Supplementary materials

Supplementary Materials and Methods.

Animals. We obtained young and aged Fischer 344 rats from Charles River Laboratories and the National Institute on Aging [NIA]. We used three months old adult rats as young control and aged rats at 22-24 months of age with or without 8-AG treatment. The 22 months old rats were treated with 8-AG (5 mg/kg body weight per day) by supplementing 8-AG in drinking water or water only for the untreated group. The C57 black 6 (C57BL/6J) mice (*Rho*$^{+/+}$, Stock no-000664) and (*Rho*$^{P23H/P23H}$ knock-in, Stock no-017628) mice (1) were purchased from Jackson Laboratory. The *Rho*$^{P23H/+}$ mice was generated by breeding the *Rho*$^{+/+}$ with *Rho*$^{P23H/P23H}$ animals. All animals were housed under standard 12-h light/12-h dark conditions in the animal facility at University of Pittsburgh. Animals were anesthetized before the experimental procedure by intraperitoneal injection (i.p.) using a cocktail of ketamine/xylazine, containing 80 mg/kg body weight (bw) ketamine and 7 mg/kg bw xylazine (Henry Schein, Dublin, OH USA). Pupils were dilated with 1% tropicamide (Akorn, Lake Forest, IL, USA) and 2.5% phenylephrine (Sigma) before spectral domain-optical coherence tomography (SD-OCT) and electroretinogram (ERG). Eyes were lubricated by the hypromellose (0.3%) (Alcon, Fort Worth, TX, USA) eye gel when the animals were anesthetized. All animal experiments followed the animal welfare act and regulations guide and approved by the University of Pittsburgh Institutional Animal Care and Use Committee (IACUC; rat protocol number 23073400, mouse protocol number 23053112).

Electroretinogram (ERG). Animals (rat & mice) were dark adapted overnight and ERG was performed using the Celeris system (Diagnosys, Lowell, MA, USA) under dim red light. Pupils were dilated with 1% tropicamide (Akorn, Lake Forest, IL, USA) and 2.5% phenylephrine (Sigma). Animals were anesthetized, pupils were dilated, and eyes were lubricated. The body temperature was maintained at 37 ^{o}C. Scotopic ERG responses of were taken from dark-adapted eyes stimulated by flash intensities from 0.0001 to 100 cd·s/m^2 and averaged from three sweeps per flash intensity with inter-sweep intervals of 10 to 30 s. Then photopic ERG responses were taken after the eyes were light adapted at 10 cd/m^2 illumination for 10 min, stimulations were flashed from 0.01 cd·s/m^2 to 100 cd·s/m^2 on top of

the 10 cd/m^2 background light. *P*-values were calculated by a two-way ANOVA to determine the statistical significance between the different treatment groups. Factor 1, treatment condition; and factor 2, flash intensity.

SD-OCT. We performed SD-OCT (Bioptigen, Morrisville, NC) to access retinal morphological changes in vivo. Animals were anesthetized, pupils were dilated, and eyes were lubricated. A heating pad was used to maintain body temperature; the A scan/B scan ratio was set at 1200 lines. Five frames of retinal B-scan images were acquired at 0°, averaged, and saved. Thickness of the ONL and OS/IS was measured along the retinal OCT scanning image at 6 points from temporal to nasal side.

Tissue collection and Immunohistochemistry (IHC). Animals were euthanized by CO_2 inhalation. The superior side of the eye was labeled with a burn mark using a cautery pen. Enucleated eye was proceeded for eye cup preparation by removing the anterior part including cornea, iris, and lens. The eye cups were fixed with 4% paraformaldehyde (PFA) for 2 hours and then dehydrated sequentially in 5, 10, 20, and 40% sucrose solutions in PBS for 30 min each followed by overnight incubation with a mixture of 40% sucrose in PBS and O.C.T. compound (1:3 ratio) (FisherScientific, Houston, TX USA). These eyes were embedded and frozen in liquid nitrogen-bathed isobutene. Twelve-micron of retinal cross-sections containing the optic nerve head were mounted onto a SuperFrost glass slide (FisherScientific, Houston, TX USA) and used for IHC. Retinal flat mounts were prepared from eye cups by making four cuts to flatten the retina followed by removal of sclera. Retinal flat mounts were fixed in 4% PFA for 2 hours and proceeded for CD68 and IBA1 immunostaining.

IHC was performed on retinal cryosections and flat mounts. The cryosections were rehydrated and permeabilized in PBST (0.1% triton-X-100) for 30 min, and then incubated with 5% goat serum in PBST for 30 min followed by incubation with corresponding primary antibodies in 5% goat serum for 2 hours. Primary antibodies applied as followed (Mouse anti-rhodopsin (1D4, 20 µg/mL), mouse anti-arrestin (MCA-S1288, Encor Biotecnology, 1 µg/ml), rabbit PDE-6B (PA1-722, Invitrogen, 4 µg/ml), Rabbit anti-TOMM20 (ab186734, Abcam, 0.5 µg/ml), rabbit anti-CD68 (ab125212, Abcam, 1 µg/mL), Rabbit SPICA DyeTM 568-conjugated anti-Iba1 (015-28011, Wako Pure Chemical Corporation, Osaka, Japan, 1:200

dilution), rabbit anti-GFAP antibody (z0334, Sigma-Aldrich, 6.4 µg/mL), anti 8-OH dG (NB110-96878, Novus Biologicals, 2 µg/ml). After three washing with PBST tissue was incubated with corresponding secondary antibodies goat anti-mouse Alexa Fluor 546 (A-11030, ThermoFisher, 5 µg/mL) and goat anti-rabbit Alexa Fluor 488 (A-11008, ThermoFisher, 2 µg/mL) in PBST having Hoechst 33342 (62249, ThermoFisher, 2 µg/mL) for 1 hour. The tissue was washed in PBST and mounted with the ProlongGold mounting solution (ThermoFisher, Waltham, MA USA) followed by confocal imaging using Olympus FV1200 confocal microscope. Click-iT plus TUNEL assay, Alexa fluor 594 kit (ThermoFisher Scienitifc, C10618) was used for in-situ apoptosis detection following the manufacture's manual. Fluorescence intensity, thickness measurements and cell counting were measured using Fiji ImageJ (imagj.nih.gov/ij) (2).

Retinal histology and H&E staining. To examine the retinal morphology, animals were euthanized as described earlier and eyes were enucleated and fixed in 4% PFA and 1% glutaraldehyde for 24 hs, before paraffin embedding. Paraffin sections (7 µm thick) were stained with H&E and imaged by light microscopy (Leica, Wetzlar, Germany). The retinal thickness measurements and cell counting were measured using Fiji ImageJ.

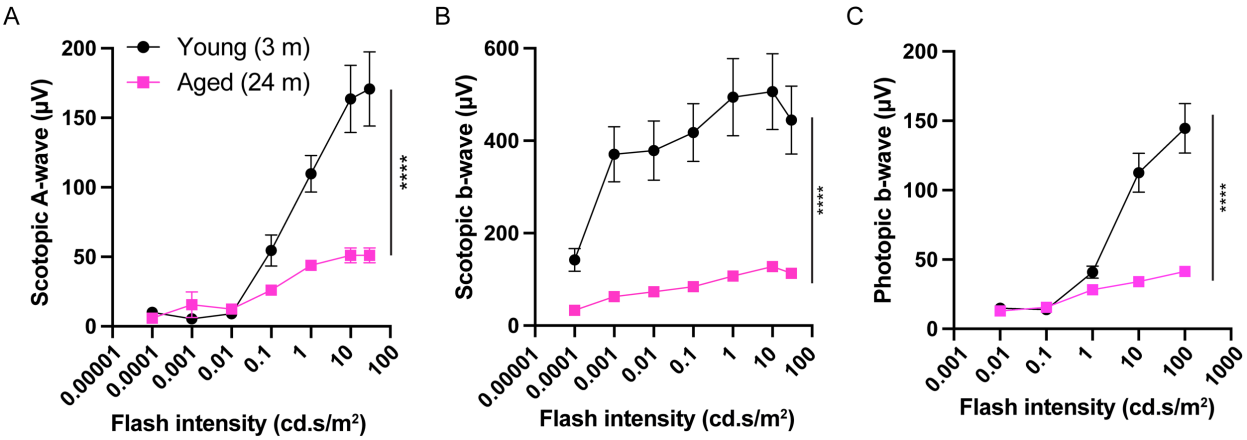
RNA-seq and transcriptome analysis. Young, aged untreated and aged 8-AG treated rats' retinae were collected in RNAzol (Sigma-Aldrich, Saint-Louis, MO) and total RNA was isolated following manufacturer's instructions. After retina isolation, RPE/choroid was isolated by washing the remaining eye cup with RNAzol. Only samples with RNA Integrity Number (RIN) >7 was selected for poly-A captured mRNA library preparation. The quality check of library size was done using bioanalyzer and RNA-seq was performed using Illumina HiSeq platform by QuickBiology Inc. The UCSC transcript set (Bowtie2 version 2.1.0) was used for the mapping of reads and RSEM v1.2.15 was used to estimate the expression level. The edgeR program was used to identify differentially expressed genes (DEGs) which was further filtered by selecting the FDR <0.05 and fold change >1.5. Gene ontology (GO) enrichment analysis and pathway analysis were done using Goseq and Kobas, respectively.

Immunoblotting. Retinae were collected from euthanized animals and homogenized using Lysing Matrix D in a FastPrep 24 instrument (MP Biomedicals, Solon, OH) in HBSS (5 mM KCl, 0.3 mM KH_2PO_4, 138 mM NaCl, 4 mM $NaHCO_3$, 0.3 mM Na_2HPO_4, 5.6 mM glucose and 10 mM Hepes, pH 7.4) containing complete protease inhibitor cocktail (1 tablet/10 mL, Roche, Indianapolis, IN) and phosphatase inhibitor cocktail (Sigma, 1:100). After centrifugation (16200g; 15 min at 4ºC), the membrane protein fraction was prepared by suspending the membrane pellets in lysis buffer containing 0.3 M NaCl, 50 mM Tris-HCl (pH 7.6) and 0.5% Triton X-100 and the same concentration of protease inhibitors as above. The suspensions were incubated on ice and centrifuged (16200 x g; 15 min at 4 ºC). The protein concentrations of the combined supernatants were determined using the Pierce BCA protein assay (Thermo Scientific, Rockford, IL). In the presence of Laemmli sample buffer, lysate from each sample was separated on a 4-15% TGX Stain-Free SDS-PAGE gel (Bio-Rad Laboratories, Hercules, CA). As a reliable loading control, total protein measurement per sample was determined using Bio-Rad Stain Free SDS-PAGE gel technology. Ultraviolet-activated protein fluorescence was imaged on a ChemiDoc MP (Bio-Rad). After proteins were transferred to polyvinylidene fluoride membranes, the membranes were incubated in 5% (w/v) dried milk dissolved in TBS-T (20 mM Trizma, 137 mM NaCl, 0.1% Tween-20, pH 7.6), rinsed with TBS-T, and incubated overnight at 4ºC with primary antibodies, including horseradish peroxidase (HRP)-conjugated 1D4 anti-RHO (0.2 µg/mL, prepared by HRP-conjugation kit on 1D4 anti-RHO antibody shared by Dr. Krzysztof Palczewski), rabbit anti-cleaved caspase-3 (Cell Signaling, 9664, 1:1000), rabbit anti-caspase 3 (Cell Signaling, 9662, 1:1000), mouse anti-arrestin (MCA-S1288, Encor Biotecnology, 0.5 µg/mL), rabbit anti-PDE-6b (PA1-722, Invitrogen, 1 µg/mL), and mouse anti-GAPDH (MA515738, Life Technologies, 0.2 µg/mL) antibodies diluted in TBS-T containing 5% (w/v) milk or 5% (w/v bovine serum albumin (BSA). After washing in TBS-T, the membranes were probed with corresponding secondary antibody (Sheep anti-mouse HRP; 1:5000; Southern Biotech, Birmingham, AL or Donkey anti-rabbit HRP; 1:10,000; Advansta, San Jose, CA) for 1 hour in 5% (w/v) Milk TBS-T, washed, and incubated in WesternBright Quantum (Advansta, Menlo Park, CA) and then imaged on a ChemiDoc MP (BioRad). After detection and analysis of cleaved caspase 3 membranes were stripped with Western Blot Recycling buffer (Alpha Diagnostics International, San Antonio, TX), rinsed with blocking buffer and probed for total caspase 3. The intensity of each protein band was determined and normalized to the total protein using Image Lab software (Bio-Rad).

Transmission Electron microscopy. Eyes were harvested and immersion fixed 2.5% glutaraldehyde and 2% paraformaldehyde in PBS overnight at 4 ^{o}C. Following fixation, tissue washed 3x in PBS then post-fixed in aqueous 1% OsO_4, 1% $K_3Fe(CN)_6$ for 1 hour. Following 3 PBS washes, the tissue was dehydrated through a graded series of 30-100% ethanol, 100% propylene oxide then infiltrated in 1:1 mixture of propylene oxide:Polybed 812 epoxy resin (Polysciences, Warrington, PA) for 1 hr. After several changes of 100% resin over 24 hours, tissue was embedded in molds, cured at 37 ^{o}C overnight, followed by additional hardening at 65 ^{o}C for two more days. Ultrathin (60 nm) cross-sections of intestine were collected on copper grids, stained with 1% uranyl acetate for 10 minutes, followed by 1% lead citrate for 7 min. Sections were imaged using a JEOL JEM 1400 Flash transmission electron microscope (Peabody, MA) at 80 kV, imaged with a bottom-mount AMT 2k digital camera (Advanced Microscopy Techniques, Danvers, MA).

Purine metabolome analyses. Retina was isolated, put in a vial and flash frozen in liquid nitrogen and stored at -80 ^{o}C until further processed. Each frozen retina was put in a 50 mL tube and rapidly submerged with 6 mL of ice-old tissue lysis buffer containing a mixture of acetonitrile, methanol and water at 1:2:2 volume ratio. The tissue was homogenized using a manual homogenizer on ice. Then, the tissue homogenate was heated at 60^{o}C for 10 min for complete enzyme denaturation. One mL of the tissue homogenate was aliquoted and centrifuged at 3000 x g for 90 minutes at 4 ^{o}C and the supernatant was transferred to a clean tube. The pellet was then mixed with additional 0.5 mL tissue lysis buffer and centrifuged at 3000 x g for 30 minutes at 4^{o}C and this supernatant was added to the supernatant collected from the first round of centrifugation. The 1.5 mL of supernatant was then dried in a sample concentrator down to about 0.3 mL, followed by an addition of 0.7 mL of water to make 1 mL. Each sample was filtered using a 30 kDa Micron YM-30 centrifugal unit (MiliPore). One hundred µL of the ultrafiltrated sample was then spiked with internal standard for UPLC-MS/MS analyses. Our UPLC-MS/MS purine metabolome assay was recently updated in reference (3).

Supplementary Figures



Supplementary Figure S1. Electroretinograms young (3 months old) and aged rats (24 months old). Aged rats were treated daily with 5 mg/kg bw of 8-AG from 22 months of age via supplementing the compound in the drinking water. ERGs were recorded at 0 and 8 weeks of treatment. Untreated age-matched animals were supplied with normal water. Four-month-old rats were used as the young control. **A-C.** The amplitudes of scotopic a-wave responses, scotopic b-wave responses, and photopic b-wave responses of rats, respectively, plotted as a function of flash intensity in semi-log format. Black circles, young rats at 3 months of age; and magenta squares, aged rats at 24 months of age. Data and error bars are means±SEM. N=6. ****, P<0.0001 by a two-way ANOVA.

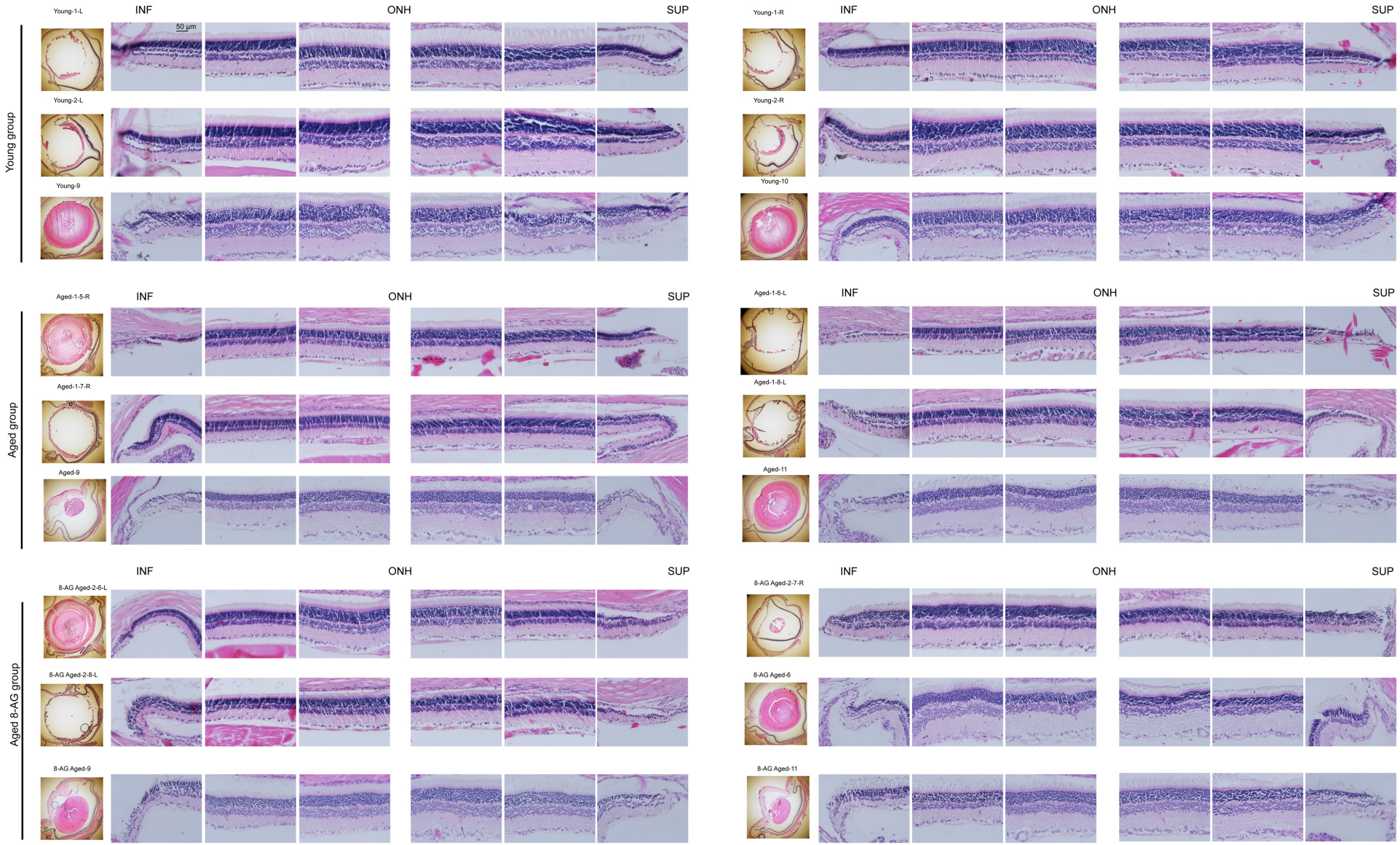
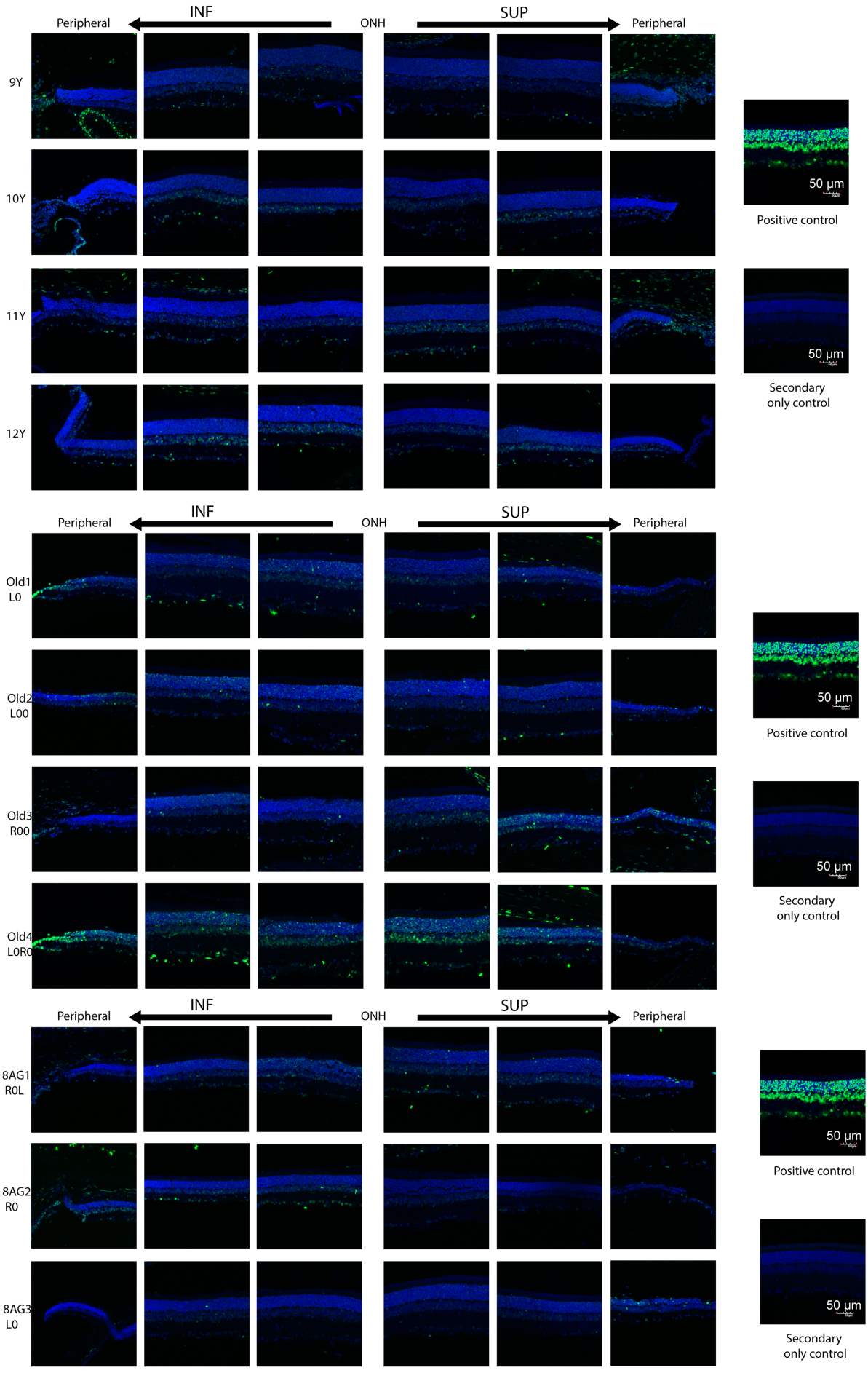
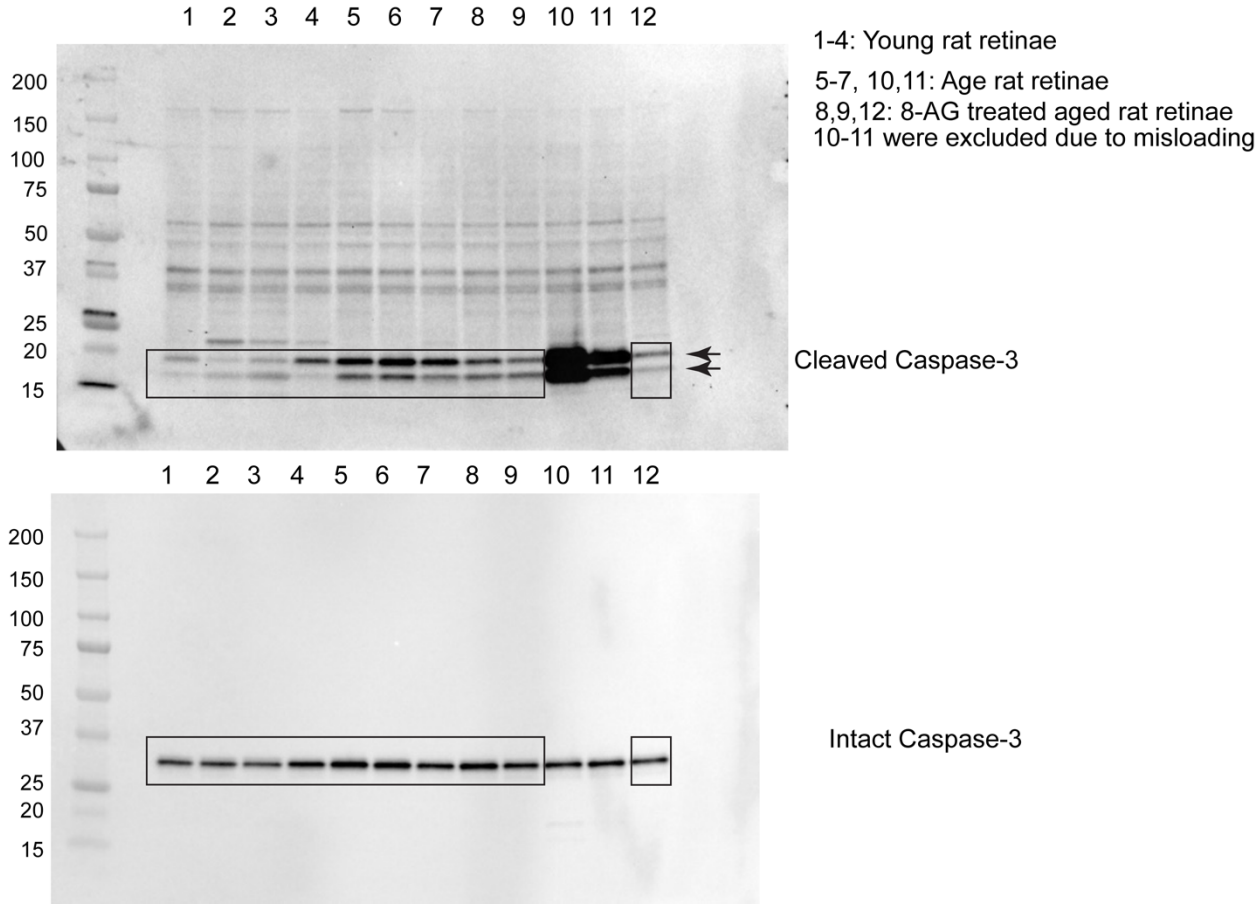

Young group
Young-1-L
INF
ONH
SUP
50 μm
Young-2-L
Young-9
Young-1-R
Young-2-R
Young-10
Aged group
Aged-1-5-R
Aged-1-7-R
Aged-9
Aged-1-6-L
Aged-1-8-L
Aged-11
Aged 8-AG group
8-AG Aged-2-6-L
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8-AG Aged-9
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8-AG Aged-6
8-AG Aged-11

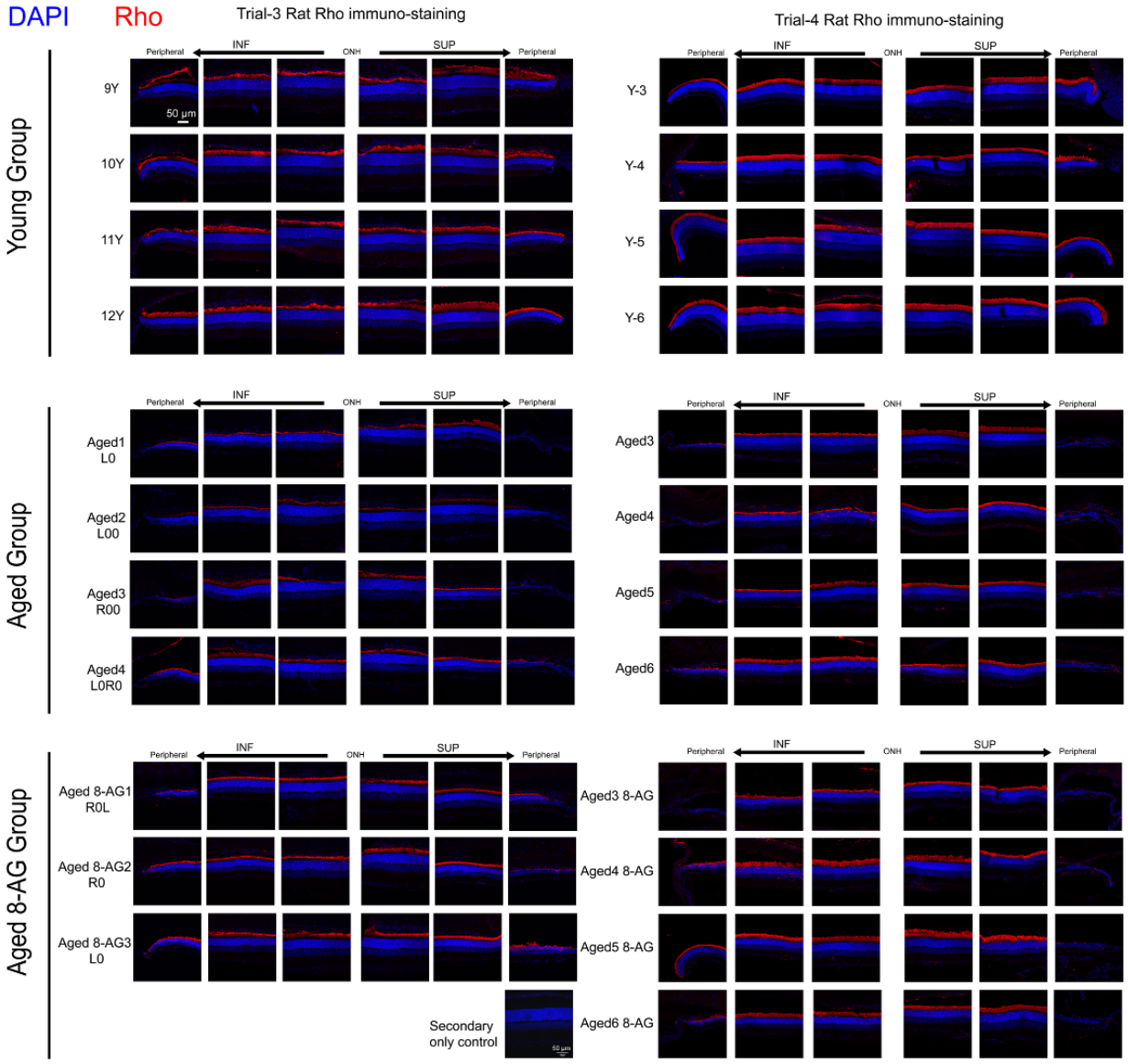
Figure S2. H&E staining of young (3 months old), aged (24 months old) and 8-AG treated aged rat (24 months old) retina cross sections taken at six positions from inferior (INF) to superior (SUP) side. Each group contains six retinae from six animals. The low magnification (2X) images of the whole cross-section are shown on the left. Scale bar for the 40x images, 50 μm.


Peripheral
INF
ONH
SUP
Peripheral
9Y
10Y
11Y
12Y
50 µm
Positive control
50 µm
Secondary only control
Peripheral
INF
ONH
SUP
Peripheral
Old1 L0
Old2 L00
Old3 R00
Old4 L0R0
50 µm
Positive control
50 µm
Secondary only control
Peripheral
INF
ONH
SUP
Peripheral
8AG1 R0L
8AG2 R0
8AG3 L0
50 µm
Positive control
50 µm
Secondary only control

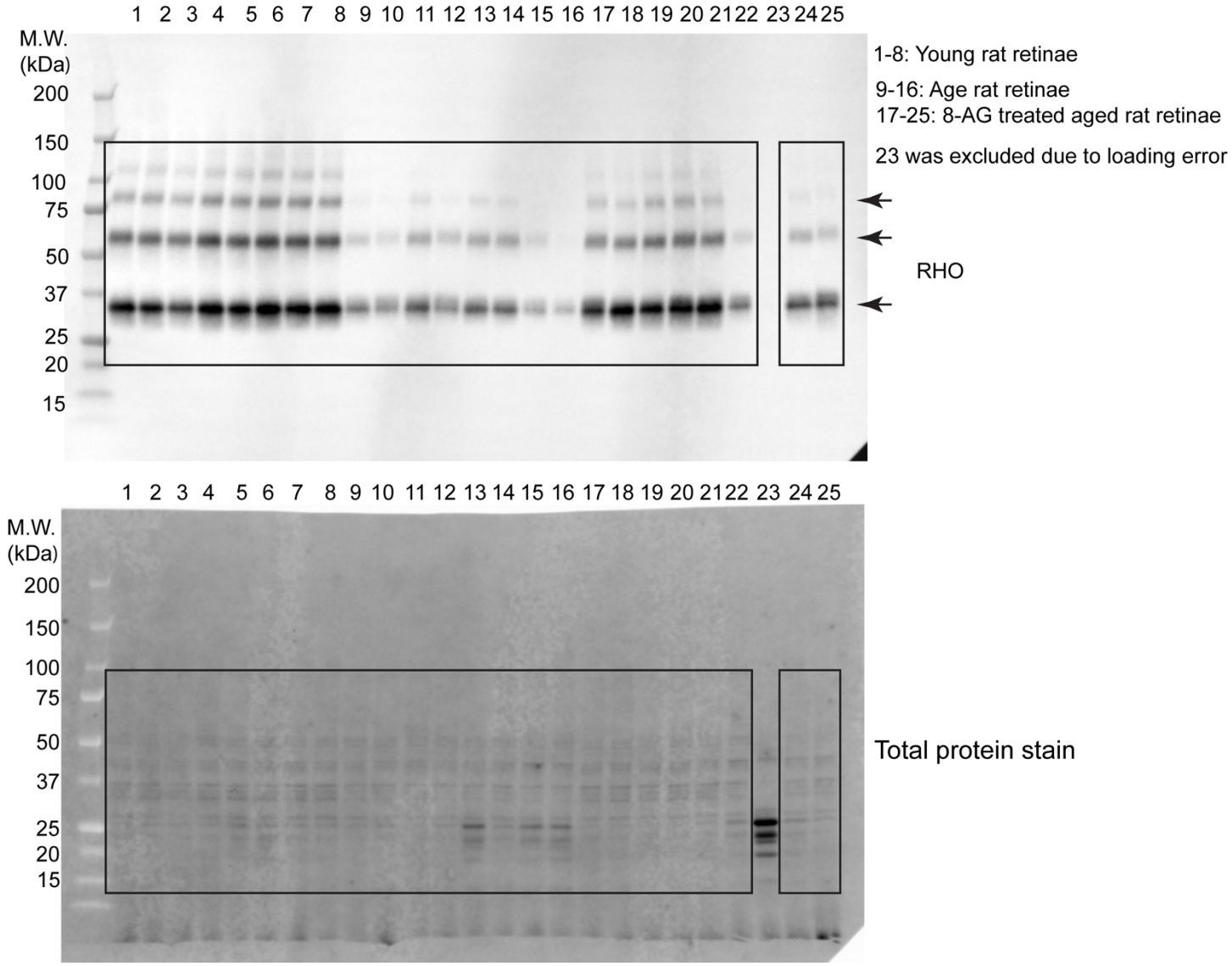
Supplementary Figure S3. TUNEL staining images of young (Y, 3 months), aged (24 months) and 8-AG-treated aged rats (8-AG). Green, TUNEL staining; blue, Hoechst33342 for staining of the nucleus. Six images were taken at the peripheral, equatorial and central areas on the superior and inferior side of each cross-section. N=3-4. Positive and secondary antibody-only controls were shown on the right side, with scale bars of 50 μm.



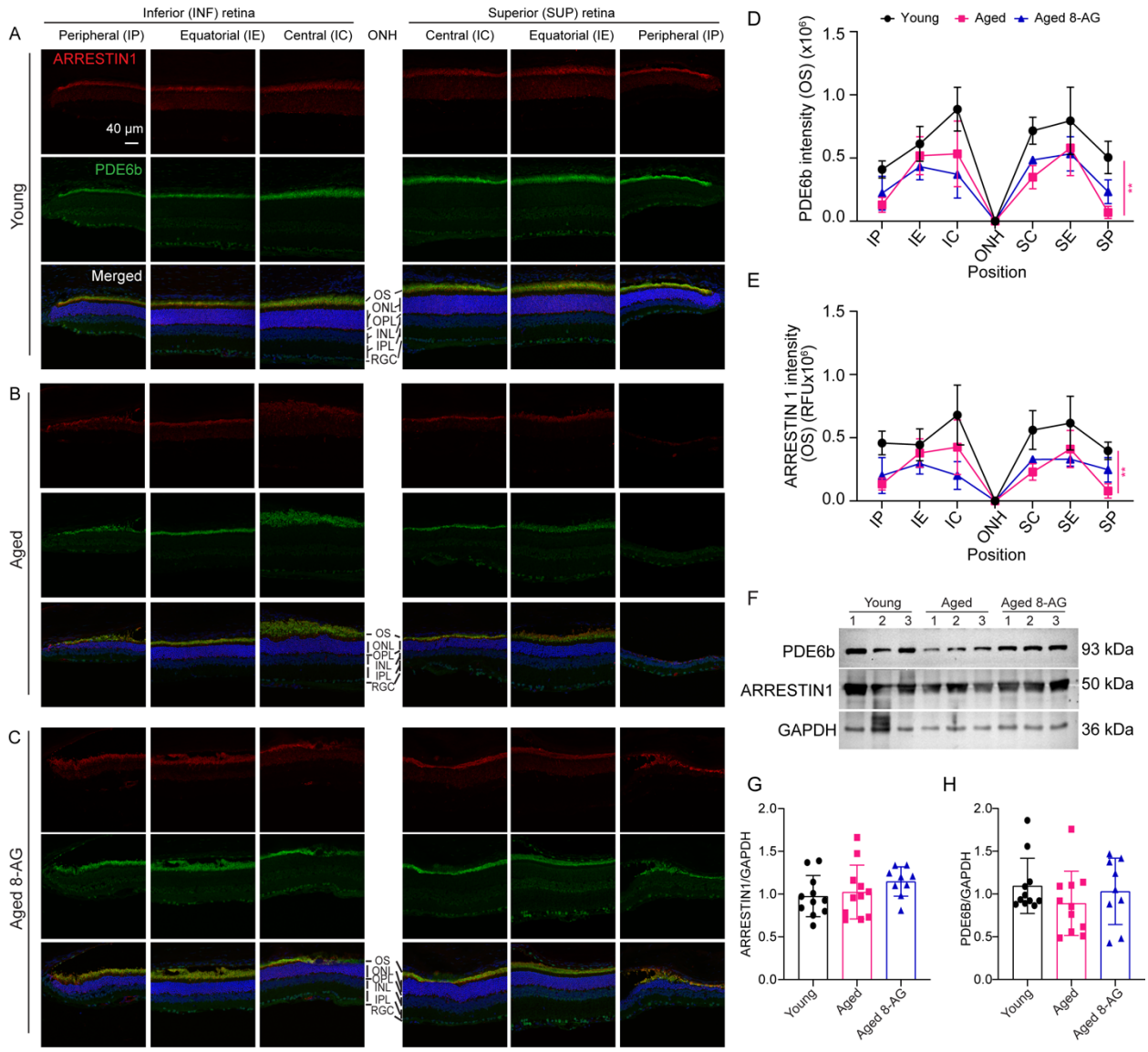
Supplementary Figure S4. Immunoblots of the cleaved and intact caspase-3 in the Fischer344 rat retinal lysates. Each sample of was loaded with 15 μg of total protein. Samples 1-4, young rat retinal lysates (3 months of age); 5-7, aged rat retinal lysates (24 months of age); and 8,9,12, 8-AG treated aged rat retinal lysates (24 months of age). Samples 10 and 11 were excluded due to abnormal bands in the loading control. Top panel is immunoblot of cleaved caspase-3; bottom panel is the immunoblot of the intact casepase-3 as control.



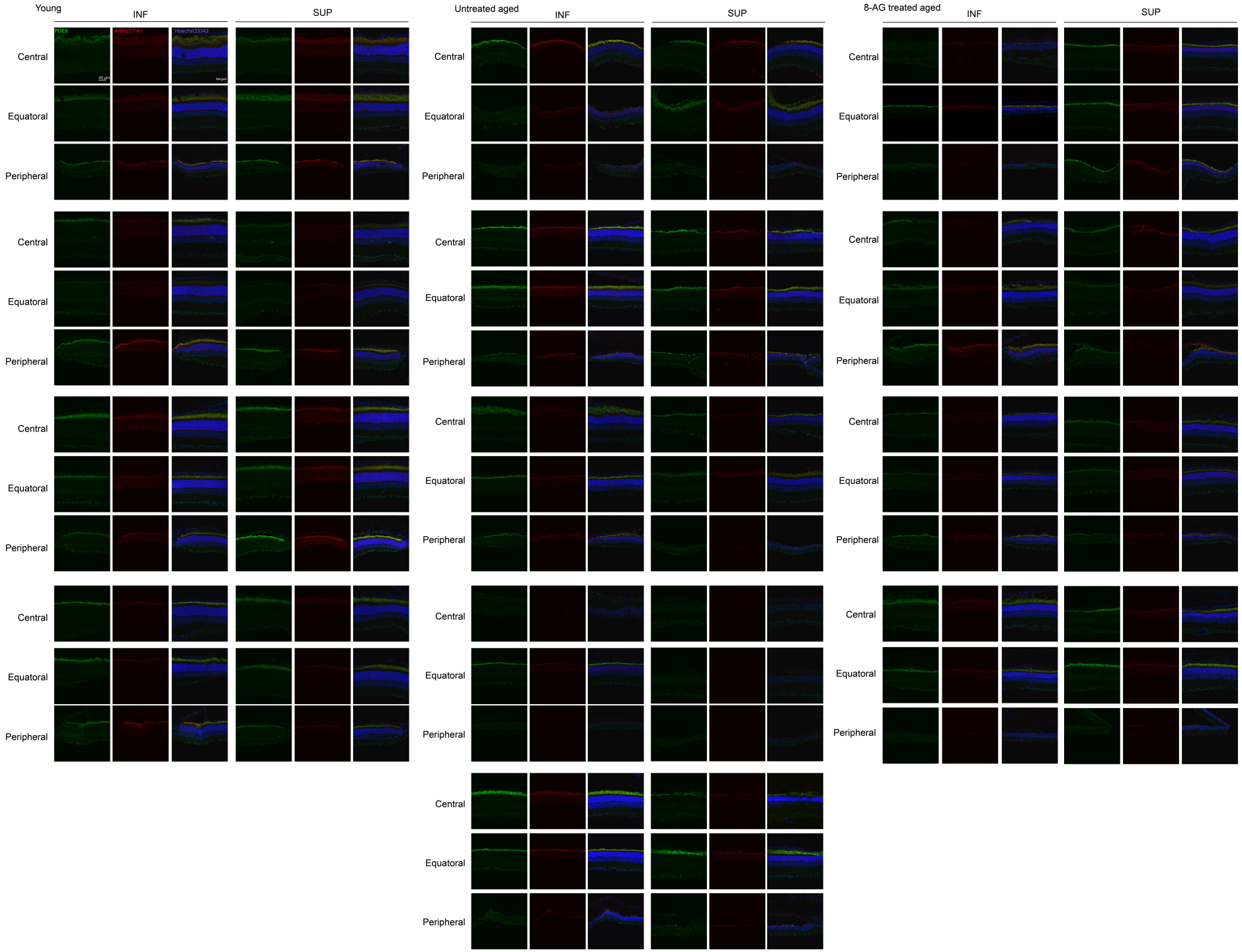
Supplementary Figure S5. Immunostaining of Rhodopsin on the retinal cryosections of the young (Y, 3 months), aged (Aged, 24 months) and 8-AG treated aged rats (Aged 8-AG, 24 months). N=7-8. Red, rhodopsin; blue, Hoechst33342. Scale bar, 50 μm. Secondary antibody only control is shown in the bottom central area.



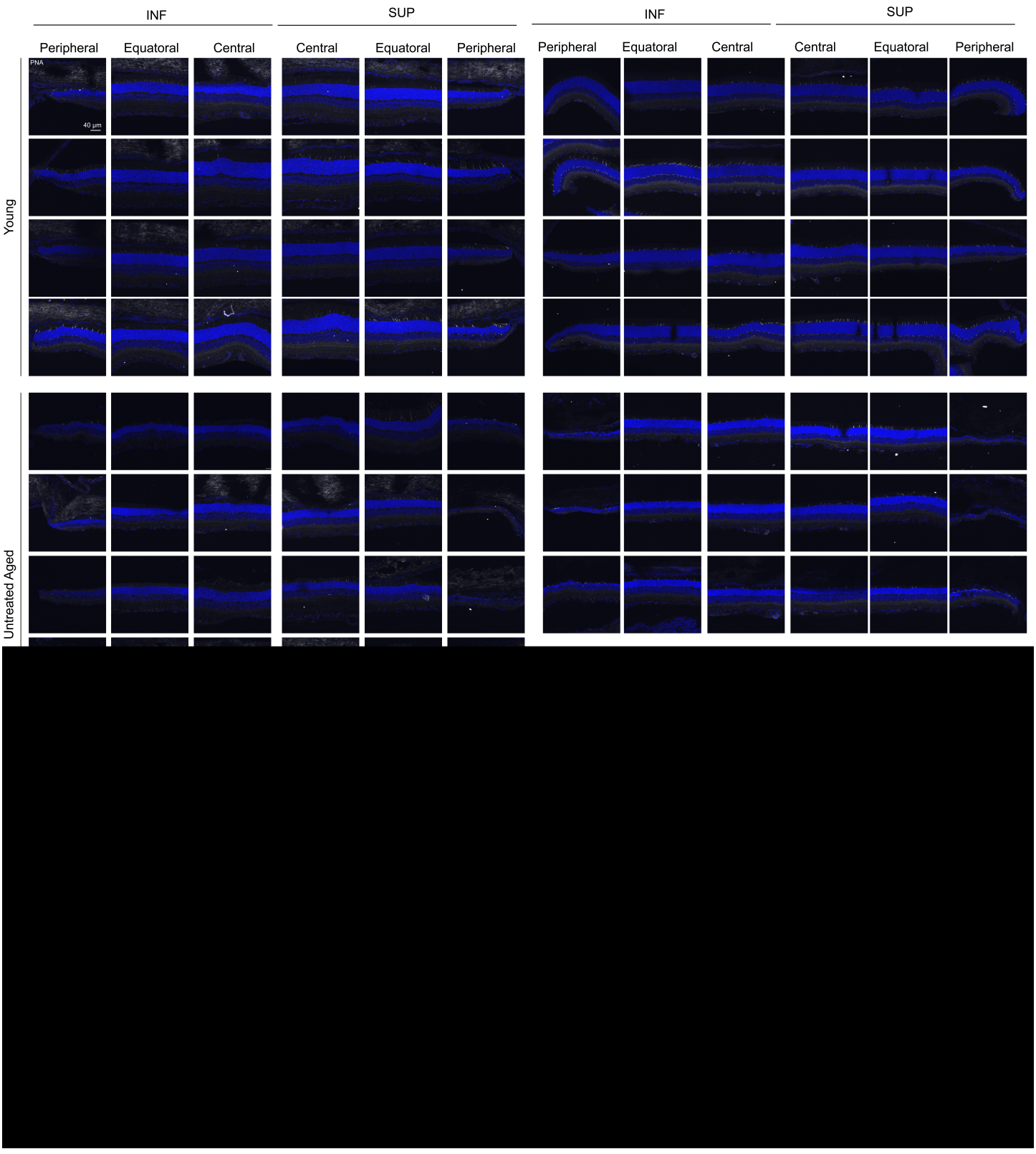
Supplementary Figure S6. Immunoblots of rhodopsin from Fischer344 rat retinal lysates. Each sample was loaded with 2 μg of total protein. Samples 1-8, young rat retinal lysates (3 months of age); 9-16, aged rat retinal lysates (24 months of age); and 17-25, 8-AG treated aged rat retinal lysates (24 months of age). Protein ladders are marked on the left. Sample 23 was excluded due to abnormal bands in the loading control. Top panel, immunoblots of rhodopsin; bottom panel, total protein staining as loading control.



Supplementary Figure S7. Immunostaining and immunoblots of ARRESTIN1 and PDE6b using the retinal cryosections and retinal lysates from the young (3 months), Aged (24 months) and 8-AG treated aged rats (Aged 8-AG, 24 months). A-C, Immunostaining of Arresting 1 (red), PDE6b (green) and Hoechst33342 (blue) on the cryosections of the young, aged and 8-AG treated aged rat retinae, respectively. Scale, 40 µm. D and E, Spidergrams of PDE6b and Arrestin1 immunofluorescence intensities in the outer segments (OS), respectively. Black, young rats; magenta, aged rats; blue, aged 8-AG. Data and errors are means±SD. **, $P<0.01$ by a two-way ANOVA. F, Immunoblots of PDE6b, ARRESTIN1 and GAPDH (loading control) from retinal lysates of young, aged and 8-AG treated aged rats. G and H, column plots of Arrestin 1 and PDE6b band intensities, respectively, normalized by GAPDH loading control.

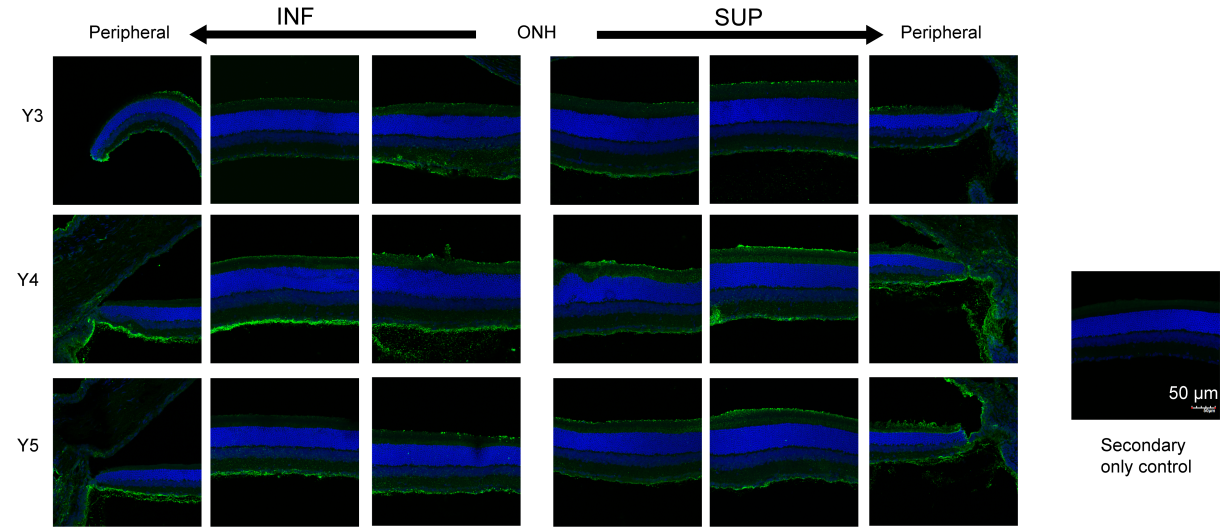

Young
INF
SUP
PDE6
ARRESTIN1
Hoechst33342
40 µm
Merged
Central
Equatoral
Peripheral
Untreated aged
8-AG treated aged

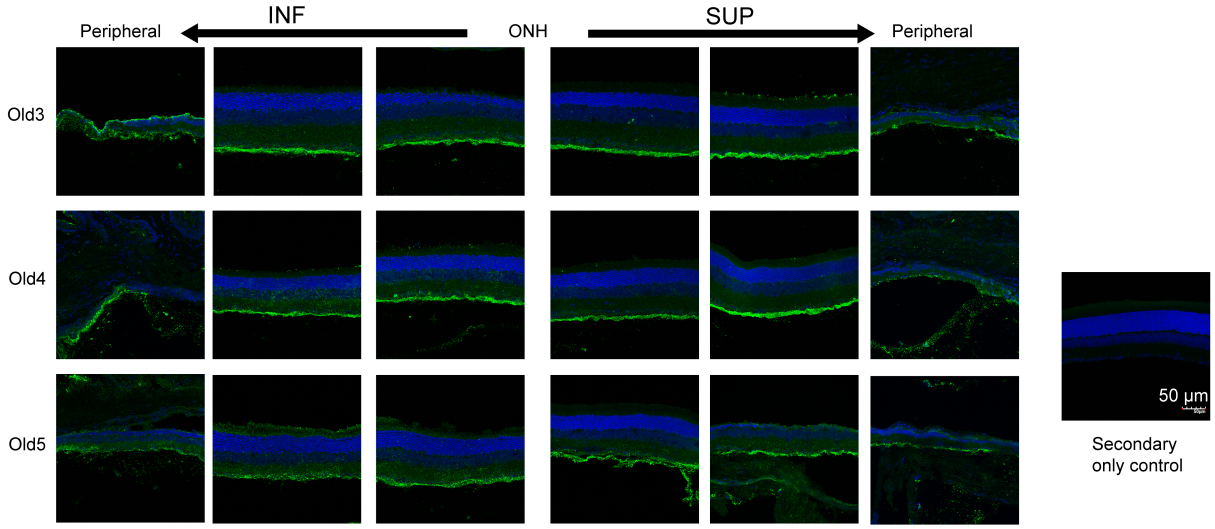
Supplementary Figure S8. Immunostaining of PDE6b and ARRESTIN1 on retinal cryosections from young (Y, 3 months), aged (Aged, 24 months) and old 8-AG treated rats (Aged 8-AG, 24 months). Green, PDE6b staining; red, ARRESTIN1 staining; blue, Hoechst33342 for nucleus staining. Secondary antibody-only controls are listed on the right side, with scale bars of 50 μm. A total of six images were taken from the superior peripheral to the inferior end on each retinal cross-section. N=4-5.

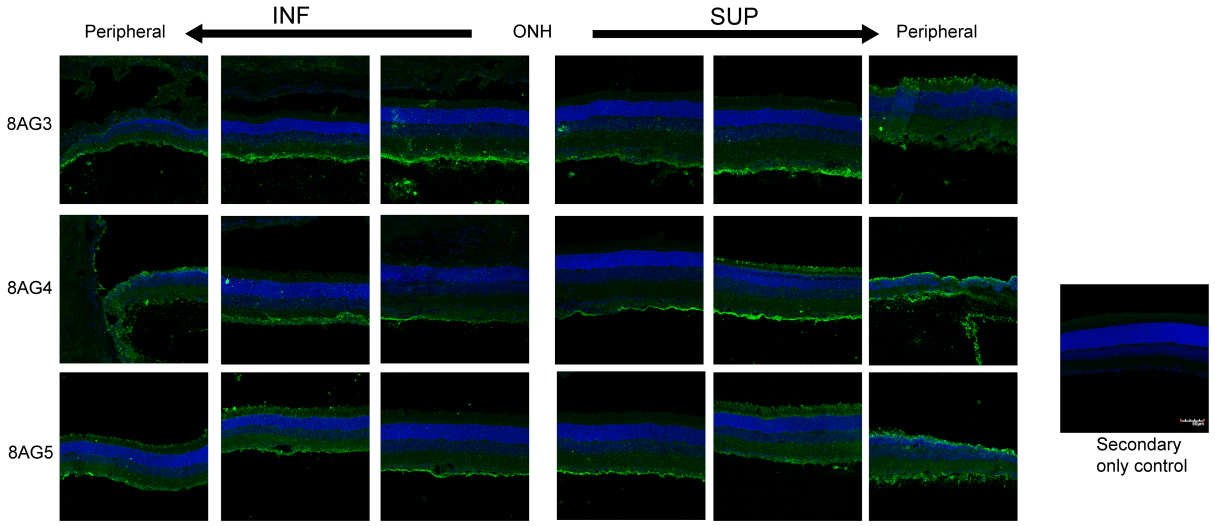

INF
SUP
INF
SUP
Peripheral
Equatoral
Central
Central
Equatoral
Peripheral
Peripheral
Equatoral
Central
Central
Equatoral
Peripheral
Young
Untreated Aged
PNA
40 µm

Supplementary Figure S9. Immunostaining of peanut agglutinin (PNA) on the retinal cryosections of the young (Y, 4 months), aged (24 months) and 8-AG treated aged rats (Aged 8-AG, 24 months). N=7-8. White, PNA; blue, Hoechst33342. Scale bar, 50 μm.

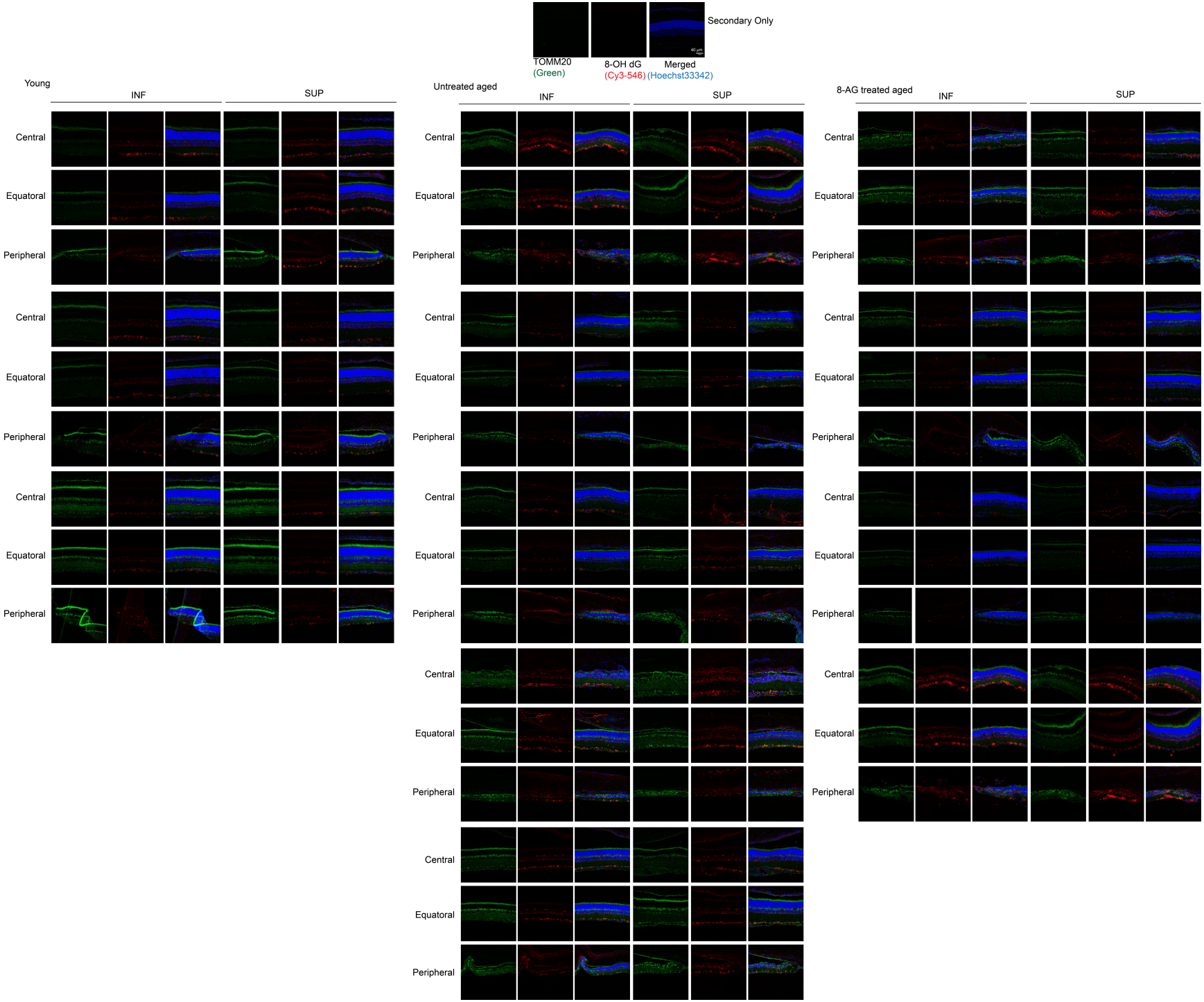
MDA


Peripheral
INF
ONH
SUP
Peripheral
Y3
Y4
Y5
50 µm
Secondary
only control

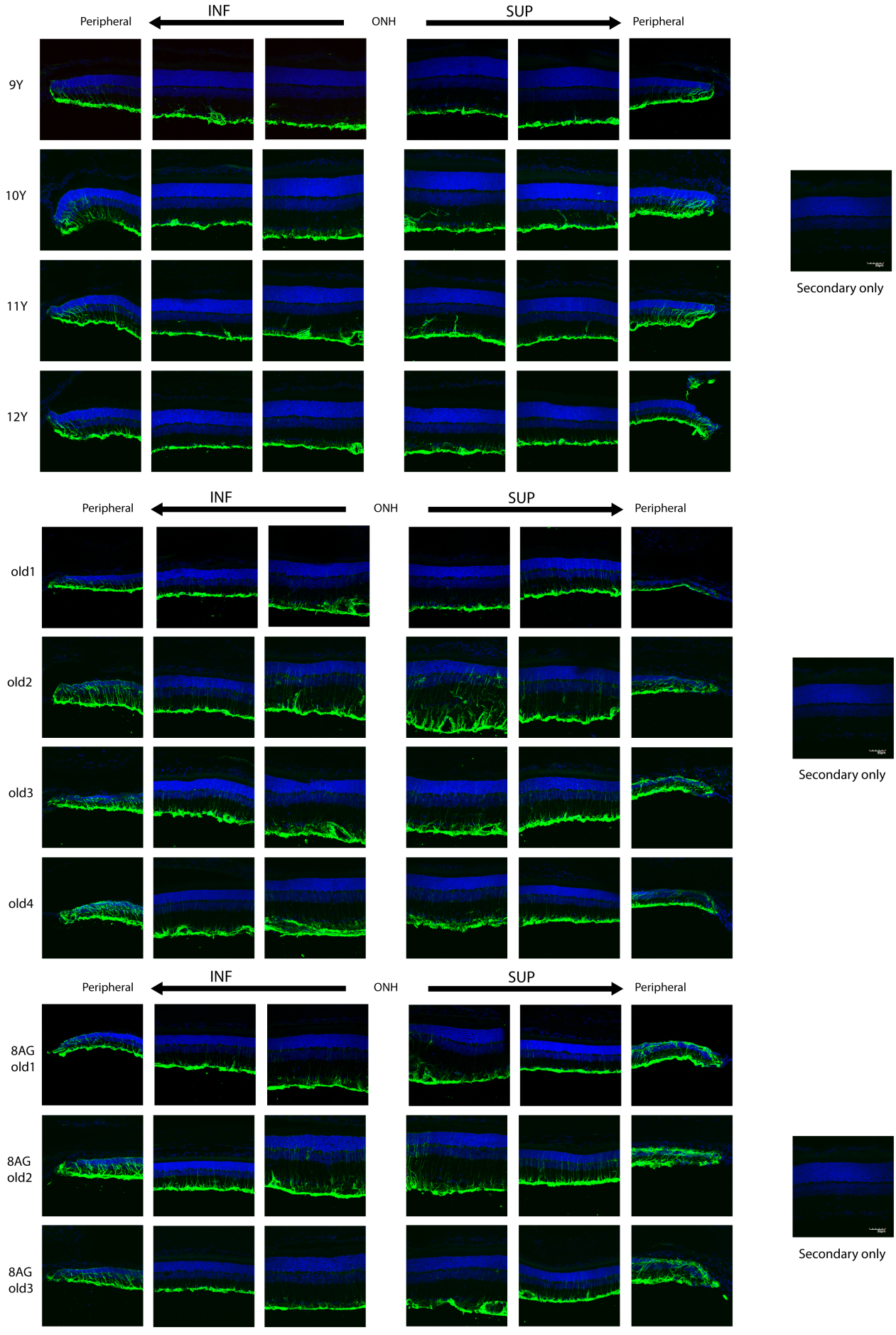

Peripheral
INF
ONH
SUP
Peripheral
Old3
Old4
Old5
50 µm
Secondary
only control


Peripheral
INF
ONH
SUP
Peripheral
8AG3
8AG4
8AG5
Secondary
only control

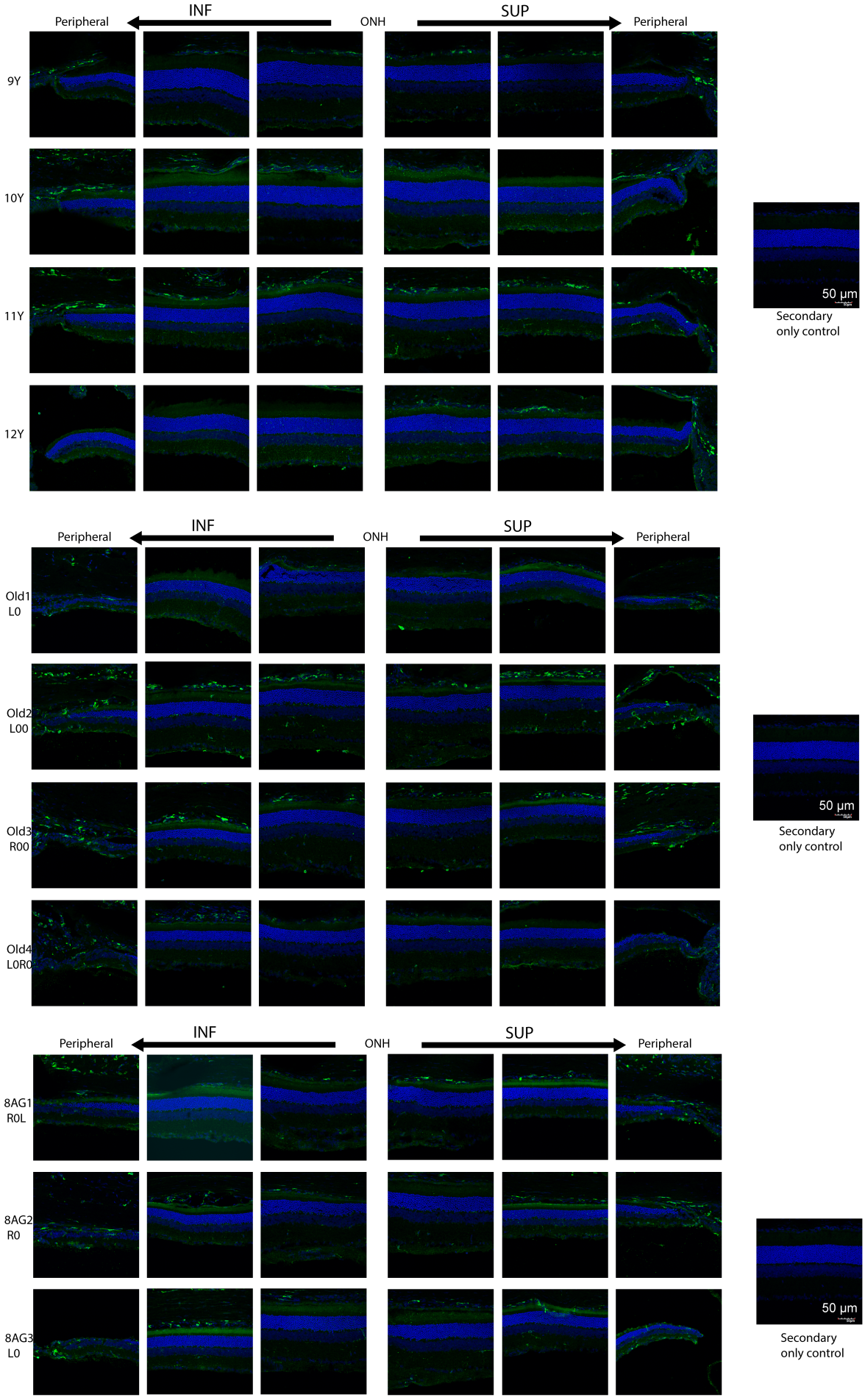
Supplementary Figure S10. Immunostaining of malondialdehyde (MDA) on retinal cryosections from young (Y, 3 months), aged (24 months) and old 8-AG treated rats (Aged 8-AG, 24 months). Green, MDA staining for lipid peroxidation; blue, Hoechst33342 for nucleus staining. Secondary antibody-only controls are listed on the right side, with scale bars of 50 μm. A total of six images were taken from the superior peripheral to the inferior end on each retinal cross-section. N=3.


Secondary Only
TOMM20
(Green)
8-OH dG
(Cy3-546)
Merged
(Hoechst33342)
Young
INF
SUP
Untreated aged
INF
SUP
8-AG treated aged
INF
SUP
Central
Equatoral
Peripheral

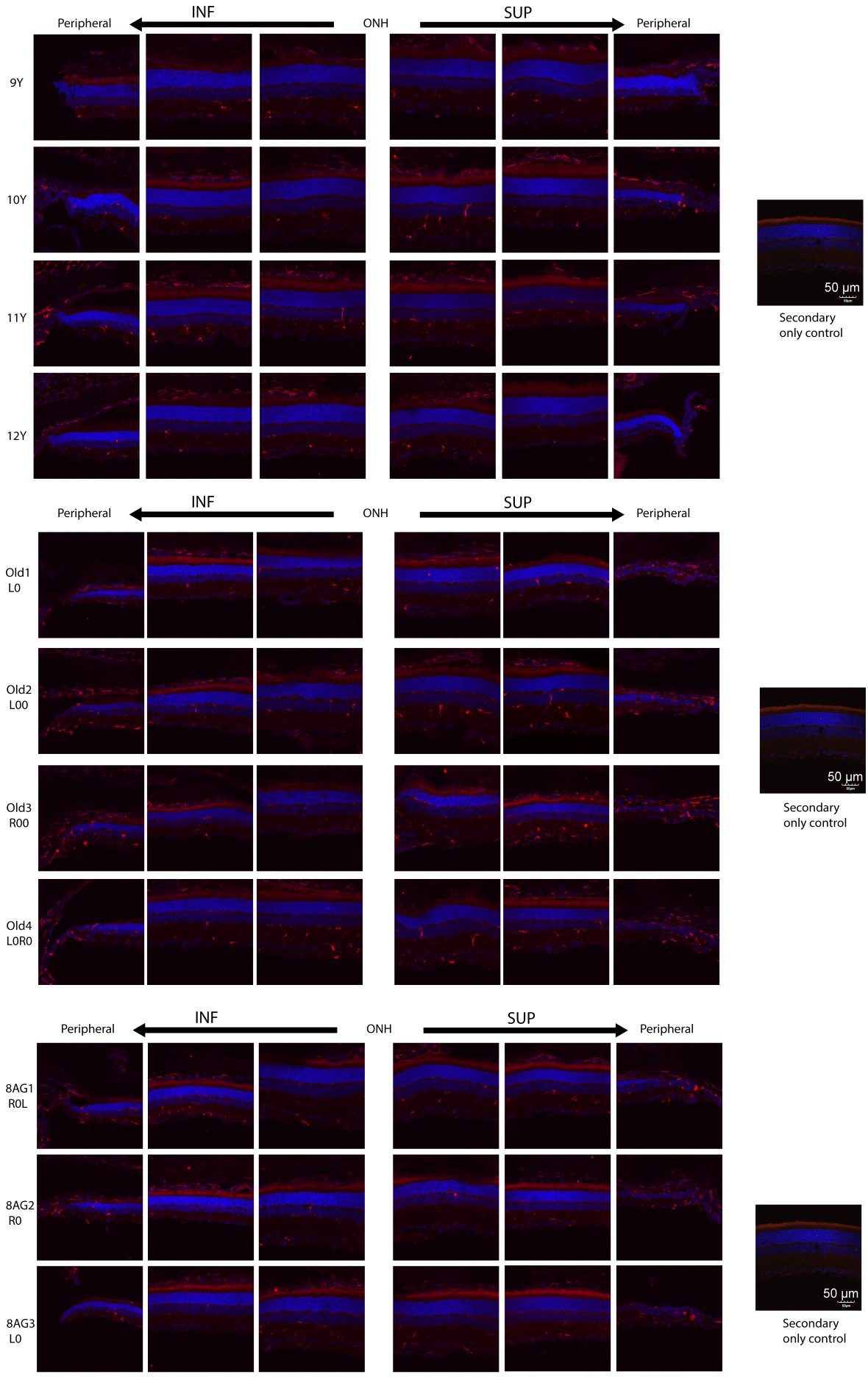
Supplementary Figure S11. Immunostaining of 8-hydroxyl deoxyguanosine (8-OH dG) and TOMM20 on retinal cryosections from young (Y, 3 months), old (24 months) and old 8-AG treated rats (Aged 8-AG, 24 months). Green, TOMM20 staining as a marker of mitochondria; red, 8-OH dG staining for DNA peroxidation; and blue, Hoechst33342 for nucleus staining. Secondary antibody-only controls are listed on the top, with scale bars of 50 μm. A total of six images were taken from the superior peripheral to the inferior end on each retinal cross-section. N=4-5.


Peripheral
INF
ONH
SUP
Peripheral
9Y
10Y
11Y
12Y
Secondary only
Peripheral
INF
ONH
SUP
Peripheral
old1
old2
old3
old4
Secondary only
Peripheral
INF
ONH
SUP
Peripheral
8AG old1
8AG old2
8AG old3
Secondary only

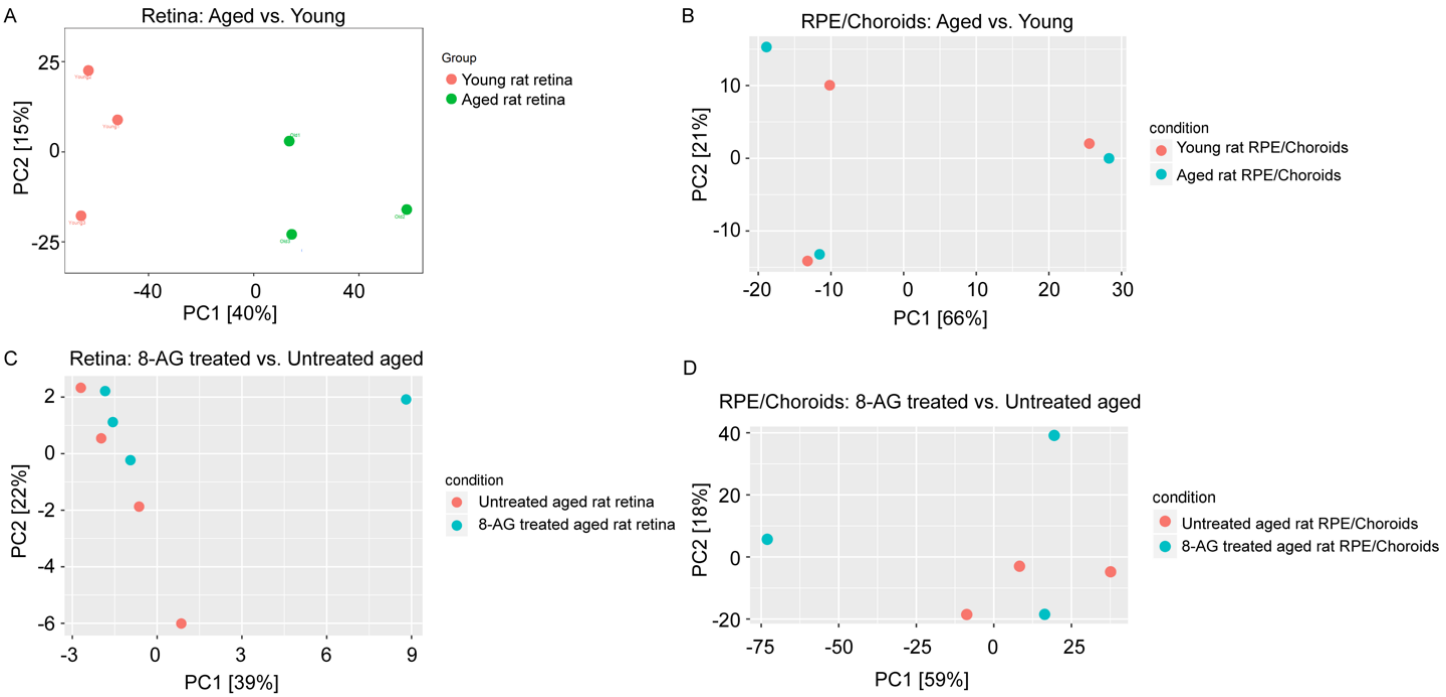
Supplementary Figure S12. Immunostaining of glial fibrillary acidic protein (GFAP) on retinal cryosections from young (Y, 3 months), aged (24 months) and 8-AG treated aged rats (Aged 8-AG, 24 months). Green, GFAP staining for the Müller glia fibrosis; blue, Hoechst33342 for nucleus staining. Secondary antibody-only controls are listed on the right side, with scale bars of 50 μm. A total of six images were taken from the superior peripheral to the inferior end on each retinal cross-section. N=3-4.


Peripheral
INF
ONH
SUP
Peripheral
9Y
10Y
11Y
12Y
50 µm
Secondary only control
Old1 L0
Old2 L00
Old3 R00
Old4 L0R0
50 µm
Secondary only control
8AG1 R0L
8AG2 R0
8AG3 L0
50 µm
Secondary only control

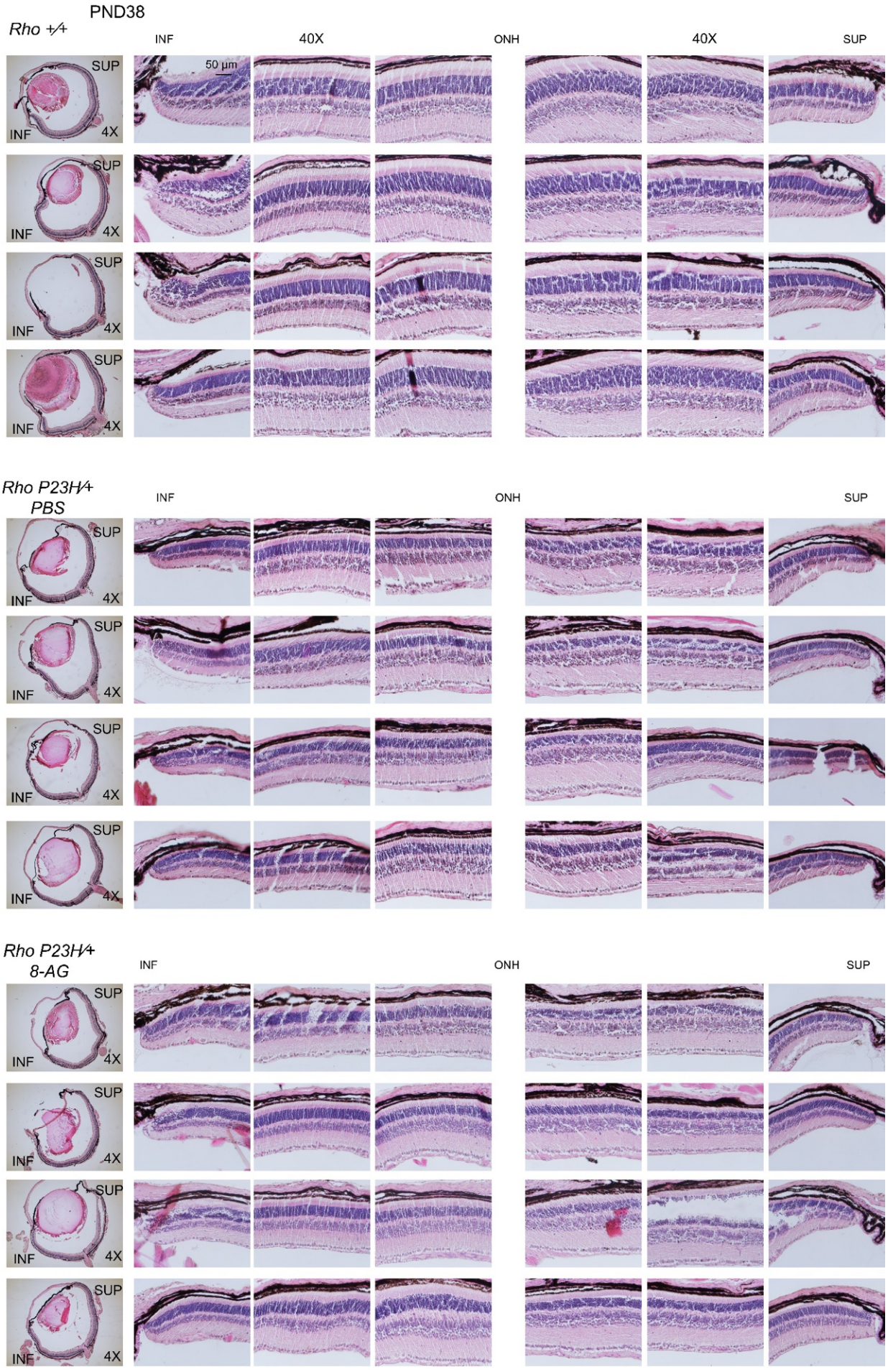
Supplementary Figure S13. Immunostaining of CD68 on retinal cryosections from young (Y, 3 months), aged (24 months) and 8-AG treated aged rats (Aged 8-AG, 24 months). Green, CD68 staining activated microglia/macrophage; blue, Hoechst33342 staining for the nucleus. Secondary antibody-only controls are listed on the right side, with scale bars of 50 μm. A total of six images were taken from the superior peripheral to the inferior end on each retinal cross-section. N=3-4.


Peripheral
INF
ONH
SUP
Peripheral
9Y
10Y
11Y
12Y
50 µm
Secondary only control
Peripheral
INF
ONH
SUP
Peripheral
Old1 L0
Old2 L00
Old3 R00
Old4 L0R0
50 µm
Secondary only control
Peripheral
INF
ONH
SUP
Peripheral
8AG1 R0L
8AG2 R0
8AG3 L0
50 µm
Secondary only control

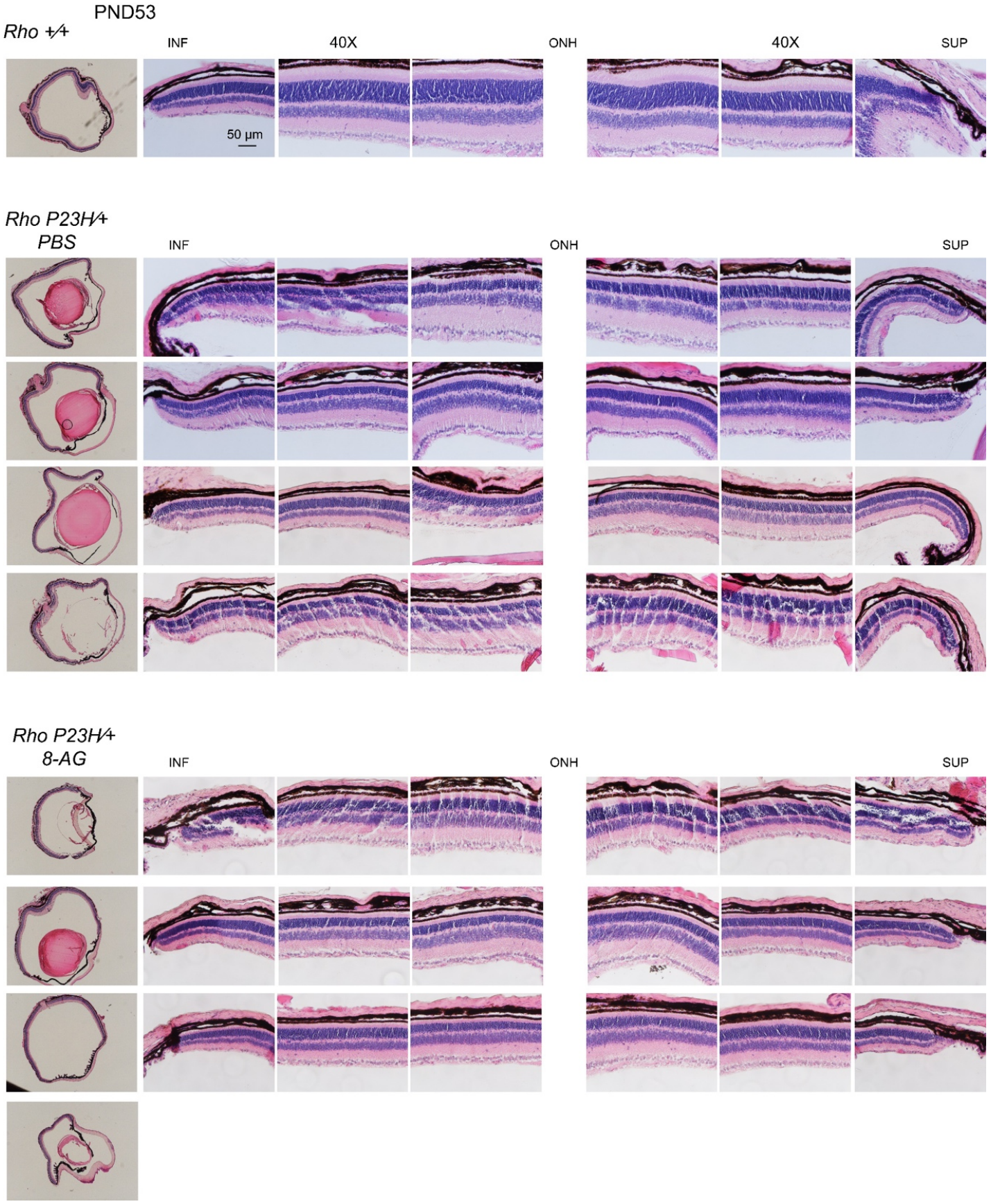
Supplementary Figure S14. Immunostaining of IBA1 on retinal cryosections from young (Y, 3 months), aged (24 months) and 8-AG treated aged rats (Aged 8-AG, 24 months). Red, IBA1 staining for the activated microglia/macrophage; blue, Hoechst33342 for nucleus staining. Secondary antibody-only controls are listed on the right side, with scale bars of 50 μm. A total of six images were taken from the superior peripheral to the inferior end on each retinal cross-section. N=3-4.



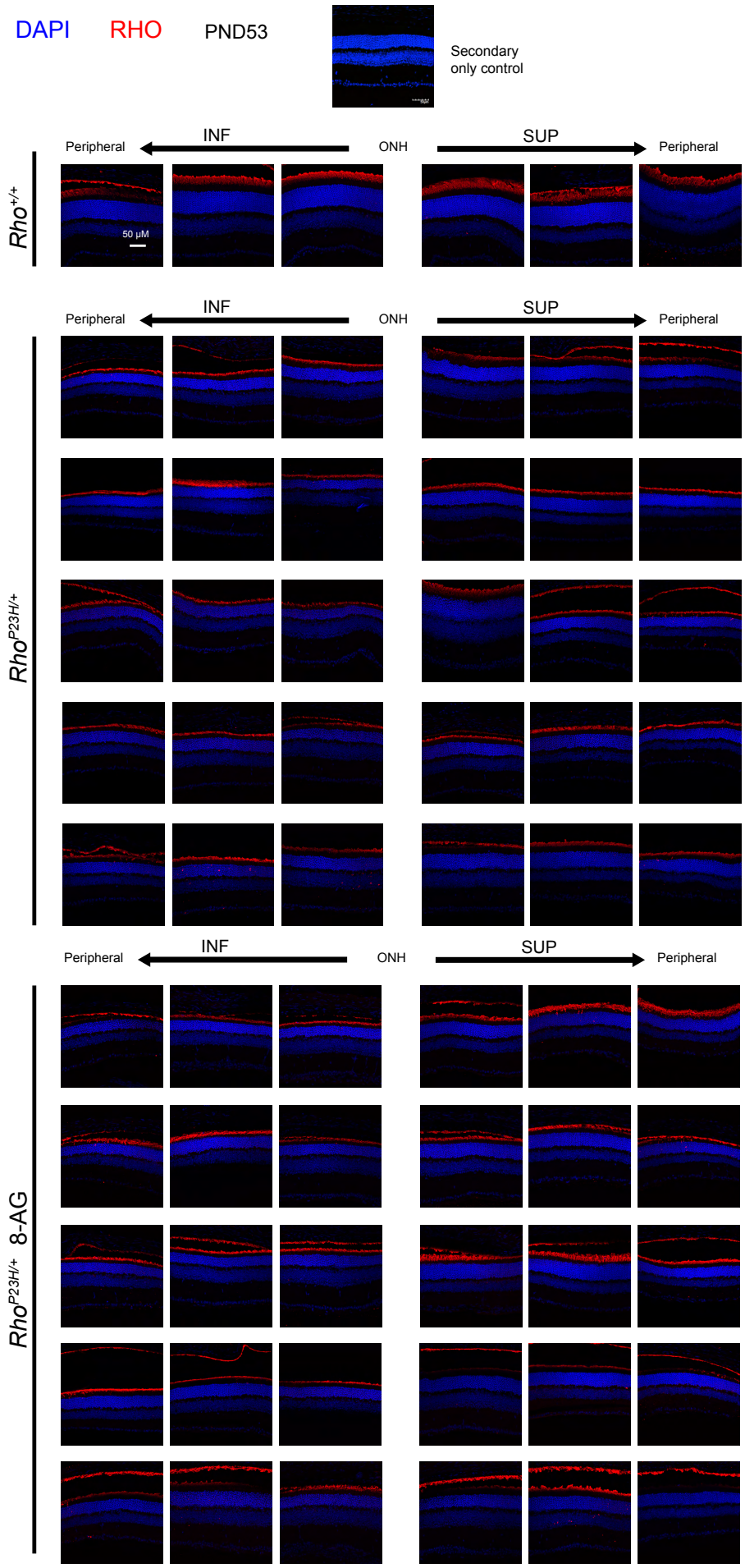
Supplementary Figure S15. Principal Component Analysis (PCA) plots of RNA-seq data from the neural retina (**A** and **C**) and RPE/choroids (**B** and **D**) comparing samples from different groups. **A.** retinae of aged (24 months) vs young rats (3 months). **B.** RPE/choroids of aged vs. young rats. **C.** retinae of 8-AG treated aged (24 months) vs untreated aged rats (24 months). **D.** RPE/choroids of 8-AG treated vs untreated aged rats. The x and y axis are the first two principal components extracted from the dataset and the percentage values in the square brackets indicate the variance in the data by each PC.


PND38
Rho +/+
INF
40X
ONH
40X
SUP
50 µm
SUP
INF
4X
Rho P23H/+
PBS
Rho P23H/+
8-AG

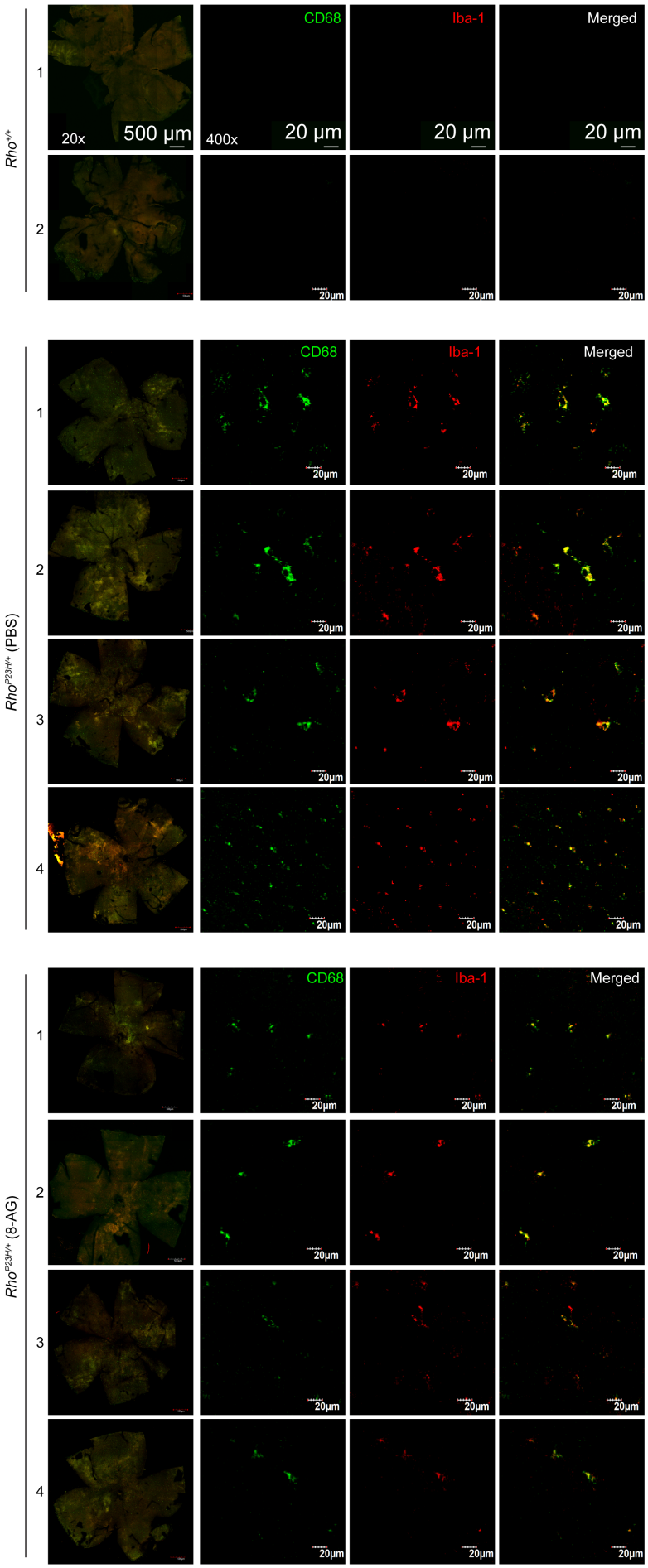
Supplementary Figure S16. H&E staining retina images from wild type (*Rho*$^{+/+}$) and *Rho*$^{P23H/+}$ knock-in mice at PND38. *Rho*$^{P23H/+}$ mice were treated with 11 mg/kg of 8-AG from P10-28 by i.p injections and then administered by supplementing 8-AG in drinking water to continue the 11 mg/kg 8-AG treatment until P38. PBS-treated *Rho*$^{P23H/+}$ mice were used as vehicle control. Scale, 50 μm.


PND53
Rho +/+
INF
40X
ONH
40X
SUP
50 µm
Rho P23H/+
PBS
INF
ONH
SUP
Rho P23H/+
8-AG
INF
ONH
SUP

Supplementary Figure S17. H&E staining retina images from wild type (*Rho*$^{+/+}$) and *Rho*$^{P23H/+}$ knock-in mice at PND53. *Rho*$^{P23H/+}$ mice were treated with 11 mg/kg of 8-AG from P10-28 by i.p injections and then administered by supplementing 8-AG in drinking water to continue the 11 mg/kg 8-AG treatment until PND, respectively. PBS-treated *Rho*$^{P23H/+}$ mice were used as vehicle control. Scale, 50 μm.


DAPI
RHO
PND53
Secondary only control
Peripheral
INF
ONH
SUP
Peripheral
Rho+/+
50 µM
Rho P23H/+
Rho P23H/+ 8-AG

Supplementary Figure S18. Immunostaining of rhodopsin (RHO) in the wildtype (*Rho*$^{+/+}$) and *Rho*$^{P23H/+}$ knock-in mice at PND 38 (left) and 53 (right). *Rho*$^{P23H/+}$ mice were treated with 11 mg/kg of 8-AG from PND 10-28 by i.p. injections and then administered by supplementing 8-AG in drinking water to continue the 11 mg/kg 8-AG treatment until PND 38 and 53, respectively. Red, rhodopsin staining for the outer segments; blue, Hoechst33342 for staining the nucleus. Secondary antibody-only controls are shown on the top row. Scale, 50 μm.


Rho+/+
1
2
20x
500 µm
400x
CD68
Iba-1
Merged
20 µm
20µm
RhoP23H/+ (PBS)
3
4
RhoP23H/+ (8-AG)

Supplementary Figure S19. Retina flat mount immunofluorescence images from wild type (*Rho*$^{+/+}$) and *Rho*$^{P23H/+}$ knock-in mice at PND 53. Rho P23H/+ mice were treated with 11 mg/kg of 8-AG from PND10-28 by i.p injections and then administered by supplementing 8-AG in drinking water to continue the 11 mg/kg 8-AG treatment until PND 53, respectively. PBS-treated *Rho*$^{P23H/+}$ mice were used as vehicle control. Low magnification images were shown on the left, followed by the green, red, and merged images at high magnification. Green, CD68 staining for the activated microglia/macrophages; red, IBA1 staining for the activated microglia/macrophages. Scale, 200 μm and 20 μm for the low and high magnification images, respectively.

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1. Sakami S, Maeda T, Bereta G, Okano K, Golczak M, Sumaroka A, et al. Probing mechanisms of photoreceptor degeneration in a new mouse model of the common form of autosomal dominant retinitis pigmentosa due to P23H opsin mutations. *J Biol Chem.* 2011;286(12):10551-67.
2. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, et al. Fiji: an open-source platform for biological-image analysis. *Nat Methods.* 2012;9(7):676-82.
3. Jackson EK, Kitsios GD, Lu MY, Schaefer CM, Kessinger CJ, McVerry BJ, et al. Suppressed renoprotective purines in COVID-19 patients with acute kidney injury. *Sci Rep.* 2022;12(1):17353.