

Reporting Summary

Nature Portfolio 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 Portfolio 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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☐	☒ 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 <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	Microarray image analysis: Feature Extraction version 10.7.3.1 (Agilent Technologies) Transcriptional regulatory network in E. coli: RegulonDB 11.0, Ecocyc RNA-seq data in the Env dataset: iModulonDB
Data analysis	Bow-tie decomposition: Python 3.6 Other statistical analysis and visualization: R 4.3.2 All custom scripts are available on GitHub (https://github.com/tsuruubi/Variability_E_coli)[https://doi.org/10.5281/zenodo.14241622].

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 Portfolio [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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

All microarray data generated by the current study are available in the NCBI GEO repository under the accession number GSE260863.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender [Reporting on sex and gender is not relevant to the current study.](#)

Reporting on race, ethnicity, or other socially relevant groupings [Reporting on race, ethnicity, or other socially relevant groupings are not relevant to the current study.](#)

Population characteristics [Population characteristics is not relevant to the current study.](#)

Recruitment [Recruitment is not relevant to the current study.](#)

Ethics oversight [Ethics oversight is not relevant to the current study.](#)

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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](https://www.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 [No statistical methods were used to predetermine sample sizes in this study. The sample size for the MA experiments \(n = 6 lineages\) was identical to that of the Mut dataset \(6 transcriptome profiles in the DNA microarray experiments\) and was chosen to match or exceed the sample sizes used in comparable studies \(n = 4 lineages in Landry et al., Science, 2007, <https://doi.org/10.1126/science.1140247>; n = 3 lineages in Tsuru et al., Genes to Cells, 2015, <https://doi.org/10.1111/gtc.12300>\).](#)

Data exclusions [All MA lineages and microarray data were used without exclusion.](#)

Replication [Reproducibility of the DNA microarray analysis in this study was checked by biological replicates \(Supplementary Fig. 1\).](#)

Randomization [Randomization is not relevant to the current study as the dataset was analyzed as a whole, rather than in subgroups.](#)

Blinding [Investigators were blinded to group allocation during all experiments.](#)

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	Involvement in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals

This study did not involve the use of laboratory animals. Escherichia coli K12 MG1655, a laboratory strain, lacking the dnaQ gene was used for the MA experiments and DNA microarray experiments in this study.

Wild animals

This study did not involve the use of wild animals.

Reporting on sex

n/a

Field-collected samples

The E. coli strains used or analyzed in this study originated from the habitats detailed in Rousset et al. (Nature Microbiology, 2021, <https://doi.org/10.1038/s41564-020-00839-y>). All samples were stored at -80°C.

Strain name of E. coli, Host, Sample origin

K12-MG1655, Homo sapiens, Faeces
 HS, Homo sapiens, Faeces
 E1114, /, Water
 E1167, /, Soil
 H120, Homo sapiens, Urine
 TA054, Isodon obesulus, Faeces
 TA447, Bettongia lesueur, Faeces
 E101, /, Water
 41-1Ti9, Homo sapiens, Crohn Disease
 TA280, Dasyurus viverrinus, Faeces
 M114, Burramys parvus, Faeces
 TA249, Dasyurus hallucatus, Faeces
 ROAR 8, Cephalophus sylvicultor, Faeces
 JJ1886, Homo sapiens, Blood
 UT189, Homo sapiens, Urine
 S88, Homo sapiens, Cerebrospinal fluid

/: not applicable

Ethics oversight

Ethical approval was not required for this study as it did not involve the use of animals.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks

n/a

Novel plant genotypes

n/a

Authentication

n/a