nature portfolio

Corresponding author(s): Fachinetti; Miroshnikova

Last updated by author(s): 14/9/2024

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	JPK NanoWizard (v4) NIS-Elements (v5.41.00) Metamorph (v7.8) softWoRx (v7.2.1) CellVoyager CV7000 (v7, Yokogawa Electric) Image Lab (v6.1.0, Bio-Rad) FACSDiva™ Software (v8, BD) Metafer4 (Metasystem v3.13.5)
Data analysis	Fiji/ImageJ (v2.9.0) JPK Data Processing Software (v6) Columbus (v2.9.1) Cellpose (v2.0) FlowJo (v10) GraphPad Prism (v10) FLIMfit (v5.1.1) Python (v3.9) Imaris (v10) ISIS (v5.8.14)

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 supporting the findings of this study are available from the corresponding authors on request. Source data are provided with this paper.

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	No statistical methods were used to pre-determine sample sizes but our sample sizes are similar to those reported in previous publications (ex: PMID: 27880912, PMID: 32302590)
Data exclusions	No data was excluded
Replication	All experiments were performed using several biological replicates. Number of replicates for each experiment is indicated in the corresponding figure legend. Several steps were taken to ensure the reproducibility of experimental findings and key results were confirmed using complementary experimental approaches.
Randomization	Sample assignment to treatment and control groups, as well as data collection, was performed randomly without prior assessment of any cell culture characteristics.
Blinding	In general, data collection and analysis were not performed blind to the conditions of the experiments since genotypes were evident by morphology, however quantitative image analyses which is at the heart of all presented data, were agnostic to experimental conditions. Thus all automated software algorithms that were used for quantification were unbiased.

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

Antibodies for Western Blot: CENP-A (1:1000 Cell Signaling #2186, RRID:AB_10828491), p53 (1:200 Millipore, OP43, RRID:AB_213402); p21 (1:200 Millipore, OP43, RRID:AB_2335868); Lamin A/C (1:1000, Santa Cruz Biotechnology, sc-7292, RRID:AB_627875); Lamin B1 (1:2000, ProteinTech #12987, RRID:AB_2136290); Vinculin (1:5000 Sigma, V9264, RRID:AB_10603627); p16 INK4A (D3W8G) (1:1000, Cell Signaling, #2186, RRID:AB_2750891); p38 MAPK (1:1000, Cell Signaling, #9212 , RRID:AB_330713); GAPDH (1:5000, Cell Signaling #2118, RRID:AB_561053); phAKT (S473) (1:1000 Cell Signaling #4060,RRID:AB_2315049); AKT (1:1000 Cell Signaling #9272, RRID:AB_329827); phCHK2 (T68) (1:1000, Cell Signaling #2661, RRID:AB_331479); CHK2 (1:1000, BD #611570, RRID:AB_399016); p70 S6 Kinase (1:1000, Cell Signaling #2708, RRID:AB_390722); php53 (S15) (1:1000, Cell Signaling #9284, RRID:AB_331464); Ph-p70 S6 Kinase (Thr389) (1:1000 Cell Signaling #9234, RRID:AB_2269803), γ-H2AX (1:1000, Cell Signaling #2718, RRID:AB_659840).

Antibodies for Immunofluorescence: ACA (1:500, Antibodies Incorporated #15-235-0001, RRID: AB_2797146); p21 (1:200, Millipore, OP43, RRID:AB_2335868); CENP-A (1:1000, Cell Signaling #2186, RRID:AB_10828491); 53BP1 (1:500, Novus-biologicals Cat# NB100-304, RRID:AB_10003037); gH2A.X (1:1000, Millipore Cat# 05-636, RRID:AB_309864); Lamin A/C (1:1000, Santa Cruz Biotechnology sc-7292, RRID:AB_627875), Lamin B1 (1:1000, Proteintech 12987-1-AP, RRID:AB_2136290); H3K9me3 (1:1000 Abcam ab8898, RRID:AB_306848); H3K27me3 (1:1000; Cell Signaling #9733S, RRID:AB_2616029); ATR (1:1000 Cell Signaling # 2790, RRID:AB_2227860); cPLA2 (1:1000; Abcam ab58375, RRID:AB_881998); phAKT (1:500 Cell Signaling #4060, RRID:AB_2315049); p53 (1:200, Millipore, OP43, RRID:AB_213402); α-tubulin (1:1000, Novus #NB100-690, RRID:AB_521686);HP1α (1:1000, gift from Aaron Straight lab, Stanford University); Alexa Fluor 647 Phalloidin (1:250, ThermoFisher Scientific #A22287, RRID AB 2620155), γ-H2AX (1:1000, Cell Signaling #2718, RRID:AB_659840).

Antibody for cytometry: BrdU (1:5, BD #347580)

Antibody for ChIP: Lamin B1 (Proteintech 12987-1-AP, RRID:AB_2136290)

Validation

CENP-A antibody was validated by loss of signal after auxin-induced CENP-A depletion. p53, php53, p21, phCHK2, 53BP1 and gH2AX antibodies were validated in cells treated with DNA damage-inducing agents. phAKT, Ph-p70 S6 Kinase (Thr389) were validated with proper controls as shown in the Extended data figure. LaminA/C and Lamin B1 antibodies were validated in lysates from cell knocked-down for their relative genes. All antibodies were purchased from vendors who had validated specificity in human cells, generally by knockdown studies. Every antibody employed in the study has been tested, validated and utilized extensively in either the Fachinetti or Miroshnikova labs. All antibodies are well characterized and widely used in the literature. They were employed according to datasheet instructions or previously published protocols.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

hTERT RPE-1 cells (female) (ATCC Cat# CRL-40000, RRID:CVCL_43888)
U-2 OS (female) (ATCC Cat#HTB-96, RRID:CVCL_0042),
h-TERT BJ (male) (ATCC Cat# CRL-4001, RRID:CVCL_6573)

Authentication

Cell lines were not authenticated but were recognizable by their morphology.

Mycoplasma contamination

Cells were regularly tested for mycoplasma contamination. Only non-contaminated cells were used in this study.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified lines per ICLAC register were used.

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	For cell cycle analysis, BrdU pulse was performed for 24 h or 30 min (10 µM final), before collection and fixation in 70% Ethanol. Denaturation was performed with 2N HCl followed by neutralization with 0.1 M Borax pH 8.5. Cells were stained with anti BrdU (1:5, BD #347580) and proper secondary antibody. Propidium iodide was added at 2.5 µg/mL final concentration in presence of 250 µg/mL RNAseA (Thermo Fisher) and incubated at least 1h before acquisition Cell staining for p21-positive or negative cell sorting was performed as follows: cells were collected, counted and fixed in 1% formaldehyde 20 min on ice. The reaction was blocked with final 125 mM Glycine for 3 min. After washes with 1X PBS, cells were permeabilized with 1X permeabilization buffer (PB) (kit Foxp3/Transcription Factor Staining Buffer kit, Thermo Fisher, 00-5523) for 30 min RT. Cells were resuspended in 1X PB containing 0.5 µg p21 (Merck, #MABE325) incubated for 1 h at room temperature. After three washes in 1X PB, cells were resuspended in PB with fluorochrome-conjugated secondary antibody (1:500 AlexaFluor-647, Jackson lab) for 30 min, at room temperature. Following 2 washes in 1XPB, cells were resuspended cells in 30 µL of 1X FACS buffer (1 % BSA, 2 mM EDTA in PBS) containing DAPI (1 µg/mL).
Instrument	LSRII or Fortessa Flow Cytometer (BD) were used for cell cycle analysis (fig1h, figS1m). SONY SH800 was used for p21-positive cell sorting (figS1g)
Software	FlowJo v10
Cell population abundance	p21 positive celle were sorted based on p21 expression detected by intracellular staining with the indicated p21 antibody. Purity of post sorted cells was assessed by FACS
Gating strategy	Fluorescence threshold to gate p21 positive cells was set based on intracellular staining with an IgG isotope control and relative secondary antibody. A log scale expression value <10^3 was considered to set p21 negative gate, and >10^3 for p21 positive one.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.