Supporting Information

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SI Text

Generation of mGluR6-cre-GFP Mice. To identify genes expressed in ON-bipolar cells that participate in the mGluR6-activated signaling cascade, we generated a transgenic line of mice expressing Cre recombinase under the control of the mGluR6 promoter. First, the codon-improved Cre recombinase (iCre) (1) was inserted upstream of the 3′ untranslated and polyadenylation site of pUHG10.3 using common *Kpn*I and *Sac*I restriction sites. Second, the 637-bp *Pme*I-*Nae*I mGluR6 promoter fragment was amplified from the mGluR6 5′ upstream region (2) and cloned in front of iCre-poly(A) in pBLSK(-) (Stratagene). Finally, the 9.5-kb *Sal*I-*Pme*I fragment of the mGluR6 5′upstream region was added to reconstitute the full-length mGluR6 promoter. The nucleotide sequence of the amplified region was verified. The promoter construct DNA, digested with *Age*I and *Not*I and size-purified to remove the plasmid vector sequence, was injected into fertilized mouse eggs. Two mouse lines were identified that transmitted the transgene to their progeny. These mice were crossed with two EGFP reporter mouse strains, a ROSA26 mouse (3) and the Z/EG marker strain (4) (Jackson Laboratory, stock no. 004077 and no. 004178, respectively). Only one transgenic mouse line expressed Cre in the retina, as determined by immunostaining for Cre and EGFP expression in double transgenic mice. Whereas EGFP fluorescence was weak in the retina of these mice when crossed with the ROSA26 marker strain, presumably because the ROSA26 promoter is not very active in retinal bipolar cells, EGFP fluorescence was clearly visible in a majority of ON-bipolar cells from double transgenic mice with the chicken actin promoter driving EGFP (Fig. S1*A*).

Genetic Profiling of Retinal ON-Bipolar Cells. Retina were dissociated by mechanical trituration after limited treatment with papain (Fig. S1*B*). Dissociated ON-bipolar cells expressing GFP were purified by fluorescence activated cell sorting (FACS; Fig. S1*C*). Total RNA was prepared from the FACS-purified ON-bipolar cells, as well as from total retina. After cDNA synthesis and linear amplification, the samples were applied to an Illumina BeadChip Expression Array, and data were analyzed using the GeneSifter program (ViZxlabs). Of the 46,642 markers analyzed, the expression of 4,097 markers was reliably detected in the ON-bipolar cell preparation ($P < 0.05$). Importantly, this analysis also demonstrated the relative purity of the ON-bipolar cell preparation. The message for mGluR6 was enriched in the ON-bipolar cell preparation by 7.5-fold over total retina, whereas photoreceptor-specific RNAs, such as that encoding rod and cone opsins, transducins, cGMP-specific phosphodiesterases, cyclic nucleotide-gated channels, and arrestin, were depleted 30- to 150-fold compared to the total retinal sample. Among the ion channels enriched in ON-bipolar cells were several TRP channels, including TRPM1 (enriched 12-fold over total retina).

SI Materials and Methods

Animals. Mice were maintained on a 12-h light-dark cycle. TRPM1$^{+/-}$ mice were generated by Lexicon Genetics Inc. and obtained from Texas A&M Institute for Genomic Medicine (Houston, TX). Exons 2–4 were replaced by a selection cassette resulting in a nonfunctional TRPM1 allele. All experiments were conducted in accordance with National Institutes of Health guidelines and approved by the Institutional Animal Care and Use Committees at Oregon Health & Science University and Washington State University.

In Situ Hybridization. Digoxigenin-labeled probes were synthesized by runoff transcription using T3 and T7 RNA polymerase and Genius Kit components (Roche). In situ hybridization methods for cryosections have been described (5). The digoxigenin probe was detected with anti-digoxygenin antibody conjugated to alkaline phosphatase, followed by Fast Red color substrate (Roche).

Immunohistochemistry. Vertical retina sections were prepared and labeled as described in ref. 29. Sections were incubated with rabbit anti-TRPM1 antibody (1:100; Sigma–Aldrich) either alone or in combination with either mouse anti-PKCα (1:5000; Novus Biologicals), sheep anti-mGluR6 (1:100; ref. 6), mouse anti-ctbp2 (anti-ribeye; 1:5,000, BD Biosciences) or mouse anti-bassoon (1:5,000; Synaptic Systems) for either 2 h at room temperature or overnight at 4 °C. After three washes in PBS, the tissue was incubated for 1 h at room temperature with anti-rabbit IgG conjugated to either Alexa Fluor 488 or 594 (1:1,000; Invitrogen) alone or in combination with anti-mouse-CY3 (1:500; Jackson Immunochemicals) or PNA-Alexa Fluor 488 (1:1,000; Invitrogen), then washed again in PBS. For wholemount macaque retina pieces, the tissue was blocked for 4 h at 4 °C, incubated with rabbit anti-TRPM1 (1:500) in combination with either mouse anti-PKCα (1:5,000) or mouse anti-Gαo (1:1,000; Biomol) for 72 h at 4 °C, washed three times for 1 h each in PBS, then incubated overnight at 4 °C in secondary antibodies, before a final three washes in PBS of 1 h each. Sections and wholemount retina pieces were coverslipped and imaged with an Olympus FluoView FV1000 confocal microscope using a 60×/1.42 oil-immersion objective.

Electroretinogram Recording. Mice were dark-adapted (>12 h) and prepared for recording under dim red light. Anesthesia was achieved via i.p. injection of ketamine:xylazine (100:10 mg/kg), and maintained with supplemental 30:3 mg/kg anesthesia injections every 30 min. Pupils were dilated with phenylephrine (2.5%) and tropicamide (1%), and the mouse was placed on a heating pad that maintained body temperature at 37–38 °C. The head was placed in a custom-made holder, which stabilized the head and delivered O_2 (≈0.25 L/min). ERGs were recorded from a custom-made contact lens electrode with its central platinum wire placed against the cornea (with a drop of methylcellulose) referenced to a platinum loop placed behind the equator of the eye. A needle electrode placed in the tail served as ground. Full-field scotopic ERGs were recorded to flashes of increasing intensity [−3.5 to 2.4 log scotopic candela-seconds/meter^2 (sc cd-s/m^2)]. For flash intensities above −1.0 log sc cd-s/m^2, rod-isolated ERGs were obtained by subtracting cone-ERGs, obtained with a paired flash protocol from the mixed rod/cone responses (7, 8). For flash intensities <1.5 log sc cd-s/m^2, the second flash was presented 0.7 s after a 1.6 log sc cd-s/m^2 conditioning flash. At higher intensities, identical paired flashes were separated by 0.7–1.5 s. Cone-driven ERGs were also obtained photopically from flashes of increasing intensity (−1.0 to 3.7 log ph cd-s/m^2) presented 20 min after the onset of a rod-saturating background light (60 cd/m^2). ERGs were low pass filtered (−3 dB) at 300 Hz for intensities <1.0 log sc cd-s/m^2, and at 1 kHz for all other recordings. ERGs were amplified (1–10 k), high pass filtered (−3 dB at 0.1 Hz), and sampled at 2.5 kHz. A P3 model was ensemble fit to the leading edges of rod-isolated ERG a-waves (7). Derived parameters were: S [(sc cd s)$^{-1}$ s^{-2}]

a sensitivity parameter; td (ms), the delay due filtering and flash duration; and $Rmax_{P3}$ (V) the maximal response.

Optokinetic Response. Visual acuity and contrast sensitivity were measured behaviorally from three $TRPM1^{-/-}$ mice, and four wild-type mice (one $TRPM1^{+/+}$ littermate of the $TRPM1^{-/-}$ mice, and three C57BL/6) using a virtual optokinetic system (9) (OptoMotry, CerebralMechanics). The responses of the $TRPM1^{+/+}$ mouse were indistinguishable from those of wild-type C57BL/6 mice.

Patch-Clamp Recordings. Detailed procedures for recording from mouse bipolar cells have been published (10). Briefly, mice were killed by cervical dislocation after isofluorane anesthesia. Eyes were removed under room light and immediately transferred to Ames solution, equilibrated with carbogen (95% O_2–5% CO_2). Retinas were dissected and adhered to nitrocellulose membrane, and approximately 200-μm slices were prepared by using a tissue chopper. Retina slices were transferred to the recording chamber on an Olympus BX51W microscope and continuously perfused with oxygenated Ames. Patch electrodes were filled with (in mM) 135 $KMeSO_4$, 6 KCl, 5 NaCl, 1 EGTA, 2 $MgCl_2$, 2 Na-ATP, and 1 Na-GTP, and 5 Na-Hepes, pH 7.4. Alexa-488 hydrazide was included to examine cell morphology at the conclusion of recording. Voltage was controlled and currents recorded with a HEKA EPC-10 amplifier and PatchMaster software. Current signals were sampled at 5 kHz and filtered at 2.5 kHz. The external Ames solution was supplemented with 4 μM L-AP4, an mGluR6 agonist to simulate the "dark" condition. A pipette attached to a Picospritzer containing Ames with 600 μM CPPG was placed on the OPL adjacent to the dendritic arbour of a target cell. After obtaining a whole-cell recording configuration, a 5-s puff application of CPPG was used to chemically simulate light responses in bipolar cells.

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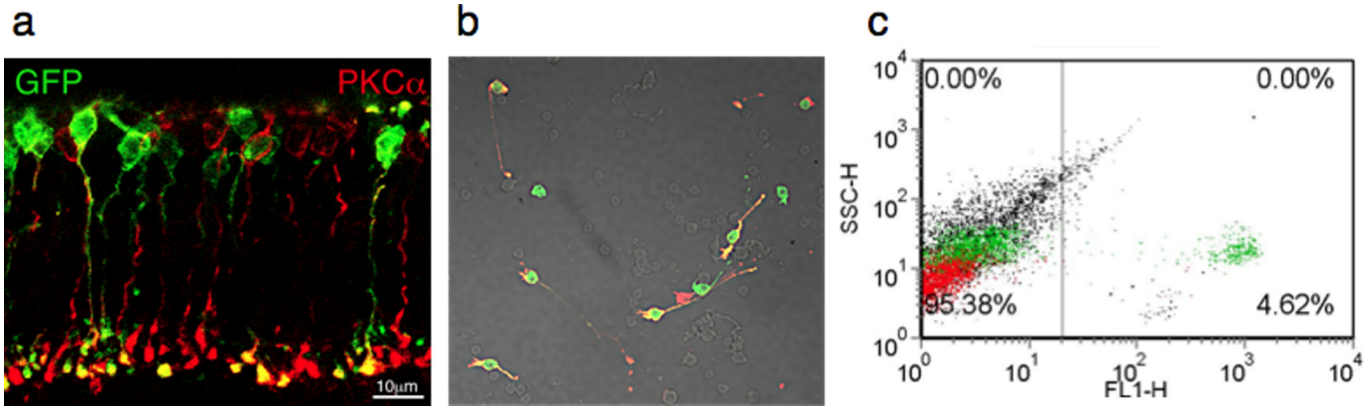

Fig. S1. GFP-tagged ON-bipolar cells in the mGluR6-Cre/ZEG mouse retina. (*A*) Vertical retina section immunolabeled for PKCα (red) and GFP (green). (*B*) Dissociated retinal neurons immunolabeled for PKCα (red) and GFP (green), superimposed on a Nomarski image. (*C*) Flow cytometry separation of GFP-labeled cells (cluster of green dots to the right) from a dissociated retina preparation. In these experiments more than half of the PKCα-labeled rod bipolar cells are GFP positive (combination of green and red fluorescence in *A* and *B*); in addition, many cone ON-bipolar cells are also GFP positive (green fluorescence only in *A* and *B*).

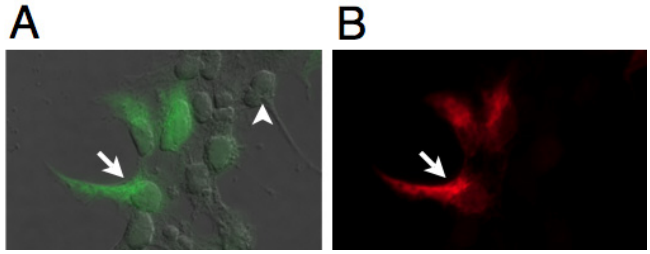

Fig. S2. Immunostaining of TRPM1-transfected cells. HEK tsA-201 cells were cotransfected with cDNAs encoding green fluorescent protein (GFP) and human TRPM1. (*A*) GFP fluorescence superimposed on a Nomarski image. (*B*) Immunolabeling with TRPM1 antibody labels transfected cells (arrow), but not untransfected cells (*A* arrowhead).

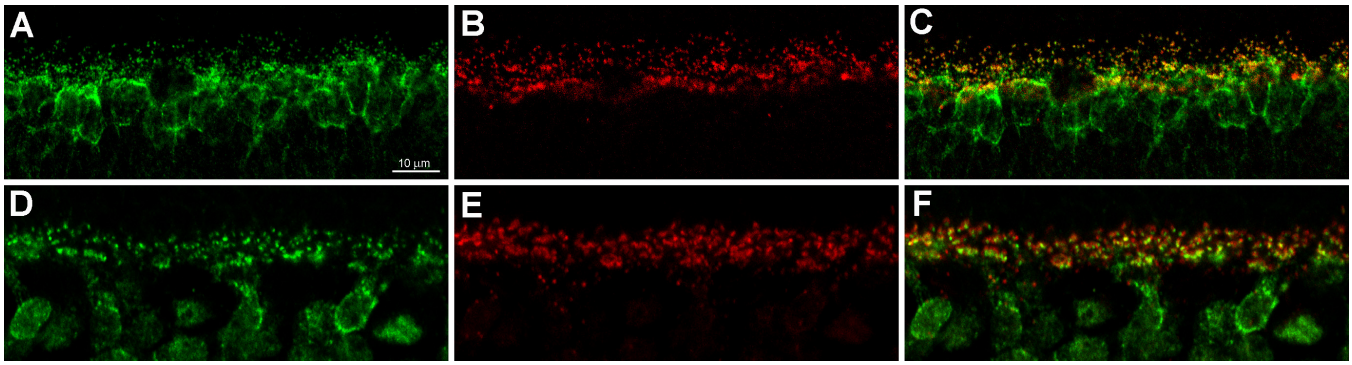

Fig. S3. TRPM1-labeled ON-bipolar cell dendrites in the OPL. Mouse retina sections were double labeled for TRPM1 (green, *A* and *D*) and either mGluR6 (red, *B*) or bassoon (red, *E*), a component of the synaptic ribbon. Merged images are shown in *C* and *F* with areas of overlap appearing yellow.

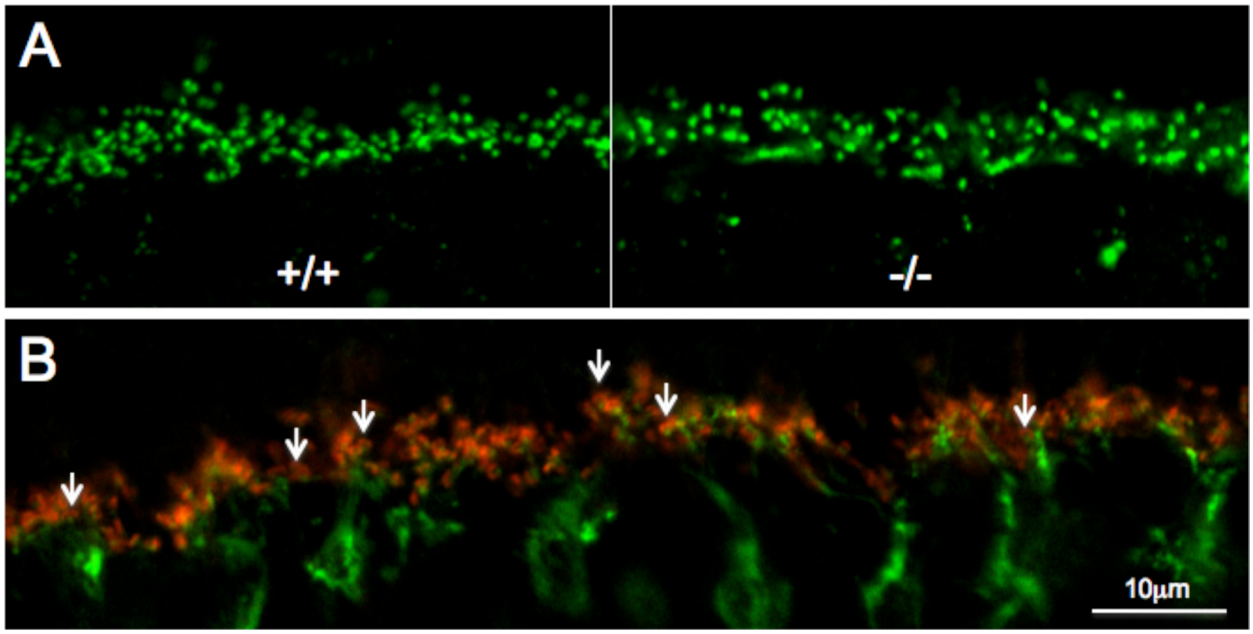

Fig. S4. ON-bipolar cell dendrites in the TRPM1$^{-/-}$ retina. (*A*) The distribution of mGluR6 in the TRPM1$^{-/-}$ outer plexiform layer (−/−, *Right*) is similar to wild-type (+/+, *Left*). (*B*) Double labeling of the TRPM1$^{-/-}$ outer plexiform layer for the rod bipolar cell marker, PKCα (green) and the synaptic ribbon protein, ribeye (red). The tips of the PKC-labeled rod bipolar cells are nested within the arcs formed by the photoreceptor synaptic ribbons (arrows).

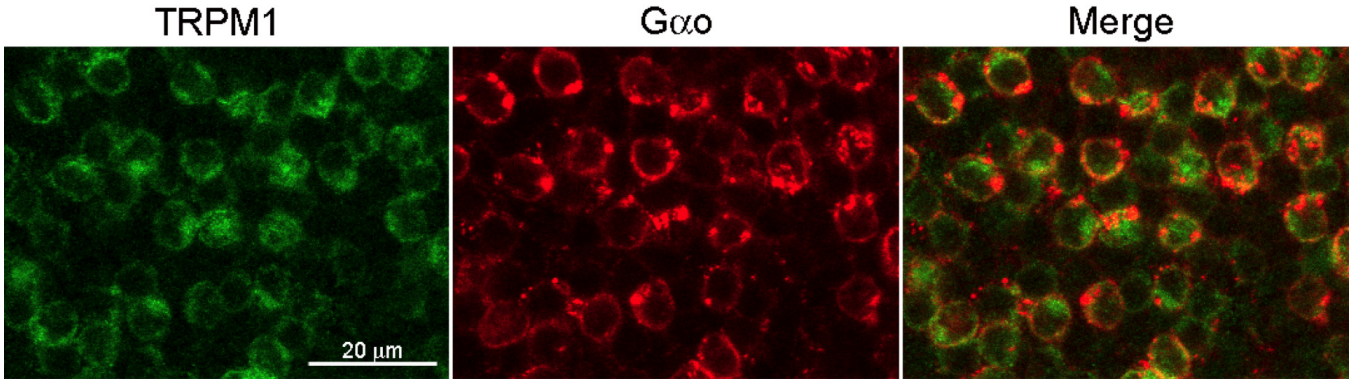

Fig. S5. All ON-bipolar cells express TRPM1. Horizontal optical section through the inner nuclear layer of a macaque retina double labeled for TRPM1 (green) and the ON-bipolar cell marker, Gαo (red). The merged image is shown on the right, with areas of colocalization appearing yellow.