Supplementary Methods

Generation of bone marrow-derived macrophages (BMDMs)

BMDMs were differentiated *in vitro* from bone marrow cells cultured with M-CSF. In brief, bone marrow was flushed from the long bones of the limbs with IMDM and centrifuged. The cell suspension was adjusted to 5×10^5/ml in culture medium containing 10 ng/ml M-CSF (GenScript). IMDM medium (Mediatech) supplemented with 10% FCS, 2 mM glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin was used for cell culture. On day 2 of culture, nonadherent cells were gently removed and fresh M-CSF-containing medium was added. On day 7, adherent cells were collected for analysis. More than 95% of cells produced in this way stained with the murine pan-macrophage cell marker, anti-F4/80 mAb (eBioscience).

KC isolation, ablation, adoptive transfer of KC, and preparation of necrotic hepatocytes

KC were isolated by collagenase (Worthington) digestion, density gradient centrifugation followed by MACS magnetic beads separation (Miltenyi Biotec). The liver was perfused with calcium-free solution and digested with a collagenase perfusion. Most hepatocytes were eliminated by taking only the supernatant after brief 50g centrifugation. The cell suspension was filtered through a 70 μm cell strainer (BD Biosciences) and the filtrate was overlaid on Histopaque 1083 (Sigma-Aldrich) and centrifuged at 1000g for 15 min to remove all non-separated hepatocytes. The nonparenchymal cell fraction was washed with buffer, and KC were isolated using anti-F4/80 magnetic beads. For further purification of KC, the small fraction of monocytes and neutrophils was depleted using an anti-Gr-1 ($Ly6G^+$ and $Ly6C^+$) mAb (BD Biosciences). This protocol provides adherent cells that are 98% positive for staining with anti-F4/80 mAb, 96% positive for nonspecific esterase staining and exhibit typical macrophage morphology. For ablation of endogenous KC, 200 μl of Clodronate-containing or PBS-containing (control) liposome suspensions (from Dr. N. van Rooijen, VUMC, Amsterdam, The Netherlands)

were administered by i.p. injection to WT and *Trem1*$^{-/-}$ mice. Depletion of KC was assessed at 48 h by immunofluorescence staining of liver sections with FITC-conjugated rat anti-mouse F4/80 (BioLegend) mAb. Donors KC were isolated from collagenase-perfused livers of WT mice as described above. Cells were washed twice with PBS and 10^5 cells were transferred i.v. at 48 h after injection of liposome into each host mouse. Adoptively transferred cells were observed by immunofluorescence staining as described above. Mice with adoptively transferred KC were given a dose of DEN (100 mg/kg) at 18h and all parameters were assessed at 4 h, 18 h, 24 h, 48 h, and 72 h after DEN injection. Necrotic hepatocytes were prepared by isolating hepatocytes by collagenase perfusion as above, and then freeze (-80^{0}C)-thawing (room temperature) 3 times. Cellular debris was filtered and the supernatant run on an agarose gel; no sign of DNA fragmentation was observed, as would be seen in apoptosis. BMDMs were incubated with the equivalent of 10^6 necrotic hepatocytes /ml in serum-free medium. Supernatants of BMDMs were collected at 4 h, 24 h, 48 h, and 72 h and used for ELISA.

Chemical crosslinking for analysis of protein-protein interactions (*in vitro)*

Crosslinking reactions with dimethyl adipimidate (DMA) or bis(sulfosuccinimidyl) suberate (BS3) with spacer arms of 8.6 Å and 11.4 Å respectively, were performed in 20 mM Hepes buffer (pH 7.5). Reaction mixtures with 5 μM interacting proteins in a total volume of 10 μl were treated with 5 μM of DMA or BS3 for 30 min at room temperature. Murine HMGB1 (eBioscience), HSP70 (Enzo Life Sciences), and TREM-1 (prepared in our laboratory) proteins were used as cross-linked proteins. The reaction was terminated by addition of the denaturing sample buffer, and the mixture was resolved by SDS-PAGE followed by silver stain.

Binding analysis using Surface Plasmon Resonance (SPR)

Murine TREM-1, RAGE and unrelated CsrA protein with similar isoelectric point were dissolved in HBS-EP buffer (10mM Hepes, pH 7.4/150 mM NaCl/3.4 mM EDTA/0.005% Surfactant P20) (GE Healthcare). SPR experiments were performed by using a BIAcore X100 system (GE Healthcare). Murine HMGB1 protein was immobilized on the CM5 sensor chip. RAGE, TREM-1, and unrelated protein CsrA flowed over at 5 μl/min. Kinetics constants were derived using the curve fitting for the bivalent analyte model or the simple 1:1 binding model by the BIAevaluation version 4.1.1 (Microcal). For equilibrium binding analyses, the equilibrating binding response at each concentration of analyte was calculated by subtracting the response measured in the control flow cell from the response in the sample flow cells. Affinity constants (K_d) were calculated by nonlinear curve fitting or by Scatchard analysis with the simple 1:1 Langmuir binding model.

NanoString analysis

NanoString nCounter gene expression assay was performed using two specific probes (capture and reporter) for each gene of interest. In brief, 200 ng of total RNA per sample were hybridized with customized Reporter CodeSet and Capture ProbeSet according to manufacturer's instructions (NanoString Technologies), for direct labeling of mRNAs of interest with molecular barcodes without the use of reverse transcription or amplification. Then, the hybridized samples were recovered with the NanoString Prep Station and the mRNA molecules counted with the NanoString nCounter. The resulting counts were corrected by subtracting the average value of the negative control (alien probes from the CodeSet, lacking spiked transcript) from the raw counts obtained for each RNA. Values less than zero were considered equal to 1. The corrected raw data were finally normalized using RPL9 as housekeeping gene.

Real-Time PCR for quantitation of *Trem1* mRNA

RNA was isolated from purified KC and cDNA synthesized as described above. Subsequent PCR analysis was performed using the manufacturer's suggested protocol. Primer oligonucleotides were synthesized by Integrated DNA Technologies: *β-actin* forward, 5'-GTGG-GCCGCTCTAGGCACCA-3' and reverse, 5'-CGGTTGGCCTTAGGGTTCAGGGG-3'; *Trem1* forward, 5′-GAGCTTGAAGGATGAGGAAGGC-3′ and reverse, 5′-CAGAGTCTGTCACT-TGAAGGTCAGTC-3′.

Light microscopy

Liver tissues were embedded in OCT compound (Miles) and frozen in liquid nitrogen. Frozen sections 7 μm thick were prepared and stained with hematoxylin and eosin (H&E) for light microscopy.

Immunohistochemistry

Liver tissues were fixed in 2% periodate-lysine-paraformaldehyde solution at 4 ^{0}C for 4 h, embedded in OCT compound (Miles), frozen in liquid nitrogen, and cut using a cryostat into 7 μm thick sections. After inhibition of endogenous peroxidase activity, an indirect immunoperoxidase staining method using a mouse anti-mouse anti-αfetoproteiṇ mAb (R&D Systems) was performed. M.O.M. (mouse on mouse) immunodetection kit (Vector Laboratories) was used to localize mouse primary antibody on mouse tissue followed by diamonobenzidine peroxidase substrate kit (Vector Laboratories). After visualization with 3.3'– diaminobenzidine, the sections were stained with hematoxylin for nuclear staining and mounted with cytoseal 60. As negative controls, the same procedures were performed but the primary antibody was omitted.

Immunoprecipitation and Western blot analysis

Hepatocytes were isolated from DEN-treated (100 mg/kg for 18 h) and non-treated WT mice. Cell lysates were pre-cleaned with immunoglobulin G (IgG) Dynabeads protein A for 10min at 4°C before incubation with recombinant mouse TREM-1-Fc fusion protein (R&D Systems)-linked Dynabeads overnight at 4°C. The immunoprecipitated Dynabeads complexes were washed 5 times with immunoprecipitation buffer (10mM Tris-HCl, pH 7.8, 1mM EDTA, 150mM NaCl, 1mM NaF, 0.5% nonidet P-40 (NP40), 0.5% glucopyranoside, 1μg/ml aprotinin, and 0.5 mM PMSF). Proteins were eluted by boiling in protein loading buffer and then processed for Western blot analysis as described below.

For Western blot analysis, liver tissues were lysed in buffer containing 50mM HEPES (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1.5 mM $MgCl_2$, 10% glycerol, 1% Triton X-100, and 100 μg/ml PMSF. Lysates were cleared by centrifugation and protein concentration was determined by the BCA protein assay kit (Pierce). Equal amounts (40 μg) were boiled for 5 min in equal volumes of sample buffer (125 mM Tris·HCl pH 6.8, 4% SDS, 15% glycerol and 10% 2-mercaptoethanol). Samples were chilled on ice and separated on a 10-12% gel (Bio-Rad) and then electroblotted onto PVDF membranes (Bio-Rad). Membranes were probed overnight with appropriate dilutions of primary Abs against -αfetoprotein (R&D Systems), anti-HMGB1, anti-HSP70 (BioLegend), and actin (Santa Gruz Biotechnology). After washing twice with TBS/0.05% Tween 20, appropriate peroxidase-conjugated secondary antibodies were applied. Blots were washed twice and incubated in enhanced chemiluminiscense detection reagent (Amersham Bioscience). Specific bands were visualized by exposure to X-ray film.