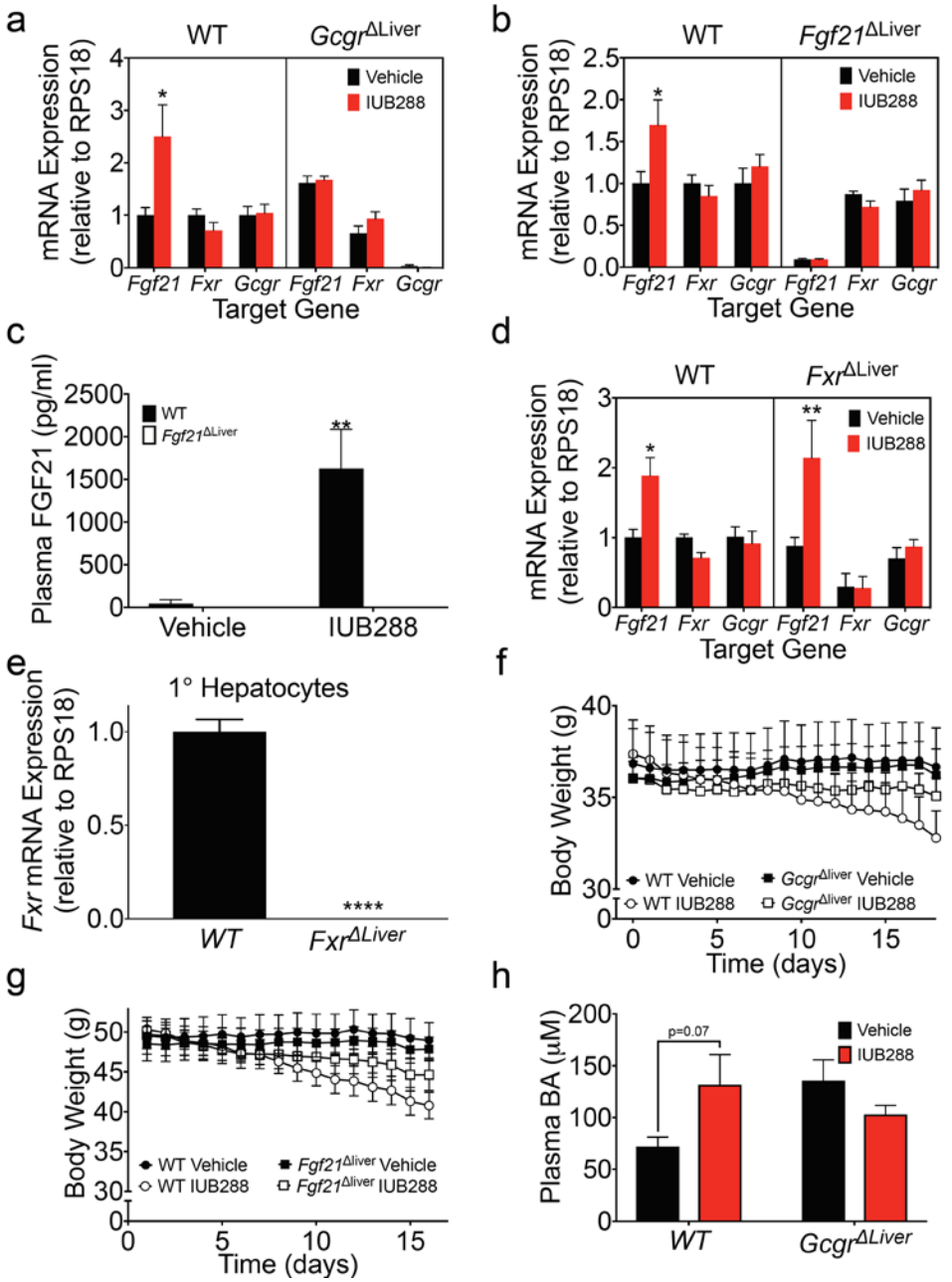
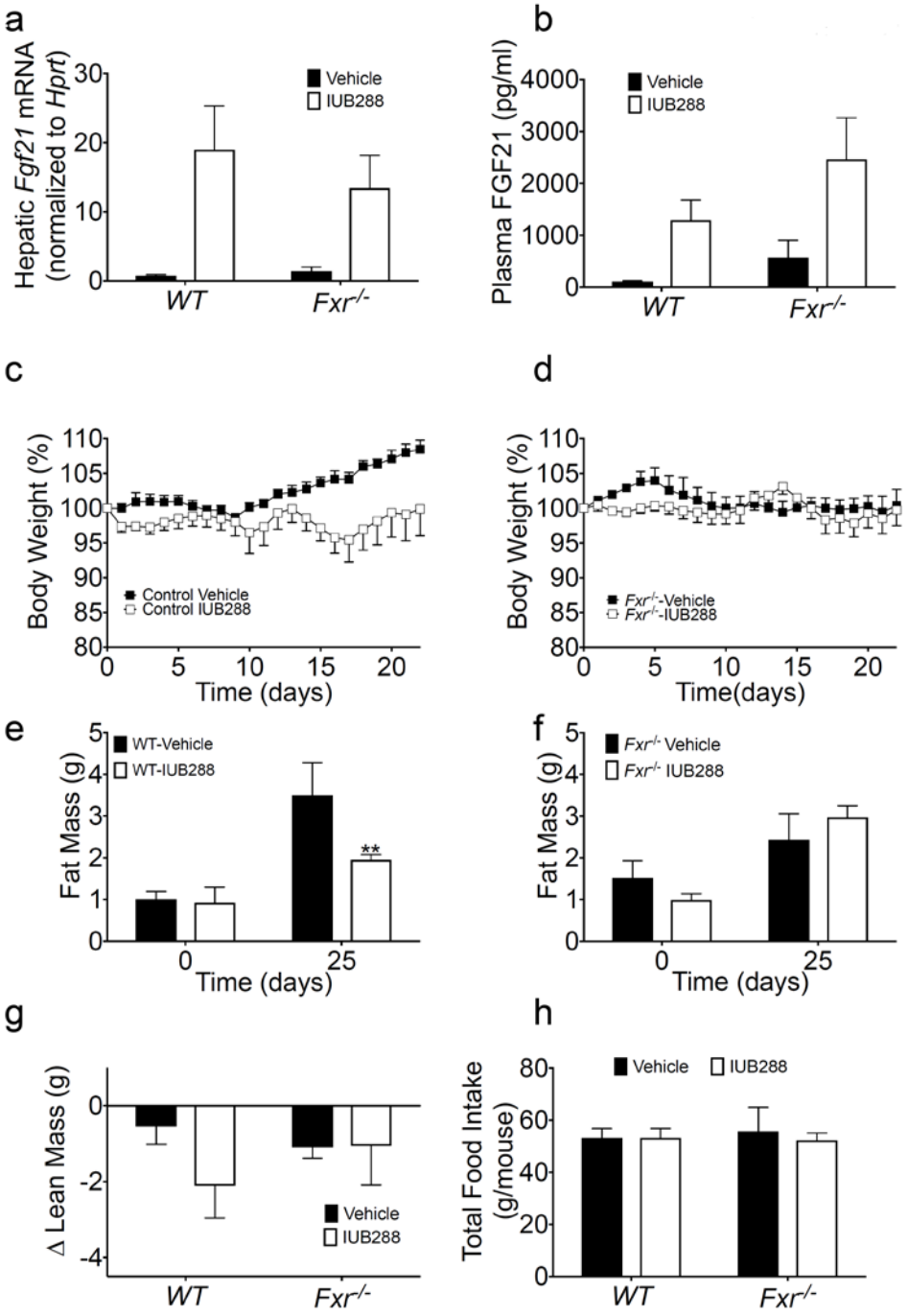
SUPPLEMENTARY DATA

Supplementary Figure S1. Model validation and GcgR agonism in GcgR and Fgf21 mice. mRNA expression of *Gcgr*, *Fgf21*, and *Fxr* in GcgRΔLiver, Fgf21ΔLiver, FxrΔLiver, and littermate Control mice (a, b, and d, n=6-10 mice/group, see Figures 2-4). Plasma FGF21 in 8-week-old, chow fed Fgf21ΔLiver and littermate Control (WT) mice following 5d Vehicle or IUB288 treatment (c, 10 nmol/kg IUB288, n=4- 10 mice/group). *Fxr* mRNA expression in primary hepatocytes isolated from 8-10 week old, chow fed FxrΔLiver or littermate control (WT) mice (e). Body weight of DIO WT and GcgRΔLiver mice (f, n=8-12 mice/group) or WT and Fgf21ΔLiver mice (g, n=5-7 mice/group) following daily GcgR agonism (10 nmol/kg IUB288). Plasma bile acid levels in IUB288-treated DIO WT and GcgRΔLiver mice after 2 h fast (h, n=8-12 mice/group, see Figure 2). All data are represented as mean +/- SEM.



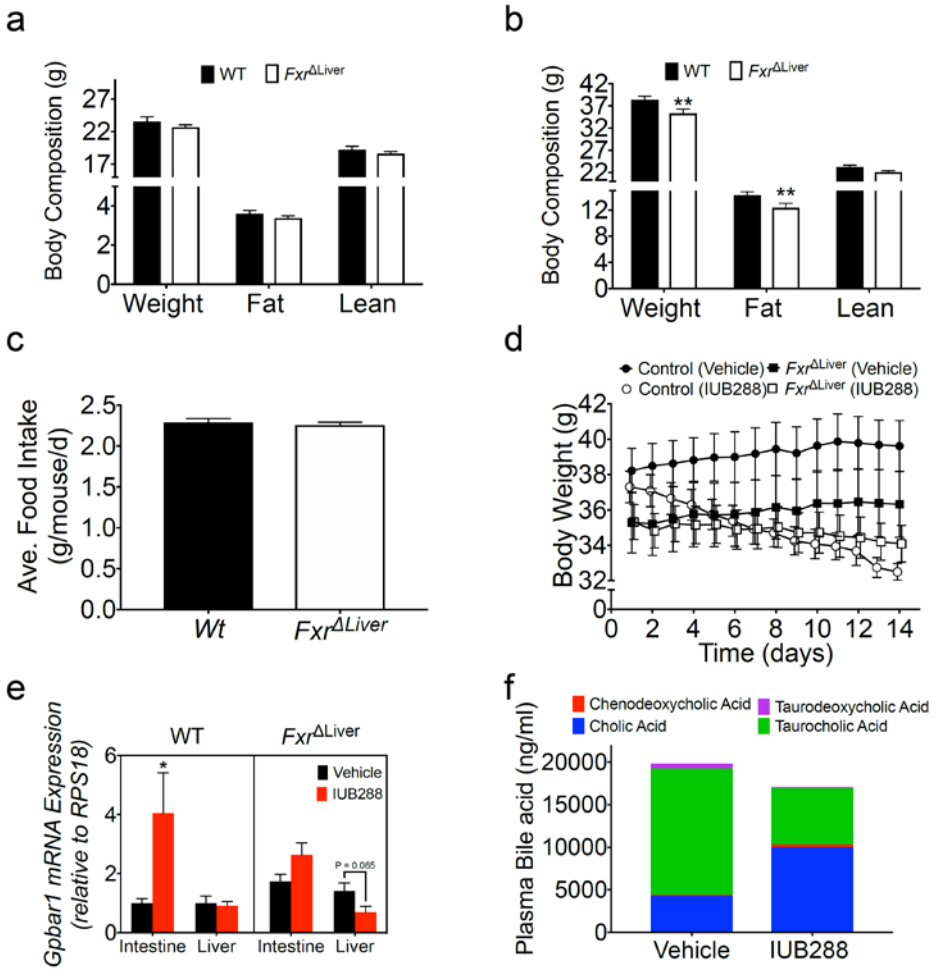
SUPPLEMENTARY DATA

Supplementary Figure S2: GcgR agonism and energy balance in FXR-/- mice. Hepatic *Fgf21* mRNA expression (a) and plasma levels (b) in HF-fed WT and FXR-/- mice. Body weight (%) and fat mass of HF-fed WT (c and e) or FXR-/- mice (d and f) following daily GcgR agonism (10 nmol/kg IUB288). Change in lean mass (g) and total food intake (h) in HF-fed WT or FXR-/- mice. All data are represented as mean +/- SEM (n=3-8 mice/group). **p< 0.01. Male, WT and Fxr-/- mice were placed on HFD at 8-10 weeks old concurrent with IUB288 treatment..



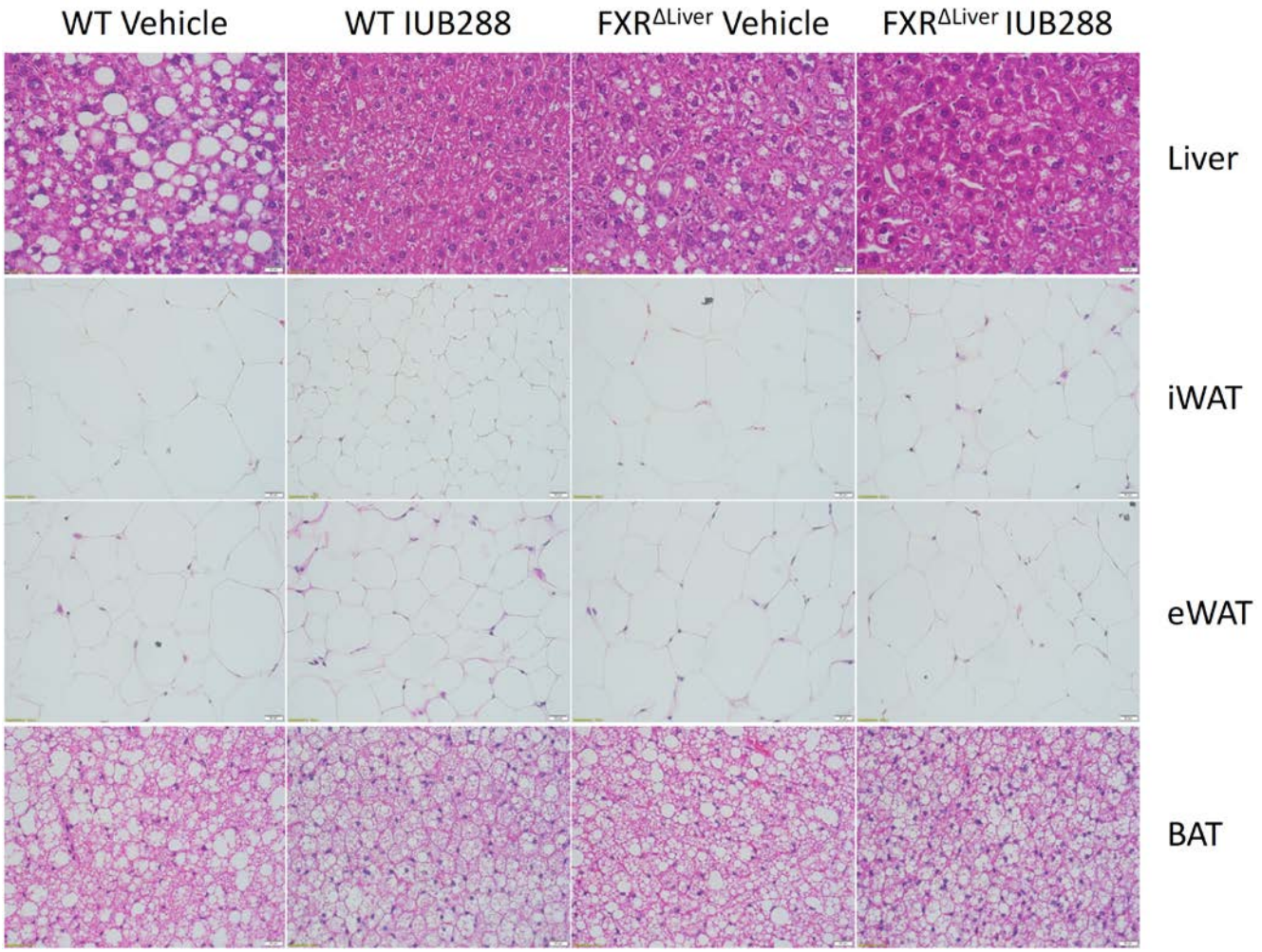
SUPPLEMENTARY DATA

Supplementary Figure S3. DIO and GcgR agonism in FXRΔLiver mice. Body composition before (a) and after (b) HF-feeding in WT and FXRΔLiver mice (n=13-15 mice/group). Average food intake (c) during HF-fat feeding in WT and FXRΔLiver mice (n=13-15 mice/group). Body weight (d) during daily GcgR agonism (10 nmol/kg IUB288) in WT and FXRΔLiver mice (n=8-10 mice/group). Intestine and liver *Gpbar1*/*Tgr5* mRNA expression (e) in 14d IUB288-treated DIO WT and FxrΔLiver mice. Plasma bile acid profile (f) in male FxrΔLiver mice following 16d GcgR agonism. *p< 0.05, **p< 0.01. Male, WT and FxrΔLiver mice were placed on HFD at 8-10 weeks old and maintained on HFD for 10 weeks to induce DIO prior to treatment.



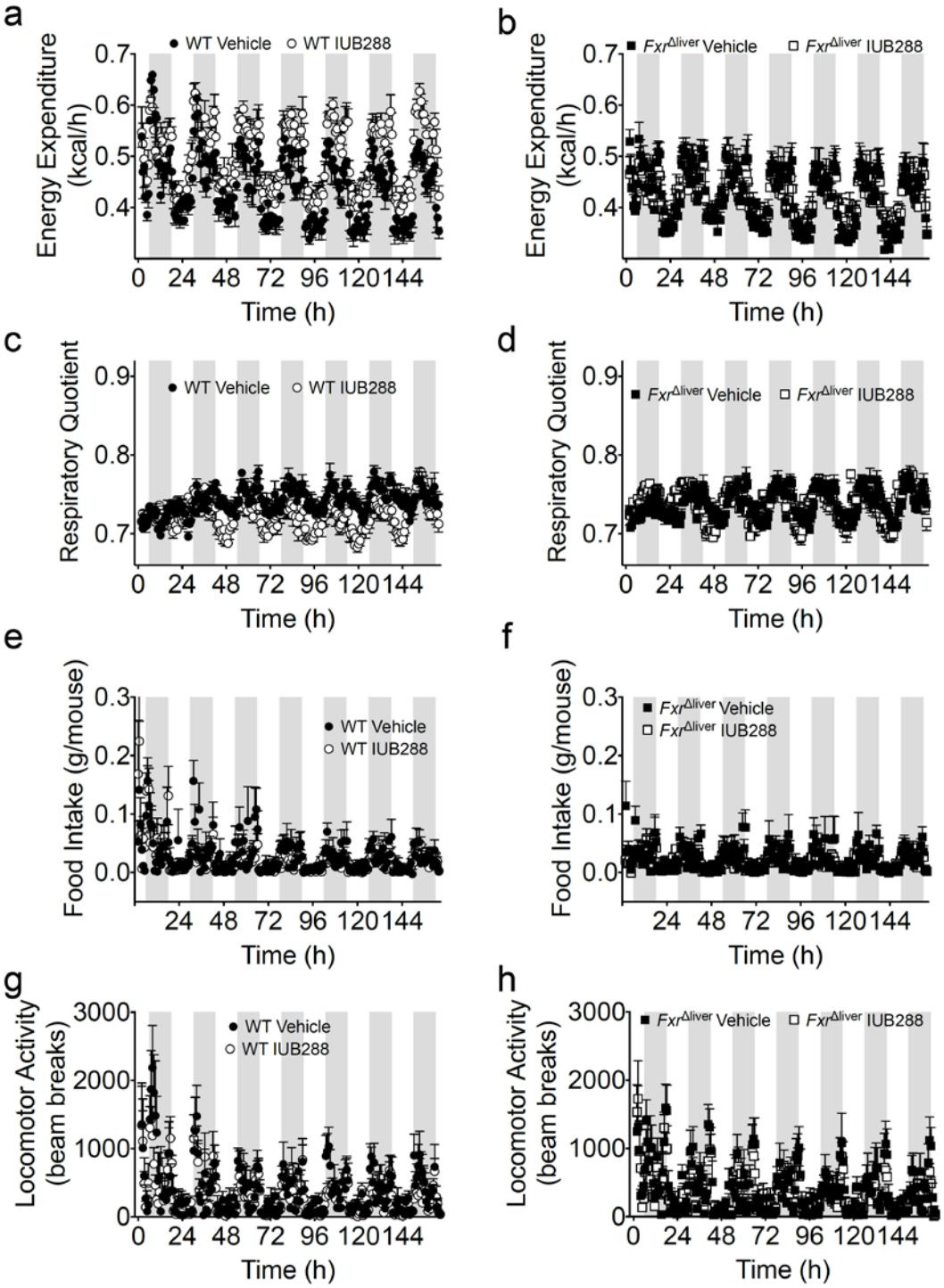
SUPPLEMENTARY DATA

Supplementary Figure S4. Liver and Adipose Tissue morphology following GcgR agonism in FXRΔLiver mice. Representative haemotoxylin and eosin (H & E) staining of liver, inguinal white adipose tissue, ependymal white adipose tissue, and interscapular brown adipose tissue following 14d IUB288 treatment. Male, WT and FxrΔLiver mice were placed on HFD at 8-10 weeks old and maintained on HFD for 10 weeks to induce DIO prior to treatment. Scale bars are 20 μm in length.



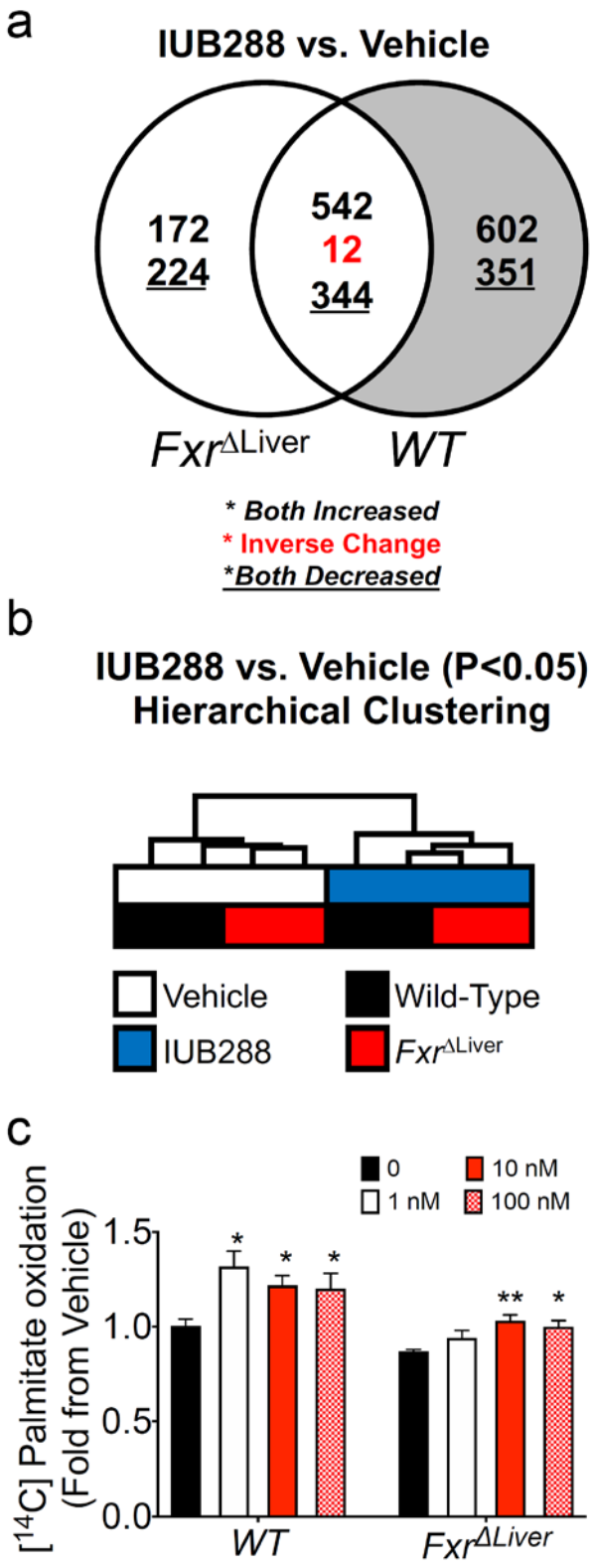
SUPPLEMENTARY DATA

Supplementary Figure S5. 7 d indirect calorimetry during GcgR agonism in FxrΔLiver mice. Energy expenditure (EE, a-b), respiratory quotient (RQ, c-d), food intake (e-f), and locomotor activity (g-h) measured during 7 d indirect calorimetry analysis (in DIO WT (a,c,e, and g) and FxrΔLiver mice (b,d,f, and h) during daily GcgR agonism (10 nmol/kg IUB288). IUB288 administered via subcutaneous injection 1hr prior to dark phase (ZT11). All data are represented as mean +/- SEM (n=6 mice/group, see Figure 5).



SUPPLEMENTARY DATA

Supplementary Figure S6. Transcriptional Analysis of IUB288 Treatment in FxrΔLiver and WT Mice. (a) Venn diagram illustrating the selection of FXR-dependent DEGs (shaded). (b) Hierarchical clustering of IUB288 vs. Vehicle comparison ($p < 0.05$). (c) [14C] Palmitate oxidation in primary hepatocytes isolated from DIO WT andFxrΔLiver mice and treated with glucagon for O.N. treatment followed by 3 hr incubation with radioactive substrate.



SUPPLEMENTARY DATA

Supplementay Table 1. qPCR primers

Gene	Forward (5'-3')	Reverse (5'-3')
Gcgr	GCCAGCGAGGTCTCCATA	ACATCATTCACCTTCTTGTGG
Fgf21	CTG CTG GGG GTC TAC CAA G	CTG CGC CTA CCA CTG TTC C
Scl10a1	GCCACACTATGTACCCTACGTCCTC	GAATGTAGCCCATCAGGAAGCCAGTG
Cyp27a1	GAAGGACCACCGAGACCACAAGG	CGT TTA AGG CAT CCG TGT AGA GCG
Hmgcr	GTGTTCAAGGAGCATGCAAAG	AGCCATCACAGTGCCACATAC
Cyp7a1	GGGATTGCTGTGGTAGTGAGC	GGTATGGAATCAACCCGTTGTC
Fxr	CACAGCGATCGTCATCCTCTCT	TCTCAGGCTGGTACATCTTGCA
Gpbar1/Tgr5	AAGAGCCAAGAGGGACAATC	GTAGCTGCTGCTTCCCTAAT
Ppargc1a	CCCTGCCATTGTTAAGACC	TGCTGCTGTTCCTGTTTTC
Ppara	AGCAGTGCTGGCTACCTTCAA	AATATGTAGCCACCCCCTTGG
Scd1	TCAGAAACACATGCTGATCCTCAT	TGGGTGTTTGCGCACAAG
Srebp1c	GAGGACCTTTGTCATTGGCTG	TACAGAGCAAGAGGGTGCCAT
Hprt	AAGGAGATGGGAGGCCAT	GTTGAGAGATCATCTCCACCAAT
Rps18	TTCTGGCCAACGGTCTAGACAAC	CCAGTGGTCTTGGTGTGCTGA
Ppia	CAGACGCCACTGTCGCTT T	TGTCTTTGGAACTTTGTCTG