## 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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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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.
$\boxtimes$	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>
$\boxtimes$	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 <u>statistics for biologists</u> contains articles on many of the points above.

## Software and code

Policy information about availability of computer code

Data collection

Metamorph (Molecular devices, 7.10.3) was used for microscopic imaging. Spark Control Dashboard (Tecan, V2.2) was used for all the OD600 measurements.

Data analysis

MassHunter Qualitative navigator (Agilent, B.08.00) was used to analyze LC/MS EIC spectra. Minitab 20 was used for Design of Experiments analyses. Graphpad Prism was used for generating graphical plots and performing statistical analyses.

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

All data generated or analysed during this study are included in this published article (and its supplementary information files).

Human rese	arch part	icipants		
Policy information	about <u>studies i</u>	involving human research participants and Sex and Gender in Research.		
Reporting on sex and gender		NA		
Population characteristics NA		NA		
Recruitment		NA		
Ethics oversight		NA		
Note that full informa	ation on the app	roval of the study protocol must also be provided in the manuscript.		
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Field-spe	ecitic re	porting		
Please select the o	ne below that	is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.		
X Life sciences		Behavioural & social sciences Ecological, evolutionary & environmental sciences		
For a reference copy of t	the document with	all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>		
Life scier	nces st	udy design		
All studies must dis	close on these	points even when the disclosure is negative.		
Sample size		x10^8 CFU/ml of spores were used for each germination experiment while spores of the order of 10^9 CFU/ml were used for eriments. This is far in excess of an amount which could cause error due to under-sampling.		
Data exclusions	_	piological replicate for L-asparagine was excluded for being a clear outlier. The criteria for outlier detection was pre-established n(xi)]/(median of all absolute deviations from the median) at a cutoff of 2.5. Including the outlier does not change the result of nt.		
Replication		at replicating our findings were successful. DOE experiments were replicated twice with two embedded technical replicates. spore germination assays were performed with 4 biological replicates. The LC-MS and imaging data were best representative of ident samples.		
Randomization	Randomization	omization was not relevant to this study.		
Blinding	Blinding is not reproducibility	s not relevant when analyzing bacteria or bacterial spores. Experimental replicates and embedded technical replicates ensured bility.		
We require informatis system or method list  Materials & ex  n/a Involved in th  Antibodies  Eukaryotic  Palaeontol  Animals ar  Clinical dat	on from authors ted is relevant to perimental size study  cell lines ogy and archaected other organism	n/a Involved in the study    ChIP-seq     Flow cytometry     MRI-based neuroimaging		