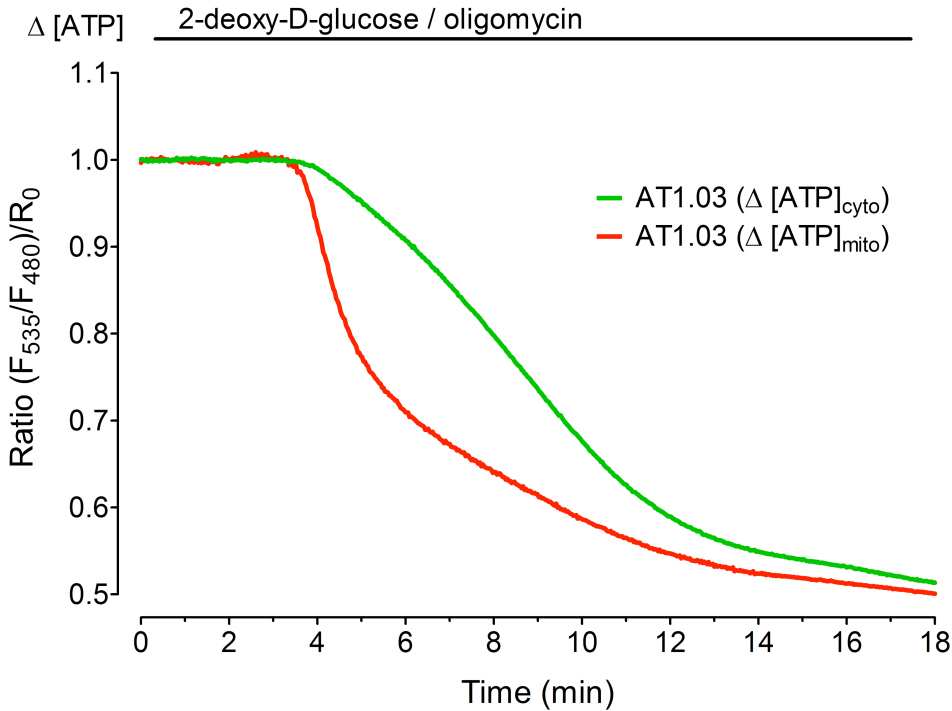


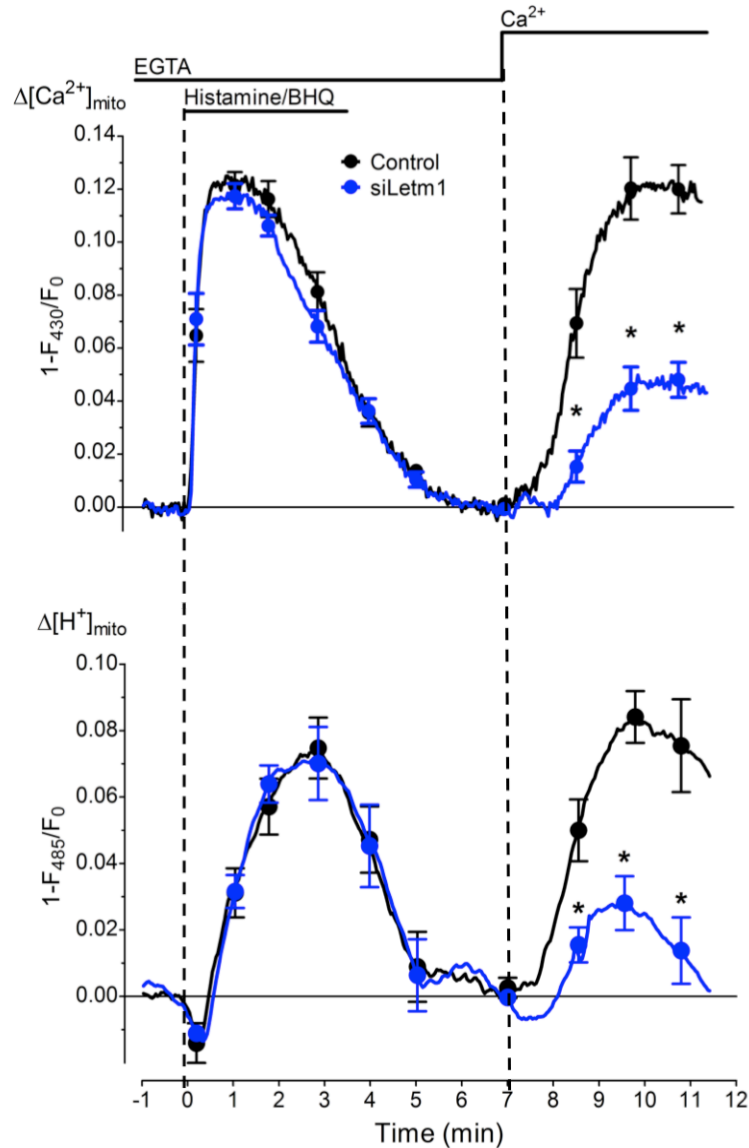
# Supplementary Figures

Supplementary Figure 1:



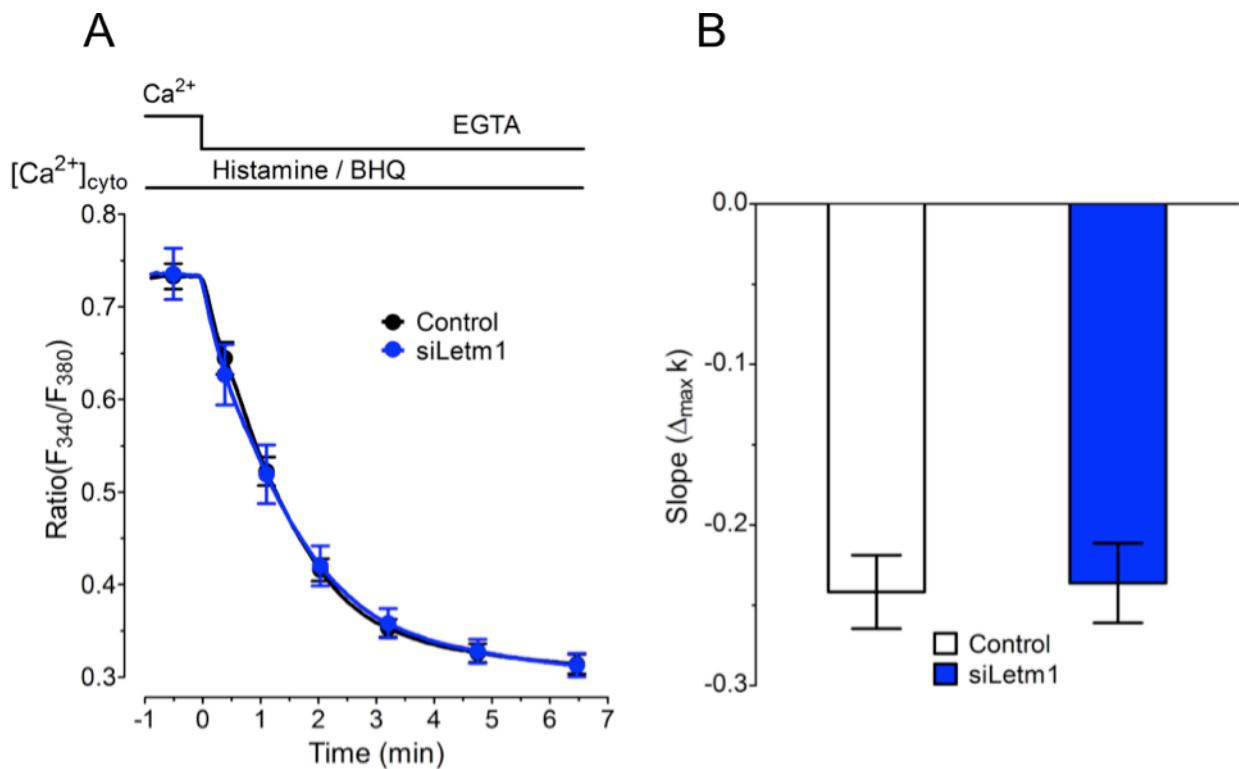
**Assesment of cytosolic and mitochondrial [ATP].** Cells were transiently transfected with AT1.03 or mt AT1.03. Curves represent the drop in [ATP]<sub>cyto</sub> (light green curve) and in [ATP]<sub>mito</sub> (red curve) by an incubation of the cells with EB containing 10 mM 2-deoxy-D-glucose instead of D-glucose and 2  $\mu$ M oligomycin.

**Supplementary Figure 2:**



**The correlation of mitochondrial  $Ca^{2+}$  and pH revealed mitochondrial pH ( $[H^+]_{mito}$ ) to be strictly associated with mitochondrial  $Ca^{2+}$  ( $[Ca^{2+}]_{mito}$ ) elevation, independently of the  $Ca^{2+}$  carrier engaged.** Mitochondrial  $Ca^{2+}$  uptake (*upper panel*) from either intracellularly released or entering  $Ca^{2+}$  yielded mitochondrial acidification (*lower panel*) using the dual ability of the RP-mt sensor to simultaneously measure  $[Ca^{2+}]_{mito}$  at  $\lambda_{exc.} = 430$  nm and  $[H^+]_{mito}$  at  $\lambda_{exc.} = 480$  nm. Knock-down of Letm1 (n=3, 13 cells) induced less acidification of mitochondria upon SOCE versus Control (n=3, 14 cells) while mitochondrial acidification in response to intracellular  $Ca^{2+}$  release remained unaffected by knock-down of Letm1. \*P < 0.05 vs. Control.

**Supplementary Figure 3:**



**The activity of the PMCA was not affected by the knock-down of Letm1.** Cells transiently transfected with nuclear GFP and either Control siRNA (Control, n=8, 28 cells) or siRNA against Letm1 (siLetm1, n=8, 13 cells) were loaded with Fura2-AM and subsequently stimulated with 100  $\mu$ M histamine and 15  $\mu$ M BHQ in low  $Na^+$  buffer (LSB) to exclusively elucidate the activity of the PMCA. **Panel A:** Cytosolic  $Ca^{2+}$  decrease after addition of 1 mM EGTA reflecting the force of the PMCA to transport the intracellular  $Ca^{2+}$  in the extracellular space. **Panel B:** Maximal slope from curves presented in *Panel A* were calculated and expressed as  $\Delta_{max}k$ .