Supplementary Information

Fibroblast growth factor 21 mediates glycemic regulation by hepatic JNK

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Supplementary Experimental Procedures

Mice

Mapk8$^{LoxP/LoxP}$ mice (Das et al. 2007), *Mapk9*$^{LoxP/LoxP}$ mice (Han et al. 2013), *Map2k4*$^{LoxP/LoxP}$ mice (Wang et al. 2007), *Map2k7*$^{LoxP/LoxP}$ mice (Hubner et al. 2012), *Map3k10*$^{-/-}$ mice (Kant et al. 2011), *Map3k11*$^{-/-}$ mice (Brancho et al. 2005), and genotyping methods were previously described. We obtained C57BL/6J mice (stock number 000664), B6.129S4-Ppara^{tm1Gonz}/J mice (stock number 008154), B6.Cg-Tg(Alb-cre)21Mgn/J mice (stock number 003574) (Postic et al. 1999), B6;FVB-Tg(Adipoq-cre)1Evdr/J mice (stock number 010803) (Eguchi et al. 2011), and B6.129S4-*Gt(ROSA)26Sor*$^{tm1(FLP1)Dym}$/RainJ mice (stock number 009086) (Farley et al. 2000) from the Jackson Laboratory. All studies were performed using mice backcrossed to the C57BL/6J strain.

Fgf21$^{LoxP/LoxP}$ mice were established using homologous recombination in embryonic stem cells (Fgf21$^{tm1a(EUCOMM)Hmgu}$) and the generation of chimeric mice that were bred to obtain germ-line transmission of the mutated *Fgf21* allele using standard procedures (Figure S1A). The *Frt-Neo*^{R}-*Frt* cassette was excised by crossing with *FLPeR* mice to obtain mice with the *Fgf21*^{LoxP} allele. These mice were backcrossed to the C57BL/6J strain. PCR analysis of genomic DNA using primers 5F (5'-CCTGACTCTTCCTGAATC-3') and 8R (5'-GAGCCCAAAATGGTG-3') was used to detect the *Fgf21*$^{+}$ (216 bp) and *Fgf21*^{LoxP} alleles (288 bp). PCR analysis using primers FGF21del2F (5'-GCCAAGTGTCAGAACTG-3') and FGF21R9 (5'-CCGTGGTACCAGGAATGC-3') was used to detect the *Fgf21*^{LoxP} (1,211bp) and *Fgf21*$^{\Delta}$ alleles (298 bp).

Male mice (8 wks old) were fed a chow diet (Iso Pro 3000, Purina) or a HFD (F3282, Bioserve). Body weight was measured with a scale. Whole body fat and lean mass were non-invasively measured using ^{1}H-MRS (Echo Medical Systems). The mice were housed in a specific pathogen-free facility accredited by the American Association for Laboratory Animal Care (AALAC). The Institutional Animal Care and Use Committee (IACUC) of the University of Massachusetts approved all studies using animals.

Primary hepatocytes

Primary hepatocytes were isolated from mice using a modified 2-step perfusion method (Seglen 1976) using Liver Perfusion Media and Liver Digest Buffer (Invitrogen). Cells were seeded on plates (pre-coated (1 h) rat tail collagen I (BD Biosciences) in DMEM plus 10% FBS, 2mM sodium pyruvate, 1 μM dexamethasone, 100 nM insulin plus 2% penicillin/streptomycin. After attachment (2 h), the medium was removed and the hepatocytes were incubated (22 h) in maintenance medium (DMEM (4.5g/L glucose) supplemented with 10% FBS, 0.2% BSA, 2mM sodium pyruvate, 2% penicillin/streptomycin, 0.1 μM dexamethasone, 1nM insulin). Where indicated, the hepatocytes were treated (30 mins) without or with 10 ng/ml TNFα (R&D Systems) or were incubated (16 h) with the PPARα agonist (50 μM) WY14043 (Sigma), the JNK inhibitor (1 μM) JNK-in-8 (Millipore), or solvent (DMSO).

Supplementary References

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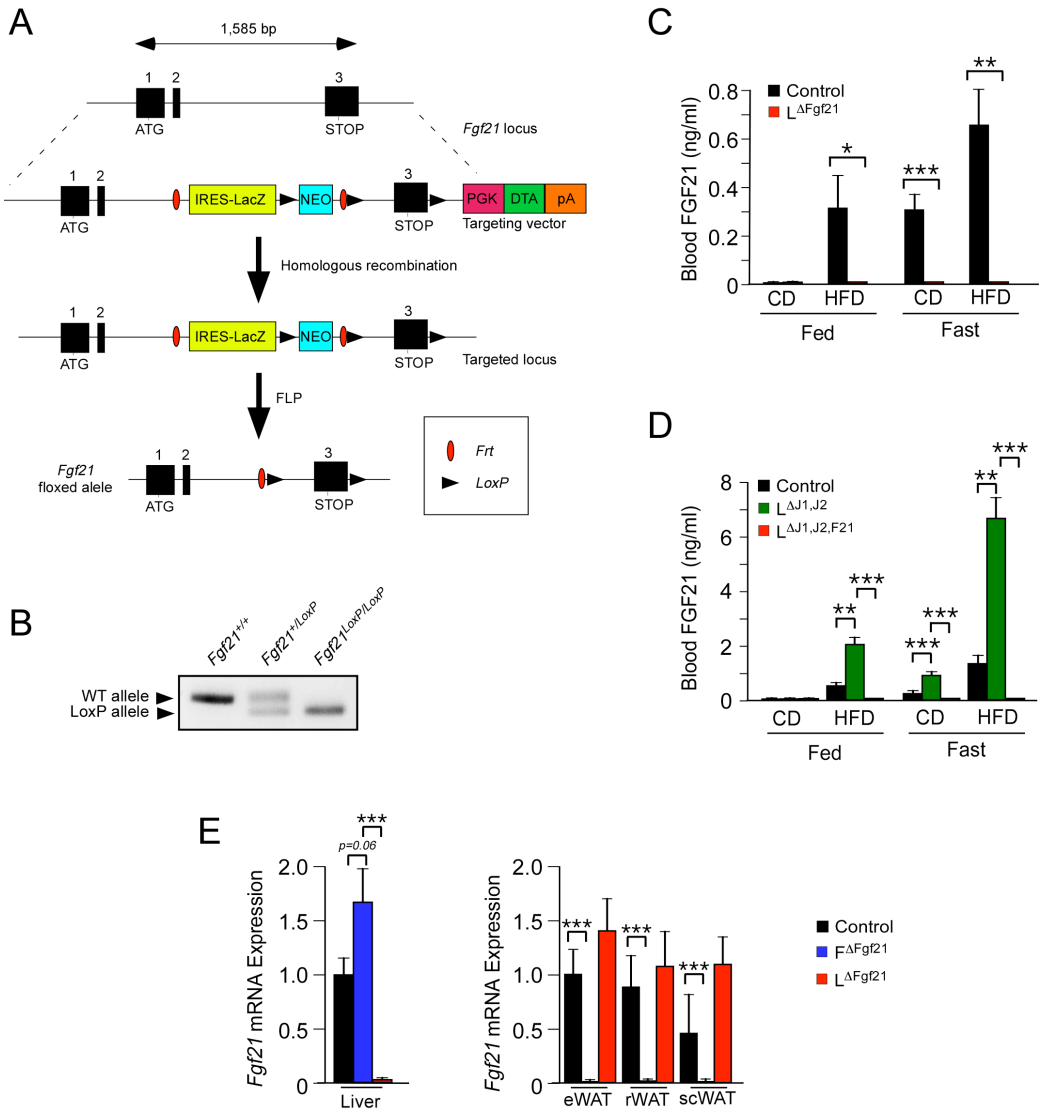

Figure S1. Liver is the major source of circulating FGF21, Related to Figure 3.

(**A**) The strategy employed to establish *Fgf21* conditional knock-out mice is illustrated in a cartoon.

(**B**) Genomic DNA from *Fgf21*$^{+/+}$ mice, *Fgf21*$^{+/LoxP}$ mice, and *Fgf21*$^{LoxP/LoxP}$ mice was examined by PCR to detect the wild-type and floxed *Fgf21* alleles.

(**C**) The blood concentration of FGF21 in CD-fed and HFD-fed control mice (*Alb-cre*$^{+}$) and liver-specific FGF21-deficient mice (*Alb-cre*$^{+}$ *Fgf21*$^{LoxP/LoxP}$) was measured by ELISA (mean ± SEM; n=10~12). The mice were fed *ad-libitum* or fasted overnight.

(**D**) The blood concentration of FGF21 was measured by ELISA (mean ± SEM; n=10~12). .

(**E**) *Fgf21* mRNA expression in liver, epididymal white adipose tissue (eWAT), retroperitoneal white adipose tissue (rWAT), and sub-cutaneous white adipose tissue (scWAT) of HFD-fed mice was measured by quantitative PCR analysis (mean ± SEM; n=8).

Statistically significant differences are indicated: * p<0.05; ** p<0.01; ***, p<0.001.

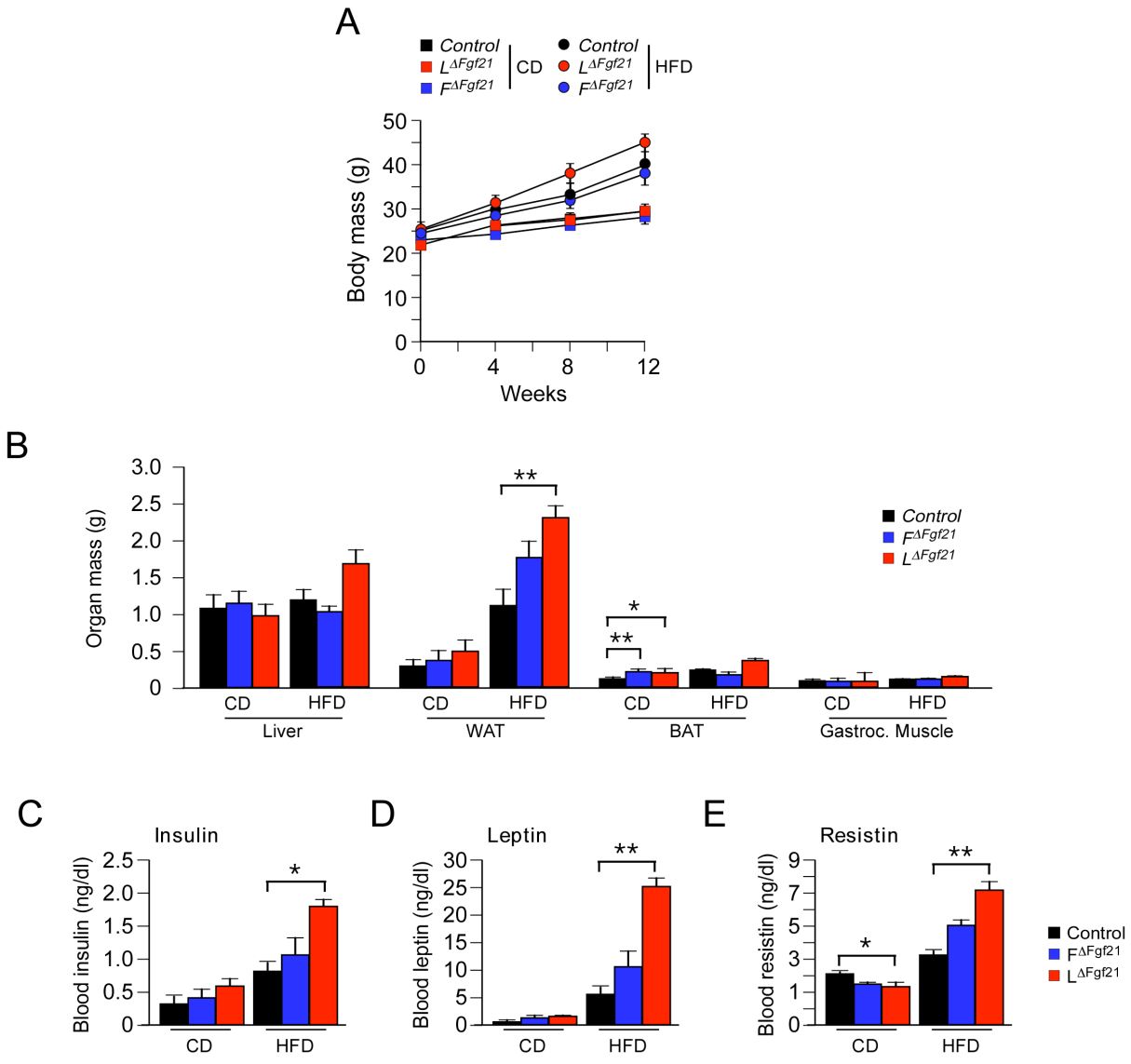

Figure S2. Deficiency of hepatic FGF21 promotes obesity, Related to Figure 3.

(**A**) The body mass of CD-fed and HFD-fed (12 wks) mice was measured (mean ± SEM; n=6~10).

(**B**) Organ mass of CD-fed and HFD-fed (12 wks) mice was measured (mean ± SEM; n=6~10).

(**C-E**) The blood concentration of insulin, leptin, and resistin in overnight fasted CD-fed and HFD-fed (12 wks) mice was measured (mean ± SEM; n = 6~10).

Statistically significant differences between control mice and mice with FGF21-deficiency are indicated: * $p<0.05$; ** $p<0.01$.

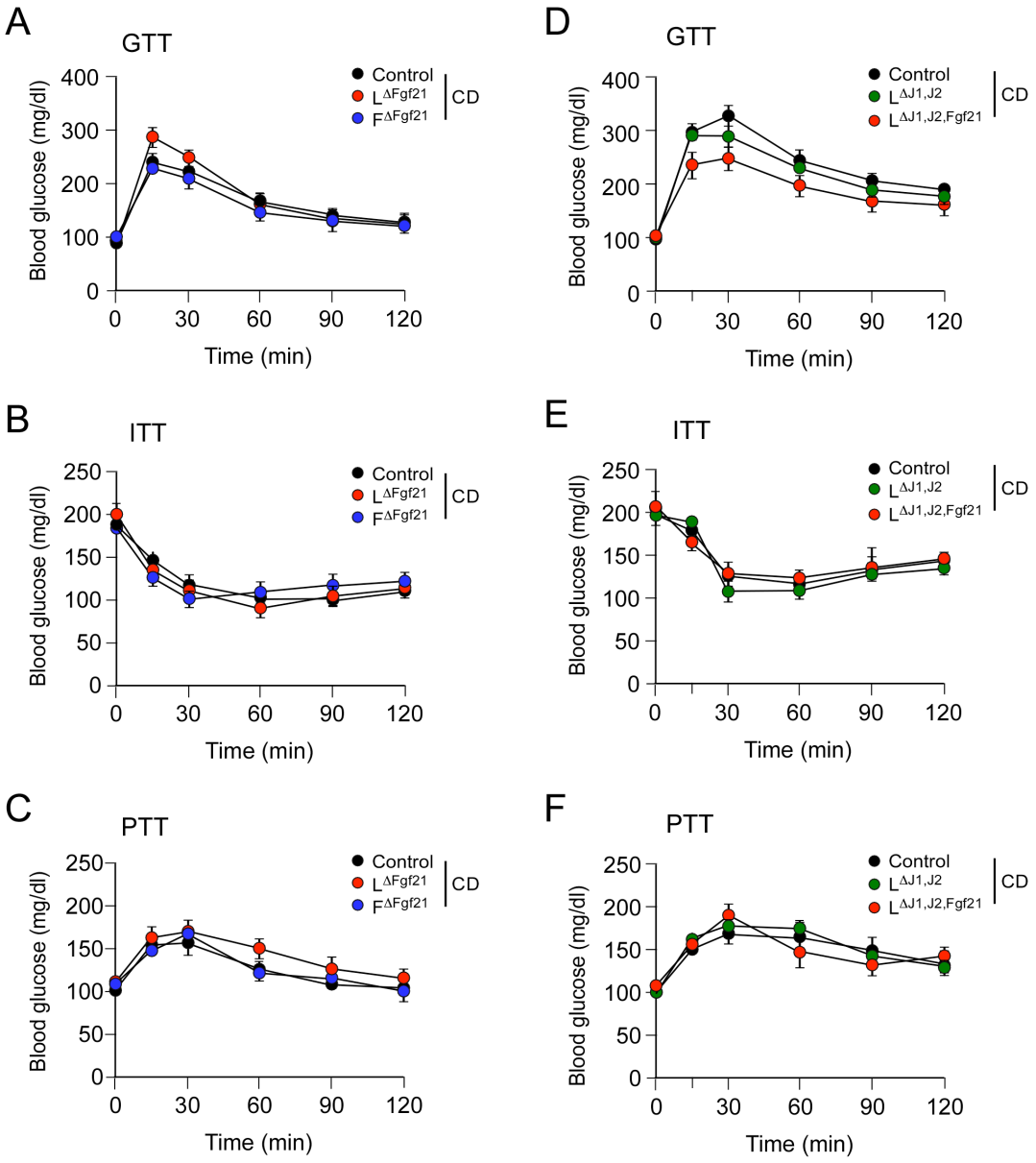

Figure S3. Effect of FGF21-deficiency on glycemic regulation in CD-fed mice, Related to Figures 3 and 4.

(**A**-**C**) Glucose tolerance (A), insulin tolerance (B), and pyruvate tolerance (C) tests using CD-fed (12 wks) control mice and mice with FGF21-deficiency in the liver ($L^{\Delta Fgf21}$) or adipose tissue ($F^{\Delta Fgf21}$) were performed (mean ± SEM; n = 6~10). No statistically significant differences between groups were detected.

(**D-F**) Glucose tolerance (D), insulin tolerance (E), and pyruvate tolerance (F) tests using CD-fed (12 wks) control mice and mice with liver-specific-deficiency of JNK ($L^{\Delta J1,J2}$) or JNK plus FGF21 ($L^{\Delta J1,J2,Fgf21}$) were performed (mean ± SEM; n = 6~10). No statistically significant differences between groups were detected.

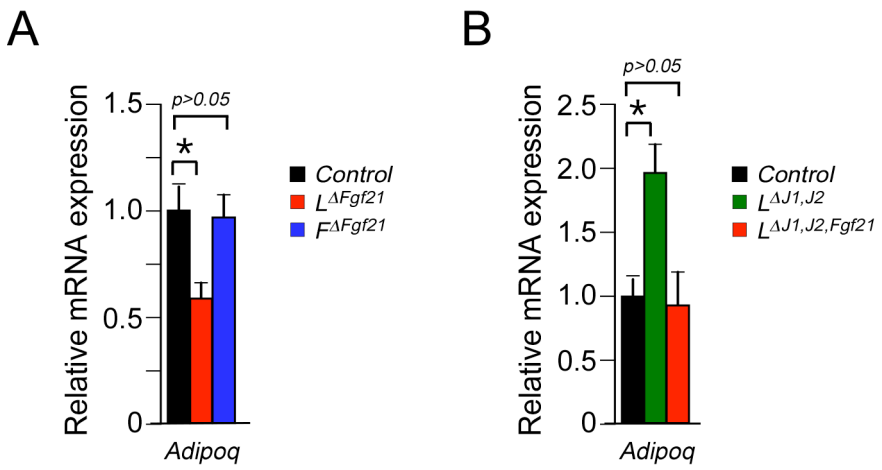

Figure S4. Effect of hepatic FGF21-deficiency on adiponectin expression, Related to Figure 4.

(**A**) The expression of *Adipoq* mRNA in the epididymal adipose tissue of CD-fed and HFD-fed (12 wks) Control mice (*Fgf21*$^{LoxP/LoxP}$) and mice with FGF21-deficiency in the liver (L$^{\Delta Fgf21}$) or adipose tissue (F$^{\Delta Fgf21}$) was measured by quantitative PCR (mean ± SEM; n=6~8).

(**B**) The expression of *Adipoq* mRNA in the epididymal adipose tissue of CD-fed and HFD-fed (12 wks) Control mice (*Alb-cre*$^{-/+}$) and mice with liver-specific deficiency of JNK (L$^{\Delta J1,J2}$) or JNK plus FGF21 (L$^{\Delta J1,J2,Fgf21}$) was measured by quantitative PCR (mean ± SEM; n=6~8).

Statistically significant differences are indicated: * p<0.05.