SUPPLEMENTAL INFORMATION

From powerhouse to processing plant: conserved roles of mitochondrial outer membrane proteins in tRNA splicing

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Supplemental Materials and Methods

Yeast strains

*S. cerevisiae* yeast strains: All *S. cerevisiae* strains were grown at 23°C unless otherwise noted. *S. cerevisiae* strains were inoculated in YEPD rich media or defined media mixing appropriate nutrients from frozen glycerol stocks and grown until saturation, and then re-inoculated in YEPD media. Early log phase cells were collected for all experiments. *los1Δ, tom70Δ, sam37Δ*, and *mip1Δ* strains were obtained from the *S. cerevisiae* MATα deletion collection (*WT BY4741* background: MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, Dharmacon, Lafayette, CO). The temperature-sensitive strain *tom22-ts* was a gift from Dr. P. Hieter (University of British Columbia, Vancouver, Canada). For endogenous C-terminal GFP-tagging of Sen15 in *WT, tom70Δ*, and *sam37Δ* strains, the knock-in cassette was cloned from pFA6a-GFP (S65T)-His3MX6, as described (Longtine et al. 1998). For endogenous N-terminal GFP-tagging of Sen2, Sen34, and Sen54 in *WT, tom70Δ*, and *sam37Δ* strains, the knock-in cassette was from plasmids digested with corresponding restriction enzymes from New England Biolabs (Ipswich, MA) and purified with QIAquick Gel Extraction Kit (28706, QIAGEN, Hilden, Germany). These plasmids contain 1kb 5’ untranslated region (UTR) of SEN subunit coding sequence, GFP without a stop codon, the SEN subunit coding sequence with stop codon, ADH1 terminator, His3MX6, and a 60-80bp recombination region sequence. The yeast transformation method was as described (Gietz and Schiestl 2008). To create mutants lacking the mitochondrial genome, the *WT BY4741* strain was treated with ethidium bromide as previously described (Ferguson and von Borstel 1992). The *Tet-Off-SEN34* strain was purchased from the Yeast Tet-Promoters Hughes Collection (yTHC) (Dharmacon, Lafayette, CO).

*S. pombe* yeast strains: All *S. pombe* strains were grown at 30°C. *S. pombe* strains were inoculated in YE5S from a frozen glycerol stock and grown until saturation, and then re-inoculated in YE5S media. Early log phase cells were collected for experiments. *WT, tom70Δ*, and *los1Δ* strains were gifts from Dr. J.-Q. Wu (The Ohio State University, Columbus, OH). The *WT* strain background is *ade6-M210 ura4-D18 leu1-32*. Gene deletions were constructed by employing kanMX4 module and verified as
described (Kim et al. 2010). The C-terminal mECitrine knock-in was carried out as described (Bähler et al. 1998) using Q5® Hot Start High-Fidelity DNA Polymerase (M0493S, New England Biolabs, Ipswich, MA) and plasmid pFA6a-mECitrine-kanMX6 (JQW228, gift from Dr. J.-Q. Wu) (Ye et al. 2012; Lee and Wu 2012) (see Table S3 and S4 for detailed plasmids and primers information). Transformation of S. pombe was conducted as described (Moreno et al. 1991). Creation of S. pombe tom70Δ strains with endogenous SEN subunits genes C-terminally tagged with mECitrine was conducted as described (Bähler et al. 1998). The knock-out cassette was cloned from plasmid pFA6a-hphMX6 (JQW272, gift from Dr. J.-Q. Wu) (Wang et al. 2014).

Plasmids construction and primers
The SEN34 plasmid was from the yeast genomic tiling collection (Dharmacon, Lafayette, CO). DNA fragments with 1kb 5’ UTR of the coding sequence, the GAL1 promoter, SEN subunits coding sequences with or without the stop codon, GFP with or without the stop codon, and ADH1 terminator followed by His3MX6 markers were cloned by Q5® Hot Start High-Fidelity DNA Polymerase (M0493S, New England Biolabs, Ipswich, MA) with restriction sites synthesized in the primers (see Table S3 and S4 for details of plasmids and primers). PCR products of the above DNA fragments were purified by QIAquick Gel Extraction Kit (28706, QIAGEN, Hilden, Germany) and digested with corresponding restriction enzymes from New England Biolabs (Ipswich, MA) and purified again with QIAquick Gel Extraction Kit (28706, QIAGEN, Hilden, Germany). Purified DNA fragments and plasmid backbone were ligated in one step with T4 DNA Ligase (M0202S, New England Biolabs, Ipswich, MA). The ligated plasmids were transformed into DH5α E.coli competent cells employing a Mix & Go! E. coli Transformation Kit and Buffer Set (T3001, Zymo Research, Irvine, CA).

Small RNA isolation
S. cerevisiae cultures were grown at 23°C (unless otherwise indicated) to early log phase. S. pombe cultures were grown at 30°C to early log phase. The temperature-sensitive mutants were shifted to non-permissive temperature (37°C) for 2 h before RNA extraction. Small RNAs (tRNAs, 5S rRNA,
5.8S rRNA, and other small RNAs) were extracted from the cells by an optimized phenol extraction method as follows. 15 ml yeast culture was collected at 4°C by centrifugation (Jouan centrifuge CR412, Jouan SA, France). The supernatants were evacuated and the cell pellets were resuspended in 0.8 ml cold TSE (0.01 M Tris, 0.01 M EDTA, 0.1 M NaCl, pH 7.5) and 1.0 ml TSE-buffered phenol. The suspensions were transferred to RNase-free microcentrifuge tubes and vortexed with vortex mixer (Fisher Scientific, S02216109) for 30 s and then incubated in a 55°C water bath for 3 min. The vortexing and incubation steps were repeated 5 additional times. The tubes were then incubated on ice for 10 min, followed by centrifugation at 21,130Xg for 10 min at 4°C. After centrifugation, the aqueous phases were transferred to new RNase-free microcentrifuge tubes containing 1.4 ml cold 100% EtOH. The samples were incubated at -80°C for at least 30 min. Small RNAs were pelleted from the sample by centrifugation at 21,130Xg for 20 min at 4°C. The supernatant was removed and the RNA pellets were washed with cold 70% ethanol. Then, the RNA pellets were air-dried and dissolved in 30 µl RNase-free water. RNA concentrations were measured by spectrophotometry at 260 nm (A260) and the RNA qualities were assessed by analyzing the A260:A280 ratios.

_Northern analysis and quantification_

5µg of small RNA samples were loaded on a 10% poly-acrylamide neutral 8 M urea gel and electrophoretically separated for 20 h at 120 V at 4°C. 10% acrylamide gels were stained with ethidium bromide and imaged using Alphalmager EP (Alpha Innotech, San Leandro, CA). RNAs were then transferred onto nylon membranes (RPN303B, GE Healthcare, Chicago, IL), first for 15 min at 15 V, and then for 2 h at 0.6 A. RNAs were fixed to the membranes with a Spectrolinker XL-100 UV crosslinker with optimal crosslinking setting with an energy dosage of 240 mJ/cm². After crosslinking, the membranes were placed in hybridization tubes (1324597j, Wheaton, Millville, NJ). The membranes were incubated at 37°C for 30 min with pre-hybridization buffer [5X saline-sodium citrate (SSC), 0.1% w/v N-lauroylsarcosine, 0.02% w/v sodium dodecyl sulphate (SDS), 1% w/v Roche Blocking Reagent (11096176001, Roche, Indianapolis, IN)] with rotation. The prehybridization buffer was then replaced by hybridization buffer (pre-hybridization buffer containing 1 nM DIG-labelled
probe) (see Table S2 for probe sequences) and the membranes were incubated at 37°C overnight with rotation. After hybridization, the membranes were washed four times with 15 ml 2X SSC containing 0.1% SDS for 10 min at 37°C and then washing buffer (0.1M maleic acid, 0.15M NaCl, pH7.5, 0.3% Tween-20) for 2 min at room temperature (RT). The membranes were incubated in blocking buffer (1% w/v Roche Blocking Reagent, 0.1M maleic acid, 0.15M NaCl, pH7.5) for 30 min at RT. After blocking, the membranes were incubated in antibody solution [1:20 000 dilution of anti-DIG antibody conjugated with alkaline phosphatase (11093274910, Roche, Indianapolis, IN) in blocking buffer] for 30 min at RT. The membranes were washed for 15 min twice with washing buffer. The membranes were incubated in detection buffer (0.1 M Tris–HCl, 0.1 M NaCl, pH9.5) for 3 min before addition of the substrate solution for alkaline phosphatase [0.125 mM CDP-Star, (11685627001, Roche, Indianapolis, IN) in detection buffer] and then incubated for 10 min at RT. The membranes were imaged using UVP ChemStudio (Analytic Jena, Jena, Germany). Signal intensities of intron-containing tRNAs (I) and primary tRNA transcript (P) were measured by Image Studio (LI-COR, Lincoln, NE) and the ratios of I/P were calculated and normalized to the appropriate controls.

FISH and quantification

*S. cerevisiae* cells were grown in 15 mL media overnight at 23 °C to early log phase. Cells were prefixed in the culture by the addition of 0.1 volume of 37% formaldehyde. After 15 min, 10 ml cells were harvested by centrifugation and resuspended in 6 ml Solution A (4% freshly prepared paraformaldehyde, 0.1 M potassium phosphate buffer (pH 6.5), and 5 mM MgCl2). After 3 h, cells were washed twice with solution B (1.2 M sorbitol and 0.1 M potassium phosphate buffer, pH 6.5) and resuspended in 0.5 ml solution B containing 0.05%(v/v) β-mercaptoethanol and 0.3 mg/ml freshly prepared 20T Zymolyase (MP Biomedicals, Santa Ana, CA). The cells can be stored at 4°C for a few days at this stage. Spheroplasting was conducted at 37°C for about 20 min. When the percentage of spheroplasts reached about 80-90%, cells were collected by centrifugation at 4°C for 3 min at 1,800Xg. Spheroplasts appear black when viewed by light microscopy. Spheroplasts were collected by centrifugation at 4°C, washed once in solution B and resuspended in 0.6 ml solution B. The volume
to resuspend cells was adjusted depending on cell density. Teflon-faced slides (Cel-Line/Erie Scientific, Portsmouth, NH) were pretreated with 0.1% (w/v) poly-L-lysine-containing solution (Sigma Chemical, St. Louis, MO) for 1 min and washed once with water. Cells were adhered to the wells of the slides by placing 20 µl resuspended cells in each well for 5 min. Non-adhered cells were removed by aspiration. The cells were treated with 70, 90, and 100% ethanol successively for a duration of 5 min each. The cells were then incubated in prehybridization buffer containing 10% dextran sulfate, 0.2% BSA (acetylated), 2X SSC (1X SSC is 0.15 M NaCl and 0.015 M Na-citrate), 1X Denhardt’s solution, 250 µg *Escherichia coli* tRNA/ml, RNasin (Ambion, Grand Island, NY) at a concentration of 1 U/µl, and 500 µg denatured salmon sperm DNA/ml (Invitrogen, Grand Island, NY) for 2 h at 39°C in a humid chamber. The hybridization buffer contained the same composition plus 0.3 pmol/µl digoxigenin-labeled probes (see Table S2 for probe sequences). All probes were labeled at their 3’ end using DIG Oligonucleotide Tailing Kit, 2nd Generation (Roche, Indianapolis, IN) according to the manufacturer’s instructions. Hybridization was carried out at 43°C overnight.

After overnight incubation, the cells were washed once with 2X SSC at 50°C for tRNA probes. The cells were then washed two times for 10 min each with 1X SSC at room temperature. The cells were briefly washed with 4X SSC containing 1% Triton X-100 and then blocked for 2.5 h using 1% BSA containing 4X SSC at room temperature. Anti-Digoxigenin-Rhodamine Fab fragment (Roche, Indianapolis, IN) was diluted according to the manufacturer’s recommendation (1:30) in solution containing 1% BSA and 4X SSC, and the cells were incubated with the diluted antibody for 2.5 h at room temperature. The cells were washed twice with 4X SSC followed by two more washes with 4X SSC containing 1% Triton X-100, each wash lasting for 10 min. After two more rapid washes with 4X SSC, DNA in cell nuclei were counterstained with 0.1 µg/ml 4’,6-diamidino-2-phenylindole dihydrochloride (DAPI) for 5 min. After two rapid washes with water, the slides were mounted under 90% glycerol and 1X PBS containing 1 mg/ml 4-phenylenediamine and covered with cover slips, sealed around the edges with nail polish. Slides can be stored at -20°C for 1 week.
tRNA signals were visualized using a 561nm (red) argon ion laser, and DNA stained by DAPI was visualized using a 405nm (blue) laser and a 60X/1.4 NA objective lens. Images were captured using Volocity software and then assembled using National Institutes of Health ImageJ and Adobe Photoshop CS6 (Adobe Systems, San Jose, CA). For Fig. 2, the ratios of cytoplasmic and nuclear FISH signals were calculated from three biological repeats. ImageJ was used to measure signal intensity from FISH images using rectangle selections of the same size in both the nucleus and cytoplasm. The cytoplasmic and nuclear FISH signals were measured from 10 randomly selected cells with background signals subtracted. The background signals were measured from 10 randomly selected areas in the background. The average signal intensities were used for calculating the ratios. For Fig. S3, the Intensity Ratio Nuclei Cytoplasm Tool (http://dev.mri.cnrs.fr/projects/imagej-macros/wiki/Intensity_Ratio_Nuclei_Cytoplasm_Tool) was used to calculate the ratio of cytoplasmic/nuclear FISH signals from all the cells in the field employing default settings.

Mitochondria isolation and protein extraction

Whole cell lysate (W), Mitochondria-enriched (M), and Cytoplasm excluding mitochondria-enriched (C) fractions from S. cerevisiae were isolated by Yeast Mitochondria Isolation Kits (MITOISO3 from Sigma-Aldrich, St. Louis, MO. or K259 from BioVision, Milpitas, CA). Proteins were extracted employing CellLytic M (C2978, Sigma-Aldrich, St. Louis, MO) supplemented with Protease Inhibitor Cocktail (1:100 [v/v]) (P8215, Sigma-Aldrich, St. Louis, MO). Total protein was isolated from S. pombe as follows: 15ml cell cultures grown at 30°C (OD600 0.35-0.45) were collected and washed once with cold water; the cells were disrupted by vortexing 3 times with the presence of an equal volume of acid-washed glass beads and CellLytic M buffer supplemented with Protease Inhibitor Cocktail. The cell extracts were cleared by centrifugation at ~12,600Xg for 5 min at 4°C. Protein concentrations were measured by Pierce™ BCA Protein Assay Kit (23225, Thermo Fisher Scientific, Waltham, MA)

Western analysis and quantification
Protein samples were analyzed employing NuPAGE™ 10% Bis-Tris Protein Gels (NP0301BOX, Thermo Fisher Scientific, Waltham, MA) and then the fractionated proteins were electrophoretically transferred to nitrocellulose membranes (162-0112, Bio-Rad, Hercules, CA). Total proteins were stained by employing the REVERT™ Total Protein Stain Kit (926-11010, LI-COR, Lincoln, NE). After blocking with 5% non-fat milk for 1 h at room temperature (RT), the blot was incubated with primary antibody overnight at 4°C, followed by incubation with the secondary antibody for 1 h at RT (see Table S5 for detailed antibody information). Total protein staining and western blots were imaged using the Odyssey® CLx Imaging System (LI-COR, Lincoln, NE). The signal intensities were measured by software Image Studio (LI-COR, Lincoln, NE).

**Microscopy**

*S. cerevisiae* mitochondrial DNA (mtDNA) was imaged in live cell after staining with DAPI (2.5 µg/ml for 30 min). *S. pombe* mitochondria were stained with 1 µM MitoView Blue (70052, Biotium, Fremont, CA) for 30 min at 30°C. Live cell and FISH images were acquired using 60X/1.4 or 100X/1.4 numerical aperture Plan-Apo objective lens (Nikon, Melville, NY) on a spinning-disk confocal system (UltraVIEW ERS; Perkin Elmer, Waltham, MA) with an ORCA-AG camera (Hamamatsu, Bridgewater, NJ) with a Nikon Eclipse TE2000-U microscope, or on a spinning-disk confocal system (UltraVIEW Vox CSUX1 system; Perkin Elmer, Waltham, MA) with a back-thinned, electron-multiplying charge-coupled device camera (C9100-13; Hamamatsu, Bridgewater, NJ) with a Nikon Ti-E microscope.

**Statistical analysis**

The ratios from FISH, northern analysis, and western analysis were reported as the mean of at least three experimental replicates (n≥3), along with standard deviation (SD). Significance was determined using paired, two-tailed t tests (ns: not significant, P > 0.05; *: P ≤ 0.05; **: P ≤ 0.01; ***: P ≤ 0.001; ****: P ≤ 0.0001). GraphPad Prism 7 was used for statistical analyses and graphing.
Supplemental References

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Supplemental Figure Legends

Figure S1. Mutation of S. cerevisiae TOM70, SAM37, or TOM22 causes accumulation of intron-containing tRNAs.
(A) Northern analysis of tRNA_{UAU}^{ile} (Probe JW0041; see Table S2) from tom22-ts cells. (B) Statistical analysis of I/P ratios of (A) from three biological repeats comparing RNA samples from tom22-ts cells growing in 23°C and 37°C (*: P ≤ 0.05). (C, D) Northern analysis of tRNA_{GUA}^{Tyr} (Probe SRIM15; see Table S2) in WT, los1Δ, tom70Δ, sam37Δ and tom22-ts cells.

ts: temperature-sensitive mutant; P: Primary tRNA transcript; I: End-processed intron-containing tRNA; 5S: 5S rRNA employed as loading control, stained with ethidium bromide; *: unidentified RNA species; I/P: Signal intensity of I/P, normalized to WT for deletion mutants, or normalized to 23°C for ts mutants; 23°C: Permissive temperature. 37°C: Non-permissive temperature for 2 h.

Figure S2. Quantitative analysis of FISH images with randomly selected cells.

FISH analysis of the subcellular localization of tRNA_{UAU}^{ile} using probe SRIM03 in WT, los1Δ, tom70Δ and sam37Δ cells from three biological repeats (#1, 2, 3), as described in Figure 2. The average signal intensities from the cytoplasm and nucleus were obtained from 10 randomly selected cells (indicated with red arrows), and statistical analysis was performed (Figure 2).

Figure S3. Quantitative analysis of FISH images using the Intensity Ratio Nuclei Cytoplasm Tool.

Original and processed images for FISH and DAPI staining from (A) WT, (B) los1Δ, (C) tom70Δ and (D) sam37Δ, as described in Figure 2. In the Processed Image for FISH, the blue area shows the background that is subtracted; red circles show the boundary between the nucleus and the cytoplasm, based on the DAPI Processed Image. Total cytoplasmic signals are measured from the area outside of red circles and excluding the blue area. Total nuclear signals are measured from the area inside red circles. (E) The average signal intensities from the cytoplasm and nucleus were obtained from all the cells in the field from (A) WT, (B) los1Δ, (C) tom70Δ and (D) sam37Δ using the Intensity Ratio Nuclei Cytoplasm Tool (See Supplemental Methods). The ratio between signal intensity of cytoplasm vs. nucleus was calculated and normalized to WT (Cyto/Nuc).
Figure S4. Ethidium bromide-treated WT cells and mip1Δ cells do not possess mitochondrial DNA (mtDNA) and are defective in respiratory metabolism.

(A) WT cells, ethidium bromide-treated WT cells and mip1Δ cells were subjected to fluorescence microscopy. Live cell DAPI staining was used to stain nuclear and mitochondrial DNA. Scale bar: 4µm. (B) Equal amounts of serially diluted WT cells, ethidium bromide-treated WT cells, and mip1Δ cells were spotted onto YEPD solid media containing glucose as the carbon source. After incubation at 23°C for 3 days, the master plate was replica plated onto YEP Gly media with non-fermentable glycerol as the carbon source, and then onto a YEPD plate; the plates were incubated at 23°C for 7 days and 4 days, respectively.

Figure S5. S. cerevisiae SEN34 tagged with GFP at the N-terminus, but not the C-terminus, maintains full activity.

(A) The Tet-Off-SEN34 strain was transformed with vectors or constructs containing the C-terminal GFP-tagged SEN34, as indicated. Equal amounts of serially diluted cells were spotted onto a plate without doxycycline. After incubation at 23°C for 3 days, the plate was replica plated to solid media with doxycycline (+DOX) first, and then to media without doxycycline (-DOX). The plates were incubated at 23°C for 4 days. Note that single copy C-terminal GFP-tagged SEN34 is defective in complementing SEN34 depletion. However, C-terminal GFP-tagged SEN34 maintains some activity because it is able to rescue the lethal phenotype of tetracycline-induced Tet-Off-SEN34 strain when over-expressed by a multi-copy plasmid. (B) Same procedures as (A) employing N-terminally GFP-tagged SEN34. Note that the single copy N-terminal GFP-tagged SEN34 complements SEN34 depletion.

Figure S6. GFP-SEN2, SEN15-GFP, GFP-SEN34, and GFP-SEN54 strains have efficient tRNA splicing, similar to the WT strain.

(A) Northern analysis for tRNA^{ile}_{UAU} (Probe JW0041) in strains with endogenously GFP-tagged SEN subunits. GFP-SEN2, GFP-SEN34, and GFP-SEN54 strains are tagged at the N-termini. SEN15-GFP
strain is tagged at the C-terminus. (B) Statistical analysis of I/P ratios of (A) from three biological repeats compared to WT (ns: P > 0.05; **: P ≤ 0.01; ***: P ≤ 0.001; ****: P ≤ 0.0001).

P: Primary tRNA transcript; I: End-processed intron-containing tRNA; 5S: 5S rRNA employed as loading control, stained with ethidium bromide; I/P: Signal intensity of I/P, normalized to WT.

Figure S7. Subcellular fractionation of endogenously tagged S. cerevisiae SEN subunits and reduced levels of MTCO1 in tom70Δ and sam37Δ cells. Panels: (A), (B), (C), and (D), are subcellular fractionations for: GFP-Sen2, Sen15-GFP, GFP-Sen34, and GFP-Sen54, respectively (as described in Figure 4). Whole cell lysate (W), Mitochondria-enriched (M) and Cytoplasm excluding mitochondria-enriched (C) fractions were isolated; extracted proteins were analyzed by western blots. Anti-GFP detects GFP-tagged SEN subunits. Anti-MTCO1, and Anti-Pgk1 were employed to assess mitochondrial and cytoplasmic proteins, respectively. Pgk1 was used as the loading control for Whole cell lysate (W) and Cytoplasm excluding mitochondria-enriched (C) fractions. MTCO1 was used as loading control for Mitochondria-enriched (M) fraction. Signal intensities were quantified from three biological repeats. The mean and standard deviation (SD) of GFP-tagged SEN subunits to Pgk1 or MTCO1 are plotted (ns: not significant, P > 0.05; *: P ≤ 0.05). Note: for the two closely migrating bands for GFP-Sen2, only the upper band was associated with mitochondria-enriched fraction and used for quantification.

(E) MTCO1 levels are reduced ~30% in tom70Δ and sam37Δ cells, compared to WT cells. Signal intensities of MTCO1 and total protein in Mitochondria-enriched (M) fraction were quantified from 13 samples. The mean and standard deviation (SD) of MTCO1 to total protein (MTCO1/Total) are plotted; paired t test was employed for statistical analysis (**: P ≤ 0.01; ***: P ≤ 0.001).

Figure S8. Sen54, but not Sen2, Sen15, or Sen34, localizes to mitochondria when over-expressed in S. cerevisiae.

Each strain containing endogenously GFP-tagged SEN subunits was transformed with a plasmid possessing the same GFP-tagged SEN subunit regulated by the GAL1 promoter. Cells were grown in
media with raffinose (Raff) as the carbon source and then expression of the GFP-tagged SEN subunits were induced by addition of 2% galactose for 1 h (Gal 1h). Raff and Gal 1h panels have the same image settings. Lower intensity images (Lower intensity) are shown for easier viewing of the SEN subunits. Scale bar: 4µm.

Figure S9. GFP antibody is specific to mECitrine-tagged SEN subunits in S. pombe and can detect mECitrine-tagged SEN subunits in WT and tom70Δ S. pombe cells. Proteins were extracted from S. pombe cells with or without endogenously mECitrine-tagged SEN subunits in WT and tom70Δ background, as indicated, and then subjected to western analysis. Anti-GFP was used to detect mECitrine-tagged SEN subunits. Total protein stain shows equal loading of the protein samples.

Figure S10. Model for the conserved role of mitochondrial outer membrane proteins in the localization of SEN subunits of S. cerevisiae and S. pombe.

(A) In WT S. cerevisiae cells, the SEN subunits efficiently localize to the mitochondrial surface to form the SEN complex. The catalytic SEN subunits are depicted as squares and the other subunits are depicted as ovals. (B) Upon mutation of TOM70 or SAM37 in S. cerevisiae (as indicated by crosses), SEN subunits are mislocalized from mitochondrial surface. (C) In WT S. pombe, the SEN subunits efficiently localize to the mitochondrial surface. The subcellular localization of Sen15 was not determined, as indicated by the question mark. (D) Upon mutation of TOM70 in S. pombe (indicated by the cross), Sen2 and Sen54 are mislocalized from mitochondrial surface and their protein levels are reduced (depicted as dotted pattern). The subcellular localizations of Sen15 and Sen34 in tom70Δ cells were not determined, as indicated by the question marks.
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E

Cyto/Nuc signal from >100 cells

|         | WT | los1Δ | tom70Δ | sam37Δ |
|---------|----|-------|--------|--------|
| Cyto/Nuc| 1.0| 0.5   | 1.5    | 1.2    |
### A

**Strain background:** Tet-Off-SEN34 with plasmid

|                | Number of cells | Number of cells |
|----------------|-----------------|-----------------|
| Single copy vector | ![Image]       |                 |
| Multiple copy vector | ![Image]       |                 |
| Single copy SEN34-GFP | ![Image]       |                 |
| Single copy SEN34-GFP (2X cell amount) | ![Image]       |                 |
| Multiple copy SEN34-GFP | ![Image]       |                 |
| Multiple copy SEN34-GFP (2X cell amount) | ![Image]       |                 |
| Untagged SEN34 #1    | ![Image]       |                 |
| Untagged SEN34 #2    | ![Image]       |                 |

### B

**Strain background:** Tet-Off-SEN34 with plasmid

|                | Number of cells | Number of cells |
|----------------|-----------------|-----------------|
| No cell control | ![Image]       |                 |
| Single copy SEN34-GFP | ![Image]       |                 |
| Multiple copy SEN34-GFP | ![Image]       |                 |
| Single copy GFP-SEN34 #1 | ![Image]       |                 |
| Single copy GFP-SEN34 #2 | ![Image]       |                 |
| Single copy GFP-SEN34 #3 | ![Image]       |                 |
| Multiple copy vector | ![Image]       |                 |
| Untagged SEN34 #1    | ![Image]       |                 |

-DOX

+DOX
Wan and Hopper_Fig. S6
Wan and Hopper_Fig. S9
Table S1: Generation Time (Hours) of GFP-tagged or untagged SEN subunits strains

| Strains      | Untagged | GFP-SEN2 | SEN15-GFP | GFP-SEN34 | GFP-SEN54 |
|--------------|----------|----------|-----------|-----------|-----------|
| WT           | 2.6      | 2.3      | 2.7       | 2.7       | 2.4       |
| *tom70Δ*     | 2.4      | 2.2      | 2.5       | 2.7       | 2.6       |
| *sam37Δ*     | 2.8      | 2.6      | 2.8       | 2.8       | 2.9       |
| Probe    | Complementary to                      | Sequences (5' – 3')                                                                 |
|----------|---------------------------------------|-------------------------------------------------------------------------------------|
| JW0041   | *S. cerevisiae* tRNA<sup>UAU</sup> 5' exon | TATAAGCAGAAGCTCTAACCACCTGAGCTACAGAGC                                              |
| SRIM15   | *S. cerevisiae* tRNA<sup>Tyr</sup> 5' exon and 3' exon | GCGAGTCGAACGCCCCGATCTCAAGATTGACGTCTTGCGCTTAAACAACCTGCTACC                           |
| SRIM03   | *S. cerevisiae* tRNA<sup>UAU</sup> intron | CGTTGCTTTGAAGCGCTTTGAAAGGTCTTTGGCAGAAACCTTGGATTTGCTAT                              |
| YW0169   | *S. pombe* tRNA<sup>Leu</sup> 5' exon  | CTTTGAGTGCTGCGCCATAGACCCTGCGGCCCAA                                                |
| YW0170   | *S. pombe* tRNA<sup>Pro</sup> 5' exon   | CCCGAAGAGGTATGTTAACCACCTACATATGGCC                                               |
| YW0202   | *S. pombe* tRNA<sup>Leu</sup> intron   | GACTATCGTCCAAAGTATTA                                                               |
| YW0203   | *S. pombe* tRNA<sup>Pro</sup> intron   | AACTCAGCATAAGTCGTTTGCTTATGGA                                                      |
| Plasmids | Construction | Purpose | Source |
|----------|--------------|---------|--------|
| YW131 | pFA6a-GFP (S65T)-His3MX6 | Endogenous C-terminal GFP-tagging of SEN15 | Gift from John Pringle (Addgene plasmid # 41598) |
| YW174 | pFA6a-His3MX6-P_{GAL1}-GFP | Cloning of GAL1 promoter | Gift from John Pringle (Addgene plasmid # 41616) |
| YW149 | pRS415-Xmal-1kb 5' UTR of SEN34-SEN34-HindIII-GFP-Xhol | Single copy SEN34-GFP | This study |
| YW150 | pRS425-Xmal-1kb 5' UTR of SEN34-SEN34-HindIII-GFP-Xhol | Multiple copy SEN34-GFP | This study |
| YW151 | Yeast tilling plasmid collection 1b3, contains intact SEN34 gene | Untagged SEN34 | This study |
| YW178 | pRS415-Xbal-1kb 5' UTR of SEN34-BamHI-GFP-Xmal-SEN34-HindIII | Single copy GFP SEN34 | This study |
| YW204 | pRS415-Xbal-1kb 5' UTR of SEN34-BamHI-GFP-Xmal-SEN34-HindIII- T_{ADH1} His3MX6-SEN34 recombine region-Xhol | Endogenous N-terminal GFP-tagging of SEN34 | This study |
| YW208 | pRS415-Eagl-1kb 5' UTR of SEN54-BamHI-GFP-Xmal-SEN54-HindIII- T_{ADH1} His3MX6-SEN54 recombine region-Xhol | Endogenous N-terminal GFP-tagging of SEN54 | This study |
| YW224 | pRS415-Eagl-1kb 5' UTR of SEN2-BamHI-GFP-Xmal-SEN2-HindIII- T_{ADH1} His3MX6-SEN2 recombine region-Xhol | Endogenous N-terminal GFP-tagging of SEN2 | This study |
| YW215 | pRS415-Eagl- P_{GAL1}-BamHI-SEN15-Xmal-GFP-HindIII- T_{ADH1} His3MX6-50bp recombine-Xhol | Over-expression of Sen15-GFP; GAL1 promoter | This study |
| YW217 | pRS415-Eagl- P_{GAL1}-BamHI-GFP-Xmal-SEN34-HindIII- T_{ADH1} His3MX6-50bp recombine-Xhol | Over-expression of GFP-Sen34; GAL1 promoter | This study |
| YW219 | pRS415-Eagl- P_{GAL1}-BamHI-GFP-Xmal-SEN54-HindIII- T_{ADH1} His3MX6-50bp recombine-Xhol | Over-expression of GFP-Sen54; GAL1 promoter | This study |
| YW230 | pRS415-Eagl- P_{GAL1}-BamHI-GFP-Xmal-SEN2-HindIII- T_{ADH1} His3MX6-50bp recombine-Xhol | Over-expression of GFP-Sen2; GAL1 promoter | This study |
| JQW228 | pFA6a-mECitrine-kanMX6. Amp\textsuperscript{R} for E.coli | Endogenous C-terminal mECitrine-tagging of SEN subunits | Gift from Dr. Jian-Qiu Wu |
| JQW272 | pFA6a-hphMX6. Kan\textsuperscript{R} for E. coli | Deletion of TOM70 in S. pombe | Gift from Dr. Jian-Qiu Wu |
Table S4: Primers list

| Primers                  | Sequence (5’ – 3’)                                      |
|--------------------------|--------------------------------------------------------|
| YW0077 XbaI 1kb 5’ UTR SEN34 F | AAAAAAAATCTAGAATCACCTCTTTGATTGTGC                      |
| YW0078 1kb 5’ UTR SEN34 BamHI R | AAAAAAAAGGATCCAAAAACCTCCTCGCCTTT                       |
| YW0079 BamHI-GFP without stop codon F | AAAAAAAAAAGGATCCATGAGTAAAGGAGAGAAGCTTTC                |
| YW0080 GFP without stop codon - Xmal R | AAAAAAAAACCCGGGTTTGTATAGTTCCATCCATGCC                  |
| YW0081 Xmal SEN34 CDS with stop codon F | AAAAAAAACCCGGGATGCAACCAGCTAGTATTTG                    |
| YW0082 SEN34 CDS with stop codon HindIII R | AAAAAAAACGGCTTTACCAACATCCAGC                            |
| YW0089 F recombinant SEN15 | TTTATTACTTCTGTTTATAAGGAGTGACAAAAACCGAAA                |
| YW0090 R recombinant SEN15 | TAAAAATAACAAAAATATGCAAGAGGTCTTAAAAAGGCTTCTTTAACC       |
| YW0105 F HindIII F3       | AAAAAAAAGCCTTTGAGGGGCGCCACTTTCTAAA                     |
| YW0106 R recombinant SEN34 With Xhol | AAAAAAAAACCTCGAGTCAATGGCAATTTAAAGTTGATACAGGGTTTAAACC |
| YW0108 F XbaI 1k 5’ UTR SEN54 | AAAAAATCTAGAATGATGTAAGAATGACTTGT                      |
| YW0109 R BamHI 1k 5’ UTR SEN54 | AAAAAAGGATCCTTTAAATGGCGAGGAAACAGA                     |
| YW0110 F BamHI GFP without stop codon | AAAAAAGGATCCACAAAAATGAGTAAGAGAAGAAGACTTTC             |
| YW0111 R Xmal GFP without stop codon | AAAAAACCCGGGTTTGTATAGTTCCATCATGCGA                    |
| YW0112 F Xmal SEN54 CDS with stop codon | AAAAAACCCGGGATGCAATTTCGTGGGAAAG                      |
| YW0113 R HindIII SEN54 CDS with stop codon | AAAAAAAGGCTTTAATGCTTTCCCATCTTTG                     |
| YW0114 F XbaI 1kb 5’ UTR SEN54 | AAAAAATCTAGAATGATGTAAGAATGACTTGT                      |
| YW0115 R HindIII GFP      | AAAAAAAAGGTTCTATTTTGTATAGTTCCACCATGC                  |
| YW0116 F XbaI 1kb 5’ UTR SEN54 | AAAAAATCTAGAATGATGTAAGAATGACTTGT                      |
| YW0117 R BamHI SEN54 without stop codon | AAAAAAGGATCCATGCTTCTTCCATCATCTTTG                    |
| YW0118 F BamHI GFP        | AAAAAAGGATCCAAAAAGGAGAAGACTTTTCTACT                  |
| YW0119 R HindIII GFP with stop codon | AAAAAAAAGGCTTTCTATTTGTATAGTTCCATCATGCG               |
| YW0125 R SEN54 recombination Xhol | AAAAAAAAACCTCGAGGAGAAGCCAGTTTTGCAAGGATA               |
| YW0126 R SEN54 recombination with Xhol and R1 | AAAAAAAAACCTCGAGGAGAAGCCAGTTTTGCAAGGATA |
| YW0129 F Eagl 1kb 5’ UTR SEN2 | AAAAAACGGCCGGCATGAGCCAAGTGACTACA                     |
| YW0130 R BamHI 1kb 5’ UTR SEN2 | AAAAAAGGATCCGCTTCTTGTAGTTCCATCCTTC                    |
| YW0131 F BamHI GFP without stop codon | AAAAAAGGATCCCAAAAAATGAGTAAGAGAAGAAGACTTTC             |
| YW0132 R Xmal GFP without stop codon | AAAAAACCCGGGTTTGTATAGTTCCATCATGCC                    |
| YW0133 F Xmal SEN2 CDS with stop codon | AAAAAACCCGGGATGCTCAAGGGAGGCTA                           |
| YW0134 R HindIII SEN2 CDS with stop codon | AAAAAAAAGCTTCTAGTCTCTATTTTCTCCGGG |
| YW0135 F HindIII F3 | AAAAAAAAAAAAAAGCTTGTAGGCGCGCCTTTCTCTAAA |
| YW0136 R SEN2 recombination with XhoI and R1 | AAAAAAAAAACTCGAGTATAAAAAATACATAACATACATACA TATATACATACGAGTTTTATTTTCTCATTGATATGGA ATTCGAGCTGTTTAAAC |
| YW0137 F Eagl P_GAL1 | AAAAAACGGGCCCGGATTAGAAGCCGC |
| YW0138 R BamHI P_GAL1 | AAAAAAGGATCCCTCCCTTGACGTTAAAGTATAGAG |
| YW0139 F BamHI SEN15 GFP | AAAAAAAAAAGGATCCATGGCAACGACAGATGATC GTCGATTAAAC |
| YW0140 R HindIII SEN15 GFP | AAAAAAAAAAGCTTCTATTTGTATAGTTCATCCATGC |
| YW0142 R XmaI SEN15 without stop codon | AAAAAAAAAACCCGGGATTTTTTCTGTTTAC |
| YW0143 F XmaI GFP with stop codon | AAAAAACCCGGCGCGATCCCCGGGTTA |
| YW0183 F S. pombe SEN2 C-terminal mECitrine tag | GTCTGTACTCTGCAGAATATTTAATTCGATGTGTAACCT TACTTTCTATTGTAACCTT |
| YW0184 R S. pombe SEN2 C-terminal mECitrine tag | GATGCTTTGAAATTTATGATCAACTTCTTTCAAAAAATAT AAAAAATCACTTTTATAGTAATAATGTGATAGAACAAATC |
| YW0187 F S. pombe SEN34 C-terminal mECitrine tag | GGAAAAAGTCTAAAGGAAGACTACTATTACTATTACAGG TGCATTATTTCCATGTAATGGGCTGTTGGTTGGCGGATC |
| YW0188 R S. pombe SEN34 C-terminal mECitrine tag | AGTTATGTAACAACTATTGTTGAAAATTTCTATTGGTATTAGTAAAT TTGTTGAACTTTAAGTGAAATATCATTGGAATTTGAGG |
| YW0189 F S. pombe SEN54 C-terminal mECitrine tag | AGTTATACAGACTTCTCAAAAAGGGATCTAAAAGGA AAAAAAGTGAATCCCAATTTCAACTTTGCCCAGATC CCGGTTAATTTA |
| YW0190 R S. pombe SEN54 C-terminal mECitrine tag | GCATAATGAAAATTCAAGATATGCACCCGAACTTTTTTC TCTCCTCTTTTTCCGAGGACATATTATTTGAGGATTCG |
| YW0211 S. pombe delete TOM70 F | TAGCAATGCAATAAGTGACTCTACTTTGCTGAAGT TAGTGTCATGAAATCGGTAACAACTATTGACAAGAATCCCAAAACCA ATACCCGATCCCGGGTTAATTT |
| YW0212 S. pombe delete TOM70 R | TTGGAAACCTTTTTAAAGCAATTCAAAATGTTTTGCGCAAAC TGCTTATTTGACTACAGTATTTCTCTCATATACAATCTC GTCGATTGAGCTGTTTAAAC |
| Antibody                                      | Company         | Catalog Number |
|------------------------------------------------|-----------------|----------------|
| Mouse monoclonal anti-GFP                    | Sigma-Aldrich   | 11814460001    |
| Mouse monoclonal anti-MTCO1                  | Abcam           | Ab110270       |
| Mouse monoclonal anti-Pgk1                   | Abcam           | Ab113687       |
| Mouse monoclonal anti-TAT1                   | Sigma-Aldrich   | 00020911       |
| IRDye® 800CW Goat anti-Mouse IgG              | LI-COR          | 926-32210      |