Supplementary information

The mitochondrial calcium uniporter is a multimer that can include a dominant-negative pore-forming subunit

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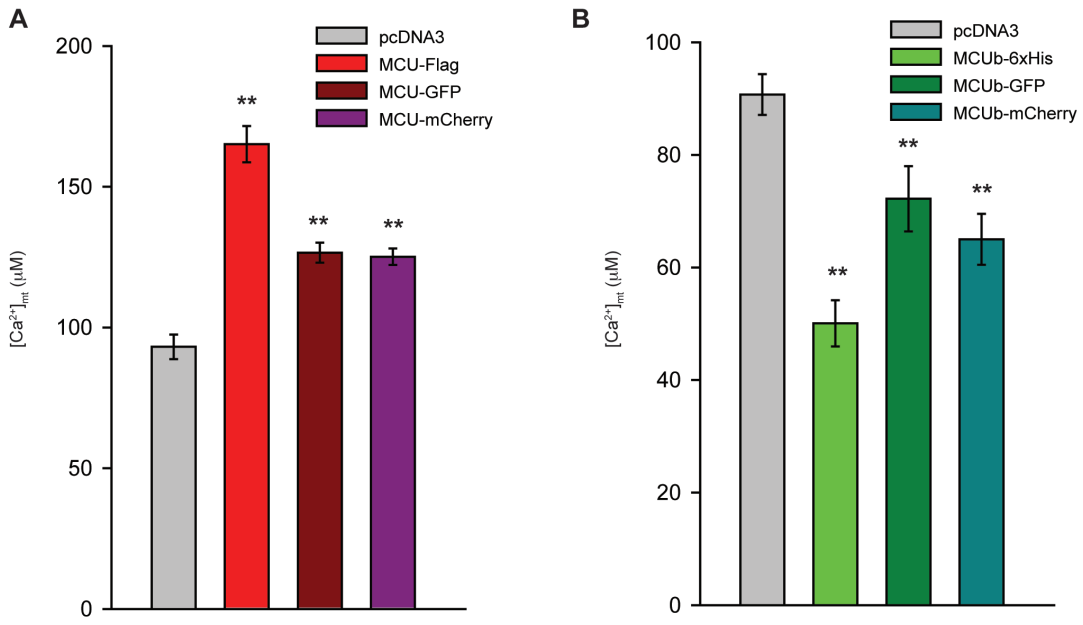
Supplementary table

Table S1

Experiment	Measurement	Mean ± S.E.M. *Mean ± S.D.	n	Figure
GFP + mCherry	% FRET efficiency	0.736 ± 0.980*	18	3C
MCU-GFP + MCU-mCherry	% FRET efficiency	9.333 ± 3.256*	18	3C
MCUb-GFP + MCUb-mCherry	% FRET efficiency	3.831 ± 1.660*	18	4B
MCU-GFP + MCUb-mCherry	% FRET efficiency	8.090 ± 3.700*	18	4B
MCUb-GFP + MCU-mCherry	% FRET efficiency	9.029 ± 4.151*	18	4B
siRNA-scrambled	$[Ca^{2+}]_{mt}$ peak value (μM)	75.410 ± 2.989	12	7A
siRNA-MCU	$[Ca^{2+}]_{mt}$ peak value (μM)	19.386 ± 2.696	12	7A
siRNA-MCUb	$[Ca^{2+}]_{mt}$ peak value (μM)	130.919 ± 4.882	16	7A
Control	$[Ca^{2+}]_{mt}$ peak value (μM)	90.511 ± 3.728	28	7B
MCU	$[Ca^{2+}]_{mt}$ peak value (μM)	157.610 ± 3.827	14	7B
MCUb	$[Ca^{2+}]_{mt}$ peak value (μM)	58.099 ± 2.807	35	7B
siRNA-scrambled	Ca^{2+} uptake speed (μmol/sec)	4.428 ± 0.664	8	7C
siRNA-MCU	Ca^{2+} uptake speed (μmol/sec)	1.102 ± 0.198	8	7C
siRNA-MCUb	Ca^{2+} uptake speed (μmol/sec)	5.638 ± 0.610	8	7C
siRNA-MCU + siRNA-MCUb	Ca^{2+} uptake speed (μmol/sec)	1.439 ± 0.0859	8	7C
Control	$[Ca^{2+}]_{mt}$ peak value (μM)	73.968 ± 7.485	5	7D
MCU	$[Ca^{2+}]_{mt}$ peak value (μM)	145.133 ± 13.974	5	7D
$MCU^{R252W,E257V}$	$[Ca^{2+}]_{mt}$ peak value (μM)	42.792 ± 4.742	11	7D
$MCU^{D260N,E263Q}$	$[Ca^{2+}]_{mt}$ peak value (μM)	34.953 ± 5.634	11	7D

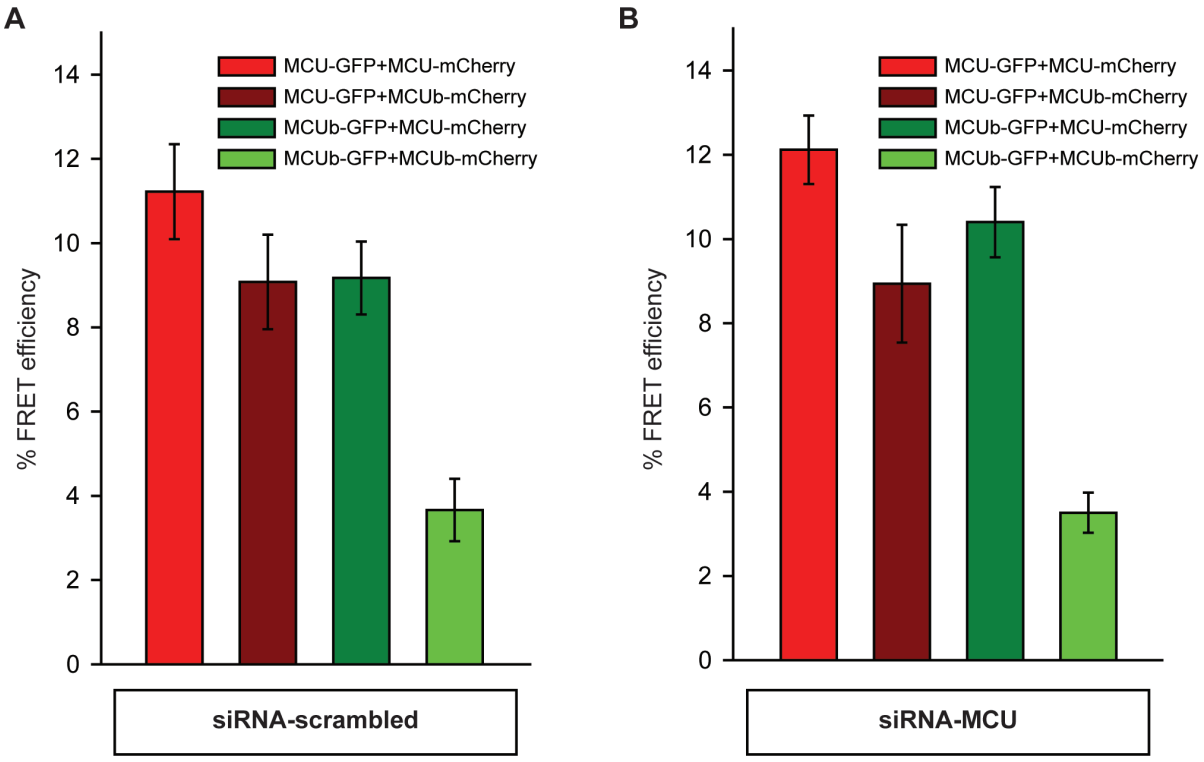
Table S1. Descriptive statistics for the experiments shown in main text figures.

Supplementary Figure 1



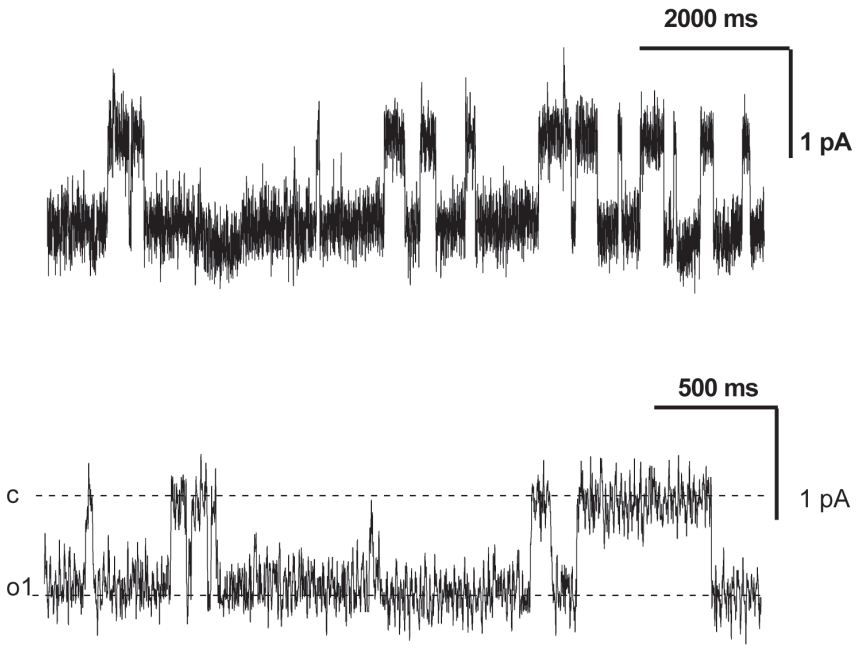
Supplementary Figure 1. (A) $[Ca^{2+}]_{mt}$ measurements in intact control and MCU-overexpressing HeLa cells with the indicated tags. Histograms indicate the mean peaks in $[Ca^{2+}]_{mt}$ after cell stimulation with 100 μM histamine (peak $[Ca^{2+}]_{mt}$ value: 93.2 μM ± 4.4, pcDNA3; 165.2 μM ± 6.4, MCU-Flag; 126.6 μM ± 3.5, MCU-GFP, 125.1 μM ± 2.9, MCU-mCherry; n >13; ** indicates $p<0.001$). (B) $[Ca^{2+}]_{mt}$ measurements in intact control and MCUb-overexpressing HeLa cells with the indicated tags. Histograms indicate the mean peaks in $[Ca^{2+}]_{mt}$ after cell stimulation with 100 μM histamine (peak $[Ca^{2+}]_{mt}$ value: 90.7 μM ± 3.6, pcDNA3; 50.1 μM ± 4.1, MCUb-6xHis; 72.2 μM ± 5.8, MCUb-GFP, 65.0 μM ± 4.5, MCUb-mCherry; n=8; ** indicates $p<0.001$).

Supplementary Figure 2



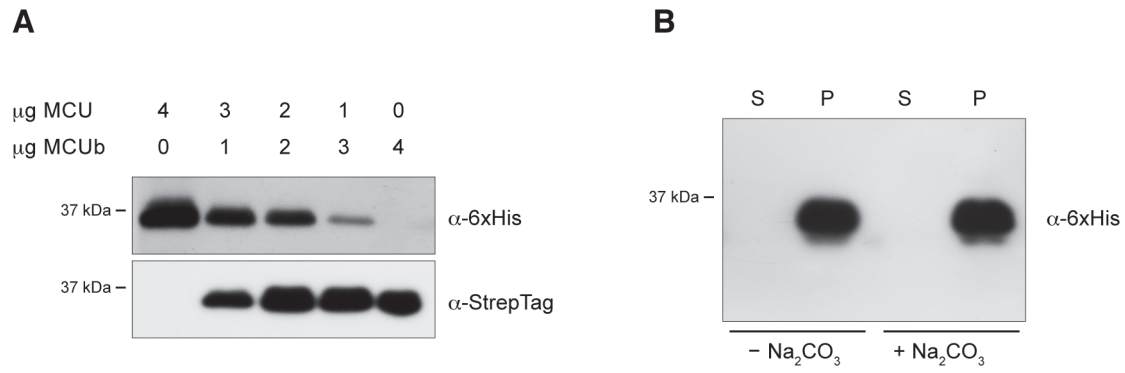
Supplementary Figure 2. HeLa cells were transfected with a siRNA-scrambled (A) or siRNA-MCU targeting the 3'UTR region (B) and analyzed after 72h. One day before the experiment, cells were transfected with the indicated FRET pair. Images of donor and acceptor were taken before and after photobleaching as in figures 3 and 4. FRET was calculated as detailed in the experimental procedures. Histogram bar diagram shows FRET efficiency of the indicated donor and acceptors pair (for siRNA-scrambled: 11.22 ± 1.13, MCU-GFP + MCU-mCherry; 9.08 ± 1.12, MCU-GFP + MCUb-mCherry; 9.17 ± 0.87, MCUb-GFP + MCU-mCherry; 3.66 ± 0.74, MCUb-GFP + MCUb-mCherry; for siRNA-MCU: 12.12 ± 0.81, MCU-GFP + MCU-mCherry; 8.94 ± 1.40, MCU-GFP + MCUb-mCherry; 10.40 ± 0.83, MCUb-GFP + MCU-mCherry; 3.50 ± 0.48, MCUb-GFP + MCUb-mCherry; n= 15).

Supplementary Figure 3



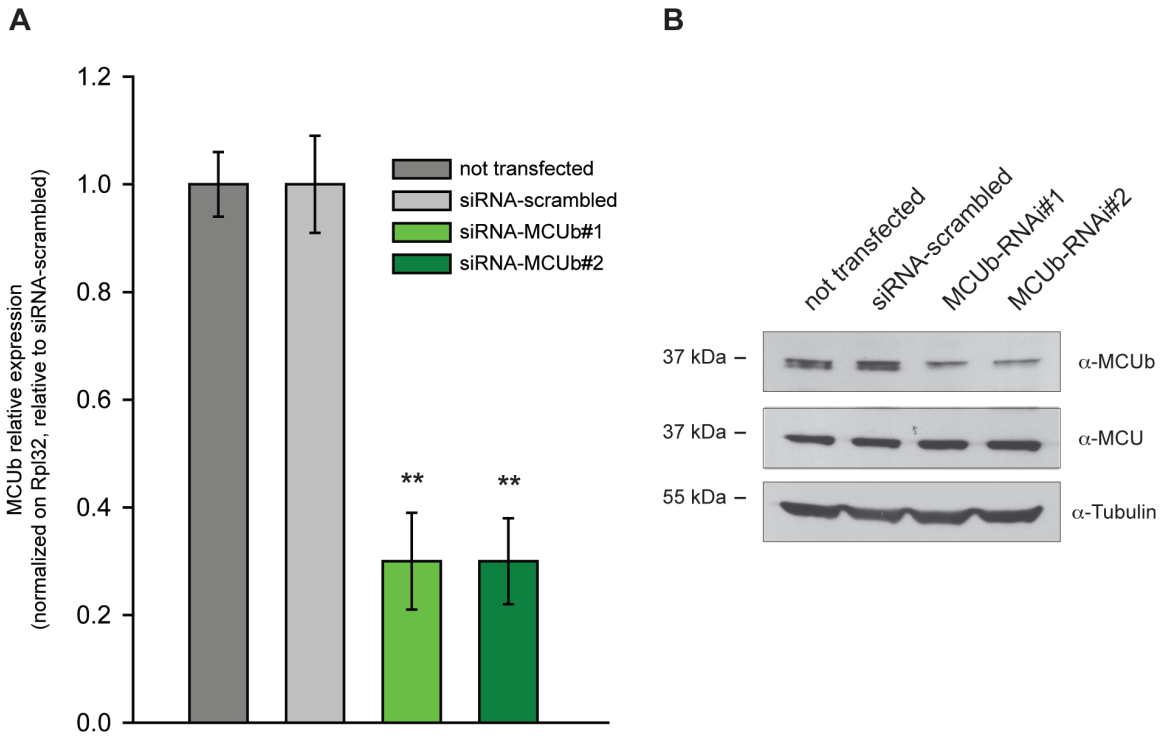
Supplementary Figure 3. MCUb activity in low divalent sodium gluconate. Representative current recordings are shown (V = -60 mV). Mean conductance is 18 pS. Similar data were obtained in other four experiments.

Supplementary Figure 4



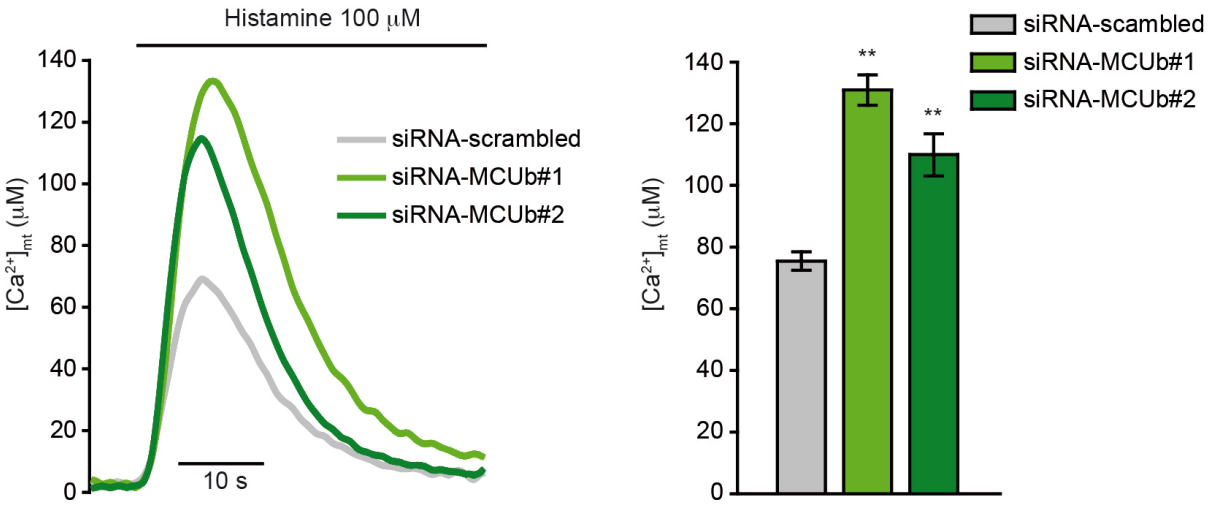
Supplementary Figure 4. (A) MCU/MCUb proteoliposomes. MCU and MCUb were *in vitro* expressed by using different plasmidic DNA ratios and incorporated into proteoliposomes. Equal amount of liposomes were loaded on SDS-PAGE and blotted with anti-6xHis (for MCU) and anti-StrepTag (for MCUb) antibodies. Densitometric analysis gave an approximate quantity of MCU homo-oligomers added in the bilayer experiments equal to 2.5 pmoles. In the case of co-expression 3:1 the estimated protein quantities in 10 μl liposomes are 1.75 and 1 pmoles for MCU and MCUb respectively. When co-expression was performed using plasmids in 2:2 ratio the protein concentrations were equal. (B) Alkaline extraction on MCU-proteoliposomes. After reconstitution, proteoliposomes were subjected to alkaline extraction to check for the correct insertion of the MCU protein. S and P: supernatant and pellet (liposomes). Blot was developed with anti-6xHis antibody.

Supplementary Figure 5



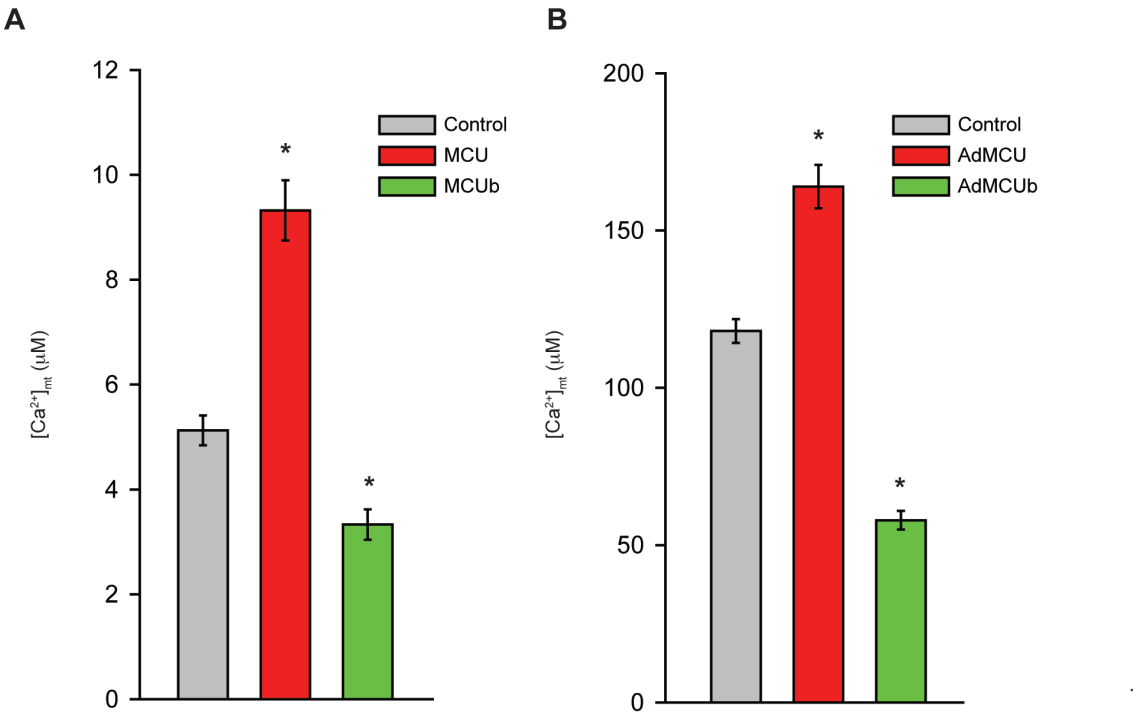
Supplementary Figure 5. Effect of two different MCUb siRNAs on (A) MCUb mRNA and (B) MCUb protein level. MCUb was silenced using two different siRNA. (A) HeLa cells were harvested 48 hours after transfection and MCUb mRNA expression was tested by Quantitative Real Time PCR using specific primers for MCUb and normalized for Rpl32 expression. Values represent the mean of three independent experiments ± S.D. (B) Hela cells were either not transfected or transfected for 72 hours with either scrambled, siRNA-MCUb#1 or siRNA-MCUb#2. Cells were harvested, total protein were extracted and subjected to Western blotting analysis with antibodies anti-MCUb, anti-MCU and anti-β-Tubulin as loading control. ** $p<0.001$.

Supplementary Figure 6



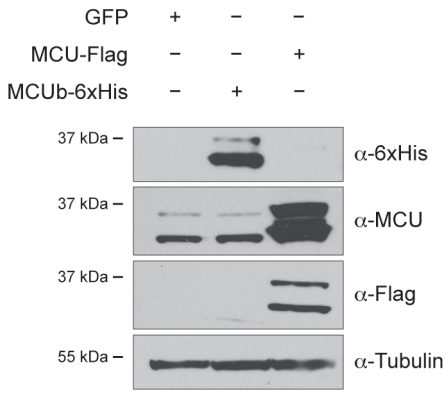
Supplementary Figure 6. Effect of two different MCUb siRNAs on mitochondrial Ca^{2+} transients. The MCUb siRNAs were transfected 48 h before the aequorin measurements. Aequorin luminescence data were collected and converted into $[Ca^{2+}]$ values as detailed in the experimental procedures. MCUb silencing by both siRNAs drastically increase the $[Ca^{2+}]_{mt}$ rise evoked by histamine stimulation (peak $[Ca^{2+}]_{mt}$ value: 75.41 μM ± 2.99, scrambled; 130.92 μM ± 4.88, siRNA-MCUb#1; 109.93 μM ± 6.79, siRNA-MCUb#2; n = 10). ** $p < 0.001$.

Supplementary Figure 7



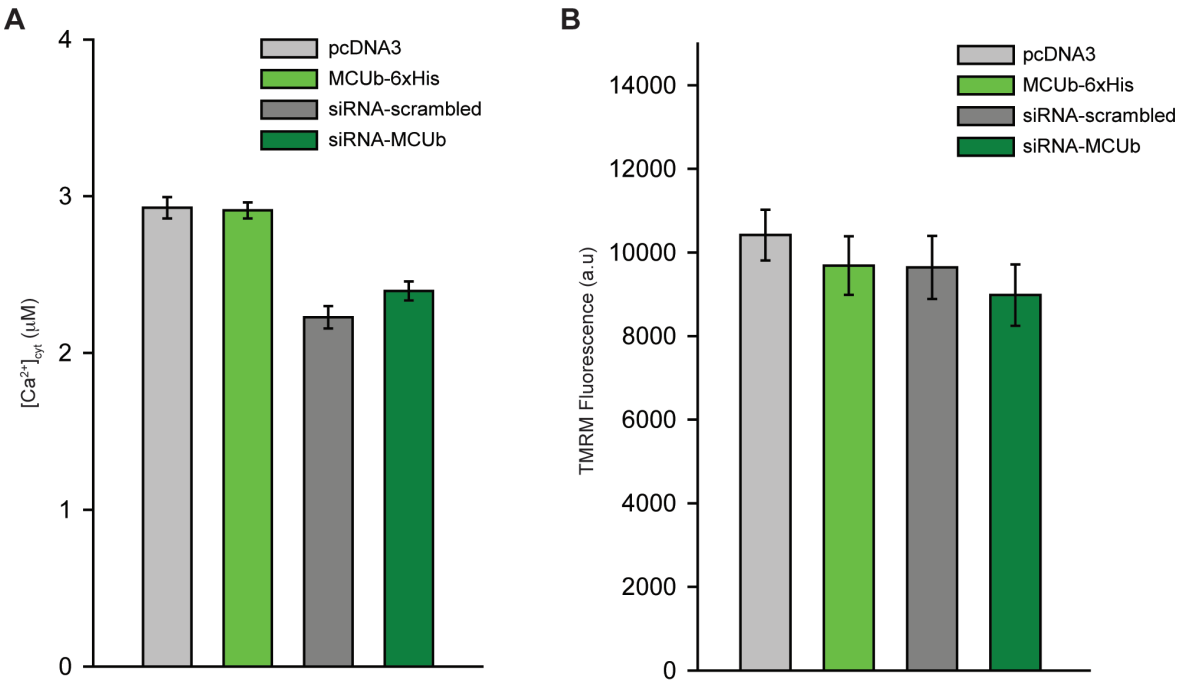
Supplementary figure 7. (A) $[Ca^{2+}]_{mt}$ measurements in intact control, MCU- and MCUb-overexpressing HEK 293 cells. Histograms indicates the mean peaks in $[Ca^{2+}]_{mt}$ after cell stimulation with 100 μM ATP (peak $[Ca^{2+}]_{mt}$ value: 5.13 μM ± 0.28, pcDNA3; 9.32 μM ± 0.57, MCU-Flag; 3.33 μM ± 0.29, MCUb-6xHis; n = 8; * indicates $p<0.05$). (B) $[Ca^{2+}]_{mt}$ measurements in intact control, MCU- and MCUb-overexpressing neonatal mouse cardiac fibroblasts. Histograms indicates the mean peaks in $[Ca^{2+}]_{mt}$ after cell stimulation with 100 μM ATP (peak $[Ca^{2+}]_{mt}$ value: 118.1 μM ± 3.7, AdGFP; 164.0 μM ± 6.9, AdMCU; 57.9 μM ± 2.9, AdMCUb; n = 32; * indicates $p<0.05$).

Supplementary Figure 8



Supplementary Figure 8. MCUb overexpression does not alter MCU protein levels. HeLa cells were infected with the indicated adenoviruses. 24 hours after infection cells were harvested, total protein were extracted and subjected to Western blotting analysis with antibodies anti-6xHis, anti-MCU, anti-Flag and anti-β-Tubulin as loading control.

Supplementary Figure 9



Supplementary Figure 9. (A) $[Ca^{2+}]_{cyt}$ measurements in intact HeLa cells transfected with the indicated plasmid or siRNA. Histograms indicates the mean peaks in $[Ca^{2+}]_{cyt}$ after cell stimulation with 100 μM histamine (peak $[Ca^{2+}]_{cyt}$ value: 2.93 μM ± 0.07, pcDNA3; 2.91 μM ± 0.05, MCUb-6xHis; 2.23M ± 0.25, siRNA-scrambled, 2.39 μM ± 0.20, siRNA-MCUb; n =12). (B) Tetramethyl rhodamine methyl ester (TMRM) fluorescence measurements. HeLa cells were co-transfected with GFP and the indicated plasmid or siRNA. TMRM was loaded and fluorescence measurements were carried out as detailed in the methods section. The histogram represents the mean basal fluorescence intensities ± S.E.M. (fluorescence a.u.: 10416 ± 607, pcDNA3; 9643 ± 699, MCUb-6xHis; 9642 ± 755, siRNA-scrambled; 8980, siRNA-MCUb; n>40, p>0.48).