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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 Authors & Referees 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	For immunostaining imaging: Zeiss LSM 710 Multiphoton Laser Scanning Confocal, Leica TCS SP5 confocal microscope (Leica Microsystems) and Nikon 80i with Neurolucida and StereoInvestigator For axon cross section: Nikon microscope (Eclipse E800, Nikon, Japan) with the DP Controller v. 1.2.1.108 and the DP Manager v. 1.2.1.107 software (Olympus) For PERG: Celeris, Full-Field and Pattern Stimulation for the rodent model For RT-qPCR: LightCycler 480 thermocycler (Roche) For RNAseq and RRBS: sequenced using on the Illumina Hiseq2500/4000
Data analysis	Statistical analyses were performed using GraphPad Prism 7/8 (GraphPad Software Inc., La Jolla, CA). For images: ImageJ Fiji, version 2.0.0-rc- 69/1.52n) For RNA-Seq analysis: R software version 3.4.2; edgeR R package version 3.26; hisat2 v2.1.0; featureCounts v1.6.4; AmiGO v2.5.12 For DNA methylation analysis: trim galore v0.4.1; Bismark v0.15.0

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/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

RRBS data for DNA methylation analysis and RNA-seq data are available in the Biosample database (NCBI) and under BioProject PRJNA655981. Illumina Human Methylation EPIC array data is available in the GEO database (NCBI) and under GSE147436. Associated figures are Fig. 2d and e, Fig. 4c-e, Extended Data Fig. 5g-k,

Extended Data Fig. 8e-g, Extended Data Fig. 9i, j, Extended Data Fig. 10a-c and e-l. All other relevant data that support the findings of this study are available from the corresponding author upon reasonable request.

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	Although no statistical methods were used to predetermine sample size, we took into account former publications of the group (Parker, et al, 2008, Science; Sun, et al, Nature, 2011; Krishnan, et al, J Immunol, 2016; Bei, et al, Cell, 2016), to estimate the number of animals needed in each experiment (e.g., n = 4-7 for optic nerve crush study, n=5-20 for elevated IOP model and optomotor).
Data exclusions	(1) Mice that had intravitreal bleeding or developed signs of inflammation (clouding or an edematous cornea) during or post intravitreal injection were excluded from vision test and further histological analysis, exclusion criteria were pre-determined before the experiments; (2) Optic nerves broken during dissection were not processed for accessing axon regeneration; (3) Retinas infected with AAV2-tTA; TRE-d2EGFP were assessed for GFP signal during FACS and 1 sample that failed to show GFP signal was excluded from the group (Fig. 2d).
Replication	For in vivo experiments using rodent, biological replicates and independent cohorts of mice were used; for in vitro experiments, biological replicates as well as technical triplicates were used to ensure reproducibility. All attempts at replication were successful.
Randomization	The mice were randomly assigned into different experimental groups whenever possible, except in experiments required specific genotypes.
Blinding	Researchers were blinded to group allocation during data collection for all animal experiment, by assigning numbers to animals without showing treatment condition. Take experiments on pattern electroretinogram and quantification of optic nerve axons in glaucoma model for example, the person performing the recording or quantification did not know the treatment group of the animal. For cell culture experiment, researchers were not blinded to the treatment condition to avoid any plate mixing. Data analysis was not blinded, as data collected from numbered animals has to be distributed to individual groups.

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
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used	Staining: Mouse anti-Oct4 (1:100, BD bioscience 611203), Rabbit anti-Sox2 (1:100, Cell signaling 14962), Goat anti-Klf4 (1:100, R&D system AF3158), Rabbit anti-phosphorylated S6 Ser235/236 (1:100, Cell Signaling 4857), Mouse anti-Brn3a (1:200, EMD Millipore MAB1585), Rabbit anti-Ki67(1:100, Abcam ab15580), Mouse anti-AP2 alpha (1:100, Developmental Studies Hybridoma Bank 3B5), Rabbit anti-pStat3 (Tyr705) (1:100, Cell signaling 9145S), Rat anti-HA (1:400, Roche 11867423001), Rabbit anti-5mC (1:100, Cell signaling 28692S), Rabbit anti-5hmC (1:100, Active Motif 39769), Rat anti-BrdU (1:200, Abcam ab6326), Rabbit anti-Olig2 (1:100, Novusbio NBP1-28667), Chicken anti-GFP (1:10,000, Aves Labs GFP-1020), Guinea pig anti-RBPMS (1:400, Raygene custom order A008712 to peptide GGKAEKENTPSEANLQEEEVRC) and Thy1.2-PE-Cy7 antibody (1:2000, Invitrogen 25-0902-81). Western Blot: Rabbit anti-Sox2 (1:100, EMD Millipore, AB5603), Mouse anti-Klf4 (1:1,000, ReproCell, 09-0021), Rabbit anti-p-S6 (S240/244) (1:1,000, CST, 2215), Mouse anti-S6 (1:1,000, CST, 2317), Mouse anti-β-Tubulin (1:1,000, Sigma-Aldrich, 05-661), Mouse anti-β-Actin–Peroxidase antibody (1:20,000, Sigma-Aldrich, A3854).
Validation	Mouse anti-Oct4 (1:100, BD bioscience 611203): Mouse and human, Immunofluorescence, validated on manufactures's website:

https://www.bdbiosciences.com/us/applications/research/stem-cell-research/cancer-research/human/purified-mouse-anti-oct-34-40oct-3/p/611203

Rabbit anti-Sox2 (1:100, Cell signaling 14962), Mouse and human, Immunohistochemistry, validated on manufactures's website: https://www.cellsignal.com/products/primary-antibodies/sox2-d1c7j-xp-rabbit-mab/14962?Ntk=Products&Ntt=14962

Goat anti-Klf4 (1:100, R&D system AF3158), Mouse, Immunohistochemistry, validated on manufactures's website: https://www.rndsystems.com/products/mouse-klf4-antibody_af3158

Rabbit anti-phosphorylated S6 Ser235/236 (1:100, Cell Signaling 4857), Mouse, Immunohistochemistry, validated on manufactures's website: https://www.cellsignal.com/products/primary-antibodies/phospho-s6-ribosomal-protein-ser235-236-91b2-rabbit-mab/4857?Ntk=Products&Ntt=4857

Mouse anti-Brn3a (1:200, EMD Millipore MAB1585), Mouse, Immunohistochemistry, validated on manufactures's website: https://www.emdmillipore.com/US/en/product/Anti-Brn-3a-Antibody-POU-domain-protein-clone-5A3.2,MM_NF-MAB1585

Rabbit anti-Ki67(1:100, Abcam ab15580), Mouse, Immunohistochemistry, validated on manufactures's website: https://www.abcam.com/ki67-antibody-ab15580.html

Mouse anti-AP2 alpha (1:100, Developmental Studies Hybridoma Bank 3B5), Mouse, Immunohistochemistry, validated on manufactures's website: https://www.labome.com/product/Developmental-Studies-Hybridoma-Bank/3B5.html

Rabbit anti-pStat3 (Tyr705) (1:100, Cell signaling 9145S), Mouse, Immunohistochemistry, validated on manufactures's website: https://www.cellsignal.com/products/primary-antibodies/phospho-stat3-tyr705-d3a7-xp-rabbit-mab/9145?Ntk=Products&Ntt=9145

Rat anti-HA (1:400, Roche 11867423001), HA peptide sequence (YPYDVPDYA), immunocytochemistry, validated on manufactures's website: https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Roche/Bulletin/1/roahahabul.pdf

Rabbit anti-5mC (1:100, Cell signaling 28692S), All, Immunohistochemistry, validated on manufactures's website: https://www.cellsignal.com/products/primary-antibodies/5-methylcytosine-5-mc-d3s2z-rabbit-mab/28692?Ntk=Products&Ntt=28692

Rabbit anti-5hmC (1:100, Active Motif 39769), All, Immunohistochemistry, validated on manufactures's website: https://www.activemotif.com/catalog/details/39769.html

Rat anti-BrdU (1:200, Abcam ab6326), Not applicable, Immunohistochemistry, validated on manufactures's website: https://www.abcam.com/brdu-antibody-bu175-icr1-proliferation-marker-ab6326.html

Rabbit anti-Olig2 (1:100, Novusbio NBP1-28667), Mouse, Immunohistochemistry, validated on manufactures's website: https://www.novusbio.com/products/olig2-antibody_nbp1-28667

Chicken anti-GFP (1:10,000, Aves Labs GFP-1020), GFP, Immunohistochemistry, validated on manufactures's website: https://www.aveslabs.com/products/green-fluorescent-protein-gfp-antibody

Guinea pig anti-RBPMS (1:400, Raygene custom order A008712 to peptide GGKAEKENTPSEANLQEEEVRC), Mouse, Immunohistochemistry, validated by co-stained with rabbit anti-RBPMS (Abcam ab194213) and observing co-localization at first layer of inner retina (RGCs).

Thy1.2-PE-Cy7 antibody (1:2000, Invitrogen 25-0902-81), Mouse, FACS, validated on manufactures's website: https://www.thermofisher.com/antibody/product/CD90-2-Thy-1-2-Antibody-clone-53-2-1-Monoclonal/25-0902-82

Rabbit anti-Sox2 (1:100, EMD Millipore, AB5603), Mouse, Western Blotting, validated on manufactures's website: https://www.emdmillipore.com/US/en/product/Anti-Sox2-Antibody,MM_NF-AB5603

Mouse anti-Klf4 (1:1,000, ReproCell, 09-0021), Mouse, Western Blotting, validated by dete ting overexpression of Klf4 in liver samples of Rosa26-M2rtTA/Col1a1-tetOP-OKS-mCherry mice treated with Doxycycline.

Rabbit anti-p-S6 (S240/244) (1:1,000, CST, 2215), Mouse, Western Blotting, validated on manufactures's website: https://www.cellsignal.com/products/primary-antibodies/phospho-s6-ribosomal-protein-ser240-244-antibody/2215?Ntk=Products&Ntt=2215&N=4294960393&Nrpp=100&fromPage=plp

Mouse anti-S6 (1:1,000, CST, 2317), Mouse, Western Blotting, validated on manufactures's website: https://www.cellsignal.com/products/primary-antibodies/s6-ribosomal-protein-54d2-mouse-mab/2317?Ntk=Products&Ntt=2317

Mouse anti-β-Tubulin (1:1,000, Sigma-Aldrich, 05-661), Mouse, Western Blotting, validated on manufactures's website: https://www.sigmaaldrich.com/catalog/product/mm/05661?lang=en&region=US

Mouse anti-β-Actin–Peroxidase antibody (1:20,000, Sigma-Aldrich, A3854), Mouse, Western Blotting, validated on manufactures's website: https://www.sigmaaldrich.com/catalog/product/sigma/a3854?lang=en&region=US

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Ear fibroblasts (EFs) were isolated from Reprogramming 4F (Jackson Laboratory 011011) or 3F (Hochedlinger lab, Harvard) mice. SH-SY5Y neuroblastoma cells were obtained from the American Tissue Culture Collection (ATCC, CRL-2266) and maintained according to ATCC recommendations.
Authentication	Primary ear fibroblasts were authenticated using genotyping and qPCR. SH-SY5Y (ATCC CRL-2266) was authenticated using RNA-seq.
Mycoplasma contamination	All cell lines were tested routinely and confirmed negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used in this study

Animals and other organisms

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

Laboratory animals	For optic nerve crush and glaucoma model experiments, C57BL6/J wild type mice were purchased from the Jackson Laboratory (000664). For ageing experiments, young and old females from the NIA Aged Rodent Colonies were used. Rosa26-M2rtTA/ Col1a1-tetOP-OKS-mCherry alleles were a gift from the Hochedlinger lab (Harvard). Rosa-CAG-lox-STOP-lox-Tomato mice were provided by Fan Wang (Duke). Vglut2-IRES-Cre (016963), Vgat-IRES-Cre (016962), Tet2flox/flox (017573) and Rosa26-M2rtTA/ Col1a1-tetOP-OSKM (011011) were purchased from the Jackson Laboratory. All animal work was approved by the Institutional Animal Care and Use Committees (IACUCs) at Harvard Medical School, Boston Children's Hospital, and the Mass Eye and Ear Institution. Animals were housed under 12-hour light/dark cycles 6am-6pm-6am, 70-72°F ambient temperature, and 40-50% humidity.
Wild animals	No wild animals were used in the study.
Field-collected samples	No field collected samples were used in the study.
Ethics oversight	All animal work was approved by the Institutional Animal Care and Use Committees (IACUCs) at Harvard Medical School, Boston Children's Hospital, and the Mass Eye and Ear Institution.

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

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	For Extended data Fig. 8c, SH-SY5Y neuroblastoma cells were obtained from the American Tissue Culture Collection (ATCC, CRL-2266) and maintained according to ATCC recommendations. SH-SY5Y cells were differentiated into neurons and transduced with corresponding AAV.DJ vectors to allow OSK to express for five days. Cells were then harvested and fixed with 70% cold ethanol for 16 hours at 4°C. After fixation, cells were washed twice with PBS and incubated with PBS containing 50 ug/mL propidium iodide (Biotium, 40017) and 100 ug/mL RNase A (Omega) for 1 hour at room temperature. PI-stained samples were then analyzed.
Instrument	BD Biosciences, LSR-II cell analyzer
Software	FCS Express 6
Cell population abundance	The nucleated cells (propidium iodide positive) were gated to get ride of debris, and only single cells (but not doublets and larger aggregates) were selected for cell cycle analysis. The single cells are 85%-97% of all nucleated cells across all samples.

Gating strategy

FSC/SSC profiles were used to exclude debris. Since nucleated cells were later gated based on propidium iodide signal, FSC/SSC gating include more than 95% of the whole cell population to include as many cells/debris as possible. The boundaries between positive and negative propidium iodide staining is clear-cut since propidium iodide is very specific to DNA. The nucleated (propidium iodide positive) cells were gated and scattered on PI-A and PI-W plots and only single cells were gated for cell cycle analysis. G1, S, and G2 gating was determined by FCS Express 6 software and verified by the investigator.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.