nature portfolio

Corresponding author(s): Toshiya Endo

Last updated by author(s): Jun 26, 2025

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

Fuji LAS-3000 & LAS 4000, Amersham Imager 680, GE Thyphoon FLA 7000.
SerialEM (ver 3.7.10) was used for cryo-EM data collection.
Data collection of gel-separated proteins are described in Methods section.
For the modeling of Por1, the phenix-1.18.2 package and the MODELLER-9.2.4 package were used. Both are available from https://www.phenix-online.org/download/phenix/ and https://salilab.org/modeller/.
For the preparation of the initial structures for molecular dynamics (MD) simulations, the SCWRL4.0, which is available from http://dunbrack.fccc.edu/lab/scwrl, was used.
For all the MD simulations, the GROMACS-2023 package, which is available from http://ftp.gromacs.org/pub/gromacs/gromacs-2023.tar.gz was used.

Data analysis	Data processing for cryo-EM and single particle analyses, RELION-3.1 and CTFFIND-4 were used. Coot 0.8.9.1 was used for model building, and Phenix version 1.20.1-4487 was used for refinement for the structural determination of the PORIN complex. For the analyses of the output of MD simulations, the analytic tools included in the GROMACS-2023 package, the analytic tool package memdian available from https://github.com/Ladme/memdian, and the Python scripts for using the Martinize2 package (https://github.com/marrink-lab/vermouth-martinize) and the MDtraj-1.9.5 package (https://github.com/mdtraj/mdtraj) were used. For the HS-AFM analysis, simulated images were generated by the Biomolecular AFM viewer. Data analysis of gel-separated proteins are described in Methods section. Data were processed using ImageQuant 5.2, ImageJ2 2.3, MultiGauge 3.0 and 3.2, Adobe Photoshop and Illustrator CS4, CS5, CS6 and CC 2017, and Affinity Photo and Designer 1.9.

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 cryo-EM density maps have been deposited in the Electron Microscopy Data Bank under accession codes 61843. The atomic coordinates have been deposited in the Protein Data Bank under accession codes 9JVQ.

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

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	The sample size was determined based on previous experience with specific types of experiments. According to this the required amount of mitochondrial or cellular proteins was selected for each experiment. For key experiments, several runs were performed to determine the optimal sample size.
Data exclusions	No data were excluded from this study. All relevant data are shown.
Replication	All biochemical and cell biological experiments in this study have been replicated using the same experimental setup (amount of cells or mitochondrial proteins, buffers, detergents, affintiy matrices, etc.) with similar results. Representative results of two or more experiments are shown. Successful replications are available for all data presented.
Randomization	Random clones of the used yeast strains were selected.
Blinding	Blinding was not performed. The used yeast strains have to be validated before use in cell biological or biochemical analysis.

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

Antisera were raised in rabbits against recombinant proteins (Por1, Tom22, Tom40) from baker´s yeast Saccharomyces cerevisiae, and in mouse against peptides (HA tag). The commercial antibodies are as follows; The antisera were used in 1:250 to 1:5,000 dilutions. Commercial antibodies are as follows. Anti-Pgk1 mAb, Abcam ab113687; Anti-HA mAb, Merck H9658 and Cell Signaling Technology 3724; Anti-Porin mAb, Thermo Fisher Scientific 459500.

Anti-mouse IgG, Thermo Fisher Scientific A10524; Anti-rabbit IgG, Thermo Fisher Scientific A10523

Validation

The specificity of the antibody raised against a protein from baker´s yeast (Saccharomyces cerevisiae) and polypeptides was controlled by comparing total cell extracts or mitochondrial lysates from wild-type yeast cells and the corresponding deletion strain or strainsexpressing a tagged version of the protein of interest via SDS-PAGE and Western blotting. Absence or size shift of the signal in cellular fractions of the mutant strain confirmed the specificity of the antibody signal.

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.