

Supplementary Materials for

A gene therapy for inherited blindness using dCas9-VPR-mediated transcriptional activation

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Table S1

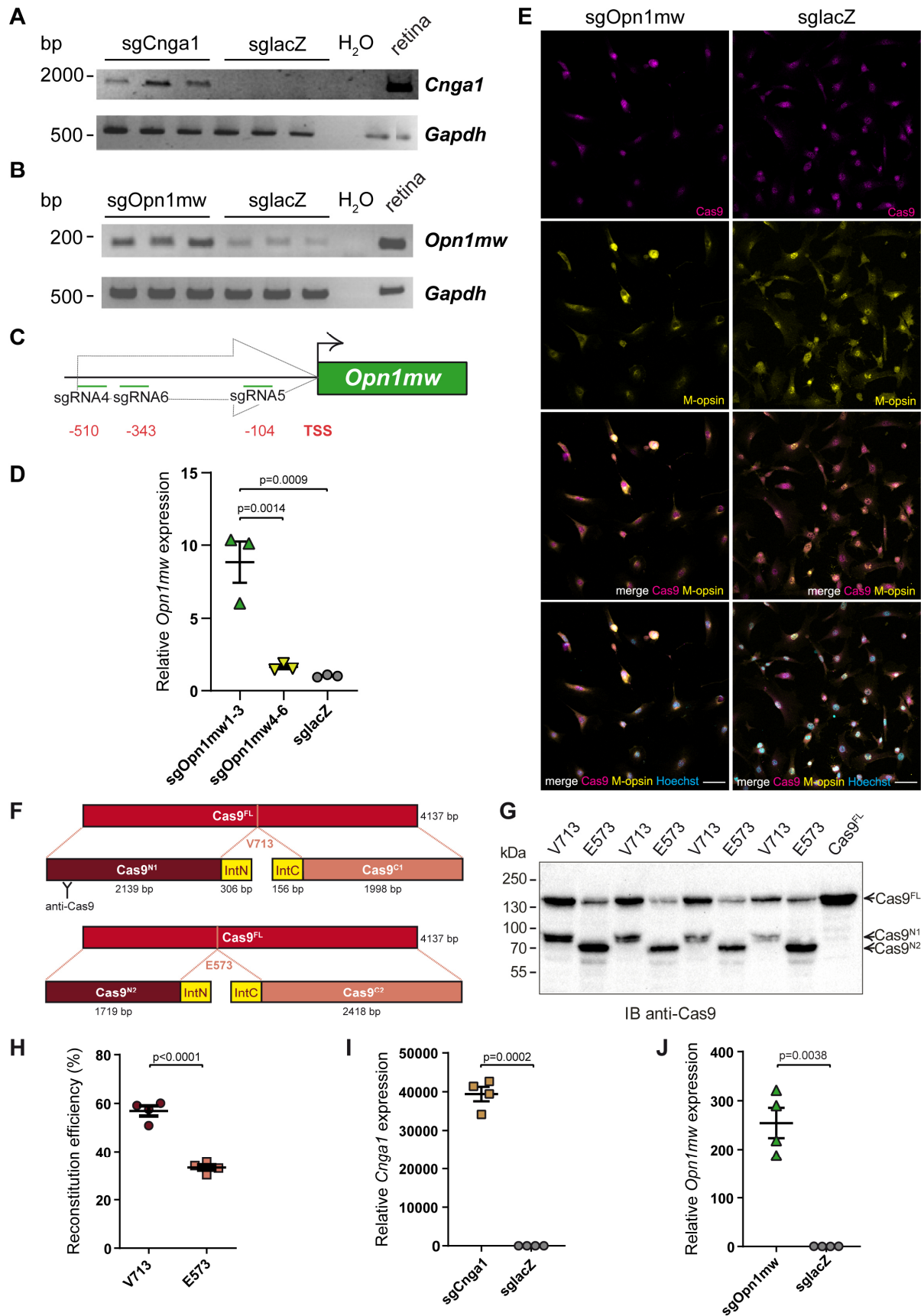


Fig. S1 *In vitro* characterization of split dCas9-VPR-mediated transactivation. **A** RT-PCR from 661W cells co-transfected with rAAV-sgRNA-CMVmini-dCas9^N and rAAV-CMVmini-dCas9^C-VPR using *Cnga1*-specific primers. *Gapdh* was used as loading control and cDNA from wild type mouse retinas served as positive control. **B** RT-PCR from MEF cells co-

transfected with rAAV-sgRNA-CMVmini-dCas9^N and rAAV-CMVmini-dCas9^C-VPR using *Opn1mw*-specific primers. Note the basal *Opn1mw* expression in MEF cells expressing the control *lacZ* sgRNA. **C** Binding position of three additional sgRNAs used for targeting dCas9-VPR to the promoter of the *Opn1mw* gene. The relative distance of each sgRNA to the transcription start site (TSS) of the target gene is given in bp. **D** Quantification of transactivation potency between the two different *Opn1mw*-specific sgRNA sets. Expression was normalized to MEF cells co-transfected with split dCas9-VPR and a *lacZ* sgRNA (One-way ANOVA with Dunnett's multiple comparisons test). **E** Immunostainings of MEF-pb cells cultured with 5 ng/ml DOX using Cas9- (magenta) or M-opsin-specific antibodies (yellow). Scale bar 30 μ m. **F** Scheme of the split Cas9 cassettes. N-intein and C-intein sequences of *Rhodothermus marinus* (*Rma*) were incorporated after amino acid position V713 or E573 of *SpCas9*. **G** Immunoblot (IB) from HEK293 cells co-transfected with the respective Cas9 halves. The uppermost band indicates successfully reconstituted full-length Cas9 (Cas9^{FL}). **H** Quantification of reconstitution efficiency by ratiometric analysis of the Cas9^{FL} and Cas9^N band intensities. (Unpaired t-test with Welch's correction, two-tailed). **I, J** qRT-PCR from 661W (**I**) and MEF (**J**) cells co-transfected with dual AAVs encoding for V713 split dCas9-VPR fragments and *Cnga1*, *Opn1mw* or *lacZ* sgRNAs (rAAV-sgRNA-CMV-dCas9^N + rAAV-CMV-dCas9^C-VPR). Expression was normalized to cells co-transfected with split dCas9-VPR and control *lacZ* sgRNA. (Unpaired t-test with Welch's correction, two-tailed).

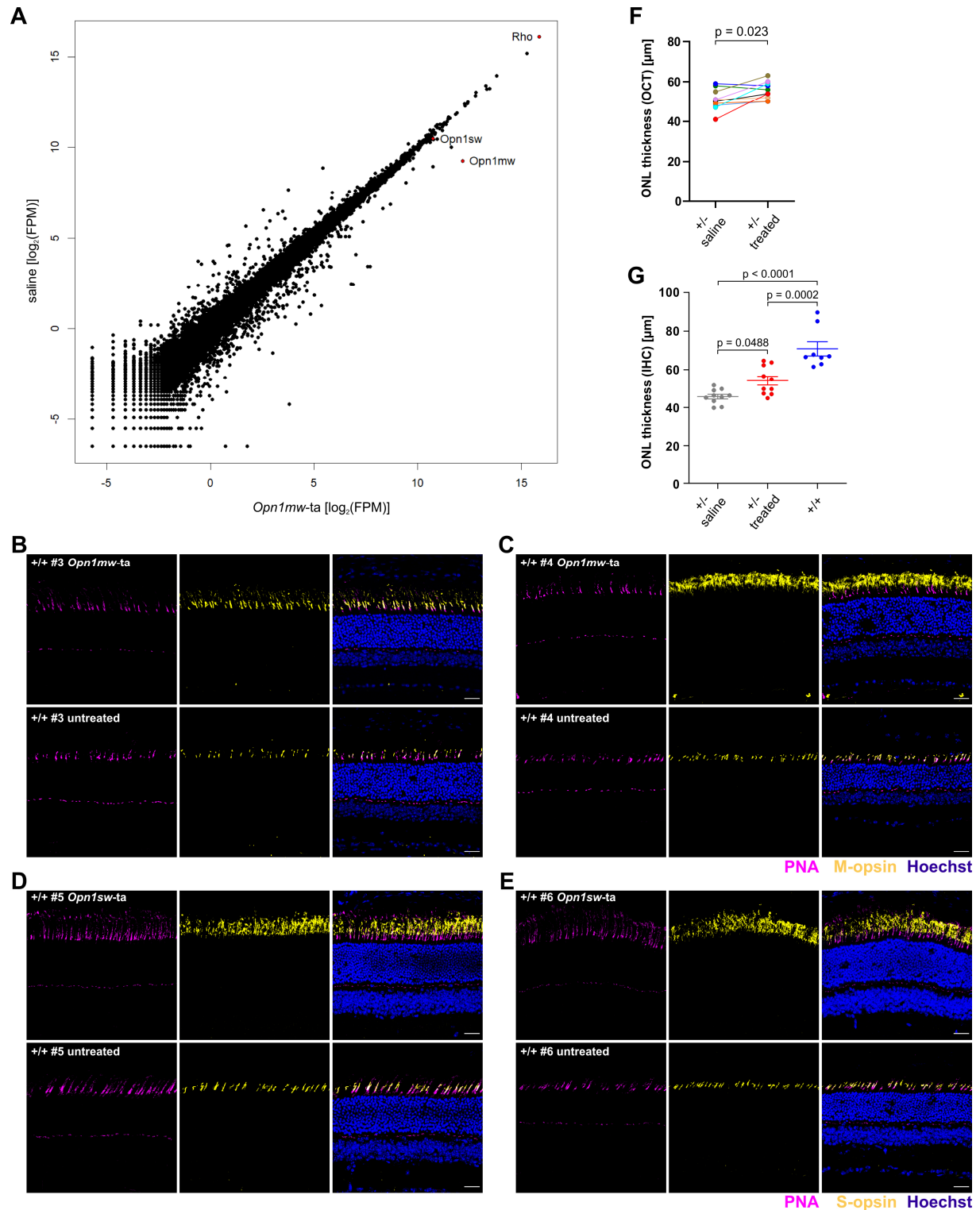


Fig. S2 Transactivation of *Opn1mw* or *Opn1sw* in wild type and ONL thickness measurements in $\text{Rho}^{+/-}$ mice. **A** The treated eye of a wild type (+/+) mouse ($n = 1$) was subretinally injected with dual rAAVs expressing split dCas9-VPR and *Opn1mw*-specific sgRNAs (*Opn1mw-ta*). The contralateral eye was injected with a NaCl solution (saline). RNA-seq was performed four weeks post-injection. Each dot represents one transcript and *Opn1mw*, *Opn1sw* and *Rho* transcripts are highlighted in red. FPM, fragments per million mapped reads. **B-E** Immunolabeling of retinas from four wild type mice (#3 - #6) injected with dual rAAVs expressing split dCas9-VPR and either *Opn1mw*-specific (B, C) or *Opn1sw*-specific sgRNAs (D, E) or untreated (lower panel) mice 4 weeks post-injection. Peanut

agglutinin (PNA, magenta) was used as marker for cones. All experiments were repeated at least once. Scale bar 30 μm . **F** Pairwise comparison of outer nuclear layer (ONL) thickness originating from OCT measurements from treated and saline-injected eyes (Paired t-test, two-tailed). **G** Histology-based (immunohistochemistry, IHC) measurements of the ONL thickness of the single groups as indicated. Statistical analysis was done using one-way ANOVA with Tukey's post-hoc test.

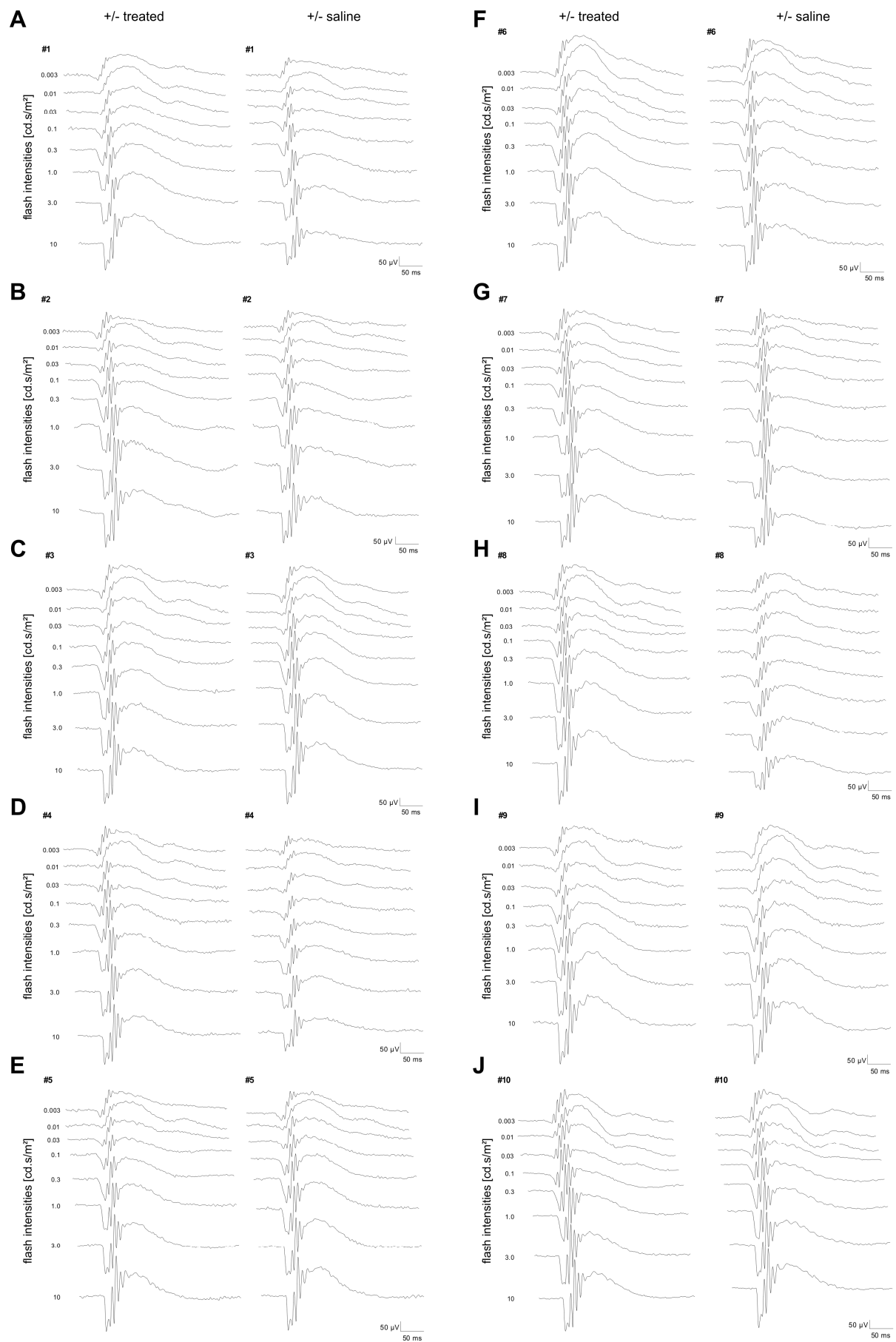


Fig. S3 Single flash scotopic ERG responses for all injected $Rho^{+/-}$ mice. A-J Serial responses to increasing flash stimuli were recorded from the *Opn1mw* expressing $Rho^{+/-}$ (+/-

treated, left) and NaCl-injected (+/- saline, right) eyes of Rho^{+/-} mice (#1-10) under dark-adapted conditions one year after injection.

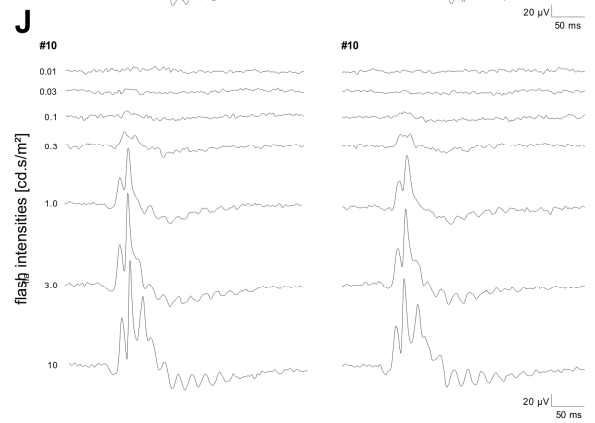
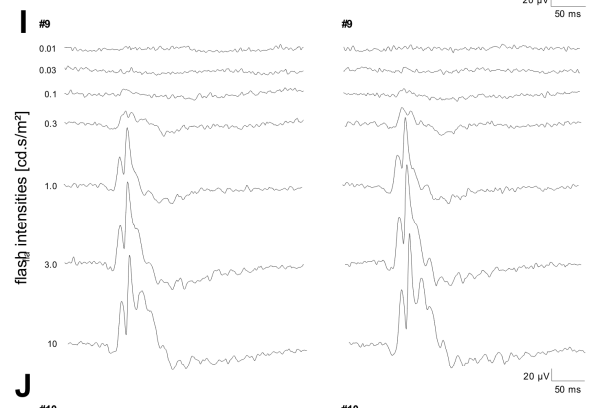
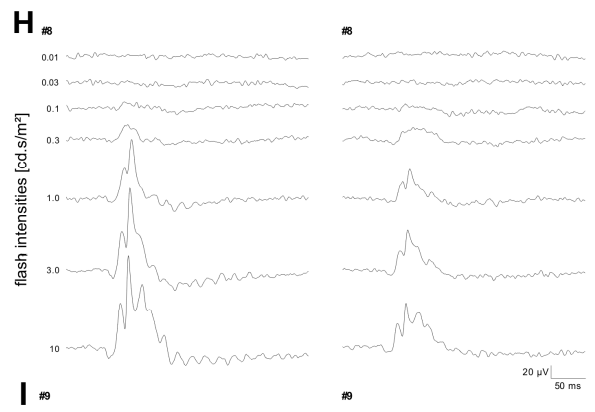
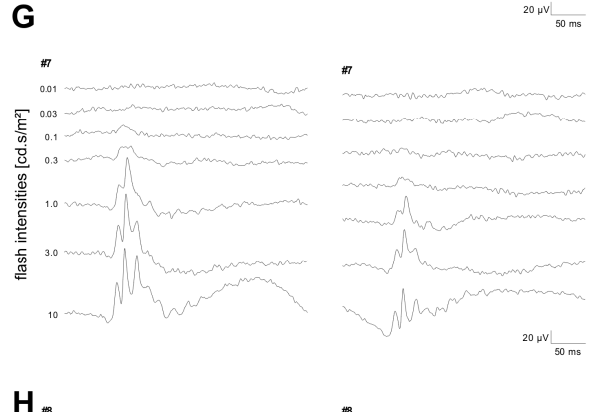
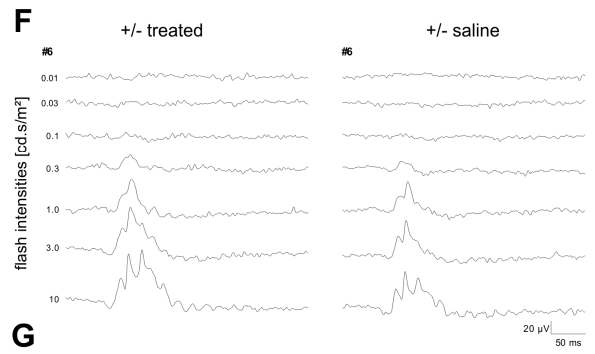
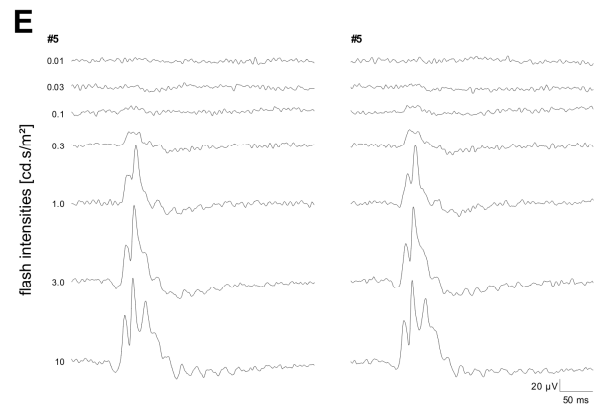
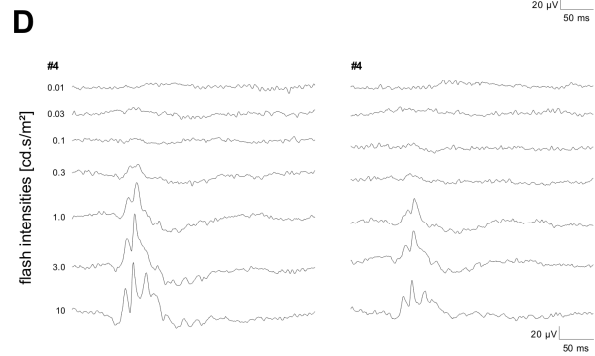
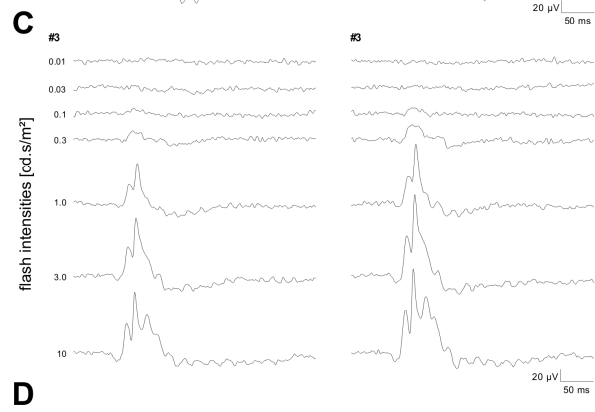
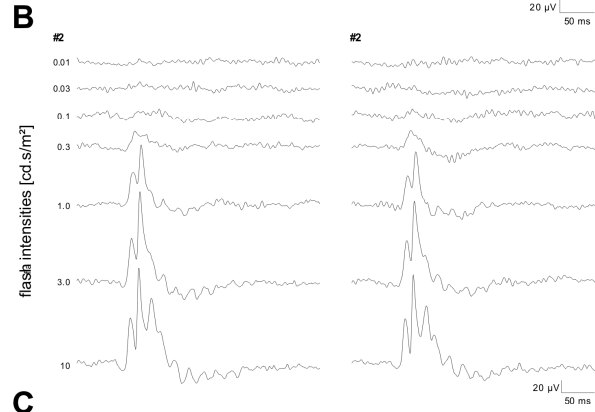
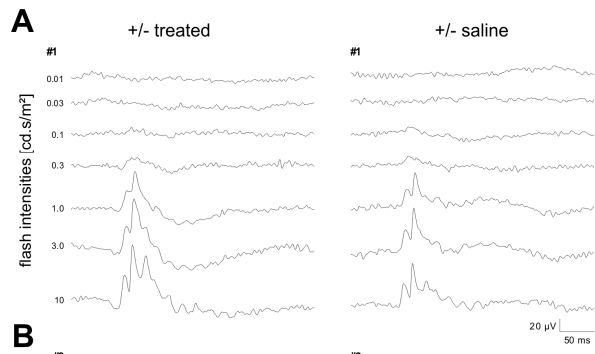
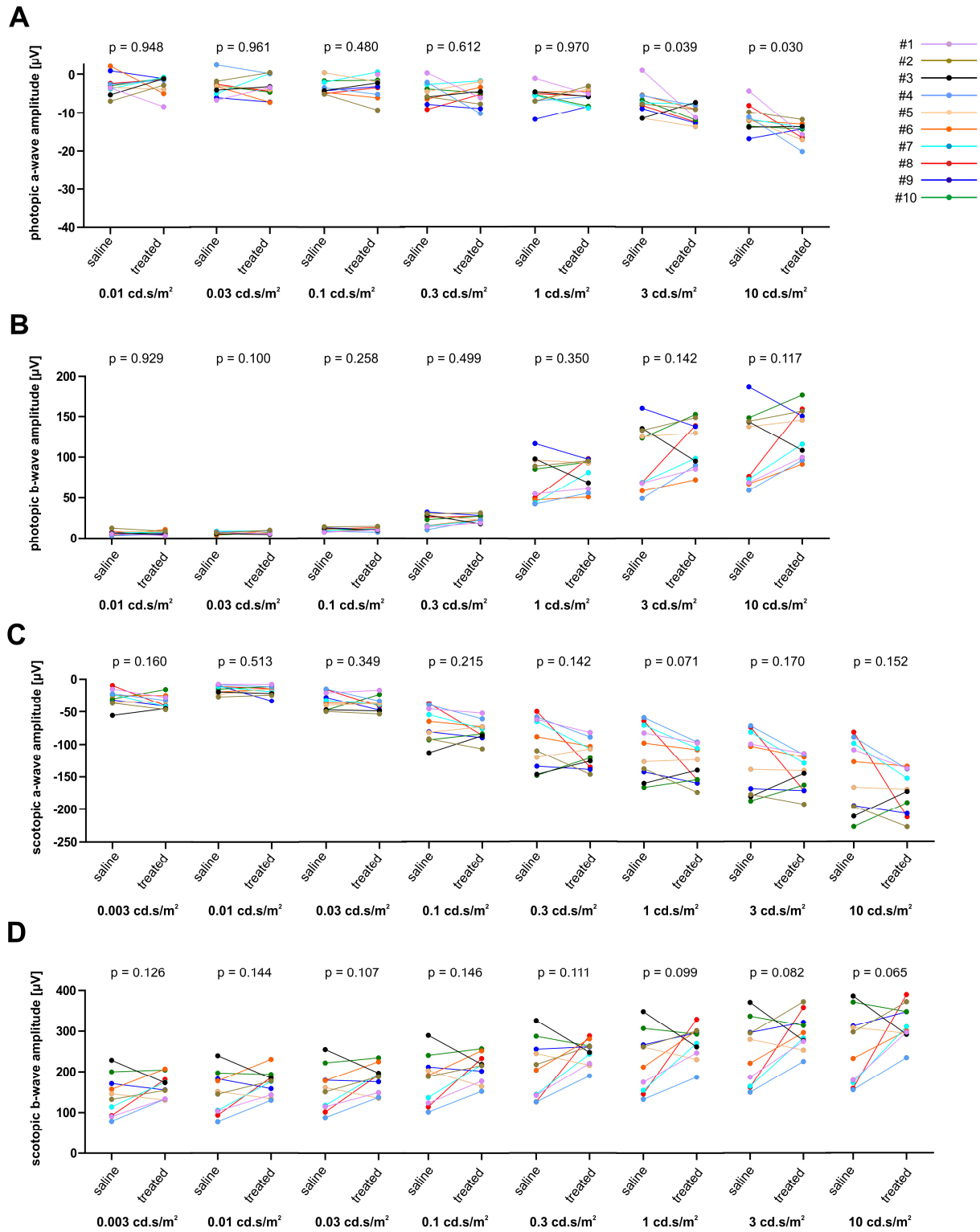


Fig. S4 Single flash photopic ERG responses for all injected $Rho^{+/-}$ mice. A-J Serial responses to increasing flash stimuli were recorded from the *Opn1mw* expressing (+/- treated, left) and control NaCl-injected (+/- saline, right) eyes of $Rho^{+/-}$ mice (#1-10) under light-adapted conditions one year after injection.



E

p-values *belonging to main Fig. 4*

Amplitude	Comparison	0.003 cd.s/m ²	0.01 cd.s/m ²	0.03 cd.s/m ²	0.1 cd.s/m ²	0.3 cd.s/m ²	1 cd.s/m ²	3 cd.s/m ²	10 cd.s/m ²
photopic a-wave	treated vs. saline		0.9973	0.9992	0.9022	0.8618	0.9994	0.0769	0.031
	treated vs. WT		0.4187	0.1489	0.8713	0.8831	0.5076	0.3640	0.0083
	saline vs. WT		0.4069	0.1342	0.6110	0.9958	0.5441	0.5019	0.9716
photopic b-wave	treated vs. saline		0.9959	0.2891	0.6838	0.8499	0.7751	0.5844	0.5219
	treated vs. WT		0.9915	0.9710	0.4000	0.3592	0.7271	0.5337	0.1500
	saline vs. WT		0.9994	0.3523	0.1477	0.3383	0.4034	0.1510	0.0492
scotopic a-wave	treated vs. saline	0.4231	0.8137	0.6938	0.6365	0.4483	0.4114	0.5635	0.4817
	treated vs. WT	0.0083	0.3847	0.0122	0.0002	0.0002	0.0018	0.0011	0.0083
	saline vs. WT	0.0019	0.0849	0.0043	0.0014	0.0014	0.0013	0.0045	0.0080
scotopic b-wave	treated vs. saline	0.4183	0.4485	0.4236	0.4226	0.2735	0.2459	0.2129	0.1665
	treated vs. WT	0.0313	0.0107	0.0193	0.0038	0.0008	0.0142	0.0658	0.0554
	saline vs. WT	0.0160	0.0091	0.0091	0.0049	0.0040	0.0059	0.0116	0.0103

Fig. S5 Pairwise comparison of ERG amplitudes between treated and saline-injected eyes. **A-D** Pairwise plot (saline vs. treated) of the ERG measurements for each individual animal (#1-10) as indicated. p-values (Paired t-test, two-tailed) are shown above the corresponding measurements. **E** p-values of single comparisons (treated vs. saline, treated vs. wild type and saline vs. wild type) for all scotopic and photopic a- and b-wave amplitudes shown in main Fig. 4 (two-way ANOVA with Tukey's post-hoc test).

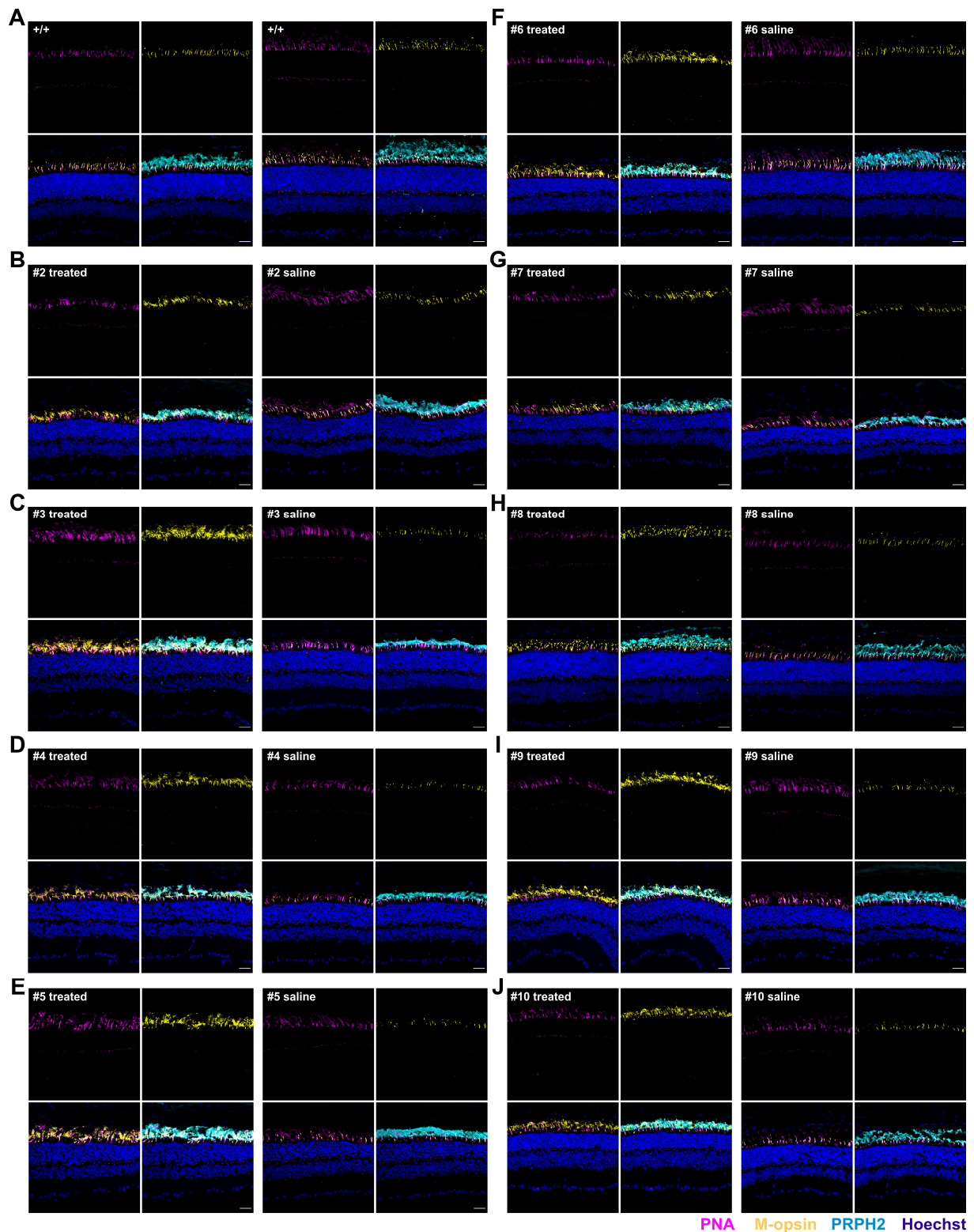
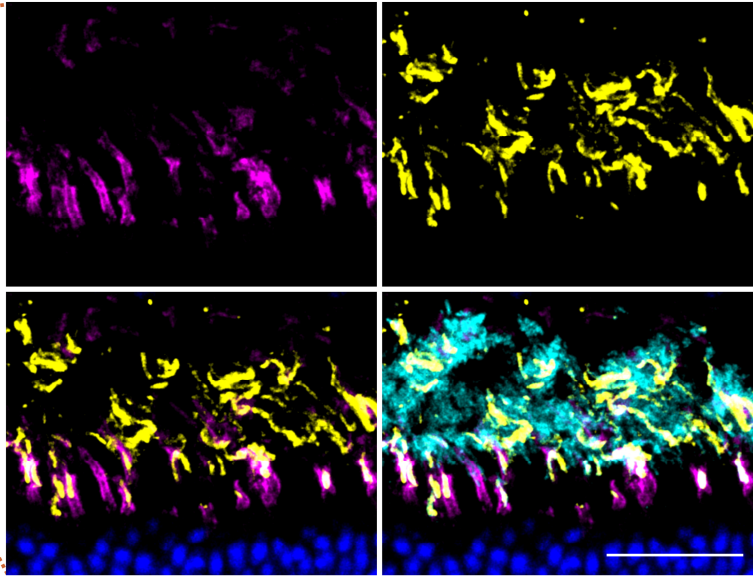
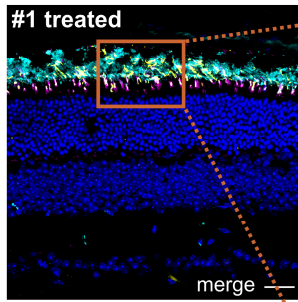
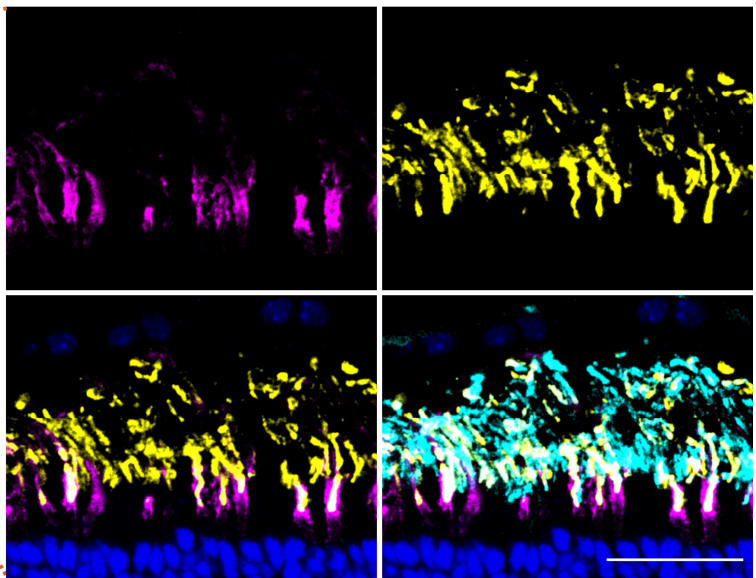
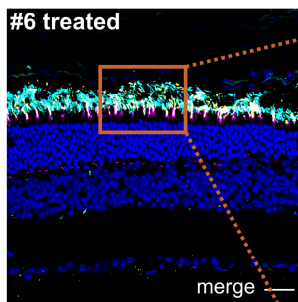


Fig. S6 Transactivation of *Opn1mw* in retinal cryosections of $Rho^{+/-}$ mice. A-J Immunolabeling of retinas from untreated WT (+/+ A) and $Rho^{+/-}$ mice #2-10 (B-J) either injected with split dCas9-VPR and *Opn1mw*-specific sgRNAs (treated, left panel) or with saline (right panel) at one year post-injection. PRPH2 (cyan) was used as rod and cone outer segment marker and PNA (magenta) was used as marker for cones. Scale bar 30 μ m.

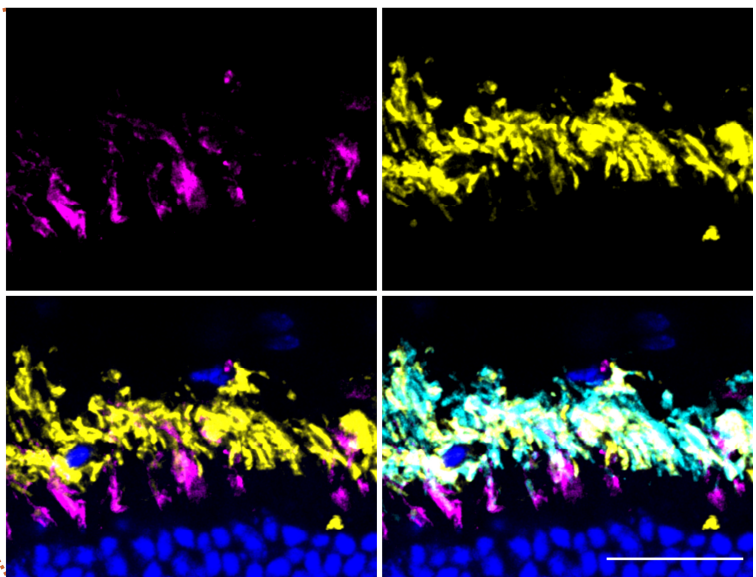
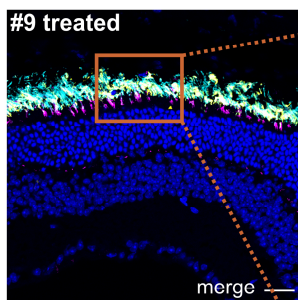
A



B



C



PNA M-opsin PRPH2 Hoechst

Fig. S7 High magnification images of retinas from treated $Rho^{+/-}$ mice. A-C Left panel, Representative sections of immunolabeled retinas from $Rho^{+/-}$ mouse #1, #6 and #9 injected with split dCas9-VPR and *Opn1mw*-specific sgRNAs (treated). Right panel, Magnifications of the areas indicated by the brown rectangles in the left panel. PRPH2 (cyan) was used as rod and cone outer segment marker and PNA (magenta) was used as marker for cones. Scale bar 30 μ m.

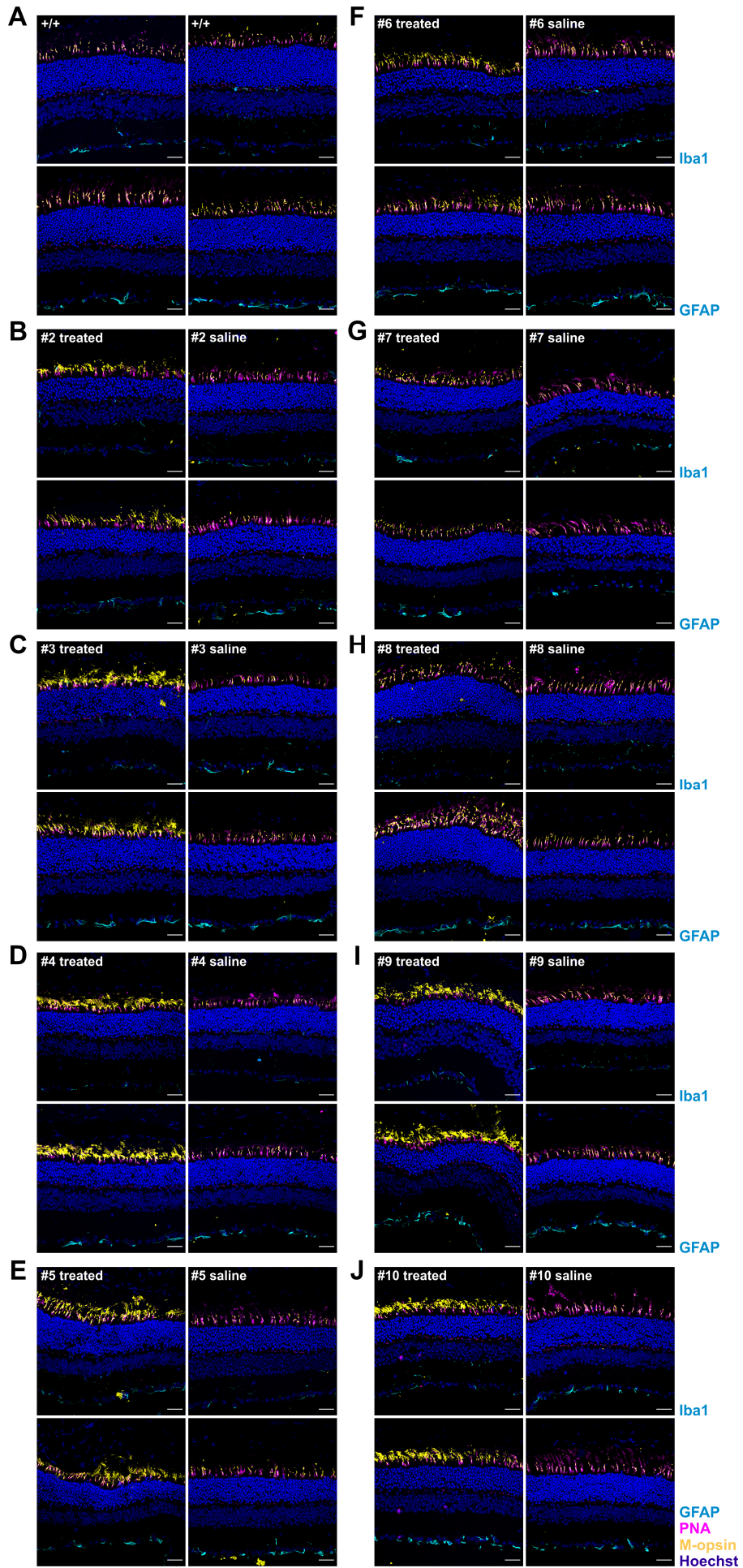
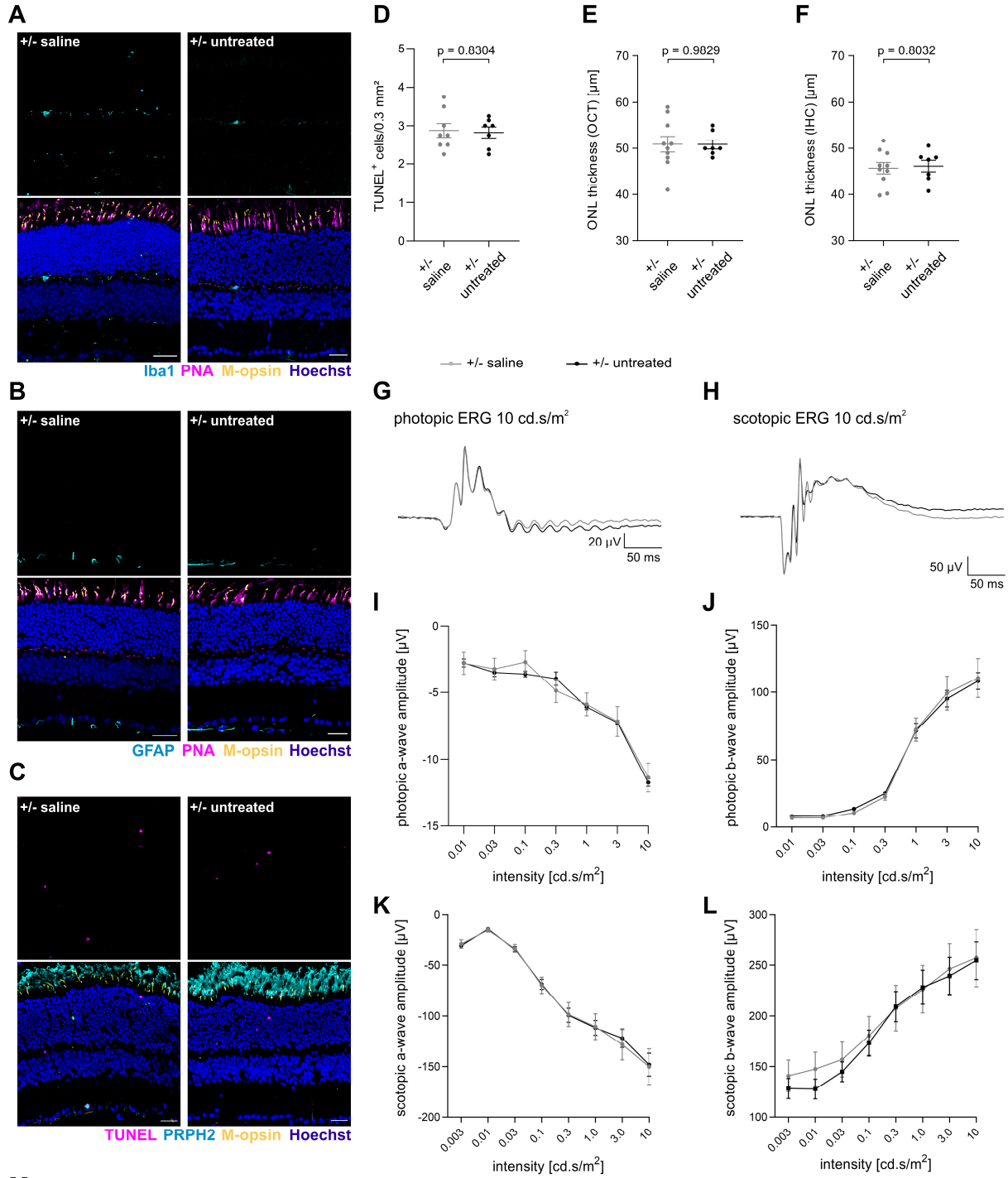


Fig. S8 Transactivation of *Opn1mw* in *Rho*^{+/-} mice does not evoke any obvious gliosis or immune response. A-J Immunolabeling of retinas from untreated wild type (+/+, A) and *Rho*^{+/-} mice #2-10 (B-J) either injected with split dCas9-VPR and *Opn1mw*-specific sgRNAs (treated, left panel) or with saline (right panel) at one year post-injection. Iba1-labeling (cyan, upper panel) was used to visualize microglial cells and GFAP staining (cyan, lower panel) to mark reactive gliosis. Scale bar 30 μ m.



M

		p-values								
		Amplitude	0.003 cd.s/m ²	0.01 cd.s/m ²	0.03 cd.s/m ²	0.1 cd.s/m ²	0.3 cd.s/m ²	1 cd.s/m ²	3 cd.s/m ²	10 cd.s/m ²
+/- saline vs. +/- untreated	photopic a-wave			0.9760	0.7682	0.3356	0.3938	0.8210	0.9428	0.7389
	photopic b-wave			0.2668	0.1298	0.0073	0.3862	0.9376	0.7721	0.8895
	scotopic a-wave	0.7114	0.7333	0.8772	0.9124	0.9544	0.9402	0.7185	0.9259	
	scotopic b-wave	0.5111	0.3220	0.5485	0.7560	0.9549	0.9418	0.8264	0.9443	

Fig. S9 Characterization of untreated one-year-old Rho^{+/-} mice. **A-C** Representative immunostainings of the retina from a NaCl-injected Rho^{+/-} mouse #8 (saline, left panel) one year post-injection and from an untreated age- and background-matched Rho^{+/-} mouse (untreated, right panel). Iba1-labeling was used to visualize microglial cells (A) and GFAP staining to mark reactive gliosis (B). **C, D** TUNEL staining and the corresponding quantification. Scale bar 30 μm. **E-F** ONL thickness measurements originating from OCT recordings (E) or

post mortem histological analysis of retinal cryosections (IHC, F). **G-H** Averaged photopic (left) or scotopic (right) ERG traces for both groups at 10 cd.s/m². **I-L** Photopic and scotopic a- and b-wave amplitudes across different light intensities. **M** Summary of the individual p-values for the a- and b-wave amplitudes shown in **I-L**. For all panels, statistical analysis was conducted using an unpaired t-test with Welch's correction (two-tailed).

Table S1 sgRNA sequences and primers used in this study.

Target gene	sgRNA sequence 5' – 3'	sgRNA position
<i>Cnga1</i> promoter	TAGGCGACCGGCTTTGAGAA CTGTGGAAGTCTCCAAACGC TCTTCTCTCTCGGCGCTATG	1 -104 bp to TSS 2 -270 bp to TSS 3 -309 bp to TSS
<i>Opn1mw</i> promoter	GTTTGGGGGCCTTTAAGGTA CCTGAGCCACCCCTGTGGAT TAGCTCTTGCTTGTTTACAA	1 -60 bp to TSS 2 -159 bp to TSS 3 -260 bp to TSS
	GCTCCCATGGAAAAGCGGGT CTGATCTCTTAATTGGGCC TTGTGGACCAGAGTGTGAGT	4 -510 bp to TSS 5 -104 bp to TSS 6 -343 bp to TSS
<i>E. coli lacZ</i>	GTCTGACCGATGATCCGCGC	Gene body
Primer name	Primer sequence 5' – 3'	
qPCR mCnga1 forward	AACGAGCCATTTGTGCTGC	
qPCR mCnga1 reverse	TGGTTAGTTTAATATCTGCGCTTGT	
RT-PCR mCnga1 forward	GTCGTGGTTATTGATCCTTCAGG	
RT-PCR mCnga1 reverse	TTGACCAGCTTTTCAGTCCTGTA	
mOpn1mw forward	GGAGCAGGTACTIONGGCCTTATG	
mOpn1mw reverse	GGAGGTAGCAGAGCACGATG	
mOpn1sw forward	ACAAAAAGTTGCGACAGCCC	
mOpn1sw reverse	CCATCCTGTCACTAGACCTGC	
Cas9 forward	AGTACAAGGTGCCGAGCAAA	
Cas9 reverse	CCGTGCTGTTCTTTTGAGCC	
mAlas forward	TCGCCGATGCCATTCTTATC	
mAlas reverse	GGCCCCAACTTCCATCATCT	