SUPPLEMENTAL INFORMATION:

Human COQ10A and COQ10B are distinct lipid-binding START domain proteins required for coenzyme Q function

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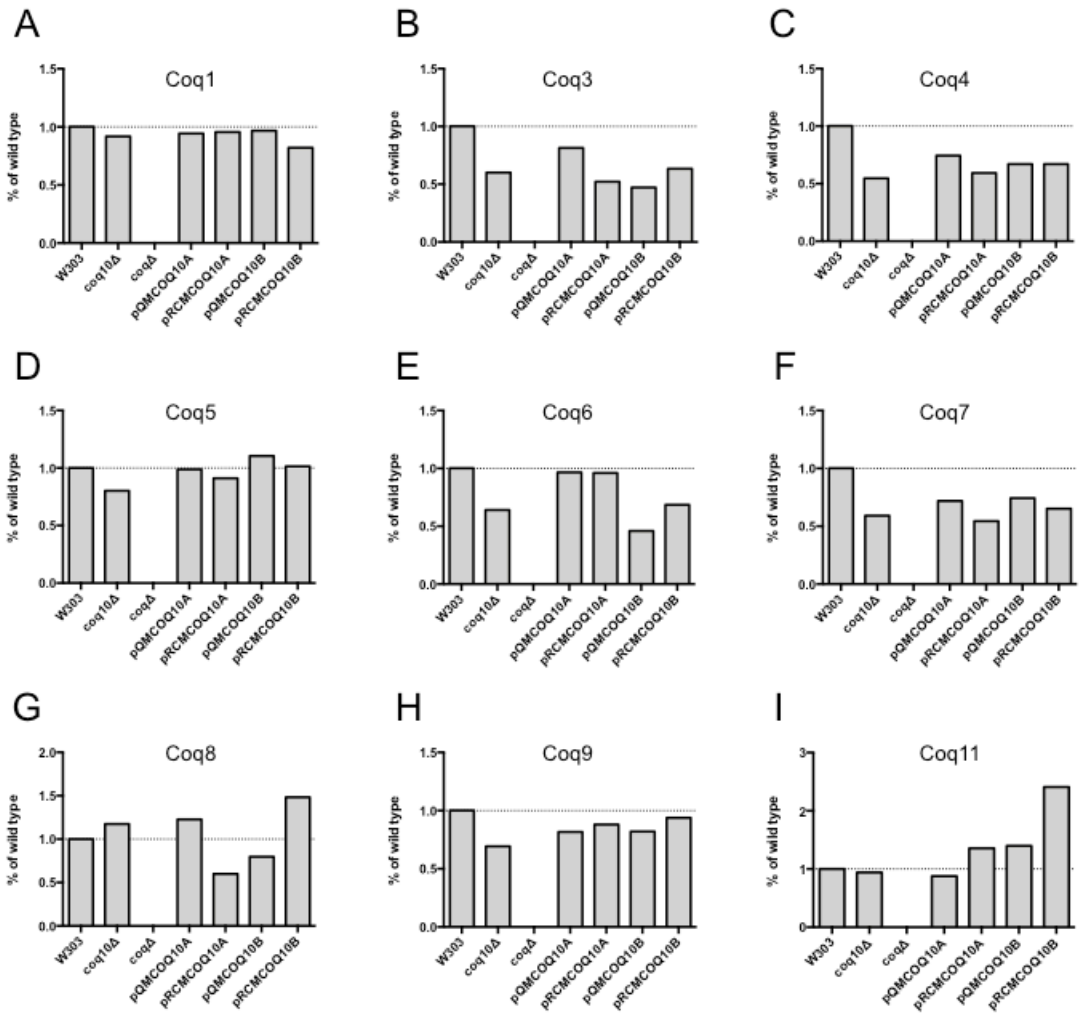
Supplemental Table S1. Predicted human COQ10A and COQ10B mitochondrial targeting sequence[d]

Protein	N-terminal sequence[e]
COQ10A isoform 1	**MAWAGSRRVPAGTRA**AAERCCRLSLSPGAQPAPPPGPLPPPRPMR[46]FLTSCS…
COQ10A isoform 2	MLLQVVREGKFSG[14]FLTSCS…
COQ10B isoform 1	**MAARTGHTALRRVVSGCRPKSATAAGAQAPVRNGRYL**ASCGILMSRTLPLHTSILPKEICARTFFKITAPLINKRKEYSERRILG[86]YSMQEM…
COQ10B isoform 2	**MAARTGHTALRRVVSGCRPKSATAAGAQAPVRNGR**----------[36]YSMQEM...
COQ10B isoform 3	MGVCVWR----------YLASCGILMSRTLPLHTSILPKEICARTFFKITAPLINKRKEYSERRILG[58]YSMQEM…
COQ10B isoform 4	MSRTLPLHTSILPKEICARTFFKITAPLINKRKEYSERRILG[43]YSMQEM…

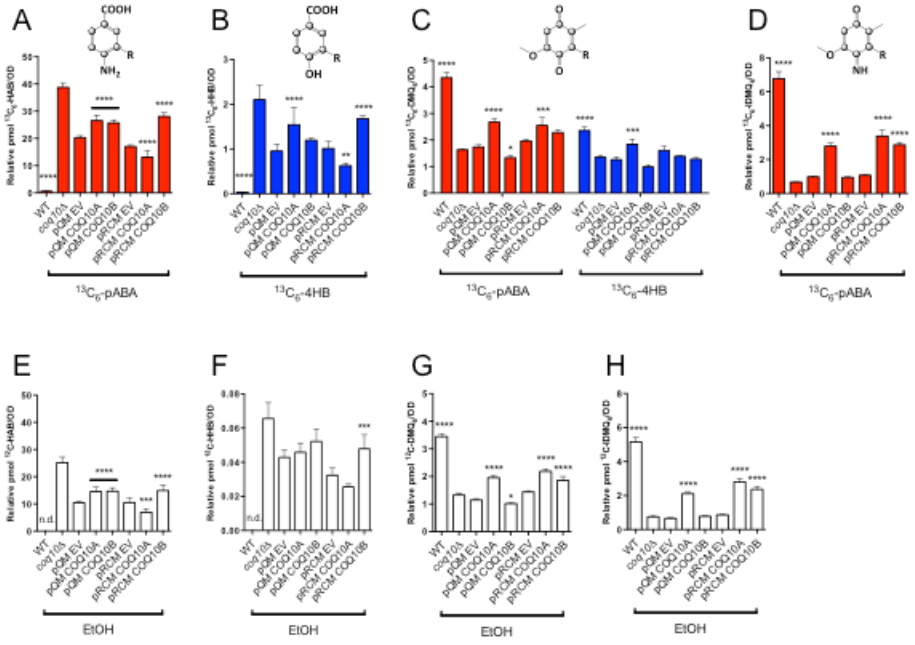
[d]UniprotKB, https://www.uniprot.org

[e]NCBI, https://www.ncbi.nlm.nih.gov

The N-terminal sequence of the human COQ10A and COQ10B isoforms were aligned with their predicted mitochondrial targeting sequence highlighted in bold. The amino acid residue number in superscript represented the beginning of the shared amino acid sequences among isoforms of either COQ10A or COQ10B.



Supplemental Figure S1. Expression of either human COQ10A or COQ10B restores steady state levels of Coq polypeptides. Relative Coq polypeptide levels shown in Figure 4 were quantified by band densitometry using Image J software. The densitometry reading of each *coqΔ* control was first subtracted from the reading of each sample blotted against the corresponding specific Coq polypeptide, then normalized by the reading of the loading control Mdh1. The percentage of signal intensity from each sample relative to the wild type control was plotted in the y-axis.



Supplemental Figure S2. Expression of either human COQ10A or COQ10B has minimal effect on *de novo* and steady state levels of CoQ_6-intermediates. The *de novo* production $^{13}C_6$-CoQ_6-intermediates, including **A)** $^{13}C_6$-HAB, **B)** $^{13}C_6$-HHB, **C)** $^{13}C_6$-DMQ_6, **D)** $^{13}C_6$-$IDMQ_6$ were measured from yeast whole cell lipid extracts from wild type, *coq10Δ*, *coq10Δ* expressing single- or multi-copy COQ10A or COQ10B, or their respective empty vector labeled with $^{13}C_6$-pABA (red) or $^{13}C_6$-4HB (blue) for 5 hours. The steady state levels of **E)** HAB, **F)** HHB, **G)** DMQ_6, **H)** $IDMQ_6$ were measured from designated samples treated with ethanol as vehicle control. The statistical analyses were performed using two-way ANOVA multiple comparisons from three biological replicates, comparing yeast *coq10Δ* expressing single- or multi-copy COQ10A or COQ10B to their respective empty vector controls, and comparing yeast *coq10Δ* mutant to the wild-type control. The error bar indicates mean ±SD, and the statistical significance is represented by *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001.