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Supplemental information

Anti-diabetic effects of GLP1 analogs are mediated by thermogenic interleukin-6 signaling in adipocytes

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n	16
Ethnicity (Caucasian/Black/Hispanic/Pacific Islander)	3/8/4/1
Gender (M/F)	9/7
Age (years)	50 ± 8
Weight (kg)	94.3 ± 11.7
BMI (kg/m^2)	32.5 ± 1.8
SBP (mm Hg)	136 ± 23
DBP (mm Hg)	79 ± 11
Fasting glucose (mg/dL)	93 ± 9
Fasting insulin (mIU/L)	8.2 ± 6.2
HOMA-IR	1.98 ± 1.85
Hemoglobin A1c (%)	5.96 ± 0.23
Triglycerides (mg/dL)	122 ± 55
Total cholesterol (mg/dL)	198 ± 44
HDL cholesterol (mg/dL)	54 ± 10
LDL cholesterol (mg/dL)	120 ± 43
AST (units/L)	21 ± 7
ALT (units/L)	22 ± 12
Creatinine (mg/dL)	0.98 ± 0.26
Hemoglobin (g/dL)	13.8 ± 1.0
Platelets ($x10^9/L$)	233 ± 54

Table S1 - **Baseline Clinical Characteristics of Study Participants.**

Related to Figure 1

Data are means ± SD.

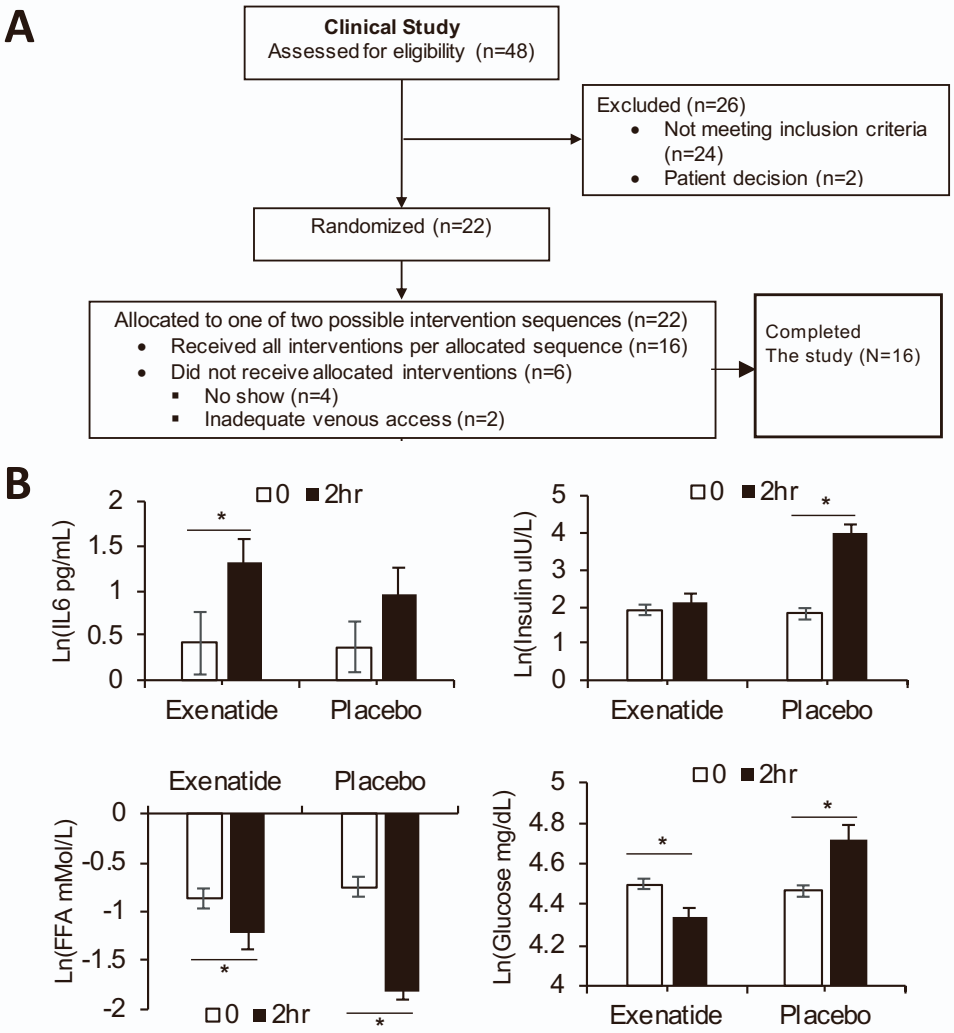

Figure S1 Clinical study. Related to Figure 1.
(A) Study design. The study was a single center, randomized, crossover, double-blinded, placebo-controlled trial. Simple randomization (for study drug order in each participant) was achieved via a computer-generated algorithm. Allocation concealment was maintained by independent study pharmacists. Blinding of intervention sequence (to investigators and subjects) was achieved through the use of an identical-appearing placebo (i.e., normal saline served as the placebo for exenatide). All analyses were conducted as intention-to-treat. Potential subjects participated in a screening visit, where a complete history and physical examination was performed, followed by laboratory testing. Qualified subjects completed two separate, daylong outpatient studies at the Clinical Research Unit (CRU). On each study day, subjects were given a single dose of exenatide 10 mcg by subcutaneous (sc) injection or the same volume of placebo by sc injection. Each study was performed at least 10 days apart to ensure adequate washout of study medication. The study day began at 8:00 AM after an overnight fast and avoidance of alcohol and excessive exercise for 24 hr. An intravenous catheter was placed in a stable vein in an upper extremity. Baseline blood draw was collected a few minutes before the scheduled 11:00 AM meal. Then study medication was given immediately. Then at 11:00 AM subject began eating a timed standardized test meal which consisted of a hamburger, French fries, small apple pies, and diet soda (1550 kcal; 700 kcal from fat; 60% carbohydrate, 30% fat, and 10% protein). The subject was given up to 30 minutes to complete the meal. Venous blood samples were collected again 2 hours later. These procedures were repeated for the second study arm. This study design is similar to our previously described protocol (Hamidi et al., 2020).
(B) Plots of natural log-transformed Figure 1A data, which are not normally distributed.
*$p<0.05$, Student's t-test.

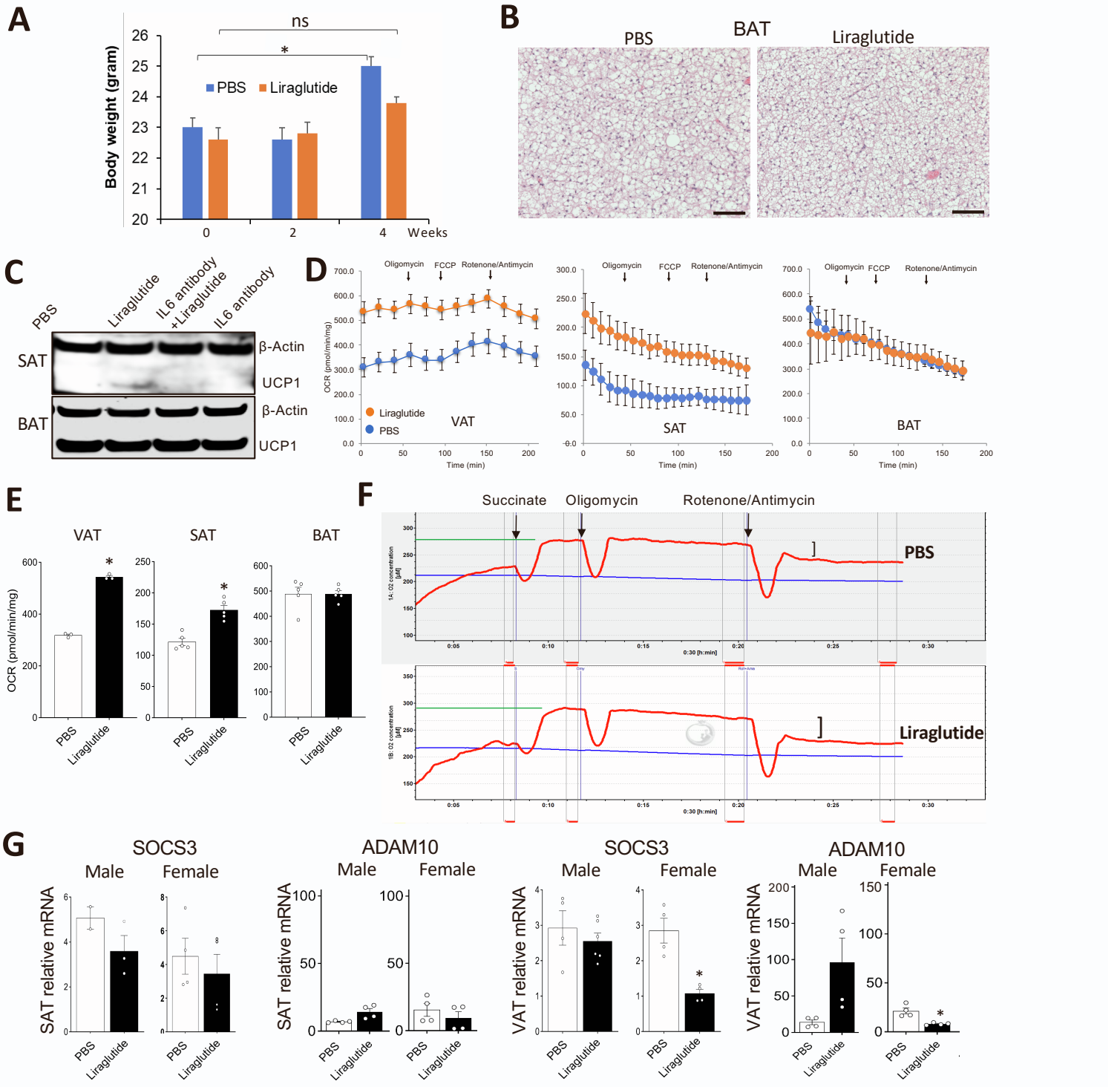

Figure S2 Liraglutide induces IL6 signaling and adipocyte browning in mice. Related to Figure 2.

(A) EchoMRI analysis of HFD-fed overweight C57BL/6 males after 2 and 4 weeks of liraglutide, treatment shows reduced body mass gain in liraglutide-treated mice. n=5. ns: non-significant; *p<0.05, Student's t-test.

(B) BAT sections from mice in (A) at week 4 stained with H&E. Scale bar: 50 µm.

(C) Analysis of mice in Fig. 3 by immunoblotting with the indicated antibodies shows liraglutide-induced UCP1 expression in SAT, but not in BAT. Actin: loading control.

(D) Increased mitochondrial Respiration in VAT, SAT, but not in BAT, of mice treated with liraglutide in a Seahorse Mito Stress Assay performed with XFe24 (Agilent Technologies). n=5 to 10 wells.

(E) Average basal oxygen consumption rate (OCR) calculated from Seahorse data in (D). *p<0.05, Student's t-test.

(F) Increased respiration in SAT of mice treated with liraglutide, compared with control mice, measured by Oroboros Oxygraph O2k Respirometer. Red: O_2 flux. Green : maximal respiration upon succinate treatment.
]: uncoupled respiration (post-Oligomycin value minus post-Rotenone/Antimycin value). Performed once.

(G) C57BL/6 males were injected with a single s.c. dose of liraglutide (0.2mg/kg BW). Analysis of SAT and VAT by RT-PCR after 24 hr shows a trend for a reduction in relative SOCS3 and ADAM10 expression (normalized to 18SRNA). *p<0.05, Student's t-test.

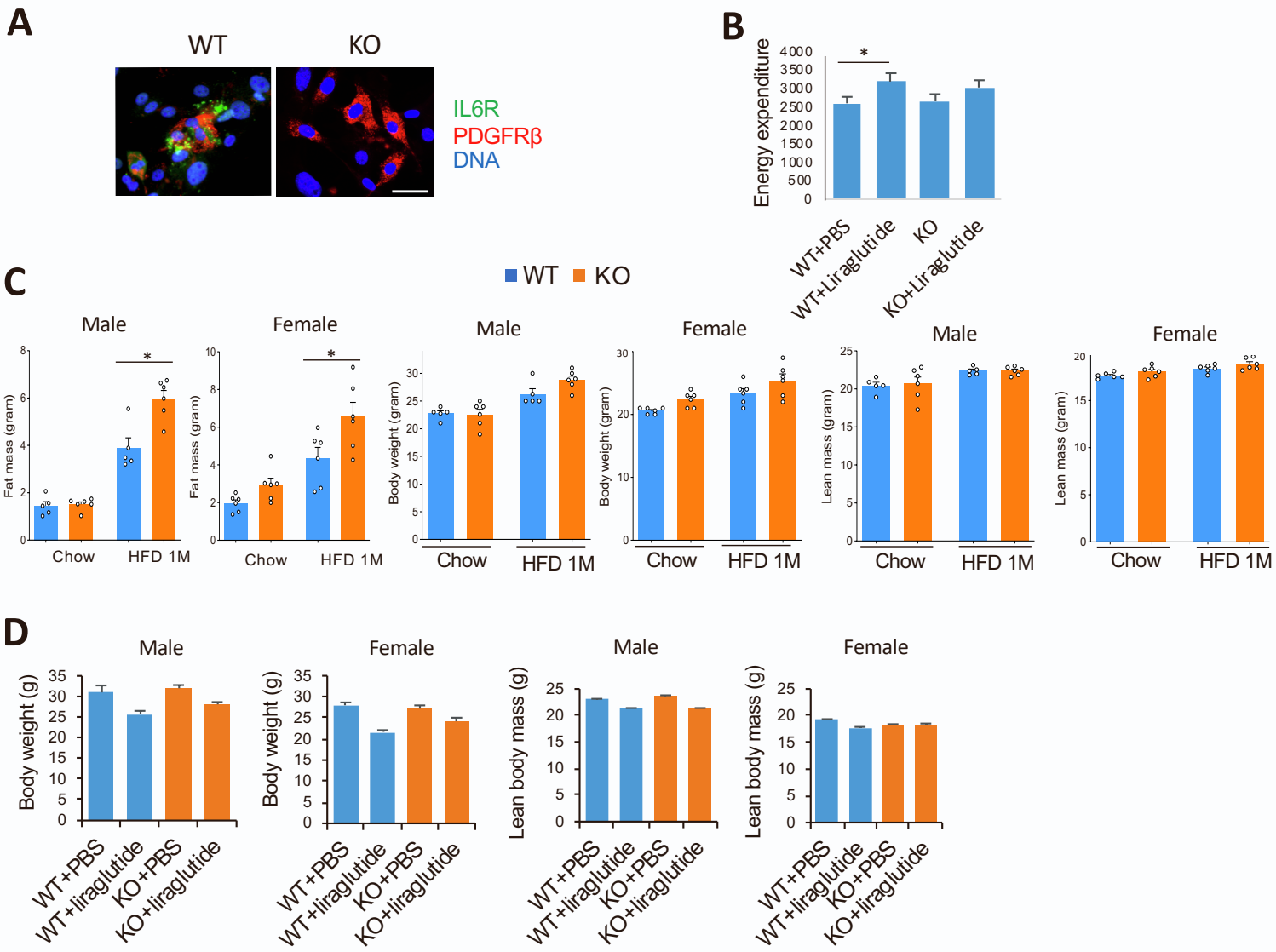

Figure S3. IL6R KO in adipocyte progenitors negates thermogenic effect of liraglutide. Related to Figure 4.
(A) SVF from AT of WT and Pdgfrb-Cre; IL6R$^{fl/fl}$ (KO) mice subjected to IF. Note that IL6R protein expression (green) is lost in PDGFRβ+ ASCs of KO mice. Scale bar: 50 µm.
(B) Energy expenditure in C57BL/6 and IL6R KO males and females after 4 weeks of liraglutide, treatment measured based on Oxygen consumption (VO_2) by indirect calorimetry during the night. Note that the liraglutide-induced increase observed in WT mice is not observed in KO mice. n=3. *p<0.05, Student's t-test.
(C) EchoMRI analysis of C57BL/6 and IL6R KO males after feeding with chow or HFD for 1 month.
(D) EchoMRI analysis of mice in (B). After 4 weeks of liraglutide, treatment (0.2 mg/kg s.c. 20 metronomic injections) the trends for lean and total body mass are similar in WT and KO mice.

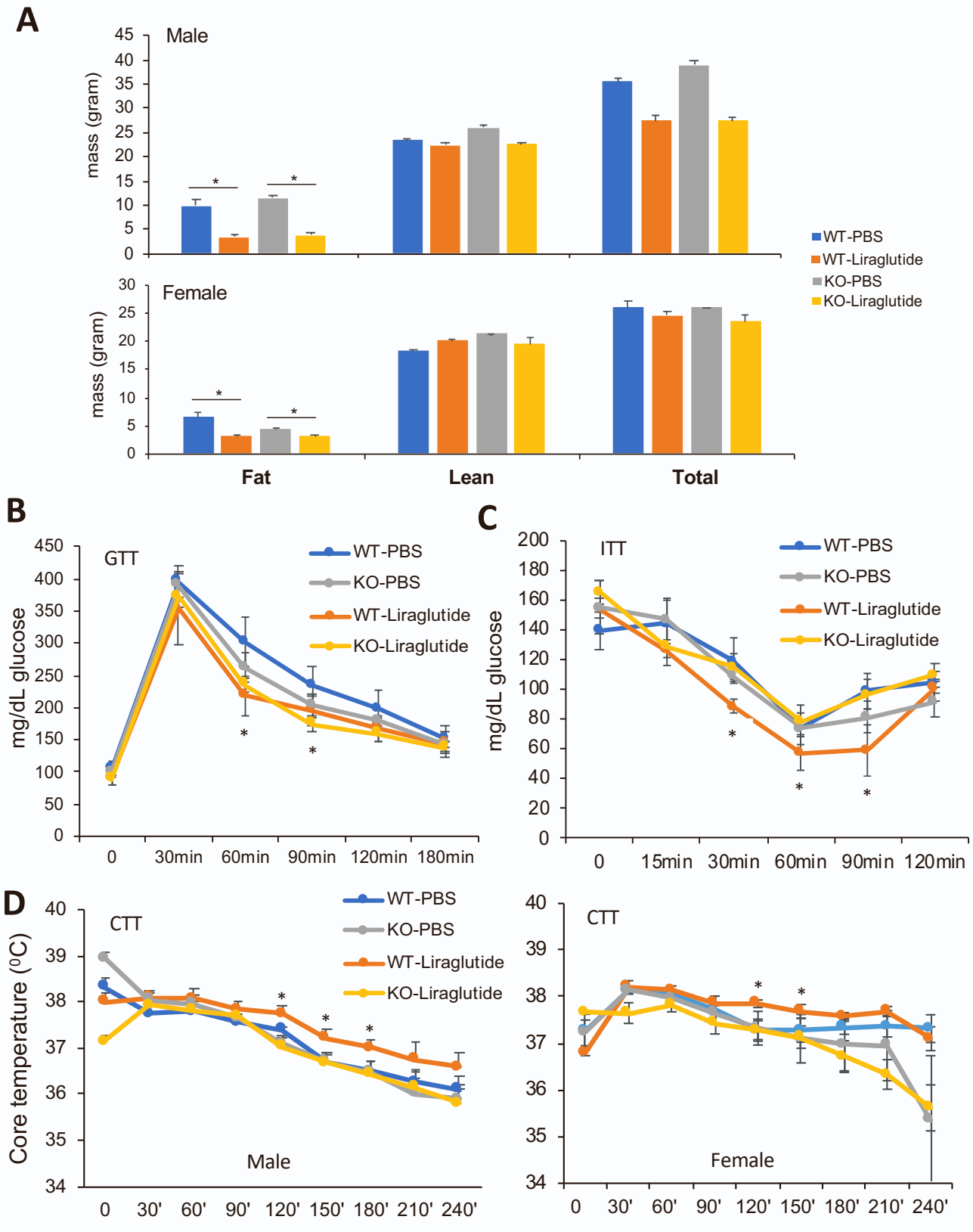

Figure S4. IL6R KO in mature adipocytes negates thermogenic effect of liraglutide. . Related to Figure 4. Adiponectin-Cre;IL6Rafl/fl (KO) and control L6Rafl/fl (WT) progeny were compared.
(A) EchoMRI analysis of HFD-fed overweight C57BL/6 and KO males and females after 4 weeks of liraglutide, treatment (0.2 mg/kg i.p. 20 metronomic injections). Note the reduction of fat mass in both WT and KO liraglutide-treated males and females.
(B) Analysis of mice in (A) at week 4 by glucose tolerance test in males.
(C) Analysis of mice in (A) at week 4 by insulin tolerance test (ITT) in males.
(D) Analysis of mice in (A) at week 4 by cold tolerance test in males and females.
(A-D): n=5. *p<0.05 (1-way ANOVA) for WT PBS vs WT-Liraglutide comparison. Not significant for KO mice.