SUPPLEMENTAL MATERIAL

Ectopic expression of cone-specific G-protein-coupled receptor kinase GRK7 in zebrafish rods leads to lower photosensitivity and altered responses

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Containing:

Supplemental Figures S1–S5
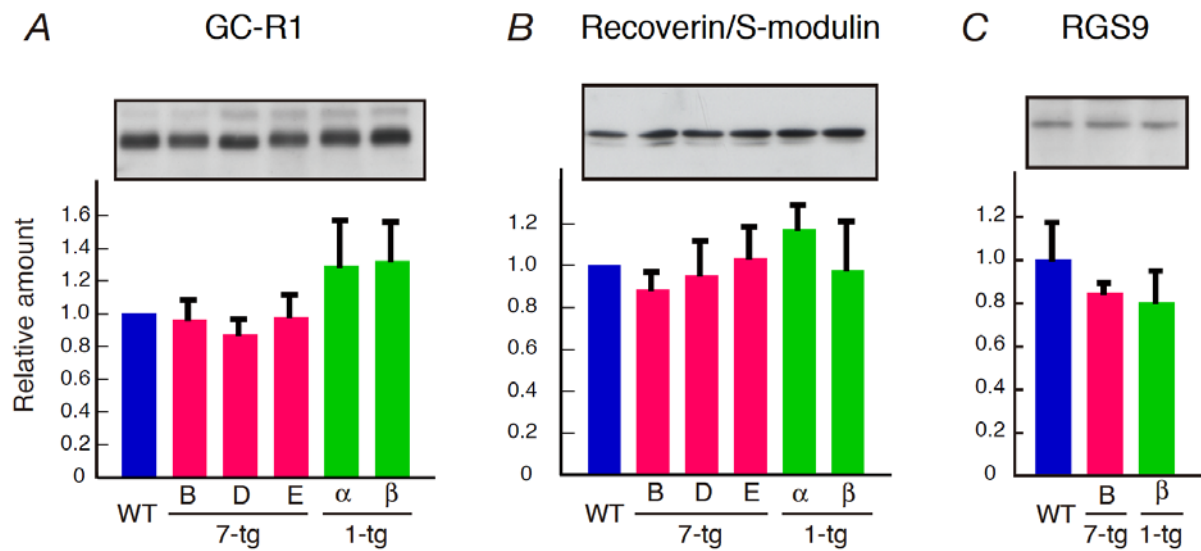
and

Captions for Movies 1 and 2

Figure S1. Expression levels of phototransduction proteins in the eyes (or the retinas) of WT, GRK7-tg and GRK1-tg zebrafish. ***A,*** rod guanylate cyclase (GC-R1) in the eyes; ***B,*** Recoverin/S-modulin in the eyes; ***C,*** RGS9 in the retinas. Typical immunoblot images from wild-type and transgenic zebrafish are shown (top panels). Protein amounts of the tissue homogenates loaded on the gels were 12.8 µg (***A***), 6.4 µg (***B***) and 8.0 µg (***C***). The band densities were quantified to estimate the protein expression levels, and the averages from three to five independent experiments are shown with SEM after normalized to those from wild-type (bottom panels).
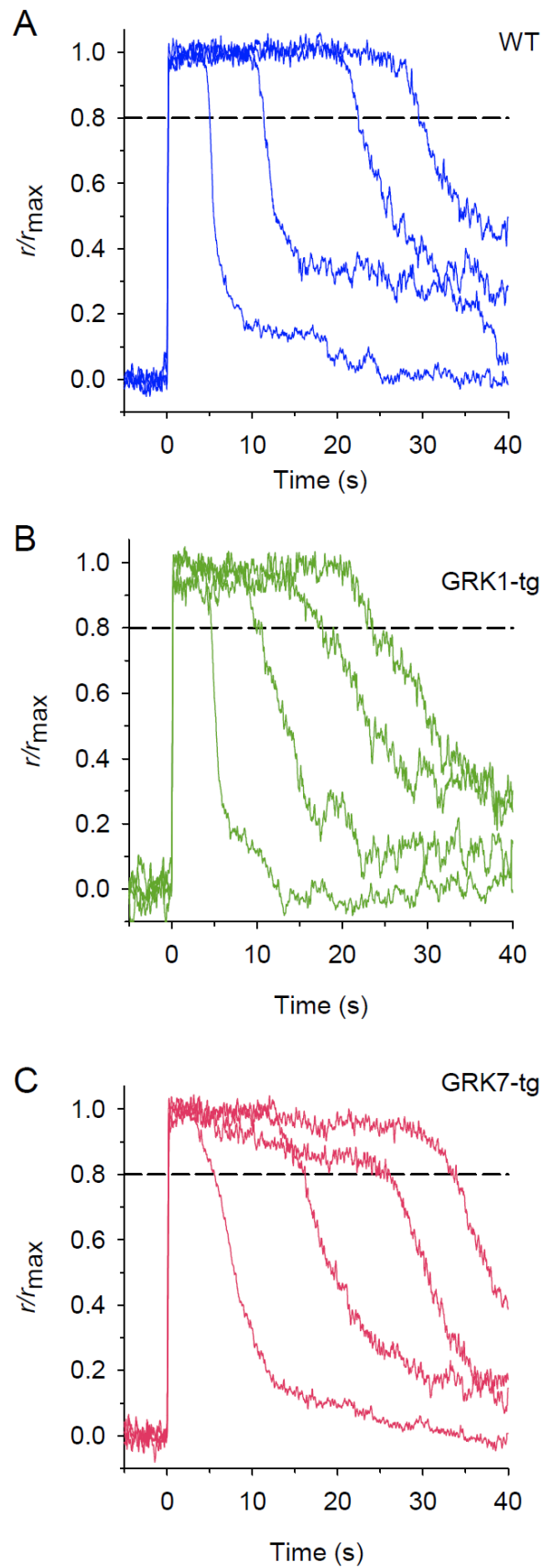
Figure S2. Responses to intense flashes from a representative rod for each genotype; these rods are the same as in Figure 3. *A*, WT; *B*, GRK1-tg; *C*, GRK7-tg. Flash intensities were: 1,800, 18,000, 70,000, and 143,000 photons μm^{-2}.
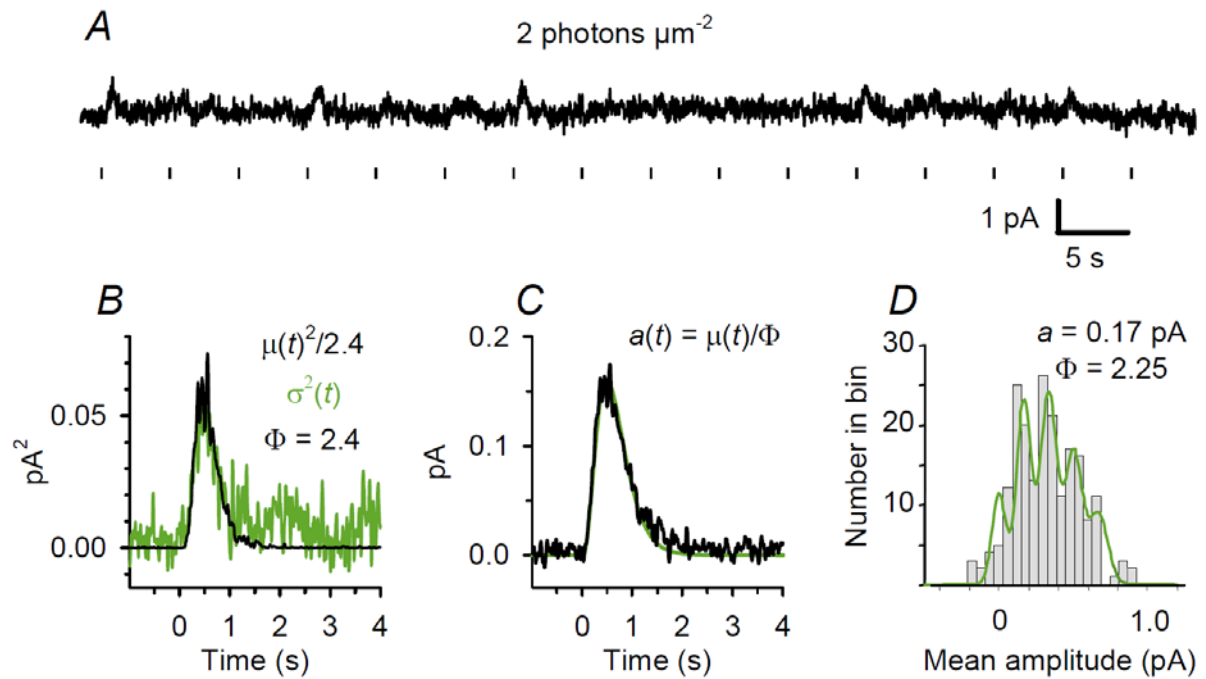
Figure S3. Analysis of quantal responses in a GRK1-tg rod. *A*, Set of 16 consecutive responses from a GRK1-tg rod for flashes of fixed dim intensity (~2 photons μm^{-2}) delivered at 5 s intervals, as indicated by vertical markers below the trace. *B–D*, Variance analysis and histogram analysis on 199 responses of the kind illustrated in *A*. *B*, Ensemble variance, $\sigma^2(t)$ (green trace), together with a scaled version of the square of the mean response, $\mu(t)^2$ (black trace); with a scaling factor of 1/2.4 the two traces coincided closely, yielding an estimate for the number of photoisomerizations as $\Phi = 2.4$; see eqn (4a). *C*, Estimated quantal response, $a(t)$, obtained by dividing the mean response by the estimate for Φ; see eqn (4b). The green curve plots the Poisson kinetics of eqn (3), with amplitude $r_{peak} = 0.16$ pA, $t_{peak} = 0.5$ s, and $n = 4$. *D*, Amplitude histogram for the 199 responses, measured over a window 0.5 s wide centered at t_{peak}, and using a bin width of 0.05 pA. The green curve plots the sum of Poisson-weighted Gaussian components, using parameters: $a = 0.17$ pA; $\Phi = 2.25$; $\sigma_0 = 0.045$ pA; $\sigma_1 = 0.02$ pA.
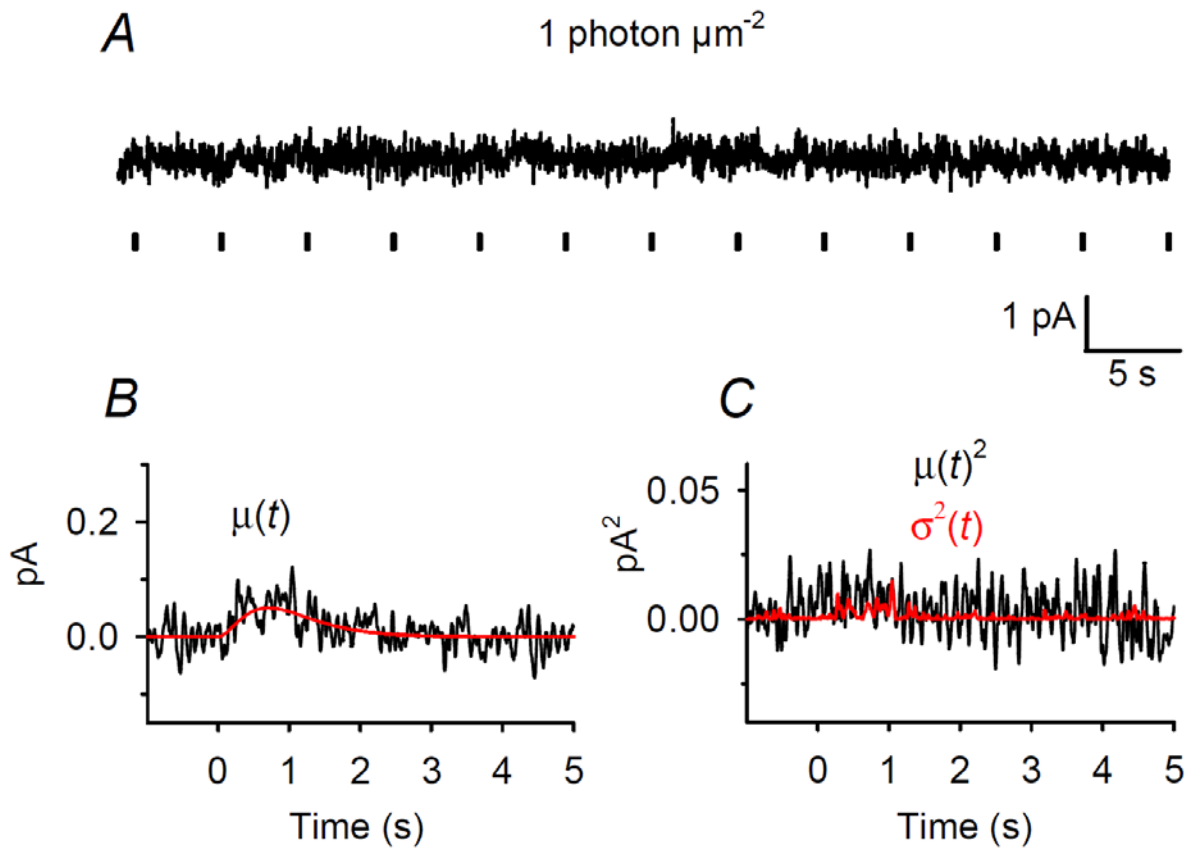
Figure S4. Preliminary analysis of quantal responses in a GRK7-tg zebrafish rod tested with 100 trials at a low intensity of ~1 photon μm^{-2}. **A**, Sample consecutive responses. **B**, Ensemble mean. The red curve plots the Poisson kinetics of eqn (3), with amplitude r_{peak} = 0.05 pA, t_{peak} = 0.71 s, and n = 4. **C**, Ensemble variance, $\sigma^2(t)$ (red trace), together with the square of the mean response, $\mu(t)^2$ (black trace).
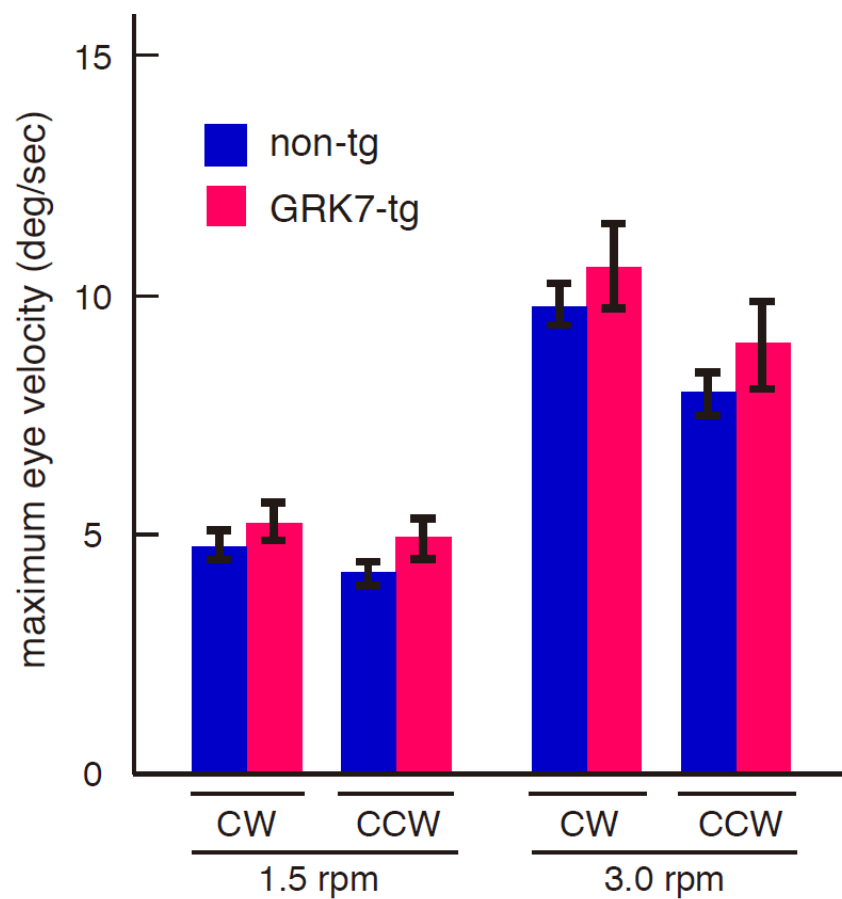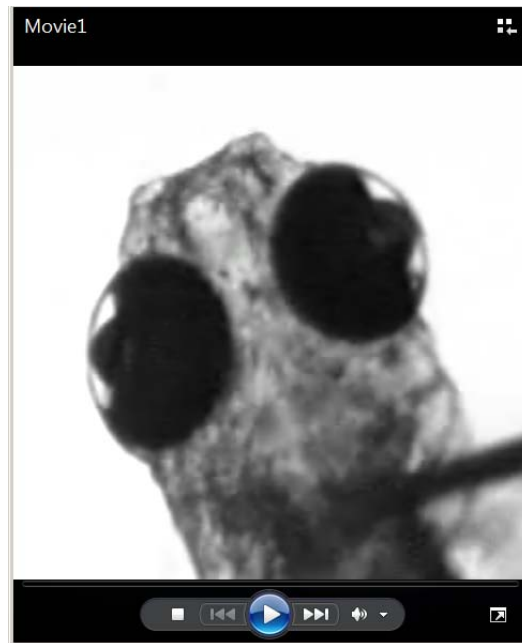
Figure S5. The maximum velocity of eye movements in rod-mediated OKR. The mean eye velocity was calculated for each intensity and the highest velocity among all the conditions was defined as the maximum velocity for each larva. Averages with SEM from 16 GRK7B-tg fish and 19 non-tg fish are shown.

Movie captions and screenshots

Movie 1. Compressed version (10 frames s^{-1}) of original OKR movie (at 29.7 frames s^{-1}) used for the calculation of eye orientation and eye velocity in the right-hand panels (3 rpm stimulus) of Figure 9*A*. This movie is for the upper row of the two eye angle panels, obtained at the dimmer level of -2.0 log relative light intensity, which elicited clear optokinetic responses.
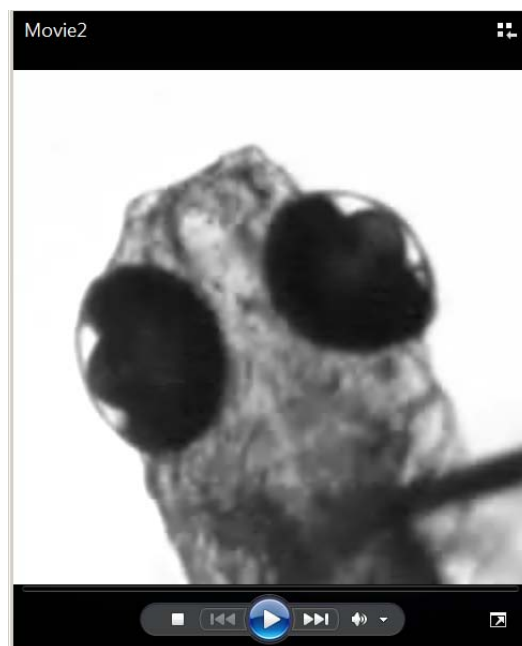
Movie 2. OKR movie, similar to Movie 1, except at the brighter level of -0.9 log relative light intensity (i.e. lower row of the two eye angle panels in Figure 9*A* right). No optokinetic response was apparent; the slight slow drift between saccades was not correlated with drum rotation.