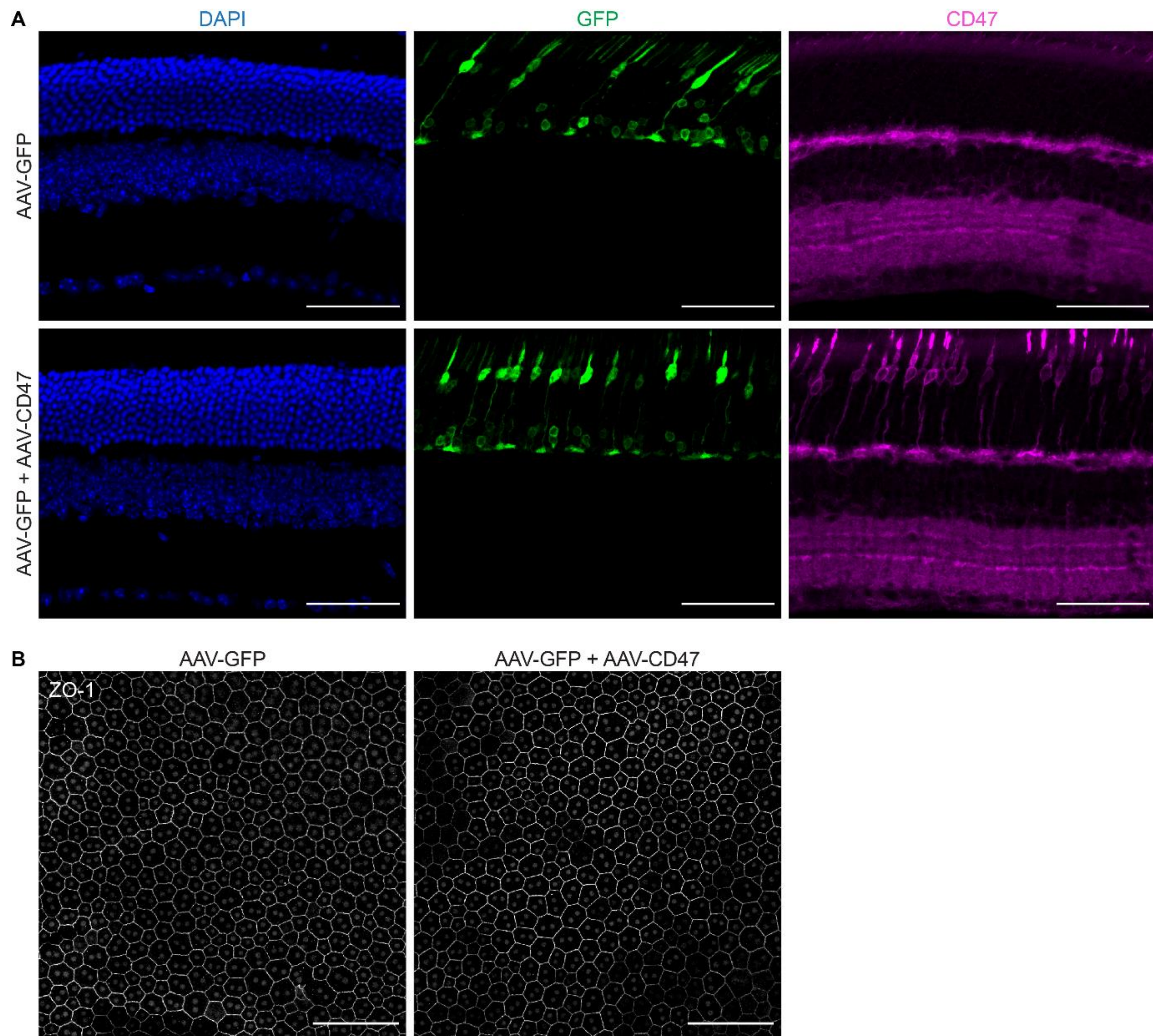
## SUPPLEMENTAL METHODS

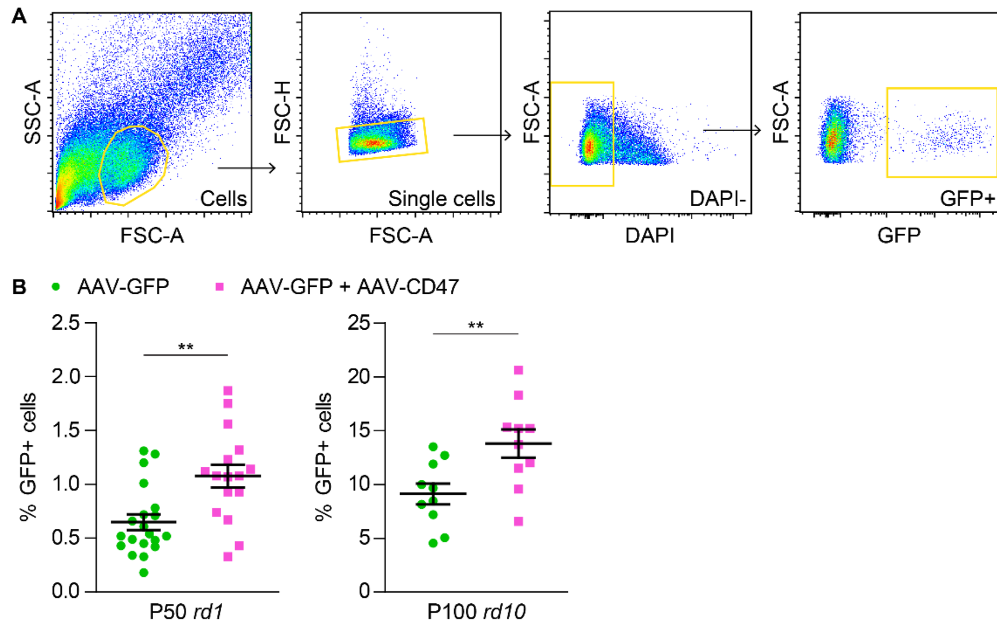
*Retinal pigment epithelium (RPE) immunostaining.* To assess the morphology of the RPE, enucleated eyes were dissected to remove the cornea, iris, lens, ciliary body, retina, and connective tissue. The remaining RPE-choroid-sclera complex was fixed in 4% paraformaldehyde for one hour at room temperature, blocked in PBS containing 5% donkey serum and 0.3% Triton X-100 for one hour, and stained with 1:100 of anti-ZO-1 (ThermoFisher Scientific, 61-7300) for two nights at 4°C. Samples were subsequently incubated with 1:1000 of donkey anti-rabbit secondary antibody (Jackson ImmunoResearch, 711-585-152) in PBS for two hours at room temperature, relaxed with four radial incisions, and flat-mounted onto microscope slides. Images of ZO-1 immunostaining in flat-mounted RPE preparations were acquired in the mid-periphery using a Zeiss LSM710 scanning confocal microscope (20x air objective) and displayed as maximum intensity projections.

*Quantitative polymerase chain reaction (qPCR).* RNA was isolated from *rd1* (FVB) and wild-type (sighted FVB) retinas using an RNeasy Micro Kit (Qiagen) with one whole retina collected per sample. cDNA was then synthesized using the SuperScript III First-Strand Synthesis System (Invitrogen) with oligo(dT) primers, and qPCR was performed using the Power SYBR Green PCR Master Mix (Applied Biosystems) on a CFX96 real-time PCR detection system (Bio-Rad). Reactions were conducted in triplicate with expression normalized to either *Gapdh*, a housekeeping gene, or *Thy1*, a marker for retinal ganglion cells. qPCR primer sequences are listed in Supplemental Table 2.



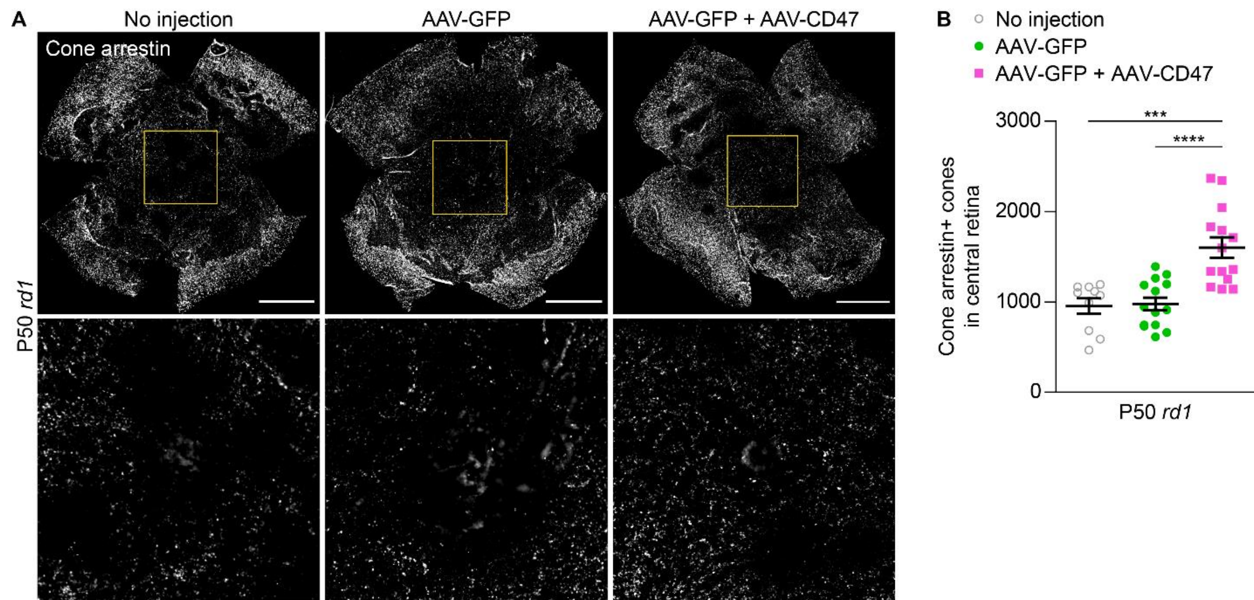

**Supplemental Figure 1. Long-term expression of CD47 in wild-type retinas.**

(**A**) Immunostaining for CD47 in P100 wild-type (CD-1) retinas following infection with AAV8-RedO-GFP or AAV8-RedO-GFP plus AAV8-RedO-CD47. Nuclei were labeled with 4′,6-diamidino-2-phenylindole (DAPI). Scale bars, 50 µm. (**B**) Immunostaining for ZO-1, a marker of epithelial tight junctions, in flat-mounted RPE preparations from P100 wild-type eyes following infection with AAV8-RedO-GFP or AAV8-RedO-GFP plus AAV8-RedO-CD47. Scale bars, 100 µm.



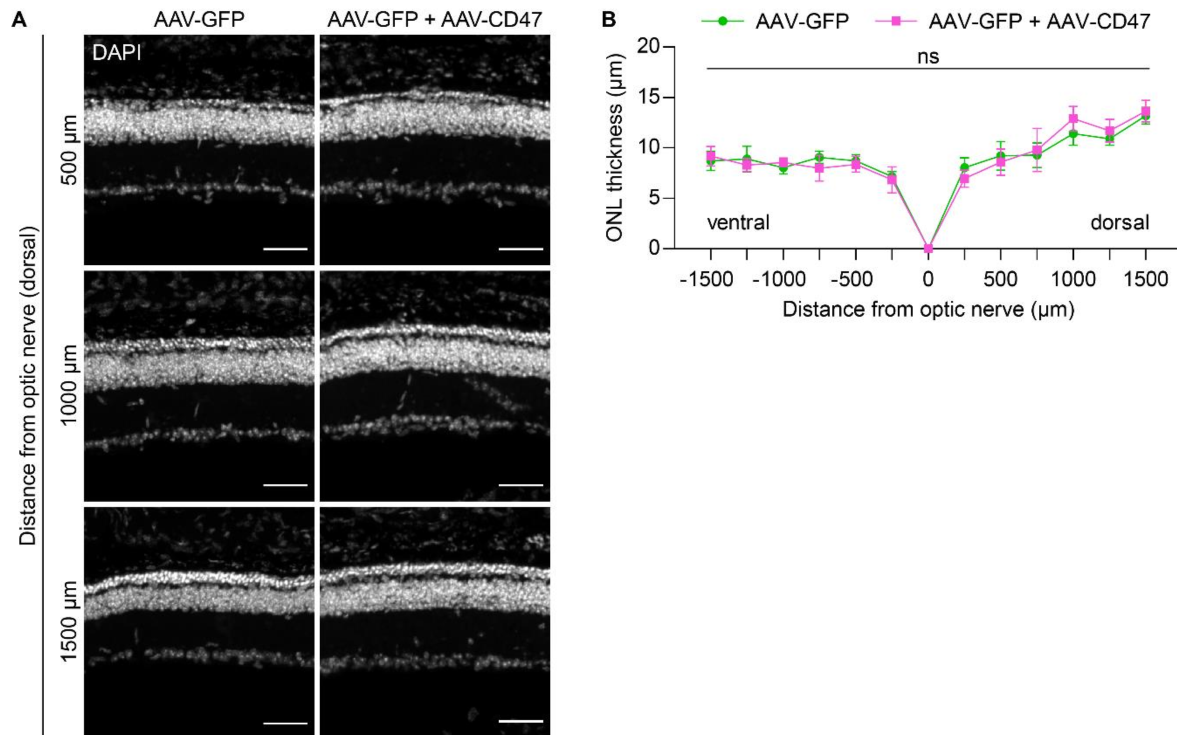

**Supplemental Figure 2. Quantification of cone survival by flow cytometry.**

(**A**) Flow cytometry gating for GFP-positive cones in *rd1* and *rd10* retinas following infection with AAV8-RedO-GFP or AAV8-RedO-GFP plus AAV8-RedO-CD47. (**B**) Quantification by flow cytometry of GFP-positive cones in P50 *rd1* ($n$ = 16-20) and P100 *rd10* ($n$ = 10) retinas following infection with AAV8-RedO-GFP or AAV8-RedO-GFP plus AAV8-RedO-CD47. Data are shown as mean ± SEM. ** $P$<0.01 by two-tailed Student's t-test.



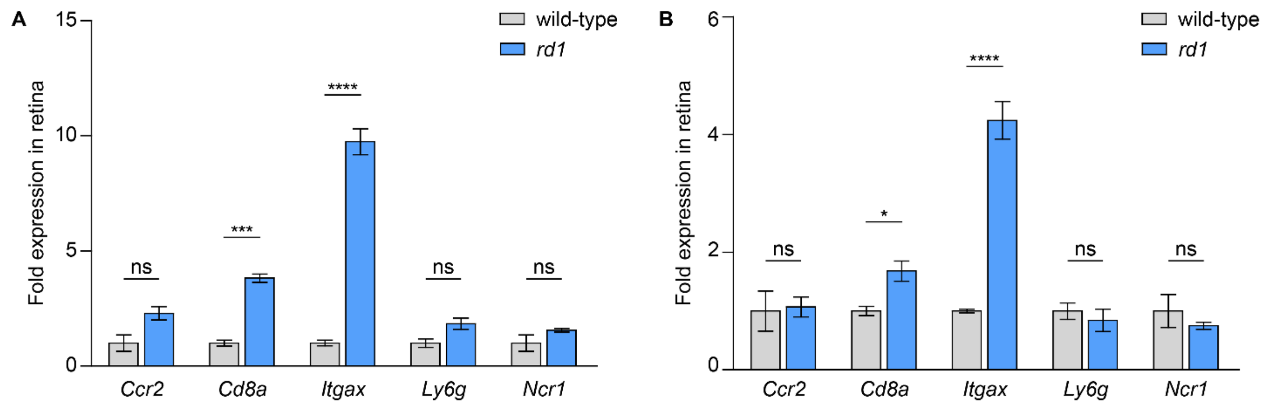

**Supplemental Figure 3. Quantification of cone survival by immunostaining.**

(**A**) Representative flat-mounts of P50 *rd1* retinas without treatment or following infection with AAV8-RedO-GFP or AAV8-RedO-GFP plus AAV8-RedO-CD47 after cone arrestin immunostaining. Paired images depict low and high magnifications. Scale bars, 1 mm. (**B**) Quantification of cone arrestin immunostaining in central retinas of *rd1* mice ($n$ = 10-14) without treatment or following infection with AAV8-RedO-GFP or AAV8-RedO-GFP plus AAV8-RedO-CD47. Data are shown as mean ± SEM. *** $P$<0.001, **** $P$<0.0001 by two-tailed Student's t-test.




**Supplemental Figure 4. Effect of CD47 expression on rod survival.**

(**A**, **B**) Representative cross-sections (A) and measurements of outer nuclear layer (ONL) thickness (B) at indicated distances from the optic nerve in P40 *rd10* retinas (n = 5) following infection with AAV8-RedO-GFP or AAV8-RedO-GFP plus AAV8-RedO-CD47. Nuclei were labeled with DAPI. Scale bars, 50 µm. Data are shown as mean ± SEM. ns, not significant.




**Supplemental Figure 5. Retinal expression of SIRPα-positive immune cell types.**
(**A**, **B**) RNA expression of marker genes for monocytes (*Ccr2*), cytotoxic T cells (*Cd8a*), dendritic cells (*Itgax*), neutrophils (*Ly6g*), and natural killer cells (*Ncr1*) in retinas (n = 4–5) from 6- to 8-week-old wild-type (sighted FVB) or P40 *rd1* mice after normalization to either the housekeeping gene *Gapdh* (A) or the retinal ganglion cell marker *Thy1* (B). Data are shown as mean ± SEM. * $P<0.05$, *** $P<0.001$, **** $P<0.0001$ by two-tailed Student's t-test with Bonferroni correction. ns, not significant.

**Supplemental Table 1. Summary of mouse experiments.**

| Mutation | Strain(s) | Assay or intervention | Age(s) |
|---|---|---|---|
| wild-type | Sighted CX3CR1$^{GFP/+}$ | Ex vivo phagocytosis | P20, P50 |
| | CD-1 | Retinal histology | P40, P100 |
| | CD-1 | RPE histology | P100 |
| | Sighted C3H | Light-dark discrimination | P30 |
| | Sighted FVB | qPCR | 6-8 weeks |
| *rd1* | *rd1*;CX3CR1$^{GFP/+}$ | Ex vivo phagocytosis | P20, P50 |
| | FVB, *rd1*;CreERT2/+, *rd1*;TSP1$^{-/-}$, *rd1*;SIRPα$^{-/-}$ | Cone survival (histology) | P50 |
| | FVB | Cone survival (flow cytometry) | P50 |
| | C3H | Light-dark discrimination | P30 |
| | *rd1*;CreERT2/+ | Tamoxifen | P19-21 |
| | FVB | Microglia depletion | P20-49 |
| | *rd1*;TSP1$^{+/-}$, *rd1*;TSP1$^{-/-}$, *rd1*;SIRPα$^{+/-}$, *rd1*;SIRPα$^{-/-}$ | Retinal histology | P30 |
| | FVB | qPCR | P40 |
| *rd10* | *rd10* | Cone survival (histology) | P130 |
| | *rd10* | Cone survival (flow cytometry) | P100 |
| | *rd10* | Rod survival | P40 |
| | *rd10* | Optomotor | P60 |
| *Rho*$^{-/-}$ | *Rho*$^{-/-}$ | Cone survival (histology) | P150 |

**Supplemental Table 2. qPCR primer sequences.**

| Gene | 5’ | 3’ |
|---|---|---|
| *Ccr2* | ATCCACGGCATACTATCAACATC | CAAGGCTCACCATCATCGTAG |
| *Cd8a* | CCGTTGACCCGCTTTCTGT | CGGCGTCCATTTTCTTTGGAA |
| *Gapdh* | AGGTCGGTGTGAACGGATTTG | TGTAGACCATGTAGTTGAGGTCA |
| *Itgax* | CTGGATAGCCTTTCTTCTGCTG | GCACACTGTGTCCGAACTCA |
| *Ly6g* | GACTTCCTGCAACACAACTACC | ACAGCATTACCAGTGATCTCAGT |
| *Ncr1* | ATGCTGCCAACACTCACTG | GATGTTCACCGAGTTTCCATTTG |
| *Thy1* | TGCTCTCAGTCTTGCAGGTG | TGGATGGAGTTATCCTTGGTGTT |