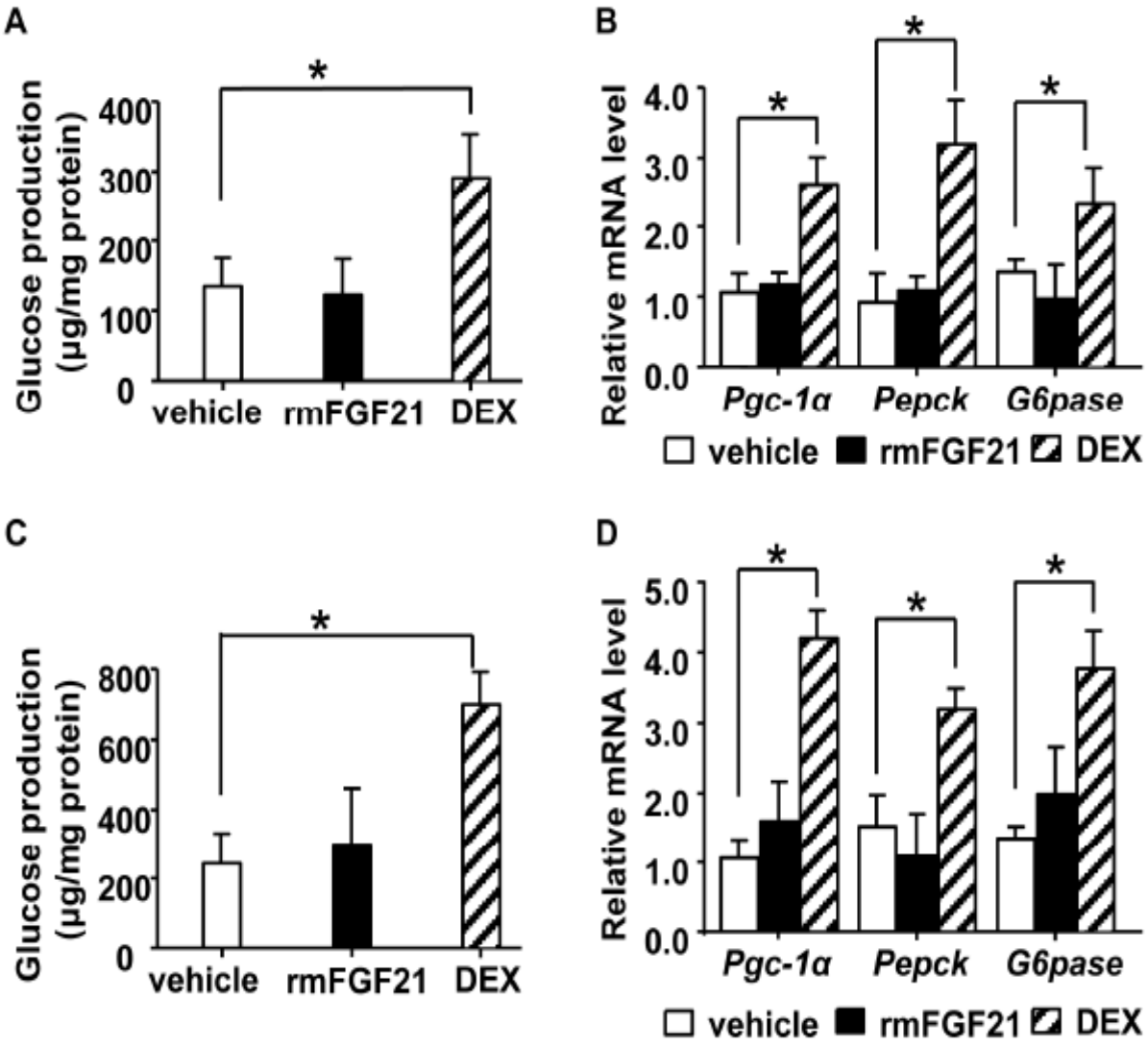
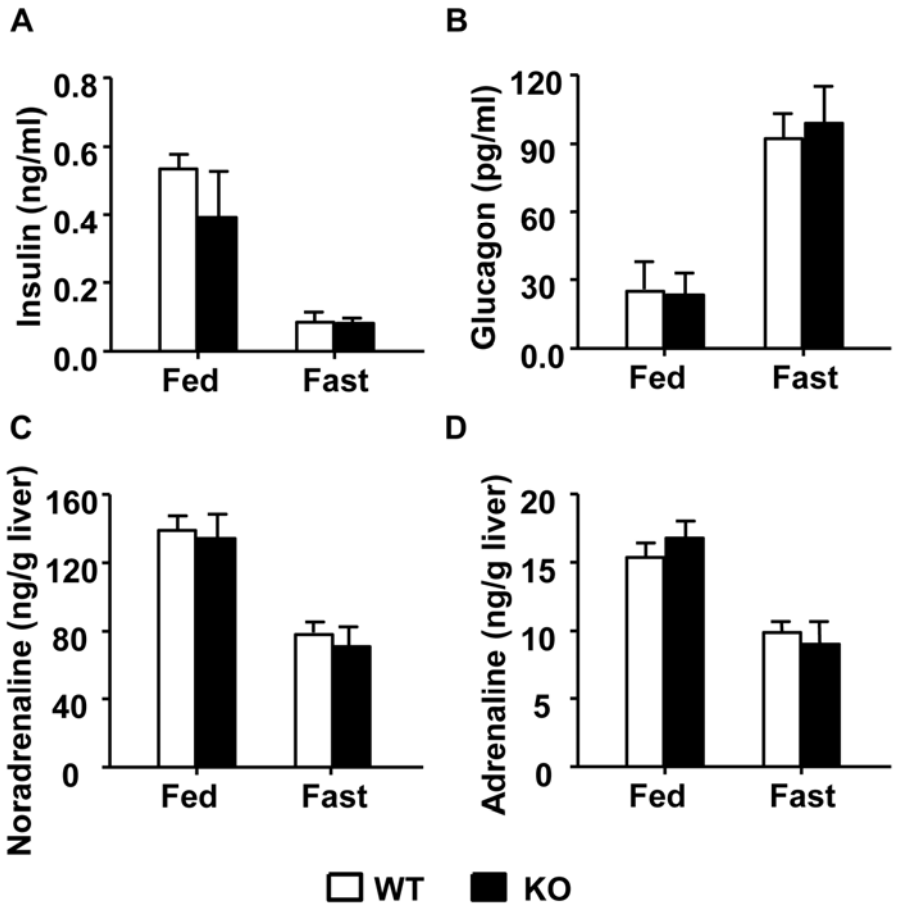
SUPPLEMENTARY DATA

Supplementary Figure 1. FGF21 does not exert direct effects on hepatic glucose production. The liver explants from C57BL/6J mice (A, B) or primary rat hepatocytes (C, D) were incubated with rmFGF21 (2 ug/ml), dexamethasone (DEX, 1μM) or vehicle for 24 hours in glucose free DMEM medium. (A, C). The glucose concentration in the conditioned medium. (B, D). The mRNA abundance of PGC-1α, PEPCK and G6Pase expressed as fold relative to vehicle-treated group. A similar effect of FGF21 and dexamethasone on expression of the three gluconeogenic genes was also observed in primary hepatocytes cultured in different glucose levels (0, 5 and 15 mM). *p<0.05 (n=5). Data were expressed as mean ± SEM.



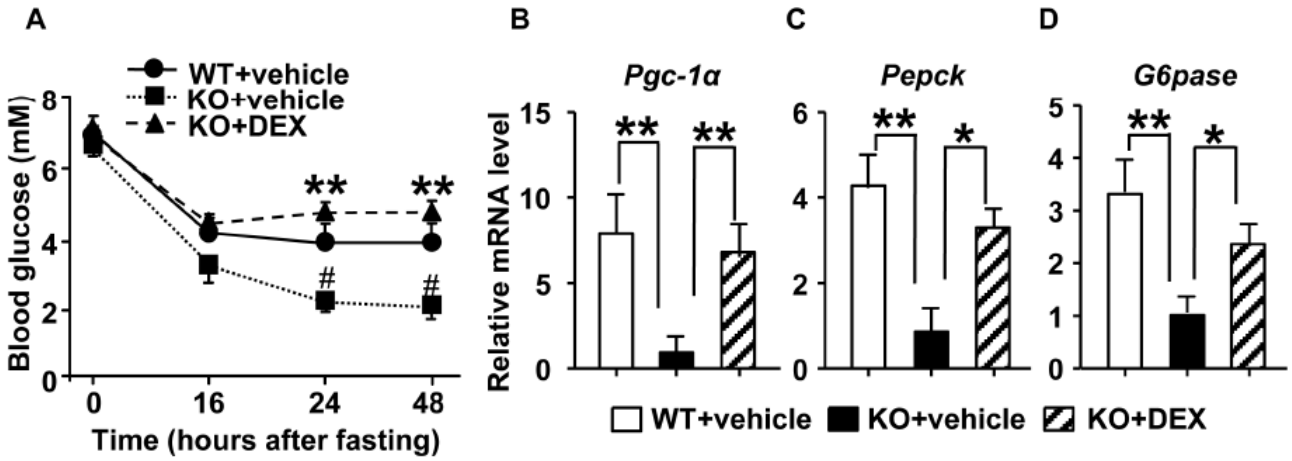
SUPPLEMENTARY DATA

Supplementary Figure 2. FGF21 deficiency does not alter serum levels of insulin, glucagon or hepatic levels of noradrenaline and adrenaline in mice. Serum levels of insulin (A), glucagon (B), hepatic noradrenaline (C) and adrenaline (D) in 10-week-old male FGF21-KO mice and WT littermates were measured at fed state or 24 hours after fasting. Data were expressed as mean ± SEM (n=8).



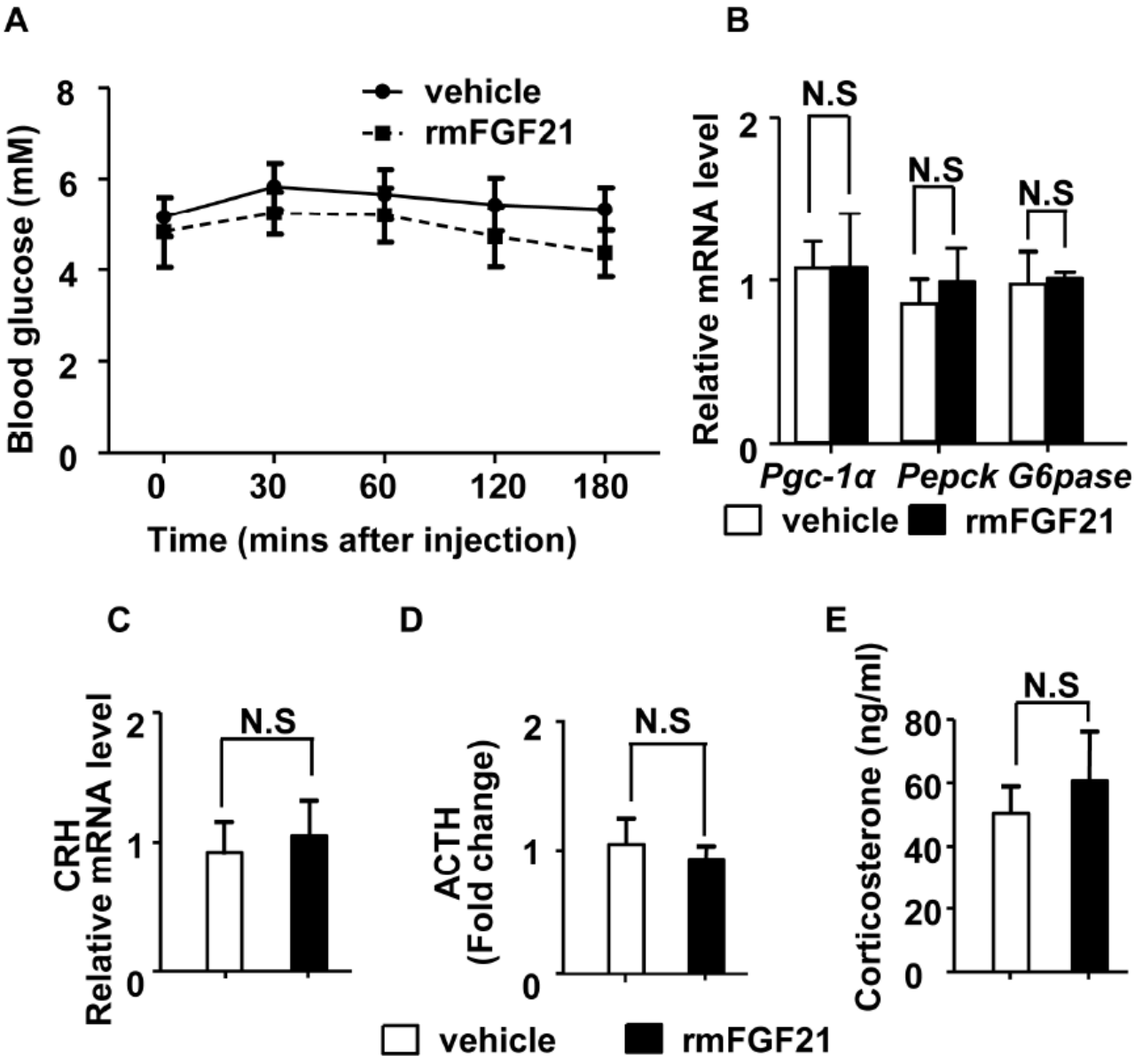
SUPPLEMENTARY DATA

Supplementary Figure 3. Dexamethasone reversed the impaired hepatic glucose production in FGF21 KO mice. 10-12-week-old FGF21 KO mice were treated with DEX (2mg/kg) or vehicle by intraperitoneal injection, followed by fasting for 48 hours. (A) Effects of dexamethasone (DEX) on blood glucose levels in FGF21 KO mice. **p<0.01 versus KO+vehicle group; #p<0.05 versus WT+vehicle group (n=6). (B-D) The hepatic mRNA abundance of PGC-1α, PEPCK and G6Pase as determined by real-time PCR. The livers were collected from mice treated with DEX or vehicle and fasted for 24 hours. *p<0.05; **p<0.01 (n=6). Data were expressed as mean ± SEM.



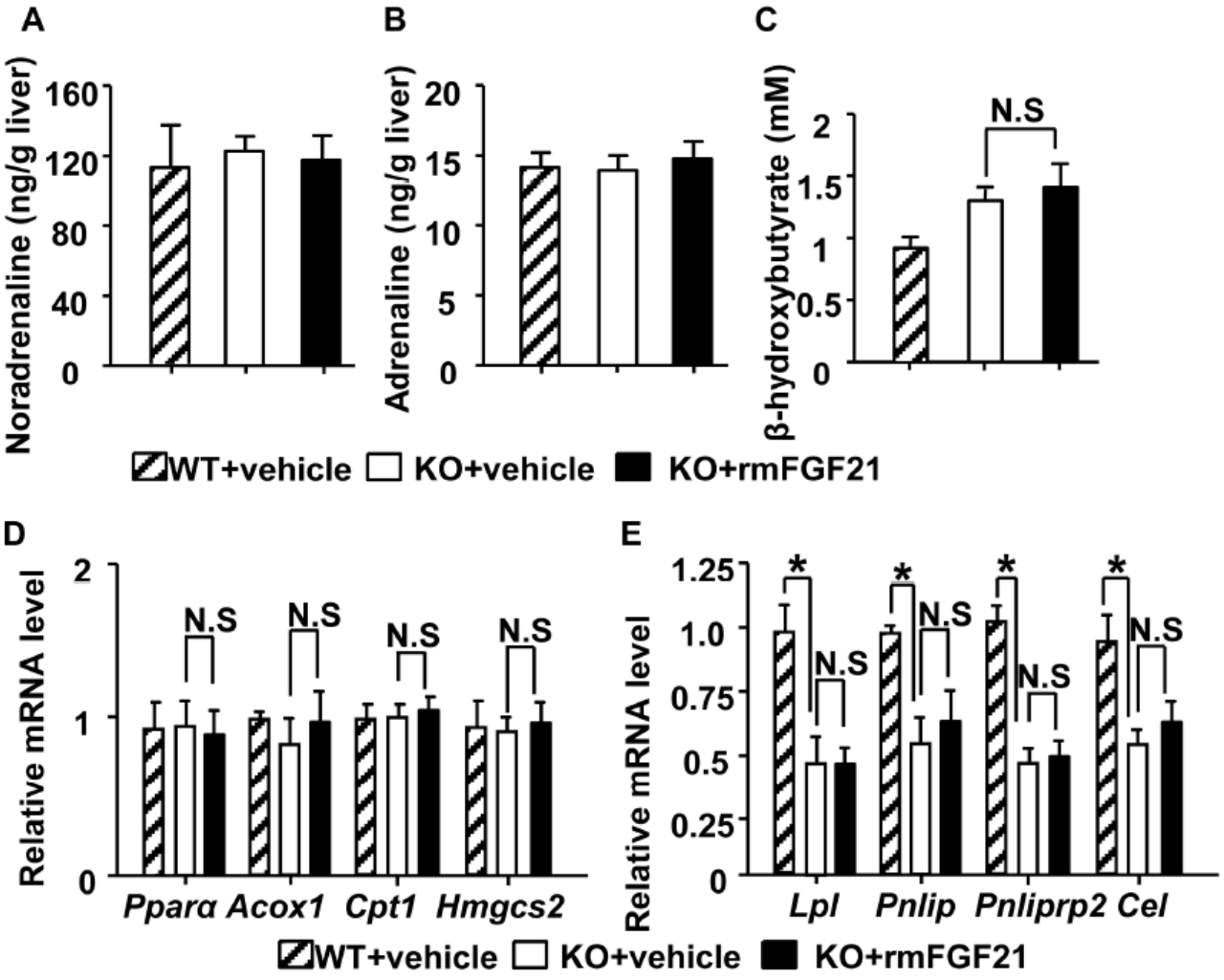
SUPPLEMENTARY DATA

Supplementary Figure 4. Peripheral administration of low-dose FGF21 does not affect the HPA axis or hepatic gluconeogenesis. FGF21 KO mice were treated with rmFGF21 (0.02μg/g) by intraperitoneal injection. (A) Blood glucose levels measured at different time points. (B) The mRNA abundance of PGC-1α, PEPCK and G6Pase in the liver, (C) the mRNA abundance of CRH in the hypothalamus, (D) plasma ACTH levels and (E) serum corticosterone levels were determined at three hours after treatment. N.S: not significant. Data were expressed as mean ± SEM (n=4).



SUPPLEMENTARY DATA

Supplementary Figure 5. Central administration of rmFGF21 has no effects on hepatic noradrenaline and adrenaline, plasma ketone body and expression of several genes involved in hepatic ketogenesis and lipid metabolism. 10-week-old male FGF21 KO mice were treated with rmFGF21 (0.02μg/g) or vehicle by intracerebroventricular (icv) injection. WT mice treated with vehicle were used as control. (A) noradrenaline levels and (B) adrenaline levels in livers. (C) Serum levels of β-hydroxybutyrate. (D and E) The hepatic mRNA abundance of ketogenic genes including peroxisome proliferator activated receptor alpha (Pparα), acyl-CoA oxidase 1 (Acox1), carnitine palmitoyl transferase 1 (Cpt1) and 3-hydroxy-3-methylglutaryl CoA synthase 2 (Hmgcs2); Genes involved in regulation of hepatic lipid metabolism, including lipoprotein lipase (Lpl), pancreatic lipase (Pnlip), pancreatic lipase-related protein 2 (Pnliprp2) and carboxyl ester lipase (Cel) were determined by real-time PCR. Data were expressed as mean ± SEM. * $p<0.05$, (n=6).



SUPPLEMENTARY DATA

Supplementary Figure 6. FGF21 activates the ERK1/2-CREB signaling pathway to stimulate CRH production. The protocol of treatment for mice is the same as described in Figure 6. Mouse hypothalamic slices (paraventricular nucleus, adjacent to the third ventricle) were immunostained for p-ERK1/2, p-CREB and CRH as indicated. The samples were taken at 30 minutes after the injection of rmFGF21 for p-ERK1/2 and p-CREB, and 3 hours for CRH. Representative images of immunostained slices are shown, n=6.