**Highlights**

- FXR activation induces expression of many transcriptional repressors including *MafG*

- MAFG represses bile acid synthetic genes and alters bile acid composition

- Bile acid synthesis and metabolism genes have MAFG response elements (MAREs)

- MAFG is an important regulator of bile acid negative feedback regulation

**In Brief**

de Aguiar Vallim et al. identify MAFG as an FXR target gene that functions as a transcriptional repressor of bile acid synthetic genes, thus altering the composition of the bile acid pool. These studies identify a molecular mechanism for the negative feedback regulation of bile acid synthesis.
MAFG Is a Transcriptional Repressor of Bile Acid Synthesis and Metabolism

Thomas Q. de Aguiar Vallim,1,* Elizabeth J. Tarling,1 Hannah Ahn,1 Lee R. Hagey,2 Casey E. Romanoski,3 Richard G. Lee,4 Mark J. Graham,4 Hozumi Motohashi,5 Masayuki Yamamoto,6 and Peter A. Edwards1,7,*

1Division of Cardiology, David Geffen School of Medicine, University of California, Los Angeles, CA 90095, USA
2Department of Medicine, University of California, San Diego, La Jolla, CA 92039, USA
3Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA 92039, USA
4ISIS Pharmaceuticals, Carlsbad, CA 92010, USA
5Department of Gene Expression Regulation, Institute of Development, Aging and Cancer, Tohoku Medical Megabank Organization, Sendai 980-8573, Japan
6Department of Medical Biochemistry, Tohoku Medical Megabank Organization, Sendai 980-8573, Japan
7Department of Biological Chemistry, University of California, Los Angeles, CA 90095, USA

*Correspondence: tvallim@mednet.ucla.edu (T.Q.d.A.V.), pedwards@mednet.ucla.edu (P.A.E.)

http://dx.doi.org/10.1016/j.cmet.2015.01.007

SUMMARY

Specific bile acids are potent signaling molecules that modulate metabolic pathways affecting lipid, glucose and bile acid homeostasis, and the microbiota. Bile acids are synthesized from cholesterol in the liver, and the key enzymes involved in bile acid synthesis (Cyp7a1, Cyp8b1) are regulated transcriptionally by the nuclear receptor FXR. We have identified an FXR-regulated pathway upstream of a transcriptional repressor that controls multiple bile acid metabolism genes. We identify MafG as an FXR target gene and show that hepatic MAFG overexpression represses genes of the bile acid synthetic pathway and modifies the biliary bile acid composition. In contrast, loss-of-function studies using MafG−/− mice cause de-repression of the same genes with concordant changes in biliary bile acid levels. Finally, we identify functional MafG response elements in bile acid metabolism genes using ChIP-seq analysis. Our studies identify a molecular mechanism for the complex feedback regulation of bile acid synthesis controlled by FXR.

INTRODUCTION

Bile acids function both as detergents that facilitate lipid absorption and as endogenous ligands that regulate metabolic pathways through activation of several nuclear receptors, including the farnesoid X receptor (FXR, Nr1h4) as well as TGR5, a G protein-coupled receptor (de Aguiar Vallim et al., 2013a). Although FXR plays a particularly important role in maintaining bile acid homeostasis, numerous studies have shown that FXR directly regulates many genes that affect multiple metabolic cascades (de Aguiar Vallim et al., 2013a; Evans and Mangelsdorf, 2014). Consistent with these findings, mice lacking FXR exhibit not only dysregulated bile acid metabolism, but also abnormal lipoprotein (Sinal et al., 2000) and glucose metabolism (Duran-Sandoval et al., 2005; Zhang et al., 2006), increased hepatic susceptibility to certain toxins (Lee et al., 2010), increased levels of ileal bacteria, and impaired barrier function of intestinal epithelia (Inagaki et al., 2006). Reduced FXR signaling is also associated with obesity, possibly as a result of bile acid-dependent modulation of the microbiota (Li et al., 2013; Ridauro et al., 2013). Moreover, a recent study demonstrated that in mice, the benefits of bariatric surgery may be mediated by FXR signaling to modulate bile acid-dependent effects on the microbiota (Ryan et al., 2014). Thus, the findings that elevated bile acid levels in humans and/or mice are associated with gastrointestinal diseases, hepatotoxicity, altered plasma lipoprotein levels, and aberrant glucose metabolism suggest that abnormal control of the bile acid pool can have broad physiological effects (de Aguiar Vallim et al., 2013a; Kuipers et al., 2014).

Although negative feedback of bile acid synthesis was first described over 50 years ago (Beher et al., 1961), the precise mechanisms by which bile acids mediate this repression are still not fully understood. The enzymatic catabolism of cholesterol, or hydroxysterols, to form primary bile acids occurs via either the classic or alternative pathways (Figure 1A). These two pathways generate approximately 75% and 25%, respectively, of the total primary bile acids and involve at least 17 enzymes (Russell, 2003) (Figure 1A). Within the classic pathway, CYP7A1 is the rate-limiting enzyme, while CYP8B1 regulates the synthesis of cholic acid (Li-Hawkins et al., 2002) and thus regulates the bile acid pool composition (Figure 1A). The transcription of both Cyp7a1 and Cyp8b1 are particularly responsive to end product feedback control (Russell, 2003). In contrast, the alternative bile acid pathway involves CYP7B1 and CYP27A1 (Figure 1A) (Russell, 2003). Little is known about the regulation of the genes that encode enzymes of the alternative pathway, or downstream of CYP7A1 and CYP8B1. Nonetheless, the finding that a number of diseases result from mutations of CYP7A1, CYP7B1, CYP27A1, HSD3B7, AMACR, or AKR1C4 (Ak1c14 in mice) (Figure 1A) emphasizes the importance of maintaining normal bile acid synthesis and homeostasis.

In humans and mice, one of the most abundant bile acids is cholic acid (CA). Humans also have high levels of chenodeoxycholic acid (CDCA). In contrast, mice almost quantitatively...
convert CDCA to muricholic acid (MCA) (Figure 1A) (de Aguiar Vallim et al., 2013a; Russell, 2003). The negative feedback regulation of bile acid synthesis is largely dependent on activation of hepatic and/or intestinal FXR (Kim et al., 2007). Such activation results in the induction of small heterodimerizing partner (SHP, Nr0b2) in the liver (Kerr et al., 2002; Wang et al., 2002) and Fgf15 (mouse) or FGF19 (human) in the small intestine (Inagaki et al., 2005, 2006). SHP does not bind DNA directly, but rather binds to other transcription factors such as HNF4α and LRH-1 to impair their function (Båvner et al., 2005; Goodwin et al., 2000; Lu et al., 2000). In contrast, intestinally derived FGF15/19 is secreted into the blood before binding to the FGFR4/Klotho receptor on the surface of hepatocytes to initiate an incompletely understood pathway that leads to repression of Cyp7a1 (Inagaki et al., 2005). In contrast to the detailed studies detailing the mechanisms that control Cyp7a1, the mechanisms involved in the repression of Cyp8b1, and thus cholic acid synthesis, are less well understood. Nonetheless, FXR activation is known to repress Cyp8b1 expression by mechanisms that may also involve SHP and FGF15/19 (Kerr et al., 2002; Kong et al., 2012; Wang et al., 2002).

Here, we identify a previously unrecognized FXR-regulated pathway involving MAFG (V-Maf Avian Musculoaponeurotic Fibrosarcoma Oncogene Homolog G), a member of the MAF family of transcription factors. We show that MafG is a direct target gene of FXR, and hepatic overexpression of MAFG in mice represses genes encoding enzymes of the classic and alternative pathways. In addition, overexpression of MAFG in mice resulted in a decrease in biliary cholic acid levels and an increase in muricholic acid levels, a finding consistent with the greater inhibition of Cyp8b1 as compared to Cyp7a1. Finally, we utilize loss-of-function approaches (knockdown of MafG with antisense oligonucleotides, MafG+/− mice) to show that a 50%–80% loss of hepatic MAFG protein results in both derepression of many of the same genes, including Cyp8b1, and an increase in biliary cholic acid levels. In conclusion, our results identify an FXR-MafG pathway that functions in the feedback repression of bile acid metabolism by modulating the composition of the bile acid pool.

Figure 1. FXR Activation Represses Most Bile Acid Synthesis Genes
(A) Schematic diagram of the major hepatic enzymes involved in bile acid synthesis. Genes in red are repressed, while genes in yellow are unchanged, and the gene in green is induced following FXR activation.

(B–D) Hepatic expression of genes encoding enzymes of the (B) classic or (C) alternative bile acid synthetic pathway or (D) remaining genes involved in primary bile acid synthesis. mRNA levels were measured by qRT-PCR following treatment of wild-type or Fxr−/− mice (7–9 mice/group) for 3 days with GW4064 or GSK2324 at 60 mpk/day. All data are shown as mean ± SEM. Asterisks indicate statistically significant differences comparing WT or KO vehicle-treated against agonist-treated mice (*p < 0.05; **p < 0.01; ***p < 0.001).
RESULTS

FXR Activation Represses Both the Classic and Alternative Bile Acid Synthetic Pathways

Multiple studies have shown that activated FXR leads to repressed transcription of both Cyp7a1 and Cyp8b1 (Kerr et al., 2002; Kong et al., 2012; Wang et al., 2002). While the mechanisms regulating Cyp7a1 expression have been extensively studied, much less is known about how FXR represses Cyp8b1 or whether FXR regulates the expression of other genes involved in the two bile acid synthetic pathways. In our initial studies, we treated wild-type and Fxr−/− mice (KO) for 3 days with either GW4064, a widely used FXR agonist (Maloney et al., 2000), or GSK2324, a water-soluble derivative of GW4064 that exhibits increased potency (Bass et al., 2011). As expected, both agonists led to robust repression of both Cyp7a1 and Cyp8b1 in wild-type, but not Fxr−/− mice (Figure 1B). Importantly, both agonists also resulted in an FXR-dependent repression of Cyp7b1 and Cyp27a1 in the alternative pathway (Figure 1C). In addition, we measured the hepatic mRNA levels of a number of additional genes that encoded enzymes involved in bile acid synthesis. We now show for the first time that FXR activation in wild-type, but not Fxr−/− mice, results in repression of numerous bile acid synthetic genes, including Accox2, Akrlc14, Hsd3b7, Hsd17b4, Smp2, and Slic2a5 (Figure 1D). In contrast, Amacr and Cyp39a1 mRNA levels are unchanged (Figure 1D), while Akrlc14 is modestly induced after FXR activation, consistent with ChIP-seq data that identify a putative intrinsic FXRE in the Akrlc14 locus (Figure S1A). Overall, the repression of most bile acid synthetic genes was not as pronounced as that observed for Cyp8b1 and Cyp7a1 (Figure 1B). Nevertheless, these studies demonstrate that treatment of mice with two different synthetic FXR agonists results in repression of genes involved in both the classic and alternative bile acid synthetic pathways, consistent with a central role for FXR in regulating all aspects of bile acid synthesis.

FXR Activation Induces the Expression of Several Transcriptional Repressors

The nuclear receptor FXR binds to its cognate response element (FXRE) as an FXR:FXR heterodimer and functions almost exclusively as a transcriptional activator (de Aguilar Vallim et al., 2013a). Nonetheless, although activation of FXR leads to induction of many hepatic genes, it also results in repression of numerous genes involved in bile acid metabolism (Figure 1D). A number of studies have demonstrated that the mechanisms involved in the repression of Cyp7a1 and Cyp8b1 are indirect and are the result of the FXR-dependent increased expression of Shp and Fgf15/19 that encode proteins that function to inhibit transcription of specific genes (de Aguilar Vallim et al., 2013a; Kuipers et al., 2014). One additional mechanism by which FXR causes a reduction in specific genes is through miRNAs. For example, we recently identified miR-144 as an FXR-regulated miRNA that subsequently targets ABCA1 (de Aguilar Vallim et al., 2013b). Based on these earlier studies, we hypothesized that FXR activation might increase the expression of additional repressors that function to control metabolic pathways.

To identify such putative transcriptional repressors, we reanalyzed the data from our prior ChIP-seq study that had been used to identify global hepatic FXR response elements (FXREs) (Chong et al., 2010). Gene ontology analysis identified a significant enrichment in transcription factors containing FXREs (Chong et al., 2010). Consequently, we searched this subset of FXRE-containing genes, focusing specifically on genes annotated to have transcriptional repressor activity. Our analysis identified four putative transcriptional repressor genes, namely Shp, a well-characterized FXR target gene, v-raf murine sarcoma viral oncogene homolog (v-Raf), and zinc finger protein 385a (Zfp385a). We also identified a fifth putative transcriptional repressor, oligodendrocyte transcription factor 1 (Olig1), which we show can be regulated by FXR agonists (Figure S1B) and contains an FXRE in its genomic loci. However, since the hepatic expression of Olig1 is very low (data not shown), we have not studied this gene further.

To confirm the presence of FXREs at the loci of these putative transcriptional repressors, we analyzed a second independent FXR ChIP-seq dataset from mouse livers (Thomas et al., 2010). This analysis verified that Crip2 (Figure 2A), MafG (Figure 2B), Zfp385a (Figure 2C), and Shp (Figure S1C) contained one or more FXREs at their genomic loci. To investigate whether these genes were regulated in response to FXR and FXR agonists, we measured the hepatic expression of Crip2, MafG, and Zfp385a in wild-type and Fxr−/− mice (KO) pre-treated for 3 days with either GW4064 or GSK2324. We utilized a dose of 60 mpk/day to directly compare the effects of the two agonists. In all experiments, Shp and/or Bsep, both well-characterized FXR-target genes (Ananthanarayanan et al., 2001; Goodwin et al., 2000), served as positive controls. Hepatic MafG, Crip2, and Zfp385a, and Shp mRNA levels were all significantly induced following treatment of wild-type mice, but not Fxr−/− mice (KO), with either FXR agonist (Figure 2D), demonstrating that induction was FXR dependent. Further, induction of each gene was greater after treatment with GSK2324 as compared to GW4064 (Figure 2D), consistent with increased potency of GSK2324. In the case of MafG, the levels of protein were induced 2- to 3-fold following treatment of mice with either GW4064 or GSK2324, and this effect was specific, as it was not observed in Fxr−/− mice treated with either agonist (Figure 2E).

In order to determine whether cholic acid, an endogenous ligand that activates a number of receptors, including FXR (Makishima et al., 1999; Parks et al., 1999), induces these same genes, we fed wild-type and Fxr−/− mice (KO) a diet containing non-toxic levels of cholic acid (0.2%) for 7 days. The hepatic expression of MafG and Zfp385a (Figure 2F), as well as the positive controls Bsep (Figure 2F) and Shp (Figure S1D), were modestly induced when wild-type, but not Fxr−/− mice, were fed the cholic acid-enriched diet. In contrast, the increase in Crip2 mRNA levels did not reach statistical significance (Figure 2F). Taken together, these results demonstrate that MafG, Crip2, and Zfp385a, which encode putative transcriptional repressors, are induced by specific FXR agonists (GSK2324 and GW4064), while MafG and Zfp385a are also induced by cholic acid, an endogenous FXR ligand.

To determine the optimal dose of GSK2324, we treated wild-type mice for 3 days with vehicle or 10, 30, or 100 mpk GSK2324. Induction of the classic FXR target genes Shp and Bsep was dependent upon the dose of GSK2324 with
Figure 2. Identification of Transcriptional Repressors as Direct FXR Target Genes
(A–C) ChIP-seq analysis of hepatic FXR from Chong et al. (2010) (top) and Thomas et al. (2010) (bottom) at (A) Crip2, (B) MafG, and (C) Zfp385a genomic loci.
(D) Hepatic expression of Shp, MafG, Crip2, and Zfp385a in C57BL/6 wild-type (WT) and Fxr−/− (KO) mice treated with vehicle, GW4064, or GSK2324 for 3 days (n = 7–9 mice/group).
(E) Western blotting analysis and quantification of MAFG protein in livers of C57BL/6 wild-type and Fxr−/− mice treated with vehicle or GW4064 (top), or GSK2324 (bottom) for 3 days.
(F) Hepatic expression of Bsep, Crip2, MafG, and Zfp385a in C57BL/6 wild-type and Fxr−/− mice fed either a control (Ctrl) or 0.2% cholic acid (CA) diet for 7 days.
(G) Hepatic expression of Crip2, MafG, and Zfp385a in C57BL/6 wild-type mice treated with vehicle (Ctrl) or 10, 30, or 100 mpk/day of GSK2324 for 3 days (n = 4–8 mice/group).
(H) Hepatic expression of Crip2, MafG, and Zfp385a following treatment of wild-type mice with a single injection of GSK2324 (30 mpk) 1, 2, or 4 hr before sacrifice (n = 6 mice/group).
(I) Hepatic expression of Crip2, MafG, and Zfp385a in littermate C57BL/6 wild-type (Flox) or liver-specific Fxr−/− mice (L-KO) treated with GSK2324 for 3 days (n = 7–9 mice/group). All data are shown as mean ± SEM. Asterisks indicate statistically significant differences comparing WT or KO vehicle-treated against agonist-treated mice (**p < 0.01; ***p < 0.001).
near-maximal effects at 30 mpk (Figure S2A). Induction of Crip2, MafG, and Zfp385a was also dependent upon the dose of GSK2324 (Figure 2G). Further, a single dose of GSK2324 at 30 mpk resulted in a significant induction of Crip2, MafG, and Zfp385a (Figure 2H) and Shp and Bsep (Figure S2B) mRNA levels within 1 hr. To investigate whether induction of Crip2, MafG, and Zfp385a in response to GSK2324 is dependent upon hepatic FXR expression, we treated wild-type (Fxr<sup>lox/lox</sup>) mice or littermates lacking hepatic Fxr (Fxr<sup>-/-</sup>; L-KO) for 3 days with the agonist at 30 mpk. While GSK2324 treatment of Fxr<sup>lox/lox</sup> mice led to increased hepatic expression of Crip2, MafG, Zfp385a, and Shp, induction of these genes was not observed following GSK2324 treatment of Fxr<sup>-/-</sup> mice (Figure 2I; Figure S2C). Taken together, our results demonstrate that Crip2, MafG, and Zfp385a are acutely and robustly induced following activation of hepatic FXR.

**MafG Overexpression In Vivo Decreases Cyp8b1 and Biliary Cholic Acid Levels**

To investigate the potential repressive effects of MafG, Crip2, and Zfp385a on gene expression, we generated, and then injected, adenoviral vectors to acutely overexpress these three proteins in the livers of wild-type mice. We then measured the hepatic mRNAs of the two major bile acid synthetic enzymes, as these are robustly repressed following activation of FXR (Figure 1B). Ad-MafG, but not Ad-Crip2 or Ad-Zfp385a treatment, resulted in a decrease in the hepatic levels of Cyp8b1 (Figure 3A). In contrast, the expression of Cyp7a1 was unaffected by any of these treatments (Figure 3A).

The MafG-dependent repression of Cyp8b1 suggests that MAFG may regulate the synthesis of cholic acid and alter the bile acid pool composition. To determine whether MAFG overexpression could indeed change the biliary pool composition, we treated a new cohort of mice with either Ad-control or Ad-MafG adenovirus. Changes in the composition of the bile acid pool are relatively slow under normal conditions, since only 5% of the bile acids are excreted each day during multiple enterohepatic cycles (de Aguiar Vallim et al., 2013a; Hofmann and Hagey, 2008). Consequently, we fed mice either control diet (chow) or the same diet supplemented with a bile acid sequestrant-containing diet increased basal expression of hepatic FXR.

Numerous Genes Involved in Bile Acid Metabolism

Numerous genes involved in bile acid metabolism, in addition to Cyp8b1, are preferentially transcribed from exon 1b (Figure 3I). Thus, the putative FXRE identified by ChIP-seq analysis (Figure 2B) would reside in the hepatic MafG proximal promoter that lies upstream of exon 1b (Figure 3I). Consequently, we generated a luciferase reporter gene controlled by the hepatic MafG promoter (upstream of exon 1b). Treatment of cells with GSK2324, following co-transfection of plasmids expressing FXR and the reporter gene, led to a robust increase in luciferase activity (Figure 3J).

To determine if MAFG overexpression regulated additional genes of bile acid metabolism, in addition to Cyp8b1, we carried out gene expression profiling of livers of mice treated with either quantitative, as total bile acid levels in the liver, intestine, or gall bladder (Figures S2E–S2G) were similar in mice treated with Ad-control, Ad-MafG, Ad-Crip2, or Ad-Zfp385a. Thus, these two studies demonstrate that the MafG-dependent repression of Cyp8b1 is sufficient to decrease cholic acid and increase muricholic acid levels, even after acute (7 days) and modest increases (2- to 3-fold) in MAFG protein.

To determine whether the pathway described here is conserved in human cells, we treated the human hepatoma cell line HepG2 with increasing concentrations of CDCA, a natural FXR agonist (Makishima et al., 1999; Parks et al., 1999). Consistent with our observations in mouse liver, CDCA treatment increased the expression of both MAFG (Figure 3F) and SHP (Figure S2H). As expected, CDCA treatment also decreased CYP7A1 and CYP8B1 expression in a dose-dependent manner (Figure 3G). Finally, Ad-MafG, but not Ad-Crip2 or Ad-Zfp385a, treatment of HepG2 cells decreased CYP8B1 expression without affecting CYP7A1 (Figure 3H). Thus, we have identified a pathway whereby MAFG represses CYP8B1 expression both in mice and in human cells.

To further characterize the FXR-dependent regulation of MafG, we carried out more in-depth analysis. The Mafg gene is reported to contain two transcriptional start sites that correspond to exon 1a or exon 1b (Katsuoka et al., 2005a) (Figure 3I). However, our analysis of hepatic RNA-seq data from Menet et al. (2012) (Figure 3I, upper panel) together with the data of Katsuoka et al. (2005a) suggest that in the liver, Mafg is preferentially transcribed from exon 1b (Figure 3I). Thus, the putative FXRE identified by ChIP-seq analysis (Figure 2B) would reside in the hepatic MafG proximal promoter that lies upstream of exon 1b (Figure 3I). Consequently, we generated a luciferase reporter gene controlled by the hepatic MafG promoter (upstream of exon 1b). Treatment of cells with GSK2324, following co-transfection of plasmids expressing FXR and the reporter gene, led to a robust increase in luciferase activity (Figure 3J). As observed, the FXR- and GSK2324-dependent increase in luciferase activity was abolished when the FXRE, which corresponds to an inverted repeat 1 (IR-1), was mutated in the Mafg promoter construct (Figure 3J). Taken together, these data demonstrate that Mafg is a bona fide FXR target gene that contains a functional FXRE in its hepatic proximal promoter.

**MAFG Overexpression in Mouse Liver Represses Numerous Genes Involved in Bile Acid Metabolism**

MAFG is a member of the small MAF family of transcription factors, composed of MAFG, MAFF, and MAFK, that lack an activation domain and therefore are considered transcriptional repressors (Motohashi et al., 2002). Small MAF proteins can bind to DNA as either homo- or hetero-dimers and function as transcriptional repressors. Alternatively, they can heterodimerize with transcriptional activators to induce gene expression (Motohashi et al., 2002). Consistent with their role as transcription factors, we show that epitope-tagged MAFG localizes to the nucleus (Figure S3A). Mafg is expressed in several metabolic tissues, including the liver (Figure S3B), the major site for bile acid synthesis.

To determine if MAFG overexpression regulated additional genes of bile acid metabolism, in addition to Cyp8b1, we carried out gene expression profiling of livers of mice treated with either
Figure 3. MafG Overexpression Represses Cyp8b1 mRNA and Reduces Biliary Cholic Acid Levels

(A) Hepatic expression of Cyp7a1 and Cyp8b1 following treatment of C57BL/6 mice with Ad-control (Ad-Ctr), Ad-Crip2, Ad-MafG, or Ad-Zfp385a adenoviruses for 5 days (n = 7–8 mice/group).

(B–E) Hepatic levels of (B) MafG mRNA, (C) MAFG protein, and (D) Cyp8b1 mRNA and (E) taurine-conjugated biliary bile acid levels in C57BL/6 wild-type treated with Ad-control or Ad-MafG adenovirus for 7 days and fed either a control or Colesevelam-containing diet (Colesev) for 7 days prior to and 7 days post-adenovirus treatment (n = 8–9 mice/group).

(F and G) Expression levels of (F) MAFG or (G) CYP7A1 and CYP8B1 in human HepG2 cells treated with 100, 150, or 200 μM chenodeoxycholic acid (CDCA) for 24 hr (n = 4 wells/condition).

(H) Expression levels of CYP7A1 and CYP8B1 in HepG2 cells infected with Ad-Control, -Crip2, -MafG, or -Zfp385a for 36 hr (n = 3–4 wells/condition).

(i) RNA-seq (Menet et al., 2012) (top) and FXR ChIP-seq (Chong et al., 2010) (bottom) analysis of the MafG genomic loci showing locations of MafG exons and FXRE in the putative MafG proximal promoter.

(J) Wild-type and FXRE mutant (mutated bases are bolded and underlined) MafG promoter (MafG prom) constructs upstream of a luciferase reporter gene were transfected into HepG2 cells with increasing amounts of a FXR expression plasmid and treated with vehicle or GSK2324 for 24 hr. Luciferase activity was normalized to β-galactosidase and expressed as fold change. All data are shown as mean ± SEM. Asterisks indicate statistically significant differences versus controls (**p < 0.01; ***p < 0.001). Different letters (a–d) indicate statistically significant differences (p < 0.05).
control or MafG adenovirus (Figure 4A). MAFG overexpression repressed 554 genes and activated 833 genes. Induction of genes in response to MAFG overexpression is not unexpected, as MAFG can heterodimerize with transcriptional activators, most notably NRF2 and NRF3, to activate specific genes (Kat-suoka et al., 2005a, 2005b). Gene ontology analysis (Huang et al., 2009) of the hepatic genes that are repressed following MAFG overexpression revealed a significant enrichment in primary bile acid synthesis genes (Figure 4B) that included Cyp8b1 and Cyp7b1, as well as the bile acid importer, sodium taurocholate co-transporting polypeptide (Ntcp, gene symbol Slc10a1). Interestingly, these same genes are also repressed in mouse liver following treatment with FXR agonists (Figures 1B–1D), suggesting that the repression of these genes by FXR agonists is indirect and likely dependent upon induction of MAFG mRNA and protein levels.

We also determined the expression of the remaining bile acid synthesis genes in Ad-control and Ad-MafG-treated mice that had been fed either normal chow or a diet supplemented with 0.25% Colesevelam. Ad-MafG treatment resulted in repression of almost all bile acid synthesis genes, including Cyp7b1 and Cyp27a1, independent of the diet (Figure 4C). Interestingly, comparison of the data shown in Figures 4C and 1D demonstrates that the bile acid synthetic genes that are repressed following FXR activation are also repressed following overexpression of MAFG. The repression of bile acid synthesis genes is not universal, since Amacr and Cyp39a1 mRNA levels were unchanged following treatment of mice with FXR agonists or following hepatic overexpression of MAFG (Figures 1D and 4C). Together, these data suggest that MAFG is an important transcriptional regulator of bile acid synthesis and may play an important role in mediating the FXR-dependent repression of genes involved in bile acid metabolism.

Loss of MAFG Causes De-Repression of Multiple Bile Acid Synthetic Genes and Increases Biliary Cholic Acid Levels

To further evaluate the role of MafG in regulating bile acid metabolism, we investigated the effect of loss of MafG. Short-term silencing of MafG in isolated mouse hepatocytes using three distinct shRNA constructs (Figure 5A) resulted in de-repression of Cyp8b1 (Figure 5B). Further, siRNA-mediated silencing of

---

**Figure 4. MAFG Regulates Several Genes Involved in Bile Acid Synthesis**

(A) Microarray analysis of livers from mice treated with either Ad-control or Ad-MafG for 7 days. Lines delineate fold-change cut-off (1.5-fold). Red and blue dots indicate genes that are repressed or induced genes, respectively (n = 3/condition).

(B) KEGG pathway analysis for categories that were significantly enriched from global analysis of repressed genes in (A).

(C) Hepatic expression of genes involved in bile acid synthesis or transport following treatment of C57BL/6 wild-type with Ad-control or Ad-MafG adenovirus for 7 days and fed either a control or Colesevelam-containing diet for 7 days prior to and 7 days post-adenovirus treatment (n = 6–9 mice/group). All data are shown as mean ± SEM. Asterisks indicate statistically significant differences versus controls on the same diet (**p < 0.01; ***p < 0.001).
MAFg mRNA and protein levels in human HepG2 cells (Figure 5C) also led to de-repression of CYP8B1 (Figure 5D). Together, these results suggest that MAFg is a critical negative regulator of CYP8B1 expression in both mice and humans. We then generated an antisense oligonucleotide (ASO) to silence MAFG in vivo. Acute treatment with the MAFG ASO significantly decreased the levels of MAFg mRNA (Figure 5E) and protein (Figure 5F), full blot in Figure S4A). Notably, Cyp8b1, but not Cyp7a1, was de-repressed in mice after MAFg silencing with the ASO treatment (Figure 5G), thus recapitulating our in vitro findings in an in vivo setting.

To determine whether complete loss of MAFg also affected bile acid homeostasis, we obtained MAFg+/− mice, which are reported to generate viable MAFg−/− mice on a mixed background (Shavit et al., 1998). We then backcrossed MAFg+/− mice onto a C57BL/6 background to be consistent with the genetic background of all other mice used in the current studies. Unexpectedly, we failed to recover any MAFg−/− mice on a C57BL/6 background (data not shown). We conclude that complete loss of MAFg on a C57BL/6 background is lethal, likely a result of pronounced neurological disorders previously reported in MAFg−/− mice. Partial loss of MAFg mRNA and protein led to a significant increase in the biliary levels of cholic acid and decreased muricholic acid levels in MAFg−/+ mice (Figure 5J), consistent with de-repression of Cyp8b1. MAFg+/− mice do not have significantly altered total bile acid levels in liver, intestine, or gall bladder (Figures S4B–S4D), suggesting that MAFG regulates the bile acid pool composition, but not the pool size. We quantified the expression of multiple genes encoding enzymes of the bile acid synthetic pathway in the livers of wild-type and MAFg−/− mice. Partial loss of MAFG caused de-repression of several genes, including Acox2, Akr1d1, Akr1c14, Hsd17b4, Ntcp, and Scp2 (Figure 5K). Collectively, these results support the hypothesis that hepatic MAFG functions as a repressor of Cyp8b1 and cholic acid synthesis as well as a regulator of bile acid metabolism in vivo.

Identification of MAFG Binding Sites at Multiple Genes Involved in Bile Acid Synthesis and Metabolism

To investigate the molecular mechanism for the MAFg-dependent repression of Cyp8b1 as well as additional target genes,
we generated an adenovirus construct to overexpress a biotin-ligase recognition peptide (BLRP)-tagged MafG. Consistent with our studies using untagged MAFG (Ad-MafG) (Figures 3A–3D), treatment of mice with Ad-BLRP-MafG resulted in increased hepatic MafG mRNA (Figure S5A) and protein (Figure 6A) and decreased Cyp8b1, Cyp7b1, and Cyp27a1 expression (Figure S5B), suggesting the BLRP epitope does not interfere with MAFG function. We then used ChIP analysis to identify MAFG bound to MAFG response elements (MAREs). First, as controls we show that in mouse liver, BLRP-tagged MAFG was enriched at MAREs that had been previously identified in Nqo and G6pdx in studies using cultured cells (Hirotsu et al., 2012) (Figure S5C). We then carried out ChIP-seq analysis from livers of mice treated with Ad-BLRP-MafG as well as Ad-BLRP (control), using the same anti-BLRP antibody. Global analysis of all peaks for MAFG revealed that 46% reside in intergenic regions, as compared to 41% in introns, while there was a modest enrichment in proximal promoters (Figure 6B). Motif enrichment analysis of sequences for the top 20,000 peaks (representing the largest number of reads per site) identified the consensus MARE (Figure 6C, top) which is highly similar to the MARE described previously for MAFG homodimers (Hirotsu et al., 2012). Analysis of all MAFG ChIP-seq peaks (n = 68,754) identified a MARE that contained a consensus sequence of GTCAGC (Figure 6C, bottom) but was otherwise different from that found in the top 20,000 peaks. Presumably, the latter
bile acid synthetic genes, including Acox2, Akr1d1, Akr1c14, Ntcp, Hsd17b4, and Scp2 (Figures S5D–S5I), but not in the 100 kb upstream of Cyp7a1 (Figure S5J). Together, these data demonstrate that MAFG directly regulates several bile acid metabolism genes.

Interestingly, liver-specific LRH-1-deficient mice also have decreased Cyp8b1, unchanged Cyp7a1, and altered bile acid composition (Lee et al., 2008; Mataki et al., 2007). We therefore investigated whether MAFG binding sites were associated with LRH-1 occupancy in the liver. Using ChIP-seq analysis for hepatic LRH-1 sites (Chong et al., 2012), we only identified a small number of genes that had both MAFG and LRH-1 binding sites (282 of 10,351; Figure S6A), and these genes were enriched in genes of negative regulation of metabolic processes, but not bile acid synthesis genes (Figure S6B). Taken together, these results suggest that MAFG is unlikely to repress transcription of multiple bile acid synthetic genes by displacing LRH-1.

In conclusion, our extensive studies identify a pathway involving the nuclear receptor FXR and the FXR-target gene MafG that functions to repress transcription of Cyp8b1 as well as multiple bile acid genes, including Acox2, Akr1d1, Akr1c14, Cyp7b1, Cyp27a1, Hsd17c14, Ntcp, and Scp2, and thus modulate bile acid homeostasis (Figure 7).

DISCUSSION

The current studies identify an FXR-MAFG pathway that controls the transcription of multiple genes involved in both the classic and alternative pathways of bile acid synthesis, and bile acid transport. We show that the MafG gene is a direct target of FXR and that MAFG subsequently represses many genes involved in bile acid synthesis and metabolism (Figures 1, 2, 3, 4, and 7). Further, we show that loss of ≥50% hepatic MAFG leads to de-repression of many of these genes (Figure 5). Importantly, we demonstrate that MAFG binds to MAREs associated with the same repressed genes (Figures 6 and 7). Cyp8b1 or Cyp7b1 promoter-reporter assays provided additional insight into the functional importance of selected MAREs (Figure 6). The identification of a MAFG-dependent regulation of Cyp8b1, Acox2, Akr1d1, Akr1c14, Cyp27a1, Cyp7b1, Hsd17c4, Ntcp, and Scp2 suggests a concerted action of MAFG in regulating various aspects of bile acid metabolism that has not been previously appreciated (Figure 1A).

Consistent with the finding that hepatic overexpression of MAFG in mice represses Cyp8b1, we show that under these conditions there is a decrease in biliary cholic acid and an increase in muricholic acid levels (Figure 3) without increasing bile acid levels in liver, intestine, or gall bladder (Figures S2E–S2G). This finding is consistent with Cyp8b1 encoding the regulatory enzyme for cholic acid synthesis from 7-hydroxycholesterol and the earlier observation that Cyp8b1−/− mice not only fail to synthesize cholic acid, but exhibit increased muricholic acid levels in bile, without a change in the bile acid pool size (Li-Hawkins et al., 2002). In contrast, loss of MAFG, as a result of partial gene ablation or silencing, caused de-repression of Cyp8b1 (Figure 5) and an increased ratio of cholic acid/muricholic acid without altering total bile acid levels in liver, intestine, and gall bladder (Figures S4B–S4D). This change in the bile acid composition is expected to alter the hydrophobicity. However, the
physiologic consequences of such a change on metabolism as a whole are unknown and will require additional studies.

We did not observe changes in Cyp7a1 mRNA levels in the MafG−/− mice or in ASO-treated wild-type mice (Figure 5), further supporting the specificity of the regulation of specific bile acid genes by MAFG. Nonetheless, after prolonged MAFG overexpression, we have observed some repression of Cyp7a1 (data not shown). This effect was not consistent across all our studies. Since MafG silencing and MafG−/− mice do not exhibit changes in Cyp7a1 expression (Figure 5), and MAFG ChIP-seq analysis at the Cyp7a1 locus did not identify MAFG binding sites (Figure S5J), we suggest that the repression of Cyp7a1 may be indirect.

The current studies suggest that MAFG represents a complimentary pathway that is critical for the regulation of bile acid homeostasis. Previous studies reported that the FXR-dependent repression of Cyp8b1 involves Shp (Kerr et al., 2002; Wang et al., 2002), although the authors suggested at that time that additional unknown pathways were likely to play a role in the repression of bile acid synthetic genes. Earlier in vitro studies had shown that SHP can repress luciferase reporter gene activity by binding to and inhibiting HNF4α and LRH-1 transcription factors that normally activate Cyp7a1 and/or Cyp8b1 (Goodwin et al., 2000; Lu et al., 2000). Nonetheless, the effects of hepatic overexpression of SHP on the expression of Cyp7a1 or Cyp8b1 in vivo are at best very modest (Kir et al., 2012; Kong et al., 2012), while the effects of SHP overexpression on other bile acid synthetic genes or bile acid composition have not been reported. Further, Kong et al. (2012) reported that treatment of Shp−/− mice with the FXR agonist GW4064 resulted in near-normal repression of both Cyp8b1 and Cyp7a1. Taken together, these results contrast with the broad and significant repression of numerous bile acid synthetic genes and the decreased levels of cholic acid we observe in mice following MAFG overexpression (Figures 3 and 5).

A second pathway that leads to repression of Cyp7a1 involves activation of FXR in enterocytes and the resulting increase in Fgf15 (mouse) or FGF19 (humans) and subsequent secretion of the protein (Inagaki et al., 2005). FGF15/19 binds to the cognate receptor, FGFR4/β-klotho, resulting in repression of Cyp7a1 (Potthoff et al., 2012). Whether the FGF15/19 pathway plays a role in the regulation of Cyp8b1 is unclear at the present time. A recent study also demonstrated a co-requirement for SHP in mediating the effects of FGF19 repression of Cyp7a1 (Kir et al., 2012). In contrast, a separate study showed that injection of FGF15 protein into Shp−/− mice resulted in near-normal repression of both Cyp7a1 and Cyp8b1 (Kong et al., 2012). Thus, it appears that the precise role of SHP in mediating the FGF15/19 and/or FXR-dependent repression of Cyp8b1 and/or Cyp7a1 remains to be established. Nonetheless, the finding that Cyp7a1 and/or Cyp8b1 mRNA levels are induced/de-repressed in cells or the livers of mice deficient for either Shp (Kerr et al., 2002; Wang et al., 2002), Fgfr4/β-klotho (Kong et al., 2012), or MafG (Figure 5) suggest that SHP, FGF15/19, and MAFG represent three complimentary pathways that control bile acid synthesis and composition. Indeed, the existence and complexity of the complementary pathways highlight the fact that tight regulation of bile acid homeostasis is required, and dysregulation can lead to various metabolic diseases.

The role of MAFG in bile acid metabolism has not been previously appreciated. The MAF family of proteins is divided into small (MAFG, MAFF, and MAFK) and large (cMAF, MAFA, MAFB) members (Kannan et al., 2012). The small members contain a DNA-binding domain and a basic leucine zipper but lack the transcriptional activation domain found in the large family members (Kannan et al., 2012; Motohashi et al., 2002). Small members of the family can form homodimers or heterodimers that bind MAREs to repress transcription of target genes (Kurokawa et al., 2009). However, the small MAF proteins can also dimerize with transcriptional activators, such as NRF2, a member of the Cap ‘n’ Collar family of transcription factors, to induce genes involved in the stress response and detoxification (Kannan et al., 2012; Motohashi et al., 2002). At the current time, the factors that control the formation of homodimers versus heterodimers of the small MAF proteins are poorly understood.

Feedback repression of bile acid synthesis in response to accumulating bile acids is critical for the normal maintenance of bile acid homeostasis and for the prevention of hepatotoxicity that occurs with elevated levels of bile acids. The identification of the pathway described here may have important implications in disease since bile acid metabolism is linked to several metabolic disorders, including cardiovascular disorders, diabetes, and specific types of cancer.

**EXPERIMENTAL PROCEDURES**

GSK2324 was dissolved in water and administered to mice via intraperitoneal (i.p.) injection at 30 mg/kg body weight (mpk) unless otherwise stated. In experiments where GW4064 and GSK2324 were compared, agonists were dissolved in water containing 0.5% Tween 80, and mice were treated once daily with either drug or vehicle alone at 60 mpk for 3 days via i.p. injection. Unless otherwise stated, mice were fasted for 4–6 hr after the last treatment with FXR agonists prior to removal of tissues. All animal experiments were carried out according to NIH guidelines and were approved by the Office of Animal Research Oversight (OARO) at UCLA. For MafG ASO studies, male 12-week-old C57BL/6 mice (Jackson Laboratory) were dosed once with either control or MafG ASO at 100 mpk, and 3 days later, mice were fasted overnight and livers collected the following morning (9–11 a.m.). All adenoviruses were prepared in BSL2 category facilities. Briefly, cDNAs for mouse MafG, Crip2, and Zfp385a were cloned from whole-liver cDNA into pAdTrack CMV plasmid and prepared as described in Bennett et al. (2013). For animal experiments, 1 x 10⁸ plaque-forming units (PFU) were used, and tissues were collected after 5–7 days, and for cell culture studies, a moi of 1–10 was used, and cells were harvested for analysis after 24–48 hr. For gene expression analysis, RNA was isolated using QIAZOL according to the manufacturer’s instructions (QIAGEN) and rDNasel treated before cDNA was synthesized (Life Technologies), qRT-PCR analysis was carried out using primers described in Table S1, and gene expression data were normalized to Tbp and/or 36B4/Rplp0. Western blotting analysis was carried out from liver samples (approximately 100 mg of tissue) homogenized in 1 ml of RIPA buffer supplemented with protease inhibitor complex (Roche). Protein was quantified using the BCA assay (Thermo Fisher Scientific), and 10–30 μg of protein was loaded on pre-cast gels (Bio-Rad). Protein was transferred to PVDF membranes (Millipore), probed with antibodies described in the Supplemental Experimental Procedures, and detected with ECL reagent (Sigma) or ECL Prime (GE Healthcare) using a GE Image Quant LAS 4000 detection system (GE Healthcare). Bile acid analysis was carried out from biliary material by HPLC, and the major taurine-conjugated bodies described in the Supplemental Experimental Procedures. For promoter-reporter studies, mouse MafG promoter (2 kb), Cyp8b1 promoter (0.5 kb), and Cyp7b1 promoter (1 kb) were amplified from mouse genomic DNA (C57BL/6) using KAPA HiFi polymerase (Kapa) and
cloned into pGL4.10[luc2] plasmid (Promega). Luciferase reporter constructs were transfected using FuGene HD (Promega) (n = 6 wells per condition) according to manufacturer’s instructions into human HepG2 or Hep3B cells (ATCC) and plated onto 48-well dishes. For MaFG ChIP analysis, mice were treated with Ad-BlRTP or Ad-BlRTP-MafG for 5 days. Livers were fixed in PBS containing 1% formaldehyde, nuclei were isolated, and chromatin was sheared by sonication for 25–30 cycles using BioRuptor Twin (Diagenode) and immunoprecipitated using a BlRTP antibody (Avi-tag, GeneScript) as described in the Supplemental Experimental Procedures. For ChIP-seq, immunoprecipitated DNA was used for library preparation (Kapa Biosystems) and sequenced by the UCLA UNG Core. Analysis of ChIP- and RNA-seq, as well as microarray analysis, is described in the Supplemental Experimental Procedures.

Statistics
All bars shown are mean ± SEM. The comparison of different groups was carried out with Student’s t test, one- and two-way ANOVA, and differences under p < 0.05 were considered statistically significant (*p < 0.05, **p < 0.01, ***p < 0.001, and different letters indicate at least p < 0.05).

SUPPLEMENTAL INFORMATION
Supplemental Information includes Supplemental Experimental Procedures, six figures, and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.cmet.2015.01.007.

AUTHOR CONTRIBUTIONS
T.Q.d.A.V. and P.A.E. conceived the project and wrote the manuscript. L.R.H. carried out bile acid analysis, H.M. and M.Y. provided MaGr−/− mice, and R.L. and M.G. provided the MaFt ASO. C.R. carried out ChIP-seq bioinformatics analysis. All authors provided feedback during preparation of the manuscript. T.Q.d.A.V. executed all experiments and was aided by H.A. and E.J.T.

ACKNOWLEDGMENTS
We thank Drs. Timothy Willson and David Deaton for the kind gift of GSK2324 and Peter Tontonoz and his lab for helpful discussions. We also thank Tieyan Han, Joan Cheng, Christina Cheung, and Elizabeth Nam for excellent technical assistance. This work was supported in part by United States Public Health Service, grants 1R01DK102559-01 and the Laubish fund at UCLA (to P.A.E.); American Heart Association (AHA) Beginning Grant In Aid (13BIA17080038) and NIH K99HL118161 (to E.J.T); and AHA Postdoctoral Fellowship (12POST1176017) and NIH NHLBI K99HL12348501 (to C.E.R.). T.Q.d.A.V. was supported by an AHA Scientist Development Grant (14SDG18440015), University of California Los Angeles (UCLA) Clinical and Translational Science Institute (CTSI) grant (UL1TR000124), a UCLA Digestive Diseases Research Center (DDRC) grant (DK41301), and a UCLA Diabetes Research Center (DRC) grant (DK033491). R.G. Lee and M.J. Graham are employees and shareholders of Isis Pharmaceuticals.

Received: September 18, 2014
Revised: November 20, 2014
Accepted: January 13, 2015
Published: February 3, 2015

REFERENCES
Cell Metabolism, Volume 21

Supplemental Information

MAFG Is a Transcriptional Repressor of Bile Acid Synthesis and Metabolism

Supplementary Figure 1
Supplementary Figure 2
Supplementary Figure 3
Supplementary Figure 4
Supplementary Figure 5
### Supplementary Figure 6

#### Term ID | Term Name | Enrichment p-value | Genes in Term | Target Genes in Term | Total Target Genes in Term | Fraction of Targets in Term
--- | --- | --- | --- | --- | --- | ---
GO:0009892 | negative regulation of metabolic process | 3.67E-11 | 1715 | 53 | 243 | 22%

A

![Venn Diagram showing overlapping sets of MafG peaks and LRH-1 peaks](image)

B

Top 20,000 MafG peaks

Top 10,633 LRH-1 peaks

19,718

282

10,351
Supplemental Figure Legends

Supplemental Figure 1 (related to Figure 2)– Identification of Transcriptional Repressors as Direct FXR Target Genes. (A) ChIP-Seq analysis of hepatic FXR from (Chong et al., 2010) (top) and (Thomas et al., 2010) (bottom) at Akr1d1 genomic locus. (B) Hepatic expression of Olig1 in C57BL/6 wild-type and Fxr−/− mice treated with vehicle, GW4064 or GSK2324 for 3 days (n=7-9 mice/group). (C) ChIP-Seq analysis of hepatic FXR from (Chong et al., 2010) (top) and (Thomas et al., 2010) (bottom) at Shp (gene symbol Nr0b2) genomic locus. (D) Hepatic expression of Shp in C57BL/6 wild-type and Fxr−/− mice fed either a control or 0.25% cholic acid (CA) diet for 7 days. All data shown as mean ± SEM. Asterisks indicate statistically significant differences comparing WT or KO vehicle treated against agonist treated mice (*p<0.05; **p<0.01; *** p<0.001).

Supplemental Figure 2 (related to Figure 3) – Regulation of Crip2, MafG and Zfp385a by FXR and Effect of MafG Overexpression on Total Bile Acid Levels. (A) Hepatic expression of Shp and Bsep in C57BL/6 wild-type mice treated daily with vehicle, or 10, 30 or 100mpk of GSK2324 for 3 days (n=4-8 mice/group). (B) Hepatic expression of Shp and Bsep in C57BL/6 wild-type treated with 30 mpk of GSK2324 for 1, 2 or 4h before sacrifice (n=6 mice/group). (C) Hepatic expression of Shp in littermate C57BL/6 wild-type (Flox) or liver-specific Fxr−/− mice (L-KO) treated with GSK2324 (n=7-9 mice/group). (D) Western blotting analysis of MafG and beta actin for Ad-control or Ad-MafG-treated livers (n=5 mice/group) fed
either control or colesevelam (Colesev) diet. Quantification of Western blots are shown in Fig 3C. (E-G) Total bile acid levels measured following extraction from liver (E), intestine (F) or directly from biliary fluid (G), normalized to body weight (n=7/8 mice/group). (H) Expression levels of SHP in human HepG2 cells treated with 100,150 or 200µM chenodeoxycholic acid (CDCA) for 4 hours (n=4 wells/condition). All data shown as mean ± SEM. Asterisks indicate statistically significant differences comparing WT or KO vehicle treated against agonist treated mice, or in H, comparing vehicle with CDCA treatments (** p<0.001).

**Supplemental Figure 3 (related to Results section: MAFG Overexpression in Mouse Liver Represses Numerous Genes Involved in Bile Acid Metabolism) – Nuclear Localization and Tissue Expression of MafG.** (A) Nuclear localization of MAFG protein following transfection of Hep3B cells with pcDNA3.1 expressing MAFG containing a FLAG peptide at either the N- or C-termini (identified with anti-FLAG antibody). (B) Expression of MafG mRNA in various tissues isolated from 8-week old C57BL/6 male mice (n=3 mice per tissue). Cp values, indicative of mRNA abundance, are listed for each tissue in white. All data are shown as mean ± SEM.

**Supplemental Figure 4 (related to Figure 5)– Loss of MafG Does not Affect Total Bile Acid Levels.** (A) Western blotting analysis of MAFG and β-ACTIN in livers of mice treated with control ASO or MafG ASO (100mpk) for 3 days. Quantification of the Western blots is shown in Fig. 5F. (B-D) Total bile acid levels measured following extraction from liver (B), intestine (C) or directly from biliary fluid (D), normalized to body weight (n=8-9 mice/group).
Supplemental Figure 5 (related to Figure 6) – Identification of MAFG-Response Elements (MAREs) in Known and Newly Identified MAFG Target Genes. (A) Hepatic levels of MafG mRNA or (B) Cyp8b1 mRNA levels in C57BL/6 mice treated with either control (Ad-BLRP) or BLRP-tagged MafG adenovirus (Ad-BLRP MafG) (n=6-7 mice/group). All data shown as mean ± SEM. Asterisks indicate statistically significant differences comparing BLRP-Control versus BLRP MafG (** p<0.01; *** p<0.001). (C) Chromatin immunoprecipitation (ChIP) analysis of hepatic BLRP-MafG occupancy at the Nqo1 and G6pdx loci determined by RT-qPCR using primers described in Hirotsu et al. (Hirotsu et al., 2012). (D-I) ChIP-Seq analysis of MAREs in chromatin isolated from livers of mice treated with Ad-BLRP (control; top) or Ad-BLRP MafG (bottom) at loci for (D) Acox2 (E) Akr1d1, (F) Akr1c14, (G) Ntcp, (H) Hsd17b4, (I) Scp2 and (J) Cyp7a1. Significant peaks are highlighted with *.

Supplemental Figure 6 (related to Results section: Identification of MAFG Binding Sites at Multiple Genes Involved in Bile Acid Synthesis and Metabolism) – Co-occupancy of Genes by MafG and LRH1. (A) Venn diagram for ChIP-Seq analysis from liver LRH1 and MafG binding sites, showing 282 genes are co-bound by MafG and LRH1. (B) Gene ontology analysis of 282 genes identifies negative metabolic processes as a significantly enriched category.

Supplemental Table 1 - RT-qPCR Primer Sequences

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward</th>
<th>Reverse</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse Crip2</td>
<td>TGCTGAGCATGATGGGAAG</td>
<td>CGGTCTGAGGCTTCTCGTAG</td>
<td></td>
</tr>
<tr>
<td>Mouse MafG</td>
<td>GACCCCCAATAAAAGGAAACAA</td>
<td>TCAACTCTCGGACCCGACAT</td>
<td></td>
</tr>
<tr>
<td>Species</td>
<td>Gene</td>
<td>Forward Primers</td>
<td>Reverse Primers</td>
</tr>
<tr>
<td>---------</td>
<td>------</td>
<td>----------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Mouse</td>
<td>Zfp385a</td>
<td>GTCTGTCAGATCCGCTTCAA</td>
<td>GGCGATTACCTTGTAGTGC</td>
</tr>
<tr>
<td>Mouse</td>
<td>Bsep (Abcb11)</td>
<td>AAGCTACATCTGCTTAGACAGAA</td>
<td>CAATACAGGTCGGACCCCTCCTCCT</td>
</tr>
<tr>
<td>Mouse</td>
<td>Shp (Nr0b2)</td>
<td>CGATCCTCTTCAAACCCAGATG</td>
<td>AGGGCTCCAAGACTTCACACA</td>
</tr>
<tr>
<td>Mouse</td>
<td>Cyp7a1</td>
<td>AGCAACTAAACACCTGCAATGTA</td>
<td>GTCCGGATATTTCAAGGATGCA</td>
</tr>
<tr>
<td>Mouse</td>
<td>Cyp8b1</td>
<td>GCCCTCAAATGTAGTGGTTCCT</td>
<td>GATCTTCTTGCCCCAGCTTGTAGA</td>
</tr>
<tr>
<td>Mouse</td>
<td>Cyp7b1</td>
<td>TAGCCCTTTTTCCACTCATA</td>
<td>GAACCGATCGAACTTAAATTCCT</td>
</tr>
<tr>
<td>Mouse</td>
<td>Cyp27a1</td>
<td>GCCTCACCTATGGCTCAAAGGTAC</td>
<td>TCAAGGCCTGACGAGATG</td>
</tr>
<tr>
<td>Mouse</td>
<td>Cyp39a1</td>
<td>ACCTATGATGAGGGCTTTGGA</td>
<td>CCATCTTTTTGGATTTTGACCA</td>
</tr>
<tr>
<td>Mouse</td>
<td>Acox2</td>
<td>AGATTGGGCCTATAGGGAAG</td>
<td>CACCCGGAGGGTACAAAGA</td>
</tr>
<tr>
<td>Mouse</td>
<td>Akr1d1</td>
<td>GAAAGATAGCAGAAGGGAAGGTT</td>
<td>GGGACATGCTTCTGTATTCATA</td>
</tr>
<tr>
<td>Mouse</td>
<td>Akr1c14</td>
<td>TTATTCATTTCCTCAATGGCTC</td>
<td>TTTCCATGTTGCTCTCGTG</td>
</tr>
<tr>
<td>Mouse</td>
<td>Amacr</td>
<td>GCCAGGTGATCGATTTCAAG</td>
<td>GCTGGGTTTTCCACAGGA</td>
</tr>
<tr>
<td>Mouse</td>
<td>Hsd17b4</td>
<td>GGGAAGCTACTTGGAGCTG</td>
<td>TCAGCAATAACTGCTTCCATTA</td>
</tr>
<tr>
<td>Mouse</td>
<td>Hsd3b7</td>
<td>CGCTTTGGAGGCTGTCTATT</td>
<td>CAGTATGTGACATTCAAAGCA</td>
</tr>
<tr>
<td>Mouse</td>
<td>Ntcp</td>
<td>GAAGTCCAAAAGGCCACACT</td>
<td>ACCAGCCACAGAGGGAAGAA</td>
</tr>
<tr>
<td>Mouse</td>
<td>Scp2</td>
<td>GATTGCTTCTTGTAATGAACCTC</td>
<td>ACCAGGGTTCCACCTTGT</td>
</tr>
<tr>
<td>Mouse</td>
<td>Slc25a7</td>
<td>AGGGTTTTGCCATTTCCTTG</td>
<td>TTGGTCTTTTCAACCTTGG</td>
</tr>
<tr>
<td>Mouse</td>
<td>36B4 (Rlplp0)</td>
<td>CACTGGTCTTGGAGCCGAAGAAG</td>
<td>GGTGCCCTCAGGATTTTTCG</td>
</tr>
<tr>
<td>Mouse</td>
<td>Tbp</td>
<td>CTCAGTTACAGGTCGGCAGCA</td>
<td>ACCAACAATCACAACAGCA</td>
</tr>
<tr>
<td>Human</td>
<td>MAFG</td>
<td>GTGGACAGGAAGCGGCTCA</td>
<td>TATTTGCGTGTCAACCA</td>
</tr>
<tr>
<td>Human</td>
<td>CYP8B1</td>
<td>CTGGGCAACATGCTTCA</td>
<td>ACTTGTCTGCATAGCTGAGG</td>
</tr>
<tr>
<td>Human</td>
<td>36B4 (RPLP0)</td>
<td>GGTGCCTCTGGAGATTTTAG</td>
<td>CACTGGTCTCGGCGCCAGAA</td>
</tr>
</tbody>
</table>
Extended Experimental Procedures

Animals. All animals were bred and housed in a pathogen-free animal facility and unless otherwise stated, were maintained on a C57BL/6 background (Jackson Laboratories). The generation of liver- and intestine-specific Fxr\(^{-/-}\) mice and their respective (littermate) wild-type (flox) controls, and whole body Fxr\(^{-/-}\) mice was previously described (de Aguiar Vallim et al., 2013). Littermate wild-
type and \( \textit{MafG}^{+/−} \) mice on a mixed background, were generated after re-
derivation at UCLA from \( \textit{MafG}^{+/−} \) mating pairs.

**Primary Hepatocyte Isolation:** Primary hepatocytes were isolated as described previously from wild-type C57BL/6 mice (de Aguiar Vallim et al., 2013).

**Bile Acid Analysis.** Gall bladders were removed from all experimental mice after a 4-6 hour fast and bile removed and stored at -80°C. Bile acid species were measured by the HPLC System. Conjugated bile acids were analyzed by high-pressure liquid chromatography using a Kinetex 5m C-18 100A 250 x 4.6 mm column (Phenomenex, Torrance, CA) with isocratic elution at 0.75 ml/min. The eluting solution was composed of a mixture of methanol and 0.01M KH\(_2\)PO\(_4\) (67.4% v/v), adjusted to an apparent pH of 5.25 with H\(_2\)PO\(_4\). Bile acids were quantified by measuring their absorbance at 205 nm and were identified by matching their relative retention times with those of known standards.

Total bile acids were extracted from liver and intestine by homogenizing a piece (approx. 250mg) of liver or the entire small intestine with contents in 96% ethanol. Samples were incubated at 55°C overnight, and centrifuged at 2000xg the following day, where bile acids in the supernatant were collected. Pellets were re-suspended in the same volumes described above of 96% ethanol and incubated at 55°C for a second overnight period. Samples were centrifuged as above, and supernatants combined, with the pellet being resuspended in half volume of chloroform:methanol (2:1 ratio) at incubated at room temperature for a final overnight period. Total bile acids were quantified with a calorimetric assay (Diazyme) calculated using a standard curve from different concentrations of Na-
tauro-cholic acid. For biliary bile acids, samples were diluted 1:1000 and assayed, for intestine, extracted samples were diluted 1:10 and for liver, undiluted extracted samples were used for the assay. Bile acid levels were then corrected for total body weight.

**RNA Isolation, RT-qPCR Quantification.** Liver samples (approximately 100mg) were removed from mice and immediately flash-frozen in liquid nitrogen and stored at -80°C. Frozen tissue was then homogenized in Qiazol (Qiagen) and extracted according to the manufacturer’s instructions. RNA was then DNase-treated (rDNaseI, Ambion/Life Technologies) for 1 hour at 37°C RNA was re-extracted by phenol:chloroform:isoamyl alcohol (25:24:1, pH 6.6, Life Technologies). Complementary DNA (cDNA) was synthesized from 500ng of RNA using the High Capacity cDNA Reverse Transcriptase kit (Applied Biosciences/Life Technologies) according to manufacturers’ instructions. RT-qPCR analysis and microarray analysis are described in Supplemental Materials.

**ChIP, ChIP-Seq and RNA-Seq Analysis.** The biotin-ligase recognition peptide (BLRP) (Heinz et al., 2010) was cloned at the N-terminus of MafG, and used to generate at BLRP-tagged MafG adenovirus. C57BL/6 mice were infused with adenovirus particles as described above. Liver chromatin was isolated as described in detail in Supplemental Materials. FXR ChIP-Seq data have been previously described (Chong et al., 2010; Thomas et al., 2010). BED files were converted to BigWig files and visualized using the IGV browser or UCSC genome browser. Publically available RNA-Seq data (Menet et al., 2012) BedGraph files were downloaded from GEO datasets and visualized using IGV browser. For
MAFG ChIP-Seq analysis, libraries from IP chromatin isolated as described above were prepared using the Kapa Library Prep kit (Kapa Bioscience), sequences using the Illumina HiSeq sequencer (Illumina) by the UNGC at UCLA. Data analysis was carried out using Galaxy suite and visualized as described above. Peak and motif analysis was carried out using HOMER as previously described (Heinz et al., 2010). LRH1 ChIP-Seq peaks (identified using MACS2) (Chong et al., 2012) were converted to coordinates in mm10 and compared with MafG peaks identified as described above.

**Western Blotting.** Antibodies used for Western Blotting were MAFG (Genetex; GTX114541; 1:1000), β-ACTIN (Sigma, 1:5000), BLRP (Avi-tag; Genescript 1:1000), GAPDH (Genetex, 1:2000), LAMIN A/C (Santa Cruz, 1:1000) and for immuno-staining M2 FLAG (Sigma, 1:1000). For ASO-treated, or Ad-MafG treated livers (Colesevelam), Western blots were quantified using HRP detection with ECL prime reagent (GE Healthcare) according to manufacturer’s instructions. HRP signal detection was determined electronically using GE Image Quant LAS 4000 system and parameters set strictly below the saturation point. Densitometric analysis was carried out using Quantity One software (BioRad) and relative protein levels expressed as fold change from control after normalization to β-actin protein levels, with vehicle, Ad-control or control ASO-treated mice set to 1.

**Promoter Reporter Analysis and Cell Culture.** Mouse MafG promoter (2kb) or Cyp8b1 promoter luciferase reporter constructs (3kb, 1kb and 0.5kb) were generated by amplifying these regions from mouse genomic DNA using KAPA
HiFi polymerase (Kapa) and cloning them into pGL4.10[luc2] plasmid (Promega). Mutations were made with a nested PCR approach, and primers were designed with the Quick Change Site-Directed Mutagenesis website (Agilent). Luciferase reporter constructs were transfected using Fugene HD (Promega) according to manufacturer’s instructions into human HepG2 cells (ATCC) or Hep3B cells (ATCC), plated onto 48-well dishes (n=6 wells/condition). After 24 hours, medium was replaced and cells were treated with either vehicle (DMSO or water) or GSK2324 (1µM in water) in medium containing 10% charcoal-stripped serum (Omega Scientific) for a further 24 hours. For siRNA transfections, HepG2 cells were seeded in 6 well plates transfected with 1µM siRNA (MafG siRNA; Cat #SI00036701, CCGGTTATTATTGCTGTACA, or Allstars negative control (SI03650318) Qiagen) for 24 hours and then medium was replaced with 10% charcoal-stripped serum for further 24 hours before cells were harvested in Qiazol for RNA analysis or RIPA buffer for protein analysis.

**Anti-sense oligonucleotides (ASO).** A chimeric 16-mer phosphorothioate oligonucleotide targeted to mouse MafG (5’- GGCCAATACGCGGTCA -3’) or control (5’- TAATGTCTGATAACTC -3’) containing 2’-4’ constrained ethyl groups at bases underlined was synthesized and purified as described in (Seth et al., 2010).

**ChIP Analysis:** One lobe of the liver was frozen in liquid nitrogen, and the remainder (~80%) of the liver was then macerated in PBS containing 1% formaldehyde and protease inhibitor cocktail 1 (PIC1, comprised of 1µg/ml leupeptin, 1.4µg/ml pepstatin, 0.2mg/ml PMSF (Sigma), 1mM EGTA, 1mM
EDTA) for 10 minutes with gentle rotation at room temperature. Crosslinking was then stopped by the addition of glycine (0.125M) and samples were further incubated for 5 minutes at room temperature. Fixed livers were then placed on ice and then centrifuged (2,000 x g for 10 minutes) and the pellet resuspended in 8ml of PBS containing protease inhibitor cocktail. Resuspended pellets were gently homogenized and again centrifuged as above. The resulting pellet was resuspended in 5ml lysis buffer (5mM PIPES, pH 8.0, 85mM KCl, 0.5% NP-40 alternate) supplemented with protease inhibitor complex 2 (PIC2, 10µg/ml leupeptin, 5µg/ml pepstatin, 0.2mg/ml PMSF, 50µg/ml ALLN, Sigma). Nuclei were released after 30 strokes using a Dounce homogenizer and collected after centrifugation as above. Pellets were resuspended in 6ml homogenization buffer (10mM HEPES, pH 7.6, 25mM KCl, 1mM EDTA, 1mM EGTA, 1M Sucrose, 10% glycerol, 0.15mM spermine, supplemented with PIC1) and layered onto 3ml of the same buffer. Nuclei were then pelleted at 26,000rpm for 1 hour (Beckman SW41 rotor) and stored at -80ºC. Nuclear pellets were re-suspended in 0.3ml nuclear lysis buffer (50mM Tris pH 7.6, 10mM EDTA and 1% SDS), and diluted with 0.6ml immunoprecipitation (IP) dilution buffer (0.01% SDS, 1.1% Triton x100, 167mM NaCl, 16.7mM Tris pH 7.6, 1.2mM EDTA). For sonication, 0.3ml (1/3) of nuclear lysate was sonicated for 25-30 cycles 30 seconds on 30 seconds off at 4ºC with BioRuptor twin sonicator (Diagenode). Sonicated chromatin was then further diluted to 1ml with IP dilution buffer, which is sufficient for three ChIP reactions. The BLRP antibody (Genescript) was conjugated to Protein G Dynabeads (Life Technologies) in PBS containing 0.5% BSA overnight at 4ºC
with gentle rotation. Sonicated DNA (0.33ml made up to 1ml in IP dilution buffer) was then incubated overnight with Protein G-BLRP beads at 4°C with gentle rotation. For input samples, 10% was used. Samples were then washed twice with wash buffer I (20mM Tris, pH 7.4, 150mM NaCl, 0.1% SDS, 1% Triton X100, 2mM EDTA), three times with alternate wash buffer III (10mM Tris, pH 7.4, 250mM LiCl, 1% NP-40 alternate, 0.7% Deoxycholate, 1mM EDTA), two washes with 0.2% Triton X100 TE buffer and two final washes with 50mM NaCl containing TE buffer. Chromatin was recovered and DNA from IP and input was isolated after reverse crosslinking (incubating at 65°C overnight in 0.3M NaCl and Proteinase K digestion) and DNA purified using gel/PCR extraction kit (Clontech). For qPCR analysis, samples were diluted 1:10 and run in triplicate as described above. A standard curve for PCR was generated from 4 log serial dilutions of input samples and data expressed as percentage of input. Primer sequences for ChIP analysis are provided in Table S2.

**RT-qPCR Analysis:** RT-qPCR standards were prepared from an aliquot from each cDNA reaction that was then pooled. Standards which were diluted over 3 log range (dilution range 1:5, 1:10, 1:50, 1:100, 1:500, 1:1000). Quantitative PCR was carried out with primers designed to cross exon-exon boundaries using the Roche UPL primer design website. RT-qPCR primer sequences are provided in Table S1. Quantitative PCR was carried out in triplicate for each sample in 384-well format using Kapa LC480 SYBR green mix (Kapa Biosciences). Quantitative PCR was carried out using a Lightcycler 480 (Roche) and concentrations were determined from the standard curve using the efficiency corrected method (2nds
derivative max, Roche). Relative quantification was determined by normalizing the expression of each gene to a housekeeper. Two housekeepers were used to normalize gene expression data (36B4 and Tbp).

**Microarray Analysis**: Hepatic RNA was isolated from 3 mice treated with Ad-Control or Ad-MafG and global gene expression measured by the UCLA Neuroscience Genomics Core (UNGC) using Illumina microarrays (Illumina). Microarray analysis was carried out using Genome Suite (Illumina) and differential gene expression determined from probes that had expression of p>0.05, (approximately 10,000 genes left over after filtering). Of those, genes that were altered 1.5 fold were considered differentially expressed. Gene enrichment analysis was carried out using DAVID and pathway analysis using KEGG (Huang da et al., 2009).

**Supplemental References**


cassette transporter A1 and plasma high-density lipoprotein after activation of the nuclear receptor farnesoid X receptor. Circ Res 112, 1602-1612.


Supplemental Figure Legends

Supplemental Figure 1 (related to Figure 2) – Identification of Transcriptional Repressors as Direct FXR Target Genes. (A) ChIP-Seq analysis of hepatic FXR from (Chong et al., 2010) (top) and (Thomas et al., 2010) (bottom) at Akr1d1 genomic locus. (B) Hepatic expression of Olig1 in C57BL/6 wild-type and Fxr−/− mice treated with vehicle, GW4064 or GSK2324 for 3 days (n=7-9 mice/group). (C) ChIP-Seq analysis of hepatic FXR from (Chong et al., 2010) (top) and (Thomas et al., 2010) (bottom) at Shp (gene symbol Nr0b2) genomic locus. (D) Hepatic expression of Shp in C57BL/6 wild-type and Fxr−/− mice fed either a control or 0.25% cholic acid (CA) diet for 7 days. All data shown as mean ± SEM. Asterisks indicate statistically significant differences comparing WT or KO vehicle treated against agonist treated mice (*p<0.05; **p<0.01; *** p<0.001).

Supplemental Figure 2 (related to Figure 3) – Regulation of Crip2, MafG and Zfp385a by FXR and Effect of MafG Overexpression on Total Bile Acid Levels.

(A) Hepatic expression of Shp and Bsep in C57BL/6 wild-type mice treated daily with vehicle, or 10, 30 or 100mpk of GSK2324 for 3 days (n=4-8 mice/group). (B) Hepatic expression of Shp and Bsep in C57BL/6 wild-type treated with 30 mpk of GSK2324 for 1, 2 or 4h before sacrifice (n=6 mice/group). (C) Hepatic expression of Shp in littermate C57BL/6 wild-type (Flox) or liver-specific Fxr−/− mice (L-KO) treated with GSK2324 (n=7-9 mice/group). (D) Western blotting analysis of MafG and beta actin for Ad-control or Ad-MafG-treated livers (n=5 mice/group) fed
either control or colesevelam (Colesev) diet. Quantification of Western blots are shown in Fig 3C. (E-G) Total bile acid levels measured following extraction from liver (E), intestine (F) or directly from biliary fluid (G), normalized to body weight (n=7/8 mice/group). (H) Expression levels of SHP in human HepG2 cells treated with 100,150 or 200\(\mu\)M chenodeoxycholic acid (CDCA) for 4 hours (n=4 wells/condition). All data shown as mean ± SEM. Asterisks indicate statistically significant differences comparing WT or KO vehicle treated against agonist treated mice, or in H, comparing vehicle with CDCA treatments (*** p<0.001).

**Supplemental Figure 3 (related to Results section: MAFG Overexpression in Mouse Liver Represses Numerous Genes Involved in Bile Acid Metabolism) – Nuclear Localization and Tissue Expression of MafG.** (A) Nuclear localization of MAFG protein following transfection of Hep3B cells with pcDNA3.1 expressing MAFG containing a FLAG peptide at either the N- or C-termini (identified with anti-FLAG antibody). (B) Expression of MafG mRNA in various tissues isolated from 8-week old C57BL/6 male mice (n=3 mice per tissue). Cp values, indicative of mRNA abundance, are listed for each tissue in white. All data are shown as mean ± SEM.

**Supplemental Figure 4 (related to Figure 5) – Loss of MafG Does not Affect Total Bile Acid Levels.** (A) Western blotting analysis of MAFG and β-ACTIN in livers of mice treated with control ASO or MafG ASO (100mpk) for 3 days. Quantification of the Western blots is shown in Fig. 5F. (B-D) Total bile acid levels measured following extraction from liver (B), intestine (C) or directly from biliary fluid (D), normalized to body weight (n=8-9 mice/group).
Supplemental Figure 5 (related to Figure 6) – Identification of MAFG-Response Elements (MAREs) in Known and Newly Identified MAFG Target Genes. (A) Hepatic levels of *MafG* mRNA or (B) *Cyp8b1* mRNA levels in C57BL/6 mice treated with either control (Ad-BLRP) or BLRP-tagged *MafG* adenovirus (Ad-BLRP MafG) (n=6-7 mice/group). All data shown as mean ± SEM. Asterisks indicate statistically significant differences comparing BLRP-Control versus BLRP MafG (** p<0.01; *** p<0.001). (C) Chromatin immunoprecipitation (ChIP) analysis of hepatic BLRP-*MafG* occupancy at the *Nqo1* and *G6pdx* loci determined by RT-qPCR using primers described in Hirotsu *et al.* (Hirotsu et al., 2012). (D-I) ChIP-Seq analysis of MAREs in chromatin isolated from livers of mice treated with Ad-BLRP (control; top) or Ad-BLRP MafG (bottom) at loci for (D) *Acox2* (E) *Akr1d1*, (F) *Akr1c14*, (G) *Ntcp*, (H) *Hsd17b4*, (I) *Scp2* and (J) *Cyp7a1*. Significant peaks are highlighted with *. 

Supplemental Figure 6 (related to Results section: Identification of MAFG Binding Sites at Multiple Genes Involved in Bile Acid Synthesis and Metabolism) – Co-occupancy of Genes by MafG and LRH1. (A) Venn diagram for ChIP-Seq analysis from liver LRH1 and MafG binding sites, showing 282 genes are co-bound by MafG and LRH1. (B) Gene ontology analysis of 282 genes identifies negative metabolic processes as a significantly enriched category.

Supplemental Table 1 - RT-qPCR Primer Sequences

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward</th>
<th>Reverse</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td><em>Crip2</em></td>
<td>TGCTGAGCATGATGGGAAG</td>
<td>CGGTCTGAGGCTTCTCGTAG</td>
</tr>
<tr>
<td>Mouse</td>
<td><em>MafG</em></td>
<td>GACCCCCAATAAGGAAACAA</td>
<td>TCAACTCTCGACCCGACAT</td>
</tr>
<tr>
<td>Gene Name</td>
<td>Reference Sequence 1</td>
<td>Reference Sequence 2</td>
<td>Reference</td>
</tr>
<tr>
<td>-------------</td>
<td>----------------------</td>
<td>----------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Mouse Zfp385a</td>
<td>GTCTGTCAGATCGCCTGATCAAT</td>
<td>GGGATTACCCCTTTGTAGTGC</td>
<td>(Schmidt et al., 2010)</td>
</tr>
<tr>
<td>Mouse Bsep (Abcb11)</td>
<td>AAGCTACATCTGCGCTTAGACAA</td>
<td>CAATACAGGTTCGGACCCTCTCT</td>
<td>(Schmidt et al., 2010)</td>
</tr>
<tr>
<td>Mouse Shp (Nr0b2)</td>
<td>CGATCCCTTCAACCCAGATGA</td>
<td>AAGGCTCAAGAATTCACACA</td>
<td>(Schmidt et al., 2010)</td>
</tr>
<tr>
<td>Mouse Cyp7a1</td>
<td>AGCAACTAAACAACCTGCGCAGTA</td>
<td>GTCCGGATATTCAAGGATGCA</td>
<td>(Schmidt et al., 2010)</td>
</tr>
<tr>
<td>Mouse Cyp8b1</td>
<td>GCCTCAAGATGATTCGGTTCTCT</td>
<td>GATCTTCTTGGCCGACTTGTAGA</td>
<td>(Schmidt et al., 2010)</td>
</tr>
<tr>
<td>Mouse Cyp7b1</td>
<td>TAGGCCCTTCTTCCACTCATA</td>
<td>GAACCGATCGAACCATATTTCT</td>
<td>(Schmidt et al., 2010)</td>
</tr>
<tr>
<td>Mouse Cyp27a1</td>
<td>GCCTCACCTATTGGGATCTCT</td>
<td>TCAAAAGCCTGAGCGAGATG</td>
<td>(Schmidt et al., 2010)</td>
</tr>
<tr>
<td>Mouse Cyp39a1</td>
<td>ACCTATGATGAGGGCTTTGATGTA</td>
<td>CCATCCTTTGGGATTTTGACCCA</td>
<td>(Schmidt et al., 2010)</td>
</tr>
<tr>
<td>Mouse Acox2</td>
<td>AGATTGGGCCTATAGGGAAGA</td>
<td>CACCGGGAGGTAACCAAGAAA</td>
<td></td>
</tr>
<tr>
<td>Mouse Akr1d1</td>
<td>GAAAAAGATAGCAGAAGGGAA</td>
<td>GGGGACATGCTGATATTCCATA</td>
<td></td>
</tr>
<tr>
<td>Mouse Akr1c14</td>
<td>TTATCTTCTTCTTATGGCTT</td>
<td>TTTTCCATGTTCTGCTCGTG</td>
<td></td>
</tr>
<tr>
<td>Mouse Amacr</td>
<td>GCCAGGTCATCGGATTTCAAG</td>
<td>GCTGGGTTTCACAGGA</td>
<td></td>
</tr>
<tr>
<td>Mouse Hsd17b4</td>
<td>GGGAGCAGTACCTGGAGCTG</td>
<td>TCAGCAATAACTGCTCATTCT</td>
<td>(Schmidt et al., 2010)</td>
</tr>
<tr>
<td>Mouse Hsd3b7</td>
<td>CGCTTTGGGAGGTGCTTATT</td>
<td>CAGTATGTGACATCCAGCAAC</td>
<td></td>
</tr>
<tr>
<td>Mouse Ntcp (Slc10a1)</td>
<td>GAAGTCACAAAAAGGCAACTATG</td>
<td>ACACAGCCAGAGGGAGAAAG</td>
<td></td>
</tr>
<tr>
<td>Mouse Scp2</td>
<td>GATGCTTCTCGTCAATGAACCTC</td>
<td>ACCAGGGGTTCCACCTTTGTC</td>
<td></td>
</tr>
<tr>
<td>Mouse Slc25a7</td>
<td>AGGGTTTGGCATTCTTGTG</td>
<td>TTGTTTCTTTCGAACCCTTG</td>
<td></td>
</tr>
<tr>
<td>Mouse 36B4 (Rplp0)</td>
<td>CACTGGTCTAGGAGCCGGAAGG</td>
<td>GGTGCTCCTGGAGATTTTCTC</td>
<td></td>
</tr>
<tr>
<td>Mouse Tbp</td>
<td>CTCAATACGGATTGGCAAGCA</td>
<td>ACCAAACATCACACCAAGCA</td>
<td></td>
</tr>
<tr>
<td>Human MAFG</td>
<td>GTGGGAGGAAGCAGCTCA</td>
<td>TATGGGGGCGTCAGCTAACC</td>
<td></td>
</tr>
<tr>
<td>Human CYP8B1</td>
<td>CTGGGCAACATGCTTCTAGT</td>
<td>ACTTTGCTCAGCTAGCTAGG</td>
<td></td>
</tr>
<tr>
<td>Human 36B4 (RPLP0)</td>
<td>GGTGCCTCCTGGAGATTTTAG</td>
<td>CACTGGTCTCGGCGCCGAGAA</td>
<td></td>
</tr>
</tbody>
</table>
Supplemental Table 2 – BLRP-"MafG ChIP qPCR Primer Sequences

<table>
<thead>
<tr>
<th>Primer Pair</th>
<th>Forward</th>
<th>Reverse</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyp8b1 -2652bp</td>
<td>AACTGCAAGCAGCATTCTTC</td>
<td>GAAGCAGGGCAGGAATC</td>
<td></td>
</tr>
<tr>
<td>Cyp8b1 -2299bp</td>
<td>GAGTTCTCCCCAGCAAGGA</td>
<td>GCCCCAGAGATGGGATAGTC</td>
<td></td>
</tr>
<tr>
<td>Cyp8b1 -1874bp</td>
<td>CTAATGGCAACTAAGGAGAC</td>
<td>TCTCCATCAGACATCTGCTAGTTT</td>
<td></td>
</tr>
<tr>
<td>Cyp8b1 -1211bp</td>
<td>GCCAGACACTGTCTAAGAGC</td>
<td>GAGTACACCCATGTCAGTGATT</td>
<td></td>
</tr>
<tr>
<td>Cyp8b1 -890bp</td>
<td>CAGGGAGAGATCCTGAGGAG</td>
<td>GGGTCTCTGGAAAAAGCAGA</td>
<td></td>
</tr>
<tr>
<td>Cyp8b1 -382bp</td>
<td>TCATTTGCTAGCTTCTAGCTAA</td>
<td>ACAGACACGCACTTTAAAAACAA</td>
<td></td>
</tr>
<tr>
<td>Cyp8b1 -83bp</td>
<td>TCCCCTGTGCCAGCTAACTAG</td>
<td>GTTCCTGCCCTTGGACTTT</td>
<td></td>
</tr>
<tr>
<td>Cyp8b1 +39bp</td>
<td>GAGCTGACAAGTGGAGCTCA</td>
<td>GCAGGGCTCTAGCAGTA</td>
<td></td>
</tr>
<tr>
<td>Cyp8b1 +485bp</td>
<td>AACTCAACCAGGCGATGC</td>
<td>GCACCAGACTCAGA</td>
<td></td>
</tr>
<tr>
<td>G6pdx ARE</td>
<td>GTCGGAGGGGACTGGTCTGC</td>
<td>TGTCAGTTGCAGGCTGGGCAAA</td>
<td>(Hirotsu et al., 2012)</td>
</tr>
<tr>
<td>G6pdx MARE #1</td>
<td>GGCCCTGAGGAGGTGAGGCA</td>
<td>AGCCGACCCCTCAGTGCAGG</td>
<td>(Hirotsu et al., 2012)</td>
</tr>
<tr>
<td>G6pdx MARE #3</td>
<td>GGGAGCCTGAGCCCAATGGC</td>
<td>AAGCCGACCTGGCAGCAAGT</td>
<td>(Hirotsu et al., 2012)</td>
</tr>
<tr>
<td>Nqo1</td>
<td>GCACGAATTCATTTACACAGGGA</td>
<td>GGAACTCAGCTTTCAGCAGTCAG</td>
<td>(Hirotsu et al., 2012)</td>
</tr>
<tr>
<td>Negative</td>
<td>CTAGGAGTAATTGAAAGATCT</td>
<td>GTAGGTTGGGAATTTTCTAGCATTCC</td>
<td></td>
</tr>
</tbody>
</table>

Extended Experimental Procedures

Animals. All animals were bred and housed in a pathogen-free animal facility and unless otherwise stated, were maintained on a C57BL/6 background (Jackson Laboratories). The generation of liver- and intestine-specific Fxr\(^{-/-}\) mice and their respective (littermate) wild-type (flox) controls, and whole body Fxr\(^{-/-}\) mice was previously described (de Aguiar Vallim et al., 2013). Littermate wild-
type and $MafG^{+/−}$ mice on a mixed background, were generated after re-
derivation at UCLA from $MafG^{+/−}$ mating pairs.

**Primary Hepatocyte Isolation:** Primary hepatocytes were isolated as described previously from wild-type C57BL/6 mice (de Aguiar Vallim et al., 2013).

**Bile Acid Analysis.** Gall bladders were removed from all experimental mice after a 4-6 hour fast and bile removed and stored at -80°C. Bile acid species were measured by the HPLC System. Conjugated bile acids were analyzed by high-pressure liquid chromatography using a Kinetex 5m C-18 100A 250 x 4.6 mm column (Phenomenex, Torrance, CA) with isocratic elution at 0.75 ml/min. The eluting solution was composed of a mixture of methanol and 0.01M KH$_2$PO$_4$ (67.4% v/v), adjusted to an apparent pH of 5.25 with H$_2$PO$_4$. Bile acids were quantified by measuring their absorbance at 205 nm and were identified by matching their relative retention times with those of known standards.

Total bile acids were extracted from liver and intestine by homogenizing a piece (approx. 250mg) of liver or the entire small intestine with contents in 96% ethanol. Samples were incubated at 55°C overnight, and centrifuged at 2000xg the following day, where bile acids in the supernatant were collected. Pellets were re-suspended in the same volumes described above of 96% ethanol and incubated at 55°C for a second overnight period. Samples were centrifuged as above, and supernatants combined, with the pellet being resuspended in half volume of chloroform:methanol (2:1 ratio) at incubated at room temperature for a final overnight period. Total bile acids were quantified with a calorimetric assay (Diazyme) calculated using a standard curve from different concentrations of Na-
tauro-cholic acid. For biliary bile acids, samples were diluted 1:1000 and assayed, for intestine, extracted samples were diluted 1:10 and for liver, undiluted extracted samples were used for the assay. Bile acid levels were then corrected for total body weight.

**RNA Isolation, RT-qPCR Quantification.** Liver samples (approximately 100mg) were removed from mice and immediately flash-frozen in liquid nitrogen and stored at -80°C. Frozen tissue was then homogenized in Qiazol (Qiagen) and extracted according to the manufacturer's instructions. RNA was then DNAses-treated (rDNAsel, Ambion/Life Technologies) for 1 hour at 37°C RNA was re-extracted by phenol:chloroform:isoamyl alcohol (25:24:1, pH 6.6, Life Technologies). Complementary DNA (cDNA) was synthesized from 500ng of RNA using the High Capacity cDNA Reverse Transcriptase kit (Applied Biosciences/Life Technologies) according to manufacturers’ instructions. RT-qPCR analysis and microarray analysis are described in Supplemental Materials.

**ChIP, ChIP-Seq and RNA-Seq Analysis.** The biotin-ligase recognition peptide (BLRP) (Heinz et al., 2010) was cloned at the N-terminus of MafG, and used to generate at BLRP-tagged MafG adenovirus. C57BL/6 mice were infused with adenovirus particles as described above. Liver chromatin was isolated as described in detail in Supplemental Materials. FXR ChIP-Seq data have been previously described (Chong et al., 2010; Thomas et al., 2010). BED files were converted to BigWig files and visualized using the IGV browser or UCSC genome browser. Publically available RNA-Seq data (Menet et al., 2012) BedGraph files were downloaded from GEO datasets and visualized using IGV browser. For
MAFG ChIP-Seq analysis, libraries from IP chromatin isolated as described above were prepared using the Kapa Library Prep kit (Kapa Bioscience), sequences using the Illumina HiSeq sequencer (Illumina) by the UNGC at UCLA. Data analysis was carried out using Galaxy suite and visualized as described above. Peak and motif analysis was carried out using HOMER as previously described (Heinz et al., 2010). LRH1 ChIP-Seq peaks (identified using MACS2) (Chong et al., 2012) were converted to coordinates in mm10 and compared with MafG peaks identified as described above.

**Western Blotting.** Antibodies used for Western Blotting were MAFG (Genetex; GTX114541; 1:1000), β-ACTIN (Sigma, 1:5000), BLRP (Avi-tag; Genescript 1:1000), GAPDH (Genetex, 1:2000), LAMIN A/C (Santa Cruz, 1:1000) and for immuno-staining M2 FLAG (Sigma, 1:1000). For ASO-treated, or Ad-MafG treated livers (Colestevamelam), Western blots were quantified using HRP detection with ECL prime reagent (GE Healthcare) according to manufacturer’s instructions. HRP signal detection was determined electronically using GE Image Quant LAS 4000 system and parameters set strictly below the saturation point. Densitometric analysis was carried out using Quantity One software (BioRad) and relative protein levels expressed as fold change from control after normalization to β-actin protein levels, with vehicle, Ad-control or control ASO-treated mice set to 1.

**Cell Culture.** For GSK2324 treatments, cells were transfected for 24 hours and then treated with either vehicle (DMSO or water) or GSK2324 (1μM in water) in medium containing 10% charcoal-stripped serum (Omega Scientific) for a further
24 hours. For siRNA transfections, HepG2 cells were seeded in 6 well plates transfected with 1µM siRNA (MafG siRNA; Cat #SI00036701, CCGGGTATTTATTGCTGTACA, or Allstars negative control (SI03650318) Qiagen) for 24 hours and then medium was replaced with 10% charcoal-stripped serum for further 24 hours before cells were harvested in Qiazol for RNA analysis or RIPA buffer for protein analysis.

**Anti-sense oligonucleotides (ASO).** A chimeric 16-mer phosphorothioate oligonucleotide targeted to mouse MafG (5’-GGCCAATACGCCGTCA-3’) or control (5’-TAATGTCTGATAACTC-3’) containing 2’-4’ constrained ethyl groups at bases underlined was synthesized and purified as described in (Seth et al., 2010).

**ChIP Analysis:** One lobe of the liver was frozen in liquid nitrogen, and the remainder (~80%) of the liver was then macerated in PBS containing 1% formaldehyde and protease inhibitor cocktail 1 (PIC1, comprised of 1µg/ml leupeptin, 1.4µg/ml pepstatin, 0.2mg/ml PMSF (Sigma), 1mM EGTA, 1mM EDTA) for 10 minutes with gentle rotation at room temperature. Crosslinking was then stopped by the addition of glycine (0.125M) and samples were further incubated for 5 minutes at room temperature. Fixed livers were then placed on ice and then centrifuged (2,000 x g for 10 minutes) and the pellet resuspended in 8ml of PBS containing protease inhibitor cocktail. Resuspended pellets were gently homogenized and again centrifuged as above. The resulting pellet was resuspended in 5ml lysis buffer (5mM PIPES, pH 8.0, 85mM KCl, 0.5% NP-40 alternate) supplemented with protease inhibitor complex 2 (PIC2, 10µg/ml
leupeptin, 5µg/ml pepstatin, 0.2mg/ml PMSF, 50µg/ml ALLN, Sigma). Nuclei were released after 30 strokes using a Dounce homogenizer and collected after centrifugation as above. Pellets were resuspended in 6ml homogenization buffer (10mM HEPES, pH 7.6, 25mM KCl, 1mM EDTA, 1mM EGTA, 1M Sucrose, 10% glycerol, 0.15mM spermine, supplemented with PIC1) and layered onto 3ml of the same buffer. Nuclei were then pelleted at 26,000rpm for 1 hour (Beckman SW41 rotor) and stored at -80°C. Nuclear pellets were re-suspended in 0.3ml nuclear lysis buffer (50mM Tris pH 7.6, 10mM EDTA and 1% SDS), and diluted with 0.6ml immunoprecipitation (IP) dilution buffer (0.01% SDS, 1.1% Triton x100, 167mM NaCl, 16.7mM Tris pH 7.6, 1.2mM EDTA). For sonication, 0.3ml (1/3) of nuclear lysate was sonicated for 25-30 cycles 30 seconds on 30 seconds off at 4°C with BioRuptor twin sonicator (Diagenode). Sonicated chromatin was then further diluted to 1ml with IP dilution buffer, which is sufficient for three ChIP reactions. The BLRP antibody (Genescript) was conjugated to Protein G Dynabeads (Life Technologies) in PBS containing 0.5% BSA overnight at 4°C with gentle rotation. Sonicated DNA (0.33ml made up to 1ml in IP dilution buffer) was then incubated overnight with Protein G-BLRP beads at 4°C with gentle rotation. For input samples, 10% was used. Samples were then washed twice with wash buffer I (20mM Tris, pH 7.4, 150mM NaCl, 0.1% SDS, 1% Triton X100, 2mM EDTA), three times with alternate wash buffer III (10mM Tris, pH 7.4, 250mM LiCl, 1% NP-40 alternate, 0.7% Deoxycholate, 1mM EDTA), two washes with 0.2% Triton X100 TE buffer and two final washes with 50mM NaCl containing TE buffer. Chromatin was recovered and DNA from IP and input was
isolated after reverse crosslinking (incubating at 65°C overnight in 0.3M NaCl and Proteinase K digestion) and DNA purified using gel/PCR extraction kit (Clontech). For qPCR analysis, samples were diluted 1:10 and run in triplicate as described above. A standard curve for PCR was generated from 4 log serial dilutions of input samples and data expressed as percentage of input. Primer sequences for ChIP analysis are provided in Table S2.

**RT-qPCR Analysis:** RT-qPCR standards were prepared from an aliquot from each cDNA reaction that was then pooled. Standards which were diluted over 3 log range (dilution range 1:5, 1:10, 1:50, 1:100, 1:500, 1:1000). Quantitative PCR was carried out with primers designed to cross exon-exon boundaries using the Roche UPL primer design website. RT-qPCR primer sequences are provided in Table S1. Quantitative PCR was carried out in triplicate for each sample in 384-well format using Kapa LC480 SYBR green mix (Kapa Biosciences). Quantitative PCR was carried out using a Lightcycler 480 (Roche) and concentrations were determined from the standard curve using the efficiency corrected method (2nd derivative max, Roche). Relative quantification was determined by normalizing the expression of each gene to a housekeeper. Two housekeepers were used to normalize gene expression data (36B4 and Tbp).

**Microarray Analysis:** Hepatic RNA was isolated from 3 mice treated with Ad-Control or Ad-MafG and global gene expression measured by the UCLA Neuroscience Genomics Core (UNGC) using Illumina microarrays (Illumina). Microarray analysis was carried out using Genome Suite (Illumina) and differential gene expression determined from probes that that had expression of
p>0.05, (approximately 10,000 genes left over after filtering). Of those, genes that were altered 1.5 fold were considered differentially expressed. Gene enrichment analysis was carried out using DAVID and pathway analysis using KEGG (Huang da et al., 2009).

**Supplemental References**


