

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 [Editorial Policies](#) 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 EM data collection Commercial software was used: EPU (version 3.4.0.5704 REL) from Thermo Fisher Scientific for automated cryo-EM data collection.

Data analysis Micrograph movie frames were aligned with MotionCor2. CTF estimation was performed using CTFFIND4 v4.1.14. Data were processed using RELION v4.0 and cryoSPARC v2.14.2. Particle picking was performed with TOPAZ v0.2.5. Models were built using AlphaFold (<https://alphafold.ebi.ac.uk/>), Chimera X v1.4 and COOT v0.8.9.2 and real-space refinement in PHENIX v1.20.1. Model validation was performed using MolProbity v4.5. Visualization was done using Chimera X v1.4. Sequence alignments were performed using Uniprot, Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) and displayed with JALVIEW v 1.0. Structure prediction was performed using local instalation of the ALPHAFOLD2 software. ITC data was analysed using MicroCal PEAQ-ITC Analysis Software 1.0.0.1258.

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.

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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

The cryoEM map has been deposited with the Electron Microscopy Data Bank (EMDB) with accession code: EMD-17814 Protein coordinate has been deposited with RCSB with accession code: 8PPR. Publicly available PDB entries used in this study are: 5LSK, 3VZA and AlphaFold2 AF-Q8NG31-F1. CENP-C uniprot entries used for sequence alignments: Q03188 (H. sapiens), P49452 (M. musculus), O57392 (G. gallus), Q66L13 (B. taurus); DSN1 uniprot entries used for sequence alignment: Q9H410 (H. sapiens), A6QQ81 (B. taurus), Q9CYC5 (M. musculus), Q1T768 (G. gallus), A0A8J0US97 (X. laevis).

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

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

c

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.

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](https://www.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

No statistical method was used for sample size calculation. For the KMN network complex, a total of 17,815 micrographs were collected. A total number of 2,549,620 particles were auto-picked, of which 599,831 were used for a final reconstruction. For cryoEM reconstructions, sample sizes were determined by available electron microscopy time and the number of particles on each micrograph obtained during the collection time.

Data exclusions

Through 2D and 3D classification procedures, broken particles or particles that do not belong to the classes of interests were discarded. This is a standard practice in cryoEM studies to obtain homogeneous cryoEM structures.

Replication

CryoEM datasets were collected with multiple samples in separate imaging sessions. All biochemical experiments were repeated at least in three independent experiments and are all reproducible.

Randomization

No randomization was performed, since this study did not allocate experimental groups.

Blinding

No blinding was performed, since it is not relevant to 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 | Involvement in the study |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Antibodies |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> Eukaryotic cell lines |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Animals and other organisms |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Plants |

- | | |
|-------------------------------------|---|
| n/a | Involvement in the study |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Flow cytometry |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging |

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	High-5 insect cell (BTI-TN-5B1-4) were obtained from ThermoFisher Scientific.
Authentication	The High-5 insect cell line was not authenticated
Mycoplasma contamination	The High-5 insect cell line was not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	None