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FRANCESC-XABIER CONTRERAS, MARCELO E. GUERIN, BEATRIZ TRASTOY AND LAURA

Corresponding author(s): $\underline{\text{ALONSO-SÁEZ}}$

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Reporting Summary

MAFFT v7 trimAl v1.4 SortMeRNA v4.3.6

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	
	The exac	xt sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
	🗶 A statem	nent on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
		stical test(s) used AND whether they are one- or two-sided mon tests should be described solely by name; describe more complex techniques in the Methods section.
X	A descrip	otion of all covariates tested
×	A descrip	otion of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
		scription of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) iation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
x		hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted whenever suitable.
x	For Baye	sian analysis, information on the choice of priors and Markov chain Monte Carlo settings
x	For hiera	archical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
x	Estimate	es of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated
'		Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.
Sof	ftware ar	nd code
Polic	cy information	about <u>availability of computer code</u>
Da	ta collection	Zeiss Zen Microscopy Software Chromeleon v 7.2 SR5 software
Da	ta analysis	ImageJ open source software, version 1.51J8, GraphPad Prism 9 xds (Jun 30, 2023) Ccp4 8.0 Phenix 1.20.1 WinCoot 0.9.8.1 Chimera 1.16 HOLLOW DALI PISA R version 4.2.1 dbCAN v3.0.7 RAXML 8.2.12

Bowtie2 2.4.2		
HTSeq 0.11.2		
ColabFold v1.5.5.		
BLAST 2.9.0.		
Peaks Studio X+		
Perseus 1.6.14.0		

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 atomic coordinates and structure factors have been deposited with the Protein Data Bank (PDB), accession code 8RG3 (PbFucA-1), 8RG4 (PbFucA-2), 8RG5 (PbFucA-3) and 9F9V (Rho5174). The sequence datasets generated during the current study are available in the European Nucleotide Archive (ENA) repository with project accession number PRJEB71092 and Zenodo (https://doi.org/10.5281/zenodo.10539754). Proteomic data has been deposited to the ProteomeXchange Consortium via the PRIDE repository with the dataset identifier PXD057797. The protein structures have been deposited in PDB under the following accession codes: 8RG3 [http://doi.org/10.2210/pdb8RG3/pdb], 8RG4 [http://doi.org/10.2210/pdb8RG5/pdb], 8RG5 [http://doi.org/10.2210/pdb8RG5/pdb], and 9F9V [http://doi.org/10.2210/pdb9F9V/pdb].

Research involving human participants, their data, or biological material

and sexual orientation and race, or	ethnicity and racism.
Reporting on sex and gender	n/a
Reporting on race, ethnicity, or other socially relevant groupings	n/a
Population characteristics	n/a
Recruitment	n/a
Ethics oversight	n/a

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation),

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	v 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 the docume	ent with all sections, see nature.com/document	:s/nr-reporting-summary-flat.pdf

Life sciences study design

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

Sample size	We performed all experiments (growth experiments, transcriptomics, proteomics, enzyme assays) in three independent biological replicates except for C-PAGE experiments that were performed in duplicates In the fluorescence microscopy analysis, sample size was empirically determined to optimize numbers based on our previous experience with equivalent experiments.
Data exclusions	No data was excluded from the analyses.
Replication	Triplicate experiments were performed in activity assays using PAHBAH methodology, growth curves, transcriptomic and proteomic assays Duplicate experiments were performed in activity assays using C-PAGE. All attempts at replicating the results in fluorescence microscopy analysis were successful.
Randomization	Data were obtained by analyzing randomly selected bacteria based only on bacterial specific fluorescence signal detections.
Blinding	Researchers analysing fluorescence microscopy images were blinded to group allocation during the experiments.

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		Me	Methods	
n/a	Involved in the study	n/a	Involved in the study	
×	Antibodies	X	ChIP-seq	
×	Eukaryotic cell lines	×	Flow cytometry	
×	Palaeontology and archaeology	×	MRI-based neuroimaging	
X	Animals and other organisms	•		
x	Clinical data			
x	Dual use research of concern			
×	Plants			

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

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.