

| Corresponding author(s):   | Joseph Bondy-Denomy |
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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 <u>Authors & Referees</u> and the <u>Editorial Policy Checklist</u>.

| 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. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted Give <i>P</i> 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 <u>statistics for biologists</u> contains articles on many of the points above.   |  |  |
| Software and code   |  |  |
| Policy information about <u>availability of computer code</u>   |  |  |
| Data collection Images were taken using Azure Biosystems 2015 cSeries Version 1.6.15.1030 and Image Lab Version 6.0.1 build 34 Standard Edition, bacteria growth curves were collected using Gen5 3.05.11, next-generation sequencing runs to assess genome editing efficiencies were performed using a MiSeq sequencer (Illumina)                                    |  |  |
| Data analysis  Data were analyzed using Microsoft Excel Version 16.30, CRISPResso2 (https://github.com/lucapinello/CRISPResso) and plotted using GraphPad Prism 6.0 or 8.2.1. Phylogenetic tree analyses were performed using FigTree v1.4.4  |  |  |
| 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/reviewer 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  |  |  |

## Data

Policy information about availability of data

All manuscripts must include a <u>data availability statement</u>. 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

This study is not associated with the generation of new datasets

| Field-specific reporting        |  |  |
|---------------------------------|--|--|
| Please select the o             | ne below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.  |  |
| \(\sum_{\text{life sciences}}\) | Behavioural & social sciences Ecological, evolutionary & environmental sciences  |  |
| For a reference copy of t       | he document with all sections, see <a href="mailto:nature.com/documents/nr-reporting-summary-flat.pdf">nature.com/documents/nr-reporting-summary-flat.pdf</a>  |  |
|                                 |  |  |
| Life scier                      | nces study design  |  |
| All studies must dis            | close on these points even when the disclosure is negative.  |  |
| Sample size                     | No sample size calculation was performed to determine sample size. Sample sizes of two or three biological replicates are standard for the types of experiment conducted in this study, as in the field of molecular microbiology  |  |
| Data exclusions                 | For Fig 1c and Extended Data 1b, only two and three induction levels respectively were shown for simplicity. For gels in Fig 3b, Fig 4a-c and Extended Data 4a-d, gels were cropped to show only lanes that are relevant to this study.  |  |
| Replication                     | In all cases, experiments were conducted in biological duplicate or triplicate as is standard in the field. All attempts displayed similar agreements.   |  |
| Randomization                   | This is not relevant to our study, as randomization is not used in small-scale studies in the field of molecular microbiology  |  |
| Blinding                        | This is not relevant to our study, as blinding is not used in small-scale studies in the field of molecular microbiology   |  |
|                                 |  |  |
| We require informati            | g for specific materials, systems and methods on from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, led 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.  |  |
| ,                               |  |  |
|                                 | e study  Methods  n/a Involved in the study  |  |
| n/a   Involved in th            |  |  |
| Eukaryotic                      |  |  |
| Palaeontol                      |  |  |
|                                 | d other organisms  |  |
|                                 | earch participants   |  |
| Clinical dat                    | a  |  |
| Antibodies                      |  |  |
| Antibodies used                 | Mouse anti-Myc (Cell Signaling Technology #2276), Rabbit anti-GST (Cell Signaling Technology #2625), Mouse anti-E.coli RNA Polymerase Beta (BioLegend #663903), HRP-conjugated Goat anti-Mouse IgG (Santa Cruz Biotechnology #sc-2005), HRP-conjugated Goat anti-Rabbit IgG (Bio-Rad #170-6515)  |  |
| Validation                      | Primary antibodies (Mouse anti-Myc, Rabbit anti-GST and Mouse anti-E.coli RNA Polymerase Beta) were used at 1:5000 in TBS with 0.1% Tween20 and 5% nonfat dry milk for 1hr at room temperature or 16hrs at 4 degrees Celsius. Secondary antibodies (Goat anti-Mouse IgG and Goat anti-Rabbit IgG) were used similarly for 1hr at room temperature. Antibodies were validated by comparing western blot results to strains with no epitope. |  |
| Eukaryotic c                    | ell lines  |  |
| Policy information              | about <u>cell lines</u>  |  |
| Cell line source(s              | HEK 293T (ATCC)  |  |

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|---|---|
| olicy information about cell lines                |   |
| Cell line source(s)                               | HEK 293T (ATCC)   |
| Authentication                                    | HEK 293T cells (ATCC) were authenticated by STR profiling   |
| Mycoplasma contamination                          | All cell cultures tested negative for contamination; media supernatant from cell cultures were analyzed monthly for the mycoplasma using MycoAlert Plus (Lonza) |
| Commonly misidentified lines (See ICLAC register) | No commonly misidentified cell lines were used  |
|   |   |