Supplementary Material for

Alternative Splicing of UCP1 by Non-Cell-Autonomous Action of PEMT

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Figure S1

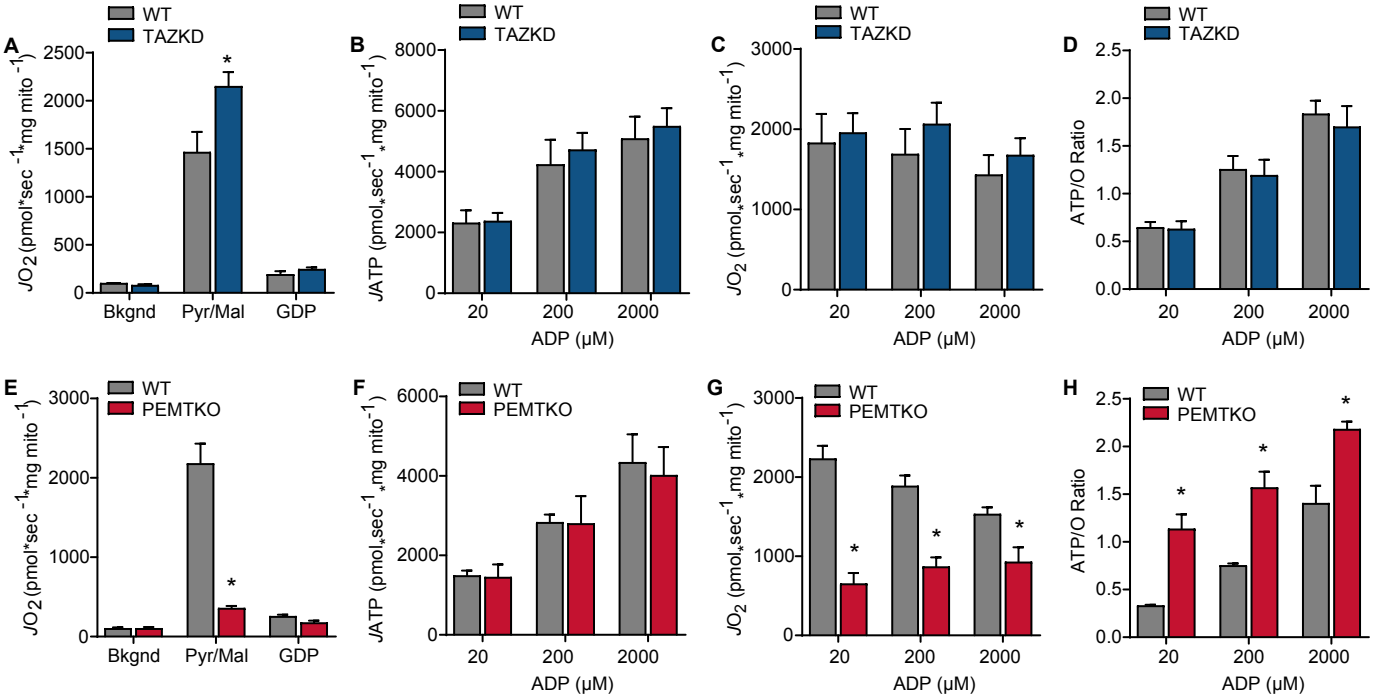

Figure S1. Mitochondrial phenotyping of TAZKD and PEMTKO mice fed a standard-chow diet. (A) UCP1-dependent respiration in BAT mitochondria from TAZKD mice, n=5-6. (B) ATP production in BAT mitochondria from TAZKD mice, n=5-6. (C) O_2 utilization in BAT mitochondria from TAZKD mice, n=5-6. (D) ATP/O ratio in BAT mitochondria from TAZKD mice, n=5-6. (E) UCP1-dependent respiration in BAT mitochondria from PEMTKO mice, n=4-5. (F) ATP production in BAT mitochondria from PEMTKO mice, n=4-5. (G) O_2 utilization in BAT mitochondria from PEMTKO mice, n=4-5. (H) ATP/O ratio in BAT mitochondria from PEMTKO mice, n=4-5. Data are expressed as mean ± SEM, * $p<0.05$.

Figure S2

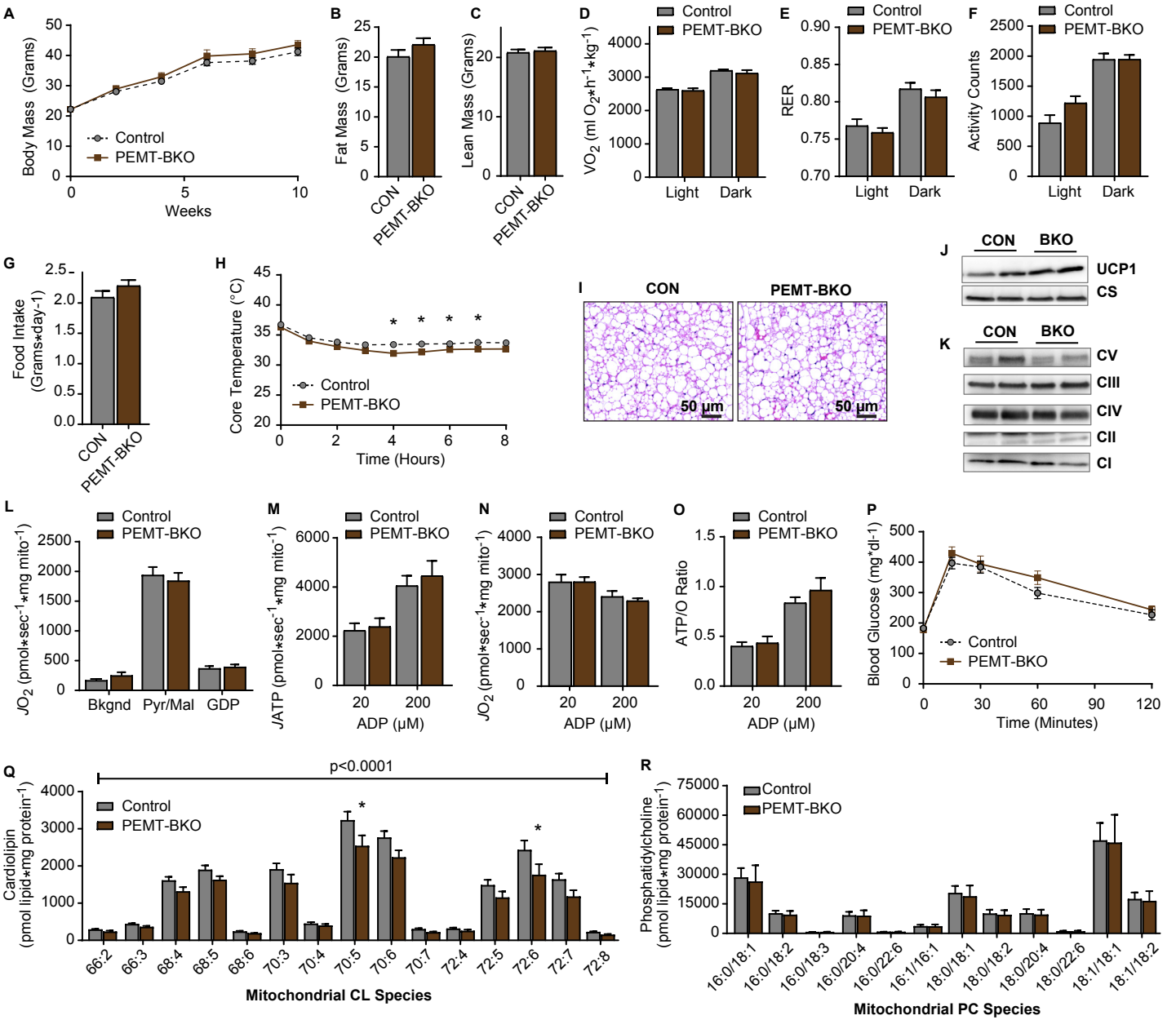

Figure S2. Phenotyping of PEMT-BKO mice fed HFD. (A) Body mass through 10 weeks of HFD in PEMT-BKO mice, n=11-14. (B-C) Fat mass and lean mass in PEMT-BKO mice following 10 weeks of HFD, n=11-13. (D) Whole-body VO_2 measured by CLAMS, n=4. (E) RER, n=4. (F) Activity counts measured by beam breaks in the CLAMS system, n=4. (G) Food intake, n=4. (H) Cold tolerance test, n=8-11. (I) Histology images of BAT sections stained with hematoxylin and eosin. (J) Protein levels of UCP1 and CS. (K) Protein levels of ETS complexes. (L) UCP1-dependent respiration in BAT mitochondria from PEMT-BKO mice, n=7-11. (M) ATP production in BAT mitochondria from PEMT-BKO mice, n=7-11. (N) O_2 consumption in BAT mitochondria from PEMT-BKO mice. n=7-11. (O) ATP/O ratio in BAT mitochondria from PEMT-BKO mice, n=7-11. (P) Glucose tolerance test in PEMT-BKO mice, n=11-14. (Q) Lipidomic analysis of CL, n=7-11. (R) Lipidomic analysis of mitochondrial PC, n=7-11. Data are expressed as mean ± SEM, * p<0.05.

Figure S3

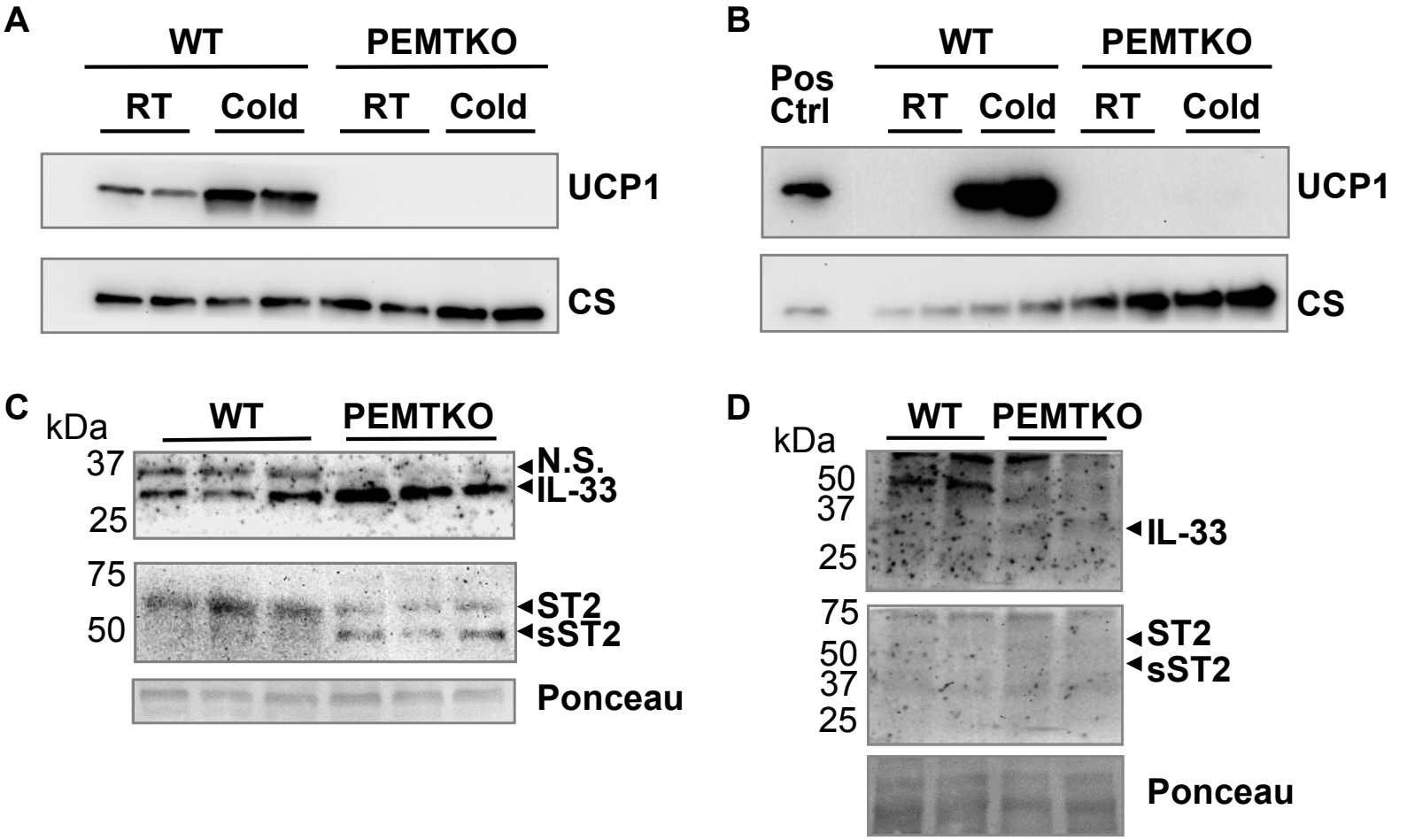

Figure S3. Prolonged cold exposure does not rescue UCP1 protein levels in PEMTKO mice. (A) UCP1 protein abundance in BAT from mice housed at 6.5° C for 7 days following cold acclimation. (B) UCP1 protein levels in iWAT following 7 days of cold exposure. 2 µg of BAT protein was used as a positive control for UCP1 expression. (C) Protein levels of membrane-bound ST2, soluble ST2 (sST2) and IL-33 in whole blood from WT and PEMTKO mice housed at room temperature. (D) IL-33 or ST2 was undetectable in BAT depots from both WT and PEMTKO mice, despite long exposure (25 mins) and concentrated protein load (60 µg).

Table S1: Antibodies used in western blotting.

Antibody	Source	Catalogue Number
Rabbit Anti-Mouse UCP1	Alpha Diagnostic	Cat# UCP11-A
Rabbit Anti-UCP1	ABCAM	Cat# ab23841
Rabbit Anti-UCP1	ABCAM	Cat# ab10983
Rabbit Anti-Mouse UCP1	Cell Signaling	Cat# 14670S
Total OXPHOS Rodent WB Antibody Cocktail	ABCAM	Cat# ab110413
Anti-Citrate Synthetase	ABCAM	Cat# ab96600
Anti-Actin	Sigma	Cat# A2228
Anti-PERK	Cell Signaling	Cat# 3192
Anti-IREα	Cell Signaling	Cat# 3294
Anti-Chop	Santa Cruz	Cat# SC-793
Anti-Tubulin	Santa Cruz	Cat# SC-9104
Anti-ST2	R&D Systems	Cat# AF1004
Anti-IL-33	R&D Systems	Cat# AF3626

Table S2: Primers used in PCR reactions.

Primers		
Gene	**Forward**	**Reverse**
UCP1	TCTCTGCCAGGACAGTACCC	AGAAGCCACAAACCCTTTGA
PEMT	GGTTACATGGACCCCACAGA	AGTTCTCTGCTCCCATCTCG
TAZ	CCCTCCATGTGAAGTGGCCATTCC	TGGTGGTTGGAGACGGTGATAAGG
CRLS1	TGACCTATGCAGATCTTATTCCA	TGGCAGAGTTCGGTATCTGA
ALCAT1	TGGACCGCCTAAGAGAAGGGAA	CGGTAACATGCAAGTTCAATGA
PRDM16	ATGGGAGATGCTGACGGATA	ACGCAGAACTTCTCGCTACC
PGC1α	TGTAGCGACCAATCGGAAAT	TGAGGACCGCTAGCAAGTTT
PGC1β	GCTCTCGTCCTTCTTCCTCA	GAGGTCAAGCTCTGGCAAGT
PPARα	AGTTCGGGAACAAGACGTTG	CAGTGGGGAGAGAGGACAGA
PPARδ	AGATGAAGACAAACCCACGG	CTGTGGCTGTTCCATGACTG
PPARγ	TGCACTGCCTATCAGCACTT	GAATGCGAGTGGTCTTCCAT
C/EBPα	CCAAGAAGTCGGTGGACAAG	TTGTTTGGCTTTATCTCGGC
C/EBPβ	GTTTCGGGACTTGATGCAAT	GGCCCGGCTAGACAGTTAC
UCP1 Splice Variant A	TGTAAACAACAAAATACTGGCAGATG	GACCCGAGTCGCAGAAAAG
UCP1 Splice Variant B	TGTAAACAACAAAATACTGGCAGCTC	GACCCGAGTCGCAGAAAAG
UCP1 Splice Variant C	TGTAAACAACAAAATACTGGCAGGAC	GACCCGAGTCGCAGAAAAG
UCP1 Splice Variant D	TGTAAACAACAAAATACTGGCAGGGT	GACCCGAGTCGCAGAAAAG
UCP1 Exon 5 genomic DNA	CGTCCCCTGCCATTTACTGT	CTTTGAAAAAGGCCGTCGGT
UCP1 Exon 5 cDNA	TGTAAACAACAAAATACTGGCAG	GACCCGAGTCGCAGAAAAG
Cre	GCAAGAACCTGATGGACAGTTCAG	GCAATCCCCAGAAATGCCAGATTAC
FABP	TGGACAGGACTGGACCTCTCGCTTTCC	TAGAGCTTTGCCACATCACAGGTCAT
LoxP PEMT	CTGGGAGTGAAAACACCATCC	GAGGTGGAGACTGGGCTGATA