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<br>*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<br>*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 | Confocal images were acquired on a Zeiss Apotome fluorescence microscope and images were collected and processed using the ZEN (ZEISS Efficient Navigation) software.<br>Stimulated-emission-depletion (STED) microscopy images were captured on a Leica TCS SP8 STED microscope, controlled by LASX software, equipped with a 660 nm depletion laser and a 100x NA1.4 objective.<br>Electron microcroscopy iImages were acquired using a Jeol 1400 TEM (Jeol, Croissy-sur-Seine, France) operating at 120 keV and equipped with a RIO CMOS camera (Ametek SAS, Elancourt, France). |
| Data analysis | Image analyses were performed using Image J version 1.52a. Statistics were performed with GraphPad Prism version 10.2.3.<br>All these information are provided in the method section |

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 primary source data have been provided with the manuscript.

## 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 | The study did not involve human research participants. |
| 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 | The statistical analyses used for each experiment can be found in the associated figure legends. No statistical methods were used to predetermine sample sizes but our sample size are similar those reported in other publications in the field.<br>In vitro experiments were performed in duplicate and were reproduced at least 3 times or in triplicates and repeated at least 2 times.<br>For each experiment, the figure legends indicate the exact sample size (n), the p values, the definition of the center values and error bars, along with the statistical method used. |
| Data exclusions | No data were excluded from analysis. |
| Replication | We have indicated the number of independent experiments performed in the figure legends. |
| Randomization | n/a |
| Blinding | The investigators were blinded during data collection and analysis where possible. |

# 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

SM1 antibody (Virji et al., 1983)
anti-PilE antibody 20D9
Alexa Fluor 488 (Molecular Probes)
Alexa Fluor 546 rabbit anti-ORF4 antibody
Alexa Fluor 514 secondary antibody fragments
ATTO550 secondary antibody fragments
Fab2' goat anti-rabbit conjugated to 10 nm diameter gold (Aurion, Wageningen, The Netherlands)

Validation

For the commercially available antibodies, validation statements are provided on the manufacturer's website, and/or relevant citations.
For antibodies developped for non-commercial-use, the specificity was assessed in previous studies, see material and methods section.

# Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Pharynx carcinoma-derived epithelial cell line (FaDu; ATCC #HTB-43)
Human umbilical vein cell line (EA.hy926; ATCC #CRL-2922)

Authentication

Cell lines has been ordered on the ATCC platform

Mycoplasma contamination

We periodically check our cell lines for potential mycoplasma contamination and they were mycoplasma free.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used in this study.

# Plants

Seed stocks

n/a

Novel plant genotypes

n/a

Authentication

n/a