Supplementary Methods

Primers used for sAC mt-sAC amplification:

Forward: 5'-3' CGCACCGGTATGAGTGCCCGAAGGCAG (*AgeI* restriction site underlined);

Reverse: 5'-3'CGCGCGGCCGCCTA**AGCGTAGTCTGGGACG**

TCGTATGGGTAGACTTTCTCATTGAGGC (*NotI* restriction site underlined, HA sequence in bold).

Primers used in RT-PCR for mtDNA quantification:

COXI Forward: 5'-3'CTAACAGACCGCAACCT

COXI Reverse: 5'-3'GTGGCGAGTCAGCTAA

28sRNA Forward: 5'-3' ATCCTTCGATGTCGGC

28sRNA Reverse: 5'-3' AGCACATACACCAAATGTCT

Primers used in RT-PCR to quantify mRNA expression:

PGC1α Forward: 5'-3' GGCACGCAATCCTATT

PGC1α Reverse: 5'-3' GCTGTAGGGCGATCTT

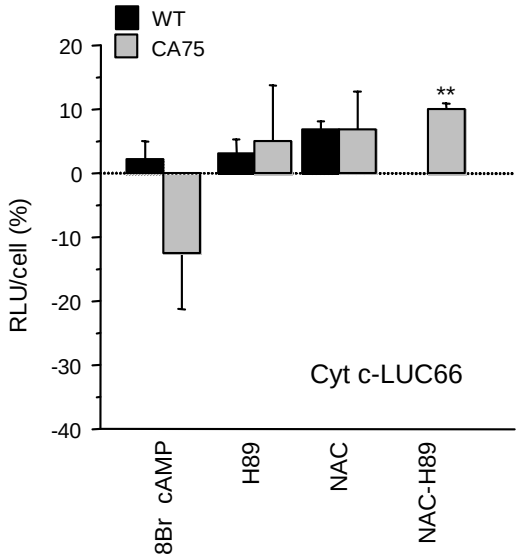
NRF-1 Forward: 5'-3'AGGTAACCGAAGTATTAGGG

NRF-1 Reverse: 5'-3' CCTTCCGCGTACTGAC

GAPDH Forward: 5'-3'AGAGCTGAACGGGAAG

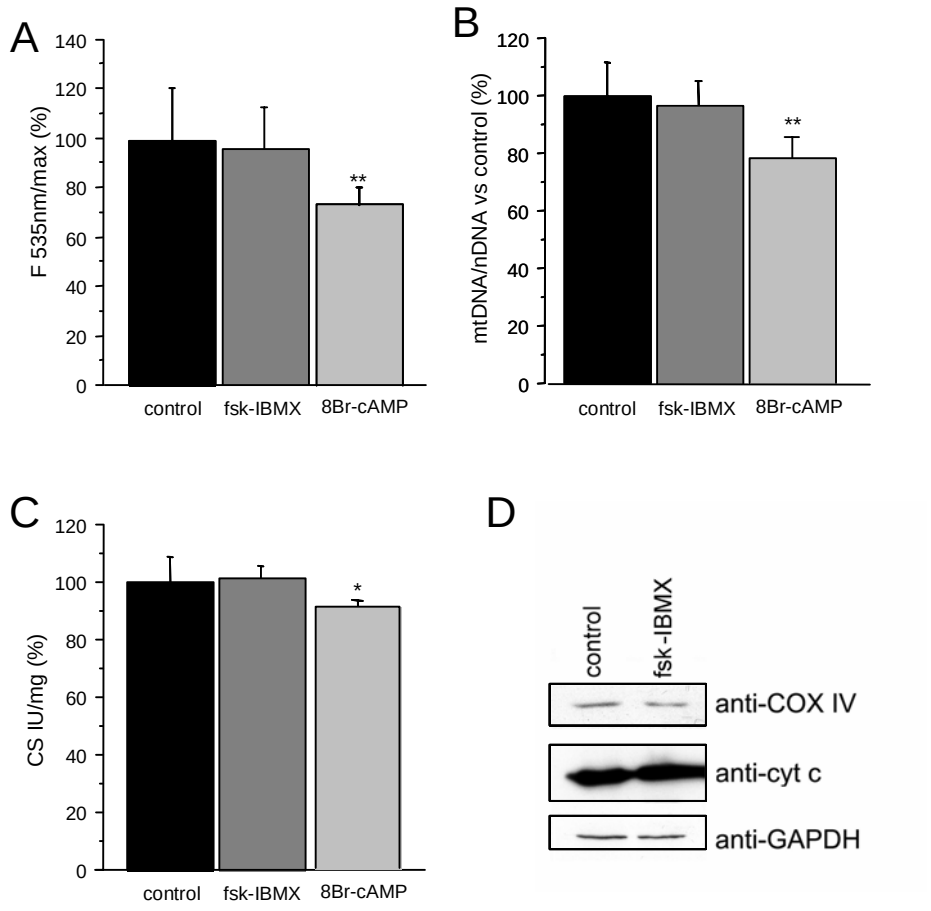
GAPDH Reverse: 5'-3' GTTGAAGTCGCAGGAG

Supplementary Figures



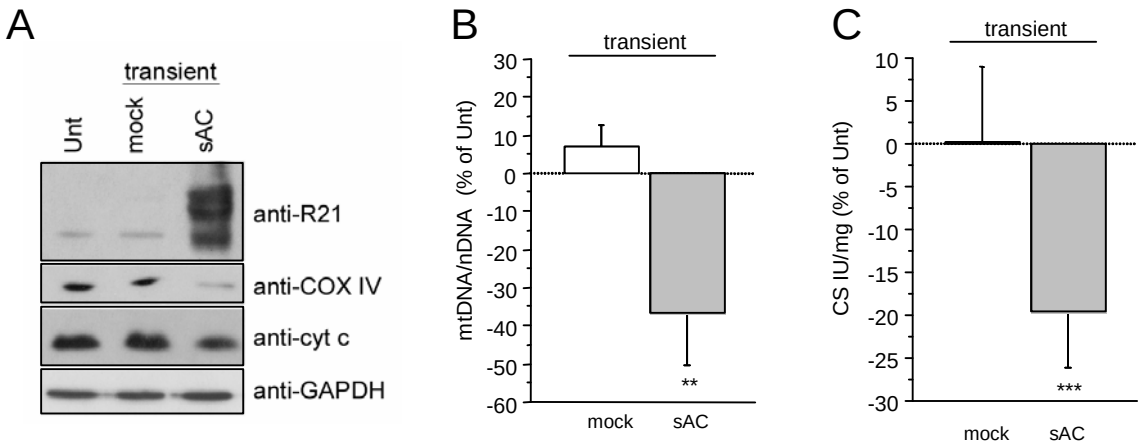
Supplementary Figure 1. Cyt c transcription regulation by PKA and ROS depends on NRF1-CREB

Luciferase activity in WT and CA75 cells transfected with Cyt c-LUC 66, whose promoter lacks the NRF-1 and CREB binding sites, after 48 hrs treatment with 8Br-cAMP, H89, NAC, or H89 plus NAC. Luminescence was normalized by the number of cells (RLU/cell ratio). Values are shown as a percentage of untreated controls for each cell line and represent the mean of three independent transfections, each measured in triplicates.



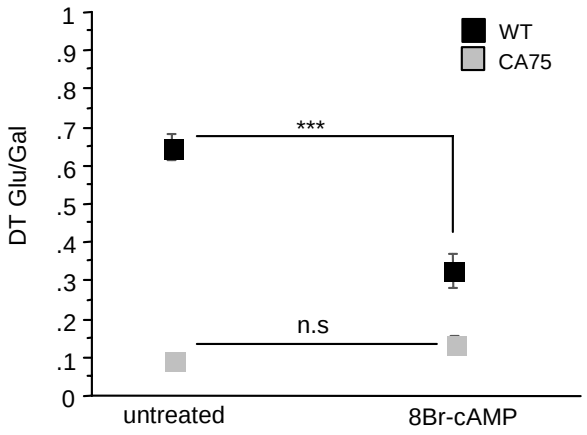
Supplementary Figure 2. Cytoplasmic cAMP does not affect ROS-dependent OXPHOS biogenesis

(**A**) ROS production (n=12), (**B**) mtDNA content (n=9), and (**C**) cytrate synthase (CS) activity (n=6) were significantly changed in cells treated with membrane permeant 8Br-cAMP for 48 hrs, but not in cells treated with forskolin-IBMX (fsk-IBMX). *, $p<0.01$; **, $p<0.001$. (**D**) Western blot analyses of Cyt c and COXIV in homogenates from cells treated with fsk-IBMX for 48 hrs. Re-probing the membrane with GAPDH showed that comparable amount of protein was loaded in the gel.



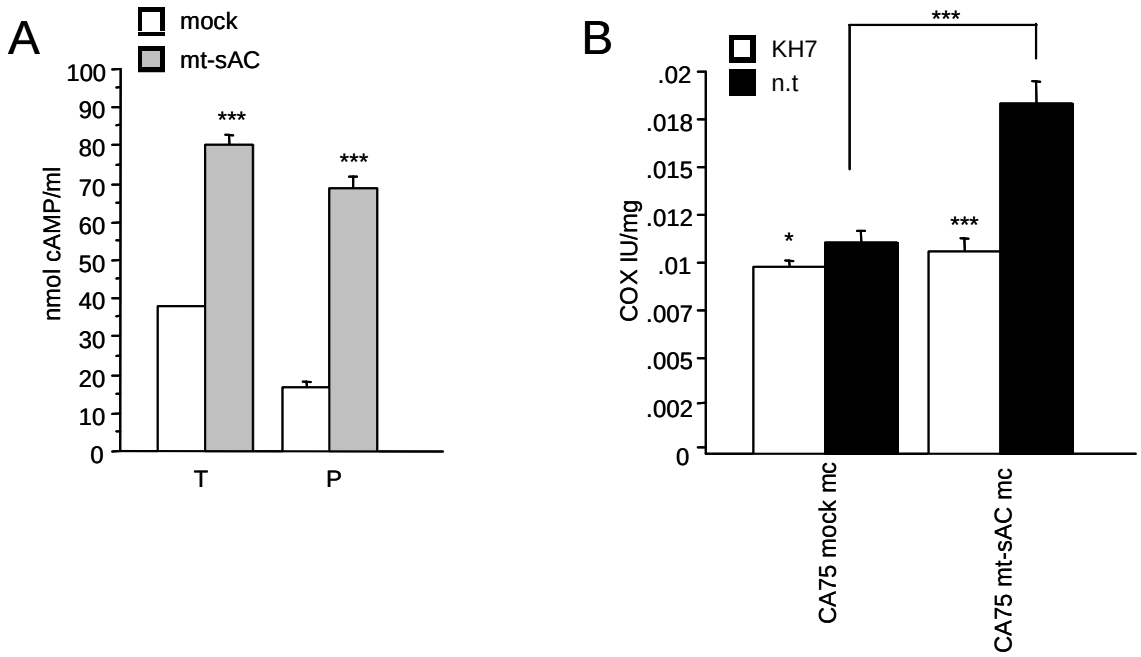
Supplementary Figure 3. Effects of sAC transient expression on OXPHOS biogenesis

(**A**) Expression of sAC detected with the R21 antibody in transiently transfected 293T HEK cells. Steady state levels of mitochondrial proteins COXIV and Cyt c were reduced in sAC expressing cells in comparison to untransfected (Unt) or mock transfected cells. GAPDH is used as protein loading control. (**B**) MtDNA content (n=6) and (**C**) cytrate synthase (CS) activity (n=9) in 293T HEK cells transiently expressing sAC and mock transfected cells. Values are shown as a percentage of Unt cells. **, $p<0.001$; ***, $p<0.0001$.



Supplementary Figure 4. **Effect of 8Br-cAMP on DT glu/gal in WT and COX deficient cells**

Treatment with 8Br-cAMP impairs growth in galactose of WT cells, whereas CA75 cells, which do not grow in galactose, were unaffected by 8Br-cAMP (n=3). ***, $p<0.0001$.



Supplementary Figure 5. Adenylyl cyclase activity and sensitivity to KH7 in COXI mutant cells stably expressing mt-sAC

(A) cAMP levels measured in total cell homogenate (T), and the pellet fraction containing mitochondrial (P) in mt-sAC CA75 expressing cells and mock transfected cells (n=3). (**B**) KH7 suppresses the increase of COX enzymatic activity in CA75 cells induced by mt-sAC expression (n=5). *, $p<0.01$; ***, $p<0.0001$.