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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](#).

Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study.

For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

### 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

- |                                     |                                     |                                                                                                                                                                                                                                                            |
|-------------------------------------|-------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement                                                                                                                                    |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly                                                                                                                                    |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>                                                               |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | A description of all covariates tested                                                                                                                                                                                                                     |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons                                                                                                                                        |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | 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) |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | For null hypothesis testing, the test statistic (e.g. $F$ , $t$ , $r$ ) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted<br><i>Give <math>P</math> values as exact values whenever suitable.</i>                            |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings                                                                                                                                                           |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes                                                                                                                                     |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | 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** Optical density and fluorescence measurements were performed using a Synergy Mx plate reader (BioTek Instruments) and the accompanying BioTek Gen5 software. RT-qPCR data was collected using a StepOnePlus Real-Time PCR machine (Applied Biosystems) and the accompanying StepOnePlus software. Growth curves were measured using a Bioscreen C device (Oy Growth Curves Ab Ltd.) and the corresponding BioScreener software. Whole-genome sequencing of the evolved biofilm populations was outsourced the GENEWIZ Europe (Leipzig, Germany), and Sanger sequencing was outsourced to Eurofins Genomics (Leuven, Belgium). The *Salmonella* Typhimurium ATCC14028 reference genome was obtained from NCBI (<https://www.ncbi.nlm.nih.gov>) with the RefSeq accession GCF\_000022165.1. Protein crystal structures were obtained from the Protein DataBase (PDB), with as accessions 3VVX (RamR), 6UTW (RpoS) and 4JKR (RNA polymerase).

**Data analysis** All data analysis was performed in Microsoft Excel and GraphPad Prism (v10). For analysis and visualisation of the Sanger sequencing data, Benchling was used. For analysis of the whole-genome sequencing results, FastQC, MultiQC, Bowtie2, Samtools, LoFreq & SnpEff were used. For visualization of protein structures, the Mol\* (WebGL) tool embedded in the PDB webpages was used.

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 raw data can be found in the supplementary information (XLSX). The datasets analyzed and processed (PRISM) in the current study are available upon request at the corresponding author.

The raw sequencing reads obtained from whole genome sequencing of the evolved biofilm populations were deposited in the NCBI Sequence Read Archive (SRA) and can be accessed through BioProject accession PRJNA1224455.

## 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                                        | No human test subjects were included in this in vitro study. |
| Reporting on race, ethnicity, or other socially relevant groupings | No human test subjects were included in this in vitro study. |
| Population characteristics                                         | No human test subjects were included in this in vitro study. |
| Recruitment                                                        | No human test subjects were included in this in vitro study. |
| Ethics oversight                                                   | No human test subjects were included in this in vitro study. |

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 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 [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     | At least three biological repeats were included in each experiment. The exact sample size is noted for each dataset in the manuscript. |
| Data exclusions | No data was excluded.                                                                                                                  |
| Replication     | At least three biological repeats were included in each experiment. The exact sample size is noted for each dataset in the manuscript. |
| Randomization   | No randomisation was implemented.                                                                                                      |
| Blinding        | No blinding was implemented.                                                                                                           |

## Behavioural & social sciences study design

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

|                   |  |
|-------------------|--|
| Study description |  |
| Research sample   |  |
| Sampling strategy |  |
| Data collection   |  |
| Timing            |  |
| Data exclusions   |  |
| Non-participation |  |
| Randomization     |  |

# Ecological, evolutionary & environmental sciences study design

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

|                          |                      |
|--------------------------|----------------------|
| Study description        | <input type="text"/> |
| Research sample          | <input type="text"/> |
| Sampling strategy        | <input type="text"/> |
| Data collection          | <input type="text"/> |
| Timing and spatial scale | <input type="text"/> |
| Data exclusions          | <input type="text"/> |
| Reproducibility          | <input type="text"/> |
| Randomization            | <input type="text"/> |
| Blinding                 | <input type="text"/> |

Did the study involve field work? ☐ Yes ☐ No

## Field work, collection and transport

|                        |                      |
|------------------------|----------------------|
| Field conditions       | <input type="text"/> |
| Location               | <input type="text"/> |
| Access & import/export | <input type="text"/> |
| Disturbance            | <input type="text"/> |

# 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                           |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Antibodies                    | <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Eukaryotic cell lines         | <input checked="" type="checkbox"/> | <input type="checkbox"/> Flow cytometry         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology | <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Animals and other organisms   |                                     |                                                 |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data                 |                                     |                                                 |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern  |                                     |                                                 |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Plants                        |                                     |                                                 |

## Antibodies

|                 |                      |
|-----------------|----------------------|
| Antibodies used | <input type="text"/> |
| Validation      | <input type="text"/> |

## Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

|                                                                      |                      |
|----------------------------------------------------------------------|----------------------|
| Cell line source(s)                                                  | <input type="text"/> |
| Authentication                                                       | <input type="text"/> |
| Mycoplasma contamination                                             | <input type="text"/> |
| Commonly misidentified lines<br>(See <a href="#">ICLAC</a> register) | <input type="text"/> |

## Palaeontology and Archaeology

Specimen provenance

Specimen deposition

Dating methods

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight

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

## Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals

Wild animals

Reporting on sex

Field-collected samples

Ethics oversight

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

## Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration

Study protocol

Data collection

Outcomes

## Dual use research of concern

Policy information about [dual use research of concern](#)

### Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

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Policy information about [dual use research of concern](#)

### Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

| No                       | Yes                                                 |
|--------------------------|-----------------------------------------------------|
| <input type="checkbox"/> | <input type="checkbox"/> Public health              |
| <input type="checkbox"/> | <input type="checkbox"/> National security          |
| <input type="checkbox"/> | <input type="checkbox"/> Crops and/or livestock     |
| <input type="checkbox"/> | <input type="checkbox"/> Ecosystems                 |
| <input type="checkbox"/> | <input type="checkbox"/> Any other significant area |

### Experiments of concern

Does the work involve any of these experiments of concern:

| No                       | Yes                                                                                                  |
|--------------------------|------------------------------------------------------------------------------------------------------|
| <input type="checkbox"/> | <input type="checkbox"/> Demonstrate how to render a vaccine ineffective                             |
| <input type="checkbox"/> | <input type="checkbox"/> Confer resistance to therapeutically useful antibiotics or antiviral agents |
| <input type="checkbox"/> | <input type="checkbox"/> Enhance the virulence of a pathogen or render a nonpathogen virulent        |
| <input type="checkbox"/> | <input type="checkbox"/> Increase transmissibility of a pathogen                                     |
| <input type="checkbox"/> | <input type="checkbox"/> Alter the host range of a pathogen                                          |
| <input type="checkbox"/> | <input type="checkbox"/> Enable evasion of diagnostic/detection modalities                           |
| <input type="checkbox"/> | <input type="checkbox"/> Enable the weaponization of a biological agent or toxin                     |
| <input type="checkbox"/> | <input type="checkbox"/> Any other potentially harmful combination of experiments and agents         |

## Plants

Seed stocks

Novel plant genotypes

Authentication

## ChIP-seq

### Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links

*May remain private before publication.*


Files in database submission

Genome browser session

(e.g. [UCSC](#))

### Methodology

Replicates

Sequencing depth

Antibodies

Peak calling parameters

Data quality

Software

## Flow Cytometry

### Plots

Confirm that:

☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ All plots are contour plots with outliers or pseudocolor plots.

☐ A numerical value for number of cells or percentage (with statistics) is provided.

### Methodology

Sample preparation

Instrument

Software

Cell population abundance

Gating strategy

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

## Magnetic resonance imaging

### Experimental design

|                                 |                                                                 |
|---------------------------------|-----------------------------------------------------------------|
| Design type                     | <input type="text"/>                                            |
| Design specifications           | <input type="text"/>                                            |
| Behavioral performance measures | <input type="text"/>                                            |
| Imaging type(s)                 | <input type="text"/>                                            |
| Field strength                  | <input type="text"/>                                            |
| Sequence & imaging parameters   | <input type="text"/>                                            |
| Area of acquisition             | <input type="text"/>                                            |
| Diffusion MRI                   | <input type="checkbox"/> Used <input type="checkbox"/> Not used |

### Preprocessing

|                            |                      |
|----------------------------|----------------------|
| Preprocessing software     | <input type="text"/> |
| Normalization              | <input type="text"/> |
| Normalization template     | <input type="text"/> |
| Noise and artifact removal | <input type="text"/> |
| Volume censoring           | <input type="text"/> |

### Statistical modeling & inference

|                                           |                                                                                                       |
|-------------------------------------------|-------------------------------------------------------------------------------------------------------|
| Model type and settings                   | <input type="text"/>                                                                                  |
| Effect(s) tested                          | <input type="text"/>                                                                                  |
| Specify type of analysis:                 | <input type="checkbox"/> Whole brain <input type="checkbox"/> ROI-based <input type="checkbox"/> Both |
| Statistic type for inference              | <input type="text"/>                                                                                  |
| (See <a href="#">Eklund et al. 2016</a> ) |                                                                                                       |
| Correction                                | <input type="text"/>                                                                                  |

### Models & analysis

|                                               |                                                                       |
|-----------------------------------------------|-----------------------------------------------------------------------|
| n/a                                           | Involved in the study                                                 |
| <input type="checkbox"/>                      | <input type="checkbox"/> Functional and/or effective connectivity     |
| <input type="checkbox"/>                      | <input type="checkbox"/> Graph analysis                               |
| <input type="checkbox"/>                      | <input type="checkbox"/> Multivariate modeling or predictive analysis |
| Functional and/or effective connectivity      | <input type="text"/>                                                  |
| Graph analysis                                | <input type="text"/>                                                  |
| Multivariate modeling and predictive analysis | <input type="text"/>                                                  |