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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.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Flow cytometry data was collected using BD FACSDIVA v8.0.1.
Data analysis	sgRNA design code is available from Github (https://github.com/traeki/sgrna_design). Data analysis was performed in Galaxy v18.01 (pooled sequencing data), FlowJo v10.4.2 and FCS Express 6 Plus (flow cytometry data), GraphPad Prism 7.0e (graphing and statistical analysis) and Excel v16.12. Plasmid sequence maps were created using SnapGene v3.1.4. Iris was used to measure colony sizes from plate images (https://github.com/critichu/Iris).

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 upon request. 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 data from this study are available upon request.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size	Sample sizes of three to four biological replicates are standard for the types of experiments shown here. Experiments with two biological replicates showed strong agreement. The use of three to four replicates is standard in the field of molecular microbiology.
Data exclusions	No data were excluded.
Replication	In all cases except the library construction experiments, replicate data were from independently constructed strains and showed excellent agreement.
Randomization	Randomization is not typically used in small-scale studies in the field of molecular microbiology.
Blinding	Blinding is not typically used in small-scale studies in the field of molecular microbiology.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

Methods

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

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	All unique materials are available upon request.

Antibodies

Antibodies used	Anti-CRISPR-Cas9 (AbCam 191468), c-Myc (Santa Cruz Biotechnology 9E10), IRDye 680RD Goat anti-Mouse IgG
Validation	Each primary antibody (Anti-CRISPR-Cas9 (AbCam 191468) and c-Myc (Santa Cruz Biotechnology 9E10)) were used at 1:1000 in PBS + 0.05% Tween80 + 3% BSA at room temperature for 2hr. Secondary antibody (IRDye 680RD Goat anti-Mouse IgG) was used

at 1:10000 in Odyssey Blocking Buffer (Licor) at room temperature for 1hr. Antibodies were validated by comparing western blot results to strains with no epitope.

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	Bacterial cells from a mid-log culture were treated with 1% formaldehyde, diluted 1:10 in PBS, and run on the flow cytometer.
Instrument	BD LSRII
Software	Flow cytometry analysis was performed using FlowJo v10.4.2, FCS Express 6 Plus, and Excel v16.12.
Cell population abundance	RFP levels were measured for at least 10,000 cells and 100% of the data collected was used in the analysis.
Gating strategy	No gating strategy was used. Flow cytometry was only used to measure RFP levels in bacterial cells, not to separate out subpopulations for further analysis. Supplemental Figure 6 shows histograms of RFP levels (Texas-red or B-A) for all cells collected.

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