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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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Cor	nfirmed
\boxtimes	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
\boxtimes	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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
\boxtimes	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 <u>statistics for biologists</u> contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

SerialEM v4.0

Data analysis

 $relion\ v4.0\ cryosparc\ v\ 3.3\ CTFFIND\ v4.1.13\ MotionCor2\ v1.5.0\ topaz\ v0.2.5\ phenix 1.19\ wincoot\ v0.9.4\ chimeraX\ v1.3\ ISOLDE1.3\ Molprobity\ EMRinger$

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 maps have been deposited in the EMDB under accession codes 34748, 34749, 34750, 34751, 34752, 34753, 34754, 34755, 34756, 34757, 34758, 34760, 34759, 34761, 34763, 34762, 34764, 34765, 34766, 34767, 35373, 34768, 34769, 34770, 34771, 34772, 34773, 34774, 34775, 34776, 34777, 34779, 34780, 34781, 34782, 34783, 34784, 34785, 34786, 34787, 34788, 34789, and 34790. The atomic models have been deposited in the Protein Data Bank accession

accession number 61	N2Y. The data th	at support the findings of this study are available from the corresponding author upon reasonable request.			
Human rese	arch part	icipants			
		involving human research participants and Sex and Gender in Research.			
Reporting on sex	and gender	N/A			
Population characteristics		N/A			
Recruitment		N/A			
Ethics oversight		N/A			
Note that full informa	ation on the app	roval of the study protocol must also be provided in the manuscript.			
Field-spe	ecific re	eporting			
-		is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.			
X Life sciences		Behavioural & social sciences Ecological, evolutionary & environmental sciences			
For a reference copy of	the document with	all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>			
Life scier	nces st	udy design			
All studies must dis	sclose on these	points even when the disclosure is negative.			
Sample size	The sample size is judged from previous studies using the same sample, and a minimum of 5000 micrographs is sufficient to maintain sufficient resolution after classification.				
Data exclusions	heterogeneou	crograph images were excluded using defocus values calculated by CTFFIND. Bad particle images were excluded by 2D classification and erogeneous refinement. Some micrograph images were largely out of focus due to acquisition errors and were excluded by the defocus ue (8000-25000Å) calculated by CTFFIND.			
Replication	At least five in successful.	dependent protein purifications have been performed, including previous studies, and all attempts at replication have been			
Randomization	This is not rele	vant to structural determination using cryo-EM, because no grouping is needed.			
Blinding	Blinding is not required because it is based on a random subset of particles.				
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Reportin	g for s	pecific materials, systems and methods			
1		about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.			
system of method ha	ted is relevant to	your stady. If you are not sure if a list item applies to your research, read the appropriate section service scienting a response.			
Materials & ex	perimental s	systems Methods			
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Animals and other organisms					
Clinical dat	_				
	esearch of conce	rn			

codes 8HH1, 8HH2, 8HH3, 8HH4, 8HH5, 8HH6, 8HH7, 8HH8, 8HH9, 8HHA, 8HHB, and 8HHC. The initial model for model building is accessible in PDB under