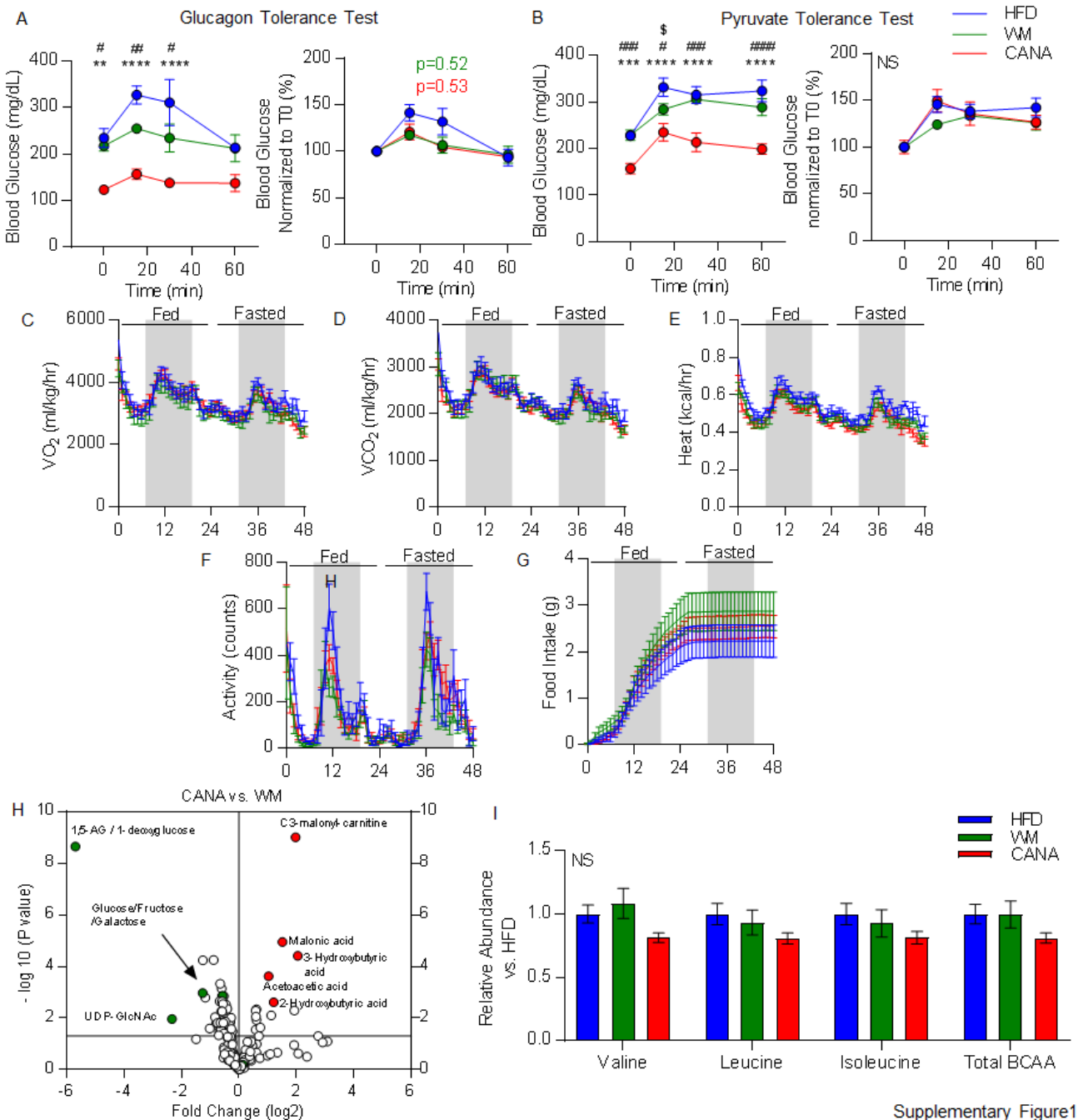
Supplemental methods and procedures

Acute cold exposure

Mice were exposed to an ambient temperature of 4^{o}C with access to water but not food. Rectal temperatures were measured hourly for 4 hours.



Supplementary Figure1

SUPPLEMENTARY FIGURES

Supplementary Figure 1. Metabolic responses to canagliflozin.

(A) Glucagon tolerance test (20 μg/kg, intraperitoneal, n=4/group). Raw glucose data are provided in the left panel, and baseline-normalized data are provided in the right panel.

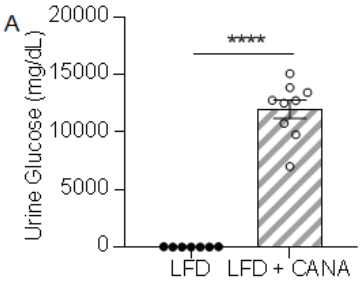
(B) Pyruvate tolerance test (1g/kg intraperitoneally, n=8/group). Raw glucose data are provided in the left panel, and baseline-normalized data are provided in the right panel.

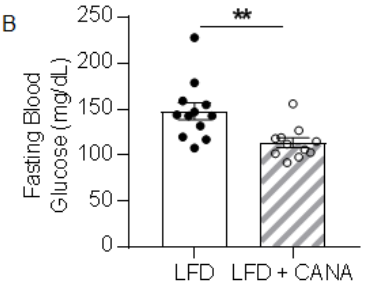
(C-G) Metabolic analysis using CLAMS. (C) Oxygen consumption (VO_2), (D) CO_2 exhaled, (E) heat production, (F) activity, (G) cumulative food intake. Data are presented as mean ± SEM, n=6-12, with p values calculated via either 1-way or 2-way ANOVA. *P<0.05, **P<0.01, ***P < 0.001 and ****P<0.0001 for HFD+CANA vs. HFD.

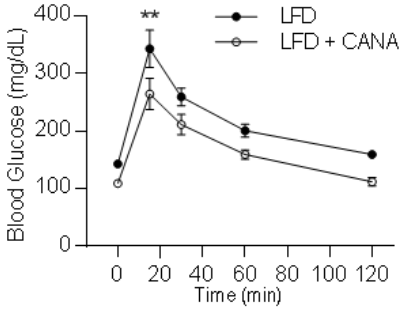
(H) Volcano plot of serum metabolites differentially abundant between CANA and HFD groups, analyzed using LC/MS. X axis indicates log2 fold change (upregulated by CANA to right, downregulated to left of 0), while y axis indicates -log 10 p value. Metabolites colored in red and green indicate selected significantly altered metabolites, increased or decreased, respectively.

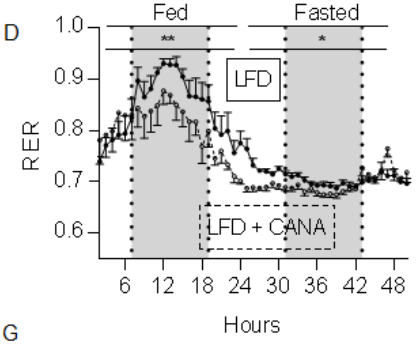
(I) Serum branched chain amino acids after 8 weeks of CANA (n=8/group)

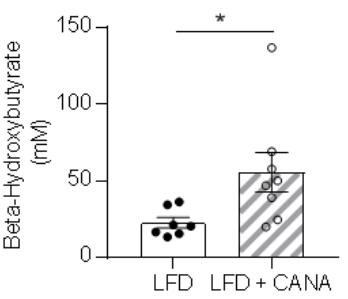
p values obtained by 1 or 2-way ANOVA. *P<0.05, **P<0.01, ***P < 0.001 and ****P<0.0001 in HFD vs. CANA; $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 and $^{\#\#\#\#}$P<0.0001 in WM vs. CANA; and $^{\$}$P<0.05, $^{\$\$}$P<0.01, $^{\$\$\$}$P<0.001 and $^{\$\$\$\$}$P<0.0001 in WM vs. HFD.

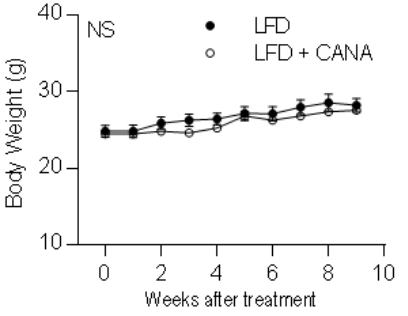


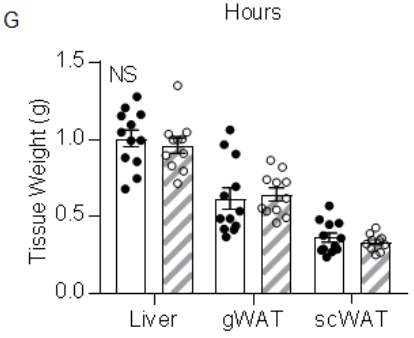












Supplementary Figure 2

Supplementary Figure 2. Metabolic response to canagliflozin in lean animals.

(A) Urine glucose concentration obtained in the fasting state in mice fed a low fat diet (LFD) or LFD + CANA (CANA) after 9 weeks of treatment (n=8-11/group).

(B) Body weight during the course of treatment (n=12 /group).

(C) Liver, gonadal and subcutaneous adipose weight after 9 weeks of treatment (n=12 /group);

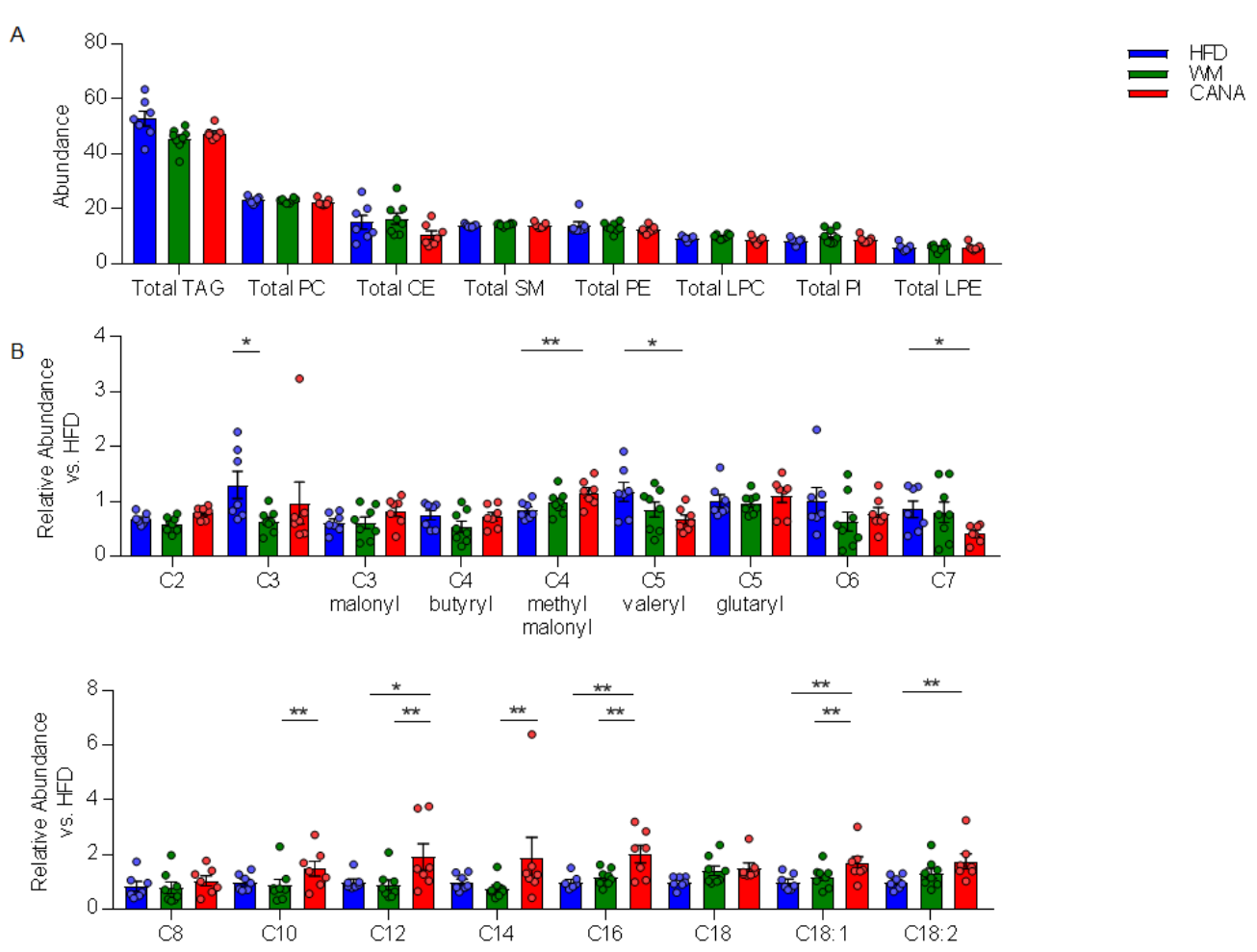
(D) Blood glucose after a 16-hour fast (n=8/group);

(E) Intraperitoneal glucose tolerance test (2g/kg, n=10/group);

(F) Respiratory exchange ratio (RER, n=6/group);

(G) Serum beta-hydroxybutyrate after a 4 hour fast (n=7-8/group).

p values obtained by 1 or 2-way ANOVA. *P<0.05, **P<0.01, ***P < 0.001 and ****P<0.0001.

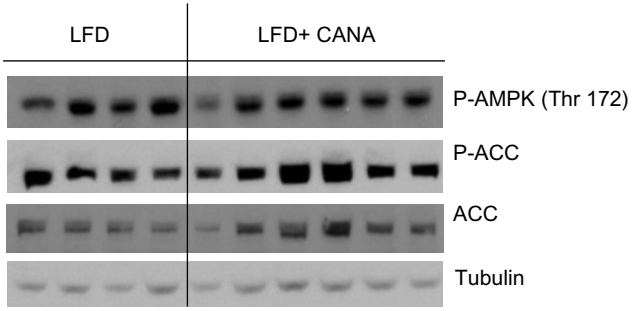


Supplementary Figure 3

Supplementary Figure 3. Hepatic lipid content and acylcarnitines.

(A) Total lipid content within various lipid species, including triacylglycerol (TAG) , lysophosphatidylethanolamine (LPE), lysophosphatidylcholine (LPC), phosphatidylinositol (PI), sphingomyelin (SM), cholesterol ester (CE), phosphatidylethanolamine (PE) and phosphatidylcholine (PC) (n=8/group).

(B) Hepatic acylcarnitine levels, measured by LC/MS (n=7-8/group).

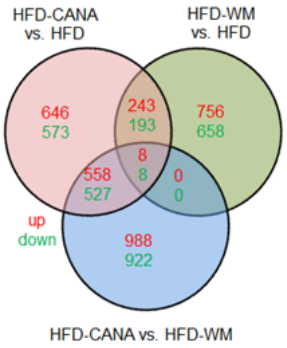


Supplementary Figure 4

Supplementary Figure 4. CANA did not alter phosphorylation of AMPK in lean animals.

Western blots showing phosphorylation of 5’ AMPK-activated protein kinase (AMPK, Thr172) and acetyl CoA carboxylase (ACC) (Ser79), total ACC, and tubulin in mice fed a low fat diet (LFD) or LFD with canagliflozin (CANA).

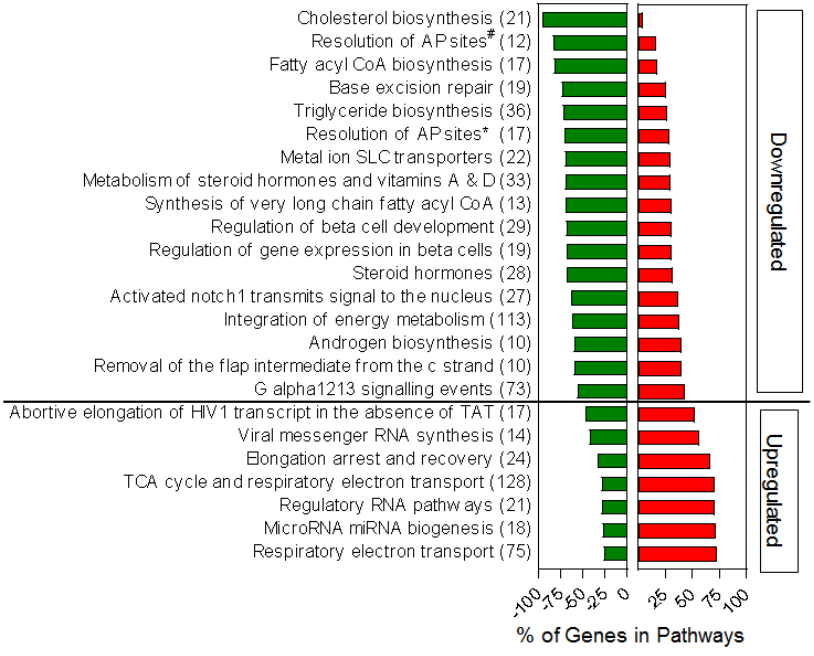
A



16 transcripts that overlap between all comparisons

Gene symbol	Gene name
Cyp4a31	cytochrome P450, family 4, subfamily a, polypeptide 31 (Cyp4a31), transcript variant 2
Lrrc16a	leucine rich repeat containing 16A (Lrrc16a), transcript variant 2
Acot9	acyl-CoA thioesterase 9 (Acot9), transcript variant 2
Prokr2	prokineticin receptor 2 (Prokr2)
Mir466h	microRNA 466h (Mir466h)
---	Non-coding transcript (Probe ID: 17203891)
---	Non-coding transcript (Probe ID: 17207229)
---	Non-coding transcript (Probe ID:17339460)
Trp53cor1	tumor protein p53 pathway corepressor 1 long non-coding RNA.
C730036E19Rik	RIKEN cDNA C730036E19 gene (C730036E19Rik), long non-coding RNA.
---	Non-coding transcript (Probe ID: 17203685)
---	Non-coding transcript (Probe ID: 17205071)
---	Non-coding transcript (Probe ID: 17209261)
---	Non-coding transcript (Probe ID: 17202079)
---	Non-coding transcript (Probe ID: 17209509)
---	Non-coding transcript (Probe ID: 17206983)

B



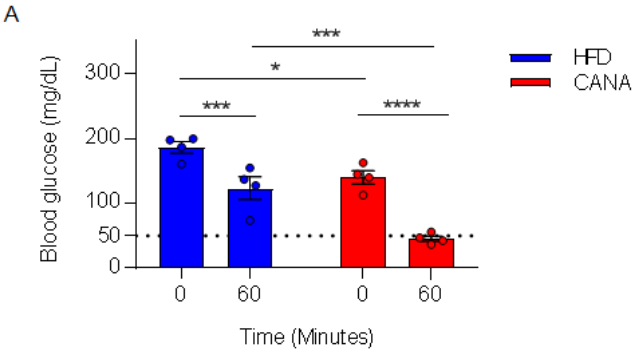
Supplementary Figure 5

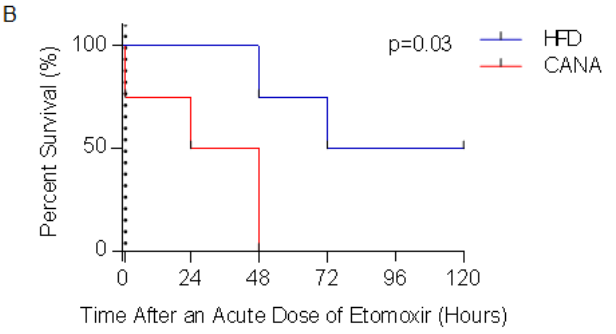
Supplementary Figure 5. Hepatic gene expression in HFD, WM, and CANA.

(A) Venn diagram indicating the number of genes altered in each of the 3 pairwise group comparisons, as determined using microarray analysis. 16 genes that were altered consistently in all three comparisons are provided in the adjacent table. Gray cells indicate upregulation, while unshaded cells indicate downregulated transcripts.

(B) Top-ranking pathways altered by CANA in comparison with WM (n=3-5/group, nominal $p<0.05$). Full name of pathway: * Resolution of AP sites via the multiple nucleotide patch replacement pathway, #Resolution of AP sites via the single nucleotide replacement pathway.

p-values were obtained by 1-way ANOVA. *P<0.05, **P<0. 01, ***P < 0.001 and ****P<0.0001





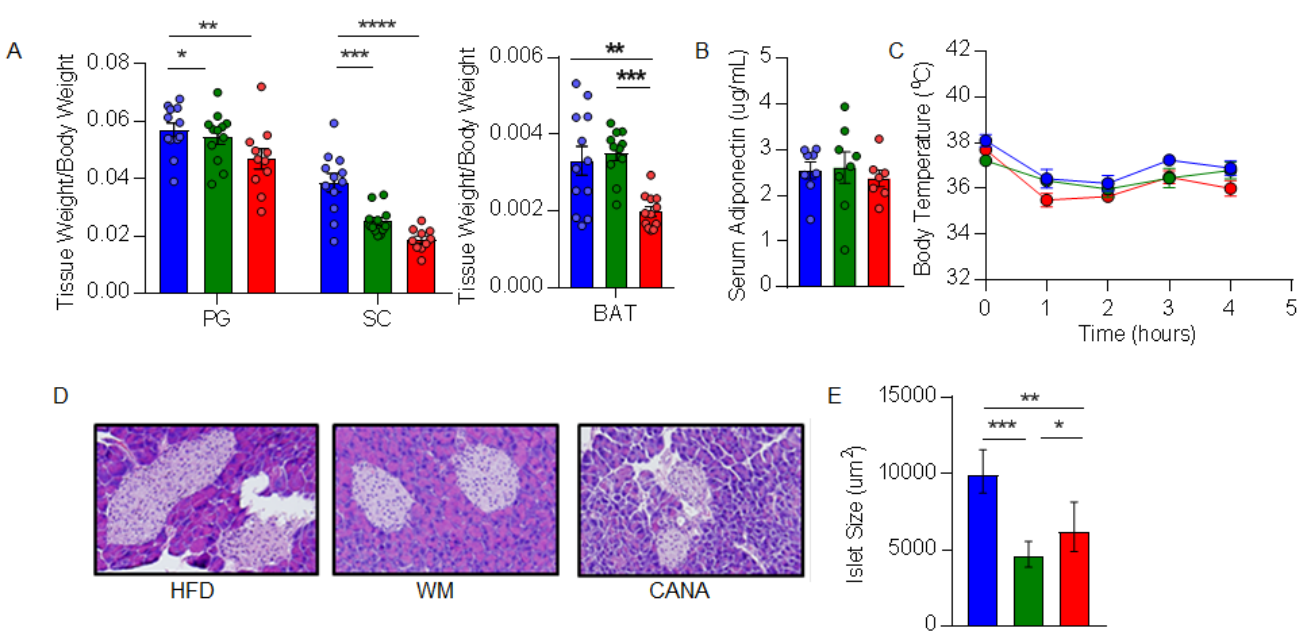
Supplementary Figure 6

Supplementary Figure 6. Fatty acid oxidation is required for successful adaptation to canagliflozin.

(A) Blood glucose level before and 1 hour after intraperitoneal injection of the Cpt1 inhibitor etomoxir (15 mg/kg).

(A) Survival curve after single-dose etomoxir injection (15mg/kg).

p-values were obtained by 1-way ANOVA. *P<0.05, **P<0. 01, ***P < 0.001 and ****P<0.0001



Supplementary Figure 7

Supplementary Figure 7. Canagliflozin reduces adipose depot weight and islet size without changes in adiponectin, or alterations in cold sensitivity.

(A) Tissue weight after 9 weeks of CANA treatment (gWAT, perigonadal; SC, subcutaneous; LIV, liver; BAT, brown adipose tissue), n=12 per group;

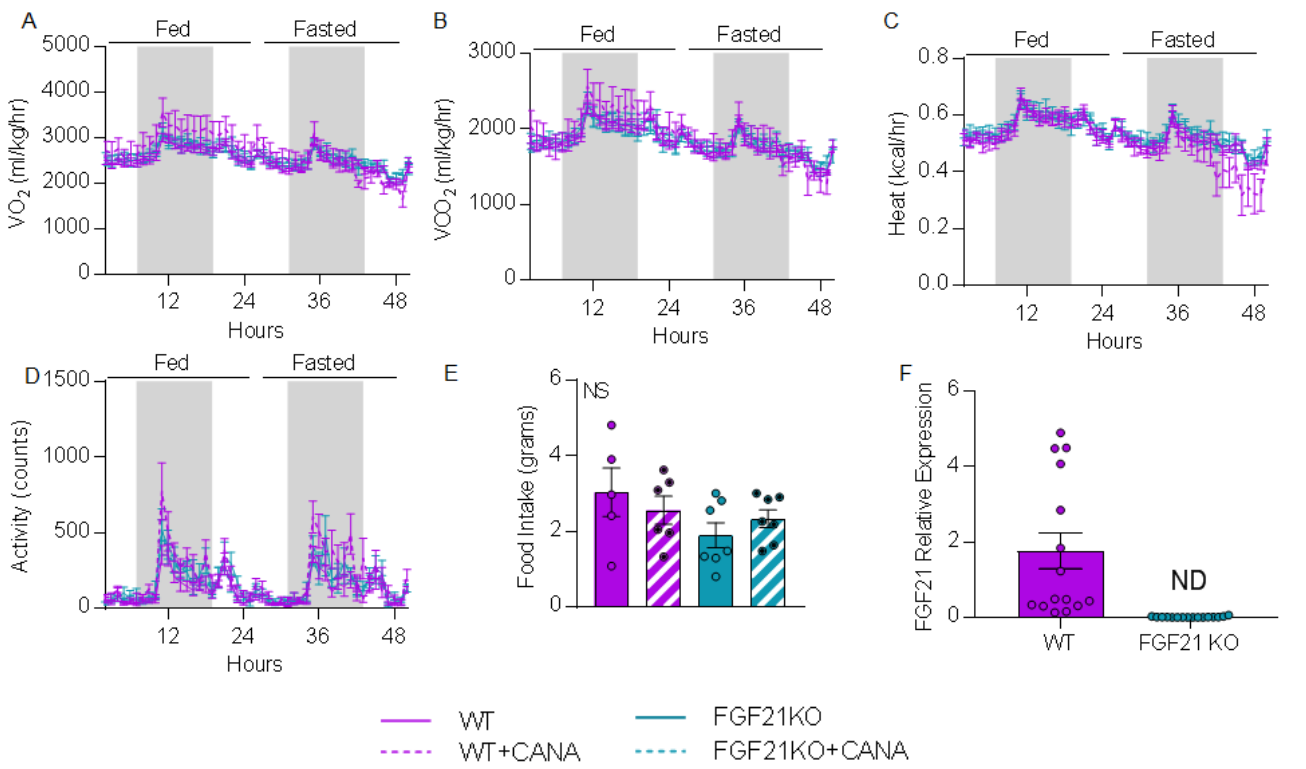
(B) Fasting serum adiponectin after 4 weeks of CANA (n=6/group);

(C) Body temperature, assessed via rectal probe, after a 4-hour cold exposure at 4^{o}C (n=4/group);

(D) Representative H&E-stained pancreatic tissue section (40x);

(E) Quantification of islet size (n=8/group).

p values obtained by 1-way ANOVA. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001



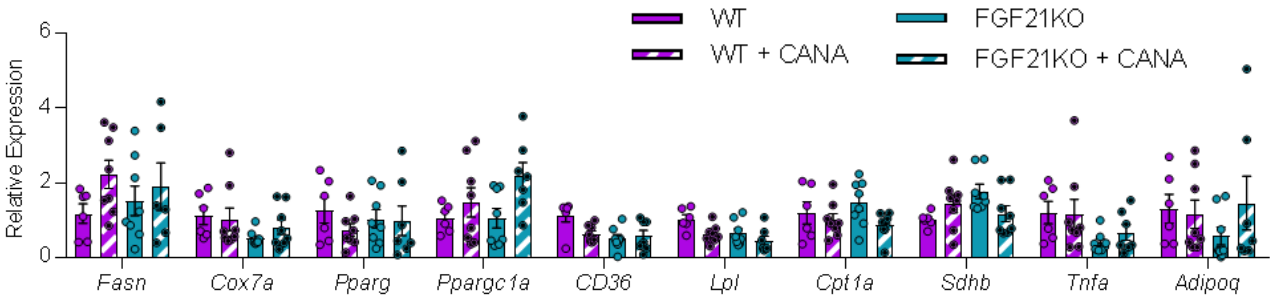
Supplementary Figure 8

Supplementary Figure 8. Metabolic phenotypes of WT and FGF21KO mice treated with CANA.

(A-E) Metabolic cage analysis. (A) Oxygen consumption (VO_2), (B) CO_2 exhaled, (C) heat production, (D) activity, and (E) cumulative food intake (n=7-9 per group)

(F) qPCR of liver tissue in wild type and FGF21 null mice (n=6-8 per group)

Data are presented as mean ± SEM, n=6-12, with p values calculated via 2-way ANOVA. *P<0.05, **P<0.01, ***P < 0.001 and ****P<0.0001



Supplementary Figure 9

Supplementary Figure 9. gWAT gene expression of key metabolic regulators.

(A) qPCR analysis of representative genes regulating adipose metabolism (n=6-7 per group).

p values obtained by 1-way ANOVA. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001