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	MATLAB (MathWorks, version 2020b) image-segmentation and nuclear tracking scripts were used on raw microscopy images as previously published (Ratnayeke et al., 2023) and publicly available on Github (https://github.com/MeyerLab/image-analysis-ratnayeke-2022).
Data analysis	MATLAB (MathWorks, version 2020b) image-processing scripts and iterative imaging analysis pipeline were previously published and publicly available on Github (https://github.com/MeyerLab/image-analysis-ratnayeke-2022). RStudio was used for RNA-sequencing analysis using published analysis pipelines (DESeq2, Love et al., 2014)

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

RNA-seq data were deposited into the Gene Expression Omnibus database. Analyzed summary data reported in this study and uncropped Western blot images are

available within the Supplementary Information. Raw multiplexed immunofluorescence data and live-cell data are not provided due to size limitations, but additional datasets are available upon reasonable 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	Sample size calculation was not performed. Single-cell experiments involved analysis of thousands of cells per condition with 2-3 well replicates per experiment. Mostly three biological replicates (with some experiments only two biological replicates, as indicated in the figure legend) were conducted per condition.
Data exclusions	Data exclusion was based on automated criteria for cell nuclear segmentation quality, presence after iterative immunofluorescence washing steps (where some cells wash off in later rounds of imaging), as described in the methods section.
Replication	Experiments were conducted in biological duplicate and triplicate, as indicated in the manuscript figure legends.
Randomization	N/A
Blinding	There was no blinding for experiments. Uniform analyses were applied to all experimental conditions.

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

For immunofluorescence: rabbit anti-CDK6 (1:1000, Abcam Cat#ab124821, RRID: AB_10999714), rabbit anti-YAP (1:400, Cell Signaling Technology Cat# 14074, RRID:AB_2650491), mouse anti-p27 (1:1000, Cell Signaling Technology Cat#3698, RRID:AB_2077832), mouse anti-p21 (1:500, BD Bioscience Cat#556430, RRID:AB_396414), rabbit phospho-AKT(S473)(1:400, Cell Signaling Technology Cat#4060, RRID:AB_2315049), rabbit phospho-MAPK1/2(Thr202/Tyr204)(1:400, Cell Signaling Technology Cat#4370, RRID:AB_2315112), mouse phospho-S6(235/236)(1:800, Cell Signaling Technology Cat#62016, RRID:AB_2799618), mouse anti-cyclin D1 (1:200, Santa Cruz Biotech Cat#sc-8396, RRID:AB_627344), mouse anti-FRA1 (1:200, Santa Cruz Biotech Cat#sc-28310, RRID: AB_ AB_627632), rabbit anti-Myc (1:800, Cell Signaling Technology Cat#5605, RRID:AB_1903938), mouse anti-Myc tag (1:8,000, Cell Signaling Technology Cat#2276, RRID:AB_331783), mouse anti-N-cadherin (1:200, Santa Cruz Biotech Cat#sc-393933, RRID: AB_2832921), rabbit anti-p27 (1:1600, Cell Signaling Technology Cat#3686, RRID:AB_2077850), rabbit anti-p21 (1:2500, Cell Signaling Technology Cat#2947, RRID:AB_823586), rabbit anti-phospho-Rb(Ser807/811)(1:2500, Cell Signaling Technology Cat#8516, RRID:AB_1117658), rabbit anti-phospho-S6(240/244)(1:2500, Cell Signaling Technology Cat#5364, RRID:AB_10694233), rabbit anti-Skp2 (1:800, Cell Signaling Technology Cat#2652, RRID:AB_11178941), mouse anti-YAP (1:200, Santa Cruz Biotech. Cat#sc-101199,RRID: AB_1131430). For Western blotting: rabbit anti-AXL (1:1000, Cell Signaling Technology Cat# 8661, RRID:AB_11217435), rabbit anti-EGFR (1:1000, Cell Signaling Technology Cat# 4267, RRID:AB_2246311), rabbit anti-GAPDH (1:1000, Cell Signaling Technology Cat# 5174, RRID:AB_10622025), mouse anti-Vinculin (1:1000, Thermo Fisher Scientific Cat# 14-9777-80, RRID:AB_2573027)

Validation

Antibodies were selected based on their prior validation in past Meyer lab and other publications. Additional validations were confirmed through inhibitor treatments, siRNA-mediated knockdown, and known cell cycle phase regulation.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

All cell lines were obtained from ATCC.

Authentication

Cells were obtained directly from ATCC.

Mycoplasma contamination

All cell lines tested negative for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

N/A

Plants

Seed stocks

N/A

Novel plant genotypes

N/A

Authentication

N/A