

**Title Page:**

Involvement of NF- $\kappa$ B, not PXR, in Inflammation-mediated Regulation of Hepatic  
Transporters

**Authors:**

Walaa A. Abualsunun and Micheline Piquette-Miller

**Affiliations of WA and MPM:**

Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada  
m.piquette.miller@utoronto.ca.

**Running Title Page:**

NF- $\kappa$ B mediated Regulation of Transporters in Inflammation

**Corresponding author:**

Micheline Piquette-Miller, PhD.

Professor, Leslie Dan Faculty of Pharmacy

University of Toronto

144 College Street, Toronto, ON, M5S 3M2

Ph.: (416) 946 3057

Fax: (416) 978-8511

E-mail: [m.piquette.miller@utoronto.ca](mailto:m.piquette.miller@utoronto.ca)

Number of text pages: 10 (from abstract to end of discussion)

Number of tables: 2

Number of figures: 6

Number of references: 38

Number of words in the Abstract: 246

Number of words in the Introduction: 745 words

Number of words in the Discussion: 1041 words

**List of Non Standard Abbreviations:**

ABC, ATP-binding cassette;

CAR, constitutive androstane receptor;

CYP or P450, cytochrome P450;

FXR, farnesoid X receptor;

IL, interleukin;

iNOS, inducible nitric oxide synthase;

LPS, lipopolysaccharide;

NF- $\kappa$ B, nuclear factor kappa B;

P-gp, p-glycoprotein;

PXR, pregnane X receptor;

RT-PCR, reverse transcription-polymerase chain reaction;

RXR, retinoid X receptor;

SLC, solute carrier;

TLR4, toll-like receptor 4;

TNF, tumor necrosis factor;

## Abstract

Endotoxin-induced inflammation decreases the hepatic expression of several drug transporters, metabolizing enzymes and nuclear transcription factors including PXR. As the nuclear factor NF- $\kappa$ B is a major mediator of inflammation, and reciprocal repression between NF- $\kappa$ B and PXR signaling has been reported, the objective of this study was to examine whether NF- $\kappa$ B directly regulates the expression of transporters or exerts its effect indirectly via PXR. PXR deficient (-/-) or wild-type (+/+) male mice were dosed with the selective NF- $\kappa$ B inhibitor PHA408 (40 mg/kg ip) or vehicle (n=5-8/group) followed by endotoxin (5 mg/kg) or saline 30 minutes later. Animals were sacrificed at 6 hours; samples were analyzed using qRT-PCR and western blots. Endotoxin induced TNF- $\alpha$ , IL-6 IL-1 $\beta$  and iNOS in PXR (+/+) and (-/-) mice. As compared to saline controls, endotoxin administration imposed 30-70% significant decreases in the expression of Abcb1a, Abcb11, Abcc2, Abcc3, Abcg2, Slc10a1, Slco2b1 and Slco1a4 in PXR (+/+) and (-/-) mice to a similar extent. Pre-administration of PHA408 attenuated endotoxin-mediated changes in both PXR (+/+) and (-/-) mice (p<0.05). Our findings demonstrate that endotoxin activates NF- $\kappa$ B and imposes a downregulation of numerous ABC and SLC transporters through NF- $\kappa$ B in liver and is independent of PXR. Moreover, inhibition of NF- $\kappa$ B attenuates the impact of endotoxin on transporter expression. As NF- $\kappa$ B activation is involved in many acute and chronic disease states, disease-induced changes in transporter function may be an important source of variability in drug response. This information may be useful in predicting potential drug-disease interactions.

## Introduction

Drug transporters play a fundamental role in the determination of drug absorption, distribution and excretion. Consequently, changes in the expression of drug transporters could impact the disposition of their substrates, thereby causing inter-individual differences in drug response (Ayrton and Morgan 2001, Petrovic, Teng, and Piquette-Miller 2007). Alterations in the expression and activity of transporters and metabolic enzymes have been frequently observed in many acute or chronic inflammatory conditions (Goralski et al. 2003, Hartmann, Vassileva, and Piquette-Miller 2005, Morgan 2009, Morgan et al. 2008). Administration of the bacterial endotoxin lipopolysaccharide (LPS) is a well-characterized model of acute inflammation that induces a systemic response that exhibits symptoms such as fever, hypotension and tachycardia among others (Copeland et al. 2005). Previous studies have found that endotoxin-induced inflammation causes the release of pro-inflammatory cytokines Interleukin (IL)- 6, IL-1 $\beta$ , and Tumor necrosis factor (TNF)- $\alpha$  and elicits pronounced changes in the hepatic expression of many transporters and metabolic enzymes (Aitken, Richardson, and Morgan 2006, Petrovic, Teng, and Piquette-Miller 2007).

The endotoxin signaling pathway is mediated by Toll-like receptor 4 (TLR4). Upon stimulation, TLR4 activates a number of signaling pathways including mitogen-activated protein kinases (MAPKs) and nuclear factor kappa b (NF- $\kappa$ B) (figure.1). NF- $\kappa$ B is an inducible transcription factor that is known to be a major mediator of LPS signaling, and plays a critical in acute and chronic inflammatory responses (Lawrence 2009). The NF- $\kappa$ B complex consists primarily of p50 and p65 sub-units, which normally exist in the cytoplasm in an inactive form, bound to the inhibitory I $\kappa$ B proteins. Exposing the cells to stimuli such as bacterial endotoxin or pro-inflammatory cytokines activates I $\kappa$ B kinases

(IKK) that phosphorylate the inhibitory I $\kappa$ B proteins. This leads to their ubiquitination, degradation and subsequent dissociation from NF- $\kappa$ B, which permits nuclear translocation of NF- $\kappa$ B. There it can regulate the transcription of many genes including proinflammatory cytokines (IL-1, IL-6, and TNF- $\alpha$ ), chemokines, and inducible effector enzymes such as inducible nitric oxide synthase (iNOS) (Hoesel and Schmid 2013, Pahl , Oeckinghaus and Ghosh 2009). NF- $\kappa$ B can be selectively suppressed during an acute inflammatory reaction through inhibition of I $\kappa$ B proteins. PHA408 is a selective small molecule inhibitor of IKK-2 and has been shown to selectively inhibit the NF- $\kappa$ B pathway *in vivo* in rodents (Fig.1) (Mbalaviele et al. 2009, Carlson et al. 2015). *In vivo* administration of PHA408 was found to decrease NF- $\kappa$ B nuclear translocation and inflammatory response in the lungs of rats exposed to endotoxin or cigarette smoke (Rajendrasozhan et al. 2010). In addition, *in vivo* oral administration of PHA408 to arthritic rats was shown to efficiently inhibit the induction of pro-inflammatory cytokines and NF- $\kappa$ B translocation in a dose dependent manner (Mbalaviele et al. 2009).

Numerous studies have demonstrated that inflammation-mediated activation of NF- $\kappa$ B decreases the expression of several nuclear receptors including pregnane X receptor (PXR), constitutive androstane receptor (CAR), and farnesoid X receptor FXR (Teng and Piquette-Miller 2008). It is believed that the downregulation of these nuclear receptors, specifically PXR and CAR, may be involved in the downregulation of the hepatic drug metabolizing enzymes and transporters during inflammation. Indeed, NF- $\kappa$ B has been shown to both directly regulate CYP gene expression through binding to the NF- $\kappa$ B response element of the promoter regions, and indirectly through NF- $\kappa$ B mediated repression of nuclear receptors (Zordoky and El-Kadi 2009).

It is well established that activation of PXR leads to the induction of several genes that

are known to be suppressed by inflammatory stimuli (Morgan et al. 2008, Teng and Piquette-Miller 2005). Moreover, it has been reported that PXR is significantly downregulated during acute inflammation and could be partially involved in the downregulation of several hepatic transporters (Teng and Piquette-Miller 2005). However, the role of PXR during inflammation is still not clearly understood. A crosstalk between PXR and NF- $\kappa$ B has been suggested from results of several studies where it is believed that PXR and NF- $\kappa$ B are mutually repressive (Xie and Tian 2006). Indeed, PXR activation has been found to significantly decrease the expression of NF- $\kappa$ B target genes (Wahli 2008) , while silencing PXR significantly increased expression of NF- $\kappa$ B target genes (Mencarelli et al. 2011). Moreover, intestinal tissues from PXR-null mice showed higher levels of NF- $\kappa$ B target genes (Zhou et al. 2006). Therefore, our objective was to clarify the relative roles of PXR and NF- $\kappa$ B in the regulation of drug transporters during acute inflammation. Using mice proficient or deficient in PXR and through administration of a selective NF- $\kappa$ B inhibitor, we examined whether the downregulation of transporters following endotoxin exposure occurs directly through NF- $\kappa$ B signaling or indirectly through PXR.

## **Materials and Methods**

**Animals.** C57BL/6 wild-type [PXR (+/+)] mice were purchased from Charles River Canada (Montreal, PQ, Canada) and PXR deficient C57BL/6 [PXR (-/-) mice, originally provided by Dr. Christopher Sinal (Dalhousie University, Halifax), were obtained from an inbred colony. Mice were housed in a temperature-controlled facility and provided with water and a standard chow diet on a 12-hour light/dark cycles. The animal study was conducted in accordance with the guidelines of the University of Toronto Animal Care Committee and the Canadian Council on Animal Care.

**Experimental Design.** Ten to twelve week-old male PXR (+/+) wild type and PXR (-/-) knockout mice were initially administered a 40 mg/kg intraperitoneal (IP) injection of the selective NF- $\kappa$ B inhibitor PHA408 (Axon Medchem, Netherlands) or vehicle (DMSO/saline). Thirty minutes later, a second injection of 5 mg/kg of endotoxin (Lipopolysaccharide from *Escherichia coli* 055:B5; Sigma-Aldrich, ON, Canada) or saline was given. Mice (n=5-8 / group) were euthanized six hours after the second injection, and serum and organs were collected, snap-frozen in liquid nitrogen and stored at -80°C until analysis.

**Quantitative Reverse Transcription Polymerase Chain reaction.** Total RNA from mouse liver samples were extracted using the TRIzol method (Invitrogen, Carlsbad, CA) according to the manufacturer's instruction. RNA concentration was measured using the Nano Drop 1000 (Thermo Fisher Scientific, Waltham, MA). cDNA was synthesized using 2 $\mu$ g of RNA, and reverse transcribed using High Capacity cDNA RT kit (Applied Biosystems, ON, Canada). The primers used are listed in Supplemental Table S1. Each RNA sample was plated in triplicate and assayed for genomic cDNA specific for each primer set using Power SYBR Green detection system (ABI 7900HT). Relative mRNA levels were calculated using the comparative threshold cycle method ( $\Delta\Delta$ Ct), where each gene of interest was normalized to the endogenous house keeping gene 36B4.

**ELISA Analysis.** Serum IL-6 levels were measured using commercially available mouse specific enzyme-linked immunosorbent assay (ELISA) kits for IL-6 (R&D Systems, Minneapolis, MN) according to manufacturer's instructions. The minimum detectable limit was 1.3 pg/ml.



**Western blotting.** Nuclear proteins were isolated from liver tissue as described in the Supplemental file, modified from (Durk et al. 2014). Total protein concentration was quantified using the Bradford assay with BSA standards. Protein samples (40 µg) in Laemmli sample buffer (Biorad, Hercules, CA, USA) were heated at 95°C for 3 minutes then separated using 10% SDS - Polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride (PVDF) membranes (Bio-Rad Laboratories Canada, Mississauga, ON). Membranes were blocked with 5% skim milk in Tris-buffered saline (TBST) and incubated over night at 4°C with anti-PXR.1 (A-20) 1: 200 and anti- p-NF-κB p65 subunit (Ser-536) 1:1000 (Santa Cruz Biotechnology) 1° antibodies in 2% skim milk TBST. Membranes were washed multiple times with TBST before incubation with 2° antibodies (anti-goat 1:3000, anti-rabbit 1:5000, anti-mouse 1:5000 Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). Immunodetectable levels were detected using Super Signal West Pico Chemiluminescent Substrate (Thermo Scientific) and Alpha Ease FC imaging software (Alpha Innotech, Santa Clara, CA). Gel to gel variability was accounted for by normalizing to a calibrator as well as β-actin (AC-15, 1:50000, Sigma-Aldrich).

**Statistics.** Statistical analysis was performed using GraphPad Prism 7 software. All results were expressed as mean ± S.E. Unpaired Student *t* test was used to compare basal gene expression in PXR (+/+) and PXR (-/-) mice. Two-way ANOVA analysis with Holm-Sidak's multiple comparison test was used to determine the effect of NF-κB and endotoxin.

## Results

**Basal Hepatic Gene Expression in PXR (+/+) and PXR (-/-).** The basal expression of inflammatory markers and hepatic transporters were examined in the PXR (+/+) and PXR (-/-) mice. The basal expression of NF- $\kappa$ B target genes IL-6 and IL-1 $\beta$  were 35-100% higher in PXR (-/-) as compared to PXR (+/+) mice ( $P < .005$ ) (Table.1). Expression of Socs3, which is an IL6 target gene, was 5 fold higher in PXR (-/-) as compared with PXR (+/+). As compared to PXR (+/+), the hepatic expression of Abcb1a, Abcb1b, Abcc2 and Abcb11, Slco1a4 were 50-90% lower in PXR (-/-) while levels of Abcg2, Slco2b1, Slc10a1 and Cyp3a were 30%-110% higher in the PXR (-/-) mice (Table1).

### **Effect of NF- $\kappa$ B Inhibition on Inflammatory Response.**

In both PXR (+/+) and PXR (-/-) endotoxin-treated animals, the mRNA levels of IL-6, IL-1 $\beta$ , TNF- $\alpha$  and iNOS were approximately 40 fold higher than saline controls. This endotoxin-mediated induction was significantly decreased in mice receiving PHA408 (Fig. 2). PHA408 also effectively inhibited endotoxin-mediated induction of NF- $\kappa$ B mRNA (Fig. 3A) as well as the expression of the active nuclear protein fraction of NF- $\kappa$ B (Fig. 3B) in both PXR (+/+) and PXR (-/-).

In PXR (+/+) mice, serum concentrations of IL-6 were increased from 17.31(pg/ml) in saline to 2408.99 (pg/ml) in endotoxin-treated animals, however, pre-administration of PHA408 did not significantly alter this induction 2410.33 (pg /ml). In PXR (-/-) mice, serum concentrations of IL-6 were increased from 373.93 (pg/ml) in saline to 1669.24 (pg/ml) in endotoxin-treated animals and pre-administration of PHA408 did not significantly alter this induction 1450.27 (pg /ml).

### **Effect of NF- $\kappa$ B Inhibition on Hepatic Gene Expression**

Administration of endotoxin led to significant 30-70% decreases in the expression of Cyp3a and the majority transporters and this occurred to a similar extent in both PXR (+/+) and PXR (-/-) mice (Fig.4.A, B, C). The inhibition of NF- $\kappa$ B with PHA408 attenuated the endotoxin-mediated changes in both PXR (+/+) and PXR (-/-). In contrast, while endotoxin administration increased the level of abc1b this change was not seen in PXR (-/-) mice and was not significantly affected by PHA 408. As shown in Table 2, pre-administration of PHA408 alone imposed significant decreases in the expression of several transporters in PXR wild-type (Abcb1a, Abcc2, Abcb11, Slco1a4) and PXR null mice (Abcb1a, Abcc2, Abcc3, Abcb11, Abcg2, Slco1a4, Slco2b1, Slco10a1). Sole administration of PHA408 was found to increase expression of Cyp3a and Abcg2 in wild-type but not knock-out mice.

Following endotoxin administration, mRNA expression of PXR, CAR, FXR, RXR and PPAR- $\gamma$  were significantly decreased in PXR (+/+) mice (Fig 5. A). Pre-administration of PHA408 significantly attenuated endotoxin-mediated effects for only PXR. Consistent with these results, the nuclear portion of the activated form of PXR showed a significant downregulation in PXR (+/+) mice following endotoxin administration and pretreatment with PHA408 significantly attenuated this effect (Fig 6A, B). While a significant increase in PXR mRNA was seen in mice treated with PHA408 alone, no changes in nuclear protein expression were seen (data not shown). Endotoxin administration also imposed significant decreases in the expression of FXR and RXR in PXR (-/-) while levels of PPAR- $\gamma$  and CAR were not significantly affected (Fig 5.B). Pre-administration of PHA408 did not significantly impact these endotoxin-mediated changes.

## Discussion

The acute inflammatory response induced by endotoxin administration has long been associated with a downregulation in the expression of numerous transporters and metabolizing enzymes (Petrovic, Teng, and Piquette-Miller 2007). The NF- $\kappa$ B signaling cascade is well recognized as the main pathway activated during the early response to endotoxin (Pan et al. 2010). However, few studies have established a direct connection between NF- $\kappa$ B and PXR signaling and regulation of transporters. Overall this study found that not only does endotoxin administration impose a pronounced downregulation of cyp3a, transporters, and nuclear receptors, but that these changes were prevented by prior administration of the NF- $\kappa$ B inhibitor PHA408. Moreover, neither the endotoxin-mediated downregulation nor the attenuation with NF- $\kappa$ B inhibition appeared to be dependent on PXR.

The NF- $\kappa$ B signaling pathway is well recognized as a major regulator of the inflammatory response (Tak and Firestein 2001, Lawrence 2009). Our findings demonstrated that endotoxin administration significantly increased nuclear protein levels of the active phosphorylated form of NF- $\kappa$ B. Moreover, a reduction of phosphorylated NF- $\kappa$ B to that of controls was seen in nuclear fractions obtained from endotoxin-treated mice that were pre-treated with PHA408, demonstrating effective inhibition of NF- $\kappa$ B activation. Likewise, induction of pro-inflammatory cytokines was effectively suppressed. The inhibition of NF- $\kappa$ B was also associated with the attenuation of endotoxin-mediated changes in the hepatic expression of Cyp3a and transporter genes. This indicates a principle role of NF- $\kappa$ B in the regulation of these genes. NF- $\kappa$ B has been previously reported to suppress the transcription of several Cyp enzymes by binding to their promoter regions (Morgan, Li-Masters, and Cheng 2002, Zordoky and El-Kadi 2009).

Similar to our findings with *Abcb1b*, TNF- $\alpha$  mediated activation of NF- $\kappa$ B signaling was found to induce levels of *Abcb1b* in primary cultures of rat hepatocytes and binding sites for NF- $\kappa$ B p50 and p65 have been identified in the promoter region (Ros et al. 2001). Moreover, in cultured human brain endothelial cells, NF- $\kappa$ B activation was reported to decrease expression of MDR1/P-gp (Fan et al. 2015). Overall these studies suggest an important role of NF- $\kappa$ B signaling in the regulation of drug transporters and metabolizing enzymes.

Although evidence of cross talk between PXR and NF- $\kappa$ B has been reported, we found that the endotoxin-mediated downregulation of hepatic transporters and *Cyp3a* generally occurred to a similar extent in wild type PXR (+/+) and PXR (-/-) mice. Moreover, the expressions of these genes were similarly attenuated in both strains after inhibition of NF- $\kappa$ B signaling with PHA408. These results indicate that PXR is not involved in endotoxin-mediated downregulation of hepatic transporters and that the downregulation occurs directly through NF- $\kappa$ B signaling rather than through PXR downregulation. Previous work in PXR (+/+) and PXR (-/-) mice found that while IL-6 administration imparted differences in transporter expression between the strains, only slight differences were seen after endotoxin administration (Teng and Piquette-Miller 2005). On the other hand, we observed higher basal levels of inflammatory markers in the PXR null mice, which were also associated with a decreased basal expression of most hepatic transporters. However, higher basal expression of *Cyp3a11*, *Abcg2* and *Slc10a1* were seen in PXR (-/-) mice. While the underlying cause of the increased inflammatory state in the PXR null mice is not known, an increased inflammation of the small bowel has been reported in PXR null mice (Zhou et al. 2006). Although it was proposed that intestinal inflammation suggested an increased NF- $\kappa$ B activity due to the absence of PXR, this observed inflammation could also be due to the dysregulation of genes

involved in maintaining intestinal homeostasis.

In addition to PXR, we observed an endotoxin-mediated downregulation in the mRNA expression of other nuclear hormone receptors that are involved in transporter regulation. For example FXR is involved in the regulation of Bsep/Abcb11, Mrp2/Abcc2, and Slco10a1 (Urquhart, Tirona, and Kim 2007, Kalaany and Mangelsdorf 2006, Tirona and Kim 2005). While RXR is a required co-dimer of numerous nuclear transcription factors, it has been previously suggested that decreased hepatic expression of RXR, along with decreased expression of LXR and PPAR $\alpha$ , could be responsible for the downregulation of multiple genes during inflammation (Beigneux et al. 2000). On the other hand, pre-administration of PHA408 did not significantly alter the endotoxin-mediated downregulation of RXR, FXR, PPAR-g and CAR. Therefore, NF- $\kappa$ B may not play a primary role in the regulation of these transcription factors after endotoxin exposure. Nevertheless, while endotoxin did not impose changes in the transcript levels of these genes, it is still possible that effects could occur on their activation.

In order to determine whether the effects of PHA-408 in endotoxin-treated animals were solely due to NF- $\kappa$ B inhibition or other drug effects, we also examined the effects of PHA-408 in saline treated mice. Of note, we found that administration of PHA408 alone caused a downregulation rather than upregulation of several transporters. As the serum concentrations of IL-6, a pro-inflammatory cytokine were significantly increased in PHA-408 treated mice, IL-6 induction could be responsible for these findings. Several *in vivo* and *in vitro* studies have reported that IL-6 treatments impose a downregulation of transporters and metabolizing enzymes (Morgan 1997, 2001). On the other hand, despite the higher levels of IL-6, PHA408 administration caused an increase in the mRNA levels of Cyp3a11 and Bcrp in PXR (+/+) but not PXR (-/-) mice. This indicates that PHA408 could be an activator of PXR. However, animals were sacrificed hours

after administration, while induction with most PXR activators requires 2-3 days of exposure (Anapolsky et al. 2006, Teng and Piquette-Miller 2005). Moreover, we did not see an increase in the protein levels of PXR in the nuclear fraction extracts obtained from the PHA408 treated mice. As only a single time point was examined, this does not fully rule out the possibility that PHA408 is an activator of PXR.

The overall findings from this study demonstrate that the nuclear factor NF- $\kappa$ B is the main signaling pathway involved in regulation of hepatic drug transporters during acute inflammation. This is the first study to demonstrate that selective *in vivo* inhibition of NF- $\kappa$ B attenuates endotoxin-mediated changes in the expression of transporters in liver. Although PXR activation is involved in the induction of several of these transporters, it does not appear to play a role in their down-regulation after endotoxin treatment. As NF- $\kappa$ B activation is linked to the pathogenesis of many acute and chronic disease states this information can be used to predict potential drug-disease interactions.

**Acknowledgement:**

The authors thank Ragia Ghoneim and Yen Ting Shen for their technical assistance.

**Authorship Contributions:**

*Participated in research design:* Abualsunun, Piquette-Miller

*Conducted experiments:* Abualsunun

*Performed data analysis:* Abualsunun

*Wrote or contributed to the writing of the manuscript:* Abualsunun, Piquette-Miller

There are no conflicts of interest to declare

## References:

- Aitken, A. E., T. A. Richardson, and E. T. Morgan. 2006. "Regulation of drug-metabolizing enzymes and transporters in inflammation." *Annu Rev Pharmacol Toxicol* 46:123-49. doi: 10.1146/annurev.pharmtox.46.120604.141059.
- Anapolsky, A., S. Teng, S. Dixit, and M. Piquette-Miller. 2006. "The role of pregnane X receptor in 2-acetylaminofluorene-mediated induction of drug transport and -metabolizing enzymes in mice." *Drug Metab Dispos* 34 (3):405-9. doi: 10.1124/dmd.105.006197.
- Ayrton, A., and P. Morgan. 2001. "Role of transport proteins in drug absorption, distribution and excretion." *Xenobiotica* 31 (8-9):469-97. doi: 10.1080/00498250110060969.
- Beigneux, A. P., A. H. Moser, J. K. Shigenaga, C. Grunfeld, and K. R. Feingold. 2000. "The acute phase response is associated with retinoid X receptor repression in rodent liver." *Journal of Biological Chemistry* 275 (21):16390-16399. doi: 10.1074/Jbc.M000953200.
- Carlson, C. G., E. Dole, C. Stefanski, and D. Bayless. 2015. "The effect of specific IKKbeta inhibitors on the cytosolic expression of I $\kappa$ B-alpha and the nuclear expression of p65 in dystrophic (MDX) muscle." *Am J Transl Res* 7 (4):670-82.
- Copeland, S., H. S. Warren, S. F. Lowry, S. E. Calvano, D. Remick, Inflammation, and Investigators the Host Response to Injury. 2005. "Acute inflammatory response to endotoxin in mice and humans." *Clin Diagn Lab Immunol* 12 (1):60-7. doi: 10.1128/CDLI.12.1.60-67.2005.
- Durk, M. R., K. Han, E. C. Chow, R. Ahrens, J. T. Henderson, P. E. Fraser, and K. S. Pang. 2014. "1 $\alpha$ ,25-Dihydroxyvitamin D3 reduces cerebral amyloid-beta accumulation and improves cognition in mouse models of Alzheimer's disease." *J Neurosci* 34 (21):7091-101. doi: 10.1523/JNEUROSCI.2711-13.2014.
- Fan, X., L. Chai, H. Zhang, Y. Wang, B. Zhang, and X. Gao. 2015. "Borneol Depresses P-Glycoprotein Function by a NF-kappaB Signaling Mediated Mechanism in a Blood Brain Barrier in Vitro Model." *Int J Mol Sci* 16 (11):27576-88. doi: 10.3390/ijms161126051.
- Goralski, K. B., G. Hartmann, M. Piquette-Miller, and K. W. Renton. 2003. "Downregulation of mdr1a expression in the brain and liver during CNS inflammation alters the in vivo disposition of digoxin." *Br J Pharmacol* 139 (1):35-48. doi: 10.1038/sj.bjp.0705227.
- Hartmann, G., V. Vassileva, and M. Piquette-Miller. 2005. "Impact of endotoxin-induced changes in P-glycoprotein expression on disposition of doxorubicin in mice." *Drug Metab Dispos* 33 (6):820-8. doi: 10.1124/dmd.104.002568.
- Hoesel, B., and J. A. Schmid. 2013. "The complexity of NF-kappaB signaling in inflammation and cancer." *Mol Cancer* 12:86. doi: 10.1186/1476-4598-12-86.
- Kalaany, N. Y., and D. J. Mangelsdorf. 2006. "LXRS and FXR: the yin and yang of cholesterol and fat metabolism." *Annu Rev Physiol* 68:159-91. doi: 10.1146/annurev.physiol.68.033104.152158.
- Lawrence, T. 2009. "The nuclear factor NF-kappaB pathway in inflammation." *Cold Spring Harb Perspect Biol* 1 (6):a001651. doi: 10.1101/cshperspect.a001651.
- Mbalaviele, G., C. D. Sommers, S. L. Bonar, S. Mathialagan, J. F. Schindler, J. A. Guzova, A. F. Shaffer, M. A. Melton, L. J. Christine, C. S. Tripp, P. C. Chiang, D. C. Thompson, Y. Hu, and N. Kishore. 2009. "A novel, highly selective, tight binding I $\kappa$ B kinase-2 (IKK-2) inhibitor: a tool to correlate IKK-2 activity to the fate and functions of the components of the nuclear factor-kappaB pathway in



- arthritis-relevant cells and animal models." *J Pharmacol Exp Ther* 329 (1):14-25. doi: 10.1124/jpet.108.143800.
- Mencarelli, A., B. Renga, G. Palladino, D. Claudio, P. Ricci, E. Distrutti, M. Barbanti, F. Baldelli, and S. Fiorucci. 2011. "Inhibition of NF-kappaB by a PXR-dependent pathway mediates counter-regulatory activities of rifaximin on innate immunity in intestinal epithelial cells." *Eur J Pharmacol* 668 (1-2):317-24. doi: 10.1016/j.ejphar.2011.06.058.
- Morgan, E. T. 1997. "Regulation of cytochromes P450 during inflammation and infection." *Drug Metab Rev* 29 (4):1129-88. doi: 10.3109/03602539709002246.
- Morgan, E. T. 2001. "Regulation of cytochrome p450 by inflammatory mediators: why and how?" *Drug Metab Dispos* 29 (3):207-12.
- Morgan, E. T. 2009. "Impact of infectious and inflammatory disease on cytochrome P450-mediated drug metabolism and pharmacokinetics." *Clin Pharmacol Ther* 85 (4):434-8. doi: 10.1038/clpt.2008.302.
- Morgan, E. T., K. B. Goralski, M. Piquette-Miller, K. W. Renton, G. R. Robertson, M. R. Chaluvadi, K. A. Charles, S. J. Clarke, M. Kacevska, C. Liddle, T. A. Richardson, R. Sharma, and C. J. Sinal. 2008. "Regulation of drug-metabolizing enzymes and transporters in infection, inflammation, and cancer." *Drug Metab Dispos* 36 (2):205-16. doi: 10.1124/dmd.107.018747.
- Morgan, E. T., T. Li-Masters, and P. Y. Cheng. 2002. "Mechanisms of cytochrome P450 regulation by inflammatory mediators." *Toxicology* 181-182:207-10.
- Oeckinghaus, A., and S. Ghosh. 2009. "The NF-kappaB family of transcription factors and its regulation." *Cold Spring Harb Perspect Biol* 1 (4):a000034. doi: 10.1101/cshperspect.a000034.
- Pahl, Heike L. "Activators and target genes of Rel/NF-kappaB transcription factors." *Oncogene* 18:6853-6866.
- Pan, W., C. Yu, H. Hsueh, and A. J. Kastin. 2010. "The role of cerebral vascular NFkappaB in LPS-induced inflammation: differential regulation of efflux transporter and transporting cytokine receptors." *Cell Physiol Biochem* 25 (6):623-30. doi: 10.1159/000315081.
- Petrovic, V., S. Teng, and M. Piquette-Miller. 2007. "Regulation of drug transporters during infection and inflammation." *Mol Interv* 7 (2):99-111. doi: 10.1124/mi.7.2.10.
- Rajendrasozhan, S., J. W. Hwang, H. Yao, N. Kishore, and I. Rahman. 2010. "Anti-inflammatory effect of a selective IkappaB kinase-beta inhibitor in rat lung in response to LPS and cigarette smoke." *Pulm Pharmacol Ther* 23 (3):172-81. doi: 10.1016/j.pupt.2010.01.002.
- Ros, J. E., J. D. Schuetz, M. Geuken, K. Streetz, H. Moshage, F. Kuipers, M. P. Manns, P. L. Jansen, C. Trautwein, and M. Muller. 2001. "Induction of Mdr1b expression by tumor necrosis factor-alpha in rat liver cells is independent of p53 but requires NF-kappaB signaling." *Hepatology* 33 (6):1425-31. doi: 10.1053/jhep.2001.24667.
- Tak, P. P., and G. S. Firestein. 2001. "NF-kappaB: a key role in inflammatory diseases." *J Clin Invest* 107 (1):7-11. doi: 10.1172/JCI11830.
- Teng, S., and M. Piquette-Miller. 2005. "The involvement of the pregnane X receptor in hepatic gene regulation during inflammation in mice." *J Pharmacol Exp Ther* 312 (2):841-8. doi: 10.1124/jpet.104.076141.
- Teng, S., and M. Piquette-Miller. 2008. "Regulation of transporters by nuclear hormone receptors: implications during inflammation." *Mol Pharm* 5 (1):67-76. doi: 10.1021/mp700102q.

- Tirona, R. G., and R. B. Kim. 2005. "Nuclear receptors and drug disposition gene regulation." *J Pharm Sci* 94 (6):1169-86. doi: 10.1002/jps.20324.
- Urquhart, B. L., R. G. Tirona, and R. B. Kim. 2007. "Nuclear receptors and the regulation of drug-metabolizing enzymes and drug transporters: implications for interindividual variability in response to drugs." *J Clin Pharmacol* 47 (5):566-78. doi: 10.1177/0091270007299930.
- Wahli, W. 2008. "A gut feeling of the PXR, PPAR and NF-kappaB connection." *J Intern Med* 263 (6):613-9. doi: 10.1111/j.1365-2796.2008.01951.x.
- Xie, W., and Y. Tian. 2006. "Xenobiotic receptor meets NF-kappaB, a collision in the small bowel." *Cell Metab* 4 (3):177-8. doi: 10.1016/j.cmet.2006.08.004.
- Zhou, Changcheng, Michelle M. Tabb, Edward L. Nelson, Felix Grün, Suman Verma, Asal Sadatrafiei, Min Lin, Shyamali Mallick, Barry M. Forman, Kenneth E. Thummel, and Bruce Blumberg. 2006. "Mutual repression between steroid and xenobiotic receptor and NF-kB signaling pathways links xenobiotic metabolism and inflammation." *The Journal of Clinical Investigation* 116 (8).
- Zordoky, B. N., and A. O. El-Kadi. 2009. "Role of NF-kappaB in the regulation of cytochrome P450 enzymes." *Curr Drug Metab* 10 (2):164-78.

## Footnotes

Funding for this study was provided by an operating grant from the Canadian Institutes of Health Research [MOP 13346]. W.A is a recipient of the King Abdul-Aziz University Scholarship for Postgraduate Studies.

This work was previously presented as a poster at the 2017 American Society of Clinical Pharmacology and Therapeutics (ASCPT) Annual Meeting, March 15-18, 2017; Washington, DC.

## Legend to Figures

**Figure 1. NF- $\kappa$ B signaling pathway.** Lipopolysaccharides (LPS) activates NF- $\kappa$ B through a series of cytoplasmic receptors that leads to activation of IKK kinases and degradation of the I $\kappa$ B inhibitory protein, resulting in translocation of NF- $\kappa$ B subunits into the nucleus. PHA408 selectively inhibits IKK kinases.

**Figure 2.** The effect of PHA408 on (A) mRNA levels of inflammatory markers following endotoxin (LPS) administration in the liver of PXR (+/+) and PXR (-/-) mice. Data is presented as % mean  $\pm$  SEM (n=5-8). Where \* significant from saline, # significant from LPS. \*\*, ## P<0.01, \*\*\*, ### P< 0.001.

**Figure 3.** Effect of PHA408 on (A) NF- $\kappa$ B mRNA expression and (B) NF- $\kappa$ B nuclear protein expression and representative western blot following endotoxin (LPS) administration in the liver of PXR (+/+) and PXR (-/-) mice, and mRNA and protein expression was determined as described in methods. Data is presented as % mean  $\pm$  SEM (n=5-8), where \* significant from saline and # significant from LPS. \*, # P<0.05, \*\*, ## P<0.01, \*\*\*, ### P<0.001.

**Figure 4.** Effect of PHA408 on hepatic mRNA expression of (A) ABC Transporters, (B) SLC Transporters, and (C) Cyp3a11 following endotoxin administration in PXR (+/+) and PXR (-/-) mice. Data is presented as % mean  $\pm$  SEM (n=5-8) where \* significant from saline and # significant from LPS. \*, # P<0.05, \*\*, ## P<0.01, \*\*\*, ### P<0.001.

**Figure 5.** Effect of PHA408 on the hepatic mRNA expression of Nuclear Hormone Receptors in (A) PXR (+/+) and (B) PXR (-/-) mice following endotoxin administration.

Data is presented as % mean  $\pm$  SEM (n=5-8) where \* significant from saline and # significant from LPS. \*,# P<0.05, \*\*,## P<0.01, \*\*\*,### P<0.001.

**Figure 6.** Effect of PHA408 on A) hepatic mRNA expression of PXR and (B) hepatic nuclear protein expression and representative western blot of PXR in wild type mice following endotoxin administration. mRNA and protein expression was determined as described in methods. Data is presented as % mean  $\pm$  SEM (n=5-8) where \* significant from saline and # significant from LPS. \*,# P<0.05, \*\*,## P<0.01, \*\*\*,### P<0.001.

**Table 1.** Basal gene expression in the liver of PXR (+/+) and PXR (-/-) mice.

Results are reported relative to control wild type PXR (+/+). Data is presented as % mean  $\pm$  SEM ( $n=5-8$ ). Where \* significant from saline control, \*\*\* $p<0.001$ .

Cytokines/Genes	PXR (+/+)	PXR (-/-)
<b>Proinflammatory Cytokines</b>		
IL-6	100 $\pm$ 0.02	194 $\pm$ 0.06***
IL-1 $\beta$	100 $\pm$ 0.02	135 $\pm$ 0.09***
TNF- $\alpha$	100 $\pm$ 0.03	36 $\pm$ 0.02***
iNOS	100 $\pm$ 0.02	105 $\pm$ 0.04
Socs3	100 $\pm$ 0.03	509 $\pm$ 0.17***
<b>ABC Transporters</b>		
Abcb1a	100 $\pm$ 0.02	34 $\pm$ 0.05***
Abcb1b	100 $\pm$ 0.02	22 $\pm$ 0.02***
Abcg2	100 $\pm$ 0.03	210 $\pm$ 0.06***
Abcc2	100 $\pm$ 0.02	55 $\pm$ 0.04***
Abcc3	100 $\pm$ 0.03	119 $\pm$ 0.08
Abcb11	100 $\pm$ 0.02	41 $\pm$ 0.01***
<b>SLC Transporters</b>		
Slc10a1	100 $\pm$ 0.02	160 $\pm$ 0.09***
Slco1a4	100 $\pm$ 0.02	3 $\pm$ 0.01***
Slco2b1	100 $\pm$ 0.02	128 $\pm$ 0.07*
<b>Metabolic Enzyme</b>		
Cyp3a11	100 $\pm$ 0.02	193 $\pm$ 0.09***

**Table 2.** Effect of PHA408 on hepatic mRNA expression in PXR (+/+) and PXR (-/-) mice. Results are reported relative to saline controls. Data is presented as % mean  $\pm$  SEM of (n=4-5) mice; \* significant from saline; # significant from LPS. \*,# P<0.05, \*\*,## P<0.01.

Genes	% Control in PXR (+/+)	% Control in PXR (-/-)
IL-6	369 **	505 **
IL-1 $\beta$	96	90
TNF- $\alpha$	84	40 #
iNOS	110	116
Socs3	194 **	69 #
Abcb1a	28 **	38 **
Abcb1b	28 **	77 #
Abcg2	168 **	33 **
Abcc2	59 *	61 *
Abcc3	84	65 *
Abcb11	56 *	76 **
Slc10a1	101	62 **
Slco2b1	90	69 *
Slco1a4	41 *	40 *
Cyp3a11	158 **	80 #
PXR	148 **	-

Figure 1.

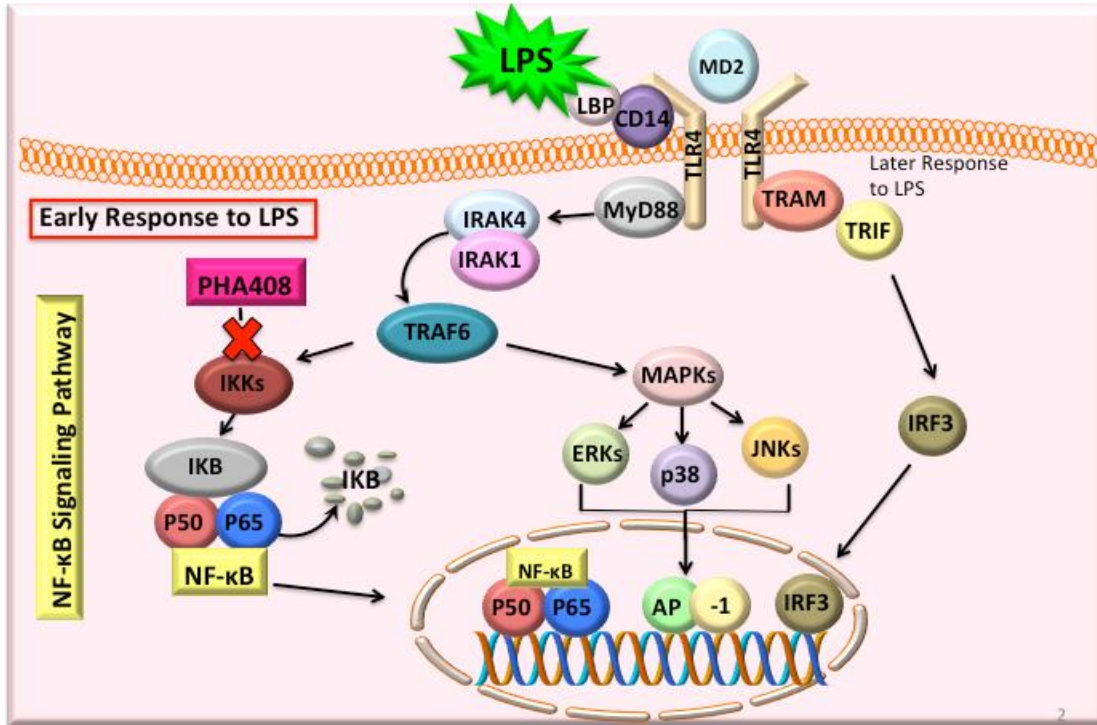




Figure 2.

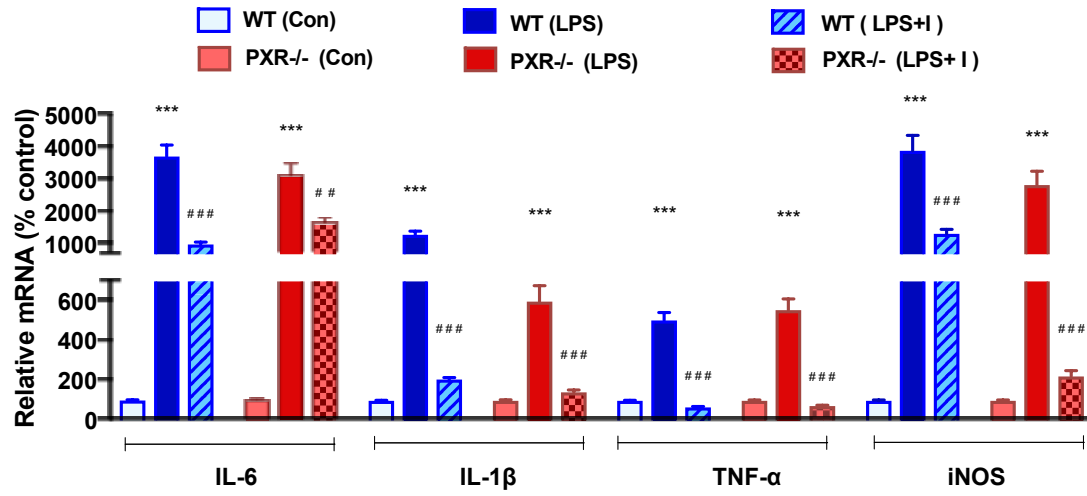


Figure 3.

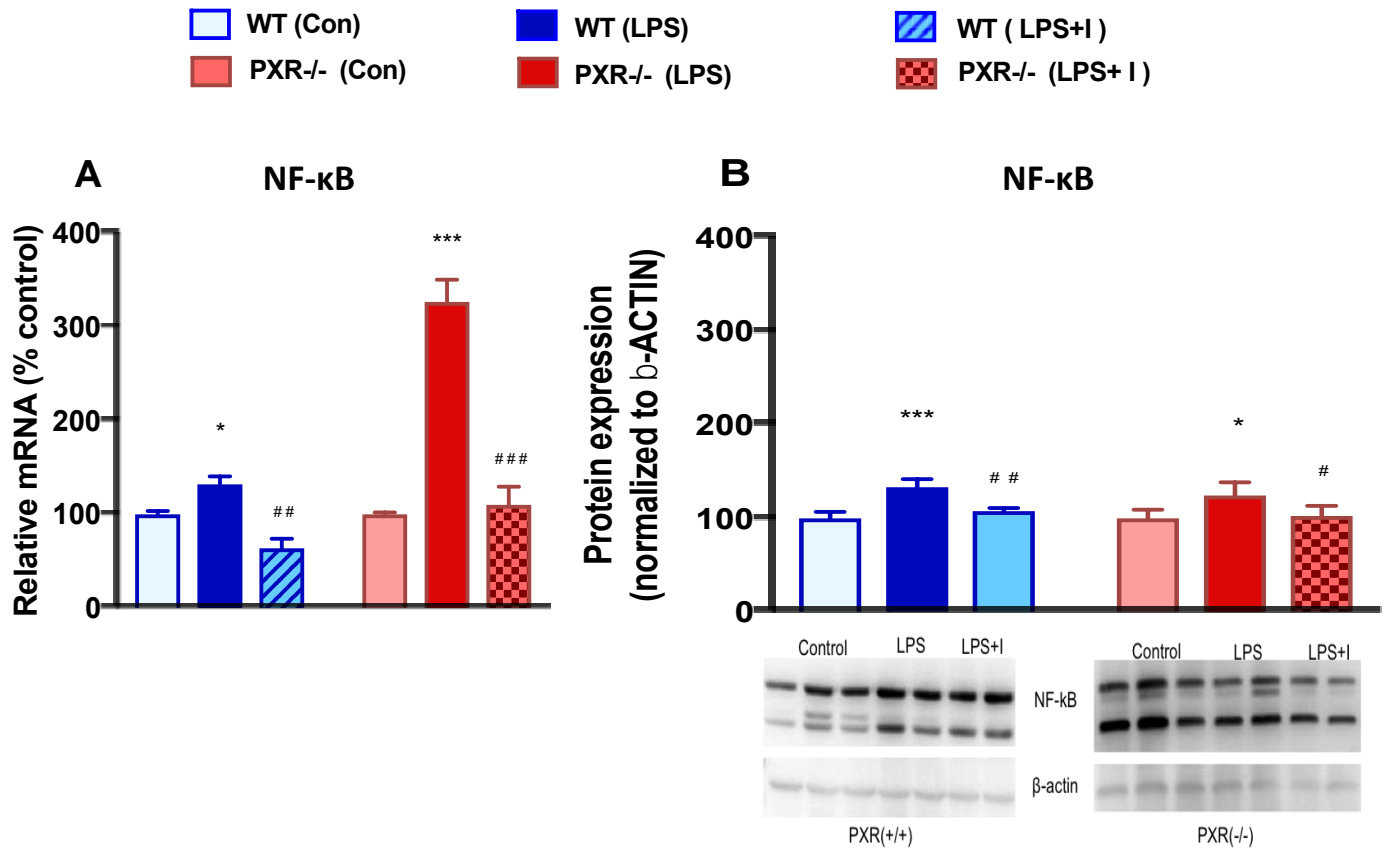


Figure 4.

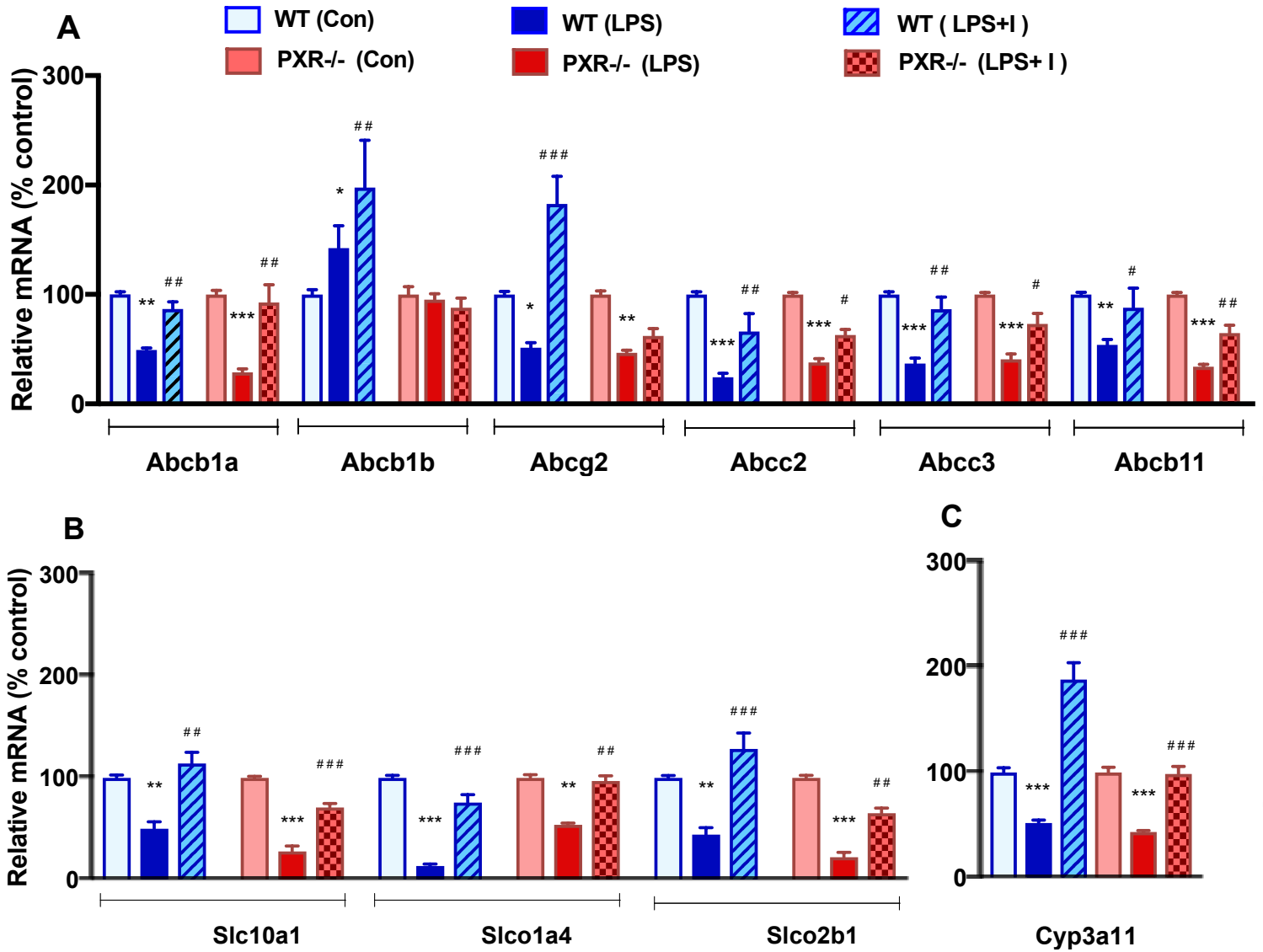


Figure 5.

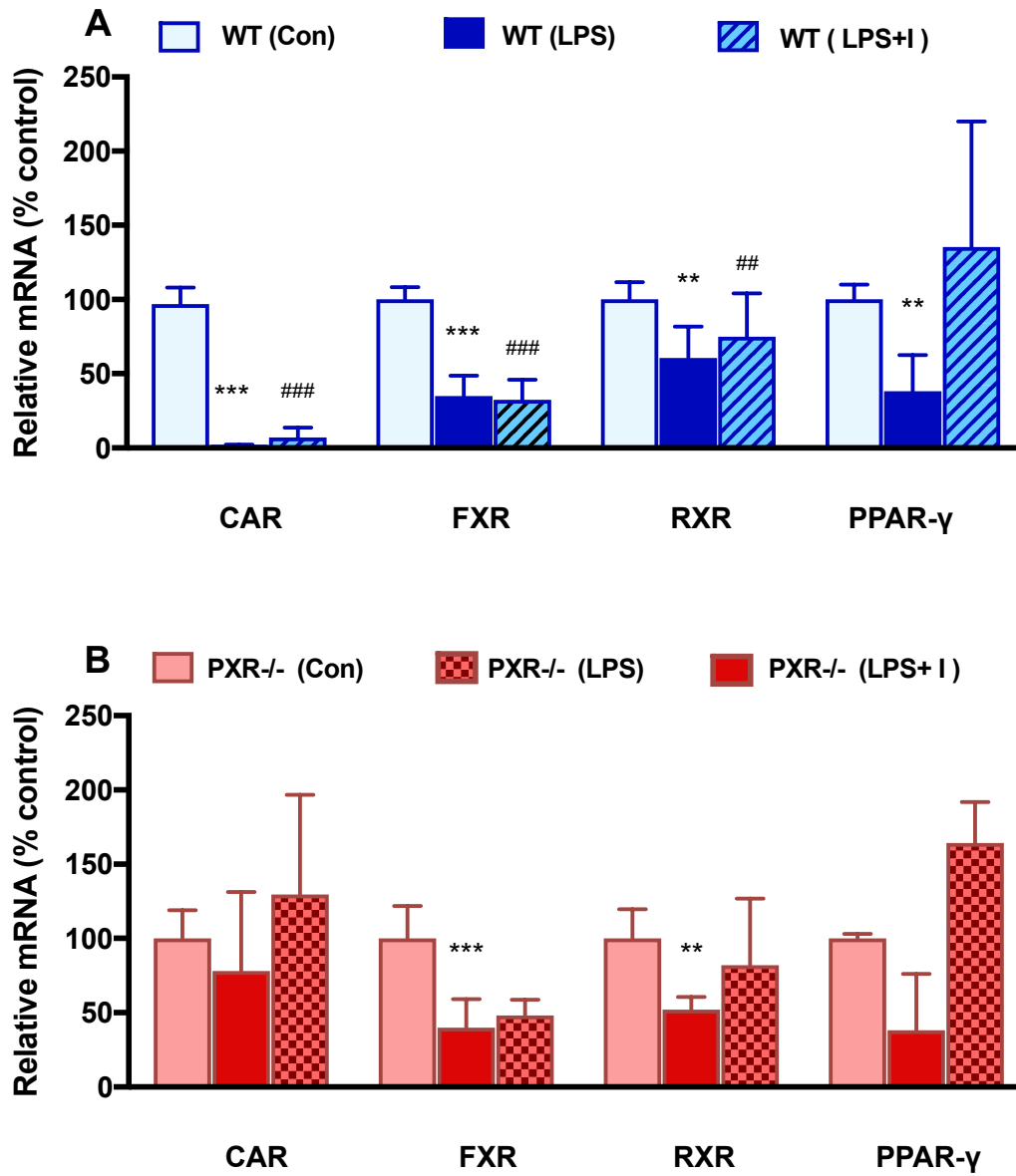


Figure 6.

