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Supplemental Information

COA6 Is Structurally Tuned to Function as a Thiol-Disulfide Oxidoreductase in Copper Delivery to Mitochondrial Cytochrome *c* Oxidase

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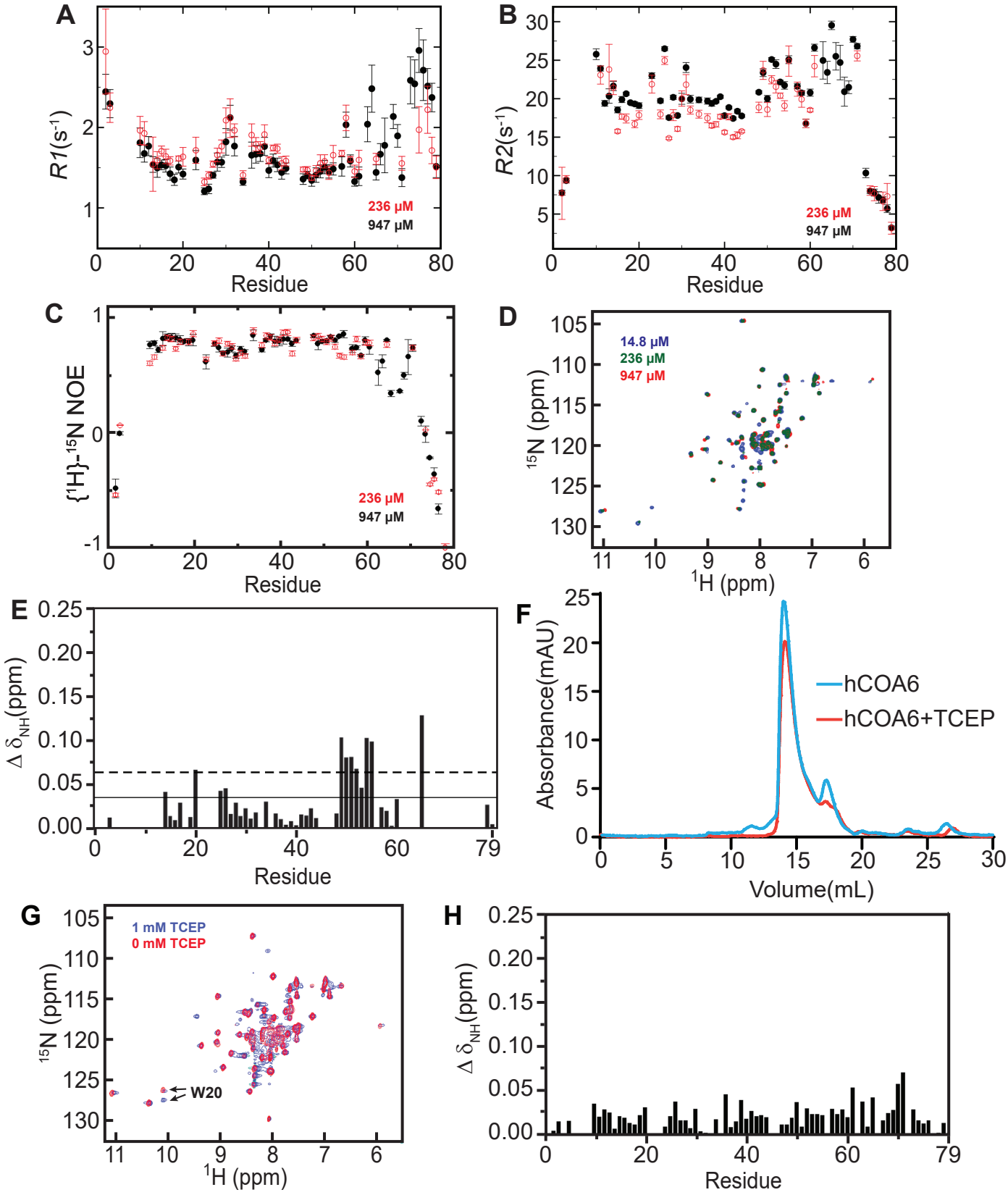

Figure S1 (related to Figure 1). Interrogation of COA6 structure by NMR.
(A) Longitudinal relaxation rates (R1), (B) transverse relaxation rates (R2), and (C) Heteronuclear ^{1}H-^{15}N NOEs, are plotted as a function of residue number for COA6 at two different protein concentrations. (D) Overlay of the ^{1}H-^{15}N HSQC spectra of human COA6 at three different protein concentrations: 14.8 μM (blue), 236 μM (green) and 947 μM (red). (E) Average chemical shift deviation of ^{15}N-labeled COA6 at 947 μM TCEP when compared to treatment with 14.8 μM TCEP. The horizontal solid line represents the mean and the dashed line represents the mean + SD of chemical shift perturbation for all residues. (F) Elution profile of COA6 from a Superdex 75 gel filtration column in the presence and absence of 1mM TCEP. (G) Overlay of the ^{1}H-^{15}N HSQC spectra of human COA6 with and without 1mM TCEP. (H) Average chemical shift deviation of ^{15}N-labeled COA6 upon addition of 1mM TCEP.

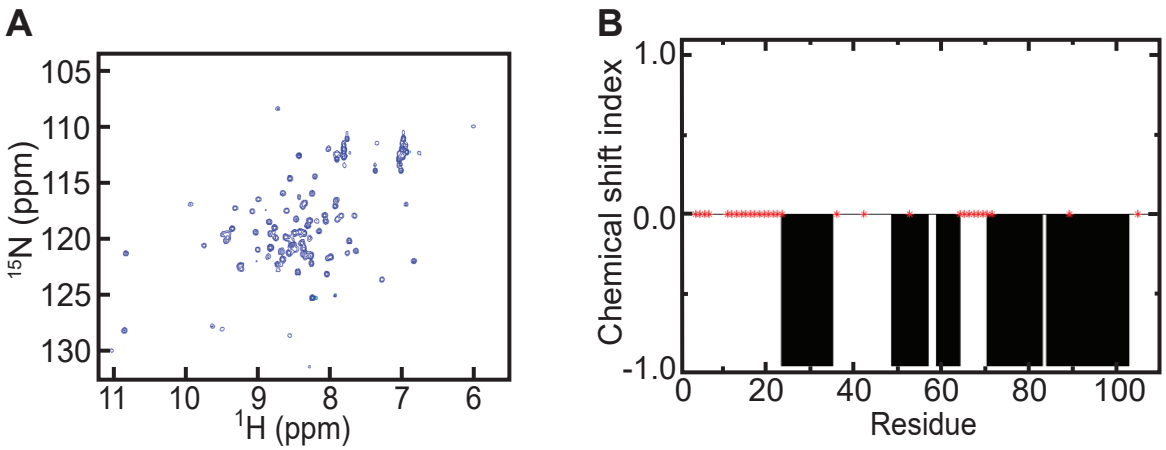

Figure S2 (related to Figure 1). Yeast Coa6 is a helical protein.
(A) ^{1}H-^{15}N HSQC spectrum of yeast Coa6. (B) The chemical shift index is shown as a function of residue number. Negative values represent an α-helical propensity, while positive values are indicative of a β-strand. A red asterisk denotes residues which were unassigned.

A

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SCO1 SFTGKPLLGGPFSLTTHTGERKTDKDYLGQWLLIYFGFTHCPDVCPEELEKMIQVVDEID 189
SCO2 ---GSFTGQGDFHLLDHRGRARCKADFRGQWVLMYFGFTHCPDICPDELEKLVQVVRQLE 152
        *.    * * *  * *. : . *: ***:*:**********:**:****::*** :::
SCO1 SITTLPDLTPLFISIDPERDTKEAIANYVKEFSPKLVGLTGTREEVDQVARAYRVYYSPG 349
SCO2 AEPGLPPVQPVFITVDPERDDVEAMARYVQDFHPRLLGLTGSTKQVAQASHSYRVYYNAG 212
     :    ** :*:**::*****  **:*.**::* *:*:****: ::* *.:::*****. *
SCO1 PKDEDEDYIVDHTIIMYLIGPDGEFLDYFGQNKRKGEIAASIATHMRPYRKKS- 301
SCO2 PKDEDQDYIVDHSIAIYLLNPDGLFTDYYGRSRSAEQISDSVRRHMAAFRSVLS 266
     *****:******:* :**:.*** * **:*:.:   :*: *:  **  :*.
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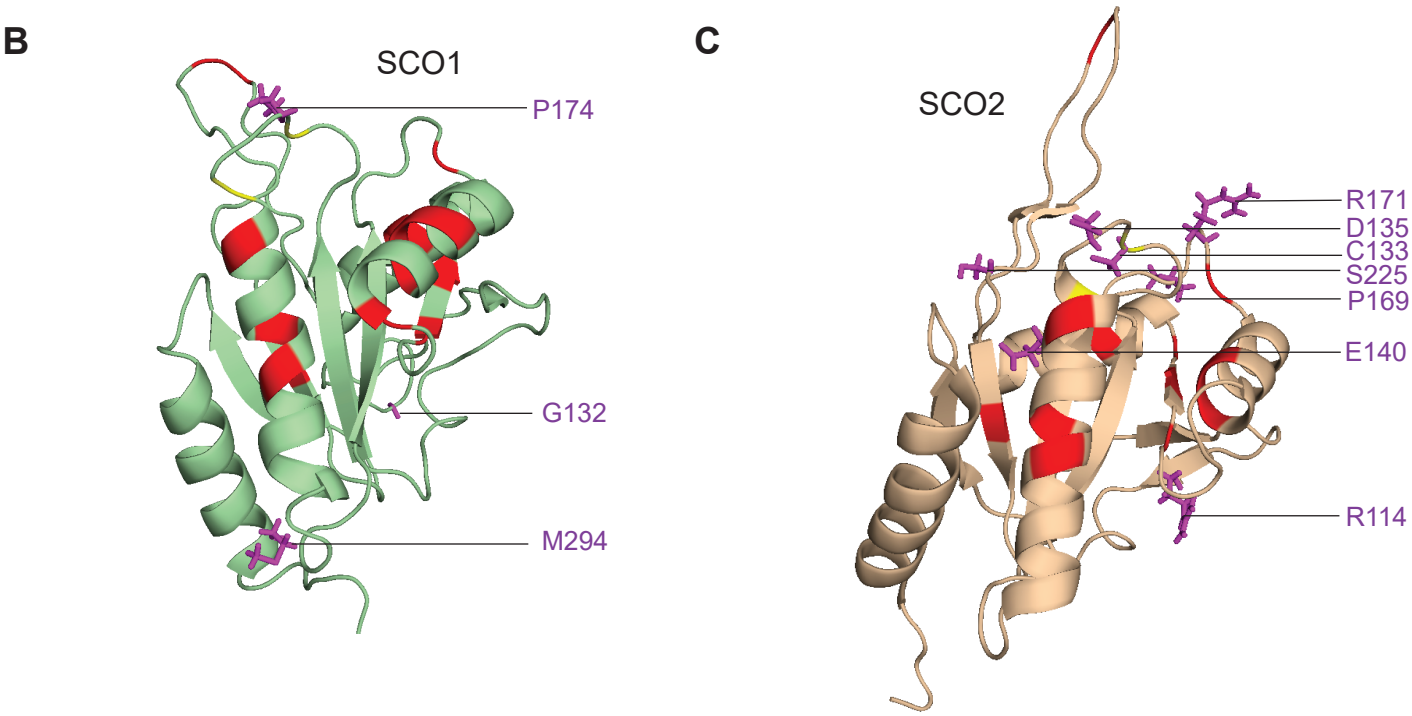

Figure S3 (related to Figure 4). A subset of human patient mutations in SCO1 and SCO2 are in close vicinity to the COA6 interaction surface.

A) Alignment of human SCO1 and SCO2 proteins. The amino acids shown in red undergo chemical shift perturbation in the presence of COA6, which is indicative of their involvement in an interaction. The residues shown in red and bold are the residues that are identical or similar in both SCO proteins. B) SCO1 (PDB: 2GVP) and SCO2 (PDB:2RLI) amino acid residues that interact with COA6 are highlighted in red and side-chains of the residues mutated in human patients are depicted in purple. Patient mutations listed here are from OMIM database (OMIM:603644, OMIM:604272) and from Leary et al., 2013 and Rebelo et al., 2018.

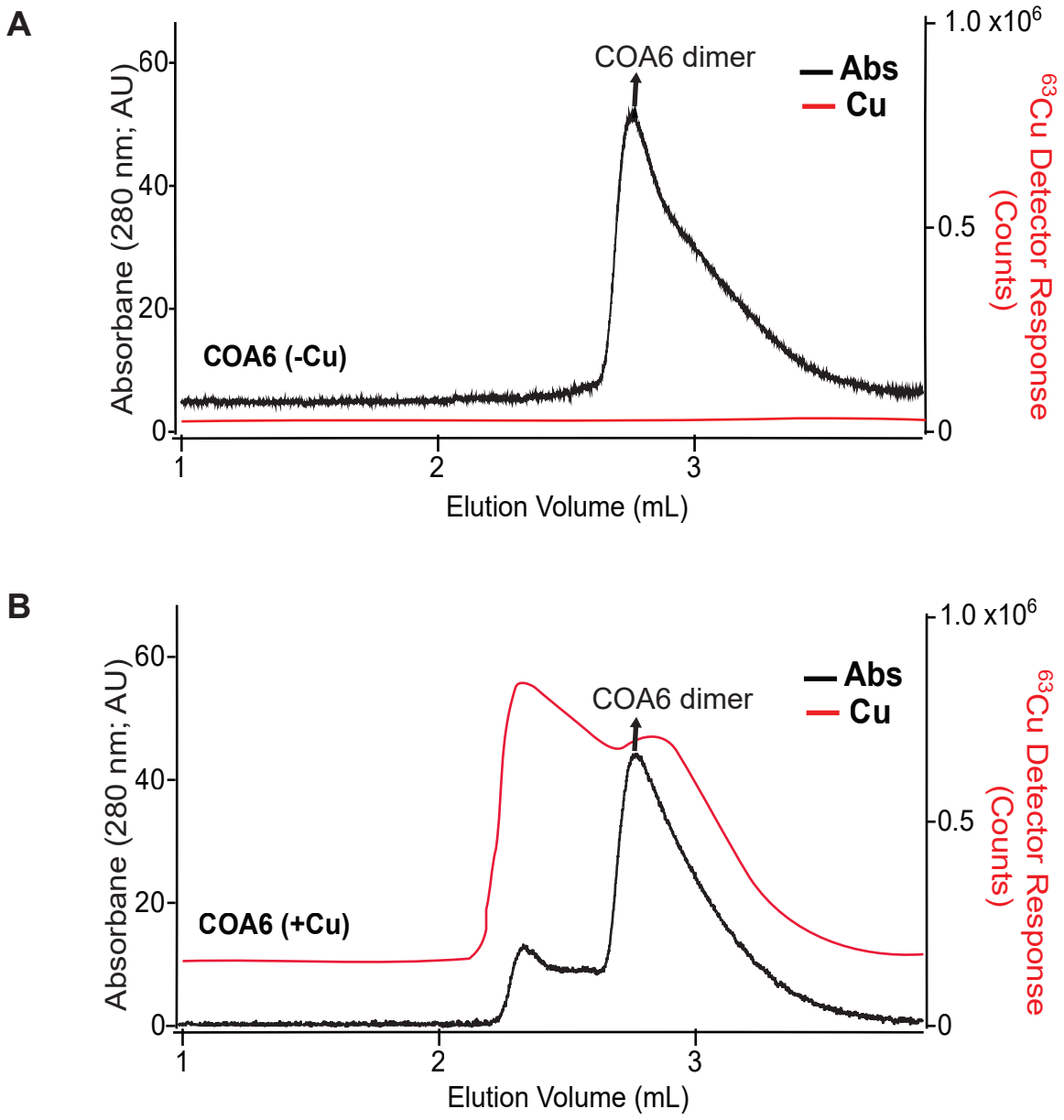

Figure S4 (related to Figure 5). Recombinant COA6 only binds copper when reconstituted with the metal ion and reductant.

(A) ^{63}Cu and protein traces of recombinant COA6 analyzed by LC-ICP-MS. (B) ^{63}Cu and protein traces of recombinant COA6 reconstituted with $CuSO_4$ and reduced glutathione.

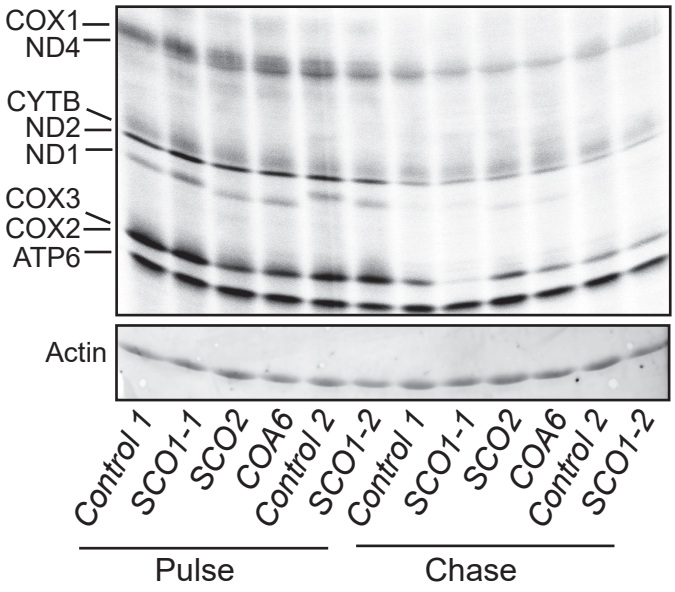

Figure S5 (related to Figure 6). Impact of SCO1, SCO2 and COA6 mutations on the synthesis and turnover of COX2.

In vivo ^{35}S-labeling of mitochondrial translation products in control and patient cell lines. Samples were pulse-chased and analyzed by SDS-PAGE and digital autoradiography.

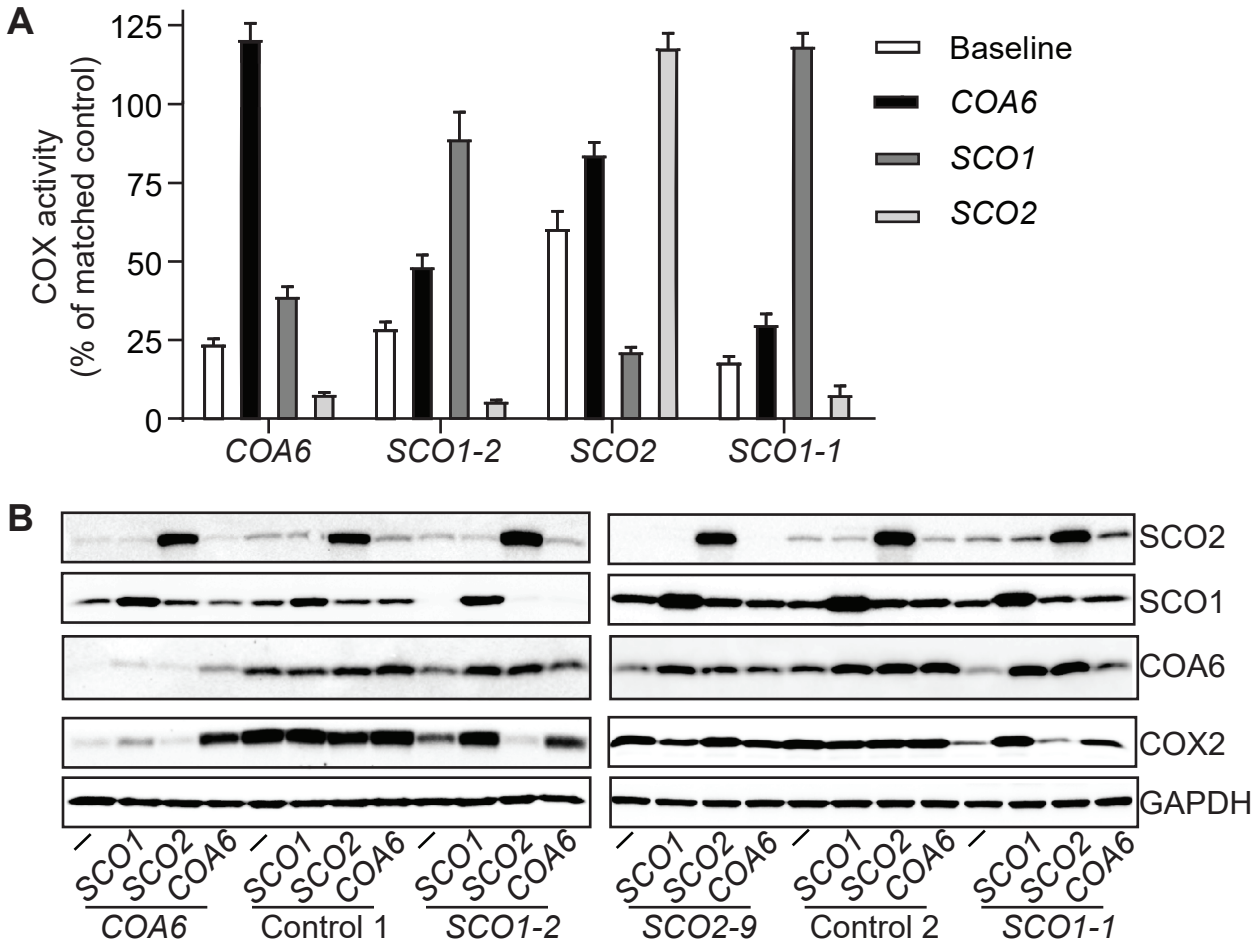

Figure S6 (related to Figure 6). Epistasis analysis of SCO1, SCO2 and COA6 overexpression on CcO activity in patient cell lines.

(A) Cytochrome c oxidase acitvity (CcO) in *COA6, SCO1* and *SCO2* patient cell lines alone (baseline) or when overexpressing COA6, SCO1 or SCO2. Data are represented as mean + SEM. (B) SDS-PAGE/Western blot analysis of COA6, SCO1, SCO2, COX2 and GAPDH (loading control) abundance in protein lysates from control and patient cells described in Figure A.

Table S1 (related to Figure 1). NMR and refinement statistics for solution structure of COA6

NMR distance and dihedral constraints	
Distance restraints	
Total NOE	554
Intra-residue	163
Inter-residue	284
Sequential ($\|i - j\| = 1$)	177
Nonsequential ($\|i - j\| > 1$)	214
Long-Range NOE	107
Hydrogen bonds	32
Total dihedral angle restraints	108
ϕ (TALOS+)	54
ψ (TALOS+)	54
Structure statistics	
Violations (mean and s.d.)	
Distance constraints (Å)	0.01 ± 0.03
Dihedral angle constraints (º)	0.37 ± 0.88
Max. dihedral angle violation (º)	6.18
Max. distance constraint violation (Å)	0.43
Deviations from idealized geometry	
Bond lengths (Å)	0.008
Bond angles (º)	0.6
Ramachandran Plot	
Most favored	79.6%
Additionally allowed	17.2%
Generously allowed	3.2%
Disallowed	0.1%
Average pairwise r.m.s. deviation* (Å)	
Protein structured region (residues 9 to 64)	
Heavy	1.35
Backbone	0.54

*Pairwise r.m.s. deviation was calculated among 20 refined structures.

Table S2. Related to STAR Methods. Primers used in this study.

Name	Sequence (5' to 3')
Protein expression primers	
Human COA6 isoform 3 Forward	GGGCCAGGATCCATGGCAGCACCGAGCATGAAA
Human COA6 isoform 3 Reverse	GGGCCACTCGAGTTAGCTTTTTGCGGTGGTTTC
Yeast Coa6 Forward	TAGCGAAAATCTGTATTTTCAGGGTAGCGAATTCATGGGC TTATTTTCATTTGATGGTGGC
Yeast Coa6_Stop Reverse	GTGGTGGTGGTGGTGCTCGAGTCACTGATTTCGTTCCCT CTGTTTAG
Yeast Coa6 Reverse	GTGGTGGTGGTGGTGCTCGAGCTGATTTCGTTCCCTCTG TTTAG
Site Directed mutagenesis primers	
HyCOA6 W26C Forward	AGTATTCATCTCTAGCACCGCAACACAATTTTCTTTGGG
HyCOA6 W26C Reverse	CCCAAAGAAAATTGTGTTGCGGTGCTAGAGATGAATACT
HyCOA6 W33R Forward	TTTTCGTCCAAACATTTCCGGTATTCATCTCTAGCACCC
HyCOA6 W33R Reverse	GGGTGCTAGAGATGAATACCGGAAATGTTTGGACGAAAA
HyCOA6 E54X Forward	CATTGTTGTGGACAAGAAGATTAGAAAGAGGATCTCAACT TCTTA
HyCOA6 E54X Reverse	TAAGAAGTTGAGATCCTCTTTCTAATCTTCTTGTCCACAAC AATG
Primers for Human COA6	
COA6-F	AAAAAGCAGGCTACCATGGGCCCGGGAGGTCCCTT
COA6-R	AGAAAGCTGGGTCTAGGATTTTGCAGTTGTTTCTGA
COA6 W59C-F	GAAAGACAGGTCTGCTGTGGGGCCCGGGATGAGTAC
COA6 W59C-R	GTACTCATCCCGGGCCCCACAGCAGACCTGTCTTTC
COA6 W66R-F	GCCCGGGATGAGTACCGGAAGTGTTTAGATG
COA6 W66R-R	CATCTAAACACTTCCGGTACTCATCCCGGGC
Protein truncation primers	
HyCOA6 SacI Forward	CCCCTGGAGCTCGCAAAGACGCGCAGCCAAAAA
HyCOA6_64 BamH1 Reverse	GCGCGCGGATCCCTTTTCTTTGAACTTCAAGTAGTCTCT
HyCOA6_72 BamH1 Reverse	GCGCGCGGATCCTGGTTCGAATTGACCGGCTTCGAACTT