

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 (<i>n</i>) 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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ 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. <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> |
| ☒ | ☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	NovoExpress (FACS) Zen (imaging) CellSens (imaging) Metamorph (imaging) Andor fusion (imaging)
Data analysis	ImageJ (image analysis) Imaris (3D reconstruction) Prism 10 (statistical 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](#)

Provide your data availability statement here.

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](https://www.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 FACS analyses the number of cells analysed per condition was 20000. For live cell imaging experiments, we chose sample sizes to be consistent with previous studies in the literature or from our lab (whenever possible n>100)

Data exclusions

No data exclusion was applied

Replication

Most of our experiments were replicated 3X to ensure reproducibility

Randomization

All samples used in this study were randomly allocated

Blinding

No blinding was applied, due to the high number of samples and 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

anti-AID (M214-3 Medical and Biological Laboratories; 1:1000)
 anti-Pgk1 (22C5D8 Invitrogen Molecular Probes, 1:40000)
 anti-GFP (3H9 Chromotek; 1:1000)
 anti-Pkc1 (sc-6801 Santa Cruz; 1:5000)
 anti-HA 12CA5 (AgroBio custom made; 1:5000)
 anti-Flag M2 (F3165 Sigma, 1:5000)
 anti-Cdc11 (sc-7170 Santa Cruz; 1:2000)

Validation

anti-AID: WB, IP, IC (<https://www.mblbio.com/bio/g/dtl/A/?pcd=M214-3>)
 anti-Pgk1: WB (<https://www.thermofisher.com/antibody/product/PGK1-Antibody-clone-22C5D8-Monoclonal/459250>)
 anti-GFP: WB (<https://www.ptglab.com/products/GFP-antibody-3H9.htm>)
 anti-Pkc1: WB; now discontinued (<https://datasheets.scbt.com/sc-6801.pdf>)
 anti-HA 12CA5: WB, IP (DOI:<https://doi.org/10.1016/j.cub.2019.11.054>)
 anti-Flag M2: WB, IP (<https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/415/240/f3165dat-mk.pdf>)
 anti-Cdc11: WB, IC (<https://www.scbt.com/fr/p/cdc11-antibody-y-415?requestFrom=search>)

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

All yeast strains are congenic to W303 (ade2-1, trp1-1, leu2-3,112, his3-11, 15 ura3) and listed in Table S1

Authentication

Yeast strains were generated by genetic crosses and meiotic recombination. Newly generated yeast mutants or tagged strains were validated by PCR and sequencing.

Mycoplasma contamination

N/A

Commonly misidentified lines (See [ICLAC](#) register)

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

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

10-20 millions of cells were collected, spun at 10000 g and fixed with 1 ml of 70% ethanol for at least 30 min at RT. After one wash with 50 mM Tris-Cl pH 7.5, cells were resuspended in 0.5 ml of the same buffer containing 0.05 ml of a preboiled 10 mg/ml RNase solution and incubated overnight at 37°C. The next day cells were spun at 10,000 g and resuspended in 0.5 ml of 5 mg/ml pepsin freshly diluted in 55 mM HCl. After 30-min incubation at 37°C, cells were washed with FACS buffer (200 mM Tris-Cl pH 7.5, 200 mM NaCl, 78 mM MgCl₂) and resuspended in the same buffer containing 50 µg/ml propidium iodide. After a short sonication, samples were diluted (1:8) in 200 µl of 50 mM Tris-Cl pH 7.5.

Instrument	ACEA NovoCyte cytometer
Software	NovoExpress software
Cell population abundance	No cell sorting was used
Gating strategy	Gating was applied only to eliminate cellular debris with minimal DNA contents.

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