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Reporting Summary

Nature Research 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 Research policies, see Authors & Referees 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	Plate reader: Tecan Sparkcontrol Method Editor V.2.2 Microscopy: Leica LAS X 3.1.1.15751 with HCSA Multiwell Full SP8 MAtrix Screener Software Module (No. 156602533, 2017), ZEN software 2012 SP1 (8.1.0.484) and Nikon Imaging Software Elements 5.02. ITC: MicroCal PEAQ-ITC (MAL1177030) Äkta Pure: using Unicorn 7.1 Build (7.1.0.378)
Data analysis	Microsoft Excel for Mac (version 16.16.16(191111)), Fiji/ImageJ (version 2.9.9-rc-69/1.52p, build: 269a0ad53f), Graphpad Prism (version 8.0.2), MicroCal PEAQ-ITC Analysis software (version 1.1.0.126), XDS (version Jan 26, 2018 BUILT=20180126), Refmac5 (versions 5.8.0222 and 5.8.0232), Phaser (version 2.8.2), PHENIX (version 1.14rc1-3177), MacPyMol (v1.8.4.0) and Coot (version 0.8.9.1).

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

There are no restrictions on data availability.

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	Sample size was based on experience in prior studies, for example Cai et al 2018 (Nature). All Mad2L1-EGFP/GFP-LAMA experiments were performed at least in three different cell cultures, which gave enough observations of mitosis for cell cycle analysis.
Data exclusions	For continuous live cell imaging of mitosis, the pre-established exclusion criteria were as follows: cells which did not express the protein of interest were excluded from the analysis (no SiR signal from labeling SNAP-tag). Cells which did not have a clear fate after mitosis and cells that underwent cell death during the time course were not included in the analysis. For perfusion experiments of GFP-LAMAs, the pre-established exclusion criteria was: data from cells out-of-focus during perfusion were not included in the analysis. No other data was excluded.
Replication	Screening of insertion positions were performed once in duplicates. All validation and further experiments of LAMAs were performed in minimum triplicates, on multiple cell culture replicates, and in multiple experiments. All replicates were successful.
Randomization	No randomization was applied, due to the nature of the experiments.
Blinding	No blinding of experimental set ups or analysis were applied, due to the nature of the experiments.

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
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK293 (CRL-3216; RRID: CVCL_0063) provided by ATCC. HeLa TZM-bl (Cat# 8129; RRID: CVCL_B478) provided by NIH AIDS Repository. Wildtype HeLa Kyoto cells (RRID: CVCL_1922; kind gift from Prof. Narumiya in Kyoto University).
Authentication	Cell lines were not further authenticated.
Mycoplasma contamination	Cell lines have been tested and are negative.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.