Figure S4!

Digitonin (%): -! -! -! -! -! -! -! -! -! -! -!
1% Triton: -! -! -! -! -! -! -! -! -! -! -!

Figure S5!

A!

B!
**Figure S4:** MICU2, MICU2\textsuperscript{EFmut} and MICU1-ΔC localize to the IMS in MICU1 KO cells

Localization of MICU2-FLAG, MICU2\textsuperscript{EFmut}-FLAG, and FLAG-MICU1-ΔC expressed in MICU1 KO cells is presented. Isolated mitochondria were exposed to proteinase K (PK) along with different concentrations of digitonin or Triton-X-100. Proteins with known localization are immunoblotted (MCU, matrix; SDHA, matrix; TIMM23, intermembrane space) along with FLAG for the experimental condition (MICU2-FLAG, MICU2\textsuperscript{EFmut}-FLAG, or FLAG-MICU1-ΔC).

**Figure S5:** Expression of mutant MICU1 in MICU1 KO cells and MCU-MYC Co-IP

(A) Immunoblot of whole cell lysates from WT or MICU1 KO HEK-293T cells stably expressing FLAG-MICU1\textsuperscript{EFmut}-ΔC or FLAG-MICU1\textsuperscript{EFmut}-ΔN with antibodies against MICU1 or SDHB. (B) c-MYC IP of MICU1 KO cells transiently expressing MCU-MYC along with stably expressing either FLAG-MICU1-ΔN or FLAG-MICU1-ΔC. Lysate and IP elution are immunoblotted for c-MYC and FLAG.