Supplemental Methods

Mice

The mice involved in our experiments were all on the C57BL/6J background, and age- and sex-mached. HCV mice expressing human TF (hTF^{+}) from a human chromosome vector (HCV), equal to 100% levels of wild-type mouse TF and Low-TF (LTF) mice expressing very low levels of human TF, equal to 1% levels of wild-type mouse TF, were donated by Professor Nigel Mackman. In order to measure human TF activity in mice, HCV mice were crossed with Caspase-11 KO or Gsdmd KO mice to generate Caspase-$11^{-/-}$ hTF^{+} and Gsdmd$^{-/-}$ hTF^{+}mice. All animals were raised in specific pathogen-free conditions at the Department of Laboratory Animals of Central South University. WT littermates were used as the controls for the transgenic mice in our study. All transgenic mice in the present study were identified using standard genomic PCR genotyping techniques. All experimental animal procedures were approved by the Institutional Animal Care and Use Committees of Central South University.

Antibodies and Reagents

Recombinant HMGB1 protein was expressed in *Escherichia coli*, purified to homogeneity as described previously[23], and contained < 500 pg endotoxin per microgram of rHMGB1 (prepared in Kevin J. Tracey Lab). SensoLyte internally quenched 5-FAM/QXL-520 FRET thrombin substrate was obtained from Anaspec

(Cat.AS-72129). AF647-labeled anti-mouse CD49b (clone HMα2) and mouse IFN-β ELISA Kit (Cat.439407) were purchased from Biolegend. The Antibody Labeling Kits (Cat.A20186, Invitrogen) were used to label anti-mouse albumin antibodies (Cat.A90-234A, Bethyl) and anti-mouse fibrin antibodies (59D8, provided by Professor Nigel Mackman) with Alexa Fluor 647. Mouse HMGB1 Elisa kits were obtained from Shino-Test Corporation (ST51011). Mouse TNF-α Elisa kit was from Invitrogen (88-7324-77). Fibrinogen Mouse ELISA Kit (Cat. ab108844), PAI-1 Mouse Simple Step ELISA Kit (Cat.ab197752), TAT Complexes Mouse ELISA Kit (Cat.ab137994) and TF Elisa Kit (Cat.ab214091) were purchased from Abcam. Mouse d-dimer (D2D) ELISA Kit (Cat.CEA506Mu) was from Cloud-Clone Corp. The Assay Sense Human Tissue Factor Chromogenic Activity Kit (CT1002b) was purchased from Assaypro. Other reagents were crude LPS from *E.coli* 0111：B4 (Sigma, L2630), ultrapure LPS (InvivoGen, tlrl-3pelps), monoclonal HMGB1 neutralizing antibody, 2G7 (prepared in Kevin J. Tracey Lab), rMFG-E8 (R&D systems), Annexin V-FITC (BD bioscience), ML120B (Tocris, Cat.4899) and Western Blot antibodies against mouse TF antibody (clone EPR8986, Abcam) and mouse β-actin antibody (clone 8H10D10, Cell Signaling Technologies).

Endotoxemia model

To induce a DIC-like response and LPS lethality in mice, we used a LPS sensitive model. Mice 25 to 30 g in weight were primed with 0.4 mg/kg LPS (intraperitoneal injection) for 7 hr, and then challenged with 10 mg/kg LPS for 8 hr. In some

experiments, mice were treated with monoclonal HMGB1 neutralizing antibody (2G7, 160 μg/mouse) or the isotype control IgG (160 μg/mouse) or 200 IU/kg low molecular weight heparin (LMWH; Enoxaparin Sodium Injection, Sanofi-Synthelabo Limited, the France) 30 min before 10 mg/kg LPS. Mice were treated with or without rMFG-E8 (160 μg/kg) 2h before 10 mg/kg LPS. As a control, mice were injected with sterile saline. Mice were sacrificed at 8 hr for subsequent experiments after 10 mg/kg LPS challenge. In the intravital microscopy experiments, mice were injected with 4mg/kg LPS for 6 hr (non-lethal dose of LPS) to ensure that most blood vessels were not completely occluded and the dynamic changes of liver and lung microcirculation can be better observed.

Cecal Ligation and Puncture (CLP) sepsis model

Polymicrobial sepsis was induced in mice by cecal ligation and puncture (CLP). The mice used were 8-10 week old, male, and raised in the same conditions. Briefly, laparotomy was performed following disinfection of skin with 2% iodine tincture after the mice were anesthetized under 2% isoflurance (Piramal Critical Care) with oxygen. 10–15 mm midline incision was made to expose the cecum. For severe polymicrobial sepsis model, 75% of the cecum was ligated, push the feces gently toward the distal cecum, and then double puncture was made with an 18-gauge needle. A small amount of feces was extruded from the double puncture holes, the cecum was relocated into the peritoneal cavity and the wound was closed. Prewarmed sterile saline (1 mL) was injected subcutaneously for fluid resuscitation immediately after

operation. The same procedure was subjected to the sham group except the ligation and puncture. All the mice did not receive antibiotics.

Preparation for Intravital Microscopy

Mice were anesthetized by intraperitoneal injection of xylazine hydrochloride (10 mg/kg) and ketamine hydrochloride (200 mg/kg). The right jugular vein was exposed and was cannulated for maintenance of anesthetic and injection of antibodies. Preparation of liver for intravital microscopy. Briefly, the left liver was exposed by electrocoagulation knife. Cut the ligaments connecting the liver with the diaphragm. A thin glass coverslip was placed on the inverted microscope heat-controlled stage with a thermostatic device and the left liver was externalized onto the coverslip. Preparation of lung for intravital microscopy. Briefly, after tracheal intubation in mice following anesthesia, mice were ventilated with ventilator. A thoracic window with 5 mm internal diameter was constructed surgically to expose the lung. Images and videos were recorded using a special vacuum-chamber with a glass slide adhered to the exposed lung surface through negative pressure with 20–25 mm Hg. This negative pressure helped to fix the alveoli for observation but it does not affect pulmonary blood flow.

Spinning Disk Confocal Intravital Microscopy and Images Analysis

Fluorescent images of livers were photographed by Spinning Disk Confocal Intravital

Microscopy (SD-IVM). The images were captured using a Nikon Ti2-E inverted microscope (Nikon Instruments) equipped with a Yokogawa CSU-W1 head (Yokogawa Electric Corporation). All images were captured with a Plan Apochromat Lambda 20X N.A. 0.75 air objective in our study. Two laser excitation wavelengths (488 and 640 nm; TOPTICA Photonics) with the appropriate long-pass filters (Semrock and Chroma) were used in rapid succession. Exposure time was set 100ms for excitation wavelengths. The fluorescence signal was detected by a scientific complementary metal oxide semiconductor (sCMOS) camera (Prime 95B, Photometrics). NIS-Elements AR software (Nikon Instruments) was used to drive the microscope.

Thrombin generation was visualized using SensoLyte internally-quenched 5-FAM/QXL-520 FRET thrombin substrate (2μL/mouse). Fibrin deposition, Platelets aggregation and perfused vessels were visualized by injection of Alexa Fluor 647-conjugated anti-mouse fibrin antibody (4μL/mouse), AF647-anti-CD49b antibody (2μg/mouse) and Alexa Fluor 647-conjugated anti-mouse albumin antibody (0.05μg/mouse) respectively. All images were saved in Tiff format, exported and analyzed in ImageJ software. Contrast was adjusted to eliminate the tissue background autofluorescence and the threshold of minimum brightness was set for emergence of the positive staining only. Apply the same parameter for all images assay. Images were grouped into positive or negative according to the threshold values, and the area per field of view covered by positive staining was analyzed. Data were presented as the percentage of area per field of view covered by positive

staining.

Multiphoton Laser Scanning Confocal Intravital Microscopy Analysis

Images of lung were captured using an Olympus multiphoton imaging system (FVMPE-RS, Tokyo, Japan) with a 25x/NA 1.05 water-dipping objective. The microscope system was equipped with a multiphoton light path installed on upright microscope (BX63, Olympus), and a tunable femtosecond pulse laser (MaiTai DeepSee, Spectra Physics, USA). The excitation wavelength was tuned to 920 nm and the emission filters were selected as 495-540nm for green channel and 575-645nm for red channel. Antibodies administration and images analysis were performed as described above.

$FeCl_3$ Induced Thrombosis Model

Male IFN-α/βR1-deficient mice of 20–24 g and matched WT controls were injected with rhodamine 6G (1 mg/ml; 200 μl) to label platelets after anesthetized with ylazine hydrochloride (10 mg/kg) and ketamine hydrochloride (200 mg/kg). Standard midline incisions were performed from xiphoid to lower abdomen. Fixed the mice to right lateral position, place the garose gel on the glass of the adapter, slightly exteriorize the intestines and spread it on the top of the gel. filter paper saturated with 15% $FeCl_3$ solution was then applied to the second branches mesenteric artery for 1 min. Thrombus formation is identified by accumulation of the fluorescent platelets. The images were recorded by Nikon fluorescence microscope. The end point of the

experiment：blood flow ceased for > 30 sec or vessels were not occluded in 30 min after injury.

Macrophages depletion

WT mice were injected with liposome-encapsulated clodronate (N.van Rooijen) intraperitoneally of 400 μl at 48 hr and intravenously of 250 μl at 24 hr before LPS injection. Lipsome-PBS was injected in the control group.

Liver and Lung Histology

The livers (the left lobe) and lungs (the left upper) were collected after perfusion with PBS containing 6.25IU/ml heparin, fixed in 10% formalin, embedded in paraffin and then cut into 3-5μm thick sections for subsequent hematoxylin and eosin staining and fibrin immunohistochemistry. Paraffin-embedded sections of livers and lungs were incubated with anti-mouse fibrin antibody at 1:500 dilution overnight at 4 °C, washed, incubated with anti-mouse secondary antibody conjugated with HRP, and detected by 3, 3′-diaminobenzidine tetrahydrochloride reaction.

ELISA Assay

Mouse blood was collected by heart puncture with plastic syringes containing 3.8% sodium citrate (9: 1. v/v) after mice being anesthetized. Plastic syringes and

tubes were pre-incubated with heparin to prevent coagulation activation. Plasma was isolated immediately after centrifugation (3000×g, 15 min) at 4℃.The plasma concentrations of TAT, PAI-1, Fibrinogen, D-dimer, IFN-β, HMGB1 and TNF-α were detected using commercially ELISA kits according to the instructions. The concentration of TF protein in lungs was measured after lungs were homogenizated in extraction buffer PTR according to the instruction of the commercially TF ELISA kit.

Bacterial counts in sepsis

Sixteen hours after CLP, mice were sacrificed and the livers, lungs and spleens were collected. These organs were homogenized, diluted serially with sterile saline, then cultured overnight on Luria-Bertani agar plates at 37 °C. Bacterial colony-forming units (CFUs) were counted.

Western blot and Quantitative RT－PCR

After the extraction from tissues and macrophages, proteins were separated by 10% SDS-PAGE and transferred onto PVDF membranes (Millipore). The membranes were incubated with rabbit anti-mouse TF antibody (1:1000) and β-actin (1:5000). Bots were visualized using Bio-Rad system after ECL treatment. The expression of TF was normalized to β-actin expression and quantified using Image Lab software. RNA was isolated using the RNeasy Kit (Qiagen) and reverse transcribed to cDNA with RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher). Quantitative PCR was

performed in LightCycler 480 (Roche) system. The TF mRNA expression was normalized to GAPDH. The TF-specific primers were as follows: TF sense: 5’-AACCCACCAACTATACCTACACT-3’, antisense: 5’-GTCTGTGAGGTCGCACTCG-3’.

Macrophage cultures and siRNA knockdown

Mice in age between 7-12 week received i**ntra**peritoneal injection with 3 mL of sterile 3% thioglycollate broth to induce peritoneal macrophages. Cells were harvested after 72 hr by peritoneal lavage with 5mL of RPMI medium 1640 (Gibco) and passed through a 40-μm cell strainer. After centrifugation (800 rpm, 5 min), cells were resuspended in RPMI medium 1640 containing 10% fetal bovine serum (Gibco) and 1% antibiotics (Gibco) and plated in 12-well or 96-well plate or 6-well slide overnight. Small interfering RNA (siRNA) targeted TMEM16F was transfected with Lipofectamine™ RNAiMAX. The sequences of two siRNAs were as follow: siRNA1: 5’-GCAUACGAAUCUAACCUUATT-3’; siRNA2: 5’-GCGAGAAGAUUGGAAUCUATT-3’.

Annexin V detection of PS exposure

Peritoneal macrophages plated on six-well slide with indicated stimuli were stained with Annexin V-FITC for 15 min protected from light at room temperature, fixed with 4% paraformaldehyde and counterstained with DAPI. Images of PS exposure were

captured by Nikon confocal microscope and analyzed using ImageJ software. Mice periphery leukocytes and splenocytes were stained with Annexin V-FITC (5μl/test) for 30 min on ice in the dark and performed on the Arai-II-Flow Cytometer (BD Biosciences). Data were analyzed with FlowJo software.

Supplementary Figure Legends

Figure S1. The bacterial loads in livers, lungs and spleens.(A-C) WT, IFN-α/βR1 deficient and TRIF deficient mice were subjected to sham operation or CLP. The bacterial counts were determined in livers (A), lungs (B) and spleens (C) after 16 hr of operation. Data are shown as mean ± SEM; **P*<0.05; N=3-9 mice per group.

Figure S2. Plasma concentration of HMGB1 in endotoxemia. Plasma concentrations of HMGB1 was measured in Myd88-deficient and WT mice primed with 0.4mg/kg LPS for 7 hr and then challenged with 10mg/kg LPS for 8 hr. Data are shown as mean ± SEM; NS: not significant; N=3-5 mice per group.

Figure S3. **Concentrations of HMGB1, TAT, tumor necrosis factor (TNF-α) in the plasma and TF mRNA expression in the lungs at different time points in endotoxemia and sepsis.** (A-C) The concentrations of TAT, HMGB1, TNF-α in plasma and TF mRNA expression in the lungs were measured at different time points in WT mice intraperitoneally injected with 0.4mg/kg LPS alone (A), 10mg/kg LPS alone (B) or 0.4mg/kg LPS for 7 hr then 10mg/kg LPS for 12 hr (C).

(D) The concentrations of TAT, HMGB1, TNF-α in plasma and TF mRNA in lungs were measured at different time points in WT mice subjected to sham operation or CLP. Data are shown as mean ± SEM.

Figure S4. Platelets are not important source of HMGB1 that contributes to DIC in both endotoxemia and bacterial sepsis. (A-C) $Hmgb1^{fl/fl}$ $Pf4\text{-}cre^{+}$ mice, $Hmgb1^{fl/fl}$ $Vav\text{-}cre^{+}$ mice, $Hmgb1^{fl/fl}$ and WT mice primed with 0.4mg/kg LPS and then challenged with 10mg/kg LPS for 8 hr. Plasma concentrations of HMGB1 and TAT were measured (A and B) and fibrin deposition in livers and lungs were detected (C,400×). (D) Plasma concentrations of HMGB1 and TAT were detected 16 hr after $Hmgb1^{fl/fl}$ $Vav\text{-}cre^{+}$ and $Hmgb1^{fl/fl}$ mice subjected to sham operation or CLP. Data are shown as mean ± SEM. Data are shown as mean ± SEM; NS = not significant; N=3-7 mice per group.

Figure S5. Depletion of macrophages significantly attenuated thrombin generation and platelets aggregation. (A-B) Mice were pretreated with liposomes-clodronate and liposomes-PBS before 4mg/kg LPS for 6 hr. Representative SD-IVM images of thrombin (green) and platelet adhesion (blue) within the liver microvasculature (A). Quantitative analysis of thrombin and platelets fluorescence intensity by ImageJ (B). Data are shown as mean ± SEM; **P*<0.05; ***P*<0.01; N=4 mice per group. Scale bar represents 50 μm.

Figure S6: TF protein expression in macrophages neutralizing PS or knocking down

TMEM16F. (A) TF protein expression in macrophages treated with HMGB1+ ultrapure LPS with or without MFG-E8. (B) TF and Tmem16F protein expression in LH-stimulated macrophages transfected with control siRNA or TMEM16F-specific siRNA.

Figure S7. **Deletion of Caspase-11 reduced plasma levels of TAT and PAI-1 in endotoxemia.** Plasma concentrations of TAT (A) and PAI-1 (B) were measured in WT and Caspase-11 KO mice primed with 0.4mg/kg LPS and then challenged with 10mg/kg LPS for 8 hr. Heparin (200 IU/kg) was injected subcutaneously 30 min before LPS injection. Data are shown as mean ± SEM; **P*<0.01; N=3-5 mice per group.

Supplementary Video Legends

Video 1. Thrombin generation and platelets adherence in WT and IFN-α/βR1 KO mice. Thrombin (green) and platelets adherence (blue) within the liver microvasculature of WT and IFN-α/βR1 KO mice injected with 4mg/kg LPS for 6 hr. Note thrombin and platelet adhesion attenuated in LPS-treated IFN-α/βR1 KO mice compared with LPS-treated WT mice. Scale bar represents 50 μm.

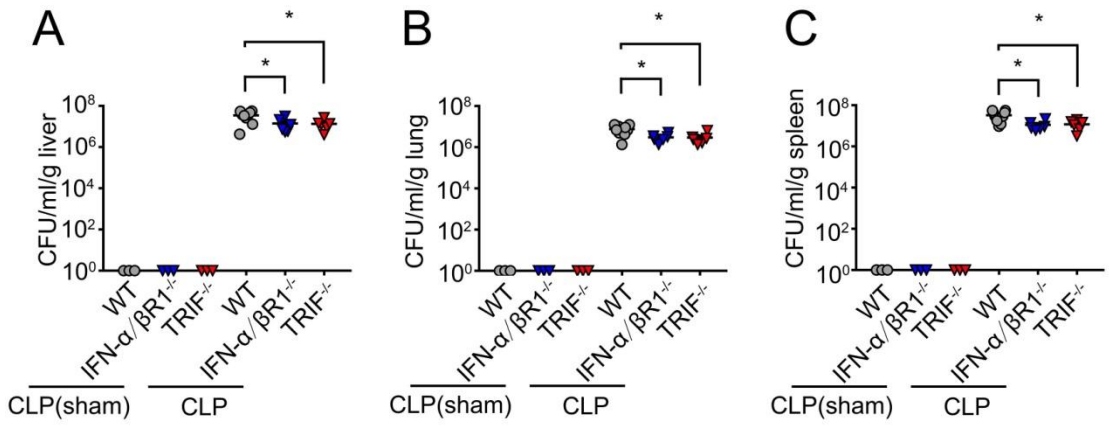
Video 2. Fibrin deposition in WT and IFN-α/βR1 KO mice. Fibrin deposition (dark red) within the liver microvasculature of WT and IFN-α/βR1 KO mice injected with 4mg/kg LPS for 6 hr. Note fibrin deposition attenuated in LPS-treated IFN-α/βR1 KO mice compared with LPS-treated WT mice. Scale bar represents 50 μm.

Video 3. Perfused vessels within the liver microvasculature in LPS-treated WT and IFN-α/βR1 KO mice. The area of perfused vessels (red) was larger in

LPS-treated IFN-α/βR1 KO mice compared with LPS-treated WT mice. Scale bar represents 50 μm.

Video 4. Perfused vessels within the lung microvasculature in LPS-treated WT and IFN-α/βR1 KO mice. The area of perfused vessels (red) within the lung microvasculature was larger in LPS-treated IFN-α/βR1 KO mice compared with LPS-treated WT mice. Scale bar represents 50 μm.

Supplementary Figure 1



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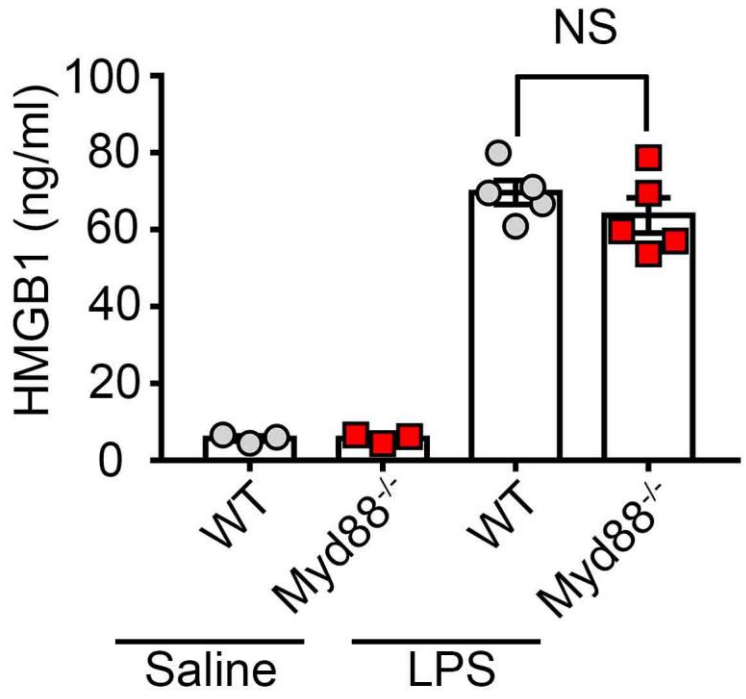
Supplementary Figure 2



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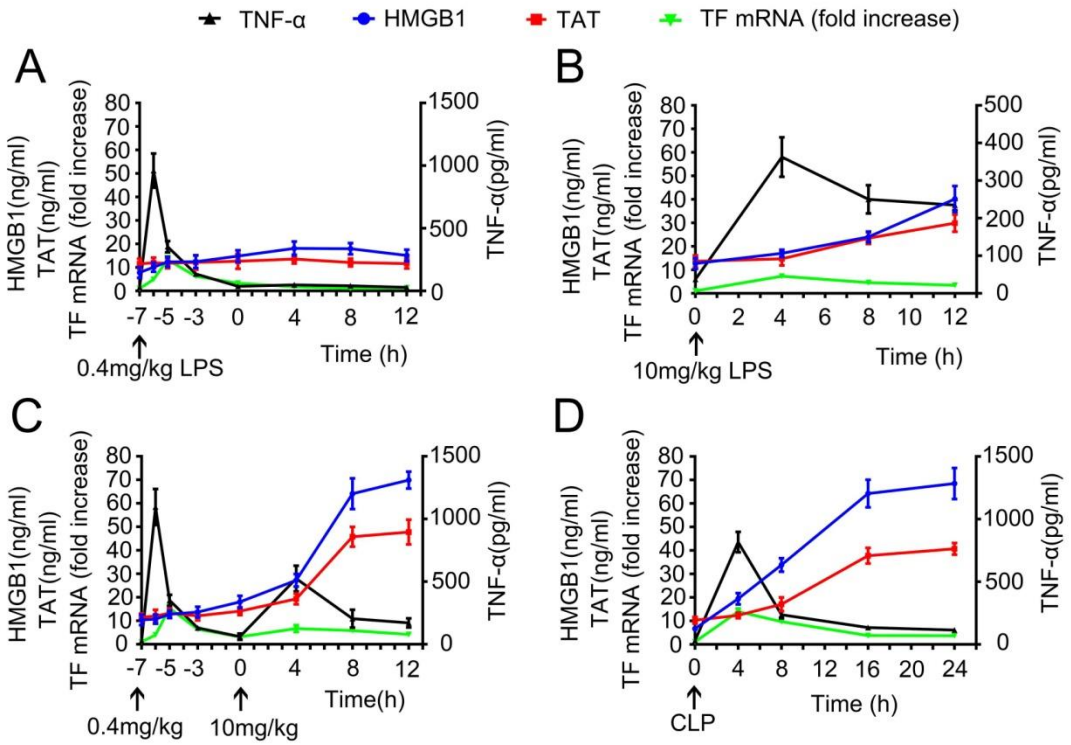
Supplementary Figure 3



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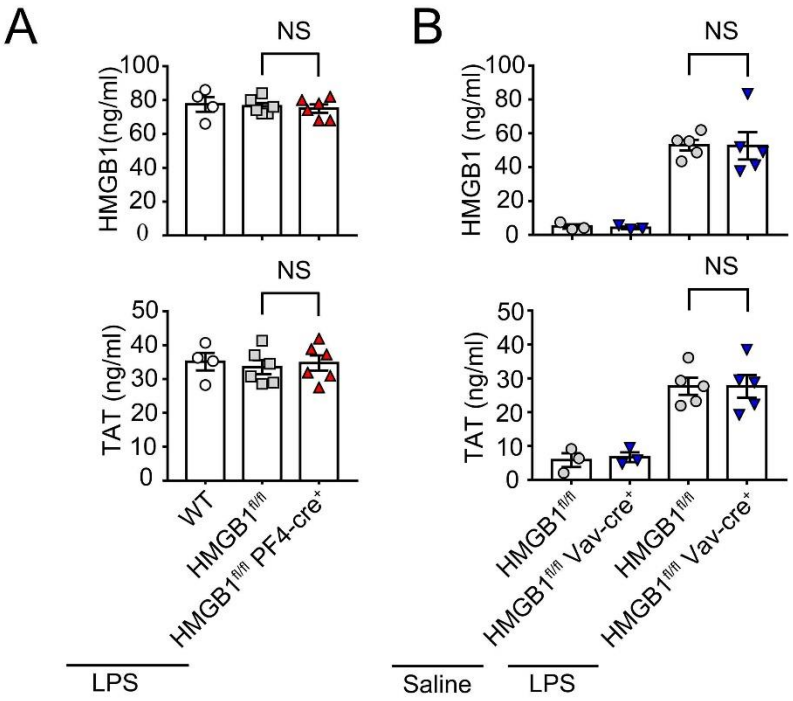
Supplementary Figure 4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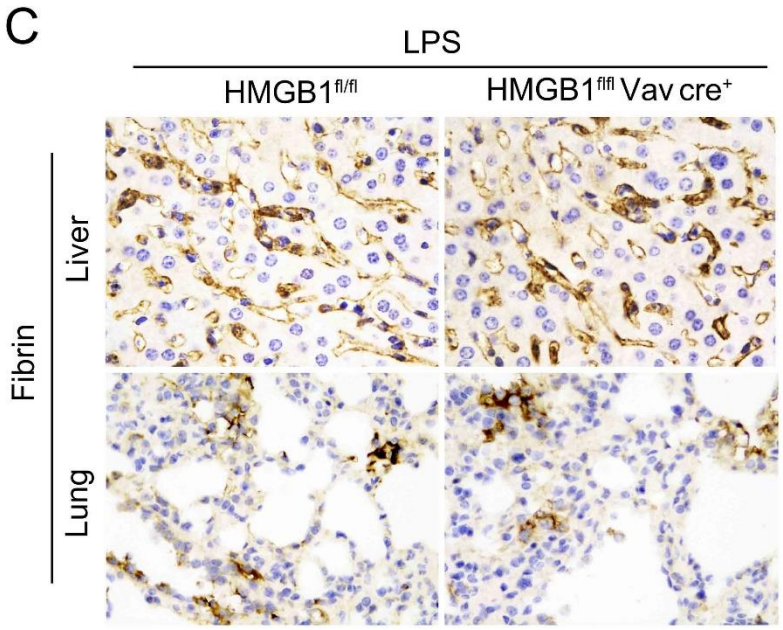
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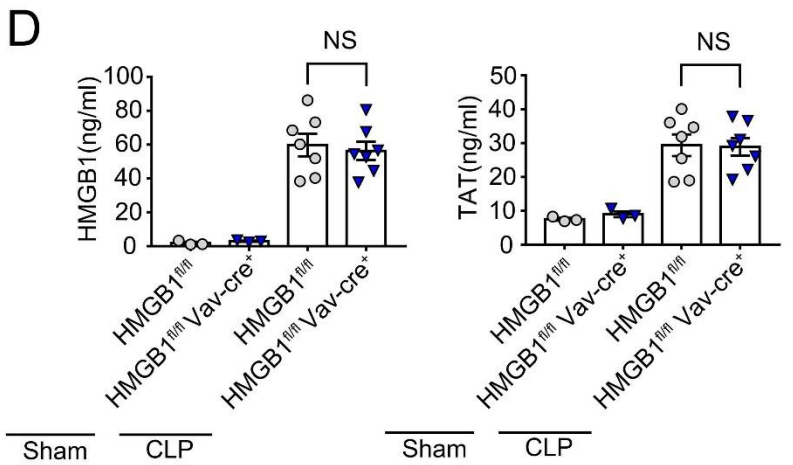
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CLP
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CLP

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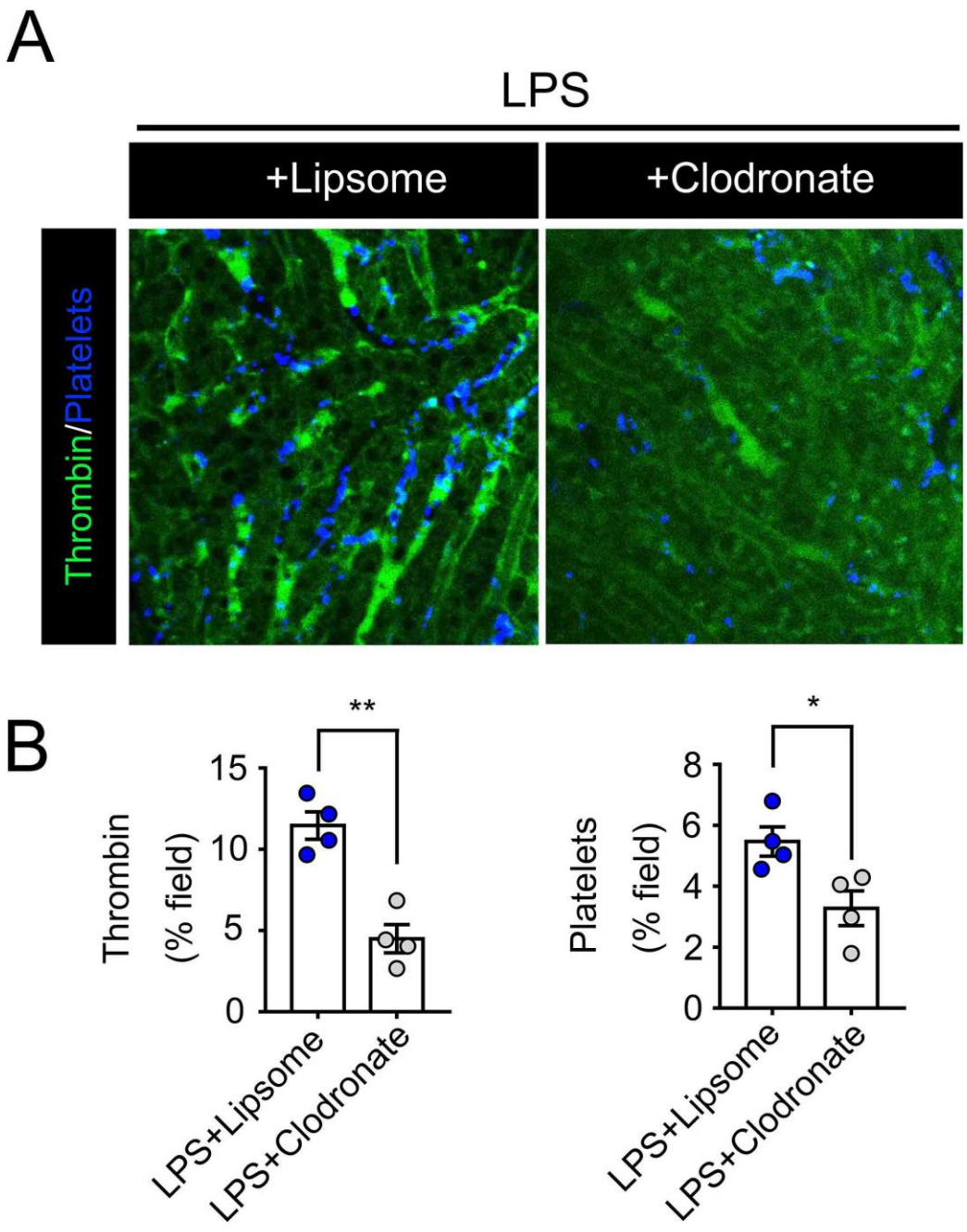
Supplementary Figure 5



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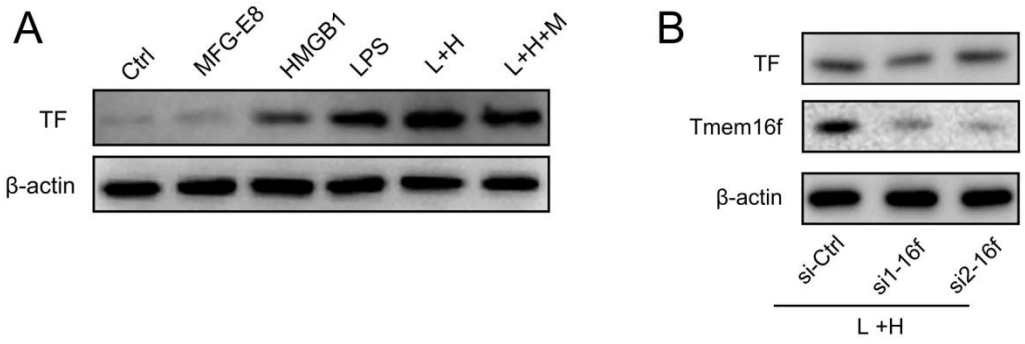
Supplementary Figure 6



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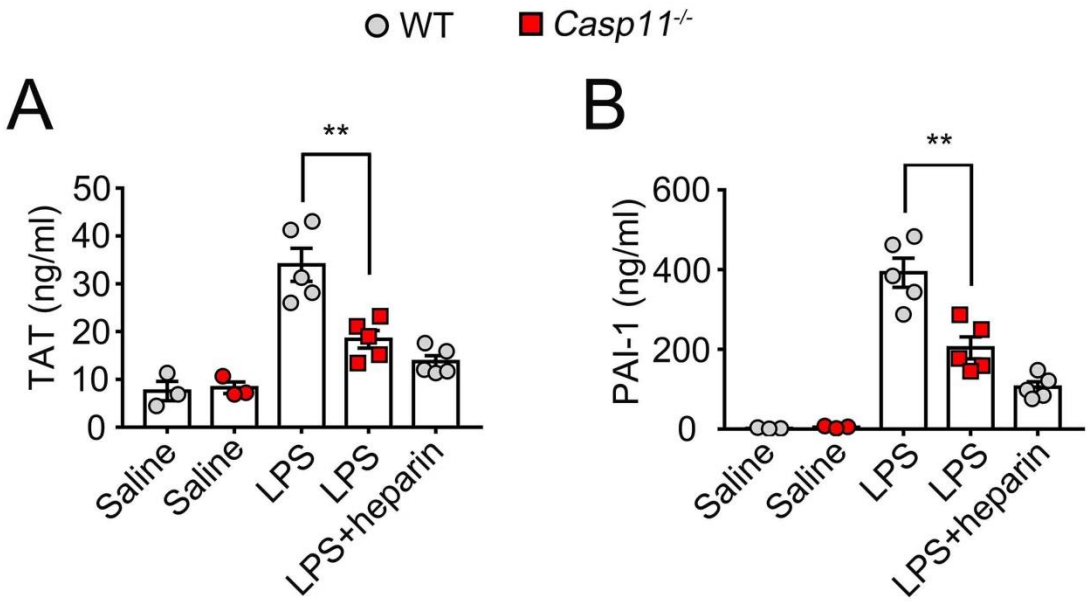

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