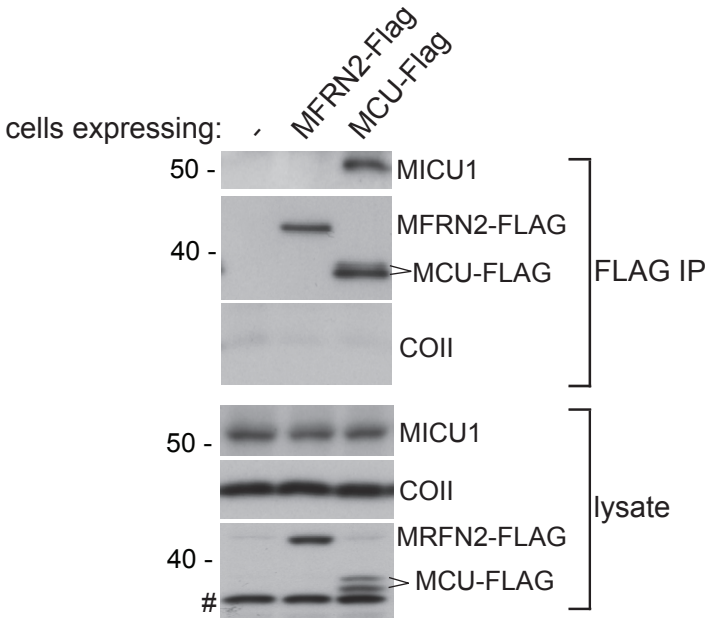
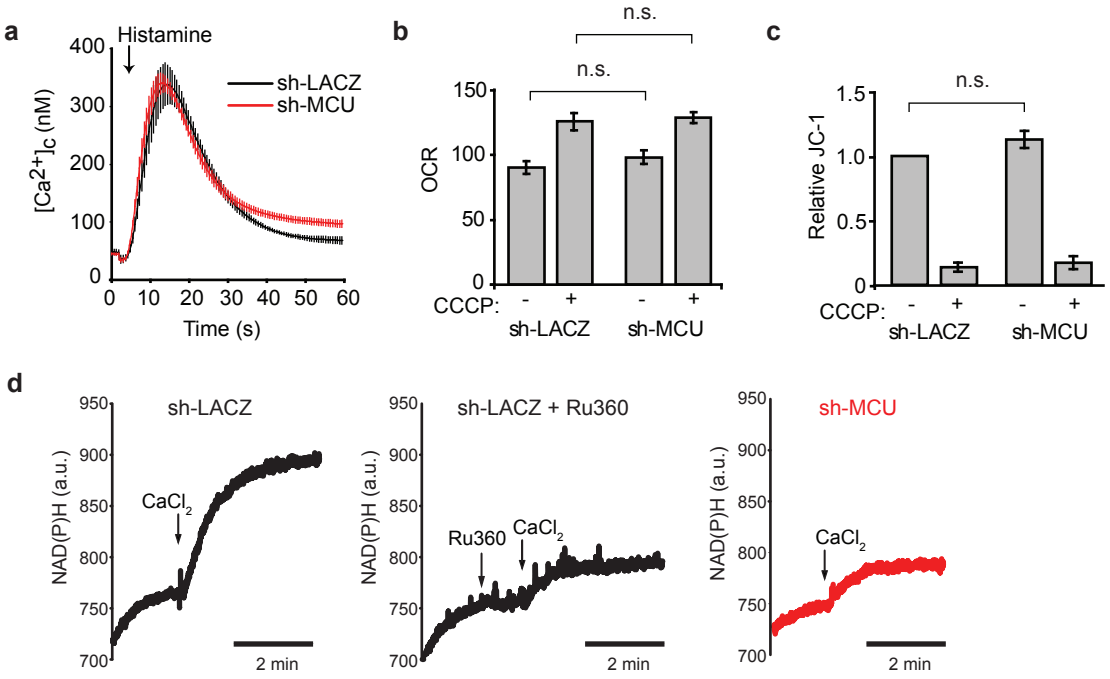
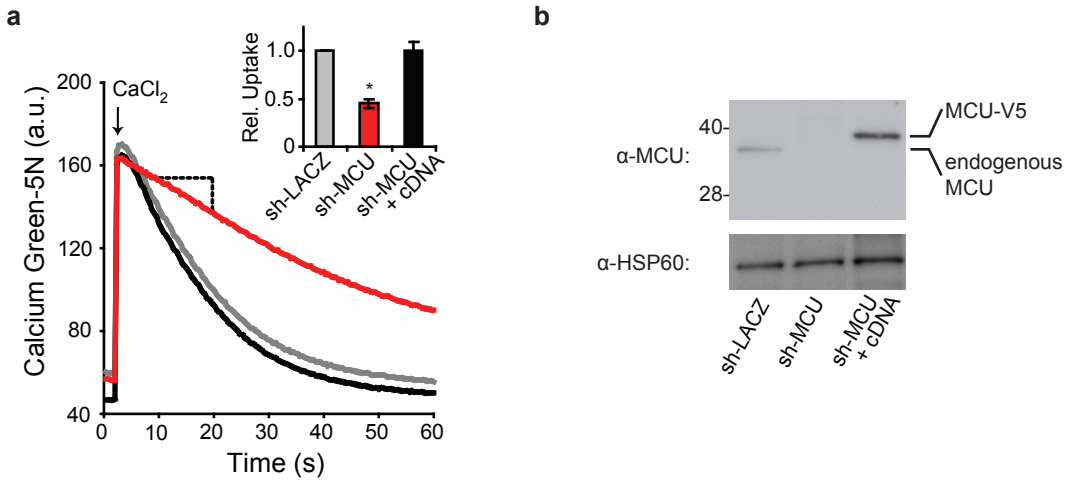
doi:10.1038/nature10234



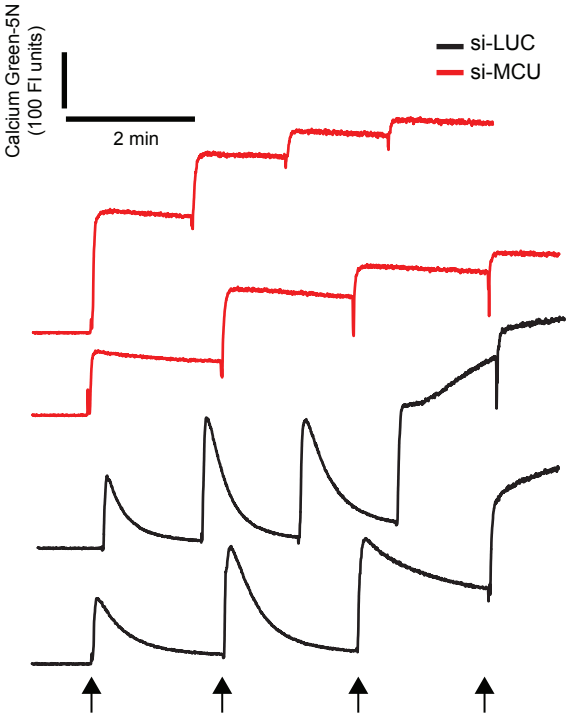
Supplementary Figure 1. Validation of MCU/MICU1 interaction using an independent tag
HEK-293 cells transiently transfected with MCU-FLAG expression plasmid or a control plasmid (MFRN2-FLAG) were lysed in the presence of 1% Triton-X-100 and subjected to anti-FLAG immunoprecipitation. Eluents and lysates were blotted with anti-FLAG, anti-MICU1 and control anti-COII antibodies. # indicates an unspecific band. Molecular weight markers (kilodalton) are indicated on the left.



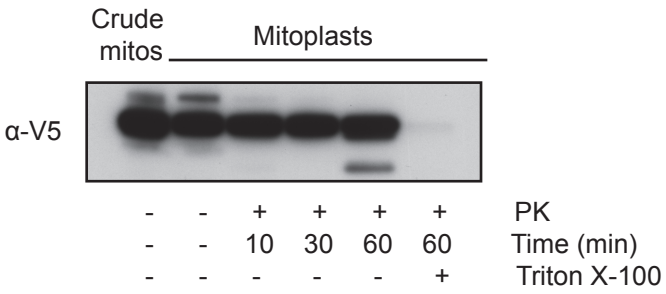
Supplementary Figure 2. Cytosolic Ca^{2+} kinetics, cellular respiration, membrane potential and NAD(P)H levels in MCU-silenced cells.
a, Cytosolic Ca^{2+} dynamics measured by Fluo-4 in populations of sh-LACZ and sh-MCU HeLa cells following treatment with 100 μM histamine (mean ± s.d., *n* = 3).
b, Basal and uncoupled oxygen consumption rate (OCR, pmol O_2 min^{-1}) in control sh-LACZ and knockdown sh-MCU HeLa cells (mean ± s.e.m., *n* = 10). Uncoupling was induced using 0.5 μM carbonyl cyanide m-chlorophenyl hydrazone (CCCP).
c, Mitochondrial membrane potential relative to control sh-LACZ HeLa cells measured by the JC-1 ratiometric dye in the presence or absence of the uncoupler CCCP (5 μM) (mean ± s.d., *n* = 8)
d, NAD(P)H fluoresence in permeabilized sh-LACZ or sh-MCU HEK-293 cells after addition of 50 uM $CaCl_2$. 1 uM Ru360 was added to HEK-293 cells as a positive control. a.u., arbitrary units.



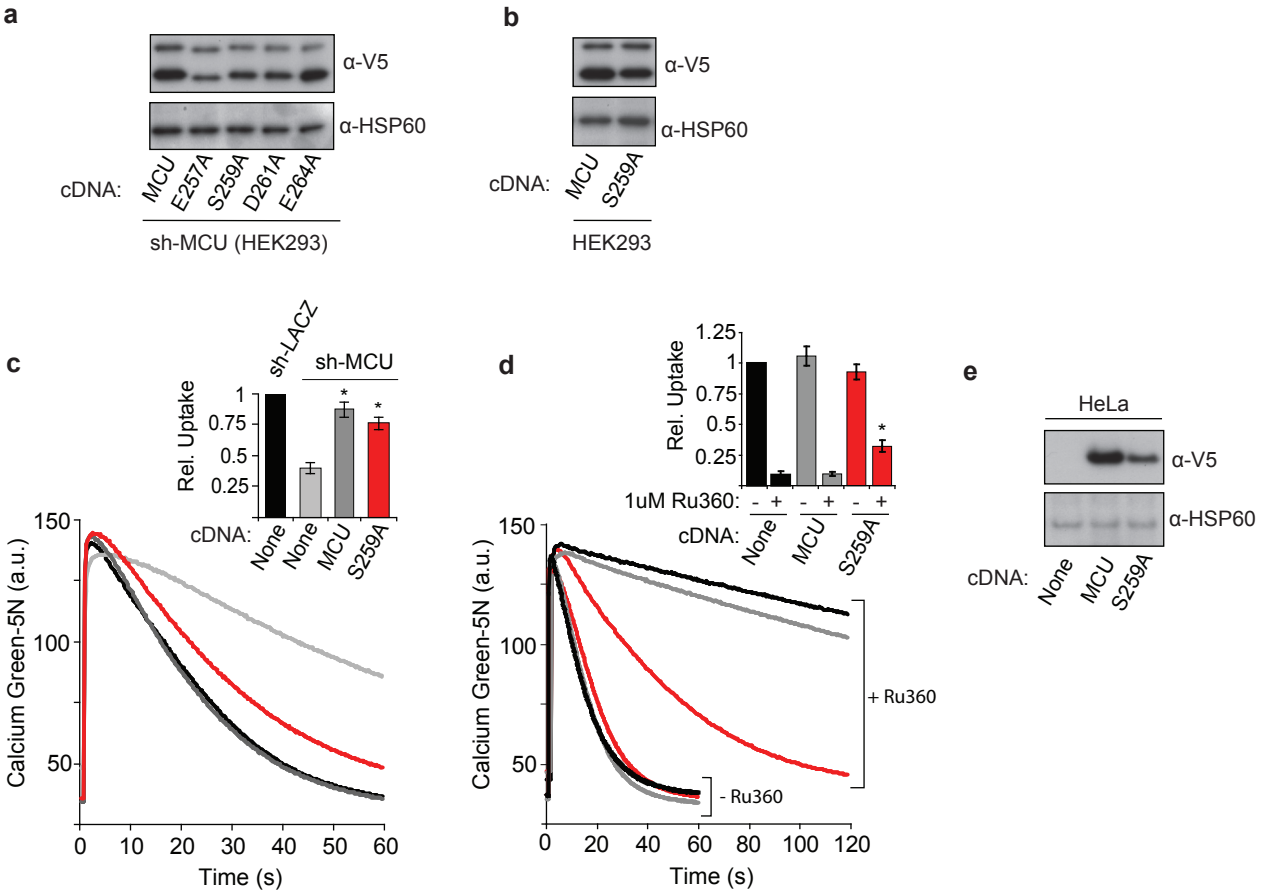
Supplementary Figure 3. Calcium uptake in permeabilized shMCU HeLa cells.
a, Representative traces of Ca^{2+} uptake kinetics in digitonin-permeabilized HeLa cells stably expressing sh-LACZ, sh-MCU or sh-MCU and RNAi-resistant MCU cDNA. Clearance of extra-mitochondrial Ca^{2+} was monitored over time by calcium green-5N fluorescent dye after addition of $CaCl_2$ (50 μM final concentration), in the presence of 2 μM thapsigargin. The dotted line indicates the linear portion used to derive relative rates of Ca^{2+} uptake. Inset: Mean Ca^{2+} uptake ± s.d. relative to sh-LACZ (n = 4, *P < .01).
b, anti-MCU immunoblot probing whole cell lysate samples collected before experiments in (a). MCU-V5 and endogenous MCU are indicated. anti-HSP60 is used as a loading control.



Supplementary Figure 4. Effect of *in vivo* silencing of MCU on Ca^{2+} uptake in mouse liver mitochondria. Representative traces of Ca^{2+} uptake in mitochondria isolated from livers of mice in which MCU was silenced (si-MCU). An siRNA duplex specific for the firefly luciferase gene (si-LUC) was used as a negative control. Clearance of extramitochondrial Ca^{2+} was monitored over time using the calcium green-5N fluorescent dye. Prior to each measurement mitochondria were energized for 2 minutes in presence of 5 mM glutamate/malate followed by multiple pulses of $CaCl_2$ (arrowheads) to a final concentration of 50 μM. Each trace is obtained from an independent mouse.



Supplementary Figure 5. MCU is a substrate for proteinase K. Immunoblot analysis of isolated mitoplasts from HEK-293 cells expressing MCU-V5 and treated with 40ug/ml proteinase K (PK) for the indicated times with or without 1% Triton X-100.



Supplementary Figure 6. Expression of MCU-V5 mutants in HEK-293 cells and Ru360 sensitivity of MCU-S259A in HeLa cells.

a, Immunoblotting of whole cell lysates from MCU-V5 expressing HEK-293 cell lines used in Figure 4b. All lines stably express sh-MCU and are transfected with either wild-type MCU-V5 or a mutant MCU-V5 (E257A, S259A, D261A, or E264A). Mitochondrial HSP60 is used as a loading control.

b, Immunoblotting of whole cell lysates from wild-type HEK-293 cells used in Figure 4c that are transiently over-expressing either wild-type MCU-V5 or the MCU-V5 S259A mutant.

c, Ca^{2+} uptake in permeabilized HeLa cells stably expressing either sh-LACZ (black), sh-MCU alone (light gray), or sh-MCU also expressing MCU-V5 (dark gray) or the MCU-V5 S259A mutant (red). Inset reports linear fits of uptake kinetics between 15s and 20s reported relative to sh-LACZ (mean ± s.e.m., $n = 6$, $^*P < .001$ compared to untransfected sh-MCU, two-tailed t-test).

d, Ca^{2+} uptake measurements in permeabilized HeLa cells stably expressing MCU-V5 or the MCU-V5 S259A mutant on a wild-type background. Inset reports linear fits of uptake kinetics between 15s and 30s for 1uM Ru360-treated samples and between 15s and 20s for untreated cells. (mean ± s.e.m., $n = 6$, $^*P < .001$ compared to untransfected cells treated with 1uM Ru360 using a two-tailed t-test).

e, Immunoblots of whole cell lysates from cell lines used in panel (d).