# 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>.

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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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			
$\boxtimes$		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 descrip	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)				
$\boxtimes$	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 Bayes	ian 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				
$\square$ 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>					
Da	ata collection	EPU			
Da	nta analysis	MotionCor2, Gctf, Relion3.0, cryoSPARC, Chimera, ChimeraX, Coot, PHENIX 1.17			
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 quidelines for submitting code & software for further information.					

#### Data

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

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 atomic coordinates for human pre-Bact, Bact-I, Bact-II, Bact-III, Bact-IV, post-Bact and C complexes have been deposited in the Protein Data Bank (PDB) under the accession codes 8I0P, 8I0R, 8I0S, 8I0T, 8I0U, 8I0V and 8I0W, respectively. The EM maps of human pre-Bact, Bact-II, Bact-III, Bact-

Research inv	olving hu	man participants, their data, or biological material		
	about studies w	vith human participants or human data. See also policy information about sex, gender (identity/presentation),		
Reporting on sex and gender		N/A		
Reporting on race other socially rele groupings		N/A		
Population chara	cteristics	N/A		
Recruitment		N/A		
Ethics oversight		N/A		
Note that full informa	ation on the appro	oval of the study protocol must also be provided in the manuscript.		
Field-spe	ecific re	porting		
Please select the or	ne below that is	the best fit for your research. If you are not sure, read the appropriate sections before making your selection.		
X Life sciences	В	ehavioural & social sciences		
For a reference copy of t	the document with a	all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>		
Life scier	nces stu	udy design		
All studies must dis	sclose on these	points even when the disclosure is negative.		
Sample size	We totally collected 18,277 images for the cryo-sample, and 17,070 good micrographs were used for the final reconstruction of EM maps. Sample size for the cryo-EM studies was determined by the microscope time availability and ensure us to obtain the high-resolution EM maps.			
Data exclusions	Bad particles were excluded from the final reconstructions in order to enhance the complex homogeneity, which enabled a higher resolution reconstruction.			
Replication	All biochemical experiments were replicated at least three times within a month. All attempts at replication are successful.			
Randomization	N/A			
Blinding	The particle assignment to different classes during reconstruction is implemented automatically by the Relion software, without direct supervision by the investigator.			
Reportin	g for sp	pecific materials, systems and methods		
		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.		
Materials & exp	perimental s	ystems Methods		
	cell lines ogy and archaeol d other organism			

## Antibodies

Antibodies used

PRP2 antibody (Proteintech, Cat# 11021-1-AP)

### Eukaryotic cell lines

Policy information about <u>cell lines and Sex and Gender in Research</u>

Cell line source(s) Hela S3 cells were originally purchased from company. Expi293F (A14527) cells were purchased from ThermoFisher Scientific. Authentication None of the cells are authenticated.

Mycoplasma contamination They were tested negative for mycoplasma contamination.

Commonly misidentified lines No commonly misidentified lines were used in this study. (See ICLAC register)

#### **Plants**

iditis		
Seed stocks	N/A	
Novel plant genotypes	N/A	
Authentication	N/A	