Supplementary Methods

Assessment of liver damage, inflammation, NASH Activity Score, and tumors

Liver damage was assessed by measuring serum alanine aminotransaminase (ALT) as was previously described, using the DRI-CHEM 4000 Chemistry Analyzer System (HESKA) (1). IL-6 levels in mouse serum were measured by a capture ELISA (R&D systems) according to the manufacturer's directions. mRNA for TNF-α from mouse liver tissues was quantified in triplicate by SYBR Green qRT-PCR as described by Huang et al (1). To determine the NASH activity scores (NAS), mouse livers were fixed in formalin, sectioned, and stained for Hematoxylin and Eosin. An experienced liver pathologist who was blinded to the disease and treatment conditions evaluated the livers for NAS, based histopathologic evaluation for degree of steatosis (scored 0-3) lobular inflammation (scored 0-3), and hepatocyte ballooning (scored 0-2), as previously described (2). Total NAS ranged from 0-8. Liver surface tumors were counted. H&E stained cross sections of livers were scanned with a Zeiss Axio Scan Z1. Tumor borders were highlighted on the obtained images and tumor area sizes were calculated using FIJI software.

Flow Cytometry Analysis of liver non-parenchymal cells

Mouse non-parenchymal liver cells (NPC) were isolated as previously described (3). Briefly, after laparotomy, the portal vein was cannulated, and the liver was flushed with HBSS (Invitrogen Life Technologies) supplemented with 0.96g sodium bicarbonate/500 ml (Perfusate I). Then, the liver was perfused with 0.2% protease (Sigma-Aldrich) in Perfusate I for 3 min, after which it was excised, placed in Perfusate II and diced into 2–3mm pieces. NPCs were separated from the hepatocytes by differential centrifugation (400 rpm for 5 min). The supernatant was centrifuged further (1500 rpm for 5 min x2) to obtain NPCs. Isolated NPCs were incubated with flurochrome-conjugated monoclonal

antibodies to mark innate immune cells. Antibodies used were Fixable Viability Dye (APC-Cy7), anti-mouse Ly-6C (PerCP-Cy5.5, clone HK1.4) (both eBioscience), anti-mouse CD45 (PE-Cy7, clone 30-F11), anti-mouse Ly-6G (BUV395, clone 1A8) (both BD Biosciences), anti-mouse F4/80 (PE, clone BM8) (BioLegend), and anti-mouse CD11b (APC, M1/70.15.11.5) (Miltenyi Biotec). Cells were analyzed using a BD LSRFortessa cell analyzer.

Free fatty acid and triglyceride measurement

Levels of free fatty acids (FFA) were measured in liver isolates using a Free Fatty Acid Quantification kit according to the manufacturer's instructions (Abcam). Serum triglycerides were measured using HESKA technology.

References

1. **Huang H, Nace GW**, McDonald KA, Tai S, Klune JR, Rosborough BR, Ding Q, et al. Hepatocyte-specific high-mobility group box 1 deletion worsens the injury in liver ischemia/reperfusion: a role for intracellular high-mobility group box 1 in cellular protection. Hepatology 2014;59:1984-1997.

2. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313-1321.

3. Huang H, Chen HW, Evankovich J, Yan W, Rosborough BR, Nace GW, Ding Q, et al. Histones activate the NLRP3 inflammasome in Kupffer cells during sterile inflammatory liver injury. J Immunol 2013;191:2665-2679.

Author names in bold designate shared co-first authorship.

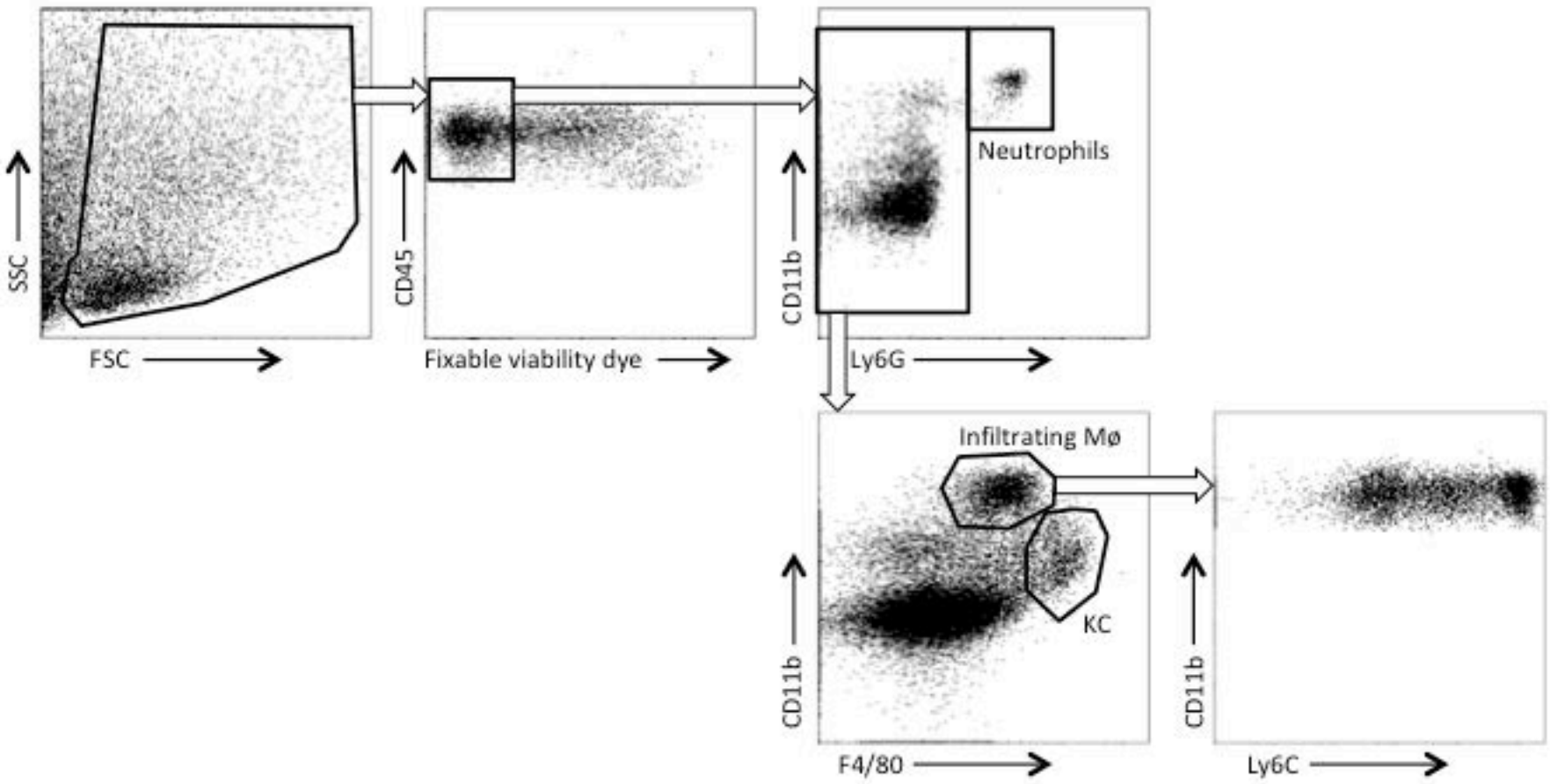
Supporting Table S1. Characteristics of patients with normal liver histology vs. patients with NASH.

	Normal liver n=30	NASH n=56	P value*
Age – median (IQR)	62 (53-72)	62 (54-69)	0.93
Female gender – n (%)	17 (57)	24 (43)	0.22
Comorbidities			
- Diabetes – n (%)	1 (3)	16 (29)**	0.004
- Hypertension – n (%)	13 (43)	31 (55)	0.29
- Hyperlipidemia – n (%)	10 (33)	10 (18)**	0.12
- Body mass index – median (IQR)	26.0 (22.5-32.2)	31.5 (26.9-33.7)**	0.012
- Alcohol abuse – n (%)	1 (3)	4 (7)	0.47
- ASA class#			
• II – n (%)	9 (31)	8 (15)	
• III – n (%)	18 (62)	43 (80)	
• IV – n (%)	2 (7)	3 (6)	0.19
Baseline laboratory values			
- AST – median (IQR)	25 (20-30)	27 (23-34)	0.24
- ALT – median (IQR)	21 (16-28)	28 (20-40)**	0.03
- Total bilirubin – median (IQR)	0.6 (0.4-0.8)	0.6 (0.4-0.9)#	0.75
- INR – median (IQR)	1.0 (1.0-1.1)**	1.1 (1.0-1.1)**	0.37
- Albumin – median (IQR)	3.8 (3.6-4.2)	4.0 (3.6-4.3)**	0.19
Indication for liver resection			
- Benign disease – n (%)	4 (13)	5 (9)	
- Primary malignant disease – n (%)	5 (17)	15 (27)	

- Metastatic disease – n (%)	21 (70)	36 (64)	0.52

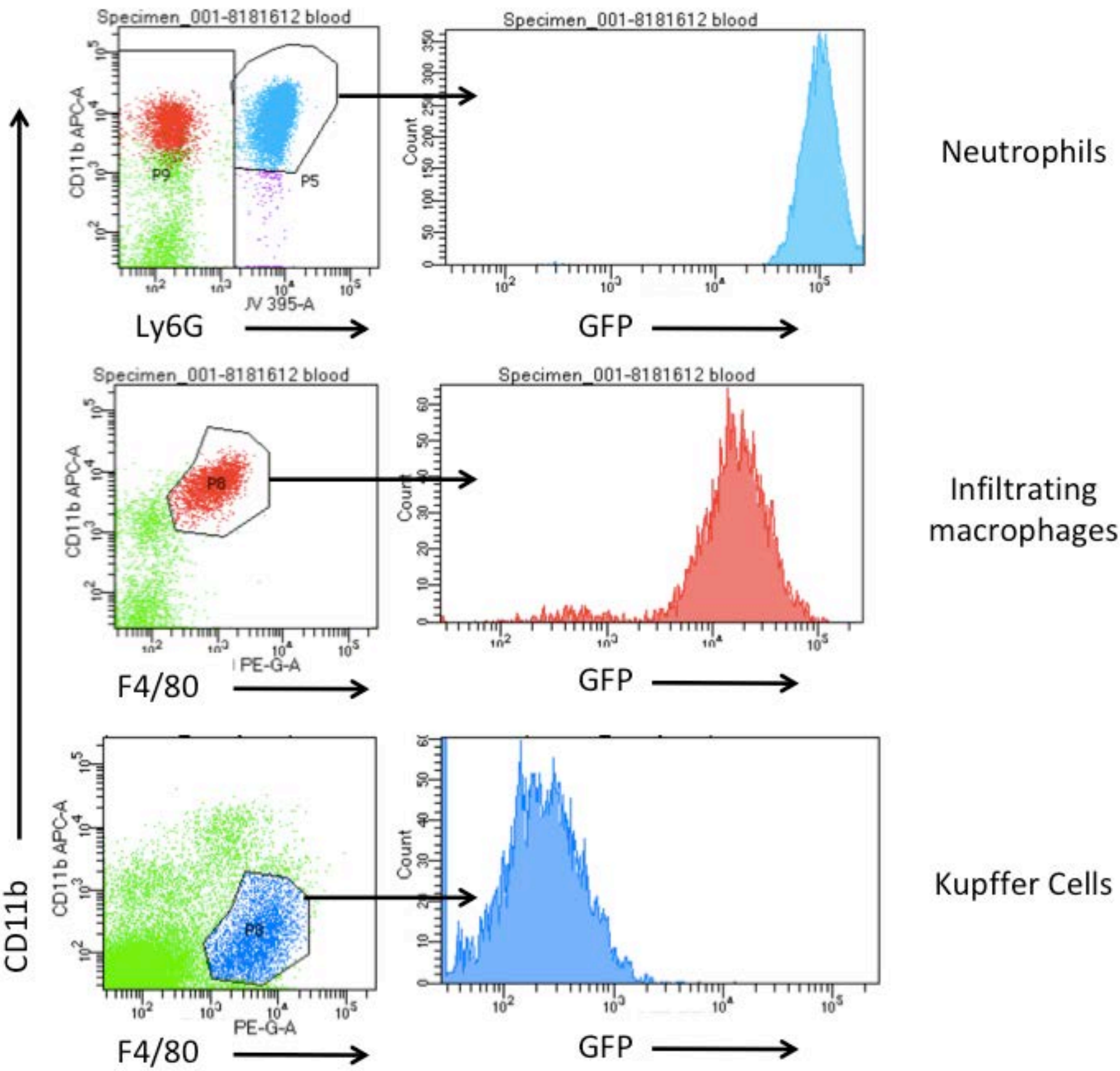
* p values obtained by Chi-square test or Mann Whitney test, ** data missing for 1 patient, # data missing for 2 patients. IQR - interquartile range, ASA - American Society of Anesthesiologists, ALT - alanine aminotransferase, AST - aspartate aminotransferase, INR - international normalized ratio.

Supporting Figure S1.



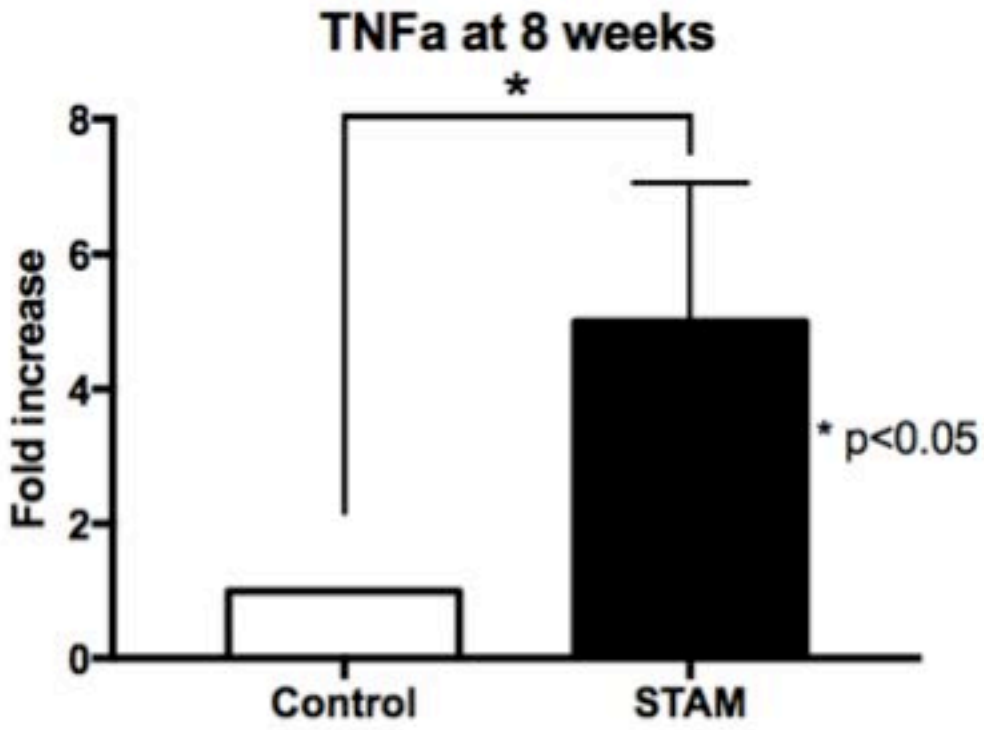
Supporting Figure S1.
Gating strategy for flow cytometric characterization of liver innate immune cells. Infiltrating Mø – infiltrating macrophages. KC – Kupffer cells.

Supporting Figure S2.



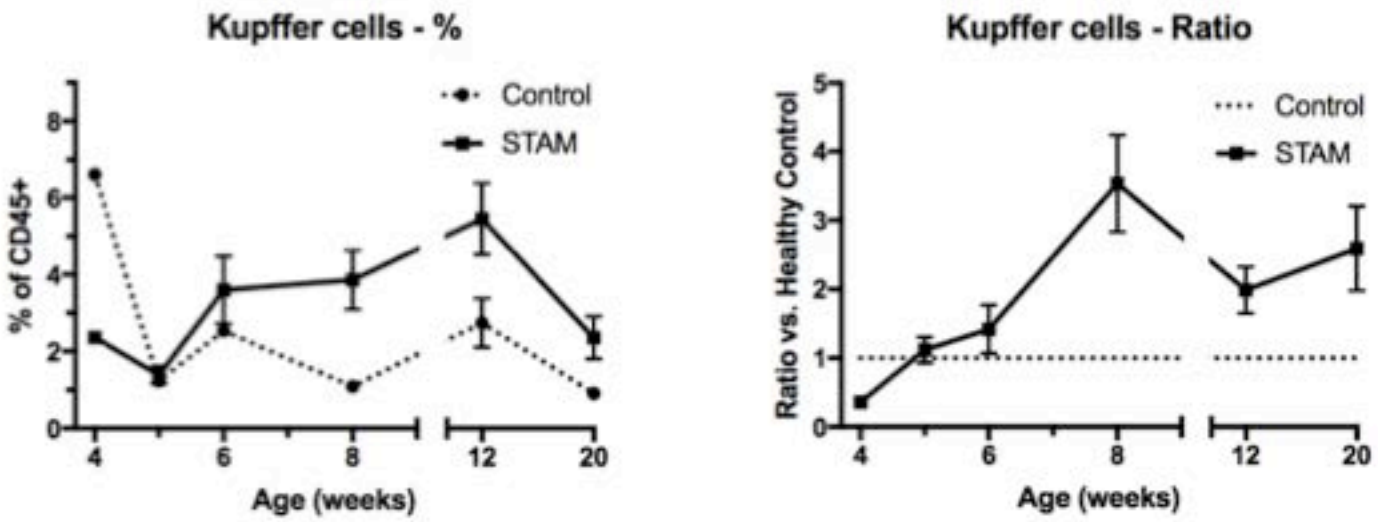
Supporting Figure S2.
In LysM^{eGFP} knockin mice mice, the expression of GFP was 10-fold higher on neutrophils compared to infiltrating macrophages, and 3 log higher on neutrophils compared to Kupffer cells.

Supporting Figure S3.



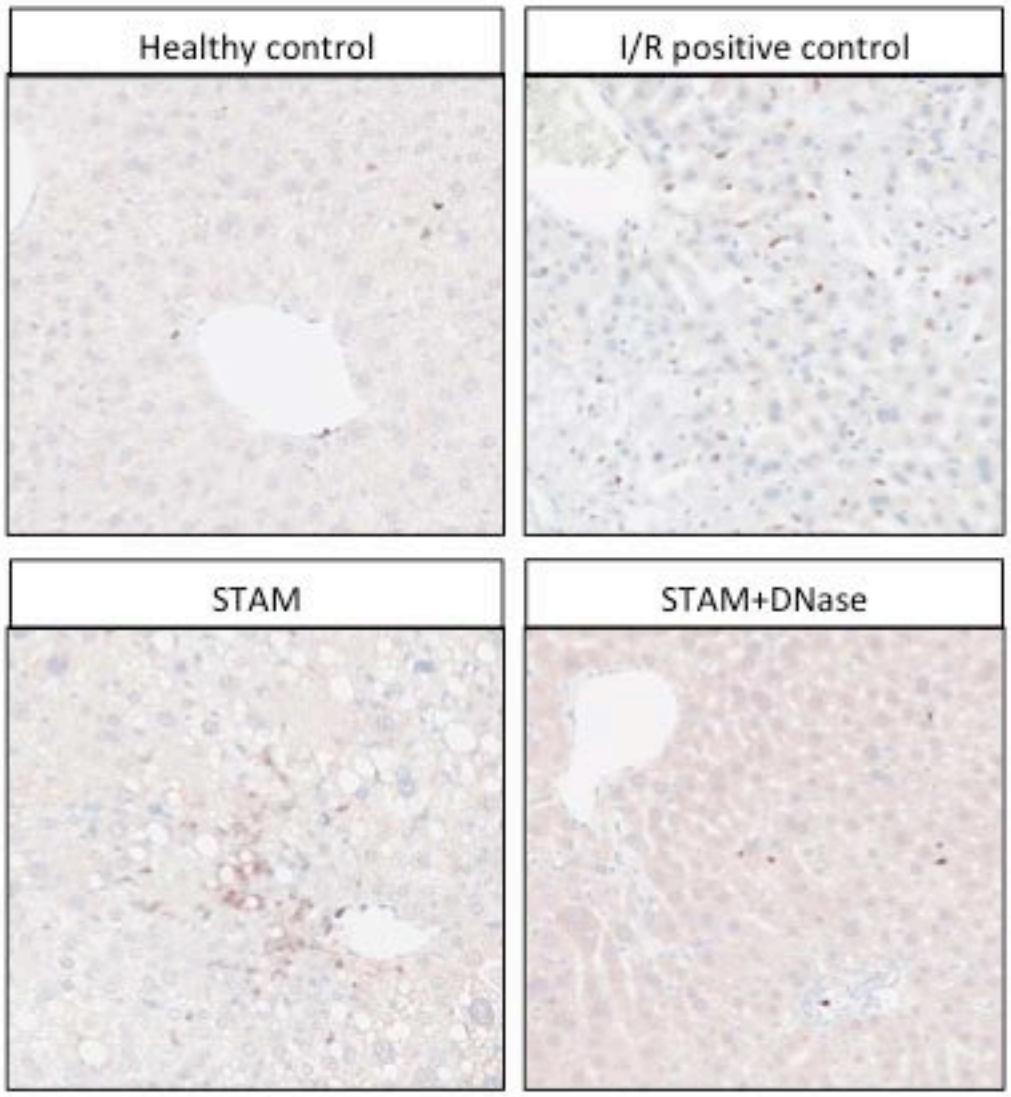
Supporting Figure S3.
RT-PCR for TNF-α mRNA in livers shows a significant increase in STAM mice at the age of 8 weeks (n=8 mice per group).

Supporting Figure S4.



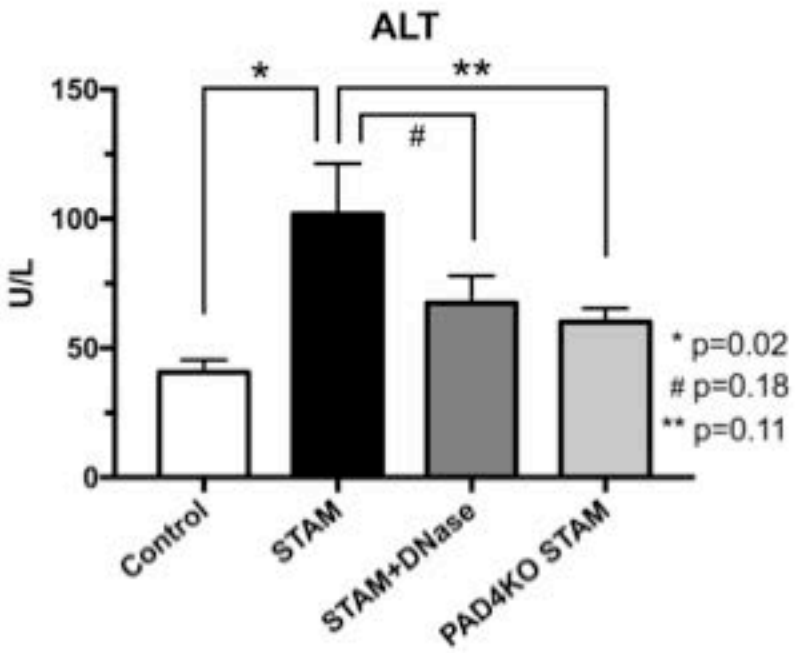
Supporting Figure S4.
Kupffer cells undergo initial depletion, followed by repopulation in STAM mice.

Supporting Figure S5.



Supporting Figure S5.
Healthy control mice demonstrated few neutrophils in the hepatic lobule that spans between portal triads and central hepatic veins. In livers exposed to ischemia and reperfusion injury (positive control), the hepatic lobules were diffusely infiltrated with neutrophils (stained for Ly6G, in red/brown). In STAM livers, focal areas of lobular infiltration were seen with a predominant presence of neutrophils. DNase treatment greatly reduced the presence of neutrophils in the hepatic lobule indicating that DNase potentially reduces the extravasation of neutrophils from the sinusoids and their infiltration into the liver lobule.

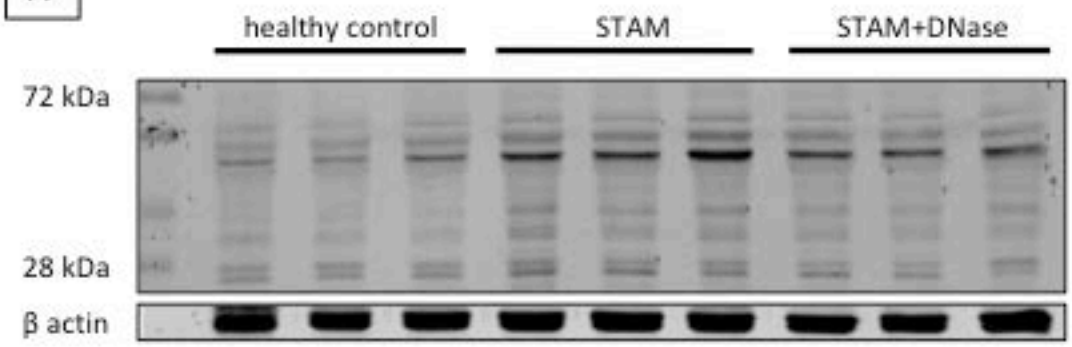
Supporting Figure S6.

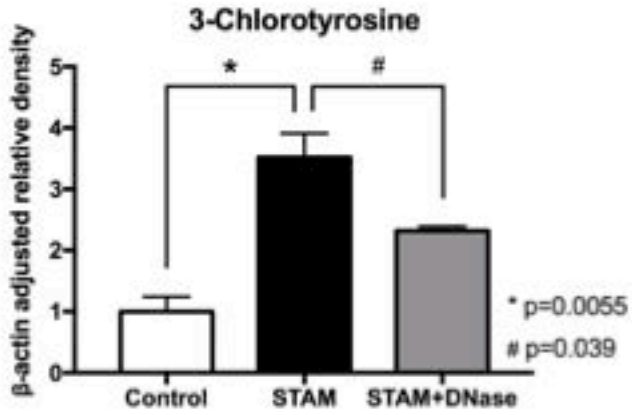


Supporting Figure S6.
Serum ALT levels in STAM mice at 8 weeks (n=9 mice per group) with or without DNase treatment, and in PAD$^{-/-}$ STAM mice.

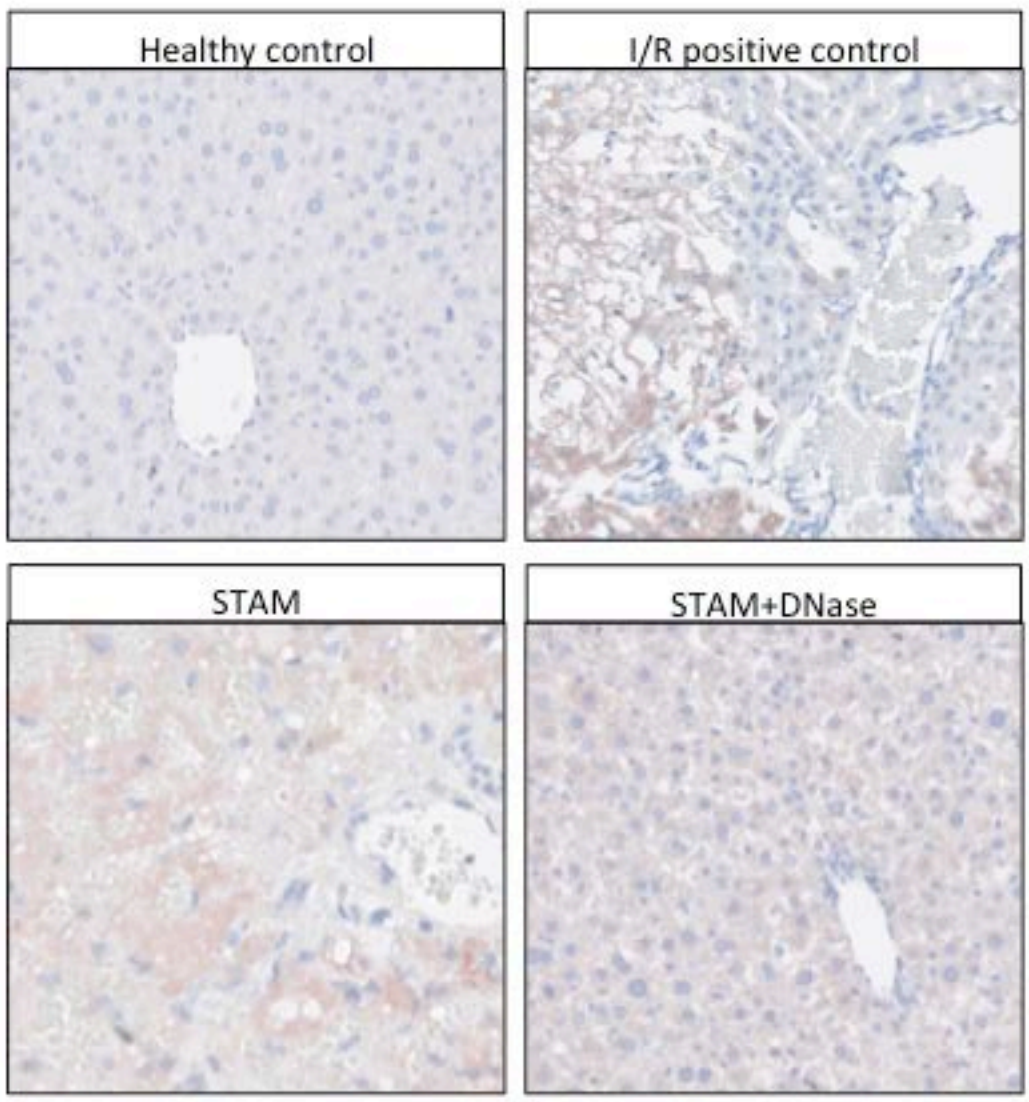
Supporting Figure S7.

A





B



Supporting Figure S7.
(A) Western blot of total liver protein isolates of 8 week old healthy control, STAM, and STAM+DNase mice, incubated with antibody against 3-chlorotyrosine. Between 28 and 72 kDA, multiple bands stained with the anti-3-chlorotyrosine antibody, indicating 3-chlorotyrosine adduct formation to various proteins. Bands in STAM mice were significantly stronger than in healthy control mice. DNase treatment of STAM mice reduced 3-chlorotyrosine adducts. **(B)** 3-chlorotyrosine staining was absent in healthy control liver, and present in necrotic areas as brown/red staining in livers exposed to ischemia and reperfusion injury, in which we have previously shown that neutrophils are strongly stimulated to form NETs (Huang et al. Hepatology. 2015;62:600-14). 3-chlorotyrosine staining was also observed in STAM liver. In contrast, DNase substantially reduced 3-chlorotyrosine staining.