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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For all	statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a C	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.
\boxtimes	A description of all covariates tested
$\boxtimes \Box$	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. <i>F, t, 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
\boxtimes	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
\boxtimes	\Box 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 LEGINON 3.5, pCLAMP 8.2

Data analysis MotionCor2, GCTF v1.

MotionCor2, GCTF v1.06, RELION 3.0.7, cryoSPARC 3.2.0, Microsoft Excel v15.26, UCSF Chimera 1.16, COOT 0.9 EL, Phenix-1.15.2-3472, Pymol 2.3.2, MolProbity 4.2, HOLE program v2.2.005, Clampfit 10.3, Prism 7, SWISS-MODEL online server (https://swissmodel.expasy.org/)

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 authors declare that the data supporting the findings of this study are available within the paper. The cryo-EM density map has been deposited in the Electron Microscopy Data Bank under the accession number EMD-24468. The coordinates of the atomic model have been deposited in the Protein Data Bank under the accession number 7RHS. Several structural coordinates in the PDB database were used in this study, which can be located by accession numbers 6WEJ and 7LFT. Source Data is available online.

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