Supplementary Materials

Engineering yeast endosymbionts as a first step towards the evolution of mitochondria

Angad P. Mehta, Lubica Supekova, Jian-Hua Chen, Kersi Pestonjamasp, Paul Webster, Yeonjin Ko, Scott Henderson, Gerry McDermott, Frantisek Supek, Peter G. Schultz.
correspondence to: schultz@scripps.edu; fsupek@gnf.org

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Movie S2

www.pnas.org/cgi/doi/10.1073/pnas.1813143115

Materials and Methods

Escherichia coli and *Saccharomyces cerevisiae* strains

E. coli strains used in his study were derived from DH10B *E. coli* (F⁻ *mcrA Δ(mrr-hsdRMS-mcrBC)* Φ80*lacZΔM15 ΔlacX74 recA1 endA1 araD139 Δ(ara leu) 7697 galU galK rpsL nupG* λ–). Additionally, we used *E. coli* JW2880-1 strain (F⁻*Δ(araD-araB)567, ΔlacZ4787*(::rrnB-3), *λ⁻, ΔserA764::kan^R, rph-1, Δ(rhaD-rhaB)568, hsdR514)* from Keio collection (CGSC # 10234, **https://cgsc2.biology.yale.edu/KeioList.php**) as a serine auxotroph. As the host for *E. coli* endosymbionts, we used *S. cerevisiae* ρ+ NB97 (*MAT***a** *leu2-3,112 lys2 ura3-52 his3ΔHindIII arg8Δ::URA3* [*cox2-60::ARG8^m*](1) and ρ^o MTCC109 (ATCC201440) strains. For isolation of mitochondria, we used *S. cerevisiae* ρ+ YPH500 strain (*MATα ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his1-Δ200 leu2-Δ1*).

Growth media

E. coli cells were routinely cultured in 2YT or LB medium. *E. coli* auxotrophy requirements were tested by culturing bacteria on plates with minimal agar medium (M9 medium containing Casamino Acids – Vitamin Assay (Fisher # DF0188156)). Where indicated, medium was supplemented with 10 µM thiamin, 100 µM NAD, 50 mg/L kanamycin, 50 mg/L chloramphenicol, 5 mg/L tetracycline and 1 mM arabinose. Yeast cells were routinely cultured in YPD medium (1% Bacto yeast extract, 2% Bacto peptone, 2% glucose). Where indicated, 2% glucose was substituted with 0.1% glucose/ 3% glycerol or 3% glycerol, and 1 M sorbitol was added to osmotically stabilize the medium. To isolate mitochondria, YPH500 cells were grown in 1% Bacto yeast extract, 2% Bacto peptone, 2% glucose medium.

In vitro ADP/ATP translocase assay

E. coli ΔthiC::gfp-kan^R was transformed with pAM136, and individual colonies were grown in 5 ml of 2YT medium in presence of kanamycin, chloramphenicol and arabinose (1 mM). The cells were harvested at OD_{600} ~ 0.8 and washed twice with Tris-HCl buffer (50 mM Tris-HCl, pH 8). The cells were resuspended in 100 µL Tris-HCl buffer and incubated with [γ-^{35}S]ATP (10 mM) for 30 min at 30 ^{o}C. The cells were harvested and washed twice with Tris-HCl buffer and resuspended in 10 µl of Tris-HCl buffer. This suspension was spotted on a filter paper (nitrocellulose filter, 0.2 µm, Whatman) and let dry. The radioactivity was measured using a scintillation counter (Packard TRI-CARB 2100TR). In parallel, radioactivity was measured for defined quantities of [γ-^{35}S]ATP to generate a calibration curve. For ATP efflux assays, *E. coli ΔthiC::gfp-kan^R*(pAM136) cells were incubated with [γ-^{35}S]ATP (10 mM) for 30 min. The cells were harvested, washed twice with Tris-HCl buffer and incubated with 10 mM of either ADP (ATP-free), AMP or potassium phosphate for up to 30 mins at 30 ^{o}C. The cells were then harvested, washed twice with Tris-HCl buffer, resuspended in 10 µl of Tris-HCl buffer and the radioactivity was measured as described above.

Measurement of ATP release by *E. coli ΔthiC::gfp-kan^R* cells expressing ATP/ADP translocase after addition of ADP to the growth medium

E. coli ΔthiC::gfp-kan^R was grown in LB medium in presence of kanamycin with or without arabinose (1 mM). *E. coli ΔthiC::gfp-kan^R*(pAM136) cells were grown in presence

of kanamycin, chloramphenicol and arabinose (1 mM). Cells were grown to OD_{600} = 0.6 and ADP (ATP-free, 20 µM final concentration) was added to the growth medium. The cells were then harvested and the growth medium was analyzed for the presence of ATP using a luciferase assay (ATP determination kit, Life Technologies - #A22066). ATP standards (provided with the kit) were used to obtain a calibration curve.

Introduction of respiration competent mitochondria into ρ^0 *S. cerevisiae* cells

Mitochondria were isolated from the YPH500 strain using the protocol described by Kucejova *et al. (2)*. To generate ρ^0 spheroplasts, MTCC109 yeast strain was grown in YPD medium overnight. The cells were harvested, washed twice with sterile water and then washed twice with sterile-filtered 1 M sorbitol solution (20 mL per gram of cells). The cells were resuspended in 1 M sorbitol solution (5 ml per gram of cells) containing Zymolase 100T (5-10 mg/g of cells) and the suspension was incubated at 37 ^oC in a water bath for 1 h. The resulting spheroplast suspension was cooled on ice for 20-30 min and then centrifuged at 1,500g, 4^oC. The harvested spheroplasts were washed twice with 1 M sorbitol solution (5 ml per gram of cells) and resuspended in 1M sorbitol solution (2 ml per gram of cells). Two hundred µl of spheroplast suspension was added to 800 µl of TSC buffer (10 mM Tris-HCl, 10 mM $CaCl_2$, 1 M sorbitol, pH 7.5), incubated at 30 ^oC for 10 min and then centrifuged at 1,500g for 10 min. The harvested spheroplasts were resuspended in 100 µl of TSC buffer. 50 µl of isolated mitochondria were mixed with 50 µl of 2 M sorbitol solution and quickly added to 100 µl of spheroplast suspension in TSC buffer. The mixture was incubated at 30 ^oC for 30 min. The entire mixture was added to 2 mL of PEG buffer (20% PEG 8000, 10 mM Tris-HCl, 2.5 mM $MgCl_2$, 10 mM $CaCl_2$, pH 8) and incubated at 30 ^oC for 45 min. After 45 min, the mixture was centrifuged at 1,500g/ 25 ^oC for 10 min. The harvested cells were then resuspended in 200 µl of 1 M sorbitol solution and 100 µl of this suspension was plated on selection medium I (0.67% Difco yeast nitrogen base without amino acids, 50 µg/mL of adenine sulfate, 50 µg/mL of uracil, 3% glycerol, 0.1% glucose, 1.5% agar, 1 M sorbitol). After plating, cells were overlayed with top agar containing the same selection medium. The plates were incubated at 30 ^oC for 3 to 4 days. Colonies were observed only on the plates where mitochondria were fused with ρ^0 spheroplasts, but not on the control plates where either only mitochondria or ρ^0 spheroplasts alone were plated. Further increasing the number of starting spheroplasts increased the number of colonies observed on the selection medium plates.

Introduction of *E. coli* into *S. cerevisiae* cells

E. coli cells (expressing GFP and containing defined gene knockout) were transformed with a fusion plasmid (pAM94, pAM126, pAM132, pAM136, or pAM150) and grown in 5 mL of 2YT medium in presence of kanamycin, chloramphenicol and arabinose (1 mM) till OD_{600} ~ 0.8. The cells were harvested (4 ^oC) and washed twice in chilled bacterial resuspension buffer (10 mM Tris-HCl, 2.5 mM $MgCl_2$, 10 mM $CaCl_2$, pH 8). The cells were then resuspened in 500 µl of resuspension buffer. For fusion, ρ^o MTCC109 or ρ+ NB97 *S. cerevisiae* were used as host strains. Yeast were grown in YPD medium overnight. To prepare spheroplasts, the yeast cells were harvested, washed twice with equal volume of sterile water and then washed twice with 1 M sorbitol solution (20 ml per gram of cells), and finally resuspended in sterile-filtered 1 M sorbitol solution (5 mL per gram of cells) containing Zymolase 100T (5 to 10 mg/g of cells). The suspension was incubated in a water

bath 37 ºC for 1 h to generate spheroplasted yeast cells. The spheroplast suspension was cooled on ice for 20 to 30 min and then centrifuged at 1,500g at 4 ºC, 10 min. The harvested spheroplasts were washed twice with 1 M sorbitol solution (5 mL per gram of cells) and then resuspended in 1M sorbitol solution (2 ml per gram of cells). 500 µl of spheroplast suspension was mixed with 500 µl of TSC buffer, incubated at 30 ºC for 10 min and the centrifuged at 1,500g for 10 min. The harvested spheroplasts were resuspended in 100 µl of TSC buffer. 150 µl of bacterial cell suspension was quickly mixed with 50 µl of 4 M sorbitol and the mixture was immediately added to 100 µl of spheroplast suspension in TSC buffer, mixed by inverting tubes and incubated at 30 ºC for 10 min. This mixture was then added to 2.5 ml of PEG buffer and incubated at 30 ºC for 45 min. After 45 min, the mixture was centrifuged at 1,500g, 25 ºC, for 10 min. The supernatant PEG solution was discarded and 1 ml of YPDS medium (YPD medium supplemented with 1 M sorbitol) was slowly added to the harvested cells pellet (without disturbing the pellet). This was then incubated at 30ºC for 2 h. After 2 h, the pellet was partially resuspended by tapping the side of the tube. This mixture was then incubated in a 30 ºC shaker at 70 rpm. After 3 h the cells were pelleted by centrifugation at 1,500g at room temperature. The cells were resuspended in 1 M sorbitol and plated on selection medium II (1% Bacto yeast extract, 2 % Bacto peptone, 3 % glycerol, 0.1% glucose, 1 M sorbitol, 1.5% agar + 1 mM arabinose). After plating, cells were overlayed with the same selection medium as a fixation agar preincubated at 50ºC (1.5 % agar). The plates were incubated at 25 ºC for 3 to 4 days. For subsequent rounds of growth the cells were plated on selection medium II or selection medium III (1% Bacto yeast extract, 2 % Bacto peptone, 3% glycerol, 1 M sorbitol, 1.5% agar + 1 mM arbinose; i.e. selection medium II without glucose) as indicated.

Total internal reflection fluorescence (TIRF) microscopy

Cells were allowed to attach for a few minutes to clean coverslips and mounted on slides in suspension medium. The samples were imaged in TIRF mode for enhanced contrast on a Nikon TI microscope with a 100X 1.49 NA TIRF objective using a 488 nm laser illumination. Images were collected with a 16-bit Hamamatsu Flash 4 camera. Images were deconvolved using a no-neighbors 2D deconvolution algorithm in the Elements software. The GFP images were overlaid with the differential interference contrast (DIC) images collected for each sample.

Confocal laser scanning fluorescence microscopy

Yeast cells, scraped from agar plates, were fixed by immersion in 4% phosphate buffered formalin and centrifuged into a pellet. Concentrated suspensions of cells were then spread over glass coverslips coated with gelled 12% gelatin and re-fixed with buffered formalin. FISH labeling on the yeast cells was performed following previously published protocols (3-5). The yeast cells on coated coverslips were immersed in 50% ethanol for 10 min. and hybridized with the EUB338-Cy3 probe *(6)* at a final concentration of 5 ng/µL for 90 min at 46°C. Hybridization was quickly stopped by adding cold H_2O and the coverslips were then labeled with FITC-labeled concanavalin A, a lectin which binds to yeast cell wall (7). The coverslips were mounted onto glass slides using Moviol as a mounting medium (8, 9) and images were collected using a laser scanning confocal microscope (LSM510; Carl Zeiss MicroImaging. Images were collected with identical microscope settings and pseudo-colored to follow journal specifications. Brightness

adjustment using similar parameters were used to modify the images and they were cropped using Photoshop (Adobe).

Soft X-ray tomography data collection

Yeast cells were collected from surface of an agar plate, washed three times with phosphate buffered saline (PBS) and then re-suspended in 50 µL of PBS. One µL of cell suspension was transferred to a thin-walled, specimen mounting capillary using a standard micropipette (10). Cells were cryopreserved by rapidly plunging the capillary into liquid propane using a custom-built apparatus. Cryopreserved specimens were transferred to a storage Dewar and held at 77K prior to being imaged. To image cells, capillaries were cryogenically transferred from the storage Dewar to the specimen cryo-stage of XM-2, a soft X-ray microscope at the Advanced Light Source of Lawrence Berkeley National Laboratory. To mitigate radiation damage during data collection, the specimen capillary was bathed in a stream of helium gas at liquid nitrogen temperature (11). Each tomographic dataset (i.e., 90 projection images spanning a range of 180^{o}) was collected using Fresnel zone plate objective lens with a resolution of 50 nm. Exposure times for each projection image ranged from 150 to 300 msec.

Soft X-ray tomographic reconstruction and image segmentation

Projection images in a rotation series were aligned to a common axis using the AREC-3D software package (12). The same software was used to automatically calculate 3D reconstructions by iterative methods. 3D-reconstructed images were segmented to identify subcellular structures. Segmentation is the process of isolating, visualizing and quantifying specific components in a tomographic reconstruction (13). Each voxel (3D equivalent of pixel) in a soft X-ray tomographic reconstruction is a direct measurement of the soft X-ray Linear Absorption Coefficient (LAC) at the corresponding location in the cell. The LAC value for a particular voxel depends solely on the concentration and composition of biomolecules in that volume in the cell. Water has an order of magnitude lower LAC than lipids and proteins. LAC values for homogeneous solutions of isolated biomolecules can be calculated using tables of known absorption coefficients. For example, pure water in the form of ice has a calculated LAC of 0.109 μm^{-1} whereas a model protein with the chemical composition $C_{94}H_{139}N_{24}O_{31}S$ has a theoretical LAC of 1.35μm^{-1} (14). In practice, most voxels in a 50 nm resolution of soft X-ray tomography reconstruction of a cell contain a heterogeneous mixture of biomolecules. However, in terms of their overall LAC value, organelles and other sub-cellular structures are sufficiently similar in their biochemical composition to allow them to be readily distinguished from the surrounding cell contents. For example, the water content in a vacuole makes it readily distinguishable from organelles with a greater density of biomolecules, for example, mitochondria. The software package Amira (**https://www.fei.com/software/amira-3d-for-life-sciences/**) was used to semi-automatically segment the major organelles in the collected cell images.

Construction of gene disruption cassettes and plasmids

All DNA oligonucleotides were purchased from IDT (Integrated DNA Technologies). Gene-coding DNA fragments and gene disruption cassettes were generated from the oligonucleotides either by PCR (Q5 Hot Start High-Fidelity 2X Master mix, using manufacturer's protocol) or by the Gibson Assembly method (Q5 Hot Start High-Fidelity

2X Master mix, using manufacturer's protocol: step 1: 98 ^{o}C, 30 sec; step 2: 98 ^{o}C, 10 sec; Step 3: 50-72 ^{o}C, 20 sec; Step 4: 72 ^{o}C, 30 sec/kb; (35 cycles step 2 to step 4); step 5: 72 ^{o}C, 2 min). All the oligonucleotides used in PCR amplification reactions are listed in Table S1. gBlock oligonucleotide sequences used for gene assembly by Gibson Assembly method are listed in Table S2. Coding sequences were codon-optimized for *E. coli* expression using IDT codon optimization software (**https://www.idtdna.com/CodonOpt**).

gfp-kan^{R} gene disruption cassettes: the cassette included the *sf-gfp* gene under control of the constitutive λ phage pL promoter (ACTGAGCACATCAGGCAGGACGCACTGACCACCATGAAGGTGACGCTCTTA AAAATTAAGCCCTGAAGAAGGGCTTTATTTGCATACATTCAATCAATTGTTAT CTAAGGAAATACTTACAT) and a kanamycin resistance gene. To construct the cassette, the *sf-gfp* gene was amplified with the AM829/AM840 primers from pBAD-sf-gfp (**https://www.addgene.org),** and then extended by three sequential PCR steps using the AM830/AM840, AM831/AM840 and AM832/AM841 primer pairs to generate the pL-*sf-gfp* fragment. Kanamycin resistance gene (*kan*^{R}) was first amplified from the pKD4 plasmid(15) using the AM838/AM833 primers and then further amplified with the AM839/AM843 primers. The two fragments, *pL-sf-gfp* and *kan*^{R}, were assembled together using the Gibson Assembly protocol(16). The resulting *pL-sf-gfp-kan*^{R} (further abbreviated as *gfp-kan*^{R}) construct was gel purified (Qiagen gel purification kit) and used as a linear DNA fragment for generation of the *E. coli thiC* knockout strain. To genetically knock out nicotinamide adenine dinucleotide (NAD) biosynthesis in *E. coli*, the above *gfp-kan*^{R} cassette was sequentially amplified using the AM1033/AM1034 and AM1035/AM1036 primer pairs to generate a *nadA* (quinolinate synthase A) knockout cassette.

tet^{R} gene disruption cassette: the cassette was used to knock out the *nadA* gene in the *E. coli ΔthiC::gfp-kan*^{R} mutant to generate *E. coli ΔnadA::tet*^{R} *ΔthiC::gfp-kan*^{R}. The tetracycline resistance cassette was sequentially amplified from plasmid pAM22 (17) using the YK44/YK45 and YK48/YK49 primer pairs to generate a linear DNA fragment used for *nadA* knockout.

pAM94: the vector pCdf-Chlor (17) was linearized by PCR using the AM152/AM153 primers. The coding sequence of the UWE25 ADP/ATP translocase (*ntt1*) was codon-optimized, and the corresponding *UWE25_ntt1* gBlock oligonucleotide was synthesized and then amplified sequentially using the AM783/AM785 and AM784/AM786 primer pairs. The amplified *UWE25-ntt1* fragment was inserted into the linearized pCdf-Chlor using the Gibson Assembly method (16) to afford pAM94.

pAM126: the plasmid pAM94 was linearized by PCR using the AM785/AM153 primers. The *C. tr. incA* coding sequence was codon-optimized and a spacer sequence (spacer-1; Table S3) was added to the 5'end of the optimized sequence. The corresponding *Ctr_incA* gBlock oligonucleotide was synthesized and then amplified using the AM906/AM907 primers. The amplified DNA fragment was inserted into the linearized pAM94 using Gibson Assembly method to afford pAM126.

pAM132: the plasmid pAM126 was linearized using the AM955/AM958 primers. The *C. ca incA* DNA sequence was codon-optimized and a spacer sequence (spacer-2; Table S3) was added to the 5'end of the optimized sequence. The corresponding *Cca_incA* gBlock was synthesized and then amplified using the AM956/AM959 primers. The

amplified fragment was inserted into the linearized pAM126 using the Gibson Assembly method to afford pAM132.

pAM136: the plasmid pAM132 was linearized using the AM988/AM153 primers. The *Chlamydia trachomatis* gene *CT_813* DNA sequence was codon-optimized and a spacer sequence (spacer-3; Table S3) was added to the 5'end of the optimized sequence. The corresponding *CT_813* gBlock was synthesized and then amplified using the AM989/AM990 primers. The amplified fragment was inserted into the linearized pAM132 using the Gibson Assembly method to afford pAM136.

pAM150: the plasmid pAM136 was linearized using the AM152/AM1139. The linearized vector was circularized using the NEB Q5 site-directed mutagenesis kit to afford pAM150.

pAM158: Introduction of C-terminal FLAG tag for *C.tr.* IncA the plasmid pAM136 was linearized using the AM1266/AM1267. The linearized vector was circularized using the NEB Q5 site-directed mutagenesis kit to afford pAM158.

pAM159: Introduction of C-terminal FLAG tag for *C.ca.* IncA the plasmid pAM136 was linearized using the AM1268/AM1269. The linearized vector was circularized using the NEB Q5 site-directed mutagenesis kit to afford pAM159.

pAM160: Introduction of C-terminal FLAG tag for CT_813 the plasmid pAM136 was linearized using the AM1270/AM1271. The linearized vector was circularized using the NEB Q5 site-directed mutagenesis kit to afford pAM160.

pAM161: Introduction of N-terminal FLAG tag for *C.ca.* IncA the plasmid pAM136 was linearized using the AM1272/AM1273. The linearized vector was circularized using the NEB Q5 site-directed mutagenesis kit to afford pAM160.

pAM162: To delete *C.ca. incA* from the plasmid pAM136, pAM136 was linearized using the AM1280/AM1281. The linearized vector was circularized using the NEB Q5 site-directed mutagenesis kit to afford pAM162.

pAM163: To delete *C.tr. incA* from the plasmid pAM162, pAM162 was linearized using the AM785/AM1281. The linearized vector was circularized using the NEB Q5 site-directed mutagenesis kit to afford pAM163.

Construction of *E. coli* genetic knockout strains

The thiamin pyrimidine synthase gene (*thiC*) was disrupted in the DH10B *E. coli* strain with a PCR-amplified *gfp-kan*^{R} gene disruption cassette using the phage λ Red recombinase method (15). The kanamycin resistant colonies were tested for the thiamin auxotrophy by culturing them in minimal medium in the presence and absence of thiamin. Growth was observed only in the thiamin-supplemented medium. The *E. coli ΔthiC::gfp-kan*^{R} strain was used for further studies. To genetically inactivate nicotinamide adenine dinucleotide (NAD) biosynthesis in *E. coli*, the *gfp-kan*^{R} fragment was PCR amplified to generate a *nadA* (quinolinate synthase A) knockout cassette as described above. The PCR product was purified (Qiagen PCR purification kit) and used as a linear DNA fragment to generate a *nadA* deletion in the DH10B *E. coli* strain by the λ Red recombinase-dependent. NAD auxotrophy was confirmed by growing the *nadA* cells in minimal medium in the presence and absence of NAD (100 μM). Similarly, the *tet*^{R} gene disruption cassette was used to generate *E. coli ΔnadA::tet*^{R} *ΔthiC::gfp-kan*^{R} from the *E. coli ΔthiC::gfp-kan*^{R} strain. Thiamin and NAD auxotrophies were confirmed by growing the *E. coli ΔnadA::tet*^{R}

ΔthiC::gfp-kan^R in minimal medium in the presence and absence of thiamin (10 µM) and NAD (100 µM).

Determination of GFP expression in *E. coli* cells by fluorimetry

E. coli DH10B and *E. coli ΔthiC::gfp-kan*^{R} strains were grown in LB medium in the absence or presence of kanamycin (40 mg/L) till OD_{600} ~ 1.0. The cells were washed twice with 1X PBS buffer (Corning # 46-013-CM), resuspended in equal volume of 1X PBS buffer at OD_{600} = 1.0 and the fluorescence was measured by SpectraMax Gemini EM fluorimeter (excitation/ emission wavelength: 485 nm/ 510 nm).

Flow cytometry analysis of *E. coli* knockout strains for GFP expression

E. coli DH10B and *E. coli ΔthiC::gfp-kan*^{R} strains were grown in LB medium in absence or presence of kanamycin, respectively. The cells were washed twice in 1X PBS buffer (Corning #46-013-CM) and analyzed by FACS (Astrios EQ) to determine the presence of GFP-positive cells. FITC fluorochrome detection settings (Ex =488 nm/ Em = 530 nm) were used for fluorescence measurement.

Total genomic DNA isolation and PCR analysis

For qualitative PCR analysis, the yeast cells were collected from the agar plates and resuspended in 200 µl of 1 M sorbitol solution. The cells were then harvested, and the total genomic DNA was isolated using Purelink Genomic DNA Mini kit (Invitrogen #K182002) using manufacturer's protocol. The isolated DNA was stored at -20 ^oC prior to PCR analysis. The *S. cerevisiae* specific oligonucleotide primers previously reported in literature (for sequences check - AM965, AM966, AM967) were used to detect the *S. cerevisiae MAT***a** gene. Similarly, to detect the *E. coli* genome, the *gfp* primers AM572/AM656 were used. To amplify bacterial 16S rDNA, 8F (AM519) and 1492R (AM520) primers were used (18). The amplified 16S rDNA template was sequenced to confirm the presence of *E. coli*. For *E. coli glyA* amplification AM391/AM392 were used.

Determination of *E. coli*:*S. cerevisiae* genome ratios in chimeras by qPCR

Total DNA from *S. cerevisiae-E. coli* chimera cells, NB97 yeast cells and *E. coli ΔthiC::gfp-kan*^{R} cells was extracted using the Master Pure Yeast DNA Purification Kit (Epicentre). Two genes - *S. cerevisiae MAT***a** (primers AM965 and AM967, Table S1) and *E. coli gfp* (primers GFP1 and GFP2, Table S1) - were quantified in parallel in the chimera DNA samples using the PowerUp SYBR Green Master Mix kit (Applied Biosystems). qPCR reactions (10 µL) included 5 µl of SYBR Green Master Mix, two primers at 0.5 µM concentration each and 2 ng of sample DNA. Each qPCR experiment included a *gfp* calibration curve (prepared from *E. coli ΔthiC::gfp-kan*^{R} total DNA) and *MAT***a** calibration curve (prepared from NB97 yeast total DNA). Amounts of yeast and *E. coli* DNAs in chimera DNA samples were quantified using the Applied Biosystems 7900HT instrument and the sample/calibration curve Ct values. Number of *E. coli* cells per one yeast cell in a chimera was calculated as the ratio of *E. coli* to yeast DNA in the chimera total DNA and multiplied by the ratio of the two genome sizes (2.7; *E. coli* K12 genome size = 4.64 Mbp; *S. cerevisiae* haploid genome size = 12.5 Mbp).

Western Blot analysis for SNARE expression analysis

E. coli DH10B ΔnadA::gfp-kan^R cells were transformed with either pAM158, pAM159, pAM160 or pAM161. The cells were grown in LB medium in the presence of kanamycin, chloramphenicol and arabinose (1 mM) till OD_{600} ~ 0.8. The cells were resuspended in a lysis buffer (50 mM Tris-HCl, pH 8, 5 % SDS, 1 mM β-mercaptoethanol) and lysed by heating them at 90^{o}C for 10 min. The cell lysate was mixed with NuPAGE LDL sample buffer (final sample buffer concentration – 1X, Invitrogen). Gel electrophoresis was performed (NuPAGE 4-12% Bis-Tris Protein gels (Invitrogen). Western blot analysis was performed according to manufacturer's protocol; primary antibody – Anti-FLAG Rabbit mAb (Cell Signaling Technology, Catalog # 14793S) and secondary antibody – Anti-Rabbit IgG (Sigma, Catalog # A0545).

Membrane fraction isolation

E. coli DH10B ΔnadA::gfp-kan^R cells were transformed with either pAM158, pAM159, pAM160 or pAM161 The cells were grown in LB medium in the presence of kanamycin, chloramphenicol and arabinose (1 mM) till OD_{600} ~ 0.8. The membrane fractions were isolated using ReadyPrep Protein Extraction Kit, Membrane II (Bio-Rad, Catalog # 1632084) following the manufacturer's protocol.

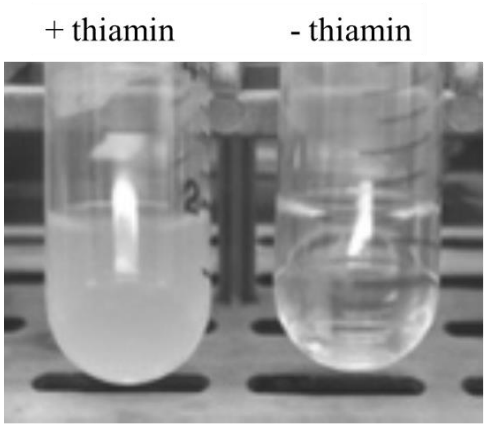

Fig. S1. ***Escherichia coli ΔthiC::gfp-kan*^{R} thiamin dependence.** Cells were cultured in M9 minimal medium at 37^{o}C, with and without thiamin. No growth was observed in medium lacking thiamin.

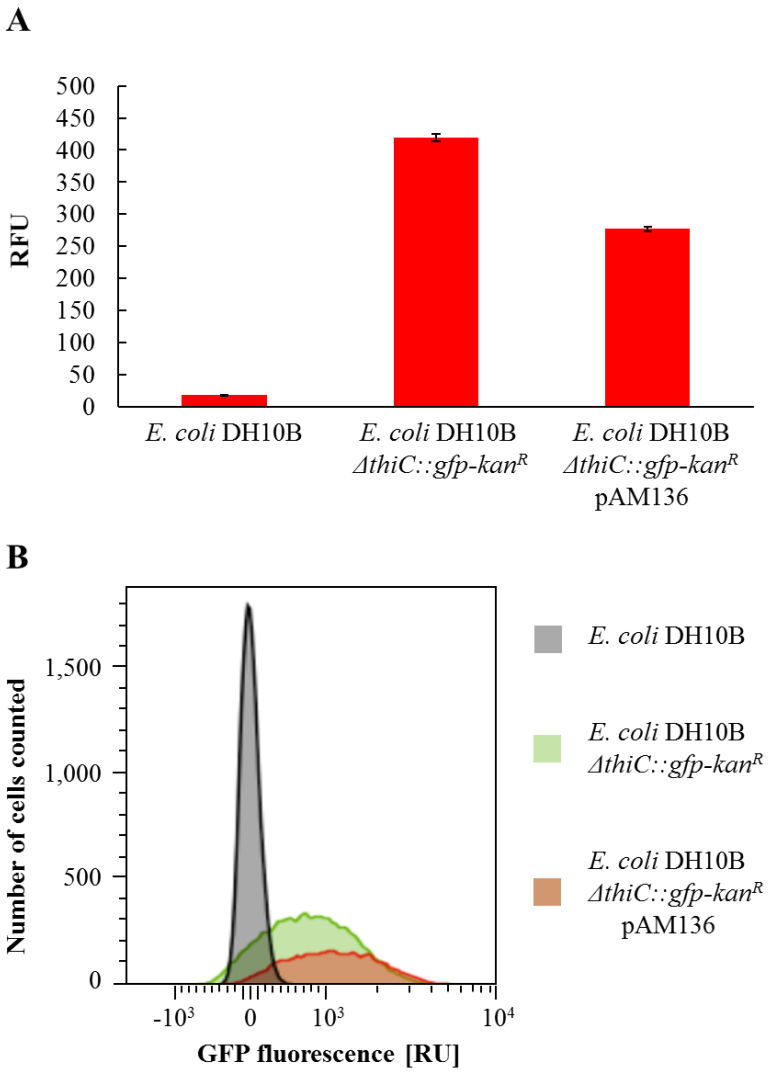

Fig. S2. *E. coli ΔthiC::gfp-kan*^{R} cells express GFP.

(**A**) Fluorescence of indicated *E. coli* cell suspensions was determined at Ex = 485 nm/ Em = 510 nm. (Data bars show a mean of 3 technical replicates; error bars represent standard errors of mean)

(**B**) Flow cytometry analysis of *E. coli* strains. Fluorescence measurements were collected at Ex =488 nm/ Em = 530 nm (FITC). A composite plot is shown that includes data from separate analyses of *E. coli* strains.

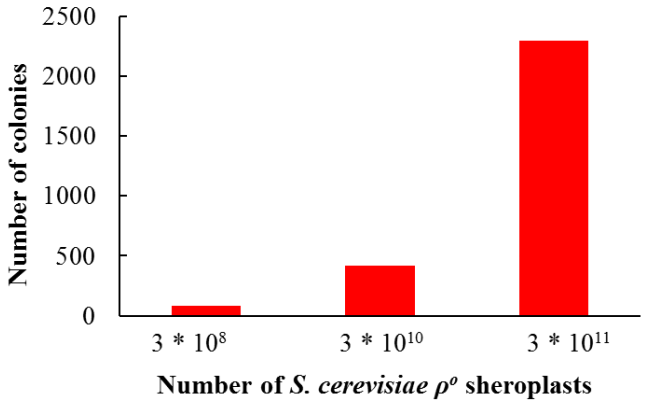

Fig. S3. Fusion of *ρ^o^ S. cerevisiae* spheroplasts with respiration-competent mitochondria yields yeast cybrids with restored respiration-competent phenotype.

Fusion of *ρ^o^* MTCC109 *S. cerevisiae* spheroplasts with respiration-competent mitochondria isolated from YPH500 *S. cerevisiae* strain yielded cybrids that grew on minimal medium containing glycerol as the sole carbon source (Selection Medium I). The number of cybrid colonies increased with increasing number of ρ^o spheroplasts used in fusion mixture.

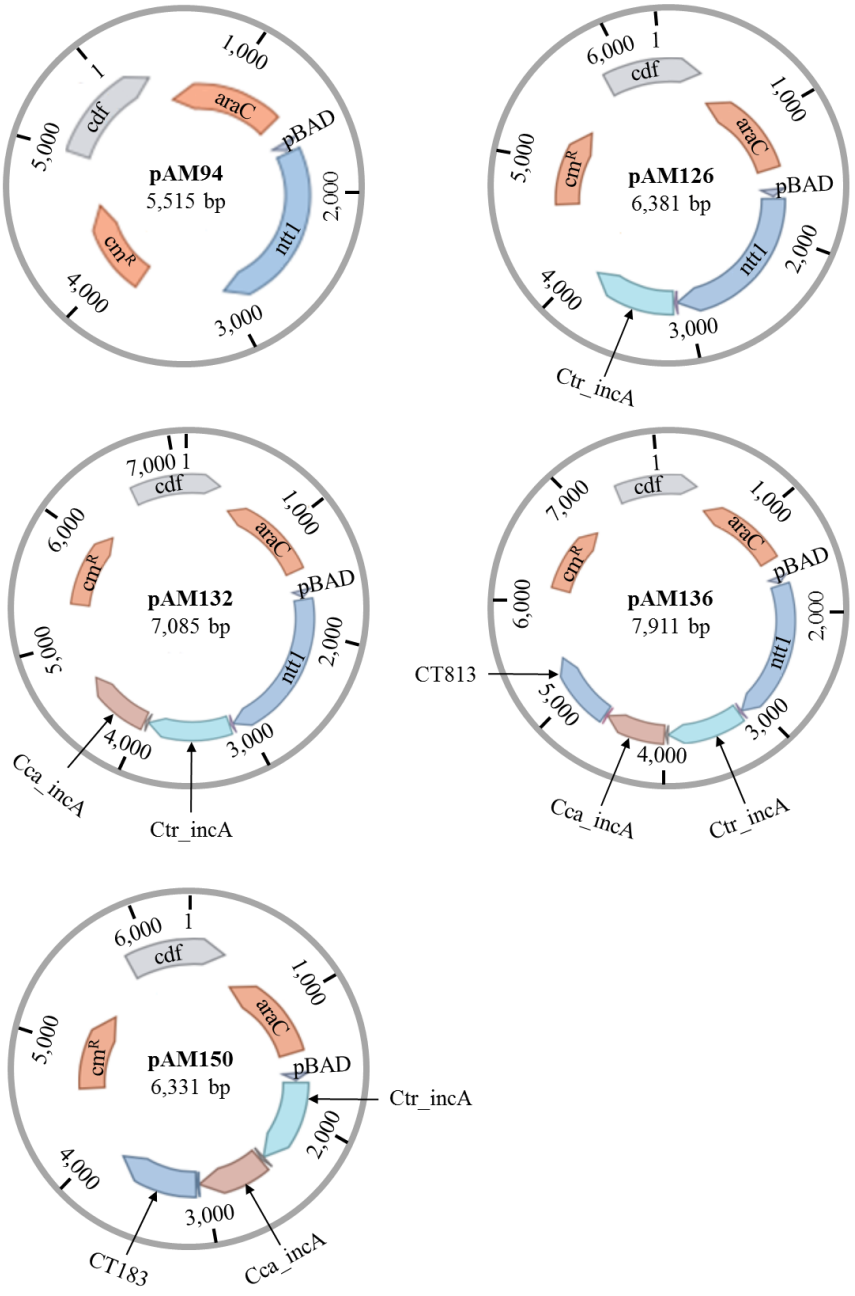

Fig. S4. Maps of plasmids used in this study.

Cdf – CloDF-13 origin; ntt1 – ADP/ATP translocase; *Ctr-incA*, *Cca_incA* and *CT_813* are genes encoding SNARE-like proteins from *C. trachomatis*, *C. caviae* and *C. trachomatis*, respectively; cm^R – chloramphenicol acetyltransferase; pBAD – promoter; *araC* – gene encoding arabinose operon regulatory protein.

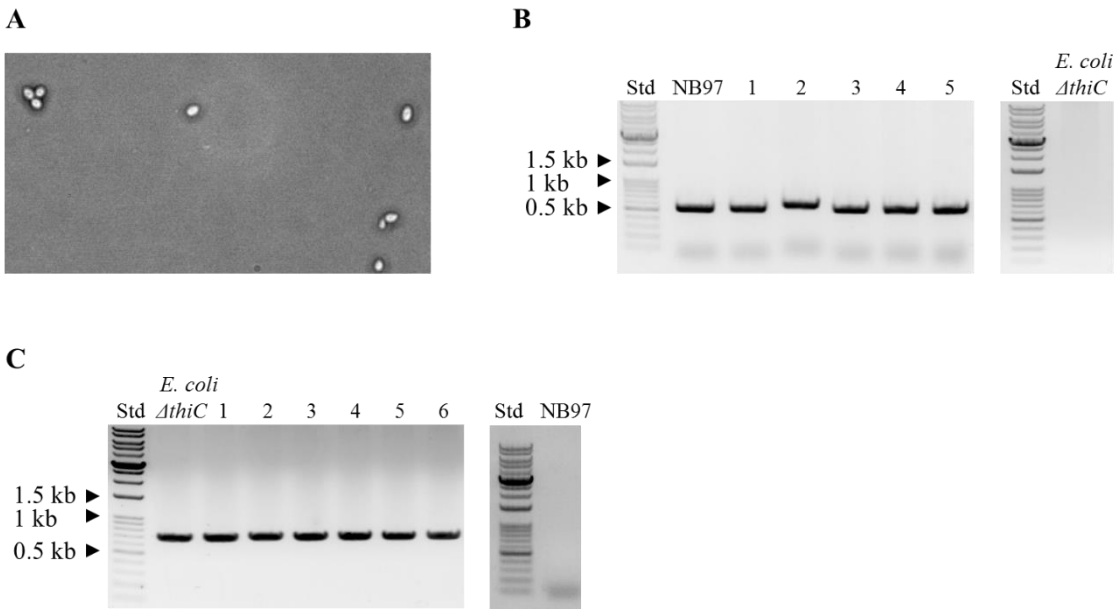

Fig. S5. Fusion of *S. cerevisiae cox2-60* mutant with *E. coli ΔthiC* (pAM132) cells yields yeast colonies that have partially restored respiration-competent phenotype.
(**A**) Fusion of *S. cerevisiae cox2-60* (NB97) with *E. coli* DH10B *ΔthiC::gfp-kan*^{R} (pAM132) cells yields yeast colonies growing on medium containing 3% glycerol as the sole carbon source (Selection Medium III + 50 mg/L carbenicillin) that lack extracellular *E. coli* cells upon microscopic examination (magnification – 40X).
(**B**) Detection of NB97-specific *MAT***a** mating type by PCR in total DNA samples isolated from NB97 yeast strain and five randomly selected yeast-*E. coli* fusion colonies. Std indicates DNA molecular weight standards. *E. coli ΔthiC* refers to PCR reaction performed with DNA isolated from *E. coli* DH10B *ΔthiC::gfp-kan*^{R} strain.
(**C**) Detection of *gfp* gene by PCR in DNAs isolated from NB97 yeast strain and five randomly selected yeast-*E. coli* fusion colonies shown in panel B. Std indicates DNA molecular weight standards. *E. coli ΔthiC* refers to DNA isolated from *E. coli* DH10B *ΔthiC::gfp-kan*^{R} strain.

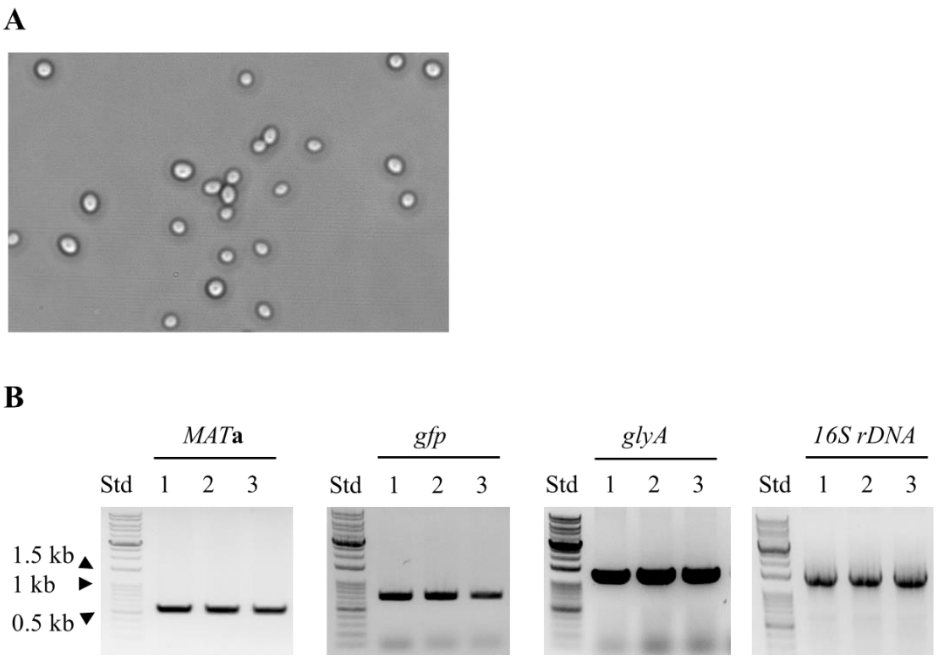

Fig. S6. Fusion of *S. cerevisiae cox2-60* mutant with *E. coli ΔthiC* (pAM136) cells yields yeast colonies that have partially restored respiration-competent phenotype.
(**A**) Fusion of *S. cerevisiae cox2-60* (NB97) with *E. coli* DH10B *ΔthiC::gfp-kan*^{R} (pAM136) cells yields yeast colonies growing on medium containing 3% glycerol as the sole carbon source (Selection Medium III + 50 mg/L carbenicillin) that lack extracellular *E. coli* cells upon microscopic examination (magnification – 40X).
(**B**) Detection of NB97-specific *MAT***a** mating type gene and *E. coli gfp*, *glyA* and *16S RNA* genes by PCR of total DNA samples isolated from three randomly selected yeast colonies.

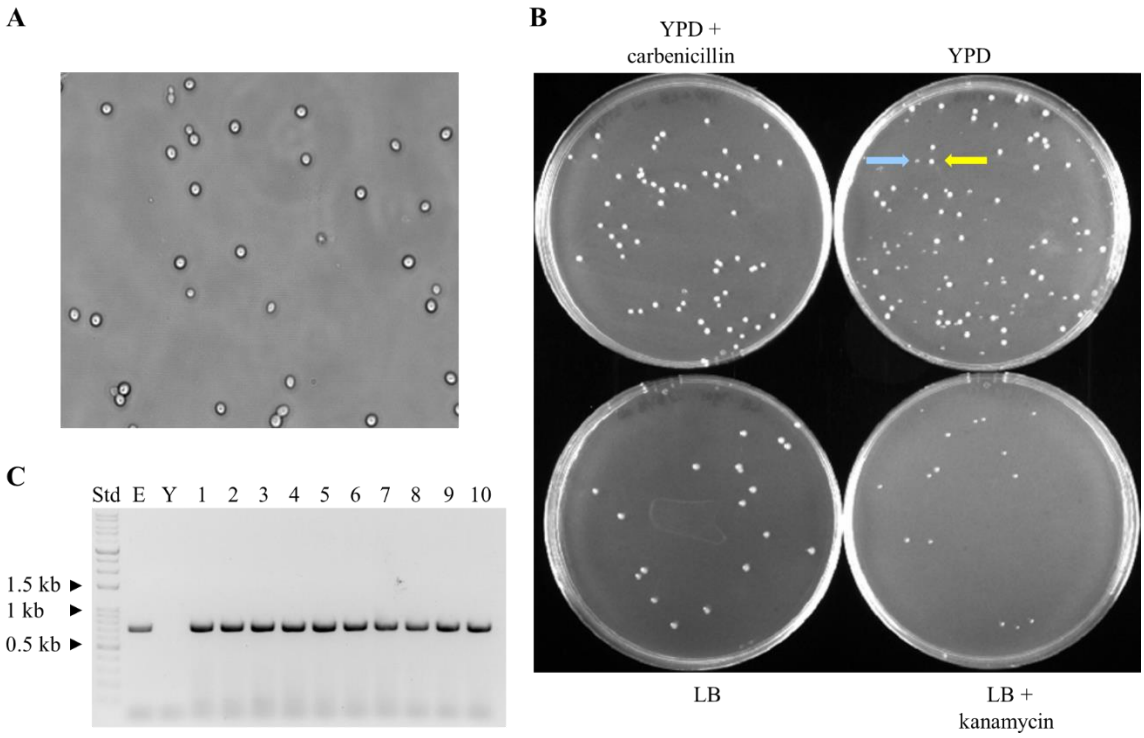

Fig. S7. *Saccharomyces cerevisiae* - *E. coli* chimeras can release *E. coli* cells when grown without selection pressure.

(**A**) *S. cerevisiae cox2-60* - *E. coli ΔnadA::gfp-kan*^{R} (pAM136) chimera culture after four rounds of re-plating on Selection Medium III with 50 mg/L carbenicillin (magnification – 40X).

(**B**) Chimera culture from panel A after overnight growth in YPD medium lacking carbenicillin. Only yeast colonies were formed on YPD + carbenicillin plate (upper left). The YPD plate with no carbenicillin (upper right) contained both yeast (larger white colonies, yellow arrow) and *E. coli* colonies (smaller gray colonies, light blue arrow). Colonies formed by released *E. coli* cells were observed on LB plates without antibiotics. Colonies formed by released *E. coli* cells were resistant to kanamycin (lower right, LB + kanamycin).

(**C**) Released *E. coli* cells encode *gfp* gene marker. Ten *E. coli* colonies from LB plate shown in panel B (lower left) were analyzed by PCR for the presence of *gfp* gene. Lane E: total DNA isolated from *E. coli ΔnadA::gfp-kan*^{R} was used for PCR; Lane Y: total DNA isolated from NB97 *S. cerevisiae* strain was used for PCR.

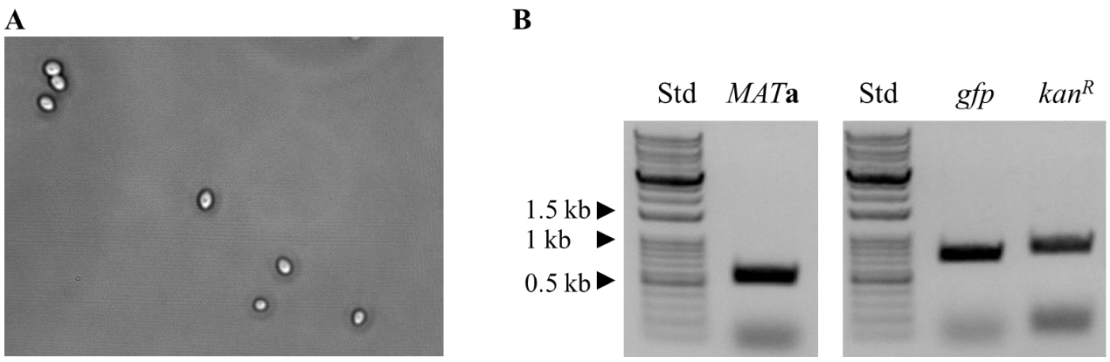

Fig. S8. Fusion of *S. cerevisiae cox2-60* mutant with *E. coli ΔnadA* (pAM136) cells yields yeast colonies that have partially restored respiration-competent phenotype.
(**A**) Fusion of *S. cerevisiae cox2-60* (NB97) with *E. coli* DH10B *ΔnadA::gfp-kan*^{R} (pAM136) cells yields yeast colonies growing on Selection Medium III + 50 mg/L carbenicillin that lack extracellular *E. coli* cells upon microscopic examination (magnification – 40X).
(**B**) Detection of NB97-specific *MAT***a** mating type gene and *E. coli gfp* and *kan*^{R} gene by PCR of total DNA samples isolated from one randomly selected yeast colony shown in panel A.

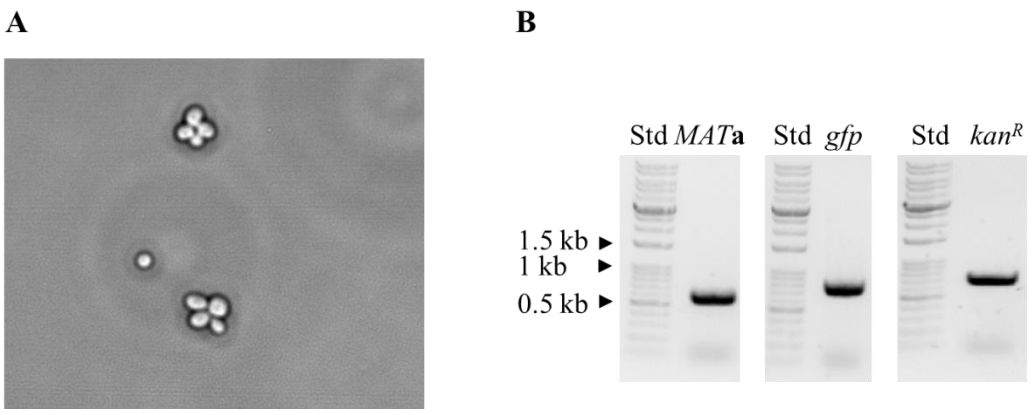

Fig. S9. Fusion of *S. cerevisiae cox2-60* mutant with *E. coli ΔthiC ΔnadA* (pAM136) cells yields yeast colonies that have partially restored respiration-competent phenotype.

(**A**) Fusion of *S. cerevisiae cox2-60* (NB97) with *E. coli* DH10B *ΔnadA::tet*^{R} *ΔthiC::gfp-kan*^{R} (pAM136) cells yields yeast colonies growing on Selection Medium III + 50 mg/L carbenicillin that lack extracellular *E. coli* cells upon microscopic examination (magnification – 40X).

(**B**) Detection of NB97-specific *MAT***a** mating type gene and *E. coli gfp* and *kan*^{R} gene by PCR of total DNA samples isolated from one randomly selected yeast colony shown in panel A.

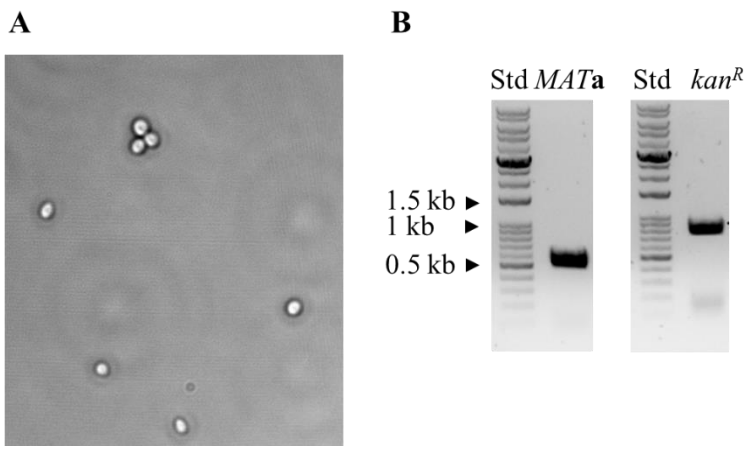

Fig. S10 Fusion of *S. cerevisiae cox2-60* mutant with *E. coli ΔserA* (pAM136) cells yields yeast colonies that have partially restored respiration-competent phenotype. (**A**) Fusion of *S. cerevisiae cox2-60* (NB97) with *E. coli* DH10B *ΔserA::kan*^{R} (pAM136) cells yields yeast colonies growing on Selection Medium III + 50 mg/L carbenicillin that lack extracellular *E. coli* cells upon microscopic examination (magnification – 40X). (**B**) Detection of NB97-specific *MAT***a** mating type gene and *E. coli kan*^{R} gene by PCR of total DNA samples isolated from one randomly selected yeast colony shown in panel A.

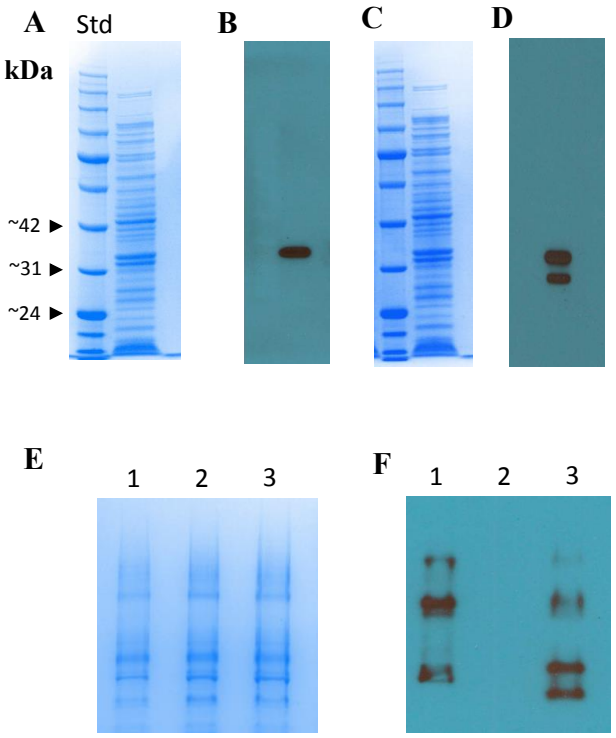

Fig. S11 Western Blot analysis to detect expression and membrane localization of *Chlamydia* SNARE proteins in *E. coli*.

(**A**) *E. coli* DH10B *ΔnadA::gfp-kan*^{R} – pAM158 lysate analyzed by SDS PAGE and Coomassie stained.

(**B**) *E. coli* DH10B *ΔnadA::gfp-kan*^{R} – pAM158 lysate analyzed by SDS PAGE gel and western blot (anti-FLAG primary antibody, anti-rabbit secondary antibody).

(**C**) *E. coli* DH10B *ΔnadA::gfp-kan*^{R} – pAM160 lysate analyzed by SDS PAGE gel and Coomassie stained.

(**D**) *E. coli* DH10B *ΔnadA::gfp-kan*^{R} – pAM160 lysate analyzed by SDS PAGE and western blot (anti-FLAG primary antibody,anti-rabbit secondary antibody).

(**E**) Membrane fractions were isolated and analyzed by Coomassie-stained SDS PAGE gels; lane 1: *E. coli* DH10B *ΔnadA::gfp-kan*^{R} – pAM158 membrane fraction; lane 2: *E. coli* DH10B *ΔnadA::gfp-kan*^{R} – pAM159 membrane fraction; lane 3: *E. coli* DH10B *ΔnadA::gfp-kan*^{R} – pAM160 membrane fraction.

(**F**) Membrane fractions were isolated and analyzed by western blot with anti-FLAG antibody; lane 1: *E. coli* DH10B *ΔnadA::gfp-kan*^{R} – pAM158 membrane fraction; lane 2: *E. coli* DH10B *ΔnadA::gfp-kan*^{R} – pAM159 membrane fraction; lane 3: *E. coli* DH10B *ΔnadA::gfp-kan*^{R} – pAM160 membrane fraction.

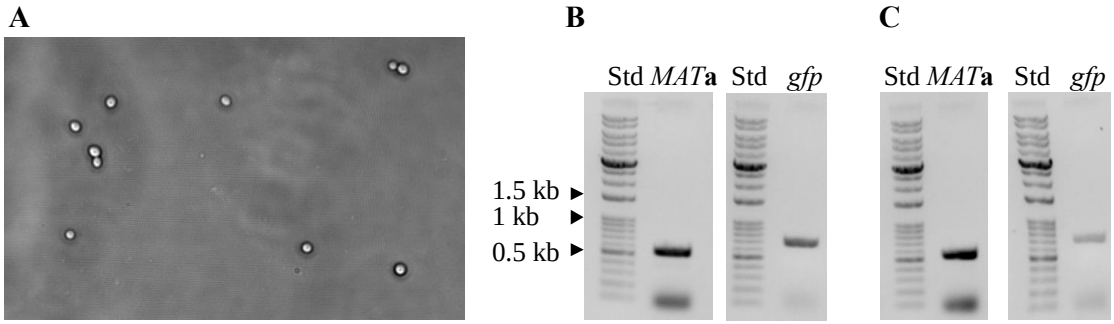

Fig. S12 Fusion of *S. cerevisiae cox2-60* mutant with *E. coli ΔthiC and E. coli ΔnadA* (pAM162) cells yields yeast colonies that have partially restored respiration-competent phenotype.

(**A**) Fusion of *S. cerevisiae cox2-60* (NB97) with *E. coli* DH10B *ΔthiC::gfp-kan*^{R} (pAM162) cells yields yeast colonies growing on Selection Medium III + 50 mg/L carbenicillin (magnification – 40X).

(**B**) Detection of NB97-specific *MAT***a** mating type gene and *E. coli gfp* gene by PCR of total DNA samples isolated from one randomly selected yeast colony of *S. cerevisiae cox2-60* (NB97) - *E. coli* DH10B *ΔthiC::gfp-kan*^{R} (pAM162) chimera.

(**C**) Detection of NB97-specific *MAT***a** mating type gene and *E. coli gfp* gene by PCR of total DNA samples isolated from one randomly selected yeast colony of *S. cerevisiae cox2-60* (NB97) - *E. coli* DH10B *ΔnadA::gfp-kan*^{R} (pAM162) chimera.

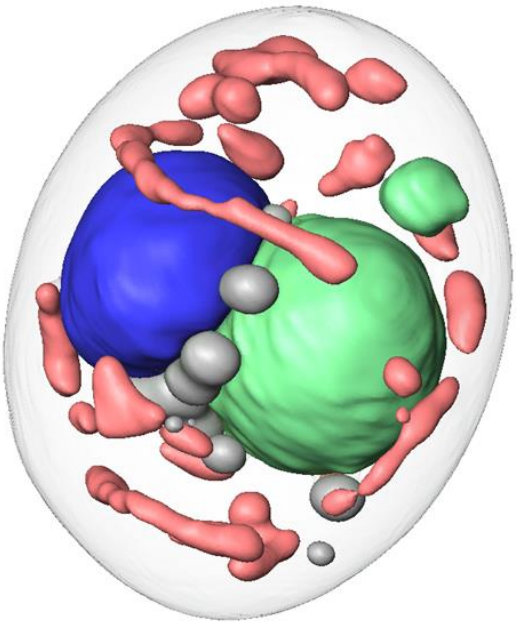

Fig. S13. Reconstruction of *S. cerevisiae cox2-60* cell from images collected by soft X-ray tomography. Organelle color key: green - vacuole; blue - nucleus; salmon – mitochondria.

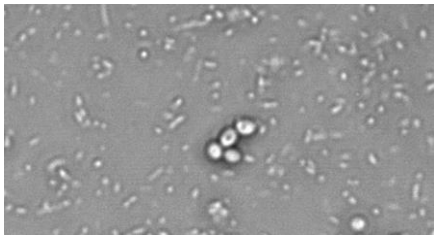

Fig. S14: A representative microscopic image of extracellular bacterium and yeast cells (magnification – 40X)

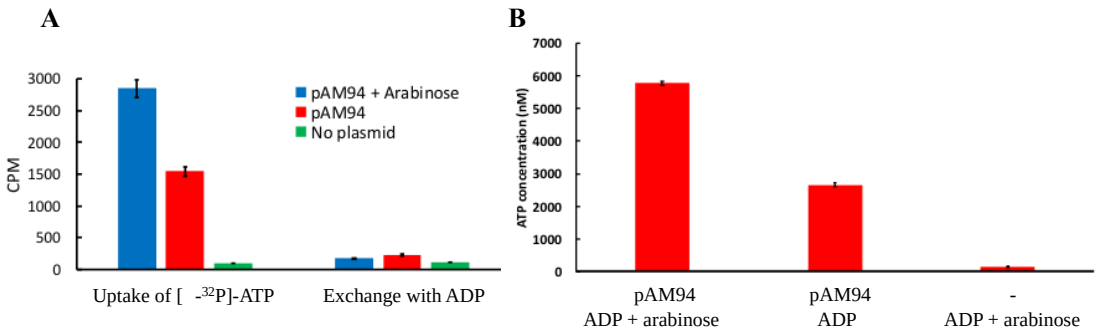

Fig. S15: Release of ATP by *E. coli* cells encoding ADP/ATP translocase. (**A**) Cellular [γ-^{32}P]ATP uptake/release by *E. coli* cells expressing the UWE25 ADP/ATP translocase (pAM94 plasmid) in the presence and absence of 1 mM arabinose. Cellular [γ-^{32}P]ATP was released when *E. coli* cells containing pAM94 were challenged with extracellular ADP (10 mM). (**B**) Release of ATP into the growth medium by *E. coli* cells containing pAM94 plasmid in presence of 20 µM ADP and in presence and absence of 1 mM arabinose. The ATP concentration in the medium was determined by luciferase assay. (Data bars show a mean of 3 technical replicates; error bars represent standard errors of mean). These data suggest significant leaky expression of UWE25 ADP/ATP translocase from plasmid pAM94.

Table S1.
PCR oligonucleotides used for construction of gene disruption cassettes and plasmids.

Oligonucleotide name	**Oligonucleotide sequence**
AM152	GGTTAATTCCTCCTGTTAGCCCAAAAAACGG
AM153	GTCGACCATCATCATCATCATCATTGAGTTTAAACG
AM391	ATGTTAAAGCGTGAAATGAACATTGCCGATTATGATGCC
AM392	TTATGCGTAAACCGGGTAACGTGCGCAGATGTCG
AM519	AGAGTTTGATCCTGGCTCAG
AM520	GGTTACCTTGTTACGACTT
AM572	ATGAGCAAAGGAGAAGAACTTTTCACTGGAGTTGTCC
AM656	GGATCCTTTGTAGAGCTCATCCATGCCATGTG
AM783	ATGTCACAAGATGCAAAGCAAGACTTTGGC
AM784	GGGCTAACAGGAGGAATTAACCATGTCACAAGATGCAAAGCAAGACTTTGGC
AM785	TTAACTAGTTGCGATCTCACTAGTCTTGTCAATA
AM786	TGATGATGATGATGATGGTCGACTTAACTAGTTGCGATCTCACTAGTCTTGTCAATA
AM829	AAGGGCTTTATTTGCATACATTCAATCAATTGTTATCTAAGGAAATACTTACATATGAGCAAAGGAGAAGAACTTTTCACTGGAGTT
AM830	TGACCACCATGAAGGTGACGCTCTTAAAAATTAAGCCCTGAAGAAGGGCTTTATTTGCATACATTCAATCAATTGTTATC
AM831	ACTGAGCACATCAGGCAGGACGCACTGACCACCATGAAGGTGACGCTCTTAAAAA
AM832	AGCGGGACTCTGGGGTTCGAAACTGAGCACATCAGGCAGGACGC
AM833	TTCGAACCCCAGAGTCCCGCTCAGA

AM838	TTATCGACACCCTGGAAGGCACCGCCAAGAGAAAGCAGGTAGCTTGCAGTGG
AM839	CGGGCCCAACATTTTATCGACACCCTGGAAGGCACCG
AM840	GGCGACTTTACCTGACTCTTGCGGCAGTTGGGCGTCGCTTGGTCGGTCATTT
AM841	GGAGCAAAAATGGGCGACTTTACCTGACTCTTGCGGC
AM843	GCGTCCTGCCTGATGTGCTCAGTTTCGAACCCCAGAGTCCCGCTCAGA
AM906	GACTAGTGAGATCGCAACTAGTTAATCTCATACCGGTACGCCCGCATGA
AM907	ATGATGATGATGATGATGGTCGACCTAGGAGCTTTTTGTAGAGGGTGATGCAGA
AM955	AATGTCAGCCACTAGGAGCTTTTTGTAGAGGGTGATGCAGA
AM956	CAAAAAGCTCCTAGTGGCTGACATTCTGCTGCTCGATAATAAGG
AM958	CAGTCTTTGTAAGTCGACCATCATCATCATCATCATTGAGTTTAAACG
AM959	TGATGATGGTCGACTTACAAAGACTGAGCTAATTTCTCAAATGGAGAGAGCA
AM965	AGTCACATCAAGATCGTTTATGG
AM966	GCACGGAATATGGGACTACTTCG
AM967	ACTCCACTTCAAGTAAGAGTTTG
AM988	CGGCTACTTACAAAGACTGAGCTAATTTCTCAAATGGAGAGAGCA
AM989	AATTAGCTCAGTCTTTGTAAGTAGCCGCATCCGGTATAGG
AM990	CAATGATGATGATGATGATGGTCGACTTAAATGCTACCACGGCGACCAGGC

AM1033	ACGGCGATTTATCCTAAGAGAAAGCAGGTAGCTTGCAGTGGG
AM1034	AAATCCAGCATACGATTTTGGGCGTCGCTTGGTCGGT
AM1035	ATGAGCGTAATGTTTGATCCAGACACGGCGATTTATCCTAAGAGAAAGCAGGTAG
AM1036	TTATCCACGTAGTGTAGCCGCAAAATCCAGCATACGATTTTGGGCGTCG
AM1139	ATGCCTATGACAACGCCTACTCTAATCGTGA
AM1266	GATGATGACAAATAGTGGCTGACATTCTGCTGCTCGATAATA
AM1267	GTCCTTATAGTCGGAGCTTTTTGTAGAGGGTGATGCAGAC
AM1268	GATGATGACAAATAAGTAGCCGCATCCGGTATAGGAGAA
AM1269	GTCCTTATAGTCCAAAGACTGAGCTAATTTCTCAAATGGAGAGAGC
AM1270	GATGATGACAAATAAGTCGACCATCATCATCATCATCATTGAGTTT
AM1271	GTCCTTATAGTCAATGCTACCACGGCGACCAG
AM1272	CGTCCTTATAGTCCATCGATTCACCCTCCTTATTATCGAGCAG
AM1273	ATGATGACAAAACAGTATCCACAGACAACACAAGTCCTG
AM1280	CTAGGAGCTTTTTGTAGAGGGTGATGC
AM1281	GTAGCCGCATCCGGTATAGGAGA
YK44	CGGCGATTTATCCTGTGTAGGCTGGAGC
YK45	ATCCAGCATACGATTATGGGAATTAGCCAT
YK48	ATGAGCGTAATGTTTGATCCAGACACGGCGATTTATCCT
YK49	TTATCCACGTAGTGTAGCCGCAAAATCCAGCATACGATT

GFP1	ATGAGCAAAGGAGAAGAACTTTTCACTGGAGTTGTCC
GFP2	GGATCCTTTGTAGAGCTCATCCATGCCATGTG

Table S2.

gBlock name	gBlock sequence
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Ctr_incA	TCTCATACCGGTACGCCCGCATGAAGGAGatcGAGCTCAT GCCTATGACAACGCCTACTCTAATCGTGACCCCTCCATCT CCCCCTGCACCTTCCTACTCAGCCAATCGCGTACCTCAAC CTTCTTTGATGGACAAAATTAAGAAAATAGCAGCCATTG CCTCCCTAATTCTTATAGGCACAATAGGCTTTTTAGCTCT TTTGGGACATCTTGTTGGCTTTCTGATCGCTCCACAAATC ACTATTGTTCTTCTTGCCCTATTCATTATCTCATTAGCAGG GAATGCTCTTTATCTACAGAAAACCGCTAATCTACATCTA TACCAGGATCTGCAAAGAGAAGTTGGGTCTCTAAAAGAA ATTAATTTCATGCTGAGCGTTCTACAGAAAGAATTTCTTC ATTTATCTAAAGAATTTGCAACGACATCTAAAGACCTCTC TGCTGTATCTCAAGATTTTTATTCTTGTTTGCAAGGATTTA GAGATAACTATAAAGGTTTTGAATCTCTTTTGGATGAGTA TAAAAACTCTACAGAAGAAATGCGCAAACTTTTTTCGCA AGAAATCATAGCAGATCTTAAAGGCTCTGTTGCCTCATTA AGAGAGGAAATCCGATTCCTAACCCCATTAGCAGAAGAA GTTCGCCGATTAGCGCATAACCAGCAATCATTAACAGTG GTTATTGAAGAATTAAAAACAATTCGTGATAGCTTACGA GATGAAATTGGACAACTTTCACAACTTTCTAAAACTCTTA CCAGTCAAATTGCATTACAACGAAAAGAGAGCTCAGATC TGTGTTCCCAGATAAGAGAGACGCTCTCCTCCCCCAGAA AGTCTGCATCACCCTCTACAAAAAGCTCCTAG
Cca_incA	TGGCTGACATTCTGCTGCTCGATAATAAGGAGGGTGAAT CGATGACAGTATCCACAGACAACACAAGTCCTGTAATAT CGAGAGCGTCCTCACCTACTTTTGGAGATCATGGTAAGG ATTTCGACAACAATAAAATTATACCCATTTCAATAGAAG CTCCAACTTCTTCAGCTGCTGCTGTAGGGGCTAAAACGGC TATCGAGCCTGAAGGAAGAAGCCCACTACTTCAAAGGAT TTGCTATCTTGTTAAAATTATCGCTGCCATCGCCCTCTTTG TTGTTGGTATCGCAGCCTTAGTTTGCTTATATCTCGGTAG CGTTATCTCAACGCCTTCTCTTATTCTTATGCTTGCGATCA TGCTTGTATCCTTTGTGATCGTTATTACGGCAATTCGAGA TGGCACACCGTCTCAAGTGGTCCGTCACATGAAACAGCA AATTCAGCAATTTGGCGAAGAAAACACGCGTTTACATAC CGCAGTAGAAAATCTAAAAGCTGTTAACGTTGAGCTCTC AGAGCAAATTAACCAACTTAAACAACTACATACTAGATT ATCGGATTTTGGTGATAGGCTTGAAGCGAATACCGGTGA TTTTACTGCACTTATTGCGGATTTCCAACTCAGTCTGGAA GAGTTTAAGTCTGTTGGTACTAAAGTTGAAACCATGCTCT CTCCATTTGAGAAATTAGCTCAGTCTTTGTAA

CT_813	CAGTCTTTGTAAGTAGCCGCATCCGGTATAGGAGAAAAC TAGTATGACCACTCTTCCCAATAACTGCACCAGTAATAGT AACAGCATCAACACATTTACAAAAGACATTGAAATGGCG AAGCAGATTCAAGGCTCACGTAAAGACCCATTGGCAAAA ACTTCGTGGATTGCGGGTCTTATCTGCGTAGTCGCAGGGG TACTGGGATTATTGGCTATCGGAATCGGAGGTTGTAGCAT GGCGTCGGGTTTAGGCCTTATCGGTGCAGTAGTCGCGGC AGTAATCGTCGCCGTTGGCCTGTGTTGCTTGGTTTCCGCA CTTTGTTTGCAAGTGGAAAAATCACAATGGTGGCAGAAG GAGTTTGAGTCATGGATCGAGCAGAAGAGCCAGTTTCGC ATCGTAATGGCAGACATGTTAAAGGCTAACCGTAAATTG CAGAGCGAAGTGGAATTCCTGAGTAAAGGGTGGTCCGAC GATACGGCGGTACATAAAGAGGATGTAACCAAGTACGAG CAAGTGGTGGAGGAATACGCCGAAAAGATTATGGAGCTT TACGAAGAAACGGGGGTTCTTACAATCGAGAAAATCAAT TTACAGAAAGAAAAGAAAGCGTGGCTGGAGGAAAAAGC TGAAATGGAGCAAAAGCTGACGACTGTAACGGACTTGGA AGCAGCGAAGCAACAGCTTGAAGAAAAAGTGACAGACT TGGAGTCAGAAAAACAGGAGCTGCGCGAAGAACTGGAT AAGGCGATCGAAAATCTGGACGAGATGGCATATGAGGCC ATGGAGTTTGAAAAGGAAAAGCACGGTATTAAGCCTGGT CGCCGTGGTAGCATTTAA

Table S3.

Oligonucleotide name	Oligonucleotide sequence
Spacer-1	TCTCATACCGGTACGCCCGCATGAAGGAGATCGAGCTC
Spacer-2	TGGCTGACATTCTGCTGCTCGATAATAAGGAGGGTGAATCG
Spacer-3	GTAGCCGCATCCGGTATAGGAGAAAACTAGT

Caption for Movie S1.

Animated sequence showing the correlation between LAC values in the tomographic reconstruction of a *S. cerevisiae cox2-60* - *E. coli ΔnadA* chimera cell and segmented structures. Orthoslices from the reconstructed cell were sampled at regular intervals throughout the volume and displayed in grey scale (dark is higher LAC value). The segmented organelle volumes were overlaid into this sequence of orthoslices and then shown in isolation. Color key: blue - nucleus; salmon - mitochondria; green - vacuole; grey - lipid; yellow - density not attributable to any other major organelle. Animation was generated using the Amira software package.

Caption for Movie S2.

Animated sequence showing the correlation between LAC values in the tomographic reconstruction of a *S. cerevisiae cox2-60* cell and segmented structures. Orthoslices from the reconstructed cell were sampled at regular intervals throughout the volume and displayed in grey scale (dark is higher LAC value). The segmented organelle volumes were overlaid into this sequence of orthoslices and then shown in isolation. Color key: blue - nucleus; salmon - mitochondria; green - vacuole; grey - lipid. Animation was generated using the Amira software package.

References:

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