Supplemental figure legends

Fig. S1. Effects of 3A-AHPC, an analogue of 3Cl-AHPC, on CYP7A1 expression.
HepG2 cells were treated with 200 nM 3-Cl-AHPC and then further treated with increasing concentrations of 3A-AHPC as indicated for 8 h and collected for qRT-PCR. The mRNA levels of CYP7A1 were measured by q-RTPCR and normalized to those of 36B4, SEM (n=3). Statistical significance was measured using the Student's t test. ** and NS, indicate $p<0.01$ and statistically not significant, respectively.

Fig. S2. Effects of 3Cl-AHPC or GW4064 on SHP expression.
HepG2 cells were treated with 200 nM 3-Cl-AHPC, 200 nM GW4064, a synthetic FXR agonist, or vehicle, for 8 h, and then cells were collected for qRT-PCR. The mRNA levels of SHP were measured by q-RTPCR and normalized to those of 36B4, SEM (n=3). Statistical significance was measured using the Student's t test. ** indicates $p<0.01$.

Fig. S3. 3Cl-AHPC increases SHP interaction selectively with Foxa2 but not with HNF-4.
A) Experimental outlines. (B, C) HepG2 cells were transfected with expression vectors for Foxa2, HNF-4, or Foxo-1 and then infected with Ad-flag-SHP. Twenty four h later, cells were treated with 200 nM 3Cl-AHPC for 2 h and cell extracts were prepared. Foxa2, HNF-4, Foxo-1, or control IgG was immunoprecipitated from HepG2 cell extracts. Flag-SHP in the immunoprecipitates was detected by western analysis using M2 antibody. Proteins in the input samples were also detected.

Fig. S4. Effects of overexpression of SHP or LRH-1 on CYP7A1 gene repression by 3-Cl-AHPC.
HepG2 cells were transfected with expression vectors for LRH-1 or SHP using the Neon transfection system (Invitrogen, Inc) and 24 h later, cells were treated with 200 nM 3-Cl-AHPC for 8 h. Then, cells were collected for qRT-PCR. The mRNA levels of CYP7A1 were measured by q-RTPCR and normalized to those of 36B4, SEM (n=3). Statistical significance was measured using the Student's t test. *, **, and NS, indicate $p<0.05$, $p<0.01$, and statistically not significant, respectively.

Fig. S5. 3Cl-AHPC treatment increases occupancy of SHP at the CYP8B1 gene.
(A) HepG2 cells were infected with Ad-flag-SHP, 24 h later, cells were treated with 100 nM 3Cl-AHPC or vehicle for 2 h, and occupancy of SHP was examined by ChIP assays. Semi-q-PCR was performed to detect occupancy at the CYP7A1 promoter. (B) HepG2 cells were treated with 100 nM 3-Cl-AHPC for 2 h, and occupancy of endogenous SHP and LRH-1 was examined by ChIP assays. Semi-q-PCR was performed to detect occupancy at the CYP7A1 promoter and the coding region of GAPDH as a negative control.

Supplemental Figures

Fig. S1

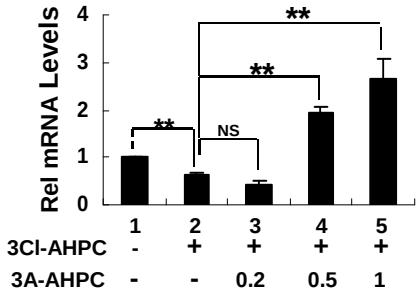

Fig. S2

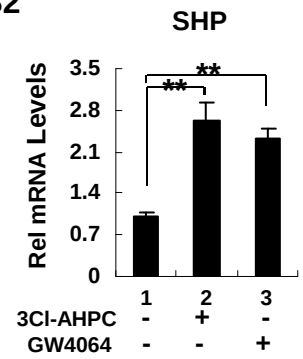

Fig. S3

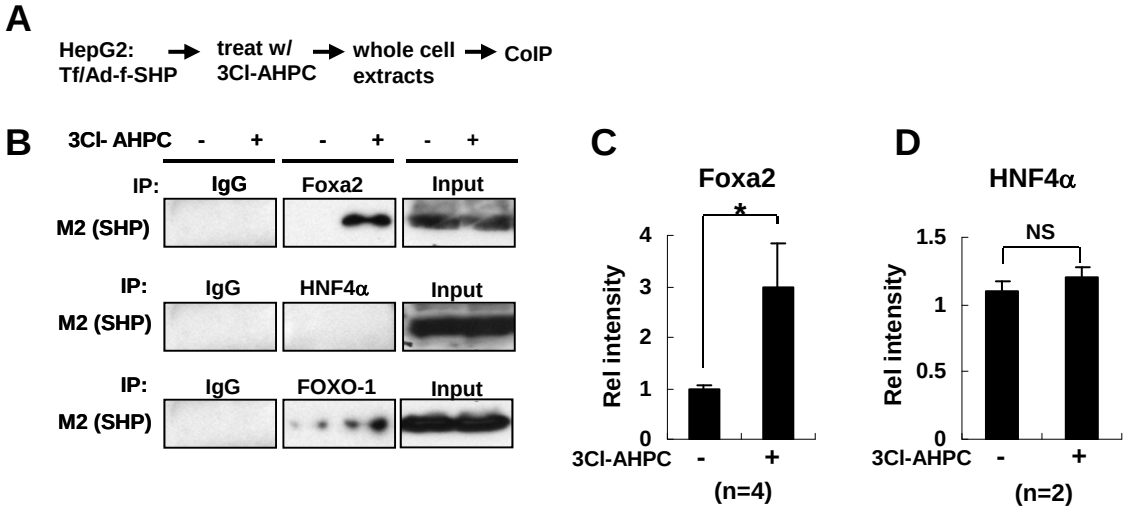

Fig. S4

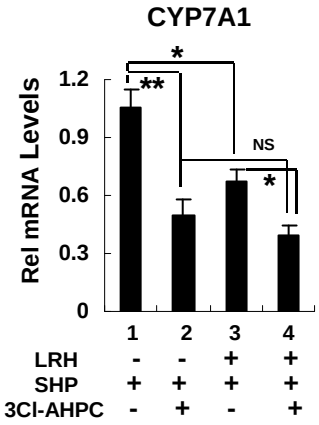

Fig. S5