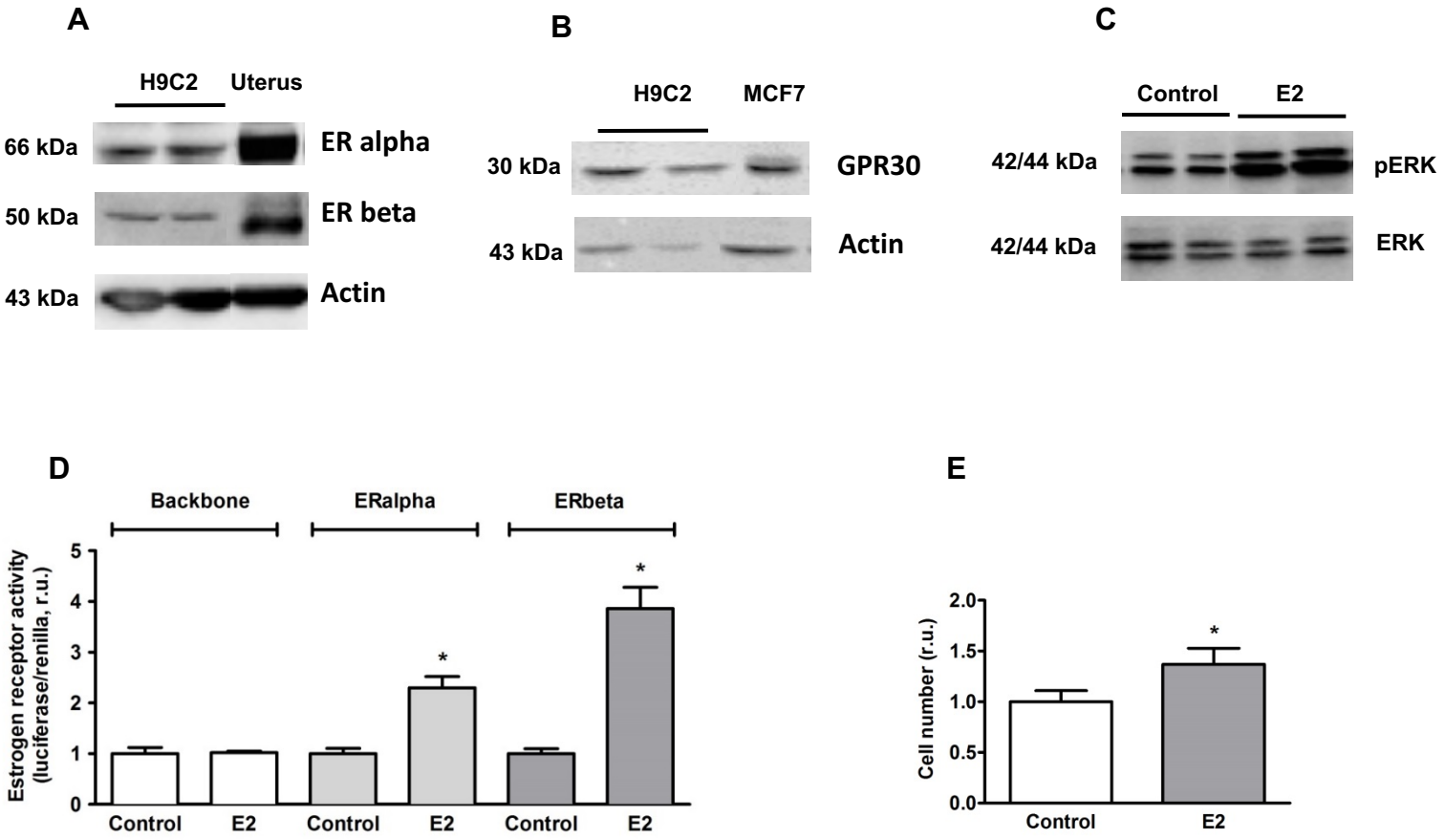
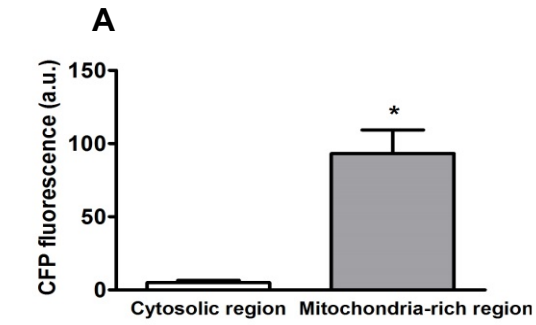
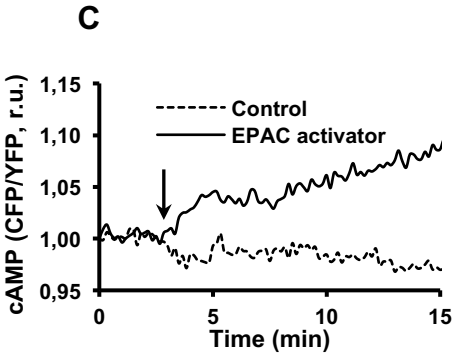
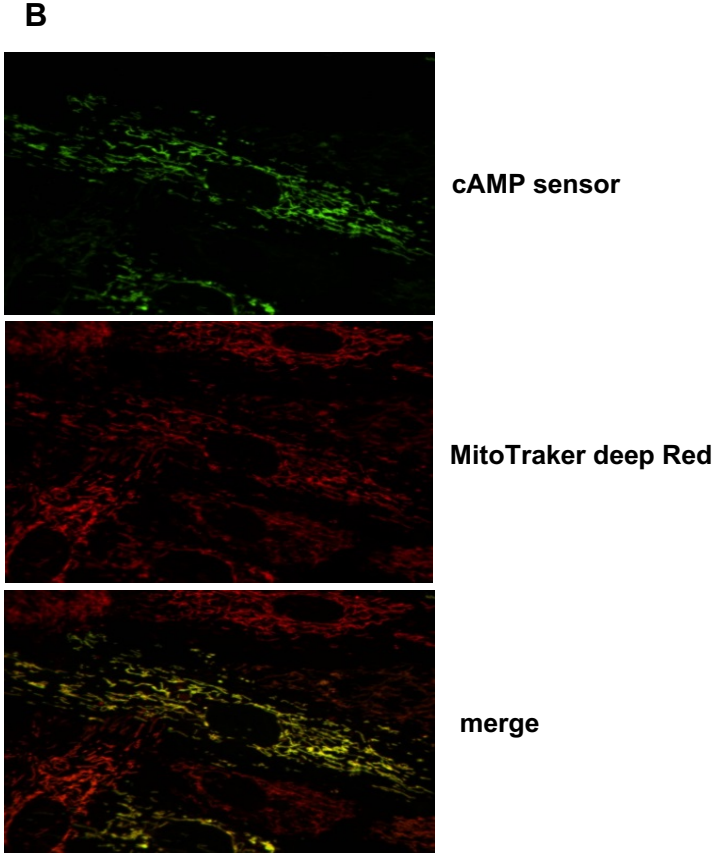
Supplementary figure 1

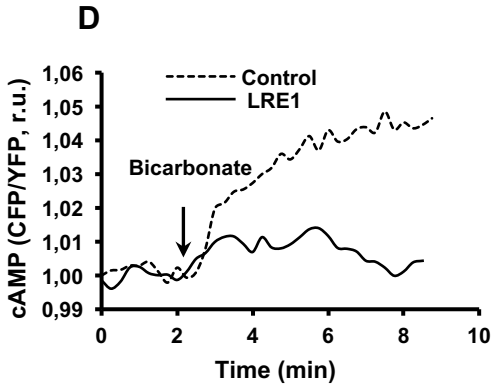


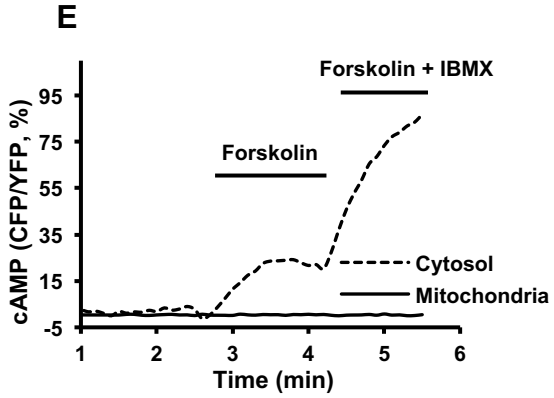
Supplementary figure 2



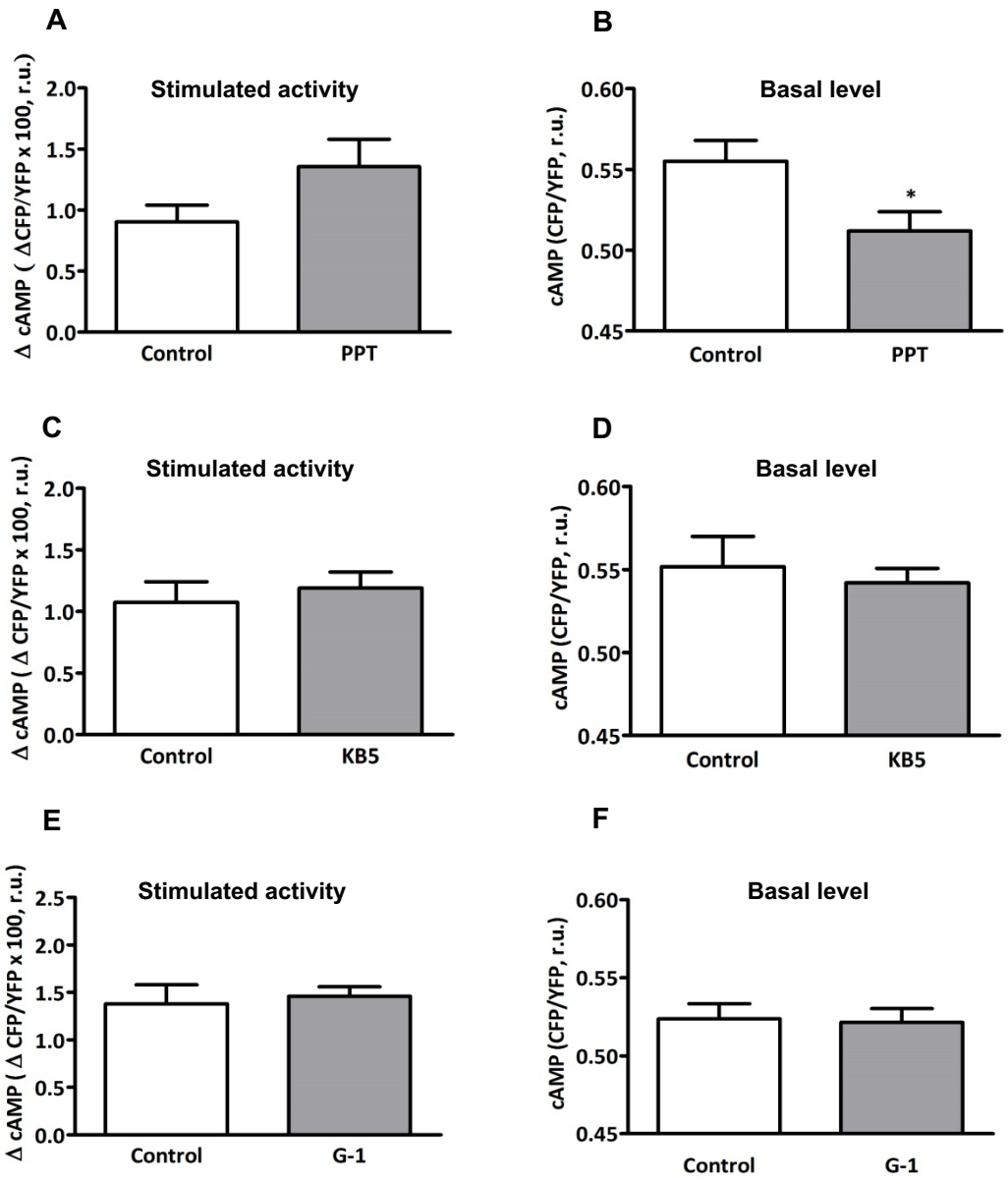






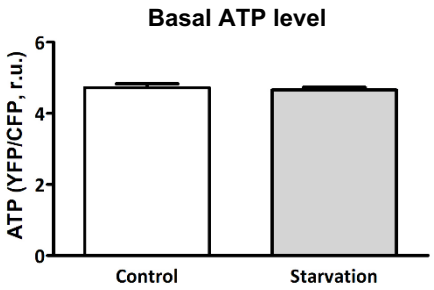


Supplementary figure 3



Supplementary figure 4

A



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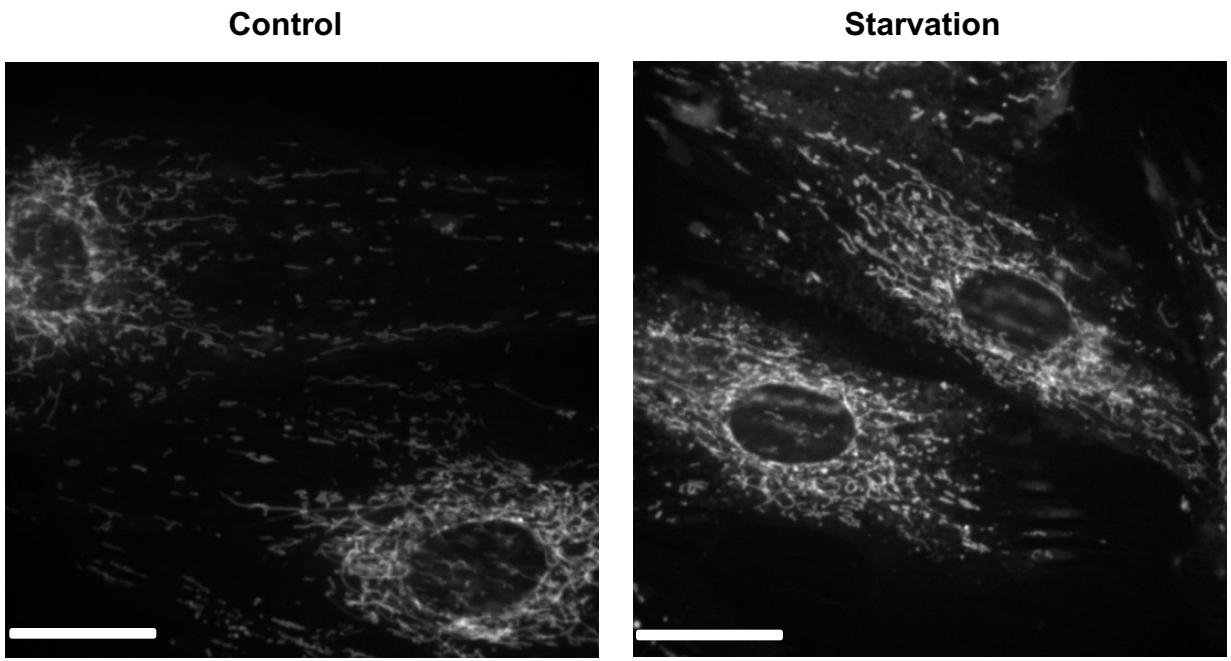

Supplementary figure legends

Supplementary Fig. 1 (A, B) Western blot expression analysis of estrogen receptor (ER) alpha, beta and GRP30 in H9C2 cells. The uterus was used as a positive control for estrogen receptors alpha and beta, while MCF7 cells were used as a positive control for GRP30. Data are representative of three independent experiments with similar results. (**C**) Western bot analysis of phosphorylated ERK1/2 (pERK) performed with H9C2 cells after treatment with 30 nmol/l estradiol (E2) or vehicle (Control) for 24 h. Data are representative of three independent experiments with similar results. **(D)** Analysis of estradiol-induced activation of estrogen receptors performed in H9C2 cells overexpressing estrogen receptor alpha or beta. Cells were treated with 30 nmol/l estradiol (E2) or vehicle (Control) for 24 h. Values are means ± SEM. n=8. *P<0.05 vs. corresponding Control. **(E)** Analysis of cell number (normalized to Control) after treatment with 30 nmol/l estradiol (E2) or vehicle (Control) for 24 h. Values are means ± SEM. n = 6. *P < 0.05 vs Control.

Supplementary Fig. 2 Characterization of mitochondria-targeted cAMP probe expressed in H9C2 cells. (**A)** Statistical analysis of CFP fluorescence obtained in H9C2 cells transfected with mitochondria-targeted cAMP probe 4mtH30 from mitochondria-free, i.e., cytosolic, or mitochondria-rich regions. Values are means ± SEM. n = 4. (**B)** Representative fluorescent images of H9C2 cells transfected with EPAC-based mitochondria-targeted cAMP probe 4mtH30 (upper panel, green) and stained with MitoTracker deep Red (middle panel, red). The merge image (lower panel) demonstrates a high level of 4mtH30 and MitoTracker deep Red co-localization (yellow). (**C)** Representative kinetics of mitochondrial cAMP level observed in H9C2 cells after treatment with either 200 µmol/l EPAC activator (8-pCPT2'-O-Me-cAMP) or vehicle (Control). Values are means ± SEM. n=4. *P<0.05. Arrow indicates the start of treatment. (**D)** Representative kinetics of mitochondrial cAMP level observed in H9C2 cells under treatment with 50 mmol/l bicarbonate. Cells were pretreated for 30 min with either 100 µmol/l LRE1 (a sAC inhibitor) or vehicle (DMSO). Values are means ± SEM. n=4. *P<0.05 (**E)** Representative kinetics of cytosolic (transfection with non-targeted H30 probe) or mitochondrial (transfection with mitochondria-targeted 4mtH30 probe) cAMP observed in a H9C2 cell under treatment with forskolin (40 µmol/l) and IBMX (200 µmol/l). Units are presented in % and were calculated as $(R-R_{min})/(R_{max}-R_{min})*100\%$, where R=CFP/YFP, R_{min}=R before treatment, R_{max}= maximal R after treatment with forskolin and IBMX. Values are means from three experiments.

Supplementary Fig. 3 Effect of ER agonists on mitochondrial cAMP. Statistical analysis of mitochondrial cAMP elevation (Stimulated activity) induced by 50 mmol/l bicarbonate **(A)** and basal cAMP level **(B)** were measured in H9C2 cells transfected with 4mtH30 sensor and either pretreated with 1 nmol/l estrogen receptor alpha agonist (PPT) or vehicle (Control) for 24 h. Values are means ± SEM. n=7. *P < 0.05. Statistical analysis of mitochondrial cAMP elevation (Stimulated activity) induced by 50 mmol/l bicarbonate **(C)** and basal cAMP level **(D)** measured in H9C2 cells, either pretreated with 10 nmol/l estrogen receptor beta agonist (KB5) or vehicle (Control) for 24 h. Values are means ± SEM. n=10. Statistical analysis of mitochondrial cAMP elevation (Stimulated activity) induced by 50 mmol/l bicarbonate **(E)** and basal cAMP level **(F)** measured in H9C2 cells, either pretreated with 10 nmol/l GRP30 agonist (G-1) or vehicle (Control) for 24 h. Values are means ± SEM. n=10.

Supplementary Fig. 4 Effect of FBS reduction on cellular ATP and mitochondrial morphology. **(A)** FRET-based assay of the basal cytosolic ATP measured in H9C2 cells transfected with ATP sensor. Cell were kept for 48 h

either in DMEM supplemented with 10% FBS (Control) or in DMEM supplemented with 2.5% FBS (Starvation). Values are means ± SEM. n=5-6. (B) Representative fluorescent images of H9C2 cells stained MitoTracker green. White scale indicate 30 μm. Note, the similar tubular structure, density and predominant perinuclear localization of mitochondria found in Control and Starvation groups. Images are representative from four independent experiments with similar results.