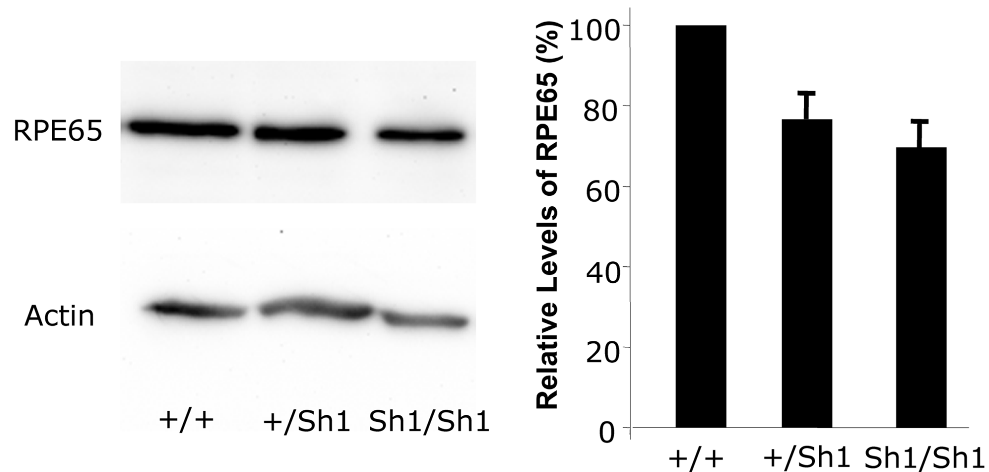
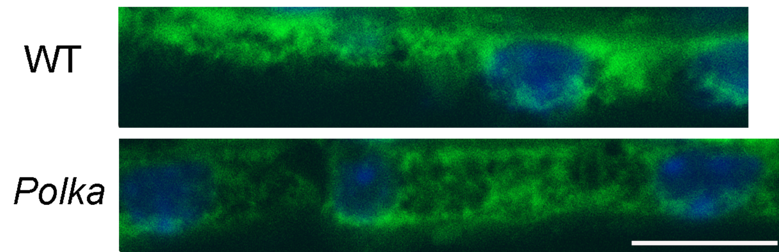
The Usher 1B protein, MYO7A, is required for normal localization and function of the visual retinoid cycle enzyme, RPE65

Lopes et al.

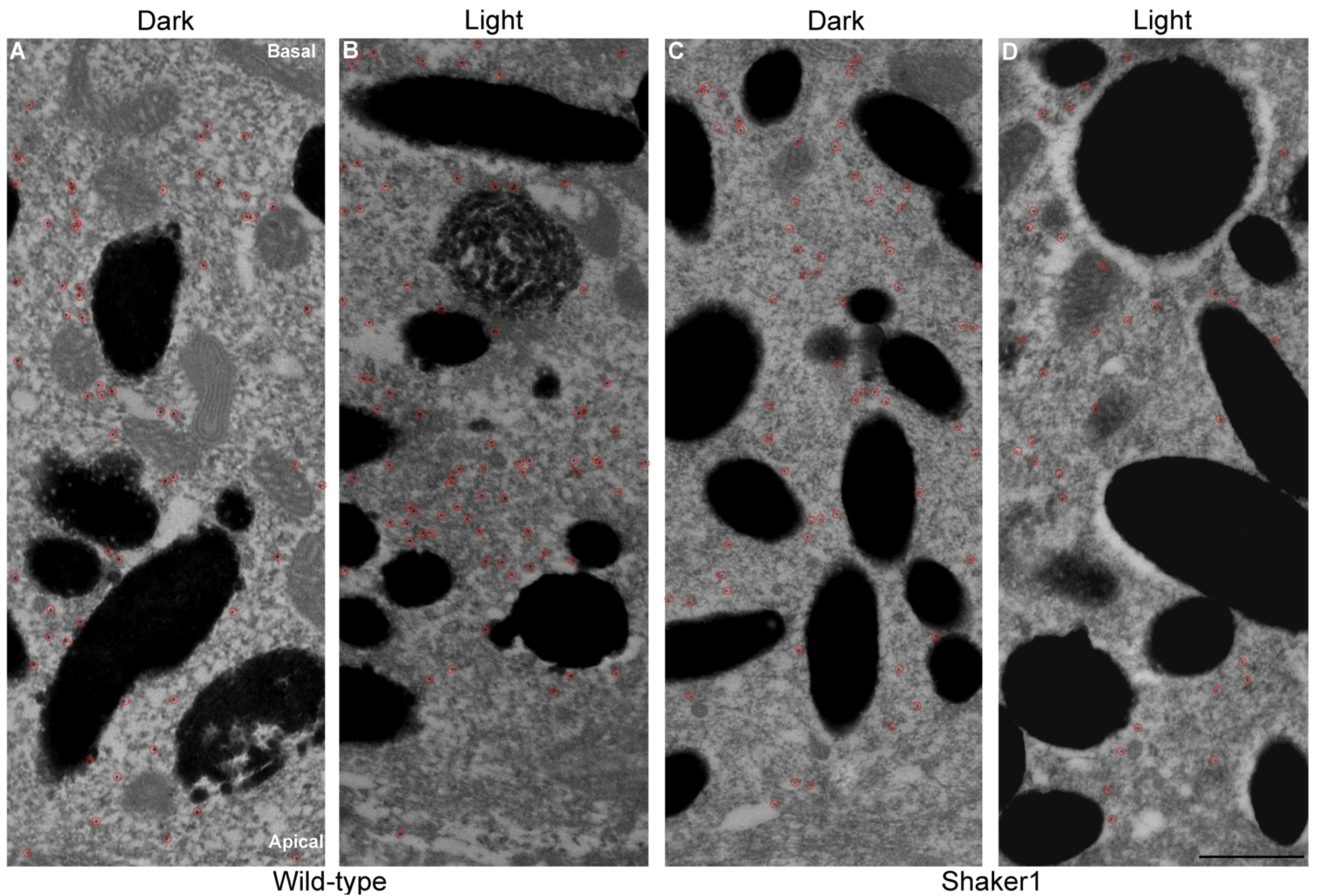
Supplementary Figures and Legends



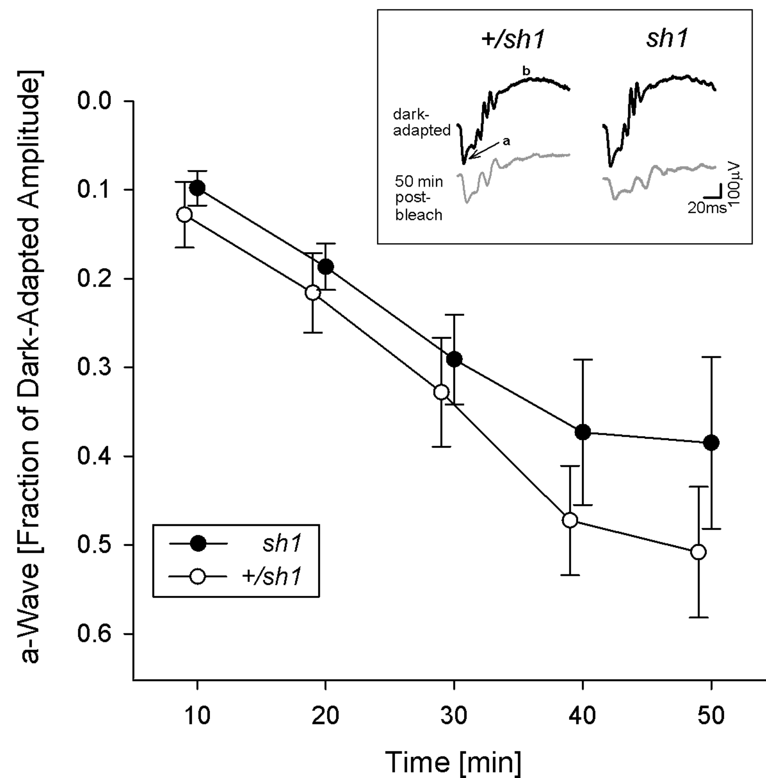
Supplementary Fig. 1. Western blot analysis of RPE65 levels in eyecups of shaker1, *+/sh1* and WT mice. Example of a western blot (left). Actin was used as a loading control. Bar graph (right) showing densitometric measurements (mean ± SEM). All mice were homozygous for RPE65 Leu450.



Supplementary Fig. 2. RPE65 immunofluorescence. Frozen sections (8 μm) of the RPE from WT and *polka* (MYO7A C-terminal mutant) light-adapted mice, labeled with anti-RPE65 (green) and DAPI (blue). DAPI staining indicates the RPE cell nuclei. Note that the immunofluorescence is more constricted in the WT RPE; in the mutant, the apical limit of the RPE65 labeling is below the nuclei. Scale bar = 10 μm.



Supplementary Fig. 3. Immunoelectron microscopy of RPE65. Images of RPE cells, including the central region and some of the basal (upper) and apical (lower) regions. WT (*A* and *B*) and shaker1 (*C* and *D*) mice were dark-adapted overnight for 16 hrs and then kept in the dark (*A* and *C*) or exposed to room lights (*B* and *D*) for 2 hrs. Each gold particle has been encircled in red to help identify it at this relatively low magnification. RPE65 is fairly evenly distributed throughout the RPE, except in WT RPE after light exposure; in this case, there is a greater concentration in the central region where there is extensive smooth endoplasmic reticulum. Ultrathin sections were stained with anti-RPE65 and secondary 12-nm gold conjugated goat anti-rabbit antibody. Scale bar = 500 nm.



Supplementary Fig. 4. Recovery of ERG photoreceptor function in shaker1 mice after exposure to adapting light. Change in mean maximum photoresponse (a-wave) amplitude as a function of time after an adapting light in shaker1 mice (*sh1/sh1*; n= 6; unfilled symbols) compared with heterozygous controls (+*/sh1*; n=7; filled symbols). Values were normalized by the maximum a-wave amplitude under fully dark-adapted conditions; error bars represent ± SEM. Inset: representative ERGs from shaker1 and *+/sh1* animals recorded 50 min after the adapting light (gray traces) compared with dark-adapted ERGs (black traces) recorded before the bleaching light exposure. ERG a- and b-wave, representing responses from outer and inner retina, respectively, are denoted with labels next to the waveform of the *+/sh1* mouse.