

Figure S1. *Opn5* alleles. Related to STAR methods.

(A) Schematic of the *Opn5* allele as targeted in ES cells by the International Knockout Mouse Consortium. Exons are numbered yellow boxes. *FRT*, FLP recombinase site-specific recombination sites. En2 SA, *Engrailed 2* splice acceptor. IRES, internal ribosome entry sequence. LacZ, β -galactosidase open reading frame. pA, polyadenylation signal. *loxP*, cre recombinase site specific recombination sequences. hbactP, human β -actin promoter. Right-facing arrow indicates the start point of transcription for hbactP. neo, the neomycin resistance gene. (B) The *Opn5*^{-/-} allele generated after germ-line deletion at *Frt* then *LoxP* sites. (C) The *Opn5*^{cre} allele was generated by targeting the cre recombinase open reading frame into Exon 1 of the *Opn5* gene using CRISPR methods.

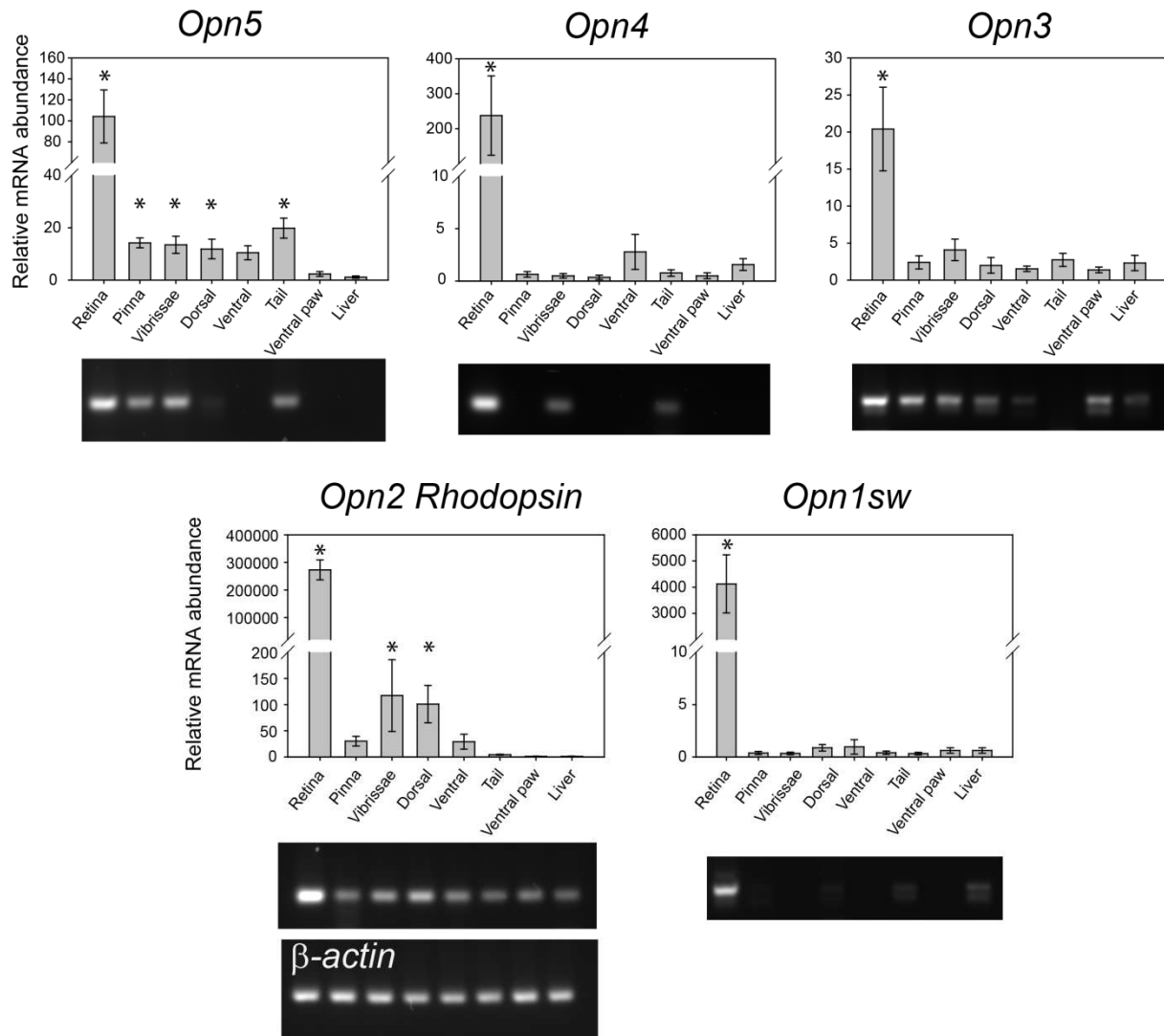


Figure S2. Opsin expression in skin areas. Related to Figure 1. Quantitative RT-PCR was used to compare levels of expression of *Opn5*, *Opn4*, *Opn3*, *Opn2* (rhodopsin), and *Opn1sw* (short wavelength cone opsin) in tissues indicated. All values represent the mean \pm SEM of $\Delta\Delta$ Ct RT-PCR comparing transcript levels to β actin relative to expression in liver. * indicates $p < 0.05$ in ANOVA Tukey post-hoc. N = 5 for each. Below each is an example of agarose gel electrophoresis of one replicate's amplicons from RT-PCR run.

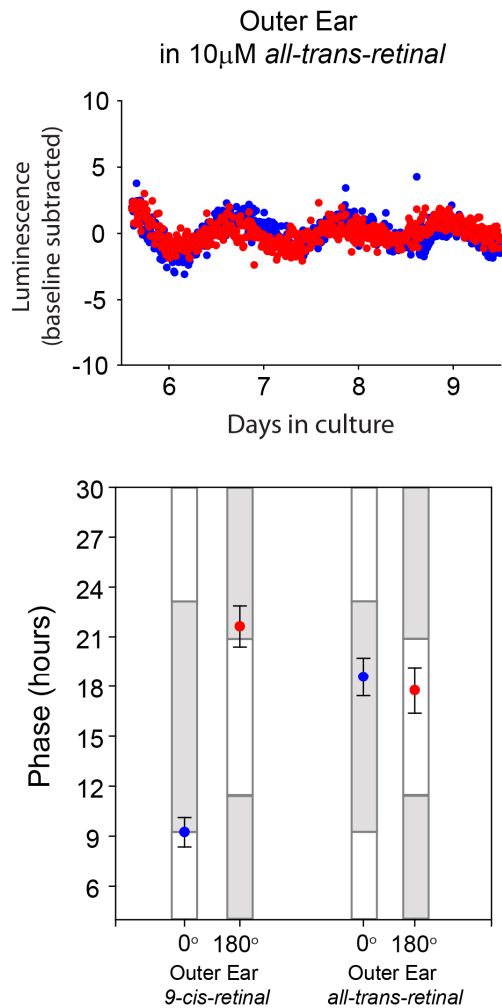


Figure S3. Cultures of outer ear do not photoentrain in *all-trans-retinaldehyde*. Related to Figure 2. Luminescence traces of cultured outer ear (pinna) from *Per2^{Luc}* mice after 5 days of an LD cycle *ex vivo* from wild-type mice with 10 μ M *all-trans-retinaldehyde* in the culture media (upper panel). Phase of the peak of *Per2^{Luc}* luminescence on the day after 5 days of LD in either the 0° or 180° position of a photoentrainment apparatus (lower panel). Points show mean \pm SEM. White and grey bars represent times at which the tissues experienced light or dark in the previous LD cycle. *9-cis-retinal* data is the same as Figure 1. *All-trans-retinal*: n = 7 pairs.

Oligonucleotides	
REAGENT or RESOURCE	SOURCE
Circadian genes for qPCR:	
<i>Per1</i> forward: 5'-CCCAGCTTTACCTGCAGAAG-3'	[S1]
<i>Per1</i> reverse: 5'-ATGGTCGAAAGGAAGCCTCT-3'	[S1]
<i>Per2</i> forward: 5'-CCAACACAGACGACAGCATC-3'	[S1]
<i>Per2</i> reverse: 5'-TCTCGCAGTAAACACAGCCT-3'	[S1]
<i>Bmal1</i> forward: 5'-GACATTTCTCAACCATCAGCG-3'	[S1]
<i>Bmal1</i> reverse: 5'-GCATTCTTGATCCTTCCTTGGT-3'	[S1]
<i>Dbp</i> forward: 5'-CGAAGAACGTCATGATGCAG-3'	[S1]
<i>Dbp</i> reverse: 5'-GGTTCCCAACATGCTAAGA-3'	[S1]
<i>Cry2</i> forward: 5'-CACTGGTTCGCAAAGGACTA-3'	[S2]
<i>Cry2</i> reverse: 5'-CCACGGGTCGAGGATGTAGA-3'	[S2]
<i>Rev-erbα</i> forward: 5'- ACCTTTGAGGTGCTGATGGT-3'	[S2]
<i>Rev-erbα</i> reverse: 5'- CTCGCTGAAGTCAAACATGG-3'	[S2]
β -actin forward: 5'-AAA GAG AAG CTG TGC TAT GTT G-3'	[S1]
β -actin reverse: 5'-CAT AGA GGT CTT TAC GGA TGT C-3'	[S1]
Opsin genes for qPCR:	
<i>Opn5</i> forward: 5'-AGCTTTTGAAGGCCAGAC-3'	[S3]
<i>Opn5</i> reverse: 5'- CAGCACAGCAGAAGACTTC-3'	[S3]
<i>Opn4</i> forward: 5'- TCTGTTAGCCCCACGACATC-3'	[S1]
<i>Opn4</i> reverse: 5'- TGAACATGTTTGCTGGTGTCC-3'	[S1]
<i>Opn3</i> forward: 5'- CTGTTCCGAGTCACCTTCAC -3'	[S1]
<i>Opn3</i> reverse: 5'- GTATGTCTAGGATGTACCTGTTC -3'	[S1]
<i>Opn2</i> forward: 5'- CTTTGCCCACTTGGAGGTGA -3'	[S1]
<i>Opn2</i> reverse: 5'- TGATCCAGGTGAAGACCACAC -3'	[S1]
<i>Opn1sw</i> forward: 5'- TCACGGATACTTCCTCTTTGGTC -3'	[S1]
<i>Opn1sw</i> reverse: 5'- GGCCAACTTTGCTAGAAGAGAC -3'	[S1]

Table S1. Primer sequences. Related to STAR Methods.

Supplemental References

- S1. Buhr, E.D., and Van Gelder, R.N. (2014). Local photic entrainment of the retinal circadian oscillator in the absence of rods, cones, and melanopsin. *Proc Natl Acad Sci U S A* *111*, 8625-8630.
- S2. Amador, A., Campbell, S., Kazantzis, M., Lan, G., Burris, T.P., and Solt, L.A. (2018). Distinct roles for REV-ERB α and REV-ERB β in oxidative capacity and mitochondrial biogenesis in skeletal muscle. *PLoS One* *13*, e0196787.
- S3. Tarttelin, E.E., Bellingham, J., Hankins, M.W., Foster, R.G., and Lucas, R.J. (2003). Neuropsin (Opn5): a novel opsin identified in mammalian neural tissue. *FEBS Lett* *554*, 410-416.