

**Prevention of spontaneous hepatocarcinogenesis in FXR null mice  
by intestinal specific FXR re-activation**

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**List of abbreviations:** AKR1B7, aldo-keto reductase 1b7; ALDH1B1, aldehyde dehydrogenase 1 family member B1; ANG1, angiogenin 1; BSEP, bile salt export pump; CCND1 and E1, cyclinD1 and E1; CYP3A11, cytochrome P450 isoform 3A11; CYP7A1, cholesterol-7alpha-hydroxylase; CYP7B1,

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cholesterol-7 $\beta$ -hydroxylase; CYP8B1, sterol-12- $\alpha$  hydroxylase; FGF15, fibroblast growth factor 15; FGFR4, FGF receptor 4; FXR, farnesoid X receptor; GSTM1, glutathione S-transferase mu1; IBABP, ileal bile acid binding protein; HCC, hepatocellular carcinoma; IL-6, interleukin 6; MDR2, multidrug resistance protein 2; MRP2-4, multidrug resistance-associated protein 2-4; NTCP, sodium taurocholate cotransporting polypeptide; OATP, organic anion transporting polypeptide; OST $\alpha$  and  $\beta$ , organic solute transporter  $\alpha$  and  $\beta$ ; PCNA, proliferating cell nuclear antigen; SHP, small heterodimer partner; STAT3, signal transducer and activator of transcription 3; TNF $\alpha$ , tumor necrosis factor  $\alpha$ .

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**ABSTRACT**

Farnesoid X receptor (FXR) is the master regulator of bile acid (BA) homeostasis since it controls BA synthesis, influx, efflux and detoxification in the gut/liver axis. Deregulation of BA homeostasis has been linked to hepatocellular carcinoma (HCC) and spontaneous hepatocarcinogenesis has been observed in FXR null mice. This dreaded liver neoplasm has been associated to both FXR gene deletion and to BA-mediated metabolic abnormalities following inactivation of FXR transcriptional activity. In the present study we address the hypothesis that intestinal selective FXR re-activation would be sufficient to restore the fibroblast growth factor 15 (FGF15)-cholesterol-7 $\alpha$ -hydroxylase (Cyp7a1) enterohepatic axis and eventually provide protection against HCC. To this end, we generated FXR null mice with re-expression of constitutively active FXR in enterocytes (FXR<sup>-/-</sup>iVP16FXR) and corresponding control mice (FXR<sup>-/-</sup>iVP16). In FXR null mice, intestinal selective FXR re-activation normalized BA enterohepatic circulation along with up-regulation of intestinal FXR transcriptome and reduction of hepatic BA synthesis. At 16 months of age, intestinal FXR re-activation protected FXR null mice from spontaneous HCC development that occurred in otherwise FXR null mice. Activation of intestinal FXR conferred hepatoprotection by restoring hepatic homeostasis, limiting cellular proliferation through reduced cyclinD1 expression, decreasing hepatic inflammation and fibrosis (decreased signal transducer and activator of transcription 3 (STAT3) activation and curtailed collagen deposition). *Conclusion:* Intestinal FXR is sufficient to restore BA homeostasis via FGF15 axis and prevent progression of liver damage to HCC also in absence of hepatic FXR. Intestinal-selective FXR modulators could stand as potential therapeutic intervention to prevent this devastating hepatic malignancy, even if carrying somatic FXR mutation.

## INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most prevalent type of cancer and the second leading cause of cancer-related death, with over 500000 new cases and more than 695000 deaths globally per year. This deadly malignancy mostly occurs within an already established chronic liver disease and displays a highly heterogeneous aetiology with hepatitis B and C, alcoholic liver disease and non alcoholic steatohepatitis being the most prominent factors. Less common aetiological factors are hereditary hemochromatosis,  $\alpha$ 1-antitrypsin deficiency and some porphyries (1). The most common and unifying condition associated with HCC is cirrhosis that unfolds after long latencies of chronic liver disease; notably HCC is the leading cause of death in patients with compensated liver cirrhosis (2).

An association between HCC development and altered bile acid (BA) metabolism has been documented in both animal and human studies (3-7). BAs are end-products of cholesterol metabolism, synthesized and conjugated in the liver and secreted via bile into small intestine where they aid solubilization and absorption of lipids, nutrients and vitamins. Gut/liver axis homeostasis relies on a tight control of BA levels to avoid BA overload. Elevated BA concentrations induce DNA oxidative damage, inflammation, nuclear factor-kappa B (NF-kB) activation, resistance to apoptosis and enhanced cell proliferation thus promoting neoplastic transformation of hepatocytes and enterocytes (8). The nuclear receptor (NR) farnesoid X receptor (FXR) is the master regulator of BA homeostasis by controlling BA synthesis, influx, efflux and detoxification in the gut/liver axis and it has been implicated in both liver and intestinal tumorigenesis (7, 9-14).

Earlier work from Dr Moore, Huang and Gonzalez's laboratories first documented the link between FXR deficiency and hepatocarcinogenesis thus electing aged FXR null mice as a valuable animal model for studying HCC development in a context of diseased liver. Aged male and female FXR null

mice develop spontaneous HCC by the age of 12-16 months and their livers exhibit increased inflammation, cell proliferation, activation of Wnt/ $\beta$ -catenin pathway and c-myc signaling (7, 13, 15). Strategies aimed at limiting BA overload are anticipated to provide hepatoprotection as earlier reported in FXR null mice treated with BA-sequestering agents (7). However, it is still unclear whether FXR-mediated BA homeostasis restoration or the effective presence of a functional hepatic FXR transcriptional machinery would prevent hepatocarcinogenesis.

Tissue-specific knockout and selective transgenic FXR constitutive activation studies revealed that some molecular pathways are primarily governed by hepatic FXR while others are preferentially controlled by intestinal FXR (16-18). Moreover, the tissue-specific pattern of FXR regulation and its patho-physiological relevance to liver regeneration capacity and ability to cope with BA overload has been documented in rodents (17, 19, 20). Indeed, there is a compelling evidence that tissue-specific FXR modulators may hold promise in the prevention and/or treatment of chronic liver disease and ultimately HCC.

In the present work we first show that intestinal FXR activation is sufficient to restore BA homeostasis in both young and aged FXR null mice thus protecting them from age-related hepatic inflammation, fibrosis and cancer.

## EXPERIMENTAL PROCEDURES

### Animals

Intestinal specific transgenic iVP16FXR and control iVP16 mice on pure FVB/N background were previously generated (17). Whole body FXR<sup>-/-</sup> mice were originally obtained from Dr. Frank J Gonzalez (National Institute of Health, USA). FXR<sup>-/-</sup>iVP16 and FXR<sup>-/-</sup>iVP16FXR mice were created

by cross-breeding for more than 8 generations C57B6J pure strain FXR<sup>-/-</sup> and FVBN pure strain iVP16 and iVP16FXR mice. FXR<sup>-/-</sup> mice carrying the transgene hFXR under the control of the villin promoter were identified by polymerase chain reaction (PCR) of tail genomic DNA to confirm the presence of hFXR coding sequence. Four-month and 16-month old male mice were used in the experiments. All mice were housed under a standard 12-hr light/dark cycle and fed standard rodent chow diet and autoclaved tap water ad libitum. All experiments were approved by the Ethical Committee of the Fondazione Mario Negri Sud (Chieti, Italy) and certified by the Italian Ministry of Health in accordance with internationally accepted guidelines for animal care.

BA measurements, serum analysis, histology and immunohistochemistry, RNA isolation, quantitative real time PCR, protein extract preparation and immunoblot analysis, intestinal permeability assay and microarray analysis are detailed in the Supporting Information.

### **Statistical analysis**

All results are expressed as means± SEM. Significant differences between two groups were determined by Mann-Whitney *U* test. Comparison between multiple groups was assessed using non parametric ANOVA i.e. Kruskal Wallis. Post-hoc multiple pair-wise comparisons were calculated with Nemenyi-Damico Wolfe-Dunn test. Comparison between proportions was evaluated using  $\chi^2$  test. All statistical analyses were performed with Graph Pad Prism v5.0 (Graph Pad Software, San Diego, CA) and conducted as a two-sided alpha level of 0.05.

## RESULTS

### Intestinal FXR re-activation restores BA enterohepatic circulation in young FXR null mice

To investigate whether intestinal constitutive FXR activation would be sufficient to recover BA enterohepatic circulation in FXR null mice we crossbred FXR null mice with intestinal specific transgenic iVP16FXR and control iVP16 mice, thus generating FXR<sup>-/-</sup>iVP16FXR and FXR<sup>-/-</sup>iVP16 mice, respectively. Gene expression analysis of the ileum of FXR<sup>-/-</sup>iVP16FXR mice revealed a significant up-regulation of intestinal FXR target genes including fibroblast growth factor 15 (*Fgf15*), ileal bile acid binding protein (*Ibabp*), organic solute transporter  $\alpha$  and  $\beta$  (*Osta* and  $\beta$ ) thus confirming the transgene expression of FXR in intestinal epithelium compared to control FXR<sup>-/-</sup>iVP16 mice (Fig.1A). *Fgf15* functions as hormone through activating FGFR4 in the liver, which triggers a signaling cascade involving mitogen-activated protein kinase (ERK1/2 MAPK) pathway and synergizes with small heterodimer partner (SHP) to repress hepatic cholesterol-7 $\alpha$ -hydroxylase (*Cyp7a1*) expression through involvement of c-Jun (NH<sub>2</sub>)-terminal kinase (JNK)-dependent pathway (16, 18, 21). *Fgf15* requires  $\beta$ -*klotho* to stably bind to FGFR4 (22, 23) and both  $\beta$ -*klotho* and *Fgfr4* transcript levels were found upregulated upon intestinal selective FXR re-activation (Fig.1B). FGFR4 activation was associated with JNK, but not ERK1/2, phosphorylation and with no changes in FRS2 protein level (Fig.1C) thus suggesting that FGF15-induced signaling cascades were partially activated. *Cyp7a1* gene and protein (Fig.1D) expression were down-regulated in FXR<sup>-/-</sup>iVP16FXR compared to control FXR<sup>-/-</sup>iVP16 mice. In agreement with previous tissue-specific knockout studies (16, 18), intestinal FXR did not impact *Cyp8b1* expression (Fig.1E) thus confirming that FXR-mediated repression of *Cyp8b1* was more dependent on the presence of FXR in liver rather than in the intestine. Interestingly, transcript levels of *Cyp7b1*, a major enzyme in the alternative BA synthetic pathway, were highly upregulated in FXR<sup>-/-</sup>iVP16FXR compared to control FXR<sup>-/-</sup>iVP16 mice (Fig.1E). Downregulation of *Cyp7a1* and

upregulation of *Cyp7b1* were associated with an increased percentage of  $\beta$ -muricholic acid ( $\beta$ MCA) at the expenses of cholic acid (CA) in the biliary BA pool as indicated in Table 1. Upregulation of intestinal FXR transcriptome along with suppression of hepatic *Cyp7a1* expression rescued young FXR null mice from a massive BA overload as indicated by the reduced BA content in serum, bile, liver and feces of FXR<sup>-/-</sup>iVP16FXR compared to control FXR<sup>-/-</sup>iVP16 mice (Fig.2A). Finally, these metabolic changes were not accompanied by significant alterations in either basolateral including *Mrp4* or canalicular (Fig.2B&C) BA transporters but in *Oatp1* and 2.

### **Intestinal FXR re-activation protects from HCC progression**

Aged FXR null mice have been elected as unique animal model for studying HCC pathogenesis as they resemble many features of human HCC progression: inflammation-steatohepatitis-fibrosis-cancer (24). In the absence of FXR, chronically higher BA levels elicit a plethora of detrimental cell responses including cell hyperproliferation, NF- $\kappa$ B activation, DNA oxidative damage thus laying the foundation to hepatocarcinogenesis. Earlier study suggested that attenuation of systemic BA overload via BA-sequestering agent treatment may reduce number and size of liver tumors (7). As FXR modulators are able to restore BA homeostasis along with repression of NF- $\kappa$ B signaling, inhibition of collagen deposition and apoptosis, one can hypothesize that FXR modulation may serve as a promising approach to prevent age-related HCC. To investigate whether the metabolic changes induced by intestinal FXR activation in young FXR null mice (Fig.1&2) would translate in hepatoprotection at older age, FXR<sup>-/-</sup>iVP16 and FXR<sup>-/-</sup>iVP16FXR mice were sacrificed at 16 months of age when HCC incidence was reported occurring in almost 100% of aged FXR null mice (7). As shown in Fig.3A, 16 month-old FXR<sup>-/-</sup>iVP16 mice developed grossly identifiable liver tumors (tumor number ranging from 4 to 12) while none of them was found in transgenic FXR<sup>-/-</sup>iVP16FXR (  $P < 0.001$ ,  $\chi^2$  test). In agreement with previous studies, FXR<sup>-/-</sup>iVP16 mice exhibited a higher liver/body weight ratio and a marked



elevation of liver enzymes (alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels) compared to FXR<sup>-/-</sup>iVP16FXR mice (Fig.3B). Importantly, restoration of BA homeostasis in aged FXR null mice (Fig.3C-D) was paralleled by HCC prevention even in absence of hepatic FXR.

### **At young age intestinal FXR re-activation induces metabolic changes that set the stage for HCC prevention**

To elucidate the early events contributing to HCC formation, we first analyzed liver morphology and compared histological changes between FXR<sup>-/-</sup>iVP16 and FXR<sup>-/-</sup>iVP16FXR at both young (4 months) and older (16 months) age. At young age, FXR<sup>-/-</sup>iVP16 livers clearly showed liver injury and a disrupted hepatic parenchymal structure while at older age lobular inflammation (score 3) and a marked necrosis (HAI score) worsened the observed phenotype (Fig.4A). As early as 4 months of age FXR<sup>-/-</sup>iVP16FXR livers displayed a more preserved hepatic parenchyma and by 16 months of age displayed less necrotic areas and inflammatory infiltrates. Finally, in contrast to aged FXR<sup>-/-</sup>iVP16 mice, no signs of lipid-containing vacuoles were found in any of FXR<sup>-/-</sup>iVP16FXR mice at same age. Previous studies have shown that during aging FXR null mice are characterized by enhanced cell proliferation, fibrogenesis and upregulation of fibrosis-related transcripts (7, 13, 24, 25). To explore the mechanisms underlying intestinal FXR-mediated hepatoprotection we examined the extent of collagen deposition in livers isolated from both young and aged mice and we found less Sirius red-positive cells in FXR<sup>-/-</sup>iVP16FXR livers (Fig.4A).

### **Intestinal FXR activation prevents FXR-deficiency-associated inflammatory status in both the liver and the gut.**

Liver histological analysis (Fig.4A) revealed that intestinal FXR activation confer benefits to liver health status as early as 4 months of age, thus suggesting that FXR may regulate cellular responses in

the initiation phases of liver tumorigenesis. HCC arises in a setting of chronic inflammatory state and accordingly tumor-bearing FXR null mice have higher serum and hepatic cytokine (IL-6, IL-1 $\beta$ , TNF $\alpha$ , IFN $\gamma$ ) levels (7, 13, 24) thus implicating a putative protective role for FXR in BA-induced hepatic inflammation. Several cytokines and their downstream mediators are able to divert inflammation to liver carcinogenesis and among them IFN $\gamma$  and signal transducer and activator of transcription 3 (STAT3) appear more prominent. IFN $\gamma$  has been found as the most upregulated cytokine in FXR null mouse livers (13) while STAT3 activation has been detected in aged FXR null livers and considered a mechanism of hepatocarcinogenesis (26-28). Conversely, FXR agonists proved to be effective at suppressing NF-kB activation and counteracting STAT3 phosphorylation in hepatoma cell lines (29, 30) thus suggesting that FXR modulation could stand as a strategy to suppress inflammation-driven hepatocarcinogenesis. To explore whether intestinal FXR activation could reverse or attenuate the chronic inflammatory state observed in FXR null mice, we first monitored cytokine gene expression levels and we found that *Il6*, *Tnfa* and *IFN $\gamma$*  transcripts were lower in FXR<sup>-/-</sup>iVP16FXR livers compared to FXR<sup>-/-</sup>iVP16 (Fig.4B). Moreover, upon intestinal FXR activation STAT3 activation, as assessed by the amount of Tyr705-phosphorylated protein, was inhibited whereas total STAT3 protein remained unchanged (Fig.4C).

Given the importance of the intestinal mucosal barrier in systemic inflammatory inputs, we examined the gene-related changes induced by intestinal FXR re-activation in FXR null enterocytes. Interestingly, genes involved in BA detoxification including aldo-keto-reductase 1B7 (*Akr1b7*) and cytochrome P450 isoform 3A11 (*Cyp3a11*) and antibacterial defense such as angiogenin 1 (*Ang1*) were found upregulated (Fig.5A), while intestinal epithelium integrity was preserved as assessed by permeability test (Fig.5B) and histological examination (Fig.5C). Our findings support the concept that intestinal FXR activation could effectively reverse FXR deficiency-associated inflammatory status in

both the liver and the gut, thus providing a contributing explanatory pathway to the hepatocarcinogenesis preventing-scenario.

### **Downregulation of cell cycle-related genes and aryl-hydrocarbon receptor signaling contribute to intestinal FXR-mediated hepatoprotection**

In order to characterize the liver-specific transcriptional scenario activated by restoration of intestinal FXR transcriptome we performed a microarray analysis (Supplementary Tables 1-4). We identified 14 upregulated and 39 downregulated genes ( $P < 0.05$ ,  $> 1.5$  fold) in both young and aged liver, we clustered them in Ingenuity canonical pathways and discussed only those biologically significant. Data obtained from microarray (Fig.6A), gene (Fig.6B) and protein (Fig.6C) analysis pointed to a downregulation of genes involved in aryl hydrocarbon receptor signaling, glutathione-mediated detoxification, IL-1-mediated inhibition of RXR function and NRF2-mediated oxidative stress response in both young and aged livers (Supplementary Table 4). One of the most significantly modulated pathway ( $P_{\text{value}} 3.02E-07$ ) is the aryl-hydrocarbon receptor signaling, whose genes are ascribed to cell cycle progression, cancer and cell proliferation, with cyclins occupying a focal position in this pathway. Alterations of cell cycle-related genes have been documented in hepatocarcinogenesis (31, 32) as well as a compensatory proliferative response to BA-induced hepatocellular damage thus providing evidence for a prognostic role of G1-S modulators in HCC (33). CyclinD1 (*Ccnd1*) is a key regulator of cell cycle progression and its overexpression has been reported to be sufficient to initiate hepatocellular carcinogenesis (34). Accordingly, mouse models of disrupted BA homeostasis, such as FXR and SHP null mice, display enhanced *Ccnd1* expression (27, 35). Intestinal FXR activation lowered cyclinD1 transcript and protein in both young and older mice (Fig.6B&C). Dysregulated cyclinE1 (*Ccne1*) expression has been shown to act as a potent oncogene and amplification of cyclinE1 promotes HCC formation (36). Interestingly, cyclinE1 transcript (Fig.6B) and protein (Fig.6C) levels

were lower in young FXR<sup>-/-</sup>iVP16FXR mice compared to FXR<sup>-/-</sup>iVP16 while being unchanged at older age, thus underscoring a direct role in the early phases of hepatocarcinogenesis. Accordingly, immunohistochemistry revealed more proliferating cell nuclear antigen (PCNA)-positive cells in FXR<sup>-/-</sup>iVP16 livers than in FXR<sup>-/-</sup>iVP16FXR at both young and older age (Fig.6D). Of note, cyclinD1 is a negative target gene of Shp (35) and hepatic Shp overexpression has been associated with lower cyclinD1 transcript levels and reduce tumor progression in FXR null mice (27). In agreement with previous data, hepatic Shp expression was found inversely associated with hepatic cyclinD1 gene and protein levels in both young and aged mice (Supplementary Fig.1). Thus, intestinal FXR-mediated hepatic Shp upregulation along with cyclinD1 downregulation may serve as a novel HCC suppressing mechanism. Finally, intestinal FXR reactivation modulated the hepatic pregnane-X-receptor (PXR) transcriptome by downregulating at both young and older age PXR target genes involved in IL-1-mediated inhibition of RXR signaling including *Gstm1* and *Aldh1b1* (Fig.6E).

## DISCUSSION

A compelling evidence stemming from animal and human studies implicates nuclear receptors as regulators of liver metabolic homeostasis and candidate biomarkers in HCC development (5, 7, 13, 24, 37). The nuclear receptor FXR is the master transcriptional regulator of BA homeostasis by controlling BA synthesis, influx, efflux and detoxification in the gut/liver axis and it has been implicated in liver tumorigenesis (7, 13). Accordingly, abnormal BA levels owing to the disruption of metabolic homeostasis has been observed in both animal model of liver tumors (7, 13, 35) and patients of hepatitis B virus infection, cirrhosis and HCC (5, 6). To date, strategies aimed at restoring BA homeostasis have the potential to pave the way for future therapy of chronic liver disease and ultimately HCC. FXR is highly expressed in the gut/liver axis and tissue-specific knockout and

transgenic FXR activation studies revealed the relative contribution of hepatic versus intestinal FXR in regulating BA and lipid homeostasis thus providing the rationale to exploit tissue-specific FXR modulation in the management of chronic liver disease. We previously reported the generation of a transgenic mouse model (iVP16FXR) able to protect mice from chemically- and genetically-induced cholestasis via downregulation of BA synthesis and upregulation of intestinal BA disposal (17).

The main goal of the present study was to investigate in newly generated transgenic tissue-specific mouse models if restoration of the FXR-Fgf15-Cyp7a1 enterohepatic regulatory axis-driven BA homeostatic processes would provide protection against HCC even in the absence of hepatic FXR. Herein we provide evidence that intestinal FXR is sufficient to protect against hepatocarcinogenesis by limiting BA overload, restoring the Fgf15/FGFR4 enterohepatic signaling axis and upregulating BA detoxification and efflux pathways. Moreover, in agreement with previous results *in vitro* showing anti-inflammatory and antifibrotic properties of FXR agonists (30, 38), we show *in vivo* that intestinal FXR re-activation confers hepatoprotection via reduced inflammation and proliferation, limited collagen deposition thus resulting in less liver injury. Our findings depict an integrated framework linking intestinal FXR-mediated transcriptional programs, endocrine Fgf15/FGFR4 signaling pathways, BA homeostasis and cellular responses such as inflammation and proliferation. Importantly, the pathophysiological scenario described in our work arises only upon aging and ascribes intestinal FXR as a crucial contributor to liver health status. Indeed, FXR tissue-specific gain and loss of function studies provide further support to the idea that Fgf15/FXR axis could serve as a promising target to promote liver regeneration/repair as well as protection against cholestasis (17, 20). Finally, the therapeutic exploitation of intestinal targets in the clinical management of liver pathological conditions has received a great deal of attention. Bacterial translocation and dysbiosis are hallmarks of chronic liver disease and cirrhosis and rifaximin, a non-absorbable antibiotic, is currently employed in patients with advanced liver disease (39). Strategies aimed at promoting antimicrobial defense and preserving

intestinal epithelial integrity and mucosa fitness are anticipated to offer protection against cirrhosis and its complications including hepatic encephalopathy and eventually hepatocarcinogenesis. Previously it has been shown that the synthetic FXR agonist GW4064 protects mice against bacterial translocation via preservation of intestinal epithelium integrity (40). In our mice, intestinal FXR re-activation induces genes involved in BA detoxification and efflux (*Akr1b7*, *Cyp3a11*, *Osta $\beta$* ), antibacterial defense (*Ang1*) and anti-inflammatory response (*Il-1 $\beta$* , *Tnf $\alpha$* ). These events, along with downregulation of genes involved in lipid metabolism, hepatic system disease and cell growth as underscored by microarray analysis, may all stand as potential underlying mechanisms contributing to the HCC prevention.

In conclusion, we show that intestinal FXR activation is able to rescue FXR null mice from BA overload and prevents hepatocarcinogenesis by controlling BA synthesis via restoration of FGF15 axis, limiting hepatic inflammation and proliferation while preserving intestinal epithelium integrity. Besides the debated positive (41) or negative (42-44) association between hepatic FXR expression and HCC development, our findings may support the therapeutic exploitation of intestinal FXR and FGF19 axis in the clinical management of HCC patients, even if carrying a somatic FXR mutation.

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## REFERENCES

1. Bruix J, Boix L, Sala M, Llovet JM. Focus on hepatocellular carcinoma. *Cancer Cell* 2004; 5(3):215-219.

2. Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology* 2004 ;127(5 Suppl 1):S35-S50.
3. Cameron R, Imaida K, Ito N. Promotive effects of deoxycholic acid on hepatocarcinogenesis initiated by diethylnitrosamine in male rats. *Gann* 1981;72(4):635-636.
4. Knisely AS, Strautnieks SS, Meier Y, Stieger B, Byrne JA, Portmann BC, et al. Hepatocellular carcinoma in ten children under five years of age with bile salt export pump deficiency. *Hepatology* 2006; 44(2):478-486.
5. Wang X, Fu X, Van NC, Meng Z, Ma X, Huang W. Bile Acid Receptors and Liver Cancer. *Curr Pathobiol Rep* 2013 ;1(1):29-35.
6. Chen T, Xie G, Wang X, Fan J, Qiu Y, Zheng X, et al. Serum and urine metabolite profiling reveals potential biomarkers of human hepatocellular carcinoma. *Mol Cell Proteomics* 2011 ;10(7):M110.
7. Yang F, Huang X, Yi T, Yen Y, Moore DD, Huang W. Spontaneous development of liver tumors in the absence of the bile acid receptor farnesoid X receptor. *Cancer Res* 2007 ;67(3):863-867.
8. Perez MJ, Briz O. Bile-acid-induced cell injury and protection. *World J Gastroenterol* 2009 ;15(14):1677-1689.
9. Makishima M, Okamoto AY, Repa JJ, Tu H, Learned RM, Luk A, et al. Identification of a nuclear receptor for bile acids. *Science* 1999 ;284(5418):1362-1365.
10. Parks DJ, Blanchard SG, Bledsoe RK, Chandra G, Consler TG, Kliewer SA, et al. Bile acids: natural ligands for an orphan nuclear receptor. *Science* 1999;284(5418):1365-1368.
11. Sinal CJ, Tohkin M, Miyata M, Ward JM, Lambert G, Gonzalez FJ. Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. *Cell* 2000 ;102(6):731-744.

12. Kok T, Hulzebos CV, Wolters H, Havinga R, Agellon LB, Stellaard F, et al. Enterohepatic circulation of bile salts in farnesoid X receptor-deficient mice: efficient intestinal bile salt absorption in the absence of ileal bile acid-binding protein. *J Biol Chem* 2003;278(43):41930-41937.
13. Kim I, Morimura K, Shah Y, Yang Q, Ward JM, Gonzalez FJ. Spontaneous hepatocarcinogenesis in farnesoid X receptor-null mice. *Carcinogenesis* 2007 ;28(5):940-946.
14. Modica S, Murzilli S, Salvatore L, Schmidt DR, Moschetta A. Nuclear bile acid receptor FXR protects against intestinal tumorigenesis. *Cancer Res* 2008;68(23):9589-9594.
15. Wolfe A, Thomas A, Edwards G, Jaseja R, Guo GL, Apte U. Increased activation of the Wnt/beta-catenin pathway in spontaneous hepatocellular carcinoma observed in farnesoid X receptor knockout mice. *J Pharmacol Exp Ther* 2011;338(1):12-21.
16. Kong B, Wang L, Chiang JY, Zhang Y, Klaassen CD, Guo GL. Mechanism of tissue-specific farnesoid X receptor in suppressing the expression of genes in bile-acid synthesis in mice. *Hepatology* 2012;56(3):1034-1043.
17. Modica S, Petruzzelli M, Bellafante E, Murzilli S, Salvatore L, Celli N, et al. Selective activation of nuclear bile acid receptor FXR in the intestine protects mice against cholestasis. *Gastroenterology* 2012;142(2):355-365.
18. Kim I, Ahn SH, Inagaki T, Choi M, Ito S, Guo GL, et al. Differential regulation of bile acid homeostasis by the farnesoid X receptor in liver and intestine. *J Lipid Res* 2007;48(12):2664-2672.
19. Borude P, Edwards G, Walesky C, Li F, Ma X, Kong B, et al. Hepatocyte-specific deletion of farnesoid X receptor delays but does not inhibit liver regeneration after partial hepatectomy in mice. *Hepatology* 2012;56(6):2344-2352.



20. Zhang L, Wang YD, Chen WD, Wang X, Lou G, Liu N, et al. Promotion of liver regeneration/repair by farnesoid X receptor in both liver and intestine in mice. *Hepatology* 2012;56(6):2336-2343.
21. Inagaki T, Choi M, Moschetta A, Peng L, Cummins CL, McDonald JG, et al. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. *Cell Metab* 2005; 2(4):217-225.
22. Ito S, Fujimori T, Furuya A, Satoh J, Nabeshima Y, Nabeshima Y. Impaired negative feedback suppression of bile acid synthesis in mice lacking betaKlotho. *J Clin Invest* 2005;115(8):2202-2208.
23. Moschetta A, Kliewer SA. Weaving betaKlotho into bile acid metabolism. *J Clin Invest* 2005 ;115(8):2075-2077.
24. Liu N, Meng Z, Lou G, Zhou W, Wang X, Zhang Y, et al. Hepatocarcinogenesis in FXR-/- mice mimics human HCC progression that operates through HNF1alpha regulation of FXR expression. *Mol Endocrinol* 2012 ;26(5):775-785.
25. Kong B, Luyendyk JP, Tawfik O, Guo GL. Farnesoid X receptor deficiency induces nonalcoholic steatohepatitis in low-density lipoprotein receptor-knockout mice fed a high-fat diet. *J Pharmacol Exp Ther* 2009 ;328(1):116-122.
26. Meng Z, Wang X, Gan Y, Zhang Y, Zhou H, Ness CV, et al. Deletion of IFNgamma enhances hepatocarcinogenesis in FXR knockout mice. *J Hepatol* 2012 ;57(5):1004-1012.
27. Li G, Kong B, Zhu Y, Zhan L, Williams JA, Tawfik O, et al. Small heterodimer partner overexpression partially protects against liver tumor development in farnesoid X receptor knockout mice. *Toxicol Appl Pharmacol* 2013; 272(2):299-305.
28. Li G, Zhu Y, Tawfik O, Kong B, Williams JA, Zhan L, et al. Mechanisms of STAT3 activation in the liver of FXR knockout mice. *Am J Physiol Gastrointest Liver Physiol* 2013; 305(11):G829-G837.

29. Wang YD, Chen WD, Wang M, Yu D, Forman BM, Huang W. Farnesoid X receptor antagonizes nuclear factor kappaB in hepatic inflammatory response. *Hepatology* 2008;48(5):1632-1643.
30. Xu Z, Huang G, Gong W, Zhou P, Zhao Y, Zhang Y, et al. FXR ligands protect against hepatocellular inflammation via SOCS3 induction. *Cell Signal* 2012 ;24(8):1658-1664.
31. Nishida N, Fukuda Y, Komeda T, Kita R, Sando T, Furukawa M, et al. Amplification and overexpression of the cyclin D1 gene in aggressive human hepatocellular carcinoma. *Cancer Res* 1994 ;54(12):3107-3110.
32. Nishida N, Fukuda Y, Ishizaki K, Nakao K. Alteration of cell cycle-related genes in hepatocarcinogenesis. *Histol Histopathol* 1997 ;12(4):1019-1025.
33. Ito Y, Matsuura N, Sakon M, Miyoshi E, Noda K, Takeda T, et al. Expression and prognostic roles of the G1-S modulators in hepatocellular carcinoma: p27 independently predicts the recurrence. *Hepatology* 1999 ;30(1):90-99.
34. Deane NG, Parker MA, Aramandla R, Diehl L, Lee WJ, Washington MK, et al. Hepatocellular carcinoma results from chronic cyclin D1 overexpression in transgenic mice. *Cancer Res* 2001;61(14):5389-5395.
35. Zhang Y, Xu P, Park K, Choi Y, Moore DD, Wang L. Orphan receptor small heterodimer partner suppresses tumorigenesis by modulating cyclin D1 expression and cellular proliferation. *Hepatology* 2008 ;48(1):289-298.
36. Ohashi R, Gao C, Miyazaki M, Hamazaki K, Tsuji T, Inoue Y, et al. Enhanced expression of cyclin E and cyclin A in human hepatocellular carcinomas. *Anticancer Res* 2001;21(1B):657-662.
37. Vacca M, Degirolamo C, Massafra V, Polimeno L, Mariani-Costantini R, Palasciano G, et al. Nuclear receptors in regenerating liver and hepatocellular carcinoma. *Mol Cell Endocrinol* 2013;368(1-2):108-119.
38. Li J, Zhang Y, Kuruba R, Gao X, Gandhi CR, Xie W, et al. Roles of microRNA-29a in the antifibrotic effect of farnesoid X receptor in hepatic stellate cells. *Mol Pharmacol* 2011;80(1):191-200.

39. Ridlon JM, Alves JM, Hylemon PB, Bajaj JS. Cirrhosis, bile acids, and gut microbiota: Unraveling a complex relationship. *Gut Microbes* 2013;4(5).
40. Inagaki T, Moschetta A, Lee YK, Peng L, Zhao G, Downes M, et al. Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. *Proc Natl Acad Sci U S A* 2006;103(10):3920-3925.
41. Kumagai A, Fukushima J, Takikawa H, Fukuda T, Fukusato T. Enhanced expression of farnesoid X receptor in human hepatocellular carcinoma. *Hepato Res* 2013 ;43(9):959-969.
42. Su H, Ma C, Liu J, Li N, Gao M, Huang A, et al. Downregulation of nuclear receptor FXR is associated with multiple malignant clinicopathological characteristics in human hepatocellular carcinoma. *Am J Physiol Gastrointest Liver Physiol* 2012;303(11):G1245-G1253.
43. Ohno T, Shirakami Y, Shimizu M, Kubota M, Sakai H, Yasuda Y, et al. Synergistic growth inhibition of human hepatocellular carcinoma cells by acyclic retinoid and GW4064, a farnesoid X receptor ligand. *Cancer Lett* 2012;323(2):215-222.
44. Zhang Y, Gong W, Dai S, Huang G, Shen X, Gao M, et al. Downregulation of human farnesoid X receptor by miR-421 promotes proliferation and migration of hepatocellular carcinoma cells. *Mol Cancer Res* 2012;10(4):516-522.

## FIGURE LEGENDS

**Figure 1.** Molecular phenotyping of 4-month old intestinal transgenic FXR<sup>-/-</sup> iVP16FXR mice. (A) Gene expression analyses of FXR-regulated genes in the ileum. (B) Gene and (C) protein expression analyses of restoration of Fgf15 signaling and downstream mediators in the liver. (D) Cyp7a1 gene and protein level in livers of FXR<sup>-/-</sup> iVP16FXR and FXR<sup>-/-</sup> iVP16 mice. (E) Gene expression analysis of BA-synthesizing enzymes. Protein expression was measured in liver extracts from n=3 animals/genotype. Cyclophilin was used as a housekeeping gene to normalize data and FXR<sup>-/-</sup> iVP16 mice were used as calibrators. All values shown were mean ± standard error of mean (n=6-8 animals/genotype). Statistical significance ( $P<0.05$ ) was assessed by Mann-Whitney U test. Cyp7a1, cholesterol-7alpha-hydroxylase; Cyp7b1, cholesterol-7beta-hydroxylase; Cyp8b1, sterol-12-alpha hydroxylase; ERK, extracellular signal-regulated kinase; Fgf15, fibroblast growth factor 15; FGFR4, FGF receptor 4; FRS2, FGF receptor substrate 2; Ibabp, ileal bile acid binding protein; JNK, c-jun N-terminal kinase; Osta and  $\beta$ , organic solute transporter  $\alpha$  and  $\beta$ .

**Figure 2.** Intestinal FXR activation effect on bile acid enterohepatic circulation (A) and basolateral and canalicular (B&C) transporter gene expression in 4-month old FXR null mice. All values shown were mean ± standard error of mean (n=6-8 animals/genotype). Statistical significance ( $P<0.05$ ) was assessed by Mann-Whitney U-test.

**Figure 3.** Prevention of spontaneous hepatocarcinogenesis in aged FXR null mice by intestinal FXR re-activation. (A) Macroscopic appearance of livers from 16-month old FXR<sup>-/-</sup> iVP16FXR mice and corresponding controls. (B) Morphological and biochemical parameters of liver damage. (C) Bile acid enterohepatic circulation in aged mice. (D) Cyp7a1 gene and protein expression in tumor-free liver extracts from 16-month old FXR<sup>-/-</sup> iVP16FXR mice and corresponding controls. All values shown were

mean  $\pm$  standard error of mean (n=7-11 animals/genotype). Statistical significance ( $P<0.05$ ) was assessed by Mann-Whitney U-test. ALT, alanine aminotransferase; AST, aspartate aminotransferase.

**Figure 4.** Prevention of hepatic inflammation and fibrosis by intestinal FXR activation. (A) Gross liver histology was assessed by H&E staining and fibrosis by Sirius Red staining (original magnification X100). (B) Gene expression analysis of inflammatory cytokines. (C) Protein levels of both total and phospho-STAT3 were measured in tumor-free liver extracts (n=3 animals/genotype/age). Cyclophilin was used as housekeeping gene to normalize data and young FXR<sup>-/-</sup> iVP16 were used as calibrators. Data from groups sharing the same lower case letters were not significantly different, while data from groups with different case letters were significantly different ( $P<0.05$ , ANOVA Kruskal Wallis followed by post hoc Dunn test). Il6, interleukin-6; Tnfa, tumor necrosis factor  $\alpha$ ; IFN $\gamma$ , interferon  $\gamma$ .

**Figure 5.** Intestinal FXR re-activation preserves intestinal epithelium integrity and BA-detoxifying function. Gene expression analysis of genes involved in BA detoxification and preservation of intestinal mucosa integrity in the ileum (A) of young FXR<sup>-/-</sup> iVP16FXR mice versus FXR<sup>-/-</sup> iVP16. (B) Permeability test and (C) ileum morphology as assessed by H&E staining (original magnification X200). Cyclophilin was used as a housekeeping gene to normalize data and FXR<sup>-/-</sup> iVP16 mice were used as calibrators. All values shown were mean  $\pm$  standard error of mean (n=5 animals/genotype). Statistical significance ( $P<0.05$ ) was assessed by Mann-Whitney U-test. Akr1b7, aldo-keto reductase 1b7; Ang1, angiogenin 1; Cyp3a11, cytochrome P450 isoform 3A11.

**Figure 6.** Network of genes modulated by intestinal FXR re-activation and identified by whole-genome microarray analysis. (A) Venn diagram of genes down-regulated in both young and aged livers of FXR<sup>-/-</sup> iVP16FXR versus FXR<sup>-/-</sup> iVP16 mice. (B-E) Gene and protein expression analysis of molecular

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contributors to intestinal transgenic FXR activation-mediated hepatoprotection in livers from both young and aged mice. Cyclophilin was used as a housekeeping gene to normalize data and young FXR<sup>-/-</sup> iVP16 mice were used as calibrators. All values shown were mean  $\pm$  standard error of mean (n=7-11 animals/genotype). Data from groups sharing the same lower case letters were not significantly different, while data from groups with different case letters were significantly different ( $P < 0.05$ , ANOVA\_Kruskal Wallis followed by post hoc Dunn test). Aldh1b1, aldehyde dehydrogenase 1 family member B1; Ccnd1, cyclin D1; Ccne1, cyclin E1; Gstm1, glutathione S-transferase mu1.

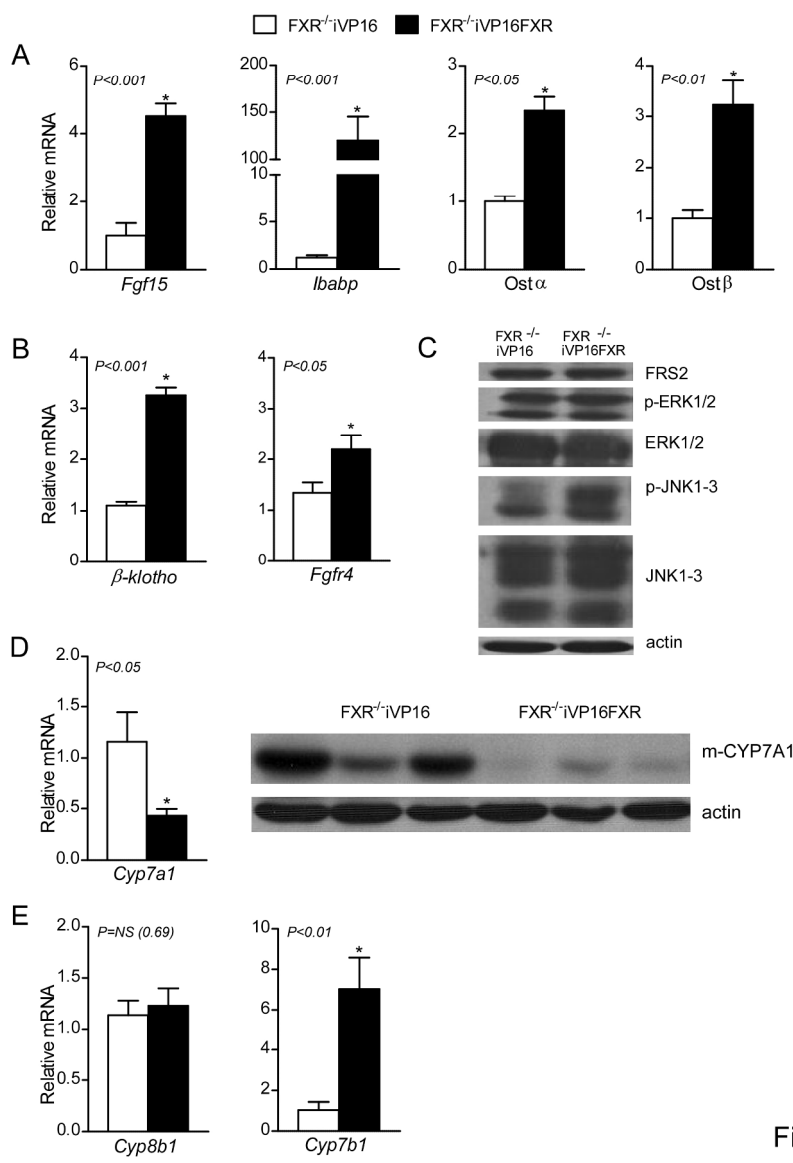


Figure 1

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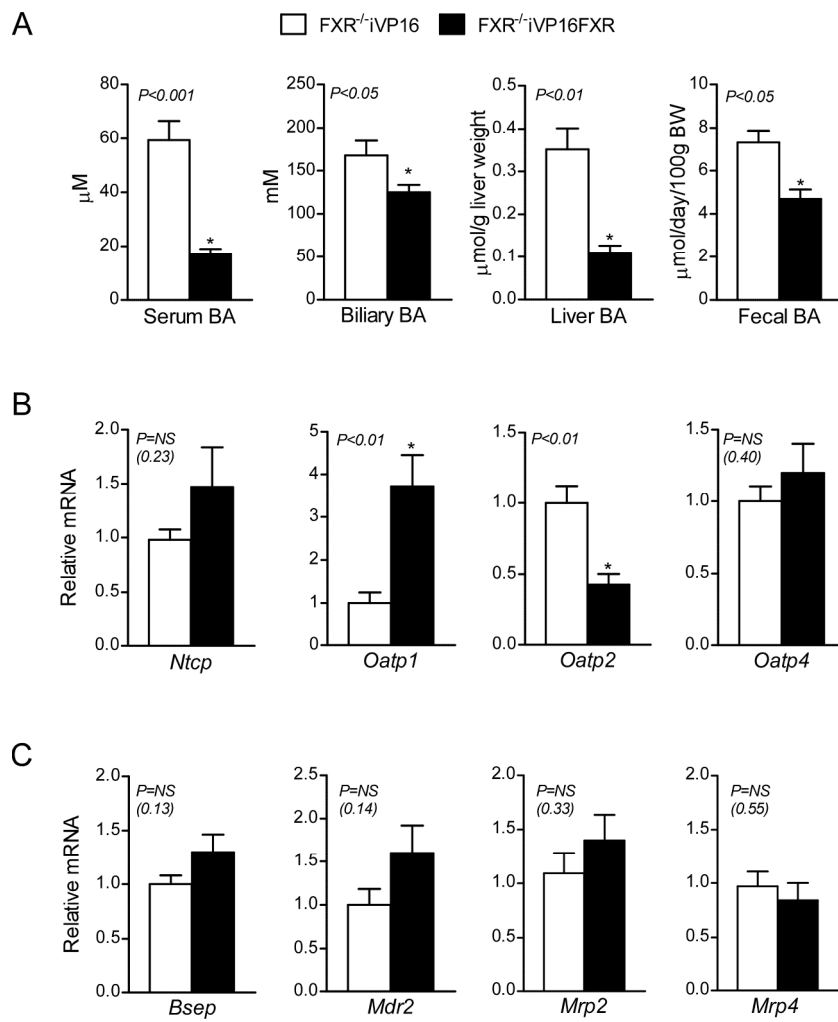


Figure 2

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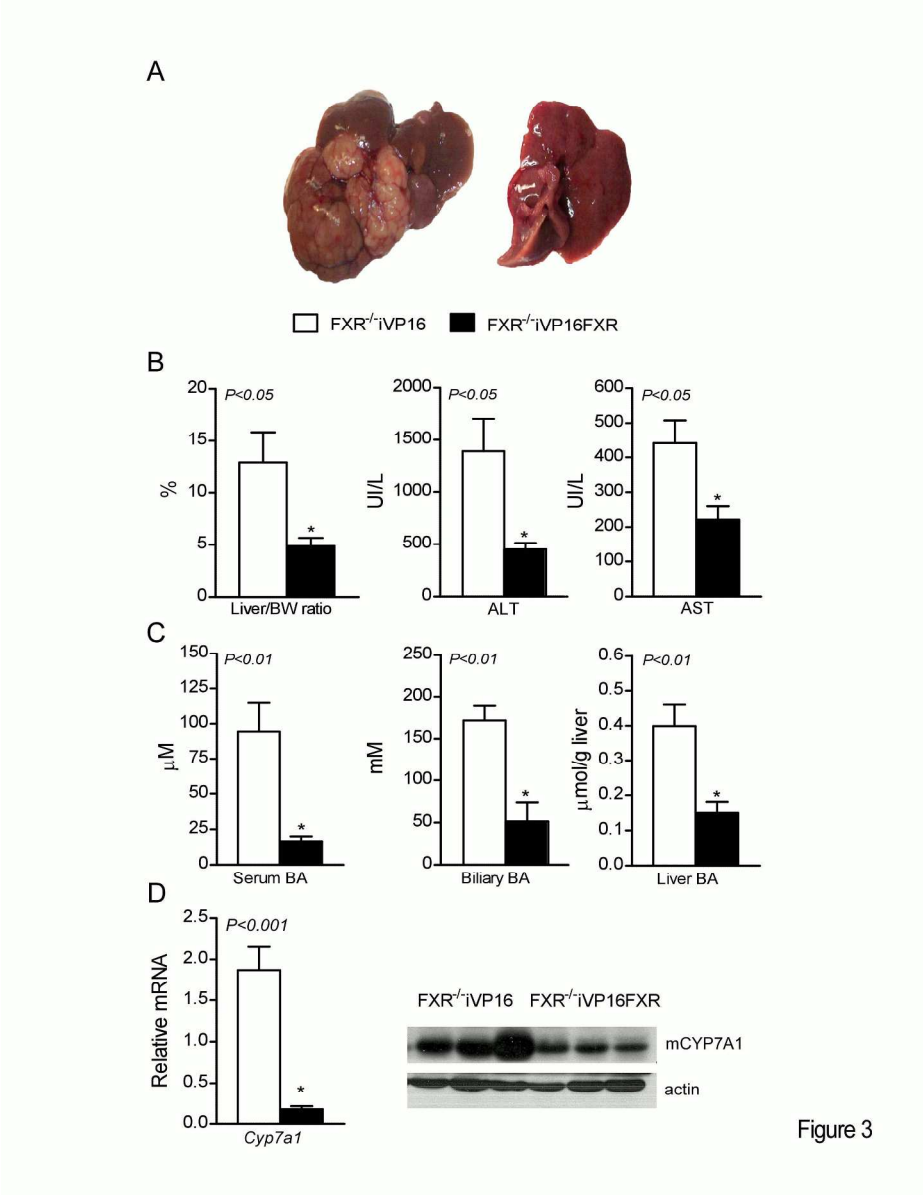


Figure 3

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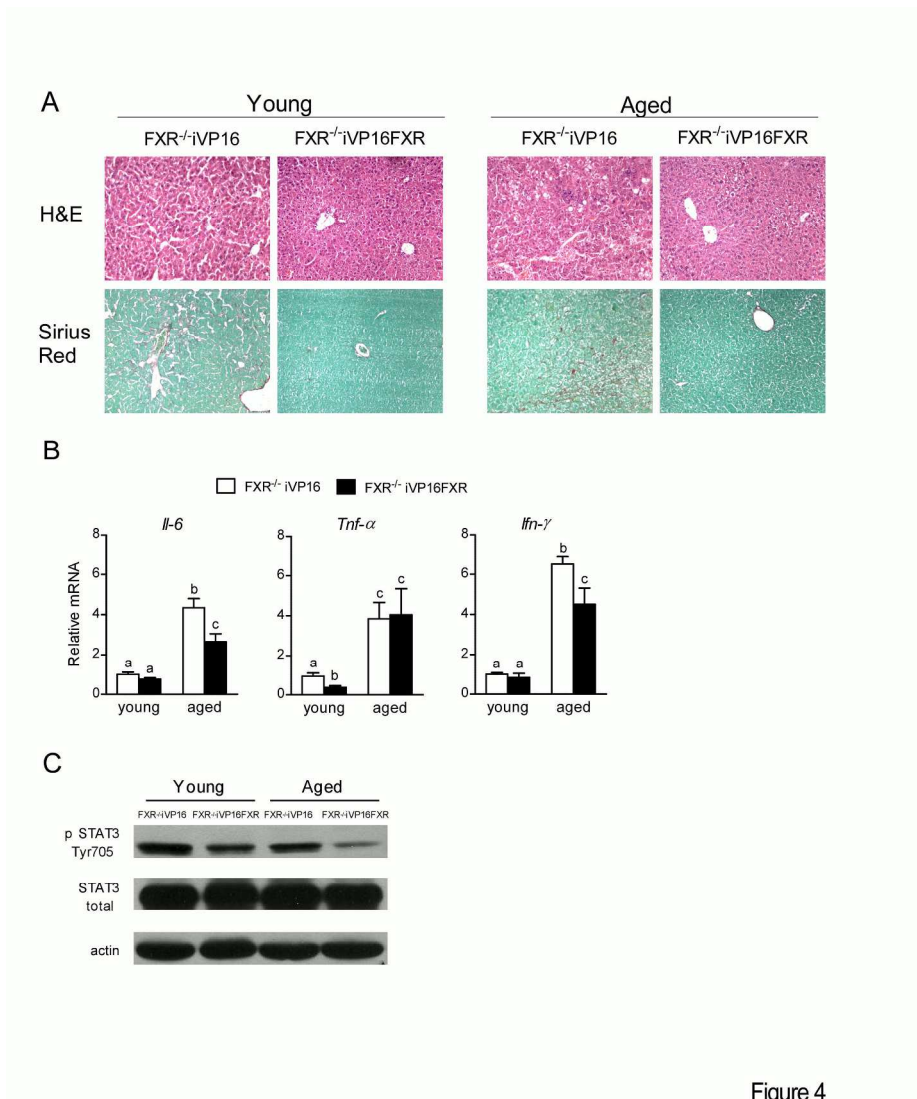


Figure 4

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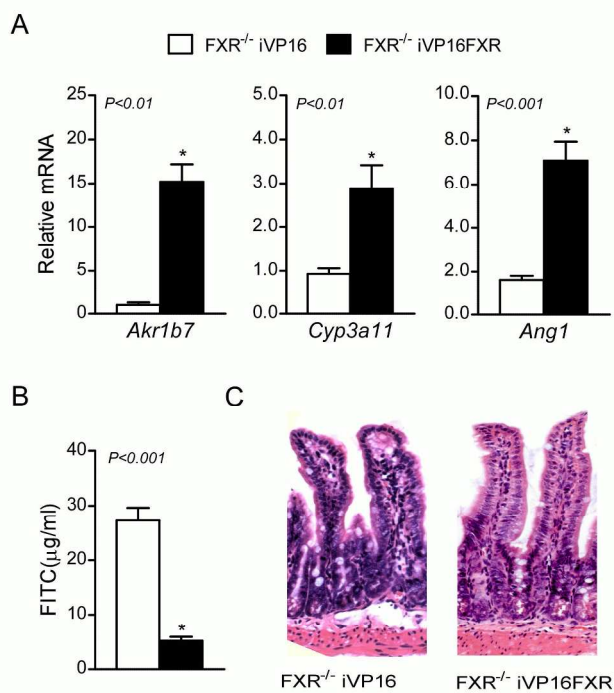


Figure 5

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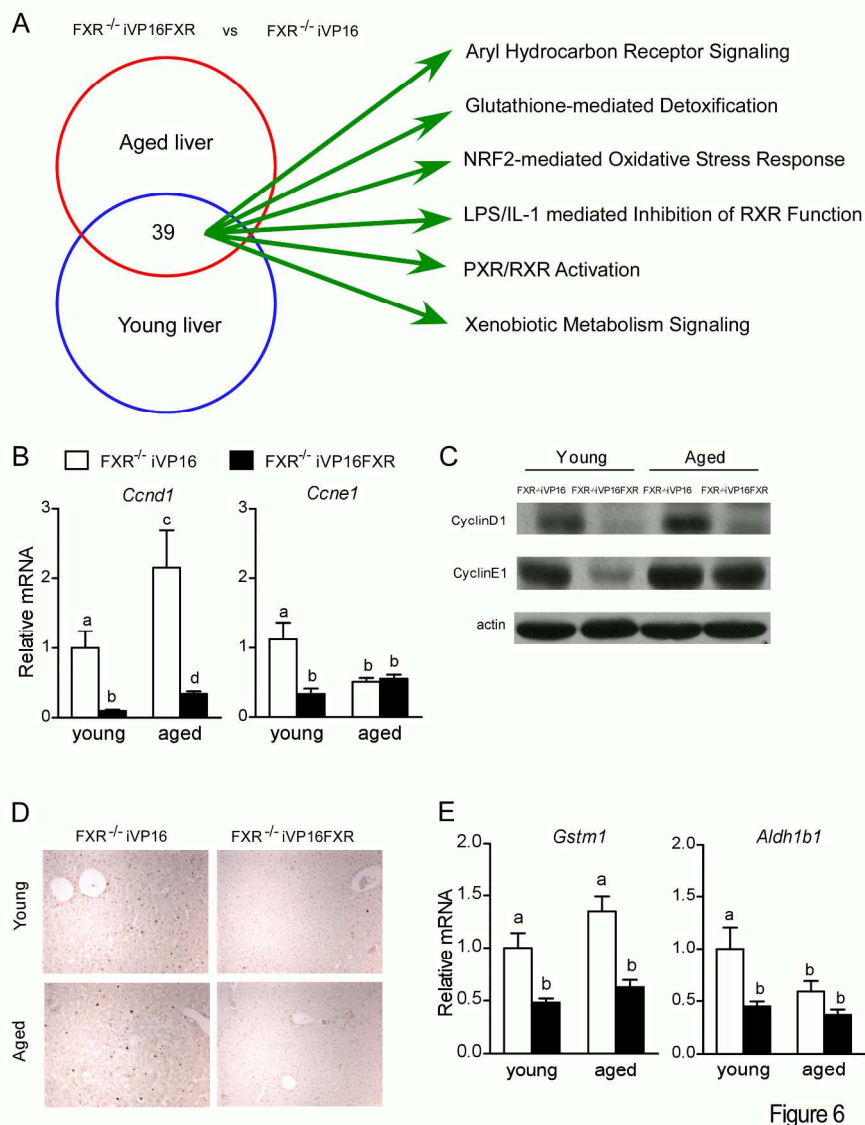


Figure 6

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Genotype	Age	CA %	$\alpha$ MCA %	$\beta$ MCA %	$\Omega$ MCA %	CDCA %	DCA %	UDCA %
FXR <sup>-/-</sup> iVP16	Y	89.7±2.3	0.8±0.15	7.2±1.7	1.0±0.25	0.2±0.1	0.73±0.5	0.27±0.1
FXR <sup>-/-</sup> iVP16FXR	Y	50.9±6.6	0.8±0.12	39.8±6.6	5.9±0.1	0.4±0.1	1.5±0.1	0.63±0.1
FXR <sup>-/-</sup> iVP16	A	70.6±11.8	1.2±0.4	23.9±13.9	1.5±0.5	0.37±0.2	1.87±0.5	0.5±0.25
FXR <sup>-/-</sup> iVP16FXR	A	40.4±6.0	2.5±0.1	49.8±5.8	4.9±0.1	0.43±0.1	0.6±0.1	1.2±0.12

Abbreviations: Y, young (4-month old); A, aged (16-month old). CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; MCA, muricholic acid; UDCA, ursodeoxycholic acid.

## Prevention of spontaneous hepatocarcinogenesis in FXR null mice by intestinal specific FXR re-activation

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### SUPPORTING INFORMATION

### SUPPLEMENTAL MATERIALS AND METHODS

#### Serum analysis

Levels of ALT and AST were measured with a colorimetric kit (BioQuant Heidelberg, Germany) according to manufacturer's instructions. Total bile acids were measured with a colorimetric kit (Diazyme, Poway, CA) according to manufacturer's instructions.

#### Bile acid measurements

BA content in bile, liver and feces was determined by enzymatic assay (Diazyme). Hepatic bile acids were measured as described by Kim et al (1) in tumor-free liver samples. For fecal BA excretion, mice were individually housed in wire bottom cages and stools were collected over 3 days and then air-dried, weighted and grounded. BAs were extracted as described by Turley et al. (2) Individual bile acid concentrations were determined by GC/MS as previously described by Angelin B et al (3). Results are expressed as molar percentage of the total BA content.

#### *In vivo* intestinal permeability assay

Male young mice (n=4/genotype) were gavaged with 0.6 mg/g body weight of fluorescein isothiocyanate (FITC)-conjugated dextran (Sigma, S Louis, Missouri, USA; molecular mass 3-5 kDa).

After 4 h blood was collected and FITC concentrations were measured in plasma (Fluorimeter Pharos FX; BioRad, Hercules, California, USA).

### **Histology and Immunohistochemistry**

Liver and ileum samples were fixed in 10% buffered formalin for 12-24 hours, dehydrated, and embedded in paraffin. Five-micrometer- thick sections were stained with hematoxylin-eosin (H&E) following standard protocols. Liver fibrosis was analyzed with Sirius Red by using Direct Red 80 and Fast Green FCF (Sigma Aldrich, Milan, Italy). Hepatocyte proliferation was assessed by immunohistochemical detection of proliferating cell nuclear antigen (PCNA) as previously described. All the stained sections were analyzed through a light microscope. Histological features of hepatic disease have been assessed according to Histological Scoring System by Kleiner DE et al. and Brunt EM et al. (4,5).

### **Western blot analysis**

Frozen tumor-free liver specimens were homogenized in RIPA (Sigma-Aldrich) lysis buffer with a protease (Roche, Indianapolis, USA) and a phosphatase inhibitor cocktail (Sigma-Aldrich). Equal amounts of total cellular proteins (50µg) were separated onto 10% SDS-poly-acrylamide gel, transferred to nitrocellulose membrane and detected by chemiluminescence (GE Healthcare, Proctor, MN). Western blotting was performed using the following primary antibodies: rabbit polyclonal Cyp7a1 (a gift from Dr. D. Russell, University of Texas Southwestern, Dallas, USA), rabbit anti-phospho-Stat3 (Cell Signaling, Danvers, MA cat.9145), rabbit anti-Stat3 (Cell Signaling, cat.4904), rabbit monoclonal antibody CyclinD1 (Abcam, Cambridge, UK, cat.ab16663), rabbit polyclonal

antibody CyclinE1 (Abcam, cat. ab7959), rabbit polyclonal p44/42 MAP kinase antibody (Cell Signaling, cat.9102), mouse monoclonal phospho-p44/42 MAP kinase antibody (Cell Signaling, cat.9106), rabbit polyclonal JNK (Santa Cruz Biotechnology Inc, cat. sc-571), mouse polyclonal phospho-JNK (Santa Cruz Biotechnology Inc, cat. sc-6254), rabbit polyclonal FRS2 antibody (Abcam, cat.ab10425), mouse  $\beta$ -actin (Sigma Aldrich, cat.A5441).

### **RNA Isolation and Quantitative Real Time PCR**

Total RNA was isolated from tumor-free livers by TRIzol reagent (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. cDNA was synthesized by retro-transcribing 4  $\mu$ g of total RNA using High Capacity DNA Archive Kit (Applied Biosystem, Foster City, CA) and following the manufacturer's instructions. Primers to detect the mRNA expression level of each gene were designed using Primer Express software (Applied Biosystem) based on Gene Bank sequence data. The mRNA expression levels were quantified by qRT-PCR using Power Syber Green chemistry and normalized to cyclophilin mRNA levels. Validated primers for real time PCR are available upon request.

### **Microarray**

Microarray gene expression analysis were conducted on RNA extracted from tumor-free livers from 4- and 16-month old male FXR<sup>-/-</sup>iVP16FXR and corresponding control mice (FXR<sup>-/-</sup>iVP16) (n=5 samples/genotype/age). After RNA quality check (RQI>8; 1.7<260/280 ratio<2), cRNA synthesis was performed with the Illumina Total Prep RNA Amplification kit (Ambion, Austin, TX) following the manufacturer's instructions. Whole-Genome gene expression experiments were conducted on the Illumina microarray platform (Illumina iScan System). Data were processed through specific



algorithms of filtration and cleaning of the signal of the Illumina Genome Studio Software. Final output consisted of fluorescence intensity of each probe (AVG signal), representing the expression levels of each gene. All the genes differentially expressed (Differential Expression Analysis with the “Illumina-custom error model” without False Discovery Rate (FDR)) between groups were analyzed using the Core Analysis function of Ingenuity Pathway Analysis (Ingenuity System Inc., USA) to identify biological functions, pathways and networks. We considered significant only genes included in significant Ingenuity Pathways as previously described by Vacca et al.(6).

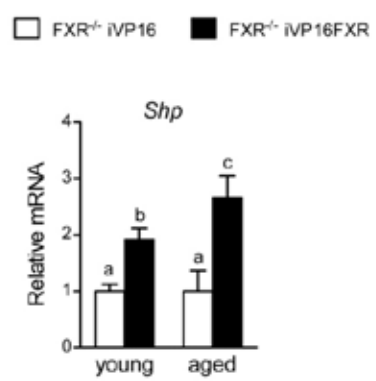
#### Supplementary reference list

1. Kim I, Ahn SH, Inagaki T, Choi M, Ito S, Guo GL, et al. Differential regulation of bile acid homeostasis by the farnesoid X receptor in liver and intestine. *J Lipid Res* 2007; 48: 2664-2672.
2. Turley SD, Herndon MW, Dietschy JM. Reevaluation and application of the dual-isotope plasma ratio method for the measurement of intestinal cholesterol absorption in the hamster. *J Lipid Res* 1994; 35: 328-339.
3. Angelin B and Leijd B. Effects of cholic acid on the metabolism of endogenous plasma triglyceride and on biliary lipid composition in hyperlipoproteinemia. *J. Lipid Res.* 1980; 21:1–9.
4. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ; Nonalcoholic Steatohepatitis Clinical. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; 41: 1313-1321
5. Brunt EM. Grading and staging the histopathological lesions of chronic hepatitis: the Knodell histology activity index and beyond. *Hepatology* 2000; 31:241-242.

6. Vacca M, Murzilli S, Salvatore L, Di Tullio G, D'Orazio A, Lo Sasso G, et al. Neuron-derived orphan receptor 1 promotes proliferation of quiescent hepatocytes. *Gastroenterology* 2013 Jun; 144(7):1518-1529.

#### **SUPPLEMENTARY FIGURE**

Supplementary Figure 1. Hepatic Shp expression in both young and aged FXR<sup>-/-</sup> iVP16FXR mice and corresponding controls. Cyclophilin was used as housekeeping gene and young FXR<sup>-/-</sup> iVP16 mice as calibrators. Columns sharing the same superscript were not significantly different while columns not sharing the same superscript were significantly different (P<0.05, ANOVA Kruskal Wallis followed by posthoc Dunn test).



Suppl Figure 1

Supplementary Table 1: Differentially expressed genes in livers from YOUNG FXR<sup>-/-</sup>iVP16FXR vs. FXR<sup>-/-</sup>iVP16 mice.  
Genes are listed from the highest fold ratio to the lowest fold ratio.

SYMBOL	KO AVG Signal	KO - SD	TG AVG Signal	TG - SD	Ratio	P	DEFINITION
Gdpd3	2,0	8,7	459,3	346,0	227,77	0,01	Mus musculus glycerophosphodiester phosphodiesterase domain containing 3 (Gdpd3), mRNA.
BC021614	366,3	95,3	4608,4	3259,5	12,58	0,01	Mus musculus cDNA sequence BC021614 (BC021614), mRNA.
Cyp2d13	126,3	86,4	596,1	306,3	4,72	0,00	Mus musculus cytochrome P450, family 2, subfamily d, polypeptide 13 (Cyp2d13), non-coding RNA.
Psmc3ip	43,4	83,5	194,3	4,2	4,48	0,00	Mus musculus proteasome (prosome, macropain) 26S subunit, ATPase 3, interacting protein (Psmc3ip), mRNA.
Parp16	272,2	133,1	1130,9	675,8	4,15	0,01	Mus musculus poly (ADP-ribose) polymerase family, member 16 (Parp16), mRNA.
Krt23	264,5	167,7	1098,3	674,1	4,15	0,02	Mus musculus keratin 23 (Krt23), mRNA.
Cyp4a12b	61,4	37,8	244,3	177,6	3,98	0,04	Mus musculus cytochrome P450, family 4, subfamily a, polypeptide 12B (Cyp4a12b), mRNA.
Sult5a1	28,5	19,6	112,8	63,5	3,96	0,02	Mus musculus sulfotransferase family 5A, member 1 (Sult5a1), mRNA.
Erdr1	32,3	5,9	122,9	71,3	3,81	0,01	Mus musculus erythroid differentiation regulator 1 (Erdr1), mRNA.
Plekhb1	129,7	41,9	436,6	226,4	3,37	0,01	Mus musculus pleckstrin homology domain containing, family B (evectins) member 1 (Plekhb1), mRNA.
Rgs16	1152,1	949,8	3854,9	1849,9	3,35	0,01	Mus musculus regulator of G-protein signaling 16 (Rgs16), mRNA.
P2ry1	67,6	14,2	224,7	131,8	3,32	0,02	Mus musculus purinergic receptor P2Y, G-protein coupled 1 (P2ry1), mRNA.
Tspan33	44,4	17,2	145,7	28,7	3,28	0,00	Mus musculus tetraspanin 33 (Tspan33), mRNA.
Serpina11	113,4	69,9	356,7	211,6	3,15	0,03	Mus musculus serine (or cysteine) peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 11 (Serpina11), mRNA.
Cyp7b1	1824,3	1127,3	5664,1	1970,7	3,10	0,00	Mus musculus cytochrome P450, family 7, subfamily b, polypeptide 1 (Cyp7b1), mRNA.
2810007J24Rik	715,9	670,3	2096,6	1226,1	2,93	0,04	Mus musculus RIKEN cDNA 2810007J24 gene (2810007J24Rik), mRNA.
Mup4	3726,0	1960,0	9677,6	1524,0	2,60	0,00	Mus musculus major urinary protein 4 (Mup4), mRNA.
Avpr1a	317,6	185,9	819,5	276,2	2,58	0,00	Mus musculus arginine vasopressin receptor 1A (Avpr1a), mRNA.
Pnpla7	718,1	311,4	1675,6	717,6	2,33	0,01	Mus musculus patatin-like phospholipase domain containing 7 (Pnpla7), mRNA.
Ccng2	99,5	91,9	231,1	28,4	2,32	0,01	Mus musculus cyclin G2 (Ccng2), mRNA.
Cyp39a1	53,6	42,5	121,5	37,6	2,27	0,02	Mus musculus cytochrome P450, family 39, subfamily a, polypeptide 1 (Cyp39a1), mRNA.
Cyp4a12a	2730,5	1090,9	6099,7	2201,8	2,23	0,01	Mus musculus cytochrome P450, family 4, subfamily a, polypeptide 12a (Cyp4a12a), mRNA.
Hao1	1089,1	369,2	2322,0	244,8	2,13	0,00	Mus musculus hydroxyacid oxidase 1, liver (Hao1), mRNA.
Slc30a10	105,9	29,1	222,5	78,2	2,10	0,01	Mus musculus solute carrier family 30, member 10 (Slc30a10), mRNA.
C6	982,3	375,3	2054,7	619,8	2,09	0,00	Mus musculus complement component 6 (C6), mRNA.

Supplementary Table 1: Differentially expressed genes in livers from YOUNG FXR<sup>-/-</sup>iVP16FXR vs. FXR<sup>-/-</sup>iVP16 mice.  
Genes are listed from the highest fold ratio to the lowest fold ratio.

SYMBOL	KO AVG Signal	KO - SD	TG AVG Signal	TG - SD	Ratio	P	DEFINITION
C9	1714,5	454,2	3559,3	888,0	2,08	0,00	Mus musculus complement component 9 (C9), mRNA.
Pigr	1132,9	550,1	2325,1	162,1	2,05	0,00	Mus musculus polymeric immunoglobulin receptor (Pigr), mRNA.
Slc25a33	445,5	168,9	890,3	401,1	2,00	0,04	Mus musculus solute carrier family 25, member 33 (Slc25a33), mRNA.
Cdk5rap1	112,4	41,0	224,0	89,2	1,99	0,03	Mus musculus CDK5 regulatory subunit associated protein 1 (Cdk5rap1), mRNA.
Mcm10	547,6	345,7	1087,4	365,5	1,99	0,02	Mus musculus minichromosome maintenance deficient 10 (S. cerevisiae) (Mcm10), mRNA.
Crim2	67,1	12,9	132,7	45,5	1,98	0,01	Mus musculus cysteine rich BMP regulator 2 (chordin like) (Crim2), mRNA.
00003I07R	124,9	37,2	246,0	111,5	1,97	0,04	Mus musculus RIKEN cDNA 1200003I07 gene (1200003I07Rik), transcript variant 3, mRNA.
Ak311	97,9	56,9	192,0	60,2	1,96	0,02	Mus musculus adenylate kinase 3-like 1 (Ak311), nuclear gene encoding mitochondrial protein, mRNA.
Amigo2	152,9	25,0	298,7	42,8	1,95	0,00	Mus musculus adhesion molecule with Ig like domain 2 (Amigo2), mRNA.
1200011O22Rik	55,8	14,6	108,8	38,8	1,95	0,01	Mus musculus RIKEN cDNA 1200011O22 gene (1200011O22Rik), mRNA.
AI317395	222,0	109,9	430,4	170,3	1,94	0,03	Mus musculus expressed sequence AI317395 (AI317395), mRNA.
Arl4a	99,7	25,7	192,2	56,0	1,93	0,02	Mus musculus ADP-ribosylation factor-like 4A (Arl4a), transcript variant 1, mRNA.
Bach2	93,7	29,7	177,3	20,6	1,89	0,00	Mus musculus BTB and CNC homology 2 (Bach2), mRNA.
3110049J23Rik	1756,7	966,8	3284,3	620,5	1,87	0,01	Mus musculus RIKEN cDNA 3110049J23 gene (3110049J23Rik), mRNA.
Serpina1e	17014,3	10865,1	31746,6	6111,3	1,87	0,01	Mus musculus serine (or cysteine) peptidase inhibitor, clade A, member 1e (Serpina1e), mRNA.
H1f0	183,8	15,7	331,3	97,6	1,80	0,00	Mus musculus H1 histone family, member 0 (H1f0), mRNA.
Neu2	137,7	59,4	243,4	56,4	1,77	0,01	Mus musculus neuraminidase 2 (Neu2), mRNA.
OTTMUSG0000007485	20012,0	6341,3	35053,1	2011,2	1,75	0,00	Mus musculus predicted gene, OTTMUSG0000007485 (OTTMUSG0000007485), mRNA.
Rab1	251,3	109,0	428,8	36,5	1,71	0,01	Mus musculus RAB1, member RAS oncogene family (Rab1), mRNA.
Mxi1	65,6	14,4	111,9	22,6	1,71	0,01	Mus musculus Max interacting protein 1 (Mxi1), transcript variant 3, mRNA.
Gys2	230,7	64,3	388,5	111,6	1,68	0,01	Mus musculus glycogen synthase 2 (Gys2), mRNA.
Slc35e1	169,8	41,7	285,1	34,7	1,68	0,00	Mus musculus solute carrier family 35, member E1 (Slc35e1), mRNA.
Igfbp2	1003,3	232,3	1683,8	394,7	1,68	0,00	Mus musculus insulin-like growth factor binding protein 2 (Igfbp2), mRNA.
LOC100047173	93,8	46,4	157,3	38,4	1,68	0,03	PREDICTED: Mus musculus similar to synaptotagmin-like 1 (LOC100047173), misc RNA.
Wnt2	61,8	28,5	102,2	16,2	1,66	0,04	Mus musculus wingless-related MMTV integration site 2 (Wnt2), mRNA.
Grtp1	73,2	9,8	121,1	35,5	1,65	0,02	Mus musculus GH regulated TBC protein 1 (Grtp1), mRNA.

Supplementary Table 1: Differentially expressed genes in livers from YOUNG FXR<sup>-/-</sup>iVP16FXR vs. FXR<sup>-/-</sup>iVP16 mice.  
Genes are listed from the highest fold ratio to the lowest fold ratio.

SYMBOL	KO AVG Signal	KO - SD	TG AVG Signal	TG - SD	Ratio	P	DEFINITION
Rab3ip	60,9	6,1	100,6	28,0	1,65	0,02	Mus musculus RAB3A interacting protein (Rab3ip), mRNA.
D9Wsu20e	165,5	64,9	271,8	80,0	1,64	0,03	
Foxa1	75,9	10,6	124,3	42,0	1,64	0,04	Mus musculus forkhead box A1 (Foxa1), mRNA.
LOC100047651	1651,4	377,6	2696,6	465,8	1,63	0,00	PREDICTED: Mus musculus similar to FOG (LOC100047651), mRNA.
Atf5	3721,8	1226,8	6052,0	2087,0	1,63	0,05	Mus musculus activating transcription factor 5 (Atf5), mRNA.
Taf15	152,3	53,5	243,8	75,0	1,60	0,04	Mus musculus TAF15 RNA polymerase II, TATA box binding protein (TBP)-associated factor (Taf15), mRNA.
Bop1	153,2	32,1	243,6	59,5	1,59	0,01	Mus musculus block of proliferation 1 (Bop1), mRNA.
Ascc3l1	97,7	18,1	155,1	27,0	1,59	0,01	Mus musculus activating signal cointegrator 1 complex subunit 3-like 1 (Ascc3l1), mRNA.
AA415398	90,2	7,7	142,5	23,3	1,58	0,01	Mus musculus expressed sequence AA415398 (AA415398), mRNA.
1810009N02Rik	143,6	30,1	226,8	76,3	1,58	0,04	Mus musculus RIKEN cDNA 1810009N02 gene (1810009N02Rik), mRNA.
Cd300lg	79,8	24,6	125,8	28,4	1,58	0,03	Mus musculus CD300 antigen like family member G (Cd300lg), mRNA.
Abce1	88,9	7,3	139,3	33,8	1,57	0,02	Mus musculus ATP-binding cassette, subfamily E (OABP), member 1 (Abce1), mRNA.
Ush2a	451,7	126,9	706,0	192,5	1,56	0,02	Mus musculus Usher syndrome 2A (autosomal recessive, mild) homolog (human) (Ush2a), mRNA.
Lman2l	201,1	24,2	314,1	87,5	1,56	0,02	Mus musculus lectin, mannose-binding 2-like (Lman2l), mRNA.
Kcmf1	112,0	21,1	173,9	33,4	1,55	0,01	
Suhw4	79,9	10,6	123,7	12,6	1,55	0,02	Mus musculus suppressor of hairy wing homolog 4 (Drosophila) (Suhw4), mRNA.
Ehd3	171,2	35,6	263,3	43,3	1,54	0,01	Mus musculus EH-domain containing 3 (Ehd3), mRNA.
Znhit2	294,3	41,9	452,4	151,6	1,54	0,05	Mus musculus zinc finger, HIT domain containing 2 (Znhit2), mRNA.
Cyp2c67	1872,9	479,2	2877,0	262,8	1,54	0,01	Mus musculus cytochrome P450, family 2, subfamily c, polypeptide 67 (Cyp2c67), mRNA.
Smarce1	121,8	20,0	185,9	39,5	1,53	0,02	Mus musculus SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily e, member 1 (Smarce1), mRNA.
Dmgdh	654,6	150,3	991,3	283,0	1,51	0,03	Mus musculus dimethylglycine dehydrogenase precursor (Dmgdh), nuclear gene encoding mitochondrial protein, mRNA.
Ttc17	243,6	46,9	367,8	60,1	1,51	0,01	Mus musculus tetratricopeptide repeat domain 17 (Ttc17), mRNA.
Ptprb	344,6	85,6	519,4	99,2	1,51	0,01	Mus musculus protein tyrosine phosphatase, receptor type, B (Ptprb), mRNA.
As3mt	298,0	120,4	449,0	83,0	1,51	0,04	
2700078K21Rik	77,2	11,9	116,2	19,9	1,51	0,03	
E330036I19Rik	624,5	165,4	935,3	190,0	1,50	0,01	Mus musculus RIKEN cDNA E330036I19 gene (E330036I19Rik), mRNA.

Supplementary Table 1: Differentially expressed genes in livers from YOUNG FXR<sup>-/-</sup>iVP16FXR vs. FXR<sup>-/-</sup>iVP16 mice. Genes are listed from the highest fold ratio to the lowest fold ratio.

SYMBOL	KO AVG Signal	KO - SD	TG AVG Signal	TG - SD	Ratio	P	DEFINITION
Gnb2l1	105,5	7,7	157,7	29,0	1,49	0,02	Mus musculus guanine nucleotide binding protein (G protein), beta polypeptide 2 like 1 (Gnb2l1), mRNA.
Tgoln1	543,0	129,7	806,0	215,0	1,48	0,03	Mus musculus trans-golgi network protein (Tgoln1), mRNA.
Gpr137	108,2	27,6	159,8	18,8	1,48	0,03	Mus musculus G protein-coupled receptor 137 (Gpr137), mRNA.
Psm9	142,1	43,0	209,7	38,3	1,48	0,02	Mus musculus proteasome (prosome, macropain) 26S subunit, non-ATPase, 9 (Psm9), mRNA.
LOC654467	188,7	39,1	277,3	76,1	1,47	0,04	Mus musculus heterogeneous nuclear ribonucleoprotein A1 pseudogene (LOC654467) on chromosome 9.
Mlxipl	871,8	128,2	1278,9	113,4	1,47	0,01	Mus musculus MLX interacting protein-like (Mlxipl), mRNA.
Asgr2	242,2	21,6	354,9	104,2	1,47	0,05	Mus musculus asialoglycoprotein receptor 2 (Asgr2), mRNA.
Abcb11	312,2	60,6	456,6	64,3	1,46	0,01	Mus musculus ATP-binding cassette, sub-family B (MDR/TAP), member 11 (Abcb11), mRNA.
2400010D15Rik	237,7	25,9	346,9	89,8	1,46	0,03	
C8b	2093,4	568,4	3052,6	834,0	1,46	0,05	Mus musculus complement component 8, beta polypeptide (C8b), mRNA.
4833420G17Rik	230,8	76,1	336,6	22,3	1,46	0,04	Mus musculus RIKEN cDNA 4833420G17 gene (4833420G17Rik), mRNA.
Ccl27	138,7	29,3	201,3	9,1	1,45	0,04	Mus musculus chemokine (C-C motif) ligand 27 (Ccl27), transcript variant 2, mRNA.
Twistnb	148,7	13,0	215,8	56,0	1,45	0,04	Mus musculus TWIST neighbor (Twistnb), mRNA.
Retsat	10539,2	1863,4	15245,1	2873,3	1,45	0,01	Mus musculus retinol saturase (all trans retinol 13,14 reductase) (Retsat), mRNA.
Tcp1	521,7	145,8	754,2	85,7	1,45	0,05	Mus musculus t-complex protein 1 (Tcp1), mRNA.
Ywhaq	244,8	30,5	353,6	96,4	1,44	0,04	Mus musculus tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta polypeptide (Ywhaq), mRNA.
Mapk8	122,9	26,1	177,5	18,0	1,44	0,03	
Maged1	1440,8	421,7	2076,2	395,1	1,44	0,03	Mus musculus melanoma antigen, family D, 1 (Maged1), mRNA.
Ncor1	123,1	25,6	177,2	40,0	1,44	0,03	Mus musculus nuclear receptor co-repressor 1 (Ncor1), mRNA.
Gcs1	415,3	93,3	597,1	88,1	1,44	0,02	Mus musculus glucosidase 1 (Gcs1), mRNA.
Creld1	847,6	150,5	1217,3	270,1	1,44	0,01	Mus musculus cysteine-rich with EGF-like domains 1 (Creld1), mRNA.
Rbp4	6485,6	1251,1	9312,9	1305,3	1,44	0,01	Mus musculus retinol binding protein 4, plasma (Rbp4), mRNA.
Cyp2d22	6139,5	692,8	8775,5	2029,2	1,43	0,02	Mus musculus cytochrome P450, family 2, subfamily d, polypeptide 22 (Cyp2d22), mRNA.
LOC100044298	601,0	98,7	858,7	150,1	1,43	0,01	PREDICTED: Mus musculus hypothetical protein LOC100044298 (LOC100044298), mRNA.
Acot4	215,2	39,5	307,5	82,6	1,43	0,04	Mus musculus acyl-CoA thioesterase 4 (Acot4), mRNA.
Il11ra1	1531,1	184,0	2184,3	167,0	1,43	0,01	Mus musculus interleukin 11 receptor, alpha chain 1 (Il11ra1), mRNA.
Hp	19821,0	2520,9	28250,4	5178,0	1,43	0,02	Mus musculus haptoglobin (Hp), mRNA.

Supplementary Table 1: Differentially expressed genes in livers from YOUNG FXR<sup>-/-</sup>iVP16FXR vs. FXR<sup>-/-</sup>iVP16 mice.  
Genes are listed from the highest fold ratio to the lowest fold ratio.

SYMBOL	KO AVG Signal	KO - SD	TG AVG Signal	TG - SD	Ratio	P	DEFINITION
Hsd17b7	1239,9	151,9	1757,5	228,2	1,42	0,01	Mus musculus hydroxysteroid (17-beta) dehydrogenase 7 (Hsd17b7), mRNA.
LOC100048301	361,5	40,8	511,9	132,0	1,42	0,04	PREDICTED: Mus musculus similar to RNA Polymerase II subunit 14.5 kD (LOC100048301), mRNA.
Nudt7	1430,0	614,6	2006,2	533,2	1,40	0,04	Mus musculus nudix (nucleoside diphosphate linked moiety X)-type motif 7 (Nudt7), transcript variant 2, mRNA.
Polr1c	291,1	52,1	406,7	104,1	1,40	0,04	Mus musculus polymerase (RNA) I polypeptide C (Polr1c), mRNA.
Nxf1	126,2	10,1	176,3	43,7	1,40	0,05	Mus musculus nuclear RNA export factor 1 homolog ( <i>S. cerevisiae</i> ) (Nxf1), mRNA.
Dhx36	371,7	78,1	517,4	34,7	1,39	0,03	Mus musculus DEAH (Asp-Glu-Ala-His) box polypeptide 36 (Dhx36), mRNA.
Cd59a	2573,9	338,4	3578,1	149,8	1,39	0,02	Mus musculus CD59a antigen (Cd59a), mRNA.
Thap4	227,4	53,9	314,1	51,3	1,38	0,05	Mus musculus THAP domain containing 4 (Thap4), mRNA.
Ugt3a1	1946,0	280,1	2683,3	467,1	1,38	0,02	Mus musculus UDP glycosyltransferases 3 family, polypeptide A1 (Ugt3a1), mRNA.
Rnuxa	282,5	51,4	389,3	88,8	1,38	0,03	Mus musculus RNA U, small nuclear RNA export adaptor (Rnuxa), mRNA.
Hemk1	164,9	20,2	227,1	46,1	1,38	0,05	Mus musculus HemK methyltransferase family member 1 (Hemk1), mRNA.
Pja1	633,1	99,0	871,2	103,9	1,38	0,03	Mus musculus praja1, RING-H2 motif containing (Pja1), mRNA.
Emg1	325,3	51,5	447,2	38,8	1,37	0,04	Mus musculus EMG1 nucleolar protein homolog ( <i>S. cerevisiae</i> ) (Emg1), mRNA.
Ccdc101	169,6	19,1	233,0	41,1	1,37	0,05	Mus musculus coiled-coil domain containing 101 (Ccdc101), mRNA.
Tspan12	721,4	159,5	990,4	77,0	1,37	0,04	Mus musculus tetraspanin 12 (Tspan12), mRNA.
Suox	257,0	40,1	352,7	66,2	1,37	0,04	Mus musculus sulfite oxidase (Suox), nuclear gene encoding mitochondrial protein, mRNA.
Chkb	926,4	214,8	1271,1	134,4	1,37	0,04	Mus musculus choline kinase beta (Chkb), mRNA.
620807	23956,6	3242,0	32834,3	1960,2	1,37	0,02	Mus musculus predicted gene, 620807 (620807), mRNA.
Ganc	425,1	63,4	579,4	100,5	1,36	0,03	Mus musculus glucosidase, alpha; neutral C (Ganc), mRNA.
Dcps	216,0	12,4	294,2	45,1	1,36	0,05	Mus musculus decapping enzyme, scavenger (Dcps), mRNA.
Ddt	1815,7	308,6	2470,3	291,4	1,36	0,03	Mus musculus D-dopachrome tautomerase (Ddt), mRNA.
AII32487	18027,7	3636,8	24496,2	2215,7	1,36	0,04	Mus musculus expressed sequence AII32487 (AII32487), mRNA.
Rhbdl2	993,9	117,0	1345,0	301,2	1,35	0,04	Mus musculus rhomboid, veinlet-like 2 ( <i>Drosophila</i> ) (Rhbdl2), mRNA.
l7Rn6	485,1	75,8	652,8	147,7	1,35	0,04	Mus musculus lethal, Chr 7, Rinchik 6 (l7Rn6), mRNA.
2010007H12Rik	196,6	23,2	258,9	17,1	1,32	0,03	Mus musculus RIKEN cDNA 2010007H12 gene (2010007H12Rik), mRNA.
Tm4sf4	1396,2	247,1	1174,3	256,1	0,84	0,03	Mus musculus transmembrane 4 superfamily member 4 (Tm4sf4), mRNA.
Kng1	18442,1	1762,8	14933,1	1034,2	0,81	0,02	Mus musculus kininogen 1 (Kng1), mRNA.
Rpl38	7849,3	1126,0	6109,2	379,6	0,78	0,02	Mus musculus ribosomal protein L38 (Rpl38), transcript variant 1, mRNA.
Rpl29	1464,6	143,3	1126,9	292,8	0,77	0,01	Mus musculus ribosomal protein L29 (Rpl29), mRNA.



Supplementary Table 1: Differentially expressed genes in livers from YOUNG FXR<sup>-/-</sup>iVP16FXR vs. FXR<sup>-/-</sup>iVP16 mice. Genes are listed from the highest fold ratio to the lowest fold ratio.

SYMBOL	KO AVG Signal	KO - SD	TG AVG Signal	TG - SD	Ratio	P	DEFINITION
Fga	24693,2	4562,0	18952,5	1506,3	0,77	0,04	
Hpx	24644,4	3715,3	18906,5	3567,3	0,77	0,04	Mus musculus hemopexin (Hpx), mRNA.
Cfi	6234,1	1182,4	4782,0	1000,3	0,77	0,05	Mus musculus complement component factor i (Cfi), mRNA.
Dhrs7b	667,7	90,8	511,7	47,2	0,77	0,04	Mus musculus dehydrogenase/reductase (SDR family) member 7B (Dhrs7b), mRNA.
Xdh	661,9	75,7	503,9	87,2	0,76	0,04	Mus musculus xanthine dehydrogenase (Xdh), mRNA.
LOC677317	731,7	138,5	556,1	99,3	0,76	0,05	PREDICTED: Mus musculus similar to Mod1 protein, transcript variant 4 (LOC677317), mRNA.
Ubl4	497,9	78,4	377,7	72,8	0,76	0,04	Mus musculus ubiquitin-like 4 (Ubl4), mRNA.
F2r	1160,9	204,3	880,2	110,8	0,76	0,03	Mus musculus coagulation factor II (thrombin) receptor (F2r), mRNA.
2310016E02Rik	2016,8	337,1	1527,6	238,0	0,76	0,03	Mus musculus RIKEN cDNA 2310016E02 gene (2310016E02Rik), transcript variant 1, mRNA.
Coasy	2461,6	477,2	1862,6	98,3	0,76	0,04	Mus musculus Coenzyme A synthase (Coasy), nuclear gene encoding mitochondrial protein, mRNA.
Prkrir	377,7	64,0	285,2	26,0	0,76	0,04	Mus musculus protein-kinase, interferon-inducible double stranded RNA dependent inhibitor, repressor of (P58 repressor) (Prkrir), mRNA.
Golim4	319,3	40,4	240,9	50,4	0,75	0,04	Mus musculus golgi integral membrane protein 4 (Golim4), mRNA.
1500032L24Rik	1628,9	254,5	1229,1	162,3	0,75	0,03	Mus musculus RIKEN cDNA 1500032L24 gene (1500032L24Rik), mRNA.
Itih4	8344,3	1127,0	6292,6	1584,5	0,75	0,03	Mus musculus inter alpha-trypsin inhibitor, heavy chain 4 (Itih4), mRNA.
Arl6ip5	216,3	24,5	163,1	17,8	0,75	0,04	Mus musculus ADP-ribosylation factor-like 6 interacting protein 5 (Arl6ip5), mRNA.
Ostm1	501,2	50,3	375,5	20,0	0,75	0,03	Mus musculus osteopetrosis associated transmembrane protein 1 (Ostm1), mRNA.
Atp6v1a	254,1	25,5	189,9	22,6	0,75	0,04	Mus musculus ATPase, H <sup>+</sup> transporting, lysosomal V1 subunit A (Atp6v1a), mRNA.
Psap	7667,4	1046,9	5683,9	784,1	0,74	0,02	Mus musculus prosaposin (Psap), mRNA.
Coro1c	427,4	87,5	316,0	47,3	0,74	0,04	Mus musculus coronin, actin binding protein 1C (Coro1c), mRNA.
Pgam1	343,9	22,4	253,9	45,5	0,74	0,03	Mus musculus phosphoglycerate mutase 1 (Pgam1), mRNA.
Hist2h2aa1	573,2	123,2	423,2	72,1	0,74	0,04	
Prdx3	1093,1	89,1	804,4	92,4	0,74	0,02	
Tesk1	309,1	29,1	227,0	44,7	0,73	0,03	Mus musculus testis specific protein kinase 1 (Tesk1), mRNA.
Ctsf	170,5	27,4	124,8	16,4	0,73	0,05	Mus musculus cathepsin F (Ctsf), mRNA.
2500002L14Rik	332,2	53,8	243,0	48,1	0,73	0,02	
Gpld1	305,8	53,4	223,6	29,0	0,73	0,03	Mus musculus glycosylphosphatidylinositol specific phospholipase D1 (Gpld1), mRNA.
LOC100046898	386,5	36,9	282,6	69,0	0,73	0,03	PREDICTED: Mus musculus similar to Cell division cycle 34 homolog ( <i>S. cerevisiae</i> ) (LOC100046898), mRNA.
Srr	965,1	114,0	704,3	183,3	0,73	0,03	Mus musculus serine racemase (Srr), mRNA.

Supplementary Table 1: Differentially expressed genes in livers from YOUNG FXR<sup>-/-</sup>iVP16FXR vs. FXR<sup>-/-</sup>iVP16 mice.  
Genes are listed from the highest fold ratio to the lowest fold ratio.

SYMBOL	KO AVG Signal	KO - SD	TG AVG Signal	TG - SD	Ratio	P	DEFINITION
Hdac3	606,5	101,1	442,6	92,6	0,73	0,02	Mus musculus histone deacetylase 3 (Hdac3), mRNA.
Habp2	1048,0	128,1	762,1	75,4	0,73	0,04	Mus musculus hyaluronic acid binding protein 2 (Habp2), mRNA.
Avpi1	267,1	15,4	194,0	32,7	0,73	0,02	Mus musculus arginine vasopressin-induced 1 (Avpi1), mRNA.
P4hb	12980,5	1810,2	9427,6	2098,6	0,73	0,01	Mus musculus prolyl 4-hydroxylase, beta polypeptide (P4hb), mRNA.
Tmem50a	1419,5	324,4	1030,9	213,1	0,73	0,03	Mus musculus transmembrane protein 50A (Tmem50a), mRNA.
Alg5	1012,9	99,1	734,2	100,0	0,72	0,02	Mus musculus asparagine-linked glycosylation 5 homolog (yeast, dolichyl-phosphate beta-glucosyltransferase) (Alg5), mRNA.
Atp6v0e	2293,4	282,9	1661,7	174,0	0,72	0,01	Mus musculus ATPase, H <sup>+</sup> transporting, lysosomal V0 subunit E (Atp6v0e), mRNA.
Nus1	253,3	34,2	183,3	26,2	0,72	0,03	Mus musculus nuclear undecaprenyl pyrophosphate synthase 1 homolog (S. cerevisiae) (Nus1), mRNA.
Nudt16l1	336,3	55,3	243,2	50,2	0,72	0,02	Mus musculus nudix (nucleoside diphosphate linked moiety X)-type motif 16-like 1 (Nudt16l1), mRNA.
Spg21	546,5	91,4	394,9	43,1	0,72	0,01	Mus musculus spastic paraplegia 21 homolog (human) (Spg21), mRNA.
LOC100047674	185,5	41,5	133,9	23,3	0,72	0,05	PREDICTED: Mus musculus similar to solute carrier family 35 (UDP-glucuronic acid/UDP-N-acetylgalactosamine dual transporter), member D1 (LOC100047674), mRNA.
Serpinb6a	401,1	65,4	289,2	77,9	0,72	0,03	Mus musculus serine (or cysteine) peptidase inhibitor, clade B, member 6a (Serpinb6a), mRNA.
Elf1	142,7	20,4	102,9	13,8	0,72	0,05	Mus musculus E74-like factor 1 (Elf1), mRNA.
Ctsb	9708,3	2088,2	6985,4	1375,3	0,72	0,02	Mus musculus cathepsin B (Ctsb), mRNA.
C4b	6117,4	862,5	4395,1	775,1	0,72	0,01	Mus musculus complement component 4B (Childo blood group) (C4b), mRNA. XM_921663 XM_921673 XM_921676 XM_921678
Nme1	5352,6	1112,7	3824,8	198,1	0,71	0,02	Mus musculus non-metastatic cells 1, protein (NM23A) expressed in (Nme1), mRNA.
Man2b1	1855,1	504,7	1324,8	244,7	0,71	0,05	Mus musculus mannosidase 2, alpha B1 (Man2b1), mRNA.
Pcbd1	1441,9	293,4	1029,1	174,0	0,71	0,02	Mus musculus pterin 4 alpha carbinolamine dehydratase/dimerization cofactor of hepatocyte nuclear factor 1 alpha (TCF1) 1 (Pcbd1), mRNA.
Mupcdh	2234,0	552,6	1587,5	315,2	0,71	0,03	Mus musculus mucin-like protocadherin (Mupcdh), mRNA.
LOC100044204	4831,4	988,5	3424,4	563,7	0,71	0,01	PREDICTED: Mus musculus hypothetical protein LOC100044204 (LOC100044204), mRNA.
Coq5	473,7	88,6	335,7	43,8	0,71	0,03	Mus musculus coenzyme Q5 homolog, methyltransferase (yeast) (Coq5), mRNA.
4833421E05Rik	696,8	71,6	493,2	104,8	0,71	0,01	
Adck5	190,5	43,5	134,7	32,5	0,71	0,04	Mus musculus aarF domain containing kinase 5 (Adck5), mRNA.

Supplementary Table 1: Differentially expressed genes in livers from YOUNG FXR<sup>-/-</sup>iVP16FXR vs. FXR<sup>-/-</sup>iVP16 mice. Genes are listed from the highest fold ratio to the lowest fold ratio.

SYMBOL	KO AVG Signal	KO - SD	TG AVG Signal	TG - SD	Ratio	P	DEFINITION
Slc25a10	1284,2	355,7	908,1	152,8	0,71	0,05	Mus musculus solute carrier family 25 (mitochondrial carrier, dicarboxylate transporter), member 10 (Slc25a10), nuclear gene encoding mitochondrial protein, mRNA.
Tuba6	1103,4	201,3	780,1	169,7	0,71	0,01	
Nudt8	422,4	46,1	296,8	74,9	0,70	0,01	Mus musculus nudix (nucleoside diphosphate linked moiety X)-type motif 8 (Nudt8), mRNA.
Kctd2	586,0	122,4	411,7	87,6	0,70	0,02	Mus musculus potassium channel tetramerisation domain containing 2 (Kctd2), mRNA.
Aim1	396,3	97,3	277,6	52,1	0,70	0,03	
Bgn	2593,5	514,9	1815,6	257,2	0,70	0,01	Mus musculus biglycan (Bgn), mRNA.
Galk1	470,2	122,8	329,1	39,0	0,70	0,04	Mus musculus galactokinase 1 (Galk1), mRNA.
Ifitm3	6830,3	1685,4	4776,8	1411,6	0,70	0,05	Mus musculus interferon induced transmembrane protein 3 (Ifitm3), mRNA.
Lmna	455,8	79,5	318,7	36,2	0,70	0,02	Mus musculus lamin A (Lmna), transcript variant 1, mRNA.
H2-Q8	925,0	186,4	644,7	160,6	0,70	0,02	Mus musculus histocompatibility 2, Q region locus 8 (H2-Q8), mRNA.
C4bp	3055,2	584,4	2126,3	186,7	0,70	0,01	Mus musculus complement component 4 binding protein (C4bp), mRNA.
Masp1	1493,7	333,1	1039,3	90,6	0,70	0,02	Mus musculus mannan-binding lectin serine peptidase 1 (Masp1), mRNA.
Iah1	1129,6	163,3	785,6	204,7	0,70	0,01	Mus musculus isoamyl acetate-hydrolyzing esterase 1 homolog ( <i>S. cerevisiae</i> ) (Iah1), mRNA.
Timd2	1449,6	245,4	1007,8	168,3	0,70	0,00	Mus musculus T-cell immunoglobulin and mucin domain containing 2 (Timd2), mRNA.
Tmem9	254,2	39,8	176,4	34,1	0,69	0,01	Mus musculus transmembrane protein 9 (Tmem9), mRNA.
Il6st	184,8	19,6	128,2	39,1	0,69	0,03	Mus musculus interleukin 6 signal transducer (Il6st), mRNA.
Npc2	454,6	77,2	314,4	114,2	0,69	0,04	Mus musculus Niemann Pick type C2 (Npc2), mRNA.
Eif6	1866,5	236,8	1288,0	159,4	0,69	0,00	Mus musculus eukaryotic translation initiation factor 6 (Eif6), mRNA.
Tuba1b	1945,6	262,4	1341,4	226,7	0,69	0,00	Mus musculus tubulin, alpha 1B (Tuba1b), mRNA.
Mrpl23	1877,3	352,8	1293,7	288,3	0,69	0,01	Mus musculus mitochondrial ribosomal protein L23 (Mrpl23), nuclear gene encoding mitochondrial protein, mRNA.
Rpain	411,7	39,1	283,4	40,2	0,69	0,01	Mus musculus RPA interacting protein (Rpain), mRNA.
Pla2g15	734,2	187,9	504,2	117,9	0,69	0,03	Mus musculus phospholipase A2, group XV (Pla2g15), mRNA.
Lgmn	624,4	165,1	427,6	101,2	0,68	0,03	Mus musculus legumain (Lgmn), mRNA.
Elov11	354,2	70,9	242,2	47,5	0,68	0,03	Mus musculus elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 1 (Elov11), transcript variant 3, mRNA.
Stbd1	1270,6	385,6	868,2	189,5	0,68	0,04	Mus musculus starch binding domain 1 (Stbd1), mRNA.
Suc1g2	813,7	171,7	555,5	100,5	0,68	0,04	Mus musculus succinate-Coenzyme A ligase, GDP-forming, beta subunit (Suc1g2), mRNA.
2310079N02Rik	329,4	48,5	224,4	67,8	0,68	0,02	Mus musculus RIKEN cDNA 2310079N02 gene (2310079N02Rik), mRNA.

Supplementary Table 1: Differentially expressed genes in livers from YOUNG FXR<sup>-/-</sup>iVP16FXR vs. FXR<sup>-/-</sup>iVP16 mice.  
Genes are listed from the highest fold ratio to the lowest fold ratio.

SYMBOL	KO AVG Signal	KO - SD	TG AVG Signal	TG - SD	Ratio	P	DEFINITION
Pacs2	600,4	163,1	408,1	39,4	0,68	0,03	Mus musculus phosphofurin acidic cluster sorting protein 2 (Pacs2), mRNA.
Rnf181	2384,3	393,1	1620,5	190,1	0,68	0,00	Mus musculus ring finger protein 181 (Rnf181), mRNA.
Rpo2tc1	169,0	31,3	114,8	27,7	0,68	0,01	
Hn1	373,5	67,8	252,5	40,7	0,68	0,02	Mus musculus hematological and neurological expressed sequence 1 (Hn1), mRNA.
33414D02R	134,4	32,6	90,8	28,3	0,68	0,04	Mus musculus RIKEN cDNA 5033414D02 gene (5033414D02Rik), mRNA.
Srxn1	367,2	69,0	248,0	50,1	0,68	0,03	Mus musculus sulfiredoxin 1 homolog (S. cerevisiae) (Srxn1), mRNA.
Tmem176b	4335,3	846,4	2927,7	345,3	0,68	0,00	Mus musculus transmembrane protein 176B (Tmem176b), mRNA.
Anxa6	1062,3	305,2	715,3	210,2	0,67	0,04	Mus musculus annexin A6 (Anxa6), mRNA.
Gnai2	316,9	56,8	212,8	39,4	0,67	0,00	Mus musculus guanine nucleotide binding protein, alpha inhibiting 2 (Gnai2), mRNA.
Pip4k2a	159,6	34,3	106,8	28,8	0,67	0,02	Mus musculus phosphatidylinositol-5-phosphate 4-kinase, type II, alpha (Pip4k2a), mRNA.
Mad2l2	141,7	24,9	94,8	32,7	0,67	0,03	Mus musculus MAD2 mitotic arrest deficient-like 2 (yeast) (Mad2l2), mRNA.
Arpc1b	228,2	61,8	152,0	55,6	0,67	0,03	Mus musculus actin related protein 2/3 complex, subunit 1B (Arpc1b), mRNA.
Rdh16	953,5	184,9	634,6	188,0	0,67	0,01	Mus musculus retinol dehydrogenase 16 (Rdh16), mRNA.
Mif4gd	1093,6	148,2	722,4	85,1	0,66	0,01	Mus musculus MIF4G domain containing (Mif4gd), mRNA.
Galm	602,0	168,5	396,0	52,6	0,66	0,02	Mus musculus galactose mutarotase (Galm), mRNA.
Ercc3	551,1	120,5	360,5	37,5	0,65	0,01	Mus musculus excision repair cross-complementing rodent repair deficiency, complementation group 3 (Ercc3), mRNA.
Axl	500,5	109,2	326,5	93,7	0,65	0,01	Mus musculus AXL receptor tyrosine kinase (Axl), mRNA.
Abhd4	356,7	108,2	231,9	32,6	0,65	0,03	Mus musculus abhydrolase domain containing 4 (Abhd4), mRNA.
Ugcg	418,9	61,7	271,3	28,4	0,65	0,00	Mus musculus UDP-glucose ceramide glucosyltransferase (Ugcg), mRNA.
Sparc	194,9	45,4	126,1	15,9	0,65	0,02	Mus musculus secreted acidic cysteine rich glycoprotein (Sparc), mRNA.
Cd99l2	103,8	23,8	66,9	27,7	0,64	0,05	Mus musculus CD99 antigen-like 2 (Cd99l2), mRNA.
Serpina10	5713,7	1309,8	3676,9	450,9	0,64	0,00	Mus musculus serine (or cysteine) peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 10 (Serpina10), mRNA.
LOC100048589	130,1	23,5	83,4	35,7	0,64	0,04	PREDICTED: Mus musculus similar to CDNA sequence BC052040, transcript variant 1 (LOC100048589), mRNA.
D0H8S2298E	102,9	19,0	65,9	13,8	0,64	0,02	
Atpif1	2098,2	656,3	1343,6	337,4	0,64	0,03	Mus musculus ATPase inhibitory factor 1 (Atpif1), nuclear gene encoding mitochondrial protein, mRNA.
Ldhd	678,7	183,4	434,5	158,2	0,64	0,04	Mus musculus lactate dehydrogenase D (Ldhd), nuclear gene encoding mitochondrial protein, mRNA.

Supplementary Table 1: Differentially expressed genes in livers from YOUNG FXR<sup>-/-</sup>iVP16FXR vs. FXR<sup>-/-</sup>iVP16 mice. Genes are listed from the highest fold ratio to the lowest fold ratio.

SYMBOL	KO AVG Signal	KO - SD	TG AVG Signal	TG - SD	Ratio	P	DEFINITION
Cap1	298,7	105,2	190,5	47,8	0,64	0,04	Mus musculus CAP, adenylate cyclase-associated protein 1 (yeast) (Cap1), mRNA.
Mvp	721,1	200,4	457,9	48,3	0,64	0,01	Mus musculus major vault protein (Mvp), mRNA.
Spata2L	277,5	58,8	176,1	46,2	0,63	0,00	Mus musculus spermatogenesis associated 2-like (Spata2L), mRNA.
Dynll1	454,0	78,8	287,9	59,0	0,63	0,00	Mus musculus dynein light chain LC8-type 1 (Dynll1), mRNA.
Capns1	807,7	139,5	511,3	51,7	0,63	0,00	
Cul7	140,6	24,1	88,9	8,4	0,63	0,01	Mus musculus cullin 7 (Cul7), mRNA.
H2-Q10	1286,3	236,5	810,8	329,2	0,63	0,02	Mus musculus histocompatibility 2, Q region locus 10 (H2-Q10), mRNA.
Stard3nl	132,4	31,9	83,4	22,3	0,63	0,01	Mus musculus STARD3 N-terminal like (Stard3nl), mRNA.
Cldn2	579,6	190,5	364,2	79,0	0,63	0,02	Mus musculus claudin 2 (Cldn2), mRNA.
Col4a1	354,7	89,9	222,0	79,1	0,63	0,02	Mus musculus procollagen, type IV, alpha 1 (Col4a1), mRNA.
Entpd5	910,6	286,9	569,1	99,9	0,62	0,04	Mus musculus ectonucleoside triphosphate diphosphohydrolase 5 (Entpd5), transcript variant 1, mRNA.
Rdh5	439,0	84,6	273,8	97,4	0,62	0,01	Mus musculus retinol dehydrogenase 5 (Rdh5), mRNA.
Atl3	156,0	18,7	97,2	21,7	0,62	0,03	Mus musculus atlastin GTPase 3 (Atl3), mRNA.
Acad9	112,7	26,0	70,2	13,4	0,62	0,01	
Hist1h2af	156,8	38,8	97,6	24,9	0,62	0,01	Mus musculus histone cluster 1, H2af (Hist1h2af), mRNA.
LOC268782	807,2	161,1	502,2	228,6	0,62	0,02	
Mfge8	846,7	309,9	524,5	97,7	0,62	0,05	
Ccng1	254,2	69,8	156,9	10,9	0,62	0,01	Mus musculus cyclin G1 (Ccng1), mRNA.
Cryz	313,3	81,1	192,9	39,3	0,62	0,01	Mus musculus crystallin, zeta (Cryz), mRNA.
Sh3bgr1	346,3	72,4	212,8	59,7	0,61	0,00	Mus musculus SH3-binding domain glutamic acid-rich protein like (Sh3bgr1), mRNA.
5830404H04Rik	774,8	102,1	474,7	68,9	0,61	0,00	Mus musculus RIKEN cDNA 5830404H04 gene (5830404H04Rik), mRNA.
2310008M10Rik	1540,0	136,3	943,5	240,7	0,61	0,00	Mus musculus RIKEN cDNA 2310008M10 gene (2310008M10Rik), mRNA.
Zdhhc21	121,4	17,8	74,2	20,1	0,61	0,01	Mus musculus zinc finger, DHHC domain containing 21 (Zdhhc21), mRNA.
Vat1	151,5	57,3	92,6	9,9	0,61	0,04	Mus musculus vesicle amine transport protein 1 homolog (T californica) (Vat1), mRNA.
Ttc23	155,1	29,9	94,8	39,2	0,61	0,01	Mus musculus tetratricopeptide repeat domain 23 (Ttc23), mRNA.
Tpm1	606,0	147,8	369,4	76,1	0,61	0,00	Mus musculus tropomyosin 1, alpha (Tpm1), mRNA.
Cpb2	2177,9	89,1	1325,4	413,2	0,61	0,00	Mus musculus carboxypeptidase B2 (plasma) (Cpb2), mRNA.
Serpina3m	8148,1	1863,7	4955,5	1771,8	0,61	0,01	Mus musculus serine (or cysteine) peptidase inhibitor, clade A, member 3M (Serpina3m), mRNA.
Crip2	2665,6	449,1	1620,5	717,3	0,61	0,01	Mus musculus cysteine rich protein 2 (Crip2), mRNA.
LOC674706	177,2	47,0	107,7	17,1	0,61	0,01	PREDICTED: Mus musculus similar to Zinc finger protein 341 (LOC674706), mRNA.

Supplementary Table 1: Differentially expressed genes in livers from YOUNG FXR<sup>-/-</sup>iVP16FXR vs. FXR<sup>-/-</sup>iVP16 mice.  
Genes are listed from the highest fold ratio to the lowest fold ratio.

SYMBOL	KO AVG Signal	KO - SD	TG AVG Signal	TG - SD	Ratio	P	DEFINITION
Ube2l6	121,2	29,6	73,5	17,2	0,61	0,01	Mus musculus ubiquitin-conjugating enzyme E2L 6 (Ube2l6), mRNA.
Pycr2	113,6	27,5	68,8	14,0	0,61	0,01	Mus musculus pyrroline-5-carboxylate reductase family, member 2 (Pycr2), mRNA.
Il13ra1	178,5	32,9	107,4	37,4	0,60	0,00	Mus musculus interleukin 13 receptor, alpha 1 (Il13ra1), mRNA.
Tgfb1	367,4	113,6	220,6	44,8	0,60	0,03	Mus musculus transforming growth factor, beta induced (Tgfb1), mRNA.
Plvap	352,8	25,8	211,5	75,0	0,60	0,00	Mus musculus plasmalemma vesicle associated protein (Plvap), mRNA.
00094K13R	751,2	188,3	448,6	53,4	0,60	0,00	Mus musculus RIKEN cDNA 2700094K13 gene (2700094K13Rik), transcript variant 2, mRNA.
Gm2a	3635,4	1329,7	2169,9	848,2	0,60	0,04	Mus musculus GM2 ganglioside activator protein (Gm2a), mRNA.
Mrap	1544,9	393,2	915,9	279,2	0,59	0,01	Mus musculus melanocortin 2 receptor accessory protein (Mrap), mRNA.
Rab31	123,0	26,5	72,9	24,6	0,59	0,01	Mus musculus RAB31, member RAS oncogene family (Rab31), mRNA.
Afm	1165,6	139,8	690,2	218,5	0,59	0,00	Mus musculus afamin (Afm), mRNA.
Serpina3n	16762,2	6372,1	9905,9	457,1	0,59	0,02	Mus musculus serine (or cysteine) peptidase inhibitor, clade A, member 3N (Serpina3n), mRNA.
2610039C10Rik	142,1	42,4	83,5	15,8	0,59	0,01	Mus musculus RIKEN cDNA 2610039C10 gene (2610039C10Rik), mRNA.
Tmem97	2353,7	889,9	1380,5	232,1	0,59	0,02	Mus musculus transmembrane protein 97 (Tmem97), mRNA.
Cyb5r3	1435,1	384,5	840,8	186,7	0,59	0,02	Mus musculus cytochrome b5 reductase 3 (Cyb5r3), mRNA.
Nqo2	943,5	279,7	550,2	132,1	0,58	0,01	Mus musculus NAD(P)H dehydrogenase, quinone 2 (Nqo2), mRNA.
Pros1	1850,5	535,3	1077,1	146,0	0,58	0,00	Mus musculus protein S (alpha) (Pros1), mRNA.
Abcc2	598,8	79,7	345,7	98,7	0,58	0,00	Mus musculus ATP-binding cassette, subfamily C (CFTR/MRP), member 2 (Abcc2), mRNA.
Rabl4	109,7	19,3	63,2	12,6	0,58	0,01	Mus musculus RAB, member of RAS oncogene family-like 4 (Rabl4), mRNA.
Lyz	1949,1	601,4	1122,6	553,9	0,58	0,03	Mus musculus lysozyme (Lyz), mRNA.
Orml	20072,4	3294,8	11539,4	4840,3	0,57	0,00	Mus musculus orosomucoid 1 (Orml), mRNA.
9330129D05Rik	206,3	57,6	118,2	65,1	0,57	0,04	
Hsd17b11	374,0	37,1	214,2	86,7	0,57	0,01	Mus musculus hydroxysteroid (17-beta) dehydrogenase 11 (Hsd17b11), mRNA.
Fbxo36	157,8	53,7	89,6	20,7	0,57	0,01	Mus musculus F-box protein 36 (Fbxo36), mRNA.
LOC100043671	1059,7	249,5	600,5	226,3	0,57	0,00	PREDICTED: Mus musculus hypothetical protein LOC100043671 (LOC100043671), mRNA.
LOC100044190	683,1	212,3	386,7	133,3	0,57	0,01	PREDICTED: Mus musculus hypothetical protein LOC100044190 (LOC100044190), mRNA.
Lrg1	15986,6	2465,3	9033,3	1676,9	0,57	0,00	Mus musculus leucine-rich alpha-2-glycoprotein 1 (Lrg1), mRNA.
Tgm2	2711,0	975,1	1517,8	200,6	0,56	0,01	Mus musculus transglutaminase 2, C polypeptide (Tgm2), mRNA.
Pgd	1300,9	332,9	724,7	62,0	0,56	0,00	
Cstb	5001,9	1739,4	2782,4	185,1	0,56	0,01	Mus musculus cystatin B (Cstb), mRNA.
BC031353	1660,1	272,6	922,2	408,6	0,56	0,00	Mus musculus cDNA sequence BC031353 (BC031353), mRNA.



Supplementary Table 1: Differentially expressed genes in livers from YOUNG FXR<sup>-/-</sup>iVP16FXR vs. FXR<sup>-/-</sup>iVP16 mice. Genes are listed from the highest fold ratio to the lowest fold ratio.

SYMBOL	KO AVG Signal	KO - SD	TG AVG Signal	TG - SD	Ratio	P	DEFINITION
Lgals3bp	557,3	69,0	309,4	119,5	0,56	0,00	Mus musculus lectin, galactoside-binding, soluble, 3 binding protein (Lgals3bp), mRNA.
Exoc7	140,6	31,7	77,9	26,3	0,55	0,00	Mus musculus exocyst complex component 7 (Exoc7), mRNA.
Anxa4	120,5	44,0	66,6	34,4	0,55	0,04	Mus musculus annexin A4 (Anxa4), mRNA.
D730039F16Rik	249,6	89,8	137,3	16,3	0,55	0,01	Mus musculus RIKEN cDNA D730039F16 gene (D730039F16Rik), mRNA.
Aldh1a7	1893,8	526,9	1036,0	583,3	0,55	0,02	Mus musculus aldehyde dehydrogenase family 1, subfamily A7 (Aldh1a7), mRNA.
Efha1	489,5	175,3	266,2	85,4	0,54	0,01	Mus musculus EF hand domain family A1 (Efha1), mRNA.
Bbs7	102,4	36,4	55,6	28,0	0,54	0,04	Mus musculus Bardet-Biedl syndrome 7 (Bbs7), mRNA.
Col4a2	190,2	42,0	103,2	21,6	0,54	0,00	Mus musculus collagen, type IV, alpha 2 (Col4a2), mRNA.
Fgl1	10094,9	3120,9	5455,8	2156,9	0,54	0,01	Mus musculus fibrinogen-like protein 1 (Fgl1), mRNA.
Vnn3	1156,6	246,2	623,3	339,3	0,54	0,01	Mus musculus vanin 3 (Vnn3), mRNA.
Adamts2	123,8	22,9	66,7	16,0	0,54	0,00	Mus musculus a disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 2 (Adamts2), mRNA.
Cxadr	492,4	77,2	265,1	60,9	0,54	0,00	Mus musculus coxsackie virus and adenovirus receptor (Cxadr), transcript variant 1, mRNA.
Rnf145	244,3	92,1	131,4	60,4	0,54	0,03	Mus musculus ring finger protein 145 (Rnf145), mRNA.
Lyz2	232,1	47,7	123,9	54,2	0,53	0,00	Mus musculus lysozyme 2 (Lyz2), mRNA.
Slc26a1	121,7	35,8	64,8	18,8	0,53	0,00	Mus musculus solute carrier family 26 (sulfate transporter), member 1 (Slc26a1), mRNA.
Slc25a19	125,9	29,9	66,7	7,2	0,53	0,01	Mus musculus solute carrier family 25 (mitochondrial thiamine pyrophosphate carrier), member 19 (Slc25a19), nuclear gene encoding mitochondrial protein, mRNA.
Snurf	255,0	33,5	134,9	24,9	0,53	0,00	Mus musculus SNRPN upstream reading frame (Snurf), mRNA.
Frag1	126,8	24,1	66,0	32,7	0,52	0,01	Mus musculus FGF receptor activating protein 1 (Frag1), mRNA.
Rab34	114,1	34,1	59,3	5,0	0,52	0,00	Mus musculus RAB34, member of RAS oncogene family (Rab34), mRNA.
Myadm	450,1	176,0	232,1	75,6	0,52	0,01	Mus musculus myeloid-associated differentiation marker (Myadm), mRNA.
Ebpl	3558,6	895,3	1823,8	670,6	0,51	0,00	Mus musculus emopamil binding protein-like (Ebpl), mRNA.
D12Erttd53e	103,7	26,1	53,0	24,2	0,51	0,00	Mus musculus DNA segment, Chr 12, ERATO Doi 553, expressed (D12Erttd53e), mRNA.
Iigp2	490,9	173,9	249,1	99,6	0,51	0,01	Mus musculus interferon inducible GTPase 2 (Iigp2), mRNA.
Serpina3g	263,7	100,1	133,4	30,5	0,51	0,01	Mus musculus serine (or cysteine) peptidase inhibitor, clade A, member 3G (Serpina3g), mRNA.
Hexa	586,7	196,2	296,3	35,1	0,50	0,00	Mus musculus hexosaminidase A (Hexa), mRNA.

Supplementary Table 1: Differentially expressed genes in livers from YOUNG FXR<sup>-/-</sup>iVP16FXR vs. FXR<sup>-/-</sup>iVP16 mice.  
Genes are listed from the highest fold ratio to the lowest fold ratio.

SYMBOL	KO AVG Signal	KO - SD	TG AVG Signal	TG - SD	Ratio	P	DEFINITION
Laptm5	938,9	351,3	473,1	192,3	0,50	0,01	Mus musculus lysosomal-associated protein transmembrane 5 (Laptm5), mRNA.
Tmem43	228,0	88,5	114,0	21,6	0,50	0,01	Mus musculus transmembrane protein 43 (Tmem43), mRNA.
Tgfbr2	441,5	158,5	219,4	83,8	0,50	0,05	Mus musculus transforming growth factor, beta receptor II (Tgfbr2), transcript variant 1, mRNA.
Cotl1	208,9	86,2	103,7	41,0	0,50	0,02	Mus musculus coactosin-like 1 (Dictyostelium) (Cotl1), mRNA.
Mpp1	166,9	27,4	82,6	29,9	0,50	0,00	Mus musculus membrane protein, palmitoylated (Mpp1), mRNA.
Wdr21	114,7	28,9	55,3	35,6	0,48	0,01	Mus musculus WD repeat domain 21 (Wdr21), mRNA.
Elov16	513,4	224,6	247,5	130,9	0,48	0,03	Mus musculus ELOVL family member 6, elongation of long chain fatty acids (yeast) (Elov16), mRNA.
Nudt18	2371,7	522,1	1140,1	237,0	0,48	0,00	Mus musculus nudix (nucleoside diphosphate linked moiety X)-type motif 18 (Nudt18), mRNA.
Cenpa	374,1	165,2	179,1	28,9	0,48	0,01	Mus musculus centromere protein A (Cenpa), mRNA.
Cyba	237,4	78,5	113,1	55,2	0,48	0,01	Mus musculus cytochrome b-245, alpha polypeptide (Cyba), mRNA.
Cyp7a1	4739,6	2264,7	2255,4	1148,5	0,48	0,03	Mus musculus cytochrome P450, family 7, subfamily a, polypeptide 1 (Cyp7a1), mRNA.
Ciapin1	718,1	143,9	337,6	79,0	0,47	0,00	Mus musculus cytokine induced apoptosis inhibitor 1 (Ciapin1), mRNA.
Ccdc120	128,5	50,8	60,4	29,7	0,47	0,01	Mus musculus coiled-coil domain containing 120 (Ccdc120), mRNA.
Gstm1	7374,2	2747,9	3445,5	452,5	0,47	0,05	
Aacs	422,1	217,9	196,8	44,1	0,47	0,02	Mus musculus acetoacetyl-CoA synthetase (Aacs), mRNA.
Plac8	129,5	57,6	59,0	15,1	0,46	0,01	Mus musculus placenta-specific 8 (Plac8), mRNA.
Tmem86b	346,8	74,6	157,6	28,5	0,45	0,00	Mus musculus transmembrane protein 86B (Tmem86b), mRNA.
Gas6	456,5	228,9	204,1	34,5	0,45	0,02	Mus musculus growth arrest specific 6 (Gas6), mRNA.
Arsb	129,7	34,2	57,5	15,3	0,44	0,01	Mus musculus arylsulfatase B (Arsb), mRNA.
Reep4	135,7	55,7	59,9	18,8	0,44	0,01	Mus musculus receptor accessory protein 4 (Reep4), mRNA.
Psm8	2130,7	936,9	937,4	532,9	0,44	0,02	Mus musculus proteasome (prosome, macropain) 26S subunit, non-ATPase, 8 (Psm8), mRNA. XM_001002011
Alas2	523,3	228,5	229,0	217,8	0,44	0,05	
Apcs	1432,6	524,6	622,3	191,9	0,43	0,00	Mus musculus serum amyloid P-component (Apcs), mRNA.
Sccpdh	131,7	45,5	56,7	28,2	0,43	0,00	Mus musculus saccharopine dehydrogenase (putative) (Sccpdh), mRNA.
Ccrn4l	178,7	79,6	76,5	53,7	0,43	0,03	Mus musculus CCR4 carbon catabolite repression 4-like ( <i>S. cerevisiae</i> ) (Ccrn4l), mRNA.
3300001G02Rik	206,8	73,9	87,8	12,0	0,42	0,00	Mus musculus RIKEN cDNA 3300001G02 gene (3300001G02Rik), mRNA.
Htatip2	3738,3	1433,5	1586,6	179,9	0,42	0,00	Mus musculus HIV-1 tat interactive protein 2, homolog (human) (Htatip2), mRNA.



Supplementary Table 1: Differentially expressed genes in livers from YOUNG FXR<sup>-/-</sup>iVP16FXR vs. FXR<sup>-/-</sup>iVP16 mice.  
Genes are listed from the highest fold ratio to the lowest fold ratio.

SYMBOL	KO AVG Signal	KO - SD	TG AVG Signal	TG - SD	Ratio	P	DEFINITION
Cyp3a11	28214,4	6606,5	11960,5	4451,8	0,42	0,00	Mus musculus cytochrome P450, family 3, subfamily a, polypeptide 11 (Cyp3a11), mRNA.
Krt8	3112,2	1035,7	1300,8	435,2	0,42	0,00	Mus musculus keratin 8 (Krt8), mRNA.
Gstm6	1209,9	550,5	505,4	162,5	0,42	0,00	
Mmp2	183,6	91,0	74,6	25,9	0,41	0,01	Mus musculus matrix metalloproteinase 2 (Mmp2), mRNA.
Mgst3	564,1	302,9	226,6	93,1	0,40	0,02	Mus musculus microsomal glutathione S-transferase 3 (Mgst3), mRNA.
Dhcr24	1293,3	545,1	518,8	70,6	0,40	0,00	
Vcam1	297,5	128,5	118,9	57,3	0,40	0,01	Mus musculus vascular cell adhesion molecule 1 (Vcam1), mRNA.
Apol9b	591,1	380,0	236,1	46,7	0,40	0,04	Mus musculus apolipoprotein L 9b (Apol9b), mRNA.
Tubb6	241,7	138,4	95,4	35,2	0,39	0,02	Mus musculus tubulin, beta 6 (Tubb6), mRNA.
Nupr1	116,6	32,6	45,7	29,8	0,39	0,00	Mus musculus nuclear protein 1 (Nupr1), mRNA.
Rdh9	307,4	187,4	118,8	71,5	0,39	0,04	Mus musculus retinol dehydrogenase 9 (Rdh9), mRNA.
Aldh1b1	471,8	280,7	179,7	53,5	0,38	0,02	Mus musculus aldehyde dehydrogenase 1 family, member B1 (Aldh1b1), nuclear gene encoding mitochondrial protein, mRNA.
2210023G05Rik	168,1	56,1	63,8	38,2	0,38	0,03	Mus musculus RIKEN cDNA 2210023G05 gene (2210023G05Rik), mRNA.
Tmprss2	289,9	154,6	109,4	50,6	0,38	0,01	Mus musculus transmembrane protease, serine 2 (Tmprss2), mRNA.
Gamt	3193,9	1263,4	1196,4	628,0	0,37	0,00	Mus musculus guanidinoacetate methyltransferase (Gamt), mRNA.
S100a11	641,1	255,4	239,5	87,6	0,37	0,00	Mus musculus S100 calcium binding protein A11 (calgizzarin) (S100a11), mRNA.
Gstm2	7671,5	3858,3	2865,7	1502,8	0,37	0,01	Mus musculus glutathione S-transferase, mu 2 (Gstm2), mRNA.
Rcan2	210,0	102,2	78,2	40,1	0,37	0,02	Mus musculus regulator of calcineurin 2 (Rcan2), transcript variant 1, mRNA.
Ddah1	800,5	280,7	290,7	118,4	0,36	0,00	
Ces6	159,5	75,3	57,1	31,5	0,36	0,01	Mus musculus carboxylesterase 6 (Ces6), mRNA.
Scd1	21031,9	4889,0	7462,6	3782,1	0,35	0,00	Mus musculus stearoyl-Coenzyme A desaturase 1 (Scd1), mRNA.
Fos	188,8	125,7	65,8	20,4	0,35	0,03	Mus musculus FBJ osteosarcoma oncogene (Fos), mRNA.
Robo1	154,9	32,7	52,6	44,2	0,34	0,00	Mus musculus roundabout homolog 1 (Drosophila) (Robo1), mRNA.
Cxcl9	170,7	84,0	57,9	54,4	0,34	0,02	Mus musculus chemokine (C-X-C motif) ligand 9 (Cxcl9), mRNA.
Cxcl1	450,5	306,5	148,3	37,6	0,33	0,03	Mus musculus chemokine (C-X-C motif) ligand 1 (Cxcl1), mRNA.
Lyve1	133,0	54,8	43,7	38,6	0,33	0,00	Mus musculus lymphatic vessel endothelial hyaluronan receptor 1 (Lyve1), mRNA.
Rsad2	379,2	127,4	123,9	54,7	0,33	0,00	Mus musculus radical S-adenosyl methionine domain containing 2 (Rsad2), mRNA.
Mod1	424,0	93,2	136,0	89,3	0,32	0,00	Mus musculus malic enzyme, supernatant (Mod1), mRNA.
Adck4	179,9	76,3	57,0	13,8	0,32	0,00	
2200001I15Rik	934,2	348,7	292,4	179,2	0,31	0,00	Mus musculus RIKEN cDNA 2200001I15 gene (2200001I15Rik), mRNA.

Supplementary Table 1: Differentially expressed genes in livers from YOUNG FXR<sup>-/-</sup>iVP16FXR vs. FXR<sup>-/-</sup>iVP16 mice.  
Genes are listed from the highest fold ratio to the lowest fold ratio.

SYMBOL	KO AVG Signal	KO - SD	TG AVG Signal	TG - SD	Ratio	P	DEFINITION
Slc39a4	513,7	197,3	160,6	101,6	0,31	0,01	Mus musculus solute carrier family 39 (zinc transporter), member 4 (Slc39a4), mRNA.
Ly6e	643,8	345,9	199,5	76,5	0,31	0,01	Mus musculus lymphocyte antigen 6 complex, locus E (Ly6e), mRNA.
Lgals3	603,2	286,7	184,0	97,9	0,31	0,00	Mus musculus lectin, galactose binding, soluble 3 (Lgals3), mRNA.
Cyb561	124,8	64,7	37,9	15,4	0,30	0,00	Mus musculus cytochrome b-561 (Cyb561), mRNA.
Anxa5	2869,5	909,0	868,3	106,7	0,30	0,00	Mus musculus annexin A5 (Anxa5), mRNA.
Uap111	433,2	232,4	130,3	45,5	0,30	0,00	Mus musculus UDP-N-acetylglucosamine pyrophosphorylase 1-like 1 (Uap111), mRNA. XM_918982
Cyp2b23	1479,6	850,6	431,6	226,5	0,29	0,01	Mus musculus cytochrome P450, family 2, subfamily b, polypeptide 23 (Cyp2b23), mRNA.
Slc25a17	390,9	111,5	113,9	112,1	0,29	0,00	Mus musculus solute carrier family 25 (mitochondrial carrier, peroxisomal membrane protein), member 17 (Slc25a17), nuclear gene encoding mitochondrial protein, mRNA.
Chac1	211,1	86,1	60,1	37,7	0,28	0,00	Mus musculus ChaC, cation transport regulator-like 1 (E. coli) (Chac1), mRNA.
Cd63	484,1	252,8	134,4	49,8	0,28	0,00	Mus musculus CD63 antigen (Cd63), transcript variant 2, mRNA.
Serpina6	1092,9	749,9	297,2	108,5	0,27	0,02	Mus musculus serine (or cysteine) peptidase inhibitor, clade A, member 6 (Serpina6), mRNA.
Mfsd2	4079,7	1900,4	1088,5	809,7	0,27	0,00	Mus musculus major facilitator superfamily domain containing 2 (Mfsd2), mRNA.
Mmd2	682,9	389,9	181,9	93,4	0,27	0,01	Mus musculus monocyte to macrophage differentiation-associated 2 (Mmd2), mRNA.
Nt5e	248,1	191,7	62,6	11,7	0,25	0,04	Mus musculus 5' nucleotidase, ecto (Nt5e), mRNA.
Saa1	241,1	99,9	58,8	73,5	0,24	0,00	Mus musculus serum amyloid A 1 (Saa1), mRNA.
Tomm22	574,0	131,3	138,5	140,2	0,24	0,00	Mus musculus translocase of outer mitochondrial membrane 22 homolog (yeast) (Tomm22), nuclear gene encoding mitochondrial protein, mRNA.
Hr	121,0	47,5	28,9	6,4	0,24	0,00	Mus musculus hairless (Hr), mRNA.
Rbp1	790,9	566,1	184,2	30,5	0,23	0,02	
Akr1b3	181,1	70,6	42,0	50,9	0,23	0,00	Mus musculus aldo-keto reductase family 1, member B3 (aldose reductase) (Akr1b3), mRNA.
Cyp2a5	8942,9	4942,5	2061,2	919,3	0,23	0,00	Mus musculus cytochrome P450, family 2, subfamily a, polypeptide 5 (Cyp2a5), mRNA.
Car3	2097,5	1424,2	439,6	457,6	0,21	0,01	Mus musculus carbonic anhydrase 3 (Car3), mRNA.
Sult1e1	203,3	168,0	35,9	35,4	0,18	0,03	Mus musculus sulfotransferase family 1E, member 1 (Sult1e1), mRNA.
BC048546	564,9	93,5	98,3	58,6	0,17	0,00	Mus musculus cDNA sequence BC048546 (BC048546), mRNA.
Gsta2	4118,1	3201,3	599,4	200,0	0,15	0,01	Mus musculus glutathione S-transferase, alpha 2 (Yc2) (Gsta2), mRNA.
Saa2	278,6	121,4	37,5	24,0	0,13	0,00	Mus musculus serum amyloid A 2 (Saa2), mRNA.
Ccnd1	494,3	286,6	64,0	27,8	0,13	0,00	Mus musculus cyclin D1 (Ccnd1), mRNA.

Supplementary Table 1: Differentially expressed genes in livers from YOUNG FXR<sup>-/-</sup>iVP16FXR vs. FXR<sup>-/-</sup>iVP16 mice.  
Genes are listed from the highest fold ratio to the lowest fold ratio.

SYMBOL	KO AVG Signal	KO - SD	TG AVG Signal	TG - SD	Ratio	P	DEFINITION
Orm2	10536,1	6608,0	1322,0	749,2	0,13	0,00	
Lcn2	5005,8	2866,2	554,6	335,1	0,11	0,00	Mus musculus lipocalin 2 (Lcn2), mRNA.
Lgals1	824,3	781,3	58,0	49,0	0,07	0,03	
Cyp2b9	2165,9	1979,9	103,5	175,8	0,05	0,02	Mus musculus cytochrome P450, family 2, subfamily b, polypeptide 9 (Cyp2b9), mRNA.
Ly6d	220,4	193,3	7,0	10,0	0,03	0,01	Mus musculus lymphocyte antigen 6 complex, locus D (Ly6d), mRNA.
Gsta1	4466,1	2994,0	113,8	62,9	0,03	0,00	Mus musculus glutathione S-transferase, alpha 1 (Ya) (Gsta1), mRNA.
Gstm3	336,5	319,0	8,1	3,4	0,02	0,02	Mus musculus glutathione S-transferase, mu 3 (Gstm3), mRNA.
Nope	107,1	94,5	2,1	7,7	0,02	0,01	Mus musculus neighbor of Punc E11 (Nope), mRNA.
Cyp2b13	1481,4	1648,8	18,7	28,0	0,01	0,05	Mus musculus cytochrome P450, family 2, subfamily b, polypeptide 13 (Cyp2b13), mRNA.
Cyp2c55	477,2	475,3	-3,5	3,0	-0,01	0,02	Mus musculus cytochrome P450, family 2, subfamily c, polypeptide 55 (Cyp2c55), mRNA.

Supplementary Table 2. Differentially expressed genes in livers from OLD FXR<sup>-/-</sup>iVP16FXR vs. FXR<sup>-/-</sup>iVP16 mice. Genes are listed from the highest fold ratio to the lowest fold ratio.

SYMBOL	KO AVG Signal	KO - SD	TG AVG Signal	TG - SD	Ratio	P	DEFINITION
Lrrc24	1.0	4.1	149.2	143.8	149,214	0.016	Mus musculus leucine rich repeat containing 24 (Lrrc24), mRNA.
Ctse	6.8	11.5	110.7	68.8	16,212	0.001	Mus musculus cathepsin E (Ctse), mRNA.
Serpina4-ps1	21.8	11.2	193.0	141.0	8,843	0.007	Mus musculus serine (or cysteine) peptidase inhibitor, clade A, member 4, pseudogene 1 (Serpina4-ps1), non-coding RNA.
Hsd3b5	453.6	928.3	3250.0	2451.6	7,165	0.017	Mus musculus hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 5 (Hsd3b5), mRNA.
Serpina12	218.8	235.3	1470.4	938.4	6,719	0.018	Mus musculus serine (or cysteine) peptidase inhibitor, clade A (alpha-1 antitrypsin, antitrypsin), member 12 (Serpina12), mRNA.
Gna14	31.0	12.9	146.2	89.0	4,709	0.005	Mus musculus guanine nucleotide binding protein, alpha 14 (Gna14), mRNA.
Tpmt	223.5	339.4	1050.5	292.9	4,700	0.000	Mus musculus thiopurine methyltransferase (Tpmt), mRNA.
Cyp2d13	145.4	135.1	681.2	344.5	4,684	0.001	Mus musculus cytochrome P450, family 2, subfamily d, polypeptide 13 (Cyp2d13), non-coding RNA.
Dbp	327.5	141.2	1462.3	587.1	4,465	0.000	Mus musculus D site albumin promoter binding protein (Dbp), mRNA.
Nr1d1	34.1	7.9	147.4	62.8	4,322	0.000	Mus musculus nuclear receptor subfamily 1, group D, member 1 (Nr1d1), mRNA.
OTTMUSG0000000231	4631.3	5244.6	19754.3	11861.1	4,265	0.009	Mus musculus predicted gene, OTTMUSG0000000231 (OTTMUSG0000000231), mRNA.
Bach2	60.3	21.8	252.5	103.6	4,184	0.000	Mus musculus BTB and CNC homology 2 (Bach2), mRNA.
2010001M09Rik	24.8	7.3	102.9	53.2	4,153	0.002	Mus musculus RIKEN cDNA 2010001M09 gene (2010001M09Rik), mRNA.
Cml1	832.2	335.1	3021.1	1088.8	3,630	0.000	Mus musculus camello-like 1 (Cml1), mRNA.
Tspan33	38.1	31.0	134.8	88.5	3,537	0.022	Mus musculus tetraspanin 33 (Tspan33), mRNA.
Krt23	52.7	30.8	182.0	132.7	3,454	0.034	Mus musculus keratin 23 (Krt23), mRNA.
Adh7	62.8	39.2	207.6	46.9	3,304	0.000	Mus musculus alcohol dehydrogenase 7 (class IV), mu or sigma polypeptide (Adh7), mRNA.
Junb	171.4	88.9	554.0	420.8	3,232	0.047	Mus musculus Jun-B oncogene (Junb), mRNA.
1190007F08Rik	87.5	21.8	282.0	134.1	3,224	0.001	Mus musculus RIKEN cDNA 1190007F08 gene (1190007F08Rik), mRNA.
2810007J24Rik	1030.9	1114.8	3071.3	1468.7	2,979	0.013	Mus musculus RIKEN cDNA 2810007J24 gene (2810007J24Rik), mRNA.
Ela1	416.2	113.8	1169.0	784.1	2,808	0.034	Mus musculus elastase 1, pancreatic (Ela1), mRNA.
Osgin1	698.6	133.1	1917.2	819.9	2,744	0.001	Mus musculus oxidative stress induced growth inhibitor 1 (Osgin1), mRNA.
Spsb4	71.1	31.2	193.4	70.0	2,720	0.000	Mus musculus sp1A/ryanodine receptor domain and SOCS box containing 4 (Spsb4), mRNA.
Cyp4a12b	252.1	251.9	683.0	390.5	2,709	0.038	Mus musculus cytochrome P450, family 4, subfamily a, polypeptide 12B (Cyp4a12b), mRNA.
2810405K02Rik	165.6	69.0	448.5	198.9	2,708	0.003	Mus musculus RIKEN cDNA 2810405K02 gene (2810405K02Rik), mRNA.
Sc5d	76.0	41.1	205.2	128.4	2,699	0.033	
Ndubf2	165.7	42.5	443.1	66.7	2,673	0.000	Mus musculus NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 2 (Ndubf2), mRNA.
Bdh2	409.1	202.1	1079.9	453.2	2,640	0.003	Mus musculus 3-hydroxybutyrate dehydrogenase, type 2 (Bdh2), mRNA.
Dclk3	71.2	17.8	186.2	102.5	2,615	0.014	Mus musculus doublecortin-like kinase 3 (Dclk3), mRNA.
Parp12	40.5	60.4	105.3	26.4	2,598	0.031	Mus musculus poly (ADP-ribose) polymerase family, member 12 (Parp12), mRNA.
Hal	439.3	206.3	1139.2	55.9	2,593	0.000	Mus musculus histidine ammonia lyase (Hal), mRNA.
LOC100047427	155.8	48.5	400.9	132.4	2,573	0.000	PREDICTED: Mus musculus similar to thyroid hormone receptor (LOC100047427), mRNA.
Cml4	340.1	276.8	849.0	399.7	2,496	0.019	Mus musculus camello-like 4 (Cml4), mRNA.
Cyp7b1	2844.2	1394.4	7014.4	2780.3	2,466	0.003	Mus musculus cytochrome P450, family 7, subfamily b, polypeptide 1 (Cyp7b1), mRNA.
Scnn1a	44.5	31.4	106.6	40.0	2,395	0.008	Mus musculus sodium channel, nonvoltage-gated 1 alpha (Scnn1a), mRNA.
Nudt7	2009.2	974.3	4800.9	2274.5	2,389	0.034	Mus musculus nudix (nucleoside diphosphate linked moiety X)-type motif 7 (Nudt7), transcript variant 2, mRNA.
Atp5e	193.9	87.0	459.1	43.9	2,368	0.000	Mus musculus ATP synthase, H+ transporting, mitochondrial F1 complex, epsilon subunit (Atp5e), nuclear gene encoding mitochondrial protein, mRNA.
Efn1	347.9	68.6	822.2	338.0	2,363	0.002	Mus musculus ephrin A1 (Efn1), mRNA.
Cox7b	119.5	41.2	282.2	19.3	2,363	0.000	Mus musculus cytochrome c oxidase subunit VIIb (Cox7b), nuclear gene encoding mitochondrial protein, mRNA.
Parp16	141.5	21.7	330.6	167.4	2,337	0.014	Mus musculus poly (ADP-ribose) polymerase family, member 16 (Parp16), mRNA.
1110001J03Rik	804.1	296.7	1843.3	293.4	2,292	0.000	Mus musculus RIKEN cDNA 1110001J03 gene (1110001J03Rik), mRNA.
Egrf	518.3	236.7	1185.9	508.5	2,288	0.034	
Gadd45a	72.0	25.4	164.4	36.9	2,284	0.000	Mus musculus growth arrest and DNA-damage-inducible 45 alpha (Gadd45a), mRNA.
Hdh3	195.2	61.5	433.3	242.5	2,219	0.034	Mus musculus haloacid dehalogenase-like hydrolase domain containing 3 (Hdh3), mRNA.
Usmg5	72.8	30.2	161.0	16.5	2,212	0.000	Mus musculus upregulated during skeletal muscle growth 5 (Usmg5), mRNA.
Mrp152	596.1	239.6	1312.5	100.4	2,202	0.000	Mus musculus mitochondrial ribosomal protein L52 (Mrp152), nuclear gene encoding mitochondrial protein, mRNA.
Plekhh1	421.3	192.1	915.9	251.0	2,174	0.000	Mus musculus pleckstrin homology domain containing, family B (evectins) member 1 (Plekhh1), mRNA.
C8a	790.9	262.2	1694.8	415.6	2,143	0.000	Mus musculus complement component 8, alpha polypeptide (C8a), mRNA.
Hyi	1059.6	325.2	2202.9	790.0	2,079	0.003	Mus musculus hydroxypyruvate isomerase homolog (E. coli) (Hyi), mRNA.
Pparg1b	84.9	10.6	176.4	41.3	2,078	0.000	Mus musculus peroxisome proliferative activated receptor, gamma, coactivator 1 beta (Pparg1b), mRNA.
Alkbh7	152.2	44.2	313.3	75.9	2,058	0.000	Mus musculus alkB, alkylation repair homolog 7 (E. coli) (Alkbh7), mRNA.
Ccl27	143.0	66.4	294.2	56.9	2,057	0.001	Mus musculus chemokine (C-C motif) ligand 27 (Ccl27), transcript variant 2, mRNA.
LOC100041703	160.0	34.7	327.4	30.6	2,046	0.000	PREDICTED: Mus musculus similar to G protein gamma-5 subunit (LOC100041703), mRNA.
Cox7a2	161.5	48.3	324.1	33.8	2,007	0.000	Mus musculus cytochrome c oxidase, subunit VIIa 2 (Cox7a2), mRNA.
BC021614	373.9	68.2	749.3	94.4	2,004	0.000	Mus musculus cDNA sequence BC021614 (BC021614), mRNA.
Slc25a25	579.9	102.7	1153.8	318.9	1,990	0.000	Mus musculus solute carrier family 25 (mitochondrial carrier, phosphate carrier), member 25 (Slc25a25), nuclear gene encoding mitochondrial protein, mRNA.
LOC668837	402.3	143.3	799.9	172.8	1,989	0.000	PREDICTED: Mus musculus similar to ATP synthase, H+ transporting, mitochondrial F0 complex, subunit G (LOC668837), misc RNA.
Sec61g	1431.0	610.1	2840.2	343.9	1,985	0.000	Mus musculus SEC61, gamma subunit (Sec61g), mRNA.

Supplementary Table 2. Differentially expressed genes in livers from OLD FXR<sup>-/-</sup>iVP16FXR vs. FXR<sup>-/-</sup>iVP16 mice. Genes are listed from the highest fold ratio to the lowest fold ratio.

SYMBOL	KO AVG Signal	KO - SD	TG AVG Signal	TG - SD	Ratio	P	DEFINITION
Slc26a1	74.9	33.6	148.1	49.1	1.978	0.007	Mus musculus solute carrier family 26 (sulfate transporter), member 1 (Slc26a1), mRNA.
Krt10	65.4	25.4	128.5	18.1	1.966	0.000	Mus musculus keratin 10 (Krt10), mRNA.
Mmp15	92.4	17.1	181.3	37.4	1.962	0.000	Mus musculus matrix metalloproteinase 15 (Mmp15), mRNA. XM_001002221
1100001G20Rik	835.3	304.4	1637.2	410.7	1.960	0.000	Mus musculus RIKEN cDNA 1100001G20 gene (1100001G20Rik), mRNA.
Selenbp2	1214.6	480.5	2372.3	1174.4	1.953	0.041	Mus musculus selenium binding protein 2 (Selenbp2), mRNA.
Gls2	1146.0	537.4	2230.7	905.4	1.947	0.022	Mus musculus glutaminase 2 (liver, mitochondrial) (Gls2), nuclear gene encoding mitochondrial protein, mRNA.
Fxyd1	97.3	32.4	189.5	18.2	1.946	0.000	Mus musculus FX/YD domain-containing ion transport regulator 1 (Fxyd1), transcript variant 5, mRNA.
Ccbl2	127.5	50.8	247.9	93.8	1.944	0.009	Mus musculus cysteine conjugate-beta lyase 2 (Ccbl2), mRNA.
Pums	92.5	28.9	177.2	84.4	1.916	0.035	Mus musculus parathyrimosin (Pums), mRNA.
Lrit1	55.2	24.5	105.3	23.2	1.907	0.002	Mus musculus leucine-rich repeat, immunoglobulin-like and transmembrane domains 1 (Lrit1), mRNA.
LOC327956	63.7	20.9	121.1	41.5	1.901	0.007	
Pcdh1	186.2	64.3	352.2	40.5	1.891	0.000	Mus musculus protocadherin 1 (Pcdh1), mRNA.
Ndubf4	120.2	51.0	226.9	17.2	1.888	0.000	Mus musculus NADH dehydrogenase (ubiquinone) 1 beta subcomplex 4 (Ndubf4), nuclear gene encoding mitochondrial protein, mRNA.
S100a13	242.5	73.0	449.8	48.9	1.855	0.000	Mus musculus S100 calcium binding protein A13 (S100a13), mRNA.
Fgd6	131.7	53.9	243.8	63.9	1.851	0.003	Mus musculus FYVE, RhoGEF and PH domain containing 6 (Fgd6), mRNA.
C8b	1754.2	848.0	3223.1	782.4	1.837	0.004	Mus musculus complement component 8, beta polypeptide (C8b), mRNA.
Dpm3	353.2	198.9	648.5	94.6	1.836	0.004	
Ddt	1187.9	230.6	2179.0	304.2	1.834	0.000	Mus musculus D-dopachrome tautomerase (Ddt), mRNA.
Inhbc	226.9	100.3	414.2	182.3	1.826	0.044	Mus musculus inhibitor beta-C (Inhbc), mRNA.
Id2	546.2	119.4	996.0	229.0	1.824	0.001	Mus musculus inhibitor of DNA binding 2 (Id2), mRNA.
Rnf186	88.5	22.8	161.1	58.1	1.820	0.011	Mus musculus ring finger protein 186 (Rnf186), mRNA.
Zfx2	98.7	35.0	179.2	51.5	1.816	0.006	Mus musculus zinc finger homeobox 2 (Zfx2), mRNA.
LOC100048301	316.2	93.7	571.7	180.5	1.808	0.005	PREDICTED: Mus musculus similar to RNA Polymerase II subunit 14.5 kD (LOC100048301), mRNA.
Vmo1	329.1	84.6	594.1	163.0	1.805	0.001	Mus musculus vitelline membrane outer layer 1 homolog (chicken) (Vmo1), mRNA.
Nox4	127.8	66.7	230.5	86.3	1.803	0.036	Mus musculus NADPH oxidase 4 (Nox4), mRNA.
LOC100047173	64.2	13.7	115.7	25.6	1.803	0.001	PREDICTED: Mus musculus similar to synaptotagmin-like 1 (LOC100047173), misc RNA.
Npm3-ps1	284.7	134.1	513.1	15.6	1.803	0.002	Mus musculus nucleoplamin 3, pseudogene 1 (Npm3-ps1), non-coding RNA.
Tomm7	590.7	186.0	1064.0	37.3	1.801	0.000	Mus musculus translocase of outer mitochondrial membrane 7 homolog (yeast) (Tomm7), mRNA.
Chchd7	234.7	106.6	422.6	49.6	1.801	0.001	Mus musculus coiled-coil-helix-coiled-coil-helix domain containing 7 (Chchd7), mRNA.
LOC100048187	4159.1	1675.0	7483.0	1328.2	1.799	0.001	PREDICTED: Mus musculus similar to ribosomal protein S27 (metallopantistimulin 1) (LOC100048187), mRNA.
Pnpla7	824.2	367.7	1479.0	466.6	1.794	0.014	Mus musculus patatin-like phospholipase domain containing 7 (Pnpla7), mRNA.
Cdk5rap1	65.6	5.7	117.6	35.7	1.793	0.017	Mus musculus CDK5 regulatory subunit associated protein 1 (Cdk5rap1), mRNA.
Bik	106.6	46.0	189.7	70.1	1.779	0.028	Mus musculus BCL2-interacting killer (Bik), mRNA.
Ndubf9	620.1	128.6	1102.5	72.7	1.778	0.000	Mus musculus NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 9 (Ndubf9), mRNA.
Aadat	77.5	29.9	137.2	26.9	1.770	0.002	Mus musculus aminoadipate aminotransferase (Aadat), mRNA.
Npm3	168.3	77.0	296.9	39.1	1.764	0.034	Mus musculus nucleoplamin 3 (Npm3), mRNA.
Ferm2	1403.5	422.7	2468.4	843.6	1.759	0.012	Mus musculus fermitin family homolog 2 (Drosophila) (Ferm2), mRNA.
Hc	1693.7	1121.4	2970.8	645.1	1.754	0.027	Mus musculus hemolytic complement (Hc), mRNA.
8430408G22Rik	1710.0	541.6	2997.1	1293.4	1.753	0.040	Mus musculus RIKEN cDNA 8430408G22 gene (8430408G22Rik), mRNA.
Ndufa3	2054.4	911.4	3588.0	247.2	1.746	0.002	Mus musculus NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 3 (Ndufa3), mRNA.
Ndubf10	472.6	151.3	824.4	175.5	1.744	0.001	Mus musculus NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 10 (Ndubf10), mRNA.
2010100012Rik	279.8	101.1	487.4	18.8	1.742	0.013	Mus musculus RIKEN cDNA 2010100012 gene (2010100012Rik), mRNA.
Pcbd2	662.6	165.7	1152.5	222.1	1.739	0.000	Mus musculus pterin 4 alpha carbinolamine dehydratase/dimerization cofactor of hepatocyte nuclear factor 1 alpha (TCF1) 2 (Pcbd2), mRNA.
Cyp2c70	10391.1	3314.6	18038.9	3799.4	1.736	0.001	Mus musculus cytochrome P450, family 2, subfamily c, polypeptide 70 (Cyp2c70), mRNA.
1810014F10Rik	226.5	83.4	393.0	61.0	1.735	0.001	Mus musculus RIKEN cDNA 1810014F10 gene (1810014F10Rik), mRNA.
Magix	94.3	39.3	163.5	21.4	1.734	0.003	Mus musculus MAGI family member, X-linked (Magix), mRNA.
Ugt2b1	1714.9	818.7	2970.6	807.9	1.732	0.015	Mus musculus UDP glucuronosyltransferase 2 family, polypeptide B1 (Ugt2b1), mRNA.
Pigr	1761.6	598.2	3044.8	414.7	1.728	0.000	Mus musculus polymeric immunoglobulin receptor (Pigr), mRNA.
Prps2	72.9	9.8	126.0	29.7	1.728	0.002	Mus musculus phosphoribosyl pyrophosphate synthetase 2 (Prps2), mRNA.
F11	700.3	207.9	1210.1	153.3	1.728	0.001	Mus musculus coagulation factor XI (F11), mRNA.
Ethe1	780.8	151.1	1347.8	234.6	1.726	0.000	Mus musculus ethylmalonic encephalopathy 1 (Ethe1), mRNA.
3010026O09Rik	163.1	46.2	281.4	97.1	1.725	0.015	Mus musculus RIKEN cDNA 3010026O09 gene (3010026O09Rik), mRNA.
Crim2	66.2	21.3	114.0	23.4	1.722	0.002	Mus musculus cysteine rich BMP regulator 2 (chordin like) (Crim2), mRNA.
Nme3	270.3	57.5	462.0	72.2	1.709	0.000	Mus musculus non-metastatic cells 3, protein expressed in (Nme3), mRNA.
Grc10	656.4	210.5	1121.7	78.2	1.709	0.000	Mus musculus gene rich cluster, C10 gene (Grc10), mRNA.
BC029214	88.6	21.0	151.3	29.1	1.708	0.001	Mus musculus cDNA sequence BC029214 (BC029214), mRNA.
Cox6c	4149.8	774.9	7048.6	871.5	1.699	0.000	Mus musculus cytochrome c oxidase, subunit VIc (Cox6c), mRNA.
mar-08	88.4	20.3	149.4	19.7	1.689	0.001	Mus musculus membrane-associated ring finger (C3HC4) 8 (March8), mRNA.
Cd300g	67.9	30.9	114.2	21.3	1.682	0.013	Mus musculus CD300 antigen like family member G (Cd300g), mRNA.
Ndufa1	2702.4	843.3	4543.6	119.5	1.681	0.000	Mus musculus NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 1 (Ndufa1), nuclear gene encoding mitochondrial protein, mRNA.
Tm7sf2	221.7	71.3	372.5	121.9	1.680	0.017	Mus musculus transmembrane 7 superfamily member 2 (Tm7sf2), mRNA.

Supplementary Table 2. Differentially expressed genes in livers from OLD FXR<sup>-/-</sup>iVP16FXR vs. FXR<sup>-/-</sup>iVP16 mice. Genes are listed from the highest fold ratio to the lowest fold ratio.

SYMBOL	KO AVG Signal	KO - SD	TG AVG Signal	TG - SD	Ratio	P	DEFINITION
Ndufb5	482.2	127.3	809.9	105.5	1.680	0.000	Mus musculus NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 5 (Ndufb5), nuclear gene encoding mitochondrial protein, mRNA.
Unc84b	798.1	296.2	1333.9	325.8	1.671	0.007	Mus musculus unc-84 homolog B (C. elegans) (Unc84b), mRNA.
Mug4	11294.9	3807.3	18876.0	2339.5	1.671	0.001	PREDICTED: Mus musculus murinoglobulin 4 (Mug4), misc RNA.
Paics	63.3	33.7	105.7	11.2	1.670	0.027	Mus musculus phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoribosylaminoimidazole, succinocarboxamide synthetase (Paics), mRNA.
Agxt	1712.4	544.9	2859.1	536.6	1.670	0.001	Mus musculus alanine-glyoxylate aminotransferase (Agxt), mRNA.
1500012F01Rik	265.2	104.4	442.6	94.6	1.669	0.005	Mus musculus RIKEN cDNA 1500012F01 gene (1500012F01Rik), mRNA.
Pigy1	110.8	27.3	184.8	15.4	1.668	0.000	Mus musculus phosphatidylinositol glycan anchor biosynthesis, class Y-like (Pigy1), mRNA.
Anapc13	1337.3	447.6	2230.7	78.6	1.668	0.001	Mus musculus anaphase promoting complex subunit 13 (Anapc13), mRNA.
Ccdc5	128.6	4.2	214.1	30.1	1.665	0.000	Mus musculus coiled-coil domain containing 5 (Ccdc5), mRNA.
Dak	631.3	96.6	1050.4	341.9	1.664	0.011	Mus musculus dihydroxyacetone kinase 2 homolog (yeast) (Dak), mRNA.
Cyp2f2	8083.1	4190.0	13448.2	3440.0	1.664	0.027	Mus musculus cytochrome P450, family 2, subfamily f, polypeptide 2 (Cyp2f2), mRNA.
Slc25a23	137.4	43.1	227.7	40.6	1.658	0.001	Mus musculus solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 23 (Slc25a23), nuclear gene encoding mitochondrial protein, mRNA.
Klf9	1796.9	416.9	2948.0	580.4	1.641	0.000	Mus musculus Kruppel-like factor 9 (Klf9), mRNA.
Osbp18	63.6	15.6	104.3	13.0	1.640	0.005	Mus musculus oxysterol binding protein-like 8 (Osbp18), transcript variant 1, mRNA.
H1f0	182.0	37.7	298.3	45.2	1.639	0.000	Mus musculus H1 histone family, member 0 (H1f0), mRNA.
BC031853	62.0	4.3	101.4	10.3	1.634	0.005	Mus musculus cDNA sequence BC031853 (BC031853), mRNA.
Spnb3	221.8	75.1	361.9	47.1	1.631	0.002	Mus musculus spectrin beta 3 (Spnb3), mRNA.
2410015M20Rik	449.0	133.6	730.8	64.4	1.628	0.001	Mus musculus RIKEN cDNA 2410015M20 gene (2410015M20Rik), mRNA.
Pla1a	200.5	66.6	326.0	44.0	1.626	0.004	Mus musculus RIKEN cDNA 2900010M23 gene (2900010M23Rik), mRNA.
2900010M23Rik	1354.1	318.7	2199.1	119.6	1.624	0.000	Mus musculus RIKEN cDNA 2410015N17 gene (2410015N17Rik), mRNA.
2410015N17Rik	483.6	126.9	784.7	141.2	1.623	0.000	Mus musculus RIKEN cDNA 1810035L17 gene (1810035L17Rik), mRNA.
1810035L17Rik	374.1	149.0	604.4	38.7	1.616	0.006	Mus musculus DnaJ (Hsp40) homolog, subfamily C, member 3 (Dnajc3), mRNA.
Dnajc3	561.8	220.2	907.0	215.4	1.615	0.012	Mus musculus acyl-Coenzyme A dehydrogenase, short/branched chain (Acadsb), nuclear gene encoding mitochondrial protein, mRNA.
Acadsb	970.9	231.9	1566.7	183.1	1.614	0.000	Mus musculus ATP-binding cassette, sub-family A (ABC1), member 6 (Abca6), mRNA.
Abca6	101.9	25.3	164.3	39.0	1.612	0.029	Mus musculus split hand/foot malformation (ectrodactyly) type 1 (Shfm1), mRNA.
Shfm1	2099.4	419.4	3379.1	407.6	1.610	0.000	Mus musculus arginine vasopressin receptor 1A (Avpr1a), mRNA.
Avpr1a	450.5	202.9	723.6	96.7	1.606	0.009	Mus musculus nasal embryonic LHRH factor (Nelf), transcript variant 2, mRNA.
Nelf	971.3	246.5	1558.6	300.4	1.605	0.001	Mus musculus SET domain containing 1B (Setd1b), mRNA.
Setd1b	265.8	63.6	426.1	74.8	1.603	0.000	Mus musculus suppressor of Ty 4 homolog 1 (S. cerevisiae) (Supt4h1), mRNA.
Supt4h1	155.2	36.2	248.7	35.4	1.602	0.001	Mus musculus nuclear factor 1X (Nfix), transcript variant 2, mRNA. XM_921228 XM_921240 XM_921250 XM_921261 XM_921270 XM_921288 XM_921293 XM_921299 XM_921300 XM_921307
Nfix	309.5	82.8	494.7	104.2	1.598	0.032	Mus musculus sPLA/ryanodine receptor domain and SOCS box containing 2 (Spsb2), mRNA.
Spsb2	81.8	5.2	130.2	14.2	1.592	0.004	Mus musculus RIKEN cDNA 2010001J22 gene (2010001J22Rik), mRNA.
2010001J22Rik	144.6	64.1	229.8	43.0	1.589	0.020	PREDICTED: Mus musculus similar to FERM/RhoGEF (Arhgef) and pleckstrin domain protein 1 (LOC100045542), mRNA.
LOC100045542	114.8	21.6	181.9	43.6	1.584	0.005	Mus musculus RIKEN cDNA 1810027O10 gene (1810027O10Rik), mRNA. XM_109683 XM_909001
1810027O10Rik	3621.6	1496.6	5731.6	557.5	1.583	0.008	PREDICTED: Mus musculus similar to Ad1 protein (LOC100046254), mRNA.
LOC100046254	178.2	39.3	281.7	46.2	1.581	0.001	Mus musculus expressed sequence C78339 (C78339), mRNA.
C78339	78.6	4.7	124.2	32.6	1.580	0.012	Mus musculus RIKEN cDNA 0610012D14 gene (0610012D14Rik), mRNA.
0610012D14Rik	1024.0	397.1	1617.8	475.7	1.580	0.036	Mus musculus NFU1 iron-sulfur cluster scaffold homolog (S. cerevisiae) (Nfu1), mRNA.
Nfu1	88.3	26.7	139.4	28.1	1.580	0.006	Mus musculus GH regulated TBC protein 1 (Grtp1), mRNA.
Grtp1	63.6	12.8	100.5	14.5	1.579	0.009	PREDICTED: Mus musculus similar to mitochondria-associated granulocyte macrophage CSF signaling cascade molecule (LOC100042179), mRNA.
LOC100042179	206.4	61.8	325.6	56.4	1.578	0.002	Mus musculus RNA terminal phosphate cyclase-like 1 (Rcl1), mRNA.
Rcl1	1701.5	356.4	2677.9	483.2	1.574	0.005	Mus musculus myeloid differentiation primary response gene 116 (Myd116), mRNA.
Myd116	80.8	19.5	127.0	37.2	1.571	0.021	Mus musculus small nuclear ribonucleoprotein polypeptide G (Snrpg), mRNA.
Snrpg	303.4	90.5	476.3	73.3	1.570	0.002	Mus musculus RIKEN cDNA 1500032D16 gene (1500032D16Rik), transcript variant 1, mRNA.
1500032D16Rik	1775.5	360.6	2783.9	200.9	1.568	0.004	Mus musculus cDNA sequence BC002163 (BC002163), non-coding RNA.
BC002163	1021.7	268.5	1601.0	189.4	1.567	0.001	Mus musculus NADH dehydrogenase (ubiquinone) 1 beta subcomplex 3 (Ndufb3), mRNA.
Ndufb3	1106.8	254.4	1729.7	370.2	1.563	0.002	Mus musculus RELT-like 1 (Rel1), mRNA.
Rel1	90.7	28.7	141.7	24.3	1.561	0.008	Mus musculus ATP-binding cassette, sub-family A (ABC1), member 8a (Abca8a), mRNA.
Abca8a	171.3	45.3	267.2	66.3	1.560	0.008	Mus musculus claudin 3 (Cldn3), mRNA.
Cldn3	697.1	108.1	1085.4	86.9	1.557	0.000	Mus musculus pyruvate dehydrogenase kinase, isoenzyme 2 (Pdk2), mRNA.
Pdk2	78.6	17.2	122.2	21.9	1.555	0.007	Mus musculus nudix (nucleoside diphosphate linked moiety X)-type motif 22 (Nudt22), mRNA.
Nudt22	176.8	40.7	274.9	31.6	1.554	0.001	Mus musculus aspartoacylase (aminoacylase) 3 (Acy3), mRNA.
Acy3	449.1	102.7	697.9	186.5	1.554	0.009	Mus musculus RIKEN cDNA 4732496O08 gene (4732496O08Rik), mRNA.
4732496O08Rik	66.6	14.0	103.5	27.1	1.554	0.019	Mus musculus solute carrier family 9 (sodium/hydrogen exchanger), member 3 regulator 1 (Slc9a3r1), mRNA.
Slc9a3r1	1098.6	286.4	1706.3	272.3	1.553	0.001	Mus musculus bolA-like 1 (E. coli) (Bola1), mRNA.
Bola1	129.8	41.5	201.5	41.3	1.552	0.007	



Supplementary Table 2. Differentially expressed genes in livers from OLD FXR<sup>-/-</sup>iVP16FXR vs. FXR<sup>-/-</sup>iVP16 mice. Genes are listed from the highest fold ratio to the lowest fold ratio.

SYMBOL	KO AVG Signal	KO - SD	TG AVG Signal	TG - SD	Ratio	P	DEFINITION
Rassf5	104.3	5.2	161.8	52.1	1,552	0.031	Mus musculus Ras association (RalGDS/AF-6) domain family 5 (Rassf5), mRNA.
Fxc1	162.8	43.0	252.1	24.5	1,549	0.003	Mus musculus fractured callus expressed transcript 1 (Fxc1), mRNA.
Rps26	2936.2	1329.9	4542.4	585.0	1,547	0.019	Mus musculus ribosomal protein S26 (Rps26), mRNA.
Znrd1	246.5	47.3	380.8	37.5	1,545	0.002	Mus musculus zinc ribbon domain containing_1 (Znrd1), mRNA.
1110019N10Rik	76.5	8.4	118.1	18.2	1,544	0.009	Mus musculus RIKEN cDNA 1110019N10 gene (1110019N10Rik), mRNA.
Lsm2	97.5	19.5	150.5	17.4	1,544	0.005	Mus musculus LSM2 homolog, U6 small nuclear RNA associated (S. cerevisiae) (Lsm2), mRNA.
Abcc6	437.0	100.1	674.6	181.8	1,544	0.011	Mus musculus ATP-binding cassette, sub-family C (CFTR/MRP), member 6 (Abcc6), mRNA.
Selk	365.0	45.0	563.3	59.4	1,543	0.000	Mus musculus hairy and enhancer of split 1 (Drosophila) (Hes1), mRNA.
Hes1	77.5	19.5	119.4	34.1	1,541	0.025	Mus musculus hairy and enhancer of split 1 (Drosophila) (Hes1), mRNA.
Cpn2	77.7	31.2	119.7	11.9	1,540	0.026	Mus musculus carboxypeptidase N, polypeptide 2 (Cpn2), mRNA.
Zfp467	76.3	18.8	117.5	31.1	1,540	0.019	Mus musculus zinc finger protein 467 (Zfp467), transcript variant 3, mRNA.
C730025P13Rik	285.9	58.6	440.4	113.8	1,540	0.008	Mus musculus RIKEN cDNA C730025P13 gene (C730025P13Rik), mRNA.
Xrcc1	69.9	7.5	107.5	20.5	1,538	0.011	Mus musculus X-ray repair complementing defective repair in Chinese hamster cells 1 (Xrcc1), mRNA.
Dgadcl	167.4	68.4	257.4	40.5	1,538	0.018	Mus musculus deaminase domain containing 1 (Dgadcl), mRNA.
1810009N02Rik	134.0	22.3	206.0	31.8	1,537	0.002	Mus musculus RIKEN cDNA 1810009N02 gene (1810009N02Rik), mRNA.
LOC100048483	4721.6	1032.4	7259.1	543.3	1,537	0.000	PREDICTED: Mus musculus similar to cytochrome c oxidase subunit VIII (LOC100048483), mRNA.
2310003F16Rik	1086.1	290.1	1668.7	81.1	1,536	0.001	Mus musculus RIKEN cDNA 2310003F16 gene (2310003F16Rik), mRNA.
Creb3l3	599.7	157.4	920.5	196.9	1,535	0.004	Mus musculus cAMP responsive element binding protein 3-like 3 (Creb3l3), mRNA.
Ssbp3	198.2	34.4	303.7	55.9	1,532	0.027	Mus musculus single-stranded DNA binding protein 3 (Ssbp3), transcript variant 1, mRNA.
Ndubf7	241.8	49.7	369.9	37.2	1,529	0.001	Mus musculus NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 7 (Ndubf7), nuclear gene encoding mitochondrial protein, mRNA.
Mrps21	572.6	143.9	875.3	117.3	1,529	0.001	Mus musculus mitochondrial ribosomal protein S21 (Mrps21), nuclear gene encoding mitochondrial protein, mRNA.
LOC100047634	74.7	17.3	114.1	18.3	1,528	0.011	PREDICTED: Mus musculus similar to Eukaryotic translation initiation factor 2 alpha kinase 3 (LOC100047634), misc RNA.
2310021P13Rik	115.1	25.3	175.5	35.6	1,525	0.004	Mus musculus similar to Eukaryotic translation initiation factor 2 alpha kinase 3 (LOC100047634), misc RNA.
Stk40	96.1	20.2	146.5	45.0	1,524	0.033	Mus musculus serine/threonine kinase 40 (Stk40), mRNA.
Ndubf8	4675.8	732.4	7111.8	552.7	1,521	0.000	Mus musculus NADH dehydrogenase (ubiquinone) 1 beta subcomplex 8 (Ndubf8), mRNA.
D2Erd391e	261.6	47.6	397.8	130.4	1,521	0.033	Mus musculus DNA segment, Chr 2, ERATO Doi 391, expressed (D2Erd391e), mRNA.
LOC100048480	1645.4	347.8	2501.8	155.9	1,520	0.000	PREDICTED: Mus musculus similar to ubiquinol-cytochrome c reductase binding protein (LOC100048480), mRNA.
Sema4g	457.1	114.7	694.9	135.8	1,520	0.003	Mus musculus sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4G (Sema4g), mRNA.
LOC100047998	3688.3	1356.6	5605.4	981.4	1,520	0.010	PREDICTED: Mus musculus similar to ribosomal protein L37a (LOC100047998), mRNA.
AU022252	358.1	62.7	544.1	103.1	1,519	0.001	Mus musculus expressed sequence AU022252 (AU022252), mRNA.
LOC100048530	89.2	32.2	135.3	22.0	1,517	0.021	PREDICTED: Mus musculus similar to coiled-coil domain containing 72 (LOC100048530), mRNA.
Dhrsx	146.2	28.8	221.6	25.7	1,516	0.001	Mus musculus similar to coiled-coil domain containing 72 (LOC100048530), mRNA.
Ndufa2	3093.4	949.0	4681.5	509.9	1,513	0.004	Mus musculus NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 2 (Ndufa2), mRNA.
Hsd17b2	925.9	229.7	1400.5	286.6	1,513	0.004	Mus musculus hydroxysteroid (17-beta) dehydrogenase 2 (Hsd17b2), mRNA.
LOC100044087	500.2	103.5	755.9	136.1	1,511	0.001	PREDICTED: Mus musculus similar to brain protein 44 (LOC100044087), misc RNA.
2310016M24Rik	885.7	274.6	1337.1	71.0	1,510	0.005	Mus musculus RIKEN cDNA 2310016M24 gene (2310016M24Rik), mRNA.
Ssr4	2639.3	683.8	3979.6	419.3	1,508	0.002	Mus musculus signal sequence receptor, delta (Ssr4), mRNA.
Mrps18c	640.3	200.7	965.5	92.8	1,508	0.005	Mus musculus mitochondrial ribosomal protein S18C (Mrps18c), nuclear gene encoding mitochondrial protein, mRNA.
Mrp133	1013.9	254.3	1526.7	74.6	1,506	0.001	Mus musculus mitochondrial ribosomal protein L33 (Mrp133), nuclear gene encoding mitochondrial protein, mRNA.
Hgfaf	2784.6	957.8	4191.6	1066.4	1,505	0.028	Mus musculus hepatocyte growth factor activator (Hgfaf), mRNA.
Snrpd2	856.3	227.5	1287.7	56.1	1,504	0.002	Mus musculus small nuclear ribonucleoprotein D2 (Snrpd2), mRNA.
LOC100039532	3673.4	952.5	5520.9	384.8	1,503	0.002	PREDICTED: Mus musculus similar to ribosomal protein L35a (LOC100039532), mRNA.
Sec14l2	1328.8	141.1	1994.1	413.3	1,501	0.003	Mus musculus SEC14-like 2 (S. cerevisiae) (Sec14l2), mRNA.
Grhpr	4204.4	896.7	6299.1	1224.0	1,498	0.002	Mus musculus glyoxylate reductase/hydroxypyruvate reductase (Grhpr), mRNA.
Ptgr2	77.0	22.2	115.3	21.8	1,498	0.016	Mus musculus prostaglandin reductase 2 (Ptgr2), mRNA.
Gm561	357.3	113.1	535.0	70.6	1,497	0.007	Mus musculus gene model 561, (NCBI) (Gm561), mRNA.
LOC100048613	4995.0	1205.1	7470.6	705.4	1,496	0.001	PREDICTED: Mus musculus similar to cytochrome c oxidase, subunit VIIc, transcript variant 1 (LOC100048613), mRNA.
LOC100046081	88.7	21.5	132.6	11.5	1,495	0.011	PREDICTED: Mus musculus similar to OTU domain, ubiquitin aldehyde binding 1 (LOC100046081), mRNA.
Ank	131.5	31.5	196.6	5.5	1,495	0.005	Mus musculus progressive ankylosis (Ank), mRNA.
Atp5j2	8348.8	1823.3	12473.2	1072.0	1,494	0.001	Mus musculus ATP synthase, H+ transporting, mitochondrial F0 complex, subunit f, isoform 2 (Atp5j2), nuclear gene encoding mitochondrial protein, mRNA.
Hmgcs2	14413.8	4751.9	21481.9	3904.2	1,490	0.010	Mus musculus 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (Hmgcs2), nuclear gene encoding mitochondrial protein, mRNA.
Nrp1	473.6	86.8	704.9	222.2	1,489	0.035	Mus musculus neuropilin 1 (Nrp1), mRNA.
1190017O12Rik	369.8	92.9	549.7	75.3	1,487	0.003	Mus musculus RIKEN cDNA 1190017O12 gene (1190017O12Rik), mRNA.
Kmo	442.0	44.1	656.7	161.5	1,486	0.010	Mus musculus kynurenine 3-monooxygenase (kynurenine 3-hydroxylase) (Kmo), mRNA.
Dsp	92.8	16.1	137.7	23.4	1,485	0.011	PREDICTED: Mus musculus desmoplakin, transcript variant 1 (Dsp), mRNA.
Nr1d2	88.2	17.9	130.5	15.8	1,479	0.047	Mus musculus nuclear receptor subfamily 1, group D, member 2 (Nr1d2), mRNA.
Setd1a	92.3	33.6	136.6	17.5	1,479	0.031	Mus musculus SET domain containing 1A (Setd1a), mRNA.
2010107E04Rik	4347.7	1011.4	6425.0	1016.5	1,478	0.002	Mus musculus RIKEN cDNA 2010107E04 gene (2010107E04Rik), mRNA.

Supplementary Table 2. Differentially expressed genes in livers from OLD FXR<sup>-/-</sup>iVP16FXR vs. FXR<sup>-/-</sup>iVP16 mice. Genes are listed from the highest fold ratio to the lowest fold ratio.

SYMBOL	KO AVG Signal	KO - SD	TG AVG Signal	TG - SD	Ratio	P	DEFINITION
Fzd5	81.4	18.4	120.3	30.1	1.477	0.026	Mus musculus frizzled homolog 5 (Drosophila) (Fzd5), transcript variant 1, mRNA.
Clcn2	90.0	10.6	132.8	40.2	1.475	0.047	Mus musculus chloride channel 2 (Clcn2), mRNA.
1700001C19Rik	163.9	41.5	241.2	27.1	1.472	0.012	Mus musculus RIKEN cDNA 1700001C19 gene (1700001C19Rik), mRNA.
Daxx	86.1	32.1	126.6	7.2	1.471	0.038	Mus musculus Fas death domain-associated protein (Daxx), mRNA.
Rps21	5426.0	2531.1	7979.6	1105.3	1.471	0.046	Mus musculus ribosomal protein S21 (Rps21), mRNA.
Rassf3	541.6	129.8	796.4	221.1	1.471	0.026	Mus musculus Ras association (RalGDS/AF-6) domain family member 3 (Rassf3), mRNA.
Ncl	91.4	11.0	134.4	4.8	1.470	0.013	Mus musculus nucleolin (Ncl), mRNA.
1700021C14Rik	137.9	40.1	202.7	25.5	1.470	0.012	Mus musculus RIKEN cDNA 1700021C14 gene (1700021C14Rik), mRNA.
Atp5h	1448.8	253.4	2129.2	198.3	1.470	0.001	Mus musculus ATP synthase, H <sup>+</sup> transporting, mitochondrial F0 complex, subunit d (Atp5h), nuclear gene encoding mitochondrial protein, mRNA.
Gnb2l1	101.9	24.4	149.2	30.2	1.465	0.012	Mus musculus guanine nucleotide binding protein (G protein), beta polypeptide 2 like 1 (Gnb2l1), mRNA.
2310005N03Rik	276.1	83.9	404.2	50.8	1.464	0.010	Mus musculus RIKEN cDNA 2310005N03 gene (2310005N03Rik), mRNA.
Ndufa12	1422.4	358.0	2082.1	201.0	1.464	0.003	Mus musculus NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 12 (Ndufa12), mRNA.
Ndufa5	1021.6	296.1	1495.2	152.7	1.464	0.000	Mus musculus NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 5 (Ndufa5), nuclear gene encoding mitochondrial protein, mRNA.
Pecr	335.8	84.0	491.2	124.4	1.463	0.021	Mus musculus peroxisomal trans-2-enoyl-CoA reductase (Pecr), mRNA.
Rpp21	442.0	83.0	646.2	51.4	1.462	0.002	Mus musculus ribonuclease P 21 subunit (human) (Rpp21), mRNA.
Rnasek	402.2	67.3	587.9	27.5	1.462	0.002	Mus musculus ribonuclease, RNase K (Rnasek), mRNA.
C8g	8046.4	1813.9	11753.7	1490.5	1.461	0.002	Mus musculus complement component 8, gamma polypeptide (C8g), mRNA.
LOC100044779	638.7	104.2	932.6	184.5	1.460	0.004	PREDICTED: Mus musculus similar to prothymosin alpha (LOC100044779), misc RNA.
BC056474	350.5	70.4	511.7	58.7	1.460	0.002	Mus musculus cDNA sequence BC056474 (BC056474), mRNA.
Sardh	266.9	102.4	389.7	68.0	1.460	0.028	Mus musculus sarcosine dehydrogenase (Sardh), nuclear gene encoding mitochondrial protein, mRNA.
Krtcap2	2588.3	896.5	3778.5	334.4	1.460	0.015	Mus musculus keratinocyte associated protein 2 (Krtcap2), mRNA.
Ndufa13	7846.9	1531.4	11445.5	1120.8	1.459	0.001	Mus musculus NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 13 (Ndufa13), mRNA.
C130074G19Rik	278.9	69.5	406.8	51.0	1.459	0.005	Mus musculus RIKEN cDNA C130074G19 gene (C130074G19Rik), mRNA.
Dexi	95.3	31.8	138.9	31.1	1.458	0.034	Mus musculus dexamethasone-induced transcript (Dexi), mRNA.
Robld3	464.5	127.4	676.9	43.5	1.457	0.006	Mus musculus roadblock domain containing 3 (Robld3), mRNA.
2210412D01Rik	373.7	29.3	544.5	50.5	1.457	0.002	Mus musculus RIKEN cDNA 2210412D01 gene (2210412D01Rik), mRNA.
Gchfr	925.8	314.5	1348.0	135.7	1.456	0.009	Mus musculus GTP cyclohydrolase 1 feedback regulator (Gchfr), mRNA.
Bad	137.5	21.9	200.1	34.8	1.455	0.008	Mus musculus BCL2-associated agonist of cell death (Bad), mRNA.
Igf1	1787.1	402.8	2600.7	382.1	1.455	0.010	Mus musculus insulin-like growth factor 1 (Igf1), transcript variant 2, mRNA.
Mrp134	471.9	115.9	686.4	103.1	1.454	0.004	Mus musculus mitochondrial ribosomal protein L34 (Mrp134), nuclear gene encoding mitochondrial protein, mRNA.
Mrps16	416.2	89.3	604.3	96.7	1.452	0.002	Mus musculus mitochondrial ribosomal protein S16 (Mrps16), nuclear gene encoding mitochondrial protein, mRNA.
C9	2443.4	1045.6	3541.2	456.9	1.449	0.026	Mus musculus complement component 9 (C9), mRNA.
Rpl38	5043.6	2187.9	7300.5	1334.7	1.447	0.041	Mus musculus ribosomal protein L38 (Rpl38), transcript variant 1, mRNA.
Uqcr	6372.0	1584.5	9209.7	899.8	1.445	0.004	Mus musculus ubiquinol-cytochrome c reductase (6.4kD) subunit (Uqcr), mRNA.
Trak1	130.8	27.3	189.1	48.3	1.445	0.027	Mus musculus trafficking protein, kinesin binding 1 (Trak1), mRNA.
Elmo3	174.6	13.4	251.6	22.5	1.441	0.041	Mus musculus engulfment and cell motility 3, ced-12 homolog (C. elegans) (Elmo3), mRNA.
Myk	227.4	40.2	327.7	95.4	1.441	0.048	Mus musculus myosin, light polypeptide kinase (Myk), mRNA.
Cdc14b	146.9	22.5	211.6	38.2	1.440	0.029	Mus musculus CDC14 cell division cycle 14 homolog B (S. cerevisiae) (Cdc14b), mRNA.
Ssca1	312.9	95.4	450.5	44.7	1.440	0.015	Mus musculus Sjogren's syndrome/scleroderma autoantigen 1 homolog (human) (Ssca1), mRNA.
LOC100046746	163.1	55.9	234.1	32.7	1.435	0.029	PREDICTED: Mus musculus similar to SMAP1 (LOC100046746), mRNA.
Abcg8	2156.8	468.1	3093.5	284.2	1.434	0.003	Mus musculus ATP-binding cassette, sub-family G (WHITE), member 8 (Abcg8), mRNA.
Vgll4	82.8	16.5	118.7	30.7	1.433	0.043	Mus musculus vestigial like 4 (Drosophila) (Vgll4), mRNA.
AA409316	80.8	12.5	115.8	29.3	1.433	0.042	Mus musculus expressed sequence AA409316 (AA409316), mRNA.
LOC100046775	385.7	86.0	552.6	34.0	1.433	0.004	PREDICTED: Mus musculus similar to CMP-sialic acid transporter (LOC100046775), mRNA.
Ndufc1	1337.2	246.5	1914.4	127.9	1.432	0.002	Mus musculus NADH dehydrogenase (ubiquinone) 1, subcomplex unknown, 1 (Ndufc1), mRNA.
Mrp14	1410.8	312.7	2019.0	161.9	1.431	0.003	Mus musculus mitochondrial ribosomal protein L14 (Mrp14), nuclear gene encoding mitochondrial protein, mRNA.
Serf2	1288.5	360.5	1838.3	238.1	1.427	0.010	Mus musculus small EDRK-rich factor 2 (Serf2), mRNA.
Leng9	124.3	14.0	177.2	22.0	1.426	0.014	Mus musculus leukocyte receptor cluster (LRC) member 9 (Leng9), mRNA.
Apo4	17060.1	4169.1	24305.4	1320.2	1.425	0.005	Mus musculus apolipoprotein C-IV (Apo4), mRNA.
Klhdc3	98.5	22.5	140.3	29.7	1.424	0.022	Mus musculus kelch domain containing 3 (Klhdc3), mRNA.
Cldn12	595.5	132.7	847.7	61.6	1.423	0.004	Mus musculus claudin 12 (Cldn12), mRNA.
Nt5c	326.8	52.3	464.6	101.2	1.422	0.013	Mus musculus 5',3'-nucleotidase, cytosolic (Nt5c), mRNA.
Rps27l	6123.1	863.2	8697.1	807.4	1.420	0.002	Mus musculus ribosomal protein S27-like (Rps27l), mRNA.
1110020P15Rik	8793.3	1841.2	12484.2	958.4	1.420	0.003	Mus musculus RIKEN cDNA 1110020P15 gene (1110020P15Rik), mRNA.
0910001L09Rik	2833.6	557.0	4022.6	467.0	1.420	0.002	Mus musculus RIKEN cDNA 0910001L09 gene (0910001L09Rik), mRNA.
LOC100041835	4066.0	665.9	5771.1	618.3	1.419	0.002	PREDICTED: Mus musculus similar to H <sup>+</sup> ATP synthase (LOC100041835), mRNA.
Tcea3	333.0	82.6	472.6	115.4	1.419	0.028	Mus musculus transcription elongation factor A (SII), 3 (Tcea3), mRNA.
Rps15	1341.7	481.7	1902.5	222.4	1.418	0.030	Mus musculus ribosomal protein S15 (Rps15), mRNA.
Apon	2139.7	335.9	3032.9	511.7	1.417	0.003	Mus musculus apolipoprotein N (Apon), mRNA.
Smarc2	141.8	22.6	200.7	38.3	1.416	0.013	Mus musculus SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily c, member 2 (Smarc2), mRNA.
Acn9	82.0	10.0	116.1	17.4	1.415	0.033	Mus musculus ACN9 homolog (S. cerevisiae) (Acn9), mRNA.
S100a1	1529.9	459.3	2163.8	250.4	1.414	0.015	Mus musculus S100 calcium binding protein A1 (S100a1), mRNA.
Ubl5	2634.6	486.4	3717.8	216.4	1.411	0.003	Mus musculus ubiquitin-like 5 (Ubl5), mRNA.



Supplementary Table 2. Differentially expressed genes in livers from OLD FXR<sup>-/-</sup>iVP16FXR vs. FXR<sup>-/-</sup>iVP16 mice. Genes are listed from the highest fold ratio to the lowest fold ratio.

SYMBOL	KO AVG Signal	KO - SD	TG AVG Signal	TG - SD	Ratio	P	DEFINITION
Ndufs6	6325.3	1582.4	8923.1	546.1	1.411	0.007	Mus musculus NADH dehydrogenase (ubiquinone) Fe-S protein 6 (Ndufs6), mRNA.
Slc25a42	282.2	68.3	398.1	49.2	1.411	0.009	Mus musculus solute carrier family 25, member 42 (Slc25a42), mRNA.
Thap7	162.9	40.1	229.4	25.5	1.408	0.014	
Crot	77.3	18.1	108.8	9.1	1.407	0.040	Mus musculus carnitine O-octanoyltransferase (Crot), mRNA.
Kpnb1	223.4	27.9	313.9	57.1	1.405	0.009	Mus musculus karyopherin (importin) beta 1 (Kpnb1), mRNA.
Rbp4	4117.6	485.6	5784.4	554.6	1.405	0.003	Mus musculus retinol binding protein 4, plasma (Rbp4), mRNA.
Khsrp	92.7	14.9	130.3	22.9	1.405	0.030	Mus musculus KH-type splicing regulatory protein (Khsrp), mRNA.
Emg1	344.1	50.6	483.3	47.9	1.405	0.008	Mus musculus EMG1 nucleolar protein homolog (S. cerevisiae) (Emg1), mRNA.
Polr2j	606.6	129.6	851.7	82.8	1.404	0.005	Mus musculus polymerase (RNA) II (DNA directed) polypeptide J (Polr2j), mRNA.
Tmem86b	219.5	61.3	308.1	60.0	1.404	0.022	Mus musculus transmembrane protein 86B (Tmem86b), mRNA.
Rpl39	4879.1	1638.8	6849.1	877.0	1.404	0.027	Mus musculus ribosomal protein L39 (Rpl39), mRNA.
LOC100041500	340.7	115.1	477.6	39.2	1.402	0.033	PREDICTED: Mus musculus similar to LSM7 homolog, U6 small nuclear RNA associated (LOC100041500), mRNA.
Cbs	1077.2	356.9	1508.4	246.6	1.400	0.033	Mus musculus cystathionine beta-synthase (Cbs), transcript variant 1, mRNA.
Dcxr	1074.8	311.6	1504.6	276.4	1.400	0.029	Mus musculus dicarbonyl L-xylulose reductase (Dcxr), mRNA.
Rpl41	15380.8	5113.1	21527.9	3038.5	1.400	0.027	Mus musculus ribosomal protein L41 (Rpl41), mRNA.
Tcfec3	82.4	11.9	115.2	12.7	1.398	0.039	Mus musculus transcription factor E3 (Tcfec3), mRNA.
Xpnppe3	157.6	38.8	220.4	33.0	1.398	0.017	Mus musculus X-prolyl aminopeptidase (aminopeptidase P) 3, putative (Xpnppe3), mRNA.
Tmem179b	139.3	37.6	194.7	20.0	1.398	0.024	Mus musculus transmembrane protein 179B (Tmem179b), mRNA.
Vcl	450.3	69.1	627.1	85.2	1.393	0.007	Mus musculus vinculin (Vcl), mRNA.
Rpain	334.1	49.7	464.7	112.0	1.391	0.029	Mus musculus RPA interacting protein (Rpain), mRNA.
Cox5b	6745.6	880.0	9381.9	335.1	1.391	0.004	
BC039093	209.9	40.0	291.8	50.9	1.390	0.012	
0610012G03Rik	1229.9	235.8	1709.4	204.4	1.390	0.005	
BC021381	111.7	24.3	155.2	33.3	1.390	0.031	Mus musculus cDNA sequence BC021381 (BC021381), mRNA.
Saps1	105.5	16.4	146.6	26.2	1.390	0.029	Mus musculus SAPS domain family, member 1 (Saps1), mRNA.
Nostrin	123.0	20.5	170.9	8.5	1.389	0.024	Mus musculus nitric oxide synthase trafficker (Nostrin), mRNA.
Mett11d1	115.6	26.9	160.6	22.3	1.389	0.026	Mus musculus methyltransferase 11 domain containing 1 (Mett11d1), mRNA.
Mrpl47	119.1	40.6	165.4	25.9	1.388	0.036	Mus musculus mitochondrial ribosomal protein L47 (Mrpl47), nuclear gene encoding mitochondrial protein, mRNA.
Cox17	1348.7	350.0	1871.9	230.4	1.388	0.013	Mus musculus cytochrome c oxidase, subunit XVII assembly protein homolog (yeast) (Cox17), mRNA.
Pgpep1	261.0	75.1	362.2	37.7	1.387	0.013	Mus musculus pyroglutamyl-peptidase I (Pgpep1), mRNA.
Zscan12	173.4	42.9	240.4	34.8	1.387	0.019	Mus musculus zinc finger and SCAN domain containing 12 (Zscan12), mRNA.
Spta2L	104.7	27.8	145.0	22.2	1.386	0.034	Mus musculus spermatogenesis associated 2-like (Spta2L), mRNA.
St6gal1	2333.1	254.3	3232.8	831.6	1.386	0.035	Mus musculus beta galactosidase alpha 2,6 sialyltransferase 1 (St6gal1), mRNA.
1600002K03Rik	142.8	41.7	197.7	11.0	1.384	0.035	Mus musculus RIKEN cDNA 1600002K03 gene (1600002K03Rik), mRNA.
2010011I20Rik	299.9	58.8	414.8	73.5	1.383	0.010	Mus musculus RIKEN cDNA 2010011I20 gene (2010011I20Rik), mRNA.
Ict1	124.4	28.2	172.1	31.4	1.383	0.025	Mus musculus immature colon carcinoma transcript 1 (Ict1), mRNA.
2510006D16Rik	720.1	96.3	995.0	154.0	1.382	0.007	Mus musculus RIKEN cDNA 2510006D16 gene (2510006D16Rik), mRNA.
Twistnb	117.4	17.0	162.0	21.6	1.380	0.028	Mus musculus TWIST neighbor (Twistnb), mRNA.
Med12	106.7	20.0	147.3	22.3	1.380	0.033	Mus musculus mediator of RNA polymerase II transcription, subunit 12 homolog (yeast) (Med12), mRNA.
Cox6b1	5362.2	1174.2	7397.2	585.8	1.380	0.007	Mus musculus cytochrome c oxidase, subunit VIb polypeptide 1 (Cox6b1), mRNA.
LOC665250	2388.9	712.9	3294.2	221.8	1.379	0.025	PREDICTED: Mus musculus similar to RNA polymerase II transcription factor SIII p18 subunit (LOC665250), mRNA.
Snrpd3	962.3	146.5	1325.8	139.3	1.378	0.007	Mus musculus small nuclear ribonucleoprotein D3 (Snrpd3), mRNA.
Ppp1r11	370.7	70.6	510.6	49.6	1.377	0.010	Mus musculus protein phosphatase 1, regulatory (inhibitor) subunit 11 (Ppp1r11), mRNA.
Arl16	94.9	18.4	130.7	19.0	1.377	0.040	Mus musculus ADP-ribosylation factor-like 16 (Arl16), mRNA.
Hemk1	181.2	12.4	249.4	41.9	1.376	0.018	Mus musculus HemK methyltransferase family member 1 (Hemk1), mRNA.
Sepx1	1806.8	271.7	2486.6	461.6	1.376	0.017	
Nudt6	237.2	38.4	325.8	54.9	1.373	0.014	Mus musculus nudix (nucleoside diphosphate linked moiety X)-type motif 6 (Nudt6), mRNA.
Grb14	126.3	23.8	173.3	27.2	1.373	0.041	Mus musculus growth factor receptor bound protein 14 (Grb14), mRNA.
LOC654426	1446.0	398.2	1984.2	179.6	1.372	0.021	Mus musculus ATP synthase, H <sup>+</sup> transporting, mitochondrial F0 complex, subunit F pseudogene (LOC654426), non-coding RNA.
LOC100044566	94.1	9.0	129.1	22.1	1.372	0.043	PREDICTED: Mus musculus similar to Nuclear receptor coactivator 1 (NCoA1) (Steroid receptor coactivator 1) (SRC-1) (Nuclear receptor coactivator protein 1) (mNRC-1) (LOC100044566), mRNA.
Ndufb6	1375.0	235.5	1884.1	183.1	1.370	0.001	Mus musculus NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 6 (Ndufb6), nuclear gene encoding mitochondrial protein, mRNA.
Sec16b	344.9	52.3	472.3	107.7	1.369	0.030	Mus musculus SEC16 homolog B (S. cerevisiae) (Sec16b), mRNA.
Bcd02	167.0	34.7	228.6	51.4	1.369	0.035	Mus musculus beta-carotene 9', 10'-dioxygenase 2 (Bcd02), mRNA.
Tmem19	437.6	124.0	599.1	122.5	1.369	0.039	Mus musculus transmembrane protein 19 (Tmem19), mRNA.
Adprh	171.0	32.5	234.0	44.2	1.369	0.021	Mus musculus ADP-ribosylarginine hydrolase (Adprh), mRNA.
Repin1	289.2	55.3	395.6	96.9	1.368	0.042	Mus musculus replication initiator 1 (Repin1), transcript variant 5, mRNA.
Rnaseh2c	132.6	36.6	181.3	39.7	1.368	0.049	Mus musculus ribonuclease H2, subunit C (Rnaseh2c), mRNA.
Rdm1	213.4	39.6	291.7	31.0	1.367	0.017	Mus musculus RAD52 motif 1 (Rdm1), mRNA.
Myst1	203.7	43.6	278.4	64.7	1.367	0.038	Mus musculus MYST histone acetyltransferase 1 (Myst1), mRNA.
Nit2	479.2	144.5	654.7	198.8	1.366	0.023	Mus musculus nitrilase family, member 2 (Nit2), mRNA.
Lrp1	272.0	62.7	371.3	64.2	1.365	0.017	Mus musculus low density lipoprotein receptor-related protein 1 (Lrp1), mRNA.
Brf1	92.5	21.7	126.1	22.1	1.364	0.048	Mus musculus BRF1 homolog, subunit of RNA polymerase III transcription initiation factor IIIB (S. cerevisiae) (Brf1), mRNA.
Rnf40	131.7	30.7	179.5	18.2	1.364	0.030	Mus musculus ring finger protein 40 (Rnf40), mRNA.
Pegf1	132.9	9.7	181.1	18.3	1.362	0.031	Mus musculus polycomb group ring finger 1 (Pegf1), mRNA.
Taf10	822.2	144.4	1120.0	136.5	1.362	0.009	Mus musculus TAF10 RNA polymerase II, TATA box binding protein (TBP) associated factor (Taf10), mRNA.
Mrpl22	448.7	43.3	610.6	36.0	1.361	0.012	Mus musculus mitochondrial ribosomal protein L22 (Mrpl22), nuclear gene encoding mitochondrial protein, mRNA.
Srp72	108.6	23.0	147.8	19.3	1.360	0.041	Mus musculus signal recognition particle 72 (Srp72), mRNA. XM_896078 XM_896090 XM_896109 XM_916867 XM_924530 XM_924532 XM_924533 XM_924534 XM_924537 XM_924542 XM_924545 XM_973233 XM_974428

Supplementary Table 2. Differentially expressed genes in livers from OLD FXR<sup>-/-</sup>iVP16FXR vs. FXR<sup>-/-</sup>iVP16 mice. Genes are listed from the highest fold ratio to the lowest fold ratio.

SYMBOL	KO AVG Signal	KO - SD	TG AVG Signal	TG - SD	Ratio	P	DEFINITION
Tceb2	1763.7	470.6	2396.0	165.1	1.358	0.038	Mus musculus transcription elongation factor B (SIID), polypeptide 2 (Tceb2), mRNA.
Phpt1	331.1	90.2	449.0	17.0	1.356	0.030	Mus musculus phosphohistidine phosphatase 1 (Phpt1), mRNA.
Tmem125	107.1	24.1	145.1	30.6	1.355	0.046	Mus musculus transmembrane protein 125 (Tmem125), mRNA.
Pccb	727.3	111.6	984.8	79.6	1.354	0.034	Mus musculus propionyl Coenzyme A carboxylase, beta polypeptide (Pccb), mRNA.
Akt1s1	216.3	45.3	292.9	39.4	1.354	0.021	Mus musculus AKT1 substrate 1 (proline-rich) (Akt1s1), mRNA.
Rpl13a	259.7	47.7	351.5	57.4	1.354	0.018	Mus musculus ribosomal protein L13a (Rpl13a), mRNA.
Deb1	1971.9	407.7	2669.2	267.8	1.354	0.010	Mus musculus differentially expressed in B16F10 I (Deb1), mRNA.
Nxf1	139.2	18.2	188.3	43.1	1.353	0.049	Mus musculus nuclear RNA export factor 1 homolog (S. cerevisiae) (Nxf1), mRNA.
Ndufa7	2179.4	711.8	2943.5	262.3	1.351	0.048	Mus musculus NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 7 (B14.5a) (Ndufa7), mRNA.
Psg23	824.5	184.0	1112.6	155.9	1.349	0.015	Mus musculus pregnancy-specific glycoprotein 23 (Psg23), mRNA.
Mrpl27	946.4	131.3	1276.2	166.2	1.349	0.011	Mus musculus mitochondrial ribosomal protein L27 (Mrpl27), nuclear gene encoding mitochondrial protein, mRNA.
Fiz1	168.5	31.3	227.1	20.8	1.348	0.029	Mus musculus Ftz3 interacting zinc finger protein 1 (Fiz1), mRNA.
Snf8	199.2	17.9	268.4	8.4	1.348	0.025	Mus musculus SNF8, ESCRT-II complex subunit, homolog (S. cerevisiae) (Snf8), mRNA.
Dusp23	174.8	37.6	235.5	40.3	1.347	0.028	Mus musculus dual specificity phosphatase 23 (Dusp23), mRNA.
Txndc12	309.3	38.9	416.7	52.9	1.347	0.018	Mus musculus thioredoxin domain containing 12 (endoplasmic reticulum) (Txndc12), mRNA.
Rpl28	4867.1	1392.3	6554.5	764.4	1.347	0.033	Mus musculus ribosomal protein L28 (Rpl28), mRNA.
Gckr	507.7	114.6	683.2	150.0	1.346	0.038	Mus musculus glucokinase regulatory protein (Gckr), mRNA.
Ankrd46	317.2	23.0	426.6	43.2	1.345	0.018	Mus musculus ankyrin repeat domain 46 (Ankrd46), mRNA.
0610006I08Rik	859.8	199.7	1155.7	73.2	1.344	0.035	Mus musculus RIKEN cDNA 0610006I08 gene (0610006I08Rik), mRNA.
Ring1	149.7	25.1	201.0	26.7	1.343	0.035	Mus musculus ring finger protein 1 (Ring1), mRNA.
Wdr23	566.7	139.4	760.5	152.9	1.342	0.037	Mus musculus WD repeat domain 23 (Wdr23), mRNA.
Hist2h2ac	1780.7	515.6	2389.0	186.7	1.342	0.037	Mus musculus histone cluster 2, H2ac (Hist2h2ac), mRNA.
1810009A15Rik	128.1	23.3	171.8	23.3	1.341	0.023	Mus musculus RIKEN cDNA 1810009A15 gene (1810009A15Rik), mRNA.
Atp5j	1641.9	287.8	2201.2	110.5	1.341	0.017	Mus musculus ATP synthase, H+ transporting, mitochondrial F0 complex, subunit J (Atp5j), nuclear gene encoding mitochondrial protein, mRNA.
Trip11	132.4	9.5	177.5	38.1	1.340	0.049	Mus musculus tuftelin interacting protein 11 (Trip11), mRNA.
Cnpy2	680.8	77.6	912.3	151.4	1.340	0.014	Mus musculus canopy 2 homolog (zebrafish) (Cnpy2), mRNA.
Mrpl53	717.5	100.9	960.9	130.5	1.339	0.014	Mus musculus mitochondrial ribosomal protein L53 (Mrpl53), nuclear gene encoding mitochondrial protein, mRNA.
a2ld1	357.3	53.9	478.2	88.3	1.338	0.021	Mus musculus AIG2-like domain 1 (a2ld1), mRNA.
Ndufs8	1855.5	205.6	2483.0	189.1	1.338	0.012	Mus musculus NADH dehydrogenase (ubiquinone) Fe-S protein 8 (Ndufs8), mRNA.
Cfb	2447.0	349.5	3266.7	545.7	1.335	0.012	Mus musculus complement factor B (Cfb), mRNA.
2010007H12Rik	193.6	30.2	258.5	39.0	1.335	0.038	Mus musculus RIKEN cDNA 2010007H12 gene (2010007H12Rik), mRNA.
Ifi20	672.3	84.7	896.7	90.5	1.334	0.005	Mus musculus intraflagellar transport 20 homolog (Chlamydomonas) (Ifi20), mRNA.
Atp5l	1594.1	427.5	2120.8	154.9	1.330	0.034	Mus musculus ATP synthase, H+ transporting, mitochondrial F0 complex, subunit g (Atp5l), nuclear gene encoding mitochondrial protein, mRNA.
Timm8b	1002.2	229.9	1332.5	42.2	1.330	0.022	Mus musculus translocase of inner mitochondrial membrane 8 homolog b (yeast) (Timm8b), mRNA.
Camk2n1	1151.0	257.2	1529.7	200.4	1.329	0.020	Mus musculus calcium/calmodulin-dependent protein kinase II inhibitor 1 (Camk2n1), mRNA.
Comm1d	425.6	75.0	565.1	76.1	1.328	0.032	Mus musculus COMM domain containing 1 (Comm1d), mRNA.
Ulk2	138.4	26.9	183.6	31.1	1.327	0.045	Mus musculus Unc-51 like kinase 2 (C. elegans) (Ulk2), mRNA.
Hamp	15754.9	4574.9	20894.0	3591.5	1.326	0.048	Mus musculus hepcidin antimicrobial peptide (Hamp), mRNA.
Cox6a1	4959.2	821.0	6566.3	244.3	1.324	0.017	Mus musculus cytochrome c oxidase, subunit VI a, polypeptide 1 (Cox6a1), nuclear gene encoding mitochondrial protein, mRNA.
Atp5g2	944.3	150.6	1250.0	69.3	1.324	0.017	Mus musculus ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c (subunit 9), isoform 2 (Atp5g2), mRNA.
Ywhaq	266.6	24.7	352.7	35.8	1.323	0.029	Mus musculus tyrosine 3-monoxygenase/tryptophan 5-monoxygenase activation protein, theta polypeptide (Ywhaq), mRNA.
Gcs1	393.4	37.0	520.1	42.5	1.322	0.023	Mus musculus glucosidase 1 (Gcs1), mRNA.
Uroc1	5570.2	590.5	7363.8	1030.5	1.322	0.015	Mus musculus urocanase domain containing 1 (Uroc1), mRNA.
Mcee	1825.7	264.4	2413.2	178.5	1.322	0.016	Mus musculus methylmalonyl CoA epimerase (Mcee), mRNA.
Dynlrb1	1077.7	217.0	1423.5	134.2	1.321	0.018	Mus musculus dynein light chain roadblock-type 1 (Dynlrb1), mRNA.
OTTMUSG0000007855	188.2	39.3	248.4	27.8	1.320	0.038	Mus musculus predicted gene, OTTMUSG0000007855 (OTTMUSG0000007855), mRNA.
Znhit2	249.6	43.3	329.6	64.1	1.320	0.036	Mus musculus zinc finger, HIT domain containing 2 (Znhit2), mRNA.
Pank4	146.3	11.1	193.1	38.4	1.320	0.049	Mus musculus pantothenate kinase 4 (Pank4), mRNA.
Ndufs4	1127.0	136.9	1486.4	175.9	1.319	0.007	Mus musculus NADH dehydrogenase (ubiquinone) Fe-S protein 4 (Ndufs4), nuclear gene encoding mitochondrial protein, mRNA.
Hint3	502.8	111.4	662.8	75.2	1.318	0.030	Mus musculus histidine triad nucleotide binding protein 3 (Hint3), mRNA.
Rpl19	930.8	208.5	1226.7	162.2	1.318	0.025	Mus musculus ribosomal protein L19 (Rpl19), mRNA.
2810432D09Rik	489.7	103.5	645.4	33.6	1.318	0.023	Mus musculus RIKEN cDNA 2810432D09 gene (2810432D09Rik), mRNA.
Haao	5399.6	820.8	7113.1	1598.4	1.317	0.047	Mus musculus 3-hydroxyanthranilate 3,4-dioxygenase (Haao), mRNA. XM_925483 XM_986039 XM_986095 XM_988905 XM_988933 XM_988974
Brp44	123.1	36.1	162.2	21.2	1.317	0.049	Mus musculus brain protein 44 (Brp44), mRNA.
Aes	1392.7	236.6	1830.8	164.4	1.315	0.019	Mus musculus amino-terminal enhancer of split (Aes), mRNA.
Baat	1935.3	239.5	2543.4	114.2	1.314	0.018	Mus musculus bile acid-Coenzyme A: amino acid N-acyltransferase (Baat), mRNA.
Ppp2r5e	321.1	72.1	421.9	84.5	1.314	0.043	Mus musculus protein phosphatase 2, regulatory subunit B (B56), epsilon isoform (Ppp2r5e), mRNA.
Fnbp11	219.5	30.7	288.0	39.6	1.312	0.038	Mus musculus formin binding protein 1-like (Fnbp11), mRNA.
Mta2	182.9	41.5	239.9	29.0	1.312	0.044	Mus musculus metastasis-associated gene family, member 2 (Mta2), mRNA.
Cyp2d22	6451.4	638.9	8458.7	1421.3	1.311	0.019	Mus musculus cytochrome P450, family 2, subfamily 4, polypeptide 22 (Cyp2d22), mRNA.
Ai662250	177.6	35.2	232.8	24.6	1.311	0.046	Mus musculus expressed sequence AI662250 (AI662250), mRNA.
Bhlhb2	967.8	187.4	1265.4	204.2	1.307	0.023	Mus musculus basic helix-loop-helix domain containing, class B2 (Bhlhb2), mRNA.
Sqrdl	693.9	124.7	906.4	189.7	1.306	0.046	Mus musculus sulfide quinone reductase-like (yeast) (Sqrdl), mRNA.
Them2	1283.5	167.1	1676.4	111.3	1.306	0.022	Mus musculus thioesterase superfamily member 2 (Them2), mRNA.
Vamp8	804.1	159.0	1050.2	163.5	1.306	0.044	Mus musculus vesicle-associated membrane protein 8 (Vamp8), mRNA.

Supplementary Table 2. Differentially expressed genes in livers from OLD FXR<sup>-/-</sup>iVP16FXR vs. FXR<sup>-/-</sup>iVP16 mice. Genes are listed from the highest fold ratio to the lowest fold ratio.

SYMBOL	KO AVG Signal	KO - SD	TG AVG Signal	TG - SD	Ratio	P	DEFINITION
Chchd1	2288,4	460,7	2986,5	264,4	1,305	0,021	Mus musculus coiled-coil-helix-coiled-coil-helix domain containing 1 (Chchd1), mRNA.
Dnajc15	630,5	140,5	822,8	81,9	1,305	0,032	Mus musculus Dnaj (Hsp40) homolog, subfamily C, member 15 (Dnajc15), mRNA.
Klhl17	201,5	16,9	262,7	40,9	1,304	0,046	Mus musculus kelch-like 17 (Drosophila) (Klhl17), mRNA.
Mrps28	465,3	84,9	606,4	29,8	1,303	0,030	Mus musculus mitochondrial ribosomal protein S28 (Mrps28), nuclear gene encoding mitochondrial protein, mRNA.
Agpat3	352,7	46,3	459,6	34,2	1,303	0,033	Mus musculus 1-acylglycerol-3-phosphate O-acyltransferase 3 (Agpat3), mRNA.
Map3k11	248,9	28,3	324,3	44,1	1,303	0,040	Mus musculus mitogen-activated protein kinase kinase 11 (Map3k11), mRNA.
2310047M10Rik	173,4	36,3	225,8	37,4	1,302	0,048	Mus musculus RIKEN cDNA 2310047M10 gene (2310047M10Rik), mRNA.
Tmem39a	197,0	27,2	256,3	34,0	1,301	0,048	Mus musculus transmembrane protein 39a (Tmem39a), mRNA.
Hint2	4636,5	899,2	6024,4	686,1	1,299	0,023	Mus musculus histidine triad nucleotide binding protein 2 (Hint2), mRNA.
Ars2	196,0	12,5	254,6	47,9	1,299	0,043	Mus musculus arsenate resistance protein 2 (Ars2), mRNA.
Ndufa6	4029,1	855,0	5223,5	492,5	1,296	0,028	Mus musculus NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 6 (B14) (Ndufa6), nuclear gene encoding mitochondrial protein, mRNA.
Fyco1	270,8	54,7	348,7	31,7	1,288	0,048	Mus musculus FYVE and coiled-coil domain containing 1 (Fyco1), mRNA.
Dpy30	639,3	63,1	823,1	113,3	1,288	0,035	Mus musculus dpy-30 homolog (C. elegans) (Dpy30), mRNA.
Rab3gap1	722,9	69,2	930,4	92,3	1,287	0,034	Mus musculus RAB3 GTPase activating protein subunit 1 (Rab3gap1), mRNA.
Vkorc1	3117,3	448,0	4009,7	442,6	1,286	0,033	Mus musculus vitamin K epoxide reductase complex, subunit 1 (Vkorc1), mRNA.
Chkb	655,1	102,4	841,7	123,3	1,285	0,036	Mus musculus choline kinase beta (Chkb), mRNA.
Nsun4	623,6	30,7	801,2	103,2	1,285	0,036	Mus musculus NOL1/NOP2/Sun domain family, member 4 (Nsun4), mRNA.
Rrbp1	651,8	131,1	836,4	160,3	1,283	0,039	Mus musculus ribosome binding protein 1 (Rrbp1), transcript variant 1, mRNA.
1110058L19Rik	539,5	76,0	692,2	81,2	1,283	0,039	Mus musculus RIKEN cDNA 1110058L19 gene (1110058L19Rik), mRNA.
Aqp11	207,1	32,5	265,6	61,6	1,283	0,037	Mus musculus aquaporin 11 (Aqp11), mRNA.
Abcf3	348,3	59,6	446,3	66,7	1,281	0,047	Mus musculus ATP-binding cassette, sub-family F (GCN20), member 3 (Abcf3), mRNA.
Sec24c	542,5	89,6	693,9	89,6	1,279	0,041	Mus musculus Sec24 related gene family, member C (S. cerevisiae) (Sec24c), mRNA.
5033414K04Rik	1141,9	178,4	1457,6	94,1	1,276	0,037	Mus musculus RIKEN cDNA 5033414K04 gene (5033414K04Rik), mRNA.
Acp6	406,2	31,7	517,4	39,7	1,274	0,049	Mus musculus acid phosphatase 6, lysophosphatidic (Acp6), mRNA.
Psenen	1266,1	244,6	1612,8	178,3	1,274	0,038	Mus musculus presenilin enhancer 2 homolog (C. elegans) (Psenen), mRNA.
3110001D03Rik	764,3	149,9	971,5	106,1	1,271	0,043	Mus musculus RIKEN cDNA 3110001D03 gene (3110001D03Rik), mRNA.
Adh4	1651,8	252,0	2098,8	219,1	1,271	0,039	Mus musculus alcohol dehydrogenase 4 (class II), pi polypeptide (Adh4), mRNA.
Mpnd	757,9	76,4	962,5	35,3	1,270	0,044	Mus musculus MPN domain containing (Mpnd), mRNA.
2310036D22Rik	594,9	96,1	754,4	95,2	1,268	0,048	Mus musculus RIKEN cDNA 2310036D22 gene (2310036D22Rik), mRNA.
1500032L24Rik	1292,7	169,5	1635,0	86,6	1,265	0,044	Mus musculus RIKEN cDNA 1500032L24 gene (1500032L24Rik), mRNA.
Prdx4	2491,2	403,0	3149,3	438,7	1,264	0,042	Mus musculus peroxiredoxin 4 (Prdx4), mRNA.
LOC100042777	1002,6	162,7	1265,2	128,1	1,262	0,048	PREDICTED: Mus musculus similar to human protein homologous to DROER protein (LOC100042777), mRNA.
MGC18837	8149,9	1216,3	10266,7	603,5	1,260	0,044	Mus musculus tetrapeptide repeat domain 36 (Ttc36), mRNA.
Ttc36	3652,1	722,9	4589,9	502,4	1,257	0,047	Mus musculus tetrapeptide repeat domain 36 (Ttc36), mRNA.
Apoel	27158,0	4022,0	33526,8	1783,3	1,235	0,025	Mus musculus apolipoprotein C-I (Apoel), mRNA.
Uox	3612,8	812,6	3921,4	548,7	1,085	0,015	Mus musculus urate oxidase (Uox), mRNA.
Atf4	985,5	129,1	979,2	168,8	0,994	0,041	Mus musculus activating transcription factor 4 (Atf4), mRNA.
Clk4	509,2	44,0	460,0	36,8	0,903	0,034	Mus musculus CDC like kinase 4 (Clk4), mRNA.
Sumo1	906,6	133,9	766,0	108,2	0,845	0,009	Mus musculus CDC like kinase 4 (Clk4), mRNA.
Cyp2d26	4458,8	523,9	3659,9	405,8	0,821	0,020	Mus musculus cytochrome P450, family 2, subfamily d, polypeptide 26 (Cyp2d26), mRNA.
Stard5	1216,1	132,7	983,4	107,7	0,809	0,048	Mus musculus STAR-related lipid transfer (START) domain containing 5 (Stard5), mRNA.
Hfe	439,7	65,0	353,6	56,3	0,804	0,028	Mus musculus hemochromatosis (Hfe), mRNA.
Ptplad1	7747,7	897,5	6119,5	692,3	0,790	0,048	Mus musculus protein tyrosine phosphatase-like A domain containing 1 (Ptplad1), mRNA.
Hspa9	6453,6	702,3	5094,0	549,8	0,789	0,019	Mus musculus heat shock protein 9 (Hspa9), mRNA.
Adh1	20755,2	2986,5	16376,2	2345,4	0,789	0,048	Mus musculus alcohol dehydrogenase 1 (class I) (Adh1), mRNA.
Ndfip1	4032,7	427,0	3170,0	519,1	0,786	0,045	Mus musculus Nedd4 family interacting protein 1 (Ndfip1), mRNA.
2310044H10Rik	2135,2	239,4	1677,6	185,8	0,786	0,046	Mus musculus RIKEN cDNA 2310044H10 gene (2310044H10Rik), mRNA.
Poldip2	3482,2	462,0	2724,5	96,8	0,782	0,042	Mus musculus polymerase (DNA-directed), delta interacting protein 2 (Poldip2), mRNA.
Nadk	3335,5	443,5	2606,7	384,4	0,781	0,041	Mus musculus NAD kinase (Nadk), mRNA.
Afiph	663,8	43,5	515,3	69,8	0,776	0,045	Mus musculus aftiphilin (Afiph), mRNA.
PsmA5	5231,6	340,3	4056,3	365,9	0,775	0,034	Mus musculus proteasome (prosome, macropain) subunit, alpha type 5 (PsmA5), mRNA.
Rnpep1	4294,7	573,5	3321,5	304,4	0,773	0,033	Mus musculus arginyl aminopeptidase (aminopeptidase B)-like 1 (Rnpep1), mRNA.
Iqgap2	2228,1	294,3	1718,4	328,5	0,771	0,040	Mus musculus IQ motif containing GTPase activating protein 2 (Iqgap2), mRNA.
PsmA4	3719,8	488,7	2865,7	207,2	0,770	0,031	Mus musculus proteasome (prosome, macropain) subunit, alpha type 4 (PsmA4), mRNA.
Syap1	774,1	56,5	595,6	68,3	0,769	0,047	Mus musculus synapse associated protein 1 (Syap1), mRNA.
Vdac3	926,5	189,3	711,1	149,0	0,768	0,046	Mus musculus voltage-dependent anion channel 3 (Vdac3), mRNA.
Fbxo4	562,8	65,3	431,8	38,2	0,767	0,038	Mus musculus F-box protein 4 (Fbxo4), mRNA.
Bxdc2	562,9	70,4	430,7	51,4	0,765	0,028	Mus musculus brix domain containing 2 (Bxdc2), mRNA.
Crcp	426,5	79,9	326,2	59,0	0,765	0,040	Mus musculus calcitonin gene-related peptide-receptor component protein (Crcp), mRNA.
Sord	4483,8	283,3	3422,4	800,1	0,763	0,048	Mus musculus sorbitol dehydrogenase (Sord), mRNA.
2700087H15Rik	358,0	55,3	273,0	50,2	0,763	0,041	Mus musculus sorbitol dehydrogenase (Sord), mRNA.
Cyp8b1	25075,7	4516,0	19092,8	3771,8	0,761	0,032	Mus musculus cytochrome P450, family 8, subfamily b, polypeptide 1 (Cyp8b1), mRNA.
Dctn3	686,8	83,6	522,5	27,8	0,761	0,021	Mus musculus dynactin 3 (Dctn3), mRNA.
Rrage	1608,3	178,4	1223,6	196,9	0,761	0,026	Mus musculus Ras-related GTP binding C (Rrage), mRNA.
201031D03Rik	352,1	74,0	267,7	52,2	0,760	0,041	Mus musculus RIKEN cDNA 201031D03 gene (201031D03Rik), mRNA.

Supplementary Table 2. Differentially expressed genes in livers from OLD FXR<sup>-/-</sup>iVP16FXR vs. FXR<sup>-/-</sup>iVP16 mice. Genes are listed from the highest fold ratio to the lowest fold ratio.

SYMBOL	KO AVG Signal	KO - SD	TG AVG Signal	TG - SD	Ratio	P	DEFINITION
8430410K20Rik	717.0	112.0	544.8	34.3	0.760	0.029	Mus musculus RIKEN cDNA 8430410K20 gene (8430410K20Rik), mRNA.
Atad3a	1665.1	125.2	1264.8	92.1	0.760	0.025	Mus musculus ATPase family, AAA domain containing 3A (Atad3a), mRNA.
Mki67ip	352.4	37.5	267.2	53.8	0.758	0.042	Mus musculus Mki67 (FHA domain) interacting nuclear phosphoprotein (Mki67ip), mRNA.
8430432M10Rik	248.8	16.3	188.5	25.0	0.758	0.046	
Slc22a1	7683.8	1647.4	5813.7	941.7	0.757	0.036	Mus musculus solute carrier family 22 (organic cation transporter), member 1 (Slc22a1), mRNA.
Tmem55b	442.9	82.4	334.8	42.4	0.756	0.032	Mus musculus transmembrane protein 55b (Tmem55b), mRNA. XM_919952 XM_919965
Slc25a5	10590.9	1269.8	7997.8	786.0	0.755	0.020	Mus musculus solute carrier family 25 (mitochondrial carrier, adenine nucleotide translocator), member 5 (Slc25a5), nuclear gene encoding mitochondrial protein, mRNA.
1110007L15Rik	638.3	84.4	481.4	34.4	0.754	0.035	Mus musculus RIKEN cDNA 1110007L15 gene (1110007L15Rik), mRNA.
Uhrf1bp11	407.2	51.6	306.8	36.5	0.754	0.031	Mus musculus UHRF1 (ICBP90) binding protein 1-like (Uhrf1bp11), mRNA.
Gars	2933.5	291.5	2205.0	131.8	0.752	0.019	Mus musculus glycyl-tRNA synthetase (Gars), mRNA.
Hsd17b4	9975.4	2091.9	7496.6	950.1	0.752	0.023	Mus musculus hydroxysteroid (17-beta) dehydrogenase 4 (Hsd17b4), mRNA.
Coasy	2991.8	405.3	2246.8	117.2	0.751	0.018	Mus musculus Coenzyme A synthase (Coasy), nuclear gene encoding mitochondrial protein, mRNA.
Dhrs7b	822.5	166.0	617.4	78.0	0.751	0.049	Mus musculus dehydrogenase/reductase (SDR family) member 7B (Dhrs7b), mRNA.
BC002199	970.3	130.7	727.6	98.2	0.750	0.021	Mus musculus cDNA sequence BC002199 (BC002199), mRNA.
Gpt2	5348.0	1197.5	4000.9	707.7	0.748	0.030	Mus musculus glutamic pyruvate transaminase (alanine aminotransferase) 2 (Gpt2), mRNA.
Nsfl1c	727.6	83.5	544.2	42.0	0.748	0.022	Mus musculus NSFL1 (p97) cofactor (p47) (Nsfl1c), mRNA.
Ppp2r1a	453.4	66.9	339.1	51.4	0.748	0.026	Mus musculus protein phosphatase 2 (formerly 2A), regulatory subunit A (PR65), alpha isoform (Ppp2r1a), mRNA.
Akr1a4	7375.8	1043.8	5507.0	487.5	0.747	0.017	Mus musculus aldo-keto reductase family 1, member A4 (aldehyde reductase) (Akr1a4), mRNA.
Nptn	1673.5	264.1	1248.5	248.8	0.746	0.023	Mus musculus neuroplastin (Nptn), mRNA.
Tra2a	296.6	67.8	221.2	51.3	0.746	0.049	Mus musculus transformer 2 alpha homolog (Drosophila) (Tra2a), mRNA.
1500031L02Rik	190.5	23.2	142.0	28.6	0.746	0.045	Mus musculus RIKEN cDNA 1500031L02 gene (1500031L02Rik), mRNA.
Zranb1	292.7	58.2	217.9	22.8	0.744	0.031	Mus musculus zinc finger, RAN-binding domain containing 1 (Zranb1), mRNA.
C4bp	4420.6	1043.8	3289.2	396.7	0.744	0.032	Mus musculus complement component 4 binding protein (C4bp), mRNA.
Zfp259	1013.9	83.2	754.4	73.2	0.744	0.018	Mus musculus zinc finger protein 259 (Zfp259), mRNA.
261010J23Rik	282.9	57.9	210.2	27.4	0.743	0.030	
Psmd7	989.2	124.9	734.8	52.8	0.743	0.003	Mus musculus proteasome (prosome, macropain) 26S subunit, non-ATPase, 7 (Psmd7), mRNA.
Ipo5	175.3	13.8	130.2	14.2	0.743	0.046	Mus musculus importin 5 (Ipo5), mRNA.
Apfp2	7757.4	1347.4	5762.0	1442.5	0.743	0.035	Mus musculus amyloid beta (A4) precursor-like protein 2 (Apfp2), transcript variant 3, mRNA.
Igf2r	199.5	30.5	148.1	14.9	0.742	0.039	Mus musculus insulin-like growth factor 2 receptor (Igf2r), mRNA.
Ssr3	3276.2	391.1	2426.5	306.9	0.741	0.014	Mus musculus signal sequence receptor, gamma (Ssr3), mRNA.
Abhd3	303.6	62.8	224.5	51.1	0.739	0.036	Mus musculus abhydrolase domain containing 3 (Abhd3), mRNA.
Sec24b	191.4	24.0	141.4	29.4	0.739	0.039	Mus musculus Sec24 related gene family, member B (S. cerevisiae) (Sec24b), mRNA.
Man2a1	2572.8	356.0	1901.3	167.2	0.739	0.013	Mus musculus mannosidase 2, alpha 1 (Man2a1), mRNA.
Mtdh	417.1	62.5	307.8	30.8	0.738	0.021	Mus musculus Metadherin (Mtdh), mRNA.
Psmd4	6604.3	518.4	4872.9	193.7	0.738	0.012	Mus musculus proteasome (prosome, macropain) 26S subunit, non-ATPase, 4 (Psmd4), mRNA.
Atp6ap2	575.2	101.0	423.4	71.3	0.736	0.031	Mus musculus ATPase, H+ transporting, lysosomal accessory protein 2 (Atp6ap2), mRNA.
Ppp2r2d	552.8	84.4	406.7	53.9	0.736	0.017	Mus musculus protein phosphatase 2, regulatory subunit B, delta isoform (Ppp2r2d), mRNA.
Entpd4	1832.9	166.3	1345.5	311.0	0.734	0.024	Mus musculus ectonucleoside triphosphate diphosphohydrolase 4 (Entpd4), mRNA.
Atp6v1h	1726.9	238.5	1266.9	350.5	0.734	0.037	Mus musculus ATPase, H+ transporting, lysosomal V1 subunit H (Atp6v1h), mRNA.
Pgrmc1	3610.8	701.1	2648.1	423.1	0.733	0.011	Mus musculus progesterone receptor membrane component 1 (Pgrmc1), mRNA.
Arntl	141.1	21.3	103.2	18.9	0.731	0.047	Mus musculus aryl hydrocarbon receptor nuclear translocator-like (Arntl), mRNA.
LOC100047579	286.6	18.0	209.5	30.5	0.731	0.023	PREDICTED: Mus musculus similar to transmembrane protein 20 (LOC100047579), mRNA.
Ppfbp2	173.2	16.5	126.5	10.7	0.730	0.014	Mus musculus protein tyrosine phosphatase, receptor-type, F interacting protein, binding protein 2 (Ppfbp2), mRNA.
Prkrir	373.4	31.8	272.3	59.8	0.729	0.024	Mus musculus protein-kinase, interferon-inducible double stranded RNA dependent inhibitor, repressor of (P58 repressor) (Prkrir), mRNA.
2310001A20Rik	1085.4	246.0	791.0	69.5	0.729	0.019	Mus musculus RIKEN cDNA 2310001A20 gene (2310001A20Rik), mRNA.
Litaf	5669.3	1088.6	4131.3	899.7	0.729	0.017	Mus musculus LPS-induced TN factor (Litaf), mRNA.
2610204L23Rik	590.7	45.3	430.3	22.1	0.728	0.014	
2310044G17Rik	263.9	39.3	192.2	36.7	0.728	0.023	Mus musculus RIKEN cDNA 2310044G17 gene (2310044G17Rik), mRNA.
mar-02	385.7	24.9	280.2	29.9	0.727	0.016	Mus musculus membrane-associated ring finger (C3HC4) 2 (March2), mRNA.
Mbnl2	703.1	94.8	510.6	84.7	0.726	0.028	Mus musculus muscleblind-like 2 (Mbnl2), transcript variant 1, mRNA.
Atp5s	132.8	23.4	95.9	20.6	0.722	0.043	Mus musculus ATP synthase, H+ transporting, mitochondrial F0 complex, subunit s (Atp5s), nuclear gene encoding mitochondrial protein, mRNA.
Ten2	414.4	77.9	299.1	65.7	0.722	0.020	Mus musculus transcalaminin 2 (Ten2), mRNA.
Slc45a3	367.5	68.3	265.1	19.1	0.721	0.015	Mus musculus solute carrier family 45, member 3 (Slc45a3), mRNA.
Fvt1	219.2	49.9	157.9	23.3	0.721	0.024	Mus musculus follicular lymphoma variant translocation 1 (Fvt1), mRNA. XM_921793 XM_985103
Prnp	2392.4	334.2	1723.7	377.8	0.721	0.014	Mus musculus prion protein (Prnp), mRNA.
Atp6v1c1	221.1	47.6	159.3	12.0	0.720	0.022	Mus musculus ATPase, H+ transporting, lysosomal V1 subunit C1 (Atp6v1c1), mRNA.
2610039C10Rik	174.3	25.1	125.5	33.0	0.720	0.041	Mus musculus RIKEN cDNA 2610039C10 gene (2610039C10Rik), mRNA.
D93000I122Rik	1347.9	272.2	968.2	119.3	0.718	0.008	Mus musculus RIKEN cDNA D93000I122 gene (D93000I122Rik), mRNA.

Supplementary Table 2. Differentially expressed genes in livers from OLD FXR<sup>-/-</sup>iVP16FXR vs. FXR<sup>-/-</sup>iVP16 mice. Genes are listed from the highest fold ratio to the lowest fold ratio.

SYMBOL	KO AVG Signal	KO - SD	TG AVG Signal	TG - SD	Ratio	P	DEFINITION
Aco2	1788,5	278,2	1283,3	269,1	0,718	0,012	Mus musculus aconitase 2, mitochondrial (Aco2), nuclear gene encoding mitochondrial protein, mRNA.
Acadm	10903,1	2088,3	7822,3	783,9	0,717	0,006	Mus musculus acyl-Coenzyme A dehydrogenase, medium chain (Acadm), mRNA.
Dpp7	1254,0	271,1	899,6	282,0	0,717	0,043	Mus musculus dipeptidylpeptidase 7 (Dpp7), mRNA.
Leprtl1	425,4	87,4	304,7	66,5	0,716	0,017	Mus musculus leptin receptor overlapping transcript-like 1 (Leprtl1), mRNA.
Taldol1	4050,8	622,8	2897,6	224,0	0,715	0,006	Mus musculus transaldolase 1 (Taldol1), mRNA.
Suelg2	1177,1	183,7	841,8	95,0	0,715	0,007	Mus musculus succinate-Coenzyme A ligase, GDP-forming, beta subunit (Suelg2), mRNA.
Actn4	308,8	56,7	220,4	43,2	0,714	0,026	Mus musculus actinin alpha 4 (Actn4), mRNA.
Fkbp1a	425,6	104,3	303,5	61,6	0,713	0,025	Mus musculus FK506 binding protein 1a (Fkbp1a), mRNA.
App11	188,9	29,0	134,6	24,9	0,713	0,022	Mus musculus adaptor protein, phosphotyrosine interaction, PH domain and leucine zipper containing 1 (App11), mRNA.
Ikbkb	137,9	31,6	98,2	10,7	0,712	0,033	Mus musculus nuclear undecaprenyl pyrophosphate synthase 1 homolog (S. cerevisiae) (Nus1), mRNA.
Nus1	204,3	40,2	145,4	32,6	0,712	0,023	Mus musculus nuclear undecaprenyl pyrophosphate synthase 1 homolog (S. cerevisiae) (Nus1), mRNA.
Aldh1a1	7758,8	939,0	5509,5	1486,8	0,710	0,019	Mus musculus aldehyde dehydrogenase family 1, subfamily A1 (Aldh1a1), mRNA.
4933407N01Rik	378,9	59,4	268,7	87,4	0,709	0,036	Mus musculus aldehyde dehydrogenase family 1, subfamily A1 (Aldh1a1), mRNA.
Aldh3a2	8969,2	1545,9	6356,7	1217,9	0,709	0,007	Mus musculus aldehyde dehydrogenase family 3, subfamily A2 (Aldh3a2), mRNA.
Ndel1	877,2	120,1	621,7	75,1	0,709	0,007	Mus musculus nuclear distribution gene E-like homolog 1 (A. nidulans) (Ndel1), mRNA.
Sparc	194,1	41,9	137,5	25,9	0,708	0,048	Mus musculus secreted acidic cysteine rich glycoprotein (Sparc), mRNA.
Grina	12517,9	2114,0	8861,7	787,8	0,708	0,005	Mus musculus glutamate receptor, ionotropic, N-methyl D-aspartate-associated protein 1 (glutamate binding) (Grina), mRNA.
Cct3	839,1	82,7	592,0	101,6	0,705	0,002	Mus musculus chaperonin containing Tcp1, subunit 3 (gamma) (Cct3), mRNA.
Gtf2e1	372,7	54,2	262,6	54,1	0,705	0,011	Mus musculus general transcription factor II E, polypeptide 1 (alpha subunit) (Gtf2e1), mRNA.
Efha1	644,9	159,7	454,3	130,6	0,704	0,042	Mus musculus EF hand domain family A1 (Efha1), mRNA.
Prmt3	476,4	76,9	335,5	10,7	0,704	0,008	Mus musculus protein arginine N-methyltransferase 3 (Prmt3), mRNA.
Qsox1	469,4	40,0	330,0	82,7	0,703	0,016	Mus musculus quiescin Q6 sulfhydryl oxidase 1 (Qsox1), transcript variant 2, mRNA.
Bet1	199,9	40,8	140,5	31,3	0,703	0,019	Mus musculus blocked early in transport 1 homolog (S. cerevisiae) (Bet1), mRNA.
Mtmr12	104,4	20,0	73,3	13,7	0,702	0,043	Mus musculus myotubularin related protein 12 (Mtmr12), mRNA.
Bag3	2104,2	629,3	1476,5	182,6	0,702	0,038	Mus musculus BCL2-associated athanogene 3 (Bag3), mRNA.
Pgam1	334,7	18,7	234,7	65,7	0,701	0,023	Mus musculus phosphoglycerate mutase 1 (Pgam1), mRNA.
5033414D02Rik	194,3	52,4	136,0	26,7	0,700	0,032	Mus musculus RIKEN cDNA 5033414D02 gene (5033414D02Rik), mRNA.
Idh1	3080,9	720,6	2155,5	450,5	0,700	0,015	Mus musculus isocitrate dehydrogenase 1 (NADP+), soluble (Idh1), mRNA.
Nudt4	1157,7	207,0	809,4	118,0	0,699	0,004	Mus musculus nudix (nucleoside diphosphate linked moiety X)-type motif 4 (Nudt4), mRNA.
Tmem120a	1826,9	248,7	1273,1	408,3	0,697	0,024	Mus musculus transmembrane protein 120A (Tmem120a), mRNA.
Hsp90ab1	1858,3	318,5	1294,0	209,7	0,696	0,001	Mus musculus heat shock protein 90kDa alpha (cytosolic), class B member 1 (Hsp90ab1), mRNA.
Atg16l1	731,9	39,5	509,5	36,8	0,696	0,005	Mus musculus autophagy-related 16-like 1 (yeast) (Atg16l1), transcript variant b, mRNA.
Pcyt1a	482,8	90,8	335,9	62,9	0,696	0,006	Mus musculus phosphate cytidylyltransferase 1, choline, alpha isoform (Pcyt1a), mRNA.
Ces3	20581,9	3126,4	14317,2	2446,1	0,696	0,003	Mus musculus carboxylesterase 3 (Ces3), mRNA.
Tspo	1565,9	391,8	1088,6	123,4	0,695	0,006	Mus musculus translocator protein (Tspo), mRNA.
Psm13	3335,3	511,6	2317,3	216,7	0,695	0,003	Mus musculus proteasome (prosome, macropain) 26S subunit, non-ATPase, 13 (Psm13), mRNA.
Dnajb6	204,1	32,3	141,8	31,5	0,695	0,028	Mus musculus DnaJ (Hsp40) homolog, subfamily B, member 6 (Dnajb6), transcript variant 2, mRNA.
Pnpo	1431,1	263,7	993,5	161,5	0,694	0,006	Mus musculus pyridoxine 5'-phosphate oxidase (Pnpo), mRNA.
Mrap	1319,0	288,8	915,6	143,6	0,694	0,006	Mus musculus melanocortin 2 receptor accessory protein (Mrap), mRNA.
BC031353	1332,7	399,8	923,6	198,3	0,693	0,040	Mus musculus cDNA sequence BC031353 (BC031353), mRNA.
Atp6v0a2	329,0	71,6	227,8	55,9	0,692	0,013	Mus musculus ATPase, H+ transporting, lysosomal V0 subunit A2 (Atp6v0a2), mRNA.
Hprt1	828,4	190,7	573,1	62,8	0,692	0,043	Mus musculus hypoxanthine guanine phosphoribosyl transferase 1 (Hprt1), mRNA.
Alg1	274,2	49,3	189,5	19,8	0,691	0,008	Mus musculus asparagine-linked glycosylation 1 homolog (yeast, beta-1,4-mannosyltransferase) (Alg1), mRNA.
Ostf1	738,2	105,7	510,0	39,7	0,691	0,004	Mus musculus osteoclast stimulating factor 1 (Ostf1), mRNA.
Atg12	754,1	120,1	519,1	52,1	0,688	0,004	Mus musculus autophagy-related 12 (yeast) (Atg12), mRNA.
Ugt1a10	8915,4	1984,2	6123,1	1303,9	0,687	0,009	Mus musculus UDP glycosyltransferase 1 family, polypeptide A10 (Ugt1a10), mRNA.
Slc19a1	103,7	17,9	71,2	7,0	0,686	0,033	Mus musculus solute carrier family 19 (sodium/hydrogen exchanger), member 1 (Slc19a1), mRNA.
Rab5a	244,0	65,1	167,1	37,3	0,685	0,049	Mus musculus RAB5A, member RAS oncogene family (Rab5a), mRNA.
LOC100047261	2259,9	461,1	1546,0	617,5	0,684	0,038	PREDICTED: Mus musculus similar to spermidine/spermine N1-acetyltransferase (LOC100047261), misc RNA.
Eif6	1405,2	334,0	960,0	143,6	0,683	0,008	Mus musculus eukaryotic translation initiation factor 6 (Eif6), mRNA.
Ikbkg	299,3	36,6	204,4	8,4	0,683	0,037	Mus musculus inhibitor of kappaB kinase gamma (Ikbkg), transcript variant 2, mRNA.
250002L14Rik	479,1	137,6	327,2	104,6	0,683	0,050	Mus musculus inhibitor of kappaB kinase gamma (Ikbkg), transcript variant 2, mRNA.
Ugt3a2	9616,2	1179,8	6563,7	582,9	0,683	0,002	Mus musculus UDP glycosyltransferases 3 family, polypeptide A2 (Ugt3a2), mRNA.
Eif2ak2	501,8	111,8	341,4	32,5	0,680	0,005	Mus musculus eukaryotic translation initiation factor 2-alpha kinase 2 (Eif2ak2), mRNA.
Marcks	264,9	81,1	180,2	34,9	0,680	0,034	Mus musculus myristoylated alanine rich protein kinase C substrate (Marcks), mRNA.
Slc46a3	1073,3	142,5	728,9	195,1	0,679	0,009	Mus musculus solute carrier family 46, member 3 (Slc46a3), mRNA.
Cyp3a25	4575,5	705,4	3095,4	460,0	0,677	0,002	Mus musculus cytochrome P450, family 3, subfamily a, polypeptide 25 (Cyp3a25), mRNA.
Smpd13a	345,2	101,2	233,4	50,3	0,676	0,028	Mus musculus sphingomyelin phosphodiesterase, acid-like 3A (Smpd13a), mRNA.
Dhrs7	504,1	58,9	340,9	53,2	0,676	0,010	Mus musculus dehydrogenase/reductase (SDR family) member 7 (Dhrs7), mRNA.
Cpb2	2707,4	492,3	1830,1	288,8	0,676	0,002	Mus musculus carboxypeptidase B2 (plasma) (Cpb2), mRNA.
Srd5a3	208,8	28,8	141,1	50,2	0,676	0,027	Mus musculus steroid 5 alpha-reductase 3 (Srd5a3), mRNA.
Mrc1	337,5	113,8	228,0	41,7	0,676	0,046	Mus musculus mannose receptor, C type 1 (Mrc1), mRNA.



Supplementary Table 2. Differentially expressed genes in livers from OLD FXR<sup>-/-</sup>iVP16FXR vs. FXR<sup>-/-</sup>iVP16 mice. Genes are listed from the highest fold ratio to the lowest fold ratio.

SYMBOL	KO AVG Signal	KO - SD	TG AVG Signal	TG - SD	Ratio	P	DEFINITION
Rnf11	1134.0	247.0	765.1	96.0	0.675	0.004	Mus musculus ring finger protein 11 (Rnf11), mRNA.
Ep300	164.7	22.4	111.1	11.9	0.674	0.011	Mus musculus E1A binding protein p300 (Ep300), mRNA.
mar-06	296.9	81.9	200.1	51.9	0.674	0.027	Mus musculus membrane-associated ring finger (C3HC4) 6 (March6), mRNA.
G3bp2	774.6	100.5	521.5	50.4	0.673	0.009	Mus musculus GTPase activating protein (SH3 domain) binding protein 2 (G3bp2), transcript variant 2, mRNA.
Tmem77	297.9	41.9	200.5	45.4	0.673	0.014	Mus musculus transmembrane protein 77 (Tmem77), transcript variant 2, mRNA.
Lmna	497.4	119.0	334.6	22.3	0.673	0.014	Mus musculus lamin A (Lmna), transcript variant 1, mRNA.
Nckap1	342.9	54.6	230.3	38.5	0.672	0.004	Mus musculus NCK-associated protein 1 (Nckap1), mRNA.
Arl8a	742.1	180.3	496.5	203.2	0.669	0.043	Mus musculus ADP-ribosylation factor-like 8A (Arl8a), mRNA.
Tex264	1054.2	221.5	704.8	88.3	0.669	0.011	Mus musculus testis expressed gene 264 (Tex264), transcript variant 1, mRNA.
Ostm1	598.1	46.7	399.3	68.6	0.668	0.002	Mus musculus osteopetrosis associated transmembrane protein 1 (Ostm1), mRNA.
Cldn2	1079.3	270.1	719.0	220.1	0.666	0.021	Mus musculus claudin 2 (Cldn2), mRNA.
Fbxo36	105.0	16.8	69.9	14.9	0.666	0.022	Mus musculus F-box protein 36 (Fbxo36), mRNA.
Sqstm1	4821.4	669.3	3210.4	263.8	0.666	0.001	Mus musculus sequestosome 1 (Sqstm1), mRNA.
Vps35	394.4	116.6	261.6	18.0	0.663	0.020	Mus musculus vacuolar protein sorting 35 (Vps35), mRNA.
LOC545056	1017.8	263.4	673.7	201.5	0.662	0.020	Mus musculus ubiquitin-conjugating enzyme E2, J2 homolog pseudogene (LOC545056) on chromosome 14.
2310076L09Rik	581.1	134.1	384.4	90.4	0.661	0.011	Mus musculus RIKEN cDNA 2310076L09 gene (2310076L09Rik), transcript variant 1, mRNA.
Ephx1	10646.4	2866.8	7041.8	2119.5	0.661	0.024	Mus musculus epoxide hydrolase 1, microsomal (Ephx1), mRNA.
Usp20	263.2	66.7	173.8	44.1	0.661	0.014	Mus musculus ubiquitin specific peptidase 20 (Usp20), mRNA.
Snx7	214.0	61.3	141.2	19.8	0.660	0.018	Mus musculus sorting nexin 7 (Snx7), mRNA.
D0H852298E	104.3	17.3	68.7	8.6	0.659	0.019	Mus musculus histocompatibility 2, T region locus 23 (H2-T23), mRNA.
H2-T23	2711.5	660.1	1786.2	784.5	0.659	0.046	Mus musculus histocompatibility 2, T region locus 23 (H2-T23), mRNA.
Tmem97	2687.6	330.9	1768.2	558.0	0.658	0.008	Mus musculus transmembrane protein 97 (Tmem97), mRNA.
Erc3	653.3	123.3	428.8	20.7	0.656	0.006	Mus musculus excision repair cross-complementing rodent repair deficiency, complementation group 3 (Erc3), mRNA.
Parp14	433.9	125.2	283.7	60.5	0.654	0.016	Mus musculus poly (ADP-ribose) polymerase family, member 14 (Parp14), mRNA. XM_901644 XM_916789 XM_924484 XM_924488
Tm6sf2	247.1	76.6	161.6	41.3	0.654	0.029	Mus musculus transmembrane 6 superfamily member 2 (Tm6sf2), mRNA.
Vat1	207.3	68.3	135.4	29.7	0.653	0.033	Mus musculus vesicle amine transport protein 1 homolog (T californica) (Vat1), mRNA.
Gnb1	379.2	95.5	247.1	27.8	0.652	0.006	Mus musculus guanine nucleotide binding protein (G protein), beta 1 (Gnb1), mRNA.
Aadacl1	1278.4	308.7	831.7	90.8	0.651	0.003	Mus musculus arylacetamide deacetylase-like 1 (Aadacl1), mRNA.
Slc40a1	6686.9	1226.7	4347.2	635.2	0.650	0.001	Mus musculus solute carrier family 40 (iron-regulated transporter), member 1 (Slc40a1), mRNA.
Bcap29	191.7	22.5	124.5	20.8	0.649	0.007	Mus musculus B-cell receptor-associated protein 29 (Bcap29), mRNA.
Ube2e2	1022.5	115.1	662.0	167.2	0.647	0.003	Mus musculus ubiquitin-conjugating enzyme E2E 2 (UBC4/5 homolog, yeast) (Ube2e2), mRNA.
Gss	156.5	27.6	101.2	18.0	0.646	0.017	Mus musculus glutathione synthetase (Gss), mRNA.
Ahcy11	434.4	82.6	280.7	43.1	0.646	0.001	Mus musculus S-adenosylhomocysteine hydrolase-like 1 (Ahcy11), mRNA.
Acad9	203.2	63.0	131.3	40.4	0.646	0.044	Mus musculus acyl-CoA dehydrogenase family, member 9 (Acad9), mRNA.
Tbc1d20	206.7	29.6	133.4	24.1	0.645	0.003	Mus musculus TBC1 domain family, member 20 (Tbc1d20), mRNA.
Prune	115.1	31.9	74.2	18.0	0.645	0.041	Mus musculus prune homolog (Drosophila) (Prune), mRNA.
Gulo	3116.0	1023.4	2000.0	429.3	0.642	0.025	Mus musculus gulonolactone (L-) oxidase (Gulo), mRNA.
Asl	790.2	185.6	505.6	223.4	0.640	0.031	Mus musculus aspartate aminotransferase-like 1 (Asl), mRNA.
EG630499	644.3	165.6	410.7	182.0	0.637	0.034	Mus musculus predicted gene, EG630499 (EG630499), mRNA.
Psm5	727.3	123.5	462.9	102.4	0.636	0.001	Mus musculus proteasome (prosome, macropain) subunit, beta type 5 (Psm5), mRNA.
Ranbp9	121.5	13.7	77.0	11.5	0.633	0.008	Mus musculus RAN binding protein 9 (Ranbp9), mRNA.
Chehd4	302.1	36.9	191.2	36.2	0.633	0.001	Mus musculus coiled-coil-helix-coiled-coil-helix domain containing 4 (Chehd4), nuclear gene encoding mitochondrial protein, mRNA.
Mpp1	247.0	55.8	155.9	33.3	0.631	0.002	Mus musculus membrane protein, palmitoylated (Mpp1), mRNA.
Akr1c12	2768.6	484.4	1746.6	442.9	0.631	0.001	Mus musculus aldo-keto reductase family 1, member C12 (Akr1c12), mRNA.
BC005537	281.3	34.4	177.1	32.6	0.629	0.001	Mus musculus cDNA sequence BC005537 (BC005537), mRNA.
Mupcdh	3251.6	695.3	2045.2	557.3	0.629	0.002	Mus musculus mucin-like protocadherin (Mupcdh), mRNA.
Picalm	697.0	95.7	438.1	137.7	0.628	0.004	Mus musculus picalmon (Picalm), mRNA.
Cd1d1	4222.5	387.5	2653.4	378.5	0.628	0.000	Mus musculus CD1d1 antigen (Cd1d1), mRNA.
Grn	3525.7	619.3	2213.5	913.5	0.628	0.011	Mus musculus granulin (Grn), mRNA.
Ugt2b35	1244.3	388.3	781.2	157.8	0.628	0.014	Mus musculus UDP glucuronosyltransferase 2 family, polypeptide B35 (Ugt2b35), mRNA.
Hlcs	119.4	27.0	74.9	11.2	0.627	0.007	Mus musculus holocarboxylase synthetase (biotin-[propionyl-Coenzyme A-carboxylase (ATP-hydrolysing)] ligase) (Hlcs), mRNA.
Ano10	144.3	39.0	90.3	15.5	0.626	0.009	Mus musculus anoctamin 10 (Ano10), mRNA.
Mir16	3636.8	402.1	2273.6	624.8	0.625	0.001	Mus musculus membrane interacting protein of RGS16 (Mir16), mRNA.
Sumo3	680.6	147.1	424.3	62.5	0.623	0.001	Mus musculus SMT3 suppressor of mif two 3 homolog 3 (yeast) (Sumo3), mRNA.
Notum	216.9	74.1	135.1	23.0	0.623	0.023	Mus musculus notum pectinacetyltransferase homolog (Drosophila) (Notum), mRNA.
Eef2	386.7	100.4	239.6	60.4	0.620	0.007	Mus musculus eukaryotic translation elongation factor 2 (Eef2), mRNA.
382044	3893.8	1073.2	2411.9	613.8	0.619	0.007	Mus musculus predicted gene, 382044 (382044), mRNA.
Crls1	180.6	11.2	111.7	15.1	0.618	0.014	Mus musculus cardiolipin synthase 1 (Crls1), transcript variant 2, mRNA.
Mertk	151.0	52.1	93.2	27.6	0.617	0.032	Mus musculus c-mer proto-oncogene tyrosine kinase (Mertk), mRNA.
Zcchc14	150.6	40.8	92.7	3.8	0.615	0.007	Mus musculus zinc finger, CCHC domain containing 14 (Zcchc14), mRNA.
Scamp5	229.2	57.6	140.6	18.9	0.613	0.036	Mus musculus secretory carrier membrane protein 5 (Scamp5), mRNA.
Pros1	1547.4	317.9	948.8	338.8	0.613	0.004	Mus musculus protein S (alpha) (Pros1), mRNA.
Pnpla2	2341.3	545.6	1435.4	404.2	0.613	0.003	Mus musculus patatin-like phospholipase domain containing 2 (Pnpla2), mRNA.
Cyp4a31	2178.8	734.6	1326.2	581.0	0.609	0.042	Mus musculus cytochrome P450, family 4, subfamily a, polypeptide 31 (Cyp4a31), mRNA.
Sccpdh	135.1	28.2	81.8	48.6	0.606	0.046	Mus musculus saccharopine dehydrogenase (putative) (Sccpdh), mRNA.
Rnf135	415.4	76.4	251.2	38.1	0.605	0.019	Mus musculus ring finger protein 135 (Rnf135), mRNA.
Insig2	3612.5	707.5	2183.1	323.1	0.604	0.001	Mus musculus insulin induced gene 2 (Insig2), mRNA.

Supplementary Table 2. Differentially expressed genes in livers from OLD FXR<sup>-/-</sup>iVP16FXR vs. FXR<sup>-/-</sup>iVP16 mice. Genes are listed from the highest fold ratio to the lowest fold ratio.

SYMBOL	KO AVG Signal	KO - SD	TG AVG Signal	TG - SD	Ratio	P	DEFINITION
Pex11a	2345.3	971.5	1416.6	181.8	0.604	0.043	Mus musculus peroxisomal biogenesis factor 11a (Pex11a), mRNA.
Aifm2	336.6	43.0	202.6	53.7	0.602	0.037	Mus musculus apoptosis-inducing factor, mitochondrion-associated 2 (Aifm2), nuclear gene encoding mitochondrial protein, transcript variant 3, mRNA.
Pef1	653.5	153.5	392.1	61.4	0.600	0.001	Mus musculus penta-EF hand domain containing 1 (Pef1), mRNA.
Hspb1	290.4	95.6	174.2	64.3	0.600	0.025	Mus musculus heat shock protein 1 (Hspb1), mRNA.
EG433923	2315.4	528.5	1386.9	286.3	0.599	0.001	Mus musculus predicted gene, EG433923 (EG433923), mRNA.
LOC100043257	651.2	146.2	388.6	238.3	0.597	0.036	PREDICTED: Mus musculus similar to RNA binding motif protein 3 (LOC100043257), mRNA.
Upp2	608.5	164.3	362.8	106.5	0.596	0.010	Mus musculus uridine phosphorylase 2 (Upp2), mRNA.
St3gal5	1290.8	501.0	767.8	240.3	0.595	0.035	Mus musculus ST3 beta-galactoside alpha-2,3-sialyltransferase 5 (St3gal5), transcript variant 2, mRNA.
Abcc2	575.1	143.0	340.2	86.3	0.591	0.003	Mus musculus ATP-binding cassette, sub-family C (CFTR/MRP), member 2 (Abcc2), mRNA.
Spg21	551.0	112.5	325.6	51.0	0.591	0.000	Mus musculus spastic paraplegia 21 homolog (human) (Spg21), mRNA.
Krt8	4195.3	1540.1	2477.4	668.3	0.591	0.022	Mus musculus keratin 8 (Krt8), mRNA.
Zfand2a	408.2	66.8	240.8	27.6	0.590	0.000	Mus musculus zinc finger, AN1-type domain 2A (Zfand2a), mRNA.
Acot4	297.9	60.1	174.8	51.8	0.587	0.001	Mus musculus acyl-CoA thioesterase 4 (Acot4), mRNA.
Gpld1	306.4	124.6	179.2	47.6	0.585	0.023	Mus musculus glycosylphosphatidylinositol specific phospholipase D1 (Gpld1), mRNA.
Bivr1	5335.5	1872.9	3112.4	423.3	0.583	0.011	Mus musculus biliverdin reductase B (flavin reductase (NADPH)) (Bivr1), mRNA.
Tesk1	327.2	50.4	190.8	20.0	0.583	0.000	Mus musculus testis specific protein kinase 1 (Tesk1), mRNA.
6720467C03Rik	283.8	25.5	165.4	40.4	0.583	0.001	Mus musculus RIKEN cDNA 6720467C03 gene (6720467C03Rik), mRNA.
Gstm2	10034.4	4383.2	5835.7	1585.9	0.582	0.043	Mus musculus glutathione S-transferase, mu 2 (Gstm2), mRNA.
Fmo1	5111.3	789.0	2964.2	555.2	0.580	0.000	Mus musculus flavin containing monooxygenase 1 (Fmo1), mRNA.
Capns1	1112.2	194.3	643.7	88.5	0.579	0.000	Mus musculus cytokine induced apoptosis inhibitor 1 (Ciapin1), mRNA.
Ciapin1	541.0	114.5	313.0	40.5	0.579	0.000	Mus musculus cytokine induced apoptosis inhibitor 1 (Ciapin1), mRNA.
Ddc	1540.9	333.4	884.7	195.9	0.574	0.000	Mus musculus dopamine beta-hydroxylase (Ddc), mRNA.
Golim4	287.4	73.6	164.9	32.4	0.574	0.001	Mus musculus golgi integral membrane protein 4 (Golim4), mRNA.
Tmed2	123.0	33.4	70.4	15.3	0.573	0.003	Mus musculus transmembrane emp24 domain trafficking protein 2 (Tmed2), mRNA.
Gde1	636.8	73.9	361.0	88.5	0.567	0.000	Mus musculus glycerophosphodiester phosphodiesterase 1 (Gde1), mRNA.
Wtip	101.2	43.2	57.3	15.7	0.566	0.038	Mus musculus WTI-interacting protein (Wtip), mRNA.
Ube2l6	323.8	127.5	183.2	47.1	0.566	0.021	Mus musculus ubiquitin-conjugating enzyme E2L6 (Ube2l6), mRNA.
LOC100044190	1106.9	353.3	622.8	299.0	0.563	0.019	PREDICTED: Mus musculus hypothetical protein LOC100044190 (LOC100044190), mRNA.
Abhd4	461.2	165.9	258.8	50.8	0.561	0.009	Mus musculus abhydrolase domain containing 4 (Abhd4), mRNA.
Nup93	170.6	40.8	95.0	18.8	0.557	0.000	Mus musculus nucleoporin 93 kDa (Nup93), mRNA.
Sgk1	1233.8	219.0	686.4	232.8	0.556	0.000	Mus musculus serum/glucocorticoid induced kinase 1 (Sgk1), mRNA.
Gstm4	1063.2	457.2	590.8	146.2	0.556	0.028	Mus musculus glutathione S-transferase, mu 4 (Gstm4), mRNA.
Cbr1	813.1	328.1	451.8	75.8	0.556	0.017	Mus musculus carbonyl reductase 1 (Cbr1), mRNA.
Ppp1r10	393.2	97.0	216.8	52.3	0.551	0.003	Mus musculus protein phosphatase 1, regulatory subunit 10 (Ppp1r10), mRNA.
LOC333331	1932.0	767.3	1064.6	318.4	0.551	0.020	PREDICTED: Mus musculus similar to medium-chain acyl-CoA dehydrogenase (LOC333331), misc RNA.
Fkbp5	150.5	55.9	82.2	30.4	0.546	0.018	Mus musculus FK506 binding protein 5 (Fkbp5), mRNA.
Tmem43	304.3	106.4	165.3	91.1	0.543	0.032	Mus musculus transmembrane protein 43 (Tmem43), mRNA.
Gja1	236.9	88.4	128.6	49.5	0.543	0.018	Mus musculus gap junction membrane channel protein alpha 1 (Gja1), mRNA.
Oat	22593.5	6512.9	12114.0	4126.2	0.536	0.002	Mus musculus ornithine aminotransferase (Oat), mRNA.
Chchd6	118.5	42.5	63.2	16.8	0.533	0.009	Mus musculus coiled-coil-helix-coiled-coil-helix domain containing 6 (Chchd6), mRNA.
Impact	512.7	166.4	272.6	44.4	0.532	0.002	Mus musculus imprinted and ancient (Impact), mRNA.
4930570C03Rik	849.0	290.3	449.5	108.9	0.529	0.004	Mus musculus RIKEN cDNA 4930570C03 gene (4930570C03Rik), mRNA.
Anxa3	241.0	121.2	127.3	29.8	0.528	0.047	Mus musculus annexin A3 (Anxa3), mRNA.
Gas6	1228.3	600.1	648.9	240.5	0.528	0.045	Mus musculus growth arrest specific 6 (Gas6), mRNA.
Mvp	1228.8	402.9	643.8	176.5	0.524	0.003	Mus musculus major vault protein (Mvp), mRNA.
Pgd	2768.7	1280.4	1446.0	475.0	0.522	0.030	Mus musculus phosphoglucoepimerase (Pgd), mRNA.
Sbk	863.9	53.2	450.5	148.5	0.522	0.000	Mus musculus serine/threonine kinase (Sbk), mRNA.
Lgals3bp	923.5	438.0	478.4	129.2	0.518	0.029	Mus musculus lectin, galactoside-binding, soluble, 3 binding protein (Lgals3bp), mRNA.
Pdgfra	167.6	73.0	86.5	28.8	0.516	0.022	Mus musculus platelet derived growth factor receptor, alpha polypeptide (Pdgfra), transcript variant 1, mRNA.
Por	1464.1	531.9	752.7	354.9	0.514	0.015	Mus musculus P450 (cytochrome) oxidoreductase (Por), mRNA.
Kif21a	196.7	34.6	100.4	26.9	0.510	0.000	Mus musculus kinesin family member 21A (Kif21a), mRNA.
Igtp	822.0	309.9	418.1	148.3	0.509	0.009	Mus musculus interferon gamma induced GTPase (Igtp), mRNA.
Cd9	363.9	118.9	184.2	84.9	0.506	0.006	Mus musculus CD9, mRNA.
Siat9	512.4	163.3	257.7	96.3	0.503	0.003	Mus musculus sialin 9 (Siat9), mRNA.
Ivns1abp	242.3	86.0	121.7	19.2	0.502	0.008	Mus musculus influenza virus NS1A binding protein (Ivns1abp), transcript variant 2, mRNA.
Hmox1	190.5	77.6	94.9	41.8	0.498	0.016	Mus musculus heme oxygenase (decycling) 1 (Hmox1), mRNA.
Rab34	123.5	49.5	60.6	16.3	0.491	0.014	Mus musculus RAB34, member of RAS oncogene family (Rab34), mRNA.
Nudt18	1536.4	468.4	752.9	606.8	0.490	0.022	Mus musculus nudix (nucleoside diphosphate linked moiety X)-type motif 18 (Nudt18), mRNA.
Mfsd7c	181.2	59.7	87.7	65.9	0.484	0.020	Mus musculus major facilitator superfamily domain containing 7C (Mfsd7c), mRNA.
Hspa8	2023.5	1002.1	972.4	456.8	0.481	0.033	Mus musculus heat shock protein 8 (Hspa8), mRNA.
As3mt	209.8	47.0	100.7	41.7	0.480	0.000	Mus musculus aspartyl aminotransferase 3 mitochondrial (As3mt), mRNA.
Ddit4	151.0	65.5	72.2	21.4	0.478	0.012	Mus musculus DNA-damage-inducible transcript 4 (Ddit4), mRNA.
Entpd5	1558.5	356.0	735.5	139.5	0.472	0.000	Mus musculus ectonucleoside triphosphate diphosphohydrolase 5 (Entpd5), transcript variant 1, mRNA.
Tyki	125.8	53.7	59.1	21.2	0.470	0.011	Mus musculus tyrosine kinase (Tyki), mRNA.
Arl6ip5	335.5	139.0	156.2	52.5	0.466	0.008	Mus musculus ADP-ribosylation factor-like 6 interacting protein 5 (Arl6ip5), mRNA.
Tmem62	350.4	90.7	162.8	30.9	0.465	0.000	Mus musculus transmembrane protein 62 (Tmem62), mRNA.
9330129D05Rik	241.2	74.8	110.4	21.4	0.458	0.000	Mus musculus RIKEN cDNA 9330129D05 gene (9330129D05Rik), mRNA.
Lgmn	1111.7	557.7	500.3	166.8	0.450	0.019	Mus musculus legumain (Lgmn), mRNA.
Ctgf	839.2	306.5	359.0	199.8	0.428	0.003	Mus musculus connective tissue growth factor (Ctgf), mRNA.
Anxa5	3934.0	1637.0	1674.2	1370.9	0.426	0.018	Mus musculus annexin A5 (Anxa5), mRNA.
Mgst3	1103.6	524.5	468.5	141.2	0.424	0.009	Mus musculus microsomal glutathione S-transferase 3 (Mgst3), mRNA.
Tomm22	562.3	96.1	237.4	189.2	0.422	0.001	Mus musculus translocase of outer mitochondrial membrane 22 homolog (yeast) (Tomm22), nuclear gene encoding mitochondrial protein, mRNA.

Supplementary Table 2. Differentially expressed genes in livers from OLD FXR<sup>-/-</sup>iVP16FXR vs. FXR<sup>-/-</sup>iVP16 mice. Genes are listed from the highest fold ratio to the lowest fold ratio.

SYMBOL	KO AVG Signal	KO - SD	TG AVG Signal	TG - SD	Ratio	P	DEFINITION
Hsd17b11	357.4	183.7	149.5	65.8	0.418	0.034	Mus musculus hydroxysteroid (17-beta) dehydrogenase 11 (Hsd17b11), mRNA.
Slc25a17	628.2	66.9	262.4	164.1	0.418	0.000	Mus musculus solute carrier family 25 (mitochondrial carrier, peroxisomal membrane protein), member 17 (Slc25a17), nuclear gene encoding mitochondrial protein, mRNA.
Nrg4	101.6	27.5	41.8	18.8	0.411	0.000	Mus musculus neuregulin 4 (Nrg4), mRNA.
Apoa4	2786.1	1706.7	1137.5	329.8	0.408	0.034	Mus musculus apolipoprotein A-IV (Apoa4), mRNA.
S100a11	1150.1	652.9	463.5	282.3	0.403	0.031	Mus musculus S100 calcium binding protein A11 (calgizzarin) (S100a11), mRNA.
Ptdx3	1678.9	383.8	668.6	115.7	0.398	0.000	
Anxa4	238.2	65.2	93.9	42.4	0.394	0.000	Mus musculus annexin A4 (Anxa4), mRNA.
Anxa2	1768.8	1010.4	681.2	326.9	0.385	0.022	Mus musculus annexin A2 (Anxa2), mRNA.
Ctpps	142.9	37.9	54.9	17.9	0.384	0.000	Mus musculus cytidine 5'-triphosphate synthase (Ctpps), mRNA.
Tgfb2	549.1	245.3	210.7	90.8	0.384	0.018	Mus musculus transforming growth factor, beta receptor II (Tgfb2), transcript variant 1, mRNA.
Vcam1	321.3	184.7	120.9	78.7	0.376	0.026	Mus musculus vascular cell adhesion molecule 1 (Vcam1), mRNA.
Fgl1	10339.3	6313.8	3825.2	3366.4	0.370	0.042	Mus musculus fibrinogen-like protein 1 (Fgl1), mRNA.
Atp10d	187.6	81.8	68.6	37.3	0.366	0.003	Mus musculus ATPase, Class V, type 10D (Atp10d), mRNA.
Spp1	982.9	505.4	357.6	127.2	0.364	0.007	Mus musculus secreted phosphoprotein 1 (Spp1), mRNA.
Drcub1a	468.9	145.1	169.6	67.7	0.362	0.000	
Lum	204.5	110.8	73.1	67.8	0.357	0.024	Mus musculus lumican (Lum), mRNA.
Htatip2	4955.6	1500.2	1770.6	518.0	0.357	0.000	Mus musculus HIV-1 tat interactive protein 2, homolog (human) (Htatip2), mRNA.
Lyz2	726.1	436.6	256.6	202.9	0.353	0.029	Mus musculus lysozyme 2 (Lyz2), mRNA.
Cyp3a11	28403.5	11367.8	9335.5	5622.9	0.329	0.001	Mus musculus cytochrome P450, family 3, subfamily a, polypeptide 11 (Cyp3a11), mRNA.
Cyp2a5	13953.1	6009.3	4539.2	2462.6	0.325	0.002	Mus musculus cytochrome P450, family 2, subfamily a, polypeptide 5 (Cyp2a5), mRNA.
Tmprss2	152.0	86.3	49.0	13.2	0.322	0.009	Mus musculus transmembrane protease, serine 2 (Tmprss2), mRNA.
Mfsd2	515.1	269.3	158.2	199.4	0.307	0.017	Mus musculus major facilitator superfamily domain containing 2 (Mfsd2), mRNA.
Snhg11	663.4	158.7	203.7	99.2	0.307	0.000	Mus musculus small nucleolar RNA host gene 11 (non-protein coding) (Snhg11), mRNA.
Oasl2	142.5	125.6	42.9	37.2	0.301	0.043	Mus musculus 2'-5' oligoadenylate synthetase-like 2 (Oasl2), mRNA.
Csad	2458.5	1404.0	731.8	412.9	0.298	0.008	Mus musculus cysteine sulfinate decarboxylase (Csad), mRNA.
Usp18	370.3	226.5	109.1	78.7	0.295	0.013	Mus musculus ubiquitin specific peptidase 18 (Usp18), mRNA.
Lgals1	1266.0	646.5	361.1	250.1	0.285	0.004	
Tceal8	143.5	101.6	40.1	21.2	0.280	0.027	Mus musculus transcription elongation factor A (SID)-like 8 (Tceal8), mRNA.
Abhd1	289.0	181.5	74.9	88.8	0.259	0.022	Mus musculus abhydrolase domain containing 1 (Abhd1), transcribed RNA.
Osblp3	102.3	52.5	25.0	15.8	0.244	0.013	Mus musculus oxysterol binding protein-like 3 (Osblp3), mRNA.
Uap111	719.8	353.9	174.2	116.1	0.242	0.001	Mus musculus UDP-N-acetylglucosamine pyrophosphorylase 1-like 1 (Uap111), mRNA. XM_918982
Cyp2b10	926.9	324.5	223.8	226.1	0.241	0.006	Mus musculus cytochrome P450, family 2, subfamily b, polypeptide 10 (Cyp2b10), transcript variant 2, mRNA.
Aco3	529.1	186.9	122.7	64.3	0.232	0.000	Mus musculus acyl-CoA thioesterase 3 (Aco3), mRNA.
Scara3	210.0	134.9	46.9	20.8	0.223	0.008	Mus musculus scavenger receptor class A, member 3 (Scara3), mRNA.
B930041F14Rik	192.5	99.3	42.6	24.7	0.221	0.001	Mus musculus RIKEN cDNA B930041F14 gene (B930041F14Rik), mRNA.
Gsta1	6687.4	5424.3	1469.4	2311.7	0.220	0.048	Mus musculus glutathione S-transferase, alpha 1 (Ya) (Gsta1), mRNA.
Cyp2b9	2954.1	2013.8	648.6	602.0	0.220	0.014	Mus musculus cytochrome P450, family 2, subfamily b, polypeptide 9 (Cyp2b9), mRNA.
LOC100048346	317.7	180.5	69.2	50.2	0.218	0.003	PREDICTED: Mus musculus similar to ubiquitin specific protease UBPA3 (LOC100048346), mRNA.
Hr	168.0	107.5	32.9	56.6	0.196	0.032	Mus musculus hairless (Hr), mRNA.
Mgst2	151.1	108.5	27.7	35.2	0.183	0.016	Mus musculus microsomal glutathione S-transferase 2 (Mgst2), mRNA.
Cyp7a1	7972.2	1795.3	1421.3	624.2	0.178	0.000	Mus musculus cytochrome P450, family 7, subfamily a, polypeptide 1 (Cyp7a1), mRNA.
Rsad2	1140.2	842.0	194.9	120.2	0.171	0.013	Mus musculus radical S-adenosyl methionine domain containing 2 (Rsad2), mRNA.
Tuba8	177.5	139.9	27.6	54.6	0.156	0.026	Mus musculus tubulin, alpha 8 (Tuba8), mRNA.
Cyp2b23	914.0	506.4	140.7	109.6	0.154	0.001	Mus musculus cytochrome P450, family 2, subfamily b, polypeptide 23 (Cyp2b23), mRNA.
Akr1b3	419.4	114.9	62.5	68.1	0.149	0.000	Mus musculus aldo-keto reductase family 1, member B3 (aldose reductase) (Akr1b3), mRNA.
Gstm3	520.1	487.9	65.8	54.6	0.127	0.039	Mus musculus glutathione S-transferase, mu 3 (Gstm3), mRNA.
Cyp2b13	1509.8	1233.3	180.8	158.5	0.120	0.017	Mus musculus cytochrome P450, family 2, subfamily b, polypeptide 13 (Cyp2b13), mRNA.
Nt5e	393.8	298.6	43.2	20.7	0.110	0.009	Mus musculus 5' nucleotidase, ecto (Nt5e), mRNA.
Ly6d	390.0	318.2	30.8	30.6	0.079	0.012	Mus musculus lymphocyte antigen 6 complex, locus D (Ly6d), mRNA.
Sdcbp2	135.0	153.7	-0.2	2.6	-0.001	0.045	Mus musculus syndecan binding protein (syntenin) 2 (Sdcbp2), mRNA.



Supplementary Table.3			
Inguinity Canonical Pathways	Age	P	Molecules
Complement System	YOUNG	3.02E-07	C9, C6
	AGED	1.45E-04	C8B, C5, C8A
Histidine Degradation VI	YOUNG	2.57E-06	CYP7B1
	AGED	5.75E-08	CYP7B1, HAL
Nicotine Degradation III	YOUNG	3.02E-06	Cyp2c40 (include others)
	AGED	3.24E-08	CYP2F1, ADH7, UGT2B17
Systemic Lupus Erythematosus Signaling	YOUNG	2.24E-05	C9, C6
	AGED	2.14E-04	SNRPG, C8B, C5, SNRPD2, C8A, LSM2

The Inguinity Canonical Pathways identify clusters of pathways related by genes that are found upregulated in the overlap of the age groups ( iVP16FXRFXR<sup>-/-</sup> vs. iVP16FXR<sup>-/-</sup> mice).

Supplementary Table.4			
Ingenuity Canonical Pathways	Age	P	Molecules
Aryl Hydrocarbon Receptor Signaling	YOUNG	3.02E-07	TGM2,ALDH1B1,GSTM1,FOS,GSTM5,Gstm3,NQO2,GSTA5,CCND1,MGST3
	AGED	1.45E-04	GSTM1,MGST2,Gstm3,GSTA5,GSTM4,MGST3,HSPB1
Glutathione-mediated Detoxification	YOUNG	2.57E-06	GSTM1,GSTM5,Gstm3,GSTA5,MGST3
	AGED	5.75E-08	GSTM1,MGST2,Gstm3,GSTA5,GSTM4,MGST3
LPS/IL-1 Mediated Inhibition of RXR Function	YOUNG	3.02E-06	ALDH1B1,GSTM1,GSTM5,Gstm3,CYP2A13,CYP3A5,ABCC2,GSTA5,SULT1E1,CYP7A1,MGST3
	AGED	3.24E-08	GSTM1,MGST2,CYP3A5,CYP2A13,Gstm3,ABCC2,GSTA5,CYP7A1,GSTM4,FMO1,CYP2B6,CYP4A11,MGST3
PXR/RXR Activation	YOUNG	2.24E-05	GSTM1,SCD,Aldh1a7,CYP3A5,ABCC2,CYP7A1
	AGED	2.14E-04	GSTM1,CYP3A5,ABCC2,CYP7A1,CYP2B6
NRF2-mediated Oxidative Stress Response	YOUNG	1.62E-04	GSTM1,FOS,GSTM5,Gstm3,NQO2,ABCC2,GSTA5,MGST3
	AGED	3.02E-09	HMOX1,GSTM1,MGST2,Gstm3,ABCC2,GSTA5,GSTM4,FMO1,SQSTM1,FKBP5,MGST3,CBR1,EPHX1
Xenobiotic Metabolism Signaling	YOUNG	1.70E-04	ALDH1B1,GSTM1,GSTM5,Gstm3,CYP3A5,NQO2,ABCC2,GSTA5,SULT1E1,MGST3
	AGED	6.31E-07	HMOX1,GSTM1,MGST2,Ces1b/Ces1c,UGT2B7,CYP3A5,Gstm3,ABCC2,GSTA5,GSTM4,FMO1,CYP2B6,MGST3

The Ingenuity Canonical Pathways identify clusters of pathways related by genes that are found downregulated in the overlap of the age groups (iVP16FXRFXR<sup>-/-</sup> vs. iVP16FXR<sup>-/-</sup> mice).