

## 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 [Authors & Referees](#) and the [Editorial Policy Checklist](#).

### Statistics

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

n/a 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.*
- ☒ ☐ A description of all covariates tested
- ☒ ☐ 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.  $F$ ,  $t$ ,  $r$ ) with confidence intervals, effect sizes, degrees of freedom and  $P$  value noted  
*Give  $P$  values as exact values whenever suitable.*
- ☒ ☐ 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 [statistics for biologists](#) contains articles on many of the points above.*

### Software and code

Policy information about [availability of computer code](#)

Data collection

SerialEM

Data analysis

MotionCor2, Relion 2 & Relion 3, Chimera, COOT, Phenix

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. 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

Final coordinates and cryo-EM maps have been submitted to the PDB and EMDB, respectively. Accession codes are provided in the revised manuscript. Coordinates and cryo-EM maps will be released prior to publication.

## 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.

- ☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

## Life sciences study design

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

|                 |                                                                                            |
|-----------------|--------------------------------------------------------------------------------------------|
| Sample size     | No sample size calculation was performed                                                   |
| Data exclusions | Data were excluded according the cutoff criteria defined in programs Relion 2 and Relion 3 |
| Replication     | Cryo-EM data analysis and reproducibility are described in detail in the methods section   |
| Randomization   | Not relevant for this study                                                                |
| Blinding        | Not relevant for this study                                                                |

## 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    |                                                           | Methods                             |                                                 |
|-------------------------------------|-----------------------------------------------------------|-------------------------------------|-------------------------------------------------|
| n/a                                 | Involved in the study                                     | n/a                                 | Involved in the study                           |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Antibodies                       | <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq               |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Eukaryotic cell lines | <input checked="" type="checkbox"/> | <input type="checkbox"/> Flow cytometry         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology                    | <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Animals and other organisms      |                                     |                                                 |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Human research participants      |                                     |                                                 |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data                    |                                     |                                                 |

## Eukaryotic cell lines

Policy information about [cell lines](#)

|                                                                      |                                                                  |
|----------------------------------------------------------------------|------------------------------------------------------------------|
| Cell line source(s)                                                  | Pol I was purified from yeast strain SC1613                      |
| Authentication                                                       | Provided by Cellzome AG                                          |
| Mycoplasma contamination                                             | Mycoplasma contamination testing is not required for this strain |
| Commonly misidentified lines<br>(See <a href="#">ICLAC</a> register) | No commonly misidentified cell line was used                     |