Supplemental Material for:

Title: The orphan nuclear receptor, DAX-1, functions as a potent co-repressor of the constitutive androstane receptor (CAR, NR1I3)

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Table S1. RT-PCR Standard Curve Target Data

Target	Efficiency	R^2	Slope
DAX1	88.4	0.999	-3.635
CYP2B6	64.5	0.998	-4.625
GAPDH	88.5	0.998	-3.631

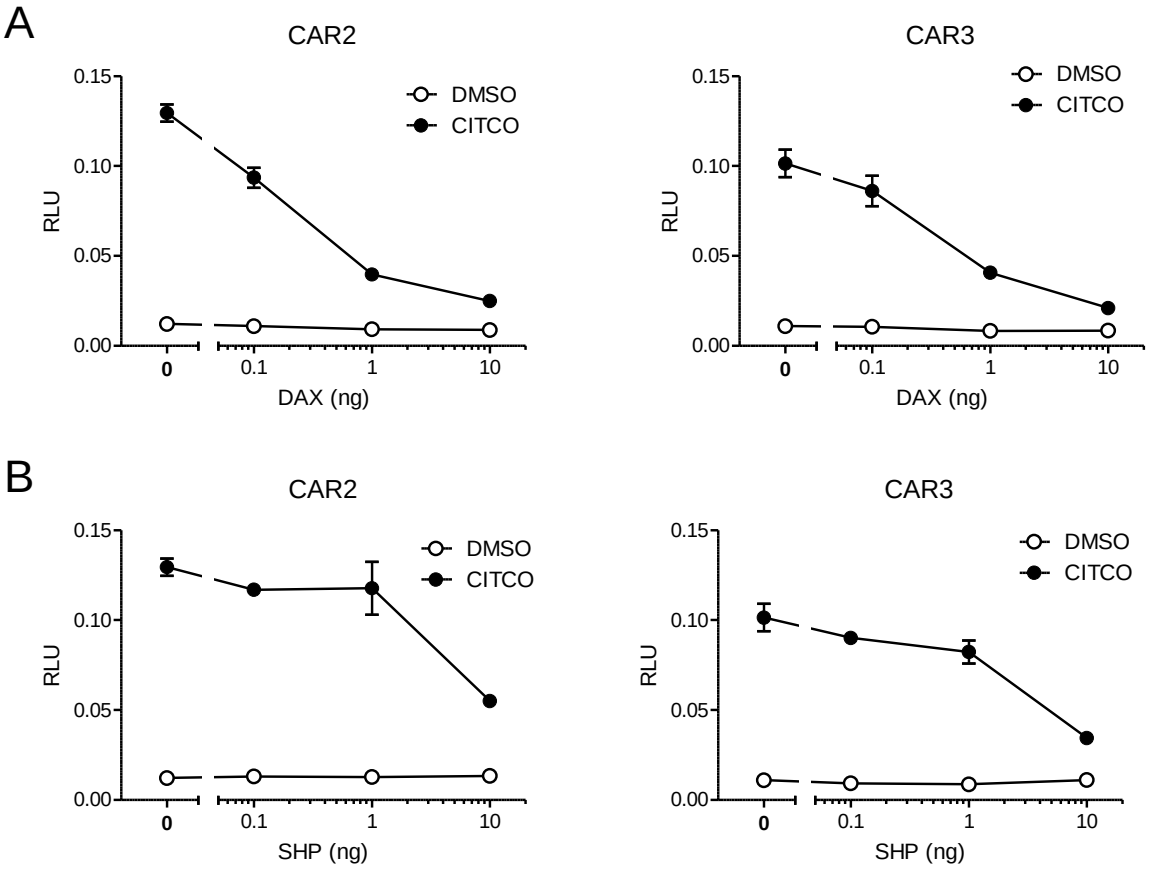
Table S2. Interaction of McDonnell[1] Panel peptides with human CAR variants

Peptide Class/#[2]		Peptide Sequence																			CAR1		CAR2		CAR3	
																					DMSO	CITCO	DMSO	CITCO	DMSO	CITCO
I	D2	G	S	E	P	K	S	**R**	**L**	L	E	**L**	**L**	S	A	P	V	T	D	V	-	-	-	-	-	-
	D11	V	E	S	G	S	S	**R**	**L**	M	Q	**L**	**L**	M	A	N	D	L	L	T	-	-	-	-	-	-
	D30	H	P	T	H	S	S	**R**	**L**	W	E	**L**	**L**	M	E	A	T	P	T	M	-	+	+	+	-	+
II	D14	Q	E	A	H	G	**P**	L	**L**	L	S	**L**	**L**	S	R	S	D	T	D	W						
	D47	H	V	Y	Q	H	**P**	L	**L**	L	S	**L**	**L**	S	S	E	H	E	S	G	+	+	-	+	-	+
	C33	H	V	E	M	H	**P**	L	**L**	M	G	**L**	**L**	M	E	S	Q	W	G	A	+	+	-	+	-	-
III	F6	G	H	E	P	L	**T**	L	**L**	E	R	**L**	**L**	M	D	D	K	Q	A	V	+	+	-	-	-	-
	D22	L	P	Y	E	G	**S**	L	**L**	L	K	**L**	**L**	R	A	P	V	E	E	V	-	-	-	-	-	+
	D48	S	G	W	E	N	**S**	I	**L**	Y	S	**L**	**L**	S	D	R	V	S	L	D	+++	+++	-	++	-	++
	D43	A	H	G	E	S	**S**	L	**L**	A	W	**L**	**L**	S	G	E	Y	S	S	A	+	+	-	+	-	++
	D40	G	V	F	C	D	**S**	I	**L**	C	Q	**L**	**L**	A	H	D	N	A	R	L	+	++	-	+	-	+
	D15	H	H	N	G	H	**S**	I	**L**	Y	G	**L**	**L**	A	G	S	D	A	P	S	-	+	-	+	-	+
	F4	L	G	E	R	A	**S**	L	**L**	D	M	**L**	**L**	R	Q	E	N	P	A	W	+	+	-	+	-	-

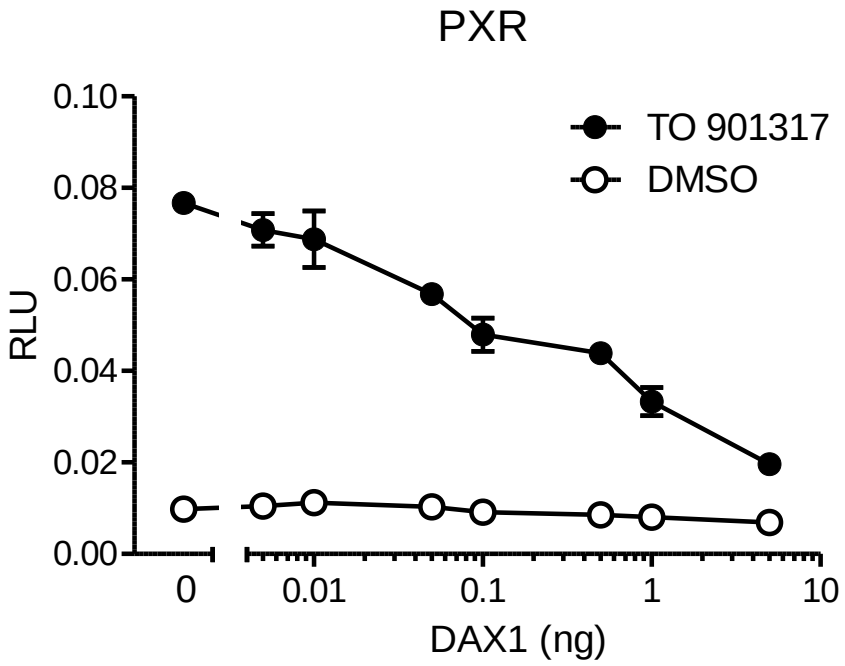
[1] Chang C, Norris J D, Gron H, Paige L A, Hamilton P T, Kenan D J, Fowlkes D and McDonnell D P (1999) Dissection of the LXXLL Nuclear Receptor-Coactivator Interaction Motif Using Combinatorial Peptide Libraries: Discovery of Peptide Antagonists of Estrogen Receptors Alpha and Beta. *Mol Cell Biol* **19**:8226-8239.

[2] Peptides were classified into three different classes based on sequences flanking the conserved LXXLL motif. The highlights showed conserved sequences in the classes and the 'LXXLL' motifs found in all of the peptides.

Supplemental Figure S1. Effect of DAX-1 and SHP on CAR2 and CAR3 activity. Results shown here represent single transfection experiments, with all treatments in quadruplicate. COS-1 cells were transfected with the CMV2-CAR2 or CMV2-CAR3 and 3.1-RXRα expression vectors, the 2B6-XREM-PBREM reporter, the pRL-CMV vector for normalization of transfection efficiency and varying amounts of PCMV6-DAX1 (Panel A) or pCMV6-SHP (Panel B). All treatments were for 24h and the data are represented as normalized luciferase values. Each data point represents the mean (+/- S.D.).



Supplemental Figure S2. Effect of DAX-1 and SHP on pregnane X receptor (PXR) activity. Results shown here represent single transfection experiments, with all treatments in quadruplicate. COS-1 cells were transfected with the CMV2-PXR and 3.1-RXRα expression vectors, the 3A4-XREM-PBREM reporter, the pRL-CMV vector for normalization of transfection efficiency and varying amounts of PCMV6-DAX1. All treatments (TO901317 is a PXR agonist; Mitro N, Vargas L, Romeo R, Koder A, Saez E. T0901317 is a potent PXR ligand: implications for the biology ascribed to LXR. FEBS Lett. 2007;581:1721-1726) were for 24h and the data are represented as normalized luciferase values. Each data point represents the mean (+/- S.D.).



Supplemental Figure S3. Effect of DAX-1 mutations on on pregnane X receptor (PXR) activity. Results shown here represent single transfection experiments, with all treatments in quadruplicate. COS-1 cells were transfected with the 3.1-RXRα expression vector, the 3A4-XREM-PBREM reporter, the pRL-CMV vector for normalization of transfection efficiency and varying amounts of CMV2-CAR1, -CAR2 or CAR3 and PCMV6-empty, -DAX1 WT, -DAX1 M1, -DAX1 M2, -DAX1 M3, or -DAX1 PCF (Panel A) or PCMV6-DAX1-TSD (Panel B). All treatments (TO901317 is a PXR agonist; Mitro N, Vargas L, Romeo R, Koder A, Saez E. T0901317 is a potent PXR ligand: implications for the biology ascribed to LXR. FEBS Lett. 2007;581:1721-1726) were for 24 h and data are represented as normalized luciferase values and each data point represents the mean (+/- S.D.). Asterisks indicate that each treatment was significantly different from its respective empty vector control ($p<0.05$).