

# Survival transcriptome in the coenzyme Q<sub>10</sub> deficiency syndrome is acquired by epigenetic modifications: a modelling study for human coenzyme Q<sub>10</sub> deficiencies

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## ABSTRACT

**Objectives:** Coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>) deficiency syndrome is a rare condition that causes mitochondrial dysfunction and includes a variety of clinical presentations as encephalomyopathy, ataxia and renal failure. First, we sought to set up what all have in common, and then investigate why CoQ<sub>10</sub> supplementation reverses the bioenergetics alterations in cultured cells but not all the cellular phenotypes.

**Design Modelling study:** This work models the transcriptome of human CoQ<sub>10</sub> deficiency syndrome in primary fibroblast from patients and study the genetic response to CoQ<sub>10</sub> treatment in these cells.

**Setting:** Four hospitals and medical centres from Spain, Italy and the USA, and two research laboratories from Spain and the USA.

**Participants:** Primary cells were collected from patients in the above centres.

**Measurements:** We characterised by microarray analysis the expression profile of fibroblasts from seven CoQ<sub>10</sub>-deficient patients (three had primary deficiency and four had a secondary form) and aged-matched controls, before and after CoQ<sub>10</sub> supplementation. Results were validated by Q-RT-PCR. The profile of DNA (CpG) methylation was evaluated for a subset of gene with displayed altered expression.

**Results:** CoQ<sub>10</sub>-deficient fibroblasts (independently from the aetiology) showed a common transcriptomic profile that promotes cell survival by activating cell cycle and growth, cell stress responses and inhibiting cell death and immune responses. Energy production was supported mainly by glycolysis while CoQ<sub>10</sub> supplementation restored oxidative phosphorylation. Expression of genes involved in cell death pathways was partially restored by treatment, while genes involved in differentiation, cell cycle and growth were not affected. Stably demethylated genes were unaffected by treatment whereas we observed restored gene expression in either non-methylated genes or those with an unchanged methylation pattern.

## ARTICLE SUMMARY

### Article focus

- To analyse the common gene expression profile in primary cell cultures of dermal fibroblasts from patients suffering any of the clinical presentation of the human syndrome of coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>) deficiency (primary or secondary CoQ<sub>10</sub> deficiency).
- To determine why CoQ<sub>10</sub> treatment, the current therapy for all forms of CoQ<sub>10</sub> deficiency, restored respiration but not all the clinical phenotypes.
- To investigate the stable genetic cause responsible for the survival adaptation to mitochondrial dysfunction owing to CoQ<sub>10</sub> deficiency.

### Key messages

- The mitochondrial dysfunction owing to CoQ<sub>10</sub> deficiency induces a stable survival adaptation of somatic cells in patients at early or postnatal development by epigenetic modifications of chromatin. Deficient cells unable to maintain this survival state during differentiation would die contributing to the pathological phenotype.
- Supplementation with CoQ<sub>10</sub> restores respiration through enhanced sugar rather than lipid metabolism; partially restores stress response, immunity, cell death and apoptotic pathways; and does not affect cell cycle, cell growth, and differentiation and development pathways.
- Survival transcriptome in the CoQ<sub>10</sub> deficiency syndrome is acquired by epigenetic modifications of DNA: DNA-demethylated genes corresponded to unaffected genes by CoQ<sub>10</sub> treatment, whereas those with unchanged DNA-methylation pattern corresponded to genes with responsive expression to CoQ<sub>10</sub> supplementation. These results would approach to explain the incomplete recovery of clinical symptoms after CoQ<sub>10</sub> treatment, at least in some patients.

## ARTICLE SUMMARY

## Strengths and limitations of this study

- Human CoQ<sub>10</sub> deficiencies are considered rare diseases with low prevalence, which limits the sample size.
- The genetic heterogeneity of this disease is owing to mutations in any of the 11 genes directly involved in the synthesis of CoQ<sub>10</sub> inside mitochondria, or other mutations altering somehow the mitochondria and its metabolism, affecting their inner CoQ<sub>10</sub> synthesis as a side effect, will course with CoQ<sub>10</sub> deficiency.
- Among this genetic heterogeneity, all cells showed a common transcriptomic profile that justified their pathological phenotype, responded equally to CoQ<sub>10</sub> treatment and presented the same DNA methylation pattern.

**Conclusions:** CoQ<sub>10</sub> deficiency induces a specific transcriptomic profile that promotes cell survival, which is only partially rescued by CoQ<sub>10</sub> supplementation.

## INTRODUCTION

Coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>) is a small electron carrier which is an essential cofactor for several mitochondrial biochemical pathways such as oxidative phosphorylation, β-oxidation and pyrimidine nucleotide biosynthesis. CoQ<sub>10</sub> biosynthesis depends on a multienzyme complex<sup>1</sup> that involves at least 11 proteins encoded by COQ genes. Mutations in any of these genes cause primary CoQ<sub>10</sub> deficiencies, which are clinically heterogeneous mitochondrial diseases.<sup>2</sup> Clinical presentations include encephalomyopathy with lipid storage myopathy and myoglobinuria,<sup>3</sup> ataxia and cerebellar atrophy,<sup>4</sup> severe infantile encephalomyopathy with renal failure,<sup>5</sup> isolated myopathy,<sup>6</sup> and nephrotic syndrome.<sup>7</sup> Secondary CoQ<sub>10</sub> deficiency has also been associated with diverse mitochondrial diseases.<sup>8–13</sup> In all of these conditions, CoQ<sub>10</sub> supplementation partially improves symptoms<sup>14 15</sup> and usually induces a return to normal growth and respiration in CoQ<sub>10</sub>-deficient fibroblasts.<sup>8 16 17</sup> Adaptation of somatic cells to CoQ<sub>10</sub> deficiency may affect both onset and course of the disease. We document common transcriptomic profile alterations in somatic cells of CoQ-deficient patients, their response to CoQ<sub>10</sub> supplementation, and the relationship with the DNA methylation status of specific genes.

## MATERIALS AND METHODS

## Cells

Primary skin fibroblasts from CoQ<sub>10</sub>-deficient patients and from aged-matched controls, at similar culture passage, were cultured at 37°C using Dulbecco's Modified Eagle Medium (DMEM) 1 g/l glucose, L-glutamine and pyruvate (Invitrogen, Prat de Llobregat, Barcelona) supplemented with an antibiotic/antimycotic solution (Sigma Chemical

Co, St Louis, Missouri) and 20% fetal bovine serum (FBS, Linus). When required, CoQ<sub>10</sub> prediluted in FBS was added to the plates at a final concentration of 30 μM (CoQ<sub>10</sub>, Synthetic Minimum 98%, high-performance liquid chromatography, Sigma). We studied five patients with primary CoQ<sub>10</sub> deficiency: two siblings harboured a homozygous p.Y297C mutation in the *COQ2* gene,<sup>5</sup> other with a pathogenic mutation (c.483G>C) in the *COQ4* gene (this paper), and another one with haploinsufficiency of *COQ4*.<sup>18</sup> Patients with secondary CoQ<sub>10</sub> deficiency included: a mitochondrial encephalopathy, lactic acidosis and stroke-like episodes patient harbouring the m.3243A>G in the mitochondrial tRNA<sup>Leu(UUR)</sup> with 43% heteroplasmy level,<sup>8</sup> a patient with mtDNA depletion syndrome<sup>12</sup> and a third patient with ataxia of unknown origin.<sup>4</sup> Table 1 summarises the clinical phenotype and biochemical studies of these patients.

## Transcriptome analysis

RNA extraction, probe synthesis and hybridisation with two independent expression arrays (GeneChip Human Genome U133 Plus 2.0 and GeneChip Human Gene 1.0 ST, Affymetrix) were used as described.<sup>19</sup> Gene expression was validated by the MyiQ Single Color Real Time PCR Detection System (Biorad). See supplementary methods for full description.

Data had been deposited with the NCBI-GEO database, at <http://www.ncbi.nlm.nih.gov/geo/>, accession number GSE33941 (this SuperSeries is composed of two subset Series, see online supplementary table S7 for an explanation).

Statistical analyses were performed comparing each signal of patient's fibroblasts RNA with the corresponding signal of control RNA by two different approaches. The main statistical analysis for both GeneChip Human Genome U133 Plus 2.0 Array and GeneChip Human Gene 1.0 ST Array was achieved as previously described,<sup>19</sup> which selects the most significant genes commonly and equally regulated in all samples using very stringent parameters. In a few special cases, other unselected but regulated genes were studied because of their role in specific processes and pathways. They were equally described in table 2. The second statistical analysis approach for the Gene Ontology (GO) study was performed as previously described<sup>20</sup> and analyses the most altered biological processes and pathways using a lower stringency analysis, which permits to select the hundred most altered GOs in different functional categories (see online supplementary table S4) and the hundred more distorted pathways (see online supplementary table S5) that had been regulated in CoQ<sub>10</sub>-deficient cells. GO regulated in both independent analysis of primary and secondary CoQ<sub>10</sub> deficiencies (see online supplementary table 3), and those regulated by CoQ<sub>10</sub> supplementation (see online supplementary table S9) were studied using the GORILLA software (Gene Ontology enrichment analysis and visualisation tool), at <http://cbl-gorilla.cs.technion>.

**Table 1** Clinical phenotype and biochemical studies performed in patients with coenzyme Q<sub>10</sub> deficiency

Patient/cells*	Clinical phenotype	Biochemical studies (% with respect to mean reference values)	Effect of CoQ <sub>10</sub> supplementation†	Reference as cited in the text	Array and epigenetic code
Human dermal skin fibroblast	Healthy volunteers	<i>Reference values</i>	<i>Reference values</i>	<sup>12</sup>	#2 #HDF #control #1
12-year-old girl	<ul style="list-style-type: none"> <li>▶ Ataxia and cerebellar atrophy</li> <li>▶ Secondary CoQ<sub>10</sub> deficiency</li> </ul>	<ul style="list-style-type: none"> <li>▶ 17% CoQ<sub>10</sub> in muscle</li> <li>▶ 31% mt-RC complex I+III (muscle)</li> <li>▶ 46% mt-RC complex II+III (muscle)</li> <li>▶ 22% CoQ<sub>10</sub> in fibroblast</li> <li>▶ 24% CoQ<sub>10</sub> biosynthesis rate</li> <li>▶ ROS production (three fold)</li> </ul>	<ul style="list-style-type: none"> <li>▶ Improvement of neurological assessment</li> <li>▶ No biochemical studies performed</li> </ul>	<sup>4</sup>	
33-month-old boy(his sister below)	<ul style="list-style-type: none"> <li>▶ Corticosteroid-resistant nephropathy</li> <li>▶ Progressive encephalomyopathy</li> <li>▶ COQ2 gene mutation (c.890A&gt;G)</li> <li>▶ Primary CoQ<sub>10</sub> deficiency</li> </ul>	<ul style="list-style-type: none"> <li>▶ 23% CoQ<sub>10</sub> in muscle</li> <li>▶ 19% mt-RC complex I+III (muscle)</li> <li>▶ 32% mt-RC complex II+III (muscle)</li> <li>▶ 17% CoQ<sub>10</sub> in fibroblast</li> <li>▶ 10% CoQ<sub>10</sub> biosynthesis rate</li> <li>▶ 57% mt-RC complex II+III (cells)</li> </ul>	<ul style="list-style-type: none"> <li>▶ Improvement of neurological assessment but not the renal dysfunction</li> <li>▶ Recovery of cell growth</li> <li>▶ Improvement of 35% complex II+III (cells)</li> </ul>	<sup>5</sup> <sup>17</sup> <sup>12</sup> case 3	#3
9-month-old girl(her brother above)	<ul style="list-style-type: none"> <li>▶ Corticosteroid-resistant nephropathy</li> <li>▶ COQ2 gene mutation (c.890A&gt;G)</li> <li>▶ Primary CoQ<sub>10</sub> deficiency</li> </ul>	<ul style="list-style-type: none"> <li>▶ 29% CoQ<sub>10</sub> in fibroblast</li> <li>▶ 15% CoQ<sub>10</sub> biosynthesis rate</li> <li>▶ 60% mt-RC complex II+III (cells)</li> </ul>	<ul style="list-style-type: none"> <li>▶ Improvement of 25% complex II+III (cells)</li> <li>▶ Recovery of cell growth</li> </ul>	<sup>17</sup> <sup>12</sup> case 4	#5
Boy	<ul style="list-style-type: none"> <li>▶ MELAS (A3243G mutation)</li> <li>▶ Secondary CoQ<sub>10</sub> deficiency</li> </ul>	<ul style="list-style-type: none"> <li>▶ 58% CoQ<sub>10</sub> in fibroblast</li> <li>▶ 35% mt-RC complex I (cells)</li> <li>▶ 41% mt-RC complex II+III (cells)</li> <li>▶ 12% mt-RC complex IV (cells)</li> <li>▶ 60% mt-ΔΨ</li> <li>▶ 70% mitochondrial mass</li> <li>▶ ROS production (&gt;2-fold)</li> <li>▶ Defective autophagosome elimination</li> </ul>	<ul style="list-style-type: none"> <li>▶ Recovery of mt-RC</li> <li>▶ Recovery of ATP production</li> <li>▶ No ROS production</li> </ul>	<sup>8</sup>	#4 #MEL+Q

Continued

Table 1 Continued

Patient/cells*	Clinical phenotype	Biochemical studies (% with respect to mean reference values)	Effect of CoQ <sub>10</sub> supplementation†	Reference as cited in the text	Array and epigenetic code
10-day-old boy	<ul style="list-style-type: none"> <li>▶ mtDNA depletion syndrome</li> <li>▶ Neonatal encephalopathy</li> <li>▶ Secondary CoQ<sub>10</sub> deficiency</li> </ul>	<ul style="list-style-type: none"> <li>▶ 20% CoQ<sub>10</sub> in muscle</li> <li>▶ 32% mt-RC complex I+III (muscle)</li> <li>▶ 19% mt-RC complex II+III (muscle)</li> <li>▶ 15% CoQ<sub>10</sub> in fibroblast</li> <li>▶ 85% mt-RC complex II+III (cells)</li> </ul>	<ul style="list-style-type: none"> <li>▶ Improvement of 41% complex II+III (cells)</li> <li>▶ Recovery of cell growth</li> </ul>	<sup>34</sup>	#ELO #ELO+Q
3-year-old boy	<ul style="list-style-type: none"> <li>▶ Dysmorphic features</li> <li>▶ Ventricular septal defect and weakness</li> <li>▶ Hypotonia and hyporeactivity</li> <li>▶ Moderate mental retardation</li> <li>▶ COQ4 gene deletion</li> <li>▶ Primary CoQ<sub>10</sub> deficiency</li> </ul>	<ul style="list-style-type: none"> <li>▶ 40% CoQ<sub>10</sub> in fibroblast</li> <li>▶ 44% CoQ<sub>10</sub> biosynthesis rate</li> <li>▶ 64% mt-RC complex I+III (cells)</li> <li>▶ 58% mt-RC complex II+III (cells)</li> </ul>	<ul style="list-style-type: none"> <li>▶ Improvement in muscle tone and strength</li> <li>▶ He began to speak and walk</li> </ul>	<sup>18</sup>	#GIO
Girl	<ul style="list-style-type: none"> <li>▶ COQ4 gene mutation (c.483G&gt;C)</li> <li>▶ Rhabdomyolysis</li> <li>▶ Primary CoQ<sub>10</sub> deficiency</li> </ul>	<ul style="list-style-type: none"> <li>▶ 18% CoQ<sub>10</sub> in fibroblast</li> </ul>	<ul style="list-style-type: none"> <li>▶ Recovery of both complex I+III activity and growth of fibroblasts</li> </ul>	This paper	#SIL+Q#epi
Girl	<ul style="list-style-type: none"> <li>▶ Ataxia</li> <li>▶ Secondary CoQ<sub>10</sub> deficiency</li> </ul>	<ul style="list-style-type: none"> <li>▶ 38% CoQ<sub>10</sub> in fibroblast</li> </ul>	<ul style="list-style-type: none"> <li>▶ Improvement of ATP synthesis</li> </ul>	<sup>12</sup> case 1	#SOF+Q#epi

\*Cultured at 37°C using DMEM 1 g/l glucose, L-glutamine, pyruvate (Invitrogen) plus antibiotic/antimycotic solution (Sigma) and 20% fetal bovine serum (FBS, Linus).

†CoQ<sub>10</sub> prediluted in FBS was added to the plates at a final concentration of 30 µM (coenzyme Q<sub>10</sub>, Synthetic Minimum 98%, high-performance liquid chromatography, Sigma).

CoQ<sub>10</sub>, Coenzyme Q<sub>10</sub>; MELAS, mitochondrial encephalopathy, lactic acidosis and stroke-like episodes; mtDNA, mitochondrial DNA; mt-RC, mitochondrial respiratory chain; ROS, reactive oxygen species.

**Table 2** Differentially expressed genes in coenzyme Q<sub>10</sub> deficiency

Gene symbol*	Gene title	FC†	FC‡	CoQ <sub>10</sub> §	Q-RT-PCR¶	CoQ <sub>10</sub> **
Mitochondrial metabolism						
C7orf55	Chromosome 7 open reading frame 55	-2.1	nc	–		
BRP44	Brain protein 44	2.0	2.3	U	8.0	-2-fold
C10orf58	Chromosome 10 open reading frame 58	-19.5	-1.6	pR		
NADH mobilisation						
CYB561	Cytochrome <i>b</i> 561	-1.3	nc	O		
CYB5A	Cytochrome <i>b</i> 5-A	-1.4	-1.5	U		
CYB5R1	Cytochrome <i>b</i> 5 reductase 1	-1.3	nc	U		
CYB5R2	Cytochrome <i>b</i> 5 reductase 2	-1.4	-1.9	U		
CYB5R3	Cytochrome <i>b</i> 5 reductase 3	-1.4	-1.6	R		
CYB5R4	Cytochrome <i>b</i> 5 reductase 4	-1.3	-1.6	R		
Lipid metabolism						
FDFT1	Farnesyl-diphosphate farnesyltransferase 1	-2.3	-1.5	U	-4.3	+2-fold
IDI1	Isopentenyl-diphosphate $\delta$ isomerase 1	-2.1	nc	U		
CH25H	Cholesterol 25-hydroxylase	-10.8	-3.2	O	-1.3	-3-fold
RSAD2	Radical <i>S</i> -adenosyl methionine domain containing 2	-6.8	1.4	pR		
INSIG1	Insulin-induced gene 1	-2.6	1.7	O		
LDLR	Low density lipoprotein receptor	-3.0	-1.8	pR		
SQLE	Squalene epoxidase	-2.5	nc	U		
SCD	Stearoyl-coenzyme A desaturase ( $\delta$ -9-desaturase)	-3.3	nc	U		
Insulin metabolism						
CPE	Carboxypeptidase E	10.0	2.5	pR		
PAPPA	Pregnancy-associated plasma protein A, pappalysin	2.5	1.7	R	4.8	-5-fold
PCSK2	Proprotein convertase subtilisin/kexin type 2	-75.5	-4.3	O		
Other metabolism						
SCIN	Scinderin	-5.4	-1.4	O		
PYGL	Phosphorylase, glycogen; liver	-2.5	-1.6	R		
SLC40A1	Solute carrier family 40 (iron-regulated transporter)	7.6	2.9	R		
QPRT	Quinolate phosphoribosyltransferase	-3.4	nc	R		
ATP8B1	ATPase, class I, type 8B and member 1	2.4	nc	pR		
Cell cycle						
POSTN	Periostin, osteoblast specific factor	73.8	153.9	U	238.2	-20%
VEGFA	Vascular endothelial growth factor A	2.9	nc	–		
SEMA5A	Semaphorin 5A, receptor for cell growth	3.6	1.6	pR		
AEBP1	AE binding protein 1	66.1	nc	R		
CSRP2	Cysteine and glycine-rich protein 2	5.3	1.5	R		
DOK5	Docking protein 5	6.5	1.6	U		
MID1	Midline 1 (Opitz/BBB syndrome)	3.9	4.4	U		
CHURC1	Churchill domain containing 1	3.5	nc	–		
CREG1	Repressor 1 of E1A-stimulated genes	3.0	1.3	R		
RUNX1	Runt-related transcription factor 1 (aml1 oncogene)	1.9	1.6	–		
BHLHB5	Basic helix-loop-helix domain containing; class B, 5	-6.1	-1.4	–		
IFITM1	Interferon induced transmembrane protein 1 (9–27)	-3.8	-3.7	O		
EDN1	Endothelin 1	-3.0	nc	U		
MATN2	Matrilin 2	-9.2	nc	U		
MCAM	Melanoma cell adhesion molecule	-6.7	-3.0	R	-10.9	+10%
MKX	Mohawk homeobox	-4.5	-1.5	–		
PSG6	Pregnancy specific $\beta$ -1-glycoprotein 6	2.6	nc	–		
DCN	Decorin	2.0	-1.6	–		
PKP4	Plakophilin 4	2.0	1.4	U		
EFEMP1	EGF-containing fibulin-like extracellular matrix protein 1	13.2	2.2	pR		
VCAN	Versican	2.8	2.7	–	4.6	+10%
SMARCA1	Component of SWI/SNF chromatin complex, member A1	-1.3	nc	pR		
SMARCA4	Component of SWI/SNF chromatin complex, member A4	-1.9	nc	pR		
CDK6	Cyclin-dependent kinase 6, overexpressed in tumour	1.4	2.9	U		
CDKN1A	P21, inhibitor of CDK	-9.2	-2.1	U		
CDKN1C	P57, inhibitor of CDK	-2.6	-1.3	R		
CDKN3	Inhibitor of CDK, overexpressed in cancer cells	1.9	2.7	U		

Continued

Table 2 Continued

Gene symbol*	Gene title	FC†	FC‡	CoQ <sub>10</sub> §	Q-RT-PCR¶	CoQ <sub>10</sub> **
CD31	Cell surface antigen	-1.8	-1.5	R		
RB1	Retinoblastoma protein	-1.4	nc	R		
E2F7	E2F transcription factor 7	3.6	nc	U		
E2F8	E2F transcription factor 8	2.2	nc	U		
FST	Follistatin	2.6	1.4	O		
Development and differentiation						
BDNF	Brain-derived neurotrophic factor	-2.9	nc	pR		
GRP	Gastrin-releasing peptide	-263.6	nc	-		
NTNG1	Netrin G1	-8.3	1.8	U		
PTN	Pleiotrophin (neurite growth-promoting factor 1)	-2.7	nc	R		
FOXQ1	Forkhead box Q1	-6.5	nc	-		
HOXA11	Homeobox A11	-4.3	-2.4	U		
HOXC9	Homeobox C9	-4.8	-2.0	U		
LHX9	LIM homeobox 9	-93.0	-1.5	U		
SP110	SP110 nuclear body protein	-2.5	nc	pR		
P2RY5	Purinergic receptor P2Y; G-protein coupled, 5	-4.4	-1.3	pR		
TSPAN10	Tetraspanin 10	-10.1	nc	-		
EPSTI1	Epithelial stromal interaction 1	-5.2	-1.4	R		
TSHZ1	Teashirt zinc finger homeobox 1	-2.8	nc	R		
KRT34	Keratin 34	-5.3	-7.6	R	-5.7	-60%
TPM1	Tropomyosin 1 ( $\alpha$ )	-1.8	1.7	-		
FOXP1	Forkhead box P1	2.3	nc	-		
LMCD1	LIM and cysteine-rich domains 1	3.8	nc	U		
Cell resistance to stress						
CYP1B1	Cytochrome P450, family 1B and polypeptide 1	4.5	1.5	-	7.0	-5-fold
MGC87042	Similar to six epithelial antigen of prostate	12.2	-	R		
TMEM49	Transmembrane protein 49/microRNA 21	1.9	nc	-		
RAD23B	RAD23 homologue B ( <i>Saccharomyces cerevisiae</i> )	2.2	nc	R		
TXNIP	Thioredoxin-interacting protein	2.0	-4.9	-		
SGK1	Serum/glucocorticoid regulated kinase 1	3.4	1.5	-		
SOCS3	Suppressor of cytokine signalling 3	-3.6	nc	R		
RHOU	Ras homologue gene family, member U	-8.3	nc	O		
Apoptosis						
AIM1	Absent in melanoma 1	-4.5	-1.4	O		
APCDD1	Adenomatosis polyposis coli down-regulated 1	-6.4	-1.8	O		
MAGED1	Melanoma antigen family D, 1	-1.7	nc	U		
MAGED4/4B	Melanoma antigen family D, 4/4B	-5.0	-1.6	U		
RAC2	Small GTP-binding protein Rac2 (rho family)	-2.3	-1.3	U		
TRIM55	Tripartite motif-containing 55	-11.7	-1.6	U		
IFI6	Interferon, $\alpha$ -inducible protein 6	-4.9	-1.3	R		
XAF1	XIAP associated factor-1	-3.0	-1.5	R		
TNFRSF10D	Tumour necrosis factor receptor superfamily 10D	2.4	2.6	U	15.1	+20%
SFRP1	Secreted frizzled-related protein 1	8.7	2.5	U	11.8	-2-fold
Signalling						
ARL4C	ADP-ribosylation factor-like 4C	3.8	1.6	pR		
USP53	Ubiquitin specific peptidase 53	4.2	1.7	-		
GABBR2	$\gamma$ -aminobutyric acid B receptor, 2	13.8	2.0	U		
CNGA3	Cyclic nucleotide gated channel $\alpha$ -3	-67.3	nc	-		
GNG2	G-protein, $\gamma$ -2	-4.2	1.4	pR		
HERC6	Hect domain and RLD 6	-7.4	-1.4	R		
MLPH	Melanophilin	-8.5	-1.9	R		
NCK2	NCK adaptor protein 2	-1.7	nc	-		
PARP14	Poly (ADP-ribose) polymerase family, member 14	-3.1	-1.5	-		
Immunity						
CDC42SE2	CDC42 small effector 2	-2.8	nc	-		
LY6K	Lymphocyte antigen 6 complex, locus K	-4.7	1.4	-		
GALNAC4S-6ST	B cell RAG associated protein	-17.3	-2.5	O		

Continued

Table 2 Continued

Gene symbol*	Gene title	FC†	FC‡	CoQ <sub>10</sub> §	Q-RT-PCR¶	CoQ <sub>10</sub> **
TNFSF4	Tumour necrosis factor superfamily, member 4	-5.9	nc	-		
TRIM14	Tripartite motif-containing 14	-4.5	nc	-		
BTN3(A2/A3)	Butyrophilin 3 (A2/A3)	-2.0	-1.3	R		
IFI27	Interferon, $\alpha$ -inducible protein 27	-9.8	nc	O		
IFI44	Interferon-induced protein 44	-3.3	-2.3	R		
IFI44L	Interferon-induced protein 44-like	-15.0	-1.9	R		
IFIT1	Interferon-induced protein (tetratricopeptide repeats 1)	-5.3	nc	-		
IFIT3	Interferon-induced protein (tetratricopeptide repeats 3)	-3.5	-1.7	R		
GBP1	Guanylate binding protein 1, interferon-inducible	-2.7	-	-		
ISG15	ISG15 ubiquitin-like modifier	-6.4	nc	R		
MX1	Myxovirus resistance 1	-7.4	-1.8	pR		
MX2	Myxovirus resistance 2	-6.1	-3.0	pR		
OAS1	2',5'-oligoadenylate synthetase 1, 40/46 kDa	-5.1	-4.9	R		
OAS2	2'-5'-oligoadenylate synthetase 2, 69/71 kDa	-6.2	-1.6	R		
OAS3	2'-5'-oligoadenylate synthetase 3, 100 kDa	-3.6	-1.3	R		
OASL	2'-5'-oligoadenylate synthetase-like	-3.1	-2.6	R		
PSMB9	Proteasome subunit, $\beta$ -type, 9	-1.8	nc	U		

\*In italic letter, biomarkers used in several types of cancer as described by Yoo and collaborators.<sup>28</sup> See the text for more information.

†Full change (FC) in the comparative analysis ran with Affymetrix GeneChip Human Genome U133 Plus 2.0 Array. Values represent the FC (mean) for each gene corresponding to different patient samples (SAM analysis; R=1.5; false discovery rate (FDR)=0%). In parenthesis, FC of non-significant genes by the statistical threshold used, which were selected owing to their role in specific processes and pathways (see the text for full details). In the case of different probes selected for one gene, values represent the mean of FC for each probe (see online supplementary table S1 for full details).

‡FC in the comparative analysis ran with Affymetrix Gene Chip Human Gene 1.0 ST Array. In parenthesis, FC of non-significant genes by the statistical threshold used. Genes with no change (nc).

§Effect of coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>) supplementation on gene expression in CoQ<sub>10</sub> deficiency: unaffected genes by CoQ<sub>10</sub> treatment (U); genes that restored the expression either partially (pR) or completely (R); genes with opposite regulation than in CoQ<sub>10</sub> deficiency (O); and specifically regulated genes only after CoQ<sub>10</sub> supplementation (S). Genes non-affected by CoQ<sub>10</sub> supplementation (-). See the text and online supplementary table S8 for full details.

¶FC in gene expression analysed by quantitative real time PCR (Q-RT-PCR). See supplementary material and table S11 for primer sequence.

\*\*Effect of CoQ<sub>10</sub> supplementation on mRNA levels analysed by Q-RT-PCR. Positive values, increase on gene expression; negative values, decrease on gene expression.

AE binding protein 1, adipocyte enhancer binding protein 1; aml1 oncogene, acute myeloid leukaemia 1 oncogene; EGF-containing fibulin-like extracellular matrix protein 1, elongation factor G-containing fibulin-like extracellular matrix protein 1; small GTP-binding protein Rac2 (rho family), small guanosine triphosphate-binding protein Rac2 (rho family); SP110 nuclear body protein, specificity protein-110 nuclear body protein.

ac.il/.<sup>21</sup> Full description of statistical analysis be found in the supplementary material.

### Epigenetic analysis

DNA (CpG) methylation analysis was performed using a base-specific cleavage reaction with bisulfite combined with mass spectrometric analysis (MassCLEAVE). For the statistical analysis, the CpGs' methylation degree for each gene was analysed with the MultiExperiment Viewer software developed by Saeed.<sup>22</sup> See supplementary methods for full description.

## RESULTS

### Transcriptome analysis

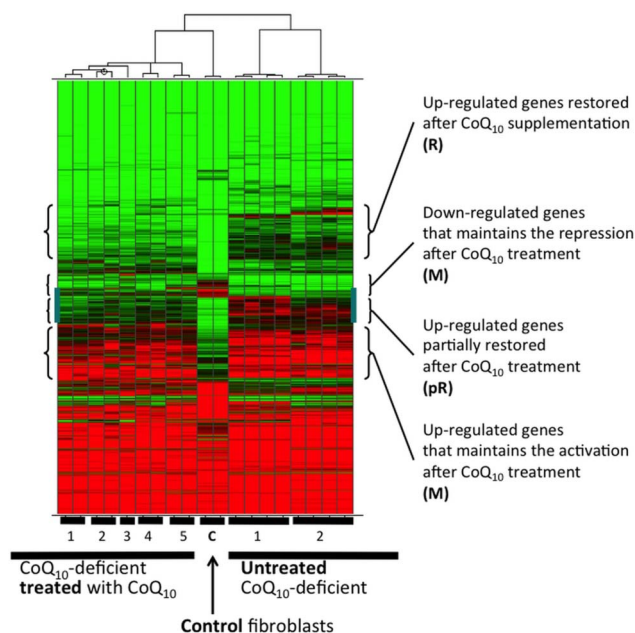
We studied skin fibroblasts from four patients with primary CoQ<sub>10</sub> deficiency and three patients with secondary CoQ<sub>10</sub> deficiency (table 1). We analysed the transcriptomic profiles and compared them with those of cells from age-matched control individuals, and evaluated the modifications induced by supplementation with 30  $\mu$ M CoQ<sub>10</sub> for 1 week to allow recovery of ATP

levels.<sup>8 16 17</sup> A very stringent analysis selected the most significant genes displaying a common and equally altered expression in all samples (summarised in table 2 and shown with full details in online supplementary table S1). Other genes unselected by this analysis, but still abnormally expressed were also included in the study because of their role in specific processes and pathways, such as NADH mobilisation, cell cycle and immunity (see online supplementary table S1) and energetic metabolism (see online supplementary table S2). GO classification of these genes showed similar profiles when comparing independently primary-deficient and secondary-deficient fibroblasts (see online supplementary table S3). A lower stringency analysis showing the most altered biological processes and pathways selected 100 most altered GO in different functional categories (see online supplementary table S4) and 100 more distorted pathways (see online supplementary table S5) in CoQ<sub>10</sub>-deficient cells. See supplementary data for description of statistical analyses.

CoQ<sub>10</sub> treatment modified the specific transcriptomic profile displayed by CoQ<sub>10</sub>-deficient fibroblasts (see

online supplementary tables S6 and S7). We classified genes into five groups according to the consequence of CoQ<sub>10</sub> treatment on gene expression (see online supplementary table S8 for a graphical view). About 54% of probes with altered expression were unaffected by CoQ<sub>10</sub> supplementation. Only 36% of probes showed partial or complete normalisation of expression and 2% showed inverse regulation (figure 1). Approximately 5% of probes were specifically altered after treatment in both deficient and non-deficient cells and 3% showed small or non-specific changes (these were not considered for further analysis). After statistical analysis, we obtained 70 altered GO with a significant p value (<0.001) and an enrichment value that represents the most altered GO within each group (see online supplementary table S9).

Data have been deposited with the NCBI-GEO database, at <http://www.ncbi.nlm.nih.gov/geo/>, accession number GSE33941 (see online supplementary table S10 for an explanation). The functional description of each gene was updated from the GeneCard of The Human Gene Compendium (Weizmann Institute of Science), <http://www.genecards.org/>. See supplementary data for a full description of genes, biological process and pathways regulated in CoQ<sub>10</sub> deficiency.



**Figure 1** Cluster of genes differentially expressed in coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>)-deficiency and after CoQ<sub>10</sub> supplementation. Four arrays of two representative fibroblasts from patients with Q deficiency were plotted with two arrays of control fibroblasts and nine arrays of five patient's fibroblasts with CoQ<sub>10</sub> deficiency treated with 30 µM CoQ<sub>10</sub>. Activated genes were coloured in red and repressed ones in green. Between parentheses—group classification of genes after CoQ<sub>10</sub> supplementation (see online supplementary Table S3).

### CoQ<sub>10</sub>-deficient fibroblasts readapt the energetic metabolism and CoQ<sub>10</sub>-treatment restores

In CoQ<sub>10</sub>-deficient fibroblasts, mitochondrial functions, including respiratory chain and tricarboxylic acid (TCA) cycle, were repressed, whereas 9 of 10 steps in glycolysis and pyruvate metabolism were activated, including lactate and pyruvate dehydrogenases (see online supplementary tables S2 and S4). Accordingly, genes involved in the negative regulation of glycolysis were downregulated, whereas those involved in its activation were upregulated (see online supplementary table S2). Furthermore, genes involved in cytosolic NADH oxidation (cytochrome *b<sub>5</sub>* and several oxidoreductases) were slightly repressed (table 2). The expression of genes involved in cholesterol and fatty acid metabolism was downregulated (table 2), as well all the GO related with lipid metabolism (see online supplementary table S5).

CoQ<sub>10</sub> supplementation normalised the expression (either partially or completely) of genes involved in the glycolytic pathway and activated the expression of repressed respiratory chain genes, whereas the TCA cycle remained unaffected (see online supplementary table S2). Most of the repressed enzymes of lipid metabolism and fatty acid β-oxidation remained downregulated (table 2), whereas several other pathways, such as mono-carboxylic acid transport and the insulin response, were normalised (see online supplementary table S9). These results are in agreement with the recovery of aerobic metabolism observed in CoQ<sub>10</sub>-deficient fibroblasts after CoQ<sub>10</sub> supplementation.<sup>8 16 17</sup>

### CoQ<sub>10</sub> deficiency induces specific adaptations of cells to promote survival

The major novel finding of transcriptome profiling in CoQ<sub>10</sub>-deficient fibroblasts was the altered expression of genes concerned with cell cycle and development and with resistance to stress and cell death (table 2). This suggests both a remodelling of differentiation and growth maintenance and an increase of cell survival mechanisms. Specifically, genes involved in cell cycle activation and maintenance were upregulated, and genes involved in cell cycle regulation increased or decreased their expression depending of their activating or repressing roles. This proliferative response was also enhanced by the repression of cellular attachment factors and by the activation of extracellular matrix proteins that reduce cell attachment and favour cell division. In parallel, GO clusters favouring cell cycle and cell division were activated, and those inhibiting cell growth were repressed (see online supplementary table S4). The differentiation of these cells was compromised because many required factors, transducers, antigens and structural proteins appeared downregulated, whereas repressors of differentiation during development were overexpressed (table 2). See supplementary material for a full description of genes, biological processes and related pathways.

Cell cycle activation was supported by the upregulation of CDK6 (table 2), a cyclin-dependent kinase that induces entry into the S-phase, and by a robust repression (more than ninefold) of p21/CDKN1A, an inhibitor of cyclin-dependent kinase that blocks cell cycle at the G1/S check point to stimulate cell differentiation. Moreover, subsequent pathways inactivated by p21<sup>23</sup> were enhanced in CoQ<sub>10</sub>-deficient cells (see online supplementary table S5), as well as both transcription factors E2F7 and E2F8 (table 2), which push the progression of the cell cycle, activate cell survival and inhibit apoptosis.<sup>24</sup>

Cell survival in CoQ<sub>10</sub>-deficient cells was improved by the induction of DNA-repairing mechanisms, and by the establishment of pathways that regulate Jun kinases and activate NAD(P)H-CoQ oxidoreductase, which are involved in stress responses (table 2 and see online supplementary table S4). Components of apoptosis and cell death pathways were systematically repressed (table 2), including tumour suppressor genes, antigens, intracellular mediators and effectors of cell death. Also, cell surface receptors and modulators that inhibit apoptosis were greatly activated.

Interestingly, CoQ<sub>10</sub> treatment did not alter the newly acquired resistance to cell death in CoQ<sub>10</sub>-deficient fibroblasts, kept cell growth activated, and allowed a higher degree of differentiation (tables 2 and see online supplementary table S8). However, genes controlling stress resistance pathways and cortical cytoskeleton were completely restored, as indicated by the shifts in gene expression listed in table 2. However, treated fibroblasts kept the DNA repair mechanism activated.

Signalling-related genes and pathways were differentially affected by CoQ<sub>10</sub> deficiency, but most of immunity-related genes showed a general downregulation (table 2). Pathways and biological processes involved in immunity regulation were restored by CoQ<sub>10</sub> supplementation (table 2 and see online supplementary table S5).

#### Stable DNA methylation profile is responsible for the specific gene expression profile in CoQ<sub>10</sub> deficiency

CoQ<sub>10</sub> supplementation modified the expression of 43% of genes that were abnormally expressed in CoQ<sub>10</sub>-deficient fibroblasts (see online supplementary table S8). In the majority of these cases, expression levels were restored to those of control fibroblasts (20%), but few showed inverse regulation (2%) and others were specifically altered after CoQ<sub>10</sub> treatment in both deficient and non-deficient cells (5%). The remaining 16% corresponded to partially restored genes, which slightly alter their expression level without changing the CoQ<sub>10</sub>-deficient pattern. These genes along with the unaffected (54%) constitute 72% of regulated genes in CoQ<sub>10</sub> deficiency, which were not significantly altered after CoQ<sub>10</sub> supplementation.

To explain this differential response to respiratory dysfunction, we analysed the DNA-methylation profile of 20

among the most altered genes listed in table 2. These genes encompass the main biological processes and pathways affected by CoQ<sub>10</sub> deficiency (table 3). Upregulated genes, which were unaffected by CoQ<sub>10</sub> supplementation, had less-defined DNA methylation sites in their promoter regions.

Genes with partial restoration of their expression after CoQ<sub>10</sub> supplementation showed precise methylation and demethylation profiles that may explain their altered expression during CoQ<sub>10</sub> deficiency. The methylation degree of these genes changed after treatment, and may be responsible for the modulation of expression (table 3). The patterns of methylation of activated and repressed genes in CoQ<sub>10</sub> deficiency that could be normalised by CoQ<sub>10</sub> supplementation, were either unaffected or only slightly affected by the treatment, and we did not detect new methylation sites after CoQ<sub>10</sub> supplementation.

However, a few genes showed significant differences in the methylation degree after the treatment, which correspond to the partially restored genes that maintain the specific expression pattern of untreated CoQ<sub>10</sub> deficient cells at a lower level.

Finally, reviewing the biological processes and molecular functions of regulated genes in CoQ<sub>10</sub> deficiency, the main adaptation for cell survival activated genes by DNA demethylation, which increased the expression of genes involved in cell cycle activation, apoptosis inhibition, and cell stress resistance, meanwhile the undifferentiated state could be owing to gene repression by DNA methylation, which decreased the expression of genes involved in cell differentiation. CoQ<sub>10</sub> treatment did not alter the methylation degree of these genes and subsequently the expression level was maintained.

## DISCUSSION

### CoQ<sub>10</sub>-deficient fibroblasts readapt the energetic metabolism and CoQ<sub>10</sub>-treatment restores

CoQ<sub>10</sub> is an essential component of the mitochondrial respiratory chain,<sup>1</sup> therefore dysfunctional mitochondria are a common finding in both primary CoQ<sub>10</sub> deficiencies<sup>3–7</sup> and secondary forms.<sup>8–13</sup> Although each form presents a specific clinical phenotype, all these conditions display a substantial reduction of cellular CoQ<sub>10</sub> content and deficit in the mitochondrial enzymatic activities of respiratory chain (table 1). Accordingly to these results, we have shown here that fibroblasts from patients with CoQ<sub>10</sub> deficiency have reorganised their genetic resources to cope with this mitochondrial dysfunction. Consistent with the role of CoQ<sub>10</sub> in bioenergetics, the lack of CoQ<sub>10</sub> would force the cell to support it mainly by glycolysis, whereas both mitochondrial lipid metabolism and respiratory chain were repressed (see online supplementary tables S2 and S4). These findings, together with the mild repression of cytosolic enzymes that oxidise NADH (cytochrome *b<sub>5</sub>* and its oxidoreductases listed in table 2) could indicate that NADH is

**Table 3** Epigenetic modifications in CoQ<sub>10</sub> deficiency owing to DNA (CpG) methylation/demethylation

Gene symbol	FC*	Q-effect†	CpGs‡	Demethylations in CoQ <sub>10</sub> deficiency			Methylations in CoQ <sub>10</sub> deficiency			Q-effect††
				CpGs§	Degree (C/P)¶	CpGs' location**	CpGs§	Degree (C/P)¶	CpGs' location**	
POSTN	73.8	U	5 (P)	2 (16 fold)	50%/3%	Close together (P)	0	–	–	–
GABBR2	13.8	U	101 (P,I)	5 (40%)	47%/37%	Close together (P)	14 (6-fold)	10%/22%	Close together (P)	–15%
VCAN	2.8	U	58 (P,E,I)	5 (2-fold)	12%/7%	Scattered groups	3 (90%)	9%/17%	Dispersed (I)	–
TNFRSF10D	2.4	U	59 (P,E,I)	27 (2-fold)	60%/25%	Scattered groups (P)	0	–	–	–
FOXP1	2.3	U	85 (I)	11 (2-fold)	57%/32%	Scattered groups	2 (3-fold)	7%/19%	Close together	–
END1	–3.0	U	25 (P)	0	–	–	0	–	–	–
PARP14	–3.1	U	29 (P)	0	–	–	3 (3-fold)	5%/19%	Dispersed	–
CPE	11.6	pR	26 (P,I)	3 (4-fold)	20%/6%	Dispersed	0	–	–	+2-fold
ARL4C	4.2	pR	63 (P,E,I)	5 (90%)	52%/28%	Scattered groups	9 (60%)	16%/28%	Scattered groups	+7%
HOXA11	–4.3	pR	17 (P)	0	–	–	8 (4-fold)	5%/20%	Close together (P)	–3-fold
AEBP1	66.1	R	80 (P,E,I)	8 (25%)	76%/61%	Scattered groups	8 (3-fold)	10%/27%	Widely dispersed	–
CYP1B1	4.7	R	24 (P)	0	–	–	0	–	–	–
CHURC1	3.5	R	20 (P,E,I)	1 (50%)	11%/7%	(I)	0	–	–	–
PYGL	–2.5	R	84 (P,E,I)	8 (2-fold)	12%/6%	Close together (E)	1 (3-fold)	4%/13%	(P)	–
XAF1	–3.0	R	25 (P,E,I)	0	–	–	0	–	–	–
EPSTI1	–5.9	R	34 (P,E)	0	–	–	7 (2-fold)	27%/37%	Scattered groups	–
MCAM	–7.7	R	74 (P,E,I)	0	–	–	0	–	–	–
MLPH	–8.5	R	8 (P)	0	–	–	0	–	–	–
PCSK2	–94.3	O	32 (P)	0	–	–	0	–	–	–
GRP	–263.6	O	73 (P,E,I)	20 (two fold)	53%/35%	Scattered groups	6 (50%)	28%/35%	Close together (P)	–

\*Full change (FC) in coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>) deficiency (patient samples (SAM) analysis; R=1.5; false discovery rate (FDR)=0%) ran with Affymetrix GeneChip Human Genome U133 Plus 2.0 Array. Full details are shown in [table 2](#).

†Effect of CoQ<sub>10</sub> supplementation on gene expression in CoQ<sub>10</sub> deficiency (for more information see online supplementary table S8): unaffected genes by CoQ<sub>10</sub> treatment (U), genes that restored the expression either partial (pR) or completely (R) and genes with opposite regulation after CoQ<sub>10</sub> supplementation than in CoQ<sub>10</sub> deficiency (O).

‡Number of CpG islands analysed. In parenthesis, gene location of CpG islands: promoter (P), first exon (E) and first intron (I).

§Significant methylated CpGs for each gene in control and CoQ<sub>10</sub>-deficient fibroblast. Significance determined by t test (p<0.01). In parenthesis, fold change in methylation degree (small changes, in %). Non-significant changes in methylation (–).

¶Methylation degree (mean of significant CpG). Values represent the % of CpG's methylation of both control (C) and patient deficient in CoQ<sub>10</sub> (P).

\*\*Location of significant CpGs. In parenthesis, gene location: promoter (P), first exon (E) and first intron (I).

††Significant changes in CpG methylation owing to CoQ<sub>10</sub> supplementation in CoQ<sub>10</sub> deficiency. Positive values, an increase in the methylation degree and negative values, demethylations. Significance determined by t test (p<0.01) between CoQ<sub>10</sub>-supplemented fibroblasts and untreated CoQ<sub>10</sub>-deficient fibroblasts. Non-significant changes in methylation (–).

mainly used for biosynthetic purposes rather than for energy production.

Supplementation with CoQ<sub>10</sub>, the current therapy for all forms of CoQ<sub>10</sub> deficiency, restored respiration through enhanced sugar utilisation, but did not stimulate lipid metabolism. The expression of genes involved in the glycolytic pathway was partially or completely normalised after CoQ<sub>10</sub> treatment, whereas the repressed genes involved in the respiratory chain were activated. The TCA cycle remained unaffected (see online supplementary table S2). These results are in agreement with the recovery of aerobic metabolism observed in CoQ<sub>10</sub>-deficient fibroblasts after CoQ<sub>10</sub> supplementation.<sup>8 16 17</sup>

### CoQ<sub>10</sub> deficiency induces a stable survival adaptation of cells

CoQ<sub>10</sub>-deficient fibroblasts adapted several physiological processes to acquire a cellular-resistance state for survival under the conditions of mitochondrial dysfunction induced by CoQ<sub>10</sub> deficiency. The new genetic pattern increases cell survival by activating cell cycle and growth, maintaining an undifferentiated phenotype, upregulating stress-induced proteins and inhibiting apoptosis and cell death pathways. These results recapitulate a survival network that can be observed in nutritional stress such as when cells are grown in galactose-enriched media.<sup>25</sup>

The survival adaptation shown by CoQ<sub>10</sub>-deficient cells included a global resistance mechanism that is observed also during the initial phase of tumorigenesis. In fact, the CoQ<sub>10</sub>-deficient expression profile was very similar to that described during myeloid cell transformation<sup>26</sup> and breast tumours.<sup>27</sup> Moreover, some of the regulated genes in CoQ<sub>10</sub> deficiency (listed in [table 2](#) as italicised letter) are used as biomarkers in several types of cancer,<sup>28</sup> like KRT34, the cell cycle-related POSTN, MCAM, EFEMP1 and VCAN, and the apoptotic and cell resistance-related CYP11B1, XAF1 and TNFRSF10D. Although these biomarkers behaved in CoQ<sub>10</sub> deficiency (increased or decreased) as described by Yoo *et al*,<sup>28</sup> there is no sign of tumour formation reported in the patients so far. In addition, cellular senescence, a defining feature of premalignant tumours,<sup>29</sup> is characterised by a gene expression pattern similar to that of CoQ<sub>10</sub>-deficient fibroblasts (see online supplementary table S5).

Supplementation with CoQ<sub>10</sub> enhanced both stress response and immunity pathways. Although the pathway of cell death was partially restored, cell cycle and growth, and the mechanisms to prevent differentiation and development were not. These results indicate that the mitochondrial dysfunction owing to CoQ<sub>10</sub> deficiency induces a stable survival adaptation of somatic cells in patients at early or postnatal development, and we speculate that cells unable to institute, or to maintain, this survival mechanism during differentiation will die, contributing to the pathological phenotype.

### A stable DNA methylation profile is responsible of specific gene expression in CoQ<sub>10</sub> deficiency

The cellular adaptation to CoQ<sub>10</sub> deficiency-enhanced DNA demethylation of genes that regulate cell cycle activation, apoptosis inhibition and cell stress resistance as part of an adaptation survival mechanism. Comparable results were observed in several models of epigenetic regulation by demethylation (see online supplementary table S5), whereas DNA methylation inhibits activation of genes related to tumorigenesis and apoptosis.<sup>30</sup>

Pathways unaffected by CoQ<sub>10</sub> treatment corresponded to stably demethylated genes, whereas those that responded to CoQ<sub>10</sub> supplementation were controlled by genes with unchanged methylation patterns.

We did not find changes in the methylation degree of all genes affected by CoQ<sub>10</sub> deficiency, suggesting that other modalities of gene regulation are responsible, including epigenetic mechanisms such as histone modifications by methylation and acetylation, or even DNA methylation in CpG islands other than those studied here. Interestingly, it has been reported that CoQ<sub>10</sub> regulates lipid metabolism in mice liver without any effect on the DNA methylation profile,<sup>31</sup> indicating that supplemented CoQ<sub>10</sub> by itself may not alter the DNA methylation pattern that cells acquired during the survival adaptation to CoQ<sub>10</sub> deficiency.

Mechanisms unaffected by therapy corresponded to stably DNA demethylated genes, which were responsible for the acquisition of the undifferentiated state for survival and resistance that cells obtain during the adaptation to CoQ<sub>10</sub> deficiency, whereas the responsive to CoQ<sub>10</sub> supplementation were controlled by genes with unchanged methylation patterns and correspond mainly to metabolic genes and those related with the restoration of mitochondrial function.

We propose that these epigenetic changes may be established as early as during the fetal life<sup>32</sup> in order to cope with CoQ<sub>10</sub> deficiency; these cells then maintain this adaptive response throughout their life. We speculate that cells unable to maintain this survival mechanism during differentiation would die contributing to the pathological phenotype.

Our model has some limits: we treated cells only for 1 week and in principle we cannot rule out that prolonged exposure to CoQ<sub>10</sub> could restore also some of the other unaffected pathways. Alternatively, incomplete recovery of the gene expression profiles could be explained by the fact that exogenous CoQ<sub>10</sub> can rescue the bioenergetic defect, but not all other functions of CoQ<sub>10</sub> in these cells, as it has been observed in other organisms.<sup>33</sup>

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## SUPPLEMENTARY DOCUMENT

**Survival transcriptome in the coenzyme Q10 deficiency syndrome is acquired by epigenetic modifications: a modelling study for human coenzyme Q10 deficiencies.**

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## SUPPLEMENTARY DATA

### **Identification of genes, biological process and pathways regulated in CoQ<sub>10</sub> deficiency.**

Data analysis for each gene in the array compared the signal of each patient's fibroblast mRNA with the signal of each control fibroblast mRNA, over which the statistical analyses were performed. MAS5 algorithm and SAM software were performed in order to select those probe sets with significant differences in gene expression. Approximately from 6% to 11% of the probe sets present in the array were picked with a significant fold change higher than 1.5, while the false discovery rate (FDR) was maintained lower than 5%, the maximum value to consider changes as significant [1]. Even more, we reduced the FDR to zero by increasing the stringency of the statistical analysis via extension of the cut off threshold ( $\Delta$ -value). After such filtering, from 842 to 1672 probe sets were identified as showing significant differences in expression between CoQ<sub>10</sub>-deficient fibroblasts and control cells, representing approximately 2% to 3% of the array (supplementary table 9 online). Later on, we compared the regulated probes in each comparative analysis in order to get a list of genes that were regulated simultaneously in all patient's fibroblasts at the same time (supplementary tables 10 and 11 online). We found 136 probes whose expression was regulated similarly in all samples, including 47 UP-regulated probes and 89 DOWN-regulated ones. Most of the other probes that were selected as significant in only one sample were no selected in other (2575 probes were regulated similarly in 3 or less of the 4 samples), and 174 probes appeared UP-regulated in some samples and DOWN-regulated in others. Taking in mind that an Affymetrix GeneChip® Human Genome U133 Plus 2.0 Array contains 54675 probes, the probability of the 136 selected probes to be randomly selected in one comparative analysis is 0.0025, which represents the 136 over the total amount of probes present in the array. This probability is reduced to 0.00000625 (less that one probe per array) if we consider two analyses to select randomly the 136 probes, and it is reduced to almost zero if we consider the four comparative

analyses over which we ran the statistical analysis. This result gave us the security that the changes in the gene expression were due to the mitochondrial of CoQ<sub>10</sub> deficiency syndrome and not to a random selection of probes. Then, the probes were grouped in a list to define the most altered genes as biological markers in the mitochondrial of CoQ<sub>10</sub> deficiency syndrome. We found that many of them represented the same gene, so that the 136 probes correspond to 116 regulated genes (Table 2). These were validated in selected representative cases by quantitative real-time PCR (Table 2 and supplementary table 1 online). To corroborate the altered gene expression, we considered to re-hybridize the same samples with another oligonucleotide array, the Affymetrix Gene Chip® Human Gene 1.0 ST Array. The analysis was performed as follows: PLIER algorithm and SAM software were performed in order to select those probe sets with significant differences in gene expression ( $s_0=50$ ;  $R=1.5$ ; cut off threshold of delta-value so that  $FDR=0\%$ ). The regulation on gene expression is listed in parallel to the main analysis in table 2 and supplementary table 1 online. Finally, we called such genes as genes regulated in the mitochondrial CoQ<sub>10</sub>-deficiency syndrome.

### **CoQ<sub>10</sub> supplementation partially restores the altered gene expression of CoQ<sub>10</sub>-deficient fibroblasts.**

We extracted total RNA from 5 different cultures of CoQ<sub>10</sub>-deficient fibroblast treated for 48 hours with 30  $\mu$ M of CoQ<sub>10</sub> and duplicate hybridizations were performed with the Affymetrix Gene Chip® Human Gene 1.0 ST Array. The statistical analysis was performed as follow, using PLIER algorithm and SAM software to select those probe sets with  $t\text{-test}<0.05$  and establishing three different thresholds of minimum fold change ( $R=1.5$ ;  $R=2.0$ ; and  $R=3.0$ ) to select a probe as significant. After that we made a comparative analysis between the three types of fibroblasts: control (C), fibroblasts from CoQ<sub>10</sub> deficient patients (P), and CoQ<sub>10</sub> supplemented fibroblasts from CoQ<sub>10</sub>-deficient patients (PQ). Finally, we obtained three different comparative analyses: treated versus non-treated fibroblasts (PQ-P), treated versus control fibroblasts (PQ-C), and non-treated

versus control fibroblasts (P-C) that represented the previous analysis done between CoQ<sub>10</sub> deficient fibroblasts and control ones.

Independently of the minimum full change threshold that we used, around a half of the probes selected with the comparative analyses between CoQ<sub>10</sub> deficient and control fibroblasts (both P-C and PQ-C) were selected in the analysis between treated and non-treated fibroblasts (supplementary table 3 online), indicating that CoQ<sub>10</sub> supplementation altered the expression of around 50% of the regulated genes by CoQ<sub>10</sub> deficiency. Also, by increasing the minimum full change threshold, the number of selected probes as significant was halved in each comparative analysis.

Considering that a regulated probe, either being up-regulated or down-regulated, in a comparative analysis can be regulated or not in the others comparative analyses, 27 possibilities can be achieved for a probe to be or not to be regulated in one, two or in all the comparative analyses (supplementary table 4 online and figure S1 for a graphical view). Because of the objective of these analyses is to study how the altered gene expression in CoQ<sub>10</sub> deficiency was regulated with the supplementation of CoQ<sub>10</sub>, we decided to not consider those probes regulated only in one comparative analysis, as it happened using a threshold of twice for the minimum full change ( $R=2.0$ ), since those genes were slightly modified with a lower R-value ( $R=1.5$ ) and became unselected and no significant by increasing the minimum full change threshold (i.e.  $R=2.0$ ).

After that, we established 5 groups of classification for a probe to be regulated after CoQ<sub>10</sub> supplementation (supplementary figure 1 online for a graphical interpretation and supplementary table 2 online for full details). Around half of the regulated probes in CoQ<sub>10</sub>-deficiency were unaffected after the treatment with CoQ<sub>10</sub>, whereas most of the 50% that changed the gene expression did not affect the relative pattern respect control fibroblasts. Only the 20% restored completely the gene expression after CoQ<sub>10</sub> supplementation and the 3% shift their expression showing an opposite regulation than in CoQ<sub>10</sub>-deficiency. The 72% of the probes in CoQ<sub>10</sub> deficiency kept a similar gene expression pattern in the presence of CoQ<sub>10</sub> respect to control fibroblasts.

Also, we looked the highly regulated genes in the CoQ<sub>10</sub>-deficiency listed in table 1 to see if the supplementation with CoQ<sub>10</sub> modify, restore or even alter more their expression. Almost all the genes were regulated in one patient or treatment, although only 73% of the known genes listed in table 2 were regulated according to the previously described groups of classification (75 classified out of 103 known genes). The 54% of the classified genes restored the control expression level after the treatment either partially (19%, 14 out of 75) or completely (35%, 26 of the 75), whereas the 27% kept their regulation as it happened in CoQ<sub>10</sub>-deficiency (20 out of 75). The 13% of the genes (10 out of 75) presented an opposite regulation in the presence of CoQ<sub>10</sub> respect to that in CoQ<sub>10</sub>-deficiency, and the 5% of them (4 out of 75) amplified the altered gene expression showed by the patient fibroblasts, whereas only 1% of the regulated in CoQ<sub>10</sub>-deficiency (1 out of 75) were CoQ<sub>10</sub>-specific regulated after the treatment. Such changes had been next discussed in the main text.

To get a biological meaning of such changes, we decided to study the gene ontologies (GO) regulated by CoQ<sub>10</sub> supplementation in CoQ<sub>10</sub> deficient fibroblasts within each group of classification, using the GORILLA software (Gene Ontology enrichment analysis and visualization tool) [2]. After the statistical analysis we got 70 regulated GO with a significant P-value<0.001 and a given enrichment value that represents the more altered GO within each group of classification (supplementary table 5 online). A positive enrichment value signified that CoQ<sub>10</sub> supplementation kept activated the expression of the gene included within the selected GO, whereas a negative value meant that the expression was kept down.

### **Full description of genes, biological process and pathways regulated in CoQ<sub>10</sub> deficiency.**

#### Related to energy production:

Three mitochondrial genes were included within the most altered listed in table 2. One of these, C7orf55, is directly involved in the formation of complex I and was down-regulated, in agreement with the general repression of mitochondrial respiration. The other two mitochondrial-

targeted genes, BRP44 and C10orf58, also showed altered expression but their functions are still unknown.

#### Related to lipid metabolism:

Cholesterol metabolism was highly repressed in CoQ<sub>10</sub>-deficient fibroblasts and multiple enzymes were affected, including enzymes involved directly in cholesterol biosynthesis (FTFT1, IDI1), their regulators (CH25H, RSAD2), regulators of cholesterol concentration inside the cell (INSIG1, LDLR), and enzymes that catalyze the rate-limiting step in the biosynthetic pathway of both sterols (SQLE) and unsaturated fatty acids (SCD). Overall,  $\beta$ -oxidation of fatty acids was unaffected, but genes acting on its regulation decreased by 50%. The repressed enzymes of lipid metabolism and fatty acids  $\beta$ -oxidation remained down-regulated after CoQ<sub>10</sub> supplementation (table 2), although the repressed key regulator proteins (CH25H and the insulin-inducible gene INSIG1) that control cholesterol concentration inside the cell inverted their expression levels and appeared highly up-regulated by treatment.

#### Related to insulin metabolism:

Two peptidases involved in the processing of both proinsulin and other prohormones (CPE, selected twice in the array with two different probes) and in the cleavage of insulin-like growth factor binding proteins (PAPPA) were highly activated, whereas a convertase (PCSK2, also selected twice) involved in the regulation of insulin biosynthesis was highly repressed (Table 2). Also, a liver-specific glycogen phosphorylase (PYGL), responsible for glucose mobilization, was repressed. These two latter genes shift their expression to either up-regulation or to control levels after CoQ<sub>10</sub> supplementation.

#### Related to cell cycle:

Specifically, genes involved in cell cycle activation and maintenance were up-regulated, including mitogen, growth factors and receptors (VEGFA, POSTN, SEMA5A), intracellular activators for MAP-kinase pathway that enhances cell proliferation and reduces differentiation (AEBP1, CSRP2, DOK5, MID1), and transcriptional activators that mediate growth factor activity and inhibit

differentiation (CHURC1, CREG1, RUNX1). In contrast, genes involved in cell cycle regulation increased or decreased their expression depending of their activating or repressing roles (Table 2). Furthermore, both a mediator of anti-proliferative signals (IFITM1) and a transcriptional repressor of cell division (BHLHB5) were down-regulated. This proliferative transcriptome is also facilitated by the repression of cellular attachment factors (EDN1, MATN2, MCAM, MKX) and by the up-regulation of extracellular matrix proteins that reduce cell attachment (PSG6, DCN, PKP4) and favor cell division (EFEMP1, VCAN).

GO clusters favoring cell cycle and cell division were activated and those inhibiting cell growth were repressed (Table 3). Thus, there was activation of DNA replication, mitosis, meiosis, synthesis of microtubule components involved in cell division, such as spindle pole, segregation of chromosomes, and the positive growth regulation. On the other hand, there was a general down-regulation of biological processes related with nucleosome assembly, and chromosome organization and biogenesis. This trend could be explained by the repression of Swi/Snf chromatin remodeling apparatus, in which SMARCA1 and SMARCA4 components were specifically down-regulated in all CoQ<sub>10</sub>-deficient fibroblasts (Table 2), thus preventing the cell cycle arrest that is induced by the activation of p21 pathway by BRG1/SMARCA4 [3].

#### Related to differentiation:

Differentiation of these cells was compromised because of the down-regulation of factors necessary for differentiation (BDNF, GRP, NTNG1, PTN), transcription factors expressed during development and morphogenesis (FOXQ1, HOXA11, HOXC9, LHX9, SP110), intracellular transducers of development and morphogenesis (P2RY5, TSPAN10), antigens expressed on the surface of differentiated cells (TSHZ1, EPSTI1), and structural proteins of cytoskeleton expressed at the end of differentiation, such as keratin (KRT34) and tropomyosin (TPM1) (Tables 2).

Conversely, repressors of differentiation during development (FOXP1, LMCD1) were activated.

Follicle-stimulating hormone (FSH) stimulates the differentiation of germ cells [4] through the activation of genes that are significantly down-regulated in CoQ<sub>10</sub>-deficient cells. Accordingly, the

FSH-suppressing hormone follistatin (FST) was increased in the same cells. FST inhibits both development and morphogenic proteins function [5]. Finally, the maintenance of cell division is supported by the fact that 47 of the 51 genes encoding for proteins involved in both DNA replication and cell cycle progression behaved similarly to those genes regulated after the addition of serum or growth factors to fibroblasts [6, 7].

The gene expression profile of CoQ<sub>10</sub>-deficient fibroblasts was similar to that shown by different cell models (supplementary table 5 online). CoQ<sub>10</sub>-deficient cells repressed CD31 and presented similar gene expression profile than cells lacking CD31 [8], which maintains the non-differentiated phenotype and stemness properties. Similarly, CoQ<sub>10</sub>-deficient cells showed the same pattern of gene expression than (1) undifferentiated tumor cells compared to well-differentiated cancer cells [9], (2) embryonic stem cells compared to differentiated brain and bone marrow cells [10], and (3) immature lymphocytes compared to both mature CD4(+) T cells [11] and B-cells [12]. Also CoQ<sub>10</sub>-deficient cells were regulated as hematopoietic stem cells, activating the mechanism to avoid the replicative stress and bypass the senescence program [13].

#### Related to cell survival and cell resistance:

Within the most regulated genes classified under these altered biological processes (Table 3), we note that several oxidoreductases that take part in xenobiotic and drug metabolism (CYP1B1, MGC87042), a stress-induced protein (TMEM49), and a gene involved in the nucleotide excision repair (RAD23B) were induced. Intracellular stress was controlled by activation of thioredoxin regulator (TXNIP) and of a kinase that activates the JAK/STAT pathway (SGK1), whereas an inhibitor of this pathway (SOCS3) and Rho GTPase (RHOU) that induces the dissolution of stress fibers through the JNK pathway were repressed. In parallel, tumor suppressor genes (AIM1 and APCDD1), cell-surface antigens that facilitate apoptosis in tumor cells (MAGED1, MAGED4/4B), and Rho GTPase (RAC2) that activates the intracellular signaling to induce apoptosis appeared down-regulated. CoQ<sub>10</sub>-deficient fibroblasts also showed activation of a cell surface receptor that inhibits apoptosis (TNFRSF10D) and of a modulator of Wnt signaling pathway that sends anti-

apoptotic and pro-proliferative paracrine signals to adjacent cells (SFRP1). Furthermore, these cells also showed repression of some effectors of cell death involved in cytoskeleton reassembly during apoptosis (TRIM55) and of the interferon-induced genes that activate apoptosis (IFI6, and XAF1). Gene expression pattern showed that CoQ<sub>10</sub>-deficient fibroblasts had a higher survival and stress resistance, a phenomenon similar to the up-regulation of the drug-resistant gene profile in cancer cells compared to chemo-sensitive cell lines [5], and to the down regulated and repressed pathways in oxidative stress induced in epithelium cells [14]. Furthermore, CoQ<sub>10</sub>-deficient fibroblasts showed a gene expression pattern that was opposite to that of fibroblasts treated with either transforming growth factor- $\beta$  (TGF- $\beta$ ) [15] or with the antitumor drug trabectedin [16, 17].

#### Related to signalling:

Signaling pathways were also affected by CoQ<sub>10</sub>-deficiency (Table 2) through the activation of genes, each selected three times in the statistical analysis, including a GTP-binding protein (ARL4C) involved in transferrin recycling from early endosomes, a ubiquitin specific peptidase with no peptidase activity (USP53), and a G-protein receptor (GABBR2) that inhibits adenilil cyclase and activates phospholipase A2. A set of signaling genes were down regulated including a G-protein (GNG2) and a guanine exchange factor for G-proteins (HERC6), a finding that agrees with the repression of GTPase activity and G-protein signaling showed in table 2. There was also repression of a Rab effector (MLPH) involved in vesicular transport, an adaptor of cell surface receptors to Tyrosine kinase (NCK2) that also interact with T-cell surface glycoproteins, and an effector protein (PARP14) induced by STAT6 during IL4 signaling in immune cells. However, the molecular activator of Rho GTPase appeared activated, which could be related to the activation of cytoskeleton for contraction as shown above.

#### Related to immunity:

CoQ<sub>10</sub>-deficient fibroblasts also showed a general inhibition of most immunity-related genes (Table 2), and of pathways and biological processes involved in immunity regulation (Table 3). Repressed genes include cell surface antigens (CDC42SE2, LY6K), receptors and cytokines for immune

activation (GALNAC4S-6ST, TNFSF4, TRIM14), butyrophilin 3, an immunoglobulin associated to milk fat droplets (BTN3A2, BTN3A3), several interferon-inducible genes of unknown function (IFI27, IFI44L, IFIT1, IFIT3), and other genes whose products bind microtubules (IFI44), take part in the immunoproteasome (PSMB9), activate immune cells during immune response (GBP1, ISG15, MX1 and MX2), or control differentiation and induce apoptosis (OAS2 and OAS3). Both OAS1 and OASL genes were also repressed but to a lesser extent. Other interferon-inducible proteins markedly repressed in CoQ<sub>10</sub>-deficient fibroblast were described above and include the two regulators of apoptosis (IFI6 and XAF1), the regulator of development (EPSTI1) and the inhibitor of cell proliferation (IFITM1). Taken together, these findings support the concept that immune response was generally repressed in CoQ<sub>10</sub>-deficient cells (Table 3), such as the response to virus and lymphocyte-T functions, including proliferation and activation.

CoQ<sub>10</sub> supplementation restored the gene expression of almost all interferon-inducible genes to control levels. This restoration mainly affects stress and immune responses and can be attributed to the anti-inflammatory properties of CoQ<sub>10</sub> [18, 19]. Treatment of fibroblasts with interleukin-6 [20] or treatment of endothelial cells with interferon [21] induced a transcriptomic profile opposite to that described for CoQ<sub>10</sub>-deficient fibroblasts because activated pathways with interleukin or interferon were repressed in CoQ<sub>10</sub> deficiency.

## **SUPPLEMENTARY METHODS**

### **RNA extraction**

Three groups of cells were cultured independently from each type of fibroblast prior to RNA extraction. Each group of cells was then homogenized in 1 ml of Tripure Isolation Reagent (Roche) and RNA was extracted with chloroform and precipitated with 2-propanol by centrifugation at 12000 g for 10 min at 4°C. After washing with 75% EtOH, RNA was resuspended in DEPC-treated water and treated with deoxyribonuclease I (Sigma) for removal of possible DNA contamination. RNA was cleaned with the RNeasy® MinElute™ Cleanup kit (Qiagen), its concentration and purity were checked spectrophotometrically, and its quality was verified electrophoretically. RNA was stored at –20 °C in RNase-free water.

### **Probe synthesis and hybridization with expression Arrays**

A cRNA probe was synthesized and fragmented from each RNA sample using Affymetrix® protocols and kits provided by Qiagen. Each cRNA probe was then hybridized to independent GeneChip® Human Genome U133 Plus 2.0 Array (Affymetrix), an expression array that covers mainly at the 3'-end over 47000 transcripts and variants, representing around 39000 of the best characterized human genes, plus another 9921 probes sets representing an extra 6500 new genes, all of them covered by 11 probes pairs per array.

Hybridization and high-quality scan of the arrays was performed with specific protocols and equipment provided by Affymetrix as described previously[22]. To corroborate the previous results, the same cDNA probes synthesized from both the control fibroblast and one of the patient samples were re-hybridizing with the GeneChip® Human Gene 1.0 ST Array (Affymetrix), an exon-expression arrays that offers whole-transcript coverage from each of the 28869 genes represented on the array, each of them covered by approximately 26 probes spread across the full length of the

gene. For CoQ<sub>10</sub> supplementation analyses, cDNA probes from treated and not supplemented patient's fibroblast were hybridized with the GeneChip® Human Gene 1.0 ST Array (Affymetrix). Data had been deposited with the NCBI-GEO database, at <http://www.ncbi.nlm.nih.gov/geo/>, accession number GSE33941 (this SuperSeries is composed of two subset Series, see supplementary table 10 online for an explanation).

## **Q-RT-PCR**

One µg of RNA was reverse-transcribed using the iScript cDNA Synthesis Kit (Biorad). Real Time PCR was performed in triplicate in a MyiQ™ Single Color Real Time PCR Detection System (Biorad) coupled to a Biorad conventional thermocycler. Primers for selected genes and the housekeeping gene were designed with the Primer Premier 5 software (see supplementary table 11 online for primer sequences). Amplification was carried on with iQ SYBR Green supermix (Biorad) with the following thermal conditions: 30 s at 95°C and 45 cycles of 30 s at 94°C, 30 s at 60°C and 30 s at 72°C. All the results were normalized to the levels of 18S rRNA. The raw fluorescence data were extracted using Light Cycler data collection software version 3.5 (Roche) and analyzed according to manufacturer's instructions for baseline adjustment and noise reduction.

## **Epigenetic analysis**

DNA (CpG) methylation analysis is based on a base-specific cleavage reaction combined with mass spectrometric analysis (MassCLEAVE™). In brief, the method employs a T7-promoter-tagged PCR amplification of bisulphite-converted DNA, followed by generation of a single-stranded RNA molecule and subsequent base-specific cleavage (3' to either rUTP or rCTP) by RNase A. The mixture of cleavage products differing in length and mass are analyzed by MALDI-TOF-MS. Differences in template DNA methylation profile will result in changes in nucleotide sequence after bisulphite treatment, which in turn will yield different fragment masses in the assay. The abundance

of each fragment (signal/noise level in the spectrum) is indicative of the amount of DNA methylation in the interrogated sequence.

The previous Bisulfite treatment was done as follow. Genomic DNA sodium bisulfite conversion was performed with 1 µg of genomic DNA by using EZ-96 DNA methylation kit (Zymo Research) manufacturer's protocol.

The methylation assay was done as follow. Sequenom's MassARRAY platform was used to perform quantitative methylation analysis. This system utilizes MALDI-TOF mass spectrometry in combination with RNA base specific cleavage (MassCLEAVE). A detectable pattern is then analyzed for methylation status. PCR primers for amplification of different regions of the genes listed in table 2 were designed by using Epidesigner (Sequenom). When feasible, amplicons were designed to cover CGIs in the same region as the 5' UTR. For each reverse primer, an additional T7 promoter tag for *in vivo* transcription was added, as well as a 10-mer tag on the forward primer to adjust for melting-temperature differences.

The PCRs were carried out in a 5 µl format with 10 ng/ml bisulfite-treated DNA, 0.2 units of TaqDNA polymerase (Sequenom), 1x supplied Taq buffer, and 200 mM PCR primers.

Amplification for the PCR was as follows: pre-activation of 95°C for 15 min, 45 cycles of 95°C denaturation for 20 s, 56°C annealing for 30 s, and 72°C extension for 30 s, finishing with a 72°C incubation for 4 min. Dephosphorylation of unincorporated dNTPs was performed by adding 1.7 ml of H<sub>2</sub>O and 0.3 units of shrimp alkaline phosphatase (Sequenom), incubating at 37°C for 20 min, then for 10 min at 85°C to deactivate the enzyme.

The MassCLEAVE biochemistry was performed as follows. Next, *in vivo* transcription and RNA cleavage was achieved by adding 2 µl of PCR product to 5 µl of transcription/cleavage reaction and incubating at 37°C for 3 h. The transcription/cleavage reaction contains 27 units of T7 R&DNA polymerase (Sequenom), 0.64x of T7 R&DNA polymerase buffer, 0.22 µl T Cleavage Mix (Sequenom), 3.14 mM DTT, 3.21 µl H<sub>2</sub>O, and 0.09 mg/ml RNase A (Sequenom). The reactions

will be additionally diluted with 20 ml of H<sub>2</sub>O and conditioned with 6 mg of CLEAN Resin (Sequenom) for optimal mass-spectra analysis.

For the statistical analysis, amplicons covering the same gene were pooled together and the CpGs' methylation degree were analyzed with the MultiExperiment Viewer software developed by Saeed[23]. ANOVA test was performed as follows: 3 groups (control, untreated CoQ<sub>10</sub> deficient, and CoQ<sub>10</sub> deficient supplemented with CoQ<sub>10</sub>); alpha (overall threshold p-value) = 0.05; significance determined by alpha with standard Bonferroni correction; and Pearson correlation for clustering of samples and CpGs.

## **SUPPLEMENTARY FIGURE LEGEND**

### **Supplementary figure 1. Graphical view of gene regulation by CoQ<sub>10</sub> supplementation in CoQ<sub>10</sub> deficiency.**

The comparative analysis between CoQ<sub>10</sub> deficient and control fibroblasts (P-Q), between untreated and CoQ<sub>10</sub> supplemented fibroblasts from patient with CoQ<sub>10</sub> deficiency (PQ-P), and between CoQ<sub>10</sub> deficient cells treated with CoQ<sub>10</sub> and control fibroblasts (PQ-P) gave 27 possibilities for a probe to be or not to be regulated in one, two or in all the three comparative analyses, considering that a regulated probe in a comparative analysis, being the probe activated or repressed, can be regulated or not in the other comparative analyses. The 5 different groups of classification, depending on effect of CoQ<sub>10</sub> supplementation on gene expression in fibroblasts of patients suffering CoQ<sub>10</sub> deficiency, are listed in parallel and numbered between parentheses. For a table with full details in number of genes in each category see supplementary table 8 online. The genes that were not classified into any of these groups were those that either slightly regulated the gene expression in only one comparative analysis or even did not change the expression in any of them.

### **Supplementary Figure 2. Regulated genes in CoQ<sub>10</sub> deficiency.**

Functional representation of regulated genes in the syndrome of CoQ<sub>10</sub> deficiency. Represented are listed in table 1 and fully described in the text. Left schema represents a cell where are located all the up-regulated genes, whereas all the repressed genes are located in the right schema. In red are shown all the genes that both kept up their activation after CoQ<sub>10</sub> supplementation and those

repressed in CoQ<sub>10</sub> deficiency that CoQ<sub>10</sub> activated so that they were up-regulated respect the control; in green are shown all the genes that kept down their repression after CoQ<sub>10</sub> supplementation and those activated in CoQ<sub>10</sub> deficiency that were repressed after the treatment with CoQ<sub>10</sub>, being repressed now respect to the control; in blue are shown the regulated genes that completely restored the expression to control level after the CoQ<sub>10</sub> supplementation.

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## SUPPLEMENTARY TABLES ONLINE

**Table 1** Regulated probes in the mitochondrial syndrome of CoQ<sub>10</sub> deficiency

**Table 2** Regulation of energetic metabolism in CoQ<sub>10</sub> deficiency

**Table 3** Regulated genes in either primary (3A) or secondary (3B) deficiency classified into Gene Ontologies

**Table 4** GO classification of regulated genes common to primary and secondary CoQ<sub>10</sub> deficiency

**Table 5** Regulated pathways in CoQ<sub>10</sub> deficiency

**Table 6** Regulated probes in CoQ<sub>10</sub>-deficient fibroblast treated with 30  $\mu$ M

**Table 7** Regulated probes in CoQ<sub>10</sub>-deficient fibroblast treated with 30  $\mu$ M (expanded table)

**Table 8** Classification groups of gene expression after CoQ<sub>10</sub> supplementation

**Table 9** GO classification of regulated genes in CoQ<sub>10</sub>-deficient fibroblasts supplemented with CoQ<sub>10</sub>

**Table 10** Sample description during submission to NCBI-GEO (SuperSeries GSE33941)

**Table 11** Primers used for Q-RT-PCR

**Table 12** Selection of probes during filtering and statistical analysis in CoQ<sub>10</sub> deficient fibroblast

**Table 13** Coherence of changes in gene expression after the comparative analysis

**Table 14** Coherence of changes in gene expression after the comparative analysis (expanded table)

**Table 15** Pathology's gene expression similar to CoQ<sub>10</sub> deficiency

Supplementary Table 1

Regulated probes in the mitochondrial syndrome of Coenzyme Q<sub>10</sub> deficiency

Gene Symbol	Gene Title	Probe ID <sup>(a)</sup>	mean FC <sup>(b)</sup>	FC <sup>(c)</sup>	CoQ <sub>10</sub> <sup>(d)</sup>	Q-RT-PCR <sup>(e)</sup>	CoQ <sub>10</sub> <sup>(f)</sup>
<b>Mitochondrial metabolism</b>							
C7orf55	chromosome 7 open reading frame 55	226780_s_at	-2,1 □ 0,4	nc	-		
BRP44	brain protein 44	202427_s_at	2,0 □ 0,4	2,3	U	8,0 □ 8,3	-2-fold
C10orf58	chromosome 10 open reading frame 58	224435_at	-20,7 □ 15,0	-1,6	pR		
		228155_at	-18,2 □ 14,7	-1,6	pR		
<b>NADH mobilization (unselected but regulated genes studied because their role in specific processes and pathways)</b>							
CYB561	cytochrome b561	209163_at	-1,4 □ 0,4	nc	O		
		207986_x_at	-1,3 □ 0,3				
		209164_s_at	-1,2 □ 0,2				
		210816_s_at	-1,2 □ 0,2				
		217200_x_at	-1,3 □ 0,1				
CYB5A	cytochrome b5-A	209366_x_at	-1,5 □ 0,2	-1,5	U		
		207843_x_at	-1,5 □ 0,3				
		215726_s_at	-1,3 □ 0,1				
		217021_at	-1,3 □ 0,1				
CYB5R1	cytochrome b5 reductase 1	202263_at	-1,3 □ 0,4	nc	U		
		1560043_at	-1,2 □ 0,0				
CYB5R2	cytochrome b5 reductase 2	220230_s_at	-1,4 □ 0,3	-1,9	U		
CYB5R3	cytochrome b5 reductase 3	201885_s_at	-1,3 □ 0,1	-1,6	R		
		1554574_a_at	-1,5 □ 0,2				
CYB5R4	cytochrome b5 reductase 4	219079_at	-1,3 □ 0,1	-1,6	R		
<b>Lipid metabolism</b>							
FDFT1	farnesyl-diphosphate farnesyltransferase 1	210950_s_at	-2,4 □ 0,2	-1,5	U	-4,3 □ 9,1	+2-fold
		208647_at	-2,2 □ 0,3	-1,5	U		
IDI1	isopentenyl-diphosphate delta isomerase 1	204615_x_at	-2,2 □ 0,2	nc	U		
		208881_x_at	-2,0 □ 0,2	nc	U		
CH25H	cholesterol 25-hydroxylase	206932_at	-10,8 □ 10,7	-3,2	O	-1,3 □ 0,1	-3-fold
RSAD2	radical S-adenosyl methionine domain containing 2	242625_at	-6,8 □ 6,0	(1,4)	pR		
INSIG1	insulin induced gene 1	201627_s_at	-2,8 □ 1,1	1,7	O		
		201626_at	-2,4 □ 0,9	1,7	O		
LDLR	low density lipoprotein receptor	202068_s_at	-3,0 □ 1,1	-1,8	pR		
SQLE	squalene epoxidase	209218_at	-2,5 □ 0,6	nc	U		
SCD	stearoyl-CoA desaturase (delta-9-desaturase)	200832_s_at	-3,3 □ 1,4	nc	U		
<b>Insulin metabolism</b>							

CPE	carboxypeptidase E	201116_s_at	8,4	□	5,1	2,5	pR			
		201117_s_at	11,6	□	6,7	2,5	pR			
PAPPA	pregnancy-associated plasma protein A, pappalysin	224941_at	2,5	□	0,7	1,7	R	4,8	□	3,1
PCSK2	proprotein convertase subtilisin/kexin type 2	204869_at	-56,6	□	48,8	-4,3	O			-5-fold
		204870_s_at	-94,3	□	107,8	-4,3	O			
<b>Other metabolism</b>										
SCIN	scinderin	1552365_at	-5,4	□	3,6	(-1,4)	O			
PYGL	phosphorylase, glycogen; liver	202990_at	-2,5	□	0,3	-1,6	R			
SLC40A1	solute carrier family 40 (iron-regulated transporter)	223044_at	7,6	□	6,4	2,9	R			
QPRT	quinolinate phosphoribosyltransferase	242414_at	-3,4	□	1,3	nc	R			
ATP8B1	ATPase, class I, type 8B, member 1	226302_at	2,4	□	0,5	nc	pR			
<b>Cell cycle</b>										
POSTN	periostin, osteoblast specific factor	210809_s_at	73,8	□	35,8	153,9	U	238,2	□	378,5
VEGFA	vascular endothelial growth factor A	210512_s_at	2,9	□	1,8	nc	-			-20%
SEMA5A	semaphorin 5A	205405_at	3,7	□	1,2	1,6	pR			
		213169_at	3,4	□	1,1					
AEBP1	AE binding protein 1	201792_at	66,1	□	38,4	nc	R			
CSRP2	cysteine and glycine-rich protein 2	207030_s_at	4,8	□	1,2	(1,5)	R			
		211126_s_at	5,6	□	1,6					
DOK5	docking protein 5	214844_s_at	6,5	□	2,0	1,6	U			
MID1	midline 1 (Opitz/BBB syndrome)	203636_at	3,9	□	1,7	4,4	U			
CHURC1	churchill domain containing 1	226736_at	3,5	□	0,4	nc	-			
CREG1	repressor 1 of E1A-stimulated genes	201200_at	3,0	□	0,8	(1,3)	R			
RUNX1	runt-related transcription factor 1 (aml1 oncogene)	209360_s_at	1,9	□	0,4	1,6	-			
BHLHB5	basic helix-loop-helix domain containing, class B, 5	228636_at	-6,1	□	3,2	(-1,4)	-			
IFITM1	interferon induced transmembrane protein 1 (9-27)	214022_s_at	-3,9	□	1,8	-3,7	O			
		201601_x_at	-3,6	□	1,6	-3,7	O			
EDN1	endothelin 1	218995_s_at	-3,0	□	1,4	nc	U			
MATN2	matrilin 2	202350_s_at	-9,2	□	1,7	nc	U			
MCAM	melanoma cell adhesion molecule	210869_s_at	-7,7	□	5,2	-3,0	R	-10,9	□	8,4
		211340_s_at	-5,6	□	2,5	-3,0	R			+10%
MKX	mohawk homeobox	241902_at	-4,5	□	2,7	(-1,5)	-			
PSG6	pregnancy specific beta-1-glycoprotein 6	209738_x_at	2,6	□	0,6	nc	-			
DCN	decorin	209335_at	2,0	□	0,4	-1,6	-			
PKP4	plakophilin 4	201928_at	2,0	□	0,2	(1,4)	U			
EFEMP1	EGF-containing fibulin-like extracellular matrix protein 1	201843_s_at	15,2	□	18,1	2,2	pR			
		201842_s_at	11,2	□	10,8	2,2	pR			
VCAN	versican	204620_s_at	2,8	□	1,0	2,7	-	4,6	□	3,8
<b>Cell cycle (unselected but regulated genes studied because their role in specific processes and pathways)</b>										
SMARCA1	component of SWI/SNF chromatin complex, member A1	203875_at	-1,3	□	0,1	nc	pR			

SMARCA4	component of SWI/SNF chromatin complex, member A4	203873_at	-1,3	□	0,1	nc	pR
		203874_s_at	-1,2	□	0,1		
		215294_s_at	-1,2	□	0,1		
		214360_at	-3,8	□	1,1		
		213719_s_at	-1,9	□	0,6		
		1569073_x_at	-1,6	□	0,2		
		215714_s_at	-1,4	□	0,2		
		212520_s_at	-1,4	□	0,2		
		208794_s_at	-1,3	□	0,1		
		213720_s_at	-1,3	□	0,2		
		214728_x_at	-1,3	□	0,0		
		208793_x_at	-1,2	□	0,0		
CDK6	Cyclin-dependent kinase 6, overexpressed in tumor	224851_at	1,3	□	0,1	2,9	U
		224847_at	1,5	□	0,4		
CDKN1A	p21, inhibitor of CDK	243000_at	1,4	□	0,1		
		202284_s_at	-15,0	□	22,2	-2,1	U
CDKN1C	p57, inhibitor of CDK	1555186_at	-3,4	□	2,8		
		213183_s_at	-3,1	□	1,4	-1,3	R
		213182_x_at	-2,9	□	1,0		
		213348_at	-2,8	□	1,9		
		219534_x_at	-2,2	□	0,9		
		216894_x_at	-2,2	□	1,0		
		209714_s_at	1,8	□	0,5	2,7	U
CD31	cell surface antigen	1555758_a_at	1,9	□	0,5		
		208982_at	-2,2	□	0,6	-1,5	R
		208983_s_at	-1,7	□	0,6		
RB1	retinoblastoma protein	1559921_at	-1,5	□	0,3		
		211540_s_at	-1,5	□	0,5	nc	R
		203132_at	-1,3	□	0,4		
E2F7	E2F transcription factor 7	228033_at	3,6	□	3,1	nc	U
E2F8	E2F transcription factor 8	219990_at	2,2	□	0,4	nc	U
FST	follistatin	226847_at	2,3	□	1,0	1,4	O
		207345_at	3,2	□	2,2		
		204948_s_at	2,4	□	1,0		
<b>Development and differentiation</b>							
BDNF	brain-derived neurotrophic factor	206382_s_at	-2,9	□	1,0	nc	pR
GRP	gastrin-releasing peptide	206326_at	-	□	396,6	nc	-
				263,6			
NTNG1	netrin G1	236088_at	-8,3	□	5,8	1,8	U
PTN	pleiotrophin (neurite growth-promoting factor 1)	211737_x_at	-2,7	□	1,3	nc	R

FOXQ1	forkhead box Q1	227475_at	-6,5	□	1,6	nc	-			
HOXA11	homeobox A11	213823_at	-4,3	□	1,8	-2,4	U			
HOXC9	homeobox C9	231936_at	-4,8	□	2,1	-2,0	U			
LHX9	LIM homeobox 9	1562736_at	-93,0	□	106,2	-1,5	U			
SP110	SP110 nuclear body protein	223980_s_at	-2,5	□	0,4	nc	pR			
P2RY5	purinergic receptor P2Y, G-protein coupled, 5	218589_at	-4,4	□	1,5	(-1,3)	pR			
TSPAN10	tetraspanin 10	223795_at	-10,1	□	4,9	nc	-			
EPSTI1	Epithelial stromal interaction 1	235276_at	-5,9	□	5,6	(-1,4)	R			
		227609_at	-4,4	□	4,3	(-1,4)	R			
TSHZ1	teashirt zinc finger homeobox 1	223283_s_at	-3,0	□	1,2	nc	R			
		223282_at	-2,5	□	0,9	nc	R			
KRT34	keratin 34	206969_at	-5,3	□	3,5	-7,6	R	-5,7	□	4,3
TPM1	tropomyosin 1 (alpha)	206117_at	-1,8	□	0,1	1,7	-			-60%
FOXP1	forkhead box P1	224837_at	2,3	□	0,3	nc	-			
LMCD1	LIM and cysteine-rich domains 1	218574_s_at	3,8	□	1,6	nc	U			
<b>Cell resistance to stress</b>										
CYP1B1	cytochrome P450, family 1B, polypeptide 1	202436_s_at	4,0	□	1,8	(1,5)	-	7,0	□	5,6
		202437_s_at	3,7	□	1,6	(1,5)	-			-5-fold
		202435_s_at	4,7	□	2,4	(1,5)	-			
MGC87042	similar to Six epithelial antigen of prostate	217553_at	12,2	□	4,9	-	R			
TMEM49	transmembrane protein 49 /// microRNA 21	220990_s_at	1,9	□	0,2	nc	-			
RAD23B	RAD23 homolog B (S. cerevisiae)	201223_s_at	2,2	□	0,4	nc	R			
TXNIP	thioredoxin interacting protein	201010_s_at	2,0	□	0,5	-4,9	-			
SGK1	serum/glucocorticoid regulated kinase 1	201739_at	3,4	□	1,2	(1,5)	-			
SOCS3	suppressor of cytokine signaling 3	227697_at	-3,6	□	1,2	nc	R			
RHOU	ras homolog gene family, member U	223168_at	-8,3	□	6,5	nc	O			
<b>Apoptosis</b>										
AIM1	absent in melanoma 1	212543_at	-4,5	□	3,2	(-1,4)	O			
APCDD1	adenomatosis polyposis coli down-regulated 1	225016_at	-6,4	□	4,5	-1,8	O			
MAGED1	melanoma antigen family D, 1	209014_at	-1,7	□	0,1	nc	U			
MAGED4 / 4B	melanoma antigen family D, 4 / 4B	223313_s_at	-5,0	□	3,2	-1,6	U			
RAC2	small GTP binding protein Rac2 (rho family)	213603_s_at	-2,3	□	0,5	(-1,3)	U			
TRIM55	tripartite motif-containing 55	236175_at	-11,7	□	1,2	-1,6	U			
IFI6	interferon, alpha-inducible protein 6	204415_at	-4,9	□	1,3	(-1,3)	R			
XAF1	XIAP associated factor-1	228617_at	-3,0	□	1,2	(-1,5)	R			
TNFRSF10D	tumor necrosis factor receptor superfamily 10D	227345_at	2,4	□	0,4	2,6	U	15,1	□	15,1
SFRP1	secreted frizzled-related protein 1	202037_s_at	8,7	□	4,3	2,5	U	11,8	□	7,4
<b>Signalling</b>										
ARL4C	ADP-ribosylation factor-like 4C	202208_s_at	3,6	□	1,3	1,6	pR			
		202206_at	3,6	□	1,1	1,6	pR			

USP53	ubiquitin specific peptidase 53	202207_at	4,2	□	1,2	1,6	pR
		237465_at	3,6	□	0,6	1,7	-
		230083_at	4,5	□	1,0	1,7	-
		231817_at	4,8	□	1,0	1,7	-
GABBR2	gamma-aminobutyric acid (GABA) B receptor, 2	209990_s_at	13,8	□	10,1	2,0	U
CNGA3	cyclic nucleotide gated channel alpha 3	207261_at	-67,3	□	73,8	nc	-
GNG2	G-protein, gamma 2	224964_s_at	-4,2	□	2,3	(1,4)	pR
HERC6	hect domain and RLD 6	219352_at	-7,4	□	3,5	(-1,4)	R
MLPH	melanophilin	218211_s_at	-8,5	□	7,2	-1,9	R
NCK2	NCK adaptor protein 2	203315_at	-1,7	□	0,1	nc	-
PARP14	poly (ADP-ribose) polymerase family, member 14	224701_at	-3,1	□	0,3	(-1,5)	-
<b>Immunity</b>							
CDC42SE2	CDC42 small effector 2	229026_at	-2,8	□	0,3	nc	-
LY6K	lymphocyte antigen 6 complex, locus K	223687_s_at	-4,7	□	2,4	(1,4)	-
GALNAC4S-6ST	B cell RAG associated protein	203066_at	-17,3	□	9,6	-2,5	O
TNFSF4	tumor necrosis factor superfamily, member 4	207426_s_at	-5,9	□	5,1	nc	-
TRIM14	tripartite motif-containing 14	203148_s_at	-4,5	□	2,2	nc	-
BTN3(A2/A3)	butyrophilin3 (A2/A3)	204820_s_at	-2,0	□	0,6	(-1,3)	R
IFI27	interferon, alpha-inducible protein 27	202411_at	-9,8	□	10,1	nc	O
IFI44	interferon-induced protein 44	214453_s_at	-3,3	□	1,0	-2,3	R
IFI44L	interferon-induced protein 44-like	204439_at	-15,0	□	12,5	-1,9	R
IFIT1	interferon-induced protein (tetratricopeptide repeats 1)	203153_at	-5,3	□	2,6	nc	-
IFIT3	interferon-induced protein (tetratricopeptide repeats 3)	204747_at	-3,5	□	1,0	-1,7	R
GBP1	guanylate binding protein 1, interferon-inducible	242907_at	-2,7	□	0,2	-	-
ISG15	ISG15 ubiquitin-like modifier	205483_s_at	-6,4	□	3,5	nc	R
MX1	myxovirus resistance 1	202086_at	-7,4	□	5,5	-1,8	pR
MX2	myxovirus resistance 2	204994_at	-6,1	□	2,3	-3,0	pR
OAS2	2'-5'-oligoadenylate synthetase 2, 69/71kDa	204972_at	-6,2	□	1,7	-1,6	R
OAS3	2'-5'-oligoadenylate synthetase 3, 100kDa	218400_at	-3,6	□	1,1	(-1,3)	R
PSMB9	proteasome subunit, beta type, 9	204279_at	-1,8	□	0,2	nc	U
<b>Immunity (unselected but regulated genes studied because their role in specific processes and pathways)</b>							
OAS1	2',5'-oligoadenylate synthetase 1, 40/46kDa	202869_at	-5,5	□	2,8	-4,9	R
		205552_s_at	-4,7	□	2,2		
OASL	2'-5'-oligoadenylate synthetase-like	205660_at	-3,4	□	0,8	-2,6	R
		210797_s_at	-2,8	□	0,8		
<b>Genes of unknown function</b>							
---	Unknown gene	1567575_at	-5,2	□	1,1	-	-
---	Unknown gene	244503_at	-4,8	□	1,0	-	-
---	Unknown gene	230175_s_at	-2,7	□	0,6	-	-
---	Unknown gene	228049_x_at	-2,5	□	1,0	-	-

---	Unknown gene	235274_at	-3,2	□	2,2	-	-
C7orf58	chromosome 7 open reading frame 58	228728_at	2,2	□	0,4	nc	-
C9orf150	chromosome 9 open reading frame 150	227443_at	2,5	□	0,5	(1,5)	-
FAM43A	family with sequence similarity 43, member A	227410_at	-4,7	□	3,0	-2,9	U
FLJ37228	Unknown gene	1556641_at	-12,5	□	9,2	-	-
FLJ41747	hypothetical gene supported by AK123741	239153_at	-27,9	□	12,3	-	-
KIAA1199	KIAA1199	212942_s_at	1,7	□	0,1	nc	-
LOC283666	Unknown gene	226682_at	2,8	□	0,5	-	-
LOC730259	Unknown gene	239624_at	-19,3	□	17,3	-	-
TMEM106B	transmembrane protein 106B	218930_s_at	-1,9	□	0,2	-	-
TMEM106B	transmembrane protein 106B	226529_at	-2,0	□	0,5	-	-

<sup>(a)</sup> Probe ID in Affymetrix GeneChip® Human Genome U133 Plus 2.0 Array

<sup>(b)</sup> mean +/- sd corresponding to the values of FC of each sample (SAM analysis; R=1,5; FDR=0%)

<sup>(c)</sup> Full change in the comparative analysis ran with Affymetrix Gene Chip® Human Gene 1.0 ST Array. In parenthesis, FC of non-significant / non-selected genes. Genes with no change (nc)

<sup>(d)</sup> Effect of coenzyme CoQ<sub>10</sub> supplementation on gene expression in CoQ<sub>10</sub> deficiency (more information in table 3 and in the text): unaffected genes by CoQ<sub>10</sub> treatment (U); genes that restored the expression either partial (pR) or completely (R); genes with opposite regulation than in CoQ<sub>10</sub> deficiency (O); and specifically regulated genes only after CoQ<sub>10</sub> supplementation (S). Genes non-affected by CoQ<sub>10</sub> supplementation (-).

<sup>(e)</sup> Full change in gene expression analyzed by quantitative real time PCR (Q-RT-PCR).

<sup>(f)</sup> Effect of coenzyme Q<sub>10</sub> supplementation on mRNA levels analyzed by Q-RT-PCR.

## Supplementary table 2

### Regulation of energetic metabolism in CoQ<sub>10</sub> deficiency

Gene Ontology / Description	mean FC <sup>(a)</sup>	# genes <sup>(b)</sup>	Q-effect <sup>(c)</sup>
<b>Glycolysis</b>			
glycolysis, overall 10 catalytic steps	1,5	27 (34)	pR
glycolysis, step 1 - HK	1,4	6 (6)	pR
glycolysis, step 2 - GPI	-1,2	1 (1)	U
glycolysis, step 3 - PFK	1,3	3 (3)	U
glycolysis, step 4 - ALDOLASE	1,7	3 (4)	pR
glycolysis, step 5 - TPI	1,4	1 (1)	pR
glycolysis, step 6 - GAPDH	1,6	2 (2)	pR
glycolysis, step 7 - PGK	1,5	2 (2)	pR
glycolysis, step 8 - PGAM	1,2	3 (6)	pR
glycolysis, step 9 - ENOLASE	2,3	4 (7)	pR
glycolysis, step 10 - PK	1,3	2 (2)	pR
Lactate dehydrogenase	1,7	5 (5)	R
Pyruvate dehydrogenase	2,2	4 (4)	pR
negative regulation of glycolysis	-1,3	1 (1)	U
positive regulation of glycolysis	2,3	4 (4)	pR
<b>TCA cycle</b>			
TCA cycle, overall	no change	29 (32)	U
cytosolic isoenzymes of TCA	no change	3 (3)	U
mitochondrial isoenzymes of TCA	-1,3	3 (3)	pR
<b>Fatty acid beta-oxidation</b>			
fatty acid beta oxidation	no change	28 (28)	U
fatty acid beta oxidation, regulation	-1,5	10 (10)	pR
<b>OXPPOS</b>			
Respiratory chain - complex I	-1,7	46 (64)	O
Respiratory chain - complex II	1,2	4 (13)	pR
Respiratory chain - complex III	-1,3	7 (14)	O
Respiratory chain - complex IV	-1,5	3 (45)	O
ATP synthase - complex V	-1,4	(64)	O

<sup>a</sup> mean of Full Change corresponding to the values of each probe included in the Affymetrix Gene Chip® Human Gene 1.0 ST Array

<sup>b</sup> Number of genes analysed within each gene ontology. In parenthesis, number of probes.

<sup>c</sup> Effect of coenzyme CoQ<sub>10</sub> supplementation on gene expression in CoQ<sub>10</sub> deficiency: unaffected genes by CoQ<sub>10</sub> treatment (U); genes that restored the expression either partial (pR) or completely (R); and genes with opposite regulation after CoQ<sub>10</sub> supplementation than in CoQ<sub>10</sub> deficiency (O).

**Supplementary table 3A**

**Regulated genes clasified in Gene Ontologies in primary CoQ<sub>10</sub> deficiency**

Gene Ontology Term		P-value	# genes <sup>(1)</sup>	# selected <sup>(2)</sup>	Enrichment <sup>(3)</sup>
<b>Immunity-related GO repressed in primary CoQ<sub>10</sub> deficiency</b>					
(*)	GO:0001730 2'-5'-oligoadenylate synthetase activity (interferon-inducible)	5,03E-07	3	3	124,8
	GO:0035457 cellular response to interferon-alpha	9,36E-04	6	2	41,6
(*)	GO:0045071 negative regulation of viral genome replication	4,30E-07	20	5	31,2
(*)	GO:0048525 negative regulation of viral reproduction	4,30E-07	20	5	31,2
(*)	GO:0060337 type I interferon-mediated signaling pathway	5,14E-20	65	16	30,7
(*)	GO:0071357 cellular response to type I interferon	5,14E-20	65	16	30,7
(*)	GO:0034340 response to type I interferon	6,74E-20	66	16	30,3
(*)	GO:0045069 regulation of viral genome replication	3,11E-06	29	5	21,5
(*)	GO:0009615 response to virus	2,76E-14	199	18	11,3
(*)	GO:0060333 interferon-gamma-mediated signaling pathway	1,85E-04	66	5	9,5
(*)	GO:0071346 cellular response to interferon-gamma	4,29E-04	79	5	7,9
	GO:0050792 regulation of viral reproduction	7,41E-04	89	5	7,0
(*)	GO:0019221 cytokine-mediated signaling pathway	1,46E-09	291	16	6,9
(*)	GO:0071345 cellular response to cytokine stimulus	4,53E-09	361	17	5,9
(*)	GO:0034097 response to cytokine stimulus	2,24E-08	454	18	5,0
	GO:0002252 immune effector process	9,60E-04	194	7	4,5
	GO:0002376 immune system process	7,36E-04	1141	20	2,2
<b>Development-related GO repressed in primary CoQ<sub>10</sub> deficiency</b>					
(*)	GO:0042474 middle ear morphogenesis	4,44E-04	19	3	19,7
	GO:0048598 embryonic morphogenesis	4,60E-04	283	9	4,0
	GO:0044087 regulation of cellular component biogenesis	6,50E-04	297	9	3,8
(*)	GO:0009653 anatomical structure morphogenesis	8,50E-07	1056	25	3,0
(*)	GO:0045595 regulation of cell differentiation	6,21E-05	869	19	2,7
(*)	GO:0050793 regulation of developmental process	3,51E-04	1246	22	2,2
<b>Cell response-related GO repressed in primary CoQ<sub>10</sub> deficiency</b>					
	GO:2000242 negative regulation of reproductive process	4,39E-05	49	5	12,7
(*)	GO:0051707 response to other organism	1,24E-10	327	18	6,9
(*)	GO:0009607 response to biotic stimulus	5,00E-10	502	21	5,2
(*)	GO:0051704 multi-organism process	3,56E-08	763	23	3,8
(*)	GO:0044419 interspecies interaction between organisms	8,61E-04	374	10	3,3
(*)	GO:0071310 cellular response to organic substance	1,16E-05	989	22	2,8
(*)	GO:0070887 cellular response to chemical stimulus	3,57E-05	1225	24	2,4
(*)	GO:0010033 response to organic substance	1,25E-04	1588	27	2,1
(*)	GO:0007166 cell surface receptor signaling pathway	5,90E-05	1875	31	2,1

(*)	GO:0042221	response to chemical stimulus	1,00E-05	2345	38	2,0
<b>Metabolism-related GO repressed in primary CoQ<sub>10</sub> deficiency</b>						
	GO:0060700	regulation of ribonuclease activity	1,90E-04	3	2	83,2
	GO:0070566	adenylyltransferase activity	4,44E-04	19	3	19,7
(*)	GO:0016126	sterol biosynthetic process	6,19E-07	39	6	19,2
(*)	GO:0006695	cholesterol biosynthetic process	6,06E-06	33	5	18,9
	GO:0008299	isoprenoid biosynthetic process	5,19E-04	20	3	18,7
	GO:0003725	double-stranded RNA binding	2,88E-04	40	4	12,5
(*)	GO:0008203	cholesterol metabolic process	1,06E-07	100	9	11,2
(*)	GO:0016125	sterol metabolic process	1,90E-07	107	9	10,5
(*)	GO:0006694	steroid biosynthetic process	2,01E-05	104	7	8,4
	GO:0008202	steroid metabolic process	9,21E-05	228	9	4,9
	GO:0006066	alcohol metabolic process	1,12E-04	234	9	4,8
	GO:0016053	organic acid biosynthetic process	5,47E-04	231	8	4,3
	GO:0046394	carboxylic acid biosynthetic process	5,47E-04	231	8	4,3
	GO:0008610	lipid biosynthetic process	7,04E-05	390	12	3,8
<b>Cellular location-related GO repressed in primary CoQ<sub>10</sub> deficiency</b>						
(*)	GO:0005829	cytosol	2,71E-05	2165	35	2,0
<b>Development-related GO activated in primary CoQ<sub>10</sub> deficiency</b>						
	GO:0060343	trabecula formation	5,07E-06	20	4	34,0
	GO:0048640	negative regulation of developmental growth	2,80E-04	22	3	23,2
	GO:0001837	epithelial to mesenchymal transition	7,90E-05	39	4	17,4
	GO:0002053	positive regulation of mesenchymal cell proliferation	9,48E-04	33	3	15,5
	GO:0060688	regulation of morphogenesis of a branching structure	1,39E-04	45	4	15,1
	GO:0045766	positive regulation of angiogenesis	8,43E-05	76	5	11,2
(*)	GO:0045765	regulation of angiogenesis	1,94E-05	141	7	8,4
(*)	GO:0001871	pattern binding	1,41E-06	185	9	8,3
(*)	GO:1901342	regulation of vasculature development	3,15E-05	152	7	7,8
	GO:2000027	regulation of organ morphogenesis	4,97E-04	111	5	7,7
	GO:0007389	pattern specification process	1,43E-04	331	9	4,6
	GO:0051093	negative regulation of developmental process	3,29E-04	453	10	3,8
(*)	GO:0022603	regulation of anatomical structure morphogenesis	2,20E-04	516	11	3,6
	GO:0009888	tissue development	6,37E-04	493	10	3,5
(*)	GO:0009653	anatomical structure morphogenesis	1,28E-04	1056	17	2,7
(*)	GO:2000026	regulation of multicellular organismal development	4,37E-04	955	15	2,7
<b>Cell response-related GO activated in primary CoQ<sub>10</sub> deficiency</b>						
	GO:0009629	response to gravity	2,30E-05	10	3	51,0
	GO:0034695	response to prostaglandin E stimulus	8,53E-05	15	3	34,0
	GO:0034694	response to prostaglandin stimulus	2,09E-04	20	3	25,5

GO:0005109	frizzled binding	3,21E-04	23	3	22,2
GO:0007565	female pregnancy	2,10E-07	72	7	16,5
GO:0032874	positive regulation of stress-activated MAPK cascade	2,45E-04	52	4	13,1
GO:0070304	positive regulation of stress-activated protein kinase signaling cascade	2,64E-04	53	4	12,8
(*) GO:0008201	heparin binding	9,32E-06	126	7	9,4
(*) GO:0005539	glycosaminoglycan binding	5,99E-07	167	9	9,2
(*) GO:0030247	polysaccharide binding	1,41E-06	185	9	8,3
GO:0070482	response to oxygen levels	8,80E-07	227	10	7,5
GO:0001666	response to hypoxia	3,66E-05	213	8	6,4
GO:0036293	response to decreased oxygen levels	3,91E-05	215	8	6,3
GO:0030246	carbohydrate binding	3,48E-04	373	9	4,1
GO:0016477	cell migration	1,68E-04	500	11	3,7
GO:0009628	response to abiotic stimulus	1,59E-05	710	15	3,6
GO:0040011	locomotion	5,70E-05	889	16	3,1
GO:0022414	reproductive process	4,57E-04	959	15	2,7
(*) GO:0048523	negative regulation of cellular process	1,90E-05	2476	31	2,1
(*) GO:0048519	negative regulation of biological process	1,54E-05	2704	33	2,1
<b>Metabolism-related GO activated in primary CoQ<sub>10</sub> deficiency</b>					
GO:0004720	protein-lysine 6-oxidase activity	2,04E-04	4	2	85,0
GO:0043627	response to estrogen stimulus	2,47E-04	149	6	6,8
GO:0045934	negative regulation of nucleobase-containing compound metabolic process	8,75E-04	808	13	2,7
GO:0051172	negative regulation of nitrogen compound metabolic process	9,79E-04	818	13	2,7
<b>Cellular location-related GO activated in primary CoQ<sub>10</sub> deficiency</b>					
GO:0030199	collagen fibril organization	8,65E-04	32	3	15,9
GO:0005604	basement membrane	6,14E-04	66	4	10,3
(*) GO:0031012	extracellular matrix	1,51E-07	294	12	6,9
(*) GO:0005578	proteinaceous extracellular matrix	2,17E-05	198	8	6,9
GO:0044420	extracellular matrix part	4,85E-04	169	6	6,0
GO:0005615	extracellular space	8,51E-06	761	16	3,6
GO:0048870	cell motility	3,74E-04	549	11	3,4
(*) GO:0044421	extracellular region part	6,38E-06	926	18	3,3
(*) GO:0007155	cell adhesion	2,91E-04	719	13	3,1
(*) GO:0022610	biological adhesion	2,91E-04	719	13	3,1
GO:0005576	extracellular region	8,03E-05	1226	19	2,6
<b>Cell signalling-related GO activated in primary CoQ<sub>10</sub> deficiency</b>					
GO:0005114	type II transforming growth factor beta receptor binding	6,79E-06	7	3	72,8
GO:0090036	regulation of protein kinase C signaling cascade	7,06E-04	7	2	48,5
GO:0090037	positive regulation of protein kinase C signaling cascade	7,06E-04	7	2	48,5

GO:0005160	transforming growth factor beta receptor binding	1,51E-04	18	3	28,3
GO:0030514	negative regulation of BMP signaling pathway	5,81E-04	28	3	18,2
GO:0071560	cellular response to transforming growth factor beta stimulus	5,81E-04	28	3	18,2
GO:0071559	response to transforming growth factor beta stimulus	8,65E-04	32	3	15,9
GO:0007179	transforming growth factor beta receptor signaling pathway	5,30E-05	69	5	12,3
GO:0090101	negative regulation of transmembrane receptor protein serine/threonine kinase signaling pathway	4,84E-04	62	4	11,0
GO:0007178	transmembrane receptor protein serine/threonine kinase signaling pathway	4,58E-04	109	5	7,8
(*) GO:0090092	regulation of transmembrane receptor protein serine/threonine kinase signaling pathway	9,16E-04	127	5	6,7

(\*) GO regulated simultaneously in primary and secondary CoQ10 deficiency

<sup>(1)</sup> number of human genes within each gene ontology

<sup>(2)</sup> number of significantly regulated genes included within each gene ontology

<sup>(3)</sup> Gene Ontology Enrichment represents the selection of genes associated with a specific GO. It is calculated by GORILLA as follows:  $E=(b/n)/(B/N)$ , being "N" the total number of genes, "B" the total number of genes associated with a particular GO, "n" the number of regulated genes for each analysis and "b" the number of regulated genes associated with a particular GO.

**Supplementary table 3B**

**Regulated genes clasified in Gene Ontologies in secondary CoQ deficiency**

Gene Ontology Term	P-value	# genes <sup>(1)</sup>	# selected <sup>(2)</sup>	Enrichment <sup>(3)</sup>
<b>Immunity-related GO repressed in secondary CoQ<sub>10</sub> deficiency</b>				
(*) GO:0001730 2'-5'-oligoadenylate synthetase activity (interferon-inducible)	2,68E-04	3	2	70,05
GO:0019060 intracellular transport of viral proteins in host cell	8,83E-04	5	2	42,03
GO:0051708 intracellular protein transport in other organism involved in symbiotic interaction	8,83E-04	5	2	42,03
(*) GO:0060337 type I interferon-mediated signaling pathway	1,09E-15	65	14	22,63
(*) GO:0071357 cellular response to type I interferon	1,09E-15	65	14	22,63
(*) GO:0034340 response to type I interferon	1,38E-15	66	14	22,29
(*) GO:0045071 negative regulation of viral genome replication	3,39E-05	20	4	21,01
(*) GO:0048525 negative regulation of viral reproduction	3,39E-05	20	4	21,01
(*) GO:0045069 regulation of viral genome replication	1,56E-04	29	4	14,49
(*) GO:0071346 cellular response to interferon-gamma	1,06E-04	79	6	7,98
(*) GO:0060333 interferon-gamma-mediated signaling pathway	4,09E-04	66	5	7,96
(*) GO:0009615 response to virus	5,67E-08	199	13	6,86
GO:0034341 response to interferon-gamma	3,08E-04	96	6	6,57
(*) GO:0071345 cellular response to cytokine stimulus	6,17E-08	361	17	4,95
(*) GO:0034097 response to cytokine stimulus	6,39E-08	454	19	4,4
<b>Development-related GO repressed in secondary CoQ<sub>10</sub> deficiency</b>				
GO:2000648 positive regulation of stem cell proliferation	8,83E-04	5	2	42,03
(*) GO:0042474 middle ear morphogenesis	7,32E-04	19	3	16,59
(*) GO:0051704 multi-organism process	1,14E-05	763	21	2,89
GO:0045597 positive regulation of cell differentiation	9,75E-04	437	12	2,89
GO:0051094 positive regulation of developmental process	5,39E-04	593	15	2,66
(*) GO:0045595 regulation of cell differentiation	2,20E-04	869	20	2,42
(*) GO:0009653 anatomical structure morphogenesis	1,62E-04	1056	23	2,29
(*) GO:2000026 regulation of multicellular organismal development	7,36E-04	955	20	2,2
(*) GO:0050793 regulation of developmental process	7,00E-04	1246	24	2,02
GO:0051239 regulation of multicellular organismal process	7,89E-04	1567	28	1,88
<b>Cell response-related GO repressed in secondary CoQ<sub>10</sub> deficiency</b>				
GO:0030581 symbiont intracellular protein transport in host	8,83E-04	5	2	42,03
GO:0071503 response to heparin	8,83E-04	5	2	42,03
GO:0071504 cellular response to heparin	8,83E-04	5	2	42,03
(*) GO:0051707 response to other organism	2,95E-06	327	14	4,5
(*) GO:0009607 response to biotic stimulus	1,39E-06	502	18	3,77
(*) GO:0044419 interspecies interaction between organisms	2,45E-04	374	12	3,37

(*)	GO:0071310	cellular response to organic substance	6,06E-05	989	23	2,44
	GO:0042981	regulation of apoptotic process	3,94E-05	1098	25	2,39
	GO:0043067	regulation of programmed cell death	4,51E-05	1107	25	2,37
	GO:0010941	regulation of cell death	6,76E-05	1135	25	2,31
(*)	GO:0070887	cellular response to chemical stimulus	8,76E-05	1225	26	2,23
(*)	GO:0010033	response to organic substance	8,00E-05	1588	31	2,05
(*)	GO:0042221	response to chemical stimulus	5,60E-05	2345	41	1,84
<b>Metabolism-related GO repressed in secondary CoQ<sub>10</sub> deficiency</b>						
(*)	GO:0016126	sterol biosynthetic process	1,69E-06	39	6	16,16
(*)	GO:0006695	cholesterol biosynthetic process	1,40E-05	33	5	15,92
	GO:0071396	cellular response to lipid	2,30E-04	32	4	13,13
(*)	GO:0008203	cholesterol metabolic process	4,97E-06	100	8	8,41
(*)	GO:0016125	sterol metabolic process	8,23E-06	107	8	7,86
(*)	GO:0006694	steroid biosynthetic process	4,74E-04	104	6	6,06
(*)	GO:0019221	cytokine-mediated signaling pathway	1,21E-07	291	15	5,42
<b>Cellular location-related GO repressed in primary CoQ<sub>10</sub> deficiency</b>						
(*)	GO:0005829	cytosol	9,97E-04	2165	35	1,7
<b>Cellular location-related GO repressed in secondary CoQ<sub>10</sub> deficiency</b>						
	GO:0003924	GTPase activity	3,30E-05	210	10	5
	GO:0019001	guanyl nucleotide binding	5,04E-04	348	11	3,32
	GO:0032561	guanyl ribonucleotide binding	5,04E-04	348	11	3,32
(*)	GO:0007166	cell surface receptor signaling pathway	6,95E-04	1875	32	1,79
<b>Development-related GO activated in primary CoQ<sub>10</sub> deficiency</b>						
	GO:0072017	distal tubule development	3,68E-04	3	2	59,82
	GO:0048569	post-embryonic organ development	7,30E-04	4	2	44,86
	GO:0003206	cardiac chamber morphogenesis	8,25E-04	17	3	15,83
	GO:0033688	regulation of osteoblast proliferation	8,25E-04	17	3	15,83
	GO:0001944	vasculature development	5,96E-04	35	4	10,25
	GO:0008406	gonad development	9,33E-07	93	9	8,68
	GO:0008584	male gonad development	1,09E-04	68	6	7,92
	GO:0048608	reproductive structure development	1,41E-05	129	9	6,26
	GO:0061138	morphogenesis of a branching epithelium	4,94E-05	117	8	6,14
(*)	GO:0045765	regulation of angiogenesis	1,83E-04	141	8	5,09
	GO:0001763	morphogenesis of a branching structure	1,92E-04	142	8	5,05
(*)	GO:1901342	regulation of vasculature development	3,05E-04	152	8	4,72
	GO:0001701	in utero embryonic development	3,79E-04	157	8	4,57
	GO:0043009	chordate embryonic development	4,31E-04	160	8	4,49
	GO:0009792	embryo development ending in birth or egg hatching	5,28E-04	165	8	4,35
	GO:0002009	morphogenesis of an epithelium	4,94E-04	205	9	3,94
	GO:0048729	tissue morphogenesis	1,99E-04	266	11	3,71

	GO:0009790	embryo development	6,44E-04	258	10	3,48
	GO:0048513	organ development	1,77E-06	831	26	2,81
(*)	GO:0022603	regulation of anatomical structure morphogenesis	6,76E-04	516	15	2,61
	GO:0048731	system development	1,95E-04	619	18	2,61
	GO:0050878	regulation of body fluid levels	8,87E-04	530	15	2,54
	GO:0051094	positive regulation of developmental process	9,93E-04	593	16	2,42
	GO:0045595	regulation of cell differentiation	1,01E-04	869	23	2,37
	GO:2000026	regulation of multicellular organismal development	3,98E-04	955	23	2,16
(*)	GO:0009653	anatomical structure morphogenesis	2,83E-04	1056	25	2,12
	GO:0042127	regulation of cell proliferation	6,43E-04	1052	24	2,05
<b>Cell response-related GO activated in secondary CoQ<sub>10</sub> deficiency</b>						
	GO:0071481	cellular response to X-ray	3,68E-04	3	2	59,82
	GO:0046882	negative regulation of follicle-stimulating hormone secretion	7,30E-04	4	2	44,86
	GO:0004364	glutathione transferase activity	9,82E-04	18	3	14,95
	GO:0017147	Wnt-protein binding	1,57E-04	25	4	14,36
	GO:0036294	cellular response to decreased oxygen levels	1,29E-05	47	6	11,45
	GO:0071456	cellular response to hypoxia	1,29E-05	47	6	11,45
	GO:0071453	cellular response to oxygen levels	2,09E-05	51	6	10,56
<b>Metabolism-related GO activated in secondary CoQ<sub>10</sub> deficiency</b>						
	GO:0007026	negative regulation of microtubule depolymerization	9,82E-04	18	3	14,95
	GO:0010951	negative regulation of endopeptidase activity	4,12E-04	121	7	5,19
	GO:0010466	negative regulation of peptidase activity	4,78E-04	124	7	5,07
<b>Cellular location-related GO activated in secondary CoQ<sub>10</sub> deficiency</b>						
	GO:0001569	patterning of blood vessels	2,48E-04	28	4	12,82
	GO:0010811	positive regulation of cell-substrate adhesion	1,54E-04	46	5	9,75
	GO:0010810	regulation of cell-substrate adhesion	6,51E-05	90	7	6,98
(*)	GO:0005539	glycosaminoglycan binding	1,80E-05	167	10	5,37
	GO:0031012	extracellular matrix	3,25E-08	294	17	5,19
(*)	GO:0005578	proteinaceous extracellular matrix	1,38E-05	198	11	4,98
(*)	GO:0008201	heparin binding	5,26E-04	126	7	4,98
(*)	GO:0001871	pattern binding	4,33E-05	185	10	4,85
(*)	GO:0030247	polysaccharide binding	4,33E-05	185	10	4,85
	GO:0030155	regulation of cell adhesion	4,04E-04	243	10	3,69
(*)	GO:0007155	cell adhesion	5,23E-05	719	21	2,62
(*)	GO:0022610	biological adhesion	5,23E-05	719	21	2,62
(*)	GO:0044421	extracellular region part	9,79E-05	926	24	2,33
<b>Cell signalling-related GO activated in primary CoQ<sub>10</sub> deficiency</b>						
(*)	GO:0090092	regulation of transmembrane receptor protein serine/threonine kinase signaling pathway	5,52E-04	127	7	4,95

(\*) GO regulated simultaneously in primary and secondary CoQ<sub>10</sub> deficiency

<sup>(1)</sup> number of human genes within each gene ontology

<sup>(2)</sup> number of significantly regulated genes included within each gene ontology

<sup>(3)</sup> Gene Ontology Enrichment represents the selection of genes associated with a specific GO. It is calculated by GORILLA as follows:  $E = (b/n) / (B/N)$ , being "N" the total number of genes, "B" the total number of genes associated with a particular GO, "n" the number of regulated genes for each analysis and "b" the number of regulated genes associated with a particular GO.

**Supplementary table 4**

**GO classification of regulated genes common to primary and secondary CoQ<sub>10</sub> deficiency**

<b>Gene Ontology Term</b>	<b>genes<sup>(a)</sup></b>	<b>significant<sup>(b)</sup></b>	<b>Z_Score</b>
<b>Mitochondrial metabolism</b>			
GO0009055 electron carrier activity	344	79	-2,2
GO0004777 succinate semialdehyde dehydrogenase activity	2	2	-2,1
GO0005739 mitochondrion	1132	625	-2,0
GO0006818 hydrogen transport	78	10	-1,7
GO0008137 NADH dehydrogenase (ubiquinone) activity	51	33	-1,7
GO0005746 mitochondrial respiratory chain	83	12	-1,6
GO0006099 tricarboxylic acid cycle	29	20	-1,3
GO0003954 NADH dehydrogenase activity	44	14	-1,3
GO0042593 glucose homeostasis	52	19	-1,2
GO0005758 mitochondrial intermembrane space	34	5	0,6
GO0031304 intrinsic to mitochondrial inner membrane	5	1	0,6
GO0042720 mitochondrial inner membrane peptidase C	1	1	1,0
<b>Lipid metabolism</b>			
GO0008395 steroid hydroxylase activity	17	1	-9,5
GO0016126 sterol biosynthetic process	26	18	-5,7
GO0006695 cholesterol biosynthetic process	32	19	-4,7
GO0006694 steroid biosynthetic process	71	34	-4,1
GO0004506 squalene monooxygenase activity	1	1	-3,1
GO0047598 7 dehydrocholesterol reductase activity	1	1	-3,1
GO0004312 fatty acid synthase activity	8	1	-2,6
GO0004313 acyl carrier protein s acetyltransferase	1	1	-2,6
GO0004314 acyl carrier protein s malonyltransferase	2	1	-2,6
GO0004315 3 oxoacyl acyl carrier protein synthase	2	1	-2,6
GO0004317 3 hydroxypalmitoyl acyl carrier protein	1	1	-2,6
GO0004319 enoyl acyl carrier protein reductase	1	1	-2,6
GO0004320 oleoyl acyl carrier protein hydrolase	2	1	-2,6
GO0010281 acyl acp thioesterase activity	2	1	-2,6
GO0004420 hydroxymethylglutaryl CoA reductase	1	1	-2,5
GO0001619 lysosphingolipid and lysophosphatidic acid receptor	15	10	-2,2
GO0030549 acetylcholine receptor activator activity	1	1	-2,2
GO0006636 unsaturated fatty acid biosynthetic process	39	1	-2,1
GO0016213 linoleoyl coa desaturase activity	1	1	-2,1
GO0006656 phosphatidylcholine biosynthetic process	14	6	-2,1
GO0006650 glycerophospholipid metabolic process	127	1	-1,9
GO0006678 glucosylceramide metabolic process	4	1	-1,9
GO0006681 galactosylceramide metabolic process	4	1	-1,9
GO0046459 short chain fatty acid metabolic process	5	1	-1,9
GO0030169 low density lipoprotein binding	21	1	-1,9
GO0030229 very low density lipoprotein receptor activity	4	1	-1,9
GO0008299 isoprenoid biosynthetic process	22	10	-1,9
GO0047012 sterol 4 alpha carboxylate 3 dehydrogenase	1	1	-1,7
<b>Cell cycle</b>			
GO0030308 negative regulation of cell growth	81	9	-1,2
GO0005720 nuclear heterochromatin	33	10	1,4
GO0006310 DNA recombination	77	28	1,5
GO0000793 condensed chromosome	28	15	1,7
GO0008094 dna dependent atpase activity	30	15	1,9
GO0005816 spindle pole body	2	1	1,5
GO0000922 spindle pole	23	7	1,9
GO0008608 attachment of spindle microtubules to kinetochore	8	1	2,0
GO0042393 histone binding	62	7	2,0
GO0007098 centrosome cycle	25	3	2,0
GO0007059 chromosome segregation	40	19	2,2

GO0000070 mitotic sister chromatid segregation	38	5	2,3
GO0007140 male meiosis	22	5	2,5
GO0005874 microtubule	266	119	2,6
GO0006270 dna replication initiation	25	10	2,7
GO0000776 kinetochore	31	10	2,8
GO0007067 mitosis	199	103	3,2
GO0007126 meiosis	120	29	3,4
GO0051301 cell division	250	157	3,6
GO0000775 chromosome pericentric region	61	30	4,2
GO0006260 DNA replication	154	88	4,2
GO0007049 cell cycle	516	318	4,2
GO0045743 positive regulation of fibroblast growth	6	1	4,9
GO0045741 positive regulation of epidermal growth	6	1	6,5
<b>Nucleosome and chromosome</b>			
GO0000786 nucleosome	120	62	-4,0
GO0007001 chromosome organization and biogenesis	93	57	-4,0
GO0006334 nucleosome assembly	142	67	-3,4
GO0005694 chromosome	222	113	-2,4
GO0016514 swi or snf complex	11	6	-1,3
<b>Development and differentiation</b>			
GO0021768 nucleus accumbens development	1	1	-5,5
GO0060166 olfactory pit development	2	1	-5,5
GO0048535 lymph node development	18	16	-3,4
GO0001886 endothelial cell morphogenesis	4	1	-2,9
GO0060014 granulosa cell differentiation	4	1	-2,2
GO0048389 intermediate mesoderm development	2	1	-2,0
GO0031017 exocrine pancreas development	6	1	-2,0
GO0045651 positive regulation of macrophage differentiation	6	2	-1,9
GO0030879 mammary gland development	8	14	-1,3
GO0045617 negative regulation of keratinocyte differentiation	3	1	2,3
GO0048706 embryonic skeletal development	85	1	4,9
<b>Cell resistance and stress response</b>			
GO0006281 DNA repair	319	137	3,1
GO0003960 NADPH quinone reductase activity	4	2	1,9
GO0006974 response to DNA damage stimulus	201	133	2,3
GO0008630 DNA damage response signal transduction	23	10	2,3
GO0007256 activation of JNKK activity	5	3	3,2
GO0043506 regulation of JNK activity	51	1	2,1
<b>Apoptosis and cell death</b>			
GO0043065 positive regulation of apoptosis	114	39	-3,4
GO0043071 positive regulation of cell death (non apoptotic)	2	1	-2,8
GO0005164 tumor necrosis factor receptor binding	93	15	-2,5
GO0008219 cell death	60	8	-1,6
GO0008629 induction of apoptosis by intracellular signals	22	8	-1,6
<b>Immune response</b>			
GO0009615 response to virus	98	27	-6,4
GO0006955 immune response	825	197	-5,5
GO0042098 T cell proliferation	27	8	-2,3
GO0019028 viral capsid	23	5	-1,3
GO0042130 negative regulation of T cell proliferation	20	10	1,2
GO0045190 isotype switching	25	6	1,5
<b>Cytosolic protein metabolism</b>			
GO0004298 threonine endopeptidase activity	36	18	-2,5
GO0005839 proteasome core complex	35	18	-2,5
GO0004298 threonine endopeptidase activity	21	18	-2,5
GO0004175 endopeptidase activity	47	31	-2,0
GO0015198 oligopeptide transporter activity	7	6	-2,0
GO0003756 protein disulfide isomerase activity	9	7	-1,8
GO0043234 protein complex	419	63	-1,7
GO0016567 protein ubiquitination	72	21	-1,2

GO0006461 protein complex assembly	171	44	-1,2
GO0008320 protein carrier activity	16	6	-1,1
GO0016023 cytoplasmic membrane-bounded vesicle	71	35	-2,5
GO0045921 positive regulation of exocytosis	15	1	-2,1
GO0006888 ER to golgi vesicle-mediated transport	41	44	-1,8
GO0030126 COPI vesicle coat	11	7	-1,4
GO0019894 kinesin binding	10	7	-1,1
GO0016939 kinesin II complex	1	1	-1,5
GO0006893 golgi to plasma membrane transport	10	3	2,9
<b>Cytoskeleton metabolism</b>			
GO0008092 cytoskeletal protein binding	79	22	-1,8
GO0005862 muscle thin filament tropomyosin	4	3	-1,7
GO0030863 cortical cytoskeleton	38	5	-1,3
GO0031430 M line	11	1	0,7
GO0005863 striated muscle thick filament	3	16	1,2
GO0006941 striated muscle contraction	45	24	1,7
GO0060052 neurofilament cytoskeleton organization	10	8	1,9
GO0031674 I band	60	4	2,2
GO0030017 sarcomere	101	11	2,3
<b>Iron metabolism</b>			
GO0006826 iron ion transport	32	18	2,5
<b>Other metabolism</b>			
GO0006164 purine nucleotide biosynthetic process	23	12	1,3
GO0008033 tRNA processing	68	29	1,4
GO0000049 tRNA binding	24	9	1,7
<b>Intracellular signaling</b>			
GO0008589 regulation of smoothened signaling pathway	24	1	-5,1
GO0003924 GTPase activity	248	106	-3,2
GO0005886 plasma membrane	2727	391	-3,1
GO0031583 G protein signaling phospholipase D activation	1	1	-2,6
GO0000186 activation of MAPKK activity	31	7	-1,9
GO0016324 apical plasma membrane	130	41	-1,6
GO0005100 rho GTPase activator activity	31	8	1,5
GO0005149 interleukin 1 receptor binding	12	12	2,9
<b>Sexual determination</b>			
GO0019101 female somatic sex determination	1	1	-2,2
GO0019100 male germ line sex determination	1	1	2,4
GO0006702 androgen biosynthetic process	7	2	2,4
GO0030238 male sex determination	10	8	3,6

<sup>a</sup> number of human genes included within each gene ontology

<sup>b</sup> number of significantly regulated genes by CoQ<sub>10</sub> deficiency included within each gene ontology

# Supplementary table 5

## Regulated pathways in CoQ<sub>10</sub>-deficiency

Pathway	Z_Score	PMID <sup>(a)</sup>	model <sup>(b)</sup>	COQ <sub>10</sub> deficiency <sup>(c)</sup>	analysis <sup>(d)</sup>	model description
<b>Lipid and liver metabolism</b>						
CHOLESTEROL_BIOSYNTHESIS	-5,3	-	-	down	-	genes included in the biosynthesis of cholesterol
BIOSYNTHESIS_OF_STEROIDS	-3,9	-	-	down	-	genes included in the biosynthesis of steroids
TERPENOID_BIOSYNTHESIS	-3,0	-	-	down	-	genes included in the biosynthesis of terpenoids
ICHIBA_GVHD	-4,3	<b>12663442</b>	up	down	opposite	genes expressed in mouse liver
CPR_LOW_LIVER_UP	-3,7	<b>16006652</b>	up	down	opposite	genes expressed in mouse liver
FETAL_LIVER_enriched_transcription_factors	-3,2	-	-	down	-	genes expressed in fetal liver
<b>Cell cycle activation and cell differentiation inhibition</b>						
P21_P53_MIDDLE_DN	3,3	<b>12138103</b>	down	up	opposite	p21 (cyclin-dependent kinase inhibitor)
P21_ANY_DN	3,3	<b>12138103</b>	down	up	opposite	inhibits cell cycle and stimulate cell
P21_P53_EARLY_DN	4,5	<b>12138103</b>	down	up	opposite	differentiation
P21_P53_ANY_DN	5,5	<b>12138103</b>	down	up	opposite	
GREENBAUM_E2A_UP	3,5	<b>15310760</b>	up	up	similar	lack of E2A (transcription factor that induce p21) - no p21 inhibition
E2F1_DNA_UP	4,0	<b>11313881</b>	up	up	similar	E2F transcription factor pushes the progression of cell cycle
CMV_IE86_UP	4,8	<b>11867723</b>	up	up	similar	cyclin overexpression by CMV infection activates CDK
VERNELL_PRB_CLSTR1	5,0	<b>12923195</b>	down	up	opposite	deregulation of pRB pathway is a hallmark of tumorigenesis
BRG1_ALAB_UP	-3,7	<b>14673169</b>	up	down	opposite	Swi/Snf chromatin remodeling apparatus arrests cell cycle
JISON_SICKLECELL_DIFF	-3,8	<b>15031206</b>	up	down	opposite	arrest of cell cycle, induction of apoptosis
LAL_KO_3MO_UP	-3,3	<b>16127159</b>	up	down	opposite	and repression of both mitogen-activated
MANALO_HYPOXIA_DN	3,3	<b>15374877</b>	down	up	opposite	pathways and cell growth and proliferation in sickle cell disease.
DNA_REPLICATION_REACTOME	5,0	-	-	up	-	genes included in the reactome for DNA replication
VEGF_MMMEC_6HRS_UP	6,1	<b>12200464</b>	up	up	similar	growth factors activate pathways that induce cell cycle
SERUM_FIBROBLAST_CELLCYCLE	6,9	<b>14737219</b>	up	up	similar	serum activate pathways that induce cell cycle

**Maintenance of cellular state of no differentiation**

BOQUEST_CD31PLUS_VS_CD31MINUS_UP	4,1	<b>15635089</b>	up	up	similar	absent of CD31 marker maintains stemness for no differentiation
CANCER_UNDIFFERENTIATED_META_UP	3,4	<b>15184677</b>	up	up	similar	gene pattern of undifferentiated tumoral cells
STEMCELL_EMBRYONIC_UP	3,5	<b>12228720</b>	up	up	similar	gene pattern of embryonic stem cells
LEE_TCELLS4_UP	-3,8	<b>15210650</b>	up	down	opposite	gene pattern of mature CD4(+) T-cells
LEE_TCELLS3_UP	6,2	<b>15210650</b>	up	up	similar	gene pattern of immature CD4(+) T-cells
HOFFMANN_BIVSBII_BI_TABLE2	4,1	<b>11779835</b>	up	up	similar	gene pattern of immature B-cells
LE_MYELIN_DN	-2,8	<b>15695336</b>	down	down	similar	gene pattern of immature Schwann cells
LIAN_MYELOID_DIFF_TF	-2,9	<b>11468144</b>	up	down	opposite	differentiation of myeloid stem cells to neutrophils
XU_ATRA_PLUSNSC_UP	-3,1	<b>16140955</b>	up	down	opposite	differentiation of myeloid stem cells by retinoic acid
LI_FETAL_VS_WT_KIDNEY_DN	4,7	<b>12057921</b>	down	up	opposite	development and differentiation of fetal structures
GAY_YY1_DN	5,1	<b>16611997</b>	down	up	opposite	development and differentiation of fetal structures
ZHAN_MULTIPLE_MYELOMA_UP	-3,2	<b>11861292</b>	up	down	opposite	development and differentiation of multiple myeloid cells compared to undifferentiated lymphocytes in bone marrow
MOREAUX_TACI_HI_VS_LOW_UP	-3,0	<b>15827134</b>	up	down	opposite	differentiation of fibroblast to adipocytes by insulin
ADIP_DIFF_CLUSTER4	3,7	<b>12137940</b>	up	up	similar	differentiation of fibroblast to adipocytes by insulin
IDX_TSA_UP_CLUSTER3	6,4	<b>15033539</b>	up	up	similar	

**Cell transformation during tumorigenesis**

ZHAN_MM_CD138_PR_VS_REST	5,4	<b>16728703</b>	up	up	similar	adaptation and resistance of myeloid cells during tumorigenesis
BRCA_PROGNOSIS_NEG	4,3	<b>11823860</b>	up	up	similar	activated markers for breast tumor
VANTVEER_BREAST_OUTCOME	3,5	<b>11823860</b>	up	up	similar	
AGUIRRE_PANCREAS_CHR19	-3,2	<b>15199222</b>	down	down	similar	repressed markers for pancreatic adenocarcinoma
SANSOM_APC_LOSS4_UP	2,9	<b>15198980</b>	up	up	similar	lost of APC

**Development**

FSH_OVARY_MCV152_UP	-3,7	<b>15386376</b>	down	up	opposite	FSH stimulates maturation of germ cells (lost of undifferentiation)
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**Aging**

MIDDLEAGE_DN	2,9	<b>10741968</b>	down	up	opposite	gene pattern on middleage humans
OLDAGE_DN	4,8	<b>10741968</b>	down	up	opposite	gene pattern on oldage humans
ROTH_HTERT_UP	-9,6	<b>15741219</b>	up	down	opposite	gene pattern of senescence T-cells

### Cell resistance

UVC_TTD_4HR_UP	-3,1	<b>15608684</b>	up	down	opposite	DNA response not induced in deficiency of
UVC_TTD_ALL_UP	-2,8	<b>15608684</b>	up	down	opposite	nucleotide excision repair after UV treatment
PENG_Glutamine_DN	-3,2	<b>12101249</b>	down	down	similar	resistance to glutamine starvation
DOX_RESIST_GASTRIC_UP	6,4	<b>14734480</b>	up	up	similar	resistance to chemotherapy in cancer cells
AGED_MOUSE_HIPPOCAMPUS_ANY_UP	4,7	<b>15960800</b>	up	up	similar	aged-mediated inflammatory response in hippocampus
KAMMINGA_EZH2_TARGETS	4,6	<b>16293602</b>	up	up	similar	hematopoietic stem cells avoid replicative stress and senescence
OXSTRESS_RPETHREE_DN	-3,2	<b>12419474</b>	down	down	similar	gene repression in epithelium cells by oxidative stress

### Apoptosis and cell cycle arrest

TGFBETA_C2_UP	-3,5	<b>11279127</b>	up	down	opposite	TGF-beta arrests cell cycle and induce apoptosis
ET743_SARCOMA_UP	-3,6	<b>15897246</b>	up	down	opposite	trabectedin (ET-743) induce cell cycle arrest and apoptosis
ET743_SARCOMA_DN	3,1	<b>15897246</b>	down	up	opposite	
ET743_SARCOMA_6HRS_UP	-3,2	<b>15897246</b>	up	down	opposite	

### Epigenetic regulation of gene expression

DAC_BLADDER_UP	-8,4	<b>11861364</b>	up	down	opposite	inhibition of DNA methylation prevents
DAC_IFN_BLADDER_UP	-7,7	<b>11861364</b>	up	down	opposite	initial phase of tumorigenesis
TSADAC_RKOEUP_UP	-4,5	<b>11992124</b>	up	down	opposite	gene regulation by demethylation of DNA
TSA_HEPATOMA_CANCER_UP	-3,2	<b>15452378</b>	up	down	opposite	histone deacetylase inhibition arrests cell cycle, induce apoptosis

### Interferon induced genes

DER_IFNA_UP	-9,0	<b>9861020</b>	up	down	opposite	interferon (alpha, beta, and gamma)-induced genes in fibrosarcoma cells treated with any interferon or with all at the same time and at different time points
DER_IFNB_UP	-8,2	<b>9861020</b>	up	down	opposite	
IFN_ALPHA_UP	-8,0	<b>9861020</b>	up	down	opposite	
IFN_ANY_UP	-7,1	<b>9861020</b>	up	down	opposite	
IFN_BETA_UP	-6,9	<b>9861020</b>	up	down	opposite	
DER_IFNG_UP	-5,1	<b>9861020</b>	up	down	opposite	
IFN_GAMMA_UP	-4,1	<b>9861020</b>	up	down	opposite	
IFN_ALL_UP	-4,1	<b>9861020</b>	up	down	opposite	interferon-inducible and cytomegalovirus-induced genes in fibroblasts
IFNA_UV-CMV_COMMON_HCMV_6HRS_UP	-9,8	<b>11711622</b>	up	down	opposite	
CMV_HCMV_TIMECOURSE_12HRS_UP	-9,7	<b>11711622</b>	up	down	opposite	
IFNA_HCMV_6HRS_UP	-9,6	<b>11711622</b>	up	down	opposite	interferon-alpha inducible genes in primary hepatocytes
RADAEVA_IFNA_UP	-11,7	<b>11910354</b>	up	down	opposite	
IFNALPHA_NL_UP	-11,2	<b>11910354</b>	up	down	opposite	
IFNALPHA_NL_HCC_UP	-9,2	<b>11910354</b>	up	down	opposite	
IFNALPHA_HCC_UP	-7,9	<b>11910354</b>	up	down	opposite	

GRANDVAUX_IFN_NOT_IRF3_UP	-9,2	<b>11991981</b>	up	down	opposite	interferon alpha and beta)-inducible genes in
GRANDVAUX_IRF3_UP	-6,3	<b>11991981</b>	up	down	opposite	Jurkat cells
NF90_DN	3,8	<b>12036489</b>	down	up	opposite	interferon-iducible by ectopic NF90 expression without viral infection
BECKER_IFN_INDUCIBLE_SUBSET_1	-7,1	<b>15657362</b>	up	down	opposite	interferon-iducible genes in cells resistant to tamoxifen therapy
SANA_IFNG_ENDOTHELIAL_UP	-8,9	<b>15749026</b>	up	down	opposite	interferon-gamma and TNF-alpha iducible
SANA_TNFA_ENDOTHELIAL_UP	-8,6	<b>15749026</b>	up	down	opposite	genes in endothelial cells
TAKEDA_NUP8_HOXA9_3D_UP	-13,2	<b>16818636</b>	up	down	opposite	interferon-iducible genes in myelodysplastic
TAKEDA_NUP8_HOXA9_10D_UP	-8,4	<b>16818636</b>	up	down	opposite	syndromes and in acute myeloid leukaemia
TAKEDA_NUP8_HOXA9_8D_UP	-7,8	<b>16818636</b>	up	down	opposite	
TAKEDA_NUP8_HOXA9_16D_UP	-7,2	<b>16818636</b>	up	down	opposite	
CIS_RESIST_LUNG_UP	-5,8	<b>14737109</b>	up	down	opposite	interferon-inducible genes by DNA damage after cisplatin treatment
<b>Interferon-induced and virus-induced genes at 48 hours</b>						
CMV_HCMV_TIMECOURSE_48HRS_UP	4,8	<b>11711622</b>	up	up	similar	cytomegalovirus-induced genes in fibroblasts at 48 hours
IFN_BETA_GLIOMA_DN	-3,0	<b>16140920</b>	down	down	similar	interferon-beta inducible genes in glioma cells at 48 hours
<b>Interleukin induced genes</b>						
IL6_FIBRO_UP	-5,1	<b>15095275</b>	up	down	opposite	IL-6 inducible genes in normal skin fibroblasts
BROCKE_IL6	-3,4	<b>12969979</b>	up	down	opposite	IL-6 inducible genes in multiple myeloma
KRETZSCHMAR_IL6_DIFF	-3,4	<b>12969979</b>	up	down	opposite	cell lines
CROONQUIST_IL6_RAS_DN	4,3	<b>12791645</b>	down	up	opposite	IL-6 repressed genes in multiple myeloma cell lines
CROONQUIST_IL6_STARVE_UP	5,0	<b>12791645</b>	up	up	similar	inducible genes in multiple myeloma cell lines IL-6 starved
<b>Immunity</b>						
CMV_8HRS_UP	-5,8	<b>9826724</b>	up	down	opposite	fibroblasts infectd with cytomegalovirus
CMV_ALL_UP	-4,8	<b>9826724</b>	up	down	opposite	
CMV_24HRS_UP	-4,0	<b>9826724</b>	up	down	opposite	
WIELAND_HEPATITIS_B_INDUCED	-5,0	<b>15100412</b>	up	down	opposite	hepatocytes infected with hepatitis B virus
HPV31_DN	-6,4	<b>10756030</b>	up	down	opposite	keratinocytes infected with human papillomavirus
BENNETT_SLE_UP	-12,2	<b>12642603</b>	up	down	opposite	genes activated in lupus (an autoimmune disease)
<b>Other markers</b>						
SLRPPATHWAY	3,0	-				

- <sup>(a)</sup> Reference listed as PubMed Identity number
- <sup>(b)</sup> Regulation of gene expression in the model described
- <sup>(c)</sup> Regulation of the same genes in CoQ<sub>10</sub> deficiency
- <sup>(d)</sup> Model vs.CoQ<sub>10</sub> deficiency comparative analysis

Supplementary table 6

Regulated probes in coenzyme Q<sub>10</sub>-deficient fibroblast treated with 30  $\mu$ M

minimum FC	comparative analysis <sup>(a)</sup>	Treated vs. No treated (PQ-P)		Treated vs. Control (PQ-C)		No treated vs. Control (P-C)	
		probes	% <sup>(b)</sup>	probes	% <sup>(b)</sup>	probes	% <sup>(b)</sup>
<b>R = 1.5</b>	regulated probes	6043	18%	7448	22%	10680	32%
	UP-regulated	2441	7%	4927	15%	7238	22%
	DOWN-regulated	3602	11%	2521	8%	3442	10%
<b>R = 2.0</b>	regulated probes	3046	9%	4824	14%	5830	18%
	UP-regulated	1257	4%	3326	10%	3868	12%
	DOWN-regulated	1789	5%	1498	4%	1962	6%
<b>R = 3.0</b>	regulated probes	1317	4%	2304	7%	2405	7%
	UP-regulated	479	1%	1597	5%	1655	5%
	DOWN-regulated	838	3%	707	2%	750	2%

<sup>(a)</sup> ITERPLIER algorithms; t-test<0.05

<sup>(b)</sup> An Affymetrix GeneChip® Human Gene ST 1.0 array contains 33297 probe sets

# Supplementary table 7

## Regulated probes in coenzyme Q<sub>10</sub>-deficient fibroblast treated with 30 $\mu$ M (expanded table) <sup>(a)</sup>

PQ - P	PQ - C	P - C	significant probes (t-test<0.05) <sup>(b)</sup>			% vs. all regulated probes <sup>(c)</sup>			# <sup>(d)</sup>
			R = 1.5	R = 2	R = 3	R = 1.5	R = 2	R = 3	
UP	UP	UP	190	151	112	1,4%	2,4%	4,5%	U
		DOWN	51	48	34	0,4%	0,8%	1,4%	O
		-	401	156	23	3,0%	2,5%	0,9%	S
	DOWN	UP	-	-	-	-	-	-	-
		DOWN	273	273	211	2,1%	4,4%	8,5%	pR
		-	-	-	-	-	-	-	-
	-	UP	-	-	-	-	-	-	-
		DOWN	1075	621	99	8,1%	10,0%	4,0%	R
		-	451	8	0	3,4%	0,1%	-	-
DOWN	UP	UP	791	741	525	6,0%	11,9%	21,1%	pR
		DOWN	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
	DOWN	UP	50	50	33	0,4%	0,8%	1,3%	O
		DOWN	165	165	141	1,2%	2,7%	5,7%	U
		-	505	183	57	3,8%	2,9%	2,3%	S
	-	UP	1516	636	82	11,4%	10,2%	3,3%	R
		DOWN	-	-	-	-	-	-	-
		-	575	14	0	4,3%	0,2%	-	-
-	UP	UP	3156	2218	903	23,7%	35,7%	36,3%	U
		DOWN	-	-	-	-	-	-	-
		-	338	12	0	2,5%	0,2%	-	-
	DOWN	UP	-	-	-	-	-	-	-
		DOWN	1187	812	265	8,9%	13,1%	10,7%	U
		-	341	15	0	2,6%	0,2%	-	-
	-	UP	1535	72	0	11,5%	1,2%	-	-
		DOWN	691	43	0	5,2%	0,7%	-	-
		-	20006	26979	30812	-	-	-	-

<sup>(a)</sup> for a graphical view, see figure S1

<sup>(b)</sup> ITERPLIER algorithms; t-test<0.05

<sup>(c)</sup> The percentaje is calculated on the total number of probes regulated at least in one comparative analysis, being: 13291 for R=1.5; 6218 for R=2.0; and 2485 for R=3.0

<sup>(d)</sup> Effect of coenzyme CoQ<sub>10</sub> supplementation on gene expression in CoQ<sub>10</sub> deficiency (more information in Supplementary Table 4 and in the text): unaffected genes by CoQ<sub>10</sub> treatment (U); genes that restored the expression either partial (pR) or completely (R); genes with opposite regulation than in CoQ<sub>10</sub> deficiency (O); and specifically regulated genes only after CoQ<sub>10</sub> supplementation (S). Genes non-affected by CoQ<sub>10</sub> supplementation (-).

## Supplementary table 8

### Classification groups of gene expression after coenzyme Q<sub>10</sub> supplementation

Classification group <sup>(c)</sup>	significant probes <sup>(a)</sup>			% vs. all regulated probes <sup>(b)</sup>		
	R = 1.5	<b>R = 2</b> <sup>(d)</sup>	R = 3	R = 1.5	<b>R = 2</b> <sup>(d)</sup>	R = 3
( U ) Unaffected by CoQ <sub>10</sub>	4698	<b>3346</b>	1421	35%	<b>54%</b>	57%
( pR ) Partial restored by CoQ <sub>10</sub>	1064	<b>1014</b>	736	8%	<b>16%</b>	30%
( R ) Completely restored by CoQ <sub>10</sub>	2591	<b>1257</b>	181	19%	<b>20%</b>	7%
( O ) Opposite regulation than in CoQ <sub>10</sub> deficiency	101	<b>98</b>	67	1%	<b>2%</b>	3%
( S ) Regulated only after CoQ <sub>10</sub> supplementation	906	<b>339</b>	80	7%	<b>5%</b>	3%
- small changes (non-specific)	3931	<b>164</b>	0	30%	<b>3%</b>	0%

<sup>(a)</sup> ITERPLIER algorithms; t-test<0.05

<sup>(b)</sup> The percentage is calculated on the total number of probes regulated at least in one comparative analysis, being: 13291 for R=1.5; 6218 for R=2.0 (in bold letter); and 2485 for R=3.0

<sup>(c)</sup> Description of the classification groups in the text (see figure S1 for a graphical representation)

<sup>(d)</sup> A minimum full change of 2-fold (R=2) was considered for a further analysis (see comment in the text)

# Supplementary table 9

## GO classification of regulated genes in CoQ<sub>10</sub>-deficient fibroblasts supplemented with CoQ<sub>10</sub>

Gene Ontology Term	P-value	# genes <sup>(a)</sup>	# selected <sup>(b)</sup>	Enrichment <sup>(c)</sup>
<b>Classification group (U): unaffected genes by CoQ<sub>10</sub> treatment</b>				
GO:0043506 regulation of JUN kinase activity	6,58E-04	10	6	-4,6
GO:0070302 regulation of stress-activated protein kinase signaling cascade	3,21E-04	23	10	-3,3
GO:0010627 regulation of intracellular protein kinase cascade	3,46E-04	74	21	-2,1
GO:0019899 enzyme binding	6,91E-04	99	25	-2,0
GO:0050789 regulation of biological process	9,16E-04	1008	60	-1,2
GO:0065007 biological regulation	8,86E-04	1081	70	-1,2
GO:0044428 nuclear part	4,21E-04	176	98	1,3
GO:0022402 cell cycle process	7,37E-05	109	67	1,4
GO:0007049 cell cycle	1,34E-05	92	60	1,5
GO:0044427 chromosomal part	8,17E-05	64	43	1,5
GO:0022403 cell cycle phase	2,43E-05	71	48	1,6
GO:0007017 microtubule-based process	1,76E-04	49	34	1,6
GO:0000280 nuclear division	1,32E-04	45	32	1,6
GO:0007067 mitosis	1,32E-04	46	32	1,6
GO:0048285 organelle fission	7,76E-05	46	33	1,7
GO:0051301 cell division	4,22E-07	65	48	1,7
GO:0006281 DNA repair	2,54E-04	32	24	1,7
GO:0005634 nucleus	7,51E-04	594	28	1,8
GO:0000777 condensed chromosome kinetochore	5,80E-04	18	15	1,9
GO:0000776 kinetochore	1,47E-04	20	17	2,0
GO:0003677 DNA binding	4,91E-04	491	23	2,1
GO:0071156 regulation of cell cycle arrest	2,69E-04	23	5	8,2
GO:0007059 chromosome segregation	8,45E-04	17	4	8,8
GO:0000910 cytokinesis	9,20E-04	8	3	14,1
GO:0009954 proximal/distal pattern formation	3,42E-04	6	3	18,8
GO:0030199 collagen fibril organization	1,74E-04	5	3	22,6
GO:0033599 regulation of mammary gland epithelial cell proliferation	6,99E-04	2	2	37,6
GO:0035264 multicellular organism growth	6,99E-04	2	2	37,6
GO:0005095 GTPase inhibitor activity	6,99E-04	2	2	37,6
GO:0010997 anaphase-promoting complex binding	5,13E-04	2	2	43,9
<b>Classification group (O): opposite regulation than in CoQ<sub>10</sub> deficiency</b>				
GO:0051240 positive regulation of multicellular organismal process	6,69E-04	61	4	-9,5

GO:0048856	anatomical structure development	7,21E-04	341	9	3,3
GO:0032269	negative regulation of cellular protein metabolic process	9,19E-04	25	3	15,0
GO:0031400	negative regulation of protein modification process	3,38E-04	18	3	20,8
GO:0010563	negative regulation of phosphorus metabolic process	1,92E-04	15	3	24,9
GO:0042326	negative regulation of phosphorylation	1,92E-04	15	3	24,9
GO:0045936	negative regulation of phosphate metabolic process	1,92E-04	15	3	24,9
GO:0001933	negative regulation of protein phosphorylation	9,41E-05	12	3	31,2
GO:0001569	patterning of blood vessels	9,04E-04	6	2	41,6
GO:0031258	lamellipodium membrane	3,65E-04	4	2	62,3
GO:0031527	filopodium membrane	1,83E-04	3	2	83,1
GO:0031952	regulation of protein autophosphorylation	6,14E-05	2	2	124,7
GO:0031953	negative regulation of protein autophosphorylation	6,14E-05	2	2	124,7
<b>Classification group (S): specific regulation in CoQ<sub>10</sub> deficiency due to CoQ<sub>10</sub> treatment</b>					
GO:0005523	tropomyosin binding	3,05E-04	5	3	-18,7
GO:0034728	nucleosome organization	8,52E-04	19	4	8,9
GO:0071824	protein-DNA complex subunit organization	8,52E-04	19	4	8,9
GO:0006334	nucleosome assembly	5,42E-04	17	4	9,9
GO:0065004	protein-DNA complex assembly	5,42E-04	17	4	9,9
GO:0032993	protein-DNA complex	4,22E-04	16	4	10,6
GO:0009898	internal side of plasma membrane	5,00E-05	4	3	31,7
<b>Classification group (pR): CoQ<sub>10</sub> partially restored the altered expression in CoQ<sub>10</sub> deficiency</b>					
GO:0060665	regulation of branching involved in salivary gland morphogenesis	8,68E-04	5	4	-6,8
GO:0005576	extracellular region	9,62E-04	424	29	1,8
GO:0032868	response to insulin stimulus	9,73E-04	21	5	6,2
GO:0015718	monocarboxylic acid transport	2,29E-04	9	4	11,5
GO:0005520	insulin-like growth factor binding	2,29E-04	9	4	11,5
GO:0048666	neuron development	3,01E-05	11	5	1,8
<b>Classification group (R): CoQ<sub>10</sub> completely restored the altered expression of CoQ<sub>10</sub> deficiency</b>					
GO:0010941	regulation of cell death	6,87E-04	144	21	2,1
GO:0043067	regulation of programmed cell death	6,25E-04	143	21	2,1
GO:0042981	regulation of apoptosis	5,67E-04	142	21	2,1
GO:0045121	membrane raft	3,46E-04	21	7	4,8
GO:0046777	protein autophosphorylation	7,14E-05	8	5	9,0
GO:0060674	placenta blood vessel development	3,27E-04	3	3	14,4

<sup>(a)</sup> Number of human genes analyzed within each gene ontology

<sup>(b)</sup> Number of significantly regulated genes by CoQ<sub>10</sub> supplementation included within each gene ontology

<sup>(c)</sup> Gene Ontology Enrichment represents the selection of genes associated with a specific GO. It is calculated by GORILLA software as follows:  $E = (b/n)/(B/N)$ , being "N" the total number of genes, "B" the total number of genes associated with a particular GO, "n" the number of genes in each group of classification and "b" the number of genes associated to a particular GO within each group of classification.

**Supplementary table 10**

**Sample description during submission to NCBI-GEO (SuperSeries GSE33941)**

SubSeries	Sample	Sample description	# <sup>(a)</sup>
<b>Series GSE33769</b>  Platform GPL570  [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	GSM833438	fibroblast_patient_1_A	P
	GSM833441	fibroblast_patient_1_B	P
	GSM833444	fibroblast_patient_1_C	P
	GSM833478	control_fibroblast_A	C
	GSM833479	control_fibroblast_B	C
	GSM833480	control_fibroblast_C	C
	GSM833490	fibroblast_patient_3_A	P
	GSM833491	fibroblast_patient_3_B	P
	GSM833492	fibroblast_patient_3_C	P
	GSM833493	fibroblast_patient_5_A	P
	GSM833494	fibroblast_patient_5_B	P
	GSM833495	fibroblast_patient_5_C	P
	GSM833502	fibroblast_patient_4_A	P
	GSM833503	fibroblast_patient_4_B	P
	GSM833504	fibroblast_patient_4_C	P
<b>Series GSE33940</b>  Platform GPL6244  [HuGene-1_0-st] Affymetrix Human Gene 1.0 ST Array (transcript gene version)	GSM839154	fibroblast_patient_ELO_1_1	P
	GSM839155	fibroblast_patient_ELO_1_2	P
	GSM839156	fibroblast_patient_ELO_2_1	P
	GSM839157	fibroblast_patient_ELO_2_2	P
	GSM839158	fibroblast_patient_GIO_1_1	P
	GSM839159	fibroblast_patient_GIO_1_2	P
	GSM839160	fibroblast_patient_GIO_2_1	P
	GSM839161	fibroblast_patient_GIO_2_2	P
	GSM839162	control_fibroblast_2c	C
	GSM839163	control_fibroblast_4a	C
	GSM839164	control_fibroblast_4b	C
	GSM839165	fibroblast_patient_SOF_Q-treated_1	P+Q
	GSM839166	fibroblast_patient_SOF_Q-treated_2	P+Q
	GSM839167	fibroblast_patient_SIL_Q-treated_1	P+Q
	GSM839168	fibroblast_patient_SIL_Q-treated_2	P+Q
	GSM839169	fibroblast_patient_MSI_Q-treated_1	P+Q
	GSM839170	fibroblast_patient_MSI_Q-treated_2	P+Q
	GSM839171	fibroblast_patient_ELO_Q	P+Q
	GSM839172	fibroblast_patient_ELO_Q	P+Q
	GSM839173	fibroblast_patient_MEL_Q	P+Q

<sup>(a)</sup> human dermal fibroblast from healthy volunteers (C), fibroblasts from CoQ<sub>10</sub> deficient patients (P), and fibroblasts from CoQ<sub>10</sub>-deficient patients treated with 30  $\mu$ M CoQ<sub>10</sub> for 48 hours (PQ) as described in the text.

supplementary table 11

Primers used for Q-RT-PCR

Gene	Forward	Reverse
BRP44	5 ' GATAAAGTGGAGCTGATGC 3 '	5 ' TTGGAGCCCAGAAGAAAA 3 '
CH25H	5 ' CCCTTGGTCCACTCACAG 3 '	5 ' GCAGCGTTCCAGTATTT 3 '
CYP1B1-1	5 ' AGGCAAAGGTCCCAGTTC 3 '	5 ' GATGGACAGCGGGTTTAG 3 '
CYP1B1-2	5 ' AGTGCAGGCAGAATTGGA 3 '	5 ' GAGGTGTTGGCAGTGGTG 3 '
EFNB2	5 ' ACCAAATCCAGGTTCTA 3 '	5 ' CTCCTCCGGTACTTCA 3 '
FDFT1	5 ' ACTTTGGCTGCCTGTTAT 3 '	5 ' CATCCATCATCAGGGTCA 3 '
KRT34	5 ' GGGAAAGTGGAGCAATG 3 '	5 ' AGAGTCTCGCAGGTTGT 3 '
MCAM - 1	5 ' GTGTAGGGAGGAACGG 3 '	5 ' CTGGGACGACTGAATGT 3 '
MCAM - 2	5 ' AGTCAGGACGAGACCATC 3 '	5 ' CTTCAGCCTTCCGAGTA 3 '
PAPPA	5 ' TGGGTCATAACTATTTTCAGG 3 '	5 ' TATTCATGTCGTCGCATT 3 '
POSTN-1	5 ' TTAAGTTTGTTTCGTGGTAG 3 '	5 ' TGTGGGTCCTTCAGTTTT 3 '
POSTN-2	5 ' CCGAGCCTTGATGTATG 3 '	5 ' TTACCAGTAAACCCACTC 3 '
SFRP1	5 ' GCTGGAGCACGAGACCA 3 '	5 ' AGCGAGCAGAGGAAGACC 3 '
TNFRSF10D	5 ' GTTCGTCTGCTTTCATCG 3 '	5 ' CCTCTGTTGCTGTGGG 3 '
VCAN	5 ' GATGAAACCTCGTTATGAA 3 '	5 ' CTAAGCACCGGATAGTTG 3 '

Supplementary table 12

Selection of probes during filtering and statistical analysis in primary and secondary CoQ<sub>10</sub> deficient fibroblasts

	Primary deficiency		Sample 3 - COQ2		Sample 5 - COQ2		Secondary deficiency		Sample 1 - ataxia		Sample 4 - MELAS	
	Probes	% <sup>(a)</sup>	Probes	% <sup>(a)</sup>	Probes	% <sup>(a)</sup>	Probes	% <sup>(a)</sup>	Probes	% <sup>(a)</sup>	Probes	% <sup>(a)</sup>
Probes with p-value<0.05	26783	49%	28407	52%	27711	51%	27042	50%	27893	51%	28130	51%
SAM analysis <sup>(b)</sup> FDR < 5%	-	-	5940	11%	3200	6%	-	-	3425	6%	3077	6%
Δ-value (FDR)	-		0,73 (4,2%)		0,81 (3,6%)		-		0,83 (3,3%)		0,82 (3,2%)	
SAM analysis <sup>(b)</sup> FDR < 1%	1162	2.1%	4127	8%	2497	5%	1217	2.2%	2697	5%	2263	4%
Δ-value (FDR)	-		1,3 (0,7%)		1,2 (0,8%)		-		1,2 (0,9%)		1,3 (0,97%)	
UP-regulated	397	0.7%	2145	3.9%	1244	2.3%	515	0.9%	1287	2.4%	1207	2.2%
DOWN-regulated	517	0.9%	1982	3.6%	1253	2.3%	628	1.1%	1410	2.6%	1056	1.9%
SAM analysis <sup>(2)</sup> FDR = 0	387	0.7%	1672	3%	915	2%	479	0.9%	1240	2%	842	2%
Δ-value (FDR)	-		3,1 (0%)		3,2 (0%)		-		2,7 (0%)		3,3 (0%)	
UP-regulated	139	0.2%	849	1.6%	544	1.0%	255	0.5%	703	1.3%	489	0.9%
DOWN-regulated	175	0.3%	823	1.5%	371	0.7%	207	0.4%	537	1.0%	353	0.6%

<sup>(a)</sup> An Affymetrix GeneChip® Human Genome U133 Plus 2.0 array contains 54675 probe sets

<sup>(b)</sup> MAS5 and RMA algorithms; two class unpaired (unlogged data); s0=50; R=1.5

# Supplementary table 13

## Coherence of changes in gene expression after the comparative analysis

		FDR = 0%		FDR < 1%	
simultaneous change in		selected probes <sup>(a)</sup>	% <sup>(b)</sup>	selected probes <sup>(a)</sup>	% <sup>(b)</sup>
4 samples	UP	47	0,09%	137	0,25%
	DOWN	89	0,16%	237	0,43%
3 samples	UP	149	0,27%	337	0,62%
	DOWN	124	0,23%	383	0,70%
2 samples	UP	334	0,61%	695	1,27%
	DOWN	203	0,37%	560	1,02%
1 sample	UP	1051	1,92%	2224	4,07%
	DOWN	714	1,31%	1726	3,16%
4 samples	2 UP + 2 DOWN	7	0,01%	18	0,03%
	3 UP + 1 DOWN	12	0,02%	34	0,06%
	1 UP + 3 DOWN	9	0,02%	47	0,09%
3 samples	2 UP + 1 DOWN	28	0,05%	85	0,16%
	1 UP + 2 DOWN	28	0,05%	105	0,19%
2 samples	1 UP + 1 DOWN	94	0,17%	252	0,46%

<sup>(a)</sup> SAM analysis (R=1,5)

<sup>(b)</sup> An Affymetrix GeneChip® Human Genome U133 Plus 2.0 array contains 54675 probe sets

Supplementary table 14

Coherence of changes in gene expression after the comparative analysis  
(expanded data)

sample 1	sample 3	sample 4	sample 5	# probes <sup>(a)</sup>	
				FDR<1%	FDR=0%
UP	UP	UP	UP	137	47
			DOWN	8	2
			-	59	32
		DOWN	UP	2	1
			DOWN	2	1
			-	6	1
		-	UP	77	28
			DOWN	6	2
			-	129	74
	DOWN	UP	UP	20	8
			DOWN	4	2
			-	17	6
		DOWN	UP	3	1
			DOWN	6	1
			-	8	2
		-	UP	17	3
			DOWN	7	2
			-	26	16
	-	UP	UP	148	75
			DOWN	5	1
			-	117	82
		DOWN	UP	2	0
			DOWN	5	0
			-	6	3
		-	UP	128	71
			DOWN	8	7
			-	335	235
DOWN	UP	UP	UP	4	1
			DOWN	4	0
			-	4	2
		DOWN	UP	3	0
			DOWN	27	5
			-	11	3
		-	UP	13	8
			DOWN	33	6
			-	41	18
	DOWN	UP	UP	2	3
			DOWN	5	0
			-	2	1
		DOWN	UP	9	3
			DOWN	237	89
			-	76	39
		-	UP	4	4
			DOWN	110	36
			-	118	58
	-	UP	UP	3	0
			DOWN	3	0
			-	7	0
		DOWN	UP	8	3
			DOWN	157	33
			-	100	32

		-	UP	8	3
			DOWN	120	47
			-	301	143
-	UP	UP	UP	53	14
			DOWN	3	2
			-	115	32
		DOWN	UP	4	1
			DOWN	18	4
			-	29	10
		-	UP	103	41
			DOWN	39	13
			-	1216	501
	DOWN	UP	UP	5	2
			DOWN	1	1
			-	25	7
		DOWN	UP	5	2
			DOWN	40	16
			-	58	26
		-	UP	44	14
			DOWN	107	25
			-	1026	456
	-	UP	UP	103	34
			DOWN	9	1
			-	344	134
		DOWN	UP	10	2
			DOWN	57	15
			-	167	60
		-	UP	329	181
			DOWN	232	55
			-	-	-

<sup>(a)</sup> selected probes by SAM analysis (R=1.5)

Supplementary table 15

Pathology's gene expression similar to CoQ<sub>10</sub> deficiency

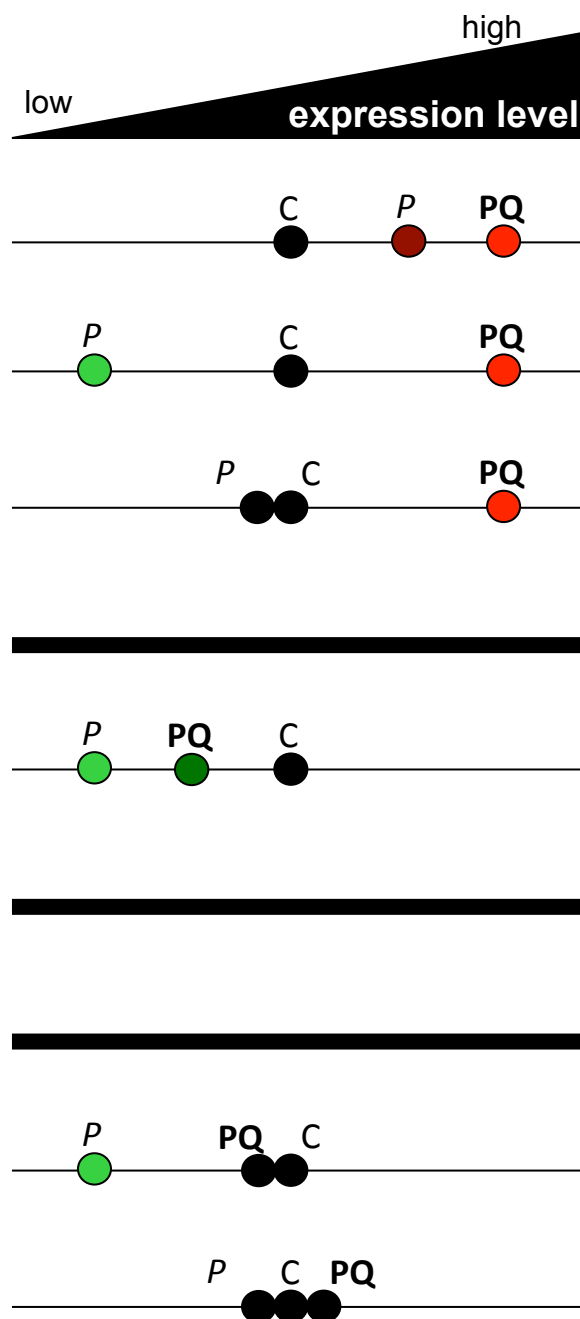
MeshTerm <sup>(a)</sup>	# genes <sup>(b)</sup>	# significant <sup>(c)</sup>	Z_Score <sup>(d)</sup>
<b>Digestive</b>			
COLITIS, ULCERATIVE	48	43	2,2
CELIAC DISEASE	19	18	1,9
DIARRHEA	9	6	1,8
<b>Embryo</b>			
FETAL MEMBRANES (PREMATURE RUPTURE)	12	12	2,7
<b>Heart</b>			
CAROTID ARTERY DISEASES	34	34	3,6
CORONARY RESTENOSIS	11	11	3,2
CORONARY DISEASE	104	100	3,2
VENTRICULAR DYSFUNCTION	9	9	2,4
VENTRICULAR HYPERTROPHY	20	20	1,9
<b>Immune</b>			
CHLAMYDIA INFECTIONS	8	8	3,2
<b>Kidney</b>			
KIDNEY FAILURE	38	37	2,5
<b>Liver</b>			
HEPATITIS C	18	17	4,5
LIVER CIRRHOSIS	30	29	2,5
<b>Neuronal</b>			
PHOBIC DISORDERS	3	3	1,7
SLEEP DISORDERS	3	3	1,7
BRAIN INFARCTION	9	9	1,8
PANIC DISORDER	11	11	1,8
MOOD DISORDERS	18	18	2,4
<b>Weight and Insulin-related</b>			
WEIGHT LOSS	13	13	2,4
DIABETES MELLITUS (TYPE 1)	100	98	1,7
<b>Lung</b>			
PULMONARY DISEASE	33	32	2,8
BRONCHIAL HYPERREACTIVITY	11	11	2,5
LUNG DISEASES (OBSTRUCTIVE)	3	3	1,7
<b>Epithelium</b>			
HYPERSENSITIVITY	32	32	2,5
RHINITIS	7	7	2,5
NASAL POLYPS	4	4	1,8
PSORIASIS	39	36	1,7
<b>Tumor</b>			
NEOPLASM INVASIVENESS	22	21	4,3
CARCINOMA (TRANSITIONAL CELL)	13	13	2,2
NEOPLASMS (SQUAMOUS CELL)	7	7	1,9
UTERINE CERVICAL NEOPLASMS	21	21	1,8
MELANOMA	23	22	1,7

<sup>(a)</sup> MeshTerm (MeSH) is the vocabulary of the National Library of Medicine used for indexing articles for MEDLINE/PubMed. MeSH terminology provides a consistent way to retrieve information.

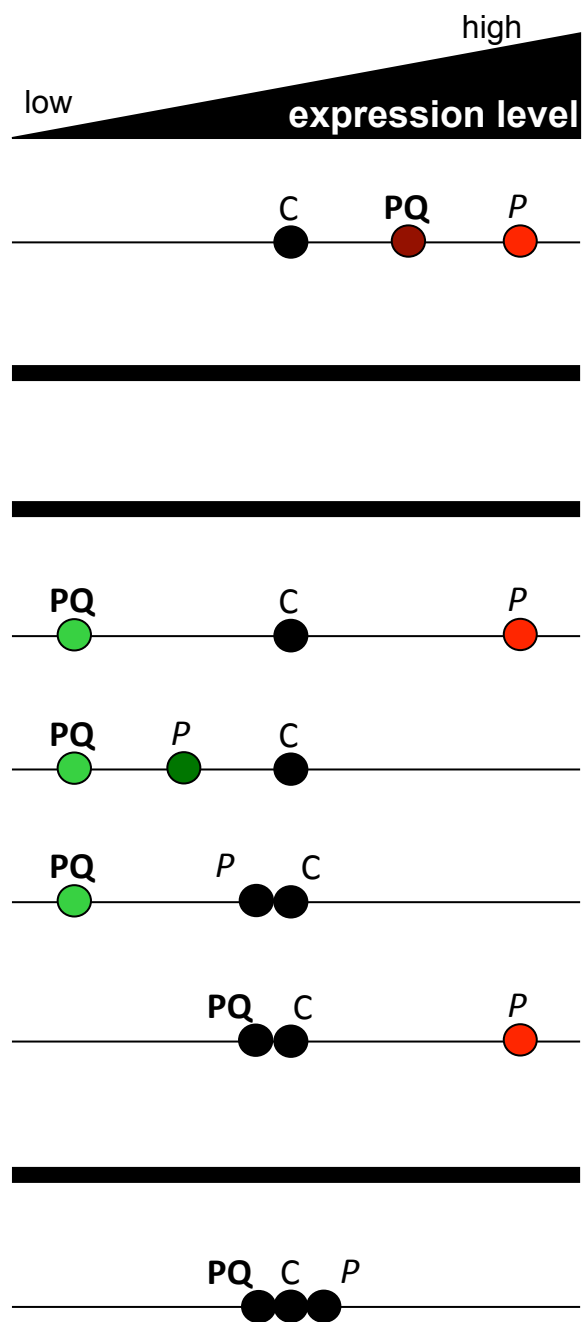
<sup>(b)</sup> number of genes regulated / specific markers of a disease given.

<sup>(c)</sup> number of genes regulated similarly in CoQ<sub>10</sub> deficiency.

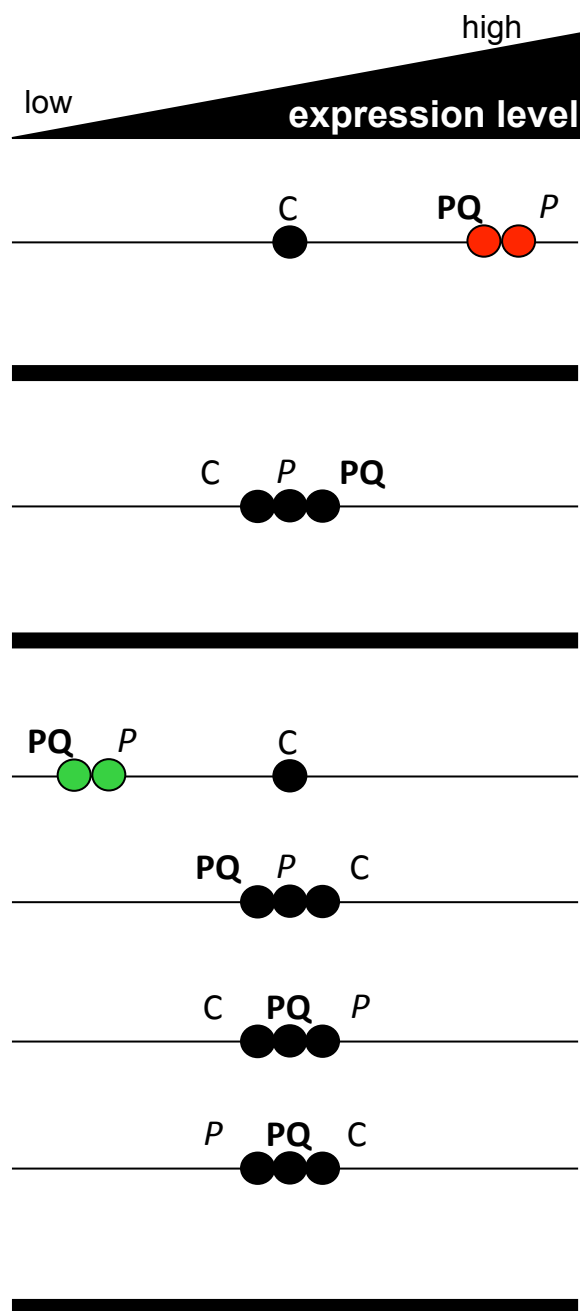
<sup>(d)</sup> Here are listed the 32th MeshTerm with Z-score higher than 1.5



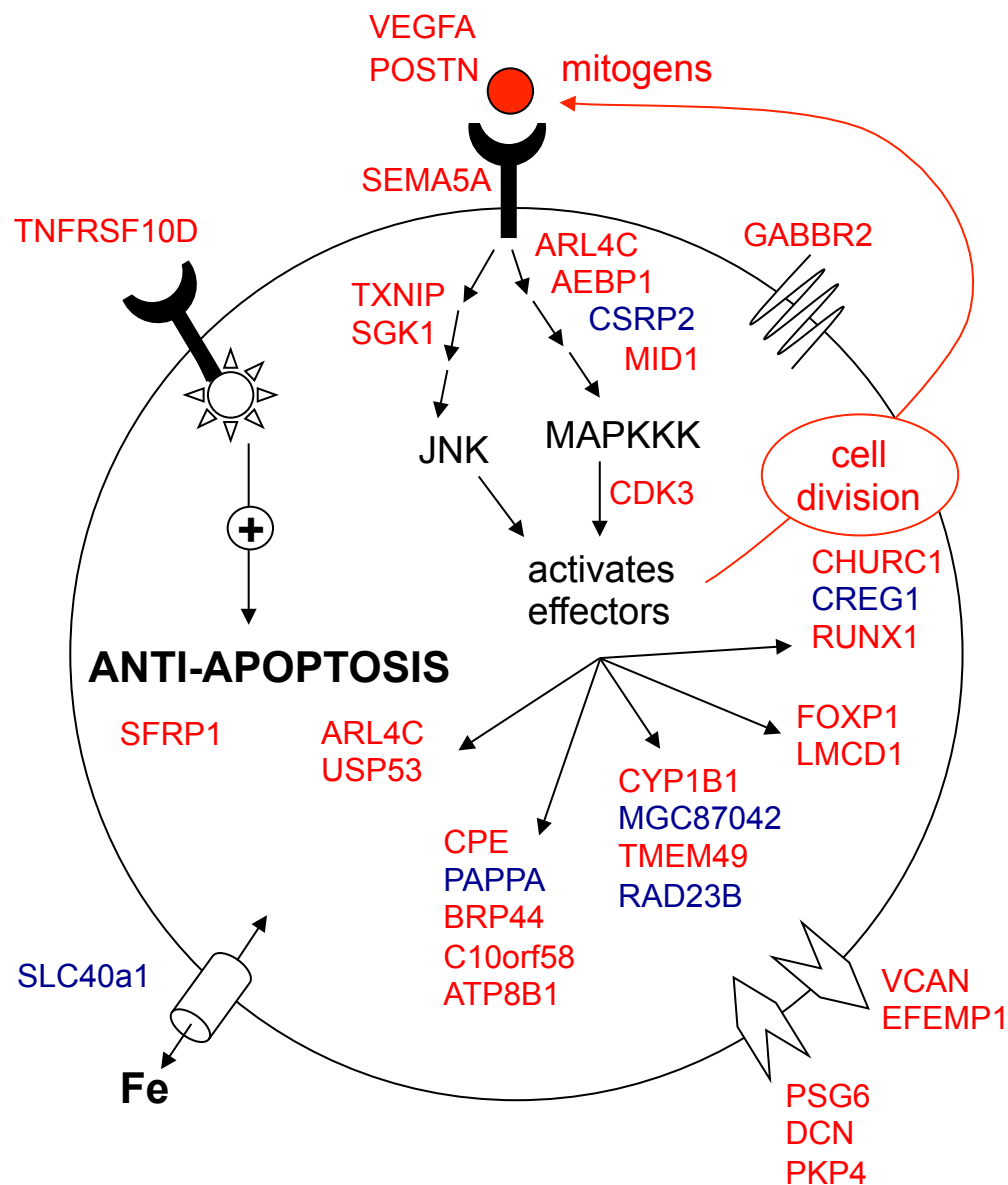
PQ-P	PQ-C	P-C	Effect of CoQ <sub>10</sub> supplementation on gene regulation in CoQ <sub>10</sub> deficiency
UP	UP	UP	<b>Unaffected genes by CoQ<sub>10</sub> (U)</b> CoQ <sub>10</sub> maintain up-regulated at higher levels
UP	UP	DOWN	<b>Opposite regulation (O)</b> CoQ <sub>10</sub> activates the repressed genes in CoQ <sub>10</sub> deficiency
UP	UP	-	<b>Regulated only after CoQ<sub>10</sub> treatment (S)</b> Genes specifically activated by CoQ <sub>10</sub> supplementation
UP	DOWN	UP	--- NOT POSSIBLE ---
UP	DOWN	DOWN	<b>Partial restored genes by CoQ<sub>10</sub> (pR)</b> CoQ <sub>10</sub> activates the repressed without reach control level
UP	DOWN	-	--- NOT POSSIBLE ---
UP	-	UP	--- NOT POSSIBLE ---
UP	-	DOWN	<b>CoQ<sub>10</sub> restores completely (R)</b> CoQ <sub>10</sub> activates the repressed to reach control level
UP	-	-	--- SMALL CHANGES (ONLY ONE COMPARATIVE ANALYSIS) ---



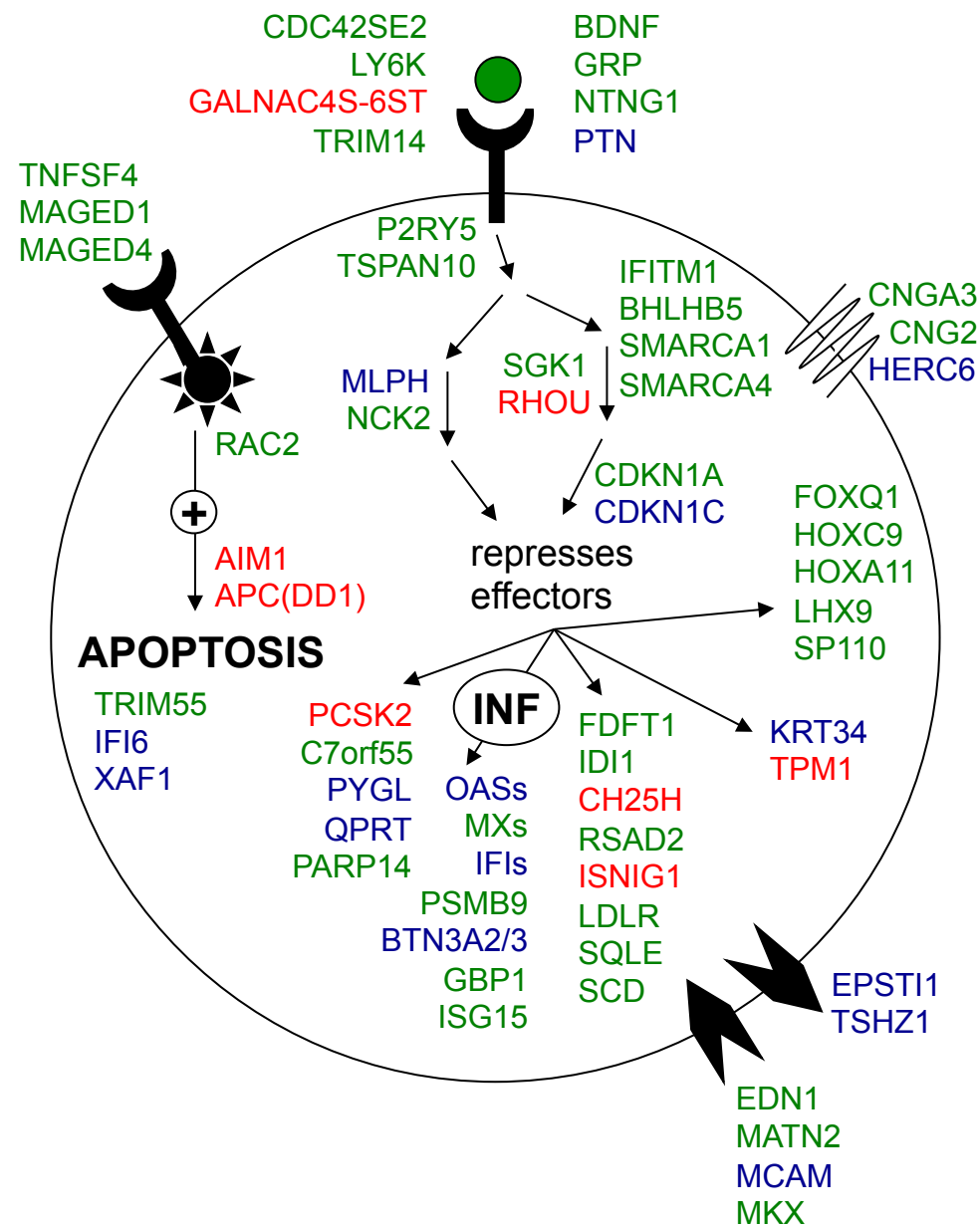
PQ-P	PQ-C	P-C	Effect of CoQ <sub>10</sub> supplementation on gene regulation in CoQ <sub>10</sub> deficiency
DOWN	UP	UP	<b>Partial restored genes by CoQ<sub>10</sub> (pR)</b> CoQ <sub>10</sub> represses the activated without reach control level
DOWN	UP	DOWN	--- NOT POSSIBLE ---
DOWN	UP	-	--- NOT POSSIBLE ---
DOWN	DOWN	UP	<b>Opposite regulation (O)</b> CoQ <sub>10</sub> represses the activated genes more than control
DOWN	DOWN	DOWN	<b>Unaffected genes by CoQ<sub>10</sub> (U)</b> CoQ <sub>10</sub> maintain down-regulated at higher levels
DOWN	DOWN	-	<b>Regulated only after CoQ<sub>10</sub> treatment (S)</b> Genes specifically repressed by CoQ <sub>10</sub> supplementation
DOWN	-	UP	<b>CoQ<sub>10</sub> restores completely (R)</b> CoQ <sub>10</sub> represses the activated to reach control level
DOWN	-	DOWN	--- NOT POSSIBLE ---
DOWN	-	-	--- SMALL CHANGES (ONLY ONE COMPARATIVE ANALYSIS) ---



PQ-P	PQ-C	P-C	Effect of CoQ <sub>10</sub> supplementation on gene regulation in CoQ <sub>10</sub> deficiency
-	UP	UP	<b>Unaffected genes by CoQ<sub>10</sub> (U)</b> CoQ <sub>10</sub> maintain up-regulated
-	UP	DOWN	--- NOT POSSIBLE ---
-	UP	-	--- SMALL CHANGES (ONLY ONE COMPARATIVE ANALYSIS) ---
-	DOWN	UP	--- NOT POSSIBLE ---
-	DOWN	DOWN	<b>Unaffected genes by CoQ<sub>10</sub> (U)</b> CoQ <sub>10</sub> maintain down-regulated
-	DOWN	-	--- SMALL CHANGES (ONLY ONE COMPARATIVE ANALYSIS) ---
-	-	UP	--- SMALL CHANGES (ONLY ONE COMPARATIVE ANALYSIS) ---
-	-	DOWN	--- SMALL CHANGES (ONLY ONE COMPARATIVE ANALYSIS) ---
-	-	-	--- NOT SELECTED FOR EXPRESSION ANALYSIS ---



## gene activation in CoQ<sub>10</sub> deficiency



## gene repression in CoQ<sub>10</sub> deficiency