Supporting Information

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SI Methods

Mice. Palm−/−, Palm−/+/, Rdh8−/−, Abca4−/−, Lrat−/−, and Gnat1−/− mice were generated as previously described (1–5), whereas Palm−/−Rdh8−/−, Palm−/−Abca4−/−, Palm−/−Lrat−/−, and Palm−/−Gnat1−/− double-knockout animals were obtained by crossbreeding these single mutant mice. All mice were genotyped by established methods (1–5) and only those with Leu450 in RPE65 were used. Mice were housed in the animal facility at the School of Medicine at Case Western Reserve University, where they were maintained in the dark or under a 12 h light (approximately 10 lx)/12 h dark cycle. Manipulations in the dark were done under dim red light transmitted through a Kodak No. 1 safelight filter (transmittance >560 nm). All animal procedures and experiments were approved by the Case Western Reserve University Institutional Animal Care and Use Committees and conformed to recommendations of both the American Veterinary Medical Association Panel on Euthanasia and the Association of Research for Vision and Ophthalmology.

Ultra–High-Resolution SD-OCT and SLO Imaging. Ultra-high resolution SD-OCT (Bioptigen) and HRAII (Heidelberg Engineering) for SLO were employed for in vivo imaging of mouse retinas. Mice were anesthetized by i.p. injection of a mixture (20 μL/g body weight) containing ketamine (6 mg/mL) and xylazine (0.44 mg/mL) in 10 mM sodium phosphate, pH 7.2, containing 100 mM NaCl. Pupils were dilated with 1% tropicamide. Four pictures acquired in the B-scan mode were used to construct each final averaged SD-OCT image.

Retinoid and A2E Analyses. All experimental procedures related to extraction, derivatization, and separation of retinoids from dissected mouse eyes were carried out as previously described (2). For A2E extraction, two eyes were homogenized in 1 mL of acetonitrile in a glass/glass homogenizer. After evaporation of solvent, extracts were dissolved in 150 μL acetonitrile with 0.1% TFA, and then passed through a Teflon syringe filter (National Scientific Company). Samples (100 μL) were loaded on C18 columns (Phenomenex) and analyzed by normal-phase HPLC with a mobile-phase gradient of acetonitrile:H2O of 100:0 and acetonitrile:H2O of 80:20 with 0.1% TFA for 30 min. Quantification of A2E by HPLC was performed by comparison with known concentrations of pure synthetic A2E prepared as previously reported (2).

Histology. Histological and immunohistochemical procedures employed were well established (2). Anti-rhodopsin 1D4 antibody was a gift from R. S. Molday (Vancouver, BC, Canada). Anti–M/Л opsin and anti–S opsin antibodies were purchased from Millipore, and antimacrophage antibodies CD169, CD11b, and F4/80 were obtained from AbD Serotec. Eyecups for histology were fixed in 2% glutaraldehyde/4% paraformaldehyde and processed for embedding in Epon. Sections were cut at 1 μm and stained with toluidine blue. EM analyses were performed as previously described (2).

ERG. All ERG procedures were performed according to published methods (2, 6). For single-flash recordings, the duration of white light flash stimuli (20 μs to 1 ms) was adjusted to provide a range of illumination intensities from −3.7 to 1.6 log cd s/m². Three to five recordings were obtained at sufficient intervals between flash stimuli (10 s to 1 min) to allow recovery from photobleaching effects.

Mouse Fundus Images. Retinal fundus images were obtained by using a surgical microscope (M651 MSD; Leica) connected to a CCD camera. Aberrant reflection from the cornea was removed by a HOYA HHV Dispo type-6d lens.

Statistical Analyses. Data representing the means ± SD for the results of at least three independent experiments were compared by one-way ANOVA.

Fig. S1. Palmitoylation of rhodopsin and visual retinoid kinetics in mice with different genetic backgrounds. (A) Secondary structure of mouse rhodopsin. Palmitoylated Cys residues are indicated in magenta. Amino acids mutated in human retinitis pigmentosa (RP) are displayed as black circles [autosomal dominant (AD)] or gray circles [autosomal recessive (AR)]. (B) C-terminal sequence of rhodopsin and cone opsins. Palmitoylated Cys residues are highlighted by black in rhodopsin. Cys residues in cone opsins aligning with palmitoylated Cys residues in rhodopsin are indicated in gray. The gray Cys residues probably are not palmitoylated because mouse S-opsin is not palmitoylated (1). (C) Kinetics of all-trans-retinal reduction (Left) and 11-cis-retinal formation (Right) in 6-week-old WT, Palm+/-, Palm−/−, Palm−/−Rdh8−/−, and Palm−/−Abca4−/− mice. Retinoids were quantified by HPLC in eye samples collected at different time points after light exposure (500 cd/m² for 3 min) that bleached approximately 60% of visual pigments. Palmitoylation deficiency did not alter these retinoid kinetics. Bars indicate SD of the means (n > 3).

Fig. S2. Retinoids in eyes of *Palm*−/− and WT mice after bright light illumination. Retinoids were quantified by HPLC (Left) in eyes from 6-week-old *Palm*−/− and WT mice collected immediately after illumination with 10,000 lx of light for the time periods indicated. Representative amounts of retinoids in eyes of *Palm*−/− mice (Upper Right) and WT mice (Lower Right) are shown. Most of the rhodopsin (approximately 80%) was bleached after 5 min illumination and amounts of retinoids were similar in *Palm*−/− and WT mice. 11cRAL, 11-cis-retinal; atRAL, all-trans-retinal; atRE, all-trans-retinyl esters. Bars indicate SD of the means (n > 3).

Fig. S3. Heterozygous *Palm*+/− retinas withstand severe light-induced retinal degeneration better than *Palm*−/− retinas. WT, *Palm*+/−, *Palm*−/−, *Palm*−/−Rdh8−/−, and *Palm*−/−Abca4−/− mice at 6 weeks of age with pupils dilated with 1% tropicamide were illuminated with 10,000 lx light for 60 min and then dark-adapted for 7 d before analyses. (A) Amounts of 11-cis-retinal (indicative of photoreceptor number) were quantified by HPLC in mice with and without illumination. Bars indicate SD of the means (n > 3). (B) Retinal histology of WT, *Palm*+/−, and *Palm*−/− mice illuminated with 10,000 lx light for 60 min. Representative images are shown (n > 3). OS, outer segment; IS, inner segment; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. (Scale bars: 10 μm.) Six-week-old WT, *Palm*+/−, and *Palm*−/− mice with pupils dilated by 1% tropicamide were illuminated with 10,000 lx light for 15 min (C) or 60 min (D) and then dark-adapted for 1 week before analysis. ONL thickness (in μm) is plotted as a function of retinal distance (in μm) away from the optic nerve head (ONH). *Palm*+/− retinas withstood light-induced severe retinal degeneration far better than *Palm*−/− retinas. Error bars indicate SD of the means (n > 3).
Retinal damage after bright light exposure is more severe in \textit{Palm}^{-/-} \textit{Rdh8}^{-/-} than \textit{Palm}^{-/-} mice. (A) Dark adapted 6-week-old \textit{Palm}^{-/-} \textit{Rdh8}^{-/-} and \textit{Palm}^{-/-} mice were illuminated with 10,000 lx light for 30 min, and retinal histology (cryosection with DAPI) was examined after they were kept in the dark for 7 d. Light-induced retinal degeneration was more severe in \textit{Palm}^{-/-} \textit{Rdh8}^{-/-} mice than in \textit{Palm}^{-/-} mice (Fig. 2 in the main text and Fig. S3). A representative image is shown \((n > 6)\). (Scale bar: 10 \(\mu\)m.) (B) \textit{Palm}^{-/-} \textit{Rdh8}^{-/-} mice at 6 months of age maintained under a 12-h light (10 lx)/12-h dark cycle displayed retinal rosette formation (yellow arrows). A representative image is shown \((n > 6)\). (Scale bar: 10 \(\mu\)m.) (C) Dark-adapted 4-week-old WT, \textit{Rdh8}^{-/-}, and \textit{Abca4}^{-/-} mice were illuminated with 10,000 lx of light for 60 min, and retinal histology (cryosection with DAPI) was examined after they were kept in the dark for 7 d. Mild changes in the ONL were observed in \textit{Rdh8}^{-/-} and \textit{Abca4}^{-/-} mice, but \textit{Rdh8}^{-/-} mice exhibited a higher prevalence of degeneration compared to \textit{Abca4}^{-/-} mice. Representative images are shown \((n > 6)\). (Scale bar: 10 \(\mu\)m.) (D) Amounts of all-trans-retinal in WT, \textit{Rdh8}^{-/-}, and \textit{Abca4}^{-/-} mouse retinas were quantified by HPLC after a flash-light exposure that bleached approximately 60\% of rhodopsin. \textit{Rdh8}^{-/-} mouse retinas exhibited delayed clearance of all-trans-retinal.

Bright light illumination causes appearance of yellow and blue deposits in the outer retinas of \textit{Palm}^{-/-} \textit{Abca4}^{-/-}, \textit{Palm}^{-/-} \textit{Rdh8}^{-/-}, and \textit{Palm}^{-/-} mice. (A) Six-week-old \textit{Palm}^{-/-} \textit{Abca4}^{-/-}, \textit{Palm}^{-/-} \textit{Rdh8}^{-/-}, and \textit{Palm}^{-/-} mice were illuminated with 10,000 lx light for periods indicated and the numbers of yellow deposits (Fig. 3A in the main text) 500–800 \(\mu\)m away from the optic nerve head in the inferior retina were counted. Numbers of yellow deposits in all three mouse models were maximally increased 5 min after light exposure. No yellow deposits were observed in WT mice illuminated for 5 min. Bars indicate SD of the means \((n > 6)\). (B) Blue deposits after illumination (Fig. 3B in the main text) stained with all tested dyes, namely DAPI and Hoechst 33342 and 33258 (DNA-binding dyes). These deposits also stained with SYTOX (Invitrogen), a nucleic acid–binding dye.
Fig. S6. Conjugate products of all-trans-retinal are unchanged in the eyes of Palm−/−Abca4−/−, Abca4−/−, and WT mice after bright light illumination. Amounts of A2E, isoA2E, and A2PE in the eyes of illuminated (10,000 lx for 15 min) 6-week-old Palm−/−Abca4−/−, Abca4−/−, and WT mice were quantified by HPLC. Bars indicate SD of the means (n > 6).

Fig. S7. Cone degeneration is detected in retinas of 6-week-old Palm−/−Lrat−/− mice. Six-week-old Palm−/−Lrat−/− mice were maintained in a 12 h light (10 lx)/12 h dark environment since birth. (A) Numbers of cone photoreceptors recognized by PNA in 100-μm-wide sections located 1,000 μm from the optic nerve head were equally decreased (approximately 55%) in Lrat−/−, Palm−/+Lrat−/−, and Palm−/−Lrat−/− mice compared with WT mice. Error bars indicate SD of the means (n > 3). (B) Immunohistochemistry with S cone opsin and M/L cone opsin antibodies failed to reveal opsin expression in 6-week-old Lrat−/−Palm−/− mouse retinas (Left) whereas these antibodies stained cone cells in WT retinas (Right). (C) EM of Palm−/−Lrat−/− and WT retinas from 6-week-old mice shows photoreceptor inner segments (IS) without outer segments (OS) (magenta arrows, Upper Left) in Palm−/−Lrat−/− retinas. Detected OS (green arrow) did not retain their disk membranes (Upper Right). OS (Left) and IS (Right) of WT retinas are shown (Lower). (Scale bars: 3 μm.) (D) Cryosection of a 3-month-old Palm−/−Lrat−/− mouse retina stained with DAPI. The inferior retina lacks photoreceptors (i.e., ONL), whereas two layers of these photoreceptors are still preserved in the superior retina. (Scale bars: 10 μm.)

Fig. S8. Characterization of retinas from Palm−/−Gnat1−/− mice. (A) Immunohistochemistry of Palm−/−Gnat1−/− retinas from mice at 6 weeks and 3 months of age stained with antirhodopsin (1D4) antibody (red), PNA (green), and DAPI (blue). No degeneration was observed. OS, outer segment; IS, inner segment; INL, inner nuclear layer; IPL, inner plexiform layer. (Scale bars: 10 μm.) (B) SD-OCT B-scan images of the same eye are shown 1, 3, and 7 d after illumination of the retinas of 6-week-old Palm−/−Gnat1−/− mice with 10,000 lx light for 30 min. Hazy changes were seen in photoreceptor-related layers on d 1 and d 3 after illumination, similar to those found in Palm−/− mice shown in Fig. 2E in the main text. ONL is mostly gone 7 d after exposure to light. INL, inner nuclear layer. (Scale bars: 10 μm.) (C) When cryosections of the same eye shown in Fig. S8B were stained with DAPI (red), autofluorescent macrophages (green) were evident 7 d after light exposure. INL, inner nuclear layer, IPL, inner plexiform layer. (Scale bar: 10 μm.) (D) SLO retinal images from 6-week-old Palm−/−Gnat1−/− mouse retina stained with DAPI. The inferior retina lacks photoreceptors (i.e., ONL), whereas two layers of these photoreceptors are still preserved in the superior retina. (Scale bar: 10 μm.)
Supplementation with the artificial chromophore, 9-cis-retinal, prevents rod and cone photoreceptor cell death in Palm−/−Lrat−/− mice. Two-week-old Palm−/−Lrat−/− mice were kept in the dark with their mothers since birth and then supplemented with 9-cis-retinyl acetate (150 μg in 50 μL DMSO) given by i.p. injection at 14, 16, and 18 d after birth. Immunohistochemical analyses were performed 21 d after birth. DMSO (50 μL) vehicle-injected mice served as untreated controls. (A) Cryosections of Palm−/−Lrat−/− retinas from 21-d-old mice with and without 9-cis-retinal supplementation were incubated with anti-rhodopsin (1D4), anti- S opsin and anti-M/L opsin antibodies. Retinas from supplemented mice showed preserved rod and cone photoreceptors. OS, outer segment; IS, inner segment; INL, inner nuclear layer; GCL, ganglion cell layer; PR, photoreceptors. (Scale bars: 10 μm.) (B) Counts of photoreceptor nuclei per row in the inferior retina 500 μm from the optic nerve head are indicated. Error bars indicate SD of the means (n > 3). (C) Numbers of cone photoreceptor cells in a 100-μm-wide section 450 to 550 μm from the optic nerve head stained with anti-S and anti-M/L cone opsin antibodies were counted in the inferior (S cones) and superior (M/L cones) retina of 3-week-old Palm−/−Lrat−/− mice. Error bars indicate SD of the means (n > 3). Supplementation with 9-cis-retinal prevented rod and cone photoreceptor cell death.