Identification of genes that link obesity and stress/emotional behaviors using outbred rats.

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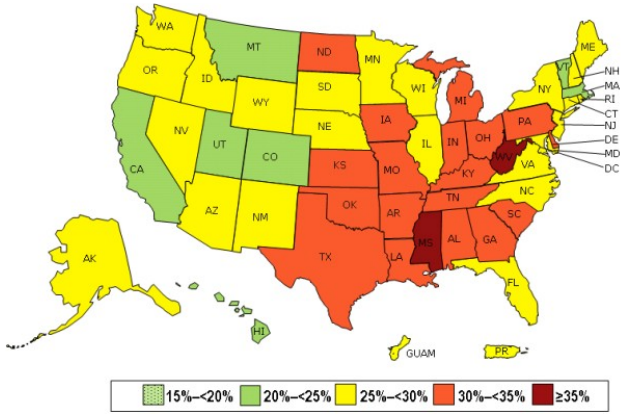
Department of Internal Medicine, Molecular Medicine

Increasing prevalence of obesity and mental health diseases over time

2013



2023

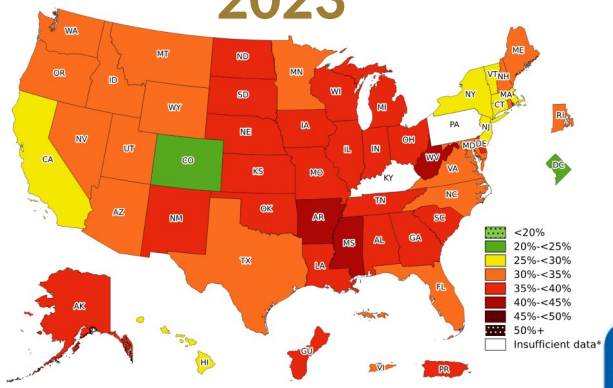
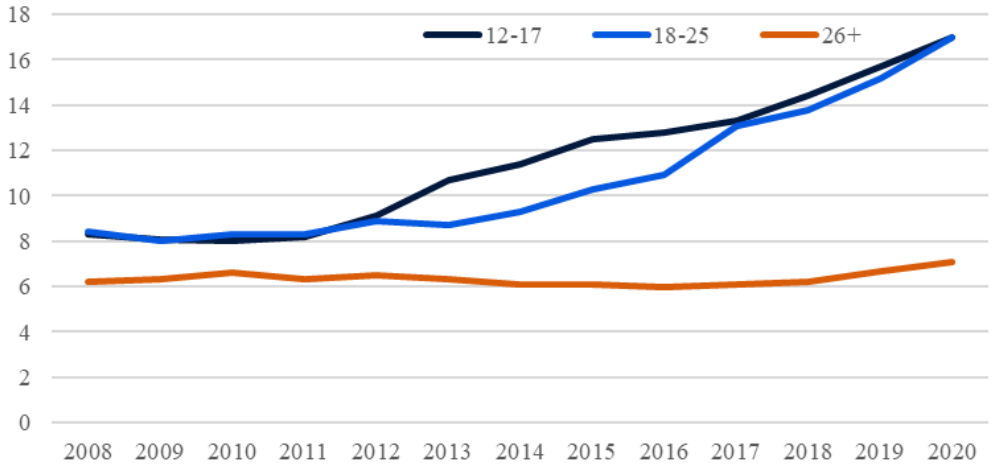
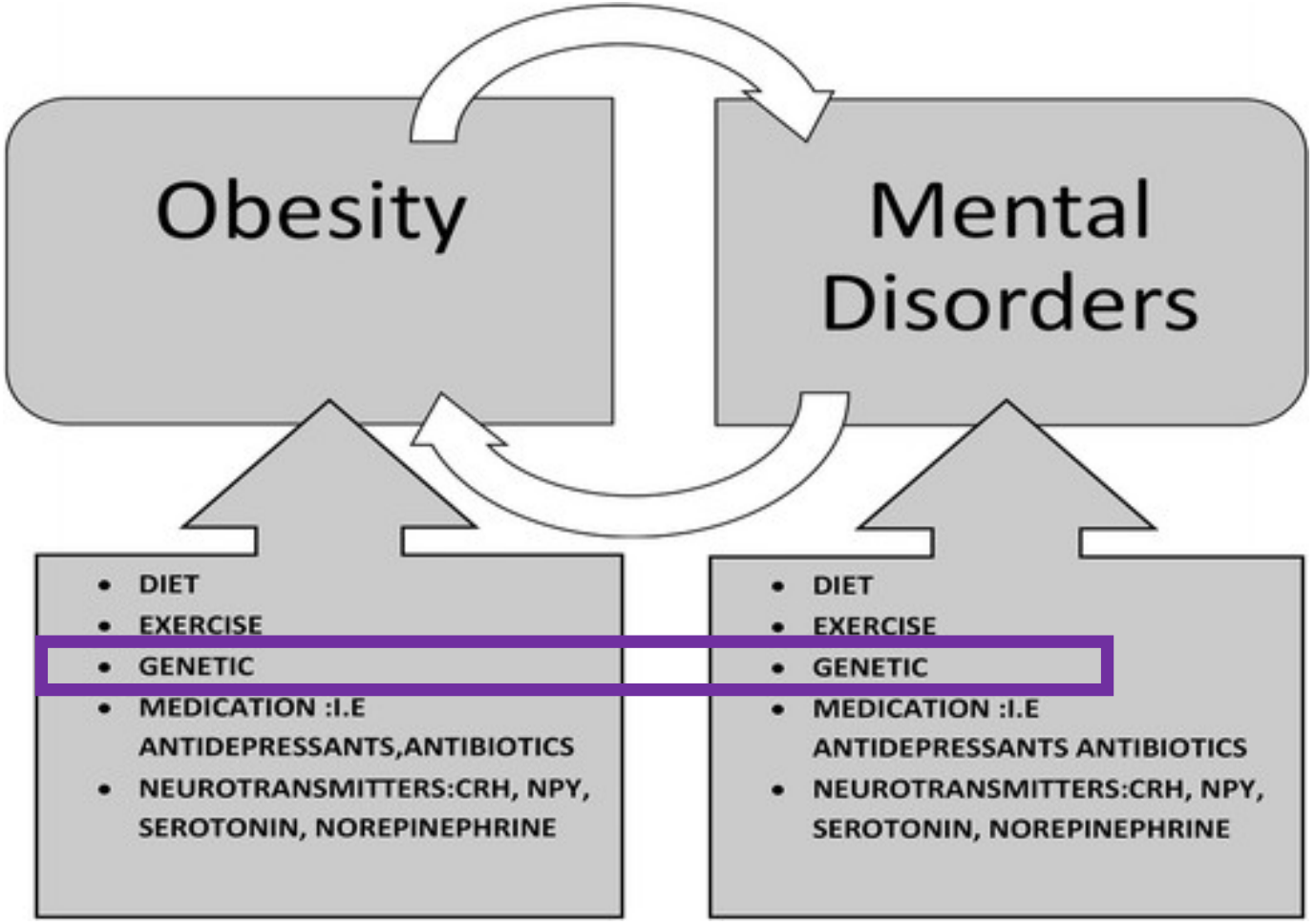


Figure 1. Percent of the population with a major depressive episode in the past year by age, 2008-2020

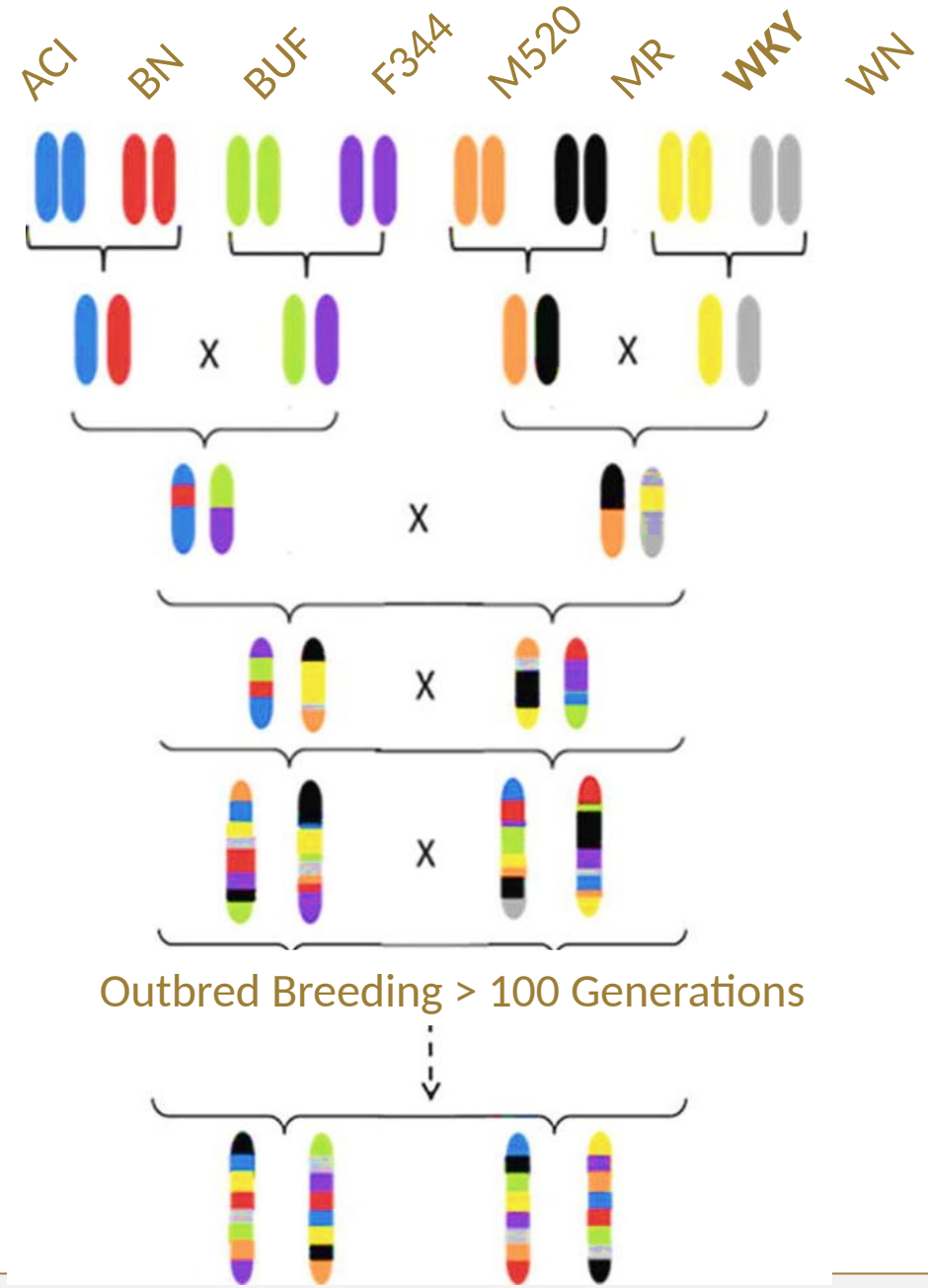


Source: Substance Abuse and Mental Healthh Services Administration

Bidirectional association between obesity and mental health disorders

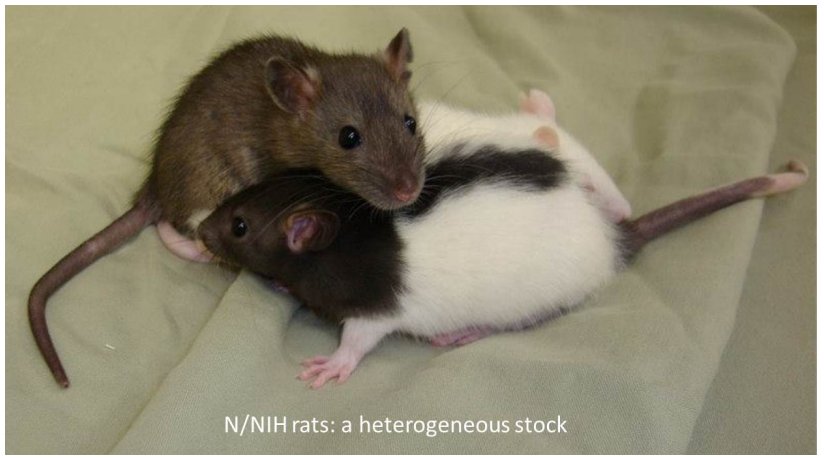


Avila et al., 2015



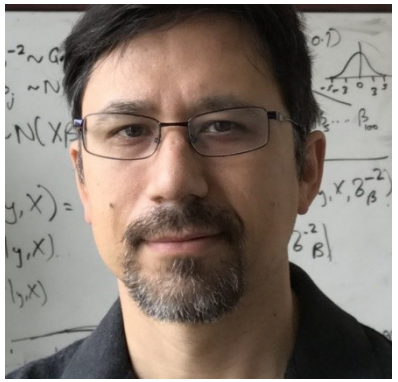
Heterogeneous Stock (HS) Rat

HS rat = outbred rat model
Genetic mapping of complex traits



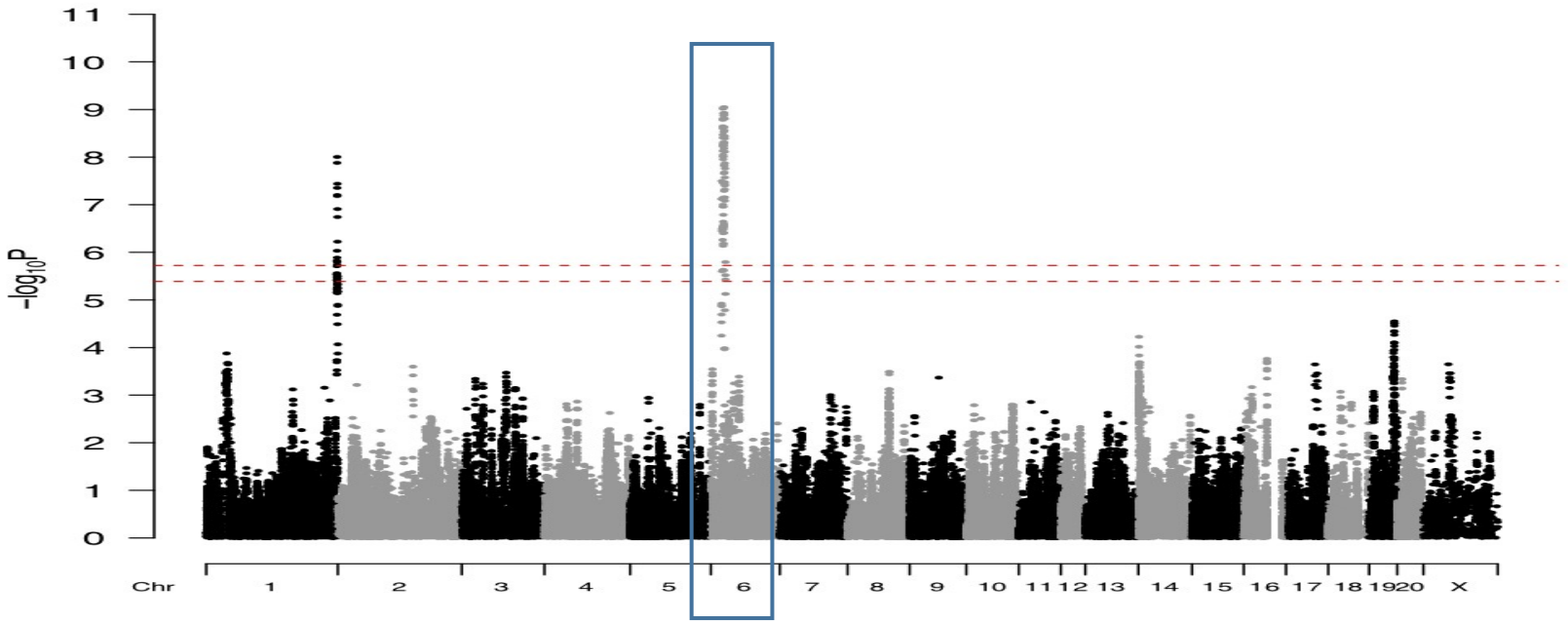
Solberg Woods and Palmer, 2019

QTL for visceral fat pad weight on rat chr. 6



William Valdar

Richard Mott



Keele et al., 2018; Chitre et al., 2020; Le et al; 2022

Two candidate genes identified

- ***Krtcap3*** –identified using mediation analysis
  - SNP alters *Krtcap3* expression to influence adiposity

- ***Adcy3*** -non-synonymous coding variant identified
  - Protein modeling supports a potentially damaging role of the variant

Two candidate genes identified

- **Krtcap3** –identified using mediation analysis
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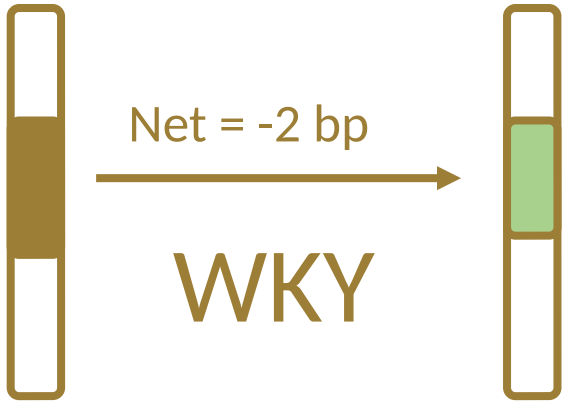
Creating a *Krtcap3*-KO

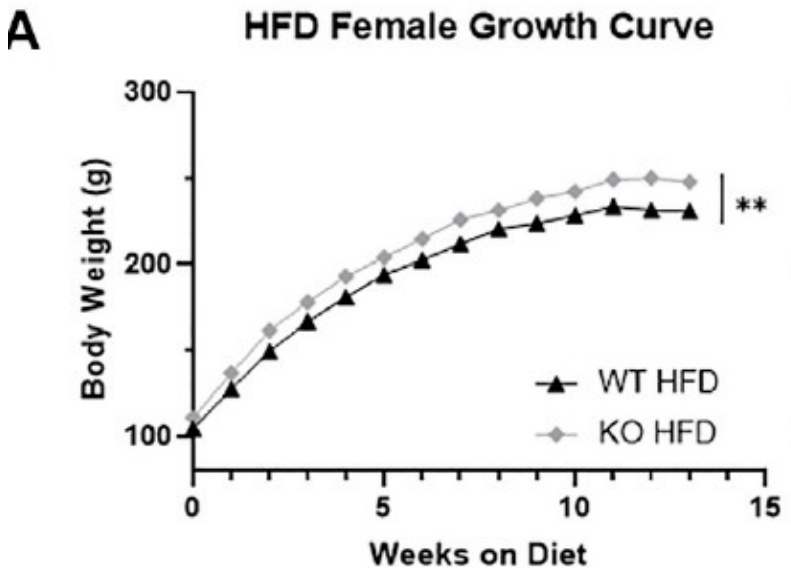
Aron Geurts

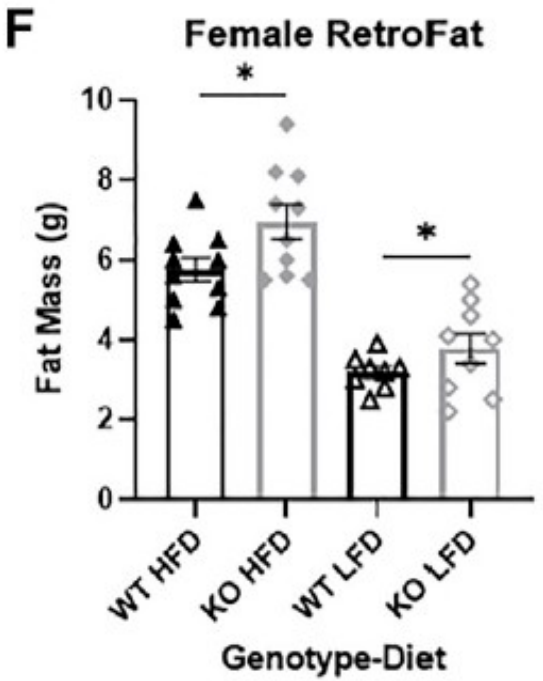


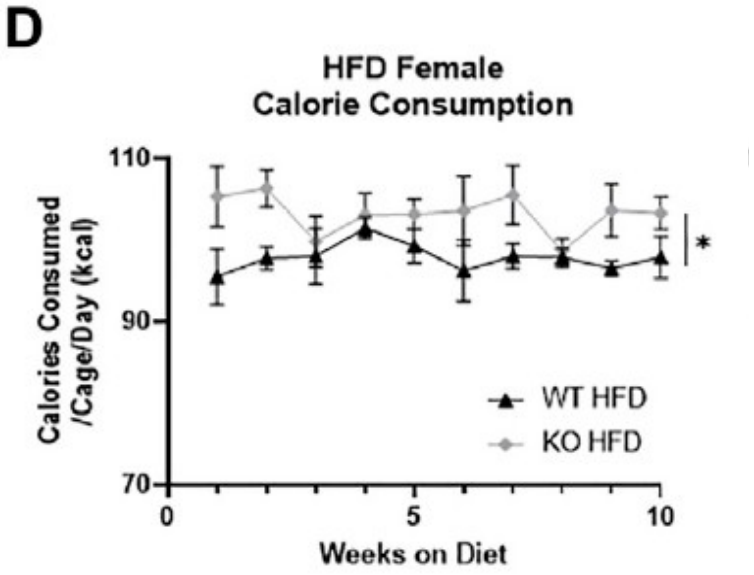
Lexie Szalanczy

***Krtcap3* expression** → **RetroFat**

Study 1: *Krtcap3* KO females on HFD have increased body weight, adiposity and food intake

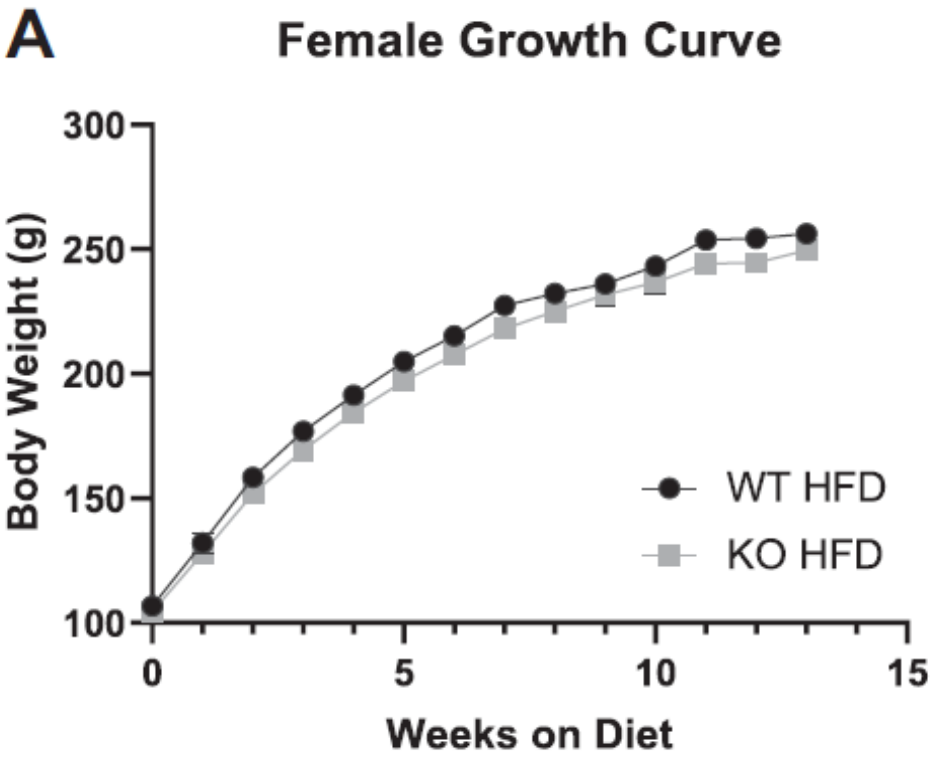


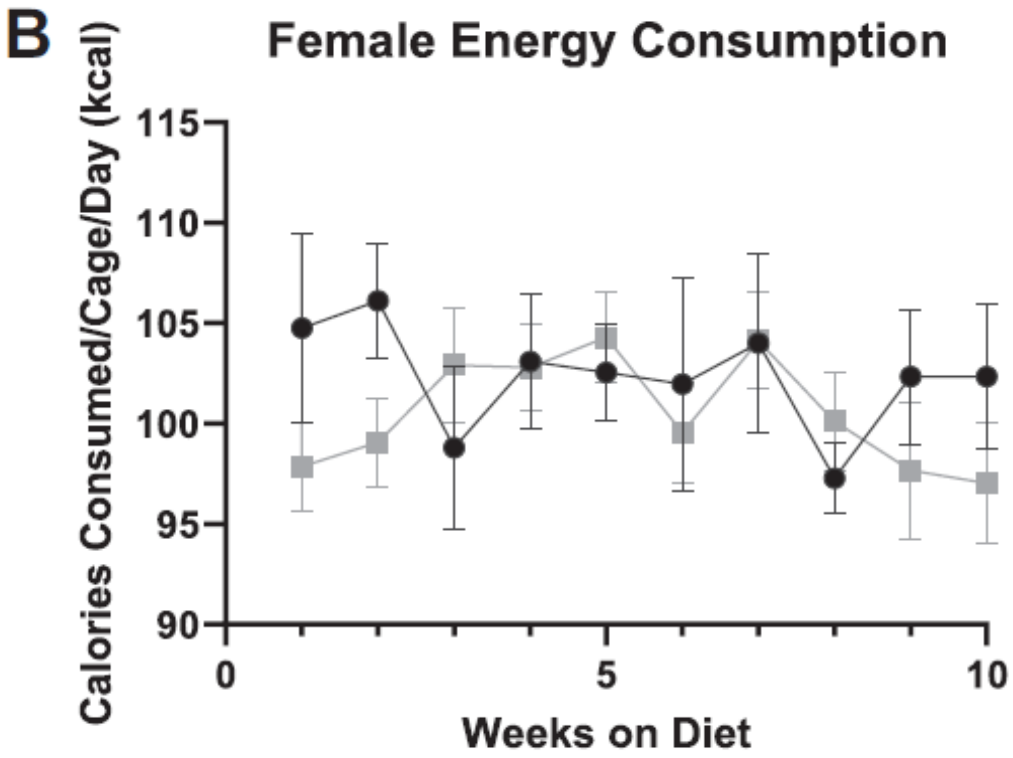




Szalanczy et al., 2022

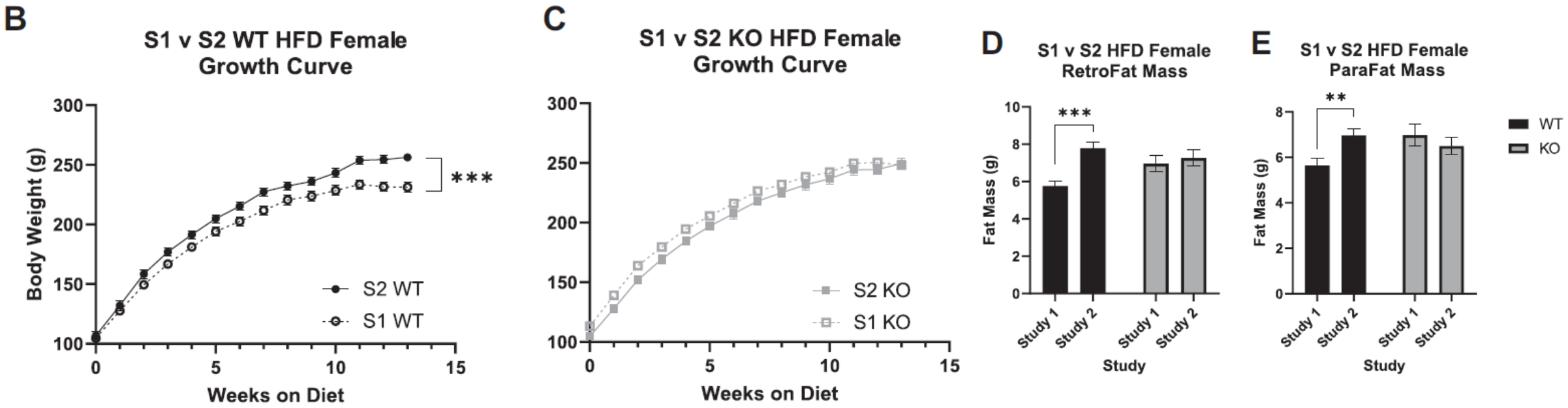
Study 2: Unable to replicate this finding in a second study





Szalanczy et al., 2023

Study 2: Lack of replication is because WT ate more and became fatter in Study 2 with no change in KO rats



Szalanczy et al., 2023

Study 1

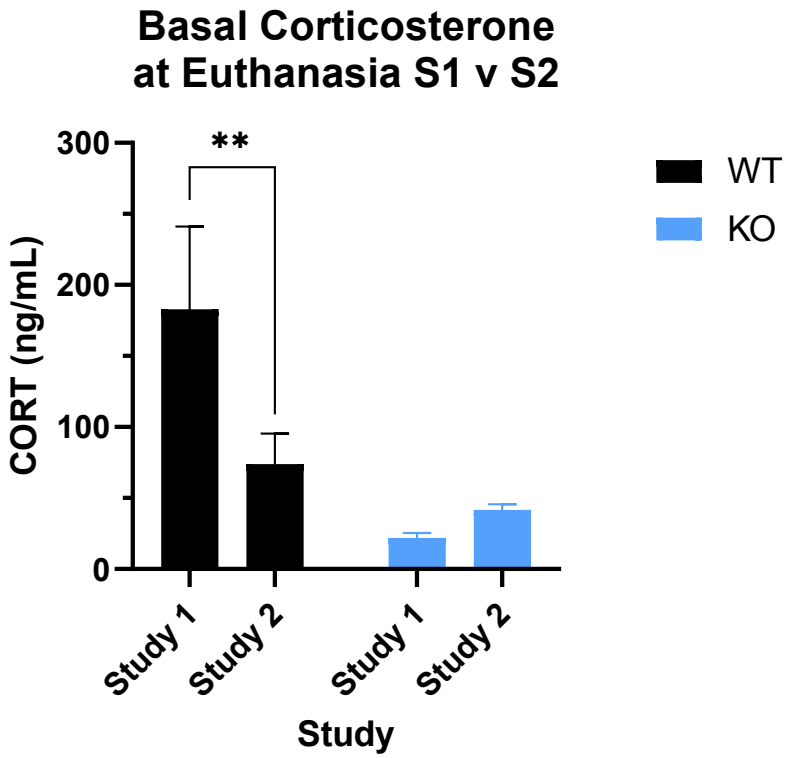
2019-2020

Study 2

2020-2021

WT and KO rats have different serum CORT responses

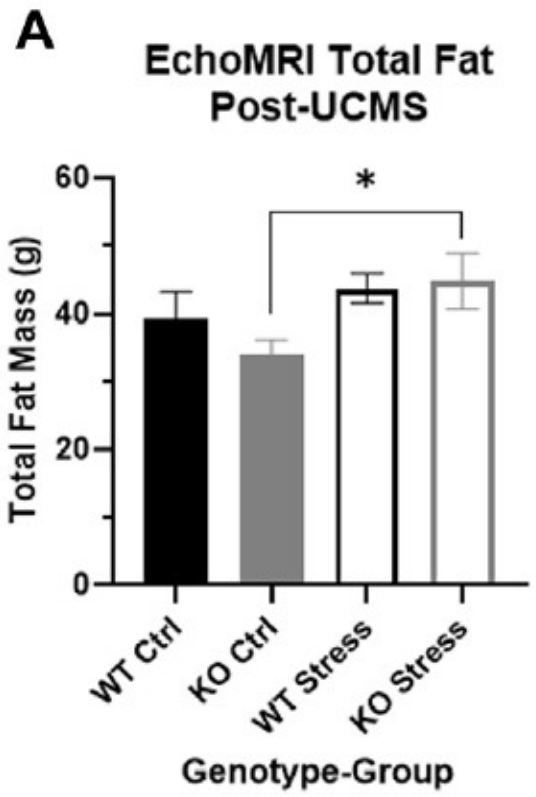
Eva Redei

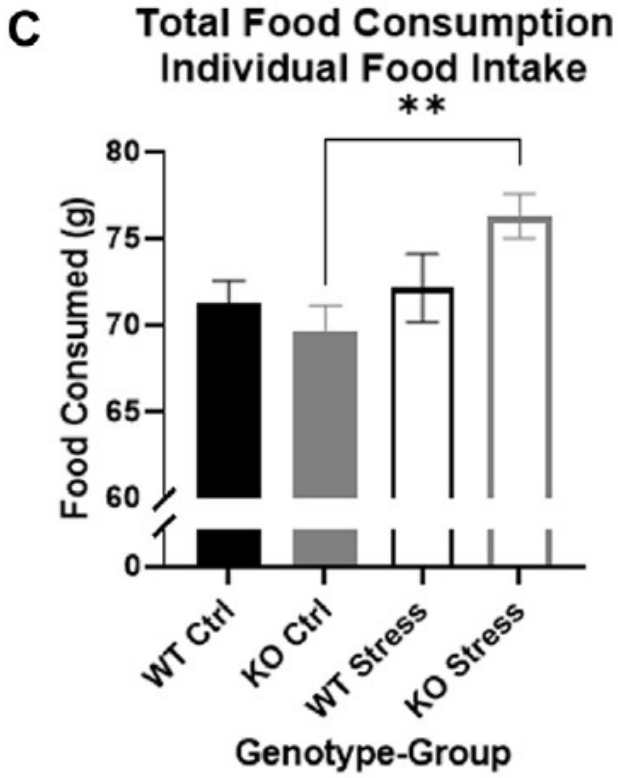


Szalanczy, 2023

n = 4-5

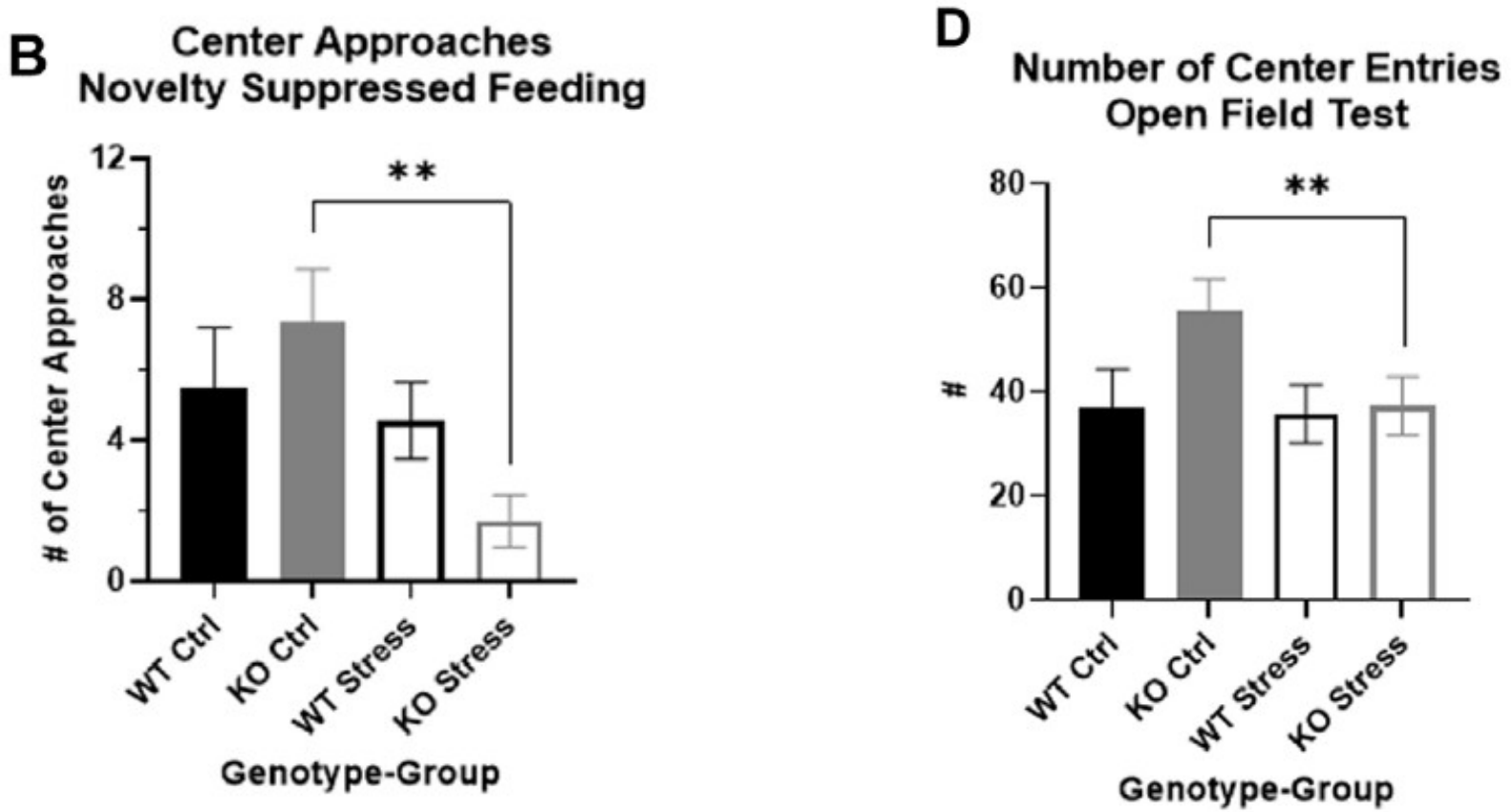
Study 3: Chronic Stress leads to increased food intake and adiposity in KO, but not WT rats





Szalanczy, 2024

Study 3: Chronic stress leads to anxiety-like behaviors in KO, not WT



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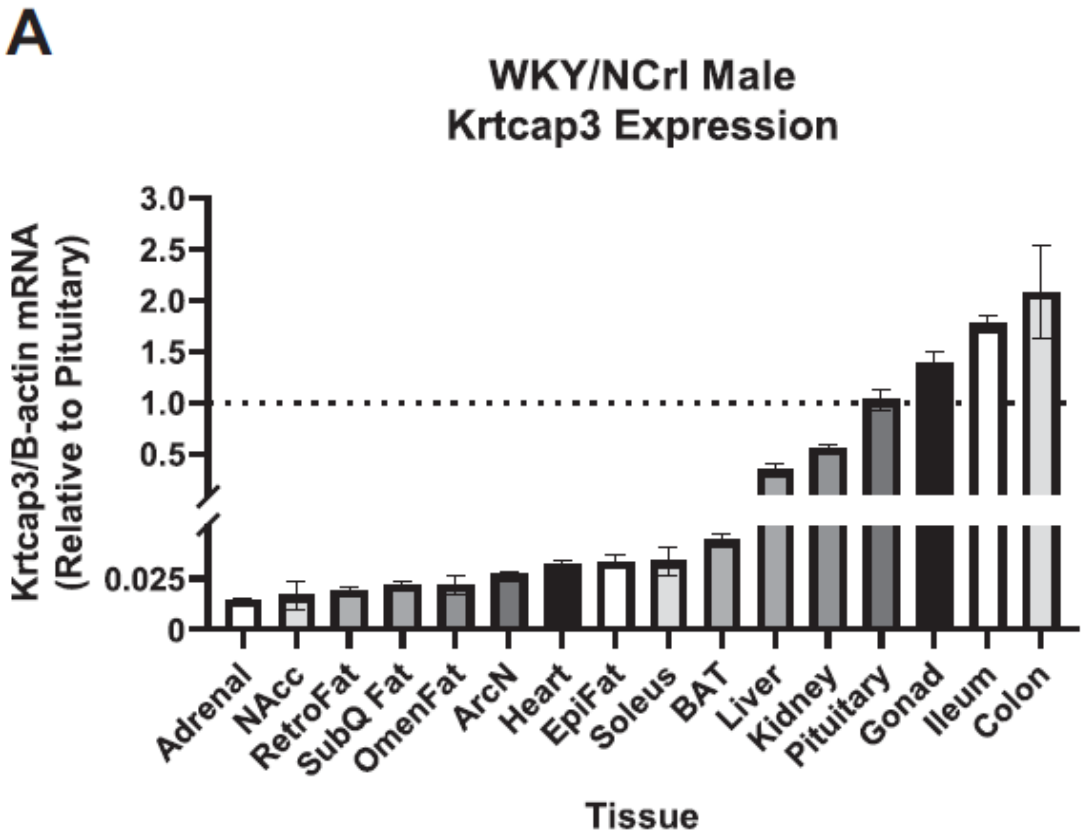
Krtcap3 is highly expressed in the pituitary and colon



Szalanczy, 2023

Krtcap3 Summary

- Studies are consistent with the hypothesis that low *Krtcap3* leads to increased adiposity under conditions of stress
- *Krtcap3* may be acting in the pituitary and colon to influence stress response and adiposity

Two candidate genes identified

- ***Krtcap3*** –identified using mediation analysis
  - SNP alters Krtcap3 expression to influence adiposity

- ***Adcy3*** -non-synonymous coding variant identified
  - WKY haplotype associates with decreased fat mass
  - L121P in WKY founder strain
  - Protein modeling supports a potentially damaging role of the variant

Jeremy Prokop

Creating an *Adcy3* mutant strain

DNA

```
Wild-type:          CGGTCTGGTGTTGGACATCATCCCTTTCGTGCTCTGCAAAAAGGGGC
Adcy3mut/mut:       CGGTCTGGTGTTGGACATCATCCCTT---TGCTCTGCAAAAAGGGGC
```

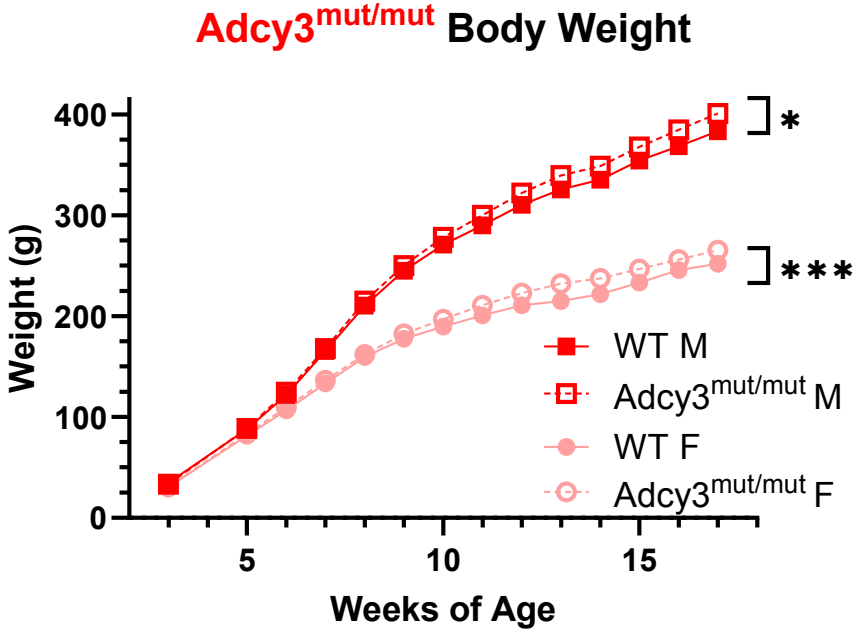
Protein

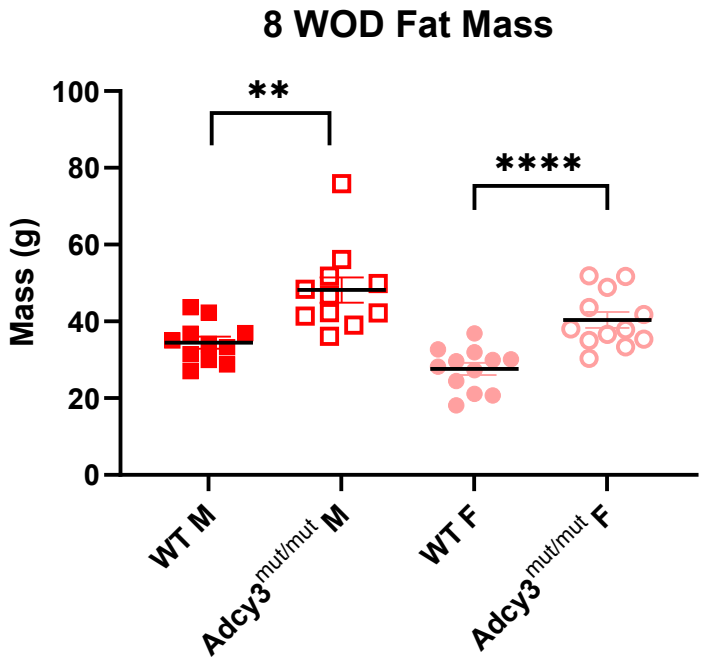
```
Wild-type:    (101)  SSDKLAPLMVAGVGLVLDIIPFVLCKKGLLPDRVSRKVVPYLLWLLITAQ  (150)
Adcy3mut/mut: (101)  SSDKLAPLMVAGVGLVLDIIP-LLCKKGLLPDRVSRKVVPYLLWLLITAQ  (150)
```

Adcy3$^{mut/mut}$ replaced the adjacent amino acids with a leucine.

Adcy3$^{mut/mut}$ → **RetroFat**

Adcy3$^{mut/mut}$ rats weigh more than WT rats due to increased fat mass



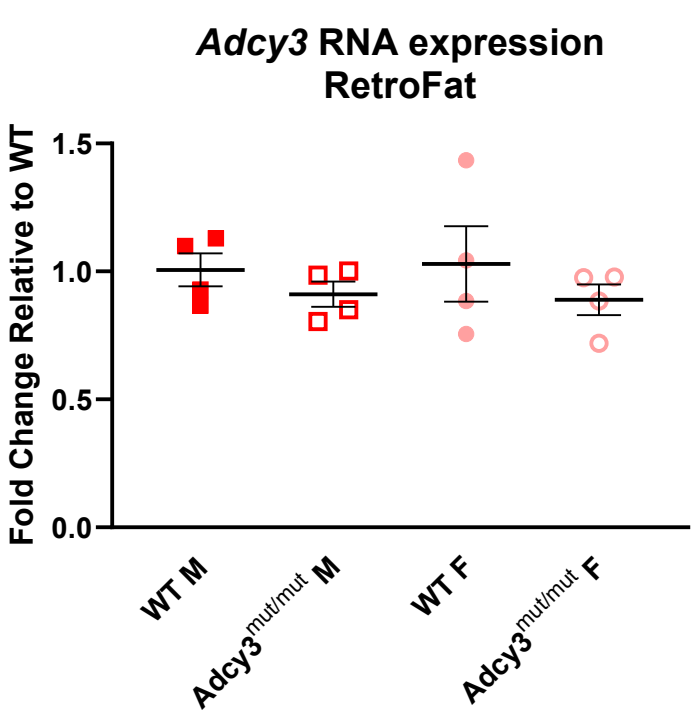


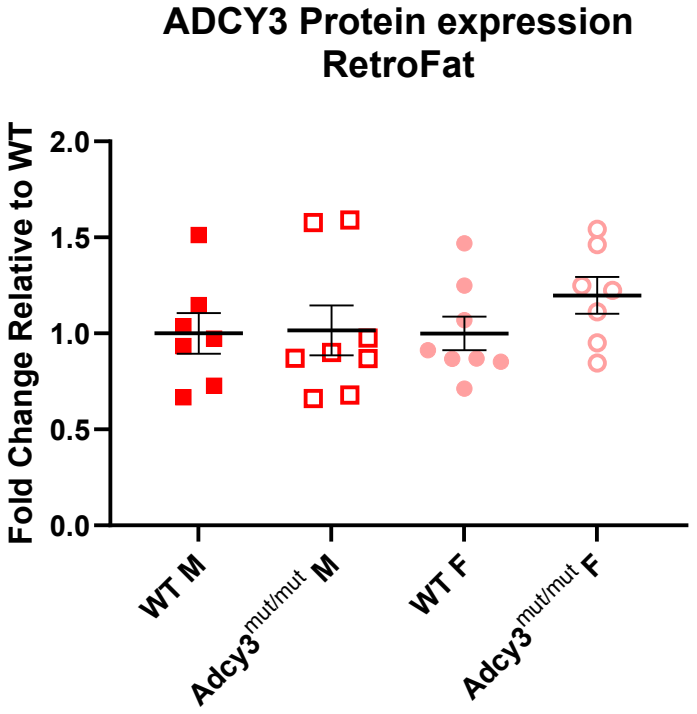
*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

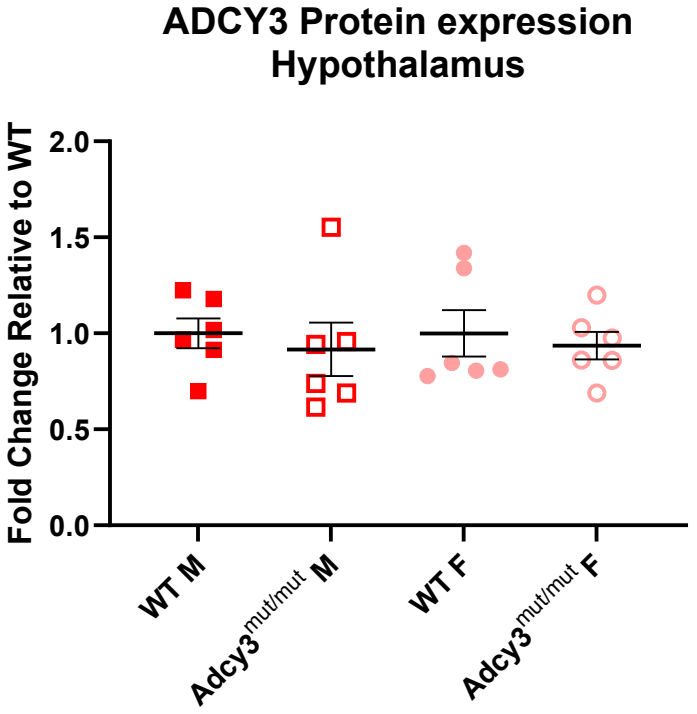
N=11-12

Fitzpatrick et al. 2024

Adcy3$^{mut/mut}$ does not alter *Adcy3* expression levels



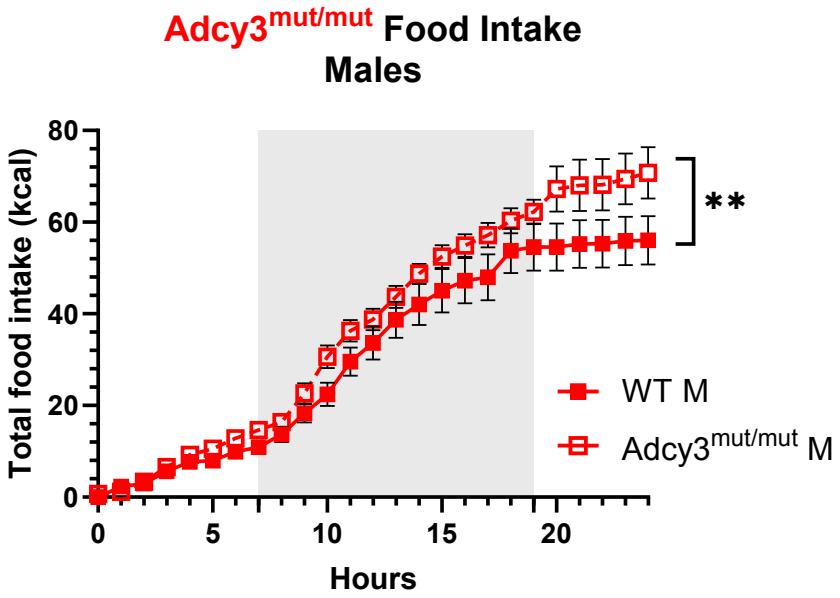


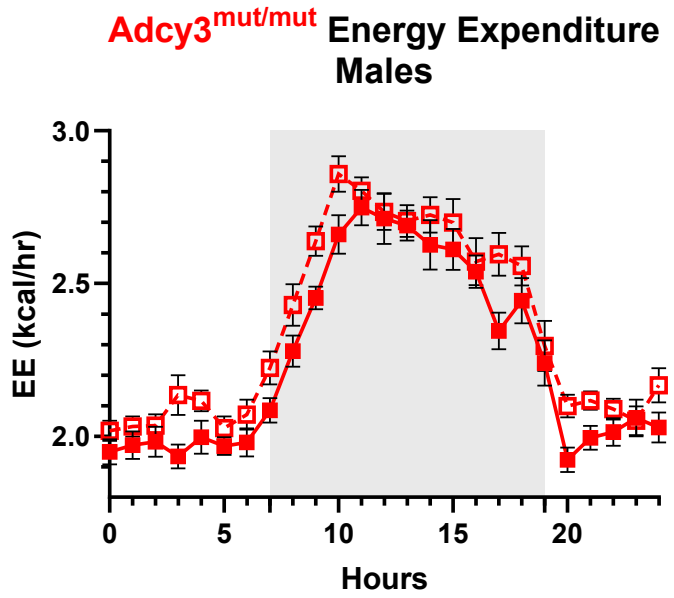


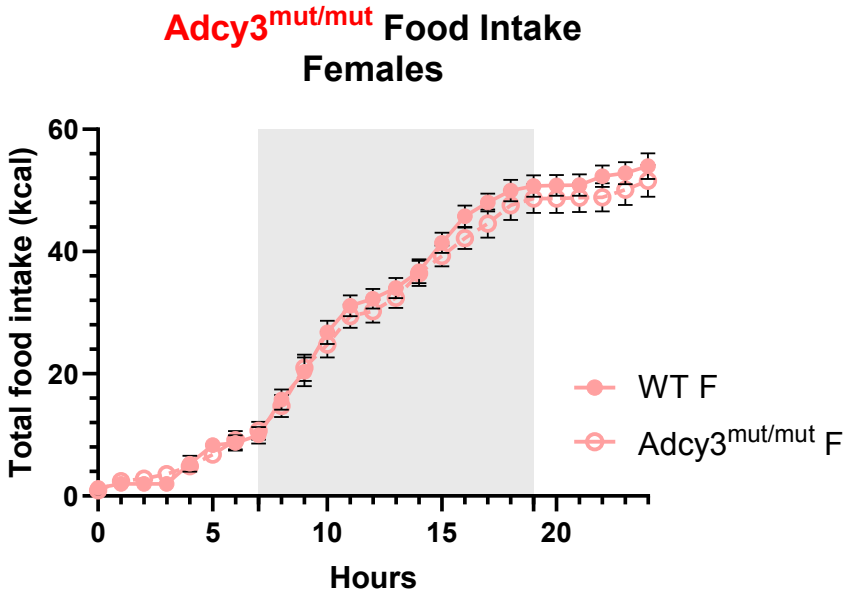
N=4-8

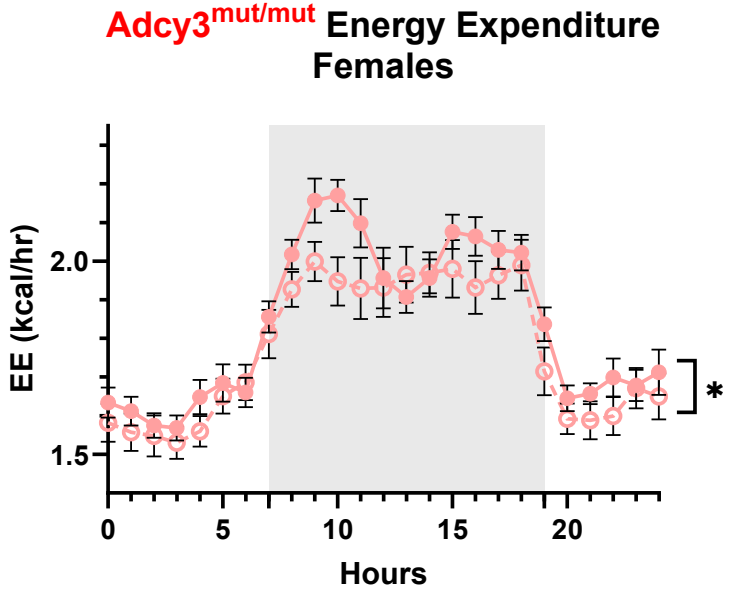
Fitzpatrick et al. 2024

Adcy3$^{mut/mut}$ males consume more food than WT, while females have decreased energy expenditure









CalR Version 1.3 (calrapp.org)
ANCOVA, **p<0.01. N=12-16

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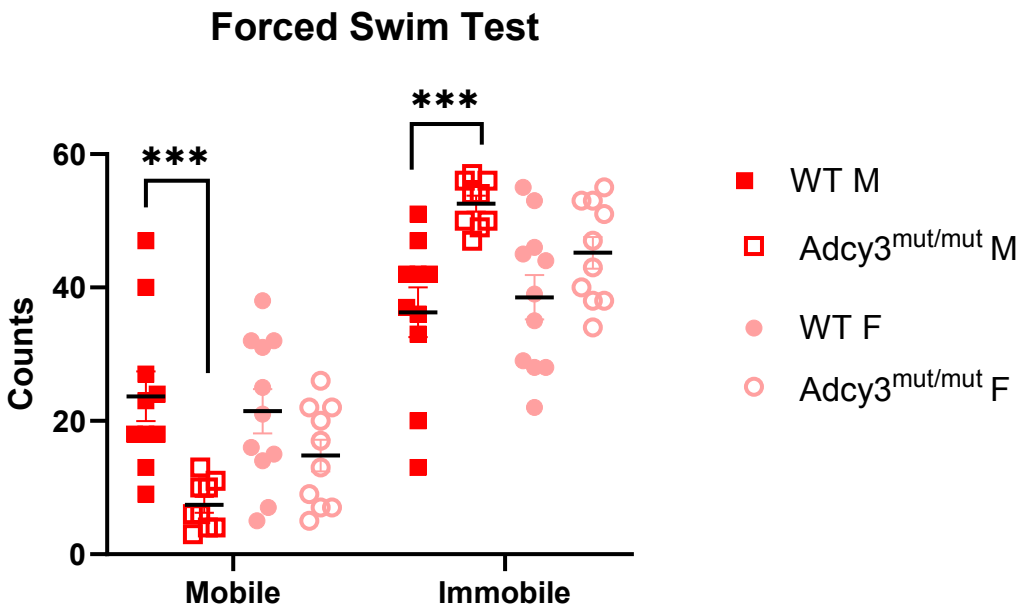
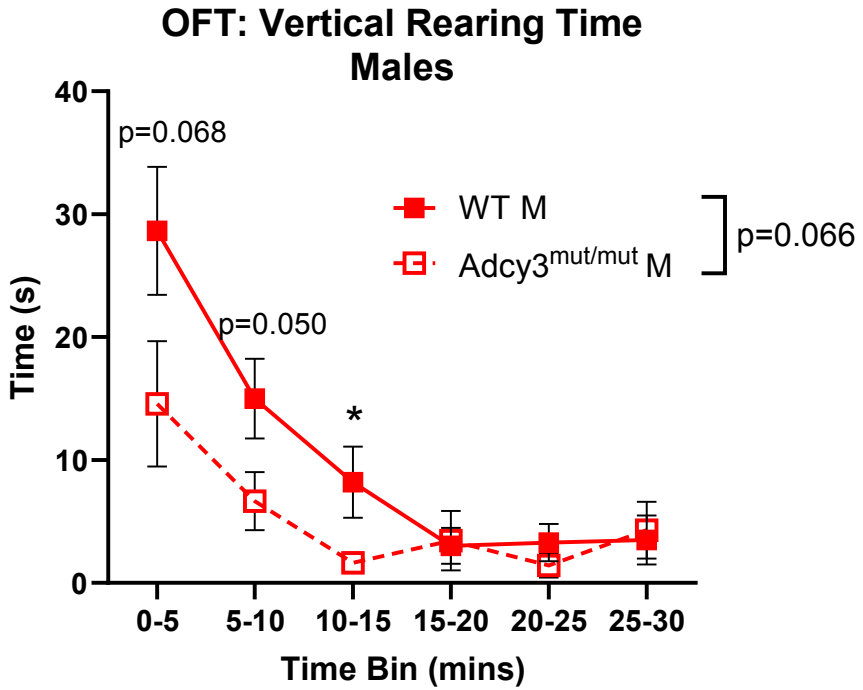
Adcy3$^{mut/mut}$ males show increased depression and anxiety-like behaviors





More despair-like = increased immobility

More anxiety-like = less time rearing

*p<0.05

Adcy3 Summary

- Mutation in the transmembrane domain of *Adcy3* leads to increased adiposity in both males and females, with increased eating and emotional behaviors only in males

Overall Summary

- **Causal genes for adiposity in HS rats also play a role in stress/emotional behaviors**
  - Low *Krtcap3* expression increases adiposity under conditions of chronic stress in females
  - *Adcy3* transmembrane mutation increases adiposity in both males and females, with changes in emotional behaviors only in males

Acknowledgments

Solberg Woods Lab

Dr. Lexie Szalanczy

Mackenzie Fitzpatrick

Christina Scott

Anusha Vora

Nataley Der

Ternya Gibson

Angie Beeson

Ozzie Seshie

Trangdai Bui

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Dr. Greg Keele

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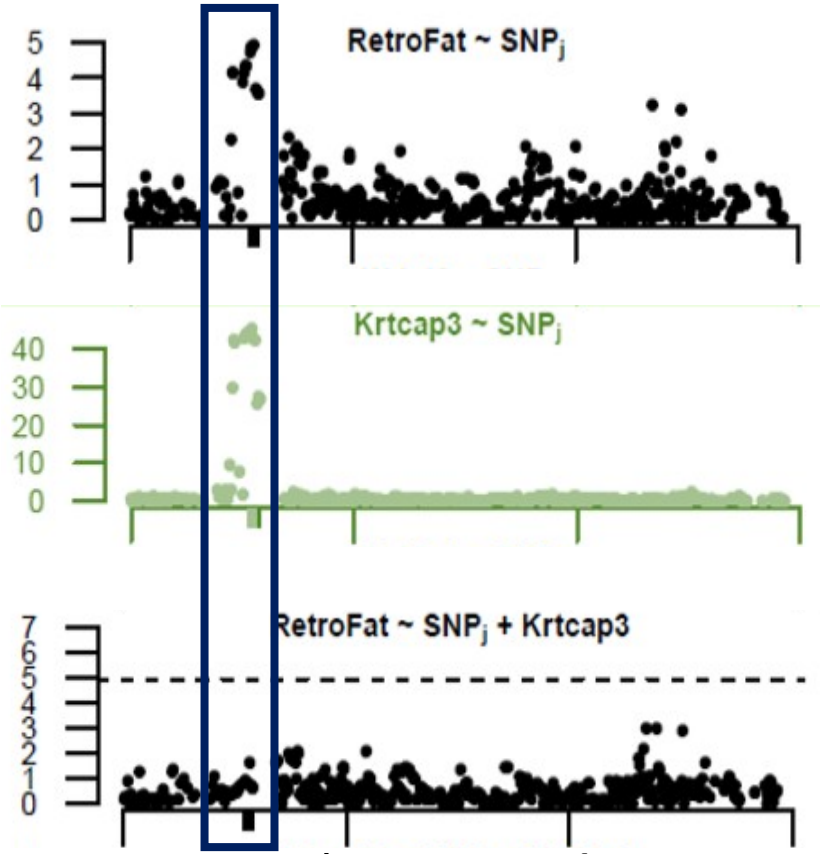
Funding Sources

R01 DK120667

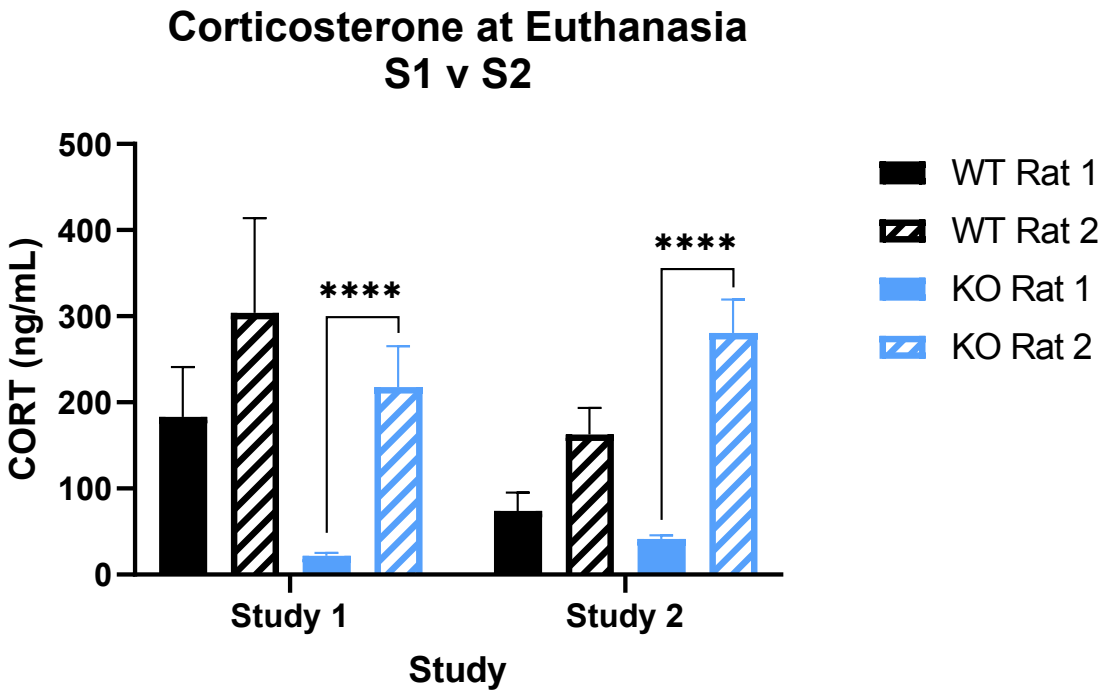
R01 DK106386

WFUSM start-up funds

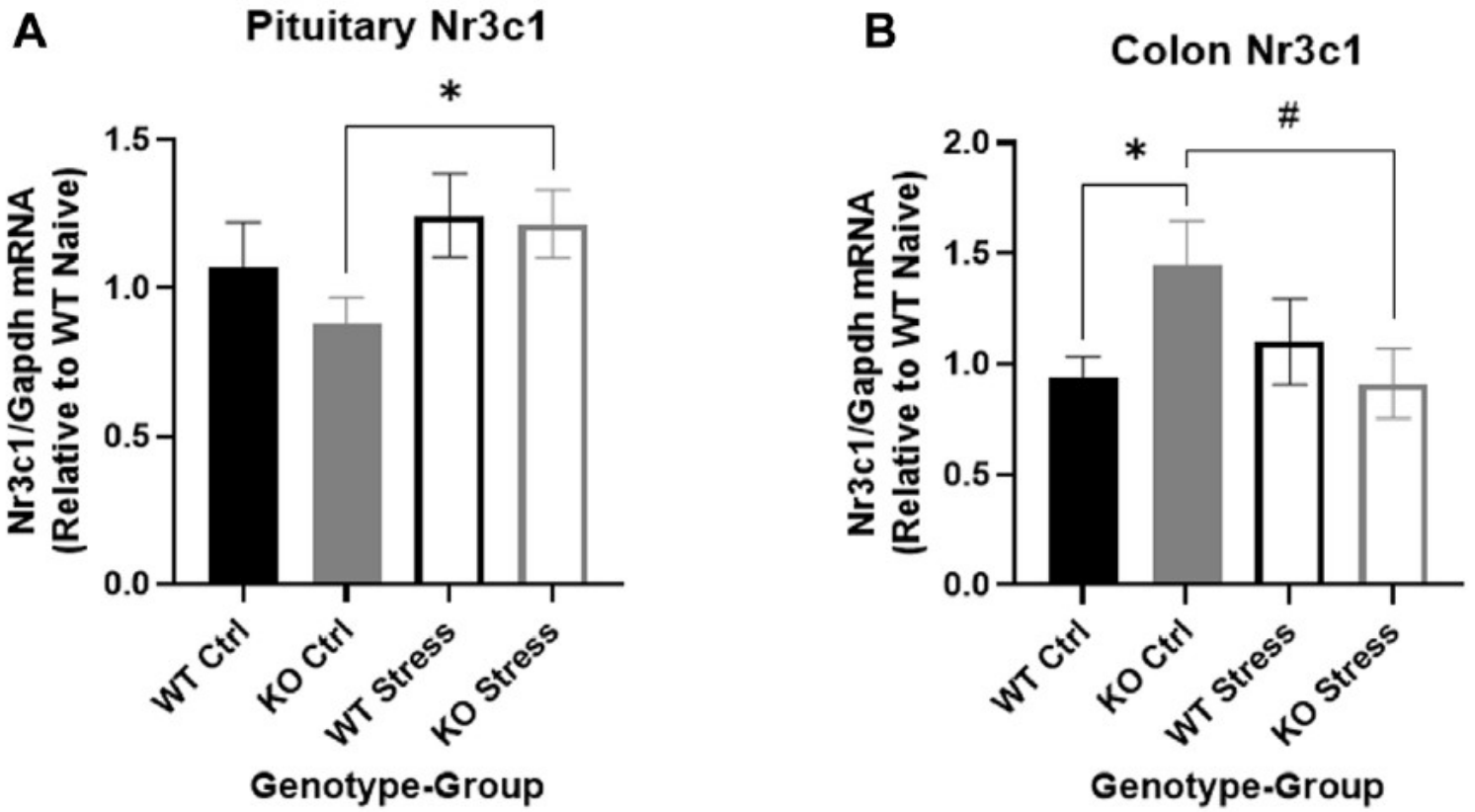
Krtcap3 identified as a candidate gene using mediation analysis



Keele et al., 2018


Corticosterone at Euthanasia
S1 v S2
CORT (ng/mL)
500
400
300
200
100
0
****
****
Study 1
Study 2
Study
WT Rat 1
WT Rat 2
KO Rat 1
KO Rat 2

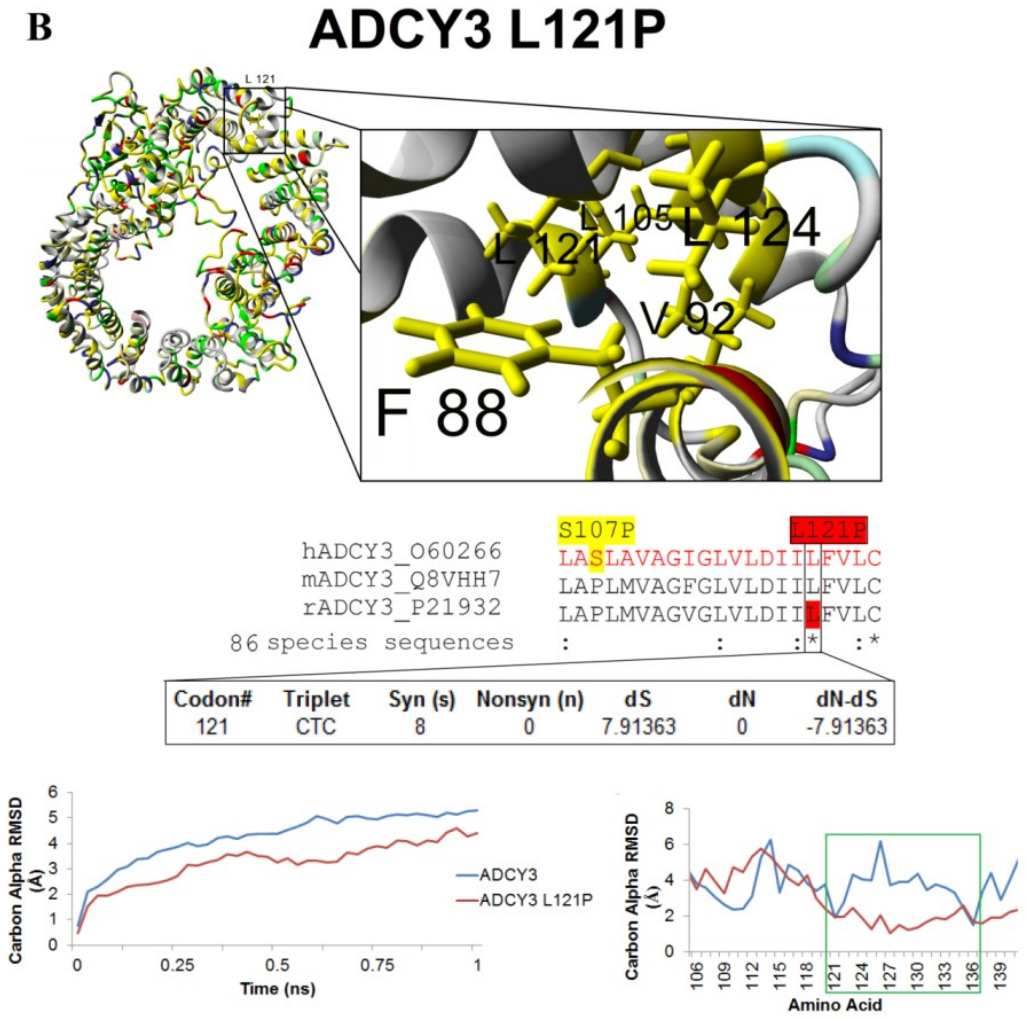
Glucocorticoid receptor levels change in pituitary and colon in KO, but not WT rats



Szalanczy, 2024

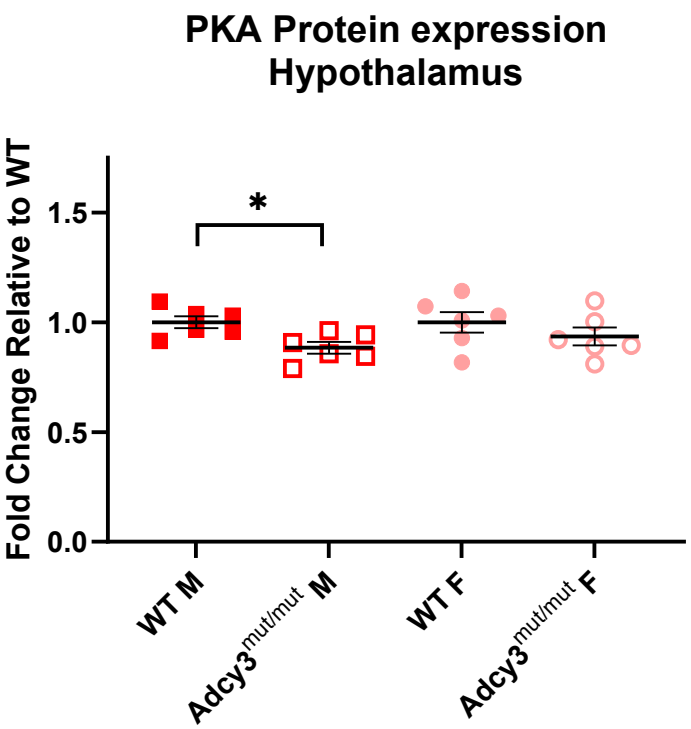
Non-synonymous coding variant identify in *Adcy3*

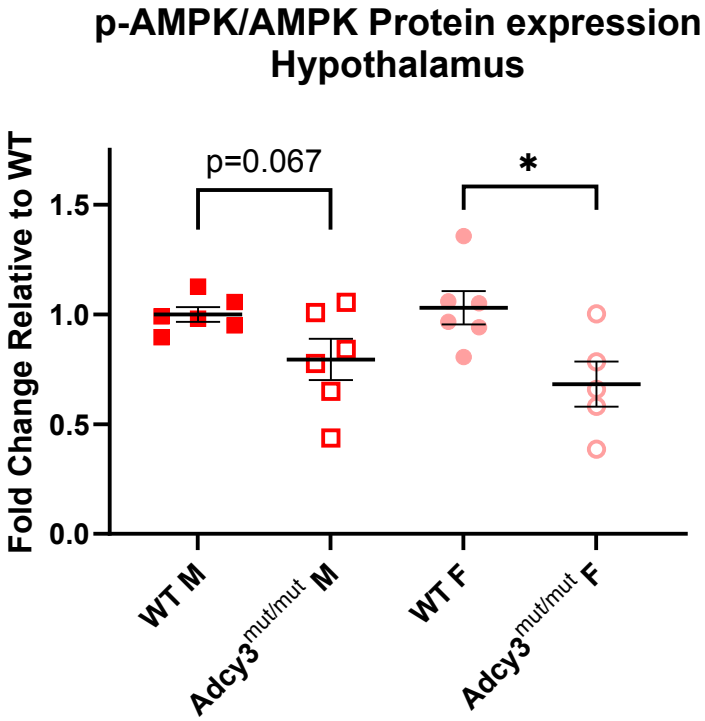
Jeremy Prokop

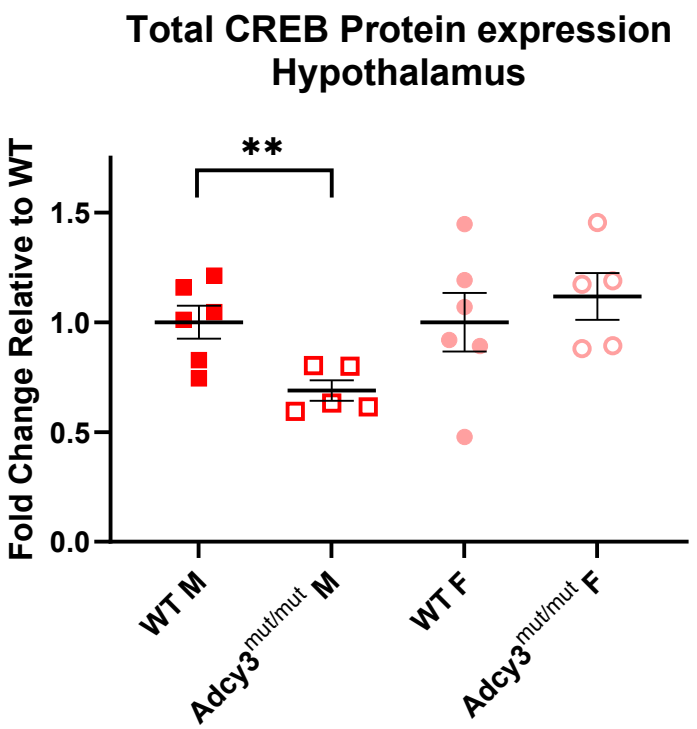


Keele et al., 2018

Adcy3$^{mut/mut}$ rats have decreased PKA, AMPK and CREB signaling





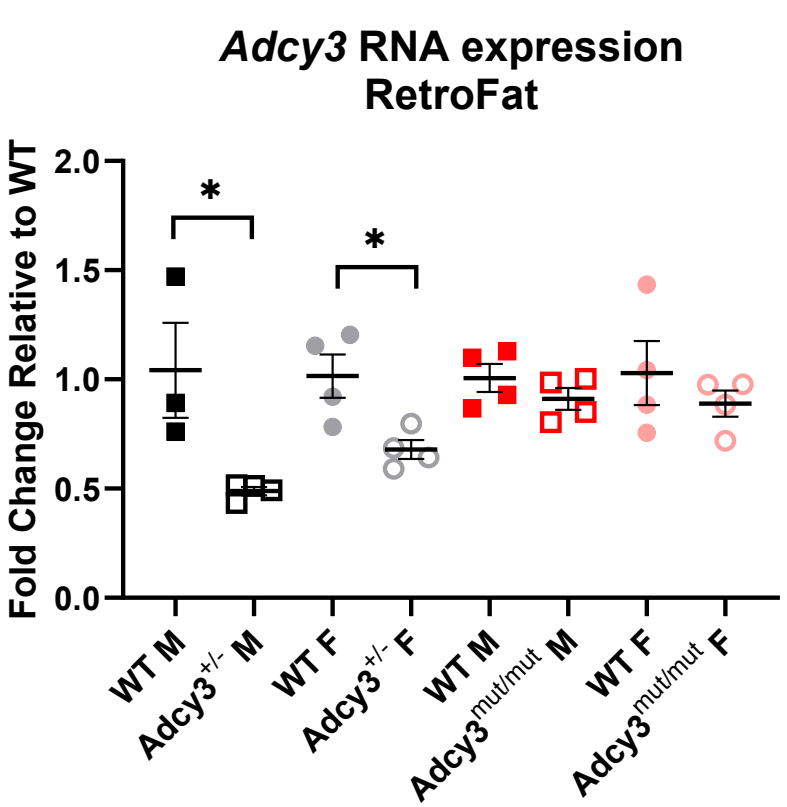


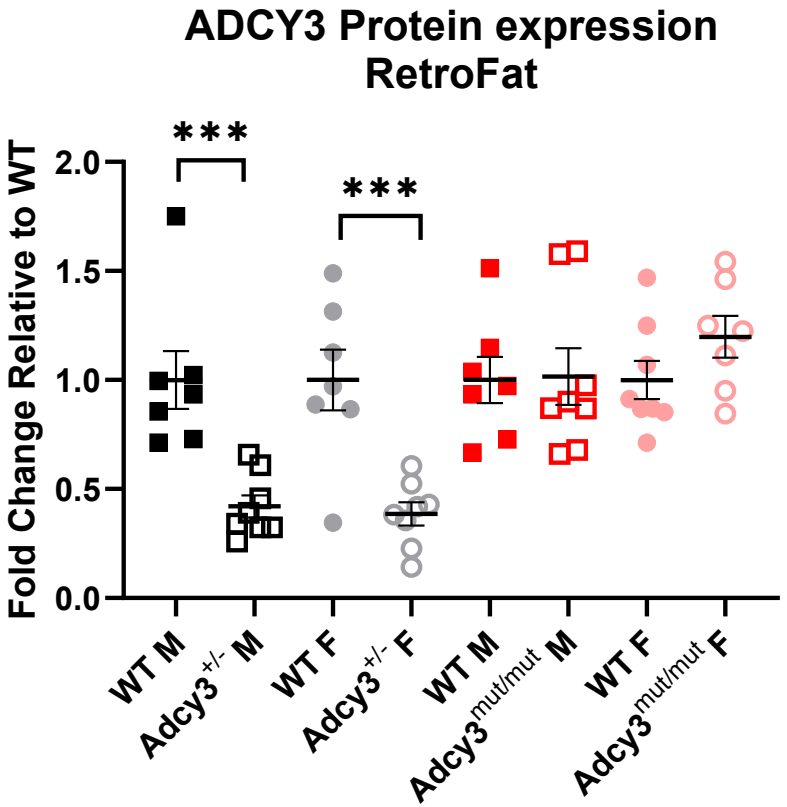
N=5-6

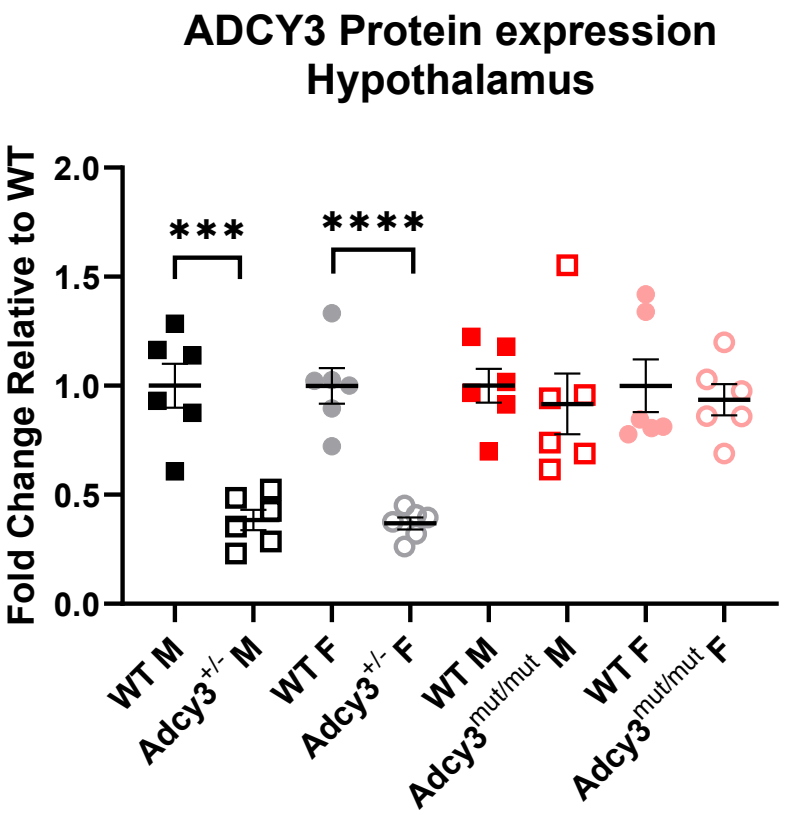
*p<0.05, **p<0.01

Fitzpatrick et al. 2024

Adcy3$^{mut/mut}$ does not alter *Adcy3* expression levels







*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

N=4-8

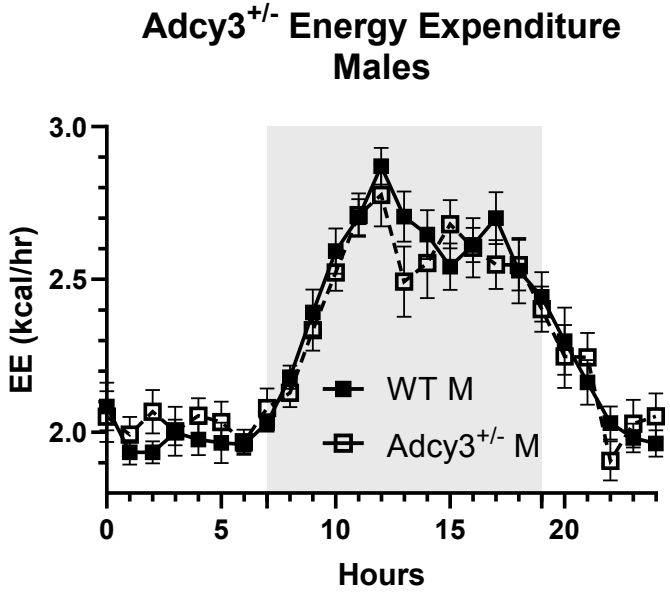
Fitzpatrick et al. 2024

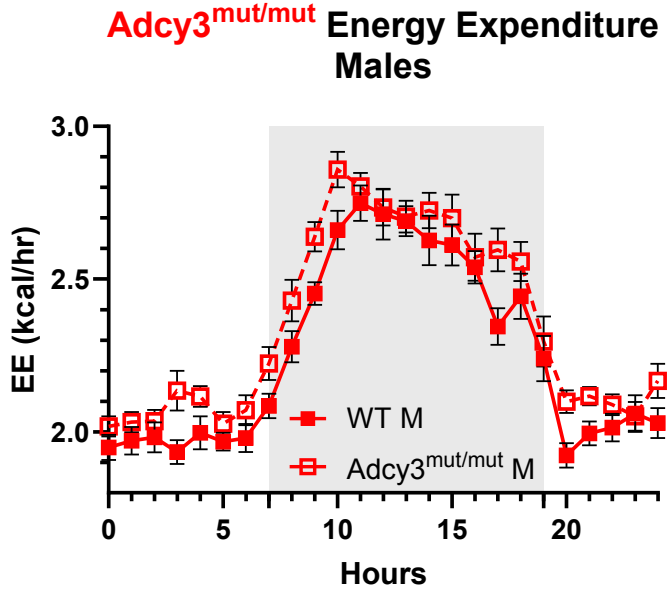
Study 1: Krtcap3 KO femaless have increased adiposity on both HFD and LFD

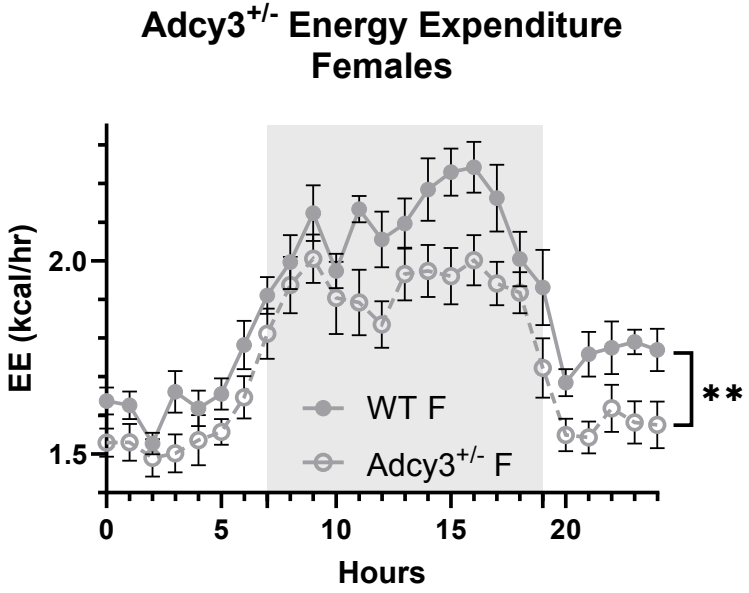
Szalanczy et al., 2022

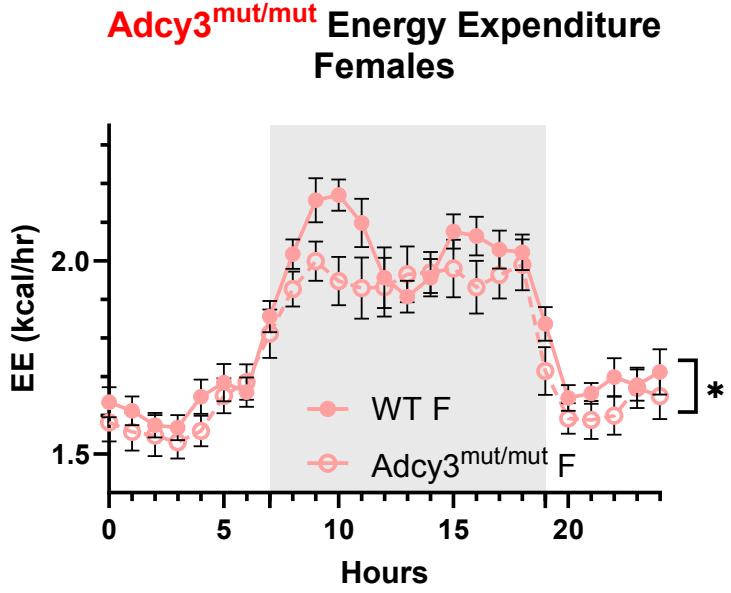
TSE Chambers

Adcy3$^{+/-}$ and Adcy3$^{mut/mut}$ females, but not males, expend less energy than WT









CalR Version 1.3 (calrapp.org)
ANCOVA, **p<0.01. N=12-16

Fitzpatrick et al. 2024, preprint

Adcy3$^{mut/mut}$ males and females show increased anxiety-like behavior in the OFT

More anxiety-like =

- **Less center time**
- **Less time rearing**
- **More time grooming**

*p<0.05