nature portfolio

Corresponding author(s):	Alan Davidson		
Last updated by author(s):	Aug 8, 2022		

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.

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section

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n/a	Confirmed
	$oxed{x}$ 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.
x	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)
x	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>
x	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
×	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.
Sof	ftware and code

Software and code

Policy information about availability of computer code

Data collection

Provide a description of all commercial, open source and custom code used to collect the data in this study, specifying the version used OR state that no software was used.

Data analysis

Photoshop CS6 v. 13.0, Zeiss Zen blue version 3.0 19154.1, GraphPad Prism 9 version 9.5.0, Image J v. 1.53, Jalview v. 2.11.10, Scaffold v.

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 <u>data availability statement</u>. 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 datasets generated during and/or analysed during the current study are available in the supplementary material of the paper.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

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.

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

Ple	ease select the one below	/ tha	t is the best fit for your research	. If yo	u are not sure,	, read the appropriate	sections before r	making your selectior	١.
X	Life sciences		Behavioural & social sciences		Ecological, ev	olutionary & environn	nental 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: No sample size calculation was performed. For EM/ microscopy assays, sample size was based on all available images collected and the presence of measurable particles or hyphae on each micrograph. Overall similar numbers of values were obtained for samples to be compared (WT vs. mutants), and significance between samples was verified using statistical tests.

For plate based assays, quantitative data was collected for a selected n number of experiments (usually 6 plates per strain, per time point). Results were reproducible and a higher n number would not have further aided our interpretation of the results.

Data exclusions

None

Replication

All findings were reproducible.

Randomization

Organisms were partitioned into wild-type and various mutants. Randomization was not required.

Blinding

Blinding was not relevant to this work. Experiments were performed on wild-type and defined mutant strains.

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	
Antibodies	▼ ChiP-seq	
x Eukaryotic cell lines	Flow cytometry	
Palaeontology and archaeology	MRI-based neuroimaging	
Animals and other organisms		
Clinical data		
Dual use research of concern		
1		
Antibodies		
Commercial antibodies used: A. Rabbit anti FLAG — Sigma Aldrich F7425, LOT: 064m4757v B. Mouse anti rabbit — Santa Cruz Biotechnology IgG HRP sc-2357 C. Goat anti-mouse — Santa Cruz Biotechnology IgG HRP sc-2005 Mouse polyclonal antibodies were produce as part of this study.		
Validation Polyclonal antibodies were validated by testing identical strains lacking the gene of the protein of interest. Anti-FLAG antibodies were validated using a strain not expressing a FLAG-tagged protein.		