nature portfolio

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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	NIS Elements software version 4.60, NIS Elements software version 5.42.06, LAS X system 3.7.6.25997, MetaXpress 6.7.2.290
Data analysis	ImageJ 1.54f, Matlab R2023a, MetaXpress 6.7.2.290, ThunderStorm 1.3-2014-11-08, Python 3, CellProfiler 4.2.6, GraphPad Prism 10.4.1. Custom scripts used for image analysis are available at Github.com (https://github.com/QilabGitHub/CRISPR-TO).

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

The RNA-seq datasets generated and analyzed during this study are available in the Gene Expression Omnibus repository under the accession number GSE278329. All RNA-seq data have been aligned to the mouse reference genome GRCm39. All data supporting the findings of this study are included in this article and Supplementary Information, and are available at https://purl.stanford.edu/rv392xg8117. Source data are provided with this paper.

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	No statistical methods were used to predetermine the sample size. At least 10 fields of view were captured per condition for imaging experiment to ensure representative population capture. For quantifying neurite outgrowth, 25 fields of view were automatically captured using ImageXpress Micro Confocal system for each well.
Data exclusions	Neurite outgrowth was automatically measured using MetaXpress software across 25 fields of view per well. Due to the automated capture by the mageXpress Micro Confocal system, some fields exhibited low imaging quality. Data from these low-quality fields were excluded from the analysis.
Replication	The application of CRISPR-TO in cell lines and primary neurons for RNA manipulation under different conditions were repeated at least 3 times as biological replicates and they were all successful. The CRISPR-TO screening experiment shown in Fig. 5a-c was performed with one biological replicate due to large number of samples. But the validation experiments for the screened candidate, Stmn2 mRNA, were repeated at least three times as biological replicates in primary mouse cortical neurons. All the flow and RT-qPCR experiments were repeated at least 3 times except the flow experiments in Extended Data Fig. 1b-c which were repeated twice, and they were all successful. The live neuron imaging experiment in Extended Data Fig. 9l was only performed once but observed similar results in many neurons.
Randomization	Randomization was used when selecting cells after transfection for image capture with microscopy. However, randomization is not applicable for other experimental design in this study, because control groups (gT+DMSO and gNT+ABA) were always included in experiments to control for conditions without inducer (ABA) or without a targeting guide RNA (gNT).
Blinding	Blinding was not used due to technical and analytical requirements and challenges. However, the use of standard imaging analysis protocols with customized codes, CellProfiler pipelines, and MetaXpress modules minimized bias in the results.

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

Mouse monoclonal anti-GAPDH (Biolegend, 649201, Clone: FF26A/F9, lot B338342, dilution 1:50)
Mouse monoclonal anti-SDHC (Santa Cruz, sc-515102, Clone: C-2, lot A3020, dilution 1:50)
Rabbit polyclonal anti-SDHD (Novus, NBP2-81948, Polyclonal, lot 6847-2001, dilution 1:50)
Mouse monoclonal anti-NDUFS2 (Santa Cruz, sc-390596, Clone: B-3, lot A2119, dilution 1:50)
Mouse monoclonal anti-beta Actin (GeneTex, GTX26276, Clone: AC-15, lot 822304562, dilution 1:1000)
Rabbit monoclonal anti-ATF4 (CST, 11815T, Clone: D4B8, dilution 1:200)
Rabbit monoclonal anti-GFAP (CST, 12389T, Clone: D1F4Q, dilution 1:400)
Rabbit polyclonal anti-IBA1 (FUJIFILM Wako Pure Chemical Corporation, 019-19741, Polyclonal, dilution 1:500)
Mouse monoclonal anti-puromycin (Kerafast, Kf-Ab02366-1.1, Clone: 3RH11, dilution 1:2,500)
Rabbit monoclonal anti-Fibrillarin (CST, 2639, Clone: C13C3, dilution 1:400)
AF647-conjugated donkey anti-mouse IgG (Thermo Fisher, A-31571, Polyclonal, lot 2555690, dilution 1:500)
AF647-conjugated donkey anti-rabbit IgG (Thermo Fisher, A-31573, Polyclonal, lot 2359136, dilution 1:500)
CF583R-conjugated donkey anti-mouse IgG (Biotium, 20794, Polyclonal, dilution 1:250)
AF555-conjugated goat anti-rabbit IgG (Thermo Fisher, A-21429, Polyclonal, lot 2354429, dilution 1:500)

Validation

All the antibodies are commercialized, and validation statements and references are provided by the vendor's websites:
https://www.biolegend.com/nl-be/products/purified-anti-gapdh-antibody-6685
https://www.scbt.com/p/sdhc-antibody-c-2
https://www.novusbio.com/products/sdhd-antibody_nbp2-81948
https://www.scbt.com/p/ndufs2-antibody-b-3
https://www.genetex.com/Product/Detail/beta-Actin-antibody-AC-15/GTX26276?srsltid=AfmBOorwUY28-9y90MHT_SH-p4rqVuRBuy00eUKC2PBxsizK6y_MflJl
https://www.thermofisher.com/antibody/product/Donkey-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31571
https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31573
https://www.cellsignal.com/products/primary-antibodies/atf-4-d4b8-rabbit-mab/11815?srsltid=AfmBOop5HYWeu2svn_KMIEDGUgV81qPJ-L-7kVAErDZGe-J3FeXpD-2L
https://www.cellsignal.com/products/primary-antibodies/gfap-d1f4q-xp-rabbit-mab/12389?srsltid=AfmBOoqrJDnkP8K792hwyut55illstIgYaU9d_xGTHdOe1zl9O9X04XO
https://labchem-wako.fujifilm.com/us/product/detail/W01W0101-1974.html
https://www.kerafast.com/productgroup/190/anti-puromycin-3rh11-antibody
https://www.cellsignal.com/products/primary-antibodies/fibrillarin-c13c3-rabbit-mab/2639?srsltid=AfmBOort07e2GEKBO7-1VSnytC_t5_ABQGYmGUb27cPCrar2jU4BMKXE
https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21429

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

HEK 293T (ATCC, CRL-3216)
Lenti-X 293T (Takara, 632180)
HeLa (Sigma, 93021013)
Neuro-2a (ATCC, 30-2003)
U2OS-2-6-3 (a gift from Dr. David L. Spector, Cold Spring Harbor Laboratory, Kumaran and Spector, 2008), originally generated and reported in this paper (doi: 10.1016/s0092-8674(04)00171-0).

Authentication

Cell lines were authenticated by the supplier. We did not perform any additional authentication upon reception. We made bulk stocks for each cell line after recovering from the original frozen vials. We discard the cells after passage for 30 days and thaw new cells from liquid nitrogen stocks. Cell morphology was monitored at each passage by microscope.

Mycoplasma contamination

Cells were routinely tested (PCR based test) and were mycoplasma negative.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used in this manuscript.

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	Primary mouse cortical neurons were dissected from cortices of newborn (P0) C57BL/6J mouse (The Jackson Laboratory, 000664) pups.
Wild animals	This study does not involve wild animals.
Reporting on sex	This study did not consider sex.
Field-collected samples	This study did not include field-collected samples.
Ethics oversight	All animals were housed and experiments were conducted in accordance with the US National Institutes of Health (NIH) guidelines for the care and use of laboratory animals. The protocols were approved by Stanford University's Administrative Panel on Laboratory Animal Care. (APLAC # APLAC-32070).

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	For splice reporter assay, cells were dissociated with 0.05% Trypsin-EDTA, neutralized with 1xDPBS supplemented with 2% FBS, passed through a 45 µm cell strainer to a 96-well round-bottom plate for flow cytometry analysis. For analyzing the translation of human ACTB and mouse Actb mRNA, cells were fixed for IF staining using Cyto-Fast Fix/Perm Buffer Set (BioLegend, 426803) following the vendor's protocol before flow cytometry analysis.
Instrument	All samples were analyzed using Beckman Coulter CytoFlex S.
Software	All flow cytometry data was analyzed using FlowJo 10.
Cell population abundance	No sorting was used in this study.
Gating strategy	Gating was based on FSC-H and SSC-H. Doublet was removed using FSC-H and FSC-A. Positive cells were indicated by gates according to negative control samples.

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