SUPPLEMENTAL INFORMATION

The supplemental data includes 5 supplemental figures with legends, 1 supplemental table, supplemental extended experimental procedures, and supplemental references.

Figure S1

A

MICU1

```
mfrlnslsal aelavgsrwy hggsqpiqir rrlmmvaflg asavtastgl
lwkrahaesp pcvdnlksdi gdkgknkdeg dvcnhekkta dlaphpeekk
kkrsgfrdrk vmeyenrira ystpdkifry fatlkvisep geaevfmtpe
dfvrsitpne kqpehlgldq yiikrfdgkt ekisqerekf adegsifytl
gecglisfsd yiflttvlst pqrnfeiafk mfdlngdgev dmeefeqvqs
iirsqtsmgm rhrdrpttgn tlksglcsal ttyffgadlk gkltiknfle
fqrklqhdvl kleferhdpv dgriterqfg gmllaysgvq skkltamqrq
lkkhfkegkg ltfqevenff tflknindvd talsfyhmag asldkvtmqq
vartvakvel sdhvcdvvfa lfdcdgngel snkefvsimk qrlmrglekp
kdmgftrlmq amwkcaqeta wdfalpkq
```

B

MICU1-Polybasic region

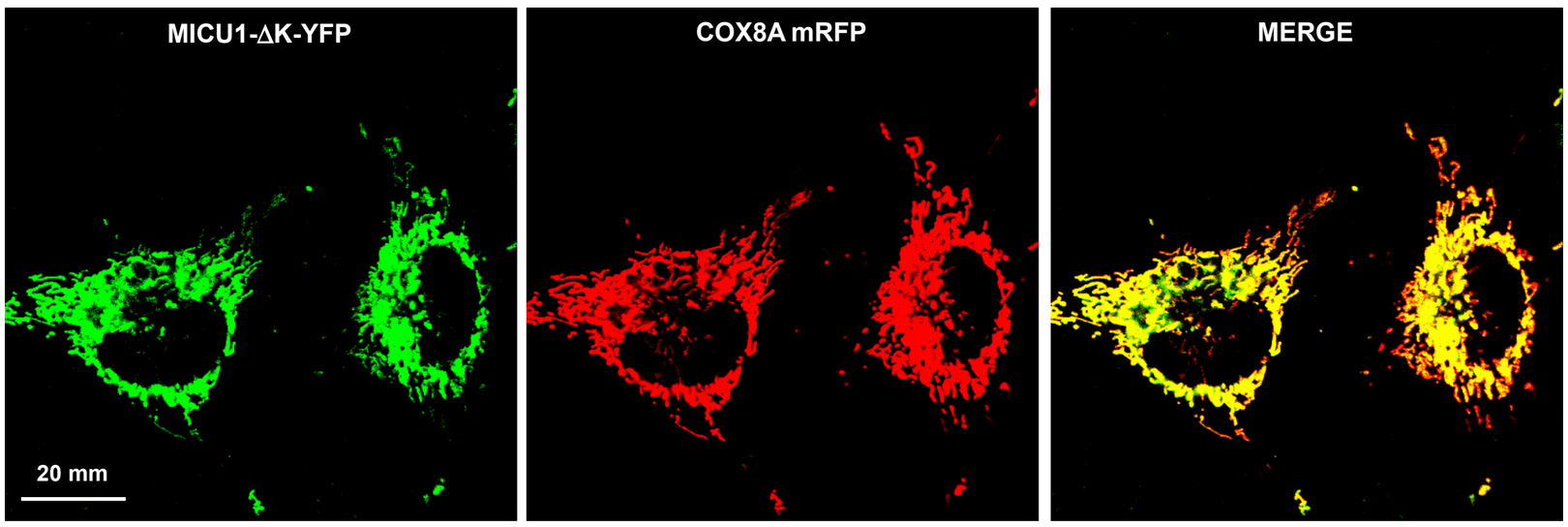
```
dvcnhekktadlaphpeekkkkrsgfrdrkvmeyenri- Human
dvcnhekktadlaphpeekkkkrsgfrdrkvmeyenri- chimpanzee
-sregraadaaaepypedkkkkrsgfrdrkvmeyenri- Mouse
cnaekkaagvclepypeekkkkrsgfrdrkvmeyenri- Bovine
hnhekkaadsclepcteekkkkrsgfrdrkvmeyenri- Horse
kdpdqavessdeeqpqegkkkkmrvgfrdrkvmeyenri- X. Laevis
----sssgededeagseekkkkqrigfrdrkvmeyenri- Zebrafish
sedsedeeagsdlhlhegkkirekvgfrerkiieyenri- D.Melagaster
```

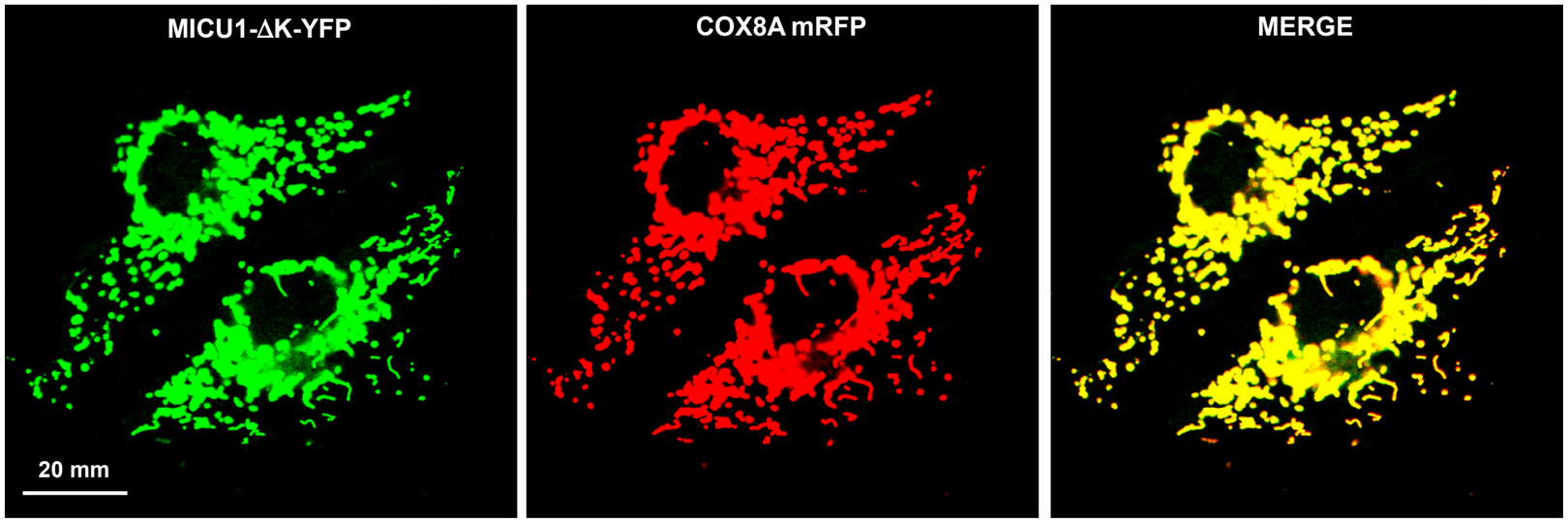
C

MCU

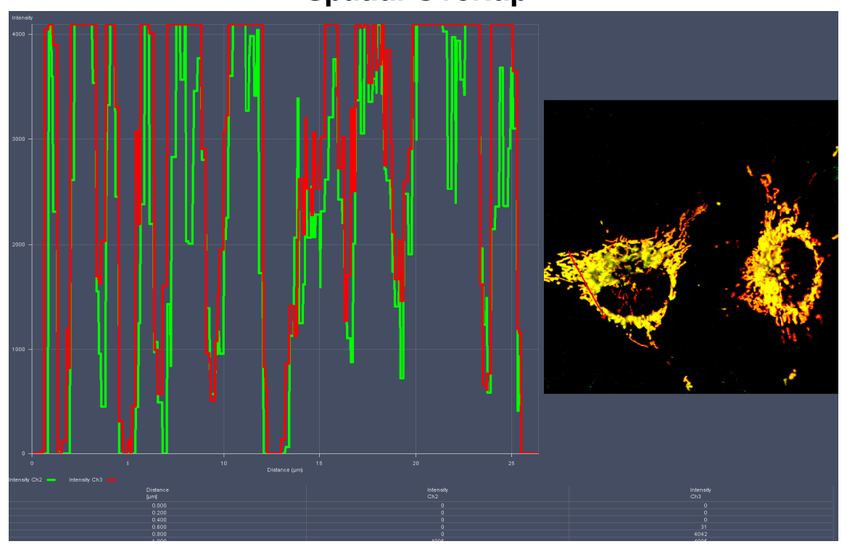
```
maaaagrsll lllssrgggg ggaggcgalt agcfpglgvs rhrqqqhhrt
vhqriaswqn lgavycstvv psddvtvvyq nglpvisvrl psrrercqft
lkpisdsvgv flrqlqeedr gidrvaiysp dgvrvaastg idllllddfk
lvindltyhv rppkrdllsh enaatlndvk tlvqqlyttl cieqhqlnke
relierledl keqlaplekv rieisrkaek rttlvlwggl aymatqfgil
arltwweysw dimepvtyfi tygsamamya yfvmtrqeyv ypeardrqyl
lffhkgakks rfdlekynql kdaiaqaemd lkrlrdplqv hlplrqigekd
```

D





E



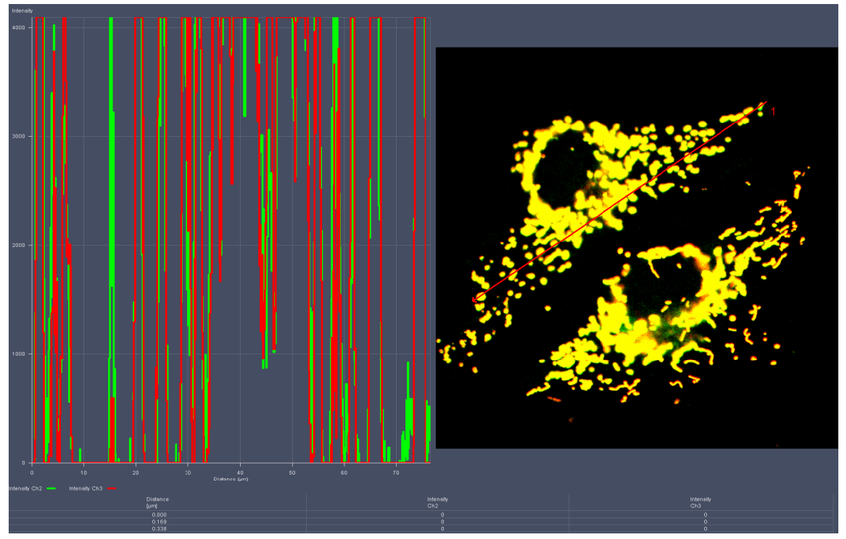


Figure S1, Related to Figure 2. MICU1 sequence alignment reveals an N-terminal conserved polybasic region, MICU1-ΔK, localizes to the mitochondria.

(A) Human MICU1 sequence with predicted mitochondrial cleavage site (green) and polybasic region (blue).

(**B)** The polybasic region is conserved from drosophila to homosapiens and contains a glycine (red) residue, which possibly undergoes myristoylation.

(C) Human MCU sequence with coiled coil domains (pink), transmembrane domains (blue) and intermembrane space loop (red).

(D) HeLa cells cotransfected with MICU1-ΔK-YFP and COX8A-mRFP were imaged with a Zeiss 510 confocal microscope using 488nm and 561nm excitation respectively.

(E) Spatial overlap demonstrates mitochondrial colocalization of MICU1-ΔK-YFP and COX8A-mRFP. n = 3-6.

Figure S2

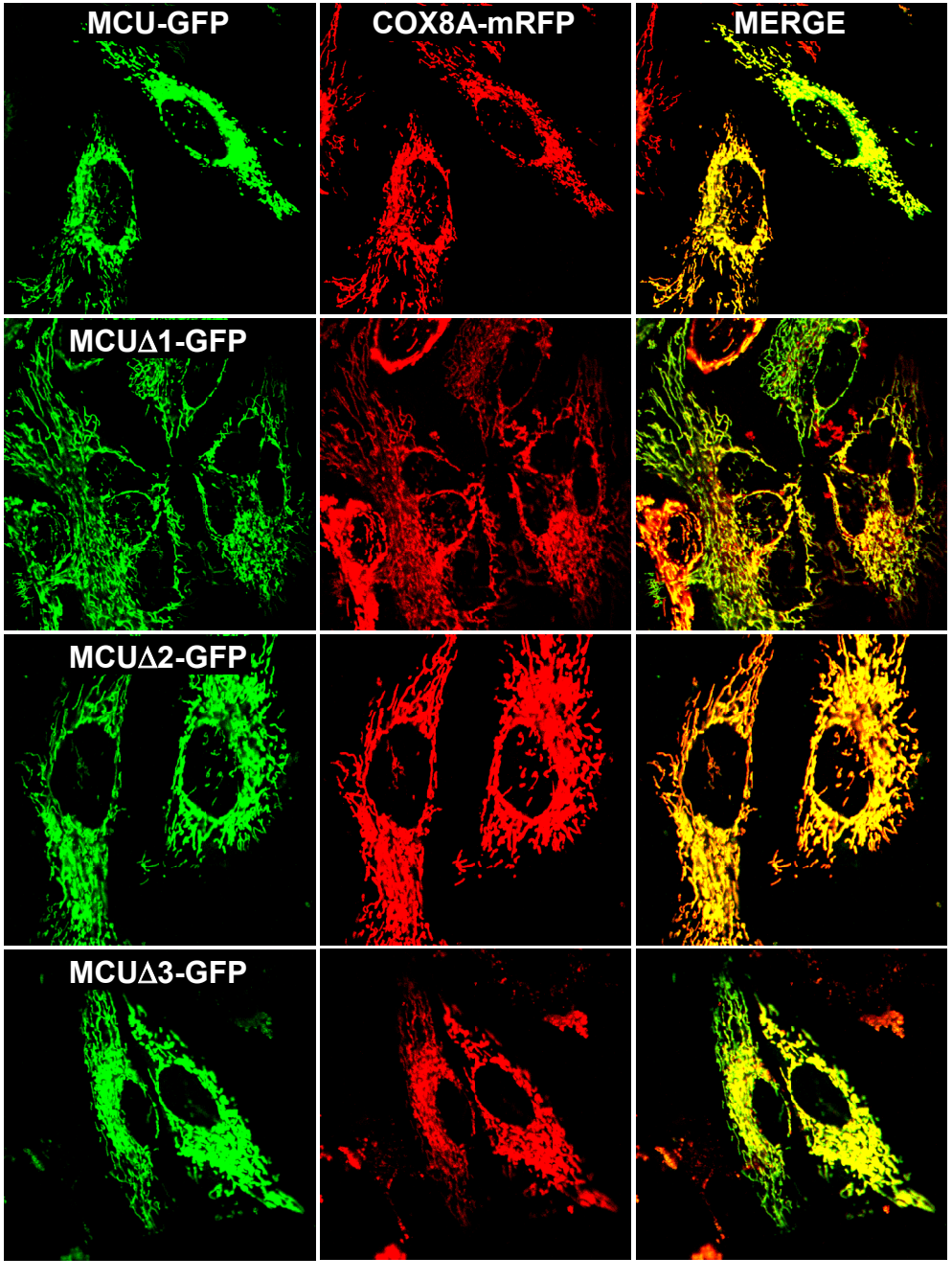
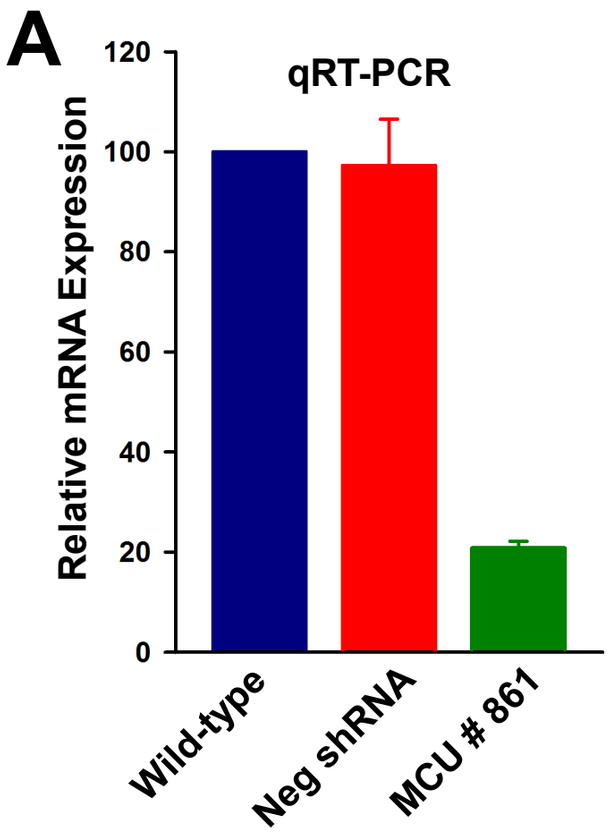
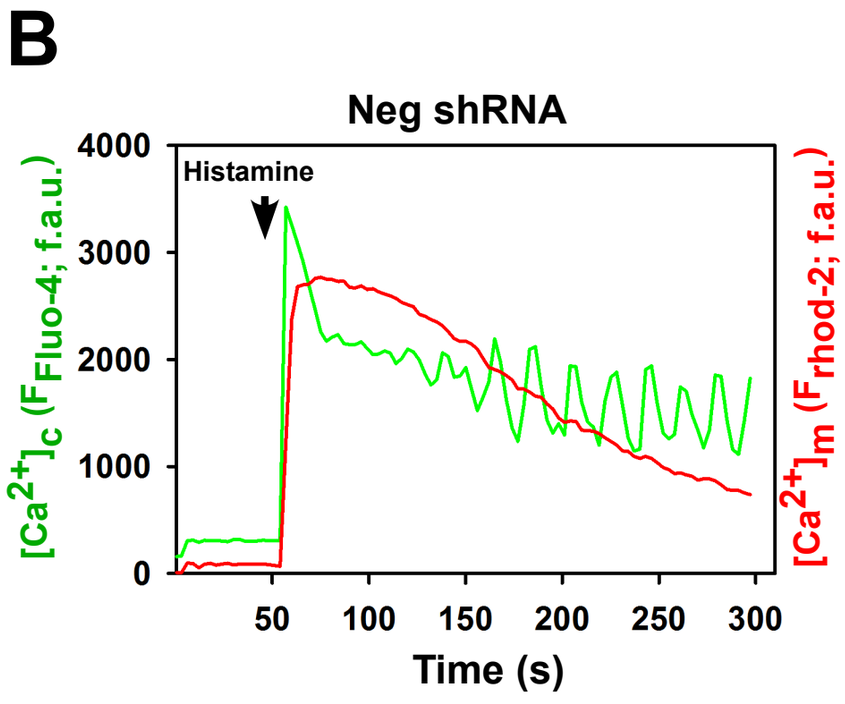


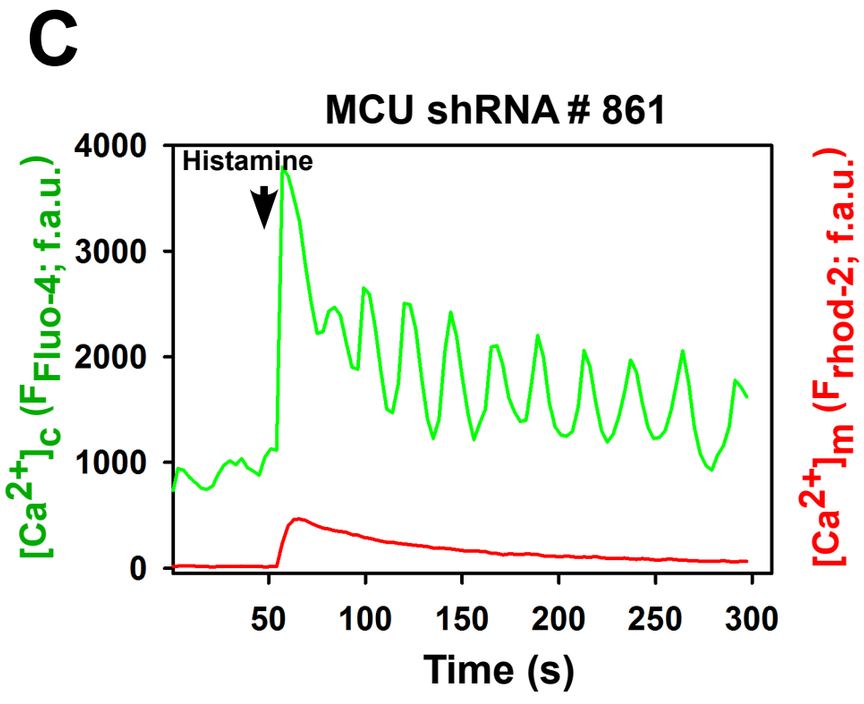
Figure S2, Related to Figure 3. Mitochondrial targeting of MCU truncation mutants.

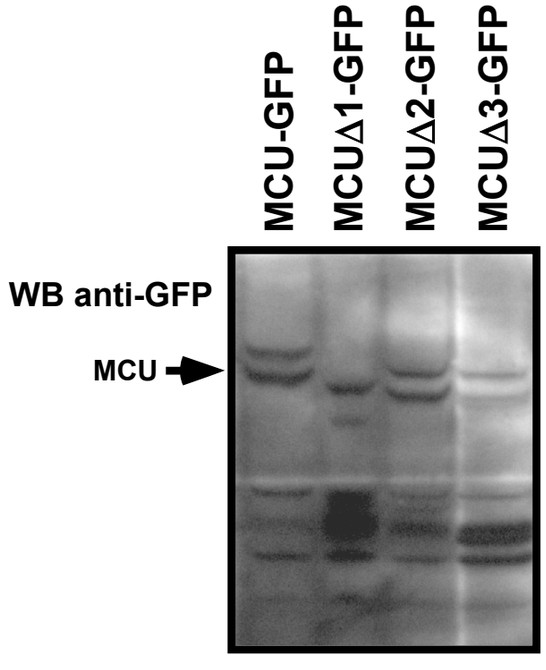
MCU knockdown HeLa cells were cotransfected with MCU full length, MCU-Δ1, MCU-Δ2, or MCU-Δ3, all with C-terminal GFP and COX8A-mRFP and imaged with a Zeiss 510 confocal microscope with 488nm and 561nm excitation respectively.

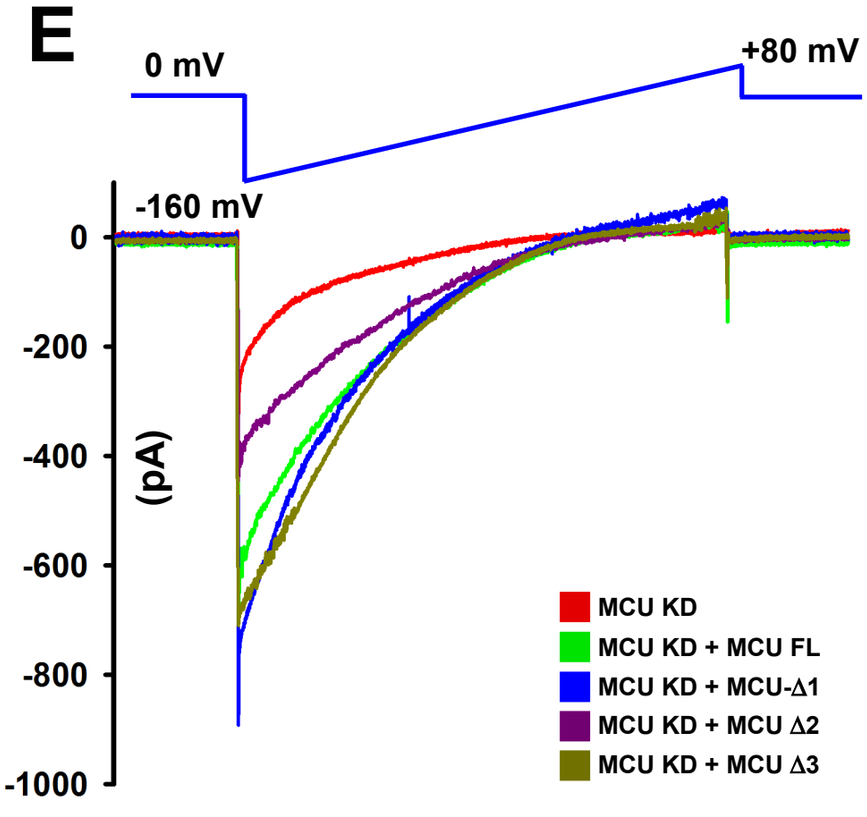
Figure S3


A
qRT-PCR
Relative mRNA Expression
120
100
80
60
40
20
0
Wild-type
Neg shRNA
MCU # 861


B
Neg shRNA
Histamine
[Ca2+]c (FFluo-4; f.a.u.)
[Ca2+]m (Frhod-2; f.a.u.)
4000
3000
2000
1000
0
50
100
150
200
250
300
Time (s)


C
MCU shRNA # 861
Histamine
[Ca2+]c (FFluo-4; f.a.u.)
[Ca2+]m (Frhod-2; f.a.u.)
4000
3000
2000
1000
0
50
100
150
200
250
300
Time (s)


D
MCU-GFP
MCUΔ1-GFP
MCUΔ2-GFP
MCUΔ3-GFP
WB anti-GFP
MCU


E
0 mV
+80 mV
-160 mV
0
-200
-400
-600
-800
-1000
(pA)
MCU KD
MCU KD + MCU FL
MCU KD + MCU-Δ1
MCU KD + MCU Δ2
MCU KD + MCU Δ3

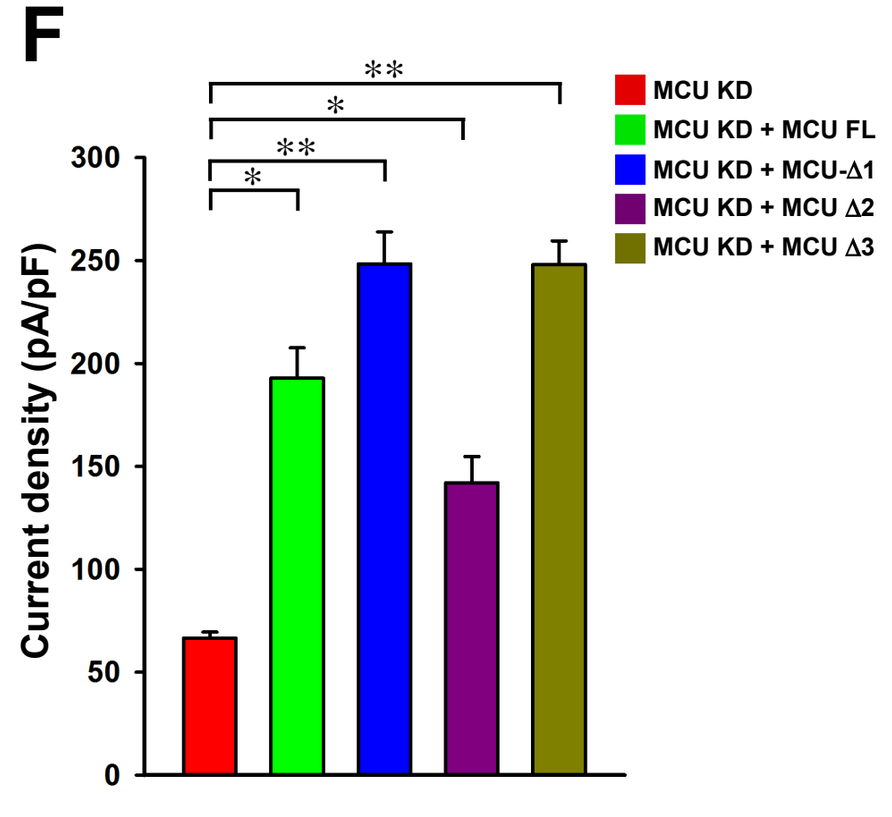

F
**
*
**
*
MCU KD
MCU KD + MCU FL
MCU KD + MCU-Δ1
MCU KD + MCU Δ2
MCU KD + MCU Δ3
Current density (pA/pF)
300
250
200
150
100
50
0

Figure S3, Related to Figure 5. Effects of MCU Silencing on Cytosolic and Mitochondrial Ca^{2+} Handling.

(A) Knockdown of MCU mRNA levels in HeLa cells after MCU shRNA lentiviral transduction. Total mRNA was isolated from HeLa cells stably expressing MCU #861 shRNA targeting MCU mRNA and qRT-PCR was performed n = 3.

(B and C) Dynamic Fluo-4 and Rhod-2 fluorescence changes were measured simultaneously by confocal microscopy after stimulation with histamine (100 μM) in negative shRNA (neg shRNA) and MCU KD cells (#861). Representative traces. n = 6.

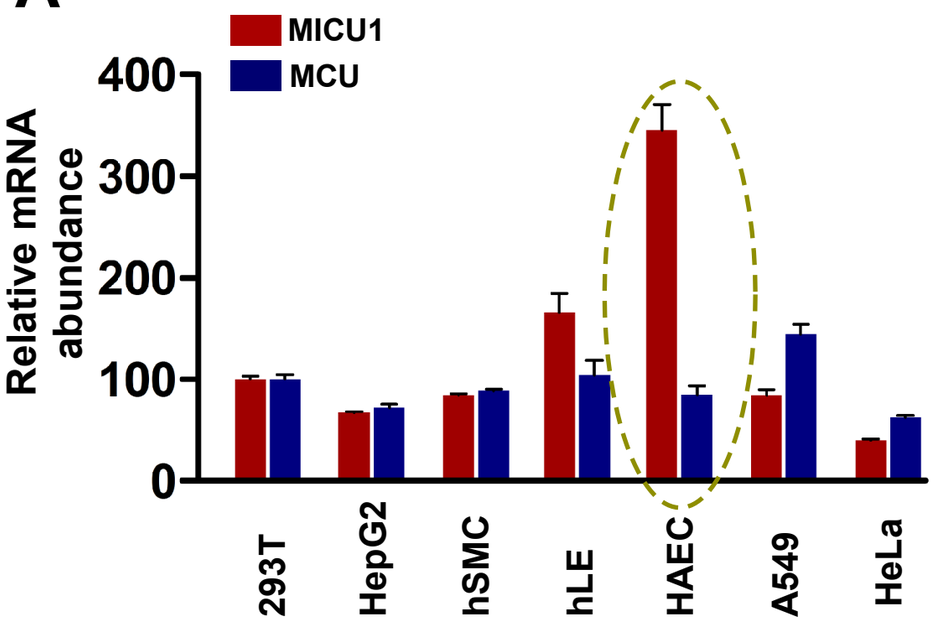
(D) Stable expression of MCU-GFP constructs in MCU KD HeLa cells. Cell lysates were subjected to Western blotting and probed with anti-GFP antibody.

(E) Representative I_{MCU} traces measured at 5 mM bath Ca^{2+} from mitoplasts generated from MCU KD HeLa cells stably expressing MCU-GFP constructs. Traces are a representative single recording of I_{MCU}. n = 5-6.

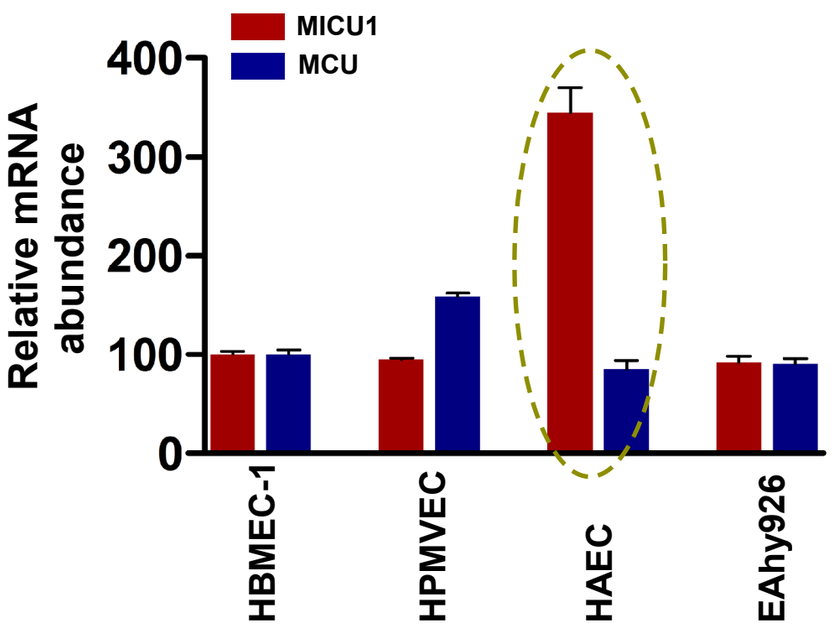
(F) I_{MCU} density (pA/pF) in MCU KD HeLa cells stably expressing MCU-GFP constructs. Mean ± SEM; *$p < 0.05$, **$p < 0.01$; n = 5-6.

Figure S4

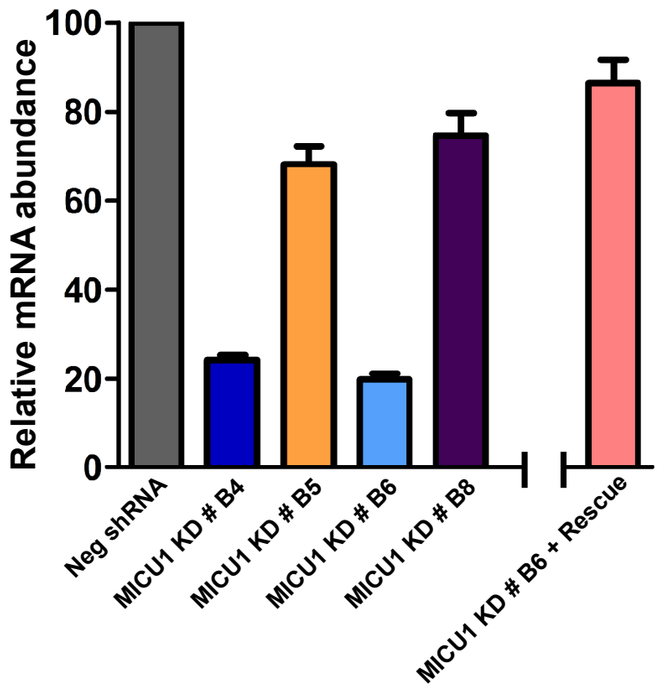
A



B



C



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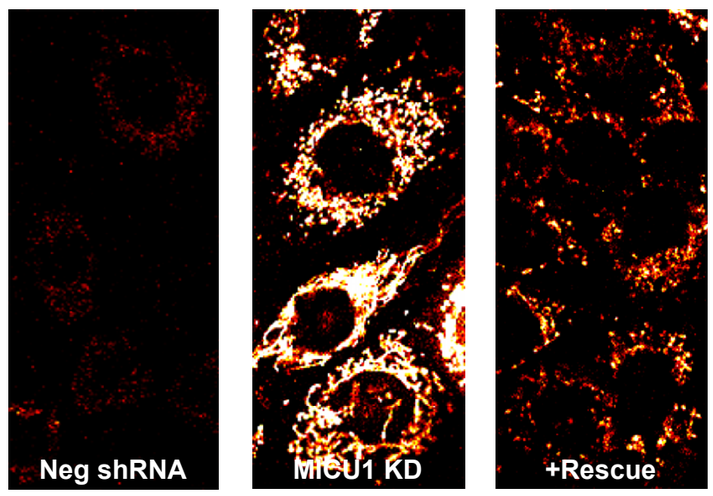
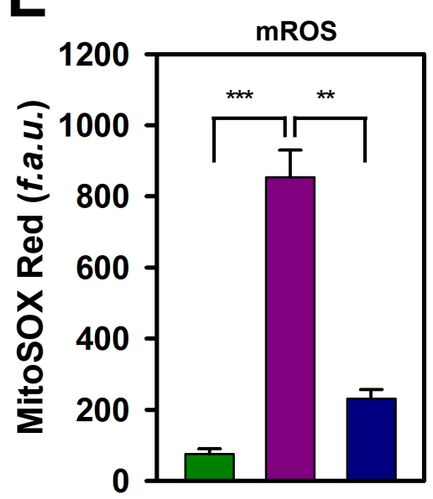
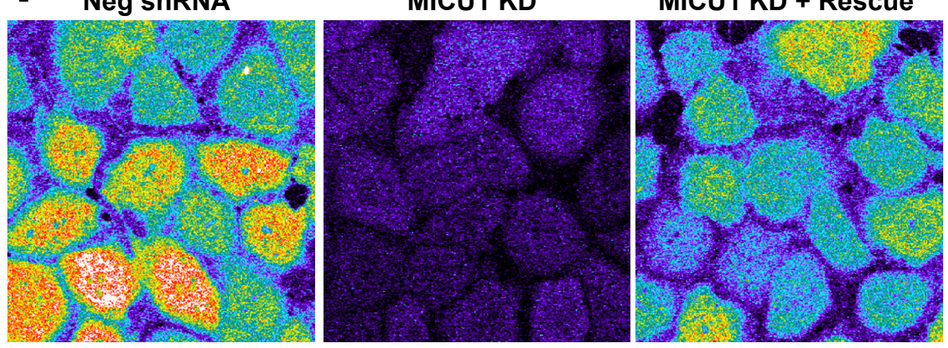


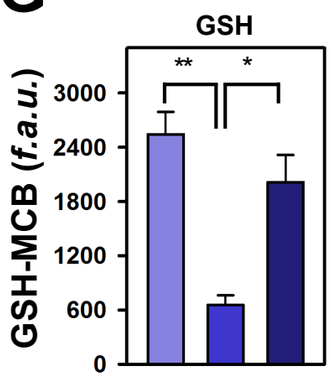
E



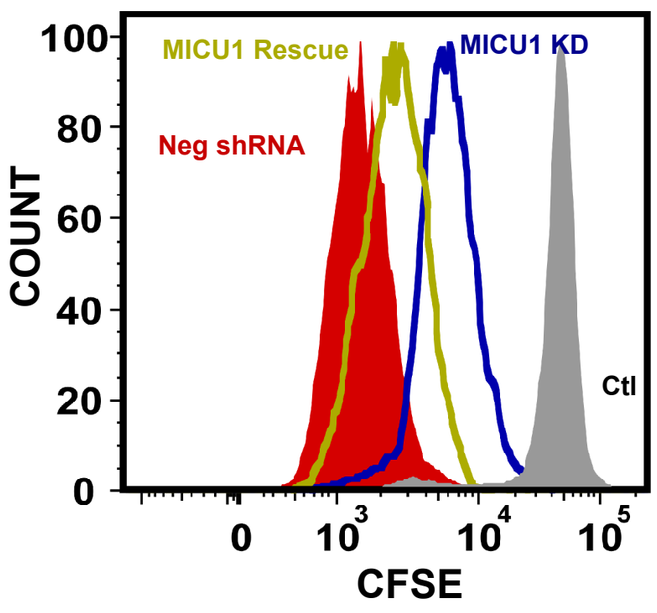
F



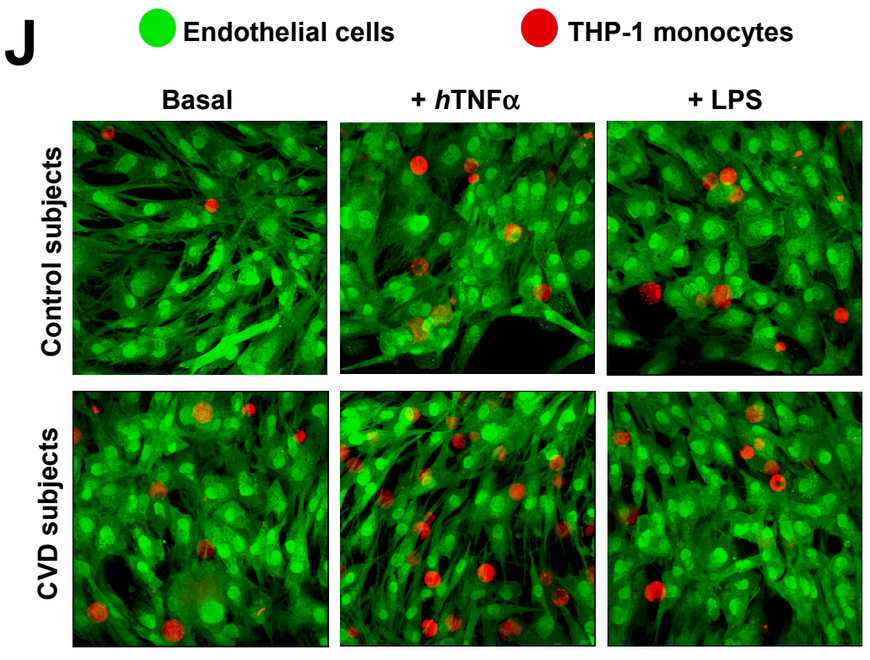
G



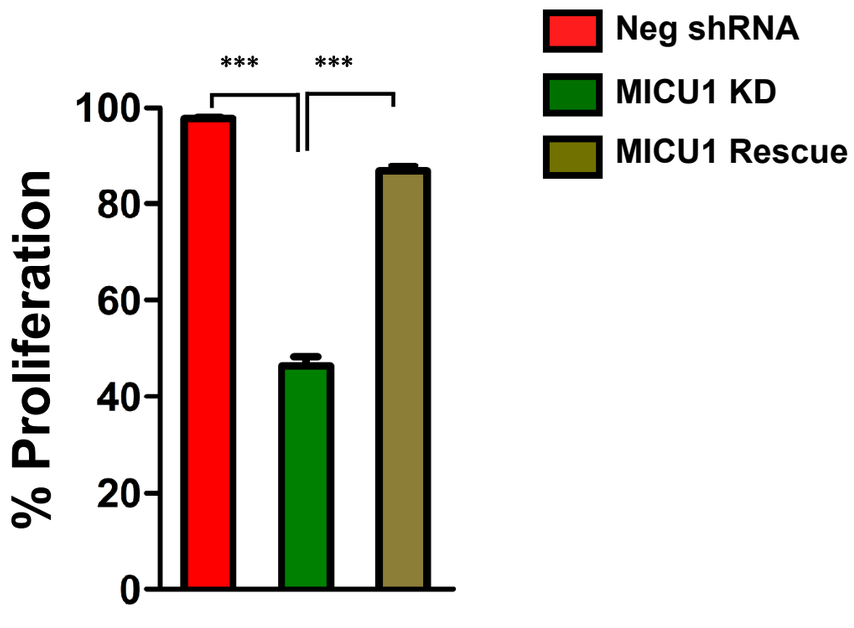
H



J



I



K

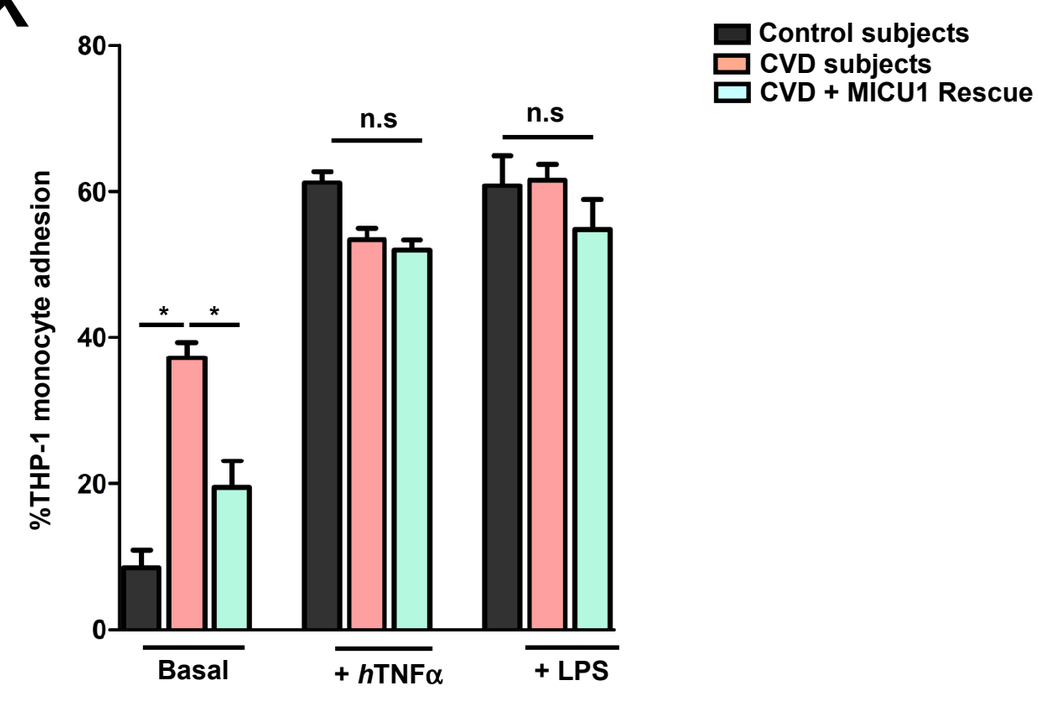


Figure S4, Related to Figure 6. MICU1 and MCU Expression in Primary Cells and Human Cell Lines and the effects of MICU1 loss on mROS production, Antioxidant Levels, EC Proliferation and Endothelial-Monocyte Adhesion.

(A) 293T, HepG2, primary human pulmonary smooth muscle cell (hSMC), epithelial cells (hLE), primary human aortic endothelial cells (HAEC), A549 and HeLa cells were cultured for 48 hours and total mRNA was isolated for qRT-PCR analyses. Mean ± SEM; n=3.

(B) Similarly, total mRNA isolated from HBMEC-1, HPMVEC, HAEC and EA.hy926 cells were subjected to qRT-PCR. Mean ± SEM; n = 3.

(C) Knockdown of MICU1 mRNA levels in endothelial cell line, EA.hy926 after MICU1 shRNA lentiviral transduction. Total mRNA was isolated from ECs stably expressing five shRNAs targeting different regions and qRT-PCR was performed. Ectopic expression of MICU1 in MICU1 knockdown ECs (right bar). Total mRNA was isolated from MICU1 #B6 cells stably expressing shRNA insensitive MICU1 cDNA and qRT-PCR was performed. Mean ± SEM; n = 3.

(D) ECs stably expressing neg shRNA, MICU #B6 shRNA and #B6 + MICU1 were loaded with mitochondrial superoxide indicator MitoSOX Red and confocal images were acquired. Images are representative of multiple independent experiments. n = 3.

(E) Quantitation of MitoSOX Red fluorescence. Mean ± SEM; $^{**}p < 0.01$, $^{***}p < 0.001$; n = 3.

(F) ECs stably expressing neg shRNA, MICU #B6 shRNA and #B6 + MICU1 were loaded with low molecular weight reduced thiol indicator monochlorobimane (mBCl) and confocal images were acquired. Representative images. n = 3.

(G) Quantitation of GSH-MCB fluorescence. Mean ± SEM; $^{*}p < 0.05$, $^{***}p < 0.01$; n = 3.

(H) Neg shRNA, #B6 and #B6 + MICU1 ECs were labeled with CFSE and cell proliferation was determined by flow cytometry.

(I) Quantitation of cell proliferation as CFSE distribution after 72 hrs. Mean ± SEM; $^{***}p < 0.001$; n = 3.

(J) To assess endothelial-monocyte adhesion, endothelial cells derived from control and CVD subjects were labeled with Cell Tracker green prior to addition of THP-1 monocytes labeled with Cell Tracker red. Images were acquired using 488 nm and 561 nm excitation on a 40x oil immersion objective (Zeiss 510 META). TNFα (10 ng/ml) and LPS (1 μg/ml) were used as positive controls. Representative images. n = 3.

(K) Quantitation of monocyte adhesion expressed as percent THP-1 monocyte/EC. Mean ± SEM; $^{*}p < 0.05$; n = 3.

Figure S5

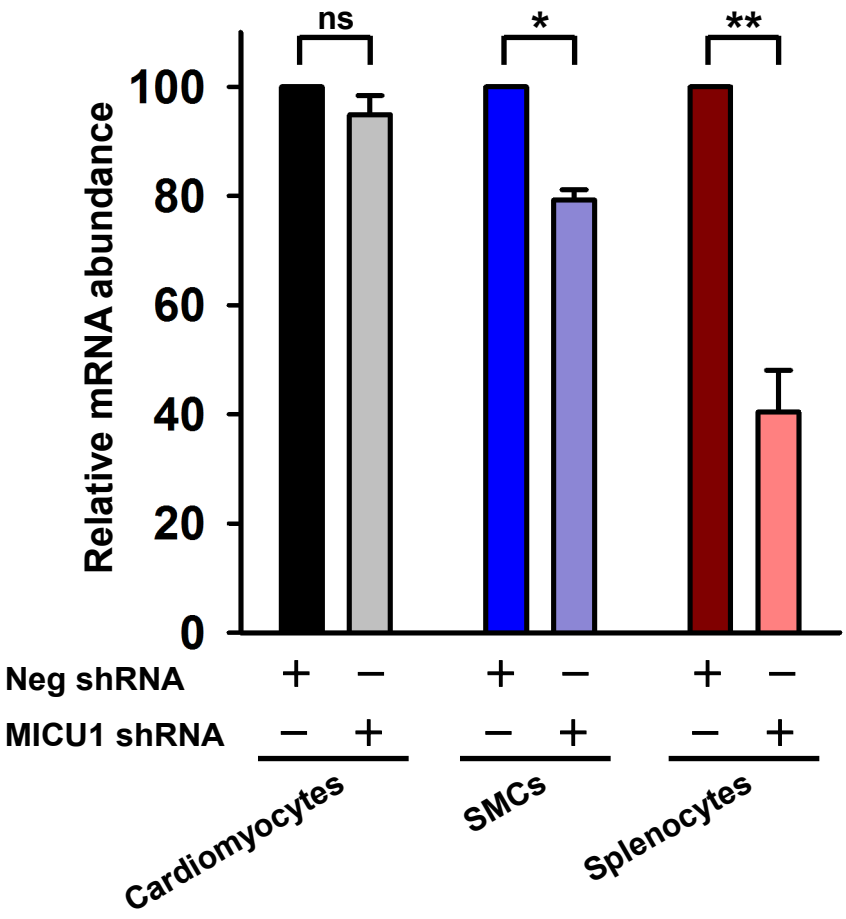

ns
*
**
Relative mRNA abundance
100
80
60
40
20
0
Neg shRNA
MICU1 shRNA
+ − + − + −
− + − + − +
Cardiomyocytes
SMCs
Splenocytes

Figure S5, Related to Figure 7. In Vivo knockdown of MICU1 in cardiomyocytes, smooth muscle cells and splenocytes.

Knockdown of MICU1 mRNA levels in mouse cardiomyocytes, SMCs and splenocytes after MICU1 shRNA lentiviral delivery. Total mRNA was isolated from cells and qRT-PCR was performed. Mean ± SEM; *$p < 0.05$, **$p < 0.01$; $n = 3$.

Supplementary Table

Supplementary Table 1 Subject characteristics –CAD/PAD (CVD)

	Control subjects *(n=3)*	**CAD/PAD (CVD)** *(n=19)*
Gender (M:F)	3 : 0	10 : 9
CVD	0	100%
Diabetes	0	68.4%
Hypertension	0	78.9%
Hypercholesterolemia	0	38.8%
Chronic kidney disease (stage 3 or greater)	0	36.8%
Stroke	0	10.5%
Smoking*	0	63.1%

*5 individuals had history of smoking (not current smokers)