

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
<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. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P 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 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 AR 5.42.01

Data analysis Picasso v0.7.4, Custom Codes for Analysis of Data (<https://github.com/ABanny/Epi-PAINT>)

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](#)

Data containing all localizations are available on Zenodo (10.5281/zenodo.19344709). Source Data for all plots in the figures are provided.

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](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	Mock: n = 7 cell, Treatment: n = 8 cells.
Data exclusions	Cells with the entire nucleus in the image were used for further analysis.
Replication	All datasets were imaged in at least three replicates to verify reproducibility.
Randomization	N/A
Blinding	N/A

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	RNA Pol II Ser5P Abcam AB5131 Polyclonal 1093785-4 RNA Pol II Ser2P Abcam AB193468 Polyclonal 10868763-2 H3K27ac Cell Signalling Technology 8173 D5E4 8 H3K27me3 Cell Signalling Technology 9751 C36B11 19 H3K9me3 Abcam AB8898 Polyclonal Lot No: Unavailable H3K4me3 Abcam AB8580 Polyclonal 1090826 CTCF Abcam AB188408 EPR18253 10141855-2
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Lamin B1 Abcam AB16048 Polyclonal 1022148-1
 SC35 Abcam AB11826 Polyclonal 1078259-8
 Fibrillarin Cell Signaling Technology 2639T Rabbit Monoclonal Antibody

Validation

RNA Pol II Ser5P: Validated for ICC/IF, western blotting, ChIP, IP and IHC. Suitable for human, mouse, and rat samples. (<https://www.abcam.com/en-us/products/primary-antibodies/rna-polymerase-ii-ctd-repeat-ysptps-phospho-s5-antibody-ab5131>)
 RNA Pol II Ser2P: Suitable for IP, ChIP, Dot, WB, ICC/IF, Flow Cyt (Intra), IHC-P and reacts with Human, Mouse, Rat, Synthetic peptide samples. Cited in 24 publications. (<https://www.abcam.com/en-us/products/primary-antibodies/rna-polymerase-ii-ctd-repeat-ysptps-phospho-s5-antibody-ab5131>)
 H3K27ac: Validated for IF/F, western blotting, ChIP, C&R and C&T. Suitable for human, mouse, rat and monkey samples. 593 citations (<https://www.cellsignal.com/products/primary-antibodies/acetyl-histone-h3-lys27-d5e4-xp-rabbit-mab/8173>)
 H3K27me3: Validated for IF/F, IHC, western blotting, ChIP, C&R and C&T. Suitable for human, mouse, rat and monkey samples. 717 citations (<https://www.cellsignal.com/products/primary-antibodies/tri-methyl-histone-h3-lys4-c42d8-rabbit-mab/9751>)
 H3K9me3: Suitable for WB, IHC and IF. Suitable for human and mouse samples. 1735 publications (<https://www.abcam.com/en-us/products/primary-antibodies/histone-h3-tri-methyl-k9-antibody-chip-grade-ab8898>)
 H3K4me3: Suitable for WB, IHC and IF. Suitable for human samples. 2132 publications (<https://www.abcam.com/en-us/products/primary-antibodies/histone-h3-tri-methyl-k4-antibody-chip-grade-ab8580>)
 CTCF: Suitable for CUT&Tag, ChIC/CUT&RUN-seq, ChIP, WB, ICC/IF, ChIP-seq, IHC-P and reacts with Mouse, Human, Rat samples. Cited in 12 publications. (<https://www.abcam.com/en-us/products/primary-antibodies/ctcf-antibody-epr18253-chip-grade-ab188408>)
 Lamin B1: Suitable for WB, IHC and IF. Suitable for human, mouse and rat samples. Specificity confirmed with LMNB1 knockout cell line validation. 1181 publications (<https://www.abcam.com/en-us/products/primary-antibodies/lamin-b1-antibody-nuclear-envelope-marker-ab16048>)
 SC35: Suitable for IF. Suitable for human, mouse and rat samples. 138 publications (<https://www.abcam.com/en-us/products/primary-antibodies/sc35-antibody-sc-35-nuclear-speckle-marker-ab11826>)
 Fibrillarin: Suitable for WB and IF, Suitable for Human Mouse Rat Monkey. 104 publications (<https://www.cellsignal.com/products/primary-antibodies/fibrillarin-c13c3-rabbit-monoclonal-antibody/2639>)

Eukaryotic cell lines

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

Cell line source(s)

HeLa Kyoto cell, Received as a gift from Dr. Shovamayee Maharana

Authentication

Not Authenticated

Mycoplasma contamination

Not tested

Commonly misidentified lines
(See [ICLAC](#) register)

N/A

Plants

Seed stocks

N/A

Novel plant genotypes

N/A

Authentication

N/A