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 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	NA
Data analysis	NA

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

The source data are available in Supplementary Data. Uncropped blot images are provided as Supplementary Figure 6 in the Supplementary Information file.

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	NA
Reporting on race, ethnicity, or other socially relevant groupings	NA
Population characteristics	NA
Recruitment	NA
Ethics oversight	NA

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	Around cells per condition were scored in 4 independent microscopy slide fields, error bars refer to variability within three independent experiments performed under similar experimental conditions.
Data exclusions	NA
Replication	error bars refer to variability within at least three independent experiments performed under similar experimental conditions
Randomization	The samples were scored blindly, the author that mounted the slides was different from the author scoring the cells
Blinding	The samples were scored blindly, the author that mounted the slides was different from the author scoring the cells

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	Primary antibodies used in this study: mouse anti-β-tubulin (Cat# T9026, clone DM1A, Sigma-Aldrich; 1:1000); mouse anti-Cdk1 (Cat# sc-54, Santa Cruz Biotechnology; 1:1000), rabbit anti-cyclin B1(Cat# A305-000A-M, Bethyl Laboratories; 1:1000), rabbit anti-Arpp-19 (Cat# 11678-1-AP, Proteintech; 1:1000), rabbit anti-Ensa (Proteintech; Cat 14518-1-AP; 1:1000), rabbit anti-phospho-Ensa (Ser67)/

Arpp-19(Ser62) (DpSG; Cat# 5240S, Cell Signaling Technology; 1:1000), rabbit anti-Fcp1 (Cat# A301-172A; Bethyl Laboratories; 1:1000), human anti-centromere (CREST; Cat# 15-234, Antibodies Incorporated; 1:100), rabbit anti-Cdk substrate motif [(K/H)pSP] (Cat# 9477, Cell Signaling Technology; 1:1000), anti-Flag Peroxidase (HRP) (Cat# A8592, Sigma-Aldrich; 1:500), mouse anti-□-tubulin (Cat# T5326, Clone GTU-88, Sigma-Aldrich; 1:1000), rabbit anti-Cdc25C (Cat# 4688S, Cell Signaling Technology; 1:1000), anti-Flag FITC (Cat# F4049, Sigma-Aldrich; 1:200). Rabbit polyclonal antibody against phosphorylated serine 23 of human Arpp-19 (rabbit anti-phospho-serine-23-Arpp-19; pS23-Arpp-19) was raised using C-EDKVT(Sp)PEK-coNH2 peptide as immunogen; peptide synthesis, rabbit inoculation, serum production and cross-affinity antibody purification were carried out by CovalAb (Bron, France; 1:100). Secondary antibodies used in this study: sheep anti-mouse IgG HRP linked (Cat# NA931; GE Healthcare; 1:3000), donkey anti-rabbit IgG HRP linked (Cat# NA934; GE Healthcare; 1:4000), goat anti-mouse IgG Alexa Fluor 488 (Cat# A11029; Invitrogen; 1:15000), goat anti-human IgG Alexa Fluor 594 (Cat# A11014; Invitrogen; 1:15000). Antibody-conjugated beads used in this study: protein A/G PLUS-agarose (Cat# sc-2003; Santa Cruz Biotechnology), mouse anti-Flag agarose (Cat# A2220; Sigma-Aldrich).

Validation

Commercially available antibodies have been validated by the manufacturers. The Rabbit polyclonal antibody against phosphorylate serine 23 of human Arpp-19 (rabbit anti-phospho-serine-23-Arpp-19; pS23-Arpp-19) was raised using C-EDKVT(Sp)PEK-coNH2 peptide as immunogen; peptide synthesis, rabbit inoculation, serum production and cross-affinity antibody purification were carried out by CovalAb (Bron, France). Validation was obtained by the positive recognition on blots of exogenously expressed wild type Arp-19 and failure to recognize an Arpp-19 version in which serine 23 was mutated into non-phosphorylatable alanine.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HeLa cells were form CEINGE Cell Culture Facility. Human Telomerase Reverse Transcriptase immortalized Retinal Pigment Epithelial (hTERT-RPE1) cells were a gift from Dr. A. Musacchio, Max Planck Institute of Molecular Physiology, Dortmund.
Authentication	HeLa cells and hTERT-RPE1 cells were originally obtained from ATCC
Mycoplasma contamination	Cells scored negative for mycoplasma in routinely performed tests.
Commonly misidentified lines (See ICLAC register)	NA

Plants

Seed stocks	NA
Novel plant genotypes	NA
Authentication	NA