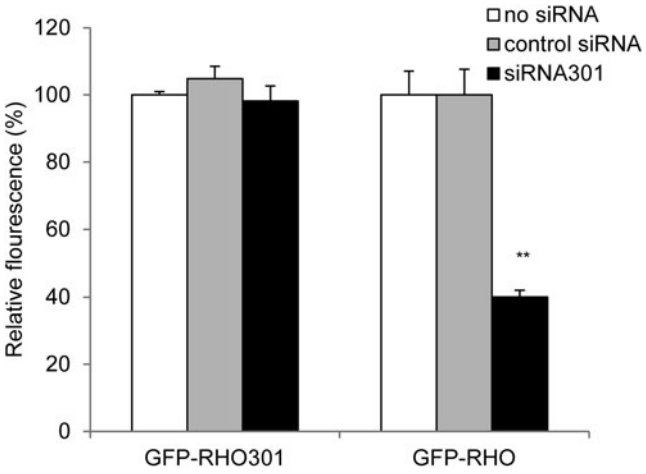
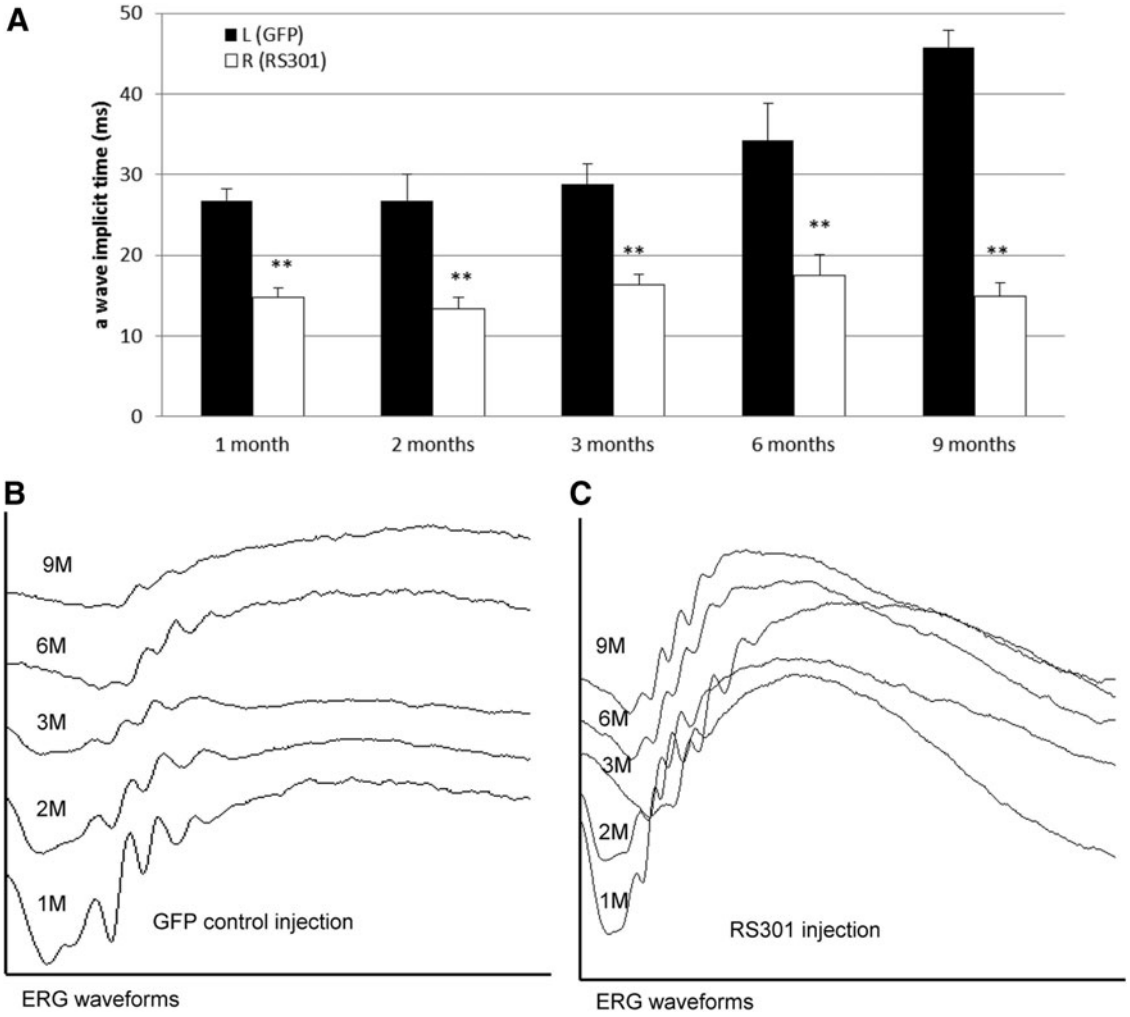
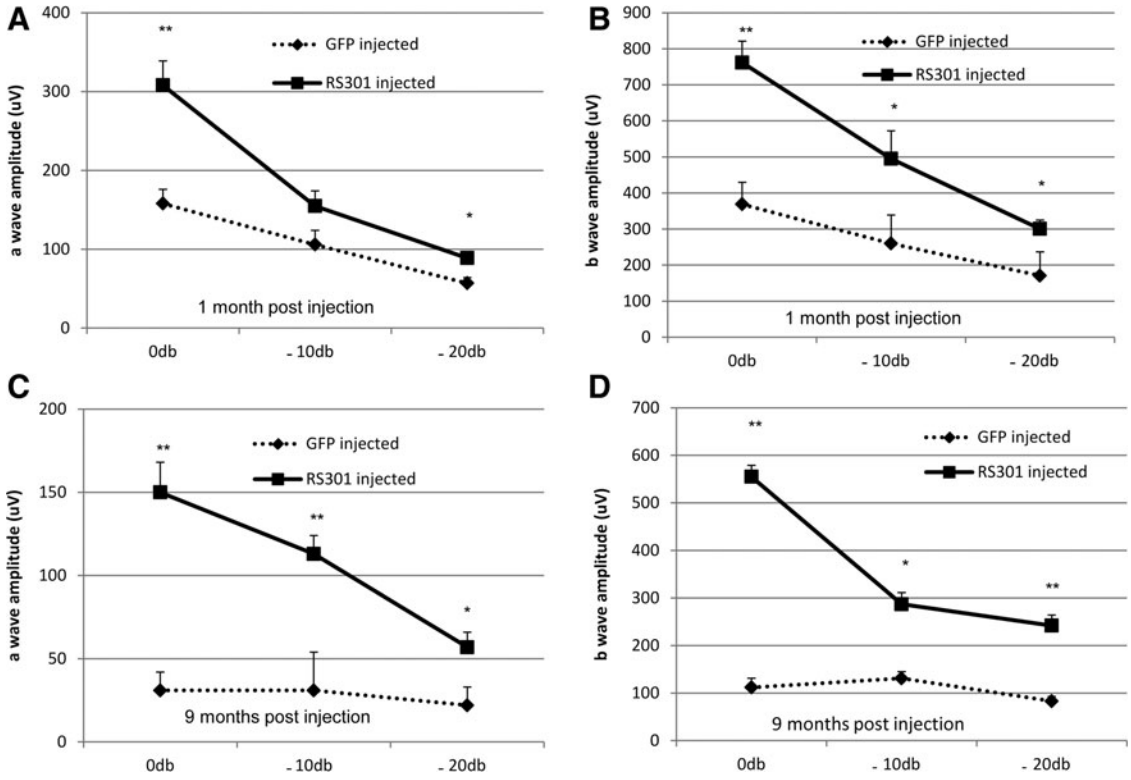
# Supplementary Data



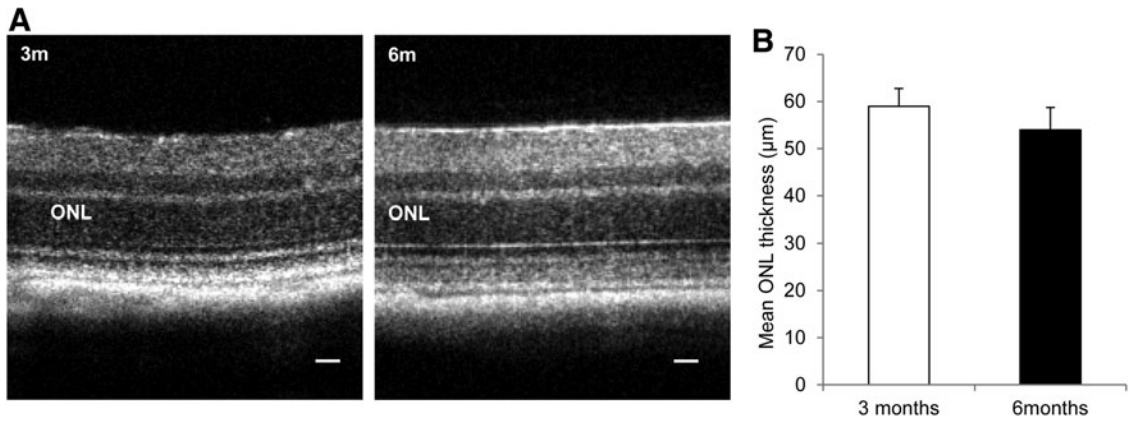

**SUPPLEMENTARY FIG. S1.** GFP fluorescence detected by flow cytometry in HEK293 cells cotransfected with plasmids expressing fused *GFP–RHO* or *GFP–RHO301* and siRNAs. Relative fluorescence of GFP from *GFP–RHO* or *GFP–RHO301* was detected in various groups (white bar, no siRNA group; gray bar, control siRNA group; black bar, siRNA301 group) (each bar, $n = 4$) (siRNA301 group compared with control siRNA group or no siRNA group; $^{**}p < 0.005$ in *GFP–RHO* cotransfection).



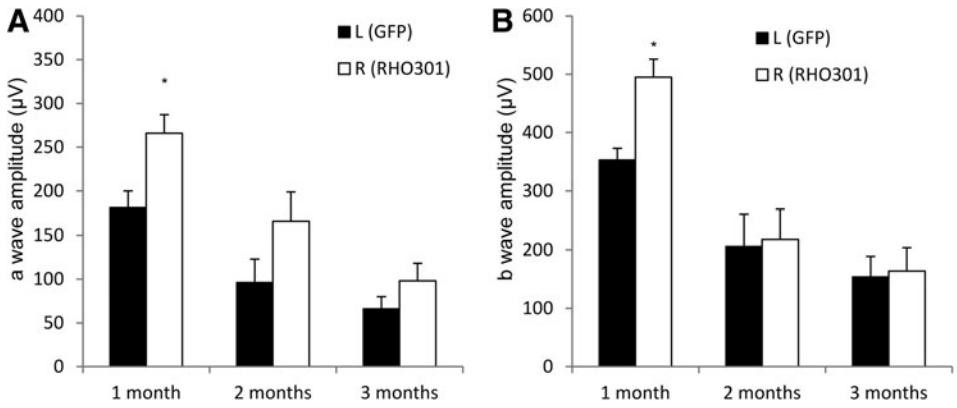

**SUPPLEMENTARY FIG. S2.** **(A)** The implicit time of the a-wave response was analyzed at 1, 2, 3, 6, and 9 months by comparing left AAV-GFP-injected eyes with right AAVRS301-injected eyes. The implicit time of RS301-injected eyes was significantly shorter at all five intervals, indicating a more rapid response to the light flash ($n = 9$; $^{**}p < 0.005$). **(B)** Representative scotopic ERG waveforms presented from left *GFP* control eyes and in **(C)** as right RS301-treated eyes at 1, 2, 3, 6, and 9 months.




**SUPPLEMENTARY FIG. S3.** Scotopic ERG responses to various intensities (0, –10, –20 dB) ($n=7$) at 1 and 9 months postinjection were measured from P23H transgenic mice after treatment with either AAV-RS301 or AAV-GFP. At 1 month postinjection, significant improvement of a-wave amplitudes from RS301-injected eyes was detected at both 0 and –20 dB **(A)**. At 9 months postinjection, RS301 treatment demonstrated a significant increase at all three intensities **(C)**. A significant increase in b-wave amplitudes was observed at all three intensities at both 1 and 9 months postinjection **(B** and **D)**.




**SUPPLEMENTARY FIG. S4.** Retinal structure and morphology in C57BL/6 wild-type mice. **(A)** Spectral domain optical coherence tomography (SD-OCT) images were captured in live mice at 3 and 6 months of age. Scale bar: 30 $\mu$m. **(B)** The thickness of the outer nuclear layer (ONL) was measured from SD-OCT images at four standard locations relative to the optic nerve head at 3 and 6 months of age ($n=8$). No statistically significant difference in ONL thickness was found between 3 and 6 months of age. These values are nearly identical to those measured in RS301-treated P23H mice of the same ages (Fig. 5).




**SUPPLEMENTARY FIG. S5.** AAV-RHO301 gene transfer showed transient rescue in retinal function in P23H transgenic mice. **(A)** a-wave amplitudes were measured 1, 2, and 3 months postinjection. The data presented here are from high-intensity flashes (0 dB) and represent both rod and cone responses. AAV-RHO301-injected eyes exhibited a significantly increased a-wave response at the 1-month postinjection time point compared with control injected eyes (AAV-GFP), but not at 2- and 3-month time points ($n = 5$; $^*p < 0.05$ at 1 month). **(B)** b-wave amplitudes in response to high-intensity flashes (0 dB) were measured 1, 2, and 3 months postinjection. AAV-RHO301-injected eyes exhibited a significantly increased b-wave response only at the 1-month postinjection time point compared with control injected eyes (AAV-GFP) ($n = 5$; $^*p < 0.05$ at 1 month).