nature portfolio

Corresponding author(s): Professor Maria Jose Gomez-Benito

Last updated by author(s): 2025/01/02

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 *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	Zeiss Axio Observer software to acquire fluorescence microscope images. Custom code in Fiji (ImageJ) to post-process the microscope images: adjust brightness/contrast/color balance, segment individual nuclei and measure morphological parameters.
Data analysis	Custom algorithm in MATLAB for image alignment, drift correction and nuclei tracking throughout the time-lapse experiment. Custom algorithm in MATLAB to segment bright areas within the nuclei in order to evaluate chromatin condensation and chromatin clusters dynamics. Custom algorithm in MATLAB to classify the nuclei according to their position in the microchannel and to represent the results.

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 needed to evaluate the conclusions in the paper are present in the manuscript and/or the Supplementary Materials. Additionally, we are submitting our

experimental results data in Figshare at https://doi.org/10.6084/m9.figshare.30657911.v1. The algorithm used to process fluorescent microscope images is available on Github (https://github.com/yablazro/Segmentation-chromatin-clusters). Any other data or code related to this project will be willingly provided upon request.

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

Life sciences study design

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

Sample size	Two to four different experiments were performed and analyze for each cell line type following the experimental protocol developed. Cells were classified according to the confining microchannel through which they migrate, either long or short channel. For PACA-H2B-GFP cell line, sample size in long channels was n=71 and in short channels it was n=65. For A549-H2B-GFP cell line, sample sizes were n=26 and n=42, respectively.
Data exclusions	Apoptotic cells were excluded from the analysis. To prevent mitosis-induced chromatin changes from influencing the analysis, dividing cells were only considered up to 20 frames (200 minutes) prior to mitotic entry and at least 20 frames after cell division were excluded. Frames in which multiple cells occupied the same microchannel region were excluded to ensure that confinement conditions remained consistent across time points.
Replication	Two to four independent experiments were conducted for each cell line, analyzing over 100 individual cells. Each experiment was evaluated separately, consistently yielding the same conclusions. The data were then combined for collective analysis and representation. Furthermore, reproducibility was confirmed between the two different cell lines.
Randomization	Cells were categorized into distinct groups based on their position within the microchannel. The geometric center of the nucleus was used to determine the specific region of the microchannel occupied by each cell in every frame throughout the migration experiment.
Blinding	Blinding was not relevant in these experiment.

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: Anti-Histone H3 tri-methyl K9 antibody - ChIP Grade (Abcam, ab8898), Anti-Histone H3 tri-methyl K27 antibody - ChIP Grade (Abcam, ab6002)

Validation: Rabbit anti-Histone H3 antibody ab8898 is a rabbit polyclonal antibody that is used in Histone H3 western blotting,IHC and immunofluorescence. Suitable for human and mouse samples. Tried and trusted by researchers since 2002. Anti-Histone H3 antibody ab8898 is cited in over 1810 publications.

Mouse Monoclonal H3 tri methyl K27 antibody. Suitable for ChIP, ELISA, WB, ICC/IF, IHC - Wmt and reacts withHuman, Mouse, Cow, Synthetic peptide - Human samples. Cited in 981 publications.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s): PACA cells are a neuroblastoma primary culture procured from a male patient's tumor with metastatic potential at University and Polytechnic La Fe Hospital (Valencia, Spain) in accordance with the ethics committee guidelines of the hospital, with reference number 2019-305-1. This cell line was generously provided by Jaime Font de Mora. Cells were transfected with H2B-GFP and sorted, they are referred to as PACA-H2B-GFP.

A549 cells are adenocarcinomic human alveolar basal epithelial cells isolated from a 58-year-old caucasian male and they were first develop to constitute a cell line in 1972 by D. J. Giard et al. The H2B-GFP transfected cells were supplied by Isabel Marzo Rubio and they are referred to as A549-H2B-GFP.

Authentication: None of the cell lines used were authenticated directly in our lab.

Mycoplasma contamination: Cell lines were proven infection-free through PCR detection for mycoplasma, performed with specific probes Myc A (5' GGCGAATGGGTGAGTAACACG 3') and Myc B (5'CGGATAACGCTTGCGACCTATG 3') using a real-time PCR thermocycler (Bio Rad: CFX96 Real Time System) and visualized with gel imaging system (Bio Rad: ChemiDoc XRS + with Image Lab Software).

Commonly misidentified lines (See ICLAC register): n/a

Plants

Seed stocks: n/a

Novel plant genotypes: n/a

Authentication: n/a