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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Fixed and live images were captured using Metamorph (Molecular Devices) and NIS-Elements 4.30 AR (Nikon). SIM images were captured using DeltaVision OMX (GE Healthcare). LM images from CLEM were collected usng NIS-Elements v.4.6. (Nikon). The EM camera was controlled by Capture Engine v.7.0. (Advanced Microscopy Techniques). Live-cell movies of furrowing were recorded under Andor's IQ3 v.4.1. (Andor).
Data analysis	Graphical data was plotted and statistical analysis was performed using Graphpad Prism 7.02 GraphPad StatMate 2.00 (Graphpad Software Inc). Linescan profiles were generated and plotted using ImageJ/FIJI and Microsoft Excel 2013. Fixed and live images were analyzed using Metamorph (Molecular Devices) and NIS-Elements AR 4.20.02 (Nikon). Quantification of fixed images was done using ImageJ/FIJI. For SIM data, experimental data sets were registered using MATLAB (MathWorks) based on axial and lateral chromatic misregistration (as described in Method section). 3D rendered images of SIM data were generated using Imaris (Bitplane). All fluorescence images from CLEM were deconvolved in SoftWoRx 5.0 software (Applied Precision).

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 upon request. 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

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Source data for all graphs (Figs. 1c, 2a, 2b, 3a, 3c, 4b, 4d, 4e; Extended Data Figs. 1d, 1f, 2b, 2c, 2d, 2f, 2g, 3a, 3b, 3c, 3e, 4c, 4d, 4f, 4g, 5b, 5c, 5e, 6a, 6b, 6c, 7b, 7d, 7e, 8d, 9a, 9d, 9e, 9f, 9h, 10c, 10d) are provided with the online version of the paper. All other datasets generated and/or analysed in the current study are available from the corresponding author upon reasonable request. Supplementary Figure 1 contains scanned complete images of western blots.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	We did not compute statistical analyses to predetermine sample sizes prior to performing each individual experiment. Our sample sizes were chosen based on similar published studies elsewhere. Additionally, we calculated the variances of the control populations from several experiments (GraphPad Prism software), and confirmed that the sample size we chose have at least a 90% power of detecting a mean difference of >50% from the controls (for a medium to large effect), with a significance level of 5% (two-tailed, GraphPad StatMate software, assuming normal distributions). In fact, because most of our data distributions are nonparametric, sample sizes we chose were larger than 115% of the necessary sample sizes for a parametric test.
Data exclusions	No experiments were excluded. No data from experiments were excluded after the data analyses except for the experiments monitoring γH2AX level (Fig. 4e, Extended Data Fig. 10c), where cells had significant DNA damage in the primary nucleus (FI of γH2AX > 3 standard deviations above the mean FI of γH2AX in the primary nuclei, see Methods for details) were excluded. Prior to data analyses, for MKLP2-depleted cells, only cells had a near-complete loss of midzone Aurora B by immunofluorescence were sampled and quantified as described in figure legend (Extended Data Fig. 4d). For SMC2-depleted cells, only cells had a near-complete loss of SMC2 by immunofluorescence were sampled and quantified as described in figure legend (Extended Data Fig. 5c). For data sampling for assessing the restoration of micronuclear defects (Fig. 4, Extended Data Figs. 9, 10), only cells that produced chromosomes that remain consistently peripheral or central relative to the mitotic spindle were sampled/monitored and quantified. For data sampling for assessing restoration of DNA replication (Extended Data Fig. 9e), only cells that progressed into the S-phase of the cell cycle were sampled and quantified (see legend).
Replication	No attempts for replication failed. The numbers of all replicates for each experiment are indicated in the figure legends. Experimental findings have been either reproduced independently by co-authors of the paper using the same experimental conditions or verified using orthogonal approaches and different cell lines.
Randomization	The cells quantified in each experiment were randomly sampled from the total population of cells.
Blinding	Investigators were not blinded. Note that almost all experimental results involved unbiased, automated image analysis.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

Methods

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

Antibodies

Antibodies used

For immunofluorescence, the following primary antibodies were used: LAP2α (1:300, Abcam, ab66588), Lap2β (1:200, Bethyl Laboratories, A304-840A), LAP2 (1:750, BD Transduction, 611000), mouse emerin (1:300, Abcam, ab54996), rabbit emerin (1:150 , Proteintech, 10351-1-AP), rabbit lamin A/C (1:1000, Abcam, ab26300), mouse lamin A (1:1000, Abcam, ab8980), rabbit LBR (1:500, Abcam, ab32535), mouse LBR (1:300, Sigma-Aldrich, SAB1400151), Nup133 (1:300, Abcam, ab155990), Nup107 (1:100, Life Technologies, 39C7), Nup62 (1:500, BD Transduction, 610497), Nup358 (1:1000, Abcam, ab64276), Tpr (1:200, Abcam, ab84516), Nup153 (1:500, Abcam, ab24700), mAb414 (1:1000 -1:2000, Abcam, ab24609), ELYS/MEL28 (1:200, Bethyl Laboratories, A300-166A), lamin B1 (1:1000, Abcam, ab16048), lamin B2 (1:1000, Abcam, ab151735), RPA1 (1:100, Cell Signaling, 2267), RPA2 (1:100, Abcam, ab2175), LSD1 (1:200, Cell Signaling, 2184), Rb (1:400, Cell Signaling, 9309), Aurora B (1:100, Abcam, ab3609), mouse phospho-H3S10 (1:10000, Abcam, ab14955), rabbit phospho-H3S10 (1:5000, Abcam, ab5176), SMC2 (1:1000, Abcam, ab10412), Aurora B Phospho pT232 (1:500, Rockland Antibodies, 600-401-677), CHMP4B (1:100, Proteintech, 13683-1-AP), CHMP2A (1:100, Proteintech, 10477-1-AP), IST1 (1:100, Proteintech, 51002-1-AP), rabbit α-Tubulin (1:1000, Abcam, ab18251), mouse α-Tubulin (1:300, Sigma-Aldrich, DM1A), CENP-A (1:300, Abcam, ab13939), rabbit γH2AX (1:500, Cell Signaling, 2577), mouse γH2AX (1:500, EMD Milipore, JBW301), FluoTag (Atto488)-X4 anti-GFP (1:250, NanoTag Biotechnologies), 53BP1 (1:100, Cell Signaling, 4937), BRCA1 (1:200, Santa Cruz, D-9) and POLD3 (1:100, Abnova, Clone 3-E2). Secondary antibodies used were Alexa Fluro 405, 488, 568 and 647 (1:500 for Alexa Fluro 405; 1:1000, Life Technologies).
For Immunoblotting, the following primary antibodies were used: rabbit anti-MKLP2 (1:1000, Bethyl Laboratories, A300-879A), mouse anti-SMC2 (1:1000, Abcam, ab10412), rabbit anti-KIF4A (1:500, Bethyl Laboratories, A301-074A), rabbit anti-TTL (1:2000, Proteintech, 13618-1-AP), and mouse anti-α-Tubulin (1:15000, Sigma-Aldrich, DM1A).

Validation

Previously validated antibodies (by western/immunofluorescence using RNAi or KO) were used for immunofluorescence in this study include: LAP2α (ab66588, Qi R et al. J Cell Sci, 2015), emerin (Proteintech 10351-1-AP, Pfaff J et al. J Cell Sci, 2016), lamin A/C (ab26300, KO validated by Abcam), lamin A (ab8980, Ranade D et al. Chromosoma, 2017), LBR (ab32535, Mimura Y et al. J Cell Sci, 2016), Nup358 (ab64276, Mamede JI et al. Sci Rep, 2017), Tpr (ab84516, Umlauf D et al. J Cell Sci, 2013), lamin B1(ab16048, KO validated by Abcam), CHMP2A (Proteintech 10477-1-AP, Vietri et al. Nature, 2015), IST1 (Proteintech 51002-1-AP, Vietri et al. Nature, 2015), POLD3 (Abnova, Minocherhomji S et al, Nature, 2015).
BRCA1 and 53BP1 was validated by immunofluorescence using aphidicolin treatment (Casta et al, Nature, 2012). γH2AX (Cell signaling 2577) was validated by western using UV irradiation (by Cell signaling). γH2AX (EMD Milipore 07-164) was validated by immunofluorescence using gamma irradiation (Hill SJ et al. Mol Biol Cell, 2014). Antibodies used for immunofluorescence for staining LAP2α, SMC2, ELYS/MEL28, and LBR were previously validated by prior studies and additionally validated in this study by western or immunofluorescence using siRNA. Aurora B Phospho pT232 (Rockland) was a previously validated antibody and validated in this study by immunofluorescence using Aurora B inhibitor (ZM447439, 5 μM and 50 μM) treatment. Both mouse pH3S10 (ab14955) and rabbit pH3S10 (ab5176) were validated by immunofluorescence using Aurora B inhibitor (ZM447439, 5 μM and 50 μM). Monoclonal antibodies, mAb414 (Aris JP, Blobel G J, Cell Biol, 1988), and DM1A (Blose SH et al, J Cell Biol, 1984) were previously validated and used for immunofluorescence. Other antibodies were previously used for immunofluorescence by prior studies and their localizations were confirmed by this study.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

HeLa Kyoto, U2OS and hTERT RPE-1 were purchased from ATCC or obtained from other laboratories as described in Method section. HeLa Kyoto cell line expressing GFP-Nup107 was purchased from CLS (cell lines service GmbH) with EMBLEM MTA (Dr. Jan Ellenberg, Otsuka et al. 2016 Elife)

Authentication

Cell line authentication was not performed.

Mycoplasma contamination

All cell lines used were regularly checked for mycoplasma contamination and no contamination was found. All cells used for experiments were stained with DAPI and examined under X60 or X100 1.4 NA objective lens and no contamination was found.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used in this study.