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Corresponding author(s): Nobuo N. Noda

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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 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	Fluorescence and DIC images were obtained by FV31S-SW ver. 2.3.1.163, SoftWoRx ver. 6.5.2, MetaMorph (64-bit) ver. 7.10.1.161 AFM images were obtained by FalconTS_1.0.0 Cryo-EM data collection: SerialEM ver. 3 Blot images were obtained by Image Studio Ver. 4.0.21, ImageQuant LAS4000 ver. 1.2 or 1.3 SDS-PAGE images were obtained by Image Lab Ver. 4.0 build 16
Data analysis	Image processing: FIJI ver. 1.52e, ImageJ ver. 1.51w, FV31S-SW ver. 2.1.1.98 AFM image processing: Falcon Viewer, IGOR Pro ver. 6.2.2.2 Cryo-EM analysis: CTFFIND4 4.1.13, Relion 3.0 or 3.1, crYOLO ver. 1.2 IDR prediction: DISOPRED ver. 3.1 (http://bioinf.cs.ucl.ac.uk/psipred/) Sequence alignment: UCSF Chimera ver. 1.13.18 (build 41965) Structural analysis: Phenix ver. 1.16_3549 or 1.14-3260, COOT ver. 0.8.9.1, PyMOL ver. 2.3.3 SEC-MALS: ASTRA ver. 7.1.2.5 Floatation assay: Gen5 ver. 2.07 Pore surface and volume calculation: CASTp 3.0

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 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

All relevant data are available upon request to the authors. The cryo-EM density maps have been deposited into the Electron Microscopy Data Bank under the accession numbers EMD-30535 (hexamer) and EMD-30545 (trimer). Coordinates are deposited into the Protein Data Bank with the accession number 7D0I (hexamer). Source data for graphs and gels/blots are provided as supplementary information.

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	Sample size for yeast experiments was determined by referring to commonly used size.
Data exclusions	No exclusion.
Replication	All attempts at replication were successful.
Randomization	Randomization is not relevant for this study because our work does not involve clinical trials or population studies.
Blinding	Blinding is not relevant for this study because no group allocation was performed.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	Anti-GFP (11814460001; Roche)(dilution 1:2000), Anti-Atg9 (J Cell Biol 148, 465, 2000)(dilution 1:2000), Anti-API-2 (Cell Struct Funct 28, 49, 2003)(dilution 1:5000), Anti-GST (A190-122A, Bethyl Laboratories, Inc)(Dilution 1:200), Peroxidase AffiniPure Rabbit Anti-Mouse IgG (H+L) (315-035-003, Jackson ImmunoResearch)(dilution 1:5000), Peroxidase AffiniPure Goat Anti-Rabbit IgG (H+L) (111-035-144, Jackson ImmunoResearch)(dilution 1:5000), Anti-Rabbit IgG (whole molecule)-Peroxidase (A6154; Sigma-Aldrich) (1:20000)
Validation	Validation for anti-GFP was described in the manuscript (Blood 118, 4305, 2011). Validation for anti-Atg9 was described in the manuscript (J Cell Biol 148, 465, 2000). Validation for Anti-API-2 was described in the manuscript (Cell Struct Funct 28, 49, 2003). Validation for Anti-GST was described in the manuscript (Proc Natl Acad Sci U S A 116, 13368, 2019), Validation for Peroxidase AffiniPure Rabbit Anti-Mouse IgG (H+L) was described in https://www.jacksonimmuno.com/catalog/products/315-035-003, Validation for Peroxidase AffiniPure Goat Anti-Rabbit IgG (H+L) was described in https://www.jacksonimmuno.com/catalog/

products/111-035-144. Validation for Anti-Rabbit IgG (whole molecule)-Peroxidase was described in the manuscript (J Virol 85, 178, 2011).