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Corresponding author(s): Nikola Pavletich

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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	SerialEM3, Digital Micrograph (GATAN), Leginon and Appion (3.2)
Data analysis	CryoEM data processing was done with MOTIONCORR2, UNBLUR, CTFFIND4 and RELION3, and cryoEM structure refinement was done with REFMAC5 and PHENIX. ImageGauge (version 4.1) was used to quantify DNA-binding data. Analyses of biochemical data were performed using Excel or GraphPad Prism 7.

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

Data availability. The ID-ICL DNA, IDUb-DNA, ID-/Ub-DNA and apoID coordinates, corresponding cryo-EM maps, including the focused reconstructions and the composite map used in refinement, have been deposited with the Protein Data Bank and the Electron Microscopy Data Bank under accession codes PDB-6VAA and EMDB-21134, PDB-6VAE and EMDB-21138, PDB-6VAF and EMDB-21139, PDB-6VAD and EMDB-21137, respectively.

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 sample size calculations were performed. For the DNA-binding (Fig. 4e, ED Figs 6a, 9g) and de-ubiquitination (Fig. 3f) assays, a sample size of 3 repeats was used based on the minimal variation of the results among repetitions. For the cryo-EM data of Extended Table 1, sample size (number of particles) was based on the reconstruction resolution plateauing as more data sets were added.
Data exclusions	No data were excluded from analyses.
Replication	All attempts at replication were successful. For DNA-binding assays (Fig. 4e, ED Figs 6a, 9g), verification was based on the quantification of the autoradiograms; for de-ubiquitination (Fig. 3f) they were judged by visual inspection. For the cryo-EM data of Extended Table 1, the particles were collected from multiple, independently prepared samples, and the individual data sets produced very similar maps with reconstruction resolution depending on the number of particles in each data set.
Randomization	The randomization used in cryoEM refinement was performed by the software RELION-3 and it was random. Randomization is not relevant for the biochemical experiments, because each repetition is an independent experiment.
Blinding	No blinding was used. It is not relevant because the samples used are reconstituted from purified components characterized by massspec, SDS PAGE and immunoblots where applicable.

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	Santa Cruz Cat#sc-271289; clone#F-11; Lot#H2019; used in 10000 X dilution (20 ng/ml)
Validation	Validation statement on vendor's website: Ub Antibody (F-11) is recommended for detection of ubiquitin and polyubiquitin of mouse, rat, human by WB. Selected citations: 1.Ning, B. et al. 2017. Nat Commun. 8: 349. 2.Song, Y. et al. 2017. Nat Commun. 8: 14654. 3.Zhou, Q. et al. 2016. Nature genetics. 48: 67-73.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	High Five insect cells(Invitrogen B85502);FreeStyle 293F cells (Invitrogen R79007)
Authentication	Vendor authenticated and tested: High Five™ Cells are a clonal isolate derived from the parental Trichoplusia ni cell line and are commonly used for the expression of recombinant proteins using the Baculovirus. The FreeStyle™ 293-F cell line is prepared from low-passage Master Cell Bank cultures derived from parental 293-F cells.
Mycoplasma contamination	Cell lines were not tested for mycoplasma contamination.

Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.