**Supplementary Methods**

***Electroporation experiments:*** Electroporations were performed following previously published protocols (1, 2). Plasmids were prepared using Qiagen Maxi prep kits and were diluted such that the working concentration was 5 μg/μl (shRNAs) or 1 μg/μl (full length Neogenin). Prior to injection, 0.1% fast green dye was added to the DNA solution to act as a tracer to monitor accurate injection location.

Mice were anesthetized by being placed in ice. A sharp 30 gauge needle was used to puncture the nasal sclera at the limbus border with the cornea. A Hamilton syringe containing a blunt end 0.5" 32 gauge needle tip was used to draw up 0.3 μl of solution and the needle tip was passed through the vitreous until resistance from the opposing scleral wall is felt. 10 mM diameter tweezer electrodes were used to apply five square pulses with each pulse consisting of 80V for 50 ms with a 950 ms interstimulus interval. The pup is then warmed up under a heating lamp until adequate recovery is achieved.

Neogenin constructs consisted of pCDNA3-hNeogenin VSVg and pCDNA3-hNeogenin D1323N described previously (3). For silencing experiments, previously validated shRNA's (Sigma) that target Mus musculus Neogenin (NM_008684.2) were used. Sequences are as follows:

shRNA 1:

CCGGCCCAGCAAACACGAAGTACAACTCGAGTTGTACTTCGTGTTTGCTGGGTTTTT

G*

shRNA 2:

CCGGCCCTCGAAACTCTCAAGATATCTCGAGATATCTTGAGAGTTTCGAGGGTTTTT

G**

*= mean knockdown level 0.68. **= mean knockdown level 0.58. Knockdown was verified using NIE115 Mus musculus neuroblastoma cell line that endogenously expresses Neogenin (data not shown).

***In situ hybridization.*** Mouse Neogenin 1231bp- 1694bp region was cloned into pBluescript II SK (+) following PCR using the following primers: mNeo-forward; gcaggaggttgcttggagat, mNeo-reverse; ttgtggctggtgctagatgg. In Situ hybridization was performed using digoxigenin (DIG) labeled anti-sense RNA probes (Roche). Alkaline phosphatase (AP)-conjugated anti-DIG antibody (Roche) was used to detect the hybridized probe. Sections were developed with 330 μg/ml 4-nitroblue tetrazolium chloride and 160 μg/ml 5-bromo-4-chloro-3-indolyl phosphate in 100 mM Tris-HCl, 5 mM MgCl2, pH 9.6. All sections were treated in tandem and stopped at the same time to allow relative mRNA quantification.

***Intravitreal Injection:*** 2 μl of solution was loaded into 10 μl gastight Hamilton syringe (7653-01) equipped with a blunt end 32G 0.5" tip (7803-04). A small incision was made in either the superior or inferior retina at the corneal-scleral border using a sharp 30G needle tip. Under a surgical microscope, the Hamilton syringe was inserted into the incision. 1 μl solution was slowly injected into the vitreous followed by careful removal of the needle tip; this resulted in efflux of approximately half of the injected solution. The needle tip was then carefully re-inserted into the eye and the remaining 1 μl injected. Care was taken to avoid unwanted elevation

of intraocular pressure. Based on tests using Fast Green dye, we calculated approximately 1 µl solution remains in the vitreous per treatment.

Following injection and prior to euthanasia or functional testing, eyes were dilated with 1% Mydriacyl and were checked under a microscope. Evidence of injection related damage to the lens or retina resulted in exclusion from analysis. The exceptions were experiments in Figure 6J-K. Here treatment related cataracts were observed and recorded in Supplementary Table 1.

***Immunohistochemistry.*** 12 µm thick serial cryosections were generated throughout the entire mouse retina, with the exception of human samples (see below). For consistency, only mouse sections from the central retina containing the optic nerve were used for imaging and subsequent quantification. Prior to blocking, optional heat induced antigen retrieval was done in 10 mM citric acid (pH6) depending on the requirements of the primary antibody. Slides were blocked in 10% serum + 0.05% tween in PBS for 1 hr at room temperature. Primary antibody incubation was in PBS + 5% serum + 0.05% tween overnight at 4°C in a humidified chamber.

Alexa conjugated secondary antibodies (LifeTechnologies) and DAPI incubation was performed the following day using standard procedures. Slides were mounted in MOWIOL mounting solution and imaged using an Olympus BX61 Spinning Disc Confocal or a Zeiss LSM780 Laser Scanning Confocal. For quantification of inner/outer segment Neogenin levels: all IHC procedures were done simultaneously under identical conditions using identical reagent preparations. All images were then taken using the Zeiss LSM780 using standardized settings (same values for averaging, pinhole size, gain, laser strength etc.) and a 63X oil immersion pan-achromat objective. Unaltered raw images were then analyzed using ImageJ (4).

For all IHC, negative controls consisted samples stained without primary antibodies. Antibodies used: Neogenin (C20, Santa Cruz; 1:200), Rhodopsin (1D4, Abcam; 1:1000) and Cone Arrestin (AB15282, Millipore; 1:1000).

Secondary Antibodies: Alexa conjugated (LifeTechnologies; 1:1000). Flat mount retina staining was performed as previously described (5).

***Tissue Preparation*.** Human eyes from donors with RP were collected through the FFB Rare Eye Donor Program (Foundation Fighting Blindness, Columbia MD). Eyes were examined, imaged and maintained by the Cole Eye Institute Tissue Repository in the Cleveland Clinic. Immuno-cytochemical analysis was performed with the approval of the Cleveland Clinic Institutional Review Board (IRB #14-057). The research adhered to the tenets of the Declaration of Helsinki. The interval between death and tissue fixation was 4-28 hours post-mortem (PMI). Globes were fixed in 4% paraformaldehyde and 0.5% glutaraldehyde made in D-PBS. Upon receiving, globes were cut through the ora serrata, imaged in D-PBS, and stored in 2% paraformaldehyde in D-PBS. The analyzed tissue included FFB donations #685, 951, 968, 1002, and 1027.

***TUNEL staining.*** For all TUNEL staining procedures, 9 μm thick cryosections of the mouse retina were prepared. Sections were first washed in PBS 3X5 min then incubated in PBS + 0.1% triton. For a positive control, a slide was incubated in DNAse I at room temperature for 30 min, followed by 3X5 min washes in PBS. TUNEL staining consisted of TDT buffer (Roche), TdT enzme (Roche), biotin d-UTP (Roche) and CaCO at 37°C for two hours. Slides were then washed 5X5 min in PBS + 0.1% triton. For TUNEL detection Streptavidin conjugated to Alexa 555 was used and imaged using an Olympus BX61 fluorescent microscope. Quantification was done using CellSens software (Olympus).

***RNA extraction, Reverse Transcription and Quantitative Real-Time PCR.*** This was done as previously described (6-8). Briefly, total RNA was isolated from retinas using the RNeasy mini kit (Qiagen), followed by digestion with RNase-Free DNase (DNA-free™, Thermo Fisher Scientific) to remove DNA contamination. After quantification by a Nanodrop (NanoDrop Technologies, USA), first-strand cDNA was synthesized from 0.2-1μg of total RNA using SuperScript II first-strand synthesis system (Invitrogen). PCR primers are as follows:

Intron-1f: 5'-tgtttcatgcattcctggag-3'

Intron-1r: 5'-cttcaagagccgagtgaacc-3'

Intron-2f: 5'-gccagctgagaaaaggaaaa-3'

Intron-2r: 5'-cccagttccacattcaggtt-3'

Last Exon-1f: 5'-TGAACCAGATGAGCTGACCA-3'

Last Exon-1r: 5'-AGGCTTGGAGTCATGTCCAG-3'

Last Exon-2f: 5'-GCCAAGGGAGTCTGTGATGT-3'

Last Exon-2r: 5'-CAATGCCTGTCAGTGGTGTC-3'

Real-time quantitative PCR was performed using the C1000 touch Thermal Cycler (Bio-Rad, USA). Tests were run in duplicate on three separate biological samples with SYBRGreen PCR Master Mix (Applied Biosystems). PCR consisted of 40 cycles of denaturation at 95°C for 15 seconds, annealing and extension at 55°C for 30 seconds. An additional cycle (95°C, 15 seconds) generated a dissociation curve to confirm a single product. Values obtained for test RNAs were normalized to β-actin mRNA levels.

## Supplementary References:


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2. Cockburn DM, Charish J, Tassew NG, Eubanks J, Bremner R, Macchi P, et al. The double-stranded RNA-binding protein Staufen 2 regulates eye size. *Mol Cell Neurosci.* 2012;51(3-4):101-11.
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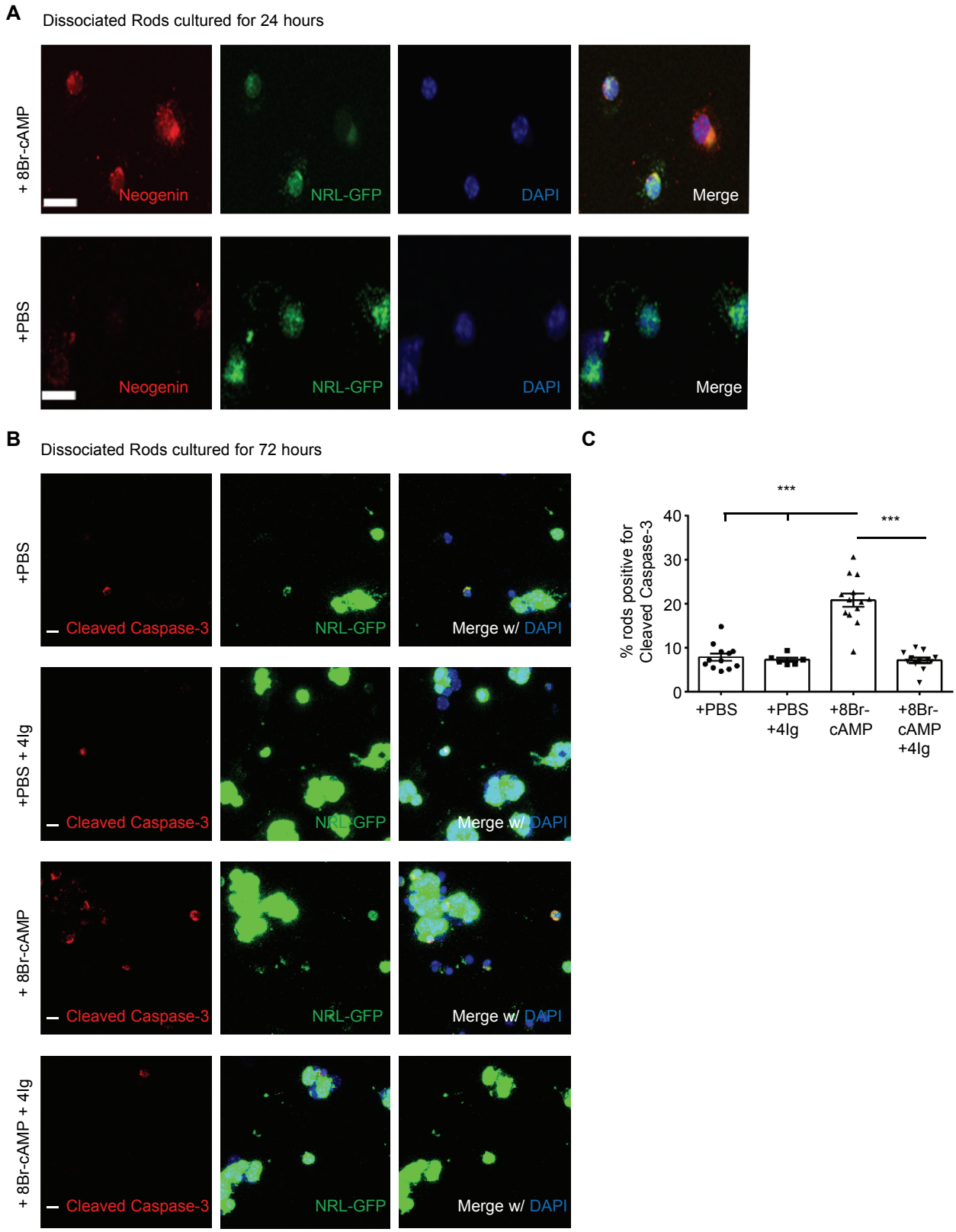
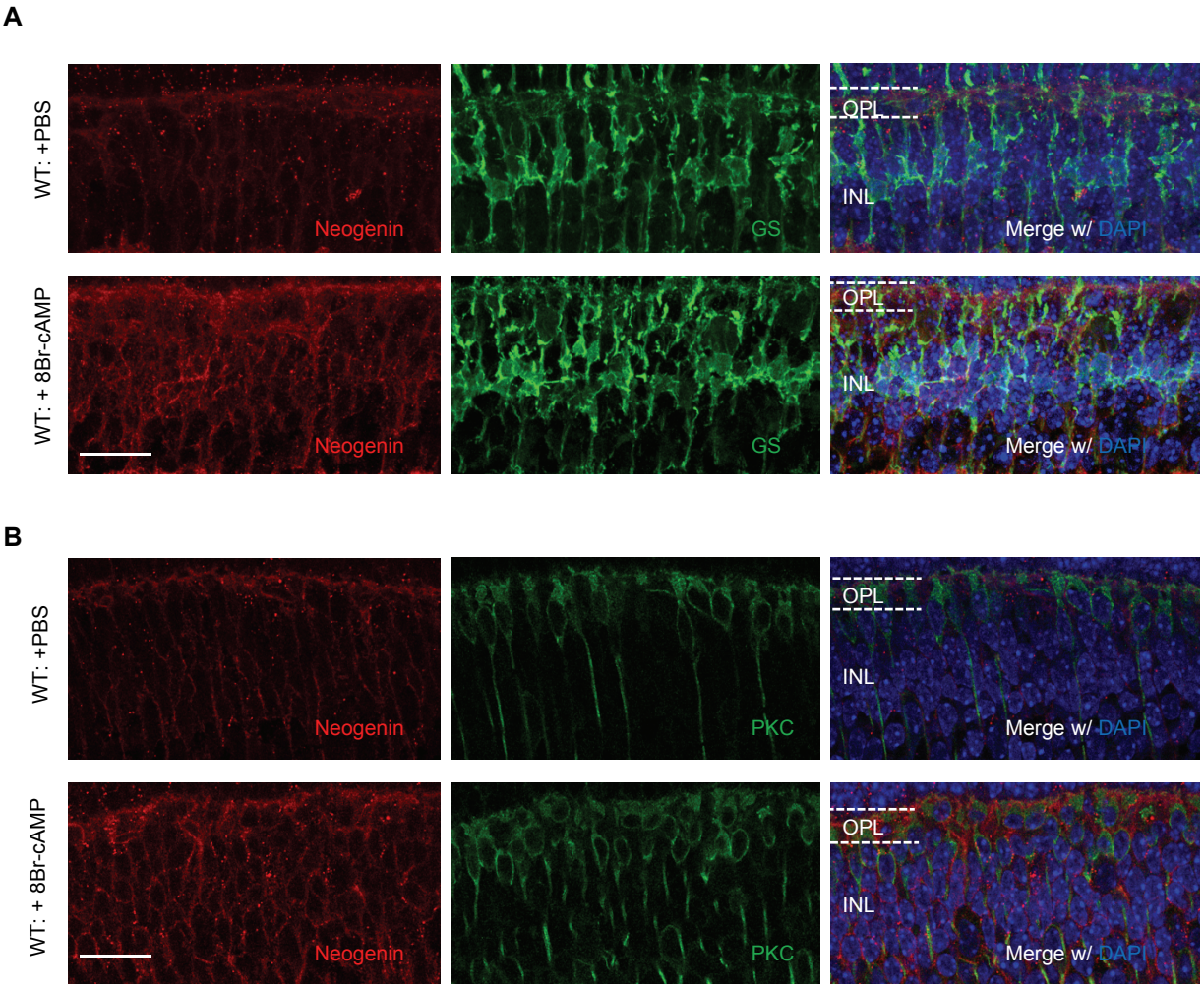
8. Wei R, Ren X, Kong H, Lv Z, Chen Y, Tang Y, et al. Rb1/Rbl1/Vhl loss induces mouse subretinal angiomatous proliferation and hemangioblastoma. *JCI Insight.* 2019;4(22).

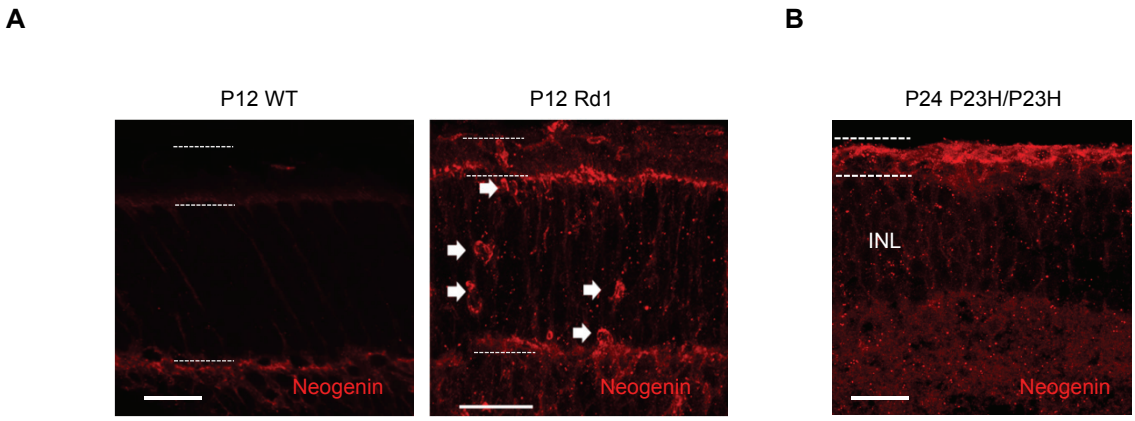



**Supplementary Figure 1. (A)** Dissociated rod photoreceptors from P3 Nrl-GFP mice were incubated in the presence of 1 mM 8Br-cAMP for 24 hrs (top) or without (bottom). In Nrl-GFP mice, rods express GFP. Cell surface Neogenin (red) staining is only seen in rods (green) when cells were treated 8Br-cAMP. Nuclear stain is in blue (DAPI). Scale: 10 μm. **(B)** Dissociated P7 photoreceptor cultures (NRL-GFP; green) were incubated in the presence or absence of 1 mM 8Br-cAMP and 50 μg/mL 4Ig. After 72 hours, cells were fixed and stained with Cleaved Caspase-3 (red) and a nuclear stain (DAPI; blue). Scale: 10 μm. (C) Quantification of (B). Addition of 8Br-cAMP (n=13) leads to a significantly increased percentage of rod

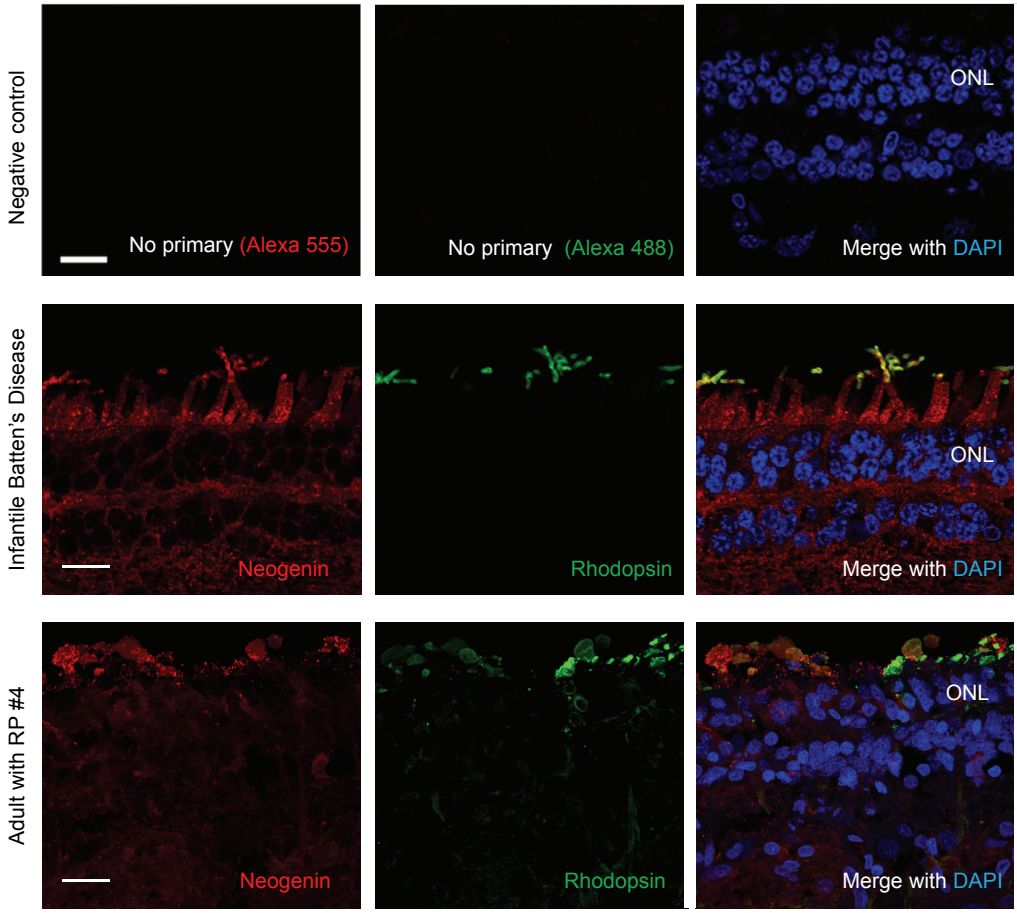
photoreceptors staining positive for Cleaved capsase-3 when compared to PBS treated of PBS + 4Ig treated controls (n= 12 and 6). This effect was completely abrogated with the addition of 4Ig to 8Br-cAMP (n=12; ***, $p<0.001$). Significance determined by one-way ANOVA followed by Bonferroni's multiple comparison test.



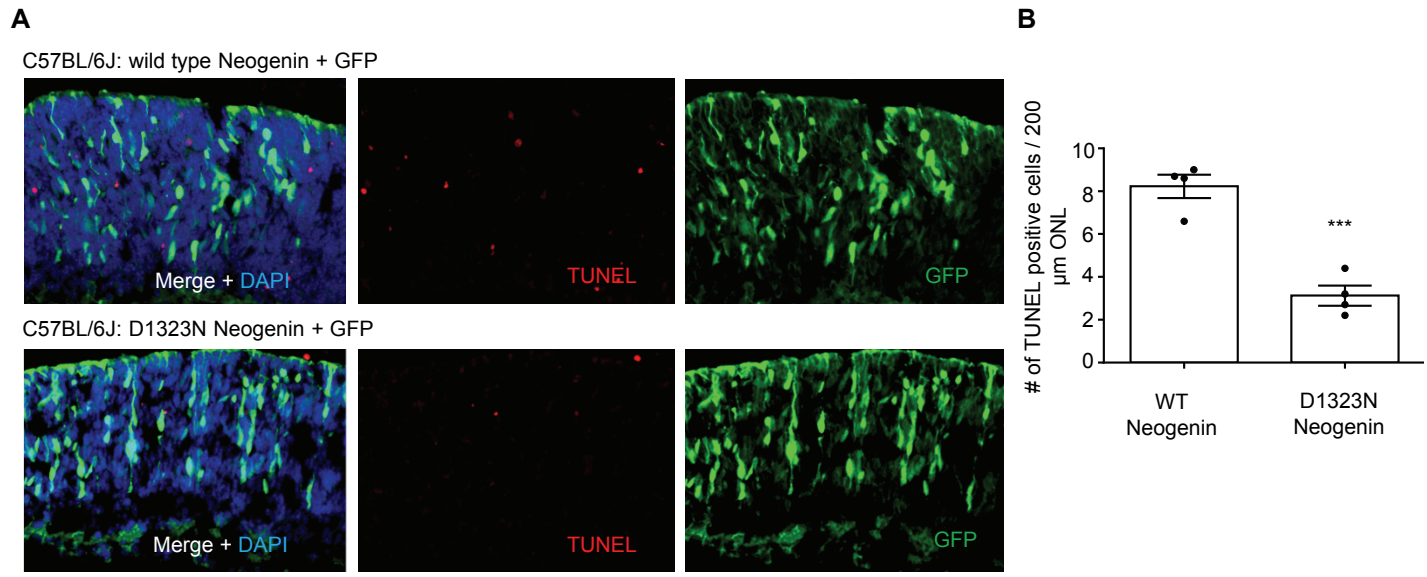

**Supplementary Figure 2. (A and B)** P9 C57BL/6J (WT: Wild Type) mice received one intravitreal injection of either 2 µl 10 mM 8Br-cAMP or 2 µl PBS. Eyes were harvested at P12 and cryosectioned. **(A)** Representative images of P12 retina treated with PBS (top) or 10 mM 8Br-cAMP (bottom) and stained for Neogenin (red), a marker for Müller glia (GS: glutamine synthetase; green) and a nuclear stain (DAPI; blue). In 8Br-cAMP treated eyes, there is increased Neogenin expression in cells corresponding to Müller glia in the inner nuclear layer (INL). Dashed lines indicate the border of the outer plexiform layer (OPL). Scale = 20 µm. **(B)** Representative images of P12 retina treated with PBS (top) or 10 mM 8-Br-cAMP (bottom) and stained for Neogenin (red), a marker for rod bipolar cells (PKC: protein kinase c; green) and a nuclear stain (DAPI; blue). Neogenin staining in the INL does not correspond to rod bipolar cells. Scale = 20 µm.

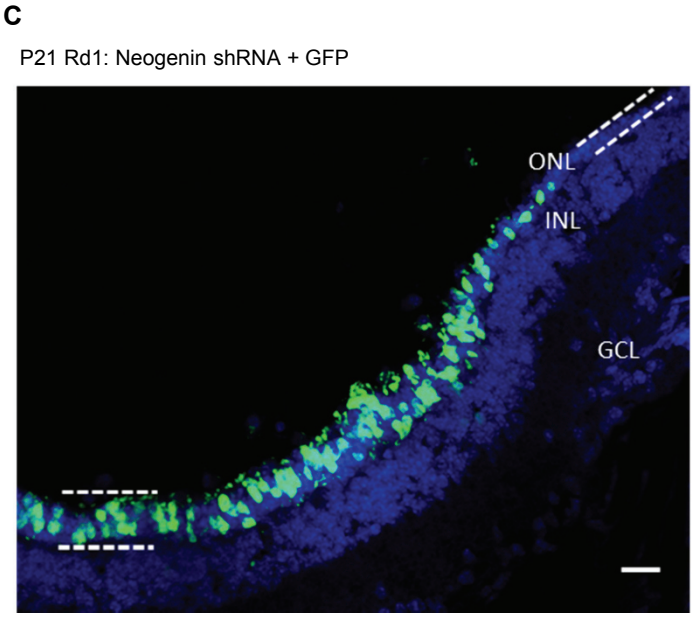



**Supplementary Figure 3. (A)** Representative image of Neogenin staining (red) in the P12 WT (Wild Type, C57BL/6J; left) and Rd1 (right) retina. At P12 in Rd1, but not WT mice, increased Neogenin (red) expression can also be seen in the soma of some degenerating photoreceptors (arrows). Dashed lines indicate the border of the Outer Nuclear Layer (ONL) and the Inner/Outer Segments (IS/OS). Note: gain settings were increased for these images. Scale = 20 µm. **(B)** Representative image of Neogenin staining in a P24 P23H/P23H retina. Similar to Rd1 and Rd10 retina, there's increased Neogenin signal in the photoreceptor layer. Dashed lines indicate the border of the Outer Nuclear Layer. INL = Inner Nuclear Layer. Scale = 20 µm.

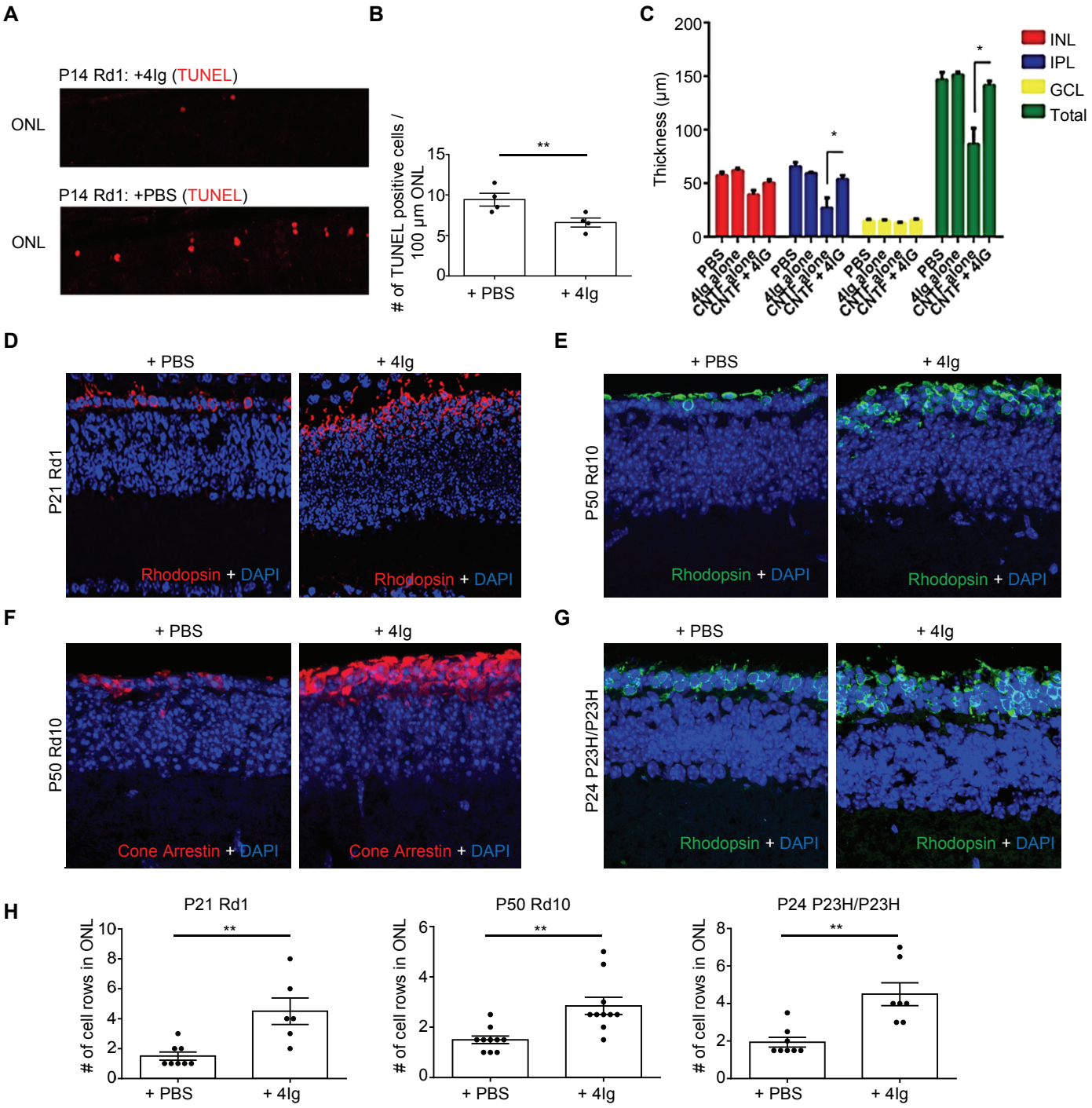



**Supplementary Figure 4.** Top: Negative control immunohisochemistry staining performed on a post-mortem cryosection from a human retina with retinal degeneration. Sections were stained without primary antibodies followed by incubation with a secondary antibody conjugated to the indicated fluorophore. Image settings correspond to those used in Figure 4. The merge image includes a nuclear stain (DAPI; blue). ONL = Outer Nuclear Layer. Middle: A representative image of an immunostained cryosection from an age 3 human retina with infantile Batten's disease. Sections are stained for Neogenin (red), Rhodopsin (green) and a nuclear stain (DAPI; blue). Note the presence Neogenin in the inner/outer segments in the diseased retina. Scale= 20 µm. Bottom: An example of an immunostained section from an age 41 human retina diagnosed with Retinitis Pigmentosa (RP) as well as displaying gross pathology consistent with diabetic retinopathy. Scale = 20 µm.




**Supplementary Figure 5. (A)** The temporal retina of P0 C57BL/6J (WT: wild type) mice were electroporated with a full-length wild type Neogenin expression plasmid or with a D1323N Neogenin mutant expression plasmid (pCDNA3-hNeogenin D1323N). D1323N Neogenin lacks the cleavage site to produce Neogenin's pro-apoptotic domain. Eyes were harvested two days later and TUNEL staining (red) performed. GFP (green) indicates electroporated cells. **(B)** Quantification of A). Overexpression of a non-cleavable Neogenin mutant significantly reduced the number of TUNEL positive cells. For each eye, TUNEL cells were counted on 3 sections and averaged. (n=4 for each; ***, $p<0.001$). Significance determined by Student's t-test. **(C)** An example of a P21 Rd1 retina electroporated at P0 with shRNA 4 against Neogenin (+GFP). Note that the thickness of the Outer Nuclear Layer (ONL) reduces as soon as non-electroporated regions of the ONL are reached. Scale 20 µm. INL= Inner Nuclear Layer; GCL= Ganglion Cell Layer.



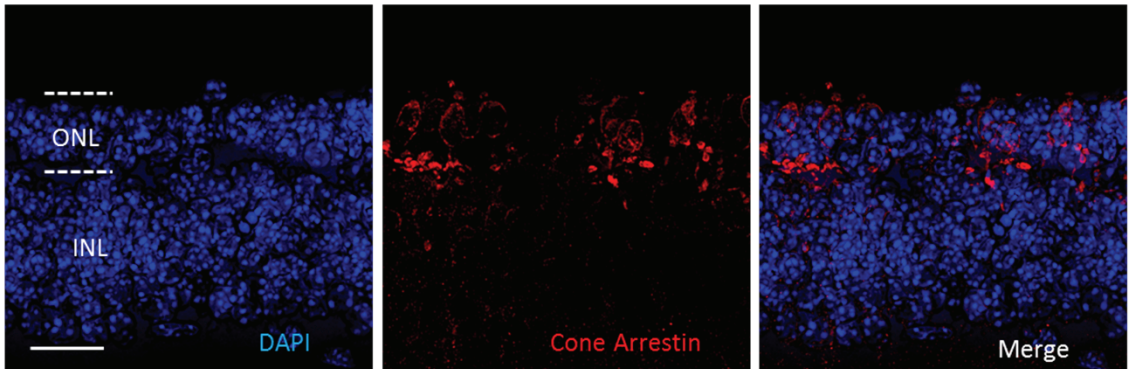

**Supplementary Figure 6. (A)** Rd1 injected with PBS or 4Ig (1 µg/µl) at P9, harvested at P14 and used for TUNEL staining (red). ONL: Outer Nuclear Layer. **(B)** Quantification of **(A)**. The number of TUNEL cells per 100 µm length of ONL of 9 µm thick sections. Three sections per eye were measured and averaged (n=4 for each; **, p<0.01). Significance determined by Student's t-test. **(C)** Quantification of the thickness of INL (inner nuclear layer), IPL (inner plexiform layer), GCL (ganglion cell layer) and total retinal thickness at 400 µm from the optic disk for eyes presented in Figure 6J. CNTF significantly reduced total retinal thickness, primarily due to thinning of the IPL. This effect was not present in the combination treatment group (n=8 for PBS; n=7 for CNTF; n=8 for 4Ig; n=8 for 4Ig+ CNTF groups; *,p<0.05). Significance determined by one-way ANOVA with Bonferroni's test for multiple comparisons. **(D)** Rd1 treated with PBS or 4Ig (1 µg/µl) at P9 and P15 and harvested at P21. Sections were stained with DAPI (nuclear stain; blue) and a marker for rods (Rhodopsin; red). **(E)** Rd10 treated with 4Ig (1 µg/µl) or PBS at P20, P30 and P40 and eyes were harvested at P50. Sections were stained with DAPI (blue) and Rhodopsin (green). **(F)** Rd10 sections stained with a marker for

cones (Cone Arrestin; red) and DAPI (blue). **(G)** Rho (P23H/P23H) treated with 4Ig (1 µg/µl) or PBS at P10 and eyes were harvested at P24. Sections were stained with a marker for rods (Rhodopsin; green) and DAPI (blue). **(H)** Additional quantifications of data presented in **(D-G)** and Figure 6 (B-D) demonstrating 4Ig treatment significantly increases the number of cell rows in the ONL compared to controls in P21 Rd1 mice (left; n=8 and 6; **, $p<0.01$), P50 Rd10 mice (middle: n=10 and 11; **, $p<0.01$) and P24 Rho (P23H/P23H) mice (right: n=8 and 6; **,$p<0.01$). Significance determined by Student's t-test.

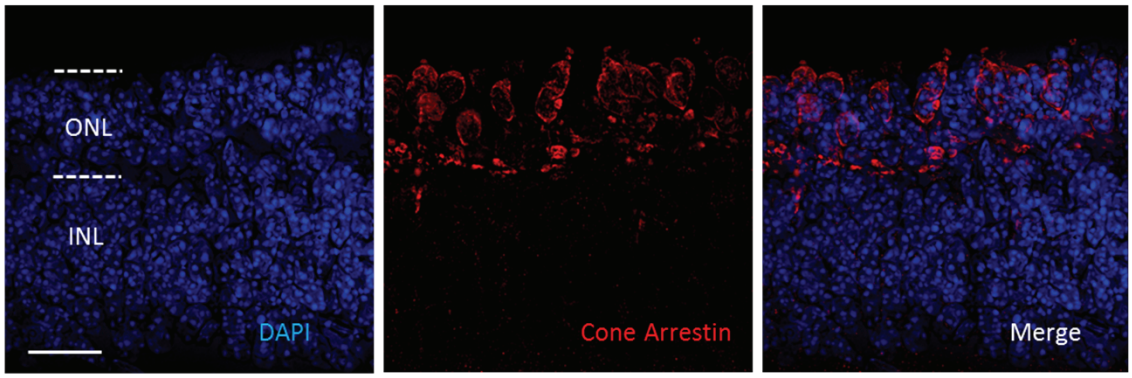
| | Severe cataract (majority of lens) | Mild cataract (injection site) | Normal |
|---|---|---|---|
| PBS | 0% (0/8) | 0% (0/8) | 100% (8/8) |
| CNTF alone | 100% (7/7) | 0% (0/7) | 0% (0/7) |
| 4Ig alone | 0% (0/8) | 0% (0/8) | 100% (8/8) |
| CNTF + 4Ig | 12.5% (1/8) | 25% (2/8) | 62.5% (5/8) |

**Supplementary Table 1.** Table illustrating the incidence of treatment related cataracts for the respective treatment groups presented in Figure 6C. CNTF alone had a 100% incidence of severe cataracts, whereas 4Ig + CNTF had a 12.5% incidence of severe cataracts.

4Ig treated Rd1 retina: Example 1

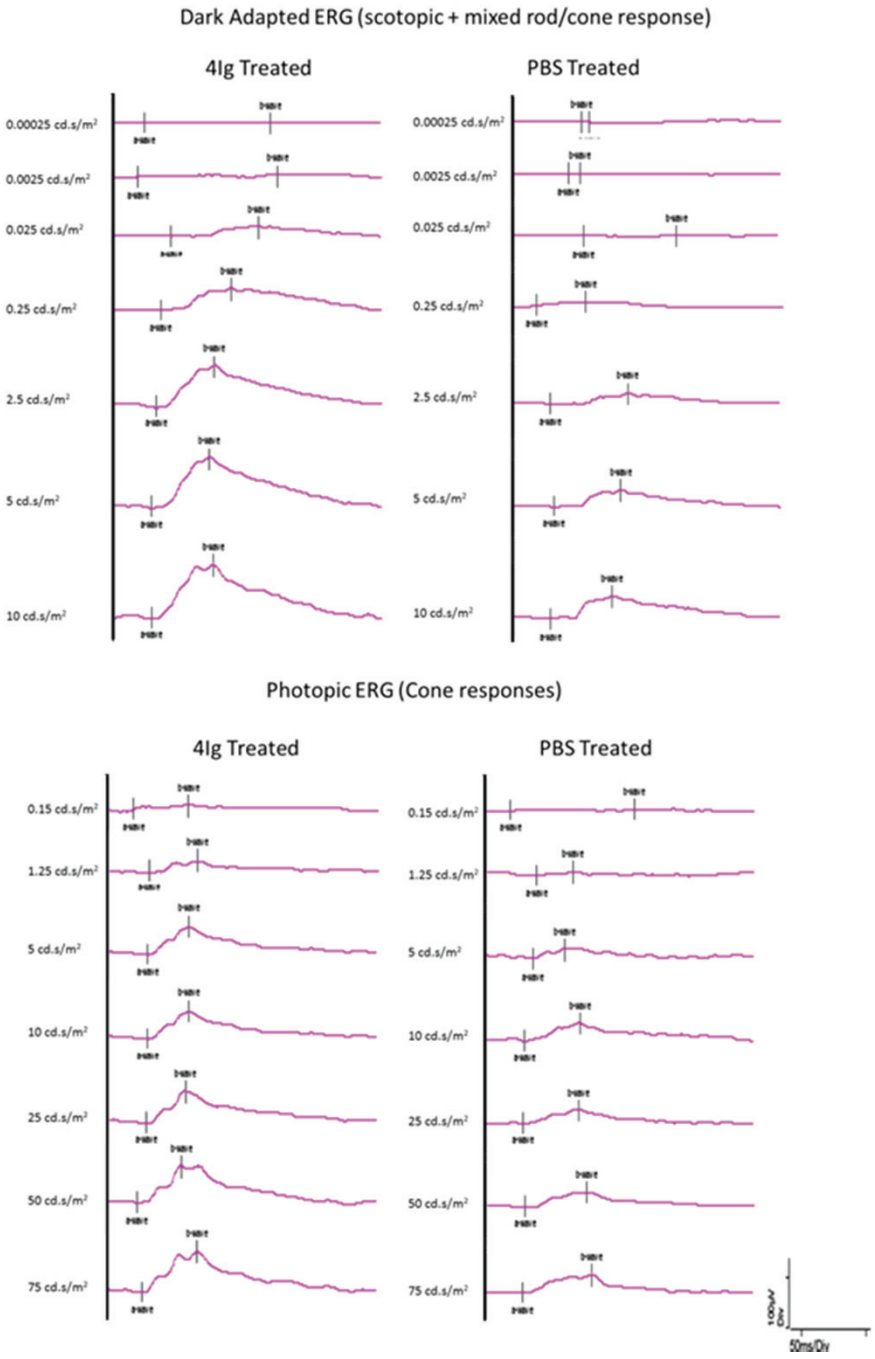



4Ig treated Rd1 retina: Example 2




4Ig treated Rd1 retina: Example 3


**Supplementary Figure 7.** Rd1 mice were treated at P8 and P13 with 2 µl of 4Ig (1 µg/µl) injected into the vitreous. Eyes were used for light-evoked retinal ganglion cell recording at P29-P35 seen in Figure 7. Following recordings, retinas were fixed and cryosectioned. Presented are examples of three treated Rd1 retinas stained with a nuclear stain (DAPI;blue) and a marker of cones (Cone Arrestin; red). Note the preservation in the size of the ONL and the number of cones at this advanced stage of degeneration. Scale = 20 µm




**Supplementary Figure 8.** Examples of ERG traces for one 4Ig treated (left) and one PBS treated (right) P30 Rd10 mouse as used for Figure 7D. Included are traces under scotopic (top) and photopic (bottom) conditions. For scotopic traces: pictured are average traces from 5-10 recording per stimulus intensity. For photopic traces: pictured are average traces from 30 recordings per stimulus intensity.