Supplemental Figure Legends:

Fig. S1: **The RPE65 substrate cavity encloses the crystal residual electron density.** The residual density seen in the RPE65 crystal structure (see also figure 5) is overlaid on the RPE65 central cavity modeled by the MarkUs cavity server (see also Fig. 3). The grey mesh represents the cavity and the dark green mesh represents the σ-A weighted difference density (contoured at 3σ) as observed in the 3KVC crystal structure. F418 (dark grey stick), 11-*cis* retinol (blue), palmitate (light grey), and iron (orange) are also depicted.

Fig. S2: **Elevated 13-*cis* retinol production by Y338A mutant is observed compared to thermal production of 13-*cis* retinol in presence of or absence of wildtype RPE65.** In Y338A-transfected cells, 11-cis retinol production is concomitantly sharply reduced. 13-*cis* retinol production by Y338A is comparable to the summed amounts for 11- and 13-*cis* of wildtype RPE65. HEK293-F cells were transfected with wildtype RPE65 pVitro2 vector, Y338A pVitro2, or mock-transfected with vector, and all-*trans* retinol added to a final concentration of 2.5 μM 24 hours later (see Experimental Procedures). Cultures were harvested at 0.25, 0.5, 1.0, 2.0, 4.0 and 8.0 hours post-retinol addition and analyzed by normal phase HPLC (see Experimental Procedures) for *cis* isomer conversion; $n \geq 3$ for each time point.