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Protection of Cells from *Alu*-RNA-Induced Degeneration and Inhibitors for Protecting Cells

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Ambati et al.

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(54) **PROTECTION OF CELLS FROM *ALU*-RNA-INDUCED DEGENERATION AND INHIBITORS FOR PROTECTING CELLS**

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(57) **ABSTRACT**

A method of protecting a cell includes inhibiting an inflammasome, MyD88, IL-18, VDAC1, VDAC2, caspase-8, and/or NFκB of the cell. Administering an inhibitor of MyD88, IL-18, VDAC1, VDAC2, caspase-8, and/or NFκB can protect the cell from Alu-RNA-induced degeneration. Protecting a cell, such as an retinal pigment epithelium (RPE), can be therapeutically useful in the context of age-related macular degeneration and geographic atrophy.

25 Claims, 23 Drawing Sheets

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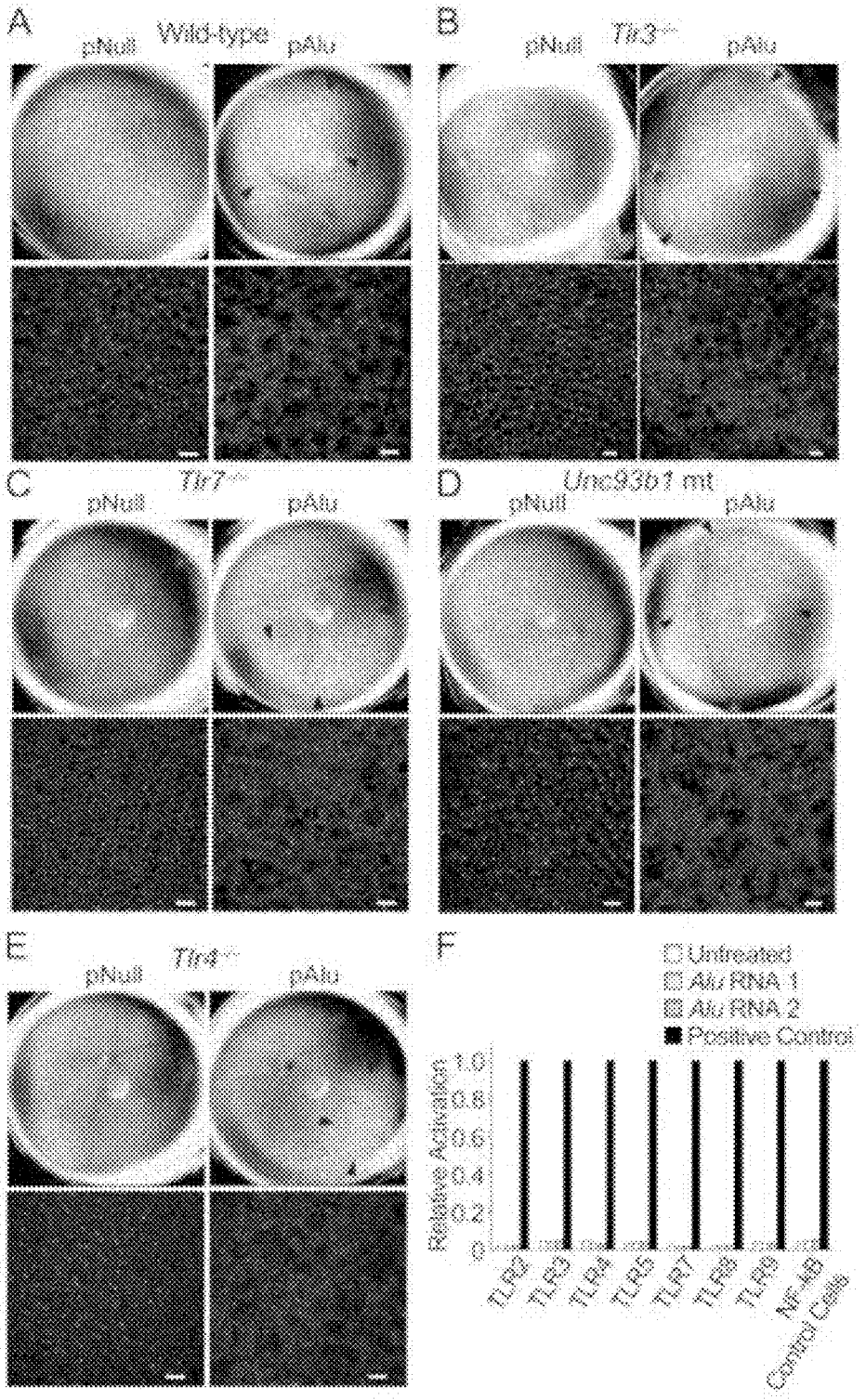
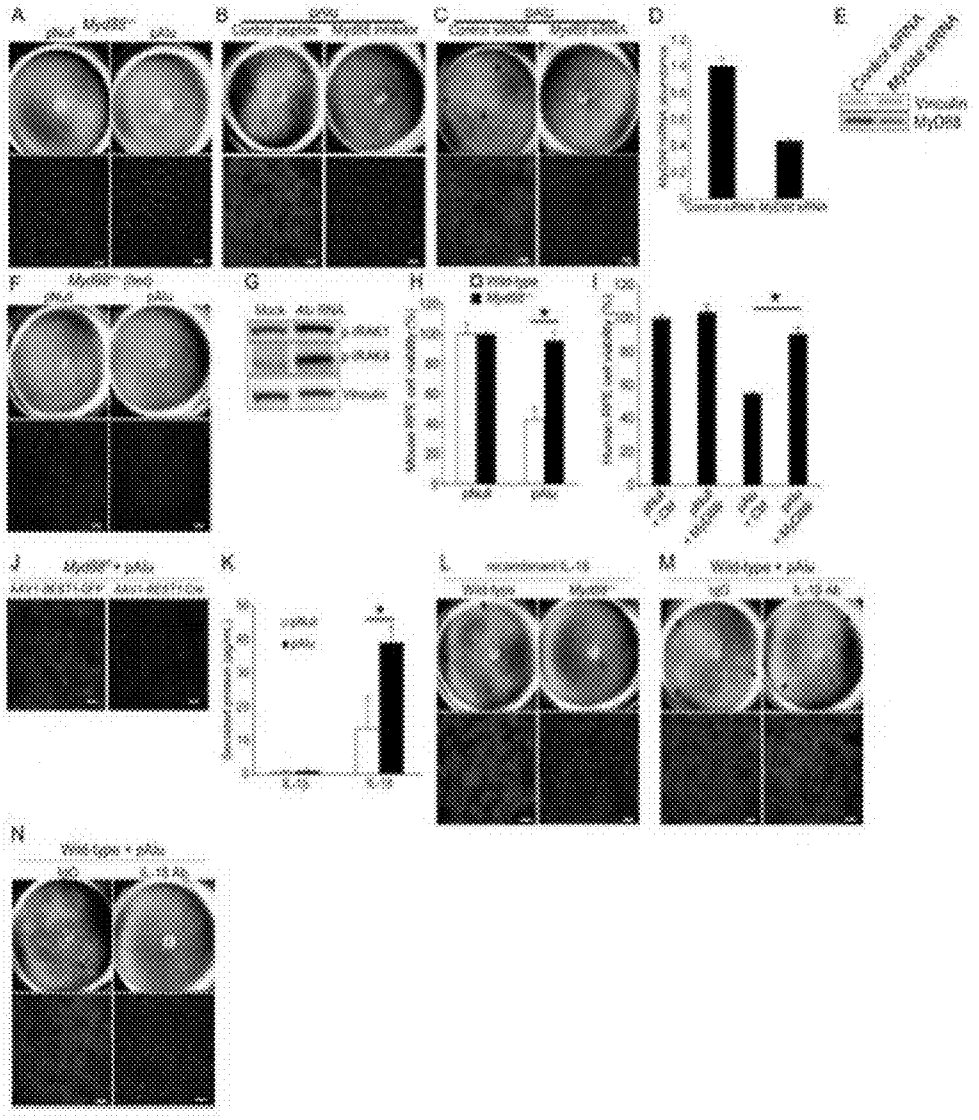

A
Wild-type
pNull
pAlu
B
Tlr3−/−
pNull
pAlu
C
Tlr7−/−
pNull
pAlu
D
Unc93b1 mt
pNull
pAlu
E
Tlr4−/−
pNull
pAlu
F
Untreated
Alu RNA 1
Alu RNA 2
Positive Control
Relative Activation
1.0
0.8
0.6
0.4
0.2
0
TLR2
TLR3
TLR4
TLR5
TLR7
TLR8
TLR9
NF-κB
Control Cells

Figure 1



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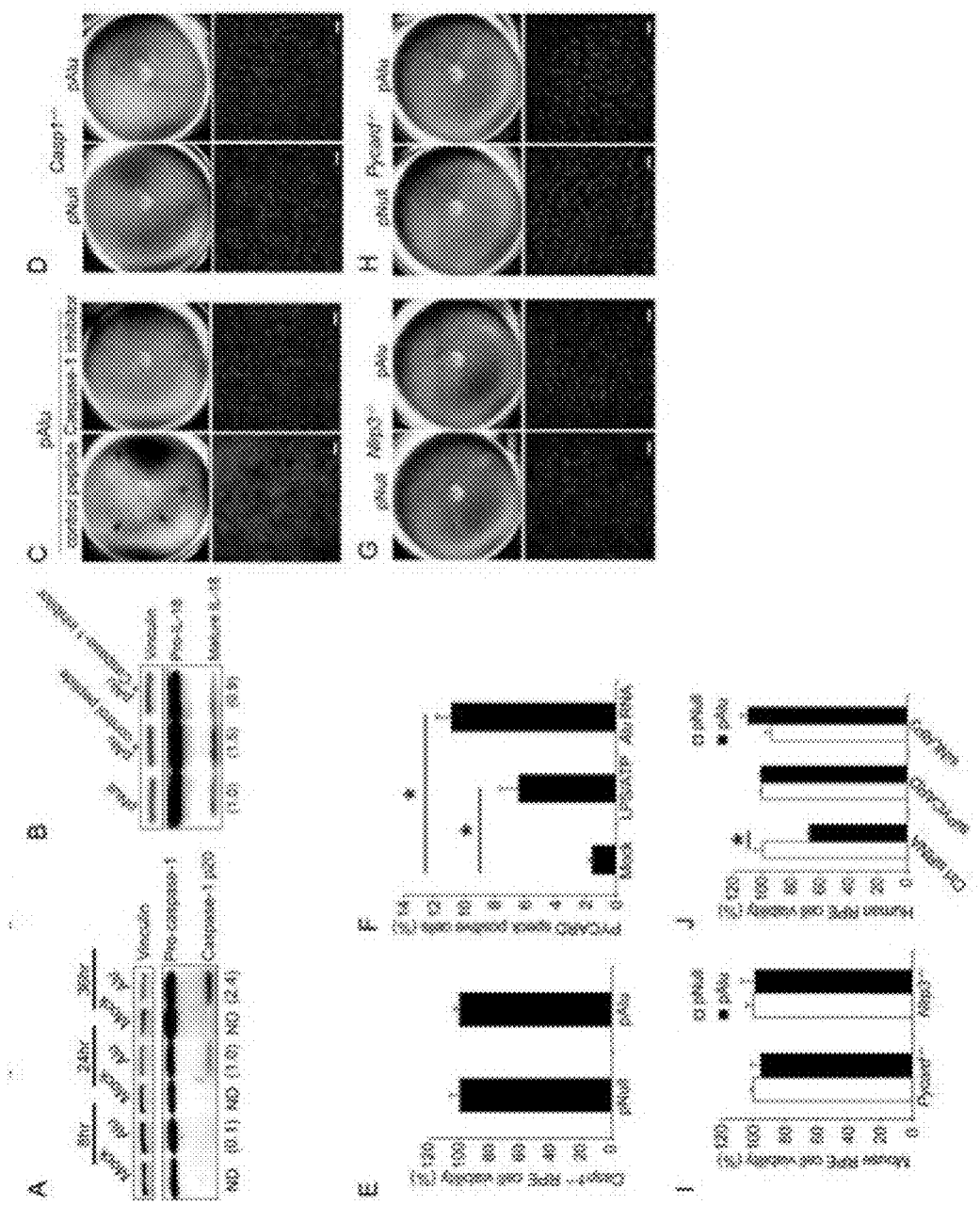
A
B
C
D
E
F
G
H
I
J
Caspase-1 protein
Caspase-1 mRNA
Caspase-1 KO
Caspase-1 KO (2.4)
Caspase-1 KO (1.5)
Caspase-1 KO (0.8)
Caspase-1 KO (1.0)
Caspase-1 KO (0.1)
Caspase-1 KO (0.6)
Caspase-1 KO (1.5)
Caspase-1 KO (0.9)
Caspase-1 KO (1.0)
Caspase-1 KO (0.8)
Caspase-1 KO (1.0

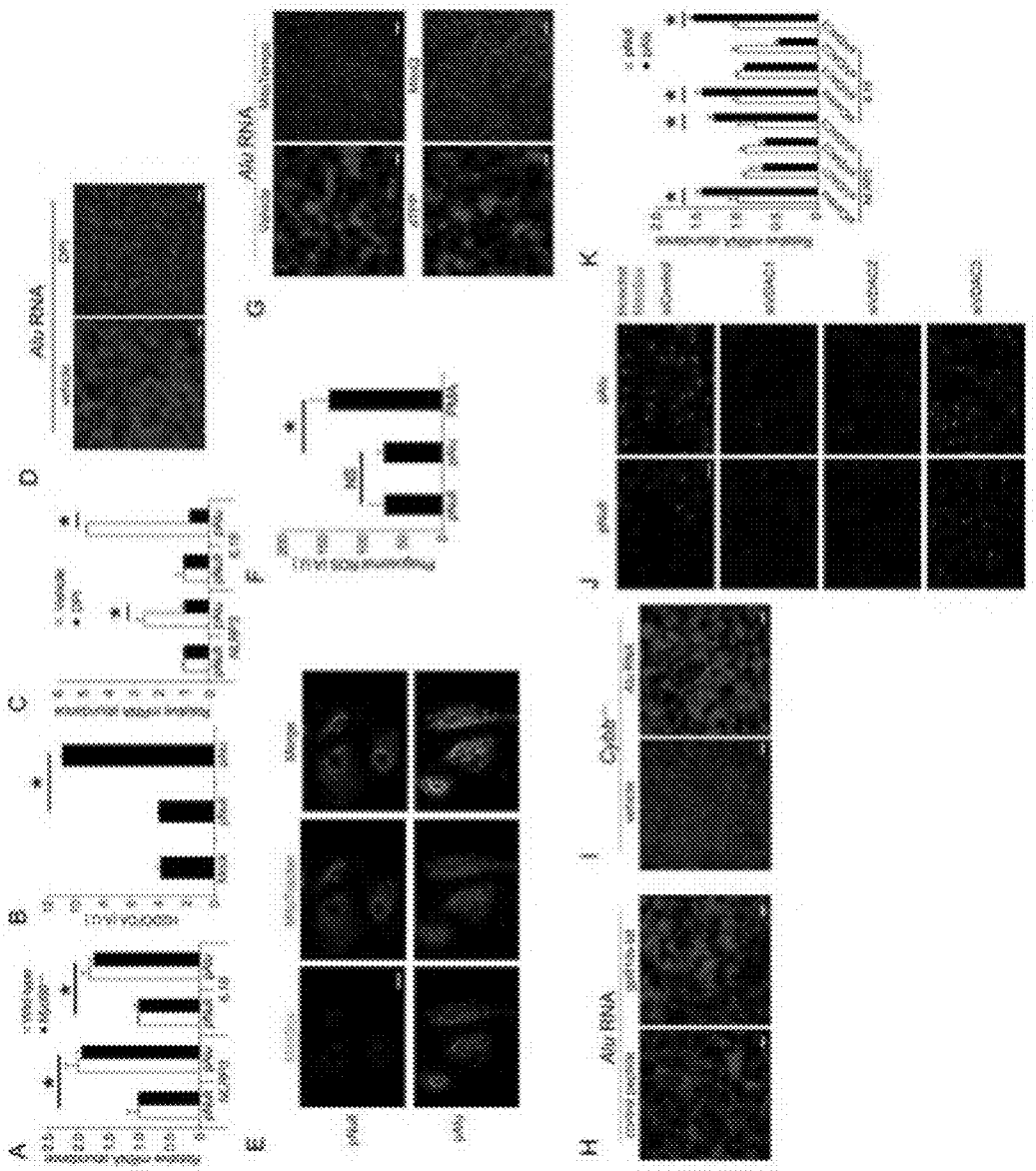

Figure 4

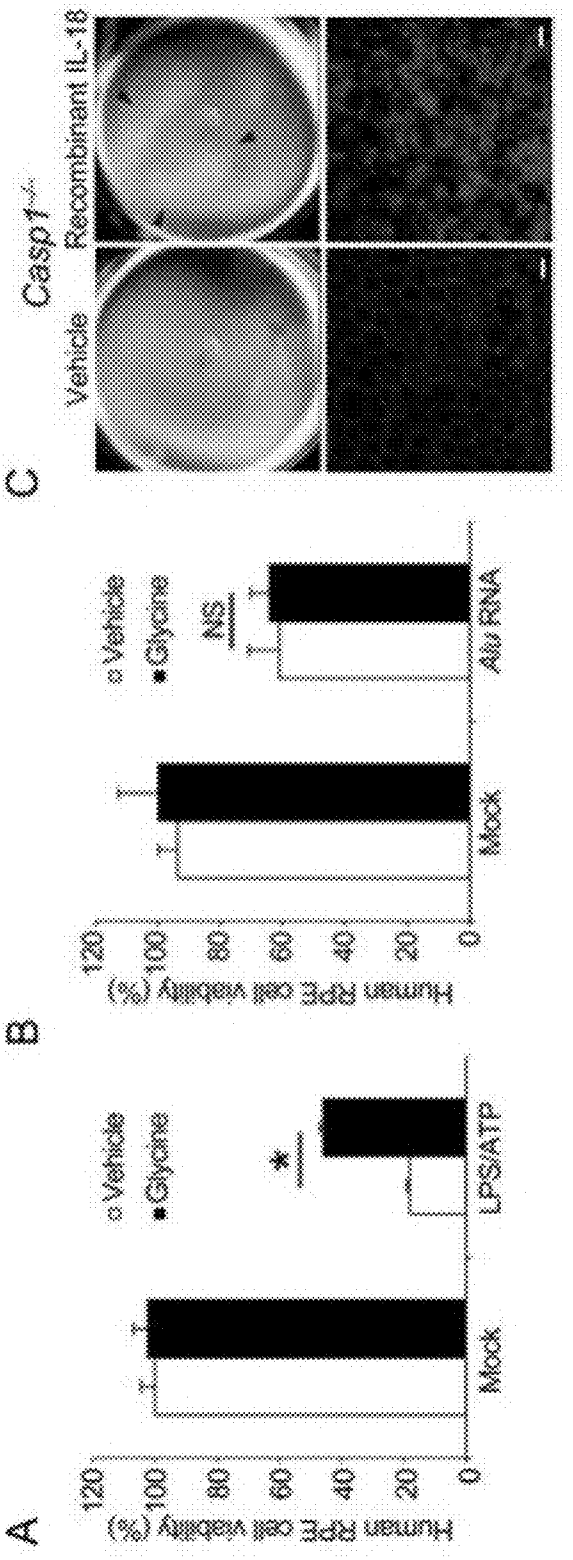
A
Human RPE cell viability (%)
120
100
80
60
40
20
0
Vehicle
Glycine
*
Mock
LPS/ATP
B
Human RPE cell viability (%)
120
100
80
60
40
20
0
Vehicle
Glycine
NS
Mock
Alu RNA
C
Casp1-/-
Vehicle
Recombinant IL-18

Figure 5

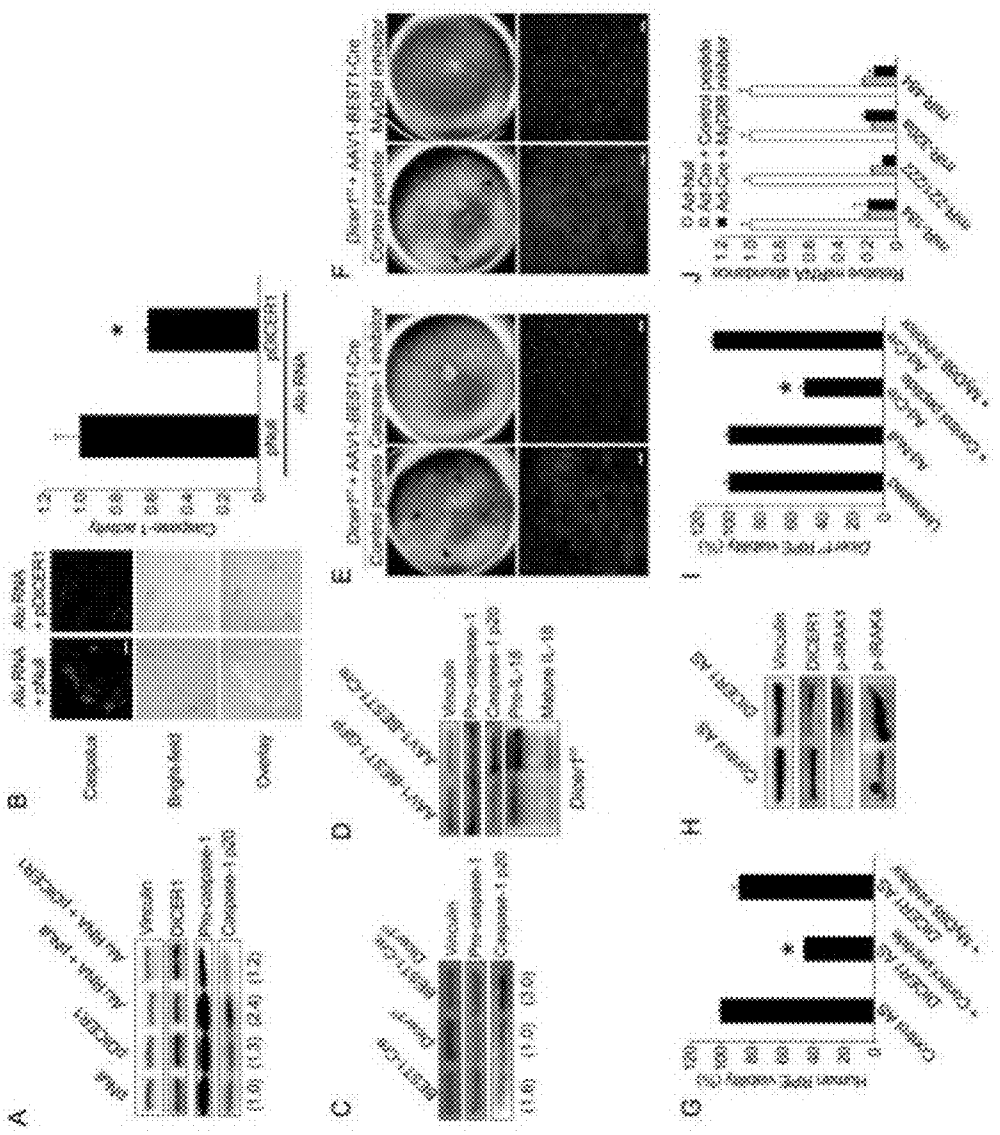

Figure 6

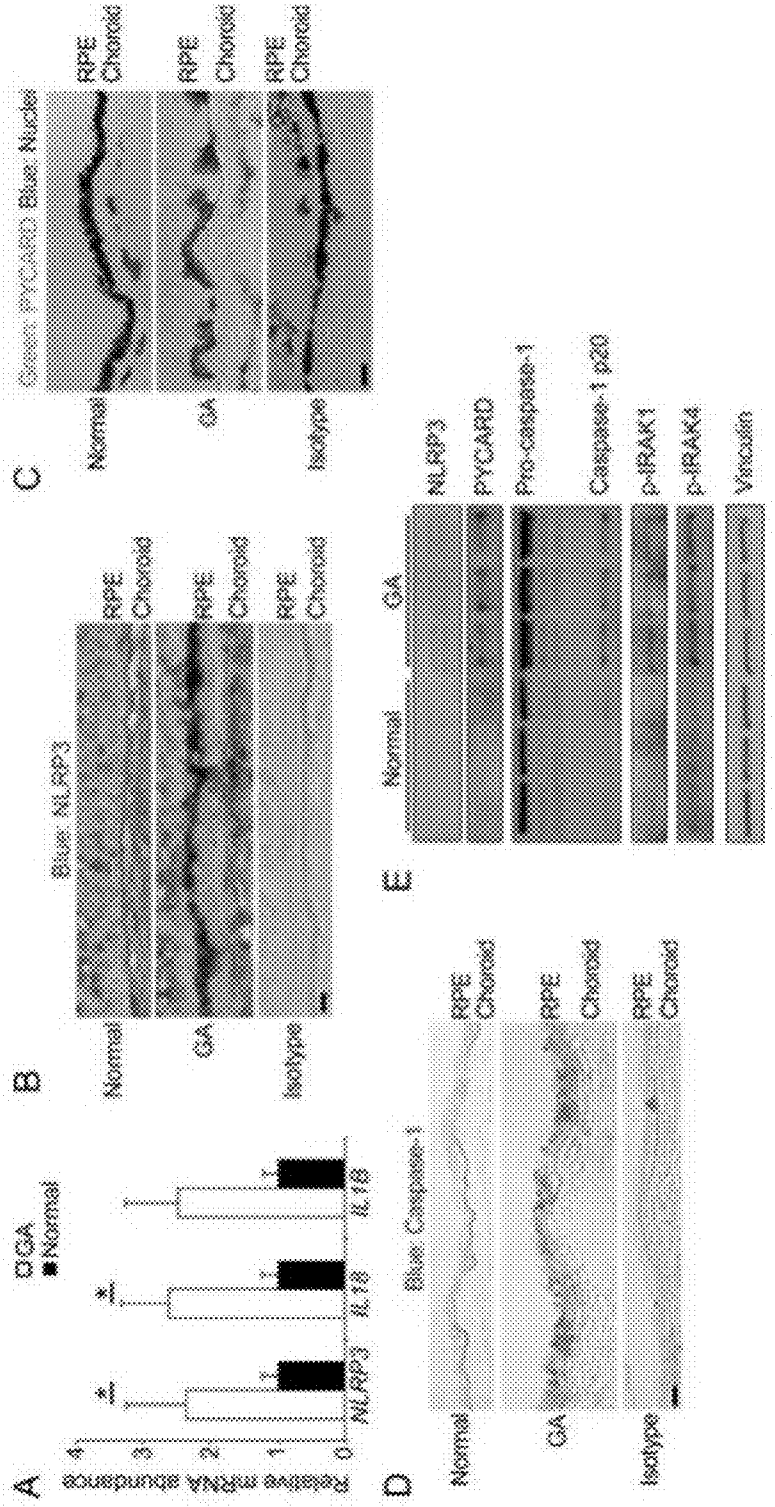
A
GA
Normal
Relative mRNA abundance
4
3
2
1
0
NLRP3
IL18
IL18
B
Blue NLRP3
Normal
GA
Isotype
RPE
Choroid
C
Green PYCARD Blue Nuclei
Normal
GA
Isotype
RPE
Choroid
D
Blue Caspase-1
Normal
GA
Isotype
RPE
Choroid
E
Normal
GA
NLRP3
PYCARD
Pro-caspase-1
Caspase-1 p20
p-IRAK1
p-IRAK4
Vinculin

Figure 7

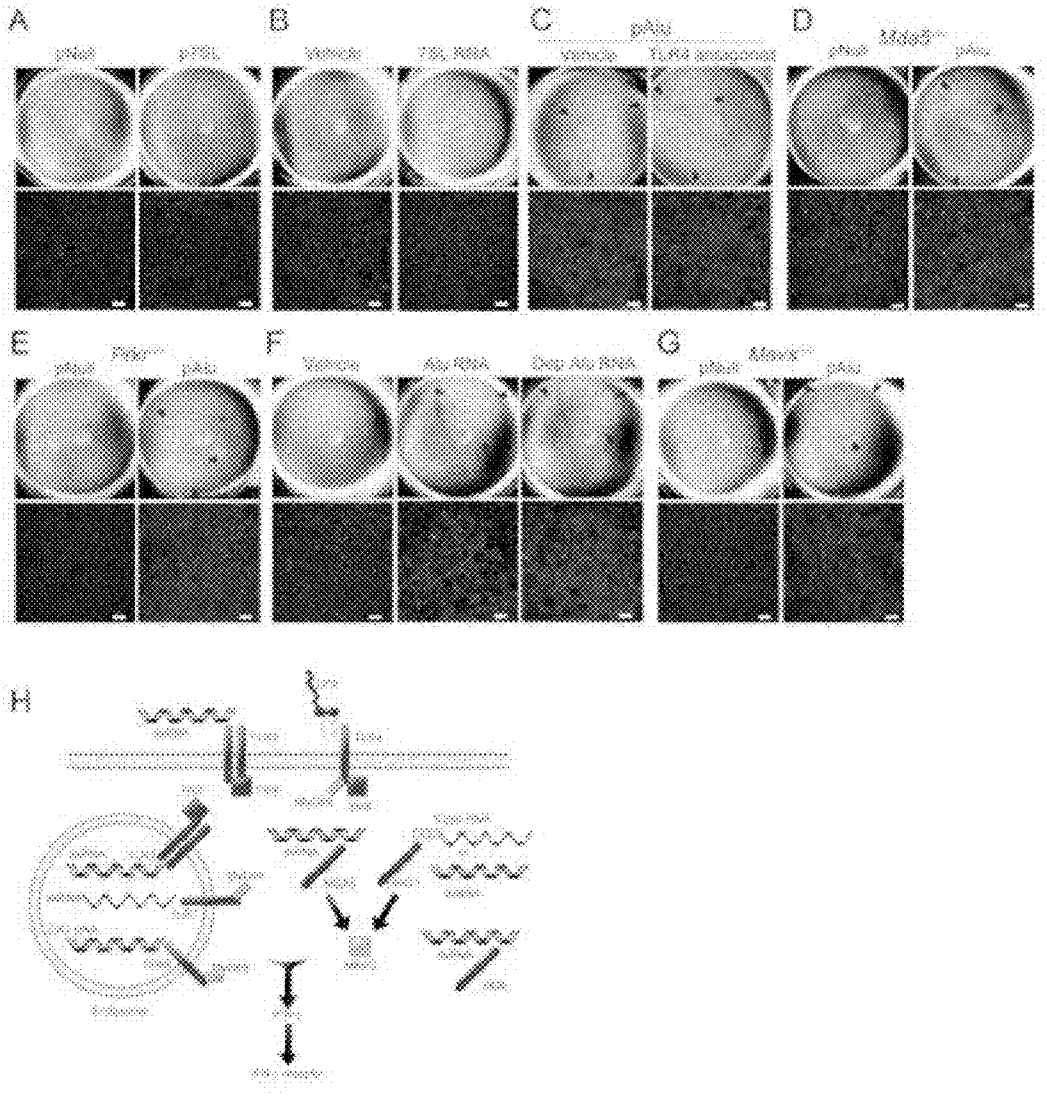

Figure 8

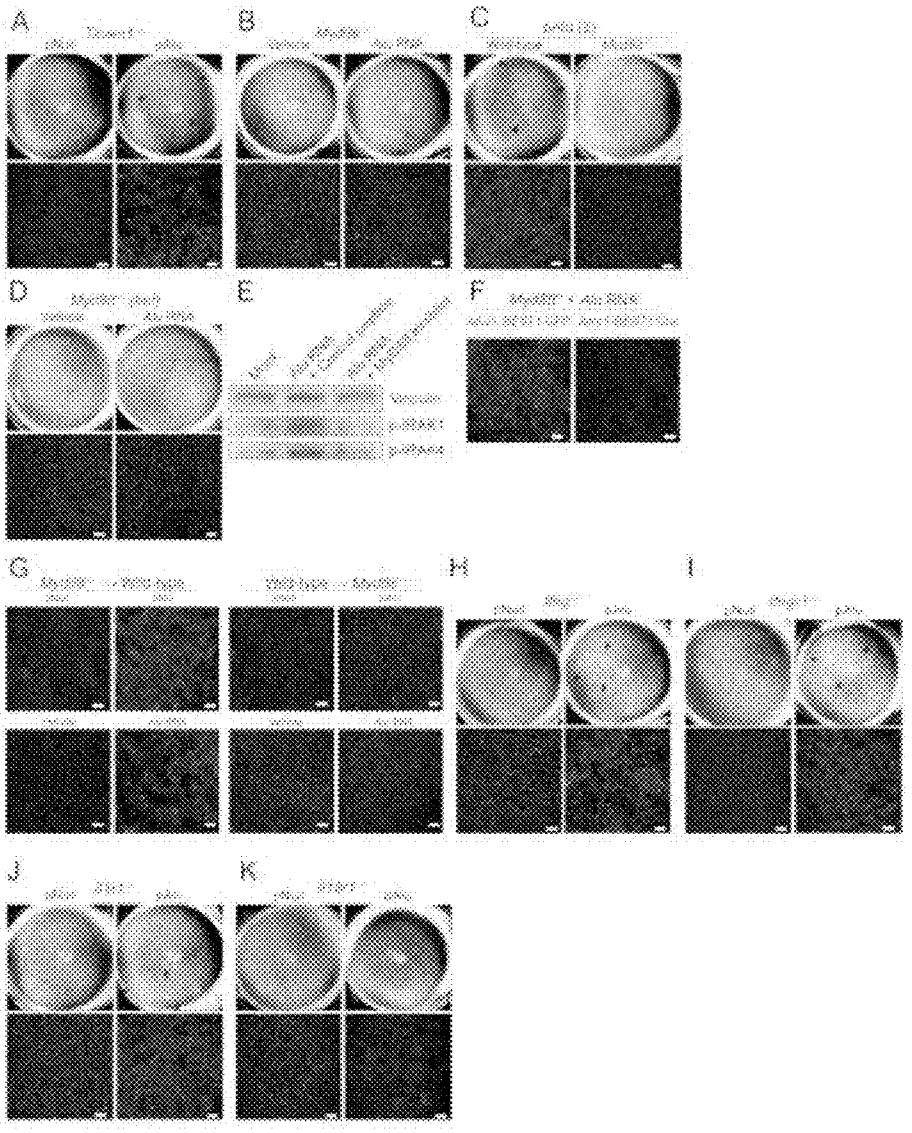

Figure 9

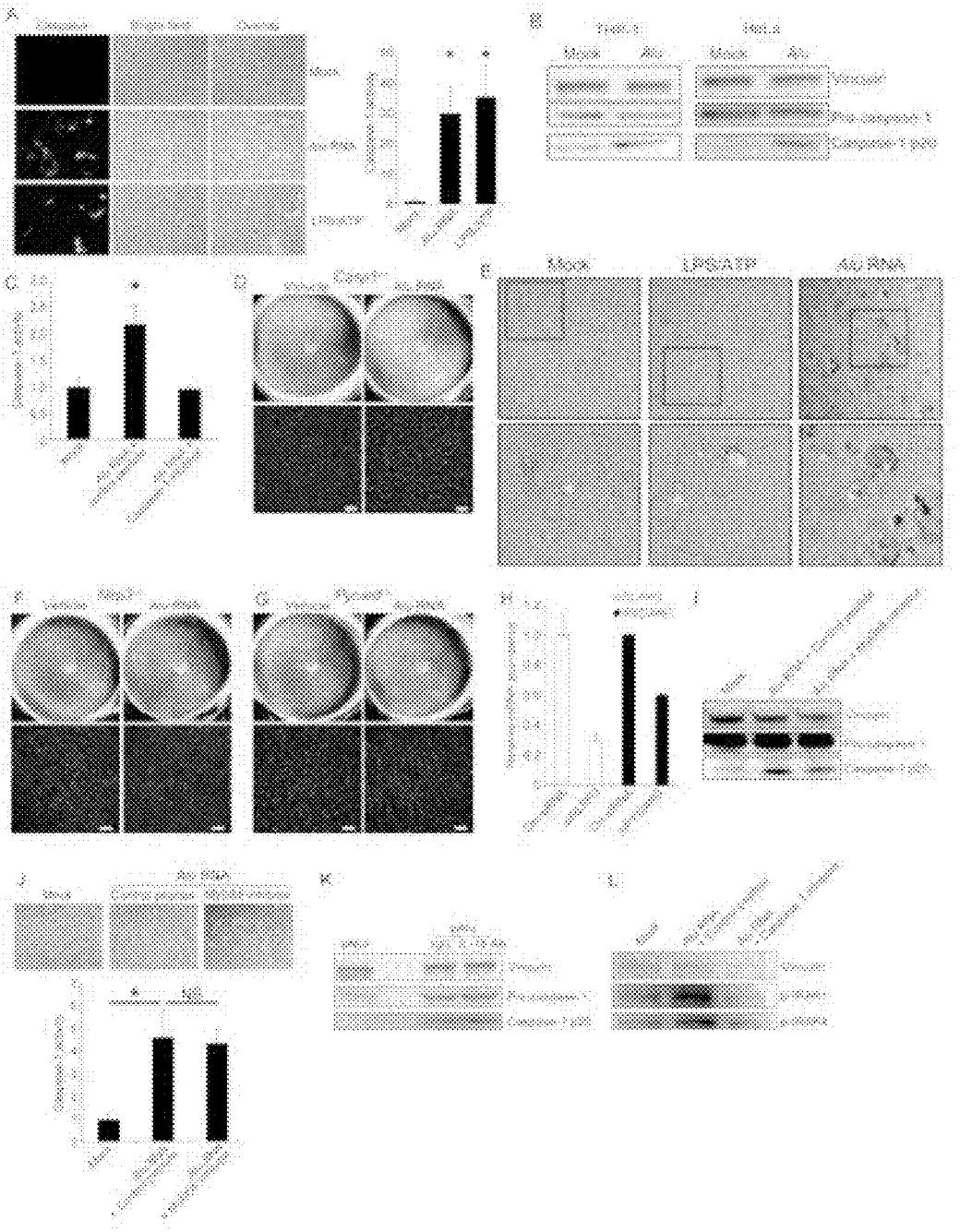

Figure 10

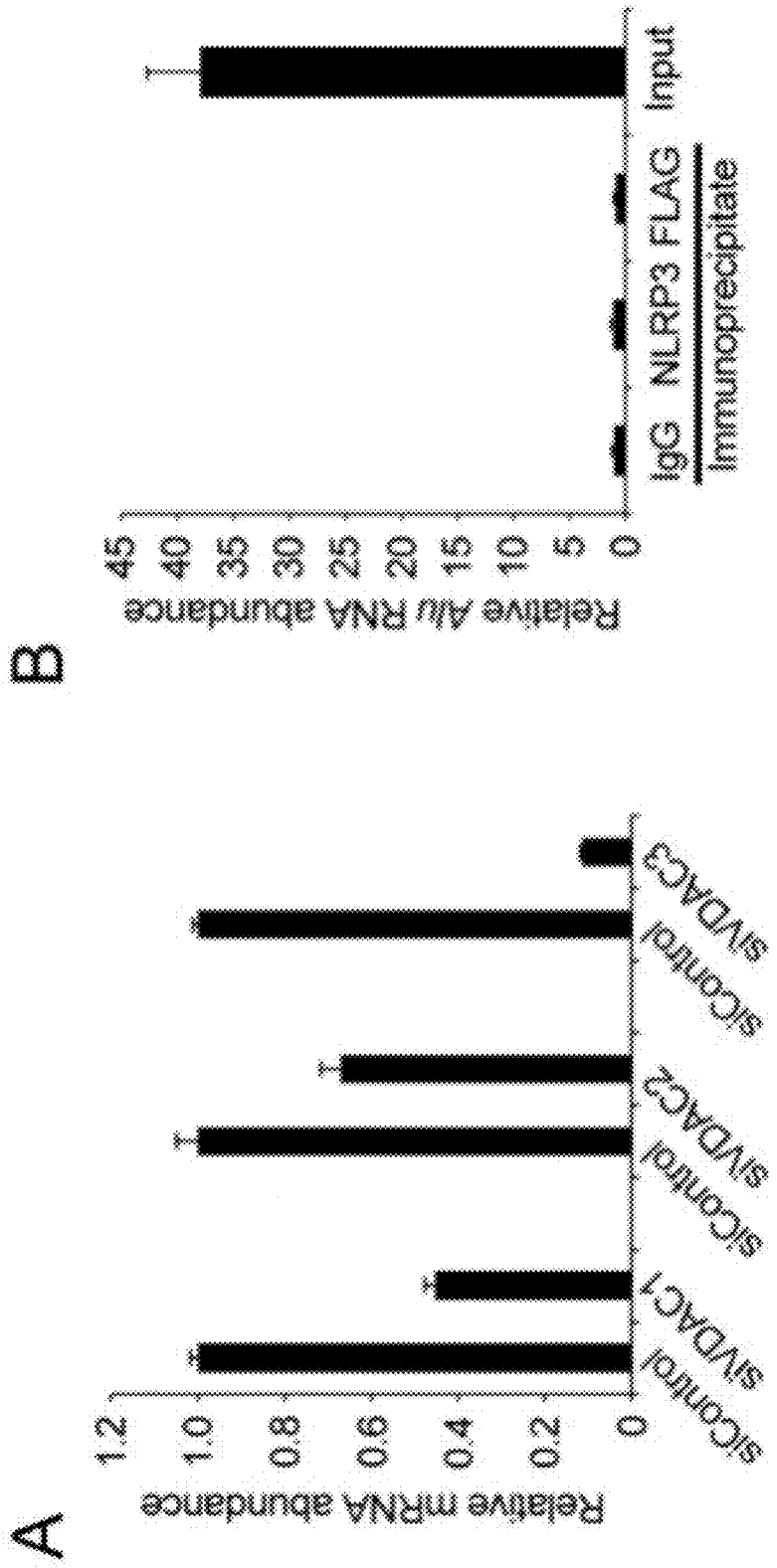
A
Relative mRNA abundance
1.2
1.0
0.8
0.6
0.4
0.2
0
siControl
siVDAC1
siControl
siVDAC2
siControl
siVDAC3
B
Relative Alu RNA abundance
45
40
35
30
25
20
15
10
5
0
IgG
NLRP3 FLAG
Immunoprecipitate
Input

Figure 11

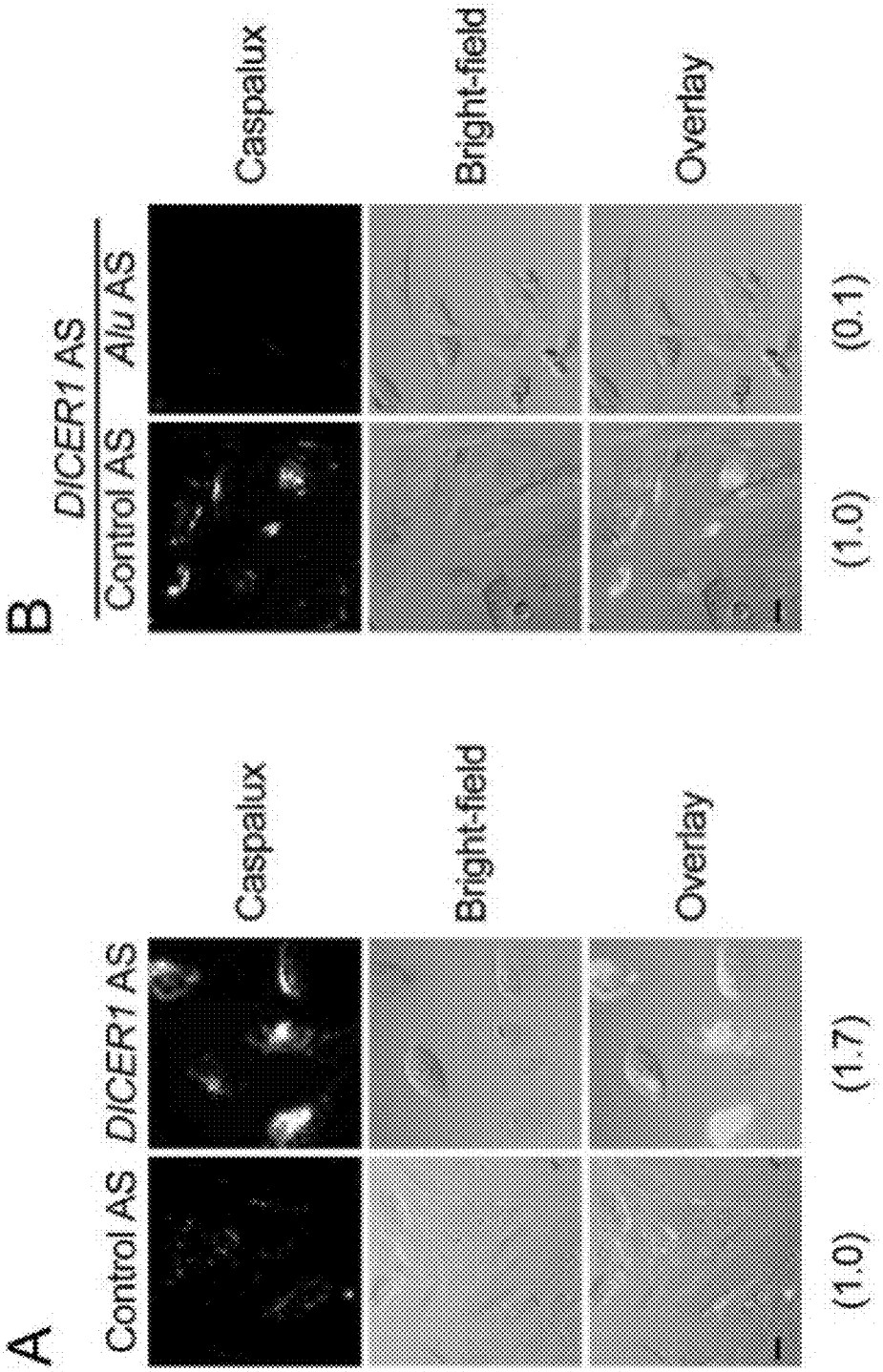
A
Control AS
DICER1 AS
Caspalux
Bright-field
Overlay
(1.0)
(1.7)
B
DICER1 AS
Control AS
Alu AS
Caspalux
Bright-field
Overlay
(1.0)
(0.1)

Figure 12

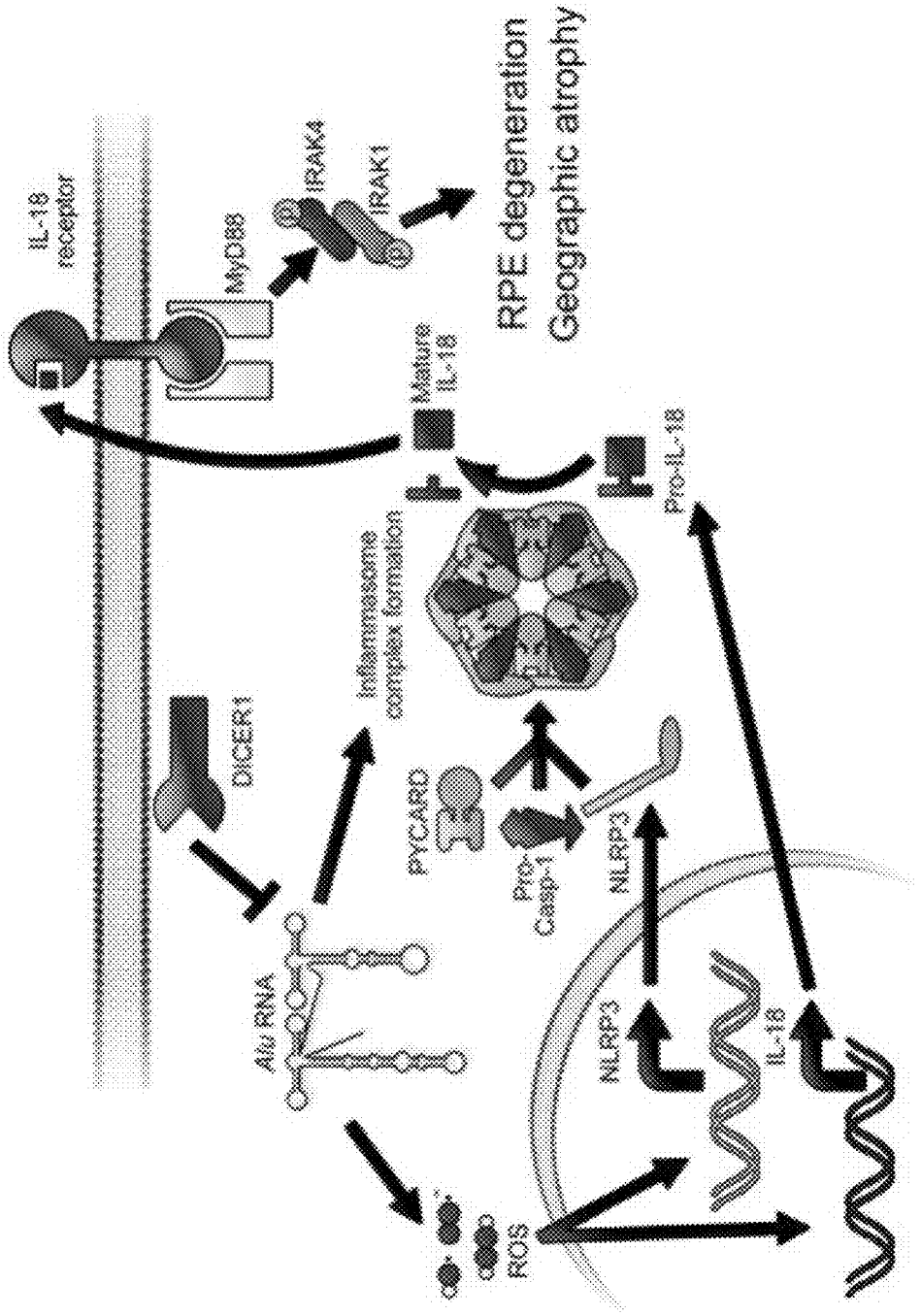
IL-18
receptor
MyD88
IRAK4
IRAK1
RPE degeneration
Geographic atrophy
Mature
IL-18
Pro-IL-18
Inflammasome
complex formation
DICER1
PYCARD
Pro-
Casp-1
NLRP3
Alu RNA
ROS
NLRP3
IL-18

Figure 13

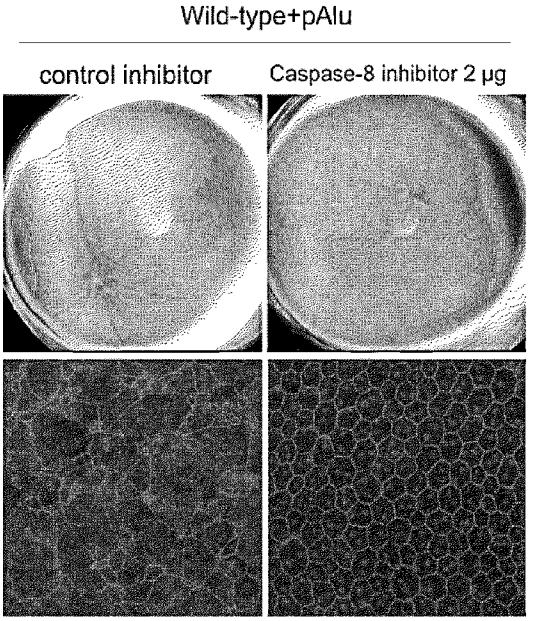

Figure 14

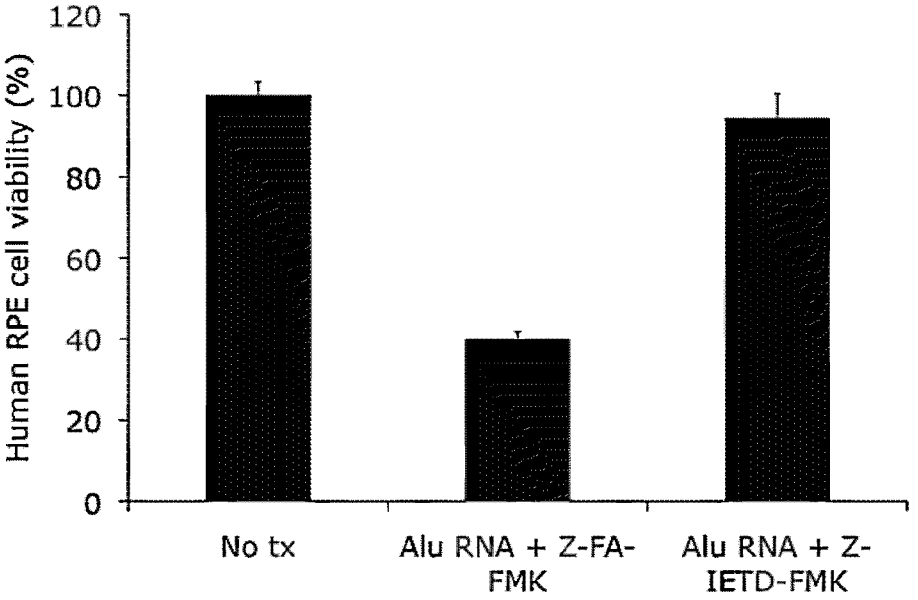

Figure 15

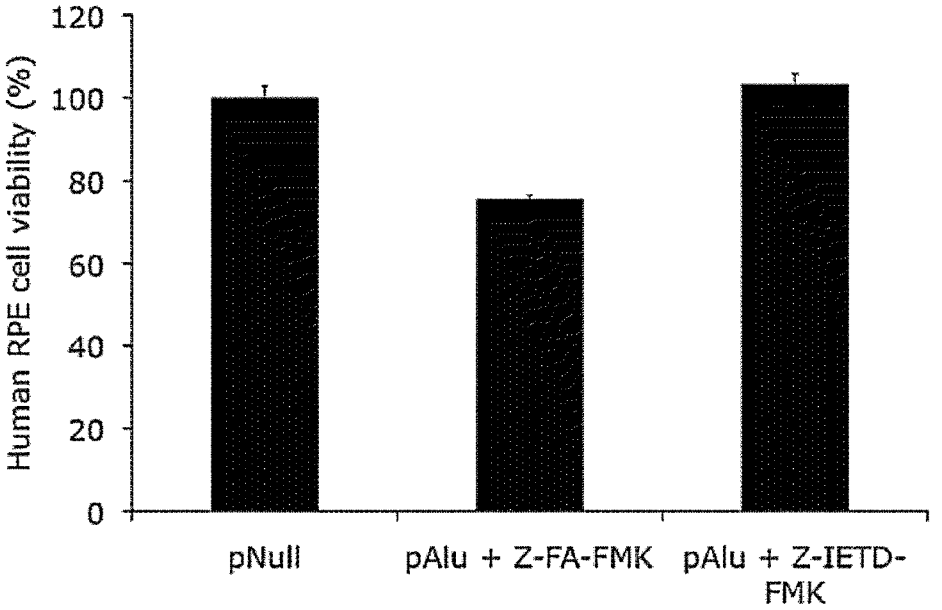

Figure 16

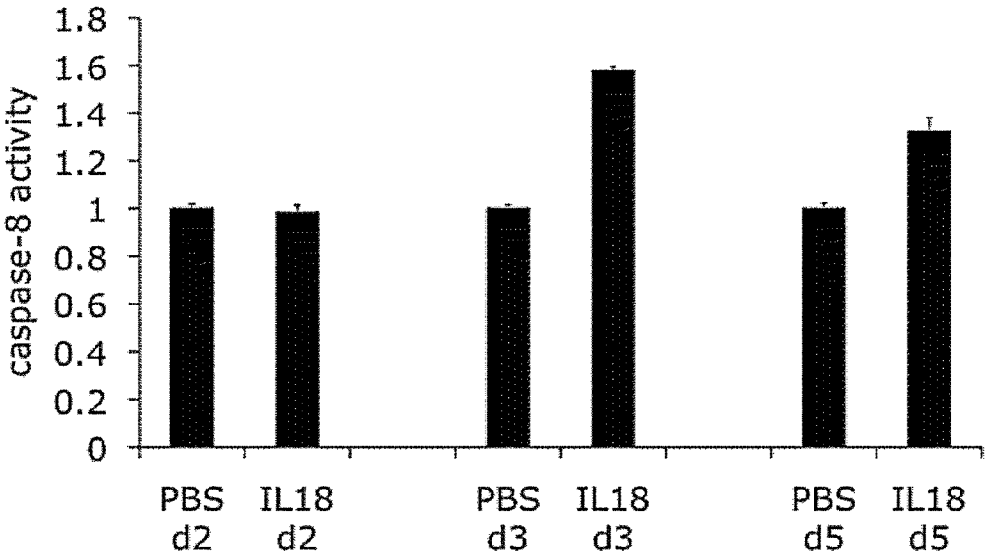

Figure 17

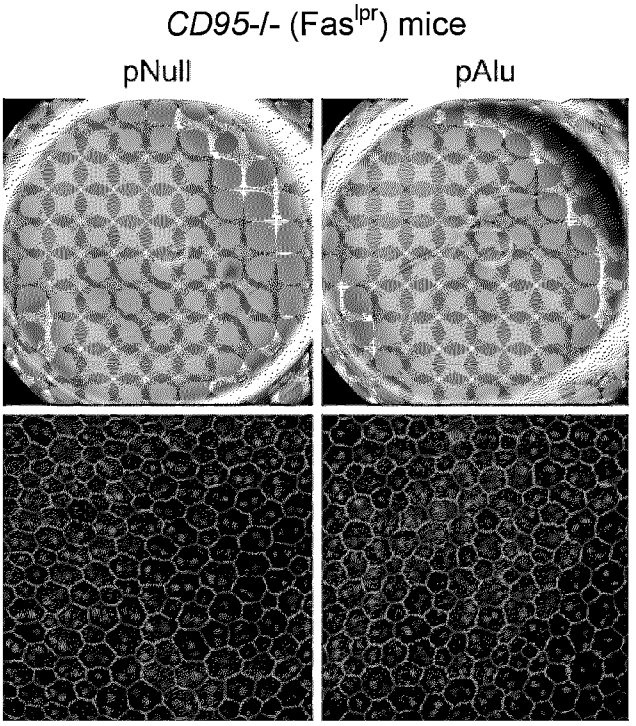

Figure 18

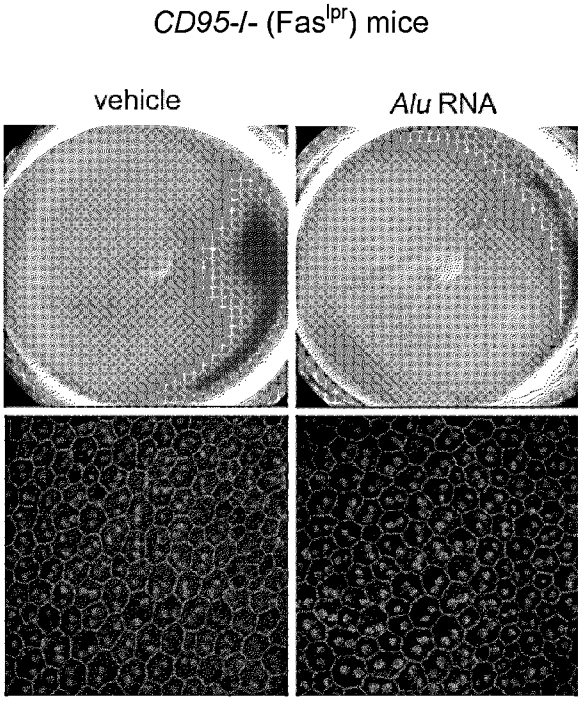

Figure 19

CD95-/- (Fas^{lpr}) mice

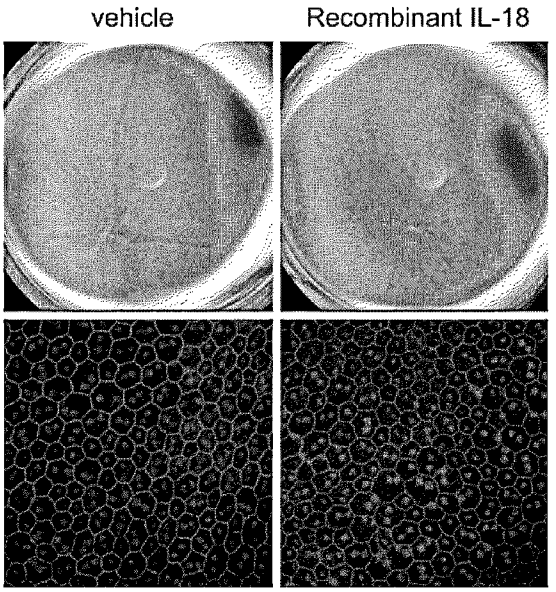

Figure 20

FasIg-/- (FasI^{gld}) mice

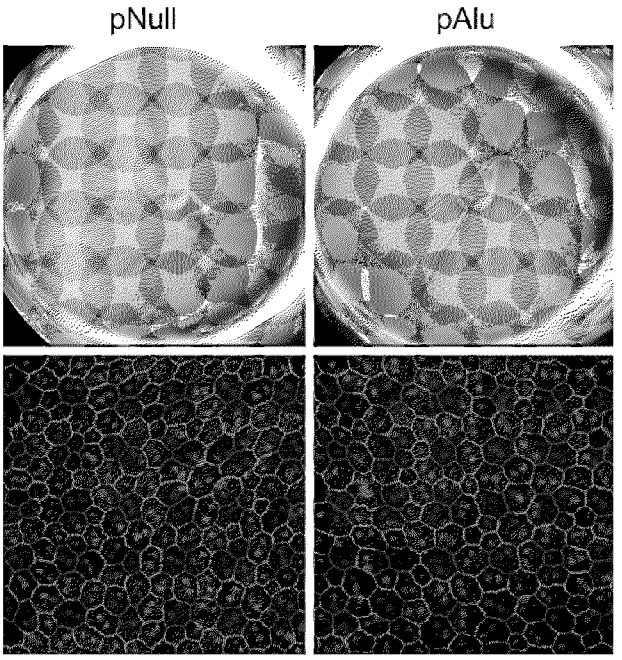

Figure 21

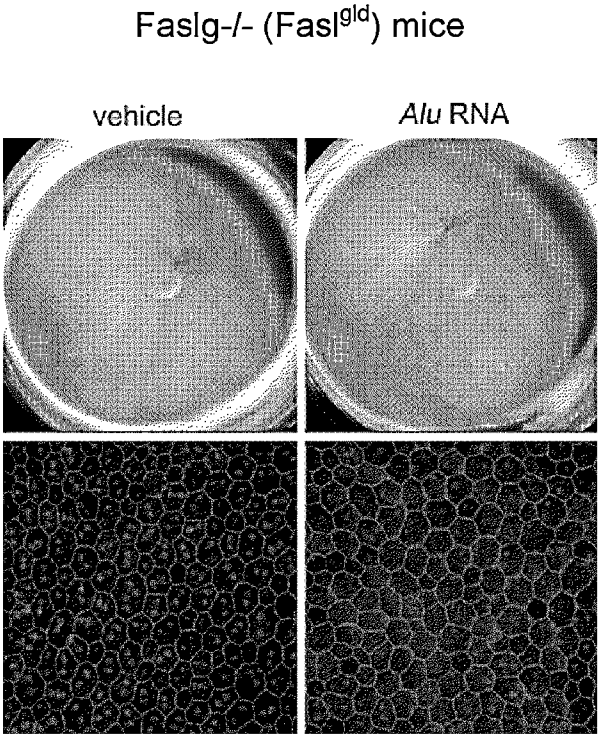

Figure 22

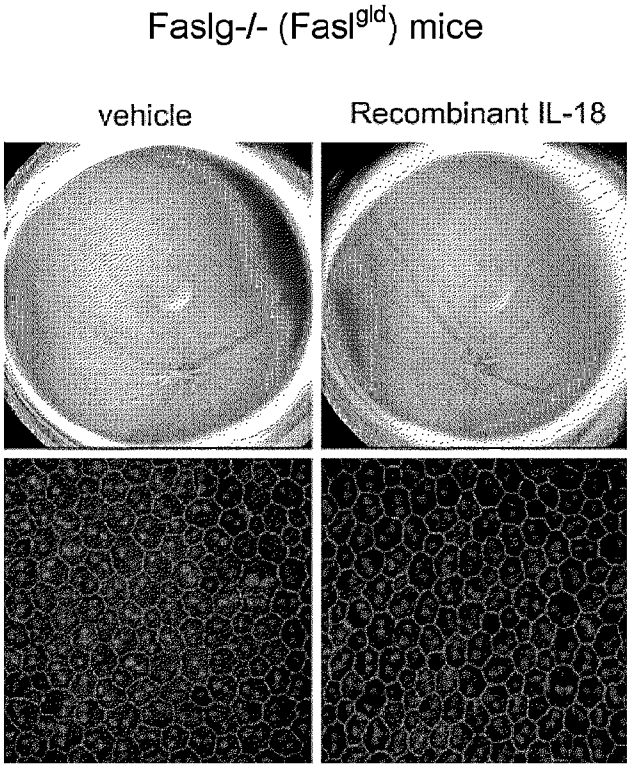

Figure 23

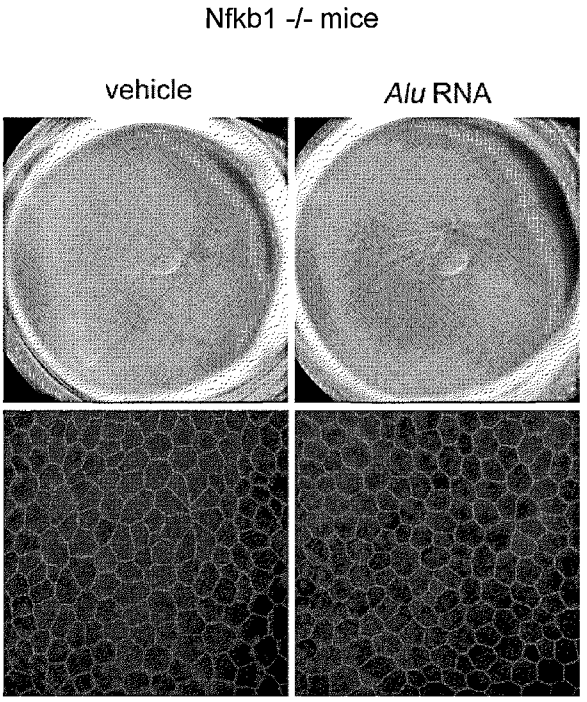

Figure 24

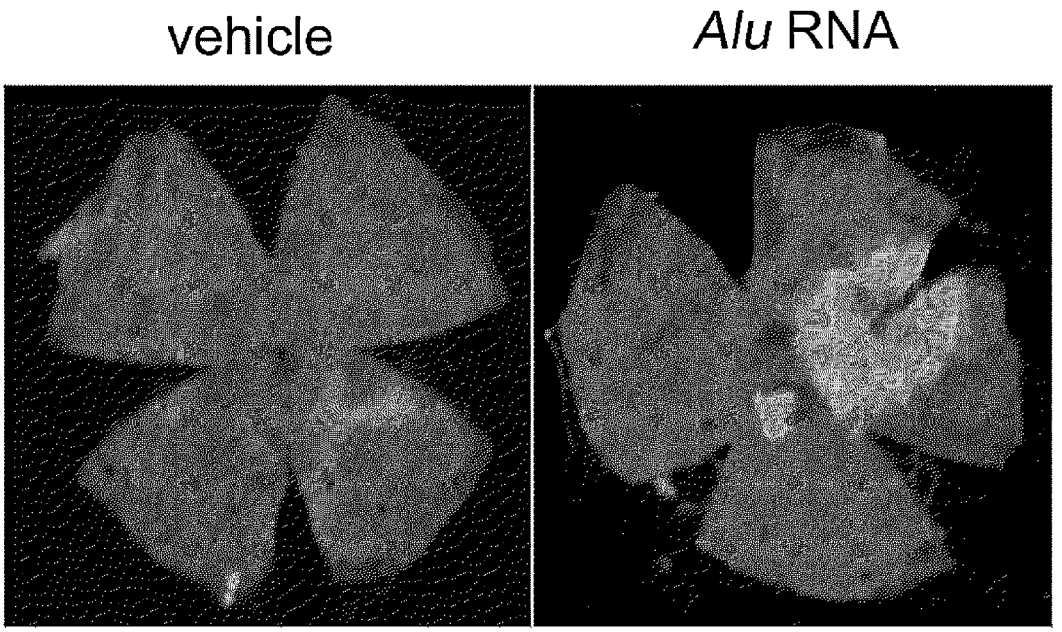

Figure 25

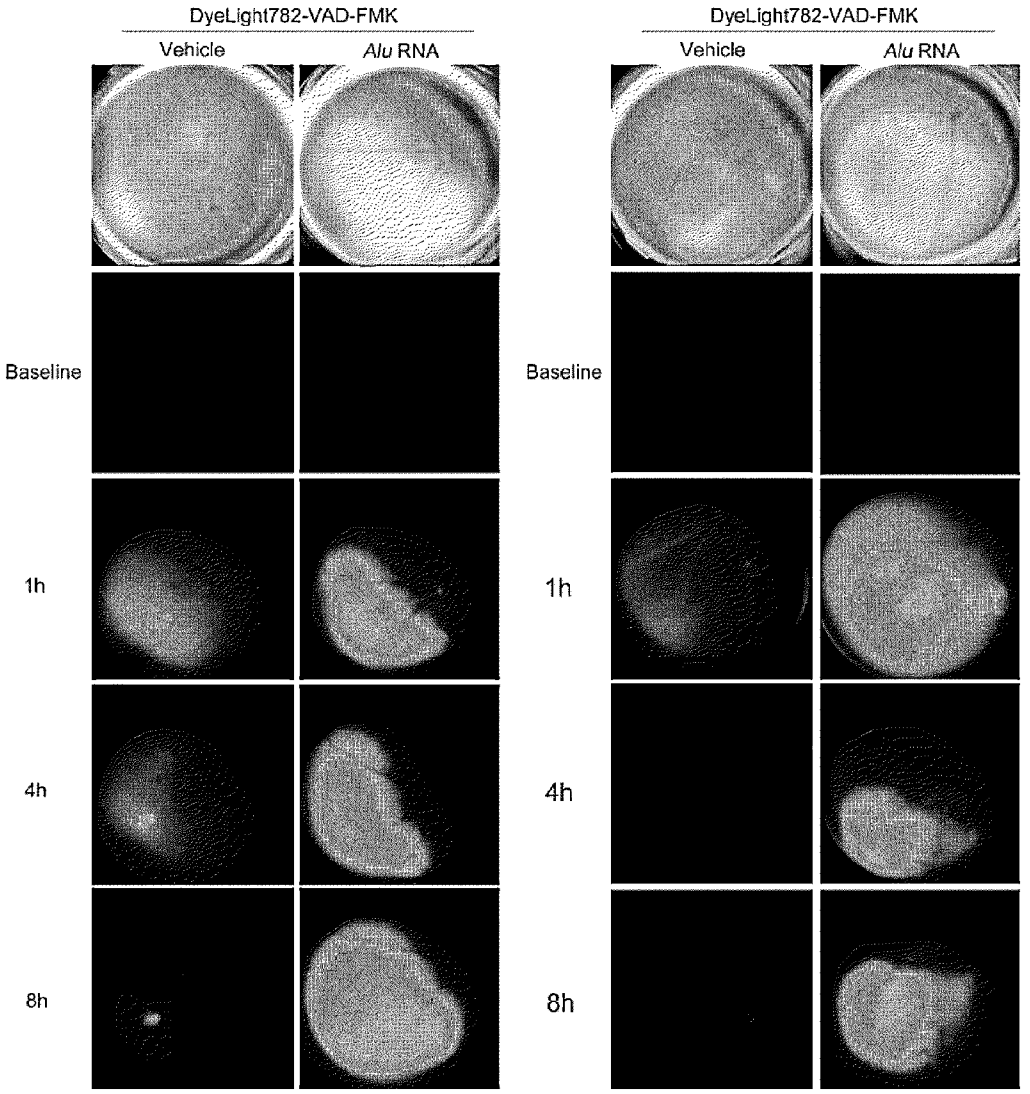
DyeLight782-VAD-FMK
Vehicle
Alu RNA
DyeLight782-VAD-FMK
Vehicle
Alu RNA
Baseline
Baseline
1h
1h
4h
4h
8h
8h

Figure 26

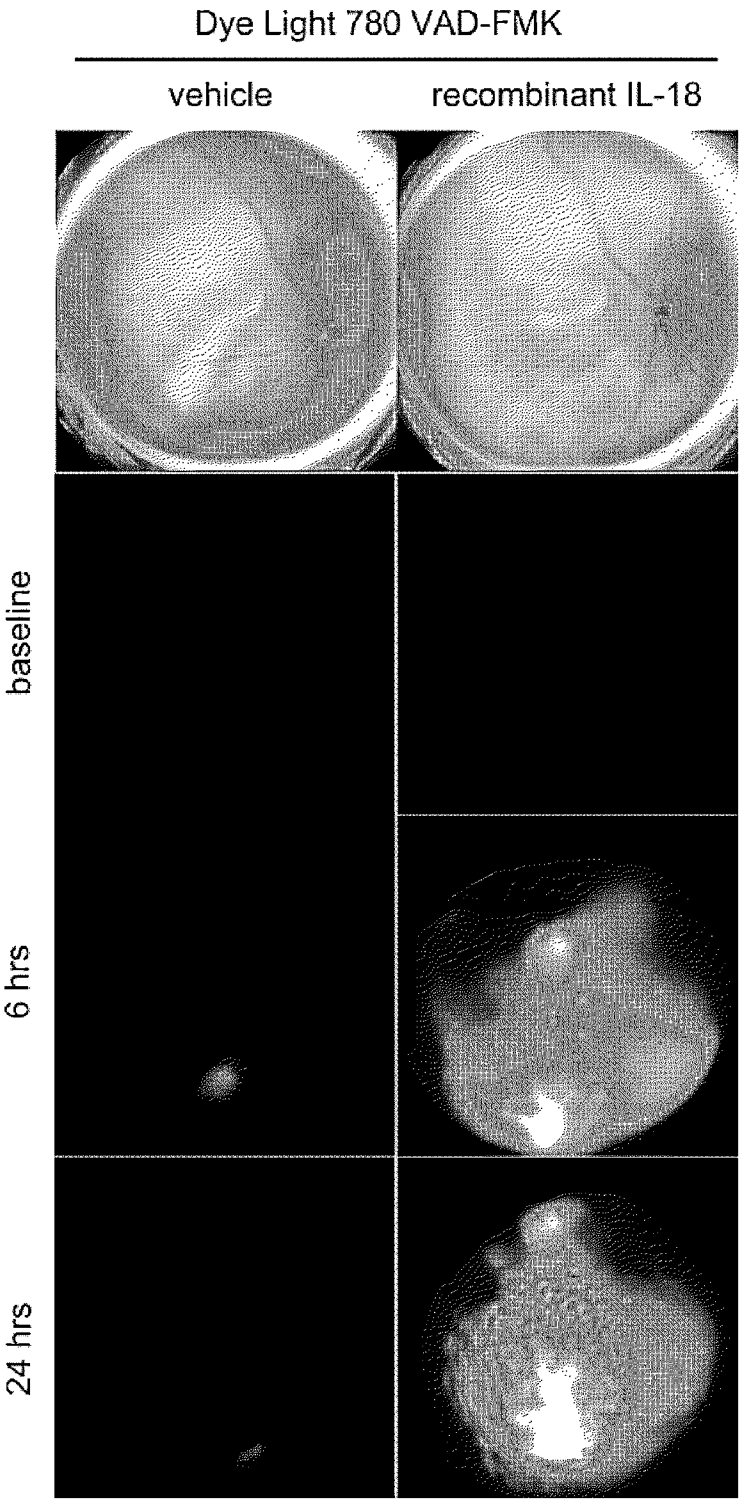
Dye Light 780 VAD-FMK
vehicle
recombinant IL-18
baseline
6 hrs
24 hrs

Figure 27

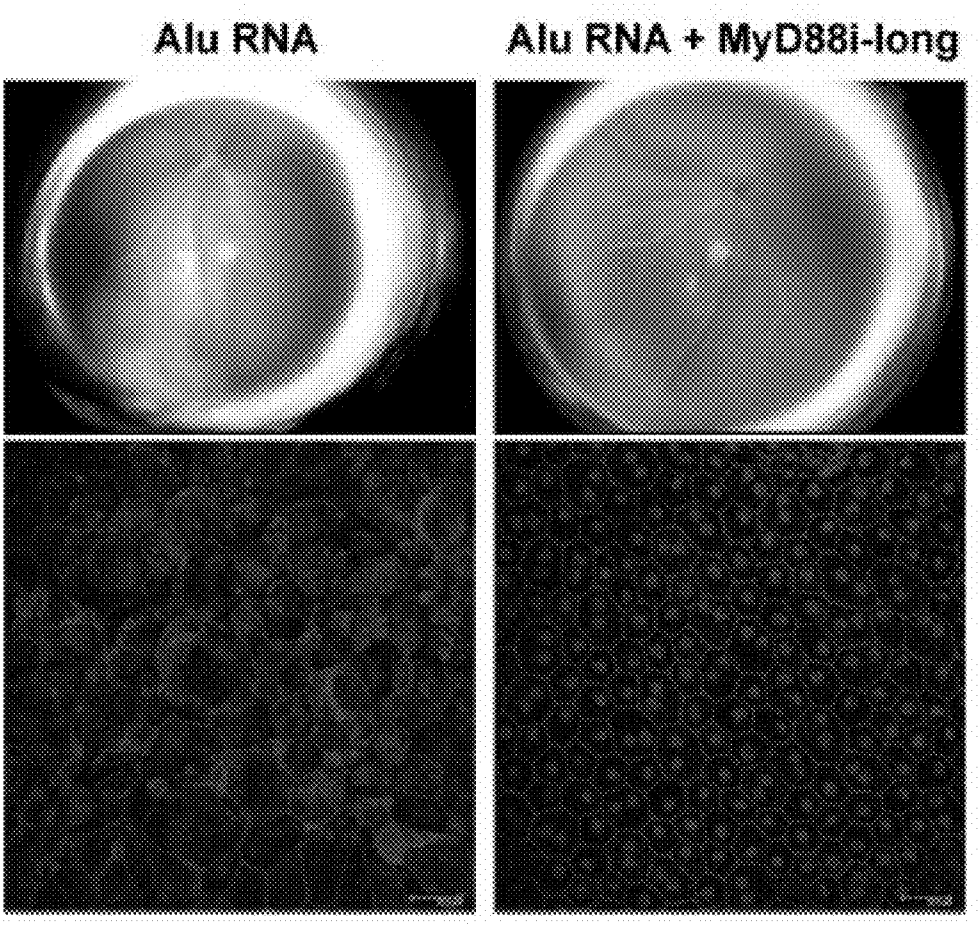

Figure 28

PROTECTION OF CELLS FROM *ALU*-RNA-INDUCED DEGENERATION AND INHIBITORS FOR PROTECTING CELLS

RELATED APPLICATIONS

This application claims priority from International Patent Application No. PCT/US2012/046928 filed Jul. 16, 2012, which claims priority to U.S. Provisional Application Ser. No. 61/508,867 filed Jul. 18, 2011 and U.S. Provisional Application Ser. No. 61/543,038 filed Oct. 4, 2011, the entire disclosures of each of which are incorporated herein by this reference.

GOVERNMENT INTEREST

This invention was made with government support under R01EY018350, R01EY018836, R01EY020672, R01EY022238, R21EY019778, RC1EY020442 awarded by the National Eye Institute of the National Institutes of Health. The government has certain rights in the invention.

TECHNICAL FIELD

The presently-disclosed subject matter relates to inhibition of inflammosome, MyD88, IL-18, VDAC1, VDAC2, Caspase-8, and NFκB; inhibitors of inflammosome, MyD88, IL-18, VDAC1, VDAC2, Caspase-8, and NFκB, methods protecting a cell, and screening methods for identifying inhibitors.

INTRODUCTION

Age-related macular degeneration (AMD), which is as prevalent as cancer in industrialized countries, is a leading cause of blindness worldwide. In contrast to the neovascular form of AMD, for which many approved treatments exist, the far more common atrophic form of AMD remains poorly understood and without effective clinical intervention. Extensive atrophy of the retinal pigment epithelium (RPE) leads to severe vision loss and is termed geographic atrophy, the pathogenesis of which is unclear. Geographic atrophy causes blindness in millions of people worldwide and there is currently no approved treatment.

The present inventors have shown a dramatic reduction of the RNase DICER1 in the retinal pigmented epithelium (RPE) of human eyes with geographic atrophy (Kaneko et al. *Nature* 2011, which is incorporated herein by this reference). The present inventors have also demonstrated that DICER1 deficiency leads to an accumulation of Alu RNA transcripts, which is also observed in the RPE of human eyes with geographic atrophy. These Alu RNA transcripts induce cell death of human RPE cells and RPE degeneration in mice. The precise mechanisms of cytotoxicity of Alu transcripts are unknown.

As described herein the present inventors have now found that DICER1 deficit or Alu RNA exposure activates the NLRP3 inflammasome and triggers toll-like receptor-independent MyD88 signalling via IL-18 both in the RPE of mice and in human and mouse RPE cells.

SUMMARY

The presently-disclosed subject matter meets some or all of the above-identified needs, as will become evident to those of ordinary skill in the art after a study of information provided in this document.

This Summary describes several embodiments of the presently-disclosed subject matter, and in many cases lists variations and permutations of these embodiments. This Summary is merely exemplary of the numerous and varied embodiments. Mention of one or more representative features of a given embodiment is likewise exemplary. Such an embodiment can typically exist with or without the feature(s) mentioned; likewise, those features can be applied to other embodiments of the presently-disclosed subject matter, whether listed in this Summary or not. To avoid excessive repetition, this Summary does not list or suggest all possible combinations of such features.

The presently-disclosed subject matter includes methods for identifying MyD88 inhibitors, and methods and compositions for inhibiting MyD88 and uses thereof. The presently-disclosed subject matter includes methods for identifying inflammasome inhibitors, and methods and compositions for inhibiting an inflammasome and uses thereof. The presently-disclosed subject matter includes methods for identifying inhibitors of components of inflammosome, and methods and compositions for inhibiting a component of inflammasome and uses thereof. Components of inflammasome include, for example, NLRP3, PYCARD, and Caspase-1. The presently-disclosed subject matter includes methods for identifying IL-18 inhibitors, and methods and compositions for inhibiting IL-18 and uses thereof. The presently-disclosed subject matter includes methods for identifying VDAC1 and VDAC2 inhibitors, and methods and compositions for inhibiting VDAC1 and VDAC2 and uses thereof. The presently-disclosed subject matter includes methods for identifying caspase-8 inhibitors, and methods and compositions for inhibiting caspase-8 and uses thereof. The presently-disclosed subject matter includes methods for identifying NFkB inhibitors, and methods and compositions for inhibiting NFkB and uses thereof. Also provided are methods and compositions for imaging activated caspase-1 in an eye of a subject.

The presently-disclosed subject matter includes methods including inhibiting one or more of an inflammasome, MyD88, and IL-18 of a cell. In some embodiments, the presently-disclosed subject matter includes methods including inhibiting one or more of MyD88, IL-18, VDAC1, VDAC2, NFκB, caspase-8, caspase-1, NLRP-3, PYCARD, and an inflammasome, including a component of an inflammasome (e.g., caspase 1, NLRP-3, PYCARD) of a cell. In some embodiments, the presently-disclosed subject matter includes methods including administering one or more inhibitors selected from inhibitors of MyD88, IL-18, VDAC1, VDAC2, NFκB, caspase-8, caspase-1, NLRP-3, PYCARD, and an inflammasome, including a component of an inflammasome (e.g., caspase 1, NLRP-3, PYCARD).

In some embodiments of the method, the cell is selected from an RPE cell, a retinal photoreceptor cell, or a choroidal cell. In some embodiments, the cell is an RPE cell. In some embodiments, the cell is the cell of a subject. In some embodiments, the cell is a cell of a subject having, suspected of having, or at risk of having a condition of interest. In some embodiments, the cell is a cell of a subject having, suspected of having, or at risk of having age-related macular degeneration. In some embodiments, the cell is a cell of a subject having, suspected of having, or at risk of having geographic atrophy. In some embodiments, the cell is a cell of a subject having, suspected of having, or at risk of having geographic atrophy and the cell is an RPE cell. In some embodiments, a subject having age-related macular degeneration can be treated using methods and compositions as disclosed herein.

In some embodiments of the method the cell is protected against Alu-RNA-induced degeneration.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1**. Alu RNA does not activate or function via toll-like receptors (TLRs) (A-E) pAlu, but not pNull, induces RPE degeneration in WT (A), Tlr3$^{-/-}$ (B), Tlr7$^{-/-}$ (C), Unc93b1 mt mice, which are functionally deficient in TLRs-3,7,9 (D), and Tlr4$^{-/-}$ mice (E). Representative images shown. n=8-12. Fundus photographs, top row; Flat mounts stained for zonula occludens-1 (ZO-1; red), bottom row. Degeneration outlined by blue arrowheads. Scale bars, 20 μm. (F) Stimulation of HEK293 cell lines expressing various TLRs with either of two different Alu RNA sequences does not elicit NF-κB activation. Positive (+) controls using TLR-specific ligands activated NF-κB. n=3. Data are represented as mean+/−SEM. See also FIG. **8**.

FIG. **2**. Alu RNA induces RPE degeneration via MyD88 (A) pAlu does not induce RPE degeneration in Myd88$^{-/-}$ mice. (B) pAlu-induced RPE degeneration in WT mice is inhibited by a MyD88 homodimerization peptide inhibitor (MyD88i), but not by a control peptide. (C) pAlu-induced RPE degeneration in WT mice is inhibited by cholesterol-conjugated Myd88 siRNA but not control siRNA. (D and E) siRNA targeting MyD88 (siMyD88) reduces target gene (D) and protein (E) abundance in mouse RPE cells compared to control siRNA. n=3, *p<0.05 by Student t-test. (F) pAlu does not induce RPE degeneration in Myd88 heterozygous (het) mice. (G) Western blot of Alu RNA-induced IRAK1 and IRAK4 phosphorylation in human RPE cells. Image representative of 3 experiments. (H) pAlu reduces cell viability of WT but not Myd88 mouse RPE cells. (I) Loss of human RPE cell viability induced by pAlu is rescued by MyD88i. (J) AAV1-BEST1-Cre, but not AAV1-BEST1-GFP, protected Myd88$^{f/f}$ mice from pAlu-induced RPE degeneration. (K) pAlu induces IL-18 secretion from human RPE cells measured by ELISA. IL-1β secretion is barely detectable. n=3, *p<0.05 by Student t-test. (L) Recombinant IL-18 induces RPE degeneration in WT but not Myd88$^{-/-}$ mice. (M and N) pAlu-induced RPE degeneration in WT mice is rescued by IL-18 neutralizing antibody (N) but not by IL-1β neutralizing antibody (M). Representative images shown. n=8-12. Fundus photographs, top row; ZO-1 stained (red) flat mounts, bottom row. Degeneration outlined by blue arrowheads. Scale bars, 20 μm (A-C,F,J,L-N). n=3, *p<0.05 by Student t-test. Data are represented as mean+/−SEM (D,E,H,I,K). See also FIG. **9**.

FIG. **3**. Alu RNA induces RPE degeneration via NLRP3 inflammasome (A) Western blot of Caspase-1 activation (p20 subunit) by Alu RNA in human RPE cells. (B) Western blot of pAlu-induced IL-18 maturation in RPE cell lysates in wild-type mice impaired by Caspase-1 peptide inhibitor. (C) Caspase-1 peptide inhibitor protects WT mice from pAlu-induced RPE degeneration. (D and E) pAlu does not induce RPE degeneration in Casp1$^{-/-}$ mice or (E) cytotoxicity in Casp1$^{-/-}$ mouse RPE cells. (F) Alu RNA and LPS+ATP induce formation of PYCARD clusters in human RPE cells transfected with GFP-PYCARD. (G and H) pAlu does not induce RPE degeneration in Nlrp3$^{-/-}$ (G) or Pycard$^{-/-}$ (H) mice. (I) Nlrp3$^{-/-}$ and Pycard$^{-/-}$ mouse RPE cells are protected against pAlu-induced loss of cell viability. (J) siRNAs targeting NLRP3 or PYCARD rescued human RPE cells from pAlu-induced cytotoxicity, compared to control siRNA. n=3-4, *p<0.05 by Student t-test (A,B,E,F,I,J). Images representative of 3 experiments. Densitometry values normalized to Vinculin are shown in parentheses (A,B). Fundus photographs, top row; ZO-1 stained (red) flat mounts, bottom row. Degeneration outlined by blue arrowheads. n=8-12. Scale bars, 20 μm (C,D,G,H). Representative images shown. See also FIG. **11**.

FIG. **4**. Alu RNA induces mitochondrial ROS production and NLRP3 priming (A) pAlu induces NLRP3 and IL18 mRNAs in WT and Myd88$^{-/-}$ mouse RPE cells. (B) pAlu induces generation of reactive oxygen species (ROS) in human RPE cells as monitored with the fluorescent probe H_2DCFDA (A.U, arbitrary units). (C) DPI blocks pAlu-induced NLRP3 and IL18 mRNAs in human RPE cells. (D) DPI protects WT mice from pAlu-induced RPE degeneration. (E) pAlu induces generation of mitochondrial reactive oxygen species in human RPE cells as detected by the fluorescence of MitoSOX Red (green pseudocolor), colocalized with respiring mitochondria labeled by MitoTracker Deep Red (red). (F) PMA, but not pAlu, induces phagosomal ROS generation, as assessed by fluorescent Fc OXYBURST Green assay in human RPE cells. (A.U, arbitrary units). (G) MitoTempo and MitoQ, but not vehicle or dTPP controls, prevent Alu RNA-induced RPE degeneration in WT mice. (H) NADPH oxidase inhibitor gp91ds-tat or a scrambled peptide do not prevent Alu RNA-induced RPE degeneration in WT mice. (I) Alu RNA induces RPE degeneration mice deficient in Cybb (which encodes the gp91^{phox} subunit of NADPH oxidase). (J and K) siRNAs targeting VDAC1 and VDAC2, but not VDAC3 or scrambled control, prevent pAlu-induced mROS generation (J) and upregulation of NLRP3 and IL18 mRNAs (K) in human RPE cells. mROS visualized with MitoSox Red dye and cell nuclei with Hoechst stain. n=3-4, *p<0.05 by Student t-test (A-C, K), NS, not significant by Student t-test (F). Representative images shown. n=8-12. ZO-1 stained (red) flat mounts. Scale bars, 20 nm (D, E, G-I), n=3-4. Scale bar, 100 μm (J). See also FIG. **11**.

FIG. **5**. RPE degeneration does not occur via pyroptosis (A and B) Glycine inhibits human RPE cell death induced by LPS+ATP (A) but not by pAlu (B). (C) Recombinant IL-18 induces RPE degeneration in Casp1$^{-/-}$ mice. n=3-4 (A,B), *p<0.05 by Student t-test. Representative images shown. n=8-12. Fundus photographs, top row; ZO-1 stained (red) flat mounts, bottom row. Degeneration outlined by blue arrowheads. Scale bars, 20 μm (C).

FIG. **6**. DICER1 loss induces cell death via inflammasome (A) Western blot of Alu RNA-induced Caspase-1 cleavage (p20) inhibited by DICER1 overexpression in human RPE cells. (B and C) DICER1 overexpression reduces Alu RNA-induced Caspase-1 activation in human RPE cells (measured by cleavage (B left panel, green) of Caspalux®1 fluorescent substrate). Fluorescence quantification shown in right panel. (C) Western blot of increased Caspase-1 activation (p20 subunit) in RPE cell lysates of BEST1-Cre; Dicer1$^{f/f}$ mice compared to BEST1-Cre or Dicer1$^{f/f}$ mice. (D) Western blot of increased Caspase-1 activation (p20 subunit) and IL-18 maturation in RPE cell lysates of Dicer1$^{f/f}$ mice treated with AAV1-BEST1-Cre. (E and F) RPE degeneration induced by AAV1-BEST1-Cre in Dicer1$^{f/f}$ mice is rescued by peptide inhibitors of either Caspase-1 (E) or MyD88 (F). (G) MyD88 inhibitor rescues loss of human RPE cell viability induced by DICER1 antisense (AS) treatment. (H) DICER1 antisense (AS) treatment of human RPE cells reduces DICER1 and increases IRAK1 and IRAK4 phosphorylation. (I) MyD88 inhibitor rescues loss of cell viability in Dicer1$^{f/f}$ mouse RPE cells treated with adenoviral vector coding for Cre recombinase (Ad-Cre). (J) Ad-Cre induced global miRNA expression deficits in Dicer1$^{f/f}$ mouse RPE cells compared to Ad-Null.

No significant difference in miRNA abundance between MyD88 inhibitor and control peptide-treated Dicer1 depleted cells. n=3 (A,B,F-H). Densitometry values normalized to Vinculin are shown in parentheses (A,C). Degeneration outlined by blue arrowheads. n=8 (E,F). *p<0.05 by Student t-test (G,I). Images representative of 3 experiments (A,B,C,D,H). See also FIG. **12**.

FIG. **7**. NLRP3 Inflammasome and MyD88 activation in human GA (A) NLRP3 and IL18 abundance was significantly elevated in macular GA RPE (n=13) compared to normal age-matched controls (n=12). *p<0.05 by Mann-Whitney U-test. There was no significant difference between groups (p=0.32 by Mann-Whitney U-test) in IL1B abundance. (B-D) Increased immunolocalization of NLRP3 (B), PYCARD (C) and Caspase-1 (D) in macular GA RPE compared to age-matched normal controls. Scale bar, 20 μm. (E) Western blots of macular RPE lysates from individual human donor eyes show that abundance of NLRP3, PYCARD, and phosphorylated IRAK1/4, normalized to the levels of the housekeeping protein Vinculin, is reduced in geographic atrophy (GA) compared to age-matched normal controls. Data are represented as mean+/−SEM (A). Representative images shown. n=6 (B-E). See also FIG. **13**.

FIG. **8**. Alu RNA does not activate several RNA sensors. (A and B) p7SL (a 7SL expression vector) (A) and in vitro synthesized 7SL RNA (B) do not induce RPE degeneration in wild-type mice. (C) RPE degeneration induced by subretinal injection of pAlu in wild-type mice is not blocked by a TLR4 antagonist. (D-E) Mice deficient in Mda5 (D) or Prkr (E) are susceptible to pAlu-induced RPE degeneration. (F) Dephosphorylated (Dep) Alu RNA induces RPE degeneration in wild-type mice just as well as Alu RNA. (G) Mice deficient in Mays are susceptible to pAlu-induced RPE degeneration. pNull does not induce RPE degeneration in any strain of mice. Degeneration outlined by blue arrowheads. Fundus photographs, top rows; ZO-1 stained (red) RPE flat mounts, bottom rows. n=8 (A-G). (H) A schematic of the innate immune pathways that are not activated by Alu RNA.

FIG. **9**. Alu RNA induces RPE degeneration via MyD88, not TRIF or IFNγ, (A) Subretinal administration of pAlu induces RPE degeneration in Ticam1$^{-/-}$ mice. (B) Alu RNA does not induce RPE degeneration in Myd88$^{-/-}$ mice. (C) Subretinal administration of a different Alu expression plasmid (pAlu(2)) also induces RPE degeneration in wild-type but not Myd88$^{-/-}$ mice. (D) Alu RNA does not induce RPE degeneration in Myd88$^{+/-}$ heterozygous (het) mice. (E) MyD88 inhibitory peptide reduces Alu RNA-induced phosphorylation of IRAK1/4, normalized to Vinculin expression. (F) Subretinal injection of AAV1-BEST1-Cre, but not AAV1-BEST1-GFP, protects Myd88$^{f/f}$ mice from Alu RNA-induced RPE degeneration. (G) pAlu and Alu RNA induces RPE degeneration in wild-type mice receiving Myd88$^{-/-}$ bone marrow (Myd88$^{-/-}$→wild-type) but did not do so in Myd88$^{-/-}$ mice receiving wild-type bone marrow (wild-type→Myd88$^{-/-}$). (H-K) Subretinal administration of pAlu induces RPE degeneration in Ifng$^{-/-}$ (H), Ifngr1$^{-/-}$ (I), and Il1r1^{-1-} mice (J) but not in Il18r1^{-1-} mice (K). pNull administration does not induce RPE degeneration in any strain of mice. Degeneration outlined by blue arrowheads. Fundus photographs, top rows; ZO-1 stained (red) RPE flat mounts, bottom rows. n=8 (A-D, F-K).

FIG. **10**. Alu RNA induces RPE degeneration via NLRP3 inflammasome activation, (A) Alu RNA or LPS+ATP induce activation of Caspase-1 in human RPE cells as assessed by increased cleavage of Caspalux®1 (green, left panel), a fluorescent-linked peptide substrate as compared to mock treatment. Fluorescence quantification shown in right panel. (B) Western blot of Alu RNA-induced Caspase-1 activation (p20 subunit) in THP-1 and HeLa cells, normalized to Vinculin expression. (C) Caspase-1 inhibitor peptide blocks Alu RNA-induced substrate cleavage in human RPE cells. n=3. (D) Subretinal injection of Alu RNA does not induce RPE degeneration in Casp1$^{-/-}$ mice. (E) Alu RNA or LPS+ATP induce the appearance of a brightly fluorescent cluster of GFP-PYCARD visible in the cytoplasm of human RPE cells. Area in insets shown in higher magnification. Images representative of 3 experiments. (F and G) Subretinal injection of Alu RNA does not induce RPE degeneration in Nlrp3$^{-/-}$ (F) or Pycard$^{-/-}$ (G) mice. (H) The abundance of NLRP3 in HEK293 cells transfected with an NLRP3 expression vector and of PYCARD in human RPE cells is reduced by transfection of siRNAs targeting these genes, compared to control (Ctrl) siRNAs. n=3, *p<0.05 compared to Ctrl siRNAs by Student t-test. (I) Alu RNA-induced Caspase-1 activation (p20 subunit) in human RPE cells is unaffected by MyD88 inhibitory peptide, normalized to Vinculin expression. (J) MyD88 inhibitory peptide does not reduce Alu RNA-induced cleavage activity of Caspase-1 in human RPE cells (top panel). Fluorescence quantification (bottom panel). (K) Caspase-1 activation (p20 subunit) in RPE cell lysates of wild-type mice treated with subretinal pAlu administration is unimpaired by intravitreous administration of anti-IL-18 neutralizing antibodies. (L) Alu RNA-induced phosphorylation of IRAK1/4 is reduced by Caspase-1 inhibitory peptide in human RPE cells, normalized to Vinculin expression. Vehicle control injections also do not damage the RPE. Fundus photographs, top rows; ZO-1 stained (red) RPE flat mounts, bottom rows. n=8 (D,F,G). Images representative of 3 experiments (A,B,I,J-L).

FIG. **11**. NLRP3 does not physically interact with Alu RNA, and VDAC knockdown by siRNA. (A) RNA-binding protein immunoprecipitation (RIP) assay in human RPE cells transfected with pAlu and pNLRP3-FLAG Immunoprecipitation of protein-RNA complexes with antibodies against NLRP3 or FLAG did not reveal interaction between NLRP3 and Alu RNA. RNA isolated from an equal amount of cell lysate (not subjected to IP) was used as input for Alu PCR. Relative abundance of Alu RNA in the immunoprecipitate, assessed by real-time RT-PCR using Alu-specific primers, was normalized to levels obtained with control IgG immunoprecipitation. N=3. (B) The abundance of VDAC1, VDAC2, and VDAC3 mRNAs in human RPE cells is reduced by transfection of siRNAs targeting these genes compared to control (targeting Luc) siRNA. N=3. *p<0.05 compared to Control siRNA by Student t-test.

FIG. **12**. DICER1 is a negative regulator of Caspase-1 activation by Alu RNA, (A) Knockdown of DICER1 by antisense oligonucleotides (AS) in human RPE cells increases cleavage activity of Caspase-1, as monitored by Caspalux, a fluorescent (green in overlay) reporter of substrate cleavage compared to control AS treatment. (B) Inhibition of Alu RNA by AS treatment reduces Caspalux fluorescence in human RPE cells treated with DICER1 AS. Mean values of Caspalux fluorescence shown in parentheses. Images representative of 3 experiments.

FIG. **13**. Schematic representation of proposed model of NLRP3 inflammasome activation by DICER1 deficit-induced Alu RNA that leads to RPE degeneration and geographic atrophy. Alu RNA induces priming of NLRP3 and IL18 mRNAs via generation of reactive oxygen species (ROS). Activation of the NLRP3 inflammasome triggers cleavage of pro-IL-18 by activated Caspase-1 to mature

IL-18. IL-18 signals via MyD88 to phosphorylate IRAK1 and IRAK4, which leads to RPE cell death.

FIG. **14**. Intravitreous administration of Caspase-8 inhibitor protects wild-type mice from pAlu-induced RPE degeneration. Representative images shown. n=8-12. Fundus photographs, top row; ZO-1 stained (red) flat mounts, bottom row.

FIG. **15**. Caspase-8 inhibitor protects human RPE cells from Alu induced cytotoxicity. Caspase-8 inhibitory peptide Z-IETD-FMK (100 μM) but not the control peptide Z-FA-FMK (100 μM) protects human RPE cells from Alu RNA-induced cytotoxicity.

FIG. **16**. Caspase-8 inhibitor protects human RPE cells from pAlu-induced cytotoxicity. Caspase-8 inhibitory peptide Z-IETD-FMK (100 μM) but not the control peptide Z-FA-FMK (100 μM) protects human RPE cells from pAlu-induced cytotoxicity.

FIG. **17**. IL-18 induced caspase-8 activation. Subretinal injection of IL-18 in wild-type mice induced activation of caspase-8, as monitored by fluorometric plate assay.

FIG. **18**. pAlu does not induce RPE degeneration in CD95−/− mice. Representative images shown. n=8-12. Fundus photographs, top row; ZO-1 stained (red) flat mounts, bottom row.

FIG. **19**. Alu RNA does not induce RPE degeneration in CD95−/− mice. Representative images shown. n=8-12. Fundus photographs, top row; ZO-1 stained (red) flat mounts, bottom row.

FIG. **20**. Recombinant IL-18 does not induce RPE degeneration in CD95−/− mice. Representative images shown. n=8-12. Fundus photographs, top row; ZO-1 stained (red) flat mounts, bottom row.

FIG. **21**. pAlu does not induce RPE degeneration in Faslg mice. Representative images shown. n=8-12. Fundus photographs, top row; ZO-1 stained (red) flat mounts, bottom row.

FIG. **22**. Alu RNA does not induce RPE degeneration in Faslg mice. Representative images shown. n=8-12. Fundus photographs, top row; ZO-1 stained (red) flat mounts, bottom row.

FIG. **23**. Recombinant IL-18 does not induce RPE degeneration in Faslg mice. Representative images shown. n=8-12. Fundus photographs, top row; ZO-1 stained (red) flat mounts, bottom row.

FIG. **24**. Alu RNA does not induce RPE degeneration in Nfkb1−/− mice. Representative images shown. n=8-12. Fundus photographs, top row; ZO-1 stained (red) flat mounts, bottom row.

FIG. **25**. Alu RNA or vehicle (PBS) was injected into the subretinal space of fellow eyes of a wild-type mouse. 3-days later, DyeLight782-VAD-FMK3 was injected into the vitreous humor of both eyes. 24-hours later, RPE flat mount preparations were visualized under fluorescent microscopy to visualize areas of bioactive caspase (green fluorescence), which corresponded to the area of Alu RNA injection.

FIG. **26**. Alu RNA or vehicle (PBS) was injected into the subretinal space of fellow eyes of two wild-type mice (left and right panels). 3-days later, DyeLight782-VAD-FMK3 was injected into the vitreous humor of both eyes. From baseline (0 hours) to 8 hours thereafter, photographs of the fundus (retina) were taken through the ICG filter of a Topcon 50IX camera. In the Alu RNA-injected eye, white fluorescent areas corresponding to bioactive caspase generation were observed in the area of Alu RNA injection. No such widespread areas were observed in the vehicle-injected eye.

FIG. **27**. Recombinant IL-18 or vehicle (PBS) was injected into the subretinal space of fellow eyes of a wild-type mouse. 2-days later, DyeLight782-VAD-FMK3 was injected into the vitreous humor of both eyes. From baseline (0 hours) to 24 hours thereafter, photographs of the fundus (retina) were taken through the ICG filter of a Topcon 50IX camera. In the IL-18-injected eye, white fluorescent areas corresponding to bioactive caspase generation were observed in the area of IL-18 injection. No such widespread areas were observed in the vehicle-injected eye.

FIG. **28**. Representative images show that subretinally injected Alu RNA (1 μg)-induced RPE degeneration is blocked by intravitreous administration of the MyD88 peptide inhibitor DRQIKIWFQNRRMKWKKRDVLPGTCVWSIASE (2 μg). Top panels show color fundus photographs. Bottom panels show retinal flat mount preparations stained with an anti-ZO1 antibody (red). Alu RNA-induced RPE degeneration (left panels) is evidenced by depigmentation seen on color photos (top left) and dysmorphic appearing RPE cells (bottom left). Treatment with the MyD88 peptide inhibitor prevents those degenerative changes and preserves normal RPE anatomy.

BRIEF DESCRIPTION OF THE SEQUENCE LISTING

SEQ ID NO: 1. IMG-2005-1 peptide sequence: DRQIKIWFQNRRMKWKKRDVLPGT, wherein the last 7 amino acids are required for inhibition of MyD88 homodimerization, while the preceding amino acid sequence is an Antennopedia cell permeation sequence that enables the inhibitory peptide to enter the cell, so that it can block MyD88.

SEQ ID NO: 2. Control peptide sequence: DRQIKIWFQNRRMKWKK

SEQ ID NO: 3. MyD88 siRNA #1 sense: 5'-GAGAAGCCUUUACAGGUdTdT-3'

SEQ ID NO: 4. MyD88 siRNA #1 antisense: 5'-ACCUGUAAAGGCUUCUCdTdT-3'

SEQ ID NO: 5. MyD88 siRNA #2 sense: 5'-CAGAGCAAGGAAUGUGAdTdT-3'

SEQ ID NO: 6. MyD88 siRNA #2 antisense: 5'-UCACAUUCCUUGCUCUGdTdT-3'

SEQ ID NO: 7 NLRP3 siRNA—5'-GUUUGACUAUCUGUUCUdTdT-3'

SEQ ID NO: 8: NLRP3 siRNA—5'-GGAUCAAACUACUCUGUGA-3'

SEQ ID NO: 9: NLRP3 siRNA—5'-UGCAAGAUCUCUCAGCAAA-3'

SEQ ID NO: 10: NLRP3 siRNA—5'-GAAGUGGGGUUCAGAUAAU-3'

SEQ ID NO: 11: NLRP3 siRNA—5'-GCAAGACCAAGACGUGUGA-3'

SEQ ID NO: 12: PYCARD siRNA—5'-GAAGCUCUUCAGUUUCAdTdT-3'

SEQ ID NO: 13: PYCARD siRNA—5'-GGCUGCUGGAUGCUCUGUACGGGAA-3'

SEQ ID NO: 14: PYCARD siRNA—5'-UUCCCGUACAGAGCAUCCAGCAGCC-3'.

SEQ ID NO: 15: siRNA of the human Pyrin coding sequence: GCTGGAGCAGGTGTACTACTTC.

SEQ ID NO: 16: siRNA of the human NLRP3 coding sequence CAGGTTTGACTATCTGTTCT.

SEQ ID NO: 17: siRNA of the 3' UTR of the human caspase-1 GTGAAGAGATCCTTCTGTA.

SEQ ID NO: 18: Oligonucleotide primer for human MB, forward 5'-TTAAAGCCCGCCTGACAGA-3'.

SEQ ID NO: 19: Oligonucleotide primer for human MB, reverse 5'-GCGAATGACAGAGGGTTTCTTAG-3').

SEQ ID NO: 20: Oligonucleotide primer for human IL18, forward 5'-ATCACTTGCACTCCGGAGGTA-3'.

SEQ ID NO: 21: Oligonucleotide primer for human IL18, reverse 5'-AGAGCGCAATGGTGCAATC-3'.

SEQ ID NO: 22: Oligonucleotide primer for human NLRP3, forward 5'-GCACCTGTTGTGCAATCTGAA-3'.

SEQ ID NO: 23: Oligonucleotide primer for human NLRP3, reverse 5'-TCCTGACAACATGCTGATGTGA-3'.

SEQ ID NO: 24: Oligonucleotide primer for human PYCARD, forward 5'-GCCAGGCCTGCACTTTATAGA-3'.

SEQ ID NO: 25: Oligonucleotide primer for human PYCARD, reverse 5'-GTTTGTGACCCTCGCGATAAG-3'.

SEQ ID NO: 26: Oligonucleotide primer for human VDAC1, forward 5'-ACTGCAAAATCCCGAGTGAC-3'.

SEQ ID NO: 27: Oligonucleotide primer for human VDAC1, reverse 5'-CTGTCCAGGCAAGATTGACA-3'.

SEQ ID NO: 28: Oligonucleotide primer for human VDAC2, forward 5'-CAGTGCCAAATCAAAGCTGA-3'.

SEQ ID NO: 29: Oligonucleotide primer for human VDAC2, reverse 5'-CCTGATGTCCAAGCAAGGTT-3').

SEQ ID NO: 30: Oligonucleotide primer for human VDAC3, forward 5'-TTGACACAGCCAAATCCAAA-3'.

SEQ ID NO: 31: Oligonucleotide primer for human VDAC3, reverse 5'-GCCAAAACGGGTGTTGTTAC-3'.

SEQ ID NO: 32: Oligonucleotide primer for human 18S rRNA, forward 5'-CGCAGCTAGGAATAATGGAATAGG-3'.

SEQ ID NO: 33: Oligonucleotide primer for human 18S rRNA, reverse 5'-GCCTCAGTTCCGAAAACCAA-3'.

SEQ ID NO: 34: Oligonucleotide primer for mouse Myd88, forward 5'-CACCTGTGTCTGGTCCATTG-3'.

SEQ ID NO: 35: Oligonucleotide primer for mouse Myd88, reverse 5'-AGGCTGAGTGCAAACTTGGT-3'.

SEQ ID NO: 36: Oligonucleotide primer for mouse Nlrp3, forward 5'-ATGCTGCTTCGACATCTCCT-3'.

SEQ ID NO: 37: Oligonucleotide primer for mouse Nlrp3, reverse 5'-AACCAATGCGAGATCCTGAC-3'.

SEQ ID NO: 38: Oligonucleotide primer for mouse Il18, forward 5'-GACAGCCTGTGTTCGAGGAT-3'.

SEQ ID NO: 39: Oligonucleotide primer for mouse Il18, reverse 5'-TGGATCCATTTCCTCAAAGG-3'.

SEQ ID NO: 40: Oligonucleotide primer for mouse 18S rRNA, forward 5'-TTCGTATTGCGCCGCTAGA-3'.

SEQ ID NO: 41: Oligonucleotide primer for mouse 18S rRNA, reverse 5'-CTTTCGCTCTGGTCCGTCTT-3'.

SEQ ID NO: 42: Mouse miR-184-5'-TGGACGGA-GAACTGATAAGGGT-3;

SEQ ID NO: 43: Mouse miR-221/222-5'-AGCTA-CATCTGGCTACTGGGT-3;

SEQ ID NO: 44: Mouse miR-320a-5'-AAAAGCTGGGT-TGAGAGGGCGA-3', and

SEQ ID NO: 45: Mouse mouse miR-484-5'-TCAG-GCTCAGTCCCCTCCCGAT-3'.

SEQ ID NO: 46: U6 snRNA-5'-AAATTCGTGAAGCGT-TCC-3'.

SEQ ID NO: 47: VDAC1 siRNA sense-5'-CG-GAAUAGCAGCCAAGUdTdT-3'.

SEQ ID NO: 48: VDAC2 siRNA sense-5'-CCCUG-GAGUUGGAGGCUdTdT-3'.

SEQ ID NO: 49: VDAC3 siRNA sense-5'-GC-UUUAAUCGAUGGGAAdTdT-3'.

SEQ ID NO: 50: DICER1 antisense oligonucleotide (AS)-5'-GCUGACCTTTTTGCTUCUCA-3'.

SEQ ID NO: 51: Control for DICER1 AS-5'-TTGG-TACGCATACGTGTTGACTGTGA-3'.

SEQ ID NO: 52: Alu AS-5'-CCCGGGTTCACGCCAT-TCTCCTGCCTCAGCCTCA-CGAGTAGCTGGGACTACAGGCGCCCGACAC-CACTCCCGGCTAATTTTTTGTATTTTT-3'.

SEQ ID NO: 53: Control for Alu AS-5'-GCATGGCCA-GTCCATTGATCTTGCACGCTTGCC-TAGTACGCTCCTCAACCTATCCTCCTAGCCCGT-TACTTGGTGCCACCGGCG-3'.

SEQ ID NO: 54: Oligopeptide for inhibiting MyD88 homodimerization: RDVLPGT.

SEQ ID NO: 55: Oligopeptide for inhibiting MyD88 homodimerization: RDVVPGG.

SEQ ID NO: 56. MyD88 siRNA: UUAUUUC-CUAAWGGGUCdTdT.

SEQ ID NO: 57. VDAC1 siRNA sense (5'-CG-GAAUAGCAGCCAAGUdTdT-3').

SEQ ID NO: 58. VDAC2 siRNA sense (5'-CCCUG-GAGUUGGAGGCUdTdT-3').

SEQ ID NO: 59. VDAC3 siRNA sense (5'-GC-UUUAAUCGAUGGGAAdTdT-3').

SEQ ID NO: 60. MyD88 inhibitor: DRQIKIWFQNRRM-KWKKRDVLPGTCVWSIASE.

SEQ ID NO: 61. MyD88 inhibitor: RDVLPGTCVW-SIASE.

DESCRIPTION OF EXEMPLARY EMBODIMENTS

The presently-disclosed subject matter includes methods for identifying MyD88 inhibitors, and methods and compositions for inhibiting MyD88 and uses thereof. The presently-disclosed subject matter includes methods for identifying inflammasome inhibitors, and methods and compositions for inhibiting an inflammasome and uses thereof. The presently-disclosed subject matter includes methods for identifying inhibitors of components of inflammosome, and methods and compositions for inhibiting a component of inflammasome and uses thereof. Components of inflammasome include, for example, NLRP3, PYCARD, and Caspase-1. The presently-disclosed subject matter includes methods for identifying IL-18 inhibitors, and methods and compositions for inhibiting IL-18 and uses thereof. The presently-disclosed subject matter includes methods for identifying VDAC1 and VDAC2 inhibitors, and methods and compositions for inhibiting VDAC1 and VDAC2 and uses thereof. The presently-disclosed subject matter includes methods for identifying caspase-8 inhibitors, and methods and compositions for inhibiting caspase-8 and uses thereof. The presently-disclosed subject matter includes methods for identifying NFkB inhibitors, and methods and compositions for inhibiting NFkB and uses thereof. Also provided are methods and compositions for imaging activated caspase-1 in an eye of a subject.

The presently-disclosed subject matter includes methods including inhibiting one or more of an inflammasome, MyD88, and IL-18 of a cell. In some embodiments, the presently-disclosed subject matter includes methods including inhibiting one or more of MyD88, IL-18, VDAC1, VDAC2, NFκB, caspase-8, caspase-1, NLRP-3, PYCARD, and an inflammasome, including a component of an inflammasome (e.g., caspase 1, NLRP-3, PYCARD) of a cell.

In some embodiments of the method, the cell is selected from an RPE cell, a retinal photoreceptor cell, or a choroidal cell. In some embodiments, the cell is an RPE cell. In some embodiments, the cell is the cell of a subject. In some embodiments, the cell is a cell of a subject having, suspected of having, or at risk of having a condition of interest. In some embodiments, the cell is a cell of a subject having, suspected of having, or at risk of having age-related macular degeneration. In some embodiments, the cell is a cell of a subject having, suspected of having, or at risk of having geographic

atrophy. In some embodiments, the cell is a cell of a subject having, suspected of having, or at risk of having geographic atrophy and the cell is an RPE cell. In some embodiments, a subject having age-related macular degeneration can be treated using methods and compositions as disclosed herein.

As used herein, the term "subject" refers to a target of treatment. The subject of the herein disclosed methods can be a vertebrate, such as a mammal, a fish, a bird, a reptile, or an amphibian. Thus, the subject of the herein disclosed methods can be a human or non human. Thus, veterinary therapeutic uses are provided in accordance with the presently disclosed subject matter.

In some embodiments, the inhibiting one or more of an inflammasome, MyD88, IL-18, VDAC1, VDAC2, NLRP3, PYCARD, caspase-1, caspase-8, and NFκB of a cell includes administering an inhibitor to the cell, or to a subject wherein the cell is the cell of a subject. Such inhibitors can be administered, for example, by intraocular injection (e.g., localized interocular therapy); intravitreous injection; subretinal injection; episcleral injection; sub-Tenon's injection; retrobulbar injection; peribulbar injection; transscleral administration; topical administration, e.g., topical eye drop application; suprachoroidal administration; release from a sustained release delivery device that is sutured to or attached to or placed on the sclera, or injected into the vitreous humor, or injected into the anterior chamber, or implanted in the lens bag or capsule; oral administration; or intravenous administration.

As used herein the term "inhibit" or "inhibiting" refers to suppressing, reducing, decreasing, or substantially eliminating the biological activity of a polypeptide, such as MyD88, IL-18, VDAC1, VDAC2, caspase-8, NFκB, or a polypeptide of an inflammasome (e.g., NLRP3, PYCARD, caspase-1). As used herein with reference to a polypeptide being inhibited, "of a cell" refers to a polypeptide that is inside the cell (inside the cell membrane), on the cell (in the cell membrane, presented on the cell membrane, otherwise on the cell), or outside of a cell, but insofar as the polypeptide is outside of the cell, it is in the extracellular mileu such that one of ordinary skill in the art would recognize the polypeptide as being associated with the cell. For example, VDAC1, VDAC2, caspase-8, NFκB, or a polypeptide of an inflammasome (e.g., NLRP3, PYCARD, caspase-1 of a cell could be in the cell. For another example MyD88 could be in the cell or on the cell. For yet another example, IL-18 could be outside the cell because it is secreted, but it would be recognized by one or ordinary skill in the art as being associated with the cell.

As will be understood by those skilled in the art upon studying this application, inhibition of an inflammasome, MyD88, IL-18, VDAC1, VDAC2, caspase-1, caspase-8, and NFκB of a cell can be achieved in a number of manners. In some embodiments the inhibition can be achieved by affecting the transcription or translation of the polypeptide, by degrading the polypeptide, by scavenging the polypeptide, or otherwise impacting the biological activity of the polypeptide Inhibition comprises administering an inhibitor. An inhibitor is a compound that affects such inhibition of the biological activity of a polypeptide. Such compounds can be, for example, a polypeptide (including oligonucleotide, and including a polypeptide that binds to the polypeptide-of-interest to affect inhibition), a small molecule (including a small chemical compound), a compound for RNA interference (including siRNA, miRNA, shRNA), an antibody (e.g., a neutralizing antibody against polypeptide of interest, an antibody that blocks polypeptide of interest from binding to a receptor), an aptamer, a dominant negative plasmid or vector, or a virus-encoded inflammasome.

The terms "polypeptide", "protein", and "peptide", which are used interchangeably herein, refer to a polymer of the 20 protein amino acids, or amino acid analogs, regardless of its size. The terms "polypeptide fragment" or "fragment", when used in reference to a reference polypeptide, refers to a polypeptide in which amino acid residues are deleted as compared to the reference polypeptide itself, but where the remaining amino acid sequence is usually identical to the corresponding positions in the reference polypeptide. Such deletions can occur at the amino-terminus or carboxy-terminus of the reference polypeptide, from internal portions of the reference polypeptide, or a combination thereof. A fragment can also be a "functional fragment," in which case the fragment retains some or all of the activity of the reference polypeptide as described herein.

The terms "modified amino acid", "modified polypeptide", and "variant" refer to an amino acid sequence that is different from the reference polypeptide by one or more amino acids, e.g., one or more amino acid substitutions. A variant of a reference polypeptide also refers to a variant of a fragment of the reference polypeptide, for example, a fragment wherein one or more amino acid substitutions have been made relative to the reference polypeptide. A variant can also be a "functional variant," in which the variant retains some or all of the activity of the reference protein as described herein. The term functional variant includes a functional variant of a functional fragment of a reference polypeptide.

In some embodiments, the methods and compositions of the presently-disclosed subject matter can be used in a subject having, suspected of having, or at risk of having a condition of interest. In some embodiments, methods and compositions of the presently-disclosed subject matter can be used for treating a condition of interest. Examples of conditions of interest include, but are not limited to: Geographic atrophy (Kaneko, Dridi et al. 2011); Macular degeneration (Kaneko, Dridi et al. 2011); Keratitis (Guo, Gao et al. 2011); Gout (Chen, Shi et al. 2006); Acne vulgaris (Terhorst, Kalali et al. 2010); Crohn's disease (Reuter and Pizarro 2004; Abreu, Fukata et al. 2005; Medvedev, Sabroe et al. 2006); Ulcerative colitis (Reuter and Pizarro 2004; Abreu, Fukata et al. 2005; Medvedev, Sabroe et al. 2006); irritable bowel disease/irritable bowel syndrome (McKernan, Nolan et al. 2009); Type I diabetes (Devaraj, Tobias et al. 2011; von Herrath, Filippi et al. 2011); Type 2 diabetes (Hutton, Soukhatcheva et al. 2010; Nogueira-Machado, Volpe et al. 2011); Insulin resistance (Ghanim, Mohanty et al. 2008; Tilich and Arora 2011); Obesity (Fresno, Alvarez et al. 2011); Hemolytic-Uremic Syndrome (Batsford, Duermueller et al. 2011); Polyoma virus infection (Batsford, Duermueller et al. 2011); Immune complex renal disease (Anders, Banas et al. 2004; Anders and Schlondorff 2007); Acute tubular injury (Anders, Banas et al. 2004; Anders and Schlondorff 2007); Lupus nephritis (Anders, Banas et al. 2004; Anders and Schlondorff 2007); Familial cold autoinflammatory syndrome (Mariathasan, Weiss et al. 2006; Meng, Zhang et al. 2009); Muckle-Wells syndrome and neonatal onset multisystem inflammatory disease (Mariathasan, Weiss et al. 2006; Meng, Zhang et al. 2009); Chronic infantile neurologic cutaneous and articular autoinflammatory diseases, Renal ischemia-perfusion injury (El-Achkar and Dagher 2006; Robson 2009); Glomerulonephritis (E1-Achkar and Dagher 2006; Robson 2009); Cryoglobulinemia (Banas, Banas et al. 2008); Systemic vasculitides (Weyand, Ma-Krupa et al. 2005; Hurtado, Jeffs

et al. 2008; Summers, Steinmetz et al. 2011); IgA nephropathy (Lim, Lee et al. 2011); Atherosclerosis (Curtiss and Tobias 2009); HIV/AIDS (Brichacek, Vanpouille et al. 2010); Malaria (Franklin, Ishizaka et al. 2011); Helminth parasites (Babu, Blauvelt et al. 2005; Venugopal, Nutman et al. 2009); Sepsis and septic shock (Knuefermann, Nemoto et al. 2002; Opal and Huber 2002; Cristofaro and Opal 2003; Chen, Koustova et al. 2007); Allergic asthma (Slater, Paupore et al. 1998; Park, Gold et al. 2001); Hay fever (Slater, Paupore et al. 1998; Park, Gold et al. 2001); Chronic obstructive pulmonary disease (Geraghty, Dabo et al. 2011); Drug-induced lung inflammation (Liu, Yang et al. 2010); Contact dermatitis (Martin, Dudda et al. 2008; Yokoi, Niizeki et al. 2009); Leprosy (Krutzik, Tan et al. 2005; Terhorst, Kalali et al. 2010); *Burkholderia cenocepacia* infection (Ventura, Balloy et al. 2009); Respiratory syncitial virus infection (Aeffner, Traylor et al. 2011); Psoriasis (Zuany-Amorim, Hastewell et al. 2002; Barrat and Coffman 2008; Li, Zhou et al. 2009); Systemic lupus erythematosus (Zuany-Amorim, Hastewell et al. 2002; Barrat and Coffman 2008; Li, Zhou et al. 2009); Scleroderma (Zuany-Amorim, Hastewell et al. 2002; Barrat and Coffman 2008; Li, Zhou et al. 2009); Reactive arthritis (Zuany-Amorim, Hastewell et al. 2002; Barrat and Coffman 2008; Li, Zhou et al. 2009); Cystic fibrosis, Syphilis, Sjögren's syndrome (Zuany-Amorim, Hastewell et al. 2002; Barrat and Coffman 2008; Li, Zhou et al. 2009); Rheumatoid arthritis (Zuany-Amorim, Hastewell et al. 2002; Barrat and Coffman 2008; Li, Zhou et al. 2009); Inflammatory joint disease (O'Neill 2008); Non-alcoholic fatty liver disease (Tan, Fiel et al. 2009); Cardiac surgery (peri-/post-operative inflammation) (Cremer, Martin et al. 1996; Taylor 1996; Dybdahl, Wahba et al. 2002); Acute and chronic organ transplant rejection (Alegre, Leemans et al. 2008; Miller, Rossini et al. 2008; Taylor, Ehrhardt et al. 2008; Krams, Wang et al. 2010; Wang, Schmaderer et al. 2010; Shin and Harris 2011; Testro, Visvanathan et al. 2011); Acute and chronic bone marrow transplant rejection (Alegre, Leemans et al. 2008; Miller, Rossini et al. 2008; Taylor, Ehrhardt et al. 2008; Krams, Wang et al. 2010; Wang, Schmaderer et al. 2010; Shin and Harris 2011; Testro, Visvanathan et al. 2011); Alzheimer's disease; and Tumor angiogenesis (Frantz, Vincent et al. 2005; Schmid, Avraamides et al. 2011).

As used herein, the terms treatment or treating relate to any treatment of a condition of interest, including but not limited to prophylactic treatment and therapeutic treatment. As such, the terms treatment or treating include, but are not limited to: preventing a condition of interest or the development of a condition of interest; inhibiting the progression of a condition of interest; arresting or preventing the development of a condition of interest; reducing the severity of a condition of interest; ameliorating or relieving symptoms associated with a condition of interest; and causing a regression of the condition of interest or one or more of the symptoms associated with the condition of interest.

In some embodiments, the methods and compositions of the presently-disclosed subject matter are useful for protecting the cell against Alu-RNA-induced degeneration. As such, in some embodiments, a method includes administering an inhibitor, wherein the cell is protected against Alu-RNA-induced degeneration.

Inhibiting Inflammasome

In some embodiments, the presently-disclosed subject matter includes a method of protecting a cell, comprising: inhibiting an inflammasome of the cell. The method of any one of the prior claims, wherein the inflammasome is selected from NLRP3 inflammasome, NLRP1 inflammasome, NLRC4 inflammasome, AIM2 inflammasome, and IFI16 inflammasome. In some embodiments, the inflammasome is the NLRP3 inflammasome.

In some embodiments the inhibiting the inflammasome includes inhibiting a component of the inflammasome. In some embodiments the inflammasome components can include a polypeptide encoded by PYCARD. In some embodiments the inflammasomse components can include a caspase. In some embodiments the inflammasome components can include PYCARD, NLRP3, and caspase-1.

In some embodiments, the inhibiting the inflammasome comprises administering an inflammasome inhibitor. The inflammasome inhibitor can be an inhibitor of a component of the inflammasome. In some embodiments, the inflammosome

As noted above, in some embodiments, inhibiting a polypeptide of interest to the presently-disclosed subject matter comprises administering an oligonucleotide or a small RNA molecule. Such small RNA molecule can target, for example, NLRP3 and/or PYCARD. Such nucleotides can target and degrade NLRP3 and/or PYCARD. In this regard, the presently-disclosed subject matter includes a isolated double-stranded RNA molecule that inhibits expression of NLRP3 and/or PYCARD, wherein a first strand of the double-stranded RNA comprises a sequence as set forth in Table A, and includes about 14 to 25 nucleotides. As noted above, in some embodiments, inhibiting comprises administering an inflammasome inhibitor that is a dominant negative vector. In some embodiments, inhibiting inflammasome comprises administering an inhibitor of Caspase-1. In some embodiments the inhibitor of Caspase-1 is a peptide inhibitor.

Examples of inflammasome inhibitors that can be used in accordance with the presently-disclosed subject matter include, but are not limited to those set forth in Table A. As such, embodiments of the presently-disclosed subject matter can include administering an inflammasome inhibitor set forth in Table A.

TABLE A

Examples of Inflammasome Inhibitors
Ion channel inhibitors, for example, glybenclamide/glyburide (CAS Number: 10238-21-8) (Lamkanfi, et al., 2009).
IkB-α inhibitors, for example, BAY11-7082 (CAS Number: 195462-67-7; also known as (E)-3-(4-Methylphenylsulfony1)-2-propenenitrile) (Juliana, et al., 2010).
Compounds similar to BAY11-7082, for example, other related vinyl sulfone compounds, as set forth in Lamkanfi, et al., 2009; Juliana, et al., 2010; deRivero Vaccari, et al., 2008; and Newman, et al., 2011, which are incorporated herein by this reference.

TABLE A-continued

Examples of Inflammasome Inhibitors
Antibodies, for example, Anti-ASC and Anti-NALP1 and antibodies based on protein sequences selected from: ASC: ALR QTQ PYL VTD LEQ S; NALP1: MEE SQS KEE SNT EG-cys (deRivero Vaccari, et al., 2008); and Anti-NALP1 (Abcam, Cambridge, MA), anti-IL-1β (Cell Signaling Technology, Beverly, MA), anti-IL-18 (R & D Systems, Minneapolis, MN), anti-caspase-1 (Millipore, Billerica, MA), anti-caspase-1 (Santa Cruz Biotechnology, Santa Cruz, CA), anti-caspase-11 (Alexis Biochemicals, San Diego, CA), anti-caspase-11 (Santa Cruz Biotechnology).
Direct inhibitors of Caspase-1 and/or NLRP3, for example, parthenolide (Juliana, et al., 2010).
Caspase-1 inhibitors, such as estrogen binding B-box protein (Munding et al., 2006); COP (Lee, et al., 2001); ICEBERG (Humke, et al., 2000); and Z-WEHD-FMK (R&D Systems).
Caspase 1 and/or 4 inhibitors, for example, Ac-YVAD-CHO (Ac-Tyr-Val-Ala-Asp-CHO) and Ac-YVAD-CMK (CAS Number: 178603-78-6; N-acetyl-L-tyrosyl-L-valyl-N-[(1S)-1-(carboxymethyl)-3-chloro-2-oxo-propyl]-L-alaninamide) (Hilbi, et al., 1997).
Caspase-12 inhibitors (Saleh, et al., 2006).
Host-derived inhibitors of Caspase-1, for example, cellular PYRIN domain (PYD)-only proteins (POP) family: cP0P1 and cPOP2 (Stehlik, et al., 2003; Dorfleutner, et al., 2007); serpin proteinase inhibitor 9 (PI-9) (Young, et al., 2000); BCL-2 and BCL-xL (Young, et al., 2000).
Inhibitors of Nlrp1b inflammasome, for example, auranofin (Newman, et al., 2011).
Virus expressed inhibitors of the inflammasome, for example, PYD homologs M13L-PYD, S013L (Benedict, et al., 2005; Dorfleutner, et al., 2007; Johnston, et al., 2005); SPI-2 homologs CrmA, Serp2, SPI-2, (Komiyama, et al., 1994; Kettle, et al., 1997; Messud-Petit, et al., 1998); NS1 (Stasakova, et al., 2005); Kaposi Sarcoma-associated Herpesvirus Orf63
(Gregory, et al., 2011).
Potassium chloride (KCl) (CAS Number: 7447-40-7 (Schorn, et al. 2011).
Cathepsin-B inhibitors, for example, CA-074 Me (L-3-trans-(Propylcarbamoyl)oxirane-2-Carbonyl)-L-Isoleucyl-L-Proline Methyl Ester (Li, et al., 2009).
Cytochalasin D (Dostert, et al., 2008).
ROS inhibitors, for example, N-acetyl-L-cysteine (NAC), and (2R,4R)-4-aminopyrrolidine-2,4-dicarboxylate (APDC) (Dostert, et al., 2008).
ASC-1 inhibitors, for example, cellular pyrin domain (PYD) superfamily proteins, also known as M013 (Rahman, et al., 2009).
NLRP3 inflammasome pan-caspase inhibitors, for example, Z-VAD-FMK (Dostert, et al., 2009).
Microtubules, for example, colchicine (CAS Number: 64-86-8) (Martinon, et al., 2006).
An isolated double-stranded RNA molecule that inhibits expression of NLRP3, and which can be conjugated to cholesterol or not, and at least one strand including the sequence: GUUUGACUAUCUGUUCUdTdT (SEQ ID NO: 7).
An isolated double-stranded RNA molecule that inhibits expression of NLRP3, at least one strand of which includes a sequence selected from: 5'-GGAUCAAACUACUCUGUGA-3' (SEQ ID NO: 8); 5'-UGCAAGAUCUCUCAGCAAA-3' (SEQ ID NO: 9); 5'-GAAGUGGGGUUCAGAUAAU-3' (SEQ ID NO: 10); and 5'-GCAAGACCAAGACGUGUGA-3')(SEQ ID NO: 11) (Wong, et al., 2011).
An isolated double-stranded RNA molecule that inhibits expression of PYCARD, at least one strand of which includes the sequence of: 5'-GAAGCUCUUCAGUUUCAdTdT-3' (SEQ ID NO: 12).
An isolated double-stranded RNA molecule that inhibits expression of PYCARD, at least one strand of which includes a sequence selected from: 5'-GAAGCUCUUCAGUUUCAdTdT-3' (SEQ ID NO: 12); 5'-GGCUGCUGGAUGCUCUGUACGGGAA-3' (SEQ ID NO: 13); and 5'-UUCCCGUACAGAGCAUCCAGCAGCC-3' (SEQ ID NO: 14). (Stealth siRNA oligos were designed and obtained with Lipofectamine 2000).

Further information regarding Caspase-1 inhibitors and probes can be found in Table B. Information found at the links set forth in Table B as of the filing date of this application is incorporated herein by this reference.

TABLE B

Peptide Sequence	Application	Link	Notes
GWEHDGK	fluorescent in vivo	http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1502090/	Gly-Trp-Glu-His-Asp-Gly-Lys
YVADAPV	fluorescent	http://www.ncbi.nlm.nih.gov/pubmed/8012123	DABCYL-Tyr-Val-Ala-Asp-Ala-Pro -Val-EDANS
GFEVD	fluorescent	http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1221285/pdf/10947972.pdf	Abz-GXEVD-GVY(NO2)D
GYEVD	fluorescent	http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1221285/pdf/10947972.pdf	Abz-GXEVD-GVY(NO2)D
YVAD	fluorescent	http://www.sciencedirect.com/science/article/pii/S1074552199800519	BFP-YVAD-GFP
	fluorescent	http://www.jbc.org/content/286/37/32513.full	Ac-YVAD-CHO
	inhibitor	http://www.jbc.org/content/273/49/32608.long	
WEHD	fluorescent	http://jem.rupress.org/content/191/11/1819/T1.expansion.html	KDPC$_5$G-WEHD-GINGC$_5$PKGY
	Inhibitor	http://www.jbc.org/content/273/49/32608.long	
YVHDAP	fluorescent	http://www.funakoshi.co.jp/data/datasheet/ONC/CPL1RE-5.pdf	Caspalux
YVADAP	fluorescent	http://www.ncbi.nlm.nih.gov/pubmed/8012123	DABCYL-YVADAP-EDANS
YEVD	fluorescent	http://www.jbc.org/content/272/15/9677.long	Ac-YVED-pNA
YVHDAPVR	Kinetic substrate	http://www.jbc.org/content/272/11/7223/T1.expansion.html	

Small molecule Sequence	Application	Link	Notes
VX-765	Inhibitor	http://www.medkoo.com/Anticancer-trials/VX-765.htm	Vertex Pharmaceuticals, Reversible, clinical trials
ML132	Inhibitor	http://www.ncbi.nlm.nih.gov/books/NBK56241/	Reversible(?), based on VX-765
VX-740	Inhibitor	http://www.ncbi.nlm.nih.gov/pubmed/17393315	Vertex Pharmaceuticals, common name: Pralnacasan clincal trials halted (liver abnormalities)
VRT-018858	Inhibitor	http://www.ncbi.nlm.nih.gov/pubmed/17845807	Active metabolite of VX-740
CM-269	Reporter	http://www.sciencedirect.com/science/article/pii/S1074552110003091#sec5.1	Luciferase based reporter

The presently-disclosed subject matter further includes compositions useful for inhibiting an inflammasome. Such compositions include an inhibitor. As noted above, such inhibitors can be, for example, a nucleotide, a polypeptide, a small (chemical) molecule, etc. In some embodiments, a composition can include an isolated RNA molecule.

The presently-disclosed subject matter includes isolated RNA molecules that inhibit expression of a component of inflammasome, e.g., NLRP3, caspase-1 and/or PYCARD. In some embodiments, a first strand of the double-stranded RNA comprises a sequence selected from the following, and including about 14 to 25 nucleotides: 5'-GUUUGACUAUCUGUUCUdTdT-3' (SEQ ID NO: 7); 5'-GGAUCAAACUACUCUGUGA-3' (SEQ ID NO: 8); 5'-UGCAAGAUCUCUCAGCAAA-3' (SEQ ID NO: 9); 5'-GAAGUGGGGUUCAGAUAAU-3' (SEQ ID NO: 10); 5'-GCAAGACCAAGACGUGUGA-3' (SEQ ID NO: 11); 5'-GAAGCUCUUCAGUUUCAdTdT-3' (SEQ ID NO: 12); 5'-GGCUGCUGGAUGCUCUGUACGGGAA-3' (SEQ ID NO: 13); and 5'-UUCCCGUACAGAGCAUCCAGCAGCC-3' (SEQ ID NO: 14).

The presently-disclosed subject matter includes isolated RNA molecules that inhibit expression of an inflammasome component. In some embodiments, the RNA molecule comprises a sequence selected from the following:

(SEQ ID NO: 15)
GCTGGAGCAGGTGTACTACTTC,

(SEQ ID NO: 16)
CAGGTTTGACTATCTGTTCT,
and

(SEQ ID NO: 17)
GTGAAGAGATCCTTCTGTA.

The presently-disclosed subject matter further includes methods of screening candidate inhibitors to identify inflammasome inhibitors. In some embodiments, a method of identifying an inflammasome inhibitor makes use of a cultured cell wherein a cell based-system is provided, which measures PYCARD aggregation, Caspase-1 cleavage, or cleavage/secretion of IL-1β or IL-18 in response to an activator of the inflammasome (e.g., Alu RNA, lipopolysaccharide+ATP).

In some embodiments, a screening method for inflammasome inhibitors includes stimulating cells (e.g., RPE cells) or a cell line (e.g., THP-1 or RAW macrophages) that has been transfected with a plasmid encoding a fluorescent-tagged PYCARD with Alu RNA or LPS+ATP; monitoring the aggregation of fluorescent PYCARD into a "speck"—an aggregosome focus using fluorescent microscopy; and testing the candidate molecules for the degree of inhibition of PYCARD "speck" formation.

In some embodiments, a screening method for inflammasome inhibitors includes stimulating cells (e.g., RPE cells) or a cell line (e.g., THP-1 or RAW macrophages with Alu RNA or LPS+ATP; monitoring Caspase-1 activity using CaspaLux®1-E2D2 assay (OncoImmunin, Inc.); and testing the candidate molecules for the degree of inhibition of Caspaslux fluorescence.

In some embodiments, a screening method for inflammasome inhibitors includes stimulating cells (e.g., RPE cells) or a cell line (e.g., THP-1 or RAW macrophages with Alu RNA or LPS+ATP; monitoring Caspase-1 activity by measuring the abundance of cleaved Caspase-1 (p10 or p20 isoforms) by Western blotting using an anti-Caspase-1 antibody; and testing the candidate molecules for the degree of inhibition of Caspase-1 cleaved fragments (p10 or p20).

In some embodiments, a screening method for inflammasome inhibitors includes stimulating HEK-Blue™ IL-1β Cells (Invivogen) with Alu RNA or LPS+ARP to detect bioactive IL-1β formation using QUANT1-Blue™ (Invivogen); and testing the candidate molecule for degree of inhibition of colometric signal.

Inhibiting MyD88

In some embodiments, the presently-disclosed subject matter includes a method of protecting a cell, comprising: inhibiting MyD88 of the cell. In some embodiments, the inhibiting MyD88 comprises administering a MyD88 inhibitor.

As noted above, in some embodiments, inhibiting a polypeptide of interest to the presently-disclosed subject matter comprises administering an oligonucleotide or a small RNA molecule. Such small RNA molecule can target MyD88. Such nucleotides can target and degrade MyD88. In this regard, the presently-disclosed subject matter includes a isolated double-stranded RNA molecule that inhibits expression of MyD88, wherein a first strand of the double-stranded RNA comprises a sequence as set forth in Table C, and includes about 14 to 25 nucleotides. Examples of MyD88 inhibitors that can be used in accordance with the presently-disclosed subject matter include, but are not limited to those set forth in Table C. As such, embodiments of the presently-disclosed subject matter can include administering a MyD88 inhibitor set forth in Table C.

TABLE C

Examples of MyD88 Inhibitors
A inhibitor comprising the polypeptide sequence of IMG-2005-1 peptide sequence: DRQIKIWFQNRRMKWKKRDVLPGT (SEQ ID NO: 1), including about 29 to 100 nucleotides.
Oligopeptide for inhibiting MyD88 homodimerization: RDVLPGT (SEQ ID NO: 54
Oligopeptide for inhibiting MyD88 homodimerization: RDVVPGG (SEQ ID NO: 55
Loiarro et al. J Biol Chem 2005; 280:15809-14.
DRQIKIWFQNRRMKWKKRDVLPGTCVWSIASE (SEQ ID NO: 60).
RDVLPGTCVWSIASE (SEQ ID NO: 61).
An isolated double-stranded RNA molecule that inhibits expression of MyD88, at least one strand of which is about 14 to 25 nucleotides and includes a sequence selected from: 5'-GAGAAGCCUUUACAGGUdTdT-3' (SEQ ID NO: 3); 5'-ACCUGUAAAGGCUUCUCdTdT-3' (SEQ ID NO: 4); 5'-CAGAGCAAGGAAUGUGAdTdT-3' (SEQ ID NO: 5); 5'-UCACAUUCCUUGCUCUGdTdT-3' (SEQ ID NO: 6); and 5'-UAUUUCCUAAWGGGUCdTdT-3' (SEQ ID NO: 56).
A homodimerization inhibitor, such as Pepinh-MYD (Invitrogen).
A a dominant negative or splice variant of MyD88, such as a MyD88 splice variants that lack exon 2 (also known as the "intermediate domain" (e.g., having sequences set for at accession numbers NM_001172566.1 and NM_001172568.1), or other splice variants of MyD88 (e.g., having sequences set for at accession numbers NM_002468.4 and NM_001172569.1).

As noted above, in some embodiments, inhibiting MyD88 comprises administering an MyD88 inhibitor that is a dominant negative vector against MyD88, e.g., a dominant negative inhibitory form of MyD88 (pMyD88-dn) that contains the truncated ΔMyD88 (amino acids 152-296) lacking the death domain of MyD88 (Muzio et al. IRAK (Pelle) Family Member IRAK-2 and MyD88 as Proximal Mediators of IL-1 Signaling. *Science* 1997; 278:1612-1615).

As noted above, in some embodiments, inhibiting MyD88 comprises administering an MyD88 inhibitor that is a small molecule (e.g., (1) hydrocinnamoyl-L-valyl pyrrolidine, referred to as compound 4a in Bartfai et al. "A low molecular

weight mimic of the Toll/IL-1 receptor/resistance domain inhibits IL-1 receptor-mediated responses." *PNAS* 2003; 100: 7971-7976; or (2) ST2825 as described in Carminati, P., Gallo, G., Ruggiero, V., Sassano, M., Mastroianni, D. "MyD88 homodimerization inhibitors" Patent No. WO2006067091 and characterized in Loiarro et al "Inhibition of MyD88 dimerization and recruitment of IRAK1 and IRAK4 by a novel peptidomimetic compound." *Journal of Leukocyte Biology.* 2007; 82:801-810; or (3) 4-[(E)-2-(1-hexylpyridin-1-ium-2-yeethenyl]-N,N-dimethylaniline iodide, also known as 4-[(E)-2-(1-hexylpyridin-6-yl)ethenyl]-N,N-dimethyl-aniline Iodide, also known as Chemical Structure CID 5716367 in PubChem which blocks MyD88 interactions, or (4) the compounds referred to as 50-F12 and 26-J10 in Lee et al. "Application of β-Lactamase Enzyme Complementation to the High-Throughput Screening of Toll-Like Receptor Signaling Inhibitors." *Molecular Pharmacology* 2007; 72:868-875), or a natural product (malyngamide F acetate as described in Villa et al. "Selective MyD88-dependent pathway inhibition by the cyanobacterial natural product malyngamide F acetate." *European Journal of Pharmacology* 2010; 629:140-146), or a DNA or RNA aptamer generated by SELEX or other screening technology that binds or blocks MyD88.

The presently-disclosed subject matter further includes compositions useful for inhibiting MyD88. Such compositions include an inhibitor. As noted above, such inhibitors can be, for example, a nucleotide, a polypeptide, a small (chemical) molecule, etc. In some embodiments, a composition can include an isolated RNA molecule.

The presently-disclosed subject matter includes isolated RNA molecules that inhibit expression of MyD88. In some embodiments, a first strand of the double-stranded RNA comprises a sequence selected from the following, and including about 14 to 25 nucleotides: 5'-GAGAAGCCUUUACAGGUdTdT-3' (SEQ ID NO: 3); 5'-ACCUGUAAAGGCUUCUCdTdT-3' (SEQ ID NO: 4); 5'-CAGAGCAAGGAAUGUGAdTdT-3' (SEQ ID NO: 5); and 5'-UCACAUUCCUUGCUCUGdTdT-3' (SEQ ID NO: 6).

The presently-disclosed subject matter includes isolated polypeptide molecules that inhibit expression of MyD88. In some embodiments, the polypeptide molecule comprises a sequence selected from the following: DRQIKIWFQNRRMKWKKRDVLPGT (SEQ ID NO: 1), including about 29 to 100 amino acids. In some embodiments, the polypeptide molecule comprises a sequence selected from the following: RDVLPGT (SEQ ID NO: 54) and RDVVPGG (SEQ ID NO: 55).

In some embodiments, a method of identifying a MyD88 inhibitor makes use of a cultured cell wherein MyD88 is upregulated. Candidate compounds can be screened using the cultured cell to determine efficacy in modulating MyD88. Candidate compounds include, for example, small molecules, biologics, and combinations thereof, such as compositions including multiple compounds. The term small molecules is inclusive of traditional pharmaceutical compounds. The term biologics is inclusive of polypeptides and nucleotides, and including siRNAs, antibodies, aptamers, and dominant negative plasmids or vectors.

In some embodiments, the screening method includes providing a cell in culture wherein MyD88 is upregulated; and contacting a candidate compound with the cell. The method can further include identifying a change in MyD88. For example, a measurable change in MyD88 levels can be indicative of efficacy associated with the candidate compound. In some embodiments, wherein the change in the MyD88 is a measurable decrease in MyD88, the change is an indication that the candidate compound is a MyD88 inhibitor. Such MyD88 inhibitors can have utility for therapeutic applications as disclosed herein.

In some embodiments, the MyD88 can be upregulated using Alu RNA or lipopolysaccharide (LPS), for example, by stimulating cells (macrophages or RPE cells) with Alu RNA or LPS. In some embodiments, the MyD88 can be upregulated using CpG nucleotides, for example, by stimulating cells (macrophages or RPE cells) with synthetic oligonucleotides containing unmethylated CpG dinucleotides, such as 5'-tcg tcg ttt tgt cgt ttt gtc gtt-3' or 5'-ggG GGA CGA TCG TCg ggg gg-3'. In some embodiments, the MyD88 can be upregulated using interleukin-1 beta or interleukin 18, for example, by stimulating cells (macrophages or RPE cells) with recombinant forms of interleukin-1 beta or interleukin 18.

In some embodiments of the method for identifying a MyD88 inhibitor, a change in the MyD88 can be monitored by measuring cell viability, measuring the expression of genes known to be induced by MyD88 signaling (e.g., Cox-2, Socs3, TNF-alpha) or using other criteria that would be recognized by one of ordinary skill in the art, using methods known to one of ordinary skill in the art. In some embodiments, the cultured cell is an RPE cell. In some embodiments, the cell is a retinal photoreceptor cell. In some embodiments, the cell is a choroidal cell.

In some embodiments, a method of identifying a MyD88 inhibitor includes providing a cultured cell wherein MyD88 is upregulated or undergoes oligomerization or induces phosphorylation of IRAK1 or of IRAK4; and contacting the cell with a candidate compound; and determining whether the candidate compound results in a change in the MyD88 levels, or a change in the abundance of dimerized or oligomerized MyD88, or a change in the abundance of phosphorylated IRAK1 or of phosphorylated IRAK4. In some embodiments, the MyD88 is upregulated by: Alu RNA, lipopolysacharide, CpG nucleotides, single-stranded RNA, interleukin-1 beta, or interleukin 18. In some embodiments, the MyD88 is monitored by measuring cell viability, or measuring the expression of a gene known to be induced by MyD88 signaling. In some embodiments, the gene known to be induced by MyD88 signaling is selected from Cox-2, Socs3, and TNF-α.

In some embodiments of a screening method for MyD88 inhibitors, cells or cell lines can be stimulated with a known activator of MyD88, e.g., Alu RNA, or LPS. The RNA levels of genes such as Cox2, Socs3, or TNF-α can be measured using quantitative real-time RT-PCR. Candidate molecules can be tested for degree of inhibition of these gene transcripts.

In some embodiments of a screening method for MyD88 inhibitors, cells or cell lines can be stimulated with a known activator of MyD88, e.g., Alu RNA, or LPS. The abundance of dimerized or oligomerized MyD88 can be measured by Western blotting under non-reducing conditions using an anti-MyD88 antibody. The candidate molecule can be tested for degree of inhibition of MyD88 dimerization or oligomerization.

In some embodiments of a screening method for MyD88 inhibitors, cells or cell lines that have been transfected with plasmids coding for a fusion MyD88 protein tagged to fragments of YFP (yellow fluorescent protein) can be stimulated with a known activator of MyD88, e.g., Alu RNA, or LPS. The fluorescent signal can be measured using bimolecular fluorescence complementation techniques. The candidate molecule can be tested for degree of inhibition of fluorescent signal.

In some embodiments of a screening method for MyD88 inhibitors, cells or cell lines can be stimulated with a known activator of MyD88, e.g., Alu RNA, or LPS. The abundance of phosphorylated forms of IRAK1 or IRAK4 can be measured by Western blotting under reducing conditions using an anti-phosphoIRAK1 or anti-phosphoIRAK4 antibodies. The candidate molecule can be tested for degree of inhibition of IRAK1 or IRAK4 phosphorylation.

Inhibiting IL-18

In some embodiments, the presently-disclosed subject matter includes a method of protecting a cell, comprising: inhibiting IL-18 of the cell. In some embodiments, the inhibiting IL-18 comprises administering an IL-18 inhibitor.

As noted above, in some embodiments, inhibiting a polypeptide of interest to the presently-disclosed subject matter comprises administering a binding protein or an antibody. Such antibodies can include a neutralizing antibody against IL-18, or an antibody that blocks IL-18 binding to the IL-18 receptor. In some embodiments, the IL-18 inhibitor can be an IL-18 binding protein (Novick, et al., 1999).

Examples of IL-18 inhibitors that can be used in accordance with the presently-disclosed subject matter include, but are not limited to those set forth in Table D. As such, embodiments of the presently-disclosed subject matter can include administering an IL-18 inhibitor set forth in Table D.

TABLE D

Examples of IL-18 Inhibitors
A neutralizing antibody against IL-18, or an antibody that blocks IL-18 binding to the IL-18 receptor, e.g., IL-18 neutralizing antibody (MBL International); IL-18 neutralizing antibody (R&D Systems); or IL-18R1 neutralizing antibody (R&D Systems); or IL-18R1 neutralizing antibody (Genetex).
An IL-18 binding protein as described by Novick, et al., 1999.
IL18BP (an endogenous, naturally occurring IL-18 binding protein)

The presently-disclosed subject matter further includes compositions useful for inhibiting IL-18. Such compositions include an inhibitor. As noted above, such inhibitors can be, for example, a nucleotide, a polypeptide, a small (chemical) molecule, etc. In some embodiments, a composition can include an isolated RNA molecule. In some embodiments, a composition can include an antibody or a binding protein.

The presently-disclosed subject matter further includes methods of screening candidate inhibitors to identify IL-18 inhibitors. In some embodiments, a method of identifying an IL-18 inhibitor includes plating recombinant IL-18R1 on a solid state surface suitable for surface plasmon resonance (SPR); exposing the plated recombinant IL-18R1 to fluorescence-labeled recombinant IL-18; further exposing the system to a putative IL-18 inhibitor which would displace IL-18:IL-18R1 binding; and measuring fluorescence to determine degree of inhibition.

In some embodiments, a method of identifying an IL-18 inhibitor includes stimulating cells (e.g., RPE cells) or a cell line (e.g., THP-1 or RAW macrophages) with recombinant IL-18; measuring MyD88 activation, e.g., by measuring increased MyD88 dimerization (through Western blotting) or by measuring increased phosphorylation of IRAK1 or of IRAK4.

Inhibiting VDAC1 and/or VDAC2

In some embodiments, the presently-disclosed subject matter includes a method of protecting a cell, comprising: inhibiting VDAC1 and/or VDAC2 of the cell. In some embodiments, the inhibiting VDAC1 and/or VDAC2 comprises administering an VDAC1 and/or VDAC2 inhibitor.

As noted above, in some embodiments, inhibiting a polypeptide of interest to the presently-disclosed subject matter comprises administering an oligonucleotide or a small RNA molecule. Such small RNA molecule can target VDAC1 and/or VDAC2. Such nucleotides can target and degrade VDAC1 and/or VDAC2. In this regard, the presently-disclosed subject matter includes a isolated double-stranded RNA molecule that inhibits expression of VDAC1 and/or VDAC2, wherein a first strand of the double-stranded RNA comprises a sequence as set forth in Table E, and includes about 14 to 25 nucleotides. Examples of VDAC1 and/or VDAC2 inhibitors that can be used in accordance with the presently-disclosed subject matter include, but are not limited to those set forth in Table E. As such, embodiments of the presently-disclosed subject matter can include administering a VDAC1 and/or VDAC2 inhibitor set forth in Table E.

TABLE E

Examples of VDAC1 and/or VDAC2 Inhibitors
An isolated double-stranded RNA molecule that inhibits expression of VDAC1, at least one strand of which is about 14 to 25 nucleotides and includes the sequence of: 5'-CGGAAUAGCAGCCAAGUdTdT-3' (SEQ ID NO: 47).
An isolated double-stranded RNA molecule that inhibits expression of VDAC2, at least one strand of which is about 14 to 25 nucleotides and includes the sequence of: 5'-CCCUGGAGUUGGAGGCUdTdT-3' (SEQ ID NO: 48).
Any phosphorothioate oligonucleotide randomer (Trilink Industries), which all inhibit VDAC (Stein & Marco Colombini. Specific VDAC inhibitors: phosphorothioate oligonucleotides. J Bioenerg Biomembr 2008; 40:157-62; Tan et al. Phosphorothioate oligonucleotides block the VDAC channel. Biophys J. 2007; 93:1184-91)
Cyclosporin A-blocks VDAC1
Superoxide dismutase 1-blocks VDAC1
4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS)-blocks VDAC1
Bcl-x(L) BH4(4-23)-blocks VDAC
TR019622-blocks VDAC

The presently-disclosed subject matter further includes compositions useful for inhibiting VDAC1 and/or VDAC2. Such compositions include an inhibitor. As noted above, such inhibitors can be, for example, a nucleotide, a polypeptide, a small (chemical) molecule, etc. In some embodiments, a composition can include an isolated RNA molecule.

The presently-disclosed subject matter includes isolated RNA molecules that inhibit expression of VDAC1 and/or VDAC2. In some embodiments, a first strand of the double-stranded RNA comprises a sequence selected from the following, and including about 14 to 25 nucleotides: 5'-CGGAAUAGCAGCCAAGUdTdT-3' (SEQ ID NO: 47) and 5'-CCCUGGAGUUGGAGGCUdTdT-3' (SEQ ID NO: 48).

The presently-disclosed subject matter further includes methods of screening candidate inhibitors to identify VDAC1 and/or VDAC2 inhibitors. In some embodiments, cell or cell line-based methods are used.

Inhibiting Caspase-8

In some embodiments, the presently-disclosed subject matter includes a method of protecting a cell, comprising: inhibiting caspase-8 of the cell. In some embodiments, the inhibiting caspase-8 comprises administering a caspase-8 inhibitor.

Examples of caspase-8 inhibitors that can be used in accordance with the presently-disclosed subject matter include, but are not limited to those set forth in Table F. As such, embodiments of the presently-disclosed subject matter can include administering a caspase 8 inhibitor set forth in Table F.

TABLE F

Examples of Caspase-8 Inhibitors
Z-IETD-FMK (BD Biosciences)
Ac-Ala-Ala-Val-Ala-Leu-Leu-Pro-Ala-Val-Leu-Leu-Ala-Leu-Leu-Ala-Pro-Ile-Glu-Thr-Asp-CHO (EMD Millipore)
Z-Ile-Glu(OMe)-Thr-Asp(OMe)-CH_2F (EMD Millipore)
Cellular fas-associated death domain-like interleukin-1-β-converting enzyme-inhibitory protein (L), i.e. c-FLIP(L), a.k.a. FLICE, a.k.a. MACH, a.k.a. Mch5

The presently-disclosed subject matter further includes compositions useful for inhibiting caspase-8. Such compositions include an inhibitor. As noted above, such inhibitors can be, for example, a nucleotide, a polypeptide, a small (chemical) molecule, etc. In some embodiments, a composition can include an isolated RNA molecule.

The presently-disclosed subject matter further includes methods of screening candidate inhibitors to identify caspase-8 inhibitors. In some embodiments, cell or cell line-based methods are used.

Inhibiting NFκB

In some embodiments, the presently-disclosed subject matter includes a method of protecting a cell, comprising: inhibiting NFκB of the cell. In some embodiments, the inhibiting NFκB comprises administering a caspase-8 inhibitor.

Examples of NFκB inhibitors that can be used in accordance with the presently-disclosed subject matter include, but are not limited to those set forth in Table G. As such, embodiments of the presently-disclosed subject matter can include administering a NFκB inhibitor set forth in Table G.

TABLE G

Examples of NFKB Inhibitors Any one or more of the following NFKB inhibitors	
Antioxidants that have been shown to inhibit activation of NF-kB	
a-Lipoic acid	Sen et al, 1998; Suzuki et al, 1992
a-tocopherol	Islam et al, 1998
Aged garlic extract (allicin)	Ide & Lau, 2001; Lang et al, 2004; Hasan et al, 2007
2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)	Yun et al, 2005
N-acetyldopamine dimers (from P. cicadae)	Xu et al, 2006
Allopurinol	Gomez-Cabrera et al, 2006
Anetholdithiolthione	Sen et al, 1996
Apocynin	Barbieri et al, 2004
Apple juice/extracts	Shi & Jiang, 2002; Daviset al, 2006; Jung et al, 2009
Aretemsia p7F (5,6,3',5'-tetramethoxy 7,4'-hydroxyflavone)	Lee et al, 2004
Astaxanthin	Lee et al, 2003
Autumn olive extracts; olive leaf extracts	Wang et al, 2007; Wanget al, 2008
Avenanthramides (from oats)	Guo et al, 2007; Sur et al, 2008
Bamboo culm extract	Lee et al, 2008
Benidipine	Matsubara & Hazegawa, 2004
bis-eugenol	Murakami et al, 2003
Bruguiera gymnorrhiza compounds	Homhual et al, 2006
Butylated hydroxyanisole (BHA)	Israel et al, 1992; Schulze-Osthoff et al, 1993
Cepharanthine	Okamoto et al, 1994;Tamatani et al, 2007
Caffeic Acid Phenethyl Ester (3,4-dihydroxycinnamic acid, CAPE)	Natarajan et al, 1996;Nagasaka et al, 2007
Carnosol	Lo et al, 2002; Huang et al, 2005
beta-Carotene	Bai et al, 2005;Guruvayoorappan & Kuttan, 2007
Carvedilol	Yang et al, 2003
Catechol Derivatives	Suzuki & Packer, 1994;Zheng et al, 2008
Centaurea L (Asteraceae) extracts	Karamenderes et al, 2007
Chalcone	Liu et al, 2007
Chlorogenic acid	Feng et al, 2005
5-chloroacetyl-2-amnio-1,3-selenazoles	Nam et al, 2008
Cholestin	Lin et al, 2007
Chroman-2-carboxylic acid N-substituted phenylamides	Kwak et al, 2008
Cocoa polyphenols	Lee et al, 2006
Coffee extract (3-methyl-1,2-cyclopentanedione)	Chung et al, 2007
Crataegus pinnatifida polyphenols	Kao et al, 2007

TABLE G-continued

Examples of NFKB Inhibitors Any one or more of the following NFKB inhibitors	
Curcumin (Diferulolylmethane); dimethoxycurcumin; EF24 analog	Singh & Aggarwal, 1995;Pae et al, 2008; Kasinskiet al, 2008
Dehydroepiandrosterone (DHEA) and	DHEA-sulfate (DHEAS) Iwasaki et al, 2004; Liu et al, 2005
Dibenzylbutyrolactone lignans	Cho et al, 2002
Diethyldithiocarbamate (DDC)	Schreck et al, 1992
Diferoxamine	Sappey et al, 1995;Schreck et al, 1992
Dihydroisoeugenol; isoeugenol; epoxypseudoisoeugenol-2-methyl butyrate	Murakami et al, 1995Tark et al, 2007; Ma et al, 2008
Dihydrolipoic Acid	Suzuki et al, 1992, 1995
Dilazep + fenofibric acid	Sonoki et al, 2003; Yanget al, 2005
Dimethyldithiocarbamates (DMDTC)	Pyatt et al, 1998
Dimethylsulfoxide (DMSO)	Kelly et al, 1994
Disulfiram	Schreck et al, 1992
Ebselen	Schreck et al, 1992
Edaravone	Kokura et al, 2005; Ariiet al, 2007; Yoshida et al, 2007
EPC-K1 (phosphodiester compound of vitamin E and vitamin C)	Hirano et al, 1998
Epigallocatechin-3-gallate (EGCG; green tea polyphenols)	Lin & Lin, 1997; Yang et a1,1998; Hou et al, 2007; Jiang et al, 2012
Ergothioneine	Rahman et al, 2003
Ethyl Pyruvate (Glutathione depletion)	Song et al, 2004; Tsung et al, 2005; Jimenez-Lopezet al, 2008
Ethylene Glycol Tetraacetic Acid (EGTA)	Janssen et al, 1999
Eupatilin	Lee et al, 2008
Exercise	Goto et al, 2007
Fisetin	Park et al, 2006; Sung et al, 2007
Flavonoids (Cmtaegus; Boerhaavia diffusa root; xanthohumol; Eupatorium arnottianum; genistein; kaempferol; quercetin, daidzein; flavone; isorhamnetin; naringenin; pelargonidin; finestin; Sophora flavescens; Seabuckthorn fruit berry)	Zhang et al, 2004; Chenet al, 2004; Pandey et al, 2005; Albini et al, 2005;Colgate et al, 2006;Clavin et al, 2007; Hamalainen et al, 2008;Zheng et al, 2008; Junget al, 2008; Mishra et al, 2008
Flavonoid-7-glycosides (chamomile flowers extract)	Bulgari et al, 2012
Folic acid	Au-Yeung et al, 2006
Gamma-glutamylcysteine synthetase (gamma-GCS)	Manna et al, 1999
Ganoderma lucidum polysaccharides	Zhang et al, 2003; Ho et al, 2007
Garcinol (from extract of Garcinia indica fruit rind)	Liao et al, 2004
Ginkgo biloba extract	Chen et al, 2003
Glutathione	Cho et al, 1998; Schrecket al, 1992; Wang et al, 2007
Guaiacol (2-methoxyphenol)	Murakami et al, 2007
Hematein	Choi et al, 2003
Hinokitiol	Byeon et al, 2008
HMCO5 herbal extract	Kim et al, 2007
Hydroquinone	Pyatt et al, 1998; Yang et al, 2006
23-hydroxyursolic acid	Shin et al, 2004
IRFI 042 (Vitamin E-like compound)	Altavilla et al, 2001
Iron tetrakis	Kang et al, 2001
Isosteviol	Xu et al, 2008
Isovitexin	Lin et al, 2005
Isoliquiritigenin	Kumar et al, 2007; Kimet al, 2008; Kim et al, 2008
Justicia gendarussa root extract	Kumar et al, 2011
Kallistatin	Shen et al, 2008
Kangen-karyu extract	Satoh et al, 2005;Yokozawa et al, 2007
L-cysteine	Mihm et al, 1991
Lacidipine	Cominacini et al, 1997
Lazaroids	Marubayashi et al, 2002
Ligonberries	Wang et al, 2005
Lupeol	Saleem et al, 2004; Lee et al, 2007
Lutein	Kim et al, 2008
Magnolol	Chen et al, 2002; Ou et al, 2006; Kim et al, 2007
Maltol	Yang et al, 2006
Manganese superoxide dismutase (Mn-SOD)	Manna et al, 1998
Extract of the stem bark of Mangifera indica L.	Leiro et al, 2004; Garridoet al, 2005
Melatonin	Gilad et al, 1998; Mohanet al, 1995; Li et al, 2005
21 (alpha, beta)-methylmelianodiol	Zhou et al, 2007
Mulberry anthocyanins	Chen et al, 2006
N-acetyl-L-cysteine (NAC)	Schreck et al, 1991
Nacyselyn (NAL)	Antonicelli et al, 2002
Nordihydroguaiaritic acid (NDGA)	Brennan & O'Neill, 1998;Israel et a1,1992; Schulze-Osthoff et al, 1993; Staalet al, 1993
Ochnaflavone	Suh et al, 2006
Onion extract (2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyranone)	Ban et al, 2007; Tang et al, 2008
Orthophenanthroline	Schreck et al, 1992
N-(3-oxo-dodecanoyl) homoserine lactone	Kravchenko et al, 2008
Paricalcitol	Tan et al, 2008
Phenolic antioxidants (Hydroquinone and tert-butyl hydroquinone)	Ma et al, 2003
Olive oil phenols (extra-virgin olive oil)	Sangiovanni et al, 2012
alkenylphenols from Piper obliquum	Valdivia et al, 2008

TABLE G-continued

Examples of NFKB Inhibitors Any one or more of the following NFKB inhibitors	
alpha-phenyl-n-tert-butyl-nitron (PBN)	Kotake et al, 1998;Lin et al, 2006
Phenylarsine oxide (PAO, tyrosine phosphatase inhibitor)	Arbault et al, 1998
Phyllanthus urinaria	Chularojmontri et al, 2005; Shen et al, 2007
Phytosteryl ferulates (rice bran)	Islam et al, 2008; Jung et al, 2008
Piper longum Linn. extract	Singh et al, 2007
Pitavastatin	Tounai et al, 2007; Wang& Kitajima, 2007
Prodelphinidin B2 3, 3' di-O-gallate	Hou et al, 2007
Pterostilbene	Cichocki et al, 2008; Panet al, 2009
Pyrrolinedithiocarbamate (PDTC)	Schreck et al, 1992
Quercetin	Musonda & Chipman, 1998; Shih et al, 2004;Garcia-Mediavilla et al, 2006; Ruiz et al, 2007;Min et al, 2007; Kim et al, 2007
Red orange extract	Cimini et al, 2008
Red wine	Blanco-Colio et al, 2000;Cui & He, 2004
Ref-1 (redox factor 1)	Ozaki et al, 2002
Rg(3), a ginseng derivative	Keum et al, 2003
Rotenone	Schulze-Osthoff et al, 1993
Roxithromycin	Ueno et al, 2005; Ou et al, 2008
Rutin	Kyung et al, 2008
S-allyl-cysteine (SAC, garlic compound)	Geng et al, 1997
Salogaviolide (Centaurea ainetensis)	Ghantous et al, 2008
Sauchinone	Lee et al, 2003; Hwang et al, 2003
Schisandrin B	Giridharan et all, 2011
Silybin	Gazak et al, 2007
Spironolactone	Han et al, 2006
Strawberry extracts	Wang et al, 2005
Sulfuretin	Lee et al, 2012
Taxifolin	Wang et al, 2005
Tempol	Cuzzocrea et al, 2004
Tepoxaline (5-(4-chlorophenyl)-N-hydroxy-(4-methoxyphenyl)-N-methyl-1H-pyrazole-3-propanamide)	Kazmi et al, 1995; Ritchieet al, 1995
Thio avarol derivatives	Amigo et al, 2007; Amigoet al, 2008
Thymoquinone	El Gazzar et al, 2007;1Sethi et al, 2008
Tocotrienol (palm oil)	Wu et al, 2008
8-(Tosylamino)quinoline	Jung et al, 2012
Tomato peel polysaccharide	De Stefano et al, 2007
UDN glycoprotein (Ulmus davidiana Nakai) Lee & Lim, 2007	
Vaccinium stamineum (deerberry) extract	Wang et al, 2007
Vanillin (2-hydroxy-3-methoxybenzaldehyde)	Murakami et al, 2007
Vitamin C	Staal et al, 1993; Son et al, 2004
Vitamin B6	Yanaka et al, 2005
Vitamin E and derivatives	Suzuki & Packer, 1993;Ekstrand-Hammarstrom et al, 2007; Glauert, 2007
a-torphryl succinate	Staal et al, 1993; Suzuki & Packer, 1993
a-torphryl acetate	Suzuki & Packer, 1993
PMC (2,2,5,7,8-pentamethyl-6-hydroxychromane)	Suzuki & Packer, 1993
Yakuchinone A and B	Chun et al, 2002
Proteasome and proteases inhibitors that inhibit Rel/NF-kB	
Proteasome inhibitors	
Peptide Aldehydes:	Palombella et al, 1994; Grisham et al, 1999; Jobin et al, 1998
ALLnL (N-acetyl-leucinyl-leucynil-norleucynal,MG101)	
LLM (N-acetyl-leucinyl-leucynil-methional)	
Z-LLnV (carbobenzoxyl-leucinyl-leucynil-norvalinal,MG115)	
Z-LLL (carbobenzoxyl-leucinyl-leucynil-leucynal, MG132)	
MG262	Pujois et al, 2012
Lactacystine, beta-lactone	Fenteany & Schreiber, 1998; Grisham et al, 1999
Boronic Acid Peptide	Grisham et al, 1999; Iqbal et al, 1995
Dithiocarbamate complexes with metals	Cvek & Dvorak, 2007
CEP-18770	Piva et al, 2007
Ubiquitin Ligase Inhibitors	Yaron et al, 1997
PS-341 (Bortezomib)	Adams, 2004
Salinosporamide A (1, NPI-0052)	Macherla et al, 2005; Ahn et al, 2007
Cyclosporin A	Frantz et al, 1994; Kunz et al, 1995; Marienfeld et al, 1997; McCaffrey et al, 1994;Meyer et al, 1997; Wechsler et al, 1994
FK506 (Tacrolimus)	Okamoto et al, 1994; Venkataraman et al, 1995
Deoxyspergualin	Tepper et al, 1995
Disulfiram	Lovborg et al, 2005
PT-110	Momose et al, 2007
Protease inhibitors	
APNE (N-acetyl-DL-phenylalanine-b-naphthylester)	Higuchi et al, 1995
B IEE (N-benzoyl L-tyrosine-ethylester)	Rossi et al, 1998
DCIC (3,4-dichloroisocoumarin)	D'Acquisto et al, 1998
DFP (diisopropyl fluorophosphate)	
TPCK (N-a-tosyl-L-phenylalanine chloromethyl ketone)	

TABLE G-continued

Examples of NFKB Inhibitors Any one or more of the following NFKB inhibitors		
TLCK (N-a-tosyl-L-lysine chloromethyl ketone)		
IkBa phosphorylation and/or degradation inhibitors		
Molecule	Point of Inhibition	References
Desloratadine; diphenhydramine Histamine H1 receptor	Wu et al, 2004; Scadding, 2005;	Roumestan et al, 2008
Bikunin	LPS receptor agonists	Kobayashi, 2006; Kanayama et al, 2007
Ron Tyrosine kinase receptor	Suppresses TNF production	Lentsch et al, 2007
TAK-242	TLR4 intracellular domain	Kawamoto et al, 2008
Salmeterol, fluticasone propionate	beta2 agonists	Baouz et al, 2005
CPU0213	Endothelin receptor antagonist	He et al, 2006
Doxazosin	alpha1-adrenergic receptor antagonist	Hui et al, 2007
Erbin overexpression	NOD2 inhibitor	McDonald et al, 2005
Protein-bound polysaccharide from basidiomycetes	LPS-CD14 interaction	Asai et al 2005
Anti-CD146 antibody AA98	upstream of IKK	Bu et al, 2006
Calagualine (fern derivative)	upstream of IKK (TRAF2-NIK)	Manna et al, 2003
NS3/4A (HCV protease)	upstream of IKK	Karayiannis, 2005
golli BG21 (product of myelin basic protein)	upstream of IKK (PKC)	Feng et al 2004
NPM-ALK oncoprotein	Traf2 inhibition	Horie et al, 2004
NS5A (Hepatitis C virus)	Traf2 inhibition	Park et al, 2002
LY29 and LY30	PI3 Kinase inhibitors	Choi et al, 2004
Shiga toxin (Enterohemorrhagic E coli)	PI3 Kinase inhibitor	Gobert et al 2007
Evodiamine (Evodiae Fructus component)	AKT-IKK interaction	Takada et al 2005
Rituximab (anti-CD20 antibody)	up-regulates Raf-1 kinase inhibitor	Jazirehi et al, 2005
Kinase suppressor of ras (KSR2)	MEKK3 inhibitor	Channavajhala et al, 2005
Cholecystokinin ocatpeptide (CCK-8)p38 kinase		Li et al, 2007
M2L (Vaccinia virus)	ERK2 inhibitor	Gedey et al, 2006;
Pefabloc (serine protease inhibitor)upstream of IKK	Tando et al, 2002	Hinthong et al, 2008
Rocaglamides (Aglaia derivatives)	upstream of IKK	Baumann et al, 2002
Ymer	Binds to Ub-RIP	Bohgaki et al, 2007
Epoxyquinol B	TAK1 crosslinker	Kamiyama et al, 2008
Betaine	NIK/IKK	Go et al, 2004, 2007
TNAP	NIK	Hu et al, 2005
Selected peptides	NEMO binding to Ub	Wyler et al, 2007
Biochanin	upstream of IKK/ phosphorylates of IκBα	Manna et al, 2012
Desflurane	IKK complex formation with TNF-Rl	Li et al, 2008
Geldanamycin	IKK complex formation	Chen et al, 2002
Grape seed proanthocyanidins	IKKa activity	Mantena & Katiyar, 2006; Sharma et al, 2007; Cheng et al, 2007; Xu et al, 2008
Laretia acaulis azorellane diterpenoids	IKKa activity	Borquez et al, 2007
MC160 (Molluscum contagiosum virus)	IKKa activity	Nichols & Shisler , 2006
NS5B (Hepatitis C protein)	IKKa activity	Choi et al, 2006
Pomegranate fruit extract	IKKa activity	Afaq et al, 2004; Khan et al, 2006
Tetrandine (plant alkaloid)	IKKa activity	Ho et al, 2004; Xue et al, 2008; Lin et al 2008
BMS-345541 (4(2'-Aminoethyl)amino-1,8-dimethylimidazo(1,2-a) quinoxaline) and 4-amino derivatives		
IKKa and IKKb kinase activity	Burke et al, 2002; Yang et al, 2006;	Beaulieu et al, 2006
1-O-acetylbritannilactone	IKKb activity	Liu et al, 2007
2-amino-3-cyano-4-aryl-6-(2-hydroxy-phenybpyridine derivatives	IKKb activity	Murata et al, 2003, 2004, 2004
Acrolein	IKKb activity/p50 DNA binding Vallacchi et al, 2005;	Lambert et al 2007
Anandamide	IKKb activity	Sancho et al, 2003
AS602868	IKKb activity	Frelin et al, 2003: Griessinger et al, 2007
benzoxathiole(6,6-dimethyl-2-(phenylimino)-6,7-dihydro-5H-benzo-[1,3]oxathio1-4-one (and its analogs)	IKKb activity	Venkateswararao et al, 2012
Cobrotoxin	IKKb activity/p50 DNA binding Park et al, 2005	
Core protein (Hepatitis C)	IKKb activity	Joo et al, 2005; Shrivastava et al, 1998
1-[2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole	IKKb activity	Yore et al, 2006
Dihydroxyphenylethanol	IKKb activity	Guichard et al, 2006
Heibimycin A	IKKb activity	Iwasaki et al, 1992; Mahon & O'Neill, 1995; Ogino et al, 2004
Inhibitor 22	IKKb activity	Baxter et al, 2004
Isorhapontigenin	IKKb activity	Li et al, 2005
Manumycin A	IKKb activity	Bernier et al, 2005; Frassanito et al, 2005
6-methyl-2-propolyimino-6,7-dihydro-5 H-benzo[1,3]oxathiol-4-one	IKKb	Kim et al, 2008

TABLE G-continued

Examples of NFKB Inhibitors Any one or more of the following NFKB inhibitors		
MLB120 (small molecule)	IKKb activity	Nagashima et al, 2006
Naphthopyrones (6-methoxycomaparvin and 6-methooxycomaparvin 5-methyl ether)	IKKb activity	Fulmer et al, 2008
Novel Inhibitor	IKKb activity	Kamon et al, 2004
vIRF3 (KSHV)	IKKb activity	Seo et al, 2004
Nitric oxide	IKKb activity/IkB phosphorylation	Katsuyama et al, 1998; Matthews et al, 1996; Spieker & Liao, 1999; Reynaert et al, 2004
SC-514 (small molecule)	IKKb activity	Kishore et al, 2003
Thienopyridine	IKKb activity	Morwick et al, 2006
Acetyl-boswellic acids	IKK activity	Syrovets et al, 2004, 2005
Amino-pyrimidine derivative	IKK activity	Karin et al, 2004
Benzoimidazole derivative	IKK activity	Karin et al, 2004
BMS-345541	IKK activity	Burke et al, 2003
Butein	IKKb activity	Pandey et al, 2007
Beta-carboline	IKK activity	Yoon et al, 2005
CYL-19s and CYL-26z, two synthetic alpha-methylene-gamma-butyrolactone derivatives	IKK activity	Huang et al, 2004
ACHP (2-amino-6-[2-(cyclopropylmethoxy)-6-hydroxyphenyl]-4-piperidin-4-yl nicotinonitrile	IKKb activity (ATP analog)	Sanda et al, 2006
Berberine	IKKb activity	Hu et al, 2007; Yi et al, 2008; Pandey et al 2008
Compound A	IKKb activity (ATP analog)	Ziegelbauer et al, 2005
Flavopiridol	IKK activity and RelA phosphor.	Takada & Aggarwal, 2003
Cyclopentones	IKKb activity	Bickley et al, 2004
Dehydroascorbic acid (Vitamin C)	IKKb activity	Carcamo et al, 2004
Gossypyin or Gossypium extracts	IKKb activity	Kunnumakkara et al, 2007; Ji et al, 2008
M protein (SARS-Cornonavirus protein)	IKKb activity	Fang et al, 2007
IMD-0354	IKKb activity	Tanaka et al, 2004, 2006; Inayama et al 2006
Jesterone dimer	IKKb activity; DNA binding	Liang et al, 2003, 2006
KINK-1	IKKb activity	Schon et al, 2008
LCY-2-CHO	IKKb activity	Ho et al, 2007
Prolyl hydroxylase-1	IKKb activity	Cummins et al, 2006
Naphthopyrones (Echinoderm Comanthus parvicirrus)	IKKb activity	Folmer et al, 2007
Neuropeptides CGRP, PACAP and VIP	IKKb activity	Ding et al, 2007
PS-1145 (MLN1145)	IKKb activity	Hideshima et al, 2002
2-[(aminocarbonyl)amino]-5-(4-fluorophenyl)-3-thiophenecarboxamides (TPCA-1)	IKKb activity	Bonafoux et al, 2005; Podolin et al, 2005
1'-Acetoxychavicol acetate (Languas galanga)	IKK activity	Ichikawa et al, 2005; Ito et al, 2005
17-Acetoxyjolkinolide B	IKK activity	Yan et al, 2008
Acute alcohol exposure	IKK activity	Mandrekar et al, 2007
Anacardic acid (6-nonadecyl-salicylic acid)	IKK activity	Sung et al, 2008
Apigenin (plant flavinoid)	IKK activity	Shukla & Gupta, 2004; Yoon et al, 2006
Asiatic acid	IKK activity	Yun et al, 2008
Cardamomin	IKK activity	Lee et al, 2005
CDDO-Me (synthetic triterpenoid)	IKK activity	Shishodia et al, 2006
CHS 828 (anticancer drug)	IKK activity	Olsen et al, 2004
CIVIL-1	IKK activity	Mo et al, 2006
Compound 5 (Uredio-thiophenecarboxamide derivative)	IKK activity	Roshak et al, 2002
CT20126	IKK activity/NIK	Lee et al, 2008
Diaylpyridine derivative	IKK activity	Murata et al, 2003
3,4-dihydroxybenzalacetone (from Chaga)	IKK activity	Sung et al, 2008
Diosgenin	IKK activity	Shishodia & Aggarwal, 2005; Liagre et al 2005
E3-14.7K (Adenovirus)	IKK activity	Li et al, 1999
E3-10.4K/14.5K (Adenovirus)	IKK activity	Friedman & Horwitz, 2002
E7 (human papillomavirus)	IKK activity	Spitkovsky et al, 2002
Furonaphthoquinone	IKK activity	Shin et al, 2006
3-Formylchromone	IKKb activity/p65 DNA binding	Yadav et al, 2011
Guggulsterone	IKK activity	Ichikawa & Aggarwal, 2006; Deng, 2007; Lv et al, 2008; Lee et al, 2008
HB-EGF (Heparin-binding epidermal growth factor-like growth factor)	IKK activity	Mehta & Besner, 2003
Falcarindol	IKK activity	Shiao et al, 2005
Hammerhead ribozyme to IKKa/b	IKK activity	Yang et al, 2007
Hepatocyte growth factor	IKK activity	Min et al, 2005; Gong et al, 2006
Honokiol	IKK activity	Tse et al, 2005; Munroe et al, 2007
Humulone	IKK activity	Lee et al, 2007
Hypoestoxide	IKK activity	Ojo-Amaize et al, 2001
Indolecarboxamide derivative	IKK activity	Karin et al, 2004
Labdane diterpenoids	IKK activity	Giron et al, 2008

TABLE G-continued

Examples of NFKB Inhibitors Any one or more of the following NFKB inhibitors		
LF15-0195 (analog of 15-deoxyspergualine)	IKK activity	Yang et al, 2003
gamma-mangostin (from Garcinia mangostana)	IKK activity	Nakatani et al, 2004
Garcinone B	IKK activity	Yamakuni et al, 2005
(Amino)imidazolylcalboxaldehyde derivative	IKK activity	Karin et al, 2004
Imidazolylquinoline-carboxaldehyde derivative	IKK activity	Karin et al, 2004
Kahweol	IKK activity	Kim et al, 2004
Kava (Piper methysticum) derivatives	IKK activity	Folmer et al, 2006
Lead	IKK activity	Xu et al, 2006
Marasmius oreades liquid extract	IKK activity	Petrova et al, 2008
Menatetrenone (vitamin K2 analogue)	IKK activity	Ozaki et al, 2007
Metformin	IKK activity	Huang et al, 2008
Mild hypothermia	IKK activity	Han et al, 2003
ML 120B	IKK activity	Catley et al, 2006
Morin (3,5,7,2',4'-Pentahydroxyflavone)	IKK activity	Manna et al, 2007
Morusin	IKK activity	Lee et al, 2008
MX781 (retinoid antagonist)	IKK activity	Bayonet al, 2003
N-acetylcysteine	IKK activity	Oka et al, 2000
Nitrosylcobalamin (vitamin B12 analog)	IKK activity	Chawla-Sarkar et al 2003
NSAIDs	IKK activity	Takada et al, 2004
Hepatits C virus NS5B	IKK activity	Choi et al, 2006
PAN1 (aka NALP2 or PYPAF2)	IKK activity	Bruey et al, 2004
Pectin (citrus)	IKK activity	Chen et al, 2006
Pinitol	IKK activity	Sethi et al, 2008
PMX464	IKK activity	Callister et al, 2008
Pyrazolo[4,3-c]quinoline derivative	IKK activity	Karin et al, 2004
Pyridooxazinone derivative	IKK activity	Karin et al, 2004
N-(4-hydroxyphenyl) retinamide	IKK activity	Shishodia et al, 2005; Kuefer et al, 2007
Scytonemin	IKK activity	Stevenson et al, 2002
Semecarpus anacardiu extract	IKK activity	Singh et al, 2006
SPC-839	IKK activity	Palanki et al, 2002
Sulforaphane and phenylisothiocyanate	IKK activity	Xu et al, 2005; Murakami et al, 2007; Liu et al, 2008: Hayes et al, 2008
Survanta (Surfactant product)	IKK activity	Raychaudhuri et al, 2003
Torque Teno virus ORF2	IKK activity	Zheng et al, 2007
Piceatannol	IKK activity	Islam et al, 2004
Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone)	IKK activity	Sandur et al, 2006
IKKb peptide to NEMO binding domain	IKK-NEMO interaction	May et al. 2000
NEMO CC2-LZ peptide	NEMO oligomerization	Agou et al, 2004
AGRO100 (G-quadraplex oligodeoxynucleotide)	NEMO binding	Girvan et al, 2006
PTEN (tumor suppressor)	Activation of IKK	Gustin et al, 2001
Theaflavin (black tea component)	Activation of IKK	Aneja et al, 2004; Ukil et al, 2006; Kalra et al, 2007
Tilianin	Activation of IKK	Nam et al, 2005
Withanolides	Activation of IKK	Ichikawa et al, 2006
Zerumbone	Activation of IKK	Takada et al, 2005
Silibinin	IKKa activity; nuclear translocation	Dhanalakshmi et al, 2002; Singh et al, 2004; Min et al, 2007
Sulfasalazine	IKKa and IKKb kinase activity	Wahl et al, 1998: Weber et al, 2000
Sulfasalazine analogs	IKK kinase activity	Habens et al, 2005
Quercetin	IKK activity	Peet & Li, 1999
Rosmarinic acid	IKK activity	Lee et al, 2006
Staurosporine	IKK activity	Peet & Li, 1999
gamma-Tocotrienol	IKK activity	Shah & Sylvester, 2005; Ahn et al, 2006
Wedelolactone	IKK activity	Kobori et al, 2003
Betulinic acid	IKKa activity and p65 phosphorylation	Takada & Aggarwal, 2003; Rabi et al, 2008
Ursolic acid	IKKa activity and p65 phosphorylation	Shishodia et al, 2003; Manu & Kuttan, 2008
Thalidomide (and thalidomideanalogs)	IKK activity	Keifer et al, 2001; Ge et al, 2006; Carcache de- Blanco et al, 2007

TABLE G-continued

Examples of NFKB Inhibitors Any one or more of the following NFKB inhibitors		
Salubrinal	IKK activity/degradation	Huang et al, 2011
Fas-associated factor-1	IKK assembly	Park et al, 2007
Interleukin-10	Reduced IKKa and IKKb expression	Tabary et al, 2003
MC160 (molluscum contagiosum virus)	Reduced IKKa expression	Nichols & Shisler, 2006
Monochloramine and glycine chloramine (NH2C1)	Oxidizes IkB	Kim et al, 2005; Midwinter et al, 2006
GS143	Blocks IkB ubiquitylation	Nakajima et al, 2008; Hirose et al, 2008
Salmonella Secreted Factor L	Blocks IkB ubiquitylation	Le Negrate et al, 2008
Anethole	Phosphorylation	Chainy et al, 2000
Anti-thrombin III	Phosphorylation	Oelschlager et al, 2002
Artemisia vestita	Phosphorylation	Sun et al, 2006
Aspirin, sodium salicylate	Phosphorylation, IKKbeta	Frantz & O'Neill, 1995; Kopp & Ghosh, 1994; Yin et al, 1998
Azidothymidine (AZT)	Phosphorylation	Ghosh et al, 2003; Kurokawa et al, 2005
Baoganning	Phosphorylation	Tan et al, 2005
BAY-11-7082 (E3((4-methylphenyl)-sulfonyl)-2-propenenitrile) Phosphorylation	Pierce et al, 1997	
BAY-117083 (E3((4-t-butylphenyl)-sulfonyl)-2-propenenitrile) Phosphorylation	Pierce et al, 1997	
Benzyl isothiocyanate	Phosphorylation	Srivastava & Singh, 2004
Black raspberry extracts (cyanidin 3-O-glucoside, cyanidin 3-O-(2(G)-xylosylrutinoside), cyanidin 3-O-(2(G)-xylosylrutinoside), cyanidin 3-O-rutinoside)	Phosphorylation	Huang et al, 2002; Hecht et al, 2006
Buddlejasaponin IV	Phosphorylation	Won et al, 2006
Cacospongionolide B	Phosphorylation	Posadas et al, 2003
Calagualine	Phosphorylation	Manna et al, 2003
Carbon monoxide	Phosphorylation	Sarady et al, 2002
Carboplatin	Phosphorylation	Singh & Bhat, 2004
Cardamonin	Phosphorylation	Israf et al, 2006
Chorionic gonadotropin	Phosphorylation	Manna et al, 2000
Cordycepin	Phosphorylation	Kim et al, 2006; Huang et al., 2007
Crassocephalum rabens galactolipid	Phosphorylation	Hou et al., 2007
Cycloepoxydon; 1-hydroxy-2-hydroxymethyl-3-pent-1-enylbenzene	Phosphorylation	Gehrt et al, 1998
Cytomegalovirus	Phosphorylation	Jarvis et al, 2006
Decursin	Phosphorylation	Kim et al, 2006
Delphinidin	Phosphorylation	Syed et al, 2008
Dexanabinol	Phosphorylation	Juttler et al, 2004
Digitoxin	Phosphorylation	Srivastava et al, 2004; Jagielska et al, 2009
Dihydrotestosterone	Phosphorylation	Xu et al, 2011
Diterpenes (synthetic)	Phosphorylation	Chao et al, 2005
Docosahexaenoic acid	Phosphorylation	Chen et al, 2005; Zand et al, 2008
Entamoeba histolytica	Phosphorylation	Kammanadiminti & Chadee, 2006
Extensively oxidized low density lipoprotein (ox-LDL), 4-Hydroxynonenal (HNE)	Phosphorylation	Brand et al, 1997; Page et al, 1999
FBD	Phosphorylation	Lin et al, 2008
FHIT (Fragile histidine triad protein)	Phosphorylation	Nakagawa & Akao, 2006
Fructus Ligustrum lucidi	Phosphorylation	An et al, 2007
Gabexate mesilate	Phosphorylation	Uchiba et al, 2003
[6]-gingerol; casparol	Phosphorylation	Kim et al, 2005; Aktan et al, 2006; Ishiguro et al, 2007
Gleditsia sinensis thorns extract	Phosphorylation	Ha et al, 2008
Gleevec (Imatanib)	Phosphorylation	Wolf et al, 2005
Glossogyne tenuifolia	Phosphorylation	Wu et al, 2004; Ha et al, 2006
Guggulsterone	Phosphorylation	Shishodia & Aggarwal, 2004
4-hydroxy-3,6,7,8,3',4'-hexamethoxyflavone	Phosphorylation	Lai et al, 2007
Hydroquinone	Phosphorylation	Kerzic et al, 2003
Ibuprofen	Phosphorylation	Palayoor et al, 1998
Indirubin-3'-oxime	Phosphorylation	Mak et al, 2004
Inonotus obliquus ethanol extract	Phosphorylation	Kim et al, 2007
Interferon-alpha	Phosphorylation	Manna et al, 2000
Inhaled isobutyl nitrite	Phosphorylation	Ponnappan et al, 2004
Kaempferol	Phosphorylation	Garcia-Mediavilla et al, 2006; Kim et al 2007
Kushen flavonoids and kurarinone	Phosphorylation	Han et al, 2006
Licorce extracts	Phosphorylation	Kim et al, 2006: Kwon et al, 2007
Melatonin	Phosphorylation	Alonso et al, 2006; Tamura et al, 2009

TABLE G-continued

Examples of NFKB Inhibitors Any one or more of the following NFKB inhibitors		
Marine natural products (several)	IKKb/proteasome	Folmer et al, 2009
Methotrexate	Phosphorylation	Majumdar & Aggarwal, 2001; Yozai et al 2005
Monochloramine	Phosphorylation	Omori et al, 2002
Nafamostat mesilate	Phosphorylation	Noguchi et al, 2003
Obovatol	Phosphorylation	Lee et al, 2008
Oleandrin	Phosphorylation	Manna et al, 2000; Sreeivasan et al, 2003
Oleanolic acid (Aralia elata)	Phosphorylation	Suh et al, 2007
Omega 3 fatty acids	Phosphorylation	Novak et al, 2003
Panduratin A (from Kaempferia pandurata, Zingiberaceae)	Phosphorylation	Yun et al, 2003
Petrosaspongiolide M	Phosphorylation	Posadas et al, 2003
Pinosylvin	Phosphorylation	Lee et al, 2006
Plagius flosculosus extract polyacetylene spiroketal	Phosphorylation	Calzado et al, 2005
Phytic acid (inositol hexakisphosphate)	Phosphorylation	Ferry et al, 2002
Pomegranate fruit extract	Phosphorylation	Ahmed et al, 2005
Prostaglandin Al	Phosphorylation/IKK	Rossi et al, 1997, 2000
Protocatechuic Aldehyde	Phosphorylation	Xu et al, 2011
20(S)-Protopanaxatriol (ginsenoside metabolite)	Phosphorylation	Oh et al, 2004; Lee et al, 2005
Rengyolone	Phosphorylation	Kim et al, 2006
Rottlerin	Phosphorylation	Kim et al, 2005; Torricelli et al, 2008
Saikosaponin-d	Phosphorylation; Increased IkBLeung et al, 2005;	Dang et al, 2007
Saline (low Na+istonic)	Phosphorylation	Tabary et al, 2003
Salvia miltiorrhizae water-soluble extract	Phosphorylation	Kim et al, 2005
Sanguinarine (pseudochelerythrine, 13-methyl-[1,3]-benzodioxolo-[5,6-c]-1,3-dioxolo-4,5phenanthridinium)	Phosphorylation	Chaturvedi et al, 1997
Scoparone	Phosphorylation	Jang et al, 2005
Sesaminol glucosides	Phosphorylation	Lee et al, 2006
Shikonins	Phosphorylation	Nam et al, 2008
Silymarin	Phosphorylation	Manna et al, 1999; Saliou et al, 1998
Snake venom toxin (Vipera lebetina turanica)	Phosphorylation	Son et al, 2007
SOCS1	Phosphorylation	Kinlyo et al, 2002; Nakagawa et al, 2002
Spilanthol	Phosphorylation	Wu et al, 2008
Statins (several)	Phosphorylation	Hilgendorff et al, 2003; Han et al, 2004; Planavila et al, 2005
Sulindac	IKK/Phosphorylation	Yamamato et al, 1999
THI 52 (1-naphthylethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline)	Phosphorylation	Kang et al, 2003
1,2,4-thiadiazolidine derivatives	Phosphorylation	Manna et al, 2004
Tomatidine	Phosphorylation	Chiu & Lin, 2008
Vesnarinone	Phosphorylation	Manna & Aggarwal, 2000; Harada et al 2005
Xanthoangelol D	Phosphorylation	Sugii et al, 2005
YC-1	Phosphorylation	Huang et al, 2005
YopJ (encoded by Yersinia pseudotuberculosis)	Deubiquintinase for IkBa;	Schesser et al, 1998; Zhou et al, 2005;
	Acetylation of IKKbeta	Mittal et al, 2006; Mukherjee & Orth, 2008
Osmotic stress	IkB ubiquitination	Huangfu et al, 2007
Acetaminophen	Degradation	Mancini et al, 2003
Activated Protein C (APC)	Degradation	Yuksel et al, 2002
Alachlor	Degradation	Shimomura-Shimizu et al, 2005
Allylpyrocatechol	Degradation	Sarkar et al, 2008
a-melanocyte-stimulating hormone (a-MSH)	Degradation	Manna & Aggarwal, 1998
Amentoflavone	Degradation	Banerjee et al, 2002; Guruvayoorappan & Kuttan, 2007
Angelica dahurica radix extract	Degradation	Kang et al, 2006
Apple extracts	Degradation/proteasome	Yoon & Liu, 2007
Artemisia capillaris Thunb extract (capillarisin)	Degradation	Hong et al, 2004; Kim et al, 2007; Lee et al, 2007
Artemisia iwayomogi extract	Degradation	Kim et al, 2005
L-ascorbic acid	Degradation	Han et al, 2004
Antrodia camphorata	Degradation	Hseu et al, 2005
Aucubin	Degradation	Jeong et al, 2002
Baicalein	Degradation	Ma et al, 2004
N-(quinolin-8-yl)benzenesulfonamindes	Degradation	Xie et al, 2007
beta-lapachone	Degradation	Manna et al, 1999
Blackberry extract	Degradation	Pergola et al, 2006

TABLE G-continued

Examples of NFKB Inhibitors Any one or more of the following NFKB inhibitors		
1-Bromopropane	Degradation	Yoshida et al, 2006
Buchang-tang	Degradation	Shin et al, 2005
Capsaicin (8-methyl-N-vanillyl-6-nonenamide)	Degradation	Singh et al, 1996; Mori et al, 2006; Kang et al, 2007
Catalposide	Degradation	Kim et al, 2004
Clerodendron trichotomum Tunberg Leaves	Degradation	Park & Kim, 2007
Clomipramine/imipramine	Degradation	Hwang et al, 2008
Coptidis rhizoma extract	Degradation	Kim et al, 2007
Cyclolinteinone (sponge sesterterpene)	Degradation	D'Acquisto et al, 2000
DA-9601 (Artemisia asiatica extract)	Degradation	Choi et al, 2006
Diamide (tyrosine phosphatase inhibitor)	Degradation	Toledano & Leonard, 1991; Singh & Aggarwal, 1995
Dihydroarteanniun	Degradation	Li et al, 2006
Dobutamine	Degradation	Loop et al, 2004
Docosahexaenoic acid	Degradation	Weldon et al, 2006
E-73 (cycloheximide analog)	Degradation	Sugimoto et al, 2000
Ecabet sodium	Degradation	Kim et al, 2003
Electrical stimulation of vagus nerve	Degradation	Guarini et al, 2003
Emodin (3-methyl-1,6,8-trihydroxyanthraquinone)	Degradation	Kumar et al, 1998; Huang et al, 2004
Ephedrae herba (Mao)	Degradation	Aoki et al, 2005
Equol	Degradation	Kang et al, 2005
Erbstatin (tyrosine kinase inhibitor)	Degradation	Natarajan et al, 1998
Estrogen (E2)	Degradation/and various other steps	Sun et al, 1998; Kalaitzidis & Gilmore, 2005; Steffan et al, 2006
Ethacrynic acid	Degradation (and DNA binding)	Han et al, 2004
Fludarabine	Degradation	Nishioka et al, 2007
Fosfomycin	Degradation	Yoneshima et al, 2003
Fungal gliotoxin	Degradation	Pahl et al, 1999
Gabexate mesilate	Degradation	Yuksel et al, 2003
Gamisanghyulyunbueum	Degradation	Shin et al, 2005
Genistein (tyrosine kinase inhibitor)	Degradation; caspase cleavage of IkBa	Natarajan et al, 1998; Baxa & Yoshimura, 2003
Genipin	Degradation	Koo et al, 2004
Glabridin	Degradation	Kang et al, 2004
Ginsenoside Re	Degradation	Zhang et al, 2007
Glimepiride	Degradation	Schiekofer et al, 2003
Glucosamine (sulfate or carboxybutyrylated)	Degradation	Largo et al, 2003; Rafi et al, 2007; Rajapakse et al, 2008
gamma-glutamylcysteine synthetase	Degradation	Manna et al, 1999
Glutamine	Degradation	Singleton et al, 2005; Fillmann et al, 2007; Chen et al, 2008
Glycochenodeoxycholate	Degradation	Bucher et al, 2006
Guave leaf extract	Degradation	Choi et al, 2008
Gumiganghwaltang	Degradation	Kim et al, 2005
Gum mastic	Degradation	He et al, 2007
Heat shock protein-70	Degradation	Chan et al, 2004; Shi et al, 2006
Herbal mixture (Cinnamomi ramulus, Anemarrheriae rhizoma, Officinari rhizoma)	Degradation	Jeong et al, 2008
Hypochlorite	Degradation	Mohri et al, 2002
Ibudilast	Degradation	Kiebala & Maggirwar, 1998
IL-13	Degradation	Manna & Aggarwal, 1998
Incensole acetate	Degradation	Moussaieff et al, 2007
Intravenous immunoglobulin	Degradation	Ichiyama et al, 2004
Isomallotochromanol and isomallotochromene	Degradation	Ishii et al, 2003
K1L (Vaccinia virus protein)	Degradation	Shisler & Jin, 2004
Kochia scoparia fruit (methanol extract)	Degradation	Shin et al, 2004
Kummerowia striata (Thunb.) Schindl (ethanol extract)	Degradation	Tao et al, 2008
Leflunomide metabolite (A77 1726)	Degradation	Manna & Aggarwal, 1999
Lidocaine	Degradation	Feng et al, 2007; Lahat et al, 2008
Lipoxin A4	Degradation	Zhang et al, 2007
Losartan	Degradation/NF-kB expression	Chen et al, 2002; Zhu et al, 2007
Low level laser therapy	Degradation	Rizzi et al, 2006
LY294002 (PI3-kinase inhibitor) [2-(4-morpholinyl)-8-phenylchromone]	Degradation	Park et al 2002
MC159 (Molluscum contagiosum virus)	Degradation of IkBb	Murao & Shisler, 2005
Melatonin	Degradation	Zhang et al, 2004
Meloxicam	Degradation	Liu et al, 2007
5'-methylthioadenosine	Degradation	Hevia et al, 2004
Midazolam	Degradation	Kim et al, 2006
Momordin I	Degradation	Hwang et al, 2005
Morinda officinalis extract	Degradation	Kim et al, 2005
Mosla dianthera extract	Degradation	Lee et al, 2006
Mume fructus extract	Degradation	Choi et al, 2007

TABLE G-continued

Examples of NFKB Inhibitors Any one or more of the following NFKB inhibitors		
Murrl gene product	Degradation	Ganesh et al, 2003
Neurofibromatosis-2 (NF-2; merlin) protein	Degradation	Kim et al, 2002
Opuntia ficus indica va saboten extract	Degradation	Lee et al, 2006
Ozone (aqueous)	Degradation	Huth et al, 2007
Paeony total glucosides	Degradation	Chen et al, 2007
Pectenotoxin-2	Degradation	Kim et al, 2008
Penetratin	Degradation	Letoya et al, 2006
Pervanadate (tyrosine phosphatase inhibitor)	Degradation	Singh & Aggarwal, 1995; Singh et al, 1996
Phenylarsine oxide (PAO, tyrosine phosphatase inhibitor)	Degradation	Mahboubi et al, 1998; Singh & Aggarwal, 1995
beta-Phenylethyl (PEITC) and 8-methylsulphinyloctyl isothiocyanates (MSO) (watercress)	Degradation	Rose et al, 2005
Phenytoin	Degradation	Kato et al, 2005
c-phycocyanin	Degradation	Cherng et al, 2007
Platycodin saponins	Degradation	Aim et al, 2005; Lee et al, 2008
Polymeric formula	Degradation	de Jong et al, 2007
Polymyxin B	Degradation	Jiang et al, 2006
Poncirus trifoliata fruit extract	Degradation; phosphorylation of IkBa	Shin et al, 2006; Kim et al 2007
Probiotics	Degradation	Petrof et al, 2004
Pituitary adenylate cyclase-activating polypeptide (PACAP)	Degradation	Delgado & Ganea, 2001
Prostaglandin 15-deoxy-Delta(12,14)-PGJ(2)	Degradation	Cuzzocrea et al, 2003; Chatterjee et al, 2004
Prodigiosin (Hahella chejuensis)	Degradation	Huh et al, 2007
PS-341	Degradation/proteasome	Hideshima et al, 2002
Radix asari extract	Degradation	Song et al, 2007
Radix clematidis extract	Degradation	Lee et al, 2009
Resiniferatoxin	Degradation	Singh et al, 1996
Sabaeksan	Degradation	Choi et al, 2005
SAIF (Saccharomyces boulardii anti-inflammatory factor)	Degradation	Sougioultzis et al 2006
Sanguis Draconis	Degradation	Choy et al, 2007
San-Huang-Xie-Xin-Tang	Degradation	Shih et al, 2007
Schisandra fructus extract	Degradation	Kang et al, 2006; Guo et al, 2008
Scutellarin	Degradation	Tan et al, 2007
Sesquiterpene lactones (parthenolide; ergolide; guaianolides; alpha-humulene; trans-caryophyllene)	Degradation	Hehner et al, 1998; Whan Han et al, 2001; Schorr et al, 2002; Medeiros et al, 2007
Sevoflurane/isoflurane	Degradation	Boost et al, 2009
Siegeskaurolic acid (from Siegesbeckia pubescens root)	Degradation	Park et al, 2007
ST2 (IL-1-like receptor secreted form)	Degradation	Takezako et al, 2006
Synadenium carinatum latex lectin	Degradation	Rogerio et al, 2007
Taiwanofungus camphoratus	Degradation	Liu et al, 2007
Taurene bromamine	Degradation	Tokunaga et al, 2007
Thiopental	Degradation	Loop et al, 2002
Tipifarnib	Degradation	Xue et al, 2005
Titanium	Degradation	Yang et al, 2003
TNP-470 (angiogenesis inhibitor)	Degradation	Mauriz et al, 2003
Stinging nettle (Urtica dioica) plant extracts	Degradation	Riehemann et al, 1999
Trichomomas vaginalis infection	Degradation	Chang et al, 2004
Triglyceride-rich lipoproteins	Degradation	Kumwenda et al, 2002
Tussilagone (Farfarae fins)	Degradation	Lim et al, 2008
U0126 (MEK inhibitor)	Degradation	Takaya et al, 2003
Ursodeoxycholic acid	Degradation	Joo et al, 2004
Xanthium strumarium L. (methanol extract)	Degradation	Kim et al, 2005; Yoon et al, 2008
Yulda-Hanso-Tang	Degradation	Jeong et al, 2007
Zinc	Degradation	Uzzo et al, 2006; Bao et al, 2006
Molluscum contagiosum virus MC159 protein	IkBbeta degradation	Murao & Shisler, 2005
Vasoactive intestinal peptide	Degradation (and CBP-RelA interaction)	Delgado & Ganea, 2001; Delgado, 2002
HIV-1 Vpu protein	TrCP ubiquitin ligase inhibitor Bour et al, 2001	
Epoxyquinone A monomer	IKKb/DNA binding	Liang et al, 2006
Ro106-9920 (small molecule)	IkBa ubiqutination inhibitor Swinney et al, 2002	
Furonaphthoquinone	IKK activity	Shin et al, 2006
β-TrCP	Degradation	Kanarek et al, 2012
Inhibitors from IMGENEX: NF-kB Pathway Inhibitory Peptides		
Cat.No	Description	Species
IMG-2009-2	Antennapedia Control Peptide	N/A
IMG-2009-5	Antennapedia Control Peptide	N/A
IMG-2000	IKKg NEMO Binding Domain (NBD) Inhibitory Peptide Set Functions as an IKKa/IKKb decoy by binding to IKKg NBD, thereby preventing formation of the IKK complex.	H, M, R

TABLE G-continued

Examples of NFKB Inhibitors Any one or more of the following NFKB inhibitors		
IMG-2000-5	IKKg NEMO Binding Domain (NBD) Inhibitory Peptide Set Functions as an IKKa/IKKb decoy by binding to IKKg NBD, thereby preventing formation of the IKK complex.	H, M, R
IMG-2005-5	MyD88 Homodimerization Inhibitory Peptide Set Functions as a decoy by binding to the MyD88 TIR domain	H, M, R, X, Z
IMG-2005-1	MyD88 Homodimerization Inhibitory Peptide Set Functions as a decoy by binding to the MyD88 TIR domain	H, M, R, X, Z
IMG-2004	NF-kB p50 (NLS) Inhibitory Peptide Set Functions as a p50 decoy by blocking the intracellular recognition mechanism of p50 NLS.	B, C, C, D, H, M, R, X
IMG-2004-5	NF-kB p50 (NLS) Inhibitory Peptide Set Functions as a p50 decoy by blocking the intracellular recognition mechanism of p50 NLS.	B, C, C, D, H, M, R, X
IMG-2001	NF-kB p65 (Ser276) Inhibitory Peptide Set Functions as a p65 decoy through phosphorylation of the Ser276 site on the peptide.	C, D, H, M, M, R
IMG-2001-5	NF-kB p65 (Ser276) Inhibitory Peptide Set Functions as a p65 decoy through phosphorylation of the Ser276 site on the peptide.	C, D, H, M, M, R
IMG-2003	NF-kB p65 (Ser529/536) Inhibitory Peptide Set Functions as a p65 decoy through phosphorylation of the Ser529/536 sites on the peptide.	C, D, H, M, M, R
IMG-2003-5	NF-kB p65 (Ser529/536) Inhibitory Peptide Set Functions as a p65 decoy through phosphorylation of the Ser529/536 sites on the peptide.	C, D, H, M, M, R
IMG-2006-5	TIRAP Inhibitory Peptide Set Functions as a TIRAP decoy by binding to TIR interacting domains on specific TLR receptors.	H, M
IMG-2006-1	TIRAP Inhibitory Peptide Set Functions as a TIRAP decoy by binding to TIR interacting domains on specific TLR receptors.	H, M
IMG-2011 set	TLR4 Peptide Inhibitor Set: VIPER	H, M
IMG-2002	TRAF6 Inhibitory Peptide Set Functions as a TRAF6 decoy by binding to the T6DP motif of RANK, thereby preventing binding of RANK to TRAF6.	H, M, R
IMG-2002-5	TRAF6 Inhibitory Peptide Set Functions as a TRAF6 decoy by binding to the T6DP motif of RANK, thereby preventing binding of. RANK to TRAF6	H, M, R
NF kappa B Inhibitors		

9-Methylstreptimidone
Z-VRPR-FMK
2-(1,8-naphthyridin-2-y1)-Phenol
5-Aminosalicylic acid
BAY 11-7082
BAY 11-7085
Caffeic acid phenethyl ester
Diethylmaleate
Ethyl 3,4-Dihydroxycinnamate
Helenalin
NFicB Activation Inhibitor II, JSH-23
NFicB Activation Inhibitor III
PPM-18
Pyrrolidinedithiocarbamic acid ammonium salt
(R)-MG-132
Rocaglamide
Sodium Salicylate

The presently-disclosed subject matter further includes compositions useful for inhibiting NFκB. Such compositions include an inhibitor. As noted above, such inhibitors can be, for example, a nucleotide, a polypeptide, a small (chemical) molecule, etc. In some embodiments, a composition can include an isolated RNA molecule.

The presently-disclosed subject matter further includes methods of screening candidate inhibitors to identify NFκB inhibitors. In some embodiments, cell or cell line-based methods are used.

Imaging Caspase in an Eye of a Subject

In some embodiments, a diagnostic composition is provided for imaging activated Caspase in an eye of a subject, comprising a fluorescent molecule conjugated to a substrate of Caspase-1 or a molecule that fluoresces following cleavage by Caspase-1. In some embodiments, a method is provided for imaging activated Caspase-1 in an eye of a subject, including administering (e.g., intraocularly or intravenously) to RPE cells of the subject the diagnostic composition, and optically monitoring the spatial clustering of fluorescence.

The details of one or more embodiments of the presently-disclosed subject matter are set forth in this document. Modifications to embodiments described in this document, and other embodiments, will be evident to those of ordinary skill in the art after a study of the information provided in this document. The information provided in this document, and particularly the specific details of the described exemplary embodiments, is provided primarily for clearness of understanding and no unnecessary limitations are to be understood therefrom. In case of conflict, the specification of this document, including definitions, will control.

Some of the polynucleotide and polypeptide sequences disclosed herein are cross-referenced to GENBANK®/GENPEPT® accession numbers. The sequences cross-referenced in the GENBANK®/GENPEPT® database are expressly incorporated by reference as are equivalent and related sequences present in GENBANK®/GENPEPT® or other public databases. Also expressly incorporated herein by reference are all annotations present in the GENBANK®/GENPEPT® database associated with the sequences disclosed herein. Unless otherwise indicated or apparent, the references to the GENBANK®/GENPEPT® database are references to the most recent version of the database as of the filing date of this Application.

While the terms used herein are believed to be well understood by one of ordinary skill in the art, definitions are set forth to facilitate explanation of the presently-disclosed subject matter.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the presently-disclosed subject matter belongs. Although any methods, devices, and materials similar or equivalent to those described herein can be used in the practice or testing of the presently-disclosed subject matter, representative methods, devices, and materials are now described.

Following long-standing patent law convention, the terms "a", "an", and "the" refer to "one or more" when used in this application, including the claims. Thus, for example, reference to "a cell" includes a plurality of such cells, and so forth.

Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term "about". Accordingly, unless indicated to the contrary, the numerical parameters set forth in this specification and claims are approximations that can vary depending upon the desired properties sought to be obtained by the presently-disclosed subject matter.

As used herein, the term "about," when referring to a value or to an amount of mass, weight, time, volume, concentration or percentage is meant to encompass variations of in some embodiments ±20%, in some embodiments ±10%, in some embodiments ±5%, in some embodiments ±1%, in some embodiments ±0.5%, and in some embodiments ±0.1% from the specified amount, as such variations are appropriate to perform the disclosed method.

As used herein, ranges can be expressed as from "about" one particular value, and/or to "about" another particular value. It is also understood that there are a number of values disclosed herein, and that each value is also herein disclosed as "about" that particular value in addition to the value itself. For example, if the value "10" is disclosed, then "about 10" is also disclosed. It is also understood that each unit between two particular units are also disclosed. For example, if 10 and 15 are disclosed, then 11, 12, 13, and 14 are also disclosed.

The presently-disclosed subject matter is further illustrated by the following specific but non-limiting examples. The following examples may include compilations of data that are representative of data gathered at various times during the course of development and experimentation related to the present invention.

EXAMPLES

Example 1

Alu RNA accumulation due to DICER1 deficiency in the retinal pigmented epithelium (RPE) is implicated in geographic atrophy (GA), an advanced form of age-related macular degeneration that causes blindness in millions of individuals. The mechanism of Alu RNA-induced cytotoxicity is unknown. Here it is shown that DICER1 deficit or Alu RNA exposure activates the NLRP3 inflammasome and triggers TLR-independent MyD88 signaling via IL-18 in the RPE. Genetic or pharmacological inhibition of inflammasome components (NLRP3, Pycard, Caspase-1), MyD88, or IL-18 prevents RPE degeneration induced by DICER1 loss or Alu RNA exposure. These findings, coupled with the observation that human GA RPE contains elevated amounts of NLRP3, PYCARD and IL-18, and evidence of increased Caspase-1 and MyD88 activation, provide a rationale for targeting this pathway in GA. The findings also reveal a novel function of the inflammasome outside the immune system and a surprising immunomodulatory action of mobile elements.

Age-related macular degeneration (AMD) affects the vision of millions of individuals (Smith et al., 2001). AMD is characterized by degeneration of the retinal pigmented epithelium (RPE), which is situated between the retinal photoreceptors and the choroidal capillaries (Ambati et al., 2003). RPE dysfunction disrupts both photoreceptors and choroidal vasculature (Blaauwgeers et al., 1999; Lopez et al., 1996; McLeod et al., 2009; Vogt et al., 2011). These tissue disruptions lead to atrophic or neovascular disease phenotypes. Although there are therapies for neovascular AMD, there is no effective treatment for the more common atrophic form. GA, the advanced stage of atrophic AMD, is characterized by degeneration of the RPE, and is the leading cause of untreatable vision loss.

Recently it was shown that a dramatic and specific reduction of the RNase DICER1 leads to accumulation of Alu RNA transcripts in the RPE of human eyes with GA (Kaneko et al., 2011). These repetitive element transcripts, which are non-coding RNAs expressed by the highly abundant Alu retrotransposon (Batzer and Deininger, 2002), induce human RPE cell death and RPE degeneration in mice. DICER1 deficit in GA RPE was not a generic cell death response because DICER1 expression was not dysregulated in other retinal diseases. Likewise, Alu RNA accumulation did not represent generalized retrotransposon activation due to a stress response in dying cells because other retrotransposons were not elevated in GA RPE.

DICER1 is central to mature microRNA biogenesis (Bernstein et al., 2001). Yet following DICER1 deficit, the accumulation of Alu RNA and not the lack of mature microRNAs was the critical determinant of RPE cell viability (Kaneko et al., 2011). Moreover, 7SL RNA, transfer RNA, and primary microRNAs do not induce RPE degeneration (Kaneko et al., 2011), ruling out a nonspecific toxicity of excess, highly structured RNA. Still, the precise mechanisms of Alu RNA cytotoxicity are unknown.

Although the retina is exceptional for its immune privilege (Streilein, 2003), insults mediated by innate immune sensors can result in profound inflammation. The three major classes of innate immune receptors include the TLRs, RIG-1-like helicases, and NLR proteins (Akira et al., 2006). Numerous innate immune receptors are expressed in the RPE (Kumar et al., 2004), and several exogenous substances can induce retinal inflammation (Allensworth et al., 2011; Kleinman et al., 2012). However, it is not known whether this surveillance machinery recognizes or responds to host endogenous RNAs. The concept was explored that innate immune machinery, whose canonical function is the detection of pathogen associated molecular patterns and other moieties from foreign organisms, might also recognize Alu RNA.

Indeed, it was shown that Alu transcripts can hijack innate immunity machinery to induce RPE cell death. Surprisingly, the data show that DICER1 deficit or Alu RNA activates the NLRP3 inflammasome in a MyD88-dependent, but TLR-independent manner. NLRP3 inflammasome activation in vivo has been largely restricted to immune cells, although the data open the possibility that NLRP3 activity may be more widespread, as reflected by examples in cell culture studies of keratinocytes (Feldmeyer et al., 2007; Keller et al., 2008). The data also broaden the scope of DICER1 function beyond microRNA biogenesis, and identify it as a guardian against aberrant accumulation of toxic retrotransposon elements that comprise roughly 50% of the human genome (Lander et al., 2001). In sum, the findings present a novel self-recognition immune response, whereby endogenous non-coding RNA-induced NLRP3 inflammasome activation results from DICER1 deficiency in a non-immune cell.

Results

Alu RNA does not Activate a Variety of TLRs or RNA Sensors

Alu RNA has single-stranded (ss) RNA and double-stranded (ds) RNA motifs (Sinnett et al., 1991). Thus it was tested whether Alu RNA induced RPE degeneration in mice deficient in toll-like receptor-3 (TLR3), a dsRNA sensor (Alexopoulou et al., 2001), or TLR7, a ssRNA sensor (Diebold et al., 2004; Heil et al., 2004). Subretinal delivery of a plasmid coding for Alu RNA (pAlu) induced RPE degeneration in $Tlr3^{-/-}$ and $Tlr7^{-/-}$ mice just as in wild-type (WT) mice (FIGS. **1**A-C). It was previously shown that ≥21-nucleotide fully complementary siRNAs activate TLR3 on RPE cells (Kleinman et al., 2011). Lack of TLR3 activation by Alu RNA is likely due to its complex structure containing multiple hairpins and bulges that might preclude TLR3 binding. Neither 7SL RNA, the evolutionary precursor of Alu RNA, nor p7SL induced RPE degeneration in WT mice (FIGS. **8**A and **8**B), suggesting that Alu RNA cytotoxicity might be due to as yet unclear structural features. pAlu induced RPE degeneration in Unc93b1 mice (FIG. **1**D), which lack TLR3, TLR7, and TLR9 signaling (Tabeta et al., 2006), indicating that these nucleic acid sensors are not activated by Alu RNA redundantly. pAlu induced RPE degeneration in $Tlr4^{-/-}$ mice (FIG. **1**E), and the TLR4 antagonist *Rhodobacter sphaeroides* LPS (Qureshi et al., 1991) did not inhibit pAlu-induced RPE degeneration in WT mice (FIG. **8**C). Thus the observed RPE cell death is not due to lipopolysaccharide contamination. Further, two different in vitro transcribed Alu RNAs (Kaneko et al., 2011) did not activate multiple TLRs (FIG. **1**F).

Next it was tested whether other dsRNA sensors such as MDA5 (Kato et al., 2006) or PKR (encoded by Prkr, (Yang et al., 1995)) might mediate Alu RNA toxicity. However, pAlu induced RPE degeneration in $Mda5^{-/-}$ and $Prkr^{-/-}$ mice (FIGS. **8**D and **8**E). It was tested whether the 5'-triphosphate on in vitro transcribed Alu RNA, which could activate RIG-I or IFIT-1 that sense this moiety (Hornung et al., 2006; Pichlmair et al., 2011), was responsible for RPE degeneration. Dephosphorylated Alu RNA induced RPE degeneration in WT mice just as well as Alu RNA not subjected to dephosphorylation (FIG. **8**F), indicating that this chemical group is not responsible for the observed cell death. Indeed a 5'-triphosphate ssRNA that activates RIG-I does not induce RPE degeneration in mice (Kleinman et al., 2011). Further, pAlu induced RPE degeneration in mice deficient in MAVS (FIG. **8**G), through which RIG-I and MDA-5 signal (Kumar et al., 2006; Sun et al., 2006). Collectively these data pointed to a novel mechanism of Alu RNA-induced RPE degeneration not mediated by a wide range of canonical RNA sensors.

Alu RNA Cytotoxicity is Mediated Via MyD88 and IL-18

The involvement of TRIF (encoded by Ticam1), an adaptor for TLR3 and TLR4 (Hoebe et al., 2003; Yamamoto et al., 2003), and MyD88, an adaptor for all TLRs except TLR3 (Akira et al., 2006; Alexopoulou et al., 2001; Suzuki et al., 2003) were then tested. Alu RNA induced RPE degeneration in $Ticam1^{-/-}$ mice (FIG. **9**A), consistent with findings in $Tlr3^{-/-}$ and $Tlr4^{-/-}$ mice. Unexpectedly, neither Alu RNA nor two different pAlu plasmids induced RPE degeneration in $Myd88^{-/-}$ mice (FIGS. **2**A, **9**B, and **9**C). Intravitreous delivery of a peptide inhibitor of MyD88 homodimerization (Loiarro et al., 2005) prevented RPE degeneration induced by Alu RNA in WT mice, whereas a control peptide did not do so (FIG. **2**B). A MyD88-targeting short interfering RNA (siRNA), which was shorter than 21 nucleotides in length to prevent TLR3 activation and conjugated to cholesterol to enable cell permeation (Kleinman et al., 2008), but not a control siRNA, inhibited RPE degeneration induced by pAlu in WT mice (FIGS. **2**C-**2**E). $Myd88^{+/-}$ heterozygous mice were protected against Alu RNA-induced RPE degeneration (FIGS. **2**F and **9**D), corroborating the siRNA studies that partial knockdown of MyD88 is therapeutically sufficient.

MyD88-mediated signal transduction induced by interleukins leads to recruitment and phosphorylation of IRAK1 and IRAK4 (Cao et al., 1996; Kanakaraj et al., 1999; Suzuki et al., 2003; Suzuki et al., 2002). Alu RNA increased IRAK1/4 phosphorylation in human RPE cells (FIG. **2**G), supporting the concept that Alu RNA triggers MyD88 sig-

naling. The MyD88 inhibitory peptide reduced Alu RNA-induced IRAK1/4 phosphorylation in human RPE cells (FIG. **9**E), confirming its mode of action.

Next it was assessed whether MyD88 activation mediates Alu RNA-induced cell death in human and mouse RPE cell culture systems. Consonant with the in vivo data, pAlu reduced cell viability in WT but not Myd88$^{-/-}$ mouse RPE cells (FIG. **2**H). The MyD88-inhibitory peptide, but not a control peptide, inhibited cell death in human RPE cells transfected with pAlu (FIG. **2**I). Together, these data indicate that MyD88 is a critical mediator of Alu RNA-induced RPE degeneration.

MyD88 is generally considered an adaptor of immune cells (O'Neill and Bowie, 2007). However, Alu RNA induced cell death via MyD88 in RPE monoculture. Thus, it was tested whether Alu RNA-induced RPE degeneration in mice was also dependent solely on MyD88 activation in RPE cells. Conditional ablation of MyD88 in the RPE by subretinal injection of AAV1-BEST1-Cre in Myd88$^{f/f}$ mice protected against Alu RNA-induced RPE degeneration (FIGS. **2**J and **9**F). Consistent with this finding, Alu RNA induced RPE degeneration in WT mice receiving Myd88$^{-/-}$ bone marrow but did not do so in Myd88$^{-/-}$ mice receiving WT bone marrow (FIG. **9**G). Collectively, these results indicate that MyD88 expression in the RPE, and not in circulating immune cells, is critical for Alu RNA-induced RPE degeneration. These findings comport with histopathological studies of human GA tissue that show no infiltration of immune cells in the area of pathology (personal communication, C. A. Curcio, H. E. Grossniklaus, G. S. Hageman, L. V. Johnson).

Although MyD88 is critical in TLR signaling (O'Neill and Bowie, 2007), MyD88 activation by Alu RNA was independent of TLR activation. Thus, other mechanisms of MyD88 involvement were examined. MyD88 can regulate IFN-γ signaling by interacting with IFN-γ receptor 1 (encoded by Ifngr1) (Sun and Ding, 2006). However, pAlu induced RPE degeneration in both Ifng$^{-/-}$ and Ifngr1$^{-/-}$ mice (FIGS. **9**H and **9**I). MyD88 is also essential in interleukin-1 signaling (Muzio et al., 1997). Thus, it was tested whether IL-1β and the related cytokine IL-18, both of which activate MyD88 (Adachi et al., 1998), mediated Alu RNA cytotoxicity. Interestingly, whereas Alu RNA overexpression in human RPE cells increased IL-18 secretion, IL-1β secretion was barely detectable (FIG. **2**K).

Recombinant IL-18 induced RPE degeneration in WT but not Myd88$^{-/-}$ mice (FIG. **2**L). IL-18 neutralization protected against pAlu-induced RPE degeneration in WT mice, but IL-1β did not (FIGS. **2**M and **2**N). Also, pAlu induced RPE degeneration in Il1r1$^{-/-}$ mice but not Il18r1$^{-/-}$ mice (FIGS. **9**J and **9**K). These data indicate that IL-18 is an effector of Alu RNA-induced cytotoxicity.

Alu RNA Activates the NLRP3 Inflammasome

It was explored whether Caspase-1 (encoded by Casp1), a protease that induces maturation of interleukins into biologically active forms (Ghayur et al., 1997; Gu et al., 1997; Thornberry et al., 1992), was involved in Alu RNA-induced RPE degeneration. Alu RNA treatment of human RPE cells led to Caspase-1 activation as measured by western blotting and by a fluorescent reporter of substrate cleavage (FIGS. **3**A and **10**A). Indeed, Alu RNA induced Caspase-1 activation in other cell types such as HeLa and THP-1 monocytic cells (FIG. **10**B), suggesting that Alu RNA cytotoxicity has potentially broad implications in many systems. Intravitreous delivery of the Caspase-1-inhibitory peptide Z-WEHD-FMK, but not a control peptide Z-FA-FMK, blocked IL-18 maturation and pAlu-induced RPE degeneration in WT mice (FIGS. **3**B and **3**C). The Caspase-1-inhibitory peptide blocked Alu RNA-induced substrate cleavage in human RPE cells (FIG. **10**C), confirming its mode of action. Similarly, Casp1$^{-/-}$ mice treated with Alu RNA or pAlu did not exhibit RPE degeneration (FIGS. **3**D and **10**D). Also, pAlu did not induce cell death in Casp1$^{-/-}$ mouse RPE cells (FIG. **3**E).

Caspase-1 can be activated within a multiprotein innate immune complex termed the inflammasome (Tschopp et al., 2003). The best-characterized inflammasome pathway is one that is activated by binding of NLRP3 to the caspase-1 adaptor ASC (encoded by PYCARD). One hallmark of inflammasome assembly is spatial clustering of PYCARD (Fernandes-Alnemri et al., 2007). In human RPE cells transfected with fluorescent tagged PYCARD (GFP-PYCARD), Alu RNA induced the appearance of a brightly fluorescent cytoplasmic cluster similar to treatment with LPS and ATP, which activates the NLRP3 inflammasome (FIGS. **3**F and **10**E) (Mariathasan et al., 2006).

Next the functional relevance of NLRP3 and PYCARD to Alu RNA cytotoxicity was tested. Neither pAlu nor Alu RNA induced RPE degeneration in either Nlrp3$^{-/-}$ or Pycard$^{-/-}$ mice (FIGS. **3**G, **3**H, **10**F and **10**G), demonstrating the critical importance of the inflammasome in Alu RNA cytotoxicity. Also, pAlu did not induce cell death in Nlrp3$^{-/-}$ or Pycard$^{-/-}$ mouse RPE cells (FIG. **31**). Moreover, knockdown of NLRP3 or PYCARD by siRNAs rescued pAlu-induced human RPE cell death (FIGS. **3**J and **10**H). These findings provide direct evidence that NLRP3 activation in response to Alu RNA occurs in RPE cells and does not require the presence of other immune cells.

It was determined that IL-18 and MyD88 activation indeed were downstream of Caspase-1 activation by showing (1) that whereas MyD88 inhibition reduced Alu RNA-induced IRAK1/4 phosphorylation in human RPE cells (FIG. **9**E), it did not reduce Alu RNA-induced Caspase-1 cleavage or fluorescent substrate cleavage (FIGS. **10**I and **10**J); (2) that IL-18 neutralization did not inhibit Alu RNA-induced Caspase-1 cleavage (FIG. **10**K); and (3) that Caspase-1 inhibition reduced Alu RNA-induced phosphorylation of IRAK1/4 (FIG. **10**L).

Alu RNA Induces Mitochondrial ROS and NLRP3 Priming

NLRP3 inflammasome function requires two signals, the first of which is termed priming. pAlu induced inflammasome priming as it upregulated both NLRP3 and IL18 mRNAs. This priming occurred equivalently in both WT and Myd88$^{-/-}$ mouse RPE cells (FIG. **4**A), further corroborating that MyD88 functions downstream of NLRP3 in this system. Akin to other inflammasome agonists that do not directly interact with NLRP3 (Tschopp and Schroder, 2010), a physical interaction between Alu RNA and NLRP3 was not observed (FIG. **11**A). To determine how Alu RNA primed the inflammasome, it was studied whether it induced reactive oxygen species (ROS) production, a signal for priming (Bauernfeind et al., 2011; Nakahira et al., 2011). pAlu induced ROS generation in human RPE cells (FIG. **4**B), and the ROS inhibitor diphenyliodonium (DPI) blocked pAlu-induced NLRP3 and IL18 mRNA upregulation and Alu RNA-induced RPE degeneration in WT mice (FIGS. **4**C and **4**D). As DPI blocks mitochondrial ROS and phagosomal ROS (Li and Trush, 1998), it was tested which pathway was triggered because there is controversy surrounding the source of ROS contributing to NLRP3 responses (Latz, 2010).

MitoSOX Red was used, which labels ROS-generating mitochondria, in combination with MitoTracker Deep Red, which labels respiring mitochondria. To monitor phago-

somal ROS generation, Fc OxyBURST Green was used, which measures activation of NADPH oxidase within the phagosome. A marked increase in ROS-generating mitochondria was observed in human RPE cells transfected with pAlu (FIG. **4**E). In contrast, whereas phorbol myristate acetate (PMA) induced phagosomal ROS as expected (Savina et al., 2006), pAlu did not do so (FIG. **4**F). These data are consistent with the findings that NLRP3 responses are impaired by mitochondrial ROS inhibitors (Nakahira et al., 2011; Zhou et al., 2011) but are preserved in cells carrying genetic mutations that impair NADPH-oxidase-dependent ROS production (Meissner et al., 2010; van Bruggen et al., 2010).

Consonant with these reports and the observation that the principal source of cellular ROS is mitochondria (Murphy, 2009), it was found that the mitochondria-targeted antioxidants Mito-TEMPO and MitoQ (Murphy and Smith, 2007; Nakahira et al., 2011) both blocked Alu RNA-induced RPE degeneration in WT mice, whereas dTPP, a structural analog of MitoQ that does not scavenge mitochondrial ROS, did not do so (FIG. **4**G). In contrast, gp91ds-tat, a cell-permeable peptide that inhibits association of two essential NADPH oxidase subunits ($gp91^{phox}$ and $p47^{phox}$) (Rey et al., 2001), did not do so (FIG. **4**H). Corroborating these data, Alu RNA induced RPE degeneration in mice deficient in Cybb (which encodes $gp91^{phox}$) just as in WT mice (FIG. **4**I). Next the voltage-dependent anion channels (VDAC) was studied because VDAC1 and VDAC2, but not VDAC3, are important in mitochondrial ROS produced by NLRP3 activators in macrophages (Zhou et al., 2011). Consistent with these observations, siRNA knockdown of VDAC1 and VDAC2, but not VDAC3, impaired pAlu-induced mitochondrial ROS (FIGS. **4**J and **11**B) and NLRP3 and IL18 mRNA induction in human RPE cells (FIG. **4**K). Collectively, these data implicate mitochondrial ROS in Alu RNA-induced NLRP3 inflammasome-mediated RPE degeneration.

Alu RNA does not Induce RPE Degeneration Via Pyroptosis

Alu RNA activates Caspase-1, which can trigger pyroptosis, a form of cell death characterized by formation of membrane pores and osmotic lysis (Fink and Cookson, 2006). The cytoprotective agent glycine, which attenuates pyroptosis (Fink et al., 2008; Fink and Cookson, 2006; Verhoef et al., 2005), inhibited human RPE cells death induced by LPS+ATP but not by Alu RNA (FIGS. **5**A and **5**B). Pyroptosis requires Caspase-1 but can proceed independent of IL-18 (Miao et al., 2010). Thus, the finding that IL-18 induced RPE degeneration in $Casp1^{-/-}$ mice (FIG. **5**C), coupled with the lack of rescue by glycine, suggests that Alu RNA-induced RPE degeneration does not occur via pyroptosis.

DICER1 Loss Induces Cell Death Via Inflammasome

It was previously demonstrated that the key role of DICER1 in maintaining RPE cell health (Kaneko et al., 2011): DICER1-cleaved Alu RNA did not induce RPE degeneration in vivo; DICER1 overexpression protected against Alu RNA-induced RPE degeneration; and DICER1 loss-induced RPE degeneration was blocked by antagonizing Alu RNA (Kaneko et al., 2011). Also, rescue of DICER1 knockdown-induced RPE degeneration by Alu RNA inhibition was not accompanied by restoration of microRNA deficits (Kaneko et al., 2011). Therefore, it was tested whether DICER1 also prevented NLRP3 inflammasome activation by Alu RNA. Alu RNA-induced Caspase-1 activation in human RPE cells was inhibited by DICER1 overexpression (FIGS. **6**A and **6**B). Conversely, Caspase-1 cleavage induced by DICER1 knockdown in human RPE cells was inhibited by simultaneous antisense knockdown of Alu RNA (FIGS. **12**A and **12**B).

Next the relevance of these pathways was tested in the context of DICER1 loss in vivo. Caspase-1 cleavage was increased in the RPE of BEST1 Cre; $Dicer1^{f/f}$ mice (FIG. **6**C), which lose DICER1 expression in the RPE during development and exhibit RPE degeneration (Kaneko et al., 2011). Subretinal delivery of AAV1-BEST1-Cre in $Dicer1^{f/f}$ mice induced Caspase-1 activation and IL-18 maturation in the RPE (FIG. **6**D). This treatment also induced RPE degeneration, which was blocked by intravitreous delivery of the Caspase-1-inhibitory peptide but not the control peptide (FIG. **6**E). AAV1-BEST1-Cre-induced RPE degeneration in $Dicer1^{f/f}$ mice was also blocked by intravitreous delivery of the MyD88-inhibitory peptide but not a control peptide (FIG. **6**F). In addition, MyD88 inhibition prevented cell death in human RPE cells treated with antisense oligonucleotides targeting DICER1 (FIG. **6**G). DICER1 knockdown in human RPE cells increased IRAK1/4 phosphorylation, providing further evidence of MyD88 activation upon loss of DICER1 (FIG. **6**H). MyD88 inhibition also prevented cell death in $Dicer1^{f/f}$ mouse RPE cells treated with an adenoviral vector coding for Cre recombinase (FIG. **6**I). MyD88 inhibition blocked RPE cell death without restoring the microRNA expression deficits induced by Dicer1 knockdown (FIG. **6**J). These findings demonstrate that DICER1 is an essential endogenous negative regulator of NLRP3 inflammasome activation, and that DICER1 deficiency leads to Alu RNA-mediated, MyD88-dependent, microRNA-independent RPE degeneration.

Inflammasome and MyD88 Activation in Human GA

Next it was tested whether human eyes with GA, which exhibit loss of DICER1 and accumulation of Alu RNA in their RPE (Kaneko et al., 2011), also display evidence of inflammasome activation. The abundance of NLRP3 mRNA in the RPE of human eyes with GA was markedly increased compared to control eyes (FIG. **7**A). IL18 and IL1B mRNA abundance also was increased in GA RPE; however, only the disparity in IL18 levels reached statistical significance (FIG. **7**A) Immunolocalization studies showed that the expression of NLRP3, PYCARD, and Caspase-1 proteins was also increased in GA RPE (FIGS. **7**B-D). Western blot analyses corroborated the increased abundance of NLRP3 and PYCARD in GA RPE, and revealed greatly increased levels of the enzymatically active cleaved Caspase-1 p20 subunit in GA RPE (FIG. **7**E). There was also an increase in the abundance of phosphorylated IRAK1 and IRAK4 in GA RPE, indicative of increased MyD88 signal transduction (FIG. **7**E). Collectively, these data provide evidence of NLRP3 inflammasome and MyD88 activation in situ in human GA, mirroring the functional data in human RPE cell culture and mice in vivo.

Discussion

The data establish a functional role for the subversion of innate immune sensing pathways by Alu RNA in the pathogenesis of GA. Collectively, the findings demonstrate that the NLRP3 inflammasome senses GA-associated Alu RNA danger signals, contributes to RPE degeneration, and potentially vision loss in AMD (FIG. **13**). To date, the function of the NLRP3 inflammasome has been largely restricted to immune cells in vivo. The finding that it plays a critical function in RPE cell survival broadens the cellular scope of this inflammasome and raises the possibility that other non-immune cells could employ this platform.

The NLRP3 inflammasome was originally recognized as a sensor of external danger signals such as microbial toxins (Kanneganti et al., 2006; Mariathasan et al., 2006; Muruve

et al., 2008). Subsequently, endogenous crystals, polypeptides, and lipids were reported to activate it in diseases such as gout, atherogenesis, Alzheimer disease, and Type 2 diabetes (Halle et al., 2008; Masters et al., 2010; Muruve et al., 2008; Wen et al., 2011). To the knowledge, Alu RNA is the first endogenous nucleic acid known to activate this immune platform. The findings expand the diversity of endogenous danger signals in chronic human diseases, and comport with the concept that this inflammasome is a sensor of metabolic danger (Schroder et al., 2010).

Dampening inflammasome activation can be essential to limiting the inflammatory response. Pathogens have evolved many strategies to inhibit inflammasome activation (Martinon et al., 2009). Likewise, host autophagy proteins (Nakahira et al., 2011), Type I interferon (Guarda et al., 2011), and T cell contact with macrophages can inhibit this process (Guarda et al., 2009). The finding that DICER1, through its cleavage of Alu RNA, prevents activation of NLRP3 adds to the repertoire of host inflammasome modulation capabilities and reveals a new facet of how dysregulation of homeostatic anti-inflammatory mechanisms can promote AMD (Ambati et al., 2003; Takeda et al., 2009).

Added to its recently described anti-apoptotic and tumor-related functions, DICER1 emerges as a multifaceted protein. It remains to be determined how this functional versatility is channeled in various states. As DICER1 dysregulation is increasingly recognized in several human diseases, it is reasonable to imagine that Alu RNA might be an inflammasome activating danger signal in those conditions too. It is also interesting that, at least in adult mice and in a variety of mouse and human cells, the microRNA biogenesis function of DICER1 is not critical for cell survival, at least in a MyD88-deficient environment (data not shown).

The data that mitochondrial ROS production is involved in Alu RNA-induced RPE degeneration comport with observations of mitochondrial DNA damage (Lin et al., 2011), downregulation of proteins involved in mitochondrial energy production and trafficking (Nordgaard et al., 2008), and reduction in the number and size of mitochondria (Feher et al., 2006) in the RPE of human eyes with AMD. Jointly, these findings suggest a potential therapeutic benefit to interfering with mitochondrial ROS generation.

Current clinical programs targeting the inflammasome largely focus on IL-1β; presently there are no IL-18 inhibitors in registered clinical trials. However, the data indicate that IL-18 is more important than IL-1β in mediating RPE cell death in GA (similar to selective IL-18 involvement in a colitis model (Zaki et al., 2010)), pointing to the existence of regulatory mechanisms by which inflammasome activation bifurcates at the level of or just preceding the interleukin effectors. Although Caspase-1 inhibition could be an attractive local therapeutic strategy, caspase inhibitors can promote alternative cell death pathways, possibly limiting their utility (Vandenabeele et al., 2006).

MyD88 is best known for transducing TLR signaling initiated by pathogen associated molecular patterns (O'Neill and Bowie, 2007), although recently it has been implicated in human cancers (Ngo et al., 2011; Puente et al., 2011). The findings introduce an unexpected new function for MyD88 in effecting death signals from mobile element transcripts that can lead to retinal degeneration and blindness, and raise the possibility that MyD88 could be a central integrator of signals from other non-NLRP3 inflammasomes that also employ Caspase-1 (Schroder and Tschopp, 2010). Since non-canonical activation of MyD88 is a critical checkpoint in RPE degeneration in GA (FIG. **13**), it represents an enticing therapeutic target. A potential concern is its important anti-microbial function in mice (O'Neill and Bowie, 2007). However, in contrast to Myd88$^{-/-}$ mice, adult humans with MyD88 deficiency are described to be generally healthy and resistant to a wide variety of microbial pathogens (von Bernuth et al., 2008). MyD88-deficient humans have a narrow susceptibility range to pyogenic bacterial infections, and that too only in early childhood and not adult life (Picard et al., 2010). Moreover, as evident from the siRNA and Myd88$^{+/-}$ studies, partial inhibition of MyD88 is sufficient to protect against Alu RNA. Localized intraocular therapy, the current standard of care in most retinal diseases, would further limit the likelihood of adverse infectious outcomes. It is reasonable to foresee development of MyD88 inhibitors for prevention or treatment of GA.

Experimental Procedures

Subretinal injection and imaging. Subretinal injections (1 μL) were performed using a Pico-Injector (PLI-100, Harvard Apparatus). Plasmids were transfected in vivo using 10% Neuroporter (Genlantis). Fundus imaging was performed on a TRC-50 IX camera (Topcon) linked to a digital imaging system (Sony). RPE flat mounts were immunolabeled using antibodies against zonula occludens-1 (Invitrogen).

mRNA Abundance.

Transcript abundance was quantified by real-time RT-PCR using an Applied Biosystems 7900 HT Fast Real-Time PCR system by the $2^{-\Delta\Delta Ct}$ method.

Protein Abundance and Activity.

Protein abundance was assessed by Western blot analysis using antibodies against Caspase-1 (1:500; Invitrogen), pIRAK1 (1:500; Thermo Scientific), pIRAK4 (1:500, Abbomax), PYCARD (1:200, Santa Cruz Biotechnology), NLRP3 (1:500, Enzo Life Sciences) and Vinculin (1:1,000; Sigma-Aldrich). Caspase-1 activity was visualized using Caspalux1 E1D2 (Oncolmmunin) according to manufacturer's instructions.

Mice.

All animal experiments were approved by institutional review committees and in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Visual Research. Wild-type C57BL/6J, Cybb$^{-/-}$, Tlr3^{-1-}, Tlr4$^{-/-}$ (C57BL/10ScNJ), Trif$^{-/-}$ (Ticam1^{Lps2}), Ifng$^{-/-}$, Ifngr1$^{-/-}$, Il1r1^{-1-}, Il18r1^{-1-}, Myd88$^{f/f}$, and Dicer1$^{f/f}$ mice were purchased from The Jackson Laboratory. Casp1^{-1-}, Nbp3$^{-/-}$, and Pycard$^{-/-}$ mice have been previously described (Kanneganti et al., 2006). Unc93b1 mutant mice were generously provided by B. A. Beutler via K. Fitzgerald. Myd88$^{-/-}$ and Tlr7$^{-/-}$ mice were generously provided by S. Akira via T. Hawn and D. T. Golenbock. Mda5$^{-/-}$ mice were generously provided by M. Colonna. mice were generously provided by B. R. Williams and R. L. Silverman. Mavs$^{-/-}$ mice were generously provided by Z. Chen via K. Fitzgerald. 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 (Alcon Laboratories).

Fundus Photography.

Retinal photographs of dilated mouse eyes were taken with a TRC-50 IX camera (Topcon) linked to a digital imaging system (Sony).

Human Tissue.

Donor eyes or ocular tissues from patients with geographic atrophy due to 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. The study followed the guidelines of the Declaration of Helsinki. Institutional review boards granted approval for allocation and histological analysis of specimens.

Immunolabeling.

Human eyes fixed in 2-4% paraformaldehyde were prepared as eyecups, cryoprotected in 30% sucrose, embedded in optimal cutting temperature compound (Tissue-Tek OCT; Sakura Finetek), and cryosectioned into 10 μm sections. Depigmentation was achieved using 0.25% potassium permanganate and 0.1% oxalic acid. Immunohistochemical staining was performed with the rabbit antibody against NLRP3 (1:100, Sigma Aldrich) or rabbit antibody against Caspase-1 (prediluted, AbCam). Isotype IgG was substituted for the primary antibody to assess the specificity of the staining. Bound antibody was detected with biotin-conjugated secondary antibodies, followed by incubation with ABC reagent and visualized by Vector Blue (Vector Laboratories). Levamisole (Vector Laboratories) was used to block endogenous alkaline phosphatase activity. Slides were washed in PBS, counterstained with neutral red (Fisher Scientific), rinsed with deionized water, air dried, and then mounted in Vectamount (Vector Laboratories). Fluorescent labeling of human tissue was performed with the rabbit antibody against PYCARD (1:50, Clone N-15, Santa Cruz Biotechnology) Immunolabeling was visualized by fluorescently conjugated anti-rabbit secondary antibody (Invitrogen). Tissue autofluorescence was quenched by incubating the sections in 0.3% Sudan black (Fisher Scientific). Sections were mounted in Vectashield with DAPI (Vector Laboratories). Mouse RPE/choroid flat mounts were fixed with 4% paraformaldehyde or 100% methanol, stained with rabbit antibodies against human zonula occludens-1 (1:100, Invitrogen) and visualized with Alexa594 (Invitrogen). All images were obtained using the Leica SP-5 or Zeiss Axio Observer Z1 microscopes.

Subretinal Injection.

Subretinal injections (1 μL) in mice were performed using a Pico-Injector (PLI-100, Harvard Apparatus). In vivo transfection of plasmids coding for two different Alu sequences (pAlu) or empty control vector (pNull) (Bennett et al., 2008; Kaneko et al., 2011; Shaikh et al., 1997) was achieved using 10% Neuroporter (Genlantis). AAV1-BEST1-Cre (Alexander and Hauswirth, 2008) or AAV1-BEST1-GFP were injected at 1.0×10^{11} pfu/mL and in vitro transcribed Alu RNA was injected at 0.3 mg/mL.

Drug Treatments.

siRNAs formulated in siRNA buffer (20 mM KCL, 0.2 mM MgCl2 in HEPES buffer at pH 7.5; Dharmacon) or phosphate buffered saline (PBS; Sigma-Aldrich); the TLR4 antagonist Ultra Pure *Rhodobacter sphaeroides* LPS (LPS-RS, InvivoGen), a peptide inhibitor of MyD88 homodimerization IMG-2005 (IMGENEX), control inhibitor (IMGENEX), recombinant IL-18 (Medical & Biological Laboratories), neutralizing rat antibodies against mouse IL-1β (IMGENEX), neutralizing rat antibodies against mouse IL-18 (Medical & Biological Laboratories), isotype control IgGs (R&D Systems or eBioscience as appropriate), Caspase-1 inhibitor Z-WEHD-FMK (R&D Systems), Caspase control inhibitor Z-FA-FMK (R&D Systems), DPI (Enzo Life Sciences), Mito-TEMPO (Enzo Life Sciences), MitoQ and dTPP (both adsorbed to cyclodextrin and provided by M.P. Murphy, MRC Mitochondrial Biology Unit), and gp91ds-tat and scrambled gp91 ds-tat (both Anaspec), were dissolved in phosphate buffered saline (PBS; Sigma-Aldrich) or dimethyl sulfoxide (DMSO; Sigma-Aldrich), and injected into the vitreous humor in a total volume of 1 μL with a 33-gauge Exmire microsyringe (Ito Corporation). To assess the effect of MyD88 blockade on pAlu-induced RPE degeneration, 1 μL of cholesterol (chol) conjugated MyD88 siRNA (17+2 nt; 2 μg/μL) was intravitreously injected 1 day after pAlu injection. As a control, Luc siRNA-chol (17+2 nt) was used with identical dosages.

Bone Marrow Chimeras.

Bone marrow transplantation was used to create $Myd88^{-/-}$ chimera mice wherein the genetic deficiency of Myd88 was confined to either circulating cells ($Myd88^{-/-}$ →WT) or nonhematopoietic tissue (WT→$Myd88^{-/-}$). Briefly, bone marrows were collected from femur and tibia of congenic WT or $Myd88^{-/-}$ donor mice by flushing with RPMI1640. After two washing steps, cells were resuspended in RPMI1640. 1×10^{7} cells in 150 μL of RPMI1640 were injected into the tail vein of irradiated donor mice. Two chimera groups were generated: WT→$Myd88^{-/-}$ (WT cells into $Myd88^{-/-}$ mice) and Myd88→WT (Myd88 cells into WT mice). 2 months after bone marrow transfer, mice were injected subretinally with Alu RNA, vehicle, pAlu, or pNull, and monitored for RPE degeneration 7 days later.

Real-Time PCR.

Total RNA was extracted from tissues or cells using Trizol reagent (Invitrogen) according to manufacturer's recommendations, DNase treated and reverse transcribed (QuantiTect, 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. Oligonucleotide primers specific for human IL1B (forward 5'-TTAAAGCCCGCCTGACAGA-3' and reverse 5'-GCGAATGACAGAGGGTTTCTTAG-3'), human IL18 (forward 5'-ATCACTTGCACTCCGGAGGTA-3' and reverse 5'-AGAGCGCAATGGTGCAATC-3'), human NLRP3 (forward 5'-GCACCTGTTGTGCAATCTGAA-3' and reverse 5'-TCCTGACAACATGCTGATGTGA-3'), human PYCARD (forward 5'-GCCAGGCCTGCACTTTATAGA-3' and reverse 5'-GTTTGTGACCCTCGCGATAAG-3'), human VDAC1 (forward 5'-ACTGCAAAATCCCGAGTGAC-3' and reverse 5'-CTGTCCAGGCAAGATTGACA-3'), human VDAC2 (forward 5'-CAGTGCCAAATCAAAGCTGA-3' and reverse 5'-CCTGATGTCCAAGCAAGGTT-3'), human VDAC3 (forward 5'-TTGACACAGCCAAATCCAAA-3' and reverse 5'-GCCAAAACGGGTGTTGTTAC-3'), human 18S rRNA (forward 5'-CGCAGCTAGGAATAATGGAATAGG-3' and reverse 5'-GCCTCAGTTCCGAAAACCAA-3'), mouse Myd88 (forward 5'-CACCTGTGTCTGGTCCATTG-3' and reverse 5'-AGGCTGAGTGCAAACTTGGT-3'), mouse Nlrp3 (forward 5'-ATGCTGCTTCGACATCTCCT-3' and reverse 5'-AACCAATGCGAGATCCTGAC-3'), mouse Il18 (forward 5'-GACAGCCTGTGTTCGAGGAT-3' and reverse 5'-TGGATCCATTTCCTCAAAGG-3'), and mouse 18S rRNA (forward 5'-TTCGTATTGCGCCGCTAGA-3' and reverse 5'-CTTTCGCTCTGGTCCGTCTT-3') were used. The QPCR cycling conditions were 50° C. for 2 min, 95° C. for 10 min followed by 40 cycles of a two-step amplification program (95° C. for 15 s and 58° C. for 1 min). At the end of the amplification, melting curve analysis was applied using the dissociation protocol from the Sequence Detection system to exclude contamination with unspecific PCR products. The PCR products were also confirmed by agarose gel and showed only one specific band of the predicted size. For negative controls, no RT products were used as templates in the QPCR and verified by the absence of gel-detected bands. Relative expressions of target genes were determined by the $2^{-\Delta\Delta Ct}$ method.

miRNA Quantification.

Total RNA containing miRNAs was polyadenylated and reverse transcribed using universal primer using the All-In-One miRNA q-RT-PCR Detection Kit (GeneCopoeia) according to the manufacturer's specifications using a universal reverse primer in combination with the following forward primers: mouse miR-184 (5'-TGGACGGAGAACTGATAAGGGT-3'); mouse miR-221/222 (5'-AGCTACATCTGGCTACTGGGT-3'); mouse miR-320a (5'-AAAAGCTGGGTTGAGAGGGCGA-3'), and mouse miR-484 (5'-TCAGGCTCAGTCCCCTCCCGAT-3'). miRNA levels were normalized to levels of U6 snRNA (5'-AAATTCGTGAAGCGTTCC-3') using the $2^{-\Delta\Delta Ct}$ method. Detection was achieved by SYBR green qPCR with the following conditions: 95° C. for 10 min followed by 40 cycles of 95° C. for 10 s, 60° C. for 20 s and 72° C. for 20 s. Amplicon specificity was assessed by melt curve analysis and unique bands by agarose gel electrophoresis.

Western Blotting.

Tissues or cells were homogenized in lysis buffer (10 mM Tris base, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 0.5% NP-40, protease and phosphatase inhibitor cocktail (Roche)). Protein concentrations were determined using a Bradford assay kit (Bio-Rad) with bovine serum albumin as a standard. Proteins (40-100 μg) were run on NuPAGE Bis-Tris gels (Invitrogen) and transferred to Immun-Blot PVDF membranes (Bio-Rad). Cells were scraped in hot Laemmli buffer (62.5 mM Tris base, pH 6.8, 2% SDS, 5% 2-Mercaptoethanol, 10% Glycerol, 0.01% Bromophenol Blue). Samples were boiled and run on 4-20% NuPAGE Tris-Glycine gels (Invitrogen). The transferred membranes were blocked for 1 h at RT and incubated with antibodies against human Caspase-1 (1:500; Invitrogen), mouse Caspase 1 (1:500; MBL), NLRP3 (1:1000; Enzo Life Sciences), PYCARD (1:1000, RayBiotech), phospho-IRAK1 (S376) (1:500, Thermo Scientific), phospho-IRAK4 (T345) (1:500, AbboMax), DICER1 (1:2,000; Bethyl), MyD88 (1:1,000; Cell Signaling), and mouse IL-18 (1:200; MBL) at 4° C. overnight. Protein loading was assessed by immunoblotting using an anti-Vinculin antibody (1:1,000; Sigma-Aldrich). The secondary antibodies were used (1:5,000) for 1 h at RT. The signal was visualized by enhanced chemiluminescence (ECL plus) and captured by VisionWorksLS Image Acquisition and Analysis software (Version 6.7.2, UVP, LLC).

Cell Culture.

All cell cultures were maintained at 37° C. and 5% CO_2. Primary mouse RPE cells were isolated as previously described (Yang et al., 2009) and grown in Dulbecco Modified Eagle Medium (DMEM) supplemented with 20% FBS and standard antibiotics concentrations. Primary human RPE cells were isolated as previously described (Yang et al., 2008) and maintained in DMEM supplemented with 10% FBS and antibiotics. HeLa cells were maintained in DMEM supplemented with 20% FBS and standard antibiotics concentrations. THP-1 cells were cultured in RPMI 1640 medium supplemented with 10% FBS and antibiotics.

In vitro transcription of Alu RNAs. Two Alu RNAs were synthesized: a 281 nt Alu sequence originating from the cDNA clone TS 103 (Shaikh et al., 1997) and a 302 nt Alu sequence isolated from the RPE of a human eye with geographic atrophy. Linearized plasmids containing these Alu sequences with an adjacent 5' T7 promoter were subjected to AmpliScribe™ T7-Flash™ Transcription Kit (Epicentre) according to the manufacturer's instructions. DNase-treated RNA was purified using MEGAclear™ (Ambion), and integrity was monitored by gel electrophoresis. This yields single stranded RNAs that fold into a defined secondary structure identical to Pol III derived transcripts. Where indicated, transcribed RNA was dephosphorylated using calf intestine alkaline phosphatase (Invitrogen) and repurified by Phenol:Chloroform:Isoamyl alcohol precipitation.

Transient Transfection.

Human or mouse RPE cells were transfected with pUC19, pAlu, pcDNA3.1/Dicer-FLAG, pcDNA3.1, Alu RNA, NLRP3 siRNA sense (5'-GUUUGACUAUCUGUUCUdTdT-3'), PYCARD siRNA sense (5'-GAAGCUCUUCAGUUUCAdTdT-3'), MyD88 siRNA sense (sense: 5'-CAGAGCAAGGAAUGUGAdTdT-3'), VDAC1 siRNA sense (5'-CGGAAUAGCAGCCAAGUdTdT-3'), VDAC2 siRNA sense (5'-CCCUGGAGUUGGAGGCUdTdT-3'), VDAC3 siRNA sense (5'-GCUUUAAUCGAUGGGAAdTdT-3'), DICER1 antisense oligonucleotide (AS) (5'-GCUGACCTTTTTGCTUCUCA-3'), control (for DICER1) AS (5'-TTGGTACGCATACGTGTTGACTGTGA-3'), Alu AS (5'-CCCGGGTTCACGCCATTCTCCTGCCTCAGCCTCACGAGTAGCTGGGACTACAGGCGCCCGACACCACTCCCGGCTAATTTTTTGTATTTTT-3'), control (for Alu) AS (5'-GCATGGCCAGTCCATTGATCTTGCACGCTTGCCTAGTACGCTCCTCAACCTATCCTCCTAGCCCGTTACTTGGTGCCACCGGCG-3') using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

Adenoviral Infection.

Cells were plated at density of $15\times10^3/cm^2$ and after 16 h, at approximately 50% confluence, were infected with AdCre or AdNull (Vector Laboratories) with a multiplicity of infection of 1,000.

Cell Viability.

MTS assays were performed using the CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega) according to the manufacturer's instructions. For examining the cytoprotective effect of glycine in Alu RNA induced cell death, human RPE cells were transfected with pNull/pAlu. At 6 h post-transfection the cells were incubated with complete media containing glycine (5 mM) or vehicle, and cell viability was assessed after 24 h. Similarly, human RPE cells primed with LPS (5 μg/ml for 6 h) were treated with ATP (25 μM) in the presence of glycine containing media (5 mM). 30 min post ATP cell viability was assessed as described above.

Caspase-1 activity. Caspase-1 activity was visualized by incubating cells with Caspalux1E1D2 reagent (Oncolmmunin) according to the manufacturer's instructions. Caspalux1E1D2 signal was quantified reading the fluorescence (excitation 552 nm, emission 580 nm) using a Synergy 4 reader (Biotek). Quantification of fluorescence from images was performed by converting images into grayscale in Adobe Photoshop CSS, and measuring the integrated density of black and white images using ImageJ software (NIH) (Bogdanovich et al., 2008).

ROS Production.

Cellular ROS production was assessed using the ROS-specific probe 2'7'-dichlorodihydrofluorescin diacetate (H_2DCFDA, BioChemica, Fluka). Mitochondrial ROS production was assessed using MitoSOX™ Red (Invitrogen). Sub-confluent human RPE cells were transfected with pNull or pAlu. After 24 h cells were loaded for 10 min at 37° C. with 10 μM H_2DCFDA or MitoSOX™ Red (Invitrogen) mitochondrial superoxide indicator for live-cell imaging and washed twice with PBS. For H_2DCFDA, fluorescence was recorded in 96-well plate using with a Synergy 4 reader (Biotek) using a FITC filter (excitation 485 nm, emission 538 nm). To visualize respiring mitochondria for colocalization with the mitochondrial ROS signal, after PBS wash

cells were incubated with MitoTracker Deep Red™ (Invitrogen) for 30 min at 37° C. and then washed twice with PBS. The fluorescent signals were detected using Leica SP-5 or Zeiss Axio Observer Z1 microscopes. Phagosomal ROS production was assessed using the Fc-OXYBURST Green™ assay (Invitrogen). Sub-confluent human RPE cells were transfected with pNull or pAlu, or treated with PMA (0.5 μg/ml; Sigma-Aldrich). The cells were incubated with Krebs-Ringer's PBS (KRP) at 37° C. for 20 min before adding Fc-OXYBURST Green™. The total fluorescence from the cells was measured immediately after adding Fc-OXYBURST Green™ with a Synergy 4 reader (Biotek) using FITC filter (excitation 485 nm, emission 538 nm).

RNA-Binding Protein Immunoprecipitation (RIP):

The physical interaction between NLRP3 and Alu RNA was examined using RNA ChIP-IT kit following the manufacturer's instructions (Active Motif). Briefly, human RPE cells were transfected with pAlu and pNLRP3-FLAG (provided by G. Núñez) and the protein-RNA complexes were immunoprecipitated with antibodies against NLRP3 (Enzo Life Sciences), FLAG (Sigma-Aldrich) or control IgG (Sigma-Aldrich). RNA isolated from these immunoprecipitates was analyzed by real-time RT-PCR using Alu-specific primers.

ELISA.

Secreted cytokine content in conditioned cell culture media was analyzed using the Human IL-1β and IL-18 ELISA Kits (R&D) according to the manufacturer's instructions.

TLR Screen.

A custom TLR ligand screen was performed by InvivoGen using HEK293 cells over-expressing individual TLR family members coupled with an AP-1/NF-κB reporter system. Cells were stimulated with each of two Alu RNAs synthesized by in vitro transcription, or a TLR-specific positive control ligand.

Statistics.

Results are expressed as mean±SEM, with $p<0.05$ considered statistically significant. Differences between groups were compared by using Mann-Whitney U test or Student t-test, as appropriate, and 2-tailed p values are reported.

Example 2

It was shown that both in vitro transcribed Alu RNA and a plasmid encoding Alu (pAlu) both induce RPE cell death by inducing IL-18 secretion, which triggers MyD88-dependent signaling that leads to Caspase-3 activation. Determine the intervening mechanistic steps in this cell death pathway were sought.

Caspase-8 is known to activate Caspase-3 (Stennicke et al. 1998). Therefore, it was tested whether Caspase-8 inhibition would inhibit RPE cell death or degeneration induced by Alu RNA or pAlu. It was found that the Caspase-8 inhibitory peptide Z-IETD-FMK, but not the control peptide Z-FA-FMK, blocked RPE degeneration induced by pAlu in wild-type mice (FIG. **14**). Z-IETD-FMK also inhibited human RPE cell death induced by Alu RNA (FIG. **15**) or pAlu (FIG. **16**). It was also found that subretinal injection of recombinant IL-18 induced activation of Caspase-8, as monitored by a fluorometric assay, in the RPE of wild-type mice (FIG. **17**). These data indicate that Alu RNA- or pAlu-induced IL-18 leads to Caspase-8 activation upstream of Caspase-3 activation.

MyD88 is known to bind Fas-associated death domain protein (FADD) and induce apoptosis via Caspase 8 (Aliprantis et al. 2000). Therefore, it was tested whether ablation of Fas (encoded by CD95) or FasL (encoded by Faslg) would inhibit RPE cell death or degeneration induced by Alu RNA, pAlu, or IL-18. It was found that neither pAlu (FIG. **18**) nor Alu RNA (FIG. **19**) induced RPE degeneration in CD95−/− (Fas^{lpr}) mice. In addition, recombinant IL-18 also did not induce RPE degeneration in CD95−/− (Fas^{lpr}) mice (FIG. **20**). Likewise, pAlu (FIG. **21**), Alu RNA (FIG. **22**), and IL-18 (FIG. **23**) did not induce RPE degeneration in Faslg−/− ($Fasl^{gld}$) mice.

It has been shown that Alu RNA induces RPE degeneration via the NLRP3 inflammasome. Because NF-κB activation is required for NLRP3 activation (Bauernfeind et al. 2009; Qiao et al. 2012), it was tested whether Alu RNA required NF-κB to induce RPE degeneration. Indeed, it was found that Alu RNA did not induce RPE degeneration in Nfkb1−/− mice, confirming that NF-κB activation is a critical step in this cell death pathway.

Experimental Procedures

Mice.

All animal experiments were approved by institutional review committees and in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Visual Research. Wild-type C57BL/6J, $Fas^{-/-}$ (a.k.a CD95 or Fas^{lpr}) $Faslg^{-/-}$ (a.k.a. Fas^{gld}) and $Nfkb1^{-/-}$ mice were purchased from The Jackson Laboratory. 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 (Alcon Laboratories).

Fundus Photography.

Retinal photographs of dilated mouse eyes were taken with a TRC-50 IX camera (Topcon) linked to a digital imaging system (Sony).

Subretinal Injection.

Subretinal injections (1 μL) in mice were performed using a Pico-Injector (PLI-100, Harvard Apparatus). In vivo transfection of plasmids coding for two different Alu sequences (pAlu) or empty control vector (pNull) (Bennett et al., 2008; Kaneko et al., 2011; Shaikh et al., 1997) was achieved using 10% Neuroporter (Genlantis). In vitro transcribed Alu RNA was injected at 0.3 mg/mL.

Drug Treatments.

Recombinant IL-18 (Medical & Biological Laboratories), Caspase-8 inhibitor Z-IETD-FMK (R&D Systems), Caspase control inhibitor Z-FA-FMK (R&D Systems), IRAK1/4 inhibitor (Calbiochem), were dissolved in phosphate buffered saline (PBS; Sigma-Aldrich) or dimethyl sulfoxide (DMSO; Sigma-Aldrich), and injected into the vitreous humor in a total volume of 1 μL with a 33-gauge Exmire microsyringe (Ito Corporation).

Cell Culture.

All cell cultures were maintained at 37° C. and 5% CO_2. Primary mouse RPE cells were isolated as previously described (Yang et al., 2009) and grown in Dulbecco Modified Eagle Medium (DMEM) supplemented with 20% FBS and standard antibiotics concentrations. Primary human RPE cells were isolated as previously described (Yang et al., 2008) and maintained in DMEM supplemented with 10% FBS and antibiotics. HeLa cells were maintained in DMEM supplemented with 20% FBS and standard antibiotics concentrations. THP-1 cells were cultured in RPMI 1640 medium supplemented with 10% FBS and antibiotics.

In Vitro Transcription of Alu RNAs.

We synthesized a 302 nt Alu sequence isolated from the RPE of a human eye with geographic atrophy. A linearized plasmid containing this Alu sequence with an adjacent 5' T7

promoter were subjected to AmpliScribe™ T7-Flash™ Transcription Kit (Epicentre) according to the manufacturer's instructions. DNase-treated RNA was purified using MEGAclear™ (Ambion), and integrity was monitored by gel electrophoresis. This yields single stranded RNAs that fold into a defined secondary structure identical to Pol III derived transcripts. Where indicated, transcribed RNA was dephosphorylated using calf intestine alkaline phosphatase (Invitrogen) and repurified by Phenol:Chloroform:Isoamyl alcohol precipitation.

Transient Transfection.

Human RPE cells were transfected with pUC19, pAlu, Alu RNA, VDAC1 siRNA sense (5'-CGGAAUAGCAGCCAAGUdTdT-3'), VDAC2 siRNA sense (5'-CCCUGGAGUUGGAGGCUdTdT-3'), VDAC3 siRNA sense (5'-GCUUUAAUCGAUGGGAAdTdT-3'), using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

Cell Viability.

MTS assays were performed using the CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega) according to the manufacturer's instructions.

Caspase-8 Activity.

RPE tissues were homogenized in lysis buffer (10 mM Tris base, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 0.5% NP-40, protease and phosphatase inhibitor cocktail (Roche)). Protein concentrations were determined using a Bradford assay kit (Bio-Rad) with bovine serum albumin as a standard. The caspase-3 activity was measured using Caspase-8 Fluorimetric Assay (R&D) in according to the manufacturer's instructions.

Statistics.

Results are expressed as mean±SEM, with $p<0.05$ considered statistically significant. Differences between groups were compared by using Mann-Whitney U test or Student t-test, as appropriate, and 2-tailed p values are reported.

Methods for Caspase Imaging

Alu RNA or recombinant IL-18 was injected into the subretinal space of wild-type mice on day 0. DyeLight782-VAD-FMK3 (ThermoScientific), a probe that fluoresces in the presence of bioactive caspases, was injected into the vitreous humor of wild-type mice on day 2 or day 3 after injection.

Flat Mount Imaging.

At 24 hours after injection of DyeLight782-VAD-FMK3, the eyecup was dissected out of mice, the neural retina was removed, and a flat mount of the RPE was prepared, and viewed under a fluorescent microscope.

In Vivo Bioimaging in the Living Eye.

At intervals from 0-24 hours after injection of DyeLight782-VAD-FMK3, fundus photographs were taken with the Topcon 50IX camera using the ICG filter.

Throughout this document, various references are mentioned. All such references are incorporated herein by reference to the same extent as if each individual reference was specifically and individually indicated to be incorporated by reference, including the references set forth in the following list:

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It will be understood that various details of the presently disclosed subject matter can be changed without departing from the scope of the subject matter disclosed herein. Furthermore, the foregoing description is for the purpose of illustration only, and not for the purpose of limitation.

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aggctgagtg caaacttggt 20

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aaccaatgcg agatcctgac 20

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Lys Arg Asp Val Leu Pro Gly Thr Cys Val Trp Ser Ile Ala Ser Glu
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What is claimed is:

1. A method of protecting an retinal pigment epithelium (RPE) cell, a retinal photoreceptor cell, or a choroidal cell, comprising:

inhibiting one or more of an inflammasome of the cell; MyD88 of the cell; IL-18 of the cell; VDAC1 of the cell; VDAC2 of the cell; caspase-8 of the cell; and NFκB of the cell;

wherein the cell is protected against Alu-RNA-induced degeneration;

wherein inhibiting the inflammasome consists essentially of administering an inflammasome inhibitor selected from the group consisting of an NLRP3 inhibitor, a PYCARD inhibitor, an inflammosome inhibitor comprising a sequence selected from the sequences of SEQ ID NOS: 7-16, an inhibitor of Caspase-1, a peptide inhibitor of Caspase-1, a Caspase-1 inhibitor comprising the sequence of SEQ ID NO: 17, and the inflammosome inhibitors selected from: ion channel inhibitors; IkB-α inhibitors; antibodies selected from: Anti-ASC, Anti-NALP1, antibodies based on protein sequences selected from: ASC: ALR QTQ PYL VTD LEQ S; NALP1: MEE SQS KEE SNT EG-cys; Anti-NALP1, anti-IL-1β, anti-IL-18, anti-caspase-1, and anti-caspase-11; direct inhibitors of Caspase-1 and/or NLRP3; parthenolide; estrogen binding B-box proteins, COP, ICEBERG, and Z-WEHD-FMK; Caspase 1 and/or 4 inhibitors; Caspase-12 inhibitors; host-derived inhibitors of caspase-1; inhibitors of Nlrp1b inflammasome; virus expressed inhibitors of the inflammasome; potassium chloride; Cathepsin-B-inhibitors; Cytochalsin D; ROS inhibitors; ASC-1 inhibitors; NLRP3 inflammasome pan-caspase inhibitors; Microtubules; an isolated double-stranded RNA molecule that inhibits expression of NLRP3, and which can be conjugated to cholesterol or not, and at least one strand including the sequence: GUUUGACUAUCUGUUCUdTdT (SEQ ID NO: 7); an isolated double-stranded RNA molecule that inhibits expression of PYCARD, at least one strand of which includes the sequence of: 5'-GAAGCUCUUCAGUUUCAdTdT-3' (SEQ ID NO: 12); and an isolated double-stranded RNA molecule that inhibits expression of PYCARD, at least one strand of which includes a sequence selected from: 5'-GAAGCUCUUCAGUUUCAdTdT-3' (SEQ ID NO: 12); 5'-GGCUGCUGGAUGCUCUGUACGGGAA-3' (SEQ ID NO: 13); and 5'-UUCCCGUACAGAGCAUCCAGCAGCC-3' (SEQ ID NO: 14).

2. The method of claim **1**, wherein the inhibiting MyD88 comprises administering a MyD88 inhibitor.

3. The method of claim **2**, wherein the inhibitor is selected from the group consisting of a MyD88 inhibitor comprising a polypeptide sequence selected from the sequences of SEQ ID NO: 1, 54, 55, 60, and 61; and a MyD88 inhibitor comprising a double-stranded RNA molecule, at least one strand of which includes a sequence selected from SEQ ID NOS: 3, 4, 5, 6, and 56.

4. The method of claim **2**, wherein the My D88 inhibitor is selected from the group consisting of a MyD88 homodimerization inhibitor; Pepinh-MYD; a dominant negative or splice variant of MyD88; a MyD88 splice variants that lack exon 2; and MyD88 inhibitors as set forth in Table C.

5. The method of claim **4**, wherein the inhibitor is administered by intravitreous injection; subretinal injection; episcleral injection; sub-Tenon's injection; retrobulbar injection; peribulbar injection; topical eye drop application; release from a sustained release implant device that is sutured to or attached to or placed on the sclera, or injected into the vitreous humor, or injected into the anterior chamber, or implanted in the lens bag or capsule; oral administration; or intravenous administration.

6. A method of protecting an RPE cell, a retinal photoreceptor cell, or a choroidal cell, comprising:

inhibiting one or more of an inflammasome of the cell; MyD88 of the cell; IL-18 of the cell; VDAC1 of the cell; VDAC2 of the cell; caspase-8 of the cell; and NFκB of the cell;

wherein the cell is protected against Alu-RNA-induced degeneration;

wherein inhibiting the inflammasome comprises administering an inflammasome inhibitor selected from the group consisting of an NLRP3 inhibitor, a PYCARD inhibitor, an inflammosome inhibitor comprising a sequence selected from the sequences of SEQ ID NOS: 7-16, an inhibitor of Caspase-1, a peptide inhibitor of Caspase-1, a Caspase-1 inhibitor comprising the sequence of SEQ ID NO: 17, and the inflammosome inhibitors selected from: ion channel inhibitors; IkB-α inhibitors; antibodies selected from: Anti-ASC, Anti-NALP1, antibodies based on protein sequences selected from: ASC: ALR QTQ PYL VTD LEQ S; NALP1: MEE SQS KEE SNT EG-cys; Anti-NALP1, anti-IL-1β, anti-IL-18, anti-caspase-1, and anti-caspase-11; direct inhibitors of Caspase-1 and/or NLRP3; parthenolide; estrogen binding B-box proteins, COP, ICEBERG, and Z-WEHD-FMK; Caspase 1 and/or 4 inhibitors; Caspase-12 inhibitors; host-derived inhibitors of caspase-1; inhibitors of Nlrp1b inflammasome; virus expressed inhibitors of the inflammasome; potassium chloride; Cathepsin-B-inhibitors; Cytochalsin D; ROS inhibitors; ASC-1 inhibitors; NLRP3 inflammasome pan-caspase inhibitors; Microtubules; an isolated double-stranded RNA molecule that inhibits expression of NLRP3, and which can be conjugated to cholesterol or not, and at least one strand including the

sequence: GUUUGACUAUCUGUUCUdTdT (SEQ ID NO: 7); an isolated double-stranded RNA molecule that inhibits expression of PYCARD, at least one strand of which includes the sequence of: 5'-GAAGCUCUUCAGUUUCAdTdT-3' (SEQ ID NO: 12); and an isolated double-stranded RNA molecule that inhibits expression of PYCARD, at least one strand of which includes a sequence selected from: 5'-GAAGCUCUUCAGUUUCAdTdT-3' (SEQ ID NO: 12); 5'-GGCUGCUGGAUGCUCUGUACGGGAA-3' (SEQ ID NO: 13); and 5'-UUCCCGUACAGAGCAUCCAGCAGCC-3' (SEQ ID NO: 14), wherein the inflammasome includes a protein encoded by PYCARD.

7. The method of claim **6**, wherein the inflammasome is selected from NLRP3 inflammasome, NLRP1 inflammasome, NLRC4 inflammasome, AIM2 inflammasome, and IFI16 inflammasome.

8. The method of claim **7**, wherein the inflammasome is the NLRP3 inflammasome.

9. The method of claim **1**, wherein the inflammasome inhibitor is selected from glybenclamide/glyburide; BAY11-7082 (CAS Number: 195462-67-7; also known as (E)-3-(4-Methylphenylsulfonyl)-2-propenenitrile); Anti-ASC and Anti-NALP1 and antibodies based on protein sequences selected from: ASC: ALR QTQ PYL VTD LEQ S; NALP1: MEE SQS KEE SNT EG-cys; Anti-NALP1, anti-IL-1β, anti-IL-18, anti-caspase-1, and anti-caspase-11; parthenolide; estrogen binding B-box proteins, COP, ICEBERG, and Z-WEHD-FMK; Ac-Tyr-Val-Ala-Asp-CHO (Ac-YVAD-CHO) or N-acetyl-L-tyrosyl-L-valyl-N-[(1S)-1-(carboxymethyl)-3-chloro-2-oxo-propyl]-L-alaninamide (Ac-YVAD-CMK); Caspase-12 inhibitors; cellular PYRIN domain (PYD)-ony proteins (POP) family: cPOP1 and cPOP2; serpin proteinase inhibitor 9 (PI-9); BCL-2 and BCL-xL; auranofin; PYD homologs M13L-PYD, S013L, SPI-2 homologs CrmA, Serp2, SPI-2, NS1, Kaposi Sarcoma-associated Herpesvirus Orf63; potassium chloride; L-3-trans-(Propylcarbamoyl)oxirane-2-Carbonyl)-L-Isoleucyl-L-Proline Methyl Ester ("CA-074 Me"); Cytochalsin D; N-acetyl-L-cysteine (NAC), (2R,4R)-4-aminopyrrolidine-2,4-dicarboxylate (APDC); cellular pyrin domain (PYD) superfamily proteins, also known as M013; Z-VAD-FMK; colchicine; an isolated double-stranded RNA molecule that inhibits expression of NLRP3, and which can be conjugated to cholesterol or not, and at least one strand including the sequence: GUUUGACUAUCUGUUCUdTdT (SEQ ID NO: 7); an isolated double-stranded RNA molecule that inhibits expression of PYCARD, at least one strand of which includes the sequence of: 5'-GAAGCUCUUCAGUUUCAdTdT-3' (SEQ ID NO: 12); and an isolated double-stranded RNA molecule that inhibits expression of PYCARD, at least one strand of which includes a sequence selected from: 5'-GAAGCUCUUCAGUUUCAdTdT-3' (SEQ ID NO: 12); 5'-GGCUGCUGGAUGCUCUGUACGGGAA-3' (SEQ ID NO: 13); and 5'-UUCCCGUACAGAGCAUCCAGCAGCC-3' (SEQ ID NO: 14).

10. The method of claim **1**, wherein the inflammasome inhibitor is selected from the group consisting of an inflammosome inhibitor comprising a sequence selected from the sequences of SEQ ID NOS: 7-16; an inhibitor of Caspase-1 selected from VX-765, ML132, VX-740, VRT-018858, YVAD, WEHD; and a Caspase-1 inhibitor comprising the sequence of SEQ ID NO: 17.

11. The method of claim **9**, wherein the inhibitor is administered by intravitreous injection; subretinal injection; episcleral injection; sub-Tenon's injection; retrobulbar injection; peribulbar injection; topical eye drop application; release from a sustained release implant device that is sutured to or attached to or placed on the sclera, or injected into the vitreous humor, or injected into the anterior chamber, or implanted in the lens bag or capsule; oral administration; or intravenous administration.

12. The method of claim **1**, wherein the inhibiting IL-18 comprises administering an IL-18 inhibitor.

13. The method of claim **12**, wherein the IL-18 inhibitor is selected from the group consisting of a neutralizing antibody against IL-18; an antibody that blocks IL-18 binding to the IL-18 receptor, IL-18 neutralizing antibodies; IL-18 binding protein; and IL18BP.

14. The method of claim **13**, wherein the inhibitor is administered by intravitreous injection; subretinal injection; episcleral injection; sub-Tenon's injection; retrobulbar injection; peribulbar injection; topical eye drop application; release from a sustained release implant device that is sutured to or attached to or placed on the sclera, or injected into the vitreous humor, or injected into the anterior chamber, or implanted in the lens bag or capsule; oral administration; or intravenous administration.

15. The method of claim **1**, wherein the inhibiting VDAC1 comprises administering a VDAC1 inhibitor and inhibiting VDAC2 comprises administering a VDAC2 inhibitor.

16. The method of claim **15**, wherein the VDAC1 inhibitor is selected from the group consisting of a VDAC1 inhibitor comprising the sequence of SEQ ID NO: 47; a VDAC2 inhibitor comprising the sequence of SEQ ID NO: 48; phosphorothioate oligonucleotide randomer (Trilink Industries) that inhibits VDAC; cyclosporin A; superoxide dismutase 1; 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS); Bcl-x(L) BH4(4-23); and TRO19622.

17. The method of claim **15**, wherein the inhibitor is administered by intravitreous injection; subretinal injection; episcleral injection; sub-Tenon's injection; retrobulbar injection; peribulbar injection; topical eye drop application; release from a sustained release implant device that is sutured to or attached to or placed on the sclera, or injected into the vitreous humor, or injected into the anterior chamber, or implanted in the lens bag or capsule; oral administration; or intravenous administration.

18. The method of claim **1**, wherein the inhibiting Caspase-8 comprises administering a Caspase-8 inhibitor.

19. The method of claim **18**, wherein the Caspase-8 inhibitor is selected from the group consisting of Z-IETD-FMK, Ac-Ala-Ala-Val-Ala-Leu-Leu-Pro-Ala-Val-Leu-Leu-Ala-Leu-Leu-Ala-Pro-Ile-Glu-Thr-Asp-CHO, Z-Ile-Glu(OMe)-Thr-Asp(OMe)-CH_2F, and Cellular fas-associated death domain-like interleukin-1-β-converting enzyme-inhibitory protein (L).

20. The method of any one of claim **18**, wherein the inhibitor is administered by intravitreous injection; subretinal injection; episcleral injection; sub-Tenon's injection; retrobulbar injection; peribulbar injection; topical eye drop application; release from a sustained release implant device that is sutured to or attached to or placed on the sclera, or injected into the vitreous humor, or injected into the anterior chamber, or implanted in the lens bag or capsule; oral administration; or intravenous administration.

21. The method of claim **1**, wherein the inhibiting NFkB comprises administering a NFkB inhibitor.

22. The method of claim **21**, wherein the inhibitor is administered by intravitreous injection; subretinal injection; episcleral injection; sub-Tenon's injection; retrobulbar injection; peribulbar injection; topical eye drop application;

release from a sustained release implant device that is sutured to or attached to or placed on the sclera, or injected into the vitreous humor, or injected into the anterior chamber, or implanted in the lens bag or capsule; oral administration; or intravenous administration.

23. The method of claim **1**, wherein the cell is in a subject.

24. The method of claim **23**, wherein the subject has age-related macular degeneration.

25. An molecule selected from the group consisting of:

an isolated double-stranded RNA molecule that inhibits expression of MyD88, wherein a first strand of the double-stranded RNA comprises a sequence selected from SEQ ID NO: 3, 4, 5, 6, and 56, and including about 11 to 27 nucleotides;

an isolated double-stranded RNA molecule that inhibits expression of NLRP3 and/or PYCARD, wherein a first strand of the double-stranded RNA comprises a sequence selected from SEQ ID NO: 7-14, and including about 11 to 27 nucleotides;

an isolated double-stranded RNA molecule that inhibits expression of Pyrin, comprising the sequence of SEQ ID NO: 15, and including about 11 to 27 nucleotides;

an isolated double-stranded RNA molecule that inhibits expression of NALP3, comprising the sequence of SEQ ID NO: 16, and including about 11 to 27 nucleotides;

an isolated double-stranded RNA molecule that inhibits expression of caspase-1, comprising the sequence of SEQ ID NO: 17, and including about 11 to 27 nucleotides;

an isolated double-stranded RNA molecule that inhibits expression of VDAC1 and/or VDAC2, wherein a first strand of the double-stranded RNA comprises a sequence selected from SEQ ID NO: 47 and 48, and including about 11 to 27 nucleotides;

a polypeptide molecule that inhibits MyD88, comprising a sequence selected from SEQ ID NO: 60 and SEQ ID NO: 61.

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