

Appendix

Ben-Kasus Nissim et al “Mitochondria control store-operated Ca²⁺ entry through Na⁺ and redox signals”

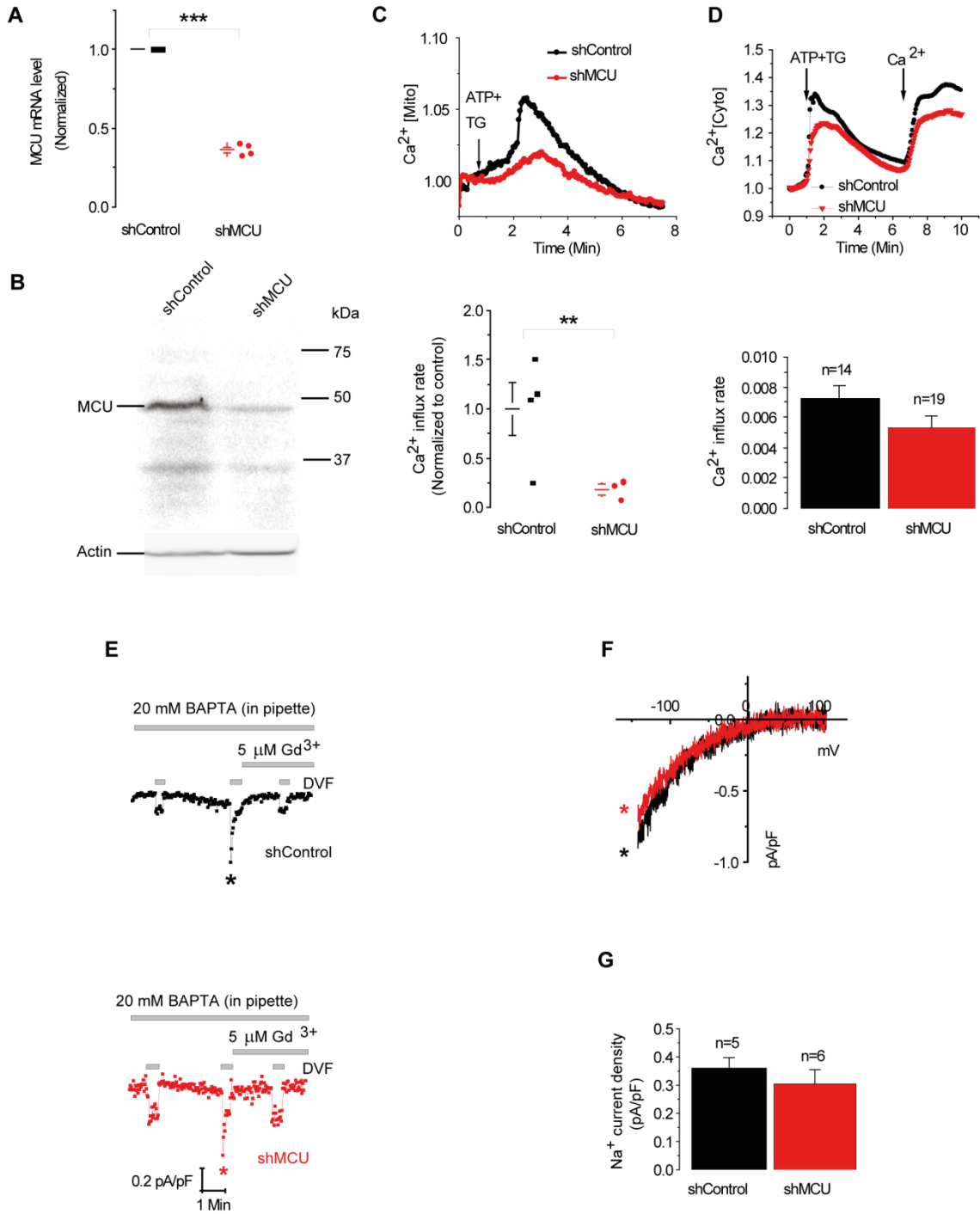
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Appendix Figure S1 – Effects of MCU Knockdown on mitochondrial Ca²⁺ transients, SOCE and CRAC in HEK293T cells.

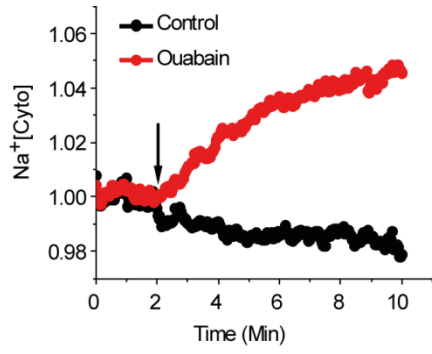
Appendix Figure S2 – Ouabain treatment triggers a cytosolic Na⁺ rise.

Appendix Figure S3 – CoroNa red and Mito Track Green colocalized in HEK293T cells.

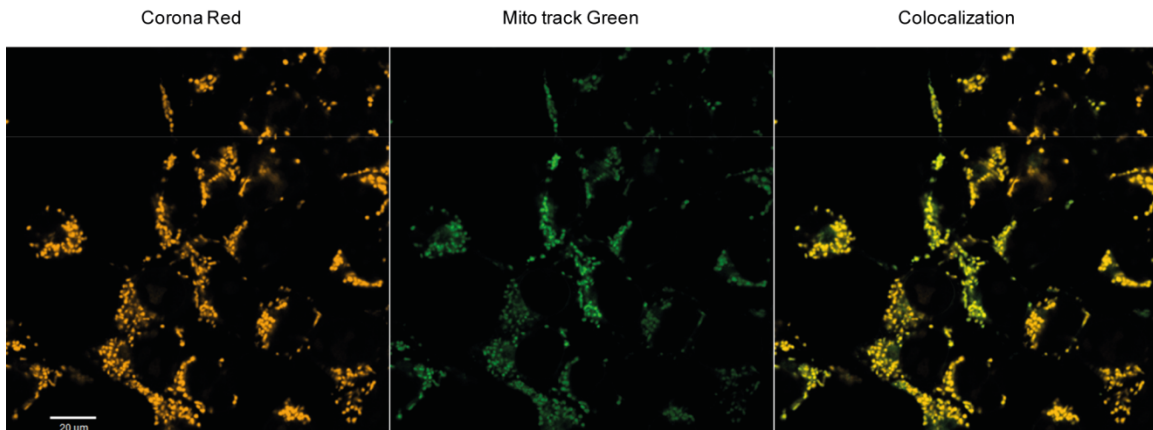
Appendix Figure S1.



Appendix Figure S2.



Appendix Figure S3.



Legends for Appendix Figures:

Appendix Figure S1. Effects of MCU Knockdown on mitochondrial Ca^{2+} transients, SOCE and CRAC in HEK293T cells. (A) Real-time PCR of MCU in shControl and shMCU treated cells. (B) Immunoblot of MCU in cells transfected with shMCU or shControl. (C) Rate of mitochondrial Ca^{2+} transients in shControl or shMCU treated cells expressing the mitochondrial Ca^{2+} sensor RP-mt. ATP and TG were added where indicated by the arrow. (C, upper panel), representative mitochondrial Ca^{2+} trace. (C, lower panel), rates of mitochondrial Ca^{2+} influx from several independent experiments. (D, upper panel), traces of cytosolic SOCE Ca^{2+} responses in HEK293T cells transfected with shMCU (red) vs. shControl (black) and loaded with Fura-2. Cells were treated as described in Fig 1B. (D, lower panel), averaged rates of Ca^{2+} rise in cells silenced MCU (n=19) vs. shControl transfected cells (n=14). (E) Electrophysiological recordings were performed on HEK293T cells transfected with shControl (black, upper panel) or shMCU (red, lower panel) and CRAC currents development are taken at -100 mV. (F), Representative I-V relationships of monovalent CRAC currents are taken from traces in (E) where indicated by color coded asterisks. (G), Statistical analysis on monovalent CRAC currents measured at -100 mV. **, p<0.01 ***, p<1E-03.

Appendix Figure S2. Ouabain treatment triggers a cytosolic Na^+ rise. HEK293T cells were loaded with the cytosolic Na^+ probe, Asante Natrium green. Then after, cells were superfused with Na^+ containing ringer solution (control) or with the same ringer containing Ouabain (100 μM). Ouabain is added where indicated by the arrow. Cells treated with Ouabain show a rise in cytosolic Na^+ .

Appendix Figure S3. CoroNa red and Mito Track Green colocalized in HEK293T cells. Cells were double stained with CoroNa red (1 μM) and Mito Track green (100 nM). Colocalization was shown using confocal microscopy. The scale bar represents 20 μm .