Integrated Data Analysis

For all ChIP-Seq data, we used BWA (version 0.5.9-r26-dev, default parameter) to align the reads to the mouse genome mm9(MGSCv37 from Sanger) and Picard(version 1.65(1160)) was used for marking duplicated reads. Duplicated reads were eliminated with samtools (parameter "-q 1 -F 1024" version 0.1.18 (r982:295)). For quality control (QC) assessment and estimation of the fragment size, non-duplicated version of SPP(version 1.11) was used for cross-correlation with support of R ((version 2.14.0) with packages caTools(version 1.17)) and bitops(version 1.0-6). All our data passed QC following ENCODE criterion. Upon manual inspection of the cross-correlation plot generated by SPP, the best fragment size estimated (the smallest fragment size estimated by SPP in all our cases) were used to extend each reads and generate bigwig files to view on IGV (version 2.3.40). We scaled the bigwig files to normalized to 15M reads so tracks are comparable across replicates. For point-source factors (Brg1-FLAG, H3K4me1, H3K4me3, H3K27Ac), MACS2 (version 2.0.9 20111102, nomodel with extsize defined as fragment size estimated above) was used to call peaks and peaks within 100bp were merged using bedtools (version 2.17.0). For broad peaks (H3K27me3), SICER (version 1.1 redundancy threshold 1, window size 200bp, effective genome fraction 0.86, gap size 600bp, FDR 0.00001 with fragment size defined above) was used for domain calling.

For each point-source histone modification with replicates, we called peaks with MACS2 twice independently with an FDR cutoff of 0.05 and 0.5. We then finalize the peaks call if they had an FDR cutoff of 0.05 in one replicate. For H3K27me3, we used the common regions from two replicates as the H3K27me3 heterochromatin domain. Brg1 promoter distal sites (not within 1kb of any TSS defined in Refseq or UCSC database) were classified into 5 classes based on whether there were overlapping histone modification regions as follows:

Active (Brg1+,H3K4me1+,K3K27ac+,H3K27me3-)

Bivalent (Brg1+,H3K4me1+,H3K27Ac+,H3K27me3+)

Isolated (Brg1+,H3K4me1-,H3K27Ac-,H3K27me3-)

Latent (Brg1+,H3K4me1+,H3K27Ac-,H3K27me3-)

Repressed (Brg1+,H3K4me1+,H3K27Ac-,H3K27me3+)

Brg1 and histone modification heatmaps were generated by ngsplot (version 2.41.3).

To compare our data to the published Brg1-FLAG data, we first downloaded Brg1-FLAG-ChIP peaks from GEO id GSE37151 for all tissues available (Heart, Limb, Hindbrain, Forebrain, Neural tube, Face for Mouse Embryonic cell at E11.5, and Mouse Embryonic stem cells). We then merged peaks across all tissues with our MACS2 called retinal P0 BRG1-FLAG-ChIP peaks if they are within 100bp to each other. Using these merged peaks as reference peaks, we calculated the correlation between two tissues as the percentage of overlapping reference peaks in both tissues divided by reference peaks in either tissue. All classification of Brg1 binding sites was made on reference peaks so the correlations could be re-calculated based on each subset.

To estimate the nucleosome depleted region size, first PyWavelets(version 0.3.0, wavelet template rbio1.3 at level 5) have been applied to the H3K27Ac bigwig profiles generated and noise have been filtered out. Then local peak summits at least 146bp (the classical DNA size wrapped a nucleosome core in mammal) to each other have been detected and filtered by higher than median of all summits signal within 2kb of TSS (python scripts are freely available upon requests). “-1” and “+1” nucleosomes then have been assigned based on the P12 CKO deregulated genes in supplementary table S9 and the nucleosome depleted region sizes are the distance between each pair of “-1” and “+1” nucleosomes. All genes have “+1” nucleosome within 2kb of TSS while less than 10% genes didn’t have detectable “-1” nucleosome within 2kb of TSS have been removed.

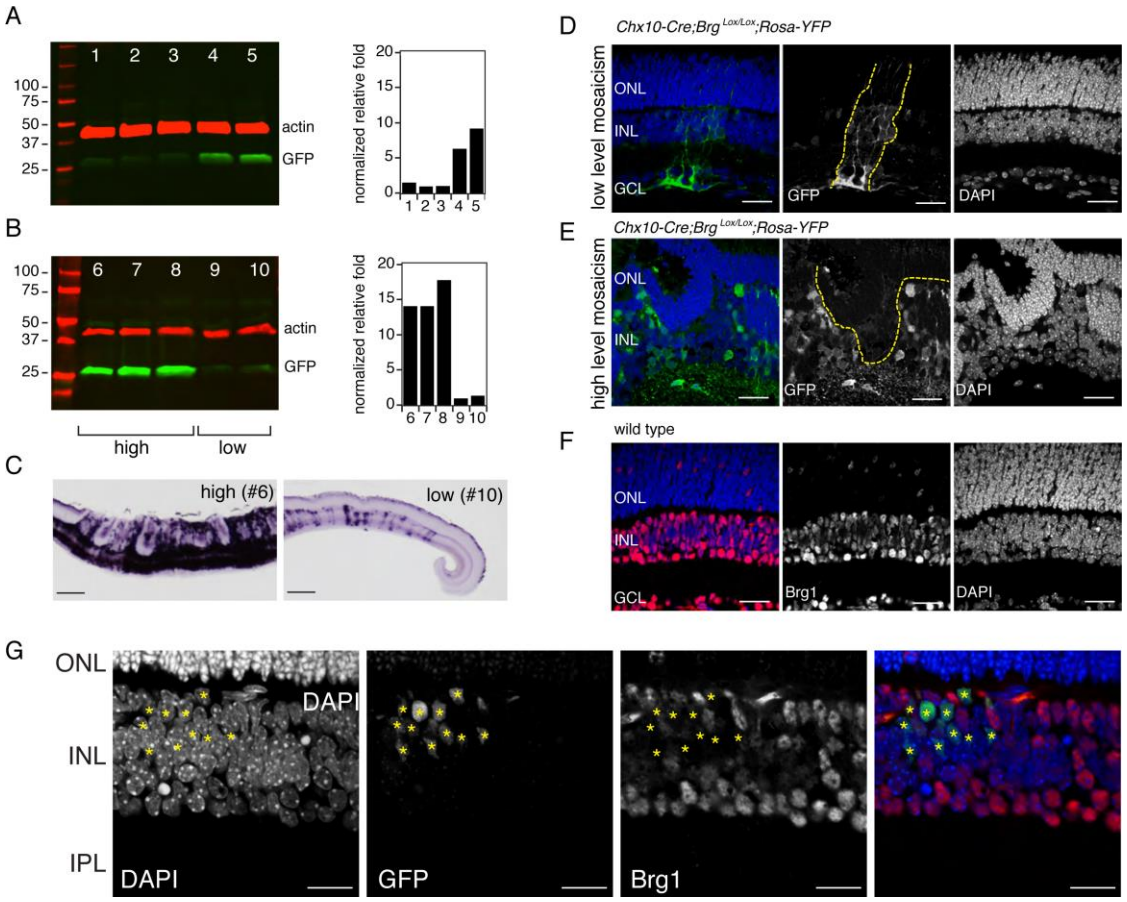


Figure S1. Mosaic inactivation of Brg1 with Chx10-Cre. A,B) Immunoblot and quantitation of GFP expression from the Chx10-Cre transgenic construct in 10 P12 *Chx10-Cre;Brg1lox/lox* pups. Normalized relative fold of GFP expression from the transgene is shown in the adjacent histograms. **C)** Representative bright field micrographs of retinal sections stained for alkaline phosphatase (expressed from the Chx10-Cre transgene). One retina with high levels of expression from the transgene and one retina with low levels of expression is shown. **D-F)** Micrographs of immunofluorescence of YFP expression from the *Rosa-YFP* Cre reporter gene. A representative retina with low level expression (D) and one with high level expression (E) is shown. The higher level of Cre expression leads to disruption in retinal lamination as shown in (E) and (C). **(F)** Immunofluorescence of Brg1 protein in P12 wild type retina showing nuclear localization to virtually all cells in the INL and GCL. **(G)** Immunostaining for Brg1 (red) and GFP (green) in *Chx10-Cre:Brg1lox/lox;Rosa-YFP* retinae showing loss of Brg1 protein (red) in the cells that had expressed Cre (green) (yellow *). Abbreviations: ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer. Scale bars: C, 100 μm; D-F, 25 μm; G, 10 μm.

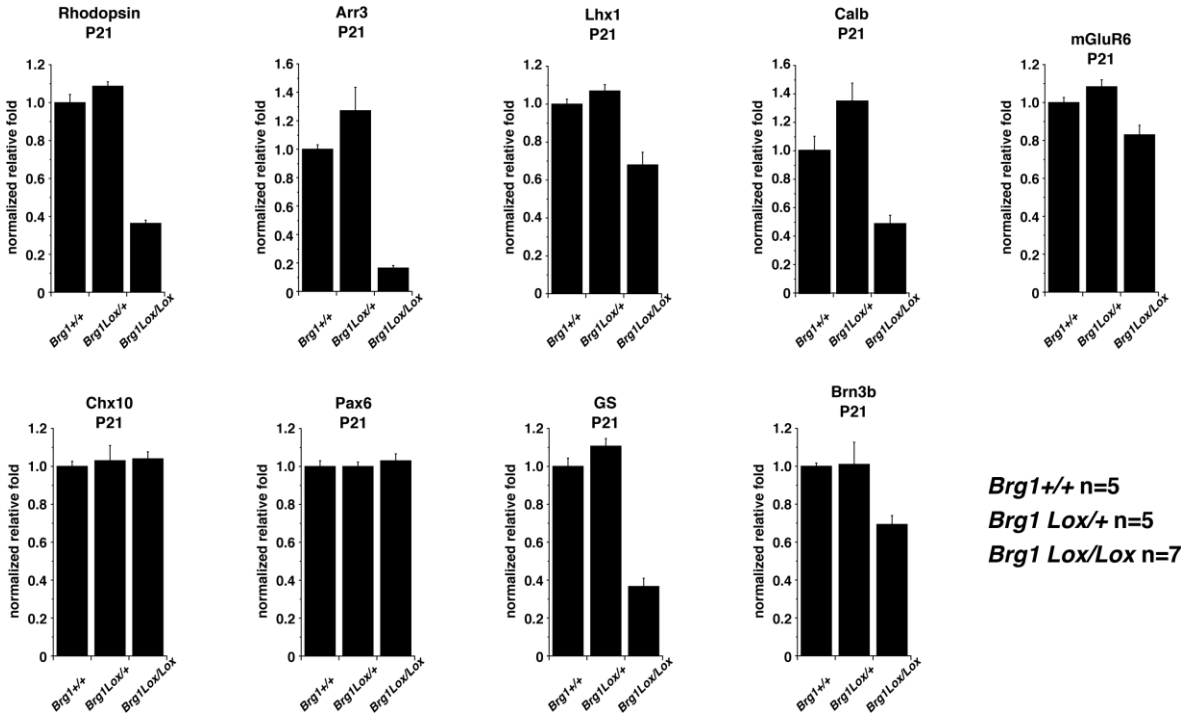


Figure S2. Gene expression analysis of wild type and Brg1-deficient retinae.

Histogram of quantitative real time PCR using Taqman probes for P21 retinae. Each bar is the mean and standard deviation of replicate PCR from 5-7 samples.

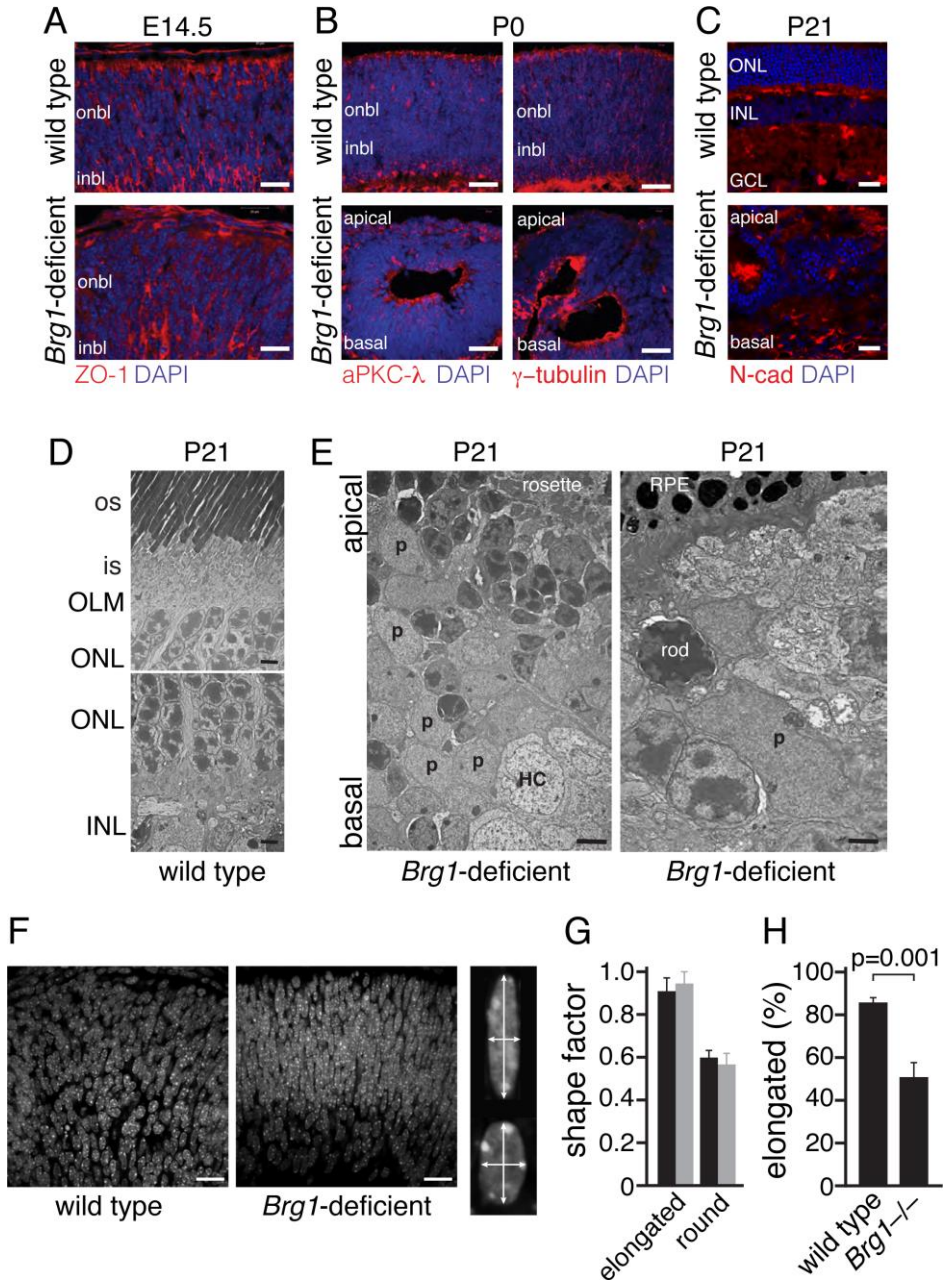


Figure S3. Brg1-deficient retinae have defects in retinal organization. A-C) Micrographs of immunofluorescent staining for ZO-1, aPKCλ, γ-tubulin and N-cadherin (red) with blue nuclear counterstain (DAPI). **D,E)** Electron micrograph of wild type P21 retina and *Brg1*-deficient P21 retinae showing immature cells with features of progenitor cells (p). **F)** Confocal micrograph of wild type and *Brg1*-deficient E14.5 retinae stained with DAPI with high magnification view of individual nuclei from the wild type (top) and *Brg1*-deficient (bottom) retinae. Arrows indicate the measurements made to determin the shape factor **(G)** and percentage of retinal progenitor cells with elongated nuclei (height/width≥2.0). Abbreviations: onbl, outer neuroblastic layer; inbl, inner neuroblastic layer; os, outer segment; is, inner segment; OLM, outer limiting membrane, ONL, outer nuclear layer; INL, inner nuclear layer. Scale bars: A,B,C, 25 μm; F, 10 μm.

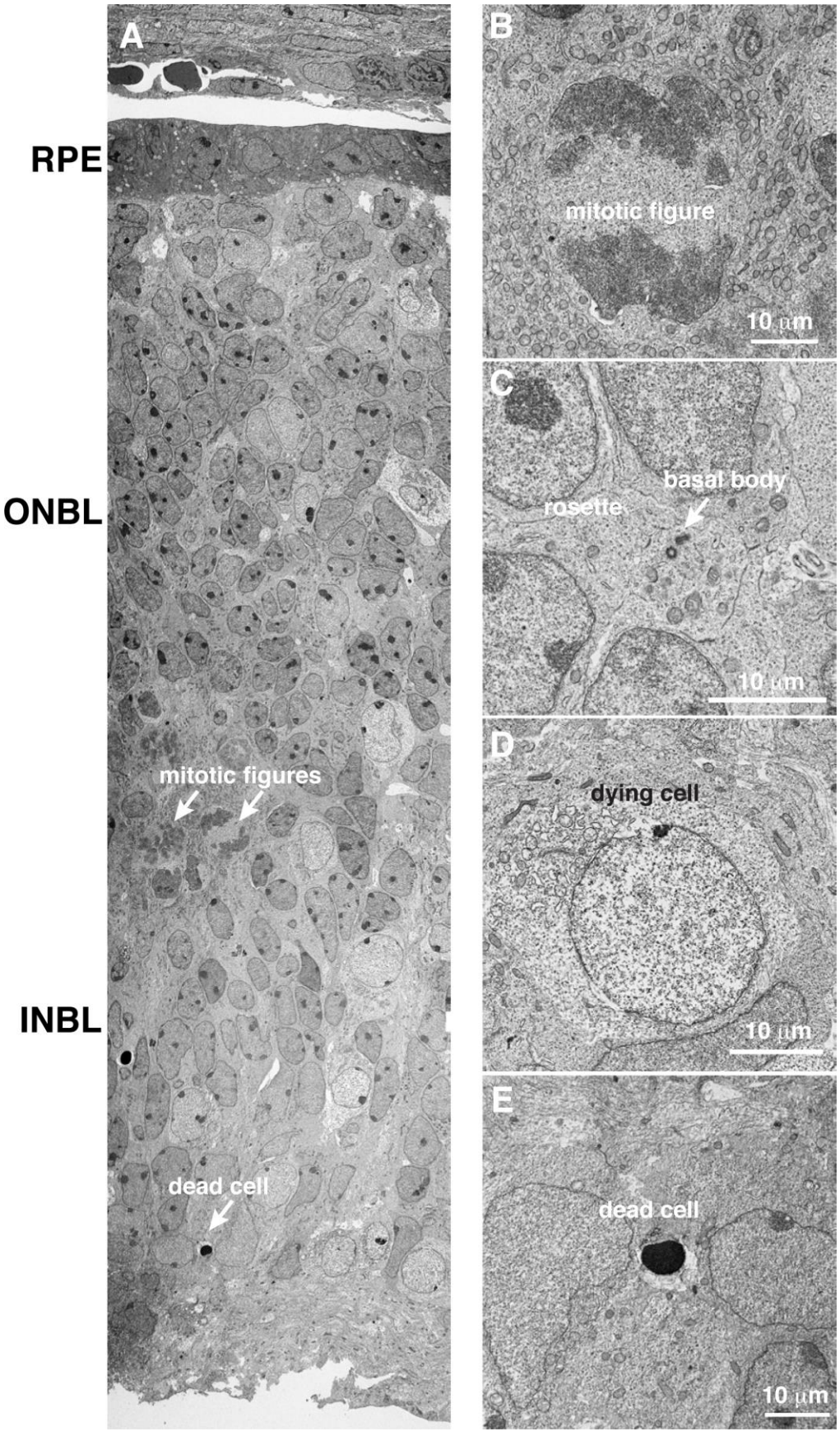


Figure S4. Electron micrographs of E14.5 Brg1-deficient retina. A) Electron micrograph montage of an E14.5 Brg1-deficient retina showing mitotic figures in the center of retina (arrows). **B-E)** High magnification electron micrographs of a mitotic figure, basal body within a rosette, dying cell and debris from a dead cell in the same E14.5 Brg1-deficient retina as shown in (A). Abbreviations: RPE, retinal pigment epithelium; ONBL, outer neuroblastic layer; INBL, inner neuroblastic layer.

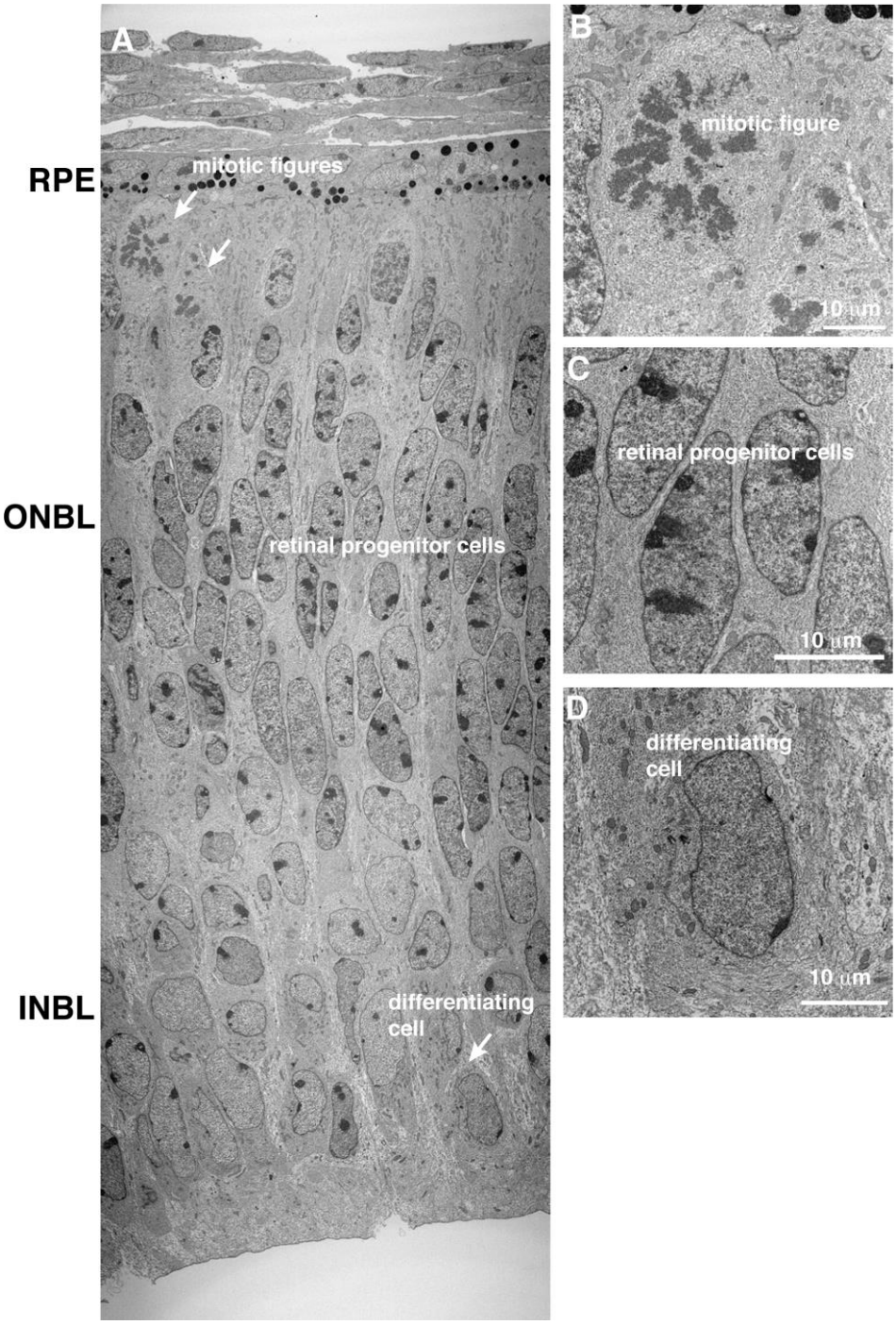


Figure S5. Electron micrographs of E14.5 wild type retina. A) Electron micrograph montage of an E14.5 Brg1-deficient retina showing mitotic figures at the apical edge and retinal progenitor cells with elongated nuclei as well as differentiating cells on the inner surface of the retina (arrow). **B-D)** High magnification electron micrographs of a mitotic figure, retinal progenitor cells and a differentiating cell from the same wild type retina as shown in (A). Abbreviations: RPE, retinal pigment epithelium; ONBL, outer neuroblastic layer; INBL, inner neuroblastic layer.

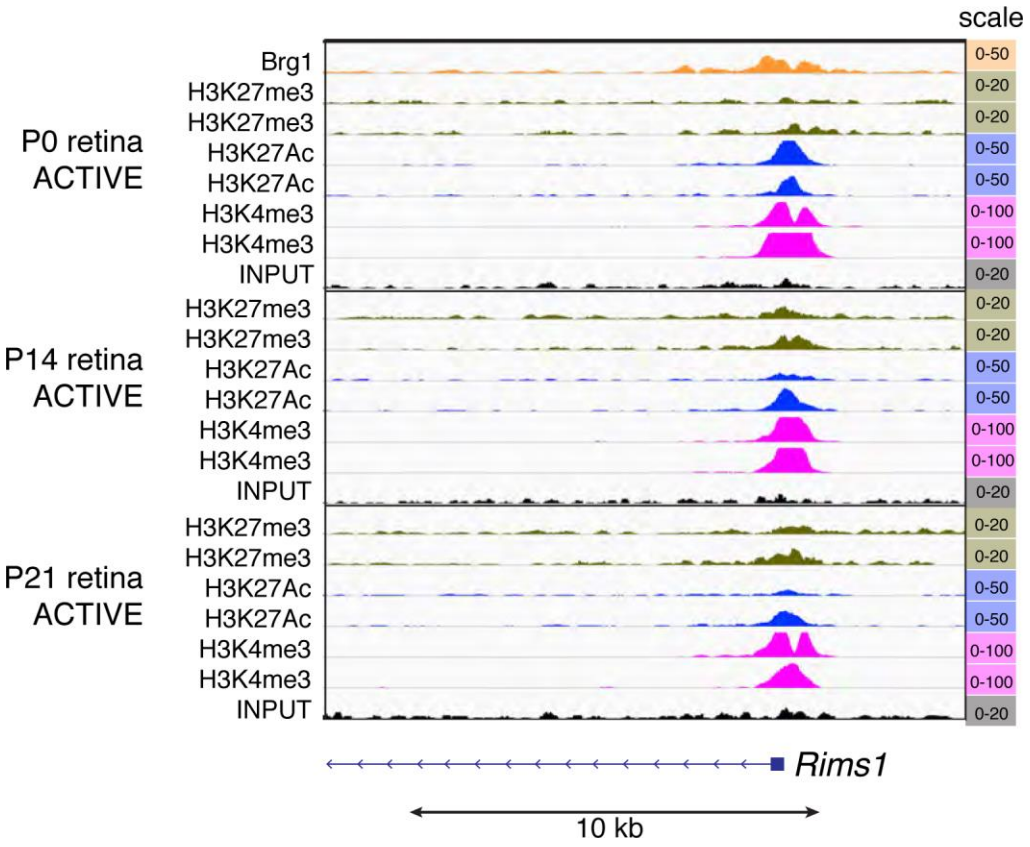
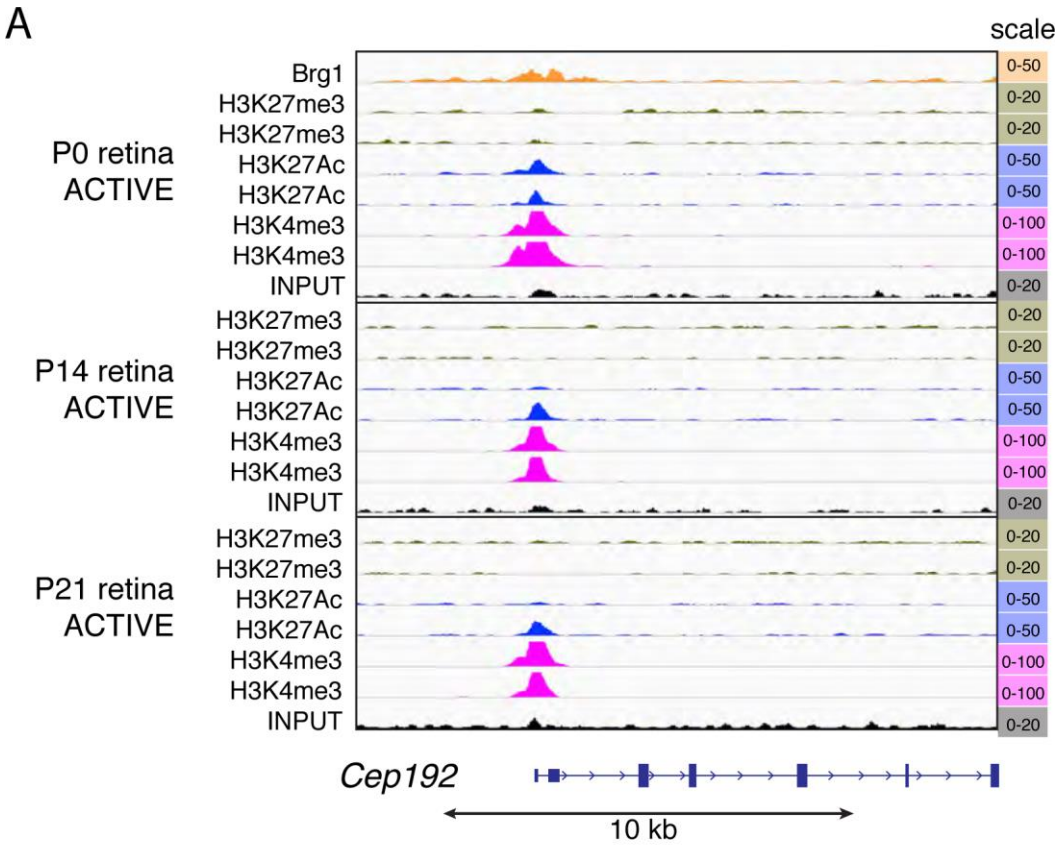


Figure S6. ChIP-seq promoter profile for *Rims1*. ChIP-Seq traces for an active Brg1 promoter proximal site in the *Rims1* promoter of the P0 retina that is also active at later stages of development (P14 and P21). Biological duplicate ChIP-Seq is shown for each histone mark at each stage.



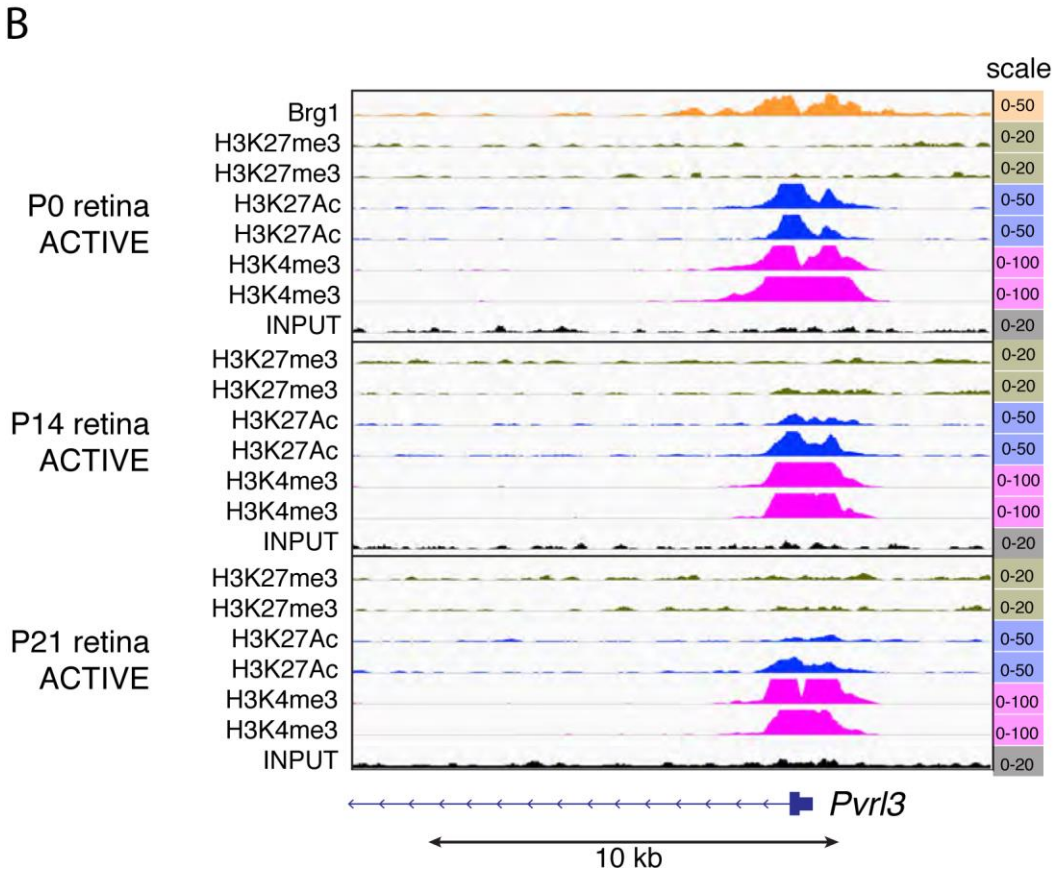
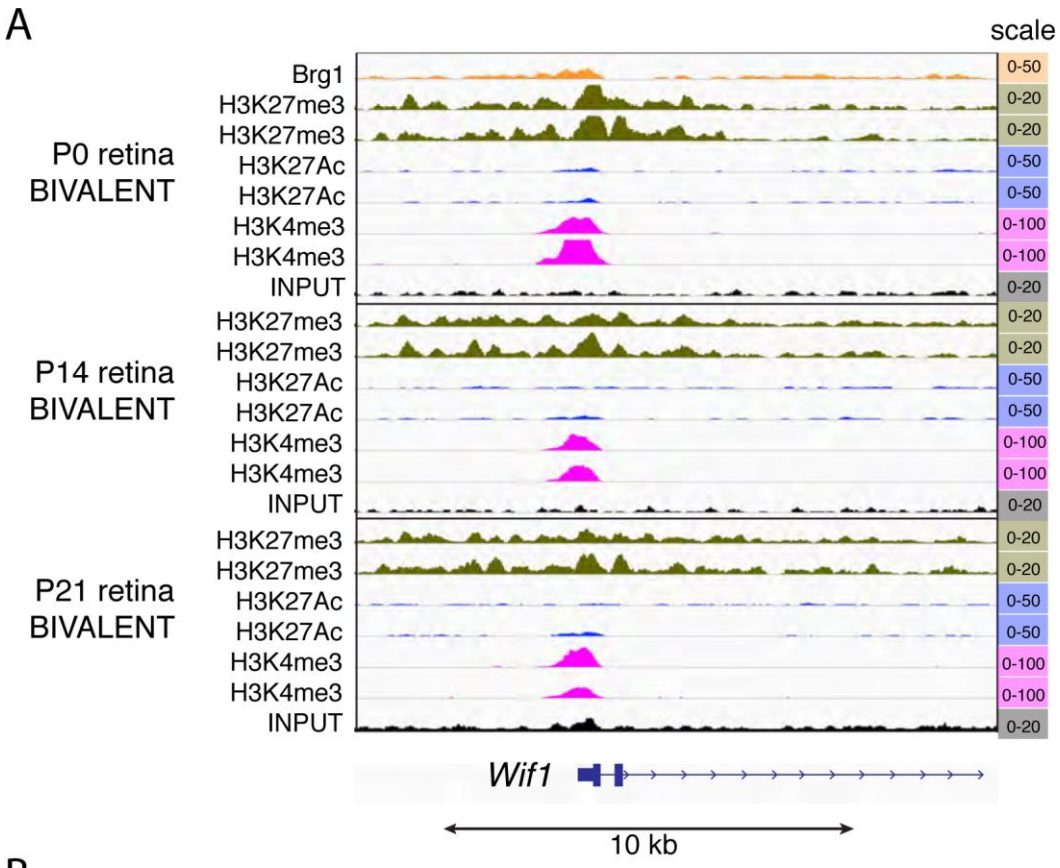


Figure S7. ChIP-seq promoter profile for *Cep192 and Pvrl3*. A) ChIP-Seq traces for active Brg1 promoter proximal site in the *Cep192* promoter of the P0 retina that is also active at later stages of development (P14 and P21). Biological duplicate ChIP-Seq is shown for each histone mark at each stage. **B)** ChIP-Seq traces for active Brg1 promoter proximal site in the *Pvrl3* promoter of the P0 retina that is also active at later stages of development (P14 and P21). Biological duplicate ChIP-Seq is shown for each histone mark at each stage.



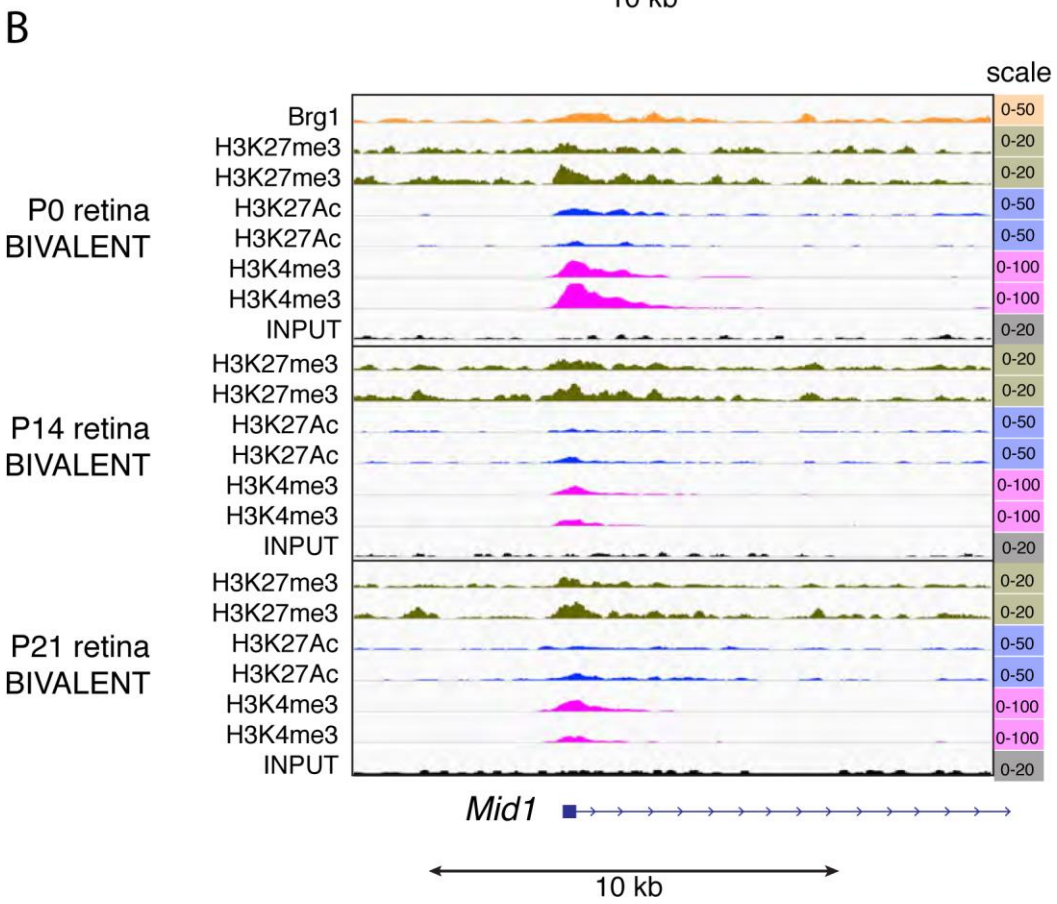


Figure S8. ChIP-seq promoter profile for *Wif1* and *Mid1*. A) ChIP-Seq traces for a bivalent Brg1 promoter proximal site in the *Wif1* promoter of the P0 retina that is also bivalent at later stages of development (P14 and P21). Biological duplicate ChIP-Seq is shown for each histone mark at each stage. **B)** ChIP-Seq traces for a bivalent Brg1 promoter proximal site in the *Mid1* promoter of the P0 retina that is also bivalent at later stages of development (P14 and P21). Biological duplicate ChIP-Seq is shown for each histone mark at each stage.

Table S1

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Table S2

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Table S11. Antibodies used in immunostaining and their dilutions

Antibody	Company-catalog #	Dilution	
		Cryosections	Dissociated cells
Anti-syntaxin	Sigma-S0664	1/100	1/1000
Anti-calbindin	Sigma-C9848	1/100	1/1000
Anti-Pax6	Hybridoma Bank	1/100	1/1000
Anti-recoverin	Millipore-AB5585	1/5000	1/1000
Anti-PKC-alpha	Millipore-05-154	1/5000	1/4000
Anti-glutamine synthase	BD Biosciences-610518	1/100	1/1000
Anti-GFAP	Sigma-S3893	1/100	1/1000
Anti-cone arrestin	Millipore-AB15282	1/5000	1/10000
Anti-Chx10	Exalpha-x1180P	1/200	1/500
Anti-Par3	Santa Cruz-SC5993	1/100	-
Anti-N-cadherin	BD-Biosciences-610920	1/1000	-
Anti-ZO1	Life Technologies-339100	1/500	-
Anti-PH3	Millipore-06-570	1/1000	-
Anti-gamma tubulin	Sigma-T6557	1/500	-
Anti-PKC-lambda	Santa Cruz-SC1091	1/1000	-

Table S12. Antibodies used in ChIP-seq experiments

Antibody	Company	Catalog #
Anti-H3K27Ac	Abcam	Ab4729
Anti-H3K4me3	Diagenode	C15410003-50
Anti-H3K27me3	Millipore	07-449
Anti-H3K4me1	Active Motif	AB8895
Anti-Flag	Sigma	F1804