SUPPLEMENTAL MATERIAL

Tables

Table S1. Human skin fibroblasts with CI or CoQ_{10} deficiency used in this study.

Defect	Gene	Mutations	Reference	Residual C-I activity
Complex I Deficiency	*ND3*	10158T>C p.Ser34Pro	(1)	52%
	ND6	14459G>A p.Ala72Val	(1)	57%
	NDUFB3	c.64T>C, p.Trp22Arg c.208G>T, p.Gly70X	(2)	17%
	NDUFB9	c.191T>C homo p.L64P	(1)	18%
	NDUFS1	c.683T>C, p.V228A c.755A>G, p.D252G	(1)	37%
	NDUFS3	c.595C>T homo; p.R199W	(3)	40%
	NDUFS8	c.187G>C homo, p.Glu63Gln	(3)	54%
				Residual CoQ_{10} levels
Primary CoQ_{10} Deficiency	*PDSS2*	c.[964C>T];[1145C>T]	(4)	15%
	COQ4	c.[155T>C];[518 520delCCA]	(5)	27%

Table S2. Levels of CoQ_{10} in untreated and CoQ_{10}-supplemented cells.

Gene	Regular Medium (A) CoQ_{10} (ng/mg prot)	Regular Medium + 100 μM CoQ_{10} (B) CoQ_{10} (ng/mg prot)	(B/A) CoQ_{10} Increase Fold
Controls	83.2 ± 13.3	715335 ± 304041 +++	8597
ND3	86.4 ± 2.1	446180 ± 166429 +++	5164
ND6	82.4 ± 30.1	450973 ± 50294 +++	4881
NDUFB3	88 ± 22.8	3260636 ± 1498894 +++	37074
NDUFB9	84.6 ± 7.5	195247 ± 31905 +++	2308
NDUFS1	75 ± 8.6	265735 ± 144425 +++	3543
NDUFS3	93 ± 9.2	446627 ± 169118 +++	4802
NDUFS8	87.4 ± 9.3	591155 ± 415712 +++	6768
PDSS2	18.8 ± 11.2 ***	2046794 ± 9957 +++	108872
COQ4	27.4 ± 13.4 ***	472774 ± 166429 +++	17255

Data are expressed as mean ± SD. ****P* < 0.001, differences versus controls; +++ P < 0.001, differences versus untreated cells (A).

Table S3. Levels of CoQ in tissues of the mouse models used in this study.

Model	Tissue	No treatment Total CoQ (ng/mg prot)	Treatment with oral $Q_{10}H_2$ Total CoQ (ng/mg prot)	
C57Bl6J	*Liver*	352.9 ± 13.3	1885.7 ± 412.6 +++	
		CoQ_9 (ng/mg prot)		
Ndufs4$^{+/+}$	*Brain*	315.3 ± 50.3		
Ndufs4$^{-/-}$	*Brain*	365.7 ± 77.8		
		Vehicle CoQ_9 (ng/mg prot)	**Treatment with NAC CoQ_9 (ng/mg prot)**	**Under SAAR CoQ_9 (ng/mg prot)**
Coq9$^{+/+}$	*Kidney*	1042.3 ± 222.4		
Coq9^{R239X}	*Kidney*	19.2 ± 2.1 ***	16.2 ± 2.1 ***	21.9 ± 4.1 ***

Data are expressed as mean ± SD. ***$P < 0.001$, differences versus wild-type; +++ $P < 0.001$, differences versus untreated animals.

Figures

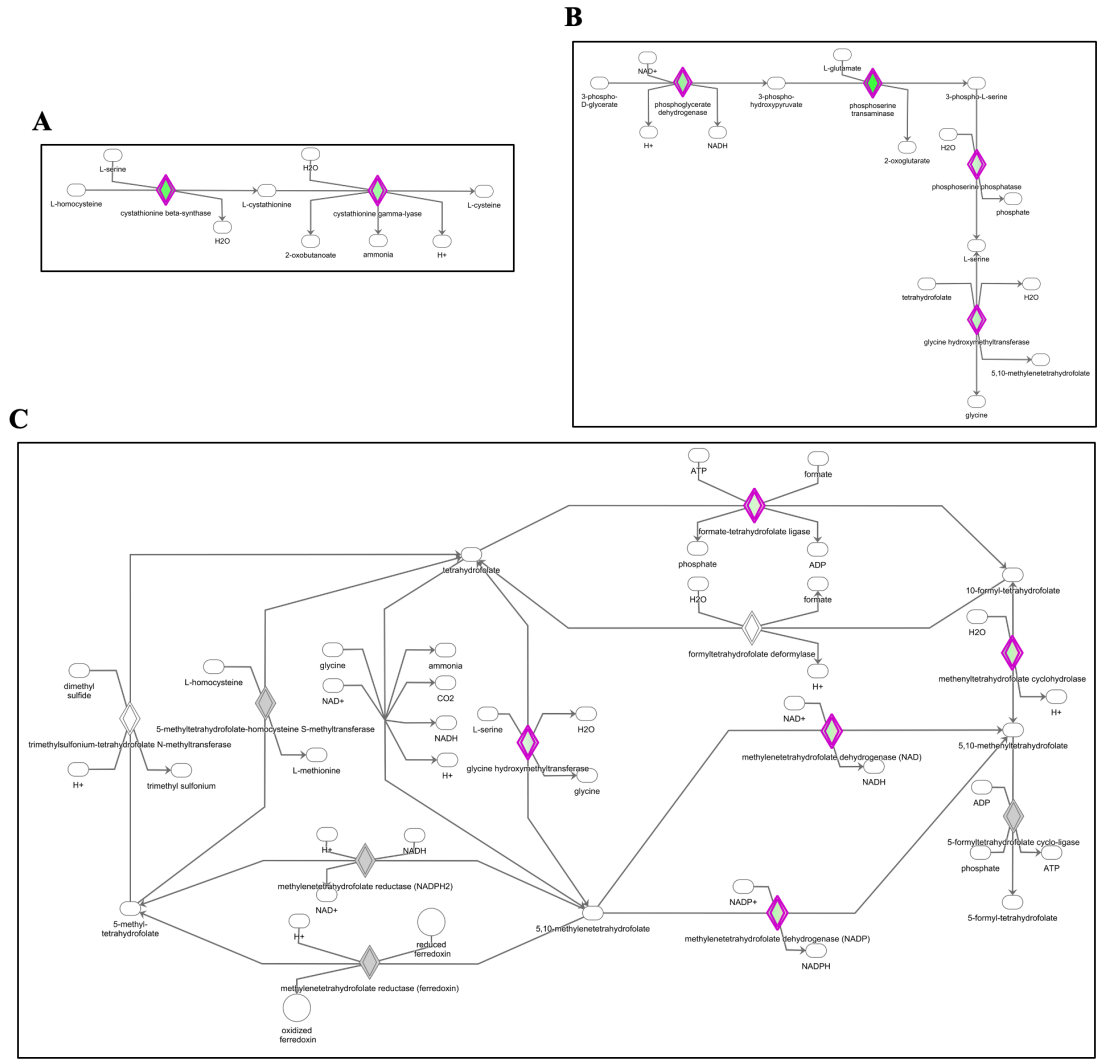

Figure S1. Representation of some of the pathways altered after CoQ_{10} supplementation.
(A) Cysteine biosynthesis/homocysteine degradation.
(B) Serine and Glycine Biosynthesis.
(C) Folate transformation.
The shape with green filling means that the gene is downregulated; the shape with pink filling means that the gene is upregulated.

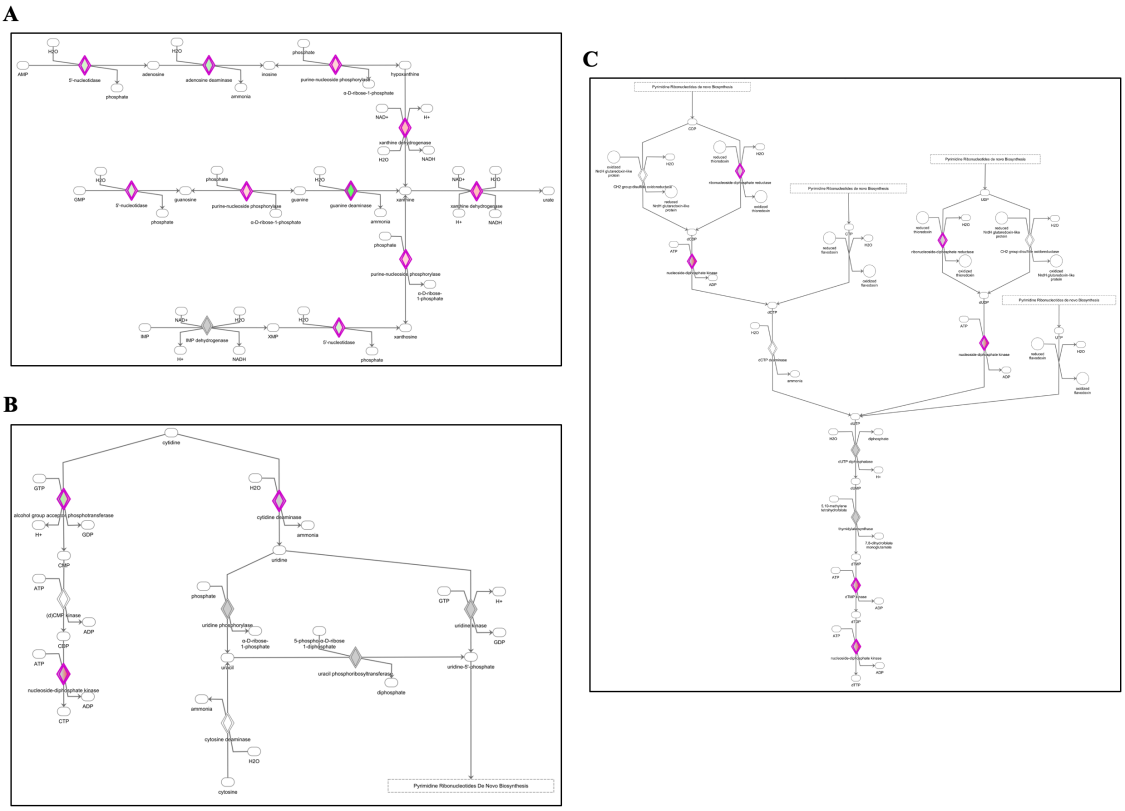

Figure S2. Representation of some of the pathways altered after CoQ_{10} supplementation.
(A) Purine nucleotides biosynthesis and urate biosynthesis.
(B) Salvage pathways of pyrimidine ribonucleotides.
(C) Pyrimidine deoxyribonucleotides *de novo* biosynthesis.
The shape with green filling means that the gene is downregulated; the shape with pink filling means that the gene is upregulated.

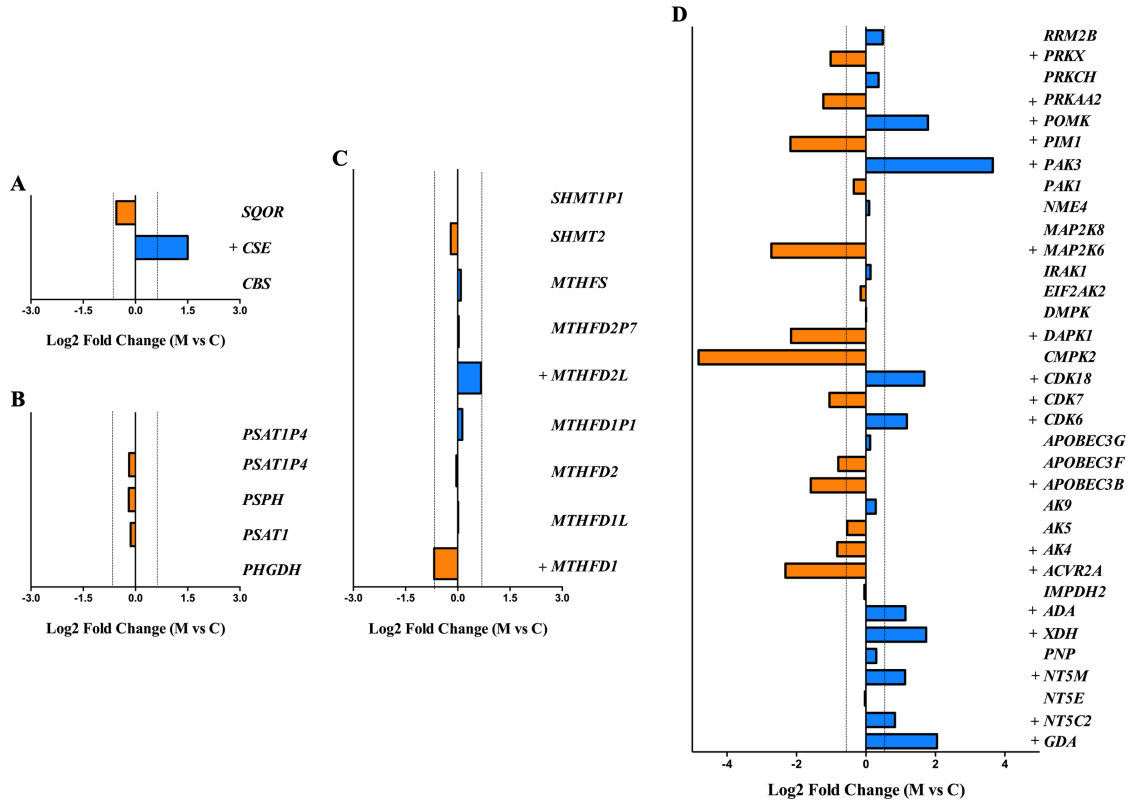

Figure S3. Changes in gene expression in the pathways related to sulfide metabolism in *NDUFS1* mutant cells.
(A) Changes in the expression of genes related to sulfide metabolism.
(B) Changes in the expression of genes related to cysteine biosynthesis.
(C) Changes in the expression of genes related to the folate cycle.
(D) Changes in the expression of genes related to nucleotides.
Bars in blue indicates genes overexpressed; bars in orange indicates genes underexpressed. Botted lines indicate the limit chosen for overexpression (log2 FC = 0.585) or underexpression (log2 FC = -0.585). Asterisks indicates the genes that exceed the chosen fold change limit and have p-values less than 0.05 in the comparison of treated and untreated cells in both control and mutant cells.

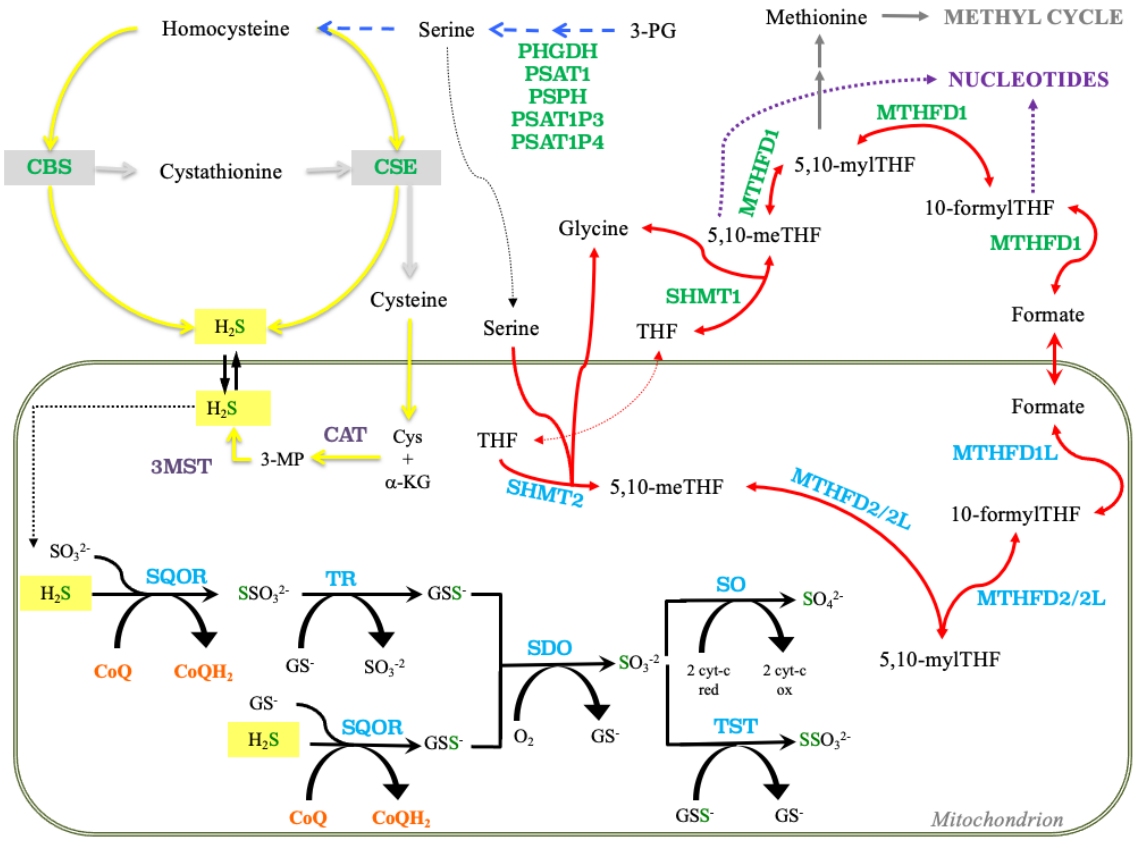

Figure S4. Relation between pathways, with some key genes indicated. Sulfide oxidation pathway (black arrows); transsulfuration pathway (grey arrows); sulfide synthesis (yellow arrows); folate cycle (red arrows); serine biosynthesis (blue arrows); nucleotides metabolism (purple arrows).

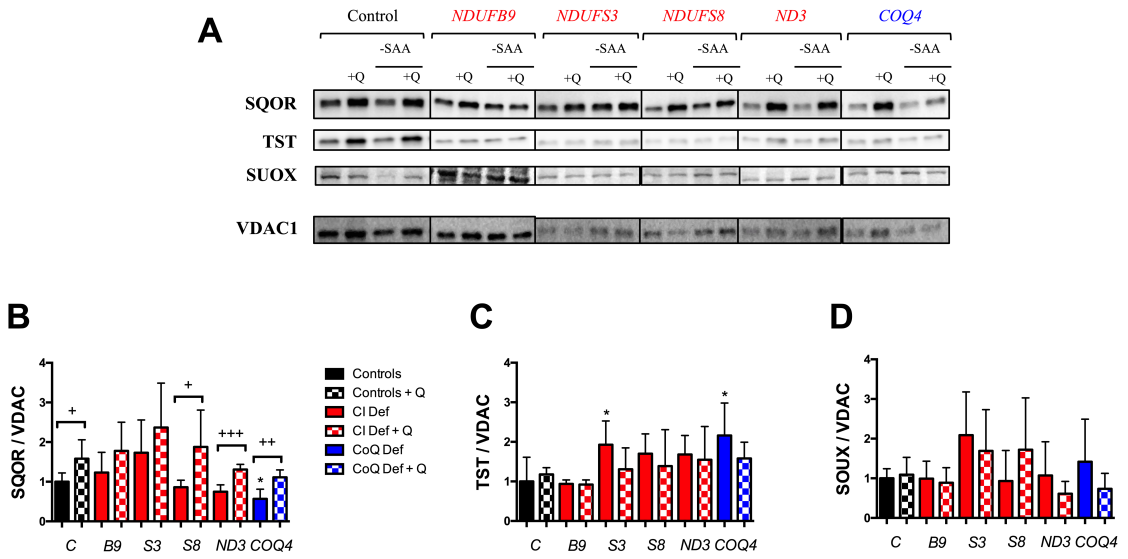

Figure S5. The levels of SQOR are modified under mitochondrial dysfunction or CoQ_{10} supplementation, independently of sulfur amino acids availability.
(A, B, C, D) Levels of the proteins of the mitochondrial hydrogen sulfide oxidation pathway in human skin fibroblasts from control and patients with mutations in Complex I subunits or CoQ_{10} biosynthetic genes. Data are expressed as mean ± SD. *P < 0.05; **P < 0.01; ***P < 0.001; differences versus Control. +P < 0.05; ++P < 0.01; +++P < 0.001; Control versus Control + CoQ_{10} or Complex I deficiency versus Complex I deficiency + CoQ_{10} or CoQ_{10} deficiency versus CoQ_{10} deficiency + CoQ_{10} (*t*-test; n = 3 for each group); n = 3 for each group). C = control; B9 = *NDUFB9* mutant; S1 = *NDUFS1* mutant; S3 = *NDUFS3* mutant; S8 = *NDUFS8* mutant; ND3 = *ND3* mutant; COQ4 = *COQ4* mutant; CI Def = cells with Complex I deficiency; CoQ Def = cells with CoQ_{10} deficiency; Q = CoQ_{10}; -SAA = medium without sulfur aminoacids. The blot image (A) has been made from three different membranes, as follow: blot 1, control; blot 2, *NDUFB9*; blot 3, *NDUFS3*, *ND3* and *COQ4*; blot 4, *NDUFS8*.

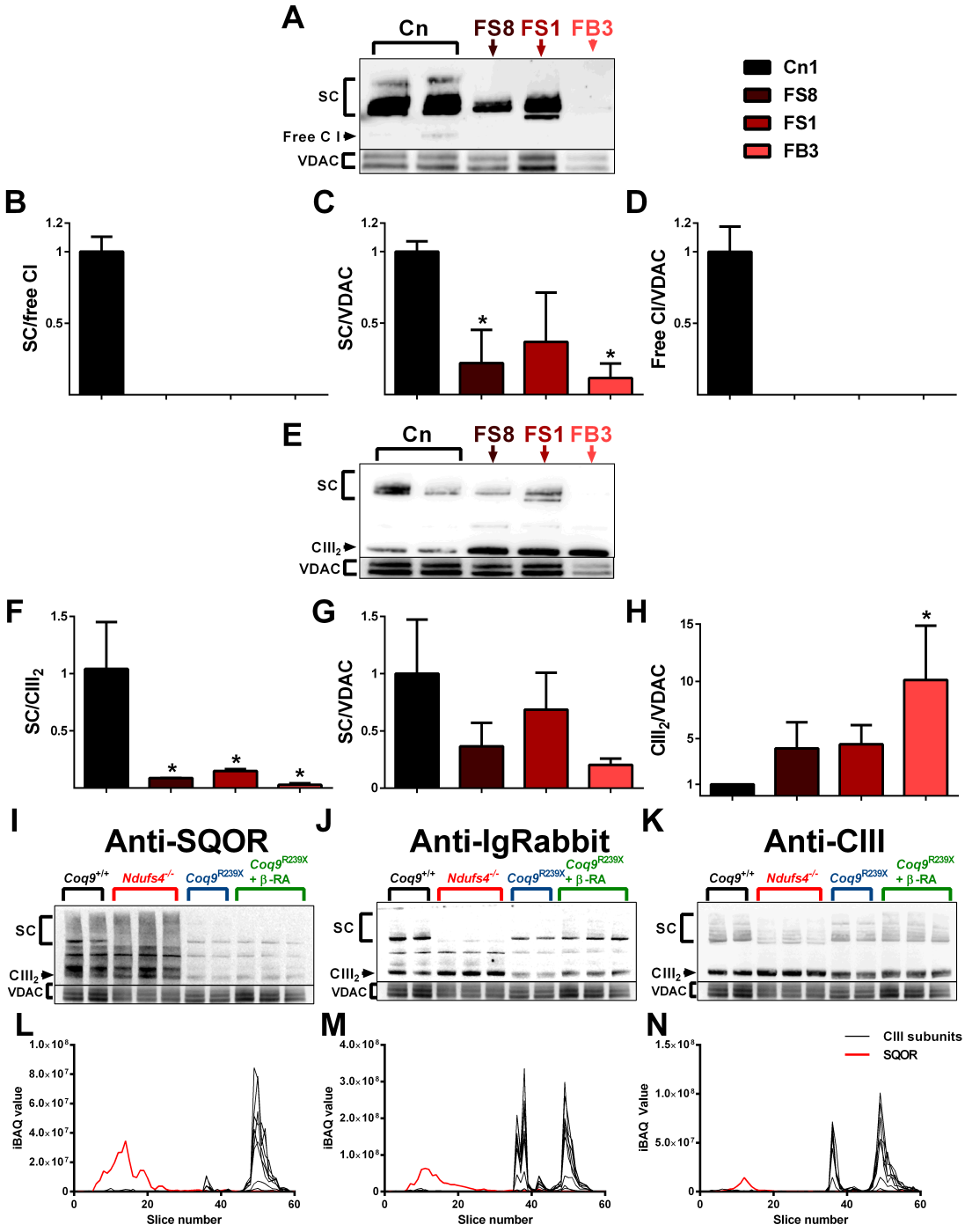

Figure S6. The changes of SQOR levels under mitochondrial dysfunction do not depend of mitochondrial supercomplexes formation.

(**A-D**) Blue-native gel electrophoresis (BNGE) followed by C-I immunoblotting analysis of mitochondrial supercomplexes in Control and CI deficiency skin fibroblasts. Anti-VDAC was used as loading control.

(**E-H**) Blue-native gel electrophoresis (BNGE) followed by C-III immunoblotting analysis of mitochondrial supercomplexes in Control and CI deficiency skin fibroblasts. Anti-VDAC was used as loading control.

(**I-K**) Blue-native gel electrophoresis (BNGE) followed by SQOR, Ig rabbit or CIII immunoblotting to check the presence of SQOR in mitochondrial supercomplexes in mitochondria isolated from kidneys of mouse models with CI or CoQ deficiency. Test made in 6 different membranes using different conditions.
(**L-N**) Migration pattern of Complex III subunits and SQOR proteins from human skin fibroblasts of three different controls. Data extracted from Van Strien, Guerrero-Castillo et al., 2019.
Data are expressed as mean SD. *P < 0.05; **P < 0.01; ***P < 0.001; Control cells versus deficiency skin fibroblasts. (*t*-test; n = 3 for each group); n = 3 for each group).

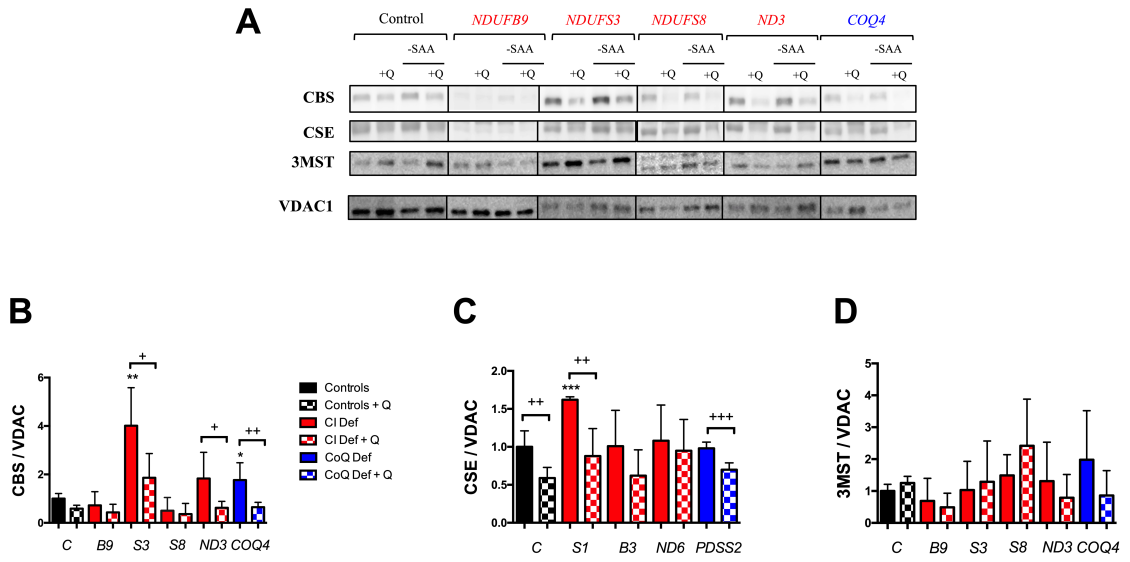

Figure S7. CoQ_{10} regulates the enzymes of transsulfuration pathway independently of sulfur amino acids availability.

(A, B, C, D) Levels of the proteins of the transsulfuration pathway in human skin fibroblasts from control and patients with mutations in Complex I subunits or CoQ_{10} biosynthetic genes. Data are expressed as mean ± SD. *P < 0.05; **P < 0.01; ***P < 0.001; differences versus Control. +P < 0.05; ++P < 0.01; +++P < 0.001; Control versus Control + CoQ_{10} or Complex I deficiency versus Complex I deficiency + CoQ_{10} or CoQ_{10} deficiency versus CoQ_{10} deficiency + CoQ_{10} (*t*-test; n = 3 for each group). C = control; B9 = *NDUFB9* mutant; S1 = *NDUFS1* mutant; S3 = *NDUFS3* mutant; S8 = *NDUFS8* mutant; ND3 = *ND3* mutant; COQ4 = *COQ4* mutant; CI Def = cells with Complex I deficiency; CoQ Def = cells with CoQ_{10} deficiency; Q = CoQ_{10}; -SAA = medium without sulfur aminoacids. The blot image (A) has been made from three different membranes, as follow: blot 1, control; blot 2, *NDUFB9*; blot 3, *NDUFS3*, *ND3* and *COQ4*; blot 4, *NDUFS8*.

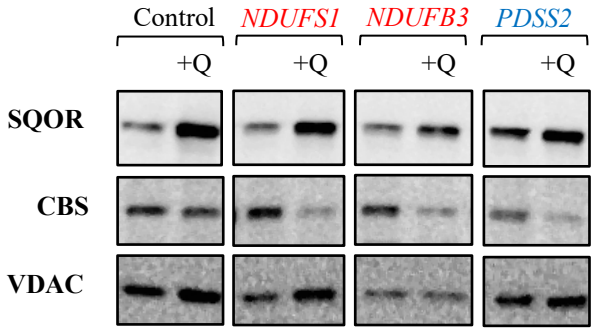

Figure S8. The levels of SQOR and CBS in control and mutant cells after supplementation with 20 μM CoQ_{10}. C = control; S1 = *NDUFS1* mutant; B3 = *NDUFB3* mutant; *PDSS2* = *PDSS2* mutant; Q = CoQ_{10}.

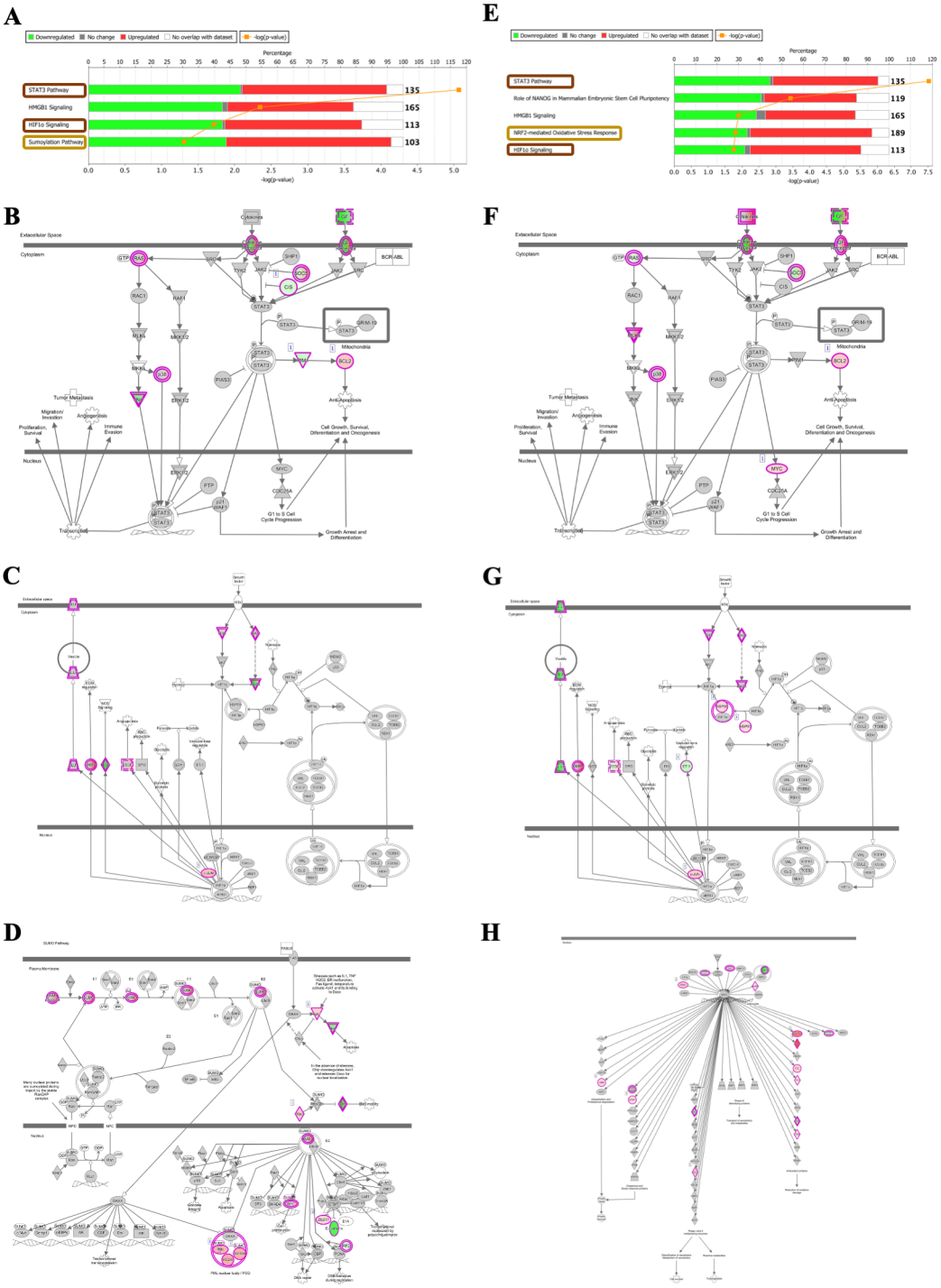

Figure S9. Transcriptional regulation and cell signaling pathways altered by CoQ_{10} supplementation.

(**A**) Canonical pathways most enriched in the differentially expressed gene list, related to transcriptional regulation and cells signaling, of the CoQ_{10} treated control cells dataset.

(B) STAT3 pathway in control cells.

(C) HIG1α signaling pathway in control cells.

(D) Sumoylation pathway in control cells.

(E) Canonical pathways most enriched in the differentially expressed gene list, related to transcriptional regulation and cells signaling, of the CoQ_{10} treated *NDUFS1* cells dataset.

(F) STAT3 pathway in *NDUFS1* mutant cells.
(G) HIG1α signaling pathway in *NDUFS1* mutant cells.
(H) NRF2 pathway in *NDUFS1* cells.
The shape with green filling means that the gene is downregulated; the shape with pink filling means that the gene is upregulated.

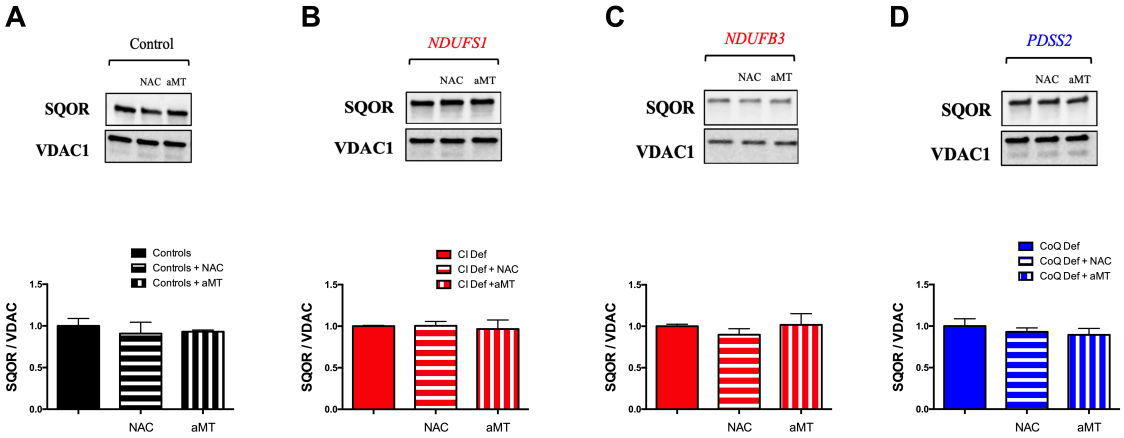

Figure S10. Levels of SQOR after the treatment with the antioxidants NAC or aMT. (A) Levels of SQOR in control cells; (B) Levels of SQOR in *NDUFS1* mutant cells; (C) Levels of SQOR in *NDUB3* mutant cells; (D) Levels of SQOR in *PDSS2* mutant cells;

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