**Supplemental material**

**Supplemental table 1.** Antibodies used for immunoblotting

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| **Antibody** | **Company** | **Catalog number** |
| ATPIF1 (human) | SCBT | sc-271614 |
| ATPIF1 (total) | Invitrogen | A-21355 |
| GAPDH | Fitzgerald | 10r-g109a |
| CAMKII (Thr286) | Cell signaling | 12716 |
| CAMKII (PAN) | Cell signaling | 4436S |
| AMPK (thr172) | Cell signaling | 2535 |
| AMPK  | Cell signaling | 2532 |
| PLN (ser16) | Badrilla | A010-12AP |
| PLN (thr17) | Badrilla | A010-13AP |
| PLN | ABCAM | ab126174 |
| PINK1 | Cell signaling | 6946 |
| PARKIN | Cell signaling | 4211 |
| DRP1 | Cell signaling | 5391 |
| COX4 | Cell signaling | 4850 |
| mtHSP70 | ThermoFisher | MA3-028 |
| OXPHOS  | ABCAM | ab110413 |
| α-actinin | ABCAM | ab72592 |
| MFN2 | Cell signaling | 11925 |

**Supplemental table 2.** Primers sequences list used for RT-qPCR

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| **Gene** |  | **Forward** | **Reverse** |
| ATP inhibitor factor-1 (IF1) | Mouse | GGAGCCTTCGGAAAACGAGA | ATGGTGTTTCCTCAGGGCAG |
| ATP inhibitor factor-1 (IF1) | Human | CAGTCCGAGAATGTCGACCG | CAGTTGTTCTCTACTCTGTG |
| ATP inhibitor factor-1 (IF1) | Rat | GTCGGAGAGCATGGATTCGG | GCCAGCTGCTCTCTAGTCTT |
| Lactate dehydrogenase (LDH) | Rat | GTGCACTAAGCGGTCCCAAA | TGTTCTGGGGGACCTGTTCT |
| Pyruvate kinase (PRK) | Rat | GCACCTGATAGCTCGAGAGG | AGGGGGTCTGTGGATTGACT |
| NAPDH oxidase 2 (NOX2) | Rat | CTCGACAAGGATTCGAAGAC | GTGCTATCATCCAAGCTACC |
| Nuclear receptor factor (NRF2) | Rat | TTGGAGCACTTACTGGAGTC | CTTCCGCCATAATGAATCCC |
| Heat shock protein 60 (HSP60) | Rat | TTCCTCAGAGGTTGGCTATG | ATTCCAGGGTCCTTCTCTTC |
| Atrium natriuretic peptide (ANP) | Rat | ATGGGCTCCTTCTCCATCAC | TCTACCGGCATCTTCTCCTC |
| Brain natriuretic peptide (BNP) | Rat | ACAATCCACGATGCAGAAGCT | GGGCCTTGGTCCTTTGAGA |
| Regulator of calcineurin 1 (RCAN1) | Rat | GTCACGGCTGTTACCTCCAA | GCCAGAGTACACACCCATCC |
| CYP (mtDNA) | Rat | CCTCCCATTCATTATCGCCGCCCTTGC | GTCTGGGTCTCCTAGTAGGTCTGGGAAA |
| TRPM2 (ncDNA) | Rat | GTACAACGAGCTGCTTCATTCC | GCACCTCTAAGAGGCATCCATC |
| Short fraction D-Loop | Rat | CCTCCCATTCATTATCGCCGCCCTTGC | GTCTGGGTCTCCTAGTAGGTCTGGGAA |
| Long fraction D-Loop | Rat | AAAATCCCCGCAAACAATGACCACCC | GGCAATTAAGAGTGGGATGGAGCCAA |
| 36B2 | Rat | GTTGCCTCAGTGCCTCACTC | GCAGCCGCAAATGCAGATGG |

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**Figure S1. IF1 gene expression during embryonic development and heart failure. (A)** IF1 protein level normalized for alpha-actinin detected by western blot in lysates of mice subjected to TAC or sham surgery (n=5). **(B)** Cardiac IF-1 mRNA expression at embryonic day (EBD) 12 and 18, post-natal day 2 and 20. IF1 is expressed at values relative to EBD12. In addition, IF1 expression was compared between 20-week-old mice with heart failure (HF) after myocardial infarction and sham controls (n=3). \*p < 0.05 vs EBD12. **(C)** IF-1 mRNA expression in neonatal rat ventricular myocytes (NRVM) infected with an adenovirus expression constitutively activated Gαq (ad-Gq) or a control virus (ad-CTL) for 48 hours (n=5). Data are presented as mean ± SEM. \*p < 0.05 and \*\*p < 0.01 vs SHAM/ ad-CTL/EBD12 by nonparametric Mann-Whitney test.

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**Figure S2. Effect of IF1 on mitochondrial membrane potential, ATP levels and cell death after respiratory collapse.** Neonatal rat ventricular myocytes (NRVM) were infected with an adenoviral vector expressing the human IF1 (ad-IF1-WT), or an empty vector control virus (ad-CTL) for 48 hrs. **(A)** Representative western blot image from whole cell lysates using an antibody specific for the human isoform of IF-1 and an antibody that recognises all IF-1 isoforms. Glyceraldehyde-3-Phosphate dehydrogenase (GAPHD) served as loading control. **(B)** Time-lapse of the mitochondrial membrane potential measured with Tetramethylrhodamine, Ethyl Ester, Perchlorate (TMRE) before and after serial addition of rotenone (rot) + Antimycin-A (AA) or FCCP. Graphs represent 5 independent experiment (n=5). **(C)** Bar graph depicting the area under the curve of TMRE fluorescence starting from the addition of rot+aa until the addition of FCCP (n=5). **(D)** Intracellular ATP levels measured in the presence or absence of incubation with rotenone for 24 h. Intracellular ATP levels were detected using microplate colorimetric reader. (n=4). **(E)** ATP-linked and maximal mitochondrial respiration of NRVC infected with different adenovirus concentrations (MOI 50, 100 and 200). **(F)** NRVM were stained with the live cell indicator Calcein® and propidium iodide after infection with ad-IF1-WT and ad-Ctl for 48 hours. A total of 5 different regions of interest were selected per well and the total number of dead cells was expressed as a percentage to the total number of cells. (n=4). **(G)** Representative immunoblot from whole cell lysate using an antibody specific total IF1 after adenovirus infection that overexpress a small interference against IF1 (siIF1) or scrambled siRNA (ad-CTL, left panel). Protein levels of IF1 normalized to total protein (n=4, right panel). **(H)** Typical SeaHorse experiment depicting oxygen consumption rate (OCR) of NRVM after serial treatments with oligomycin (oligo), FCCP and rot + AA. The graph represents 4 independent experiments. \* p < 0.05, \*\* p < 0.01 and \*\*\* p < 0.001 vs ad-CTL using the ad-CTL using the Mann-Whitney U test or T-test where appropriate.



**Figure S3. Effect of IF1 expression on phenylephrine-induced cardiomyocyte hypertrophy.** NRVMs were infected with ad-IF1-E55A or ad-CTL for 48 hrs. **(A)** Bar graph depicting differences in cardiomyocyte size in the presence or absence of phenylephrine (50 µM) (n=4). **(B)** ANP mRNA expression of cells infected as in **A**. (n=4). \* p < 0.05, \*\* p < 0.01 and \*\*\* p < 0.001 vs ad-CTL using the ad-CTL using Kruskal Wallis followed by the Mann-Whitney U post-hoc test.



**Figure S4. An IF-1 mutant incapable of binding to ATP-synthase still downregulates respiratory chain complexes and stimulates natriuretic peptides.** NRVMs were infected with ad-IF1-E55A and ad-CTL for 48 hrs. **(A)** Representative western blot image from whole cell lysate using an antibody that recognises all IF-1 isoforms (top) from cells infected with ad-CTL (left), ad-IF1-WT (middle) and ad-IF1-E55A (right). Total protein stained using Fluorescence REVERT® solution (bottom). **(B)** Representative traces ofmitochondrial membrane potential from cells infected with ad-IF1-E55A or ad-CTL measured with TMRE before and after serial addition of rotenone (rot) + Antimycin-A (AA) and FCCP. **(C)** Changes in electron transport chain protein complexes detected with western blot (n=3). **(D)** mRNA expression of the natriuretic peptides ANP and BNP (n=4). \* p < 0.05, \*\* p < 0.01 and \*\*\* p < 0.001 vs ad-CTL using the ad-CTL using the Mann-Whitney U test or T-test where appropriate.



**Figure S5. IF1 overexpression does not affect mitochondrial content.** NRVMs were infected with ad-IF1-WT or ad-CTL for 48 hrs. **(A)** Bar graph depicting changes in the total mitochondrial volume per cell. (ad-CTL; n=17 cells and ad-IF1-WT; n=18 cells) using confocal microscope as described in Fig 5. **(B)** Bar graph depicting the ratio between mitochondrial DNA (cytochrome B, CYTB) and nuclear DNA (Transient receptor potential cation channel subfamily M member-2, TRPM-2) in cells infected with ad-IF1-WT or ad-CTL (n=4).



**Figure S6. Effects of IF-1 CaMKII-dependent and PKA-dependent phosphorylation of Phospholambam.** NRVMs were infected with ad-IF1-WT or ad-CTL for 48 hrs. **(A)** Right panel: Representative immunoblot from whole cell lysate using specifics antibodies to detect total Phospholambam (PLN) as well as PLN phosphorylation at the CaMKII specific (threonine 17) and the protein kinase A specific (serine 16) phosphorylation sites. Left Pannel: Bar graph depicting changes in phospholamban phosphorylation (n=4). **(B)** mRNA levels of the Regulator of calcineurin- 1 (RCAN1) (n=4). \*\*p < 0.01 vs ad-Ctrl\* using the Mann-Whitney U test.



**Figure S7. IF-1 mutant sufficient to reduce mitochondrial calcium and activates CAMKII signaling.** NRVMs were infected with ad-IF1-E55A and ad-CTL for 48 hrs. **(A)** Bar graph depicting basal mitochondrial Ca2+ labelled with Rhod-2 AM. (n=3). **(B and C)** Protein levels of phosphorylated Phospholamban (threonine 17) (n=3) and AMPK (threonine 172) (n=5) were assessed with western blot. \*p < 0.05 vs ad-Ctrl using the Mann-Whitney U test.