SUPPLEMENTARY INFORMATION

SUPPLEMENTARY MATERIAL AND METHODS

Electroretinogram (ERG): Mice were dark adapted overnight and were prepared for ERG under dim red light. They were anesthetized with ketamine (100mg/kg) and xylazine (5mg/kg) body weight via intraperitoneal (IP) injection. For dilation of the pupil, a drop of 1% (w/v) Atropine and 1% (w/v) Tropicamide (Akorn Inc., Lake Forest, IL) along with a drop of 0.5% (w/v) Proparacaine HCl (Alkon Lab, Fort Worth, TX) as local anesthetics were applied on the cornea. Throughout the whole procedure heating pad at 37ºC was used to keep them warm and maintain their body temperature. For ERG measurement, a designated gold electrode was placed on the cornea, a reference electrode was positioned on the head, a ground electrode was placed at the tail, and the mouse was placed in a Ganzfeld illuminating sphere. For scotopic ERG, three strobe flash stimuli were presented at flash intensities 1, 2.88 and 3.3 log cd.s/m^2. The amplitude of the a-wave was measured from the pre-stimulus baseline to the a-wave trough and the amplitude of b-wave was measured from the trough of the a-wave to the peak of the b-wave. Flicker response was measured at 3, 10 and 20 Hz.

Western Blot Analysis: Two retinas from each animal were placed in 100µl of T-PER tissue protein extraction reagent containing protease inhibitor cocktail on ice, briefly sonicated, and centrifuged at 12000 rpm for 20 min at 4°C. Protein concentration of the supernatant was measured by BCA assay (Thermo Scientific) and separated by gel electrophoresis using pre-cast Tris-Glycine gels (Novex; Life Technologies). The proteins were transferred to polyvinylidene difluoride (PVDF) membranes using Bio-Rad Trans-Blot turbo transfer system. For immunodetection, the membrane was blocked by 5% BSA in tris buffered saline (TBS) containing 0.1% Tween-20 (TBS-T) for 1h and incubated with primary antibodies (1:2000) for LC3B (Sigma, L7543), p62 (Sigma, P0067), Rab7 (Sigma R8779), Rab5 (Abcam Ab-18211), Lamp1 (Santa Cruz, sc-17768) and GAPDH (Invitrogen, PA1-987) overnight at 4°C. After multiple washes with TBS-T membrane was incubated with either anti-mouse-HRP (PerkinElmer NEF82200) or anti-rabbit-HRP (PerkinElmer NEF81200) for 2h at 4°C washed with TBS-T, detected by ECL detection reagent (Pierce; Thermo Scientific) and imaged using Odyssey Imaging system (LI-COR). Densitometric analysis was performed using manufacturer's analysis software and were normalized to GAPDH. For rat retinal muller cell lines, similar procedure was followed.

Immunofluorescence Analysis: For immunohistochemistry, paraffin embedded retinal sections were de-paraffinized by washing in 100% xylene twice for 2 min each, xylene, and ethyl alcohol (1:1) for 2 min, followed by washing with 100%, 95%, 85%, 75%, 50% of ethyl alcohol for 2 min each and finally in de-ionized water for twice for 2 min each. After de-paraffinization, tissue sections were washed with phosphate buffered saline (PBS) containing 0.1% Tween 20 and blocked with 10% horse serum in 0.1% PBS-T for 1h. Tissue sections were incubated with primary antibodies (1:200) in 0.05% PBS-T at 4ºC overnight. For immunocytochemistry, coverslips with HCQ treated and untreated cells were washed with PBS and fixed with 4% paraformaldehyde for 20 min at room temperature. They were blocked with 10% horse serum in 0.1% PBS-T for 1h and incubated with primary antibodies (1:400) in 0.05% PBS-T at 4ºC overnight.

After incubation with primary antibodies, both tissue sections and fixed cells were washed thrice with 0.05% PBS-T and incubated with Alexa Fluor conjugated respective secondary antibodies for 2h. Following washing with 0.05% PBS-T they were mounted using ProLong Diamond Antifade with DAPI (Invitrogen) and covered by glass coverslips. They were imaged using confocal laser-scanning microscope (LSM 720, Carl Zeiss AG) and analyzed using Image J software.

In addition to the antibodies described in previous section, Lamp1 (Invitrogen MA1164) and ssDNA (Millipore MAB3868) were used.

Sphingolipid Analysis: Sphingolipids from mice retinas were analyzed at the Lipidomic core at the Virginia Commonwealth University, Richmond, VA following a previously published protocol (1). Samples were dissolved in a cocktail of CH_3OH:$CHCl_3$ (2:1). Internal standards for sphingolipid metabolites were purchased from Avanti Polar Lipids. The internal standards of 500 pmol each were dissolved in the cocktail of C_2H_5OH:CH_3OH:H_2O (7:2:1) and added to the sample having the final volume of 20 µl. The detailed method of sample preparation and processing was followed as in our previously published article (1). Sphingolipids were separated by reverse phase liquid chromatography (LC) using a Supelco 2.1 (i.d.) x 50mm Ascentis Express C18 column (Sigma, St. Louis, MO) and a binary solvent system at a flow rate of 0.5 mL/min. Before running and eluting the samples in the LC, the column was equilibrated for 30 s with a solvent mixture of 95% Mobile phase A1 (CH_3OH/H_2O/HCOOH, 58/41/1, v/v/v, with 5mM ammonium formate) and 5% Mobile phase B1 (CH_3OH/HCOOH, 99/1, v/v, with 5mM ammonium formate). After injecting the sample in the column, the A1/B1 ratio was maintained at 95/5 for 2.25 min, followed by a linear gradient to 100% B1 over 1.5 min, which was held at 100% B1 for 5.5 min, followed by a 0.5 min gradient return to 95/5 A1/B1. The column was re-equilibrated with 95:5 A1/B1 for 30 s

before the next run. The species of ceramide (Cer), sphingomyelin (SM), sphingoid lipids such as sphingosine (Sph), S1P were identified based on their retention time and *m/z* ratio (mass-to-charge ratio) and quantified as described in the aforementioned article (1).

1. V. Paranjpe *et al.*, Clinical signs of meibomian gland dysfunction (MGD) are associated with changes in meibum sphingolipid composition. *Ocul Surf* **17**, 318-326 (2019).

Supplementary Figures

Supplementary Figure 1

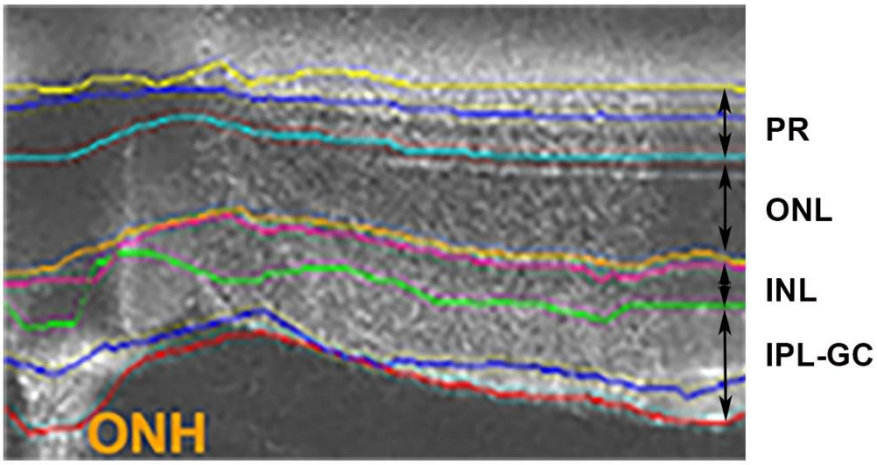

Fig. *S1*. Identification and stratification of retinal layers in SD-OCT image from mouse retina. SD-OCT image of mouse retina was captured by Bioptigen Envisu Image Guided SD-OCT system (Lecia Microsystems, Buffalo Grove, IL). The images are then analyzed using 'Diver Analysis software for small animal models' provided by the manufacturer that generated the full-volume, automated retinal thickness analysis and thickness results. We analyzed the compared the following layers between the control and HCQ-treated mice- PR, photoreceptor; ONL, outer nuclear layer; INL, inner nuclear layer; IPL-GC, inner plexiform-ganglion cell layer. ONH: Optic Nerve Head.

Supplementary Figure 2

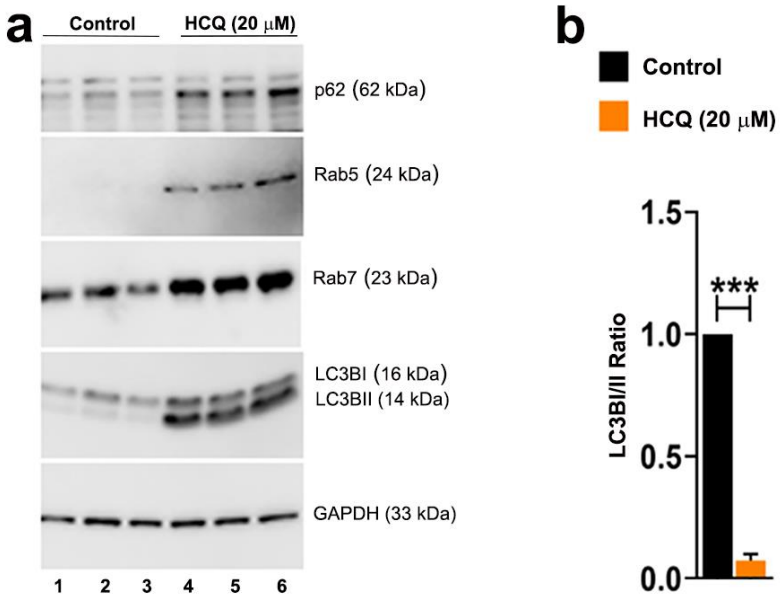

Fig. *S2*. HCQ treatment alters autophagosome-lysosomal pathway in cultured Muller cells. (***a***) Western blot analysis of relevant autophagosome-lysosomal pathway proteins from rat Muller cells treated with and without 20μM HCQ for 24 hrs. (n=3 per group). Increase in protein levels of autophagy markers p62 and LC3BII and endosomal markers Rab5 and Rab7 were observed in the treated cells. (***b***) Quantification analysis of LC3B protein expression in (***a***) showing significant decrease in the ratio of LC3B I and II. (Values are mean ±SEM, ***p≤0.001).

Supplementary Table 1: Primers used and their sequences.

Name of the Genes	Sequence (5'-3')
Rod	Forward: CTTCCTGATCTGCTGGCTTC Reverse: ACAGTCTCTGGCCAGGCTTA
Opn1sw	Forward: CATCATTCCTCTTTCCCTCAT Reverse: TGTTTTCTGAGAGCCAGACAC
Opn1mw	Forward: TGAGATTTGATGCTAAGCTGG Reverse: TGCCGGTTCATAAAGACATAG
Arr3	Forward: GGGTCAATGCCTATCCTTTT Reverse: TTACTGCAAAGGTCTGGGAG
Sag1	Forward: CTGGCAGTTCTTCATGTCTG Reverse: ATGCTTGATCTTCCCATCCA
Rpe65	Forward: GAAATCTGGATGTGGCAAGA Reverse: AGTCCATGGAAGGTCACAGG
PKCα	Forward: TCAATGTCCCTAGCCTCTGC Reverse: GCTCTCATTCTTGGGGTCAG
Mrtk	Forward: AACAACACGGGGAATGACTC Reverse: TCACGAAGGTTGTTCTCGTG
Gfap	Forward: GCCAGTTACCAGGAGGCACT Reverse: ATGGTGATGCGGTTTTCTTC
Ccl2	Forward: GTTAATGCCCCACTCACCTG Reverse: TTCCTTATTGGGGTCAGCAC
Egr1	Forward: CTACGAGCACCTGACCACAG Reverse: AGGCCACTGACTAGGCTGAA
Atg4b	Forward: GTTGCATCCAGACTTTGGTTTAC Reverse: GATCATCTGTCCACACCGAAG
Lamp2	Forward: CAAAGGGTACTTGCCTTTATGC Reverse: TGTCAGGTACTGCAATGGTTAT
TcFEB	Forward: CAACAGTCCCAGCATCAGAA Reverse: GGCGCATAATGTTGTCAATG
Smpd1	Forward: CAGTTCTTTGGCCACACTCA Reverse: TCCGGGGTAGTTTCCATCTA
Smpd2	Forward: ACGTGCTTTACAAGGCAGTC Reverse: AGGACACACAGCAACACCAG
Asah1	Forward: GCCCAGTGGGTAGGGTTTAT Reverse: CACATACCATCTGCCATGCT
Sphk1	Forward: GATGCATGAGGTGGTGAATG Reverse: AACAGCAGTGTGCAGTTGAT
Sphk2	Forward: GAAGGCATTGTCACTGTGTC Reverse: GCAGAGAAGAAGCGAGCAGT
Rpl19	Forward: TCACAGCCTGTACCTGAAGG Reverse: TCGTGCTTCCTTGGTCTTAG
Gapdh	Forward: CTGAACGGGAAGCTCACTG Reverse: ACCACCCTGTTGCTGTAGC

Supplementary Table 2: Multivariable regression to test age effect on the outcome of interest.

Outcome	Predictors	p-value for age effect
Centre-cube-vol	Duration (Year) + Age	0.51
Centre-cube-thickness	Duration (Year) + Age	0.90
GC-IPL-thickness	Duration (Year) + Age	0.55
Centre-cube-vol	Total-accumulative-doses + Age	0.42
Centre-cube-thickness	Total-accumulative-doses + Age	0.50
GC-IPL-thickness	Total-accumulative-doses + Age	0.47