# nature portfolio

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## **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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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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.
$\boxtimes$	A description of all covariates tested
$\boxtimes$	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. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
$\times$	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
X	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
X	Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i> ), indicating how they were calculated
	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.

### Software and code

Policy information about availability of computer code

Data collection

CellVoyager CV1000 Software (Yokogawa), CQ1 Software (Yokogawa), OPTIMA (BMG Labtech), Muse Cell Analyzer (Luminex)

Data analysis

All data of live cell imaging were analyzed by using Fiji. Data of cell-cycle analysis were analyzed by using FlowJo software (BD Biosciences). GraphPad Prism9 (GraphPad Software) were used for all stastical analysis.

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

All data supporting the findings of this study are available from the corresponding author on reasonable request.

### Human research participants

	Policy	y information	about studies	involving hur	nan research	participan	ts and Sex and	l Gender in Research
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Reporting on sex and gender	Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data where this information has been collected, and consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.
Population characteristics	Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."
Recruitment	Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.

Identify the organization(s) that approved the study protocol.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

# Field-specific reporting

Ethics oversight

Blinding

Life sciences	Behavioural & social sciences Ecological, evolutionary & environmental sciences				
For a reference copy of t	For a reference copy of the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>				
Life scier	Life sciences study design				
All studies must disclose on these points even when the disclosure is negative.					
Sample size	No statistical method was used to predetermine sample size.				
Data exclusions	No data were excluded.				
Replication	All experiments were successfully replicated at least two times except for Fig. 4b,c which were performed once.				
Randomization	No randomized experiments were performed.				

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.

# 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			Methods		
n/a	Involved in the study	n/a	Involved in the study		
$\boxtimes$	Antibodies	$\boxtimes$	ChIP-seq		
	Eukaryotic cell lines				
$\boxtimes$	Palaeontology and archaeology	$\boxtimes$	MRI-based neuroimaging		
$\boxtimes$	Animals and other organisms				
$\boxtimes$	Clinical data				
$\boxtimes$	Dual use research of concern				

No blinded experiments were performed.

### Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

RPE1 and BJ-5ta cell lines we

RPE1 and BJ-5ta cell lines were obtained from ATCC. RPE1 cell line stably expressing mNG-H2B and TUBG1-mRuby2 were established in our previous research (Ref. 15). RPE1 cell line expressing TUBB5-mNG and H2B-mScarlet, and BJ-5ta cell line expressing mNG-H2B and TUBG1-mRuby2 were established in this study.

Authentication Cell lines were examined for their morphology by microscopy.

Mycoplasma contamination All cell lines were tested for mycoplasma contamination and found negative. Test for mycoplasma is conducted every 6 months.

Commonly misidentified lines (See ICLAC register)

No commonly used misidentified cell lines were used.

### 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 RPE1 cells were seeded at a density of  $1\times10^4$  cells/ml in a 6-well plate. One day later, the medium was replaced with the one containing ascorbic acid (500  $\mu$ M). After 24 hr incubation, the cells were trypsinized, fixed in ice-cold 70% Ethanol, and frozen at -20°C overnight. Fixed cells were pelleted and washed three times in PBS. 200  $\mu$ L of Muse Cell Cycle Reagent

(Luminex) was added to the cell pellet and incubated for 30 min at room temperature in the dark.

Instrument Muse cell analyzer (Luminex, 0500-3115) was used for data collection.

Software (BD Biosciences) was used to analyzed the flow cytometry data.

Cell population abundance The purity of the samples was not determined.

Gating strategy The gating strategy is based on cell size and DNA content index.

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