Supplemental Material

Table S1. Genotypes and Sources of *Saccharomyces cerevisiae* Strains

Strain	Genotype	mtDNA	Source
W303-1A	**MAT**a *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1*	ρ^+	a
W303-1B	**MAT**α *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1*	ρ^+	a
MR6	**MAT**a *ade2-1 arg8::HIS3 his3-1,15 leu2-3,112 trp1-1 ura3-1*	ρ^+ (S288C)	Rak *et al.* 2007
MRS-3A	**MAT**a *ade2-1 arg8::HIS3 his3-1,15 leu2-3,112 trp1-1 ura3-1*	ρ^+ (S288C)	(McStay *et al.*, 2013)
MRS-3B	**MAT**α *ade2-1 arg8::HIS3 his3-1,15 leu2-3,112 trp1-1 ura3-1*	ρ^+ (S288C)	(McStay *et al.*, 2013)
MRS-3Bρ^0	**MAT**α *ade2-1 arg8::HIS3 his3-1,15 leu2-3,112 trp1-1 ura3-1*	ρ^0	(McStay *et al.*, 2013)
aDFKρ^0	**MAT**a *kar1-1 ade2-10 arg8::URA3 leu2Δ ura3-52 lys2*	ρ^o	(McStay *et al.*, 2013)
DFKρ^0	**MAT**α *kar1-1 ade2-10 arg8::URA3 leu2Δ ura3-52 lys2*	ρ^o	(McStay *et al.*, 2013)
SCS9S	**MAT**α *kar1ade2-101 arg8::URA3 leu2Δ ura3-52 lys2*	ρ^+ *COX2-HAC*	b
HMD22	**MATa** *leu2-3,112 ura3-52 lys2 his3Δ αrg8::hisG*	ρ^+ cox2*::ARG8*^{m}	b
MRSΔCOX2	**MAT**α *ade2-1 arg8::HIS3 his3-1,15 leu2-3,112 trp1-1 ura3-1*	ρ^+ cox2*::ARG8*^{m}	HMD22 x MRS-3Bρ^0

MRSI^{o}/COX1-HAC	**MAT**α *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1arg8::HIS3*	Intronless mtDNA with*COX3-HAC*	(McStay *et al.*, 2013)
MRS/COX2-pH	**MAT**α *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1arg8::HIS3*	*COX2-pH*	This study
MRS/COX2-HA	**MAT**α *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1arg8::HIS3*	*COX2-HA*	This study
MRS/COX2-HAC	**MAT**α *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1arg8::HIS3*	*COX2-HAC*	This study
W303/COX2-HAC	**MAT**α *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1*	*COX2-HAC*	This study
W303ΔCOX1/COX2-HAC	**MAT**α *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1*	*COX2-HAC, Δcox1::ARG8^{m}*	This study
W303/COX9-HAC	**MATα** *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 cox12::HIS3 leu2::pG16/ST14*	ρ^{+}	(McStay et al, 2013)
W303ΔCOX1/COX9-HAC	**MATa** *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 cox12::HIS3 cox1::ARG8^{m} leu2::pG16/ST14*	ρ^{+}	This study
aW303/SCO1-CH	**MATa** *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 sco1::URA3 trp1::pG41/ST50*	ρ^{+}	This study
W303ΔSCO1/COX2-HAC	**MATα** *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 sco1::URA3 trp1::pG41/ST50*	*COX2-HAC*	This study
aW303/COA6-CH	**MATa** *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 coa6::HIS3 trp1::pCOA6/ST3*	ρ^{+}	This study
aW303ΔMSS51/COX12-HAC	**MATa** *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 cox12::HIS3 mss51::HIS3 trp1::pCOX12/ST4*	ρ^{+}	This study

aW303/COX13-HAC	**MATa** *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 cox12::HIS3 trp1::pCOX13/ST4*	ρ^+	(McStay et al, 2013a)
aW303ΔMSS51/COX13-HAC	**MATa** *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 cox12::HIS3 mss51::HIS3 trp1::pCOX13/ST4*	ρ^+	This study
aW303ΔCOX16	**MATa** *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 cox16::URA3*	ρ^+	(Carlson et al, 2003)
aW303/COX16-CH	**MATa** *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 cox16::URA3 trp1::pG22/ST13*	ρ^+	This study
aW303ΔMSS51/COX16-CH	**MATa** *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 cox16::URA3 trp1::pG22/ST13 mss51::HIS3*	ρ^+	This study
aW303ΔCOX18	**MATa** *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 cox18::URA3*	ρ^+	(Souza et al, 2000)
aW303ΔCOX18-HAC	**MATα** *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 cox18::URA3 leu2::pG34/ST13*	ρ^+	This study
aW303ΔCOX20	**MATa** *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 cox20::URA3*	ρ^+	(Hell et al, 2000)
aW303ΔCOX20-HAC	**MATa** *a ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1cox20::URA3 leu2::pG92/ST20*	ρ^+	This study
aW303ΔPET100	**MATa** *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 pet100::URA3*	ρ^+	This study
aW303ΔPET100-CH	**MATa** *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 pet100::URA3 trp1::pG135/ST6*	ρ^+	This study
W303ΔPET117	**MATα** *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 pet117::HIS3*	ρ^+	This study

W303/PET117-CH	**MATα** *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 pet117::HIS3 trp1::pG8/ST10*	ρ^+	This study

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Table S2. Sequences of primers

Primer name	Primer sequence (5’ -> 3’)
	Primer used to amplify a COX2-HA hybrid gene
cox2-5	ggcgagctccttgctgtcatacctttat
cox2-13	ggcggatccttattaagcgtagtctgggacgtcgtatgggtattgttcatttaatcattccaaaaattagg
cox2-11	ggcggatccttaatatttacttattattaatatttttaa
cox2-12	ggcctgcagcaaggatacttatcaaaaatagttac
	Primers used to amplify the mitochondrial COX2-HAC hybrid gene
cox2-5	ggcgagctccttgctgtcatacctttat
HA-proC	ggcggatccttacttaccatcgattaaccgtggatctacctgatcttcacctccagcgtagtctgggacgtcgta
	Primers used to construct nuclear genes expressing HAC or CH tagged proteins
cox18-1	gaaaggcggatcccaaatgagtttaatagagatg
cox18-2	ggcgacgtctcaagcgtagtctgggacgtcgtatcgttggtaaggataaatccaa
C-phis	ggcctgcagtcatttaccatcgattaatcttggatctacttgatcttctcctccagcgtagtctgggacgtcgtatgg
cox20-1	ggcggatcccaactagctctattagtt
cox20-2	ggcgacgtctcaagcgtagtctgggacgtcgtaatgggaccagaacttgtaccatttctt
cox16-1	ggcgagctctctgggaaggtttcaactcat
cox16-2	ggcctgcagccagacattctcagattcatc
pet100-3	ggcgagctccgctccatcattagtatccttc
pet100-5	ggcctgcagtccttttttgagagccaaatcccg
pet117-1	ggcgagctccgctccatcattagtatccttc
pet117-2	ggcctgcagtccttttttgagagccaaatcccg
cyc1-1	ggcgagctcatggagaaaggagttataatg
cyc1-2	ggcctgcagtttcttagattctttaaccac
sco1-1	ggcgagctccaatatcgatgctatcatactc
sco1-2	ggcggatcctttgaataagaaggagtaccatgc
coa6-5	ggcgagctcgatggtatattggcacctgagc
coa6-6	ggcctgcagctgatttcgttccctctgtttag

Franco et al, Fig. S1

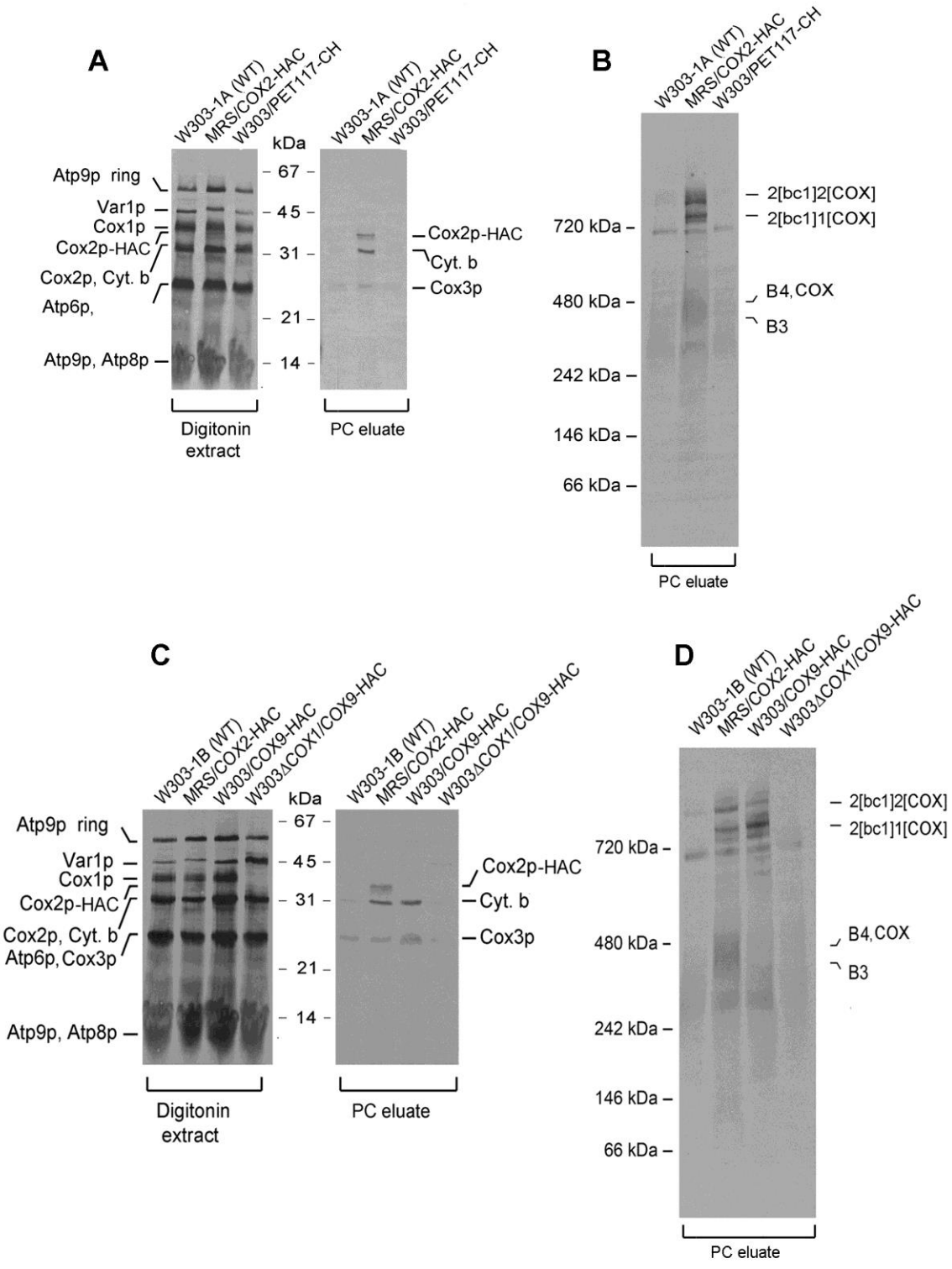

Fig. S1. Pull-down assays of radiolabeled mitochondrial gene products of strains expressing Cox9p-HAC and Pet117p-CH. Mitochondria of the respiratory competent strain W303-1B and of strains expressing tagged Cox2 (MRS/COX2-HAC), Cox9 (W303/COX9-HAC) and Pet117 (W303/PET117-CH) with and without an additonal *mss51* mutation, were grown in 2% YPGal to early stationary phase. The cells were harvested and inoculated into the same volume of fresh YPGal plus 2 mg/ml chloramphenicol and shaken for an additonal 2 hours at 30^{o}C. **A.** Mitochondria were labeled for 20 min and chased for10 min after addition of puromycin and excess cold methionine. The labeled mitochondria were extracted with digitonin and protein C tagged proteins were purified on protein C antibody beads. The digitonin extracts and eluates from the protein C antibody beads (PC eluates) were separated by SDS-PAGE on a 12% polyacrylamide gel. Proteins were blotted onto nitrocellulose and exposed to X-ray film **B.** The PC eluates from **A** were separated by BN-PAGE a 4-13% polycrylamide. Proteins were transferred to a PVDF membrane and exposed to X-ray film. **C**. Mitochondria from the indicated strains were labeled and further processed as in **A**. **D**. The PC eluates from **C** were transferred to a PVDF membrane and exposed to X-ray film. The radiolabeled mitochondrial products and assembly intermediates are marked in the margins of the autoradiograms.