# natureresearch

Corresponding author(s): Adam Frost

### **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 <u>Authors & Referees</u> and the <u>Editorial Policy Checklist</u>.

#### 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	Cor	nfirmed
	$\boxtimes$	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
	$\boxtimes$	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
$\boxtimes$		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.
$\boxtimes$		A description of all covariates tested
$\boxtimes$		A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
$\boxtimes$		A full description of the statistics including <u>central tendency</u> (e.g. means) or other basic estimates (e.g. regression coefficient) AND <u>variation</u> (e.g. standard deviation) or associated <u>estimates of uncertainty</u> (e.g. confidence intervals)
$\boxtimes$		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 Give <i>P</i> values as exact values whenever suitable.
$\boxtimes$		For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
$\ge$		For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
$\boxtimes$		Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated
$\boxtimes$		Clearly defined error bars State explicitly what error bars represent (e.g. SD, SE, Cl)

Our web collection on statistics for biologists may be useful.

### Software and code

Policy information about availability of computer code							
Data collection	Serial EM was used to collect cryoEM data.						
Data analysis	RELION (version 1.3, 1.4, 1.2ah), EMAN2, Rosetta, Gautomach, GCTF, MotionCor2, CTFFIND4, UCSF Chimera were used for analysis. All are open source softwares. All softwares were cited in the methods section.						

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

All of the 3D cryoEM density maps associated with this study have be deposited in the EMDB with accession numbers EMD-8874. The atomic coordinates have been deposited in the PDB as 5WP9. Raw data, models and image processing scripts are also available from Adam Frost (adam.frost@ucsf.edu) upon request.

## 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

s Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see <u>nature.com/authors/policies/ReportingSummary-flat.pdf</u>

## Life sciences study design

All studies must disclose on these points even when the disclosure is negative.						
Sample size	Data collection was performed until the sample size comprised all necessary views for 3D reconstruction. Given the uncertainty and lack of experimental control of preferred orientation, no predetermination of sample size could be estimated.					
Data exclusions	Single particle images of damaged, incomplete, or contaminated structures were filtered out by 2D and 3D classification procedures.					
Replication	All attempts at replication were successful.					
Randomization	Random assignments of orientation parameters and classification parameters is integral to the reconstruction process.					
Blinding	All data points were treated equally. The investigators were blinded to the group allocation and outcomes.					

# Reporting for specific materials, systems and methods

#### Materials & experimental systems

n/a	Involved in the study	n
$\boxtimes$	Unique biological materials	
$\ge$	Antibodies	
$\ge$	Eukaryotic cell lines	
$\ge$	Palaeontology	
$\square$	Animals and other organisms	

Human research participants

#### Methods

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