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Supplemental information

Identification and functional validation of FDA-approved positive and negative modulators of the mitochondrial calcium uniporter

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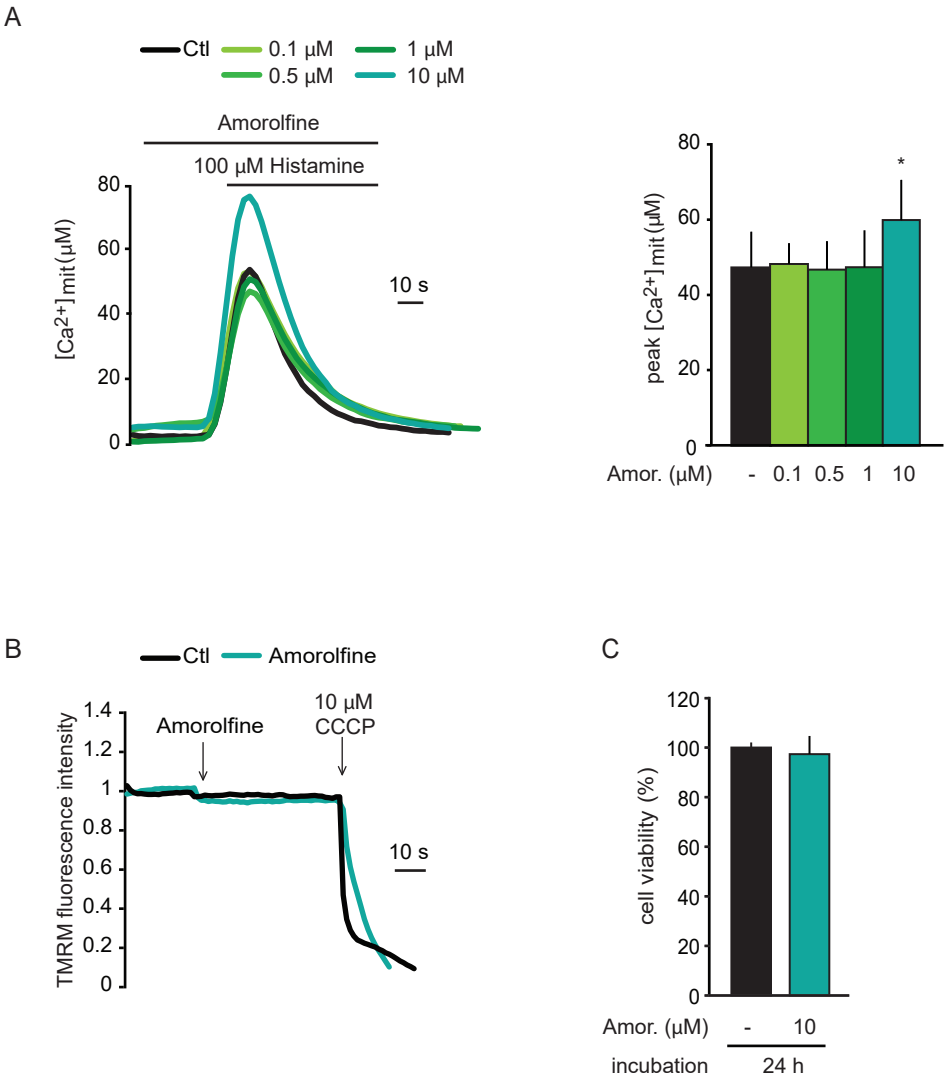

Figure S1. Effects of amorolfine on HeLa cells. Related to Figure 2.
A) Agonist-induced mitochondrial Ca^{2+} uptake in intact HeLa cells. Cells were treated with amorolfine or DMSO before (30 s) and during histamine stimulation. Left: representative traces. Right: mean $[Ca^{2+}]$mit peaks.
B) $\Delta\Psi_m$ measurements in HeLa cells treated with amorolfine or DMSO. At the end of the experiment CCCP was added to dissipate $\Delta\Psi_m$.
C) HeLa cell viability after amorolfine treatment. HeLa cells were treated with amorolfine or DMSO for 24 h and then incubated for 1 h with MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium]. During incubation, the metabolically active cells convert MTS into its reduced form formazan, which is released in the extracellular medium and has maximal absorbance at 490nm. The percent of viable cells was calculated by measuring the absorbance of cell media at 490nm.

Data are presented as mean ± SD. *p<0.05, Student's two-tailed t-test except one-way ANOVA for panel A.

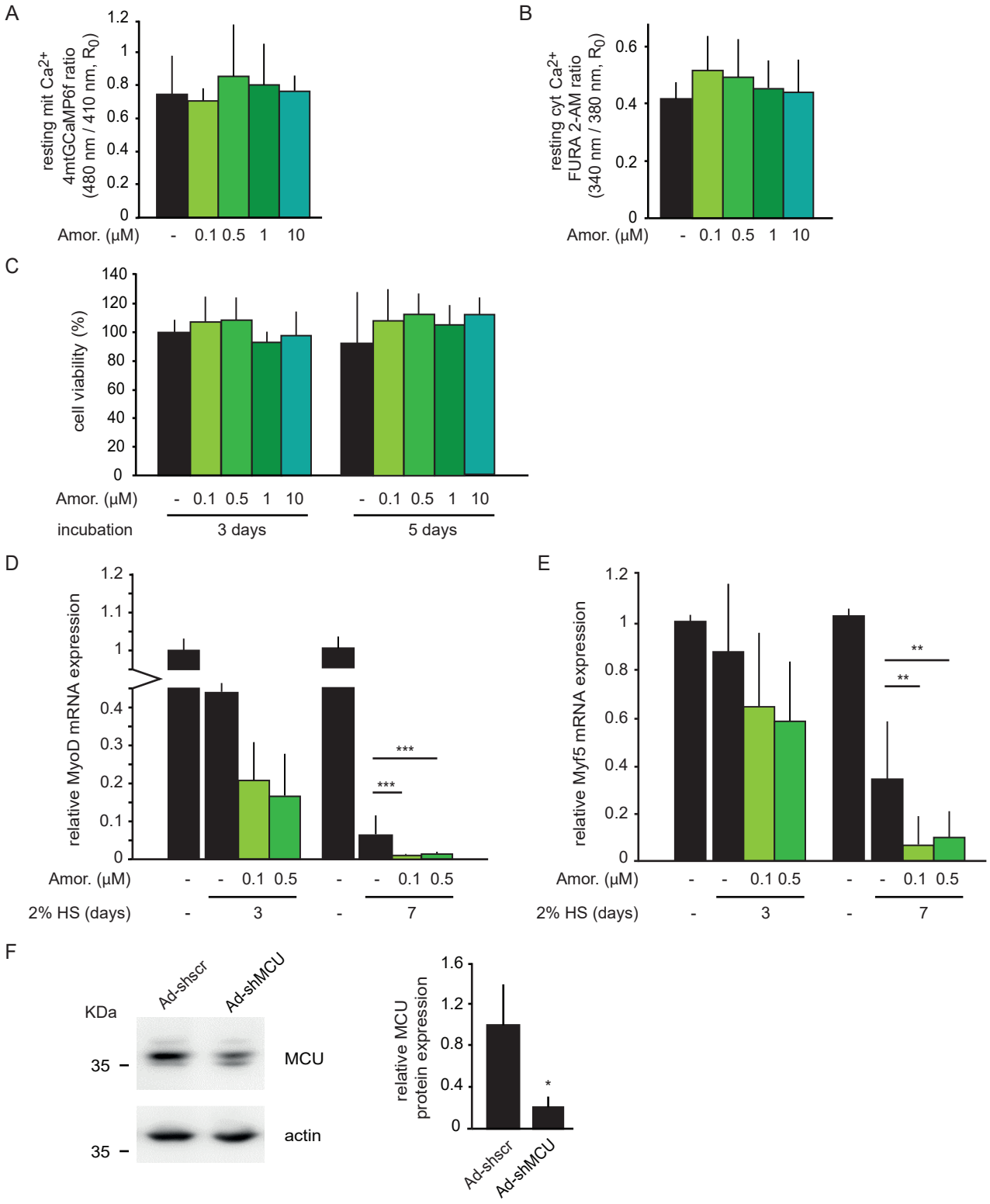

Figure S2. Amorolfine promotes C2C12 differentiation. Related to Figure 3.

A) Resting mitochondrial Ca^{2+} levels of C2C12 myoblasts. Cells were treated immediately before detection with amorolfine or DMSO and resting mitochondrial Ca^{2+} was measured.

B) Resting cytosolic Ca^{2+} levels of C2C12 myoblasts. Cells were treated immediately before detection with amorolfine or DMSO and resting cytosolic Ca^{2+} was measured.

C) C2C12 myoblasts viability after amorolfine treatment. C2C12 cells treated with amorolfine or DMSO for 3 or 5 days were incubated for 1 h with MTS. The percent of viable cells was calculated as reported in S1C.

D) Relative MyoD mRNA expression in C2C12 after amorolfine incubation. mRNA was isolated from C2C12 myoblasts from myotubes treated with amorolfine or DMSO. Gene expression was measured by real-time RT-PCR analysis. GAPDH was used as control.

E) Relative Myf5 mRNA expression in C2C12 after amorolfine incubation. mRNA was isolated from C2C12 myoblasts from myotubes treated with amorolfine or DMSO. Gene expression was measured by real-time RT-PCR analysis. GAPDH was used as control.

F) Western blot analysis of MCU in C2C12 myotubes infected with Ad-shMCU or Ad-shscr as control. Actin was used as loading control. Left: representative images. Right: quantification.

Data are presented as mean ± SD. *p<0.05, **p<0.01, ***p<0.001, two-way ANOVA except one-way ANOVA for panels A and B, and Student's two-tailed t-test for panel F.

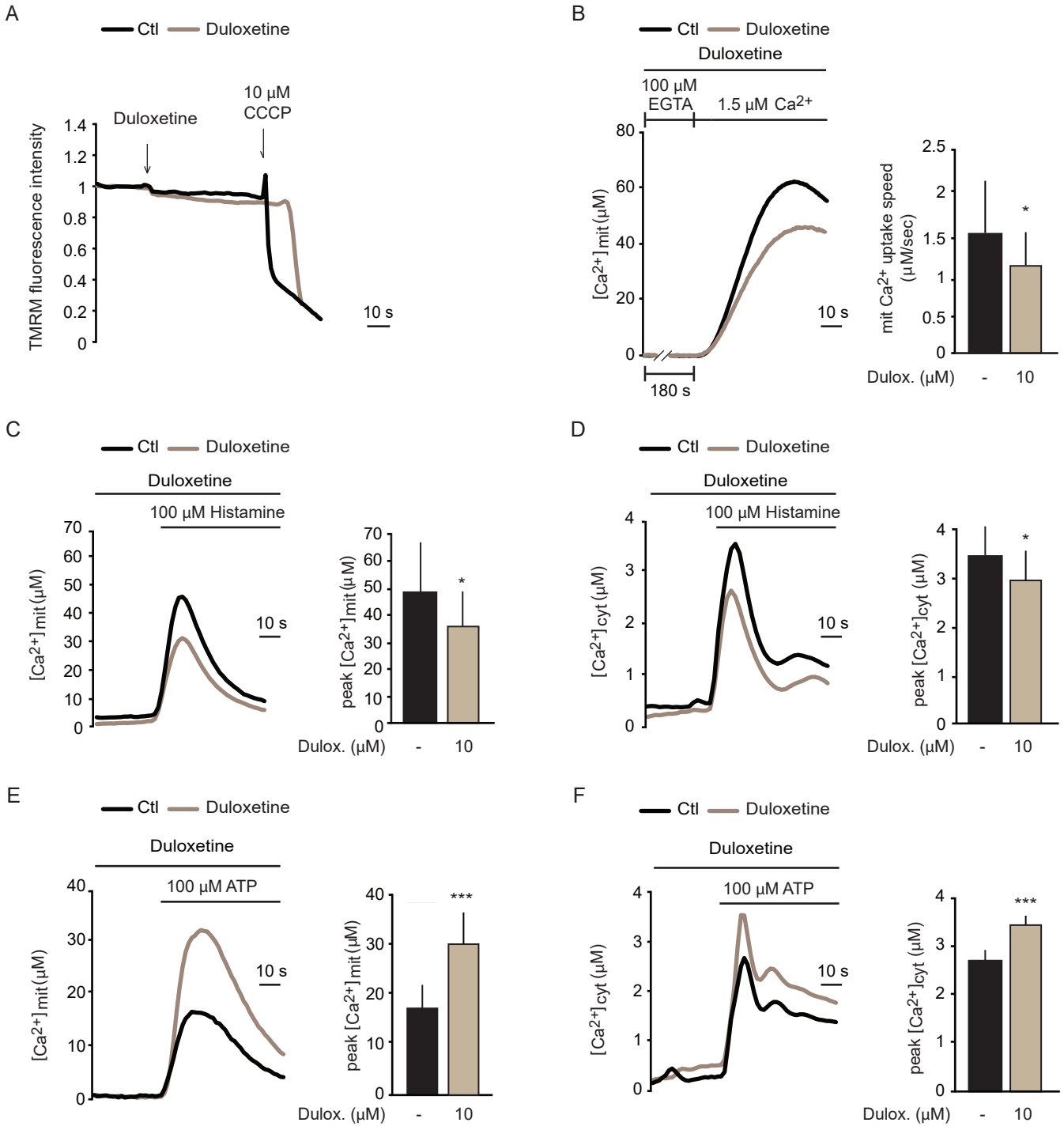

Figure S3. Duloxetine impinges on global Ca^{2+} homeostasis. Related to Figure 5.

A) $\Delta\Psi_m$ measurements in HeLa cells upon treatment with duloxetine or DMSO. CCCP was added at the end of the experiment to dissipate $\Delta\Psi_m$.

B) Mitochondrial Ca^{2+} uptake measurements in permeabilized HeLa cells treated with duloxetine or DMSO before (180 s) and during Ca^{2+} perfusion. Left: representative traces. Right: mean $[Ca^{2+}]$mit speed.

C) Agonist-induced mitochondrial Ca^{2+} uptake in intact HeLa cells. Cells were treated with duloxetine or DMSO before (30 s) and during histamine stimulation. Left: representative traces. Right: mean $[Ca^{2+}]$mit peaks.

D) Agonist-induced cytosolic Ca^{2+} transients in intact HeLa cells. Cells were treated with duloxetine or DMSO before (30 s) and during histamine stimulation. Left: representative traces. Right: mean $[Ca^{2+}]$cyt peaks.

E) Agonist-induced mitochondrial Ca^{2+} uptake in intact HeLa cells. Cells were treated with duloxetine or DMSO before (30 s) and during ATP stimulation. Left: representative traces. Right: mean $[Ca^{2+}]$mit peaks.

F) Agonist-induced cytosolic Ca^{2+} transients in intact HeLa cells. Cells were treated with duloxetine or DMSO before (30 s) and during ATP stimulation. Left: representative traces . Right: mean $[Ca^{2+}]$cyt peaks.

Data are presented as mean ± SD. *p<0.05, ***p<0.001, Student's two-tailed t-test except Mann-Whitney Rank Sum Test for panels A and D.

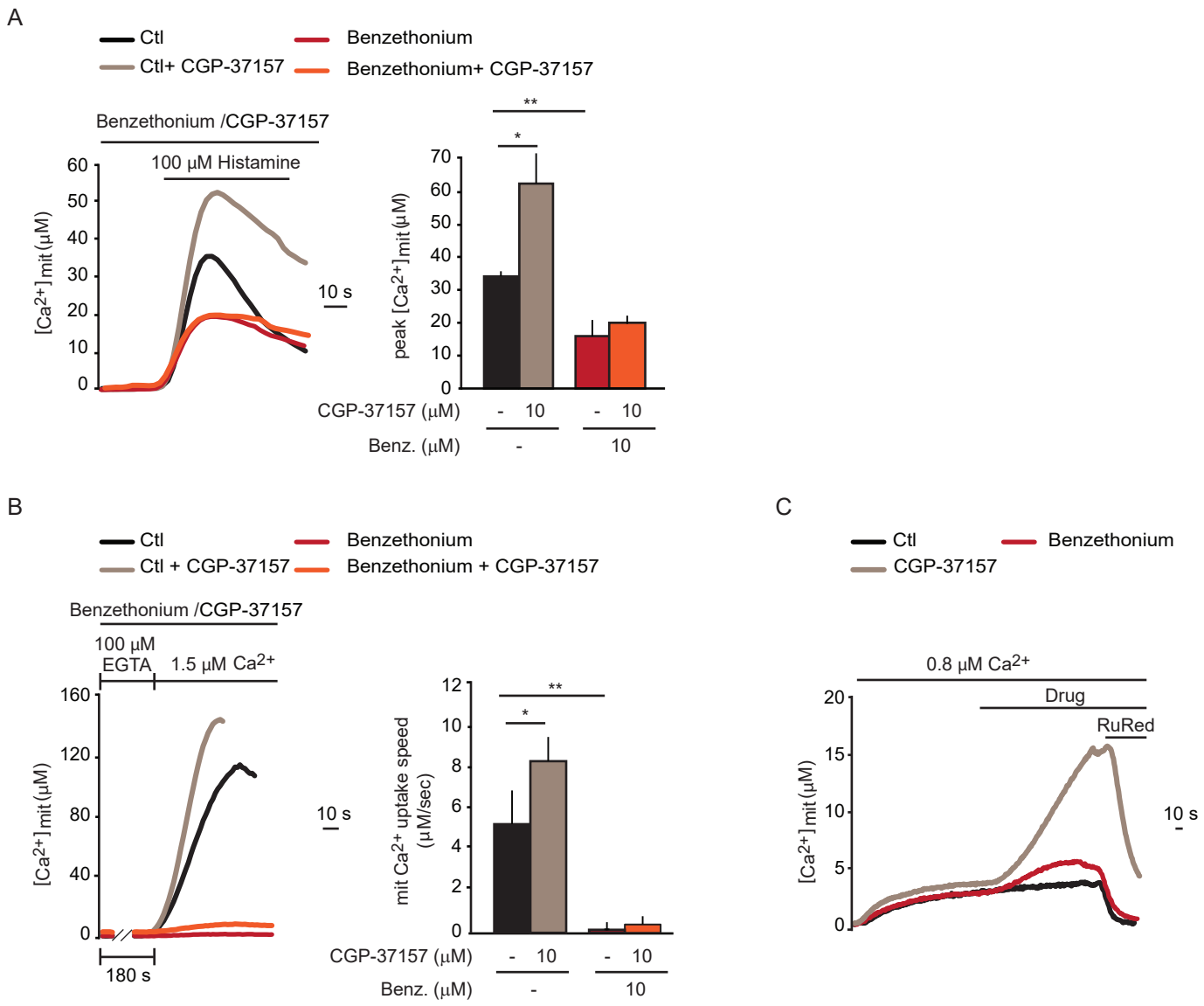

Figure S4. Benzethonium reduces mitochondrial Ca^{2+} accumulation independently on NCLX activity. Related to Figure 5.

A) Agonist-induced mitochondrial Ca^{2+} uptake in intact HeLa cells. Before measurements, cell were treated with benzethonium or DMSO for 1 h. Cells were then kept in benzethonium or DMSO with or without CGP-37157 before (30 s) and during histamine stimulation. Left: representative traces. Right: mean $[Ca^{2+}]$mit peaks.

B) Mitochondrial Ca^{2+} uptake measurements in permeabilized HeLa cells. Cell were treated with benzethonium or DMSO with or without CGP-37157 before (180 s) and during Ca^{2+} perfusion. Left: representative traces. Right: mean $[Ca^{2+}]$mit speed.

C) Mitochondrial Ca^{2+} uptake measurements in permeabilized HeLa cells in the presence Ca^{2+}. Once mitochondrial $[Ca^{2+}]$ reached the steady state, benzethonium, DMSO or CGP-37157 were added and maintained for 180 s. Ruthenium Red (10 µM) was finally added. Representative traces are reported.

Data are presented as mean ± SD. *p<0.05, **p<0.01, two-way ANOVA, except one-way ANOVA for panel C.

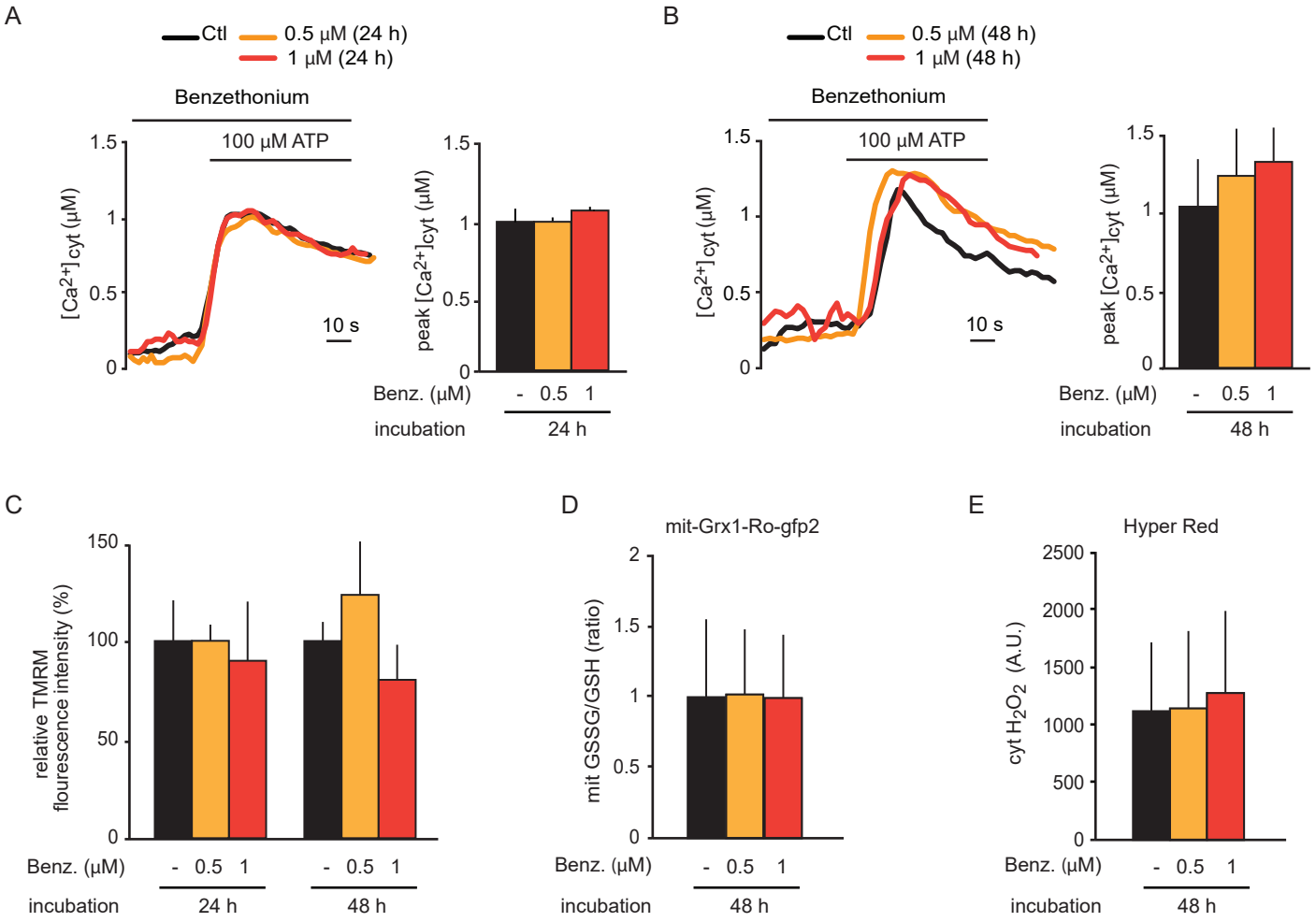

Figure S5. Benzethonium does not affect cytosolic Ca^{2+} transients, $\Delta\Psi_m$, GSSG/GSH ratio and cytosolic H_2O_2 in MDA-MB-231 cells. Related to Figure 6.

A) Agonist-induced cytosolic Ca^{2+} transients in MDA-MB-231 cells after 24 h benzethonium treatment. Before measurements, cell were treated with benzethonium or DMSO for 24 h. Cells were then kept in benzethonium or DMSO before (30 s) and during ATP stimulation. Left: representative traces. Right: mean $[Ca^{2+}]$cyt peaks.

B) Agonist-induced cytosolic Ca^{2+} transients in MDA-MB-231 cells after 48 h benzethonium treatment. Before measurements, cell were treated with benzethonium or DMSO for 48 h. Cells were then kept in benzethonium or DMSO before (30 s) and during ATP stimulation. Left: representative traces. Right: mean $[Ca^{2+}]$cyt peaks.

C) $\Delta\Psi_m$ measurements in MDA-MB-231 cells after benzethonium treatment. Cells were treated with benzethonium or DMSO.

D) Mitochondrial GSSG/GSH in MDA-MB-231 cells after benzethonium treatment. MDA-MB-231 cells were transfected with plasmids encoding pLPCXmitGrx1-roGFP2 and treated with benzethonium or DMSO. 48 h later mitochondrial GSSG/GSH was measured. Results are expressed as GSSG/GSH ratio.

E) Cytosolic H_2O_2 in MDA-MB-231 cells after benzethonium treatment. MDA-MB-231 cells were transfected with plasmids encoding Hyper Red and treated with benzethonium or DMSO. 48 h later cytosolic H_2O_2 was measured. Results are expressed as Hyper Red fluorescence.

Data are presented as mean ± SD. One-way ANOVA, except two-way ANOVA for panel C.