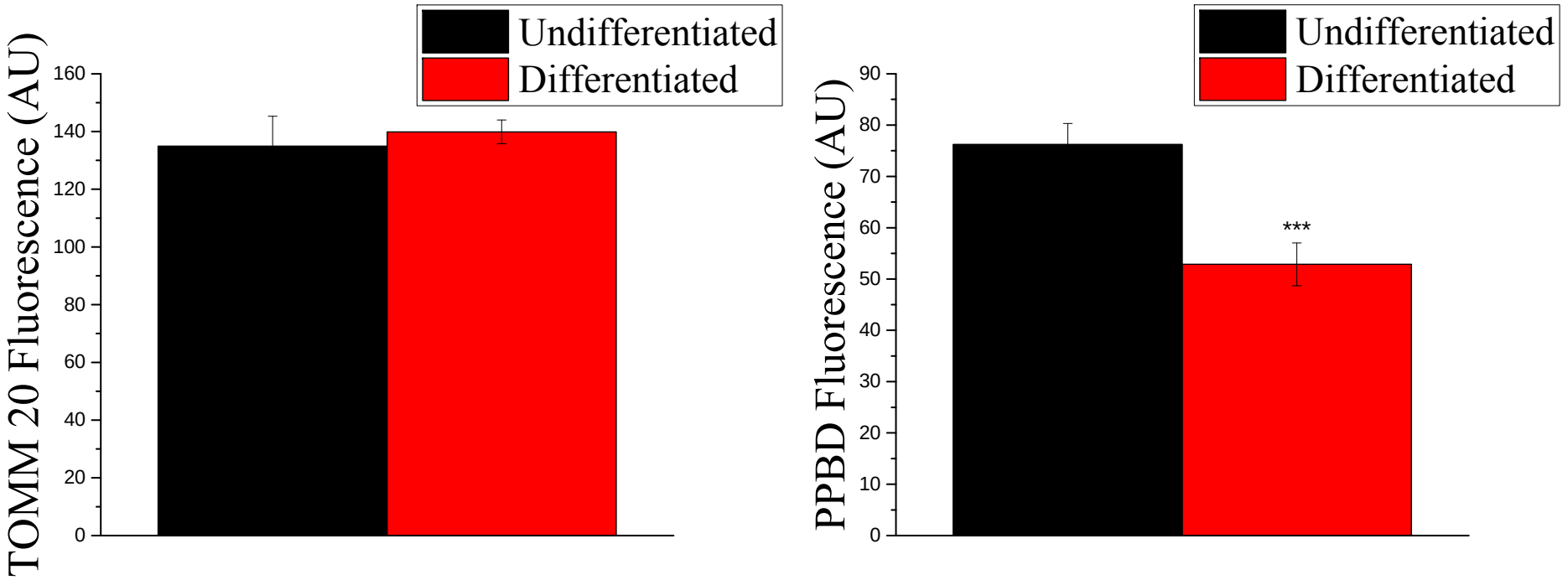
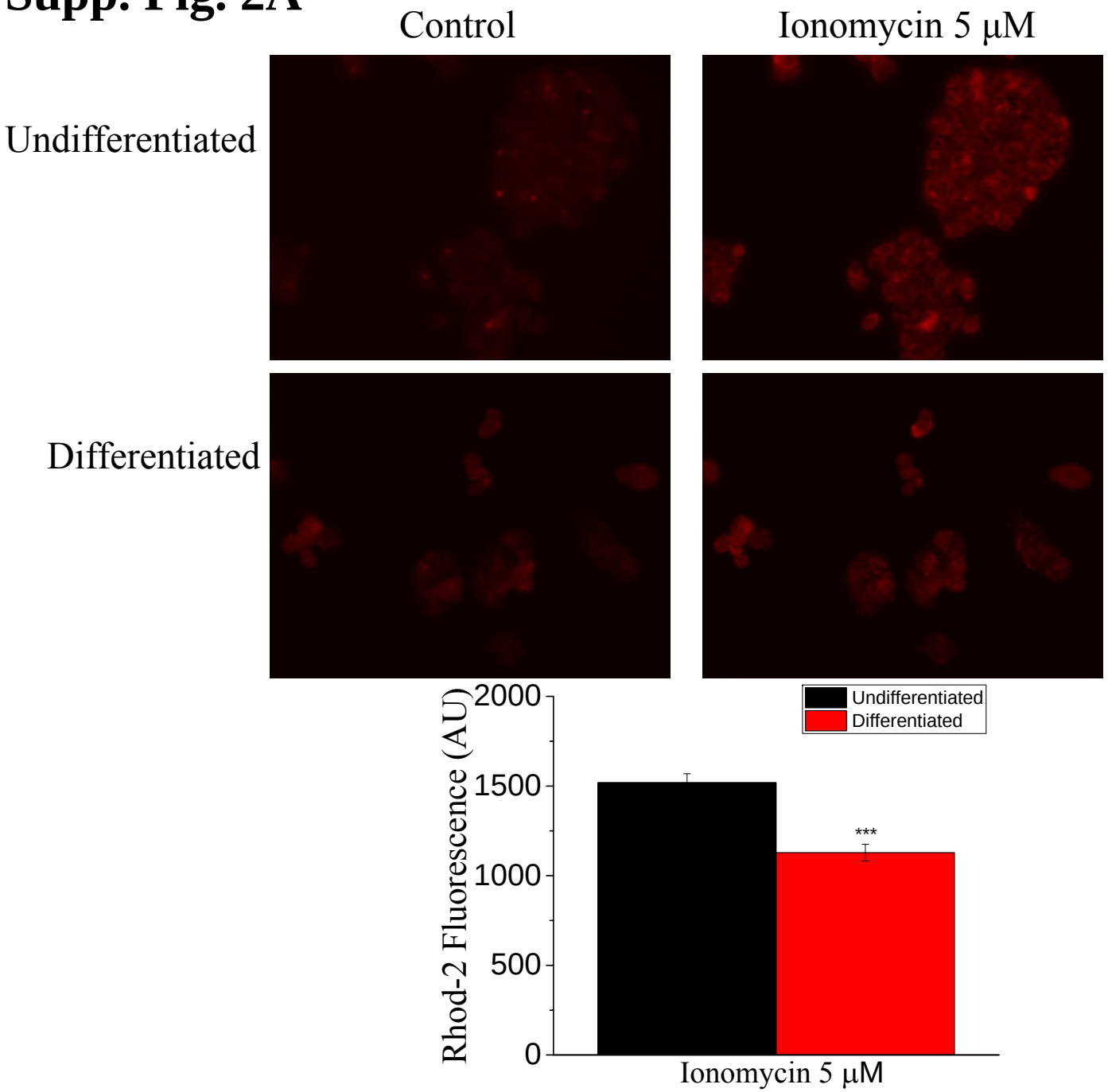
Supp. Fig. 1

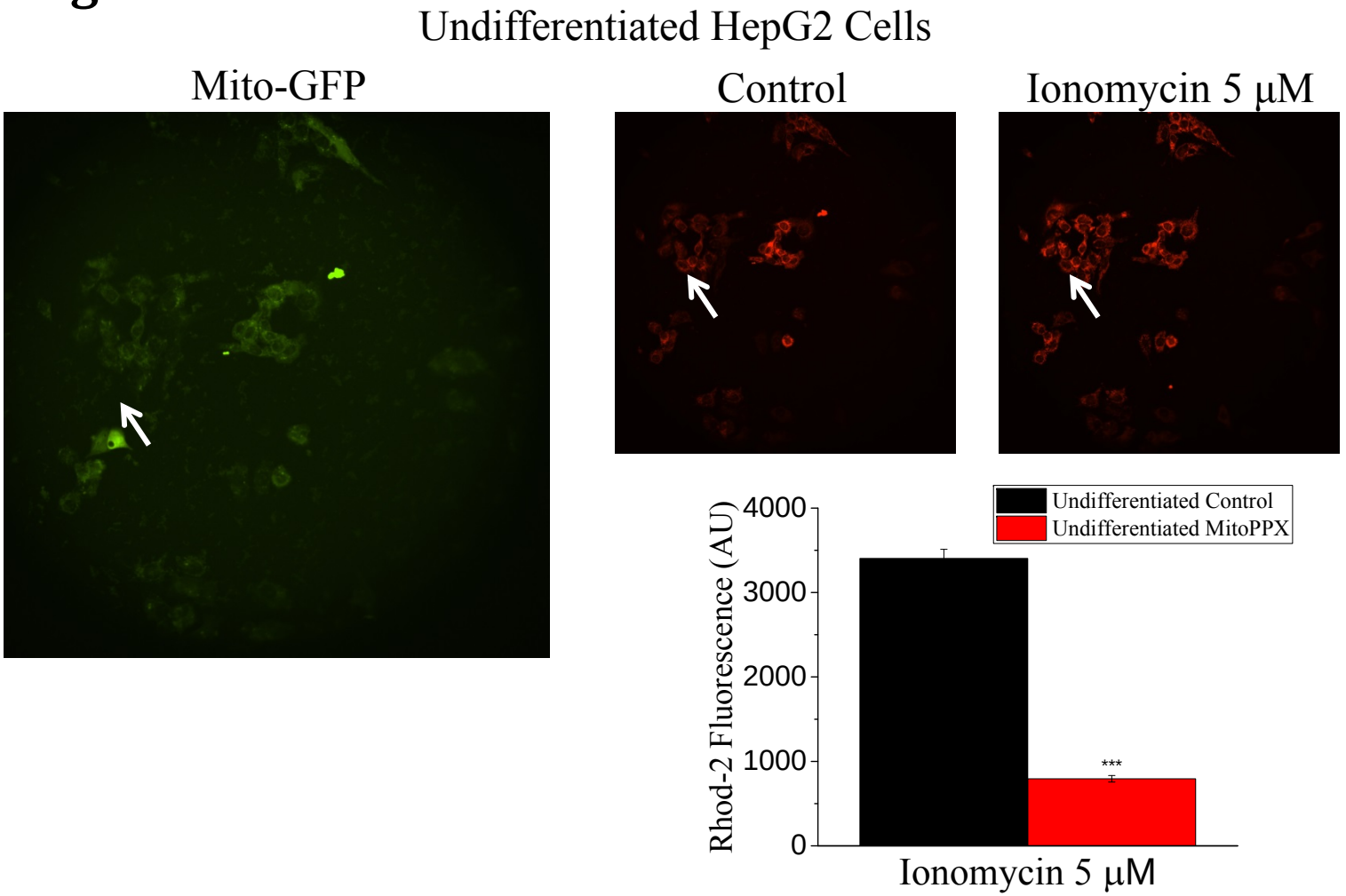


Supplementary Figure 1. PolyP levels in mitochondria of undifferentiated and differentiated HepG2 cells. Histograms showing fluorescence measurements from both, **(A)** TOMM20 (mitochondrial marker) and **(B)** PPBD (polyP marker) signal. ROIs were performed on mitochondrial regions selected using TOMM20 fluorescence. Data shown as mean ± SEM of, at least ten different cells from, at least, three independent experiments. Student's test: ***$p<0.001$.

Supp. Fig. 2A

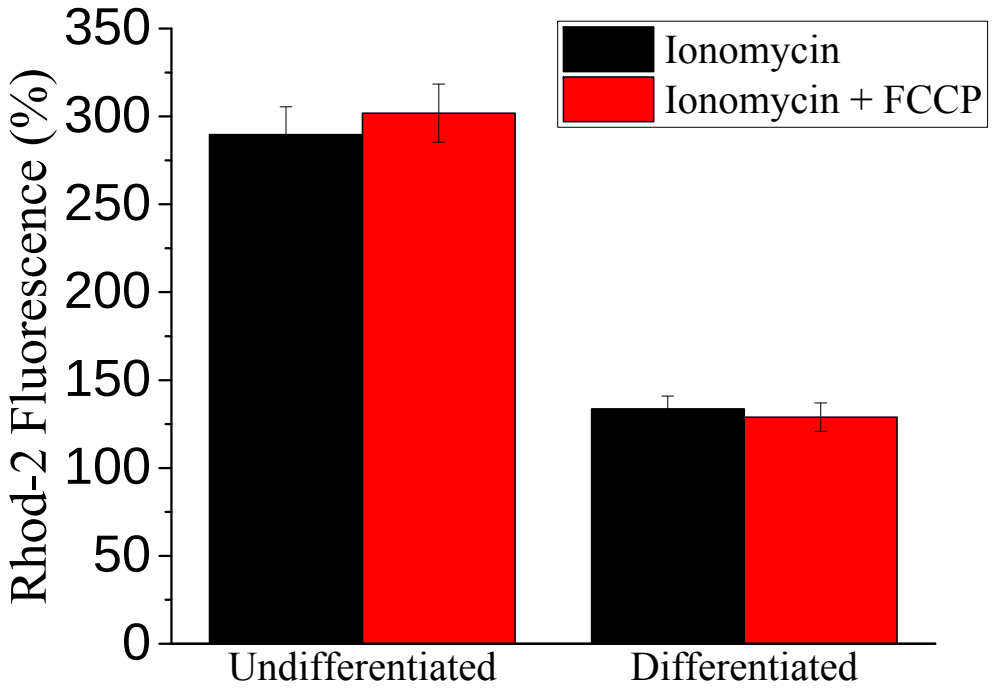

Control
Ionomycin 5 μM
Undifferentiated
Differentiated
Undifferentiated
Differentiated
Rhod-2 Fluorescence (AU)
2000
1500
1000
500
0
***
Ionomycin 5 μM

Supp. Fig. 2B



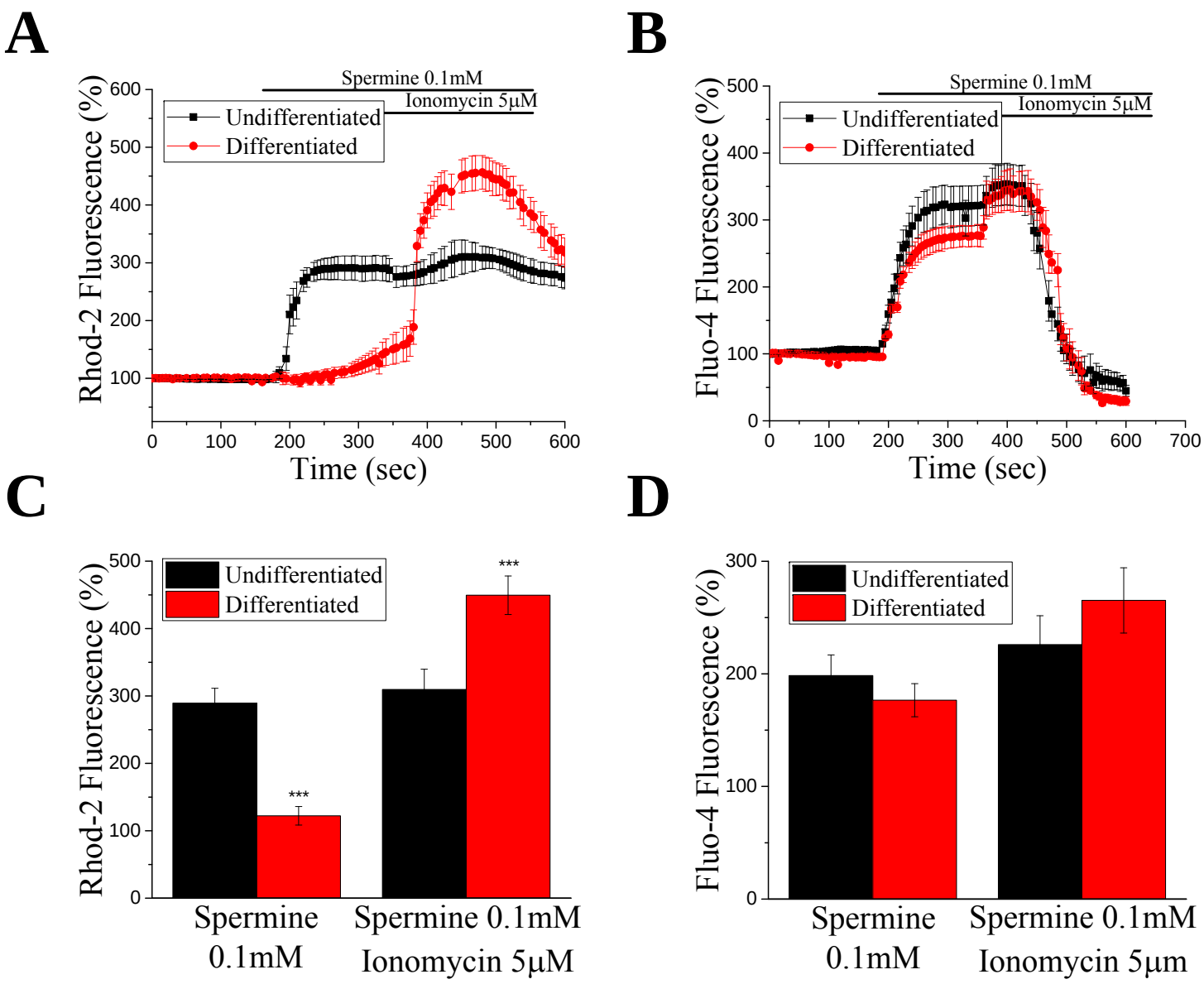
Supplementary Figure 2. Effect of polyP on HepG2 undifferentiated and differentiated cells. Role of MitoPPX. A. Images showing Rhod-2 fluorescence before and after perfunding with ionomycin 5 µM, in both undifferentiated and differentiated HepG2 cells. Histogram showing absolute values for real-time measurement of $[Ca^{+2}]_{mito\text{-}free}$ using Rhod-2 fluorescence. Data is showed as mean ± SEM, n=10 cells, Student's test: ***p<0.001. **B.** Images showing Rhod-2 fluorescence in Mito-PPX-transfected HepG2 undifferentiated cells, in the presence of ionomycin 5 µM. Histogram showing absolute values for real-time measurement of $[Ca^{+2}]_{mito\text{-}free}$ in control and transfected cells (MitoPPX). Data is showed as mean ± SEM, n=10 cells, Student's test: ***p<0.001.

Supp. Fig. 3

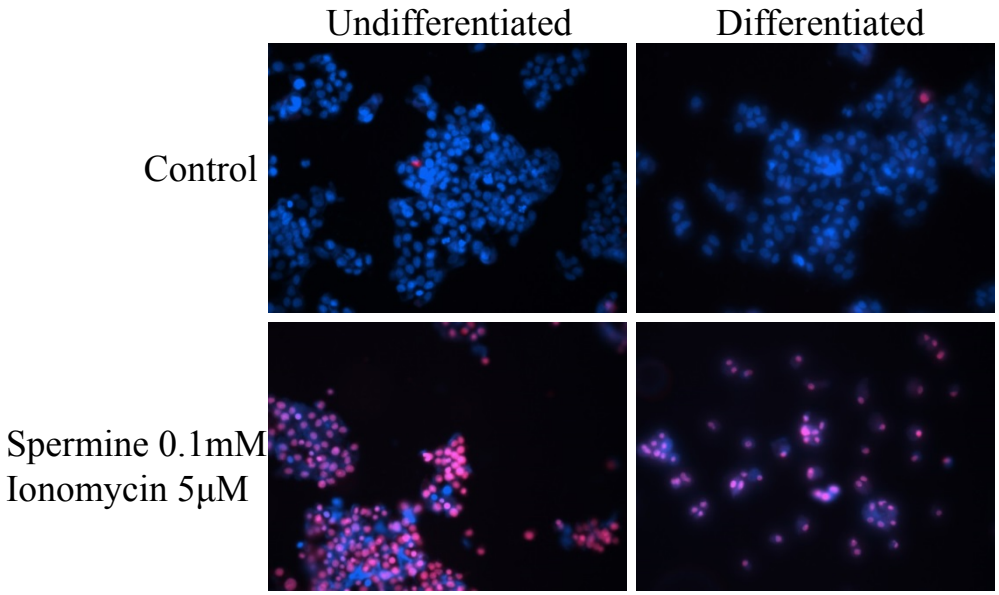


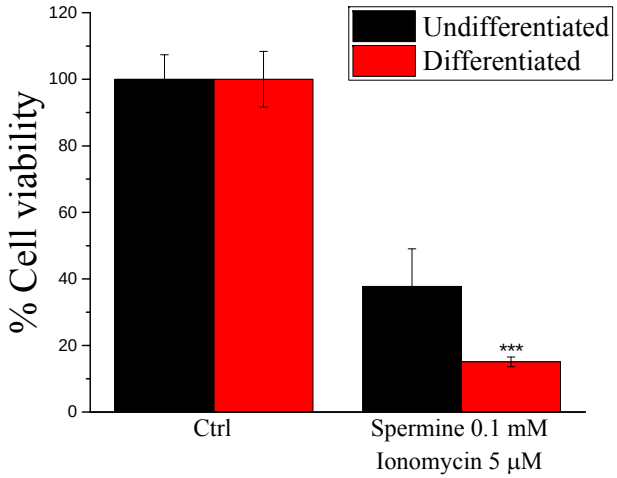
Supplementary Figure 3. Lack of effect of FCCP on HepG2 undifferentiated and differentiated cells. Histogram showing Rhod-2 fluorescence before and after prefunding FCCP 20 μM, in both undifferentiated and differentiated HepG2 cells. Cells were previously treated for 5 min with ionomycin 5μM. Data is expressed as a percentage of the initial fluorescence, before any treatment, at time=0 sec. Data is showed as mean ± SEM, n=10 cells.

Supp. Fig. 4A, B, C, D.



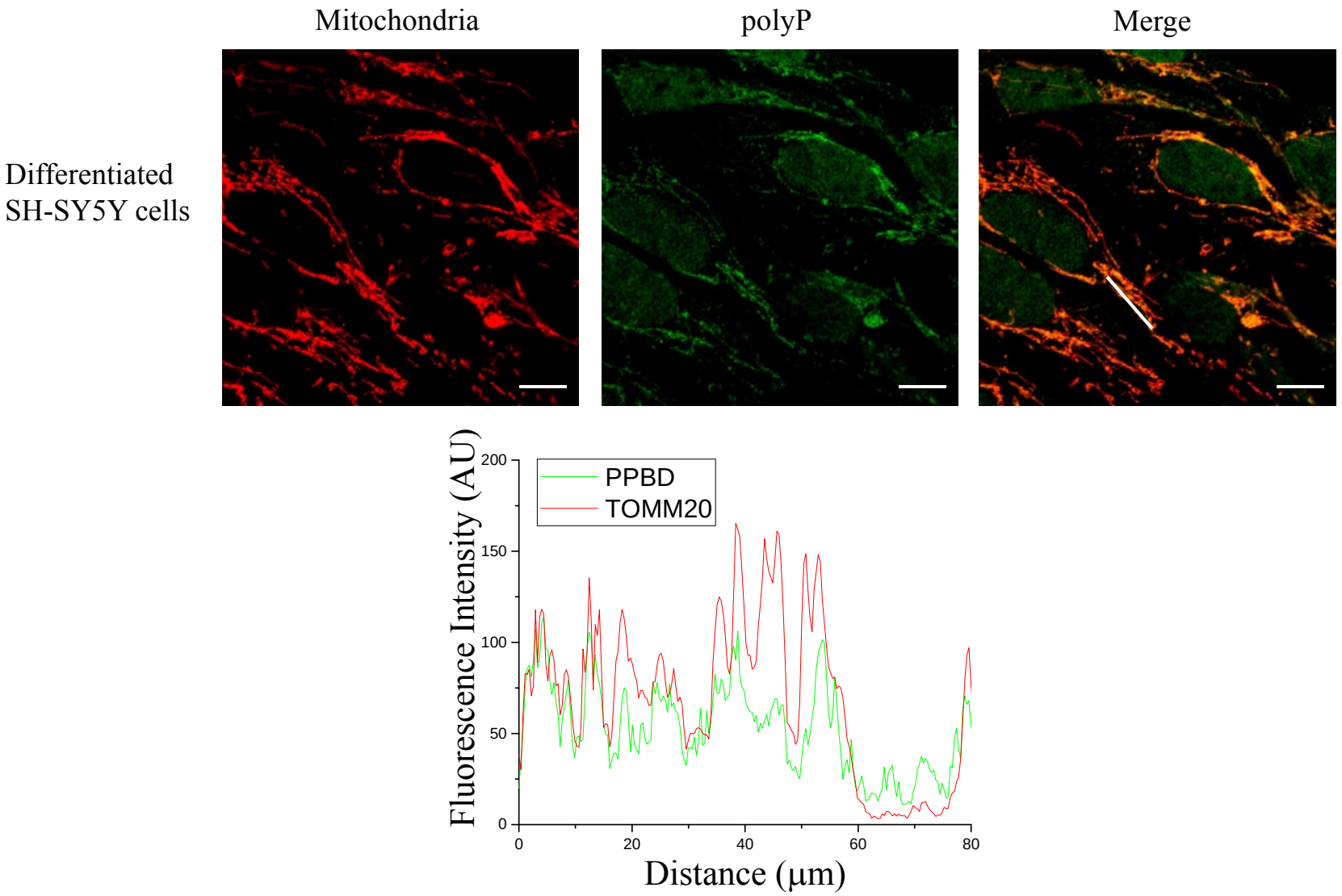
Supp. Fig. 4E



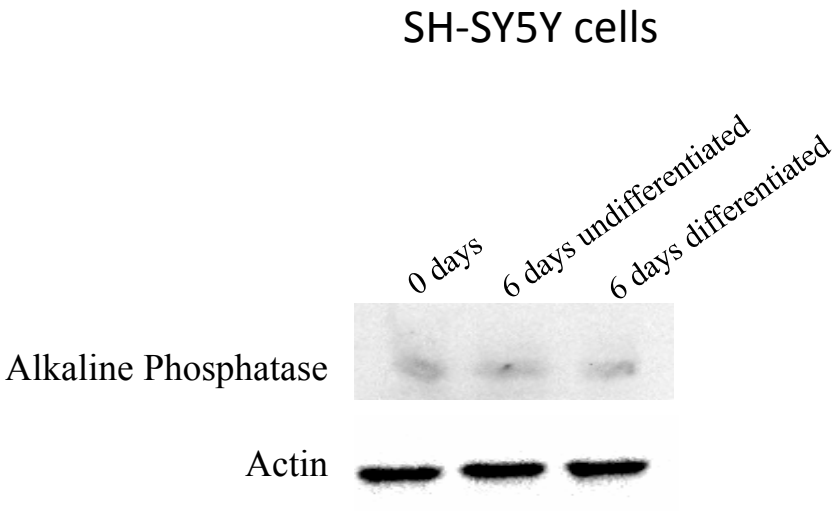


Supplementary Figure 4. Spermine effect on $[Ca^{+2}]_{mito\text{-}free}$ dynamics. Graphs showing real-time measurement of (**A**) mitochondrial (Rhod-2) and (**B**) cytoplasmic (Fluo-4) fluorescence, in response to the perfusion of spermine 0.1 mM, followed by the ionomycin 5 µM. Every point is the average of, at least, ten individual cells from the same experiment. Data are shown as mean±SEM. Bar graph showing (**C**) Rhod-2 and (**D**) Fluo-4 signal after 300 sec and 450 sec (spermine was perfunded after 180 sec and spermine and ionomycin after 360 sec) on undifferentiated and differentiated HepG2 cells. Data show combined average fluorescence values from, at least, ten cells from, at least, three independent glasses, mean ± SEM. Student's t-test: ***p<0.001. **E.** Representative images of undifferentiated and differentiated HepG2 cells, loaded with propidium iodide and with Hoechst 333258, after 3 h of treatment with spermine and ionomycin. Note the dramatic increase in cell death induced by the treatment, confirmed by the MTT-cell viability assay. Data shown as average of at least ten cells per experiment from, at least, three independent experiments ± SEM. Student's test: ***p<0.001.

Supp. Fig. 5A


Mitochondria
polyP
Merge
Differentiated
SH-SY5Y cells
PPBD
TOMM20
Fluorescence Intensity (AU)
Distance (μm)
0
50
100
150
200
0
20
40
60
80

Supp. Fig. 5B



Supplementary Figure 5. Alkaline phosphatase expression in SH-SY5Y neuroblastoma cells. A. Representative confocal images of the double immunocytochemistry assay against polyP (PPBD, green) and mitochondria, (TOMM20, red) in SH-SY5Y differentiated cells, shown the mitochondrial localization of PPBD. The histogram shows the fluorescence profile corresponding to the white line at the "Merge" image. Scale bar=5μm. **B.** Western blot showing same relative levels of alkaline phosphatase, in both undifferentiated and differentiated SH-SY5Y dopaminergic neurons.