Figure S1. Experimental protocol. OCA, obeticholic acid; TC, total cholesterol; HDL, high-density lipoprotein; qPCR, quantitative polymerase chain reaction; WB, western blotting.

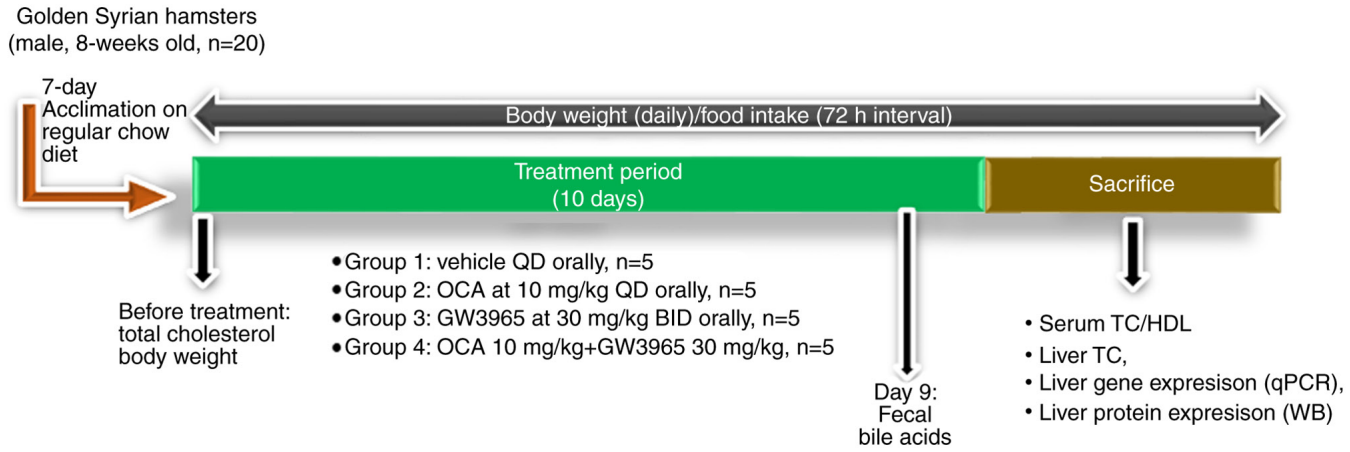

Figure S2. Synergistic activation of the mouse SR-BI reporter gene by LXR and FXR agonist combined treatment. (A) HepG2 cells were transfected with mouse SR-BI reporter gene plasmids containing site A, site B and site C segment of the first intron of mouse SR-BI gene. Transfected cells were treated with GW4064 (1 μM), OCA (10 μM) for 24 h prior to cell lysis. The data shown are summarized results of four separate transfection experiments. Statistical significance among all groups were assessed by one-way ANOVA with Tukey's multiple comparison test. **P<0.01 and P<0.001 compared with DMSO. (B) HepG2 cells were transfected with mouse SR-BI reporter gene plasmids. Transfected cells were treated with GW4064 (1 μM), OCA (10 μM), GW3965 (5 μM), GW3965 + GW4064 or GW3965 + OCA for 24 h prior to cell lysis. Data are presented as the mean ± standard error of the mean of four replicates per treatment and are expressed as the ratio of firefly/Renilla activity from each sample where the relative luminescence from DMSO-treated cells is set to 1. Statistical significance among all groups were assessed by one-way ANOVA with Tukey's multiple comparison rest. *P<0.05, **P<0.01 and ***P<0.001 compared with DMSO-treated samples; $^{\#\#\#}$P<0.001 compared with the cotreated samples. FXR, farnesoid X receptor; LXR, liver X receptor; LXRE, LXR response element; SR-BI, scavenger receptor class B type I; OCA, obeticholic acid; ANOVA, analysis of variance; n.s., not statistically significant.

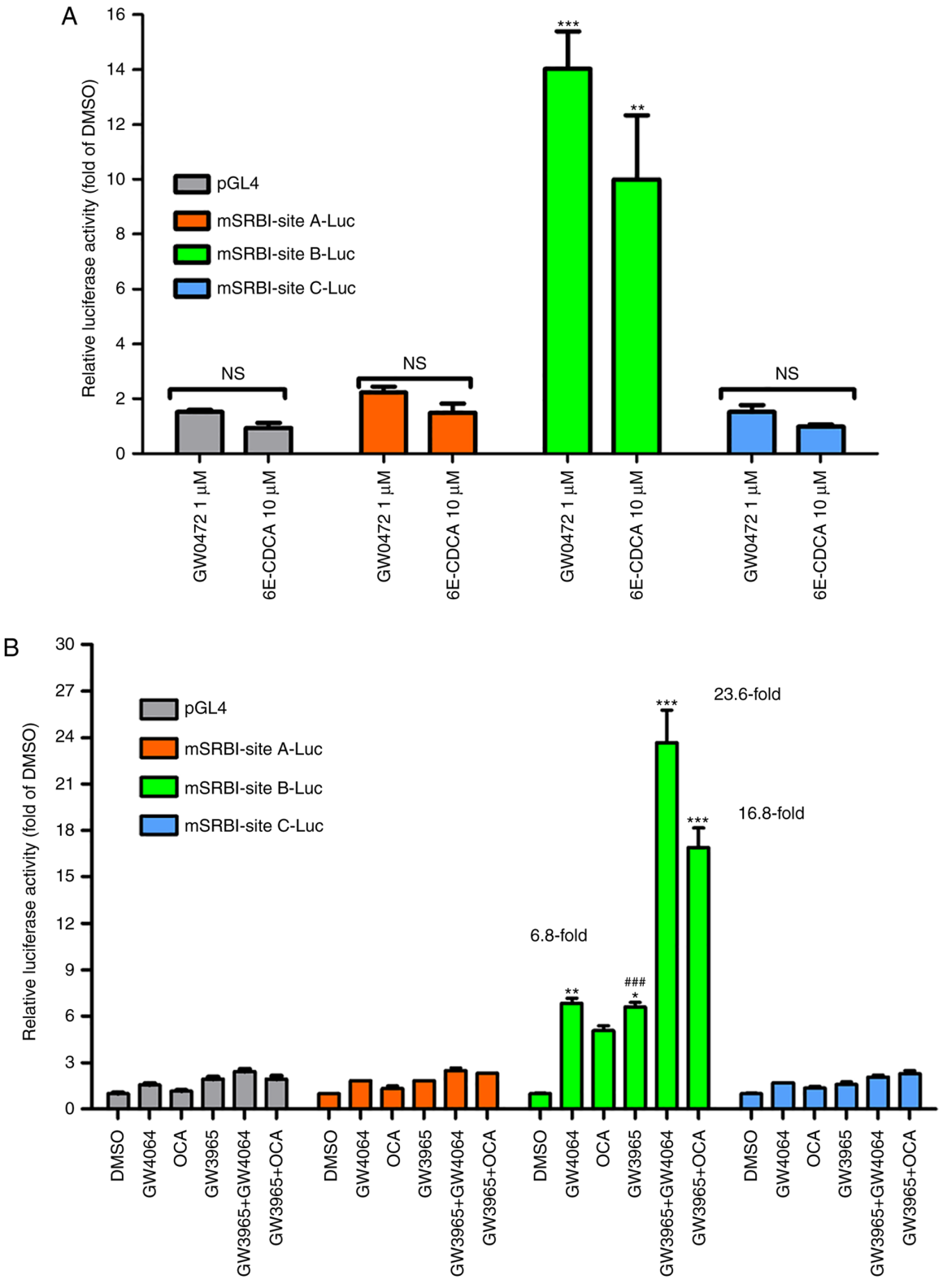

Figure S3. Examination of human SR-BI promoter constructs to ligand induced activation of farnesoid X receptor or liver X receptor. (A) Schematic presentation of human SR-BI promoter luciferase reporter constructs. (B) Control vector pGL3-basic and human SR-BI promoter reporters were transiently cotransfected with the pRL-TK vector into HepG2 cells with four wells per condition. At 1 day post-transfection, cells were incubated in 0.5% fetal bovine serum medium overnight, followed by treatment with OCA (10 μM) GW3965 (2 μM) or OCA + GW3965 for 24 h. Data are presented as the mean ± standard error of the mean of four replicates per treatment condition and are expressed as ratio of firefly/Renilla activity from each sample where the relative luminescence from pGL3-basic transfected cells without ligand treatment is set to 1. Statistical significance among all groups in each reporter were assessed by one-way ANOVA with Tukey's multiple comparison test. **P<0.05 and ***P<0.001 compared with DMSO-treated samples. (C) Data are presented as the mean ± standard error of the mean of four replicates per treatment and are expressed as ratio of firefly/Renilla activity from each sample where the relative luminescence from DMSO-treated cells is set to 1. Statistical significance among all groups from each reporter were assessed by one-way ANOVA with Tukey's multiple comparison test. **P<0.01 and ***P<0.001 compared with DMSO-treated samples. The data shown are representative of three separate transfection experiments. SR-BI, scavenger receptor class B type I; LXRE, LXR response element; TSS, transcription start site; OCA, obeticholic acid; ANOVA, analysis of variance.

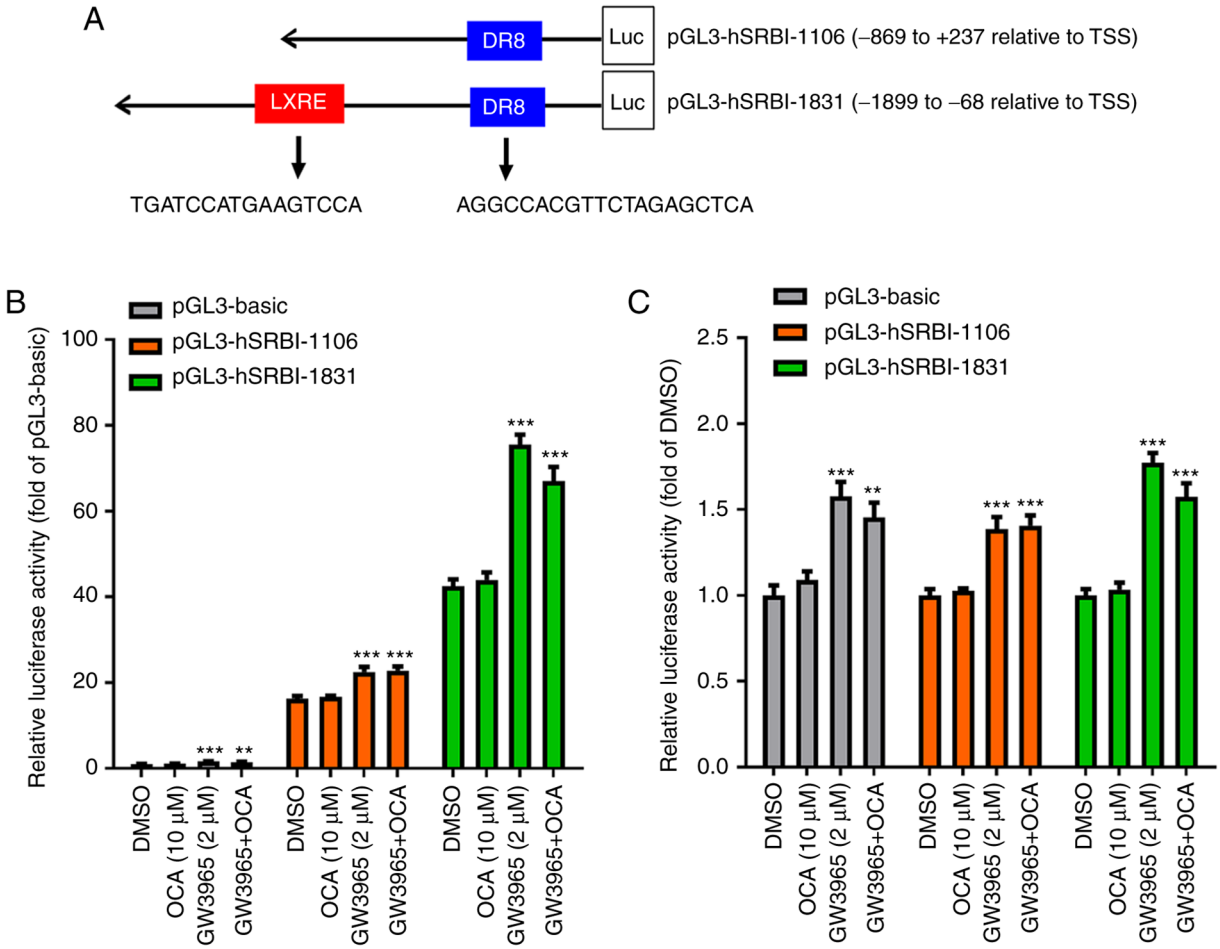

Figure S4. Effects of OCA, GW3965 and combined treatment on the body weight, food intake, liver weight and the liver index of hamsters fed a normal chow diet. Male hamsters fed a normal chow diet were treated by daily gavage with vehicle (n=5) or 10 mg/kg OCA (n=5), GW3965 30 mg/kg (n=5) or OCA + GW3965 for 10 days. Body weight and food intake were recorded throughout the treatment duration. Hamsters were fasted for 16 h prior to sacrifice for serum and liver tissue collection. Liver weight and body weight were measured. (A) Body weight measurement. (B) Food intake. *P<0.05 compared with Day 9-GW3965. (C) Liver weight. (D) Liver index. OCA, obeticholic acid; n.s., not statistically significant.

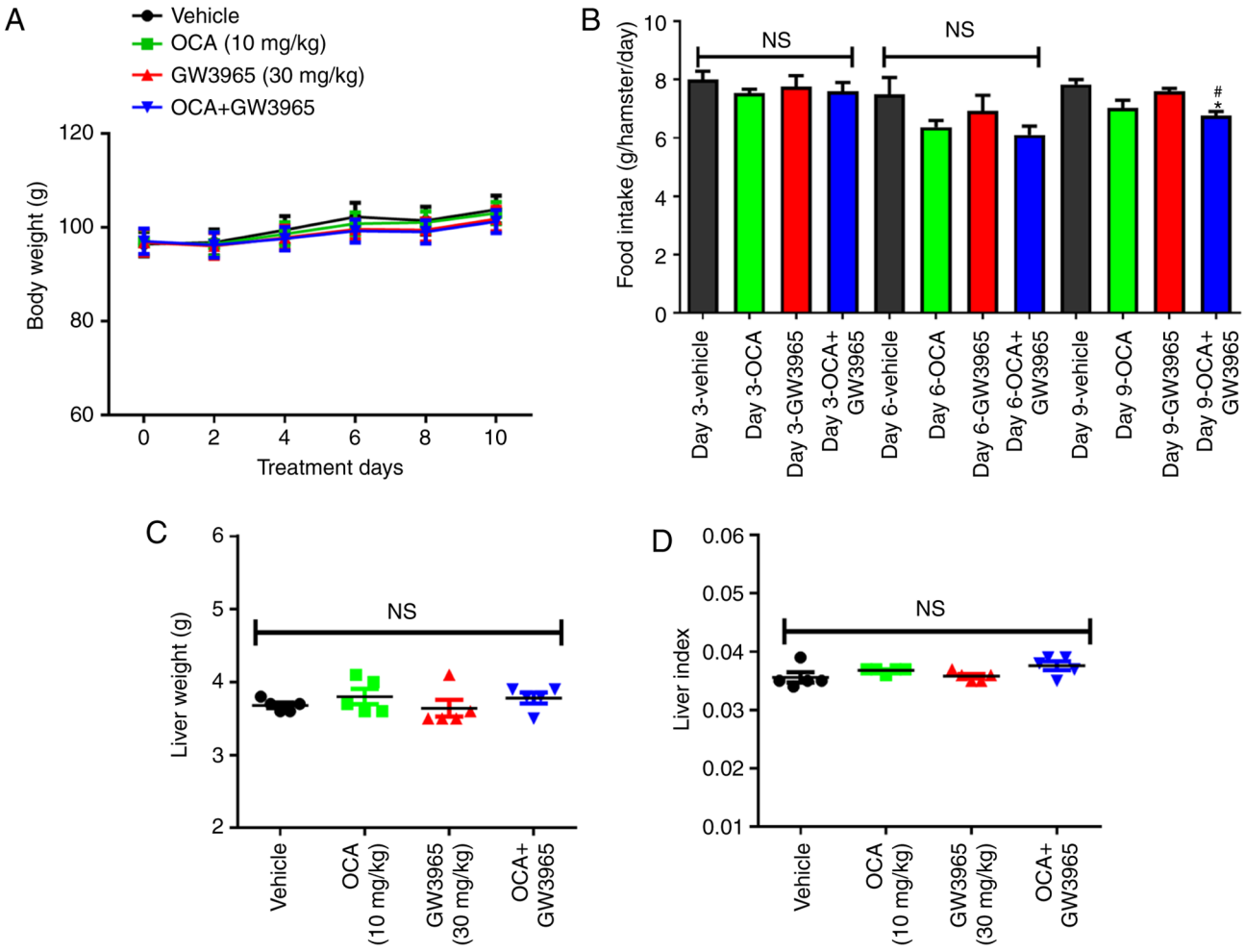

Figure S5. Treatment effects of OCA, GW3965 and combination on serum, hepatic and fecal lipid levels. Male hamsters fed a normal chow diet were treated by daily gavage with vehicle (n=5) or 10 mg/kg OCA (n=5), GW3965 30 mg/kg (n=5) and OCA 10 mg/kg + GW3965 30 mg/kg (n=5) for 10 days. Feces samples were collected on day 9 of treatment, dried and weighed. Hamsters were sacrificed and overnight fasting serum and livers were isolated at the termination of the experiment (day 11). Statistical significance among all groups were assessed by one-way ANOVA with Tukey's multiple comparison test. (A) TC levels were measured from all serum samples. Values are presented as the mean ± standard error of the mean of five hamsters per group. *P<0.05 compared with the vehicle control group; $^{\#\#}$P<0.01 compared with the GW3965 group. (B) HDL-C levels were measured from all serum samples. Values are presented as the mean ± standard error of the mean of five hamsters per group. $^{\#}$P<0.05 compared with the GW3965 group. (C) Lipids were extracted from individual liver samples, and TC was measured. Values are presented as the mean ± standard error of the mean of five hamsters per group. *P<0.05 and ***P<0.001 compared with the vehicle control group; $^{\#}$P<0.05 and $^{\#\#\#}$P<0.001 compared with the indicated treatment group. N=5 per group. (D) Lipids were also extracted from dried feces on day 9 and bile acids were measured. Values are presented as the mean ± standard error of the mean of three fecal samples per group. *P<0.05 and **P<0.01 compared with the control group; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 compared with the indicated treatment group. N=3 per group. OCA, obeticholic acid; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; ANOVA, analysis of variance.

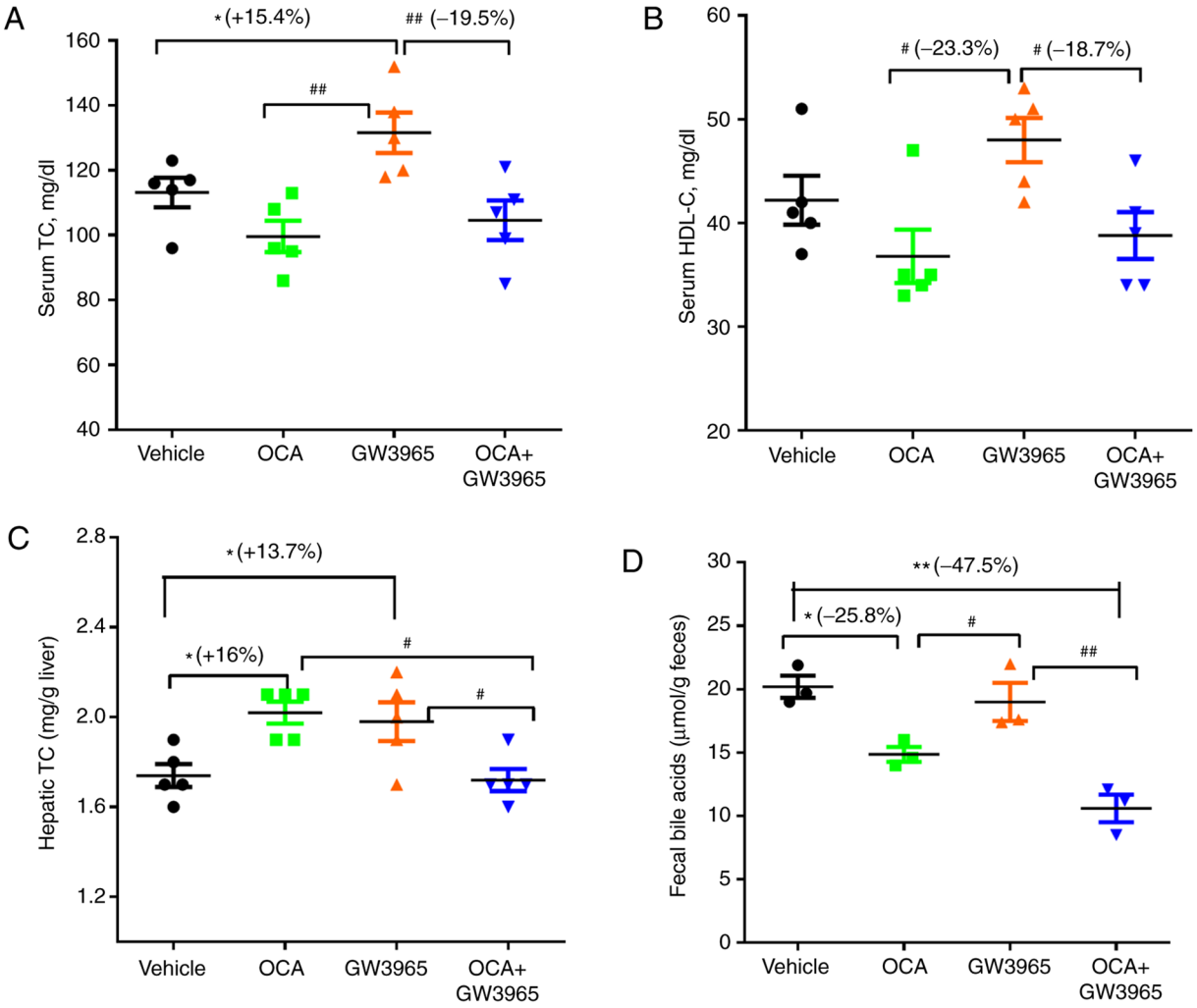

Table SI. Primers used in RT-qPCR analysis, cloning and EMSA.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
Hamster RT-qPCR		
SR-BI	GCGTGGACCCTATGTCTACAG	GTCAGGCTGGAAATGGAGGC
EL	ACGCTGGCAACTTTGTGAAA	AGGTATGCAGGACATCCACA
CYP7A1	TTCCTGCAACCTTCTGGAGC	GCCTCCTTGATGATGCTATCTAGT
SHP	AGGGAGGCCTTGGATGTC	AGAAGGACGGCAGGTTCC
GAPDH	AACTTTGGCATTGTGGAAGG	GGATGCAGGGATGATGTTCT
Human RT-qPCR		
SR-BI	ATGACTCCTGAGTCCTCGCT	AAACAGGGTTTTGGGAGCCA
ABCA1	AACTCTACATCTCCCTTCCCG	CTCCTGTCGCATGTCACTCC
ABCB4	GCGGTTCTACGACCCCTT	CCTGAGACACGATTCCGAGT
ABCG5	TGTCCTGAACATTCAAACCCT	CATCTGGACTCTCTTGGAGGT
BSEP	CATGGTGCAAGAAGTGCTGAGT	AAGCGATGAGCAACTGAAATGAT
CYP7A1	CACCTTGAGGACGGTTCCTA	CGATCCAAAGGGCATGTAGT
GAPDH	ATGGGGAAGGTGAAGGTCG	GGGGTCATTGATGGCAACAATA
Hamster intron-region cloning primers		
hamSRBI site-A	CATCTGCCTCCCACACTTGA	ATAGGGTCTCTGCTCAGCCT
hamSRBI site-B	GGTTTGGGTGGGCATCAACT	AGCCCTAGTGGCACCTTAAC
hamSRBI site-C	GTGCTTCCTGTCCCTGAGTC	ACTGCCAGTGGGTTTCTCTG
hamSRBI site-B FXR mu	AGTTTGAAGTCAGCCCAGATTGGTAT GACGCTTTGGGAAGACAAAGGTG	CACCTTTGTCTTCCCAAAGCGTCA TACCAATCTGGGCTGACTTCAAACT
hamSRBI site-B LXR mu	CCCCAGGGTCCCAAGCACAGACCTAACTT TGGTTCATGGCTTTTGTTCTTTCCTGC	GCAGGAAAGAACAAAAGCCATGAA CCAAAGTTAGGTCTGTGCTTGG GACCCTGGGG
EMSA probes		
hamSRBI-FXRE WT	AGTTTGAAGTCAGCCCAGAGGGGAATG ACCCTTTGGGAAGACAAAGGTG	CACCTTTGTCTTCCCAAAGGGTCATT CCCCTCTGGGCTGACTTCAAACT
hamSRBI-FXRE Mu	AGTTTGAAGTCAGCCCAGATTGGTATGA CGCTTTGGGAAGACAAAGGTG	CACCTTTGTCTTCCCAAAGCGTCATA CCAATCTGGGCTGACTTCAAACT
hamSRBI-LXRE WT	CAGGGTCCCAAGCACAGAGGTAACT TTGGTTCATGGCTTTTGTTCTTTCC	GGAAAGAACAAAAGCCATGAACCAA AGTTACCTCTGTGCTTGGGACCCTG
hamSRBI-LXRE Mu	CAGGGTCCCAAGCACAGACCTAACT TTGGTTCATGGCTTTTGTTCTTTCC	GGAAAGAACAAAAGCCATGAACCAA AGTTAGGTCTGTGCTTGGGACCCTG
SR-BI promoter cloning primers		
pGL3-hSRBI-1106	CCACGGTACCGGCTCCTCAGTCATTCA TTCTAG	CGAGGCTAGCGCACCATCACGATCA TGACAG
pGL3-hSRBI-1831	ACCCTCCTGTCCCTGTGTAA	CAGCCTTGGGCTTCAGGATT

RT-qPCR, reverse transcription-quantitative polymerase chain reaction; EMSA, electrophoresis mobility shift assay; SR-BI, scavenger receptor class B type I; EL, endothelial lipase; SHP, small heterodimer partner; BSEP, bile salt export pump; ABC, ABC, ATP binding cassette; FXR, farnesoid X receptor; FXRE, FXR response element; HPH, human primary hepatocytes; LXR, liver X receptor; LXRE, LXR response element; WT, wild-type; Mu, mutant.