nature portfolio

Corresponding author(s): Alexandre Persat

Last updated by author(s): September 4th, 2024

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	Software used: Microsoft Excel (version 16.39); Python (version 3.9); MATLAB (2017a), IBM ILOG Cplex (12.7.1)., TPP and TRANSIT (version 3.2.7); NIS Elements (v.5.21.02); Fiji (v.2.0).
Data analysis	Metabolic modeling: All simulations for this study were performed on Mac Pro 32 GB in MATLAB 2017a and IBM ILOG Cplex 12.7.1 as a solver. Tn-seq: Tn-seq data was analyzed using TPP and TRANSIT (version 3.2.7) as described in the Methods. General data processing and plotting: Data processing was performed in Python (version 3.9), using a combination of the Pandas (version 1.4.4), NumPy (version 1.21.5), SciPy (version 1.7.3), and scikit-learn (version 1.3.2) libraries. Microsoft Excel (version 16.39) was also used. Plots displayed were prepared using Matplotlib (version 3.5.2) and Seaborn (version 0.12.0). Statistical analysis: all performed in Python. 1-way ANOVA with Tukey HSD multiple comparison test, we used f_oneway() from scipy.stats and pairwise_tukeyhsd() from statsmodels.stats.multicomp in Python. For Welch's unpaired t-test (two-sided), we used ttest_ind() from scipy.stats in Python (parameters: alternative='two-sided', equal_var=False). Imaging data: imaging data was processed in NIS Elements (v.5.21.02) or Fiji (v.2.0), as described in the Methods. We also generated a custom image analysis software for the competition assays imaged in Transwells. This software is available on GitHub as indicated in the Data Availability session in the manuscript.

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

Spreadsheets containing the source data used to generate each plot (main and extended data figures), the code used during metabolic modeling, the microscopy data displayed in all the figures, and all files related to Tn-seq (i.e., WIG and annotation files used during analyses with TRANSIT) are openly available in Zenodo (10.5281/zenodo.13629466). The Tn-seq sequencing data has been submitted to the NCBI Sequence Read Archive under accession number PRJNA1156351. The custom image analysis software used for quantification of fitness ratios and cluster sizes distribution is available on GitHub under the following repository: https://github.com/PersatLab/CompetitionAssay. Additional files, such as the raw files of the dozens of movies recorded during AirGel experiments as well as all strains and plasmids used in this study, are available from the corresponding author upon request.

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	N/A.
Reporting on race, ethnicity, or other socially relevant groupings	N/A.
Population characteristics	N/A.
Recruitment	N/A.
Ethics oversight	N/A.

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	All sample sizes are described in the Methods section of the paper. We used a number of replicates that we believe were sufficient to capture the variability and heterogeneity of the studied phenomenon. This was not possible to know before running preliminary experiments, which we used as a base for estimation of data variability (e.g., data typically showed less variability for CFUs compared to microscopy assays). This depended on the specific experiment performed, and the details for each are extensively described in the Methods section.
Data exclusions	Data exclusion involved data that did not pass the quality control in HBE cultures and AirGels. We describe the details in the Methods section of the paper. Overall, few HBE cultures that showed too much background on the far red channel (due to cell death) and overlapped with the Cy3 channel had to be excluded from competition experiments dataset ("Competition assays in Transwells" session in the Methods). In addition, for tolerance experiments, only AirGels that did not showed tissue damage by the time we started the imaging were used . This is important since severe damage leads to leakage inside the lumen and disruption of the ALI.
Replication	All the experiments presented contained multiple biological replicates (at least two, but often more, depending on the experiment). The exact sample size can be found in the legends of each figure. We always report the data point for each replicate and explain in the legend and Methods how many times the experiment was performed. As described in the Methods, each Tn-seq experiment was ran only once, but with data pooled from multiple replicates and validated with individual follow-up experiments for the genes studied in the paper.
Randomization	N/A for this study. Experimental design does not involved use of animals or participants.
Blinding	Blinding was not possible since the investigator collecting the data performed the analyses.

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

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	We used primary cells (human bronchial epithelial cells) that are commercially available from Lonza (21TL200815 for healthy donor; 00196979 for the CF donor).
Authentication	*Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.*
Mycoplasma contamination	*Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.*
Commonly misidentified lines (See ICLAC register)	*Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*