nature portfolio

Corresponding author(s): Liselot Dewachter

Last updated by author(s): Oct 8, 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	The following software was used for data collection: CytExpert 2.4, VisionCapt v16.08g, Tecan i-control 2.0.10.0, FlexControl 3.4
Data analysis	The following software/code was used for data analysis: Galaxy suite (Trimmomatic Galaxy Version 0.36.6, FastQC Galaxy Version 0.72 +galaxy1, Bowtie2 Galaxy Version 2.4.2+galaxy0, Qualimap BamQC Galaxy Version 2.2.2c+galaxy1, htseq-count Galaxy Version 0.9.1+galaxy1), R 4.2.0, DESeq2 1.38.3, CytExpert 2.4, PyMol 2.5.2 and 2.5.7, STRINGdbR 2.10.1, MicrobeJ 5.13o, GraphPad Prism 8-10, FlexAnalysis 3.4, BioPharma Compass 4.0.1, MASCOT server 2.8.2. Code for analysis of our CRISPRi screening data was custom made and has been deposited on Zenodo as record 13902656 with DOI https://doi.org/10.5281/zenodo.13902656.

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 raw data included in this manuscript are available. The sequencing data generated in this study have been deposited in the Sequence Read Archive (SRA) with

BioProject accession number PRJNA1061367 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1061367), microscopy images and videos have been deposited on the EMBL-EBI BioImages Archive with accession number S-BIAD1002 (https://www.ebi.ac.uk/biostudies/BioImages/studies/S-BIAD1002). The output of AlphaFold-Multimer and all other data presented here (including raw gel images and MS data) are deposited on Zenodo as record 13902471 with DOI https://doi.org/10.5281/zenodo.13902471.

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	For all assays, we collected at least three completely independent biological repeats. One exception are the LpxA activity assays were limiting amounts of substrates prevented us from performing three repeats for all conditions.
Data exclusions	No data were excluded from the analyses.
Replication	All experiments were repeated and all repeats are included in our manuscript, highlighting the reproducibility of our findings.
Randomization	NA, no experimental groups were constructed for this study.
Blinding	Investigators were not blinded for this study due to practicality issues.

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

Plants

Seed stocks	NA
Novel plant genotypes	NA
Authentication	NA

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ All plots are contour plots with outliers or pseudocolor plots.

☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Cultures were grown overnight, diluted 100x in selective LB medium, grown for 2h, induced with 0.2% w/v arabinose and incubated for 2h. Cultures were then diluted 1000x in PBS and subjected to flow cytometry.
Instrument	CytoFLEX S (Beckman Coulter Life Sciences) equipped with 405 nm, 488 nm and 561 nm lasers.
Software	CytExpert 2.4
Cell population abundance	NA, no populations were recorded.
Gating strategy	NA, no gating was performed.

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.