Figure S1

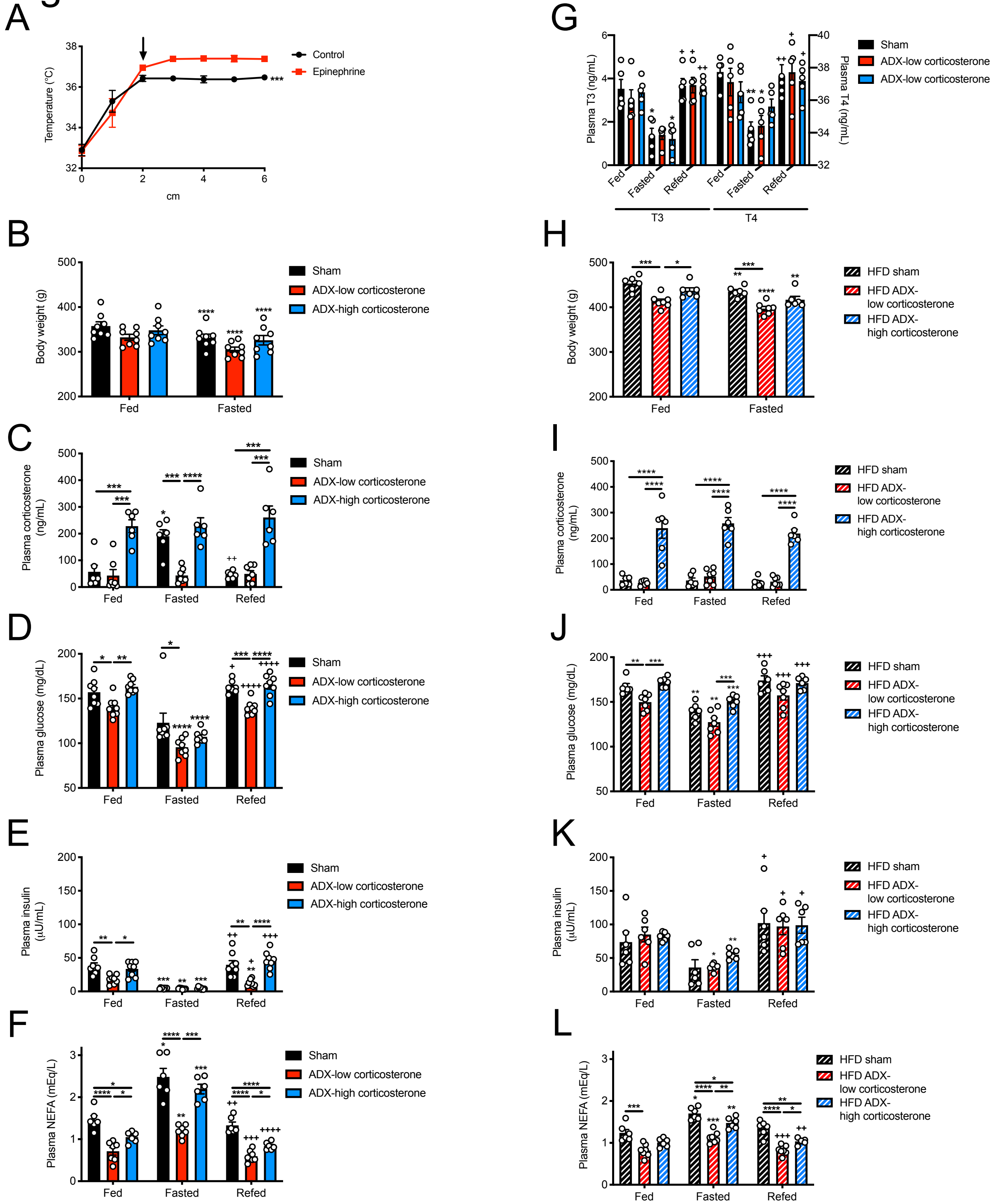


Figure S1. Adrenalectomized rats lack meal thermogenesis. (A) Temperature measured 0-6 cm into the rectum in 48 hr fasted rats±a bolus injection of epinephrine. N=4 per group. All other body temperature data in this manuscript were obtained 2 cm into the rectum (arrow). (B) Body weight. In panels (B) and (H), ***P*<0.01, *****P*<0.0001 vs. fed rats by paired Student's t-test. (C)-

(G) Plasma corticosterone, glucose, insulin, NEFA, T3, and T4 concentrations. *$P<0.05$, **$P<0.01$, ***$P<0.001$, ****$P<0.0001$, +$P<0.05$, ++$P<0.01$, +++$P<0.001$, ++++$P<0.0001$. In panels (C)-(L), * directly over bars denotes comparisons to fed rats, and + directly over bars denotes comparisons to fasted rats. The same rats were compared under fed, fasted, and refed conditions using repeated measures ANOVA with Bonferroni's multiple comparisons test, while groups were compared by ANOVA with Bonferroni's multiple comparisons test. (H) Body weight. Panels (H)-(L) show data from the same rats in panels (A)-(G), following 10 days of high fat feeding. (I)-(L) Plasma corticosterone, glucose, insulin, and NEFA concentrations. In all panels, data are the mean±S.E.M. If no symbol appears, groups and time points are not statistically different.

Figure S2

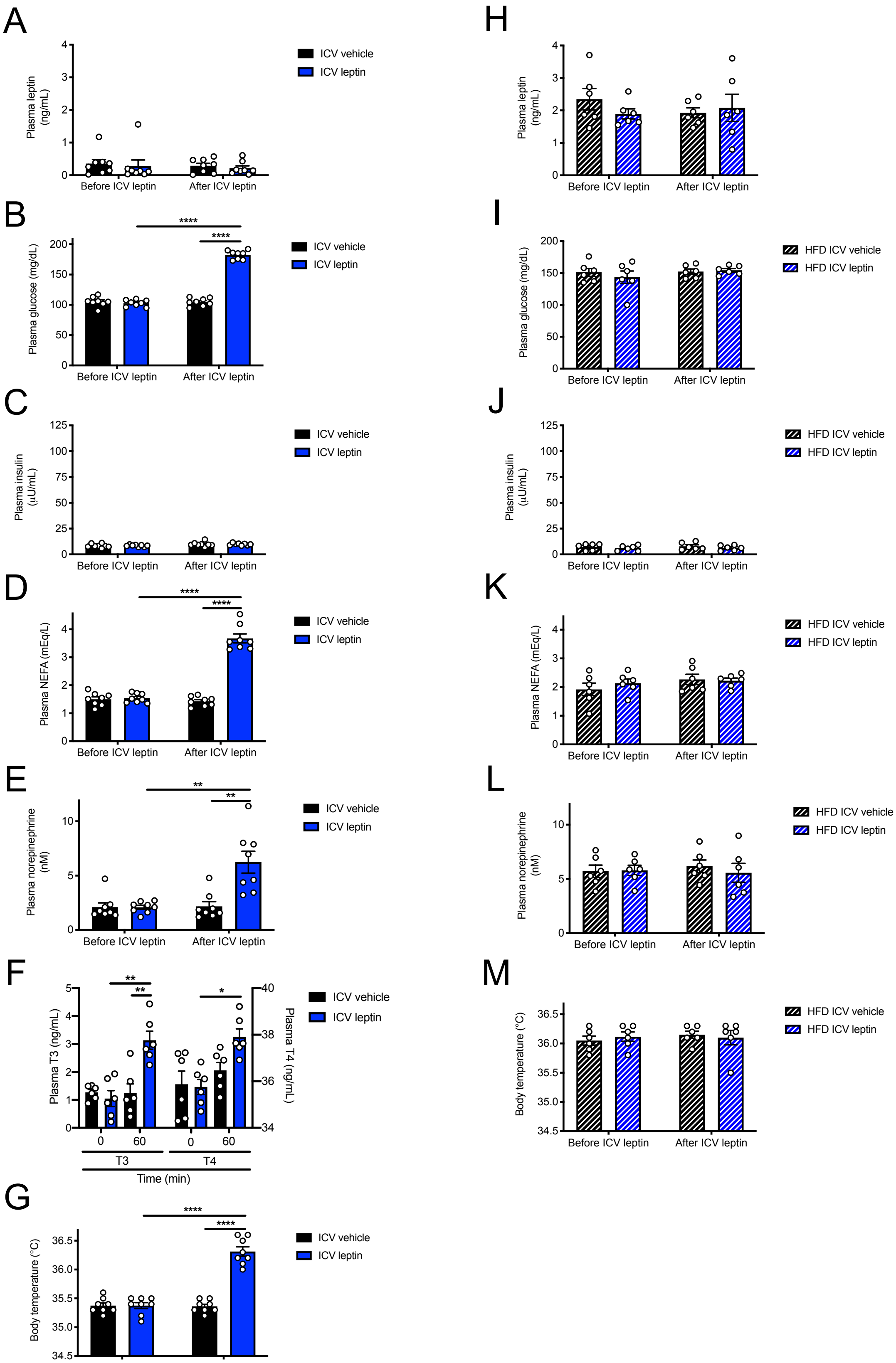

A
Plasma leptin (ng/mL)
ICV vehicle
ICV leptin
Before ICV leptin
After ICV leptin
B
Plasma glucose (mg/dL)
****
****
C
Plasma insulin (µU/mL)
D
Plasma NEFA (mEq/L)
E
Plasma norepinephrine (nM)
**
**
F
Plasma T3 (ng/mL)
Plasma T4 (ng/mL)
Time (min)
T3
T4
*
G
Body temperature (°C)
H
I
HFD ICV vehicle
HFD ICV leptin
J
K
L
M

Figure S2. Leptin promotes thermogenesis through a central effect. (A)-(F) Plasma leptin, glucose, insulin, NEFA, norepinephrine, T3, and T4 concentrations in 48 hr fasted rats. All “before ICV leptin” data were obtained 30 min after the initiation of an infusion of somatostatin, and “after ICV leptin” data were obtained 30 min after an ICV injection of leptin (or PBS control), with somatostatin infusion continuing. Rats were compared before and after leptin by the 2-tailed paired Student’s t-test, and groups were compared by the 2-tailed unpaired Student’s t-test. In all panels, $^{**}P<0.01$, $^{****}P<0.0001$. (G) Body temperature. (H)-(L) Plasma leptin, glucose, insulin, NEFA, and norepinephrine concentrations in the same rats shown in panels (A)-(G), after 10 days of high fat feeding. (M) Body temperature. In all panels, the mean±S.E.M. is shown. If no symbol appears, groups and time points are not statistically different.

Figure S3

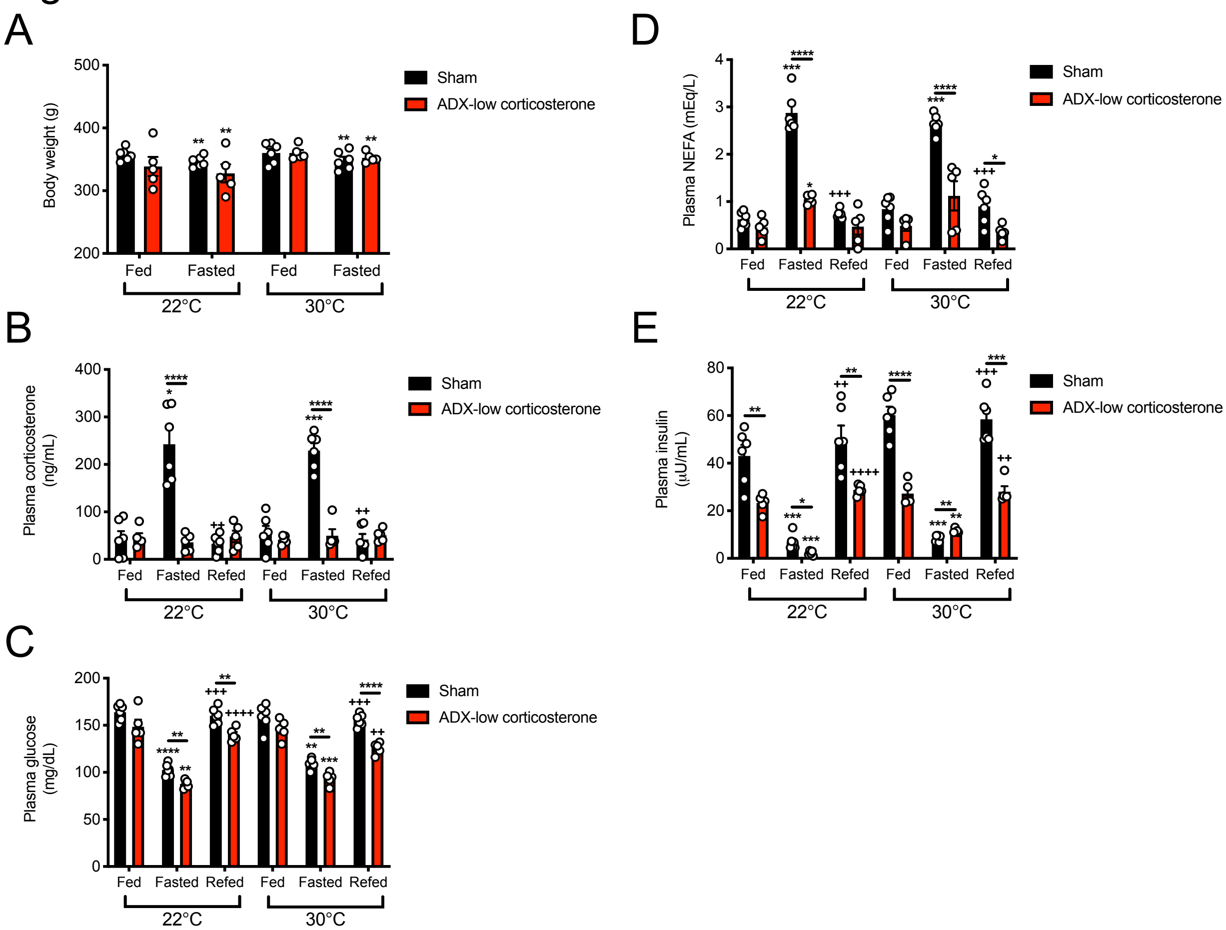


Figure S3. Ambient temperature does not affect the impact of refeeding on postprandial increases in body temperature. (A) Body weight in rats housed at thermoneutrality for one week. (B)-(E) Plasma corticosterone, glucose, insulin, and NEFA concentrations. In all panels, **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001; ++*P*<0.01, +++*P*<0.001. * directly over bars denotes comparisons to fed rats, and + directly over bars denotes comparisons to fasted rats. If no symbol appears, groups and time points are not statistically different.

Figure S4

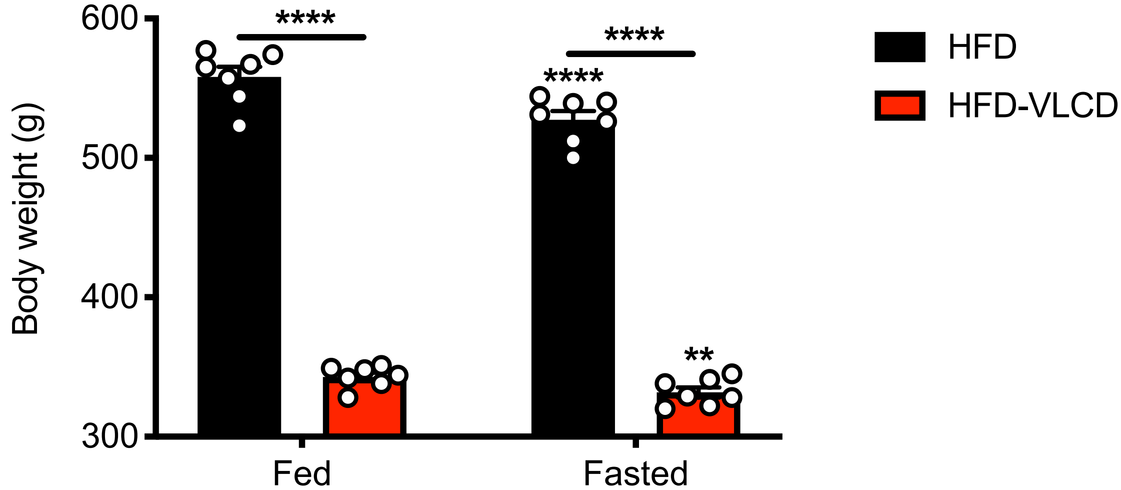
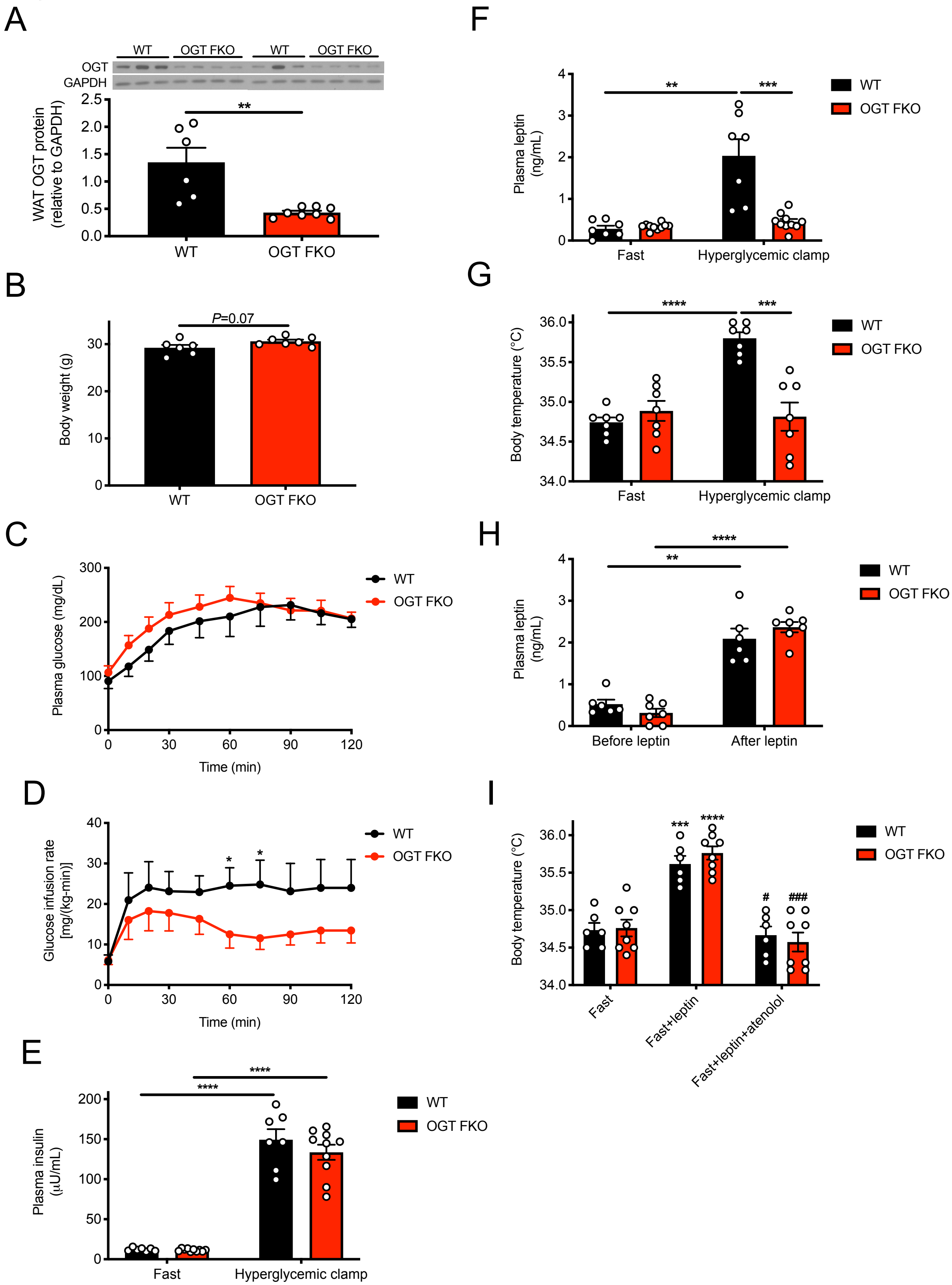


Figure S4. Normalizing body weight restores the leptin-catecholamine-thermogenic response to fasting and refeeding. Body weight before and after weight normalization via a very low calorie diet.

Figure S5



A
WT
OGT FKO
OGT
GAPDH
WAT OGT protein (relative to GAPDH)
2.5
2.0
1.5
1.0
0.5
0.0
**
WT
OGT FKO
B
Body weight (g)
30
20
10
0
P=0.07
WT
OGT FKO
C
Plasma glucose (mg/dL)
300
200
100
0
0
30
60
90
120
Time (min)
WT
OGT FKO
D
Glucose infusion rate [mg/(kg·min)]
40
30
20
10
0
*
*
0
30
60
90
120
Time (min)
WT
OGT FKO
E
Plasma insulin (μU/mL)
200
150
100
50
0
****
****
Fast
Hyperglycemic clamp
WT
OGT FKO
F
Plasma leptin (ng/mL)
4
3
2
1
0
**
***
Fast
Hyperglycemic clamp
WT
OGT FKO
G
Body temperature (°C)
36.0
35.5
35.0
34.5
34.0
****
***
Fast
Hyperglycemic clamp
WT
OGT FKO
H
Plasma leptin (ng/mL)
4
3
2
1
0
****
**
Before leptin
After leptin
WT
OGT FKO
I
Body temperature (°C)
36.0
35.5
35.0
34.5
34.0
***
****
#
###
Fast
Fast+leptin
Fast+leptin+atenolol
WT
OGT FKO

Figure S5. Leptin is required for postprandial increases in body temperature. (A) Epididymal white adipose tissue OGT expression in inducible OGT FKO mice and their WT littermate controls. Full, uncut gels are shown in Fig. S15. In all panels, **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001. Unpaired t-tests were used for comparisons between genotypes and paired t-tests (panels (E)-(H)) or ANOVA with Bonferroni's multiple comparisons test (panel (I)) were used for within-genotype comparisons. (B) Body weight. (C)-(D) Plasma glucose and glucose infusion rate during a hyperglycemic clamp. (E)-(F) Plasma insulin and leptin concentrations at time 0 and 120 min of the hyperglycemic clamp. (G) Body temperature and time 0 and 120 min of the hyperglycemic clamp. (H) Plasma leptin concentrations before and 30 min after a bolus injection of leptin in fasted mice. (I) Body temperature. ****P*<0.001, *****P*<0.0001 vs. fasted mice; #*P*<0.05, ###*P*<0.001 vs. fasted, leptin-injected mice. Data are the mean±S.E.M. If no symbol appears, groups and time points are not statistically different.

Figure S6

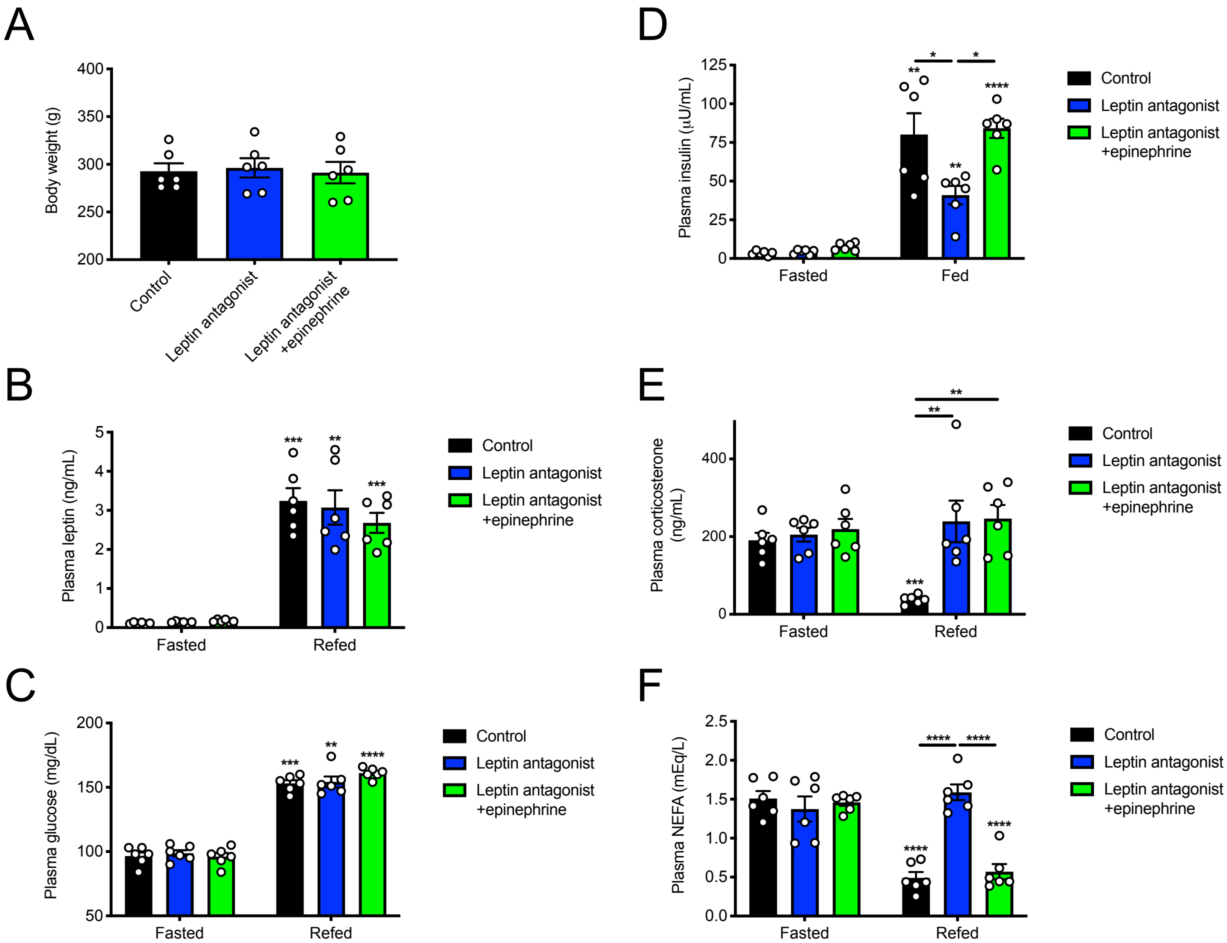


Figure S6. Leptin is required for postprandial increases in body temperature. (A) Body weight in rats treated with a single injection of a small molecule leptin antagonist. (B)-(E) Plasma leptin, glucose, insulin, and NEFA concentrations. In all panels, **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001. Asterisks directly over bars denote comparisons to fasted rats. In all panels, the mean ± S.E.M. are shown. If no symbol appears, groups and time points are not statistically different.

Figure S7

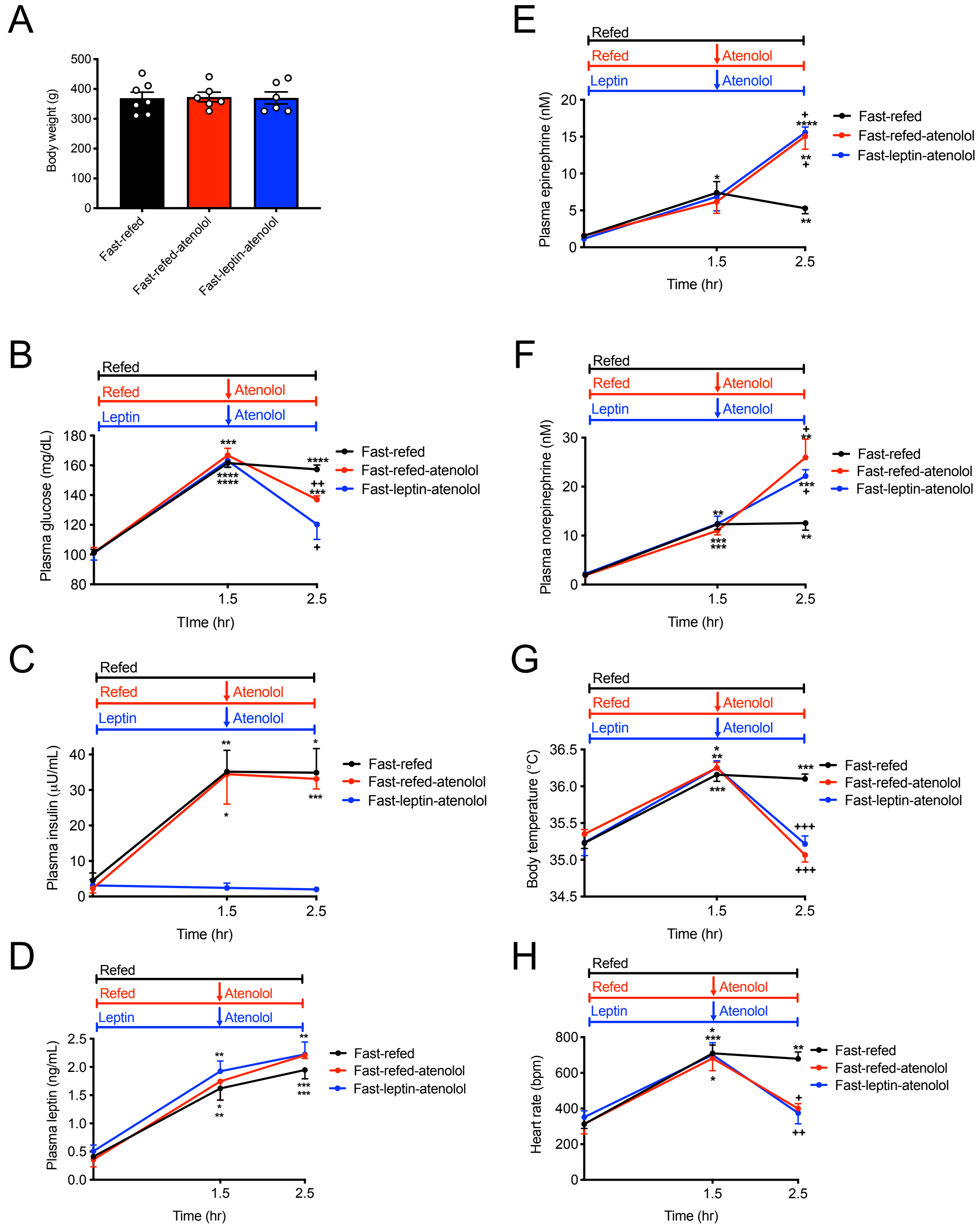


Figure S7. β-adrenergic activity mediates leptin's effect to drive postprandial increases in body temperature. (A) Body weight. (B)-(F) Plasma glucose, insulin, leptin, epinephrine, and norepinephrine concentrations. (G) Body temperature. (H) Heart rate. In all panels, **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001 vs. time 0, +*P*<0.05, ++*P*<0.01 vs. 90 min by repeated-measures ANOVA with Bonferroni's multiple comparisons test. Data are the mean±S.E.M. of n=6-7 per group. If no symbol appears, groups and time points are not statistically different.

Figure S8

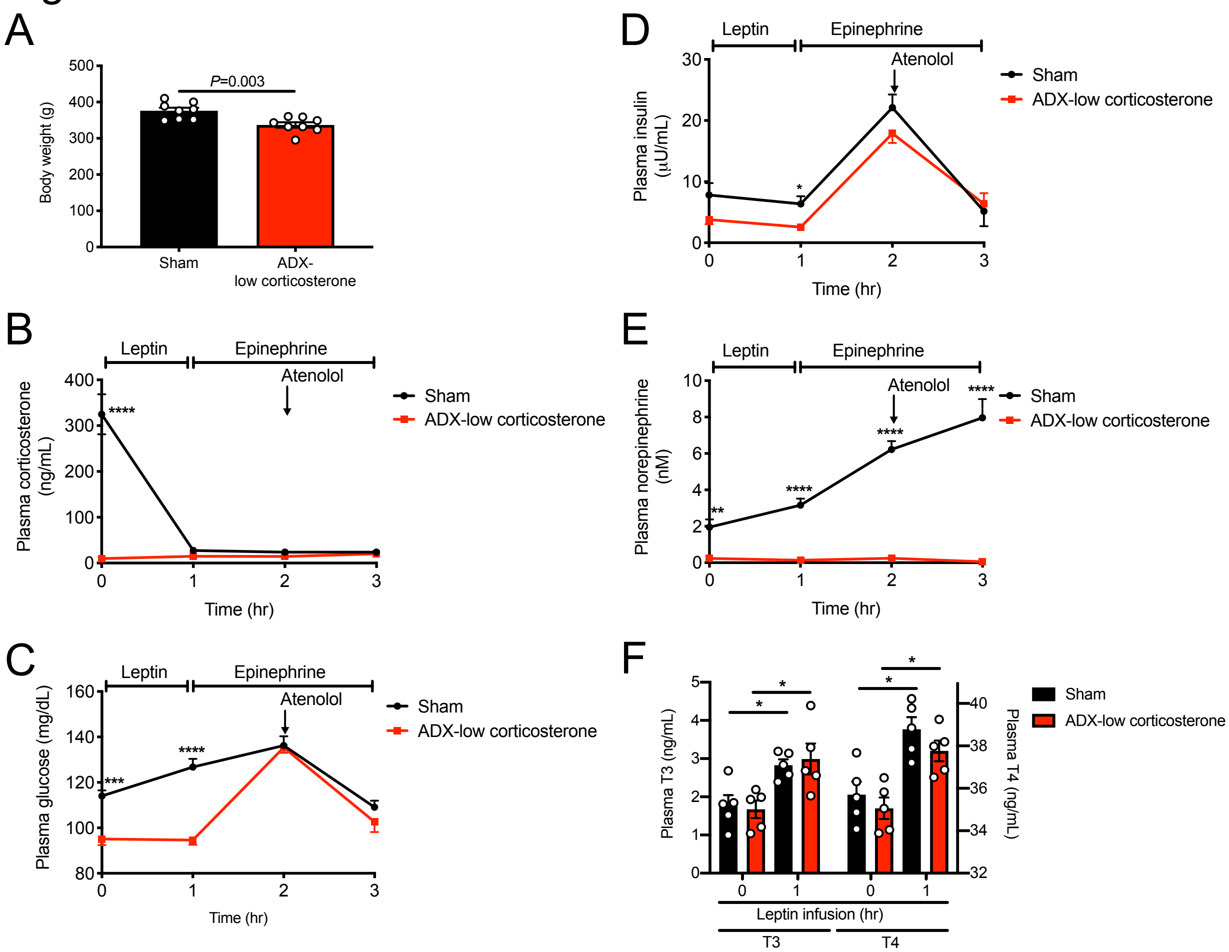


Figure S8. β-adrenergic activity mediates leptin's effect to drive postprandial increases in body temperature. (A) Body weight. (B)-(F) Plasma corticosterone, glucose, insulin, norepinephrine, T3, and T4 concentrations. **P*<0.05, ***P*<0.01, *****P*<0.0001 by the 2-tailed unpaired Student's t-test. Data are the mean±S.E.M. of n=8 per group. If no symbol appears, groups and time points are not statistically different.

Figure S9

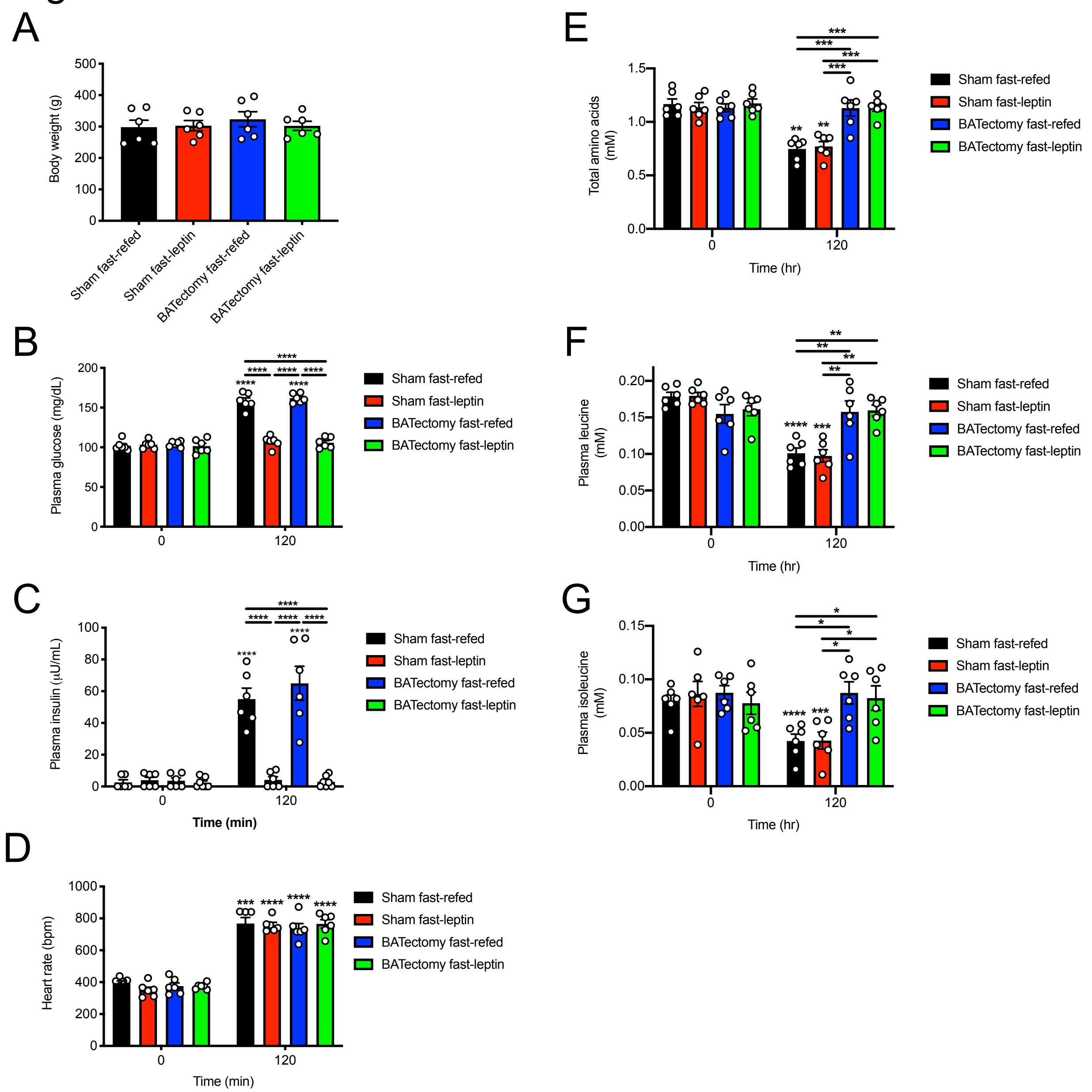


Figure S9. BAT accounts for most if not all of feeding- and leptin-induced increases in body temperature. (A) Body weight one week following BATectomy or sham surgery. (B)-(C) Plasma glucose and insulin concentrations. (D) Heart rate. (E) Total plasma amino acid concentrations. (F)-(G) Plasma branched-chain amino acid (leucine and isoleucine, respectively) concentrations. In all panels, data are presented as the mean±S.E.M. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001. Symbols over bars refer to the same group at time zero by the 2-tailed paired Student's t-test. Comparisons between groups were performed by ANOVA with Bonferroni's multiple comparisons test. If no symbol appears, groups and time points are not statistically different.

Figure S10

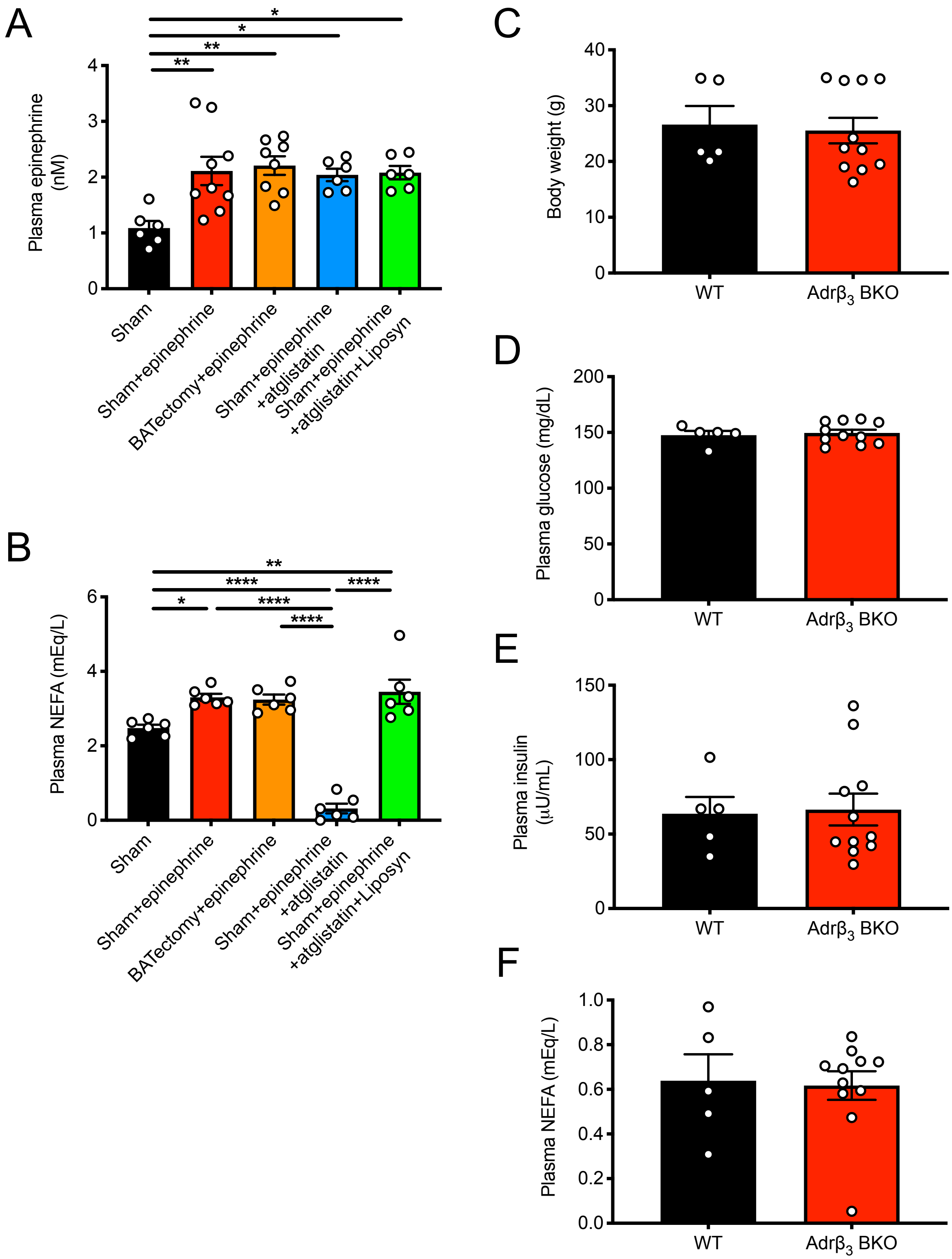

A
B
C
D
E
F
Plasma epinephrine (nM)
Plasma NEFA (mEq/L)
Sham
Sham+epinephrine
BATectomy+epinephrine
Sham+epinephrine +atglistatin
Sham+epinephrine +atglistatin+Liposyn
Body weight (g)
Plasma glucose (mg/dL)
Plasma insulin (µU/mL)
WT
Adrβ3 BKO

Figure S10. BAT β_3 adrenergic activity-mediated lipolysis is required for feeding-induced increases in body temperature. (A)-(B) Plasma epinephrine and NEFA concentrations in 48 hr fasted rats treated with the perturbations listed. Groups were compared by ANOVA with Bonferroni's multiple comparisons test. (C) Body weight in inducible BAT-specific Adrβ_3 knockout mice. (D)-(F) Plasma glucose, insulin, and NEFA concentrations. n=5 (WT) and 11 (Adrβ_3 BKO). Data are the mean±S.E.M. No significant differences were observed between groups using the 2-tailed unpaired Student's t-test.

Figure S11

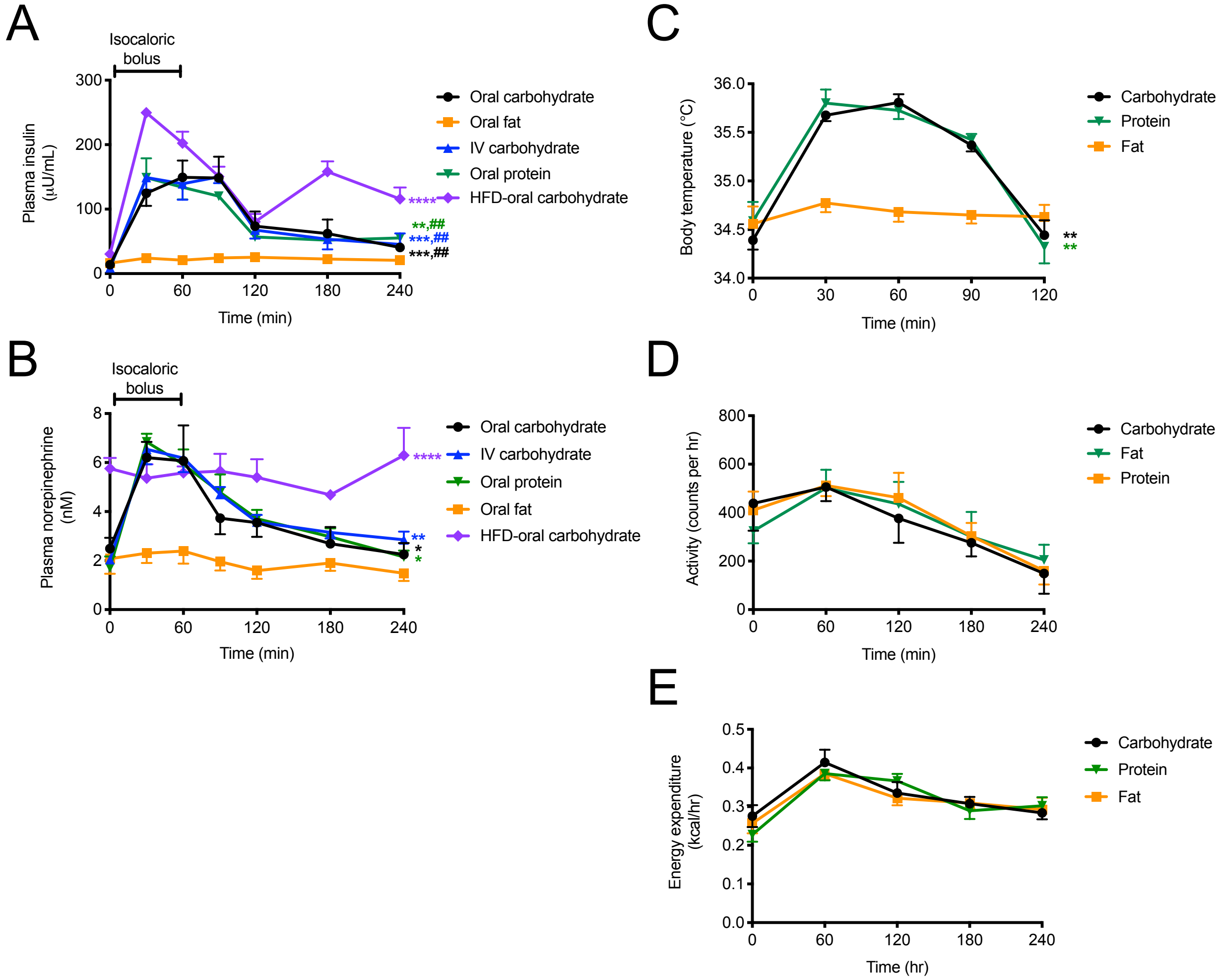


Figure S11. Increases in postprandial body temperature, but not energy expenditure, depend on meal composition. (A)-(B) Plasma insulin and norepinephrine concentrations in rats. n=4 (oral protein), 5 (HFD-oral carbohydrate), 6 (oral carbohydrate, oral fat), or 8 (IV carbohydrate). The area under the curve was compared by ANOVA with Bonferroni's multiple comparisons test. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 vs. oral fat, ##P<0.01 vs. HFD-oral carbohydrate. (C)-(D) Body temperature, activity, and energy expenditure following an oral gavage of an isocaloric carbohydrate (dextrose), fat (canola oil), or protein (casein) meal in 24 hr fasted mice. **P<0.01 vs. fat. n=8 (protein) or 12 (carbohydrate, fat). In all panels, data are the mean±S.E.M. If no symbol appears, groups are not statistically different.

Figure S12

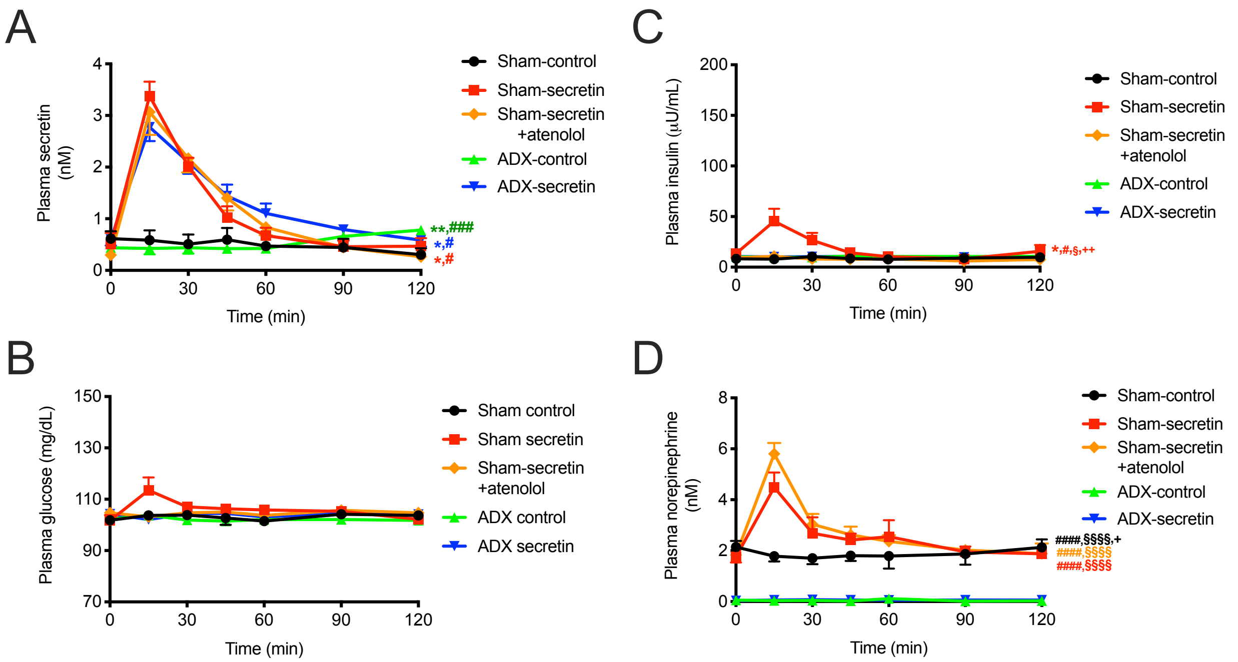


Figure S12. Secretin has a modest, catecholamine-dependent effect to cause thermogenesis. (A)-(D) Plasma secretin, glucose, insulin, and norepinephrine concentrations. **P*<0.05, ***P*<0.01 vs. sham-control, #*P*<0.05, ###*P*<0.001, ####*P*<0.0001 vs. ADX-control, §*P*<0.05, §§§§*P*<0.0001 vs. ADX-secretin, +*P*<0.05, ++*P*<0.01 vs. sham-secretin+atenolol by ANOVA with Bonferroni's multiple comparisons test (comparison of AUC). In all panels, data are the mean±S.E.M. of n=6-8 per group. If no symbol appears, groups are not statistically different.

Figure S13

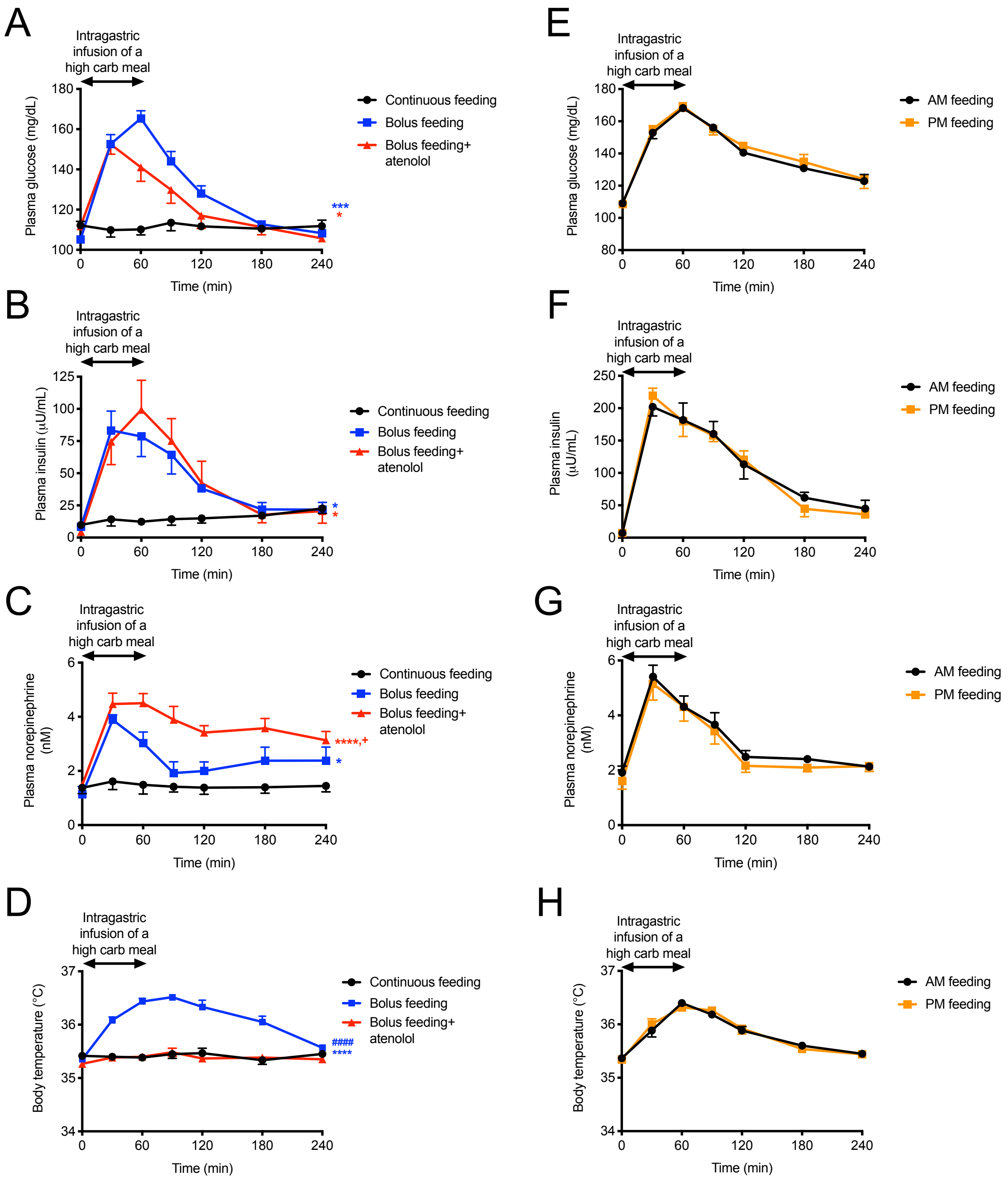


Figure S13. Bolus feeding minimizes weight gain through a β-1 adrenergic effect to promote thermogenesis. (A)-(C) Plasma glucose, insulin, and norepinephrine concentrations during and after the last meal (day 10), or during continuous feeding (day 10). In panels (A)-(D), **P*<0.05, ****P*<0.001, *****P*<0.0001 vs. continuous feeding and ####*P*<0.0001 vs. bolus feeding+atenolol comparing the 240 min AUC by ANOVA with Bonferroni's multiple comparisons test. If no symbol appears, groups are not statistically different. (D) Body temperature. (E)-(G) Plasma glucose, insulin, and norepinephrine concentrations during the last meal after 10 days of morning or evening feeding. (H) Body temperature. In panels (E)-(H), no differences were observed in AUC compared by the 2-tailed unpaired Student's t-test.

Figure S14

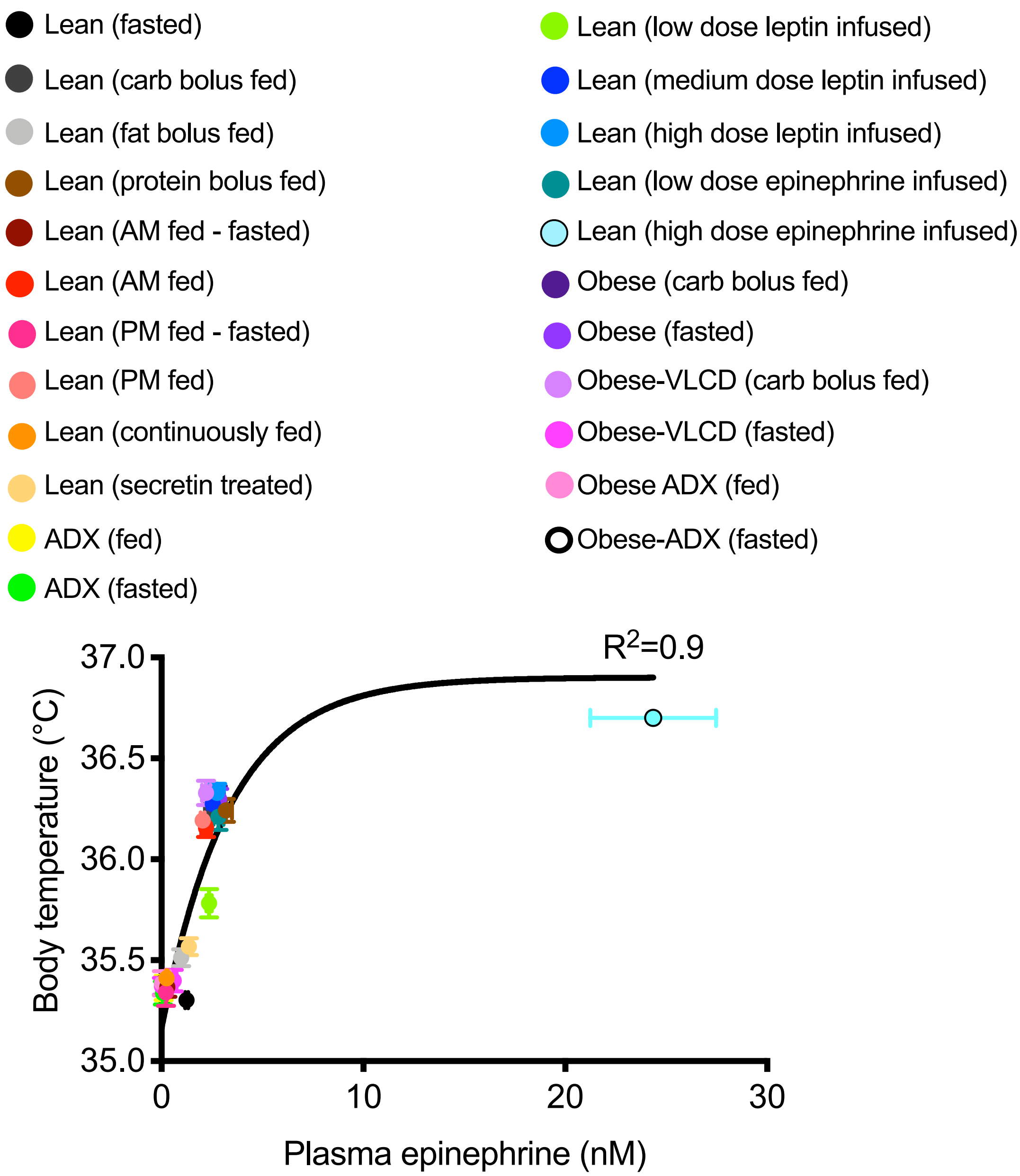
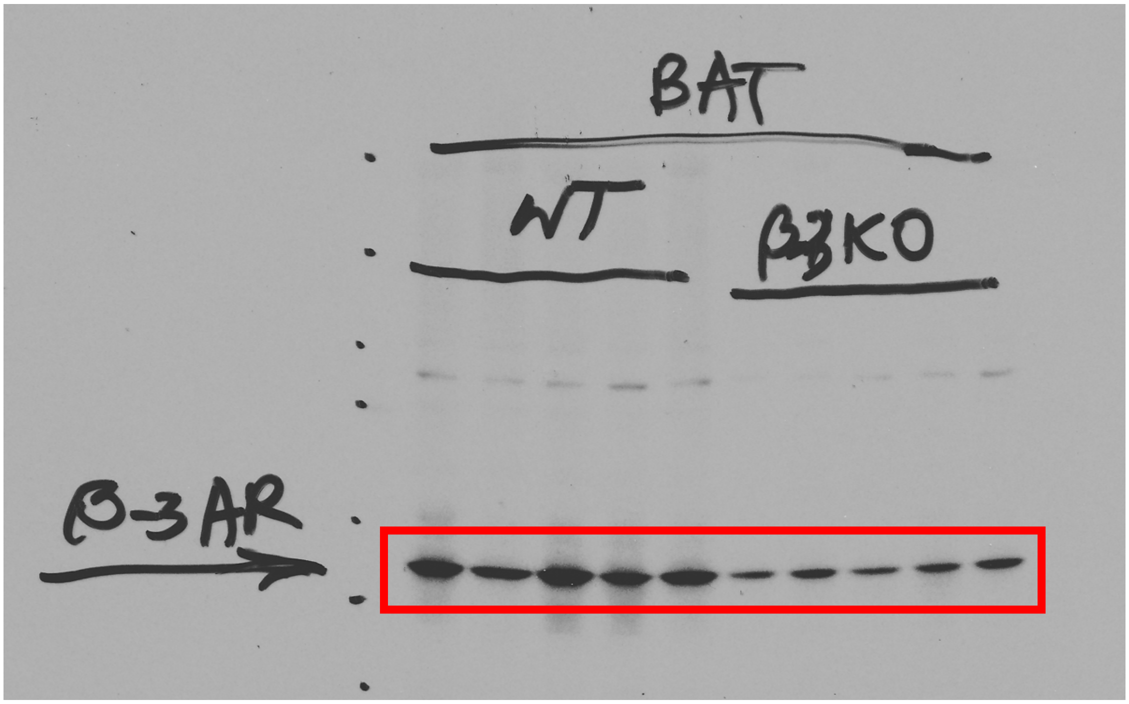


Figure S14. The adrenomedullary response to leptin plateaus at levels observed in obese rodents, obviating the need for leptin resistance. (A) Correlation between plasma epinephrine and plasma leptin concentrations. (B) Correlation between body temperature and plasma leptin concentrations. (C) Correlation between body temperature and plasma epinephrine concentrations. In all cases, correlations refer to the group mean of each analyte (all data points shown in previous figures). Data are the group mean±S.E.M.

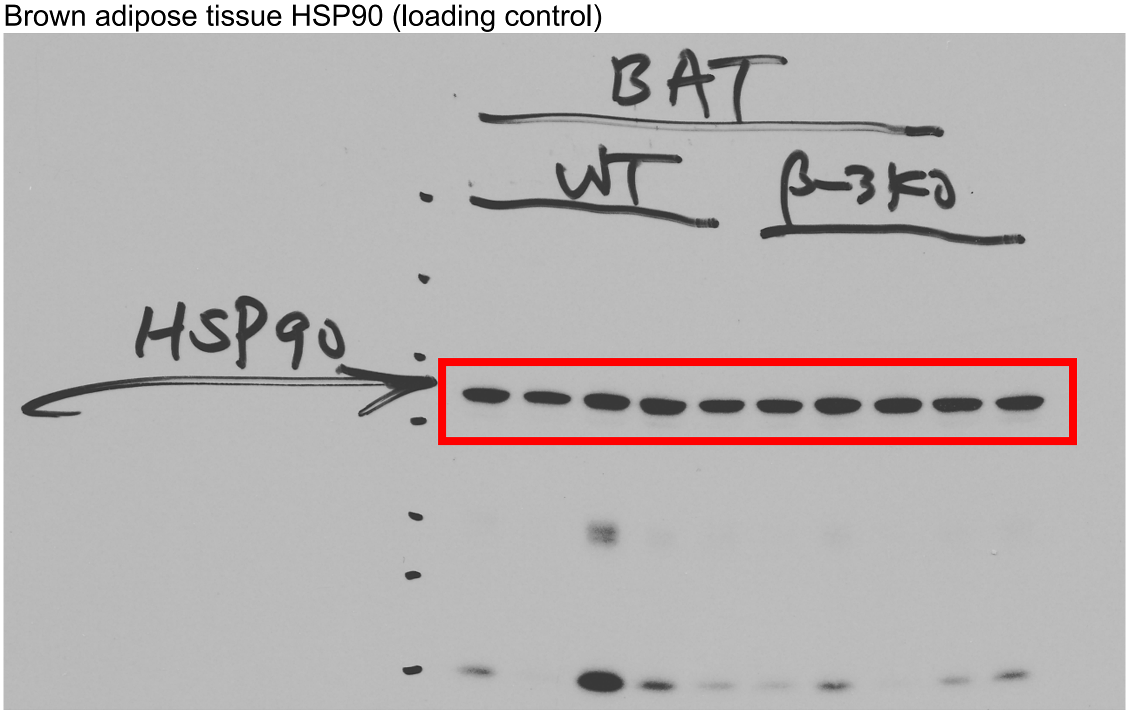
Figure S15

Original, uncut gels for Figure 5D

Brown adipose tissue Adrβ3

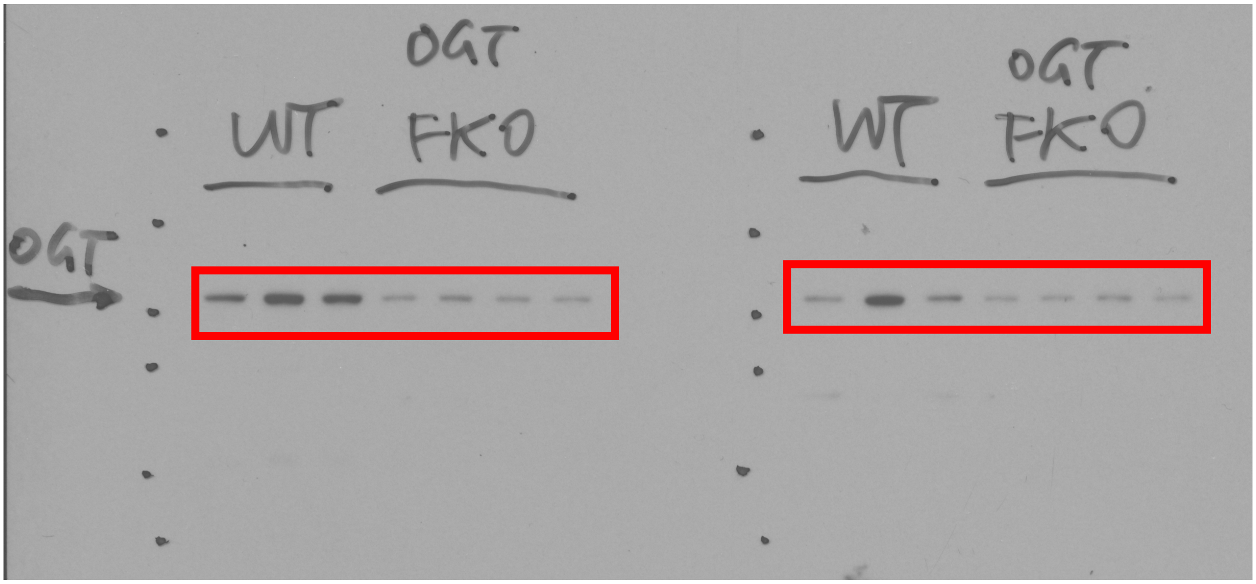


Brown adipose tissue HSP90 (loading control)

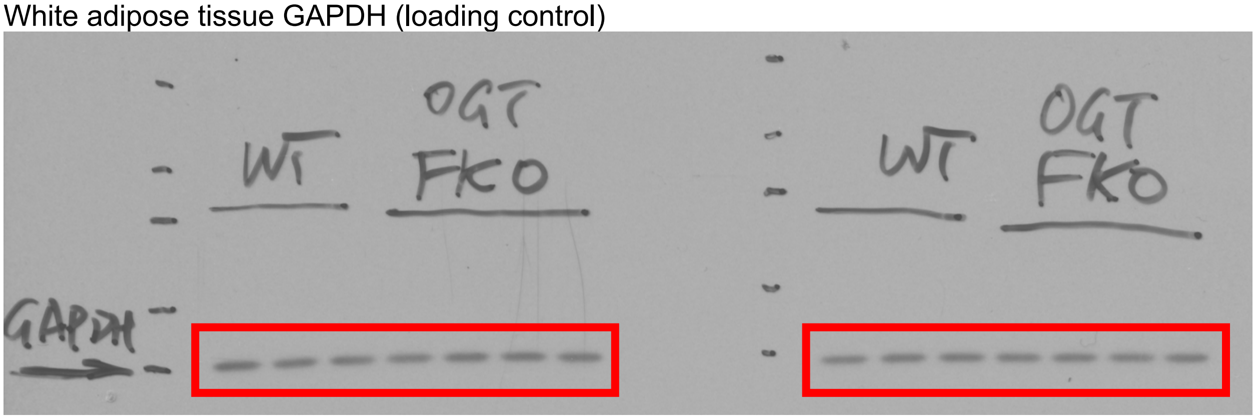


Original, uncut gels for Figure S5A

White adipose tissue OGT



White adipose tissue GAPDH (loading control)



CoA	Sham	Sham+epinephrine	Sham+epinephrine+ atglistatin	Sham+epinephrine+ atglistatin+Liposyn
C10	0.10±0.03	0.26±0.07	0.05±0.01^{+}	0.27±0.04§
C12:3	0.08±0.02	0.35±0.11	0.07±0.01	0.44±0.19
C12:2	0.17±0.03	0.37±0.05**	0.16±0.01^{+}	0.43±0.08§§
C12:1	0.11±0.02	0.90±0.30	0.07±0.01	0.98±0.47
C12	0.20±0.07	1.11±0.33*	0.11±0.02^{+}	0.96±0.22§
C14:3	0.19±0.02	0.52±0.06*	0.17±0.01^{+}	0.61±0.14**,§§
C14:2	0.30±0.05	1.02±0.21	0.18±0.02	1.19±0.39§
C14:1	0.38±0.10	2.69±0.86	0.23±0.04	3.08±1.24
C14	0.84±0.24	2.92±0.69*	0.52±0.07^{+}	3.13±0.60*,§§
C16:3	0.12±0.02	0.78±0.26	0.08±0.01	1.21±0.60
C16:2	0.43±0.09	2.63±0.98	0.22±0.04	3.01±1.35
C16:1	1.47±0.32	7.68±2.12	1.18±0.14	8.32±2.78
C16	4.58±0.79	10.02±1.21**	5.05±0.47^{++}	11.14±1.15***,§§
C18:3	0.70±0.08	2.55±0.89	0.61±0.09	3.79±1.65
C18:2	6.03±1.23	8.95±1.37	4.53±0.92	14.66±3.44*,§
C18:1	6.25±0.76	15.36±2.00**	6.38±0.69^{++}	17.13±2.65**,§§
C18	2.62±0.39	6.77±0.89**	2.25±0.17^{++}	7.23±1.07**,§§§
C20:5	0.23±0.05	0.43±0.09	0.15±0.03	0.60±0.13*,§§
C20:4	1.15±0.33	1.69±0.29	0.70±0.14	2.33±0.36§§
C22:6	0.21±0.08	0.27±0.07	0.15±0.03	0.33±0.10

Table S1. Lipolysis is required for the thermogenic response to epinephrine. BAT long-chain acyl-CoA species. *P<0.05, **P<0.01, ***P<0.001 vs. sham; +P<0.05, ++P<0.01 vs. sham+epinephrine; §P<0.05, §§P<0.01, §§§P<0.001 vs. sham+epinephrine+atglistatin. The predominant lipid species in Liposyn is soybean oil (C18:2). Total long-chain acyl-CoA concentrations from these rats can be found in Fig. 5D. n=6 per group, with data representing the mean±S.E.M. If no symbol appears, groups are not statistically different.