

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 [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 SerialEM V 4.0

Data analysis CryoSPARC v3.3.2, DeepEMhancer v1, UCSF ChimeraX v1.4, NAMDINATOR web service (<https://namdinator.au.dk/namdinator/>), iMODFIT v1.51, AlphaFold Colab v2, Phenix v1.20.1, Coot v0.9.5, Molprobit (included in Phenix v1.20.1 package), ISOLDE v1.4.

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 [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

Data availability. Structural data that support the findings of this study on the structure of the human cardiac thick filament have been deposited in the Electron Microscopy Data Bank. One 2-crown and two 5-crown maps have been deposited, with accession codes EMD-29734, EMD-29722 and EMD-29726, respectively. An atomic model of the full 3-crown structure, with myosin heads, tails, titins, and cMyBP-C, has been deposited in wwPDB with accession code 8G4L. Atomic models from the Protein Data Bank (pdb) with accession codes 2fxo and 5n69, and from AlphaFold database (<https://alphafold.ebi.ac.uk/>) with Uniprot ids P10916 (RLC) (<https://alphafold.ebi.ac.uk/entry/P10916>); P12883 (MYH7) (<https://alphafold.ebi.ac.uk/entry/P12883>); Q14896 (cMyBP-C) (<https://alphafold.ebi.ac.uk/entry/Q14896>), were used for model building.

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	In total 56,489 microscope raw movies collected from three different human heart samples were used for data processing, sufficient to provide a high resolution structure.
Data exclusions	Micrographs that had a defocus > 2 um were excluded. Particles were removed if their 2D class averages didn't show secondary structure or the 3D reconstruction had low resolution.
Replication	Four imaging sessions were carried out, on two different microscopes at different accelerating voltages. The first dataset, from sample 1, on a Talos Arctica, produced a reconstruction at 7.9 A resolution. The second, third and fourth datasets, from new sets of grids (Sample 1 and two additional samples), were on a Titan Krios, and combined produced a 6 A resolution reconstruction. The latter reconstruction reproduced the former, but with more detail. Two individual Krios datasets processed separately also produced the same reconstruction.
Randomization	In the Fourier shell correlation (FSC) measurement step of the CryoSPARC 3.3.2 data processing pipeline, data were randomly divided into two halves resulting in two independently determined 3D volumes that were used for the FSC calculation.
Blinding	Data division in the FSC calculation step is a computer-based, unbiased process. Individual processing of different datasets collected from different human heart samples gave rise to the same 3D structures.

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

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Human research participants

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

Population characteristics	Cardiac muscle samples came from patients who had been declared brain-dead at the University of Kentucky Chandler Medical Center and were enrolled in an organ donation program. Tissue was obtained from three patients with the following characteristics. Patient 5155D: male, age 36, white (not Hispanic), diabetic, BMI 22.2. Patient 78952: female, age 47, white (not Hispanic), non-diabetic, BMI 38.3. Patient D0F54: male, age 23, white (unknown ethnicity), non-diabetic, BMI 20.5.
Recruitment	Kentucky Organ Donor Affiliates, the regional Organ Procurement Organization, obtained informed consent from the legally authorized representative (LAR) of each organ donor. The LAR gave permission for the heart to be used for research if it could not be used for transplant.
Ethics oversight	The protocols were approved by Kentucky Organ Donor Affiliates and by the University of Kentucky Institutional Review Board.

Note that full information on the approval of the study protocol must also be provided in the manuscript.