nature portfolio

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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 | Leginon v. 3.5 and EPU v. 2.12.1.2782REL for cryo-EM data collection |
| Data analysis | Relion v. 4.0; cryoSPARC v. 3.3.2; GraphPad Prism v. 8.0, PHENIX v. 1.19.2, UCSF Chimera v. 1.14, UCSF ChimeraX v. 1.3 |

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 and associated electron microscopy maps for the six structures reported in this publication have been deposited in the Protein Data Bank (PDB) and Electron Microscopy Data Bank (EMDB) under the following accession numbers: Nucleotide-free BceAB-S TM State 1 (PDB 8G3A [https://www.rcsb.org/structure/8G3A], EMDB 29690 [https://www.ebi.ac.uk/emdb/EMD-29690]), Nucleotide-free BceAB-S TM State 2 (PDB 8G3B [https://www.rcsb.org/structure/8G3B], EMDB 29691 [https://www.ebi.ac.uk/emdb/EMD-29691]), Nucleotide-free BceAB-S BceS State 1 (PDB 8G3F [https://www.rcsb.org/structure/8G3F], EMDB 29694 [https://www.ebi.ac.uk/emdb/EMD-29694]), Nucleotide-free BceAB-S BceS State 2 (PDB 8G3L [https://www.rcsb.org/structure/8G3L], EMDB 29701 [https://

www.ebi.ac.uk/emdb/EMD-29701]), ATP S bound BceAB-S High-res TM (PDB 8G4C [https://www.rcsb.org/structure/8G4C], EMDB 29716 [https://www.ebi.ac.uk/emdb/EMD-29716]), ATP S bound BceAB-S Kinked BceS (PDB 8G4D [https://www.rcsb.org/structure/8G4D], EMDB 29717 [https://www.ebi.ac.uk/emdb/EMD-29717]). The initial model of BceAB used as a reference for model building is available at the PDB under the following accession number (PDB 7TCG [https://www.rcsb.org/structure/7TCG]). The AlphaFold model of BceS used as an initial template for model building is available at the AlphaFold Protein Structure Database [https://alphafold.ebi.ac.uk/entry/O35044]. Source data are provided with this paper.

## Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

| | |
|---|---|
| Reporting on sex and gender | Not applicable |
| Population characteristics | Not applicable |
| Recruitment | Not applicable |
| Ethics oversight | Not applicable |

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 | Biochemical experiments were performed in triplicate to provide appropriate estimates of error. No sample size calculation was used to predetermine sample size, and triplicate measurements were chosen to provide an adequate estimate of error associated with each measurement. |
| Data exclusions | No data was excluded. |
| Replication | Biochemical assays (ATPase measurements) were repeated with individual protein preparations from independent purifications (n=2), yielding similar and reproducible results from each preparation. Electron microscopy experiments were performed once. |
| Randomization | Not applicable, for the biochemical experiments described in this manuscript it is not possible or relevant to allocate samples into random experimental groups. |
| Blinding | Not applicable, for the biochemical experiments described in this manuscript it is not possible or relevant to blind investigators to group allocation of samples. |

# 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 |

## Methods

| n/a | Involved in the study | |
|---|---|---|
| ☒ | ☐ | ChIP-seq |
| ☒ | ☐ | Flow cytometry |
| ☒ | ☐ | MRI-based neuroimaging |