Supporting Information

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SI Materials and Methods

Biotinylation of Cell-Surface Protein. The cell-surface TRPM1-L was measured by biotinylation according to the modified method of Cayouette et al. (1). Cells grown in 60-mm dishes were washed with PBS (containing 137 mM NaCl, 3.5 mM KCl, 0.9 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM sodium phosphate buffer, pH adjusted to 7.4) and then incubated with Sulfo-NHS-SS-Biotin (Thermo Scientific) for 30 min at 4°C. The cells were washed with PBS containing 100 mM glycine three times to stop the biotinylation reaction and to remove free biotin. The cells were then lysed with 300 μL of ice-cold RIPA buffer [150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 1.7 μg/mL aprotinin, 1 μg/mL leupeptin, 1 mM phenylmethylsulfonyl fluoride, 50 mM Tris·HCl (pH 8.0)] and sonicated for 5 s. After centrifugation, the supernatant was collected and incubated with streptoavidin-agarose beads (Thermo Scientific) for 2 h at 4°C. The samples were washed with RIPA buffer three times and resuspended in SDS sample buffer before SDS/PAGE fractionation and Western blotting.

Animal Care. All procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and these procedures were approved by the Institutional Safety Committee on Recombinant DNA Experiments and the Animal Research Committee of Osaka Bioscience Institute. Mice were housed in a temperature-controlled room with a 12 h light/dark cycle. Fresh water and rodent diet were available at all times.

Quantification of Colocalization. Images were analyzed for colocalization by using the Just Another Colocalization Program (JACoP) plug-in on ImageJ (National Institutes of Health), and statistical data are reported from the Costes randomization-based colocalization module (2). Colocalization analysis with TRPM1-L and mGluR6 puncta was performed essentially as outlined with analysis of both separate and merged channels in outlined areas of the OPL using MetaMorph image analysis.

1. Cayouette S, Lussier MP, Mathieu EL, Bousquet SM, Boulay G (2004) Exocytotic insertion of TRPC6 channel into the plasma membrane upon Gq protein-coupled receptor activation. *J Biol Chem* 279:7241–7246.

2. Bolte S, Cordelières FP (2006) A guided tour into subcellular colocalization analysis in light microscopy. *J Microsc* 224:213–232.

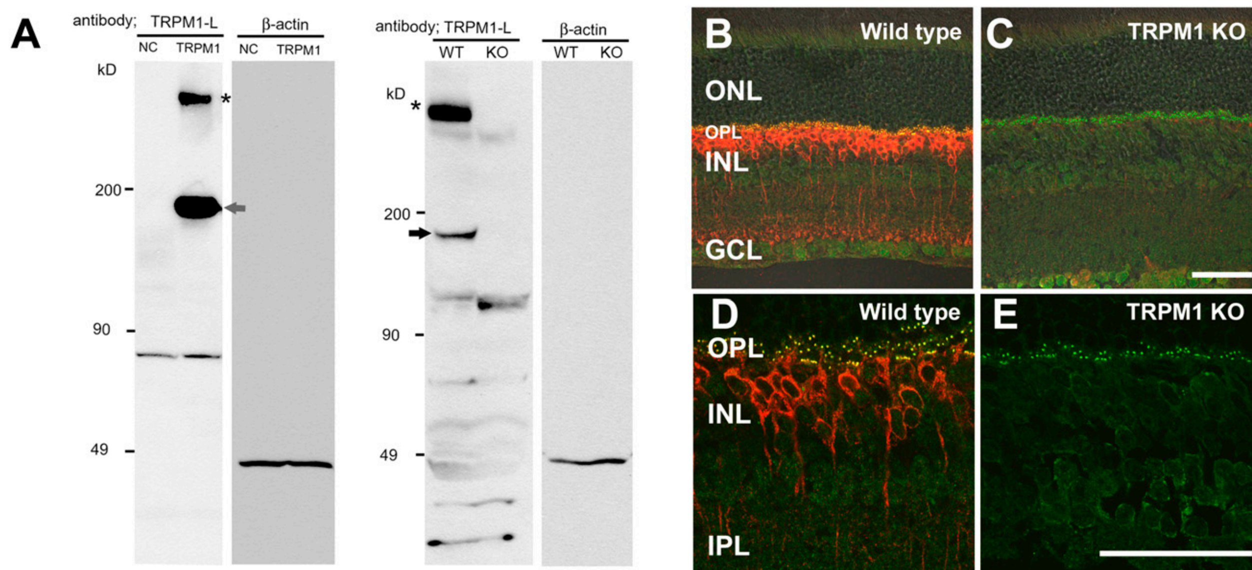

Fig. S1. Characterization of anti-TRPM1-L antibody. (*A*) Anti-TRPM1 antibody recognizes endogenous TRPM1-L protein in wild type retina. Western blot analysis was performed on cell lysate obtained from HEK293 cells transiently transfected with vector plasmid (NC) or *TRPM1-L* cDNA (TRPM1-L) (*Left*), or tissue homogenate from wild-type (WT) and *TRPM1* KO (KO) retina (*Right*). Samples, normalized to protein contents (20 μg for cell lysate, 600 μg for tissue homogenate), were analyzed by SDS-PAGE (2~15% gradient gel), transferred to a nitrocellulose membrane, and immunoblotted with an anti-TRPM1-L or anti-β-actin antibody. Immunoreactive bands of a size of ~180 kDa (arrow) and larger (star), which are presumably anti-TRPM1-L monomer and unresolved dimer, were detected with an anti-TRPM1-L antibody. A 47-kDa band was detected with an anti-β-actin antibody. (*B–E*) TRPM1-L immunohistochemistry on retinal sections of wild-type or *TRPM1* KO mice. The TRPM1-L signal (red) in the OPL and INL of WT mouse retina (*B* and *D*) was essentially undetectable in the $TRPM1^{-/-}$ mouse retina (*C* and *E*). mGluR6 signals (green) were detected in the OPL of WT and $TRPM1^{-/-}$ mouse retinas. Confocal images were taken with (*B* and *C*) or without transmission light (*D* and *E*). (Scale bar: 50 μm.) ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer.

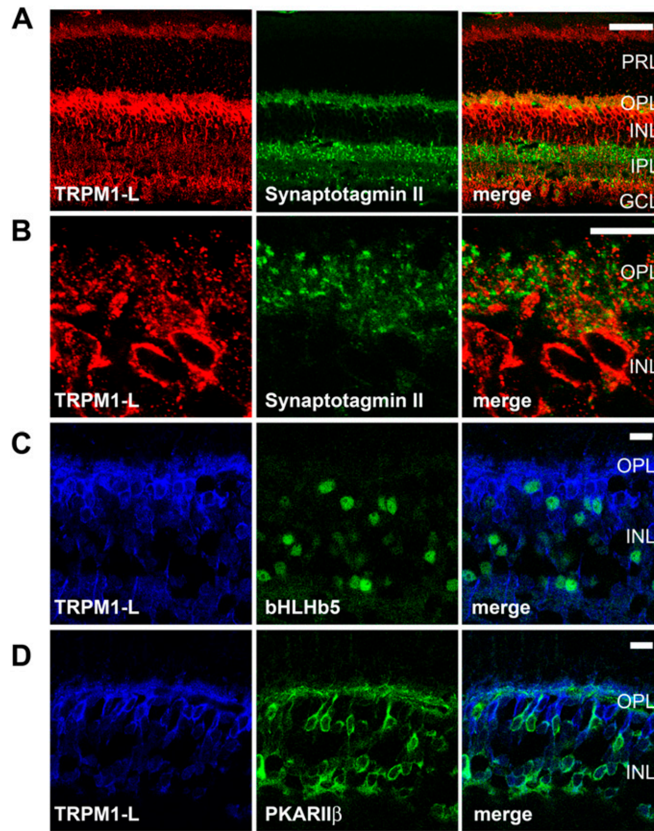

Fig. S2. Confocal images of INLs that were double-labeled with antibody to TRPM1-L and OFF cone bipolar markers. (*A* and *B*) Synaptotagmin II is a marker of OFF cone bipolar and horizontal cells. TRPM1-L signals (red) were not colocalized with synaptotagmin II signals in IPL (*A*) and OPL (*B*). (Scale bars: *A*, 50 μm; *B*, 10 μm.) (*C* and *D*) bHLHb5 and PKARIIβ are markers of type 2 and 3b OFF cone bipolar, and amacrine cells, respectively (green). TRPM1-L signals (blue) were not colocalized with bHLHb5-positive cells (*C*) or with PKARIIβ-positive cells (*D*). (Scale bars: 10 μm.) PRL, photoreceptor layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer.

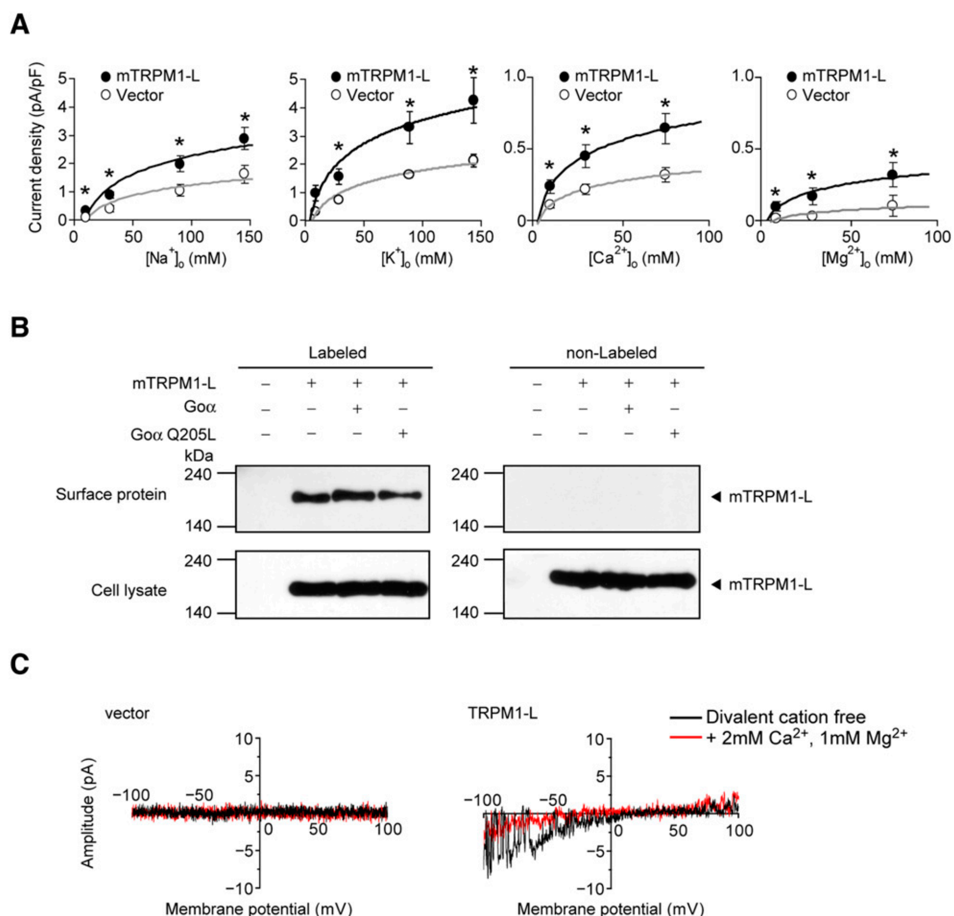

Fig. S3. Functional characterization and surface expression of TRPM1-L. (*A*) Dependence of whole-cell current densities on external Na^+, K^+, Ca^{2+}, and Mg^{2+} in *TRPM1-L*-transfected cells. Current densities were measured in extracellular solutions, which were made by mixing the cation (either Na^+, K^+, Ca^{2+}, or Mg^{2+}) solution and the solution, where cations were totally replaced with $NMDG^+$, at various ratios (V_h is −50 mV). Filled circles, *TRPM1-L*-transfected cells; open circles, vector-transfected cells. Data points are mean ± SEM. *, $P < 0.05$. (*B*) Cell-surface expression of TRPM1-L. CHO cells were transfected with the constructs indicated. The cell lysate prepared after exposure to NHS-SS-biotin was incubated with streptavidin-agarose to collect cell-surface proteins, and then the lysate was analyzed by Western blotting with an anti-TRPM1-L antibody. (*C*) *I–V* relationships obtained from outside-out patches. Voltage ramps were applied from −100 to +100 mV ($V_h = -60$ mV) for 200 ms in either divalent cation-free (black traces) or 2 mM Ca^{2+} and 1 mM Mg^{2+}-containing (red traces) bath solution. CHO cells stably expressing mGluR6 were transfected transiently with *TRPM1-L* and *Go*α, or vector and *Go*α.

Table S1. Results of colocalization test based on the Costes randomization by shuffling *z*-axis confocal images in two channels

Samples	*R* (obs)	*R* (rand)	% [*R* (obs) > *R* (rand)]
TRPM1-L and mGluR6 (*n* = 6)	0.495 ± 0.130	0 ± 0	100
TRPM1-L and Bassoon (*n* = 7)	0.074 ± 0.045	0.295 ± 0.063	0
TRPM1-L and Goα (*n* = 6)	0.413 ± 0.067	0.214 ± 0.109	100
mGluR6 and Goα (*n* = 7)	0.401 ± 0.041	0.029 ± 0.014	100
TRPM1-L and PNA (*n* = 6)	0.334 ± 0.050	0.033 ± 0.019	100

R(obs) is Pearson's coefficient for ch1 vs. ch2. *R* (rand) is Pearson's coefficient for ch1 vs. randomized ch2. Colocalization of TRPM1-L to other signals was statistically quantified by using the JACoP program from six or seven images. Significant colocalizations were observed for TRPM1-L and mGluR6, Goα or PNA with the average Pearson's colocalization coefficient [*R* (obs)], and statistically significant colocalization obtained with the Costes randomization-based colocalization for each individual image [*R* (obs) > *R* (rand)]. Significant colocalizations were observed for mGluR6 and Goα as well. No colocalization was observed for TRPM1-L and Bassoon.