

Strain	YPD	YPGal	YPGE	SCD	SCGal	SCG
BY4741 WT	++++	++++	++++	++++	++++	++++
BY4742 <i>coa6</i> Δ	++++	++++	++++	++++	++++	++++
BY4741 <i>cox11</i> Δ	++++	++++	-	++++	++	-
STY2 <i>coa6</i> Δ <i>cox11</i> Δ	++++	++++	-	++++	++	-
BY4741 <i>cox12</i> Δ	++++	++++	-	++++	+++	-
STY3 <i>coa6</i> Δ <i>cox12</i> Δ	++++	++++	-	++++	++	-
BY4741 <i>cox17</i> Δ	++++	++++	-	++++	-	-
STY1 <i>coa6</i> Δ <i>cox17</i> Δ	++++	++++	-	++++	-	-
BY4741 <i>cox19</i> Δ	++++	+++	-	++++	++	-
STY4 <i>coa6</i> Δ <i>cox19</i> Δ	++++	+++	-	++++	++	-
BY4741 <i>cox23</i> Δ	++++	+++	-	++++	++	-
STY5 <i>coa6</i> Δ <i>cox23</i> Δ	++++	+++	-	++++	++	-
BY4741 <i>cmc1</i> Δ	++++	++++	++++	++++	++++	++
STY6 <i>coa6</i> Δ <i>cmc1</i> Δ	++++	++++	++++	++++	++++	++++
BY4741 <i>sco1</i> Δ	++++	+++	-	++++	++	-
STY9 <i>coa6</i> Δ <i>sco1</i> Δ	++++	+++	-	++++	++	-
BY4741 <i>sco2</i> Δ	++++	++++	++++	++++	++++	++++
STY10 <i>coa6</i> Δ <i>sco2</i> Δ	++++	++++	-	++++	++++	++
BY4741 <i>pic2</i> Δ	++++	++++	++++	++++	++++	++++
STY11 <i>coa6</i> Δ <i>pic2</i> Δ	++++	++++	+++	++++	++++	+++

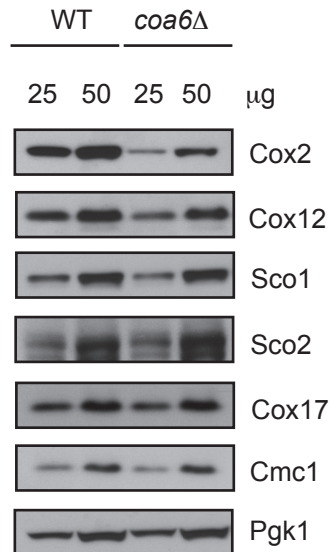
Supplementary Table 1. Genetic interaction study of Coa6 with known CcO assembly factors involved in copper metabolism. Serial dilutions of wild type (WT), single knockouts, and double knockouts were spotted on rich (YP) or synthetic complete (SC) media with different carbon sources as indicated and incubated at 30°C for 2-5 days. The plates were scored according to the number of dilutions that grew. Maximal growth of all four dilutions is indicated using “++++,” while no growth is indicated by “-”.

Strain	Genotype	Source
BY4741 WT	MAT a, <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>met15Δ0</i> , <i>ura3Δ0</i>	Greenberg lab
BY4742 WT	MAT α, <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>lys2Δ0</i> , <i>ura3Δ0</i>	Greenberg lab
BY4742 <i>coa6Δ</i>	MAT α, <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>lys2Δ0</i> , <i>ura3Δ0</i> , <i>coa6Δ::NatMX4</i>	This study
BY4741 <i>COA6^{HIA}</i>	MAT a, <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>met15Δ0</i> , <i>ura3Δ0</i>	This study
BY4741 ρ^0	MAT a, <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>met15Δ0</i> , <i>ura3Δ0</i>	This study
BY4741 <i>coa6Δ</i>	MAT a, <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>met15Δ0</i> , <i>ura3Δ0</i> , <i>coa6Δ::KanMX4</i>	Open Biosystems
BY4741 <i>sco1Δ</i>	MAT a, <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>met15Δ0</i> , <i>ura3Δ0</i> , <i>sco1Δ::KanMX4</i>	Open Biosystems
BY4741 <i>sco2Δ</i>	MAT a, <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>met15Δ0</i> , <i>ura3Δ0</i> , <i>sco2Δ::KanMX4</i>	Open Biosystems
BY4741 <i>cox11Δ</i>	MAT a, <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>met15Δ0</i> , <i>ura3Δ0</i> , <i>cox11Δ::KanMX4</i>	Open Biosystems
BY4741 <i>cox12Δ</i>	MAT a, <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>met15Δ0</i> , <i>ura3Δ0</i> , <i>cox12Δ::KanMX4</i>	Open Biosystems
BY4741 <i>cox17Δ</i>	MAT a, <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>met15Δ0</i> , <i>ura3Δ0</i> , <i>cox17Δ::HphMX4</i>	This study
BY4741 <i>cox19Δ</i>	MAT a, <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>met15Δ0</i> , <i>ura3Δ0</i> , <i>cox19Δ::KanMX4</i>	Open Biosystems
BY4741 <i>cox23Δ</i>	MAT a, <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>met15Δ0</i> , <i>ura3Δ0</i> , <i>cox23Δ::KanMX4</i>	Open Biosystems
BY4741 <i>cmc1Δ</i>	MAT a, <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>met15Δ0</i> , <i>ura3Δ0</i> , <i>cmc1Δ::KanMX4</i>	Open Biosystems
BY4741 <i>cmc2Δ</i>	MAT a, <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>met15Δ0</i> , <i>ura3Δ0</i> , <i>cmc2Δ::KanMX4</i>	Barrientos Lab
BY4741 <i>pic2Δ</i>	MAT a, <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>met15Δ0</i> , <i>ura3Δ0</i> , <i>pic2Δ::KanMX4</i>	This study
STY9 <i>sco1Δcoa6Δ</i>	MAT a, <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>ura3Δ0</i> , <i>met15Δ0</i> , <i>sco1Δ::KanMX4</i> , <i>coa6Δ::clonNAT</i>	This study
STY10 <i>sco2Δcoa6Δ</i>	MAT a, <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>ura3Δ0</i> , <i>lys2Δ0</i> , <i>sco2Δ::KanMX4</i> , <i>coa6Δ::NatMX4</i>	This study
STY2 <i>cox11Δcoa6Δ</i>	MAT a, <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>ura3Δ0</i> , <i>cox11Δ::KanMX4</i> , <i>coa6Δ::NatMX4</i>	This study
STY3 <i>cox12Δcoa6Δ</i>	MAT a, <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>met15Δ0</i> , <i>ura3Δ0</i> , <i>lys2Δ0</i> , <i>cox12Δ::KanMX4</i> , <i>coa6Δ::NatMX4</i>	This study
STY1 <i>cox17Δcoa6Δ</i>	MAT a, <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>ura3Δ0</i> , <i>lys2Δ0</i> , <i>met15Δ0</i> , <i>cox17Δ::HphMX4</i> , <i>coa6Δ::NatMX4</i>	This study
STY4 <i>cox19Δcoa6Δ</i>	MAT a, <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>ura3Δ0</i> , <i>lys2Δ0</i> , <i>cox19Δ::KanMX4</i> , <i>coa6Δ::NatMX4</i>	This study
STY5 <i>cox23Δcoa6Δ</i>	MAT a, <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>ura3Δ0</i> , <i>cox23Δ::KanMX4</i> , <i>coa6Δ::NatMX4</i>	This study
STY6 <i>cmc1Δcoa6Δ</i>	MAT a, <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>ura3Δ0</i> , <i>lys2Δ0</i> , <i>cmc1Δ::KanMX4</i> , <i>coa6Δ::NatMX4</i>	This study
STY11 <i>pic2Δcoa6Δ</i>	MAT a, <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>ura3Δ0</i> , <i>met15Δ0</i> , <i>pic2Δ::KanMX4</i> , <i>coa6Δ::NatMX4</i>	This study

Supplementary Table 2. Yeast strains used in this study.

Name	Sequence (5' → 3')
Cloning in pRS416	
<i>SacI</i> yCOA6+500bp upstream Forward	ccctgg GAGCTC gcaaagacgcgcagccaaaaagccgaacagtta
<i>Bam</i> HI yCOA6 Reverse	gggact GGATCC tactgatttcgctccctctgttagcttctgctcgat
<i>Xho</i> I 500bp upstream Reverse	ccctaa CTCGAG cgctatattactactattccttc
<i>Xho</i> I hCOA6 Forward	cccggc CTCGAG atgggtccagggtggtccattattgtc
<i>Xho</i> I HyCOA6 Forward	cccggc CTCGAG atgggttgttctctttcgacgg
<i>Bam</i> HI h/HyCOA6 Reverse	cccatt GGATCC caggacttagcagtagtttcag
Cloning in pAG423GPD	
AttB1 yCOA6 Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCatgggctattttcatttgatgggtggc
AttB2 yCOA6 Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTCcgtatttcgctccctctgttagc
Cloning in pYM14 chromosomal	
HA tag	
S3 yCOA6 Forward	aagagaatcgagcaggaagctaacagaggggaacgaaatcag CGTACGCTGCAGGTCGAC
S2 yCOA6 Reverse	atatatatgtaatatgagccaataactcactaaaaactca ATCGATGAATTCGAGCTCG
Site Directed mutagenesis primers	
yCOA6 W26C Forward	agaaagttgtgctgagtgccagggacgc
yCOA6 W26C Reverse	gcgccctggactcgagcacaactttct
yCOA6 C68A Forward	gtggaaaatgaaaatttgaggagaatgccgccatagctgga
yCOA6 C68A Reverse	tccagctatgggctgattctcctcaaattttcattttccac
yCOA6 C25A Forward	gttcacagagaaagttggcctgggagtgccagggacg
yCOA6 C25A Reverse	cgctccctggactcccaggccaactttctctgtgaac
ySCO2 E161K Forward	taatctgtcaagctttctggacaaatgtcggggc
ySCO2 E161K Reverse	gccccgacattgtccagaaaagcttgacagatta
ySCO2 S246F Forward	gggtcgatcaaatagaaaaatgaaatggccactaagtaaatcctgg
ySCO2 S246F Reverse	ccaggattacttagtgaccatttcattttctattgatcgacct

Supplementary Table 3. Primers used in this study.



Supplementary Figure 1. Levels of CcO assembly factors do not change in *coa6Δ* cells.

Western blot analysis of the indicated CcO subunits and assembly factors in total cellular protein extract from WT and *coa6Δ* cells grown to mid-logarithmic growth phase in YPGal medium. The blot was probed with the indicated antibodies. Pgk1 was used as a loading control.

A

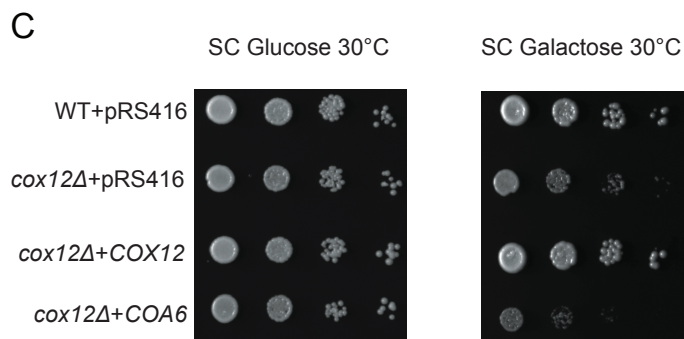
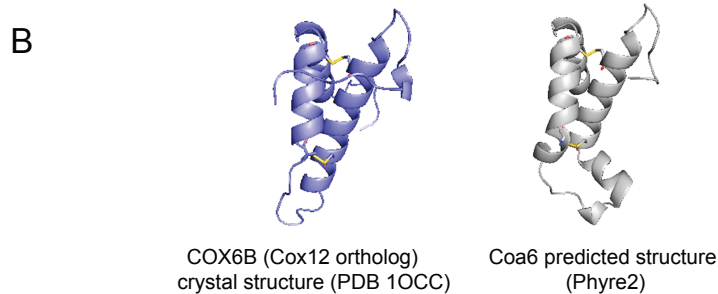
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hCOX6B -----MAED-METKIKNYK-TAPFDSRFPNQN-QTRNCW 31
yCOX12 -----MADQ-ENSPLH-----TVGFDARFPQQN-QTKHCW 28
hCOA6      MGGPGPLLSPSRGFLLCKTGWHSNRLLGDCGPHTPVSTALSFIAVGMAAPSMK-ERQVCW 59
yCOA6      -----MGLFSFDGGKK-----ESQPPNTRSQRKLCW 26

hCOX6B      QNYLDFHRCQKAM-----TAKGGDIS-VCEWYQRVYQSLQPTSMWTDWDEQR----- 77
yCOX12      QSYVDYHKCVN-----MKGEDFA-PCKVFWKTYNALCPLDWIEKWDDOR----- 71
hCOA6      GARDEYWKCLD-----ENLEDAS-QCKLRSSFESSCPQQWIKYFDKRRDYLFK 108
yCOA6      ESRDAFFQCLDKADILDAMPKNSKSIKSHCKVENEKFEENC AHSWIKYFKEKR-VIDFK 85

hCOX6B      ---AEGTF-PGKI----- 86
yCOX12      ---EKGIF-AGDINSD--- 83
hCOA6      EKFEAGQFEPSETTAKS-- 125
yCOA6      REQTIKRIEQAQQRERNQ 104

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Supplementary Figure 2. Coa6 and Cox12 exhibit sequence and structural similarity but Coa6 overexpression retards *cox12Δ* growth.

(A) Sequence alignment of human COX6B, yeast Cox12, human COA6, and yeast Coa6 proteins performed using ClustalW. (B) The yeast Coa6 structure predicted on the basis of the bovine COX6B structure (Protein Data Bank ID 1OCC) using Phyre2 software and was visualized using PyMol. (C) WT and *cox12Δ* cells were transformed with empty vector, pRS416-COX12, or pRS416-COA6 and spotted on SC Galactose plates. Cells were grown at 30°C and images were taken after 3 days.