**Supplementary Data:**

Table ST1. Primers used for qPCR analysis

| Gene name | Direction | Sequence 5' -------- 3' | Accession No. |
|---|---|---|---|
| hBSEP | Forward | aca tgc ttg cga gga cct tta | NM003472 |
| | Reverse | gga ggt tcg tgc acc agg ta | |
| hNTCP | Forward | ctc aaa tcc aaa cgg cca caa tac | NM003049 |
| | Reverse | cac act gca caa aga gaa tga tga tc | |
| hMLL3 | Forward | gaa aat gac aca atg tcg aat gc | NM170606 |
| | Reverse | ttc acc cag agc ctc ctc tt | |
| hMLL4(ALR) | Forward | gca aat cgc tag cat cat tca g | NM014727 |
| | Reverse | ggc act atg aa gtc agc cat ct | |
| mMLL4 | Forward | ttg ccc caa tgt cta cca ttt t | BC058659 |
| | Reverse | gca tgg tct tgt cct tga aga ac | |
| hNCOA6 | Forward | tgg tct gga aga ggc tga tca | NM014071 |
| | Reverse | tta ggg cct gag tta tcc aag tta a | |
| mNCOA6 | Forward | ccc acc agt gta cgc tca ata g | NM019825 |
| | Reverse | ttg gcg ctg tgg aga tga | |
| mTNF-$\alpha$ | Forward | agg ctg ccc cga cta cgt | NM013693 |
| | Reverse | atg ggc tca tac cag ggt ttg | |
| mIL-6 | Forward | tgg gac tga tgc tgg tga ca | BC132458 |
| | Reverse | ttt cca cga ttt ccc aga gaa | |

Table ST2. Primers used in ChIP analysis

| Gene name | Direction | Sequence 5’ ---- 3’ |
|---|---|---|
| mBsep | Forward | ggt ccc cac gca ctc tgg gtt |
| | Reverse | gtc ctc ttc cgc tca gac gcc act g |
| mMrp2 | Forward | cca ctt agc act act gct gaa tt |
| | Reverse | gtg cca cca ccg cct ggc |
| hBSEP | Forward | ggg ttt ccc aag cac act ctg tgt tt |
| | Reverse | gag gaa gcc aga gga aat ggt gg |
| hNTCP | Forward | ggc gac agc cag aga aat agc tca |
| | Reverse | gtg gca ggg tga agt tga at |

Table ST3 Sequences of siRNAs used in this study

| Gene name | Source-Catalog No | Sequence (Sense strand - 5' to 3') |
|---|---|---|
| NCOA6 | sc-61401 – Santa Cruz | Pool of 3 siRNAs |
| 61401-A | | CUCCGAACAUGCAAGGAAAtt |
| 61401-B | | GGAAGCACCAACAUCGUUAtt |
| 61401-C | | GUUGCGAGUUGAAGUUCAAtt |
| MLL3 | sc-62623 – Santa Cruz | Pool of 3 siRNAs |
| 62623-A | | CAAGGCUACUCAACCUUGAtt |
| 62623-B | | GAACGCACCUUAUAGUAAAtt |
| 62623-C | | CCAUUCGUGUGCACCUAAUtt |
| MLL4 | D-009670- Dharmacon | Pool of 4 siRNAs |
| D-009670-01 | | UAAGGAGGAUUGUGAUUUA |
| D-009670-02 | | GAAGAAAGAAGAAGAAGAA |
| D-009670-03 | | GCACCCAGCUAUAUGAGAA |
| D-009670-04 | | CAGCGACCCUCCUAUGAUA |

Fig. S1. Serum Bile acid analysis in sham-operated and bile duct-ligated (CBDL) mice at 1, 3 and 7 days post-ligation.

In order to verify the patency of bile duct ligation, blood from sham and CBDL mice was collected by heart puncture at the time of sacrifice. Serum was separated after clotting by centrifugation and total 3-hydroxy bile acids were estimated using a kit from Trinity Biotech, NJ, following instructions from manufacturer. As seen from the Figure below, serum bile acids (in μM) were

significantly increased following CBDL surgery at 1 day (sham 60.8 ± 1.2 vs CBDL 674.8 ± 26.8) , 3 days ( sham 204.4 ± 3 vs CBDL 984.7 ± 50.3) and 7 days (sham 87.9 ± 5.35 vs CBDL 1052.9 ± 20.4) suggesting that CBDL mice were indeed cholestatic.

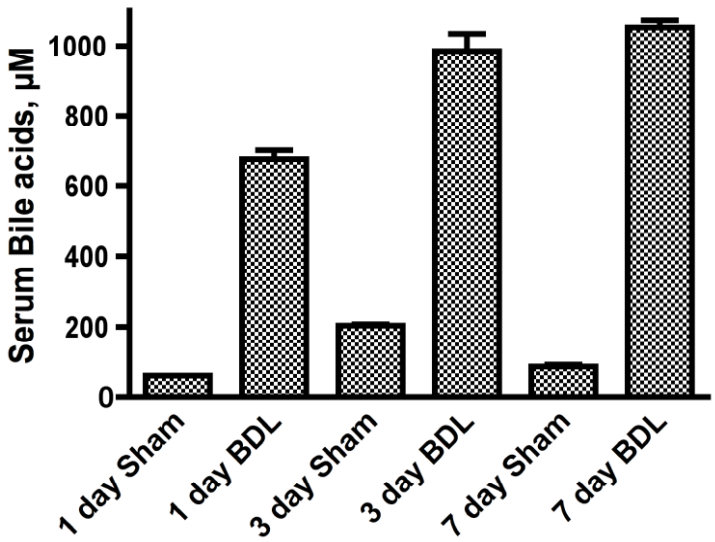


Fig. S1. Serum Bile acids in sham-operated and CBDL mice 1, 3 and 7 days post surgery

Fig. S2. Custom $RT^2$ Profiler Array analysis of RNA from sham-operated and common bile duct-ligated (CBDL) mice at 1 and 3 days post-BDL.

An unbiased array containing oligonucleotide primers corresponding to 84 mouse genes encoding a) histone lysine acetylation/deacetylation, b) lysine/arginine methylation/demethylation c) coactivators d) corepressor and nuclear receptors was custom-made and plated by SA Bioscience Corporation, Rockville. MD. The array also contained oligos for positive controls (beta-actin, glyceraldehydes 3-phosphate dehydrogenase (GAPDH) , heat shock protein 90 kDa, Acidic

ribosomal phosphoprotein (36B4) and positive PCR control in addition to negative controls (mouse genomic DNA) to detect contamination, and reverse transcription control. qPCR was conducted using $RT^2$Profiler PCR Array System reagents from SA Biosciences Corporation, Frederick, MD using an ABI 7900HT machine. $C^T$ values were obtained using ABI Software SDS Version 2.1. An example of the layout of the 96 well format of the genes is given below.

Fig. S2. Layout of the 96 well format of the custom array. Names of the individual genes correspond to gene symbols or aliases in the NCBI database

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Hat1 | Cebpγ | Cebpα | Cebpε | Ep300 | PCAF | GCN5l2 | Myst2 | Myst1 | Myst3 | NCoA1 | NCoA2 |
| NCoA3 | NCoA4 | NCoA5 | HDAC1 | HDAC2 | HDAC3 | HDAC4 | HDAC5 | HDAC6 | HDAC7a | HDAC10 | HDAC11 |
| SIRT1 | SIRT2 | SIRT3 | SIRT5 | SIRT6 | SIRT7 | ChD1 | ChD2 | ChD3 | ChD4 | ChD6 | ChD7 |
| ChD8 | Trim28 | Utx | PGC1α | PGC1β | NCoR1 | NCoR2 | NR0b2 | NR1h4 | HNF4α | IL4i | RXRα |
| RXRβ | RARα | RORγ | NR1i3 | NR1i2 | Fbx11 | Jmcd2a | Jmcd2b | Jmcd2c | PRMT1 | PRMT2 | PRMT3 |
| CARM1 | PRMT5 | PRMT7 | Ehmt2 | Ehmt1 | Suv39h1 | MLL1 | MLL3 | MLL5 | SETd1a | SETd7 | SETd8 |
| Suv420h1 | Suv420h2 | Ezh2 | ASH1L | ASH2L | TRα | TRβ | NR3c1 | PPARBP | NR1h3 | ERα | NR1h2 |
| AhR | NR5A2 | MED4 | Gusβ | HPRT1 | Hsp90αB1 | GAPDH | Actβ | ARBP(36B4) | MGDC | RTC | |

**Functional Classes of Individual Genes analyzed in the Custom Array (Fig. S2):**

*a) Transcription factors including Nuclear receptors, Coactivators and Corepressors:*

Cepbg, Cebpa, Ncoa1, Ncoa1,Ncoa3,Ncoa4, Ncoa5, Ncor1, Ncor2, Nrob2, Nr1h4, Hnf4a, Rxra, Rxrb, Rara, Rorc, Nr1i3, Nr1i2, Thra, Thrb, Nr3c1, Pparbp, Nr1h3, Esr, Nr1h2, Ahr, Nr5a2.

*B) Histone modifiying enzymes ( lysine acetylases and deacetylases, lysine methylases and demethylases, arginine methylases and demethylases):*

Ep300, Pcaf, Gcn5l2, Myst2, Myst3, Hdac1, Hdac2, Hdac3, Hdac4Hdac5, Hdac6, Hdac7a, Hdac10, Hdac11, Sirt1, Sirt2, Sirt5, Sirt6, Sirt7, Il4i1, Fbxl11, Jmjd2a, Jmjd2b, Jmjd2c, Prmt1, Prmt2, Prmt3, Carm1, Prmt5, Prmt7, Ehmt1, Ehmt2, Suv39h1, Mll1, Mll3, Mll5, Setd1a, Setd7, Setd8, Suv420h1, Suv420h2, Ezh2, Ash1l, Ash2l, Med4

*c) DNA structure modication enzymes:*

Chd1, Chd2, Chd3, Chd4, Chd6, Chd7, Chd8.

*d) Controls:*

Gusb, Hprt1, Hsp90ab1, Gapdh, Actb, Arbp.. MGDC, RTC, PPC.

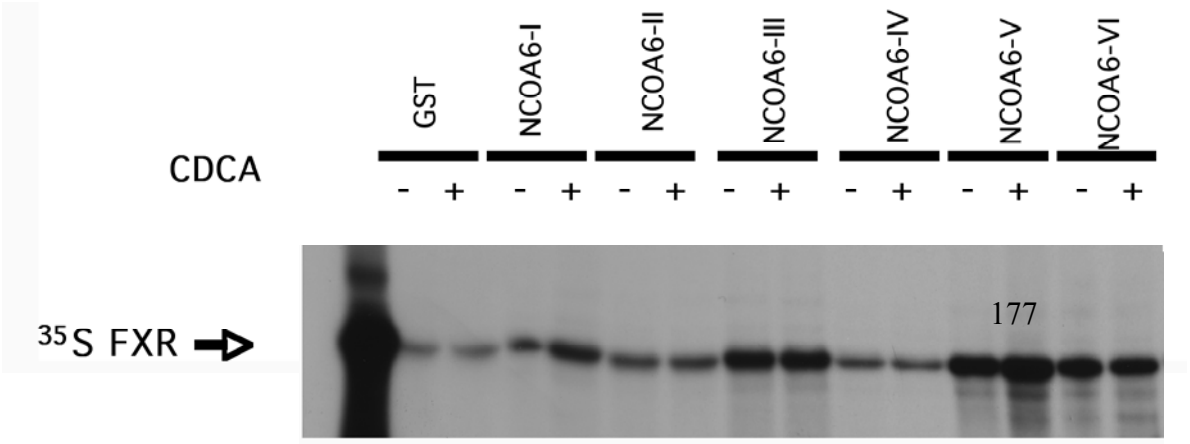


Fig.S3. Longer exposure of the audiogram showing stronger interaction of domains III and V of NCOA6 with $^{35}$FXR employing a GST-pulldown assay. Details of the methods are found Materials and Methods.

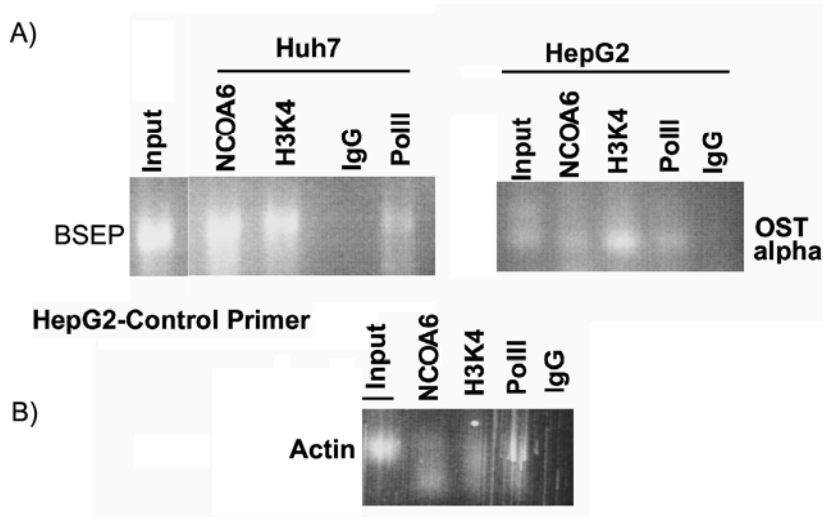


Fig.S4. A) ChIP analysis of BSEP and OST-α FXRE loci in HepG2 cells in the presence of ligand for recruitment of NCOA6, PolII and H3K4 methylation. B) ChIP analysis of a control Actin promoter shows that the recruitment of NCOA6 is specific to BSEP promoter.

**Supplementary Methods:**

*Preparation and analysis of customized epigenetic array:* A customized RT$^2$ Profiler array was used to monitor the changes in expression of nuclear receptors, histone modifying enzymes, coactivators and corepressors after CBDL. This array was created in a 96-well format by design and synthesis of primers corresponding to 87 mouse genes for the above families of proteins along with positive and negative controls by Super Array Biosciences, Frederick, MD. A layout of the 96 well plate indicating the genes represented is shown in the Supplement section. Total RNA was prepared from livers of sham-operated and bile duct-ligated mice and reverse transcribed using RT$^2$ First Strand kit (Super Array BioSciences, MD) according to instructions supplied with the kit. Real time PCR analysis of the expression of the genes corresponding to the epigenetic regulators was carried out on 384-well plates (4 samples/plate) using

ABI 7900 HT System (ABI, CA) at the Mount Sinai Core facility. Relative expression levels in bile duct-ligated samples compared to controls were computed using the $\Delta\Delta C_t$ method. For this purpose $C_t$ values from the plates were loaded onto Excel-based PCR Array Data Analysis template using the web portal http://www.superarray.com/pcrarraydataanalysis.php following instructions at this web site.

*Real time PCR analysis:* qPCR analysis of message levels for BSEP, NTCP, NCOA6, MLL3 and MLL4 were conducted as follows. Briefly, total RNA was prepared from cells or liver tissue using Trizol reagent (Invitrogen, CA) according to manufacturer's instructions. To remove genomic DNA, the RNA was digested using RNAase-free DNAase using instructions and reagents from Qiagen RNAeasy kit (Qiagen, CA). 5 µg of total RNA was used for reverse transcription in a total volume of 21 µl using 1$^{st}$ strand Synthesis kit from Invitrogen according to instructions from the manufacturer. cDNA was diluted 10-fold and used at a concentration of 38 ng/ well in triplicate for qPCR analysis. qPCR was conducted using Quantitect SYBR Green kit in a Bio Rad Mini Opticon 3 (BioRad, CA) or Step One Plus Real time PCR system (ABI, CA). qPCR analysis of 36B4 or cyclophilin (as a control for MLL4) message was used for normalization. Primers were designed using Primer Express analysis software (ABI, CA) and dissociation curves after each set of primer use was checked to verify that a single PCR amplicon was obtained and no primer-dimers were formed. PCR products were also run on agarose gels to further check the amplicon size. List of primers used in real time PCR are provided in Table ST1 (supplement).

Quantitation of message levels were achieved by the relative quantitation method expressed as fold change compared to untreated/control siRNA-treated samples according to ABI Reference Manual.

*Western Blot analysis:* Western blot analysis of NCOA6, MLL3 and MLL4 protein levels were examined after siRNA treatments to verify that cognate protein levels were reduced after the treatment. For this purpose, cell lysates prepared as described above in the siRNA treatment section were run (50 µg protein/lane) on 7.5% SDS-PAGE gels and were transferred to PVDF membranes following standard protocols. After blocking with 5% nonfat milk-containing buffer for 2 hrs at room temp., primary antibodies against NCOA6 (1:2000) , MLL3 (1:1000) and MLL4 (1.2000) in blocking buffer were added and incubated overnight at $4^0$C. Following 4 x 15 min washes, the blots were incubated with anti-rabbit/mouse/goat secondary antibodies conjugated to horseradish peroxidase (1:2000) for 1 hr at room temp. The blots were washed as before and incubated with peroxidase substrate (Femto, Pierce) and exposed to X-ray films for various periods until a suitable image was obtained. The same blots were stripped and reprobed with an antibody to β-actin and actin signals were used to normalize differences in protein levels in the different samples. The blots were scanned in a Fuji LAS3000 scanner and the bands were quantified using NIH Image J.