

Dipeptidyl Peptidase-4 (CD26/DPP-4) is a Pro-recovery Mediator During Acute Hepatotoxic Damage And Mirrors Severe Shifts in Kupffer Cells.

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SUPPLEMENTARY DATA

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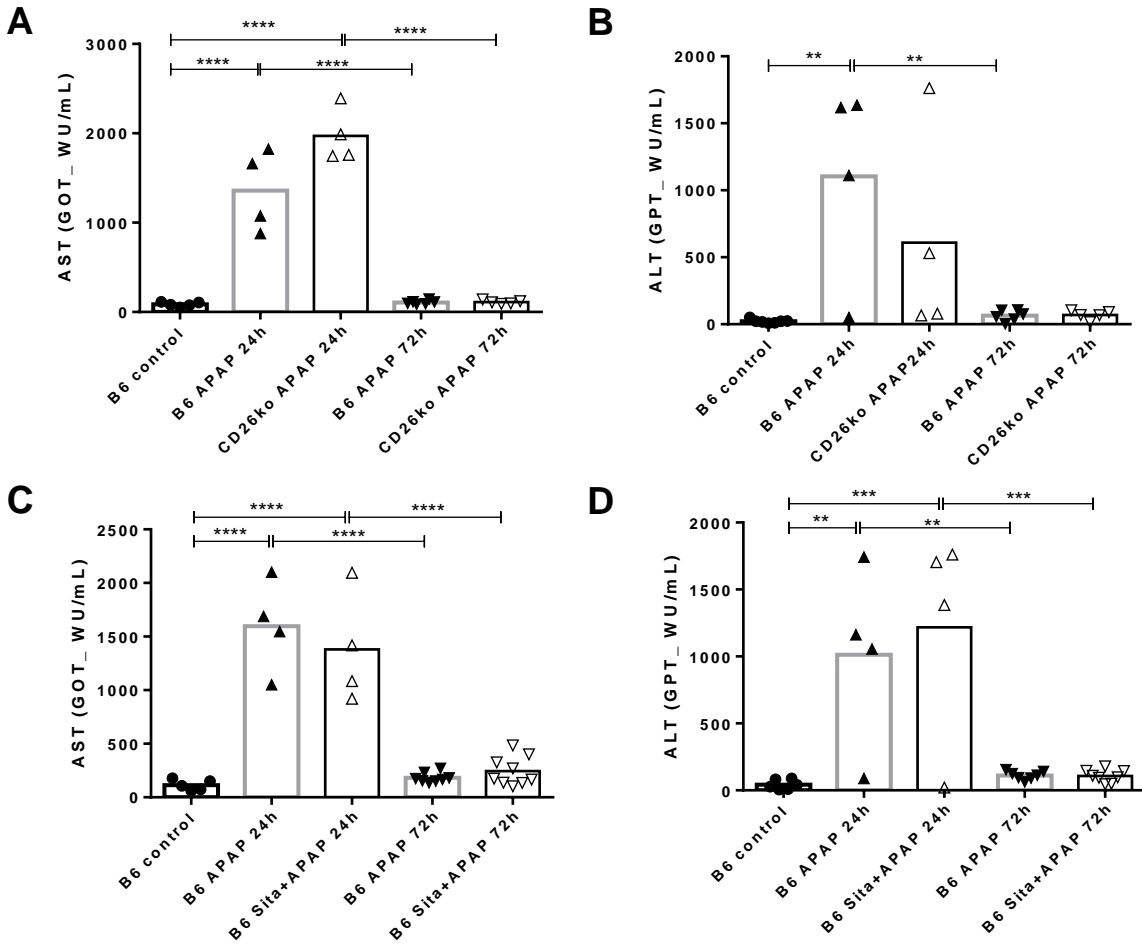
SUPPLEMENTARY MATERIAL AND METHODS

Serum levels of AST and ALT

The serum levels of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were determined by a colorimetric enzymatic assay using the GOT-GPT kit (Spinreact S.A., Spain) according to manufacture instructions.

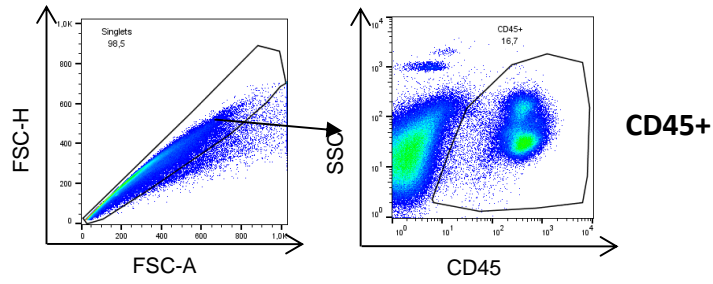
Flow cytometry

Non-parenchymal cells (NPCs) from liver were prepared as described and single cell suspensions were stained according to standard procedures. Non-specific binding to FcγIII, FcγII, and FcγI receptors was prevented by incubating the cells with unlabeled anti-mouse-Fc-block/CD16/32 (clone 2.4G2, BD Pharmingen). Liver Kupffer cells were identified using fluorescein or allophycocyanin anti-mouse F4/80 Ab (clone BM8, Biolegend). Fluorescein or brilliant violet 785 labelled, anti-mouse CD11b/Mac-1 (clone M1/70, Biolegend), efluor450 labelled anti-mouse LY6C (clone HK1.4, eBiosciences), allophycocyanin or phycoerythrin labelled anti-mouse CD45 (Clone 30-F11, Biolegend) and phycoerythrin labelled anti-mouse Tim4 (clone F31-5G3) were further used to characterize liver macrophages. Traceable latex beads (Beckman coulter Inc,) were added for counting cells. Stained cell suspensions were analyzed by flow cytometry (LSR Fortessa X20™ or CyAn ADP) and the data acquired with DIVA software (Becton Dickinson). Analysis was performed with FlowJo software (TreeStar Inc.).

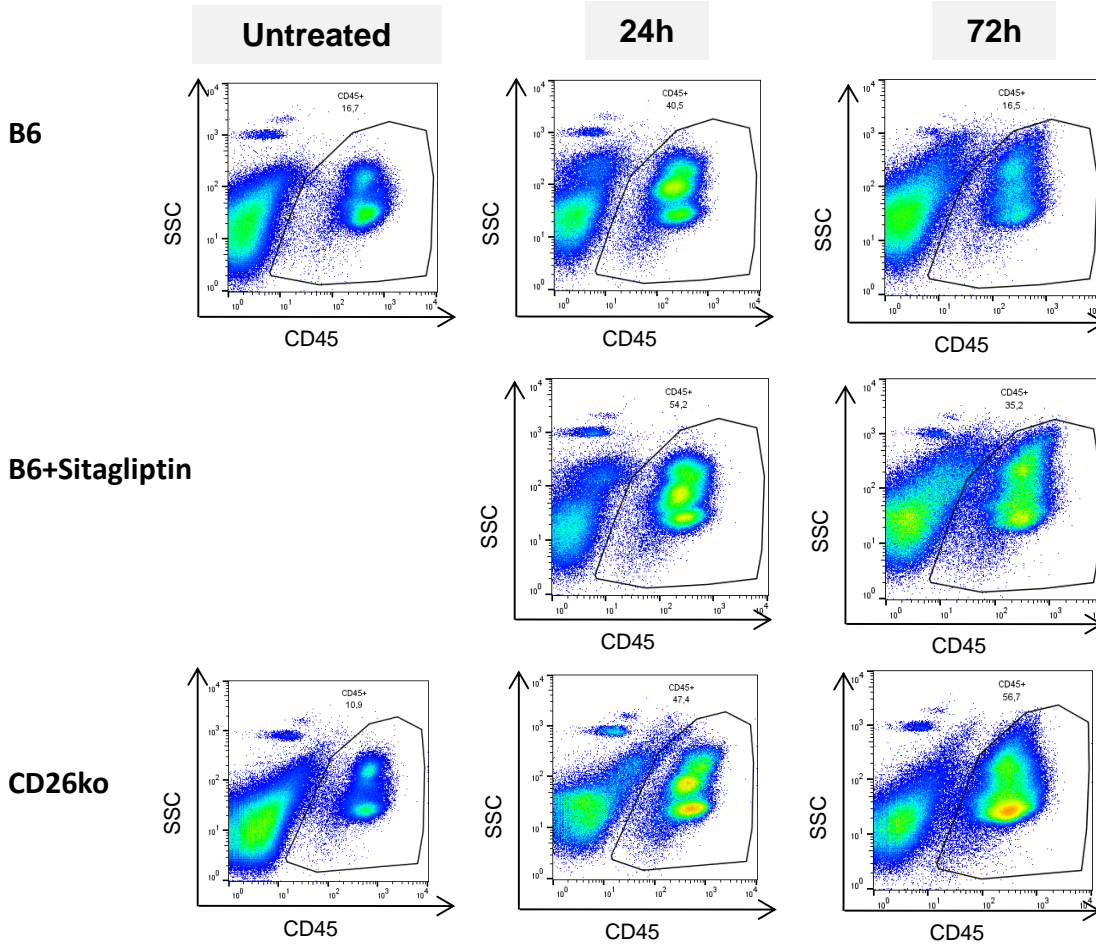


Supplementary Fig. 1. Serum levels of AST and ALT after APAP injection. The serum levels of Aspartate amino transferase (AST) and Alanine aminotransferase (ALT) were increased at 24h and reduced at 72h post acetaminophen (APAP 300mg/kg) i.p. injection in C57BL/6 (B6) and CD26ko mice (A and B) and in untreated and Sitagliptin treated (100mg/kg) B6 mice (C and D). Bars indicate mean values; **p<0.01, ***p<0.001 and ****p<0.0001 in one-way ANOVA using Tukey’s correction. Plots depict cumulative data from two independent experiments, n=4-9 mice/group.

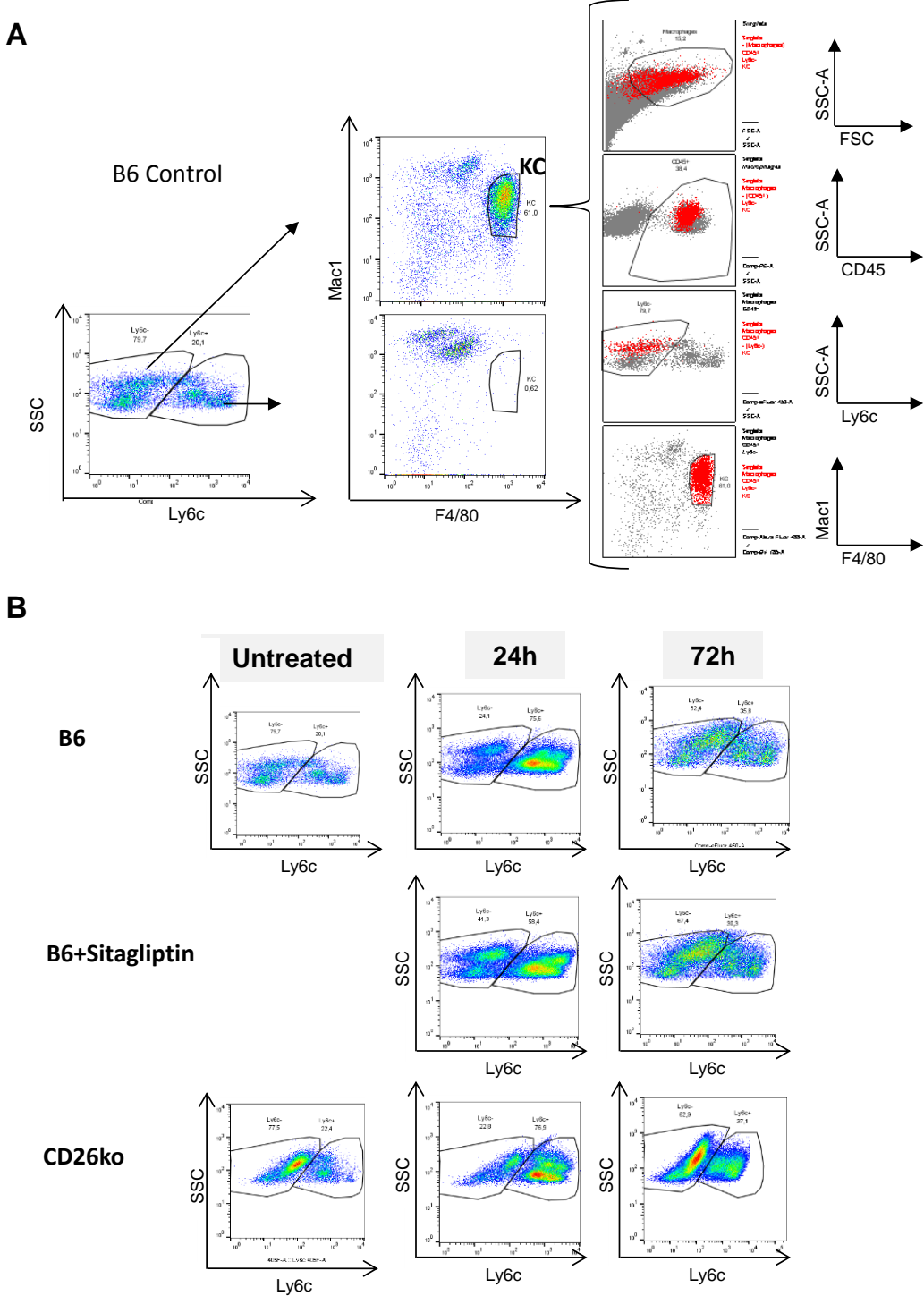
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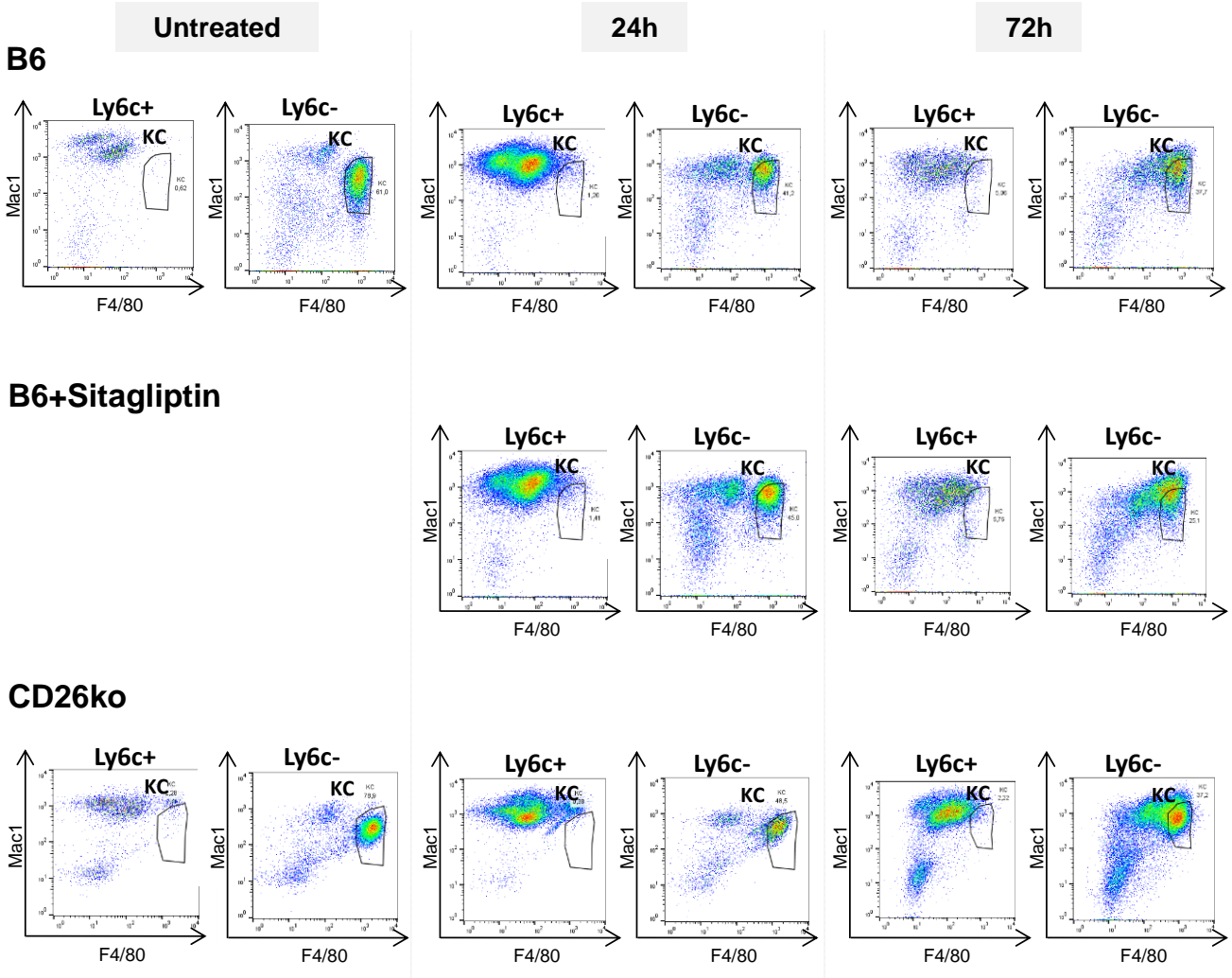
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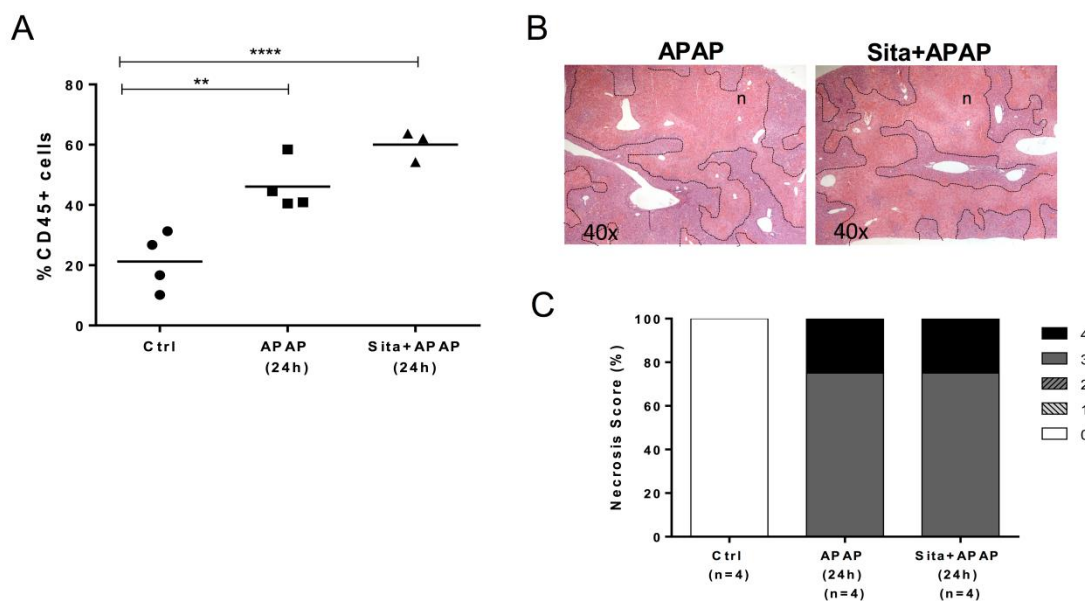
Supplementary Fig 2. Gating strategy for flow-cytometry analysis of liver CD45+ cells during liver injury. (A) Total CD45+ cells in liver non-parenchymal cell preparations were identified within single cells upon doublets exclusion. (B) Representative plots of total liver CD45+ cells in C57BL/6 (B6), B6 receiving daily gavages of Sitagliptin (100mg/Kg) and CD26ko mice, either untreated or 24h and 72h hours after intra-peritoneal injection with acetaminophen (APAP, 300mg/Kg).



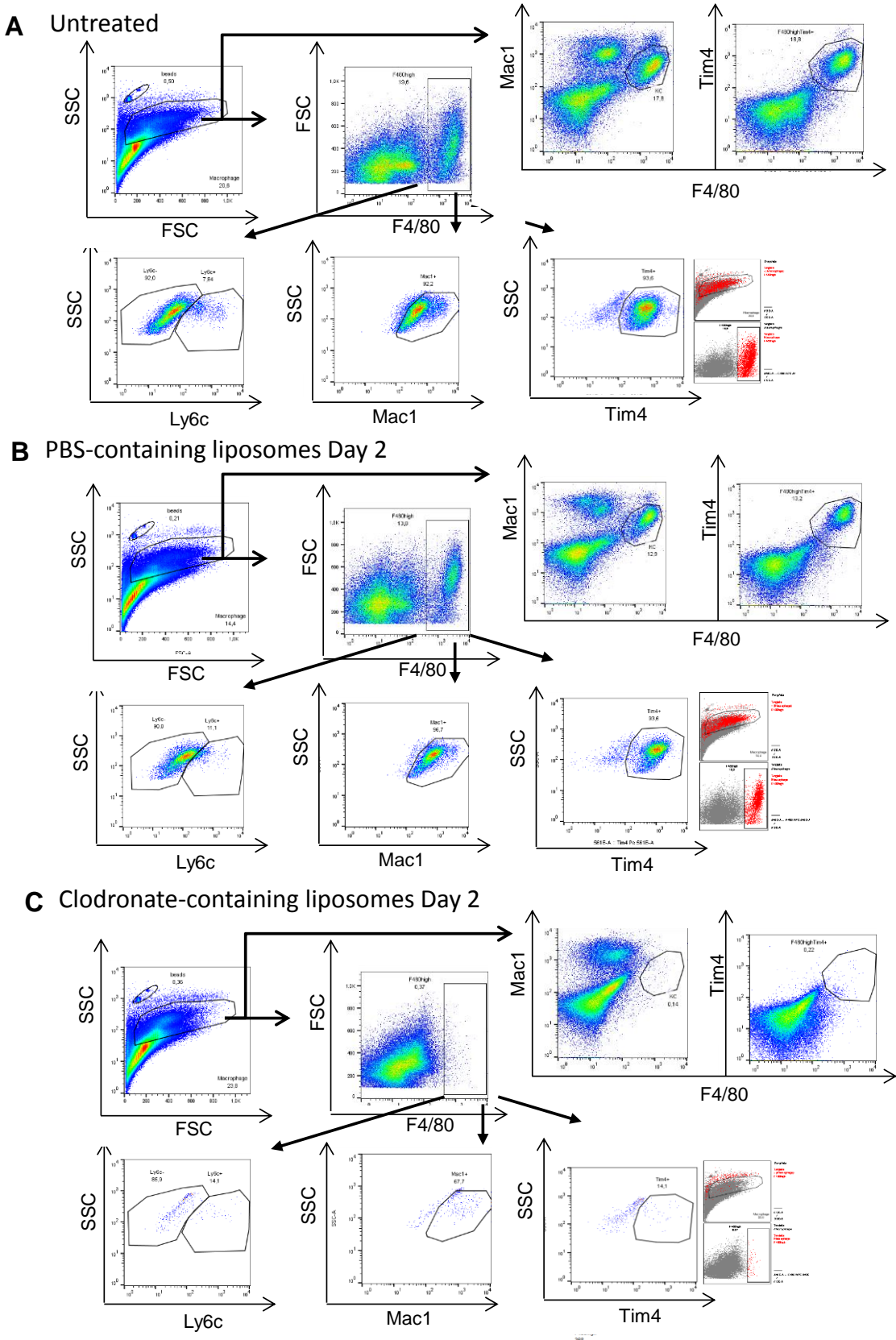
Supplementary Fig 3. Gating strategy for flow-cytometry analysis of Kupffer cells during liver injury. (A) Back-gating analysis of Kupffer cells (KC, in red), identified as Mac1+F4/80high cells validated the gating strategy used for analysis of macrophages in liver non-parenchymal cells preparations. (B) Representative plots of Ly6C- and Ly6c+ cells in C57BL/6 (B6), B6 receiving daily gavages of Sitagliptin (100mg/Kg) and CD26ko mice, either untreated or 24h and 72h hours after intra-peritoneal injection with acetaminophen (APAP, 300mg/Kg).



Supplementary Fig 4. Liver macrophages during acute liver injury. Plots represent Kupffer cells (KC) identified as Mac1+F4/80high cells within Ly6c+ or Ly6c- cells among CD45+ liver non-parenchymal cells, prepared from C57BL/6 (B6), B6 receiving daily gavages of Sitagliptin (100mg/Kg) and CD26ko mice, either untreated or 24h and 72h hours after intra-peritoneal injection of acetaminophen (APAP, 300mg/Kg).



Supplementary Fig. 5. Pharmacological inhibition of CD26/DPP4 enzymatic activity during acute liver injury does not prevent hematopoietic cell recruitment and development of necrosis. Sitagliptin (100mg/Kg, Sita+APAP group) or water (APAP group) was administered by daily gavage to C57BL/6 (B6) mice before and after acetaminophen (APAP) injection (see fig 3 A). Control B6 mice (Ctrl group) received water gavage and PBS injections. Livers were analyzed 24h after APAP and compared with controls. (A) Proportion of recruited hematopoietic CD45+ cells measured by flow cytometry. (B) Necrosis was examined on liver histological sections and (C) scored from 0 to 4 according to location and extension of necrotic lesions. Scale bar=100µm. Horizontal bars indicate mean values ** $p < 0.01$ and *** $p < 0.001$ in one-way ANOVA using Tukey's correction. $n = 3-4$ per group.



Supplementary Fig 6. Depletion of Kupffer cells by clodronate-liposome injection. F4/80high cells in liver non-parenchymal cells preparations from untreated (A) and PBS-liposome treated C57BL/6 mice (B) highly correlate with a typical Kupffer cell phenotype

(Ly6c-, Mac1+ and Tim4+). Back-gating of Tim4+ cells identifies the morphological gate used in analysis of macrophage populations (in red). (C) Similar analysis at two days after i.v. injection with clodronate-containing liposomes evidences selective depletion of Kupffer cells, presumably dependent on phagocytic activity.