

Respiratory deficiency caused by mutations in the coenzyme Q chaperone protein Coq10 is mitigated by deletion of *COQ11*

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SUPPORTING INFORMATION

Table S1. Description and source of antibodies.

<i>Antibody</i>	<i>Working dilution</i>	<i>Source</i>
Coq1	1:10,000	(1)
Coq2	1:1,000	(2)
Coq3	1:200	(3)
Coq4	1:2,000	(4)
Coq5	1:5,000	(5)
Coq6	1:200	(6)
Coq7	1:1,000	(7)
Coq8	Affinity purified, 1:30	(2)
Coq9	1:1,000	(2)
Coq10	Affinity purified, 1:400	(8)
Coq11	1:500	(8)
Mdh1	1:10,000	Lee McAlister-Henn ^b
Atp2	1:1,000	Carla M. Koehler ^c
Cyt ₁	1:1,000	Carla M. Koehler ^c
Cyb ₂	1:1,000	Carla M. Koehler ^c
Hsp60	1:5,000	Carla M. Koehler ^c
Porin	1:5,000	Carla M. Koehler ^c

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Table S2. Quantitative real-time PCR primers.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>COQ1</i>	CCCGAAGTCGTAGAACTAATG	GGAACCGGAAGTAGCTTATG
<i>COQ2</i>	CAGCTGGTATGTTGGGTATTT	GACGGACCTGATAACTCTTTG
<i>COQ3</i>	CATGCTGGAGGGAAAGATAAA	TCGACCAACAATGCCTTAAA
<i>COQ4</i>	GTGGTATCCTTGCACCTTTAC	CCAGCATTTCTCCCAATAC
<i>COQ5</i>	TGCTTAAAGAAGGTGAGAAGAG	TACCGAAGGAGACTGTGTAG
<i>COQ6</i>	TGAAGGACGAGTCGGATATT	CCAACAAGGGCAACTCTATC
<i>COQ7</i>	GCTCCCAAGTGTCAGAATTTA	CTGGTCCCATATGTGCTTTAG
<i>COQ8</i>	CGTATGGAGGGAACTGAAATAA	GAGGCACCGAAATCCAATAA
<i>COQ9</i>	CGCTGTCATGGAAGCTGATAAA	GAGAAAGGCGCTTGAATAG
<i>COQ10</i>	GCGGTACCAATCACACTATTA	GAGAGGCTTGTTATCCACAG
<i>COQ11</i>	GCAGAGATATTTTCAGGCCTATTA	CTGCTGAGTGGATACTGTTG
<i>ACT1</i>	TATGTTCTAGCGCTTGCACCA	CCAAAGCAGCAACCTCTAAA

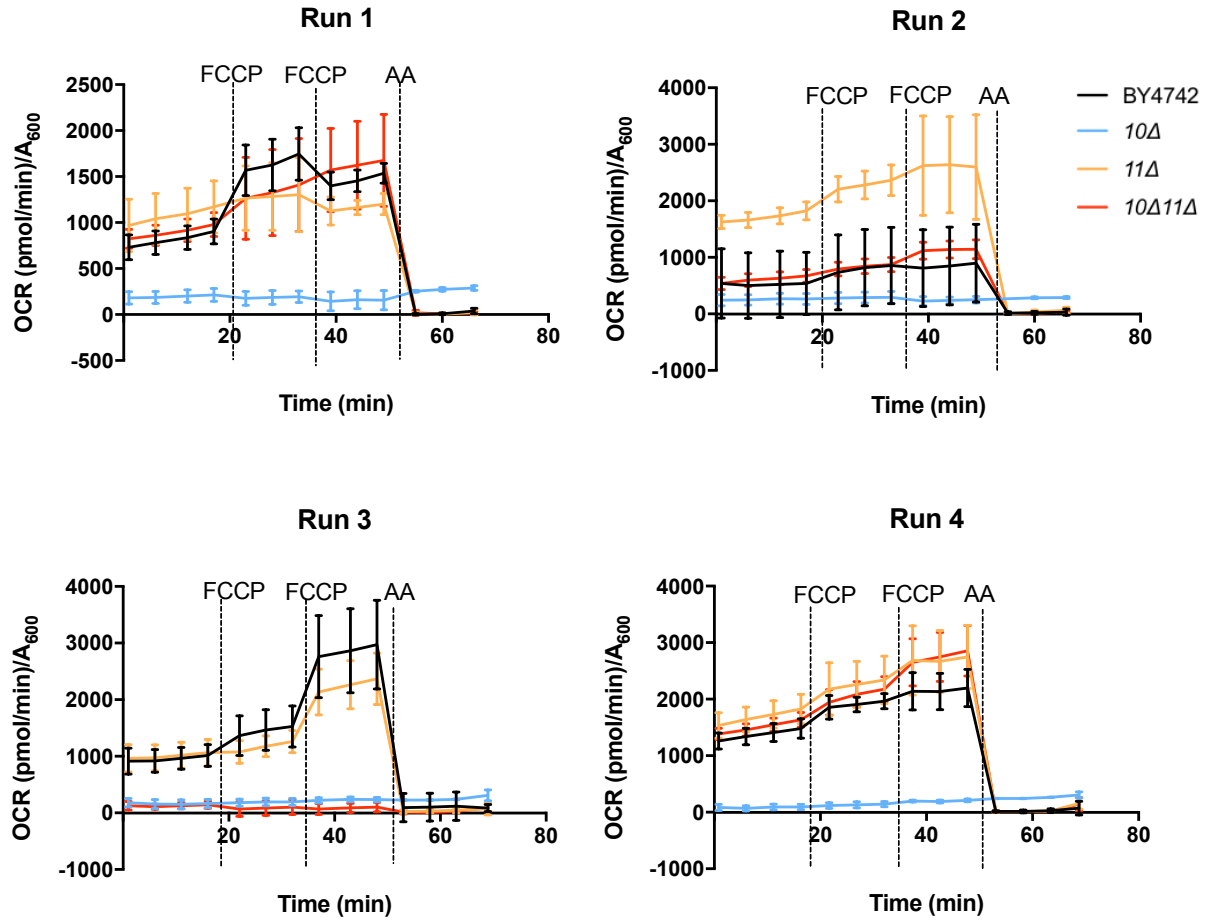


Figure S1. Oxygen consumption rates (OCRs) of yeast strains were determined with the XF96 Extracellular Flux Analyzer. The graphs depict traces from four independent experiments (Run 1 – Run 4). FCCP and Antimycin A (AA) were sequentially added to evaluate mitochondrial respiratory states. Each graph represents an individual experiment from the representative average traces (Fig. 2B), and each group represents 8–10 technical replicates.

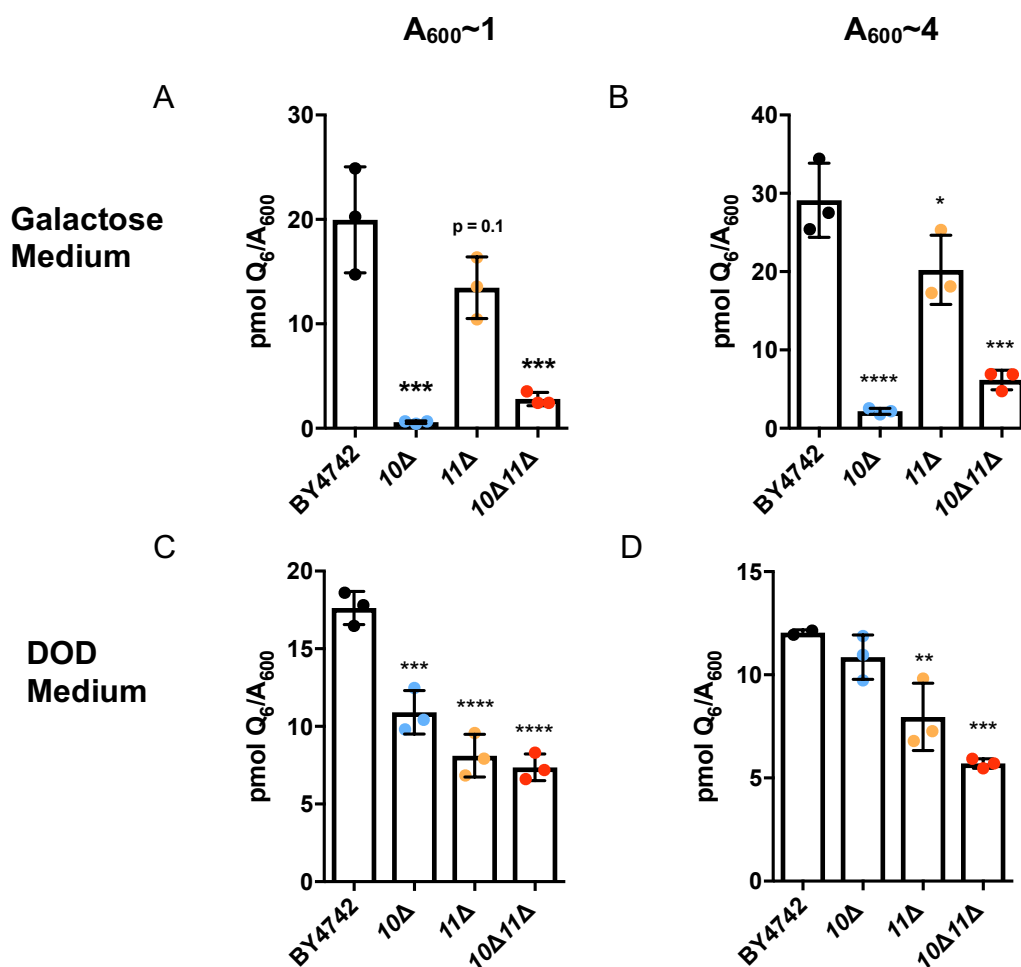


Figure S2. Q₆ content of whole cells have similar trends based on type of growth medium, rather than A₆₀₀ at time of harvest. Triplicates of 30 mL yeast cultures of wild type, *coq10*Δ, *coq11*Δ, and *coq10*Δ*coq11*Δ were grown in *A-B*, YPGal or *C-D*, dextrose-containing synthetic, minimal-medium (DOD) until they reached *A,C*, A₆₀₀~1 or *B,D*, A₆₀₀~4. Lipids from 10 mL of each culture were analyzed by LC-MS/MS. The data show mean ± SD, and the statistical significance as compared to wild type is represented by *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001. Panel B is a re-print from Fig. 5A in the main text.

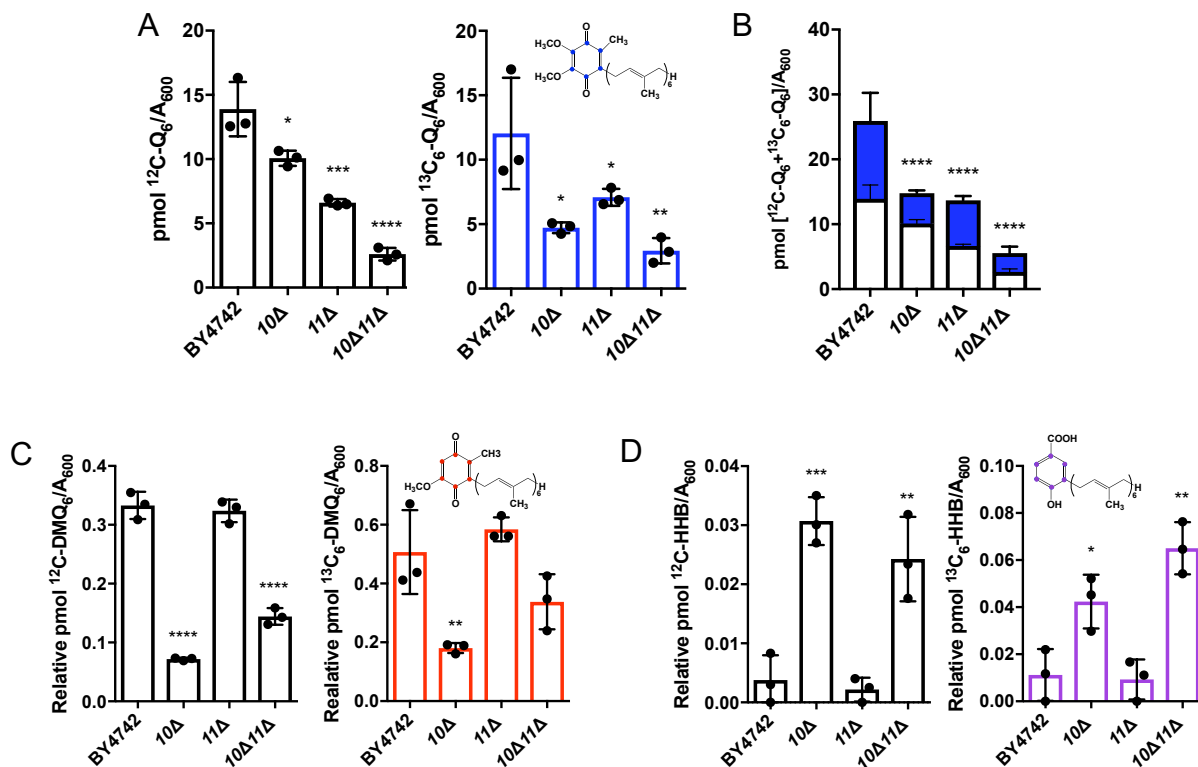


Figure S3. The *coq10Δcoq11Δ* double mutant the lowest total Q_6 compared to the single mutants and wild type in minimal medium. Triplicates of 6 mL cultures in DOD minimal medium were labeled with 5 $\mu\text{g/mL}$ $^{13}\text{C}_6\text{-4HB}$. After 4 h, lipid extracts from 5 mL of each were analyzed by LC-MS/MS. *A*, Amount of $^{12}\text{C}_6\text{-Q}_6$ and $^{13}\text{C}_6\text{-Q}_6$ (blue); *B*, Total amount of Q_6 , from the sum of $^{13}\text{C}_6\text{-Q}_6$ and $^{12}\text{C}_6\text{-Q}_6$; *C*, $^{12}\text{C}_6\text{-DMQ}_6$ and $^{13}\text{C}_6\text{-DMQ}_6$ (red); and *D*, $^{12}\text{C}_6\text{-HHB}$ and $^{13}\text{C}_6\text{-HHB}$ (purple), were measured from the whole cell lipid extracts of wild type, *coq10Δ*, *coq11Δ*, and *coq10Δcoq11Δ* mutants. The data show mean \pm SD, and the statistical significance as compared to wild type is represented by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$.

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