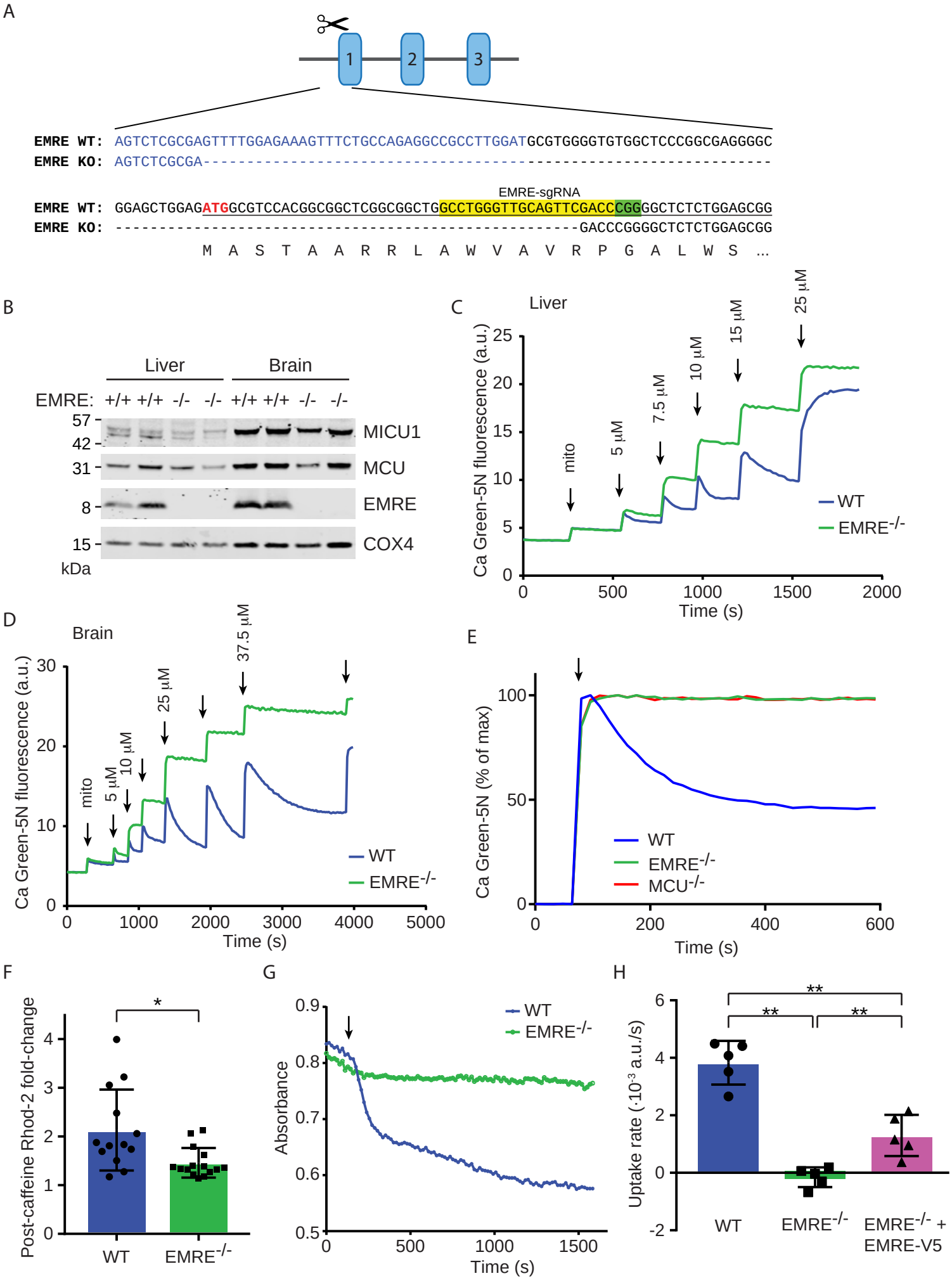
Supplemental Figure 1



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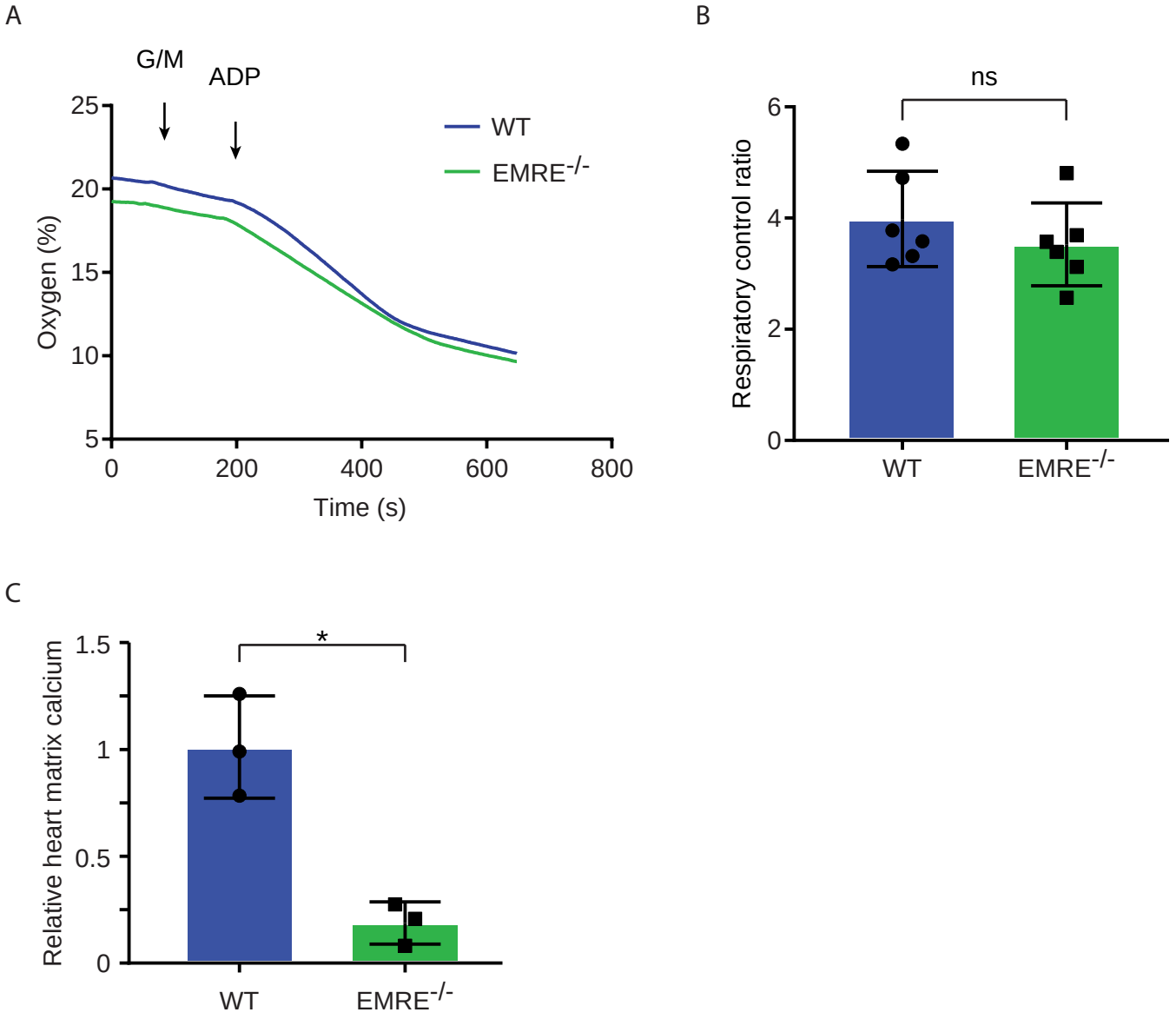
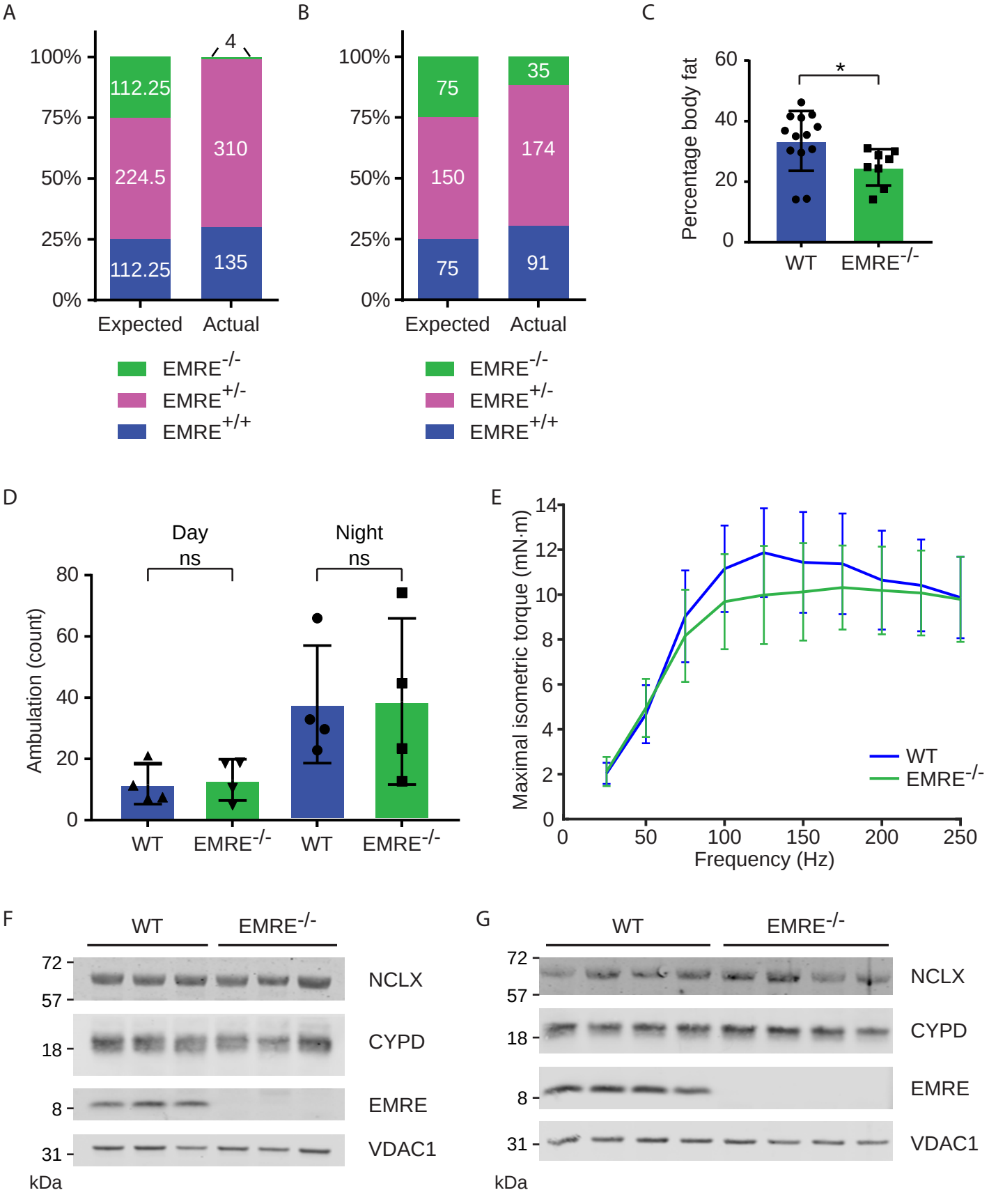
Supplemental Figure 1. EMRE is required for rapid mitochondrial calcium uptake across multiple tissues. A) Diagram of CRISPR-mediated deletion in the *EMRE* locus (dashes). The sequence of *EMRE* exon 1 is shown in black and the upstream noncoding region is depicted in blue. The CRISPR binding site is highlighted in yellow, and its PAM is highlighted in green. The translational initiation codon (ATG) is shown in red, with the coding region underlined and the amino acid sequence underneath. B) Western blot analysis of MICU1, MCU, and EMRE protein expression in isolated mitochondria from *WT* and *EMRE*$^{-/-}$ livers and brains (n = 2 mice per group, all biological replicates). COX4 serves as a mitochondrial loading control. Molecular weights from the protein ladder are indicated on the left. C-D) Extramitochondrial calcium traces with isolated mitochondria from *WT* and *EMRE*$^{-/-}$ livers (C) and brains (D) using the calcium indicator dye Calcium Green-5N. Arrows represent calcium addition of the concentration last indicated. Data are representative of at least n = 3 experiments on biological replicates. E) Extramitochondrial calcium traces of isolated heart mitochondria from *WT*, *MCU*$^{-/-}$, and *EMRE*$^{-/-}$ mice using the calcium indicator dye Calcium Green-5N in response to a 25 μM addition of calcium (arrow). Data are representative of at least n = 3 experiments on biological replicates. F) Peak mitochondrial calcium after stimulation with 20 mM caffeine (fold-change relative to baseline) measured by Rhod-2 fluorescence in FDB muscle fibers isolated from *WT* (n = 13 cells from 4 mice) and *EMRE*$^{-/-}$ (n = 15 cells from 4 mice) mice (biological replicates). G) Mitochondrial swelling assay measured by absorbance of isolated mitochondria from *WT* and *EMRE*$^{-/-}$ livers in response to a 250 μM addition of calcium. Data are representative of at least n = 3 experiments. H) Rate of calcium uptake after the first 10 μM calcium addition in *WT*, *EMRE*$^{-/-}$, and *EMRE*$^{-/-}$ MEFs reconstituted with V5 epitope-tagged EMRE transgene, as calculated by taking the slopes from linear fits of Calcium Green-5N traces during the first 2 minutes post-peak. Data were averaged from n = 5 experiments.

Supplemental Figure 2



Supplemental Figure 2. Basal metabolism is not markedly altered with loss of EMRE in multiple tissues. A) Traces of the oxygen consumption observed by Clark-type electrode in isolated *WT* and *EMRE*$^{-/-}$ liver mitochondria given glutamate and malate as substrates (G/M) followed by ADP. Data are representative of n = 6 experiments on biological replicates. B) Quantification of the respiratory control ratio (RCR; state 3/ state 4 respiration) in *WT* and *EMRE*$^{-/-}$ liver mitochondria (n = 6 biological replicates per group). C) Relative levels of matrix free calcium in isolated heart mitochondria from *WT* and *EMRE*$^{-/-}$ mice (n = 3 biological replicates per group), measured with Fluo-4 AM fluorescence. Data are represented as mean +/- SD; results of unpaired t-test with Welch's correction: ns, not significant, *$p < 0.05$.

Supplemental Figure 3



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Supplemental Figure 3. Further characterization of *EMRE*$^{-/-}$ phenotype. A) Expected and observed percentage of *EMRE*$^{+/+}$, *EMRE*$^{+/-}$, and *EMRE*$^{-/-}$ mice born to heterozygous C57BL/6N parents. The actual number of expected and observed mice is shown within the corresponding bar from a total of 449 offspring. B) Expected and observed percentage of *EMRE*$^{+/+}$, *EMRE*$^{+/-}$, and *EMRE*$^{-/-}$ mice born to heterozygous F2 (C57BL/6N crossed twice to CD-1) parents. The actual number of expected and observed mice is shown within the corresponding bar from a total of 300 offspring. C) Percentage body fat measured by Bruker body composition analyzer for 6-month-old female *WT* (n = 13 biological replicates) and *EMRE*$^{-/-}$ (n = 8 biological replicates) mice. D) Average counts of ambulation (consecutive beam breaks along the x-axis of the cage) in *WT* and *EMRE*$^{-/-}$ (n = 4 biological replicates per group) mice during day and night conditions. E) Maximal isometric torque generated by in vivo gastrocnemius muscle contraction at a series of stimulation frequencies in *WT* and *EMRE*$^{-/-}$ mice (n = 6 biological replicates per group). F) Western blot analysis of NCLX, CYPD, and EMRE protein expression in isolated mitochondria from *WT* and *EMRE*$^{-/-}$ hearts (n = 3 biological replicates per group). VDAC1 serves as a mitochondrial loading control. Molecular weights from the protein ladder are indicated on the left. G) Western blot analysis of NCLX, CYPD, and EMRE protein expression in isolated mitochondria from *WT* and *EMRE*$^{-/-}$ brains (n = 4 biological replicates per group). VDAC1 serves as a mitochondrial loading control. Molecular weights from the protein ladder are indicated on the left. Data are represented as mean +/- SD. *WT* and *EMRE*$^{-/-}$ mice were compared directly (C) or within day or night conditions (D) using unpaired t-test with Welch's correction: *$p < 0.05$, ns, not significant.

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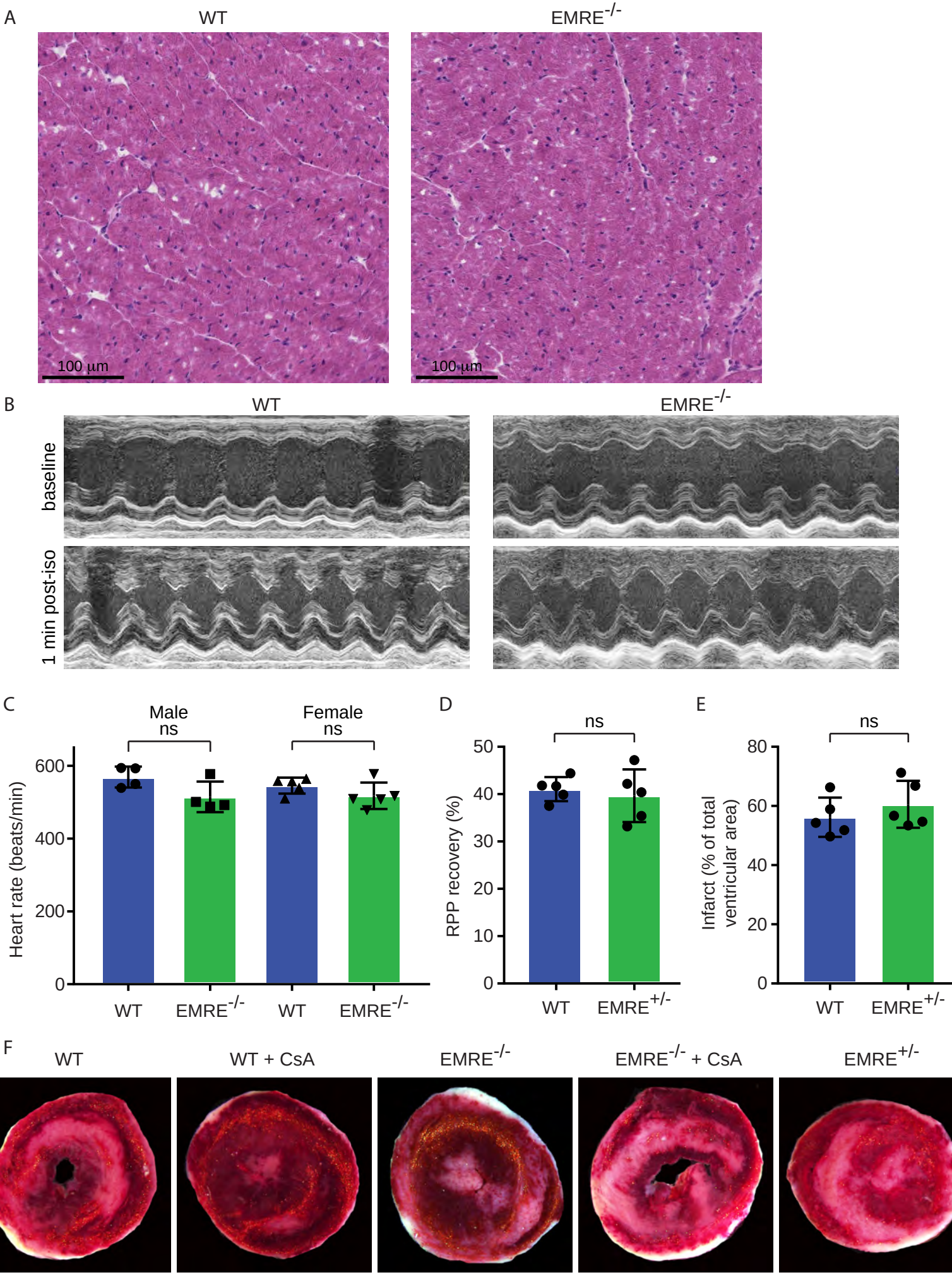
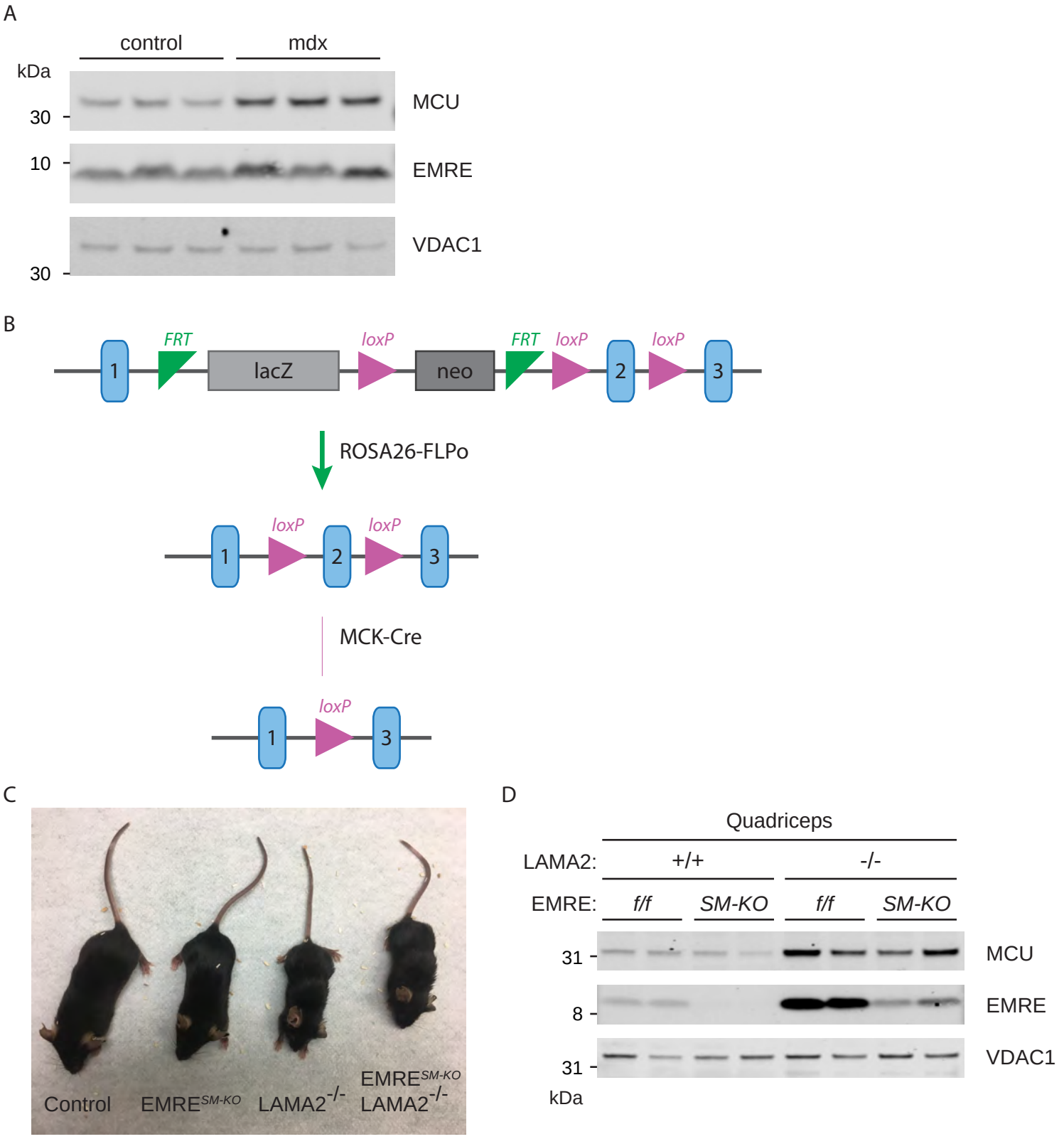
Supplemental Figure 4. Deletion of EMRE has minimal effect on cardiac structure and function. A) Histology of *WT* and *EMRE*$^{-/-}$ cardiac architecture using H&E staining. B) Representative images from echocardiography experiments at baseline and one minute following isoproterenol (2 mg/kg) injection; each is representative of n = 6 mice analyzed in Figure 4C-D. C) Echocardiography analysis of *WT* and *EMRE*$^{-/-}$ male (n = 4 biological replicates per group) and female (n = 5 biological replicates per group) mice assessing heart rate. D-E) Assessment in the hearts of *WT* (n = 5 biological replicates, included in Figure 4E-F as these experiments were done together) and *EMRE*$^{+/-}$ mice (n = 5 biological replicates) for 5 minutes prior to ischemia, following 20 minutes of global ischemia and 90 min of reperfusion of the rate pressure product (RPP; heart rate times systolic blood pressure) (D) and infarct size relative to the area at risk, which is the entire left ventricle (E). F) Representative cross-sections of TTC-stained hearts post-I/R (white area, infarcted tissue; red area, viable myocardium). Each image is representative of at least n = 5 mice analyzed in Figure 4E-F and S4D-E. Data are represented as mean +/- SD. *WT* and *EMRE*$^{-/-}$ mice were compared within each sex (C) or directly (D-E) using unpaired t-test with Welch's correction: ns, not significant.

Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 5. Further description of the role of EMRE in a muscular dystrophy mouse model. A) Western blot analysis of MCU and EMRE protein expression in quadriceps muscle from control and *mdx* (n = 3 biological replicates per group) mice. VDAC1 serves as a mitochondrial loading control. Molecular weights from the protein ladder are indicated on the left. B) Diagram of *EMRE* targeting construct. Mutant mice were crossed with ROSA26-FLPo mice to excise the selection marker and lacZ reporter gene, then $EMRE^{flox/flox}$ mice were subsequently crossed to MCK-Cre mice resulting in deletion of *EMRE*. C) Photograph of 7-week-old female control, $EMRE^{SM\text{-}KO}$, $LAMA2^{-/-}$, and $EMRE^{SM\text{-}KO}$ $LAMA2^{-/-}$ mice, representative of at least 9 mice per genotype (Figure 5F). D) Western blot analysis of MCU and EMRE protein expression in quadriceps muscle from control, $EMRE^{SM\text{-}KO}$, $LAMA2^{-/-}$, and $EMRE^{SM\text{-}KO}$ $LAMA2^{-/-}$ mice (n = 2 biological replicates per group). VDAC1 serves as a mitochondrial loading control. Molecular weights from the protein ladder are indicated on the left.