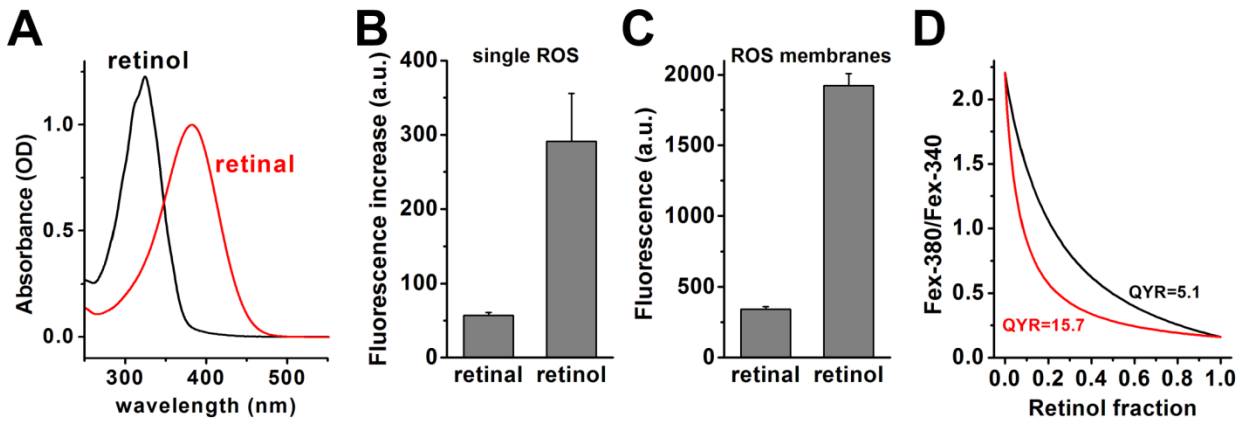
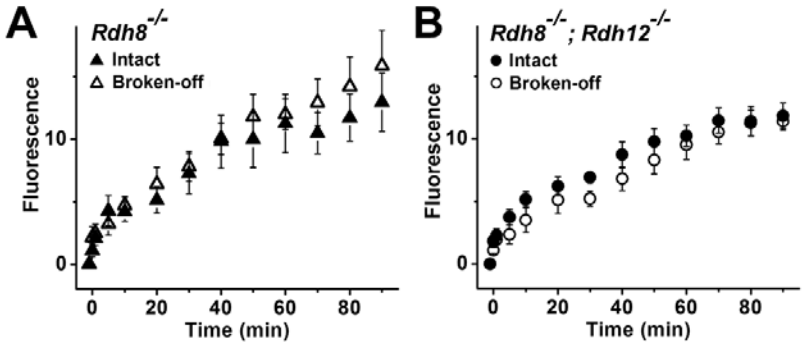
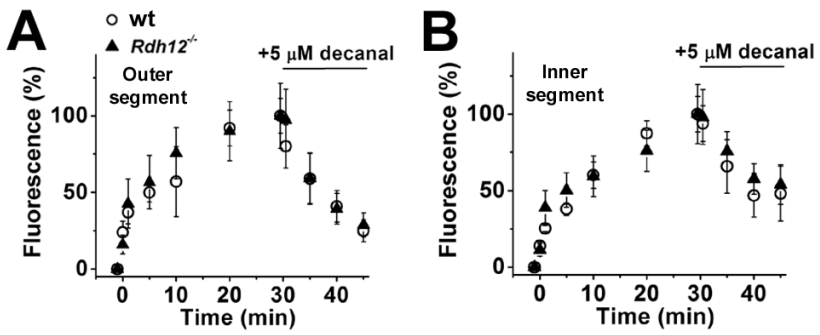


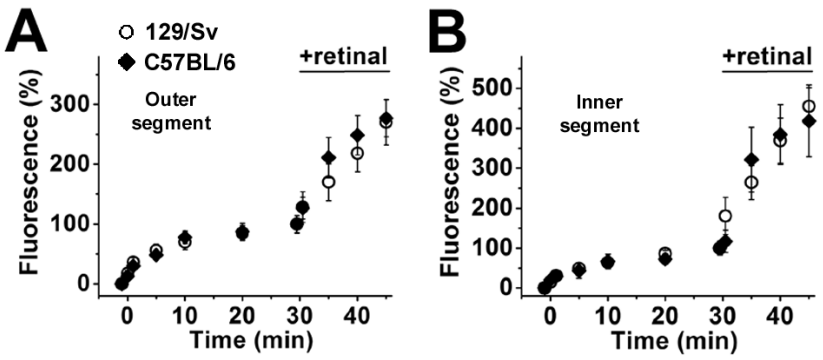
Supplemental Figure 1: Dependence of the excitation properties of the fluorescence of a retinal/retinol mixture on the fraction of retinol present. (A) Absorption spectra of equimolar amounts of retinol and retinal in ethanol. (B) Fluorescence increases in mouse broken off rod outer segments loaded with retinal (n = 8 cells) or retinol (n= 9 cells) for 10 min. Excitation 340 nm. Experiments at 37 ^{0}C. (C) Fluorescence of purified dark-adapted bovine rod outer segment membranes, containing 60 μM rhodopsin, with 0.8 mM retinal (n=3) or retinol (n =3) added. Excitation 340 nm. Experiments at 37 ^{0}C. (D) Calculated dependence of the ratio Fex-380/Fex-340 of the fluorescence of a retinal/retinol mixture on retinol fraction. The dependence has been calculated from Equation 4a for the two different values, 5.1 and 15.7, of the quantum yield ratio QYR = Q(ROL)/Q(RAL), of retinol over that of retinal. Fex-380 and Fex-340 are the fluorescence intensities excited by 380 and 340 nm light respectively.



Supplemental Figure 2: The increases in fluorescence after bleaching in the outer segments of rod photoreceptors that lack Rdh8 are the same in the presence and absence of the cell inner segment. (A) Fluorescence (excitation 360 nm; emission >420 nm) increases after bleaching in the outer segments of intact rods (from Figure 2; ▲, n = 12) and broken-off rod outer segments (△, n = 9) from *Rdh8*$^{-/-}$ mice. (B) Fluorescence increases after bleaching in the outer segments of intact rods (from Figure 2; ●, n = 14) and broken-off rod outer segments (○, n = 8) from *Rdh8*$^{-/-}$*;Rdh12*$^{-/-}$ mice. Error bars, SE. All experiments at 37 ^{0}C.



Supplemental Figure 3: Control experiments showing that changes in rod outer and inner segment fluorescence after addition of decanal are the same in wild type and Rdh12-deficient mice. (A) Fluorescence (excitation 360 nm; emission >420 nm) change in the outer segment of rod photoreceptors from 129/Sv (○, n = 5) and Rdh12-deficient (▲, n = 7) mice, at different times after bleaching. Decanal (5 μM) was added at 30 min after bleaching. (B) Fluorescence in the inner segment of the cells shown in (A). Fluorescence values have been normalized to that immediately before the addition of decanal. Error bars, SE. All experiments at 37 ^{0}C.



Supplemental Figure 4: The rod outer and inner segments of 129/Sv and C57BL/6 wild-type mice have similar capacities for reducing exogenously added retinal. (A) Fluorescence (excitation 360 nm; emission >420 nm) change in the outer segment of rod photoreceptors from 129/Sv (from Figure 6; ○, n = 7) and C57BL/6 (◆, n = 6) mice, at different times after bleaching. Retinal (5 μM) was added at 30 min after bleaching. (B) Fluorescence in the inner segment of the cells shown in (A). Fluorescence values have been normalized to that immediately before the addition of retinal. Error bars, SE. All experiments at 37 ^{0}C.

Supplemental Table 1: Fraction of retinoid in the form of retinol calculated from the fluorescence data of Figure 5. Retinol fractions are shown for the two different values of the ratio of the fluorescence quantum yields of retinol and retinal obtained from single mouse ROS and purified bovine ROS membranes (Supplemental Fig. 1). Retinol fractions were calculated from Equation 4b.

Quantum Yield Ratio Q(ROL) / Q(RAL) = 5.1

	Outer Segment			
	wildtype	***Rdh12*$^{-/-}$**	***Rdh8*$^{-/-}$**	***Rdh8*$^{-/-}$*;Rdh12*$^{-/-}$**
Intact	> 0.80	0.78	0.13	0.11
5 µM RAL	0.75	0.77	0.12	0.01
50 µM RAL	0.14	0.01	0.03	0.00

	Inner segment			
	wildtype	***Rdh12*$^{-/-}$**	***Rdh8*$^{-/-}$**	***Rdh8*$^{-/-}$*;Rdh12*$^{-/-}$**
Intact	0.75	0.40	0.25	0.01
5 µM RAL	0.84	0.04	0.25	0.04
50 µM RAL	0.32	0.00	0.06	0.00

Quantum Yield Ratio Q(ROL) / Q(RAL) = 15.7

	Outer segment			
	wildtype	***Rdh12*$^{-/-}$**	***Rdh8*$^{-/-}$**	***Rdh8*$^{-/-}$*;Rdh12*$^{-/-}$**
Intact	> 0.80	0.54	0.05	0.04
5 µM RAL	0.50	0.53	0.04	0.00
50 µM RAL	0.05	0.00	0.01	0.00

	Inner segment			
	wildtype	***Rdh12*$^{-/-}$**	***Rdh8*$^{-/-}$**	***Rdh8*$^{-/-}$*;Rdh12*$^{-/-}$**
Intact	0.50	0.18	0.10	0.00
5 µM RAL	0.63	0.01	0.10	0.01
50 µM RAL	0.13	0.00	0.02	0.00