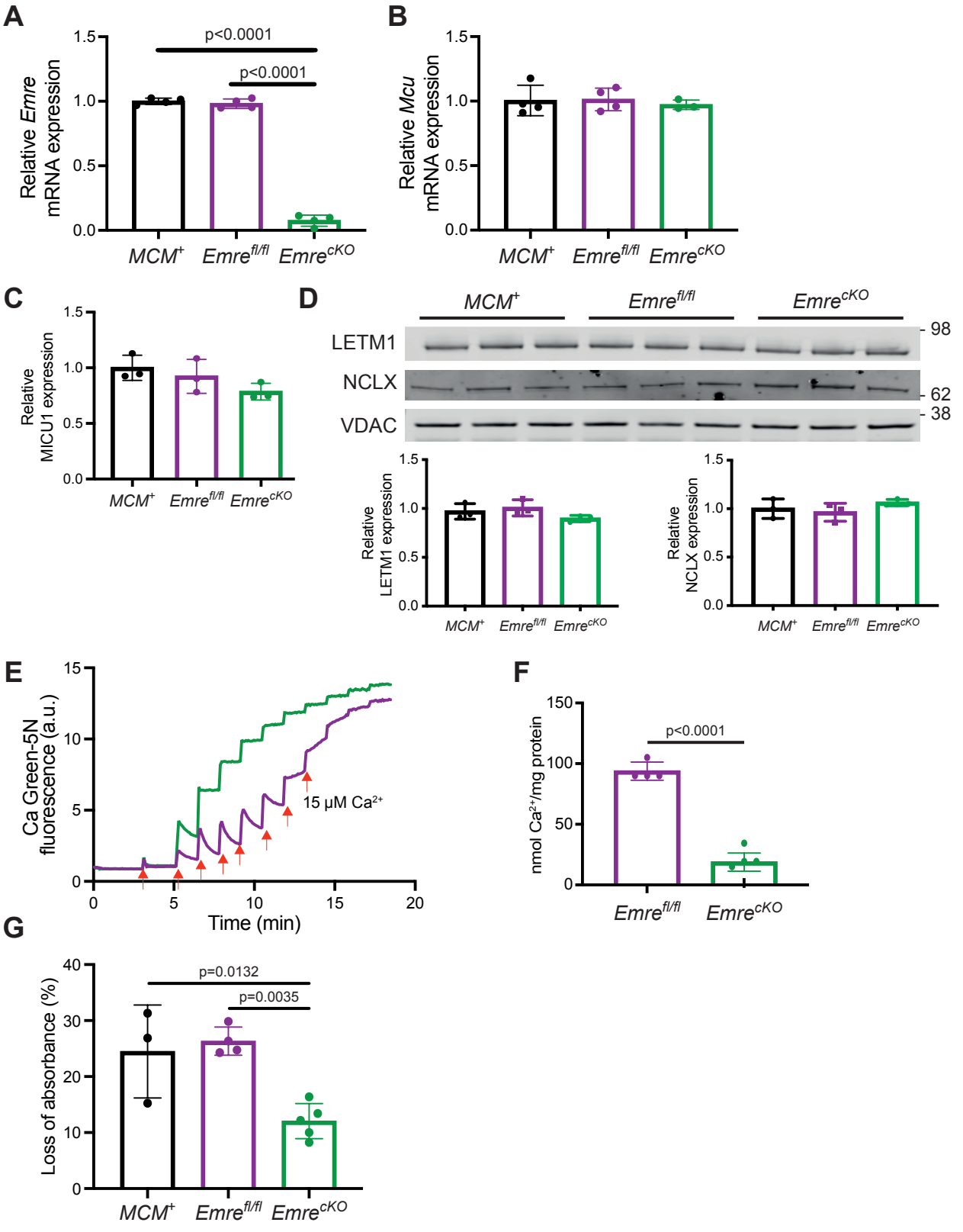
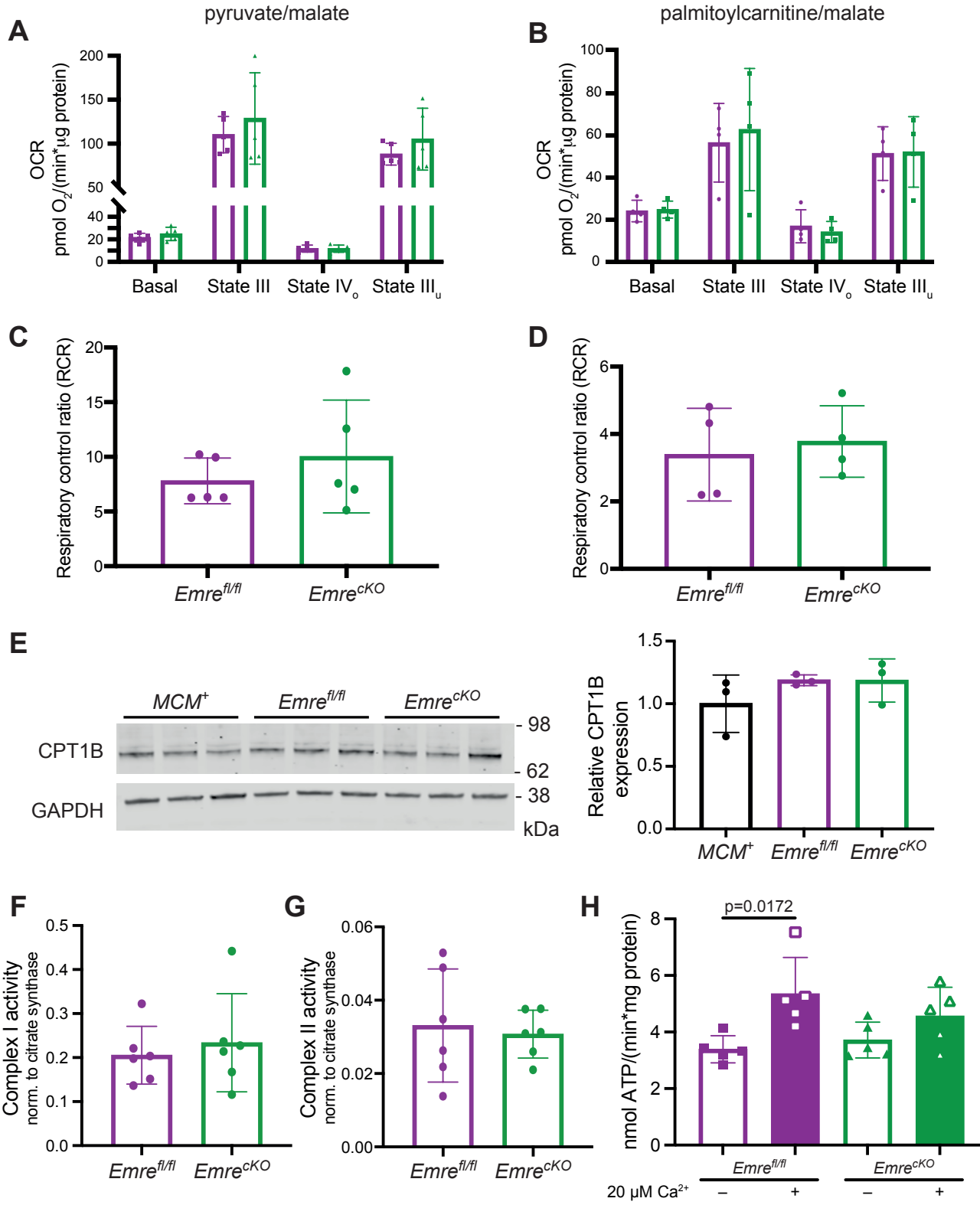
Supplementary Figure 1



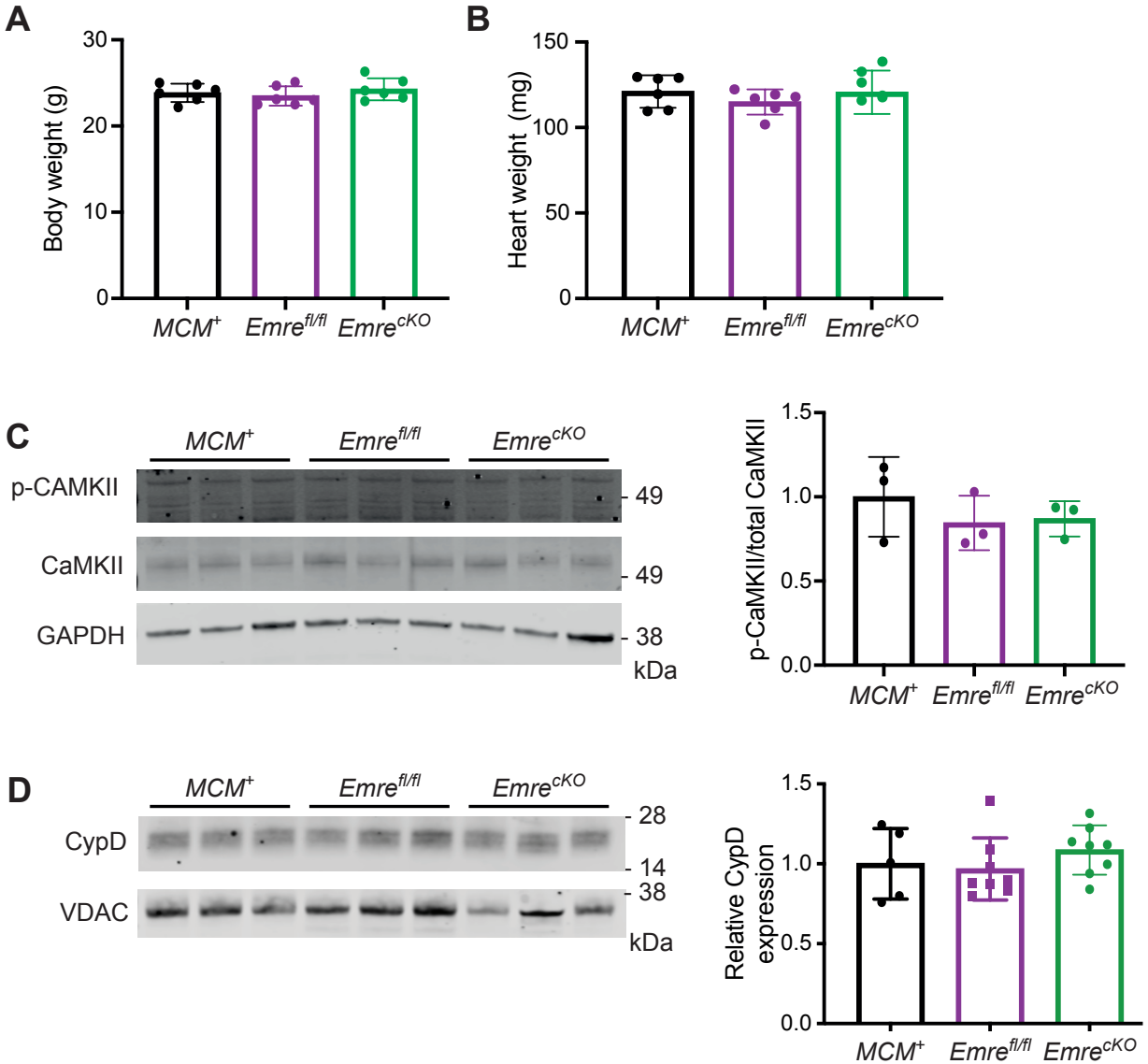
Supplementary Figure 1. *Short-term Emre deletion affects mitochondrial Ca^{2+} uptake but not the expression of mitochondrial Ca^{2+} efflux proteins.* Analyses were performed 3 weeks post-tamoxifen. **A**) Relative *Emre* mRNA expression in heart tissue from MCM^{+}: 1.00 ± 0.02, $Emre^{fl/fl}$: 0.98 ± 0.03 and $Emre^{cKO}$: 0.07 ± 0.04. Values represent mean ± SD. **p<0.0001, n = 4 per group. One-way ANOVA test was used for statistical analysis. **B**) Relative *Mcu* mRNA expression in heart tissue from MCM^{+}: 1.00 ± 0.11, $Emre^{fl/fl}$: 1.01 ± 0.08 and $Emre^{cKO}$: 0.97 ± 0.03. Values represent mean ± SD. n = 4 per group. **C**) Quantification of MICU1 protein levels from Western blot (Fig. 1A), where bars represent mean ± SD. p-values were not significant. n = 3 per group. One-way ANOVA test was used for statistical analysis. **D**) Western blot of LETM1, NCLX, and VDAC in cardiac mitochondria from MCM^{+}, $Emre^{fl/fl}$, and $Emre^{cKO}$ mice at 3 weeks post-tamoxifen. Quantification is shown below, where bars represent mean ± SD. p-values were not significant. n = 3 per group. One-way ANOVA test was used for statistical analysis. **E**) Representative Ca^{2+} retention capacity (CRC) assay in isolated heart mitochondria from $Emre^{fl/fl}$ (purple line) and $Emre^{cKO}$ (green line) mice. Mitochondria were energized with 10 mM glutamate/5 mM malate. The fluorescent Ca^{2+} indicator Calcium Green-5N was used to monitor extramitochondrial Ca^{2+}. The arrows represent 15 μM Ca^{2+} additions. Traces are representative of n = 4 independent experiments. **F**) Ca^{2+} retention capacity calculated from independent traces as shown in (E). The estimated mean Ca^{2+}/mg protein was for $Emre^{fl/fl}$: 93.8 ± 7.5 and $Emre^{cKO}$: 18.8 ± 7.5 nmol. Values represent mean ± SD. **p<0.0001, n = 4 in each group. Student's t-test was used for statistical analysis. **G**) Quantitative analysis of swelling calculated via loss of absorbance (%) 15 minutes after Ca^{2+} addition. MCM^{+} : 24.48% ± 8.29, $Emre^{fl/fl}$: 26.32% ± 2.52 and $Emre^{cKO}$: 12.04% ± 3.12. Values represent mean ± SD.**p<0.0001, n = 4-5 in each group. One-way ANOVA test was used for statistical analysis.

Supplementary Figure 2



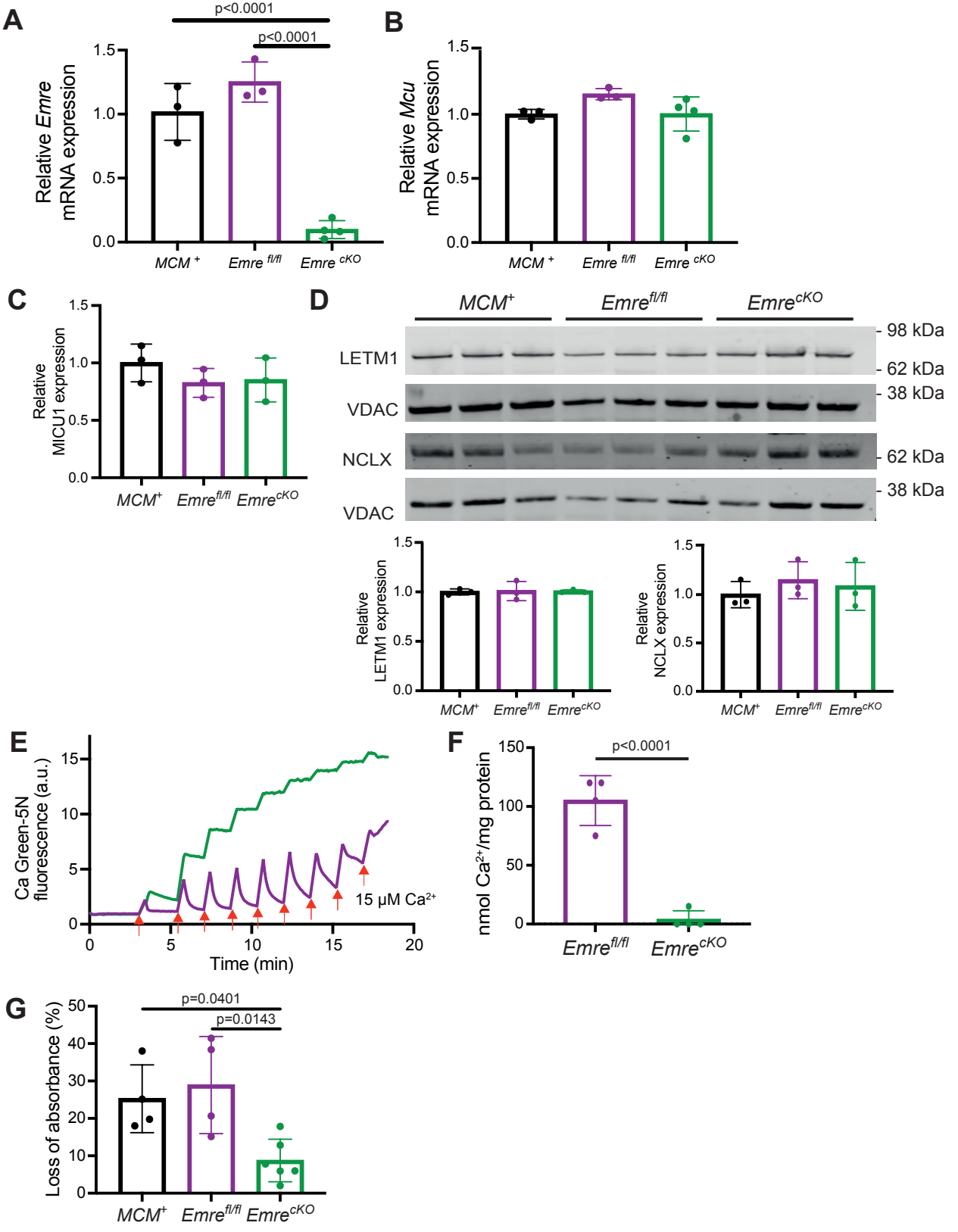
Supplementary Figure 2. *Effects of short-term Emre deletion on bioenergetic capacity*. Analyses were performed 3 weeks post-tamoxifen. **A-B**) Seahorse analysis of oxygen consumption rate (OCR) in cardiac mitochondria was measured following sequential injections: 4 mM ADP (state III), 2.5 μg/mL oligomycin (state IV_o), 6 μM FCCP (state III_u) and 4 μM rotenone/4 μM antimycin A. Mitochondria were supplied with 5 mM pyruvate/0.5 mM malate (A) or 40 μM palmitoylcarnitine/0.5 mM malate (B). **C**) Respiratory control ratio (RCR; OCR in state III_u/stateIV_o) in mitochondria supplied with 5 mM pyruvate/0.5 mM malate: $Emre^{fl/fl}$: 7.8 ± 2.08 and $Emre^{cKO}$: 10.02 ± 5.16. Values represent mean ± SD., n = 5 per group (combined males and females). Student's t-test was used for statistical analysis. **D**) RCR in mitochondria supplied with 40 μM palmitoylcarnitine/0.5 mM malate, $Emre^{fl/fl}$: 3.38 ± 1.37 and $Emre^{cKO}$: 3.77 ± 1.06. Values represent mean ± SD, n = 4 per group (combined males and females). Student's t-test was used for statistical analysis. **E**) Western blot for CPT1B in cardiac tissue: MCM^{+}: 1.00 ± 0.22, $Emre^{fl/fl}$: 1.18 ± 0.04 and $Emre^{cKO}$: 1.18 ± 0.17, n = 3 in each group. **F-G**) Complex I (NADH ubiquinone oxidoreductase) activity (F) and Complex II (succinate dehydrogenase) activity (G) normalized to citrate synthase (CS) activity. Values represent mean ± SD, n = 6 per group, Student's t-test was used for statistical analysis. **H**) ATP production rate in isolated heart mitochondria stimulated with 50 μM ADP and 10 mM glutamate/5 mM malate ± 20 μM Ca^{2+}, $Emre^{fl/fl}$: 3.39 ± 0.47 *vs* $Emre^{fl/fl}$ + 20 μM Ca^{2+}: 5.35 ± 1.28, and $Emre^{cKO}$: 3.71 ± 0.63 *vs* $Emre^{cKO}$ + 20 μM Ca^{2+}: 4.56 ± 1.01 nmol ATP/(min*mg) protein. Values represent mean ± SD. *$p<0.05$, n = 5 per group (combined males and females). One-way ANOVA was used for statistical analysis.

Supplementary Figure 3



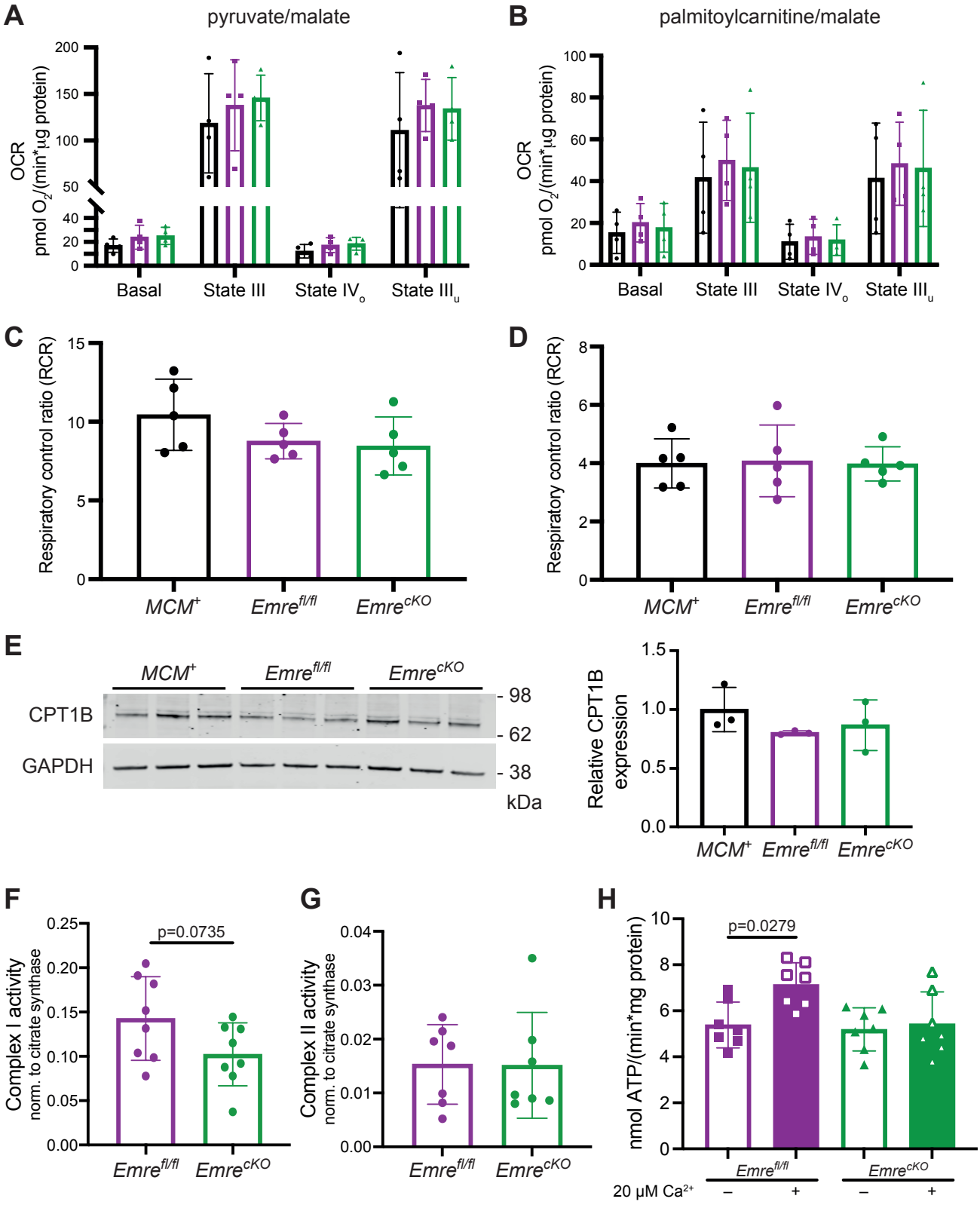
Supplementary Figure 3. *Effects of short-term Emre deletion on body and heart weight and protein expression.* Analyses were performed 3 weeks post-tamoxifen. **A-B**) Body weight (A) and heart weight (B) from MCM^{+}, $Emre^{fl/fl}$, and $Emre^{cKO}$ mice at 3 weeks post-tamoxifen. Values represent mean ± SD, n = 6 per group. **C**) Western blot for phosphorylated CaMKII level relative to total CaMKIIδ from cardiac tissue, quantified to the right in MCM^{+}: 1.00 ± 0.23, $Emre^{fl/fl}$: 0.84 ± 0.16 and $Emre^{cKO}$: 0.87 ± 0.10. Values represent mean ± SD, n = 3 in each group. **D**) Western blot for Cyclophilin D (CypD) expression from cardiac mitochondria, quantified to the right in MCM^{+}: 1.00 ± 0.22, $Emre^{fl/fl}$: 0.96 ± 0.19 and $Emre^{cKO}$: 1.08 ± 0.15. Values represent mean ± SD, n = 5/8/8 in each group. One-way ANOVA was used for statistical analysis in (A)-(D); p-values were not significant.

Supplementary Figure 4



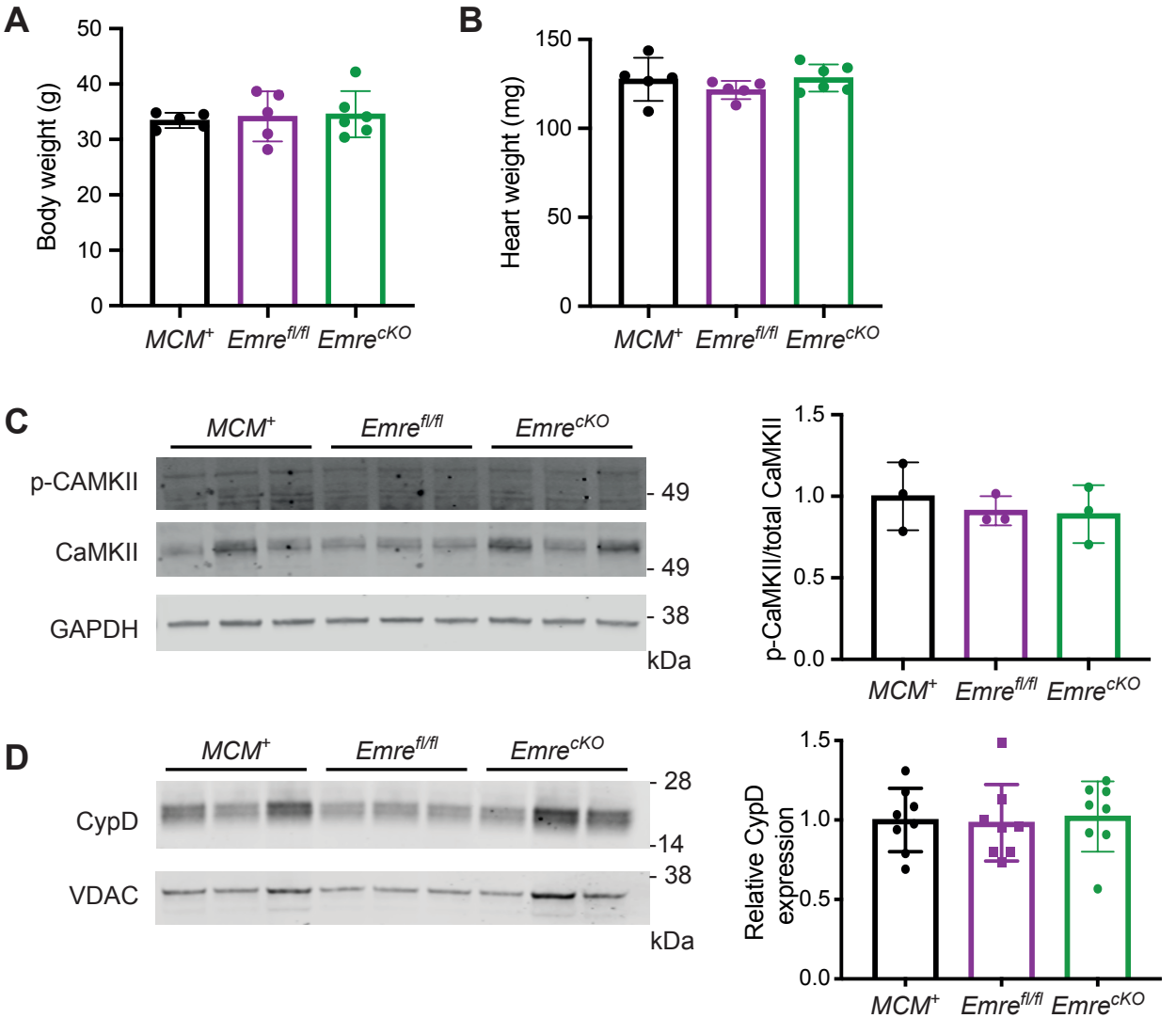
Supplementary Figure 4. *Long-term Emre deletion affects mitochondrial Ca^{2+} uptake but not the expression of mitochondrial Ca^{2+} efflux proteins.* Analyses were performed 3 months post-tamoxifen. **A**) Relative *Emre* mRNA expression in heart tissue from MCM^{+}: 1.00 ± 0.22, $Emre^{fl/fl}$: 1.25 ± 0.15 and $Emre^{cKO}$: 0.09 ± 0.06. Values represent mean ± SD. **p<0.0001, n = 3-4 per group. One-way ANOVA test was used for statistical analysis. **B**) Relative *Mcu* mRNA expression levels in heart tissue from MCM^{+}: 1.00 ± 0.03, $Emre^{fl/fl}$: 1.15 ± 0.04 and $Emre^{cKO}$: 1.00 ± 0.13. Values represent mean ± SD. n = 3-4 per group. **C**) Quantification of MICU1 expression from Western blot (Fig. 5A), where bars represent mean ± SD. p-values were not significant. n = 3 per group. One-way ANOVA test was used for statistical analysis. **D**) Western blot of LETM and NCLX in cardiac mitochondria. Quantification is shown below, where bars represent mean ± SD. p-values were not significant. n = 3 per group. One-way ANOVA test was used for statistical analysis. **E**) Representative Ca^{2+} retention capacity (CRC) assay in isolated heart mitochondria from $Emre^{fl/fl}$ (purple line) and $Emre^{cKO}$ (green line) mice. Mitochondria were energized with 10 mM glutamate/5 mM malate. The fluorescent Ca^{2+} indicator Calcium Green-5N was used to monitor extramitochondrial Ca^{2+}. The arrows represent 15 μM Ca^{2+} additions. Traces are representative of n = 4 independent experiments. **F**) Ca^{2+} retention capacity calculated from independent traces as shown in (E). The estimated mean Ca^{2+}/mg protein was for $Emre^{fl/fl}$: 105 ± 21.21 and $Emre^{cKO}$: 3.75 ± 7.5 nmol. Values represent mean ± SD.**p<0.0001, n = 4 in each group. Student's t-test was used for statistical analysis. **G**) Quantitative analysis of swelling calculated via loss of absorbance (%) 15 minutes after Ca^{2+} addition. MCM^{+}: 25.25% ± 9.04, $Emre^{fl/fl}$: 28.91% ± 12.91 and $Emre^{cKO}$: 8.74% ± 5.68. Values represent mean ± SD.**p<0.0001, n = 4-6 in each group. One-way ANOVA test was used for statistical analysis.

Supplementary Figure 5



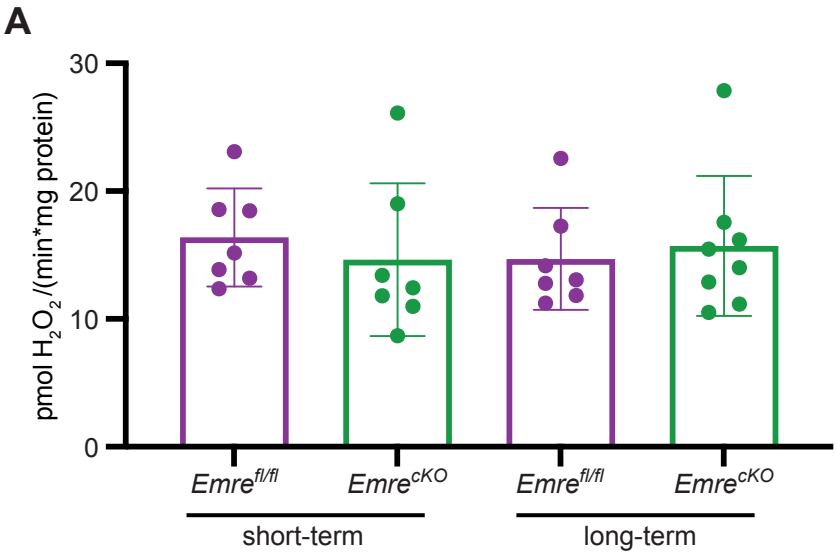
Supplementary Figure 5. *Effects of long-term Emre deletion on bioenergetic capacity*. Analyses were performed 3 months post-tamoxifen. **A-B**) Seahorse analysis of oxygen consumption rate (OCR) in cardiac mitochondria was measured by sequential injections: 4 mM ADP (state III), 2.5 μg/mL oligomycin (state IV_o), 6 μM FCCP (state III_u) and 4 μM rotenone/4 μM antimycin A. Mitochondria were supplied with 5 mM pyruvate/0.5 mM malate (A) or 40 μM palmitoylcarnitine/0.5 mM malate (B). **C**) Respiratory control ratio (RCR; OCR in state III_u/stateIV_o) in mitochondria supplied with 5 mM pyruvate/0.5 mM malate: MCM^+: 10.44 ± 2.26, $Emre^{fl/fl}$: 8.76 ± 1.12 and $Emre^{cKO}$: 8.46 ± 1.84. Values represent mean ± SD, n = 5 per group (combined males and females). One-way ANOVA test was used for statistical analysis. **D**) RCR in mitochondria supplied with 40 μM palmitoylcarnitine/0.5 mM malate, MCM^+: 3.99 ± 0.85, $Emre^{fl/fl}$: 4.07 ± 1.22 and $Emre^{cKO}$: 3.97 ± 0.58. Values represent mean ± SD, n = 5 per group (combined males and females). One-way ANOVA test was used for statistical analysis. **E**) Western blot for CPT1B in cardiac tissue: MCM^+: 1.00 ± 0.18, $Emre^{fl/fl}$: 0.80 ± 0.01 and $Emre^{cKO}$: 0.86 ± 0.21, n = 3 per group. **F-G**) Complex I (NADH ubiquinone oxidoreductase) activity (n = 8 per group) (F) and Complex II (succinate dehydrogenase) activity (n = 6 per group) (G) normalized to citrate synthase (CS) activity. Values represent mean ± SD, Student's t-test was used for statistical analysis. **H**) ATP production rate in isolated heart mitochondria stimulated with 50 μM ADP and 10 mM glutamate/5 mM malate ± 20 μM Ca^{2+}, $Emre^{fl/fl}$: 5.38 ± 0.99 *vs* $Emre^{fl/fl}$ + 20 μM Ca^{2+}: 7.13 ± 0.94, and $Emre^{cKO}$*:* 5.19 ± 0.93 *vs* $Emre^{cKO}$ + 20 μM Ca^{2+}: 5.43 ± 1.38 nmol ATP/(min*mg) protein. Values represent mean ± SD. *$p<0.05$, n = 7 per group (combined males and females). One-way ANOVA was used for statistical analysis.

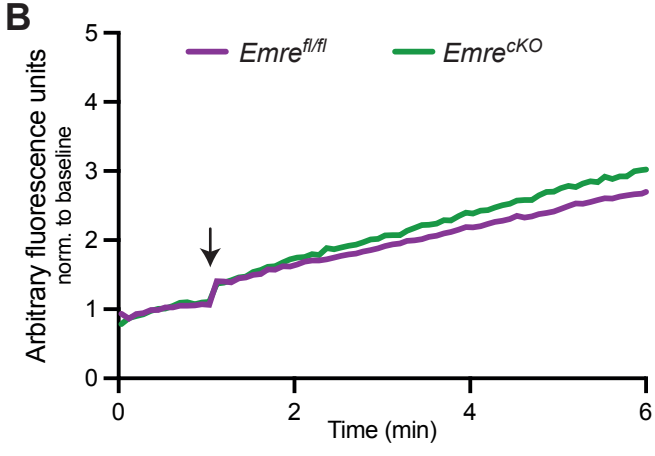
Supplementary Figure 6

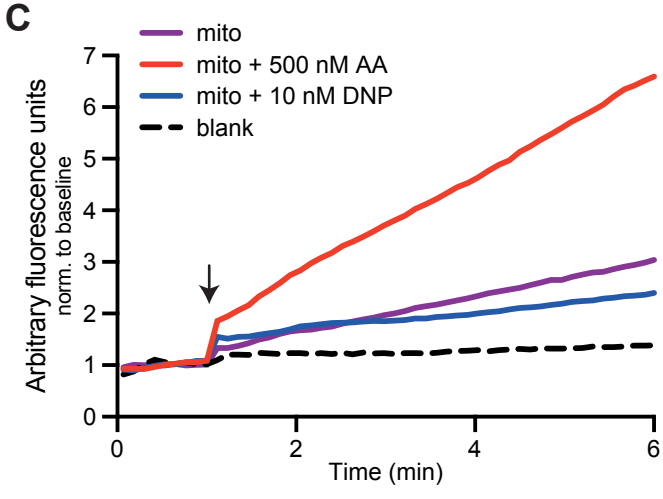


Supplementary Figure 6. *Effects of long-term Emre deletion on body and heart weight and protein expression.* Analyses were performed 3 months post-tamoxifen. **A-B**) Body weight (A) and heart weight (B) from MCM^{+}, $Emre^{fl/fl}$, and $Emre^{cKO}$ mice at 3 months post-tamoxifen. Values represent mean ± SD, n = 5-6 per group. **C**) Western blot for phosphorylated CaMKII level relative to total CaMKIIδ from cardiac tissue, quantified to the right in MCM^{+}: 1.00 ± 0.20, $Emre^{fl/fl}$: 0.91 ± 0.08 and $Emre^{cKO}$: 0.89 ± 0.17. Values represent mean ± SD, n = 3 in each group. **D**) Western blot for Cyclophilin D (CypD) expression from cardiac mitochondria, quantified to the right in MCM^{+}: 1.00 ± 0.19, $Emre^{fl/fl}$: 0.98 ± 0.24 and $Emre^{cKO}$: 1.02 ± 0.22. Values represent mean ± SD, n = 8 in each group. One-way ANOVA was used for statistical analysis in (A)-(D); p-values were not significant.

Supplementary Figure 7







Supplementary Figure 7. *Short-term and long-term Emre deletion have little effect on mitochondrial H_2O_2 production*. **A**) H_2O_2 production rates measured by Amplex Red in short- and long-term $Emre^{fl/fl}$ and $Emre^{cKO}$ cardiac mitochondria supplied 10 mM glutamate/5 mM malate. For short-term, $Emre^{fl/fl}$: 16.38 ± 3.83, $Emre^{cKO}$: 14.64 ± 5.96, and for long-term, $Emre^{fl/fl}$: 14.69 ± 3.98, $Emre^{cKO}$: 15.70 ± 5.47 pmol H_2O_2/(min*mg protein). Values represent mean ± SD, n = 7-8 in each group. One-way ANOVA was used for statistical analysis. **B**) Representative traces obtained by measuring Amplex Red fluorescence in long-term $Emre^{fl/fl}$ (purple line) and $Emre^{cKO}$ (green line) mitochondria. Arrow denotes the addition of 10 mM glutamate/5 mM malate. **C**) Representative traces confirming the sensitivity of Amplex Red to mitochondrial ROS production, using long-term $Emre^{fl/fl}$ mitochondria alone (purple line), with 500 nM antimycin A (AA, red line) as a positive control, or with 10 nM 2,4-dinitrophenol (DNP, blue line) as a negative control. A blank well without mitochondria is also shown (dashed black line).