Supplemental Figure S1.

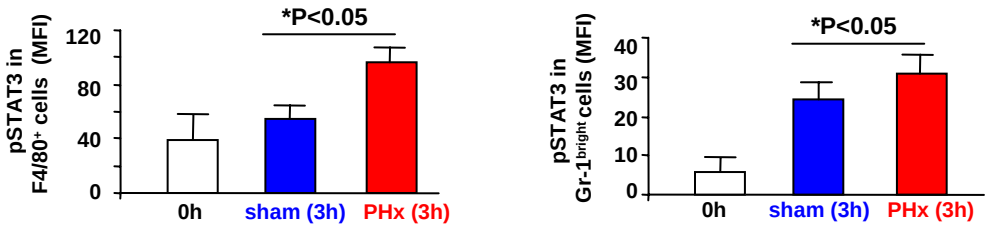

Fig. S1. Mean fluorescence intensity (MFI) of pSTAT3 in F4/80^{+} cells and Gr1^{bright} cells qualified from the figures in Fig. 1E. *Left panel* shows that STAT3 activation (pSTAT3) in F4/80^{+} macrophages from the liver 3 hrs post sham and PHx. *Right panel* shows STAT3 activation in Gr1^{bright} neutrophils from the liver 3 hrs post sham or PHx. Values represent means ± SD (n=4).

Supplemental Figure S2.

Fig. S2a

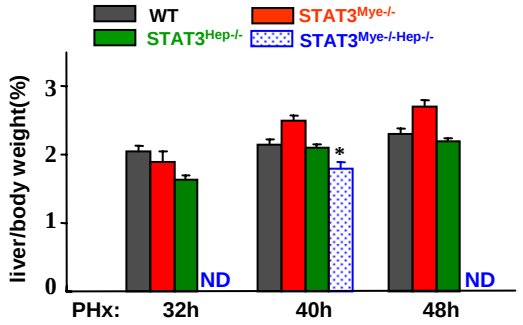

Fig. S2a. The ratio of liver/body weight after PHx. ND. Not done in STAT3$^{Mye-/-Hep-/-}$ group.

Fig. S2b

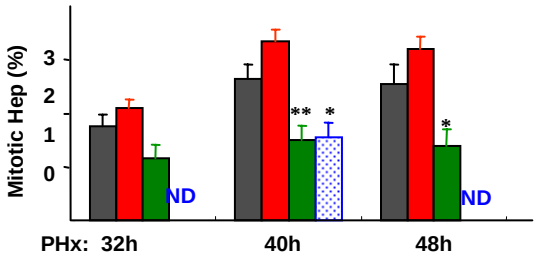

Fig. S2b. The percentage of mitotic Hepatocytes post PHx

Fig. S2c

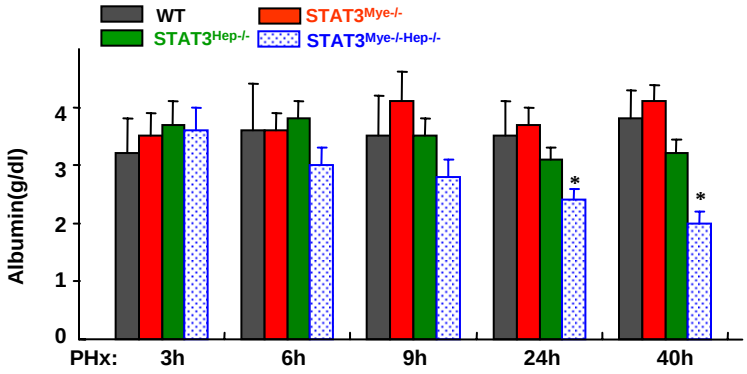

Fig. S2c: Serum levels of albumin post PHx

Supplemental Figure S3.

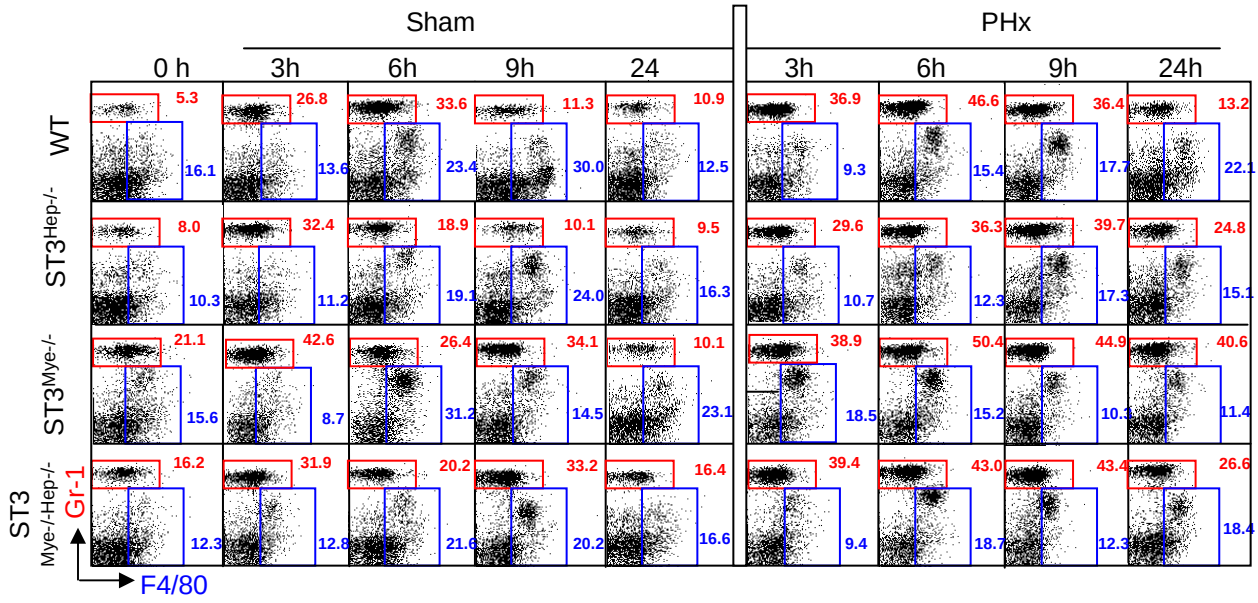

Fig. S3: Depletion of STAT3 in myeloid cells enhances inflammation after PHx. Representative flow cytometric analyses of liver leukocytes with anti-Gr1 and anti-F4/80 antibodies post sham or PHx. Gr1high cells in red gates represent neutrophils. F4/80+ cells represent macrophages.

Supplemental Figure S4.

Fig. S4a

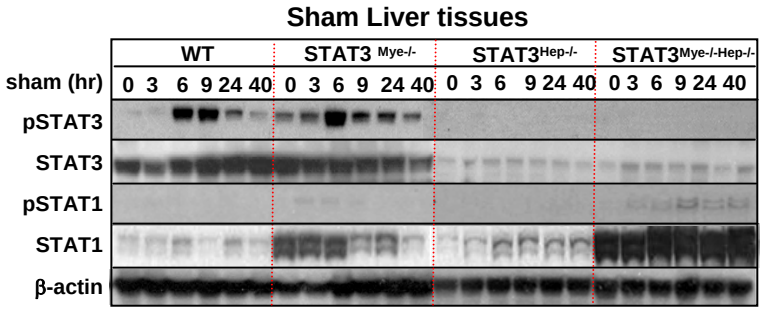

Fig. S4a. Activation of STAT1 and STAT3 in the liver from sham groups.

Fig. S4b

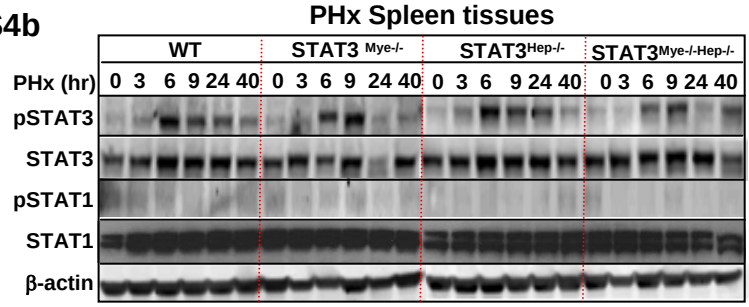

Fig. S4b. Activation of STAT1 and STAT3 in the spleen tissues from partially hepateomized mice.

Supplemental Figure S5.

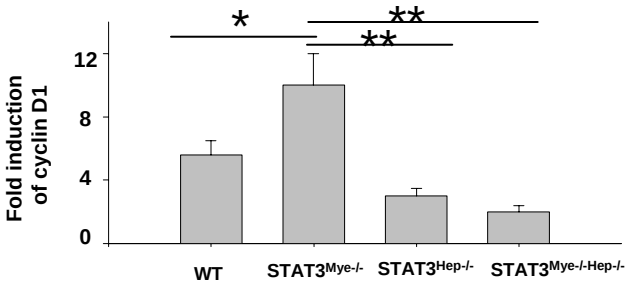

Fig. S5: Cycline D protein levels 40 h post PHx from 3 independent experiments were Quantified. Values represent means ± SD. $P<0.05$, $^{**}P<0.01$

Supporting Materials

Interplay of hepatic and myeloid STAT3 in facilitating liver regeneration via tempering innate immunity

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Materials and Methods:

Materials. Anti-STAT3, anti-phospho-STAT3 (Tyr705), anti-pSTAT1, anti-STAT1, anti-cyclin D antibodies were purchased from Cell Signaling Technology (Beverly, MA).

Isolation of liver leukocytes. Liver leukocytes were isolated as described previously [1]. Briefly, mouse livers were pressed through a 200-gauge stainless steel mesh. The liver cell suspension was suspended in RPMI 1640 media (Invitrogen, Carlsbad, CA) and centrifuged at 50×*g* for 5 minutes. Supernatants containing leukocytes were collected, washed in PBS, and resuspended in 35% Percoll (Sigma) containing 100 U/mL heparin. The cell suspension was centrifuged for 15 minutes at 500x*g*. The cell pellet containing leukocytes was collected and re-suspended in 5 mL red blood cell lysis solution (155 mmol/L NH_4CL, 10 mmol/L $KHCO_3$, 1 mmol/L EDTA, 170 mmol/L Tris, pH 7.3). After incubation for 5 minutes on ice, cells were washed twice in RPMI 1640 containing 5% fetal bovine serum.

Flow cytometry analysis of neutrophils and macrophages in the liver. Neutrophils and macrophages in the livers were determined by anti-Gr1 and anti-F4/80 antibodies (PharMingen, San Diego, CA) through a fluorescent-activated

cell sorter (FACScalibur, Becton Dickinson, Mountain View, CA). $Gr1^{high}$ cells mainly represent neutrophils, where $Gr1^{intermediate}$ cells represent monocytes and eosinophils [2].

Determination of the rate of liver regeneration. Liver regeneration rate was determined by the BrdU incorporation assay. Briefly, partially hepatectomized mice were injected intraperitoneally with BrdU (50 μg/g body weight) at various time points post PHx. Mice were euthanized 2 hours later and the livers were harvested and fixed in 10% neutral buffered formalin for 24 hours. Fixed livers were embedded in paraffin, cut into 5-μm tissue sections by a microtome, and then adhered to poly-L-lysine-coated glass slides. The slides were dried overnight at 37°C. BrdU incorporation was visualized immunohistochemically, using a BrdU immunostaining kit (BD Biosciences) according to the manufacturer's instructions. BrdU-labeled hepatocytes were quantified by counting positively stained hepatocyte nuclei in 3 to 6 low-power (100×) microscope fields, and the mean was calculated.

Histology and immunohistochemistry. Formalin-fixed liver samples were processed, and paraffin sections of 5-μm thickness were stained with hematoxylin and eosin (H&E). Inflammatory foci were counted in 10 randomly selected fields (X100 magnification).

TUNEL assay. Apoptotic cells in sections of mouse livers were detected using the ApopTag Peroxidase In Situ Apoptosis Detection Kit (Chemicon International Inc., Temecula, CA) according to the manufacturer's instructions. Numbers of $TUNEL^{+}$ hepatocytes were determined by counting positively stained hepatocyte nuclei in 5 high-power (200×) microscope fields, and the mean was calculated.

Serum cytokine levels. Serum cytokine levels were measured using cytometry bead array (BD Bioscience) according to the manufacturer's protocol.

Serum albumin levels. Serum levels of albumin were measured using a kit from Drew scientific Ltd (Barrow-in-Furness, UK).

Western blotting. Western blot analyses were performed with proteins from liver homogenates (80 μg) using various primary antibodies. Briefly, 50-80 μg of proteins were loaded. Immunoreactive bands were visualized on nitrocellulose membranes using alkaline-phosphotase-linked anti-mouse or rabbit antibody and the ECF detection system with a PhosphorImager (GE Healthcare, Piscataway, NJ).

RT-PCR: RT-PCR was used to determine the expression of SOCS1 and SOCS3 in the liver. The primer sequences include SOCS1 (forward): GAA GCC ATC TTC ACG CTG, (reverse): ACA CTC ACT TCC GCA CCT TC (PCR product 210 bp); SOCS3 (forward): GGG TGG CAA AGA AAA GGA G, (reverse) GTT GAG CGT CAA GAC CCA GT (PCR product 450bp).

Statistical Analysis. Data are expressed as means ± SEM. To compare values obtained from 2 groups, the Student *t* test was performed. To compare values obtained from three or more groups, 1-factor analysis of variance (ANOVA) was used, followed by Tukey's post hoc test. Statistical significance was taken at the $P < 0.05$ level.

References:

1. Liu ZX, Han D, Gunawan B, Kaplowitz N. Neutrophil depletion protects against murine acetaminophen hepatotoxicity. Hepatology 2006;43:1220-1230.
2. Daley JM, Thomay AA, Connolly MD, Reichner JS, Albina JE. Use of Ly6G-specific monoclonal antibody to deplete neutrophils in mice. J Leukoc Biol 2008;83:64-70.
3. Fujita J, Marino MW, Wada H, Jungbluth AA, Mackrell PJ, Rivadeneira DE, et al. Effect of TNF gene depletion on liver regeneration after partial hepatectomy in mice. Surgery 2001;129:48-54.

Supplemental TABEL S1:

Serum levels of cytokines after PHx. Values represent means ± SEM (ng/ml) (n=3-6)

	PHx 0h	PHx 1h	PHx 3h	PHx 6h	PHx 9h	PHx 24h	PHx 40h
IL-6	0.0±0.0	23.6±7.8	825.0±26.5	494.0±68.5	113.3±38.1	28.3±10.8	27.0±7.9
TNF-α	5.8±2.9	7.8±2.2	8.8±1.2	6.4±0.4	4.4±1.2	5.7±0.7	6.2±0.7
IFN-γ	0.3±0.3	0.4±0.4	1.2±0.1	0.5±0.4	0±0	0.6±0.4	0.6±0.4
IL-12p70	7.2±2.7	8.3±1.1	8.9±1.5	5.5±2.8	2.8±1.6	6.2±1.2	5.2±0.6
IL-10	0.0±0.0	0.0±0.0	0.0±0.0	4.0±0.6	0.0±0.0	0.0±0.0	0.0±0.0
IL-2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
IL-4	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

After PHx, serum levels of IL-6 were significantly elevated, while serum levels of TNF-α were slightly elevated, which is consistent with earlier findings that the increase of serum TFN-α has not been universally observed in mice.[3]