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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	Sel-mitoRP reads were filtered and aligned to the sacCer 3 genome, which is described in detail in the Methods section. Cryo-EM data was collected using EPU (Thermo Fisher Scientific)
Data analysis	Cryo-EM image processing and analysis was performed using cryoSPARC (v4.3.1), RELION (v.4.0.1), CTF Find (v4.1), AlphaFold2/3, ChimeraX (v1.6), PHENIX (v1.21-5207), and Coot (v0.9.8.1). Sel-mitoRP data was processed using samtools (v1.9), bedtools (v2.27.1), cutadapt (v1.14), bowtie (v1.2.1.1), bamtools (v2.4.1), fastqc (v0.11.3), fastx (v0.0.13), igvtools (v2.3.88), star (v2.7.3a), and picard (v2.8.0). Custom scripts used to analyze and visualized the sel-mitoRP data are available at https://github.com/churchmanlab/Yeast_selective_mitoRP.

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

Raw and processed sequencing data generated in this study were deposited in the GEO database under the accession number GSE282943. Mitoribosome footprints without crosslinking (steady-state glycerol growth) are available under accession number GSE74454.
Cryo-EM densities have been deposited at the Electron Microscopy Data Bank (EMDB) under accession codes EMD-52281 (Aep1-Aep2-Atp25C, composite), EMD-52235 (Aep1-Aep2-Atp25C, consensus), EMD-52270 (Aep1-Aep2-Atp25C, TA), EMD-52252, (Aep1-Aep2-Atp25C, SSU body), EMD-52256 (Aep1-Aep2-Atp25C, SSU head), EMD-52257 (Aep1-Aep2-Atp25C, LSU), EMD-52283 (Aep3, composite), EMD-52277 (Aep3, consensus), EMD-52276 (Aep3, TA), EMD-52278 (Aep3, SSU body), EMD-52279 (Aep3, SSU head), EMD-52280 (Aep3, LSU), EMD-48387 (Aep2-FLAG affinity purified, TA), EMD-48388 (Aep2-FLAG affinity purified, SSU), EMD-48390 (Aep2-FLAG affinity purified, LSU), and EMD-48700 (Aep2-FLAG affinity purified, composite map of SSU and Aep1-Aep2-Atp25C complex). Atomic models were deposited at the Protein Data Bank (PDB) under accession codes PDB 9HLZ (Aep1-Aep2-Atp25C, composite) and PDB 9HM0 (Aep3, composite). Other models utilized in this study are PDB 5MRC (yeast mitoribosome) and PDB 8OM4 (yeast mitoribosome, SSU).

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	This is not relevant to the present study; human participants, their data, or biological material were not used.
Reporting on race, ethnicity, or other socially relevant groupings	This is not relevant to the present study; human participants, their data, or biological material were not used.
Population characteristics	This is not relevant to the present study; human participants, their data, or biological material were not used.
Recruitment	This is not relevant to the present study; human participants, their data, or biological material were not used.
Ethics oversight	This is not relevant to the present study; human participants, their data, or biological material were not used.

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 were not predetermined. At least two biological replicates were obtained for each western blot and sequencing experiment in line with other studies in this field and the data were found to be highly reproducible.
Data exclusions	No data were excluded from the study. Criteria for filtering of reads are included in the Methods section.
Replication	All experiments were performed in at least two biological replicates as is common in this field. All results were found to be highly similar and reproducible across replicates.
Randomization	Randomization is not relevant to our study. All experiments were carried out in yeast cell lines and sel-mitoRP for translational activators was compared to a control strain where the total mitoribosome population was isolated and footprints sequenced.
Blinding	Blinding was not relevant to our study. All sequencing and structure data was analyzed using unbiased computational approaches and software.

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

Anti-FLAG (Millipore-Sigma, F1804, 1:1000), Anti-HA (Invitrogen, 26183, 1:1000), Anti-Myc (Millipore-Sigma, 05-724, 1:500), Anti-Mrp20 (Gruschke et al, 2010; PMID: 20404317), Anti-Mrpl4 (Gruschke et al, 2010; PMID: 20404317), Anti-Mrpl36 (Prestele et al, 2009; PMID: 19339279), Anti-Cox2 (Neupert lab, Munich, Germany), Anti-Tom70 (Rapaport lab, Tübingen, Germany), Anti-Tuf1 (This study), Anti-ALFA (NanoTag Biotechnologies GmbH, Göttingen, Germany. ID:N1580). Secondary antibodies used include IRDye 800CW goat anti-mouse secondary (LICORbio, 925-32210, 1:5000) and goat anti-rabbit IgG H L-HRP conjugate (BioRad, 1706515).

Validation

All commercially available antibodies have been validated by the manufacturer with validation data available on the respective manufacturer website. Anti-Mrp20 and Anti-Mrpl4 were previously published in Gruschke et al, 2010; PMID: 20404317 and were validated using deletion mutants. Anti-Mrpl36 was published in Prestele et al, 2009; PMID: 19339279 and was validated with deletion mutants. Anti-Cox2 was provided by the Neupert lab in Munich Germany and was validated using deletion mutants. Anti-Tom70 was a gift from the Rapaport lab in Tübingen, Germany and was validated using deletion mutants. Anti-Tuf1 was generated for this study and was obtained by immunizing rabbits with purified mature Tuf1. The anti-Tuf1 antibody was validated with deletion mutants of Tuf1 as can be seen in Extended Data Figure 5b.

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.