Supplemental Materials and Methods

Animal experiments

FACS analysis of primary mouse liver mononuclear cells

Isolated LMNC from WT mice (n=3) were stained with FITC-conjugated CD3 (Santa Cruz; sc18843), APC-conjugated PBS57-loaded-CD1d-tetramers (provided by NIH, Atlanta, GA, USA), and PE-conjugated Shh (R&D, IC4641P), and analyzed using the BD^{TM} LSR II (BD Biosciences) at the flow cytometry core facility at the Duke Human Vaccine Institute Flow Cytometry Core Facility, Duke University Medical Center.

Mouse primary hepatic stellate cell isolation

Hepatic stellate cells (HSC) were isolated from C57BL/6 mice as previously described (1). Briefly, after *in situ* perfusion of the liver with pronase (Roche, Indianapolis, IN) followed by collagenase (Roche), cell suspensions were layered on a discontinuous density gradient of Larcoll (Sigma-Aldrich, St. Louis, MO) and Histodenz (Sigma-Aldrich). The resulting upper layer consisted of >95% HSC. Viability of all cells was verified by phase contrast microscopy and the ability to exclude propidium iodide. The viability of all cells was >95%. Isolated HSC were seeded at a density of 5×10^3 cells/mm^2 in DMEM supplemented with 10% fetal bovine serum, streptomycin and penicillin. Day 3 HSC was used in all experiments.

Human tissues:

Double Immunolabeling of CD57 (+) cells

CD57 and Indian Hedgehog (Inh), or CD57 and Osteopontin (OPN) double immunostaining were demonstrated using DAB (DAKO) and Ferangi Blue™ (Biocare Med, Cat no FB8125, Concord, CA).

Analysis of NKT cells subsets in human liver

Non-diseased liver tissues removed during resection for colorectal hepatic metastases or from split-liver grafts, were used to isolate liver-derived lymphocytes as described (1). Primary hepatic lymphocytes were stained with Pacific Blue-conjugated anti-CD3 (Biolegend; Cat: 300-417), FITC-conjugated anti-CD56 (BD; cat no 34058; Oxford, UK), and PE-conjugated anti-OPN (R&D; cat no IC14331P). CD3-CD56 double positive cells are recognized as NKT cells[1]. Liver samples were obtained with informed consent of donors and in accordance with local ethical approval 04/Q2708/41 and REC 2003/242 from the South Birmingham Research Ethics Committee, UK.

References

1. Syn WK, Oo YH, Pereira TA, Karaca GF, Jung Y, Omenetti A, Witek RP, et al. Accumulation of natural killer T cells in progressive nonalcoholic fatty liver disease. Hepatology; 51:1998-2007.