## Supplementary Figure Legend

**Figure S1. Targeting strategy for the *Trem1* genomic locus and generation of *Trem1*-deficient mice.** (A) Schematic structure of WT *Trem1* genomic locus, targeting vector, and predicted targeted locus following homologous recombination. To delete the *Trem1* gene, the targeting vector was constructed by replacing 4583 bp of genomic DNA, including all of exon 1 (from 11 bp upstream of the ATG start codon) together with the entire first intron and exon 2 of the *Trem1* allele, with a neomycin resistance (neo) gene flanked by loxP sequence and EGFP reporter gene. Open boxes with numbers indicate exons. The vector was designed so that the promoter of the *Trem1* gene drives expression of the reporter gene and predicted targeted allele following homologous recombination. This construct was electroporated into C57BL/6 embryonic stem cells. The locations of neo and thymidine kinase (TK) genes and probe for Southern blotting are indicated. Restriction sites are designated: B, Bgl II; C, Cla I. (B) Southern blotting analyses of genomic DNA digested with Bgl II and hybridized with a probe external to the targeting vector yields 8764 bp and 6424 bp fragments for the WT and targeted allele, respectively. Genotypes are indicated as follows: +/+, WT; +/-, heterozygous; -/-, *Trem1*$^{-/-}$. (C) PCR genotyping of offspring from mutants and WT mice. The sizes of PCR products are 300 and 506 bp for the WT and mutant alleles, respectively. Chimeras were bred to transgenic mice expressing *Cre* to delete the neomycin-resistant gene. (D) Constitutive expression of TREM-1 on neutrophils and inducible expression on blood monocytes and peritoneal macrophages in WT mice. PBMC from WT and *Trem1*$^{-/-}$ mice were isolated from mice treated for 18 h with LPS (+LPS, 10 μg/g of LPS, i.p. injection) or untreated (-LPS), stained with APC-conjugated anti-CD11b, PE-Cy7-conjugated anti-Ly6C, PerCP-Cy5.5-conjugated anti-Ly6G, and PE-conjugated anti-TREM-1 mAbs followed by flow cytometry analysis. Neutrophils are defined as CD11b$^{+}$ Ly6C$^{low}$ Ly6G$^{+}$. Blood monocytes are defined as CD11b$^{+}$ Ly6C$^{high}$ Ly6G$^{-}$. Cells shown were gated on CD11b$^{+}$ Ly6G$^{+}$ Ly6C$^{low}$ population (for neutrophils), on CD11b$^{+}$ Ly6C$^{high}$ Ly6G$^{-}$ (for

monocytes). Peritoneal lavage cells from indicated mice treated for 18h with LPS (+LPS, 10 μg/g of LPS, i.p. injection) or untreated (-LPS) were stained with APC-conjugated F4/80, PE-Cy7-conjugated anti-Ly6C, PerCP-Cy5.5-conjugated anti-CD11b, and PE-conjugated anti-TREM-1 mAbs. Macrophages are defined as $F4/80^+$ $CD11b^+$ $Ly6C^-$. Filled gray histograms represent isotype control. Numbers indicate percentage of positive cells of total gated cells. Data are from one representative experiment out of five independent experiments with similar results.

*Trem1*$^{-/-}$ mice were born at Mendelian frequencies and were equivalent in size, weight, and fertility to littermate controls. No abnormalities in organ development or inflammation were found upon histological examination. Comprehensive flow cytometric analysis of thymocytes, splenocytes, lymph node cells, and peripheral blood leukocytes from 6-week-old mice did not indicate any substantial differences in the composition of immune cells in these compartments between naïve WT and *Trem1*$^{-/-}$ mice.

**Figure S2. TREM-1 expression on KC of WT mice increased after DEN administration.** (A) *Trem1* mRNA levels in purified KC. WT mice (n=10 male mice per timepoint) were treated with DEN. Livers were removed at indicated times for isolation of KC and RNA extraction. cDNA was assayed by Real-Time PCR for *Trem1* mRNA level relative to naïve WT mice. (B, C, and D) WT and *Trem1*$^{-/-}$ mice were treated with DEN for 18 h or left untreated. Liver cells were stained with anti-CD11b, -F4/80 and -TREM-1 mAbs (B and C) followed by flow cytometry analysis. Histograms are gated on $CD11b^+$ $F4/80^+$ cells. (D) Liver cells from *Trem1*$^{-/-}$ mice were stained with anti-CD11b, -F4/80 mAbs and analyzed for GFP expression. Data are from one representative experiment out of three independent experiments for *Trem1* mRNA analysis and of four independent experiments for assay of surface expression of TREM-1. Statistical significance is indicated (*$p < 0.05$, ** $p < 0.01$).

**Figure S3. Deletion of TREM-1 down regulates genes associated with DNA repair and genes involved in hepatocellular carcinoma development.**

WT and *Trem1*$^{-/-}$ mice were treated with DEN for 4h or left untreated. RNA samples extracted from livers were subjected to NanoString analysis. Decreased expression in genes associated with DNA repair (A), and genes involved in hepatocellular carcinoma development including the Akt, Met and β-catenin pathways (B) was observed in *Trem1*$^{-/-}$ mice after DEN treatment. Results are mean ± SE (n=4 male mice per group). Statistical significance is indicated.

**Figure S4**. **Deletion of TREM-1 inhibits cytokine and chemokine production by bone marrow-derived macrophages stimulated with the product of necrotic hepatocytes (PNH).**

Bone marrow-derived macrophages from WT and *Trem1*$^{-/-}$ mice were exposed to PNH for indicated times and CCL2 (A), IL-1β (B), IL-6 (C), and TNF (D) in supernatants were measured by ELISA (triplicates per timepoint). All results are means ± SD. Statistical significance is indicated (* $p < 0.05$, ** $p < 0.01$). Data are from one representative experiment out of three independent experiments with similar results.

**Figure S5. The expression of TREM-1 in human liver KC.**

Indirect TREM-1 and CD68 immunofluorescence staining was performed on normal liver tissue samples from 36 individuals and on liver tissue samples from 103 patients with HCC (normal tissue and HCC tissue arrays LV809, LVN801, T031, and BS0313, US Biomax). Histological analysis of representative normal livers (A, B) and surrounding nontumor liver tissues from HCC patients (C, D) (H&E staining, x40 in column 1, bars 5 μm; and H&E staining, x100 in column 2, bars 2 μm). Sections from both groups were double-stained with anti-TREM-1 (Prestige Antibodies, Sigma) and anti-CD68 (KP1, Thermo Scientific) antibodies followed by Alexa Fluor

555-conjugated donkey anti-rabbit IgG (third column) and Alexa Fluor 488-conjugated goat anti-mouse IgG (fourth column). Nuclear counterstain was performed with DAPI (Vector Laboratories). Stained cells were imaged with the Zeiss Axio Imager. M1 Microscope (Obj. EC Plan-Neofluar 100X/1.3). Bars 2 μm. Most of the TREM-1-positive cells (red staining) also reacted with antibody to CD68, a specific marker for macrophages/monocytes (green staining). The immunofluorescence showed that KC in both groups are positive for TREM-1.