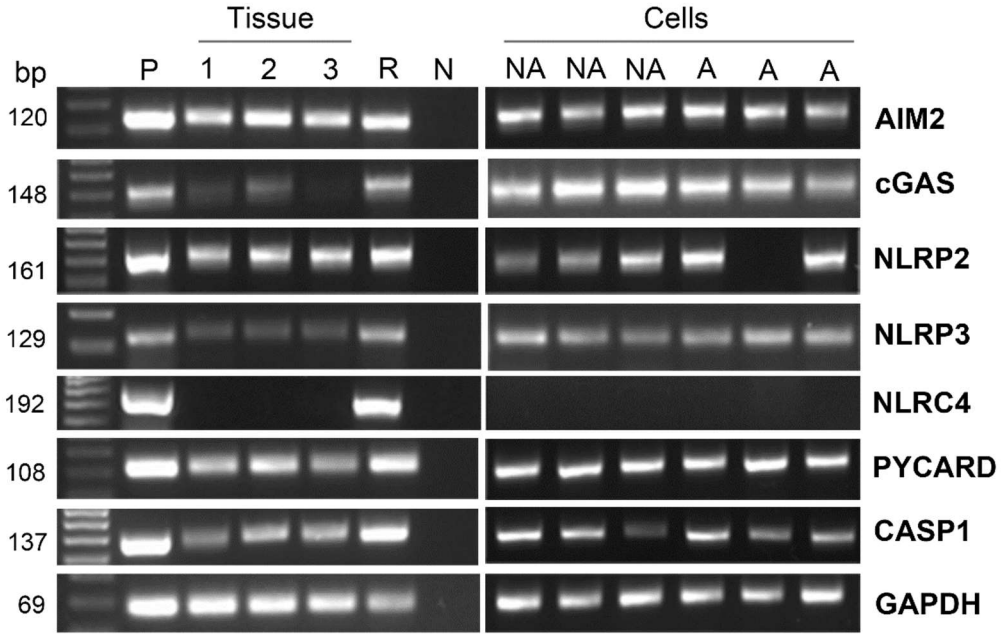


**Figure S1. Inflammasome gene expression in haRPE tissue and cell cultures.** (A) Gel images showing PCR products of inflammasome components in haRPE tissue (1 = MGS4, 87 year old female; 2 = MGS2, 87 year old male; 3 = MGS1, 56 year old male) and cell cultures from donors with (A) and without AMD (NA). 50bp ladder was used as reference for PCR product size. P = positive control, N = negative control (no DNA), R = retina. GAPDH is loading control.

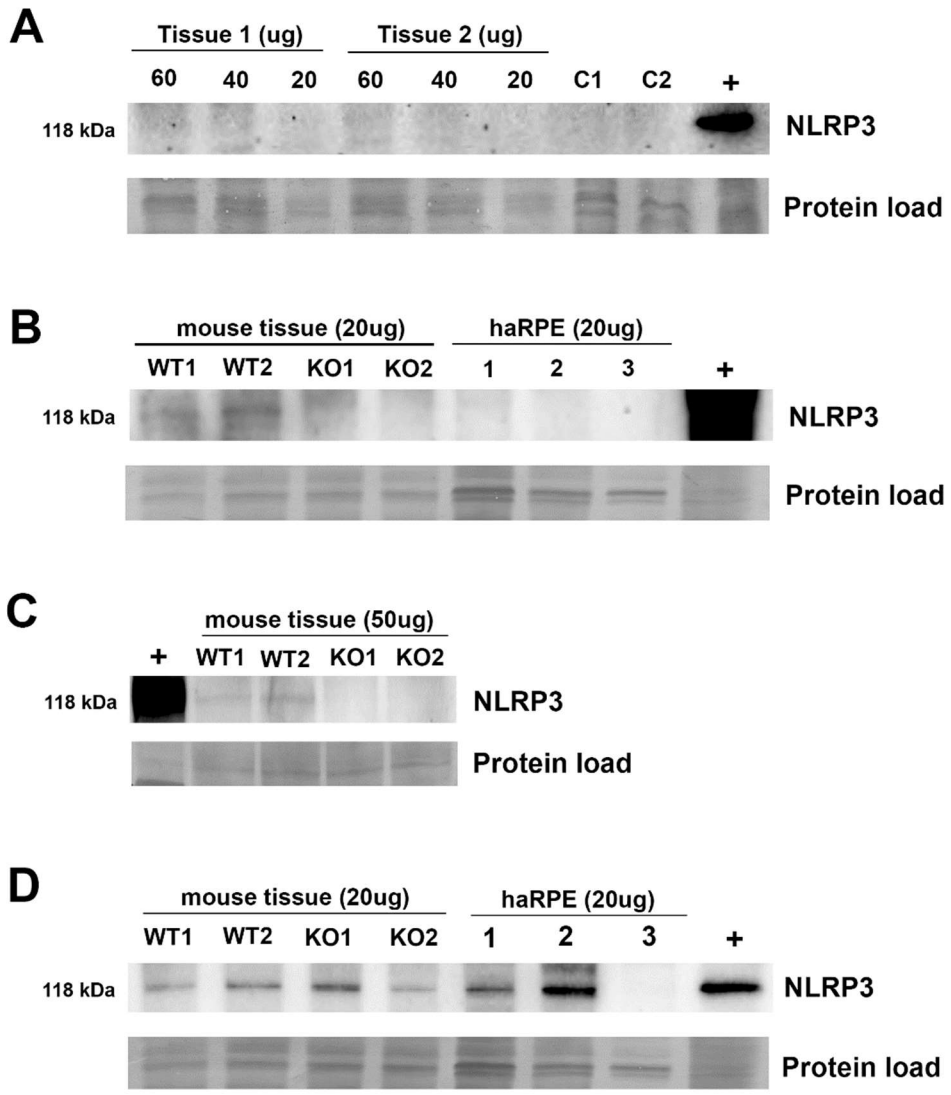


**Figure S2. Checking for NLRP3 by Western blot. (A)** Varying amounts RPE tissue lysates were run on Western blot to check for NLRP3 content using Cell Signaling antibody (#15101). C1 = Daudi cell lysate (x Ug), C2 = HeLa cell lysate (x ug). **(B)** Protein lysates (20µg/well) of wild-type mouse (WT) and NLRP3-/- mouse (KO) tissue was run on a Western blot along with haRPE cell lysates (1-3) to check for NLRP3 content using Cell Signaling antibody. **(C)** A larger amount (50µg) of mouse tissue lysates were run on Western Blot to confirm NLRP3 Cell Signaling antibody specificity. **(D)** Mouse tissue and haRPE cell lysates were run on Western blot to show the non-specificity of NLRP3 antibody from abcam (ab214185). Positive control (+) = THP1 cell lysate (x ug). Blots were stained with Coomassie Blue to show protein load.

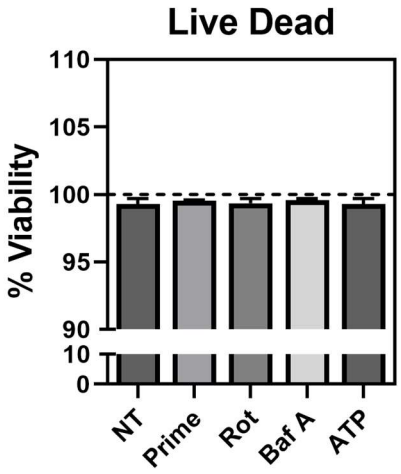


**Figure S3. Measuring cell viability after inflammasome activation.** Live/Dead Assay was used to measure cell viability in confluent haRPE cells (n=3) after inflammasome activation. Data was normalized to lysis (0% viability). Graph shows mean ± SEM. Dashed line = 100% viability. NT = no treatment, prime = IL-1α + LPS only, Rot = rotenone, Baf A = Bafilomycin A.

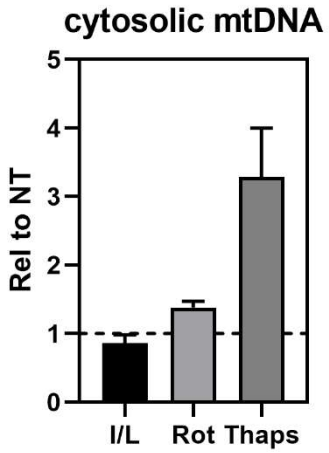


**Figure S4: Determining the amount of mtDNA in the cytosol.** mtDNA was extracted from the cytosol and quantified using qPCR. Data shown is fold change relative to NT =1 (dashed line). NT = no treatment, I/L = IL-1α + LPS only (prime), Rot = rotenone, Thaps = Thapsigargin (positive control for mtDNA release into the cytosol).

**Table S1. Donor Demographics – haRPE tissue samples used in Mass Spec[A]**

| Disease State[B]/ Genotype[C] | Sample[D] (n) | Sex Male (n) | Sex Female (n) | Age[E] (Mean ± SD) | Time[F] (Mean ± SD) | Cause of Death[G] (n) |
|---|---|---|---|---|---|---|
| **No AMD** CFH CT | 32 | 16 | 16 | 77 ± 10.6 | 19 ± 4.2 | Cancer (6), CVA (2), Dementia (1), Heart failure (4), Hemorrhage (3), Hypoxia (1), Multi-system failure (2), Natural causes (1), Pneumonia (3), Pulmonary fibrosis (1), Respiratory failure (2), Sepsis (5), TBI (1) |
| **AMD** CFH CT | 45 | 22 | 23 | 80 ± 10.4 | 19 ± 4.4 | AAA (1), ALS (1), Alzheimer's disease (3), CAD (1), Cancer (17), CVA (2), ESRD (1), Heart failure (3), Hemorrhage (1), High potassium (1), Natural causes (1), PE (1), Pneumonia (4), Pulmonary fibrosis (1), Respiratory failure (1), Sepsis (5), TBI (1) |

AAA = abdominal aortic aneurysm; ALS = amyotrophic lateral sclerosis; CAD = coronary artery disease; CVA = cerebrovascular accident; ESRD = end-stage renal disease; PE = pulmonary embolism; TBI = traumatic brain injury.

[A] Information supplied by Lions Gift of Sight (St. Paul, MN).

[B] Minnesota Grading System (MGS) was used to evaluate the stage of AMD in eye bank eyes (Olsen and Feng, 2004). No AMD = MGS1; AMD = MGS2

[C] Complement Factor H (CFH) genotype for rs1061170; low risk = TT, high risk = CT and CC. Note: all donors were high risk = CT.

[D] Sample number indicates the total donors used in the current study for each disease state.

[E] Age of donor (in years).

[F] The time from death to RPE harvest in hours.

[G] The number of donors for each cause of death is indicated in parentheses.

**Table S2. Donor Characteristics and Clinical Information – haRPE cell cultures**[A]

| Disease State[B]/ Genotype[C] | Sample[D] (n) | Sex Male (n) | Sex Female (n) | Age[E] (Mean ± SD) | Time[F] (Mean ± SD) | Cause of Death[G] (n) |
|---|---|---|---|---|---|---|
| **No AMD** | 23 | 12 | 11 | 63 ± 12.7 | 19 ± 4.2 | AAA (1), ABI (3), ACE (3), Cancer (5), ESRD (1), Heart Failure (3), ICB/ICH (1), Pneumonia (1), PE (1), Sepsis (4) |
| Low risk | 9 | 6 | 3 | 60 ± 12.5 | 20 ± 4.2 | |
| High risk | 14 | 6 | 8 | 65 ± 12.8 | 19 ± 4.3 | |
| **AMD** | 39 | 26 | 13 | 74 ± 9.5 | 20 ± 3.5 | ACE (3), Cancer (9), Cirrhosis (1), COPD (2), CVA (5), Heart Failure (5), Pneumonia (5), PE (1), Respiratory disease (1), Sepsis (7) |
| Low risk | 15 | 10 | 5 | 74 ± 10 | 18 ± 3.8 | |
| High risk | 24 | 16 | 8 | 74 ± 9.4 | 20 ± 4.1 | |

AAA = abdominal aortic aneurysm; ABI = Anoxic Brain Injury; ACE = acute myocardial event; COPD = chronic obstructive pulmonary disease; CVA = cerebrovascular accident (stroke); ESRD = end-stage renal disease; Heart failure = Myocardial infarction or cardiac arrest; ICH/ ICB = Intracerebral hemorrhage/ Intracerebral bleed; PE= pulmonary embolism.

[A] Information supplied by Lions Gift of Sight (St. Paul, MN).

[B] Minnesota Grading System (MGS) was used to evaluate the stage of AMD in eye bank eyes (Olsen and Feng, 2004). No AMD = MGS1; AMD = MGS2 and MGS3.

[C] Complement Factor H (CFH) genotype for rs1061170; low risk = TT, high risk = CT or CC.

[D] Sample number indicates the total donors used in the current study for each disease state.

[E] Age of donor (in years).

[F] The time from death to RPE harvest in hours.

[G] The number of donors for each cause of death is indicated in parentheses.

**Table S3. Antibodies used in this study**

| Antibody | | Dilution | Company | Product number |
|---|---|---|---|---|
| RPE65 | WB | 1:1000 | Novus Biologicals; Centennial, CO, USA | NB100-355 |
| CRALBP | WB | 1:1000 | Novus Biologicals; Centennial, CO, USA | NB100-74392 |
| NaK ATPase | WB | 1:1000 | Cell Signaling; Danvers, MA, USA | 23565 |
| Keratin 18 | WB | 1:1000 | Cell Signaling; Danvers, MA, USA | 4548 |
| NLRP3 | WB | 1:1000 | Cell Signaling; Danvers, MA, USA | 15101 |
| NLRP3 | WB | 1:1000 | Abcam; Cambridge, United Kingdom | Ab214185 |
| AIM2 | WB | 1:1000 | Cell Signaling;, Danvers, MA, USA | 12948 |
| IL-1β | WB | 1:500 | R & D Systems; Minneapolis, MN, USA | MAB601 |
| Caspase-1 | WB | 1:1000 | Cell Signaling; Danvers, MA, USA | 2225 |
| β-Actin | WB | 1:5000 | Santa Cruz Biotechnology; Dallas, TX, USA | Sc47778 |
| Bestrophin | IF | 1:250 | Novus Biologicals; Centennial, CO, USA | NB300-164 |
| Ezrin | IF | 1:200 | Cell Signaling; Danvers, MA, USA | 3145 |
| Alexa Fluor® 488 anti-mouse | IF | 1:800 | Life Technologies; Carlsbad, CA, USA | A21202 |
| Alexa Fluor® 594 anti-rabbit | IF | 1:800 | Life Technologies; Carlsbad, CA, USA | A21207 |

**Table S4. Primers for Real Time PCR analysis**

| Gene | Forward Primer | Reverse Primer |
|---|---|---|
| NLRP1 | AATGGCCTCTGGATGAAACGT | CTCTCACAGAAGGCTCCCATG |
| NLRP2 | ACAACTTTGAGACACCCCAAGT | TTCACCCCTGTATTCCCAATGG |
| NLRP3 | TTCAAACGACTCCCTGGAAC | AAAGGAAGTGGACTGCGAGA |
| NLRC4 | TGTCAAGTGAACCCTGTGACC | CTAGCACGTTCATCCTGTCGA |
| cGAS | CCTGCTGTAACACTTCTTAT | TTAGTCGTAGTTGCTTCCT |
| AIM2 | AGCCTGAACAGAAACAGATGG | CTTCTTGGGTCTCAAACGTGA |
| PYCARD | CTGACGGATGAGCAGTACCA | CAAGTCCTTGCAGGTCCAGT |
| CASP1 | GGGGTACAGCGTAGATGTGAA | CTTCCCGAATACCATGAGACA |
| IL-1β | CCACAGACCTTCCAGGAGAA | GTGATCGTACAGGTGCATCG |
| PPIG | CTTGTCAATGGCCAACAGAGG | GCCCATCTAAATGAGGAGTTGGT |
| ARBP | CGACCTGGAAGTCCAACTAC | ATCTGCTGCATCTGCTTG |
| GAPDH | TGCACCACCAACTGCTTCGC | GGCATGGACTGTGGTCATGAG |
| C3 | GCTACATCATCGGGAAGGAC | CTGGCATTGTTTCTGGTTCTC |
| C3AR | CCTGCTGATGTGGTCTCACCT | CCTTGTGGTAGCTCAGACTCGT |
| CFB | CCCTATGCTGACCCCAATAC | GATTACACCAACTTGAATGAAACG |
| CFHv1 | AACAGATTGTCTCAGTTTACCTAGC | ACCCGCCTTATACACATCCTTC |
| CFHv2 | CTTTACCCTCTGAACTTCTGATCG | TCTGGCTGGAATAATACACACATAAC |
| CFI | TTGGATTCTGACTGCTGCAC | TTGTCCATATTTGGTAACGATGA |
| CD46 | TGGCTACCTGTCTCAGATGACG | GCATCTGATAACCAAACTCGTAAG |
| CD55 | CTGCTGGTGCTGTTGTGC | TCCTCGGGAAAACTTGTACG |
| CD59 | GAGCCCAGGGAGGGAAAGGTT | CGAGGTTAAGGCAAAACCCTACGG |
| MCP1 | CTCATAGCAGCCACCTTCATTC | TCACAGCTTCTTTGGGACACTT |
| IL6 | GGTACATCCTCGACGGCATCT | GTGCCTCTTTGCTGCTTTCAC |
| mt191 | CAGTGAAATTGACCTGCCCGTGAA | TCTTAGCATGTACTGCTCGGAGGT |
| 18S rDNA | GGCCCTGTAATTGGAATGAGTC | CCAAGATCCAACTACGAGCTT |