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 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
  - 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 possible.
- 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**

No software was used in data collection

**Data analysis**

cutadapt (v1.18), DESeq2 (v1.26.0), vegan (v.2.5.7), Trimmomatic (v0.32), BWA-MEM (v0.7.7), featureCounts (v1.6.0), GAGE package (v2.36.0), Pathview (v1.26.0)

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 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

**Data Availability**

All raw count data for and log2FC with fdr-adjusted p-values for every guide by CRISPRi screening is available in the Supplemental Data. Condition-specific hits and putative essential genes identified by CRISPRi screening are available as Supplemental Tables. Raw count data and log2FC with fdr-adjusted p-values for RNA-sequencing is available in the Supplemental Data and Tables respectively. pidCas9 vector design, Tu3298 annotation, PCR and sequencing primers are available in the Supplemental Data. Custom R scripts for guide design is available in the Supplemental Data and custom scripts for analysis can be made available upon request.

All raw and processed sequencing data generated in this study will be available in the Short Read Archive (currently in submission SRR9563942).
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/or-reporting-summary-flat.pdf

Life sciences study design

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

Sample size | Based our pilot data indicating high correlation between CRISPRi biological replicates and between RNA-seq biological replicates, we believe performing these experiments in at least triplicate is appropriate.

Data exclusions | Second time points for the RNA-seq data were excluded based on the fact that one of the baseline media conditions (TSB) was sampled outside of the range that we selected for the remaining samples (i.e. TSB time point #2 was sampled at late exponential phase rather than mid exponential phase). To avoid comparing mid-exponential samples to this late-exponential sample, we excluded this late phase sample from our study, by extension, excluded comparing any condition to this time point. Additionally, we excluded 3 samples from our CRISPRi screening experiments (Defined media alkaline, Defined media 42C and Human serum), based on the data characteristics (either no growth during the incubation or stochastic outgrowth of a handful of strains)

Replication | All CRISPRi experiments were performed at least in biological triplicate and often in quadruplicate (separate bacterial cultures grown up independently). As described in the manuscript, correlation between replicates is high. RNA seq experiments were performed in biological triplicate (separate bacterial cultures grown up independently)

Randomization | Randomization is not relevant to our study. Our results are based on bacterial cultures grown in different conditions.

Blinding | Blinding was not relevant to this study. Researchers were aware which samples were grown in which media conditions, but the outcome data (either CRISPRi count data or RNA-seq count data) is not dependent on any subjective researcher analysis

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

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Methods

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