
Figures and figure supplements

Small molecule Photoregulin3 prevents retinal degeneration in the *Rho*^{P23H} mouse model of retinitis pigmentosa

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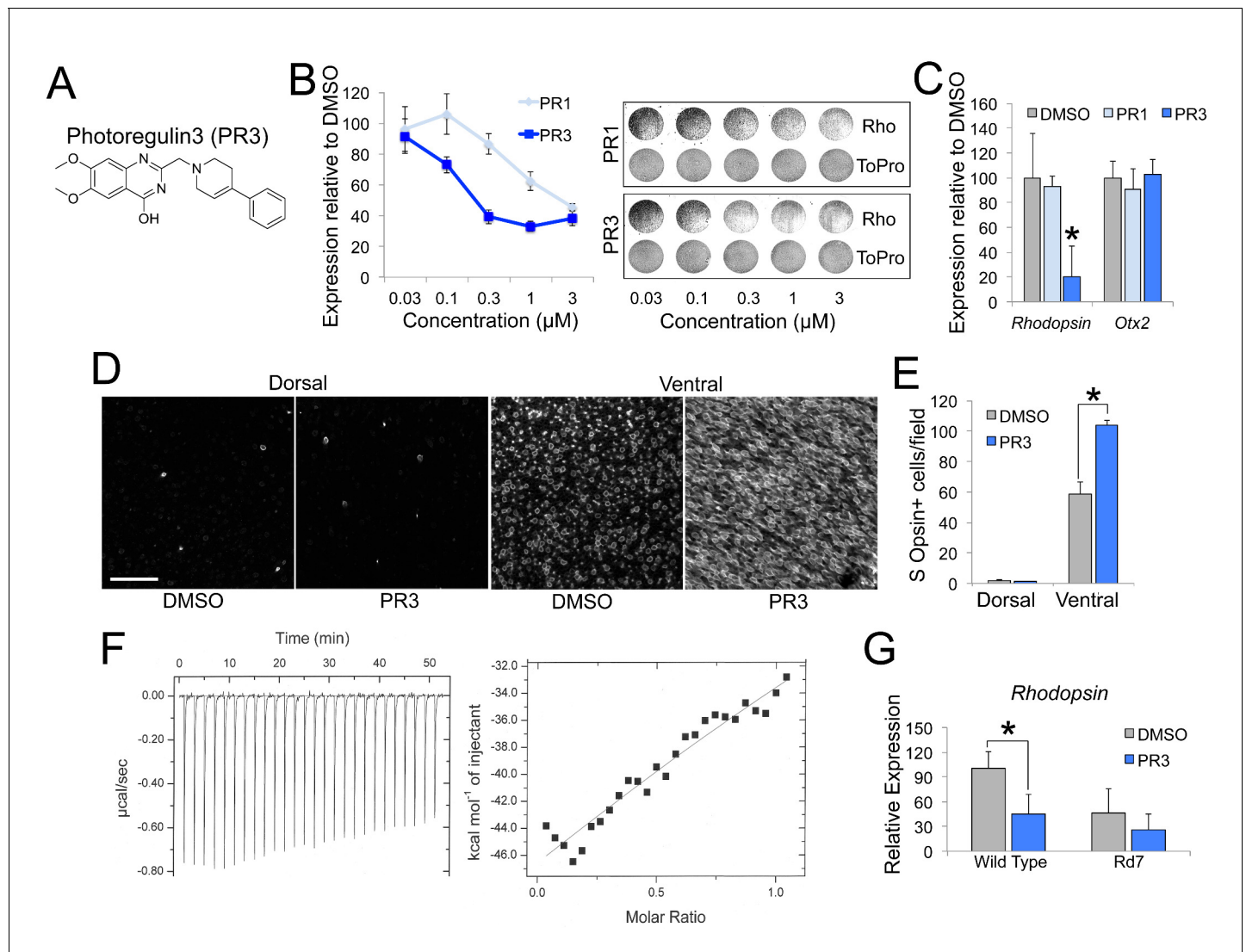


Figure 1. PR3 reduces rod gene expression via Nr2e3. (A) Chemical structure of Photoregulin3 (PR3). (B) Dose-response relationship of PR1 and PR3 on Rhodopsin expression in dissociated retinal cell cultures ($n = 3$ for each concentration for each compound; value graphed is the mean \pm SEM of 3 biological replicates). Example scans of Rhodopsin and ToPro3 staining from retinal cell cultures are shown on the right. (C) Confirmation of PR3's potency by qPCR from intact retinal explant cultures from P4 mice treated with DMSO or 0.3 μ M PR1 or PR3 for 2 days ($n = 3$ –4 for each compound; value graphed is the mean \pm SEM of the biological replicates, * $p=0.0075$ for DMSO vs. PR3, two-tailed t -test assuming equal variance using ΔC_T values). (D) Intact retinas from P11 mice were explanted in media containing DMSO or 0.3 μ M PR3 for 3 DIV and then stained for S Opsin in a whole-mount preparation. Scale bar represents 50 μ m. (E) PR3-treated retinas had more S Opsin+ cells per 100 μ m x 100 μ m field in the ventral, but not dorsal, retina compared to DMSO-treated retinas ($n = 3$ biological replicates; value graphed is the mean \pm SEM of the biological replicates, * $p=0.00063$, two-tailed t -test assuming equal variance). (F) Isothermal titration calorimetric study of PR3 binding to Nr2e3. (G) Intact retinas from adult (P23–P35) wild type and Rd7 were explanted in media containing DMSO or 1 μ M PR3 for 2 days and Rhodopsin expression was measured by qPCR. PR3 decreased Rhodopsin in wild type retinas but not in Rd7 retinas ($n = 4$ biological replicates; value graphed is the mean \pm SEM of the biological replicates, * $p=0.033$, two-tailed t -test assuming equal variance for wild type and $p=0.13$, two-tailed t -test assuming equal variance for Rd7 using ΔC_T values).

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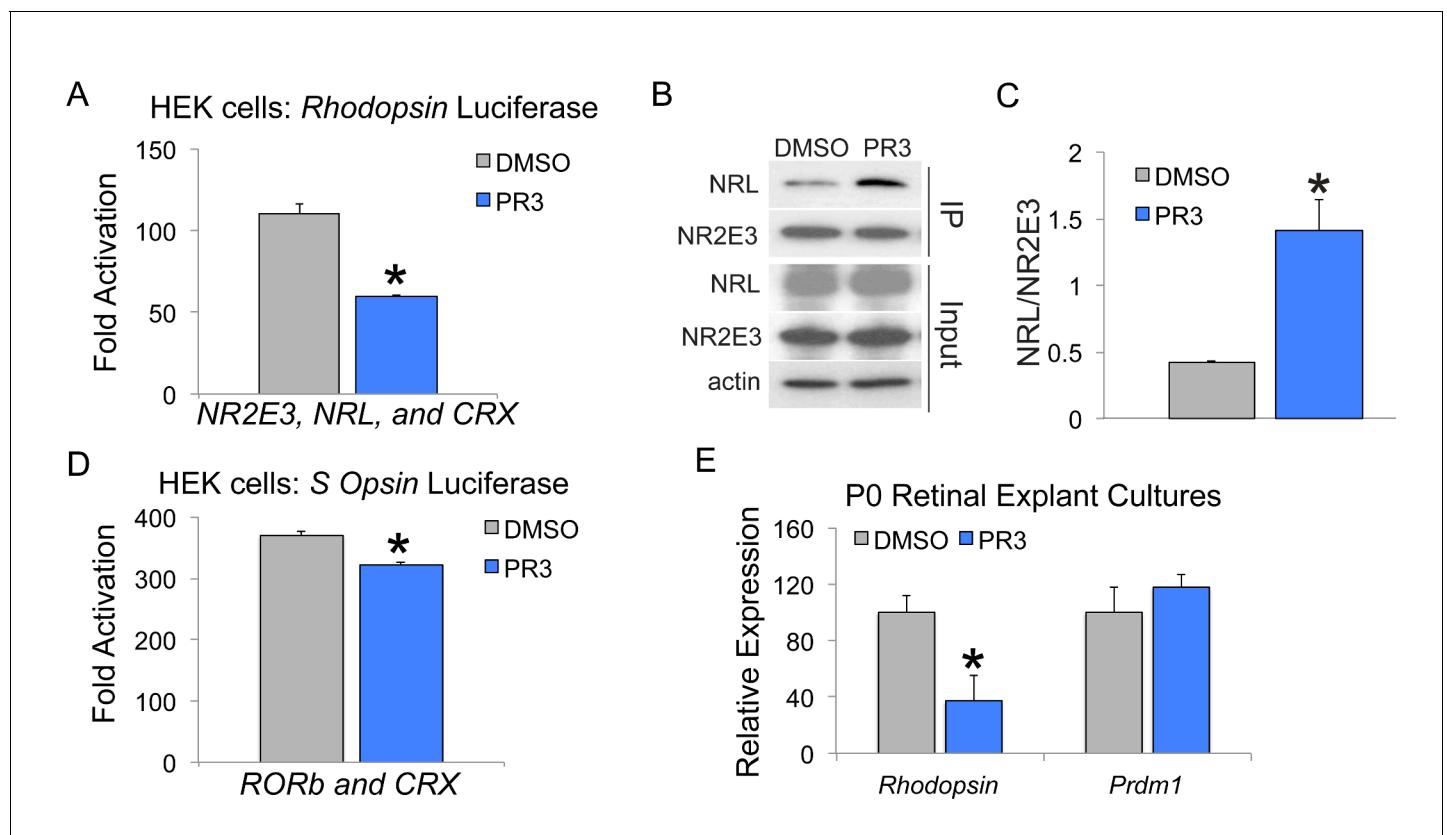


Figure 1—figure supplement 1. Effects of PR3 on photoreceptor promoter activation and co-activator binding. (A) HEK 293 cells were transfected with *NR2E3*, *CRX*, and *NRL* and BR-225Luc (firefly luciferase driven by the bovine *Rhodopsin* promoter) and pRL-CMV (renilla luciferase driven by the CMV promoter; internal transfection control) and then treated with DMSO or 1 μ M PR3 for 2 days. PR3 decreased *Rhodopsin* promoter activity after transfection of *NR2E3*, *CRX*, and *NRL* ($n = 4$ separate transfections per condition, values graphed are the mean \pm SEM of the biological replicates, $p < 0.001$, two-tailed t -test assuming equal variance). (B) HEK 293 cells were transfected with *NR2E3* and *NRL* and then treated with DMSO or 1 μ M PR3 for 2 days. Lysates were immunoprecipitated with an anti-Nr2e3 antibody and then analyzed by Western blot analysis. (C) PR3 treatment increased the binding between Nr2e3 and Nrl ($n = 3$ separate transfections per condition, values graphed are the mean \pm SEM of the biological replicates, $p = 0.013$, two-tailed t -test assuming equal variance). (D) HEK 293 cells were transfected with *RORb*, *CRX*, and *S Opsin Luc* (firefly luciferase driven by the *S Opsin* promoter) and pRL-CMV (renilla luciferase driven by the CMV promoter; internal transfection control) and then treated with DMSO or 1 μ M PR3 for 2 days. PR3 decreased *S Opsin* promoter activity ($n = 4$ separate transfections per condition, values graphed are the mean \pm SEM of the biological replicates, $p = 0.0014$, two-tailed t -test assuming equal variance). (E) Retinas from P0 mice were explanted in media containing DMSO or 0.3 μ M PR3 for 2 days. PR3 treatment reduced *Rhodopsin* expression ($n = 4$ biological replicates, values graphed are the mean \pm SEM of the biological replicates, $p = 0.00081$, two-tailed t -test assuming equal variance), but not *Prdm1* expression, a direct target of *RORb* ($n = 4$ biological replicates, values graphed are the mean \pm SEM of the biological replicates, $p = 0.41$, two-tailed t -test assuming equal variance).

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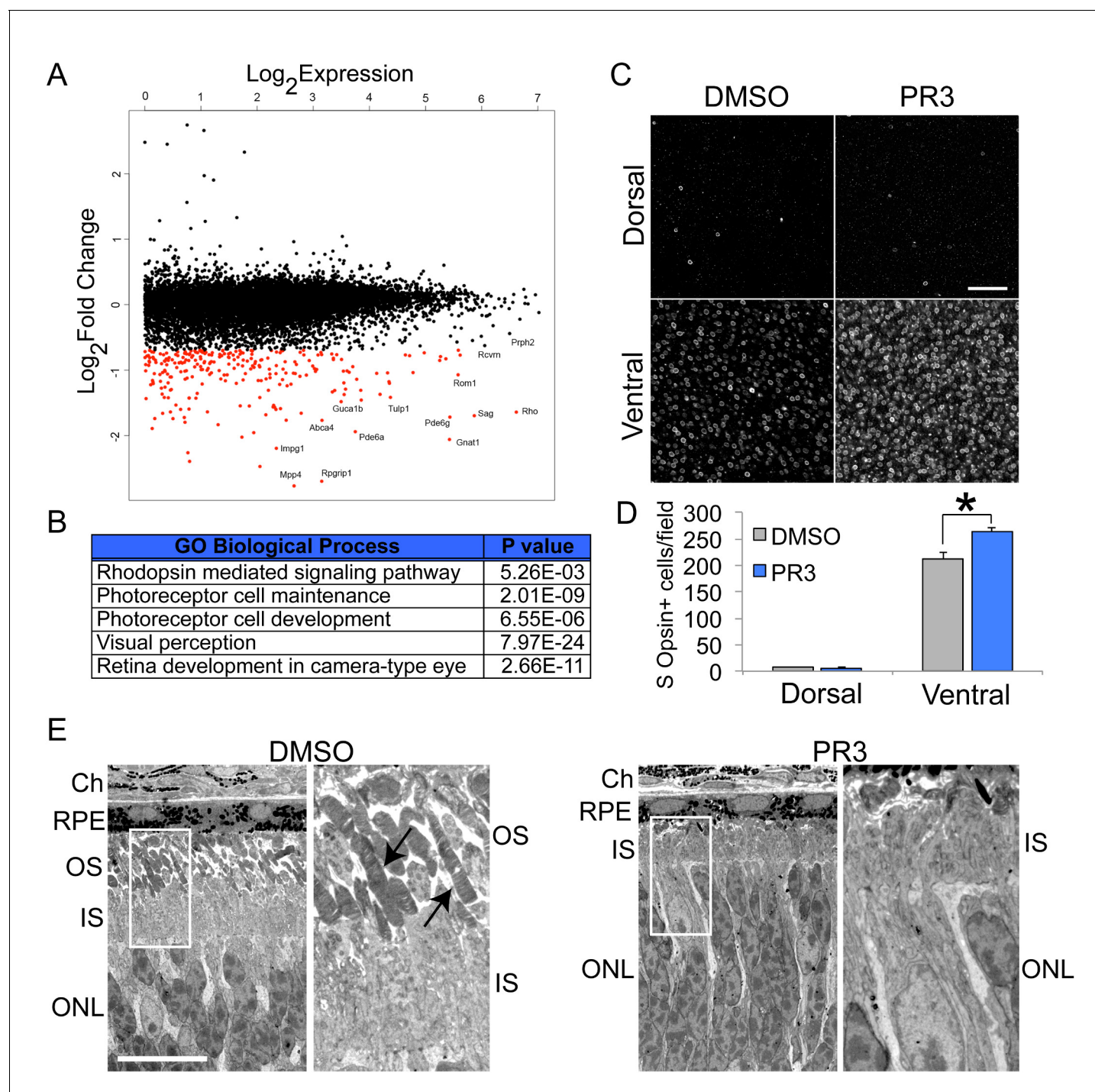


Figure 2. In vivo characterization of PR3 treatment. (A) RNA sequencing results plotting log2FoldChange against log2Expression (RPKM) of wild type mice treated with DMSO vehicle or 10 mg/kg PR3 shows robust reduction in rod photoreceptor genes. (n = 2 mice per condition; values graphed are the mean of the two biological replicates) (B) Gene ontology analysis (<http://geneontology.org/page/go-enrichment-analysis>) results for largest changes (top 100) in gene expression assessed by RNA sequencing. (C) Whole mount S Opsin staining of retinas from mice treated with 10 mg/kg PR3 or DMSO vehicle for 3 days. Scale bar represents 25 μ m. (D) Retinas from mice treated with PR3 had more S Opsin+ cells per 100 μ m x 100 μ m field in the ventral, but not dorsal, retina compared to controls (n = 4 biological replicates; value graphed is the mean \pm SEM of the biological replicates, *p=0.0086, two-tailed t-test assuming equal variance). (E) Electron microscope micrographs of retinal sections of wild type mice treated with DMSO or 10 mg/kg PR3 (n = 2 mice per condition). Compared to DMSO controls, PR3 retinas have arrested outer segment development (arrows indicate outer segments in DMSO treated mice). Scale bar represents 10 μ m.

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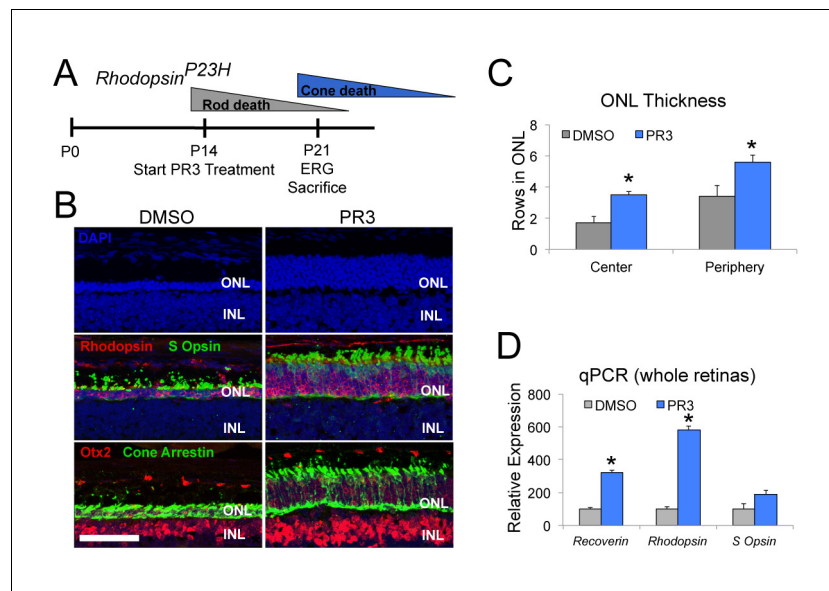


Figure 3. PR3 slows the progression of photoreceptor degeneration in the *RhoP23H* mouse model of RP. (A) Timeline of photoreceptor degeneration in the *Rhodopsin*^{P23H} mouse and experimental design. (B) Immunofluorescence staining for Rhodopsin, S Opsin, Otx2, and Cone Arrestin on retinal sections from *Rhodopsin*^{P23H} mice demonstrate preservation of photoreceptors with PR3 treatment. Scale bar represents 50 μ m. (C) Counts for rows of DAPI+ cells in the central and peripheral ONL show greater survival of photoreceptors with PR3 treatment ($n = 7$ mice for DMSO treatment and 8 mice for PR3; values graphed are the mean \pm SEM of the biological replicates, $p=0.0014$ for center and 0.015 for periphery, two-tailed t-test assuming equal variance). (D) qPCR on whole retinas from *Rhodopsin*^{P23H} mice treated with DMSO or PR3 shows greater expression of photoreceptor genes *Recoverin*, *Rhodopsin*, and *S Opsin* with PR3 treatment ($n = 6$ mice for DMSO and 7 mice for PR3; values graphed are the mean \pm SEM of the biological replicates, $p=1.73E-05$ for *Recoverin*, 3.13E-05 for *Rhodopsin*, and 0.076 for *S Opsin*, two-tailed t-test assuming equal variance on ΔC_T values).

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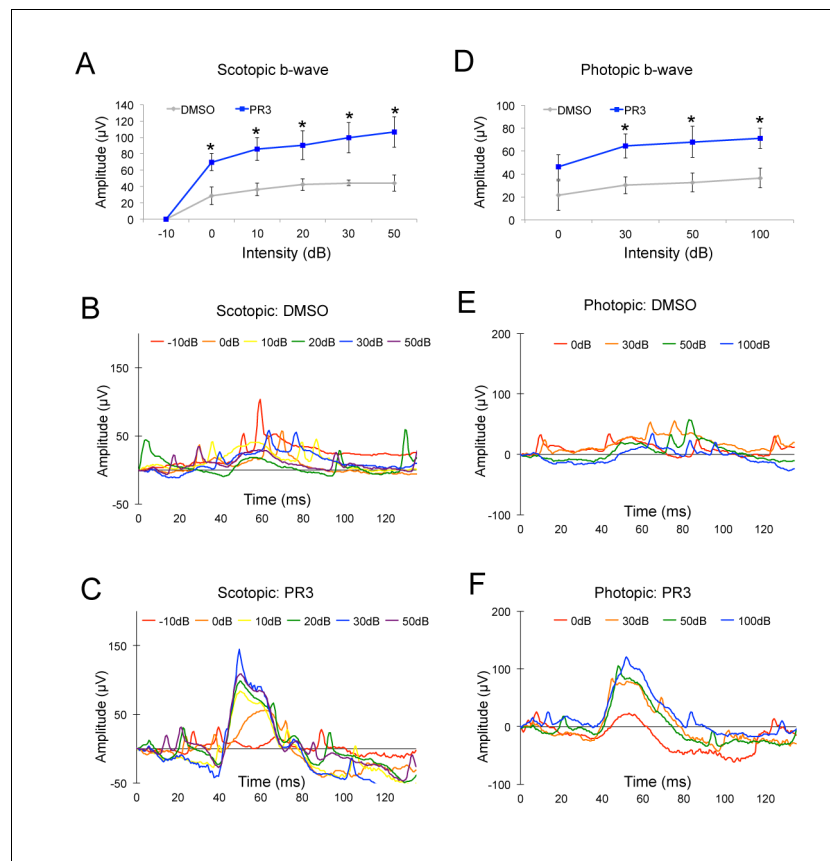


Figure 4. PR3 preserves visual function in the *Rhodopsin^{P23H}* mouse. (A) Scotopic b-wave amplitudes from P21 *Rhodopsin^{P23H}* mice treated with 10 mg/kg PR3 or DMSO vehicle (n = 4 mice for DMSO treatment and 8 mice for PR3 treatment, values graphed are the mean \pm SEM of the biological replicates, p=0.025, 0.012, 0.032, 0.021, and 0.015 for 0, 10, 20, 30, and 50 Decibels (dB) respectively, two-tailed t-test assuming unequal variance). (B) Representative scotopic ERG waveforms from a single DMSO vehicle mouse. (C) Representative scotopic ERG waveforms from a single PR3-treated mouse. (D) Photopic b-wave amplitudes from P21 *Rhodopsin^{P23H}* mice treated with 10 mg/kg PR3 or DMSO vehicle (n = 4 mice for DMSO treatment and 8 mice for PR3 treatment, values graphed are the mean \pm SEM of the biological replicates, p=0.18, 0.025, 0.048, and 0.021 for 0, 30, 50, and 100 dB respectively, two-tailed t-test assuming unequal variance). (E) Representative photopic ERG waveforms from a single control mouse. (F) Representative photopic ERG waveforms from a single PR3-treated mouse.

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