Figure S3

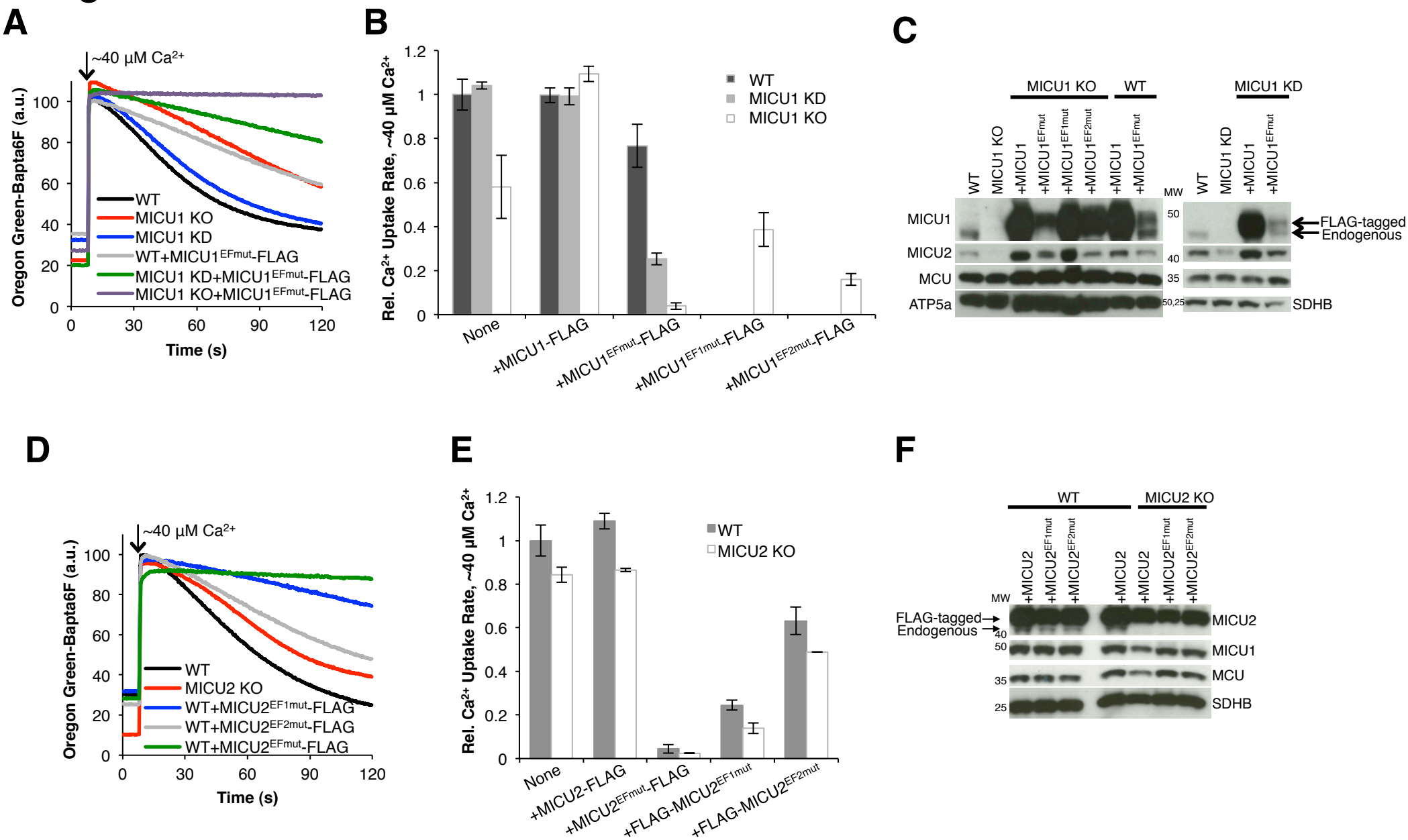
A
~40 μM Ca2+
Oregon Green-Bapta6F (a.u.)
Time (s)
WT
MICU1 KO
MICU1 KD
WT+MICU1EFmut-FLAG
MICU1 KD+MICU1EFmut-FLAG
MICU1 KO+MICU1EFmut-FLAG
B
Rel. Ca2+ Uptake Rate, ~40 μM Ca2+
WT
MICU1 KD
MICU1 KO
None
+MICU1-FLAG
+MICU1EFmut-FLAG
+MICU1EF1mut-FLAG
+MICU1EF2mut-FLAG
C
MICU1 KO
WT
MICU1 KD
MW
MICU1
MICU2
MCU
ATP5a
SDHB
FLAG-tagged
Endogenous
D
~40 μM Ca2+
Oregon Green-Bapta6F (a.u.)
Time (s)
WT
MICU2 KO
WT+MICU2EF1mut-FLAG
WT+MICU2EF2mut-FLAG
WT+MICU2EFmut-FLAG
E
Rel. Ca2+ Uptake Rate, ~40 μM Ca2+
WT
MICU2 KO
None
+MICU2-FLAG
+MICU2EFmut-FLAG
+FLAG-MICU2EF1mut
+FLAG-MICU2EF2mut
F
WT
MICU2 KO
MW
FLAG-tagged
Endogenous
MICU2
MICU1
MCU
SDHB

Figure S3: Mutation of the EF hands of MICU1 or MICU2 reduces mitochondrial Ca^{2+} uptake (A) Response of digitonin-permeabilized cells to $CaCl_2$ pulses, monitoring extramitochondrial Ca^{2+} with Oregon Green-Bapta6F. Representative traces of WT, MICU1 KO, or MICU1 KD cells with or without expression of MICU1^{EFmut}-FLAG given a large (~40 μM) pulse of Ca^{2+}. (B) Quantification of the data in (A) including data shown in Fig 1D and data for MICU1 KO cells expressing MICU1^{EF1mut}-FLAG or MICU1^{EF2mut}-FLAG. Data are presented as the rate of Ca^{2+} uptake from linear fits between 50–60 s (**n**≥3). Note that WT cells expressing each EF hand mutated separately are not shown. (C) Immunoblot of whole cell lysates from cells used in (A) and (B) for MICU1, MICU2, MCU, and loading controls ATP5a or SDHB. (D) Response of digitonin-permeabilized cells to $CaCl_2$ pulses, monitoring extramitochondrial Ca^{2+} with Oregon Green-Bapta6F. Representative traces of WT or MICU2 KO cells with or without expression of MICU2^{EFmut}-FLAG, MICU2^{EF1mut}-FLAG, or MICU2^{EF2mut}-FLAG given a ~40 μM pulse of Ca^{2+}. (E) Quantification of the data in (D) including data from Fig 1G and for expression of MICU2^{EF1mut}-FLAG or MICU2^{EF2mut}-FLAG, showing the rate of Ca^{2+} uptake from linear fits between 50−60 s (**n**≥3). (F) Immunoblot of whole cell lysates from cells used in (D) and (E) for MICU1, MICU2, MCU, and the loading control SDHB.