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Supplemental Information

Exploring the *In Vivo* Role of the Mitochondrial Calcium Uniporter in Brown Fat Bioenergetics

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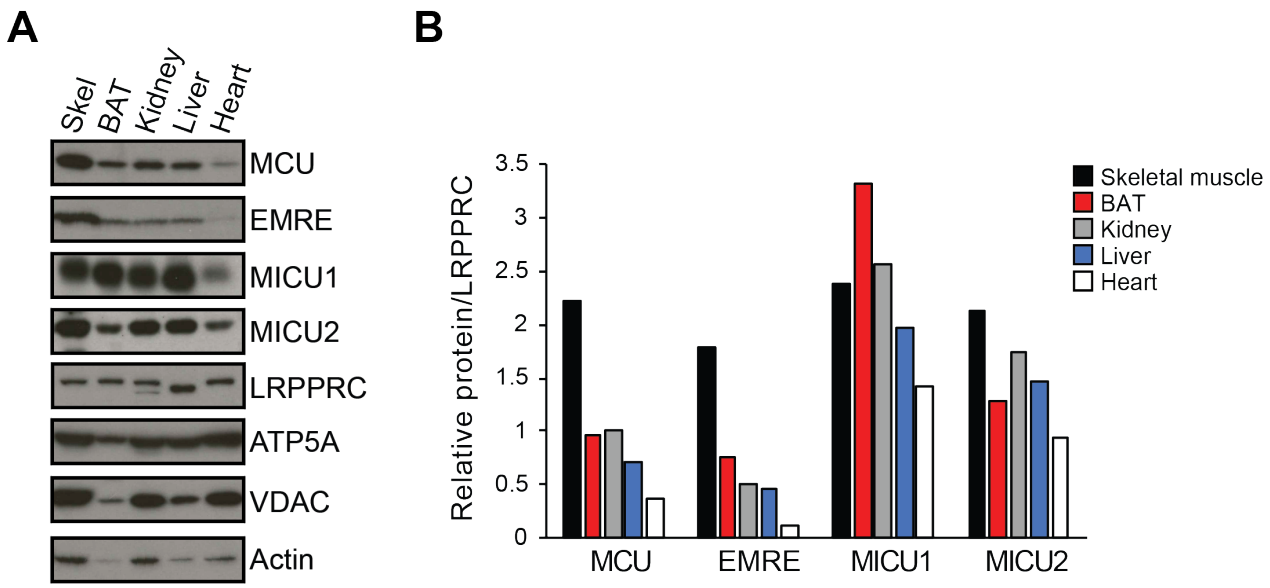

Figure S1. Tissue distribution of uniporter component proteins.
Related to Figure 1.
(A) Mitochondria were isolated from mouse skeletal muscle (gastrocnemius + soleus), BAT, kidney, liver, and heart, and lysed in RIPA buffer. Equal amounts of protein were loaded in each lane.
(B) Quantification of the uniporter component proteins in (A) relative to LRPPRC, a soluble matrix protein.

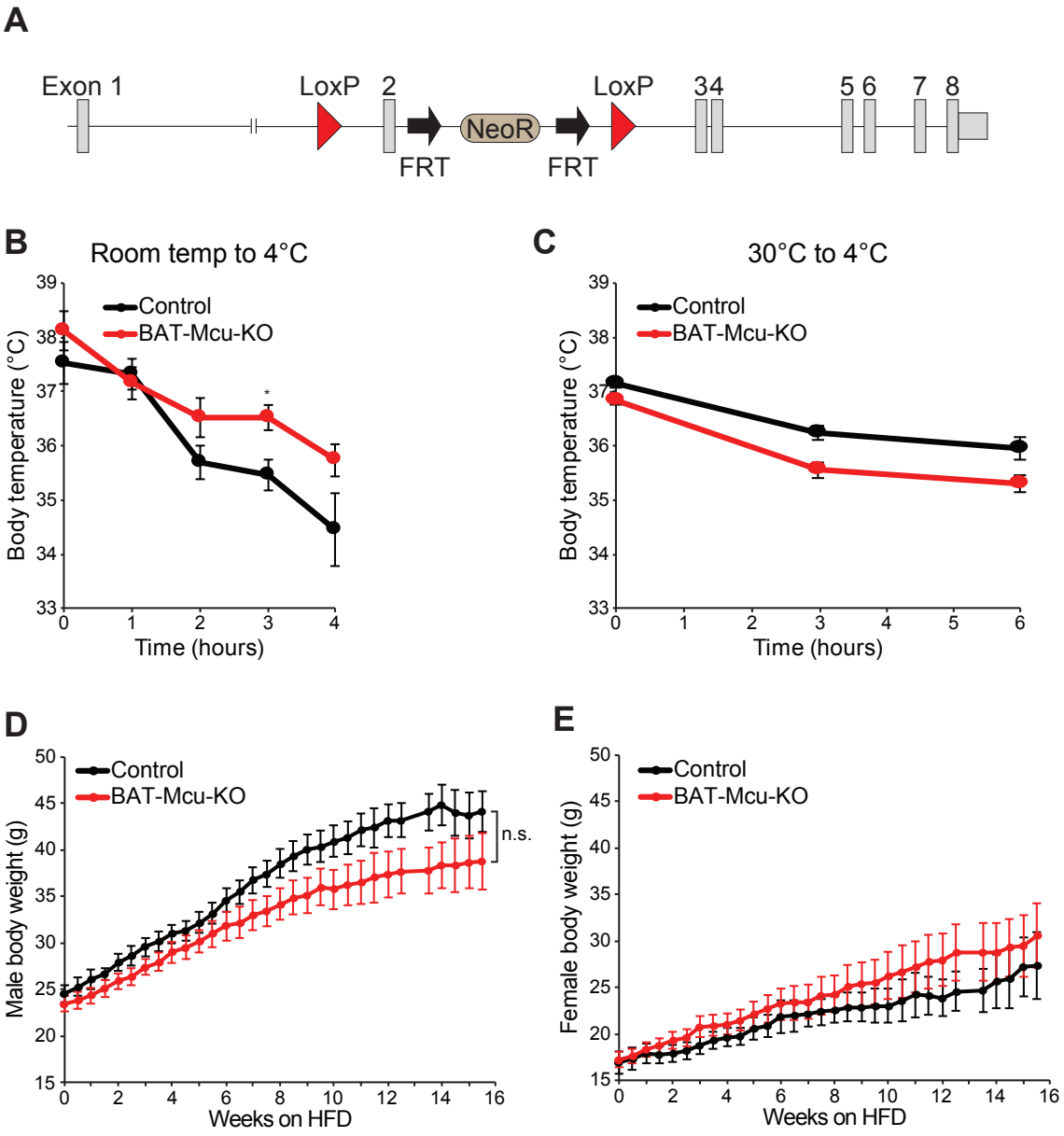

Figure S2. Construction and metabolic phenotyping of BAT-Mcu-KO mice.
Related to Figure 2.
(A) Conditional *Mcu* allele utilized to generate the *Mcu*$^{fl/fl}$ mice,
(B) Core body temperature of mice transferred from room temperature to 4°C (n = 5-6 per group).
(C) Core body temperature of mice transferred to 4°C following 1 week habituation to 30°C (n = 7 per group).
(D-E) Body mass of male (C) and female (D) -Cre and +Cre animals fed high-fat diet.

Results are reported as mean + SEM. Statistical significance is indicated as * $p < 0.05$ (student's t-test).

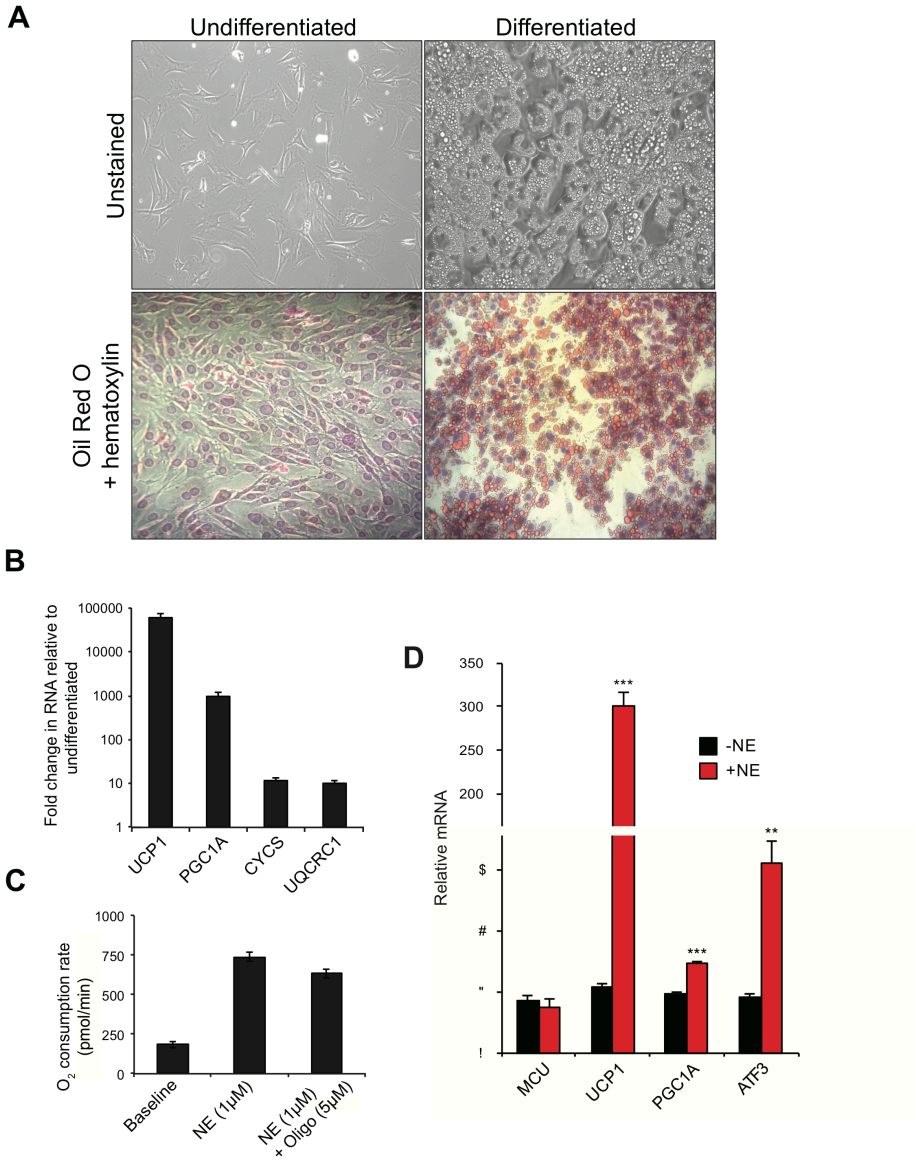

Figure S3. Transcriptional response to norepinephrine in immortalized brown adipocytes.
Related to Figure 4.
(A-C) Validation of DE-2-3 cells as an *in vitro* model of BAT physiology.
(A) Light microscopy of D-E-23 cells before and after differentiation. Oil Red O (red color) stains lipid droplets and hematoxylin (purple color) stains nuclei.
(B) Fold change of BAT marker genes in DE-2-3 cells after vs. before differentiation, as assayed by qPCR.
(C) Oxygen consumption rate in DE-2-3 cells at baseline, in response to norepinephrine (NE) treatment, and in response to NE and oligomycin (oligo) treatment.
(D) qPCR of select genes in DE-2-3 cells following stimulation with 1μM NE for 4 hours.

Results are indicated as mean + S.E.M. Statistical significance is indicated as ** $p < 0.01$, *** $p < 0.001$ (student's t-test).

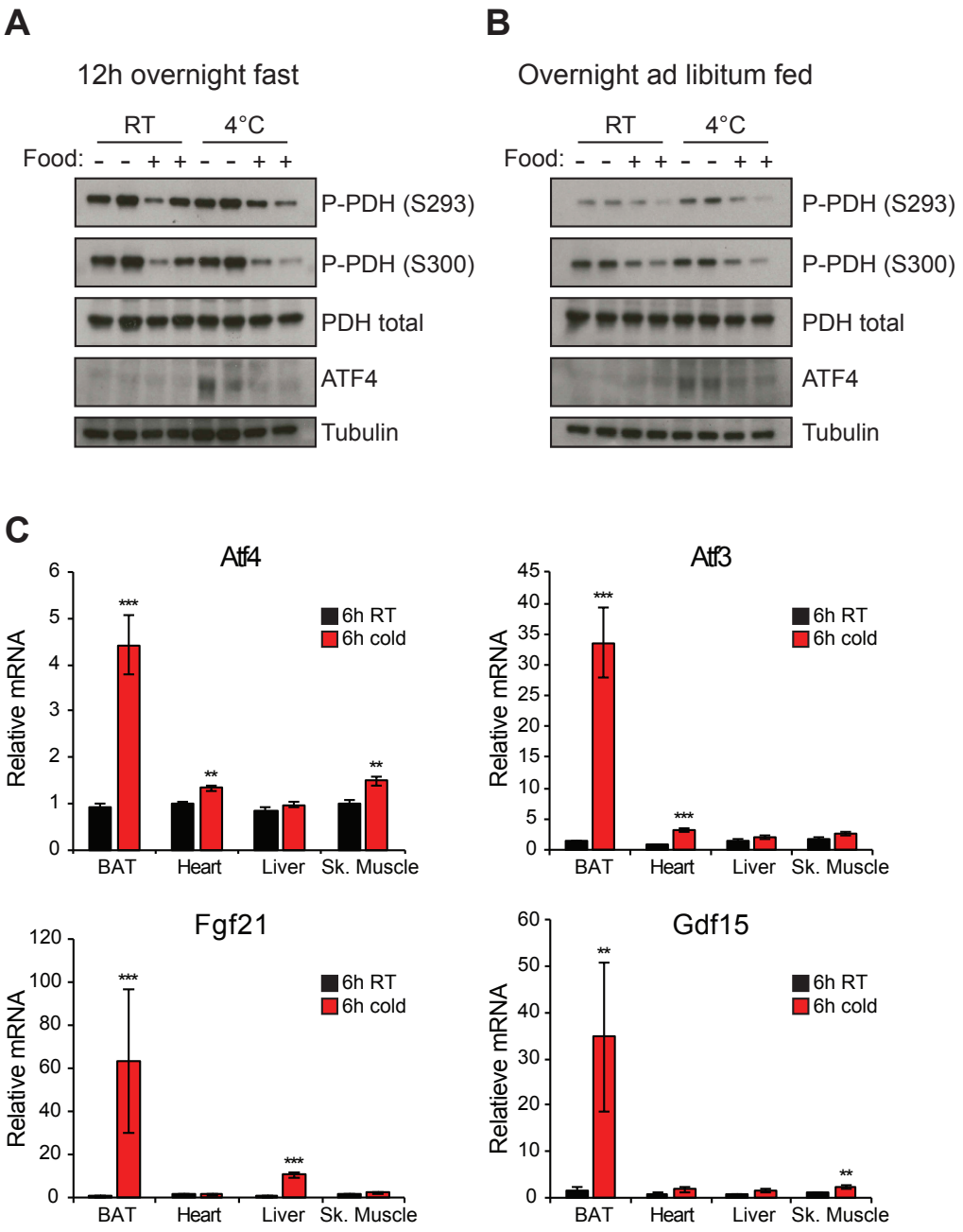

Figure S4. Cold induces the ATF4-mediated integrated stress response in BAT under fasting conditions. Related to Figure 4.

(A-B) Feeding suppresses cold-induced ATF4 accumulation. Wild-type mice were either fasted overnight (A) or provided *ad libitum* access to food (B) at room temperature (RT). Mice were then transferred to new cages at RT or 4°C, either with or without *ad libitum* access to food.

(C) Integrated stress response target gene expression in response to cold across different tissues. Wild-type C57BL6/J mice (n = 4) per group were fasted at room temperature or 4°C for 6 hours, and the indicated transcripts were quantified by qPCR.

Results are reported as mean ± SEM. Statistical significance is indicated as ** $p < 0.01$, *** $p < 0.001$ (student's t-test).