Supporting Information

Kim and Lee 10.1073/pnas.1019678108

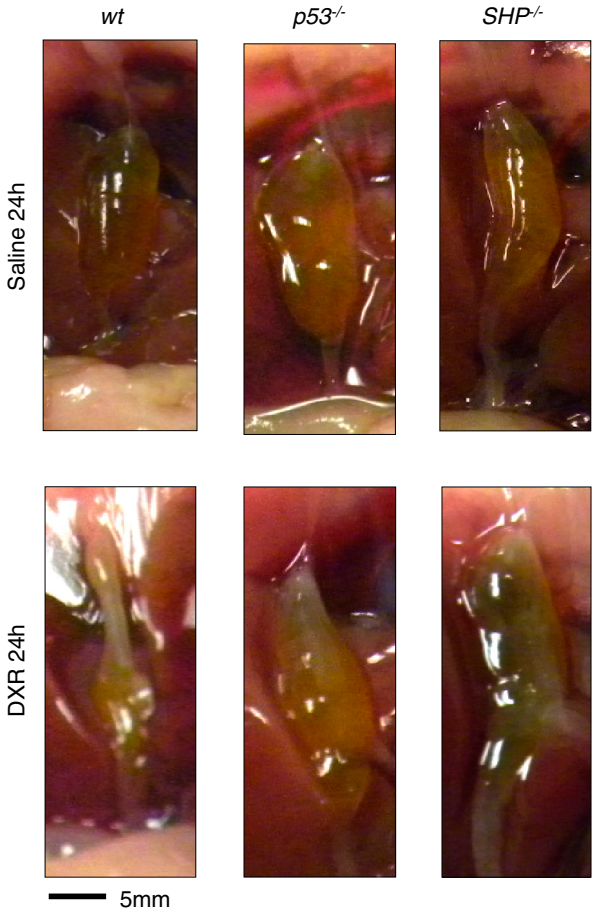

Fig. S1. Doxorubicin (DXR)-mediate shrinkage of gallbladders missing in p53-null and SHP (small heterodimer partner)-null mice. Representative pictures of gallbladders of wild-type, p53-null, and SHP-null male mice ($n = 3$ for each genotype) intraperintoneally injected with either saline or DXR for 24 h. Gallbladders of p53-null and SHP-null mice were significantly larger than those of wild-type mice in the absence of DXR. DXR treatment failed to elicit any significant changes with p53-null and SHP-null mice, whereas it resulted in empty gallbladders in wild-type mice. Similar results were obtained with female mice.

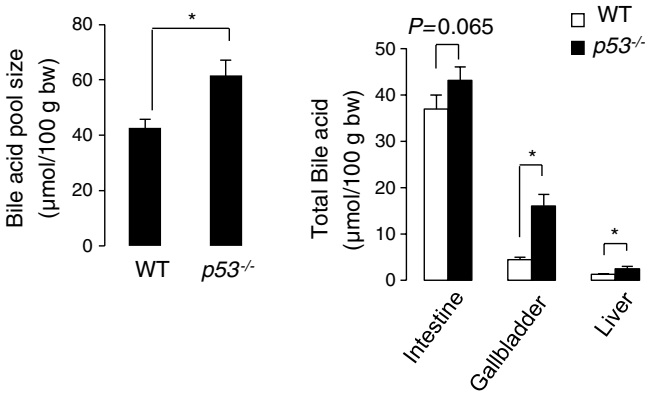

Fig. S2. An increased bile acid (BA) pool size in p53-null mice. Mice ($n = 3$ for each genotype) were treated with 10 mg/kg of DXR for 24 h before sacrificed and measured for BA levels in the intestine, gallbladder, and liver. BA levels in all three tissues were combined together (*Left*) or shown separately (*Right*). Upon deletion of *p*53, BA levels were increased more significantly in the gallbladder than in the liver and intestine (*Right*).

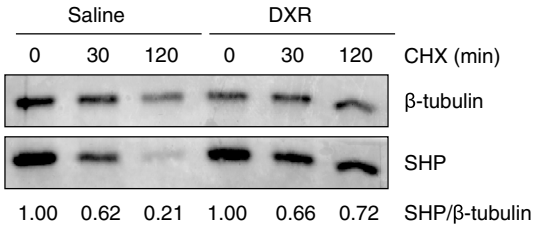

Fig. S3. Regulation of SHP protein stability by p53. HepG2 cells transfected with an expression vector for Flag-tagged SHP were treated with 10 μM of cyclohexamide (CHX), an inhibitor of protein synthesis, for 0, 30, and 120 min in the presence of either vehicle (saline) or 1 μM of DXR. The relative ratio of SHP to control protein (β-tubulin) is as shown below each SHP band.

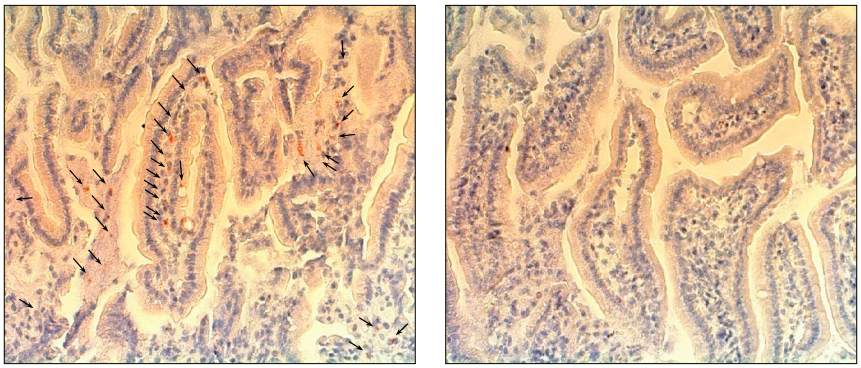

Fig. S4. Impaired lipid absorption in DXR-treated mice. By intraperitoneal injection 10 mg/kg of DXR dissolved in saline was administered. After 3 d, mice were fed triacylglycerol 1,2-dioleoyl-3-(pyren-1-yl) decanoyl-rac-glycerol using oral gavage. Small intestines of sacrificed mice were collected after 2 h, rinsed, and divided into three segments, duodenum, jejunum, and ileum. Tissues were fixed in 4% paraformaldehyde overnight at 4 °C and embedded in paraffin. Six-micrometer sections were stained with Oil-Red-O and hematoxylin. Arrowheads indicate Oil-Red-O-stained lipid droplets in a duodenum. No Oil-Red-O staining was visible in jejunum and ileum.

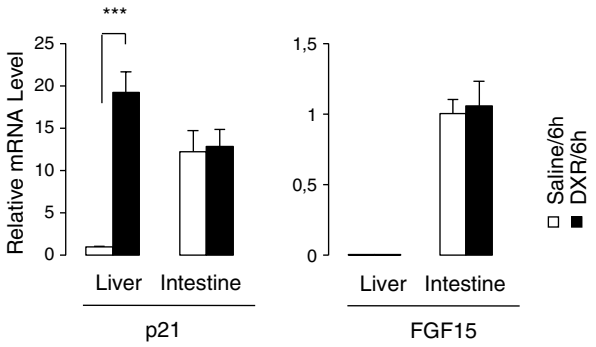

Fig. S5. DXR fails to regulate FGF15 expression. Mice ($n = 4$) were treated with 10 mg/kg of DXR for 6 h before being sacrificed and measured for mRNA levels of p21 (positive control) and FGF15 in the liver and intestine. FGF15 was expressed only in the intestine. No significant changes were observed for FGF15 levels in the intestine at least upon 6 h DXR treatment. Interestingly, levels of p21 in the intestine were much higher than those in the liver.

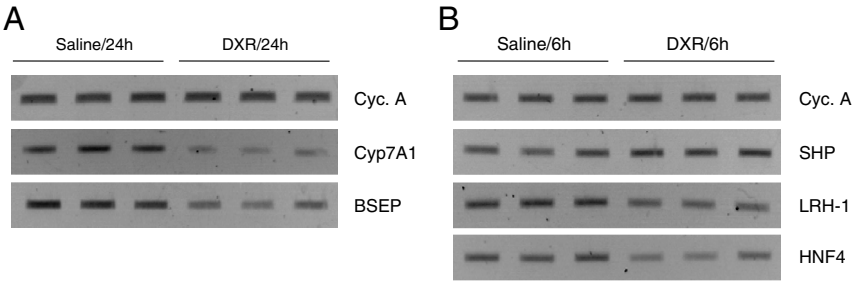

Fig. S6. Down-regulation of bile salt export pump (BSEP), liver receptor homolog 1 (LRH-1), and HNF4 by DXR. (*A*) By intraperitoneal injection 10 mg/kg of DXR dissolved in saline was administered into wild-type mice ($n = 3$). After 24 h, mice were sacrificed and examined for hepatic levels of mRNAs for Cyclophilin A (Cyc. A; control), Cyp7A1, and BSEP using RT-PCR. Expression of BSEP and Cyp7A1 was similarly down-regulated by DXR. (*B*) By intraperitoneal injection 10 mg/kg of DXR dissolved in saline was administered into wild-type mice ($n = 3$). After 6 h, mice were sacrificed and examined for hepatic levels of mRNAs for Cyc. A (control), SHP, LRH-1, and HNF4 using RT-PCR. Although SHP expression in DXR-treated mice appeared to be still higher than that of saline-treated mice, expression of LRH1 and HNF4 was clearly inhibited by DXR.

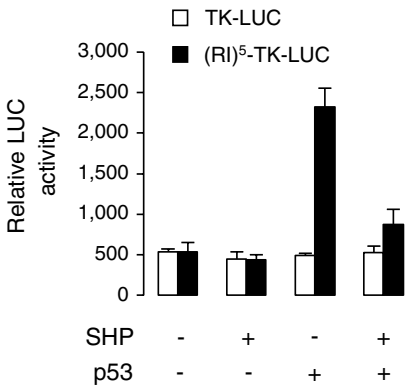

Fig. S7. Inhibition of p53 transactivation of $(RI)^5$-TK-LUC (TK, thymidine kinase; LUC, luciferase) by SHP. In HEK293 cells transfected with expression vectors for SHP and p53 as well as p53-responsive $(RI)^5$-TK-LUC and control TK-LUC reporters were monitored for their luciferase activities. In these experiments, SHP represses p53 transactivation of $(RI)^5$-TK-LUC but not of TK-LUC.