

Cbp3 and Cbp6 are dispensable for synthesis regulation of Cytochrome *b* in yeast mitochondria

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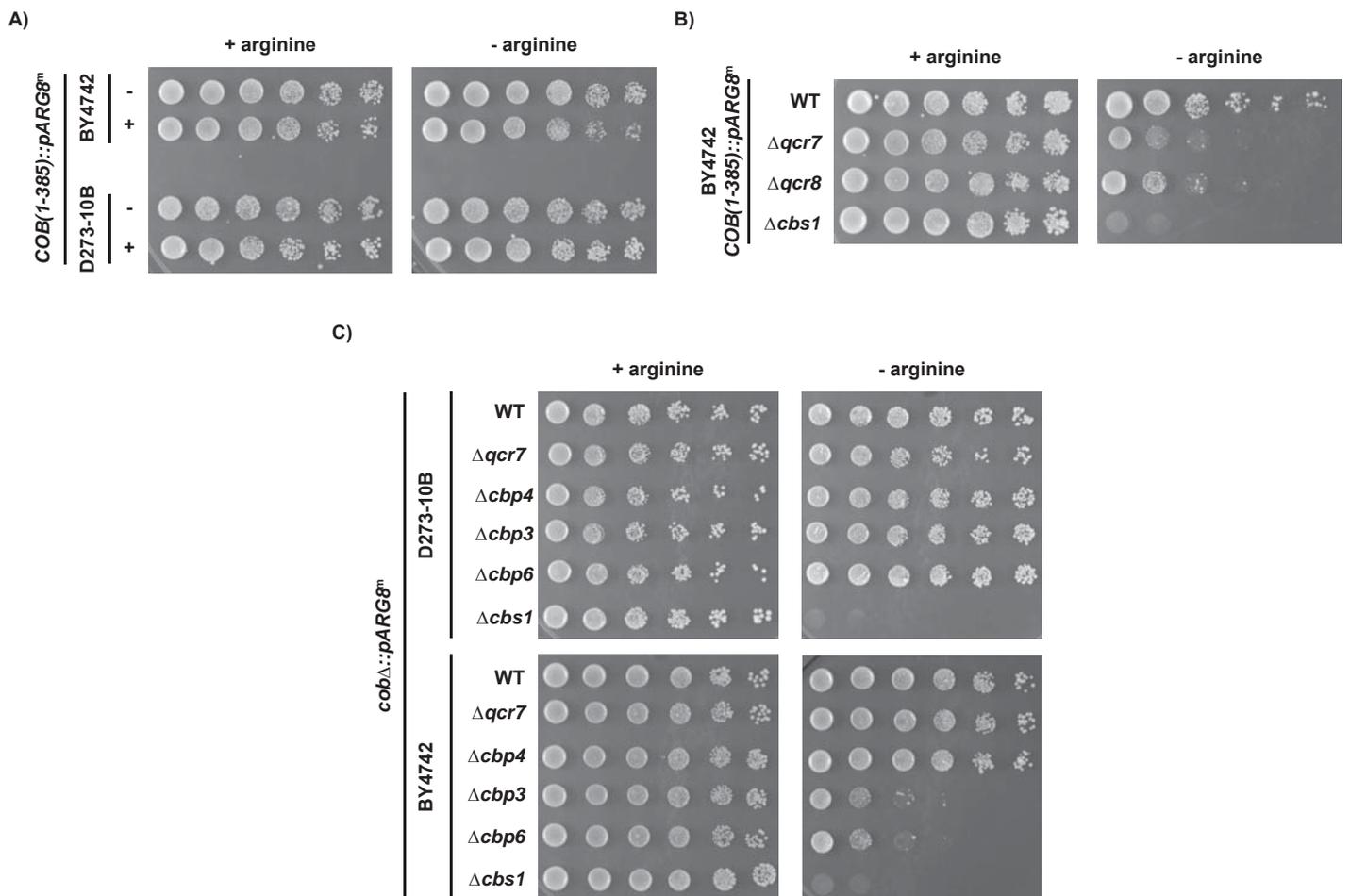
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Running title: *Cbp3 and Cbp6 are dispensable for Cytb synthesis*

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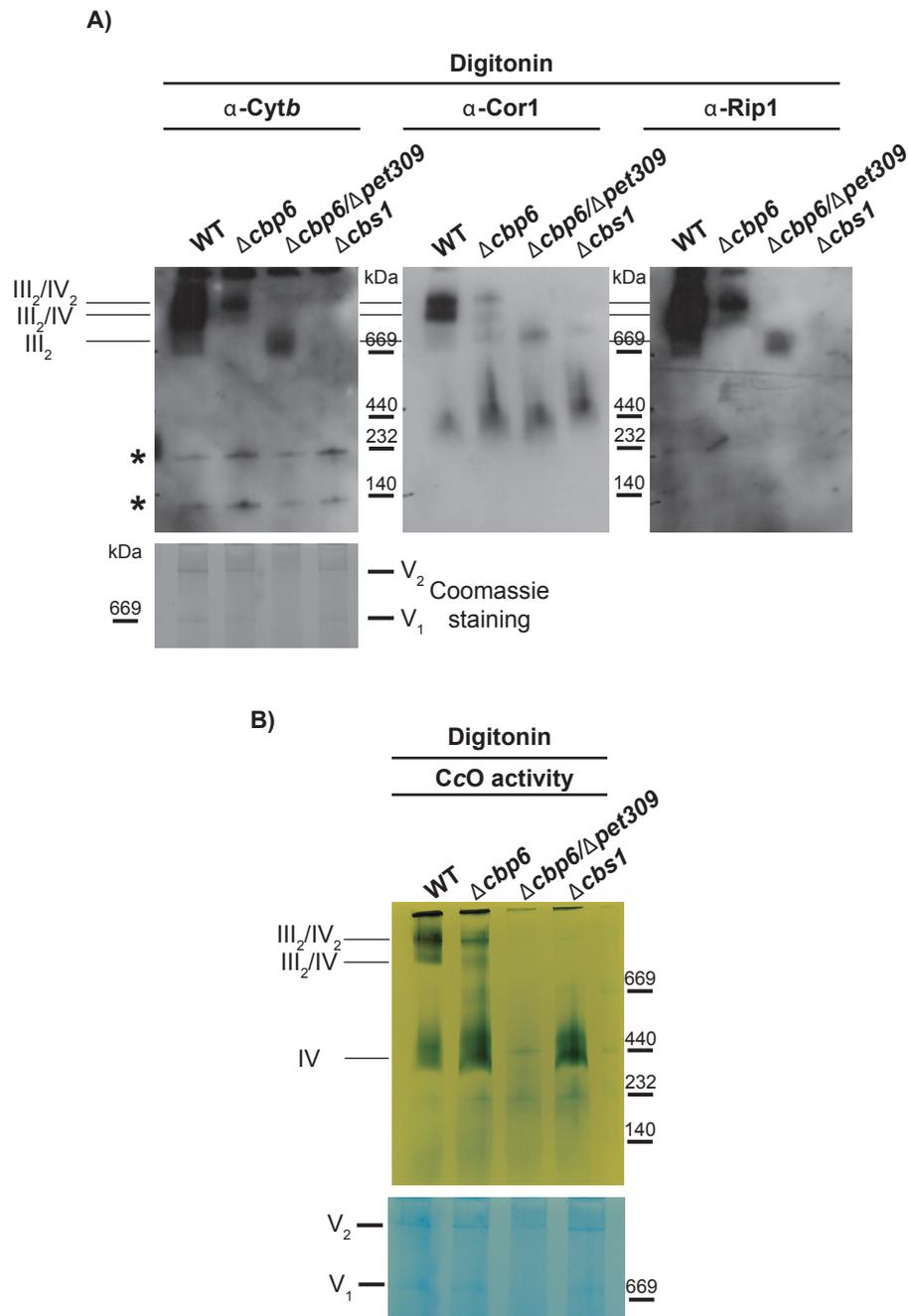
Keywords: *bc<sub>1</sub>* complex, Cbp3, Cpb6, Cytochrome *b*, mitochondria, mitochondrial DNA, Qcr7, translation

## Supporting Figure 1



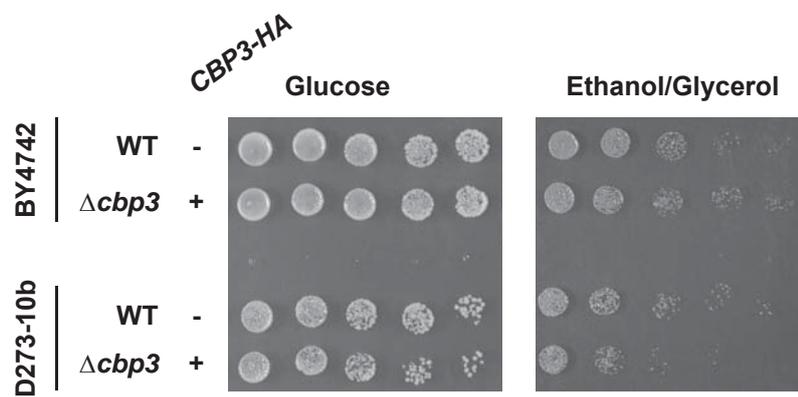
**Supporting Figure 1.** The construct *COB(1-385)::pARG8<sup>m</sup>* was used as reporter for *COB* mRNA translation. (A) Cells carrying the *COB(1-385)::pARG8<sup>m</sup>* construct in mitochondria or the wild-type nuclear *ARG8* gene were spotted on 10-fold serial dilutions on complete media or media lacking arginine, and were incubated for 3 days at 30°C. (B) Cells carrying the *COB(1-385)::pARG8<sup>m</sup>* mitochondrial reporter and the indicated mutations were grown on serial dilutions as in (A). (C) *COB* codons were replaced by *pARG8<sup>m</sup>* to create the construct *cob $\Delta$ ::pARG8<sup>m</sup>*. D273-10B or BY4742 cells with the indicated mutations were grown on serial dilutions as in (A).

## Supporting Figure 2



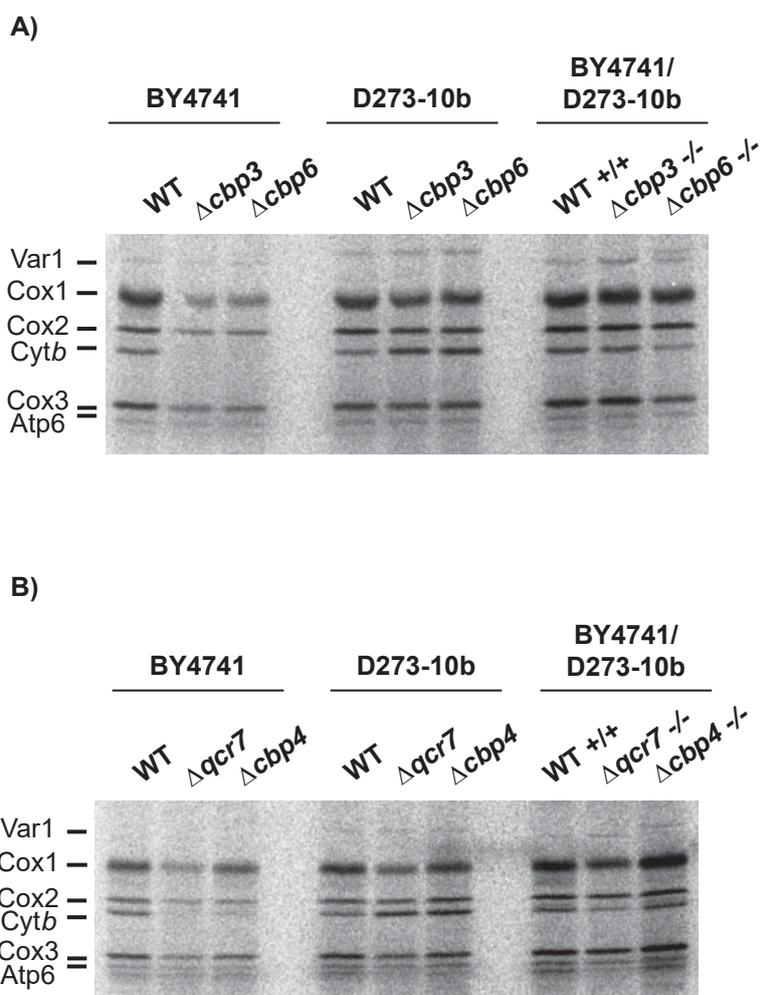
**Supporting Figure 2.** The supercomplex-like bands observed in the absence of Cbp6 depend on the cytochrome *c* oxidase. Mitochondria (200  $\mu$ g) from wild-type,  $\Delta cbp6$  and  $\Delta cbp6/\Delta pet309$  mutants were solubilized with digitonin, loaded in a BN-PAGE system and either analyzed by western blot with the indicated antibodies (A) or stained for CcO oxidase activity (B). Coomassie-stained ATP synthase dimer and monomer were used as loading control.

### Supporting Figure 3



**Supporting Figure 3.** The hemagglutinin epitope (HA) fused to the carboxyl-terminal end of Cbp3 does not interfere with yeast cells respiratory growth. Cells carrying wild-type Cbp3 or Cbp3-HA expressed from centromeric plasmids were spotted on ten-fold serial dilutions glucose or ethanol/glycerol media lacking uracil, and were grown as 10-fold serial dilutions for 3 days at 30°C.

## Supporting Figure 4



**Supporting Figure 4.** The phenotype over *Cytb* synthesis of D273-10b cells is dominant with respect to the BY4742 strain phenotype. Mitochondrial translation products from whole cells of the indicated mutants in the presence of cycloheximide and [<sup>35</sup>S]-methionine were analyzed. Haploid, D273-10b and BY4742 cells, as well as diploids obtained from a cross between D273-10b and BY4742 cells were used.