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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.

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	Agilent OpenLab CDS (C.01.07), BD FACSDiva (8.0.2), Bio-Rad Image Lab (6.0.1)
Data analysis	Microsoft Excel (2019), ImageJ (Version 1.52q)

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

We have the following statement in the manuscript: "The authors declare that all data supporting the findings of this study are available within the paper, supplementary information files, and source data. Statistical source data for Figures 1-5 and Extended Data Figures 1-5, 7-8, 9d, and 10 are provided with the paper. Uncropped gels and blots for Figure 5 and Extended Data Figures 2, 9 are provided as source data in a Source Data file."

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	Sample sizes were n >= 3; this is common practice in the fields of microbial optogenetics (e.g. Ohlendorf et al. J Mol Biol 2012, Jayaraman et al. Nuc Acids Res 2016) and metabolic engineering (e.g. Santos et al. Proc Nat Acad Sci 2012, Liu et al. Nat Comms 2019)
Data exclusions	We did not exclude any data from the analyses.
Replication	All attempts at replication were successful.
Randomization	We did not randomize allocations as statistics were compiled using genetically identical E. coli samples; no human or animal participants were used.
Blinding	Investigators were blinded to group allocation for all experiments except for the bioreactor experiments in Figure 4d, for which data was taken using defined strains/process conditions/illumination scheduling and thus blinding was not possible.

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	-Anti-His tag antibody (HRP), mAb, Mouse (GenScript A00612), Lot: 19K001984 -Anti-LacI antibody [9A5], Mouse (abcam, ab33832), Lot: GR3279941-1 + Goat Anti-Mouse IgG H&L antibody (HRP) (abcam ab205719), Lot: GR3279214-2
Validation	Anti-His tag antibody has been validated by peer-reviewed literature (e.g. Isik et al. PLoS One 2014 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4172553/). Anti-LacI antibody has been validated by the manufacturer (https://www.abcam.com/laci-antibody-9a5-ab33832.html) as well as peer-reviewed literature (e.g. Purcell et al. J Biol Eng 2012 https://pubmed.ncbi.nlm.nih.gov/22824000/). Anti-Mouse antibody has been validated by the manufacturer (https://www.abcam.com/Goat-Mouse-IgG-HL-HRP-ab205719.html) as well as peer-reviewed literature (e.g. Cai et al. J Cell Biochem 2020 https://pubmed.ncbi.nlm.nih.gov/31190436/).

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	E. coli samples were diluted 200-fold into 199 uL of ice-cold PBS solution for GFP measurement.
Instrument	LSRII flow cytometer (BD Biosciences, San Jose, CA)
Software	BD FACSDiva 8.0.2
Cell population abundance	10,000 single-cell E. coli events were collected for analysis. Single-cell events comprised 95% of total events for all samples.
Gating strategy	Total E. coli events were visualized and gated by plotting forward vs. side scatter. Single-cell E. coli events were further gated using side scatter area vs. height. GFP gating was set using E. coli strains that either didn't express GFP (negative control) or constitutively expressed GFP (positive control).

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