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 *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	Thermo Scientific EPU Software, Thermo Scientific Tomography v5.12.0
Data analysis	AlphaFold2, MotionCor2, RELION v3.1&4.0, Prism v10.4.0, Chimera v1.16 & ChimeraX v1.8, CTFFIND4, Coot v0.9, Phenix v1.21, IMOD v 4.11.3, Amira v2022.2, PyTom v0.981a&1.1, Warp v1.0.9, LipidSearch v4.2, Xcalibur v4.4

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

The cryo-EM map generated in this study have been deposited in the Electron Microscopy Data Bank (EMDB) under accession code EMD-62785 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-62785] (structure of the flotillin complex) ; EMD-67802 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-67802] (structure of

the flotillin complex in situ);. The atomic coordinate generated in this study have been deposited in the Protein Data Bank (PDB) under accession code 9L3G [https://doi.org/10.2210/pdb9L3G/pdb] (structure of the flotillin complex).

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	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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	The sample sizes for the Cryo-EM datasets were determined based on two factors: the requirement to achieve structures with sufficient resolution and the availability of cryo-EM instrument time. Detailed information on sample sizes, including the number of micrographs and particles, is provided in the accompanying EXD figures.
Data exclusions	Micrographs showing clear signs of astigmatism, extreme defocus values, uncorrectable image drift, ice contamination, or warm-up artifacts were excluded from the datasets. Additionally, particles from 2D classes without discernible secondary structural features and those from 3D classes with blurry structural details were omitted from the final reconstructions.
Replication	High-resolution structures were obtained using a 300-kV Titan Krios electron microscope. Initial small datasets were followed by additional data collection to validate and refine the structures. Structural informations were successfully replicated in all attempts.
Randomization	During 3D refinement, particle images were randomly split into two groups. In 2D classification, each particle was randomly assigned to a class with a random orientation.
Blinding	Blinding is not applicable in single-particle cryo-EM structure determination.

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	mouse-FLAG (1:8,000, Sigma-Aldrich, F3165)
Validation	mouse-FLAG validated PubMed: 31417089.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HEK293F (ATCC CRL-1573), Jurkat Clone E6-1 (ATCC TIB-152) were used in this study.
Authentication	No further authentication procedures were conducted for the commercially available cell lines used in the study.
Mycoplasma contamination	The cell lines used in the study were not tested for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were utilized in the study.

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A