Hoffart et al. Supplemental Data

Supplemental Methods

Mammalian Two-Hybrid CAR Coactivator/Corepressor Interaction Assays

Two hybrid assays for the analysis of CAR interactions with coactivators were performed as described previously (Arnold et al., 2004). For the analysis of the interaction of CAR with corepressor SMRT, COS1 cells, plated at 3 x 10^4 cells per well of a 24-well plate the day before, were transfected with 120 ng of reporter gene plasmid pGL3-G5, 10 ng of expression plasmid encoding a fusion protein of the GAL4-DBD and RID of human corepressor SMRT (NCOR2, amino acids 1109-1330), and 80 ng of expression plasmid encoding a fusion protein of VP16-AD and CAR-LBD (amino acids 105-348) or 80 ng of empty vector pVP16-AD. 20 ng of β-galactosidase expression plasmid pCMVβ and 10 ng of an expression plasmid encoding human RXRα were always co-transfected.

Plasmids

The expression plasmids encoding fusion proteins VP16-AD/CAR-LBD (105-348) and VP16-AD/CAR-SV2-LBD (105-353) have been described previously (Arnold et al., 2004). The human RXRα expression plasmid pcDhRXRα(orf) was constructed as follows: the 5'-part of human RXRα open reading frame was amplified by PCR out of pCMX-hRXRα (Geick et al., 2001) with primers 5'-ACA GAA TTC CAC CAT GGA CAC CAA ACA TTT CCT GCC G-3', introducing EcoRI restriction site and optimized Kozak consensus sequence and 5'-GTC AAT CAG GCA GTC CTT GTT GTC G-3', residing 3' of an internal BsrGI restriction site. EcoRI/BsrGI digested PCR fragment was ligated together with BsrGI/ApaI fragment (3' part of open reading frame; ApaI site 11 bp 3' of stop codon) of pCMX-hRXRα into EcoRI/ApaI digested vector pcDNA3. The identity of the PCR-derived part was verified by sequencing.

Supplemental References

Arnold KA, Eichelbaum M, Burk O (2004). Alternative splicing affects the function and tissue-specific expression of the human constitutive androstane receptor. *Nuclear Receptor* **2:** 1.

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Geick A, Eichelbaum M, Burk O (2001). Nuclear receptor response elements mediate induction of intestinal MDR1 by rifampin. *J Biol Chem* **276:** 14581-14587.

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Wolbold R, Klein K, Burk O, Nüssler AK, Neuhaus P, Eichelbaum M, Schwab M, and Zanger UM (2003). Sex is a major determinant of CYP3A4 expression in human liver. *Hepatology* **38:**978-988.

Supplemental Table 1: Hepatocyte donor data

ID	Sex	Age	Diagnosis
BH-36	M	?	liver tumour
BH-38	M	65	liver metastasis CRC
BH-42[a]	F	69	liver tumour
BH-43[a]	M	60	liver metastasis CRC
BH-53	F	86	extrahepatic bile duct carcinoma
BH-54	M	53	liver metastasis CRC
GH-07	M	65	liver metastasis CRC
GH-13[a]	M	66	liver metastasis CRC
GH-14[a]	F	20	HCC
GH-19[b]	F	62	HCC
RH-06	F	21	FNH
RH-07	M	56	HCC
RH-14[b]	F	45	liver metastasis CRC
RH-15[b]	F	71	HCC

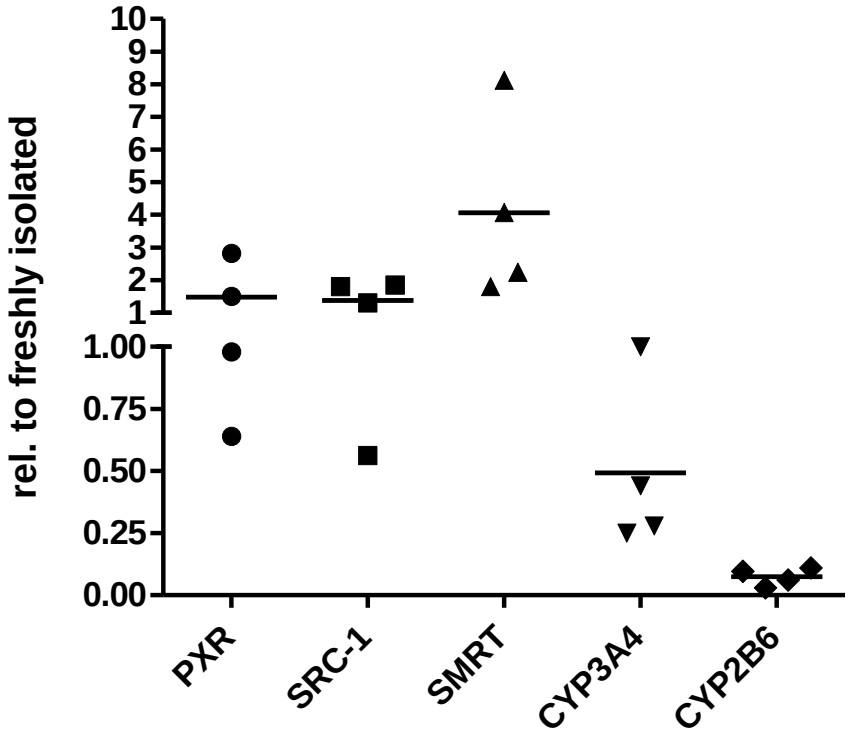
?, unknown; CRC, colorectal carcinoma; FNH, follicular nodular haemangioma; HCC, hepatocellular carcinoma; [a], used in gene expression analysis of freshly isolated and plated hepatocytes (Supplemental Figure S1); [b], used in protein analysis by Western blotting (Figure 6)

Supplemental Table 2: Oligonucleotides for quantitative real-time RT-PCR

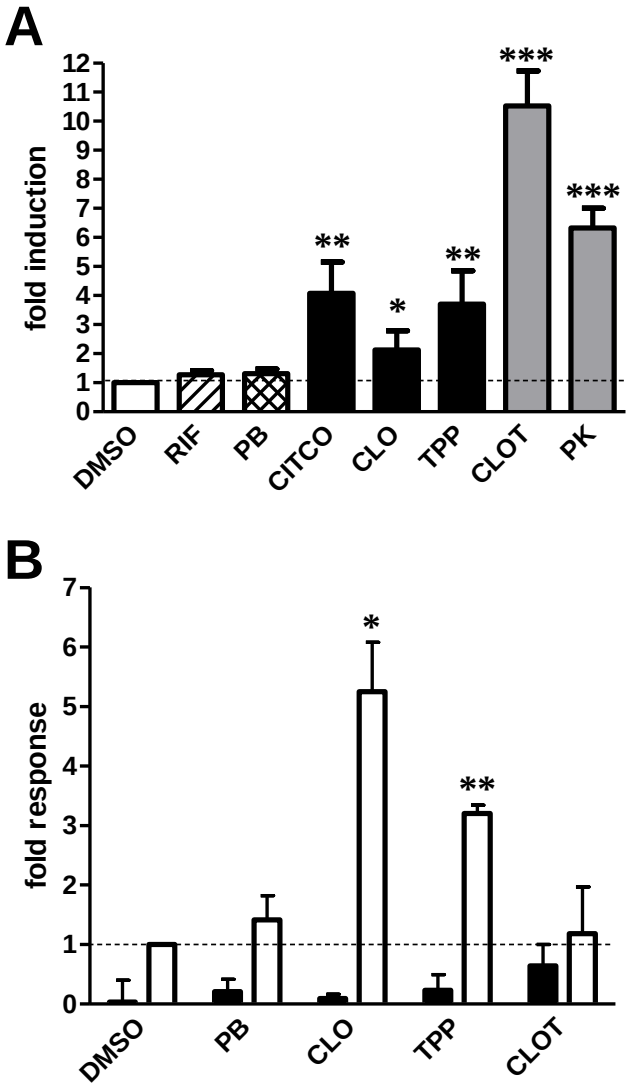
Gene	Primer/ Probe	Sequence	Concentration (nM)
18S	forward primer	5'-accgcagctaggaataatgga-3'	400
	reverse primer	5'-gcctcagttccgaaaacca-3'	400
	probe	5'-VIC-accgcggttctattt-MGB-3'	200
ABCB1[a]	forward primer	5'-ctggtgtttggagaaatgacagata-3'	900
	reverse primer	5'-tggtcatgtcttcctccagattc-3'	900
	probe	5'-FAM-tcaaacatcactaatagaag-MGB-3'	240
CYP2B6[a]	forward primer	5'-gctgaacttgttctaccagacttttc-3'	400
	reverse primer	5'-gaaagtatttcaagaagccagagaagag-3'	400
	probe	5'-FAM-tgtattcggccagctgt-MGB-3'	400
CYP3A4[b]	forward primer	5'-tgtcctaccataagggcttttgtat-3'	400
	reverse primer	5'-ttcactagcactgttttgatcatgtc-3'	400
	probe	5'-FAM-cttttatgatggtcaacagcctgtgctg-TAMRA-3'	200
EPHX1[c]	forward primer	5'-gaagcgacagcagtgcttctc-3'	300
	reverse primer	5'- gcaaagcccagcactgaagt-3'	300
	probe	5'-FAM- tgaggtacaggagccatg-MGB-3'	250
PXR[d]	forward primer	5'--tcctttgcaccggattgttc--3'	400
	reverse primer	5'-tccagctttctttgggtctca-3'	400
	probe	5'-FAM-caccaagcagtccaaga-MGB-3'	200
SLCO1B1	forward primer	5'-caacagtatggtcagccttcatct-3'	400
	reverse primer	5'- ttccacttgcaaaaataggtatgg-3'	400
	probe	5'-FAM- ctaacatcttattgggagtc-MGB-3'	200
SRC1	forward primer	5'-tgaaagtggaaaagaaagaacagatg-3'	300
	reverse primer	5'-tctccagcgtgggcagtaac-3'	300
	probe	5'-FAM-ccagagccagtttacagc-MGB-3'	150

UGT1A3[e]	forward primer	5'-ggatattctcagtcatgcatctgtgt-3'	500
	reverse primer	5'-ttcgatggtcgggttcca-3'	500
	probe	5'-FAM-ccccaggccaatc-MGB-3'	250

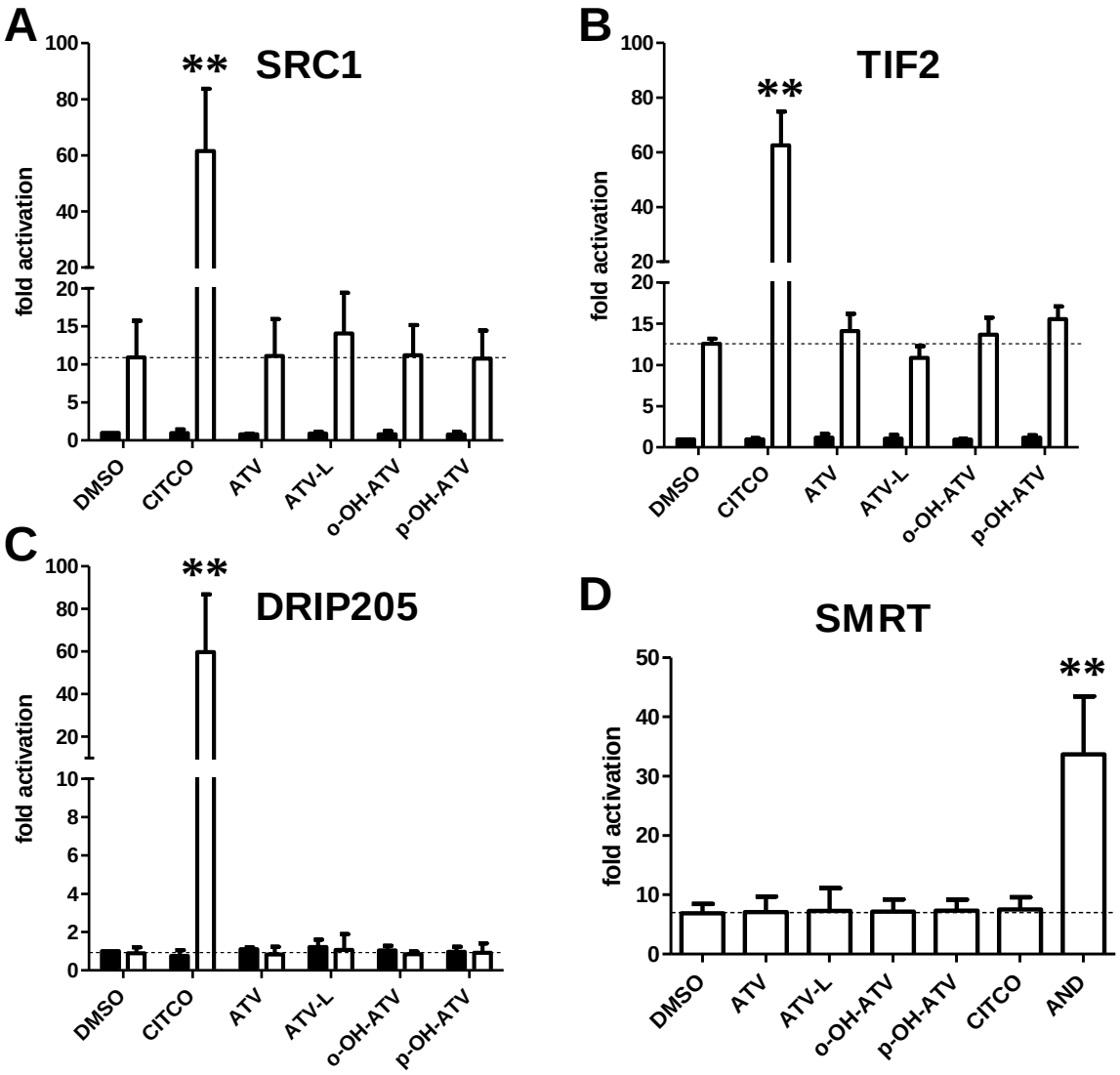
FAM, 6-carboxyflourescein; TAMRA, 6-carboxytetramethylrhodamin; VIC, proprietary fluorescent dye of Applied Biosystems; MGB, minor groove binder / non-fluorescent quencher; [a], previously published in Burk et al., 2005; [b], previously published in Wolbold et al., 2003; [c], specific for the exon 1 containing transcript; [d], specific for PXR-1 [e], previously published in Riedmaier et al., 2010.



Supplemental Figure S1 Expression of PXR and cofactors is maintained in plated primary human hepatocytes. Expression levels of the indicated genes, as determined by TaqMan real-time RT-PCR, were compared between human hepatocytes grown for 5 days as monolayer on collagen I-coated plates and matched freshly isolated cells (n=4). During the last 2 days of culture, hepatocytes were treated with 0.1% DMSO. Data are shown as relative to the expression level in freshly isolated cells, which was designated as 1. Lines indicate means.



Supplemental Figure S2 CAR ligands induce the assembly of CAR-LBD and in vitro interaction with co-activator SRC-1. **(A)** CAR-assembly assay in COS1 cells, which were co-transfected with expression plasmids encoding GAL4-DBD/hCAR-LBD (105-150) and VP16-AD/hCAR-LBD (151-348) fusion proteins. Cells were treated for 40 h with 10 µM rifampin (RIF), 1 mM Phenobarbital (PB), 1 µM CITCO, 100 µM clofibrate (CLO), 30 µM triphenyphosphate (TPP), 10 µM clotrimazole (CLOT), 10 µM PK11195 (PK) or 0.1% DMSO only. Mean fold induction (± S.D.) of the normalized activity of co-transfected reporter plasmid pGL3-G5 by treatment with the indicated chemicals is shown. Hatched and cross-hatched columns indicate treatment with negative controls. Columns in black or grey indicate treatment with agonists or inverse agonists, respectively. The respective activity of cells treated with DMSO only, was designated as 1. **(B)** Biacore analysis of ligand-induced interaction of coactivator SRC-1 with CAR in vitro. CAR-LBD protein, pre-incubated with 100 µM of the indicated chemicals or 1% DMSO only, was injected onto immobilized SRC-1-RID protein (open columns). As a control, chemicals were also injected alone (filled columns). Data are shown as means ± S.D. of 3-6 independent experiments. Open columns show increase in binding by pre-incubation of CAR with chemicals. Binding of CAR to SRC-1 in the presence of DMSO only, was designated as 1**. (A-B)** Statistically significant differences were analysed by one sample t-test, resulting p-values were corrected for multiple testing by the method of Bonferroni, and indicated by asterisks (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$).



Supplemental Figure S3 Atorvastatin and its metabolites do not change the interaction of CAR with coregulators. **(A-C)** COS1 cells were co-transfected with expression plasmids encoding GAL4-DBD/coactivator-RID fusion proteins, as indicated, and expression plasmids encoding VP16-AD/CAR-LBD (105-348) **(A, B)** or VP16-AD/CAR-SV2-LBD (105-353) **(C)** fusion proteins (open columns) or empty expression vector pVP16-AD (filled columns). Cells were treated for 40 h with 1 µM CITCO, 30 µM of atorvastatin metabolites or 0.1% DMSO only. Mean fold activation (± S.D.) of the normalized activity of co-transfected reporter plasmid pGL3-G5 by treatment with the indicated chenmicals is shown. The respective activity of cells transfected with the particular GAL4-DBD/coactivator-RID fusion protein expression plasmids and pVP16-AD, treated with DMSO only, was designated as 1. **(D)** COS1 cells were co-transfected with plasmids encoding GAL4-DBD/SMRT-RID fusion protein and VP16-AD/CAR-LBD (105-348) fusion protein. An expression plasmid encoding human RXRα was additionally co-transfected. Columns show the mean fold activation (± S.D.) of normalized pGL3-G5 reporter activity after 40 h of treatment with chemicals (AND, 10 µM androstenol), as compared to the respective activity of cells transfected with GAL4-DBD/SMRT-RID

plasmid and pVP16-AD, treated with DMSO only, which was designated as 1. **(A-D)** Statistical significant differences to DMSO vehicle treatments were analysed by repeated measures one-way analysis of variance with Dunett multiple comparisons test and indicated by asterisks (**, $p < 0.01$).