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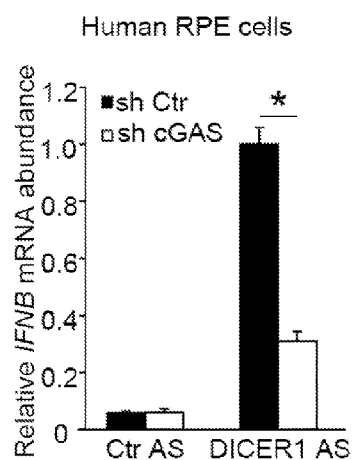


FIG. 4E

(57) Abstract: It is disclosed herein that RPE degeneration in human cell culture and in mouse models is driven by a non-canonical inflammasome pathway that results in activation of caspase-4 (also known as caspase-11 in mouse) and caspase-1, and requires cyclic GMP-AMP synthase (cGAS)-dependent interferon- β (IFN- β) production and gasdermin D-dependent interleukin-18 (IL-18) secretion. Reduction of DICER1 or accumulation of Alu RNA triggers cytosolic escape of mitochondrial DNA, which engages cGAS. Collectively, these data highlight an unexpected role for cGAS in responding to mobile element transcripts, reveal cGAS-driven interferon signaling as a conduit for mitochondrial damage-induced NLRP3 activation, and expand the immune sensing repertoire of cGAS and caspase-4 to non-infectious human disease. Coupled with the unexpected result that caspase-4, gasdermin D, IFN- β , and cGAS are elevated in the RPE of human eyes with geographic atrophy, these findings also identify new targets for a major cause of blindness.

COMPOSITIONS AND METHODS FOR TREATING AGE-RELATED MACULAR DEGENERATION AND GEOGRAPHIC ATROPHY

CROSS REFERENCE TO RELATED APPLICATIONS

5 This application claims priority under 35 U.S.C. §119(e) to U.S. Provisional Application Serial No. 62/570,207 filed October 10, 2017, the disclosure of which is incorporated by reference in its entirety herein.

10 STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

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BACKGROUND

 Age-related macular degeneration (AMD) affects over 180 million people⁴, and is increasingly the leading cause of blindness among the growing numbers of elderly across the world. Degeneration and death of the retinal pigmented epithelium (RPE), a monolayer of cells that provide trophic support to the photoreceptors⁵, is the hallmark of geographic atrophy and leads to vision loss. The RNase DICER1 is reduced in the RPE of human geographic atrophy eyes, leading to accumulation of toxic mobile element *Alu* RNA transcripts³; these *Alu* transcripts induce RPE cell death by activating the NLRP3 inflammasome². Although NLRP3 inflammasome activation has been widely implicated in macular degeneration⁶⁻¹⁰, the mechanisms regulating the inflammasome in this disease remain elusive.

 There is a long felt need in the art for compositions and methods useful for treating diseases and disorders of the retinal pigmented epithelium such as age-related macular degeneration and geographic atrophy. The present invention satisfies these needs.

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SUMMARY OF THE INVENTION

 The present application claims priority to a provisional application that was filed based on the draft of a manuscript by Kerur et al., which has since published in

Nature Medicine as "cGAS drives non-canonical NLRP3 (NLR family pyrin domain containing 3) inflammasome in age-related macular degeneration" (Nat. Med. 2018, 24(1):50-61; available as epub. on Nov. 27, 2017).

It is disclosed herein that DICER1 deficit/*Alu* RNA-driven RPE degeneration in mouse models of macular degeneration is unexpectedly mediated by caspase-4- and gasdermin D-dependent NLRP3 inflammasome activation. Unexpectedly, it is also disclosed that this non-canonical inflammasome is dependent on the activation of the DNA sensor cyclic GMP-AMP synthase (cGAS)-driven type I interferon (IFN) signaling by cytosolic mitochondrial DNA (mtDNA).

Based on the unexpected results described above, further work disclosed herein demonstrates that RPE degeneration in macular degeneration can be inhibited or prevented by targeting the alternative, non-canonical inflammasome signaling molecules, protein complexes, or their signal transduction pathways. In one aspect, the molecules and pathways include, but are not limited to, cGAS, Caspase-4/11, Gasdermin D (GSDMD), stimulator of interferon genes (STING), peptidyl-prolyl cis-trans isomerase F (PPIF), mitochondrial permeability transition pore (MPTP), IFN- β , or interferon- α/β receptor (IFNAR). Administering an inhibitor or blocking the activity of Caspase-4/11, cGAS, GSDMD STING, PPIF, MPTP, IFN- β , or IFNAR, or their signal transduction pathways, can protect RPE cells from death, and be therapeutically useful for diseases such as geographic atrophy and age-related macular degeneration. In one aspect, the protein complex is MPTP (see FIG. 21/Supplementary Fig. 15).

Data disclosed herein highlight an unexpected role for cGAS in responding to mobile element transcripts, reveal cGAS-driven interferon signaling as a conduit for mitochondrial damage-induced NLRP3 activation, and expand the immune sensing repertoire of cGAS and caspase-4 to non-infectious human disease. Coupled with the unexpected result that caspase-4, gasdermin D, IFN- β , and cGAS are elevated in the RPE of human eyes with geographic atrophy, these findings also identify new targets for a major cause of blindness.

In one embodiment, the present application discloses compositions and methods useful for inhibiting mitochondrial damage-induced NLRP3 activation.

Therefore, the present invention provides compositions and methods for protecting RPE cells against death or degeneration. In one aspect, the compositions and methods of the invention protect RPE cells by inhibiting one or more of the

molecules of the non-canonical inflammasome signaling pathway as disclosed herein. In one aspect, the compositions and methods of the invention protect RPE cells by inhibiting one or more of Caspase-4 (a.k.a. Caspase-11 in mice), Gasdermin D, IFN- β , IFNAR, STING, cGAS, PPIF, or mitochondrial permeability transition pore (mPTP) opening. In one aspect, each is inhibited.

The present application demonstrates that, unexpectedly, cGAS is increased in geographic atrophy RPE cells and that inhibiting cGAS can be useful for inhibiting Alu RNA-induced RPE cell death. The present application discloses that cGAS-driven interferon signaling is a conduit for mitochondrial-damage-induced inflammasome activation. In one embodiment, the application provides compositions and methods useful for inhibiting cGAS-driven signaling and inflammasome activation.

It is further disclosed herein that many signaling molecules can be targeted for preventing or slowing down RPE cell death in geographic atrophy and age-related macular degeneration (AMD). These signaling molecules have not been previously reported to play a role mediating RPE death. The present invention encompasses molecules, compositions, and methods useful to block the key signaling molecules including cGAS, Caspase-11/4, STING, MPTP, PPIF, Gasdermin D, IFNAR, or IFN- β . For example, it is disclosed herein that Gasdermin D is required for Alu RNA-induced RPE degeneration and inflammasome activation. In fact, it is disclosed herein for the first time that Gasdermin D is involved in a non-infectious human disease.

In one embodiment, the present invention provides compositions and methods for inhibiting Caspase-4 (Caspase-11). It is disclosed herein that Caspase-4 is required for Alu RNA-induced RPE degeneration and inflammasome activation.

In one embodiment, the compositions and methods of the invention are useful for inhibiting RPE cell death. In one aspect, the RPE cell death is Alu RNA-induced cell death. In one aspect, the compositions and methods of the invention are useful for inhibiting RPE cell death associated with age-related macular degeneration.

In one embodiment, the present invention provides compositions and methods for inhibiting GSDMD.

In one embodiment, the present invention provides compositions and methods for inhibiting STING.

In one embodiment, the present invention provides compositions and methods for inhibiting cGAS. In one aspect, inhibiting cGAS inhibits the cGAS-driven interferon signaling as disclosed herein. In one aspect, inhibiting cGAS-driven interferon signaling inhibits mitochondrial damage-induced NLRP3 activation.

In one embodiment, the present invention provides compositions and methods for inhibiting IFNAR.

In one embodiment, the present invention provides compositions and methods for inhibiting IFN- β .

In one embodiment, the present invention provides compositions and methods for inhibiting PPIF.

In one embodiment, the present invention provides compositions and methods for inhibiting mPTP.

The present application encompasses the use of multiple types of inhibitors.

In one aspect, a useful inhibitor can be an antisense oligonucleotide, small interfering RNA (siRNA), short hairpin RNA (shRNA), antibody, and biologically active fragments or homologs of the antibody. In one aspect, a useful inhibitor of the invention is cGAS shRNA (shcGAS), cGAS siRNA, Caspase-4 shRNA, caspase-4 siRNA, or an IFN- β neutralizing antibody. In one aspect, a useful inhibitor of the invention is GSDMD shRNA, STING shRNA, PPIF shRNA, IFNB shRNA, or IFNAR1 shRNA.

Useful antibodies include monoclonal antibody, humanized antibody, chimeric antibody, single chain antibody, and biologically active fragments and homologs thereof.

In one embodiment, an inhibitor of INF- β is administered to a subject in need thereof. In one aspect, the inhibitor is an IFN- β neutralizing antibody or a biologically active fragment or homolog thereof.

In one embodiment, an inhibitor of cGAS is administered to a subject in need thereof. In one aspect, the inhibitor is an shRNA. In another aspect, it is an siRNA.

By "inhibiting" a molecule is meant that its expression, activity, or levels are decreased relative to what it would be in the diseased state or that it is blocked from increasing to what is found in the disease state.

A homolog of a protein or peptide (including antibodies) of the invention may comprise one or more conservative amino substitutions relative to the parent protein or peptide.

5 In one aspect, a homolog has sequence identity of about 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% with the parent. In one aspect, a homolog has sequence identity of at least about 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% with the parent molecule.

10 In one embodiment, a pharmaceutical composition comprising an effective amount of at least one therapeutic agent (inhibitor) of the invention is administered to a subject in need thereof. A subject in need thereof is one diagnosed with a disease or disorder such as age-related macular degeneration or geographic atrophy or is one who has been determined to be susceptible to a disease or disorder such as age-related macular degeneration or geographic atrophy. Methods and biomarkers are available for predicting whether a subject is susceptible to AMD, including, for
15 example, genetic variants of complement factor H (CFH) and high-temperature requirement factor A-1 (HTRA1), smoking, and age.

In one aspect, at least two therapeutic agents or methods of the present invention are administered to a subject in need thereof. In one aspect, the at least two therapeutic agents are directed to one of the target molecules disclosed herein.
20 In another aspect, a combination therapy is administered to target at least two different alternative, non-canonical inflammasome signaling molecules or protein complexes to prevent or inhibit RPE degeneration. When a subject has been diagnosed to be susceptible to an RPE disease or disorder, one or more of the therapeutic agents of the invention can be administered prophylactically. In one
25 embodiment, administration of a therapeutic agent of the invention inhibits RPE degeneration.

In one aspect, the present application provides for treatment using at least one agent or method of the present invention regulating the non-canonical pathway in combination with other agents or methods known to be useful for treating or
30 preventing Alu RNA induced RPE degeneration in AMD or geographic atrophy. Other agents and methods that are known include, but are not limited to, the use of agents to inhibit Alu RNA, stimulate DICER1, and inhibit IL-18, MyD88, the NLRP3 inflammasome, or Caspase 1. In one aspect, the inhibitor of Alu RNA is an siRNA or an antisense oligonucleotide.

The dose administered to a subject in need thereof can vary depending on the disease state as well as on the age, sex, weight, and health of the subject.

The model as presented in FIG. 21 (also referred to as Supplementary Figure 15) summarizes the unexpected role of cGAS, Caspase 4/11, and Gasdermin D in RPE cell degeneration and also demonstrates the useful targets disclosed herein.

siRNA and shRNA targeting the cGAS, Caspase-4 and Gasdermin D are available and can also be made based on the known sequences of cGAS, Caspase-4, and Gasdermin D.

In one aspect, the human cGAS shRNA (TRCN0000146282) is 5'-
 CCGGCTTTGATAACTGCGTGACATACTCGAGTATGTCACGCAGTTATCAA
 AGTTTTTTG-3' (SEQ ID NO:1). In one aspect, the cGAS shRNA is SEQ ID
 NO:15
 (CCGGCCTGCTGTAACTTCTTATTCTCGAGAATAAGAAGTGTTACAGC
 AGGTTTTTTG).

In one aspect, the human cGAS siRNA is human cGAS siRNA
 (SASI_Hs01_
 AAGAAGAAACATGGCGGCTATCCTTCTCTCACATCGAAAAGGAAATTT)
 (SEQ ID NO:2).

In one aspect, an shRNA directed against Caspase-4 has SEQ ID NO:16.

In one aspect, a human siRNA directed against Caspase-4 has a sequence selected from the group consisting of SEQ ID NOs: 9, 10, 11, 12, 13, and 14.

In one aspect, an shRNA directed against GSDMD has SEQ ID NO:19.

In one aspect, an shRNA directed against STING has SEQ ID NO:17.

In one aspect, an shRNA directed against PPIF has SEQ ID NO:18.

In one aspect, an shRNA directed against IFNB has SEQ ID NO:20.

In one aspect, an shRNA directed against IFNAR1 has SEQ ID NO:21.

In one aspect, two or more different shRNAs are administered to a subject.

In one aspect, two or more different siRNAs are administered to a subject.

In one aspect, one shRNA and one siRNA are administered to a subject.

In one aspect, an inhibitory antibody, or a biologically active fragment or analog thereof, is administered to a subject in combination with an shRNA or an siRNA of the invention.

In one embodiment, the compositions and methods disclosed in the present application are useful for preventing or inhibiting blindness.

In one embodiment, additional therapeutic agents are administered in addition to the inhibitors of the invention. For example, additional therapeutic agent include, but are not limited to, cyclosporin A, Alu RNA antisense oligonucleotide, and a reverse transcriptase inhibitor.

5 Other agents can be administered, including antimicrobials.

The present invention further provides compositions and methods for method for inhibiting a non-canonical inflammasome signaling molecule, protein complex, or pathway in an RPE cell. In one aspect, the method comprises contacting an RPE cell with an effective amount of an inhibitor of at least one a non-canonical
10 inflammasome molecule, protein complex, or pathway. In one aspect, the method comprises contacting the RPE cell with an inhibitor of at least one molecule or complex selected from the group consisting of cGAS, caspase-4, STING, PPIF, MPTP, GSDMD, IFN- β , and IFNAR. In one aspect, the type of inhibitor includes, but is not limited to, antisense oligonucleotide, small interfering RNA (siRNA),
15 short hairpin RNA (shRNA), antibody, and biologically active fragments or homologs of the antibody. In one aspect, a homolog of an antibody or useful protein or peptide of the invention comprises at least 95% sequence identity with antibody, protein, or peptide. In one aspect, the antibody is a monoclonal antibody, humanized antibody, chimeric antibody, or single chain antibody. In one aspect, the
20 useful inhibitors include, but are not limited to, shcGAS, cGAS siRNA, caspase-4 shRNA, caspase-4 siRNA and an IFN- β neutralizing antibody. In one aspect, shcGAS is SEQ ID NO:1 or SEQ ID NO:15 and cGAS siRNA is SEQ ID NO:2. In one aspect, the inhibitor is Caspase-4 shRNA or Caspase-4 siRNA. In one aspect, the Caspase-4 shRNA is SEQ ID NO:16. In one aspect, the Caspase-4 siRNA has a
25 sequence selected from the group consisting of SEQ ID NOs: 9, 10, 11, 12, 13, and 14. In one aspect, the method protects said RPE cell from cell death. In one aspect, the method inhibits Alu RNA-induced RPE degeneration.

Also provided are compositions and methods useful for determining whether a subject has age-related macular degeneration or is susceptible to age-related
30 macular degeneration. For example, the present application discloses an increase in each of Caspase-4, cGAS, and Gasdermin D to be associated with macular degeneration. One or more of these markers can be measured to determine whether a subject has macular degeneration. These methods can be used with known

diagnostic methods previously in use for diagnosing macular degeneration, particularly age-related macular degeneration.

In one embodiment, the compositions and methods of the present application provide for treating a subject for age-related macular degeneration once the subject
5 has been diagnosed with age-related macular degeneration.

In one embodiment, the levels of caspase-4, cGAS, or Gasdermin D, or at least two of the three, are determined in a subject and when the levels are found to be higher than control levels the subject is treated to inhibit RPE cell degeneration. In one aspect, it is suspected that the subject has or is developing age-related
10 macular degeneration when one or more of the assays is performed. In one aspect, the determination of the levels of one or more of caspase-4, cGAS, or Gasdermin D is made as a routine preventative measure or checkup.

Kits are encompassed by the present invention and can include one of more of the therapeutic agents, optionally additional therapeutic compounds, an
15 applicator, and an instructional material.

Various aspects and embodiments of the invention are described in further detail below.

Some useful sequences of the invention-

20 SEQ ID NO:1- human cGAS shRNA-
CCGGCTTTGATAACTGCGTGACATACTCGAGTATGTCACGCAGTTATCAA
AGTTTTTTTG

SEQ ID NO:2- human cGAS siRNA-
25 AAGAAGAAACATGGCGGCTATCCTTCTCTCACATCGAAAAGGAAATTT

SEQ ID NO:3- human cGAS protein-
MQPWHGKAMQRASEAGATAPKASARNARGAPMDPTESPAAPEAALPKAG
KFGPARKSGSRQKKSAPDTQERPPVRATGARAKKAPQRAQDTQPSDATSAP
30 GAEGLEPPAAREPALS RAGSCRQRGARCSTKPRPPPGPVDVPSGLPVSAPIL
VRRDAAPGASKLRAVLEKLKLSRDDISTAAGMVKGVDHLLRLKCDSAF
RGVGLLNTGSYYEHVKISAPNEFDVMFKLEVPRIQLEEYSNTRAYYFVKFKR
NPKENPLSQFLEGEILSASKMLSKFRKIIKEEINDIKD TDVIMKRKRGGSPAVT
LLISEKISVDITLALESKSSWPASTQEGLRIQNWLSAKVRKQLRLKPFYLVPK

HAKEGNFQEETWRLSFSHIEKEILNNHGKSKTCCENKEEKCCRKDCLKLM
 KYLLEQLKERFKDKKHLDKFSSYHVKTAFFHVCTQNPQDSQWDRKDLGLC
 FDNCVTYFLQCLRTEKLENYFIPEFNLFSSNLIDKRSKEFLTKQIEYERNNEFP
 VFDEF

5

SEQ ID NO:4- human cGAS mRNA/nucleotide-

AGCCTGGGGTTCCCCTTCGGGTCGCAGACTCTTGTGTGCCCCGCCAGTAGT
 GCTTGTTTCCAACAGCTGCTGCTGGCTCTTCCTCTTGCGGCCTTTTCCTG
 AAACGGATTCTTCTTTTCGGGGAACAGAAAGCGCCAGCCATGCAGCCTTG
 10 GCACGGAAAGGCCATGCAGAGAGCTTCCGAGGCCGGAGCCACTGCCCCC
 AAGGCTTCCGCACGAATGCCAGGGGCGCCCCGATGGATCCCACCGAGT
 CTCCGGCTGCCCCCGAGGCCGCCCTGCCTAAGGCGGGAAAGTTCGGCCC
 CGCCAGGAAGTCGGGATCCCGGCAGAAAAAGAGCGCCCCGGACACCCA
 GGAGAGGCCGCCCCGTCCGCGCAACTGGGGCCCGCGCCAAAAAGGCCCT
 15 CAGCGCGCCCAGGACACGCAGCCGTCTGACGCCACCAGCGCCCCTGGGG
 CAGAGGGGCTGGAGCCTCCTGCGGCTCGGGAGCCGGCTCTTTCCAGGGC
 TGGTTCTTGCCGCCAGAGGGGCGCGCGCTGCTCCACGAAGCCAAGACCT
 CCGCCCCGGGCCCTGGGACGTGCCAGCCCCGGCCTGCCGGTCTCGGCCC
 CCATTCTCGTACGGAGGGATGCGGCGCCTGGGGCCTCGAAGCTCCGGGC
 20 GGTTTTGGAGAAGTTGAAGCTCAGCCGCGATGATATCTCCACGGCGGCG
 GGGATGGTGAAAGGGGTTGTGGACCACCTGCTGCTCAGACTGAAGTGCG
 ACTCCGCGTTCAGAGGCGTCGGGCTGCTGAACACCGGGAGCTACTATGA
 GCACGTGAAGATTTCTGCACCTAATGAATTTGATGTCATGTTTAAACTGG
 AAGTCCCCAGAATTCAACTAGAAGAATATTCCAACACTCGTGATATTA
 25 CTTTGTGAAATTTAAAAGAAATCCGAAAGAAAATCCTCTGAGTCAGTTTT
 TAGAAGGTGAAATATTATCAGCTTCTAAGATGCTGTCAAAGTTTAGGAA
 AATCATTAAGGAAGAAATTAACGACATTAAAGATACAGATGTCATCATG
 AAGAGGAAAAGAGGAGGGAGCCCTGCTGTAACACTTCTTATTAGTGAAA
 AAATATCTGTGGATATAACCCTGGCTTTGGAATCAAAAAGTAGCTGGCC
 30 TGCTAGCACCCAAGAAGGCCTGCGCATTCAAACTGGCTTTTCAGCAAAA
 GTTAGGAAGCAACTACGACTAAAGCCATTTTACCTTGTACCCAAGCATG
 CAAAGGAAGGAAATGGTTTCCAAGAAGAAACATGGCGGCTATCCTTCTC
 TCACATCGAAAAGGAAATTTTGAACAATCATGGAAAATCTAAAACGTGC
 TGTGAAAACAAAGAAGAGAAATGTTGCAGGAAAGATTGTTTAAACTAA

TGAAATACCTTTTAGAACAGCTGAAAGAAAGGTTTAAAGACAAAAACA
 TCTGGATAAATTCTCTTCTTATCATGTGAAAACCTGCCTTCTTTCACGTATG
 TACCCAGAACCTCAAGACAGTCAGTGGGACCGCAAAGACCTGGGCCTC
 TGCTTTGATAACTGCGTGACATACTTTCTTCAGTGCCTCAGGACAGAAAA
 5 ACTTGAGAATTATTTTATTCCTGAATTCAATCTATTCTCTAGCAACTTAAT
 TGACAAAAGAAGTAAGGAATTTCTGACAAAGCAAATTGAATATGAAAG
 AAACAATGAGTTTCCAGTTTTTGGATGAATTTTGAGATTGTATTTTAGAA
 AGATCTAAGAACTAGAGTCACCCTAAATCCTGGAGAATACAAGAAAAAT
 TTGAAAAGGGGCCAGACGCTGTGGCTCAC

10

SEQ ID NO:5- human Caspase-4 protein-

MAEGNHRKKPLKVLESLGKDFLTGVLDNLVEQNVLNWKEEEKKKYYDAK
 TEDKVRVMADSMQEKQRMAGQMLLQTFNIDQISPNKKAHPNMEAGPPES
 GESTDALKLCPHEEFLRLCKERAEEIYPIKERNNRTRLALIICNTEFDHLPPRN
 15 GADFDITGMKELLEGLDYSVDVEENLTARDMESALRAFATRPEHKSSDSTF
 LVLMSHGILEGICGTVHDEKKPDVLLYDTIFQIFNNRNCLSLKDKPKVIIVQA
 CRGANRGELWVRDSPASLEVASSQSENLEEDAVYKTHVEKDFIAFCSSTPH
 NVSWRDSTMGSIFITQLITCFQKYSWCCHLEEVFRKVQQSFETPRAKAQMPT
 IERLSMTRYFYLFPGN

20

SEQ ID NO:6- human Caspase-4 mRNA/nucleotide-

ATACATAGTTTACTTTTCAATTTTGGACTCTGAGGCTCTTTCCAACGCTGTAA
 AAAAGGACAGAGGCTGTTCCCTATGGCAGAAGGCAACCACAGAAAAAA
 GCCACTTAAGGTGTTGGAATCCCTGGGCAAAGATTTCTCTACTGGTGTTT
 25 TGGATAACTTGGTGGAAACAAAATGTACTGAACTGGAAGGAAGAGGAAA
 AAAAGAAATATTACGATGCTAAACTGAAGACAAAGTTCGGGTCATGGC
 AGACTCTATGCAAGAGAAGCAACGTATGGCAGGACAAATGCTTCTTCAA
 ACCTTTTTTAACATAGACCAAATATCCCCCAATAAAAAAGCTCATCCGAA
 TATGGAGGCTGGACCACCTGAGTCAGGAGAATCTACAGATGCCCTCAAG
 30 CTTTGTCCTCATGAAGAATTCCTGAGACTATGTAAAGAAAGAGCTGAAG
 AGATCTATCCAATAAAGGAGAGAAACAACCGCACACGCCTGGCTCTCAT
 CATATGCAATACAGAGTTTGACCATCTGCCTCCGAGGAATGGAGCTGAC
 TTTGACATCACAGGGATGAAGGAGCTACTTGAGGGTCTGGACTATAGTG
 TAGATGTAGAAGAGAATCTGACAGCCAGGGATATGGAGTCAGCGCTGAG

GGCATTTGCTACCAGACCAGAGCACAAGTCCTCTGACAGCACATTCTTG
 GTACTCATGTCTCATGGCATCCTGGAGGGAATCTGCGGAACTGTGCATG
 ATGAGAAAAAACCAGATGTGCTGCTTTATGACACCATCTTCCAGATATTC
 AACAAACCGCAACTGCCTCAGTCTGAAGGACAAACCCAAGGTCATCATTG
 5 TCCAGGCCTGCAGAGGTGCAAACCGTGGGGAACGTGTGGGTCAGAGACTC
 TCCAGCATCCTTGGAAGTGGCCTCTTCACAGTCATCTGAGAACCTAGAGG
 AAGATGCTGTTTACAAGACCCACGTGGAGAAGGACTTCATTGCTTTCTGC
 TCTTCAACGCCACACAACGTGTCCTGGAGAGACAGCACAATGGGCTCTA
 TCTTCATCACACAACCTCATCACATGCTTCCAGAAATATTCTTGGTGCTGC
 10 CACCTAGAGGAAGTATTTTCGGAAGGTACAGCAATCATTGAACTCCAA
 GGGCCAAAGCTCAAATGCCACCATAGAACGACTGTCCATGACAAGATA
 TTTCTACCTCTTTCCTGGCAATTGAAAATGGAAGCCACAAGCAGCCCAGC
 CCTCCTTAATCAACTTCAAGGAGCACCTTCATTAGTACAGCTTGCATATT
 TAACATTTTGTATTTCAATAAAAGTGAAGACAAACGA

15

SEQ ID NO:7- human Gasdermin D protein-

MGSAFERVRRVVQELDHGGEFIPVTSLSSTGFQPYCLVVRKPSSSWFWK
 PRYKCVNLSIKDILEPDAAEPDVQRGRSFHFYDAMDGQIQGSVELAAPGQA
 KIAGGAAVSDSSSTSMNVYSLSDPNTWQTLLHERHLRQPEHKVLQQLRSR
 20 GDNVYVVTEVLQTQKEVEVTRTHKREGSGRFSLPGATCLQGEGQGHLSQK
 KTVTIPSGSTLAFRVAQLVIDSDLVDVLLFPDKKQRTFQPPATGHRSTSEGA
 WPQLPSGLSMMRCLHNFLTDGVPAEGAFTEDFQGLRAEVETISKELELLDRE
 LCQLLLEGLEGVLRDQLALRALEEALQGGQSLGPVEPLDGPAGAVLECLVL
 SSGMLVPELAIPVVYLLGALTMLSETQHKLLEALESQTLLGPLELVGSLLE
 25 QSAPWQERSTMSLPPGLLGNWGE GAPAVVLLDECGLLELGEDTPHVCWEP
 QAQGRMCALYASLALLSGLSQEPH

SEQ ID NO:8- human Gasdermin D mRNA/nucleotide-

CCTGGGCGGGCCCTGCGTCAGGTTGCAGTTTCACTTTTAGCTCTGGGCAC
 30 CTCCAGCTCCTGCTCGCCGACGGCTCCCAGGGAGAGCAGACGCGCCAG
 ACGCGCCACCCTCGGGGCGCCGACGGTCACGGAGCATGGGGTCGGCCTT
 TGAGCGGGTAGTCCGGAGAGTGGTCCAGGAGCTGGACCATGGTGGGGA
 GTTCATCCCTGTGACCAGCCTGCAGAGCTCCACTGGCTTCCAGCCCTACT
 GCCTGGTGGTTAGGAAGCCCTCAAGCTCATGGTTCTGGAAACCCCGTTAT

AAGTGTGTCAACCTGTCTATCAAGGACATCCTGGAGCCGGATGCCGCGG
AACCAGACGTGCAGCGTGGCAGGAGCTTCCACTTCTACGATGCCATGGA
TGGGCAGATACAGGGCAGCGTGGAGCTGGCAGCCCCAGGACAGGCAAA
GATCGCAGGCGGGGCCGCGGTGTCTGACAGCTCCAGCACCTCAATGAAT
5 GTGTACTCGCTGAGTGTGGACCCTAACACCTGGCAGACTCTGCTCCATGA
GAGGCACCTGCGGCAGCCAGAACACAAAGTCCTGCAGCAGCTGCGCAGC
CGCGGGGACAACGTGTACGTGGTGAAGTGGTGTACAGACACAGAAG
GAGGTGGAAGTCACGCGCACCCACAAGCGGGAGGGGCTCGGGCCGGTTTT
CCCTGCCCCGAGCCACGTGCTTGCAGGGTGAGGGCCAGGGCCATCTGAG
10 CCAGAAGAAGACGGTCACCATCCCCCTCAGGCAGCACCCCTCGCATTCGGG
GTGGCCCAGCTGGTTATTGACTCTGACTTGGACGTCTTCTCTTCCCGGA
TAAGAAGCAGAGGACCTTCCAGCCACCCGCGACAGGCCACAAGCGTTCC
ACGAGCGAAGGCGCCTGGCCACAGCTGCCCTCTGGCCTCTCCATGATGA
GGTGCCTCCACAACCTTCCTGACAGATGGGGTCCCTGCGGAGGGGGCGTT
15 CACTGAAGACTTCCAGGGCCTACGGGCAGAGGTGGAGACCATCTCCAAG
GAACTGGAGCTTTTGGACAGAGAGCTGTGCCAGCTGCTGCTGGAGGGCC
TGGAGGGGGTGCTGCGGGACCAGCTGGCCCTGCGAGCCTTGGAGGAGGC
GCTGGAGCAGGGCCAGAGCCTTGGGCCGGTGGAGCCCCTGGACGGTCCA
GCAGGTGCTGTCCTGGAGTGCCTGGTGTGTCCTCCGGAATGCTGGTGCC
20 GGAATCGCTATCCCTGTTGTCTACCTGCTGGGGGCACTGACCATGCTGA
GTGAAACGCAGCACAAAGCTGCTGGCGGAGGCGCTGGAGTCGCAGACCCT
GTTGGGGCCGCTCGAGCTGGTGGGCAGCCTCTTGGAGCAGAGTGCCCCG
TGGCAGGAGCGCAGCACCATGTCCCTGCCCCCGGGCTCCTGGGGAACA
GCTGGGGGCGAAGGAGCACCGGCCTGGGTCTTGCTGGACGAGTGTGGCCT
25 AGAGCTGGGGGAGGACACTCCCCACGTGTGCTGGGAGCCGCAGGCCAG
GGCCGCATGTGTGCACTCTACGCCTCCCTGGCACTGCTATCAGGACTGAG
CCAGGAGCCCCACTAGCCTGTGCCCGGGCATGGCCTGGCAGCTCTCCAG
CAGGGCAGAGTGTTTGCCACCAGCTGCTAGCCCTAGGAAGGCCAGGAG
CCCAGTAGCCATGTGGCCAGTCTACCATGGGGCCCAGGAGTTGGGGAAA
30 CACAATAAAGGTGGCATAACGAAGGAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAA

SEQ ID NO:9- siRNA directed against Caspase-4, S1

GUGUAGAUGUAGAAGAGAATT

SEQ ID NO:10- siRNA directed against Caspase-4, S2
CCUAGAGGAAGAUGCUGUUTT

5

SEQ ID NO:11- siRNA directed against Caspase-4
CUACACUGUGGUUGACGAA

SEQ ID NO:12- siRNA directed against Caspase-4
10 CCAUAGAACGAGCAACCUU

SEQ ID NO:13- siRNA directed against Caspase-4
CAGCAGAAUCUACAAUAU

15 SEQ ID NO:14- siRNA directed against Caspase-4
CGGAUGUGCUGCUUUAUGA

SEQ ID NO:15- shRNA against cGAS
CCGGCCTGCTGTAAACACTTCTTATTCTCGAGAATAAGAAGTGTTACAG
20 CAGGTTTTTTTG

SEQ ID NO:16- shRNA against Caspase-4
CCGGAGACTATGTAAAGAAAGAGCTCTCGAGAGCTCTTTCTTTACATA
GTCTTTTTT

25

SEQ ID NO:17- shRNA against STING
CCGGCCAACATTCGCTTCCTGGATACTCGAGTATCCAGGAAGCGAATG
TTGGTTTTTTTG

30 SEQ ID NO:18- shRNA against PPIF
CCGGCTGTGGCCAGTTGAGCTAATCCTCGAGGATTAGCTCAACTGGCC
ACAGTTTTTTG

SEQ ID NO:19- shRNA against GSDMD

CCGGCAACCTGTCTATCAAGGACATCTCGAGATGTCCTTGATAGACAG
GTTGTTTTTTTG

5 SEQ ID NO:20- shRNA against IFNB

CCGGCAGAGTGGAAATCCTAAGGAACTCGAGTTCCTTAGGATTCCAC
TCTGTTTTT

SEQ ID NO:21- shRNA against IFNAR1

10 CCGGGCCAAGATTCAGGAAATTATTCTCGAGAATAATTCCTGAATCT
TGGCTTTTTG

The present application provides for the preparation and use of homologs
and fragments of the sequences disclosed herein where the homologs and fragments
15 have similar activity to the parent as disclosed herein.

SiRNAs and shRNAs encompassed by the invention can be prepared based
on the nucleic acid sequences provided above and herein.

BRIEF DESCRIPTION OF THE DRAWINGS

20 The patent or application file contains at least one drawing executed in
color. Copies of this patent or patent application publication with color drawing(s)
will be provided by the Office upon request and payment of the necessary fee.

**Figure 1, comprising FIGS 1A-1H. Caspase-4/11 in geographic atrophy
and RPE degeneration.**

25 (a) Immunoblots show increased caspase-4 activation (p30 subunits) in the RPE of
human eyes with geographic atrophy compared to unaffected controls (Ctr).
Specific bands of interest are indicated by an arrow head. Bar graph shows
densitometry of the bands corresponding to caspase-4 p30 normalized to loading
control. (b) Activation of caspase-4 (p30 subunit) in human RPE cells by *Alu* RNA,
30 pAlu, or DICER1 anti-sense oligonucleotides (DICER1 AS). Specific bands of
interest are indicated by arrowheads. (c) Immunoblot shows subretinal injection of
Alu RNA in WT mice induces activation of caspase-11 in the RPE. $n = 3$. (d) *Alu*
RNA induces RPE degeneration in WT but not *Casp11*^{-/-} mice. $n = 8-10$. (e)
Casp11^{-/-} mice expressing human caspase-4 transgene (*Casp11*^{-/-} *hCasp4*^{Tg}) are

susceptible to *Alu* RNA-induced RPE degeneration. $n = 8$. In fundus photographs (upper row), the degenerated retinal area is outlined by blue arrowheads. RPE cellular boundaries are visualized by immunostaining with zonula occludens-1 (ZO-1) antibody. Loss of regular hexagonal cellular boundaries represents degenerated RPE. (f) *Alu* RNA induced caspase-1 activation (p20 subunit) in the RPE of WT but not *Casp11*^{-/-} mice, $n = 3$. (g) *Alu* RNA activates caspase-1 (p20 subunit) in WT but not in *Casp11*^{-/-} mouse RPE cells. (h) Induction of IL-18 secretion by *Alu* RNA in mouse WT and *Casp11*^{-/-} RPE cells. $n = 3$; * $P < 0.05$. Error bars denote SD. (i) Caspase-1 and caspase-11 deficient mice (*Casp1*^{-/-} *Casp11*^{129mt/129mt}) as well as *Casp1*^{-/-} *Casp11*^{129mt/129mt} mice expressing functional mouse caspase-11 from bacterial artificial chromosome transgene (*Casp1*^{-/-} *Casp11*^{129mt/129mt} *Casp11*^{Tg}) were not susceptible to *Alu*-induced RPE degeneration. $n = 7-8$. Representative immunoblots of three independent experiments and densitometric analysis (mean (SEM)) are shown. Binary and morphometric quantification of RPE degeneration are shown (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). PM, polymegathism (mean (SEM)).

Figure 2, comprising FIGS 2A-2I. Gasdermin D in geographic atrophy and RPE degeneration.

(a) *Alu* RNA induced RPE degeneration in WT but not *Gsdmd*^{-/-} mice. $n = 6-10$. (b) Induction of IL-18 secretion by *Alu* RNA in WT and *Gsdmd*^{-/-} mouse RPE cells. $n = 3$; * $P < 0.05$. Error bars denote SD. (c) *Alu* RNA activates caspase-1 (p10 subunit) in WT but not in *Gsdmd*^{-/-} mouse RPE cells. (d) Similar activation of caspase-11 by *Alu* RNA in WT and *Gsdmd*^{-/-} mouse RPE cells. (e) Gasdermin D p30 cleavage occurs in WT mouse BMDMs following intracellular LPS exposure but not in primary human RPE cells and WT mouse RPE cells following *Alu* RNA exposure. (f) *In vivo* subretinal transfection of plasmids expressing wild type gasdermin D (pGSDMD-WT) or mutant gasdermin D incapable of undergoing p30 cleavage (pGSDMD-D276A), but not of control vector (pNull), restored *Alu* RNA-induced RPE degeneration in *Gsdmd*^{-/-} mice. $n = 4-5$. (g) Resistance of *Gsdmd*^{-/-} mice to *Alu* RNA-induced RPE degeneration was overcome by subretinal administration of recombinant mature IL-18 (recIL-18) or enforced expression by pIL-18ss. $n = 7-8$. (h) *GSDMD* mRNA abundance was greater in the RPE of human AMD eyes ($n = 7$) than in healthy age-matched control eyes ($n = 8$); * $P < 0.05$. Geometric means with 95% confidence intervals are depicted. (i) Increased

immunolocalization of gasdermin D in the RPE of human geographic atrophy eyes compared to age-matched healthy controls. Representative immunoblots of three independent experiments and densitometric analysis (mean (SEM)) are shown.

Binary and morphometric quantification of RPE degeneration are shown (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). PM, polymegethism (mean (SEM)).

Figure 3, comprising FIGS 3A-3I. Non-canonical inflammasome activation and RPE degeneration induced by *Alu* RNA is mediated via interferon signaling. (a) pAlu induces RPE degeneration in WT but not *Ifnar1*^{-/-} mice. $n = 7$. (b) Caspase-11 activation by *Alu* RNA is abrogated in *Ifnar1*^{-/-} mouse

RPE cells. (c) IFN- β treatment of human RPE cells induces caspase-4 abundance. (d) pAlu induces IFN- β secretion by human RPE cells. $n = 3$; * $P < 0.05$. Error bars denote SD. (e) Induction of STAT2 phosphorylation in human RPE cells by DICER1 antisense oligonucleotides (DICER1 AS) and pAlu. (f) pAlu induces RPE degeneration in WT but not *Irf3*^{-/-} or *Stat2*^{-/-} mice. $n = 6-7$. (g) IFN- β neutralizing antibody, but not isotype control IgG, confers protection against *Alu* RNA-induced RPE degeneration in WT mice. $n = 10$. (h) Increased immunolocalization of IFN- β in the RPE of human geographic atrophy eyes compared to age-matched unaffected controls. (i) Increased abundance of IFN- β mRNA in the RPE of human GA eyes compared to age-matched healthy controls, $n = 4$, * $P < 0.05$. Error bars denote SEM. Representative immunoblots of three independent experiments and densitometric analysis (mean (SEM)) are shown. Binary and morphometric quantification of RPE degeneration are shown (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). PM, polymegethism (mean (SEM)).

Figure 4, comprising FIGS 4A-4I. CGAS driven signaling licenses non-canonical inflammasome and RPE degeneration. (a) *Alu* RNA-induced *Ifnb* mRNA in WT mouse RPE cells is markedly depressed in *Mb21d1*^{-/-} mouse RPE cells. $n = 3$, * $P < 0.05$. Error bars denote SEM. (b) Caspase-1 activation (p20 subunit) by pAlu in WT but not *Mb21d1*^{-/-} mouse RPE cells. (c) Caspase-11 activation (p30 subunit) by pAlu in WT but not *Mb21d1*^{-/-} mouse RPE cells. (d) Impaired IL-18 secretion in *Alu* RNA-treated *Mb21d1*^{-/-} compared to WT mouse RPE cells. $n = 3$; * $P < 0.05$, Error bars denote SD. (e) DICER1 antisense oligonucleotides (DICER1 AS)-induced *IFNB* mRNA in human RPE cells is markedly depressed by cGAS shRNA (shcGAS). $n = 3$; * $P < 0.05$. Error bars denote SEM. (f) *Alu* RNA-induced phosphorylation of STAT2 and activation of

caspase-4 (p30 subunit) and caspase-1 (p20 subunit) are suppressed in human RPE cells treated with cGAS shRNA. (g) *Alu* RNA induces RPE degeneration in WT but not *Mb21d1*^{-/-} mice. *n* = 6–8. (h) Reconstitution of *Mb21d1*^{-/-} mice with *in vivo* transfection of Flag-cGAS, but not of control Flag-GFP, restored their susceptibility to *Alu* RNA-induced RPE degeneration, *n* = 7. (i) Resistance of *Mb21d1*^{-/-} mice to *Alu* RNA-induced RPE degeneration was overcome by subretinal administration of recombinant IFN- β (rec IFN- β) or enforced expression by pIFNB. *n* = 10–12. Representative immunoblots of three independent experiments and densitometric analysis (mean (SEM)) are shown. Binary and morphometric quantification of RPE degeneration are shown (*, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001). PM, polymegethism (mean (SEM)).

Figure 5, comprising FIGS. 5A-5E. CGAS in geographic atrophy and cGAS signaling in RPE degeneration. (a) Increased immunolocalization of cGAS in the RPE of human geographic atrophy eyes compared to age-matched healthy controls. (b) Caspase-1 activation (p20 subunit) by pAlu is impaired in *Tmem173*^{-/-} mouse RPE cells. (c) Caspase-11 activation (p30 subunit) by *Alu* RNA is impaired in *Tmem173*^{-/-} mouse RPE cells. (d) *Alu* RNA induces RPE degeneration in WT but not *Tmem173*^{-/-} mice. *n* = 6–10. (e) Resistance of *Tmem173*^{-/-} mice to *Alu* RNA-induced RPE degeneration was overcome by subretinal administration of recombinant IFN- β (rec IFN- β) or enforced expression by pIFNB. *n* = 8. Representative immunoblots of three independent experiments and densitometric analysis (mean (SEM)) are shown. Binary and morphometric quantification of RPE degeneration are shown (*, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001). PM, polymegethism (mean (SEM)).

Figure 6, comprising FIGS 6A-6J. MtDNA in non-canonical inflammasome activation and RPE degeneration. (a) Increased cytosolic mitochondrial DNA (mtDNA) abundance in *Alu* RNA-treated human RPE cells. *n* = 3; **P* < 0.05, Error bars denote SEM. (b) *Alu* RNA induces engagement of cGAS with mtDNA as demonstrated in a ChIP-like cGAS pull-down experiment. *n* = 3; **P* < 0.05, Error bars denote SEM. (c) *Alu* RNA induces cytosolic release of mtDNA in WT but not *Ppif*^{-/-} mouse RPE cells. (d) *Alu* RNA induces RPE degeneration in WT but not *Ppif*^{-/-} mice. *n* = 6–12. (e) *Alu* RNA induces caspase-1 activation in WT but not *Ppif*^{-/-} mouse RPE cells. (f) *Alu* RNA induces caspase-11

activation in WT but not *Ppif*^{-/-} mouse RPE cells. (g) Caspase-4 activation by *Alu* RNA is abrogated in Rho⁰ human ARPE19 cells lacking mtDNA. (h) *Alu* RNA-induced secretion of IL-18 is blunted in Rho⁰ human ARPE 19 cells. $n = 3$; $*P < 0.05$, Error bars denote SD. (i) *Alu* RNA-induced IFN- β secretion is blunted in Rho⁰ human ARPE19 cells. $n = 3$; $*P < 0.05$, Error bars denote SD. (j) Resistance of *Ppif*^{-/-} mice to *Alu* RNA-induced RPE degeneration was overcome by subretinal administration of recombinant IFN- β (rec IFN- β) or enforced expression by pIFNB. $n = 10-11$. All immunoblots are representative of three independent experiments. Representative immunoblots of three independent experiments and densitometric analysis (mean (SEM)) are shown. Binary and morphometric quantification of RPE degeneration are shown (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). PM, polymegethism (mean (SEM)).

Figure 7A-7H (also referred to as Supplementary Figure 1). Caspase-4/11 is required for *Alu*-induced RPE degeneration. (a) *Alu* RNA induces caspase-4 mRNA in human RPE cells. $n = 3$; $*P < 0.05$, Error bars denote SEM. (b) Protein lysates from RPE of human donor eyes were immunoblotted with an isotype antibody (control for anti-caspase-4 immunoblotting antibody in Fig 1a). No immunoreactive bands were observed in isotype control immunoblot. (c) Human RPE cells mock treated or stimulated with *Alu* RNA. Protein lysates were immunoblotted with secondary antibody alone, an isotype antibody, or an anti-caspase-4 antibody; caspase-4 activation (p30 subunits) was observed in *Alu* RNA stimulated cells; no bands were observed in secondary alone or isotype control immunoblots. Specific bands of interest are indicated by arrowheads. (d) Caspase-4 activation (p30 subunit) in human RPE cells treated with DICER1 antisense oligonucleotides (DICER1 AS) compared to control oligonucleotides (Ctrl AS) is abrogated by simultaneous treatment with *Alu* RNA antisense oligonucleotides (*Alu* AS) but not by scrambled oligonucleotide (Scr). (e) Activation of caspase-11 (p26 subunit) in mouse RPE cells by *Alu* RNA and p*Alu*. (f) p*Alu*, but not the control plasmid (pNull), induces RPE degeneration in WT but not *Casp11*^{-/-} mice, $n = 6-10$. (g) 129S6 mice which carry a caspase-11 inactivating passenger mutation are not susceptible to *Alu* RNA- or p*Alu*-induced RPE degeneration, $n = 5-7$. (h) *Dicer1* siRNA induces RPE degeneration in WT but not 129S6 mice, $n = 7-10$. Binary and

morphometric quantification of RPE degeneration are shown (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). PM, polymegethism (mean (SEM)).

FIGS 8A-8E (also referred to as Supplementary Figure 2). Caspase-11 is required for *Alu* RNA-induced caspase-1 activation and RPE degeneration.

- 5 (a) *Casp11*^{-/-} mouse RPE cells reconstituted via transfecting caspase-11 expression plasmid or control plasmid were stimulated with *Alu* RNA. Caspase-1 activity was assessed using CaspaLux®1-E₁D₂. *Alu* RNA-induced relative caspase-1 activity is presented; error bars denote SEM.
- (b) Caspase-1 activation (p10 subunit) by *Alu* RNA in *Casp11*^{-/-} mouse RPE cells is increased following lentiviral vector delivered caspase-11 reconstitution.
- 10 (c) pAlu induces RPE degeneration in WT mice but not in caspase-1 and caspase-11 deficient mice (*Casp1*^{-/-} *Casp11*^{129mt/129mt}) or in *Casp1*^{-/-} *Casp11*^{129mt/129mt} mice expressing functional mouse caspase-11 from a bacterial artificial chromosome transgene (*Casp1*^{-/-} *Casp11*^{129mt/129mt} *Casp11*^{Tg}), $n = 5-6$. (d) *Alu* RNA-induced caspase-11 activation is impaired in *P2rx7*^{-/-} mouse RPE cells compared to WT mouse RPE cells. (e) *Alu* RNA-induced caspase-11 activation is not impaired in *Pycard*^{-/-} mouse RPE cells. In fundus photographs, RPE degeneration is outlined by blue arrowheads. Representative immunoblots of three independent experiments and densitometric analysis (mean (SEM)) are shown. Binary and morphometric
- 15 quantification of RPE degeneration are shown (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). PM, polymegethism (mean (SEM)).

FIGS. 9A-9F (also referred to as Supplementary Figure 3). Cellular morphometry analysis. Wild-type mouse RPE flat mount images were analyzed in semi-automated fashion by 3 masked raters. There was a significant difference in

25 cell density, mean cell area, and polymegethism (coefficient of variation in cell size) between *Alu* RNA- and pAlu-treated eyes compared with their respective controls. ***, $P < 0.0001$, t test. Box plot shows median (red line), interquartile range (box), and the extremes (line segments).

FIGS 10A-10D (also referred to as Supplementary Figure 4). Increased

30 **abundance of phospholipid oxidation products in *Alu* RNA-stimulated RPE cells.** (a) Brief schematic highlighting select products of PAPC (1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphocholine) oxidation, collectively referred to as oxPAPC. (b) Representative mass scan of pure unoxidized PAPC and oxPAPC using an ABI Sciex 4000 QTrap mass spectrometer. (c) Representative mass scan of

oxPAPC, formed from air-oxidized PAPC. (d) Quantification of individual species of oxPAPC by liquid chromatography-mass spectrometry. Human RPE cells stimulated with *Alu* RNA had higher levels of PGPC (1-palmitoyl-2-glutaryl-sn-glycero-3-phosphocholine) and LysoPC (1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine) levels, indicative of extended oxidation, concomitant with a trending decrease of precursor PAPC and intermediate POVPC. $n = 6$; * $P < 0.05$, Error bars denote standard error.

FIGS. 11A-11D (also referred to as Supplementary Figure 5).

Gasdermin D is required for *Alu*-induced RPE degeneration. (a) p*Alu* induces RPE degeneration in WT but not *Gsdmd*^{-/-} mice, $n = 7-9$. (b) *Dicer1* siRNA induces RPE degeneration in WT but not *Gsdmd*^{-/-} mice, $n = 7-10$. (c) *Alu* RNA induced secretion of IL-18 in *Gsdmd*^{-/-} mouse RPE cells reconstituted with either pGSDMD-WT or pGSDMD-D276A. (d) IL-8, IL-6, and MIP-1 α mRNA abundance in the RPE was not different between human AMD eyes ($n = 4$) and healthy age-matched control eyes ($n = 4$); Error bars denote SEM. Binary and morphometric quantification of RPE degeneration are shown (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). PM, polymegethism (mean (SEM)).

FIGS. 12A and 12B (also referred to as Supplementary Figure 6), each comprising 16 panels. *Alu* RNA induces apoptotic cell death in human RPE cells. Human RPE cells mock treated or stimulated with *Alu* RNA were incubated with FITC-conjugated annexin V (green) and propidium iodide (PI, red). Staining by annexin V and PI uptake was monitored by time-lapse imaging. Representative images at various time points showing annexin V and PI staining is presented for (12a) *Alu* RNA stimulated and (12b) mock treated human RPE cells.

FIGS. 13A-13B (also referred to as Supplementary Figure 7). *Alu* RNA induces apoptotic RPE cell death in mice and in human cell culture. (13a) Annexin V (periwinkle blue) and propidium iodide (PI; red) staining of RPE flat mounts from WT mice treated with *Alu* RNA. The area of *Alu* RNA-induced RPE degeneration contained predominantly annexin-V⁺ PI⁻ cells, consistent with apoptosis. The RPE in regions of the eye distant from the site of *Alu* RNA exposure was healthy and negative for both annexin V and propidium iodide staining. (13b) Immunoblots show that cleaved caspase-3 and PARP-1 were increased in human RPE cells exposed to *Alu* RNA. ON, optic nerve.

FIG 14 (also referred to as Supplementary Figure 8), comprises 18 panels. Resistance of the RPE in *Gsdmd*^{-/-} mice to *Alu* RNA-induced apoptotic cell death is overcome by IL-18. Lack of annexin V (periwinkle blue; middle column) and propidium iodide (PI; red, left column) staining in RPE flat mounts of *Gsdmd*^{-/-} mice treated with *Alu* RNA. Administration of recombinant mature IL-18 led to the appearance of numerous annexin-V⁺ PI⁻ cells in the area of RPE degeneration. ON, optic nerve.

FIGS. 15A-15E (also referred to as Supplementary Figure 9). Interferon signaling in RPE toxicity. (a) Induction of IRF3 and STAT2 phosphorylation by *Alu* RNA in human RPE cells. (b) pAlu induces STAT2 phosphorylation in WT but not *Ifnar1*^{-/-} mouse RPE cells. (c) *Dicer1* siRNA, but not control siRNA, induces RPE degeneration in WT but not *Stat2*^{-/-} mice. *n* = 7. (d) *Alu* RNA induces RPE degeneration in WT but not *Stat2*^{-/-} mice. *n* = 6–7. (e) *Alu* RNA induces caspase-11 activation in WT but not *Stat2*^{-/-} mouse RPE cells. Expression of representative immunoblots of three independent experiments and densitometric analysis (mean (SEM)) are shown. Binary and morphometric quantification of RPE degeneration are shown (*, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001). PM, polymegathism (mean (SEM)).

FIGS. 16A-16I (also referred to as Supplementary Figure 10). cGAS driven signaling licenses non-canonical inflammasome. (a) *Alu* RNA increased abundance of cGAS mRNA in human RPE cells. *n* = 3; * *P* < 0.05, Error bars denote SEM. (b) Increased cGAS protein abundance in human RPE cells exposed to *Alu* expression plasmid (pAlu) and *Alu* RNA. (c) *Alu* RNA-induced caspase-1 activation (p20 subunit) is suppressed in *Mb21d1*^{-/-} mouse RPE cells compared to WT cells. (d) Secretion of IL-18 by canonical inflammasome activating monosodium urate (MSU) crystals is unaffected in *Casp11*^{-/-} and *Mb21d1*^{-/-} mouse RPE cells. (e) Confirmation of shRNA-mediated knockdown of cGAS mRNA in human RPE cells transfected with control or DICER1 targeted antisense oligonucleotides. Representative data of three experiments presented. Error bars denote SEM of technical replicates. (f) Confirmation of antisense oligonucleotide-mediated DICER1 knockdown in human RPE cells transduced with lentiviral vectors expressing control and cGAS targeted shRNA sequences. Representative data of three experiments presented. Error bars denote SEM of technical replicates. (g) pAlu induces RPE degeneration in WT but not *Mb21d1*^{-/-} mice. *n* = 6–8. (h)

Dicer1 siRNA induces RPE degeneration in WT but not *Mb21dl*^{-/-} mice. *n* = 6–9.

(i) *Alu* RNA-induced *Ifnb* mRNA expression is increased in *Mb21dl*^{-/-} mouse RPE cells reconstituted with cGAS expression plasmid compared to empty vector treated cells. *n* = 3. Error bars denote SEM. Representative immunoblots of three independent experiments and densitometric analysis (mean (SEM)) are shown. Binary and morphometric quantification of RPE degeneration are shown (*, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001). PM, polymegathism (mean (SEM)).

FIGS. 17A-17C (also referred to as Supplementary Figure 11). CGAS expression validation, and STING involvement in *Alu* RNA-induced IRF3

activation. (a) Immunoblot shows successful enforced cGAS expression in the RPE, in *in vitro* and *in vivo* reconstitution experiments using plasmid transfection described in Supplementary Fig. 10i and Fig. 4h, respectively. (b) Increased immunofluorescent localization of phosphorylated IRF3 (pIRF3) in the nucleus of wild-type compared to *Tmem173*^{-/-} mouse RPE cells. (c) Immunoblot shows *Alu* RNA-induced pIRF3 in wild-type mouse RPE cells is impaired in *Tmem173*^{-/-} mouse RPE cells.

FIGS. 18A-18H (also referred to as Supplementary Figure 12).

Activation of cGAS driven signaling by *Alu* RNA is mediated by cytosolic mtDNA. (a) Increased cytosolic mtDNA abundance in human RPE cells treated

with *DICER1* antisense oligonucleotides (DICER1 AS) compared to control oligonucleotides (Ctr AS). *n* = 3; * *P* < 0.05, error bars denote SEM. (b) Western blot shows the purity of the mitochondria-free cytosolic fractions used for measuring mtDNA abundance in cytosolic fractions; VDAC-1 is a mitochondrial marker. (c) Stimulation of HA-cGAS cells (*Mb21dl*^{-/-} MEF reconstituted with HA-mouse cGAS) with poly I:C (which does not activate cGAS signaling) does not induce engagement of cGAS with mtDNA, as demonstrated in a ChIP-like cGAS pull-down experiment. (d) Positive control for ChIP-like cGAS pull-down assay wherein HA-cGAS cells were transfected with plasmid DNA pUC19, followed by assaying of interaction between cGAS and pUC19 plasmid. (e) Subretinal administration of mtDNA induces RPE degeneration in WT but not *Mb21dl*^{-/-} mice. *n* = 4–6. (f) *Alu* RNA-induced abundance of *Ifnb* mRNA is reduced in *Mb21dl*^{-/-} mouse RPE cells. (g) Mitochondrial membrane potential ($\Delta\Psi$ m), assessed by the potential-sensitive fluorochrome JC-1, was significantly reduced by *Alu* RNA in WT but not *Ppif*^{-/-} mouse RPE cells. Cyclosporin A (CsA) inhibited the reduction in $\Delta\Psi$ m in WT

cells. $n = 5$, $*P < 0.05$, error bars denote SEM. (h) Mitochondrial permeabilization, assessed by the quenching of calcein-AM fluorescence by cobalt chloride, was significantly increased by *Alu* RNA in WT but not *Ppif*^{-/-} mouse RPE cells. Cyclosporin A (CsA) blocked the mitochondrial permeabilization in WT cells. $n = 5$, $*P < 0.05$, error bars denote SEM. Binary and morphometric quantification of RPE degeneration are shown (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). PM, polymegethism (mean (SEM)).

FIGS. 19A-19C (also referred to as Supplementary Figure 13).

Macrophages and microglia are dispensable for *Alu* RNA-induced RPE degeneration. (a) *Alu* RNA induces RPE degeneration in WT mice treated with clodronate liposomes. $n = 6$. (b) *Alu* RNA induces RPE degeneration in *Cx3cr1*^{CreER} ROSA-DTA mice treated with tamoxifen. $n = 4-11$. (c) Tamoxifen-induced depletion of microglia in *Cx3cr1*^{CreER} ROSA-DTA mice was confirmed by staining for microglial marker F4/80 superimposed with endothelial cell staining with isolectin B4 in retinal flat mounts. Binary and morphometric quantification of RPE degeneration are shown (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). PM, polymegethism (mean (SEM)).

FIG. 20 (also referred to as Supplementary Figure 14). Activation of caspase-1 by *Alu* RNA in bone marrow derived macrophages (BMDMs) is dependent on caspase-11, cGAS, and gasdermin D. Immunoblots show *Alu* RNA induced caspase-1 activation (Casp1 p10) in WT but not *Casp11*^{-/-}, *Mb21d1*^{-/-}, or *Gsdmd*^{-/-} BMDMs.

FIG. 21 (also referred to as Supplementary Figure 15). Schematic Model of the presently disclosed cGAS-mediated licensing of non-canonical NLRP3 inflammasome activation by DICER deficit/*Alu* RNA. Elevated *Alu* RNA triggers release of mitochondrial DNA (mtDNA) into the cytosol. Cytosolic mtDNA subsequently activates cGAS-driven type I interferons (IFNs). The resulting IFN signaling via interferon- α/β receptor (IFNAR) and STATs triggers caspase-4/11 priming and activation that, in turn, dictates gasdermin D and NLRP3 inflammasome-mediated secretion of IL-18. Secreted IL-18 drives RPE degeneration via a mechanism involving Myd88, FAS/FasL, and caspase-8^{2,21}. Three different RPE cells are depicted in the schematic model to illustrate the mechanism of *Alu* RNA-induced inflammasome

activation and the autocrine and paracrine IL-18 signaling leading to RPE cell death via Myd88, Fas/FasL, Caspase-8, and Caspase-3.

DETAILED DESCRIPTION

5 **Abbreviation and Acronyms**

AMD- age-related macular degeneration

AS- antisense

BMDM- bone marrow derived macrophage

caspase- a family of cysteine-aspartic acid protease proteases

10 caspase-4- a human caspase (the murine homolog is caspase 11)

caspase-11- a murine caspase (the human homolog is caspase 4)

cGAS- cyclic GMP-AMP synthase

CFH- complement factor H

CsA- Cyclosporin A (CAS No. 59865-13-3)

15 DAMPs- damage-associated molecular patterns

DICER1 AS- DICER1 antisense

GSDMD- Gasdermin D

HTRA1- high-temperature requirement factor A-1

IFN- interferon

20 IFN- β - interferon beta (also referred to as IFNB)

IFNAR- interferon- α/β receptor

IRF3- interferon regulatory factor 3

LPS- lipopolysaccharide

LRR- leucine-rich repeat

25 LysoPC- 1-palmitoyl-2-hydroxy-3-phosphatidylcholine

MRM- multiple reaction monitoring

MSU- monosodium urate

mtDNA- mitochondrial DNA

mPTP- mitochondrial permeability transition pore (a complex of proteins

30 often referred to as just "a" protein)

NLR- nucleotide-binding domain and leucine-rich repeat containing protein

NLRP3- NLR family pyrin domain containing 3; also known as NALP3 and cryoporin;

- ON- optic nerve
- pALU- plasmid-mediated enforced expression of *Alu* RNA
- PAMP- pathogen-associated molecular pattern
- PAPC - 1-palmitoyl-2-arachidonoyl-3-phosphatidylcholine
- 5 PARP-1- poly(ADP-ribose) polymerase 1
- PGPC- 1-palmitoyl-2-glutaryl-3-phosphatidylcholine
- POVPC- 1-palmitoyl-2-(5-oxovaleryl)-3-phosphatidylcholine
- PI- propidium iodide
- PM- polymethism
- 10 PPIF- peptidyl-prolyl cis-trans isomerase F
- RPE- retinal pigmented epithelium
- Scr- scrambled oligonucleotide
- shcGAS- cGAS shRNA
- shRNA- short hairpin RNA
- 15 siRNA- small interfering RNA
- STING- stimulator of interferon genes
- TAM- tamoxifen
- WT- wild type
- ZO-1- zonula occludens-1

20 **Definitions**

In describing and claiming the invention, the following terminology will be used in accordance with the definitions set forth below.

The articles “a” and “an” are used herein to refer to one or to more than one (i.e. to at least one) of the grammatical object of the article. By way of example, “an
25 element” means one element or more than one element.

The term “about,” as used herein, means approximately, in the region of, roughly, or around. When the term “about” is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term “about” is used herein to modify a
30 numerical value above and below the stated value by a variance of 10%. In one aspect, the term “about” means plus or minus 10% of the numerical value of the number with which it is being used. Therefore, about 50% means in the range of 45%-55%. Numerical ranges recited herein by endpoints include all numbers and fractions subsumed within that range (e.g. 1 to 5 includes 1, 1.5, 2, 2.75, 3, 3.90, 4,

and 5). It is also to be understood that all numbers and fractions thereof are presumed to be modified by the term “about.”

The terms “additional therapeutically active compound” or “additional therapeutic agent”, as used in the context of the present invention, refers to the use or administration of a compound for an additional therapeutic use for a particular injury, disease, or disorder being treated. Such a compound, for example, could include one being used to treat an unrelated disease or disorder, or a disease or disorder which may not be responsive to the primary treatment for the injury, disease or disorder being treated.

As use herein, the terms “administration of” and or “administering” a compound should be understood to mean providing a compound of the invention or a prodrug of a compound of the invention to a subject in need of treatment.

As used herein, an “agonist” is a composition of matter which, when administered to a mammal such as a human, enhances or extends a biological activity attributable to the level or presence of a target compound or molecule of interest in the mammal.

The term “alterations in peptide structure” as used herein refers to changes including, but not limited to, changes in sequence, and post-translational modification.

The term "the alternative, non-canonical inflammasome signaling molecules, protein complex, or signal transduction pathways in retinal pigment epithelium (RPE)" as used herein refers to the pathway and molecules disclosed herein and to their signal transduction pathways. Regarding the signal transduction pathways of the alternative, non-canonical inflammasome, this includes both upstream and downstream regulation that can be regulated or inhibited in RPE to inhibit or prevent RPE degeneration and age-related macular degeneration or its progression to geographic atrophy.

An “antagonist” is a composition of matter which when administered to a mammal such as a human, inhibits a biological activity attributable to the level or presence of a compound or molecule of interest in the mammal.

As used herein, “alleviating a disease or disorder symptom,” means reducing the severity of the symptom or the frequency with which such a symptom is experienced by a patient, or both.

As used herein, amino acids are represented by the full name thereof, by the three letter code corresponding thereto, or by the one-letter code corresponding thereto, as indicated in the following table:

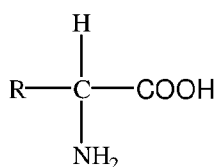
	<u>Full Name</u>	<u>Three-Letter Code</u>	<u>One-Letter Code</u>
5	Aspartic Acid	Asp	D
	Glutamic Acid	Glu	E
	Lysine	Lys	K
	Arginine	Arg	R
	Histidine	His	H
10	Tyrosine	Tyr	Y
	Cysteine	Cys	C
	Asparagine	Asn	N
	Glutamine	Gln	Q
	Serine	Ser	S
15	Threonine	Thr	T
	Glycine	Gly	G
	Alanine	Ala	A
	Valine	Val	V
	Leucine	Leu	L
20	Isoleucine	Ile	I
	Methionine	Met	M
	Proline	Pro	P
	Phenylalanine	Phe	F
	Tryptophan	Trp	W
25			

The term “amino acid” is used interchangeably with “amino acid residue,” and may refer to a free amino acid and to an amino acid residue of a peptide. It will be apparent from the context in which the term is used whether it refers to a free amino acid or a residue of a peptide.

30 The expression “amino acid” as used herein is meant to include both natural and synthetic amino acids, and both D and L amino acids. “Standard amino acid” means any of the twenty standard L-amino acids commonly found in naturally occurring peptides. “Nonstandard amino acid residue” means any amino acid, other than the standard amino acids, regardless of whether it is prepared synthetically or

derived from a natural source. As used herein, “synthetic amino acid” also encompasses chemically modified amino acids, including but not limited to salts, amino acid derivatives (such as amides), and substitutions. Amino acids contained within the peptides of the present invention, and particularly at the carboxy- or amino-terminus, can be modified by methylation, amidation, acetylation or substitution with other chemical groups which can change the peptide’s circulating half-life without adversely affecting their activity. Additionally, a disulfide linkage may be present or absent in the peptides of the invention.

Amino acids have the following general structure:



Amino acids may be classified into seven groups on the basis of the side chain R: (1) aliphatic side chains, (2) side chains containing a hydroxylic (OH) group, (3) side chains containing sulfur atoms, (4) side chains containing an acidic or amide group, (5) side chains containing a basic group, (6) side chains containing an aromatic ring, and (7) proline, an imino acid in which the side chain is fused to the amino group.

The nomenclature used to describe the peptide compounds of the present invention follows the conventional practice wherein the amino group is presented to the left and the carboxy group to the right of each amino acid residue. In the formulae representing selected specific embodiments of the present invention, the amino- and carboxy-terminal groups, although not specifically shown, will be understood to be in the form they would assume at physiologic pH values, unless otherwise specified.

The term “basic” or “positively charged” amino acid as used herein, refers to amino acids in which the R groups have a net positive charge at pH 7.0, and include, but are not limited to, the standard amino acids lysine, arginine, and histidine.

As used herein, an “analog” of a chemical compound is a compound that, by way of example, resembles another in structure but is not necessarily an isomer (e.g., 5-fluorouracil is an analog of thymine).

The term “antagomir” refers to a small RNA or DNA (or chimeric) molecule to antagonize endogenous small RNA regulators like microRNA (miRNA). These antagonists bear complementary nucleotide sequences for the most part, which means that antagomirs should hybridize to the mature microRNA (miRNA). They prevent other molecules from binding to a desired site on an mRNA molecule and are used to silence endogenous microRNA (miR). Antagomirs are therefore designed to block biological activity of these post-transcriptional molecular switches. Like the preferred target ligands (microRNA, miRNA), antagomirs have to cross membranes to enter a cell. Antagomirs also known as anti-miRs or blockmirs.

An “antagonist” is a composition of matter which when administered to a mammal such as a human, inhibits a biological activity attributable to the level or presence of a compound or molecule of interest in the mammal.

The term “antibody,” as used herein, refers to an immunoglobulin molecule which is able to specifically bind to a specific epitope on an antigen. Antibodies can be intact immunoglobulins derived from natural sources or from recombinant sources and can be immunoreactive portions of intact immunoglobulins. Antibodies are typically tetramers of immunoglobulin molecules. The antibodies in the present invention may exist in a variety of forms including, for example, polyclonal antibodies, monoclonal antibodies, Fv, Fab and F(ab)₂, as well as single chain antibodies and humanized antibodies.

An “antibody heavy chain,” as used herein, refers to the larger of the two types of polypeptide chains present in all antibody molecules.

An “antibody light chain,” as used herein, refers to the smaller of the two types of polypeptide chains present in all antibody molecules.

By the term “synthetic antibody” as used herein, is meant an antibody which is generated using recombinant DNA technology, such as, for example, an antibody expressed by a bacteriophage as described herein. The term should also be construed to mean an antibody which has been generated by the synthesis of a DNA molecule encoding the antibody and which DNA molecule expresses an antibody protein, or an amino acid sequence specifying the antibody, wherein the DNA or amino acid sequence has been obtained using synthetic DNA or amino acid sequence technology which is available and well known in the art.

The term “antigen” as used herein is defined as a molecule that provokes an immune response. This immune response may involve either antibody production, or the activation of specific immunologically-competent cells, or both. An antigen can be derived from organisms, subunits of proteins/antigens, killed or inactivated whole cells or lysates.

The term “antigenic determinant” as used herein refers to that portion of an antigen that makes contact with a particular antibody (i.e., an epitope). When a protein or fragment of a protein, or chemical moiety is used to immunize a host animal, numerous regions of the antigen may induce the production of antibodies that bind specifically to a given region or three-dimensional structure on the protein; these regions or structures are referred to as antigenic determinants. An antigenic determinant may compete with the intact antigen (i.e., the “immunogen” used to elicit the immune response) for binding to an antibody.

The term “antimicrobial agents” as used herein refers to any naturally-occurring, synthetic, or semi-synthetic compound or composition or mixture thereof, which is safe for human or animal use as practiced in the methods of this invention, and is effective in killing or substantially inhibiting the growth of microbes. “Antimicrobial” as used herein, includes antibacterial, antifungal, and antiviral agents.

As used herein, the term “antisense oligonucleotide” or antisense nucleic acid means a nucleic acid polymer, at least a portion of which is complementary to a nucleic acid which is present in a normal cell or in an affected cell. “Antisense” refers particularly to the nucleic acid sequence of the non-coding strand of a double stranded DNA molecule encoding a protein, or to a sequence which is substantially homologous to the non-coding strand. As defined herein, an antisense sequence is complementary to the sequence of a double stranded DNA molecule encoding a protein. It is not necessary that the antisense sequence be complementary solely to the coding portion of the coding strand of the DNA molecule. The antisense sequence may be complementary to regulatory sequences specified on the coding strand of a DNA molecule encoding a protein, which regulatory sequences control expression of the coding sequences. The antisense oligonucleotides of the invention include, but are not limited to, phosphorothioate oligonucleotides and other modifications of oligonucleotides.

An “aptamer” is a compound that is selected in vitro to bind preferentially to another compound (for example, the identified proteins herein). Often, aptamers are nucleic acids or peptides because random sequences can be readily generated from nucleotides or amino acids (both naturally occurring or synthetically made) in large numbers but of course they need not be limited to these.

As used herein, the term "attach", or "attachment", or "attached", or "attaching", used herein interchangeably with "bind", or "binding" or "binds" or "bound" refers to any physical relationship between molecules that results in forming a stable complex, such as a physical relationship between a ligand, such as a peptide or small molecule, with a "binding partner" or "receptor molecule." The relationship may be mediated by physicochemical interactions including, but not limited to, a selective noncovalent association, ionic attraction, hydrogen bonding, covalent bonding, Van der Waals forces or hydrophobic attraction.

As used herein, the term "avidity" refers to a total binding strength of a ligand with a receptor molecule, such that the strength of an interaction comprises multiple independent binding interactions between partners, which can be derived from multiple low affinity interactions or a small number of high affinity interactions.

The term “binding” refers to the adherence of molecules to one another, such as, but not limited to, enzymes to substrates, ligands to receptors, antibodies to antigens, DNA binding domains of proteins to DNA, and DNA or RNA strands to complementary strands.

“Binding partner,” as used herein, refers to a molecule capable of binding to another molecule.

The term “biocompatible”, as used herein, refers to a material that does not elicit a substantial detrimental response in the host.

As used herein, the term “biologically active fragments” or “bioactive fragment” of the proteins or polypeptides encompasses natural or synthetic portions of the full-length protein that are capable of specific binding to their natural ligand or of performing the function of the protein.

The term “biological sample,” as used herein, refers to samples obtained from a subject, including, but not limited to, skin, hair, tissue, blood, plasma, cells, sweat and urine.

As used herein, the term "biopsy tissue" refers to a sample of tissue that is removed from a subject for the purpose of determining if the sample contains cancerous tissue. In some embodiment, biopsy tissue is obtained because a subject is suspected of having cancer. The biopsy tissue is then examined for the presence or absence of cancer.

As used herein, the term "carrier molecule" refers to any molecule that is chemically conjugated to a molecule of interest.

Caspase-4 is a human protein and is known as caspase-11 in the mouse. Caspases (cysteine-aspartic proteases/cysteine aspartases/cysteine-dependent aspartate-directed proteases) are a family of protease enzymes playing a role in programmed cell death. There is a precursor and isoforms for caspase-4. Isoform alpha is known as the canonical sequence.

Caspase-11 is a mouse protein and is known as caspase-4 in humans.

The terms "cell," "cell line," and "cell culture" as used herein may be used interchangeably. All of these terms also include their progeny, which are any and all subsequent generations. It is understood that all progeny may not be identical due to deliberate or inadvertent mutations.

As used herein, the term "chemically conjugated," or "conjugating chemically" refers to linking the antigen to the carrier molecule. This linking can occur on the genetic level using recombinant technology, wherein a hybrid protein may be produced containing the amino acid sequences, or portions thereof, of both the antigen and the carrier molecule. This hybrid protein is produced by an oligonucleotide sequence encoding both the antigen and the carrier molecule, or portions thereof. This linking also includes covalent bonds created between the antigen and the carrier protein using other chemical reactions, such as, but not limited to glutaraldehyde reactions. Covalent bonds may also be created using a third molecule bridging the antigen to the carrier molecule. These cross-linkers are able to react with groups, such as but not limited to, primary amines, sulfhydryls, carbonyls, carbohydrates, or carboxylic acids, on the antigen and the carrier molecule. Chemical conjugation also includes non-covalent linkage between the antigen and the carrier molecule.

A "coding region" of a gene consists of the nucleotide residues of the coding strand of the gene and the nucleotides of the non-coding strand of the gene which

are homologous with or complementary to, respectively, the coding region of an mRNA molecule which is produced by transcription of the gene.

The term “competitive sequence” refers to a peptide or a modification, fragment, derivative, or homolog thereof that competes with another peptide for its cognate binding site.

“Complementary” as used herein refers to the broad concept of subunit sequence complementarity between two nucleic acids, e.g., two DNA molecules. When a nucleotide position in both of the molecules is occupied by nucleotides normally capable of base pairing with each other, then the nucleic acids are considered to be complementary to each other at this position. Thus, two nucleic acids are complementary to each other when a substantial number (at least 50%) of corresponding positions in each of the molecules are occupied by nucleotides which normally base pair with each other (e.g., A:T and G:C nucleotide pairs). Thus, it is known that an adenine residue of a first nucleic acid region is capable of forming specific hydrogen bonds (“base pairing”) with a residue of a second nucleic acid region which is antiparallel to the first region if the residue is thymine or uracil. Similarly, it is known that a cytosine residue of a first nucleic acid strand is capable of base pairing with a residue of a second nucleic acid strand which is antiparallel to the first strand if the residue is guanine. A first region of a nucleic acid is complementary to a second region of the same or a different nucleic acid if, when the two regions are arranged in an antiparallel fashion, at least one nucleotide residue of the first region is capable of base pairing with a residue of the second region. Preferably, the first region comprises a first portion and the second region comprises a second portion, whereby, when the first and second portions are arranged in an antiparallel fashion, at least about 50%, and preferably at least about 75%, at least about 90%, or at least about 95% of the nucleotide residues of the first portion are capable of base pairing with nucleotide residues in the second portion. More preferably, all nucleotide residues of the first portion are capable of base pairing with nucleotide residues in the second portion.

A “compound,” as used herein, refers to any type of substance or agent that is commonly considered a drug, or a candidate for use as a drug, as well as combinations and mixtures of the above, and can also include biologics that are used for a treatment or effect in the context of the uses described herein.

As used herein, the term “conservative amino acid substitution” is defined

herein as an amino acid exchange within one of the following five groups:

- I. Small aliphatic, nonpolar or slightly polar residues:
Ala, Ser, Thr, Pro, Gly;
- II. Polar, negatively charged residues and their amides:
5 Asp, Asn, Glu, Gln;
- III. Polar, positively charged residues:
His, Arg, Lys;
- IV. Large, aliphatic, nonpolar residues:
Met Leu, Ile, Val, Cys
- 10 V. Large, aromatic residues:
Phe, Tyr, Trp

A “control” cell is a cell having the same cell type as a test cell. The control cell may, for example, be examined at precisely or nearly the same time the test cell
15 is examined. The control cell may also, for example, be examined at a time distant from the time at which the test cell is examined, and the results of the examination of the control cell may be recorded so that the recorded results may be compared with results obtained by examination of a test cell.

A “test” cell is a cell being examined.
20 “Cytokine,” as used herein, refers to intercellular signaling molecules, the best known of which are involved in the regulation of mammalian somatic cells. A number of families of cytokines, both growth promoting and growth inhibitory in their effects, have been characterized including, for example, interleukins, interferons, and transforming growth factors. A number of other cytokines are
25 known to those of skill in the art. The sources, characteristics, targets and effector activities of these cytokines have been described.

As used herein, a “derivative” of a compound refers to a chemical compound that may be produced from another compound of similar structure in one or more steps, as in replacement of H by an alkyl, acyl, or amino group.

30 The use of the word “detect” and its grammatical variants refers to measurement of the species without quantification, whereas use of the word “determine” or “measure” with their grammatical variants are meant to refer to measurement of the species with quantification. The terms “detect” and “identify” are used interchangeably herein.

As used herein, a “detectable marker” or a “reporter molecule” is an atom or a molecule that permits the specific detection of a compound comprising the marker in the presence of similar compounds without a marker. Detectable markers or reporter molecules include, e.g., radioactive isotopes, antigenic determinants, enzymes, nucleic acids available for hybridization, chromophores, fluorophores, chemiluminescent molecules, electrochemically detectable molecules, and molecules that provide for altered fluorescence-polarization or altered light-scattering.

A “disease” is a state of health of an animal wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated then the animal’s health continues to deteriorate.

In contrast, a “disorder” in an animal is a state of health in which the animal is able to maintain homeostasis, but in which the animal’s state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the animal’s state of health.

As used herein, the term “domain” refers to a part of a molecule or structure that shares common physicochemical features, such as, but not limited to, hydrophobic, polar, globular and helical domains or properties such as ligand binding, signal transduction, cell penetration and the like. Specific examples of binding domains include, but are not limited to, DNA binding domains and ATP binding domains.

As used herein, an “effective amount” or “therapeutically effective amount” means an amount sufficient to produce a selected effect, such as alleviating symptoms of a disease or disorder. In the context of administering compounds in the form of a combination, such as multiple compounds, the amount of each compound, when administered in combination with another compound(s), may be different from when that compound is administered alone. Thus, an effective amount of a combination of compounds refers collectively to the combination as a whole, although the actual amounts of each compound may vary. The term “more effective” means that the selected effect is alleviated to a greater extent by one treatment relative to the second treatment to which it is being compared.

As used herein, the term “effector domain” refers to a domain capable of directly interacting with an effector molecule, chemical, or structure in the cytoplasm which is capable of regulating a biochemical pathway.

The term “elixir,” as used herein, refers in general to a clear, sweetened, alcohol-containing, usually hydroalcoholic liquid containing flavoring substances and sometimes active medicinal agents.

“Encoding” refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (i.e., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA.

An “enhancer” is a DNA regulatory element that can increase the efficiency of transcription, regardless of the distance or orientation of the enhancer relative to the start site of transcription.

The term “epitope” as used herein is defined as small chemical groups on the antigen molecule that can elicit and react with an antibody. An antigen can have one or more epitopes. Most antigens have many epitopes; i.e., they are multivalent. In general, an epitope is roughly five amino acids or sugars in size. One skilled in the art understands that generally the overall three-dimensional structure, rather than the specific linear sequence of the molecule, is the main criterion of antigenic specificity.

As used herein, an “essentially pure” preparation of a particular protein or peptide is a preparation wherein at least about 95%, and preferably at least about 99%, by weight, of the protein or peptide in the preparation is the particular protein or peptide.

A “fragment” or “segment” is a portion of an amino acid sequence, comprising at least one amino acid, or a portion of a nucleic acid sequence comprising at least one nucleotide. The terms “fragment” and “segment” are used interchangeably herein.

As used herein, the term “fragment,” as applied to a protein or peptide, can ordinarily be at least about 3-15 amino acids in length, at least about 15-25 amino

acids, at least about 25-50 amino acids in length, at least about 50-75 amino acids in length, at least about 75-100 amino acids in length, and greater than 100 amino acids in length.

As used herein, the term “fragment” as applied to a nucleic acid, may
ordinarily be at least about 20 nucleotides in length, typically, at least about 50
nucleotides, more typically, from about 50 to about 100 nucleotides, preferably, at
least about 100 to about 200 nucleotides, even more preferably, at least about 200
nucleotides to about 300 nucleotides, yet even more preferably, at least about 300 to
about 350, even more preferably, at least about 350 nucleotides to about 500
nucleotides, yet even more preferably, at least about 500 to about 600, even more
preferably, at least about 600 nucleotides to about 620 nucleotides, yet even more
preferably, at least about 620 to about 650, and most preferably, the nucleic acid
fragment will be greater than about 650 nucleotides in length.

As used herein, a “functional” biological molecule is a biological molecule
in a form in which it exhibits a property by which it is characterized. A functional
enzyme, for example, is one which exhibits the characteristic catalytic activity by
which the enzyme is characterized.

“Homologous” as used herein, refers to the subunit sequence similarity
between two polymeric molecules, e.g., between two nucleic acid molecules, e.g.,
two DNA molecules or two RNA molecules, or between two polypeptide molecules.
When a subunit position in both of the two molecules is occupied by the same
monomeric subunit, e.g., if a position in each of two DNA molecules is occupied by
adenine, then they are homologous at that position. The homology between two
sequences is a direct function of the number of matching or homologous positions,
e.g., if half (e.g., five positions in a polymer ten subunits in length) of the positions
in two compound sequences are homologous then the two sequences are 50%
homologous, if 90% of the positions, e.g., 9 of 10, are matched or homologous, the
two sequences share 90% homology. By way of example, the DNA sequences
3’ATTGCC5’ and 3’TATGGC share 50% homology.

As used herein, “homology” is used synonymously with “identity.”

The determination of percent identity between two nucleotide or amino acid
sequences can be accomplished using a mathematical algorithm. For example, a
mathematical algorithm useful for comparing two sequences is the algorithm of
Karlin and Altschul (1990, Proc. Natl. Acad. Sci. USA 87:2264-2268), modified as

in Karlin and Altschul (1993, Proc. Natl. Acad. Sci. USA 90:5873-5877). This algorithm is incorporated into the NBLAST and XBLAST programs of Altschul, et al. (1990, J. Mol. Biol. 215:403-410), and can be accessed, for example at the National Center for Biotechnology Information (NCBI) world wide web site having the universal resource locator using the BLAST tool at the NCBI website. BLAST nucleotide searches can be performed with the NBLAST program (designated “blastn” at the NCBI web site), using the following parameters: gap penalty = 5; gap extension penalty = 2; mismatch penalty = 3; match reward = 1; expectation value 10.0; and word size = 11 to obtain nucleotide sequences homologous to a nucleic acid described herein. BLAST protein searches can be performed with the XBLAST program (designated “blastp” at the NCBI web site) or the NCBI “blastp” program, using the following parameters: expectation value 10.0, BLOSUM62 scoring matrix to obtain amino acid sequences homologous to a protein molecule described herein. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al. (1997, Nucleic Acids Res. 25:3389-3402). Alternatively, PSI-Blast or PHI-Blast can be used to perform an iterated search which detects distant relationships between molecules (Id.) and relationships between molecules which share a common pattern. When utilizing BLAST, Gapped BLAST, PSI-Blast, and PHI-Blast programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used.

The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically exact matches are counted.

As used herein, the term “hybridization” is used in reference to the pairing of complementary nucleic acids. Hybridization and the strength of hybridization (i.e., the strength of the association between the nucleic acids) is impacted by such factors as the degree of complementarity between the nucleic acids, stringency of the conditions involved, the length of the formed hybrid, and the G:C ratio within the nucleic acids.

As used herein, the term “inhaler” refers both to devices for nasal and pulmonary administration of a drug, e.g., in solution, powder and the like. For example, the term “inhaler” is intended to encompass a propellant driven inhaler, such as is used to administer antihistamine for acute asthma attacks, and plastic spray bottles, such as are used to administer decongestants.

The term “inhibit”, as used herein, refers to the ability of a compound, agent, or method to reduce or impede a described function, level, activity, rate, etc., based on the context in which the term “inhibit” is used. The term also refers to inhibiting any metabolic or regulatory pathway which can regulate the synthesis, levels, activity, or function of a protein, mRNA, or other molecule of interest. Preferably, inhibition is by at least 10%. The term “inhibit” is used interchangeably with “reduce” and “block.”

The term “inhibit a complex,” as used herein, refers to inhibiting the formation of a complex or interaction of two or more proteins, as well as inhibiting the function or activity of the complex. The term also encompasses disrupting a formed complex. However, the term does not imply that each and every one of these functions must be inhibited at the same time.

The term “inhibit a protein,” as used herein, refers to any method or technique which inhibits protein synthesis, levels, activity, or function, as well as methods of inhibiting the induction or stimulation of synthesis, levels, activity, or function of the protein of interest. The term also refers to any metabolic or regulatory pathway which can regulate the synthesis, levels, activity, or function of the protein of interest. The term includes binding with other molecules and complex formation. Therefore, the term “protein inhibitor” refers to any agent or compound, the application of which results in the inhibition of protein function or protein pathway function. However, the term does not imply that each and every one of these functions must be inhibited at the same time.

As used herein “injecting or applying” includes administration of a compound of the invention by any number of routes and means including, but not limited to, topical, oral, buccal, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, vaginal, ophthalmic, pulmonary, or rectal means.

As used herein, an “instructional material” includes a publication, a recording, a diagram, or any other medium of expression which can be used to communicate the usefulness of the peptide of the invention in the kit for effecting alleviation of the various diseases or disorders recited herein. Optionally, or alternately, the instructional material may describe one or more methods of alleviating the diseases or disorders in a cell or a tissue of a mammal. The

instructional material of the kit of the invention may, for example, be affixed to a container which contains the identified compound invention or be shipped together with a container which contains the identified compound. Alternatively, the instructional material may be shipped separately from the container with the
5 intention that the instructional material and the compound be used cooperatively by the recipient.

An "isolated nucleic acid" refers to a nucleic acid segment or fragment which has been separated from sequences which flank it in a naturally occurring state, e.g., a DNA fragment which has been removed from the sequences which are
10 normally adjacent to the fragment, e.g., the sequences adjacent to the fragment in a genome in which it naturally occurs. The term also applies to nucleic acids which have been substantially purified from other components which naturally accompany the nucleic acid, e.g., RNA or DNA or proteins, which naturally accompany it in the cell. The term therefore includes, for example, a recombinant DNA which is
15 incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., as a cDNA or a genomic or cDNA fragment produced by PCR or restriction enzyme digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide
20 sequence.

A "ligand" is a compound that specifically binds to a target receptor or target molecule.

A "receptor" or target molecule is a compound that specifically binds to a ligand.

25 A ligand or a receptor "specifically binds to" a compound when the ligand or receptor functions in a binding reaction which is determinative of the presence of the compound in a sample of heterogeneous compounds. Thus, under designated assay (e.g., immunoassay) conditions, the ligand or receptor binds preferentially to a particular compound and does not bind in a significant amount to other compounds
30 present in the sample. For example, a polynucleotide specifically binds under hybridization conditions to a compound polynucleotide comprising a complementary sequence; an antibody specifically binds under immunoassay conditions to an antigen bearing an epitope against which the antibody was raised.

As used herein, the term “linkage” refers to a connection between two groups. The connection can be either covalent or non-covalent, including but not limited to ionic bonds, hydrogen bonding, and hydrophobic/hydrophilic interactions.

As used herein, the term “linker” refers to a molecule that joins two other molecules either covalently or noncovalently, e.g., through ionic or hydrogen bonds or van der Waals interactions, e.g., a nucleic acid molecule that hybridizes to one complementary sequence at the 5' end and to another complementary sequence at the 3' end, thus joining two non-complementary sequences.

“Malexpression” of a gene means expression of a gene in a cell of a patient afflicted with a disease or disorder, wherein the level of expression (including non-expression), the portion of the gene expressed, or the timing of the expression of the gene with regard to the cell cycle, differs from expression of the same gene in a cell of a patient not afflicted with the disease or disorder. It is understood that malexpression may cause or contribute to the disease or disorder, be a symptom of the disease or disorder, or both.

The term “measuring the level of expression” or “determining the level of expression” as used herein refers to any measure or assay which can be used to correlate the results of the assay with the level of expression of a gene or protein of interest. Such assays include measuring the level of mRNA, protein levels, etc. and can be performed by assays such as northern and western blot analyses, binding assays, immunoblots, etc. The level of expression can include rates of expression and can be measured in terms of the actual amount of an mRNA or protein present.

The term “method of identifying peptides in a sample”, as used herein, refers to identifying small and large peptides, including proteins.

Micro-RNAs are generally about 16-25 nucleotides in length. In one aspect, miRNAs are RNA molecules of 22 nucleotides or less in length. These molecules have been found to be highly involved in the pathology of several types of cancer. Although the miRNA molecules are generally found to be stable when associated with blood serum and its components after EDTA treatment, introduction of locked nucleic acids (LNAs) to the miRNAs via PCR further increases stability of the miRNAs. LNAs are a class of nucleic acid analogues in which the ribose ring is “locked” by a methylene bridge connecting the 2'-O atom and the 4'-C atom of the ribose ring, which increases the molecule's affinity for other molecules. miRNAs are species of small non-coding single-stranded regulatory RNAs that interact with

the 3'-untranslated region (3'-UTR) of target mRNA molecules through partial sequence homology. They participate in regulatory networks as controlling elements that direct comprehensive gene expression. Bioinformatics analysis has predicted that a single miRNA can regulate hundreds of target genes, contributing to the combinational and subtle regulation of numerous genetic pathways.

The term "nucleic acid" typically refers to large polynucleotides. By "nucleic acid" is meant any nucleic acid, whether composed of deoxyribonucleosides or ribonucleosides, and whether composed of phosphodiester linkages or modified linkages such as phosphotriester, phosphoramidate, siloxane, carbonate, carboxymethylester, acetamidate, carbamate, thioether, bridged phosphoramidate, bridged methylene phosphonate, bridged phosphoramidate, bridged phosphoramidate, bridged methylene phosphonate, phosphorothioate, methylphosphonate, phosphorodithioate, bridged phosphorothioate or sulfone linkages, and combinations of such linkages. The term nucleic acid also specifically includes nucleic acids composed of bases other than the five biologically occurring bases (adenine, guanine, thymine, cytosine and uracil).

As used herein, the term "nucleic acid" encompasses RNA as well as single and double-stranded DNA and cDNA. Furthermore, the terms, "nucleic acid," "DNA," "RNA" and similar terms also include nucleic acid analogs, i.e. analogs having other than a phosphodiester backbone. For example, the so-called "peptide nucleic acids," which are known in the art and have peptide bonds instead of phosphodiester bonds in the backbone, are considered within the scope of the present invention. By "nucleic acid" is meant any nucleic acid, whether composed of deoxyribonucleosides or ribonucleosides, and whether composed of phosphodiester linkages or modified linkages such as phosphotriester, phosphoramidate, siloxane, carbonate, carboxymethylester, acetamidate, carbamate, thioether, bridged phosphoramidate, bridged methylene phosphonate, bridged phosphoramidate, bridged phosphoramidate, bridged methylene phosphonate, phosphorothioate, methylphosphonate, phosphorodithioate, bridged phosphorothioate or sulfone linkages, and combinations of such linkages. The term nucleic acid also specifically includes nucleic acids composed of bases other than the five biologically occurring bases (adenine, guanine, thymine, cytosine, and uracil). Conventional notation is used herein to describe polynucleotide sequences: the left-hand end of a single-stranded polynucleotide sequence is the 5'-end; the left-

hand direction of a double-stranded polynucleotide sequence is referred to as the 5'-direction. The direction of 5' to 3' addition of nucleotides to nascent RNA transcripts is referred to as the transcription direction. The DNA strand having the same sequence as an mRNA is referred to as the "coding strand"; sequences on the
5 DNA strand which are located 5' to a reference point on the DNA are referred to as "upstream sequences"; sequences on the DNA strand which are 3' to a reference point on the DNA are referred to as "downstream sequences."

The term "nucleic acid construct," as used herein, encompasses DNA and RNA sequences encoding the particular gene or gene fragment desired, whether
10 obtained by genomic or synthetic methods.

Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. Nucleotide sequences that encode proteins and RNA may include introns.

15 The term "oligonucleotide" typically refers to short polynucleotides, generally, no greater than about 50 nucleotides. It will be understood that when a nucleotide sequence is represented by a DNA sequence (i.e., A, T, G, C), this also includes an RNA sequence (i.e., A, U, G, C) in which "U" replaces "T."

The term "otherwise identical sample", as used herein, refers to a sample
20 similar to a first sample, that is, it is obtained in the same manner from the same subject from the same tissue or fluid, or it refers a similar sample obtained from a different subject. The term "otherwise identical sample from an unaffected subject" refers to a sample obtained from a subject not known to have the disease or disorder being examined. The sample may of course be a standard sample. By analogy, the
25 term "otherwise identical" can also be used regarding regions or tissues in a subject or in an unaffected subject. These can be used as controls, as can standard samples comprising known amounts of the target to be detected or measured.

By describing two polynucleotides as "operably linked" is meant that a single-stranded or double-stranded nucleic acid moiety comprises the two
30 polynucleotides arranged within the nucleic acid moiety in such a manner that at least one of the two polynucleotides is able to exert a physiological effect by which it is characterized upon the other. By way of example, a promoter operably linked to the coding region of a gene is able to promote transcription of the coding region.

As used herein, “parenteral administration” of a pharmaceutical composition includes any route of administration characterized by physical breaching of a tissue of a subject and administration of the pharmaceutical composition through the breach in the tissue. Parenteral administration thus includes, but is not limited to, administration of a pharmaceutical composition by injection of the composition, by application of the composition through a surgical incision, by application of the composition through a tissue-penetrating non-surgical wound, and the like. In particular, parenteral administration is contemplated to include, but is not limited to, subcutaneous, intraperitoneal, intramuscular, intrasternal injection, and kidney dialytic infusion techniques.

The term “peptide” typically refers to short polypeptides.

As used herein, the term “peptide ligand” (or the word “ligand” in reference to a peptide) refers to a peptide or fragment of a protein that specifically binds to a molecule, such as a protein, carbohydrate, and the like. A receptor or binding partner of the peptide ligand can be essentially any type of molecule such as polypeptide, nucleic acid, carbohydrate, lipid, or any organic derived compound. Specific examples of ligands are peptide ligands of the present inventions.

The term “per application” as used herein refers to administration of a drug or compound to a subject.

The term “pharmaceutical composition” shall mean a composition comprising at least one active ingredient, whereby the composition is amenable to investigation for a specified, efficacious outcome in a mammal (for example, without limitation, a human). Those of ordinary skill in the art will understand and appreciate the techniques appropriate for determining whether an active ingredient has a desired efficacious outcome based upon the needs of the artisan.

As used herein, the term “pharmaceutically-acceptable carrier” means a chemical composition with which an appropriate compound or derivative can be combined and which, following the combination, can be used to administer the appropriate compound to a subject.

As used herein, the term “physiologically acceptable” ester or salt means an ester or salt form of the active ingredient which is compatible with any other ingredients of the pharmaceutical composition, which is not deleterious to the subject to which the composition is to be administered.

“Pharmaceutically acceptable” means physiologically tolerable, for either human or veterinary application.

As used herein, “pharmaceutical compositions” include formulations for human and veterinary use.

5 “Plurality” means at least two.

A “polynucleotide” means a single strand or parallel and anti-parallel strands of a nucleic acid. Thus, a polynucleotide may be either a single-stranded or a double-stranded nucleic acid.

10 “Polypeptide” refers to a polymer composed of amino acid residues, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof linked via peptide bonds, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof.

15 “Synthetic peptides or polypeptides” means a non-naturally occurring peptide or polypeptide. Synthetic peptides or polypeptides can be synthesized, for example, using an automated polypeptide synthesizer. Various solid phase peptide synthesis methods are known to those of skill in the art.

20 The term “prevent,” as used herein, means to stop something from happening, or taking advance measures against something possible or probable from happening. In the context of medicine, “prevention” generally refers to action taken to decrease the chance of getting a disease or condition.

25 A “preventive” or “prophylactic” treatment is a treatment administered to a subject who does not exhibit signs, or exhibits only early signs, of a disease or disorder. A prophylactic or preventative treatment is administered for the purpose of decreasing the risk of developing pathology associated with developing the disease or disorder.

30 “Primer” refers to a polynucleotide that is capable of specifically hybridizing to a designated polynucleotide template and providing a point of initiation for synthesis of a complementary polynucleotide. Such synthesis occurs when the polynucleotide primer is placed under conditions in which synthesis is induced, i.e., in the presence of nucleotides, a complementary polynucleotide template, and an agent for polymerization such as DNA polymerase. A primer is typically single-stranded, but may be double-stranded. Primers are typically deoxyribonucleic acids, but a wide variety of synthetic and naturally occurring primers are useful for many applications. A primer is complementary to the template to which it is designed to

hybridize to serve as a site for the initiation of synthesis, but need not reflect the exact sequence of the template. In such a case, specific hybridization of the primer to the template depends on the stringency of the hybridization conditions. Primers can be labeled with, e.g., chromogenic, radioactive, or fluorescent moieties and used as detectable moieties.

As used herein, the term “promoter/regulatory sequence” means a nucleic acid sequence which is required for expression of a gene product operably linked to the promoter/regulator sequence. In some instances, this sequence may be the core promoter sequence and in other instances, this sequence may also include an enhancer sequence and other regulatory elements which are required for expression of the gene product. The promoter/regulatory sequence may, for example, be one which expresses the gene product in a tissue specific manner.

A “constitutive” promoter is a promoter which drives expression of a gene to which it is operably linked, in a constant manner in a cell. By way of example, promoters which drive expression of cellular housekeeping genes are considered to be constitutive promoters.

An “inducible” promoter is a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a living cell substantially only when an inducer which corresponds to the promoter is present in the cell.

A “tissue-specific” promoter is a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a living cell substantially only if the cell is a cell of the tissue type corresponding to the promoter.

A “prophylactic” treatment is a treatment administered to a subject who does not exhibit signs of a disease or exhibits only early signs of the disease for the purpose of decreasing the risk of developing pathology associated with the disease.

As used herein, “protecting group” with respect to a terminal amino group refers to a terminal amino group of a peptide, which terminal amino group is coupled with any of various amino-terminal protecting groups traditionally employed in peptide synthesis. Such protecting groups include, for example, acyl protecting groups such as formyl, acetyl, benzoyl, trifluoroacetyl, succinyl, and methoxysuccinyl; aromatic urethane protecting groups such as benzyloxycarbonyl; and aliphatic urethane protecting groups, for example, tert-butoxycarbonyl or

adamantylloxycarbonyl. See Gross and Mienhofer, eds., *The Peptides*, vol. 3, pp. 3-88 (Academic Press, New York, 1981) for suitable protecting groups.

As used herein, “protecting group” with respect to a terminal carboxy group refers to a terminal carboxyl group of a peptide, which terminal carboxyl group is coupled with any of various carboxyl-terminal protecting groups. Such protecting groups include, for example, tert-butyl, benzyl or other acceptable groups linked to the terminal carboxyl group through an ester or ether bond.

The term “protein” typically refers to large polypeptides. Conventional notation is used herein to portray polypeptide sequences: the left-hand end of a polypeptide sequence is the amino-terminus; the right-hand end of a polypeptide sequence is the carboxyl-terminus.

The term “protein regulatory pathway”, as used herein, refers to both the upstream regulatory pathway which regulates a protein, as well as the downstream events which that protein regulates. Such regulation includes, but is not limited to, transcription, translation, levels, activity, posttranslational modification, and function of the protein of interest, as well as the downstream events which the protein regulates.

The terms “protein pathway” and “protein regulatory pathway” are used interchangeably herein.

As used herein, the term "providing a prognosis" refers to providing information regarding the impact of the presence of or susceptibility to, for example, age-related macular degeneration (e.g., as determined by the diagnostic methods of the present invention) on a subject's future health (e.g., age, risks such as smoking, the likelihood of getting AMD or geographic atrophy).

As used herein, the term “purified” and like terms relate to an enrichment of a molecule or compound relative to other components normally associated with the molecule or compound in a native environment. The term “purified” does not necessarily indicate that complete purity of the particular molecule has been achieved during the process. A “highly purified” compound as used herein refers to a compound that is greater than 90% pure. In particular, purified sperm cell DNA refers to DNA that does not produce significant detectable levels of non-sperm cell DNA upon PCR amplification of the purified sperm cell DNA and subsequent analysis of that amplified DNA. A “significant detectable level” is an amount of contaminate that would be visible in the presented data and would need to be

addressed/explained during analysis of the forensic evidence.

"Pyroptosis" as used herein is a highly inflammatory form of programmed cell death that occurs most frequently upon infection with intracellular pathogens and is likely to form part of the antimicrobial response. "Pyroptotic" refers to an agent or process that can induce pyroptosis.

A "recombinant adeno-associated viral (AAV) vector comprising a regulatory element active in RPE cells" refers to an AAV that has been constructed to comprise a new regulatory element to drive expression or tissue-specific expression in RPE of a gene of choice or interest. As described herein such a constructed vector may also contain at least one promoter and optionally at least one enhancer as part of the regulatory element, and the recombinant vector may further comprise additional nucleic acid sequences, including those for other genes, including therapeutic genes of interest.

"Recombinant polynucleotide" refers to a polynucleotide having sequences that are not naturally joined together. An amplified or assembled recombinant polynucleotide may be included in a suitable vector, and the vector can be used to transform a suitable host cell.

A recombinant polynucleotide may serve a non-coding function (e.g., promoter, origin of replication, ribosome-binding site, etc.) as well.

A host cell that comprises a recombinant polynucleotide is referred to as a "recombinant host cell." A gene which is expressed in a recombinant host cell wherein the gene comprises a recombinant polynucleotide, produces a "recombinant polypeptide."

A "recombinant polypeptide" is one which is produced upon expression of a recombinant polynucleotide.

A "recombinant cell" is a cell that comprises a transgene. Such a cell may be a eukaryotic or a prokaryotic cell. Also, the transgenic cell encompasses, but is not limited to, an embryonic stem cell comprising the transgene, a cell obtained from a chimeric mammal derived from a transgenic embryonic stem cell where the cell comprises the transgene, a cell obtained from a transgenic mammal, or fetal or placental tissue thereof, and a prokaryotic cell comprising the transgene.

The term "regulate" refers to either stimulating or inhibiting a function or activity of interest.

As used herein, term "regulatory elements" is used interchangeably with "regulatory sequences" and refers to promoters, enhancers, and other expression control elements, or any combination of such elements.

As used herein, the term "reporter gene" means a gene, the expression of which can be detected using a known method. By way of example, the *Escherichia coli* lacZ gene may be used as a reporter gene in a medium because expression of the lacZ gene can be detected using known methods by adding the chromogenic substrate o-nitrophenyl- β -galactoside to the medium (Gerhardt et al., eds., 1994, Methods for General and Molecular Bacteriology, American Society for Microbiology, Washington, DC, p. 574).

A "sample," as used herein, refers preferably to a biological sample from a subject, including, but not limited to, normal tissue samples, diseased tissue samples, biopsies, blood, saliva, feces, semen, tears, and urine. A sample can also be any other source of material obtained from a subject which contains cells, tissues, or fluid of interest. A sample can also be obtained from cell or tissue culture.

As used herein, the term "secondary antibody" refers to an antibody that binds to the constant region of another antibody (the primary antibody).

A "short hairpin RNA" (shRNA) as used herein is an artificial RNA molecule with a tight hairpin turn that can be used to silence target gene expression via RNA interference (RNAi). Expression of shRNA in cells is typically accomplished by delivery of plasmids or through viral or bacterial vectors. shRNA is an advantageous mediator of RNAi in that it has a relatively low rate of degradation and turnover.

By the term "signal sequence" is meant a polynucleotide sequence which encodes a peptide that directs the path a polypeptide takes within a cell, i.e., it directs the cellular processing of a polypeptide in a cell, including, but not limited to, eventual secretion of a polypeptide from a cell. A signal sequence is a sequence of amino acids which are typically, but not exclusively, found at the amino terminus of a polypeptide which targets the synthesis of the polypeptide to the endoplasmic reticulum. In some instances, the signal peptide is proteolytically removed from the polypeptide and is thus absent from the mature protein.

By "small interfering RNAs (siRNAs)" is meant, inter alia, an isolated dsRNA molecule comprised of both a sense and an anti-sense strand. In one aspect, it is greater than 10 nucleotides in length. siRNA also refers to a single transcript

which has both the sense and complementary antisense sequences from the target gene, e.g., a hairpin. siRNA further includes any form of dsRNA (proteolytically cleaved products of larger dsRNA, partially purified RNA, essentially pure RNA, synthetic RNA, recombinantly produced RNA) as well as altered RNA that differs
5 from naturally occurring RNA by the addition, deletion, substitution, and/or alteration of one or more nucleotides.

As used herein, the term "solid support" relates to a solvent insoluble substrate that is capable of forming linkages (preferably covalent bonds) with various compounds. The support can be either biological in nature, such as, without
10 limitation, a cell or bacteriophage particle, or synthetic, such as, without limitation, an acrylamide derivative, agarose, cellulose, nylon, silica, or magnetized particles.

By the term "specifically binds to", as used herein, is meant when a compound or ligand functions in a binding reaction or assay conditions which is determinative of the presence of the compound in a sample of heterogeneous
15 compounds, or it means that one molecule, such as a binding moiety, e.g., an oligonucleotide or antibody, binds preferentially to another molecule, such as a target molecule, e.g., a nucleic acid or a protein, in the presence of other molecules in a sample..

The terms "specific binding" or "specifically binding" when used in reference to the interaction of a peptide (ligand) and a receptor (molecule) also refers
20 to an interaction that is dependent upon the presence of a particular structure (i.e., an amino sequence of a ligand or a ligand binding domain within a protein); in other words the peptide comprises a structure allowing recognition and binding to a specific protein structure within a binding partner rather than to molecules in general. For example, if a ligand is specific for binding pocket "A," in a reaction
25 containing labeled peptide ligand "A" (such as an isolated phage displayed peptide or isolated synthetic peptide) and unlabeled "A" in the presence of a protein comprising a binding pocket A the unlabeled peptide ligand will reduce the amount of labeled peptide ligand bound to the binding partner, in other words a competitive
30 binding assay.

The term "standard," as used herein, refers to something used for comparison. For example, it can be a known standard agent or compound which is administered and used for comparing results when administering a test compound, or it can be a standard parameter or function which is measured to obtain a control

value when measuring an effect of an agent or compound on a parameter or function. Standard can also refer to an “internal standard”, such as an agent or compound which is added at known amounts to a sample and is useful in determining such things as purification or recovery rates when a sample is processed or subjected to purification or extraction procedures before a marker of interest is measured. Internal standards are often a purified marker of interest which has been labeled, such as with a radioactive isotope, allowing it to be distinguished from an endogenous marker.

A “subject” of analysis, diagnosis, or treatment is an animal. Such animals include mammals, preferably a human.

As used herein, a “subject in need thereof” is a patient, animal, mammal, or human, who will benefit from the method of this invention.

As used herein, the term “subject at risk for AMD” refers to a subject with one or more risk factors for developing AMD. Risk factors may include, but are not limited to, gender, age, genetic predisposition, environmental expose, and lifestyle.

As used herein, a “substantially homologous amino acid sequences” includes those amino acid sequences which have at least about 95% homology, preferably at least about 96% homology, more preferably at least about 97% homology, even more preferably at least about 98% homology, and most preferably at least about 99% or more homology to an amino acid sequence of a reference antibody chain. Amino acid sequence similarity or identity can be computed by using the BLASTP and TBLASTN programs which employ the BLAST (basic local alignment search tool) 2.0.14 algorithm. The default settings used for these programs are suitable for identifying substantially similar amino acid sequences for purposes of the present invention.

“Substantially homologous nucleic acid sequence” means a nucleic acid sequence corresponding to a reference nucleic acid sequence wherein the corresponding sequence encodes a peptide having substantially the same structure and function as the peptide encoded by the reference nucleic acid sequence; e.g., where only changes in amino acids not significantly affecting the peptide function occur. Preferably, the substantially identical nucleic acid sequence encodes the peptide encoded by the reference nucleic acid sequence. The percentage of identity between the substantially similar nucleic acid sequence and the reference nucleic acid sequence is at least about 50%, 65%, 75%, 85%, 95%, 99% or more.

Substantial identity of nucleic acid sequences can be determined by comparing the sequence identity of two sequences, for example by physical/chemical methods (i.e., hybridization) or by sequence alignment via computer algorithm. Suitable nucleic acid hybridization conditions to determine if a nucleotide sequence is substantially similar to a reference nucleotide sequence are: 7% sodium dodecyl sulfate SDS, 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 2X standard saline citrate (SSC), 0.1% SDS at 50°C; preferably in 7% (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 1X SSC, 0.1% SDS at 50°C; preferably 7% SDS, 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 0.5X SSC, 0.1% SDS at 50°C; and more preferably in 7% SDS, 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 0.1X SSC, 0.1% SDS at 65°C. Suitable computer algorithms to determine substantial similarity between two nucleic acid sequences include, GCS program package (Devereux et al., 1984 Nucl. Acids Res. 12:387), and the BLASTN or FASTA programs (Altschul et al., 1990 Proc. Natl. Acad. Sci. USA. 1990 87:14:5509-13; Altschul et al., J. Mol. Biol. 1990 215:3:403-10; Altschul et al., 1997 Nucleic Acids Res. 25:3389-3402). The default settings provided with these programs are suitable for determining substantial similarity of nucleic acid sequences for purposes of the present invention.

The term "substantially pure" describes a compound, e.g., a protein or polypeptide which has been separated from components which naturally accompany it. Typically, a compound is substantially pure when at least 10%, more preferably at least 20%, more preferably at least 50%, more preferably at least 60%, more preferably at least 75%, more preferably at least 90%, and most preferably at least 99% of the total material (by volume, by wet or dry weight, or by mole percent or mole fraction) in a sample is the compound of interest. Purity can be measured by any appropriate method, e.g., in the case of polypeptides by column chromatography, gel electrophoresis, or HPLC analysis. A compound, e.g., a protein, is also substantially purified when it is essentially free of naturally associated components or when it is separated from the native contaminants which accompany it in its natural state.

The term "susceptible to age-related macular degeneration or geographic atrophy" or "age-related macular degeneration or geographic atrophy" is not meant to infer that geographic atrophy is not a form or stage of age-related macular degeneration, but that a treatment or diagnosis can be in reference to the two.

The term “symptom,” as used herein, refers to any morbid phenomenon or departure from the normal in structure, function, or sensation, experienced by the patient and indicative of disease. In contrast, a “sign” is objective evidence of disease. For example, a bloody nose is a sign. It is evident to the patient, doctor,
5 nurse and other observers.

By "targeting at least one of the alternative, non-canonical inflammasome signaling molecules or pathways in retinal pigment epithelium (RPE)" is meant either targeting one of the molecules directly (see Schematic of FIG. 21), such as with an inhibitor, or by targeting part of the molecule's signal transduction pathway
10 that also impacts the alternative, non-canonical inflammasome signaling in RPE that leads to degeneration. The targeting and treatment encompass administering an effective amount of an inhibitor of noncanonical-inflammasome activation in RPE. Also, reference to a "protein complex" includes the mPTP, which is also sometimes just referred to as a protein. The targeting can include the use of agents that are
15 stimulatory or inhibitory, depending on the context of the particular molecule being targeted and the result desired.

A “therapeutic” treatment is a treatment administered to a subject who exhibits signs of pathology for the purpose of diminishing or eliminating those signs.

20 A “therapeutically effective amount” of a compound is that amount of compound which is sufficient to provide a beneficial effect to the subject to which the compound is administered.

The term to “treat,” as used herein, means reducing the frequency with which symptoms are experienced by a patient or subject or administering an agent or
25 compound to reduce the frequency with which symptoms are experienced.

A “prophylactic” treatment is a treatment administered to a subject who does not exhibit signs of a disease or exhibits only early signs of the disease for the purpose of decreasing the risk of developing pathology associated with the disease.

The term "transfection" is used interchangeably with the terms "gene
30 transfer", "transformation", and "transduction", and means the intracellular introduction of a polynucleotide. "Transfection efficiency" refers to the relative amount of the transgene taken up by the cells subjected to transfection. In practice, transfection efficiency is estimated by the amount of the reporter gene product expressed following the transfection procedure.

The term "transgene" is used interchangeably with "inserted gene," or "expressed gene" and, where appropriate, "gene". "Transgene" refers to a polynucleotide that, when introduced into a cell, is capable of being transcribed under appropriate conditions so as to confer a beneficial property to the cell such as, for example, expression of a therapeutically useful protein. It is an exogenous nucleic acid sequence comprising a nucleic acid which encodes a promoter/regulatory sequence operably linked to nucleic acid which encodes an amino acid sequence, which exogenous nucleic acid is encoded by a transgenic mammal.

As used herein, the term "transgenic mammal" means a mammal, the germ cells of which comprise an exogenous nucleic acid.

As used herein, a "transgenic cell" is any cell that comprises a nucleic acid sequence that has been introduced into the cell in a manner that allows expression of a gene encoded by the introduced nucleic acid sequence.

Where appropriate, the term "transgene" should be understood to include a combination of a coding sequence and optional non-coding regulatory sequences, such as a polyadenylation signal, a promoter, an enhancer, a repressor, etc.

A "vector" is a composition of matter which comprises an isolated nucleic acid and which can be used to deliver the isolated nucleic acid to the interior of a cell. Numerous vectors are known in the art including, but not limited to, linear polynucleotides, polynucleotides associated with ionic or amphiphilic compounds, plasmids, and viruses. Thus, the term "vector" includes an autonomously replicating plasmid or a virus. The term should also be construed to include non-plasmid and non-viral compounds which facilitate transfer or delivery of nucleic acid to cells, such as, for example, polylysine compounds, liposomes, and the like. Examples of viral vectors include, but are not limited to, adenoviral vectors, adeno-associated virus vectors, retroviral vectors, recombinant viral vectors, and the like. Examples of non-viral vectors include, but are not limited to, liposomes, polyamine derivatives of DNA and the like.

"Expression vector" refers to a vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis-acting elements for expression; other elements for expression can be supplied by the host cell or in an in vitro expression system. Expression vectors include all those

known in the art, such as cosmid, plasmids (e.g., naked or contained in liposomes) and viruses that incorporate the recombinant polynucleotide.

Embodiments-

5 The present application discloses a new and unexpected pathway that regulates RPE cell degeneration and provides compositions and method for regulating that pathway. That is, it is disclosed herein that RPE degeneration is regulated by the non-canonical inflammasome, aspects of which were previously known only to function in inflammation subsequent to infections.

10 Useful inhibitory molecules of the invention for inhibiting the activity and levels of the target molecules described herein include, but are not limited to, drugs, antibodies and biologically active fragments and homologs thereof, monoclonal antibodies and biologically active fragments and homologs thereof, humanized antibodies, antisense oligonucleotides, shRNA, siRNA, aptamers, and anti-oxidants.

15 By biologically active is meant that they have the intended function as described herein for inhibiting the target molecule of interest.

 The present invention also provides for homologs of proteins and peptides. Homologs can differ from naturally occurring proteins or peptides by conservative amino acid sequence differences or by modifications which do not affect sequence, or by both.

20

 For example, conservative amino acid changes may be made, which although they alter the primary sequence of the protein or peptide, do not normally alter its function. To that end, 10 or more conservative amino acid changes typically have no effect on protein function.

25 In another embodiment, the methods of the present invention may be used to prevent or treat macular degeneration. In one embodiment, macular degeneration is characterized by damage to or breakdown of the macula, which in one embodiment, is a small area at the back of the eye. In one embodiment, macular degeneration causes a progressive loss of central sight, but not complete blindness. In one

30 embodiment, macular degeneration is of the dry type, while in another embodiment, it is of the wet type. In one embodiment, the dry type is characterized by the thinning and loss of function of the macula tissue. In one embodiment, the wet type is characterized by the growth of abnormal blood vessels behind the macula. In one embodiment, the abnormal blood vessels hemorrhage or leak, resulting in the

formation of scar tissue if untreated. In some embodiments, the dry type of macular degeneration can turn into the wet type. In one embodiment, macular degeneration is age-related, which in one embodiment is caused by an ingrowth of choroidal capillaries through defects in Bruch's membrane with proliferation of fibrovascular tissue beneath the retinal pigment epithelium.

Diagnosis of AMD using the compositions and methods of the present invention can be coupled with known methods. For example, the early and intermediate stages of AMD usually start without symptoms. A comprehensive dilated eye exam can detect AMD. The eye exam may include the following:

1. Visual acuity test. An eye chart measure is used to measure vision at distances.

2. Dilated eye exam. The eye care professional places drops in the eyes to widen or dilate the pupils. This provides a better view of the back of the eye. Using a special magnifying lens, he or she then looks at your retina and optic nerve for signs of AMD and other eye problems.

3. Amsler grid. The eye care professional also may ask you to look at an Amsler grid. Changes in central vision may cause the lines in the grid to disappear or appear wavy, a sign of AMD.

4. Fluorescein angiogram. In this test, which is performed by an ophthalmologist, a fluorescent dye is injected into the subject's arm. Pictures are taken as the dye passes through the blood vessels in the eye. This makes it possible to see leaking blood vessels, which occur in a severe, rapidly progressive type of AMD (see below).

5. Optical coherence tomography. This technique uses light waves, and can achieve very high-resolution images of any tissues that can be penetrated by light—such as the eyes.

There are also multiple methods available for predicting susceptibility to age-related macular degeneration or geographic atrophy. As mentioned above, for example, "age-related macular degeneration or geographic atrophy" is not meant to infer that geographic atrophy is not a form or stage of age-related macular degeneration, but that a treatment or diagnosis can be in reference to the two. Methods and biomarkers are available for predicting whether a subject is susceptible to AMD, including, for example, the existence genetic variants of complement factor H (CFH) and high-temperature requirement factor A-1 (HTRA1) that can be

detected, smoking, and, of course, age. When a subject has been tested and is diagnosed or predicted to be susceptible to an RPE disease or disorder, one or more of the therapeutic agents of the invention can be administered prophylactically.

5 Caspases (cysteine-aspartic proteases/cysteine aspartases/cysteine-dependent aspartate-directed proteases) are a family of protease enzymes playing a role in programmed cell death. There is a precursor and isoforms for caspase-4. Isoform alpha is known as the canonical sequence.

Modifications (which do not normally alter primary sequence) include in vivo, or in vitro chemical derivatization of polypeptides, e.g., acetylation, or
10 carboxylation. Also included are modifications of glycosylation, e.g., those made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing or in further processing steps; e.g., by exposing the polypeptide to enzymes which affect glycosylation, e.g., mammalian glycosylating or deglycosylating enzymes. Also embraced are sequences which have phosphorylated
15 amino acid residues, e.g., phosphotyrosine, phosphoserine, or phosphothreonine.

Also included are polypeptides or antibody fragments which have been modified using ordinary molecular biological techniques so as to improve their resistance to proteolytic degradation or to optimize solubility properties or to render them more suitable as a therapeutic agent. Homologs of such polypeptides include
20 those containing residues other than naturally occurring L-amino acids, e.g., D-amino acids or non-naturally occurring synthetic amino acids. The peptides of the invention are not limited to products of any of the specific exemplary processes listed herein.

Substantially pure protein or peptide obtained as described herein may be
25 purified by following known procedures for protein purification, wherein an immunological, enzymatic, or other assay is used to monitor purification at each stage in the procedure. Protein purification methods are well known in the art, and are described, for example in Deutscher et al. (ed., 1990, Guide to Protein Purification, Harcourt Brace Jovanovich, San Diego).

30 RNA interference (RNAi) is a commonly used method to regulate gene expression. This effect is often achieved by using small interfering RNA (siRNA) or short hairpin RNA (shRNA). Applying these small RNAs to cells under in vitro conditions is relatively easy but this application under in vivo conditions is difficult due to various issues, such as short life of these molecules and their inability to

access target cells. In one aspect, these issues can be solved by plasmid or vector-mediated delivery.

In one aspect, AAV can be used. The natural tissue tropism of the various AAV serotypes can be exploited to favor gene delivery to one organ over another.

5 This tropism is based on the viral capsids recognizing specific viral receptors expressed on specific cell types, thus allowing a degree of cell specific targeting within a given organ. Cell-specific expression may be further aided by the use of tissue-specific promoters conferring gene expression restricted to a specific cell type. This is desirable for gene therapy applications targeting organ specific
10 diseases, as this will help avoid any possible harmful side effects due to gene expression in off target organs.

In one embodiment, an isolated nucleic acid of the invention is encoded by a vector.

15 In one aspect, the isolated nucleic acid is operably-linked to a cell-specific promoter.

In one aspect, a lipid vehicle comprises said isolated nucleic acid.

In one aspect, the vectors further comprise a gene of interest, which may be a therapeutic gene. The regulatory element may include an enhancer and/or a promoter. The combination of specific vectors, including AAV vectors, enhancers,
20 promoters, and therapeutic genes, and fragments and homologs thereof that are used can be modified to ensure a high rate of targeting cells and tissues of interest and expression of therapeutic genes and genes of interest in the target cell of tissue of interest.

25 The present invention does not just encompass administering pharmaceutical compositions comprising an effective amount of a compound of the invention. The present invention further encompasses targeting RPE cells.

In one embodiment, the present invention provides for the administration of at least one miRNA, including pre-miRNA and mature miRNA, or a mimic thereof. "miRNA mimics" are chemically synthesized nucleic acid based molecules,
30 preferably double-stranded RNAs which mimic mature endogenous miRNAs after transfection into cells. In one aspect, an antagonist of the miRNA can be used. In another aspect, an agonist of the miRNA can be used. The type of regulator can be chosen depending on the role of the molecule(s) or pathway to be targeted in the RPE cell.

miRNAs are transcribed by RNA polymerase II (pol II) or RNA polymerase III and arise from initial transcripts, termed primary miRNA transcripts (pri-miRNAs), that are generally several thousand bases long. Pri-miRNAs are processed in the nucleus by the RNase Drosha into about 70- to about 100-
5 nucleotide hairpin-shaped precursors (pre-miRNAs). Following transport to the cytoplasm, the hairpin pre-miRNA is further processed by Dicer to produce a double-stranded miRNA. The mature miRNA strand is then incorporated into the RNA-induced silencing complex (RISC), where it associates with its target mRNAs by base-pair complementarity. In the relatively rare cases in which a miRNA base
10 pairs perfectly with an mRNA target, it promotes mRNA degradation. More commonly, miRNAs form imperfect heteroduplexes with target mRNAs, affecting either mRNA stability or inhibiting mRNA translation.

In one aspect, an miR-specific inhibitor may be an anti-miRNA (anti-miR) oligonucleotide (for example, see WO2005054494).

15 An administered miRNA may be the naturally occurring miRNA or it may be an analogue or homologue of the miRNA. In one aspect, the miRNA, or analogue or homologues, are modified to increase the stability thereof in the cellular milieu. In an alternative aspect the miRNA is encoded by an expression vector and may be delivered to the target cell in a liposome or microvesicle.

20 **Amino Acid Substitutions**

In certain embodiments, the disclosed methods and compositions may involve preparing peptides with one or more substituted amino acid residues. In various embodiments, the structural, physical and/or therapeutic characteristics of peptide sequences may be optimized by replacing one or more amino acid residues.

25 In one embodiment, the invention encompasses the substitution of a serine or an alanine residue for a cysteine residue in a peptide of the invention. Support for this includes what is known in the art. For example, see the following citation for justification of such a serine or alanine substitution: Kittlesen et al., 1998 Human melanoma patients recognize an HLA-A1-restricted CTL epitope from tyrosinase
30 containing two cysteine residues: implications for tumor vaccine development J Immunol., 60, 2099-2106.

Other modifications can also be incorporated without adversely affecting the activity and these include, but are not limited to, substitution of one or more of the amino acids in the natural L-isomeric form with amino acids in the D-isomeric form.

Thus, the peptide may include one or more D-amino acid residues, or may comprise amino acids which are all in the D-form. Retro-inverso forms of peptides in accordance with the present invention are also contemplated, for example, inverted peptides in which all amino acids are substituted with D-amino acid forms.

5 The skilled artisan will be aware that, in general, amino acid substitutions in a peptide typically involve the replacement of an amino acid with another amino acid of relatively similar properties (i.e., conservative amino acid substitutions). The properties of the various amino acids and effect of amino acid substitution on protein structure and function have been the subject of extensive study and
10 knowledge in the art. For example, one can make the following isosteric and/or conservative amino acid changes in the parent polypeptide sequence with the expectation that the resulting polypeptides would have a similar or improved profile of the properties described above:

Substitution of alkyl-substituted hydrophobic amino acids: including alanine,
15 leucine, isoleucine, valine, norleucine, S-2-aminobutyric acid, S-cyclohexylalanine or other simple alpha-amino acids substituted by an aliphatic side chain from C1-10 carbons including branched, cyclic and straight chain alkyl, alkenyl or alkynyl substitutions.

Substitution of aromatic-substituted hydrophobic amino acids: including
20 phenylalanine, tryptophan, tyrosine, biphenylalanine, 1-naphthylalanine, 2-naphthylalanine, 2-benzothierylalanine, 3-benzothierylalanine, histidine, amino, alkylamino, dialkylamino, aza, halogenated (fluoro, chloro, bromo, or iodo) or alkoxy-substituted forms of the previous listed aromatic amino acids, illustrative examples of which are: 2-,3- or 4-aminophenylalanine, 2-,3- or 4-
25 chlorophenylalanine, 2-,3- or 4-methylphenylalanine, 2-,3- or 4-methoxyphenylalanine, 5-amino-, 5-chloro-, 5-methyl- or 5-methoxytryptophan, 2', 3'-, or 4'-amino-, 2', 3'-, or 4'-chloro-, 2,3, or 4-biphenylalanine, 2',-3',- or 4'-methyl- 2, 3 or 4-biphenylalanine, and 2- or 3-pyridylalanine.

Substitution of amino acids containing basic functions: including arginine,
30 lysine, histidine, ornithine, 2,3-diaminopropionic acid, homoarginine, alkyl, alkenyl, or aryl-substituted (from C₁-C₁₀ branched, linear, or cyclic) derivatives of the previous amino acids, whether the substituent is on the heteroatoms (such as the alpha nitrogen, or the distal nitrogen or nitrogens, or on the alpha carbon, in the pro-R position for example. Compounds that serve as illustrative examples include: N-

epsilon-isopropyl-lysine, 3-(4-tetrahydropyridyl)-glycine, 3-(4-tetrahydropyridyl)-alanine, N,N-gamma, gamma'-diethyl-homoarginine. Included also are compounds such as alpha methyl arginine, alpha methyl 2,3-diaminopropionic acid, alpha methyl histidine, alpha methyl ornithine where alkyl group occupies the pro-R position of the alpha carbon. Also included are the amides formed from alkyl, aromatic, heteroaromatic (where the heteroaromatic group has one or more nitrogens, oxygens, or sulfur atoms singly or in combination) carboxylic acids or any of the many well-known activated derivatives such as acid chlorides, active esters, active azolides and related derivatives) and lysine, ornithine, or 2,3-diaminopropionic acid.

Substitution of acidic amino acids: including aspartic acid, glutamic acid, homoglutamic acid, tyrosine, alkyl, aryl, arylalkyl, and heteroaryl sulfonamides of 2,4-diaminopropionic acid, ornithine or lysine and tetrazole-substituted alkyl amino acids.

Substitution of side chain amide residues: including asparagine, glutamine, and alkyl or aromatic substituted derivatives of asparagine or glutamine.

Substitution of hydroxyl containing amino acids: including serine, threonine, homoserine, 2,3-diaminopropionic acid, and alkyl or aromatic substituted derivatives of serine or threonine. It is also understood that the amino acids within each of the categories listed above can be substituted for another of the same group.

For example, the hydropathic index of amino acids may be considered (Kyte & Doolittle, 1982, J. Mol. Biol., 157:105-132). The relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics (Kyte & Doolittle, 1982), these are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5). In making conservative substitutions, the use of amino acids whose hydropathic indices are within +/-2 is preferred, within +/-1 are more preferred, and within +/- 0.5 are even more preferred.

Amino acid substitution may also take into account the hydrophilicity of the amino acid residue (e.g., U.S. Pat. No. 4,554,101). Hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0); glutamate (+3.0); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0);
 5 threonine (-0.4); proline (-0.5.+0.1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). Replacement of amino acids with others of similar hydrophilicity is preferred.

Other considerations include the size of the amino acid side chain. For
 10 example, it would generally not be preferred to replace an amino acid with a compact side chain, such as glycine or serine, with an amino acid with a bulky side chain, e.g., tryptophan or tyrosine. The effect of various amino acid residues on protein secondary structure is also a consideration. Through empirical study, the effect of different amino acid residues on the tendency of protein domains to adopt
 15 an alpha-helical, beta-sheet or reverse turn secondary structure has been determined and is known in the art (see, e.g., Chou & Fasman, 1974, *Biochemistry*, 13:222-245; 1978, *Ann. Rev. Biochem.*, 47: 251-276; 1979, *Biophys. J.*, 26:367-384).

Based on such considerations and extensive empirical study, tables of conservative amino acid substitutions have been constructed and are known in the
 20 art. For example: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine. Alternatively: Ala (A) leu, ile, val; Arg (R) gln, asn, lys; Asn (N) his, asp, lys, arg, gln; Asp (D) asn, glu; Cys (C) ala, ser; Gln (Q) glu, asn; Glu (E) gln, asp; Gly (G) ala; His (H) asn, gln, lys, arg; Ile (I) val, met, ala, phe, leu; Leu (L) val, met, ala, phe, ile; Lys (K) gln,
 25 asn, arg; Met (M) phe, ile, leu; Phe (F) leu, val, ile, ala, tyr; Pro (P) ala; Ser (S), thr; Thr (T) ser; Trp (W) phe, tyr; Tyr (Y) trp, phe, thr, ser; Val (V) ile, leu, met, phe, ala.

Other considerations for amino acid substitutions include whether or not the residue is located in the interior of a protein or is solvent exposed. For interior
 30 residues, conservative substitutions would include: Asp and Asn; Ser and Thr; Ser and Ala; Thr and Ala; Ala and Gly; Ile and Val; Val and Leu; Leu and Ile; Leu and Met; Phe and Tyr; Tyr and Trp. (See, e.g., PROWL Rockefeller University website). For solvent exposed residues, conservative substitutions would include: Asp and Asn; Asp and Glu; Glu and Gln; Glu and Ala; Gly and Asn; Ala and Pro; Ala and

Gly; Ala and Ser; Ala and Lys; Ser and Thr; Lys and Arg; Val and Leu; Leu and Ile; Ile and Val; Phe and Tyr. (Id.) Various matrices have been constructed to assist in selection of amino acid substitutions, such as the PAM250 scoring matrix, Dayhoff matrix, Grantham matrix, McLachlan matrix, Doolittle matrix, Henikoff matrix, 5 Miyata matrix, Fitch matrix, Jones matrix, Rao matrix, Levin matrix and Risler matrix (Idem.)

In determining amino acid substitutions, one may also consider the existence of intermolecular or intramolecular bonds, such as formation of ionic bonds (salt bridges) between positively charged residues (e.g., His, Arg, Lys) and negatively 10 charged residues (e.g., Asp, Glu) or disulfide bonds between nearby cysteine residues.

Methods of substituting any amino acid for any other amino acid in an encoded peptide sequence are well known and a matter of routine experimentation for the skilled artisan, for example by the technique of site-directed mutagenesis or 15 by synthesis and assembly of oligonucleotides encoding an amino acid substitution and splicing into an expression vector construct.

The invention is also directed to methods of administering the compounds of the invention to a subject.

Pharmaceutical compositions comprising the present compounds are 20 administered to an individual in need thereof by any number of routes including, but not limited to, topical, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

The present invention is also directed to pharmaceutical compositions 25 comprising the peptides of the present invention. More particularly, such compounds can be formulated as pharmaceutical compositions using standard pharmaceutically acceptable carriers, fillers, solubilizing agents and stabilizers known to those skilled in the art.

The invention also encompasses the use pharmaceutical compositions of an 30 appropriate compound, homolog, fragment, analog, or derivative thereof to practice the methods of the invention, the composition comprising at least one appropriate compound, homolog, fragment, analog, or derivative thereof and a pharmaceutically-acceptable carrier.

The pharmaceutical compositions useful for practicing the invention may be administered to deliver a dose of between 1 ng/kg/day and 100 mg/kg/day.

Pharmaceutical compositions that are useful in the methods of the invention may be administered systemically in oral solid formulations, ophthalmic, suppository, aerosol, topical or other similar formulations. In addition to the appropriate compound, such pharmaceutical compositions may contain pharmaceutically-acceptable carriers and other ingredients known to enhance and facilitate drug administration. Other possible formulations, such as nanoparticles, liposomes, resealed erythrocytes, and immunologically based systems may also be used to administer an appropriate compound according to the methods of the invention.

The invention encompasses the preparation and use of pharmaceutical compositions comprising a compound useful for treatment of the conditions, disorders, and diseases disclosed herein as an active ingredient. Such a pharmaceutical composition may consist of the active ingredient alone, in a form suitable for administration to a subject, or the pharmaceutical composition may comprise the active ingredient and one or more pharmaceutically acceptable carriers, one or more additional ingredients, or some combination of these. The active ingredient may be present in the pharmaceutical composition in the form of a physiologically acceptable ester or salt, such as in combination with a physiologically acceptable cation or anion, as is well known in the art.

As used herein, the term “physiologically acceptable” ester or salt means an ester or salt form of the active ingredient which is compatible with any other ingredients of the pharmaceutical composition, which is not deleterious to the subject to which the composition is to be administered.

The formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with a carrier or one or more other accessory ingredients, and then, if necessary or desirable, shaping or packaging the product into a desired single- or multi-dose unit.

Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for ethical administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to animals of all sorts.

Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and perform such modification with merely ordinary, if any, experimentation.

5 Subjects to which administration of the pharmaceutical compositions of the invention is contemplated include, but are not limited to, humans and other primates, mammals including commercially relevant mammals such as cattle, pigs, horses, sheep, cats, and dogs, birds including commercially relevant birds such as chickens, ducks, geese, and turkeys.

10 Pharmaceutical compositions that are useful in the methods of the invention may be prepared, packaged, or sold in formulations suitable for oral, rectal, vaginal, parenteral, topical, pulmonary, intranasal, buccal, ophthalmic, intrathecal or another route of administration. Other contemplated formulations include projected nanoparticles, liposomal preparations, resealed erythrocytes containing the active
15 ingredient, and immunologically-based formulations.

 A pharmaceutical composition of the invention may be prepared, packaged, or sold in bulk, as a single unit dose, or as a plurality of single unit doses. As used herein, a “unit dose” is discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the
20 active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

 The relative amounts of the active ingredient, the pharmaceutically acceptable carrier, and any additional ingredients in a pharmaceutical composition
25 of the invention will vary, depending upon the identity, size, and condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) active ingredient.

 In addition to the active ingredient, a pharmaceutical composition of the
30 invention may further comprise one or more additional pharmaceutically active agents. Particularly contemplated additional agents include anti-emetics and scavengers such as cyanide and cyanate scavengers.

 Controlled- or sustained-release formulations of a pharmaceutical composition of the invention may be made using conventional technology. A

formulation of a pharmaceutical composition of the invention suitable for oral administration may be prepared, packaged, or sold in the form of a discrete solid dose unit including, but not limited to, a tablet, a hard or soft capsule, a cachet, a troche, or a lozenge, each containing a predetermined amount of the active
5 ingredient. Other formulations suitable for oral administration include, but are not limited to, a powdered or granular formulation, an aqueous or oily suspension, an aqueous or oily solution, or an emulsion.

As used herein, an “oily” liquid is one which comprises a carbon-containing liquid molecule and which exhibits a less polar character than water.

10 Liquid formulations of a pharmaceutical composition of the invention which are suitable for oral administration may be prepared, packaged, and sold either in liquid form or in the form of a dry product intended for reconstitution with water or another suitable vehicle prior to use.

Liquid suspensions may be prepared using conventional methods to achieve
15 suspension of the active ingredient in an aqueous or oily vehicle. Aqueous vehicles include, for example, water and isotonic saline. Oily vehicles include, for example, almond oil, oily esters, ethyl alcohol, vegetable oils such as arachis, olive, sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin. Liquid suspensions may further comprise one or more additional ingredients
20 including, but not limited to, suspending agents, dispersing or wetting agents, emulsifying agents, demulcents, preservatives, buffers, salts, flavorings, coloring agents, and sweetening agents. Oily suspensions may further comprise a thickening agent. Known suspending agents include, but are not limited to, sorbitol syrup, hydrogenated edible fats, sodium alginate, polyvinylpyrrolidone, gum tragacanth,
25 gum acacia, and cellulose derivatives such as sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose.

Known dispersing or wetting agents include, but are not limited to, naturally occurring phosphatides such as lecithin, condensation products of an alkylene oxide with a fatty acid, with a long chain aliphatic alcohol, with a partial ester derived
30 from a fatty acid and a hexitol, or with a partial ester derived from a fatty acid and a hexitol anhydride (e.g. polyoxyethylene stearate, heptadecaethyleneoxycetanol, polyoxyethylene sorbitol monooleate, and polyoxyethylene sorbitan monooleate, respectively).

Known emulsifying agents include, but are not limited to, lecithin and acacia. Known preservatives include, but are not limited to, methyl, ethyl, or n-propyl para hydroxybenzoates, ascorbic acid, and sorbic acid. Known sweetening agents include, for example, glycerol, propylene glycol, sorbitol, sucrose, and
5 saccharin. Known thickening agents for oily suspensions include, for example, beeswax, hard paraffin, and cetyl alcohol.

Liquid solutions of the active ingredient in aqueous or oily solvents may be prepared in substantially the same manner as liquid suspensions, the primary difference being that the active ingredient is dissolved, rather than suspended in the
10 solvent. Liquid solutions of the pharmaceutical composition of the invention may comprise each of the components described with regard to liquid suspensions, it being understood that suspending agents will not necessarily aid dissolution of the active ingredient in the solvent. Aqueous solvents include, for example, water and isotonic saline. Oily solvents include, for example, almond oil, oily esters, ethyl
15 alcohol, vegetable oils such as arachis, olive, sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin.

Powdered and granular formulations of a pharmaceutical preparation of the invention may be prepared using known methods. Such formulations may be administered directly to a subject, used, for example, to form tablets, to fill capsules,
20 or to prepare an aqueous or oily suspension or solution by addition of an aqueous or oily vehicle thereto. Each of these formulations may further comprise one or more of dispersing or wetting agent, a suspending agent, and a preservative. Additional excipients, such as fillers and sweetening, flavoring, or coloring agents, may also be included in these formulations.

25 A pharmaceutical composition of the invention may also be prepared, packaged, or sold in the form of oil in water emulsion or a water-in-oil emulsion. The oily phase may be a vegetable oil such as olive or arachis oil, a mineral oil such as liquid paraffin, or a combination of these. Such compositions may further comprise one or more emulsifying agents such as naturally occurring gums such as
30 gum acacia or gum tragacanth, naturally occurring phosphatides such as soybean or lecithin phosphatide, esters or partial esters derived from combinations of fatty acids and hexitol anhydrides such as sorbitan monooleate, and condensation products of such partial esters with ethylene oxide such as polyoxyethylene sorbitan

monooleate. These emulsions may also contain additional ingredients including, for example, sweetening or flavoring agents.

A pharmaceutical composition of the invention may also be prepared, packaged, or sold in a formulation suitable for rectal administration, vaginal
5 administration, parenteral administration

The pharmaceutical compositions may be prepared, packaged, or sold in the form of a sterile injectable aqueous or oily suspension or solution. This suspension or solution may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents,
10 wetting agents, or suspending agents described herein. Such sterile injectable formulations may be prepared using a non-toxic parenterally acceptable diluent or solvent, such as water or 1,3 butane diol, for example.

Other acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic
15 mono or di-glycerides. Other parentally-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form, in a liposomal preparation, or as a component of a biodegradable polymer systems. Compositions for sustained release or implantation may comprise pharmaceutically acceptable polymeric or hydrophobic materials such as an emulsion, an ion
20 exchange resin, a sparingly soluble polymer, or a sparingly soluble salt.

Formulations suitable for nasal administration may, for example, comprise from about as little as 0.1% (w/w) and as much as 100% (w/w) of the active ingredient, and may further comprise one or more of the additional ingredients described herein.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for buccal administration. Such formulations may, for example, be in the form of tablets or lozenges made using conventional methods, and may, for example, 0.1 to 20% (w/w) active ingredient, the balance comprising an orally dissolvable or degradable composition and, optionally, one or more of the
30 additional ingredients described herein. Alternately, formulations suitable for buccal administration may comprise a powder or an aerosolized or atomized solution or suspension comprising the active ingredient. Such powdered, aerosolized, or aerosolized formulations, when dispersed, preferably have an

average particle or droplet size in the range from about 0.1 to about 200 nanometers, and may further comprise one or more of the additional ingredients described herein.

As used herein, "additional ingredients" include, but are not limited to, one or more of the following: excipients; surface active agents; dispersing agents; inert
5 diluents; granulating and disintegrating agents; binding agents; lubricating agents; sweetening agents; flavoring agents; coloring agents; preservatives; physiologically degradable compositions such as gelatin; aqueous vehicles and solvents; oily vehicles and solvents; suspending agents; dispersing or wetting agents; emulsifying agents, demulcents; buffers; salts; thickening agents; fillers; emulsifying agents;
10 antioxidants; antibiotics; antifungal agents; stabilizing agents; and pharmaceutically acceptable polymeric or hydrophobic materials. Other "additional ingredients" which may be included in the pharmaceutical compositions of the invention are known in the art and described, for example in Genaro, ed., 1985, Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA, which is incorporated
15 herein by reference.

Typically, dosages of the compound of the invention which may be administered to an animal, preferably a human, range in amount from 1 μ g to about 100 g per kilogram of body weight of the subject. While the precise dosage administered will vary depending upon any number of factors, including but not
20 limited to, the type of animal and type of disease state being treated, the age of the animal and the route of administration. In one embodiment, the dosage of the compound will vary from about 10 μ g to about 10 g per kilogram of body weight of the animal. In another embodiment, the dosage will vary from about 10 mg to about 1 g per kilogram of body weight of the subject.

25 The compound may be administered to a subject as frequently as several times daily, or it may be administered less frequently, such as once a day, once a week, once every two weeks, once a month, or even less frequently, such as once every several months or even once a year or less. The frequency of the dose will be readily apparent to the skilled artisan and will depend upon any number of factors,
30 such as, but not limited to, the type and severity of the disease being treated, the type and age of the subject, etc.

In one aspect, additional therapeutic agents of the pharmaceutical compositions of the invention are anti-ischemia agents. One of ordinary skill in the art will

appreciate that the composition may further comprise an effective amount of at least one additional therapeutic agents which may be useful for the type of injury, disease, or disorder being treated. Additional therapeutic agents include, but are not limited to, anesthetic, analgesic, antimicrobial, steroid, growth factor, cytokine, and anti-inflammatory agents. Useful anesthetic agents include benzocaine, lidocaine, bupivocaine, dibucaine, mepivocaine, etidocaine, tetracaine, butanilcaine, and trimecaine.

In another aspect, the agent is at least one analgesic. In yet another aspect, the agent is an additional therapeutic drug.

In a further aspect, the additional therapeutic agent is an antimicrobial agent. In one aspect, the antimicrobial agent is an antibacterial agent. In another aspect, the antimicrobial agent is an antifungal agent. In yet another aspect, the antimicrobial agent is an antiviral agent. Antimicrobial agents useful in the practice of the invention include, but are not limited to, silver sulfadiazine, Nystatin, Nystatin/triamcinolone, Bacitracin, nitrofurazone, nitrofurantoin, a polymyxin (*e.g.*, Colistin, Surfactin, Polymyxin E, and Polymyxin B), doxycycline, antimicrobial peptides (*e.g.*, natural and synthetic origin), Neosporin (*i.e.*, Bacitracin, Polymyxin B, and Neomycin), Polysporin (*i.e.*, Bacitracin and Polymyxin B). Additional antimicrobials include topical antimicrobials (*i.e.*, antiseptics), examples of which include silver salts, iodine, benzalkonium chloride, alcohol, hydrogen peroxide, and chlorhexidine. It may be desirable for the antimicrobial to be other than Nystatin.

In another aspect, the agent is selected from aspirin, pentoxifylline, and clopidogrel bisulfate, or other angiogenic, or a rheologic active agent.

The invention also includes a kit comprising a compound of the invention and an instructional material which describes administering the composition to a cell or a tissue of a subject. In another embodiment, this kit comprises a (preferably sterile) solvent suitable for dissolving or suspending the composition of the invention prior to administering the compound to the subject. The invention also provides an applicator, and an instructional material for the use thereof.

Examples

Methods-

Mice

All animal experiments were approved by institutional review committees and performed in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Visual Research. Both male and female mice between 6–10 weeks of age were used in the study. Wild-type C57BL/6J, *Ppif*^{+/−}, *P2rx7*^{+/−}, *Stat2*^{+/−} mice were purchased from The Jackson Laboratory. *Gsdmd*^{+/−}, *Pycard*^{+/−}, *Casp11*^{+/−}, *Casp1*^{+/−} *Casp11*^{129mt/129mt} and *Casp1*^{+/−} *Casp11*^{129mt/129mt} *Casp11*^{Tg} mice, described elsewhere^{1–4}, were a generous gift from V.M Dixit (Genentech). Caspase-11 deficient mouse transgenically expressing human caspase-4 (*Casp11*^{+/−} *hCasp4*^{Tg}) were described earlier⁵. Wild type 129S6 mice (that carry an inactivating passenger mutation in caspase-11) were purchased from Taconic Biosciences. *Ifnar1*^{+/−} mice described earlier⁶ were generous gift from M. Aguet. *Irf3*^{+/−} mice were a generous gift from T. Taniguchi via M. David⁷. *Mb21d1*^{+/−} mice were generated by K.A. Fitzgerald (University of Massachusetts Medical School) on a C57BL/6 background using cryopreserved embryos obtained from the European Conditional Mouse Mutagenesis Program (EUCOMM)⁸. *Tmem173*^{+/−} mice were described earlier⁹. For all procedures, anesthesia was achieved by intraperitoneal injection of 100 mg/kg ketamine hydrochloride (Ft. Dodge Animal Health) and 10 mg/kg xylazine (Phoenix Scientific), and pupils were dilated with topical 1% tropicamide and 2.5% phenylephrine (Alcon Laboratories).

Fundus Photography

TRC-50 IX camera (Topcon) linked to a digital imaging system (Sony) was used for fundus photographs of dilated mouse eyes.

Subretinal Injection

Subretinal injections (1 µl) in mice were performed using a Pico-Injector (PLI-100, Harvard Apparatus) or using a 35-gauge needle (Ito Co. Fuji, Japan). *In vivo* transfection of plasmids expressing *Alu* sequences (pAlu)^{10,11}, empty control vector (pNull), Flag-cGAS (pFlag-cGAS), Flag-GFP, mouse mature IL-18 (pIL-18ss)^{12,13}, wildtype mouse gasdermin D (pGSDMD-WT), the p30 cleavage incompetent mutant mouse gasdermin D ((pGSMDD-D276A)², IFN-β (Origene Cat# MR226101), or mtDNA (10 ng) was achieved using 10% Neuroporter (Genlantis) as previously described^{14,15}. *In vitro* transcribed *Alu* RNA (0.15–0.3 µg/µl), IFN-β neutralizing antibody (10 ng; Abcam Cat# ab24324), control isotype

IgG, recombinant IL-18 (100 ng/ μ l, MBL Cat#B002-5), or IFN- β (500 mUnit/ μ l, PBL Cat#12410-1) were administered via subretinal injection^{14,15}. Similarly, to knock down *Dicer1*, 1 μ l of cholesterol conjugated siRNA (1 μ g/ μ l) targeting mouse *Dicer1* or scrambled control siRNAs were injected. The choice of eye for active versus control injection was chosen randomly.

Assessment of RPE Degeneration

Alu-mediated RPE degeneration was induced by exposing mice to *Alu* RNA as previously described¹⁴⁻¹⁸. Seven days later, RPE health was assessed by fundus photography and immunofluorescence staining of zonula occludens-1 (ZO-1) on RPE flat mounts (whole mount of posterior eye cup containing RPE and choroid layers). Mouse RPE and choroid flat mounts were fixed with 4% paraformaldehyde or 100% methanol, stained with rabbit polyclonal antibodies against mouse ZO-1 (1:100, Invitrogen), and visualized with Alexa-594 (Invitrogen). All images were obtained by microscopy (model SP-5, Leica; or Axio Observer Z1, Zeiss). Imaging was performed by an operator masked to the group assignments.

Quantification of RPE Degeneration

Binary Assignment: Healthy RPE cells form a polygonal tessellation with a principally hexagonal “honeycomb” formation. RPE degeneration was assessed as a disruption of this uniformity of this polygonal sheet. Thus, RPE health was assessed as the presence or absence of morphological disruption in RPE flat mounts by two independent raters who were masked to the group assignments. Both raters deemed 100% of images as gradable. Inter-rater reliability was measured by agreement on assignments, Pearson coefficient of determination, and Fleiss κ . Fisher’s exact test was used to determine statistical significance between the fractions of healthy RPE sheets across groups.

Cellular Morphometry: Quantifying cellular morphometry for hexagonally packed cells was performed in semi-automated fashion by three masked graders by adapting our previous analysis of corneal endothelial cell density¹⁹. As RPE cells when viewed *en face* typically exhibit a principally hexagonal morphology similar to the corneal endothelium, they readily lend themselves to a similar analysis strategy. We obtained measures of cell size, polymegethism (coefficient of variation of cell size), and cell density. For this analysis, microscopy images of the RPE were captured and transmitted in deidentified fashion to the Doheny Image Reading &

Research Lab (DIRRL). Images in which no cell borders could be seen were excluded from further analysis (1.8%). All images were rescaled to 304×446 pixels to permit importation into the Konan CellCheck software (Ver. 4.0.1), a commercial U.S. FDA-cleared software that has been used for registration clinical trials. RPE cell metrics were generated by three certified reading center graders in an independent, masked fashion. Inter-grader agreement was assessed for all three metrics by computing the multiple adjusted coefficient of determination. The previously published center method was utilized which entails the user selecting the center of each identifiable cell in the image²⁰⁻²³. Once the cell centers were defined, the software automatically generated the mean cell area, cell density, and polymegethism values. By default, the Konan software assumes a scaling factor of 124 pixels per 100 μm . Based on the dimensions of the original RPE image ($1,024 \times 1,024$ pixels, 0.21 $\mu\text{m}/\text{pixel}$), the Konan provided values were converted to the actual physical values in μm .

Human Tissue

All studies on human tissue followed the guidelines of the Declaration of Helsinki. Institutional review boards granted approval for allocation and histological analysis of specimens. Donor eyes from patients with geographic atrophy, an advanced form of AMD or age-matched patients without AMD were obtained from various eye banks. These diagnoses were confirmed by dilated ophthalmic examination prior to acquisition of the tissues or eyes or upon examination of the eye globes post mortem. Enucleated donor eyes isolated within six hours post mortem were immediately preserved in RNALater (ThermoFisher). The neural retina was removed and tissue consisting of macular RPE and choroid were snap frozen in liquid nitrogen. For eyes with GA, the RPE and choroidal tissue was collected comprising of both atrophic and marginal areas.

Immunohistochemistry

Human eyes fixed in 2–4% paraformaldehyde were prepared for immunohistochemistry, and stained as described earlier^{14,15}. Briefly, immunohistochemical staining of fixed human eyes was performed with rabbit antibody against cGAS (0.1 $\mu\text{g}/\text{ml}$, Sigma-Aldrich, Cat# HPA031700) or interferon β (0.2 $\mu\text{g}/\text{ml}$, Santa Cruz Biotechnology, Cat# sc-20107) and mouse antibody against gasdermin D (1.5 $\mu\text{g}/\text{ml}$, Abcam, Cat# ab57785). Rabbit or mouse IgG controls were used to ascertain the specificity of the staining. Biotin-conjugated

secondary antibodies, followed by incubation with VECTASTAIN® ABC reagent and development using Vector Blue (Vector Laboratories) were utilized to detect the bound primary antibody. Slides were washed in PBS, and then mounted in Vectamount (Vector Laboratories). All images were obtained using Zeiss Axio Observer Z1 microscope.

Real-time PCR

Total RNA purified from cell using Trizol reagent (Invitrogen) following manufacturer's recommendation was DNase treated and reverse transcribed using QuantiTect® Reverse Transcription kit (QIAGEN). The RT products (cDNA) were amplified by real-time quantitative PCR (Applied Biosystems 7900 HT Fast Real-Time PCR system) with Power SYBR green Master Mix. Relative gene expression was determined by $2^{-\Delta\Delta C_t}$ method using 18S rRNA or GAPDH as internal control. Primers were used as described in the provisional patent application from which this application depends and as in Kerur et al., 2018, Nature, the publication resulting from the data in the provisional application. The primers were for human IFNB1, CASP4, DICER, cGAS, 18s rRNA, mitochondrial DNA, GAPDH, and GSDMD, MIP1 α , IL6, IL8, and mouse Ifnb1, Gapdh, 18s rRNA, and mitochondrial DNA.

Mitochondrial DNA Preparation

Total DNA extracted from ARPE19 cells was used to PCR-amplify mtDNA segments as described earlier²⁴. The purified mtDNA PCR products were subretinally delivered using 10% Neuroporter (Genlantis) as described above.

Western Blotting

Cell and tissue lysates prepared in RIPA buffer were homogenized by sonication. Protein concentration was determined using Pierce BCA Protein Assay Kit (Thermo Fisher Scientific Inc.). Equal quantity of protein (10–50 μ g) prepared in Laemmli buffer were resolved by SDS-PAGE on Novex® Tris-Glycine Gels (Invitrogen), and transferred onto Immobilon-FL PVDF membranes (Millipore). The transferred membranes were blocked in Odyssey® Blocking Buffer (PBS) or 5% nonfat dry skim milk for 1 hour at RT and then incubated with primary antibody at 4 °C overnight. The immunoreactive bands were visualized with help of species specific secondary antibodies conjugated with IRDye® or HRP. The blot image were either captured on Odyssey® imaging systems or on an autoradiography film. Rabbit polyclonal anti-human and mouse caspase-1 antibodies (1:500, Biovision

Cat# 3019-100; 1:1000, Invitrogen Cat#AHZ0082; 1:200 Santa Cruz Biotechnology Cat#sc-514), rabbit anti-caspase-1 mAb (1:1000, Abcam Cat# ab108362), anti-human caspase-4 (1:200, Santa Cruz Cat#1229), anti-mouse caspase-11 (1:200, Novus Rat mAb 17D9 Cat#NB120-10454, or 1: 1000 Abcam Rabbit mAb Cat# ab180673 1:500), anti-STAT2 (1:500, Cell Signaling, Cat #72604), anti-pSTAT2 (1:250, Millipore Cat# 07-224), anti-human cGAS (1:1000, Cell Signaling Cat# 15102), anti-VDAC-1 (1:1000, Cell Signaling Cat# #4661), anti-mouse IRF3 (1:500, Novus Biologicals, Cat#NBP1-78769), anti-phospho-IRF3 (1:500, Cell Signaling Cat# 4947S, Cat# 29047), anti-HA-tag (1:1000; Cell Signaling Cat# 2367), anti- α -tubulin mouse mAb (1:50000, Sigma-Aldrich), anti- β -actin mouse mAb (1:50000, Sigma-Aldrich), anti-vinculin (1:2000, Sigma-Aldrich), anti-cleaved caspase-3 (1:500, Cell Signaling Cat#9661), anti-PARP (1:1000, Cell Signaling Cat#9542), anti-human GSDMD gasdermin D (1:500, Abcam Cat#ab57785) and anti-mouse gasdermin D mAb (1 μ g/mL; a generous gift from V.M Dixit (Genentech))

Immunoblotting for activated Caspase 1 in the supernatant was performed as described earlier². Briefly, supernatants collected were briefly spun down to remove floating cells. Proteins from cell-free supernatant were precipitated by adding sodium deoxycholate (0.15% final) followed by adding TCA (7.2% final) and incubating on ice for 30 mins to overnight. Samples were spun down at 13000g for 30 mins and pellets were washed 2 times with ice-cold acetone. Precipitated proteins solubilized in 4X LDS Buffer with 2-mercaptoethanol was used for immunoblotting.

Cell Culture:

Primary mouse and human RPE cells were isolated as previously described^{25,26}. All cells were maintained at 37 °C and 5% CO₂ environment. Mouse RPE were cultured in Dulbecco Modified Eagle Medium (DMEM) supplemented with 20% FBS and standard penicillin/streptomycin antibiotics concentrations and primary human RPE cells were maintained in DMEM supplemented with 10% FBS and antibiotics. The human RPE cell line ARPE19 and those lacking mitochondrial DNA (Rho⁰ ARPE19) were cultured as described earlier²⁷. Rho⁰ ARPE19 cells were maintained at 37 °C in 24 mM Na₂HCO₃, 10% FBS, 50 μ g/ml uridine, 1 mM sodium pyruvate in DMEM-F12 (Gibco, Cat#11320-033) containing pen/strep, Fungizone, and gentamicin. ARPE19 cells were maintained in DMEM-

F12 containing pen/strep, Fungizone, and gentamicin. Bone marrow derived macrophages (BMDM) were cultured in DMEM with 10% fetal bovine serum and 20% L929 supernatants. *Mb21d1*^{-/-} and HA-cGAS reconstituted *Mb21d1*^{-/-} mouse embryonic fibroblasts were cultured in DMEM with 10% FBS and antibiotics²⁸.

5 **Synthesis of *in vitro* transcribed *Alu* RNA**

T7 promoter containing *Alu* expression plasmid was linearized and used for making *in vitro* transcribed *Alu* RNA using AmpliScribe T7-Flash Transcription Kit (Epicenter) following manufacturer's instructions. The resulting *Alu* RNA was DNase treated and purified using MEGAclear (Ambion), and integrity was
10 monitored by gel electrophoresis^{14,15}.

Transfection

Alu expression plasmid (pAlu), empty vector control (pNull) or *in vitro* transcribed *Alu* RNA were transfected in human and mouse RPE using Lipofectamine 2000 (Invitrogen) following the manufacturer's instructions.

15 **LPS transfection in BMDM**

Approximately 2×10⁶ BMDM cells were cultured overnight at 37 °C in a 60-mm dish. After 4–6 h of priming with 1 µg/ml Pam3CSK4 (Invivogen, Cat# tlr1-pms), cells were transfected with LPS (5 µg/ml final concentration, Invivogen, Cat# tlr1-3pelps, ultrapure) with EugeneHD (Promega, Cat# E2311) using standard
20 transfection protocol. 16 h post-transfection, cell lysates were collected and analyzed.

Extraction of mitochondria-free cytosolic fractions:

Human and mouse RPE cells either mock treated or stimulated with *Alu* RNA. 24 h post *Alu* RNA transfection or 48 h post scrambled or DICER1 AS
25 oligonucleotide transfection, cells were harvested by trypsinization. 2×10⁶ cells were used for collecting mitochondria free cytosolic fractions using Mitochondrial Isolation kit (Thermo Scientific Cat#89874). Briefly, cells resuspended in 800 µl of Reagent A and placed on ice for 2 min, the suspension was dounce homogenized (10 strokes) to lyse the cells release nuclei or incubated for 5 min on ice, vortexing every
30 minute after adding 10 µL Reagent B. To this suspension 800 µl Mitochondria Isolation Reagent C was added and the resulting suspension was centrifuged at 700 g for 10 min at 4 °C to pellet the nuclei. The supernatant containing cytoplasmic fraction was centrifuged at 700 g for 10 min at 4 °C total of five times to completely

remove nuclei and or any unlysed cells. The resulting nuclei-free cytoplasmic fraction was centrifuged at 13,000g for 15 min at 4 °C to pellet the mitochondria. The resulting supernatant was further centrifuged at 13,000g for 15 min at 4 °C a total of six times to remove all the mitochondria. The supernatant was next tested for absence of mitochondria by immunoblotting for the mitochondrial marker protein VDAC and cytosolic marker protein tubulin.

Reconstitution experiment

Mb21d1^{-/-} mouse RPE cells were transfected with 2 µg cGAS expression plasmid²⁹ or empty vector in a 60 mm dish at 70-80% confluency. 24 h post transfection, cells were plated on 6-well dishes. 24 h post plating, cells were transfected with *Alu* RNA (50 pmol) or mock transfected using Lipofectamine 2000. 18 h post *Alu* RNA transfection, cells were collected for RNA extraction to examine induction IFN-β mRNA. For Caspase-11 reconstitution. *Casp11*^{-/-} mouse RPE cells were transduced with control or caspase-11 expressing lentiviral particles. The transduced cells were allowed to rest for three days and the cells were then plated in 60 mm dish at 70-80% confluency. Control or Caspase-11 reconstituted *Casp11*^{-/-} cells were mock treated or stimulated with *Alu* RNA as described above and activation of caspase-1 was assessed by western blotting. For caspase-1 activity assay, *Casp11*^{-/-} mouse RPE cells transfected with caspase-11 expression plasmid (pCasp11) or empty vector (pNull) were exposed to *Alu* RNA as described above and caspase-1 activity was assessed using CaspaLux[®]1-E₁D₂ kit (OncoImmunin Cat # CPL1R1E-5). Quantification of the CaspaLux signal was performed by a blinded operator measuring the integrated density of fluorescent micrographs using Image J software (NIH) and normalizing to the number of cells.

Lentiviral transduction

Lentivirus articles were either produced by the University of Kentucky Viral Production Core facilities or in house. Lentivirus vector plasmids expressing scrambled sequences or shRNA sequences targeting human caspase-4 and cGAS were purchased (MISSION[®]shRNA, Sigma-Aldrich) to produce lentiviral particles. Human RPE cells at passage 3 were incubated with lentiviral particles at multiplicity of infection (MOI) of 5 overnight in regular growth media containing polybrene (4 µg/ml). On day 2 cells were washed and incubated in regular growth media allowed to rest for 24 h. Lentivirus transduced cells were then cultured under puromycin (5

µg/ml) selection pressure for 5 days. Knock-down of the target proteins was determined by immunoblotting.

ShRNA Sequences

Some of the shRNAs used in the present application are provided below.

5 All were obtained from Sigma-Aldrich.

shcGAS- Catalog no. TRCN0000150010 (see genes/MB21D1)- SEQ ID NO:15-

CCGGCCTGCTGTAACACTTCTTATTCTCGAGAATAAGAAGTGTTACAG
CAGGTTTTTTTG

10 shCasp4- Catalog no. TRCN0000003511 (see /genes/CASP4)- SEQ ID NO:16-

CCGGAGACTATGTAAAGAAAGAGCTCTCGAGAGCTCTTTCTTTACATA
GTCTTTTTT

shSTING- Catalog no. TRCN0000164628 (see /genes/TMEM173)- SEQ ID NO:17-

CCGGCCAACATTCGCTTCCTGGATACTCGAGTATCCAGGAAGCGAATG
TTGGTTTTTTTG

shPPIF- Catalog no. TRCN0000232684 (see /genes/PPIF)- SEQ ID NO:18-

20 CCGGCTGTGGCCAGTTGAGCTAATCCTCGAGGATTAGCTCAACTGGCC
ACAGTTTTTTG

shGSDMD- Catalog no. TRCN0000178784 (see /genes/GSDMD)- SEQ ID NO:19-

25 CCGGCAACCTGTCTATCAAGGACATCTCGAGATGTCCTTGATAGACAG
GTTGTTTTTTTG

shIFNB- Catalog no. TRCN0000005803 (see /genes/IFNB1)- SEQ ID NO:20-

CCGGCAGAGTGGAATCCTAAGGAACTCGAGTTCCTTAGGATTTCAC
TCTGTTTTT

30 shIFNAR1- Catalog no. TRCN0000059013 (see /genes/IFNAR1)- SEQ ID NO:21-

CCGGGCCAAGATTCAGGAAATTATTCTCGAGAATAATTCCTGAATCTTG
GCTTTTTTG

Enzyme-linked immunosorbent assay (ELISA)

Secreted human and mouse interferon- β and IL-18 in the media were detected using the ELISA kits (mouse IFN- β , R&D Systems Cat# 42400-1; mouse IL-18, R&D Systems Cat# 7625; human IFN- β , R&D Systems Cat# 41410; human IL-18, R&D systems Cat# DY318-05) according to the manufacturer's instructions.

- 5 Primary mouse cells cultured as above. WT, *Gsdmd*^{-/-}, *Casp11*^{-/-}, and *Mb21d1*^{-/-} mouse RPE cells were seeded at a density of 250,000 cells/well in a 12-well plate. When confluency reached 60-70%, cells were transfected with 20 pmol of *in vitro* transcribed *Alu* RNA or mock using Lipofectamine 2000 reagent (Life Technologies, Carlsbad, CA) following the manufacturer protocol. Media was collected to detect secreted cytokine content at 8 to 24 h post-transfection. For examining the induction of IL-18 secretion by monosodium urate (MSU) crystals (Invivogen Cat# tlr1-msu), mouse RPE cells were primed with LPS (500 ng/ml) for 6 h and exposed to MSU (250 μ g/ml) for 16 h, and media were collected to detect secreted cytokine.

15 **cGAS-mtDNA interaction Immunoprecipitation assay**

- Immortalized *cGAS*^{-/-} mouse embryonic fibroblasts (MEF) reconstituted with HA-tagged mouse cGAS (HA-cGAS) were described earlier²⁸. Interaction between mtDNA and cGAS was monitored using Express Chromatin Immunoprecipitation Kit (Active Motif, ChIP-IT® Express, cat# 53008). Briefly mock, *Alu* RNA, poly I: C or plasmid DNA (pUC19) transfected HA-cGAS reconstituted *cGAS*^{-/-} MEFs were fixed with 1% formaldehyde per manufacturer's instructions. The cells were then lysed by sonication in the shearing buffer, centrifuged for 10 min at 18,000g in a 4 °C microfuge. The supernatant containing the cell lysate was collected and cGAS was immunoprecipitated from each sample using anti-HA tag antibody (Abcam, cat# ab9110). DNA in the IP was eluted, reverse crosslinked and purified using Chromatin IP DNA Purification Kit (Active Motif, cat# 58002). Purified DNA was analyzed by qPCR using mouse mtDNA specific primer pairs. Fold enrichment of mtDNA in HA-cGAS IP, in cells exposed to *Alu* RNA was calculated compared to mock transfected cells.

30 **Quantification of PAPC and oxPAPC by LC-MS**

Human RPE cells, mock or stimulated with *Alu* RNA, were washed with cold PBS and trypsinized at 24 h post-stimulation. The cells were washed with cold PBS and 2 $\times$ 10⁶ cells were used for lipid extraction by a modified Bligh-Dyer

extraction method³⁰. Briefly, the cell pellet was manually homogenized and then mixed in a glass tube with 700 μ L HPLC-grade chloroform and 300 μ L HPLC-grade Methanol (Sigma) supplemented with 0.01% butylated hydroxytoluene (BHT from Sigma) and 189 nmol of the internal standard, di-nonanoyl-phosphatidylcholine (DNPC from Avanti). 1 mL of HPLC-grade water was added and the mixture was vigorously vortexed for 60 sec. Next, the mixture was centrifuged (1,000 rpm for 10 min) to separate the fractions and the organic layer (bottom) was removed and placed into a fresh glass tube. 1 mL of chloroform was added to the aqueous fraction and the extraction was performed once more. The organic layer of the second extraction was combined with the first, and then dried down under nitrogen. Upon complete evaporation of the organic solvent, the lipids were suspended in 300 μ L of Solvent A (69% water; 31% methanol; 10 mM ammonium acetate) and stored at -80°C . The determination and quantification of oxidized phosphatidylcholine and phosphatidylethanolamine species was performed by liquid chromatography-linked ESI mass spectrometry, using an ABI Sciex 4000 QTrap. Separation of the phospholipids was achieved by loading samples onto a C8 column (Kinetex 5 μ m, 150 $\times$ 4.6 mm from Phenomenex). Elution of the phospholipids was achieved using a binary gradient with Solvent A (69% water; 31% methanol; 10 mM ammonium acetate) and Solvent B (50% methanol; 50% isopropanol; 10 mM ammonium acetate) as the mobile phases. Detection for phosphatidylcholine (PC) was conducted using multiple reaction monitoring (MRM) in positive mode by identification of two transition states for each analyte. Quantification of each analyte was performed based on the peak area of the 184 m/z fragment ion for PC.

Determination of mitochondrial permeability transition pore opening.

Mitochondrial permeability transition pore (mPTP) opening in WT and *Ppif*^{-/-} mouse RPE cells was monitored by the calcein- Co^{2+} technique³¹ using the Mitochondrial Permeability Transition Pore Assay Kit (Biovision Inc Cat# K239-100). Mitochondrial membrane potential was evaluated with the JC-1 fluorochrome-based MITO-ID® Membrane Potential Cytotoxicity Kit (Enzo Cat# ENZ-51019-KP002). mPTP opening was inhibited by performing the above assays using cyclosporine A (10 μ M)-containing media. The assay was performed in a 96-well microtiter plate according to the manufacturer's instruction.

Live Cell Imaging

2×10⁴ human RPE cells were plated on each well of an 8-well chambered slide (Thermo Scientific Cat#155411). 18–24 h after plating, cells were transfected with 11.5 pmol *in vitro* transcribed *Alu* RNA (using Lipofectamine 2000) in a media supplemented with 2.5 mM CaCl₂ and annexin-V 488 (1:200, Invitrogen V13241) and propidium iodide (1:1500, Invitrogen Cat#P3566). Immediately following transfection, annexin V, propidium iodide, and DIC signals were acquired using a Nikon A1R confocal microscope equipped with an automated stage. Images were captured at 3 min intervals for a total duration of 50 h. Cells were maintained in at 37 °C and 5% CO₂ for the duration of the imaging study via a stage top incubator.

10 **RPE flat mount annexin V/PI-staining**

Mouse RPE/choroid flat mounts prepared in DMEM with 10% FBS were washed with binding buffer once and then incubated with Alexa Fluor™ 647 conjugated Annexin V (Invitrogen) for 15 min. The annexin V stained mouse RPE/choroid flat mounts were fixed with 2% paraformaldehyde for 30 min, stained with propidium iodide (PI) containing RNase (Invitrogen) for 30 min and mounted using ProLong™ Gold Antifade Mountant solution (Thermo Fisher Scientific).

Microglia depletion

Microglia were depleted via administering tamoxifen to *CX3CR1^{CreER}*; *DTA^{flox}* mice which express Cre-ER under control of microglia specific *CX3CR1* promoter and also contain flox-STOP-flox diphtheria toxin subunit α (*DTA*) gene cassette in the *ROSA26* locus (*DTA^{flox}*). *Cx3cr1^{CreER}*; *DTA^{flox}* mice were generated by breeding heterozygous *Cx3cr1^{CreER}* mice with *DTA^{flox}* mice (both mice generous gifts from Wai T. Wong and Lian Zhao, NIH). To deplete microglia, tamoxifen was administered to *Cx3cr1^{CreER}*; *DTA^{flox}* mice as described earlier³². Briefly, adult 2- to 3-month-old TG mice were administered with tamoxifen (TAM) dissolved in corn oil (Sigma-Aldrich; 500 mg/kg dose of a 20 mg/ml solution) via oral gavage (Schedule: days –2, 0, 5, 10, and 15). On day 11, *Alu* RNA was delivered via subretinal injection. *Alu* RNA-induced RPE degeneration was assessed as described above. Microglial depletion was confirmed by staining retinal flat mounts for F4/80. Briefly retinal flat mounts were prepared and fixed in 2% paraformaldehyde for 1 h, and stained with RPE conjugated F4/80 (Bio-Rad, Cat# MCA497PET) and fluorescein labeled Griffonia Simplicifolia Lectin isolectin B4 (IB4, Vector

Laboratories, Cat# FL-1201). All images were obtained using Zeiss Axio Observer Z1 microscope.

Macrophage depletion

Depletion of macrophages was achieved via administering clodronate liposomes, which eliminates macrophages, in wild-type mice³³. Briefly, animals received 200 μ l clodronate liposomes (Liposoma Cat# LIP-01) through the tail vein on days -2 and day 0. *Alu* RNA or vehicle control were subretinally injected immediately after the day 0 tail vein injection.

Statistical Analyses

Real-time qPCR and ELISA data are expressed as means $\pm$ standard error of the mean (SEM) were analyzed using Student t test. The binary readouts of RPE degeneration (i.e., presence or absence of RPE degeneration on fundus and ZO-1-stained flat mount images) were analyzed using Fisher's exact test. Cell morphometry data were assessed using Student t test. *P* values < 0.05 were deemed statistically significant. Sample sizes were selected based on power analysis $\alpha=5\%$; $1-\beta = 80\%$, such that we could detect a minimum of 50% change assuming a sample SD based on Bayesian inference. Outliers were assessed by Grubbs' test. Based on this analysis no outliers were detected and no data were excluded. Fewer than 5% of subretinal injection recipient tissues were excluded based on prescribed exclusion criteria relating to the technical challenges of this delicate procedure.

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- 10

Summary of some of the useful sequences of the invention

- 15 SEQ ID NO:1- human cGAS shRNA (TRCN0000146282)- 5'-3'

CCGGCTTTGATAACTGCGTGACATACTCGAGTATGTCACGCAGTTATCAA
AGTTTTTTTG.

- 20 SEQ ID NO:2- human cGAS siRNA (SASI_Hs01; see for example Sigma catalog number for EHU015231-20UG and the target sequence provided).

AAGAAGAAACATGGCGGCTATCCTTCTCTCACATCGAAAAGGAAATTT.

- 25 SEQ ID NO:3- Human cGAS (cyclic GMP-AMP synthase) Protein- "cyclic GMP-AMP synthase" [Homo sapiens], GenBank: AGB51853.1, 522 a.a.

MQPWHGKAMQRASEAGATAPKASARNARGAPMDPTESPAAPEAALPKAG
KFGPARKSGSRQKK SAPDTQERPPVRATGARAKKAPQRAQDTQPSDATSAP
30 GAEGLEPPAAREPALS RAGSCRQRGARCSTKPRPPPGPVDVPSPGLPVSAPIL
VRRDAAPGASKLRAVLEKLKLSRDDISTAAGMVKG VVDHLLLRLKCDSAF
RGVGLLNTGSYYEHVKISAPNEFDVMFKLEV PRIQLEEYSNTRAYYFVKFKR
NPKENPLSQFLEGEILSASKMLSKFRKIIKEEINDIKD TDVIMKRKRGGSPAVT

LLISEKISVDITLALESKSSWPASTQEGLRIQNWLSAKVRKQLRLKPFYLVPK
 HAKEGNFQEETWRLSFSHIEKEILNNHGKSKTCCENKEEKCCRKDCLKLM
 KYLLEQLKERFKDKKHLDDKFSSYHVKTAFHFVCTQNPQDSQWDRKDLGLC
 FDNCVTYFLQCLRTEKLENYFIPEFNLFSSNLIDKRSKEFLTKQIEYERNNEFP
 5 VFDEF

SEQ ID NO:4- Human cGAS (cyclic GMP-AMP synthase) mRNA- NCBI

Reference Sequence: NM_138441.2, 1802 bp

10 AGCCTGGGGTTCCCCCTTCGGGTCGCAGACTCTTGTGTGCCCCGCCAGTAGT
 GCTTGGTTTCCAACAGCTGCTGCTGGCTCTTCCTCTTGCGGCCTTTTCCTG
 AAACGGATTCTTCTTTTCGGGGAACAGAAAGCGCCAGCCATGCAGCCTTG
 GCACGGAAAGGCCATGCAGAGAGCTTCCGAGGCCGGAGCCACTGCCCCC
 AAGGCTTCCGCACGGAATGCCAGGGGCGCCCCGATGGATCCCACCGAGT
 15 CTCCGGCTGCCCCCGAGGCCGCCCTGCCTAAGGCGGGAAAGTTCGGCCC
 CGCCAGGAAGTCGGGATCCCGGCAGAAAAAGAGCGCCCCGGACACCCA
 GGAGAGGCCGCCCCGTCCGCGCAACTGGGGCCCGCGCCAAAAAGGCCCT
 CAGCGCGCCCAGGACACGCAGCCGTCTGACGCCACCAGCGCCCCCTGGGG
 CAGAGGGGCTGGAGCCTCCTGCGGCTCGGGAGCCGGCTCTTTCCAGGGC
 20 TGGTTCTTGCCGCCAGAGGGGCGCGCGCTGCTCCACGAAGCCAAGACCT
 CCGCCCCGGGCCCTGGGACGTGCCAGCCCCGGCCTGCCGGTCTCGGCCC
 CCATTCTCGTACGGAGGGATGCGGCGCCTGGGGCCTCGAAGCTCCGGGC
 GGTTTTGGAGAAGTTGAAGCTCAGCCGCGATGATATCTCCACGGCGGCG
 GGGATGGTGAAAGGGGTTGTGGACCACCTGCTGCTCAGACTGAAGTGCG
 25 ACTCCGCGTTCAGAGGCGTCGGGCTGCTGAACACCGGGAGCTACTATGA
 GCACGTGAAGATTTCTGCACCTAATGAATTTGATGTCATGTTTAAACTGG
 AAGTCCCCAGAATTCAACTAGAAGAATATTCCAACACTCGTGCAATTA
 CTTTGTGAAATTTAAAAGAAATCCGAAAGAAAATCCTCTGAGTCAGTTTT
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 GTTAGGAAGCAACTACGACTAAAGCCATTTTACCTTGTACCCAAGCATG

CAAAGGAAGGAAATGGTTTCCAAGAAGAAACATGGCGGCTATCCTTCTC
 TCACATCGAAAAGGAAATTTTGAACAATCATGGAAAATCTAAAACGTGC
 TGTGAAAACAAAGAAGAGAAATGTTGCAGGAAAGATTGTTTAAACTAA
 TGAAATACCTTTTAGAACAGCTGAAAGAAAGGTTTAAAGACAAAAACA
 5 TCTGGATAAATTCTCTTCTTATCATGTGAAAACCTGCCTTCTTTCACGTATG
 TACCCAGAACCCTCAAGACAGTCAGTGGGACCGCAAAGACCTGGGCCTC
 TGCTTTGATAACTGCGTGACATACTTTCTTCAGTGCCTCAGGACAGAAAA
 ACTTGAGAATTATTTTATTCCTGAATTCAATCTATTCTCTAGCAACTTAAT
 TGACAAAAGAAGTAAGGAATTTCTGACAAAGCAAATTGAATATGAAAG
 10 AAACAATGAGTTTCCAGTTTTTGATGAATTTTGAGATTGTATTTTAGAA
 AGATCTAAGAACTAGAGTCACCCTAAATCCTGGAGAATACAAGAAAAAT
 TTGAAAAGGGGCCAGACGCTGTGGCTCAC

SEQ ID NO:5- Human Caspase-4- Protein- Full length caspase-4- 377 a.a.-
 15 caspase-4 isoform alpha precursor [Homo sapiens], NCBI Reference Sequence:
 NP_001216.1 (for a fragment see caspase-4 isoform X1 [Homo sapiens], NCBI
 Reference Sequence: XP_016873886.1, 286 a.a.)

MAEGNHRKKPLKVLESLGKDFLTGVLDNLVEQNVLNWKEEEKKKYYDAK
 20 TEDKVRVMADSMQEKQRMAGQMLLQTFNIDQISPNKKAHPNMEAGPPES
 GESTDALKLCPHEEFLRLCKERAEEIYPIKERNNRTRLALIICNTEFDHLPPRN
 GADFDITGMKELLEGLDYSVDVEENLTARDMESALRAFATRPEHKSSDSTF
 LVLMSHGILEGICGTVHDEKKPDVLLYDTIFQIFNNRNCLSLKDKPKVIIVQA
 CRGANRGELWVRDSPASLEVASSQSENLEEDAVYKTHVEKDFIAFCSSTPH
 25 NVSWRDSTMGSIFITQLITCFQKYSWCCHLEEVFRKVQQSFETPRAKAQMPT
 IERLSMTRYFYLFPGN

SEQ ID NO:6- Human Caspase-4 mRNA/nucleotide-1319 bp, Homo sapiens
 caspase 4 (CASP4), transcript variant alpha, mRNA, NCBI Reference Sequence:
 30 NM_001225.3

ATACATAGTTTACTTTCATTTTGGACTCTGAGGCTCTTCCAACGCTGTAA
 AAAAGGACAGAGGCTGTTCCCTATGGCAGAAGGCAACCACAGAAAAAA
 GCCACTTAAGGTGTTGGAATCCCTGGGCAAAGATTTCCTCACTGGTGTTT

TGGATAACTTGGTGGAAACAAAATGTACTGAACTGGAAGGAAGAGGAAA
 AAAAGAAATATTACGATGCTAAACTGAAGACAAAGTTCGGGTCATGGC
 AGACTCTATGCAAGAGAAGCAACGTATGGCAGGACAAATGCTTCTTCAA
 ACCTTTTTTAACATAGACCAAATATCCCCCAATAAAAAAGCTCATCCGAA
 5 TATGGAGGCTGGACCACCTGAGTCAGGAGAATCTACAGATGCCCTCAAG
 CTTTGTCTCATGAAGAATTCCTGAGACTATGTAAAGAAAGAGCTGAAG
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 TTTGACATCACAGGGATGAAGGAGCTACTTGAGGGTCTGGACTATAGTG
 10 TAGATGTAGAAGAGAATCTGACAGCCAGGGATATGGAGTCAGCGCTGAG
 GGCATTTGCTACCAGACCAGAGCACAAAGTCCTCTGACAGCACATTCTTG
 GTACTCATGTCTCATGGCATCCTGGAGGGAATCTGCGGAACTGTGCATG
 ATGAGAAAAAACCAGATGTGCTGCTTTATGACACCATCTTCCAGATATTC
 AACAACCGCAACTGCCTCAGTCTGAAGGACAAACCCAAGGTCATCATTG
 15 TCCAGGCCTGCAGAGGTGCAAACCGTGGGGAAGTGTGGGTCAGAGACTC
 TCCAGCATCCTTGGAAGTGGCCTCTTCACAGTCATCTGAGAACCTAGAGG
 AAGATGCTGTTTACAAGACCCACGTGGAGAAGGACTTCATTGCTTTCTGC
 TCTTCAACGCCACACAACGTGTCCTGGAGAGACAGCACAATGGGCTCTA
 TCTTCATCACACAACCTCATCACATGCTTCCAGAAATATTCTTGGTGCTGC
 20 CACCTAGAGGAAGTATTTTCGGAAGGTACAGCAATCATTGAACTCCAA
 GGGCCAAAGCTCAAATGCCCACCATAGAACGACTGTCCATGACAAGATA
 TTTCTACCTCTTTCCTGGCAATTGAAAATGGAAGCCACAAGCAGCCCAGC
 CCTCCTTAATCAACTTCAAGGAGCACCTTCATTAGTACAGCTTGCATATT
 TAACATTTTGTATTTCAATAAAAAGTGAAGACAAACGA

25

SEQ ID NO:7- Human Gasdermin D (Also known as: DF5L, DFNA5L, FKSG10,
 GSDMDC1) Protein- 484 a.a., Gasdermin D [Homo sapiens], GenBank:
 AAH08904.1 (also NP_001159709)

30 MGSAFERVVRVQELDHGGEFIPVTSLSSTGFQPYCLVVRKPSSSWFWK
 PRYKCVNLSIKDILEPDAAEPDVQRGRSFHFYDAMDGQIQGSVELAAPGQA
 KIAGGAAVSDSSSTSMNVYSLSDPNTWQTLHERHLRQPEHKVLQQLRSR
 GDNVYVVTEVLQTQKEVEVTRTHKREGSGRFSLPGATCLQGEGQHLSQK
 KTVTIPSGSTLAFRVAQLVIDSDLVLLFPDKKQRTFQPPATGHRSTSEGA

WPQLPSGLSMMRCLHNFLTDGVPAEGAFTEDFQGLRAEVETISKELELLDRE
 LCQLLEGLEGLVLRDQLALRALEEALQGGQSLGPVEPLDGPAGAVLECLVL
 SSGMLVPELAIPVVYLLGALTMLSETQHKLAEALQSTLLGPLELVGSLLE
 QSAPWQERSTMSLPPGLLGNSWGEGAPAWVLLDECGLELGEDTPHVCWEP
 5 QAQGRMCALYASLALLSGLSQEPH

SEQ ID NO:8- Human Gasdermin D (Also known as: DF5L, DFNA5L, FKSG10, GSDMDC1) mRNA/nucleotide- GenBank Accession numbers include XM_011517301.2, NM_001166237.1, NM_024736.6, BC069000.1, and
 10 BC008904.2.; Homo sapiens gasdermin D, mRNA (cDNA clone MGC:15043 IMAGE:3634992), complete cds, GenBank: BC008904.2, 1823 bp

CCTGGGCGGGCCCTGCGTCAGGTTGCAGTTTCACTTTTAGCTCTGGGCA
 CCTCCAGCTCCTGCTCGCCGGACGGCTCCCAGGGAGAGCAGACGCGCC
 15 AGACGCGCCACCCTCGGGGCGCCGACGGTCACGGAGCATGGGGTCGG
 CCTTTGAGCGGGTAGTCCGGAGAGTGGTCCAGGAGCTGGACCATGGTG
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 CCTACTGCCTGGTGGTTAGGAAGCCCTCAAGCTCATGGTTCTGGAAAC
 CCCGTTATAAGTGTGTCAACCTGTCTATCAAGGACATCCTGGAGCCGG
 20 ATGCCGCGGAACCAGACGTGCAGCGTGGCAGGAGCTTCCACTTCTACG
 ATGCCATGGATGGGCAGATACAGGGCAGCGTGGAGCTGGCAGCCCCA
 GGACAGGCAAAGATCGCAGGCGGGGCGCGGTGTCTGACAGCTCCAG
 CACCTCAATGAATGTGTACTCGCTGAGTGTGGACCCTAACACCTGGCA
 GACTCTGCTCCATGAGAGGCACCTGCGGCAGCCAGAACACAAAGTCCT
 25 GCAGCAGCTGCGCAGCCGCGGGGACAAACGTGTACGTGGTGAAGG
 TGCTACAGACACAGAAGGAGGTGGAAGTCACGCGCACCCACAAGCGG
 GAGGGCTCGGGCCGGTTTTCCCTGCCCGGAGCCACGTGCTTGAGGGT
 GAGGGCCAGGGCCATCTGAGCCAGAAGAAGACGGTCACCATCCCCTC
 AGGCAGCACCCCTCGCATTCCGGGTGGCCCAGCTGGTTATTGACTCTGA
 30 CTTGGACGTCCTTCTCTTCCCGGATAAGAAGCAGAGGACCTTCCAGCC
 ACCCGCGACAGGCCACAAGCGTTCCACGAGCGAAGGCGCCTGGCCAC
 AGCTGCCCTCTGGCCTCTCCATGATGAGGTGCCTCCACAACCTCCTGAC
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GAGAGCTGTGCCAGCTGCTGCTGGAGGGCCTGGAGGGGGTGCTGCGG
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 5 CCCTGTTGTCTACCTGCTGGGGGCACTGACCATGCTGAGTGAAACGCA
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 GCGAAGGAGCACCGGCCTGGGTCTTGCTGGACGAGTGTGGCCTAGAG
 10 CTGGGGGAGGACACTCCCCACGTGTGCTGGGAGCCGCAGGCCCAGGG
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 15 GAAACACAATAAAGGTGGCATAACGAAGGAAAAAAAAAAAAAAAAAAAA
 AAACCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
 AAAAAAAAAAAAAAAAAAAAA

SEQ ID NO:9- siRNA directed against caspase-4, S1

20 5'-GUGUAGAUGUAGAAGAGAATT-3'

SEQ ID NO:10- siRNA directed against caspase-4, S2

5'-CCUAGAGGAAGAUGCUGUUTT-3'

25 SEQ ID NO:11- siRNA directed against caspase-4

5'-CUACACUGUGGUUGACGAA-3'

SEQ ID NO:12- siRNA directed against caspase-4

5'-CCAUAGAACGAGCAACCUU-3'

30

SEQ ID NO:13- siRNA directed against caspase-4

5'-CAGCAGAAUCUACAAAUAU-3'

SEQ ID NO:14- siRNA directed against caspase-4

5'-CGGAUGUGCUGCUUUAUGA-3'

SEQ ID NO:15- shRNA against cGAS

CCGGCCTGCTGTAACACTTCTTATTCTCGAGAATAAGAAGTGTTACAG
5 CAGGTTTTTTTG

SEQ ID NO:16- shRNA against Caspase-4

CCGGAGACTATGTAAAGAAAGAGCTCTCGAGAGCTCTTTCTTTACATA
GTCTTTTTT
10

SEQ ID NO:17- shRNA against STING

CCGGCCAACATTCGCTTCCTGGATACTCGAGTATCCAGGAAGCGAATG
TTGGTTTTTTTG

15 SEQ ID NO:18- shRNA against PPIF

CCGGCTGTGGCCAGTTGAGCTAATCCTCGAGGATTAGCTCAACTGGCC
ACAGTTTTTTG

SEQ ID NO:19- shRNA against GSDMD

20 CCGGCAACCTGTCTATCAAGGACATCTCGAGATGTCCTTGATAGACAG
GTTGTTTTTTTG

SEQ ID NO:20- shRNA against IFNB

CCGGCAGAGTGGAATCCTAAGGAACTCGAGTTCCTTAGGATTTCAC
25 TCTGTTTTT

SEQ ID NO:21- shRNA against IFNAR1

CCGGGCCAAGATTCAGGAAATTATTCTCGAGAATAATTCCTGAATCTTG
GCTTTTTTG
30

Examples

Results

Caspase-4 is activated in AMD

Caspase-4 (a human protein- also known as caspase-11 in mouse), which governs non-canonical inflammasome activation, was recently implicated in the immune response to exogenous pathogen-associated molecular patterns (PAMPs) such as intracellular LPS¹¹⁻¹⁷ and endogenously produced oxidized phospholipids (*oxPAPC*)¹¹⁻¹⁸. Caspase-4 abundance in the RPE and choroid of human geographic atrophy eyes is significantly increased compared to aged normal human eyes, as monitored by western blotting (Fig. 1a and Supplementary Fig. 1b). Introduction of *in vitro* transcribed *Alu* RNA or plasmid-mediated enforced expression of *Alu* RNA (pAlu) induced and activated caspase-4 in primary human RPE cells (Fig. 1b and Supplementary Fig. 1a, c). Anti-sense oligonucleotide-mediated knockdown of DICER1 similarly induced caspase-4 activation in human RPE cells (Fig. 1b), which was blocked by concomitant anti-sense mediated inhibition of *Alu* RNA (Supplementary Fig. 1d). Caspase-11 activation was induced by subretinal injection of *Alu* RNA in wild-type (WT) C57BL/6J mice (Fig. 1c), and by *Alu* RNA transfection in primary RPE cells isolated from WT mice (Supplementary Fig. 1e). Collectively, these data identify caspase-4 as being preferentially activated in human AMD, and dysregulation of DICER1 and *Alu* RNA as novel endogenous agonists of caspase-4.

Caspase-4 is required for *Alu* RNA-induced RPE degeneration and inflammasome activation

RPE degeneration was quantified based on zonula occludens (ZO)-1-stained flat mount images using two strategies (Supplementary Text):

1. Binary assignment (healthy versus unhealthy)¹⁹⁻²¹ by two masked raters (inter-rater agreement = 98.6%; Pearson $r^2 = 0.95$, $P < 0.0001$; Fleiss $\kappa = 0.97$, $P < 0.0001$).
2. Semi-automated cellular morphometry analysis by three masked raters adapting our prior analysis of the planar architecture of the corneal endothelium²², which resembles the RPE in its polygonal tessellation. Inter-rater agreement was high for all three metrics (multiple adjusted $r^2 = 0.99$ (cell size), 0.72 (polymegethism, i.e., coefficient of variation of cell size), 0.99 (cell density)).

For eyes treated with *Alu* RNA, pAlu, and their respective controls, inter-rater agreement on binary assignment was 100%, and the fraction of eyes classified as healthy was 100% for both control groups versus 0% for *Alu* RNA or pAlu

treatments ($P < 0.0001$ for both comparisons, Fisher exact test). All three morphometric features were significantly different between control treatments versus *Alu* RNA or pAlu treatments ($P < 0.0001$, t test; Supplementary Fig. 3). Given the similarity among all three features in differentiating healthy versus

5 degenerated RPE cells, for all remaining groups, we quantified polymegathism, a prominent geometric feature of RPE cells in human geographic atrophy^{3,23-25}.

Exogenous delivery or endogenous over-expression of *Alu* RNA induces RPE degeneration in WT mice (Fig. 1d and Supplementary Fig. 1f). In contrast, neither subretinal injection of *Alu* RNA nor pAlu induced RPE degeneration in

10 *Casp11*^{-/-} mice (Fig. 1d and Supplementary Fig. 1f). 129S6 mice, which lack functional caspase-11 due to a passenger mutation¹⁴, also were resistant to RPE degeneration induced by *Alu* RNA or pAlu (Supplementary Fig. 1g). Subretinal delivery of a cell-permeable, non-immunogenic 17+2-nt cholesterol-conjugated siRNA^{26,27} targeting *Dicer1* induced RPE degeneration in WT but not 129S6 mice

15 (Supplementary Fig. 1h). Transgenic expression of human caspase-4 on the caspase-11 deficient background (*Casp11*^{-/-} *hCASP4*^{TG})²⁸ restored the susceptibility of these mice to *Alu* RNA-induced RPE degeneration (Fig. 1e), demonstrating that human caspase-4 can compensate for mouse caspase-11 in this system. Collectively, these data demonstrate the critical role of caspase-4 (or mouse caspase-

20 11) in responding to pathological accumulation of endogenous *Alu* mobile element transcripts.

Previously we reported that *Alu* RNA does not induce RPE degeneration in caspase-1 deficient mice². However, this *Casp1*^{-/-} strain was subsequently reported to also lack functional caspase-11 as a result of a passenger mutation in their 129S6

25 background¹⁴; thus, they are properly referred to as *Casp1*^{-/-} *Casp11*^{129mt/129mt} mice. We sought to clarify the molecular hierarchy of caspases-1 and 11 in response to *Alu* RNA. Whereas *Alu* RNA treatment induced caspase-1 activation in WT systems, *Alu* RNA failed to stimulate caspase-1 activation in *Casp11*^{-/-} mice (Fig. 1f), or in primary RPE cells isolated from *Casp11*^{-/-} mice (Fig. 1g). Reconstitution of

30 caspase-11 into *Casp11*^{-/-} mouse RPE cells restored caspase-1 activation by *Alu* RNA (Supplementary Fig. 2a, b). *Alu* RNA failed to induce IL-18 secretion in *Casp11*^{-/-} mouse RPE cells (Fig. 1h). In contrast, caspase-11 was dispensable for IL-18 secretion induced by the canonical inflammasome agonist monosodium urate (MSU) crystals (Supplementary Fig. 10d).

Alu RNA did not induce RPE degeneration in *Casp1*^{-/-} *Casp11*^{129mt/129mt} *Casp11*^{Tg} mice¹⁴, in which mouse caspase-11 was functionally reconstituted by a bacterial artificial chromosome transgene (Fig. 1i and Supplementary Fig. 2c. Additionally, as previously observed², *Casp1*^{-/-} *Casp11*^{129mt/129mt} mice were not
 5 susceptible to *Alu*-induced RPE degeneration (Fig. 1i and Supplementary Fig. 2c, suggesting that both caspase-4/11 and caspase-1 are required for *Alu* toxicity.

Previously we demonstrated that PYCARD, an adaptor protein involved in inflammasome activation, and the purinoceptor P2X7 (encoded by *P2rx7*) were required for *Alu* toxicity^{2,19,20}. We assessed whether PYCARD and P2X7 were also
 10 required for *Alu* RNA-induced caspase-11 activation. *Alu* RNA-induced activation of caspase-11 was reduced in *P2rx7*^{-/-} but not *Pycard*^{-/-} mouse RPE cells, suggesting that caspase-11 is mechanistically positioned between these signaling molecules (Supplementary Fig. 2d, e). Collectively these findings support a model of non-canonical inflammasome activation by *Alu* RNA wherein caspase-4/11 lies
 15 upstream of caspase-1 activation²⁹.

Recent studies implicated the endogenous lipid molecule, oxidized phospholipid oxPAPC (oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine) in caspase-11-mediated non-canonical NLRP3 inflammasome activation¹⁸. To test whether *Alu* RNA promotes accumulation of these endogenous
 20 ligands, we extracted lipids from *Alu* RNA-treated human RPE cells and used liquid chromatography-mass spectrometry to quantify the following products of 1-palmitoyl-2-arachidonoyl-3-phosphatidylcholine (PAPC): 1-palmitoyl-2-glutaryl-3-phosphatidylcholine (PGPC), 1-palmitoyl-2-(5-oxovaleryl)-3-phosphatidylcholine (POVPC), and 1-palmitoyl-2-hydroxy-3-phosphatidylcholine (LysoPC)
 25 (Supplementary Fig. 4a-c). Compared to control cells, *Alu* RNA-treated human RPE cells exhibited a two-fold increase in oxPAPC- PGPC and LysoPC levels (Supplementary Fig. 4d), concomitant with a trend towards a reduction in precursor PAPC (Supplementary Fig. 4d). These results suggest an indirect mechanism of *Alu*-driven caspase-11 engagement possibly via oxidized phospholipid-derived
 30 damage-associated molecular patterns (DAMPs).

Gasdermin D is required for *Alu* RNA-induced RPE degeneration and inflammasome activation

Caspase-11-and caspase-1-dependent pyroptotic cell death can be executed by a pore-forming protein, gasdermin D (encoded by *Gsdmd*)³⁰⁻³³. *Gsdmd*^{-/-} mice

were resistant to *Alu* RNA-induced RPE degeneration (Fig. 2a and Supplementary Fig. 5a, b). Consistent with the role of gasdermin D in non-canonical inflammasome activation by intracellular LPS³¹, *Alu* RNA-induced caspase-1 activation and IL-18 secretion were reduced in *Gsdmd*^{-/-} mouse RPE cells (Fig. 2b, c). However, caspase-11 activation in *Gsdmd*^{-/-} mice was not impaired (Fig. 2d), suggesting that loss of caspase-1 activation in gasdermin D is not due an indirect effect of gasdermin D on caspase-11, and that caspase-11 lies mechanistically upstream of gasdermin D.

Execution of pyroptosis by gasdermin D requires its cleavage into a pore-forming p30 fragment³²⁻³⁵. Interestingly, although gasdermin D is required for *Alu* RNA-induced RPE degeneration and IL-18 secretion, we did not observe its cleavage into a p30 fragment in RPE cells either in cell culture or *in vivo* (Fig. 2e); however, as reported earlier³¹, intracellular LPS induced p30 cleavage in mouse bone marrow derived macrophages (BMDMs) (Fig. 2e).

Next, we directly tested whether gasdermin D p30 cleavage was dispensable for the toxicity of *Alu* RNA by reconstituting *Gsdmd*^{-/-} mice with WT gasdermin D (pGSDMD-WT) or mutant gasdermin D (pGSDMD-D276A), which is unable to undergo cleavage into the pyroptotic p30 fragment³¹. Interestingly, expression of either WT or the p30-cleavage incompetent mutant restored susceptibility to *Alu* RNA-induced RPE degeneration in *Gsdmd*^{-/-} mice, suggesting a non-pyroptotic function for gasdermin D in this system (Fig. 2f).

Previously we demonstrated that *Alu* RNA induced activation of caspase-3 (refs. 3,21) as well as caspase-8, Fas, and FasL²¹, and that this well-characterized pathway of apoptosis inducers was critical for its RPE toxicity. In addition, we and others have shown molecular evidence consistent with apoptosis in the RPE in human eyes with geographic atrophy^{3,36}. To further clarify the precise route of *Alu* RNA-induced cell death, we performed live-cell imaging of annexin-V and propidium iodide (PI) in primary human RPE cells. Cells treated with *Alu* RNA developed plasma membrane blebs and displayed an annexin-V⁺ PI⁻ staining pattern, findings that are consistent with early apoptosis. After several hours of annexin-V positivity, cells frequently swelled and became PI-positive, consistent with late apoptosis or secondary necrosis^{37,38} (Supplementary Fig. 6a, b). *In vivo* studies recapitulated our cell culture findings: RPE flat mounts from *Alu* RNA-exposed WT mice displayed a prominence of annexin-V⁺ PI⁻ cell death (Supplementary Fig. 7a).

Alu RNA stimulation of RPE cells induced cleavage of caspase-3 and poly(ADP-ribose) polymerase 1 (PARP-1) (Supplementary Fig. 7b), further supporting an apoptotic cell death pathway. These findings, coupled with our earlier demonstration that neither necrostatin-1, an inhibitor of primary necrosis³⁹, nor
 5 glycine, an inhibitor of pyroptosis⁴⁰, blocks *Alu* RNA-induced RPE degeneration^{2,21}, suggest that *Alu* RNA promotes cell death primarily via apoptosis rather than pyroptosis or necrosis in RPE cells.

We further explored the inter-relationship of IL-18 and gasdermin D in *Alu* RNA-induced cell death. The resistance of *Gsdmd*^{-/-} mice to *Alu* RNA-induced
 10 RPE degeneration was overcome by recombinant mature IL-18 or mature IL-18 expression plasmid, suggesting that the absence of RPE degeneration in *Gsdmd*^{-/-} mice is due to loss of IL-18 secretion, and not due to lack of pyroptosis (Fig. 2g). Supportive of this concept, *Alu* RNA induced secretion of IL-18 in *Gsdmd*^{-/-} mouse RPE cells that were reconstituted with either pGSDMD-WT or the p30 cleavage-
 15 incompetent pGSDMD-D276A (Supplementary Fig. 5c). Additionally, whereas annexin-V⁺ cells were not visible in RPE flat mounts of *Alu* RNA-treated *Gsdmd*^{-/-} mice, administration of recombinant mature IL-18 led to the appearance of numerous annexin-V⁺ PI⁻ cells (Supplementary Fig. 8). Taken together with our earlier demonstration that IL-18 neutralization or IL-18 receptor ablation in mice
 20 with intact gasdermin D blocks *Alu* RNA toxicity *in vivo*², these findings suggest that gasdermin D is required for *Alu* RNA-induced inflammasome activation, and for RPE toxicity driven via IL-18-dependent apoptosis.

Gasdermin D mRNA abundance was elevated in the RPE of human geographic atrophy eyes compared to unaffected age-matched controls (Fig. 2h). In
 25 contrast, we observed similar levels of MIP-1 α , IL-8, and IL-6 in human geographic atrophy and normal specimens (Supplementary Fig. 5d), suggesting there is no global elevation of pro-inflammatory cytokines in GA, but rather a more specific increase in inflammasome pathway genes. We also observed increased gasdermin D immunolocalization in the RPE of human geographic atrophy eyes compared to
 30 unaffected age-matched controls (Fig. 2i).

***Alu* RNA-induced non-canonical inflammasome activation is driven by Type I interferon (IFN) signaling**

To interrogate the upstream regulation of caspase-4, we focused on interferon signaling, which is involved in activation of the caspase-11 driven non-canonical inflammasome^{11,17}. *Alu* RNA did not induce RPE degeneration or caspase-11 activation in *Ifnar1*^{-/-} mice or *Ifnar1*^{-/-} mouse RPE cells (Fig. 3a, b), which are deficient in the type I interferon- α/β receptor (IFNAR). Recombinant interferon- β increased caspase-4 abundance in human RPE cells (Fig. 3c). *Alu* RNA induced secretion of interferon- β (Fig. 3d) and phosphorylation of IRF3 (Supplementary Fig. 9a), a transcription factor that induces production of interferon- β . *Alu* RNA also induced phosphorylation of STAT2 (Fig. 3e and Supplementary Fig. 9a, b), a signaling molecule activated by type-I interferons downstream of IFNAR. *Alu* RNA also did not induce RPE degeneration in *Irf3*^{-/-} or *Stat2*^{-/-} mice (Fig. 3f, and Supplementary Fig. 9c, d), and its induction of caspase-11 activation was reduced in *Stat2*^{-/-} mouse RPE cells (Supplementary Fig. 9e). *Alu* RNA-induced RPE degeneration was blocked by administration of an IFN- β neutralizing antibody (Fig. 3g), demonstrating that IFN- β is critical in *Alu* toxicity.

Human eyes with geographic atrophy displayed pronounced IFN- β expression in the RPE compared to unaffected aged human eyes (Fig. 3h, i). Collectively these findings suggest that *Alu* RNA-induced RPE degeneration is dependent on type I interferon signaling-regulated non-canonical NLRP3 inflammasome, and that this signaling provides the conditions required for caspase-11 induction and activation.

cGAS-driven IFN signaling licenses non-canonical NLRP3 inflammasome

We sought to identify the upstream activator of IRF3-driven interferon signaling induced by *Alu* RNA. *Alu* RNA-induced RPE degeneration is independent of several IRF3-activating signaling molecules including various RNA sensors: TLR3, TLR4, TLR9, RIG-I, MDA5, MAVS, and TRIF². Cyclic GMP-AMP synthase (cGAS; encoded by *Mb21d1*), has emerged as an innate immune sensor that can activate type I interferon signaling⁴¹⁻⁴³. Additionally, a role for cGAS in setting the type I IFN threshold to RNA virus infection has also been reported^{44,45}.

Alu RNA upregulated cGAS mRNA and protein in human RPE cells (Supplementary Fig. 10a, b). In contrast to WT mouse RPE cells, *Alu* RNA did not induce interferon- β (Fig. 4a), activate caspase-1 (Fig. 4b and Supplementary Fig. 10c) or caspase-11 (Fig. 4c), or induce IL-18 secretion (Fig. 4d) in *Mb21d1*^{-/-} mouse

RPE cells. Inflammasome activation by MSU crystals remained unaffected in *Mb21d1*^{-/-} mouse RPE cells (Supplementary Fig. 10d). DICER1 knockdown in human RPE cells, which leads to interferon- β induction, STAT2 phosphorylation, and activation of caspase-4 and caspase-1, were all inhibited by knockdown of cGAS (Fig. 4e, f and Supplementary Fig. 10e, f). Corroborating these data, *Alu* RNA did not induce RPE degeneration in *Mb21d1*^{-/-} mice (Fig. 4g and Supplementary Fig. 10g, h). Additionally, reconstitution with ectopic mouse cGAS restored IFN- β induction in *Mb21d1*^{-/-} mouse RPE cells and RPE degeneration in *Mb21d1*^{-/-} mice (Fig. 4h, Supplementary Fig. 10i, and Supplementary Fig. 11a). The resistance of *Mb21d1*^{-/-} mice to *Alu* RNA-induced RPE degeneration was overcome by recombinant IFN- β administration or IFN- β expression via subretinal plasmid transfection, suggesting that the loss of susceptibility to *Alu* RNA in these mice is indeed due to lack of IFN signaling (Fig. 4i).

We observed increased abundance of cGAS protein in the RPE of human geographic atrophy eyes compared to unaffected aged eyes (Fig. 5a). cGAS-driven interferon signaling can be transduced by the adaptor protein STING (encoded by *Tmem173*)^{42,43,46}. *Alu* RNA did not induce IRF3 phosphorylation (Supplementary Fig. 11b, c) or activation of caspase-1 (Fig. 5b) and caspase-11 (Fig. 5c) in *Tmem173*^{-/-} mouse RPE cells, and did not induce RPE degeneration in *Tmem173*^{-/-} mice (Fig. 5d), pointing to the involvement of the cGAS-STING signaling axis in this system. The resistance of *Tmem173*^{-/-} mice to *Alu* RNA-induced RPE degeneration was overcome by recombinant IFN- β administration or IFN- β expression via subretinal plasmid transfection, suggesting that the loss of susceptibility to *Alu* RNA in these mice is indeed due to lack of IFN signaling (Fig. 5e).

***Alu*-driven cGAS activation is triggered by engagement with mtDNA**

cGAS is activated by cytosolic DNA but not by poly(I:C), a synthetic double stranded RNA analog⁴³. Consistent with the notion that cGAS does not recognize RNA directly, *Alu* RNA did not bind cGAS in an RNA immunoprecipitation assay. Previous studies have implicated mitochondrial dysfunction in macular degeneration including mitochondrial DNA (mtDNA) damage, reactive oxygen species (ROS) production, and downregulation of proteins involved in mitochondrial energy production and trafficking^{2,47,48}. Cytosolic escape

of mitochondrial components such as DNA and formyl peptides activates innate immune pathways including cGAS (refs. 49,50).

Both *Alu* RNA stimulation and DICER1 knockdown in human RPE cells resulted in increased cytosolic abundance of mtDNA (Fig. 6a and Supplementary Fig. 12a, b). To examine whether *Alu* RNA triggers engagement of mtDNA by cGAS, we performed a DNA-protein interaction pull down assay in HA-tagged cGAS reconstituted *Mb21d1*^{-/-} immortalized mouse embryonic fibroblasts⁴⁹. As these cells express HA-cGAS from a genomically integrated DNA sequence, they would be expected to mimic endogenous cGAS expression. We observed enrichment of mtDNA in cGAS immunoprecipitate of *Alu* RNA-stimulated but not mock- or poly(I:C)-stimulated cells (Fig. 6b and Supplementary Fig. 12c), suggesting that mtDNA in the cytosol engages cGAS. As a positive control, transfected plasmid DNA in this assay was also enriched in the cGAS immunoprecipitate (Supplementary Fig. 12d). Additionally, subretinal delivery of mtDNA induced RPE degeneration in WT but not in *Mb21d1*^{-/-} mice (Supplementary Fig. 12e). Similarly, in cell culture studies, mtDNA-induced *Ifnb* mRNA was reduced in *Mb21d1*^{-/-} compared to WT mouse RPE cells (Supplementary Fig. 12f).

Mitochondrial permeability transition pore is required for *Alu*-driven mtDNA release During conditions of cellular stress, opening of the mitochondrial permeability transition pore (mPTP) leads to mitochondrial swelling, rupture, and release of mitochondrial contents into the cytosol^{51,52}. In cells lacking mitochondrial peptidyl-prolyl cis-trans isomerase F (PPIF, also known as cyclophilin D), a key enzyme involved in mPTP, mitochondria are resistant to swelling and permeability transition^{53,54}. We assessed whether *Alu* RNA induced mPTP opening using the JC-1 and cobalt-calcein assays⁵⁵. *Alu* RNA induced a reduction of mitochondrial membrane potential ($\Delta\Psi_m$), as determined by the potential-sensitive fluorochrome JC-1, and quenching of the calcein signal in wild-type but not *Ppif*^{-/-} mouse RPE cells (Supplementary Fig. 12g, h). In addition, cyclosporine A, which inhibits mPTP opening via binding to PPIF, blocked *Alu* RNA-induced mPTP opening in wild-type cells, but did not alter $\Delta\Psi_m$ or calcein intensity in *Ppif*^{-/-} cells (Supplementary Fig. 12g, h). Collectively, these findings suggest that *Alu* RNA induces *Ppif*-dependent mPTP opening in RPE cells.

Alu RNA triggered mtDNA release into the cytosol in WT but not *Ppif*^{-/-} mouse RPE cells (Fig. 6c). *Ppif*^{-/-} mice were protected against *Alu* RNA-induced RPE degeneration (Fig. 6d), confirming the *in vivo* importance of mPTP in *Alu* toxicity. *Alu* RNA-induced activation of caspase-1 and caspase-11 were reduced in *Ppif*^{-/-} mouse RPE cells (Fig. 6e, f). In human RPE cells lacking mitochondrial DNA (Rho⁰ ARPE19)⁵⁶, *Alu* RNA no longer activated caspase-4 (Fig. 6g) or induced secretion of IL-18 (Fig. 6h) or IFN-β (Fig. 6i). Furthermore, the resistance of *Ppif*^{-/-} mice to *Alu* RNA-induced RPE degeneration was overcome by recombinant IFN-β administration or IFN-β expression via subretinal plasmid transfection, suggesting that the loss of susceptibility to *Alu* RNA in these mice is indeed due to lack of IFN signaling (Fig. 6j). Collectively these data support a model wherein mPTP-driven mitochondrial permeability mediates cytosolic release of mtDNA, which in turn promotes non-canonical NLRP3 inflammasome via engaging the cytosolic DNA sensor cGAS-driven IFN signaling (Supplementary Fig. 15).

***Alu* driven RPE toxicity does not require macrophages or microglia**

We focused on the RPE as the cellular locus of inflammasome activation because we previously demonstrated the localization of DICER1 deficiency, *Alu* RNA accumulation, and increased abundance of NLRP3, PYCARD, cleaved caspase-1, and phosphorylated IRAK1/4 to the RPE layer of human eyes with geographic atrophy^{2,3}. Our current observations of elevated cGAS, gasdermin D, cleaved caspase-4, and IFN-β in the RPE in diseased eyes further buttress this cell layer as the locus of molecular perturbations in the non-canonical inflammasome pathway in geographic atrophy.

However, there are recent reports that macrophages and microglia can be observed in the vicinity of pathology in human eyes with geographic atrophy⁵⁷⁻⁵⁹. Previously we demonstrated that RPE cell-specific ablation of Myd88, the adaptor critical for IL-18-induced RPE cell death in this system, was sufficient to prevent *Alu* RNA-induced RPE degeneration in mice². We also demonstrated using mouse chimeras that ablation of Myd88 in circulating bone marrow derived cells did not prevent *Alu* RNA-induced RPE degeneration². Nevertheless, given that these professional immune cells are capable of inflammasome signaling, we studied their involvement more directly. We depleted macrophages using clodronate liposomes⁶⁰

and depleted microglia by administering tamoxifen to *Cx3cr1^{CreER}* ROSA-DTA mice⁶¹. *Alu* RNA induced RPE degeneration in mice despite depleting macrophages or microglia, providing direct evidence that these two cell populations are dispensable for RPE toxicity in this system (Supplementary Fig. 13).

5 Although these two cell types are apparently not required by *Alu* RNA to elicit RPE degeneration in mice, it is possible that they might subtly influence disease pathology in this system. Indeed we found that *Alu* RNA activates the non-canonical inflammasome in mouse BMDMs (Supplementary Fig. 14). Alike in RPE cells, *Alu* RNA-induced caspase-1 activation was reduced in *Casp11^{-/-}*, *Mb21d1^{-/-}*,
10 and *Gsdmd^{-/-}* BMDMs (Supplementary Fig. 14). Collectively these findings suggest that cGAS-driven licensing of the non-canonical NLRP3 inflammasome by *Alu* RNA is not restricted to RPE cells.

DISCUSSION

15 Our data identify an unexpected role for the DNA sensor cGAS, the non-canonical caspase-4 inflammasome, and gasdermin D in mediating *Alu* RNA-induced RPE cell death in various mouse models and human cell culture systems. Coupled with the increased abundance of cGAS, interferon- β , caspase-4, and gasdermin D in the RPE of human geographic atrophy eyes, our findings point to
20 their involvement in the pathogenesis of this form of age-related macular degeneration and provide new targets for treatment and prevention.

 cGAS was originally recognized as sensor of exogenous and endogenous cytosolic DNA that mediates IRF3-driven interferon signaling, and previous studies demonstrated that the enzymatic activity of cGAS could not be activated by an RNA
25 stimulus⁴³. Nonetheless, cGAS has been reported to be critical for the antiviral response to multiple RNA viruses^{44,45}; although the mechanistic underpinnings of this effect are not fully understood, our work defines a novel pathway by which endogenous RNAs can activate cGAS in a model of a prevalent human disease.

 Our findings also raise the possibility that cGAS-driven antiviral immunity
30 involves *Alu* RNA, which can be stimulated by viral infections⁶²⁻⁶⁶. Mitochondria have been increasingly implicated as gatekeepers of cell fate with decisive roles in diverse cellular responses including apoptosis, autophagy, and innate immunity^{67,68}. Mitochondria can facilitate the innate immune response to infection and injury via release of mitochondrial components as DAMPs that can be recognized by the cell's

innate immune components. Of note, mtDNA can activate multiple arms of innate immunity including the NLRP3 inflammasome, TLR9, and cGAS/STING-driven IFN signaling^{50,69}. mtDNA can activate the NLRP3 inflammasome by directly interacting with NLRP3 (ref. 70) or amplifying the response to an initial trigger such as ATP or ROS⁷¹.

In addition, mtDNA can activate TLR9 on neutrophils triggering systemic lung and liver inflammation⁷²⁻⁷⁴. Besides engaging TLR9 and NLRP3 signaling, mtDNA has also recently been reported in the activation of cGAS signaling by cytosolic escape of mtDNA as a consequence of mitochondrial stress⁴⁹.

Interestingly we previously demonstrated that TLR9 signaling is dispensable for *Alu* RNA-induced RPE degeneration², and that NLRP3 inflammasome priming is unaffected in mouse RPE cells lacking TLR9 (ref. 20). Therefore our findings implicating activation of both NLRP3 and cGAS signaling pathways under the conditions of *Alu* RNA-driven cytosolic mtDNA release highlight the significance of the mitochondria as a signaling platform that integrates various cellular stress cues into innate immune signaling in autoimmune and chronic inflammatory diseases.

In addition to its role in responding to infections, cGAS has been implicated in mouse models of autoimmune diseases and mouse tumor models. Our findings that cGAS is elevated in the RPE of human eyes with AMD and is critical for *Alu* RNA-induced RPE degeneration in mice and in human cells expands the functional repertoire of this innate immune sensor to chronic degenerative diseases.

Caspase-4/11-mediated activation of the non-canonical NLRP3 inflammasome has been implicated in gram-negative bacterial infection, sepsis, and antimicrobial defense at the mucosal surface^{11-17,28}. To our knowledge, our report is the first example of caspase-4-driven non-canonical inflammasome activation in a non-infectious human disease. Also it bears investigating whether caspase-4 and cGAS are involved in other conditions such as systemic lupus erythematosus and diabetes mellitus, wherein *Alu* RNA accumulation has been observed^{75,76}.

Activation of caspase-4 has been observed in conditions of endoplasmic reticulum (ER) stress⁷⁷; interestingly, several human diseases including Alzheimer's disease, and obesity driven-type 2 diabetes, which are also driven by hyperactive inflammasome, are associated with ER stress^{78,79}. It would be revealing to explore whether DICER1 deficit or *Alu* RNA-induced mitochondrial dysfunction and cGAS- and caspase-4 dependent-inflammasome activation are linked to ER stress.

Our studies also reveal that gasdermin D lies mechanistically downstream of caspase-11 activation and is required for *Alu* toxicity. Of note, the role of gasdermin D in this system appears not to be induction of pyroptosis, as it is in response to exogenous triggers such as intracellular LPS. Instead, gasdermin D supports *Alu* RNA-induced RPE cell apoptosis by promoting IL-18 secretion without being cleaved into its p30 fragment, which is required for its pyroptotic effect. To our knowledge, this is the first report of gasdermin D involvement in a non-infectious human disease. Interestingly, Fas/FasL are thought to play a critical role in limiting inflammation in immune-privileged sites such as the eye⁸⁰. Therefore it is conceivable that additional mechanisms have evolved to limit inflammasome-driven gasdermin D-mediated pore formation and pyroptotic cell death that would otherwise exacerbate inflammation via release of DAMPs leading to recruitment of inflammatory cells into the eye. Hence inflammasomes in the eye might be geared towards engaging IL-18-promoted, Fas/FasL-driven pro-apoptotic cell death. Additional studies are required to dissect the mechanisms that disengage the pore-forming function of gasdermin D from the inflammasome-activating function, i.e., caspase-1 activation and IL-18 secretion. Furthermore pyroptosis induction via gasdermin D upon non-canonical inflammasome activation could be dictated by the activating trigger (e.g. exogenous versus host) or the cell type involved. For instance, the only other endogenous molecule known to activate caspase-11, oxPAPC, also did not induce pyroptosis, yet triggered IL-1 β release from dendritic cells¹⁸. Interestingly, *Alu* RNA induces oxPAPC synthesis, raising the possibility that *Alu* RNA might recruit other DAMPs to induce RPE toxicity.

The molecular mechanism by which caspase-8 influences inflammasome activation is elusive. Caspase-8 has been reported to both prime the NLRP3 inflammasome as well as trigger cleavage of pro-IL-18 and pro-IL-1 β , either with or without caspase-1 (ref. 81). In our model, caspase-8 mediated *Alu* toxicity was mediated by IL-18-driven Fas/FasL²¹ (see also FIG. 21/Supplementary Fig. 15). In another study, both canonical and non-canonical inflammasome were regulated by FADD and caspase-8 signaling at the level of both inflammasome priming and activation⁸². Therefore a model is conceivable wherein an initial round of caspase-1 activation and IL-18 secretion set in motion a cascade of events that can be further

modulated to achieve signal amplification in a feed-forward manner by recruiting additional molecules such as FasL and caspase-8.

The mechanisms underlying regulation of the NLRP3 inflammasome by caspase-11/4 have been elusive. Previously we demonstrated that *Alu* RNA-induced RPE degeneration and NLRP3 inflammasome activation depended on NF- κ B and P2X7 (refs. 19, 20). Surprisingly in our studies *Alu* RNA-induced caspase-11 activation was subdued in *P2rx7*^{-/-} mouse RPE cells, suggesting that P2X7 is required for both caspase-1 and caspase-11 activation. A role for P2X7 also has been reported in a mouse model of caspase-11-dependent endotoxic shock⁸³. Taken together, our observations suggest that *Alu* RNA-driven NLRP3 inflammasome activation engages both caspase-11/4 and P2X7. We also observed that *Alu* RNA induces oxPAPC synthesis, suggesting that *Alu* RNA might recruit other DAMPs to activate the non-canonical inflammasome during RPE cell toxicity. Earlier reports have implicated oxidized phospholipids in the pathophysiology of age-related macular degeneration⁸⁴⁻⁸⁶; future studies should unravel the molecular details of this pathway.

In recent years, numerous groups employing a variety of cell culture systems, animal models, and human donor eyes have reported an important role for the inflammasome in AMD^{2,6-10,87-89}. Collectively these studies suggest that NLRP3 pathway is an important responder to a panoply of AMD-related molecular stressors and toxins in RPE cells. Thus there is great interest in inflammasome inhibition as a therapeutic for AMD¹⁹. Our identification of cGAS, interferon- β , caspase-4, and gasdermin D as critical mediators in inflammasome-driven RPE degeneration expands the array of therapeutic targets for AMD.

Although there is consensus that NLRP3 inflammasome activation is detrimental to RPE cell health and survival, akin to that of other cell types^{81,90}, there is controversy about the role of this pathway in neovascular AMD. It has been reported that IL-18, a cytokine produced by NLRP3 inflammasome activation, inhibits angiogenesis and that IL-18 neutralization augments angiogenesis in a laser injury model of choroidal neovascularization^{88,89}. However, an international consortium did not replicate this anti-angiogenic effect of IL-18 and also demonstrated that the promotion of angiogenesis by an IL-18 antibody was due to an excipient in its preparation⁹¹. These conflicting data in neovascular AMD models do not, however, impact the conclusion that inflammasome activation promotes RPE

degeneration, which provides a mechanistic rationale for testing inflammasome inhibition in geographic atrophy⁹¹.

Our finding that DICER1, through its cleavage of *Alu* RNA, can prevent activation of the non-canonical inflammasome adds to the functionality of this multifaceted protein that has microRNA biogenesis, anti-apoptotic, and tumor-related functions. Although it is unknown why DICER1 levels might be reduced in AMD³, DICER1 protein levels are suppressed by hypoxia, type I interferons, dsRNAs, and reactive oxygen species^{92,93}, all of which are thought to contribute to AMD pathogenesis^{94,95}.

In summary, our studies have uncovered a novel effect of cGAS signaling in response to endogenous retroelement transcripts, which involves the unexpected collaboration between mitochondrial dysfunction, cGAS-driven interferon signaling, gasdermin D, and NLRP3 inflammasome activation (See FIG. 21/Supplementary Fig. 15). Targeting this pathway or various components of the pathway presents potentially a new therapeutic approach to preserve RPE health in age-related macular degeneration and a host of other inflammasome-driven diseases.

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated by reference herein in their entirety.

Headings are included herein for reference and to aid in locating certain sections.

These headings are not intended to limit the scope of the concepts described therein under, and these concepts may have applicability in other sections throughout the entire specification.

While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention.

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CLAIMS

What is claimed is:

1. A method for preventing or treating age-related macular degeneration in a subject in need thereof by targeting at least one of the alternative, non-canonical
5 inflammasome signaling molecules, protein complex, or signal transduction pathways in retinal pigment epithelium (RPE), the method comprising administering to the subject a pharmaceutical composition comprising a pharmaceutically-acceptable carrier, an effective amount of an inhibitor of noncanonical-inflammasome activation in RPE, and optionally an additional therapeutic agent,
10 thereby preventing or treating age-related macular degeneration.
2. The method of claim 1, wherein said alternative, non-canonical inflammasome signaling molecule, protein complex, or signal transduction pathway is selected from the group consisting of cyclic GMP-AMP synthase (cGAS), Caspase-4, stimulator
15 of interferon genes (STING), peptidyl-prolyl cis-trans isomerase F (PPIF), mitochondrial permeability transition pore (MPTP), Gasdermin D (GSDMD), interferon beta (IFN- β), and interferon- α/β receptor (IFNAR).
3. The method of claim 1, wherein said age-related macular degeneration is
20 geographic atrophy.
4. The method of claim 1, wherein said inhibitor is selected from the group consisting of antisense oligonucleotide, small interfering RNA (siRNA), short hairpin RNA (shRNA), antibody, and biologically active fragments or homologs of
25 said antibody.
5. The method of claim 4, wherein said antibody is selected from the group consisting of monoclonal antibody, humanized antibody, chimeric antibody, single chain antibody, and biologically active fragments and homologs thereof.
30
6. The method of claim 5, wherein said homolog comprises at least 95% sequence identity with said monoclonal antibody, humanized antibody, chimeric antibody, or single chain antibody.

7. The method of claim 1, wherein said inhibitor is selected from the group consisting of cGAS shRNA (shcGAS), cGAS siRNA, Caspase-4 shRNA, Caspase-4 siRNA, GSDMD shRNA, STING shRNA, PPIF shRNA, IFNB shRNA, IFN- β shRNA, IFNAR1 shRNA, and an IFN- β neutralizing antibody.
- 5 8. The method of claim 7, wherein said inhibitor is shcGAS or cGAS siRNA.
9. The method of claim 8, wherein said shcGAS is SEQ ID NO:15 or SEQ ID NO:1.
- 10 10. The method of claim 8, wherein said cGAS siRNA is SEQ ID NO:2.
11. The method of claim 7, wherein said inhibitor is Caspase-4 shRNA or Caspase-4 siRNA.
- 15 12. The method of claim 11, wherein said Caspase-4 shRNA is SEQ ID NO:16 and said Caspase-4 siRNA has a sequence selected from the group consisting of SEQ ID NOs: 9, 10, 11, 12, 13, and 14.
- 20 13. The method of claim 7, wherein said inhibitor is an IFN- β neutralizing antibody
14. The method of claim 1, wherein said method protects RPE cells from cell death.
- 25 15. The method of claim 1, wherein said subject has been diagnosed with age-related macular degeneration or geographic atrophy.
16. The method of claim 1, wherein said subject is susceptible to age-related macular degeneration or geographic atrophy.
- 30 17. The method of claim 1, wherein two of said inhibitors are administered.
18. The method of claim 1, wherein said method inhibits Alu RNA induced RPE degeneration.

19. The method of claim 1, wherein an effective amount of an additional therapeutic agent is administered and said additional therapeutic agent is selected from the group consisting of cyclosporin A, Alu RNA antisense oligonucleotide, reverse transcriptase inhibitor, and IL-18 neutralizing antibody.
20. A method for preventing or inhibiting Alu RNA-induced retinal pigment epithelium (RPE) cell degeneration by targeting at least one of the alternative, non-canonical inflammasome signaling molecules or protein complex, the method comprising contacting said RPE cell with an inhibitor of at least one molecule or complex selected from the group consisting of cGAS, Caspase-4, STING, PPIF, MPTP, Gasdermin D, IFN- β , and IFNAR.
21. The method of claim 20, wherein said RPE degeneration is in age-related macular degeneration.
22. The method of claim 21, wherein said age-related macular degeneration is geographic atrophy.
23. The method of claim 20, where said cell is contacted with an inhibitor selected from the group consisting of inhibitors of cGAS, Caspase-4, GSDMD, STING, MPTP, PPIF, IFN- β , and IFNAR.
24. The method of claim 20, wherein said inhibitor is selected from the group consisting of antisense oligonucleotide, small interfering RNA (siRNA), short hairpin RNA (shRNA), antibody, and biologically active fragments or homologs of said antibody.
25. The method of claim 24, wherein said antibody is selected from the group consisting of monoclonal antibody, humanized antibody, chimeric antibody, single chain antibody, and biologically active fragments and homologs thereof.

26. The method of claim 25, wherein said homolog comprises at least 95% sequence identity with said monoclonal antibody, humanized antibody, chimeric antibody, or single chain antibody.
- 5 27. The method of claim 24, wherein said inhibitor is selected from the group consisting of shcGAS, cGAS siRNA, Caspase-4 shRNA, Caspase-4 siRNA, GSDMD shRNA, STING shRNA, PPIF shRNA, IFNB shRNA, IFN- β shRNA, IFNAR1 shRNA, and an IFN- β neutralizing antibody.
- 10 28. The method of claim 27, wherein said inhibitor is shcGAS or cGAS siRNA.
29. The method of claim 28, wherein said shcGAS is SEQ ID NO:15 or SEQ ID NO:1.
- 15 30. The method of claim 28, wherein said cGAS siRNA is SEQ ID NO:2.
31. The method of claim 27, wherein said inhibitor is Caspase-4 shRNA or caspase-4 siRNA.
- 20 32. The method of claim 31, wherein said Caspase-4 shRNA is SEQ ID NO:16 and said Caspase-4 siRNA has a sequence selected from the group consisting of SEQ ID NOs: 9, 10, 11, 12, 13, and 14.
33. The method of claim 27, wherein said inhibitor is an IFN- β neutralizing
25 antibody
34. The method of claim 20, wherein said method protects RPE cells from cell death.
- 30 35. The method of claim 20, further wherein said cell is contacted with an additional agent selected from the group consisting of cyclosporin A, Alu RNA antisense oligonucleotide, and a reverse transcriptase inhibitor.

36. The method of claim 20, wherein two inhibitors are administered.
37. A method for inhibiting a non-canonical inflammasome signaling molecule, protein complex, or pathway in an RPE cell, the method comprising contacting said
5 RPE cell with an inhibitor of at least one a non-canonical inflammasome molecule or protein complex, the method comprising contacting said RPE cell with an inhibitor of at least one molecule or complex selected from the group consisting of cGAS, Caspase-4, STING, PPIF, MPTP, GSDMD, IFN- β , and IFNAR.
- 10 38. The method of claim 37, where said cell is contacted with an inhibitor selected from the group consisting of inhibitors of cGAS, Caspase-4, GSDMD, STING, MPTP, PPIF, IFN- β , and IFNAR.
- 15 39. The method of claim 37, wherein said inhibitor is selected from the group consisting of antisense oligonucleotide, small interfering RNA (siRNA), short hairpin RNA (shRNA), antibody, and biologically active fragments or homologs of said antibody.
- 20 40. The method of claim 39, wherein said antibody is selected from the group consisting of monoclonal antibody, humanized antibody, chimeric antibody, single chain antibody, and biologically active fragments and homologs thereof.
- 25 41. The method of claim 40, wherein said homolog comprises at least 95% sequence identity with said monoclonal antibody, humanized antibody, chimeric antibody, or single chain antibody.
- 30 42. The method of claim 38, wherein said inhibitor is selected from the group consisting of shcGAS, cGAS siRNA, Caspase-4 shRNA, Caspase-4 siRNA, GSDMD shRNA, STING shRNA, PPIF shRNA, IFNB shRNA, IFN- β shRNA, IFNAR1 shRNA, and an IFN- β neutralizing antibody.
43. The method of claim 42, wherein said inhibitor is shcGAS or cGAS siRNA.

44. The method of claim 42, wherein said shcGAS is SEQ ID NO:15 or SEQ ID NO:1.

45. The method of claim 43, wherein said cGAS siRNA is SEQ ID NO:2.

5

46. The method of claim 42, wherein said inhibitor is Caspase-4 shRNA or Caspase-4 siRNA.

47. The method of claim 46, wherein said Caspase-4 shRNA has SEQ ID NO:16 and said Caspase-4 siRNA has a sequence selected from the group consisting of SEQ ID NOs: 9, 10, 11, 12, 13, and 14.

10

48. The method of claim 42, wherein said inhibitor is an IFN- β neutralizing antibody

15

49. The method of claim 37, wherein said method protects said RPE cell from cell death.

50. The method of claim 49, wherein said method inhibits Alu RNA-induced RPE degeneration.

20

51. The method of claim 37, wherein two of said inhibitors are administered.

52. A method of determining whether a subject should be treated for age-related macular degeneration, said method comprising measuring the levels of at least one of Caspase-4, cGAS, and Gasdermin D in RPE of said subject, wherein an increase in the levels of one or more of Caspase-4, cGAS, and Gasdermin D compared to control levels is an indication that the subject should be treated for said age-related macular degeneration by administering an effective amount of an inhibitor of at least one of the alternative, non-canonical inflammasome signaling molecules or protein complex in RPE.

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53. The method of claim 52, wherein each of Caspase-4, cGAS, and Gasdermin D is measured and determined to be increased compared to control levels of Caspase-4, cGAS, and Gasdermin D.

5 54. The method of claim 52, wherein when it is determined that said subject has an increase in the levels of one or more of Caspase-4, cGAS, and Gasdermin D, said subject is then treated by administering an effective amount of an inhibitor targeting at least one of the alternative, non-canonical inflammasome signaling molecules or alternative, non-canonical inflammasome protein complexes in RPE.

10

55. The method of claim 7, wherein said GSDMD shRNA has SEQ ID NO:19, said STING shRNA has SEQ ID NO:17, said PPIF shRNA has SEQ ID NO:18, said IFN- β shRNA has SEQ ID NO:20, and said IFNAR1 shRNA has SEQ ID NO:21.

15 56. The method of claim 27, wherein said GSDMD shRNA has SEQ ID NO:19, said STING shRNA has SEQ ID NO:17, said PPIF shRNA has SEQ ID NO:18, said IFN- β shRNA has SEQ ID NO:20, and said IFNAR1 shRNA has SEQ ID NO:21.

20 57. The method of claim 42, wherein said GSDMD shRNA has SEQ ID NO:19, said STING shRNA has SEQ ID NO:17, said PPIF shRNA has SEQ ID NO:18, said IFN- β shRNA has SEQ ID NO:20, and said IFNAR1 shRNA has SEQ ID NO:21.

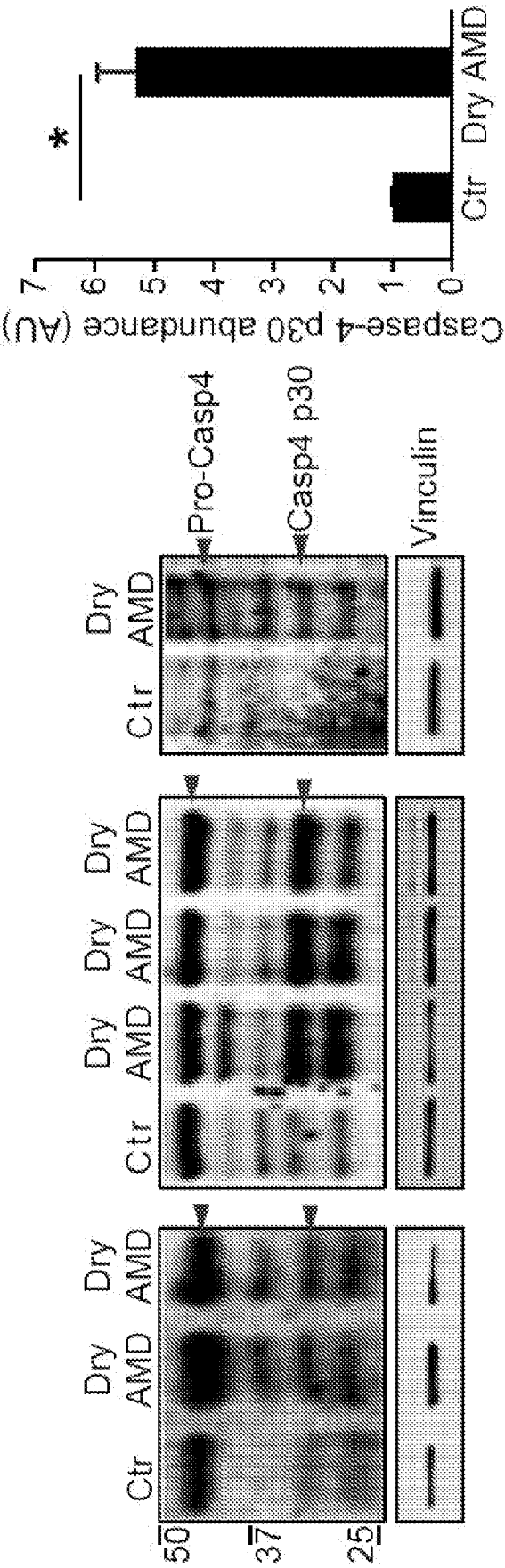


FIG. 1A

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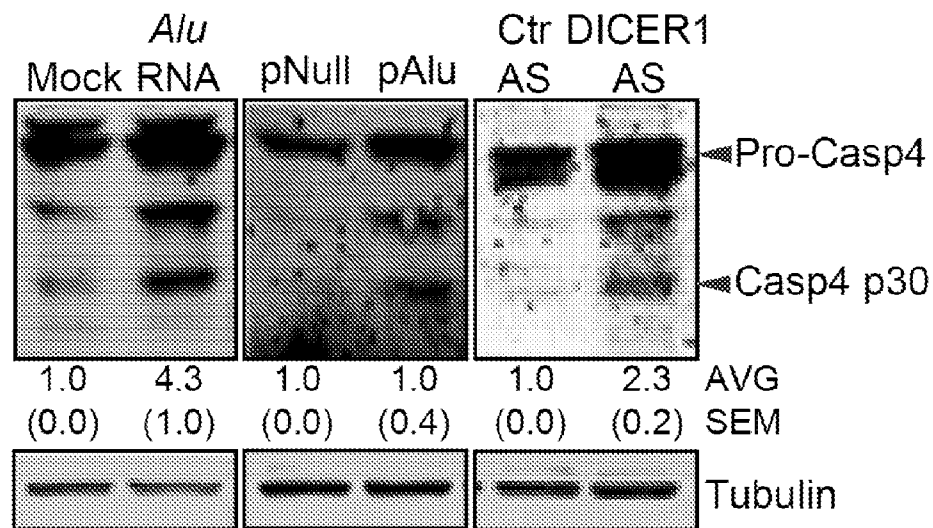


FIG. 1B

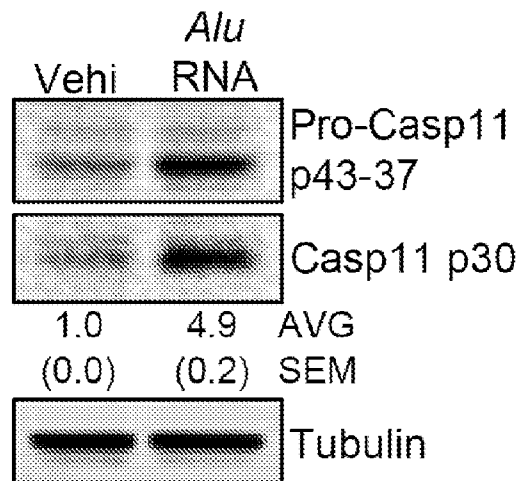
WT Mouse RPE
tissue

FIG. 1C

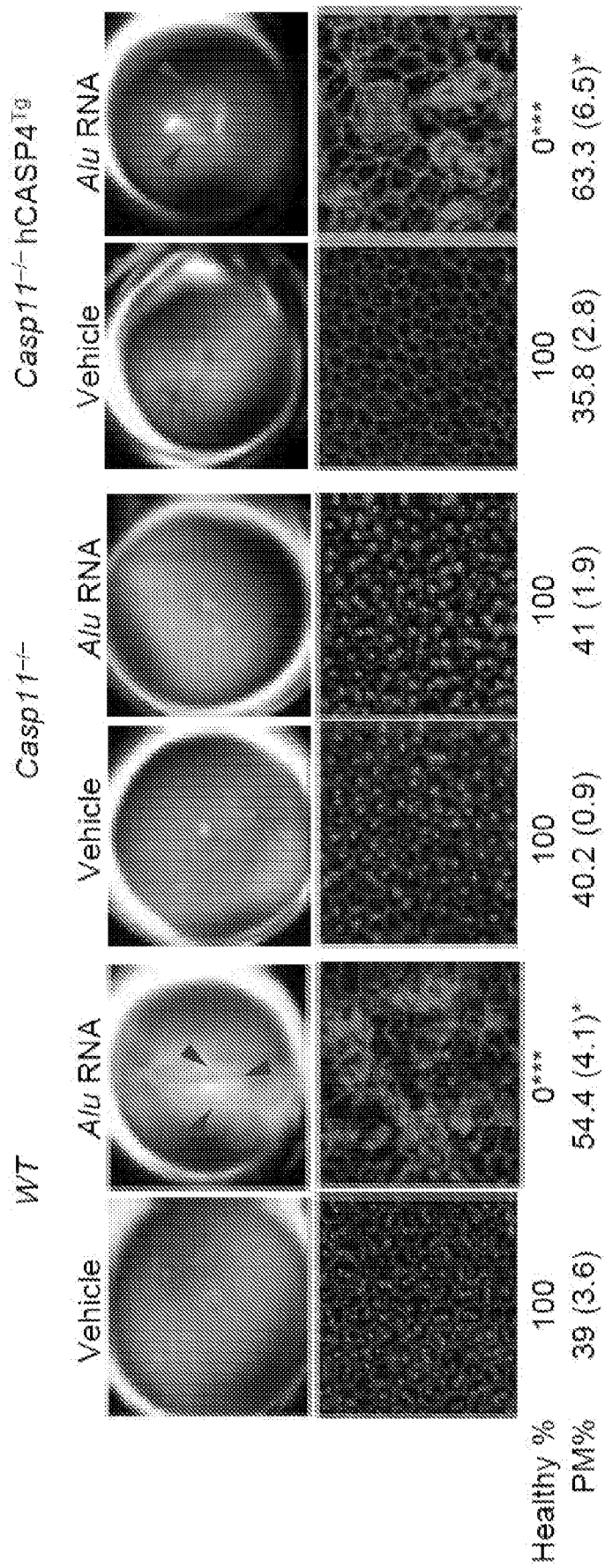


FIG. 1D

FIG. 1E

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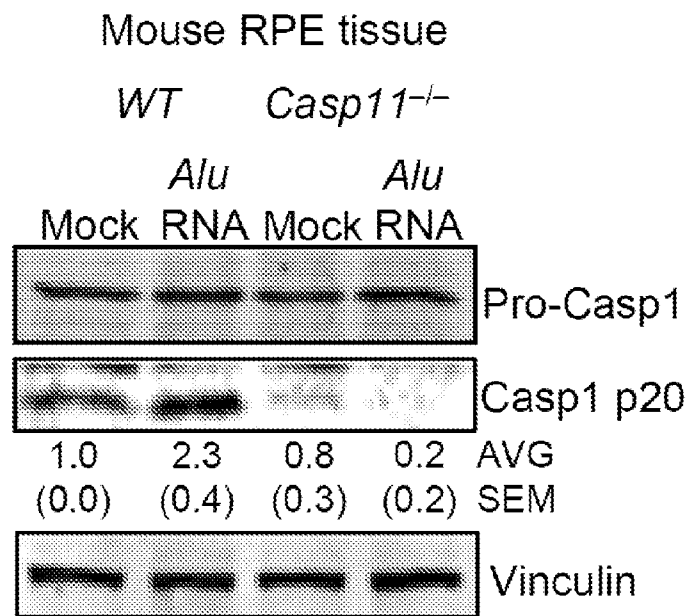


FIG. 1F

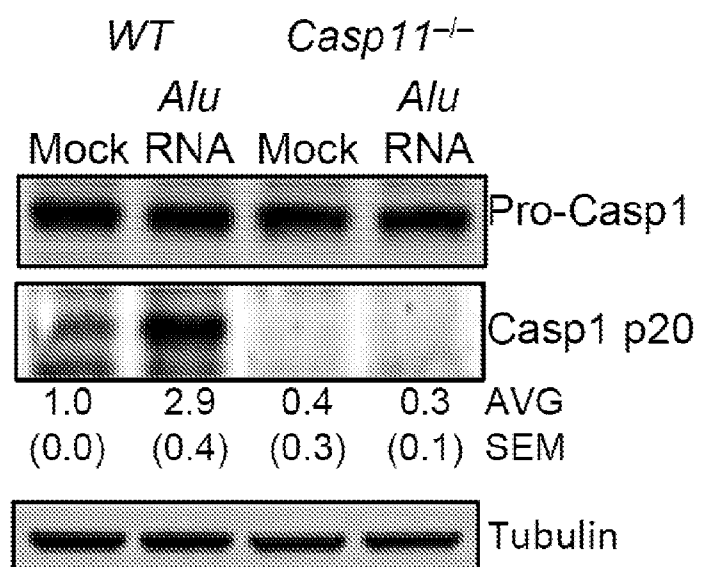


FIG. 1G

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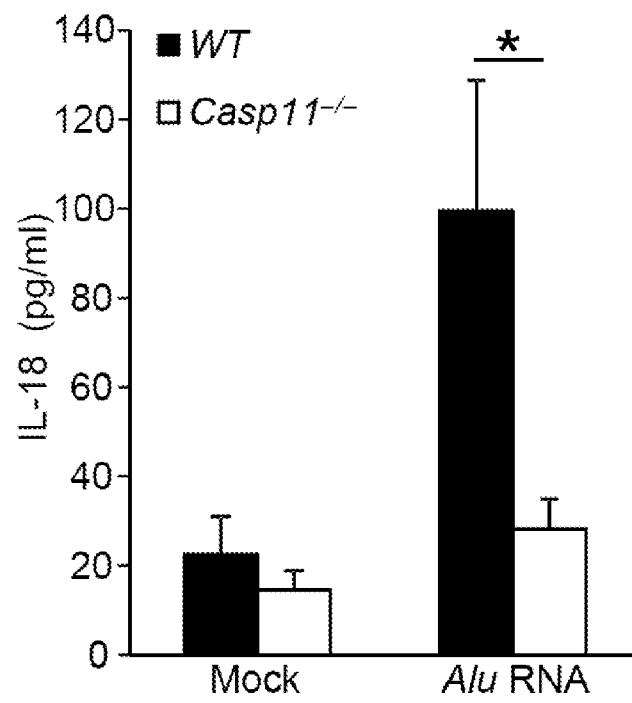


FIG. 1H

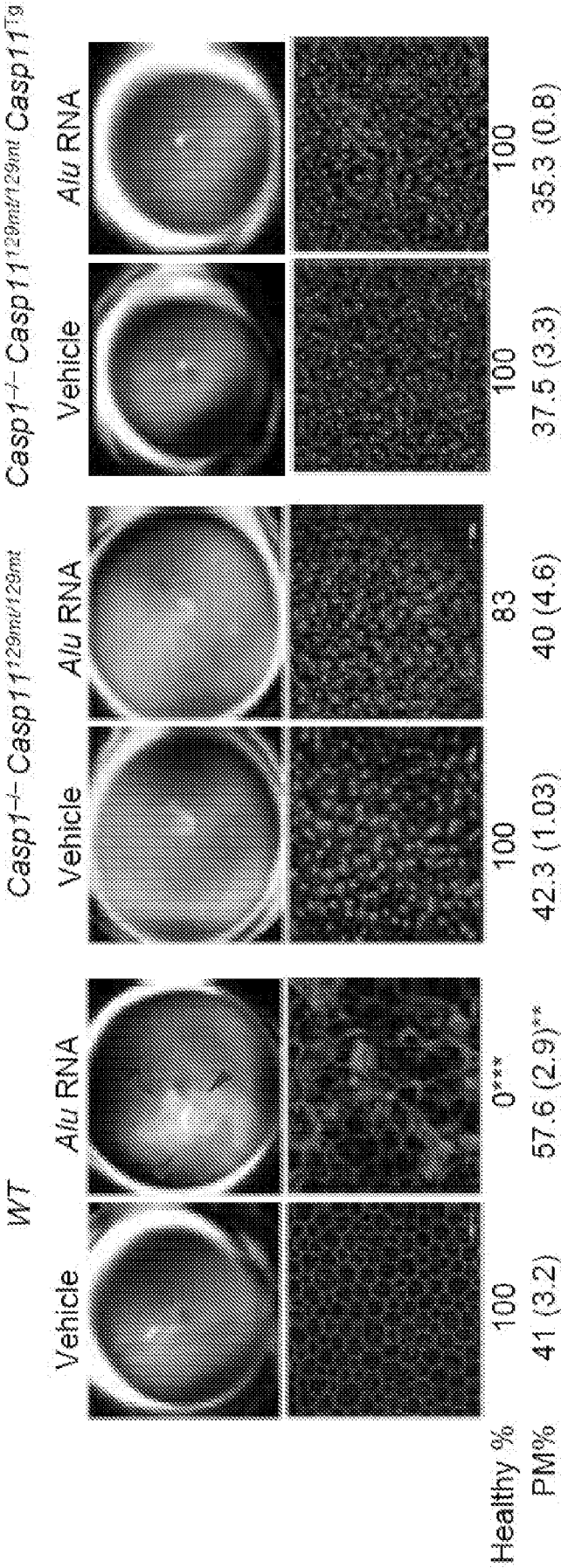


FIG. 1I

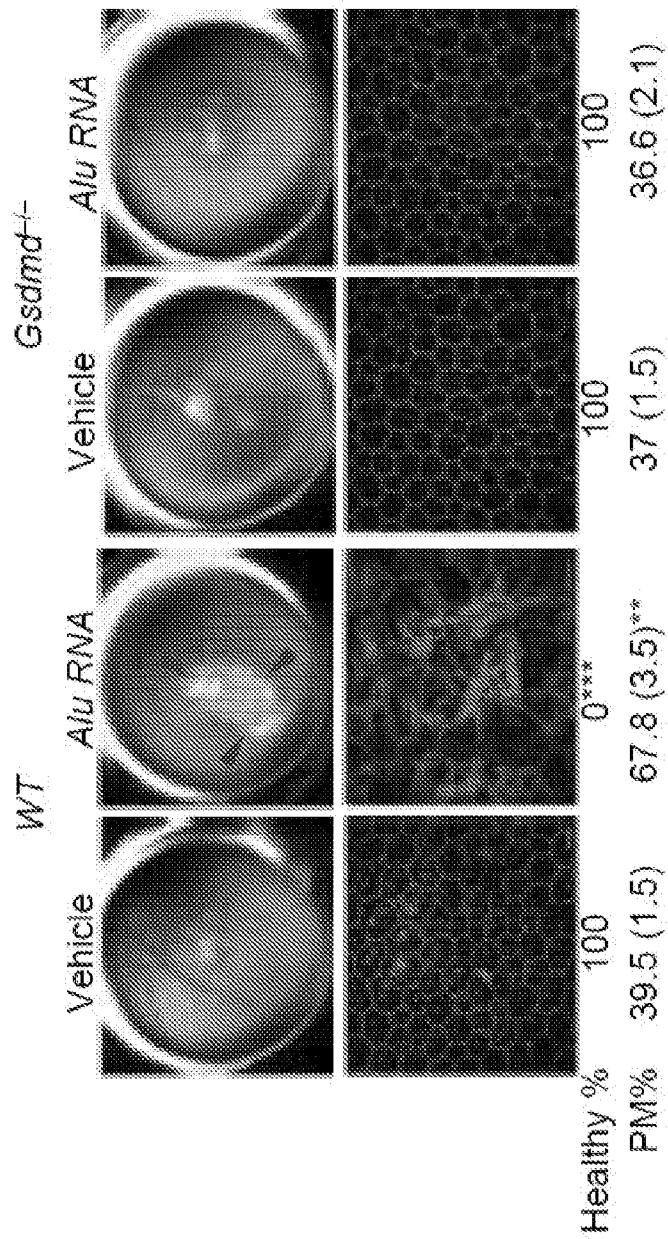


FIG. 2A

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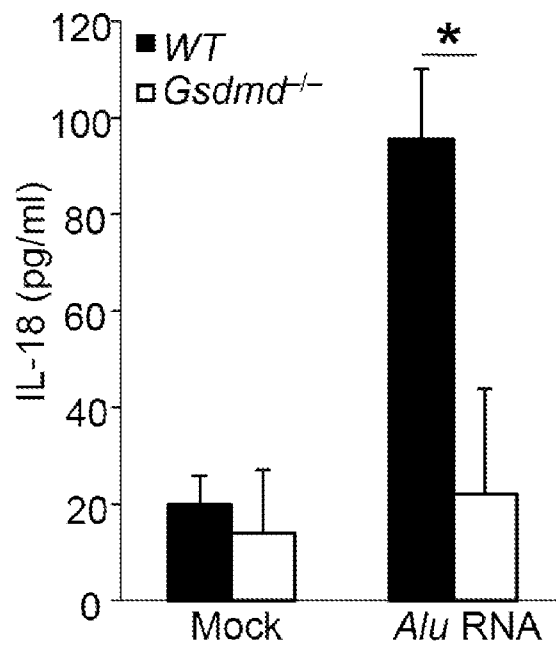


FIG. 2B

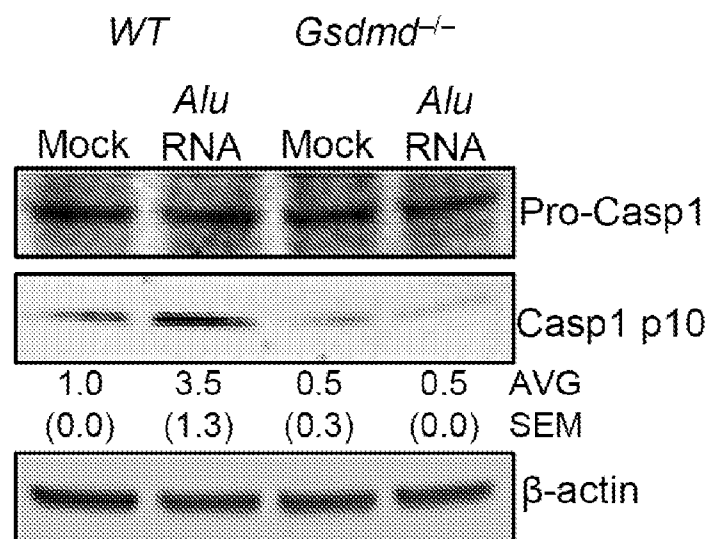


FIG. 2C

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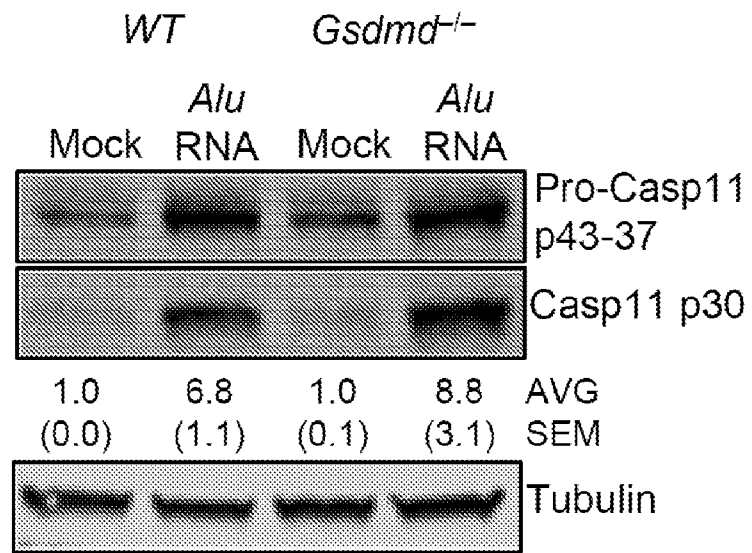


FIG. 2D

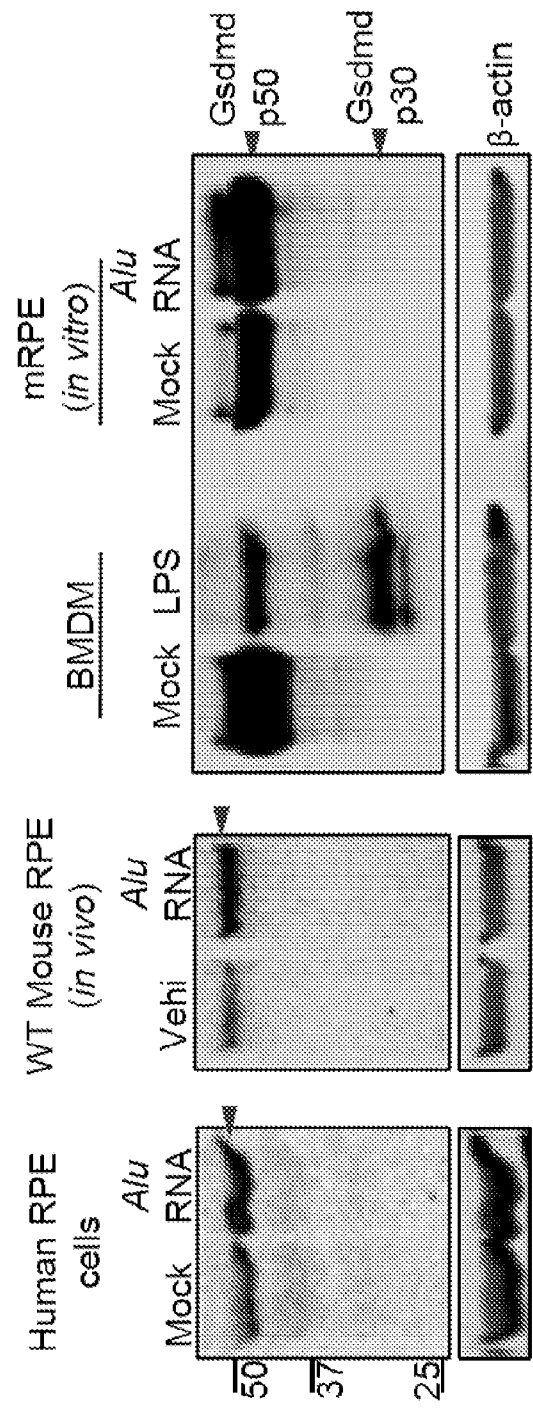
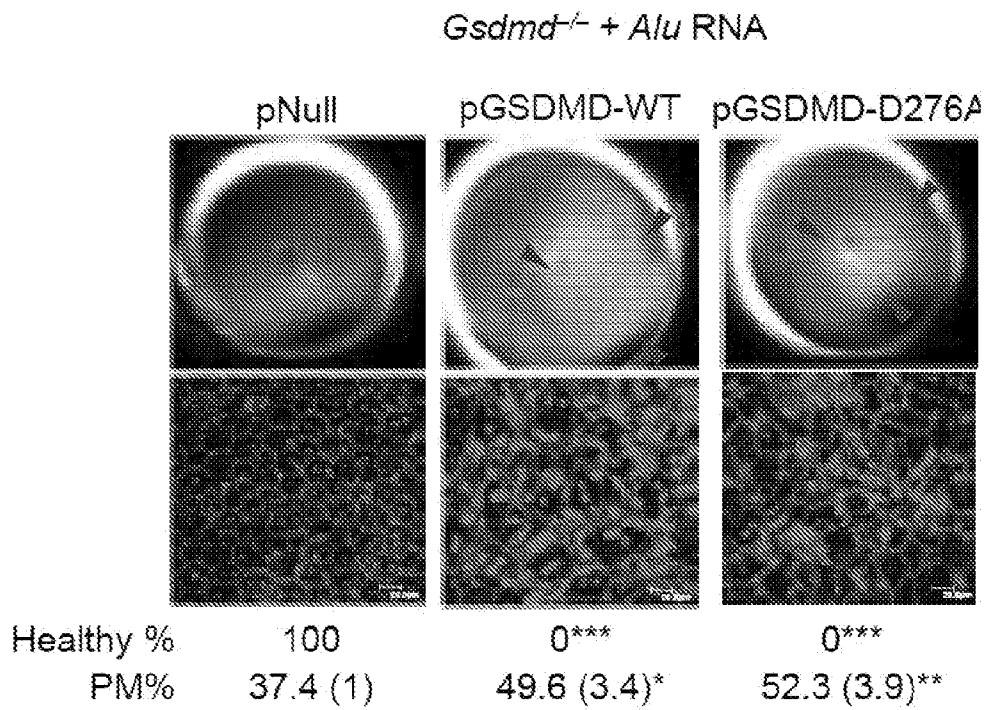


FIG. 2E

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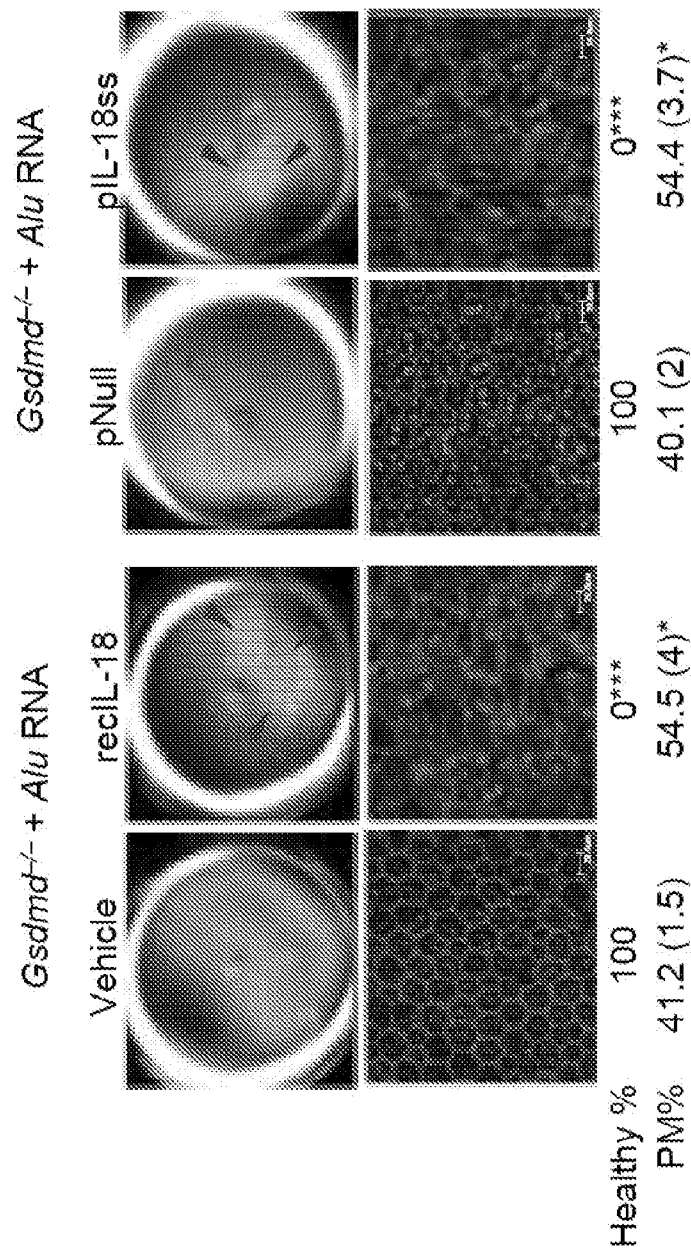


FIG. 2G

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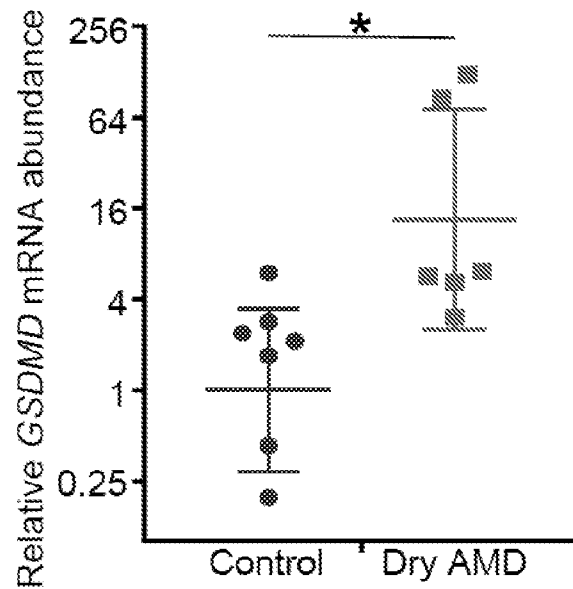


FIG. 2H

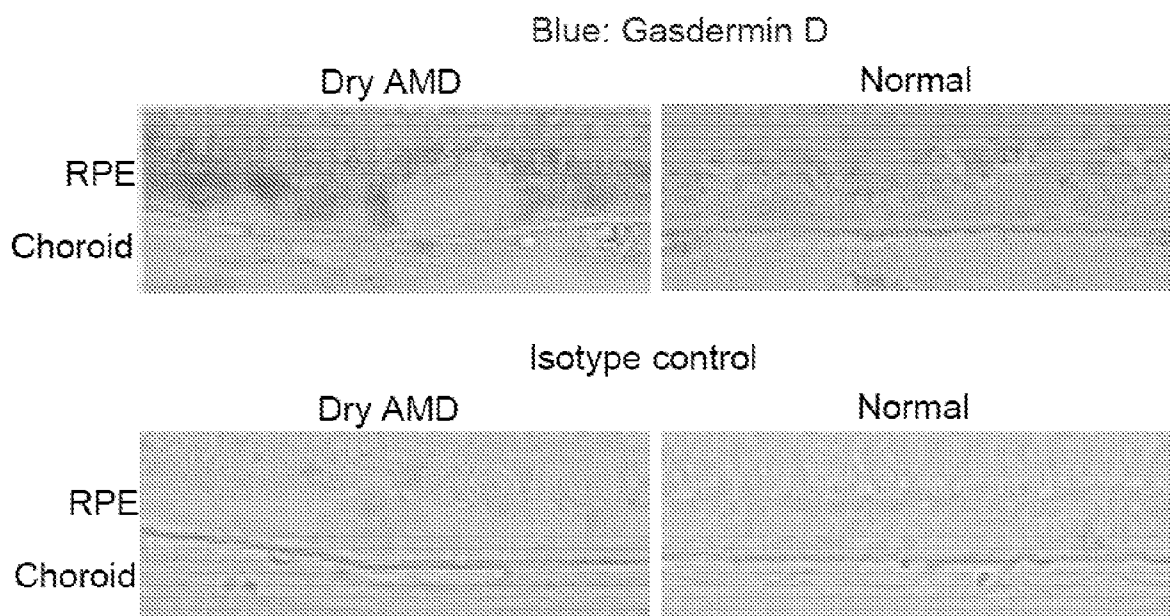


FIG. 2I

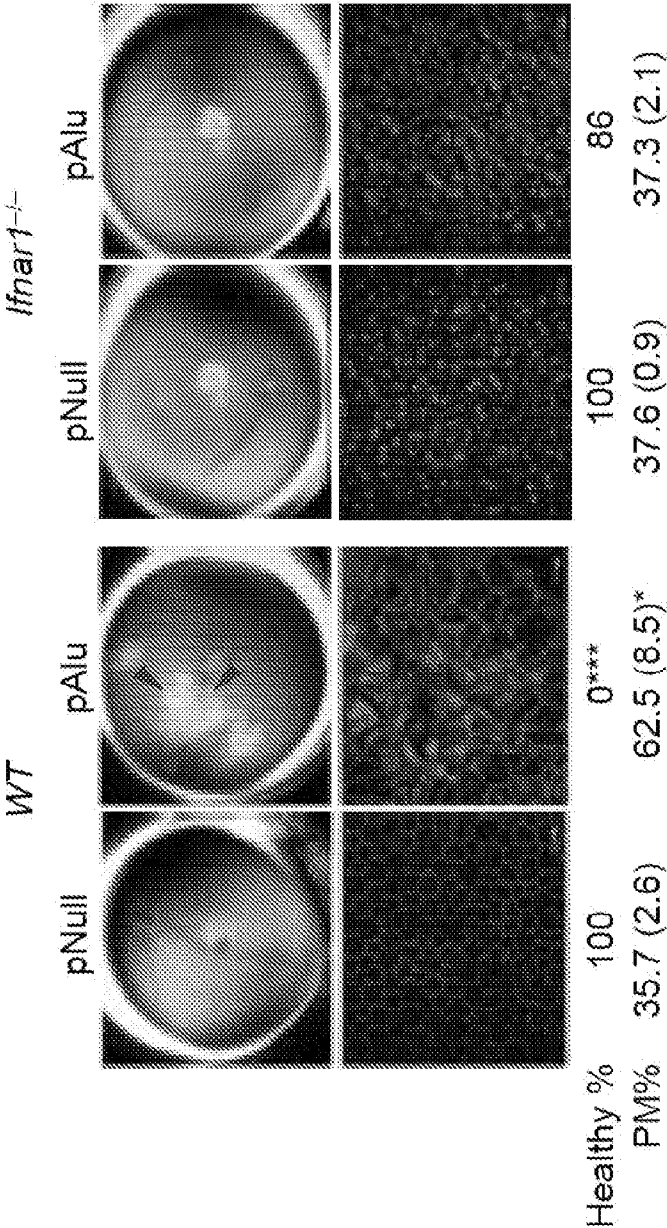


FIG. 3A

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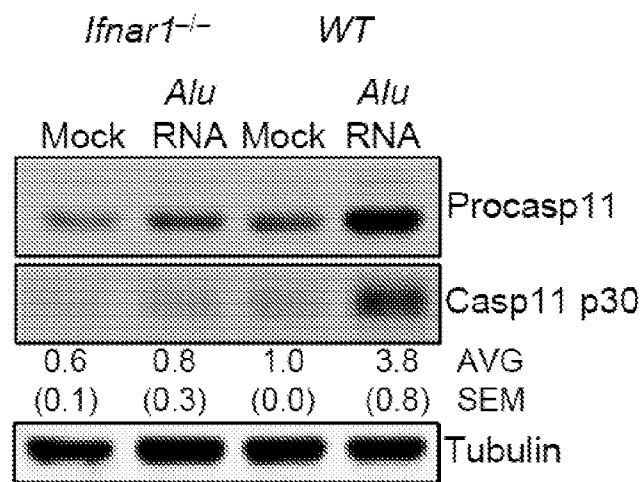


FIG. 3B

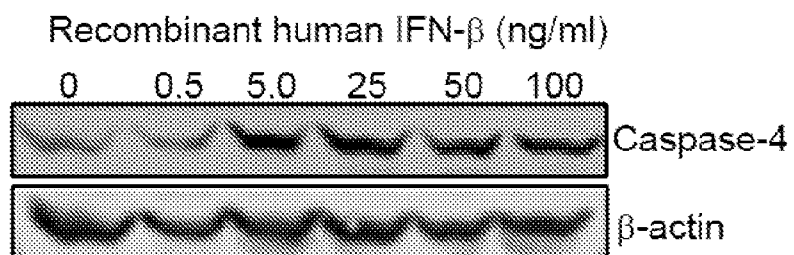


FIG. 3C

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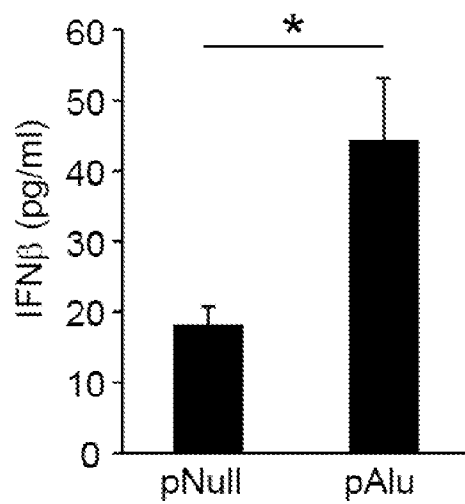


FIG. 3D

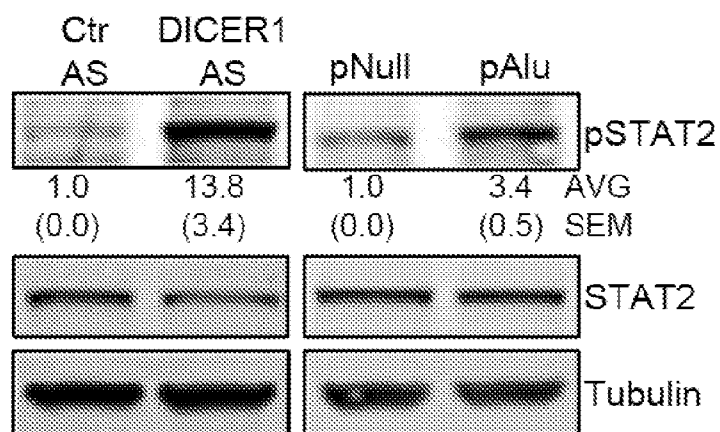


FIG. 3E

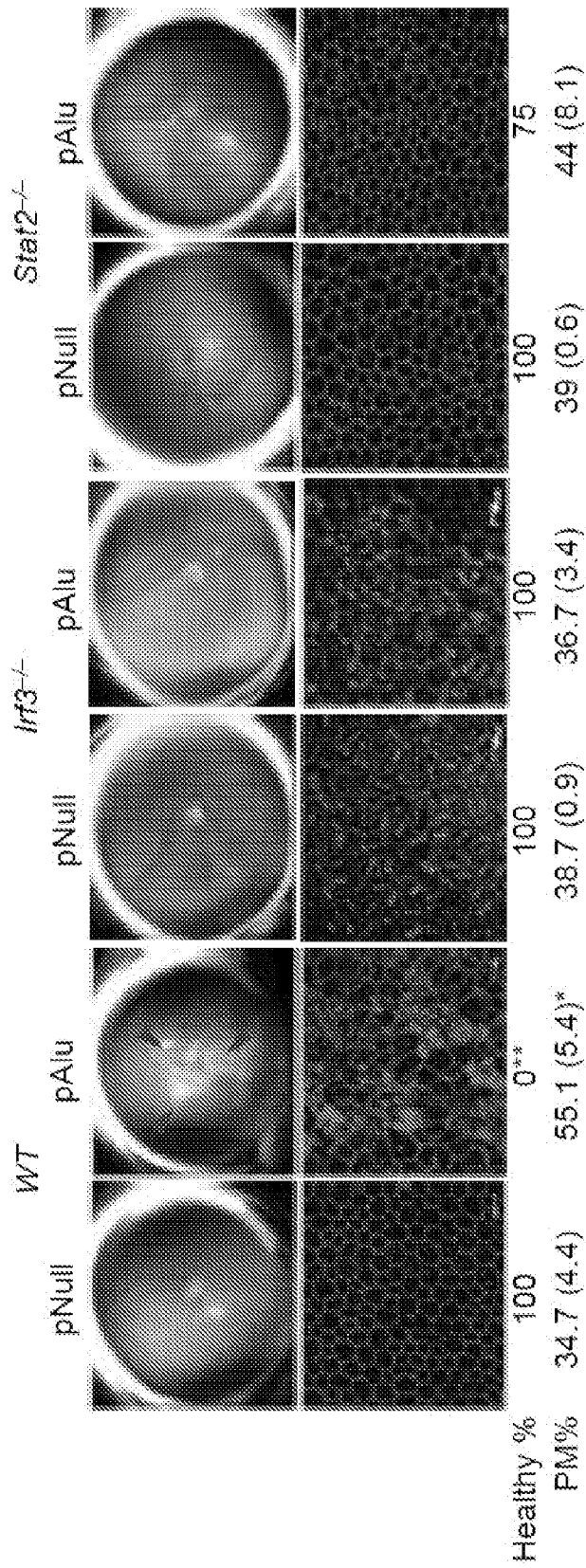
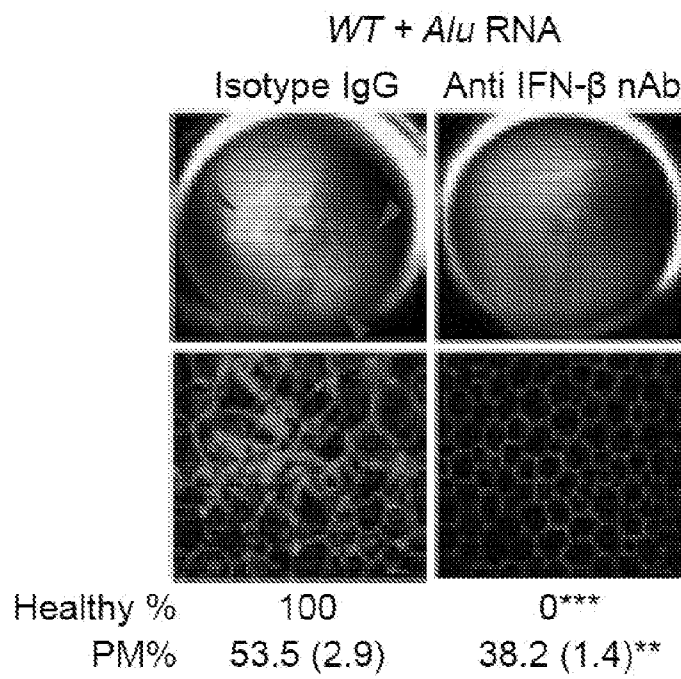


FIG. 3F

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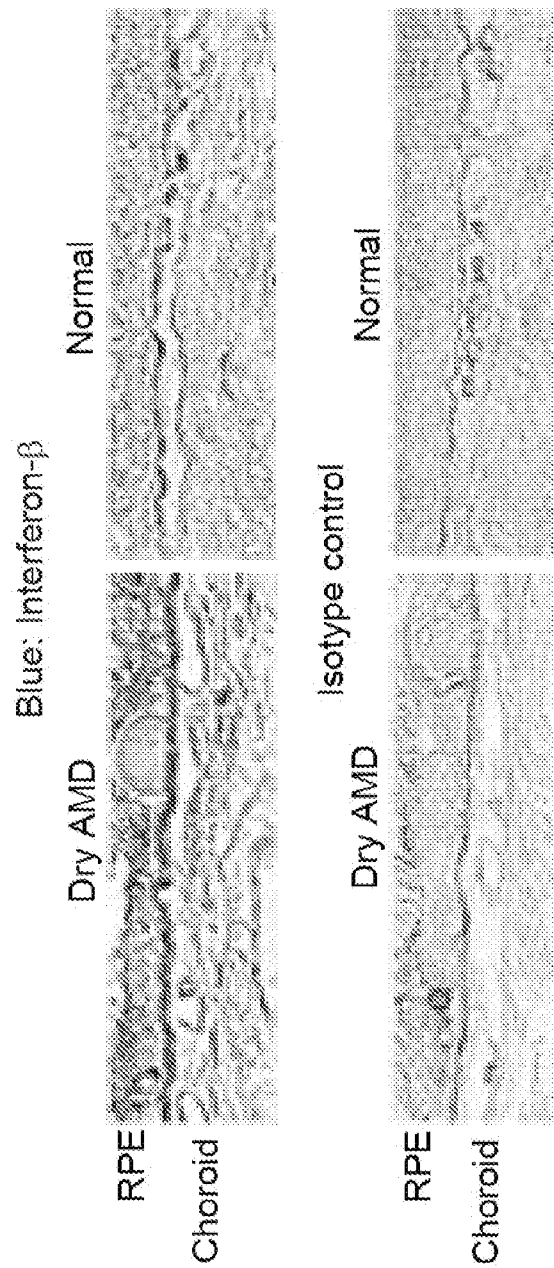


FIG. 3H

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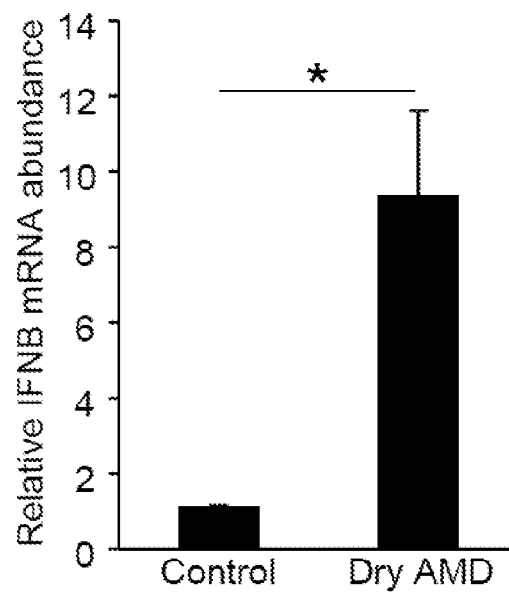


FIG. 3I

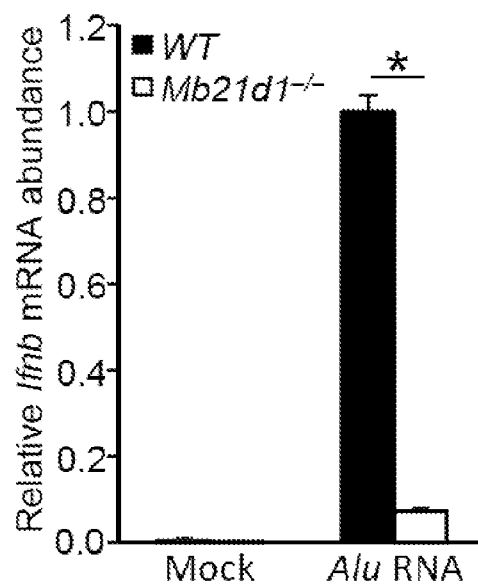


FIG. 4A

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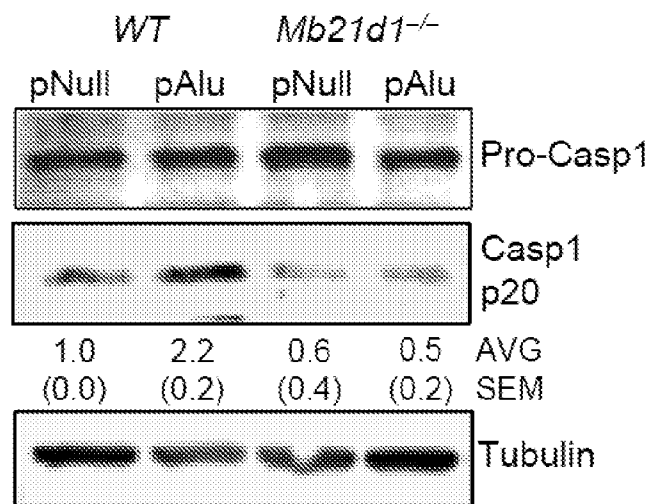


FIG. 4B

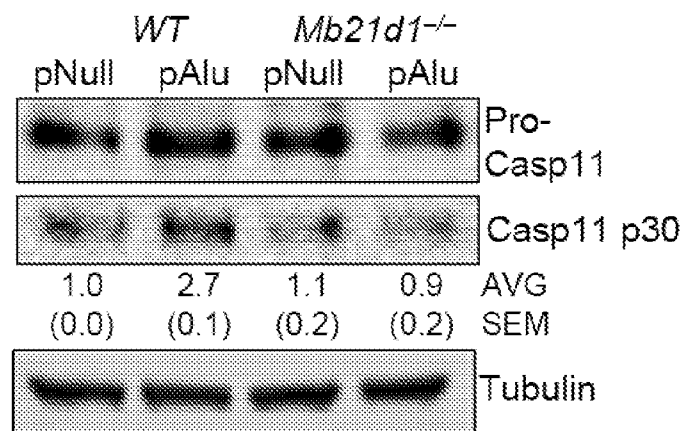


FIG. 4C

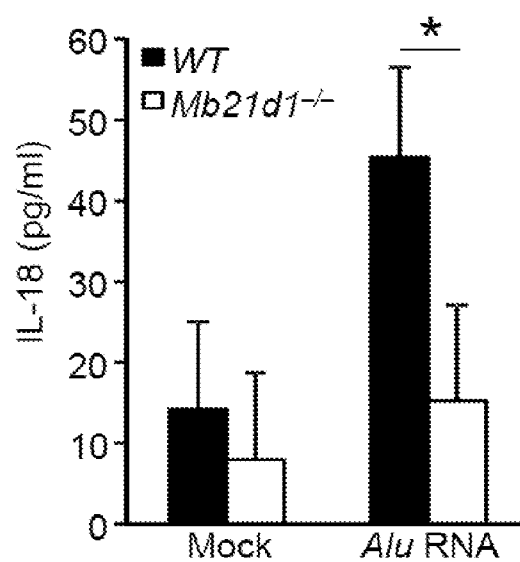


FIG. 4D

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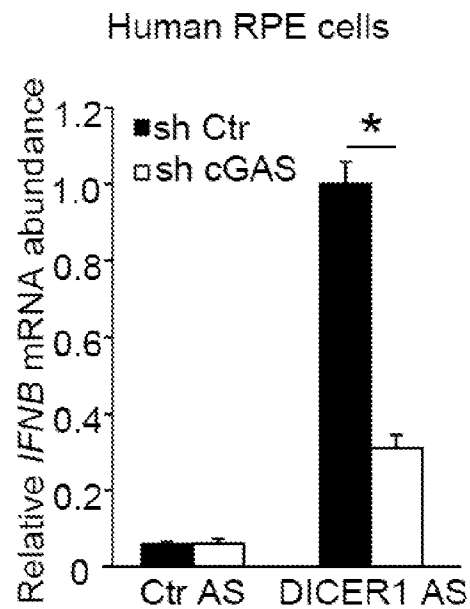


FIG. 4E

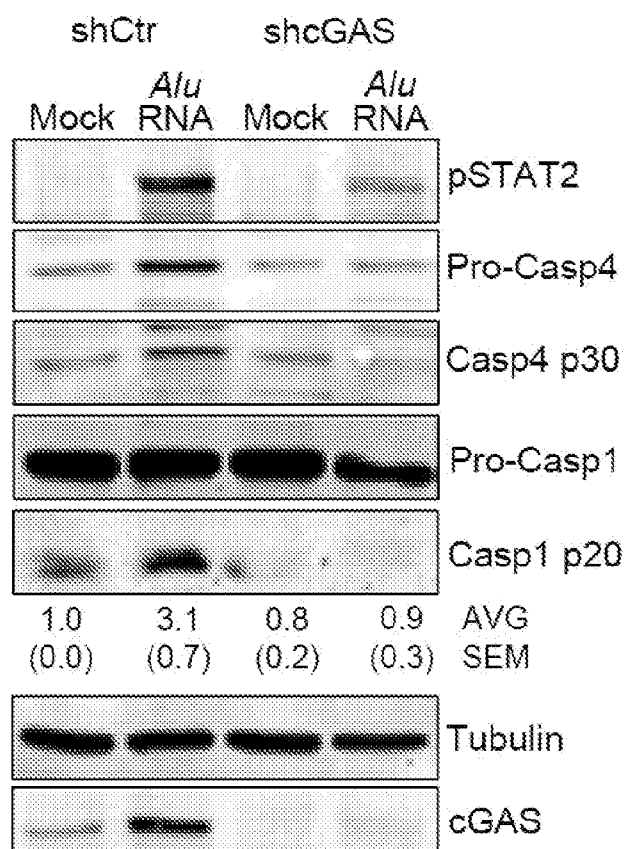
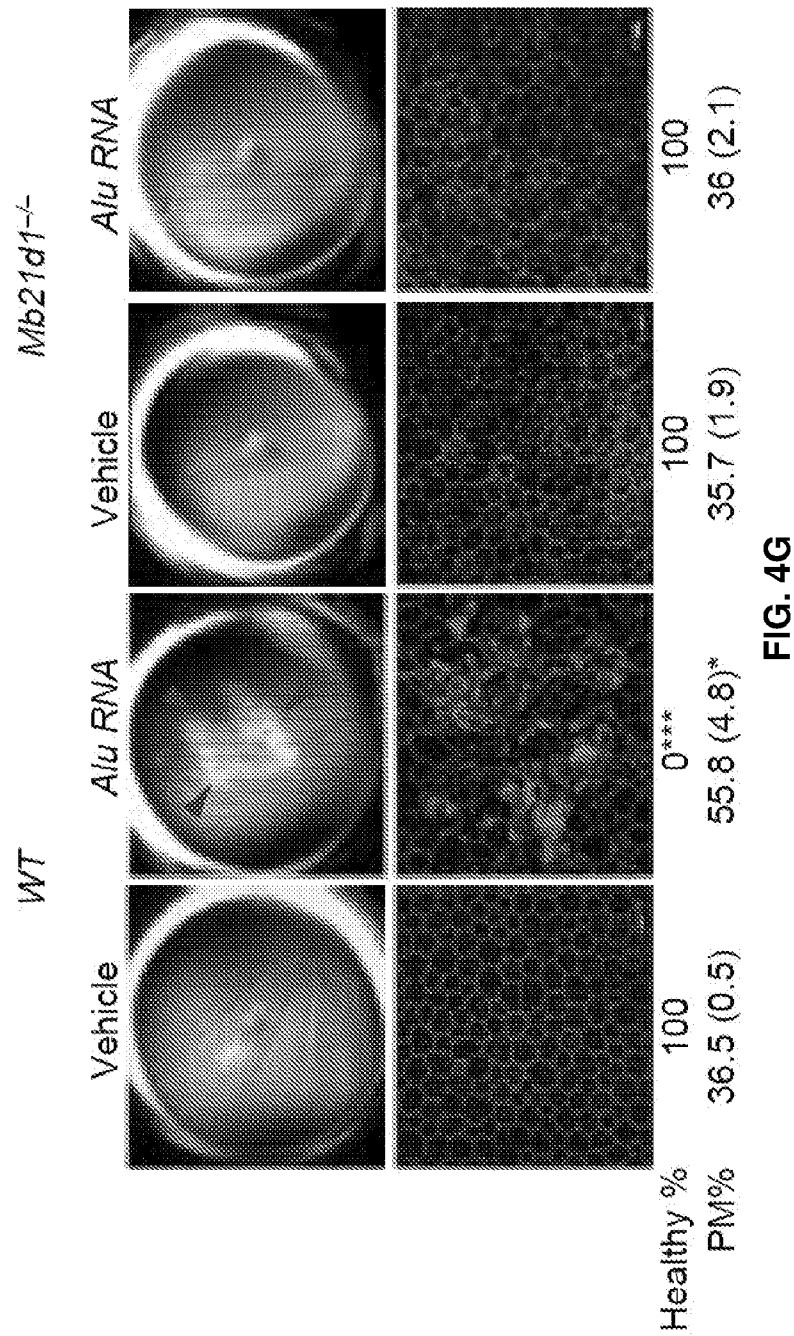


FIG. 4F



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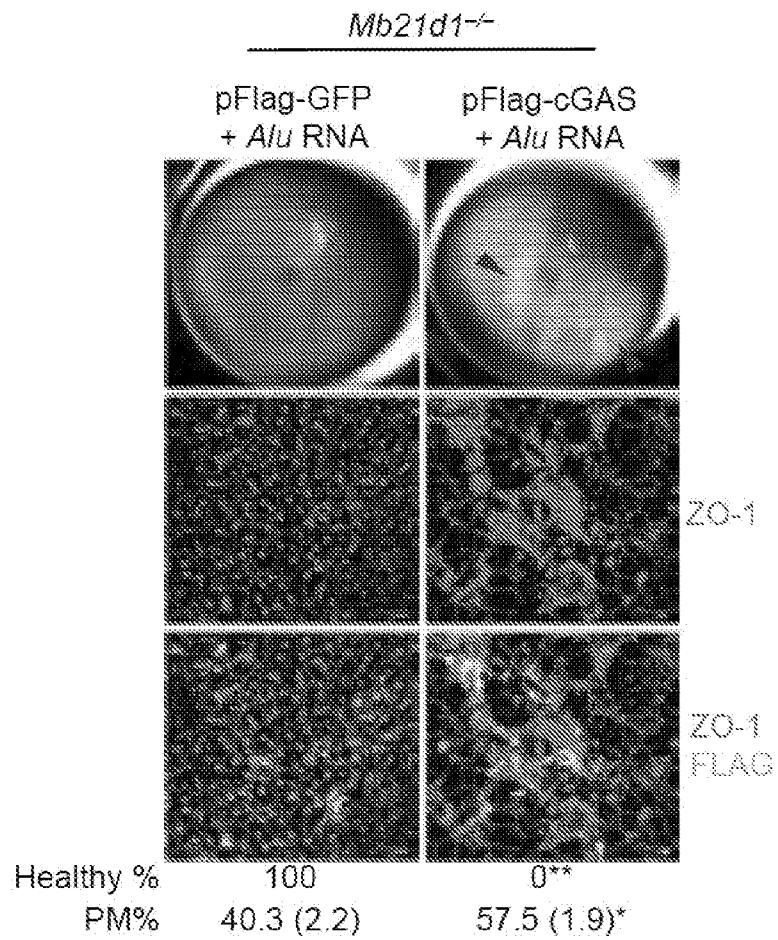
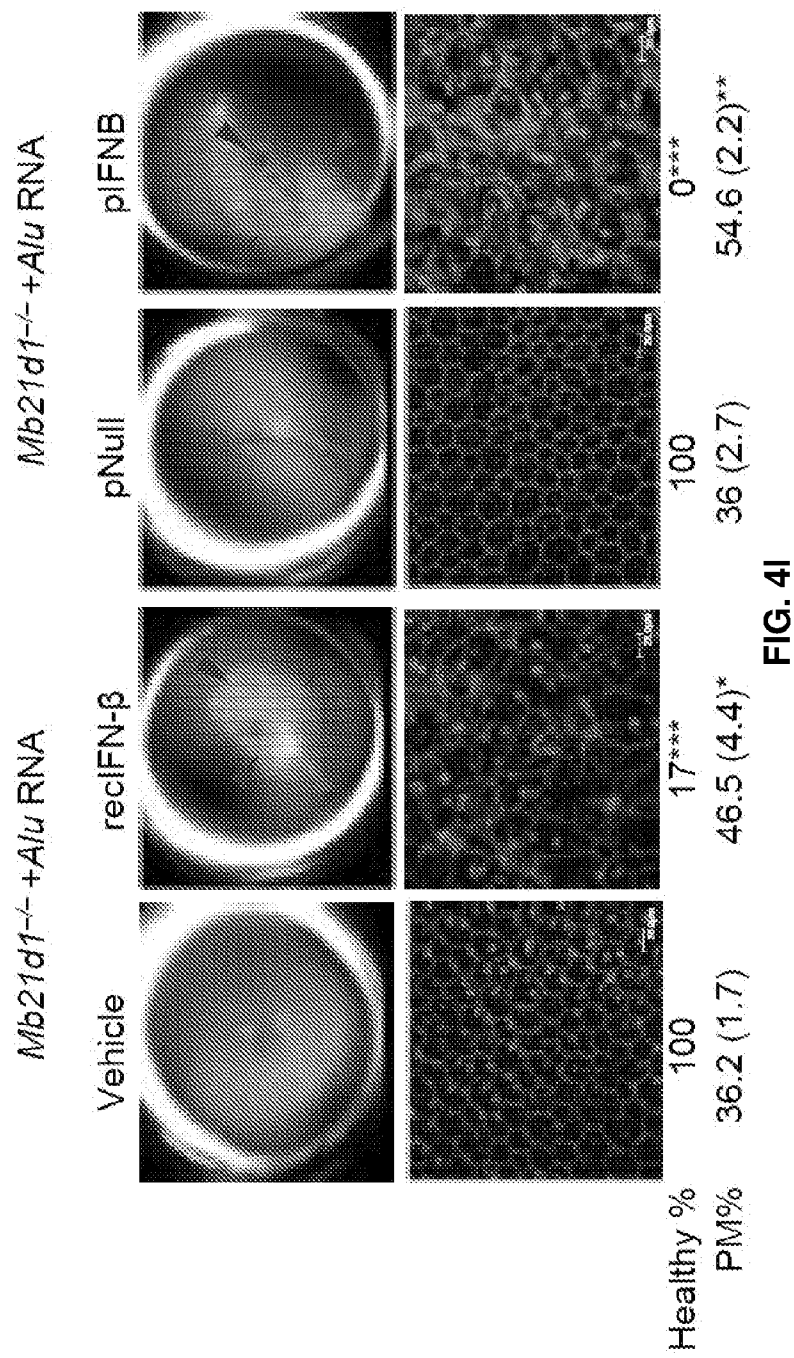


FIG. 4H



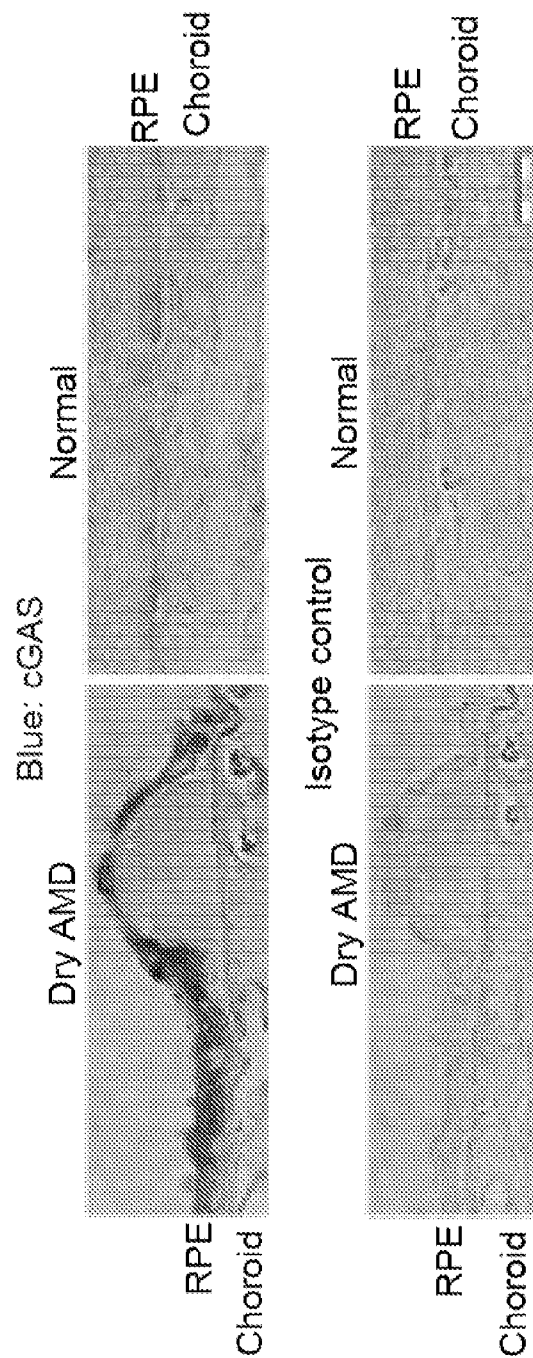


FIG. 5A

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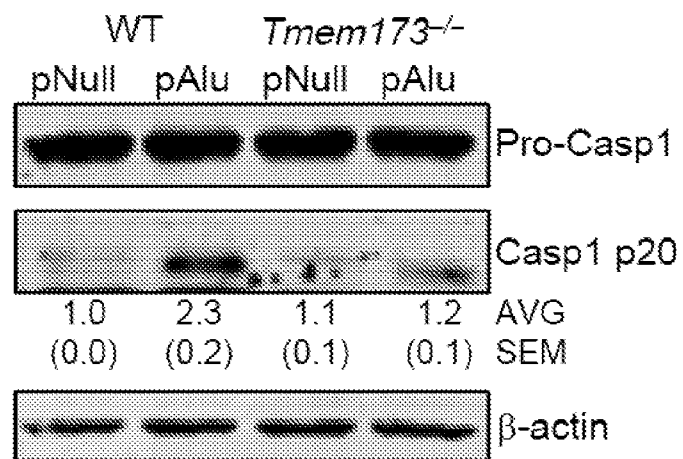


FIG. 5B

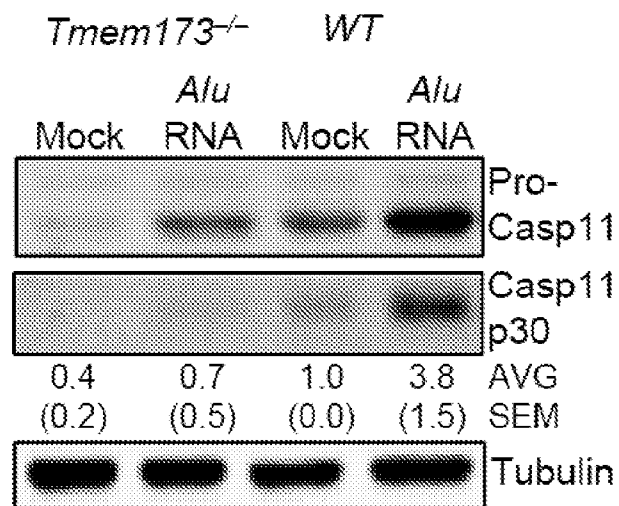
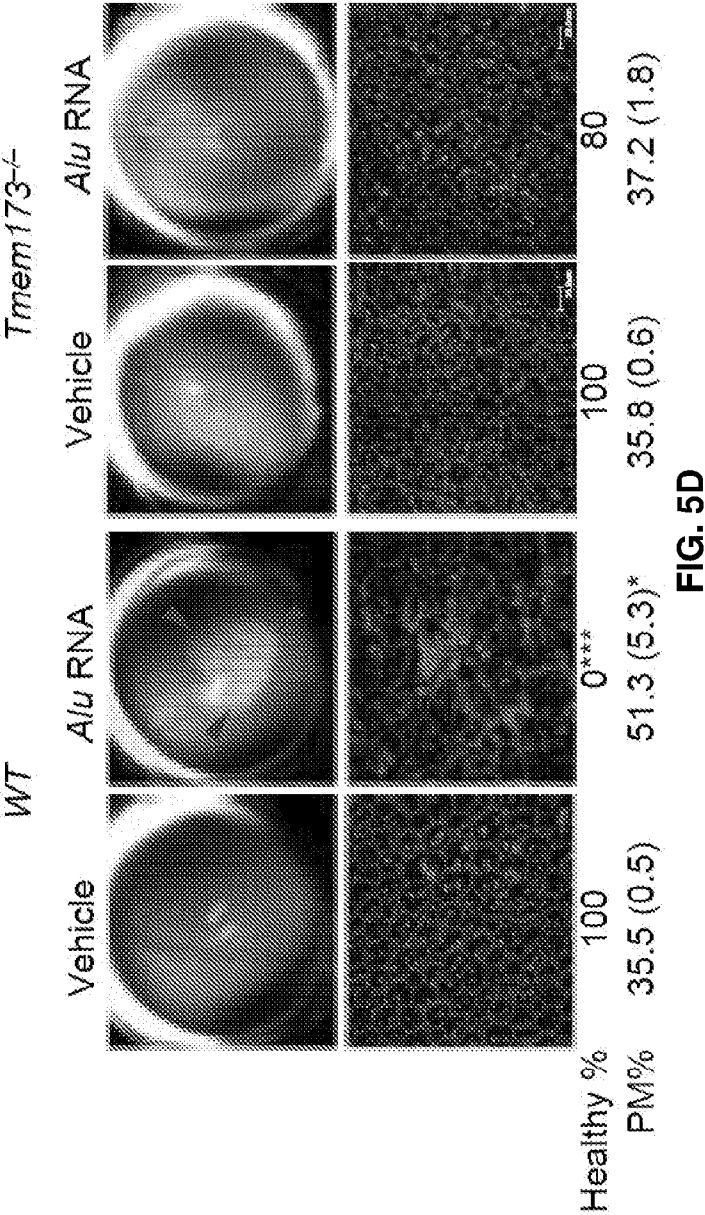
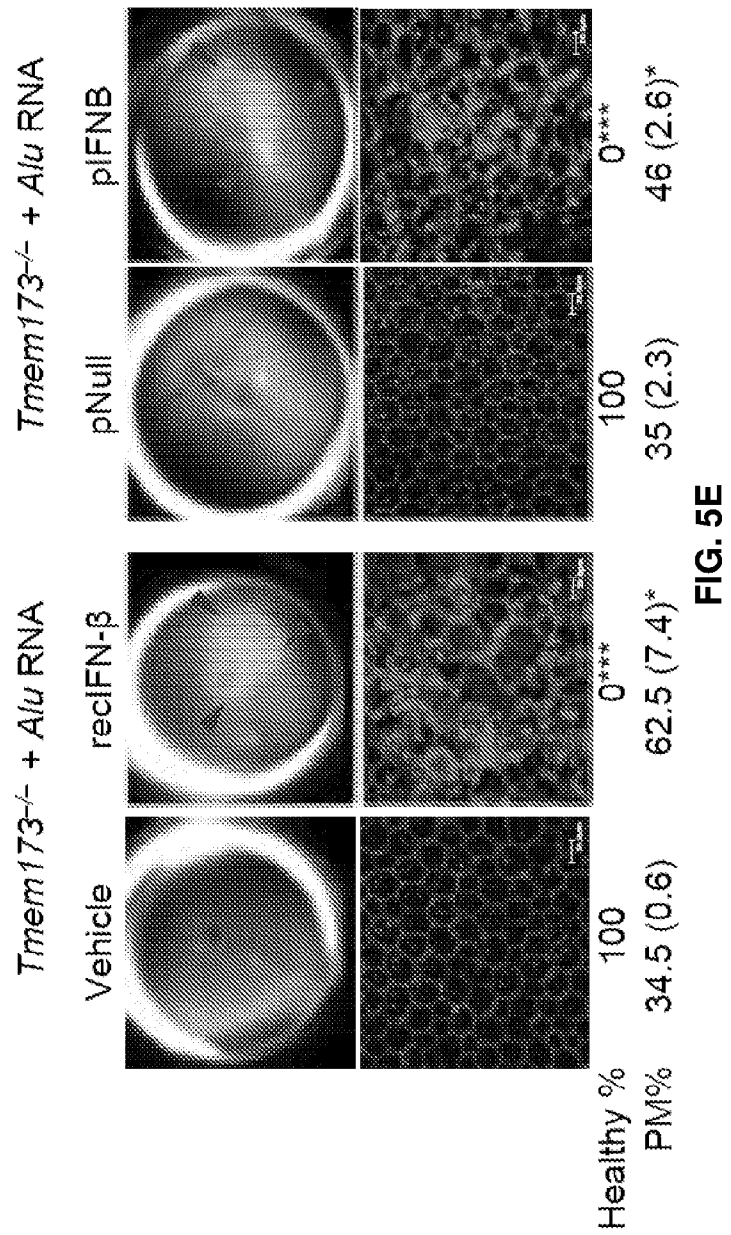


FIG. 5C





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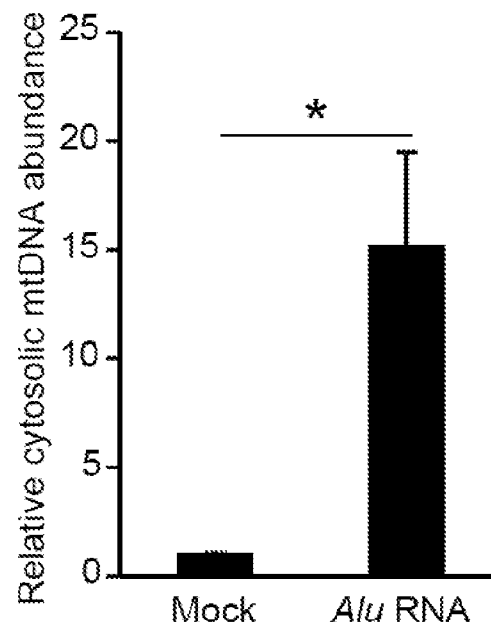


FIG. 6A

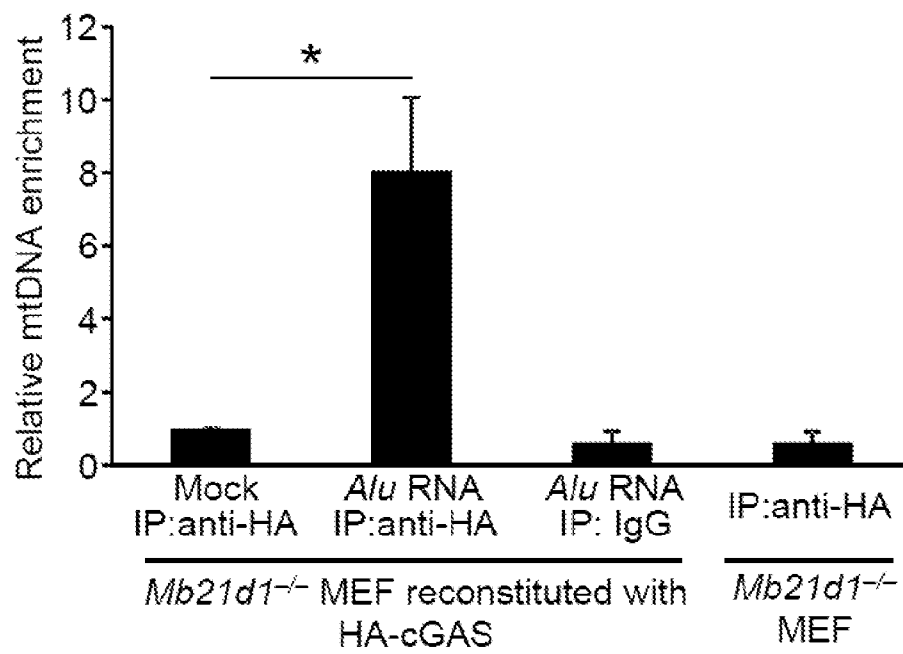


FIG. 6B

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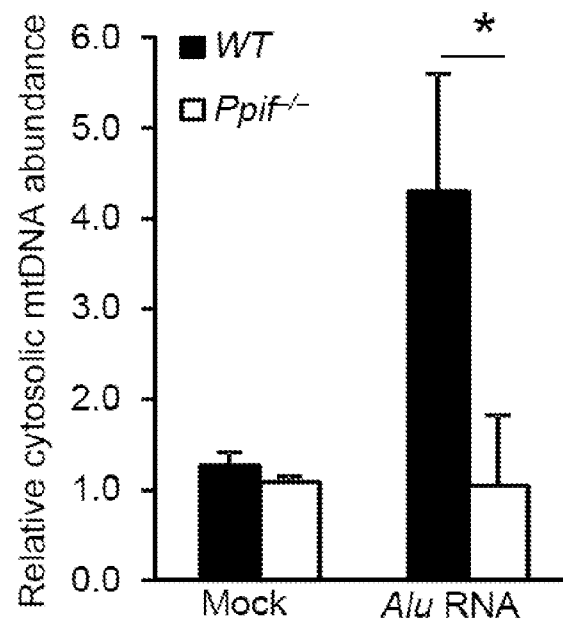


FIG. 6C

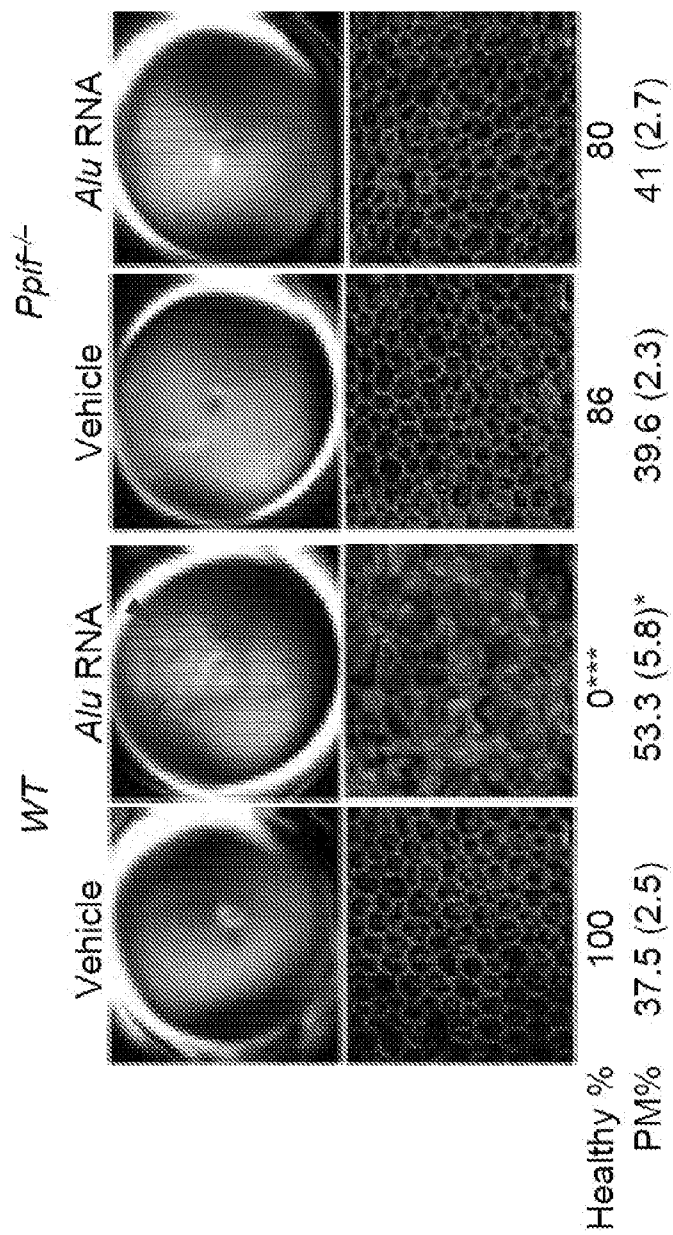


FIG. 6D

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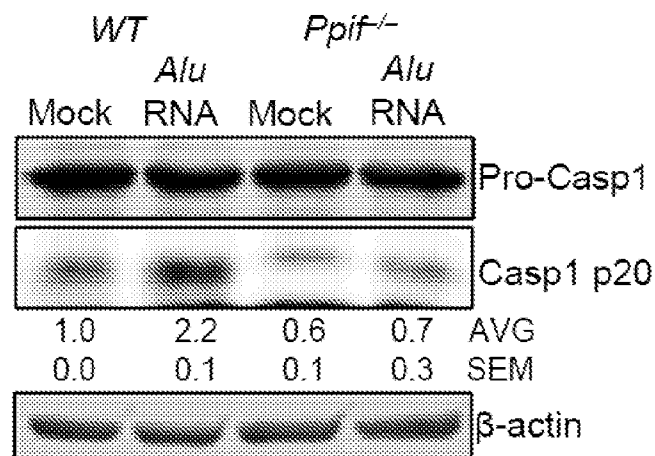


FIG. 6E

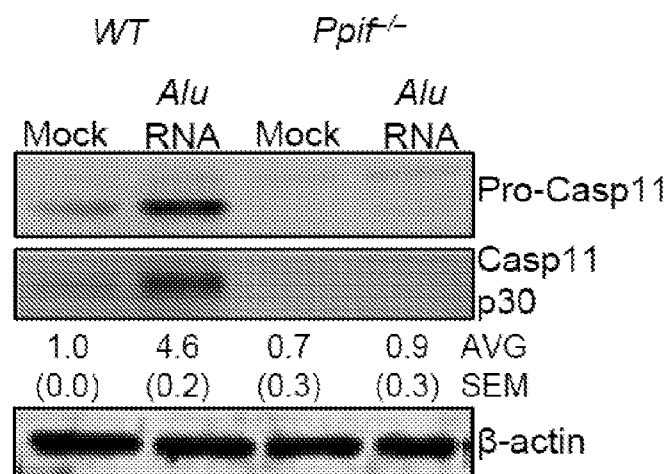


FIG. 6F

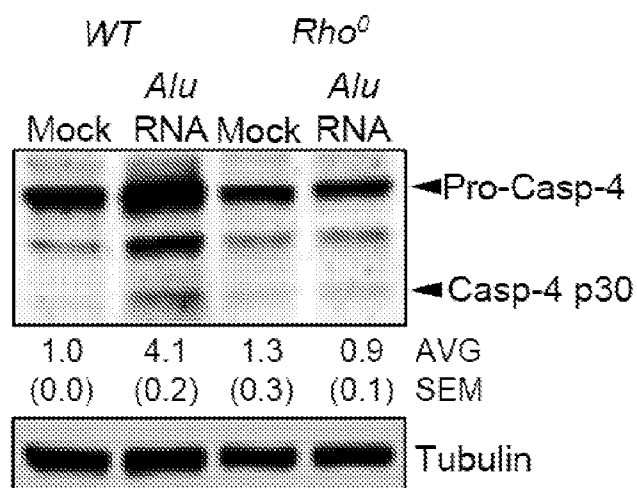


FIG. 6G

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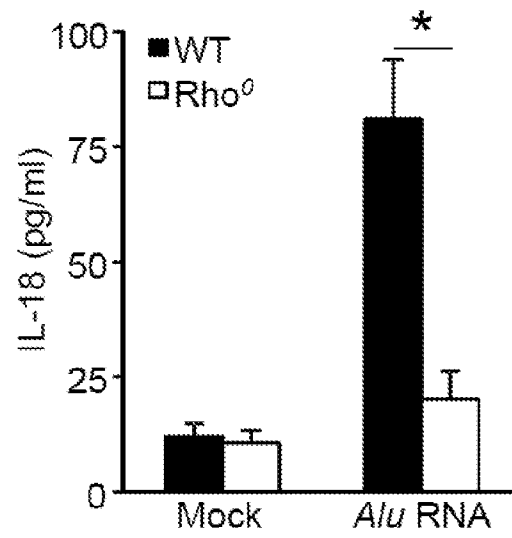


FIG. 6H

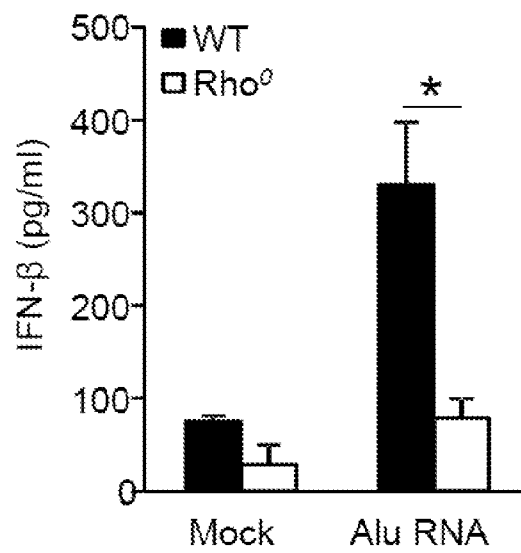


FIG. 6I

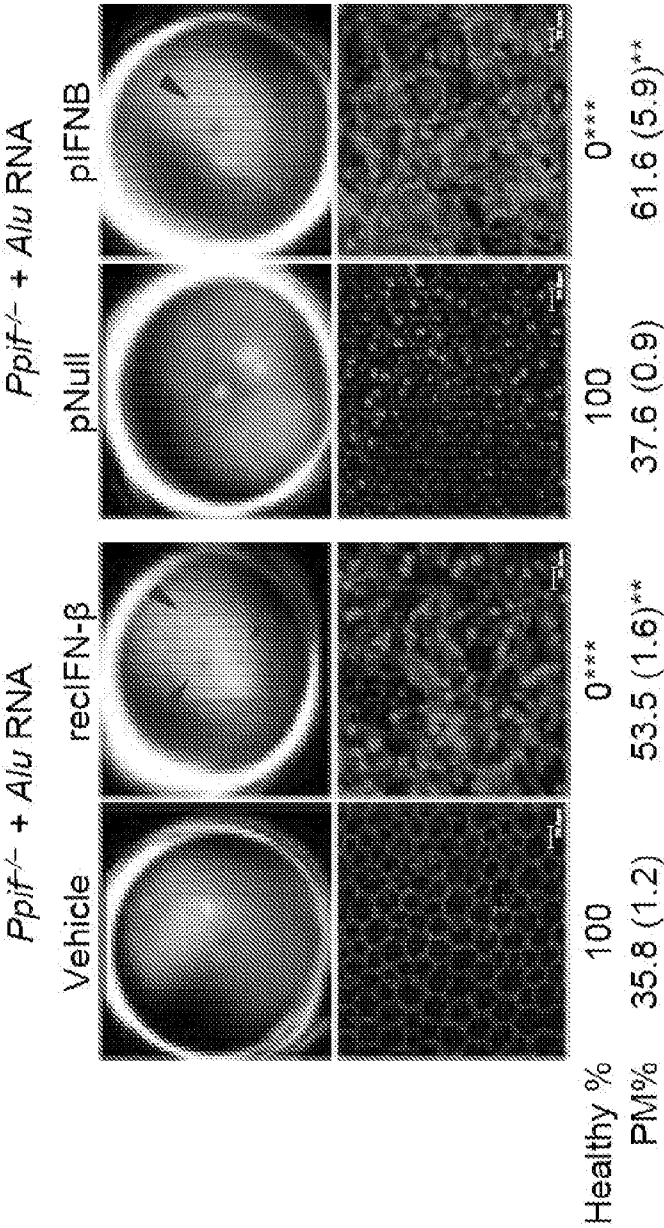


FIG. 6J

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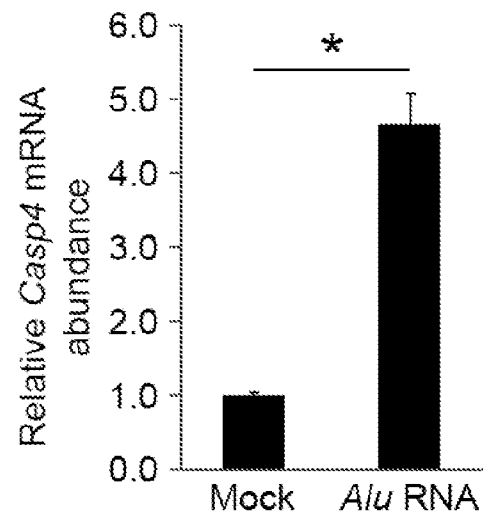


FIG. 7A

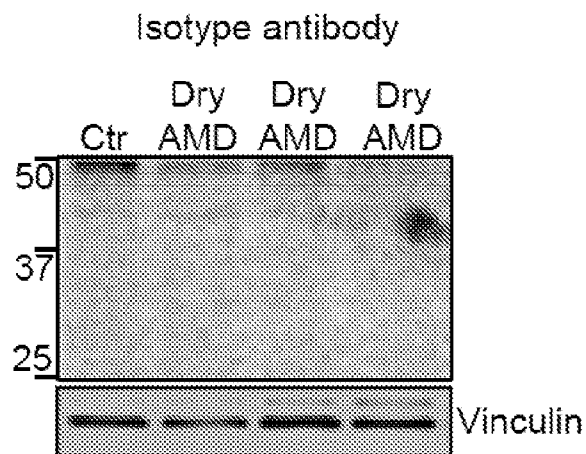


FIG. 7B

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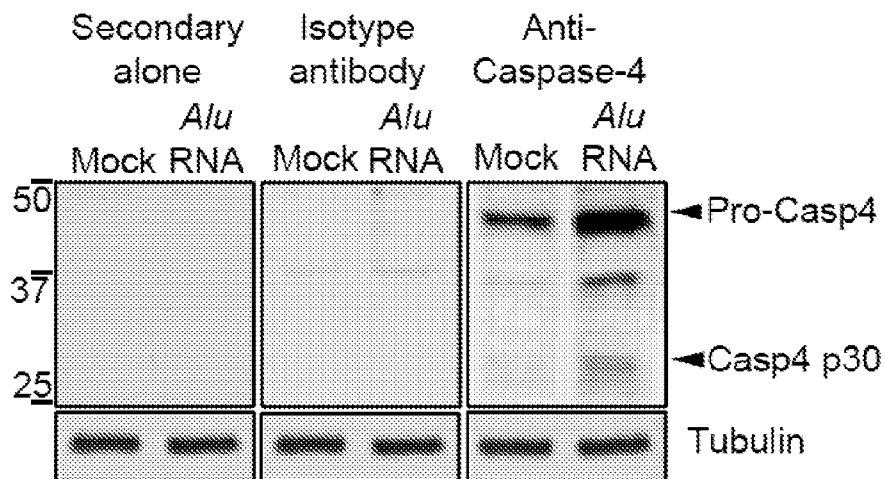


FIG. 7C

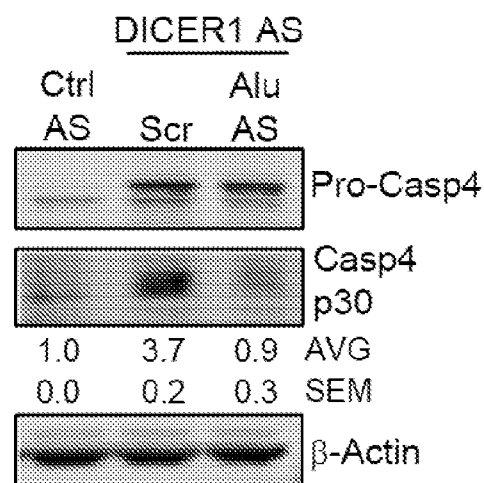


FIG. 7D

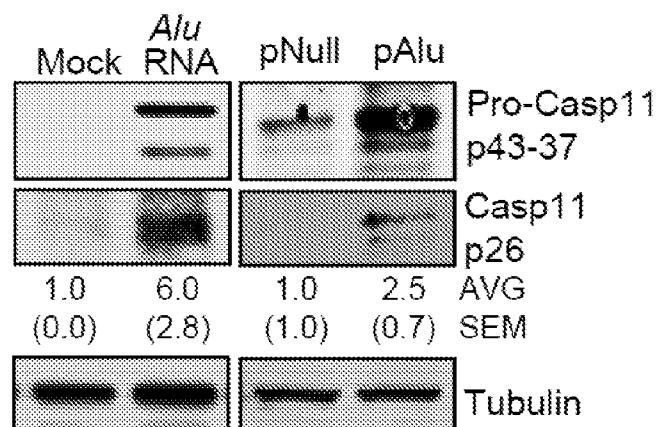
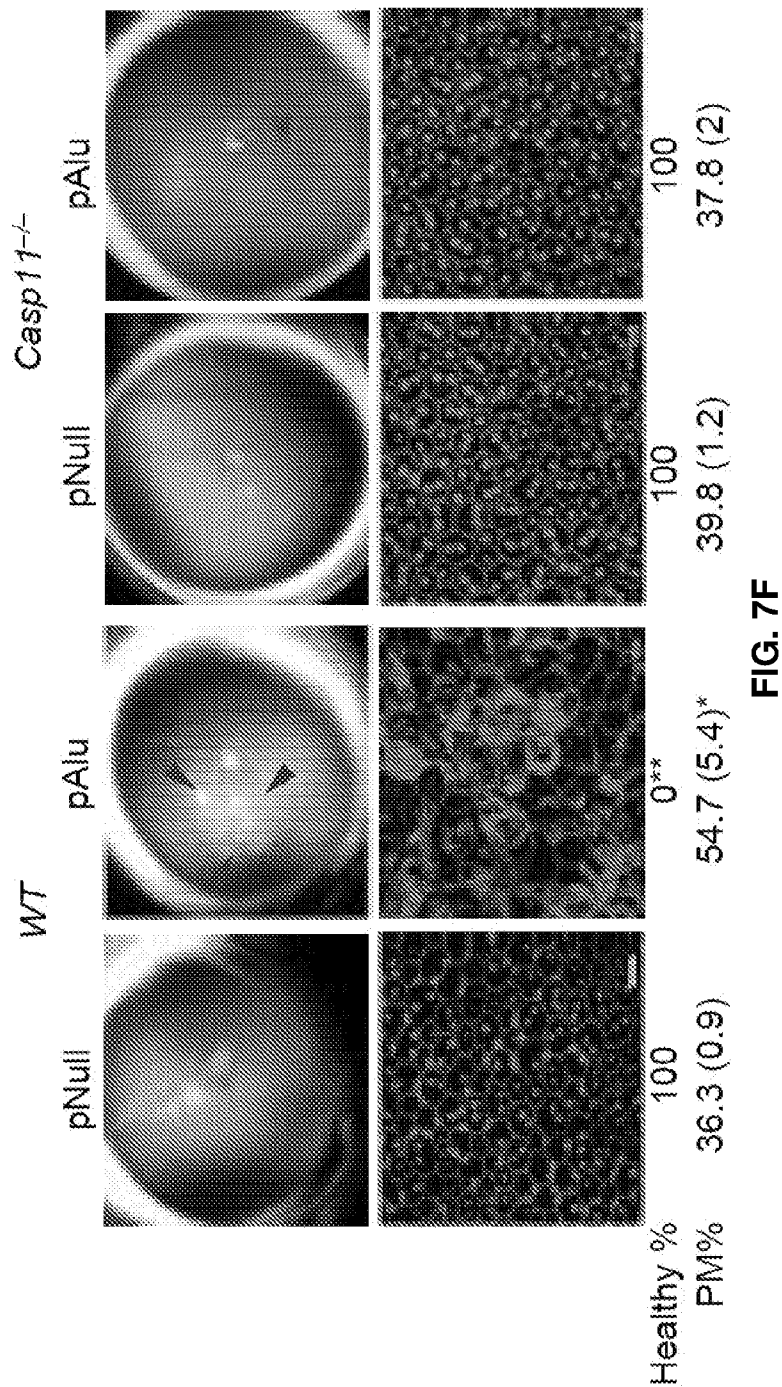


FIG. 7E



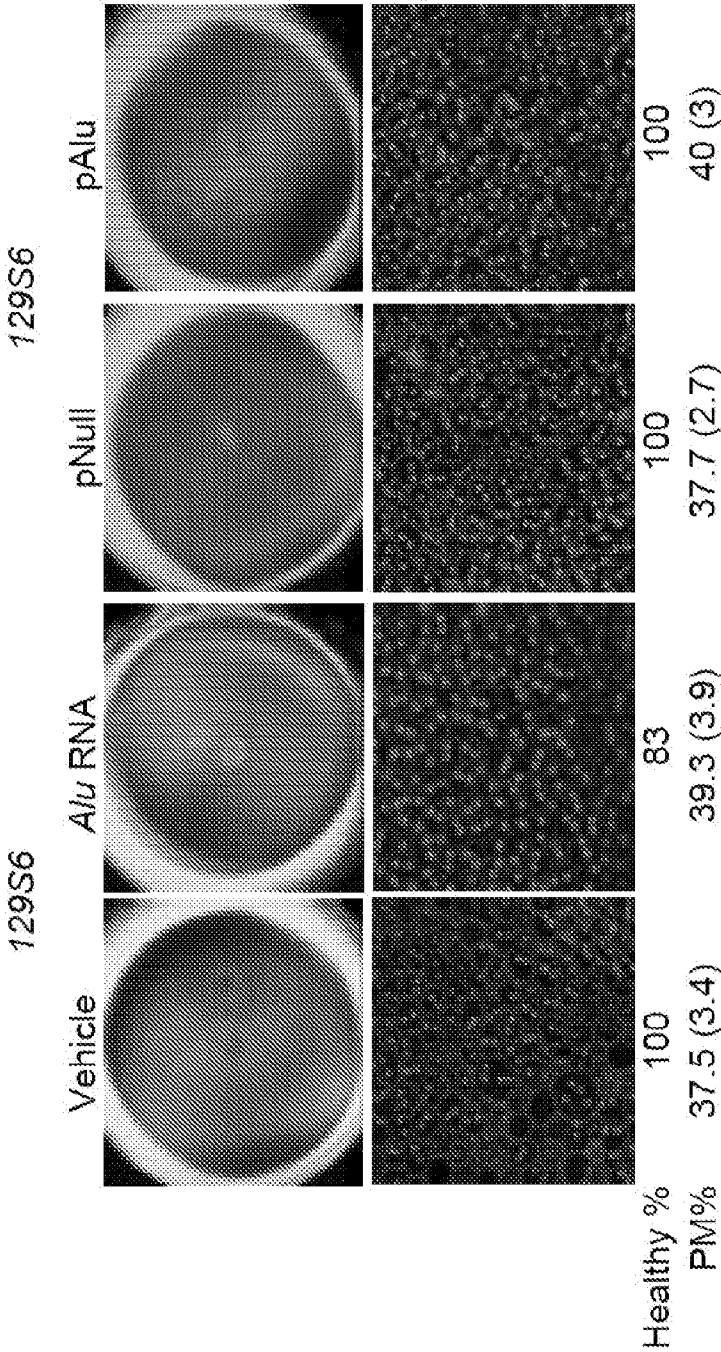


FIG. 7G

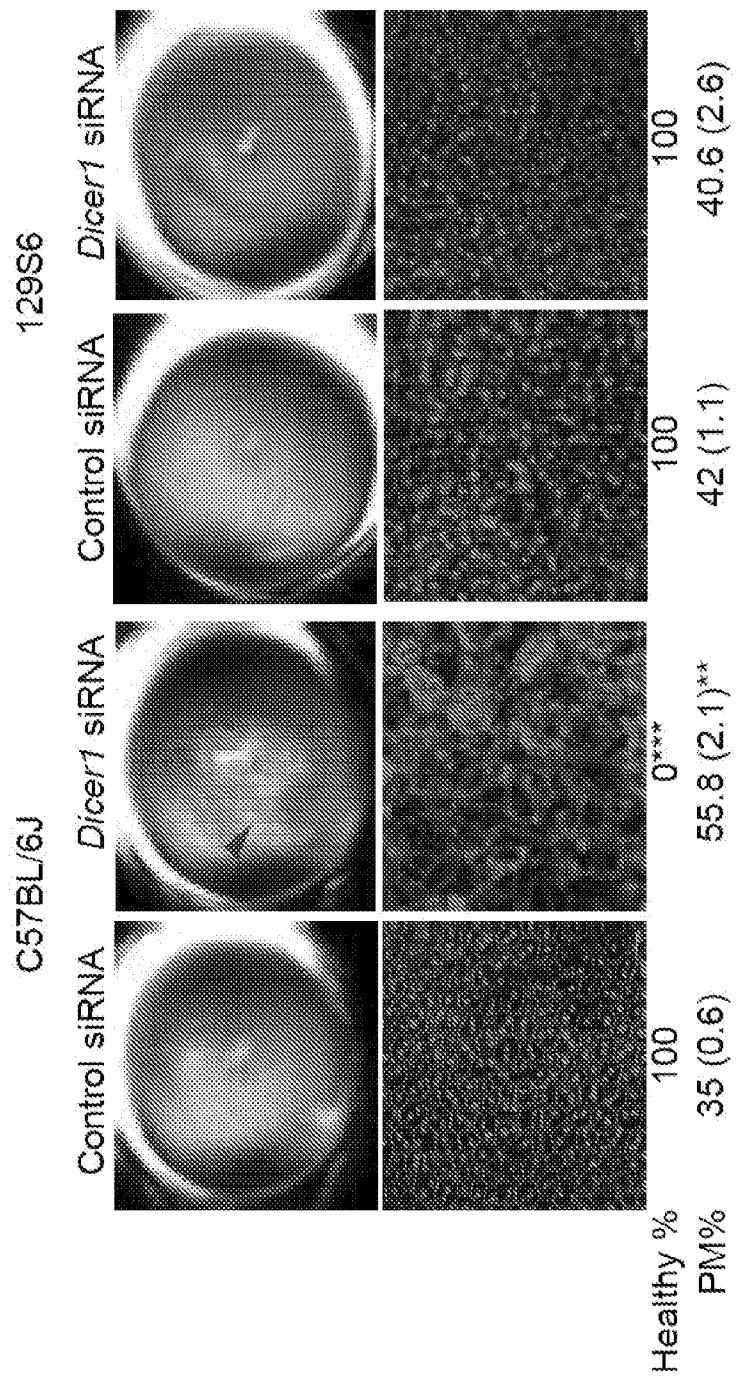


FIG. 7H

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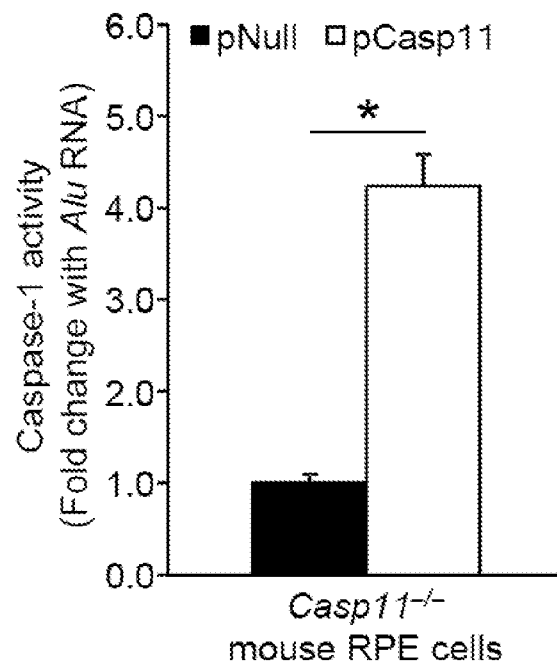


FIG. 8A

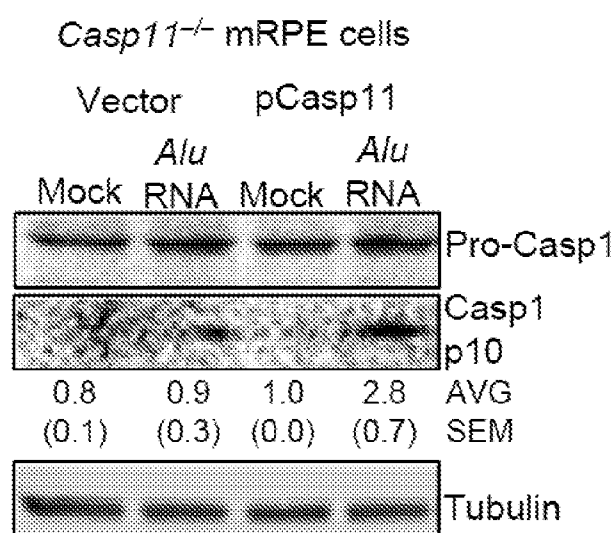


FIG. 8B

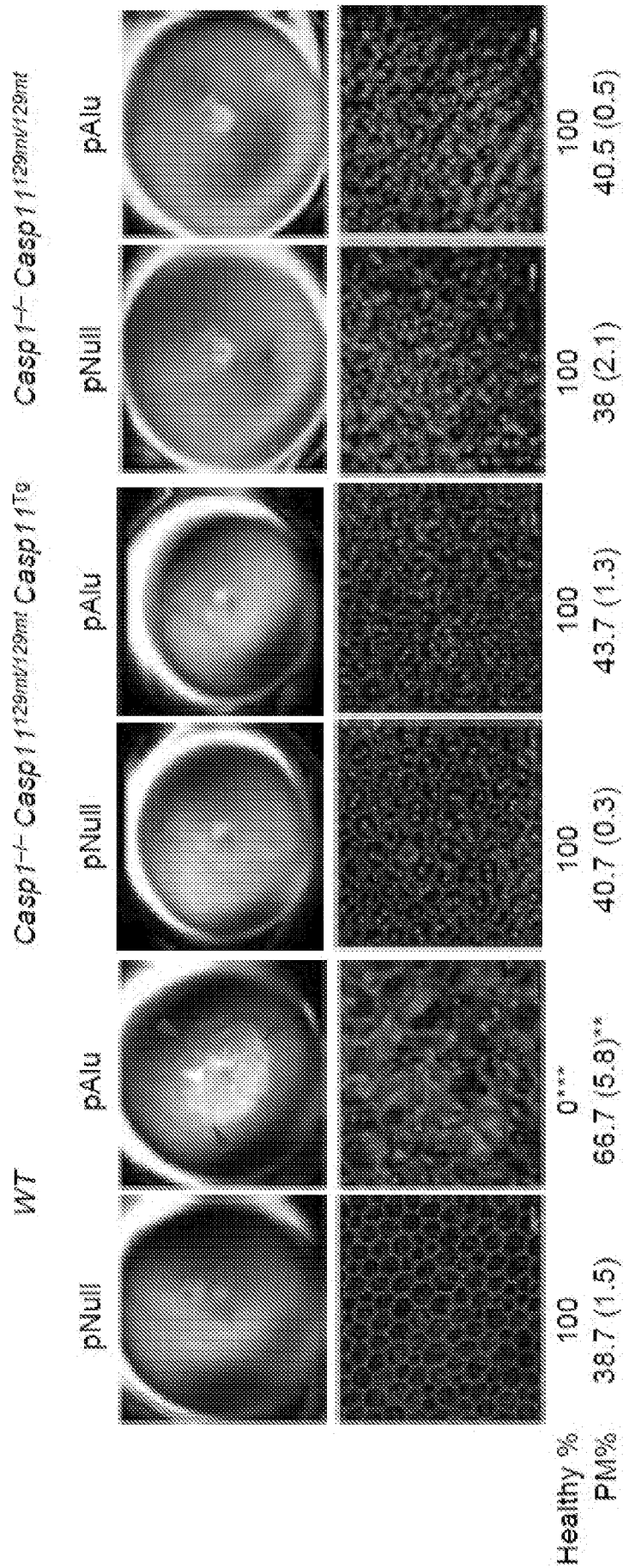


FIG. 8C

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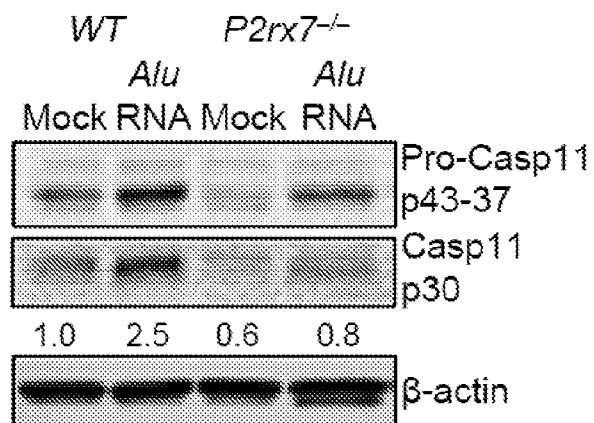


FIG. 8D

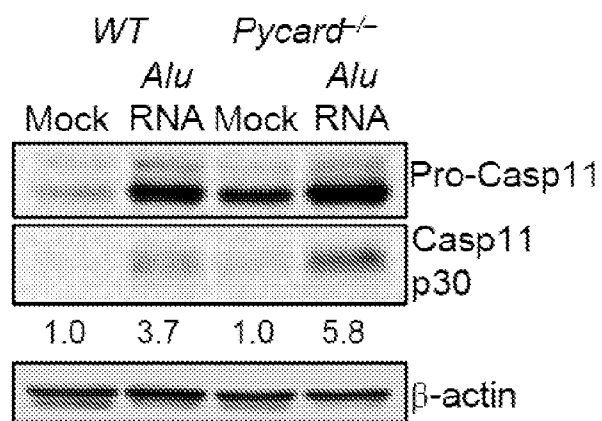


FIG. 8E

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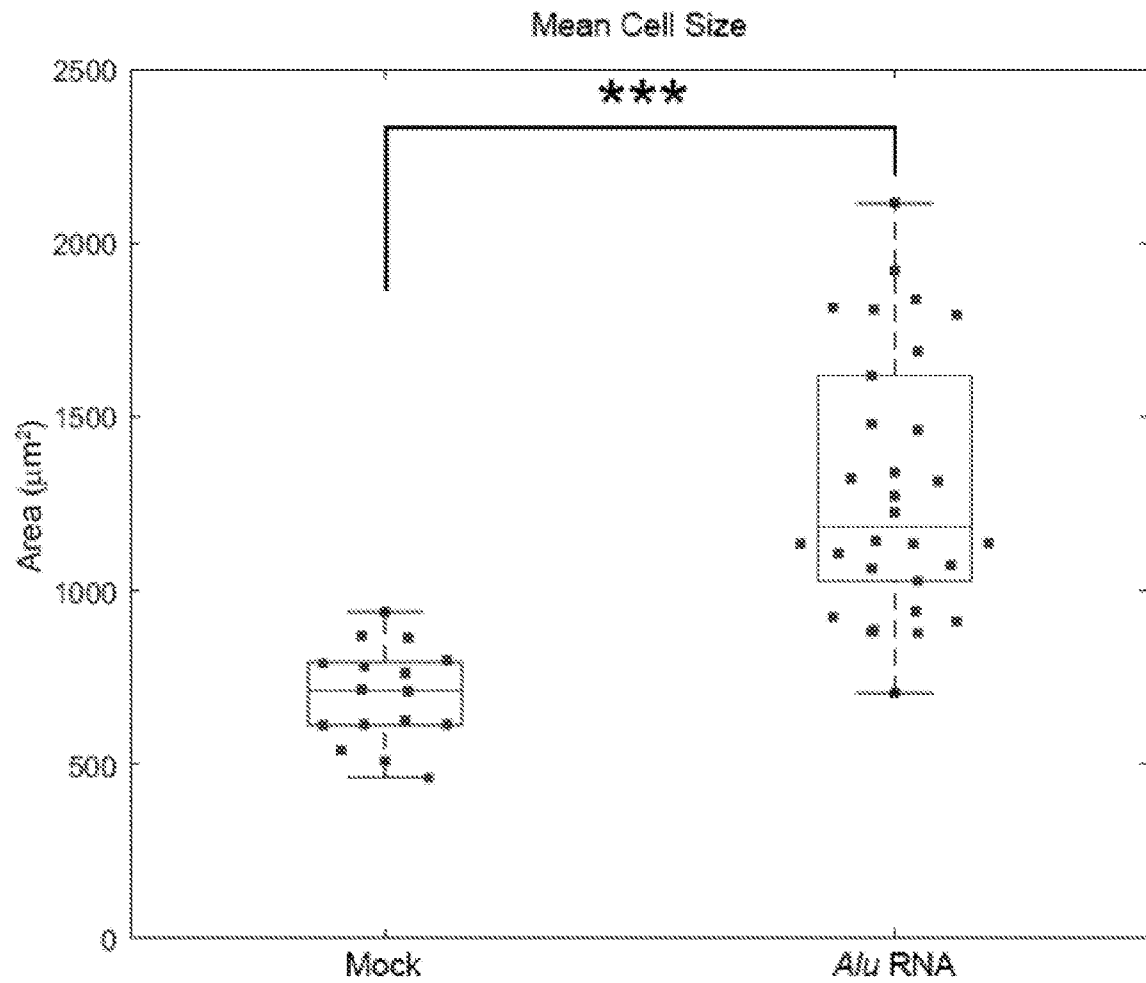


FIG. 9A

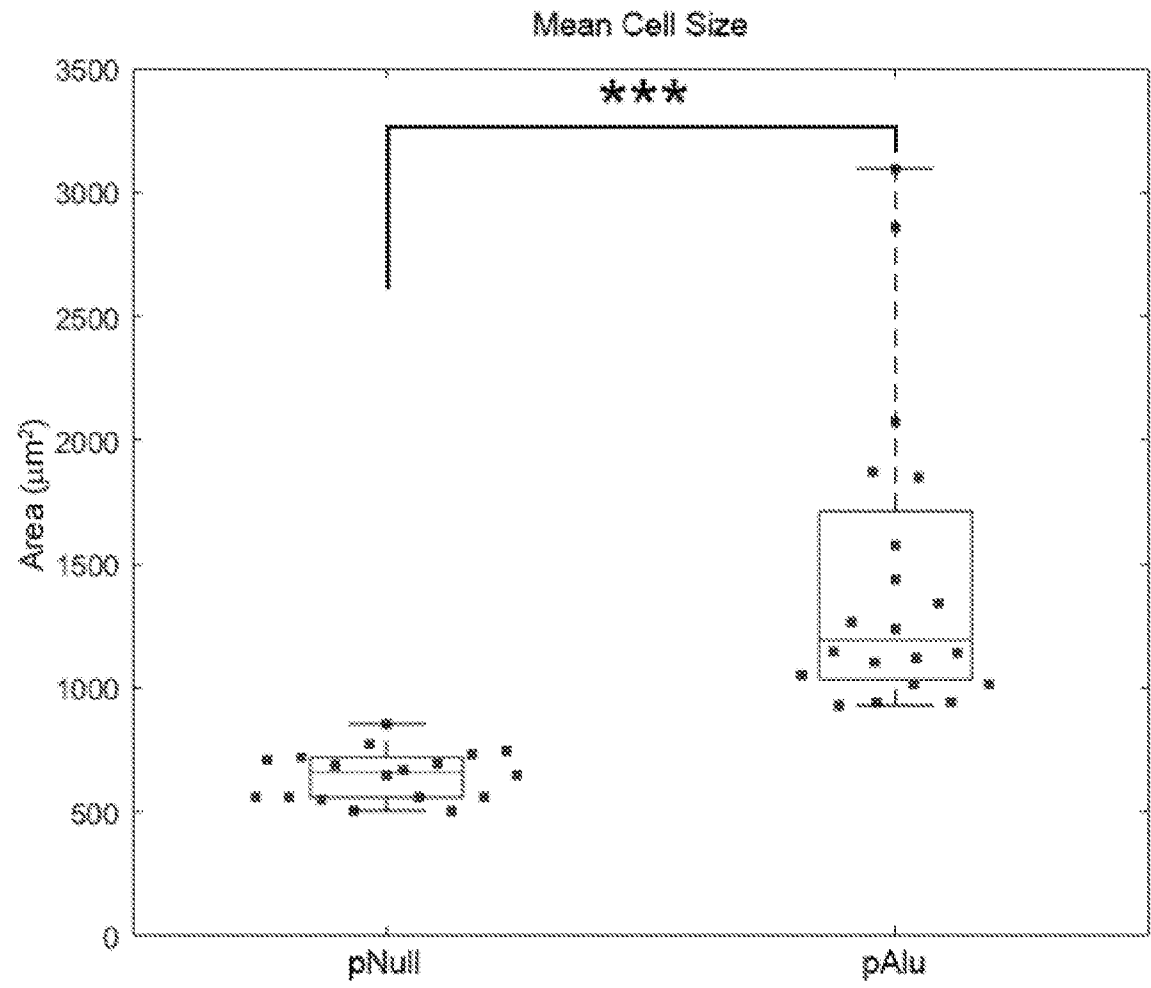


FIG. 9B

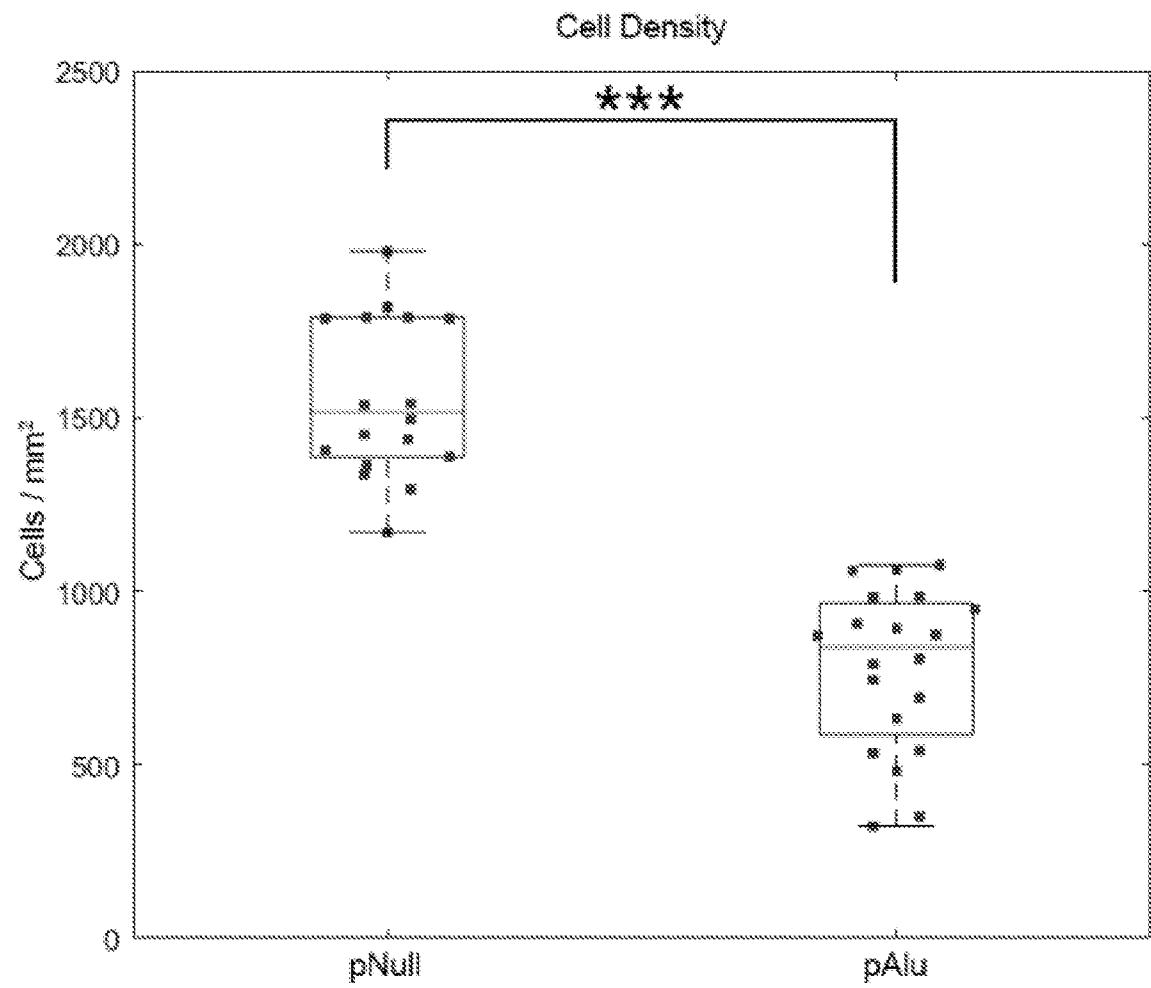


FIG. 9C

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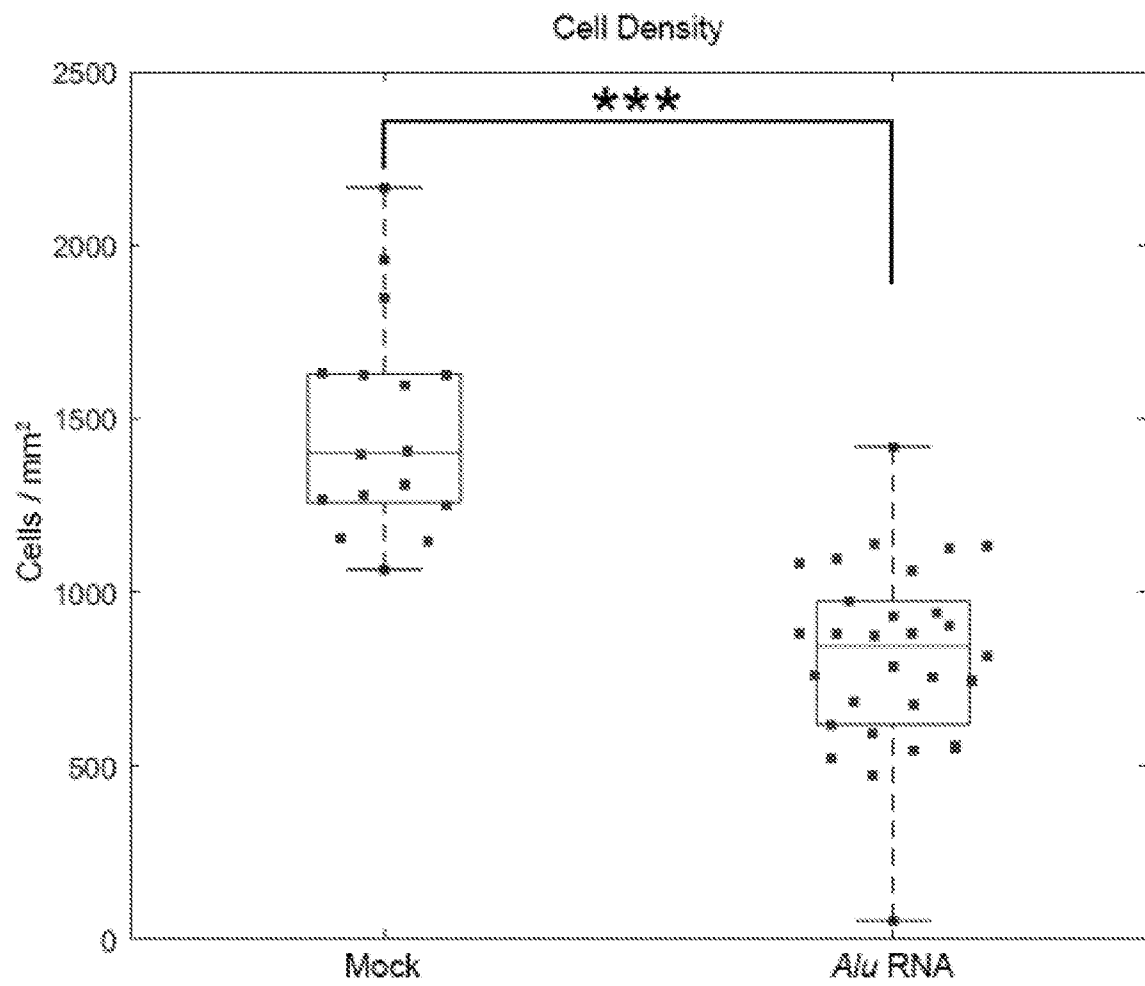


FIG. 9D

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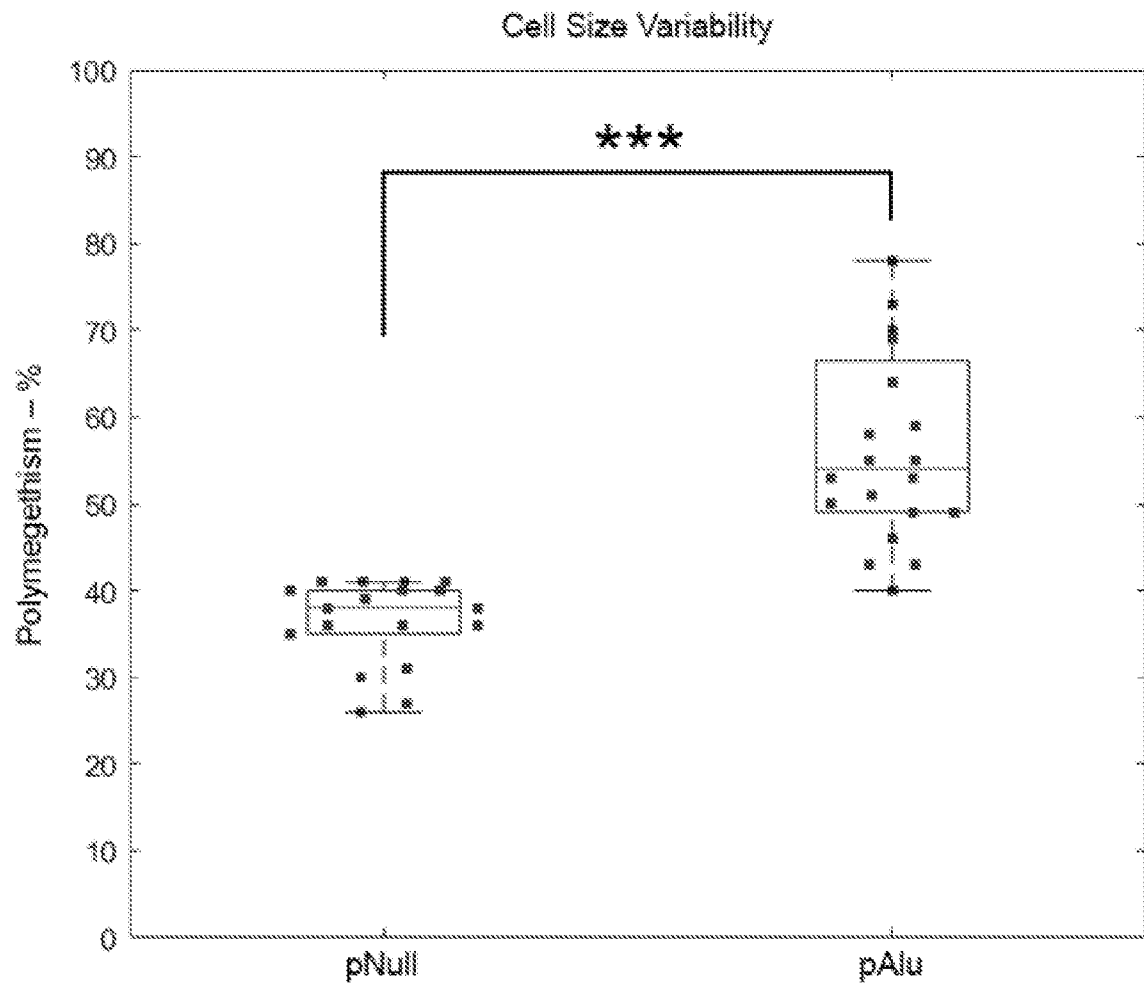


FIG. 9E

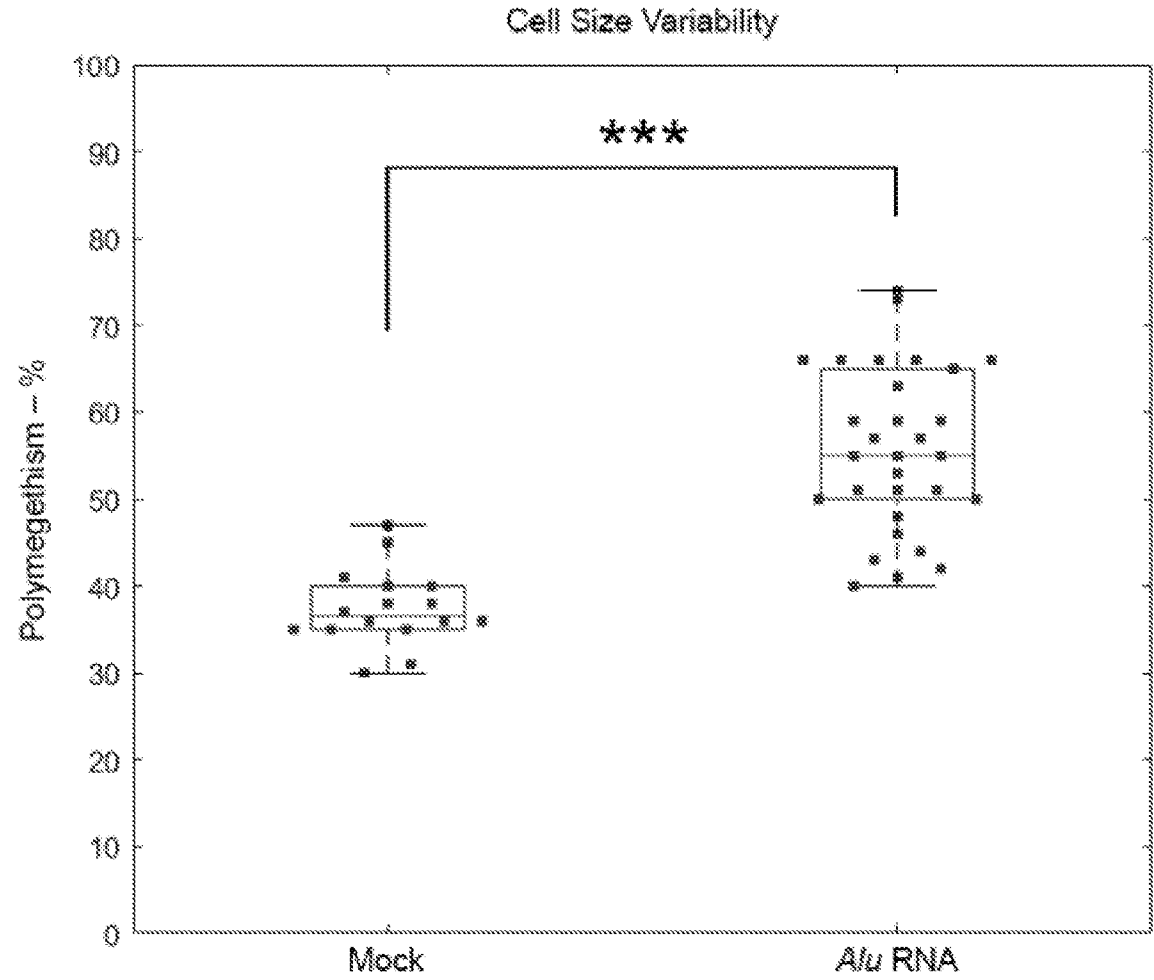


FIG. 9F

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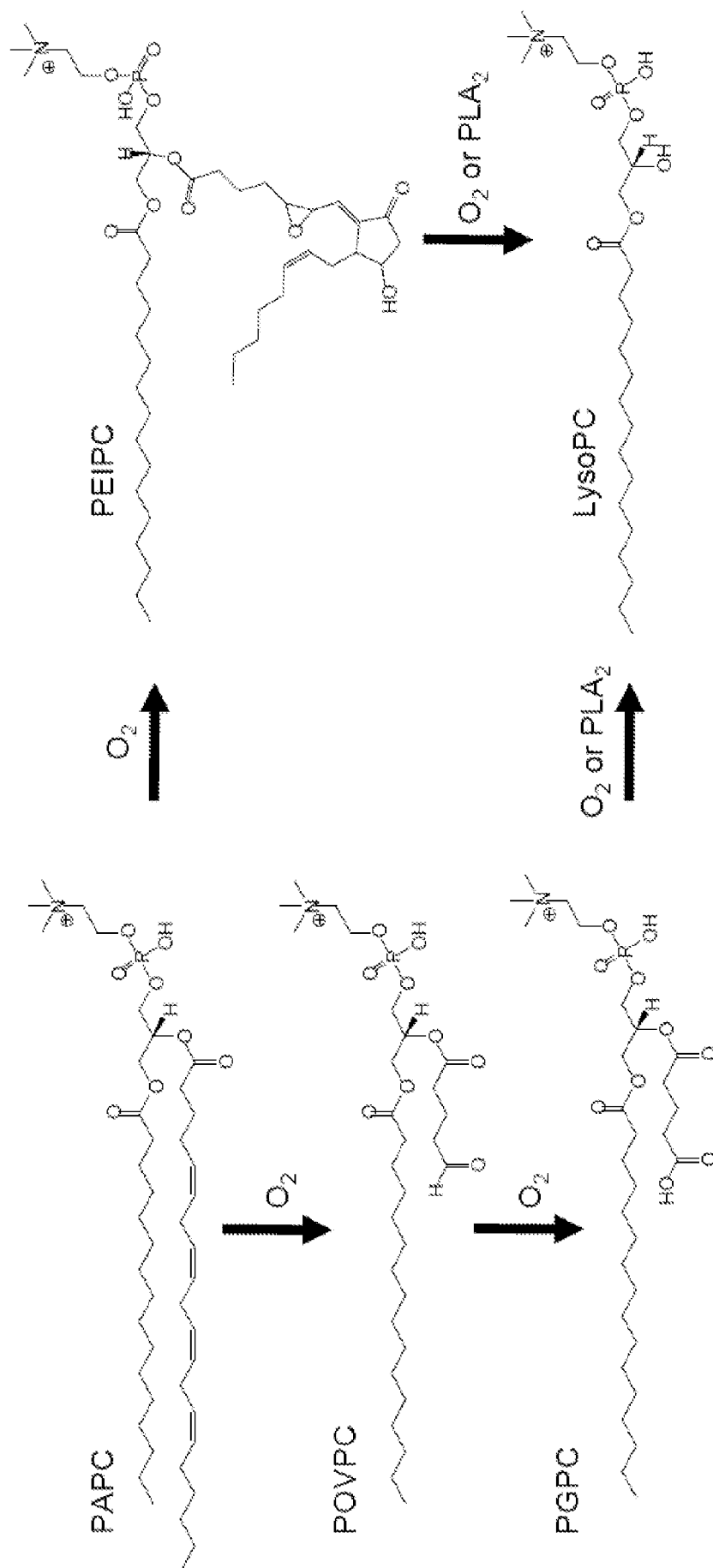


FIG. 10A

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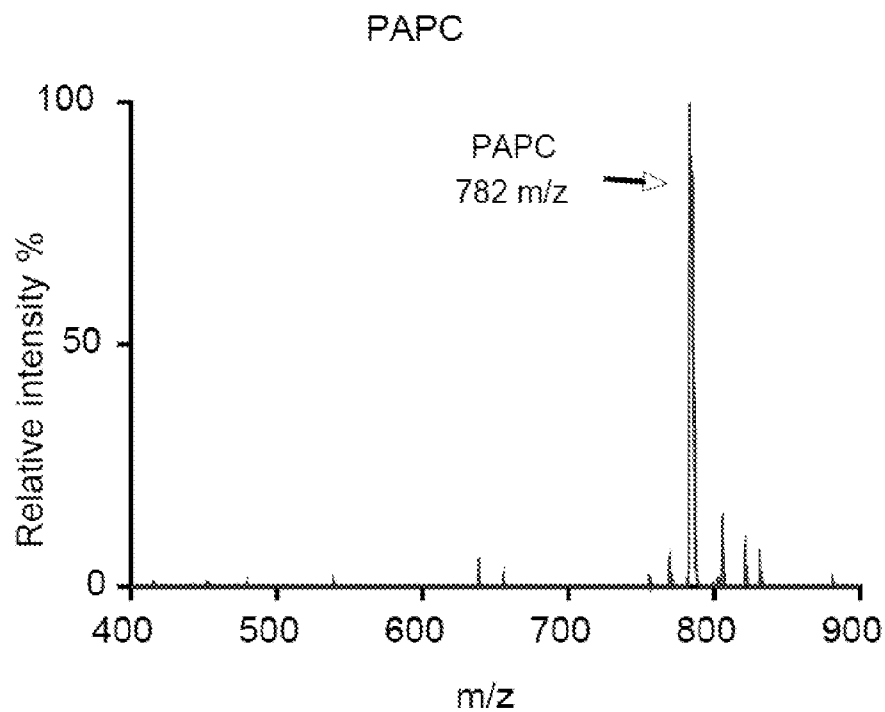


FIG. 10B

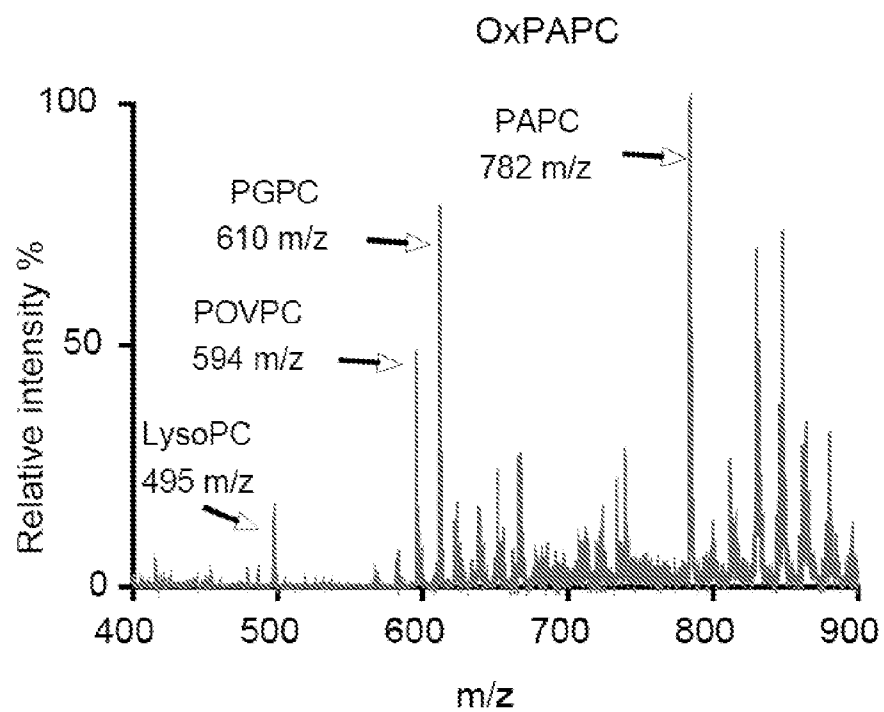


FIG. 10C

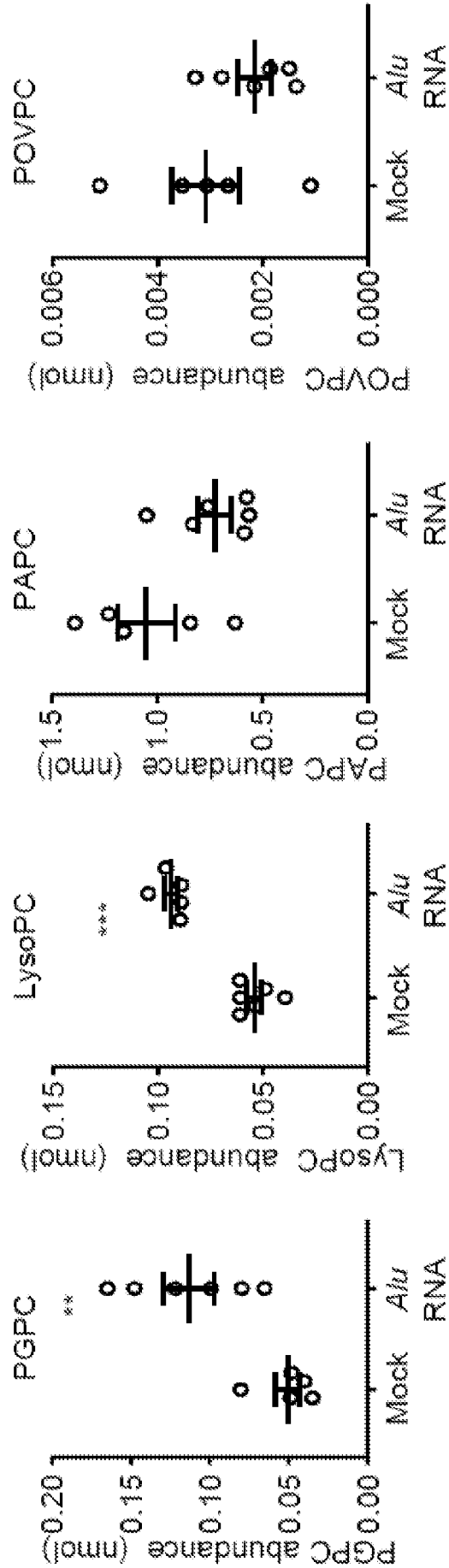
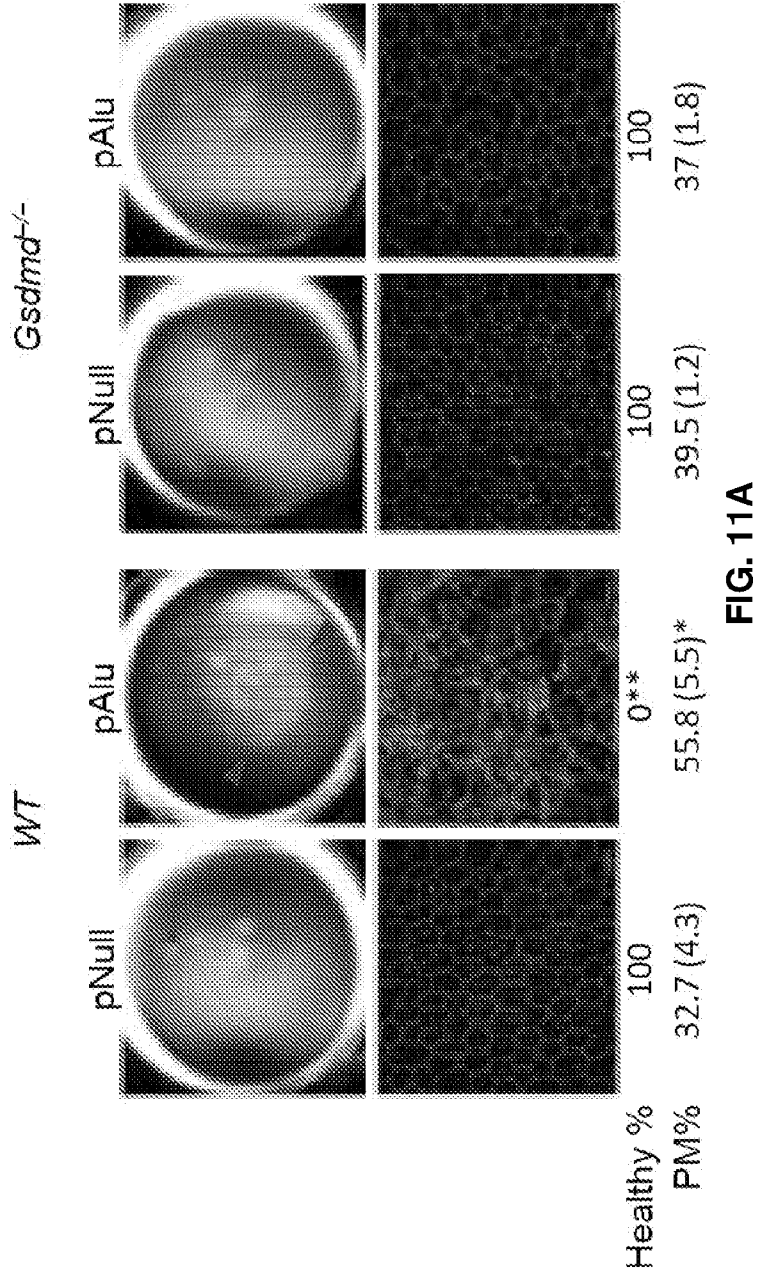


FIG. 10D



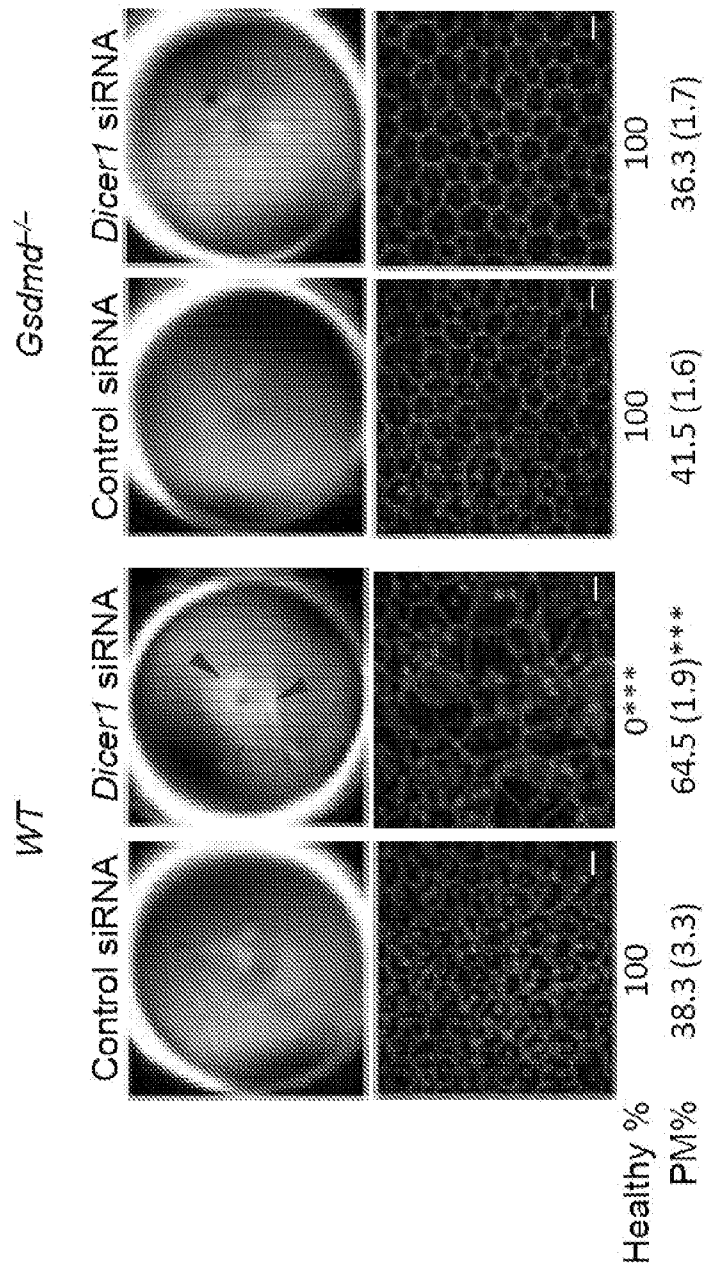


FIG. 11B

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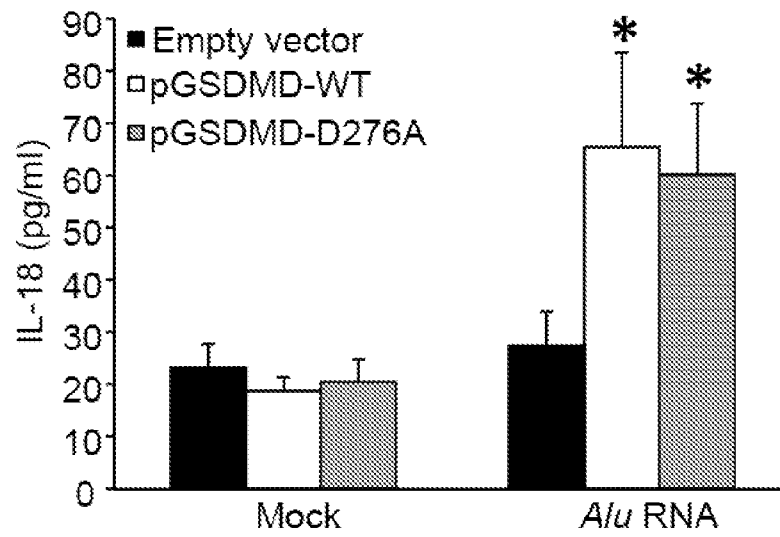


FIG. 11C

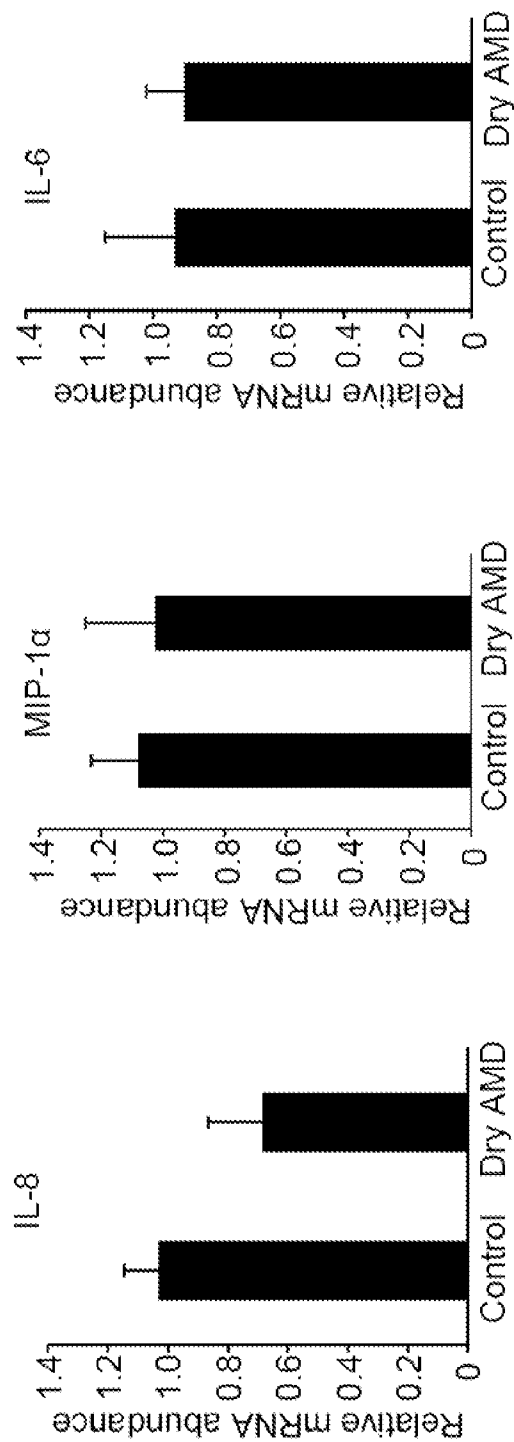


FIG. 11D

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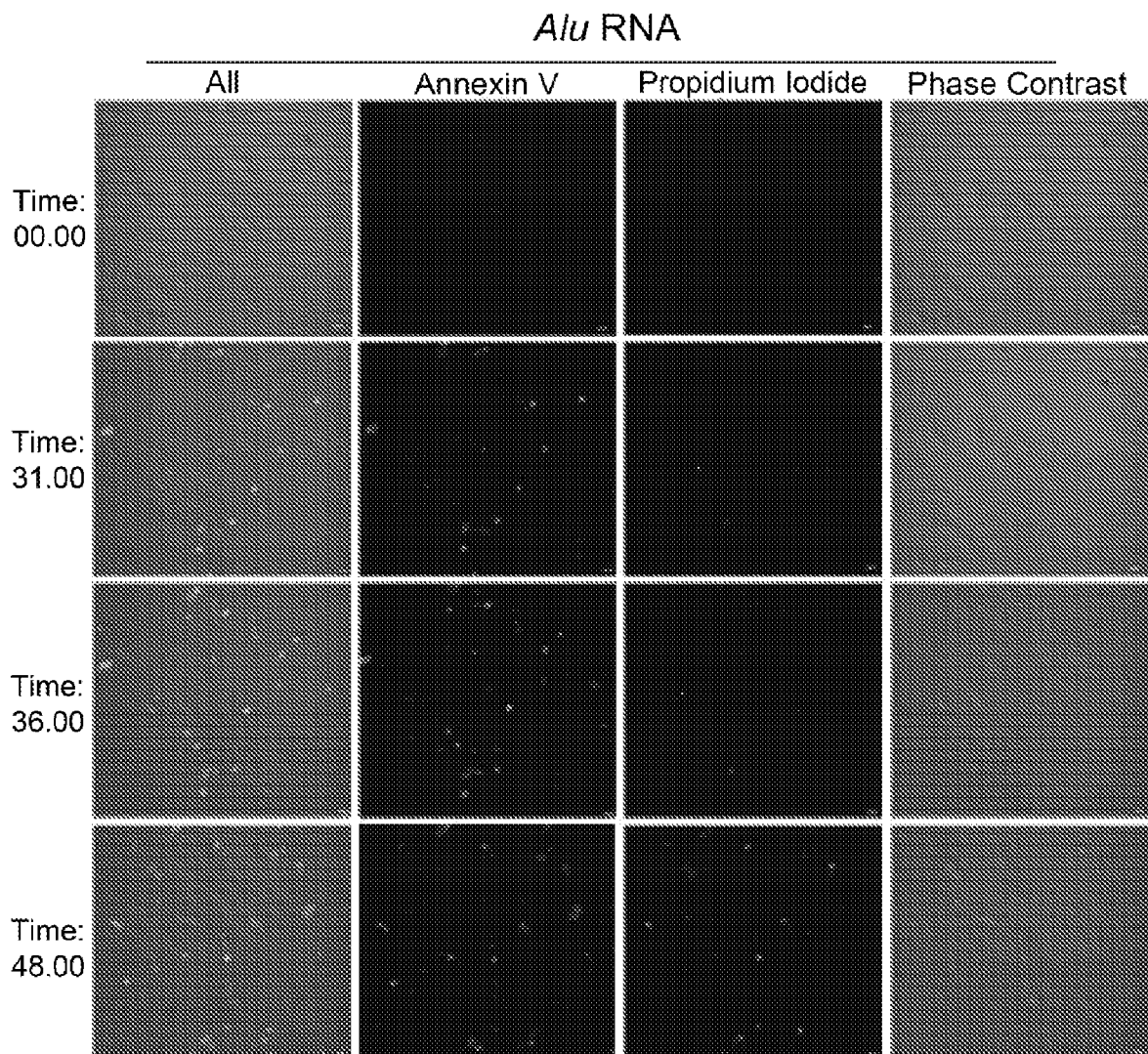


FIG. 12A

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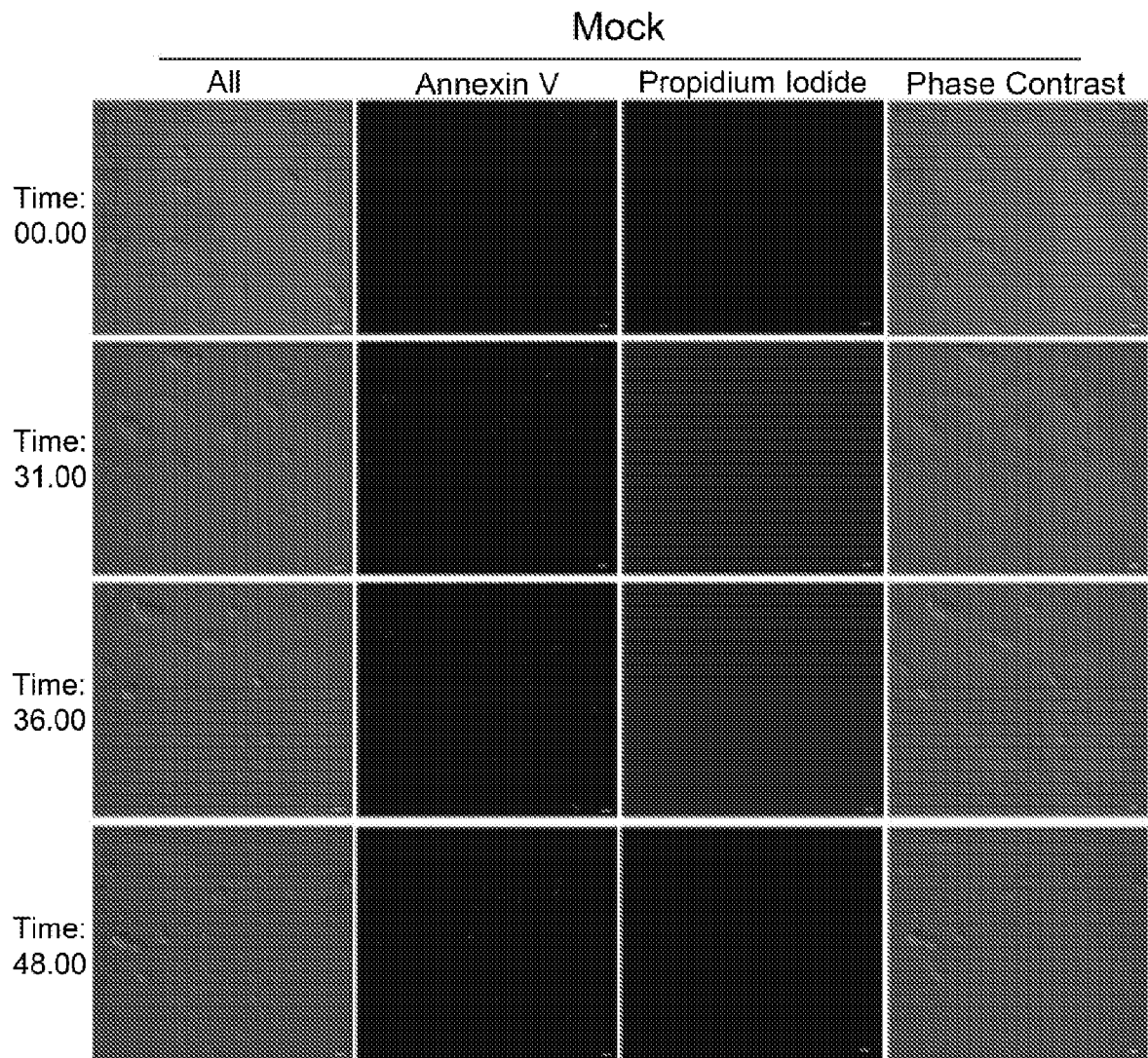


FIG. 12B

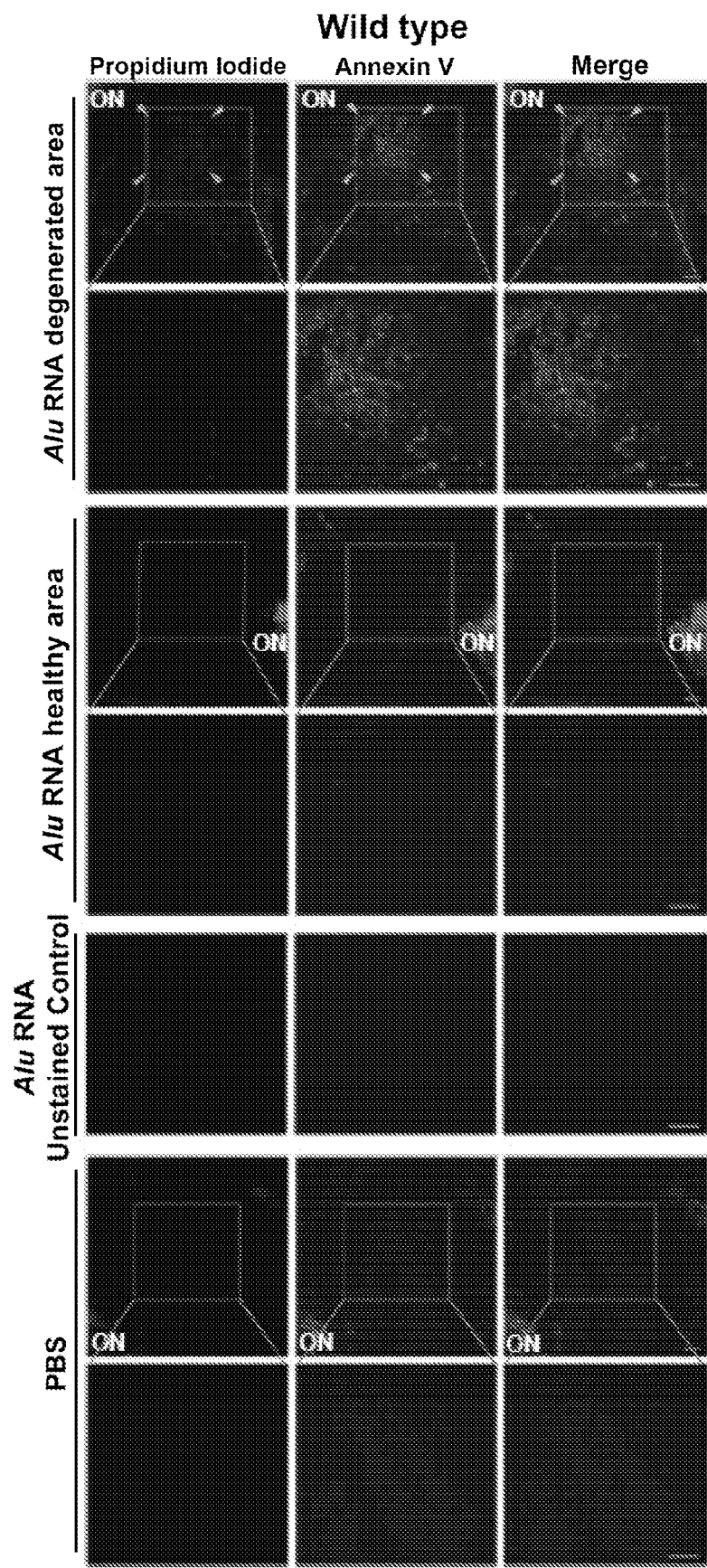


FIG. 13A

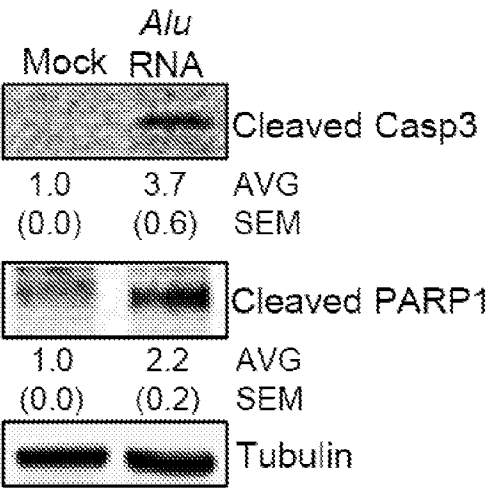


FIG. 13B

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Gsdmd^{-/-}

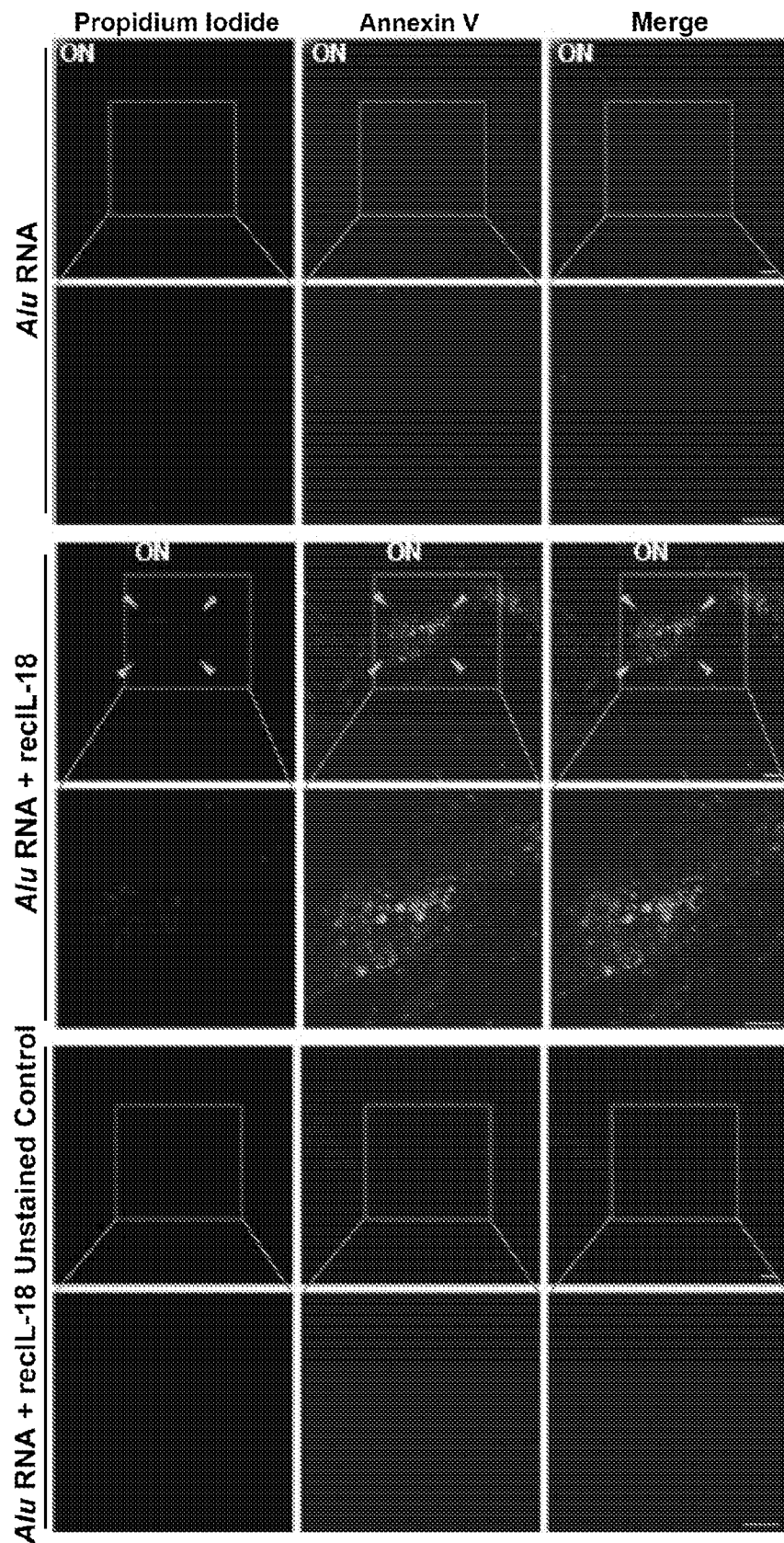


FIG. 14

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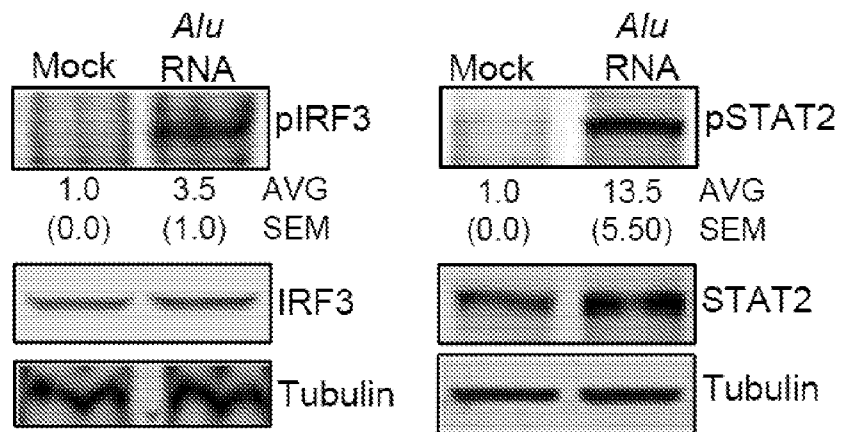


FIG. 15A

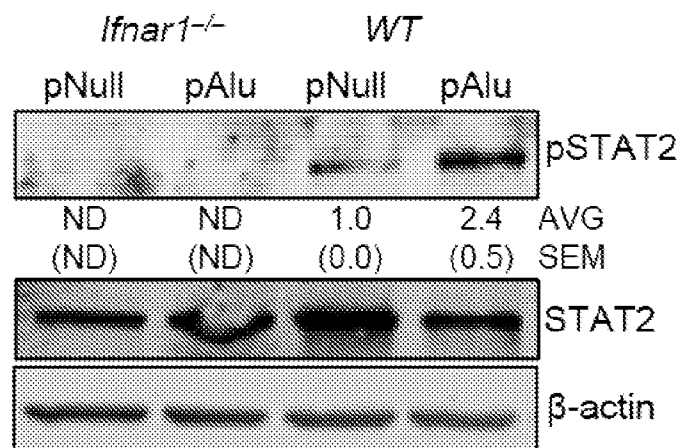
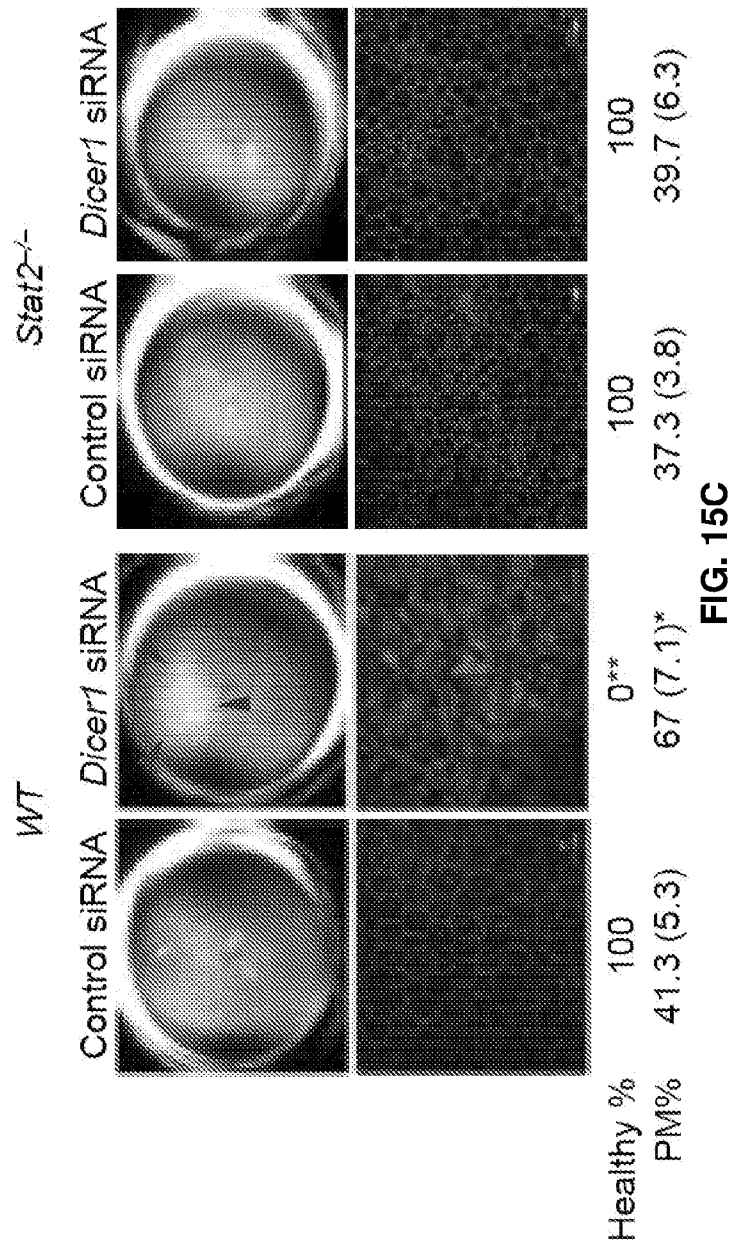
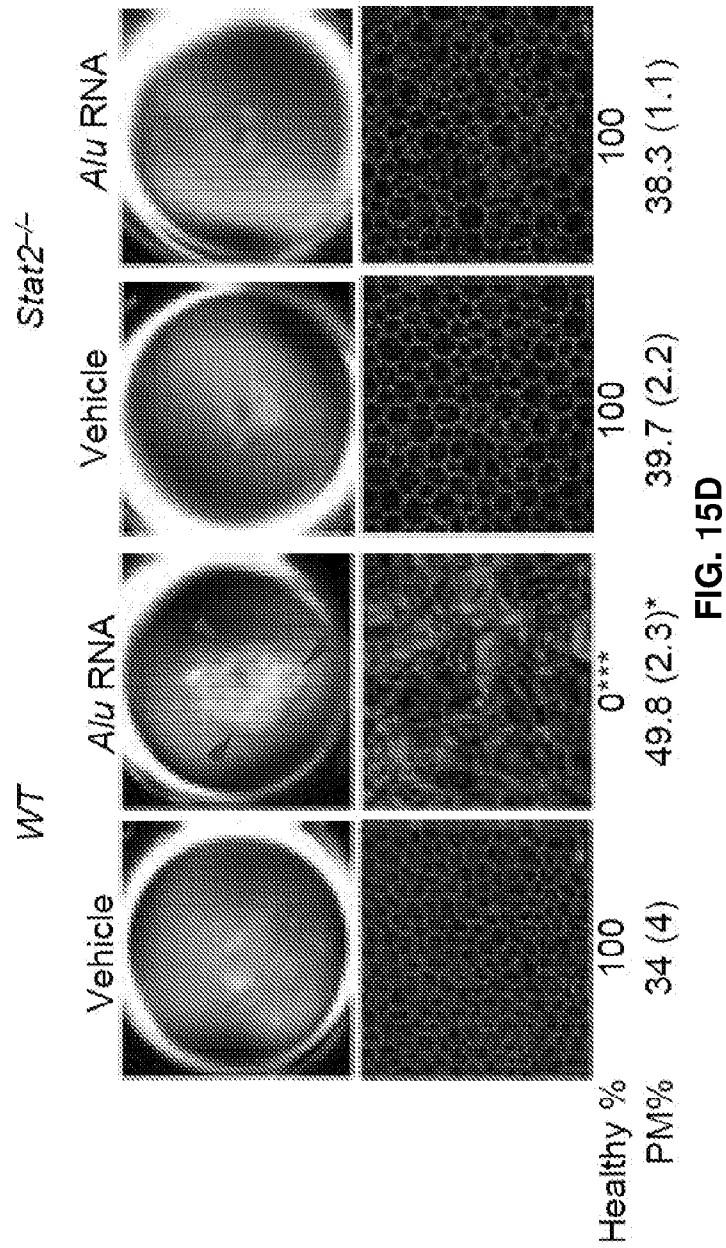


FIG. 15B





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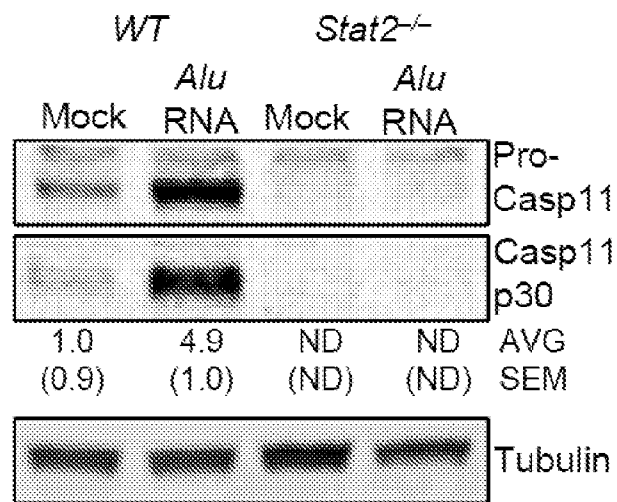


FIG. 15E

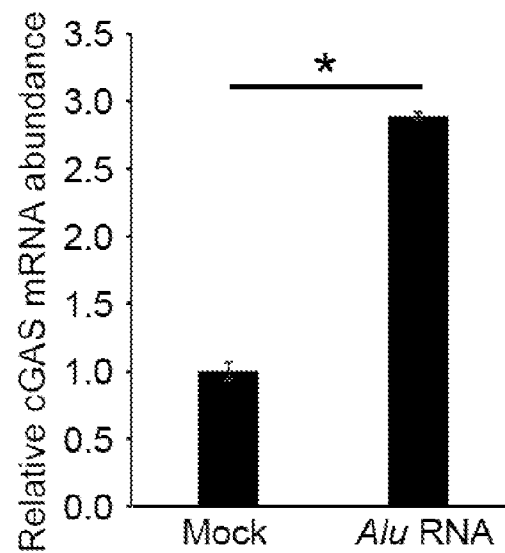


FIG. 16A

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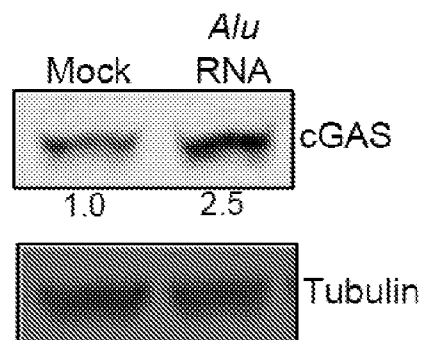
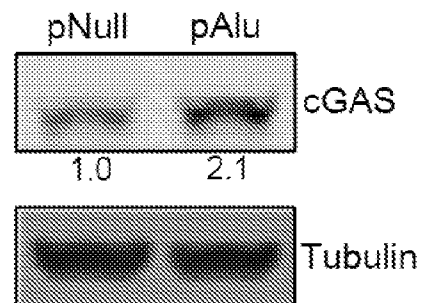


FIG. 16B

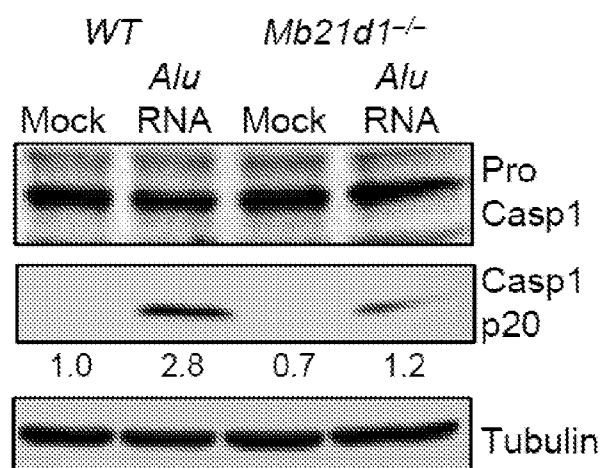


FIG. 16C

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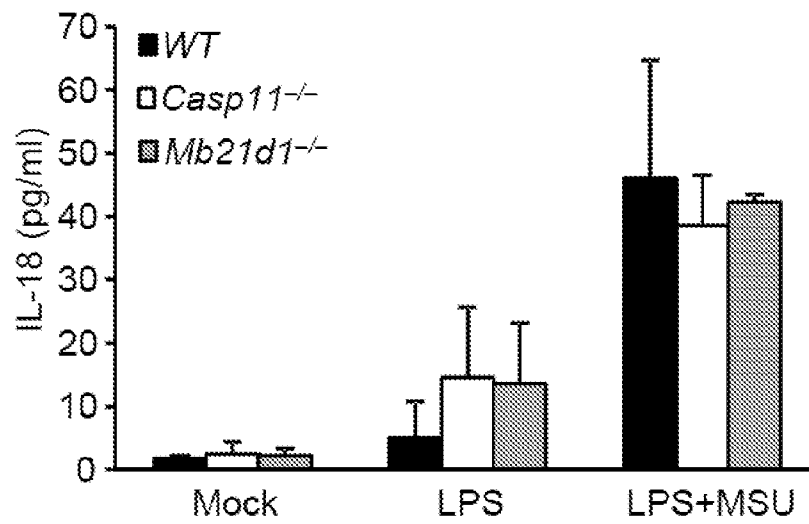


FIG. 16D

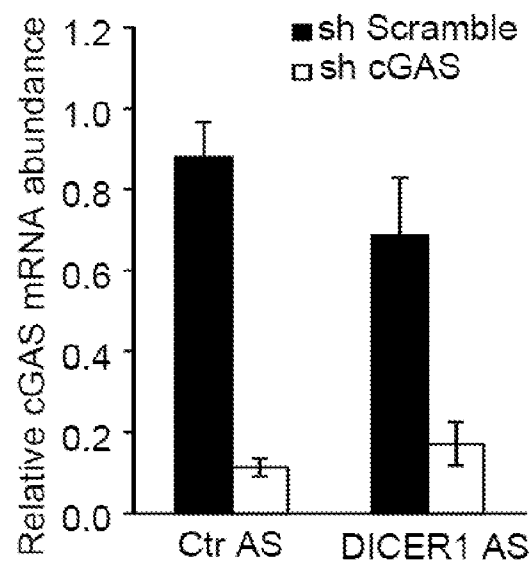


FIG. 16E

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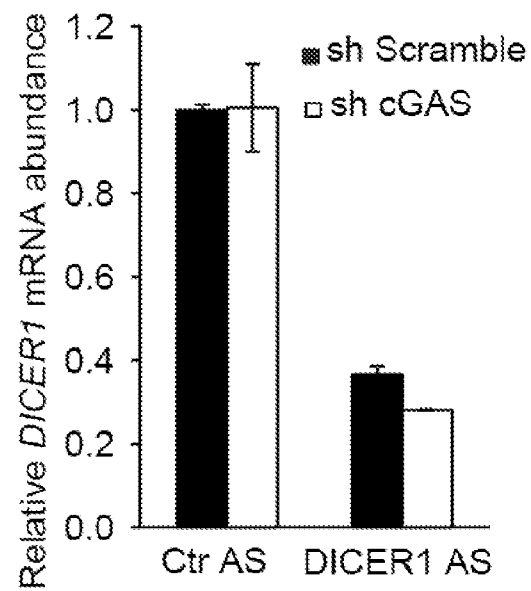


FIG. 16F

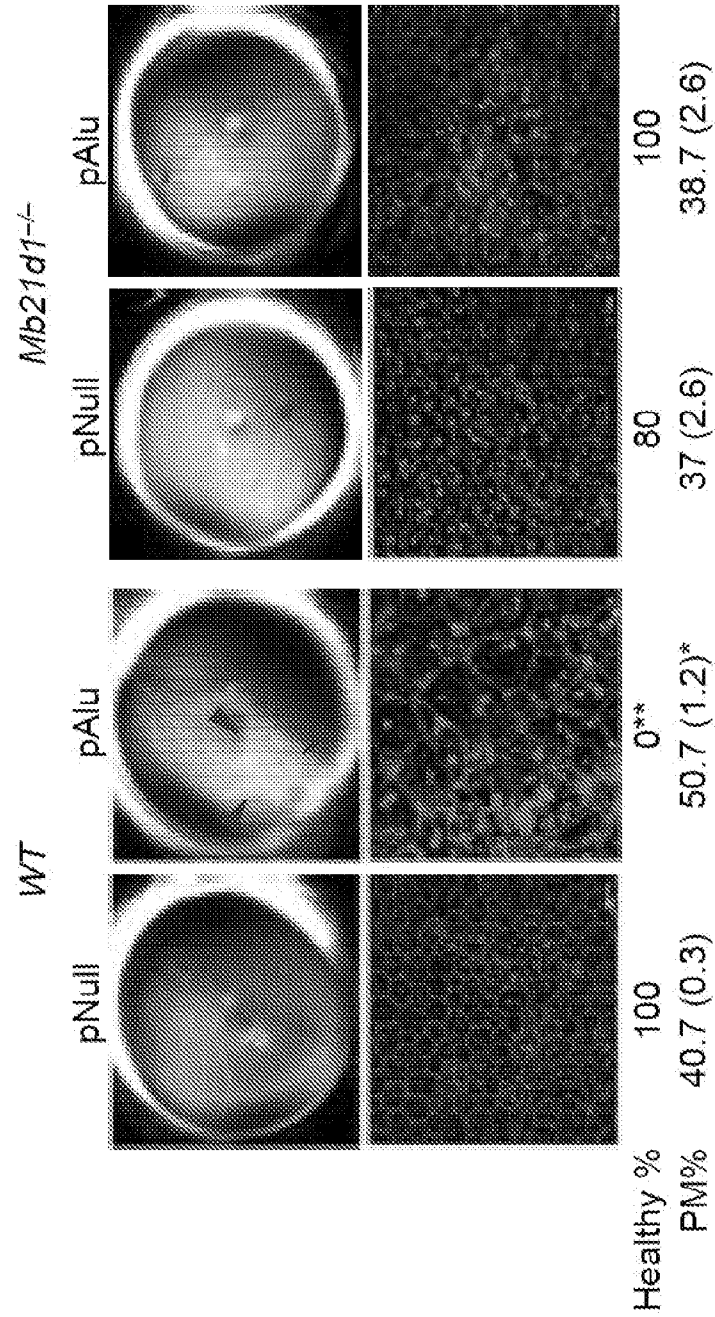


FIG. 16G

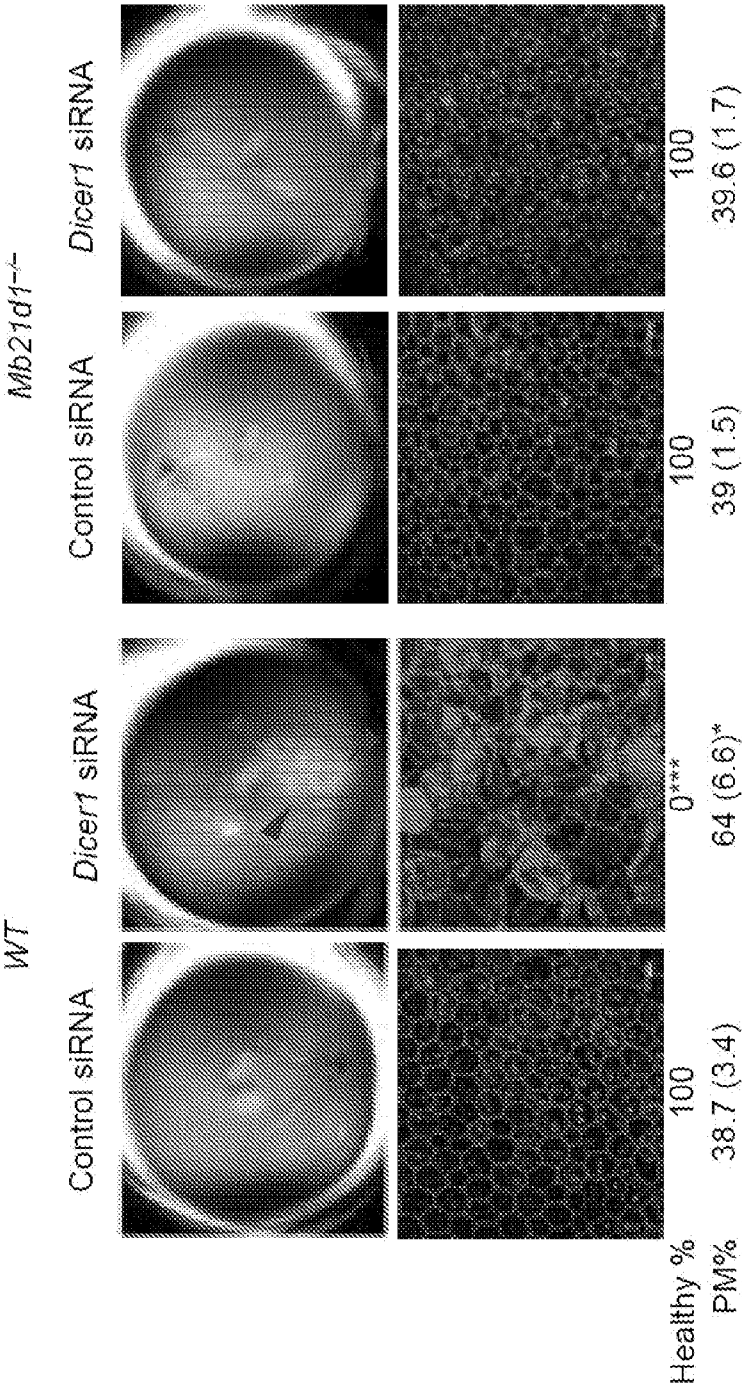


FIG. 16H

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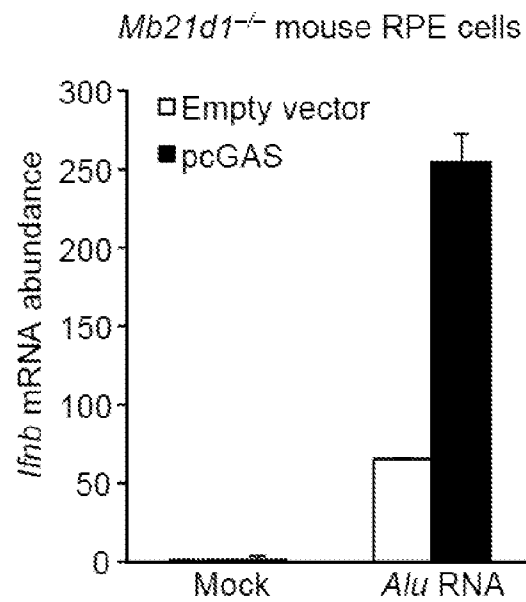


FIG. 16I

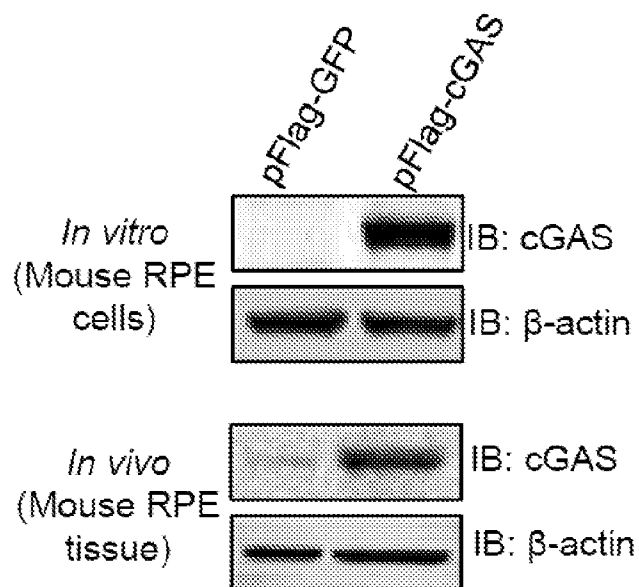


FIG. 17A

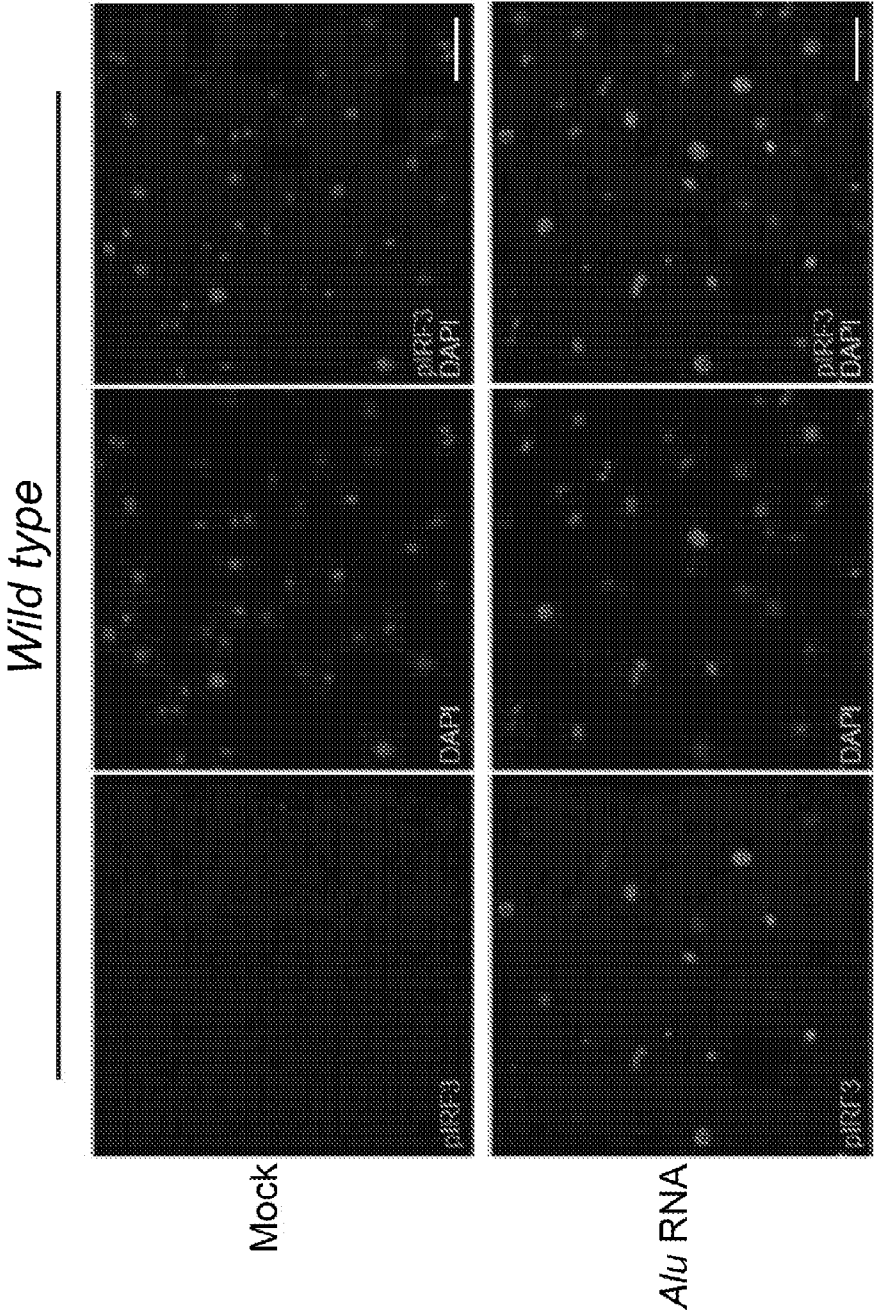


FIG. 17B

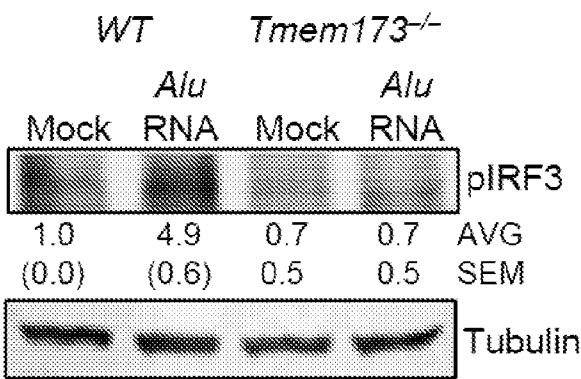


FIG. 17C

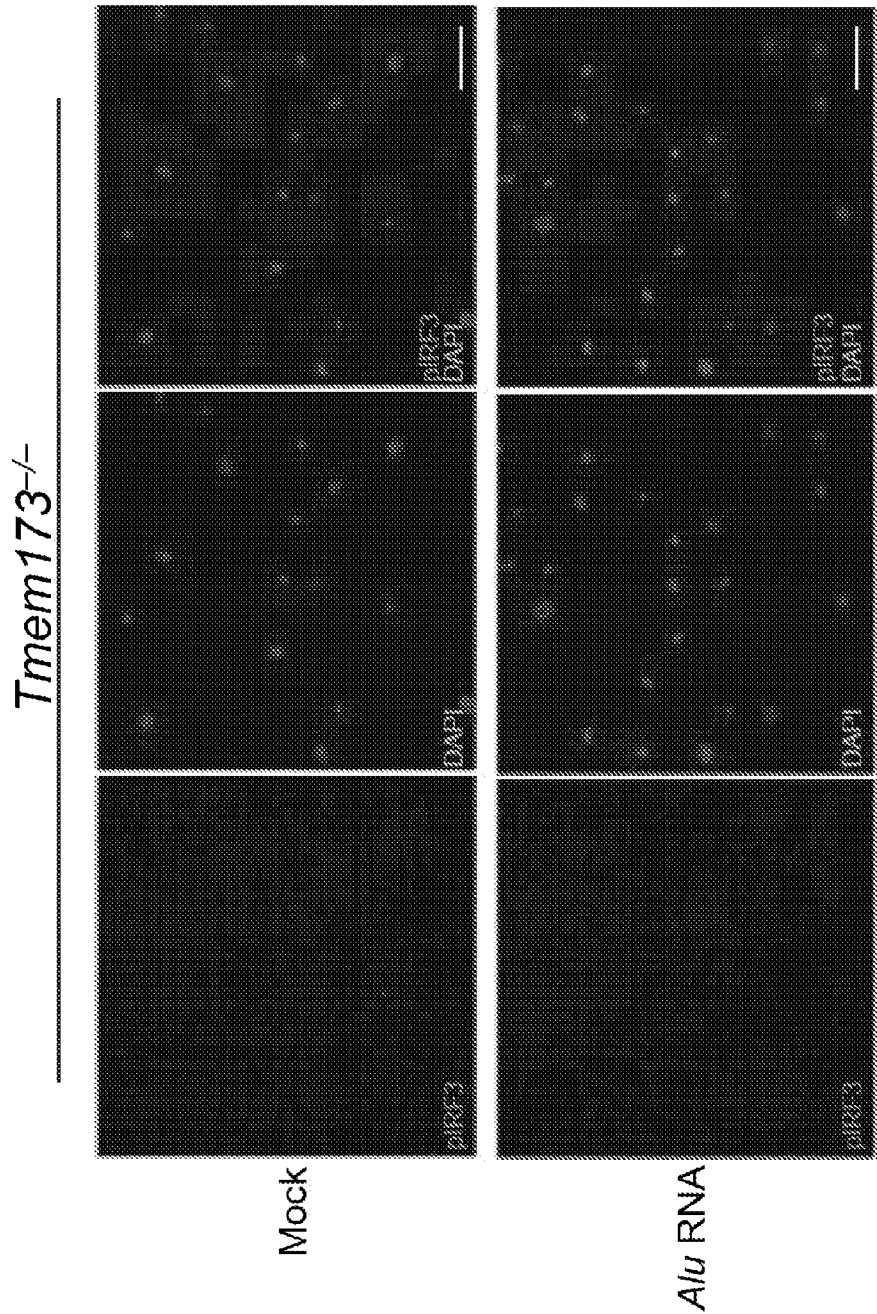


FIG. 17D

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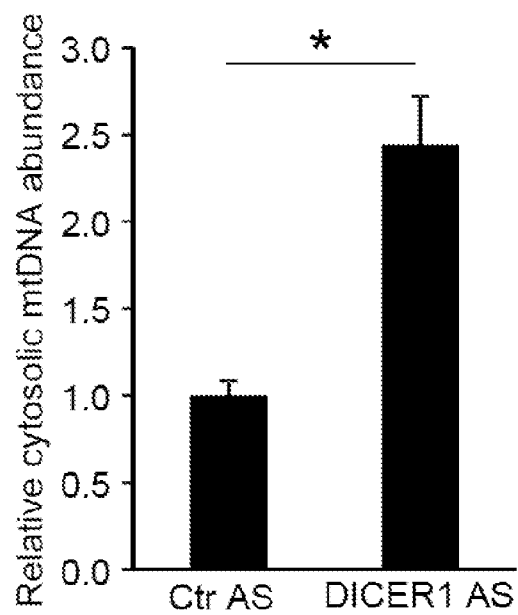


FIG. 18A

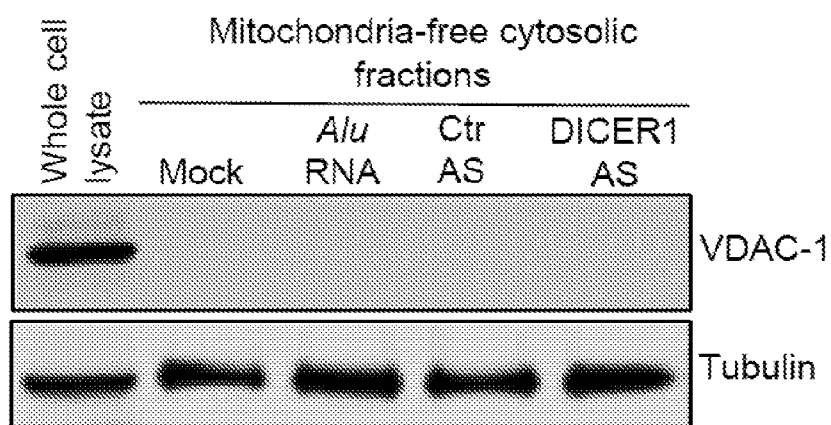


FIG. 18B

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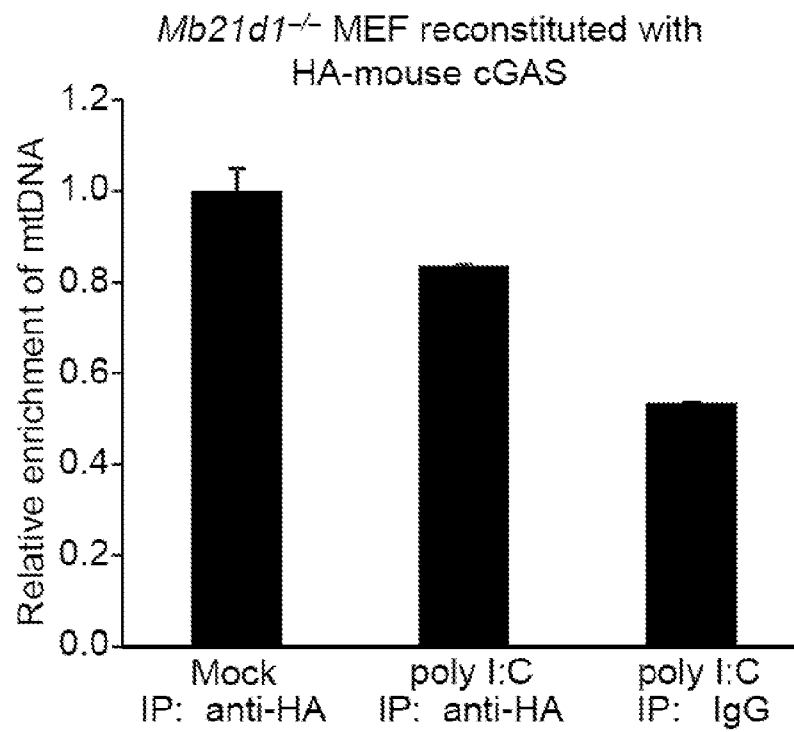


FIG. 18C

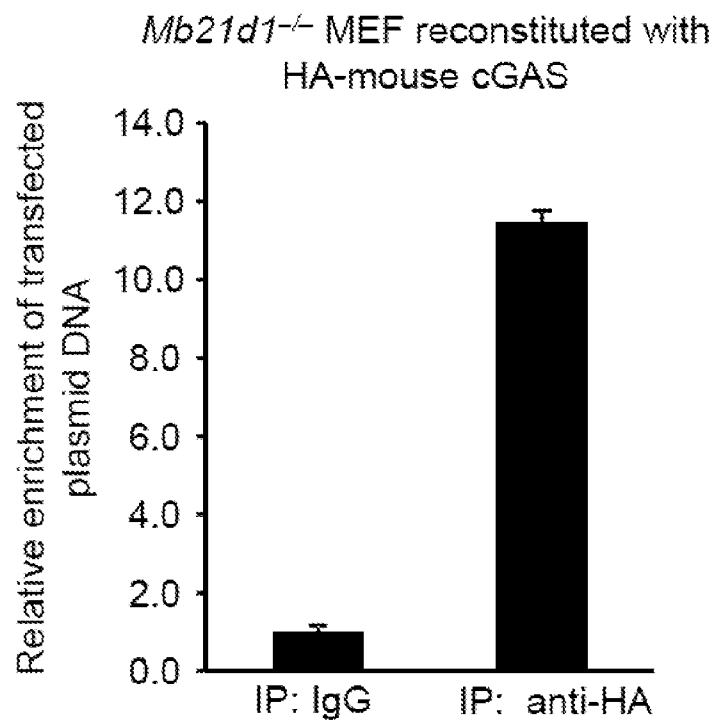


FIG. 18D

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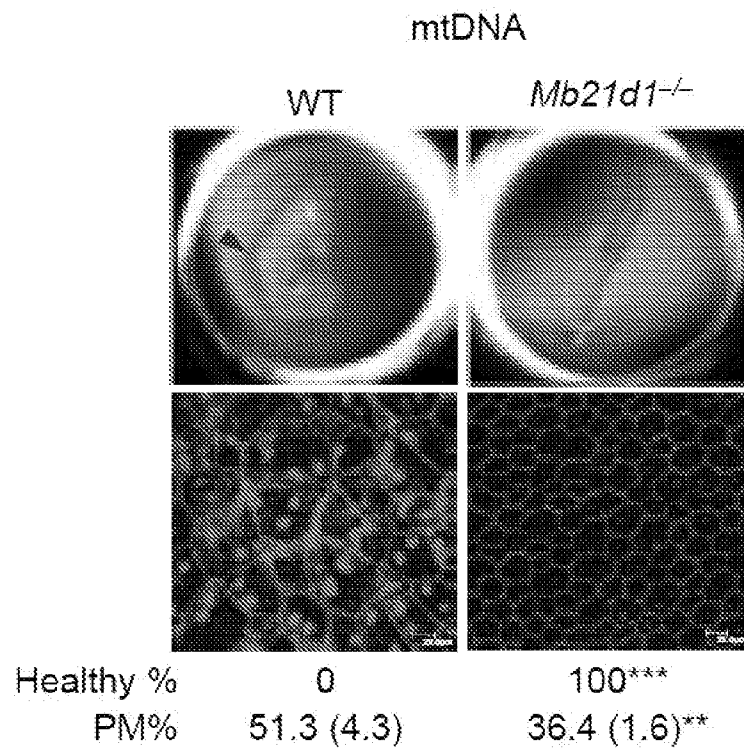


FIG. 18E

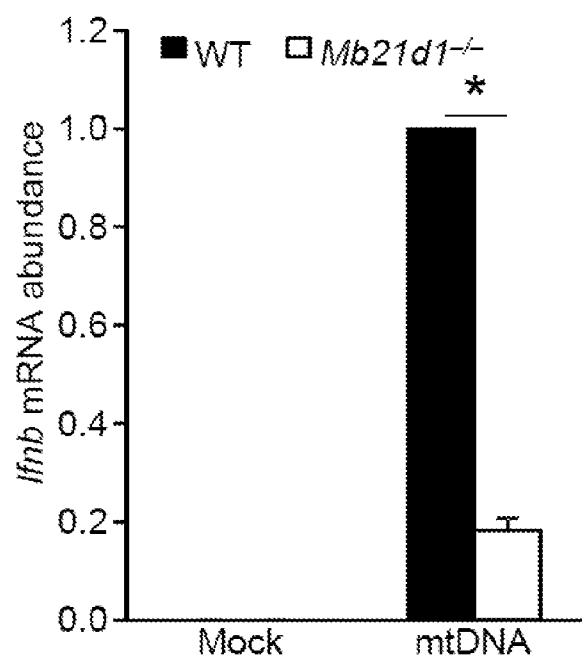


FIG. 18F

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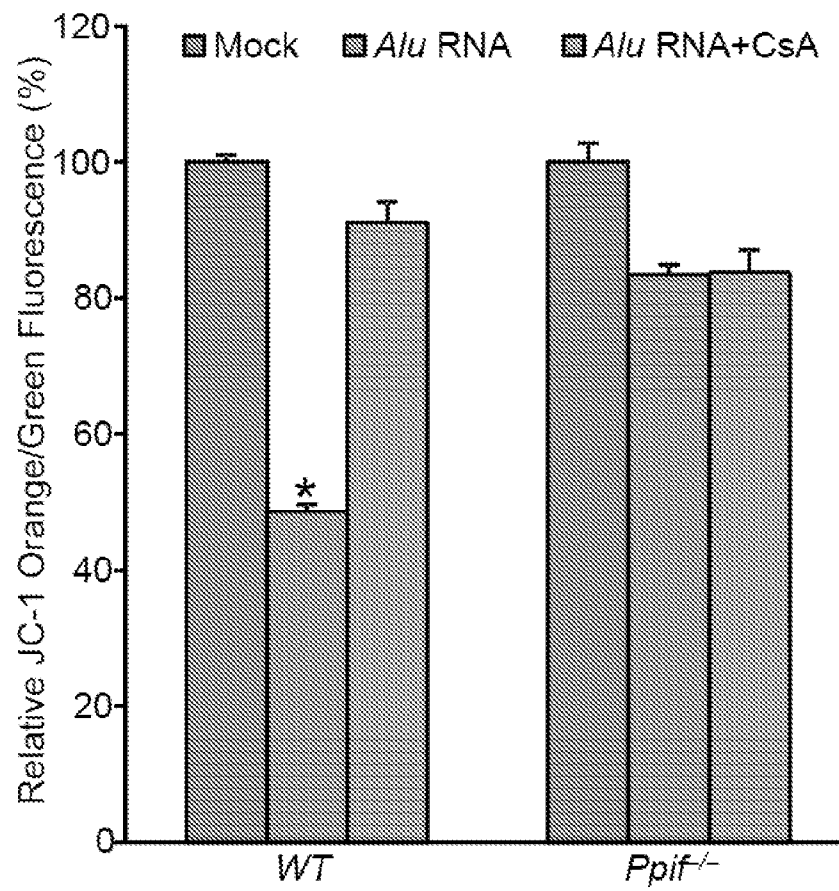


FIG. 18G

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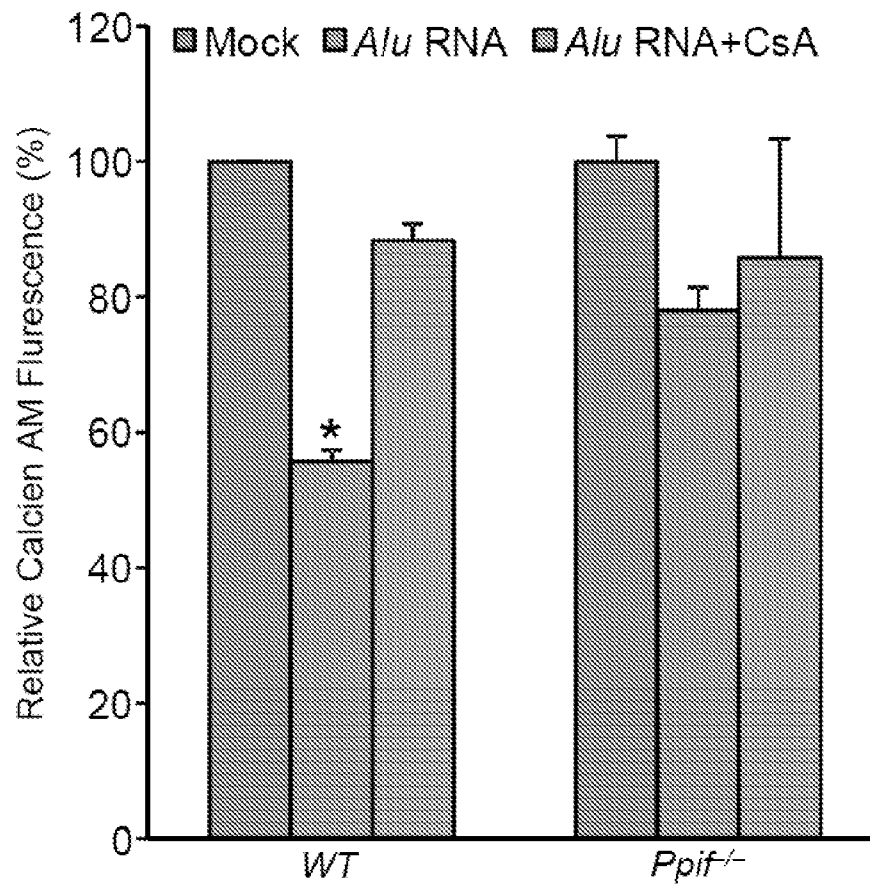


FIG. 18H

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Clodronate Liposomes

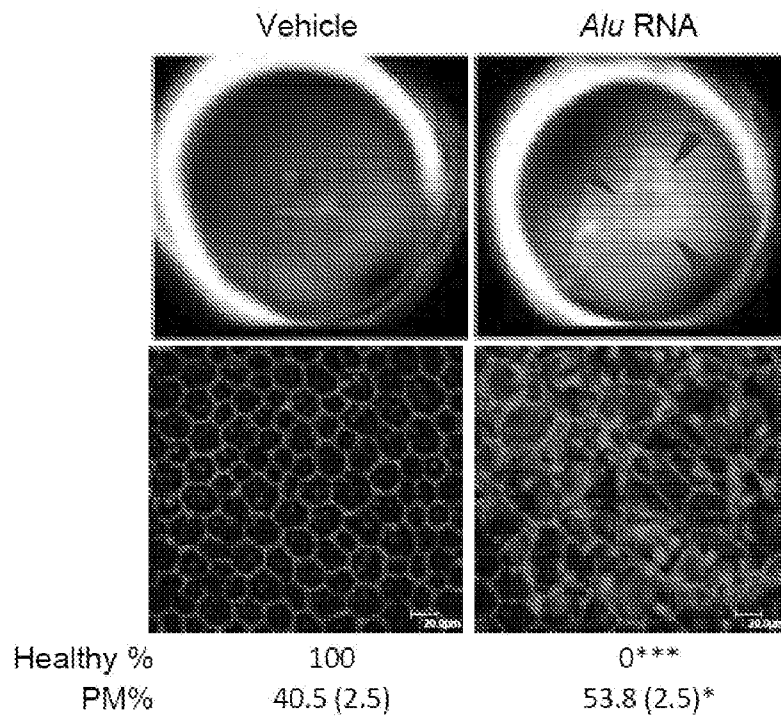


FIG. 19A

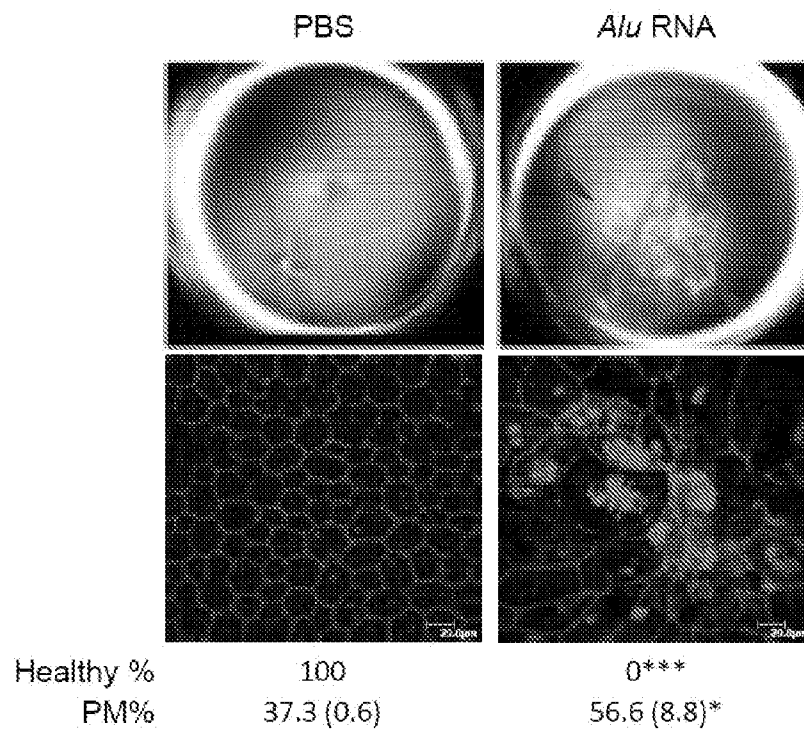
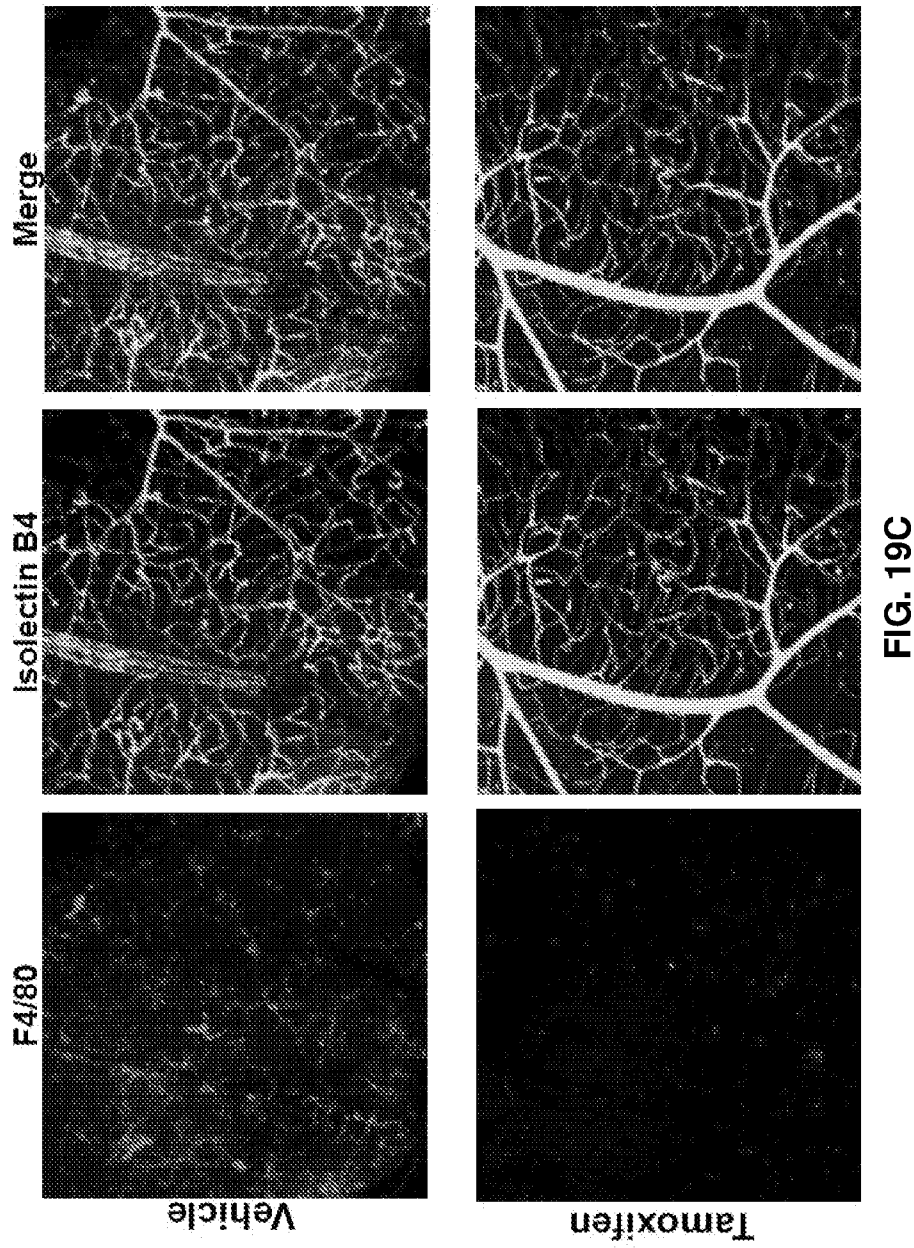
CX3CR1^{CreER};DTA^{fllox}
+ Tamoxifen

FIG. 19B



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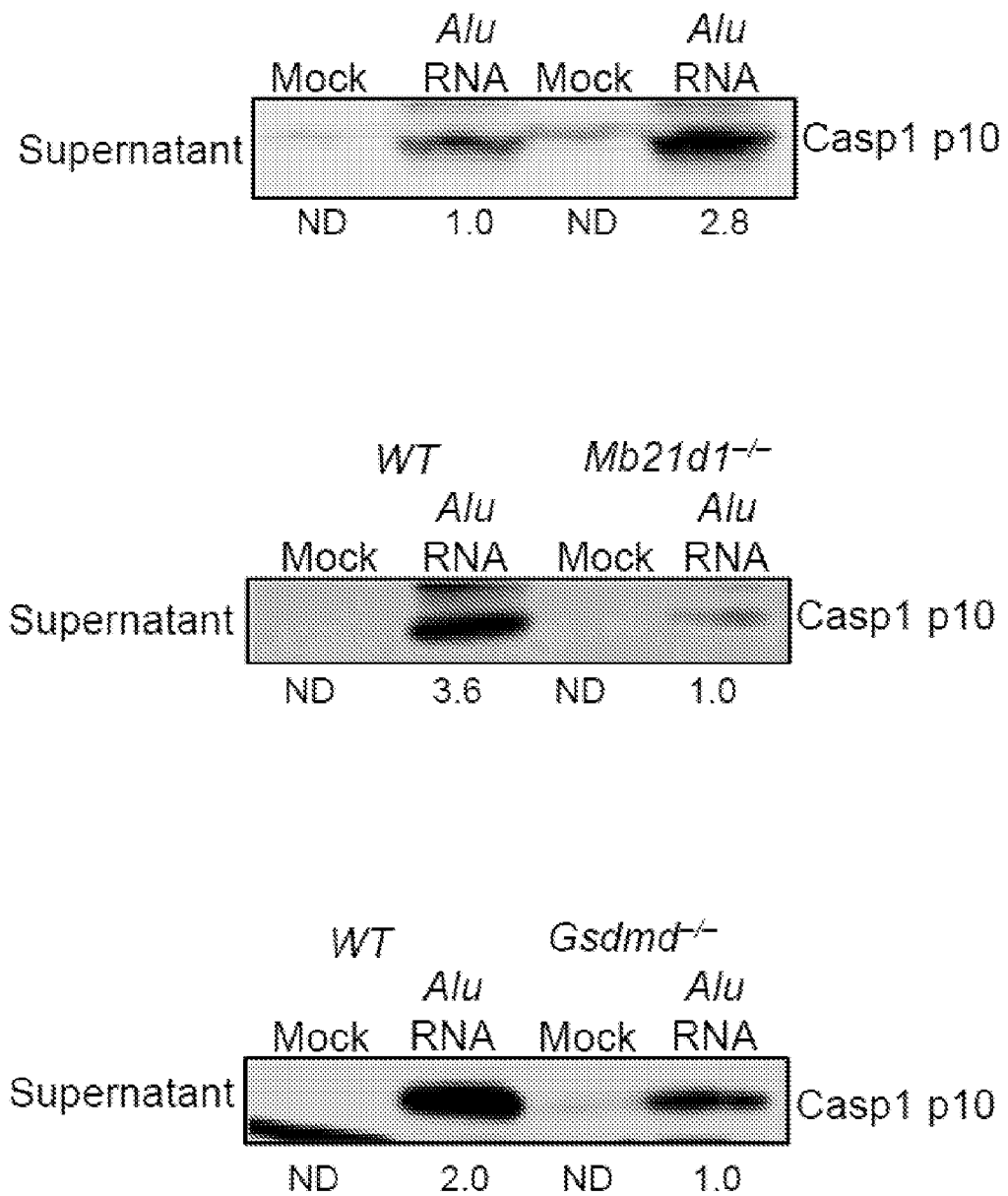


FIG. 20

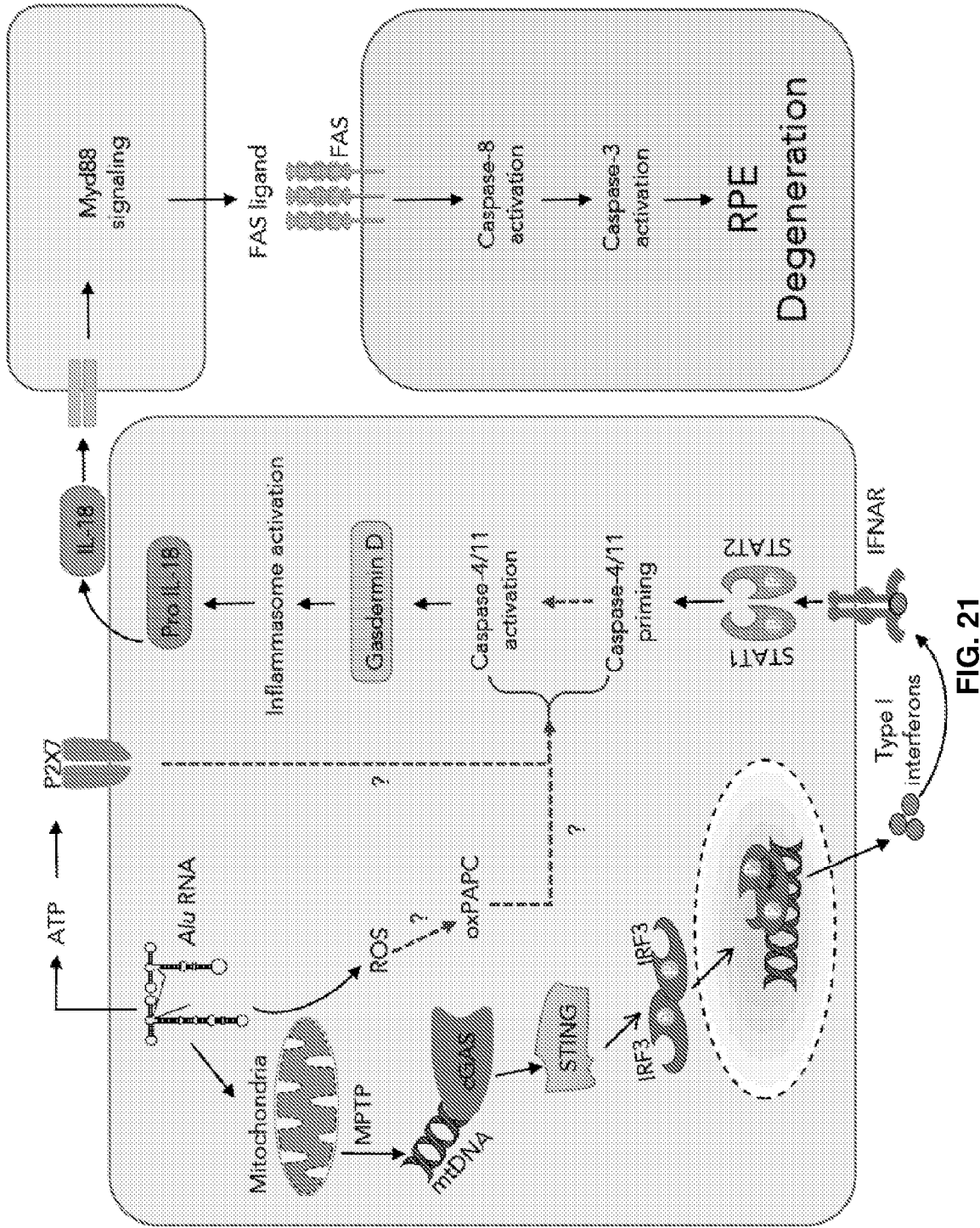


FIG. 21