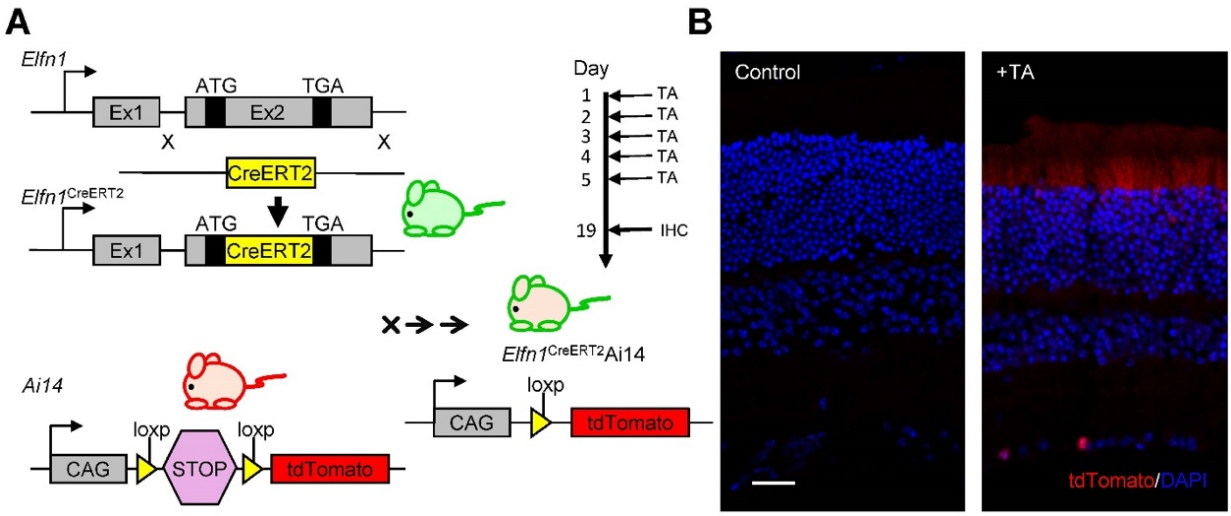

Figure S1. Characterization of *Elfn1*^{CreERT2} mice, related to Figure 1.

(A) Schematic illustration of the strategy for conditional deletion of *Elfn1* in the retina. *Elfn1*^{CreERT2} mouse was generated by recombination replacing of exon 2 of *Elfn1* gene with CreERT2 cassette. The specificity and efficiency of Cre recombination in *Elfn1*^{CreERT2} lines are evaluated by crossing with Ai14 strain which contains a CAG-loxP-Stop-loxP-tdTomato cassette at the Rosa locus of its genome. At two weeks post-injection., an induced Cre expression by 5-day tamoxifen injection removes the stop codon and restores tdTomato expression in the 2-month-old *Elfn1*^{CreERT2}Ai14 mice. TA, tamoxifen.

(B) The tamoxifen-induced Cre expression efficiently and specifically edits the photoreceptor in Ai14 Mice. At two weeks post-injection, retina cross-sections were examined under direct fluorescence microscopy. Red indicates tamoxifen injection resulted in expression of tdTomato. Scale bar, 25 μm.

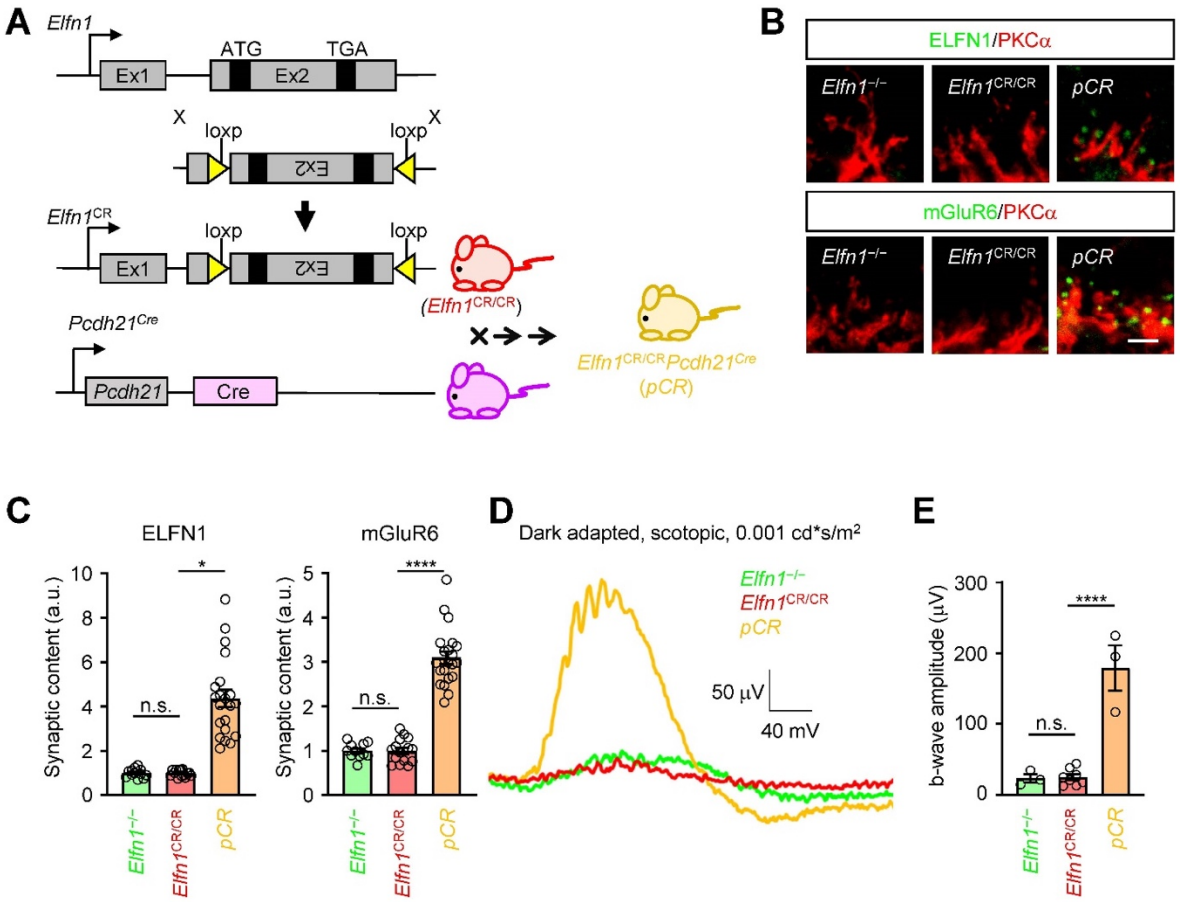

Figure S2. Characterization of conditional rescue *Elfn1*^{CR} mice, related to Figure 2.

(A) Schematic illustration of the strategy for the conditional rescue of *Elfn1* in the retina. The mice carrying the conditional rescue *Elfn1* allele (*Elfn1*^{CR}) are obtained by inverting the exon 2 of the *Elfn1* gene relative to their normal endogenous orientation and are flanked with loxP66 and loxP71 sequences inserted in an inverted orientation relative to each other. *Elfn1*$^{CR/CR}$ mice were then crossed to a photoreceptor-specific Cre driver line *Pcdh21*^{Cre} to generate *Elfn1*$^{CR/CR}$ *Pcdh21*^{Cre} (*pCR*) mice.

(B) Analysis of the effect of Elfn1 restoration on synaptic content of mGluR6 in rod terminals. Retina cross-sections were double-immunostained with antibody against Elfn1 or mGluR6 (green) and rod ON-BC marker PKCa (red). (Scale bar, 2.5 μm.).

(C) Quantification of Elfn1 and mGluR6 content in rod ON-BC synapses. Mean fluorescence intensity from 10 puncta per retina section was normalized by background. Data are from six to seven images per retina collected from two to three separate mice and represented as mean ± SEM; n = 12 to 21. *P < 0.05, ****P < 0.0001, one-way ANOVA followed by Tukey's multiple comparisons test.

(D) Analysis of rod synaptic transmission after Elfn1 restoration by ERG. Representative traces of responses to a scotopic flash of 0.001 cd*s/m^2 (~0.6 R* per rod) are shown.

(E) Quantification of b-wave ERG amplitudes in *Elfn1*$^{CR/CR}$*Pcdh21*^{Cre} (n=3) and *Elfn1*$^{CR/CR}$ (n=7) in comparison with *Elfn1*$^{-/-}$ (n=3) under scotopic conditions. Data are represented as mean ± SEM. ****P < 0.0001, one-way ANOVA followed by Tukey's multiple comparisons test.

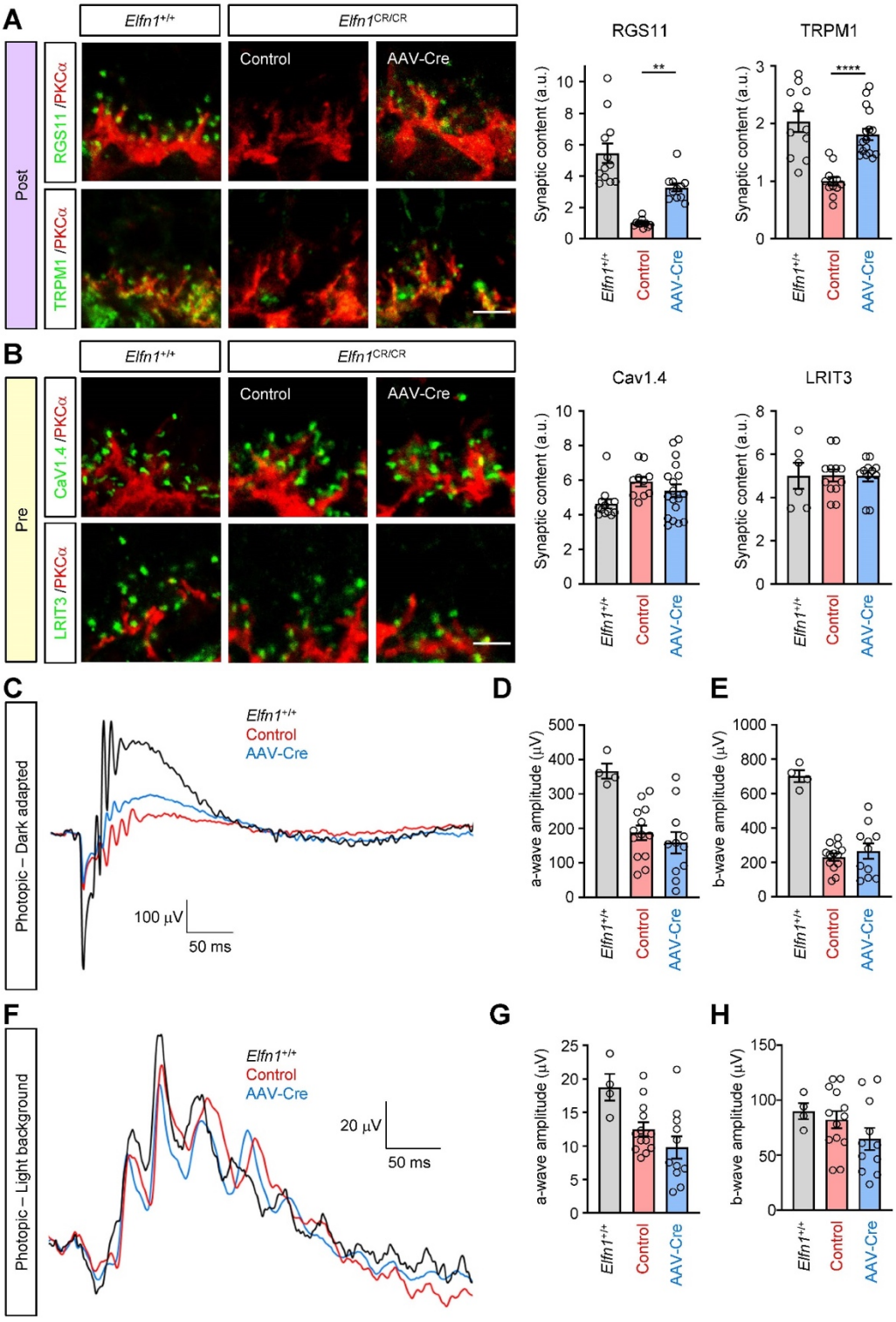

Figure S3. Characterization of conditional rescue *Elfn1*^{CR} mouse model following AAV sub-retinal injections, related to Figure 2.

(A) Staining of retina sections for postsynaptic markers. Counter-staining with rod ON-BC PKCα was used to determine the location of rod ON-BC synapses. (Scale bar, 2.5 µm.). Quantification

of synaptic content of indicated proteins. Mean fluorescence intensity from 10 puncta per retina section was normalized by background. Data are from three images per retina collected from four to six separate mice and represented as mean ± SEM; n = 12 to 18. **P < 0.01, ****P < 0.0001, one-way ANOVA followed by Tukey's multiple comparisons.
(B) Staining of retina sections for presynaptic markers. Counter-staining with rod ON-BC PKCα was used to determine the location of rod ON-BC synapses. (Scale bar, 2.5 μm.). Quantification of synaptic content of indicated proteins. Mean fluorescence intensity from 10 puncta per retina section was normalized by background. Data are from three images per retina collected from four to six separate mice and represented as mean ± SEM; n = 12 to 18.
(C) Representative ERG traces to a photopic flash of 100 cd*s/m^2 (~58,000 R*/rod) to activate both rod and cone pathways.
(D) Quantification of a-wave ERG amplitudes under photopic conditions.
(E) Quantification of b-wave ERG amplitudes under photopic conditions.
(F) Representative ERG traces to a photopic flash of 100 cd*s/m^2 (~58,000 R*/rod) under a 32 cd*s/ m^2 (~18,500 R*/rod/s) light background to activate the cone pathway only.
(G) Quantification of a-wave ERG amplitudes under light adapted photopic conditions.
(H) Quantification of b-wave ERG amplitudes under light adapted photopic conditions. Data are represented as mean ± SEM. n=4-13.

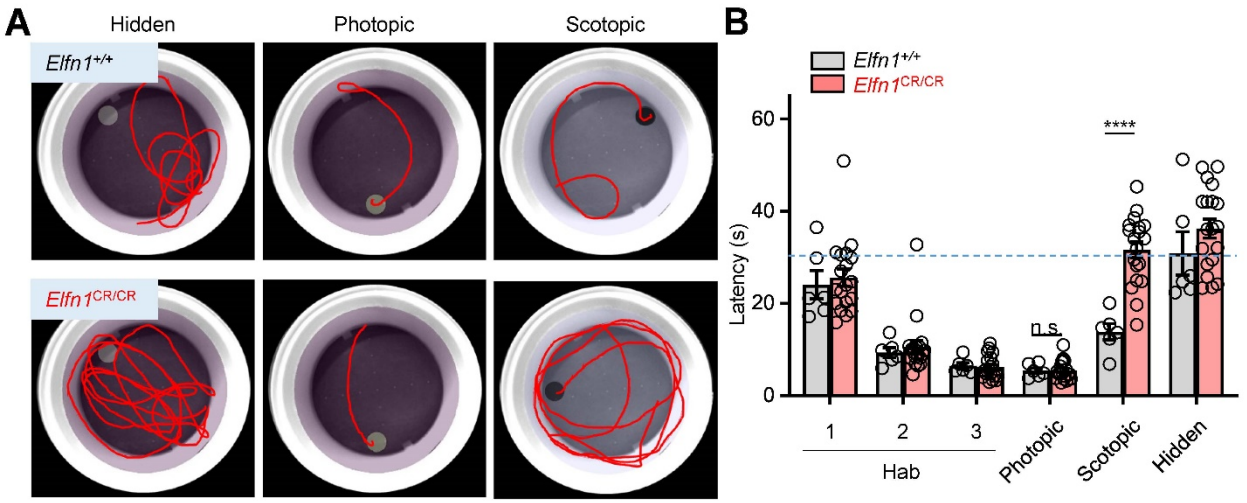

Figure S4. Visual behavior testing of conditional rescue *Elfn1*^{CR} mice before AAV sub-retinal injections, related to Figure 3.

(A) Swimming tracks of representative animals during sessions at the scotopic condition (0.001 cd*s/m^2), photopic condition (100 cd*s/m^2), and hidden platform before AAV subretinal injections. (B) Summary of platform finding latencies during the training sections and the different illumination conditions (scotopic, 0.001 cd*s/m^2; photopic, 100 cd*s/m^2) before AAV subretinal injections. Statistical analysis was performed using a two-way ANOVA test followed by Sidak's multiple comparisons test. Data are represented as mean ± SEM, n=6 to 19, ****$p < 0.0001$.

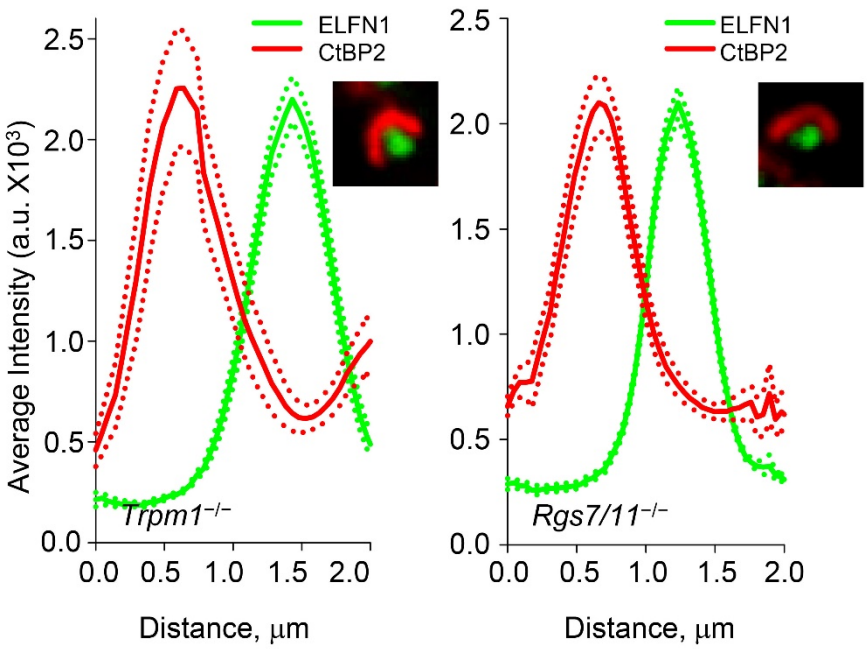

Figure S5. Analysis of Efln1 localization in postsynaptic signaling components knockout retina, related to Figure 5.

Plots show quantification of relative fluorescence intensity distributions from 10 synaptic puncta. Dashed lines are plots of respective SEM values.