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
	\square 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. <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
\boxtimes	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
\boxtimes	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

No data collection software was used.

Data analysis

R 3.6.3 stampy 1.0.23 edgeR 3.28.1 stats 3.6.1 multimode 1.4 KEGGREST 1.26.1 ggplot2 SAMtools 0.1.19 GraphPad Prism 8.3.0

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

Transcriptome data have been deposited to NCBI Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/) under the GenBank accession numbers GSE134231 (biofilm transcriptomes) and GSE123544 (planktonic transcriptomes). DNA-seq data are available in the Sequence Read Archive (SRA; https://www.ncbi.nlm.nih.gov/sra) under the reference number PRJNA526797.

ield-specific reporting					
ease 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 <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>					
ife sciences study design					
studies must disclose on these points even when the disclosure is negative.					
Sample size No sample-size calculation was performed.					
Data exclusions No data exclusions					
Replication For robust transcriptome analyses, genetically diverse isolates with identical features were regarded as replicates:					
group of lasR_WT: n = 28; group of lasR* (inactivating mutations in lasR): n = 21;					
group of lasR**/rhlR* (InDel/stop in rhlR and InDel/stop and/or non-synonymous mutations in lasR): n = 7					
For phenotypic characterization studies, a random subset from the above mentioned groups was selected and clinical strains were regarded as replicates:					
group of lasR_WT: n = 10;					
group of lasR* (inactivating mutations in lasR): n = 8;					
group of lasR**/rhlR* (InDel/stop in rhlR and InDel/stop and/or non-synonymous mutations in lasR): n = 4					
Randomization Not relevant to the study					
Blinding Not relevant to the study					

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	
\boxtimes	Antibodies	\boxtimes	ChIP-seq	
\boxtimes	Eukaryotic cell lines	\times	Flow cytometry	
\boxtimes	Palaeontology and archaeology	\times	MRI-based neuroimaging	
\boxtimes	Animals and other organisms			
\boxtimes	Human research participants			
\boxtimes	Clinical data			
\boxtimes	Dual use research of concern			