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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 Authors & Referees 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	Cryo-EM data are collected on a 300kV Titan Krios microscope with a Gatan K3 Summit direct electron detector; Gel Imaging: Image Lab Touch Software-2.4.0.03;
Data analysis	Cryo-EM:Motion Cor2(1.2.6), RELION-3.1.1, KaiZhang's gctf(v1.18); Model Building: PHENIX(1.19.2), COOT(0.8.2) ; Molecular Visualization: :ChimeraX(1.7.1) ; GraphPad Prism (v10).

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/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

The cryo-EM maps and atomic model of Ufd2/k29diUb-Ubc4-Ub and Ufd2/k29triUb-Ubc4-Ub complex have been deposited in the Electron Microscopy Data Bank (EMDB: www.ebi.ac.uk/pdbe/emdb/) and Protein Data Bank (PDB:www.rcsb.org) under accession codes: EMD-62353 and PDB-9KHS (diUb monomeric complex), EMD-62356 (diUb dimeric complex), EMD-62354 and PDB-9KHT (triUb dimeric complex), EMD-62355 (triUb monoimeric complex) and 9M7O (TriUb monomeric complex) respectively. The source data for the mass spectrometry in Extended Data Figures 2f-h has been uploaded to the ProteomeXchange Consortium (https://proteomecentral.proteomexchange.org/cgi/GetDataset?ID=PXD062799) under the accession number PXD062799.

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	No statistical methods were used to predetermine the sample size. Biochemical experiments producing qualitative results were performed independently twice or more to ensure reproducibility. For cryo-EM,the sample sizes were determined by available electron microscopy measuring time and density of the particles on the sample grids, the representative micrograph was shown, with other micrographs exhibiting similar particle density and dispersion.
Data exclusions	No data were excluded from the analysis.
Replication	The experiments including in vitro ubiquitination assays were repeated independently three times. Expression shutoff Analysis and mass spectrometry analysis was repeat two times. Surface plasmon resonance (SPR) was not repeat. Structure determination does not require replication, the 3D reconstitution of particles at different angles gives consistent results. All replication attempts were successful.
Randomization	Randomization was not relevant to this study because no grouped samples were involved.
Blinding	Blinding was not relevant to this study because no grouped samples were involved.

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
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used	Anti-Myc antibody (Cell Signaling Technology, 2272), Anti-Flag antibody (Cell Signaling Technology, 14793) , Anti-GAPDH antibody (Abcam, ab125247)
Validation	Validation of anti-Myc antibody: https://www.cellsignal.com/products/primary-antibodies/myc-tag-antibody/2272 Validation of anti-Flag antibody: https://www.cellsignal.com/products/primary-antibodies/dykddddk-tag-d6w5b-rabbit-mab-binds-to-same-epitope-as-sigma-s-anti-flag-m2-antibody/14793 Validation of anti-GAPDH antibody: https://www.abcam.cn/products/primary-antibodies/gapdh-antibody-ga1r-loading-control-ab125247.html