Plasmids

The expression plasmids for the human PXR receptor pSG5-hPXR were kindly provided by Dr. S. Kliewer (University of Texas, Dallas, TX, USA). The pGL5-luc (a luciferase gene reporter vector with five copies of the GAL4 consensus binding site), pGL4.10 and pRL-TK constructs were purchased from Promega (Madison, WI, USA). The mammalian two-hybrid fusion plasmids pM-GAL4-PXR LBDwt along with a pM-GAL4-PXR LBDmut (S247W/C284W) mutant construct have previously been described (Takeshita *et al.*, 2002; Wang *et al.*, 2007). The constitutively active PXR double mutant (S247W/C284W) was generated by substituting serine at position 247, located in the ligand binding pocket of PXR, with the larger tryptophan, which sterically obstructs the ligand binding pocket of PXR (Wang *et al.*, 2007). The PXR T422 and T248 mutants were described in our previous report (Doricakova *et al.*, 2013). PXR T248D mutant has a significant constitutive activity. PXR T422D mutant has a phospho-mimetic mutation in tyrosine residue in the activation function-2 (AF2) domain; the domain is responsible for interaction with coactivators (Doricakova *et al.*, 2013). PXR responsive construct p3A4-luc has been described in our previous report (Doricakova *et al.*, 2013). pcDNA3/HA-hPGC1α expression construct for peroxisome proliferator-activated receptor γ coactivator 1α (PPARGC1A-PGC1α) was kindly donated by Prof. Ramiro Jover (Valencia, Spain). pSG5-FLAG-hSRC-1 expression construct tagged with FLAG was graciously donated by Prof. B.W O'Malley (Baylor College of Medicine, Houston, TX). pcDNA3-HNF4a2 expression plasmid was kindly donated by Dr. B. Laine (INSERM Unit 459, Lille, France). siRNA targeting PXR and non-targeting siRNA (scrambled, siSCT) were purchased from Dharmacon (Lafayette, CO, USA). siRNA targeting HNF4 were from Origene.

The site-directed mutations in the E-box (Kajiwara *et al.*, 2008) at -95/-90 and HNF4α-responsive DR-2 motifs (Saborowski *et al.*, 2006) were introduced into the pOCT1 1.8 kb-luc and/or pOCT1(-99/+102)-luc constructs using the GENEART Site-Directed Mutagenesis System (Invitrogen, Life Technologies), with the primers listed in Table 1. All mutation constructs for the reporter assays were sequenced using an ABI PRISM^{TM} 3100 Genetic Analyzer. Other plasmids are described in Supplementary material.

In silico computational sequence analysis of the OCT1 (SLC22A1) gene promoter region has been performed using TRANSFAC 6.0, but we did not find any putative PXR binding sites.

Cell lines, HeparRG and primary human hepatocyte cells cultivation protocols

Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% FBS. The media for HepG2 and HeLa cells were supplemented with 1% nonessential amino acids. Fetal bovine serum (FBS) was purchased from PAA (Pasching, Austria). Phenol red-free Opti-MEM® medium was purchased from Invitrogen/Life Technologies (Carlsbad, CA, USA). All other chemicals were of the highest quality commercially available. Since the hepatic tumour cell lines HepG2 and HuH7 lack appropriate hepatic phenotype of differentiated hepatocytes and do not express enough functional PXR under normal conditions, we transfected the cell lines with exogenous PXR. In addition, we perform experiments in parallel in differentiated HepaRG cells and primary human hepatocyte models, which express endogenous PXR.
HepaRG cell line was cultivated in William's Medium E (WME) containing 10% FBS, 2 mM glutamine (Sigma-Aldrich, St. Louis, MO), 1% Pen/Strep, 5 μg/μl insulin (Sigma-Aldrich, St. Louis, MO) and 50 μM hydrocortisone (Sigma-Aldrich, St. Louis, MO). For the induction of differentiation, a two-step protocol was used as previously described (Gripon *et al.*, 2002). The HepaRG cells were seeded at low density (2.6×10^4 cells/cm^2) and maintained in culture medium for two weeks. After this period HepaRG cells were incubated for another two weeks in the same cultivating medium with the addition of 1.5% DMSO to promote their differentiation.
All cell lines except the HepaRG one were cultivated in antibiotic-free medium and used within 25 passages after delivery.

In the Czech Republic the primary human hepatocytes were isolated, cultivated and treated as previously described (Vrzal *et al.*, 2009). Following isolation, the cells were plated on collagen-coated culture dishes at a density of 1.4×10^5 cells/cm^2. The medium was replaced with serum-free medium the day after delivery, and the cultures were allowed to stabilize for an additional 48–72 hours prior to treatment. The cultures were maintained at 37°C in 5% CO_2 in a humidified incubator.
In France the primary human hepatocytes were isolated as previously described (Pichard-Garcia *et al.*, 2002). The hepatocytes were taken from human donor.

Preparations LH18-LH21 have been described in (Vrzal *et al.*, 2009). LH42 and commercial hepatocytes Batch No. HEP220797 (Biopredic International, Rennes, France) have been described in our latest report (Smutny *et al.*, 2014). Commercial batches of long-term primary human hepatocytes grown in monolayers HEP220650 (female, 25 years, not available), HEP220879 (female, 65 years, liver metastases from colorectal cancer), HEP220797 (female, 66 years liver metastases from colorectal cancer) were obtained from Biopredic. Primary human hepatocytes batch no. Lot 140-130614-2 (male, 54 years) were purchased from Primacyte, Schwerin, Germany. Primary hepatocytes isolated in Montpellier were FH 397 (female, from Marseille 65 years), FH 398 (male, Nimes, 76 years), FT403 (female, Montpellier, 45 years) and FT404 (female, Montpelier, data not available).

Transient gene reporter assays

All the gene reporter assays were performed in antibiotic-free medium. The cells were seeded into 48-well plates and transfected with a pOCT1-luc gene promoter luciferase reporter construct (150 ng/well), a PXR expression plasmid, the HNF4α expression construct (100 ng/well) or a co-activator expression vector (100, 200, 400 ng/well) and the *Renilla reniformis* luciferase transfection control plasmid (pRL-TK) (30 ng/well) after 24 hours. The next step consisted of stabilizing the cells for 24 hours prior to treatment and continuing to maintain them in medium (200 µl) containing rifampicin or hyperforin at the indicated concentrations for an additional 24 hours. Rifampicin and hyperforin have been used in the standard concentration known to activate PXR (10 µM). Higher concentrations saturate PXR without further significant activation of PXR target genes. Standard treatment interval for induction studies have been used for gene reporter, RT-PCR (24 h) and western blotting experiments (48 and 96 h). These intervals reflect maximal endpoint activation/induction. After the treatment, the cells were lysed and luciferase activity was measured with the Dual Luciferase Assay Kit (Promega). The data are expressed as the fold change in firefly luciferase activity normalized to *Renilla* luciferase activity in each sample relative to the vehicle (DMSO 0.1 %)-treated controls, which were normalized to 1. When necessary, an equivalent quantity of empty vector (pSG5, pDNA3) was co-transfected to maintain a constant amount of DNA in the transfection mixture.

Adherent human hepatocytes were transfected with 10 nM or 20 nM non-targeting siRNA (scrambled, siSC) or 10 nM or 20 nM siRNAs specific for *PXR* or *HNF4*, respectively at day 1

and day 3 after seeding using Lipofectamine RNAiMAX (Life Technologies, Carlsbad, CA). At day 5 post-seeding, human hepatocytes were treated with 10 μM rifampicin for 24 h. Adherent differentiated HepaRG cells were transfected with 10 nM siPXR three times within a 48 hours interval and treated 24 hours later with rifampicin for 24 h. All cells were maintained in the same cultivating medium as described above.

qRT-PCR

qRT-PCR in Czech Republic

At this point the cells were carefully washed with phosphate buffered saline (PBS) and treated with 800 μl of TRI Reagent (Sigma-Aldrich, St. Louis, MO). RNA isolation was performed according to manufacturer's protocol. After diluting the mRNA to an appropriate concentration (350 ng/μl), all the samples were transcribed to cDNA with the use of a Tetro cDNA synthesis kit (Bioline, Taunton, MA) according to manufacturer's protocol. qRT-PCR reactions were performed on a StepOnePlus system (Life Technologies, Carlsbad, CA) with the commercially available master mix SensiFAST Probe Hi-ROX Kit (Bioline, Tauton, MA). All used primers/TaqMan probe systems were purchased from Generi Biotech (Hradec Králové, Czech Republic)(hHPRT_Q1; hGADPH_Q1; hCYP3A4_Q1, hPXR_Q1) or from Life Technologies (Carslbad, CA) (SLC22A1 Cat No RPLP0, PXR, HNF4α). The expressions of the OCT1 (SLC22A1) gene were normalized to at least two reference genes, with data presented as fold activation relative to the control (vehicle-treated) samples (set to 1). All samples were run simultaneously in triplicates with negative controls. Data are means ± standard deviations (SDs) from at least three independent experiments (n = 3). The delta-delta method was used to calculate relative expression of tested genes mRNAs normalized to reference genes.

qRT-PCR in France

After extraction with Trizol reagent (Life Technologies), 500 ng of total RNA was reverse-transcribed using a random hexaprimer and the MMLV Reverse Transcriptase Kit (Life Technologies). Quantitative polymerase chain reactions were performed using the Roche SYBR Green reagent and a LightCycler 480 apparatus (Roche Diagnostic, Meylan, France). The amplification specificity was evaluated by determining the product melting curve. Results are expressed as indicated in the figure legends. The following program was used: one step at

95°C for 10 min and then 50 cycles of denaturation at 95°C for 10 s, annealing at 65°C for 15 s and elongation at 72°C for 15 s.

Table 1. Primers sequences

RPLP0	TCGACAATGGCAGCATCTAC	GCCTTGACCTTTTCAGCAAG
CYP3A4	GCCTGGTGCTCCTCTATCTA	GGCTGTTGACCATCATAAAAG
PXR	GGACCAGCTGCAGGAGCAAT	CATGAGGGGCGTAGCAAAGG
HNF4	CGCAGATGTGTGTGAGTCCA	CAGTGCCGAGGGACAATGTA

Table 2. Primer sequences

Primers for OCT1 reporter vectors construction

(KpnI)	5'-GGGGTACCGCCATGCCAAATGGCCAG-3'	-1649/+102
(KpnI)	5'-CCGGTACCTCCCTTCTTGTGTCAGTAG-3'	-1458/+102
(KpnI)	5'-GGGGTACCGTATACTGCTTGGGAGATGG-3'	-430/+102
(KpnI)	5'-GGGGTACCCACTGACTCGCTCCCGGGC-3'	-99/+102
(XhoI)	3'-GGCTCGAGGTCTCCCTCAGAGATCTTTG-5'	

Primers for the site-directed mutagenesis

HNF4α response elements mutagenesis primers

Forward

5'-CAGCTATGGA**C**CCCTATT**C**ACCCTGGAGTCCTGTTCATCTCTTCTCCTTCCCTTC-3'

Reverse

3'-GAAGGGAAGGAGAAGAGTAACAGGACTCCAGGGT**G**AATAGGG**G**TCCATAGCTG-5'

E-box mutagenesis primers

Forward 5'-CGATTTGATCAGATGGCCA**AA**TGCATTCTTCCTTTTCCTG

Reverse 3'-CAGGAAAAGGAAGAATGCA**TT**TGGCCATCTGATCAAATCG

Primers and probes for qPCR in CHIP

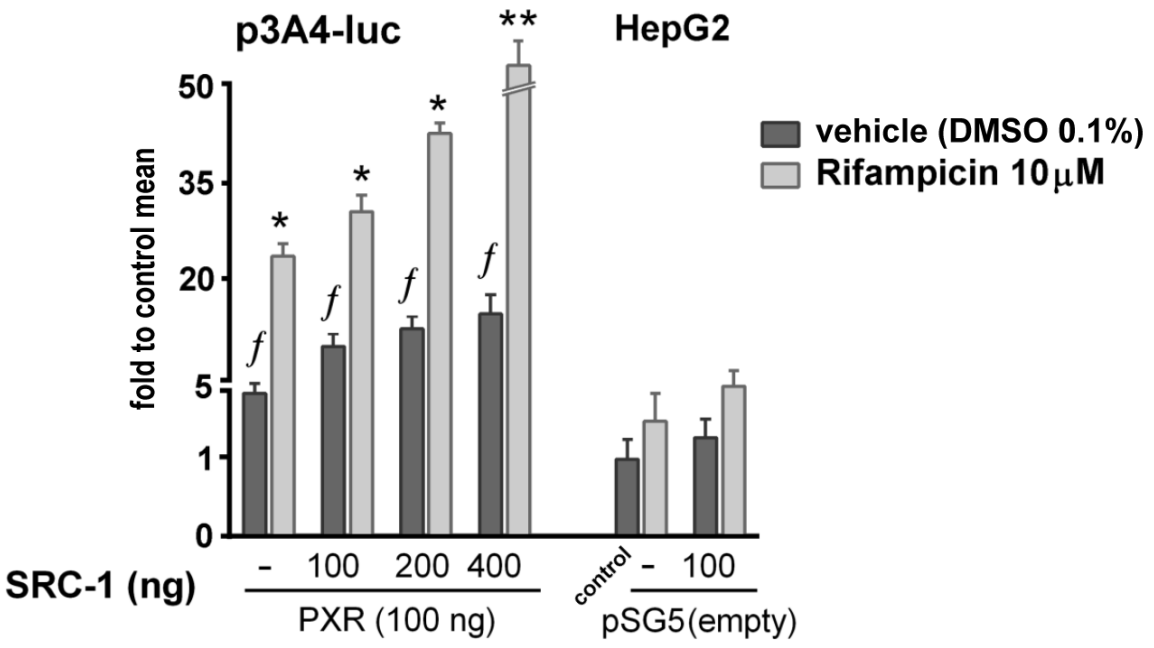
HNF4α response element

Forward primer	5'-CCTTCCCCTCAGCTATGG-3'
Reverse primer	3'-AGCTACTGACACAAGAAGGGA -5'
TaqMan probe	5'-CCCTATTGACCCTGGAGTCC-3'

E-box

Forward primer	5'-TGAGGTCTAACTATTTCCAGCAT-3'
Reverse primer	3'-AACTGCTTTGGCTTTTGAA-5'
TaqMan probe	5'-CAGGCCCTACCAAACTGCAA-3'

Figure Supplementary data



Cotransfection of SRC-1 significantly stimulates PXR-mediated transactivation of CYP3A4 gene promoter p3A4-luc construct. HepG2 cells were co-transfected with the p3A4-luc reporter construct (which is highly responsive to PXR activation), pSG5-hPXR expression construct and with increasing amount (100, 200 or 400 ng per well) of SCR-1. Cells were then treated with rifampicin (10 μM) or with vehicle (-) for 24 h. Activity of p3A4-luc gene reporter construct was detected with the use of Dual-Luciferase Reporter Assay (Promega). All data are

expressed as the fold change in activation relative to control vehicle-treated cells transfected with only empty pSG5 plasmid (normalized to 1).

*$p<0.05$ indicates statistically significant inducible activation of p3A4-luc construct by rifampicin in comparison with samples transfected with the same expression constructs; $^{f}p < 0.05$ indicates statistically significant augmentation of the *basal* p3A4-luc promoter construct activity by PXR and SRC1 expression vectors in the absence of rifampicin. ANOVA with Dunnett's *posthoc* test was used.

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