Supplemental material and methods

Cell culture

For the HEK293T cell line, cells were cultured with DMEM base medium contained 10% FBS. The lipofectamine transfection kit was used for gene delivery in the HEK293T cells according to the protocol. Briefly, the 2 μg of plasmid DNA was mixed with 2 μl of lipofectamine solution and then added into a well of 6-well-plate with about 70% confluent of HEK293T cells. After 2 days of transfection, cells were lysed for protein extraction or fixed for the immunostainings.

The procedure of iPSC induced RPE (iRPE) cells was performed as previously report (58). Briefly, the wildtype iPSC line was seeded in 6-well-plate (200k/well) with E8 completed medium at day1. From day 2 to day 10, the cells were cultured in RDM media, and the media was changed every day. From day 11 to day 20, RMNA media was used and changed every day. Day 21–25, the cells were cultured in 5% RPE media to get the RPE precursors. At Day 25, the PRE precursors were seeded at 24–30 million in T75 flask in 20 mL of RPE plating media. At day 40, immature RPE cells were purified by the MACS using anti-CD24 (1:500) and anti-CD56 (1:500). The purified immature RPE cells were seeded on transwells and continuously cultured in 5% RPE medium. At 10 days post- seeding, change the 5% RPE maturation media to 5% RPE media + PGE2 and continue for 5 weeks with PGE2-supplemented media. For knocking down SERPINE3, the iRPE cells were cultured in the 5% RPE medium containing the lenti-shSERPINE3 virus ($1x10^{10}$ genome copies / well) for 12 hours, then the medium was switched to the 5% RPE medium without virus. In addition, 10 mM of sodium iodate was used to induce iRPE degeneration as shown in Figure 4I, and the 2 nM of AC-YVAD-CMK was used to inhibit Caspase-1 activity.

EdU and TUNEL assay

The Edu (Thermofish Scientific, A10044) was dissolved in 1xPBS and intraperitoneally injected into the mice (30 mg/kg). After 4 hours of injection, the eyeballs were dissected in 1xPBS to remove the anterior segment tissues, lenses and neural retinas, and the eyecups were then fixed in 4% PFA at 4°C for 2 hours. The Edu staining was performed according to the kit protocol (Click-iT™ EdU Cell Proliferation Kit for Imaging, Alexa Fluor™ 594 dye, C10339,

Thermofish Scientific). For the TUNEL assay, the eyecups were fixed in 4% PFA 4°C overnight and then subjected to TUNEL examination. The TUNEL staining was performed according to the kit protocol (Click-iT™ Plus TUNEL Assay Kits for In Situ Apoptosis Detection, C10618, ThermoFisher).

Immunostaining

Mouse eyeballs were marked at noon and dissected in cold 1xPBS for RPE flat-mount immunostaining. The anterior segment tissues, lenses and neural retinas were removed, and the eyecups were then cut radially to make RPE flat. The dissected eyecups were fixed in 4% PFA at 4°C overnight and then subjected to immunostaining. When mounting samples, the RPE side was made sure to be apical. For retinal sectional immunostaining, eyeballs were dissected in 4% PFA, and the anterior segment tissues and lenses were removed. The eyecups were fixed again in 4% PFA at room temperature for 2 hours followed by dehydration with 20% sucrose at 4°C and being embedded in OCT compound. Then 14 μm of frozen sections were used for immunostaining. Human eyecups were obtained from the Eye Bank of Wenzhou Medical University & Wenzhou Eye Bank and fixed in 4% PFA for 12 hours at 4°C. 5 μm paraffin sections were used for immunostaining. For cell immunostaining, cells were fixed in 4% PFA at RT for 1 hour and then subjected to immunostaining. For human retinal immunostaining, the paraffin section was deparaffined at room temperature and then treated by TrueBlack to remove the autofluorescence of RPE. The primary and secondary antibodies are listed in Table 1. The images were taken by the microscope Zeiss Air scanner 800.

Caspase-1 inflammasome activity assay

The activity of Caspase1 was measured with the Caspase-Glo® 1 Inflammasome Assay kit (Promega, G9951) according to the protocol. Briefly, 25μl of the recombinant Caspase1 (Sigma, C5482) was mixed with the same volume of recombinant SERPINE3 solution (Cedarlane, 116484) and incubated for 5min at room temperature. Then, the 50μl of reconstituted Z-WEHD Substrate was added into the mixed solution. Luminescence was recorded after 30 minutes.

Co-Immunoprecipitation

The protein binding ability was analyzed by the co-immunoprecipitation kit (Thermo Scientific, 26149), and the co-IP was performed according to the standard protocol. Briefly, the IP Lysis buffer contained protease inhibitors cocktails (Sigma, P1860) was applicated to extract the protein of HEK293T cells transfected by the *SERPINE3-Myc* (Origene, RC225688) and *CASPASE1-HA* plasmids in which the Myc tag of Human CASP1 Clone (Origene, RC218364) was replaced with HA tag). In addition, 100μg of primary antibody was coupled with 100μl of the Resin in the reaction hood at room temperature for 1.5 hours. The protein samples (400μl) were added into the hood with the Resin conjugated the antibody and shook overnight at 4°C. The target proteins recognized by the antibody was separated by the filter and subjected to the western blot assay.

Western blot

For protein extraction, RPE tissue or RPE cells were lysed by RAPI buffer containing cocktail proteinase inhibitor on ice for 10 min. Samples were separated by 10% SDS-PAGE. Table 1 shows information on antibodies used in western blot. Primary antibodies were incubated at 4°C overnight. The quantification of the protein bands was done using Image J software.