

## Supporting Information

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Pregnane X Receptor Activation in Liver Macrophages Protects against Endotoxin-Induced Liver Injury

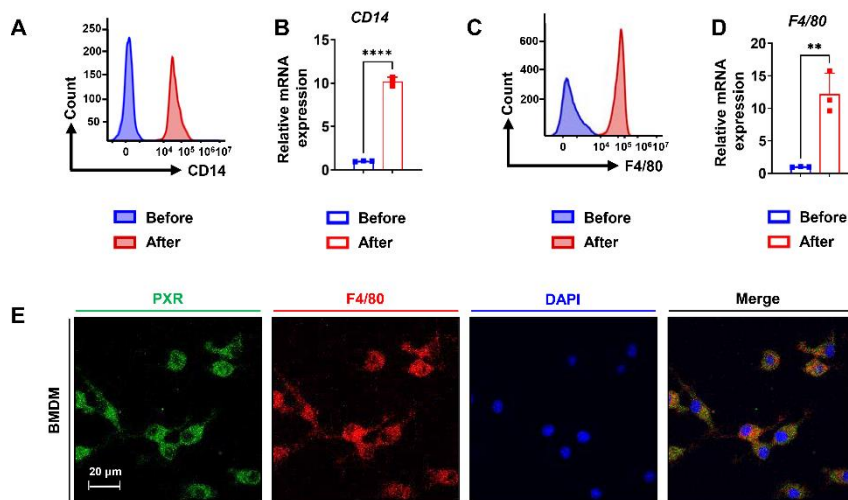
*Tingting Zhao, Guoping Zhong, Ying Wang, Renjie Cao, Shaofei Song, Yuan Li, Guohui Wan, Haiyan Sun, Min Huang\*, Huichang Bi\* and Yiming Jiang\**

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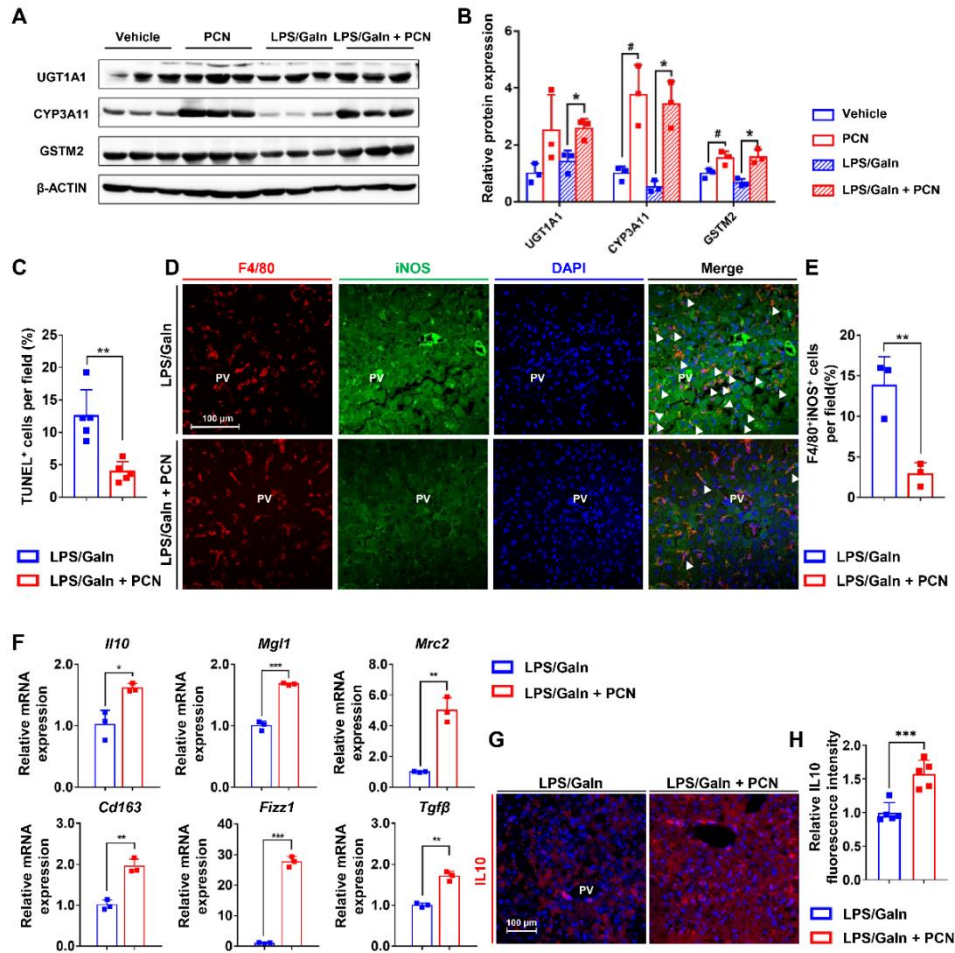
### Pregnane X receptor activation in liver macrophages protects against endotoxin-induced liver injury

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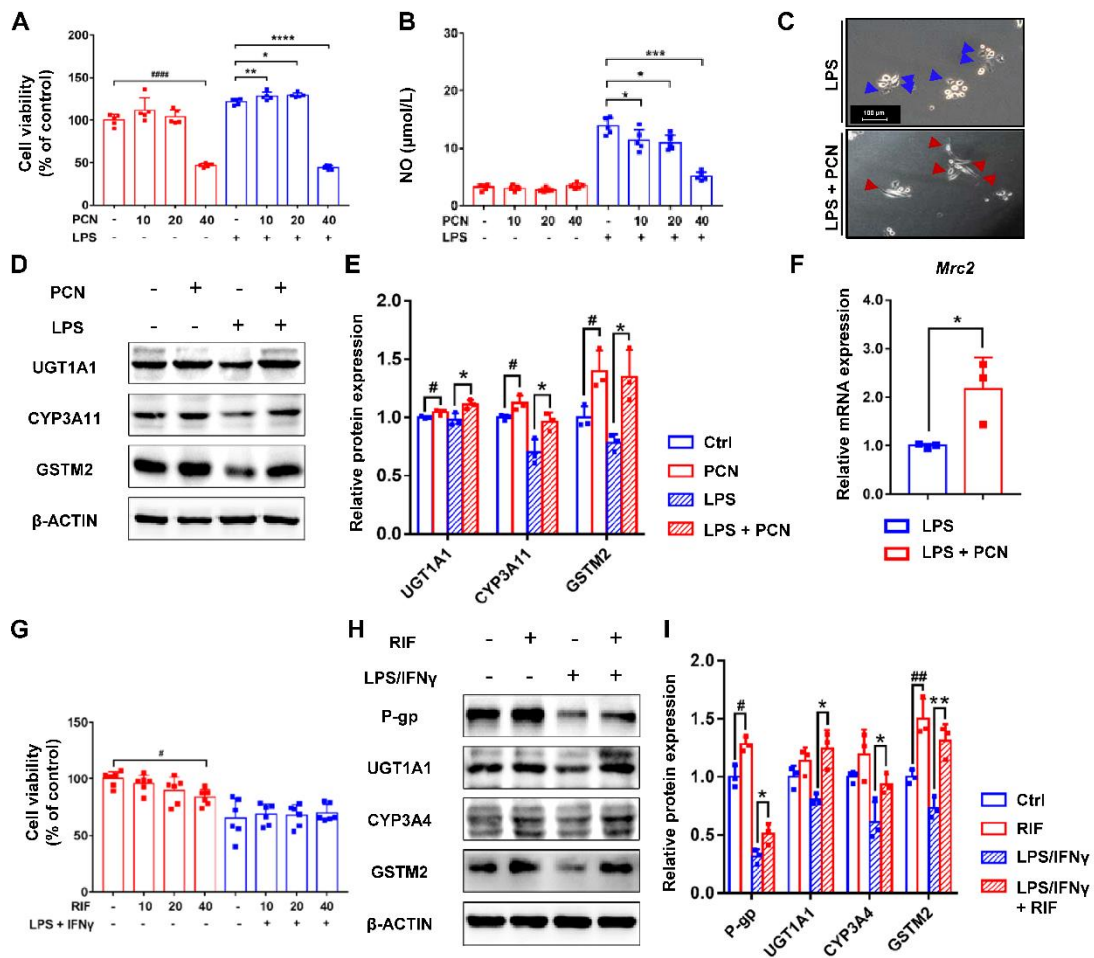
#### 1. Supplemental Figures



**Figure S1.** Detection of macrophage markers and PXR expression. A) Histogram of  $CD14^+$  cells before and after differentiation (100 ng/mL GM-CSF for 7 days) of hMDMs. B) The mRNA level of *CD14* in hMDMs before and after differentiation ( $n = 3$ ). C) Histogram of  $F4/80^+$  cells before and after differentiation (25 ng/mL M-CSF for 7 days) of BMDMs. D) The mRNA level of *F4/80* in BMDMs before and after differentiation ( $n = 3$ ). E) Immunofluorescence staining of BMDMs with PXR (green) and F4/80 (red). The data are expressed as the mean  $\pm$  SD. \*\* $P < 0.01$  and \*\*\*\* $P < 0.0001$  as hMDMs or BMDMs after differentiation compared with before.

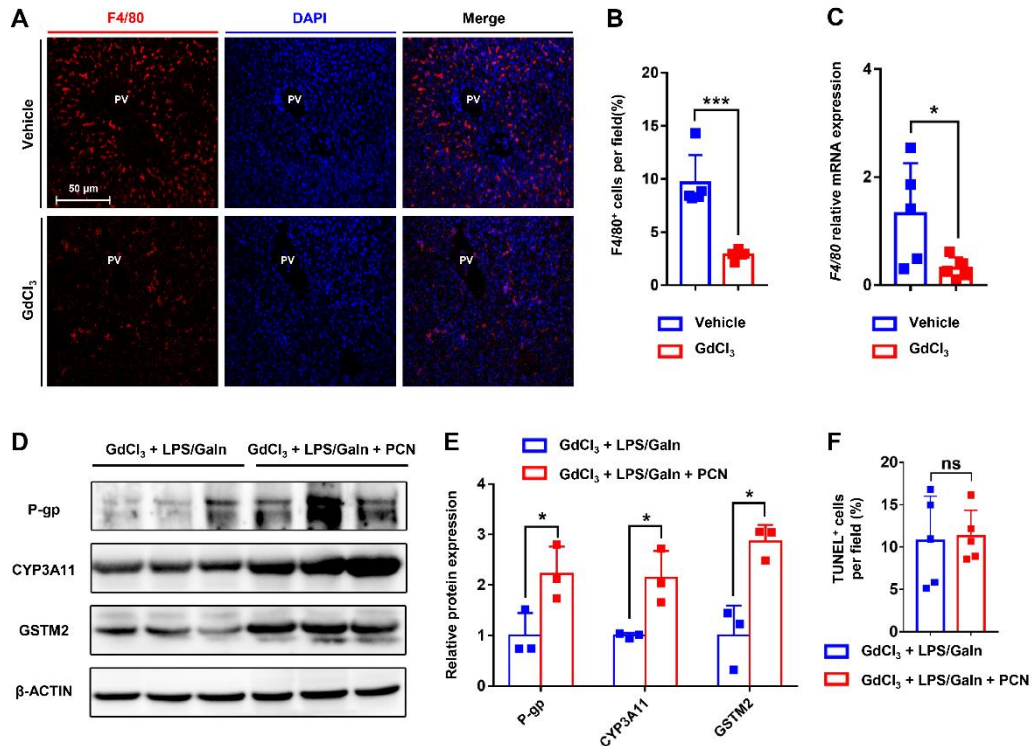


**Figure S2.** PXR activation protects against LPS/GaIn-induced liver injury and promotes macrophage M2 polarization. A, B) Western blotting and quantification of the protein expression of PXR downstream proteins in the liver (n = 3). C) Quantification of TUNEL<sup>+</sup> cells in liver sections (n = 5). D, E) Immunofluorescence staining and quantification of F4/80 (red) and iNOS (green) in LPS/GaIn and LPS/GaIn + PCN groups (n = 3). Filled triangles indicate the F4/80<sup>+</sup>iNOS<sup>+</sup> cells. F) qRT-PCR analysis of M2 polarization-related genes (*Il10*, *Mgl1*, *Mrc2*, *Cd163*, *Fizz1* and *Tgfβ*) in primary Kupffer cells isolated from mice treated with LPS/GaIn and LPS/GaIn + PCN (n = 3). G, H) Immunofluorescence staining and quantification of IL10 in LPS/GaIn and LPS/GaIn + PCN groups (n = 5). Data are expressed as the mean ± SD. #*P* < 0.05 compared with the vehicle group. \**P* < 0.05, \*\**P* < 0.01 and \*\*\**P* < 0.001 compared with the LPS/GaIn group.

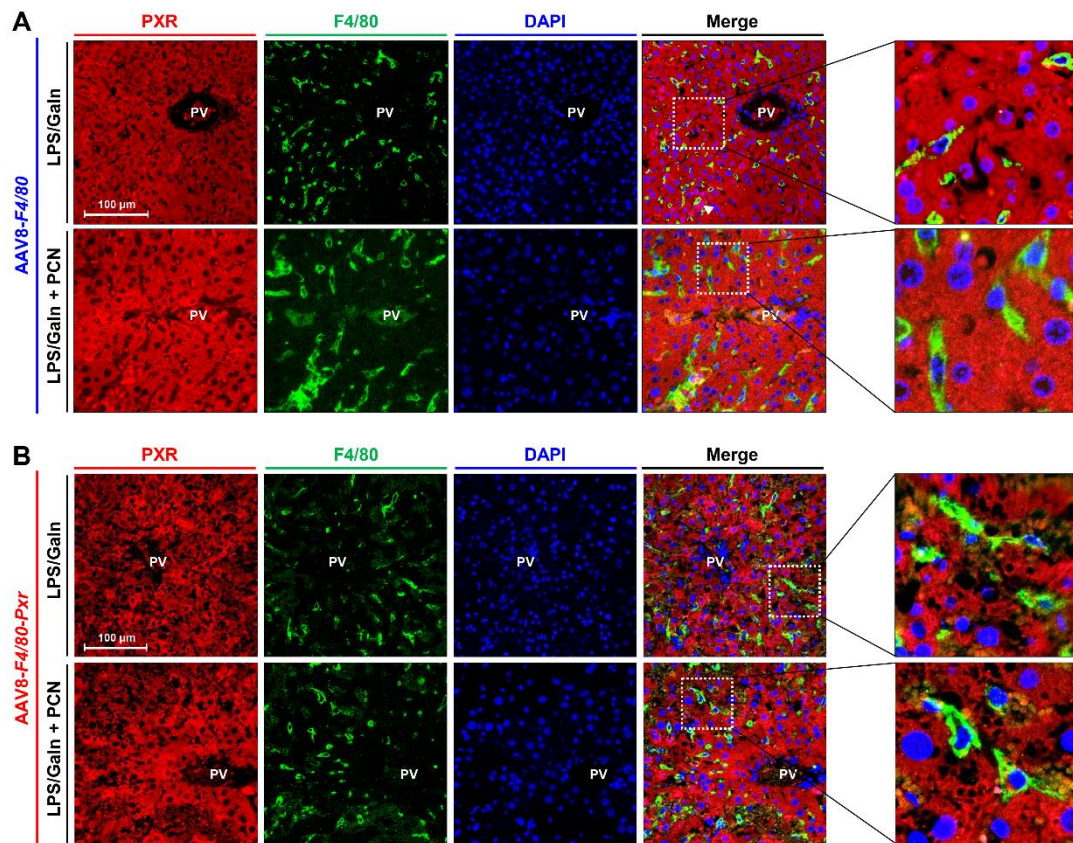


**Figure S3.** The effects of PXR activation on macrophages *in vitro*. A) The viability of Raw264.7 cells after incubation with various concentrations of PCN (0-40  $\mu$ M, for 48 h) with or without LPS (1  $\mu$ g/mL, for 24 h) (n = 5). B) NO levels in the supernatants of Raw264.7 cells incubated with PCN (0-40  $\mu$ M, for 48 h) with or without LPS (1  $\mu$ g/mL, for 24 h) (n = 5). C-F) Raw264.7 cells were incubated with LPS (1  $\mu$ g/mL, for 24 h) with or without PCN (20  $\mu$ M, for 48 h). C) Cell morphological changes of Raw264.7 cells after incubation with LPS or LPS + PCN. Blue arrows indicate M1 polarization and red arrows indicate M2 polarization. D, E) Western blotting and quantification of the protein expression of PXR downstream proteins in Raw264.7 cells (n = 3). F) qRT-PCR analysis of *Mrc2* expression in Raw264.7 cells incubated with LPS and LPS + PCN. (n = 3). G) The viability of THP-1 macrophages after incubation with various concentrations of RIF

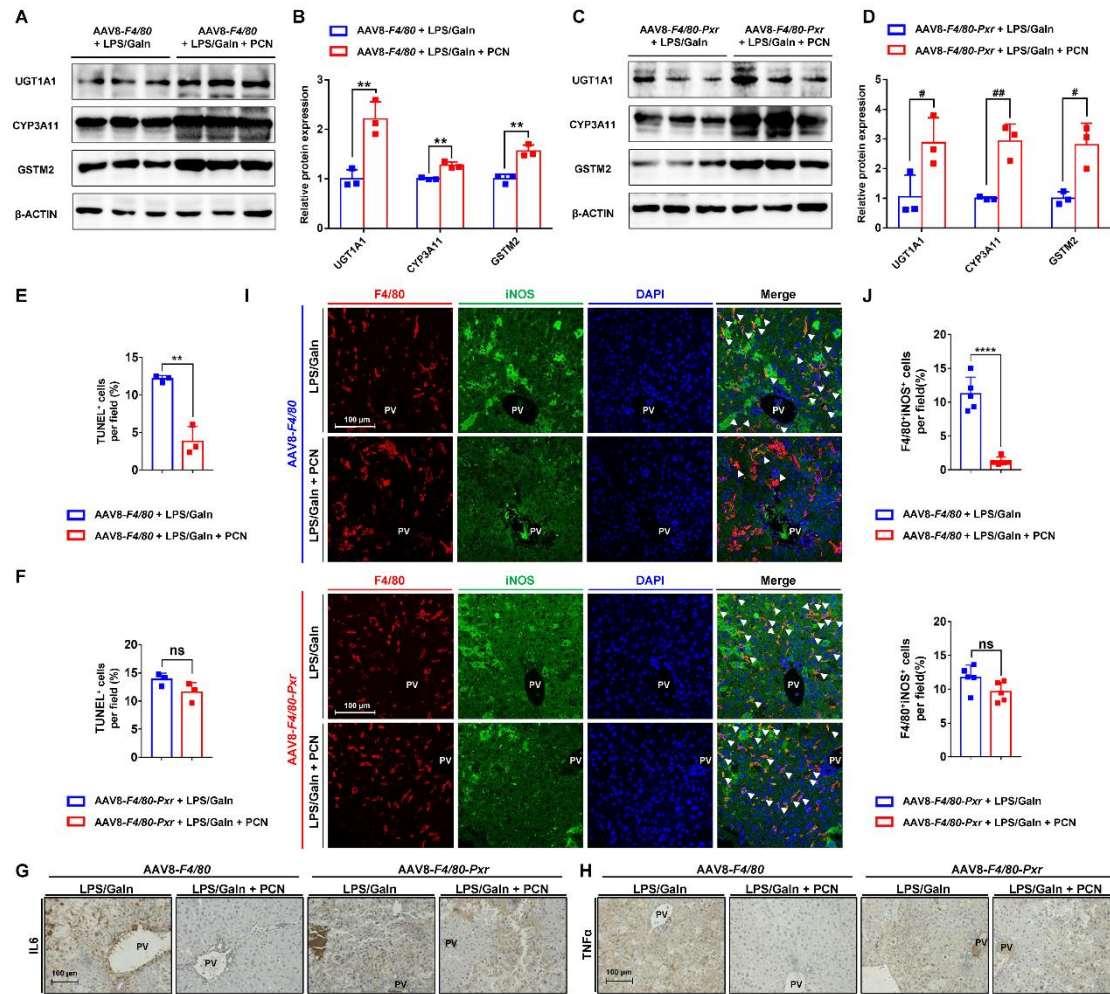
(0-40  $\mu$ M, for 48 h) with or without LPS/IFN $\gamma$  (100 ng/mL and 20 ng/mL, for 24 h) (n = 5). H, I) Western blotting and quantification of the protein expression of PXR downstream proteins in THP-1 macrophages (n = 3). THP-1 macrophages were incubated with LPS/IFN $\gamma$  (100 ng/mL and 20 ng/mL, respectively, for 24 h) with or without RIF (20  $\mu$ M, for 48 h). Data are expressed as the mean  $\pm$  SD. # $P$  < 0.05, ## $P$  < 0.01 and #### $P$  < 0.0001 compared with the control group. \* $P$  < 0.05, \*\* $P$  < 0.01, \*\*\* $P$  < 0.001 and \*\*\*\* $P$  < 0.0001 compared with the LPS or LPS/IFN $\gamma$  group.



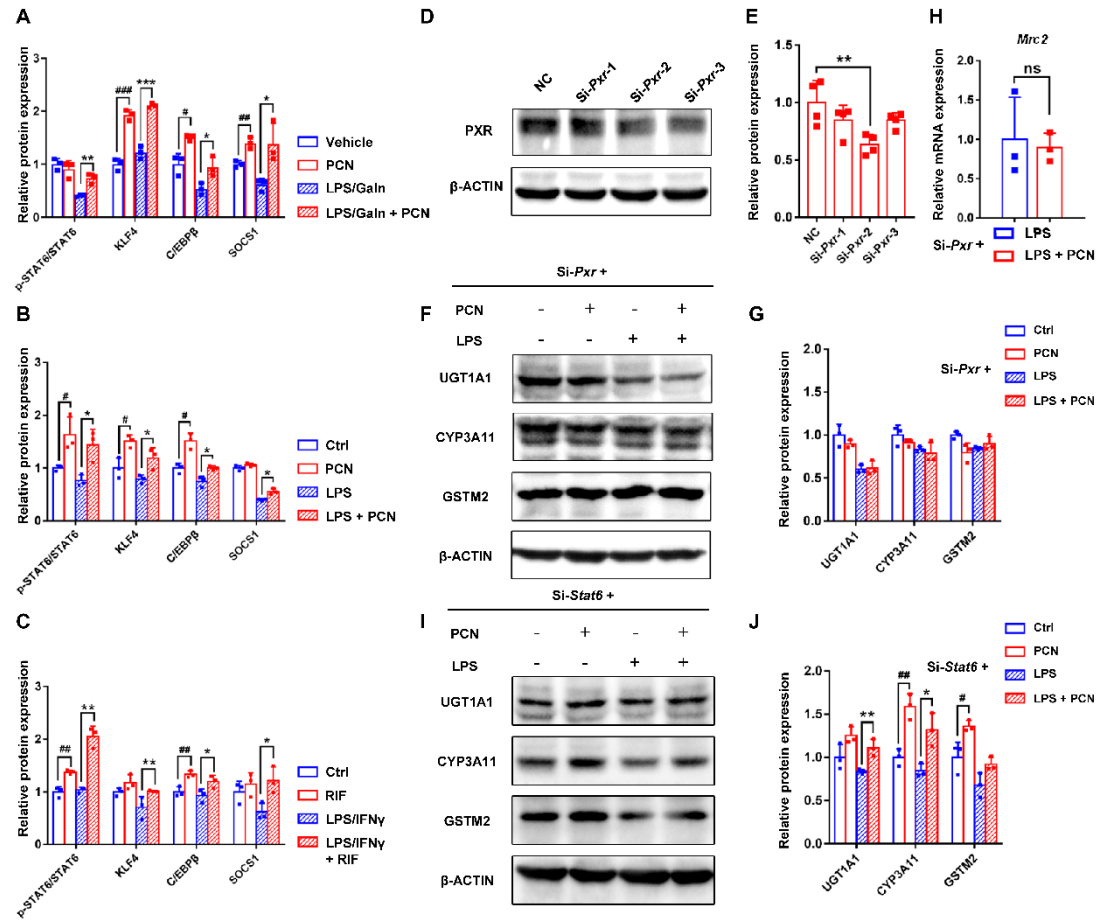
**Figure S4.** The effects of PXR activation after macrophage deletion by GdCl<sub>3</sub> on LPS/Galn-induced liver injury. A, B) Immunofluorescence staining and quantification of F4/80 (red) in the liver (n = 5). C) qRT-PCR analysis of *F4/80* expression in liver tissue after GdCl<sub>3</sub> injection. The data are expressed as the mean ± SD. \**P* < 0.05 and \*\*\**P* < 0.001 compared with the vehicle group (n = 5). D, E) Western blotting and quantification of the expression of PXR downstream proteins after PCN treatment and macrophage deletion using GdCl<sub>3</sub> (n = 3). F) Quantification of TUNEL<sup>+</sup> cells of liver sections (n = 5). The data are expressed as the mean ± SD. \**P* < 0.05 compared with the GdCl<sub>3</sub> + LPS/Galn group. *P* > 0.05 compared with the GdCl<sub>3</sub> + LPS/Galn group, ns: not significant.



**Figure S5.** The expression of PXR in AAV-*F4/80*- and AAV-*F4/80-Pxr*-treated mice. A) Immunofluorescence staining of PXR (red) and F4/80 (green) of liver sections of the AAV-*F4/80* group. B) Immunofluorescence staining of PXR (red) and F4/80 (green) of liver sections of the AAV-*F4/80-Pxr* group.



**Figure S6.** The effects of PXR activation after treatment with AAV8-F4/80 and AAV8-F4/80-Pxr. A-D) Western blotting and quantification of PXR downstream protein expressions ( $n = 3$ ). E, F) Quantification of TUNEL<sup>+</sup> cells in liver sections ( $n = 3$ ). G, H) Immunohistochemical staining of IL6 and TNF $\alpha$  in mice of AAV8-F4/80 or AAV8-F4/80-Pxr groups treated with LPS/Galn or LPS/Galn + PCN. I, J) Immunofluorescence staining and quantification of F4/80 (red) and iNOS (green) in mice of AAV8-F4/80 or AAV8-F4/80-Pxr groups treated with LPS/Galn or LPS/Galn + PCN ( $n = 5$ ). Filled triangles indicate the F4/80<sup>+</sup>iNOS<sup>+</sup> cells. The data are expressed as the mean  $\pm$  SD. \*\* $P < 0.01$  and \*\*\*\* $P < 0.0001$  compared with the AAV8-F4/80 + LPS/Galn group. # $P < 0.05$  and ## $P < 0.01$  compared to the AAV8-F4/80-Pxr + LPS/Galn group.  $P > 0.05$  compared with the AAV8-F4/80-Pxr + LPS/Galn group, ns: not significant.



**Figure S7.** The effects of *Pxr* or *Stat6* silencing on the expressions of the PXR downstream proteins. A) Quantification of p-STAT6/STAT6, KLF4, C/EBP $\beta$  and SOCS1 expression in the livers. The data are expressed as the mean  $\pm$  SD. # $P$  < 0.05, ## $P$  < 0.01 and ### $P$  < 0.001 compared with the vehicle group. \* $P$  < 0.05, \*\* $P$  < 0.01 and \*\*\* $P$  < 0.001 compared with the LPS/Galn group. B, C) Quantification of p-STAT6/STAT6, KLF4, C/EBP $\beta$  and SOCS1 expression in B) Raw264.7 cells and C) THP-1 macrophages. The data are expressed as the mean  $\pm$  SD. # $P$  < 0.05 and ## $P$  < 0.01 compared with the control group. \* $P$  < 0.05 and \*\* $P$  < 0.01 compared with the LPS or LPS/IFN $\gamma$  group. D, E) Western blotting and quantification analysis of the interference efficiency of si-*Pxr* on PXR protein expression (n = 3). The data are expressed as the mean  $\pm$  SD. \*\* $P$  < 0.01 compared with the negative control group. F, G) Western blotting and quantification of the expressions of PXR downstream proteins after *Pxr* silencing (n = 3). H) qRT-PCR

analysis of *Mrc2* expression in Raw264.7 cells after *Pxr* silencing (n = 3). I, J) Western blotting and quantification of PXR downstream protein expressions after *Stat6* silencing (n = 3). The data are expressed as the mean  $\pm$  SD. #*P* < 0.05 and ##*P* < 0.01 compared with the control group. \**P* < 0.05 and \*\**P* < 0.01 compared with the LPS group. *P* > 0.05 compared with the LPS group, ns: not significant.

## 2. Supplemental tables

**Table S1.** Sequences for siRNA and shRNA.

<b>SiRNA or shRNA</b>	<b>Sequence (5'-3')</b>
<i>Pxr</i> siRNA-1	GCCGATGTGTCAACCTACA
<i>Pxr</i> siRNA-2	GGAGGAAGATGGAGGTCTT
<i>Pxr</i> siRNA-3	GCGTCATCAACTTCGCCAA
<i>Stat6</i> siRNA-1	CCAAGACAACAACGCCAAA
<i>Stat6</i> siRNA-2	GCTGATCATTGGCTTTATT
<i>Stat6</i> siRNA-3	CCTGCAACCATCTCCTTAT

**Table S2.** List of mouse primers used in this study.

<b>Gene</b>	<b>Forward (5'-3')</b>	<b>Reverse (5'-3')</b>
<i>Tnfa</i>	AATGGCCTCCCTCTCATCAGTT	CCACTTGGTGGTTTGCTACGA
<i>Nos2</i>	AATCTTGGAGCGAGTTGTGG	CAGGAAGTAGGTGAGGGCTTG
<i>Ccl3</i>	TGAGAGTCTTGGAGGCAGCGA	TGTGGCTACTTGGCAGCAAACA
<i>Arg1</i>	CTCCAAGCCAAAGTCCTTAGAG	AGGAGCTGTCATTAGGGACATC
<i>Mgl1</i>	TGGCCTGAAGCTGACAAGTA	AGGCCGATCCAACCTAACCACAT
<i>Fizz1</i>	CCCTTCTCATCTGCATCTCC	CAGTAGCAGTCATCCCAGCA
<i>Mrc2</i>	TGCAAGCAATGCATCCAAGCCT	ACGGCTTTCCGTGTGAGTTT
<i>18s</i>	CCTGGATACCGCAGCTAGGA	GCGGCGCAATACGAATGCCCC

**Table S3.** List of human primers used in this study.

<b>Gene</b>	<b>Forward (5'-3')</b>	<b>Reverse (5'-3')</b>
<i>TNF<math>\alpha</math></i>	CCTCTCTCTAATCAGCCCTCTG	GAGGACCTGGGAGTAGATGAG
<i>NOS2</i>	TTCAGTATCACAACTCAGCAAG	TGGACCTGCAAGTTAAAATCCC
<i>CCL2</i>	GATCTCAGTGCAGAGGCTCG	TTTGCTTGTCCAGGTGGTCC
<i>TGM2</i>	CGTGACCAACTACAACCTCGG	CATCCACGACTCCACCCAG
<i>MRC1</i>	CTACAAGGGATCGGGTTTATGGA	TTGGCATTGCCTAGTAGCGTA
<i>CCL22</i>	TGGGTGAAGATGATTCTCAATAAGC	CTATAATGGCAGGGAGGTAGGG
<i>18S</i>	ATCCCTGAAAAGTTCCAGCA	CCCTCTTGGTGAGGTCAATG