SUPPLEMENTAL DATA

Supplemental Material and Methods

Generation of *Mclk1* knockout MEFs expressing either wild-type or mutant MCLK1

Full-length wild-type MCLK1 cDNA was generated by PCR amplification using wild type C57BL/6 (B6) mouse embryo cDNA as a template. The primers containing added *Bam*HI and *EcoRI* sites, 5'-CGATGA GGATCCACCATGAGCGCCGCCGGAGCCATA-3' and 5'-TGTGCTGAATTCCTAAAACCTTTCTGATAAATATATGGCTGC-3', were used to perform PCR. To construct pBabe-hygro-MCLK1, the resulting PCR products were double-digested with *Bam*HI and *EcoRI* (Invitrogen) and ligated into the *Bam*HI/EcoRI site of the retroviral vector pBabe-hygro (obtained from Addgene). pBabe-hygro-MCLK1 (E178K) construct which contains a single point mutation (a G-to-A transition) at codon 178 was generated by performing site-direct mutagenesis on pBabe-hygro-MCLK1, using a QuikChange XL site-directed mutagenesis kit (Stratagene) according to the manufacturer's directions. Constructs were verified by sequencing. The procedure for introducing pBabe-hygro, pBabe-hygro-MCLK1 or pBabe-hygro-MCLK1 (E178K) DNA into $Mclk1^{\Delta/\Delta}$-*Cre* MEFs (described in the main text) was the same as described for retroviral delivery of Cre gene into $Mclk1^{loxP/loxP}$ MEFs. Cells infected with the pBabe-hygro vectors were selected by hygromycin (200 μg/ml) for 2 days.

Measurement of cellular ATP levels

MEFs were rinsed twice with ice cold PBS and intracellular ATP was extracted in 0.5% trichloroacetic acid. The supernatants obtained following a 5-min spin at 13,000 rpm were diluted 50 times in HEPES buffer (25 mM, pH 7.75) for bioluminescent ATP content measurement using Promega ENLITEN®rLuciferase/Luciferin reagent as instructed by the manufacturer. The pellet was dissolved in 0.5 N sodium hydroxide for total protein measurement using the Bradford Protein Assay (Bio-Rad Laboratories, Inc.). For treatment with oligomcyin, cells were treated with 10 μg/ml oligomycin for 2 h and then processed for measurement of ATP content.

Exposure of cells to 2-deoxyglucose

MEFs were seeded in 48-well plates overnight and then incubated with different concentrations of 2-deoxyglucose (2-DG). 48 h later, cell viability was quantitatively determined by alamarBlue Cell Viability Assay (Invitrogen).

Determination of aconitase activity

Aconitase activity in liver mitochondria was measured spectrophotometrically as described previously.[1]

Supplemental Figures

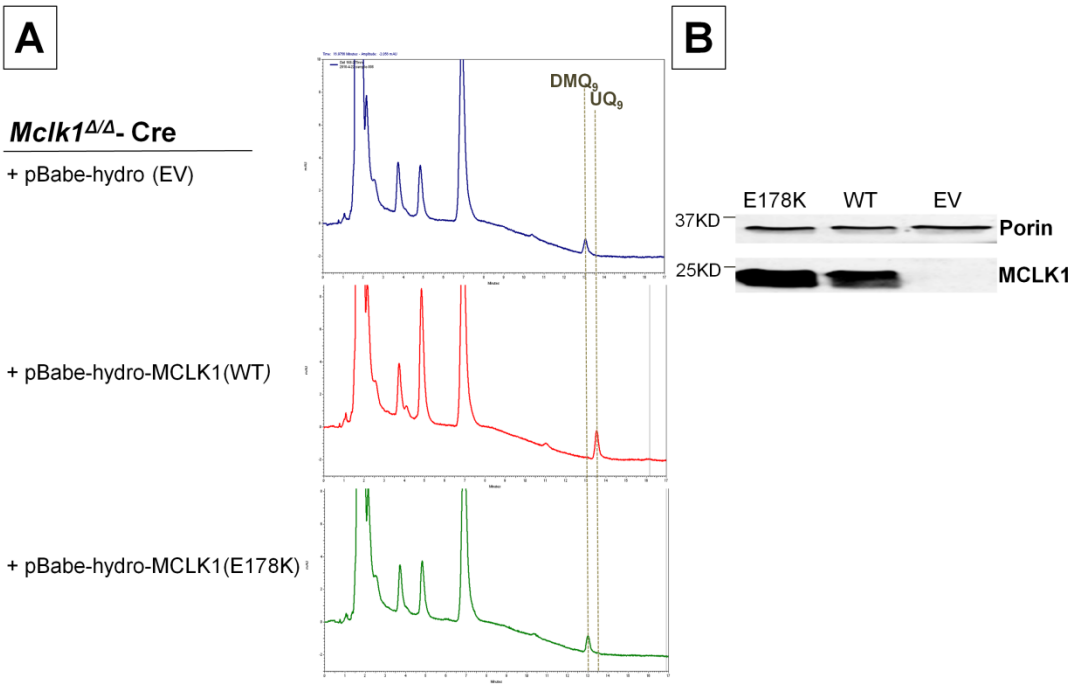

Figure S1. Restoration of UQ biosynthesis in *Mclk1* knockout MEFs by re-expression of MCLK1.

(A) *Mclk1*$^{\Delta/\Delta}$-Cre MEFs infected with empty vector (EV) or pBabe-hydro retroviral vectors expressing either wild-type MCLK1 (WT) or the MCLK1 E178K mutant. MCLK1 (E178K) contains a point mutation equivalent to the Glu-to-Lys substitution in the worm *clk-1(e2519)* allele. UQ_9 synthesis is restored only in the *Mclk1*$^{\Delta/\Delta}$-Cre MEFs infected with the retroviral vector expressing wild-type MCLK1.

(B) Western blotting of MCLK1 in *Mclk1*$^{\Delta/\Delta}$-Cre MEFs infected with retroviruses encoding empty vector (EV), wild-type MCLK1 (WT) or the MCLK1 (E178K) mutant. MCLK1 expression is restored in the cells infected with the retroviral vector encoding wild-type MCLK1. Cells infected with pBabe-hydro-MCLK1 (E178K) produce a nonfunctional mutant MCLK1 protein. The outer mitochondrial membrane porin served as a loading control.

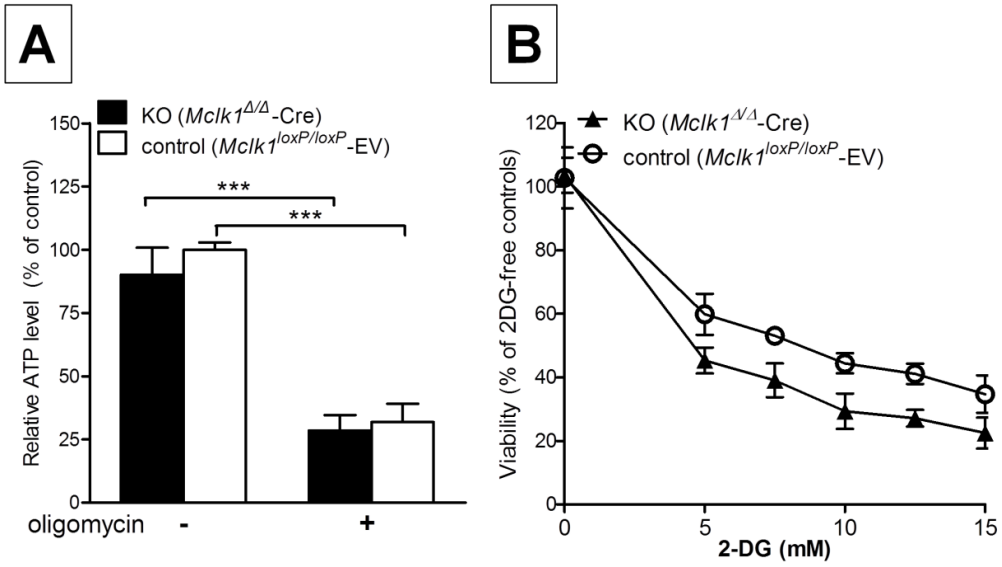

Figure S2. ATP levels and sensitivity to glycolytic inhibition.

(A) Intracellular ATP content before and after oligomycin. Cellular ATP level was measured with or without exposure to oligomycin (10 μg/ml) for 2 h. Results were normalized to cellular protein concentration and reported as a percentage of the level in control cells not treated with oligomycin. ATP content in whole cell lysates is not different between *Mclk1* knockout MEFs (*Mclk1*$^{\Delta/\Delta}$-Cre) and controls (the same MEF lines infected with empty retroviral vector, *Mclk1*$^{loxP/loxP}$-EV). Data are expressed as mean ± SEM obtained from 3 independent MEF lines. A paired *t* test was performed to identify statistically significant differences. *** significance of difference at $p<0.001$.

(B) Survival percentages of *Mclk1* knockout (*Mclk1*$^{\Delta/\Delta}$-Cre) and control MEFs (*Mclk1*$^{loxP/loxP}$-EV) after exposure to glycolytic inhibition by 2-deoxyl-D-glucose (2-DG). Cells were treated with the indicated concentrations of 2-DG for over 48 h. Survival was then measured using alamarBlue Cell Viability Assay. Data are expressed as percent cell survival relative to that of untreated (mean ± SD from 3 replicate wells). The measurement was repeated with three matched pairs of MEFs lines, and a representative result is shown. *Mclk1* knockout MEFs showed a higher sensitivity to 2-DG-induced toxicity than controls ($p<0.001$, two-way ANOVA, the effect of 2-DG vs. genotype comparison).

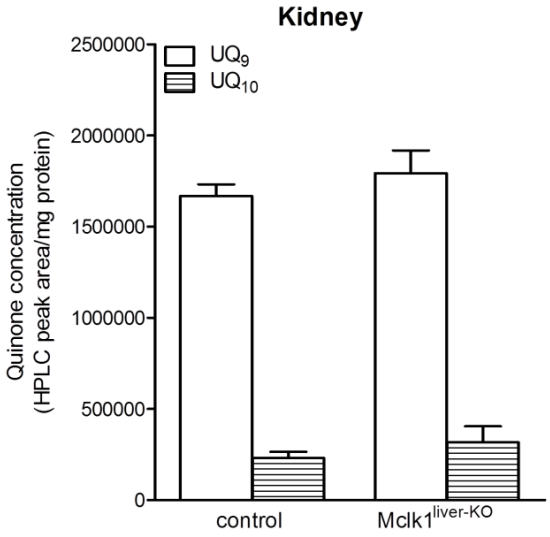

Figure S3. UQ content is not changed in the kidney of *Mclk1*$^{liver\text{-}KO}$ mice.
Quinones were extracted from the kidney of 15-month-old female *Mclk1*$^{liver\text{-}KO}$ mice (*Mclk1*$^{loxP/-}$, *AlbCre+*) or their control littermates (*Mclk1*$^{loxP/-}$). No difference in kidney ubiquinone content was found between the two groups of mice. Columns represent the mean ± SEM of peak areas for UQs on HPLC chromatographs normalized to total protein amount. DMQ was not detected in the kidney of *Mclk1*$^{liver\text{-}KO}$ or control mice. n = 5 mice per group.

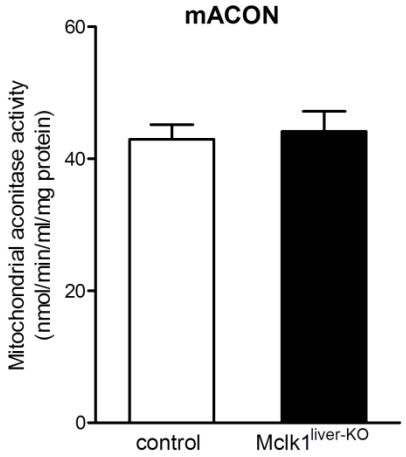

Figure S4. Mitochondrial aconitase activity in *Mclk1* knockout liver.
Aconitase activity in the liver mitochondria of 8-month-old female *Mclk1*$^{liver\text{-}KO}$ mice (*Mclk1*$^{loxP/-}$, *AlbCre+)* is not different from that of *Mclk1*$^{loxP/-}$ littermate controls. The columns are the means ± SEM for 9 mice per group. A paired *t* test was used to determine statistical significance of the difference between *Mclk1* knockout livers and controls.

Table S1. Respiratory parameters of isolated liver mitochondria. All measurements were done on liver mitochondria isolated from 15-month-old *Mclk1*$^{liver\text{-}KO}$ mice (*Mclk1*$^{lox/-}$,*AlbCre*+) or their littermate controls (*Mclk1*$^{lox/-}$). State 3 respiration rate was recorded with the addition of ADP. After exhaustion of ADP, state 4 respiration rate was determined. RCR (Respiratory Control Ratio) was calculated as the ratio of state 3 over state 4. The ADP/O ratio is the ratio of nmoles of ADP phosphorylated to nmoles of oxygen consumed. Values are mean ± SEM (n = 8 mice per group). A paired *t* test was used to determine statistical significance of the difference between the knockout livers and controls. *p<0.05;*** p<0.001. With either Complex I (glutamate plus malate) or Complex II (succinate plus rotenone) substrates, state 3 respiration rate is significantly lower in the mitochondria from *Mclk1* knockout livers compared to controls, whereas no difference is observed for state 4 respiration. RCR in *Mclk1* knockout liver mitochondria tends to be reduced but not significantly. ADP/O ratio is similar in liver mitochondria from *Mclk1*$^{liver\text{-}KO}$ or control mice.

Substrates	State 3	State 4	RCR	ADP/O
Glutamate + Malate				
Liver KO	131.7 ± 5.6*	30.6 ± 1.7	5.2 ± 0.2	2.5 ± 0.1
Control	150.6 ± 5.3	30.1 ± 1.7	5.6 ± 0.2	2.4 ± 0.1
Succinate + Rotenone				
Liver KO	221.5 ± 11.1***	49.2 ± 5.0	4.3 ± 0.2	1.4 ± 0.1
Control	267.0 ± 8.2	53.6 ± 3.1	4.9 ± 0.2	1.5 ± 0.0

Supplemental References

1. Lapointe, J., Wang, Y., Bigras, E., and Hekimi, S. (2012). The submitochondrial distribution of ubiquinone affects respiration in long-lived Mclk1+/- mice. J Cell Biol 199, 215-224.