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 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	Actin-activated ATPase data was collected using the SkanItTM software for microplate readers. Single turnover data was collected using the i-controlTM software. ATP binding data was collected using the Bio-Kine software on a BioLogic stopped-flow machine. In-vitro motility data was collected using the Micro-Manager software.
Data analysis	In-vitro motility movies were analyzed using the previously reported custom code FAST. All biochemical data were analyzed and visualized using GraphPad Prism versions 9 and 10. X-ray diffraction data reduction was performed using the XDS package (v.1.12.15). Data scaling was performed using AutoPROC (v.1.0.5) and STARANISO (released with AutoPROC v.1.0.5) for anisotropic processing. Molecular replacement was achieved using Phaser from the Phenix program suite (v.1.20.1-4487-000 for E497D and Y115H models and v.1.21.2-5419-000 for the WT model). Homology modelling for molecular replacement was done using the SWISS-MODEL server. Iterative model building was completed using Coot (v.0.9.8.92 for E497D and Y115H models and v.0.9.8.95 for the WT model). Automatic Refinement was performed using BUSTER (v.2.10.4).

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 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 atomic model and X-ray maps generated in this study are available in the PDB under accession codes 9HTF [https://doi.org/10.2210/pdb9htf/pdb] (Y115H_MD structure); 9HTG [https://doi.org/10.2210/pdb9htg/pdb] (E497D_MD structure); 9I8P [https://doi.org/10.2210/pdb9i8p/pdb] (WT_MD structure). The biochemical data generated in this study are provided in the Source Data file.

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

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

Life sciences study design

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

Sample size	Based on previous knowledge of protein prep-to-prep variability, data was collected from 2 or more independent protein preparations (3 or more technical replicates for each preparation). If data from 2 biological preps was similar, a third independent preparation was deemed unnecessary.
Data exclusions	For single turnover experiments, ambiguous fits occasionally resulting from mixing artefacts or a bubble in the optical path (< 5%) were discarded.
Replication	All attempts at replication were successful. Any steps critical for such replication are noted in the methods.
Randomization	n/a to in vitro protein based studies
Blinding	n/a to in vitro protein based studies

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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HEK 293 and C2C12 were both from ATCC
Authentication	Neither cell line was authenticated as they are both used only for reagent generation and not for cell biological studies. The HEK293 cells are used only to make adenovirus. The C2C12 cells underwent appropriate differentiation upon starvation and produced the functional recombinant myosin proteins that were used in this study.
Mycoplasma contamination	Cells were not routinely tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	*Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*

Plants

Seed stocks	n/a
Novel plant genotypes	n/a
Authentication	n/a