

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	NA
Data analysis	NA

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 authors declare that the data supporting the findings of this study are available within the paper and the source data for figures are provided with the 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

NA

Reporting on race, ethnicity, or other socially relevant groupings

NA

Population characteristics

NA

Recruitment

NA

Ethics oversight

NA

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](https://www.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 each sample, two batches, at least 10 brain lobes in total were imaged. For single neural stem cell analysis, at least 20 cells were examined. For S2 cells, there are at least two replicates.

Data exclusions

No data was excluded

Replication

At least 2 replicates were performed for all experiments. Data was reproducible.

Randomization

No.

Blinding

No.

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

Methods

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

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

Antibodies

Antibodies used

The primary antibodies used in this paper were guinea pig anti-Dpn (1:1,000), rabbit anti-Dpn (1:200), mouse anti-Mira (1:50, F. Matsuzaki), rabbit anti-GFP (1:3,000; F. Yu), mouse anti-GFP (1:3000; F. Yu), mouse anti- α -tubulin (Sigma, Cat#: T6199), rabbit anti-Msps (1:1,000, J. Raff), rabbit anti-PH3 (1:200, Sigma, Cat#: 06-570), rabbit anti-Flag (1:1,000; Sigma-Aldrich), mouse anti-Myc

(1:1,000; abcam), rabbit anti-HA (1:1000, Sigma-Aldrich), rabbit anti-SUMO (1:500, Albert J. Courey), rabbit anti-Wts (1:100, generated in this work by Abmart), rabbit anti-Yki (1:100, generated by GenScript), mouse anti- β -gal (1:100, Promega) and mouse anti-CycE (1:200, H. Richardson).

Validation

guinea pig anti-Dpn (1:1,000) validated in Deng et al., 2021 EMBO J.
 mouse anti-Mira (1:50, F. Matsuzaki) validated in Deng et al., 2021 EMBO J.
 rabbit anti-GFP (1:3,000; F. Yu) validated in Deng et al., 2021 EMBO J.
 mouse anti-GFP (1:3000; F. Yu) validated in Deng et al., 2021 EMBO J.
 mouse anti- α -tubulin (1:200, Sigma, Cat#: T6199) validated in Deng et al., 2021 EMBO J.
 rabbit anti-Msps (1:1,000, J. Raff) validated in Deng et al., 2021 EMBO J.
 rabbit anti-PH3 (1:200, Sigma, Cat#: 06-570) validated in Deng et al., 2021 EMBO J.
 rabbit anti-Flag (1:1,000; Sigma-Aldrich), validated in Koe et al. 2018, eLife
 mouse anti-Myc (1:1,000; abcam), validated in Koe et al. 2018, eLife
 rabbit anti-HA (1:1000, Sigma-Aldrich), validated in Mahekta et al. 2023, EMBO Reports.
 rabbit anti-SUMO (1:500, Albert J. Courey), validated in Yoko et al. 2005, Mechanisms of Development.
 rabbit anti-Wts (1:100, generated in this work by Abmart), validated in this paper.
 rabbit anti-Yki (1:100, generated by GenScript), validated in Ly et al. 2019, Plos Biology.
 mouse anti- β -gal (1:100, Promega), validated in Heerssen et al. 2004, Nat. Neurosci.
 mouse anti-CycE (1:200, H. Richardson), validated in Christian et al. 2010, Developmental Biology.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	Drosophila S2 cells (Schneider 2 (RRID:CVCL_Z232)), from whole embryo, originally from William Chia's laboratory.
Authentication	Schneider 2 (RRID:CVCL_Z232)
Mycoplasma contamination	No mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No.

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	Drosophila melanogaster
Wild animals	NA
Reporting on sex	Sex was not considered in this study.
Field-collected samples	No.
Ethics oversight	No.

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

Plants

Seed stocks	NA
Novel plant genotypes	NA
Authentication	NA