nature portfolio

Corresponding author(s): Jeremy M. Rock, Elizabeth A. Campbell

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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 (*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 collection was done with custom scripts dependent on third-party tools. All source code will be made publicly available online(GitHub: https://github.com/rock-lab/nusg_paper_2023).
Data analysis	GraphPad Prism (version 10.0.2) Microsoft Excel(365) bwa (version 0.7.17-r1188 or v1.3.1, depending on operating system requirements) phyOverlap algorithm (https://github.com/Nathan-d-hicks/phyOverlap, Commit 20ae57d) HaplotypeCaller tool Genome Analysis Toolkit (version 3.5) samtools (version 1.7) Mykrobe (version 0.9.012) FastTree (version 2.1.11) figTree (version 1.4.4) Snippy9 (version 3.2-dev or v4.6.0, depending on operating system requirements) QualiMap (version 2.2.2-dev) FastTree (version 2.1.11 SSE3) iTol (https://itol.embl.de/) freebayes (version 1.3.1) Image J software (NIH) viridis (version 0.6.3) reshape (version 0.8.9) Epi (version 2.47.1)

readxl (version 1.4.2)
RColorBrewer (version 1.1-3)
tidyverse (version 2.0.0)
ggplot2 (version 3.4.2)
subread-align (version 1.6.0)
Python (version 3.11.2)
SciPy (version 1.10.1)
numpy (version 1.23.5)
R (version 4.2.1)
Stan (version 2.21.8)
Rstan (version 2.21.8)

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

Raw sequencing data are deposited to the NCBI Short Read Archive under project number PRJNA1021243. The H37Rv reference genome (CP003248.2) was applied for alignments and SNP calling. Manually curated pathway calls were derived from KEGG (https://www.genome.jp/kegg-bin/show_organism?org=mtu) and PATRIC databases (https://www.bv-brc.org/search/?keyword(tuberculosis)) databases.

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	NA
Reporting on race, ethnicity, or other socially relevant groupings	NA
Population characteristics	NA
Recruitment	NA
Ethics oversight	NA

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

Life sciences study design

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

Sample size	The passaging and competitive growth data presented in the manuscript were conducted in biological triplicate. The termination efficiency and termination rates presented in Fig 4D,E,F consist of three experimental replicates. Previous work on libraries these size (Bosch et al., 2021 and Li et al., 2022) showed that 3 biological replicates are enough to detect statistically significant differences between conditions.
Data exclusions	None
Replication	We have indicated the number of times experiments were independently preformed in the figure legends and their corresponding methods.
Randomization	Strains were sequenced to determine that no other SNPs were different between the WT and S450L strains. As this was the only relevant variable, one was assigned as control (WT) and one as experimental (S450L) and no randomization was needed.

Blinding	Blinding was not performed for any experiments as measurements of optical density, spot assays, and in vitro termination quantification do not require researcher-based judgments and therefore blinding was not deemed necessary.

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 and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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