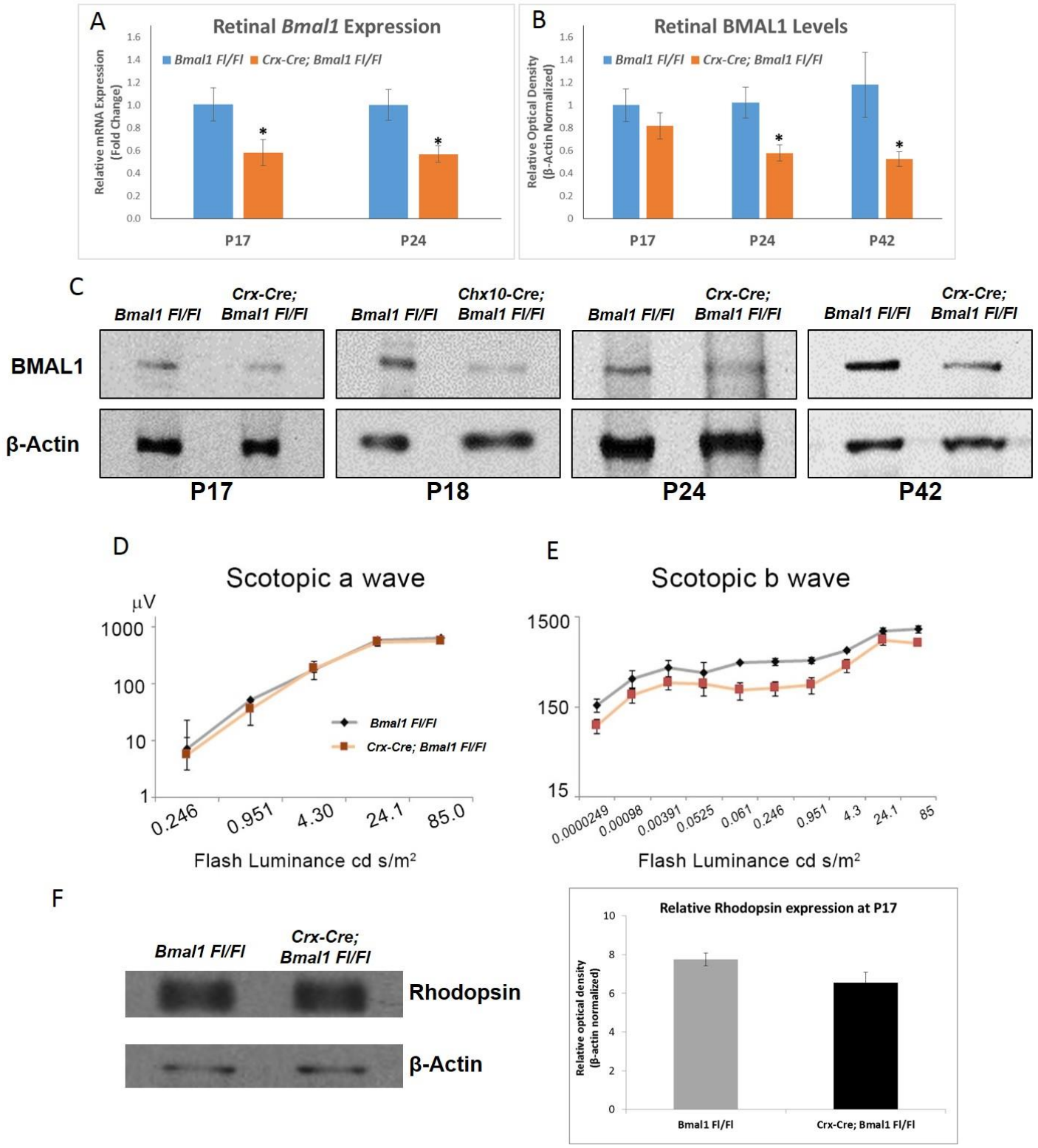

Figure S1, related to Figure 1 and 7. Ablation of *Bmal1* does not affect rod photoreceptor functioning. Relative retinal *Bmal1* expression in control and *Crx-Cre; Bmal1 Fl/Fl* animals at P17 and P24 (A). Error bars are ± SEM and n=3-4. Results were analyzed using Student's t-test. * indicates $P<0.05$.

Quantification of relative optical densities (B) using western blot technique for retinal BMAL1 expression (C) in control (*Bmal1 Fl/Fl*) and conditional mutants (*Crx-Cre; Bmal1 Fl/Fl* at P17, P24 and P42 and *Chx10-cre; Bmal1 Fl/Fl* at P18). Error bars are ± SEM and n=3-4. Results were analyzed using Student's t-test. * indicates $P<0.05$.

Dark-adapted (scotopic) a-wave ERG responses (D) were unaltered but b-wave ERG responses (E) were significantly reduced in the *Crx-Cre; Bmal1*$^{Fl/Fl}$ group ($F_{1, 119}=100.02$; $P<0.001$) compared to the control group at P24. Error bars are ± SEM and n=6. Results were analyzed using two-way ANOVA followed by Tukey post-hoc test with genotype and flash intensity as two independent factors.

Western blot (F) for rhodopsin protein validated dark-adapted a-wave ERG responses and no alterations in rhodopsin levels were observed. Error bars are ± SEM and n=3. Results were analyzed using Student's t-test.

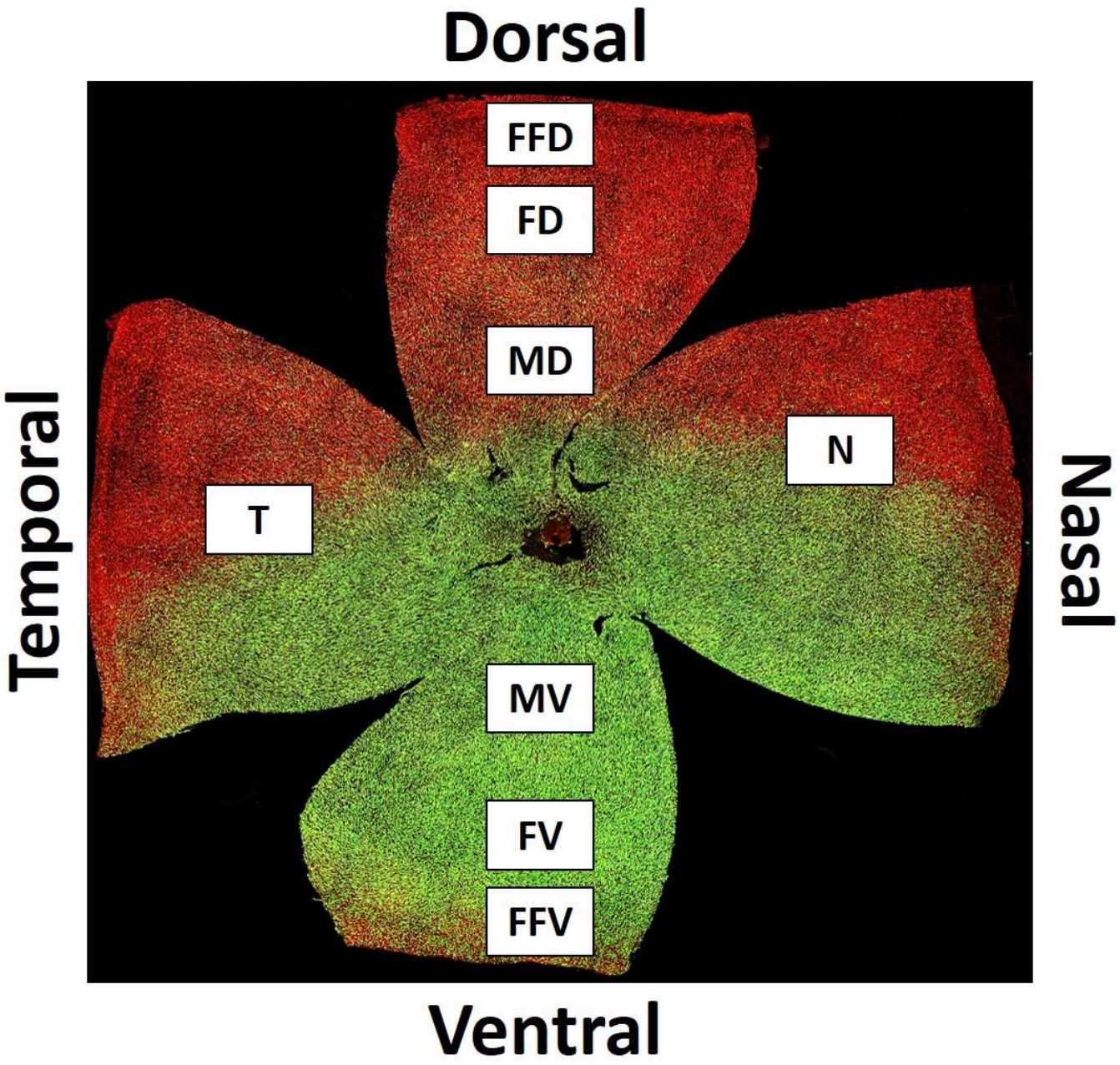

Figure S2, related to Figures 1, 2, 4, 5 and 6. Representation of various spatial regions across a flat mounted retinal preparation co-immunolabeled with S opsin (green) and M opsin (red). Quantification of cone densities and subtypes were performed across these regions. FFD-far far dorsal, FD-far dorsal, MD-mid dorsal, MV-mid ventral, FV-far ventral, FFV-far ventral, N-nasal, T-temporal. Image is created from 20x tiles.

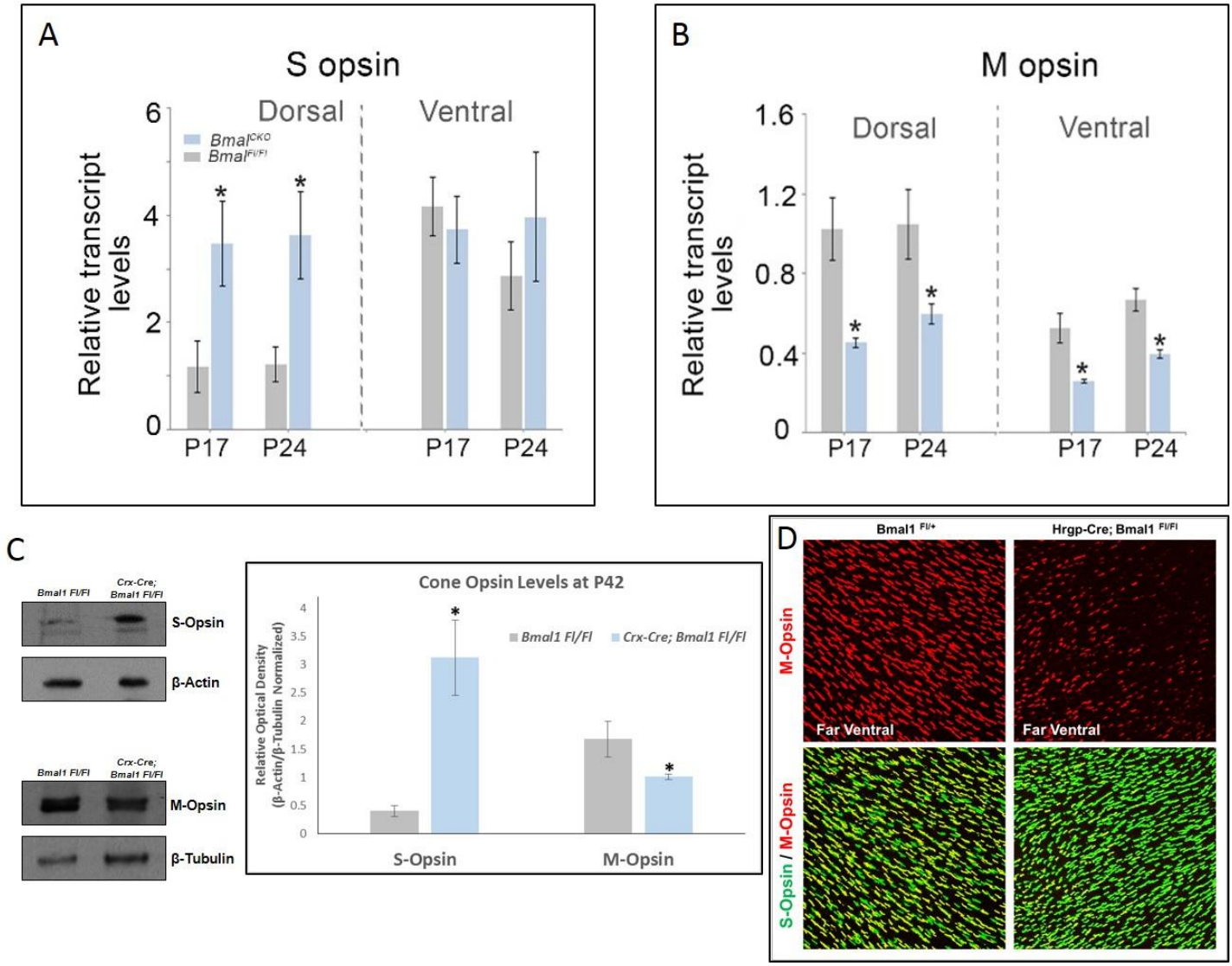

Figure S3, related to Figure 1. *Bmal1* is required for suppressing S opsin expression in dorsal retina and maintaining M opsin expression throughout the retina. Relative transcript expression in dorsal and ventral retina for S opsin (A) and M opsin (B) at P17 and P24. (C) Western blots for S opsin and M opsin proteins and (D) quantification of their relative optical density at P42 in dorsal retina and whole retina, respectively. Immunofluorescence images for M opsin (red) and S opsin (green) in far ventral region. These results indicate that suppression of S opsin in dorsal retina was abolished, and M opsin expression was decreased throughout the retina in *Bmal1* conditional mutants compared to littermate controls. Error bars are ± SEM and n=3-4. Results were analyzed using Student's t-test. * indicates $P<0.05$.

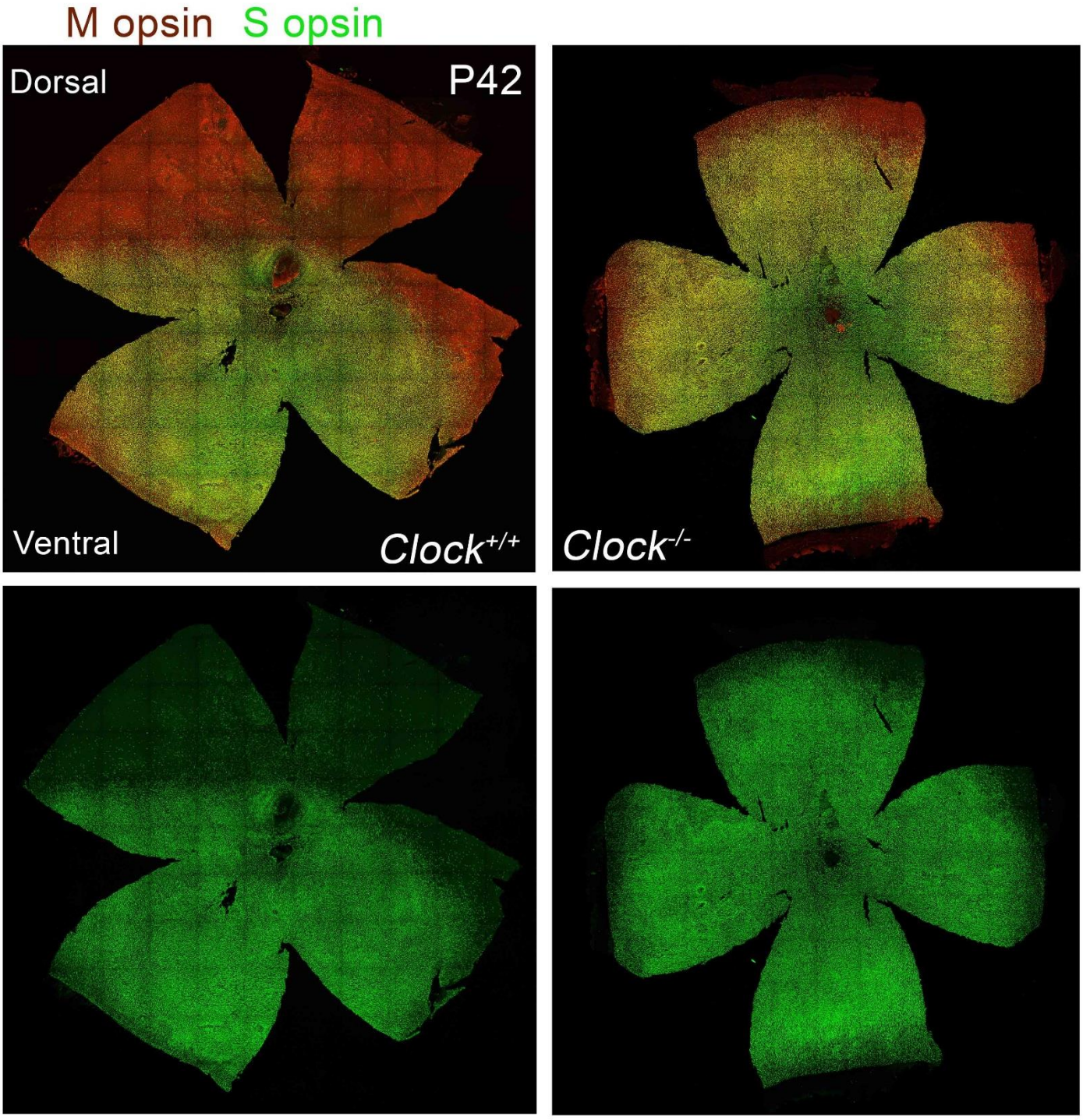

Figure S4, related to Figure 1. *Clock* is required for suppressing S opsin expression in dorsal retina. Retinal flat mount preparations from control and *Clock* mutant animals coimmunolabeled with S opsin (green) and M opsin (red). Each panel is created from 20x tiles.

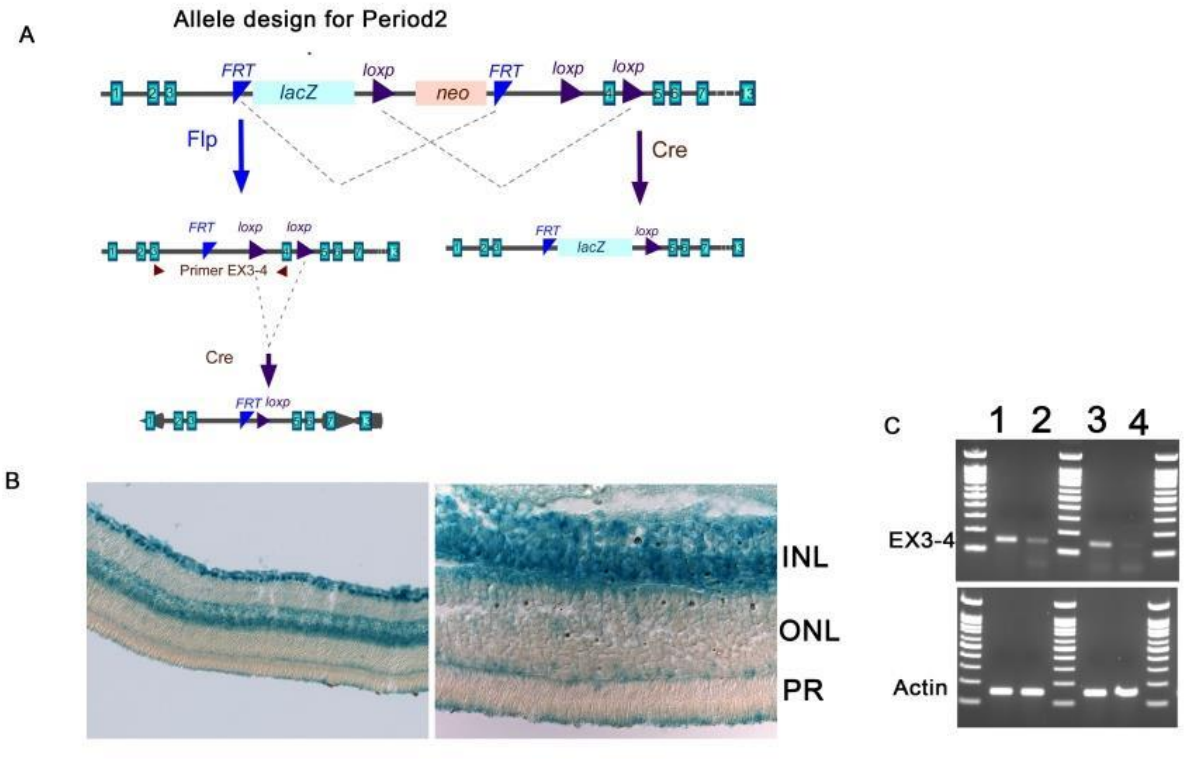

Figure S5, related to Figure 2. *Per2* is expressed in cone photoreceptors. β-galactosidase (lacZ gene) was inserted in *Per2* gene and LoxP sites before and after exon 4 were used to achieve Cre mediated deletion of *Per2* gene (A). LacZ staining demonstrating *Per2* expression in ganglion cell layer (GCL), inner nuclear layer (INL) and towards apical edge of outer nuclear layer (ONL) indicating cone nuclei (B). PCR products of primers designed around exon 4 confirming the *Per2* deletion (C). Lane 1 and 3 - *Per2* $^{Fl/Fl}$, lane 2 – *Crx-Cre; Per2* $^{Fl/Fl}$, lane 4 – *Chx10-Cre; Per2* $^{Fl/Fl}$.

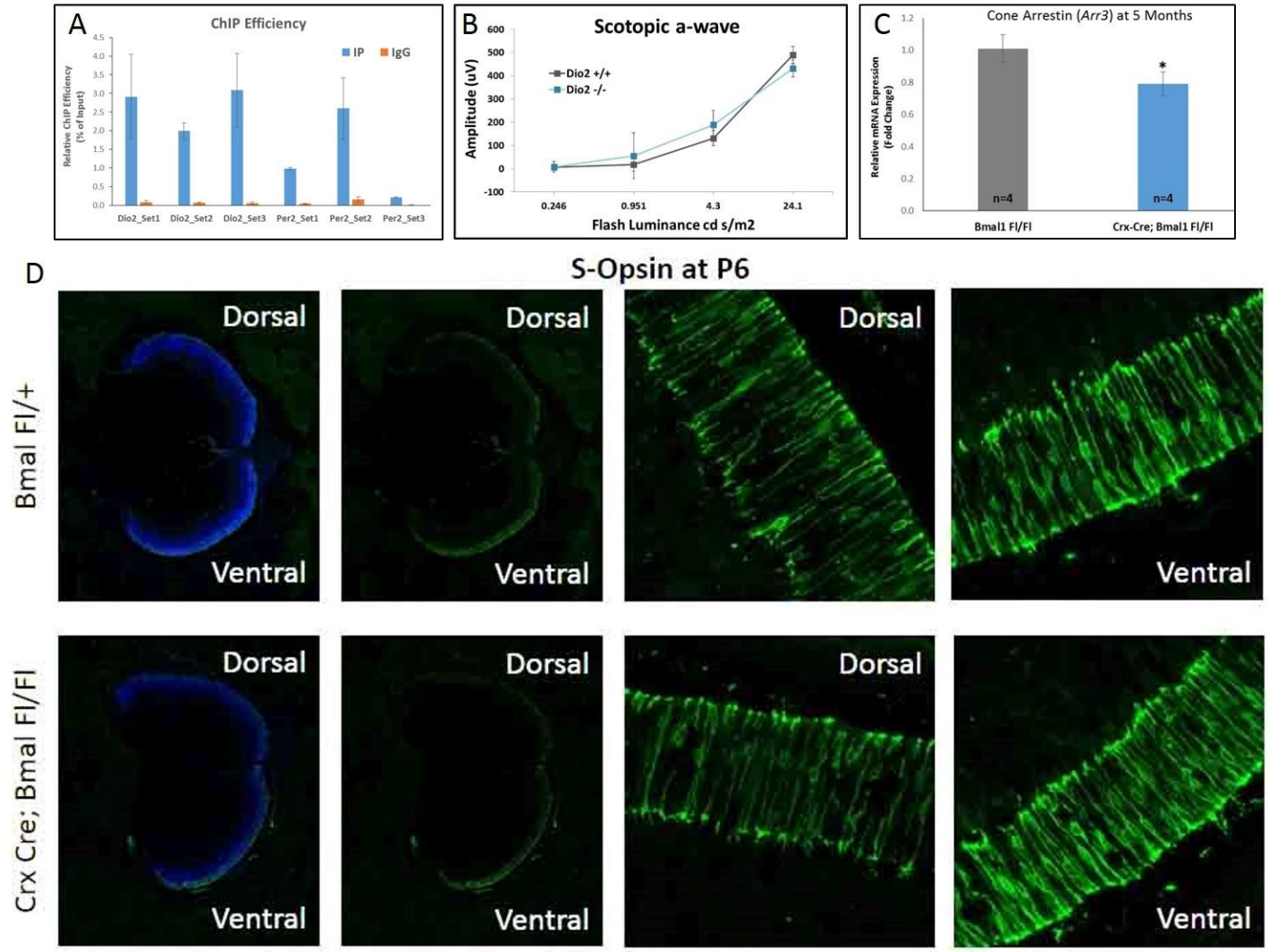

Figure S6, related to Figure 1, 5 and 7. (A) ChIP efficiency for primers designed against known BMAL1 binding sites around *Dio2* and *Per2* genes. Error bars are ± SEM. (B) Dark-adapted (scotopic) a-wave ERG responses were unaltered in *Dio2* mutant animals at 6 weeks of age. Error bars are ± SEM and n=4-5. Results were analyzed using two-way ANOVA followed by Tukey post-hoc test with genotype and flash intensity as two independent factors. (C) Cone arrestin (*Arr3*) transcript levels were decreased in *Bmal1* conditional mutants at 5 months of age. Error bars are ± SEM and n=4. Results were analyzed using Student's t-test. * indicates $P<0.05$. (D) *Bmal1* may not be required for suppressing S opsin expression in dorsal retina before P6. Retinal cryosections from control and *Bmal1* conditional mutant showing nuclear S opsin (green) expression at P6. Dorso-ventral gradient in S opsin expression was intact in the control as well as in the *Bmal1* conditional mutant retinas.

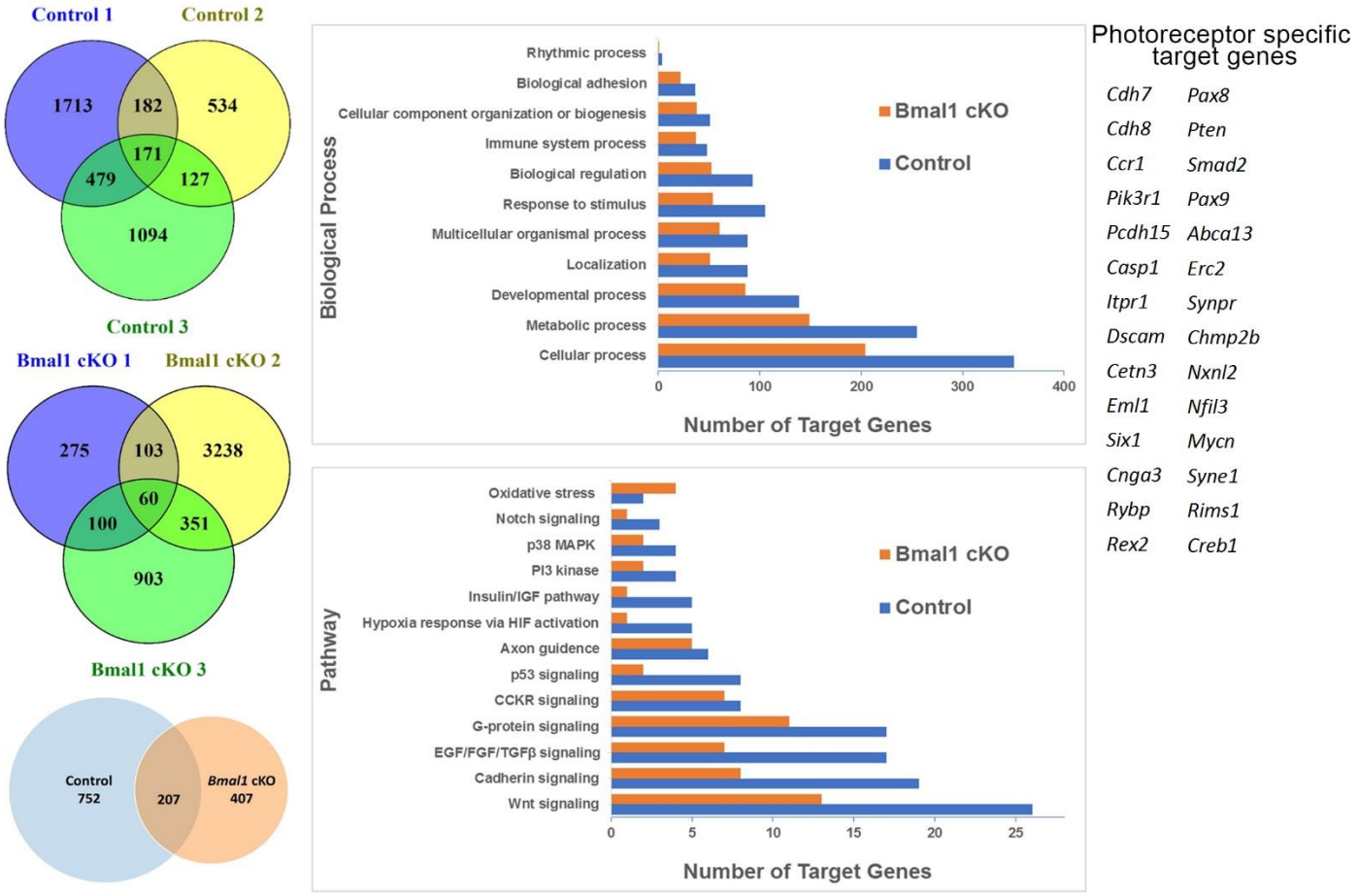

Figure S7, related to Figure 7. Identification of number of target genes, biological processes and signaling pathways from ChIP-Seq experiments from control and *Bmal1* conditional mutant (CKO) retinas. Target genes identified in at least 2 out of 3 samples were used for biological process and signaling pathway analysis.

Table S1, Related to Figure 2, 3, 5 and 6. List or PCR primers used in this study

Gene	Use	Forward (5'–3')	Reverse (5'–3')	Size (bp)
β-actin	qPCR	TTCTTTGCAGCTCCTTCGTT	ATGGAGGGGAATACAGCCC	149
Bmal1	qPCR	TGGAACCCTAGGCCTTCATT	TTCGATCCAGTGTGGGAGAT	128
Dio1	qPCR	CCACCTTCTTCAGCATCC	AGTCATCTACGAGTCTCTTG	240
Dio2	qPCR	GTCCGCAAATGACCCCTTT	CCCACCCACTCTCTGACTTTC	65
	qPCR	ATGCTGACCTCAGAAGGGC	AGTCAAGAAGGTGGCATTCG	181
	ChIP	GCTCTTAACCACTGAGCCATC	ATGCCATAGACATACAAGCAACC	150
	ChIP	GTTCGCTTTCAGGTCATCAGA	GGCAGGACCCAGGGAAAA	231
	ChIP	GCAGAGGATGGTTTAGTTGGTC	AGGATTGGCAAGGAGTGGAA	346
Dio3	qPCR	GTTTTTGGCTTGCTCTCAGG	CAACAAGTCCGAGCTGTGAA	152
Opn1mw	qPCR	GCTACTTCGTTCTGGGACAC	CAAATCTCACATTGCCAAAGGG	144
Opn1sw	qPCR	ATGGTCAACAATCGGAACCA	TGAGAGCCAGACACGTCAGA	185
Pax8	qPCR	CGTAGGAAAGCTGCGAGTGT	TGGCGAGAAATATCACAGGG	197
Per1	qPCR	AAACCTCTGGCTGTTCCTACCA	AATGTTGCAGCTCTCCAAATACC	74
Per2	qPCR	ATGACAGAGGCAGAGCACAA	TTCCCCTTGGCCTTCTTGT	140
	qPCR	ATGCTCGCCATCCACAAGA	GCGGAATCGAATGGGAGAAT	72
	ChIP	AAGCTTGTCTAGTCTCCAGTGT	GTTGCCGTCTCCTGCTGA	150
	ChIP	TGTTCCAGATGCACACCCC	GCCGTGCAGAGATACAAAGG	155
	ChIP	CGCAGCATCTTCATTGAGGA	GCCATTGGTCGGAGTGCC	154
Per3	qPCR	GTGGGCCAACAGCTCTACAT	CCAGTATCCGTGGTGCTTTTA	146
Rxrγ	qPCR	GAAGTTTCCCACCGGCTTT	TACGGAGAGCCAAGAGCATT	183
Trβ2	qPCR	GAGGAGTGGGAGCTCATCAA	AATGGGTGCTTGTCCAATGT	117

Supplemental Experimental Procedures

Mouse Strains

Mice carrying *Dio2* mutation were (Stock No: 018985, The Jackson Laboratory, Bar Harbor, ME) backcrossed onto a C57BL/6J background. *Dio2*$^{+/-}$ parents were crossed to generate +/+ and -/- offspring for experimental purpose. *HRGP-Cre* mice were crossed to *Bmal1*$^{flox/flox}$ mice (Stock No: 018985, The Jackson Laboratory, Bar Harbor, ME) to generate *HRGP-Cre; Bmal1*$^{flox/+}$. *HRGP-Cre; Bmal1*$^{flox/+}$ mice were crossed to *Bmal1*$^{flox/flox}$ and *Bmal1*$^{flox/+}$ mice to generate *HRGP-Cre; Bmal1*$^{flox/flox}$ (*Bmal1*^{CKO}) and *Bmal1*$^{flox/flox}$ or *Bmal1*$^{flox/+}$ (controls) mice. Similarly, *Crx-Cre*$^{/+}$ mice were also used to achieve conditional deletion of *Bmal1*. Mouse line expressing tdTomato (Stock No: 007914, The Jackson Laboratory, Bar Harbor, ME) was crossed to *HRGP-Cre; Bmal1*$^{flox/flox}$ to generate cones expressing red fluorescence for the purpose of cell sorting. No phenotypical differences were observed between females and males of same genotype and both genders were represented in each group. Appropriate ages of animals are mentioned in the figure legends.

Quantitative Real-Time PCR

Total RNA was extracted from neural retina using RNeasy® mini kit (Qiagen, Germantown, MD, USA) according to the manufacturer's instructions. The Verso cDNA kit (Thermo Scientific, Rockford, IL, USA) was used to generate cDNA for RT-PCR from 250 ng of total RNA. All primers used in this study are listed in Table S1. Real-time PCR was performed on the 7900 HT fast real-time PCR system (Life Technologies) using a Bullseye EvaGreen qPCR master mix (Midwest Scientific). Relative fold changes in mRNA expression were determined using the comparative Ct method ($2^{-\Delta\Delta Ct}$ method) and considering β-actin as a reference gene. For circadian oscillations of a transcript, fold change at any ZT was calculated with respect to that of ZT0 with the same genotype.

Immunohistochemistry

For cryosections, enucleated eyes were fixed in 4% PFA for 90 min at room temperature (RT), washed in PBS several times and cryoprotected using sucrose gradient (10%:20%:30%) and kept overnight in 30% sucrose at 4ºC. Eyes were mounted in mounting media by orientating them dorso-ventrally. Cryosections (12 µm) were cut with or without the CryoJane Tape-Transfer system (Leica Inc., Buffalo Grove, IL, USA). Sections were washed in PBS several times, permeabilized for 5 min using 1% Triton X-100 and blocked for 1 hr at RT with PBS containing 3% BSA and 0.03% Triton X-100. Cryosections were incubated with primary antibodies (1:500) for an overnight at 4ºC. Antibody used in this study are following: goat anti-opsin blue (S Opsin) (sc-14363, Santa Cruz Biotechnology, Dallas, TX, USA), rabbit anti-opsin red/green (M opsin) (AB5405, Millipore, Billerica, MA, USA) and secondary antibodies were Alexa Fluor donkey anti-goat 488 and Alexa Fluor donkey anti-rabbit 594 (Life Technologies, Carlsbad, CA, USA). Imaging was done using a Leica laser scanning confocal miscroscope (TCSSP2, Leica, Exton, PA, USA). Entire retinal flat mount or cross sectional images were composite images that were created from stiching individual 20x tile images.

Cell Isolation by FACS

Retinas were collected in ice-cold PBS from either P11 or P24 mouse pups. Retinal dissociation was done using 0.5 mg/ml of liberase (Roche Diagnostics, Indianapolis, IN, USA) and DNase for 40 mins at 37ºC. Resuspended cells were stained with primary antibody against rhodopsin (1:500, ab98887; Abcam, Cambridge, UK) and with PNA Alexa Fluor 488 (1:500, L21409, Life Technologies, Carlsbad, CA, USA) followed by staining for secondary antibody Alexa Fluor donkey anti-mouse 594 (Life Technologies, Carlsbad, CA, USA). DAPI was added for dead cell identification. Resuspended cells from tdTomato; Hrgp-Cre retinas were directly subjected to cell isolation. Sorted cones and rods were collected directly in RLT buffer for RNA isolation using RNeasy mini kit (Qiagen, Germantown, MD, USA).

ChIP-Seq and Data Analysis

Rubicon Genomics ThruPlex® DNA kit (Rubicon Genomics, Ann Arbor, MI, USA) or TruSeq ChIP Library Preparation Kit - Set A (Illumina, San Diego, CA, USA) was used for making the ChIP-seq libraries on samples generated from ChIP assays. QC for final libraries were performed on the Agilent 2100 Bioanalyzer, quantified via qPCR, diluted, and pooled at equimolar ratios (Agilent Technologies, Santa Clara, CA, USA). The sample pools were assayed on a single read HiSeq 2500 or HiSeq 4000 (Illumina, San Diego, CA, USA). Sequencing was performed at Genomics Core-Case Western Reserve University, Cleveland, OH, USA and at Genomics Facility-University of Chicago, Chicago, IL, USA. Sequences generated from ChIPseq experiment were filtered for quality and adapter sequences using TrimGalore! (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/). Sequences from ChIP samples and input controls were aligned to the GRCm38 mouse reference genome (mm10) with bowtie2. Peak calling was done using the aligned read files as input in HOMER peak caller for broad regions of enrichment with variable-width peaks found in ChIP-Seq experiments. Peak regions were annotated using HOMER annotation scripts and the gene list was manually curated for genes of interest. For P24 control and *Bmal1* CKO samples, target genes that appeared in at least 2 out of 3 samples were referred as trusted target. Additionally, all trusted target genes present in control group only were evaluated using Panther –gene list analysis tool (http://www.pantherdb.org).

Electroretinogram Recordings

Rod responses (scotopic) were recorded in the dark in response to a green light-emitting diode (LED) stimulus at 520nm, half-bandwidth of 35nm. To measure cone responses, rods were first saturated with a steady-state green + blue stimulus (10cd/m^2) for 7 minutes. Subsequently, to measure S opsin responses, a UV LED stimulus (peak at 365 nm, half bandwidth of 9 nm) was superimposed on the steady state background at increasing intensities of 0.0001. 0.0005, 0.002, 0.005, and 0.01 photopic (P) cd/m^2. A blue light emitting LED (peak at 445 nm, half-bandwidth of 20 nm) at intensities of 0.05, 0.2, 0.5, 1, and 5 (P)cd/m^2 was also used. To measure M/L-opsin responses, a green LED (peak at 520 nm, half-bandwidth of 35 nm) at intensities of 2, 5, 10, 20, and 80 (P)cd/m^2 was used and an amber LED (peak at 590 nm, half-bandwidth of 20 nm) at intensities of 1, 5, 15, 25, and 50 (P)cd/m^2 was used.

Sampling frequency was 100 Hz with 20 responses averaged per recorded trace. Amplitude of the response was determined by measuring the minimum to the maximum of the waveform at each stimulus intensity. All ERG testing were performed between ZT4 and ZT8.