SUPPLEMENTAL MATERIALS AND METHODS

Flow cytometry of non-retinal populations. All flow cytometry was performed on a BD FACSAria II and analyzed using FlowJo 10 (Tree Star). For peripheral blood cells, 50 µL of tail vein blood from each mouse was collected in phosphate-buffered saline (PBS) containing 2mM ethylenediaminetetraacetic acid (EDTA) and red blood cells lysed using BD Pharm Lysing Buffer according to manufacturer's instructions. For peritoneal cells, 10 mL of FACS buffer (PBS containing 2% fetal bovine serum and 2mM EDTA) was injected into the peritoneal cavity of each animal shortly after sacrifice. Following peritoneal massage, the buffer was collected and centrifuged at 400 x g for ten minutes to precipitate peritoneal cells. Harvested peritoneal cells were blocked for five minutes with 1:100 of rat anti-mouse CD16/32 (BD Pharmingen) and incubated for 20 minutes on ice with the antibodies listed in Supplemental Table 1. Prior to analysis, all samples were passed through a 40 µm filter and stained with 0.5 µg/mL of 4′,6-diamidino-2-phenylindole (DAPI) (Thermo Fisher Scientific) in FACS buffer to exclude non-viable cells.

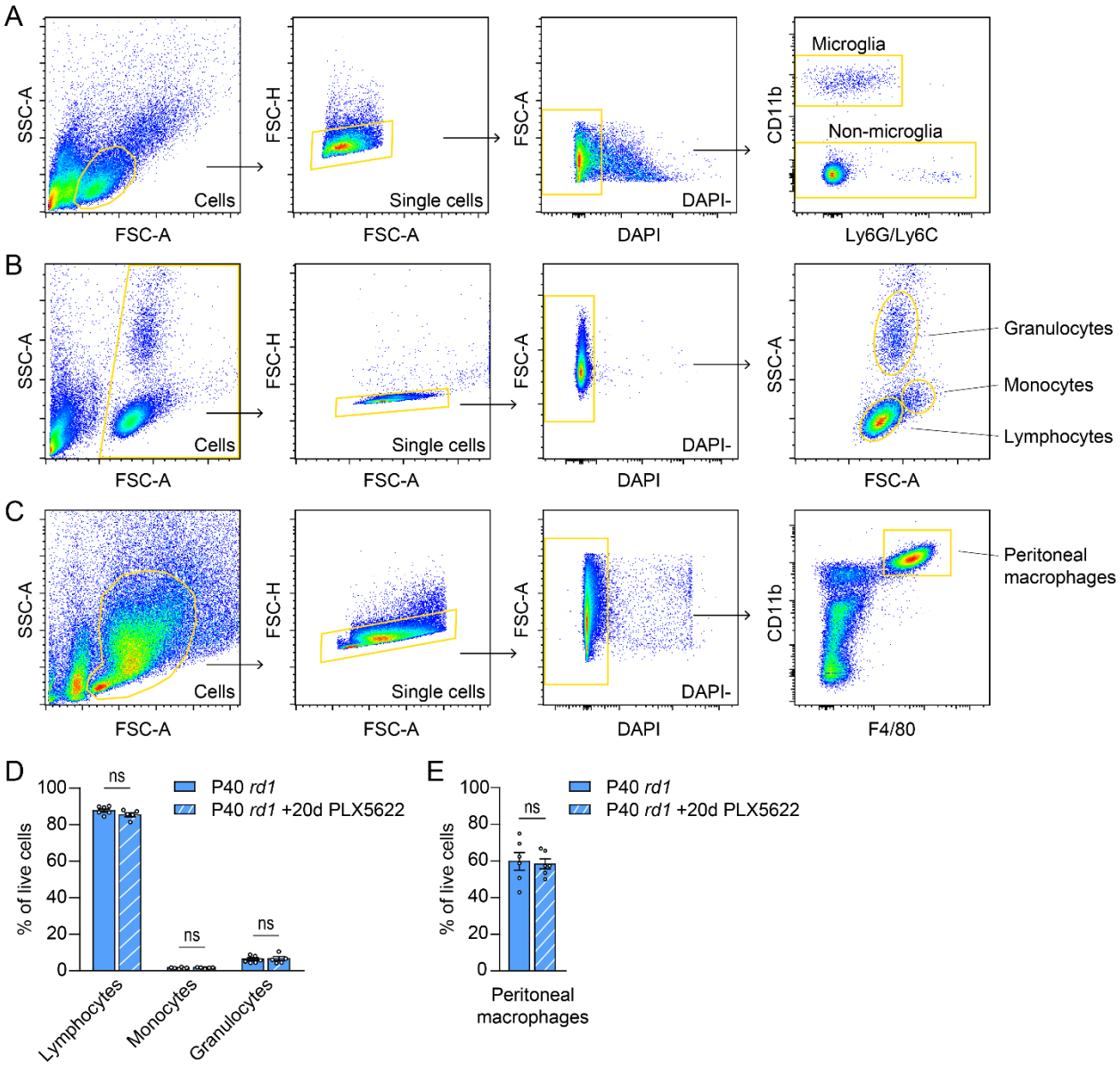
Ex vivo retinal culture. Freshly isolated retinas were relaxed with four radial incisions and placed on a 12 mm Millicell cell culture insert (Millipore) resting on 2 mL of prewarmed culture media with the ganglion cell layer facing up. Culture media consisted of a 1:1 ratio of DMEM and F-12 supplemented with L-glutamine, B27, N2, and penicillin-streptomycin. Explants were maintained in humidified incubators at 37°C and 5% CO2 for 48-72 hours, after which the media was assayed for TGF-β1, TGF-β2, or TGF-β3 protein using commercial ELISA kits (R&D Systems). All ELISA reactions were performed in triplicate.

Immunostaining of flat-mounts. Freshly dissected retinas were fixed in 4% paraformaldehyde for 30 minutes at room temperature. After PBS washes, retinas were blocked for one hour in PBS containing 5% donkey serum and 0.3% Triton X-100 and stained with either anti-cone arrestin overnight or anti-BRN3A for two nights at 4^{o}C (see Supplemental Table 1 for additional details). For retinal pigment epithelium (RPE) preparations, enucleated eyes were dissected to remove the cornea, iris, lens, ciliary body, retina, and connective tissue. The remaining RPE-choroid-sclera complex was fixed in 4% paraformaldehyde for one hour at room temperature, blocked in PBS containing 5% donkey serum and 0.3% Triton X-100 for one hour, and stained with anti-ZO-1 for two nights at 4^{o}C (see Supplemental Table 1 for additional details). All samples were subsequently incubated with the appropriate secondary antibody in PBS for two hours at room temperature, relaxed with four radial incisions, and flat-mounted onto microscope slides. Images of cone arrestin immunostaining in retinal flat-mounts were acquired using a Nikon Ti inverted widefield microscope (20x air objective). Images of BRN3A and ZO-1 immunostaining in flat-mounted retinas and RPE preparations, respectively, were acquired in the mid-periphery using a Zeiss LSM710 scanning confocal microscope (20x air objective) and displayed as maximum intensity projections.

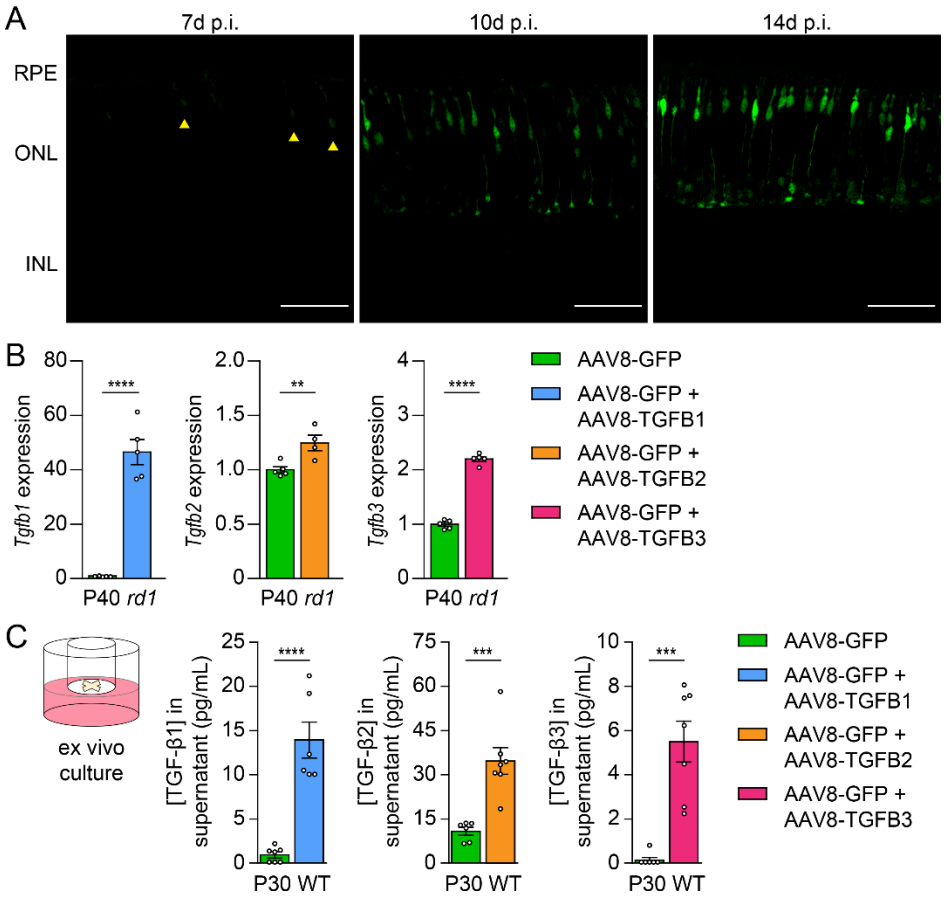
Cone arrestin quantification. Quantification of cone arrestin (CAR)-positive cones was performed similarly to that of GFP-positive cones using a custom ImageJ module (available at https://sites.imagej.net/Seankuwang/). For each flat-mount, the user indicated the location of the optic nerve head and each of the four retinal leaflets. The module then automatically defined the region corresponding to the central retina and counted the number of CAR-positive objects

within the region. This value was used to represent the number of CAR-positive cones in the central retina for each sample.

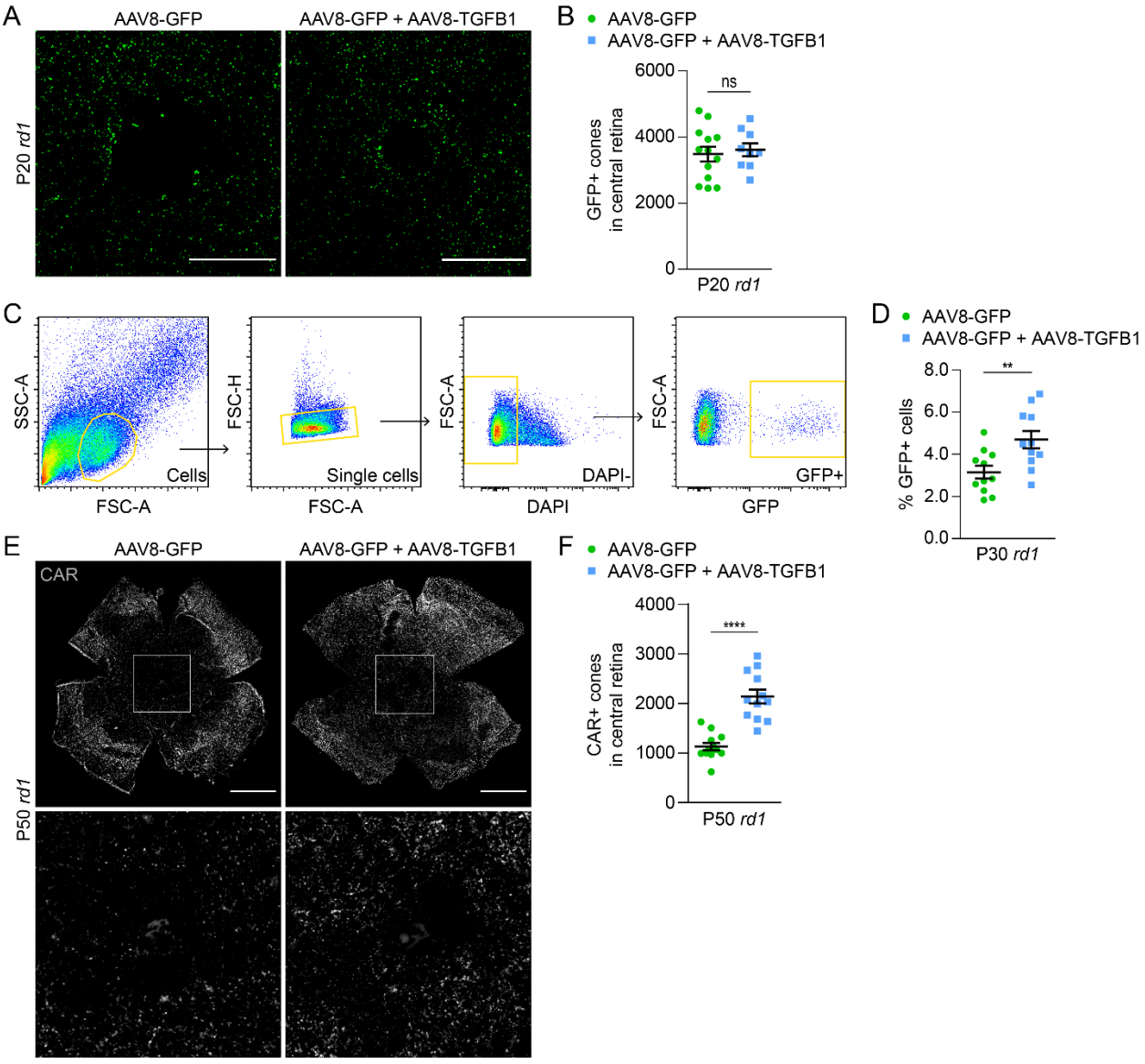
Cataract examination. Mice were examined for cataracts in vivo by a blinded observer using the Micron IV fundus imaging system (Phoenix Research Labs). Following anesthetization of animals with a mixture of ketamine/xylazine (100/10 mg/kg), pupils were dilated with a drop of 0.5% tropicamide and eyes hydrated with Gonak 2.5% hypromellose solution (Akorn). Images of isolated lenses in PBS were acquired using a Leica M165 FC dissecting microscope.



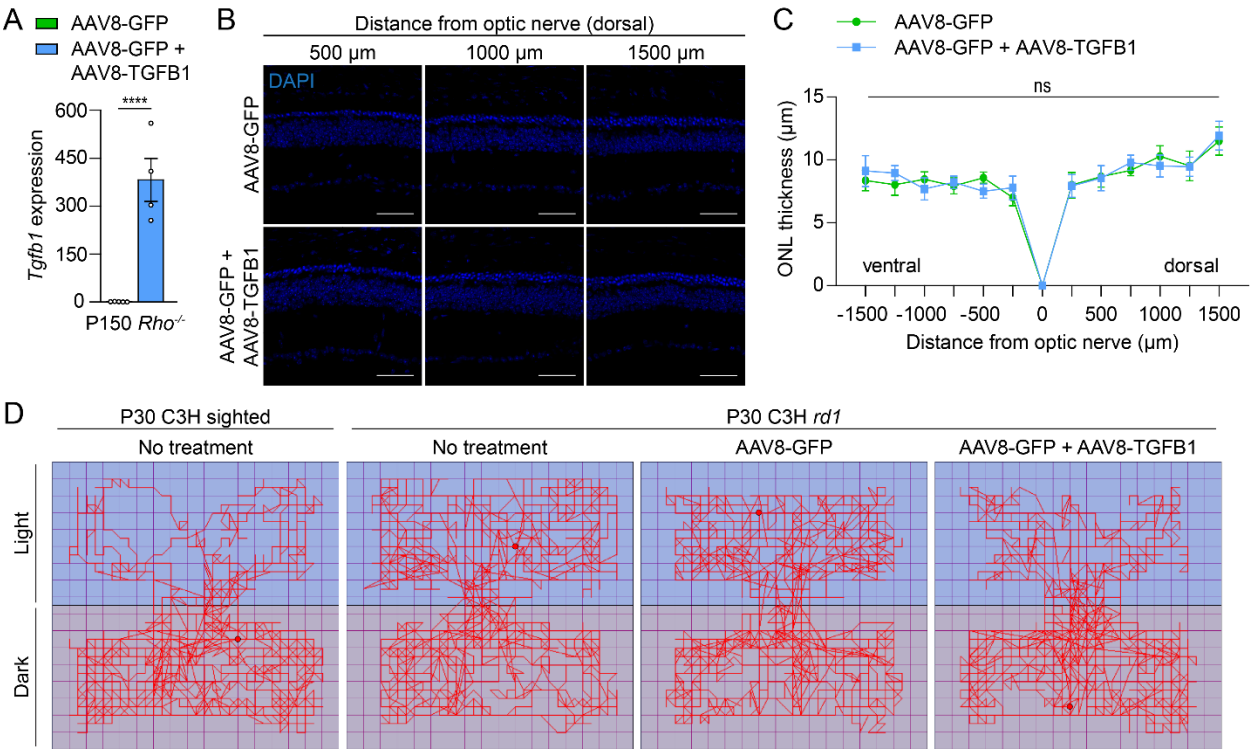
Supplemental Figure 1. (**A**) Representative flow cytometry gating for microglia and non-microglia cells in the retina. Microglia were defined as CD11b-positive Ly6G/Ly6C-negative cells. Non-microglia were defined as CD11b-negative cells. (**B**) Representative flow cytometry gating for lymphocytes, monocytes, and granulocytes from peripheral blood. Each population was defined based on its characteristic forward scatter (FSC) and side scatter (SSC) profile as previously described (1). (**C**) Representative flow cytometry gating for peritoneal macrophages isolated from the peritoneal cavity. Peritoneal macrophages were defined as CD11b-positive F4/80-positive cells. (**D**, **E**) Quantification of peripheral blood immune populations (n = 5-6) (**D**) and peritoneal macrophages (n = 6) (**E**) from P40 FVB (*rd1*) mice with or without 20 days of PLX5622. Data shown are mean ± SEM. ns, not significant by two-tailed Student's t-test.



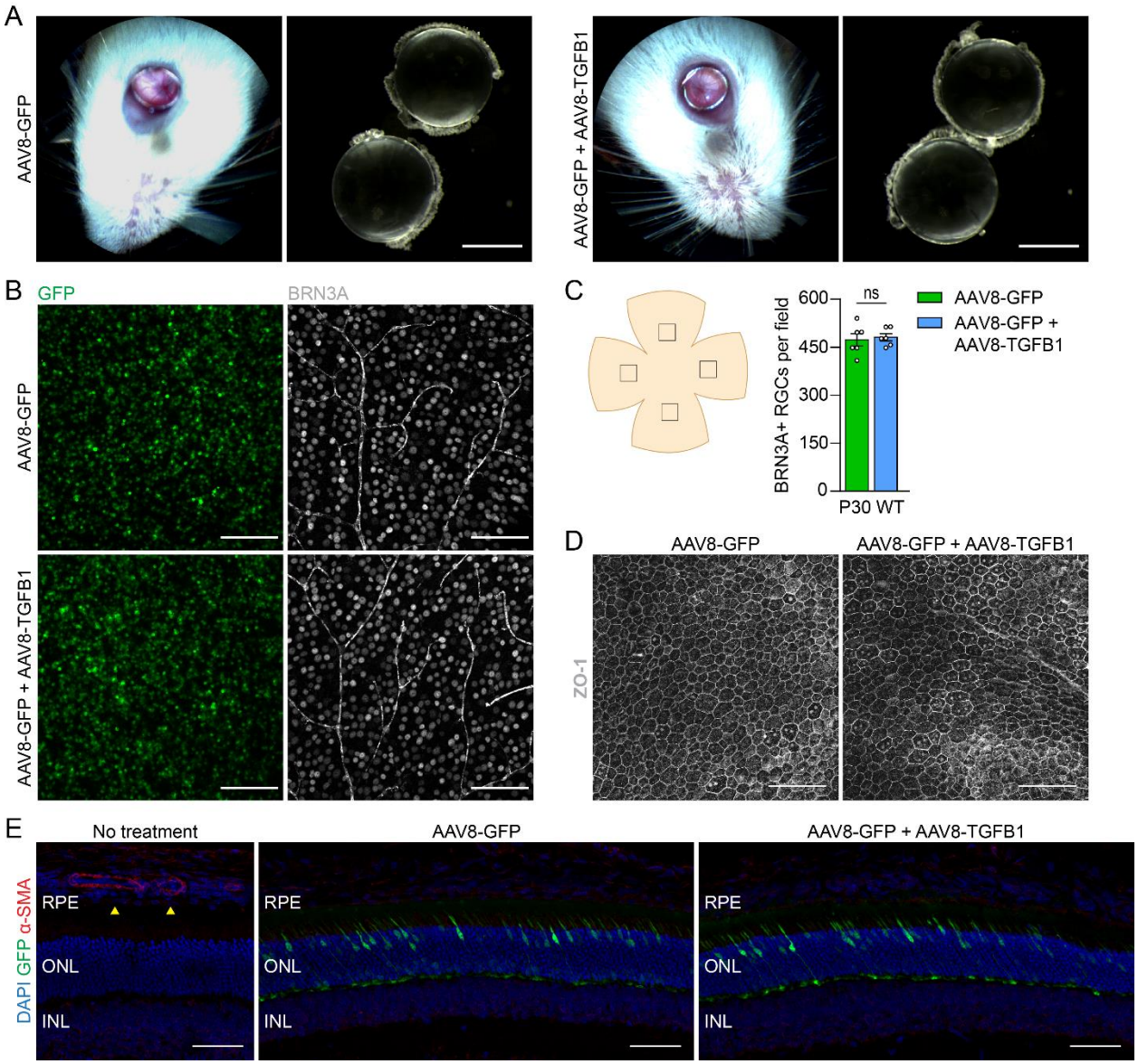
Supplemental Figure 2. (**A**) Kinetics of GFP expression in cones after subretinal delivery of AAV8-GFP. Arrowheads indicate faint GFP expression. Scale bar, 50 µm. (**B**) mRNA expression of *Tgfb1*, *Tgfb2*, and *Tgfb3* in FVB (*rd1*) retinas (n = 4-5) after treatment with AAV8-GFP plus AAV8-TGFB1, AAV8-TGFB2, or AAV8-TGFB3, respectively. Fold changes are relative to AAV8-GFP. (**C**) Quantification of TGFB1, TGFB2, and TGFB3 secreted during ex vivo culture from WT (CD-1) retinas (n = 6-7) treated with AAV8-GFP plus AAV8-TGFB1, AAV8-TGFB2, or AAV8-TGFB3, respectively. Data shown are mean ± SEM. ** P<0.01, *** P<0.001, **** P<0.0001 by two-tailed Student's t-test. RPE, retinal pigment epithelium; INL, inner nuclear layer; ns, not significant.



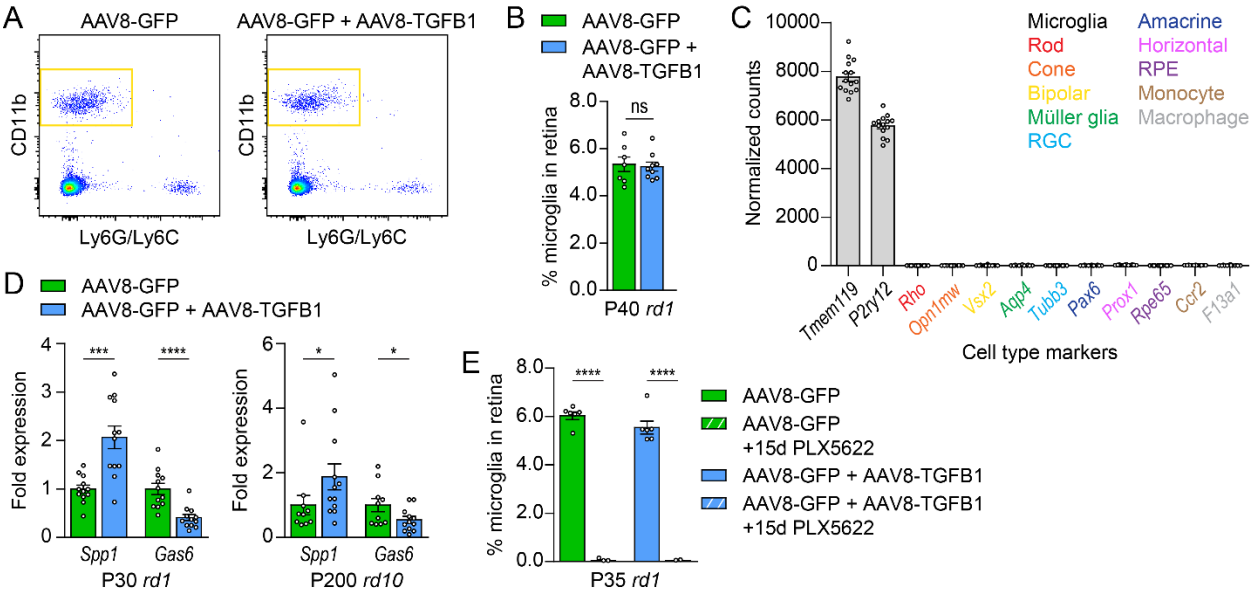
Supplemental Figure 3. (**A**, **B**) Representative images (**A**) and quantification (**B**) of cone survival in central retinas of P20 FVB (*rd1*) mice (n = 9-13) treated with AAV8-GFP or AAV8-GFP plus AAV8-TGFB1. Scale bar, 500 µm. (**C**, **D**) Representative gating (**C**) and quantification (**D**) by flow cytometry of GFP-positive cones from P30 *rd1* retinas (n = 11) treated with AAV8-GFP or AAV8-GFP plus AAV8-TGFB1. (**E**) Immunostaining for cone arrestin (CAR) in flat-mounts of P50 *rd1* retinas treated with AAV8-GFP or AAV8-GFP plus AAV8-TGFB1. Paired images depict low and high magnifications (boxed areas). Scale bar, 1 mm. (**F**) Quantification of CAR-positive cones in central retinas of P50 *rd1* mice (n = 12) treated with AAV8-GFP or AAV8-GFP plus AAV8-TGFB1. Data shown are mean ± SEM. ** P<0.01, **** P<0.0001 by two-tailed Student's t-test. ns, not significant.



Supplemental Figure 4. (A) mRNA expression of *Tgfb1* in *Rho*$^{-/-}$ retinas (n = 4-5) after treatment with AAV8-GFP plus AAV8-TGFB1 relative to AAV8-GFP only. (**B**, **C**) Representative cross-sections (**B**) and measurements of ONL thickness (**C**) at indicated distances from the optic nerve in P40 *rd10* retinas (n = 6) treated with AAV8-GFP or AAV8-GFP plus AAV8-TGFB1. Scale bar, 50 µm. (**D**) Representative movement tracks during light-dark box testing from untreated animals (n = 8-10) and C3H (*rd1*) mice (n = 11-14) treated with AAV8-GFP or AAV8-GFP plus AAV8-TGFB1. Data shown are mean ± SEM. **** P<0.0001 by two-tailed Student's t-test. ns, not significant.



Supplemental Figure 5. (A) Representative images of the ocular fundus and lens from P30 WT (CD-1) eyes (*n* = 14) treated with AAV8-GFP or AAV8-GFP plus AAV8-TGFB1. Scale bar, 1 mm. (**B**, **C**) Representative images (**B**) and quantification (**C**) of BRN3A-positive retinal ganglion cells (RGCs) in P30 WT retinas (*n* = 6) treated with AAV8-GFP or AAV8-GFP plus AAV8-TGFB1. RGCs were counted in four 20x fields per retina with the mean used to represent each sample. Scale bar, 100 µm. (**D**) Immunostaining for ZO-1, a component of epithelial tight junctions (2), in flat-mounted RPE preparations from P30 WT eyes (*n* = 4) treated with AAV8-GFP or AAV8-GFP plus AAV8-TGFB1. Scale bar, 100 µm. (**E**) Immunostaining for α-smooth muscle actin (α-SMA) in P30 WT eyes (*n* = 2-3) without treatment or treated with AAV8-GFP or AAV8-GFP plus AAV8-TGFB1. Arrowheads indicate α-SMA-positive cells in vessel walls. Scale bar, 50 µm. Data shown are mean ± SEM. ns, not significant; INL, inner nuclear layer.



Supplemental Figure 6. (**A**, **B**) Representative gating (**A**) and quantification (**B**) by flow cytometry of microglia as a percentage of all retinal cells in P40 FVB (*rd1*) retinas (n = 7-9) treated with AAV8-GFP or AAV8-GFP plus AAV8-TGFB1. (**C**) Normalized RNA-seq counts for indicated cell type markers in microglia sorted from P30 *rd1* retinas (n = 14). (**D**) mRNA expression of *Spp1* and *Gas6* in sorted microglia from P30 *rd1* retinas (n = 12) and P200 *rd10* retinas (n = 10-12) after treatment with AAV8-GFP plus AAV8-TGFB1 relative to AAV8-GFP only. (**E**) Quantification by flow cytometry of retinal microglia from P35 *rd1* retinas (n = 2-6) treated with AAV8-GFP or AAV8-GFP plus AAV8-TGFB1 with or without 15 days of PLX5622. Data shown are mean ± SEM. * P<0.05, *** P<0.001, **** P<0.0001 by two-tailed Student's t-test. RGC, retinal ganglion cell; ns, not significant.

Supplemental Table 1. List of antibodies.

Antibody	Vendor	Catalog #	Application	Dilution
PE-Cy5-conjugated anti-CD11b	BioLegend	101209	FC	1:200
FITC-conjugated anti-F4/80	BioLegend	123107	FC	1:200
APC-Cy7-conjugated anti-Ly6C	BioLegend	128025	FC	1:200
APC-Cy7-conjugated anti-Ly6G	BioLegend	127623	FC	1:200
Rabbit anti-IBA1	Thermo Fisher Scientific	PA5-21274	IHC (section)	1:1000
Rabbit anti-TGFBR2	Abcam	ab61213	IHC (section)	1:100
Mouse anti-α-smooth muscle actin	Sigma-Aldrich	A5228	IHC (section)	1:1000
Goat anti-rabbit Alexa Fluor 594	Jackson ImmunoResearch	111-585-144	IHC (section)	1:1000
Donkey anti-mouse Alexa Fluor 594	Jackson ImmunoResearch	715-585-150	IHC (section and flat-mount)	1:1000
Rabbit anti-cone arrestin	EMD Millipore	AB15282	IHC (flat-mount)	1:3000
Mouse anti-BRN3A	Santa Cruz Biotechnology	sc-8429	IHC (flat-mount)	1:100
Rabbit anti-ZO-1	Thermo Fisher Scientific	61-7300	IHC (flat-mount)	1:100
Donkey anti-rabbit Alexa Fluor 594	Jackson ImmunoResearch	711-585-152	IHC (flat-mount)	1:1000

FC, flow cytometry; IHC, immunohistochemistry

Supplemental Table 2. List of RT-PCR primers.

Gene	5’	3’
C1qa	AAAGGCAATCCAGGCAATATCA	TGGTTCTGGTATGGACTCTCC
Gapdh	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
Gapdh-s	TGACCTCAACTACATGGTCTACA	CTTCCCATTCTCGGCCTTG
Gas6	TGCTGGCTTCCGAGTCTTC	CGGGGTCGTTCTCGAACAC
Il1a	CGAAGACTACAGTTCTGCCATT	GACGTTTCAGAGGTTCTCAGAG
Il1b	GCAACTGTTCCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
Il6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
Spp1	AGCAAGAAACTCTTCCAAGCAA	GTGAGATTCGTCAGATTCATCCG
Tgfb1	CTCCCGTGGCTTCTAGTGC	GCCTTAGTTTGGACAGGATCTG
Tgfb1-s	GAGCCCGAAGCGGACTACTA	TGGTTTTCTCATAGATGGCGTTG
Tgfb2	CTTCGACGTGACAGACGCT	GCAGGGGCAGTGTAAACTTATT
Tgfb3	CCTGGCCCTGCTGAACTTG	TTGATGTGGCCGAAGTCCAAC
Tmem119	CCTACTCTGTGTCACTCCCG	CACGTACTGCCGGAAGAAATC
Tnf	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG

SUPPLEMENTAL REFERENCES

1. Rothe G et al. Peripheral blood mononuclear phagocyte subpopulations as cellular markers in hypercholesterolemia. *Arterioscler Thromb Vasc Biol.* 1996;16(12):1437–1447.

2. Georgiadis A et al. The tight junction associated signalling proteins ZO-1 and ZONAB regulate retinal pigment epithelium homeostasis in mice. *PLoS One.* 2010;5(12):e15730.