# Cox25 TEAMS UP WITH Mss51, Ssc1 AND Cox14 TO REGULATE MITOCHONDRIAL CYTOCHROME *C* OXIDASE SUBUNIT 1 EXPRESSION AND ASSEMBLY IN *SACCHAROMYCES CEREVISIAE*

**Flavia Fontanesi[1], Paula Clemente[3], Antoni Barrientos[1,2]**

[1] Department of Neurology, [2] Department of Biochemistry and Molecular Biology. University of Miami Miller School of Medicine, Miami, FL 33136. [3] Departamento de Bioquímica, Instituto de Investigaciones Biomédicas Alberto Sols CSIC-UAM Facultad de Medicina, Universidad Autónoma de Madrid, Arzobispo Morcillo 4, E-28029 Madrid, Spain.

Running head: Regulation of mitochondrial Cox1 expression

**Address correspondence to**: Antoni Barrientos, Dept. of Neurology and Biochemistry & Molecular Biology; Universtiy of Miami. Miller School of Medicine; 1600 NW 10th Ave.; RMSB # 2067; Miami, FL-33136; Phone: (305) 243 86 83; FAX: (305) 243 39 14; E. mail: abarrientos@med.miami.edu

## Supplemental Material

### Supplemental Figure Legends

Fig. S1. A GST tagged Cox25 protein is functional. (**A**) Scheme depicting the *COX25-GST* construct described in the Experimental Procedures section. Thr: thrombin cleavage site. (**B**) The wild-type strain W303, a *cox25* null mutant and three independent clones obtained by transformation of the *cox25* strain with the integrative plasmid carrying the *COX25-GST* fusion gene, as shown in panel (A), were grown overnight in liquid YPD media. Ten fold serial dilutions of the strains were plated on solid YPD or YPEG media and incubated at 30°C. Pictures were taken after three days of incubation.

Fig. S2. *S. cerevisiae COX25* codes for a protein required for respiratory growth. The respiratory competent wild type strain W303 and the strain carrying a null allele of *cox25* in the same genetic background were grown overnight in liquid YPD media. Ten fold serial dilutions of the strains were plated on solid complete media containing the indicated carbon source at 2% concentration if not differently indicated. Plates were incubated at 30°C and pictures were taken after three days of incubation.

Fig. S3. Effect of overexpression of *COX25* on *in vivo* Cox1 synthesis. (**A**) Steady-state levels of Cox25 and Cox14 in the indicated strains analyzed by Western-blotting. (**B**) Wild-type W303 and Δ*cox25* cells over-expressing or not *COX25* or *COX14* were pulse-labeled with $^{35}$S-methionine for 15 min, chased for 60 min and analyzed as in Fig. 3.

Fig. S4. Western-blot analyses of Cox25 steady state concentrations in wild-type cells, cells devoid of mtDNA (rho$^0$) or carrying null alleles of the indicated COX assembly genes (Table I). An antibody against porin was used to normalize the signals for protein loading. Quantification of the signals is shown in the lower panel. The bars indicate the mean ± SD from at least three independent sets of measurements.

**Supplemental Figures**

**Figure S1**

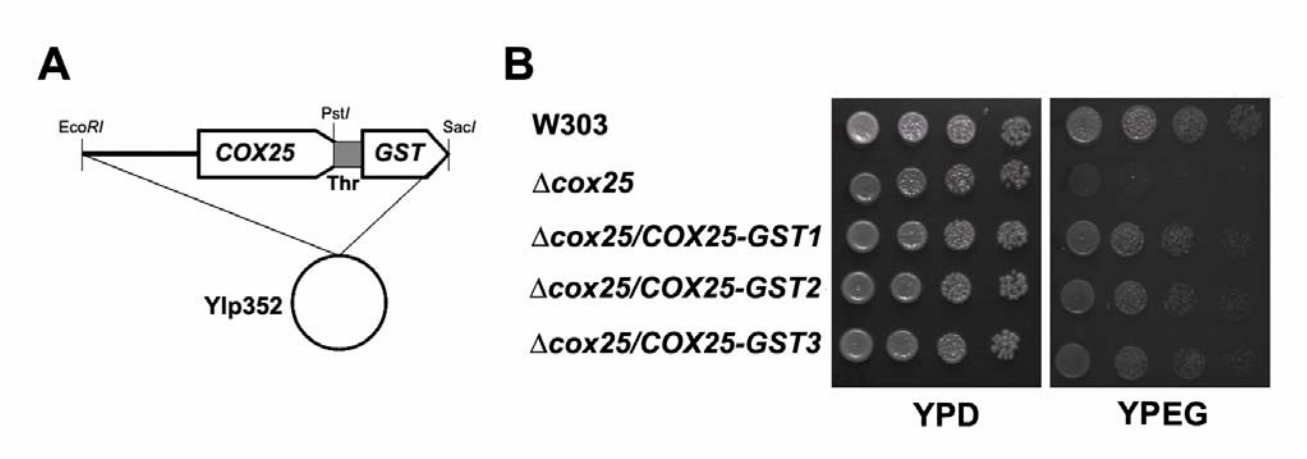

**Figure S2**

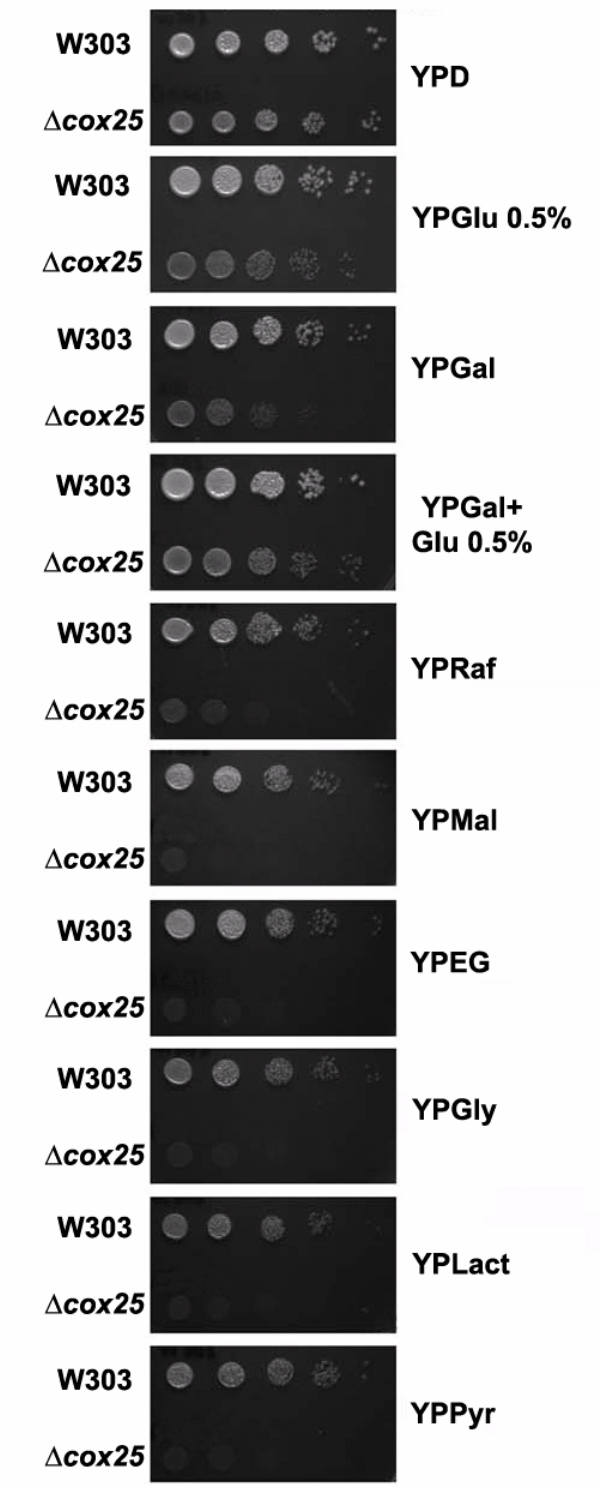
W303
Δcox25
YPD
W303
Δcox25
YPGlu 0.5%
W303
Δcox25
YPGal
W303
Δcox25
YPGal+
Glu 0.5%
W303
Δcox25
YPRaf
W303
Δcox25
YPMal
W303
Δcox25
YPEG
W303
Δcox25
YPGly
W303
Δcox25
YPLact
W303
Δcox25
YPPyr

**Figure S3**

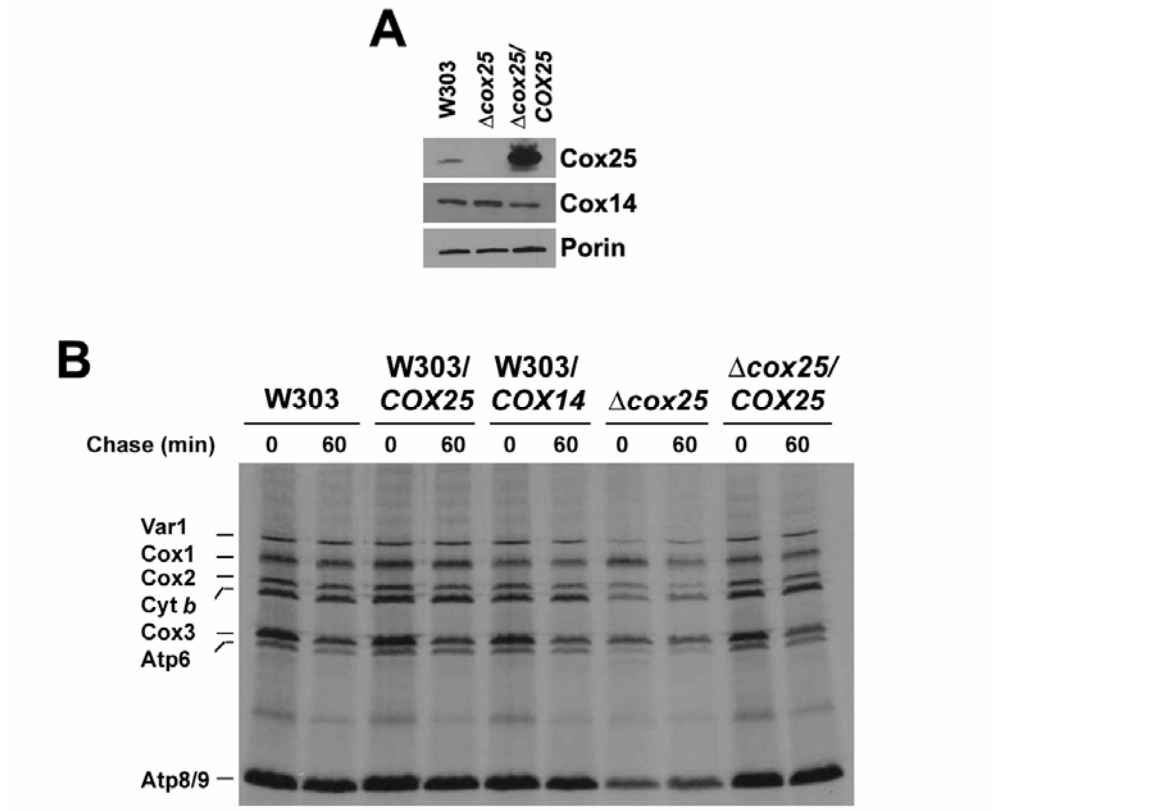


**Figure S4**