

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 analysis

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](#)

Models and cryo-EM reconstructions for BlaR1WT, BlaR1F284A ampicillin-free and BlaR1F284A ampicillin bound samples have been deposited to the PDB and the EMDB with the accession codes 8EXP [10.2210/pdb8exp/pdb], 8EXQ [10.2210/pdb8exq/pdb], 8EXR [10.2210/pdb8exr/pdb], 8EXS [10.2210/pdb8exs/pdb], 8EXT [10.2210/pdb8ext/pdb] and EMD-28658, EMD-28659, EMD-28660, EMD-28660, EMD-28660.

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	16,599 movies were collected for the BlaR1WT dataset, 11,844 movies were collected for the BlaR1F28A dataset, 10,872 movies were collected for the BlaR1F284+ampicillin dataset. Number of movies was determined by data collection time and assessed to be sufficient based on final cryo-EM analyses.
Data exclusions	During cryo-EM data processing particles not contributed to classes of interest were not included in final reconstructions as is standard in the processing strategies.
Replication	For the MRSA growth assays, three technical replicates and three biological replicates were performed. For Blal and SpsB cleavage assays, three biological replicates were performed. All replicates were successful.
Randomization	Not applicable to this study. Samples of known composition were required and used.
Blinding	Not applicable to this study. Samples of known composition were required and used.

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

Methods

n/a	Involved in the study	n/a	Involved in the study
☐	☒ Antibodies	☒	☐ ChIP-seq
☒	☐ Eukaryotic cell lines	☒	☐ Flow cytometry
☒	☐ Palaeontology and archaeology	☒	☐ MRI-based neuroimaging
☒	☐ Animals and other organisms		
☒	☐ Human research participants		
☒	☐ Clinical data		
☒	☐ Dual use research of concern		

Antibodies

Antibodies used	Anti-T7-tag Antibody HRP Conjugate (Novagen Cat No. 69048-3 Lot D00132880)
Validation	Anti-T7-Tag Antibody HRP Conjugate was from Novagen. This is commercially available Western blot antibody against a common tag epitope and validated by the supplier (Lot D00132880).