Supplement Figures

Figure S1. Comparison of mouse retinas with different photoreceptor topologies: WT (both cones and rods), cone−DTA (rod−only) and Nrl−/− (cone−like−only) retinas from mice at 6 weeks of age.

Morphology of the rod− and cone−like−only mice was evaluated by plastic sectioning and immunohistochemistry (A). The morphology of cone−DTA mice was comparable with WT mice whereas alignment of the nuclei was mildly disrupted in Nrl−/− mice. Cryo sections from these mouse models were used for immunohistochemistry (A lower panels). The outer segments (OS) of photoreceptor cells in WT retina were stained with both anti−rhodopsin antibody (1D4: red) and a biomarker for cone sheaths (PNA: green). The OS in cone−DTA and Nrl−/− retinas were stained with 1D4 and PNA, respectively. (B) ONL thicknesses are shown that reflect the photoreceptor population measured at 400 µm intervals away from the optic nerve head (ONH) in the superior and inferior retina using IHC images stained with 4−6−diamidino−2−phenylindole (DAPI: blue). Numbers of photoreceptor cells were similar in WT and cone−DTA retinas, but were 20% less in Nrl−/− as compared with WT retinas. Single−flash ERG responses were obtained by stimulating the retina with linearly−increasing flash stimuli under scotopic and photopic conditions, and representative wave forms from each mouse model (C) and amplitudes of functional b−wave under scotopic and photopic conditions (D) are shown. Under scotopic conditions, cone−DTA mice showed ERG responses with a sensitivity and amplitude comparable to WT, whereas Nrl−/− mice evidenced a more than three log unit higher threshold relative to WT (D left). Under photopic
conditions, ERG responses from cones were obtained in WT and Nrl<sup>−/−</sup> mice whereas no functional response was obtained from cone–DTA mice (D right). The visual chromophore, 11–cis–retinal, was extracted from whole mouse eyes and quantified by normal phase silica HPLC (E). There was no significant difference between the amounts of 11–cis–retinal in WT and cone–DTA mice whereas amounts in Nrl<sup>−/−</sup> mice were less than 15% of those in WT mice. Bars in A, 20 µm. Representative images of plastic section, immunohistochemistry and SD-OCT were obtained in the inferior retina. ONL, outer nuclear layer; OS, outer segment; RPE, retinal pigmented epithelium. Error bars indicate SDs in B, D and E (n> 4).

Figure S2. Cone–like–only mouse retina resists acute light–induced degeneration. Cone–like–only mice were challenged with intense light exposure (10,000 lux of light for 1 h) and then kept in the dark. At day 7 after light exposure, scotopic-ERG recordings showed only mild retinal dysfunction (<15% b–wave response decrease at high–intensity stimulation) in Nrl<sup>−/−</sup>Rdh8<sup>−/−</sup>Abca4<sup>−/−</sup> mice as compared with Nrl<sup>−/−</sup> mice (A). Bars indicate SE of means (n > 3). No significant morphological changes were seen in retinas of Nrl<sup>−/−</sup> mice with deletion of the Abca4 and Rdh8 genes (B). Representative images of SD-OCT were obtained in the superior retina. Bars in B, 40 µm.
Figure S3. All-trans-retinal clearance is affected in rods rather than cones by Abca4 and Rdh8

genes. Accumulation of toxic levels of the retinoid cycle by-product, all-trans-retinal, in the mouse eyes (6 weeks of age) after light exposure was quantified with normal phase HPLC. More than 3-fold higher amounts of all-trans-retinal was detected in eyes of cone-DTA Rdh8−/+Abca4−/+ mice as compared with eyes from cone-DTA mice, whereas amounts in Nrl−/− mouse eyes were less than 5% of those in cone-DTA Rdh8−/+Abca4−/+ mouse eyes and the latter were not significantly affected by deletion of the Abca4 and Rdh8 genes. Bars indicate SDs of the means (n > 3).

Figure S4. Fundus autofluorescence (AF) in vivo increases significantly in rod- and cone-like-only mouse retinas upon deletion of Abca4 and Rdh8 genes and this increase correlated with A2E accumulation. Fundus images were obtained from mice and the intensity of AF was evaluated with SLO. Representative fundus images from each mouse model at 6 weeks and 6 months of age are shown (upper panels). AF signals were increased in the entire fundus of both cone-DTA Rdh8−/+Abca4−/+ and Rdh8−/+Abca4−/− mice at 6 months of age whereas retinas of Nrl−/−Rdh8−/+Abca4−/+ mice displayed only mild increase with a granular pattern (A). Moreover, the mean gray value of AF levels in SLO images of fundus was increased more in cone-DTA mice than in Nrl−/− mice by deletion of Abca4 and Rdh8 genes at 6 months of age compared with those at 6 weeks of age (B). A2E levels, quantified by reverse phase
HPLC with a C18 column, were significantly increased due to deletion of *Abca4* and *Rdh8* genes, a result corresponding to the increase in fundus AF (C). Error bars indicate SDs of the means (n >3).

**Figure S1**

A. WT, cone-DTA, and Nrt+/- retinal sections showing photoreceptor layers.

B. Graph showing ONL thickness across the ONH.

C. Scotopic and photopic responses for WT, cone-DTA, and Nrt+/-.

D. Scotopic and photopic b-wave amplitude for WT, cone-DTA, and Nrt+/-.

E. Comparison of 11-cis-retinal levels in WT, cone-DTA, and Nrt+/-.
Figure S2

A

![Graph showing b-Wave amplitude vs. Intensity (log cd·s/m²)]

- `Nrf+` (pre-bleach)
- `Nrf+` (after bleach)
- `Nrf+ Rdh8+ Abca4+` (after bleach)

B

![Images showing ONL (Outer Nuclear Layer) in different conditions]

- `Rdh8+ Abca4+`
- `Rdh8+ Abca4+`
Figure S3

![Graph showing all-trans-retinal (pmol/eye) for different genotypes: Rdh8+/+Abca4++, Rdh8-/-Abca4++, Rdh8-/Abca4++, and Rdh8-/-Abca4-/- with cone-DTA.](image)
Figure S4

A

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B

![Graph](image9.png)

C

![Graph](image10.png)