SUPPLEMENTAL METHODS

Animal and Diets: *MTTP-IKO* mice and *MTTP-floxed* littermates were maintained in a mixed C57BL/6, 129/SvJ background. All animals were maintained on a 12h light-dark cycle, in a full barrier facility. 8-10 week old male mice were fed a standard rodent chow (PicoLab Rodent Diet 20, fat 4.5%, cholesterol (0.015%) for the indicated periods in the figure legend.

LXR agonist treatment: 8-10 week old male chow-fed mice were gavaged daily for 7days with either vehicle alone (below) or vehicle solution containing 25mg/kg BW of T0901317 (Cayman Chemical Company). T0901317 was dissolved in dimethyl sulfoxide and Chremophor (Sigma) in 5% mannitol/water to a final concentration of 2.5mg/ml. All studies were conducted after a 4-hr fast.

Cholesterol Absorption and Fecal fat determination: Cholesterol absorption was measured by a fecal dual isotope ratio method, as described previously [1, 2]. Animals were gavaged with 150 µl of corn oil mixed with 1 µCi of [^{14}C] cholesterol (PerkinElmer Life Sciences) and 2 µCi of [^{3}H]sitostanol (American Radiolabeled Chemicals, Inc.). Feces were collected from individually housed mice in wire bottom cages for 48 h after label administration and processed as previously described following homogenization and neutral sterol extraction of fecal samples [1, 2]. The ratio of ^{14}C and ^{3}H sterol in each sample was determined by liquid scintillation counting and cholesterol absorption percentage calculated as described

previously [2]. Fecal fat excretion (percentage) was determined gravimetrically following chloroform/methanol extraction of fecal homogenates.

Bile Flow and Biliary Lipid Output—Mice were anesthetized after a 4h fast using a ketamine/xylazine cocktail. Following induction of surgical anesthesia, the abdomen was opened using a midline incision and an external bile fistula was established using PE10 tubing that was implanted surgically via the gallbladder fundus. Hepatic bile was collected for up to 60 minutes while maintaining the body temperature at 37 °C on a heated blanket. Hepatic bile volume was determined gravimetrically, assuming a density of 1g/ml. Bile samples were stored at-80 °C until analyzed. Biliary phospholipid, cholesterol, and total bile acid content were determined enzymatically using a phospholipid B kit (catalog number 990-54009, Wako Chemicals), cholesterol E kit (catalog number 439-17501, Wako Chemicals), and total bile acids kit (catalog number BQ092A-EALD, Bioquant), respectively. Cholesterol saturation indices in hepatic bile were calculated using published parameters [3].

Radiolabeled cholesterol turnover: 8-10 week old male chow-fed mice were housed individually in wire bottom cages and gavaged daily for 8 days with either vehicle or 25mg/kg BW of T0901317 (Cayman Chemical Company) dissolved in vehicle solution. On the 4th day of T0901317 treatment, mice received an intravenous tail vein injection of 5µCi [^{3}H]-cholesterol dissolved in 100µl Intralipid (20%, Fresenius Kabi, IL, USA). Blood samples were collected at 15 minutes and at 1, 2, 3, and 4 days after injection by orbital bleed and 10µl serum counted in a liquid scintillation counter (LSC). To check the 3H-cholesterol distribution in different serum lipoproteins after intravenous bolus of

radioactive cholesterol, 40ul of pooled serum from 4-5 mice of each experimental group at 15min and 1 day after injection was mixed well with 4ul of a mixture of equal parts of 40mg/ml sodium heparinate solution (196 USP units /mg; Sigma: H3393) and 1mol/L MnCl2 solution[Demacker, Pierre N.M, 1997]. The samples were centrifuged for 15min at 4000g, 4^0C after 10min incubation at room temperature. The supernatant was transfer to a new tube. The cholesterol content and 3H-cholesterol in serum and the above supernatant was measured enzymatically or counted by LSC respectively. The percentile of HDL-cholesterol or HDL-3H-cholesterol in total serum cholesterol or 3H-cholesterol was calculated. Feces were collected at days 1, 2, 3, and 4 after injection. The total fecal collection from day 1 to day 4 was weighed and soaked in Millipore water (1ml per 100mg feces) overnight at 4 ^{o}C. The next day the samples were homogenized using a Polytron dispersion homogenizer and aliquots subjected to total neutral and acidic sterol extraction. For neutral sterol extraction, 100µl homogenate was mixed with 2ml ethanol and 200µl 45% KOH. The samples were saponified at 80^{o}C for 2 hours and cooled to the room temperature, after which the neutral sterol fraction (containing ^{3}H-cholesterol) was extracted 4 times with 2ml hexane. The extracts were pooled, evaporated, resuspended in 100µl methanol and counted in a LSC. To extract ^{3}H-bile acids, the remaining aqueous portion of the feces was acidified with concentrated HCL up to pH <1 and then extracted 3 times with 4ml ethyl acetate. The extracts were pooled, evaporated, resuspended in 100µl methanol, and counted in a LSC. Liver samples were collected at sacrifice (the day 5th after 3H-cholesterol injection). For hepatic sterol extraction, 30-50mg liver was homogenated in 500ul 1xPBS. The sterol was extracted 3 time by 5ml chloroform/methanol (2:1). The extracts (chloroform phase containing 3H-cholesterol) was pooled, evaporated,

resuspended in 100ul methanol and counted in LSC. For hepatic bile acid, the remaining aqueous portion (containing 3H-bile acid) was extracted 3 times by 5ml methanol. The extracts (supernatant) were pooled, evaporated, and resuspended in 100ul methonal and count in a LSC.

Hepatic and Intestinal Lipid Determination*:* Animals were sacrificed after a 4h fast. Aliquots of liver were taken and mucosal scrapings collected from the small intestine and frozen at -80 °C until analyzed. Tissues were homogenized and lipids extracted into chloroform/methanol (2:1), after which triglyceride, cholesterol, free fatty acids, and phospholipids were analyzed enzymatically with an L-type triglyceride H kit (catalog number 993-37592, 993-37492), cholesterol E, HR series NRFA-HR(2) (catalog number 995-34691, 995-34791), and phospholipids B, respectively (Wako Chemicals).

Gas Chromatography-Mass Spectrometry (GC-MS) determination of oxysterol abundance: Lipid extraction of cell monolayers and solid phase purification were performed as described [4]. Lipid extracts were derivatized with Sigma Sil-A for 1 h at 60°C and the mass of 24-, 25- and 27-hydroxycholesterol quantified as described using d_5-27-hydroxycholesterol as internal standard [5]

Serum lipoprotein profile and lipids quantitation: Blood was obtained and serum collected after a 4hr fast. Lipoproteins were size-fractionated by fast-performance liquid chromatography (FPLC) using tandem Superose 12 columns. 40 fractions were collected. Cholesterol and triglyceride content of serum and fractions were analyzed using Wako

enzymatic kits. Apolipoprotein B, E and A1 in the indicated fractions were separated by 4-20% Tris-HCL SDS-PAGE gel, and detected by Western blot using rabbit anti-mouse apoB (1:4000), apoE (1:2000) and rabbit anti-rat apoA1(1:500) respectively [Xie, Y ; 2003].

Gene expression analysis: RNA was extracted from liver or mucosal scrapings using TRIzolP®P Reagent (Invitrogen Life Technologies, Carlsbad, CA) and treated with DNase. Reverse-transcription was performed using the ABI high capacity cDNA reverse transcription kit (Catalog Number 4368814), with 1 μg of total RNA and random hexamers, to generate cDNA. qRT-PCR assays were performed in triplicate on an ABI Prism7000 sequence detection systemusing SYBR Green PCR Master Mix (Applied Biosystems) and primer pairs designed by Primer Express software (Applied Biosystems). Relative mRNA abundance is expressed as fold change compared to mRNA levels in vehicle-treated Mttp-Control mice, normalized to Gapdh.

References:

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5. Frolov A, Zielinski SE, Crowley JR, Dudley-Rucker N, Schaffer JE, Ory DS. Npc1 and npc2 regulate cellular cholesterol homeostasis through generation of low density lipoprotein cholesterol-derived oxysterols. *The Journal of biological chemistry*. 2003;278:25517-25525

SUPPLEMENTAL TABLE I

Intestinal gene expression

Cholesterol transporter	Control-V	Control-T	Mttp IKO-V	MttpIKO-T
Npc1l1	1.03±0.17[a]	0.45±0.10 [b]	0.17±0.05 [c]	0.18±0.04 [c]
Abca1	1.10±0.20[a]	7.30±0.87[b]	0.57±0.07[c]	3.92±0.52[d]
Abcg5	1.02±0.13[a]	3.34±0.09[b]	0.53±0.09[c]	1.81±0.24[a]
Abcg8	1.02±0.14[a]	3.38±0.22[b]	0.50±0.07[c]	2.08±0.24[d]
Srb1	1.00	1.84	1.29	2.99
Abcg1	1.01±0.11[a]	6.51±0.93[b]	0.76±0.04[a]	2.34±0.25[c]
Cd36	1.02±0.14[a]	0.97±0.2[a]	0.44±0.05[b]	0.80±0.21[a]
Cholesterol Synthesis/ metabolism				
Hmgcr	1.00±0.01[a]	0.81±0.10[ab]	0.78±0.15[ab]	0.67±0.05[b]
Acat2	1.03±0.20[a]	1.54±0.25[a]	1.64±0.24[a]	1.10±0.29[a]
Acat1	1.02±0.15[a]	2.88±0.20[b]	0.69±0.03[a]	1.34±0.41[a]
Cyp27a1	1.16±0.46[a]	1.01±0.11[a]	0.44±0.15[ac]	0.24±0.09[bc]
Cyp46a1	1.03±0.16[a]	0.57±0.20[a]	1.16±0.50[a]	0.61±0.12[a]
Ch25h	1.03±0.17[a]	0.70±0.13[a]	1.40±0.36[a]	1.02±0.17[a]
Fatty acid metabolism				
Scd1	1.03±0.17[a]	737.6±204 [b]	48.20±9.62[c]	500.9±83.5[d]
Fas	1.00	2.22	2.76	2.68
Acc	1.00	2.57	1.51	3.43

Lipoprotein related				
ApoA1	1.02±0.12[a]	0.75±0.13[a]	0.72±0.13[a]	0.38±0.05 [b]
ApoE	1.00	0.30	0.75	1.10
Mttp	1.00	0.67	0.07	0.04
Pcsk9	1.06±0.15[a]	0.68±0.08[a]	1.89±0.18[c]	1.12±0.06[a]
ApoB	1.00	1.80	0.98	1.08
Ldlr	1.00	0.85	1.24	1.23
Transcription factors				
Lxra1	1.00	1.25	1.13	1.09
Srebp1c	1.08±0.28[a]	38.73±6.85 [b]	1.66±0.35[a]	33.65±8.48 [b]
Srebp2	1.00	0.82	1.02	1.18
Ppara	1.00	4.56	1.23	4.35
Fgf15	1.00	1.95	0.94	0.96

Intestinal mucosa mRNA was extracted from livers of Control and MttpIKO mice (n=4) treated either vehicle or T0901317(n=4). The mRNA expression of indicated gene was quantitated by qRT-PCR and the value is expressed as fold change related to vehicle-treated control, which was defined as 1. The difference between values associated with different superscript letters in each parameter is statistically significant ($p<0.05$). Where no standard error is indicated, the values represent the mean from pooled samples.

SUPPLEMENTAL TABLE II

Hepatic gene expression

Cholesterol transporter	Control-V	Control-T	MttpIKO-V	MttpIKO-T
Abca1	1.01±0.11[a]	1.99±0.17 [b]	1.61±0.38[ac]	2.85±0.25[bc]
Abcg5	1.11±0.17[a]	2.17±0.17 [b]	2.17±0.25 [b]	3.55±0.58 [c]
Abcg8	1.09±0.15[a]	1.92±0.24 [b]	2.48±0.29 [bc]	3.45±0.54 [c]
Srb1	1.00±0.06	1.24±0.09	0.81±0.08	1.04±0.07
Abcg1	1.00±0.05[a]	3.83±0.34[b]	1.45±0.18[a]	3.47±0.23[b]
Cd36	1.06±0.26[a]	6.22±0.58[b]	1.73±0.35[a]	6.91±0.62[b]
Cholesterol synthesis				
Hmgcr	1.01±0.11[a]	2.53±0.44[b]	11.88±4.17[c]	4.91±0.55[c]
Bile acid /oxysterol synthesis				
Cyp7a1	1.07±0.29[a]	3.11±0.56[b]	0.33±0.10[c]	2.86±0.38[b]
Cyp27a1	1.00±0.04[a]	NA	1.09±0.10[a]	NA
Cyp46a1	1.01±0.10[a]	NA	1.91±0.54[a]	NA
Ch25h	1.09±0.32[a]	NA	0.59±0.16[a]	NA
Fatty acid metabolism				
Fas	1.04±0.21[a]	42.85±4.54 [b]	3.07±0.69 [c]	36.03±5.95 [b]
Acaa1	1.03±0.17[a]	2.34±0.08 [b]	0.56±0.16[c]	1.64±0.19 [a]
Scd1	1.32±0.51[a]	4.14±0.64[b]	1.28±0.24[a]	6.93±0.90[c]
Acc	1.00	2.08	1.15	4.92
Acox	1.00	0.71	0.63	1.74

Mcad	1.00	1.76	1.11	2.52
Vlcad	1.00	1.18	0.9	1.29
Cyp4A14	1.00	13.44	1.67	5.86
Glucose metabolism				
Pdk4	1.13±0.41[a]	2.71±1.59[a]	0.64±0.15[a]	0.84±0.08[a]
Lipoprotein related				
ApoA1	1.03±0.18[a]	0.83±0.22[a]	2.23±0.3[b]	2.01±0.35 [b]
ApoE	1.02±0.12[a]	0.94±0.06[a]	0.92±0.07[a]	1.25±0.12[a]
Pltp	1.15±0.36[a]	7.52±0.91 [b]	8.35±0.74[b]	8.74±1.28[b]
Lpl	1.02±0.14[a]	6.09±2.06[b]	0.92±0.34[a]	9.60±2.67[b]
Mttp	1.00	0.88	0.76	1.69
Pcsk9	1.07±0.16[a]	2.74±0.27[b]	7.06±0.88[c]	5.22±0.37[c]
ApoB	1.00	0.58	0.87	1.06
Ldlr	1.00	1.67	1.25	2.72
apoA5	1.01±0.10[a]	0.51±0.09[b]	1.43±0.20[a]	0.84±0.25[a]
Transcription factors				
Lxra1	1.00	1.16	0.95	2.45
Srebp1c	1.54±0.96[a]	12.38±2.51[b]	2.89±1.03[a]	15.82±2.90[b]
Srebp2	1.00	1.31	2.66	3.34
Ppara	1.00	0.95	0.75	1.39

Hepatic mRNA was extracted from livers of Control and MttpIKO mice treated either vehicle or T0901317(n=4/group). The mRNA expression of indicated gene was quantitated by qRT-PCR and the value is expressed as fold change related to vehicle-treated control,

which was defined as 1. The difference between values associated with different superscript letters in each parameter is statistically significant ($p<0.05$). Where no standard error is indicated, the values represent the mean from pooled samples.

SUPPLEMENTAL TABLE III

Serum and tissue oxysterol content

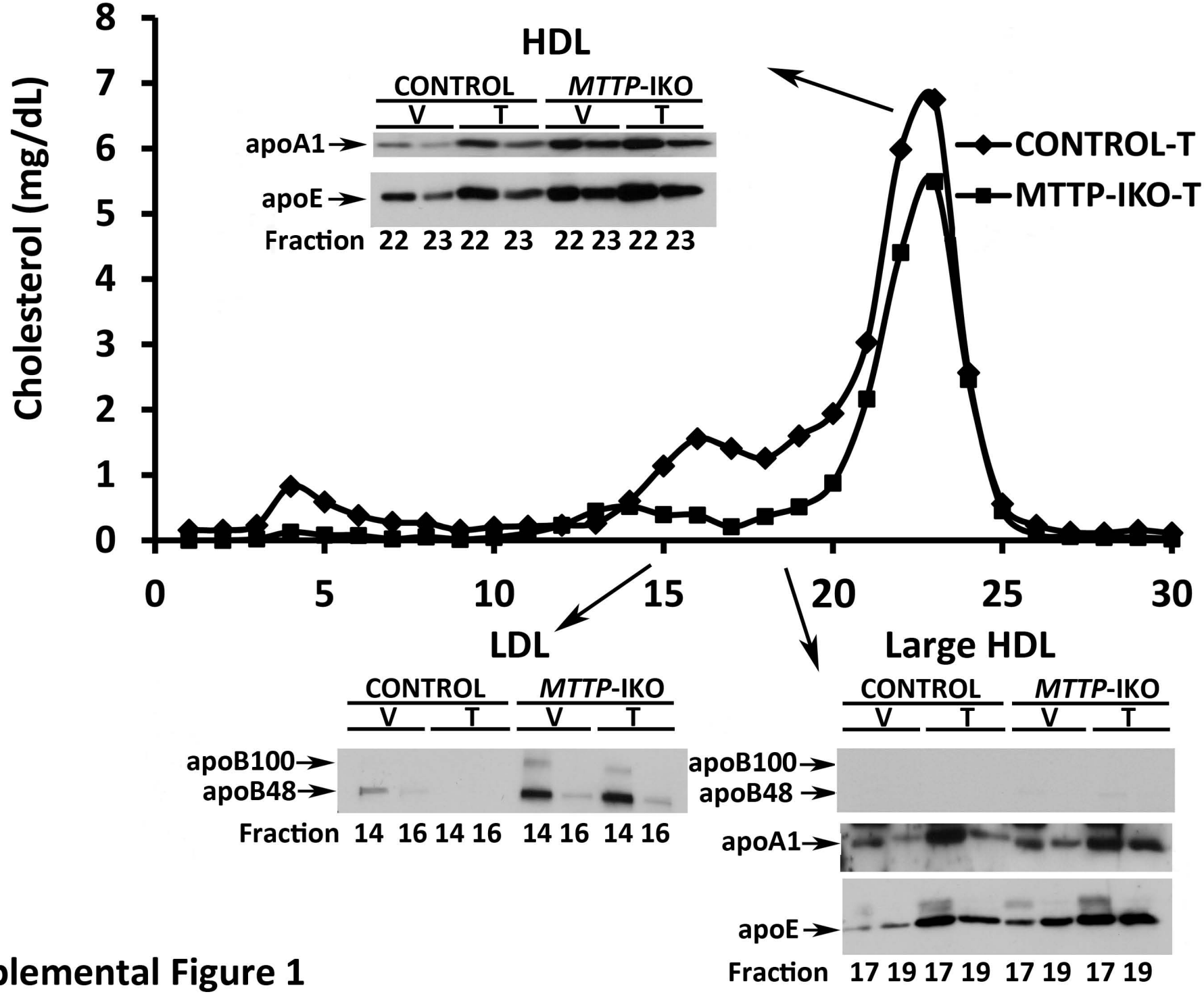
Genotype	27-hydroxycholesterol	24-hydroxycholesterol	25-hydroxycholesterol
Serum			
Control	35.28±0.89	18.78±2.98	11.27±0.72
MttpIKO	28.89±3.06	17.21±0.44	12.34±1.02
Liver			
Control	1.59±0.35	0.18±0.01	0.14±0.02
MttpIKO	1.42±0.12	0.17±0.02	0.26±0.04[a]
Intestine			
Control	0.2±0.02	0.26±0.04	0.37±0.04
MttpIKO	0.37±0.09[a]	0.83±0.28[a]	0.93±0.10[a]

Serum and tissue oxysterol content from control and MttpIKO mice (n=4 per group) were measured by GC- MassSpec (SUPPLEMENTAL METHODS). Oxysterol content was expressed as the mean ±SE ng/mg protein, except serum oxysterol, which is ng/ml. The difference between values associated with different superscript letters in each parameter is statistically significant ($p<0.05$).

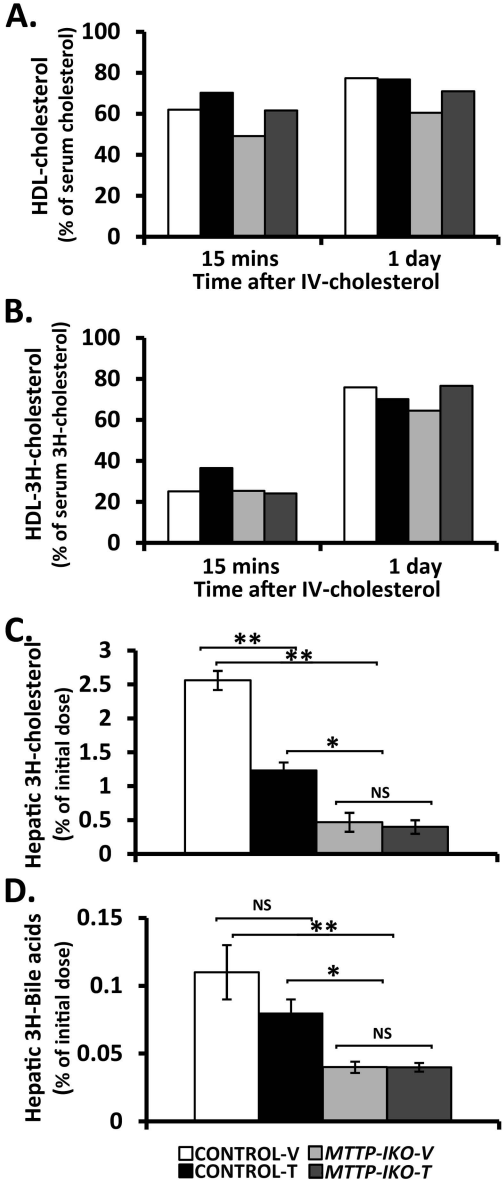
Supplemental FIGURE LEGENDS

Figure I. LXR agonist administration increases apoE-riched HDL. Lipoprotein in pooled serum of each experimental group (4-5 mice each group) was separated by FPLC. Apolipoprotein in the indicated fractions was detected by Western blot with corresponding antibodies. Apo B was detected in fraction 14 and 16, but not in Fraction 17 and 19, while apoA1 and apoE were detected in Fraction 17 to 23. Combined with the cholesterol profile, there is an increase in apoE-riched HDL by T0901317 treatment.

Figure II. A. and B. The lipoprotein distribution profile of intravenously injected ^{3}H-cholesterol was different from that of endogenous serum cholesterol. Total serum cholesterol content and HDL-cholesterol content were enzymatically measured in an aliquot of whole serum or serum depleted apoB-containing lipoprotein by heparine/manganese precipitation, respectively Pooled serum of 4-5 mice from each group was used for the measurement. ^{3}H-cholesterol in the same samples was quantified by LSC. **A.** Endogenous cholesterol distribution in HDL lipoprotein at 15mins- and 1 day after intravenous administration of ^{3}H-cholesterol. **B.** 3H-cholesterol distribution in HDL lipoprotein at 15mins- and 1 day after intravenous administration of ^{3}H-cholesterol. **C. and D. hepatic ^{3}H-cholesterol and ^{3}H-bile acids content 5 days after intravenous administration of 3H-cholesterol**. Hepatic radioactive cholesterol (C) and bile acids (D) were extracted and quantified in 4-5 samples from each group. Symbols * and ** indicate $p<0.05$ or $p<0.01$ respectively.



Supplemental Figure 1



Supplemental Figure 2