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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<br>*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<br>*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 | All equipment specifications and experimental parameters have been detailed in the manuscript. SerialEM 3.7.11 was used for cryo-EM data collection. |
| Data analysis | All software and data analyses methods have been described and references appropriately cited in the manuscript. The following software (version numbers as appropriate) were used: AutoProc (1.1.7), MotionCor2, CTFFIND 4.1.13, Phenix (1.19.1), Coot (0.9), cisTEM (version 2), RELION ( 3.1-beta), cryoSPARC (3.1), ModelAngelo (1.0) UCSF Chimera (1.14), UCSF ChimeraX 1.2, PyMol (2.4.1), Prism9 (Graphpad version 9.3.1 for Mac), Biacore S200 Biaevaluation software (Cytiva 100 Results), and Protein Deconvolution (v4.0 ), Schrodinger Suite (2022-3), Python Seaborn (0.12.2) |

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

As noted in the Data Availability statement, all data included in the paper and the Supplementary Information files are available. The 3D cryo-EM map of PTB1-1-BAM, Apo BAM-DDM, PTB2-BAM-DDM, Apo BAM-SMA and PTB2-BAM-SMA have been deposited into the Electron Microscopy Data Bank [https://www.ebi.ac.uk/emdb/] under accession code EMD-45765 [https://www.ebi.ac.uk/emdb/EMD-45765], EMD-45764 [https://www.ebi.ac.uk/emdb/EMD-45764], EMD-45767 [https://www.ebi.ac.uk/emdb/EMD-45767], EMD-45766 [https://www.ebi.ac.uk/emdb/EMD-45766], and EMD-45768 [https://www.ebi.ac.uk/emdb/EMD-45768]. The coordinates of PTB1-1-BAM, Apo BAM-DDM, PTB2-BAM-DDM, Apo BAM-SMA and PTB2-BAM-SMA have been deposited in the Protein Data Bank [https://www.rcsb.org/] with accession codes 9CNX [https://www.rcsb.org/structure/unreleased/9CNX], 9CNW [https://www.rcsb.org/structure/unreleased/9CNW], 9CNZ [https://www.rcsb.org/structure/unreleased/9CNZ], 9CNY [https://www.rcsb.org/structure/unreleased/9CNY], and 9CO0 [https://www.rcsb.org/structure/unreleased/9CO0], respectively. The crystal structure of PTB2-BamA has been deposited in the Protein Data Bank (https://www.rcsb.org/) under accession code 9CO2 [https://www.rcsb.org/structure/unreleased/9CO2]. Mass spectrometry data have been deposited in MassIVE under accession code MSV000095321 [https://doi.org/10.25345/C5QV3CF81].

## 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 | Whole human blood samples were donated by volunteers as detailed in the Methods. No information about sex or gender was communicated. No personal or medical history was specified, provided, or collected. |
| Reporting on race, ethnicity, or other socially relevant groupings | Whole human blood samples were donated by volunteers as detailed in the Methods. No information about race, ethnicity, or other socially relevant grouping was communicated. No personal or medical history was specified, provided, or collected. |
| Population characteristics | No personal or medical history was specified, provided, or collected. |
| Recruitment | All participants self-volunteered. |
| Ethics oversight | Genentech Samples for Science program protocols approved by the Western Institutional Review Board. |

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 | Samples sizes were not statistically predetermined. For cryo-EM experiments, cryo-EM images were collected until a structure of sufficient quality was obtained. |
| Data exclusions | During cryo-EM processing, poor quality particles that did not yield a clear 2D class average and useful 3D reconstruction were discarded. |
| Replication | Experiments were replicated as described in the Figure legends and Methods. All gel images shown are representative of replicates. |
| Randomization | Randomization is not relevant to the growth, or biochemical experiments described in this work. For cryo-EM data processing, particles were randomly split into half-sets and processed independently to enable resolution estimation through Fourier Shell Correlation. |
| Blinding | Data were not blinded. Blinding is not relevant to the growth, structural, or biochemical experiments described in this work as subjective analyses were not 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

| 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: Rat anti-BamA MAB2 (Genentech), human anti-LptD 3D11 (Genentech), Rabbit anti-MsbA (Genentech), and rabbit anti-GroEL (Enzo), appropriate IRDye-linked secondary antibodies (Li-Cor), and anti-Flag-HRP antibody (Sigma) as indicated in the Methods.

Validation: All antibodies used are commercially available (as indicated) or generated at Genentech and validated in previous publications (Storek et al. PNAS (2018) 115:3692; Storek et al. eLife (201) 8:e46258; Alexander et al. Antimicro Agent Chemo (2018) 62:2561).

# Plants

Seed stocks: No plants or seeds were used in this study.

Novel plant genotypes: No plants or seeds were used in this study.

Authentication: No plants or seeds were used in this study.