	ERα		GPER1		ERβ	
	Females	Males	Females	Males	Females	Males
Total VMH	71.9 ± 9.7	93.9 ± 1.7	95.9 ± 1.1	98.8 ± 0.7	92.2 ± 1.9	96.8 ± 1.3
Dorsomedial VMH	72.6 ± 9.8	93.0 ± 2.4	97.5 ± 0.7	98.5 ± 0.9	92.0 ± 2.6	96.6 ± 1.5
Ventrolateral VMH	71.0 ± 9.7	95.3 ± 2.4	93.7 ± 1.7	99.3 ± 0.4	91.0 ± 3.1	98.0 ± 1.0

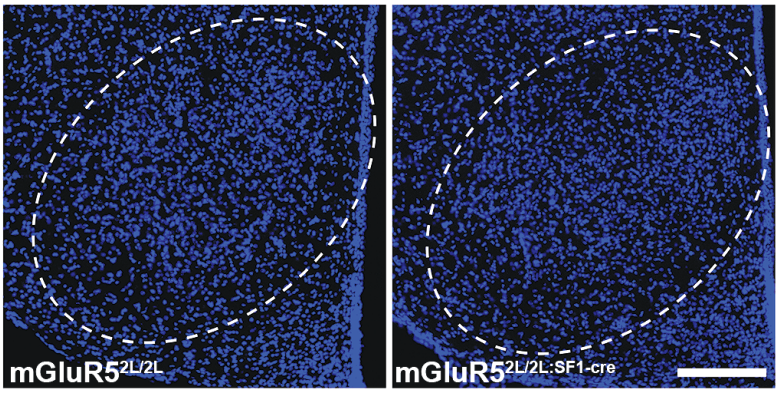
Supplementary Table 1

Percentage of $SF1^{+}$ neurons containing mGluR5 and ERα, GPER1 or ERβ in the VMH of wild type females and males (n = 3)

	% ERα+/SF1+		% SF1+/ERα+	
	Females	Males	Females	Males
Total VMH	81.0 ± 1.1	85.8 ± 3.2	70.2 ± 9.6	92.1 ± 2.2
Dorsomedial VMH	94.5 ± 0.5	97.1 ± 1.2	70.8 ± 9.5	90.5 ± 2.9
Ventrolateral VMH	65.8 ± 2.2	73.2 ± 5.2	69.4 ± 9.9	94.4 ± 2.4

Supplementary Table 2

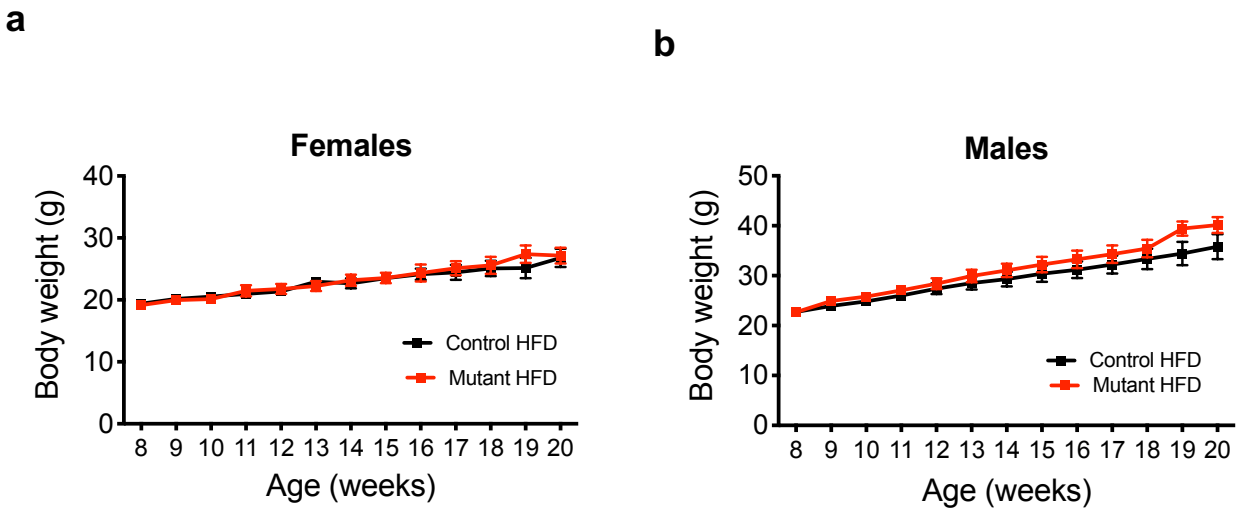
Percentage of ERα+ neurons containing SF1 (left) and percentage of SF1+ neurons containing ERα in the VMH of wild type females and males (n = 3)



Supplementary Figure 1

Gross VMH cytoarchitecture is preserved in the absence of mGluR5 in SF1 neurons

Representative brain sections obtained from mGluR5$^{2L/2L}$ control and mGluR5$^{2L/2L:SF1\text{-}cre}$ mutant females containing VMH (dashed lines) and stained with DAPI. Scale bar: 250 μM

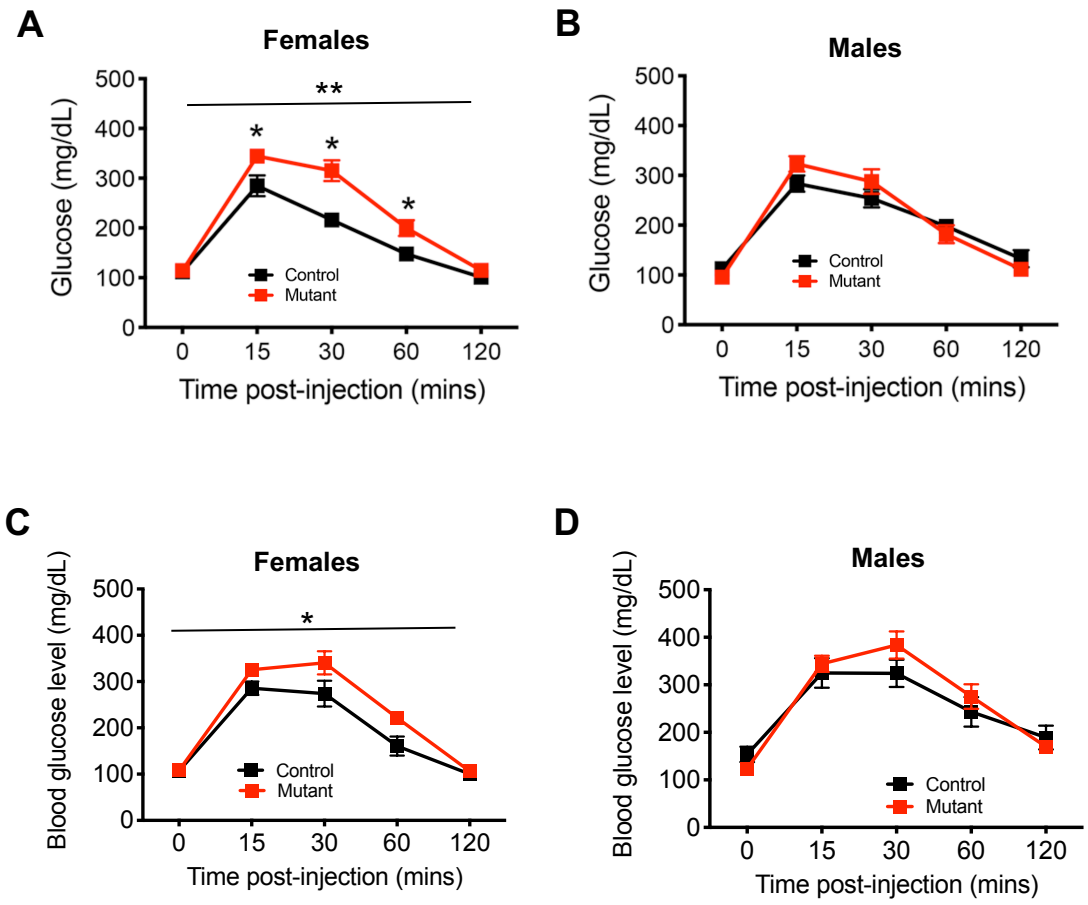


Supplementary Figure 2

Normal body weights in female and male mGluR5$^{2L/2L:SF1\text{-}cre}$ mice challenged with a high fat diet

a. Body weights of mGluR5$^{2L/2L}$ and mGluR5$^{2L/2L:SF1\text{-}cre}$ females (n = 6) fed a high fat diet starting at 8 weeks of age.

b. Body weights of mGluR5$^{2L/2L}$ (n = 7) and mGluR5$^{2L/2L:SF1\text{-}cre}$ (n = 9) males fed a high fat diet starting at 8 weeks of age.



Supplementary Figure 3

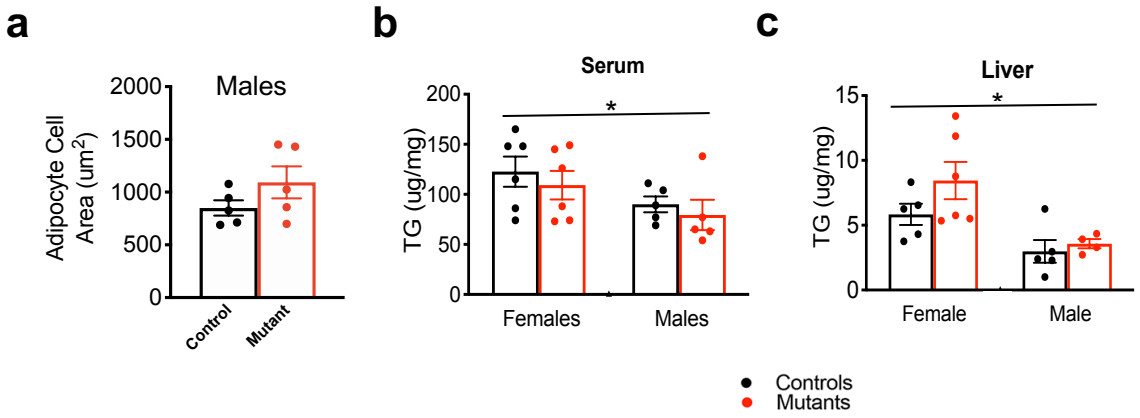
Abnormal responses of female but not male mGluR5$^{2L/2L:SF1\text{-}cre}$ in the glucose tolerance test

A. Glucose tolerance test (GTT) in mGluR5$^{2L/2L:SF1\text{-}cre}$ mutant (n = 11) and mGluR5$^{2L/2L}$ control (n = 10) females fed a chow diet at 8 weeks of age. **, Two-way *ANOVA*: Genotype, $p = 0.002$; Time, $p < 0.0001$; Interaction of time and genotype, $p = 0.002$. Bonferroni's multiple comparisons test: *, $p < 0.05$.

B. Glucose tolerance test in mGluR5$^{2L/2L:SF1\text{-}cre}$ (n = 13) and mGluR5$^{2L/2L}$ (n = 11) males fed a chow diet at 8 weeks of age. Two-way *ANOVA*: Genotype, NS. Time, $p < 0.0001$

C. Glucose tolerance test in mGluR5$^{2L/2L:SF1\text{-}cre}$ (n = 8) and mGluR5$^{2L/2L}$ (n = 7) females, 20 weeks of age fed a high fat diet starting at 8 weeks of age. *, Two-way *ANOVA*: Genotype, $p = 0.05$; Time, $p < 0.0001$; Interaction of genotype and time, $p = 0.02$.

D. Glucose tolerance test in mGluR5$^{2L/2L:SF1\text{-}cre}$ (n = 8) and mGluR5$^{2L/2L}$ (n = 8) males 20 weeks of age fed a high fat diet starting at 8 weeks of age. Two-way *ANOVA*: Genotype, NS. Time, $p < 0.0001$



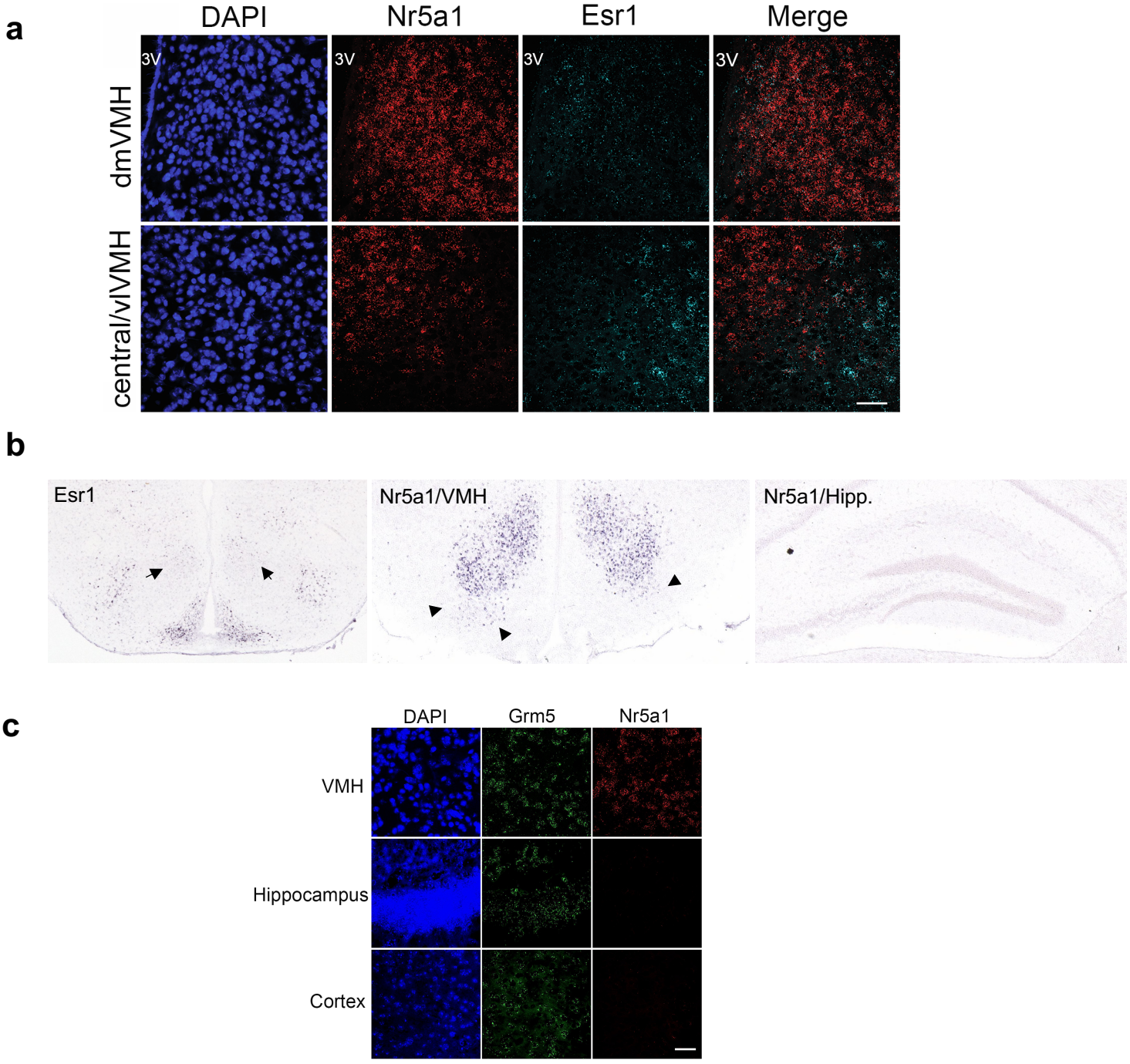
Supplementary Figure 4

Adipocyte size and levels of triglycerides in serum and liver of mGluR5$^{2L/2L:SF1\text{-}cre}$ and mGluR5$^{2L/2L}$ mice.

a. Adipocyte cell area in mGluR5$^{2L/2L:SF1\text{-}cre}$ and mGluR5$^{2L/2L}$ (n = 5) males. Two tailed unpaired t-test: NS

b. Triglyceride (TG) levels in serum of mGluR5$^{2L/2L}$ and mGluR5$^{2L/2L:SF1\text{-}cre}$ (females, n = 6 and males, n = 5). *, Two-way *ANOVA*: Genotype, NS. Sex, $p = 0.04$.

c. Triglyceride (TG) levels in liver of mGluR5$^{2L/2L}$ and mGluR5$^{2L/2L:SF1\text{-}cre}$ (females, n = 6 and males, n = 4-5). *, Two-way *ANOVA*: Genotype, NS. Sex, $p = 0.003$.



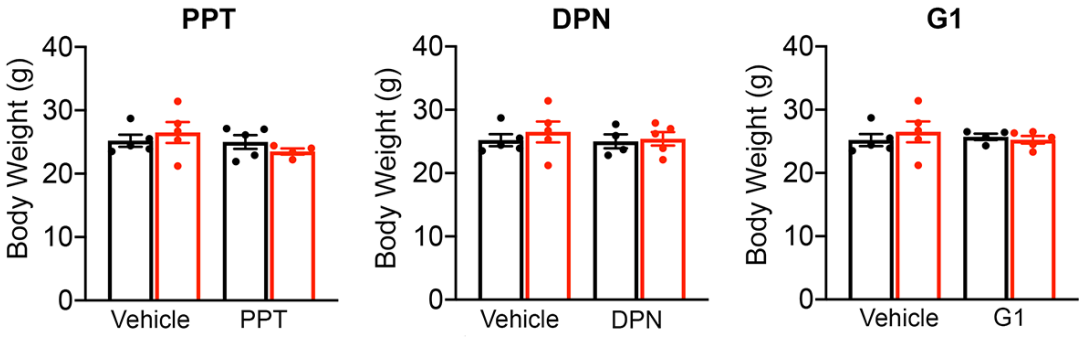
Supplementary Figure 5

ERα and SF1 mRNA expression in the adult VMH

a. Representative brain sections from adult female mice containing dorsomedial (dmVMH), central and ventrolateral (vlVMH) showing detection of SF1 (Nr5a1) and ERα (Esr1) transcripts using RNAscope. 3V, third ventricle. Scale bar = 50uM.

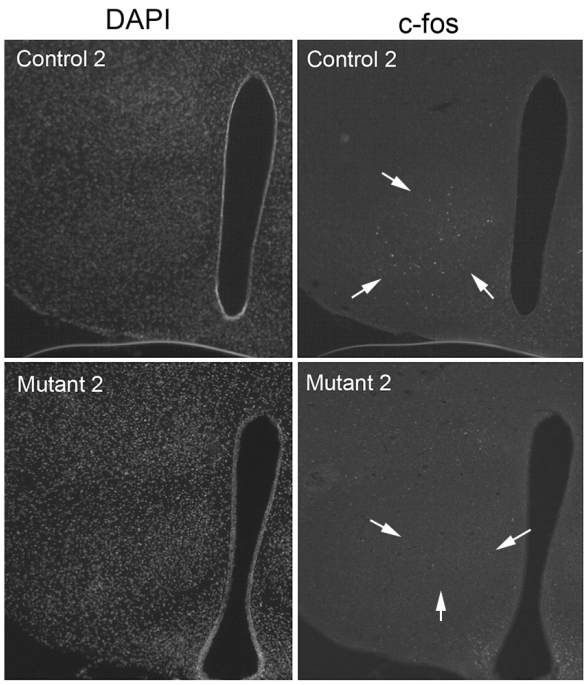
b. Examination of ERα (Experiment 79591677) and SF1 (Experiment 734) mRNA expression from the Allen Brain Institute. Black arrows: ERα mRNA in dmVMH; Black arrowheads: SF1 mRNA in vlVMH

c. RNAscope detection of mGluR5 (Grm5) and SF1 (Nr5a1) transcripts dmVMH, hippocampus and cortex. Scale bar = 50 um



Supplementary Figure 6

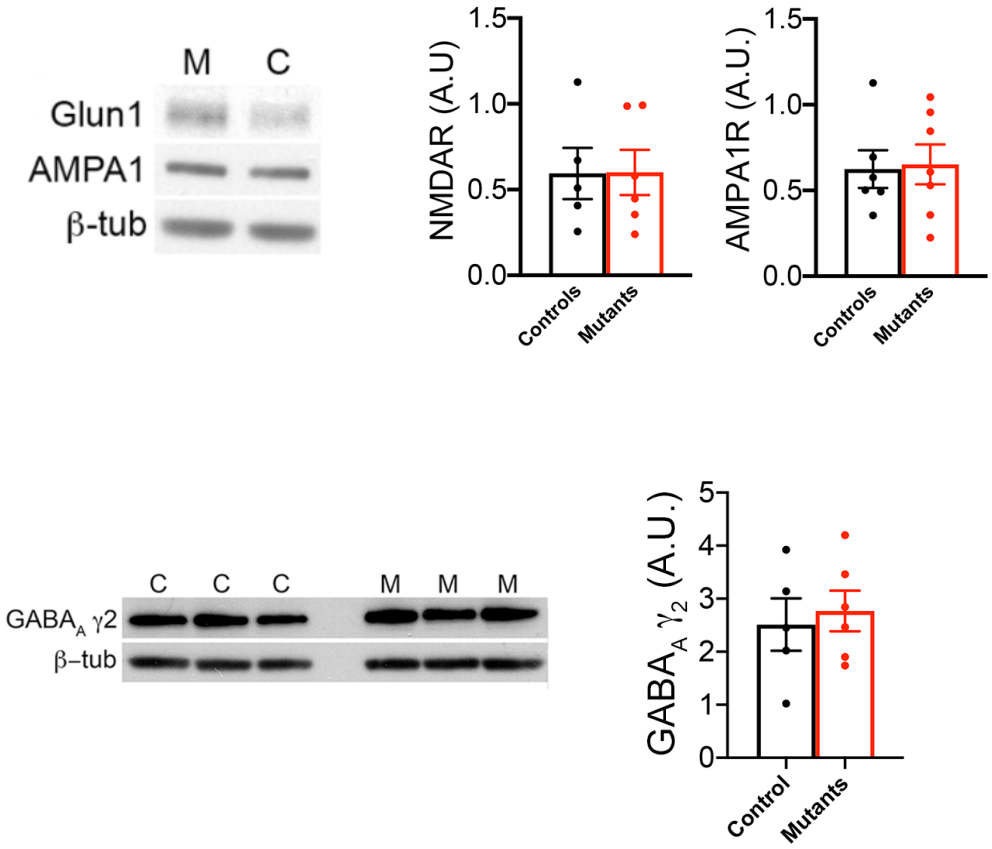
Body weights of mGluR5$^{2L/2L}$ (black) and mGluR5$^{2L/2L:SF1\text{-}cre}$ (red) females treated with estrogen receptor selective agonists PPT, DPN and G1.



Supplementary Figure 7

Diminished VMH neuronal activity following depletion of mGluR5 in SF1 neurons

c-fos^{+} cells in VMH (arrows) of fasted (16 hours) in a second set of mGluR5$^{2L/2L:SF1\text{-}cre}$ and mGluR5$^{2L/2L:SF1\text{-}cre}$ females 30 minutes following injection of a bolus of glucose.



Supplementary Figure 8

Normal expression of NMDA, AMPA1 and GABA$_A$ receptors in the VMH of mGluR5$^{2L/2L:SF1\text{-}cre}$ mutant females.

a. Glun1 and AMPA1 protein expression in the VMH of mGluR5$^{2L/2L}$ control (C) (n = 5) and mGluR5$^{2L/2L:SF1\text{-}cre}$ mutant (M) (n = 6) females. Two-tailed unpaired t-test: NS. Data presented as means ± SEM

b. Western blot analysis and quantification of total levels of GABA$_A$ γ2 protein in the VMH of mGluR5$^{2L/2L}$ control (C) (n = 5) and mGluR5$^{2L/2L:SF1\text{-}cre}$ mutant (M) (n = 6) females. Two-tailed unpaired t-test: NS. Data presented as means ± SEM