

Reporting Summary

Nature Research 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 Research policies, see [Authors & Referees](#) 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
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
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

Flow cytometry software: FACSDiva 8.0.1, CytExpert 2.1.0.92. Western blot: Li-Cor Image Studio 2.1.10.

Data analysis

Flow cytometry data was analyzed with FlowJo 10 software (Treestar).
R 3.5.0 was used to calculate growth rates by calculating the log of the absolute cell counts and performing linear regressions.
Western blot data was analyzed with Image Studio 2.1.10 software (Li-Cor).
Microsoft Excel for Mac version 15.31 was used for basic statistical analysis.
Graphpad Prism 7 was used for plotting data and cubic spline fitting.
Custom Matlab 2014a and R 3.5.0 code was used for simulations and parameter identification (available from the corresponding author on reasonable request).

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [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 list of figures that have associated raw data
- A description of any restrictions on data availability

All relevant data are included as source data and/or are available from the corresponding author on reasonable request. Plasmid sequences are deposited in GenBank under the accession codes MK775703-MK775710. Strains and plasmids used in this study are available from the corresponding author on reasonable request.

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	Each experiment was performed at least three times and is on par with standard bacterial experiments reported in the field.
Data exclusions	No data were excluded from analysis.
Replication	A minimum of three biological replicates were used and are indicated in the figure legends. All findings were reliably reproducible.
Randomization	Bacterial cultures were propagated from isogenic stocks derived from randomly selected colonies. When splitting cultures into different conditions as well as sampling cultures for measurements, cells within the cultures are randomly sampled as there is no control over which cells are selected.
Blinding	Researchers were not blinded during experiments because the experiments were not based on qualitative scoring metrics. In addition, each bacterial culture measurement is a random sampling of cells within a culture with no selection bias.

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
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used	Mouse anti-V5 tag primary antibody [E10] (AB53418, Abcam) at a 1:5,000 dilution, Goat anti-Mouse IRDye 800CW secondary antibody (925-32210, Li-Cor) was used at a 1:10,000 dilution.
Validation	Mouse anti-V5 tag primary antibody [E10] (AB53418, Abcam) was validated by the manufacturer for species-independent use for Western blots (https://www.abcam.com/v5-tag-antibody-e10-ab53418.html). Primary antibody specificity was also verified by the absence of signal in the Western blot of a V5-null E. coli lysate (See Extended Data Figure 5c, neg. ctrl.).

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	Fluorescence measurements: Cells grown in minimal media were sampled and immediately read undiluted on the flow cytometer. Absolute cell count measurements: 75 µl samples of cells grown in methionine-dropout media were mixed with 79 µl of 500 µg/ml rifampicin in phosphate-buffered saline and 21 µl of 2 µm AccuCount Blank Particles (Spherotech) before being measured on a flow cytometer.
Instrument	The samples from the high throughput titrations in 96-well plates were analyzed on a LSRII Fortessa flow cytometer (BD Biosciences) equipped with a high throughput sampler. sfGFP was measured with a 488 nm laser and 530/30 and 505 LP emission filters; the voltage gains of the instrument were set as follows: forward scatter 500 V, side scatter 300 V, sfGFP 900 V. A minimum of 5,000 events were collected for each well using thresholds of 500 FSC-H and 500 SSC-H. Fluorescence measurements for the immunoblot lysate preparation cultures, step responses, steady-state negative perturbation, and absolute cell count measurements for the growth rate control experiments were performed using a CytoFlex S flow cytometer (Beckman Coulter) with a 488 nm laser and 525/40 BP filter; the gain settings of the instrument were as follows: forward scatter 100, side scatter 100, sfGFP 500. Thresholds of 2,500 FSC-H and 1,000 SSC-H were used for all samples. 50,000 events were collected for the lysate preparation, step response experiments, and steady-state disturbance rejection experiments and 1,000 AccuCount Blank Particles were collected for the growth rate control experiments..
Software	LSRII Fortessa data was collected with FACSDiva 8.0.1 software. CytoFlex S data was collected with CytExpert 2.1.0.92 software. FlowJo 10 (Treestar) was used for data analysis.
Cell population abundance	No cell sorting was performed.
Gating strategy	For fluorescence measurements, cells were initially separated from particles that were too small to be living bacterial cells using forward and side scatter thresholds (LSRII Fortessa: 500 FSC-H/500 SSC-H; CytoFlex S: 2,500 FSC-H/1,000 SSC-H). This population was further gated to exclude particles too small and too large to be single cells based on side-scatter (SSC-H) vs forward scatter (FSC-H) and side scatter (SSC-H) vs width (SSC-width). For absolute cell count measurements, cells and beads were initially separated from particles that were too small to be living bacterial cells or beads using forward and side scatter thresholds (CytoFlex S: 2,500 FSC-H/1,000 SSC-H). This population was further gated for cells based on side-scatter (SSC-H) vs forward scatter (FSC-H) and beads based on side-scatter (SSC-H) vs forward scatter (FSC-H).

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