Expanded View Figures

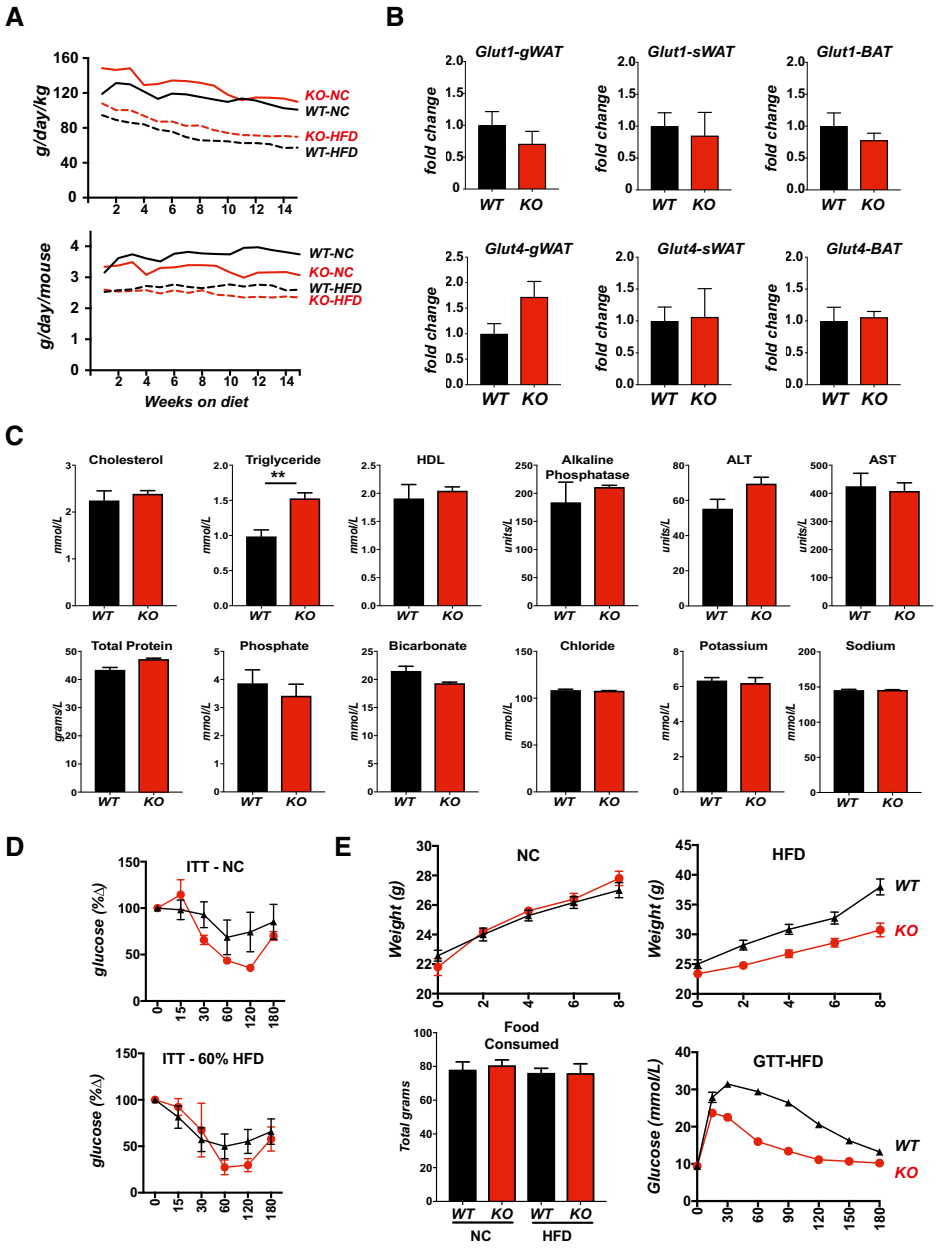


Figure EV1. ***Rcan1-KO*** **mice are resistant to diet-induced obesity.**

A Food consumption of animals in Fig 1C, calculated and tracked weekly over the first 15 weeks on NC or a 60% fat HFD, starting at 8 weeks of age (male, n = 9 each). Data are presented as grams consumed per day per kilogram body weight (g/day/kg) and grams consumed per day per mouse (g/day/mouse).

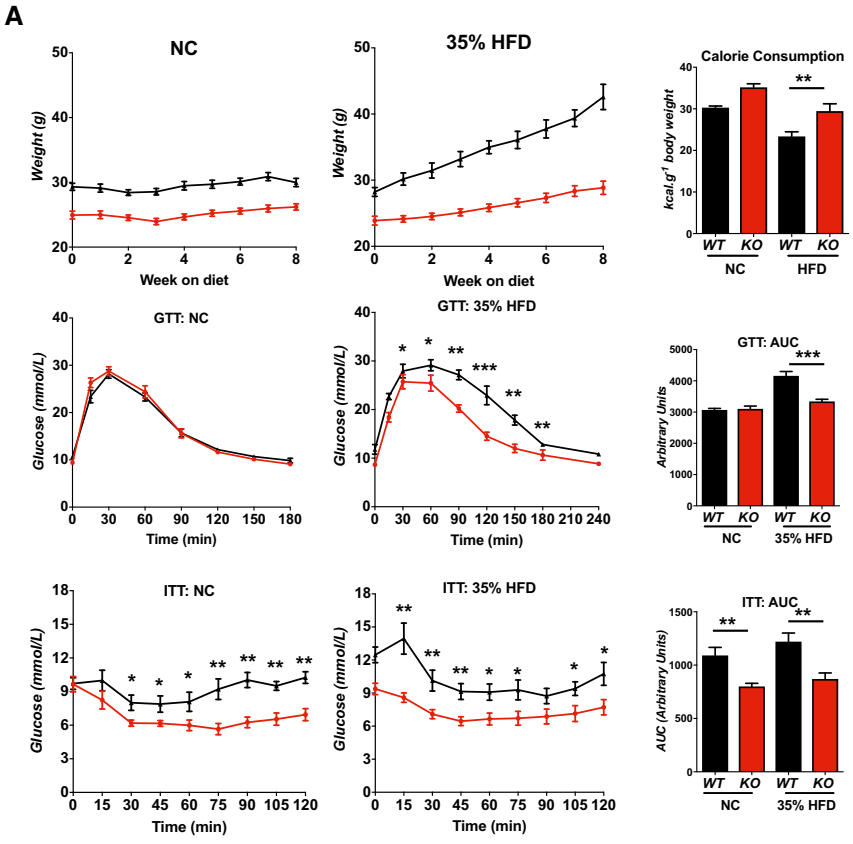
B Transcript levels for *Glut1* and *Glut4* in gWAT, sWAT, and BAT of *WT* and *KO* males after 8 weeks on a 60% fat HFD. Transcript levels were normalized to 18S.

C Assessment of standard blood parameters in fasted, 18-week-old *KO* males after 8 weeks on a 60% fat HFD (n = 4 per group).

D Insulin tolerance tests (ITT) from Fig 1H, with blood glucose levels plotted as a percentage of starting glucose levels (glucose %Δ) after 25 weeks on NC or a 60% fat HFD. GTT was performed following an overnight fast, ITT following a 3-h fast (males, n = 6).

E Body weights of *WT* and *KO* mice fed NC or a 60% fat HFD for 8 weeks starting at 3 weeks of age (males, n = 15 each). Total food consumed per animal over the course of 8 weeks. Glucose tolerance test (GTT) was given after 8 weeks on the HFD.

Data information: Values shown are mean ± SEM. An unpaired Student's t-test was used on data in (B, C). $^{**}P < 0.01$.



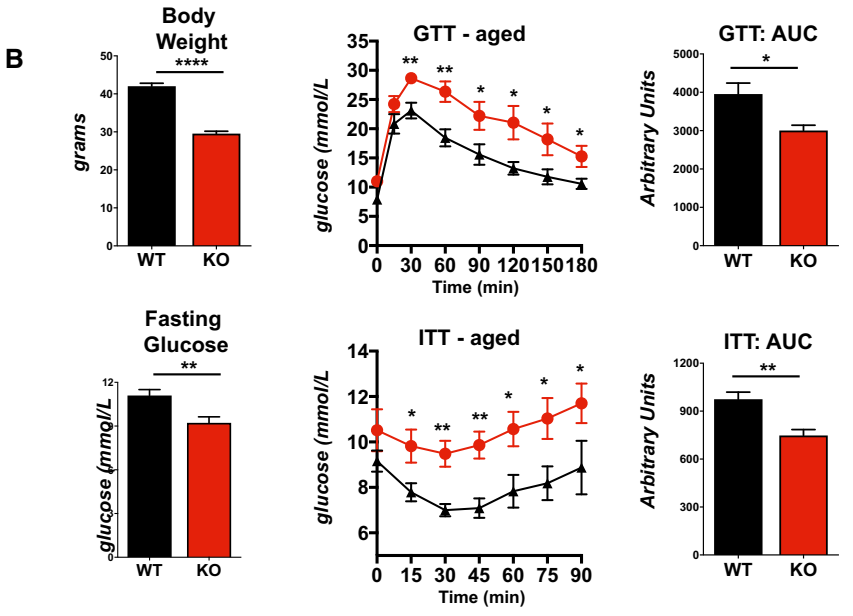


Figure EV2. *Rcan1-KO* mice are resistant to age-related obesity and insulin resistance.

A Body weights (n = 8), food consumption (n = 8), glucose tolerance tests (GTT) (n = 5), and insulin tolerance tests (ITT) (n = 5) for *WT* and *KO* male mice fed either a normal chow diet (NC) or a 35% fat HFD for 8 weeks starting at 8–10 weeks of age. Food consumption was measured as kilocalories consumed per gram change in body weight (kcal/g body weight) consumption per animal over the course of 8 weeks.

B Body weights, fasting blood glucose levels, glucose tolerance test (GTT), and insulin tolerance test (ITT) for 1-year-old *WT* and KO mice maintained on a normal chow diet (males, n = 5).

Data information: Values shown are mean ± SEM. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$ (multiple t-tests for time course plots, two-way ANOVA with multiple comparisons for bar graphs in A, t-tests for bar graphs in B).

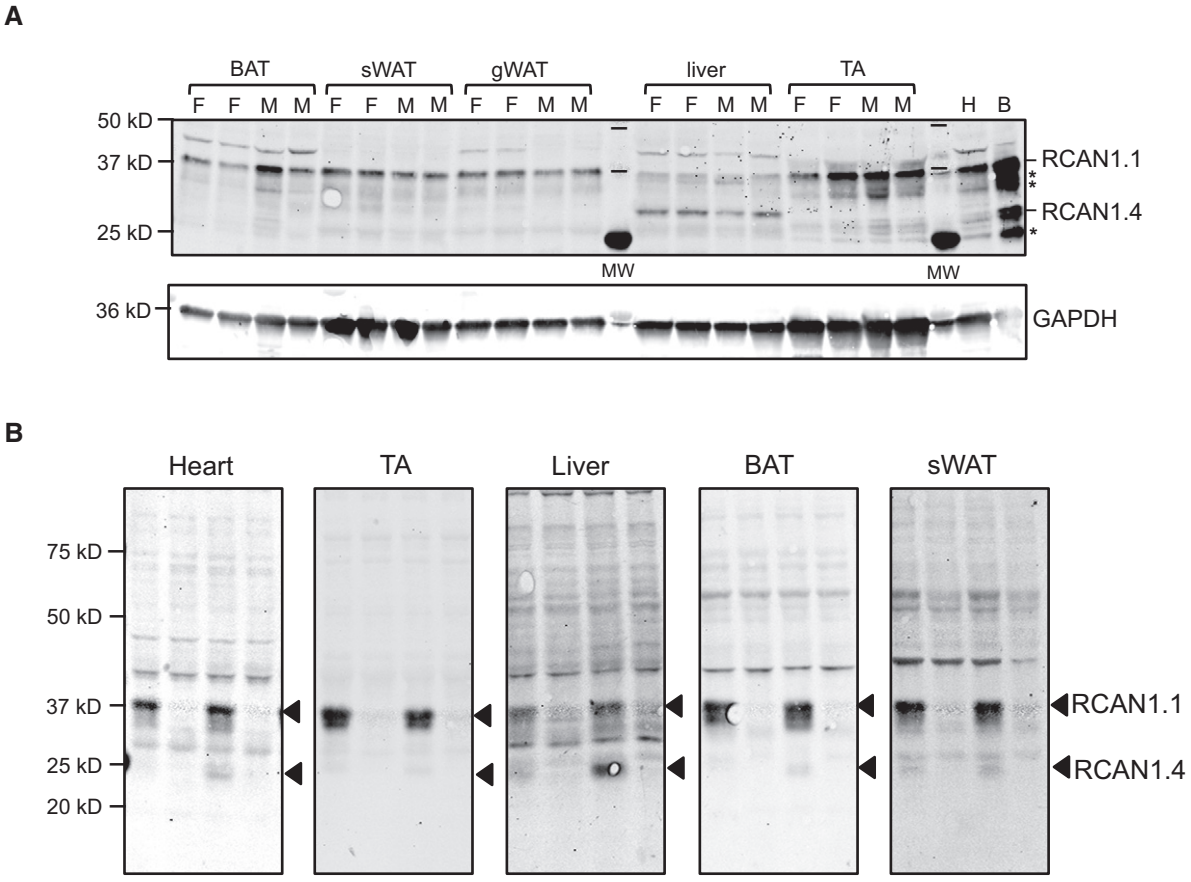


Figure EV3. RCAN1 protein is present in adipose tissues.

A Western blot analysis for RCAN1.4 and RCAN1.1 proteins in extracts from BAT, sWAT, gWAT, liver, and tibialis anterior skeletal muscle (TA) extracted from 12-week-old *WT*, mixed background females (F) and males (M) on normal chow, housed at vivarium temperatures (20 μg per lane). Protein extracts from heart (H, 20 μg) and brain (B, 10 μg) are included for comparison. The locations of potential cleavage products of RCAN1 are marked with an asterisk (*). GAPDH is provided as a loading control within each tissue type.

B Western blot to validate specificity of RCAN1 antibody. Protein extracts from heart, TA, liver, BAT, and sWAT were probed with anti-RCAN1 (20 μg per lane). The first two lanes are from animals housed at room temperature. The second two lanes are from animals housed at 4°C for 4–5 h. Tissues were harvested at 2 PM.

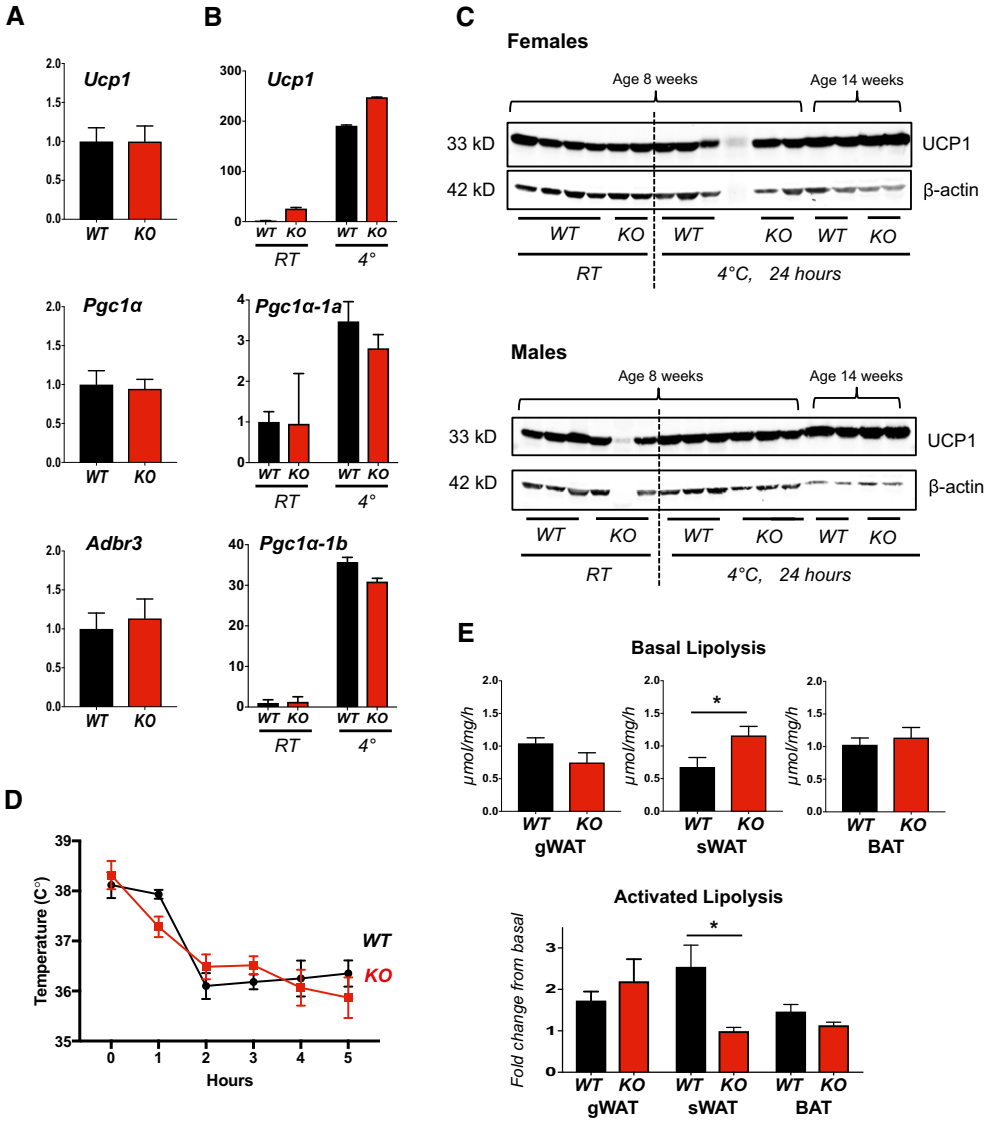


Figure EV4. The thermogenic responses of *KO* BAT are similar to those of *WT*.

A Transcript levels for *Ucp1*, *Pgc1α*, and *Adr-b3* in BAT from 18-week-old male *WT* and *KO* animals on normal chow, housed at normal vivarium temperatures (n = 5).

B Transcript levels for *Ucp1*, *Pgc1α-1a*, and *Pgc1α-1b* in BAT of WT and KO mice following cold exposure. 10- to 12-week-old *WT* and *Rcan1 KO* females were housed at 24°C (RT) or shifted to 4°C for 24 h. Tissues were harvested between 10 and 12 AM (n = 5).

C Western blot analysis for UCP1 and β-actin in protein extracts of *WT* and *KO* BAT from animals housed at 24°C (RT) or shifted to 4°C for 24 h. The upper panel contains protein extracted from females. The lower panel is from males (20 µg protein per lane).

D Change in body temperature following shift of *WT* and *KO* mice to 4°C measured using a rodent rectal temperature probe (World Precision Instruments) (females, n = 3, ± SD).

E Upper panel shows basal rates of lipolysis measured in gWAT, sWAT, and BAT tissue explants from *WT* and *KO* animals. Fold change in lipolysis following adrenergic stimulation is compared in the lower panel (males, n = 5, ± SEM). *P < 0.05 (t-tests in E, upper panel; two-way ANOVA with multiple comparisons in E, lower panel).

Figure EV5. Skeletal muscle parameters and changes in RCAN1 protein levels.

A Representative image showing metachromatic fiber-type stain of the gastrocnemius muscle proximal to the plantaris muscle in 12-week-old, male *WT* and *KO* animals.

B Silver stain of high-resolution myosin heavy stain SDS–PAGE gels of soleus muscle. Location of oxidative type 1 (1), oxidative type 2A (2A/2X) and glycolytic 2B (2B) myosin heavy chain proteins are indicated by arrows. Lower panel provides a Western blot of the same extracts probed for oxidative, type 1 myosin heavy chain (Myh-1).

C Western blot analysis for phosphorylated AKT, total AKT, and GAPDH in extracts from gastrocnemius skeletal muscle of 10-week-old female *WT* and *KO* animals house at RT on NC. Insulin was injected 10 min prior to harvesting tissue. Graph at right provides quantification of Akt phosphorylation (n = 3–4, ± SEM).

D Correlation of *Rcan1* expression in soleus and gastrocnemius skeletal muscle with body weight, plasma insulin, and plasma triglycerides, in a data set comparing backgrounds susceptible (BTBR background) or resistant (C57BL/6 background) to diabetes when carrying the *leptin*$^{ob/ob}$ (*ob*) mutation. Measures are reported as the ratio of the mean $\log_{10}$ intensity (ml ratio). Regression line (black), r = linear regression, P = P-value.

E Western blot analysis for RCAN1 and α-tubulin (αTUB) proteins in gWAT (n = 6) and liver (n = 3) of *WT*, males on normal chow (N) or high-fat diet (H) for 8 weeks starting at 10 weeks of age. $^{**}P < 0.01$ (two-way ANOVA with multiple comparisons).

F Western blot analysis for RCAN1 and GAPDH proteins in gastrocnemius skeletal muscle of 3-month-old, *WT*, mixed background, males maintained at vivarium temperatures (RT) or housed at 4°C for either 3 or 72 h as indicated. The lower blot shows extracts of gastrocnemius from *WT* C57BL/6 males fed normal chow (NC) or high-fat diet (HFD) for 15 weeks, starting at 10 weeks of age (20 μg protein per lane).

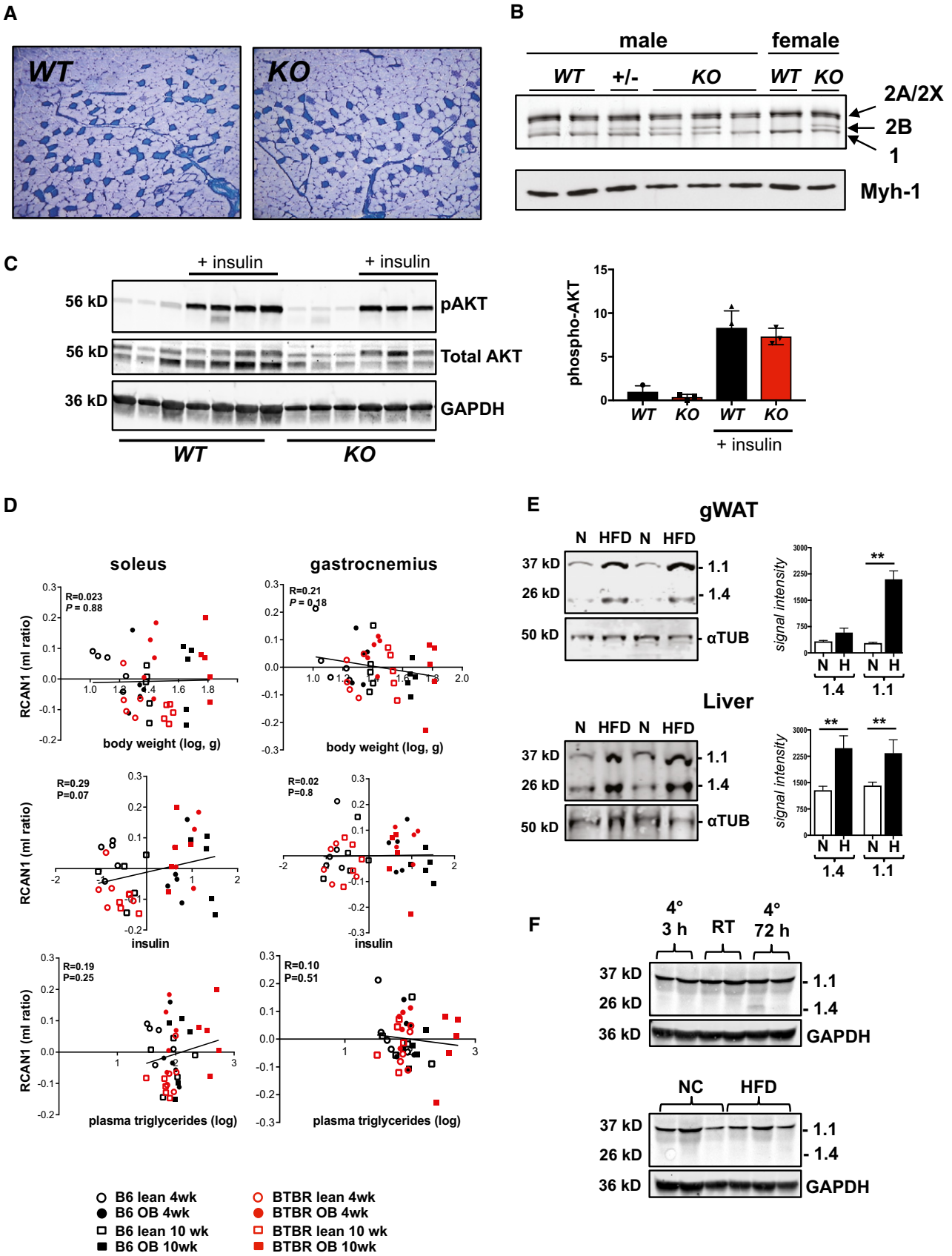

A
WT
KO
B
male
female
WT
+/-
KO
WT
KO
2A/2X
2B
1
Myh-1
C
+ insulin
+ insulin
56 kD
pAKT
56 kD
Total AKT
36 kD
GAPDH
WT
KO
phospho-AKT
WT
KO
WT
KO
+ insulin
D
soleus
gastrocnemius
R=0.023
P = 0.88
R=0.21
P = 0.18
RCAN1 (ml ratio)
body weight (log, g)
R=0.29
P=0.07
R=0.02
P=0.8
insulin
R=0.19
P=0.25
R=0.10
P=0.51
plasma triglycerides (log)
B6 lean 4wk
B6 OB 4wk
B6 lean 10 wk
B6 OB 10wk
BTBR lean 4wk
BTBR OB 4wk
BTBR lean 10 wk
BTBR OB 10wk
E
gWAT
N HFD N HFD
37 kD
26 kD
50 kD
1.1
1.4
αTUB
signal intensity
Liver
F
4° 3 h
RT
4° 72 h
37 kD
26 kD
36 kD
GAPDH
NC
HFD

Figure EV5.