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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	Data from plate reader experiments was collected with Gen5 v. 3.04. Microscopy images were collected with MicroManager v. 1.4. Sequencing reads were collected with an Illumina HiSeq 4000 (BioSample SAMN12807646) or an Illumina MiSeq (SAMN12809978).
Data analysis	Data from plate reader experiments were analyzed using custom code in MATLAB 2018b. Microscopy images were processed with FIJI v. 2.0.0-rc-69/1.52i. Data from barcode sequencing experiments were analyzed with custom perl scripts (https://bitbucket.org/berkeleylab/feba/src) and by custom code in Python3 and MATLAB 2018b (https://bitbucket.org/kchuanglab/resolve_barcode_position/src).

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

The sequencing data used in this study are publicly available on NCBI as part of the BioProject PRJNA573294. The sequence reads from the Bar-seq experiment on pools of the ordered library are available as part of BioSample SAMN12807646. The sequence reads from the RB-TnSeq experiment on the ordered library are available as part of BioSample SAMN12809978. Plate reader output files, microscopy images, and extracted growth parameters that support the findings of this study (Fig. 2, 3, 6) are available from the corresponding author (K.C.H.) upon 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

Life sciences study design

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

Sample size	Sample sizes for plate reader experiments were chosen based on the total number of samples, along with relevant controls, that could be fit within a 96-well plate format.
Data exclusions	The differential plot of individual growth curves was fit with a model function to estimate the maximum growth rate. The fit was examined for every sample, and if the fit was clearly poor the sample was excluded from analysis. Because the lag time calculation depends on the fit of maximum growth rate, if the maximum growth rate measurement for a sample was excluded, so was the lag time measurement. 3 samples were excluded from the max growth rate and lag time parameters plotted in Figure 6D.
Replication	Independent measurements were taken for every measurement with sample size dictated by the parameters of the experiment.
Randomization	Where relevant, data collection was conducted on the same day to prevent batch effects. No further randomization was conducted.
Blinding	Investigators were not blinded during data collection or 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

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