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>.

Statistics

For	all st	atistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a	Cor	firmed
	\square	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
	\boxtimes	A statement on 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
		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)
\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 <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		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 4.0
Data analysis	RELION 3.1
	MotionCorr2 1.1
	CTFFIND 4.1.13.
	Coot 0.9.8.7
	PHENIX 1.20
	UCSF ChimeraX 1.4.
	ImageJ 12.24.12
	Matlab R2022B
	Prism9 9.5.1
	DeepEMhancer
	Image Lab v6.1

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.

Policy information about <u>availability of data</u>

All manuscripts must include a <u>data availability statement</u>. 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 protein structure data reported in this study have been deposited in PDB under the accession codes PDB ID 8PP6 and 8PP7 and in the EMDB under accession codes EMD-17796 and EMD-17797. Initial models used for CryoEM model building (PDB code): 6PWE, 4R8P, 1Q5W

Research involving human participants, their data, or biological material

Policy information about studies with <u>human participants or human data</u>. See also policy information about <u>sex, gender (identity/presentation)</u>, <u>and sexual orientation</u> and <u>race</u>, <u>ethnicity and racism</u>.

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.

K 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 numbers of particles used for cryo-EM reconstructions are indicated in Extended Data Table 1 For all EMSAs and enzyme activity assays, we conducted experiments with three or more replicates to ensure reproducibility. The consistent results across all replicates suggests that the number of replicates was sufficient in all experiments. No statistical methods were used to predetermine the sample size.
Data exclusions	No data were excluded
Replication	For quantitative measurements, experiments were performed at least in triplicate. All of them were successful.
Randomization	not relevant, no grouped samples
Blinding	not relevant, no grouped samples

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

Materials & experimental systems		Me	Methods	
n/a	Involved in the study	n/a	Involved in the study	
\ge	Antibodies	\boxtimes	ChIP-seq	
	Eukaryotic cell lines	\boxtimes	Flow cytometry	
\boxtimes	Palaeontology and archaeology	\boxtimes	MRI-based neuroimaging	
\boxtimes	Animals and other organisms			
\ge	Clinical data			
\ge	Dual use research of concern			
\boxtimes	Plants			

Eukaryotic cell lines

	Policy information abo	out <u>cell lines a</u>	and Sex and Ger	der in Research
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Cell line source(s)	Cell line (Trichoplusia ni) HighFive cell line for expression Invitrogen Product nr.: B85502 BTI-Tn-5B1-4 (RRID:CVCL_C190) Protein expression Cell line (Spodoptera frugiperda) Sf21 cell line for Baculovirus production Invitrogen Product nr.: 1149701 (RRID:CVCL_0518) Baculovirus production for protein expression
Authentication	Commercial cell lines. Authentication is performed by the manufacturer
Mycoplasma contamination	The cell line were not tested for mycoplasma contamination
Commonly misidentified lines (See <u>ICLAC</u> register)	No commonly misidentified lines were used

Plants

Seed stocks	Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.
Novel plant genotypes	Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor upon which experiments were dited for editing, the editor were dited for editing guide RNA sequence (if applicable) and how the editor
Authentication	was applied. Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.