

## 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 ( <i>n</i> ) 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. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted<br><i>Give P 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 type="checkbox"/>            | <input checked="" type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes                                                                                                                                     |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> 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 [statistics for biologists](#) contains articles on many of the points above.*

### Software and code

Policy information about [availability of computer code](#)

Data collection: Biotek Synergy 4, Agilent MassHunter software package Workstation B.08.00

Data analysis: Graph Pad 10.4.1, Agilent MassHunter software package Workstation B.08.00., software suite DIA-NN, Morpheus: <https://software.broadinstitute.org/morpheus>

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](#)

Data availability statement:

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE14 partner repository with the dataset identifier PXD060741. DIA-NN is freely available for download from <https://github.com/vdemichev/DiaNN>.

All strains and their corresponding registry IDs used in this study are listed in Supplementary Table 1 and are available through the Joint BioEnergy Institute's Inventory of Composable Elements (ICE), an open-source registry software and platform for managing information about biological parts (<https://public-registry.jbei.org/folders/894>). Additionally whole genome sequencing files of the evolved mutants are linked to the corresponding strains.

Data is available from the corresponding authors upon request.

## 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                                        | N/A |
| Reporting on race, ethnicity, or other socially relevant groupings | N/A |
| Population characteristics                                         | N/A |
| Recruitment                                                        | N/A |
| Ethics oversight                                                   | N/A |

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](https://www.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     | for most experiments between 3-6 replicates are used to basic statistical analysis, including the calculation of standard deviation and error bars                                                                                                                                              |
| Data exclusions | only one data point is excluded in this text, 1 replicate out of 6 was physically dropped on the floor prior to GCMS                                                                                                                                                                            |
| Replication     | Experiments on production titers were repeated multiple times ( $\geq 2$ ), and while exact titers varied, the trends across strains and supplementation conditions remained consistent. Experiments not replicated included proteomics, whole genome sequencing, adaptive laboratory evolution |
| Randomization   | Samples were allocated based on genetic composition (genomic or plasmid-bearing). Experimental conditions, including media composition and additives, were standardized across all strains to ensure consistency and control for potential confounding variables.                               |
| Blinding        | Blinding was not implemented in this study as the data collected was primarily empirical and not subject to interpretation by the researchers                                                                                                                                                   |

## 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

| n/a                                 | Involved in the study                                  |
|-------------------------------------|--------------------------------------------------------|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Antibodies                    |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Eukaryotic cell lines         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology |
| <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                        |

### Methods

| n/a                                 | Involved in the study                           |
|-------------------------------------|-------------------------------------------------|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Flow cytometry         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging |

### Antibodies

|                 |                                  |
|-----------------|----------------------------------|
| Antibodies used | <input type="text" value="N/A"/> |
| Validation      | <input type="text" value="N.A"/> |

## 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                                                                                                                             | <input type="text"/> |
| Specimen deposition                                                                                                                             | <input type="text"/> |
| Dating methods                                                                                                                                  | <input type="text"/> |
| <input type="checkbox"/> Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information. |                      |
| Ethics oversight                                                                                                                                | <input type="text"/> |

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      | <input type="text"/> |
| Wild animals            | <input type="text"/> |
| Reporting on sex        | <input type="text"/> |
| Field-collected samples | <input type="text"/> |
| Ethics oversight        | <input type="text"/> |

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 | <input type="text"/> |
| Study protocol              | <input type="text"/> |
| Data collection             | <input type="text"/> |
| Outcomes                    | <input type="text"/> |

## 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:

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

## Plants

|                       |                      |
|-----------------------|----------------------|
| Seed stocks           | <input type="text"/> |
| Novel plant genotypes | <input type="text"/> |
| Authentication        | <input type="text"/> |

## 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<br><i>May remain private before publication.</i> | <input type="text"/> |
| Files in database submission                                       | <input type="text"/> |
| Genome browser session<br>(e.g. <a href="#">UCSC</a> )             | <input type="text"/> |

### Methodology

|                         |                      |
|-------------------------|----------------------|
| Replicates              | <input type="text"/> |
| Sequencing depth        | <input type="text"/> |
| Antibodies              | <input type="text"/> |
| Peak calling parameters | <input type="text"/> |
| Data quality            | <input type="text"/> |

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

Design specifications

Behavioral performance measures

Imaging type(s)

Field strength

Sequence &amp; imaging parameters

Area of acquisition

Diffusion MRI ☐ Used ☐ Not used

### Preprocessing

Preprocessing software

Normalization

Normalization template

Noise and artifact removal

Volume censoring

### Statistical modeling & inference

Model type and settings

Effect(s) tested

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference

(See [Eklund et al. 2016](#))

Correction

## Models & analysis

n/a | Involved in the study

☐ ☐ Functional and/or effective connectivity

☐ ☐ Graph analysis

☐ ☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Graph analysis

Multivariate modeling and predictive analysis