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Last updated by author(s): 9/25/2025

Reporting Summary

Nature Portfolio 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 Portfolio 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	Ex vivo microscopy: Zeiss LSM700 and 900 with ZEN blue (v3.1), Zeiss LSM880 Airyscan with ZEN black (v2.3) Zeiss AxioImagerZ.2 with Apotome2 with Zen blue (v3.1). Acquisition of in vivo calcium imaging data was done using a custom Neurolabware scope running MATLAB 2022b and the latest version of Scanbox. Electrophysiology data were acquired using a custom Scientifica patch-clamp rig running Clampex10.5. For flow cytometry, a BD FACS Aria Fusion was used. Bulk sequencing runs were acquired using an Illumina HiSeq 2500. Single-cell sequencing was acquired using an Illumina NovaSeqX. For Western blot image acquisition, a Licor Odyssey Clx running Image Studio v6.0 was used. Visual cliff assay trajectories were recorded with AnyMaze v7.49.
Data analysis	For bulk RNA sequencing data, raw data was converted into FASTQ files using CASAVA (v1.8.2) and tested using FASTQC v0.11.2. Alignment was performed to the mm10 genome using STAR aligner v2.5.1b. Gene expression was quantified using HOMER v4.10. The DESeq2 package (v1.14.1) in R 3.4.3 was run to obtain DEGs. For selection of DEGs , R studio v4.3.1 was used for subsequent analyses. To create heatmaps pheatmapv1.0.12 was used. To perform GSEA and ORA the clusterProfiler package v4.10.0 was used. ReactomePA v1.46.0, GSEAmining v1.12.0, and UpSetR v1.4.0 were also used. eulerr v7.0.2 was used to generate the Venn diagrams. ggplot2 v3.5.2 was used for all other plots. For analysis of Arc smFISH signal widths, the distance tool in ZEN blue (v4.3) was used. For other analysis of smFISH, FIJI (v1.54f) was used. For analysis of WFA, a custom pipeline in CellProfiler (v4.2.5 and v4.2.6) was used. Viral vector and cloning sequences were checked using SnapGene 4.0.0. For cell counting using FIJI, FIJI v1.54f was used with Java v1.8.0_265 and BioFormats v7.1.0. For analysis of Western blots, Image Studio v6.0 was used. For analysis of microglial phagocytosis of WFA, an Anaconda distribution of Python (Python v3.9.16) was used. The segmentations were

created using Napari-assistant-clesperanto (v0.22.1) in a Napari Viewer (v0.4.17) with OpenGL (v4.6.0 NVIDIA 536.19). The GPU used was an NVIDIA GeForce RTX3060. To extract the volumes of the segmentations, the segmentations were saved as tiffs and analyzed in FIJI v1.54f using 3D Object Counter (v2.0) plugin.

For analysis of microglial morphology, a custom pipeline in CellProfiler (v4.2.5 and v4.2.6) was used. R studio (v2023.06.1 Build 524) was used to write the microglial classification code. glmmTMB v1.1.11 and DHARMa v0.4.7 libraries were used for statistical analysis of morphotype proportions.

The mouse trajectories in the visual cliff assay were tracked and analyzed with AnyMaze v7.49.

Electrophysiology data was analyzed using MiniAnalysis from Synaptosoft (v6.0.7) and Clampfit v11.2.

For analysis of two-photon in vivo imaging data, MATLAB R2022a and b and an Anaconda distribution of Python (Python v3.8.8) were both used. For motion correction and ROI detection, suite2p (v0.11.2) was run on a Python v3.8.13.
For FACS diagram in Fig 4, FlowJo v10.10.0 was used to make the exemplary plots in Supplementary Figure 2.
For analysis of single-nucleus RNA sequencing data, reads were mapped onto the mouse genome (mm10) using CellRanger count v8.0.1, and biological replicates were aggregated using CellRanger Aggr v8.0.1. Subsequent analyses were run in R Studio (R version 4.4.1) using Seurat (v5.1.0). Over-representation analysis was performed using clusterProfiler v4.12.6. UCell package v2.8.0 , Augur v1.0.3, scProportiontest v0.0.0.9000, annData v0.8.0, Nebulosa v1.14.0, and scCustomize v3.0.1 were used for subsequent analysis and visualization.
All custom code has been deposited at https://doi.org/10.5281/zenodo./15785197.

GraphPad Prism v8.4.3 and v10.3.0 was used for other statistical analyses not specifically listed above.

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 Portfolio 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Bulk RNA sequencing is deposited at GEO GSE161398 (P7, P14, P28, P120 RiboSeq), GSE99791 (P120 RiboSeq), and GSE259341 (P45 NR, P45 DR, P28 contra and ipsi MD RiboSeq). Single-nucleus RNA sequencing is deposited at GEO GSE298814. Source data are provided with this paper. Two-photon data processed with suite2p is available at https://doi.org/10.5281/zenodo.15785197. Other data available on reasonable request from the authors.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	n/a
Reporting on race, ethnicity, or other socially relevant groupings	n/a
Population characteristics	n/a
Recruitment	n/a
Ethics oversight	n/a

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

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	For bulk RiboSeq, 3-6 mice per sequenced per age or condition based on Boisvert el. 2018 Cell Reports. For smFISH, Western blotting, immunohistochemistry experiments, 2-3 technical replicates (slices or blots) were obtained per mouse per experimental group. 3-6 mice were obtained per condition per experiment. These sample sizes were based on pilot experiments and previous published work from the lab (e.g. Blanco-Suarez et al.,2018 Neuron). For Arc induction smFISH experiments, sample size was based on Blanco-Suarez et al. 2018 Neuron and confirmed with a power analysis with power = 0.80. For electrophysiology, sample size was mice per experimental group. Number of cells and mice were determined by similar studies in the field (Kannan et al., 2016, J Neurosci; Lambo and Turrigiano 2013 J Neurosci; Xin et al. 2024 Nature). For two-photon imaging, sample size was 4 mice per genotype for the monocular deprivation experiments. This was based on relevant literature in the field (e.g. Tan et al., 2020 Neuron). For single-cell RNA sequencing experiments, 2 cortices were pooled per mouse and 2 mice were pooled per biological replicate as described in Farhy-Tselnicker et al. 2021 Elife. Two biological replicates per viral group were collected and sequenced independently to ensure enough cells for analysis (>6500k per group using https://satijalab.org/howmanycells/). Sequencing was performed as a paired-end experiment.
Data exclusions	Any exclusions of mice or tissue were done based on quality control standards detailed in Methods. For electrophysiology, recordings were excluded if the series resistances were > 25 MΩ or changed > 25% during the entire recording. For ex vivo microscopy, data were excluded if viral injection was off-target.
Replication	Reported n's for each experiment reflect biological replicates. Each biological replicate represents at least two technical replicates as noted in each section of the Methods, with the exception of the visual cliff assay which was run once per mouse. Each reported or represented experiment was repeated at least twice in separate cohorts. All conditions within each experiment were processed at the same time (e.g. WT no MD, WT MD, cKO no MD, cKO MD).The findings describing the increased proportion of contralateral cells and reduced proportion of binocular cells in CCN1 cKO mice vs WT mice were also replicated in a second set of experiments done in naive mice (Extended Data Fig.8).
Randomization	Mice were randomly assigned to experimental conditions. For in vivo calcium imaging, the contralateral or ipsilateral eye visual stimulus presentation order was randomized.
Blinding	Experimenters were blinded to the mouse genotype during data analysis and quantification. Blinding to viral group during data analysis/ quantification for microscopy was not possible given that the control group was expressing tdTomato. Blinding to MD vs no MD upon tissue collection was not possible given the visible nature of the manipulation.

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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used

rabbit anti-HA (CST #3724) Clone C29F4
sheep anti-CCN1 (R&D System AF4055) LOT CALW011901A
mouse anti-GFAP (Millipore Sigma 360) clone GA5
goat anti-SOX9 (R&D Systems af3075) LOT WIL0421041
mouse anti-NEUN (Millipore #MAB377) LOT 3248401
mouse anti-parvalbumin (Millipore Sigma p3088) LOT 048M4753V
rabbit anti-IBA1 (Fuji Film Wako #162001) WTF5396, SKG2278
rat anti-CD68 (BioRad #MCA1957GA) Clone FA-11BB
rabbit anti-aggrecan (Millipore AB1031) LOT 4113382
rat anti-myelin basic protein (Millipore MAB386) Clone 82-87, lot 4192189
mouse anti CC-1 (APC) (Millipore OP80-100ug) LOT 4158894
mouse anti BCAS1/NaBC1(5) (Santa Cruz SC-136342) LOT J3124
rabbit anti-OLIG2 (Millipore AB9610) LOT 4112463

rabbit anti-NG2 (Millipore AB5320) LOT 4129719
rat anti -HA (Sigma 11867423001) Clone 3F10
mouse anti-beta actin (Sigma A1978) Batch#000124305
Biotinylated wisteria floribunda agglutinin (WFA, Vector Laboratories B-1355-2) LOT ZG0826

donkey anti-goat Alexa Fluor 488 (Jackson ImmunoResearch 705-545-147) LOT 153047
donkey anti-rabbit Alexa Fluor 568 (ThermoFisher A-10042) LOT 1826664
donkey anti-mouse Alexa Fluor 647 (Jackson ImmunoResearch 715-605-150) LOT 153967
donkey anti-sheep Alexa Fluor 680 (ThermoFisher A21102) LOT 1671889
donkey anti-mouse Alexa Fluor 800 (ThermoFisher A32789) LOT Y1377825

goat anti-mouse Alexa Fluor 647 (ThermoFisher A-21236) LOT 2382182 and 2850263
goat anti-mouse Alexa Fluor 555 (ThermoFisher A21424) LOT 2480093
goat anti-mouse Alexa Fluor Plus 488 (ThermoFisher A32723) LOT ZH395844
goat anti-mouse Alexa Fluor 488 (ThermoFisher A-11001) LOT 2551357

goat anti-rabbit Alexa Fluor 647 (ThermoFisher A-21245) LOT 2497486
goat anti-rabbit Alexa Fluor 555 (ThermoFisher A21429) LOT 2156529
goat anti-rabbit Alexa Fluor 488 (ThermoFisher A-11034) LOT 2752650

goat anti-rat Alexa Fluor 594 (ThermoFisher A-11007) LOT 2300214
goat anti-rat Alexa Fluor Plus 555 (ThermoFisher A48263) LOT ZG393645
goat anti-rat Alexa Fluor Plus 647 (ThermoFisher A48265) LOT WB324586

streptavidin conjugated Alexa Fluor 568 (adult mice, ThermoFisher S-11226) LOT 2506077
streptavidin conjugated Alexa Fluor 647 (adult mice, ThermoFisher S-21374) LOT 2352166

Validation

rabbit anti-HA (CST #3724) - https://www.cellsignal.com/products/primary-antibodies/ha-tag-c29f4-rabbit-mab/3724

sheep anti-CCN1 (R&D System AF4055) - https://www.rndsystems.com/products/mouse-cyr61-ccn1-antibody_af4055

mouse anti-GFAP (Millipore Sigma 360) -https://www.sigmaaldrich.com/US/en/product/sigma/g6171

goat anti-SOX9 (R&D Systems af3075) - https://www.rndsystems.com/products/human-sox9-antibody_af3075

mouse anti-NEUN (Millipore #MAB377) https://www.sigmaaldrich.com/US/en/product/sigma/ZMS377

mouse anti-parvalbumin (Millipore Sigma p3088) https://www.sigmaaldrich.com/US/en/product/sigma/p3088

rabbit anti-IBA1 (Fuji Film Wako #162001) https://labchem-wako.fujifilm.com/us/product/detail/W01W0101-2000.html

rat anti-CD68 (BioRad #MCA1957GA) https://www.bio-rad-antibodies.com/monoclonal/mouse-cd68-antibody-fa-11-mca1957.html?f=purified

rabbit anti-aggrecan (Millipore AB1031) https://www.sigmaaldrich.com/US/en/product/mm/ab1031?mmredirect=1

rat anti-myelin basic protein (Millipore MAB386) Clone 82-87, lot 4192189
https://www.sigmaaldrich.com/US/en/substance/antimyelinbasicproteinantibody1234598765?utm_source=google&utm_medium=cpc&utm_campaign=9416925828&utm_content=95899039392&gad_source=1&gad_campaignid=9416925828&gbraid=0AAAAD8kLQSDRSyDU4DAuGbwqMbXhvAJh&gclid=CjwKCAjwx8nCBhAwEiwA_z__02GvH0-edpztGiJytxgnoEix_KnRj99rE2kPNxPuPCW4cDKQr35UrhoCkk8QAvD_BwE

mouse anti CC-1 (APC) (Millipore OP80-100ug) LOT 4158894 https://www.sigmaaldrich.com/US/en/product/mm/op80?mmredirect=1

mouse anti BCAS1/NaBC1(5) (Santa Cruz SC-136342) LOT J3124
https://www.scbt.com/p/nabc1-antibody-5?srsltid=AfmBOopQ08BW8mfX47Y4tfL6_vgy9C_vmDn_Mq13vmFaRrJ1n8y-pxq1

rabbit anti-OLIG2 (Millipore AB9610) LOT 4112463
https://www.sigmaaldrich.com/US/en/product/mm/ab9610

rabbit anti-NG2 (Millipore AB5320) LOT 4129719
https://www.sigmaaldrich.com/US/en/product/mm/ab5320?srsltid=AfmBOooRC799faSkB2fEXjbn62eO5MKFJwW4OJglTU4ldw-Jjdyo1y_F

rat anti -HA (Sigma 11867423001) Clone 3F10
https://www.sigmaaldrich.com/US/en/product/roche/roahaha

mouse anti-beta actin (Sigma A1978) Batch#000124305
https://www.sigmaaldrich.com/US/en/product/sigma/a1978?utm_source=google&utm_medium=cpc&utm_campaign=20849931024&utm_content=171133062889&gad_source=1&gad_campaignid=20849931024&gbraid=0AAAAD8kLQSuBdw97E3BJq52qhynJZSnn&gclid=CjwKCAjwx8nCBhAwEiwA_z__0_sDNsTEBV04KkP8ydC8Bv2lcIook-pcbD-FIUcjvLFUIslezUSDDxoCpJQQAvD_BwE

Biotinylated wisteria floribunda agglutinin (WFA, Vector Laboratories B-1355-2) https://vectorlabs.com/products/biotinylated-wisteria-floribunda-lectin

donkey anti-goat Alexa Fluor 488 (Jackson ImmunoResearch 705-545-147) https://www.jacksonimmuno.com/catalog/products/705-545-147

donkey anti-rabbit Alexa Fluor 568 (ThermoFisher A-10042) https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A10042

donkey anti-mouse Alexa Fluor 647 (Jackson ImmunoResearch 715-605-150) https://www.jacksonimmuno.com/catalog/products/715-605-150

goat anti-mouse Alexa Fluor 647 (ThermoFisher A-21236) https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21236

goat anti-rabbit Alexa Fluor 647 (ThermoFisher A-21245) https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21245

goat anti-mouse Alexa Fluor 488 (ThermoFisher A-11001) https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11001

goat anti-rabbit Alexa Fluor 488 (ThermoFisher A-11034) https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11034

goat anti-rat Alexa Fluor 594 (ThermoFisher A-11007) https://www.thermofisher.com/antibody/product/Goat-anti-Rat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11007

donkey anti-sheep Alexa Fluor 680 (ThermoFisher A21102) https://www.thermofisher.com/antibody/product/Donkey-anti-Sheep-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21102

donkey anti-mouse Alexa Fluor 800 (ThermoFisher A32789) https://www.thermofisher.com/antibody/product/Donkey-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A32789

goat anti-mouse Alexa Fluor 555 (ThermoFisher A21424) https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21424

goat anti-mouse Alexa Fluor Plus 488 (ThermoFisher A32723) https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A32723

goat anti-rabbit Alexa Fluor 555 (ThermoFisher A21429) https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21429

goat anti-rat Alexa Fluor Plus 555 (ThermoFisher A48263) https://www.thermofisher.com/antibody/product/Goat-anti-Rat-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A48263

goat anti-rat Alexa Fluor Plus 647 (ThermoFisher A48265) https://www.thermofisher.com/antibody/product/Goat-anti-Rat-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A48265

streptavidin conjugated Alexa Fluor 568 (adult mice, ThermoFisher S-11226) https://www.thermofisher.com/order/catalog/product/S11226

streptavidin conjugated Alexa Fluor 647 (adult mice, ThermoFisher S-21374) https://www.thermofisher.com/order/catalog/product/S21374

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HEK293T (ATCC# CRL-3216) modified to constitutively express Cre recombinase were obtained from Dr. Gerald Pao from the Salk Institute for Biological Studies.
Authentication	HEK293T (ATCC# CRL-3216) are authenticated by the vendor and confirmed in house by morphology.
Mycoplasma contamination	Mycoplasma contamination was not routinely tested.
Commonly misidentified lines (See ICLAC register)	No cross-contaminated or misidentified cell lines were identified.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Rats: Sprague-Dawley rats (Charles Rivers) were used at P1-2 for the preparation of primary cortical astrocyte cultures. Mus musculus: Astrocyte-Ribotag mice: generated by crossing GFAP-cre hemizygous females (B6.Cg-Tg (GFAP-cre)73.12Mvs/J, Jax #012886) to homozygous flox-Rpl22-HA males (B6N.129-Rpl22tm1.1Psam/J, Jax #011029). Male mice hemizygous for Cre and heterozygous for flox-Rpl22-HA (Rpl22-HA+; GFAPcre+) were used for all experiments. Mice used at P28, P45 normal reared or dark reared, and P120. Wild-type (WT) C57Bl6/J mice were used (Jax 000664) for experiments. For single nucleus RNA (snRNA) sequencing, male WT mice were used at P28. For single molecular fluorescence in situ hybridization experiments, mice were used at P7, P14, P28, P45 (normal and dark rearing), and P120. For experiments validating the RiboSeq, only male mice were used. For juvenile overexpression, injections were done at P14 or P11. For adult overexpression, injections were done at 3 months of age. MD/ME was performed at P23 or P28 for juvenile mice and 4 months of age for adult mice. For adult knock-out experiments, CCN1fl/fl mice were a gift from Dr. Lester Lau 52 and were maintained on a C57Bl6/J background. These mice were crossed to mice expressing tamoxifen inducible Cre recombinase under an astrocytic promoter for temporal elimination (Aldh1l1cre-Ert2, Jax 02965553). Experimental mice were homozygous for the CCN1 floxed allele and either Cre- or Cre+ (WT or CCN1 cKO). For adult cKO experiments, mice were injected intraperitoneally (i.p.) with 75 mg/kg of tamoxifen (MP Biomedicals #156738) for 5 consecutive days at 1 month of age and collected at 4 months of age. For juvenile cKO experiments, mice were injected i.p. once at P3-4 with 100 mg/kg of tamoxifen and collected at P28. Mice of both sexes were used and sexes were noted. Cranial window implantations were done at 3 months of age. MD/ME was performed at P28 for juvenile mice and 4 months of age for adult mice. Housing conditions: All animal experiments were approved by the Salk Institute Institutional Animal Care and Use Committee (IACUC). Rats and mice were typically housed with a standard 12-hour light/dark cycle in the Salk Institute animal facilities, with lights on at 6am and lights off at 6pm. Dark reared mice were housed in 24-hour dark cycle since birth. Mice and rats were provided access to food and water ad libitum. Humidity ranged from 38-62% and temperature from 68 to 72F.
Wild animals	No wild animals were used in this study.
Reporting on sex	Animals of both sexes were used except for smFISH validation studies of RiboSeq, RiboSeq, and single-nucleus RNA sequencing (only males). Sexes were noted.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	All animal experiments and procedures were approved by the Salk Institute Institutional Animal Care and Use Committee (IACUC)

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

Plants

Seed stocks	n/a
Novel plant genotypes	n/a
Authentication	n/a

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	Nuclei were extracted from the dissected visual cortices using the protocol in Farhy-Tselnicker et al., 2021 and nuclei labeled with Hoechst 33342 solution. Briefly, Tissue was manually homogenized on ice using a two-step Dounce homogenizer (A and B) in NIMT buffer, containing (in mM): 250 sucrose, 25 KCl, 5 MgCl2, 10 Tris-Cl pH 8, 1 DTT; a 1:100 dilution of Triton X100, Protease Inhibitor Cocktail; a 1:1000 dilution of RNaseOUT Recombinant Ribonuclease Inhibitor; and SUPERase In RNase Inhibitor. Homogenized samples were mixed with 50% iodixanol (OptiPrep Density Gradient Medium) and loaded onto 25% iodixanol cushion, and centrifuged at 10,000 g for 20 min at 4°C in a swinging bucket rotor (Sorval HS-4). Pellets resuspended in ice-cold DPBS with 1:1000 dilution of RNaseOUT Recombinant Ribonuclease Inhibitor; SUPERase In RNase Inhibitor (Thermo #AM2694). Nuclei were then incubated for 7 min on ice with Hoechst 33342 solution (final concentration 0.5 µM), followed by centrifugation at 1000 g for 10 min at 4°C to pellet nuclei. Pellets were resuspended in blocking buffer containing DPBS with RNAse inhibitors, and 1:10 dilution of pure BSA, and blocked for 30 min on ice. 50,000 single nuclei were purified using Fluorescence-assisted cell sorting (FACS) using a BD FACS Aria Fusion with a 70um nozzle. Single Hoechst- positive nuclei were gated using fluorescence measured in the BV421 channel, and debris was excluded using forward and side scatter area and width parameters (FSC-A vs FSC-W, and SSC-A vs SSC-W). Nuclei were kept on ice for all the steps. TdT and CCN1 injected samples were processed in parallel on the same day for each repeat.
Instrument	BD Biosciences FACS Aria Fusion
Software	FlowJo v10.10.0 software was used to make the example plots in Supplementary Data Figure 2
Cell population abundance	200,000 nuclei in total were sorted for snRNASeq
Gating strategy	Single Hoechst- positive nuclei were gated using fluorescence measured in the BV421 channel, and debris was excluded using forward and side scatter area and width parameters (FSC-A vs FSC-W, and SSC-A vs SSC-W)

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