Supplementary Online Material

Detailed Methods

Ethics Statement

For animal study

All experiments and protocols were approved by the national animal experimentation ethics committee (Comité d'éthique pour l'expérimentation animale Charles Darwin, agreement Ce5/2011/060) which received the agreement from the French Minister, Ministère de l'Enseignement et de la Recherche, following the European guidelines 2010/63/UE.

For human study

The study's approval by the AP-HP Cochin Hospital Ethics Committee (# CCPPRB 2061) was valid for all AP-HP hospitals. Written informed consent was obtained for each patient from the patient or next-of-kin

Mice. Male C57BL/6 mice were purchased from Elevage Janvier (Le Genest, Saint Isle, France). CX3CR1$^{gfp/+}$-CCR2$^{rfp/+}$ and *CCR2*$^{-/-}$ mice were kindly provided by Israel Charo (Gladstone Institute, San Francisco, USA)[47]. *Cx3cr1*$^{-/-}$ C57BL/6 mice[48], *Cx3crR1*$^{gfp/+}$ and *Cx3cr1*$^{gfp/gfp}$ [49], *Csf1r*-Gal4VP16/UAS-ECFP (MacBlue)[50], MacBlue×*Cx3cr1*$^{gfp/+}$, MacBlue×*Cx3cr1*$^{gfp/gfp}$ and MacBlue×*Cx3cr1*$^{gfp/+}$-*CCR2*$^{rfp/-}$ mice were bred in the Nouvelle Animalerie

Commune animal facility at Pitié-Salpêtrière. All mice were aged between 8 and 16 weeks old.

Human cell preparation

Peripheral blood mononuclear cells (PBMC) were obtained from healthy donors by venipuncture, using EDTA as anticoagulant. PBMC isolated by the Ficoll–Hypaque gradient technique contained 20–30% monocytes, 60–70% lymphocytes. Human embryonic Kidney (HEK) cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum, 1 mM sodium pyruvate, 2 mM glutamine, 100 units/ml penicillin, and 100 µg/ml streptomycin (Invitrogen). Human aortic SMC from Clonetics (San Diego, CA) were cultured as recommended by the manufacturer. Subconfluent SMC were starved for 16 h before experiments, in the absence of fetal calf serum. All cells were grown in suspension in RPMI 1640 medium (Life Technologies, Cergy Pontoise, France) supplemented with 10% inactivated fetal calf serum (FCS), 2mM L -glutamine, 100U/ ml penicillin, 100 µg/ml streptomycin and 9 µg/ml insulin.

Flow chamber adhesion of PBMC

Adhesion experiments were done as previously described [30]. Briefly, coverslips were cultured with either adherent HEK cells (HEK-pBlast or HEK-CX3CL1 clones) or aortic primary smooth muscle cells (SMC) treated with 20ng/ml TNFα and 500u/ml IFNγ (R&D systems, Lille, France) as described [51]. The coverslip was mounted in a flow chamber set on the stage of an

inverted microscope (TE300, Nikon, France) equipped with a phase contrast 10x objective (Nikon, n.a. 0.25) and a cooled CCD camera (Sensicam, PCO, Kelheim, Germany). PBMC were incubated for 30 min at 37°C with 1 µM 5-(and-6)-carboxyfluorescein diacetate, succinimidyl ester (Molecular Probes, Leiden, Netherlands) for labeling and resuspended in flow buffer (HBSS supplemented with, 0.5 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM HEPES, 2 mg/ml BSA) at 2.10^6 cells per ml. A syringe pump (PHD 2000; Harvard Apparatus, Les Ulis, France) drove 0.5 ml of cell suspension through the chamber at a wall shear stress of 1.5 dynes.cm^{-2}. Fluorescent images were recorded to count the adherent cells (excitation 450-500 nm, emission 510-560 nm, dichroic filter Q505lp, Chroma, Brattleboro, VT, USA). Specific adhesion to CX3CL1-HEK was calculated by subtracting the number of PBMCs adhering to the control HEK from the number of cells adhering to HEK-CX3CL1. Specific adhesion to activated SMC was calculated by subtracting the number of PBMCs adhering to the untreated SMC from the number of cells adhering to TNFα/IFNγ treated SMC.

Human Polymorphism study

We retrospectively studied DNA from patients included in previous studies [52]. These studies involved patients from 4 ICUs in Paris, France, 2 medical and 2 surgical. Patients meeting the criteria for severe sepsis or septic shock, defined according to the ACCP/SCCM consensus conference [1] and who had at least two organ failures defined by the SOFA (sequential organ failure assessment) score (values **>=** 1 for each organ failure were considered)[25]

were included between January 2004 and December 2005. Patients with chronic renal failure were excluded. Since the studies were designed before the publication of the RIFLE and AKIN scores [53, 54], patients with renal failure were classified on the basis of the renal item of the SOFA score, AKI was defined by a SOFA score >= 1 (creatinine value > 1.2 (110) mg/dl (µmol/l). The *Cx3cr1 V249I* polymorphism (referred in dbSNP as rs3732379) was detected as previously described [55], with a TaqMan™ fluorogenic 5'-exonuclease assay. Primer and probe sets were designed and manufactured with Applied Biosystems Assay-by-Design' custom service (Applera, Vienna, Austria).

Polymicrobial sepsis induction

Polymicrobial sepsis was generated after a caecal ligation and puncture (CLP) procedure. After anaesthesia by an intraperitoneal injection of a mixture of ketamine/xylazine (respectively 100 and 10 mg/kg body weight), the mice were put on a heating plate, and CLP was performed as previously described to induce midgrade sepsis [21]. Briefly, a midline incision (1 cm) was made below the diaphragm, exposing the caecum; the caecum was then exteriorized and ligated at 50% of its length, and punctured through and through with a 21-gauge needle; a small amount of feces was extruded through each needle puncture. In the control (sham) animals, the caecum was located, exteriorized, and simply reinserted in the abdomen. The abdominal incision was then closed in layers. After surgery, mice were resuscitated with 1 ml of sterile saline s.c. and received buprenorphine s.c. (0.05 mg/kg body

weight) every 12 h for 48 h for analgaesia. For the survival study, animals were monitored every 12 h for 7 days. We chose to ligate 50% of the caecum to obtain, as reported in the literature, a mortality rate around 50-60% of male mice. Mice were all kept in the animal facility for at least one week before the procedure to ensure relatively homogeneous microbiota. For some experiments, 3 µg in 50 µl of CX3CL1 (full length CX3CL1, R&D Systems, Lille, France) was injected i.p. 30 minutes before surgery. For F1 (kindly provided by A. Proudfoot, Merck-Serono), 50 µg was injected i.p. 30 minutes before and then 6 hours after surgery.

Sterile inflammation model

LPS (*E. coli* O111:B4) (Sigma-Aldrich) was injected at a dose of 15 mg/kg i.p., previously determined to induce 50% mortality according to the CLP model. Survival was monitored every 12 h for 7 days after the LPS challenge.

Histological analysis

After mice were euthanized, the whole body vasculature was flushed with a 10-ml injection of fresh PBS through a cardiac puncture. Kidneys were harvested 24 h after CLP, sliced in half after the capsule was removed, and incubated in 4% paraformaldehyde (PFA) for 24 h at 4°C. The kidneys were then washed and stored in ethanol 70°. Sections 3-5 µm in thickness were prepared from paraffin-embedded tissue; they were dewaxed and stained with haematoxylin-eosin (H&E) or periodic acid Schiff (PAS). Pathologists with

expertise in kidney disease (J.A and N.T.) performed the histological analyses blinded to treatment groups. Quantification was performed on at least 3 mice per condition. For each mouse, the percentage of damaged tubules was calculated on 3-5 different randomly chosen fields comprising 200-300 tubules.

Mouse Cell preparation

Blood was drawn via retroorbital puncture with heparin. After mice were euthanized, the whole mouse vasculature was flushed by intracardiac injection of 10 ml of PBS. Bone marrow and kidneys were then harvested. Bone marrow cells were collected by flushing the thigh and shin bones with PBS 0.5% BSA 2 mM EDTA. Kidneys were weighed and then cut in small pieces and digested in DMEM containing 400 IU/ml collagenase IV (Sigma Aldrich), 0.1 mg/ml DNAse I (Roche) and 0.05 M EDTA for 1 h at 37°C. The solution was then filtered through a 40 μm cell strainer (BD) and washed with cold PBS. Tubes were then centrifuged, the supernatant was discarded and cells were resuspended in 500 μl of PBS 0.5% BSA 2 mM EDTA. We stained 50 μl ($1/10^{th}$) of each cell suspension for flow cytometry.

Flow cytometry

Before antibody staining, erythrocytes were lysed with buffer containing 0.15 M NH4Cl, 0.01 mM KHCO3 and 0.1 mM EDTA and then resuspended in 50 ml PBS 0.5% BSA EDTA 2 mM with 1 μg/ml purified anti-CD16/32 (2.4G2, BD Biosciences) for 15 minutes at 4°C and for an additional 20 minutes with

the appropriate dilution of specific antibodies. The panel of antibodies comprised: anti-CD11b (clone M1/70), anti-Ly6C (clone AL-21), anti-Ly6G (clone 1A8), NK1.1 (clone PK136) and CD11c (clone HL3) (Becton Dickinson, San Jose, CA, USA) and F4/80 (clone BM8) and CD115 (Clone AFS98) (eBioscience, San Diego, CA, USA). For intracellular staining, cells were incubated in DMEM with Brefeldin A (10 µg/mL) for 2h at 37°C. Surface staining was done as described above. Cells were then fixed in paraformaldehyde 4% for 30 min then permeabilized with in PBS BSA 0.5% with 0.1% saponin in the presence of 1 µg/ml purified anti-CD16/32 for 10 min. IL-1ra antibody (Abcam, clone ab124962) was added for an additional 30 min at room temperature. Cells were washed in PBS-BSA 0.5% - 0.1% saponin and incubated in the same buffer with a secondary antibody (goat anti-rabbit IgG, clone ab96899). Secondary antibody alone was used as a control and for baseline fluorescence determination. Flow cytometry was performed with the FACScanto® (BD, Franklin Lakes, NJ, USA) flow cytometer and DIVA® Flow Cytometry acquisition software. Analysis was performed with FlowJo software (Tree Star, Inc, Ashland, OR, USA). Absolute numbers were calculated by adding to each vial a fixed number (10,000) of nonfluorescent 10-µm polybead® carboxylate microspheres (Polysciences, Niles, IL, USA) according to the formula: No. cells = (No. acquired cells×10,000)/(No. acquired beads).

qPCR

Kidneys were harvested 6h or 24h after sham surgery or CLP after vascular flush as described above. Total RNA was extracted using the RNeasy Mini Kit with on-column DNase digestion (QIAGEN) according to manufacturer instructions. RNA concentration was determined by fluorescent absorption 260 nm. cDNA synthesis was performed with SuperScript VILO cDNA Synthesis Kit (Invitrogen). Polymerase chain reaction was performed on an ABI prizm 7300 using Power SYBR Green PCR Master Mix (Life technologie). Primers for TNFα forward : 5' ACGGCATGGATCTCAAAGAC 3' , reverse : 5' AGATAGCAAATCGGCTGACG 3', TGFβ forward : 5' ATGCTAAAGAGGTCACCCGC 3', reverse : 5' GTATCAGTGGGGGTCAGCAG 3' , IL-1ra forward :5' TCACCCATGGCTTCAGAGGCAGCC 3', reverse : 5' GGCCTTTCTCAGAGCGGATGAAGG 3' and GAPDH forward : 5' CCTGGAGAAACCTGCCAAGTATG 3', reverse : 5' AGAGTGGGAGTTGCTGTTGAACTC 3'. GAPDH gene expression was used as control. Relative expression was calculated using $\Delta\Delta$Ct method.

Adoptive transfer experiments

For intravital imaging experiments, bone marrow cells were isolated from MacBlue-*Cx3cr1*$^{gfp/+}$ and MacBlue-*Cx3cr1*$^{gfp/gfp}$ mice; the latter were labelled in PBS with CMTMR (10 μM). Twenty millions cells were then co-transferred at a 1 to 1 ratio to C57Bl/6 recipient mice 30 minutes before the CLP procedure. The proportions of Ly6C^{high} monocytes adoptively transferred from each strain were controlled before transfer by flow cytometry and were

identical. Comparative adhesion of $ECFP^+$ subset (WT phenotype) and $ECFP^+CMTMR^+$ subset ($Cx3cr1^{-/-}$ phenotype) to the renal endothelium was measured by intravital imaging 6 h after the CLP**.** The number of $ECFP^+$ and $ECFP^+CMTMR^+$ cells were calculated on static 3D images and normalized to the renal cortex volume and expressed as a percent of the number of $Ly6C^{high}$ monocytes transferred. Fields were chosen when at least one adoptively transferred cell (ECFP+ or CMTMR/ECFP) was present. For kidney lesion measurement: bone marrow cells were extracted as previously described. Monocytes were negatively selected after removal of other cell types, with Ly6G, CD3, CD4, B220, DX5, and NK1.1 PE-labelled antibodies. Marked cells were then captured via a magnetic device for cell separation and anti-PE magnetic beads, according to the manufacturer's instructions (Miltenyi Biotec). In all experimental conditions, mice were injected with $4\text{-}5\times10^6$ monocytes just before the CLP procedure and monitored every 12 h for 7 days for survival or euthanized 24 h after the procedure for histological analysis.

Creatinine and urea quantifications

Creatinine concentrations in serum samples were determined by the Jaffe method with the QuantiChrom Creatinine Assay Kit (BioAssay Systems, Hayward, CA, USA), and serum urea nitrogen (BUN) concentrations by the Jung method with the QuantiChrom Urea Assay Kit (BioAssay Systems).

Multi-photon Imaging

Intravital imaging was performed on mouse kidneys. Briefly, mice were anaesthetized with isofluran 2.5% vaporized in a 70/30 mixture of O_2/N_2O. Their temperatures were monitored and maintained at 37°C. An incision was made in the flank, and the kidney was exposed gently. It was placed between two plates. The upper plate had an aperture to allow light transmission, and a coverslip was fixed to the lower face of the upper plate, in contact with the kidney. In some experiments before the imaging session, 2×10^6 MW tetramethylrhodamine-Dextran (Invitrogen) was injected to stain the vasculature and define the parenchymal areas and vessels.

The two-photon laser scanning microscopy (TPLSM) set-up used was a Zeiss LSM 710 NLO multiphoton microscope (Carl Zeiss, Germany) coupled to a titanium:sapphire crystal laser (Coherent Chameleon, CA, USA), which provides 140 fs pulses of NIR light, selectively tunable between 680 and 1080 nm, and an acousto-optic modulator to control laser power. The system included three external non-descanned detectors with a combination of 2 dichroic mirrors (565 nm and 690 nm) with 565/610 and 500/550 bandpass filters and a 485 lowpass filter to enable the simultaneous recording of three fluorescent channels. The excitation wavelength was 870 nm. Cell motility was measured every 30 s by 5 consecutive 3-µm z spacing stacks (total 12-µm thickness allowing sufficient 3D cell tracking in the renal microvasculature) with a plan apochromat ×20 (NA = 1) water immersion objective.

$ECFP^+$ and/or GFP^+ cells were tracked for 30 minutes with 3-D automatic tracking and manual correction with Imaris software. Cells were defined as

circulating if they appeared on only one picture, crawling if they were on more than 2 pictures with an estimated speed greater than 2 μm/min, and adhering if at any time their speed was lower than 2 μm/min (no movement of more than 1 μm between 2 pictures). Dwell time was defined as the total length of time for which the cell was tracked. Interaction time was defined as the total time in an adherent state. The arrest coefficient was defined as interaction time divided by dwell time.

Track straightness was defined by the ratio of the distance between the initial and the final positions of each cell to the total distance covered by that cell. Velocity was calculated at any time by measuring the mobility of cells every 30 seconds. In some experiment before the imaging session, 10 μg Ly6G-PE antibody was injected i.v. to image the neutrophils, and the laser excitation wavelength was set at 810 nm. For some experiments, F1 (50 μg) was injected i.v. 5 min before the imaging session. 2D time lapse imaging of *Cx3cr1*$^{gfp/+}$*-CCR2*$^{rfp/+}$ cells was possible by sequential exposure with alternance of 810 and 900-nm excitation wavelengths at each time point.

Statistical analysis

Group were compared with Prism software (Graphpad) with two tailed unpaired t test. For multiple comparison analysis Non-parametrics Mann-Whitney test or ANOVA with adjustments were performed according to Gaussian distribution of each sample. Survival curves were compared with a log-rank test. Multivariate analysis of the human cohort was made with JMP (SAS institute Inc.) and included relevant parameters associated with the

occurrence of Acute Kidney Injury. Samples or animals were excluded if they appeared as true outlier as defined by a value lesser or higher than mean +/- 5 SD. No blinding was used for in vivo experiments; Histological analyses and monocyte tracking were done blinded. * for $p<0.05$; ** for $p<0.01$; *** for $p<0.001$; **** for $p<0.0001$.

Supplemental figure, table and video legends

Figure S1. Effect of CLP on survival and myeloid cell mobilization.

(a) Survival of sham- and CLP-operated WT mice (n= 10 (SHAM) and 15 (CLP). **(b)** Number of $Ly6C^{high}$, $Ly6C^{low}$ monocytes, $Ly6G^{+}$ (neutrophils) and $CD11c^{+}$ Renal DCs in bone marrow, blood and kidney of sham- vs CLP-operated mice at different time points, determined by flow cytometry gated on $CD11b^{+}NK1.1^{neg}F4/80^{+}$ cells. Bars represents means±SD (n=6 from 2 independent experiments, ANOVA analyses adjusted for multiple comparisons (Bonferroni) were used. No differences were observed between time points in sham-operated mice).

Figure S2. Comparative analysis of mononuclear phagocyte subsets in the mouse strains

(a) Number of $Ly6C^{high}$ and $Ly6C^{low}$ monocytes in blood and kidney of sham- vs CLP-operated WT and *Ccr2*$^{-/-}$ mice 6h after surgery. **(b)** Number of renal DC of sham- vs CLP-operated WT, *Cx3cr1*$^{-/-}$ and *Ccr2*$^{-/-}$ mice 6h and 24h after surgery. (n= 4 to 6 mice from two independent experiments, ANOVA analyses adjusted for multiple comparisons (Bonferroni) were used.

Figure S3. Role of CX3CR1 in mouse survival during sterile lethal inflammation.

(a) Survival after CLP in *Cx3cr1*$^{+/+}$ (blue line) and *Cx3cr1*$^{-/-}$ mice (red line). n=10 mice per group. Survival curves were compared with a log-rank test. *, $p<0.05$). Data are the results of 2 experiments. **(b)** Survival in WT (blue line) versus *Cx3cr1*$^{-/-}$ (red line) mice after lethal dose of LPS (i.p. injection, 15 mg/kg). n=13 mice per group. Survival curves were compared with a log-rank test. Presented data are the results of 2 experiments. **(c)** TPLSM pictures of kidney cortex from *Cx3cr1*$^{gfp/+}$ mice 6 h after CLP show neutrophil (red) adhesion. Neutrophils were visualized by injection of 10 μg of Ly6G-PE antibody 5 minutes before the imaging session. Examples of circulating (blue squares), crawling (green squares) and adhering neutrophils (purple squares) are indicated. PE signals are in red, GFP signals in green, and renal tubules are autofluorescent. **(d)** Relative frequency of the three neutrophil behaviours in *Cx3cr1*$^{gfp/+}$ and *Cx3cr1*$^{gfp/gfp}$ mice. Bars represent means±SEM (n=2 mice per group). **(e)** Neutrophil dwell time and contact duration with renal endothelium in CLP-operated *Cx3cr1*$^{gfp/+}$ and *Cx3cr1*$^{gfp/gfp}$ mice. Red bars indicate means. Data represent a pool of cells from 2 mice per group. Two-sided t-tests and ANOVA analyses adjusted for multiple comparisons (Bonferroni) were used as statistical tests. **f)** 1:1 ratio of MacBlue×*CX3CR1*$^{gfp/+}$ and CMTMR-labelled MacBlue×*Cx3cr1*$^{gfp/gfp}$ bone marrow cells were injected into WT mice before CLP. Intravital imaging of kidney cortex was performed 6 h after CLP, and the number of ECFP^{+} and ECFP^{+}/CMTMR^{+} adherent cells/mm^{3} was counted in 20 different fields in 2 independent experiments. Two-sided t-test was used for statistical analysis.

Table S1. Demographic and clinical characteristics of the human cohort.

Demographic and clinical characteristics of septic patient at the time of inclusion. Organ failure is defined by a sequential organ failure assessment (SOFA) score ≥ 1 for each organ. Results are expressed as median (IQR). Two-sided Student t-test and Chi-square tests with yates correction were used to compare characteristics and proportions between the two groups. SAPS 2: Simplified Acute Physiology Score 2. ***: p<0.001.

Supplementary video 1

In vivo 3D-imaging of a sham-operated MacBlue×*Cx3cr1*$^{gfp/+}$ mouse showing monocyte behaviour in the vascular kidney cortex 6 h after surgery. ECFP^{+} signals are in cyan, GFP signals in green, and 2×10^{6} MW rhodamine-dextran was injected before the imaging session to visualize vasculature (red) around autofluorescent kidney tubules.

Supplementary video 2

In vivo 3D-imaging of a CLP-operated MacBlue×*Cx3cr1*$^{gfp/+}$ mouse showing monocyte behaviour in the vascular kidney cortex 6 h after surgery. ECFP^{+} signals are in cyan, GFP signals in green, and 2×10^{6} MW rhodamine-dextran was injected before the imaging session to visualize vasculature (red) around autofluorescent kidney tubules.

Supplementary video 3

In vivo 3D-imaging of a CLP-operated MacBluex*Cx3cr1*$^{gfp/+}$ *Ccr2*$^{-/-}$ mouse showing monocyte behaviour in the vascular kidney cortex, 6 h after surgery. ECFP^{+} signals are in cyan, GFP signals in green, and kidney tubules are visualized by autofluorescence.

Supplementary video 4

In vivo imaging of a CLP-operated *Cx3cr1*$^{gfp/+}$*Ccr2*$^{RFP/+}$ mouse showing monocyte behaviour in the vascular kidney cortex, 6 h after surgery. The RFP^{+} signals are in red, GFP signals in green, and kidney tubules are visualized by autofluorescence. RFP and GFP are detected by sequential excitation at 750 nm and 900 nm respectively on a 2D-field.

Supplementary video 5

In vivo 3D-imaging of a CLP-operated MacBlue×*Cx3cr1*$^{gfp/gfp}$ mouse showing monocyte behaviour in the vascular kidney cortex, 6 h after surgery. ECFP^{+} signals are in cyan, GFP signals in green, and 2×10^{6} MW rhodamine-dextran was injected before the imaging session to visualize vasculature (red) around autofluorescent kidney tubules.

Supplementary video 6

In vivo 3D-imaging of a CLP-operated MacBlue×*Cx3cr1*$^{gfp/+}$ mouse showing monocyte behaviour in the vascular kidney cortex, 6 h after surgery. ECFP^{+}

signals are in cyan, GFP signals in green, and kidney tubules are visualized by autofluorescence. The mouse was treated with 50 µg F1 before surgery and 10 min before the imaging session.

Supplementary video 7

In vivo 3D-imaging of a CLP-operated MacBlue×*Cx3cr1*$^{gfp/+}$ mouse showing monocyte behaviour in the vascular kidney cortex, 6 h after surgery. ECFP^{+} signals are in cyan, GFP signals in green, and kidney tubules are visualized by autofluorescence. The mouse was treated with 50 µg CX3CL1 before surgery.

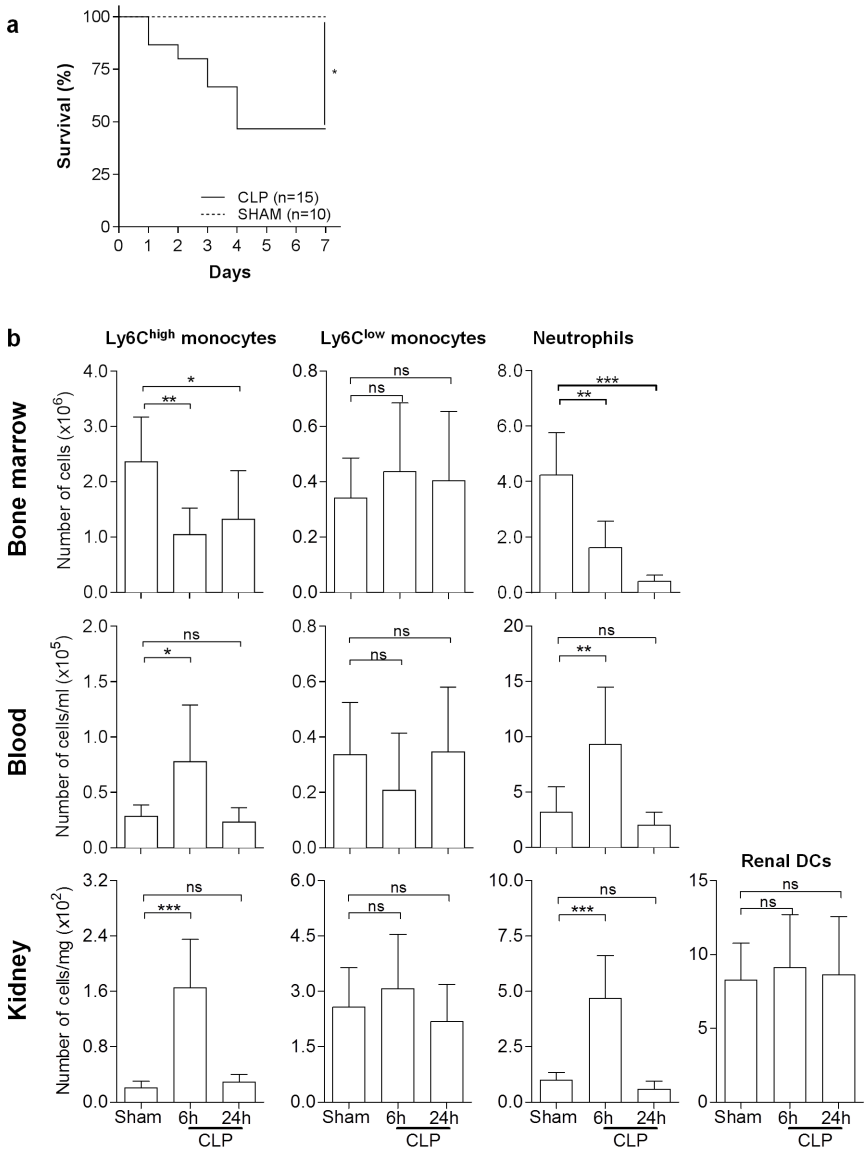

Figure S1 related to Figure 1. Effect of CLP on survival and myeloid cell mobilization

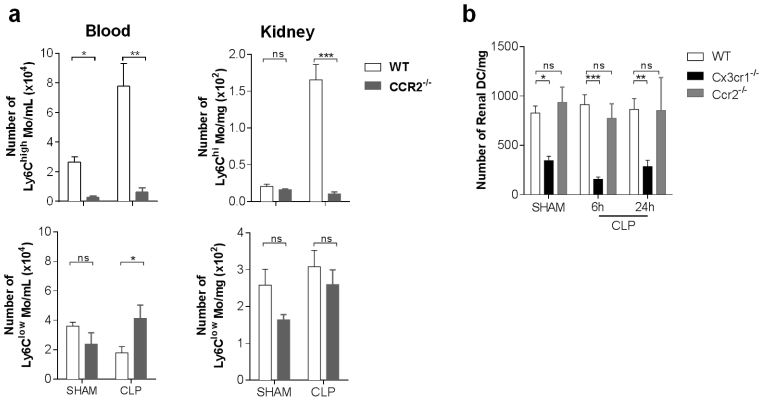

Figure S2 related to Figure 1 and 3. Comparative analysis of mononuclear phagocyte subsets in the mouse strains

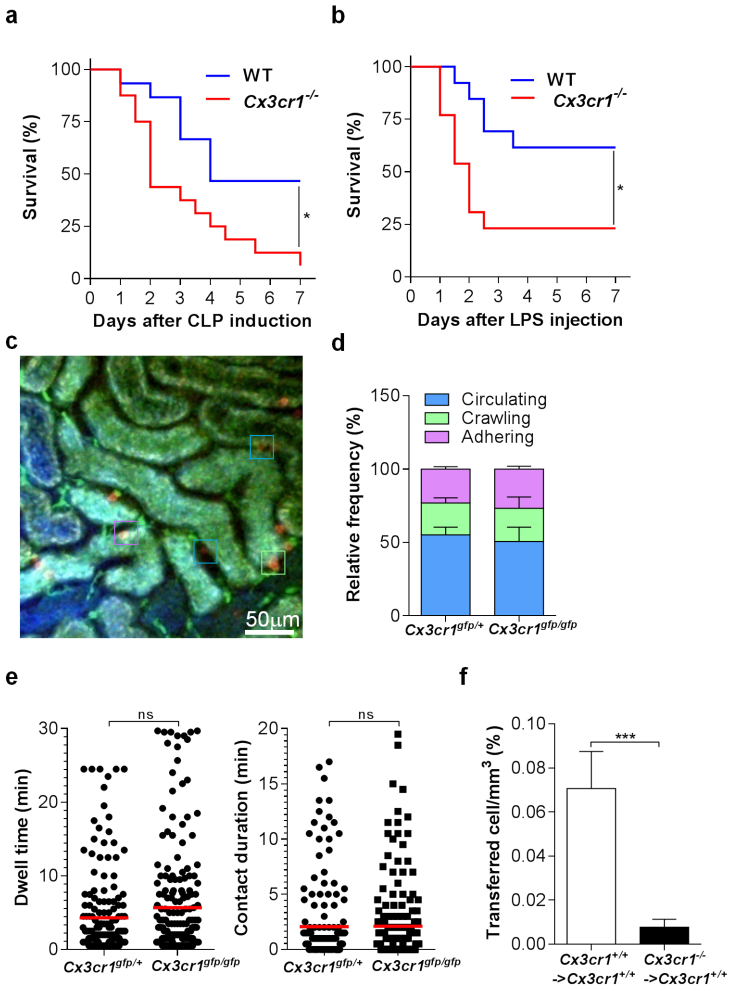

Figure S3 related to Figure 2. Role of CX3CR1 during polymicrobial sepsis

	CX3CR1-VV n = 125	CX3CR1-VI/II n = 114	p
Age, n	63 (52-78)	65 (53-75.25)	0.65
SAPS 2, n	50 (36-64.5)	48 (40.5-59)	0.85
28-day mortality (%)	30.4	31.6	0.95
IL-6 pg/mL	573 (235-5547)	890 (216-9345)	0.60
Comorbidities, n	1 (0.5-3)	2 (1-3)	0.37
Organ failure :			
- Cardiovascular, %	87.2	94	0.12
- Respiratory, %	85.6	92.9	0.10
- Coagulation, %	56.8	35.1	0.001***
- Liver, %	38.4	30.7	0.27

Table S1. Demographic and clinical characteristics of the human cohort.