Supplemental Information

Loss of FXR Protects against Diet-induced Obesity and Accelerates Liver Carcinogenesis in *ob*/*ob* Mice

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Materials and Methods

24-hour Measurement of Energy Expenditure and Activity. 24-hr activity and energy expenditure was measured in each mouse during baseline and after 12 weeks on the high-fat diet. Energy expenditure was measured in groups of 4 using a 4-chamber system. All acclimation and measurements of energy expenditure were completed in an isolation chamber kept at a thermoneutral temperature for mice (26°C±0.5°C) 40-48 hours before the start of the measurement, mice were measured for body composition and housed in the acclimation chamber, a Lexan Polycarbonate enclosure (20.2 cm long x 10.4 cm wide x 12.5 cm high) with a raised perforated floor (2.7 cm high) over absorbent paper (<1 mm thick). After the acclimation period, at approximately 0800 EST, mice were weighed and placed back into the acclimation cage with pre-weighed food. The cage tops were replaced with one allowing for and air-tight seal for calorimetric measurement. Using Columbus Instruments hardware and Oxymax software (Columbus Instruments, Columbus, OH), gas exchange was measured in mice every 30 seconds. Room air was referenced after every 60 samples, with reference and cage settle times of 90 sec. Room

air was pumped into the mouse calorimetry cages at 0.472-0.600 liters per minute (LPM; larger mice need a higher air flow, but all 4 mice measured at one time were given the same air flow), and cage air was sampled at 0.4 LPM. Physical activity was measured simultaneously using 16 infrared sensors spread over 19 cm. We measured horizontal, vertical (rearing; 16 beams over 19 cm, 3.35 cm vertical distance between lower and upper rows of sensors), and ambulatory (non-stationary counts) activity, all in the x-axis. Activity measures continued during the calorimetry reference period. Mice were removed from the cages and calorimetry and activity data were saved the following day; data from noon on day 1 to noon on day 2 (EST) were selected for analysis as the first hour or more of data may be compromised from experimenter handling and lack of air equilibrium in the calorimetry cages.

VO_{2max} Treadmill Exercise Test. Each mouse underwent testing for treadmill VO_{2max} during baseline and after 12 weeks on the high-fat diet. Mice were weighed and placed into the treadmill, which was then sealed. Air was delivered to the mice at 0.6-0.9LPM and sampled at 0.4 LPM. Mice were allowed to rest in the treadmill enclosure for 20 min; energy expenditure was measured during this time and throughout the test, ending roughly 4-5 min after the end of the treadmill endurance test. To start the test, the mouse ran on the treadmill at 10 m/min at 0° slope for 5 min. The treadmill slope was increased to 15° for 5 min, and then changed to 25° for 2 min. After this, the speed was increased to 15 m/min for 2 min, then increased 2 m/min every 2 min thereafter until the mouse reached exhaustion. For the ob/ob mice, the procedure was modified to accommodate their limitations: 20 min rest; 6 m/min at 0° slope for 5 min; then 2 min each for 6 m/min

at 10°, 7 m/min at 10°, 7 m/min at 15°, 8 m/min at 15°, 8 m/min at 20°, and 9 m/min at 20° (the peak level of difficulty attained by any ob/ob mouse).

Figure Legends

Supplemental Fig. 1 Loss of FXR in *Ldlr*$^{-/-}$ mice has no effect on food intake. *Ldlr*$^{-/-}$ mice and *Ldlr*$^{-/-}$*Fxr*$^{-/-}$ mice were fed a Western diet (n=8 per group). Food intake was measured and expressed as grams per day per mouse (g/d/mouse).

Supplemental Fig. 2 Loss of FXR in *Ldlr*$^{-/-}$ mice does not affect glucose tolerance. (A) Representative image of H & E staining of WAT from each genotype. (B) *Ldlr*$^{-/-}$ mice and *Ldlr*$^{-/-}$*Fxr*$^{-/-}$ mice were fed a Western diet (n=8 per group). After 9 weeks, glucose tolerance test was performed after an overnight fast. (C) Plasma bile acid levels were determined after fed a Western diet for 16 weeks (n=8 per group).

Supplemental Fig. 3 Male *Fxr* $^{-/-}$ mice have unchanged energy expenditure. Male wild-type and *Fxr*$^{-/-}$ mice were fed a high fat diet for 14 weeks (n=8 per group). (A-C) O_2 consumption (A), CO_2 production (B), and heat production (C) were measured.

Supplemental Fig. 4 *Fxr* $^{-/-}$ mice have increased plasma lipid levels and unchanged gene expression in brown adipose tissue. Female wild-type and *Fxr*$^{-/-}$ mice were fed a high fat diet for 14 weeks (n=8 per group). mRNA levels in the liver (A) and brown adipose tissue (B) were determined by qRT-PCR. Hepatic cholesterol levels (C) and plasma triglycerides (D) and cholesterol (E) were quantified. *P<0.05, ** P<0.01

Supplemental Fig. 5 Loss of FXR in *ob* /*ob* mice does not affect fat content or oxygen consumption on a chow diet. The double knockout ($ob^{-/-}Fxr^{-/-}$) mice and their controls ($ob^{-/-}Fxr^{+/+}$) were fed a chow diet (n=7-8 mice per group). The fat content (A), O_2 consumption (B), CO_2 production (C), and respiration quotient (D) were determined.

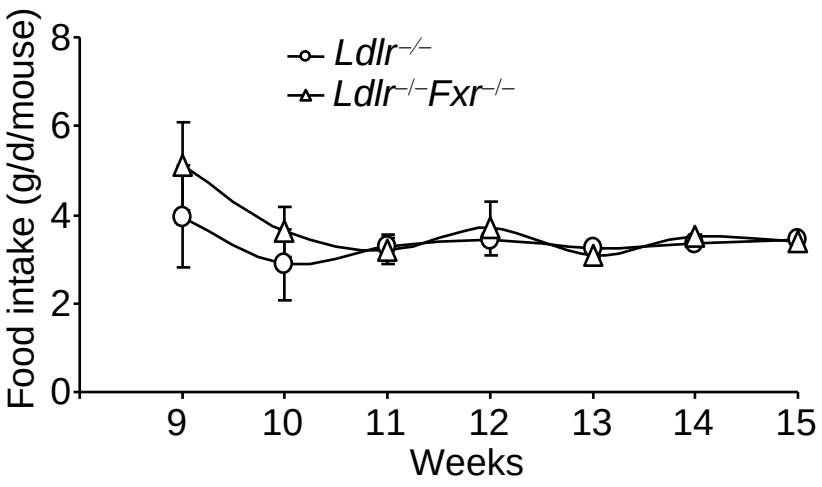
Supplemental Fig. 6 Gene expression profile in the liver and skeletal muscle of male $Fxr^{-/-}$ mice. Male wild-type mice and male $Fxr^{-/-}$ mice were fed a high fat for 14 weeks (n=8 mice per group). The mRNA levels in the liver (A) and skeletal muscle (B) were quantified by qRT-PCR. *P<0.05, ** P<0.01

Supplemental Fig. 7 FXR protein levels are not detectable in WAT of wild-type mice. (A) Western blot assays were performed to determine the expression of FXR in the liver, skeletal muscle and white adipose tissue (WAT) of wild-type mice. (B) Fat content was determined in chow-fed wild-type and $Fxr^{-/-}$ mice using a minispec NMR analyzer (n=8 per group).

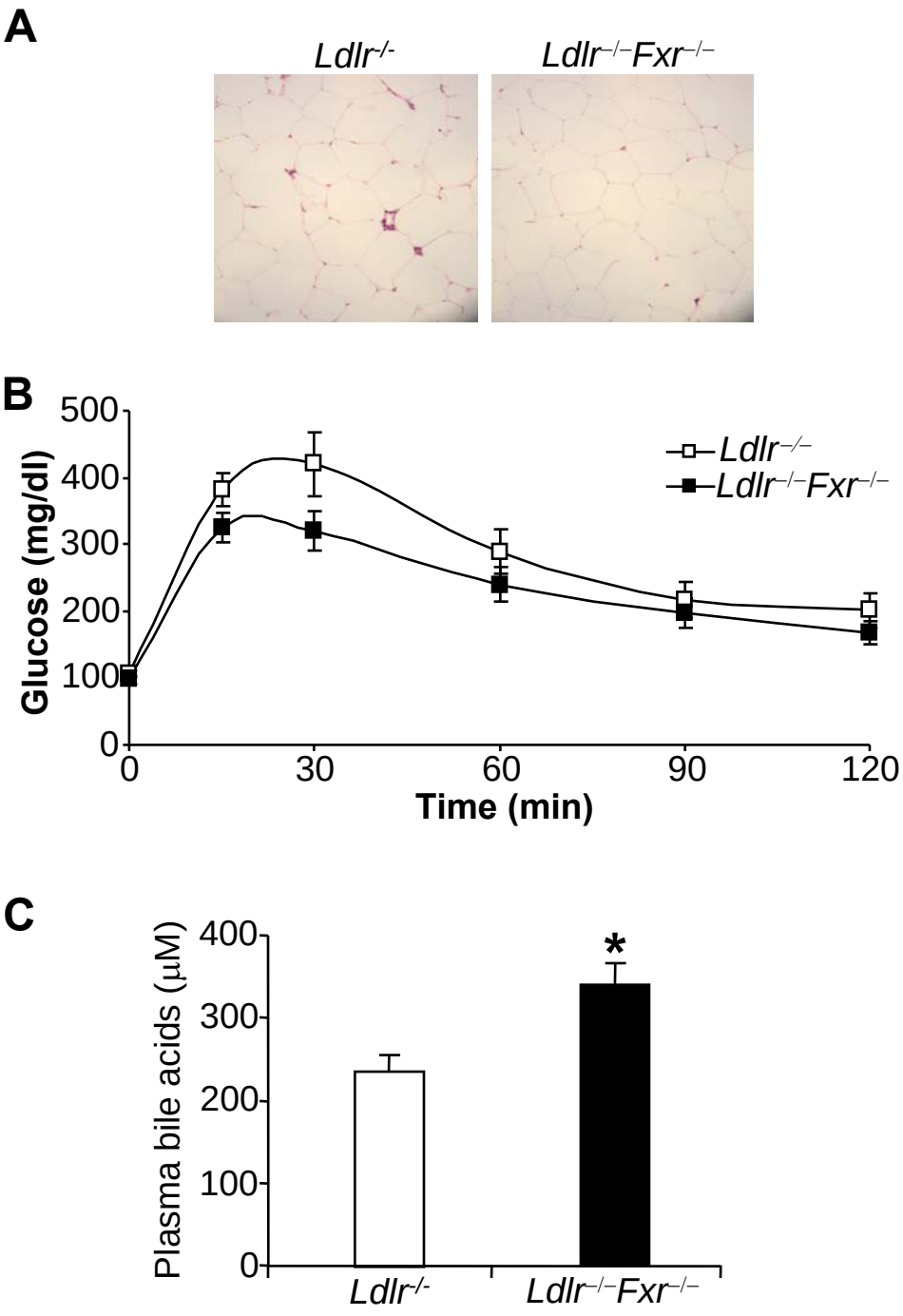
Supplemental Table 1. Physical activity of wild-type and *Fxr* $^{-/-}$ mice fed a high-fat diet. Wild-type and $Fxr^{-/-}$ mice were fed a high fat diet for 14 weeks (n=8 per group). Physical activities were measured.

Supplemental Table 2. Physical activity of *ob* $^{-/-}$*Fxr*$^{+/+}$mice and *ob*$^{-/-}$*Fxr*$^{-/-}$ mice. *ob*$^{-/-}$*Fxr*$^{+/+}$mice and *ob*$^{-/-}$*Fxr*$^{-/-}$ mice were fed a chow diet (n=3-5 per group). Physical activities were measured.

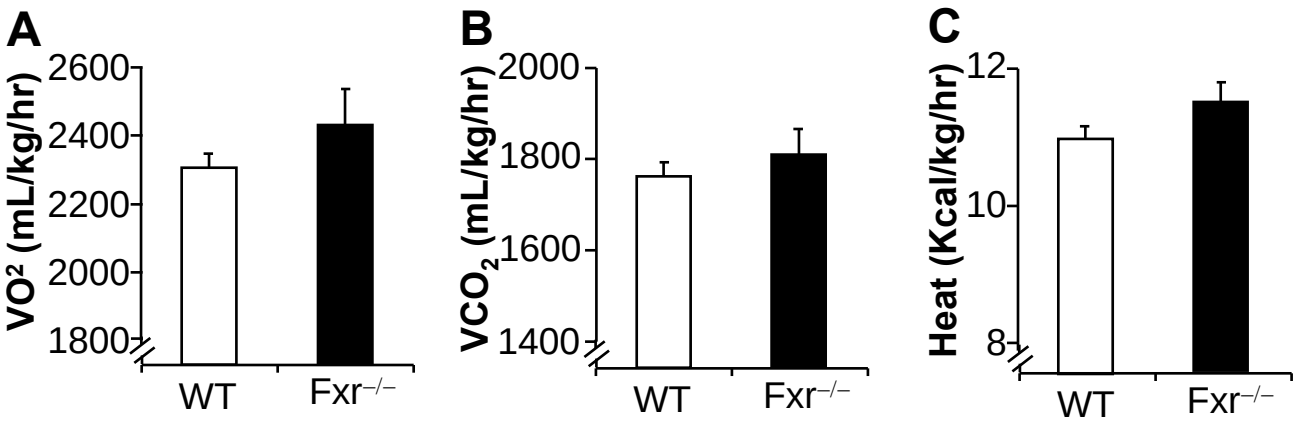
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



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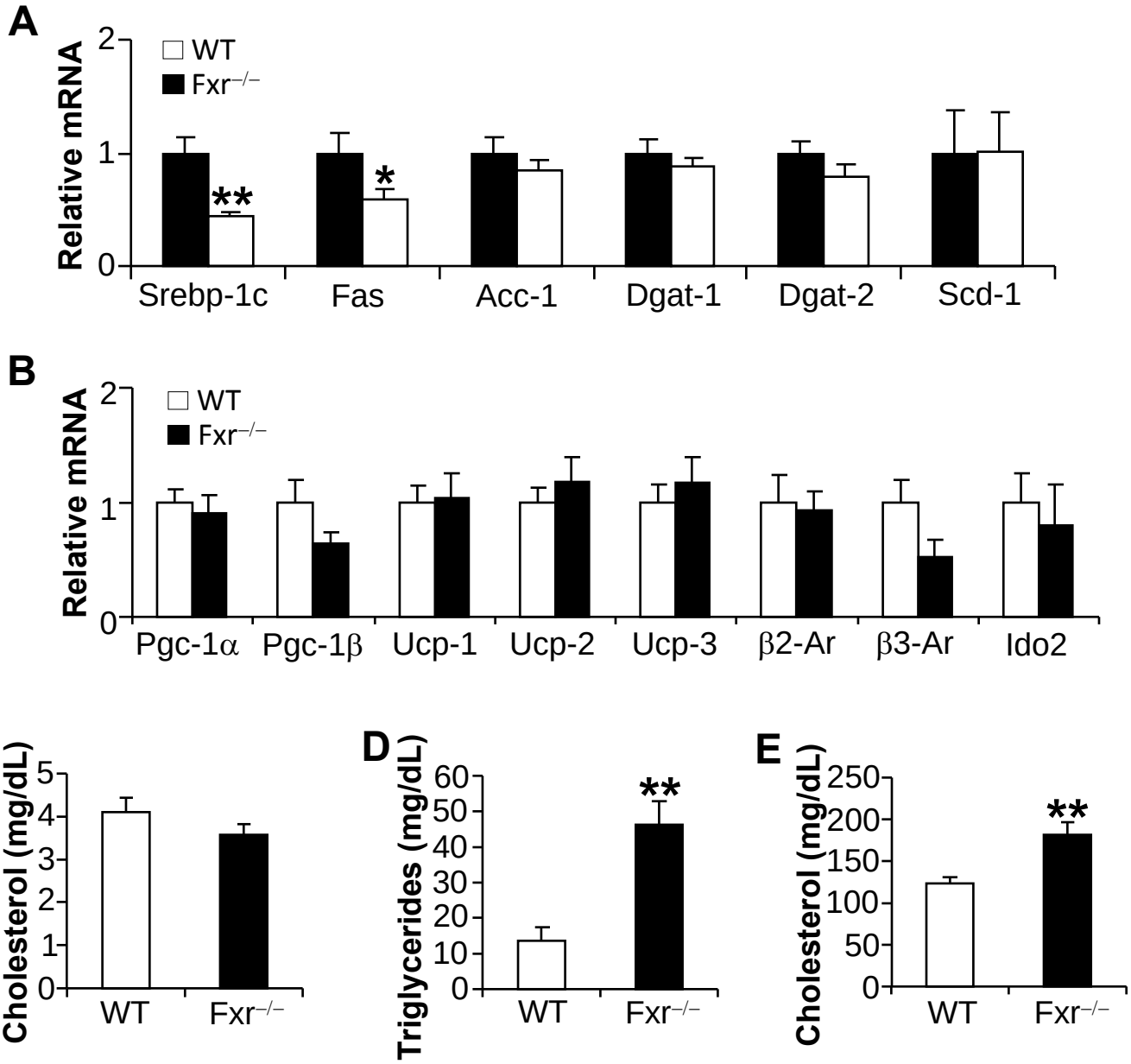

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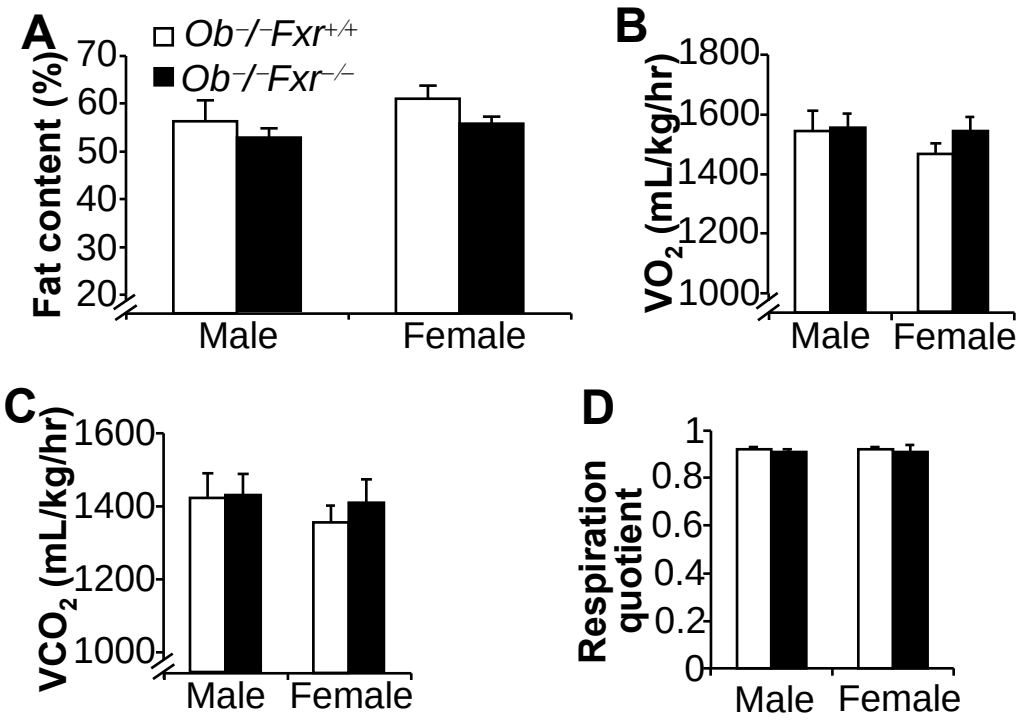
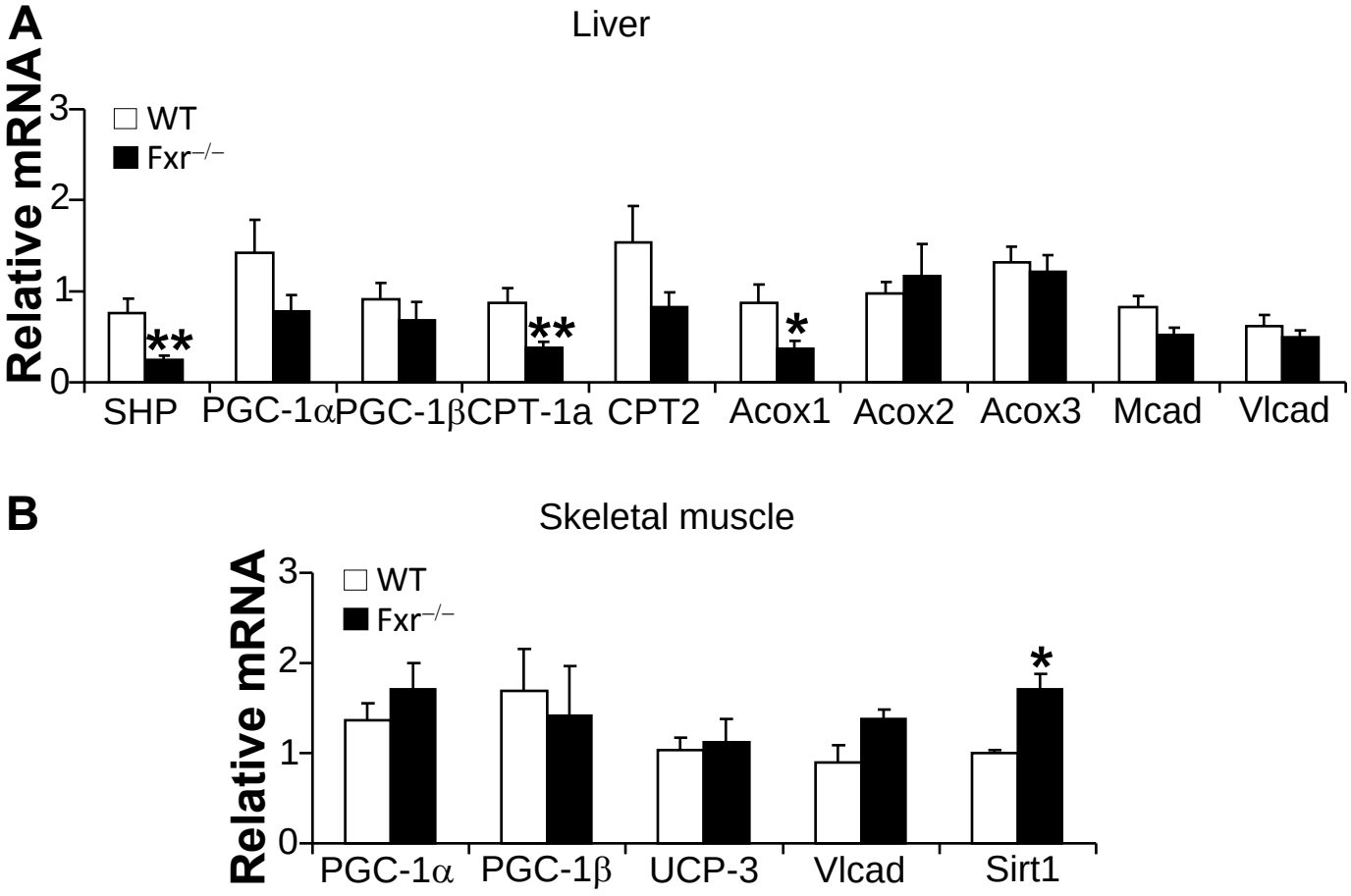
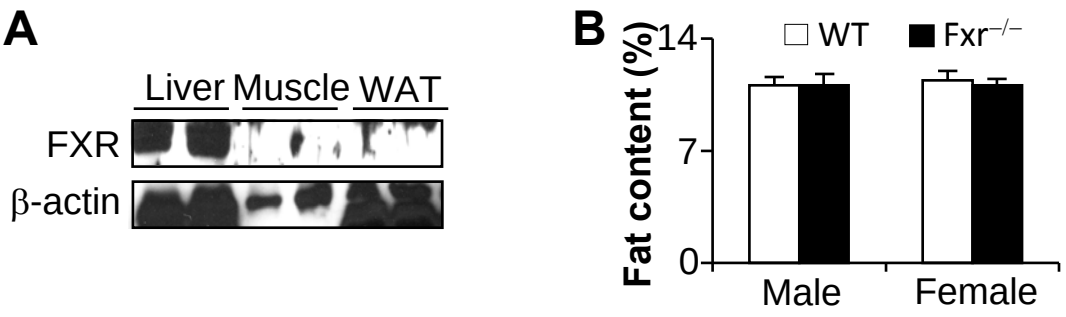


Supplemental Fig. 6



Supplemental Fig. 7



Supplemental Table 1

Table 1. Physical activity of wild-type and *Fxr*$^{-/-}$ mice fed a high-fat diet

Sex	Genotype	Diet	Horizontal activity (counts/min)	Ambulatory activity (counts/min)	Vertical activity (counts/min)
Male	WT	High-fat	16.35±0.84	5.13±0.44	1.95±0.49
Male	Fxr$^{-/-}$	High-fat	16.19±0.45	5.22±0.47	2.04±0.37
Female	WT	High-fat	22.74±2.25	8.15±1.57	3.60±0.98
Female	Fxr$^{-/-}$	High-fat	24.75±1.18	9.23±0.82	4.53±0.74

n=8 mice per group

Supplemental Table 2

Table 2. Physical activity of *ob*$^{-/-}$*Fxr*$^{+/+}$ and *ob*$^{-/-}$*Fxr*$^{-/-}$ mice fed a chow diet

Sex	Genotype	Diet	Horizontal activity (counts/min)	Ambulatory activity (counts/min)	Vertical activity (counts/min)
Male	*ob*$^{-/-}$*Fxr*$^{+/+}$	Chow	8.97±1.16	2.07±0.50	0.18±0.17
Male	*ob*$^{-/-}$*Fxr*$^{-/-}$	Chow	8.06±0.20	1.95±0.09	0.06±0.02
Female	*ob*$^{-/-}$*Fxr*$^{+/+}$	Chow	8.58±0.42	1.96±0.23	0.07±0.02
Female	*ob*$^{-/-}$*Fxr*$^{-/-}$	Chow	8.25±0.32	1.97±0.15	0.09±0.04

n=3-5 mice per group