nature portfolio

Corresponding author(s): Ian T. Paulsen

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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.

## 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 | |
|---|---|---|
| ☐ | ☒ | 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<br>*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. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted<br>*Give P values as exact values whenever suitable.* |
| ☒ | ☐ | 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 *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 | Tranposon insertion site reads were mapped by Bio-Tradis (available in GitHub). Flow cytometry data were collected through BD Influx Sortware. Growth curve data were collected through "Reader Control" and "MARS Data Analysis Sofware 3.20 R2". qPCR data were collected through ROCHE "LightCycler 480 Software release 1.5.1.62 SP2". |
| Data analysis | Time kill curve data and flow cytometry data were analyzed through R with custom codes. The data of growth curves, qPCR, and membrane potential are analyzed by Graph Pad Prism 10.0.0 (131).. |

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

TraDIS sequencing data was deposited in the European Nucleotide Database under project number PRJEB8707

## Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

| | |
|---|---|
| Reporting on sex and gender | Not applicable |
| Population characteristics | Not applicable |
| Recruitment | Not applicable |
| Ethics oversight | Not applicable |

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.

| | |
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| Sample size | Three biological replicates were included in all the experiments in this study, except for TraDIS assays. TraDIS assays used two biological replicates for each condition. This is because each TraDIS biological replicate contained more than 100k unique transposon insertion mutants, which itself will provide very high statistical confidence. |
| Data exclusions | No data was excluded. |
| Replication | Three biological replicates were included in all of the experiments in this study, except for TraDIS assays. There were some variations among the biological replicate data, but the differences among the experimental groups are statistically significant. Two independent TraDIS biological replicates were performed for each condition. Both of the TraDIS replicates of each condition produced data of high statistical confidence. |
| Randomization | The biological replicates were derived from individual colonies that were randomly picked from an agar plate. |
| Blinding | Blinding was not relevant to this study. The key criteria in determining whether the observations were reproducible is that different batch of cells can produce consistent data/results on different days, which do not need blinding. |

# 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 |
|---|---|
| ☒ | ☐ Antibodies |
| ☒ | ☐ Eukaryotic cell lines |
| ☒ | ☐ Palaeontology and archaeology |
| ☒ | ☐ Animals and other organisms |
| ☒ | ☐ Clinical data |
| ☒ | ☐ Dual use research of concern |

### Methods

| n/a | Involved in the study |
|---|---|
| ☒ | ☐ ChIP-seq |
| ☐ | ☒ Flow cytometry |
| ☒ | ☐ MRI-based neuroimaging |

# 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 | Multiple colonies were picked from an agar plate. The colonies were inoculated in MH broth and grew overnight at 37C and with shaking at 200rpm. The overnight cultures were diluted 1/100 in fresh MH broth and subcultured at the same condition till OD600 reached 0.4. The subcultures were treated by different conditions for 2-5 minutes at 37C with shaking at 200rpm. After this the subcultures were diluted to density around 10^6 cell per ml before flow cytometry. More information please refer to the "Methods and Materials" section in the article. |
| Instrument | BD Influx |
| Software | BD Influx Sortware |
| Cell population abundance | Pure bacterial cultures were analyzed. The culture density was around 10^6 cells per ml. |
| Gating strategy | We gated 50,000 cells based on their FSC and SSC to exclude the minority elongated cells and cell debris, and recorded all the events for the final data analysis. Please refer Extended Data Figure S6 and S7 for more detail. |

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