Supplementary Table 1

Details of primers used in quantitative RT-PCR analysis

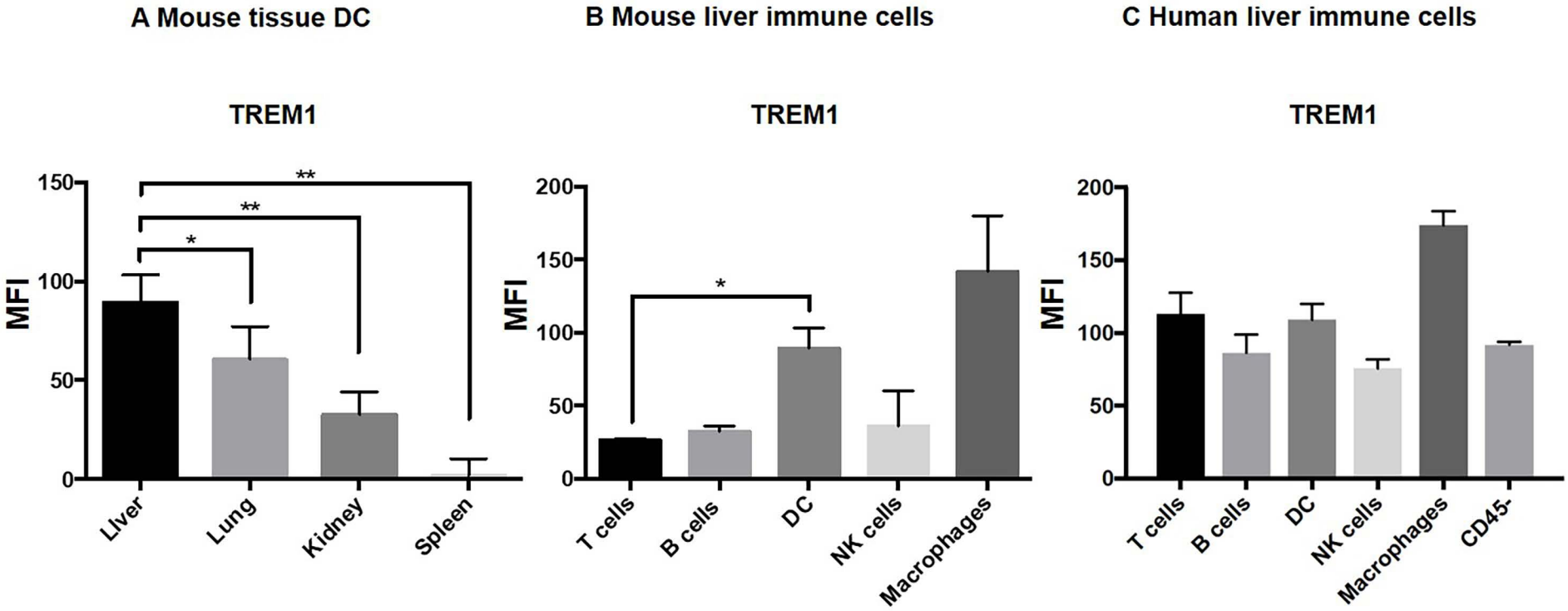
Species	Gene name	Primer Sequence [5'→3'] or product name		Supplier
mouse	DAP12	Mm_Tyrobp_1_SG QuantiTect Primer Assay QT00129514		Qiagen
	TREM1	Mm_Trem1_1_SG QuantiTect Primer Assay, QT00153979		Qiagen
	TREM2	Mm_Trem2_1_SG QuantiTect Primer Assay, QT00157969		Qiagen
	IDO	F	GACCACCACATAGATGAAG	Invitrogen
		R	GGCCCAACTTCTCTGAGAGC	
	arginase-1	F	CAGAAGAATGGAAGAGTCG	Invitrogen
		R	CAGATATGCAGGGAGTCAC	
	GAPDH	F	GCATTGTGGAAGGGCTCA	Invitrogen
		R	AAGTCGCAGGAGACAACC	
human	DAP12	Hs_TYROBP_1_SG QuantiTect Primer Assay QT00077518		Qiagen
	TREM1	Hs_TREM1_1_SG QuantiTect Primer Assay QT00046284		Qiagen
	TREM2	Hs_TREM2_1_SG QuantiTect Primer Assay QT00063868		Qiagen
	GAPDH	Hs_GAPDH_1_SG QuantiTect Primer Assay QT00079247		Qiagen

Abbreviations: F, Forward; R, Reverse

Supplementary Figure

Supplementary Fig. 1

Expression of TREM1 by (A) conventional myeloid DC freshly-isolated from various mouse tissues, (B) various mouse liver immune cells compared with DC and (C) DC and other human liver immune cells. Data are means + 1 SEM obtained from 6-8 mice and from histologically normal human liver specimens of 3 individuals. *$p<0.05$; **$p<0.01$



Supplementary Fig. 1

Supplementary Methods

REAGENTS

Complete culture medium comprised RPMI-1640 (BioWhittaker, Walkersville, MD) supplemented with 10% (v/v) fetal calf serum (Nalgene, Miami, FL) and non-essential amino acids, L-glutamine, sodium pyruvate, penicillin–streptomycin and 2-mercaptoethanol all from Life Technologies (Gaithersburg, MD). Type I collagenase was from Worthington Biochemical Co. (Lakewood, NJ). *E. coli* LPS was from InvivoGen (San Diego, CA).

MIXED LEUKOCYTE REACTIONS (MLR)

For mouse MLR, T cells were negatively selected from bulk splenocytes by magnetic-activated cell sorting (MACS) using Dynabeads (Invitrogen, Grand Island, NY) following incubation with an antibody (Ab) cocktail comprising anti(α)-CD45R/B220 (clone#: RA3-6B2), α-CD16/CD32 (2.4G2), α-TER-119, α-CD11b (M1/70) and α-Ly6G (RB-8C5) (all BD Bioscience, San Diego, CA). They were then stained with carboxyfluorescein diacetate succinimidyl ester (CFSE) (Invitrogen, Waltham, MA), as described[(1)]. Liver and spleen DC were freshly-isolated using α-CD11c immunobeads (Miltenyi Biotec, San Diego, CA), γ-irradiated (30Gy) and used as stimulators or regulators at a 1 DC : 10 T cell ratio. Cultures were maintained in RPMI-1640 complete medium for 5 days in 5% CO_2-in-air, using 96-well, round-bottom plates (Corning, NY), at a concentration of 2 x 10^5 DC and 2 x 10^6 T cells/well. T cell proliferation was determined by CFSE dilution. For human MLR, T cells were isolated from peripheral blood mononuclear cells (PBMC) using Pan T cell kits (BD) and liver DC isolated using Pan DC kits (BD). The DC were γ-irradiated (30Gy) and tested as regulators at a ratio of 1 DC:10 T cell ratio. Cultures were maintained in RPMI-1640 complete medium, as described for mouse MLR.

REAL-TIME REVERSE-TRANSCRIPTION POLYMERASE CHAIN REACTION (RT-PCR)

Total RNA was extracted using Qiagen RNeasy Mini Kits (Qiagen, Hilden, Germany) as per the manufacturer's instructions. RNA was quantified using a Take3 Gen5 spectrophotometer (BioTek, Winooski, VT). One μg of RNA was treated with DNase I (amplification grade; Invitrogen) then

reverse-transcribed using Superscript III First Strand Synthesis SuperMix (Invitrogen). cDNA was amplified using Platinum Quantitative PCR SuperMix-UDG (Invitrogen) in 20 μl volumes in quadruplicate with gene-specific primers and probed on the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Thermal cycling conditions were 50°C for 2 min then 95°C for 2 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min. Data were analyzed using the $^{\Delta\Delta}$Ct method with expression normalized to the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Primer sequences used are shown in Supplementary Table 1.

Reference

1. Yoshida O, Kimura S, Dou L, Matta BM, Yokota S, Ross MA, Geller DA, et al. DAP12 deficiency in liver allografts results in enhanced donor DC migration, augmented effector T cell responses and abrogation of transplant tolerance. Am J Transplant 2014;14:1791-1805.