

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Ex vivo activity of MitoPPX and localization in HEK 293 cells.

A. Lysates of Wt and MitoPPX cells were incubated with exogenous polyP. The decrease in polyP levels was monitored using DAPI fluorescence measurements. Incubation with alkaline phosphatase, a known polyP hydrolyzing enzyme was used as control. **B.** Subcellular localization of MitoPPX-GFP. Wt and MitoPPX cells were imaged using a fluorescence microscope. **C.** Confocal images showing co-localization of MitoPPX-GFP and mitochondria in HEK 293 MitoPPX cells. Mitochondria were labeled using TMRM. Data in the graphs are shown as average \pm SEM.

Supplementary Figure 2. Schematic representation of mitochondrial bioenergetics.

OXPPOS and tricarboxylic acids cycle (TCA) cycle are the main sources of energy within the organelle.

Supplementary Figure 3. The respiratory chain is functional in MitoPPX cells.

explain what you did – use the same lingo as above. Lower concentrations of FCCP (0,25 μ M) were able to stimulate the mitochondrial respiration and increase the OCR in MitoPPX cells. This evidence proves that the respiratory chain is functional in MitoPPX cells.

Supplementary Figure 4. Wt and MitoPPX cells show similar cell density before and after experiments using Seahorse technology.

Representative images of Wt and MitoPPX cells before and after the experiments with the **A.** Mito Stress and, **B.** Glycolytic Rate Assay.

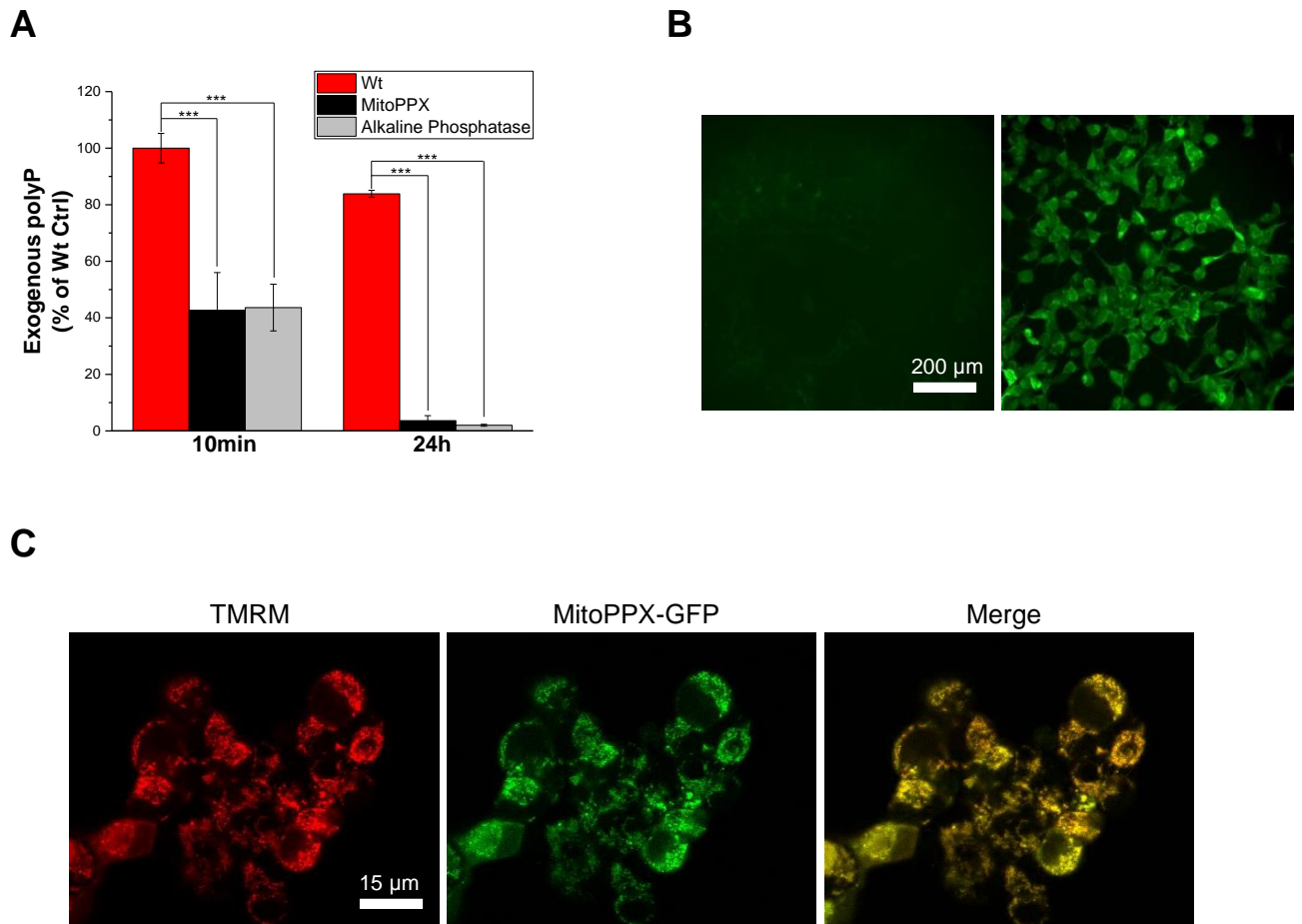
Supplementary Figure 5. Wt and MitoPPX cells show similar levels of mitochondrial Drp1 and of phospho-Drp1 (Ser 616).

Representative immunoblots showing **A.** levels of Drp1 in mitochondrial and cytosolic fractions prepared from Wt and MitoPPX cells and, **B.** phospho-Drp1 (Ser 616) in cell lysates. β actine was used as a loading control for the cellular extracts and the cytosolic fractions, while TOMM 20 was used as a loading control in the case of the mitochondrial fractions.

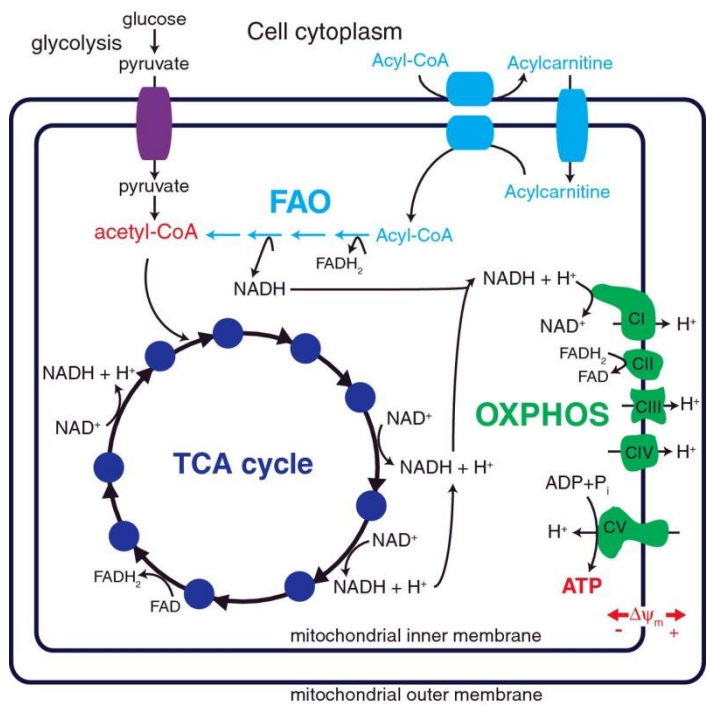
SUPPLEMENTARY TABLE LEGENDS

Supplementary Table 1: List of all the metabolites analyzed in the Wt and MitoPPX samples. Table showing all the metabolites analyzed in the samples, organized from higher to lower p value.

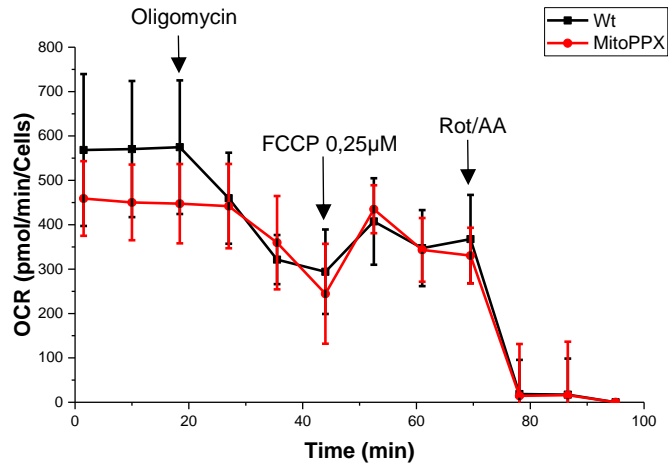
Supplem. Fig. 1



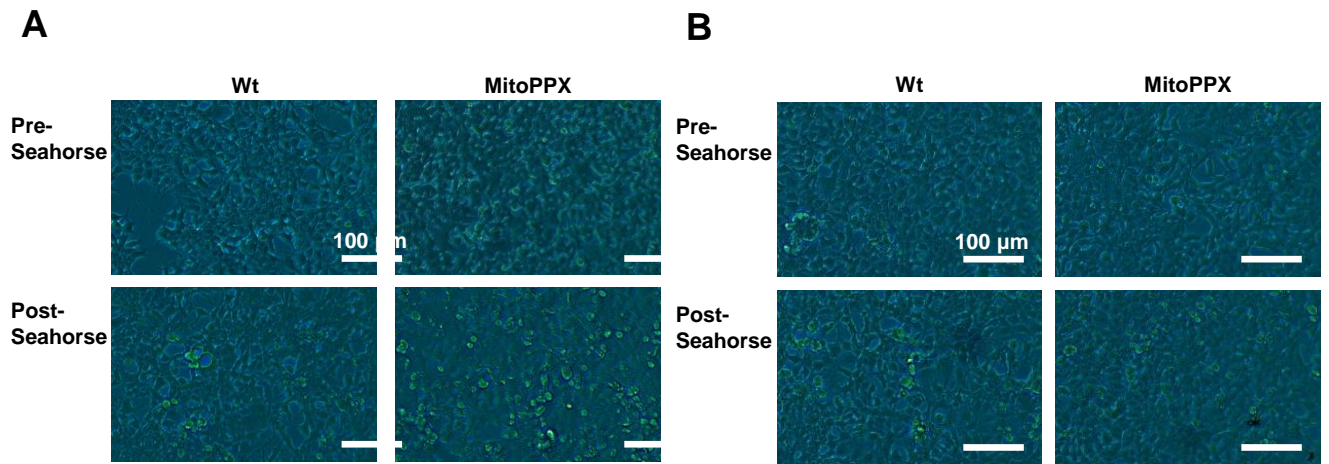
Supplem. Fig. 2



Supplem. Fig. 3

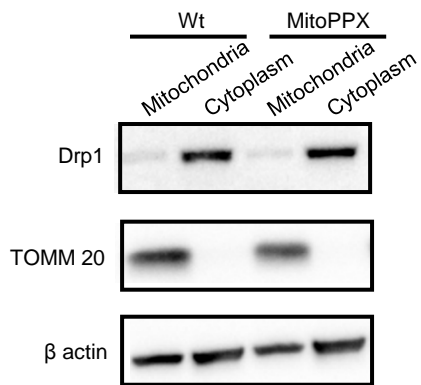


Supplem. Fig. 4



Supplem. Fig. 5

A



B

