SUPPORTING INFORMATION

Interplay between cell adhesion molecules governs synaptic wiring of cone photoreceptors.

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www.pnas.org/cgi/doi/10.1073/pnas.2009940117

Supplementary Materials and Methods

Mice, antibodies and genetic constructs

Generation of sheep anti-RGS11, rabbit anti-RGS11, sheep anti-TRPM1, sheep anti-mGluR6 and rabbit anti-ELFN1 antibodies were described previously (1, 2). Rabbit anti-R7BP (TRS) and rabbit anti-Gβ5 (ATDG) was a generous gift from Dr. William Simonds (National Institute of Diabetes and Digestive and Kidney Disease, National Institutes of Health, Bethesda, MD). Rabbit anti-$Ca_V1.4$ was generous gift from Dr. Amy Lee (University of Iowa, IA). Commercial antibodies were: rabbit anti ELFN2 (PA5-43521, Thermo Fisher Scientific), rabbit anti-ELFN2 (HPA000781, Sigma-Aldrich), rabbit anti α2δ4 (OAAF04451, Aviva Systems Biology), mouse anti-β-actin (AC-15, Sigma), rabbit anti-c-myc (A00172, Genscript), mouse anti-CtBP2 (612044, BD Biosciences), mouse anti-PKCα (ab11723; Abcam), mouse anti-GPR179 (Primm Biotech; Ab887), rabbit anti-Gαo (K-20, Santa Cruz), goat anti-Arrestin-C (I-17, Santa Cruz), rabbit anti-cone arrestin (AB15282, Millipore), Peanut Agglutinin (PNA) Alexa Fluor 647 conjugate (Life Technologies) and rabbit anti-RFP (Rockland).

Cloning of full-length rat mGluR6, mouse ELFN1, mouse ELFN2 into a mammalian expression vector pcDNA3.1 was described previously (1, 3, 4). Cone PR2.1 promotor rAAV virus (cAAV-ELFN1) was obtained from the Ocular Gene Therapy Core, Department of Ophthalmology of University of Florida. The AAV2/5 vector is carrying a full-length mouse ELFN1 cDNA under the control 2.1-kb of the human red/green opsin promoter construct (PR2.1) to provide cone specificity for ELFN1 expression (5).

Rgs7$^{-/-}$*Rgs*11$^{-/-}$, *Trpm1*$^{-/-}$, *Elfn1*$^{-/-}$, *Elfn2*$^{-/-}$, *Lrit3*$^{-/-}$, *Cacna1f*$^{-/-}$ and *Cacna2d4*$^{-/-}$ mouse lines were described previously (1, 2, 4, 6-9). The *Grm6*$^{-/-}$ mouse line (129S6.129S(Cg)-Grm6<tm1Nak> (RBRC05552)) was obtained from RIKEN BioResource Center. The Grm6-tdTomato line, in which a sparse population of ON bipolar cells expresses tdTomato (10) was obtained from Dr. Rachel Wong (University of Washington). Conditional targeting of ELFN1 was achieved by flanking exon 2 with LoxP sites (*Elfn1*$^{flox/flox}$). The resulting mice were crossed with *Elfn2*$^{-/-}$ line to generate cDKO:Cre- strain (*Elfn2*$^{-/-}$: *Elfn1*$^{flox/flox}$), and further with two different Cre driver lines. One Cre driver line is expressing Cre recombinase controlled by a ~10-kb fragment immediately upstream of the putative translation initiation site of the mouse *pcdh21* gene, and is selectively expressed in photoreceptor (11, 12). Another Cre driver line is expressing ubiquitously expressing tamoxifen-inducible Cre-ERT2 recombinase B6.Cg-Tg (CAG-cre/Esr1*)5Amc/J to produce cDKO:Cre+ mice (*Elfn2*$^{-/-}$: *Elfn1*$^{flx/flx}$: *CAG*^{CreERT2}). To induce Cre expression, tamoxifen (Sigma, St. Louis, MO), was dissolved in ethanol, then mixed with corn oil. The ethanol was removed by evaporator (SAVANT ISS110, Thermo Scientific). 75 mg/kg was administered by intraperitoneal injection with a 27 gauge smooth tip needle. Tamoxifen was administered once daily for 5 consecutive days. Mice used in the study were 1–3 months old and ground squirrels were one year old and tree shrews were five to six years old. Procedures involving animals strictly followed NIH guidelines and were approved by the Institutional Animal Care and Use Committees at the Scripps Research, National Institutes of Health and the Max Planck Florida Institute for Neuroscience.

Subretinal Injections

Subretinal injections were performed as previously published (13). The newborn pups were anesthetized on ice for several minutes. A small incision was made in the sclera near the cornea through the skin using the tip of a sharp 30-gauge needle. An injection needle was carefully insert into the eyeball through the incision into the subterinal space between RPE and retina. 0.5 μl rAAV virus (4×10^{10} vg) was slowly injected into the subretinal space. The operated pups were warmed up by a heat blanket until they recovered from the anesthetic before returning to their mother. The retinas were harvested at P21.

Preparative immunoprecipitation of mGluR6 complexes from ground squirrel or tree threw retinas and mass-spectrometry

Ground squirrel or tree shrew retinas were lysed by sonication in ice-cold PBS (Invitrogen) supplemented with complete protease inhibitor tablets (Roche). The lysate was centrifuged at 100,000 x g, 4 °C for 30 minutes. Then the pellet was then resuspended ice-cold PBS IP buffer supplemented with 150 mM NaCl, 1% Triton X-100, and complete protease inhibitor tablets (Roche). After a 30-minute incubation at 4 °C, the resuspended lysate was cleared by centrifugation at 100,000 x g, 4 ° C for 30 minutes. Supernatants were incubated with 10 μL of protein G beads (GE Healthcare) covalently coupled with either anti-mGluR6 antibodies or IgG (100 μg each) by Bis (Sulfosuccinimidyl) suberate (BS3) (Thermo Scientific) for 1 hour at 4 °C as described previously (14). After three washes with IP buffer, proteins were eluted with 50 μL SDS sample buffer (62 mM Tris, 10% glycerol, 2% SDS, and 5% β-mercaptoethanol), and analyzed by SDS-PAGE at ~ 150 mV for 15-20 minutes. Gels were fixed with using 5% acetic acid in 50% methanol, stained by NOVEX colloidal blue (Invitrogen). Stained areas were cut out, digested with trypsin (Promega), and alkylated as described previously (15). The resulting peptide mixtures were desalted, resolved by high-pressure liquid chromatography, and analyzed using LTQ-Orbitrap XL mass spectrometer, as previously described (16).

Cell culture and transfection

HEK293T cells were obtained from Clontech and maintained at 37 °C and 5% CO_2 in DMEM supplemented with antibiotics, 10% FBS. HEK293T cells were transfected at ~70% confluency using Lipofectamine LTX (Invitrogen) according to the protocol of the manufacturer. To test whether ELFN2 can interact with mGluR6 *in trans*, HEK293T cells transfected with cmyc-tagged ELFN2 were trypsinized and co-cultured with cells transfected with mGluR6 or empty vector. After two-days of incubation, the cells were harvested and proceeded to co-immunoprecipitation.

Co-immunoprecipitation and Western blotting

Cells or retina lysates were prepared in ice-cold PBS IP buffer by sonication, then incubated at 4 °C for 30 minute. After 15-minute centrifugation at 14,000 rpm, the supernatant was incubated with 20 μl of 50% protein G slurry (GE Healthcare) and 5 μg of antibodies on a rocker at 4°C for 1 hour. The beads were washed with IP buffer three times. Then proteins were eluted from beads with 50 μl of SDS sample buffer. Proteins retained by the beads were analyzed with SDS-PAGE, followed by Western blotting using HRP conjugated secondary antibodies and an ECL West Pico (Thermo Scientific) detection system. Signals were captured on film and scanned by densitometer. For quantification, band intensities were determined by using NIH ImageJ software. The integrated intensity of β-actin was used for data normalization.

Transcellular GPCR complex signaling platform

The methodology for this assay was extensively described previously (17). Cells were separated into two general categories: (1) cells expressing mGluR6 and biosensor construct(s), and (2) cells expressing empty pcDNA3.1 vector (control) or ELFN2-myc (ELFN2) without any biosensor. 0.42 µg of mGluR6 was transfected compared with 5 µg of vector/ELFN2. Approximately 24 h after transfection, cells were lifted with PBS and centrifuged at 500 × g for 5 min at room temperature. PBS was removed and replaced with Tyrode's solution (137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 0.2 mM Na_2HPO_4, 12 mM $NaHCO_3$, 5.5 mM D-glucose). Pelleted cells were dissociated via pipetting and centrifuged at 500 × g for 5 min at room temperature. Buffer was removed and replaced with fresh buffer. mGluR6/biosensor cells were plated at ~100,000 cells/well in white 96-well plates and control/ELFN2 cells were co-cultured with these cells at 4:1 for ~2 h. Experiments were performed in suspension with control/ELFN2 constructs and cells outnumbering the mGluR6 construct to maximize mGluR6 saturation. Importantly, each biological replicate represents measurements derived from the same homogenous cell population run in parallel and are identical with exception of the experimental control or ELFN2 co-culture conditions.

Measurements of cAMP dynamics

-22F pGloSensor Format: Cells were prepared using the transcellular GPCR complex signaling platform with 2.52µg of Promega -22F cAMP pGloSensor in mGluR-expressing cells. Cells were incubated in Promega GLO reagent during ~2-hour co-culture period with control/ELFN2-cells. Cells were pre-treated with various concentrations of L-glutamic acid (L-Glu) or L-2-amino-4-phosphonobutyric acid (L-AP4) and baseline luminescence was read on a BMG LabTech PHERAstar FSX. After 5 minutes, cells were treated with 1µM of forskolin (FSK) and readings were continued for up to 20 minutes. mGluR activation was calculated as the decrease in FSK-mediated luminescence amplitude.

BRET-based EPAC format: Cells were prepared using the transcellular GPCR complex signaling platform with 0.84µg of Nluc-EPAC-VV in mGluR6-expressing cells. Cells were incubated for ~2-hour with either control or ELFN2-cells prior to stimulation. For desensitization experiments, Gαq/PKC-dependent group III mGluR desensitization was facilitated with 1 µM adenosine-5'-triphosphate (ATP) pre-treatment to pre-activate endogenous Gαq-coupled purinergic receptors (18, 19). Cells were treated with 300 µM L-Glu for 5, 30, or 60 minutes and luminescence and fluorescence were read on a BMG LabTech PHERAstar FSX to attain a baseline the bioluminescence resonance energy transfer (BRET) ratio (535nm/460nm). Cells were then treated with 1µM of forskolin and readings were continued for up to 20 minutes. mGluR6 activation was calculated as the inhibition of forskolin-mediated decrease in BRET ratio. For desensitization experiments, mGluR6 activation was normalized to 5 minutes L-Glu treatment to compare desensitization rates.

Real-time kinetic BRET assays for G protein activation

Cells were prepared using the transcellular GPCR complex signaling platform with 0.84 µg of Gαo, 0.42 µg of Venus 156-239-Gβ1, 0.42 µg of Venus 1-155-Gγ2, and 0.42 µg of masGRK3ctNluc (20) in mGluR6-expressing cells. Cells were incubated for ~2 h with either control or ELFN2 cells prior to stimulation. Cells were injected with Promega Nano-Glo® Luciferase Assay Reagent to 0.067% and luminescence and fluorescence were read on a BMG LabTech PHERAstar FSX to attain a baseline BRET ratio (535 nm/460 nm). Upon stabilization

of baseline BRET ratios, cells were injected with 100 µM L-Glu and continually read every 60 ms for up to 20 s. mGluR activation was calculated as the change in BRET ratio (ΔBRET) following agonist treatment. Rates of activation (1/τ) were calculated using ClampFit 10.3 software to fit exponential curves and calculate τ.

In situ hybridization

The mRNA expression of Elfn2 and Gnat2 in the retina was evaluated with a ViewRNA™ 2-plex *In Situ* Hybridization Assay (Panomics; Santa Clara, USA). The probe sets used to detect the following mouse mRNAs were designed by Panomics (Santa Clara, USA): Elfn2 (Elfn2 NM_183141.2; Cat# VB6-17473) and GNAT2 (NM_008141; Cat#VB6-17820). The whole eye bulb was extracted, embedded in OCT and flash frozen in liquid nitrogen. 12 µm sections were cut using a Leica CM3050 S cryostat, rapidly post-fixed in 4% paraformaldehyde for 10 minutes, washed twice in phosphate buffered saline (PBS) and incubated for 2h in pre-hybridization mix (50% deionized formamide, 5X SSC, 5X Denhardt's solution, 250 µg/ml yeast tRNA, 500µg/ml sonicated salmon sperm DNA) at room temperature. Each section was incubated overnight with Panomics hybridization solution (using an incubator set to 40ºC, no CO_2 and humidity higher than 85%) containing both the QuantiGene View RNA probe sets TYPE 1 and TYPE 6 diluted 1:75 in Probe Set Diluent QT. On the second day, each section was processed according to the manufacturer's instructions provided with the ViewRNA ISH Tissue Assay Kit (QVT0012). Briefly, sections were successively incubated with PreAmplifier Mix QT, Amplifier Mix QT, Label Probe 6-AP (1:1000), Fast Blue Substrate, AP Stop QT, Label Probe 1-AP (1:1000), AP-Enhancer Solution and Fast Red Substrate. Finally the nuclei were counterstained with DAPI and mounted using Fluoromont-G (SouthernBiotech). Confocal images were generated at The Light Microscopy Facility, the Max Planck Florida Institute, using a LSM 780 Zeiss confocal microscope. Image acquisition and processing were accomplished using ZEN 2011 (64 bit) software (Carl Zeiss) with only minor manipulations of the images setting the fluorescence intensity in non-saturating conditions and maintaining similar parameters for each acquired image.

Immunohistochemistry

Dissected eyecups were fixed for 15 min in 4% paraformaldehyde, cryoprotected with 30% sucrose in PBS for 2 h at room temperature, and embedded in optimal cutting temperature medium. Twelve-micron thick frozen sections were cut, mounted onto slides and blocked in PBS with 0.1% Triton X-100 and 10% donkey serum for 1 hour. They then were incubated with primary antibody in PBS with 0.1% Triton X-100 and 2% donkey serum for at least 1 hour. After four washes with PBS with 0.1% Triton, sections were incubated with fluorophore-conjugated secondary antibodies in PT2 for 1 h. After four washes, sections were mounted in Fluoromount (Sigma). Images were taken with Zeiss LSM 780 (or 880) confocal microscope. Quantitative analysis of mGluR6/ELFN1 immunofluorescence from confocal images was performed using Metamorph software. In Amira (Thermo Fisher Scientific), the dendrites and soma of the bipolar cell were masked and used to identify the connection between cone terminal and the bipolar cell of interest.

Electroretinography (ERG)

Electroretinograms were recorded by using the UTA system and a Big-Shot Ganzfeld (LKC Technologies). Mice (21days for *pDKO* line and 6~8 weeks old for all other genotypes) were

dark-adapted (≥6 h) and prepared for recordings using a red dim light. Mice were anesthetized with an i.p. injection of ketamine and xylazine mixture containing 100 and 10 mg/kg, respectively. All procedures were approved by the Institutional Animal Care and Use committee at the Scripps Florida Research Institute. Recordings were obtained from the right eye only, and the pupil was dilated with 10% phenylephrine hydrochloride (Paragon), followed by the application of 0.5% Tropicamide (Akorn). Recordings were performed with a gold loop electrode supplemented with contact lenses to keep the eyes immersed in solution. The reference electrode was a stainless steel needle electrode placed subcutaneously in the neck area. The mouse body temperature was maintained at 37 °C by using a heating pad controlled by ATC 1000 temperature controller (World Precision Instruments). ERG signals were sampled at 1 kHz and recorded with 0.3-Hz low-frequency and 300-Hz high-frequency cut-offs.

Full field white flashes were produced by a set of LEDs (duration < 5 ms) for flash strengths ≤ 2.5 cd·s/m^2 or by a Xenon light source for flashes > 2.5 cd·s/m^2 (flash duration < 5 ms). ERG responses were elicited by a series of flashes ranging from $1\times 10^{-5}$to 800 cd*s/m^2 in 10-fold increments. Ten trials were averaged for responses evoked by flashes up to 0.1 cd*s/m^2, and three trials were averaged for responses evoked by 0.5 and 1 cd*s/m^2 flashes. Single flash responses were recorded for brighter stimuli. To allow for recovery, interval times between single flashes were as follows: 5 s for 1×10^{-5} to 0.1 cd*s/m^2, 30 s for 0.5 and 1 cd*s/m^2, 60 s for 5 and 10 cd*s/m^2, and 120 s for 100 and 800 cd*s/m^2 flashes. Rod-saturating light background of 32 cd/m^2 was administered for recording cone-only ERGs. Ten trials elicited from 100 cd*s/m^2 flashes were averaged at an interval recovery time of 1 second between flashes.

ERG traces were analyzed using the EM LKC Technologies software and Microsoft Excel. The a-wave amplitude was calculated as the value difference between the onset of light flash and the peak of the deflection wave for both dark and light-adapted ERG. The b-wave amplitude was calculated from the bottom of the a-wave response to the peak of the b-wave for both dark and light-adapted ERG. The b-wave implicit time was calculated as the time span from flash onset to the onset of the b-wave peak. The data points from the b-wave stimulus–response curves were fitted by Equation 1 using the least-square fitting method in GraphPad Prism6.

(1) $\mathbf{R=R_{max,r}*I/(I+I_{0.5,r})+R_{max,c}*I/(I+I_{0.5,c})}$

The first term of this equation describes rod-mediated responses (r), and the second term accounts primarily for responses that were cone mediated (usually at flash intensities ≥1 cd*s/m^2 for dark-adapted mice; index c). $R_{max,r}$ and $R_{max,c}$ are maximal response amplitudes, and $I_{0.5,}$r and $I_{0.5,c}$ are the half-maximal flash intensities. Stimulus responses of retinal cells increase in proportion to stimulus strength and then saturate, this is appropriately described by the hyperbolic curves of this function.

Electron Microscopy

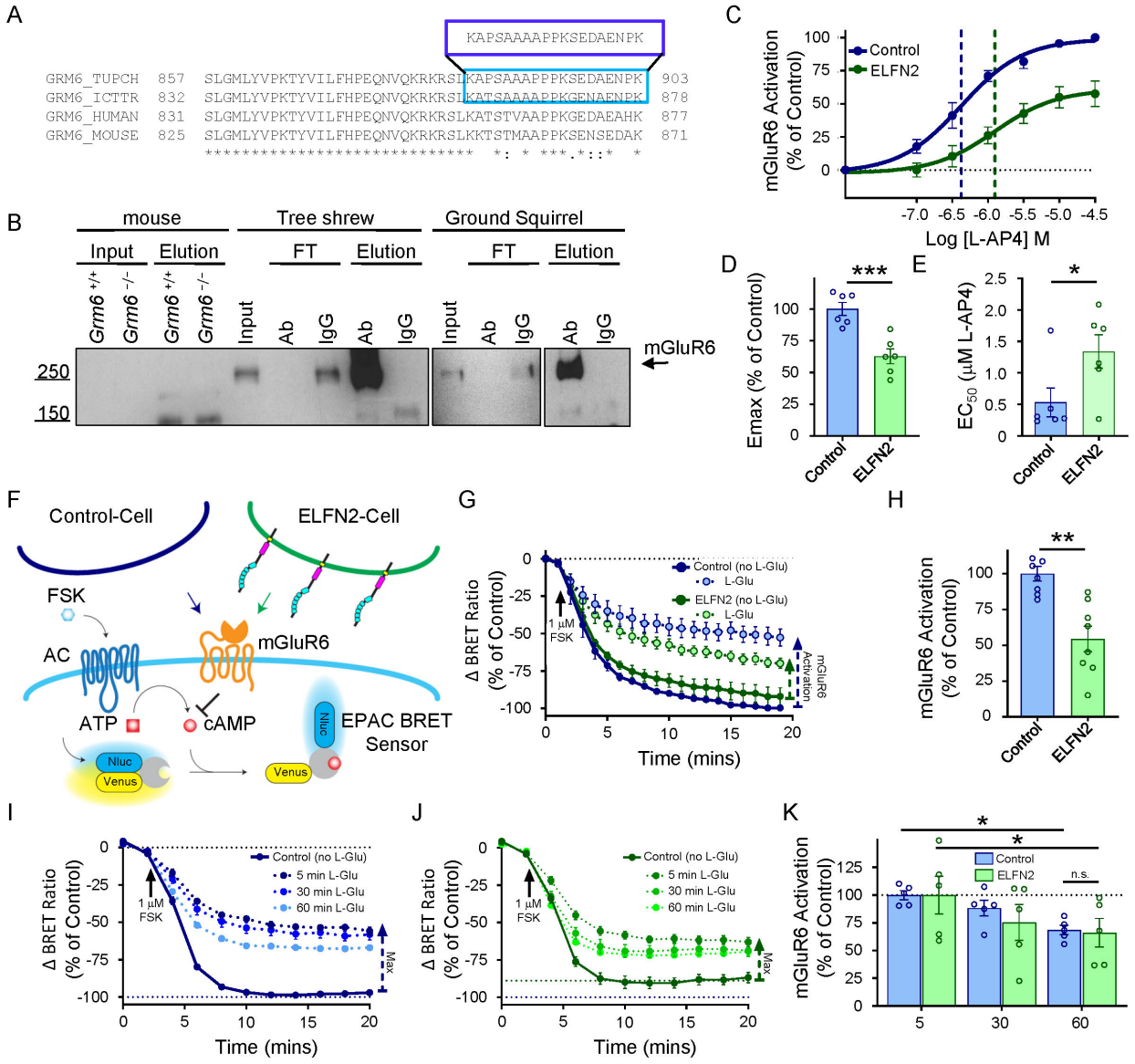
Eyes were enucleated, cleaned of extra-ocular tissue, and pre-fixed for 15 min in cacodylate-buffered half-Karnovsky's fixative containing 2mM calcium chloride. Then the eyecups were hemisected along the vertical meridian and fixed overnight in the same fixative. The specimens were rinsed with cacodylate buffer and postfixed in 2% osmium tetroxide in water for 1 hour, en-block stained with 1% uranyl acetate for 25 minutes, then gradually dehydrated in an increasing ethanol and acetone series (30–100%), and embedded in Durcupan ACM resin (Electron Microscopy Sciences, PA). Blocks were cut with 60-nm-thickness, and were stained with 3% uranyl acetate and 0.5% lead citrate. Sections were examined in a Tecnai G2 spirit BioTwin

(FEI) transmission electron microscope at 80 or 100 kV accelerating voltage. Images were captured with a Veleta CCD camera (Olympus) operated by TIA software (FEI).

Statistics

Statistics were performed using GraphPad Prism 8.3 software. Student's t-test was used for performing all pairwise comparisons unless otherwise noted. Dose response of ERG experiment utilized multiple t-test using the Holm-Sidak method mGluR6 signaling experiments utilized identical cell populations in each biological replicate and a parallel experimental design, therefore all statistical tests on mGluR6 signaling utilized two-tailed, paired t-tests (related samples t tests) as described previously (4, 17), and Supplemental Figure 1K utilized a repeated-measures (matched) two-way ANOVA with Sidak's multiple comparisons tests. All experiments passed a Shapiro-Wilk normality test justifying parametric tests, with exception of Figure 7E and Supplemental Figures S6B and S6C where a nonparametric Mann-Whitney test was performed, and Supplemental Figure S1E where a nonparametric two-tailed Wilcoxon matched-pairs signed-rank test was used as recommended. Statistical significance was expressed as * $P<0.05$, ** $P<0.01$, and *** $P<0.001$. Not significant (n.s.) was indicated where appropriate.

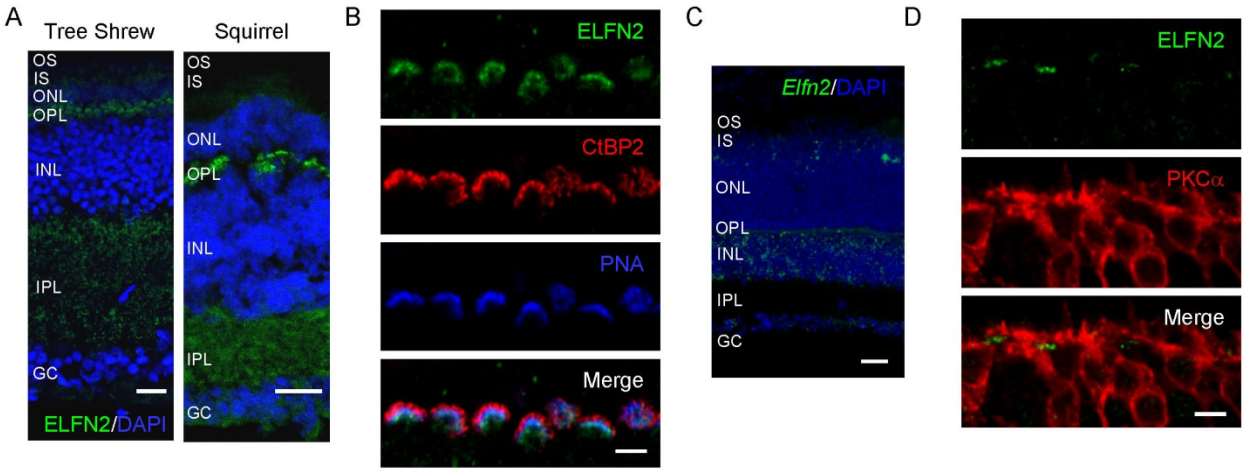
Supplementary Figures



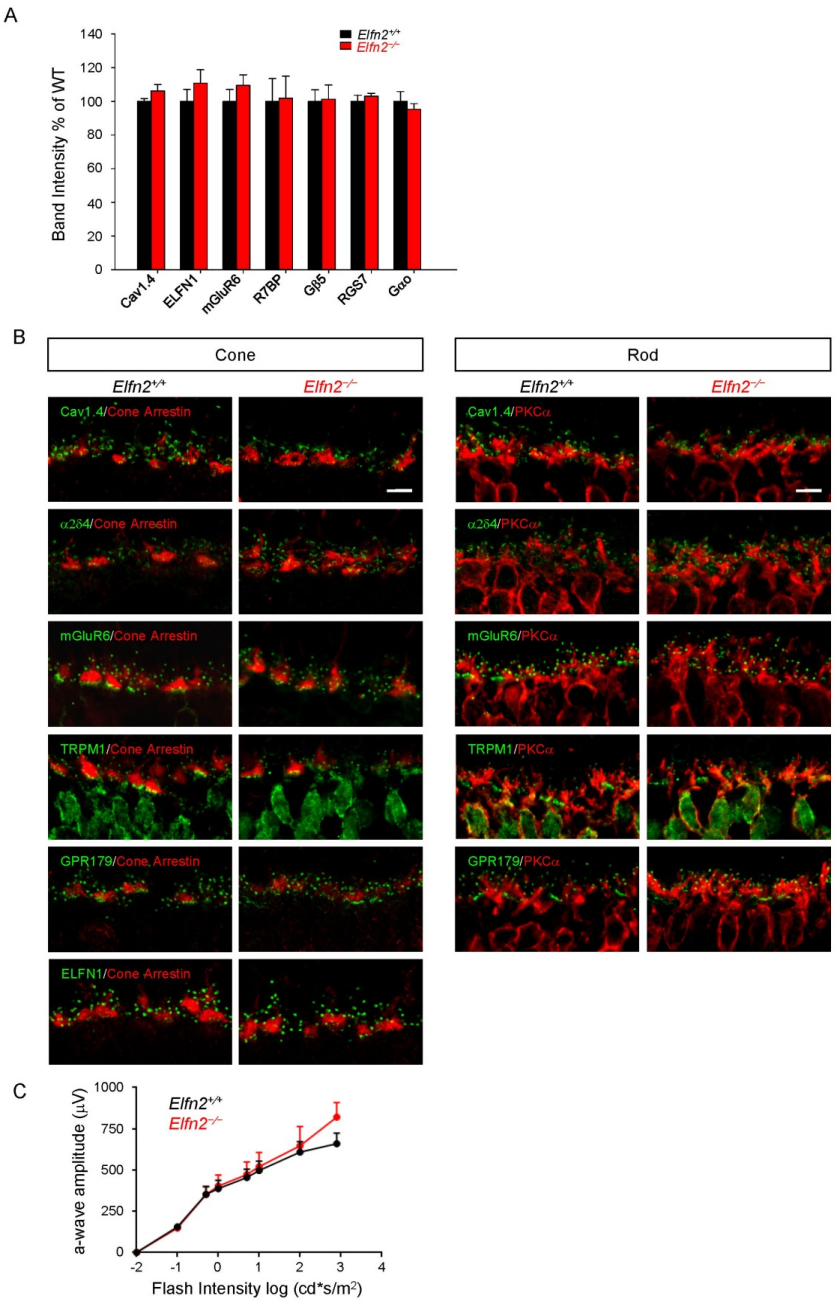
Supplementary Figure S1. ELFN2 is a novel binding partner of mGluR6 in cone dominant retinas.

A, Generation of anti-mGluR6 antibodies to recognize protein in tree shrew and ground squirrel. Sequence alignment of mGluR6 proteins from different species (cone dominant tree shrew and ground squirrel vs rod dominant retina-mouse). Antibody was generated against the synthetic peptide with sequence KAPSAAAAPPKSEDAENPK highlighted by the blue box. **B,** Anti-mGluR6 antibody specifically immunoprecipitates mGluR6 from tree shrew and ground squirrel retinas. **C,** ELFN2 modulates pharmacological properties of mGluR6 *in trans* by transcellular GPCR signaling assay utilizing cAMP pGloSensor in mGluR6-expressing cells exposed to control or ELFN2-expressing cells that lack biosensor. Dose–response curves were plotted for

the same mGluR6- and biosensor-expressing cell population, differing only by exposure to either control or ELFN2-expressing cells. **D**. Quantification of maximal efficacy (E_{max}) for L-AP4 in Figure S1C. ***P < 0.001, paired t-test. **E,** Quantification of half-maximal effective concentration (EC_{50}) for L-AP4 in Figure S1C. *P < 0.05, Wilcoxon signed-rank test. **F**, Schematic representation of transcellular GPCR signaling assay involving co-culture of control cells or ELFN2-expressing cells with mGluR6-expressing cells co-expressing BRET-based Nluc-EPAC-VV cAMP biosensor. **G**, Change in BRET ratio measurements over time of cells pretreated with buffer or 300 μM L-glutamic acid for 5 min and then stimulated with 1 μM forskolin, with increased cAMP equating to decreased BRET ratio. mGluR6 activation was quantified as a decrease in forskolin response. **H,** Quantification of mGluR6 activation following co-culture with control or ELFN2-expressing cells. *P < 0.05, paired t-test. **I,** Change in BRET ratio readings following adaptation of assay to accommodate longitudinal pre-stimulations with 300 μM L-glutamic acid. The solid control line corresponds to no prestimulation/baseline in the presence of control cells, followed by 5, 30, or 60 min of L-glutamic acid prestimulation, with 5 min providing the maximum (Max) response. **J,** Change in BRET ratio under the same parameters in parallel in the presence of ELFN2 expressing cells. **K,** Quantification of mGluR6 activation normalizing for Max response (5 min). *P < 0.05, matched two-way ANOVA with Sidak's multiple comparison test.

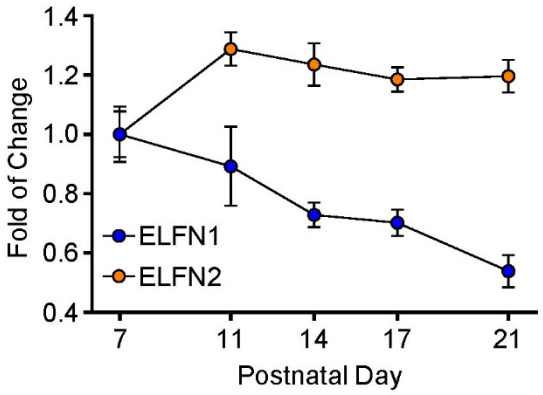


Supplementary Figure S2. ELFN2 is a synaptic protein localized in cone terminals.
A, Immunostaining of ELFN2 in tree shrew and ground squirrel cross sections. Scale bar is 20 μm. **B**, High magnification image of triple-immunostaining of ELFN2 (green), presynaptic marker CtBP2 (red) and cone synapse marker PNA (blue) in tree shrew retina. Scale bar is 5 μm. **C,** mRNA for *Elfn2* detected in ONL and INL by fluorescent *in situ* hybridization on retina cross-sections of a mouse. Scale bar is 20 μm. **D,** ELFN2 protein (green) is not localized with postsynaptic ON-RBC marker (PKCα, red). Mouse retina cross-sections were co-stained with anti-PKCα antibody. Scale bar is 5 μm.

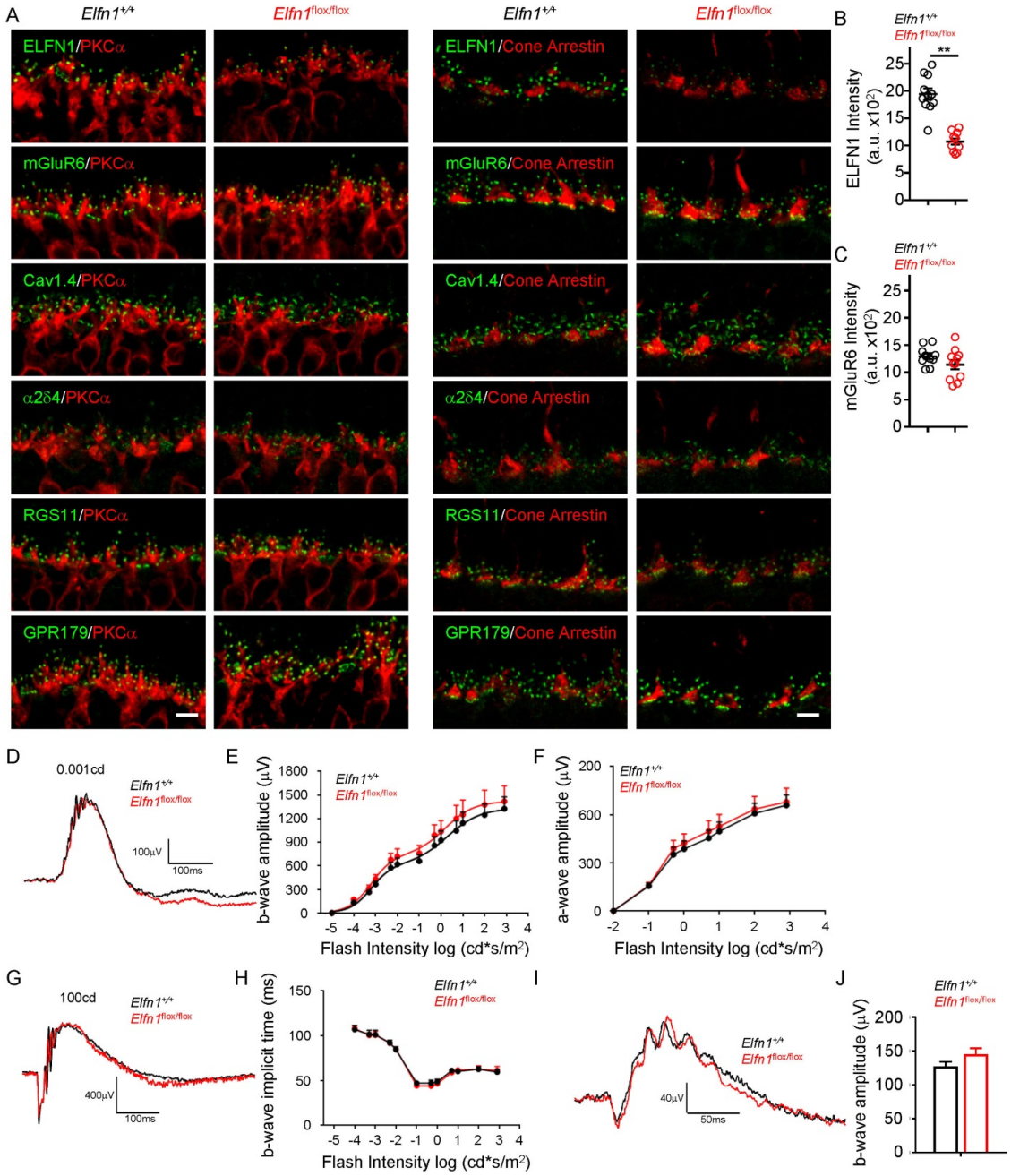


Supplementary Figure S3. Loss of ELFN2 has no impact on the key synaptic proteins and ERG in *Elfn2*$^{-/-}$ mice.

A, Quantification of synaptic protein expression level as analyzed by Western blotting followed by quantification. Data are represented as mean ± SEM. (n=3-4). **B,** Localization of synaptic signaling components in both rod and cone photoreceptors of *Elfn2*$^{-/-}$ mice. Retina sections were stained with the indicated antibodies and counter-stained with PKCα to identify the position of the rod synapses, or cone arrestin to determine the location of cone synapses. Scale bar is 5 μm. **C,** Dose response plot of maximal a-wave amplitudes from *Elfn2*$^{+/+}$ (n=7) and *Elfn2*$^{-/-}$ (n=4) mice plotted against their eliciting flash-intensities as determined by the ERG recordings. No significant difference was observed, $p>0.05$ based on multiple t-test.



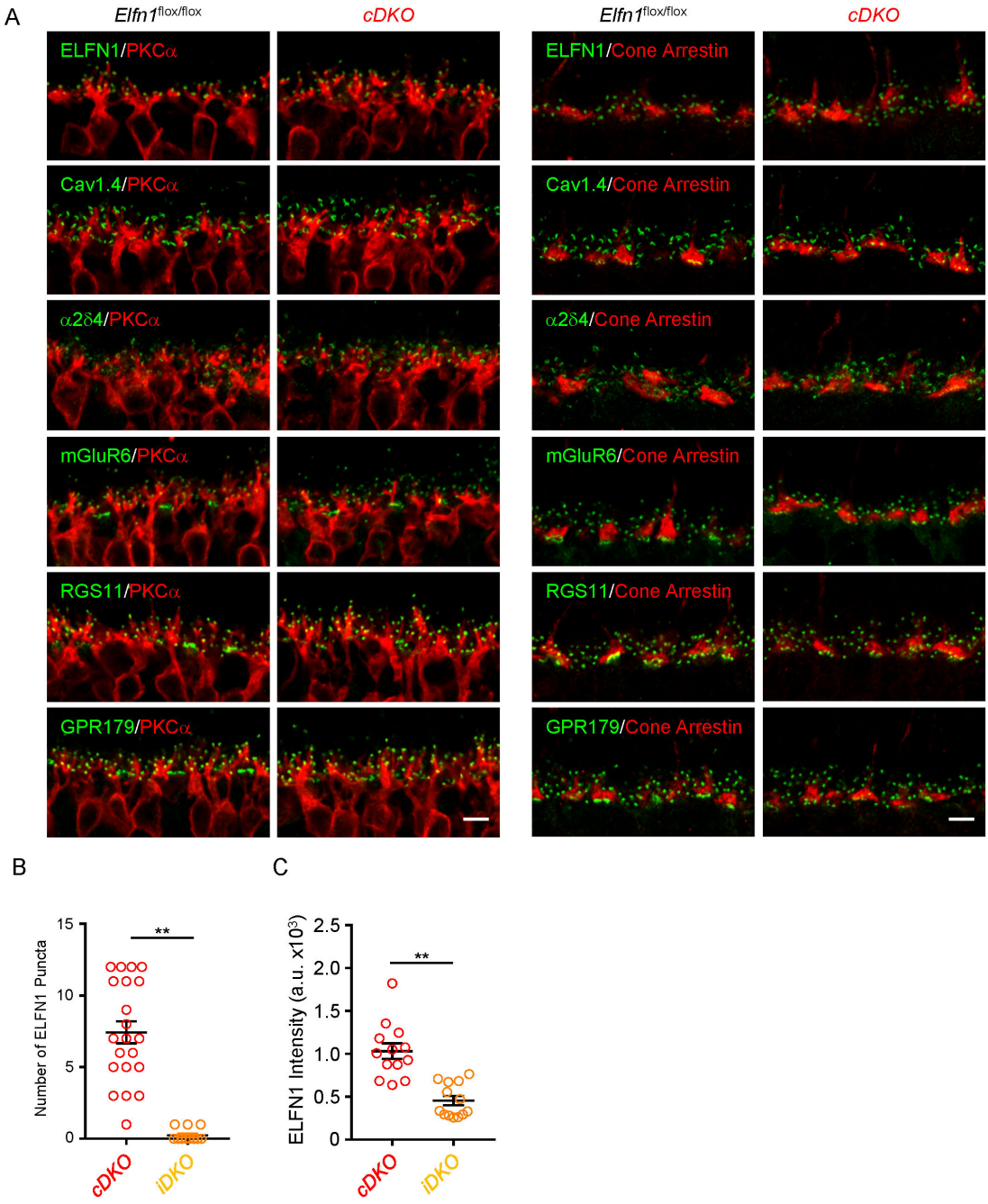
Supplementary Figure S4. Comparison of the development dynamics of ELFN1 and ELFN2 accumulation at cone synapse in wild type retina. Mean fluorescence intensity from 10 puncta per retinal section were measured from 3-6 images per retina collected from two separate mice at each development time point. Protein levels were calculated as the fold change from the mean fluorescence intensity at the indicated time point to the mean fluorescence intensity at P7. Data are represented as the mean ± SEM. n=12-18.



Supplementary Figure S5. Characterization of *Elfn1*$^{flox/flox}$ mice.

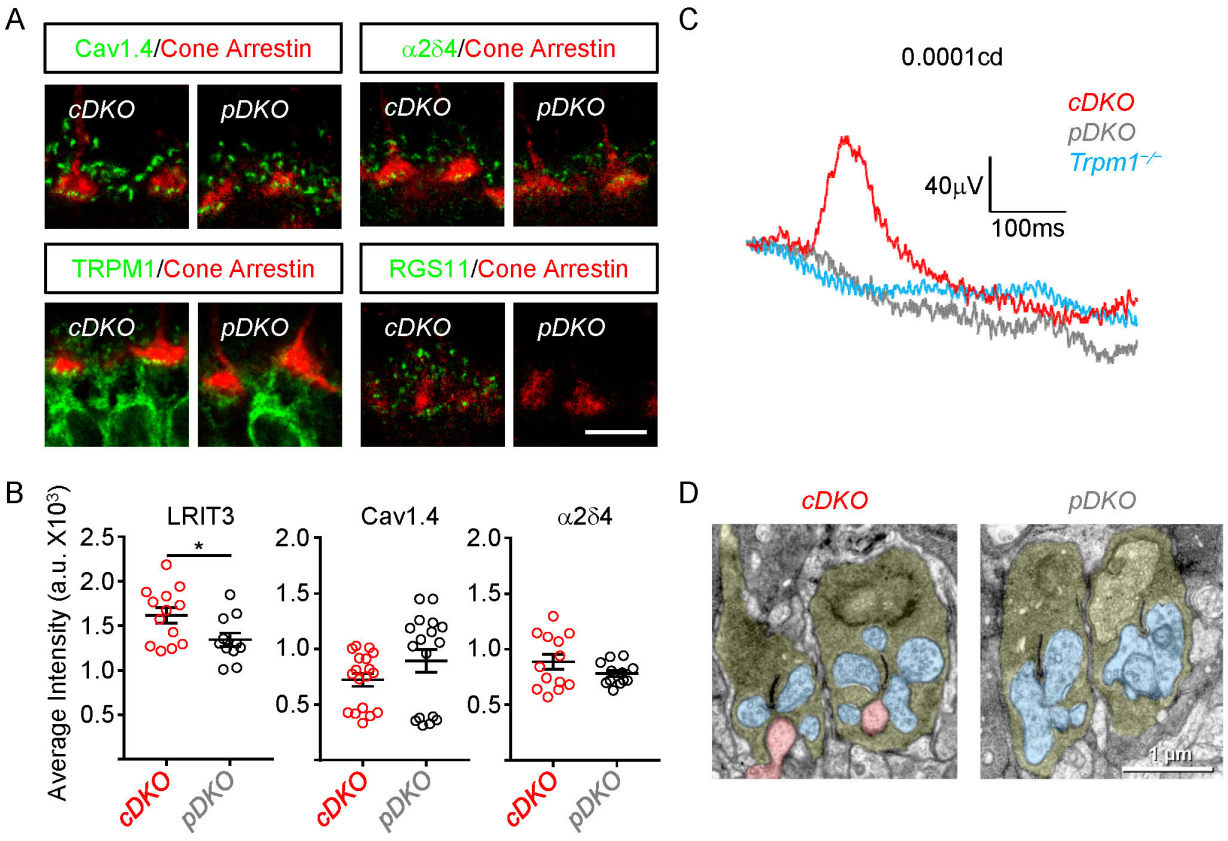
A, Synaptic organization of the *Elfn1*$^{flox/flox}$ retinas. Retina sections were stained with the indicated antibodies and counter-stained with PKCα to identify the position of the rod synapses, or cone arrestin to determine the location of cone synapses. Scale bar is 5 μm. **B**, Quantification of the synaptic puncta of ELFN1. Mean fluorescence intensity from 10 puncta per retina section were measured from 3–6 sections per retina collected from two separate mice. Data are represented as mean ± SEM. (n=10-11) **p < 0.01, t-test. **C**, Quantification of the synaptic puncta of mGluR6. Mean fluorescence intensity from 10 puncta per retina section were measured from 3–6 sections per retina collected from two separate mice. Data are represented as mean ± SEM. (n=10-11) **D**, Rod photoreceptor synaptic transmission in the *Elfn1*$^{flox/flox}$ mice.

Representative ERG traces elicited by a scotopic flash of 0.001 cd∗s/m^2 (~0.6 R∗/rod) to activate the primary rod pathway only. **E,** Dose response plot of maximal ERG b-wave amplitudes from *Elfn1*$^{+/+}$ and *Elfn1*$^{flox/flox}$ mice plotted against their eliciting flash-intensities. **F**, Dose response plot of maximal ERG a-wave amplitudes from *Elfn1*$^{+/+}$ and *Elfn1*$^{flox/flox}$ mice plotted against their eliciting flash-intensities. **G,** Effect of introducing *Elfn1* floxed allele on cone photoreceptor synaptic transmission. Representative ERG traces elicited by a photopic flash of 100 cd∗s/m2 (~58,000 R∗/rod). **H**, Dose response plot of maximal b-wave implicit times from *Elfn1*$^{+/+}$ and *Elfn1*$^{flox/flox}$ mice plotted against their eliciting flash-intensities. **I,** Representative ERG traces to a photopic flash of 100 cd∗s/m^2 (~58,000 R∗/rod) under a 32 cd∗s/m^2 (~18,500 R∗/rod/s) light background to activate cone pathway only. **J**, Quantification of ERG b-wave amplitudes under photopic conditions. The data are averaged from five and four mice for *Elfn1*$^{+/+}$ and *Elfn1*$^{flox/flox}$ mice, respectively. There were no significant genotype differences in data reported in panels E,F,H and J, $p>0.05$ based on multiple t-test.



Supplementary Figure S6. Characterization of *cDKO* mice.

A. Synaptic organization of conditional double knockout *Elfn1*$^{flox/flox}$: *Elfn2*$^{-/-}$ retinas (*cDKO*). Retina sections were stained for indicated antibodies and counter-stained with PKCα to identify the position of the rod synapses, or cone arrestin to determine the location of cone synapses. Scale bar is 5 μm. **B,** Quantification of synaptic ELFN1 puncta per terminal. Images were acquired from 3–6 sections per retina collected from two separate mice. Data are represented as mean ± SEM. n=13-21. **P < 0.01, Mann-Whitney test. **C,** Quantification of synaptic intensity of ELFN1. Mean fluorescence intensity from 10 puncta per retina section were measured from 3–6 sections per retina collected from two separate mice. Data are represented as mean ± SEM. n=14. **P < 0.01, Mann-Whitney test.



Supplementary Figure S7. Characterization of *pDKO* mice.
A, Staining of retina sections for synaptic markers. Counter-staining with cone arrestin was used to determine the location of cone synapses. Scale bar is 5 μm. **B**, Quantification of synaptic content of indicated proteins. Mean fluorescence intensity from 10 puncta per retina section were measured from 3–6 sections per retina collected from two separate mice. Data are represented as mean ± SEM. *P < 0.05, t-test. **C,** Analysis of rod photoreceptor synaptic transmission in *pDKO* mice by ERG analysis. Representative ERG traces to a scotopic flash of 0.0001 cd*s/m^2 (~0.06 R*/rod) to activate the primary rod pathway only. **D.** Examination of rod synaptic morphology by electron microscopy. Photoreceptor axonal terminals are colored in yellow, processes of horizontal cells in blue, and ON-RBC dendrites in pink.

Supplementary References

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