Supplementary Table 1. Baseline Characteristics of Patients with AH.

Characteristics	N= 54 Median (25-75 IQR)
Age (y)	54 (46-56)
Male n (%)	42 (78)
Corticosteroids n (%)	30 (56)
SIRS[+] (%)	19 (35)
Laboratory and hemodynamic parameters	
Hemoglobin (g/dL)	11 (10-13)
Leukocyte count $x10^9$ /L	8.5 (6.3-12.5)
Platelet count $x10^9$/L	112 (81-207)
AST (U/L)	117 (68-151)
ALT (U/L)	41 (26-60)
Serum albumin (g/dL)	2.6 (2.3-3.0)
Serum creatinine (mg/dL)	1.0 (0.7-1.3)
Serum bilirubin (mg/dL)	8.0 (4.0-21.4)
International normalized ratio	1.7 (1.4-2.0)
HVPG (mmHg)	19 (15-23)
Alcoholic hepatitis severity scores at admission	
MELD score	21 (16-28)
ABIC score	8.0 (6.7-8.7)
Clinical decompensations during hospitalization	
AKI n (%)**	23 (43)
Infection n (%)	25 (46)
Mortality at 90 days n (%)	17 (32)
Mortality at 180 days n (%)	19 (35)

** Abbreviations: SIRS, Systemic inflammatory response syndrome, MELD, Model for End Stage Liver disease, ABIC, Age-Bilirubin-INR-Creatinine, HVPG, Hepatic venous pressure gradient, AH, Alcoholic hepatitis.

[+] SIRS was defined as 2 or more of the following variables: temperatura > 38°C (100.4°F) or < 36°C (96.8°F), heart rate > 90 x`, respiratory >20 x' or $PaCO_2$<32mm Hg and Leucocytes >12,000/mm^3 or < 4,000/mm^3.

**Acute kidney injury was defined as an abrupt (within 48 hours) reduction in kidney function currently defined as an absolute increase in serum creatinine of more than or equal to 0.3 mg/dl (≥ 26.4 µmol/l), a percentage increase in serum creatinine of more than or equal to 50% (1.5-fold from baseline) based on AKIN (acute kidney injury network) criteria.

Supplementary Table 2. Cox Regression models for 180-day mortality in patients with AH.

Bivariate models for prediction of 180-day mortality (events=19)	***HR***	***95% CI***	***P value***
Plasma PTX3 levels	1.015	1.005-1.024	**0.002**
ABIC score	1.475	1.081-2.012	**0.014**
Plasma PTX3 levels	1.012	1.002-1.021	**0.02**
MELD score	1.010	1.031-1.180	**0.004**
Plasma PTX3 levels	1.011	1.002-1.021	**0.018**
Lille Model*	31.667	3.807-263.412	**0.001**

*30 patients treated with steroids and 15 events at 180 days.

Abbreviations: HR: hazard ratio; CI: confidence interval; ABIC: age, bilirubin, INR and creatinine score; MELD: moder for end-stage liver disease.

In vitro studies

Primary HSC assays

To asses PTX3 induction, HSC were starved overnight and treated with TNF-α 20 ng/mL (R&D Systems, Minneapolis, USA), IL-1β 20 ng/mL (Sigma-Aldrich, St Louis, Missouri, USA) and LPS 100 ng/mL (Sigma-Aldrich). To determine the effect of PTX3 on HSC activation, cultured HSC were serum starved and incubated with human recombinant PTX3 (rPTX3) from R&D Systems (Endotoxin free <1.0 EU per 1 μg of the protein by the LAL method) for 24h.

HSC signaling

Total protein was extracted from human primary HSC incubated during 15 min or 60 min with rPTX3 (200 ng/mL) and PDGFBB (25 ng/mL). Western blot analysis for phosphorilated ERK, and AKT were performed with anti-p-ERK, ERK total, p-AKT and AKT total antibodies from Cell Signaling Technologies. Protein analysis was performed using enhanced chemiluminescence (Biological Industries, Beit-Haemek, Israel) and were visualized using Las4000 Imaging system (GE Healthcare Life Sciences, Piscataway, NJ).

Wound-healing assay

10^5 cells per well were plated in a 12 well plate and serum starved 24 hours before performing the assay. Cell monolayers were carefully wounded using a 10μL pipette tip, followed by treatment of rPTX3 (1 μg/mL) or PDGFBB (25 ng/mL) (1). Pictures were taken, 8 and 24 hours after scratching. Pictures were analyzed using ImageJ software to define wound closure percentage.

Gene silencing and transfection

Three small interfering RNAs (siRNAs) specific for PTX3 were tested in cultured human HSC (LX2 cell line). Efficiency of transfection was evaluated with positive control (siRNA of GAPDH) and negative controls (scramble sequence), all siRNAs are from Life Technologies Corporation, Carlsbad, California, USA. The siRNA that induced a higher inhibition of PTX3 transcript, assessed by qPCR, was used for the PTX3 inhibition experiments. The siRNA transfection of LX2 was performed with JetPrime Transfection Reagent Kit (Polyplus Transfection, Illkirch, France). Western blot with total cell lysate was performed using PTX3 antibody from Enzo Life Sciences and GAPDH (Abcam) was used as endogenous control. Lx2 were a kind gift from Dr Friedman (Mount Sinai School of Medicine, New York, NY, USA).

FCyRs expression in HSC

Primary HSC isolated from different patients were used to evaluate expression of FCγ receptors. *FCyR1* and 2 expression was assessed by qPCR in quiescent HSC and their respective activated HSC. Cultured human primary HSC were incubated with PDGFRβ-PE (BD Pharmingen ^{TM}) as a positive marker for HSC and CD16-PE-Cy7 (BD Pharmingen ^{TM}) and analyzed using a FACS-Canto (Becton, Dickinson and Company, BD, New Jersey, NJ, USA) to quantify PDGFRβ/ CD16 positive cells.

HEK293 studies

HEK293 cells stably transfected with the genes encoding TLR4 (HEK-TLR4) or TLR4 and CD14 (HEK-TLR4/CD14) were a kind gift from Dr Golenbock (University of Massachusetts Medical School, Worcester, MA, USA). Both HEK-TLR4 and HEK-TLR4/CD14 cells were grown in DMEM/F12 (Lonza) supplemented with antibiotics (250 µg/ml Geneticin, Gibco; 100 U/ml penicillin and 100 µg/ml streptomycin, Sigma) and 10% FBS. 5x104 cells/well were plated in 96-well plates, and transfected, when

indicated, with 0.1 µg of pCMV6-Entry vector containing the cDNA encoding MD2 (LY96, Myc-DDK-tagged-Origene) using jetPrime transfection reagent (Polyplus,) following the manufacturer's instructions. After 24h, cells were incubated 1h with rPTX3 (1µg/mL) followed by 24h LPS stimulation at the indicated concentrations (from E. coli 0111:B4, Sigma-Aldrich). IL-8 production was measured with the IL-8 OptEIA ELISA kit (BD Biosciences,).

Effect of PTX3 in human monocyte inflammatory responses and polarization

To measure cytokine secretion, PB monocytes (10^5 cells/well) were plated in 96 well plates and cultured with RPMI, 10% FBS (Lonza,) for 24h. Cells were then treated for 1h with 1 µg/mL PTX3, followed by stimulation with 10 ng/mL LPS (from *E. coli* 0111:B4, Sigma-Aldrich). After 4h and 24h LPS stimulation, culture supernatant fractions were collected and assayed for TNF-α or IL-1β and CCL5 measurement, respectively with the OptEIA ELISA kit (BD Biosciences) and Quantikine ELISA Kit (R&D Systems).

For immunophenotyping, PB monocytes (10^6 cells/well) were plated in 6 well plates and cultured with RPMI, 10% FBS during 24h. Then, cells were incubated during 3 days with INF/LPS (50/100ng/mL, Preprotech /Sigma-Aldrich;), IL4 (40ng/mL, Preprotech; 200-04), IL10 (50ng/mL, Preprotech;), human albumin (hSA, 1µg/mL, Grifols,) or PTX3 (1µg/mL) at a 5% FBS final concentration. Cells were then stained with a combination of fluorescently conjugated monoclonal antibodies against CD80, CD23, CD206, CD163 and CD36 (BD Biosciences). Flow cytometry analysis was performed on a BD LSRFortessa instrument using FACSDiva software (BD Biosciences), with 10000 events acquired for each sample. CD36 surface expression was analyzed separately by staining with a specific antibody (FITC, Biotools) using a FACSCanto II instrument and FACSDiva software (BD Biosciences).

Isolation of human liver macrophages and the effect of PTX3 on inflammatory responses and polarization.

Liver tissue samples from patients were obtained during hepatectomy for colorectal metastatic disease at the Liver Unit, Hospital Clinic, Barcelona, Spain. All samples were collected with informed consent from patients and approval by the ethical committee of Hospital Clinic of Barcelona. Macrophages were isolated by adapting a previously described protocol (2). Liver samples were mechanical and enzymatic digested in order to disaggregate the tissue, then a Nycodenz density gradient of 16% was performed to isolated the fraction of macrophages. This fraction was then purified by plastic adherence as described (2).

To measure cytokine release, isolated human liver macrophages were plated in 96 well plate and cultured with RPMI 10% FBS. Macrophages were then treated for 1h with 1 μg/mL PTX3, followed by stimulation with 10 ng/mL LPS (from *E. coli* 0111:B4, Sigma-Aldrich). After 4h and 24h LPS stimulation, culture supernatant fractions were collected and assayed for TNF-α or IL-1β and CCL5 measurement, respectively with the OptEIA ELISA kit (BD Biosciences) and Quantikine ELISA Kit (R&D Systems). After 24h of LPS stimulation, cells were harvested for RNA isolation using RNeasy Micro Kit (Quiagen). Macrophage polarization was assessed by real time PCR with the following primers: CD80 Fw CTGCCTGACCTACTGCTTTG, Rv GGCGTACACTTTCCCTTCTC; CD163 Fw CACCAGTTCTCTTGGAGGAACA, Rv TTTCACTTCCACTCTCCCGC; MertK Fw CTCTGGCGTAGAGCTATCACT, Rv AGGCTGGGTTGGTGAAAACA; CD206 Fw ACACAAACTGGGGGAAAGGTT, Rv TCAAGGAAGGGTCGGATCG.

Immunohistochemistry.

Liver and lungs were fixed with 4% paraformaldehyde and embedded in paraffin. Paraffin-embedded liver sections were stained with primary PTX3 (1:500, Enzo Life Sciences), MPO (1:50, Abcam), and F4/80 (1:200, Serotec, Oxford, UK), antibodies overnight at 4°C. In order to quantify the recruitment of inflammatory cells, sections were visualized at magnification x200 and macrophages (F4/80-positive) and neutrophils (MPO-positive) staining was quantified in 10 different fields for each section by histomorphometry software. Results were expressed as percentage (%) of F4/80-positive and MPO-positive area.

ELISAs

PTX3 plasma levels from patients were determined in duplicate with a PTX3-specific ELISA (DPTX30 from R&D Systems, Minneapolis, USA) according with manufacturer's protocol. LPS serum levels were evaluated by the limulus amoebocyte lysate QCL-1000 test analysis (3).

IL-10 plasma levels from AH patients were analyzed with an IL-10 Ultrasensitive ELISA Kit (Invitrogen).

Experimental mouse models of liver damage

Mouse hepatic cell populations were FACS-Sorted from the livers of controls and mice treated with CCl_4+LPS. Cells were isolated by a two-step collagenase-pronase perfusion of livers followed by Nycodenz density gradient centrifugation as previously described (4). Cells obtained were incubated with CD3- Alexa Fluor 700 (T cells), F4/80-Alexa Fluor 647 (Serotec) (macrophages), Ly6G-APC (eBioscience, Affymetrix, San Diego, CA, USA) (neutrophils), and HSC were purified by vitamin A-based flow cytometry as previously described (4). All samples were purified by high-speed sorting using a

FACS-Aria cell sorter (Becton, Dickinson and Company, BD, New Jersey, NJ, USA). *Ptx3* gene expression from whole liver and cell populations was assessed.

Ex vivo experiments were performed in high-precision cut slices (250 μm) from controls mice and chronic CCl_4 treated mice (5). To mimic the acute-on-chronic liver injury, the high-precision cut slices were incubated with LPS 25 μg/mL in the presence or absence of rPTX3 500 ng/mL. Tissues were harvested for RNA isolation 6 hours after the addition of LPS.

Patients

The baseline clinical, demographical and biochemical characteristics of the study cohort of AH patients are listed in supplementary Table 1. The median alcohol consumption was 100 g/day (IQR 25-75: 100-150). A total of 46 patients (85%) showed advanced fibrosis (bridging fibrosis or cirrhosis) on liver biopsy. Thirty patients (56%) were treated with corticosteroids and 30% were classified as responders according to the Lille Model at day 7. At admission, systemic inflammatory response syndrome (SIRS) criteria were fulfilled by 35% (n=19) of the patients. Thirty-one patients (57%) developed at least one clinical complication during hospitalization including: bacterial infections (46%), hepatic encephalopathy (HE) (22%), ascites (11%), and acute kidney injury (43%). Subsequently, 30 liver samples from patients with AH were selected, based on total RNA availability and quality and were used to perform the hepatic RNA expression studies. Human hepatic macrophages were isolated from hepatectomy resections for colorectal liver metastasis, and from one patient who underwent liver surgery for resection of a hepatocellular carcinoma in a non-cirrhotic liver at the Liver Unit, Hospital Clinic, Barcelona, Spain. Control livers were obtained from patients with hepatectomy for colorectal metastatic disease. All patients showed normal liver

histology (no fibrosis, no inflammation, and steatosis < 10%) as described in detail previously (6)

Statistical Analysis

Continuous variables were described as means (95% confidence interval) or medians (interquartile range). Categorical variables were described by means of counts and percentages. Comparisons between groups were performed using the Student´s *t* test or the Mann-Whitney *U* test when appropriate. Differences between categorical variables were assessed or the Fisher exact test. The main end point was death at 180 days. Correlations between variables were evaluated using Spearman's *rho* or Pearson's *r*, when appropriate. The area under the receiver characteristic curve (AUROC) analysis was used to determine the best cut-off value and the accuracy (sensitivity and specificity) of continuous variables associated with mortality end points. To evaluate the potential prognostic impact of PTX3 on 180-day mortality a bivariate Cox regression (HR -hazard ratio-) analysis adjusted for prognostic scoring systems (e.g. ABIC score, MELD and Lille Model) was fitted. This approach was performed to avoid over-fitting of the different models (events n=19), a pre-defined ratio of candidate prognostic variables to the number of observed events (deaths) was set at ≈1:10. Finally, we performed a comparative risk analysis using the Kaplan-Meier method. Comparisons were performed by the log-rank test. All statistical analyses were performed using SPSS version 14.0 for Windows (SPSS Inc., Chicago, IL). Results are presented as means (± SEM) of at least 3 independent experiments with replicates.

Supplementary Figure 1. *Tlr4* gene expression in experimental model of chronic and acute-on-chronic liver injury. Hepatic gene expression of *Tlr4* in mice treated with oil+vehicle, chronically with CCl_4 during two weeks and with CCL_4+LPS (n=6 per group) (*p<0,05 compared with control group mice).

Supplementary Figure 2. *Alb, Mpo, Cd3e, F4/80* and *Col1a1* gene expression in sorted hepatic cell populations: neutrophils (Ly6G+), macrophages (F4/80+), T cells (CD3+), hepatocytes isolated by low centrifugation, and HSC (VitA+). Cell populations were compared with whole liver of oil control or CCl_4+LPS respectively.

Supplementary Figure 3. a) Evaluation of FCγRIII (CD16) expression in primary human HSC. Representative FACS plot of human primary HSC incubated with PDGFRβ-PE and CD16-PE-Cy7. Experiment was performed in cells isolated from three patients. b) hepatic gene expression of FCγ receptor 1-2-3 in mice treated with oil, CCl_4+LPS or CCl_4+rPTX3+LPS.

Supplementary Figure 4. Extra hepatic effects of PTX3 treatment in acute-on-chronic experimental model. a) Kidney and b) Lung gene expression of inflammatory mediators in CCl_4+LPS mice treated with recombinant (rPTX3) or vehicle. #p<0,05 compared with Control mice treated with corn oil+vehicle; *p<0,05 compared with CCL_4+LPS treated mice). c) Representative pictures of myeloperoxidase (MPO) immunostaining of lung sections of mice treated with CCl_4+LPS or CCl_4+rPTX3+LPS (×200 magnification). The graph showe quantification of the percentage of positive stained area.

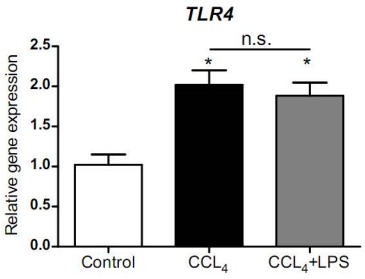
Supplementary Figure 5. Response of HEK-293 transfected with TLR4-CD14-MD2 to LPS in the presence of PTX3 or vehicle. a) HEK-TLR4 and b) HEK-TLR4/CD14 were transfected with 0,1 μg of pCMV6-Entry-MD2. IL-8 production in response to

LPS (1ng/mL or 10ng/mL) in the presence of rPTX3 assessed by ELISA. Results are shown as absorbance at 450nm.

Supplementary Figure 6. Association of PTX3 plasma levels with clinical parameters.

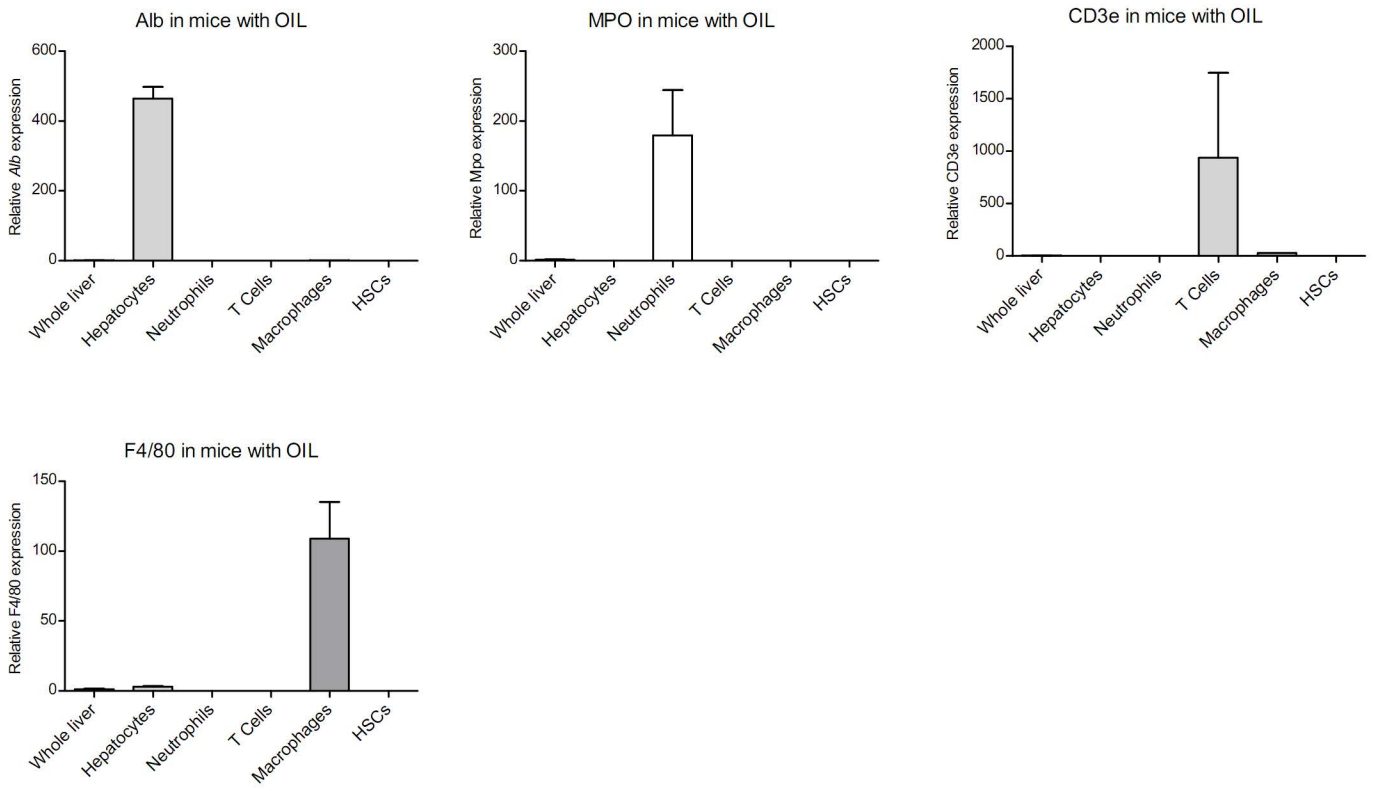
a) Correlation of PTX3 plasma level with Maddrey score (n=53)(p=0,004), procalcitonin (n=49) (p=0,001), ultrasensitive protein C-reactive (usCRP) serum levels, hepatic venous pressure gradient (HVPG). b) PTX3 plasma levels were significantly higher in patients with infections at admission (p=0,02) and in patients that developed infections during hospitalization (p=0,02). c) Correlation of PTX3 plasma levels with IL-10 plasma levels (n=21).

Supplementary Figure 1



Supplementary Figure 2

A.



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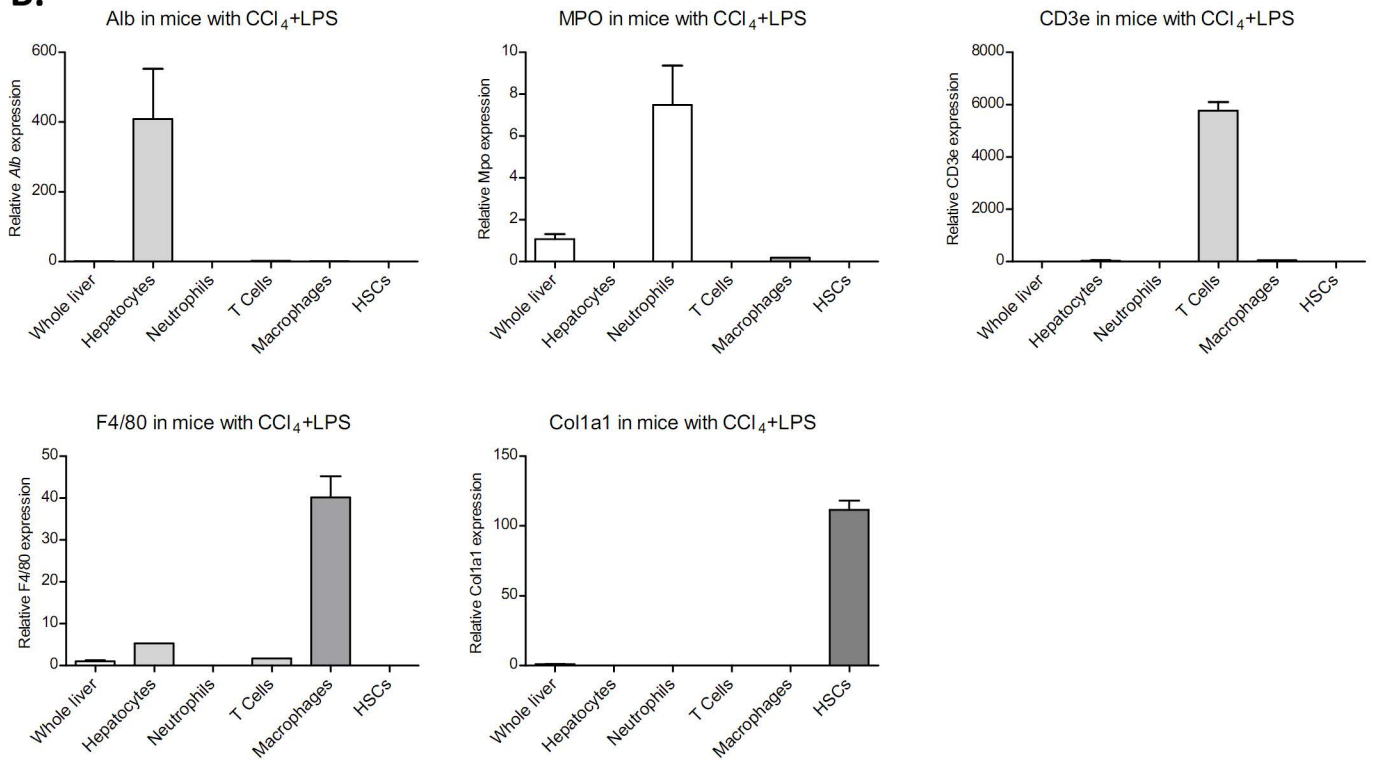
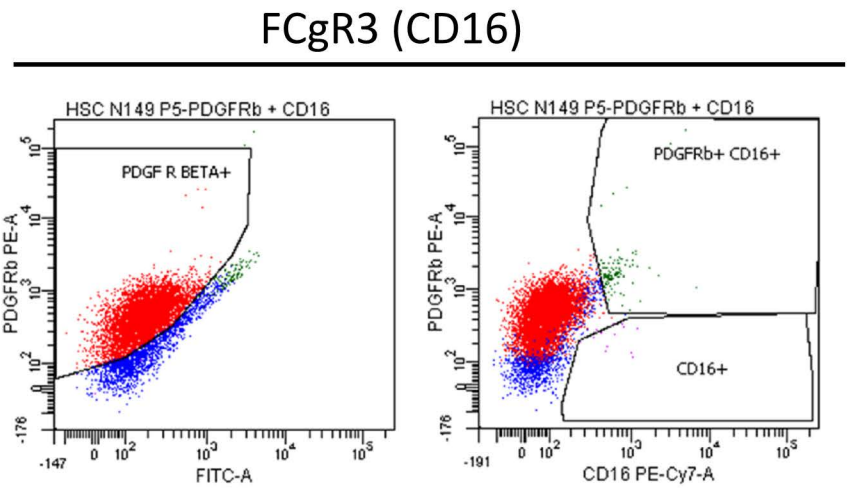


Supplementary Figure 3

A.



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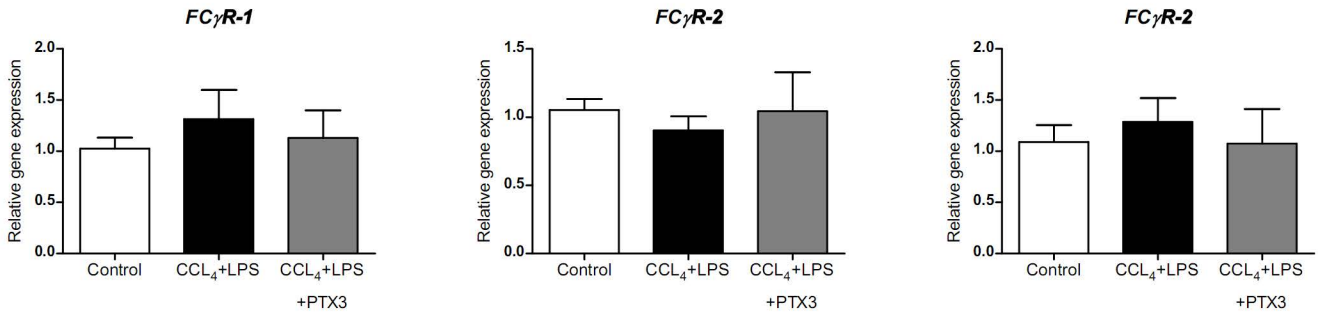
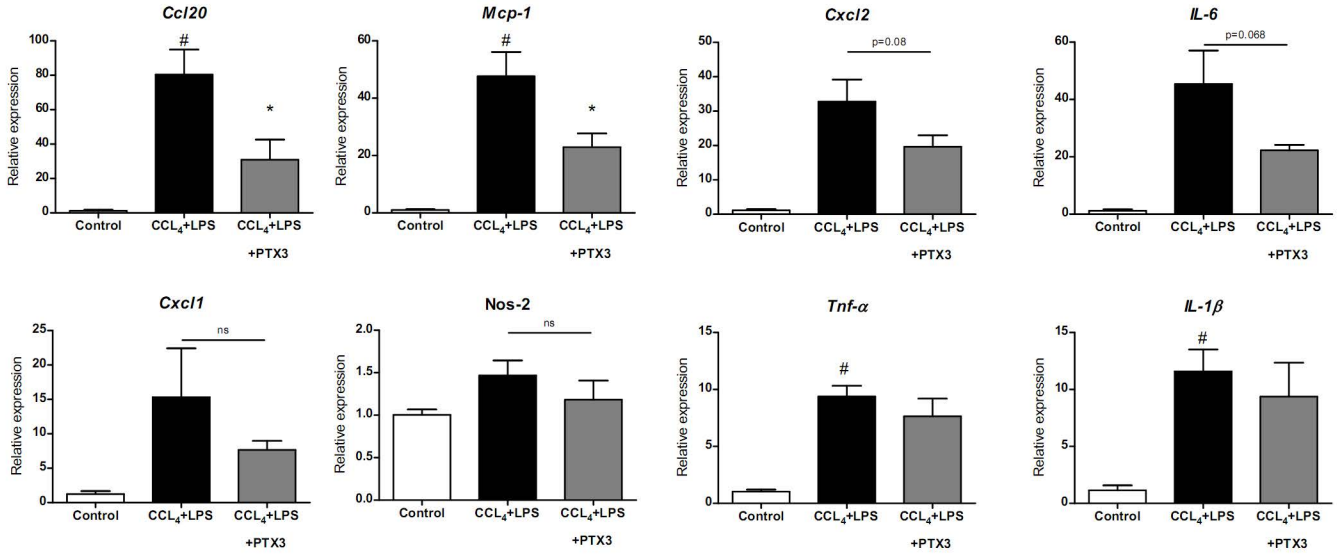
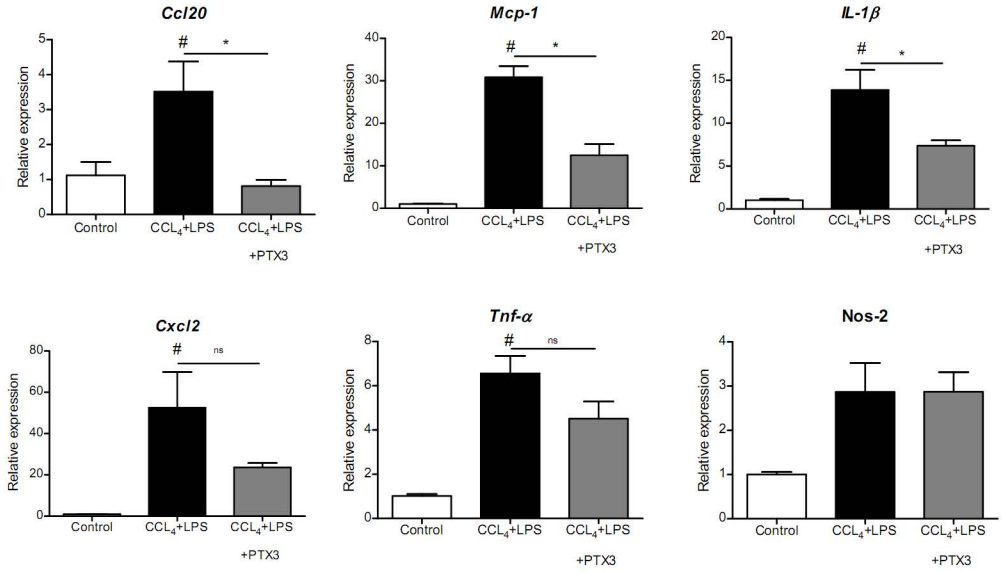


Supplementary Figure 4

A. Kidney



B. Lung



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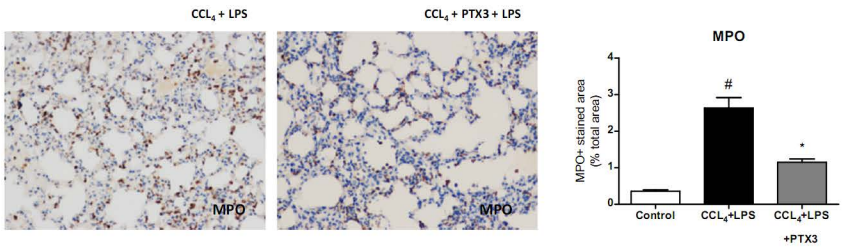
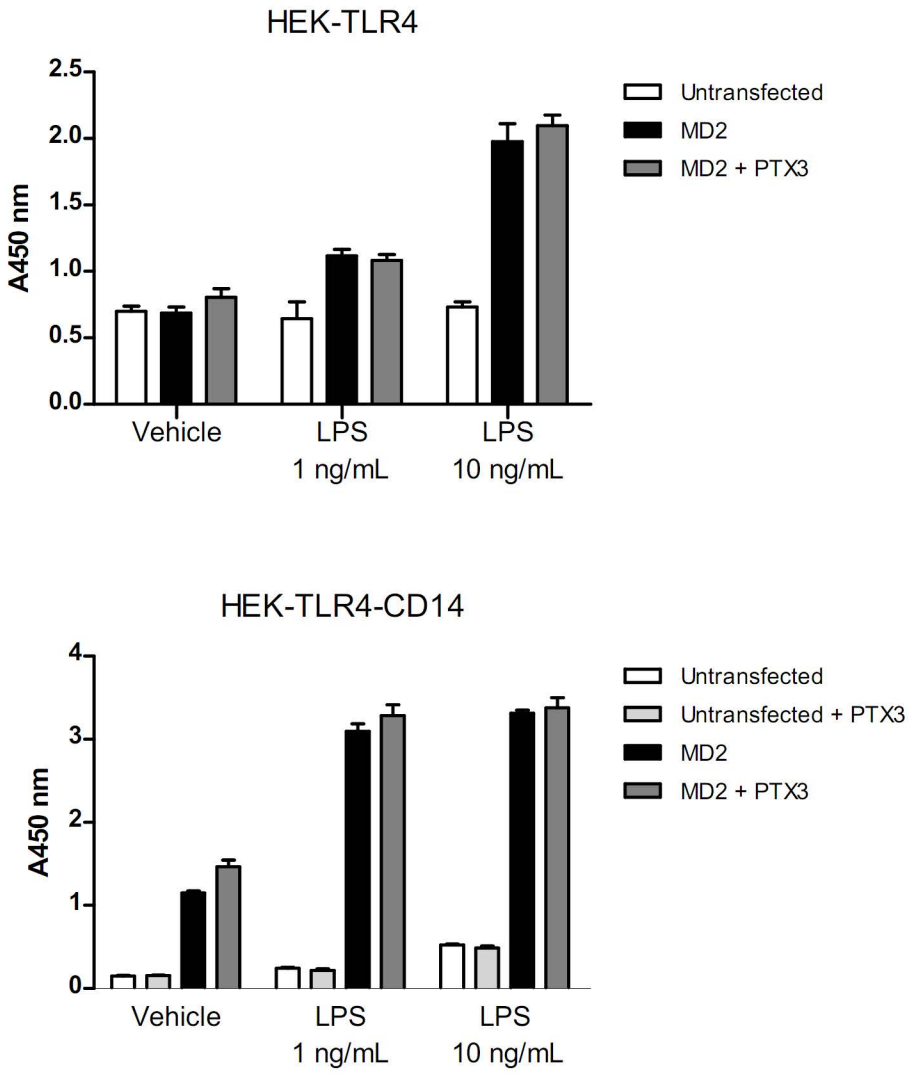


Supplementary Figure 5



Supplementary Figure 6