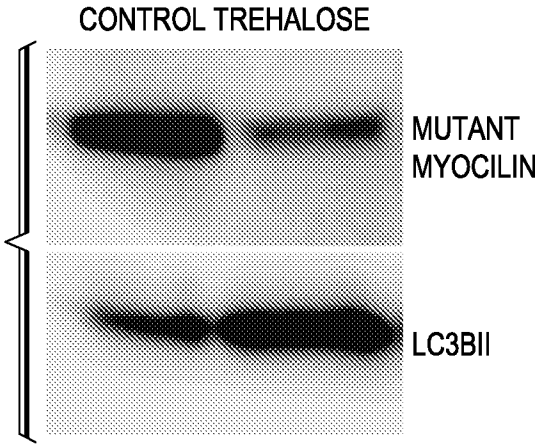


FIG. 2A

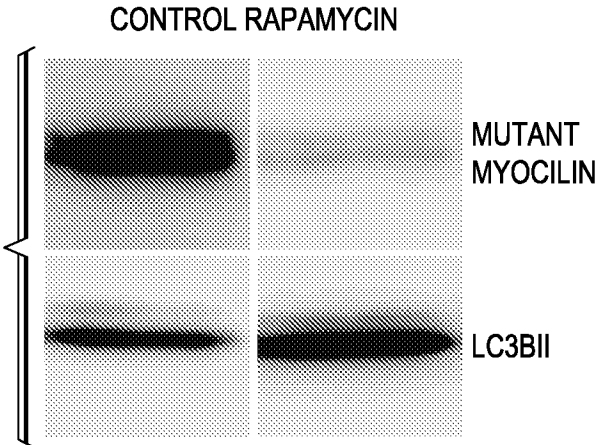


FIG. 2B

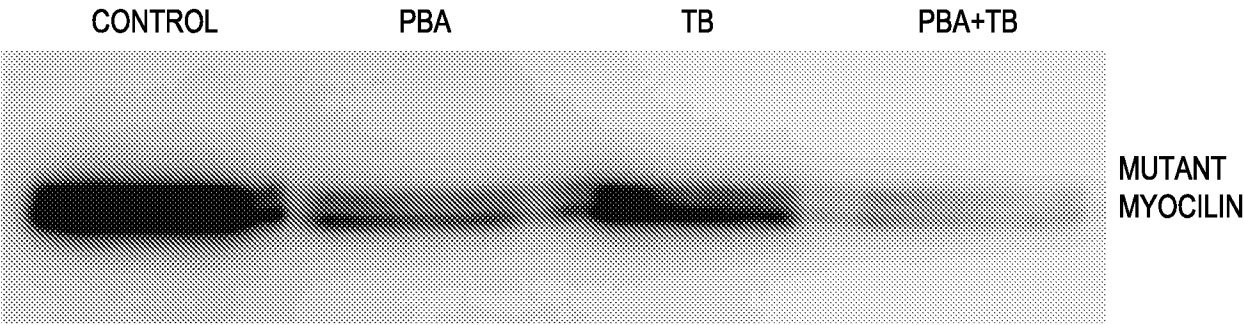


FIG. 3

4/16

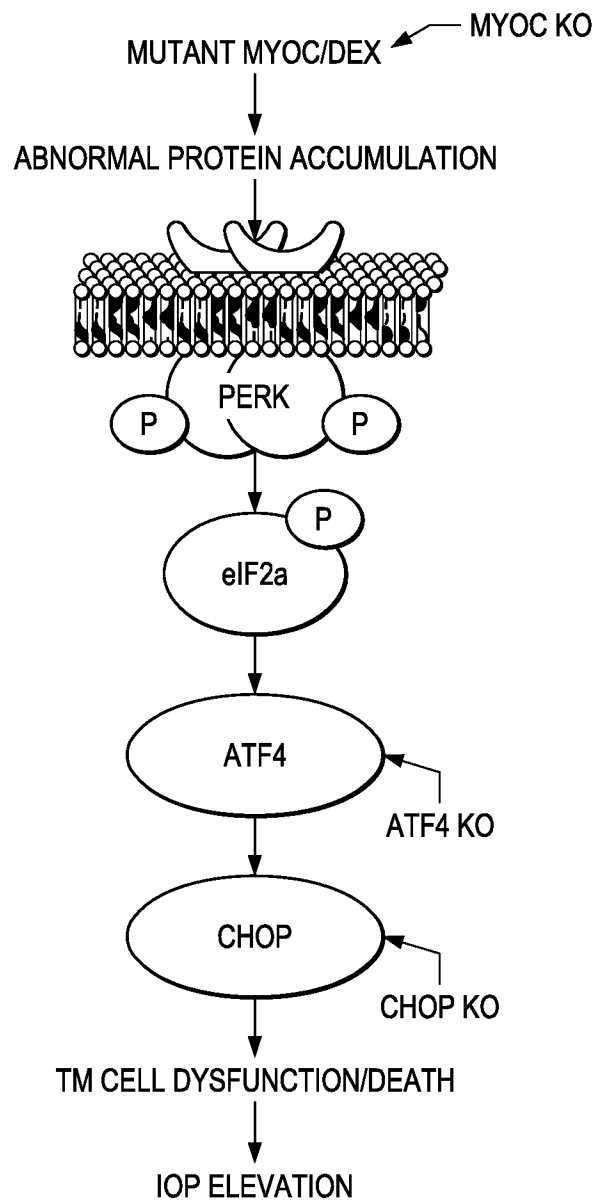


FIG. 4

5/16

FIG. 5A

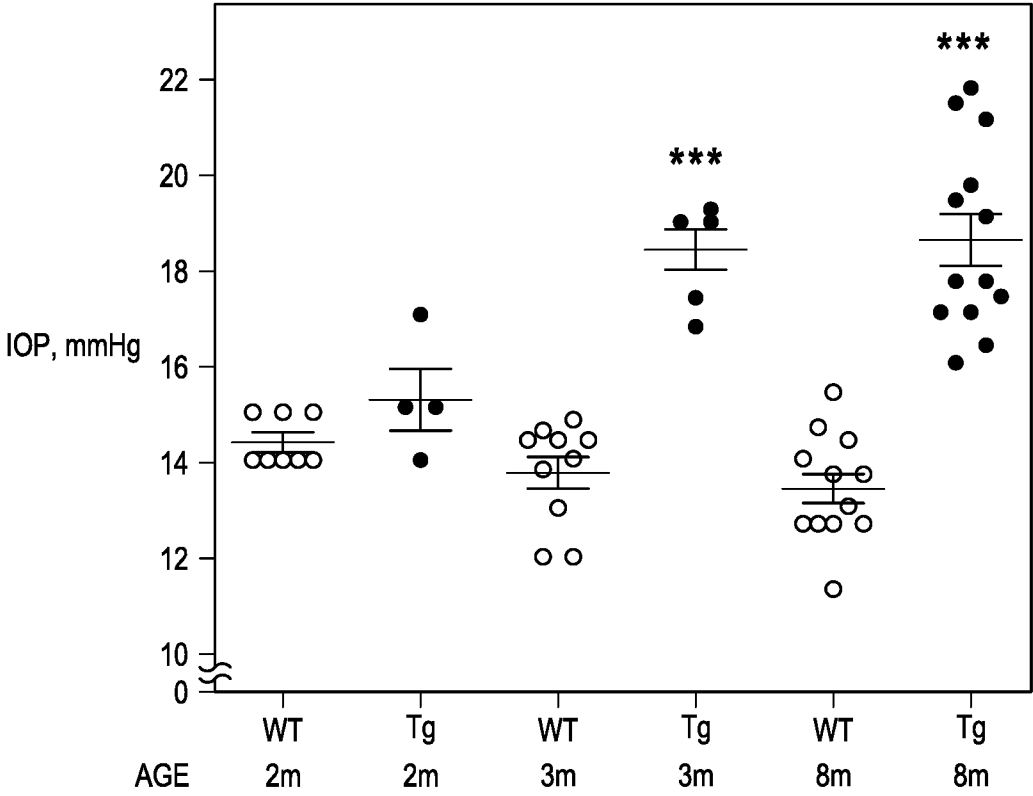
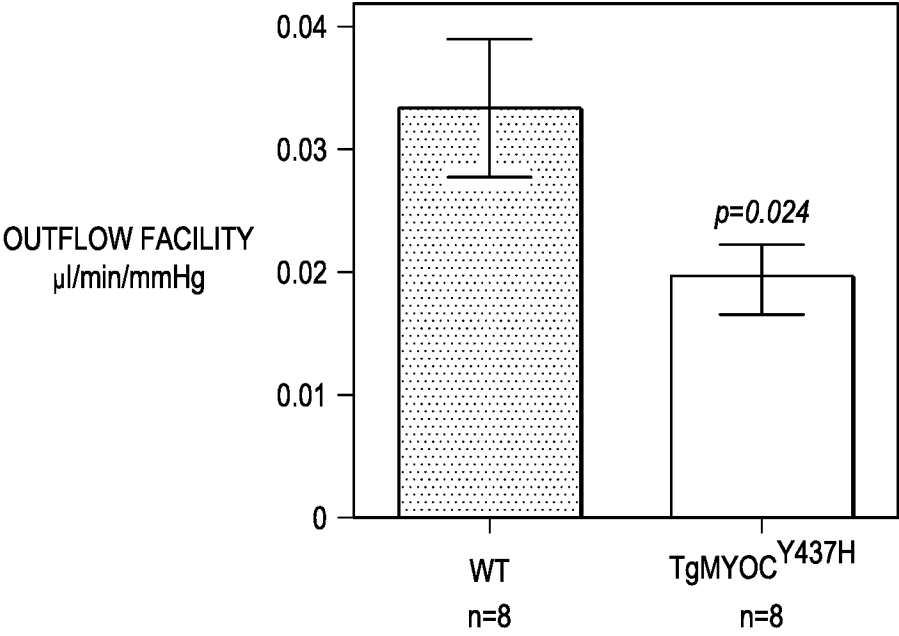


FIG. 5B



6/16

FIG. 5C

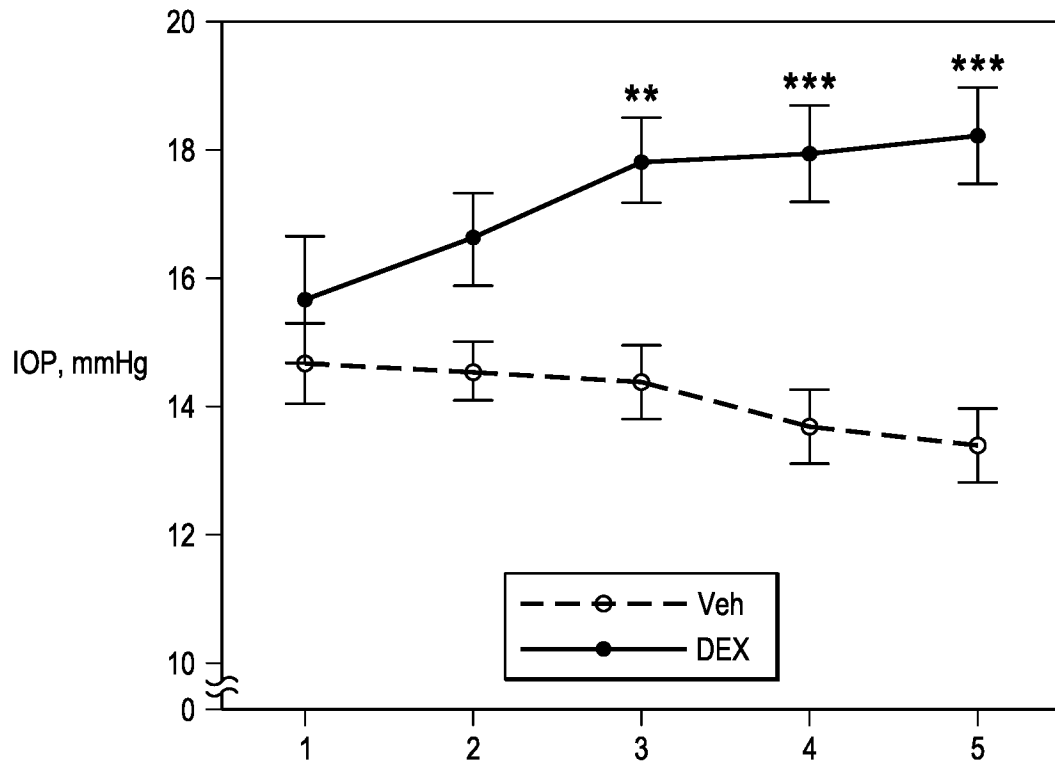
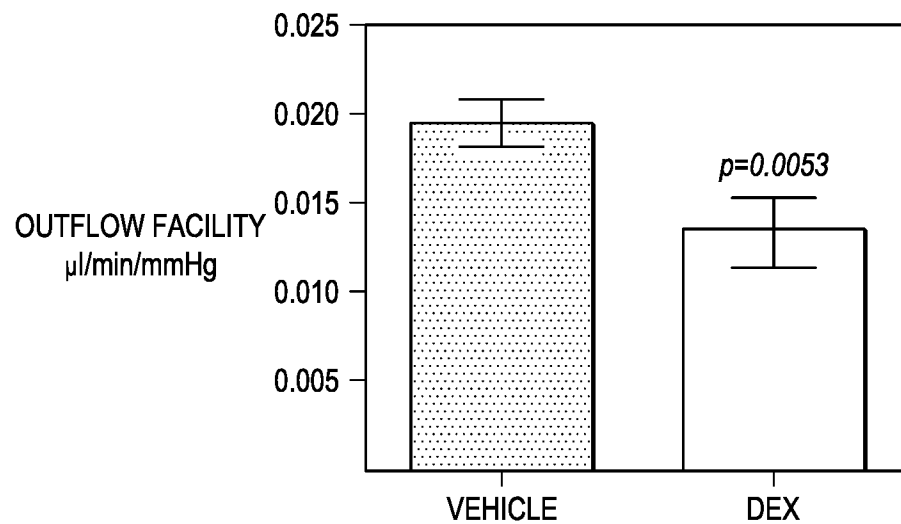


FIG. 5D



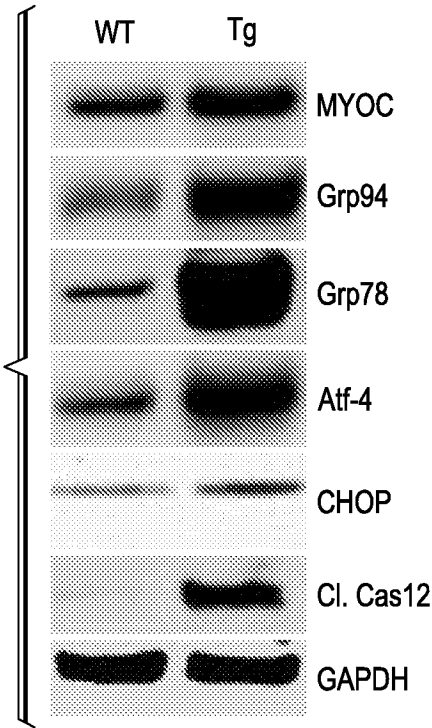


FIG. 6A

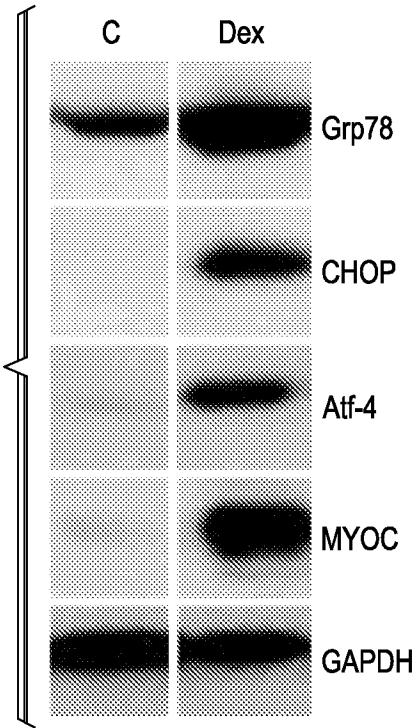


FIG. 6B

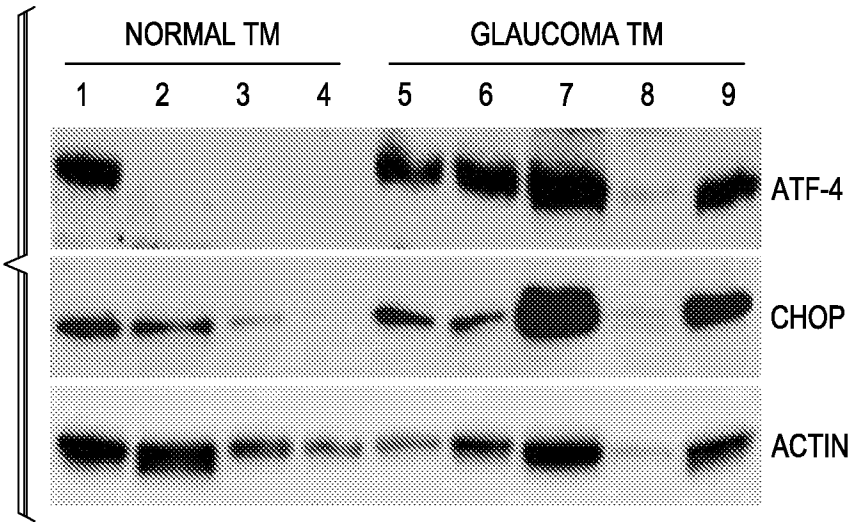


FIG. 6C

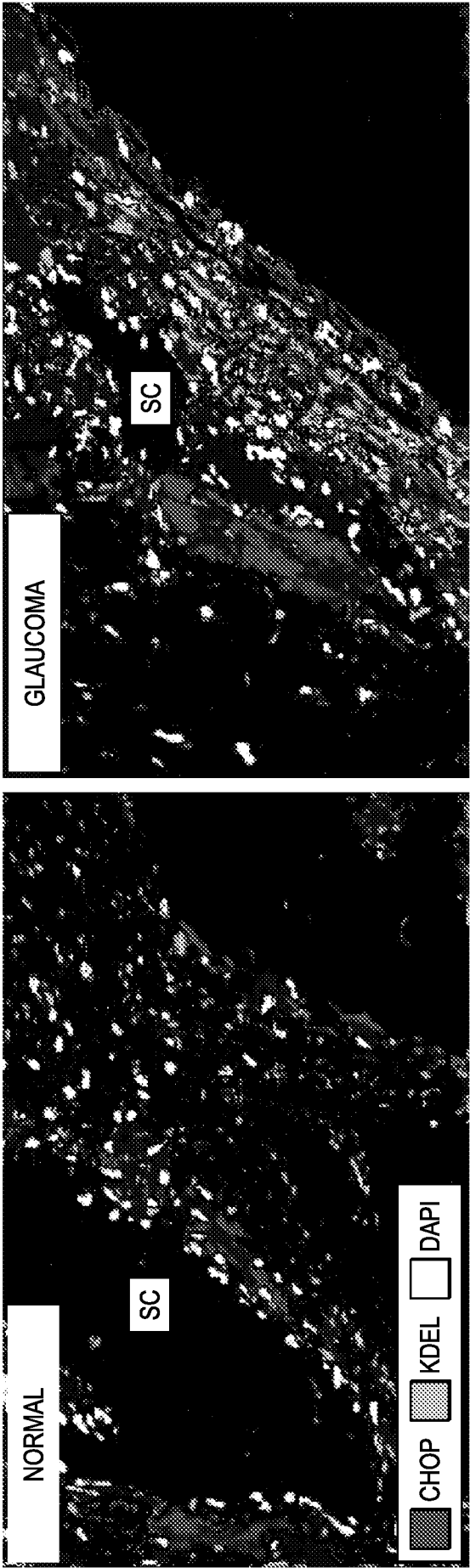


FIG. 6D

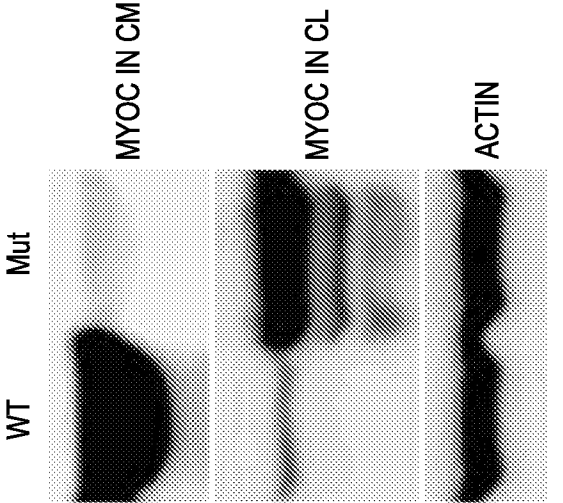


FIG. 7A

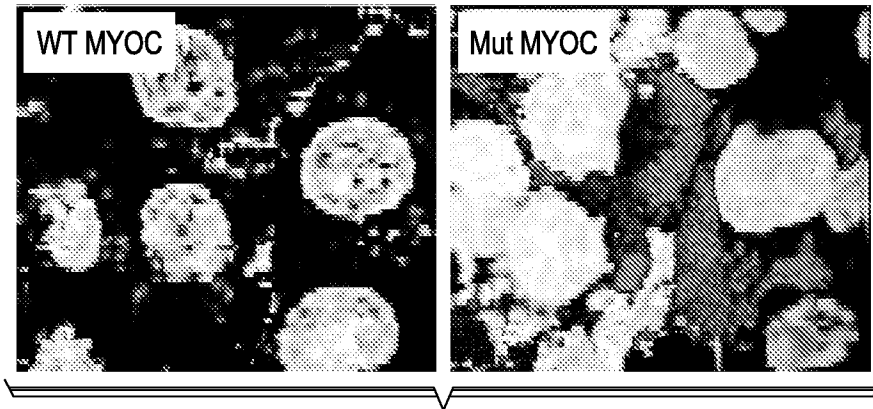


FIG. 7B

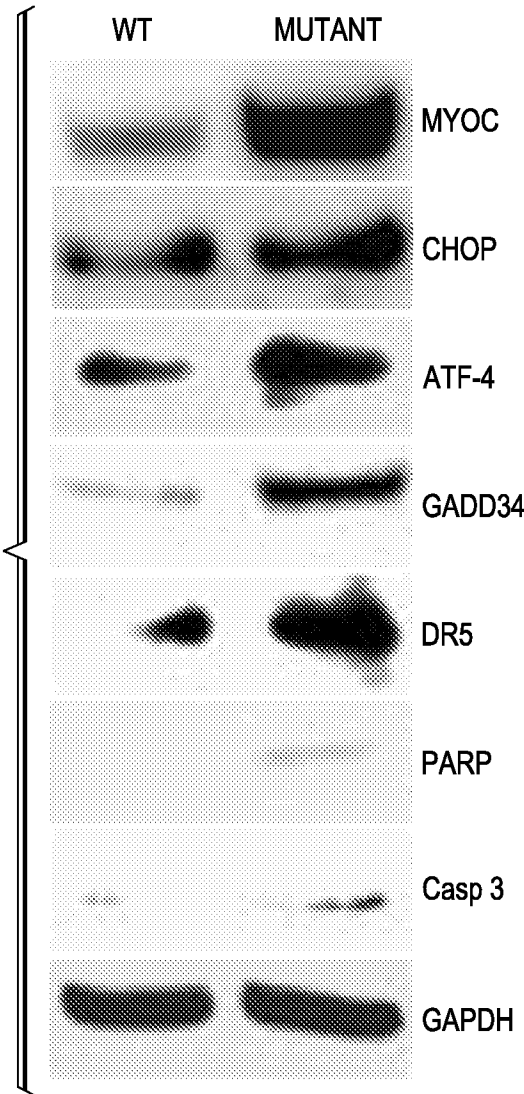


FIG. 7C

10/16

FIG. 8

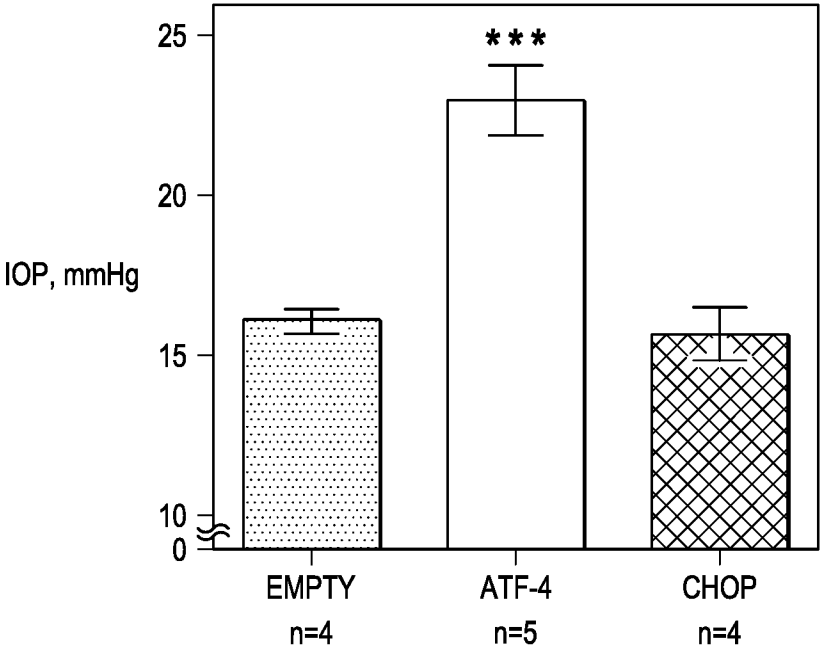
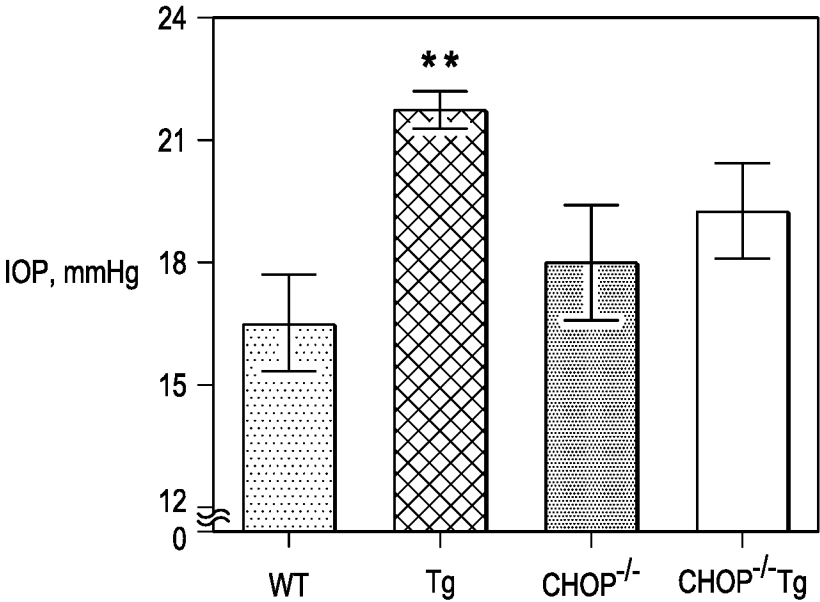


FIG. 9A



11/16

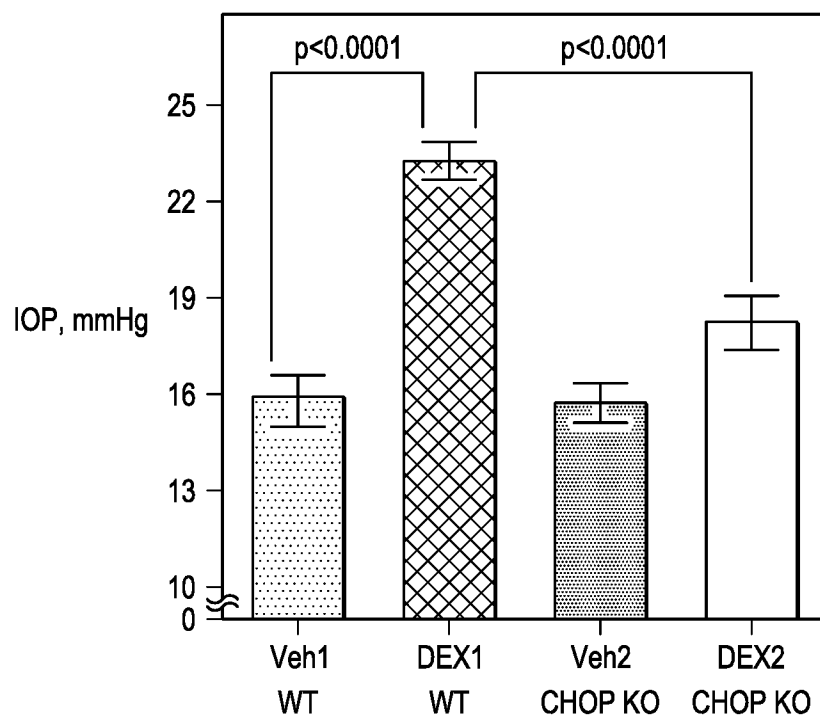


FIG. 9B

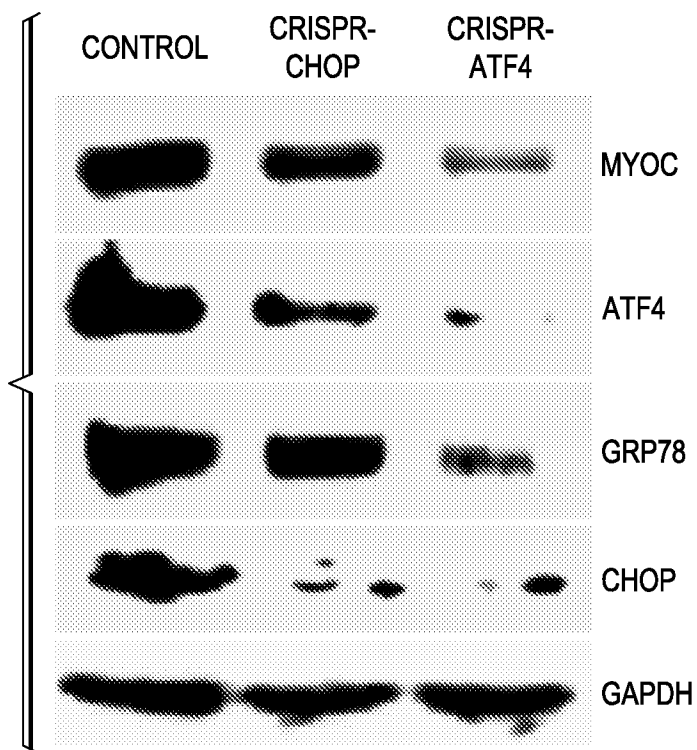


FIG. 10A

12/16

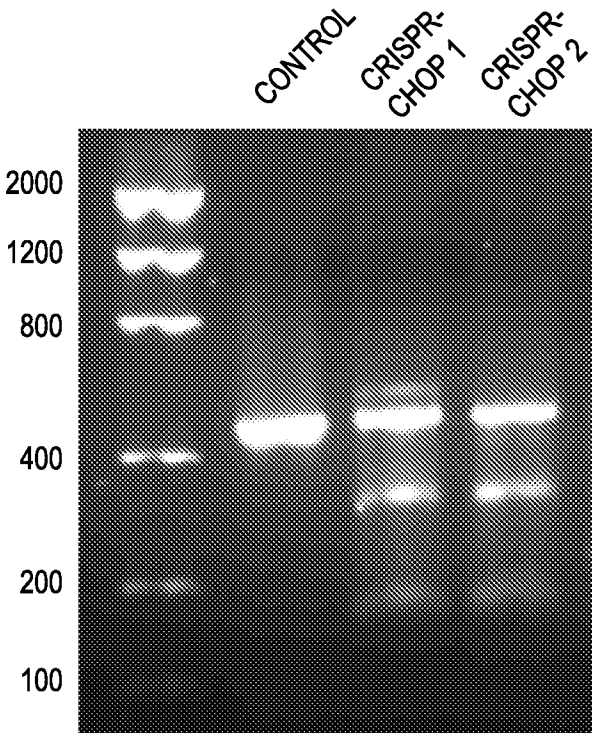


FIG. 10B

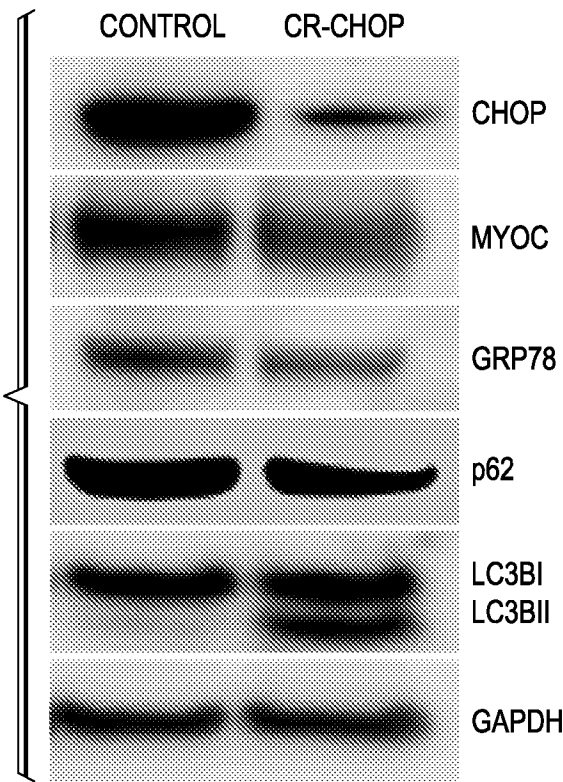


FIG. 11A

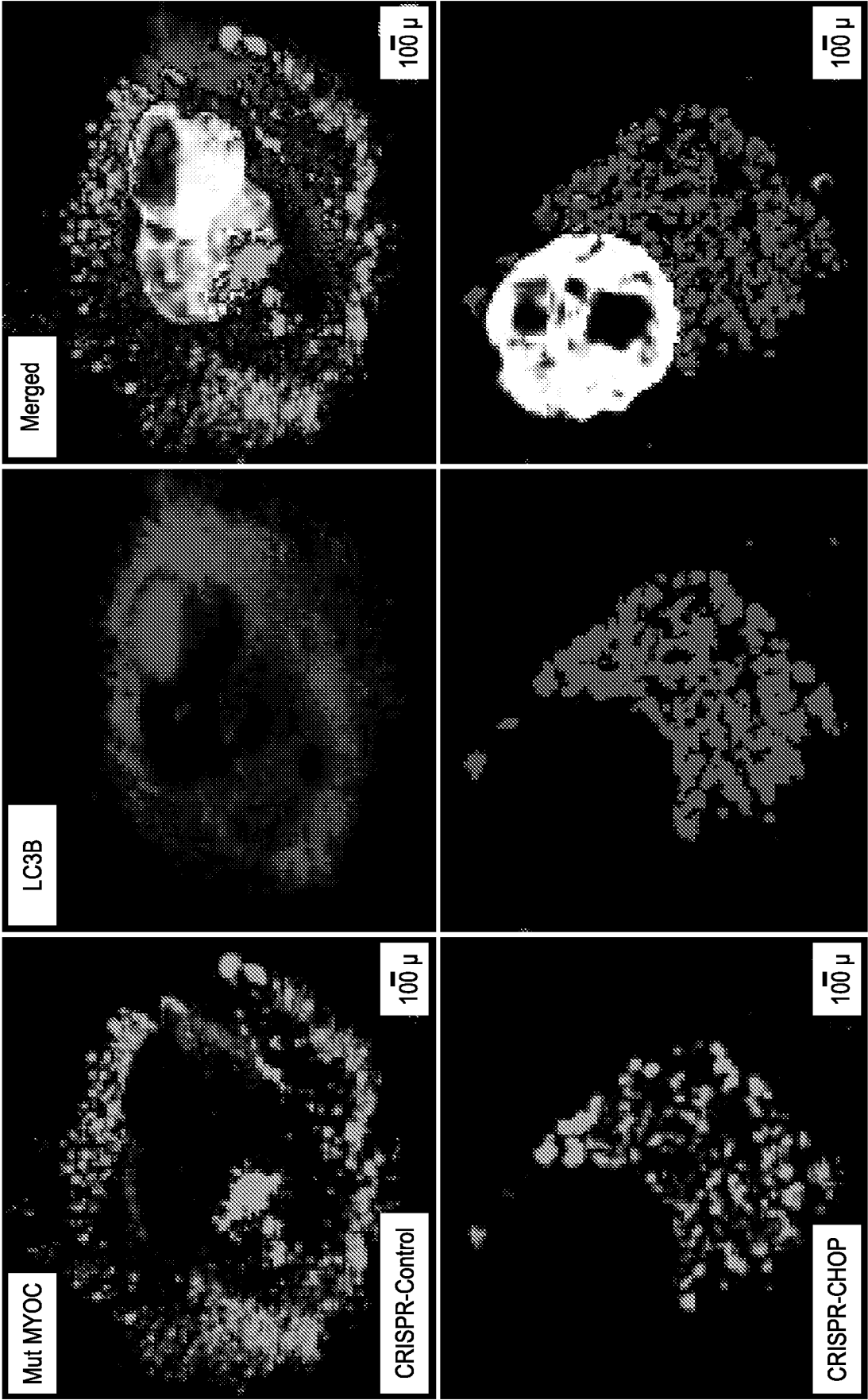


FIG. 11B

FIG. 12A

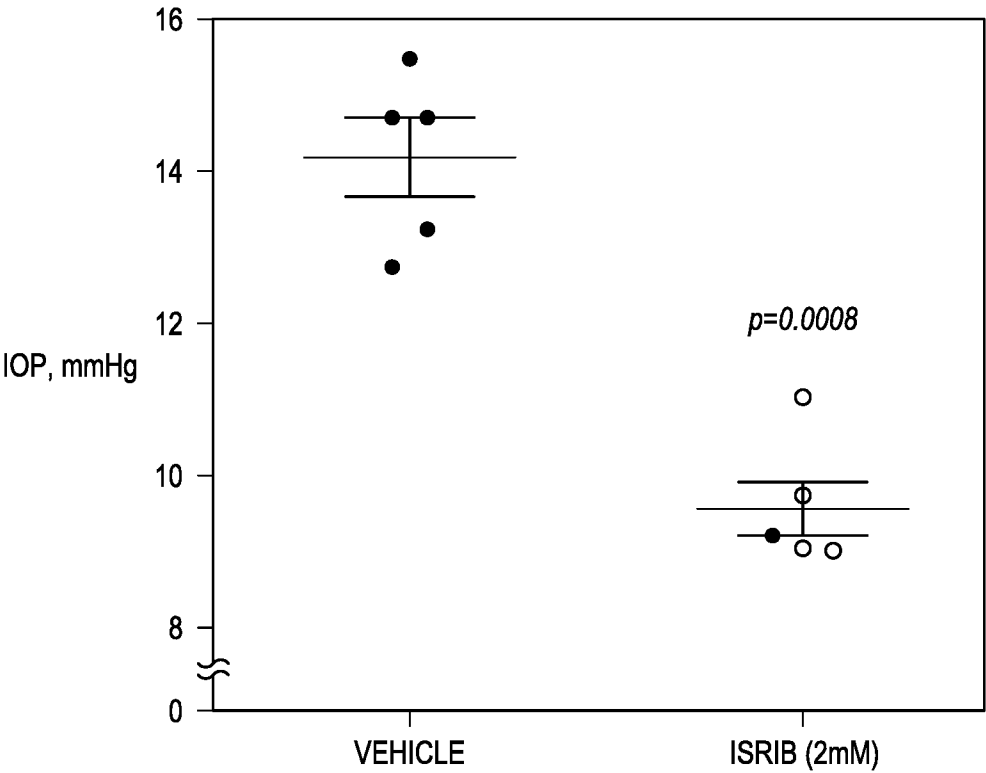
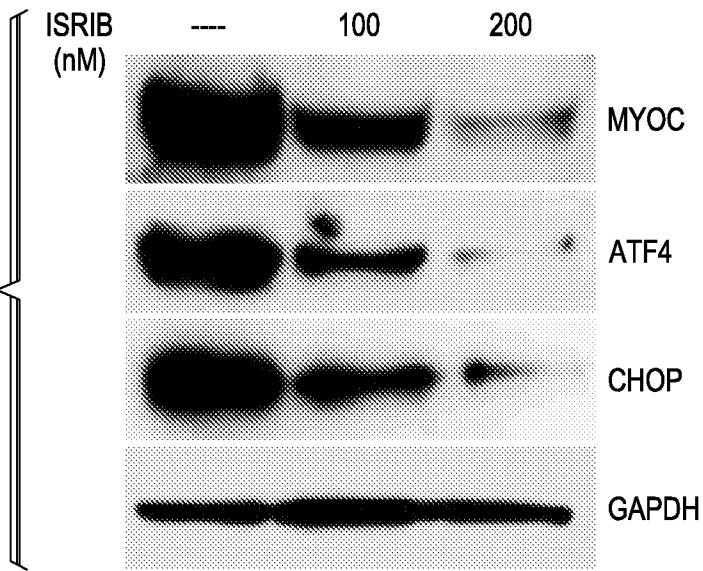
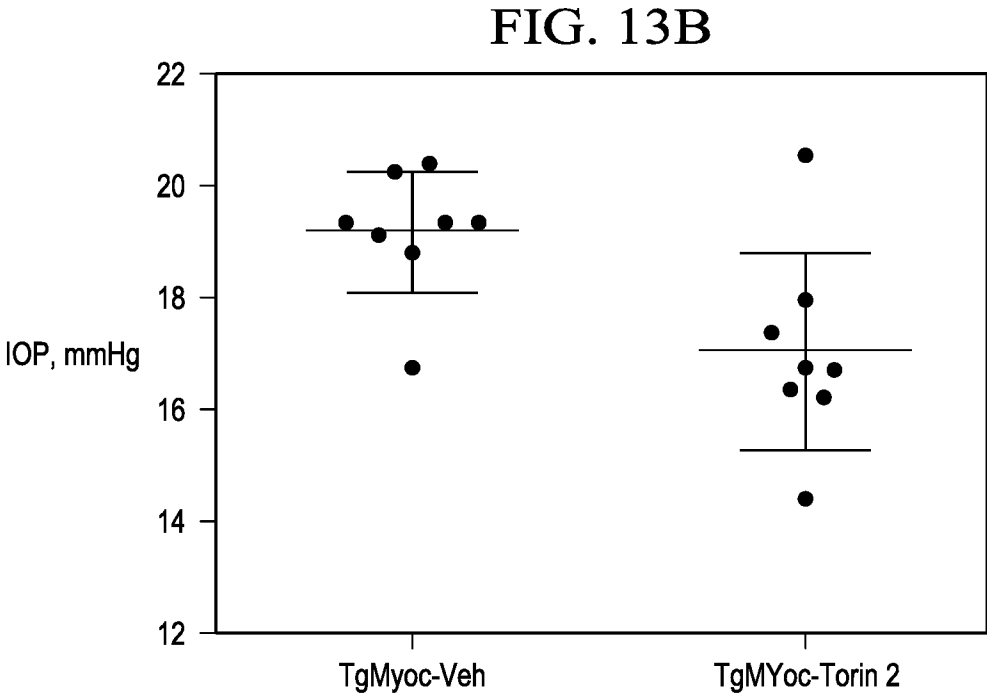
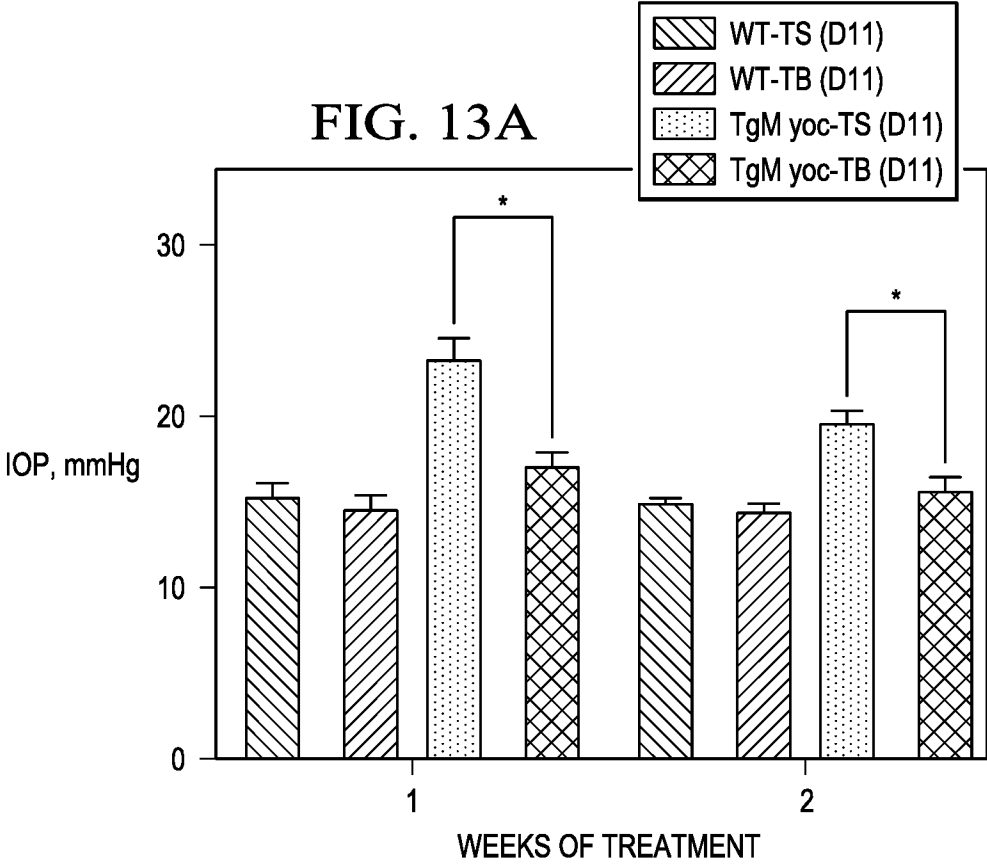


FIG. 12B



16/16

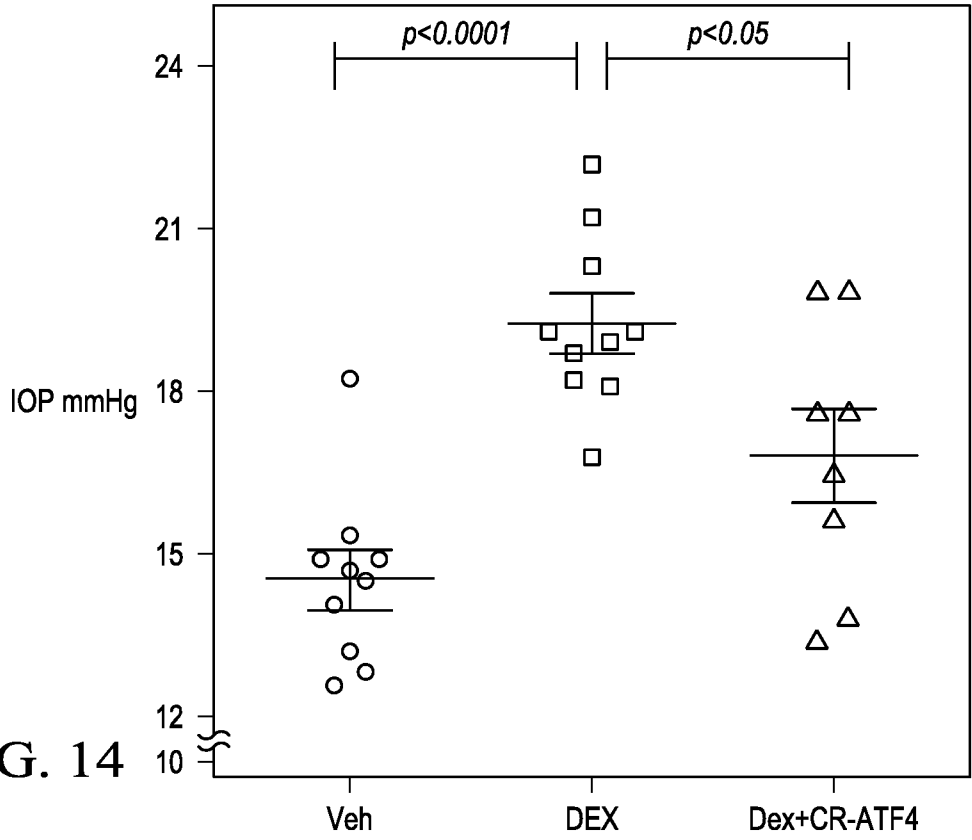


FIG. 14

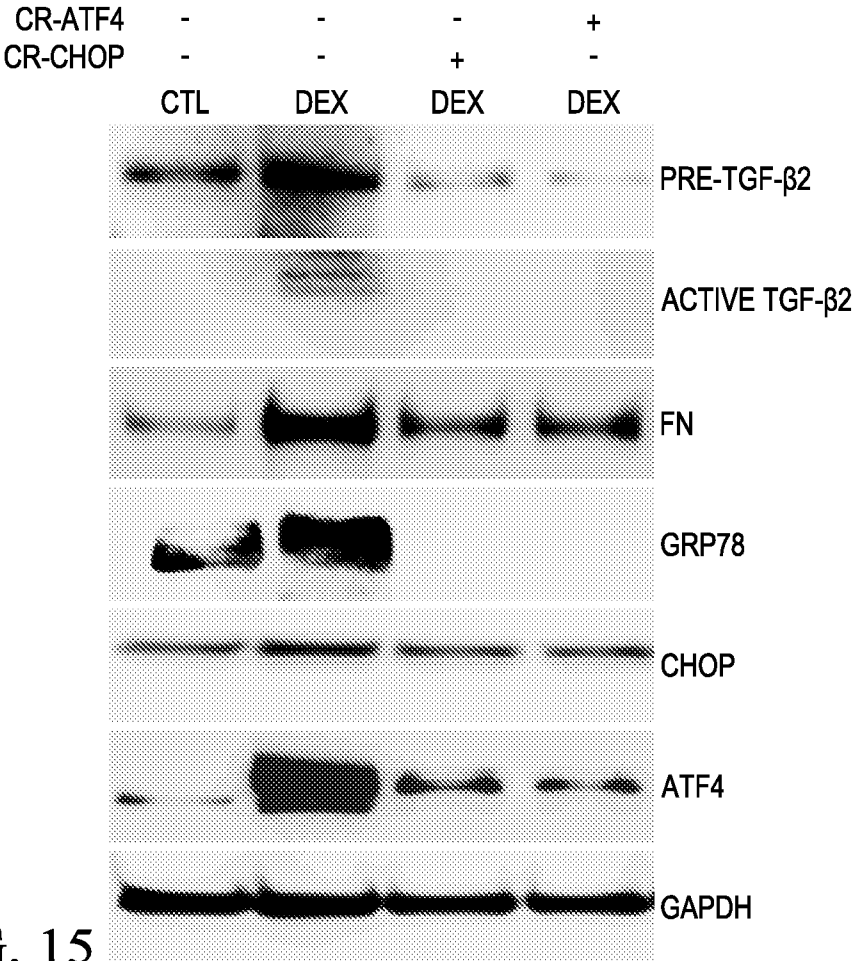


FIG. 15

A. CLASSIFICATION OF SUBJECT MATTER**A61K 38/17(2006.01)i, A61K 31/436(2006.01)i, A61K 31/216(2006.01)i, A61K 48/00(2006.01)i, A61K 45/06(2006.01)i**

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K 38/17; A61K 31/015; A61K 31/7048; A61K 31/04; A61K 31/37; A61K 31/436; A61K 31/216; A61K 48/00; A61K 45/06

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

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

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

eKOMPASS(KIPO internal) & Keywords: autophagy, eye disease, chaperone, sgRNA, evaluation

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SANO, RENATA et al., 'ER stress-induced cell death mechanisms', Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 10 July 2013(online), Vol. 1833, pp. 3460-3470 See abstract; page 3464, right column, lines 25-26; page 3465; page 3466, left column, lines 1-18 and right column, line 50.	1-12, 15-26, 47
Y		13-14
Y	ZHANG, QI et al., 'Zebrafish genome targeting with CRISPR-Cas9 endonuclease system at the nmnat1 locus', Investigative Ophthalmology & Visual Science, April 2014, Vol. 55, page 1346 See abstract.	13-14
X	US 2016-0000753 A1 (AMAZENTIS SA) 07 January 2016 See paragraphs [0003] and [0004]; claim 114.	1, 7-10, 15, 20-23
A	RULO, ALEXANDER H. et al., 'Reduction of intraocular pressure with treatment of latanoprost once daily in patients with normal-pressure glaucoma', Ophthalmology, August 1996, Vol. 103, No. 8, pp. 1276-1282 See page 1276; page 1280, left column, lines 6-11.	1-26, 47



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

25 October 2017 (25.10.2017)

Date of mailing of the international search report

25 October 2017 (25.10.2017)

Name and mailing address of the ISA/KR

International Application Division

Korean Intellectual Property Office

189 Cheongsu-ro, Seo-gu, Daejeon, 35208, Republic of Korea

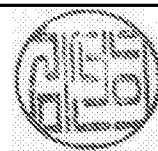


Facsimile No. +82-42-481-8578

Authorized officer

KIM, Sun Hee

Telephone No. +82-42-481-5405



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/041539

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 13ter.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 13ter.1(a)).

☐ on paper or in the form of an image file (Rule 13ter.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 REPORTInternational application No.
PCT/US2017/041539**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

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

1. ☒ Claims Nos.: 27-46
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 27-46 pertain to a method for treatment of the human body by therapy and thus relate to a subject matter which this International Searching Authority is not required to search under PCT Article 17(2)(a)(i) and Rule 39.1(iv).
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

Remark on Protest

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

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/041539

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KR 10-2012-0058886 A (INDUSTRY-ACADEMIC COOPERATION FOUNDATION, YONSEI UNIVERSITY) 08 June 2012 See abstract; paragraphs [0001], [0003] and [0058].	1-26, 47

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2017/041539

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2016-0000753 A1	07/01/2016	AR 091604 A1 AU 2013-284419 A1 CA 2877718 A1 CN 105142632 A EP 2866804 A2 JP 2015-523362 A KR 10-2015-0036224 A MX 2014016044 A SG 10201610798 QA SG 11201408539 UA US 2014-0018415 A1 WO 2014-004902 A2 WO 2014-004902 A3	18/02/2015 12/02/2015 03/01/2014 09/12/2015 06/05/2015 13/08/2015 07/04/2015 12/05/2015 27/02/2017 29/01/2015 16/01/2014 03/01/2014 06/03/2014
KR 10-2012-0058886 A	08/06/2012	KR 10-1692047 B1 US 2013-0251654 A1 US 9328059 B2 WO 2012-074187 A1	04/01/2017 26/09/2013 03/05/2016 07/06/2012