Supplementary Materials

Towards a synthetic yeast endosymbiont with a minimal genome

Angad P. Mehta, Yeonjin Ko, Lubica Supekova, Kersi Pestonjamasp, Jack Li, Peter G. Schultz.
correspondence to: schultz@scripps.edu

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Materials and Methods

Growth media

E. coli cells were routinely cultured in 2YT or LB medium. *E. coli* auxotrophy was 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. In order to calculate the growth rate of the chimeras, one spot of the cells were scraped off the growth medium plates and resuspended in 1 M sorbitol. The cells were counted by countess II automated cell counter (Thermo Fisher Scientific).

Construction of gene disruption cassettes

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, 30 sec; Step 4: 72 ^{o}C, 30 sec/kb; (35 cycles step 2 to step 4); step 5: 72 ^{o}C, 2 min).

tet^{R} gene disruption cassette: the cassette was used to knock out the essential genes in the amino acid biosynthetic pathway. The linear DNA fragment used for knockout was amplified from either plasmid pAM22[2] or the genomic DNA of the *E. coli* mutant with the tetracycline resistance cassette. The PCR product was purified by gel extraction (Macherey-Nagel Nucleospin gel and PCR clean-up kit). Primer used for *ΔmetA*: YK95/YK96; *ΔilvD*: YK54/YK55 and then YK56/YK57; *ΔargA*: YK180/YK181; *ΔproA*: YK190/YK191; *ΔglnA*: YK186/YK187; *ΔilvLXGMEDAYC*: YK216/YK217. All oligonucleotides used in PCR amplification reactions are listed in Table S1.

Construction of *E. coli* genetic knockout strains

The multiple amino acid auxotrophic strains were generated by sequentially disrupting genes involved in the amino acid biosynthesis. The *tet*^{R} gene of *E. coli ΔnadA::tet*^{R} *ΔthiC::gfp-kan*^{R} that was previously reported[3] was eliminated using plasmid pCP20 encoding the FLP recombinase as described[1]. Then gene deletion was performed using the phage λ Red recombinase method with a PCR-amplified *tet*^{R} cassette[1]. The removal of *tet*^{R} gene and genetic inactivation by adding *tet*^{R} cassette were repeated to generate multiple gene deletion mutants. Multiple amino acid auxotrophy was confirmed by growing the mutant *E. coli* strain in M9 minimal media in the presence and absence of 0.1 mM thiamin, 0.1 mM NAD, and 0.3 mM corresponding amino acids. All the single amino acid auxotrophs were obtained from the CGSC Keio collection.[1]

Total genomic DNA isolation and PCR analysis

For qualitative PCR analysis, yeast cells were collected from the agar plates and resuspended in 300 µl of 1 M sorbitol solution. 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 ^{o}C prior to PCR analysis. The *S. cerevisiae* specific oligonucleotide primers previously reported were used to detect the *S. cerevisiae MAT***a** gene.[3-4] Similarly, to detect the *E. coli* genome, the *gfp* primers (AM572/AM656), the *kan* primers (AM1039/AM1040), or the *tet* primers (AM162/AM163 or YK61/YK62) were used.[3] All oligonucleotides used in PCR amplification reactions are listed in Table S2.

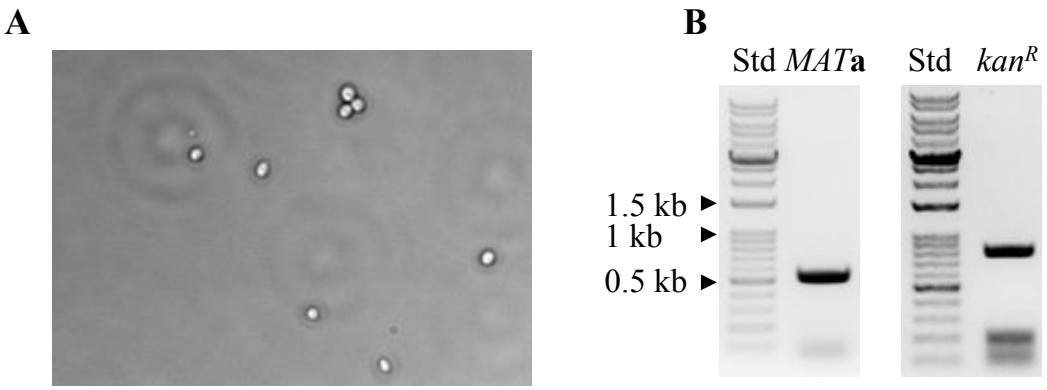

Fig. S1. Fusion of *S. cerevisiae cox2-60* mutant with *E. coli ΔilvD::kan*^{R} (pAM136) cells yields yeast colonies that have partially restored respiration-competent phenotype.

(**A**) Fusion of *S. cerevisiae cox2-60* (NB97) with *E. coli* DH10B *ΔilvD::kan*^{R} (pAM136) cells yields yeast colonies growing on medium containing 3% glycerol and 0.1% glucose (Selection Medium I + 50 mg/L carbenicillin) that lack extracellular *E. coli* cells upon microscopic examination.

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

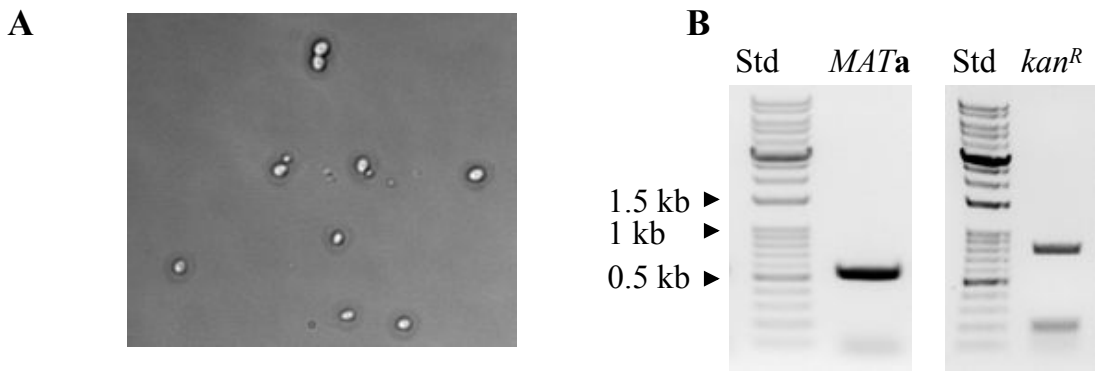

Fig. S2. Fusion of *S. cerevisiae cox2-60* mutant with *E. coli ΔtrpC::kan*^{R} (pAM136) cells yields yeast colonies that have partially restored respiration-competent phenotype.

(**A**) Fusion of *S. cerevisiae cox2-60* (NB97) with *E. coli* DH10B *ΔtrpC::kan*^{R} (pAM136) cells yields yeast colonies growing on medium containing 3% glycerol and 0.1% glucose (Selection Medium I + 50 mg/L carbenicillin) that lack extracellular *E. coli* cells upon microscopic examination.

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

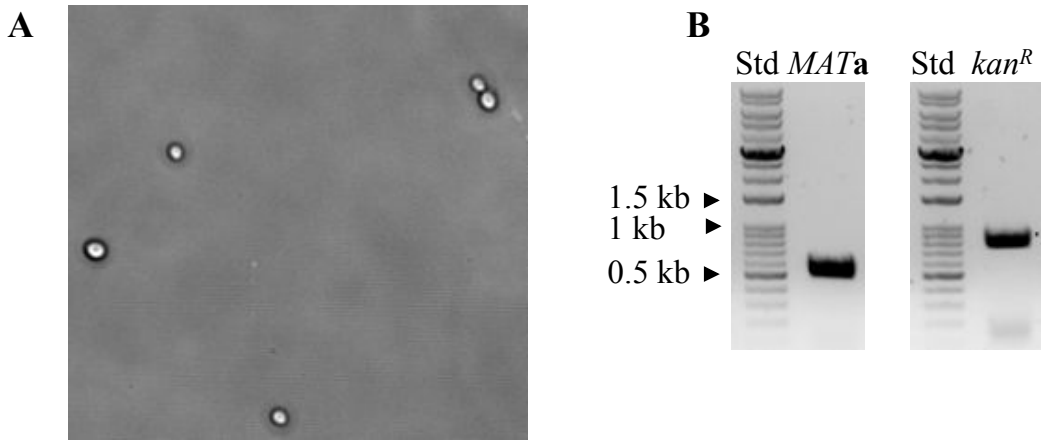

Fig. S3. Fusion of *S. cerevisiae cox2-60* mutant with *E. coli ΔserA::kan*^{R} (pAM136) cells yields yeast colonies that have partially restored respiration-competent phenotype.

(**A**) Fusion of *S. cerevisiae cox2-60* (NB97) with *E. coli* DH10B *ΔserA::kan*^{R} (pAM136) cells yields yeast colonies growing on medium containing 3% glycerol and 0.1% glucose (Selection Medium I + 50 mg/L carbenicillin) that lack extracellular *E. coli* cells upon microscopic examination.

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

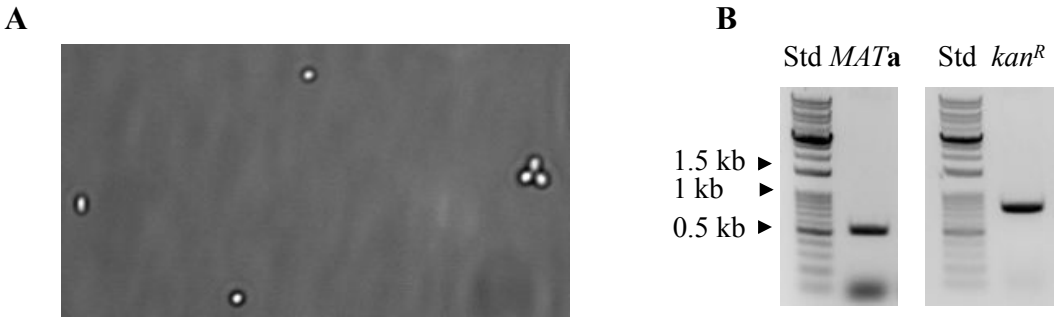

Fig. S4. Fusion of *S. cerevisiae cox2-60* mutant with *E. coli ΔmetA::kan*^{R} (pAM136) cells yields yeast colonies that have partially restored respiration-competent phenotype.

(**A**) Fusion of *S. cerevisiae cox2-60* (NB97) with *E. coli* DH10B *ΔmetA::kan*^{R} (pAM136) cells yields yeast colonies growing on medium containing 3% glycerol and 0.1% glucose (Selection Medium I + 50 mg/L carbenicillin) that lack extracellular *E. coli* cells upon microscopic examination.

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

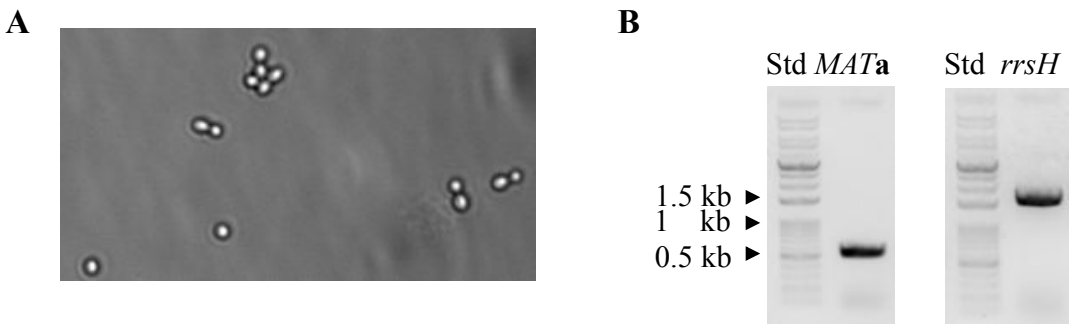

Fig. S5. Fusion of *S. cerevisiae cox2-60* mutant with *E. coli* PA340 (pAM136) cells yields yeast colonies that have partially restored respiration-competent phenotype.
(**A**) Fusion of *S. cerevisiae cox2-60* (NB97) with *E. coli* DH10B PA340 (pAM136) cells yields yeast colonies growing on medium containing 3% glycerol and 0.1% glucose (Selection Medium I + 50 mg/L carbenicillin) that lack extracellular *E. coli* cells upon microscopic examination.
(**B**) Detection of NB97-specific *MAT***a** mating type gene and *E. coli rrsH* gene by PCR of total DNA isolated from a randomly selected yeast colony. The PCR product of the *E. coli rrsH* gene was sequenced to confirm its identity.

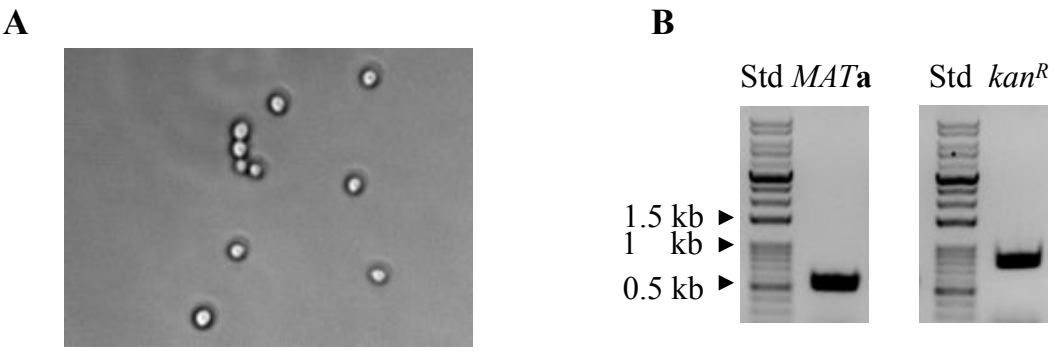

Fig. S6. Fusion of *S. cerevisiae cox2-60* mutant with *E. coli ΔcysE::kan*^{R} (pAM136) cells yields yeast colonies that have partially restored respiration-competent phenotype.

(**A**) Fusion of *S. cerevisiae cox2-60* (NB97) with *E. coli* DH10B *ΔcysE::kan*^{R} (pAM136) cells yields yeast colonies growing on medium containing 3% glycerol and 0.1% glucose (Selection Medium I + 50 mg/L carbenicillin) that lack extracellular *E. coli* cells upon microscopic examination.

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

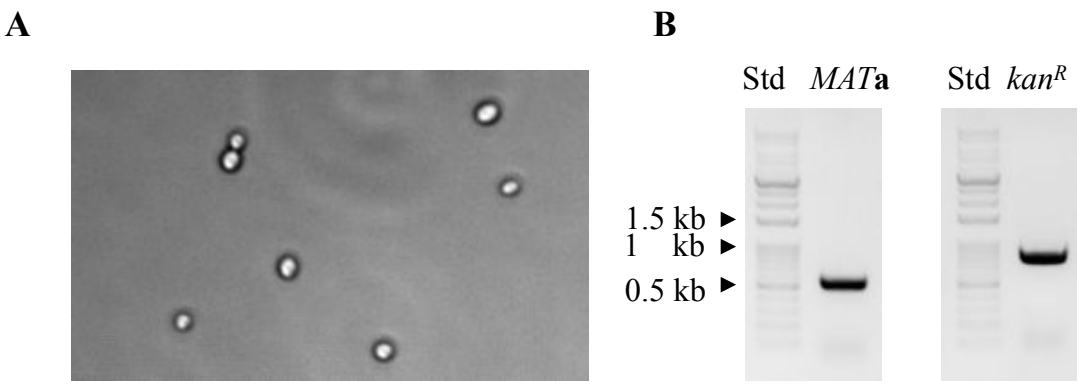

Fig. S7. Fusion of *S. cerevisiae cox2-60* mutant with *E. coli ΔglnA::kan*^{R} (pAM136) cells yields yeast colonies that have partially restored respiration-competent phenotype.

(**A**) Fusion of *S. cerevisiae cox2-60* (NB97) with *E. coli* DH10B *ΔglnA::kan*^{R} (pAM136) cells yields yeast colonies growing on medium containing 3% glycerol and 0.1% glucose (Selection Medium I + 50 mg/L carbenicillin) that lack extracellular *E. coli* cells upon microscopic examination.

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

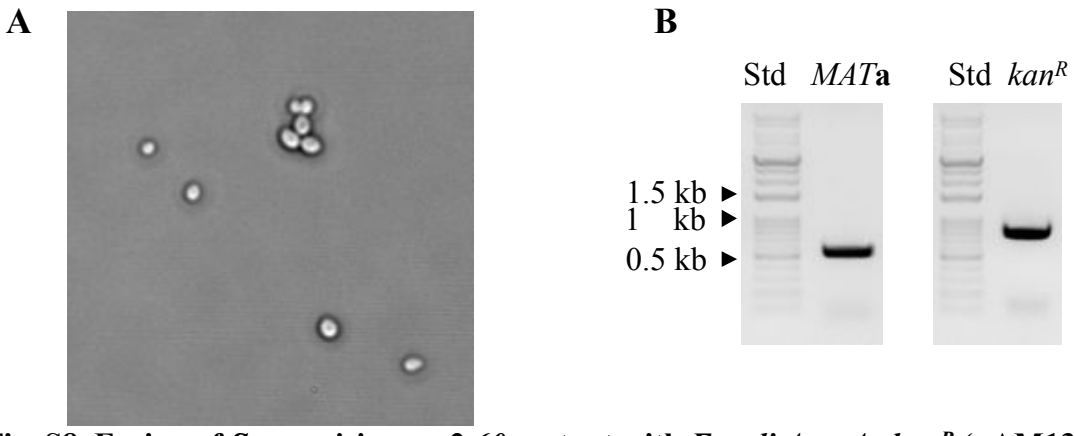

Fig. S8. Fusion of *S. cerevisiae cox2-60* mutant with *E. coli ΔargA::kan*^{R} (pAM136) cells yields yeast colonies that have partially restored respiration-competent phenotype.

(**A**) Fusion of *S. cerevisiae cox2-60* (NB97) with *E. coli* DH10B *ΔargA::kan*^{R} (pAM136) cells yields yeast colonies growing on medium containing 3% glycerol and 0.1% glucose (Selection Medium I + 50 mg/L carbenicillin) that lack extracellular *E. coli* cells upon microscopic examination.

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

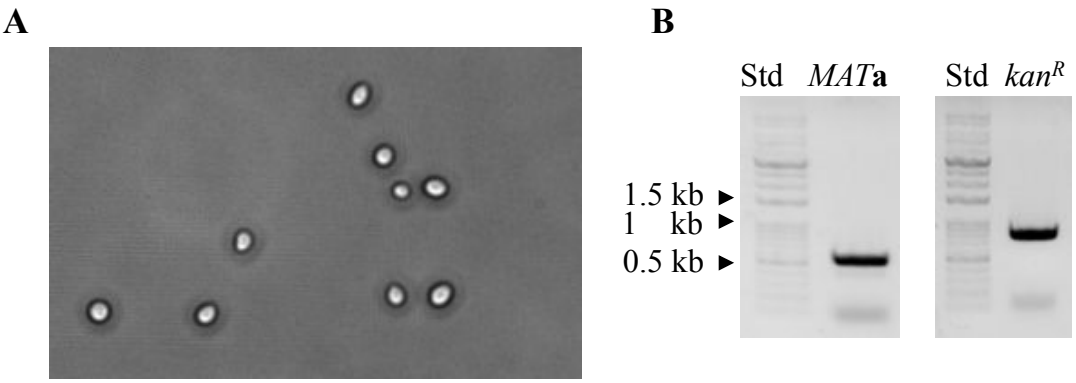

Fig. S9. Fusion of *S. cerevisiae cox2-60* mutant with *E. coli ΔproA::kan*^{R} (pAM136) cells yields yeast colonies that have partially restored respiration-competent phenotype.

(**A**) Fusion of *S. cerevisiae cox2-60* (NB97) with *E. coli* DH10B *ΔproA::kan*^{R} (pAM136) cells yields yeast colonies growing on medium containing 3% glycerol and 0.1% glucose (Selection Medium I + 50 mg/L carbenicillin) that lack extracellular *E. coli* cells upon microscopic examination.

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

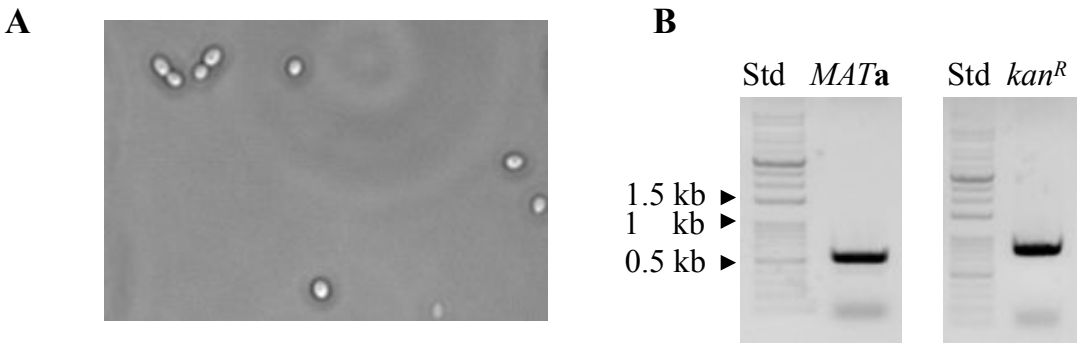

Fig. S10. Fusion of *S. cerevisiae cox2-60* mutant with *E. coli ΔlysA::kan*^{R} (pAM136) cells yields yeast colonies that have partially restored respiration-competent phenotype.

(**A**) Fusion of *S. cerevisiae cox2-60* (NB97) with *E. coli* DH10B *ΔlysA::kan*^{R} (pAM136) cells yields yeast colonies growing on medium containing 3% glycerol and 0.1% glucose (Selection Medium I + 50 mg/L carbenicillin) that lack extracellular *E. coli* cells upon microscopic examination.

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

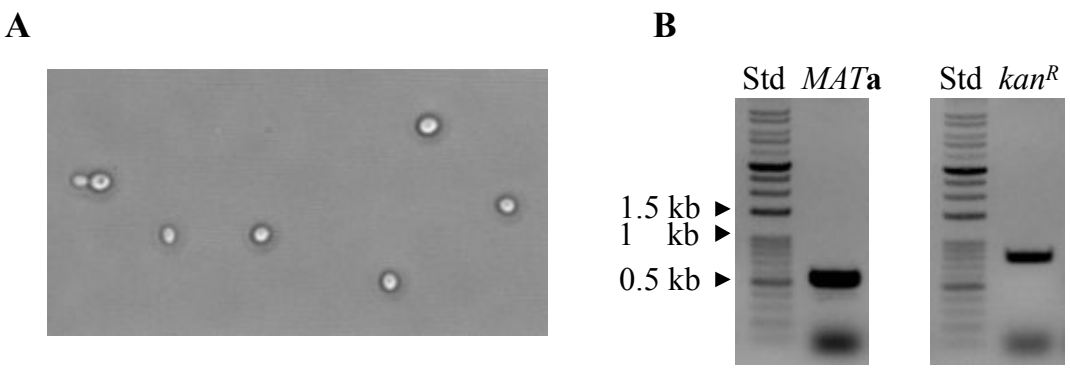

Fig. S11. Fusion of *S. cerevisiae cox2-60* mutant with *E. coli ΔthrC::kan*^{R} (pAM136) cells yields yeast colonies that have partially restored respiration-competent phenotype.

(**A**) Fusion of *S. cerevisiae cox2-60* (NB97) with *E. coli* DH10B *ΔthrC::kan*^{R} (pAM136) cells yields yeast colonies growing on medium containing 3% glycerol and 0.1% glucose (Selection Medium I + 50 mg/L carbenicillin) that lack extracellular *E. coli* cells upon microscopic examination.

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

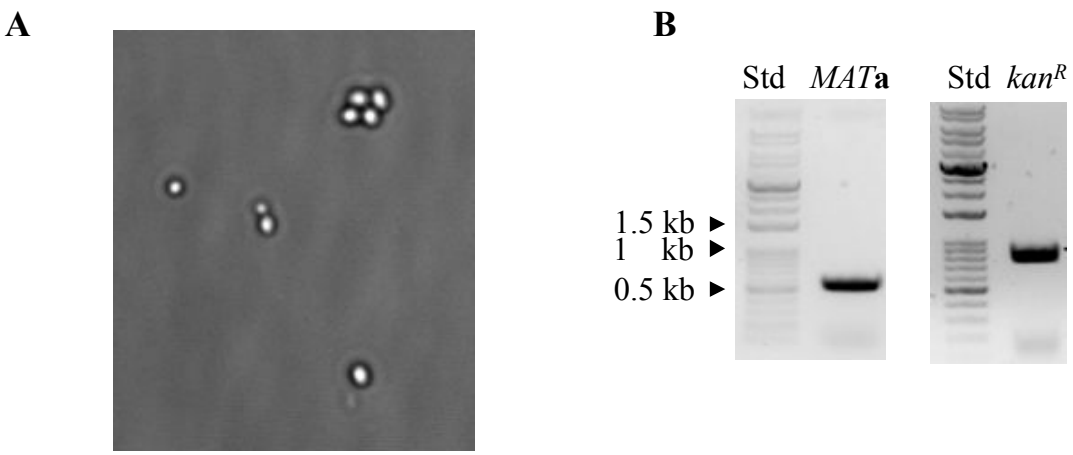

Fig. S12. Fusion of *S. cerevisiae cox2-60* mutant with *E. coli ΔhisB::kan*^{R} (pAM136) cells yields yeast colonies that have partially restored respiration-competent phenotype.

(**A**) Fusion of *S. cerevisiae cox2-60* (NB97) with *E. coli* DH10B *ΔhisB::kan*^{R} (pAM136) cells yields yeast colonies growing on medium containing 3% glycerol and 0.1% glucose (Selection Medium I + 50 mg/L carbenicillin) that lack extracellular *E. coli* cells upon microscopic examination.

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

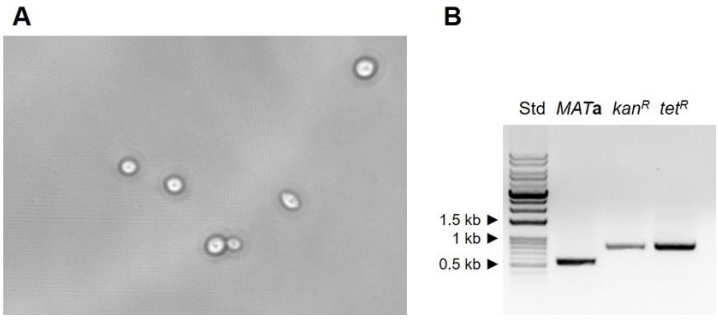

Fig. S13. Fusion of *S. cerevisiae cox2-60* mutant with *E. coli ΔthiC ΔnadA ΔmetA* (pAM136) cells yields yeast colonies that have partially restored respiration-competent phenotype.

(**A**) Fusion of *S. cerevisiae cox2-60* (NB97) with *E. coli* DH10B *ΔnadA ΔmetA::tet*^{R} *ΔthiC::gfp-kan*^{R} (pAM136) cells yields yeast colonies growing on medium containing 3% glycerol and 0.1% glucose (Selection Medium I + 50 mg/L carbenicillin) that lack extracellular *E. coli* cells upon microscopic examination.

(**B**) Detection of NB97-specific *MAT***a** mating type gene and *E. coli kan*^{R} and *tet*^{R} genes by PCR of total DNA isolated from a randomly selected yeast colony. The gene corresponding to *tet*^{R} was amplified by using primers YK61 and YK62.

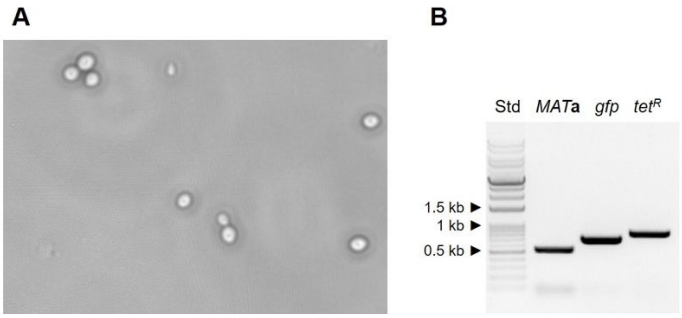

Fig. S14. Fusion of *S. cerevisiae cox2-60* mutant with *E. coli ΔthiC ΔnadA ΔmetA ΔilvD* (pAM136) cells yields yeast colonies that have partially restored respiration-competent phenotype.

(**A**) Fusion of *S. cerevisiae cox2-60* (NB97) with *E. coli* DH10B *ΔnadA ΔmetA ΔilvD::tet*^{R} *ΔthiC::gfp-kan*^{R} (pAM136) cells yields yeast colonies growing on medium containing 3% glycerol and 0.1% glucose (Selection Medium I + 50 mg/L carbenicillin) that lack extracellular *E. coli* cells upon microscopic examination.

(**B**) Detection of NB97-specific *MAT***a** mating type gene and *E. coli gfp* and *tet*^{R} genes by PCR of total DNA isolated from a randomly selected yeast colony. The gene corresponding to *tet*^{R} was amplified by using primers YK61 and YK62.

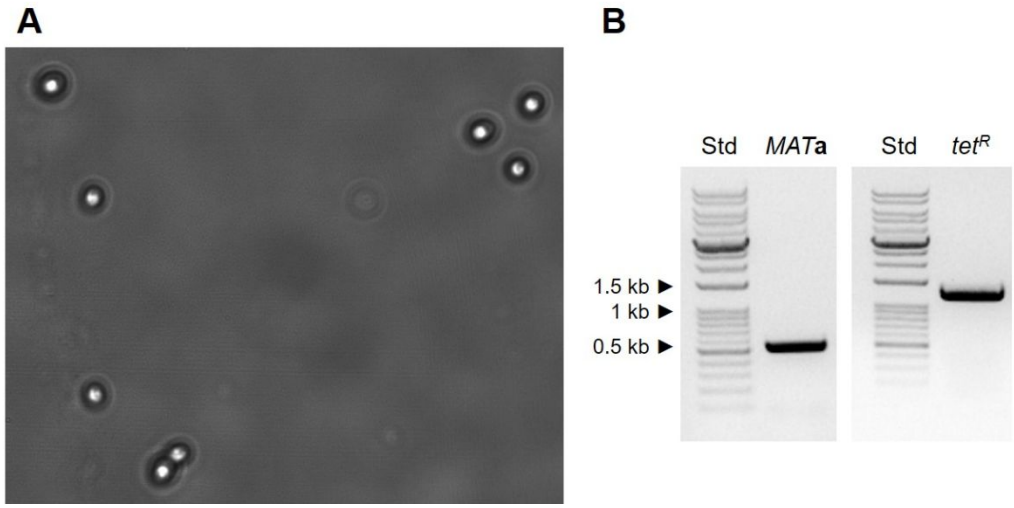

Fig. S15. Fusion of *S. cerevisiae cox2-60* mutant with *E. coli ΔthiC ΔnadA ΔmetA ΔilvD ΔargA* (pAM136) cells yields yeast colonies that have partially restored respiration-competent phenotype.

(**A**) Fusion of *S. cerevisiae cox2-60* (NB97) with *E. coli* DH10B *ΔnadA ΔmetA ΔilvD ΔargA::tet*^{R} *ΔthiC::gfp-kan*^{R} (pAM136) cells yields yeast colonies growing on medium containing 3% glycerol as the sole carbon source (Selection Medium II + 50 mg/L carbenicillin) that lack extracellular *E. coli* cells upon microscopic examination.

(**B**) Detection of NB97-specific *MAT***a** mating type gene and *E. coli tet*^{R} genes by PCR of total DNA isolated from a randomly selected yeast colony. The gene corresponding to *tet*^{R} was amplified by using primers AM162 and AM163.

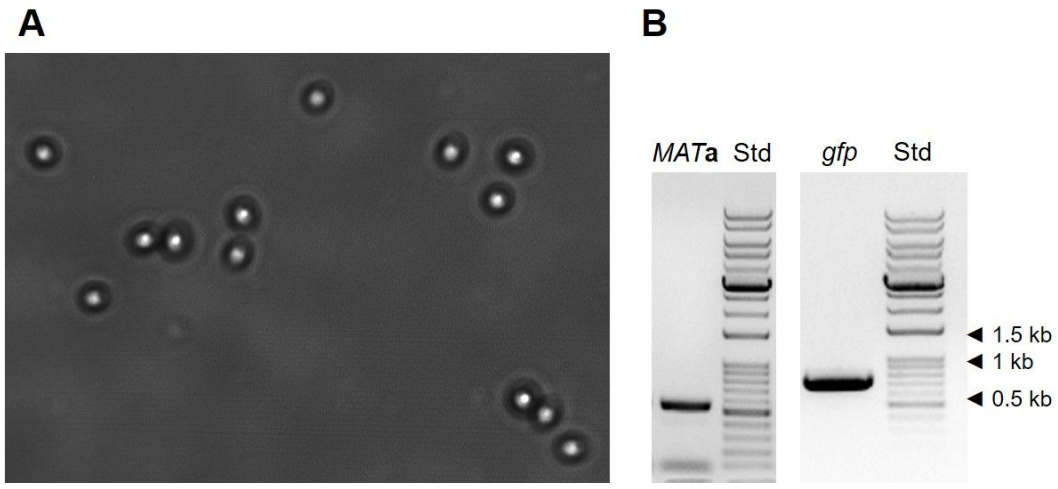

Fig. S16. Fusion of *S. cerevisiae cox2-60* mutant with *E. coli ΔthiC ΔnadA ΔmetA ΔilvD ΔargA ΔproA* (pAM136) cells yields yeast colonies that have partially restored respiration-competent phenotype.
(**A**) Fusion of *S. cerevisiae cox2-60* (NB97) with *E. coli* DH10B *ΔnadA ΔmetA ΔilvD ΔargA ΔproA::tet*^{R} *ΔthiC::gfp-kan*^{R} (pAM136) cells yields yeast colonies growing on medium containing 3% glycerol as the sole carbon source (Selection Medium II + 50 mg/L carbenicillin) that lack extracellular *E. coli* cells upon microscopic examination.
(**B**) Detection of NB97-specific *MAT***a** mating type gene and *E. coli gfp* genes by PCR of total DNA isolated from a randomly selected yeast colony.

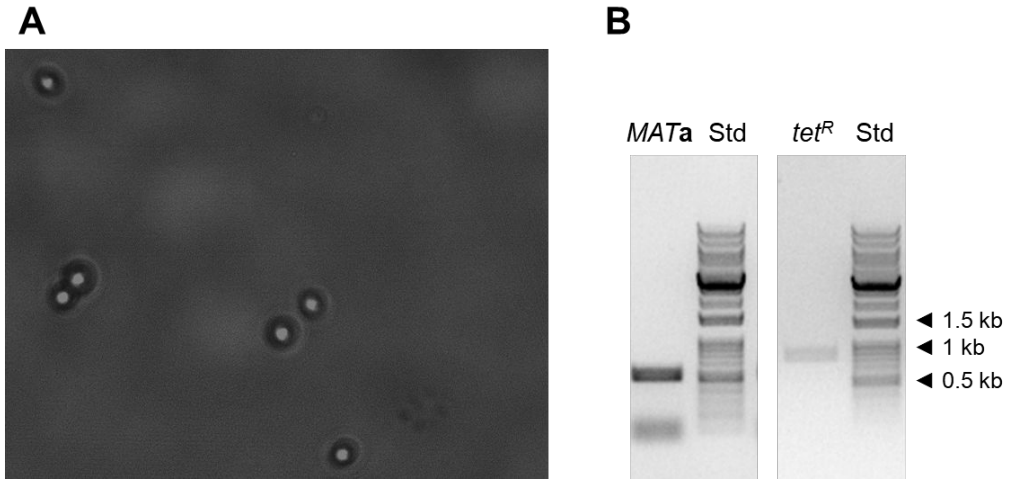

Fig. S17. Fusion of *S. cerevisiae cox2-60* mutant with *E. coli ΔthiC ΔnadA ΔmetA ΔilvD ΔargA ΔproA ΔglnA* (pAM136) cells yields yeast colonies that have partially restored respiration-competent phenotype.

(**A**) Fusion of *S. cerevisiae cox2-60* (NB97) with *E. coli* DH10B *ΔnadA ΔmetA ΔilvD ΔargA ΔproA ΔglnA::tet*^{R} *ΔthiC::gfp-kan*^{R} (pAM136) cells yields yeast colonies growing on medium containing 3% glycerol as the sole carbon source (Selection Medium II + 50 mg/L carbenicillin) that lack extracellular *E. coli* cells upon microscopic examination.
(**B**) Detection of NB97-specific *MAT***a** mating type gene and *E. coli tet*^{R} genes by PCR of total DNA isolated from a randomly selected yeast colony. The gene corresponding to *tet*^{R} was amplified by using primers YK61 and YK62.

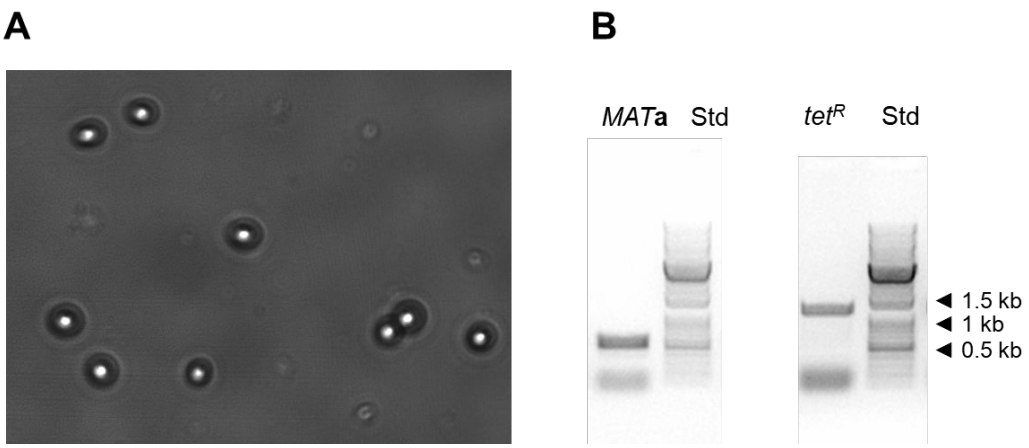

Fig. S18. Fusion of *S. cerevisiae cox2-60* mutant with *E. coli ΔthiC ΔilvLXGMEDAYC* (pAM136) cells yields yeast colonies that have partially restored respiration-competent phenotype.
(**A**) Fusion of *S. cerevisiae cox2-60* (NB97) with *E. coli* DH10B *ΔilvLXGMEDAYC::tet*^{R} *ΔthiC::gfp-kan*^{R} (pAM136) cells yields yeast colonies growing on medium containing 3% glycerol as the sole carbon source (Selection Medium II + 50 mg/L carbenicillin) that lack extracellular *E. coli* cells upon microscopic examination.
(**B**) Detection of NB97-specific *MAT***a** mating type gene and *E. coli tet*^{R} genes by PCR of total DNA isolated from a randomly selected yeast colony. The gene corresponding to *tet*^{R} was amplified by using primers AM162 and AM163.

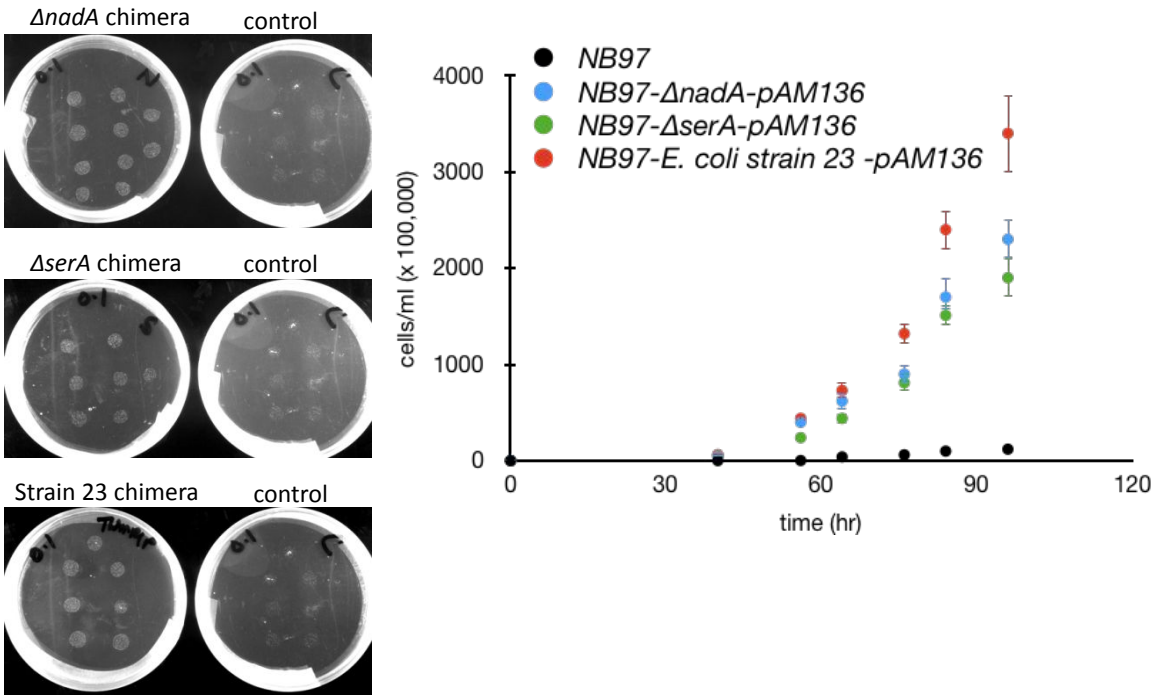

Figure S19: Growth rates for *S. cerevisiae cox2-60* - *E. coli* chimeras on selection medium I: Plates show significant growth (40 h time point) observed for *S. cerevisiae cox2-60* - *E. coli* Δ*nadA*:*gfp*-pAM136 chimeras (Δ*nadA* chimera), *S. cerevisiae cox2-60* - *E. coli* Δ*serA*-pAM136 chimeras (Δ*serA* chimera) and *S. cerevisiae cox2-60* - *E. coli* Δ Δ*thiC*:*gfp* Δ*nadA* Δ*metA* Δ*ilvD* Δ*argA* Δ*proA* -pAM136 chimeras (Strain 23 chimera). On the other hand, no significant growth was observed for *S. cerevisiae cox2-60* fused to *E. coli* Δ*nadA* lacking pAM136 (control). The plot shows growth rates for *S. cerevisiae cox2-60* - *E. coli* Δ*nadA*-pAM136 chimeras (blue), *S. cerevisiae cox2-60* - *E. coli* ΔserA-pAM136 chimeras (green), *S. cerevisiae cox2-60* - *E. coli* Δ*thiC*:*gfp* Δ*nadA* Δ*metA* Δ*ilvD* Δ*argA* Δ*proA* -pAM136 chimeras (red) and control *S. cerevisiae cox2-60* fused to *E. coli* Δ*nadA* lacking pAM136 (black). The cells were counted by countess II automated cell counter (Thermo Fisher Scientific).

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

Deletion target	Oligonucleotide name	Oligonucleotide sequence
ilvD	**YK54**	ACCACCACTCATGGTCGTGTGTAGGCTGGAG
	YK55	ATCACGCACCGCGCCTTTATGGGAATTAGC
	YK56	ATGCCTAAGTACCGTTCCGCCACCACCACTCAT
	YK57	TTAACCCCCCAGTTTCGATTTATCACGCACCGC
metA	**YK95**	TAAACGTATAAGCGTATGTAGTGAGGTAATCAGGTTATGGTGTAGGCTGGAGCTGCTTC
	YK96	ATCGACTATCACAGAAGATTAATCCAGCGTTGGATTCATATGGGAATTAGCCATGGTCC
argA	**YK180**	ACACTAATTTCGAATAATCATGCAAAGAGGTGTGCCGTGGTGTAGGCTGGAGCTGCTTC
	YK181	TCCGACGATTTTCATCGCTTACCCTAAATCCGCCATCAAATGGGAATTAGCCATGGTCC
glnA	**YK186**	CCACGACGACCATGACCAATCCAGGAGAGTTAAAGTATGGTGTAGGCTGGAGCTGCTTC
	YK187	CCACGGCAACTAAAACACTTAGACGCTGTAGTACAGCTCATGGGAATTAGCCATGGTCC
proA	**YK190**	CCGTGATGACATGATTACCCGTTAAGGAGCAGGCTGATGGTGTAGGCTGGAGCTGCTTC
	YK191	TGCATCACCCGGTTTTATTTACGCACGAATGGTGTAATCATGGGAATTAGCCATGGTCC
ilvLXGMEDAYC	**YK216**	TCGCAAAAATGCAGCGGACAAAGGATGAACTACGAGGAAGTGTAGGCTGGAGCTGCTTC
	YK217	CGCACTTAACCCGCAACAGCAATACGTTTCATATCTGTCATGGGAATTAGCCATGGTCC

Table S2.

PCR oligonucleotides used for genome analysis.

Oligonucleotide name	Oligonucleotide sequence
AM572	ATGAGCAAAGGAGAAGAACTTTTCACTGGAGT TGTCC
AM656	GGATCCTTTGTAGAGCTCATCCATGCCATGTG
AM965	AGTCACATCAAGATCGTTTATGG
AM966	GCACGGAATATGGGACTACTTCG
AM967	ACTCCACTTCAAGTAAGAGTTTG
AM1039	ATGATTGAACAAGATGGATTGCACGCAGG
AM1040	TCAGAAGAACTCGTCAAGAAGGCGATAGAAGG
AM162	ATGAAATCTAACAATGCGCTCATCGTCATCCTC
AM163	TCAGGTCGAGGTGGCCCGGCTCCATG
YK61	GCTCATCGTCATCCTCGGCACC
YK62	GCCTGCTTCTCGCCGAAACG

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