Supplementary methods

Generation of FXR mutant mice. For the generation of *Fxr* knock-out ($Fxr^{-/-}$) mice, genomic DNA covering the *Fxr* locus was amplified from the 129Sv strain using high fidelity PCR. The resulting DNA fragments were assembled into the targeting vector that after linearization was electroporated into 129Sv ES cells. G418-resistant colonies were selected and analyzed for homologous recombination. The karyotype was verified and several correctly targeted ES cell clones were injected into blastocysts from C57BL/6J mice. These blastocysts were transferred into pseudopregnant females, resulting in chimeric offspring that were mated to female C57BL/6J mice that express Flp recombinase under the control of the ubiquitous CMV promoter. Offspring that transmitted the mutated allele, in which the selection marker was excised and that lost the Flp transgene were selected, mated with CMV-Cre mice to excise the LoxP site ($Fxr^{+/-}$ mice) and intercrossed to generate wild type ($Fxr^{+/+}$) and knock-out ($Fxr^{-/-}$) mice. For mice genotyping, PCR primer pair AHX118: 5´GAAGCACACTCACAGATGTCA3´, AHX124: 5'ATGTGTTCTAAGCTAGACATGG3' (product size: 550 bp) and primer pair AHX118, AHX121: 5'AGATGCTGTTAGGTGGTCAGC3´ (product size: 380 bp) were used to identify excised (L-) and premutant (L2) alleles, respectively. RNA from liver was isolated using trizol method (Invitrogen, Carsland, CA). cDNAs were synthesized from total RNA with SuperScript^{TM} II Reverse Transcriptase (Invitrogen) and random hexamer primers (Roche, Basel, Switzerland). The real-time PCR measurement of individual cDNAs was performed using SYBR green dye (Qiagen, Courtaboeuf, France) to measure duplex DNA formation with Roche Lightcycler. Sequences of primers used to amplify *Fxr* are 5'ACAGCTAATGAGGACGACAG 3'

and 5’ GATTTCCTGAGGCATTCTCTG 3’. Nuclear extracts (100 µg) from liver of *Fxr*$^{+/+}$, *Fxr*$^{+/-}$ and *Fxr*$^{-/-}$ mice were transferred to membrane following standard procedures. The membrane was then incubated overnight at 4°C with an anti-Fxr antibody (PPMX Perseus Proteomics Inc) and next for 1 hour at 21°C with a peroxidase conjugate secondary antibody. Membranes were washed and proteins were visualized with the enhanced chemiluminescence (ECL) kit (Amersham Pharmacia Biotech).

FACS analysis of nuclear DNA content. Freshly isolated liver tissue was gently crushed in a phosphate buffered saline solution and passed through a fine mesh. Cells were stained with propriduim iodide (PI) and subjected to FACS analysis.

Quantitative Real-Time PCR: Gene expression analysis was performed using the DNA Engine Opticon-2 Real Time PCR Detection System. Each 25µl reaction mix comprised 0.8 µM primers, 1µl cDNA template and 12.5µl SYBR Green (Sigma-Genosys, Haverhill, UK). The PCR primers for *Cyp2b10* were 5’-CTGAATCCGCTCCTCCACACTC-3’ (forward) and 5’-TGAGCCAACCTTCAAGGAATAT-3’ (reverse) and for *cyclophilin*, 5’-TGGAGAGCACCAAGACAGACA-3’ (forward) and 5’-TGCCGGAGTCGACAATGAT’-3’ (reverse). Data are expressed as relative expression normalized to values obtained for *cyclophilin*.

Supplementary figure legends

Supplementary figure 1: Generation and validation of mice with a targeted mutation in the *Fxr* gene. (A) Map of the *Fxr* genomic locus. LoxP sites (grey triangles) are indicated. Boxes represent the respective exons. (B-C) Efficient recombination of the *Fxr* locus as indicated by the almost complete absence of *Fxr* mRNA (n=5)(B) and protein (n=3) (C) levels in the liver of $Fxr^{-/-}$ mice.

Supplementary figure 2: *Pregnancy increases average hepatocyte nuclear DNA content.* Isolated hepatocytes were stained with propridium iodide and subjected to FACS analysis. (A) Example traces of non-pregnant and pregnant wild type mice. (B) Average proportion of hepatocytes in Bin 1 (low DNA content) and Bin 2 (high DNA content). n=6, *$p<0.05$.

Supplementary figure 3: *Adaptive activation of CAR is unlikely to drive gestational liver growth in* $Fxr^{-/-}$ *mice.* Relative expression of hepatic *Cyp2b10*. Results are shown as mean ±S.E.M (n=6). 2-way ANOVA *$p<0.05$ compared to wild type, #$p<0.05$ compared to non-pregnant.