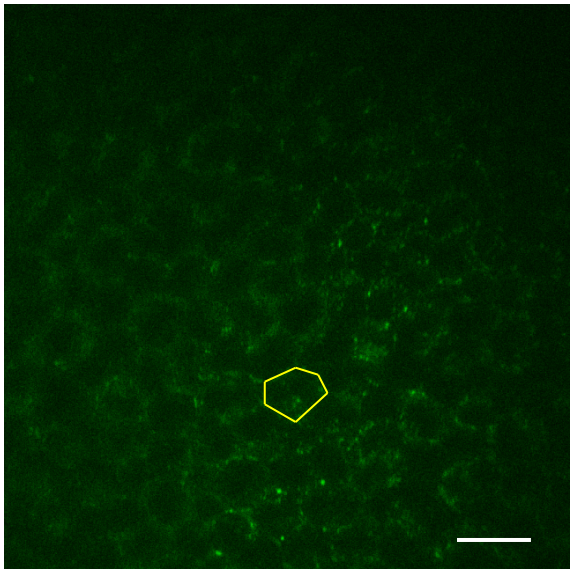
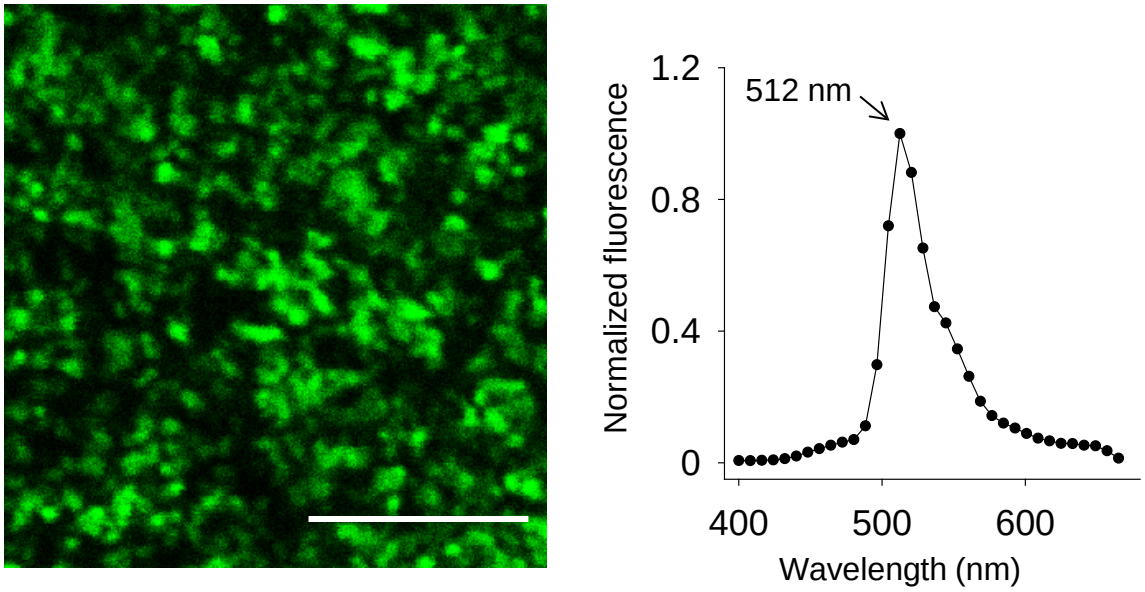
Supplementary Information

Noninvasive two–photon fluorescence microscopy imaging of mouse retina and RPE through the pupil of the eye

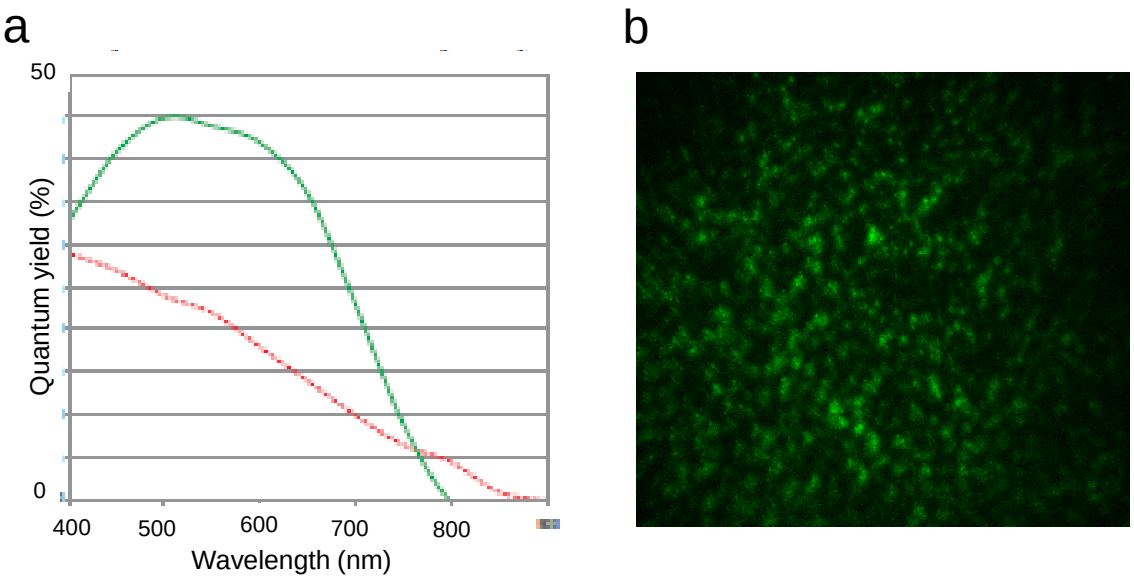
Grazyna Palczewska, Zhiqian Dong, Marcin Golczak, Jennifer J. Hunter, David R. Williams, Nathan S. Alexander, and Krzysztof Palczewski



Supplementary Figure 1. Through the pupil TPM image of the RPE in a Ret-NH_2-pretreated WT mouse. The cell membranes of one RPE cell are outlined in yellow. Black nuclei are visible. Scale bar represents 50 μm.

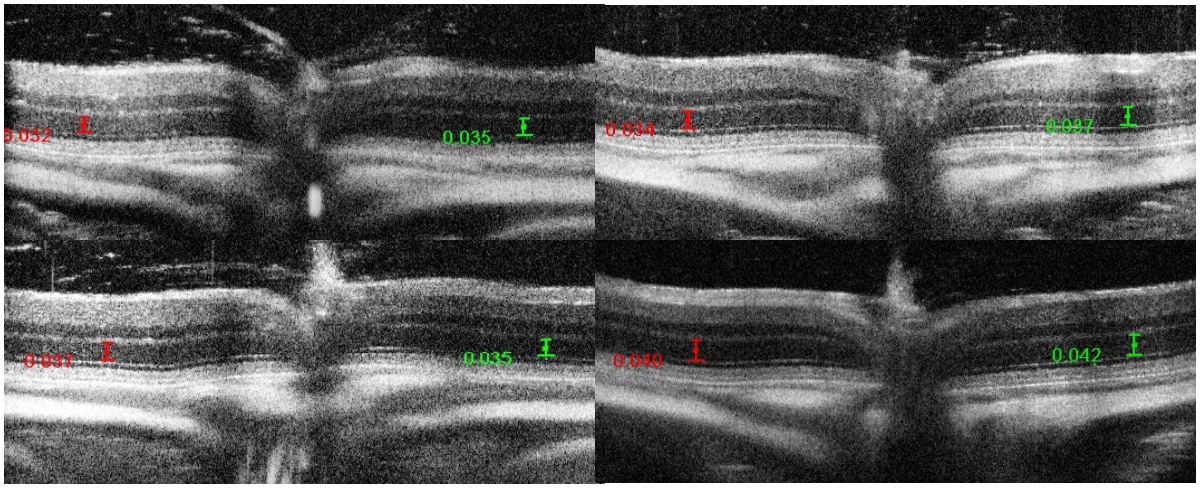


Supplementary Figure 2. TPM imaging through the sclera of *hrhoG/hrhoG* mice. (**a**) Image of photoreceptors in an *hrhoG/hrhoG* mouse. Scale bar represents 30 µm. (**b**) Emission spectrum has a maximum at 512 nm.

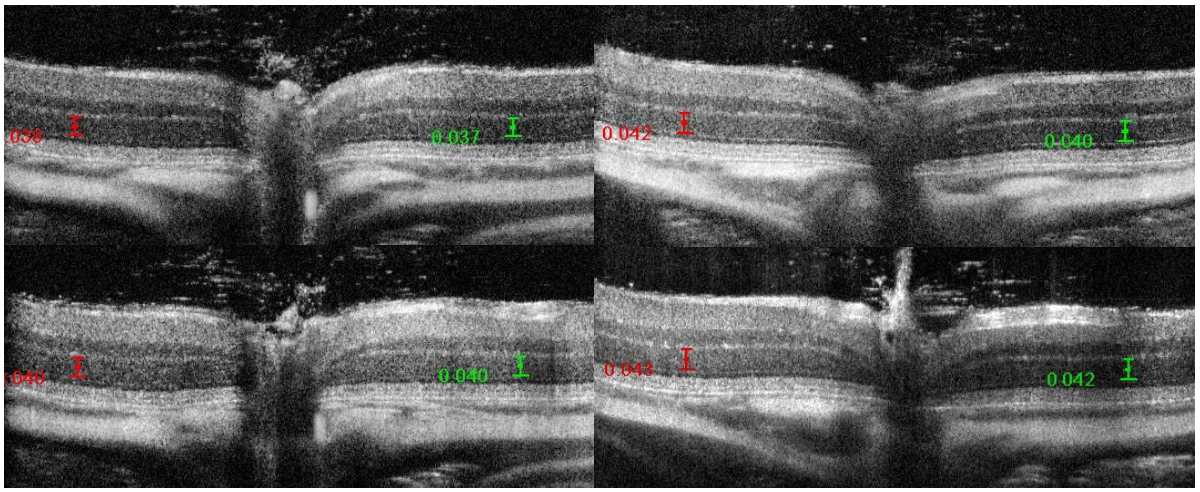


Supplementary Figure 3. New HYD Leica detector. (**a**) Quantum yield is shown as a function of wavelength for Hamamatsu R6357 (red) and HYD (green), courtesy of Leica. (**b**) TPM image of RPE obtained through the pupil of *Rpe65*$^{-/-}$ mouse using 6.3 mW of laser power.

a



b



Supplementary Figure 4. *In vivo* imaging of mouse retinas by OCT. (**a**) Images of retina in *Rpe65*$^{-/-}$ mice that were not imaged by TPM. (**b**) Images of retina in *Rpe65*$^{-/-}$ mice 4 weeks after *in vivo* TPM imaging. The ONL thicknesses are indicated in each image either in red or green font. No structural changes were observed.