An *Adcy3* coding mutation causes partial loss of enzymatic function, contributing to obesity in a rat model by reducing lipolysis

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Supplemental Figures

Figure S1. Body weight curves and tissue weights at sac in Adcy3$^{mut/mut}$ vs WT rats. (a) Adcy3$^{mut/mut}$ males (M) and females (F) gain more weight than wild-type (WT) rats during the study. (**b**) Adcy3$^{mut/mut}$ M and F have more retroperitoneal fat (RetroFat), gonadal fat, and omental fat (OmenFat) than WT rats at necropsy. (**c)** Adcy3$^{mut/mut}$ M both have larger pancreas at necropsy than WT M with no significant differences in the necropsy weights of the other non-adipose tissues. Mean ± SEM. T-test or rmANOVA, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Figure S2. *Ex vivo* cAMP production in response to stimulation with a β3-AR agonist in RetroFat of Adcy3$^{mut/mut}$ vs WT rats, separated by diet. (a) cAMP production in response to β3-AR agonist in RetroFat is blunted in males (M) on high fat diet (HFD) for nine weeks relative to those on standard diet (SD) (p=0.042) (**b)** There is not a significant effect of diet in females (F). Mean ± SEM. rmANOVA

Figure S3. Body weight curves, fat and lean mass, tissue weights at sac, fasting serum FFA and body temperature during acute cold exposure in Adcy3$^{+/-}$ vs WT rats. (a) Adcy3$^{+/-}$ males (M) and females (F) gain more weight than wild-type (WT) rats during the study. (**b**) Adcy3$^{+/-}$ M and F have increased fat mass with no differences in lean mass relative to WT rats. **(c)** Adcy3$^{+/-}$ M and F have more retroperitoneal fat (RetroFat), gonadal fat, and omental fat (OmenFat) than WT rats at necropsy. **(d)** Adcy3$^{+/-}$ M have larger pancreas at necropsy than wild-type (WT) M with no significant differences in the necropsy weights of any other non-adipose tissues. **(e)** During a 48-hour fast, Adcy3$^{+/-}$ M and F have less serum FFA than WT rats as shown by a significant genotype by time interaction in the two-way ANOVA. **(f)** During an acute 5-hour cold

exposure at 4°C, there were no significant differences in body temperature between Adcy3$^{+/-}$ M and WT M. Adcy3$^{+/-}$ F maintain a significantly lower body temperature during acute cold exposure than WT F. Mean ± SEM. T-test or rmANOVA, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

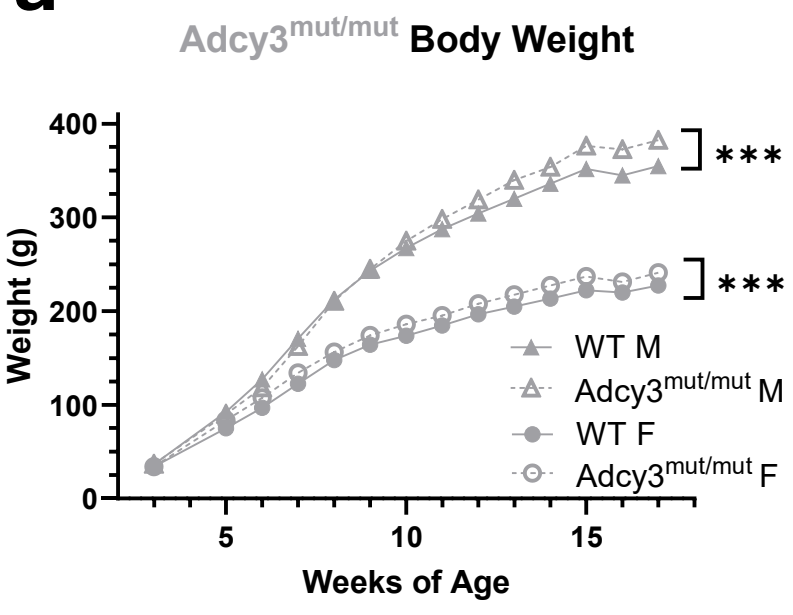
Figure S4. Gene expression changes in retroperitoneal fat (RetroFat), subcutaneous white adipose tissue (SubQ), and brown adipose tissue (BAT) in Adcy3$^{+/-}$ vs WT rats. (a) After a prolonged 48-hour cold exposure at 4°C, Adcy3$^{+/-}$ males (M) and females (F) generally have lower lipase gene expression (*Lipase E (Lipe)* and *Patatin-like phospholipase domain containing 2* (*Pnpla2))* in retroperitoneal adipose (RetroFat) and subcutaneous adipose (SubQ) than wild-type (WT) rats, as shown by both three-way ANOVA (results on legend) and stratified analyses (results in graph). There are no genotypic differences in general lipase gene expression in brown adipose tissue (BAT) after prolonged cold exposure, except that Adcy3$^{+/-}$ M express less *Pnpla2* than WT rats. **(b)** After a prolonged 48-hour cold exposure at 4°C, there were no significant differences in *Uncoupling protein 1* (*Ucp1)* expression in Adcy3$^{+/-}$ M or F in BAT or SubQ. **(c)** After standard necropsy (no cold exposure), there were no significant differences in *Peroxisome proliferator-activated receptor gamma* (*Pparg)* expression in RetroFat between genotypes in males, while Adcy3$^{+/-}$ F express less *Pparg* than WT F. There were also no significant differences in *CCAAT/enhancer-binding protein alpha* (*Cebpa)* expression in RetroFat between genotypes in either sex. Mean ± SEM. T-test, *p<0.05, ***p<0.001

Table S1. Primer sequences used in rt-qPCR.

Primer	Forward Sequence	Reverse Sequence
β-actin	TGAGGTAGTCTGTCAGGTCCCG	ACCACTGGCATTGTGATGGACT
Ucp1	GCAGAACACAGGTCTGGATG	ACGATCGTGCCTCTTCACTG
Lipe	AACTCACCCTGACCGTGAAC	CTTTGTTCGCCTGTGGGTAT
Pnpla2	GCACACAACGTTCTTCCTCA	GGGTGAACTCCAGGTACCAA
Pparg	AGGGCTGATCAACTTCTCCA	GCAGGGGAATGTCTGTGTCT
Cebpa	ATTCTGGTGTCGCCTGAAAG	CCCCCTACTCGGTAGGAAAA

Figure S1

a



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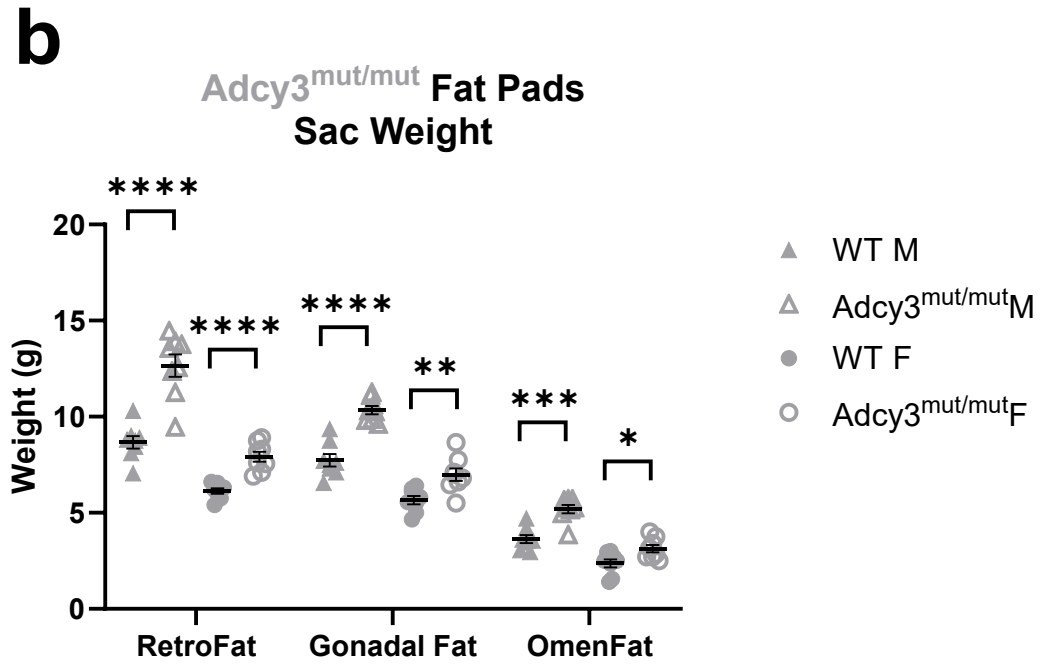

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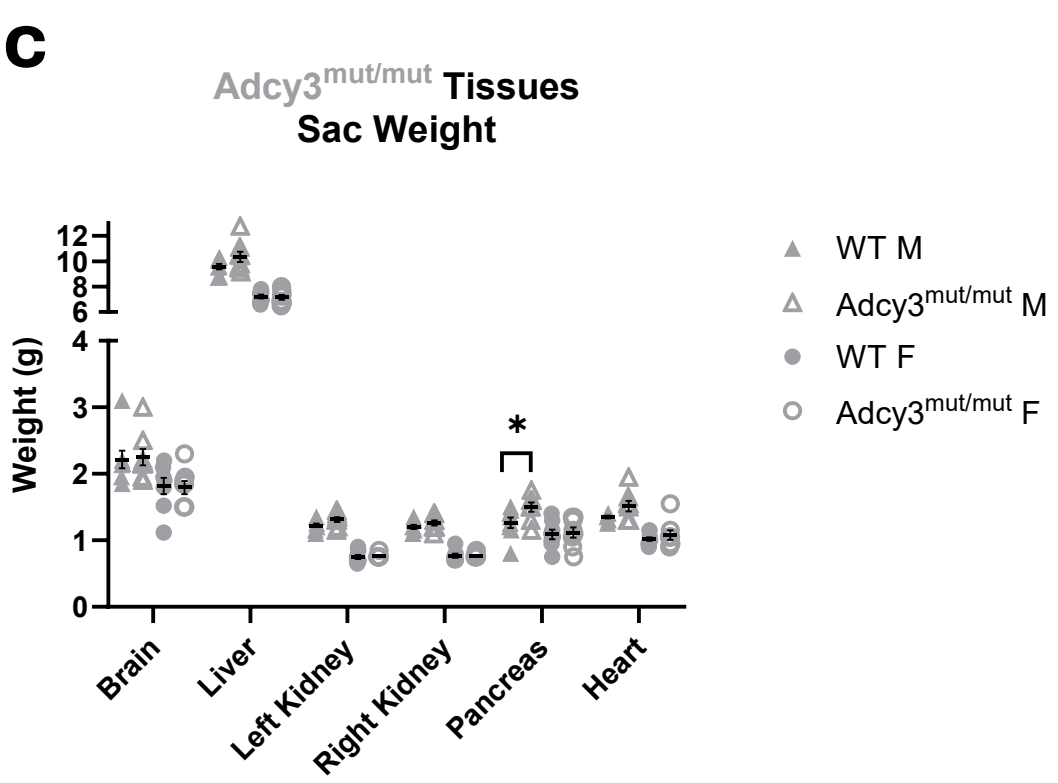

Figure S2

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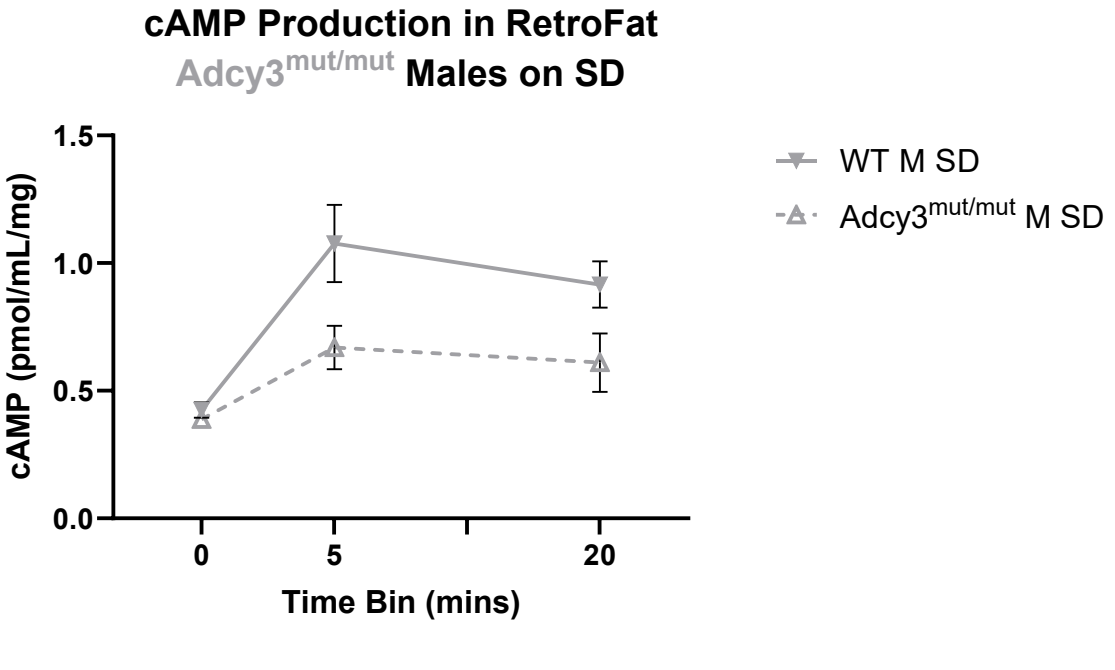
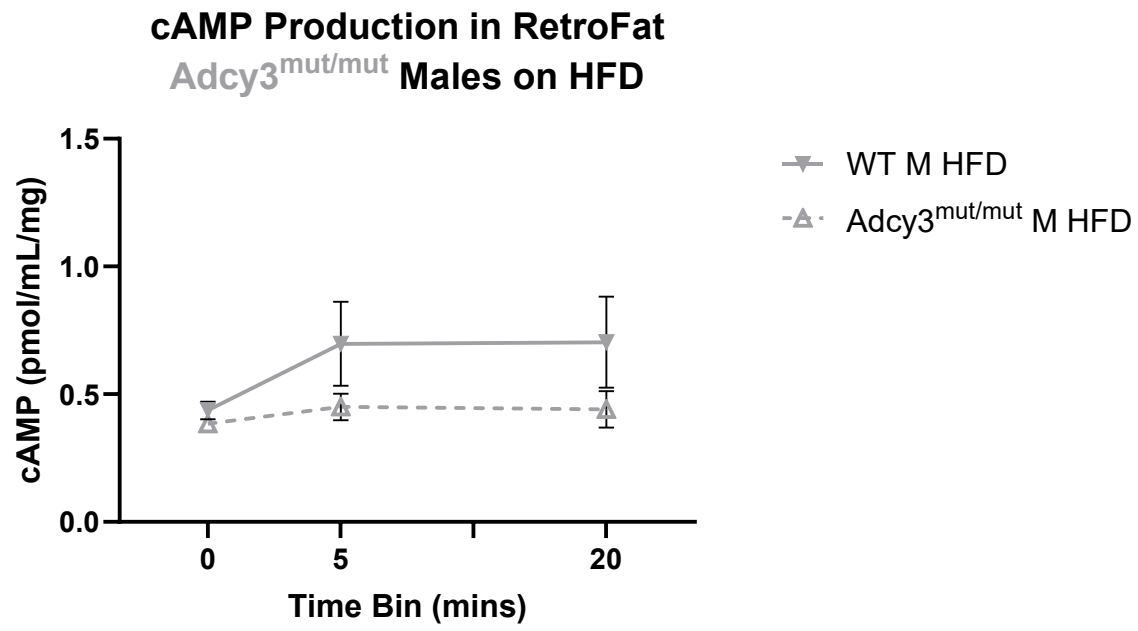
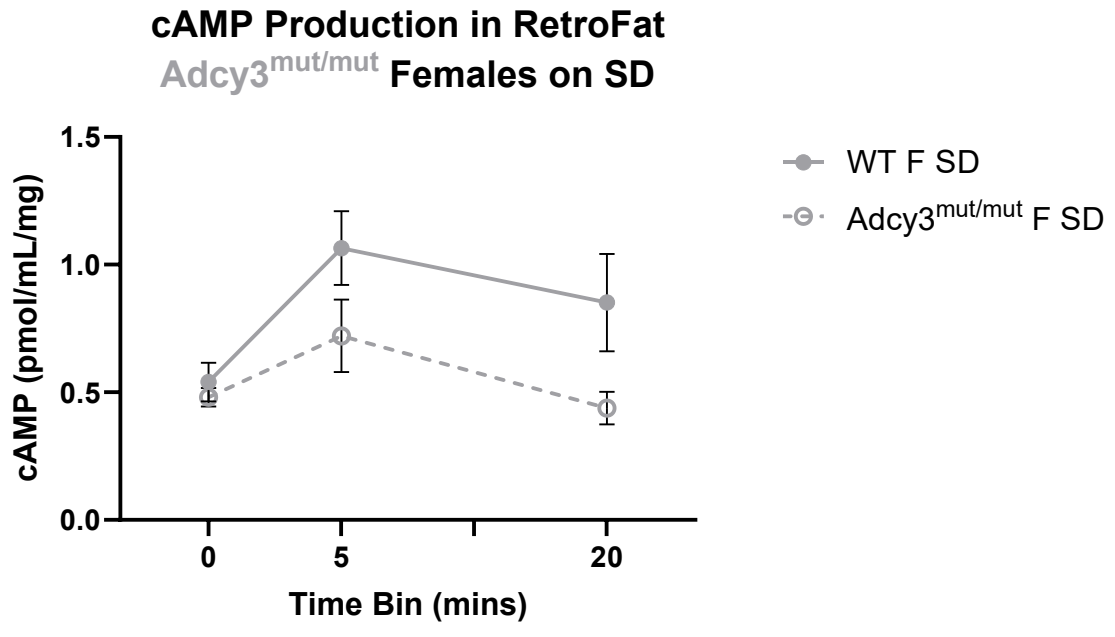

cAMP Production in RetroFat
Adcy3mut/mut Males on SD
cAMP (pmol/mL/mg)
1.5
1.0
0.5
0.0
0
5
20
Time Bin (mins)
WT M SD
Adcy3mut/mut M SD


cAMP Production in RetroFat
Adcy3mut/mut Males on HFD
cAMP (pmol/mL/mg)
1.5
1.0
0.5
0.0
0
5
20
Time Bin (mins)
WT M HFD
Adcy3mut/mut M HFD

b


cAMP Production in RetroFat
Adcy3mut/mut Females on SD
cAMP (pmol/mL/mg)
1.5
1.0
0.5
0.0
0
5
20
Time Bin (mins)
WT F SD
Adcy3mut/mut F SD

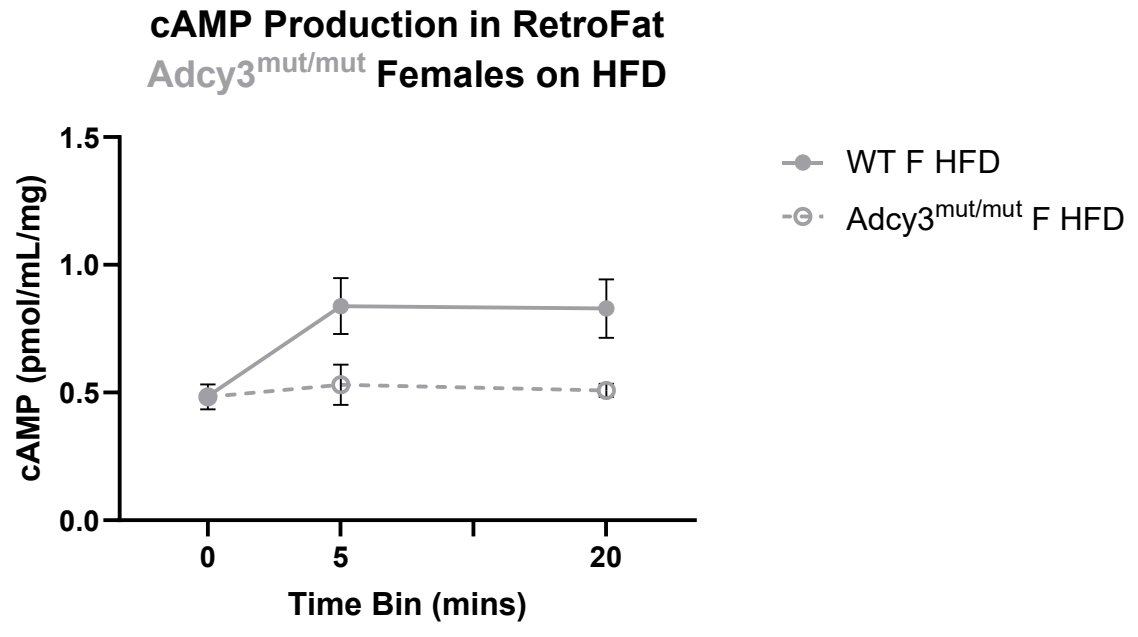
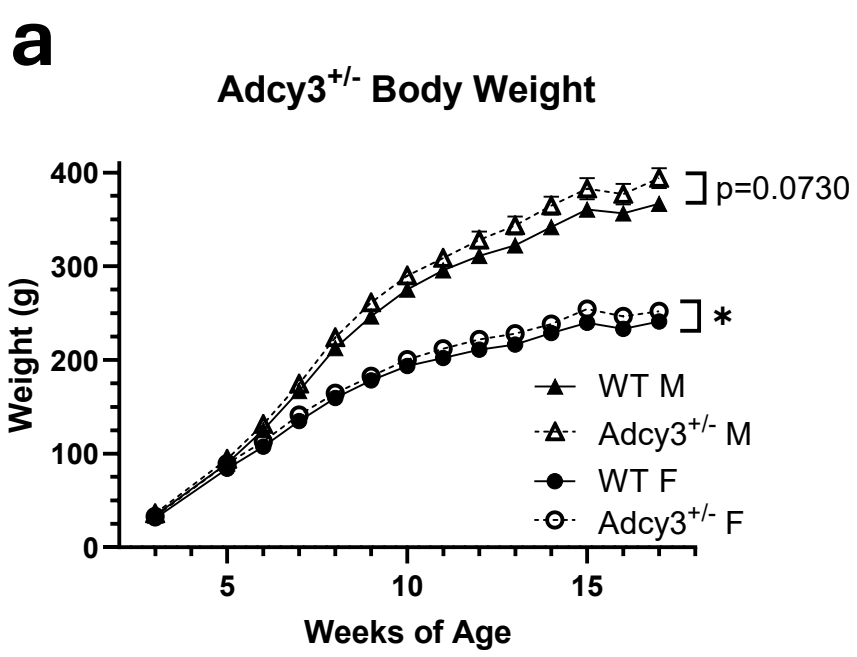
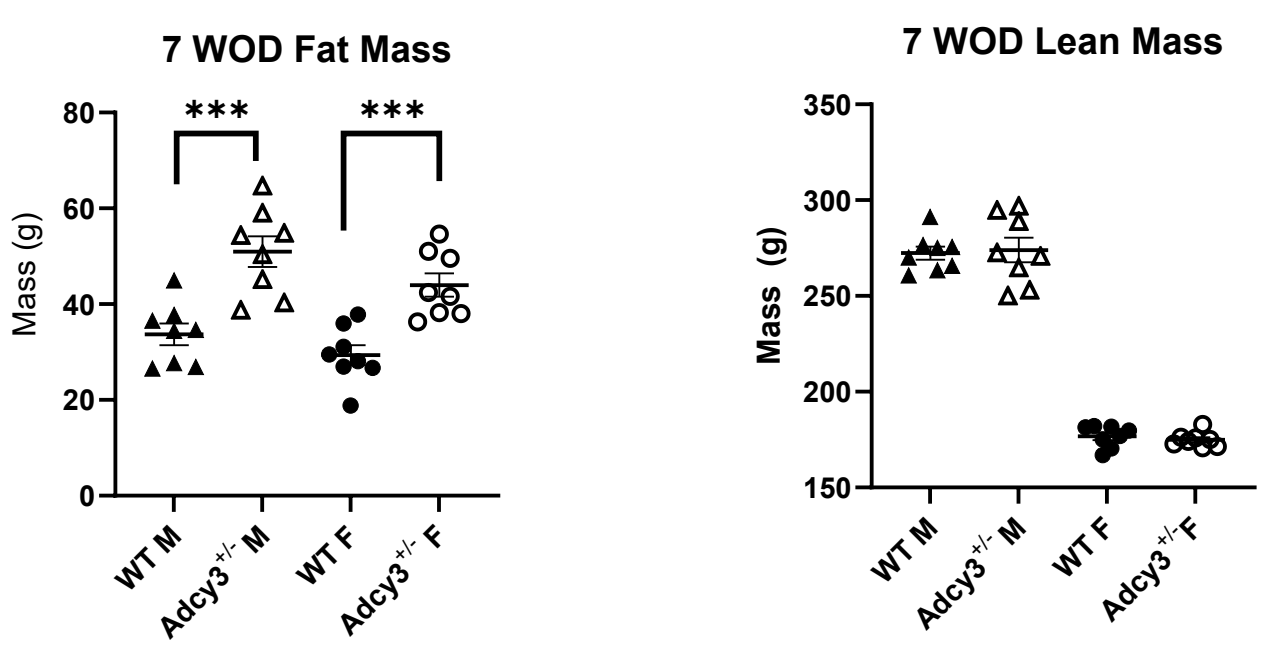
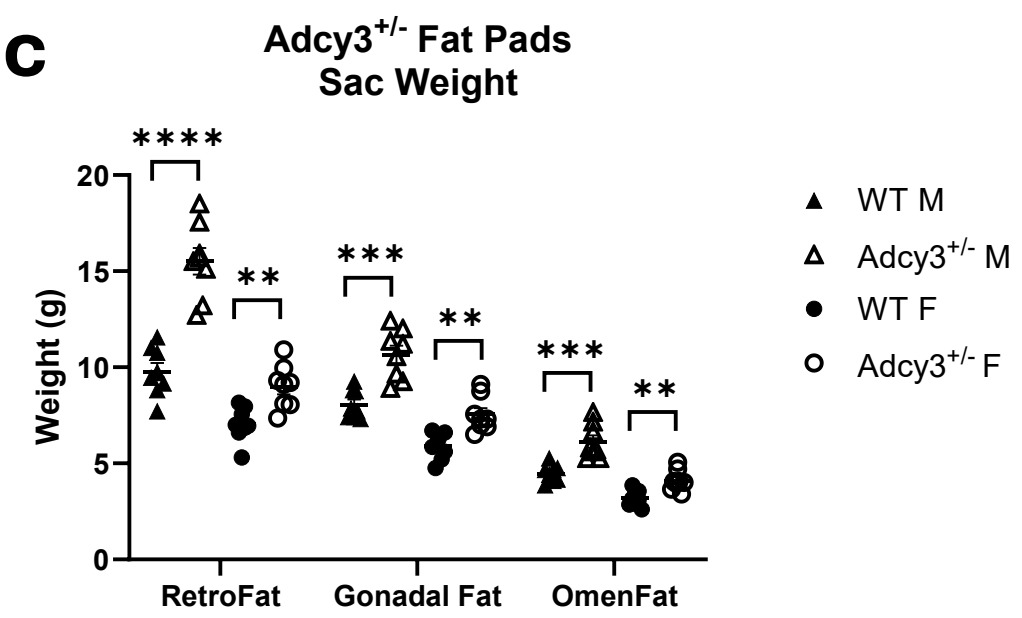
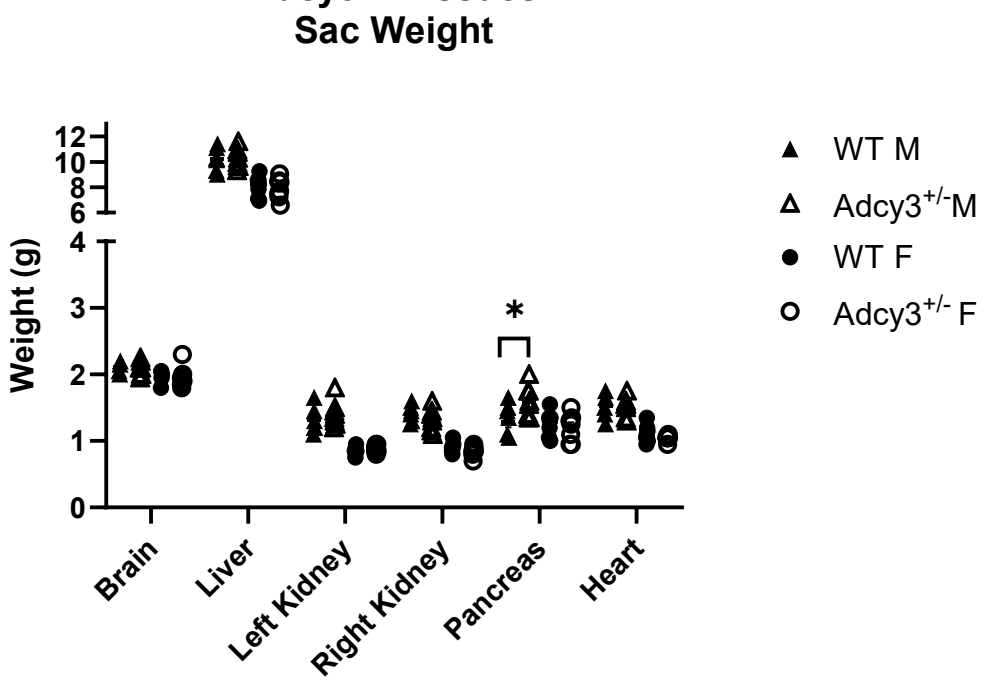

cAMP Production in RetroFat
Adcy3mut/mut Females on HFD
cAMP (pmol/mL/mg)
1.5
1.0
0.5
0.0
0
5
20
Time Bin (mins)
WT F HFD
Adcy3mut/mut F HFD

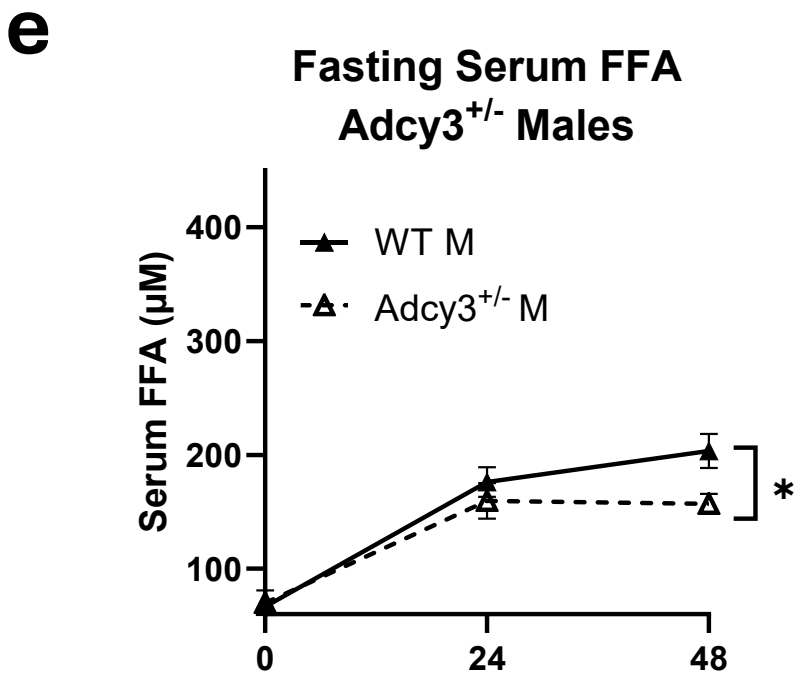
Figure S3

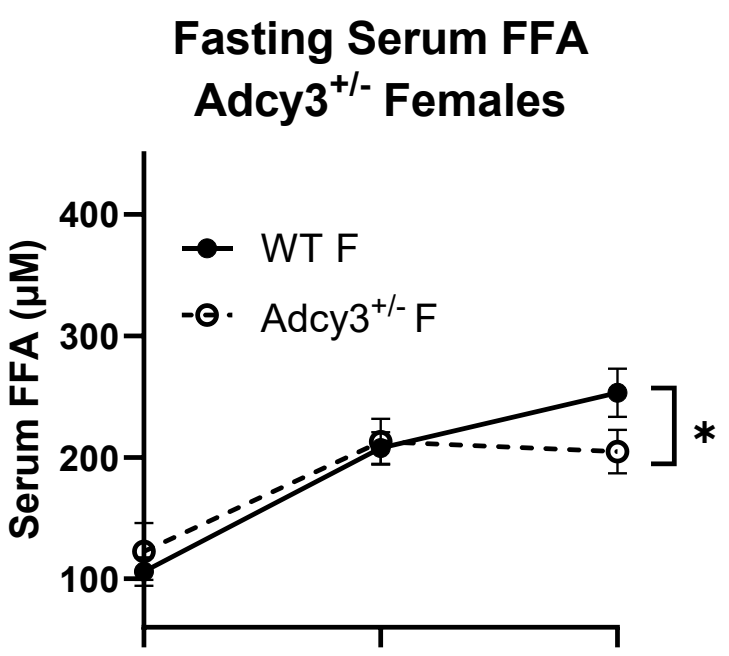

a
Adcy3+/- Body Weight
Weight (g)
Weeks of Age
p=0.0730
*
WT M
Adcy3+/- M
WT F
Adcy3+/- F

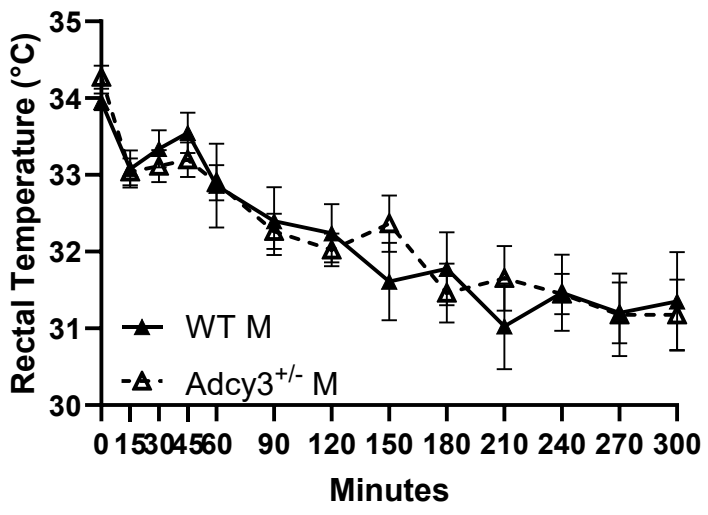

b
7 WOD Fat Mass
Mass (g)
***
***
WT M
Adcy3+/- M
WT F
Adcy3+/- F
7 WOD Lean Mass
Mass (g)


c
Adcy3+/- Fat Pads
Sac Weight
Weight (g)
RetroFat
Gonadal Fat
OmenFat
WT M
Adcy3+/- M
WT F
Adcy3+/- F


d
Adcy3+/- Tissues
Sac Weight
Weight (g)
*
Brain
Liver
Left Kidney
Right Kidney
Pancreas
Heart
WT M
Adcy3+/-M
WT F
Adcy3+/- F


e
Fasting Serum FFA
Adcy3+/- Males
Serum FFA (µM)
WT M
Adcy3+/- M
*
Hours


Fasting Serum FFA
Adcy3+/- Females
Serum FFA (µM)
WT F
Adcy3+/- F
*
Hours


f
Acute Cold Exposure
Adcy3+/- Males
Rectal Temperature (°C)
WT M
Adcy3+/- M
Minutes

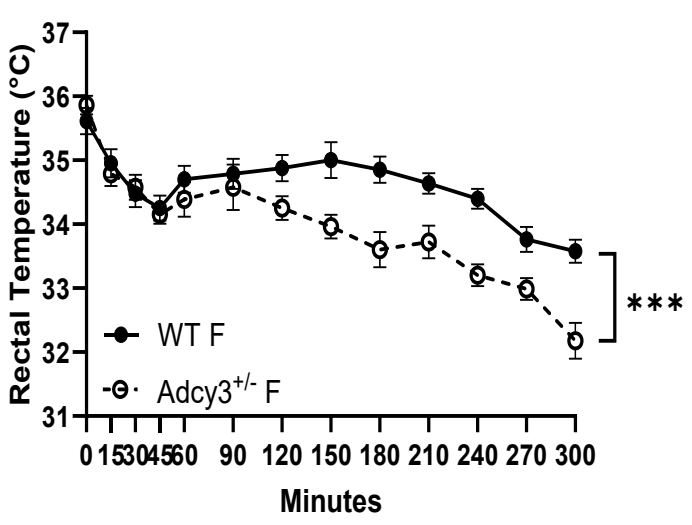
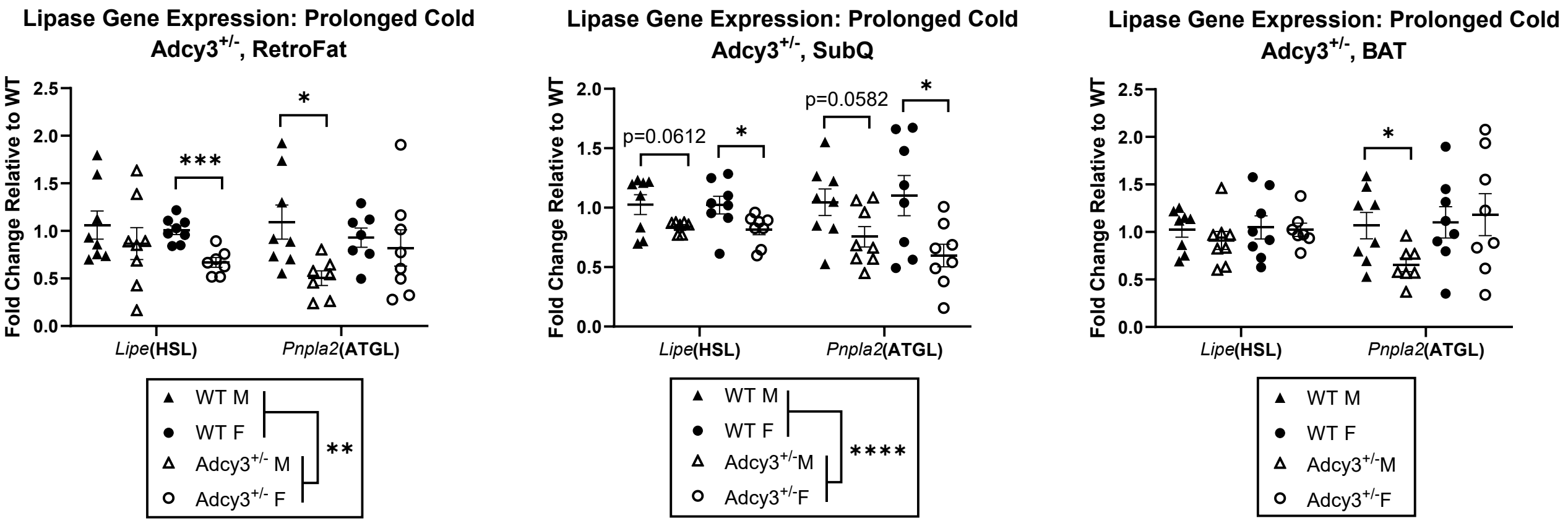

Acute Cold Exposure
Adcy3+/- Females
Rectal Temperature (°C)
WT F
Adcy3+/- F
***
Minutes

Figure S4

a



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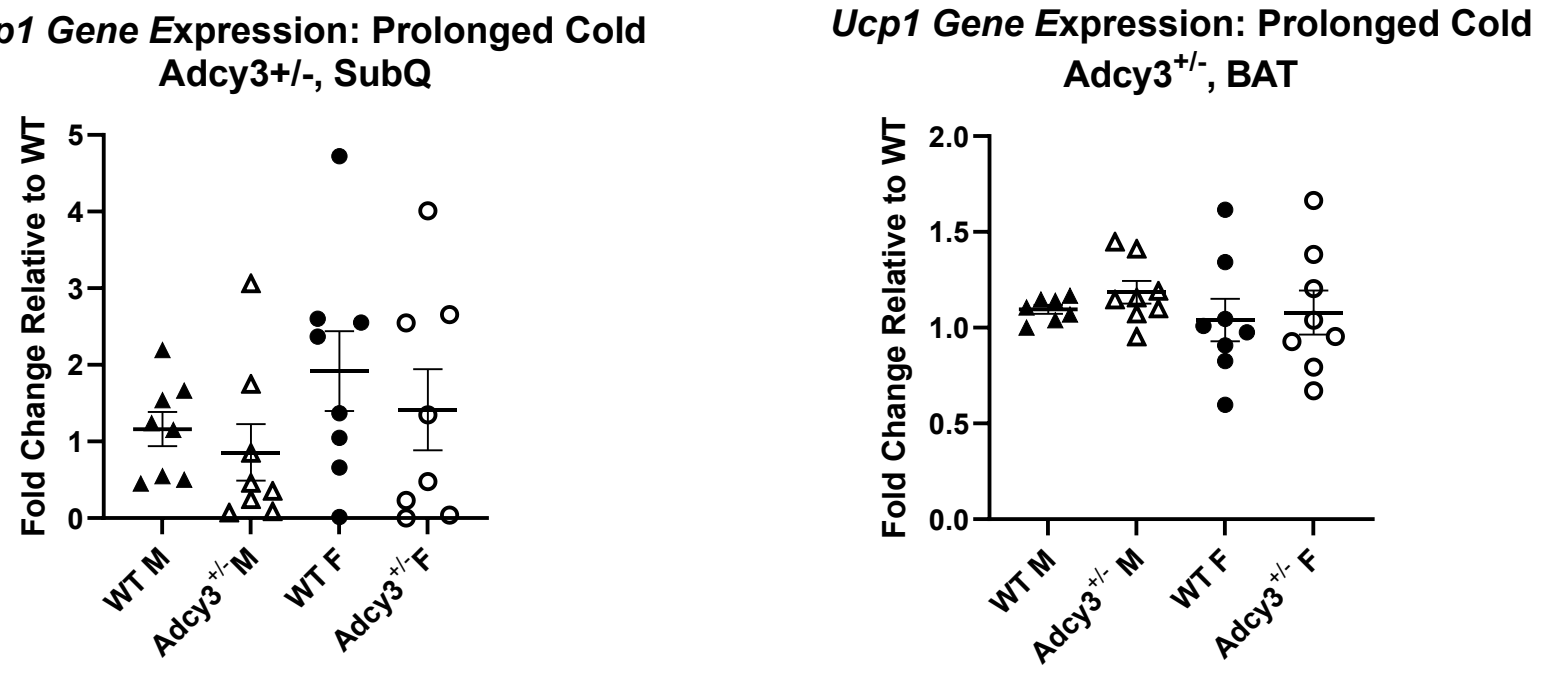

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