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Supplemental information

MCUb is an inducible regulator of calcium-dependent mitochondrial metabolism and substrate utilization in muscle

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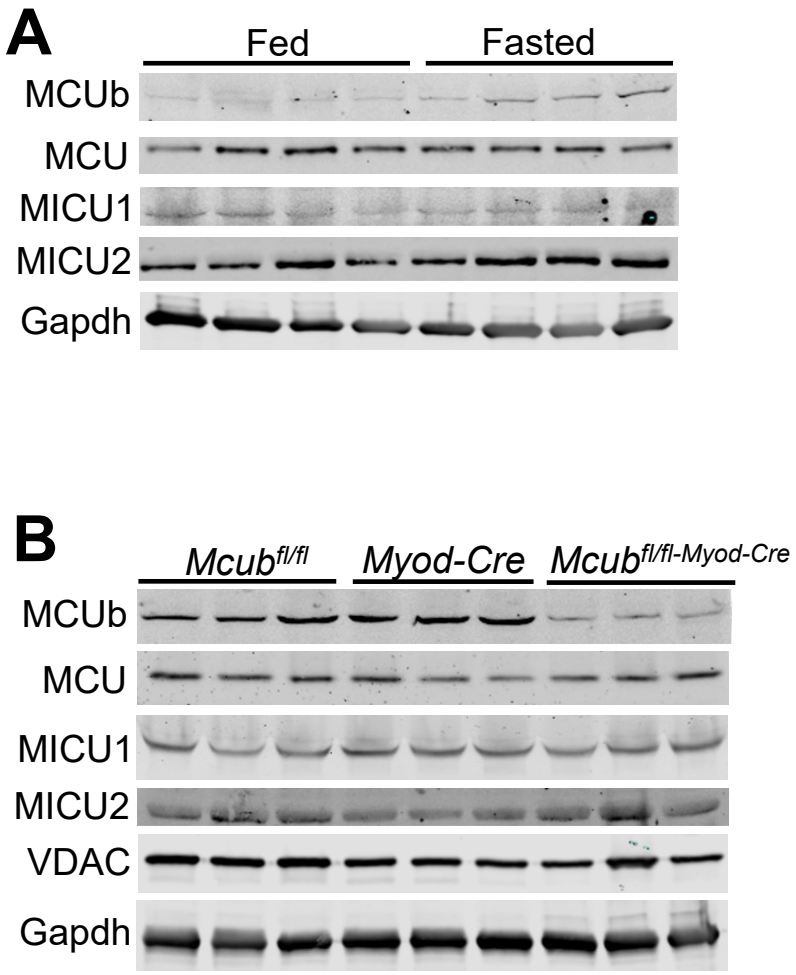

Figure S1. Protein expression for MCU complex protein in fed and fasted states or without Mcub in skeletal muscle. (A) Western blotting of the indicated proteins from quadriceps of adult mice under fed or 48 h fasted conditions. Gapdh was used as a control. Four mice in each group were analyzed. (B) Western blot for the indicated proteins from quadriceps protein extract in the indicated 2 control groups of mice and mice lacking *Mcub* in skeletal muscle. Gapdh and voltage-dependent anion channel (VDAC) were used as controls. Three mice were analyzed in each group.

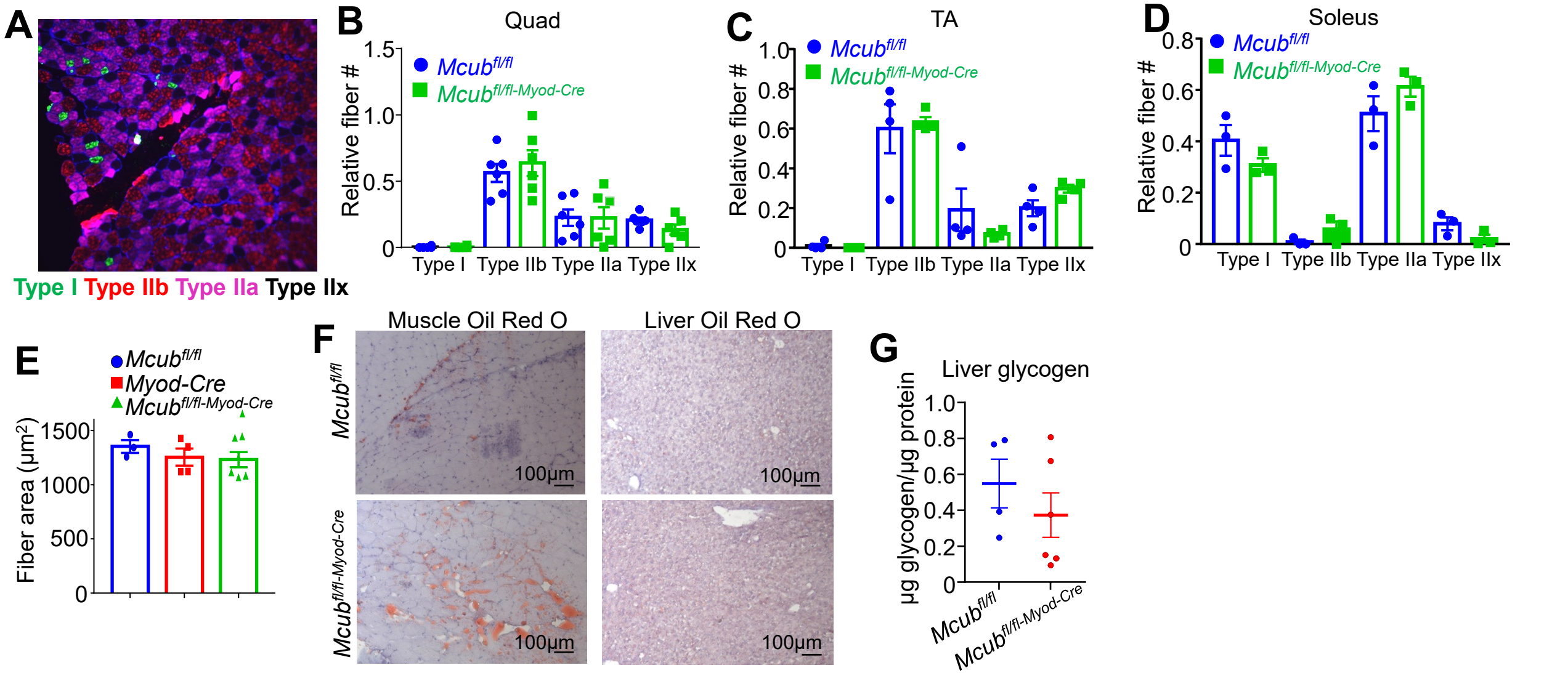

Figure S2. **Analysis of muscle fiber type distribution, muscle and liver lipid accumulation and liver glycogen with *Mcub* gene deletion in skeletal muscle**. (A) Representative immunohistochemistry of fiber type staining as indicated from quadriceps. (B-D) Quantification of fiber type distribution from the indicated groups of mice among different muscle types shown. Data presented as mean ± SEM. (E) Quantification of fiber area from quadriceps muscle from the indicated groups of the mice. n=3 in *Mcub*$^{fl/fl}$ group, n=4 in *Myod-Cre* group, n=6 in *Mcub*$^{fl/fl\text{-}Myod\text{-}Cre}$ group. (F) Representative Oil Red O-stained histological muscle and liver sections at 100 X magnification from the 2 genotypes of mice shown at 6 months of age. Scale bars are shown in each panel. (G) Liver glycogen measured from the 2 genotypes of mice shown at 6 months of age. Data presented as mean ± SEM. No difference was observed in liver glycogen or liver Oil Red O staining, but skeletal muscle Oil Red O staining was prominent in *Mcub*$^{fl/fl\text{-}Myod\text{-}Cre}$ mice but not in the control *Mcub*$^{fl/fl}$ mice. Data presented as mean ± SEM. Quad, quadriceps, TA, tibialis anterior.

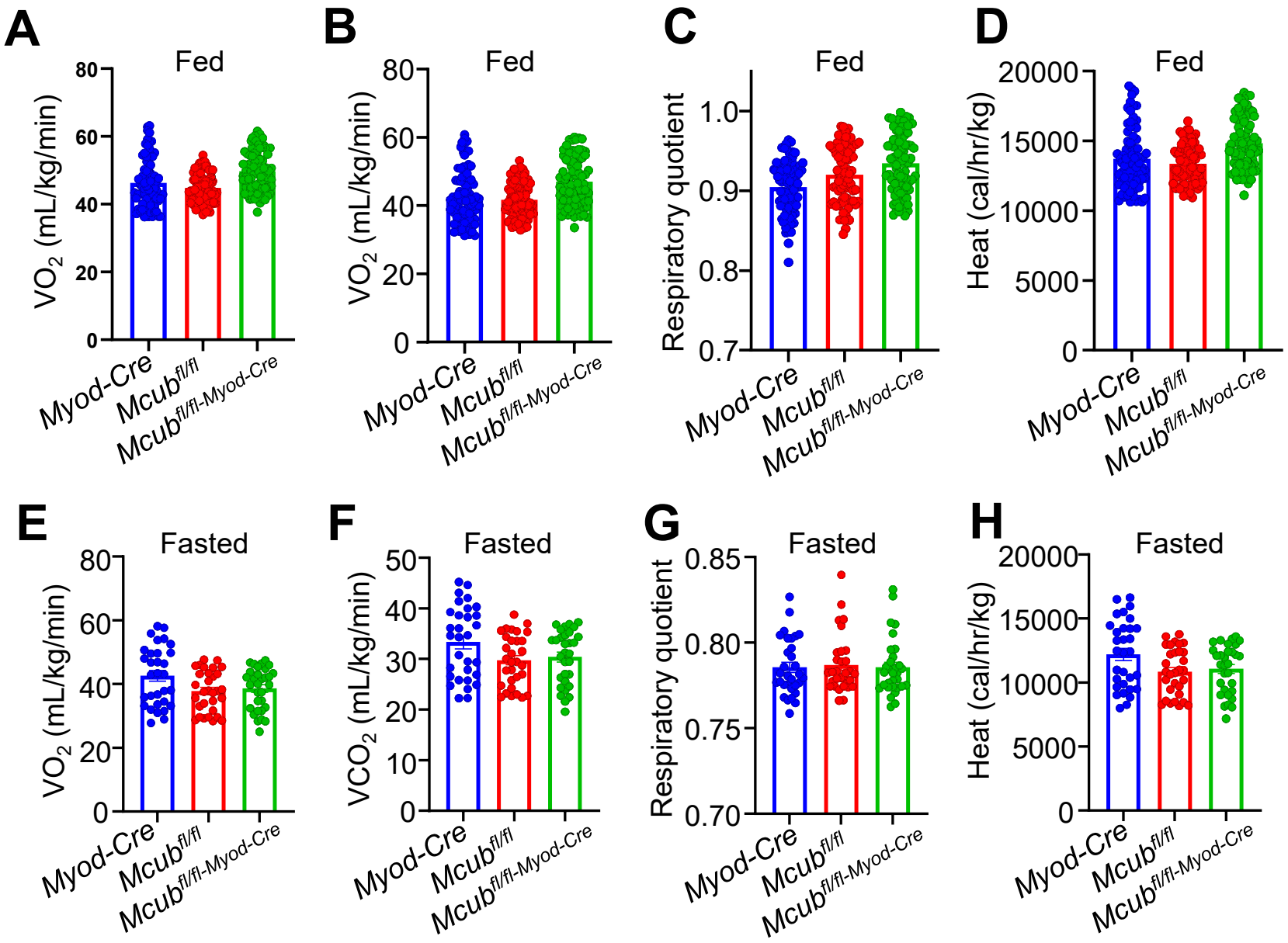

Figure S3. No changes were observed in indirect calorimetry measurement with *Mcub* muscle-specific deletion. (A-D) Mice of the indicated genotypes with full access to food for 48 hrs and assessed by indirect calorimetry with the indicated experimental assays. (E-H) Same genotypes of mice and the same indirect calorimetery assessments but with 48 hrs of food restriction (fasted). Data presented as mean ± SEM. The results show no significant differences between the 3 groups in either fed or fasted states.

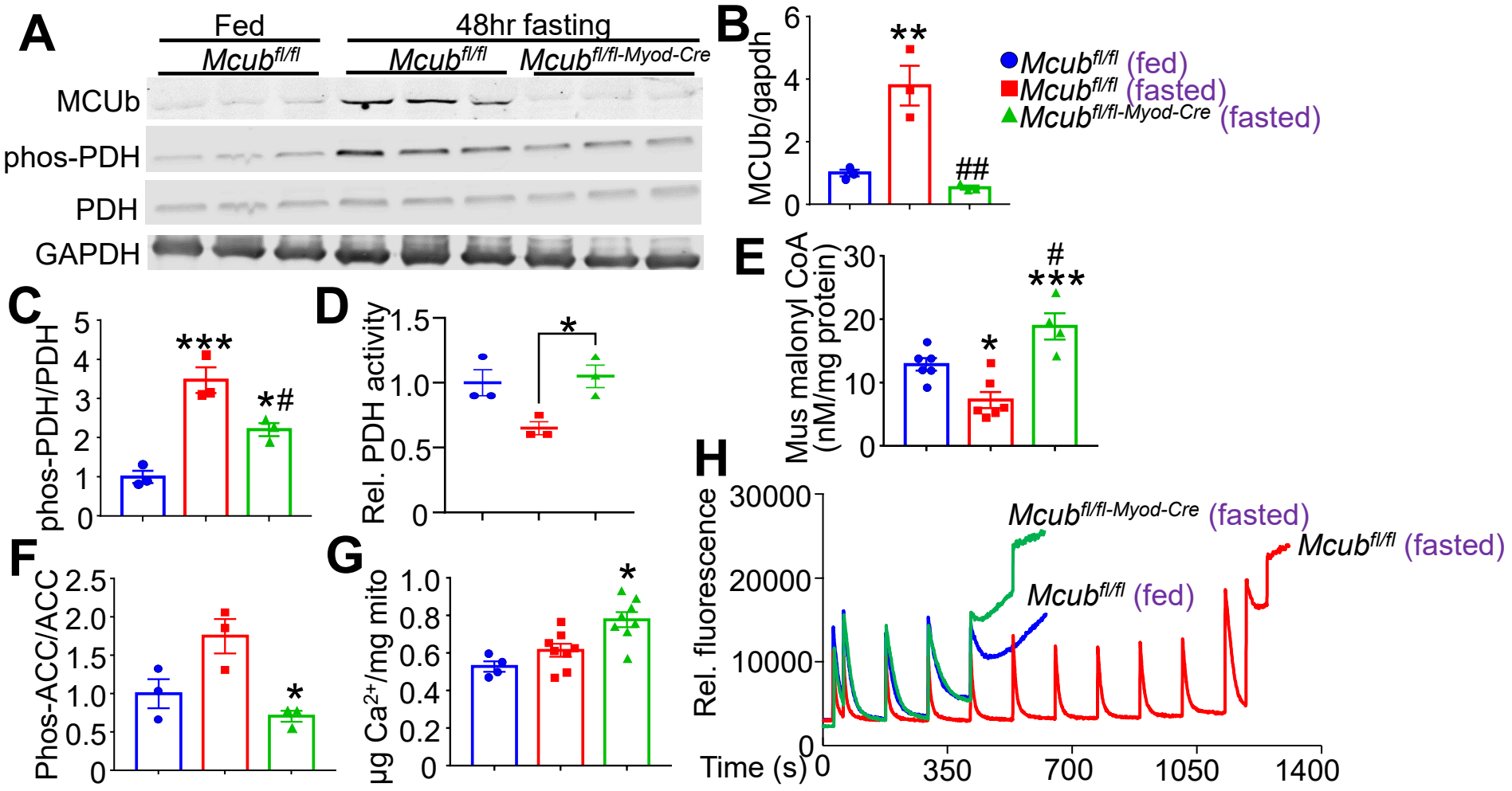

Figure S4. MCUb induction with fasting enhances fatty acid oxidation and mitochondrial Ca^{2+} uptake capacity. (A) Western blots of the indicated proteins from quadriceps in the indicated groups of mice. GAPDH was used as a control. (B) Quantification of Gapdh normalized MCUb expression as shown in A. Data presented as mean ± SEM. One-way ANOVA and post-hoc Bonferroni test was used for statistical analysis. **p<0.01 versus *Mcub*$^{fl/fl}$ fed; $^{\#\#}$P<0.01 versus *Mcub*$^{fl/fl}$ fasted. (C) Quantification of phosphorylated-PDH/PDH ratio in muscle from the groups of mice as shown in A. Data presented as mean ± SEM. One-way ANOVA and Bonferroni's multiple comparison test was used for statistical analysis. *p<0.05 versus *Mcub*$^{fl/fl}$ fed, ***p<0.001 versus *Mcub*$^{fl/fl}$ fed; $^{\#}$P<0.05 versus *Mcub*$^{fl/fl}$ fasted. (D) Relative PDH enzymatic activity from quadriceps muscle from the indicated genotypes of mice at 6 months of age. N=3 per group. Data presented as mean ± SEM. One-way ANOVA and Bonferroni's multiple comparison test was used for statistical analysis. *p<0.05 (E) Quadriceps malonyl CoA levels from the indicated groups of mice at 6 months of age. N=6 in *Mcub*$^{fl/fl}$ control fed group, n=6 in *Mcub*$^{fl/fl}$ fasting group, n=4 in *Mcub*$^{fl/fl\text{-}Myod\text{-}Cre}$ fasting group. Data presented as mean ± SEM. One-way ANOVA and Bonferroni's multiple comparison test was used for statistical analysis. *p<0.05 versus *Mcub*$^{fl/fl}$ fed, ***p<0.001 versus *Mcub*$^{fl/fl}$ fed; $^{\#}$P<0.05 versus *Mcub*$^{fl/fl}$ fasted (F) Quantification of phosphorylation-ACC/ACC ratio in quadriceps at 6 months of age from the indicated groups of mice. n=3 per group. Data presented as mean ± SEM. One-way ANOVA and Bonferroni's multiple comparison test was used for statistical analysis. *p<0.05 versus either control groups. (G) Quantification of mitochondrial Ca^{2+} levels in isolated quadriceps mitochondria from the indicated groups of mice at 6 months of age. N=4 in *Mcub*$^{fl/fl}$ fed control group, n=8 in *Mcub*$^{fl/fl}$ fasting group, n=8 in *Mcub*$^{fl/fl\text{-}Myod\text{-}Cre}$ fasting group. Data presented as mean ± SEM. One-way ANOVA and Bonferroni's multiple comparison test was used for statistical analysis. *p<0.05 versus either control groups. (H) Mitochondrial Ca^{2+} retention capacity assay in isolated quadriceps mitochondria from the indicated groups at 6 months of age. Calcium Green-5N was used as the Ca^{2+} indicator in solution. Ca^{2+} was added at each inflection pulse point until mitochondrial pore transition at end of each tracing.