Supplemental information

Treatment of autosomal dominant retinitis

pigmentosa caused by RHO-P23H mutation

with high-fidelity Cas13X in mice

Zixiang Yan, Yuqin Yao, Luyao Li, Lingqiong Cai, Haiwei Zhang, Shenghai Zhang, Qingquan Xiao, Xing Wang, Erwei Zuo, Chunlong Xu, Jihong Wu, and Hui Yang
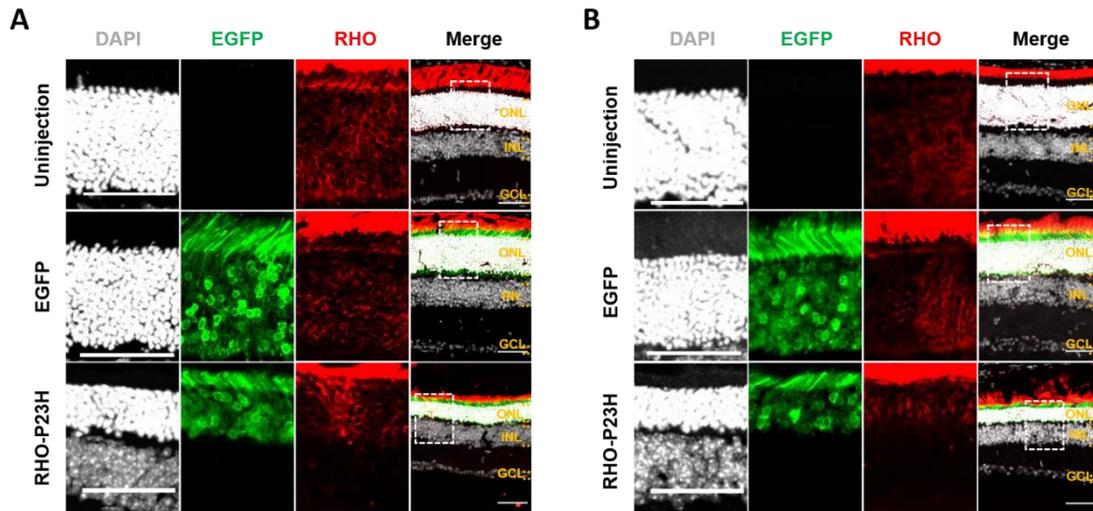
Figure S1. Human RHO-P23H overexpression in C57BL/6 mice.

(A) Representative images of ten-week-old C57BL/6 retinas uninjected or injected with AAV-EGFP and AAV-RHO(P23H)-T2A-EGFP, respectively. (B) Representative images of twelve-week-old C57BL/6 retinas uninjected or injected with AAV-EGFP and AAV-RHO(P23H)-T2A-EGFP, respectively. Individual channels zoomed in from the dashed boxes in the corresponding merged images. Scale bar, 50 μm. ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer.
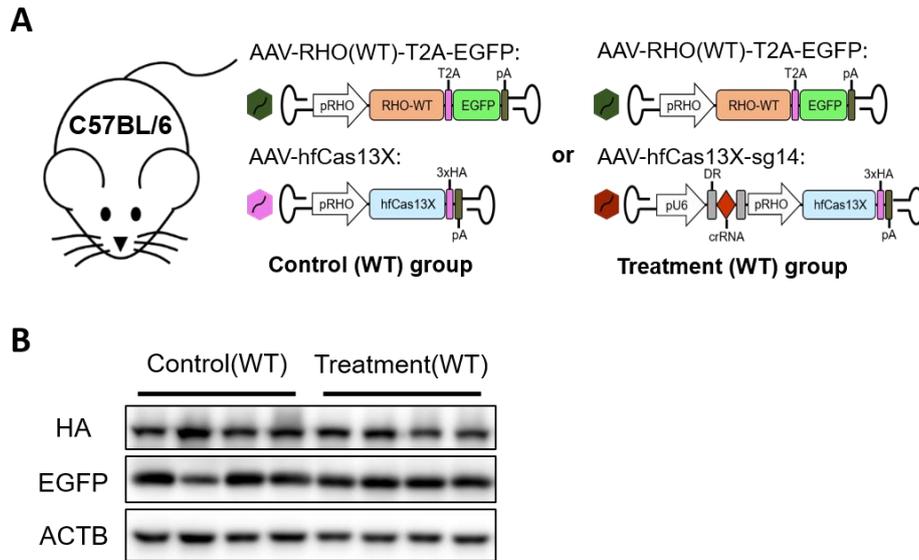
Figure S2. Human RHO-WT was not affected by hfCas13X *in vivo*.

(A) Schematic diagram of strategy for experiments of human RHO-WT knockdown in C57BL/6 mice. (B) Western blot of eight-week-old C57BL/6 retinas injected with AAVs of control (WT), and treatment (WT) groups, respectively. Each group presented four retinas.
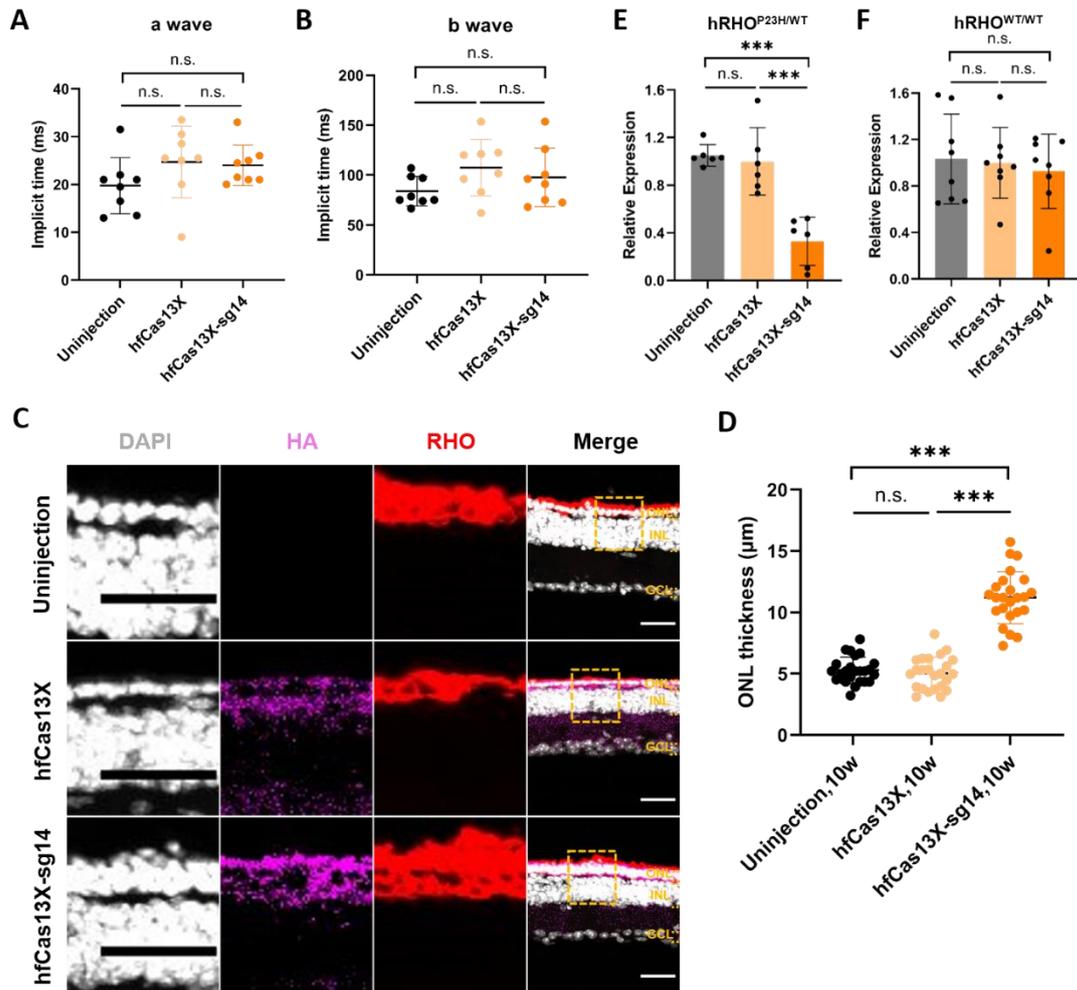
Figure S3. Treatment of humanized hRHO$^{P23H/WT}$ mice with hfCas13X.

(A) Implicit time statistics of a-wave of four-week-old hRHO$^{P23H/WT}$ mice uninjected or injected with AAV-hfCas13X and AAV-hfCas13X-sg14, respectively. n.s., not significant; one-way ANOVA test. Eight retinas for each group. (B) Implicit time statistics of b-wave of four-week-old hRHO$^{P23H/WT}$ mice uninjected or injected with AAV-hfCas13X and AAV-hfCas13X-sg14, respectively. n.s., not significant; one-way ANOVA test. Eight retinas for each group. (C) Representative images of ten-week-old hRHO$^{P23H/WT}$ retinas uninjected or injected with AAV-hfCas13X and AAV-hfCas13X-sg14, respectively. Individual channels zoomed in from the dashed boxes in the corresponding merged images. Scale bar, 30 μm. ONL, outer nuclear layer; INL, inner

nuclear layer; GCL, ganglion cell layer. (D) ONL thickness statistics of ten-week-old hRHO$^{P23H/WT}$ retinas uninjected or injected with AAV-hfCas13X and AAV-hfCas13X-sg14, respectively. ***, $P < 0.001$; n.s., not significant; one-way ANOVA test. Six retinas for each group. (E) Relative degradation of total RHO transcripts induced by hfCas13X-sg14 in hRHO$^{P23H/WT}$ mice. ***, $P < 0.001$; n.s., not significant; one-way ANOVA test. Gene expression was relative to the hfCas13X group. Six retinas for each group. (F) Relative expression of total RHO transcripts in hRHO$^{WT/WT}$ mice infected by AAV-hfCas13X-sg14. n.s., not significant; one-way ANOVA test. Gene expression was relative to the hfCas13X group. Eight retinas for each group.