nature portfolio

Corresponding author(s): Shaun K. Olsen

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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	CryoEM data was collected at Sauer Structural Biology Laboratory, University of Texas at Austin, Simons Electron Microscopy Center, NYSBC and at Structural Biology Core, UT Health San Antonio, using EPU software v2.13, ThermoScientific. Thioester activity data was imaged using BioRad GelDoc Go Gel Imaging system (12009077EDU)
Data analysis	CryoEM data was processed by using the cryoSPARC 3.3.1. Subsequent refinement, model building and visualization was performed by using the Phenix1.20.1-4487, Coot0.9.8.7, PyMOL v2.0 and ChimeraX1.5. Thioester transfer activity data analysis was done using ImageJ 1.53t and Graphpad Prism (versions 9) was used for statistical analyses

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

Atomic coordinates and their corresponding maps were deposited in RCSB under the accession code PDB: 9YKV and EMD-73059 for UBA6-UBE2Z-FAT10(t)/FAT10

(a), PDB: 9YLB and EMD-73079 for UBA6-UBE2Z/FAT10(a), PDB: 9YKW and EMD-73060 for UBA6-UBE2Z-Ub(t)/Ub(a), PDB: 9YLF and EMD-73081 for UBA6-UBE2Z/Ub(a). The structural data used from RCSB for analysis are listed here- PDB: 7SOL, 5A4P, 8SV8, 6NYA, 5KNL, 9DRJ, 4AUQ, 7K5J.
The source data and uncropped gels for underlying Fig. 1d, Fig. 3c, d, e, Fig. 4b, c, d Fig. 5b, Fig. 7c, f and Supplementary Fig. 3, 4 are provided as a 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	NA
Reporting on race, ethnicity, or other socially relevant groupings	NA
Population characteristics	NA
Recruitment	NA
Ethics oversight	NA

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	All biochemical assays were conducted in triplicate since this sample size has been broadly accepted by the scientific community for such experiments. Data are represented as mean ± SD of three independent experiments.
Data exclusions	No data were excluded from any of the analysis except junk 2D and 3D classes in CryoSPARC during CryoEM data processing.
Replication	All the biochemical assays were repeated as three distinct technical replicates. All of the data were consistent and were included.
Randomization	In these investigations, experimental grouping was not used, hence randomization was not required. All in vitro assays were done in triplicate with no randomization.
Blinding	Not relevant for this study since no bias could be introduced by the subject in the experiments performed.

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)	High Five (Cat. No.-B85502), Sf9 (Cat. No.-11496015) and Expi293F (Cat. No.-A14635)cells were purchased from Thermo Fisher Scientific
Authentication	High Five, Sf9 and Expi293F cells were directly purchased from vendor, where they were validated. These cell lines were not authenticated by authors. SDS-PAGE results, biochemical and cryoEM studies confirmed the identity of the expected recombinant proteins expressed in the High Five and Expi293F cells.
Mycoplasma contamination	High Five, Sf9 and Expi293F cells were certified as testing negative for Mycoplasma by the vendor. No testing was done by the authors. The cells exhibited normal growth pattern.
Commonly misidentified lines (See ICLAC register)	N/A