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

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For all statistical an	alyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.				
n/a Confirmed					
☐ ☐ The exact	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement				
A stateme	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly				
IVIII I	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 descript	A description of all covariates tested				
A descript	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</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>				
For Bayes	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 <u>availability of computer code</u>					
Data collection	AutoEMation 1.0				
Nata analysis	Relian 2.0 Relian 3.0 heta MationCor2.1.1.0 FMAN2.1 CTFFIND4 Phenix 1.14-3260 Coot 0.8.9.1 Pymol 1.8.2.1 Chimera 1.12				

Relion 2.0, Relion 3.0 beta, MotionCor2 1.1.0, EMAN2.1, CTFFIND4, Phenix 1.14-3260, Coot 0.8.9.1, Pymol 1.8.2.1, Chimera 1.12, CLUSTAL 2.0, ResMap v1.1, MolProbity 4.4, ENDscript 2.0

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 Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

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 list of figures that have associated raw data
- A description of any restrictions on data availability

The atomic coordinates have been deposited in the Protein Data Bank with the accession code 6LY5. The EM map have been deposited in the Electron Microscopy Data Bank with the accession codes EMD-30012. All other data and materials are available from the corresponding authors upon reasonable request.

Field-specific reporting					
Please select the one be	elow that is the best fit for your research. If you a	are not sure, read the appropriate sections before making your selection.			
X Life sciences	Behavioural & social sciences	cological, 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

Replication

Blinding

All studies must disclose on these points even when the disclosure is negative.

Sample size Amount of cryo-EM micrographs collected was based on the previous knowledge that the reconstruction of the protein particles picked from these micrographs could reach to an atomic resolution.

Data exclusions

The exclusion criteria were not pre-established. 2D and 3D classification yielded multiple classes. Only the particles in the classes that showed clear structural signals and intact structures were selected, combined and used in the final reconstruction and refinement. Details are described in the flowchart of Extended Data Figure 2 and Methods.

Multiple rounds of structural refinement have been performed and all resulted in same density maps, although at different resolutions. The purification and characterization of PSI-FCPI (detergent-solubilization, sucrose density gradient centrifugation, SDS-PAGE, absorption spectrum, fluorescence emission spectra) have been repeated for more than times, and all showed the similar results.

Randomization Randomization of samples is not relevant for structural analysis by the single particle electron microscopic technique because the study focused on a specific protein complex.

Binding is not relevant because we are studying a specific protein complex.

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.

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