Electronic supplementary material

Loss of mitochondrial calcium uniporter rewires skeletal muscle metabolism and substrate preference

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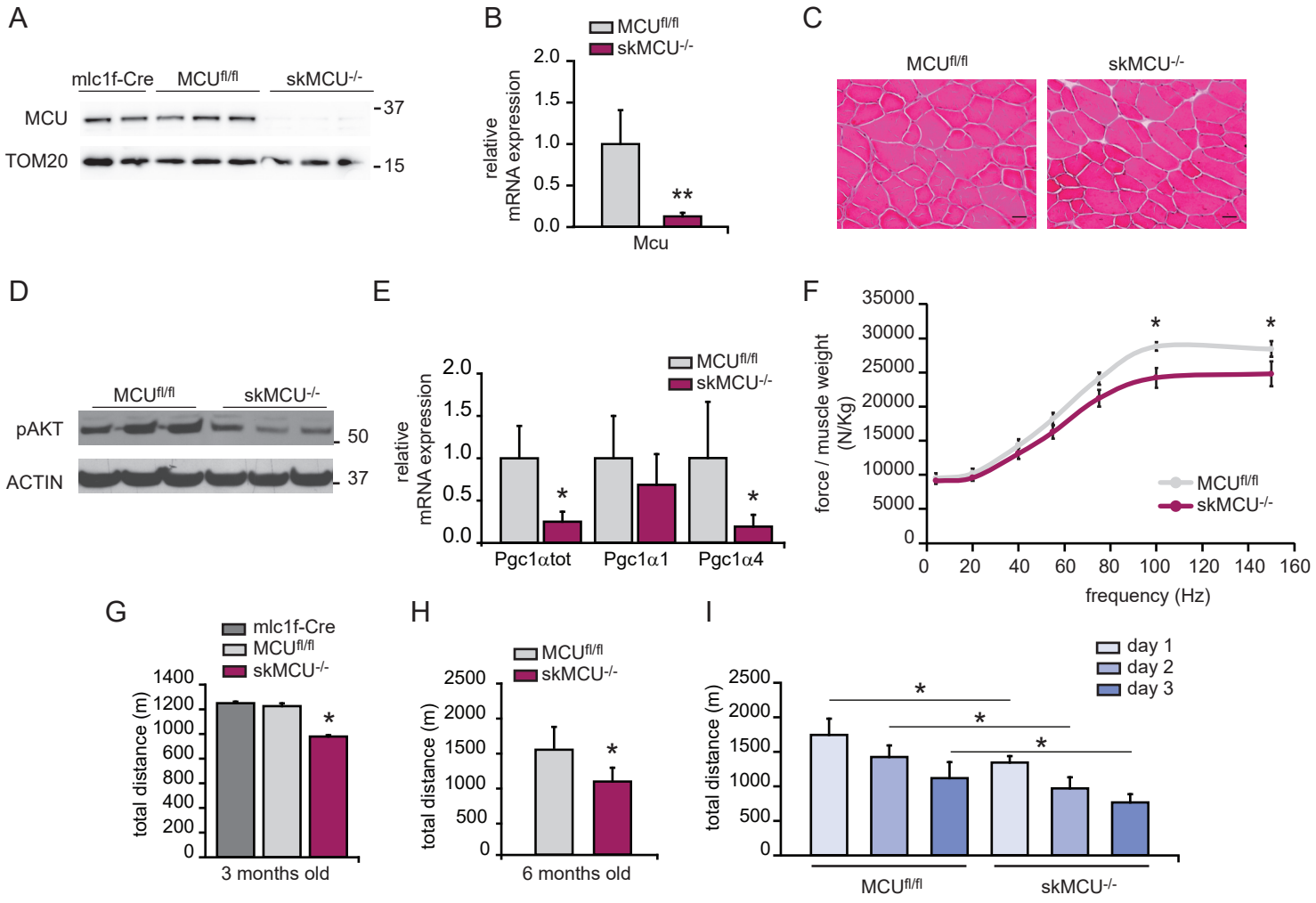

Fig S1. Anabolic pathways are downregulated in skMCU$^{-/-}$ mice, related to Figure 1

A Western blot analysis demonstrated efficient MCU deletion in skMCU$^{-/-}$ TA muscles. MCU$^{fl/fl}$ and mlc1f-Cre mice were used as controls. **B** MCU mRNA expression levels were suppressed in skMCU$^{-/-}$ TA muscles. $p < 0.05$, t- test (two-tailed, unpaired) of 4 animals per condition. Data are presented as mean ±SD. **C** Hematoxylin & Eosin staining showed unaltered histological structure of skMCU$^{-/-}$ TA muscles. Scale bar 50µm. **D** Phosphorylation levels of AKT were decreased in skMCU$^{-/-}$ TA muscles compared to MCU$^{fl/fl}$ muscles. **E** Relative mRNA levels of PGC1α4 were decreased in skMCU$^{-/-}$ TA muscles compared to controls. *$p < 0.05$, t- test (two-tailed, unpaired) of 4 animals per condition. Data are presented as mean ±SD. **F** Reduced *in vivo* force production from gastrocnemius muscles was measured in skMCU$^{-/-}$ mice compared to MCU$^{fl/fl}$ mice. *$p < 0.05$, Mann-Whitney rank sum test (two-tailed, unpaired) of 6 animals per condition. Data are presented as mean ±SD. **G** Mean maximal running distance in a single bout of run on a treadmill of 3 month old mlc1f-Cre, MCU$^{fl/fl}$ and skMCU$^{-/-}$ mice indicated that MCU deletion negatively affects exercise performance. In addition, MCU$^{fl/fl}$ and mlc1f-Cre mice had no differences in running capacity. *$p < 0.05$, t- test (two-tailed, unpaired) of 4 animals per condition. Data are presented as mean ±SD. **H** Mean maximal running distance in a single bout of run on a treadmill of 6 month old MCU$^{fl/fl}$ and skMCU$^{-/-}$ mice. *$p < 0.05$, t- test (two-tailed, unpaired) of 5 animals per condition. Data are presented as mean ±SD. **I** Mean maximal running distances after each of 3 days of downhill run on a treadmill of MCU$^{fl/fl}$ and skMCU$^{-/-}$ mice. *$p < 0.05$, t- test (two-tailed, unpaired) of 5 animals per condition. Data are presented as mean ±SD.

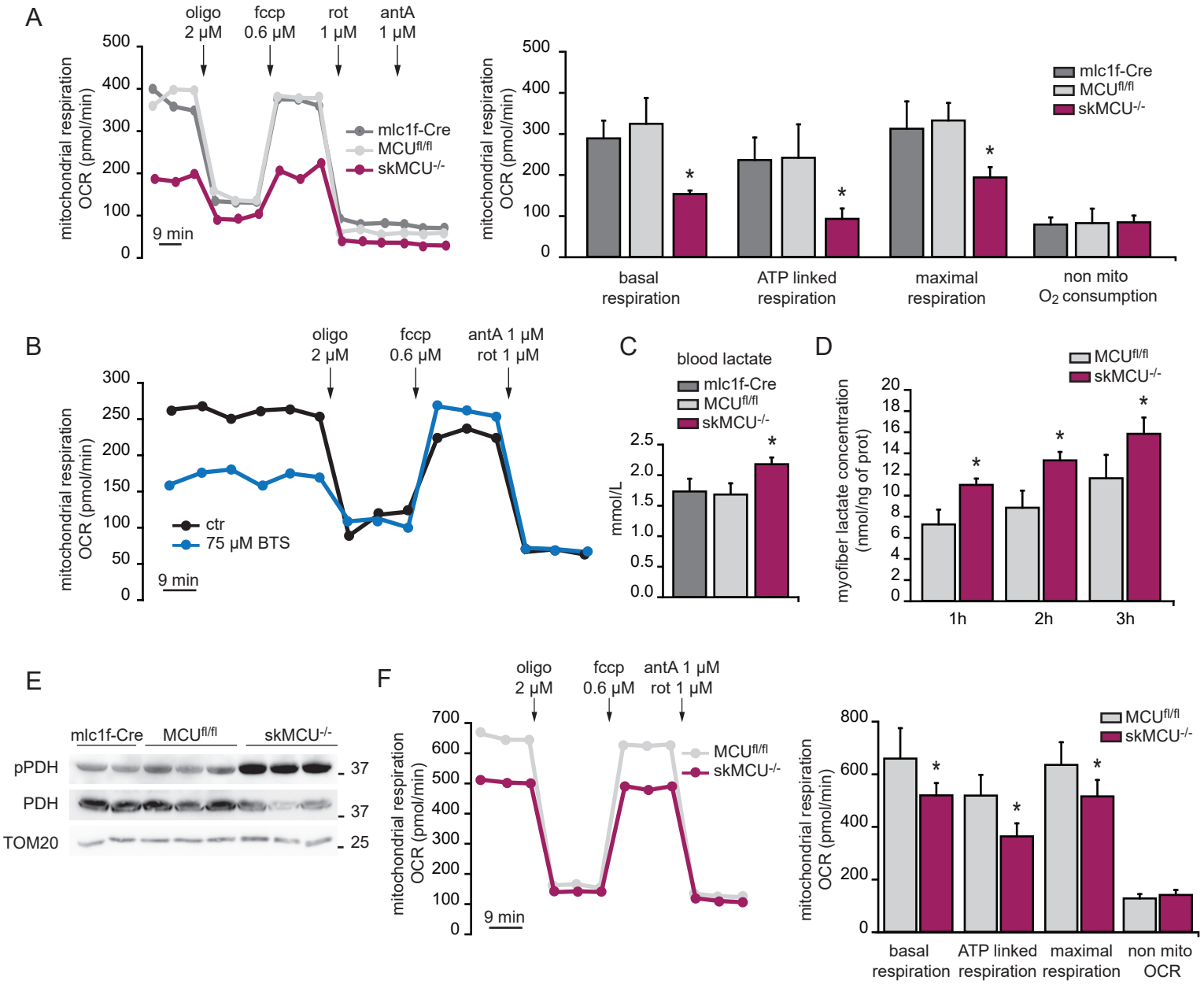

Fig S2. skMCU$^{-/-}$ mice show a metabolic rewiring toward β-oxidation, related to Figure 2

A OCR measurements indicated reduced respiratory capacity in skMCU$^{-/-}$ FDB myofibers compared to two different controls (mlc1f-Cre and MCU$^{fl/fl}$). Left: representative traces. Right: quantification. To calculate basal and maximal respiration, non-mitochondrial O_2 consumption was subtracted from absolute values. ATP linked respiration was calculated as the difference between basal and oligomycin-insensitive O_2 consumption. Data are normalized on mean Calcein fluorescence. *p <0.05, t- test (two-tailed, unpaired) of 10 samples per condition. Data are presented as mean ±SD. **B** Treatment of freshly isolated myofibers with 75µM N-benzyl-P-toluenesulfonamide (BTS) decreased basal OCR. **C** Blood lactate concentration was increased in skMCU$^{-/-}$ mice compared to controls (mlc1f-Cre and MCU$^{fl/fl}$). *p <0.05, t- test (two-tailed, unpaired) of 4 animals per condition. Data are presented as mean ±SD. **D** Lactate production was increased in skMCU$^{-/-}$ TA myofibers. *p <0.05, t- test (two-tailed, unpaired) of 4 animals per condition. Data are presented as mean ±SD. **E** Phosphorylation levels of PDH were increased in skMCU$^{-/-}$ TA muscles compared to controls (mlc1f-Cre and MCU$^{fl/fl}$) **F**. Exogenous FA oxidation was decreased in skMCU$^{-/-}$ FDB myofibers compared to controls. Left: 1 hour after the addition of palmitate to the medium, OCR measurements were performed as in A. Right: quantification. Data are normalized on mean Calcein fluorescence. *p <0.05, t- test (two-tailed, unpaired) of 5 samples per condition. Data are presented as mean ±SD.

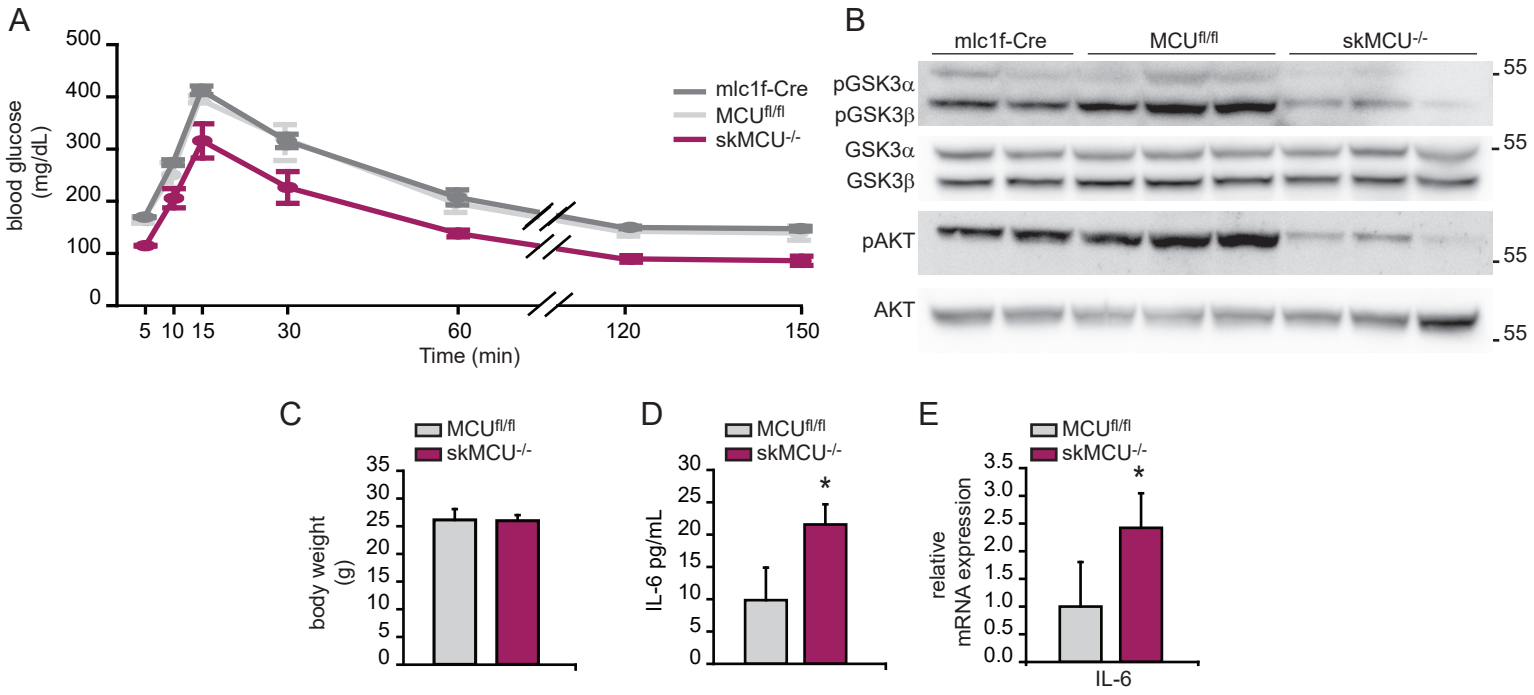

Fig S3. Skeletal muscle-specific MCU deletion triggers a whole body metabolic adaptation, related to Figure 4

A Glucose tolerance test of mlc1f-Cre, $MCU^{fl/fl}$ and $skMCU^{-/-}$ mice. $skMCU^{-/-}$ mice had decreased glycemia at all time points compared to $MCU^{fl/fl}$ and mlc1f-Cre. *p <0.05, t- test (two-tailed, unpaired) of 5 animals per condition. Data are presented as mean ±SD. **B** Phosphorylation levels of GSK3 α/β and AKT were decreased in $skMCU^{-/-}$ livers compared to controls (mlc1f-Cre and $MCU^{fl/fl}$). Total AKT and GSK3 α/β were used as control. **C** Total body weight was unchanged between $skMCU^{-/-}$ and $MCU^{fl/fl}$ mice. Data are presented as mean ±SD (4 animals per condition). **D** Plasma levels of IL-6 were increased in $skMCU^{-/-}$ mice compered to controls. *p <0.05, t- test (two-tailed, unpaired) of 5 animals per condition. Data are presented as mean ±SD. **E** Relative mRNA expression levels of the IL-6 in $skMCU^{-/-}$ compared to $MCU^{fl/fl}$ TA muscles. *p <0.05, t- test (two-tailed, unpaired) of 5 animals per condition. Data are presented as mean ±SD.

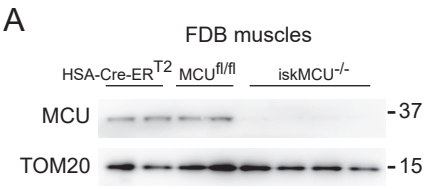

Fig S4. Downregulation of MCU expression during adulthood, related to Figure 5
A Western blot analysis demonstrated the MCU deletion in iskMCU$^{-/-}$ FDB muscles compared to controls (HSA-Cre-ER^{T2} and MCU$^{fl/fl}$). All animals received tamoxifen treatment.

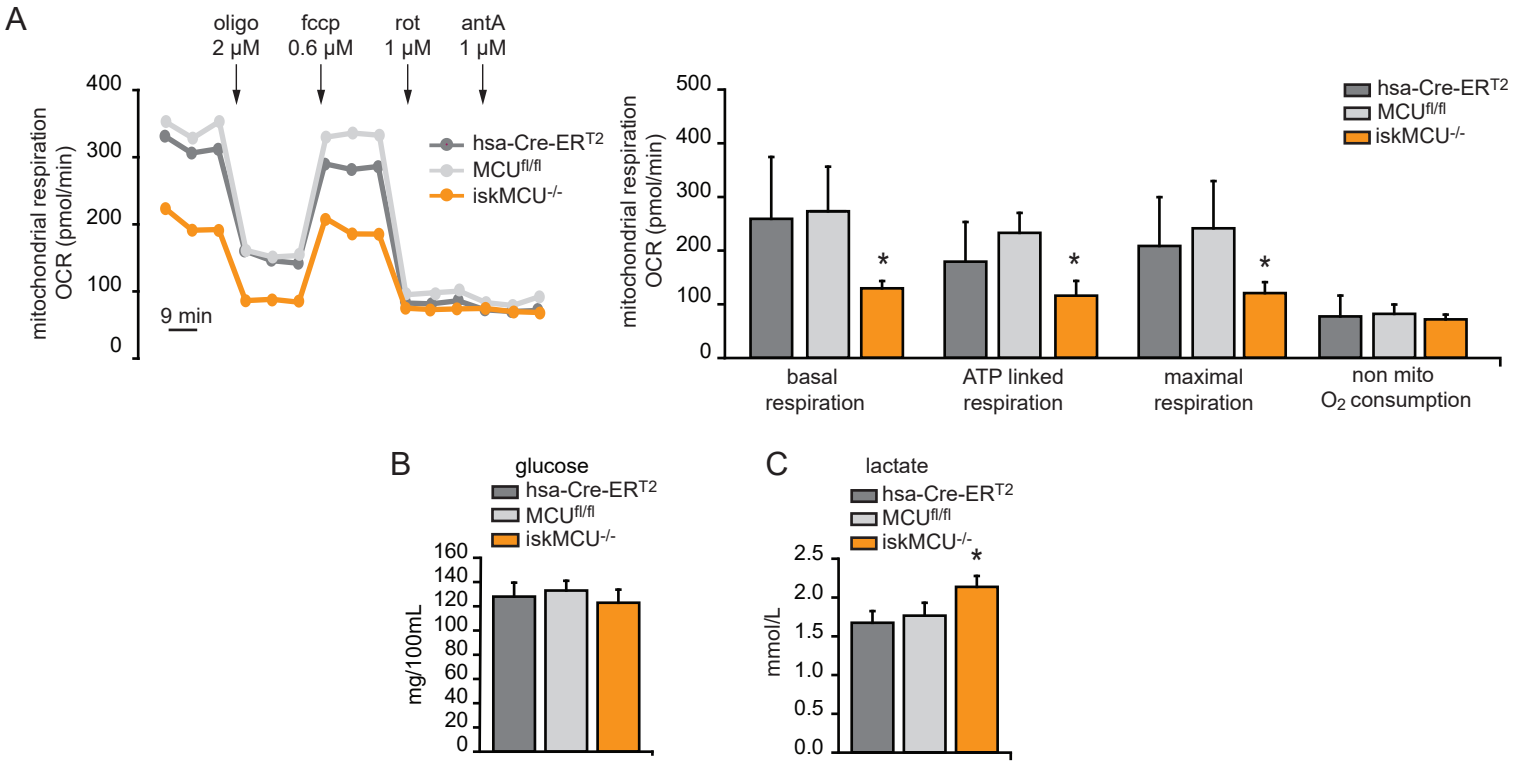

Fig S5. iskMCU$^{-/-}$ mice showed impaired metabolism, related to Figure 6

A OCR measurements indicated reduced respiratory capacity in iskMCU$^{-/-}$ FDB myofibers compared to two different controls (HSA-Cre-ER^{T2} and MCU$^{fl/fl}$). All animals received tamoxifen treatment. Left: representative traces. Right: quantification. To calculate basal and maximal respiration, non mitochondrial O_2 consumption was subtracted from absolute values. ATP linked respiration was calculated as the difference between basal and oligomycin-insensitive O_2 consumption. Data are normalized on mean Calcein fluorescence. *p <0.05, t- test (two-tailed, unpaired) of 10 samples per condition. Data are presented as mean ±SD. **B** iskMCU$^{-/-}$ mice had unaltered fasting glycemia compared to HSA-Cre-ER^{T2} and MCU$^{fl/fl}$ controls. All animals received tamoxifen treatment. Data are presented as mean ±SD (5 animals per condition). **C** Blood lactate concentration was increased in iskMCU$^{-/-}$ mice compared to controls (HSA-Cre-ER^{T2} and MCU$^{fl/fl}$). All animals received tamoxifen treatment. *p <0.05, t- test (two-tailed, unpaired) of 4 animals per condition. Data are presented as mean ±SD.