

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see [Reporting Life Sciences Research](#). For further information on Nature Research policies, including our [data availability policy](#), see [Authors & Referees](#) and the [Editorial Policy Checklist](#).

► Experimental design

1. Sample size

Describe how sample size was determined.

No statistical methods were used to predetermine sample size except sufficient images were collected to ensure adequate reconstructions can be obtained

2. Data exclusions

Describe any data exclusions.

No data were initially excluded from the analysis although final reconstructions were obtained from subset of the data as detailed in Methods and Extended Data

3. Replication

Describe whether the experimental findings were reliably reproduced.

Yes. Thousands of micrographs with this sample were consistent in quality. Several independent cryoEM datasets were used as well as different software which deal with large dataset in a statistically significant manner. Other results from solution studies have been reliably reproduced.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

All data were used in an unbiased way for data analysis and image reconstruction. Final quality control (such as resolution estimation) were conducted by randomly separating data into two halves and to calculate their correlation coefficients.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

There are no bias or randomization involved in the analysis so blinding is not relevant to this type of study.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a Confirmed

- | | | |
|-------------------------------------|-------------------------------------|--|
| ☐ | ☒ | The <u>exact sample size</u> (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.) |
| ☐ | ☒ | A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ | A statement indicating how many times each experiment was replicated |
| ☒ | ☐ | The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section) |
| ☒ | ☐ | A description of any assumptions or corrections, such as an adjustment for multiple comparisons |
| ☒ | ☐ | The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted |
| ☐ | ☒ | A clear description of statistics including <u>central tendency</u> (e.g. median, mean) and <u>variation</u> (e.g. standard deviation, interquartile range) |
| ☐ | ☒ | Clearly defined error bars |

See the web collection on [statistics for biologists](#) for further resources and guidance.

► Software

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

7. Software

Describe the software used to analyze the data in this study.

Cryo-EM analysis was performed in MotionCor2, Gctf, RELION 2.0 and cryoSPARC 0.3.6. Coot v0.8.1 was used model building, Phenix and Refmac for refinement. Pymol and Chimera for visualization and analysis.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* [guidance for providing algorithms and software for publication](#) provides further information on this topic.

► Materials and reagents

Policy information about [availability of materials](#)

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

no restrictions apply

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

No antibodies were used

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

N/A

b. Describe the method of cell line authentication used.

Describe the authentication procedures for each cell line used OR declare that none of the cell lines used have been authenticated OR state that no eukaryotic cell lines were used.

c. Report whether the cell lines were tested for mycoplasma contamination.

Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination OR state that no eukaryotic cell lines were used.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by [ICLAC](#), provide a scientific rationale for their use.

Provide a rationale for the use of commonly misidentified cell lines OR state that no commonly misidentified cell lines were used.

► Animals and human research participants

Policy information about [studies involving animals](#); when reporting animal research, follow the [ARRIVE guidelines](#)

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

No animals were used in this study

Policy information about [studies involving human research participants](#)

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

This study did not involve human research participants