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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	Confocal microscopy was carried out with an inverted confocal system Nikon C1. Images were taken with Plan-Apo VC 100x/1.40 oil immersion objective. GFP/Alexa 488 were excited at 488 nm, DAPI at 408 nm, FM4-64 at 543 nm. The fluorescence signals were collected using filter sets: 515/30 nm for GFP/Alexa 488, 480/40 nm for DAPI and 610LP nm for FM4-64. Time Lapse Microfluidics Microscopy (TLMM) experiments were performed using a Delta Vision Elite inverted microscope equipped with a 100×/1.4 Oil objective. Immediately after sporulation induction cells were loaded into the ONIX observation chamber (B04A-03-5PK CellASIC ONIX plates for bacteria cells) and further cultured in sporulation medium with addition of 0.57 – 3% of the rich medium. Images were recorded at 5 min intervals for 6 h (35 ms exposure time, 10% transmittance on transmitted light channel, 15 ms exposure time and 10% of transmittance on EGFP channel). The temperature during the experiment was maintained at 37°C. Lattice SIM Imaging was performed using an Elyra 7 (Zeiss) inverted microscope equipped with an sCMOS 4.2 CL HS camera and an alpha Plan-Apochromat 100×/1.46 Oil DIC M27 objective with an Optovar 1.6x magnification changer. Fluorescence was excited with 488 nm laser (100 mW), the signals were observed through multiple beam splitter (405/488/561/641 nm) and laser block filters (405/488/561/641 nm). Cells were immobilised on 1% agarose pads (1.0×1.0-cm GeneFrames; Thermo Fisher Scientific). Cells were illuminated with 488 nm laser with 0.9% intensity and 150 ms exposure time and with 0.5% intensity and 100 ms exposure time for respectively RpsB-GFP and RpoC-GFP fusions. Each Z-plane was illuminated in Lattice-SIM mode comprising 13 phases. Image reconstruction was performed with the ZEN 3.0 SR software (Zeiss) using SIM2 reconstruction algorithm with standard parameters. Images were viewed and analysed in ImageJ and the middle planes were selected for visualisation. Mass spectrometry was performed using UltiMate 3000 nano-LC system coupled to a Q Exactive HF-X mass spectrometer via an easy-spray source (all Thermo Fisher Scientific) as service.
Data analysis	Analyses of microscopic data were performed using: Fiji: ImageJ v.1.54f and R v.4.3.1. Analyses of mass spectrometry data were performed using: MaxQuant v.1.6.17.0, Perseus v. 1.6.10.0 and R v.4.3.1

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 mass spectrometry proteomics data generated in this study have been deposited in the ProteomeXchange Consortium via the PRIDE partner repository under accession code PXD047497 [http://proteomecentral.proteomexchange.org/cgi/GetDataset?ID=PXD047497].
Source data are provided with this paper. The microscopic measurements and log2FoldChange values for the protein mass spectrometry data generated in this study, for all data presented in graphs within the Figures are provided in the Source Data file. The calculated log2FoldChange values for the protein mass spectrometry data generated in this study are provided in the Supplementary Data 1 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	Not involved.
Reporting on race, ethnicity, or other socially relevant groupings	Not involved.
Population characteristics	Not involved.
Recruitment	Not involved.
Ethics oversight	Not involved.

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

Field-specific reporting

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	For microscopic observations, the exact numbers of cells for each experiment are given in the figures legends and Methods section.
Data exclusions	No data was excluded from the analyses.
Replication	All experiments were performed in duplicates, including mass spectrometry and microscopy. For mass spectrometry experiments, the duplicates are described in the manuscript and all calculations are based on the entire dataset (both replicates). For microscopic observations, the experiments were performed in biological duplicates or triplicates, however, data from one replicate is shown in the manuscript, as the biological repeats were successful and confirmed the observations from the first experiment.
Randomization	Randomization is not applicable in this study because there is no statistic test that requires randomization of samples.
Blinding	Samples were processed and analysed in exact same manner, thus, no blinding was required.

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

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.*