SUPPLEMENTAL MATERIAL

Supplemental Methods

Mitochondrial oxygen consumption was measured at 37^{o}C by polarography using a Clark type electrode (Oxygraph, Hansatech, Norfolk, UK). Experiments were initiated by addition of ~0.2 mg mitochondria in 1 ml of respiration buffer (in mM; 100 KCL, 5 KH_2PO_4, 1 EGTA, 50 MOPS, 10 $MgCl_2$) containing 0.2% BSA. 2mM pyruvate and 2 mM malate (final concentration) were used as a complex I respiratory substrate. The maximal respiration (state 3), defined as the rate of respiration in the presence of ADP was initiated by adding 0.25 mM ADP to the respiration chamber containing mitochondria and respiratory substrates. State 4 (resting respiration) was obtained when the complete conversion (phosphorylation) of ADP to ATP was reached. The respiratory control ratio (RCR) commonly referred to as an index of mitochondrial integrity (coupled respiration) was calculated from the ratio of state 3 to state 4 respiration.

Figure Legends

Supplemental Figure S1. Measurement of respiration in isolated rat heart mitochondria. **A.** Representative O2 electrode tracing obtained with rat heart mitochondria. Inflections represent changes in respiration rates. P/M = Pyruvate/Malate. Respiration rates were determined from tracings (2 = state 3 respiration rate, 3= state 4 respiration). **B.** State 3 and state 4 respiration rates. **C.** Respiratory control ratio.

Supplemental Figure S2. Cytochrome *c* release in WT and cypD-/- MEFs. **A.** Cells infected with an adenovirus encoding β-gal or Bnip3 for 24 h were stained with anti-

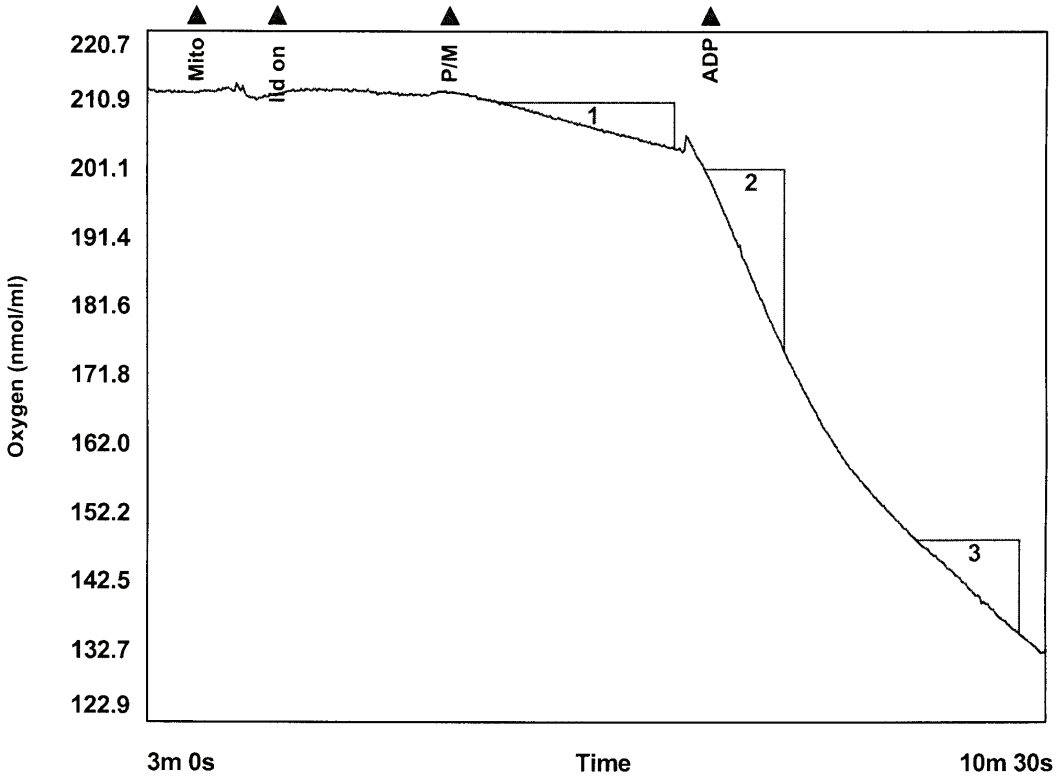
COX IV and anti-cytochrome *c* and visualized by fluorescence microscopy. **B.** Quantitative analysis of cells exhibiting release of cytochrome *c* (n=4).

Supplemental Figure S3. Cytochrome *c* release in cypD-/- MEFs reconstituted with cyclophilin D. **A.** Cells infected with adenoviruses encoding β-gal or Bnip3 plus β-gal or cypD for 24 h were stained with anti-COX IV and anti-cytochrome *c* and visualized by fluorescence microscopy. **B.** Quantitative analysis of cells exhibiting release of cytochrome *c* (n=4).

Supplemental Figure S4. Reconstitution of cyclophilin D restores H_2O_2,-mediated release of calcein from mitochondria in cypD-/- MEFs. Cells were infected with β-gal or Bnip3 plus β-gal or cypD for 24 h and then loaded with 1 μM calcein-AM and 5 mM $CoCl_2$. Alternatively, the cells were infected with β-gal or cypD for 24 h and then treated with 250 μM H_2O_2.

Supplemental Figure S1

A.



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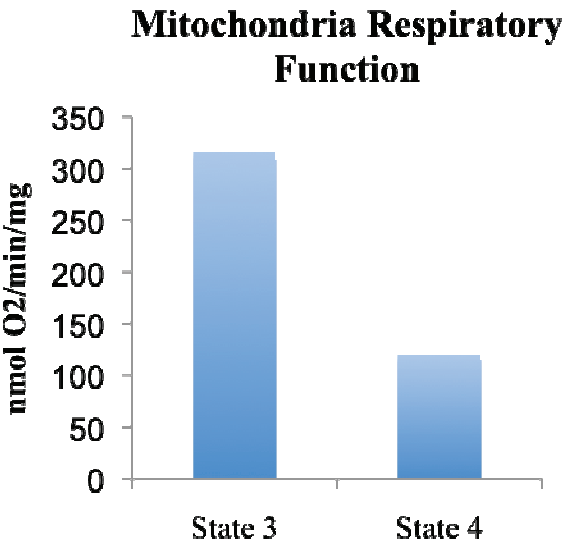

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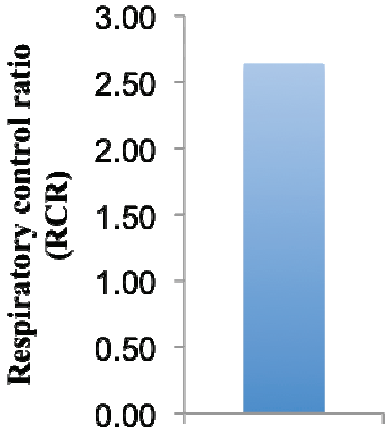
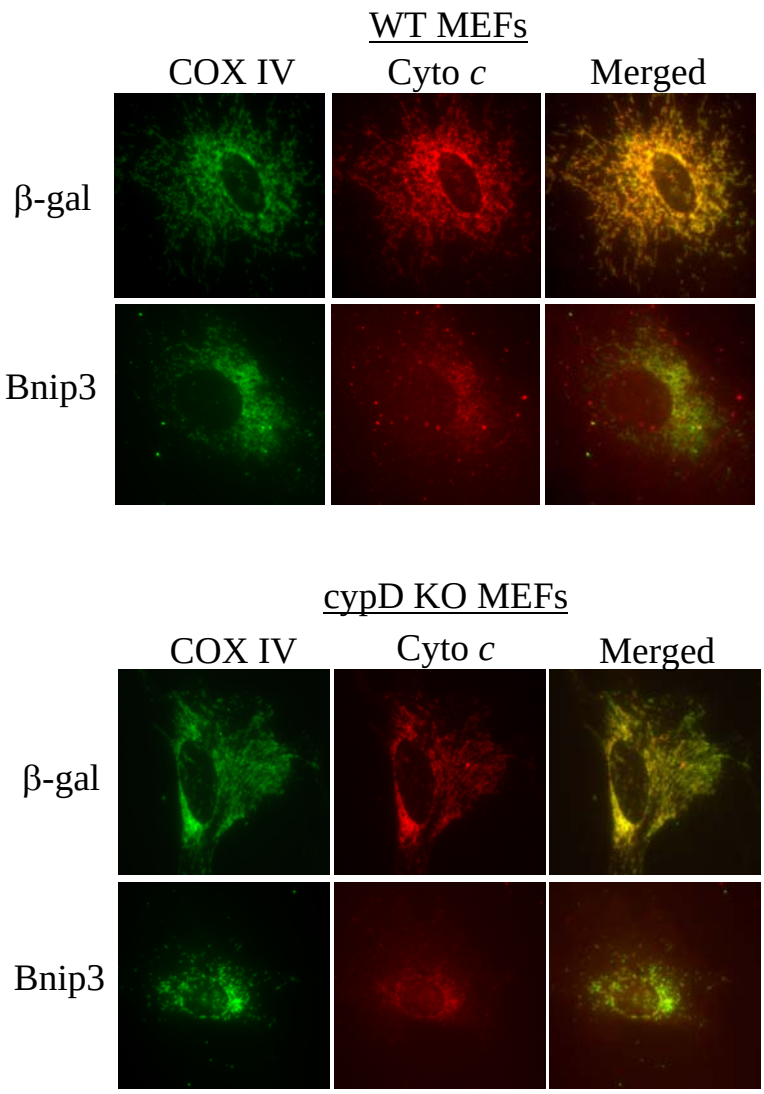


Supplemental Figure S2

A.



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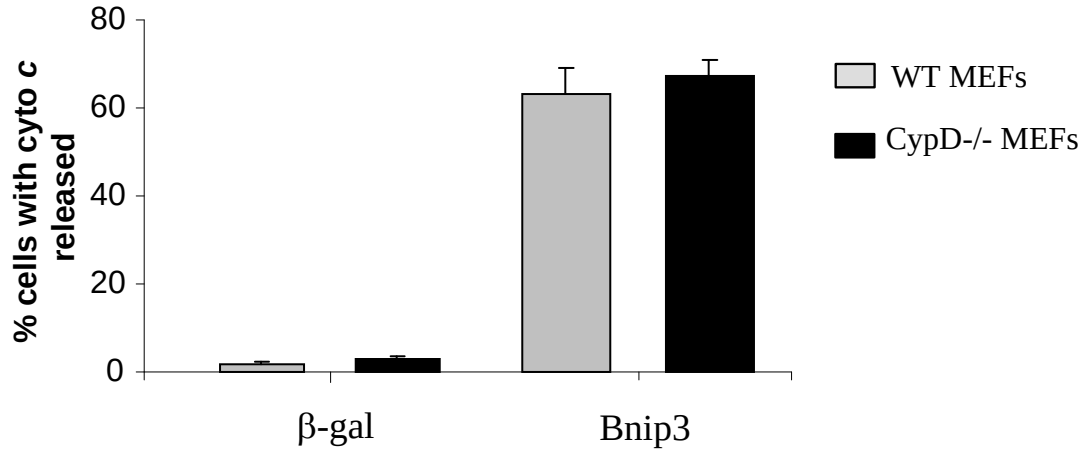
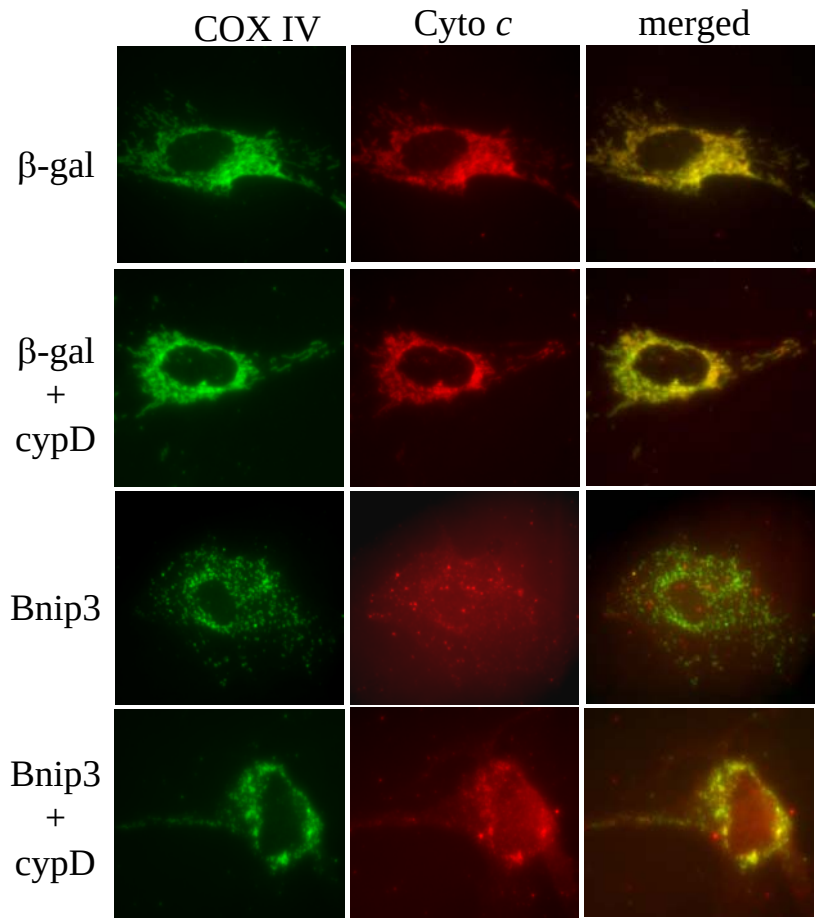


Supplemental Figure S3

A.



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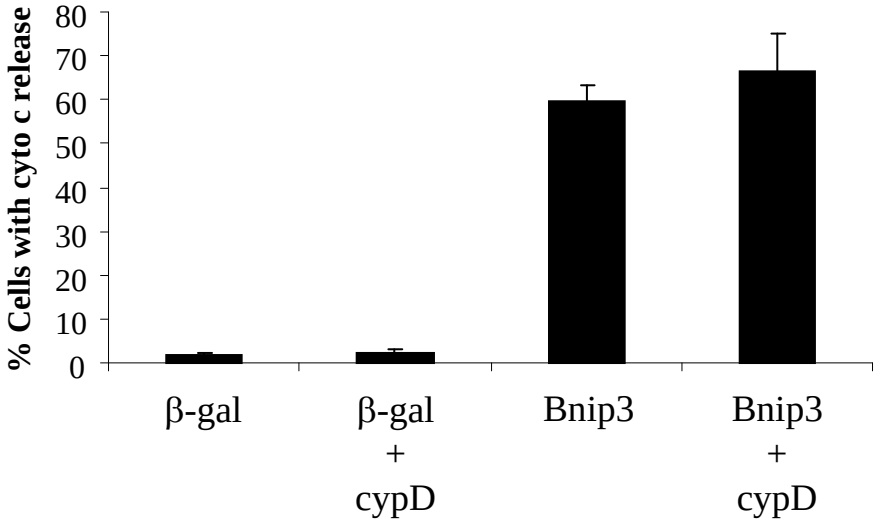

Supplemental Figure S4