Supplementary Materials for

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

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Figs. S1 to S9
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-dCas9N and rAAV-CMVmini-dCas9C-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 µm. 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 (Cas9FL). H Quantification of reconstitution efficiency by ratiometric analysis of the Cas9FL and Cas9N 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-dCas9N + rAAV-CMV-dCas9C-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 Rho<sup>−/−</sup> 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.
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<sup>+/−</sup> mice. A-J Serial responses to increasing flash stimuli were recorded from the Opn1mw expressing Rho<sup>+/−</sup> (+/−...
treated, left) and NaCl-injected (+/- saline, right) eyes of Rho<sup>+/−</sup> mice (#1-10) under dark-adapted conditions one year after injection.
Fig. S4 Single flash photopic ERG responses for all injected Rho<sup>+</sup> 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<sup>+</sup> mice (#1-10) under light-adapted conditions one year after injection.
### p-values belonging to main Fig. 4

| Amplitude   | Comparison | 0.003 cd.s/m² | 0.01 cd.s/m² | 0.03 cd.s/m² | 0.1 cd.s/m² | 0.3 cd.s/m² | 1 cd.s/m² | 3 cd.s/m² | 10 cd.s/m² |
|-------------|------------|---------------|--------------|--------------|-------------|-------------|-----------|-----------|-----------|
| Photopic a-wave | treated vs. saline | 0.9673 | 0.9992 | 0.9022 | 0.8618 | 0.9944 | 0.0769 | 0.031 |
|              | treated vs. WT       | 0.4187 | 0.1489 | 0.6713 | 0.8383 | 0.5076 | 0.340 | 0.063 |
|              | saline vs. WT        | 0.4069 | 0.1342 | 0.6110 | 0.9586 | 0.5414 | 0.5019 | 0.9716 |
| Photopic b-wave | treated vs. saline | 0.9959 | 0.2951 | 0.6838 | 0.8409 | 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        | 0.9944 | 0.3023 | 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 | 0.6635 | 0.4817 |
|              | treated vs. WT       | 0.0083 | 0.3647 | 0.0122 | 0.0002 | 0.0002 | 0.0018 | 0.0011 | 0.0083 |
|              | saline vs. WT        | 0.0019 | 0.0949 | 0.0043 | 0.0014 | 0.0013 | 0.0045 | 0.0080 |
| Scotopic b-wave | treated vs. saline | 0.4183 | 0.4489 | 0.4266 | 0.4226 | 0.2735 | 0.2499 | 0.2129 | 0.1905 |
|              | treated vs. WT       | 0.0313 | 0.0107 | 0.0193 | 0.0036 | 0.0008 | 0.0142 | 0.0058 | 0.0054 |
|              | saline vs. WT        | 0.0180 | 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<sup>−/−</sup> mice. A-J Immunolabeling of retinas from untreated WT (+/+ A) and Rho<sup>−/−</sup> 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 µm.
Fig. S7 High magnification images of retinas from treated Rho<sup>−/−</sup> mice. A-C Left panel, Representative sections of immunolabeled retinas from Rho<sup>−/−</sup> 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 µm.
**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 µm.
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 |
|------------------|------------------------|----------------|
| Cnga1 promoter   | TAGGCGACCGGCTTTTGAGAA  | 1 -104 bp to TSS|
|                  | CTGTGGAAGTCTCACAACGC   | 2 -270 bp to TSS|
|                  | TCTTCTCTCTCGGCACCTATG  | 3 -309 bp to TSS|
| Opn1mw promoter  | GTTTGGGGGCTCTTTAAGGTA  | 1 -60 bp to TSS |
|                  | CCTGAGCCACCCCTGTGGAT   | 2 -159 bp to TSS|
|                  | TAGCTCTTGCTTTTTTACA    | 3 -260 bp to TSS|
|                  | GCTCCCATGGAAAAAGCGG    | 4 -510 bp to TSS|
|                  | GCTGATCTCTTAAATGGGGCC  | 5 -104 bp to TSS|
|                  | TTGTGGGACCAGAGTGTGAGT  | 6 -343 bp to TSS|
| E. coli lacZ     | GTCTGACCGATGATCCGC    | Gene body      |

| Primer name      | Primer sequence 5’ – 3’ |
|------------------|-------------------------|
| qPCR mCnga1 forward | AACGAGCCATTTGTGCTGC    |
| qPCR mCnga1 reverse  | TGGTTAGTTTAATATCTCGCCTTGT|
| RT-PCR mCnga1 forward | GTCGTGTTATTGATCCTCAGG  |
| RT-PCR mCnga1 reverse  | TTGACCAGTTTTTTCAGTCCTGTA|
| mOpn1mw forward     | GGAGCAGGTACTGCCCTTATG  |
| mOpn1mw reverse      | GGAGGTAGCAGACAGCATG    |
| mOpn1sw forward      | ACAAAGGTTGGCGACAGCCC   |
| mOpn1sw reverse      | CCATCCTGTACATGAGCTGC   |
| Cas9 forward         | AGTACAAGGTGCCAGCAAA    |
| Cas9 reverse         | CCGTGCTGTCTTTTGAGCC    |
| mAlas forward        | TCGCCGATGCCCATTTATC    |
| mAlas reverse        | GGCCCCAACTTTCCATCTCT   |