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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.

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	ZEN software 2012 SP1 (8,1,0,484) (https://www.zeiss.com) Micronaut v0.7.0 (data acquisition, upon request Gerlich group, IMBA, Vienna) MyPiC v0.5-v0.8 (https://git.embl.de/grp-ellenberg/mypic) FCSRunner v0.6-0.7 (https://git.embl.de/grp-ellenberg/fcsrunner)
Data analysis	MATLAB 2017a (https://de.mathworks.com) Mitotic cell atlas (v1.0.1) (https://git.embl.de/grp-ellenberg/mitotic_cell_atlas) Fluctuation Analyzer 4G 150223 (https://www-ellenberg.embl.de/resources/data-analysis) FCSFitM v0.8 (https://git.embl.de/grp-ellenberg/FCSAnalyze) FCSCalibration v0.4.2 (https://git.embl.de/grp-ellenberg/FCSAnalyze) Cellcognition 1.5.2 (http://www.cellcognition-project.org/) RStudio 1.1.383 (https://www.rstudio.com/) R 3.4.1 (https://www.r-project.org/)

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

All images processed in this study including original images, concentration maps, segmentation mask for both cellular and chromosomal volume and concentration maps are available in the Image Data Resource (http://idr.openmicroscopy.org) under DOI: 10.17867/10000112.
All data supporting the spatiotemporal mitotic cell model and the analysis is available from the mitotic cell atlas website: http://www.mitocheck.org/mitotic_cell_atlas/downloads/v1.0.1
There are no restriction on material availability.

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 validated that our sample size concerning how many cells per protein need to be measured is indeed sufficient to address cell-to-cell variability and be representative for each protein. If we assume a normal distribution of protein abundance (which is what we have found in previous studies, see e.g. Mahen, Mol Biol Cell 2014) simple simulations show that the difference between the sample mean and the population mean decrease very slowly beyond 10 samples. Employing bootstrap analysis of our image based features showed that the variance of the feature mean does not improve significantly beyond 10 cells and therefore little precision is gained beyond that point. Sampling between 10-35 cells per protein is a fair representation of each protein investigated.
Data exclusions	Videos of cells with no expression of the protein of interest, with wrongly selected FCS positions (e.g. outside of the cell) or without anaphase onset were excluded from further processing. After automated segmentation of landmarks visual inspection was used to remove cells with strong over- or under-segmentation. Cells which couldn't be aligned to the mitotic standard time were removed.
Replication	The computational pipeline for generating standard mitotic time was validated using dropout datasets from HeLa Kyoto cells. We also built a standard time model for a second human cell type, U2OS cells.
Randomization	Unless stated otherwise in the text, all available cells for a given protein were used to extract quantitative information.
Blinding	There was no blinded group allocation. All data that passed QC (described in method section/data exclusion section above) were analyzed.

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

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	Genome-edited HeLa Kyoto cell lines are available from the authors upon request.

Antibodies

Antibodies used	The antibody used for knock-in validation was purchased from Roche (cat#11814460001).
Validation	Antibodies from different companies were validated by Western Blot for sensitivity and specificity (see Koch et al. Nature Protocols, vol. 13: 1465–1487 (2018), doi: 10.1038/nprot.2018.042).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Wildtype HeLa Kyoto cells (RRID: CVCL_1922) were a kind gift from Prof. S. Narumiya, Kyoto University. U2OS cells were obtained from the ATCC (HTB-96).
Authentication	HeLa Kyoto cells were authenticated by sequencing. U2OS cells were not further authenticated.
Mycoplasma contamination	PCR-based mycoplasma tests were performed every 2 or 3 months and were always negative for all cell lines.
Commonly misidentified lines (See ICLAC register)	Both cell lines are not listed in the list of commonly misidentified cell lines.