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Corresponding author(s): Youdong Mao

Reporting Summary

Nature Research 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 Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒ An indication of 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 statistics 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
☒	☐ Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Leginon and SerialEM
Data analysis	MotionCor2, GCTF, CTFFIND4, deepEM, SPIDER, Relion, ROME, Pymol, UCSF Chimera, COOT, Phenix, MolProbity, ResMap, PISA.

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/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Cryo-EM maps have been deposited in the Electron Microscopy Data Bank under accession codes EMD-9215 (the combined EA refined with CP-ATPase mask), EMD-9216 (whole EA1), EMD-9217 (whole EA2), EMD-9218 (whole EB), EMD-9219 (whole EC1), EMD-9220 (whole EC2), EMD-9221 (whole ED1), EMD-9222 (whole ED2), EMD-9223 (RP of EA1), EMD-9224 (RP of EA2), EMD-9225 (RP of EB), EMD-9226 (RP of EC1), EMD-9227 (RP of EC2), EMD-9228 (RP of ED1) and EMD-9229 (RP

of ED2). Coordinates are available from the RCSB Protein Data Bank under accession codes 6MSB (whole EA1), 6MSD (whole EA2), 6MSE (whole EB), 6MSG (whole EC1), 6MSH (whole EC2), 6MSJ (whole ED1), 6MSK (whole ED2). Raw data are available from Y.M.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were applied to pre-determine sample sizes. The actual sample size was increased until the finally achieved resolution and quality of 3D reconstructions of the cryo-EM structures meet the expectation, that is, no worse than 3.6 Å by gold-standard Fourier shell correlation (FSC) measurement.
Data exclusions	No data were excluded from the analysis. During cryo-EM data clustering, good cryo-EM images were chosen for further 3D analysis based on their achieved resolution and reconstruction quality. Poorer images were excluded in the final reconstructions based on the criteria of maximizing the map resolution and quality.
Replication	Biochemical experiments, including protein purification, and SDS-PAGE were done in biological replicates for more than 10 times. All attempts of replication were successful.
Randomization	This study did not allocate experimental groups thus no randomization was required for the reported experiments.
Blinding	Blinding was not required for the reported experiments, because all functional and structural data were analyzed using the same methods.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

Methods

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

Antibodies

Antibodies used	Anti-T7 antibody (abcam, cat#: ab9115, Rabbit polyconal, 250 µg at 1 mg/ml) was used in Western blot assay.
Validation	Information of the antibody validation is available through manufacturer's online database (https://www.abcam.com/t7-tag-antibody-ab9115.html). No further validation was done on the antibody in the reported experiments.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	A stable HEK293 cell line harboring HTBH (hexahistidine, TEV cleavage site, biotin, and hexahistidine) tagged hRPN11 , a gift from L. Huang, Departments of Physiology and Biophysics and of Developmental and Cell Biology, University of California, Irvine, California 92697
Authentication	Not further authenticated, only used for proteasome purification.
Mycoplasma contamination	Cell lines for purification were not tested.

Commonly misidentified lines (See ICLAC register)	No cell lines used in this study were commonly misidentified lines.