Supplemental information

FoxO1 suppresses Fgf21 during hepatic insulin resistance to impair peripheral glucose utilization and acute cold tolerance

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Figure S1

A

![Graph showing eWAT weight (mg) and iWAT weight (mg) for different genotypes.](image)

B

![Images of iWAT for CTRL, LDKO, and LTKO genotypes.](image)

C

![Bar graph showing iWAT adipocyte size (µm²) for CTRL, LDKO, and LTKO genotypes.](image)

D

![Images of tissue sections for CTRL, LDKO, and LTKO genotypes.](image)
Figure S1. Characteristics of visceral and subcutaneous WAT (eWAT, iWAT) in LDKO vs LTKO mice. Related to Figure 1.

(A) Weights of dissected eWAT and iWAT depots in 14 to 16-week-old male LDKO and LTKO mice and their respective double-floxed or triple-floxed controls (n=26, 8, 11, 8 mice).

(B) Representative H&E stained iWAT sections from 16-week-old CTRL, LDKO and LTKO male mice. Scale bar, 50 μm.

(C) Quantification of adipocyte sizes in 16-week-old CTRL, LDKO and LTKO male mice. Points represent the average area of individual adipocytes (n ~ 20 to 30) in iWAT samples from unique mice (n=4, 3, 2 mice).

(D) Representative high-magnification images of H&E-stained BAT sections from CTRL, LDKO and LTKO mice. Scale bar, 20 μm.

Bars and error bars represent mean values ±SD. Significant differences between means were assessed by unpaired t tests versus respective floxed control mice (A) or by one-way ANOVA (C) followed by Tukey’s HSD-based comparison of each group to every other: * p<.05, ** p<.01 between indicated groups; ns, not significantly different.
Figure S2

Graph A shows the relative Fgf21 mRNA levels in different tissues and conditions:
- CTRL
- LDKO
- LTKO

Conditions:
- fasted
- refed

Graph B compares the relative Fgf21 mRNA levels across different tissues:
- liver
- BAT
- iWAT
- eWAT
- Sk Musc

The graphs illustrate significant differences in Fgf21 expression levels between the groups under different conditions.
Figure S2. *Fgf21* mRNA expression in liver of fasted-refed mice and in liver and non-liver tissues of *Fgf21*<sup>AdV</sup>-infected mice. Related to Figure 2.

(A) RT-qPCR-determined expression of hepatic *Fgf21* mRNA in mice fasted overnight for 16 hours (fasted), or fasted and then allowed to refeed for 4 hours (refed).

(B) RT-qPCR-determined expression of *Fgf21* mRNA in liver and non-liver tissues of adenovirus-infected mice. Skeletal muscle samples (Sk. Musc.) are mixed fibers of quadriceps.

Bars and error bars represent mean values ±SD. Significant differences between means were assessed one-way ANOVA per nutritional state or tissue, followed by Tukey’s HSD-based comparison of each group to every other: * p<.05, ** p<.01, *** p<.001, **** p<.0001 between indicated groups; ns, not significantly different.
Figure S3. Fgf21 mRNA expression in liver and non-liver tissues of shFgf21AdV-infected mice and Foxo1 expression in primary hepatocytes infected with Foxo1AdV or shFoxo1AdV. Related to Figure 3.

(A) RT-qPCR-determined expression of Fgf21 mRNA in liver and non-liver tissues of scRNAAdV and shFgf21AdV-infected mice. Skeletal muscle samples (Sk. Musc.) are mixed fibers of quadriceps.

(B) RT-qPCR-determined expression of Foxo1 mRNA in primary hepatocytes infected at high (600) or low (200) moi with Foxo1AdV to over-express FoxO1, or with shFoxo1AdV to knock down Foxo1 expression. Note control viruses Foxo1AdV and scRNAAdV overexpress GFP or target non-genomic RNA respectively. All data are expressed relative to the Foxo1 mRNA level in hepatocytes infected with control GFPAdV at low moi.

Bars and error bars represent mean values ±SD. Significant differences between means were assessed by one-way ANOVA, followed by (A) Tukey’s HSD-based comparison of each group to every other or (B) comparison of each group to the average of the four control groups (open bars): ** p<.01, *** p<.001, **** p<.0001 between indicated groups; ns, not significantly different.
Figure S4

A. Adipose (g) vs. HFD, CTRL, LDKO, LTKO

B. % Lean mass vs. HFD, CTRL, LDKO, LTKO

C. Lean mass (g) vs. HFD, CTRL, LDKO, LTKO

D. 2DOG (ng·mg⁻¹·min⁻¹) in Sk. Muscle vs. GFP^AdV, Fgf21^AdV, CTRL, HFD, LDKO, HFD

E. 2DOG (ng·mg⁻¹·min⁻¹) in eWAT vs. GFP^AdV, Fgf21^AdV, CTRL, HFD, LDKO, HFD

F. 2DOG (ng·mg⁻¹·min⁻¹) in iWAT vs. GFP^AdV, Fgf21^AdV, CTRL, HFD, LDKO, HFD

G. Hepatic TG (µmol/g) vs. CTRL, LDKO, CD

H. Hepatic TG (µmol/g) vs. GFP^AdV, Fgf21^AdV, GFP^AdV, Fgf21^AdV, CTRL, HFD, LDKO, HFD
Figure S4. Fat and lean body mass of HFD-fed LDKO vs LTKO mice and \([^{14}\text{C}]2\text{DOG}\) uptake into skeletal muscles and WAT of HFD-fed CTRL and LDKO mice infected with GFP\textsuperscript{Adv} or Fgf21\textsuperscript{Adv}. Related to Figure 4.

(A) DEXA-determined fat mass (in grams) in 24-week-old HFD-fed male CTRL, LDKO and LTKO mice (n=28, 21, 22 mice).

(B) DEXA-determined lean mass, expressed as percent of body weight, in 24-week-old HFD-fed male CTRL, LDKO and LTKO mice (n=28, 21, 22 mice).

(C) DEXA-determined lean mass (in grams) in 24-week-old HFD-fed male CTRL, LDKO and LTKO mice (n=28, 21, 22 mice).

(D-F) Basal and/or insulin-stimulated uptake of \([^{14}\text{C}]2\text{DOG}\) into (D) hindlimb skeletal muscles, (E) eWAT, or (F) iWAT of HFD-fed CTRL and LDKO male mice 12 days after infection with GFP\textsuperscript{Adv} or Fgf21\textsuperscript{Adv} (n=4, 5, 3, 5, 3 mice).

(G) Hepatic triglyceride accumulation in 24-week-old male CTRL and LDKO mice on chow diet.

(H) Hepatic triglyceride accumulation in 24-week-old male CTRL and LDKO mice on HFD, 40 days after infection with GFP\textsuperscript{Adv} or Fgf21\textsuperscript{Adv}.

Bars and error bars represent mean values ±SD. Significant differences between means were assessed by unpaired t test (G) or by one-way ANOVA (A-F, H), followed by Tukey’s HSD-based comparison of each group to every other: * p<.05, ** p<.01, *** p<.001, **** p<.0001 between indicated groups; ns, not significantly different. In D-F, ## p<.01, ### p<.001 versus CTRL•GFP\textsuperscript{Adv} mice in basal condition.
Figure S5

A

B

CTRL
GFPAdV
LDKO
GFPAdV
LDKO
Fgf21AdV

CTRL + PBS
LTKO + PBS
LTKO + CL316,243 (2mg/ml)

C

D

CTRL + PBS
LTKO + PBS
LTKO + CL316,243 (1mg/ml)

CTRL + PBS
LTKO + PBS
LTKO + CL316,243 (2mg/ml)

E

CTRL
LTKO
LDKO

β3AR
GAPDH

CTRL
LTKO
LDKO
Figure S5. Impaired cold tolerance in LDKO mice and its normalization by Fgf21Adv. Related to Figure 5.

(A) Highlight of BAT Ucp1 mRNA expression shown in Figure 5J. Relative Ucp1 expression was measured by RT-qPCR in BAT of 14-week-old CTRL and LDKO male mice, 12 days after injection with GFPAdv or Fgf21Adv (n=5 mice per group). Data are normalized by the CTRL mean.

(B) Relative expression of thermogenic genes, determined by RT-qPCR, in iWAT of CTRL and LDKO mice 10 days after infection with GFPAdv or Fgf21Adv (n=5 mice per group). Data are normalized by the CTRL mean.

(C) Body core temperature (TCore) profiles of 14-week-old CTRL and LTKO male mice injected with PBS (vehicle control) or β3-adrenergic agonist CL316,243 (2 mg/ml) one hour before acute exposure to 4° cold for 2 hours (n=5 mice per group). Slopes of the fitted lines equal the dTCore values in Figure 5G.

(D) Body core temperature (TCore) profiles of 14-week-old CD-fed male CTRL, and LDKO mice injected with PBS (vehicle control) or β3-adrenergic agonist CL316,243 (1 or 2 mg/ml) one hour before acute exposure to 4° cold for 2 hours (n=5). Slopes of the fitted lines equal dTCore values in Figure 5H.

(E) Western blot showing equivalent expression of β3 adrenoreceptors (target of CL316,243) in BAT of adult CTRL and LDKO mice.

Bars and associated error bars in A-D represent mean values ±SD. Significant differences between means were assessed, per gene, by one-way ANOVA, followed by Tukey's HSD-based comparison of each group to every other: * p<.05, ** p<.01, *** p<.001, between indicated groups.