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	PrarieView 4.5 was used to drive the two photon microscope. Viventis Microscope Control 2.0 was used to drive the light-sheet microscope. Zeiss Systems (2.3) was used to drive the confocal microscope. Micromanager 1.4 was used to drive the Zeiss Axio Observer Z1.
Data analysis	Custom Python scripts (3.9) were used to analyze organoid and two photon microscopy images. NumPy (1.23.5), SciPy (1.9), pandas (1.5) Scikit-image (0.19), trimesh (3.18) Python libraries were used. Huygens Software was used for deconvolution. Scikit-learn (1.1.3) and statsmodels (0.13) libraries were used for statistical modeling. The code is available at: https://github.com/xies/mouse_skin_size_control Other significant image analysis tools used include: -Cellpose2.0 -StarDist=0.9.1 -Trimesh=4.4.9

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

A minimum dataset generated in this study have been deposited in https://github.com/skotheimlab/xie_etal_2024_autonomous_cell_size_control/test_data.zip. The raw microscopy is available upon request to Jan Skotheim. The raw microscopy data used in Figure 1 was previously published in 33. The raw microscopy data used in Figure 3 was previously published in 44. 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	*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 if 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.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status). Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.) Please provide details about how you controlled for confounding variables in your analyses.*
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.*
Ethics oversight	*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

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	For WT and Rb1-null experiments, data from two pairs of littermates were collected. Sample size for DKO data collection was was limited by the availability of the DKO mice, which breeds poorly and only yielded one litter per breeding pair.
Data exclusions	No data was excluded from the analysis.
Replication	For WT and Rb1-null experiments, robust reproducibility was confirmed across the experimental replicates. No replication estimate has been done yet for the DKO mouse due to animal availability, but multiple tissue regions on the same animal yield quantitatively similar results.
Randomization	Littermate pairs were randomly allocated to be WT or Rb1-null experimental group.
Blinding	Blinding was not possible due to the nature of the imaging experiment and image analysis and very obvious visual difference between WT, Rb1-null, and DKO tissues.

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:
β-actin (Sigma-Aldrich # A2103, rabbit monoclonal)
Rb1 (H-2) (Santa Cruz Biotechnology #sc-74570, mouse monoclonal)
β-Catenin (BD Transduction Laboratories # 610154, monoclonal mouse)
RNA Polymerase RPB1 (1C7) (Abcam, monoclonal rat, #ab252854)
Histone H3 (D1H2) (Cell Signaling Technology, monoclonal rabbit, #4499)
Rbl1 (Abcam, polyclonal rabbit, #209546).
phospho-RB1[S807/S811] (Cell Signaling Technology #9308, monoclonal rabbit 1:200)
53BP1 (Novus Biologicals #NB100-304, monoclonal rabbit 1:200)
Keratin 10 (Biolegends #19054, polyclonal rabbit 1:200)

Secondary antibodies:
IRDye 680LT goat anti-mouse IgG (LI-COR, 926-68020) and IRDye 800CW goat anti-rabbit IgG (LI-COR, 926-32211); Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 647 (Invitrogen #A32728), Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 488 (Invitrogen #A11034), and Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 488 (Invitrogen #A11029).

Validation

Validation done in study
-Rbl1: validated by 48h Rbl1 knockdown followed by immunofluorescence staining in NIH3T3s, which showed the disappearance of the expected nuclear signal
-Rb1: validated by Western blotting against mouse NIH3T3 lysates, showing single band at the expected molecular weight, and nuclear immunofluorescence staining which goes away upon 48h Rb1 knockdown by shRNA
phospho-RB1[S807/S811]: validated by the expected cell-cycle profile against mouse cells, showing high signal in small 2n cells and low signal in 4n cells.

Validated by manufacturer
-beta-Actin: validated by comparison with multiple independent epitopes against actin using IHC against multiple tissues, as well as Western blotting against mouse lysates showing a single band at the expected molecular weight https://www.sigmaaldrich.com/US/en/product/sigma/a2103#product-documentation
-beta-Catenin: validated by immunofluorescence staining showing cell boundary signal https://www.bdbiosciences.com/en-us/products/reagents/microscopy-imaging-reagents/immunofluorescence-reagents/purified-mouse-anti-catenin.610154?tab=product_details
-RNA Polymerase II CTD repeats: validated by Western blotting against mouse and human whole cell lysates showing a single band at the expected molecular weight https://www.abcam.com/en-us/products/primary-antibodies/rna-polymerase-ii-ctd-repeat-ysptsps-antibody-1c7-ab252854
-Histone H3: validated by comparison with multiple independent epitopes against histone H3 showing the expected nuclear signal in IHC or ICC against multiple tissues, as well as Western blotting against mouse lysates showing a single band at the expected molecular weight https://www.cellsignal.com/products/primary-antibodies/histone-h3-d1h2-xp-rabbit-mab/4499
53BP1: immunofluorescence on knock out HeLa cells, as well as normal and irradiated mouse tissues https://www.novusbio.com/products/53bp1-antibody_nb100-304
Keratin 10: Western blotting on mouse skin tissue and mouse tissues that do not express keratin 10 https://www.biolegend.com/de-de/products/purified-anti-keratin-10-antibody-13377

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Cell lines:
NIH3T3: ATCC

HEK293T: ATCC
mESCs: Dr. Marius Wernig (Stanford Medical School)
hRPE-1: Dr. Tim Stearns (Stanford University)

Primary cells:
A 2 month old male Lgr5-DTR-GFP mouse was used to generate primary intestinal organoids
E13 wildtype embryos were used to generate MEFs
Primary hepatocytes were isolated from FUCCI2 mice

Authentication

Primary cells were not further authenticated after generation

Cell lines were checked by morphology under microscopy. RPE1 cells were checked by relative flat and 'cobblestone' morphology and their inability to be passaged at low density. mESCs were checked by their domed colonies and ability to proliferate under MEK inhibition. NIH3T3s were checked by their spindly morphology.

293T cells were not further authenticated

Mycoplasma contamination

Cell media was tested for mycoplasma contamination every 2 months by PCR

Commonly misidentified lines
(See ICLAC register)

Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals

All mouse used in the study were adults between 3-7 months of age.
Mouse line Catalog number
Rbl1-/- JAX: 008178
Rb1-floxP JAX: 008186
Rosa26-CreERT2 JAX, 008463
K14-H2B-Cerulean N/A
Lgr5-DTR-GFP N/A
R26p-FUCCI2 RIKEN, CDB0203T

Wild animals

None

Reporting on sex

Although both male and female animals were included in the experimental design, due to the challenging nature of the data collection, only two pairs of male litteramates were used in the WT and Rb1-null experiments, and only a male animal was included in the DKO experiment. The ablation experiments included 1 female and 3 male animals.

Field-collected samples

None

Ethics oversight

All mice were handled in accordance with the guidelines of the Institutional Animal Care and Use Committee at Stanford University.

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

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.