# Supporting Information

## Wang et al. 10.1073/pnas.1312065110

### SI Materials and Methods

**Cell Culture and Reagents.** Human umbilical vein endothelial cells (HUVECs) were harvested by collagenase treatment of umbilical cord veins and cultured on plates coated with collagen. Cells were maintained in M199 supplemented with 20% (vol/vol) FBS, 20 mM Hepes at pH 7.4, 1 ng/mL recombinant human fibroblast growth factor, and 90 μg/mL heparin and antibiotics. Bovine aortic endothelial cells were isolated from freshly harvested aortas and cultured in DMEM (Invitrogen) supplemented with 10% (vol/vol) FBS. THP-1, a human acute monocytic leukemia cell line, were cultured in medium RPMI 1640 (Invitrogen) supplemented with 10% (vol/vol) FBS. Rifampicin and staurosporine were from Calbiochem Inc. The antibodies against pregnane X receptor (PXR), vascular adhesion molecule 1 (VCAM-1), E-selectin, p65 NF-κB, IκBα, histone H1, or β-actin were from Santa Cruz Biotechnology Inc. Antibodies against the herpes virus transcription factor VP16, procaspase 3, and cleaved caspase3 were from Abcam. Human recombinant TNF-α was from R&D Systems. 6-[(2S,4R,6E)-4-methyl-2-(methylamino)-3-oxo-6-octenoic acid]-7-L-valine-cyclosporin A (PSC833) and doxorubicin were from Tocris. Other reagents, unless specified, were from Sigma-Aldrich.

***Monocyte adhesion assay.*** Calcein-AM–labeled (Molecular Probes) THP-1 cells ($1 \times 10^5$ cells per mL) were added to pretreated or stimulated ECs and incubated for 30 min at 37 °C. After washing off the nonadherent cells, ECs with adherent THP-1 cells were fixed with 4% (vol/vol) paraformaldehyde in the dark and counted in 8 randomly selected microscopic fields.

***Plasmids and transfection.*** The plasmids expressing a constitutively active PXR (VP-PXR) (1), pCYP3A4XREM-362/+53 (2), 5×NF-κB-Luc (3), the yeast transcription factor GAL4-PXR-LBD (ligand-binding domain) (4), and a GAL4 reporter (5), were previously described. Gal4-SXR-LBDmutant was made by generating the double mutations, which changed highly conserved hydrophobic leucine (424) and charged glutamate (427) residues to alanine at the PXR ligand-binding domain, as previously described (4). The plasmids were transfected into bovine aortic endothelial cells by using lipofectamine 2000 (Invitrogen). After treatments, cell lysates were harvested to measure luciferase activity. The results were normalized to β-galactosidase activity.

**Analysis of Apoptosis.** Cultured HUVECs were subjected to doxorubicin (5 μM, 12 or 24 h) or staurosporine (500 nM, 6 h). Cells were trypsinized, washed with PBS, resuspended in binding buffer (10 mM Hepes at pH 7.4, 140 mM NaCl, 1 mM $MgCl_2$, 5 mM KCl, and 2.5 mM $CaCl_2$), and then incubated with FITC-conjugated annexin V (0.5 μg/mL) on ice in darkness for 30 min. DNA was stained with propidium iodide (50 μg/mL). Samples were analyzed using a FACS Calibur flow cytometer with CellQuest (Becton Dickinson) and FlowJo (TreeStar) software for acquisition and analysis.

**RNA Interference.** Small interfering RNA for PXR (PXR-siRNA) or scrambled control siRNA (Santa Cruz) was transfected into HUVECs at 100 nM, using siPORT NeoFX transfection agent (Ambion). Cells were assayed 48 h posttransfection.

**Quantitative Reverse-Transcriptase PCR.** Total RNA was isolated using a TRIzol reagent (Invitrogen). The cDNAs were synthesized with the use of SuperScript$^{III}$ reverse transcriptase (Invitrogen) and oligo-(dT) primer (Promega). Quantitative reverse-transcriptase PCR (qRT-PCR) was performed using iQ SYBR Green PCR Supermix in the DNA Engine Opticon realtime system (Bio-Rad Laboratories), with GAPDH as an internal control. Primer sequences are shown in Table S1.

**Animal Experiments.** All animal procedures were approved by the Animal Care and Use Committee of Peking University Health Science Center. Male C57/BL6 mice and Sprague-Dawley rats were fed with standard chow and water ad libitum. After euthanization, the intima layer of different parts of the rat aorta was isolated for RNA extraction. Adenoviral infection of rat carotid arteries was performed as previously reported (3). Briefly, rats were anesthetized with sodium pentobarbital (40 mg/kg). The left common carotid artery was surgically exposed, and an arteriotomy was made on the external carotid artery. Viral solution containing Ad-tTA and Ad-VP-PXR or Ad-LacZ at a combined titer of $5 \times 10^8$ pfu/mL was infused into the isolated carotid segment for 15 min. Then the external carotid artery was ligated and the blood flow to the common and internal carotid arteries was restored. After recovery, LPS (2 mg/kg) was intraperitoneally injected. After 24 h, rats were transcardially perfused with saline, and subsequently with 4% (vol/vol) paraformaldehyde solution. Then the common carotid arteries were removed for further study.

**Immunohistochemical Staining.** The *en face* staining was performed immediately after excision of rat carotid aortas, as previously described (6). Briefly, the aortic segments were longitudinally dissected. After pretreatment with 10% (vol/vol) goat or rabbit serum, the dissected segments were incubated with the primary antibodies against VCAM-1 or E-selectin (1:100 dilution) at 4 °C overnight, followed by incubation with the TRITC-conjugated secondary antibody for 1 h. After counterstaining the nuclei with 4′,6-diamidino-2-phenylindole, the segments were mounted on slides and examined under a confocal laser microscope (Leica, TCS-NT SP5). Expression of VP-PXR was examined for immunostaining of VP16 on cryosectioned carotid segments with the use of a rabbit antibody against VP16 and a TRITC-conjugated goat anti-rabbit secondary antibody.

**Microarray Analysis.** HUVECs were infected with adenoviruses for VP-PXR and tTA with (mock) or without tetracycline. After 36 h, total RNA was extracted and two independent sets of microarray experiments were performed. Gene expression was analyzed with the use of Affymetrix U133 plus 2.0 chips. RNA labeling, hybridization, washing, and scanning of the microarray were performed following the manufacturer's specifications, as previously described (7). The results were analyzed with GeneChip Operating Software 1.4. Expression patterns of up-regulated genes responsible for the detoxification-related processes were graphically represented in a heat map.


1. Xie W, et al. (2000) Reciprocal activation of xenobiotic response genes by nuclear receptors SXR/PXR and CAR. *Genes Dev* 14(23):3014–3023.
2. Zhou C, Tabb MM, Sadatrafiei A, Grün F, Blumberg B (2004) Tocotrienols activate the steroid and xenobiotic receptor, SXR, and selectively regulate expression of its target genes. *Drug Metab Dispos* 32(10):1075–1082.
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4. Wentworth JM, Agostini M, Love J, Schwabe JW, Chatterjee VK (2000) St John's wort, a herbal antidepressant, activates the steroid X receptor. *J Endocrinol* 166(3):R11–R16.
5. Zhu Y, et al. (2005) Oxidized LDL downregulates ATP-binding cassette transporter-1 in human vascular endothelial cells via inhibiting liver X receptor (LXR). *Cardiovasc Res* 68(3):425–432.
6. Iiyama K, et al. (1999) Patterns of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 expression in rabbit and mouse atherosclerotic lesions and at sites predisposed to lesion formation. *Circ Res* 85(2):199–207.
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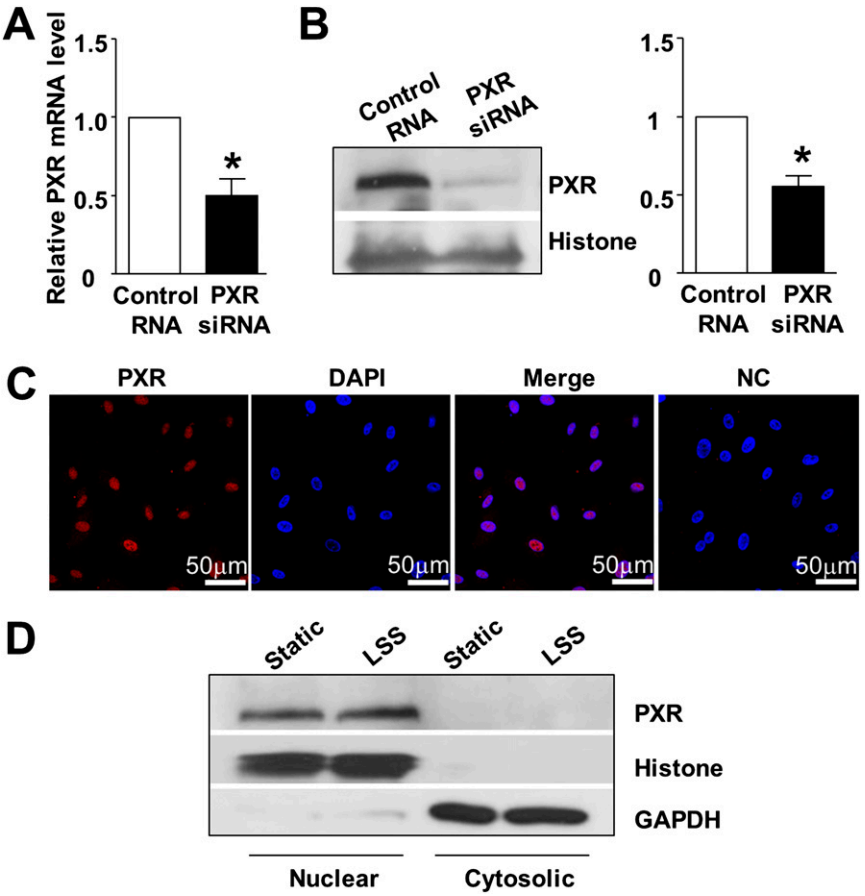


**Fig. S1.** Knockdown of PXR expression by siRNA. (*A*) HUVECs were transfected with PXR siRNA or control siRNA (100 nM, 48 h). Efficiency of siRNA-mediated PXR knockdown was measured by qRT-PCR and Western blotting (*B*). (*C*) Immunofluorescence staining showed the nuclear localization of PXR in HUVECs. (*D*) ECs were exposed to LSS or kept static for 24 h. Nuclear and cytosolic proteins were extracted and subjected to Western blotting for PXR, histone, and GAPDH. Data are shown as mean ± SEM of three independent experiments. $^*P < 0.05$ vs. control.

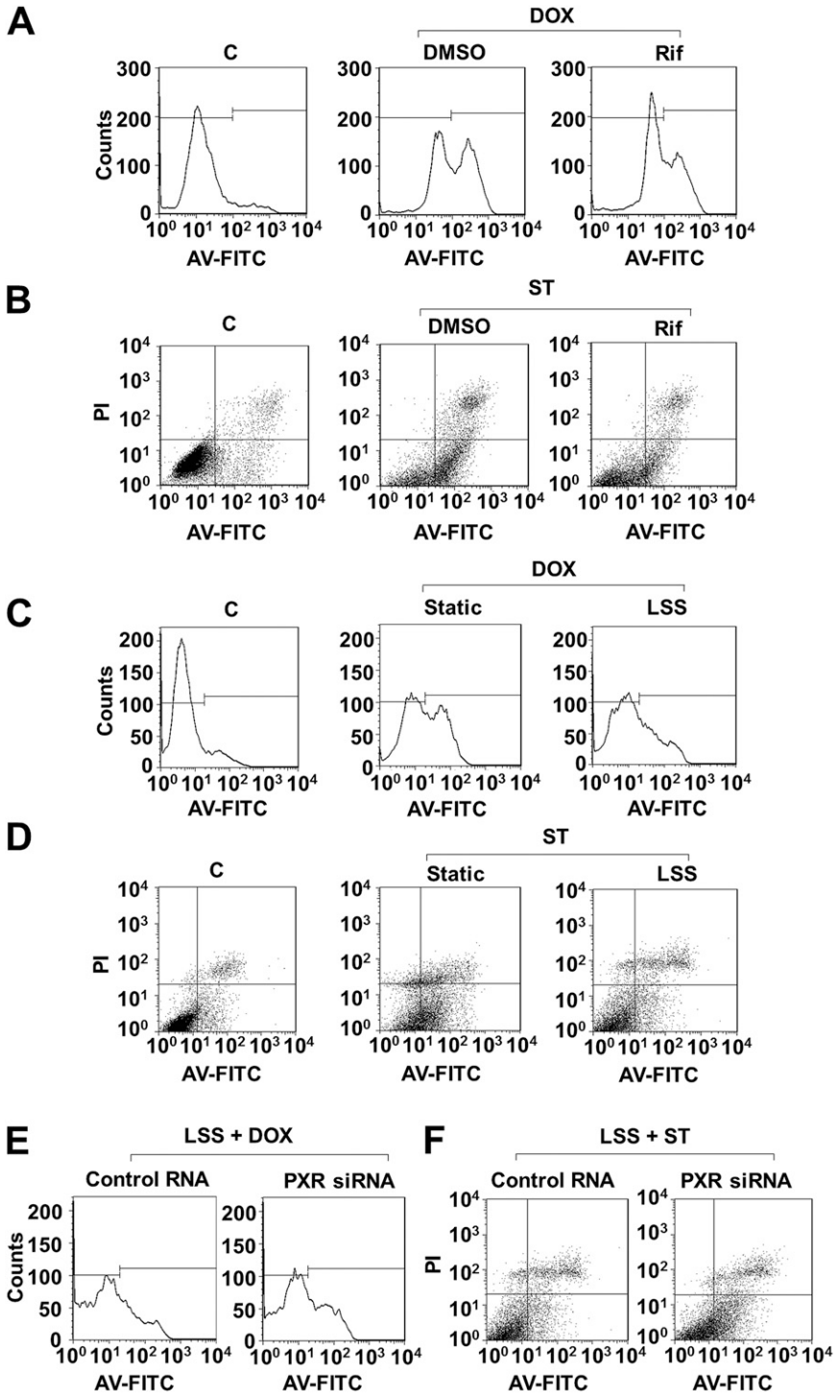


**Fig. S2.** PXR activation attenuates doxorubicin (DOX)- or staurosporine (ST)-induced apoptosis. HUVECs were treated with rifampicin (20 μM) or vehicle (DMSO) for 24 h before exposed to DOX (*A*) (5 μM, 24 h) or ST (*B*) (500 nM, 6 h). Apoptosis was assessed by using flow cytometry for annexin V-FITC. Alternatively, ECs were pretreated with LSS or kept static for 12 h before exposure to DOX (*C*) or ST (*D*). In addition, ECs were transfected with PXR or control siRNA for 48 h prior to LSS and, then, treatment with DOX (*E*) and ST (*F*). Representative flow cytometry plots are shown.

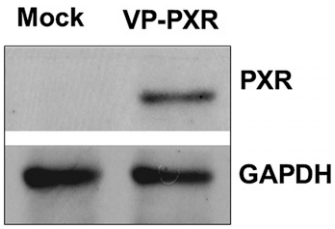


**Fig. S3.** Overexpression of VP-PXR in ECs. Confluent HUVECs were coinfected with Ad-VP-PXR together with Ad-tTA in the presence (mock) or absence of tetracycline. Protein was extracted and subjected to Western blotting for PXR or GAPDH. Blots shown are representative of three independent experiments.

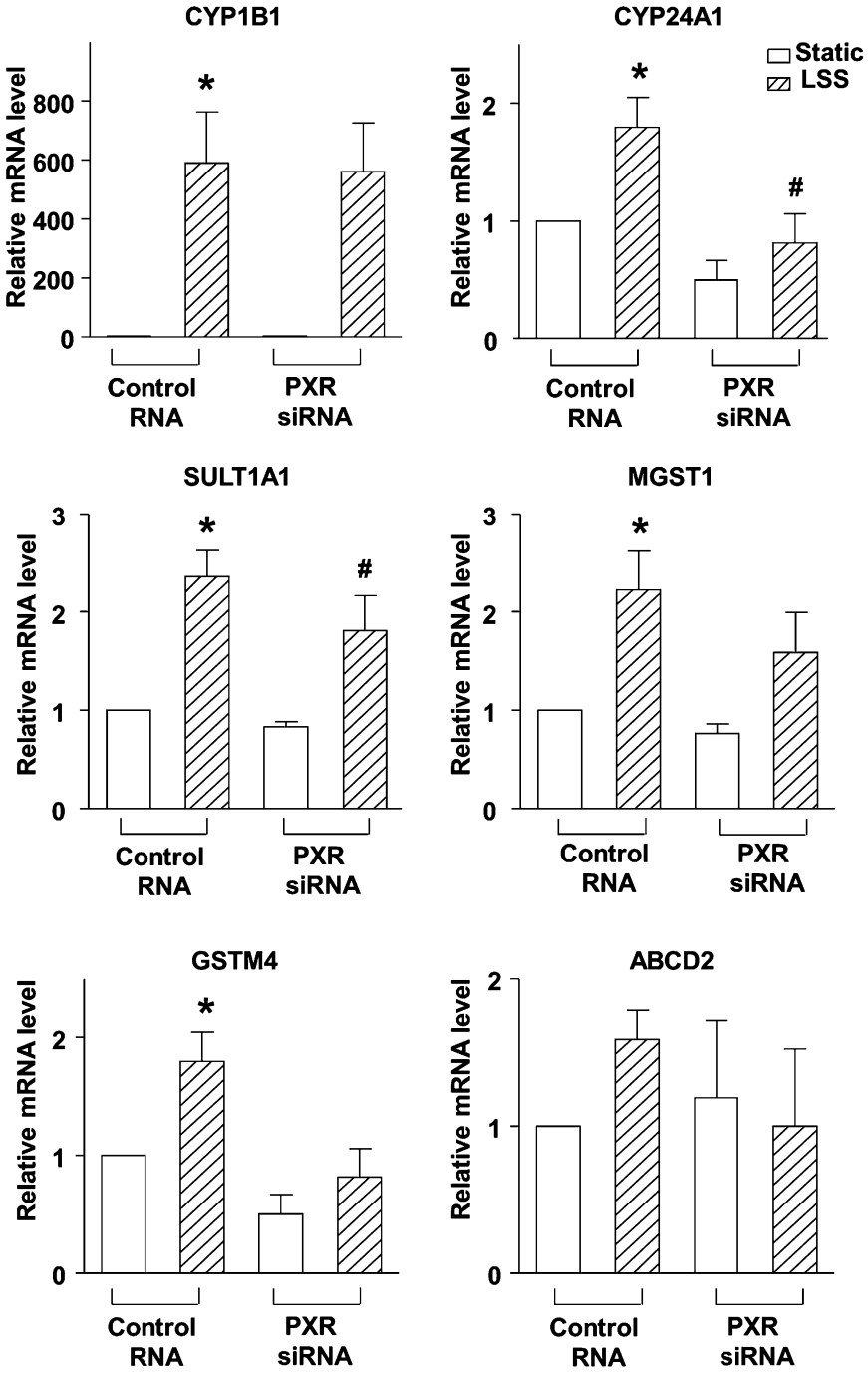


**Fig. S4.** SiRNA-mediated PXR knockdown abrogated the induction of effect detoxification genes by shear stress. HUVECs were transfected with PXR or control siRNA for 48 h and then exposed to LSS or static condition for another 24 h. RNA was extracted and analyzed by qRT-PCR. Data are shown as mean ± SEM from three independent experiments. *$P < 0.05$ vs. control.

**Table S1. Primer sequences used for qRT-PCR**

| Species and genes | Oligonucleotide sequences (5′-3′) Forward | Reverse |
|---|---|---|
| Human | | |
| MDR1 | AGGCCAACATACATGCCTTC | AGGGCTTCTTGGACAACCTT |
| VCAM-1 | GGAAGTGGAATTAATTATCCAA | GTACTGTGTCTCCTGTCTCC |
| E-selectin | GTGAACCCAACAATAGGCAA | AGCTTCTTCTTGCTGCACCT |
| CYP1B1 | TGATGGACGCCTTTATCCTC | CCACGACCTGATCCAATTCT |
| CYP27A1 | AAGCGATACCTGGATGGTTG | TGTTGGATGTCGTGTCCACT |
| CYP24A1 | AGGCCACGTTGAAGACTTGT | TTGGTGTTGAGGCTCTTGTG |
| CYP51A1 | TAGTGCTTGGATGGGCTTCT | TGGGAGAAACACACACCTGA |
| CYP26B1 | CCTTCGAGCTTGATGGTTTC | AGCACCTTCAGGAACAGCTT |
| MAOA | ACTGCTAGGCGGTTTGCTTA | CCATTATCCGTTCGCTCACT |
| ALDH2 | ACAATGGCAAGCCCTATGTC | CATCACAACCACGTTTCCAG |
| GSTM4 | GTCTGCAGAATCGACACCAA | GTGAGCCCCATCAATCAAGT |
| MGST | TGCCAATCCAGAAGACTGTG | GAATACAGGAGGCCAATTCC |
| SULT1A1 | CACGTCGTTCAAGGAGATGA | AGGTTTGATTCGCACACTCC |
| SULT1C2 | CATGGGAAAGAAGGTGGATG | GGGCAACAGTGAAGTGGTTT |
| ABCB2 | CAGGAGACGGAGTTTTTCCA | GCATGATCCCCAAGAGACAT |
| ABCB4 | AACCCCAAGATCCTTCTGCT | TCTTCATCAGTTCGCTGTGG |
| ABCC1 | CTGGCATTCAAGGACAAGGT | ACCGGAGGATGTTGAACAAG |
| ABCD2 | CTTGGAAGTCTTCGGGATCA | CCATAACAGCATCCCATCCT |
| ABCG2 | TTATCCGTGGTGTGTCTGGA | TTCCTGAGGCCAATAAGGTG |
| Mouse | | |
| CYP1B1 | CCACCAGCCTTAGTGCAGAC | GGCCAGGACGGAGAAGAGT |
| ALDH2 | AAGGGAACAAGGAGGACGTAG | CCAATCGGTACAACAGCCG |
| MGST | GACCGCATTCCAGAGGATAA | TGAAGTGCATGAGGGCTGTA |
| ABCD2 | AGAAGCATGGCTGGACAAGT | GTCAAGCAACAGCCAGAACA |
| Rat | | |
| MDR1 | ACTCGGGAGCAGAAGTTTGA | GCACCAAAGACAACAGCAGA |
| Human/mouse/rat | | |
| GAPDH | ACCACAGTCCATGCCATCAC | TCCACCACCCTGTTGCTGTA |