Table IS. Yeast strains used in this study.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Reference</th>
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<tbody>
<tr>
<td>DAU1</td>
<td>Mat[, ade2, ura3] [][7]</td>
<td>(Costanzo and Fox, 1988)</td>
</tr>
<tr>
<td>CAB9</td>
<td>Mata, lys2, leu2-3,112, ura3-52, his3] [][7]</td>
<td>C.A. Butler</td>
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<tr>
<td>NAB69</td>
<td>Mata, ade2-101, arg8::hisG, ura3-52, kar1-1 [][7]</td>
<td>N. Bsat</td>
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<tr>
<td>NB40-36a</td>
<td>Mat[, lys2, leu2-3,112, arg8::hisG, ura3-52 [][7]</td>
<td>N. Bonnefoy</td>
</tr>
<tr>
<td>HMD22</td>
<td>Mata, lys2, leu2-3,112, arg8::hisG, ura3-52, his3] [][7],</td>
<td>(Bonnefoy and Fox, 2000)</td>
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<tr>
<td></td>
<td>cox2::ARG8m]</td>
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<tr>
<td>EHW337</td>
<td>Mata, lys2, leu2-3,112, arg8::hisG, ura3-52, his3] [][7],</td>
<td>E.H. Williams</td>
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<tr>
<td></td>
<td>cox3::ARG8m]</td>
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<td>SB7</td>
<td>Mat[, ade2, , ura3], MSS51::3xHA [][7]</td>
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<td>SB15-1</td>
<td>Mat[, his4-580, leu2-3,112, trp1-289, ura3-52, pet309]::URA3 [], SUP1</td>
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<td>SB15B</td>
<td>Mat[, his4-580, leu2-3,112, trp1-289, ura3-52, pet309]::URA3, mss51:::LEU2 [], SUP1</td>
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<td>SB14a</td>
<td>Mata, ade2-101, leu2[, ura3-52, mss51:::LEU2 [][7]</td>
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<td>DFS137</td>
<td>Mata, ade2, ura3[, pet309]::[][7]</td>
<td>D.F. Steele</td>
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<td>XPM10b</td>
<td>Mat[, lys2, leu2-3,112, arg8::hisG, ura3-52 [], cox1]::ARG8m]</td>
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<td>XPM13a</td>
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<td>XPM20a</td>
<td>Mat[, lys2, leu2-3,112, arg8::hisG, ura3-52, pet309]::URA3 [], cox1]::ARG8m[, cox1]::ARG8m]</td>
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<td>XPM46</td>
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<td>XPM63a</td>
<td>Mat[], lys2, leu2-3,112, arg8::hisG, ura3-52, mss51[]::LEU2 [[], cox1[]::ARG8[]]</td>
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<td>XPM91a</td>
<td>Mat[], lys2, leu2-3, 112, arg8::hisG, ura3-52, his3[], mss51[]::LEU2 [[], cox2[]::ARG8[]]</td>
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<td>XPM93a</td>
<td>Mat[], lys2, leu2-3,112, arg8::hisG, ura3-52, pet309[]::URA3 [[], COX1(1-512)::ARG8[], DS[]]</td>
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<td>XPM119</td>
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<td>XPM126</td>
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<td>XPM131</td>
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<td>XPM142</td>
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<td>XPM155</td>
<td>Mat[], lys2, leu2-3,112, arg8::hisG, ura3-52, mss51[]::LEU2 [[], pXPM42]</td>
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<td>XPM157</td>
<td>Mat[], lys2, leu2-3,112, arg8::hisG, ura3-52, pet309[]:URA3 [[], pXPM42]</td>
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<td>XPM171a</td>
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<td>XPM174a</td>
<td>Mat[], lys2, leu2-3,112, arg8::hisG, ura3-52, mss51[]:LEU2 [[], cox1\Δ::ARG8[]m, cox2[[::COX1[]c, COX2]</td>
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<td>XPM175a</td>
<td>Mat[], lys2, leu2-3,112, arg8::hisG, ura3-52, pet309[]:URA3 [[], cox1\Δ::ARG8[]m, cox2[[::COX1[]c, COX2]</td>
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<td>XPM182a</td>
<td>Mat[], lys2, leu2-3,112, arg8::hisG, ura3-52, pet111[]:URA3 [[], cox1\Δ::ARG8[]m, cox2[[::COX1[]c, COX2]</td>
<td>This study</td>
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</table>

\(^a\) All strains are congenic or isogenic to D273-10B, except for NAB69 and SB14a, which are isogenic to DBY947. SB15-1 and SB15B are derived from GM11 (Manthey and McEwen, 1995).

\(^b\) Mitochondrial genotypes are shown in brackets. DS\[\]al DS\[\]bl refers to an intronless mitochondria derived from CK520 (Labouesse, 1990).

\(^c\) Ectopic insertion of the chimeric COX1 upstream of COX2.
Yeast strains used in the figures:

Fig. 2. **MSS51** is required for translation of **ARG8**<sup>m</sup> when it is inserted at **COX1**.

The **ARG8** strain was DAU1. Strains carrying the **COX1(1-512)::ARG8**<sup>m</sup> construct were: wild-type, XPM78a; **mss51**<sup>D</sup>, XPM76a; **pet309**<sup>D</sup>, XPM93a. Strains carrying the **cox1Δ::ARG8**<sup>m</sup> construct were: wild-type, XPM10b; **mss51**<sup>D</sup>, XPM63a; **pet309**<sup>D</sup>, XPM20a. The **arg8** null mutant strain (**arg8**<sup>D</sup>) was NB40-36a.

Fig. 3. The **shy1**<sup>D</sup> mutation does not affect the expression of **ARG8**<sup>m</sup> in the **COX1(1-512)::ARG8**<sup>m</sup> and **cox1Δ::ARG8**<sup>m</sup> mitochondrial genes.

Strains carrying the **COX1(1-512)::ARG8**<sup>m</sup> gene were: **SHY1 MSS51**, XPM78a; **shy1**<sup>D</sup>, XPM142; **mss51**<sup>D</sup>, XPM76a. Strains carrying the **cox1Δ::ARG8**<sup>m</sup> gene were: **SHY1 MSS51**, XPM10b; **shy1**<sup>D</sup>, XPM65; **mss51**<sup>D</sup>, XPM63a. The **ARG8** strain was CAB9, and **arg8**<sup>D</sup> strain was NB40-36a.

Fig. 4B. **MSS51** function cannot be bypassed by a **pet309**<sup>D</sup> suppressor.

Haploid cells containing a **SUP1** mtDNA bearing the **SUP1** gene were patched on YPD in vertical stripes. Their relevant nuclear phenotypes were **MSS51 pet309**<sup>D</sup> (SB15-1) and **mss51**<sup>D</sup> **pet309**<sup>D</sup> (SB15B). Cells containing wild-type, **PET** mitochondria were patched on horizontal stripes. Their relevant nuclear phenotypes were **MSS51 pet309**<sup>D</sup> (DFS137) and **mss51**<sup>D</sup> **PET309** (SB14a).
Fig. 5. *MSS51* is required to express the *COX1* coding sequence when it is fused to the *COX2* 5'-UTL and 3'-UTR at an ectopic locus.

Strain carrying the chimeric ectopic *COX1* gene from Fig. 5A were: wild-type, XPM171a; *mss51Δ*, XPM174a; *pet309Δ*, XPM175a; *pet111Δ*, XPM182a. Strains bearing wild-type mtDNA were: *wild-type*, NB40-36a; *mss51Δ*, XPM126; *pet309Δ*, XPM119; *pet111Δ*, XPM131.

Fig. 6B. Mss51p acts through the *COX1* coding sequence to promote *COX1* translation.

Synthetic Δ, haploid strains carrying pXPM42 DNA were patched in vertical stripes. Their relevant nuclear phenotypes were: *MSS51 pet309Δ* (XPM157) and *mss51Δ PET309* (XPM155). Cells containing wild-type Δ+ mitochondria were patched on horizontal stripes. Their relevant phenotypes were: *MSS51 pet309Δ* (XPM119) and *mss51Δ PET309* (XPM126).

Fig. 7. Mss51p interacts with newly synthesized Cox1p. The Mss51p (WT) strain was DAU1. The Mss51p-HA (HA) strain was SB7.

**LEGEND TO FIGURE 1S**

Fig. 1S. The *mss51Δ* mutation specifically prevents the accumulation of the reporter *ARG8*<sup>m</sup> when it is inserted at the *COX1* locus. *ARG8*<sup>m</sup> was inserted in place of the *COX1* (*cox1Δ::ARG8*<sup>m</sup>, Figure 1), *COX2* (*cox2Δ::ARG8*<sup>m</sup>), or *COX3* (*cox3Δ::ARG8*<sup>m</sup>) coding sequences. The *mss51Δ* mutation was introduced (Δ) and
ARG8<sup>m</sup> expression was compared to a MSS51 strain (WT). Cells containing wild-type mtDNA and either the nuclear encoded ARG8 gene (ARG8) or an arg8 null mutation (arg8<sup>D</sup>) were also included. A) Serial dilutions of the indicated strains were spotted on raffinose minimal medium containing (+Arg) or lacking (-Arg) arginine. Cells were incubated at 30°C for 3 days. B) Steady-state concentration of Arg8p. Cells were grown on raffinose complete medium and mitochondrial proteins were separated by 12.5% SDS-PAGE and probed with anti-Arg8p antibody and anti-citrate synthase antibody as in Figure 2. Strains containing the cox1<sup>Δ::ARG8<sup>m</sup></sup> gene were: MSS51, XPM10b; mss51<sup>D</sup>, XPM63a. Strains with the cox2<sup>Δ::ARG8<sup>m</sup></sup> construct were: MSS51, HMD22; mss51<sup>D</sup>, XPM91a. Strains carrying the cox3<sup>Δ::ARG8<sup>m</sup></sup> construct were: MSS51, EHW337; mss51<sup>D</sup>, XPM92. Strains bearing wild-type mtDNA were: ARG8, CAB9; arg8<sup>Δ</sup>, NB40-36a.
Figure 1S

A

+ Arg

- Arg

MSS51

ARG8

cox1Δ::ARG8m

cox2Δ::ARG8m

cox3Δ::ARG8m

arg8Δ

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