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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☒	☐	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☒	☐	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	To identify functionally equivalent genes for trans-activation, the software and search algorithms like InterPro (https://www.ebi.ac.uk/interpro/) or NCBI protein blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins) were used. The amino acid identity can be evaluated using alignment tools like ApE (https://jorgensen.biology.utah.edu/wayned/ape/) or Benchling (https://www.benchling.com/) software. To design sgRNAs we suggest using CRISPOR (http://crispor.tefor.net/). The PrimeView 5.31 software (GE Healthcare) was used to monitor the purification of the rAAVs with the ÄKTA system. The QuantStudioTM design & analysis software (Thermo Fisher Scientific) was used to prepare and analyze the qRT-PCR experiments. For confocal microscopy and imaging the Las X software (Leica Microsystems) was used.
Data analysis	To analyze the images acquired by confocal microscopy we recommend using Fiji (https://fiji.sc), an open-source image processing software. Plotting was performed with GraphPad Prism 9 (GraphPad Software).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Fig. 5 shows example data that was obtained with this protocol. Source data are provided with this protocol. Additional data related to this protocol can be found in the original paper or may be requested from the authors.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to predetermine sample size. Sample sizes were chosen based on previous experience. At least triplicates were used to meet the minimal requirements for statistical analysis. Effort was taken to keep the number of animals at a minimum.
Data exclusions	No data was excluded from the analyses.
Replication	At least 3 biological replicates were used for each experiment except for the RNAseq. All attempts at replication were successful.
Randomization	All animals and cells were randomly assigned to the experimental or control group.
Blinding	qPCR values were normalized to the internal control, thus blinded experiment is not necessary. All other experiments were not blinded.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Rabbit anti-opsin red/green (M-opsin) antibody (1:300), supplier: Merck, catalog number: AB5405, polyclonal antibody Validation: The antibody was validated by the manufacturer for Immunohistochemistry by stainings of mouse retina tissue. Mouse anti-Rhodopsin Antibody, CT, last 9 amino acids (1:2000), supplier: Sigma Aldrich, catalog number: MAB5356, clone Rho 1D4 Validation: The antibody was validated by the manufacturer by Westernblot using isolated bovine rod outer segment and immunohisto/cytochemistry with fixed frozen tissue sections. Mouse anti-Cnga1 monoclonal antibody (1:30), supplier: Molday laboratory, clone: PMc1D1 Validation: Cnga1 expression was only detected in 661W in which Cnga1 was transactivated by dCas9-VPR and was absent in

untreated 661W cells. Additionally the antibody was validated by the manufacturer.

Rabbit anti-SpCas9 antibody (1:1000), supplier: Diagenode, catalog number: C15310258, polyclonal antibody
Validation: The antibody was validated by the manufacturer by Westernblot using protein lysates of HEK293T cells stably expressing nuclease dead Cas9 and HEK293 cells transfected with dCas9. Immunocytochemistry was performed with HeLa cells expressing Cas9

Goat Alexa488 anti-mouse IgG (1:800), supplier: Thermo Fisher, catalog number: A-11001, polyclonal antibody

Donkey Cy3 anti-rabbit IgG (1:400), supplier: Merck Millipore, catalog number: AP182C, polyclonal antibody, Lot 2912152

Goat Cy5 anti-mouse IgG (1:400), supplier: Jackson, catalog number: 115-175-146, polyclonal antibody

Validation: please see above

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	661W cells were kindly provided by Prof. Muayyad Al-Ubaidi, University of Housten, US. HEK293T cells were ordered from DSMZ - German Collection of Microorganisms and Cell Cultures GmbH (cat. no. ACC305). Mouse embryonic fibroblasts (MEFs) were generated as described in Xu J. Preparation, culture, and immortalization of mouse embryonic fibroblasts. Curr Protoc Mol Biol. 2005 May;Chapter 28, doi: 10.1002/0471142727.mb2801s70.
Authentication	661W: cell lines were not authenticated. HEK293T: DSMZ states that they comprehensively perform authentication (Short Tandem Repeat profiling) and quality control tests on all distribution lots of cells lines. In cell culture the HEK293T cells had shown typical morphology and cell growth. MEFs: cell lines were not authenticated.
Mycoplasma contamination	Cell lines were not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Rho P23H/+ mice were initially bougth from the Jackson Laboratory. Rho P23H/+ and C57BL/6J WT mice were obtained by in-house breeding and maintained on a C57BL/6J background. Experiments were performed with both female and male mice. Age of mice used in the animal experience: qRT-PCR: injection at 3 weeks, Retina isolation 4 weeks after injection RNA-seq: injection at 3 weeks, Retina isolation 4 weeks after injection IHC: injection at 3 weeks, Eye cup isolation 4 weeks after injection
Wild animals	No wild animals were used.
Field-collected samples	No field-collected samples were used.
Ethics oversight	All animal studies were approved by the Regierung von Oberbayern, were in accordance with German laws on animal welfare (Tierschutzgesetz), and were performed in compliance with widely accepted ethical standards. Effort was taken to keep the number of animals at a minimum.

Note that full information on the approval of the study protocol must also be provided in the manuscript.