

Supporting Information

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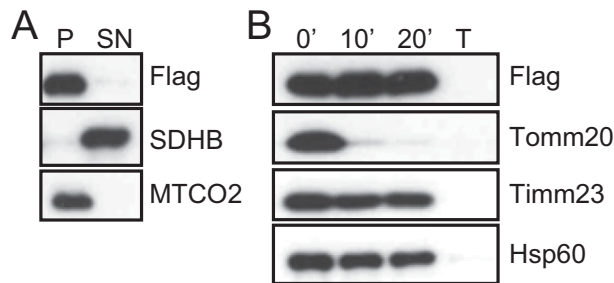


Fig. S1. Expression, localization, and topology of the DIME motif mutant of the *D. discoideum* mitochondrial calcium uniporter, DdMCU(AIIA), in HEK-293T MCU KO cells. (A) Carbonate extraction of mitochondria isolated from DdMCU(AIIA) expressing HEK-293T MCU KO cells. P, pellet, SN, supernatant fractions; SDHB, succinate dehydrogenase subunit B, peripheral membrane protein control; MTCO2, cytochrome c oxidase subunit II, membrane protein control. (B) Protease K treatment of mitoplasts from DdMCU(AIIA) expressing HEK-293T MCU KO cells. Tomm20, Timm23, and Hsp60 are mitochondrial outer membrane, inner membrane, and matrix protein controls, respectively. T, pretreatment with 1% Triton X-100. 0', 10', and 20' indicate the duration of incubation with proteasease K.

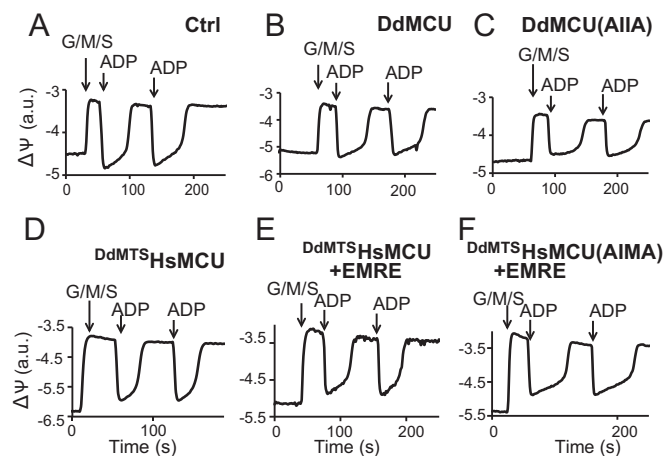


Fig. S2. Membrane potential measurements of yeast mitochondria. Representative traces show membrane potential ($\Delta\Psi$, TMRM fluorescence) measurements in isolated yeast mitochondria from control (Ctrl) (A) and DdMCU-expressing (B), DdMCU(AIIA)-expressing (C), DdMTS^{HsMCU}-expressing (D), DdMTS^{HsMCU} + EMRE-expressing (E), and DdMTS^{HsMCU(AIIA)} + EMRE-expressing (F) cells during the addition of 10 mM glutamate, malate, and succinate (G/M/S) respiratory substrates and 0.3 nmol ADP. EMRE, essential MCU regulator.

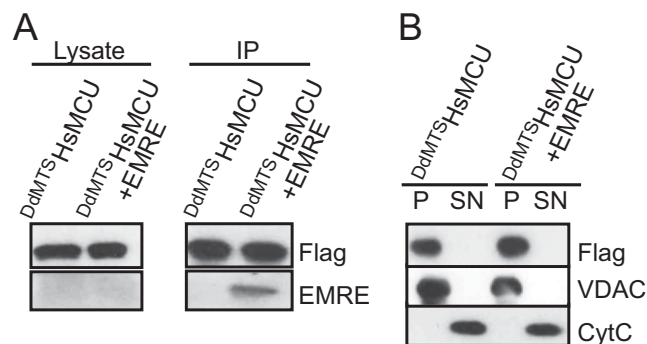


Fig. S3. Expression of EMRE and membrane localization of HsMCU in yeast cells. (A) Coimmunoprecipitation of HsMCU and EMRE using the FLAG tag fused to DdMTS^{HsMCU}. (B) Carbonate extraction of mitochondria isolated from yeast cells expressing DdMTS^{HsMCU} or DdMTS^{HsMCU} + EMRE. VDAC and cytochrome c (CytC) are mitochondrial membrane and soluble protein controls, respectively.