Supplementary data

Supplementary Table 1. List of tested genes of interest in different tissues

Gene	BAT	scWAT	gWAT	liver
acetyl-CoA carboxylase 1 (*Acaca*)		x	x	x
adiponectin		x	x	
adrenoreceptor 3β (*Adr3b*)	x	x	x	
3-hydroxybutyrate dehydrogenase 1 (Bdh1)				x
carbohydrate-responsive element-binding protein (*Chrebp*)		x	x	
carnitine palmitoyltransferase 1A (*Cpt1α*)		x	x	x
fatty acid synthase (*Fasn*)		x	x	
fibroblast growth factor 21 (*Fgf21*)				x
glucose transporter type 4 (*Glut4*)		x	x	
glycogen synthase kinase-3 β (*Gsk3β*)				x
3-hydroxy-3-methylglutaryl-CoA lyase (*Hmgcl*)				x
3-hydroxy-3-methylglutaryl-CoA synthase 1 (*Hmgcs1*)				x
insulin receptor substrate 1 (*Irs1*)		x	x	x
insulin receptor substrate 2 (*Irs2*)		x	x	x
lipase E, hormone sensitive type (*Lipe*)		x	x	
patatin like phospholipase domain containing 2 (*Pnpla2*)		x	x	
peroxisome proliferator-activated receptor alpha (*Pparα*)				x
peroxisome proliferator-activated receptor gamma (*Pparγ*)		x	x	
peroxisome proliferator activator receptor gamma coactivator 1 alpha (*Pparγc1a*)	x	x	x	x
PR/SET domain 16 (*Prdm16*)	x			
stearoyl-CoA desaturase 1 (*Scd1*)		x	x	x
sterol regulatory element-binding transcription factor 1 (*Srebp1*)		x	x	
uncoupling protein 1 (*Ucp1*)	x			
vascular endothelial growth factor alpha (*Vegfα*)	x			
tumor necrosis factor alpha (*TNFα*)	x	x	x	

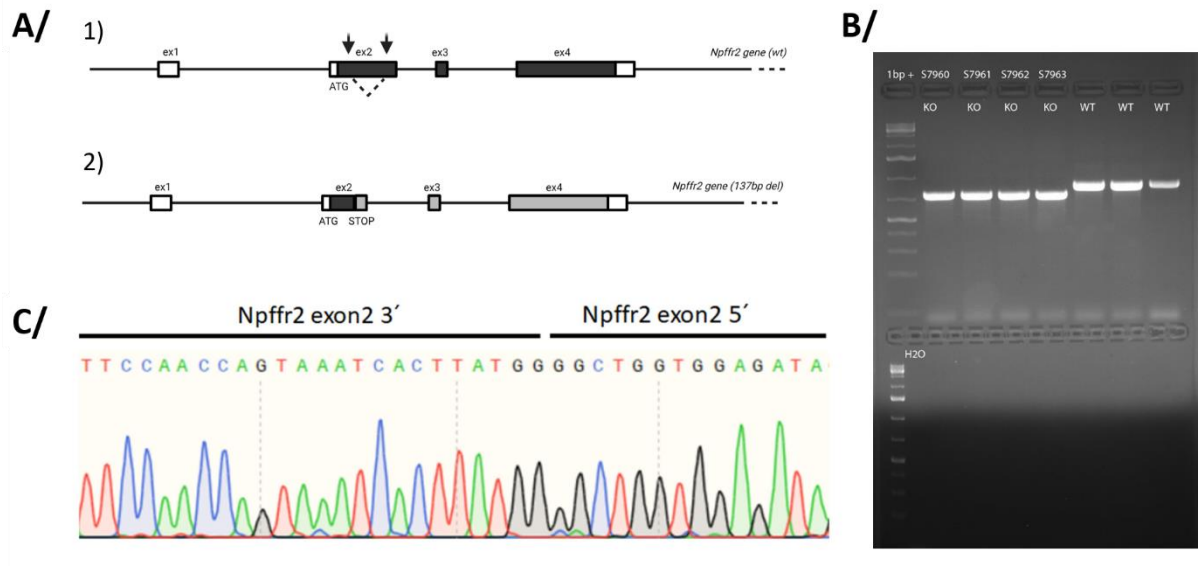
Supplementary Table 2. List of used primary antibodies

Abbreviation	Antibody	MW kDa	Dilution	Provider
p-Akt S473	Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb #4060	60	1:1000	Cell Signaling Technology
Akt	Akt (pan) (C67E7) Rabbit mAb #4691	60	1:1000	Cell Signaling Technology
p-AS160 S588	Phospho-AS160 (Ser588) (D8E4) Rabbit mAb #8730	160	1:1000	Cell Signaling Technology
AS160	AS160 (C69A7) Rabbit mAb #2670	160	1:1000	Cell Signaling Technology
GAPDH	GAPDH (D4C6R) Mouse mAb #97166	37	1:1000	Cell Signaling Technology
JNK	SAPK/JNK Antibody Rabbit #9252	46, 54	1:1000	Cell Signaling Technology
p-PDK	Phospho-PDK1 (Ser241) (C49H2) Rabbit mAb #3438	58-68	1:0000	Cell Signaling Technology
PDK	PDK1 Antibody Rabbit Ab #3062	58-68	1:0000	Cell Signaling Technology
PI3K p85	PI3 Kinase p85 (19H8) Rabbit mAb #4257	85	1:1000	Cell Signaling Technology
PI3K p110α	PI3 Kinase p110α (C73F8) Rabbit mAb #4249	110	1:1000	Cell Signaling Technology
PPARα	PPAα Polyclonal Rabbit Ab #PA1-822A	52	1:1000	Thermo Fisher Scientific Inc.

Supplementary Table 3. Morphometric analysis in NPFFR2 KO and WT mice at 26 weeks and plasma metabolic profile at 24 weeks

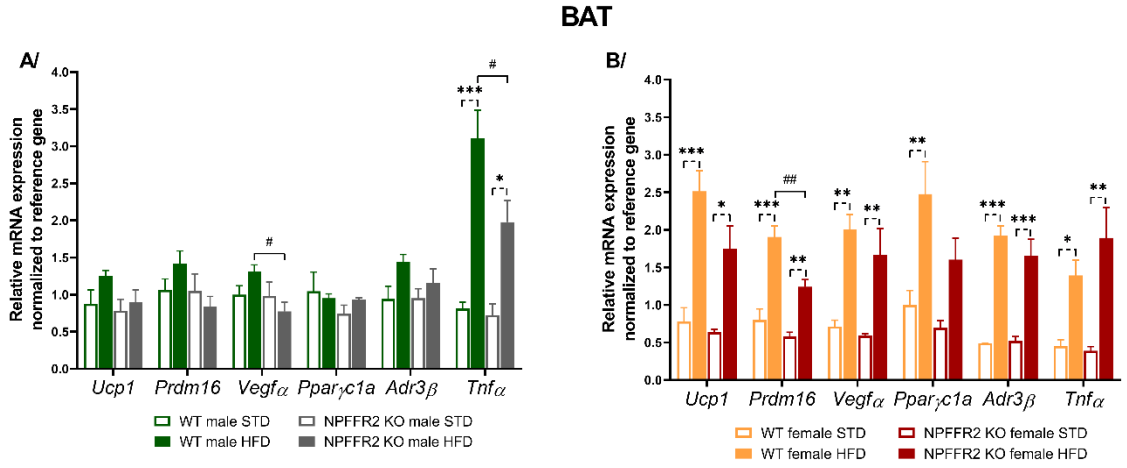
group	BW [g]	BAT [g]	scWAT [g]	gWAT [g]	liver [g]	TAG	FFA	cholesterol	leptin
WT male STD	29.96 ± 0.32	0.11 ± 0.01	0.24 ± 0.02	0.40 ± 0.02	1.33 ± 0.05	0.45 ± 0.02	0.73 ± 0.10	1.95 ± 0.12	1.85 ± 0.30
NPFFR2 KO male STD	30.34 ± 0.63	0.14 ± 0.01	0.30 ± 0.02	0.47 ± 0.06	1.38 ± 0.05	0.43 ± 0.03	0.57 ± 0.06	1.89 ± 0.12	2.92 ± 0.78
WT male HFD	51.33 ± 0.43 ###	0.33 ± 0.02 ###	2.17 ± 0.12 ###	2.30 ± 0.12 ###	1.79 ± 0.05 ###	0.54 ± 0.04	0.60 ± 0.04	3.98 ± 0.07 ###	44.85 ± 1.61 ###
NPFFR2 KO male HFD	44.65 ± 0.77 ###, ***	0.35 ± 0.03 ###	1.39 ± 0.10 ###, ***	1.87 ± 0.06 ###, ***	1.51 ± 0.08 **	0.55 ± 0.04 #	0.61 ± 0.06	3.87 ± 0.18 ###	30.14 ± 1.84 ###, ***
WT female STD	24.55 ± 0.19	0.08 ± 0.01	0.18 ± 0.02	0.22 ± 0.03	1.10 ± 0.01	0.45 ± 0.03	0.47 ± 0.06	3.36 ± 0.21	1.11 ± 0.09
NPFFR2 KO female STD	24.34 ± 0.19	0.09 ± 0.01	0.25 ± 0.03	0.30 ± 0.03	1.02 ± 0.03	0.35 ± 0.01 *	0.44 ± 0.04	2.17 ± 0.18 **	2.55 ± 0.52
WT female HFD	42.44 ± 1.18 ###	0.21 ± 0.02 ###	1.98 ± 0.16 ###	2.99 ± 0.19 ###	1.28 ± 0.07	0.58 ± 0.02 ##	0.39 ± 0.03	5.49 ± 0.23 ###	44.12 ± 3.59 ###
NPFFR2 KO female HFD	45.55 ± 1.22 ###	0.26 ± 0.02 ###	2.15 ± 0.17 ###	3.27 ± 0.18 ###	1.46 ± 0.10 ###	0.51 ± 0.04 ###	0.38 ± 0.04	6.14 ± 0.28 ###	51.00 ± 3.89 ###

A morphometric analysis was performed on NPFFR2 KO and WT mice at the end of the experiment, which took place at 26 weeks of age. Additionally, a plasma metabolic profile was assessed at 24 weeks of age. Data are expressed as mean ± SEM (n = 8-10), as determined by One-way ANOVA with Bonferroni post hoc test. Significance is * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ NPFFR2 KO vs WT mice on the same diet, # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ HFD vs STD of the same genotype.Body weight (BW), interscapular brown adipose tissue (BAT), subcutaneous white adipose tissue (scWAT), gonadal white adipose tissue (gWAT), triglycerides (TAG), free fatty acids (FFA).



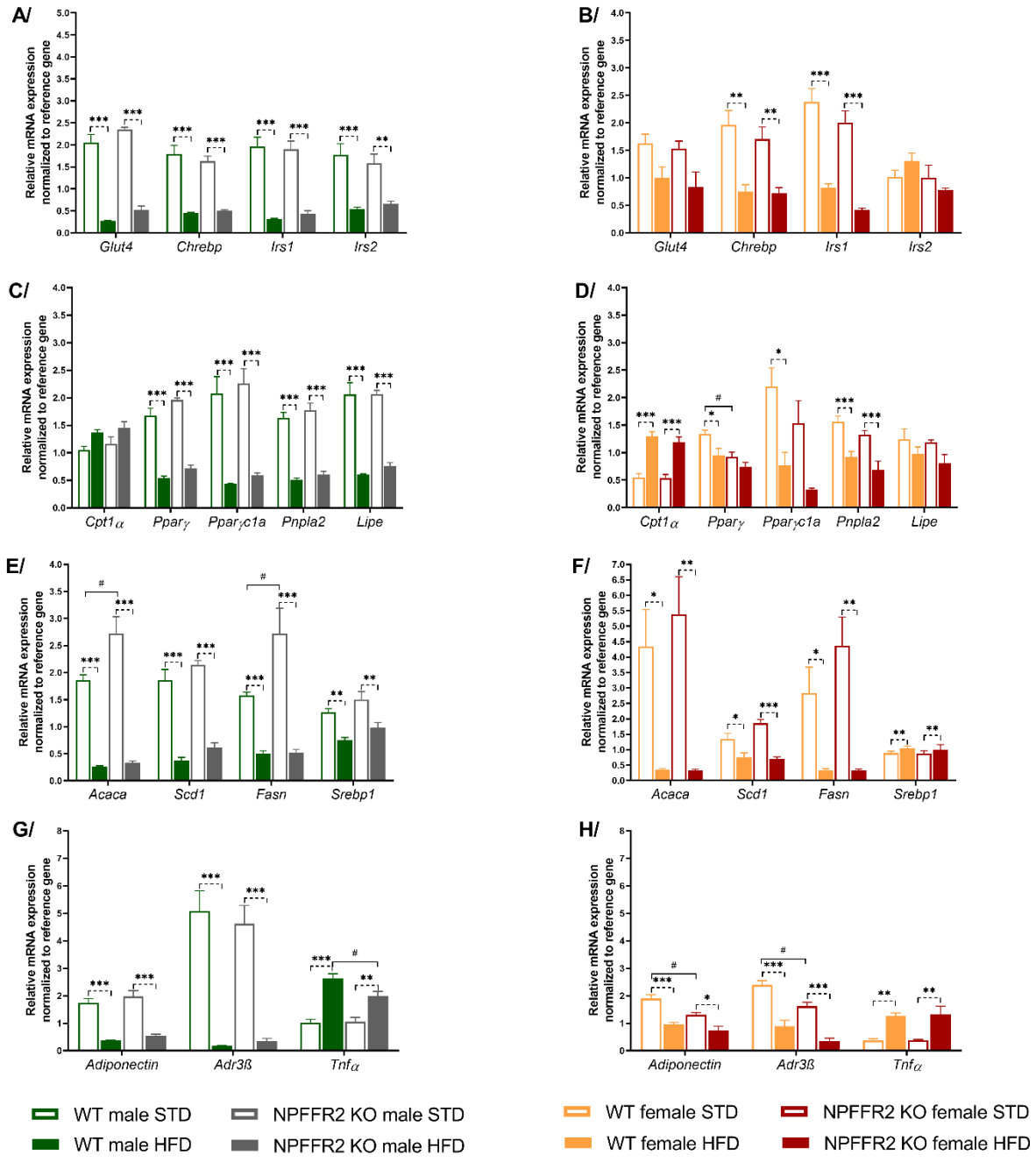
Supplementary Figure 1. Deletion in Npffr2 coding sequence

Schematic representation of the Npffr2 gene targeting strategy (**A**). The wild-type mouse Npffr2 gene locus. Intronic regions are represented as lines, protein-coding exons as black boxes, and untranslated regions as white boxes. The gRNA target sites are marked with black arrows (**1**). Schematics of Npffr2 gene structure after CRISPR-mediated gene targeting in the selected founder line. A 137 bp deletion within exon 2 of Npffr2 leads to a frameshift and generation of a premature STOP codon located in exon 2. This rearrangement results in the elimination of the vast majority of the Npffr2 protein-coding sequence (grey box) (**2**). Gel showing deletion in G1 animals S7960, 61, 62 and 63 and WT animals at 137bp using primers Npffr2-F and R (**B**).Sanger sequencing of the CRISPR target site performed in G1 mouse derived from the founder carrying 137 bp deletion in exon2 of *Npffr2* gene (**C**).



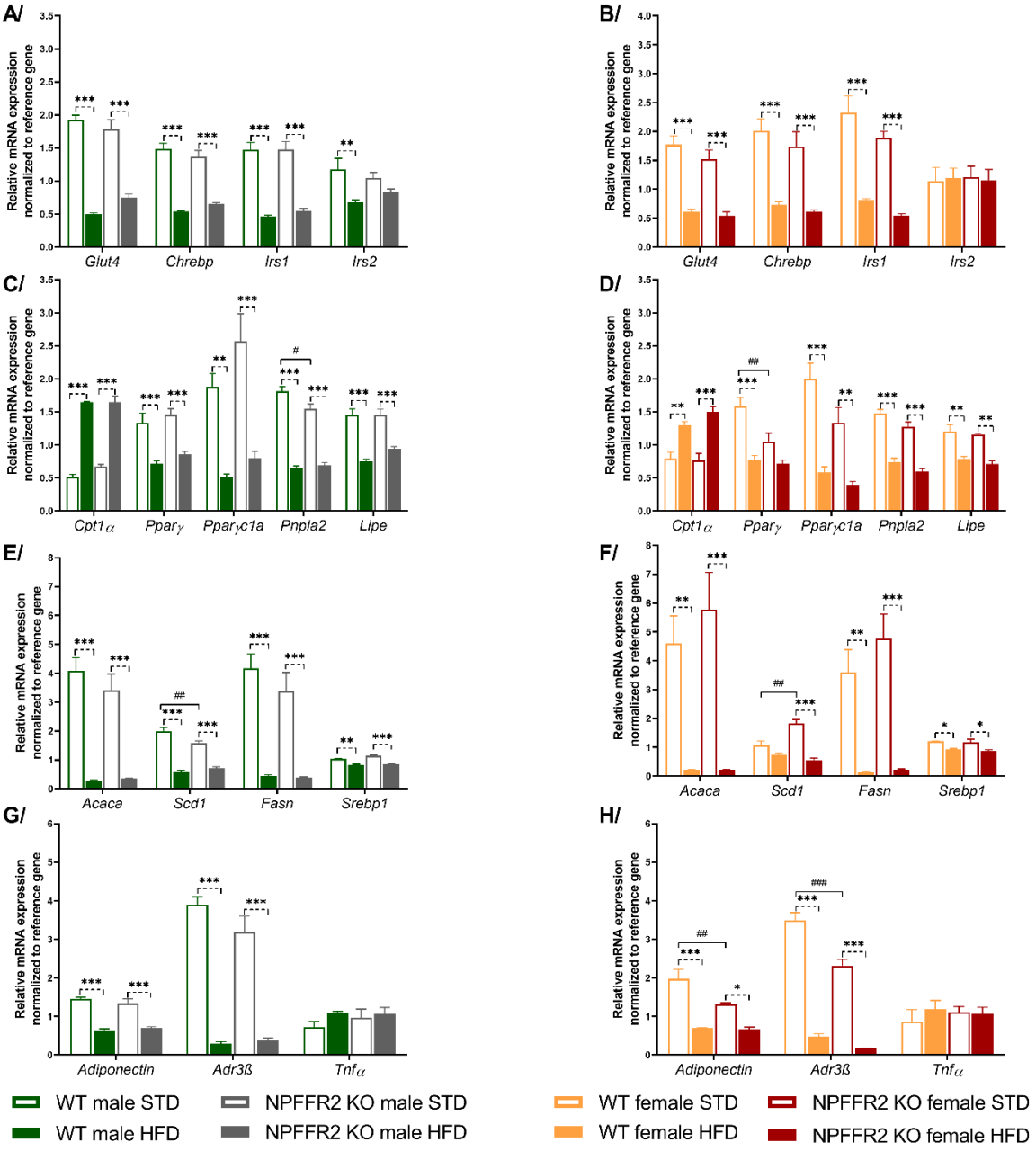
Supplementary Figure 2. Changes in the mRNA expression in the BAT of NPFFR2 KO and WT mice

mRNA expression of *Ucp1*, *Prdm16*, *Vegfα, Pparγc1a, Adr3β*, and *Tnfα* in the BAT of males (**A**)and females (**B**). Data are expressed as the mean ± SEM (n = 5). Significance was determined by one-way ANOVA with Bonferroni post hoc test. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ for HFD vs. STD mice of the same genotype; # $p < 0.05$ and ## $p < 0.01$ for NPFFR2 KO vs. WT mice on the same diet.



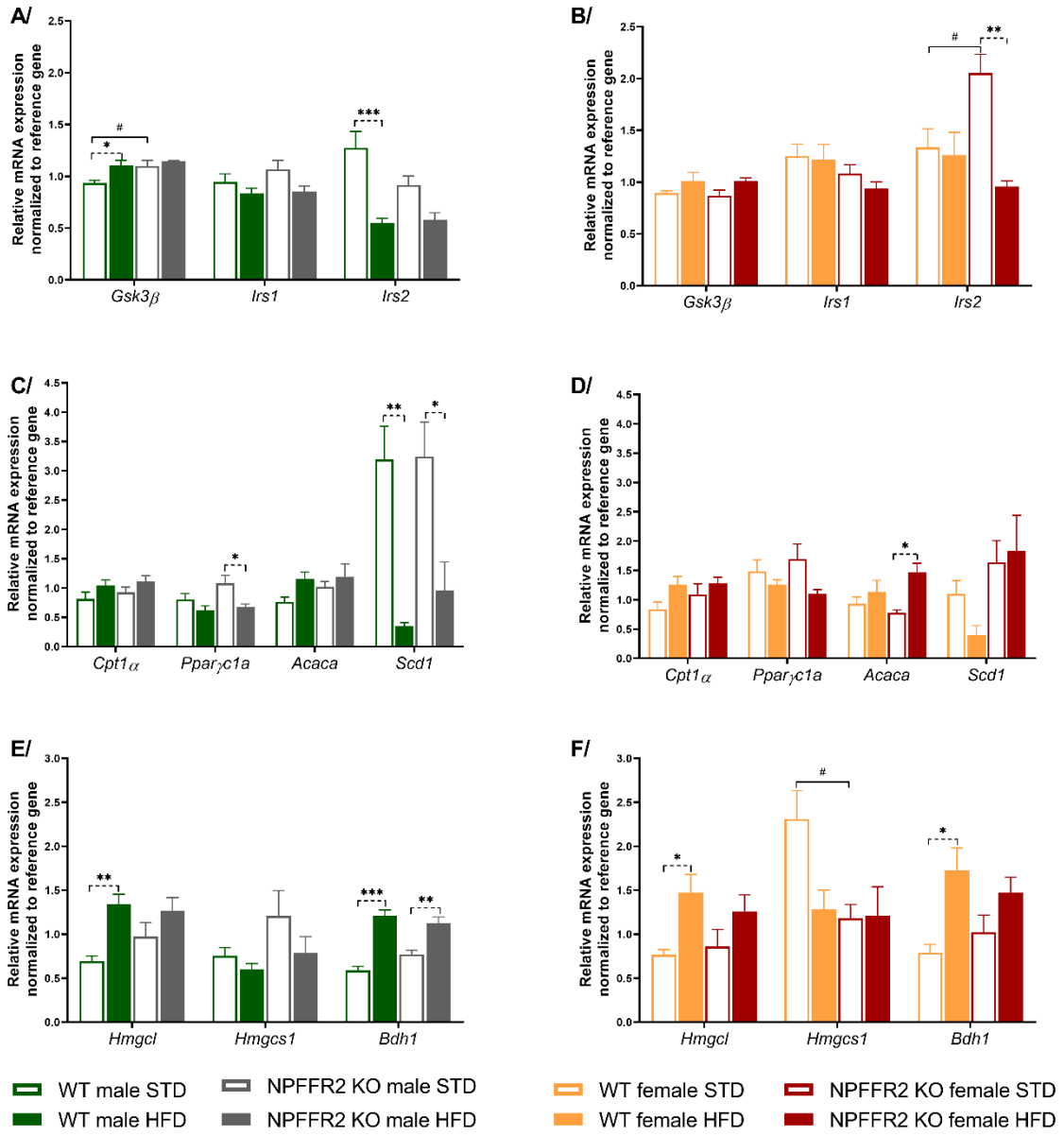
Supplementary Figure 3. Changes in mRNA expression in the gWAT of NPFFR2 KO and WT mice

mRNA expression of genes involved in glucose metabolism in the gWAT in (**A**)males and (**B**) females. mRNA expression of genes involved in lipid metabolism in the gWAT in (**C, E**)males and (**D, F**) females. mRNA expression of *adiponectin*, *Adr3β*, and *Tnfα* in (**G**)males and (**H**) females. Data are expressed as the mean ± SEM (n = 4–5). Significance was determined by one-way ANOVA with Bonferroni post hoc test. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ for HFD vs. STD mice of the same genotype; # $p < 0.05$ for NPFFR2 KO vs. WT mice on the same diet.



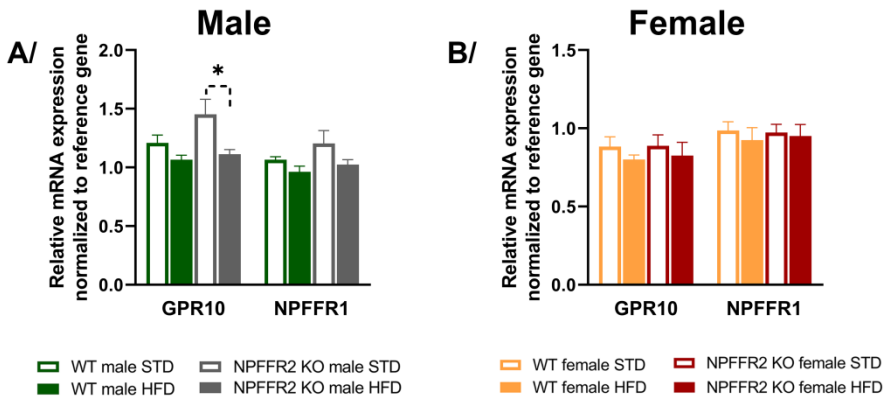
Supplementary Figure 4. Changes in the mRNA expression in the scWAT of NPFFR2 KO and WT mice

mRNA expression of genes involved in glucose metabolism in the scWAT in (**A**)males and (**B**) females. mRNA expression of genes involved in lipid metabolism in the scWAT in (**C, E**)males and (**D, F**) females. mRNA expression of *adiponectin*, *Adr3β*,and *Tnfα* in the scWAT in (**G**)males and (**H**) females. Data are expressed as the mean ± SEM (n = 4–5). Significance was determined by one-way ANOVA with Bonferroni post hoc test. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ for HFD vs. STD mice of the same genotype; # $p < 0.05$, ## $p < 0.01$, and ### $p < 0.001$ for NPFFR2 KO vs. WT mice on the same diet.

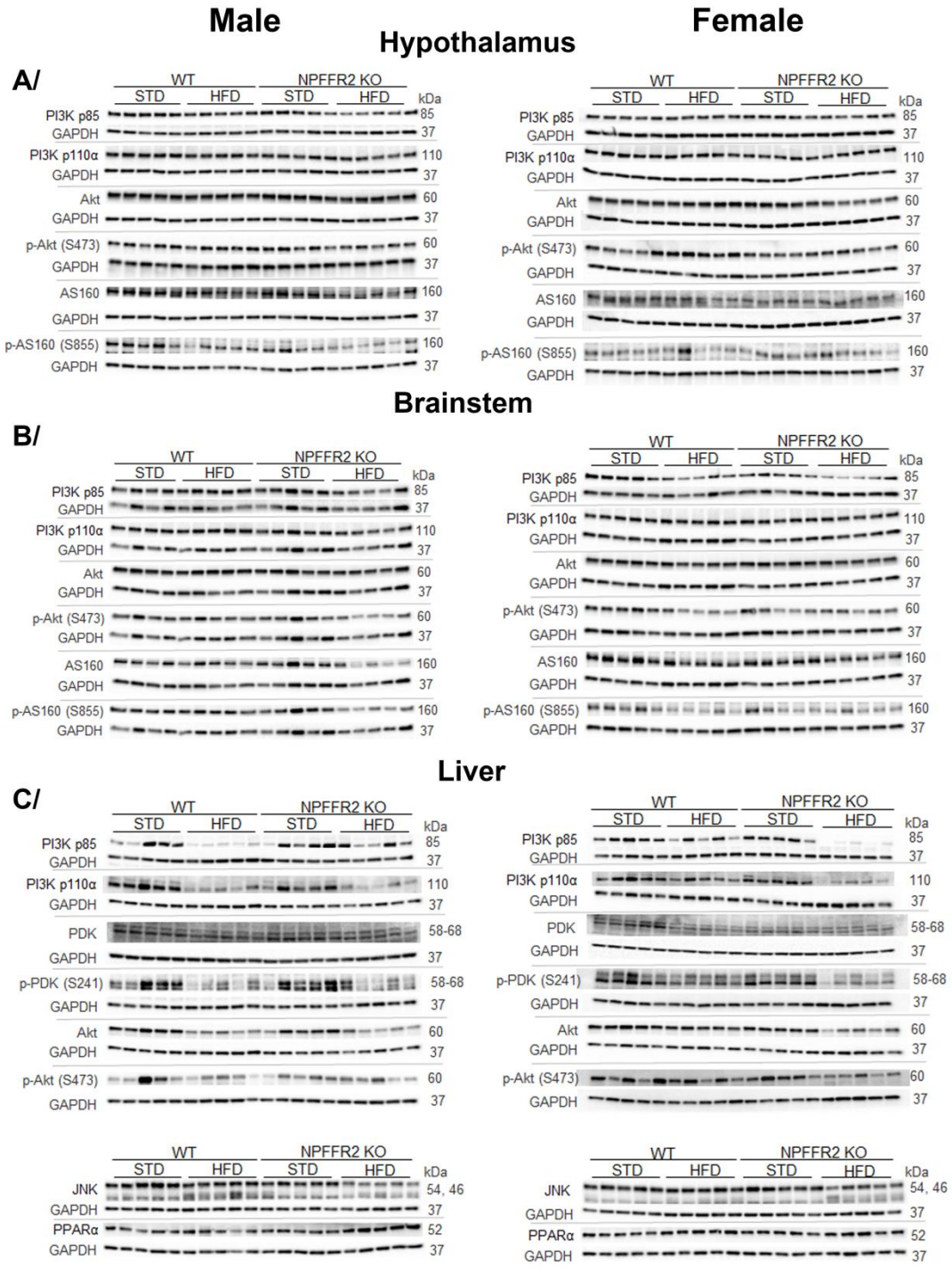


Supplementary Figure 5. Changes in mRNA expression in the livers of NPFFR2 KO and WT mice

mRNA expression of genes involved in glucose metabolism in the liver in (**A**)males and (**B**) females. mRNA expression of genes involved in lipid metabolism in the liver in (**C**)males and (**D**) females. mRNA expression of genes involved in ketogenesis in the liver in (**E**) males and (**F**) females. mRNA expression of *Fgf21* in the liver in (**G**)males and (**H**) females. Data are expressed as the mean ± SEM (n = 5). Significance was determined by one-way ANOVA with Bonferroni post hoc test. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ for HFD vs. STD mice of the same genotype; # $p < 0.05$ for NPFFR2 KO vs. WT mice on the same diet.



Supplementary Figure 6. Changes in mRNA expression in the hypothalamus of NPFFR2 KO and WT mice. mRNA expression of genes for GPR10 and NPFFR1 in (**A**)males and (**B**) females. Data are expressed as the mean ± SEM (n = 5). Significance was determined by one-way ANOVA with Bonferroni post hoc test. * $p < 0.05$ for HFD vs. STD mice of the same genotype.



Supplementary Figure 7. Overview of Western Blot analyses from Figures 5, 6 and 7.

WB analysisofhypothalamic (**A**), brainstem (**B**) and liver (**C**) signalingincludingcontrol protein (GAPDH).