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Abbreviations: Hprt1, hypoxanthine phosphoribosyl transferase 1; HSC, hepatic stellate cells; KC, Kupffer cells; NR, nuclear receptors; SEC, sinusoidal endothelial cells; Nuclear receptor names and their abbreviations are provided in Supplementary Table 1.

Abstract

Nuclear receptors (NR), the largest family of transcription factors, control many physiological and pathological processes. To gain insight into hepatic NR and their potential as therapeutic targets in cholestatis, we determined their expression in individual cell types of the mouse liver in normal and cholestatic conditions. Hepatocytes, cholangiocytes, hepatic stellate cells (HSC), sinusoidal endothelial cells (SEC) and Kupffer cells (KC) were isolated from the liver of mice with acute or chronic cholestasis (*i.e.* bile duct-ligated or *Abcb4*$^{-/-}$ mice, respectively) and healthy controls. The expression of 43 out of the 49 NR was evidenced by RT-qPCR in one or several liver cell types. Expression of four NR was restricted to non-parenchymal liver cells. In normal conditions, NR were expressed at higher levels in individual cell types when compared to total liver. Half of the NR expressed in the liver had maximal expression in non-parenchymal cells. After bile duct ligation, NR mRNA changes occurred mostly in non-parenchymal cells and mainly consisted in down-regulations. In *Abcb4*$^{-/-}$ mice, NR mRNA changes were equally frequent in hepatocytes and non-parenchymal cells. Essentially down-regulations were found in hepatocytes, HSC and cholangiocytes, as opposed to up-regulations in SEC and KC. While undetectable in total liver, Vdr expression was up-regulated in all non-parenchymal cells in *Abcb4*$^{-/-}$ mice. In conclusion, non-parenchymal liver cells are a major site of NR expression. During cholestasis, NR expression is markedly altered mainly by down-regulations, suggesting major changes in metabolic activity. Thus, non-parenchymal cells are important new targets to consider in NR-directed therapies.

Keywords: *Abcb4* knockout mice; bile duct ligation; hepatocytes; non-parenchymal liver cells.

1. Introduction

Nuclear receptors (NR) form the largest family of transcription factors with 48 members in humans and 49 in rodents [1] . NR bind a variety of natural and synthetic lipophilic ligands that include steroid hormones, fat-soluble vitamins, bile and fatty acids. NR bind to DNA response elements as monomers, homodimers or heterodimers with RXR to regulate the expression of multiple genes implicated in metabolism, reproduction or development. In recent years, systematic analysis of the expression pattern and modulation of NR has proven a powerful strategy to elucidate their biological functions and uncover their therapeutic potential [2-6].

NR play key roles in liver functions, such as glucose metabolism, cholesterol and bile acid homeostasis [7]. NR also control hepatoprotective mechanisms, notably against bile acid toxicity. As such, they are important targets for pharmacological therapies in metabolic and cholestatic liver diseases [8]. Expression profile of NR in mouse liver has shown that the most highly expressed NR belong to the physiological clusters defined as "Bile acids and xenobiotic metabolism" and "Lipid metabolism and energy homeostasis" [2, 9]. Because hepatocytes represent approximately 70% of total liver cells in normal condition, analysis of NR expression in the liver mainly reflects hepatocyte profile, and may mask NR expression in non-parenchymal cells. As an example, VDR, a therapeutic target of particular interest in cholestatic liver diseases and liver fibrosis [10-14], is exclusively expressed in non-parenchymal cells in the liver, *i.e.* cholangiocytes, hepatic stellate cells (HSC), sinusoidal endothelial cells (SEC) and Kupffer cells (KC) [15]. Non-parenchymal cells also express other NR, such as FXR or PPARs, which control inflammatory and fibrotic responses in the liver [7, 16].

In view of the importance of non-parenchymal cells in the homeostasis of liver tissue and in liver physiology, we herein determined the expression profile of NR in all major cell types of

the mouse liver in basal conditions. To gain more insight into the transcriptional regulatory networks governed by NR in cholestatic conditions, we then analyzed the changes in the NR transcriptome induced by cholestasis among individual liver cell types, in acute (bile duct ligation) and chronic (*Abcb4*$^{-/-}$) mouse models of cholestatic liver injury.

2. Materials and methods

2.1. Animals

C57BL/6J mice were purchased from Janvier Europe (Saint-Berthevin, France). *Abcb4*$^{+/-}$ mice in a FVB/N background (FVB.129P2-Abcb4tm1Bor/J) were obtained from Sanofi R&D (Chilly-Mazarin, France) in order to generate *Abcb4*$^{-/-}$ and *Abcb4*$^{+/+}$ mice by heterozygous breeding. Mice were housed in a Specific and Opportunistic Pathogen Free facility, at a controlled temperature (20-24° C), on a 12-hour light/dark cycle. They were fed a standard ROD16 chow (Genobios, Laval, France) and had free access to water. All experiments complied with the European Communities Council Directive (2010/63/UE) and were approved by the French Ethics Committee in Animal Experiment « Charles Darwin » (Ile-de-France, Paris, no 5).

2.2. Bile duct ligation

Twelve weeks old C57BL/6J mice underwent double ligation and section of the common bile duct or Sham operation, under isoflurane anesthesia. Sham operation consisted in laparotomy with exposure of the common bile duct, without ligation. Liver tissue/cell collection was performed 72 hours after surgery.

2.3. Liver tissue and cell collection

Liver tissue and cells were collected from bile duct-ligated and Sham-operated mice, *Abcb4*$^{-/-}$ mice and their *Abcb4*$^{+/+}$ littermates. All mice, only males, were 12 weeks old at the time of liver tissue/cell collection, which was performed in non-fasted animals, between 9:00 and 11:00 AM, to avoid circadian variations of NR expression [17]. Intact liver was harvested from mice (n=5-6 per group) under isoflurane anesthesia, and liver tissue samples were snap-frozen in liquid nitrogen. The different liver cell populations (n=6-8 per group) were isolated, using different methods. *In situ* perfusion-based protocols were used to isolate hepatocytes, cholangiocytes and HSC, as previously described [18]. In these protocols, mice were

anesthetized by intra-peritoneal injection of xylazine and ketamine and the liver was perfused *in situ via* the inferior vena cava. For hepatocyte isolation, the liver was perfused with collagenase P (Sigma, Saint-Louis, MO, USA), the cell suspension thus obtained was filtered through a 100-µm strainer and centrifuged twice at 70xg to eliminate non-parenchymal cells. For cholangiocyte isolation, the liver was perfused with collagenase P, after which the liver was collected and parenchymal liver tissue was mechanically removed. The remaining biliary tree was further digested in a solution containing both pronase (Sigma-Aldrich, Saint-Quentin Fallavier, France) and collagenase P, and the resulting cell suspension was filtered through 100-µm and 40-µm strainers. For HSC isolation, the liver was perfused first with pronase and then with collagenase P. Thereafter, the liver tissue was mechanically disaggregated to obtain a cell suspension, which was then submitted to centrifugation at 70000xg, on Nycodenz density gradient at 8.2% (Sigma-Aldrich). HSC selected on the basis of their lipid droplet content, were recovered from the top of the Nycodenz gradient after centrifugation. SEC and KC were isolated by a method combining mechanical dissociation and enzymatic digestion. Intact liver was harvested from mice under isoflurane anesthesia. Then, the liver was dissociated *ex situ* using a gentleMACs dissociator (Miltenyi, Bergisch Giadbach, Germany) and further digested by incubation in a collagenase P solution to obtain a single-liver cell suspension. A non-parenchymal cell fraction was obtained by centrifugation, from which SEC and KC were magnetically selected, using Miltenyi CD146 and anti-F4-80 antibodies, respectively. The purity of isolated cell fractions was ascertained by cell type marker analyses (Supplementary Figure 1).

2.4. Reverse Transcription-quantitative Polymerase Chain Reaction

Total RNA was extracted from total liver and isolated liver cell fractions, using RNeasy MiniKit and Microkit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. All RNA samples were treated with DNase I (Qiagen), to prevent genomic DNA

contamination. Reverse transcription was achieved, using the SuperScript II Reverse Transcriptase (Thermo Fisher Scientific, Carlsbad, CA, USA) and equal quantities of cDNA for each type of sample were pooled. Real-time quantitative PCR was performed using the Light Cycler 4800 Sybr Green Master Mix on a Light-Cycler 4800 (Roche Diagnostics, Basel, Switzerland) with 10 ng of cDNA per reaction. The sequences of primers are shown in Supplementary table 2, and their efficiency was validated as previously described [19]. The analysis of NR expression was performed using the efficiency-corrected ΔCt method, which permits receptor-to-receptor comparisons [2, 20]. Data expressed in arbitrary units, represent averaged, efficiency-corrected, values of nuclear receptor mRNA levels, that were normalized for those of hypoxanthine phosphoribosyl transferase 1 (*Hprt1*), used as reference gene [20]. Mean values from triplicate sample wells are shown.

2.5. Data analysis

NR expression was defined as absent (Ct ≥ 33), low, moderate or high, for mRNA levels < 0.2, between 0.2 and 1.6, and > 1.6 arbitrary units, respectively (adapted from [2]). To visualize differences in expression levels between the liver cell types, the relative mRNA levels of each NR were compared using the webtool ClustVis and were plotted in heatmaps. Before being plotted, data were pre-processed using the "Unit Variance Scaling Method", which divides the values by standard deviation so that each row has a variance equal to one [21]. To determine the most prominent changes induced by cholestatic injury, a cut-off was arbitrarily set at 2-fold change, for the comparison of NR expression in cells from normal and cholestatic liver.

3. Results

3.1. Expression pattern of nuclear receptors in liver cell types

First, transcript levels of the 49 mouse NR were analyzed in the total liver and individual liver cell types of C57BL/6J and FVB/N mice (Figure 1 and Supplementary Figure 2). In C57BL/6J mice (Figure 1), all NR with the exception of Tlx, Rorβ, Pr, Dax-1, Sf1 and Pnr were expressed in one or several liver cell types. Four NR, Vdr, Nor-1, Nurr1 and Erβ, were expressed exclusively in non-parenchymal cells and were thus undetectable in total liver. NR were expressed at higher levels in individual cell types, including hepatocytes, cholangiocytes, HSC or SEC, when compared to total liver (Figure 1). The latter observation may account for the presence of non-hepatic cells in the liver. Indeed, as shown in Supplementary Figure 3, red blood cell markers are expressed in total liver while nearly absent from isolated hepatocytes. Thus, mRNA levels in total liver accounts for parenchymal, non-parenchymal and red blood cells, a cell type in which NR expression was not detected [22]. To a large extent, the expression patterns were similar in the FVB/N strain with only small differences in the expression levels of NR compared to C57BL/6J (Supplementary Figure 2). The progesterone receptor, Pr, was the only NR undetectable in C57BL/6J that was detected at low levels in non-parenchymal cells in the FVB/N strain (Supplementary Figure 2).

A cell-type specific signature of NR expression was observed when comparing mRNA levels in the different liver cell types (Figure 2 and Supplementary Figure 4). Classification of NR by physiological clusters showed that NR belonging to the cluster “Bile Acids and Xenobiotic Metabolism” had maximal level of expression in hepatocytes, with the exception of Hnf4γ and Vdr, which were more abundant in cholangiocytes. Surprisingly, the highest expression of Fxrα, the principal regulator of bile acid homeostasis, was not detected in hepatocytes but in HSC. NR from the cluster “Lipid Metabolism and Energy Homeostasis” showed their

highest levels of expression in hepatocytes and HSC, two cell types that both control lipid metabolism and storage in the liver, or cholangiocytes. Most members of the "CNS, Circadian and Basal Metabolic Functions" cluster, a cluster related to energy homeostasis, were also expressed at maximal levels in hepatocytes or HSC. Four members of this cluster, Nor-1 and Nurr1, which regulate proliferation and inflammation, as well as Rev-Erbα and β, that are key regulators of circadian gene expression in the liver, displayed their highest expression level in cholangiocytes. The "CNS, Circadian and Basal Metabolic Functions" cluster, contains the rare NR showing a maximal expression in SEC (Errβ, Tr4) or KC (Coup-TFI). NR of the "Reproduction and Development" cluster were expressed at maximal levels in HSC, with some exceptions like Ar, expressed at highest levels in hepatocytes. Within the "Steroidogenesis" cluster, only Fxrβ was expressed in the liver, predominantly in HSC (Figure 2E). Overall, approximately half of NR were expressed at maximal level in non-parenchymal cells, most notably in HSC and cholangiocytes.

3.2. Changes in nuclear receptor transcriptome induced by cholestatic injury in liver cells

Modulations in NR expression were previously reported in human cholestatic liver diseases, such as up-regulation of PXR in obstructive cholestasis [23] or down-regulation of FXR, SHP and CAR in chronic cholestatic liver diseases [24-26]. However, modifications in the expression of NR in the cholestasic liver remain poorly defined and the cell types involved unknown. Thus, we analyzed NR expression modulations in mouse models of acute and chronic cholestasis.

In acute cholestasis, three days after bile duct ligation, at the peak of hepatocellular injury (Supplementary Figure 5) [27], the number of NR displaying ≥ 2-fold changes in mRNA levels was of 32 in HSC, 21 in KC, 19 in cholangiocytes, 12 in SEC and 6 in hepatocytes (Figure 3). Figure 4 shows changes in the 24 most abundant NR (*i.e.* expressed at moderate to

high level) in total liver of C57BL/6J mice. All 24 receptors displayed ≥ 2-fold changes in at least one liver cell type. Among them, Pxr, Shp, Pparδ and Tr4 were modified in both hepatocytes and non-parenchymal cells, whereas the other 20 NR were exclusively modified in non-parenchymal cells. The most prominent changes in NR expression were found in HSC, in which Fxrα, Pparδ, Rxrα, Gr, Mr, Erα, Coup-TFII and Rarα mRNA levels were markedly reduced (fold change ≥ 3). Although expression of most NR was reduced in acute cholestasis, Pxr, Shp and Tr4 were up-regulated in hepatocytes, whereas Lxrα and Rarα were up-regulated in SEC (Figure 4). These results therefore indicate that the NR signaling network response to acute cholestasis is essentially characterized by a reduction in NR expression, especially important in non-parenchymal cells.

In chronic cholestatic liver diseases, prolonged cellular injury may induce secondary changes in NR-mediated signaling. We thus analyzed NR expression in *Abcb4*$^{-/-}$ mice, an experimental model that closely resembles human sclerosing cholangitis, at 12 weeks, when fibrosis has reached a plateau [28] (Supplementary Figure 5). In *Abcb4*$^{-/-}$ mice, the number of NR displaying changes in mRNA levels ≥ 2-fold was of 26 in HSC, 22 in hepatocytes, 14 in SEC, 9 in KC and 6 in cholangiocytes (Figure 5). Among the 26 most abundant NR in FVB/N mouse total liver (Supplementary Figure 2), 20 disclosed ≥ 2-fold expression changes in liver cells in *Abcb4*$^{-/-}$ mice (Figure 6). A majority of them were down-regulated in hepatocytes and HSC. Namely, Car, Trβ, Pparα, Rxrα, Gr, Mr and Erα mRNA levels were decreased only in these cell types, whereas Rev-Erbα was also reduced in cholangiocytes. Moreover, mRNA levels of Hnf4α, Lrh-1, Shp, Errα, Rorα and Ar in hepatocytes and Fxrα, Tr4 and Rev-Erbβ in HSC were also decreased. Lrh-1 was the only NR to show an increased expression in HSC, while Pparα mRNA levels were increased in cholangiocytes. In both SEC and KC, mRNA levels of Pxr, Fxrα, Hnf4α, Rorγ, Rorα and Ar were up-regulated in *Abcb4*$^{-/-}$ mice. Furthermore, the expression of Car, Trβ and Pparα was also increased in SEC.

In summary, in *Abcb4*$^{-/-}$ mice, changes in a larger number of genes were detected in hepatocytes, whereas in cholangiocytes and KC the number of modified NR was lower than in bile duct ligated mice. Interestingly, SEC and KC only displayed increases in NR mRNA levels in *Abcb4*$^{-/-}$ mice. As SEC and KC play a prominent role in inflammation and vascular remodeling, our results suggest that NR-mediated signaling may participate to the inflammatory and angiogenic response of the liver in chronic cholestatic disease.

NR that displayed the most profound changes in at least one liver cell type in either acute or chronic cholestasis are recapitulated in Figure 7. Vdr, Rxrγ, Coup-TFI, Fxrα, Rorγ, Ngf1b, and Nurr1 were exclusively modified in non-parenchymal cells. Car, Pxr, Hnf4α and Pparα, all known to participate to the adaptive response of the liver to cholestasis, as well as, Erα, Rev-Erbα, Trβ, Nor-1, Errγ, Ar and Fxrβ were modulated both in hepatocytes and non-parenchymal cells. In chronic cholestasis, Vdr expression was increased in all non-parenchymal cells (Figure 7A). The expression of Nor-1 and Ngf1b was markedly up-regulated in SEC in acute cholestasis and to a lesser extent in chronic cholestasis (Figure 7C). Important reductions in the expression of Erα, Rev-Erbα, Coup-TFI, Car, Fxrα, Trβ, Ppparα, Nor-1, Ngf1b, Errγ and Fxrβ, were found in cholangiocytes and HSC (Figure 7B and C). These results indicate that the most dramatic changes elicited by cholestasis in NR expression occur in non-parenchymal cells. Among non-parenchymal cells, the most pronounced changes were found in HSC and SEC, which suggests a central role of NR in controlling features of cholestatic liver injury that involve these cell types, such as fibrosis, inflammation and angiogenesis. Taken together, these results indicate that modulation of NR expression in non-parenchymal cells may be a key event in the liver response to cholestasis.

In order to assess the functional impact of the changes observed in NR expression, we examined the mRNA levels of Cyp3a11 and Vcam-1 in all liver cell types in acute and chronic cholestatic settings. Cyp3a11 and Vcam-1 are targets of Pxr and Nor-1 respectively,

two NR subjected to important changes during cholestasis (Figure 7). As shown in Supplementary Figure 6, Cyp3a11 and Vcam-1 underwent mRNA changes that were correlated with those of Pxr and Nor-1 during acute and chronic cholestasis, respectively. Differences in the fold changes between NR and their target genes may be due to additional mechanisms of regulation, such as posttranscriptional modifications or ligand availability. These results therefore support the assumption that transcriptional changes in NR expression translate into biological effects in cholestasis.

4. Discussion

In this study, we defined the NR transcriptome in the different liver cell types in normal, acute and chronic cholestatic settings. We show that both hepatocytes and non-parenchymal cells are important sites of NR expression in the liver. Cell type-specific modulations in NR expression were demonstrated in cholestasis, which should help identifying new therapeutic targets in cholestatic liver diseases.

In keeping with previous reports [6, 17], we show that 9 and 10 NR are undetectable in total liver of C57BL/6J and FVB/N mice, respectively. Furthermore, we show that four of these receptors, Vdr, Nurr1, Nor-1 and Erβ are expressed in non-parenchymal liver cells. Among all NR expressed in at least one liver cell type, half disclosed their maximal expression levels in non-parenchymal cells. Therefore, these results suggest that NR signaling in non-parenchymal cells may be more important than previously anticipated for liver function.

We also show that acute cholestasis induced by bile duct ligation caused a reduction in the expression of a large number of NR in liver cells. Similarly, NR transcriptome in total liver showed many reductions in the initial phases of liver regeneration after partial hepatectomy [6], a situation also characterized by an acute bile acid overload [29]. In our study, the most dramatic changes in NR expression pattern occurred in HSC. This observation may indicate a central role of NR in HSC transdifferentiation to myofibroblasts, as previously suggested [30-33]. Consistently, we detected important reductions in Rxrα and Rarα mRNA levels in HSC during acute cholestasis, which is in accordance with previous observations showing a reduction in the expression of genes involved in lipid metabolism during HSC activation to myofibroblasts [33, 34]. Because HSC undergo early phenotypic changes before they fully convert into myofibroblasts [35], our results suggest that NR down-regulation may be an early transcriptional event controlling HSC activation. Although in normal liver Fxrα displayed maximal expression levels in HSC, its expression was markedly reduced in cholestasis, as

previously reported in culture activated mouse HSC [36]. However, our results clearly showed that Fxrα was still expressed in HSC from cholestatic liver, and therefore do not rule out a direct anti-fibrotic effect of FXR activation in these cells as previously reported [37]. Rev-Erbα mRNA levels were also dramatically reduced in HSC in our study. Rev-Erbα is a key regulator of circadian gene expression and a modulator of lipid and bile acid metabolism [38, 39]. In Rev-Erbα knock-out mice, bile acid synthesis is repressed and lipid accumulation altered, following a reduction in the expression levels of Cyp7a1 and Srebp-1c target genes, respectively [39]. Interestingly, reduction of Srepb-1c signaling has been described to be a central event in HSC transactivation [33]. Taken together, our results highlight the prominent role of the global NR signaling pathway in the regulation of HSC activation and thus the progression of fibrosis in cholestasis.

In chronic cholestatic liver diseases, prolonged cell injury may induce secondary changes in NR-mediated signaling [8]. Consistent with this assumption, we evidenced a NR response in chronic cholestasis, which was different from acute cholestasis. In hepatocytes, mRNA levels of Car, Shp and Hnf4α, which all mediate protective mechanisms in response to cholestasis, were dramatically reduced in *Abcb4*$^{-/-}$ mice as previously described in primary biliary cholangitis, progressive familial intrahepatic cholestasis or biliary atresia [24-26]. This suggests that adaptive mechanisms may become less efficient in chronic cholestasis. However, down-regulation of other NR expression in hepatocytes, on the contrary, may promote protection in chronic cholestasis. Thus, we observed an important decrease in the expression of Pparα in hepatocytes from *Abcb4*$^{-/-}$ mice, while it was previously reported that silencing Pparα attenuates chronic liver injury, reducing bile duct proliferation and fibrosis in *Abcb4*$^{-/-}$ mice [40]. We also evidenced that Lrh-1 expression was dramatically reduced in hepatocytes from *Abcb4*$^{-/-}$ mice. As the repression of Lrh-1 signaling leads to the generation

of a more hydrophilic bile acid pool [41], Lrh-1 down-regulation may provide protection against bile acid-induced toxicity in chronic cholestasis.

Important reductions in NR expression have been detected in chronic cholestasis such as primary biliary cholangitis, a disease predominantly occuring in women [42]. Interestingly, *Abcb4*$^{-/-}$ female mice display a more severe liver phenotype than males [43]. Moreover, the expression of NR, such as Car, Pxr or Pparα, is differently modulated in female and male C57BL/6J mice [44]. In our study, we did not directly address a potential gender disparity of NR modulation, which may however contribute to the observed gender-related differential progression in chronic cholestasis.

In both acute and chronic cholestasis, we observed important changes in the expression of NR that are not or poorly expressed in total liver. Among them, Vdr showed an increased expression in all non-parenchymal cells in chronic cholestasis, while remaining undetectable in hepatocytes. Because Vdr is involved in innate immunity [10] and has anti-inflammatory and anti-fibrotic effects [12-14, 45], our results reinforce the interest for Vdr as a therapeutic target in chronic cholestatic liver diseases. Our study also revealed a specific pattern of NR modulation in SEC and KC during cholestasis including, Nor-1, Ngfb1 and Nurr1 overexpression. Because Nor-1, Ngfb1 and Nurr1, are key regulators of angiogenesis and pro-inflammatory cytokines production [46], these up-regulations may promote the angioarchitectural changes occurring in cholestatic liver injury [18, 47, 48]. The latter assumption is further supported by our observation that Nor-1 target gene Vcam-1 is highly correlated with Nor-1 expression changes in cholestasis. Overall, our results suggest that NR with low expression levels in total liver may be of high interest for the treatment of cholestatic diseases.

In conclusion, our results highlight the importance of all non-parenchymal cells as potential targets for NR-directed therapies.

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Figure legends

Figure 1. NR expression in C57BL/6J mouse total liver and liver cells

The expression of the 49 NR was analyzed by RT-qPCR in total liver and individual liver cell populations from 12 weeks old C57BL/6J mice. The mRNA levels were measured in pooled samples from 5-8 mice, normalized for Hprt1 and expressed as arbitrary units. NR expression was defined as absent for Ct ≥ 33, low, moderate or high, for mRNA levels < 0.2, between 0.2 and 1.6, and > 1.6 arbitrary units, respectively. HSC, hepatic stellate cells; KC, Kupffer cells; SEC, sinusoidal endothelial cells.

Figure 2. NR expression patterns in the different liver cell types

The expression of the 49 NR was analyzed by RT-qPCR in liver cell populations from 12 weeks old C57BL/6J mice. The mRNA levels were measured in pooled samples from 6-8 mice and normalized for Hprt1. NR mRNA levels were compared using the webtool ClustVis and data were plotted in heatmaps in which each row has a variance of one. Tlx, Rorβ, Pr, Dax-1, Sf1 and Pnr, absent from all samples, are not represented. NR were classified according to physiological clusters [2]. Chol, cholangiocytes; CNS, Central Nervous System; Hep, hepatocytes; HSC, hepatic stellate cells; KC, Kupffer cells; SEC, sinusoidal endothelial cells.

Figure 3. Changes in NR expression patterns induced by acute cholestasis in the different liver cell types

Liver cells were isolated from 12 weeks old bile duct-ligated (BDL) or Sham-operated mice 3 days post-surgery. NR mRNA levels were measured by RT-qPCR in pooled samples from 6-8 mice and normalized for Hprt1. NR expression was compared using the webtool ClustVis and data were plotted in heatmaps. Arrowheads point to ≥ 2-fold changes. Tlx, Rorβ, Pr, Dax-1, Sf1 and Pnr, absent from all samples, are not represented. NR were classified according to physiological clusters [2]. Chol, cholangiocytes; CNS, Central Nervous System; Hep,

hepatocytes; HSC, hepatic stellate cells; KC, Kupffer cells; SEC, sinusoidal endothelial cells; St, Steroidogenesis.

Figure 4. Changes induced by acute cholestasis in the expression of the most abundant NR in liver

Liver cells were isolated from 12 weeks old, bile duct-ligated (BDL) or Sham-operated mice 3 das post-surgery. NR mRNA levels were measured by RT-qPCR in pooled samples from 6-8 mice and normalized for Hprt1. NR expressed at moderate to high level in C57BL/6J mice total liver were selected. Mean values of mRNA levels from triplicate analyses ± SD are shown. Arrowheads point to ≥ 2-fold changes in mRNA levels. Those with double arrowheads are shown in insets. Chol, cholangiocytes; Hep, Hepatocytes; HSC, hepatic stellate cells; KC, Kupffer cells; SEC, sinusoidal endothelial cells. Changes induced by acute cholestasis in NR expressed at low levels or absent from total liver are summarized in Supplementary Table 3.

Figure 5. Changes in NR expression patterns induced by chronic cholestasis in the different liver cell types

Liver cells were isolated from 12 weeks old *Abcb4*$^{-/-}$ (KO) or *Abcb4*$^{+/+}$ (WT) mice. The NR mRNA levels were measured by RT-qPCR in pooled samples from 6-8 mice and normalized to Hprt1. NR mRNA levels were compared using the webtool ClustVis and data were plotted in heatmaps. Arrowheads point to ≥ 2-fold changes. Tlx, Rorβ, Dax-1, Sf1 and Pnr, absent from all samples, are not represented. NR were classified according to physiological clusters [2]. Chol, cholangiocytes; CNS, Central Nervous System; Hep, Hepatocytes; HSC, hepatic stellate cells; KC, Kupffer cells; SEC, sinusoidal endothelial cells; St, Steroidogenesis.

Figure 6. Changes induced by chronic cholestasis in the expression of the most abundant NR in liver

Liver cells were isolated from 12 weeks old *Abcb4*$^{-/-}$ or *Abcb4*$^{+/+}$ mice. The mRNA levels were measured by RT-qPCR in pooled samples from 6-8 mice and normalized for Hprt1. The NR expressed at moderate to high level in FVB/N mice total liver showing ≥ 2-fold changes in mRNA levels were represented. Mean values of mRNA levels from triplicate analyses ± SD are shown. Arrowheads point to NR with ≥ 2-fold changes in mRNA. Those with double arrowheads are shown in insets. Chol, cholangiocytes; Hep, hepatocytes; HSC, hepatic stellate cells; KC, Kupffer cells; SEC, sinusoidal endothelial cells. Changes induced by chronic cholestasis in NR expressed at low levels or absent from total liver are summarized Supplementary Table 4.

Figure 7. NR showing maximal expression modifications in acute or chronic cholestasis in different liver cell types

Relative NR expression is shown in acute cholestasis (bile duct ligation, BDL) or chronic cholestasis (*Abcb4*$^{-/-}$) and is expressed as fold-changes compared to Sham or *Abcb4*$^{+/+}$ mice, respectively. The ten most up-regulated and down-regulated genes were selected irrespective of the model and cell type. Mean values obtained from triplicate sample analyses ± SD are shown. The dashed line represents expression level set up at one in controls. (A) Vdr expression was exclusively up-regulated in non-parenchymal cells in chronic cholestasis whereas Nurr1 up-regulation was restricted to KC in acute cholestasis. (B) Changes in Erα, Rxrγ, Rev-Erbα and Coup-TFI mRNA levels were exclusively down-regulations that were detected in at least one cell type in any of the cholestasis models. (C) Car, Pxr, Fxrα, Hnf4α, Rorγ, Trβ, Ppparα, Nor-1, Ngf1b, Errγ, Ar and Fxrβ showed both up and down-regulations in mRNA levels in cholestasic settings. N.A., not applicable for expressions that were absent. Chol, cholangiocytes; Hep, hepatocytes; HSC, hepatic stellate cells; KC, Kupffer cells; SEC, sinusoidal endothelial cells.

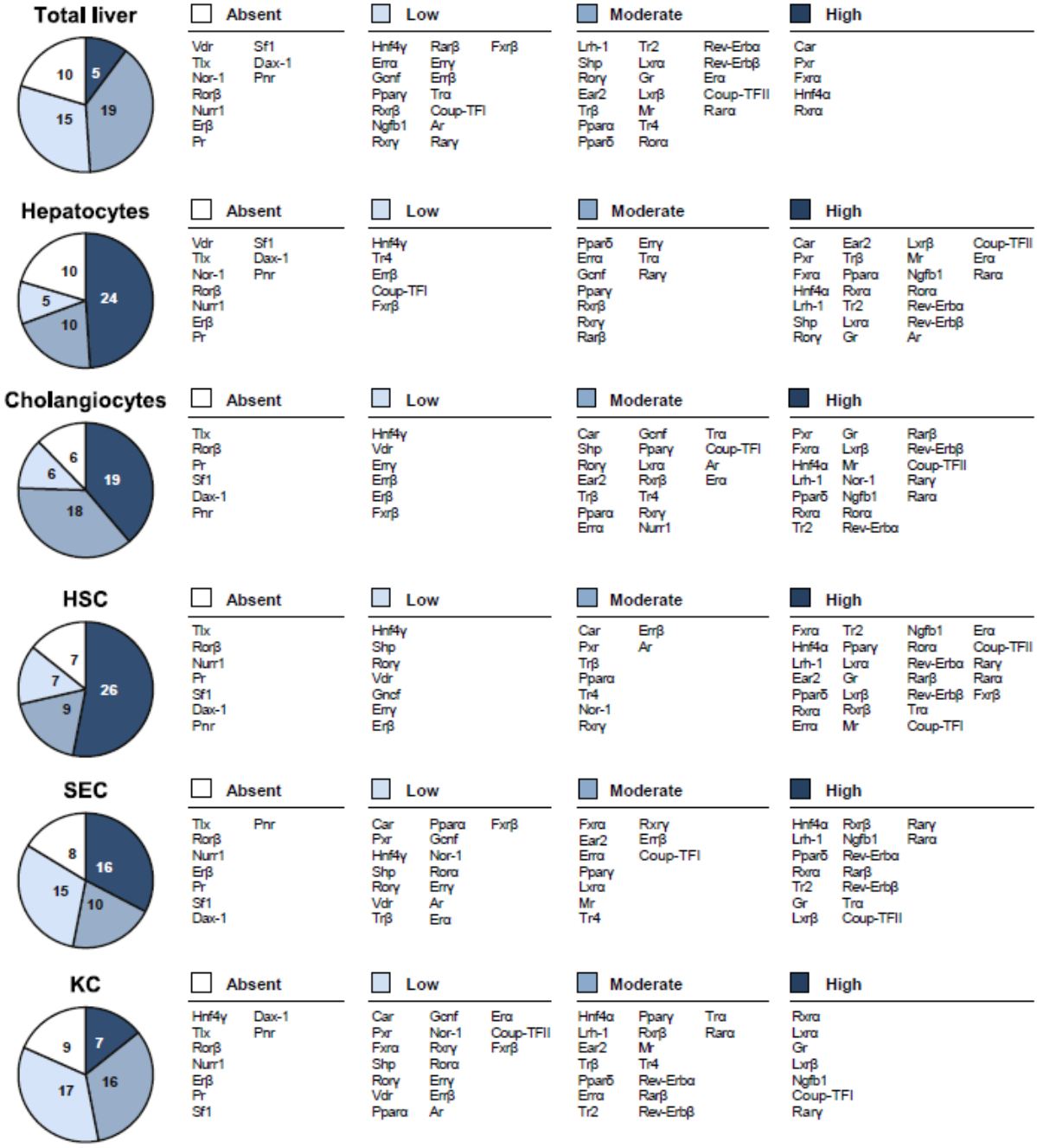

Figure 1

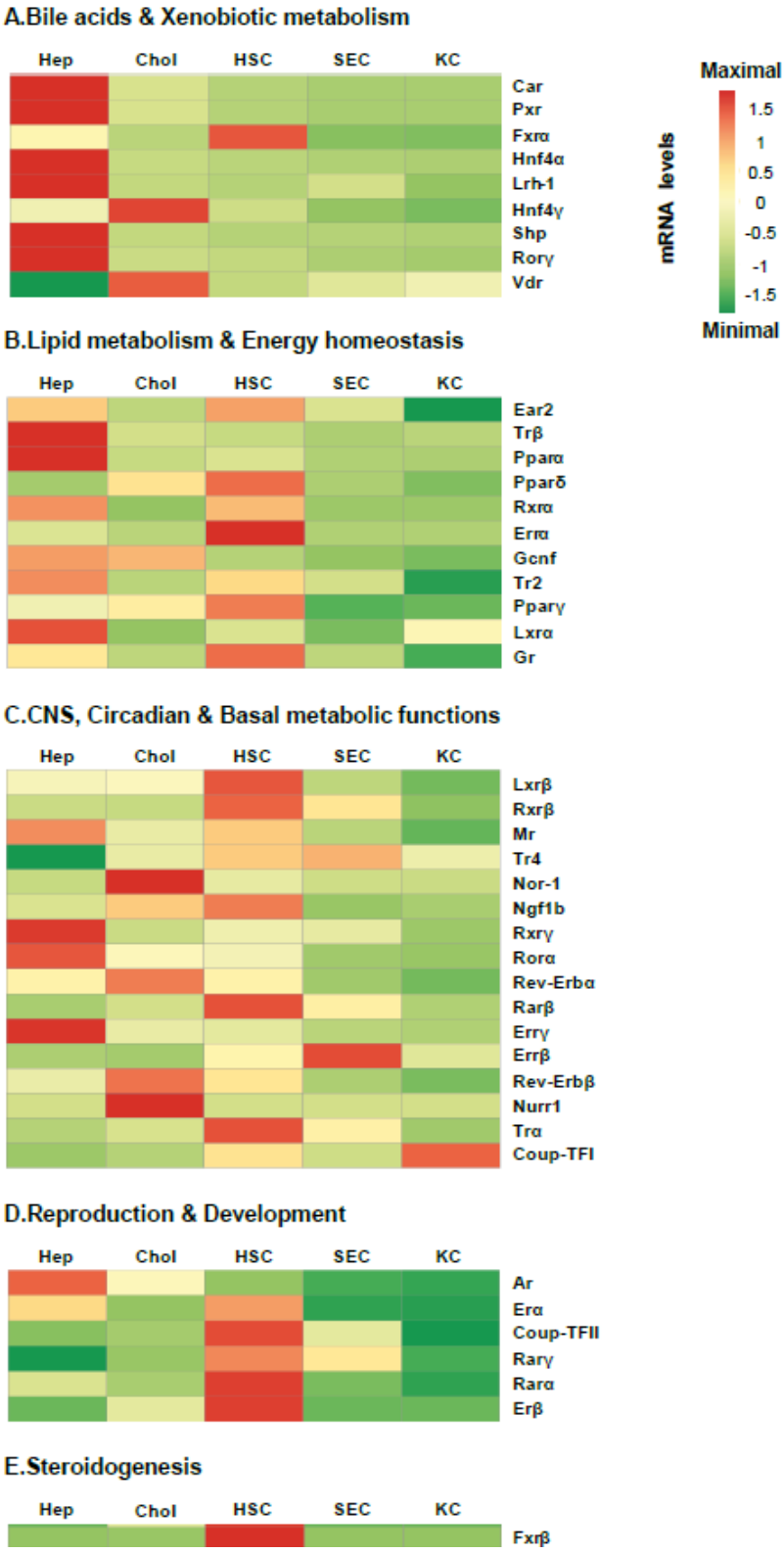

Figure 2

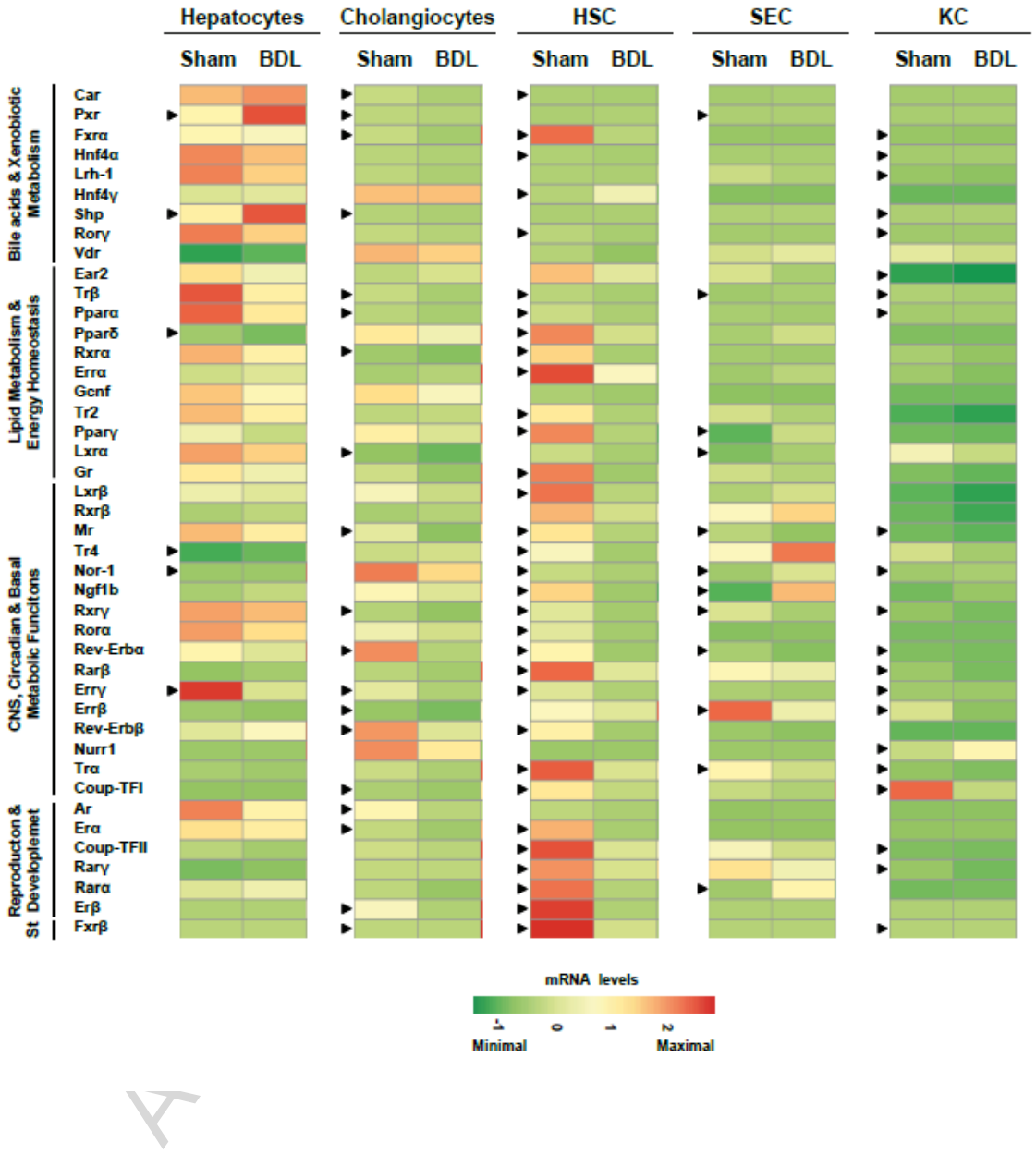

Figure 3

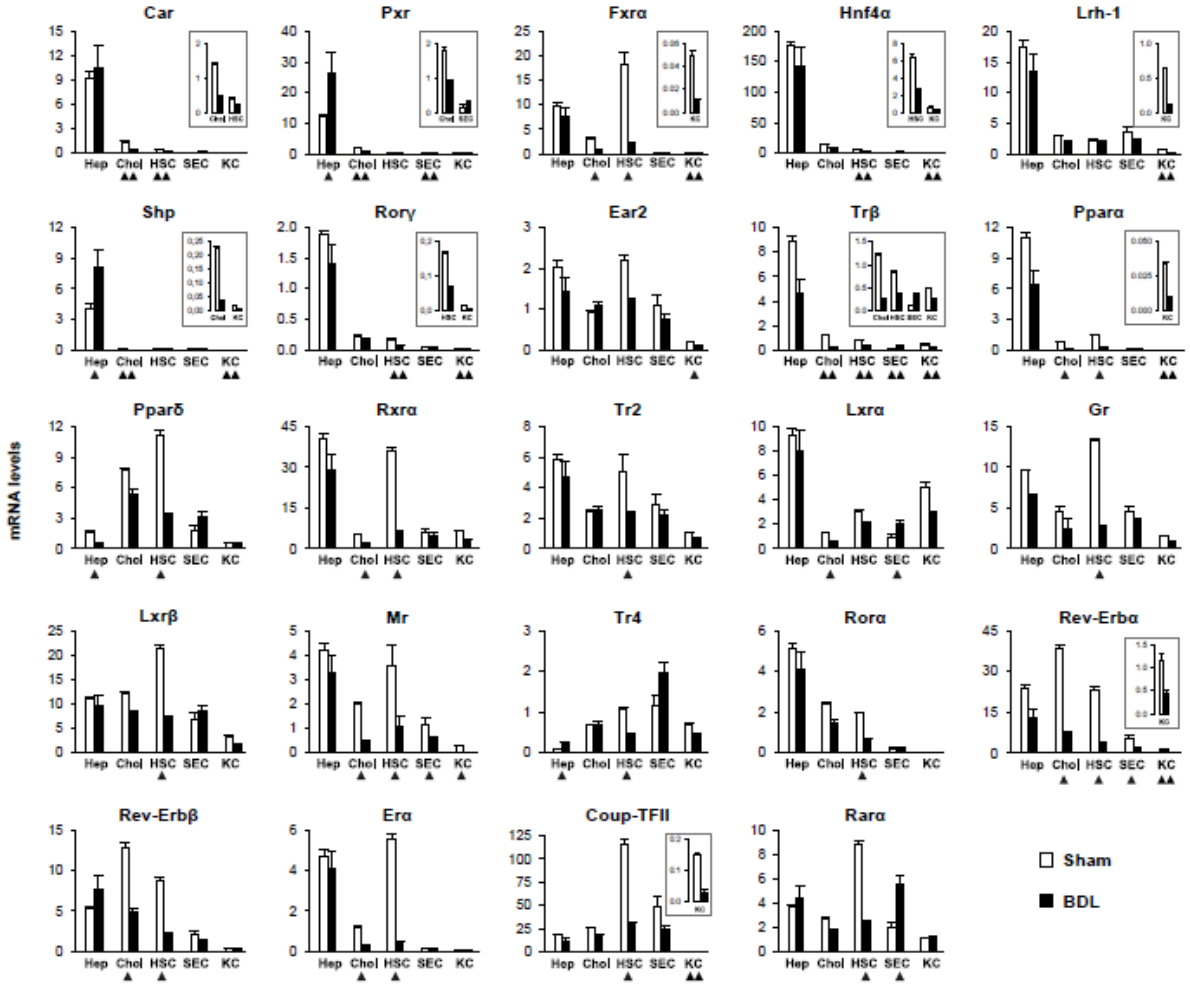

Figure 4

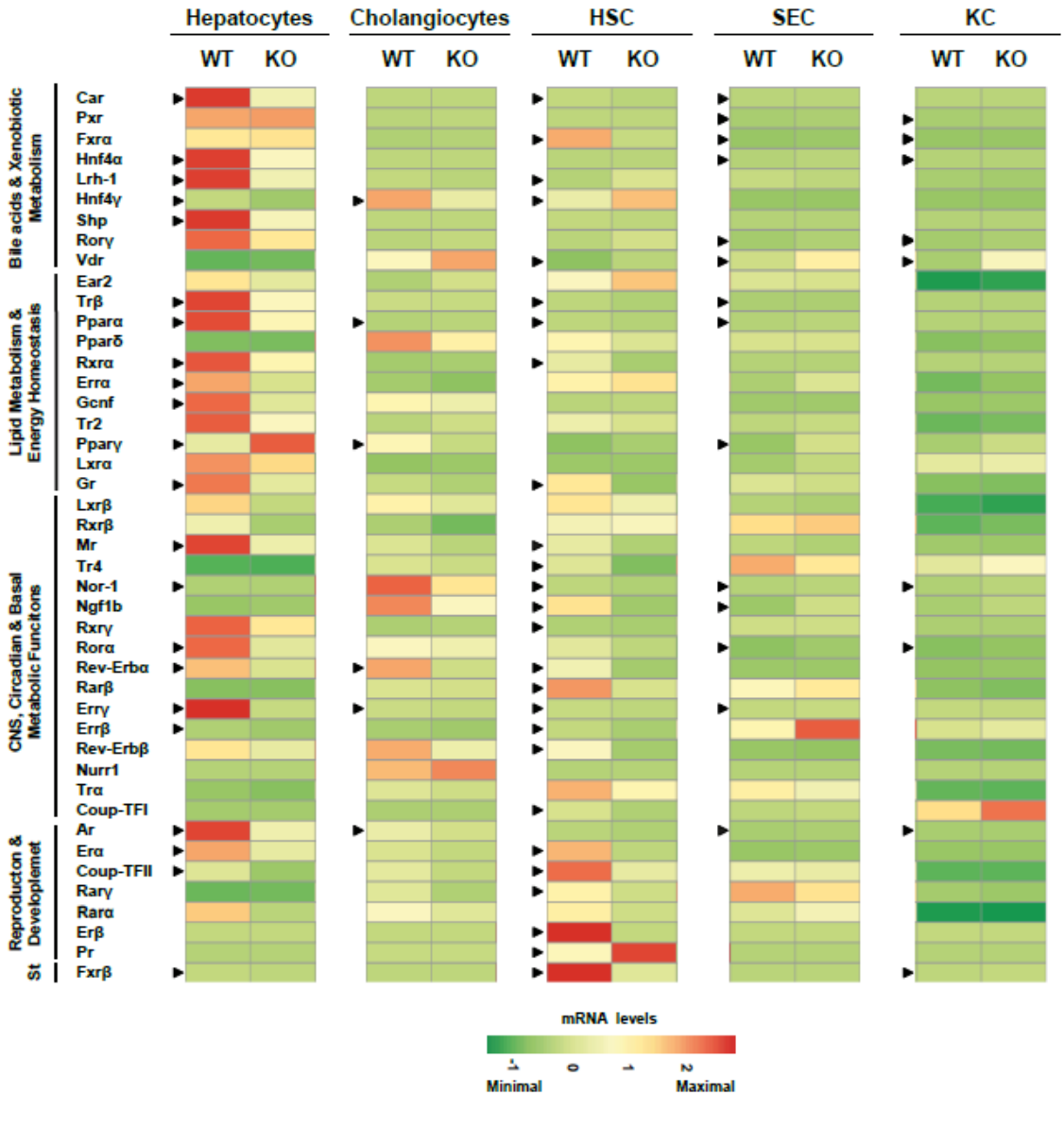

Figure 5

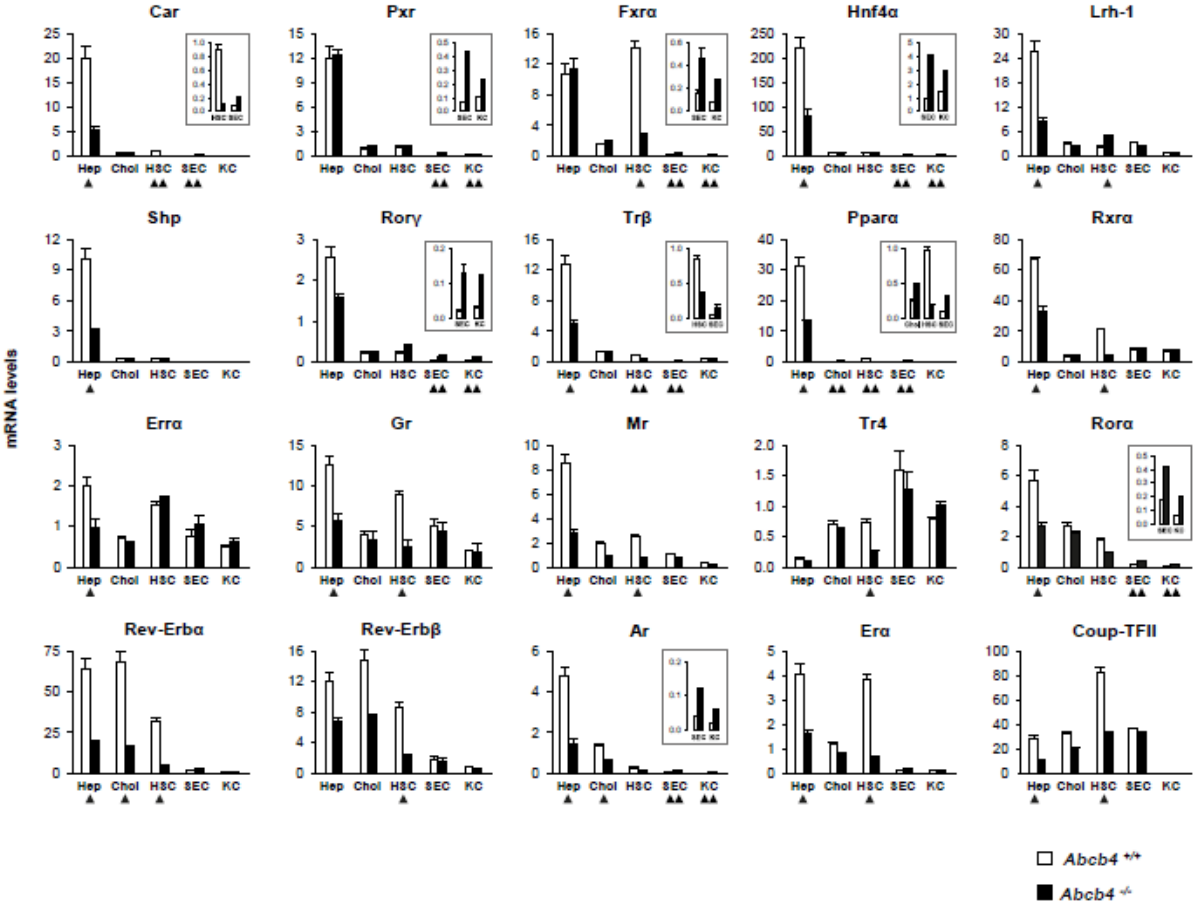

Figure 6

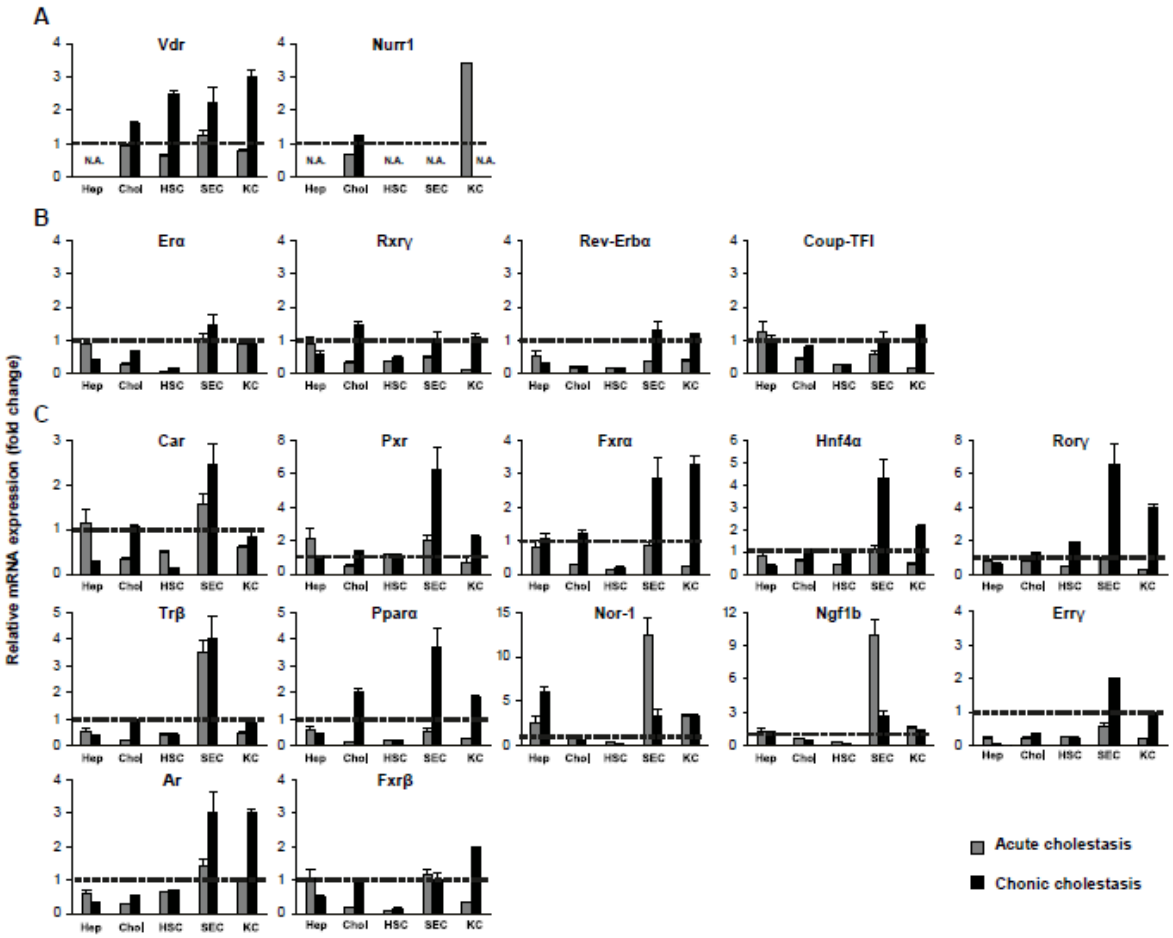

Figure 7

Highlights

- Non-parenchymal cells are major sites of nuclear receptor expression in the liver
- Modulation of nuclear receptor expression in non-parenchymal liver cells is a key event in response to cholestasis
- Non-parenchymal liver cells are important targets to consider in nuclear receptor-directed therapies