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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	No non-standard software was used.
Data analysis	The following software was used: Fiji (v 1.54p), basicdrm (v 0.3.0), AlphaFold3 (v 3.0.1), ChimeraX (v 1.10.1), GROMACS (v 2025.1), ACPYPE (v 20250519.1), STAR (v 2.7.11b), RSEM (v1.3.3), maSigPro (v 1.84.0), clusterProfiler (v 4.10.1), MaxQuant (v 2.4.2.0), TBLASTN (v 2.17.0), ape (v 5.8-1), R (v 4.5.1), R Studio (v 2025.05.1) and Inkscape (v 1.4.2).

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

Source Data are provided with this paper. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD068274 [https://www.ebi.ac.uk/pride/archive/projects/PXD068274]. The RNA sequencing data after rifampicin treatment have been deposited to the GEO database with accession number GSE315070 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE315070]. The data for

high-throughput sequencing of RNA isolated by crosslinking immunoprecipitation with PNPase were obtained through the GEO database entry with accession number GSE315071 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE315071]. The data for E. coli reference genome, transcriptome and proteome were obtained through the NCBI database entry with accession number GCF_000005845.2 [https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_000005845.2].

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	This study did not involve human participants
Reporting on race, ethnicity, or other socially relevant groupings	See above
Population characteristics	See above
Recruitment	See above
Ethics oversight	See above

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 experiments were biologically replicated at least three times (n=3), where indicated, experiments were replicated four (n=4) or five times (n=5), these sample sizes were chosen based on common practices in molecular biology. We adopted standard practices for biological replication and reproducibility.
Data exclusions	No data were excluded.
Replication	Experiments were typically conducted in triplicate and all attempts at replication were successful.
Randomization	To minimize technical bias during experimentation, the order of sample preparation was varied between biological replicates.
Blinding	Data acquisition and analysis was based on numerical values generated by the equipment used during exeprimentation. Therefore, given that blinding would not impact data acquisition or analysis, blinding was not performed in this study.

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

Antibodies

Antibodies used	Membranes were individually hybridized with primary polyclonal antibodies specific for the FLAG epitope (dilution 1:5000, produced

in mouse, Sigma, Cat. No. F1804), E. coli PNPase (dilution 1:2000, produced in rabbit, in-house), and the T25 and T18 fragments of Bordetella pertusis adenynate cyclase (dilution 1:1000, produced in rabbit, in-house). Then, membranes were hybridized with HRP-conjugated anti-IgG secondary antibodies (dilution 1:10000, anti-rabbit produced in donkey, Amersham, Cat. No. NA934V) (dilution 1:10000, anti-mouse produced in sheep, Amersham, Cat. No. NA931V).

Validation

Specificity of all primary antibodies was determined by western blot analysis of protein samples in which the target protein was absent. For primary polyclonal antibodies specific for the FLAG epitope see Fig. 6, for E. coli PNPase see Fig. 6, for the T25 and T18 fragments of Bordetella pertusis adenynate cyclase see Supplementary Fig. 6.

Plants

Seed stocks

This study did not involve the use of plants, this field cannot be disabled

Novel plant genotypes

See above

Authentication

See above