nature portfolio

Corresponding author(s): Nicholas M.I. Taylor

Last updated by author(s): Nov 19, 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	For cryo-EM: EPU; for TIRF microscopy: NIS-Elements 5.42.04
Data analysis	For cryo-EM: CryoSPARC 4.x, Other: Coot 0.9-pre, Phenix 1.13, Chimera X 1.8, Pymol 2.5.4 and 3.0.2, Bioinformatics Toolkit server (MUSCLE alignment), , MMseqs2, Python 3, ImageJ 1.53t, MicrobeJ 5.13j, GraphPad Prism 9.4.1 and GraphPad Prisim 10. Python code used to generate Extended Data Videos 1 - 6 and plot graphs is available at: https://github.com/SalmoLab/Zorya_Nature2024

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

Atomic coordinates for ZorAB WT, ZorA_E86A/E89A_ZorB, ZorA_delta_359-592_ZorB and ZorA_delta_435-729_ZorB were deposited in the Protein Data Bank (PDB) under accession codes 8QYD, 8QYH, 8QYK, 8QYY, respectively. The corresponding electrostatic potential maps were deposited in the Electron Microscopy Data Bank (EMDB) under accession codes EMD-18751, EMD-18754, EMD-18756, EMD-18766, respectively. The local refinement map of ZorB PGBD in ZorAB WT were deposited in the EMDB under accession codes EMD-18752. Atomic coordinates for ZorC were deposited in the PDB under accession codes PDB: 8R68. The corresponding electrostatic potential maps was deposited in the EMDB under accession codes EMDB: EMD-18848. Atomic coordinates for ZorD apo form and its complex with ATP-γ-S were deposited in the PDB under accession codes PDB: 8QY7 and 8QYC, respectively. The corresponding electrostatic potential maps were deposited in the EMDB under accession codes EMD-18747 and EMD-18750. Official validation reports from wwPDB for all macromolecular structures studied in the paper have been provided. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE67 partner repository with the dataset identifier PXD047450.

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	For cryo-EM: No sample size was calculated. Sample size was based on experience to have a number of micrographs/particles that, if possible, would result in a cryo-EM reconstruction that would allow atomic model construction. For microscopy experiments, no a priori sample sizes were calculated. Sample size were chosen according to our experience concerning quantitative single cell microscopy (e.g. 10.1038/s41467-024-50278-0;10.1073/pnas.2310842120). Exact sample size are provided in the figure legends.
Data exclusions	Junk particles were removed during cryo-EM data processing in CryoSPARC. No microscopy data was excluded from the analysis.
Replication	TIRF: Data derived from at least three independent clones of bacteria and two sets of phage isolations. All attempts at replication were successful. Allocating experimental groups was not relevant for this study, as all bacterial cells of a particular strain are genetic clones. We performed 3 replicates under similar conditions for both the ZorAB full length and ZorB PGBD fragment PG pull-down experiments. The experimental results were consistent. For ZorB PGBD fragment experiments, we selected the experiment with the lowest non-specific binding (as indicated by amount of ZorE pulled down
Randomization	No experimental groups were formed/compared. Data was collected randomly in each set of experiments.
Blinding	Image and data analysis was automated whenever possible. Blinding was neither possible nor necessary for this study, as all bacterial cells of a particular strain are genetic clones and analyses were not sufficiently subjective to require researcher blinding.

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: Promega Anti-Halo Tag Monoclonal Antibody #G921A 1:1,000

Validation: Mouse monoclonal antibody was raised against the Halo Tag protein and validated using E.coli cell lysates expressing Halo Tag as positive control and E.coli lysate not expressing any HaloTag protein as negative control.

Plants

Seed stocks: N/A

Novel plant genotypes: N/A

Authentication: N/A