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Corresponding author(s): Helmut Bergler, David Haselbach

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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	Cryo-EM data colelction: SerialEM v3.8 (https://bio3d.colorado.edu/SerialEM/). Fluorescence Microscopy: Leica DM6 B Microscope equipped with a ×100/1.4 Plan APO objective and a high-resolution DFC9000GT camera, LASX premium software (Leica). Western blots: ChemiDoc™ Touch Imaging System (Bio-Rad). Yeast growth curves: Bioscreen C Pro Automated Microbiology Growth Curve Analysis system (Oy Growth Curves Ab Ltd. qMS: QExactive HF or HF-X mass spectrometer (Thermo Fisher Scientific) coupled online to a Ultimate 3000 RSLC nano-HPLC (Dionex).
Data analysis	Cryo-EM structure analysis: CryoDRGN v0.3.2 & 3.4 (https://github.com/zhonge/cryodrgn), Cryosparc v3.2, crYOLO v 1.6 (Wagner et al., 2019), CTFFIND4 (Rohou and Grigorieff 2015), DeepEMhancer (https://github.com/rsanchezgarc/deepEMhancer), UCSF ChimeraX v1.6, namdinator web server (https://namdinator.au.dk/namdinator/). ImageLab software v.2.2.0.08 (BioRad). qMS: Mascot (Matrix Science, v 2.6.2), Progenesis QI software, Statgraphics v18.

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 mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE [1] partner repository with the dataset identifier PXD046691. For cryo-EM model building, published pre-60S models PDB-6EM3, PDB-6EM4, PDB-6C0F, PDB-6EM1, PDB-6EM5 and PDB-6C0F were used. Yeast strains generated in this study are available from the corresponding authors upon request.

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	not applicable
Reporting on race, ethnicity, or other socially relevant groupings	not applicable
Population characteristics	not applicable
Recruitment	not applicable
Ethics oversight	not applicable

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	Sample sizes (n) are supplied in the figure legends No mathematical sample size calculation was performed. All biochemical and yeast growth experiments were performed with multiple biological and technical replicates to allow estimation of the data distribution of the data. Sample sizes were determined by number of replicates necessary to ensure reproducibility. Detailed information for the individual experiments including sample size and replicates are stated in the figure legends, the methods section as well as the source data file provided along with this paper.
Data exclusions	during pre-processing, individual cryo-EM micrographs were discarded due to strong drift, devitrification or ice contamination after manual inspection. During the first training round of the cryoDRGN analysis, ice-contaminated and outlier particles were filtered by the absolute of the latent variable $\|\|z\|\| > 2$ stdev($\|\|z\|\|$) + mean($\|\|z\|\|$). For the biochemical measurements and yeast growth experiments no datasets were excluded.
Replication	For biochemical measurements, 2-3 biological replicates were tested, each measured with at least two technical replications. All attempts at replication were successful. Detailed information for the individual experiments, including exact sample number (n) are stated in the figure legends, the methods section as well as the source data file provided along with this paper. For the quantitative cryoDRGN analysis, the particles were randomly split into three batches, which were individually processed in cryoDRGN using the same settings.
Randomization	For the cryoDRGN For biochemical/biophysical analyses, samples from each biological replicate were randomly assigned to the tested conditions. To calculate the Fourier Shell Correlation of the final maps using Cryosparc, the cryo-EM particles were automatically split into two random halves by the software. For growth comparison experiments of different yeast strains, randomization was not applicable.
Blinding	For biochemical, yeast growth and cryo-EM experiments, the same investigators performed data collection and/or analysis. Data acquisition and analyses were performed using constant parameters across all conditions being tested. The quantitative readout of the biochemical/ biophysical assays in this study did not require subjective interpretation of the results and thus blinding was not applied.

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

Antibodies

Antibodies used

(Antibody/dilution/source)
α-Arx1 1:5 000 M. Fromont-Racine
α-Cbp 1:5 000 Sigma – Aldrich
α-Cic1 1:5 000 University of Stuttgart
α-Crm1 1:10 000 C. Yam
α-Ebp2 1:5 000 M.A. McAlear
α-Erb1 1:5 000 J. d. l. Cruz
α-GAPDH 1:20 000 G. Daum
α-Has1 1:5 000 J. d. l. Cruz
α-Hrr25 1:5 000 W. Zachariae
α-Mex67 1:10 000 E. Hurt
α-Mrt4 1:1 000 E. Hurt
α-Nmd3 1:4 000 A. W. Johnson
α-Noc1 1:5 000 P. Milkereit
α-Noc2 1:5 000 P. Milkereit
α-Noc3 1:5 000 P. Milkereit
α-Nog1 1:5 000 M. Fromont-Racine
α-Nog2 1:5 000 M. Fromont-Racine
α-Nop2 1:2 000 Invitrogen
α-Nsa2 1:5 000 M. Fromont-Racine
α-Nug1 1:10 000 E. Hurt
α-Prp43 1:4 000 E. Hurt
α-Rlp24 1:5 000 M. Fromont-Racine
α-Rok1 1:5 000 K. Karbstein
α-Rpp0 1:2 000 J. Ballesta
α-Rpl16 1:40 000 S. Rospert
α-Rrp12 1:5 000 M. Dosil
α-Rsa4 1:10 000 M. Remacha
α-Sof1 1:300 E. Hurt
α-Ytm1 (Nop7) 1:5 000 J. d. l. Cruz
α-rabbit 1:15.000 Merck/Sigma-Aldrich

Validation

Commercially available antibodies were validated by the manufacturer. All antibodies are from published studies and were used previously (Pertschy et al., 2007, Kappel et al., 2012, Bassler et al., 2012, Zisser et al., 2018).

Plants

Seed stocks	not applicable
Novel plant genotypes	not applicable
Authentication	not applicable