

## **Mitochondrial Complex I activity signals antioxidant response through ERK5**

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## Patients samples with mitochondrial diseases

Gene	Description	Number
WFS1	Wolfram syndrome	01
FXN	Friedreich's ataxia	01
OPA3	Optic Atrophy 3	01
mtDNA	Leber's hereditary optic neuropathy	01
RTN4IP1	Optic Atrophy-10 with or without ataxia, mental retardation and seizures	01
NDUFA13	Hypotonia, Dyskinesia and sensorial Deficiencies	01
Unidentified	Unidentified mito disease	01
OPA1	Optic Atrophy 1	01
	Healthy	08

**Supplemental Table 1.** List of patients suffering from various mitochondrial diseases.

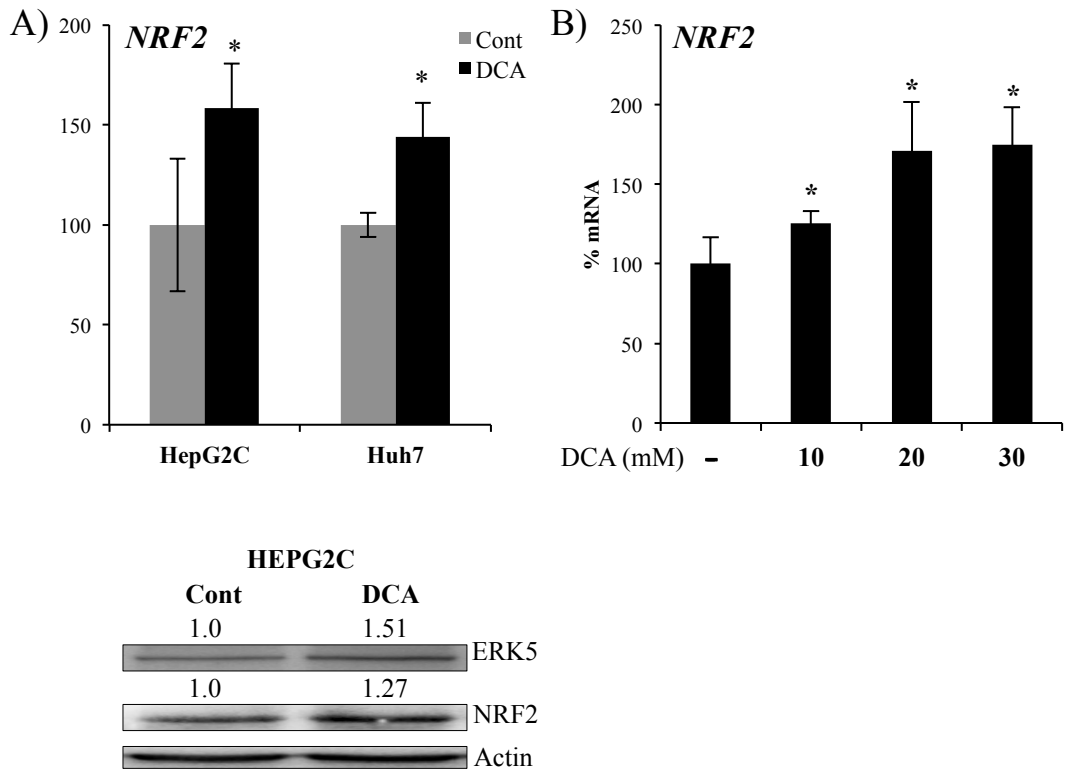
Name	Nuclear Background	mtDNA Background	Mutation	Oxphos Complex
L929	L929 (C3H/An)	L929 (C3H/An)	Wt	N/A
L929-Rho0	L929 (C3H/An)	no mtDNA	no mtDNA	No mETC
E9	L929 (C3H/An)	L929 (C3H/An)	mt-COXI (T6589C)	Complex IV
A22	L929 (C3H/An)	L929 (C3H/An)	mt-Cytb (G15263A)	Complex III
FL929	L929 (C3H/An)	L929 (C3H/An)	Wt	N/A
FG-23.1	L929 (C3H/An)	L929 (C3H/An)	mt-ND6 (iC13887)	Complex I
FBalbc/J	L929 (C3H/An)	Balb/cJ	WT	N/A
mB77	L929 (C3H/An)	Balb/cJ	mt-tRNA Ile (G3739GA)	tRNA Ile, more ROS
Bc-TMP-II	L929 (C3H/An)	Balb/cJ	mt-ATP6 (A8414G)	Complex V
FBalb/cJ- ShFp	L929 (C3H/An)	Balb/cJ	ShRNA-FpSDH	Complex II
FMI.12	L929 (C3H/An)	NIH.3T3	mt-ND4 (delA10227)	Complex I
FMI.5	L929 (C3H/An)	NIH.3T3	mt-ND6 (delC13887)	Complex I
F3T3.6	L929 (C3H/An)	NIH.3T3	Wt	N/A

**Supplemental Table 2.** Mice fibroblasts with different mitochondrial mutations.

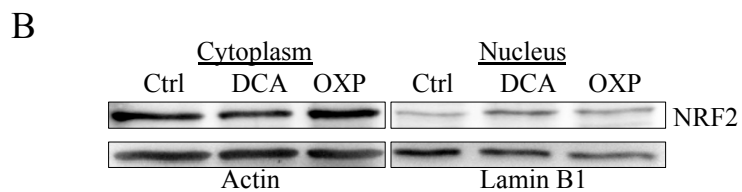
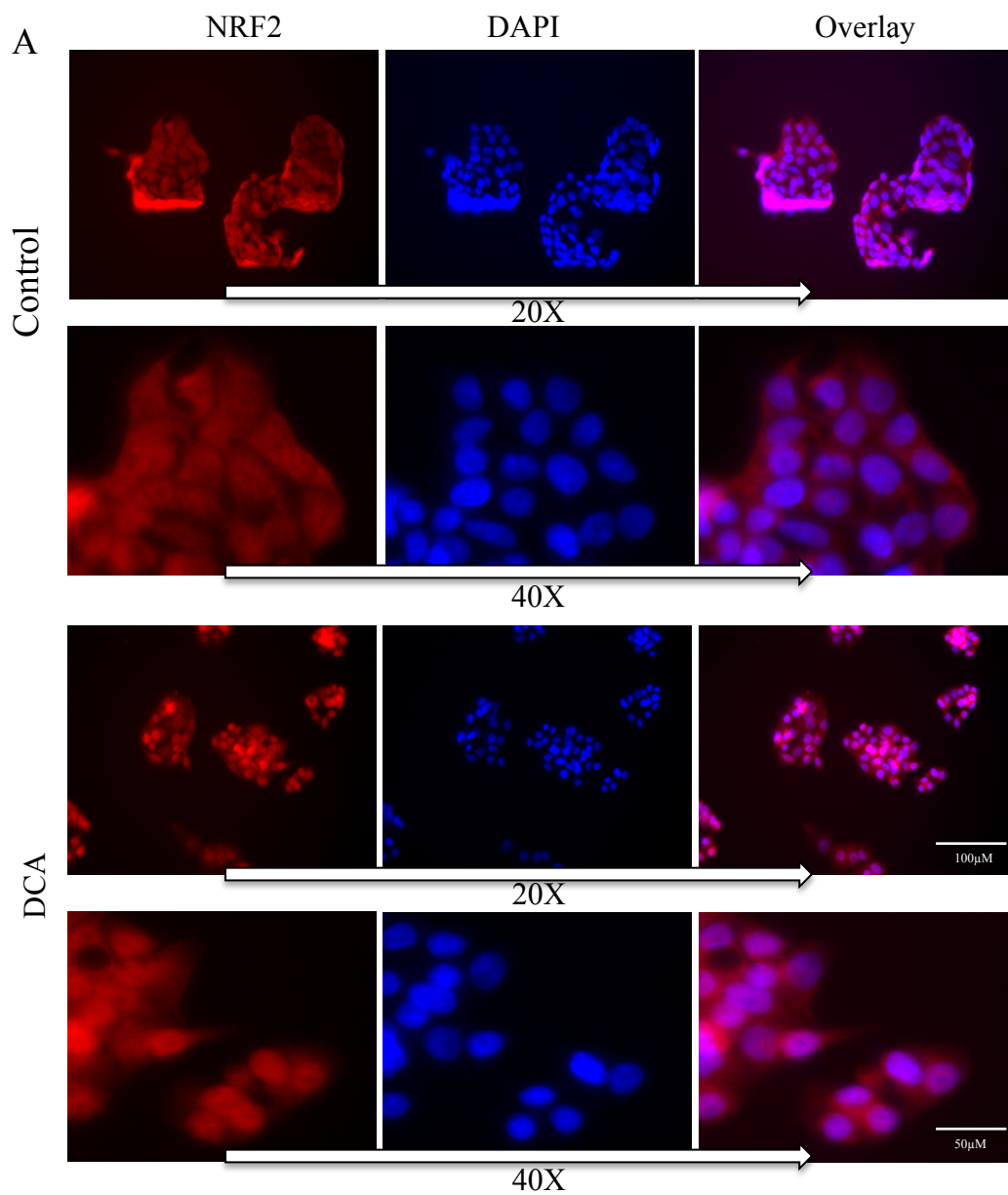
## List of Primers

<b><i>ERK5</i> (H):</b>	Forward: CGCTACTTCCTGTACCAACTGC Reverse: AGCCATACCAAAGTCACCAATC
<b><i>NRF2</i> (H):</b>	Forward: AAA CCA CCC TGA AAG CAC AG Reverse: AGT GTT CTG GTG ATG CCA CA
<b><i>HO-1</i> (H):</b>	Forward: ACA AGG AGA GCC CAG TCT TC Reverse: AGA CAG GTC ACC CAG GTA GC
<b><i>NQO-1</i> (H):</b>	Forward: CCT CTA TGC CAT GAA CTT Reverse: TAT AAG CCA GAA CAG ACTC
<b><i>ACTIN</i> (H):</b>	Forward: GAGGGAAATCGTGCGTGACA Reverse: AATAGTGATGACCTGGCCGT
<b><i>Erk5</i> (M):</b>	Forward: TATCATGGCCATCAAGGACA Reverse: AGCGGCTGTGAAGAGTGAAT
<b><i>Nrf2</i> (M):</b>	Forward: CCATTACGGAGACCCACCGCCTG Reverse: CTCGTGTGAGATGAGCCTCTAAGCGG
<b><i>Nqo-1</i> (M):</b>	Forward: GGCATCCTGCGTTTCTGTG Reverse: GGTTTCCAGACGTTTCTTCCAT

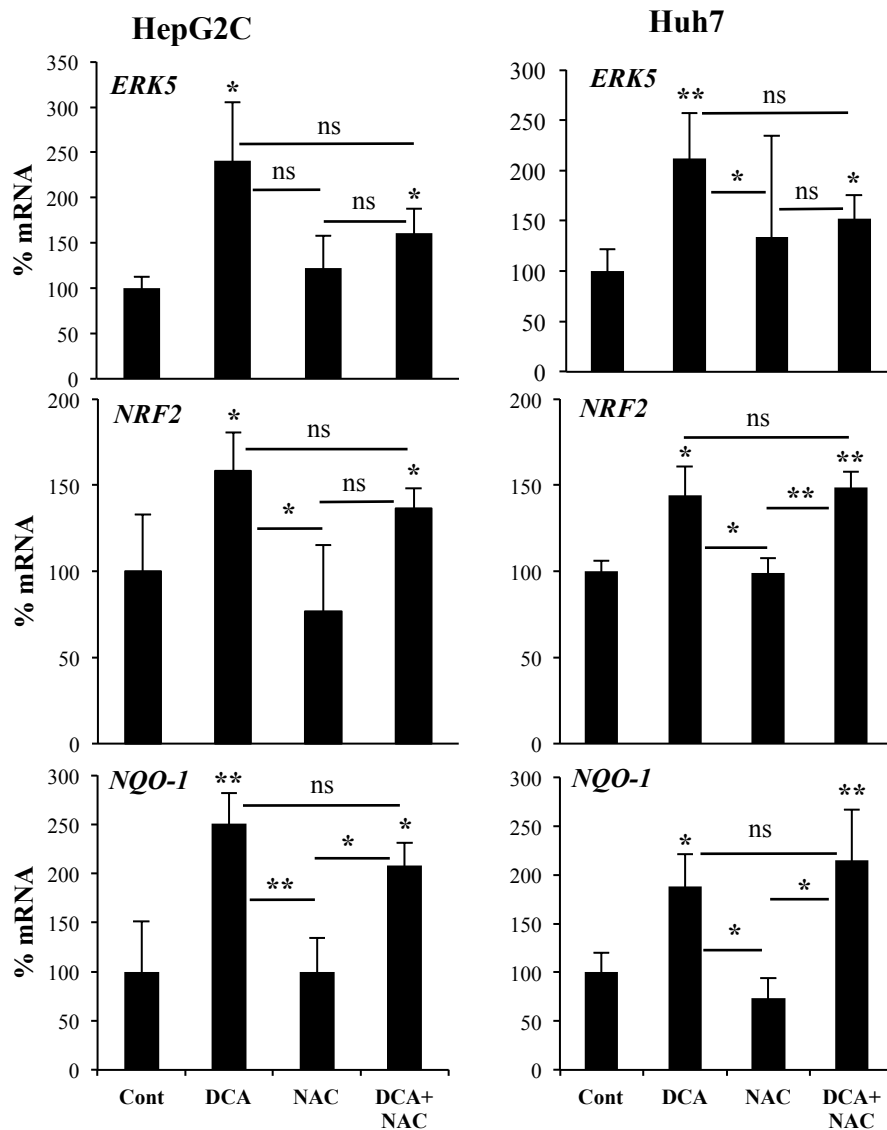
**Supplemental Table 3.** Primers Used in this study



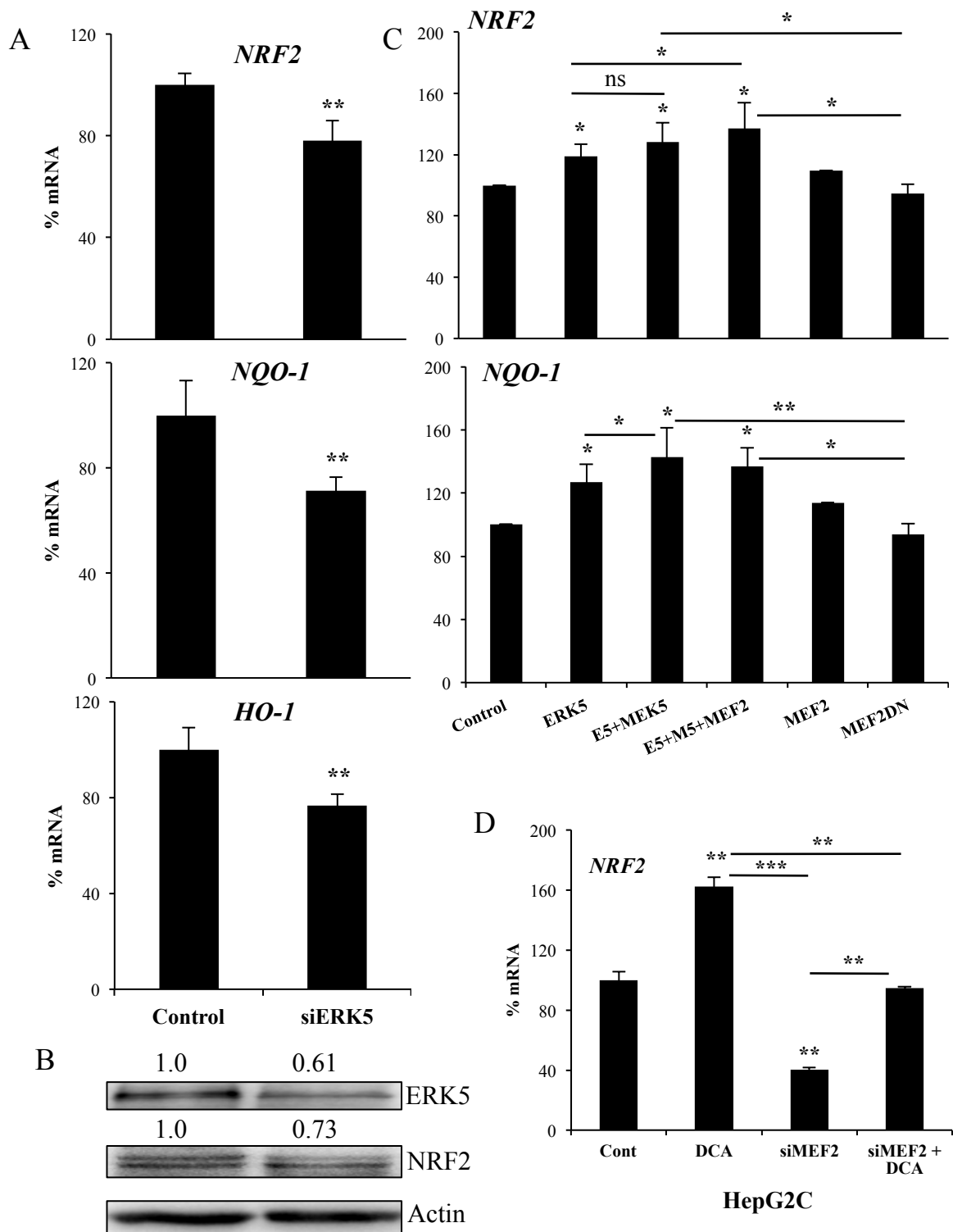
**Supplemental Fig. 1.** A) Hepatic Cells were treated with 10 mM DCA for 24h and *NRF2* mRNA (upper) and protein (lower) were measured. The ratio ERK5/actin and NRF2/actin is depicted in the figure. Results represent the means  $\pm$  SD of 3 independent experiments performed in triplicate. B) Tumor cells from hematological cancer patients (2 MM, 1 B-CLL and 1 T Lymphoma) were treated with various concentration of DCA for 24 h and *NRF2* mRNA was analyzed. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.005$  student t-test compare to non-treated cells.



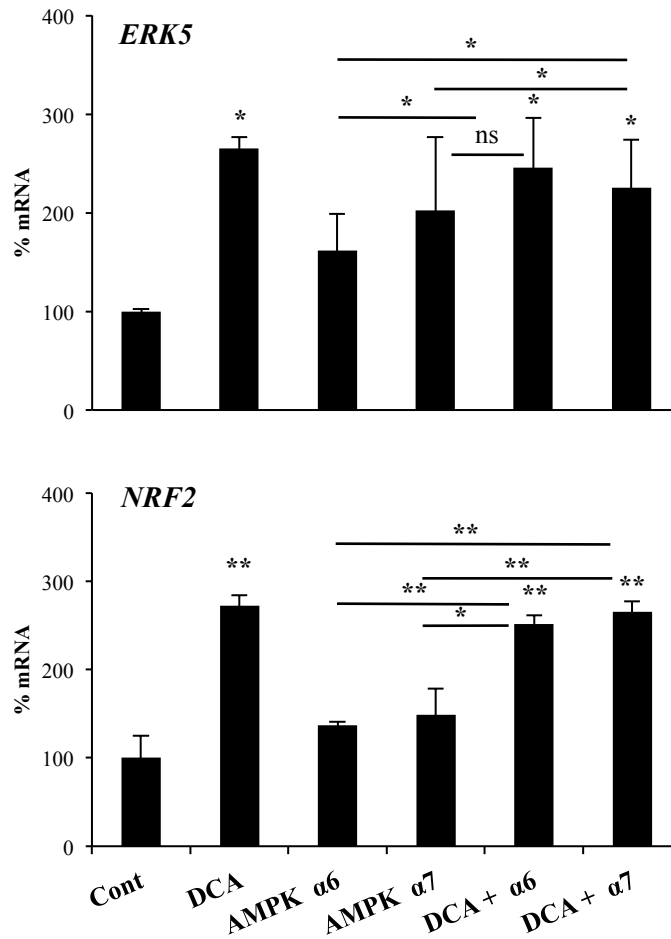
**Supplemental Fig. 2.** OXPHOS induced NRF2 translocation into the nucleus. A) HepG2C cells were treated with 10 mM DCA for 48 h and nuclear translocation was revealed by immunofluorescence. B) Jurkat cells were treated with 10 mM DCA for 48h or kept in OXPHOS for 1 week and NRF2 nuclear translocation was revealed by subcellular fractionation and WB.



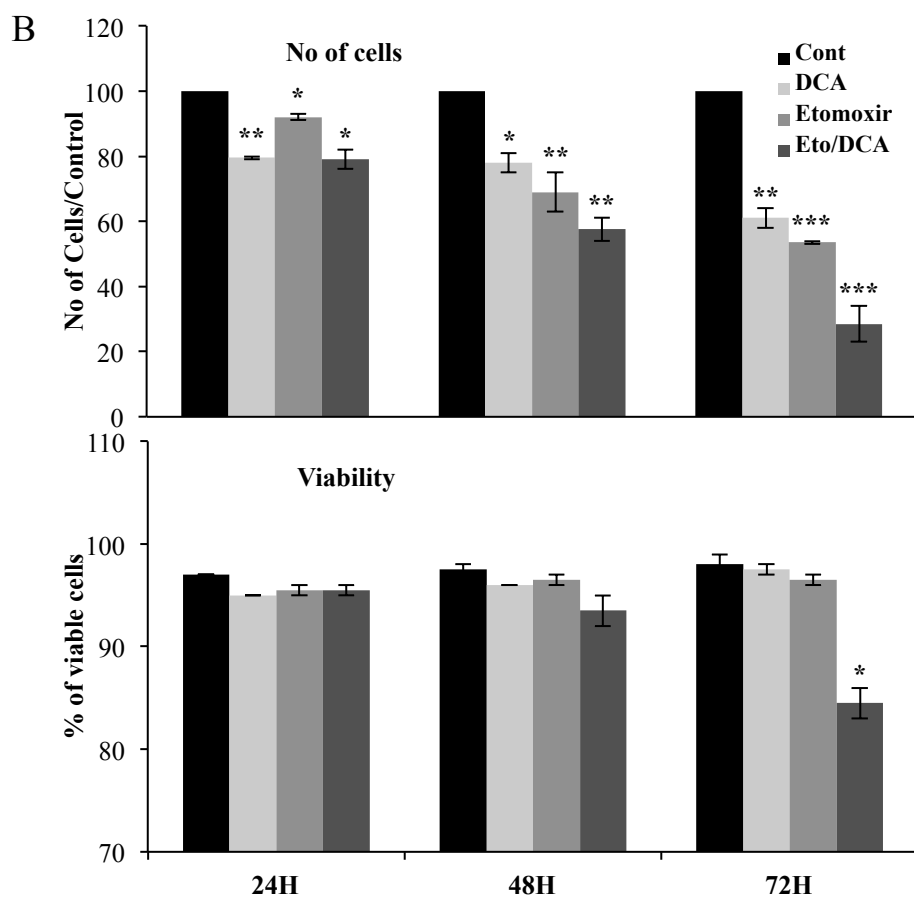
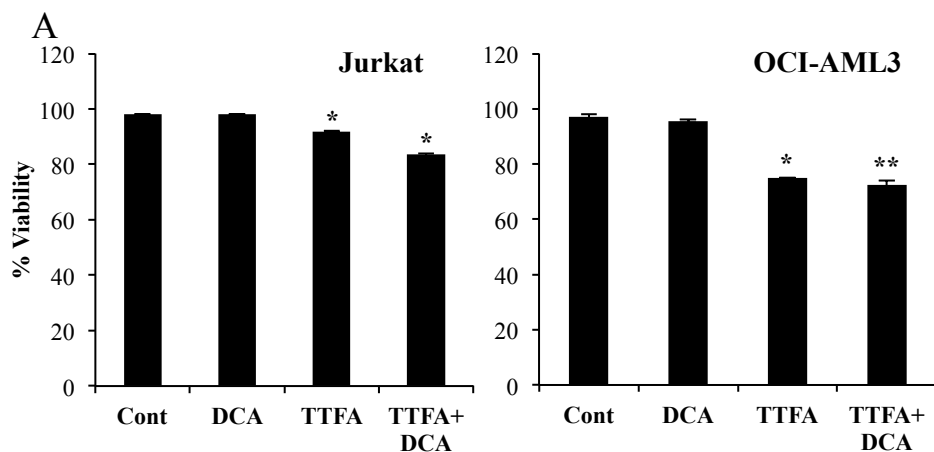
**Supplemental Fig. 3. A)** Jurkat and HepG2 cell lines were treated with 2 mM NAC 1 h before adding DCA (10 mM) for 24 h. mRNA was analyzed as described in Fig. 1. Results represent the means  $\pm$  SD of 3 independent experiments performed in triplicate; statistics were performed using One-way ANOVA with post-hoc Tukey test; \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.005$ . Treatments were compared to empty vector transfected cells (control) if not specified in the graph.



**Supplemental Fig. 4.** A) Huh7 cells were transfected with control siRNA or siRNA for ERK5. 72 h later *NRF2*, *NQO-1* and *HO-1* mRNA were analyzed. B) HepG2C cells were similarly transfected and ERK5 and NRF2 protein were analyzed. Numbers represent the ratio ERK5/actin. C)  $10^7$  Jurkat-TAG cells were co-transfected with 5  $\mu$ g of the following vectors ERK5 wild type, a constitutively active MEK5 mutant (MEK5D), MEF2C and MEF2C with dominant negative function (MEF2-DN). 72 h after *NRF2* and *NQO-1* mRNA were analyzed. D) HepG2C cells were transfected with siMEF2 and 24h later were treated with DCA 72h later *NRF2* mRNA were analyzed. Data represent means  $\pm$  SD; \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.005$  student t-test compare to empty vector transfected cells (control).



**Supplemental Fig. 5.** HCT116 cells were transfected with 2 small interference RNA (siRNA) for AMPK $\alpha$  or with control siRNA and treated with 20 mM DCA for 6 h before mRNA analysis. Data represent means  $\pm$  SD; statistics were performed using One-way ANOVA with post-hoc Tukey test; \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.005$ . Treatments were compared to empty vector transfected cells (control) if not specified in the graph.



**Supplemental Fig. 6.** A) Jurkat and OCI-AML3 cells were treated with 10 mM DCA and/or 300  $\mu$ M TTFA for 24 h and viability was quantified. B) OCI-AML3 cells were treated with 5 mM DCA and/or 100  $\mu$ M etomoxir for the indicated times and cell number (upper) and viability (lower) were calculated.