Cell Reports, Volume *35*

Supplemental information

Hepatic AKT orchestrates adipose tissue thermogenesis via FGF21-dependent and -independent mechanisms

Jaimarie Sostre-Colón, Kahealani Uehara, Anna E. Garcia Whitlock, Matthew J. Gavin, Jeff Ishibashi, Matthew J. Potthoff, Patrick Seale, and Paul M. Titchenell

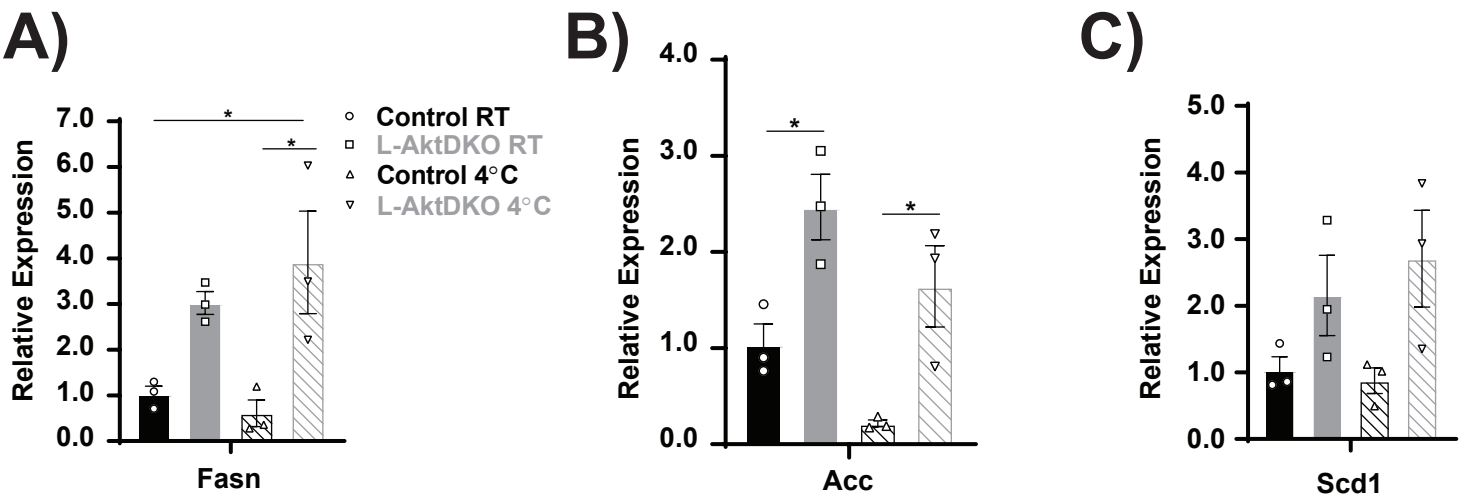

Figure S1. The loss of liver AKT increases BAT fatty acids synthesis genes, Related to Figure 1.
A-C) Relative mRNA levels of *Fasn*, *Acc* and *Scd1* in BAT from Control and L-AktDKO mice housed at RT or 4°C for 3 h (n=3 per group).
Data are presented as means ±SEM. *p<0.05, **p<0.01, ***p<0.001.

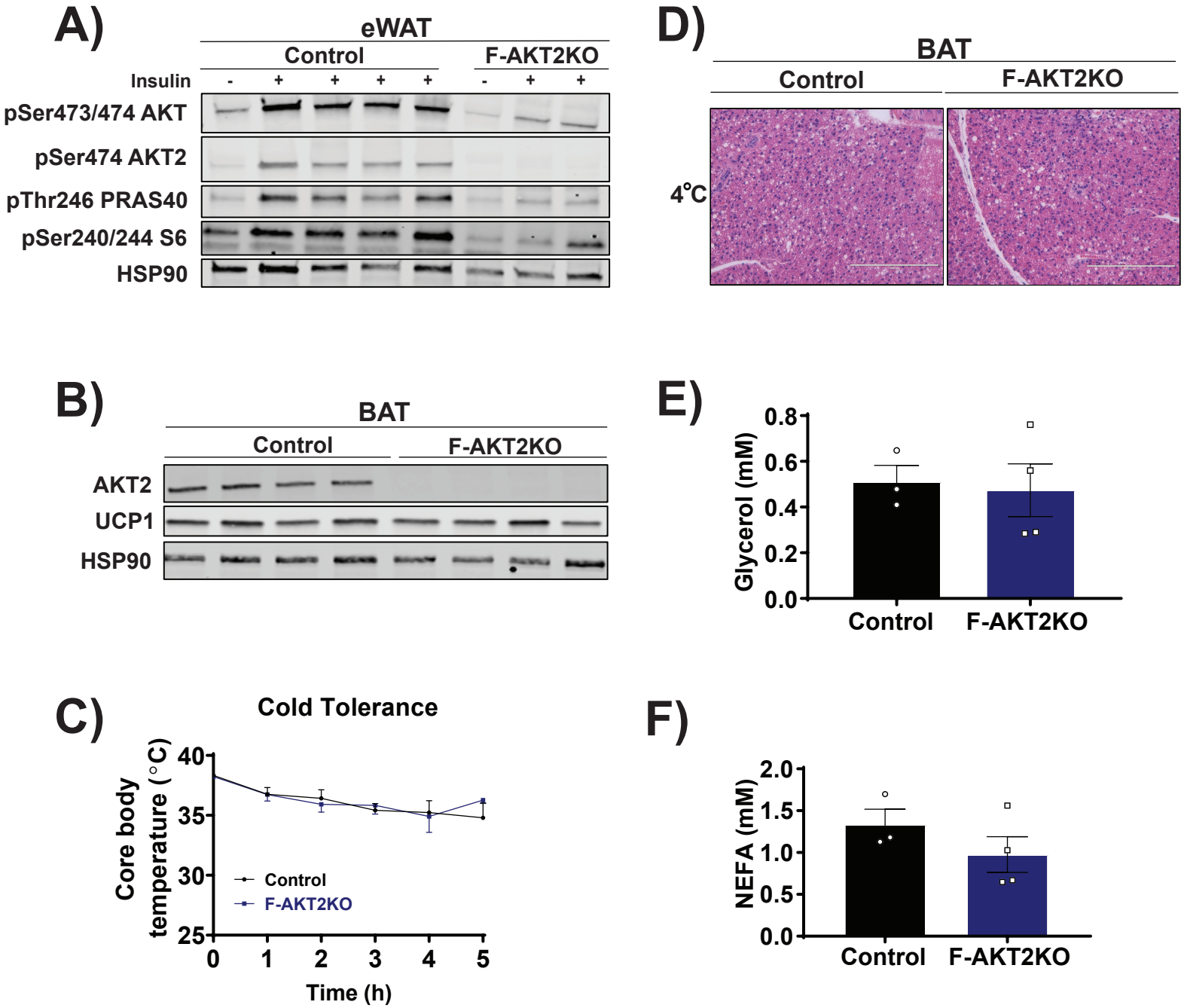

Figure S2. The acute cold thermogenic response is independent of adipocyte AKT2 signaling, Related to Figure 1.

A) Western Blot analysis of p-AKT (Ser473/474), p-AKT2 (474), p-PRAS40 (Thr246), and p-S6 (Ser240/244) of eWAT from Control and F-AKT2KO mice under unstimulated or stimulated conditions with insulin (2 U/kg) for 20 min after overnight fasting.

B) Western Blot analysis of AKT2 and UCP1 of BAT from Control and F-AKT2KO after a fasting cold tolerance test for 5 h.

C) Core body temperature of Control and F-AKT2KO mice during a fasting acute cold tolerance test for 5 h (RT→4°C) (n=4 per group).

D) H&E staining of BAT from Control and F-AKT2KO after a fasting cold tolerance test for 5 h. (Scale Bar= 200μm)

E) Glycerol serum levels of Control and F-AKT2KO after a fasting cold tolerance test for 5 h (n=3 for Control, and n=4 for F-AKT2KO mice).

F) NEFA serum levels of Control and F-AKT2KO after a fasting cold tolerance test for 5 h (n=3 for Control, and n=4 for F-AKT2KO mice).

Data are presented as means ±SEM. *p<0.05, **p<0.01, ***p<0.001.

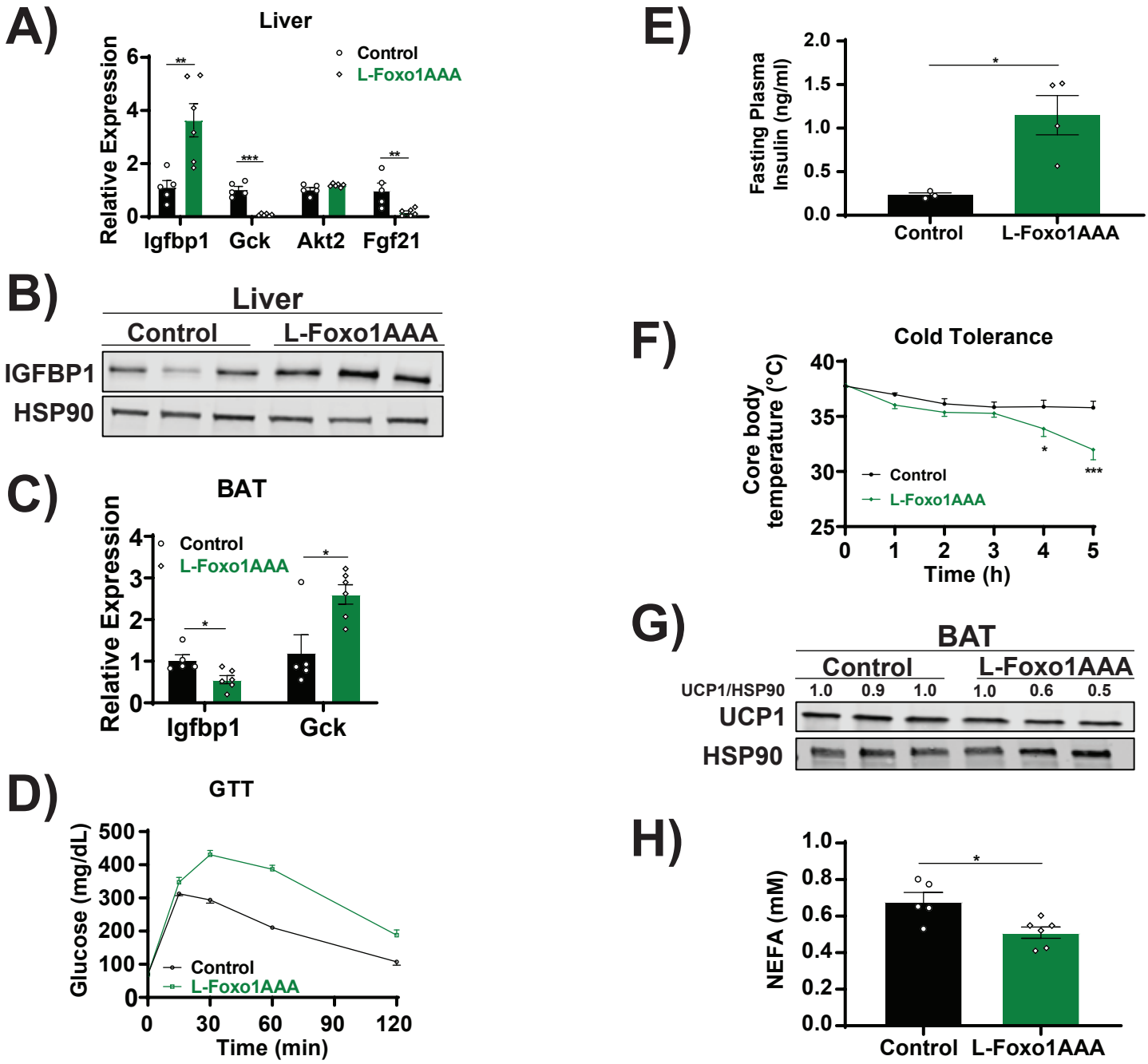

Figure S3. Hepatic FOXO1 is sufficient to impair cold tolerance, Related to Figure 3.

A) Relative mRNA levels of *Igfbp1*, *Gck*, *Akt2*, and *Fgf21* in liver from Control and L-Foxo1AAA mice (n=5 for Control mice and n=6 for L-Foxo1AAA mice).

B) Western Blot analysis of IGFBP1 of liver from Control and L-Foxo1AAA mice.

C) Relative mRNA levels of *Igfbp1*, and *Gck* in BAT from Control and L-Foxo1AAA mice (n=5 for Control mice and n=6 for L-Foxo1AAA mice).

D) Glucose levels of Control and L-Foxo1AAA mice housed at RT after i.p. injection of 2 mg/kg of glucose solution (n=3 for Control mice and n=4 for L-Foxo1AAA mice).

E) Fasting insulin levels of Control and L-Foxo1AAA mice housed at RT (n=3 for Control mice and n=4 for L-Foxo1AAA mice).

F) Core body temperature of Control and L-Foxo1AAA mice during a fasting acute cold tolerance test for 5 h (RT→4°C) (n=5 for Control mice and n=6 for L-Foxo1AAA mice).

G) Western Blot analysis of UCP1 of BAT from Control and L-Foxo1AAA mice after a fasting acute cold tolerance test for 5 h.

H) NEFA serum levels of Control and L-Foxo1AAA mice after a fasting acute cold tolerance test for 5 h (n=5 for Control mice and n=6 for L-Foxo1AAA mice).

Data are presented as means ±SEM. *p<0.05, **p<0.01, ***p<0.001.

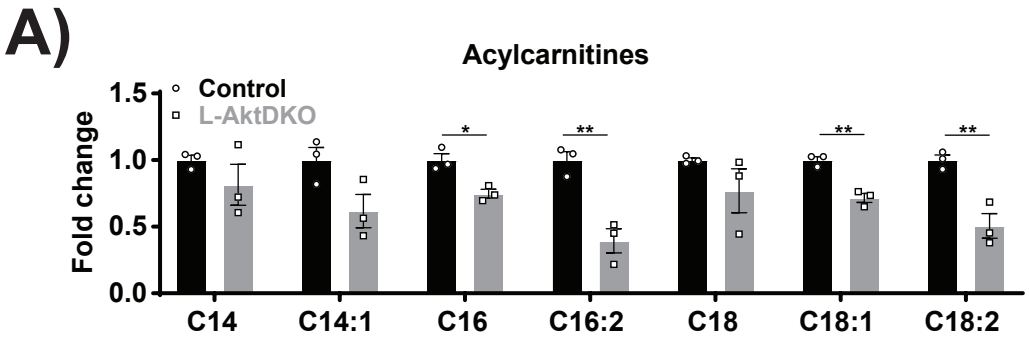

Figure S4. The inhibition of liver AKT decreases serum acylcarnitines levels, Related to Figure 4.
A) Acylcarnitines serum levels of Control and L-AktDKO mice after a fasting acute cold tolerance test for 5 h (n=3 per group).
Data are presented as means ±SEM. *p<0.05, **p<0.01, ***p<0.001.

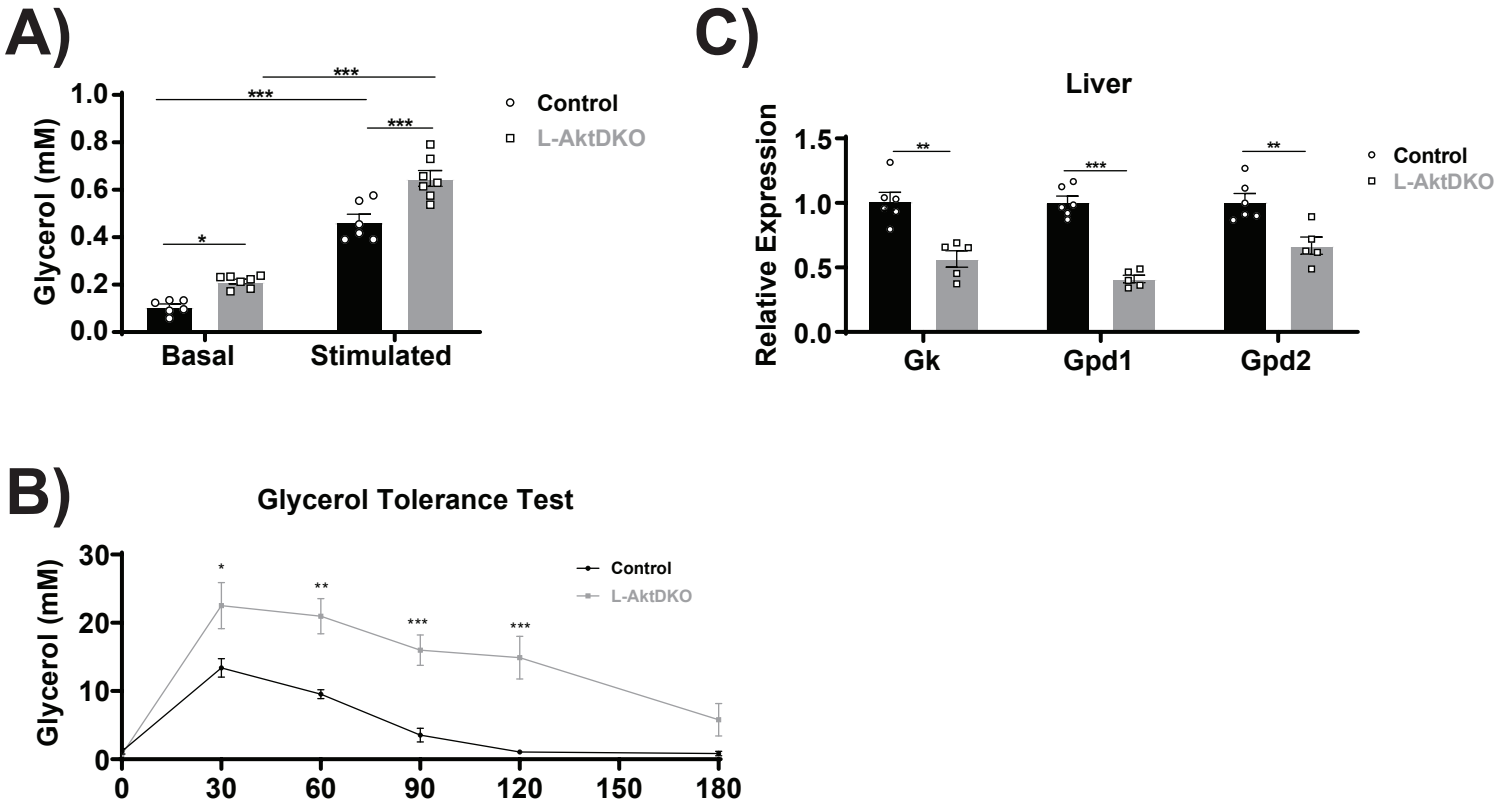

Figure S5. The loss of hepatic AKT impairs glycerol metabolism, Related to Figure 4.
A) Glycerol serum levels of Control and L-AktDKO mice housed at RT before and after 30 min of CL 316,243 i.p. injection (n=6 for Control mice and n=7 for L-AktDKO mice).
B) Glycerol serum levels of Control and L-AktDKO mice housed at RT after i.p. injection of 2 mg/kg glycerol solution (n=3 per group).
C) Relative mRNA levels of *Gk*, *Gpd1* and *Gpd2* in liver from Control and L-AktDKO mice after a fasting cold tolerance test for 5 h (n=6 for Control mice and n=5 for L-AktDKO mice).
Data are presented as means ±SEM. *p<0.05, **p<0.01, ***p<0.001.

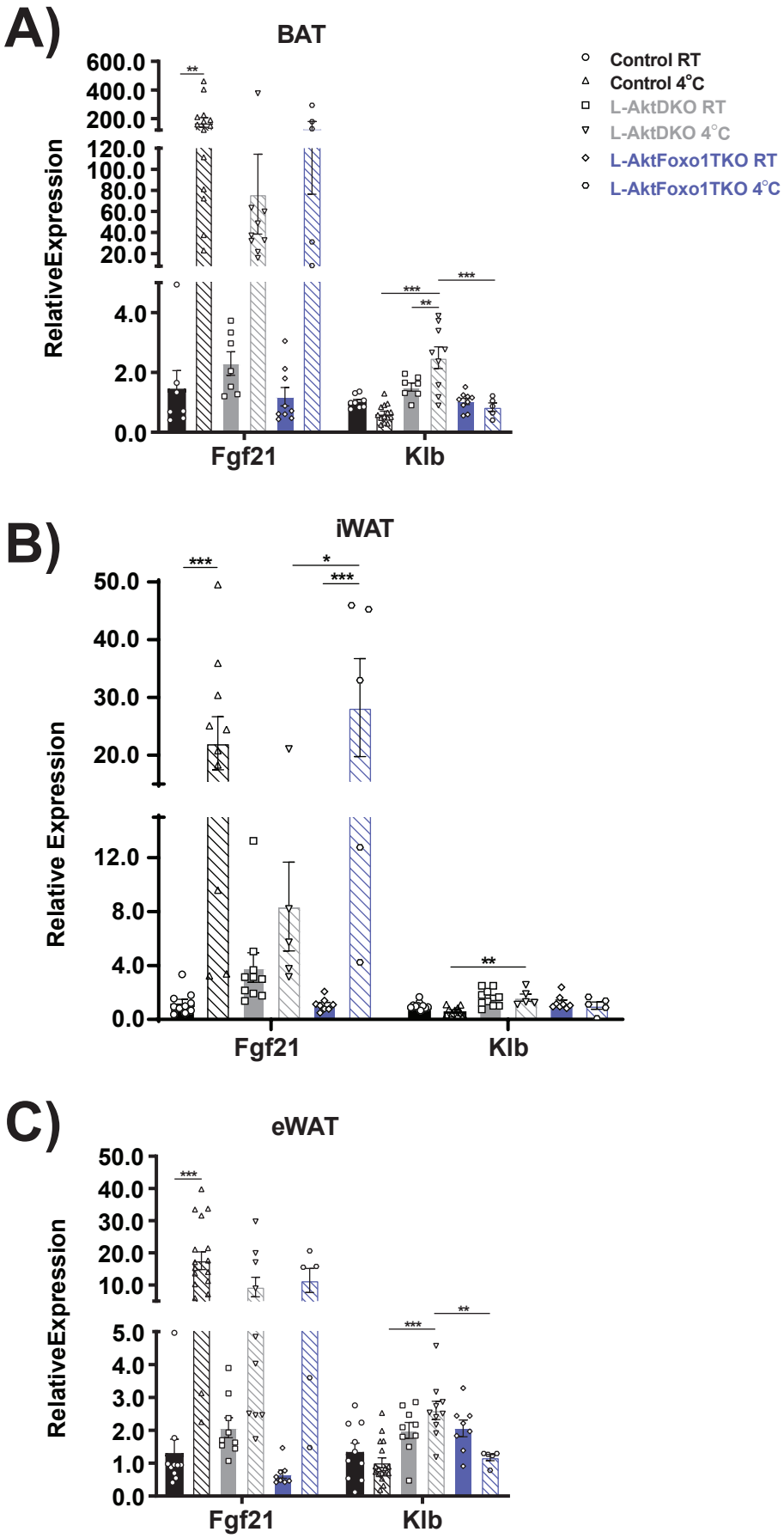

Figure S6. *Fgf21* and *β-Klotho* gene expression in BAT and WAT, Related to Figure 5.
A-C) Relative mRNA levels of *Fgf21*, and *Klb* from BAT, iWAT and eWAT of Control, L-AktDKO and L-AktFoxo1TKO mice housed at RT or 4°C for 5 h (n=5-17 per group).
Data are presented as means ±SEM. *p<0.05, **p<0.01, ***p<0.001.

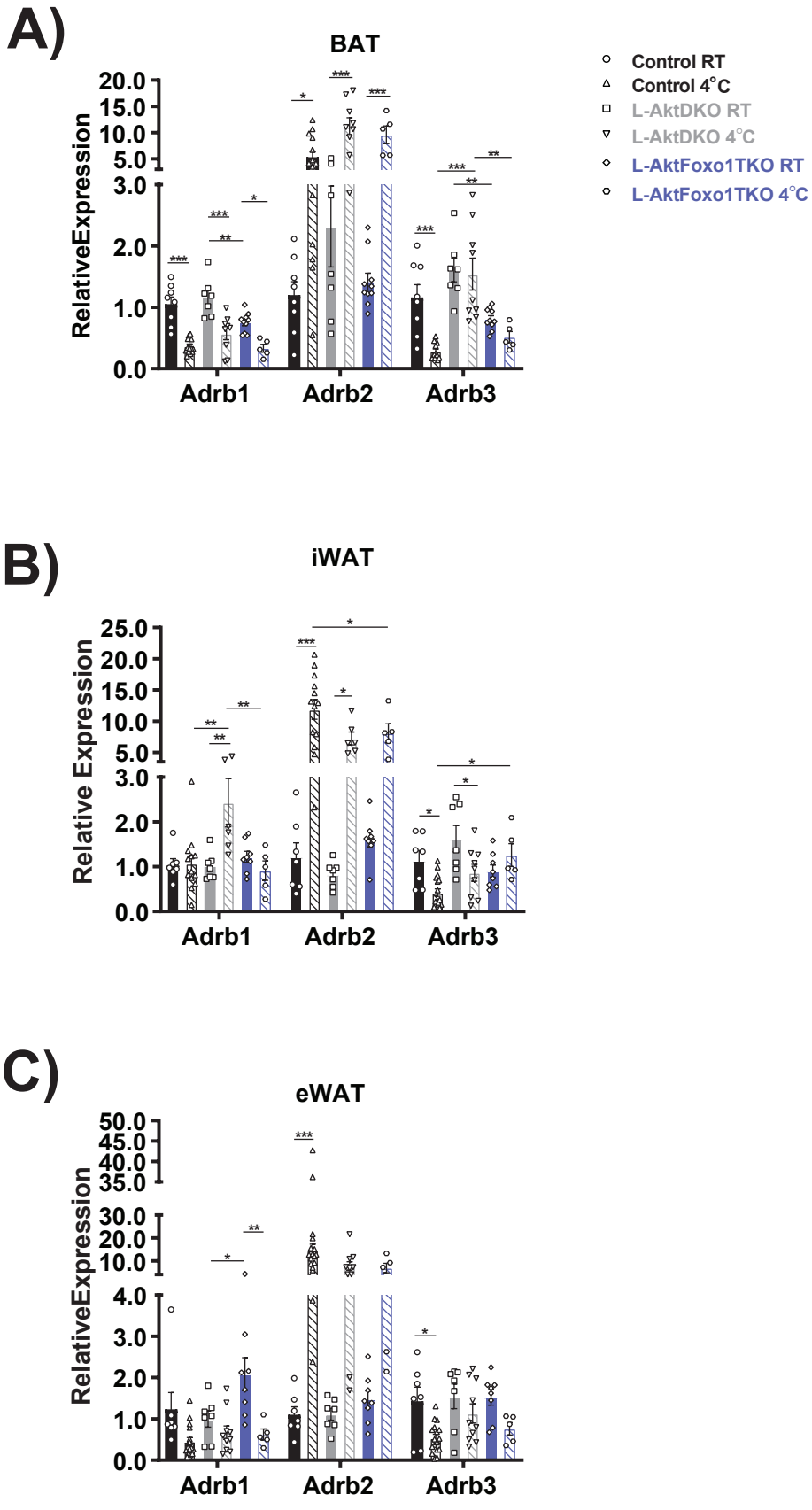

Figure S7. *β-Adrenergic* receptors gene expression in BAT and WAT, Related to Figure 4.
A-C) Relative mRNA levels of *Adrb1*, *Adrb2*, and *Adrb3* from BAT, iWAT and eWAT of Control, L-AktDKO and L-AktFoxo1TKO mice housed at RT or 4°C for 5 h (n=5-17 per group).
Data are presented as means ±SEM. *p<0.05, **p<0.01, ***p<0.001

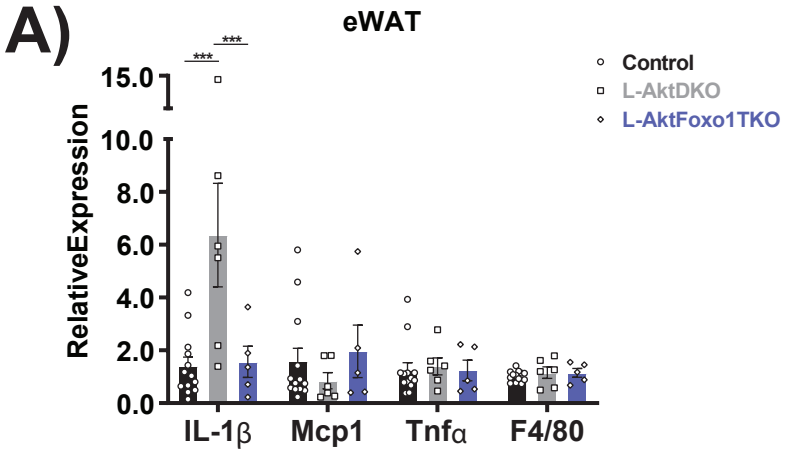

Figure S8. The inhibition of hepatic AKT increases IL-1β cytokine levels in WAT, Related to Figure 4.

A) Relative mRNA levels of *IL-1β*, *Mcp1*, *Tnfα*, and *F4/80* in eWAT from Control, L-AktDKO and L-AktFoxo1TKO mice after a fasting acute cold tolerance test for 5 h (RT→4°C) (n=13 for Control mice and n=6 for L-AktDKO mice and n=5 for L-AktFoxo1TKO mice).

Data are presented as means ±SEM. *p<0.05, **p<0.01, ***p<0.001.

Table S1. List of real-time PCR Primers listed 5' to 3', Related to the STAR Methods section.

Gene	Forward	Reverse
Ucp1	TCAGGATTGGCCTCTACGAC	TGCCACACCTCCAGTCATTA
Dio2	AATTATGCCTCGGAGAAGACCG	GGCAGTTGCCTAGTGAAAGGT
Fgf21	GCTGCTGGAGGACGGTTACA	CACAGGTCCCCAGGATGTTG
Akt2	ATGGATTACAAGTGTGGCTCCCC	GTGCCTGGTATTCTGCAGAACC
Foxo1	CTGGGTGTCAGGCTAAGAGT	GGGGTGAAGGGCATCTTT
Fasn	GCTGCGGAAACTTCAGGAAAT	AGAGACGTGTCACTCCTGGACTT
Acc	TGACAGACTGATCGCAGAGAAAG	TGGAGAGCCCCACACACA
Scd1	CCGGAGACCCCTTAGATCGA	TAGCCTGTAAAAGATTTCTGCAAA
Igfbp1	GGAGATCGCCGACCTCAAG	CTGCAGCTAATCTCTCTAGCACTTTATAG
Gck	CCCTGAGTGGCTTACAGTTC	ACGGATGTGAGTGTTGAAGC
Gk	CCCAAGAGAAGGATGGGTAGAACA	GGTCAAGCCACACCACGGCATTAT
Gpd1	TTCACTGCGGTGTACAAAGTGTGC	CATTCACATGTGTTCCGGGTGGTT
Gpd2	GAAGGGGACTATTCTTGTGGGT	GGATGTCAAATTCGGGTGTGT
Klb	GATGAAGAATTTCCTAAACCAGGTT	AACCAAACACGCGGATTTC
Adrb1	GAAAGCAGGTGAATGCAAAGC	CCGAACCTCAGAGAGAAAGGA
Adrb2	TTGCAGTGGATCGCTATGTTG	CGATAGCTTTCTTGTGGGTGG
Adrb3	TCGACATGTTCCTCCACCAA	GATGGTCCAAGATGGTGCTT
IL-1b	TGGAGAGTGTGGATCCCAAGCAAT	TGTCCTGACCACTGTTGTTTCCCA
Mcp1	TCACCTGCTGCTACTCATTCACCA	TACAGCTTCTTTGGGACACCTGCT
Tnfa	TCTCATGCACCACCATCAAGGACT	TGACCACTCTCCCTTTGCAGAACT
F4/80	TCAAATGGATCCAGAAGGCTCCCA	TGCACTGCTTGGCATTGCTGTATC