Supplemental data

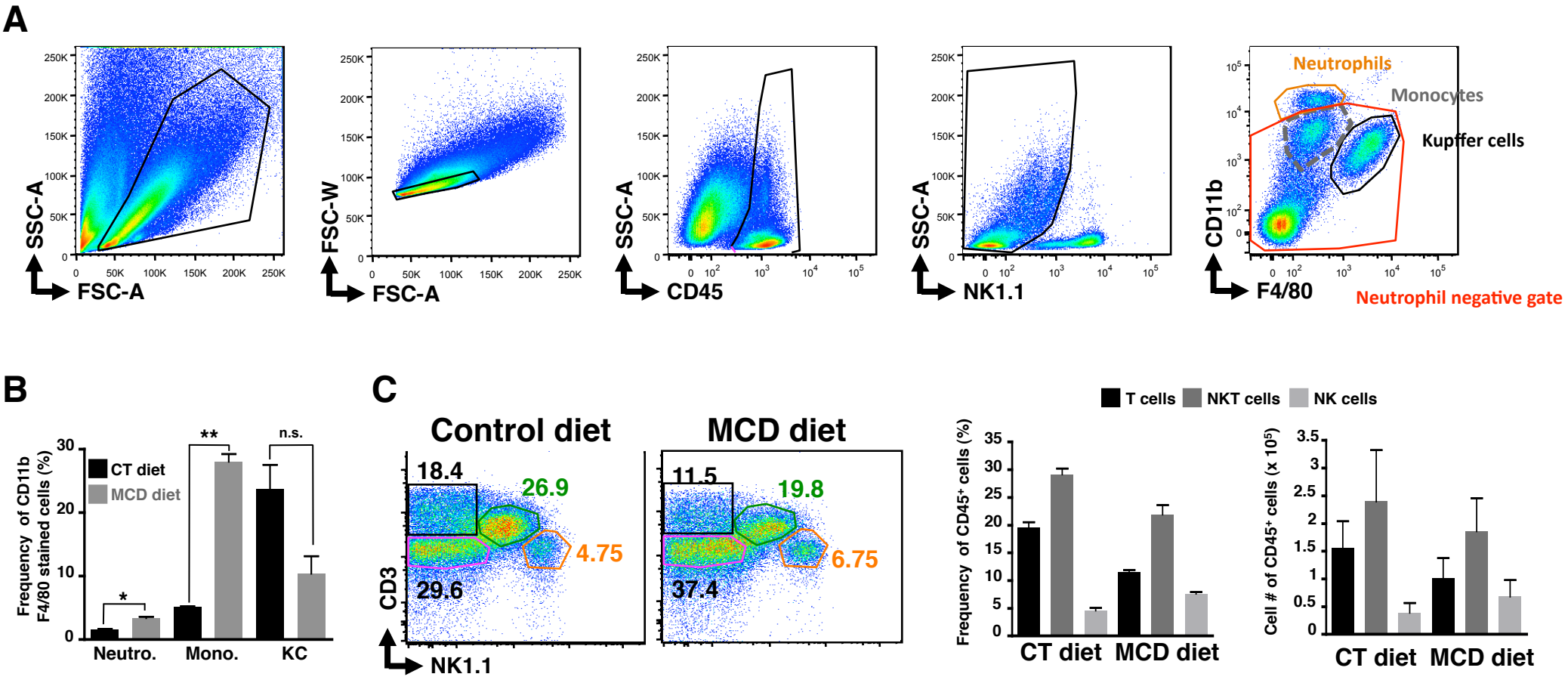
Supplemental Figure legends

Supplemental Figure 1. Monocytes are major sources of infiltrated leukocytes in early NASH. (A) Gating strategy of flow cytometry. (B) Frequency of neutrophils ($CD11b^{hi}F4/80^{-}$), monocytes ($CD11b^{int}F4/80^{low}$) and Kupffer cells ($CD11b^{+}F4/80^{hi}$) are shown in $CD45^{+}NK1.1^{-}$-gated whole liver cells isolated from CT diet- and MCD diet-fed mice. Data are representative of at least four independent experiments with a total of n=8 mice per condition. (C) No change in frequency and cell numbers of T, NK, and NKT cells in C57BL/6 mice fed with MCD diet compared to ones fed with control diet. FACS plots, frequency and cell numbers of T cells ($CD3^{+}NK1.1^{-}$), NK cells ($CD3^{-}NK1.1^{+}$), and NKT cells ($CD3^{+}NK1.1^{+}$) are shown in $CD45^{+}$ total liver cells isolated from mice fed with CT and MCD diet for 10 days. Data are representative of three independent experiments with n=2 per condition. *$p<0.05$ and ** $p<0.005$ as determined by two-tailed *t* test.

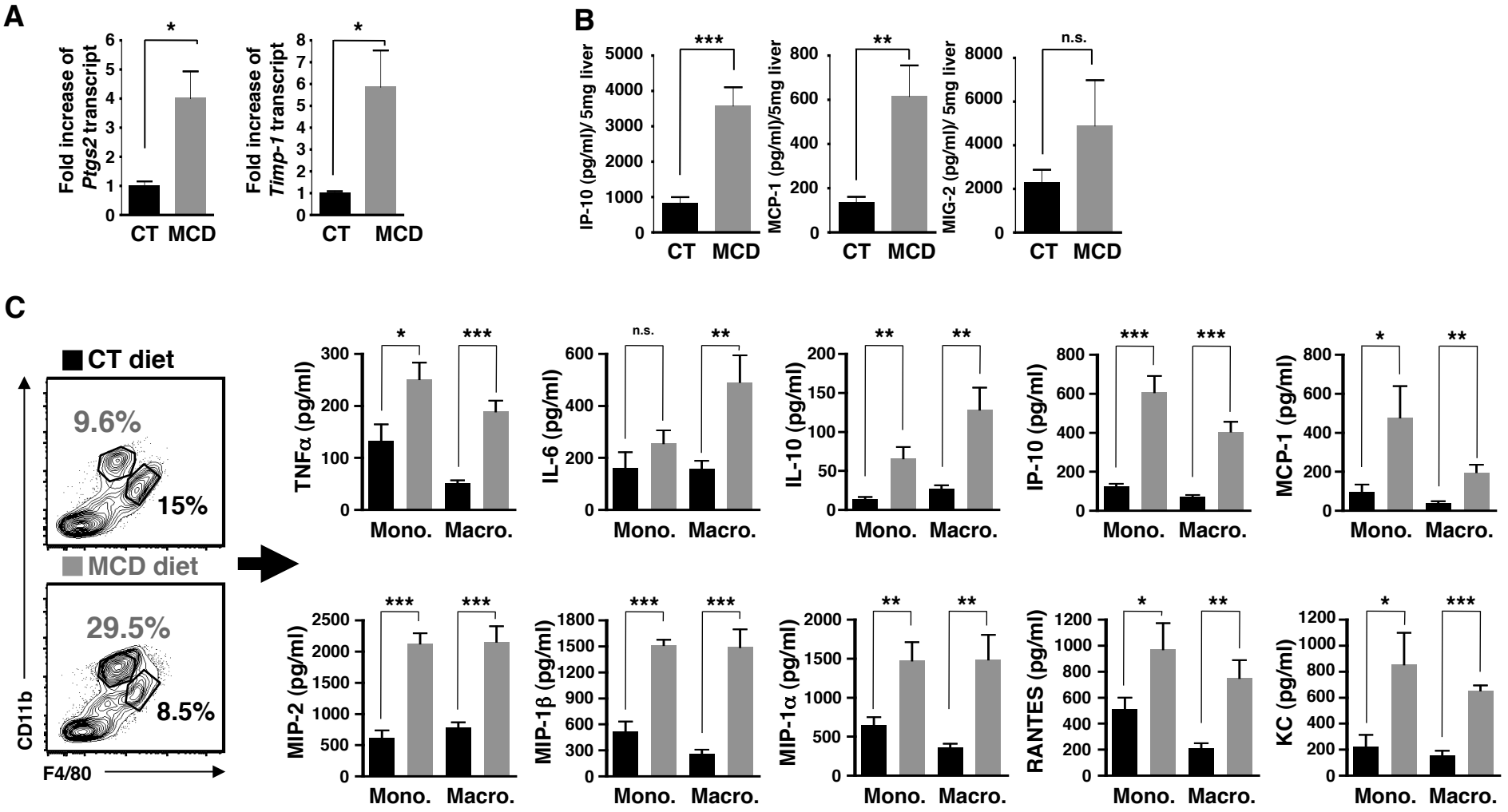
Supplemental Figure 2. Increase of hepatic inflammation after 10 days of MCD diet. (A) Hepatic *Ptgs2* (encodes for COX-2*)* and *Timp-1* (early marker for fibrosis) transcript expressions were determined by qPCR from liver samples of CT diet- and MCD diet-fed mice. The RNA level is expressed as fold increase compared with control diet. (B) Dosage of chemokine/cytokine from whole liver cell lysates of CT diet- and MCD diet-fed mice was determined by luminex assays. These results are compiling data from three independent experiments with a total of n=3 to 7 mice per condition. (C) Multiplex luminex assays for chemokines/cytokines were performed on the culture supernatants of electronically sorted monocytes ($CD45^{+}NK1.1^{-}CD11b^{int}F4/80^{low}$) and Kupffer cells ($CD45^{+}NK1.1^{-}CD11b^{low}F4/80^{hi}$) purified from mononuclear liver cells extracted from CT diet and MCD diet fed mice. Compiled data from 3 independent experiments with a total of n=6 to 8 mice. Statistical analysis was determined two-tailed *t* test (*$p<0.05$; **$p<0.005$ and *** $p<0.0005$).

Supplemental Figure 3. Clodronate-liposomes treatment did not deplete bone marrow and blood monocytes. CT and MCD-fed C57BL/6 mice were treated once either with control liposomes (CT Lipo.) or clodronate-containing liposomes (clod. Lipo.) at 2 days before (A) or 5 days after (B) starting feeding. The frequency of $Ly6C^{hi}$ and $Ly6C^{low}$ from bone marrow (BM) and blood are assessed by flux cytometry by staining of cells with CD45, NK1.1, Ly6G, CD11b, Ly6C, and F4/80.

Tosello-Trampont *et al.*, Supplemental Figure 1



Tosello-Trampont *et al.*, Supplemental Figure 2



Tosello-Trampont *et al.*, Supplemental Figure 3