**Supporting Information for** 

**Original article** 

Understanding the physiological functions of the host xenobioticsensing nuclear receptors PXR and CAR on the gut microbiome using genetically modified mice

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# Section 1 Supporting tables.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
	(10 mmol/L ammonium	(10 mmol/L ammonium
	acetate with 20% acetonitrile	acetate with 80% acetonitrile
	in water)	in water)
0.00	95	5
5.00	95	5
14.00	86	14
14.50	75	25
17.50	75	25
18.00	50	50
22.00	50	50
22.50	20	80
24.50	20	80
25.00	95	5

Table S1 Optimized UPLC–MS/MS gradient for bile acid chromatography.

 Table S2 Primer sequences.

qPCR Target	Forward primer (5'–3')	Reverse primer $(5'-3')$
Bile salt hydrolase (Bsh)	ATGGGCGGACTAGGATTACC	TGCCACTCTCTGTCTGCATC
L. acidophilus	AGCGAGCTGAACCAACAGAT	TGATCATGCGATCTGCTTTC
L. johnsonii	GAGCGAGCTTGCCTAGATGA	ATCGCCTTGGTAAGCCATTA
Il-6	CCGGAGAGGAGACTTCACAG	TCCACGATTTCCCAGAGAAC
<i>Il-2</i>	GAGTCAGCAACTGTGGTGGA	AGGGCTTGTTGAGATGATGC
Il-12p35	CTCCTGTGGGGAGAAGCAGAC	CAGATAGCCCATCACCCTGT

Table S3 Bacteria commonly regulated across PXR-null vs. WT and CAR-null vs. WT

comparisons.

Adolescent males	Adult males	Adolescent females	Adult females
Anaerostipes sp.	Anaerostipes sp.	Anaerostipes sp.	Anaerostipes sp.
Akkermansia muciniphila	Dehalobacterium sp.	Akkermansia muciniphila	Mogibacteriaceae family
Mucispirillum shaedleri	Bacteroides acidifaciens	Bacteroides sp.	Bacteroides sp.
Helicobacter sp.	Helicobacter sp.	Anaeroplasma sp.	Helicobacter sp.
Helicobacteraceae family	Helicobacteraceae family	Enterococcus sp.	Helicobacteraceae family
Oscillospira sp.	Sutterella sp.	Lactococcus sp.	Lactococcus sp.
Clostridiales order	Parabacteroides sp.	Carnobacterium sp.	Parabacteroides sp.
	S24-7 family (now		
	Muribaculaceae)		

# **Section 2 Supporting figures**



**Figure S1 Differentially abundant taxa in C57BL/6 WT, PXR-null, CAR-null, and PXR-***CAR-null mice.* Two-way hierarchical clustering of the mean differentially abundant taxa in fecal samples from adolescent- and adult-aged male and female C57BL/6 WT, *PXR*-null, *CAR*null, and *PXR-CAR*-null mice, as generated by the R packages gplots and RColorBrewer.

Asterisks (\*) represent statistically significant differences compared to WT mice (one-way ANOVA, Duncan's post hoc, P < 0.05).



Figure S2 BA concentrations in C57BL/6 WT, *PXR*-null, *CAR*-null, and *PXR*-CAR-null adolescent male mice. Individual bar plots of mean (SE) BA concentrations (ng/g) in adolescent male, mice as generated by the R package ggplot2. BAs were quantified by LC MS/MS as described in Materials and methods. Asterisks (\*) represent statistically significant differences compared to WT mice (one-way ANOVA, Duncan's post hoc, P < 0.05).



Figure S3 BA concentrations in C57BL/6 WT, *PXR*-null, *CAR*-null, and *PXR*-CAR-null adult male mice. Individual bar plots of mean (SE) BA concentrations (ng/g) in adult male, mice as generated by the R package ggplot2. BAs were quantified by LC–MS/MS as described in Materials and methods. Asterisks (\*) represent statistically significant differences compared to WT mice (one-way ANOVA, Duncan's post hoc, P < 0.05).



Figure S4 BA concentrations in C57BL/6 WT, *PXR*-null, *CAR*-null, and *PXR*-CAR-null adolescent female mice. Individual bar plots of mean (SE) BA concentrations (ng/g) in adolescent female, mice as generated by the R package ggplot2. BAs were quantified by LC MS/MS as described in Materials and methods. Asterisks (\*) represent statistically significant differences compared to WT mice (one-way ANOVA, Duncan's post hoc, P < 0.05).



Figure S5 BA concentrations in C57BL/6 WT, *PXR*-null, *CAR*-null, and *PXR*-CAR-null adult female mice. Individual bar plots of mean (SE) BA concentrations (ng/g) in adult female, mice as generated by the R package ggplot2. BAs were quantified by LC–MS/MS as described in Materials and methods. Asterisks (\*) represent statistically significant differences compared to WT mice (one-way ANOVA, Duncan's post hoc, P < 0.05).



**Figure S6 A. Comparison between physiological activation of PXR and pharmacological activation of PXR by its prototypical ligand PCN.** Differentially regulated bacteria between *PXR*-null and WT mice of the present study (adult males only) were cross-referenced with a previous 16S rDNA sequencing study where adult C57BL/6 male mice were orally gavaged with the prototypical PXR ligand PCN (75 mg/kg) once daily for 4 consecutive days (PCN *vs.* vehicle [corn oil])<sup>30</sup>. Commonly and uniquely regulated bacteria between the two models are shown in a venn diagram. **B. Comparison between physiological activation of CAR and pharmacological activation of CAR by its prototypical ligand TCPOBOP.** Differentially regulated bacteria between *CAR*-null and WT mice of the present study (adult males only) were cross-referenced with a previous 16S rDNA sequencing study where adult C57BL/6J male mice were orally gavaged with the prototypical CAR ligand TCPOBOP (3 mg/kg) once daily for 4 consecutive days (TCPOBOP vs. vehicle [corn oil])<sup>30</sup>. Commonly and uniquely regulated bacteria between the two models are shown in a venn diagram. The filtering criteria for both A and B are: average % OTUs across all groups > 0.001% in each study, and *P* < 0.1.

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**Figure S7 A. Comparison between physiological activation of PXR and toxicological activation of PXR by BDE-47 and BDE-99.** Differentially regulated bacteria between *PXR*-null and WT mice of the present study (adult males only) were cross-referenced with a previous

16S rDNA sequencing study where adult C57BL/6 male mice were orally gavaged with BDE-47 or BDE-99 (known activators of PXR, 100 µmol/kg) once daily for 4 consecutive days (PBDEs *vs.* vehicle [corn oil])<sup>9,13,37</sup>. Commonly and uniquely regulated bacteria between the two models are shown in a venn diagram. **B. Comparison between physiological activation of CAR and toxicological activation of CAR by BDE-47 and BDE-99.** Differentially regulated bacteria between *PXR*-null and WT mice of the present study (adult males only) were cross-referenced with a previous 16S rDNA sequencing study where adult C57BL/6 male mice were orally gavaged with BDE-47 or BDE-99 (known activators of CAR, 100 µmol/kg) once daily for 4 consecutive days (PBDEs vs. vehicle [corn oil])<sup>9,13,37</sup>. Commonly and uniquely regulated bacteria between the two models are shown in a venn diagram. The filtering criteria for both A and B are: average % OTUs across all groups > 0.001% in each study, and P < 0.1.





sequencing study where adult C57BL/6 female mice were orally gavaged with the Fox River PCB mixture (known activators of PXR, 6 mg/kg) once daily for 3 consecutive days (PCBs *vs.* vehicle [corn oil])<sup>31,40</sup>. Commonly and uniquely regulated bacteria between the two models are shown in a venn diagram. **B. Comparison between physiological activation of CAR and toxicological activation of CAR by PCBs.** Differentially regulated bacteria between *CAR*-null and WT mice of the present study (adult females only) were cross-referenced with a previous 16S rDNA sequencing study where adult C57BL/6 female mice were orally gavaged with the Fox River PCB mixture (known activators of PXR, 6 mg/kg) once daily for 3 consecutive days (PCBs vs. vehicle [corn oil])<sup>31,40</sup>. Commonly and uniquely regulated bacteria between the two models are shown in a venn diagram. The filtering criteria for both A and B are: average % OTUs across all groups > 0.001% in each study, and *P* < 0.1.



Figure S9 Differentially abundant taxa in WT and h*PXR*-TG FVB/NJ mice. Two-way hierarchical clustering of the mean differentially abundant taxa in fecal samples from adolescent- and adult-aged male and female WT and h*PXR*-TG FVB/NJ mice, as generated by the R packages gplots and RColorBrewer. Asterisks (\*) represent statistically significant differences compared to WT mice (*t*-test, P < 0.05).

**Functional predictions** 



Adult male

Figure S10. Functional predictions in WT and h*PXR*-TG FVB/NJ mice. Two-way hierarchical clustering dendrograms of differentially regulated KEGG pathways predicted by PICRUSt, as described in Materials and methods, from adolescent- and adult-aged male and female WT and h*PXR*-TG FVB/NJ mice (*t*-test, P < 0.05). Generated by the R packages gplots and RColorBrewer.



**Figure S11 Alpha and beta diversities of C57BL/6 and FVB/NJ mice. A.** Mean (SE) alpha diversity of gut microbiota within the low PXR/CAR expressers (C57BL/6) and high PXR/CAR expressers (FVB/NJ mice). Line plots were generated using the R package ggplot.

Asterisks (\*) represent statistically significant differences compared to C57BL/6 mice (*t*-test, P < 0.05). **B.** Principal coordinate analysis (PCoA) plots showing the beta diversities of adolescent male, adult male, adolescent female, and adult female C57BL/6 and FVB/NJ mice.



Figure S12 Percentage OTUs of *Lactococcus sp.*, *Acinetobacter sp.*, and *E. dolichum*, and cytokine concentrations in C57BL/6 and FVB/NJ mice. A. Individual bar plots of mean (SE) percentage OTUs of *Lactococcus sp.*, *Acinetobacter sp.*, and *E. dolichum*, are generated by the

R package ggplot2. Asterisks (\*) represent statistically significant differences compared to C57BL/6 mice (*t*-test, P < 0.05). **B.** Bar plots of cytokine concentrations (pg/mL) are generated by the R package ggplot2. Asterisks (\*) represent statistically significant differences compared to C57BL/6 mice (*t*-test, P < 0.05).

# Adolescent male

p Bacteroidetes;c Bacteroidia;o Bacteroidales;f Bacteroidaceae;g Bacteroides;s
p Bacteroidetes;c Bacteroidia;o Bacteroidales;f Prevotellaceae;g Prevotella;s
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Rikenellaceae;g_;s_
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_S24-7;g_;s_
<b></b> p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus;s
p_Firmicutes;c_Clostridia;o_Clostridiales;f_;g_;s_
Description p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_;s_
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_[Ruminococcus];s_gnavus
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_;s_
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Oscillospira;s_
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Ruminococcus;s_
p_Firmicutes;c_Erysipelotrichi;o_Erysipelotrichales;f_Erysipelotrichaceae;g_Allobaculum;s_
p_Proteobacteria;c_Betaproteobacteria;o_Burkholderiales;f_Alcaligenaceae;g_Sutterella;s_
<b></b> p_Proteobacteria;c_Epsilonproteobacteria;o_Campylobacterales;f_Helicobacteraceae;g_;s
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### Adolescent female

Bacteroidetes; c Bacteroidia; o Bacteroidales; f Bacteroidaceae; g Bacteroides; s
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Prevotellaceae;g_Prevotella;s_
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Rikenellaceae;g_;s_
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_S24-7;g_;s_
p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus;s_
p_Firmicutes;c_Clostridia;o_Clostridiales;f_;g_;s_
p_Firmicutes;cClostridia;oClostridiales;f_Lachnospiraceae;g_;s
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_[Ruminococcus];s_gnavus
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_;s_
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Oscillospira;s_
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Ruminococcus;s_
p_Firmicutes;c_Erysipelotrichi;o_Erysipelotrichales;f_Erysipelotrichaceae;g_Allobaculum;s_
Proteobacteria;c_Betaproteobacteria;o_Burkholderiales;f_Alcaligenaceae;g_Sutterella;s_
p Proteobacteria;c Epsilonproteobacteria;o Campylobacterales;f Helicobacteraceae;g ;s
Other Other

# Adult male

p Bacteroidetes;c Bacteroidia;o Bacteroidales;f Bacteroidaceae;g Bacteroides;s
p Bacteroidetes;c Bacteroidia;o Bacteroidales;f Prevotellaceae;g Prevotella;s
p Bacteroidetes;c Bacteroidia;o Bacteroidales;f Rikenellaceae;g ;s
p Bacteroidetes;c Bacteroidia;o Bacteroidales;f S24-7;g ;s
p Firmicutes; c Bacilli; o Lactobacillales; f Lactobacillaceae; g Lactobacillus; s
p Firmicutes;c Clostridia;o Clostridiales;f ;g ;s
p Firmicutes; c Clostridia; o Clostridiales; f Lachnospiraceae; g ; s
p Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; [Ruminococcus]; gnavus
p Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; ;s
p Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Oscillospira; s
p Bacteroidetes;c Bacteroidia;o Bacteroidales;f [Paraprevotellaceae];g [Prevotella];s
p Firmicutes; c Erysipelotrichi; o Erysipelotrichales; f Erysipelotrichaceae; g Allobaculum; s
p Proteobacteria;c Betaproteobacteria;o Burkholderiales;f Alcaligenaceae;g Sutterella;s
p Proteobacteria;c Epsilonproteobacteria;o Campylobacterales;f Helicobacteraceae;g ;s
Cother Cother

#### Adult female

- Restavaidateura Restavaidiana Restavaidaleuri Restavaidaeura Restavaidaeura
p_bacteroidates;c_bacteroidia;o_bacteroidales;i_bacteroidaceae;g_bacteroides;s_
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Prevotellaceae;g_Prevotella;s_
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Rikenellaceae;g_;s_
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_S24-7;g_;s_
p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus;s_
p_Firmicutes;c_Clostridia;o_Clostridiales;f_;g_;s_
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_;s_
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_[Ruminococcus];s_gnavus
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_;s_
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Oscillospira;s_
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_[Paraprevotellaceae];g_[Prevotella];s_
p_Firmicutes;c_Erysipelotrichi;o_Erysipelotrichales;f_Erysipelotrichaceae;g_Allobaculum;s_
p_Proteobacteria;c_Betaproteobacteria;o_Burkholderiales;f_Alcaligenaceae;g_Sutterella;s_
p_Proteobacteria;c_Epsilonproteobacteria;o_Campylobacterales;f_Helicobacteraceae;g_;s_
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Figure S13 Figure legend for Fig. 3.

#### Adolescent male

p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_S24-7;g_;s_
p_Firmicutes;c_Clostridia;o_Clostridiales;f_;g_;s_
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_[Paraprevotellaceae];g_[Prevotella];s_
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Oscillospira;s_
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_;s_
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_;s_
p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus;s_
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Rikenellaceae;g_;s_
p_Verrucomicrobia;c_Verrucomicrobiae;o_Verrucomicrobiales;f_Verrucomicrobiaceae;g_Akkermansia;s_muciniphila
Tenericutes;c_Mollicutes;o_Anaeroplasmatales;f_Anaeroplasmataceae;g_Anaeroplasma;s
p_Firmicutes;c_Bacilli;o_Turicibacterales;f_Turicibacteraceae;g_Turicibacter;s_
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Ruminococcus;s_
p_Proteobacteria;c_Betaproteobacteria;o_Burkholderiales;f_Alcaligenaceae;g_Sutterella;s_
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_[Ruminococcus];s_gnavus
Conter Other

#### Adolescent female

p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_S24-7;g_;s_
p_Firmicutes;c_Clostridia;o_Clostridiales;f_;g_;s_
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_[Paraprevotellaceae];g_[Prevotella];s_
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_;s_
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Oscillospira;s_
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Rikenellaceae;g_;s_
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_;s_
p_Verrucomicrobia;c_Verrucomicrobiae;o_Verrucomicrobiales;f_Verrucomicrobiaceae;g_Akkermansia;s_muciniphila
Tenericutes;c_Mollicutes;o_Anaeroplasmatales;f_Anaeroplasmataceae;g_Anaeroplasma;s_
p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus;s_
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_[Ruminococcus];s_gnavus
P_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Prevotellaceae;g_Prevotella;s_
p_Firmicutes;c_Bacilli;o_Turicibacterales;f_Turicibacteraceae;g_Turicibacter;s_
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Ruminococcus;s_
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### Adult male

p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_S24-7;g_;s_
p_Firmicutes;c_Clostridia;o_Clostridiales;f_;g_;s_
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_[Paraprevotellaceae];g_[Prevotella];s_
p Bacteroidetes; c Bacteroidia; o Bacteroidales; f Rikenellaceae; g; s
p Verrucomicrobia; verrucomicrobiae; verrucomicrobiales; Verrucomicrobiales; Akkermansia; muciniphila
p Firmicutes;c Clostridia;o Clostridiales;f Ruminococcaceae;g Oscillospira;s
p Firmicutes;c Clostridia;o Clostridiales;f Lachnospiraceae;g ;s
p Firmicutes;c Clostridia;o Clostridiales;f Ruminococcaceae;g ;s
p Firmicutes;c Bacilli;o Turicibacterales;f Turicibacteraceae;g Turicibacter;s
p Tenericutes;c Mollicutes;o Anaeroplasmatales;f Anaeroplasmataceae;g Anaeroplasma;s
p Firmicutes;c Bacilli;o Lactobacillales;f Lactobacillaceae;g Lactobacillus;s
p Bacteroidetes;c Bacteroidia;o Bacteroidales;f Bacteroidaceae;g Bacteroides;s
p Firmicutes:c Clostridia:o Clostridiales:f Ruminococcaceae:g Ruminococcus:s
p Proteobacteria;c Betaproteobacteria;o Burkholderiales;f Alcaligenaceae;g Sutterella;s
Other

### Adult female

p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_S24-7;g_;s_
p_Firmicutes;c_Clostridia;o_Clostridiales;f_;g_;s_
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_[Paraprevotellaceae];g_[Prevotella];s_
p_Verrucomicrobia;c_Verrucomicrobiae;o_Verrucomicrobiales;f_Verrucomicrobiaceae;g_Akkermansia;s_muciniphila
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Rikenellaceae;g_;s_
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Oscillospira;s_
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_;s_
p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_;s_
p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus;s_
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Bacteroidaceae;g_Bacteroides;s_
p_Tenericutes;c_Mollicutes;o_Anaeroplasmatales;f_Anaeroplasmataceae;g_Anaeroplasma;s_
p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Prevotellaceae;g_Prevotella;s_
p_Firmicutes;c_Bacilli;o_Turicibacterales;f_Turicibacteraceae;g_Turicibacter;s_
p_Proteobacteria;c_Betaproteobacteria;o_Burkholderiales;f_Alcaligenaceae;g_Sutterelia;s_
Other

Figure S14 Figure legend for Fig. 8.

Figure S15



Figure S15 RT-qPCR of liver cytokines in adult WT, *CAR*-null, and *PXR*-null mice. Mean (SE) data are expressed at % of the house-keeping gene *Gapdh*. Asterisks (\*) represent statistically significant differences compared to controls, and pound symbols (#) represent statistically significant sex differences (two-way ANOVA, P < 0.05).

Figure S16



Figure S16 RT-qPCR of BA synthesis enzymes in adult WT, CAR-null, and PXR-null mice.

Mean (SE) data are expressed at % of the house-keeping gene *Gapdh*. Asterisks (\*) represent statistically significant differences compared to controls, and pound symbols (#) represent statistically significant sex differences (two-way ANOVA, P < 0.05).