Supplemental Information

Supplemental Methods

Animal experiments and sample collection

Alb-Cre Ncor$^{flox/flox}$ mice generated as described previously (20) were crossed to *Lxra* $^{-/-}$ mice (kind gift of Dr. David J. Mangelsdorf, Department of Pharmacology, University of Texas Southwestern Medical Center, Dallas, Texas, USA) to obtain *Lxra* $^{+/-}$ *Alb-Cre Ncor*$^{flox/flox}$ animals that were used as breeders to generate *Lxra*$^{+/+}$ *Ncor*$^{flox/flox}$ (control), *Lxra*$^{+/+}$ *Alb-Cre Ncor*$^{flox/flox}$ (*L-ΔID*), *Lxra*$^{-/-}$ *Ncor*$^{flox/flox}$ (*Lxra*$^{-/-}$ *control)* and *Lxra*$^{-/-}$ *Alb-Cre Ncor*$^{flox/flox}$ (*Lxra*$^{-/-}$ *L-ΔID*) as littermates. Mice were maintained on mixed C57BL/6;129S background.

All experiments described were performed in male mice between 10 and 16 weeks of age. Animals were housed in the BIDMC animal research facility at 22-24°C on a 12-h light, 12-h dark cycle and given standard rodent chow (Harlan Teklad F6 Rodent Diet 8664) or the same diet supplemented with 2% cholesterol for indicated periods of time, and water *ad libitum*. For manipulations with TH levels wild type mice were fed a low iodine diet supplemented with 0.15% PTU (LoI/PTU-Harlan Teklad TD.95125) for 3 weeks. At the end of this period, mice received either PBS (PTU group) or T3 (Sigma) injections (25 μg/100 g of body weight in PBS) i.p. for 4 additional days. At the end of experiments the mice were sacrificed by asphyxiation with CO2. Blood samples were taken by cardiac puncture and plasma was separated by centrifugation. Tissues were rapidly collected, flash-frozen in liquid nitrogen and stored at -80°C.

Intestinal cholesterol absorption

Cholesterol absorption was measured using plasma dual isotope method as described (26-28). Non-fasted awake mice were given an intravenous injection of 2.5 μCi of [^{3}H] cholesterol dissolved in 100 μl of 20% Intralipid through the tail vein, followed immediately by a gavage of 1 μCi of [^{14}C] cholesterol in 200 μl of medium-chain triglyceride oil. Blood samples were taken 6, 26, 49 and 72 hours after dosing with radioactive cholesterol. Activity in the aliquots of plasma and original dosing mixtures was counted in a scintillation counter (Beckman Coulter) and used to calculate percent cholesterol absorption at each time point as follows:

% absorption = (%i g dose [^{14}C] per ml plasma/ % iv dose [^{3}H] per ml plasma) x100

Presented in the Results is the % cholesterol absorption calculated for the 72 hours post-dosing, as this time point was validated in previous experiments (26, 27).

Primary hepatocyte culture and cholesterol synthesis rate

Primary hepatocytes were isolated from 9-12 weeks old male mice with indicated genotypes. All tissue culture media and reagents were purchased from Invitrogen (Life Technologies). Animals were anesthetized with ketamine/xylazine and livers were perfused through portal vein with 20 ml of Liver Perfusion Medium followed by 20 ml of Liver Digestion Medium at flow rate 1.6 ml/min. Isolated hepatocytes were collected in Leibovitz's L-15 medium supplemented with 10% FBS, washed with Hepatocyte Wash Media, centrifuged in 25% Percoll beads, washed and resuspended in William's E Medium supplemented with 10% FBS and 1 x Antibiotic-Antimycotic, and plated onto collagen-coated 6-well plates at 1 x 10^{6} cells/well. After a 4-hour incubation at 37°C and 5% CO_2 the medium was changed to FBS-free William's E Medium supplemented with

1 x Antibiotic-Antimycotic, 100 nM dexamethasone, and 1 x Insulin-Transferrin-Selenium. After additional 16 hours of incubation the lipid synthesis rate was measured in hepatocyte cultures using the tritiated water method (64). The hepatocytes were incubated in the culture media with addition of 0.2 mCi of ^3H_2O per well for 6 hours. At the end of this period the cells were harvested in 0.8 ml of 8 N KOH and transferred to screw-cap tubes. An equal volume of ethanol was added, and the tubes were incubated at 90°C for 2 h with periodic vortexing. Nonsaponifiable lipids were extracted two times with 2 ml of petroleum ether. Aliquots of the pooled extracts were placed in glass scintillation vials and dried under the chemical hood. The ^3H radioactivity was determined by scintillation counting and used to calculate the rate of cholesterol synthesis. Experiments were performed in at least 3 wells containing hepatocytes isolated from the same animals.

Real time quantitative PCR

Total RNA was extracted from frozen tissues with STAT-60 reagent (Teltest) according to the manufacture's instructions. 0.5 μg of total RNA was reverse-transcribed using **SuperScript® VILO™ cDNA Synthesis Kit** (Life Technologies). TaqMan Gene Expression Assays were purchased from Life Technologies. Each reaction contained 15-30 ng cDNA, 5 μl of TaqMan Universal PCR Master Mix, No AmpErase UNG (Life Technologies) and 0.25 μl of TaqMan Gene Expression Assay in total volume 10 μl. Quantitative PCR were performed in duplicates using the 7900HT Fast Real-Time PCR System (Applied Biosystems). Relative mRNA levels were calculated using standard

curve method and normalized to cyclophilin mRNA. All data are presented as fold over expression in control *Lxra*$^{+/+}$ *Ncor1*$^{lox/lox}$ chow-fed group.

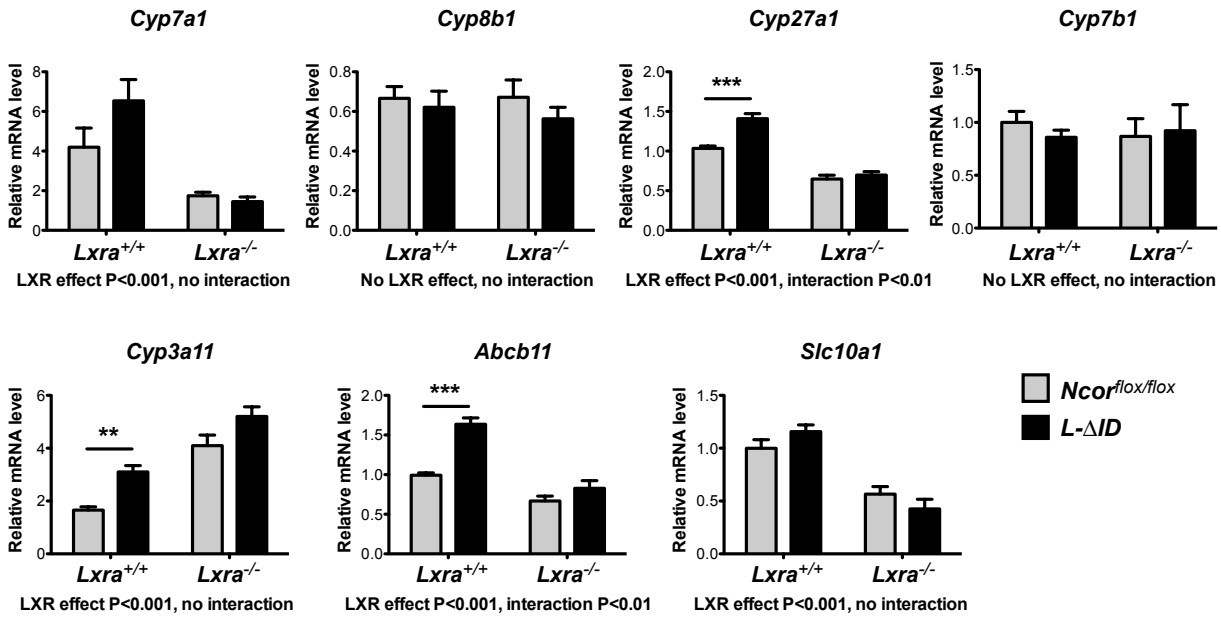
Generation of animals overexpressing biotinylated hTRβ1

The details of the study design, generation of the virus and animal treatments are described in detail by Ramadoss et al., 2013 (41). hTRβ1 cDNA obtained from Origene was cloned into the Blrp-TEV vector to add the biotinylation Blrp signal (kindly provided by Dr. Christopher K. Glass, Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA, USA). The fragment containing Blrp-TEV hTRβ1 was subcloned into the Dual-GFP-CCM adenoviral shuttle vector from Vector Biolabs, and Ad-Blrp-TEV- hTRβ1 (Ad-TRβ1) adenovirus was then generated at Vector Biolabs. Gt(ROSA)26Sortm1(birA)Mejr/J (referred to as the BirA mouse) mice that ubiquitously express biotinylation enzyme, were obtained from Jackson labs and crossed with FVB mice. 9-10 week old male heterozygous BirA mice were maintained on a low iodine propyl-thiouracil (PTU) diet (Halan Teklad) for 3 weeks to render them hypothyroid. At the end of 3 weeks, mice were injected with either Ad-GFP ($1x10^9$ pfu/mouse) or Ad-TRβ1($5 x10^9$ pfu/mouse) via the tail-vein. Half of the mice in each group were given T3 injections intraperitoneally at a dose of 25 μg/100g body weight for 4 days starting on the day of adenoviral injection to render them hyperthyroid, while the remaining half were given saline injections. Mice were euthanized on Day 5 after adenoviral injection and livers were collected and snap-frozen in liquid nitrogen and used for ChIP assays.

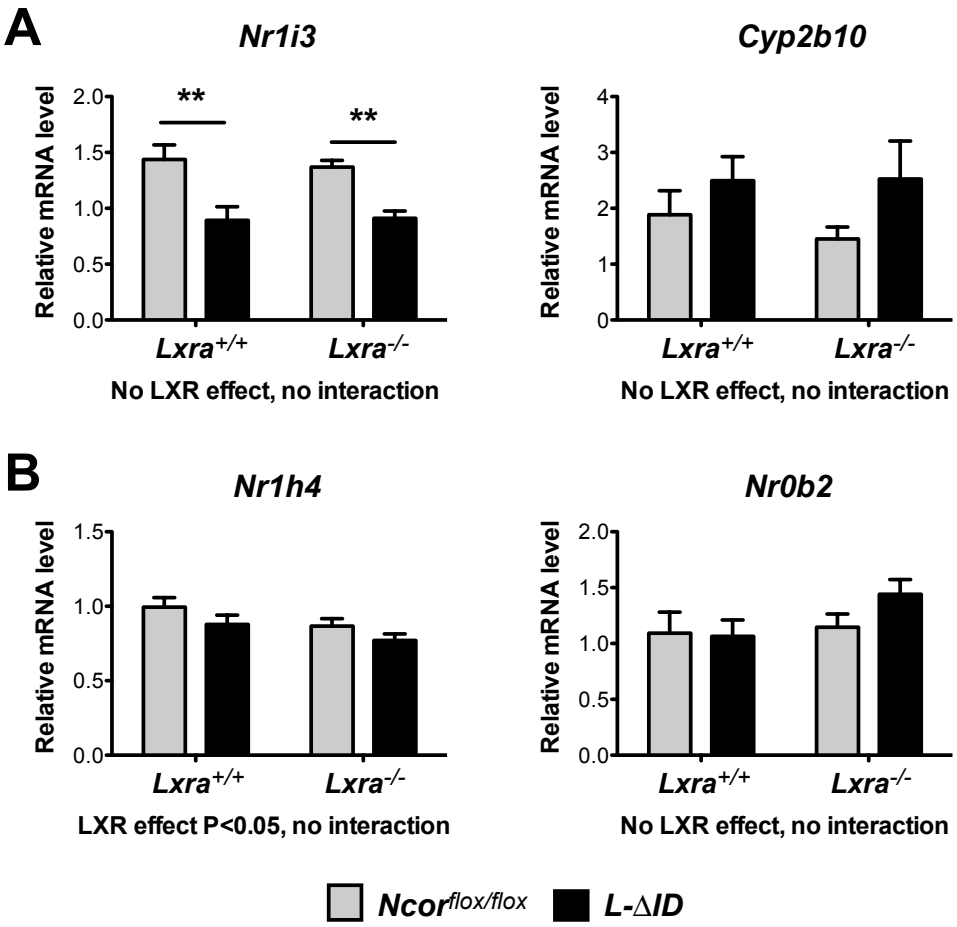
Chromatin immunoprecipitation (ChIP) assays

In this study we used chromatin affinity purification to capture protein-DNA complexes however for the purpose of this study we have referred to the method as "ChIP", rather than "ChAP". For each experiment, 4 livers per group of mice were used (groups: Ad-GFP-PTU, Ad-TRβ1-PTU and Ad-TRβ1-T3), and the chromatin was pooled before the affinity purification step. A total of 5 affinity precipitation reactions were carried out per each chromatin pool. The detailed protocol was as published by Ramdoss et al., 2013 (53). Briefly, 30 mg of liver was minced and cross-linked using 2 mM disuccinimidyl glutarate (DSG) followed by 1% formaldehyde. Crude nuclei were isolated from the crosslinked tissue, re-suspended in 50 mM Tris pH 8.0, 10 mM EDTA, 025% SDS with protease inhibitors and sonicated in 1mL AFA tubes using a Covaris sonicator to achieve a DNA shear size of 200-500 bp. Chromatin was diluted in 0.5X RIPA buffer with protease inhibitors and 20 μg glycogen and used for precipitation with streptavidin agarose beads (ThermoFisher Scientific). After the reversal of the crosslink DNA was extracted and used in qPCR reactions with SYBR chemistry to detect enrichment DNA from 5 replicate precipitation reactions was used in qPCR reactions. All primer sequences can are provided in Supplemental Table 2.

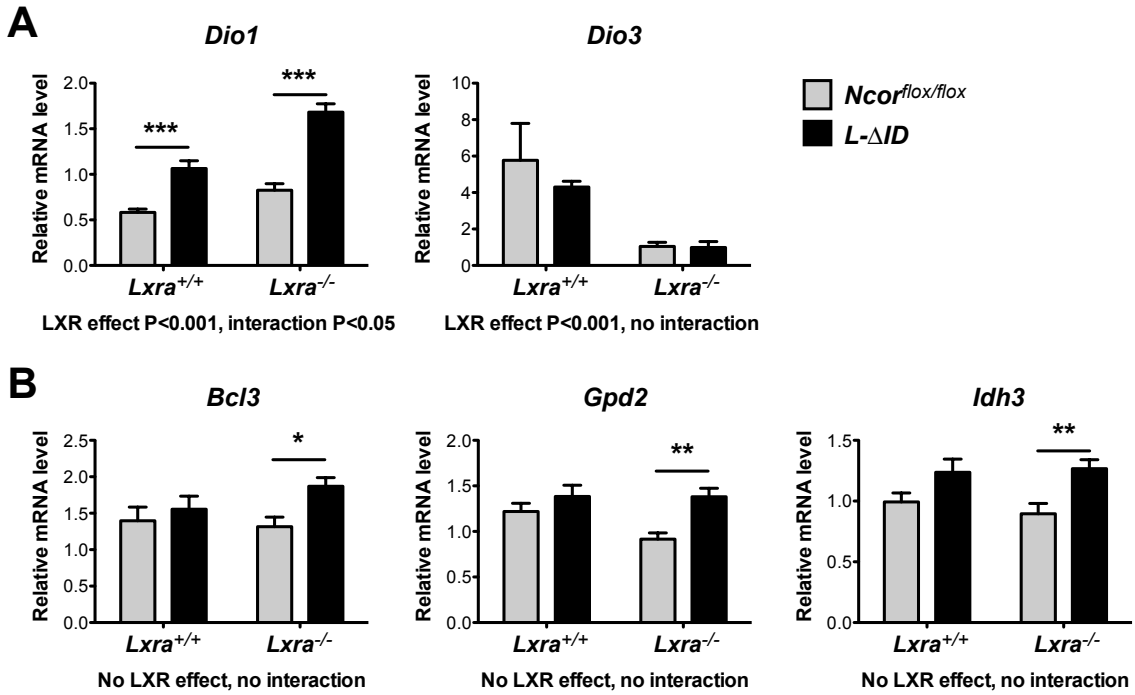
Supplemental Figures



Supplemental Figure 1. Hepatic expression of bile acid metabolism enzymes and bile salt transporters in *L-ΔID* animals after 3 week on 2% cholesterol diet. mRNA expression levels were quantified by QPCR (N=5-11 animals per group). Statistical analysis was performed using two-way ANOVA with Bonferroni posttests; * P≤ 0.05; ** P≤ 0.01; *** P≤ 0.001.



Supplemental Figure 2. Expression levels of *Car* (*Nr1i3)* and its target *Cyp2b10* (**A**), *Fxr* (*Nr1h4*) and its target *Shp* (*Nr0b2*) (**B**) were quantified in the livers of control and *L-ΔID* animals on $Lxra^{+/+}$ and $Lxra^{-/-}$ background fed with 2% cholesterol diet for 3 days (N=7-9 animals per group). Statistical analysis was performed using two-way ANOVA with Bonferroni posttests; ** P≤ 0.01.



Supplemental Figure 3. Expression levels of *Dio1* and *Dio3* (**A**) and thyroid hormone target genes (**B**) were quantified in the livers of control and *L-ΔID* animals on $Lxra^{+/+}$ and $Lxra^{-/-}$ background fed with 2% cholesterol diet for 3 days (N=7-9 animals per group). Statistical analysis was performed using two-way ANOVA with Bonferroni posttests; ** P≤ 0.01.

Supplemental Table 1. Analysis of plasma from mice with different genotypes after 2% cholesterol feeding

Measurement	Genotype		Time on 2% cholesterol diet		
			0 days	3 days	21 days
Cholesterol, mg/dL	*Lxra*$^{+/+}$	*Ncor*$^{flox/flox}$	104.8±6.7	129.6±3.9	121.7±4.6
		L-ΔID	104.3±6.8	125.2±6.0	124.4±6.0
	Lxra$^{-/-}$	*Ncor*$^{flox/flox}$	101.4±6.0	121.4±6.7	119.7±7.9
		L-ΔID	104.8±6.7	129.6±3.9	135.3±7.5
Triglycerides, mg/dL	*Lxra*$^{+/+}$	*Ncor*$^{flox/flox}$	111.5±6.3	91.4±8.7	82.4±5.2
		L-ΔID	141.1±6.2**	149.8±6.5**	143.9±17.3**
	Lxra$^{-/-}$	*Ncor*$^{flox/flox}$	71.1±4.0‡	57.0±7.6‡	25.6±2.7‡
		L-ΔID	93.5±4.2‡**	81.1±7.8‡*	40. 5±5.9‡*
AST, U/L	*Lxra*$^{+/+}$	*Ncor*$^{flox/flox}$	35.6±5.0	17.2±2.7	25.8±3.9
		L-ΔID	32.7±5.0	19.0±1.9	35.6±3.0
	Lxra$^{-/-}$	*Ncor*$^{flox/flox}$	28.7±4.5	18.8±2.8	45.8±4.4‡
		L-ΔID	26.4±2.1	22.9±2.5	50.5±6.3‡
ALT, U/L	*Lxra*$^{+/+}$	*Ncor*$^{flox/flox}$	14.8±1.7	5.0±0.5	13.9±2.7
		L-ΔID	22.2±5.6	8.2±1.1	17.1±5.2
	Lxra$^{-/-}$	*Ncor*$^{flox/flox}$	17.2±2.3	7.5±1.5	42.6±5.3‡
		L-ΔID	11.0±1.6	6.5±0.6	45.5±8.9‡

Data are presented as mean ± SEM. N=5-7 animals per group.
†P<0.01; ‡P<0.001 significant difference between *Lxra*$^{+/+}$ and *Lxra*$^{-/-}$ under the same feeding conditions.
*P<0.05; **P<0.01; ***P<0.001 significant difference between *L-ΔID* and ***Ncor***$^{flox/flox}$ on the same LXR background and feeding conditions.

Supplemental Table 2. Primers used in ChIP QPCR

Gene	Site	Forward primer	Reverse primer
Cyp27a1	**Prom/ -700 bp**	5'-TCTAGCTGACCTGGAACTTGC-3'	5'-CTCAATCCTCCACCTTCCTG-3'
	Int 1/ 5.9 kb	5'-TCCAGGATGAGCCATTTTTC-3'	5'-GCTCACAAATGCCAAGTACG-3'
	Int 1/ 8.2 kb	5'-CCAGCATTTTGCCCTTATGT-3'	5'-GCTGGCATCAAGCATAGAGA-3'
Cyp3a11	**Prom/ -120 bp**	5'-ACCCATCTGGCACTGTTGTT-3'	5-'CCAGGGATCAAGCCAGTAGA-3'
	Upst/ -1.6 kb	5'-TCAGTTATCATTTGGGGTATGG-3'	5'-TCCTGAGAACTTTGCCCTCA-3'
Abcb11	**Prom/ -410 bp**	5'-TCTCACCAGGCTCTCTACCA-3'	5'-TTGCTTATAGGTCAATGGCCT-3'
	Int 12/ 53.4 kb	5'-GGCCTGATGTTTTGGACTCT-3'	5'-AGTTCCACCCAGGGAGATTT-3'