Supporting Information

Buhr and Van Gelder 10.1073/pnas.1323350111

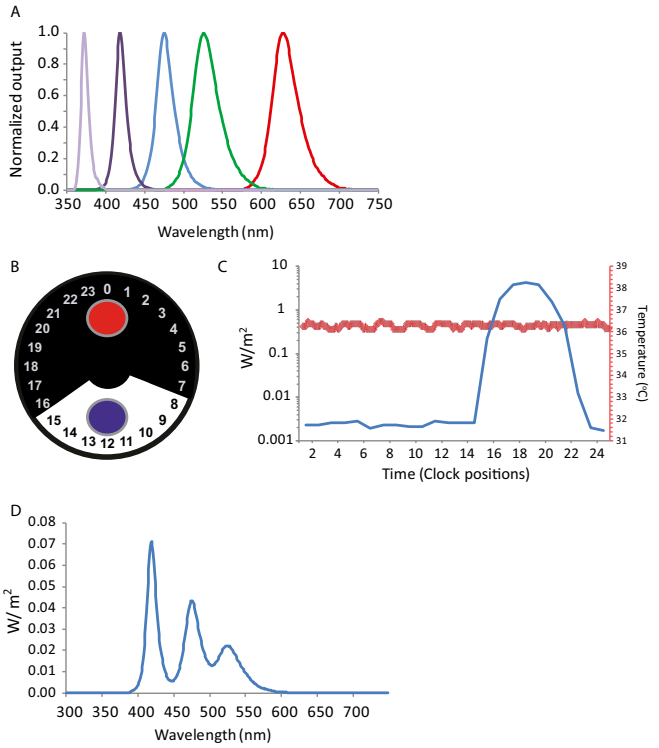

Fig. S1. (*A*) The peak and bandwidth of the LEDs used in this study. All values are normalized. (*B*) Schematic of clock apparatus. Blue and red circles represent the 0° and 180° positions of culture dishes. The transparent window on an opaque disk is rotated with a 24-h period. (*C*) Total irradiance and temperature at each time point across a 24-h day as experienced by culture dishes inside the clock apparatus. (*D*) Spectral irradiance of light experienced by cultured tissues and mice in running wheels.

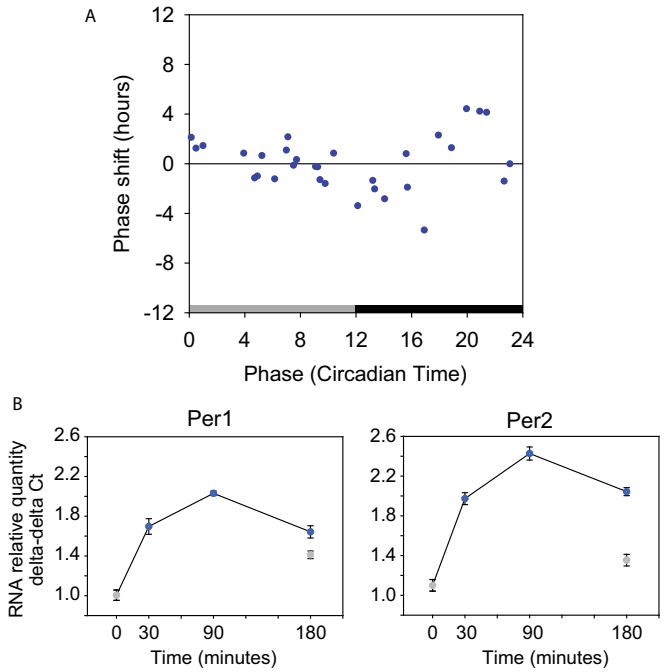

Fig. S2. A phase–response curve of cultured WT retinas to light pulses. (*A*) Retina cultures were exposed to 3 h of 3×10^{15} photons per square meter per second of 475-nm light. Each point represents the phase change of an individual retina after a single light exposure given at the circadian time indicated. The light/dark bars at bottom indicate previous phases of light (gray) and dark (black) based on RT-PCR phases. (*B*) Acute induction of Per1 and Per2 to acute light pulses of indicated durations as measured by RT-PCR from cultured retinas (blue points). Gray points indicate retinas collected as dark controls at the indicated times. Pulses began in the advance portion of the phase response curve or circadian time ~22–24.

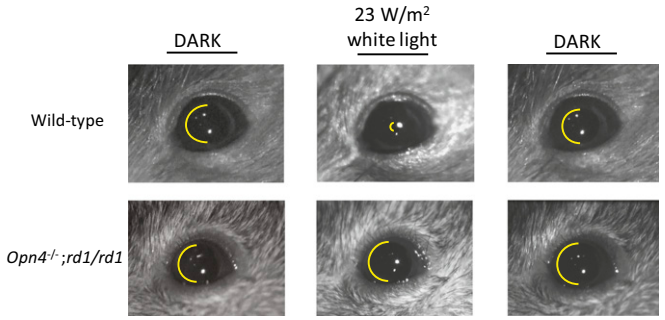

Fig. S3. Pupillary light reflex in melanopsin (*Opn4*)$^{-/-}$;*rd1/rd1* mice. Videos of the eyes of awake mice were made by using a digital video camera with IR recording. Images show pupillary responses of WT and *Opn4*$^{-/-}$;*rd1/rd1* mice in darkness, after 30 s of 23 W/m^2 white light from a xenon source, and returned to darkness. Yellow half-circles trace half of the pupil for better visualization.

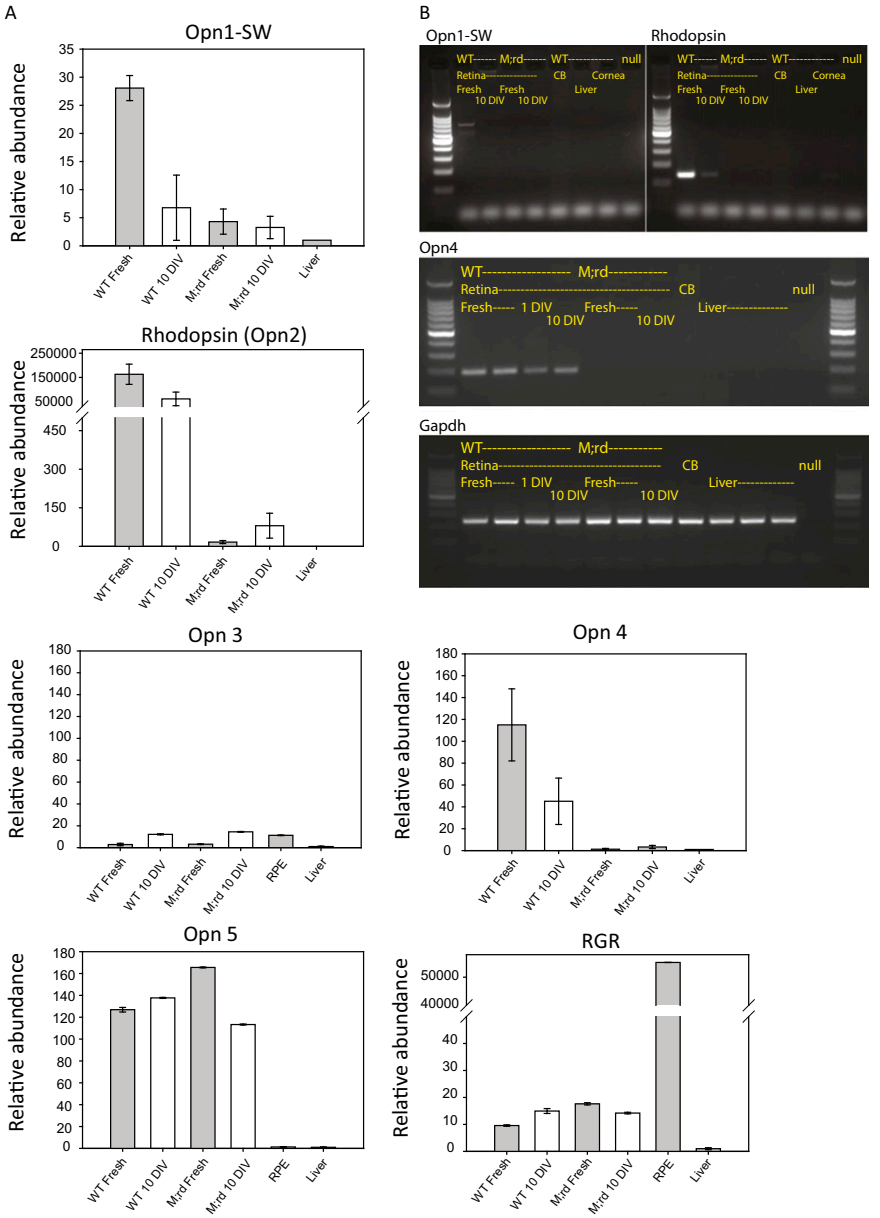

Fig. S4. RT-PCR on opsins in the retina. (*A*) Total RNA from retinas and liver was reverse-transcribed to cDNA, and RNA abundance of opsin genes was compared with Gapdh levels and compared with liver expression by using delta-delta cT method. DIV, days in vitro; M;rd, *Opn4*$^{-/-}$;*rd1*/*rd1* . Error bars represent 1 SEM ($n = 3$ for each). For Opn3, Opn5, and RGR, fresh retinal pigment epithelium (RPE) was also tested. (*B*) Products of cDNA amplification of the same retina targets in *A* run on a 2% (wt/vol) agarose gel. Opn1 and Rhodopsin are shown after 20 amplification cycles (because of the high abundance of rhodopsin) and Opn4 and Gapdh are shown after 30 amplification cycles. CB, cerebellum; null, water only/no template control.

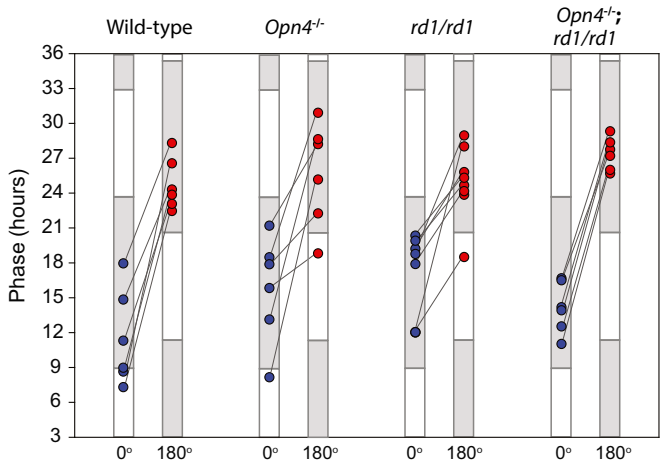

Fig. S5. Phases of individual retina cultures after 4 d in the 0° (blue) or 180° (red) positions of the clock apparatus of various genotypes. A line connects the right and left retinas from the same animal.