**Supplementary Material**

*Calcium measured with aequorin:*

Non quantitative $[\text{Ca}^{2+}]_{\text{cyt}}$ and $[\text{Ca}^{2+}]_{\text{mt}}$ calcium concentrations were determined in living HEK 293 cells using the photoprotein aequorin. Briefly, HEK 293 were transiently transfected with expression vectors encoding apoaequorin targeted to the cytoplasm (cytAEQ/pcDNA1) or the mitochondria (mtAEQ/pcDNA1) (Molecular Probes). After 24 h, inhibitors were added for 6h before the assay and the aequorin complex was reconstituted by the addition of 2.5 µM coelenterazine for 5 h. Cells were then harvested and resuspended in 100 µl HBSS containing 1 mM CaCl$_2$ and light emission was quantitated in a luminometer.

*In vitro kinase assay:*

L929, mtDNA-depleted L929 and 293 cells treated or not with mitochondrial inhibitors were lysed and endogenous CaMKs were immunoprecipitated with 10 µg of an anti-CaMKII or anti-CaMKIV antibody (Santa Cruz) for 2 h at 4°C. Immobilized immune complexes were washed three times in lysis buffer and twice in kinase reaction buffer (20 mM Tris-HCl, pH 7.5, 0.5 mM DTT, 0.1 % Tween-20, 8 mM MgCl$_2$, 1 mM CaCl$_2$, 1 µM CaM). The immunoprecipitates were resuspended in 40 µl of kinase reaction buffer containing 3 µg of CREB phosphopeptide(NEB) in the presence of 20 µM of ATP and 10 µCi [$\gamma$-$^{32}$P]ATP (NEN) for 30 min at 30 °C. The reaction was stopped by adding 25 µl on a phosphocellulose membrane spinzyme system (Pierce) and washed twice with 500 µl of phosphoric acid (75 mM). Phosphate incorporation was quantitated in a liquid scintillation counter. Amounts of immunoprecipitated kinases were controlled by Western blotting analyses.
Plasmids, reagents and cell lines

The Jun2TRE construct (kindly provided by S. Lewis and S. Hyman) and the c-myc construct (kindly provided by R.N. Eisenman) contain consensus DNA-binding sites followed by either a minimal thymidine kinase or Rous sarcoma virus promoter directing the expression of luciferase. The c-fos construct (generously given by Prof. Bender) and the cAMP-responsive element construct provided by K. Mayo contain promoter regions of c-fos promoter and α-inhibin, respectively. The MMCP-6 construct contains 3 consensus binding sequences of the mouse mast cell protease-6 promoter for transcription factors belonging to the bHLH-Zip family members (a gift from E. Morii. Molecular reagents were from Promega and control vectors were either pGL2, pGL3 or pcDNA3.

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Figure I: effect of EB-treatment on mitochondrial respiration. Mitochondrial respiration was measured *in situ* on L929 and mtDNA-depleted cells after digitonin permeabilization. Cell rate of oxygen consumption by mitochondria from cells resuspended in DHG at $2 \times 10^6$ cells/ml was assayed by an oxypolarographic method using a Clark-type electrode and the respiratory control ratio (RCR) was calculated from States 3 and 4 of the respiration ($n=4$).
Figure II: PKA activity was measured in cell homogenates of mtDNA-depleted or L929 cells treated or not with 100 μM of db-cAMP for 15, 30 or 60 min using a colorimetric SpinZyme PKA Assay Kit (Pierce) and a synthetic peptide (Kemptide, LRRASLG) as an exogenous substrate. As controls, effect of EB addition (+EB) or removal (-EB) was tested on L929 and mtDNA-depleted L929 cells for 24 h before PKA activity was determined. Phosphate incorporation was quantitated following manufacturer’s instructions and the results are expressed as O.D. at 570 nm/mg of proteins (n=3).
Figure III: (A) HEK 293 cells were transiently transfected with cytAEQ/pcDNA1 or mtAEQ/pcDNA1 expression vectors encoding HA-tagged apoaequorin targeted to the cytoplasm (a,b) or to the mitochondria (c,d). Expression of both proteins (green) was visualized by confocal microscopy after nuclei staining with propidium iodide (red). 24 h post-transfection, cells were treated for 6 h with 10 µM FCCP, 1 µM antimycin A or 8 µM oligomycin while aequorin was reconstituted with 2.5 µM coelenterazine. Metabolic inhibitions all resulted in a statistically significant increased RLU signals from cells transfected with cytAEQ/pcDNA1 (B) that are concomitant with decreased values for the mitochondrial targeted aequorin (C). Apoaequorin expression controlled by Western blot analysis (Bb,Cb) using an anti-HA antibody. These data suggest that the increase in the [Ca^{2+}]_cyt is accompanied by a decrease in the mitochondrial calcium pool.
Figure IV: (A) CaMKIV activity in 293 cells treated with 8 µM oligomycin, 1 µM antimycin A and 10 µM FCCP for 6 h followed by 16 h of recovery. CaMKIV was immunoprecipitated (IP) from cleared cell lysates, incubated with a synthetic CREB peptide in the presence of $[^{32}P]ATP$. Incorporated radioactivity was counted in a scintillation counter and expressed in cpm (a) and the amount of immunoprecipitated kinase in the different conditions was controlled by Western blotting analysis (b).