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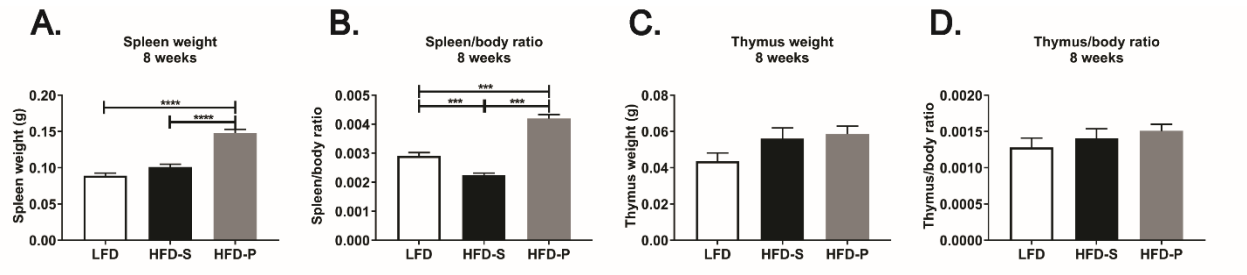
Dietary polyunsaturated fatty acids promote neutrophil accumulation in spleen by altering chemotaxis and delaying cell death

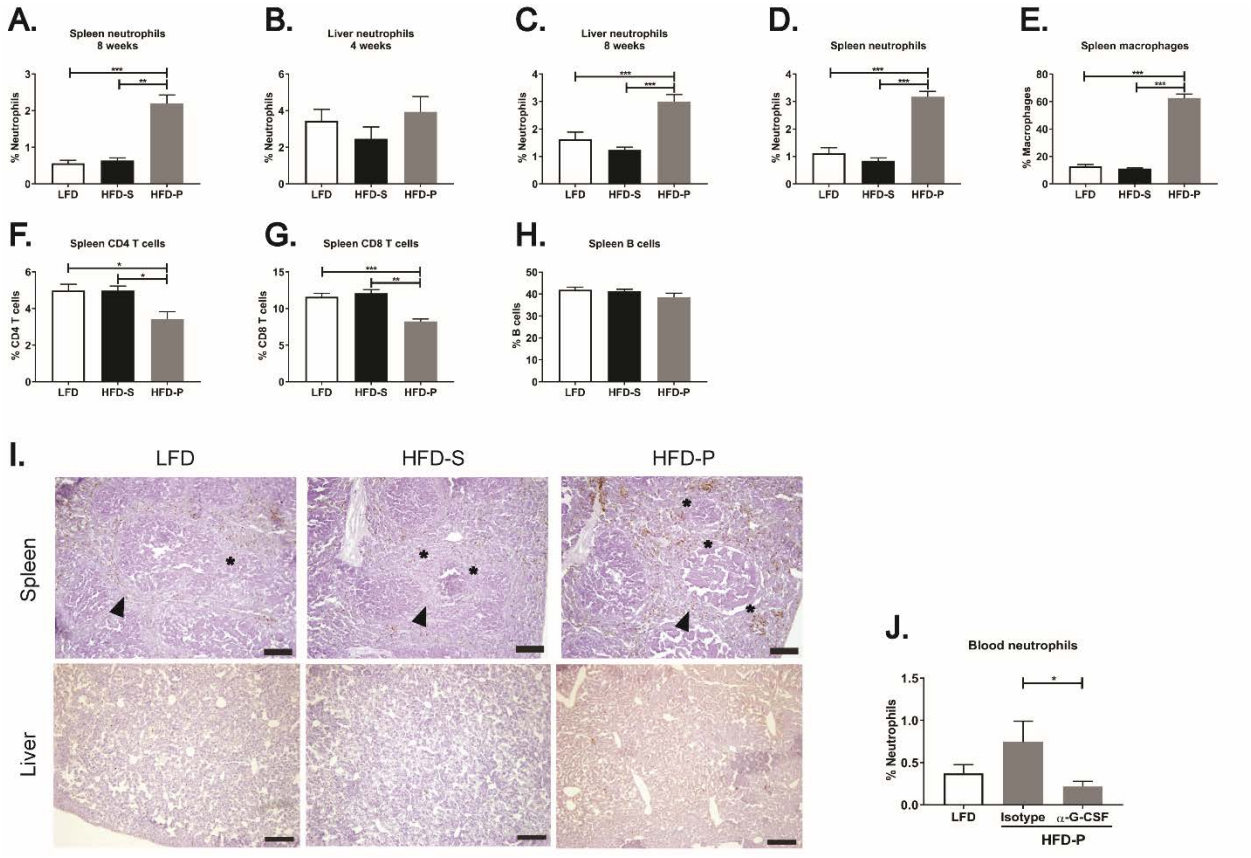
Supplemental figures

Supplemental Figure 1



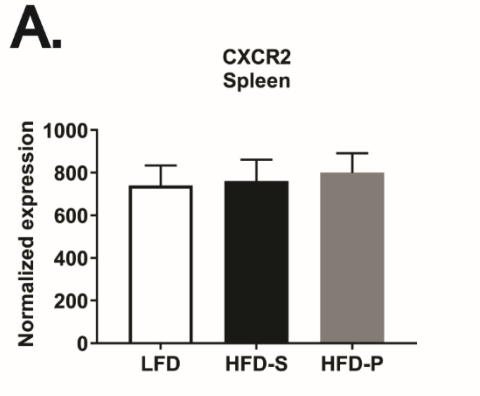
Supplementary figure 1. High fat diet rich in polyunsaturated fatty acids (HFD-P) increases spleen size. (A) Spleen weight, (B) spleen weight normalized to body weight, (C) thymus weight and (D) thymus weight normalized to body weight in mice fed LFD, HFD-S and HFD-P for 8 weeks. n=18 mice per group for A and B; n=8 mice per group for C and D. Data shown as mean + SEM. *** $p \leq 0.001$, **** $p \leq 0.0001$.

Supplemental Figure 2



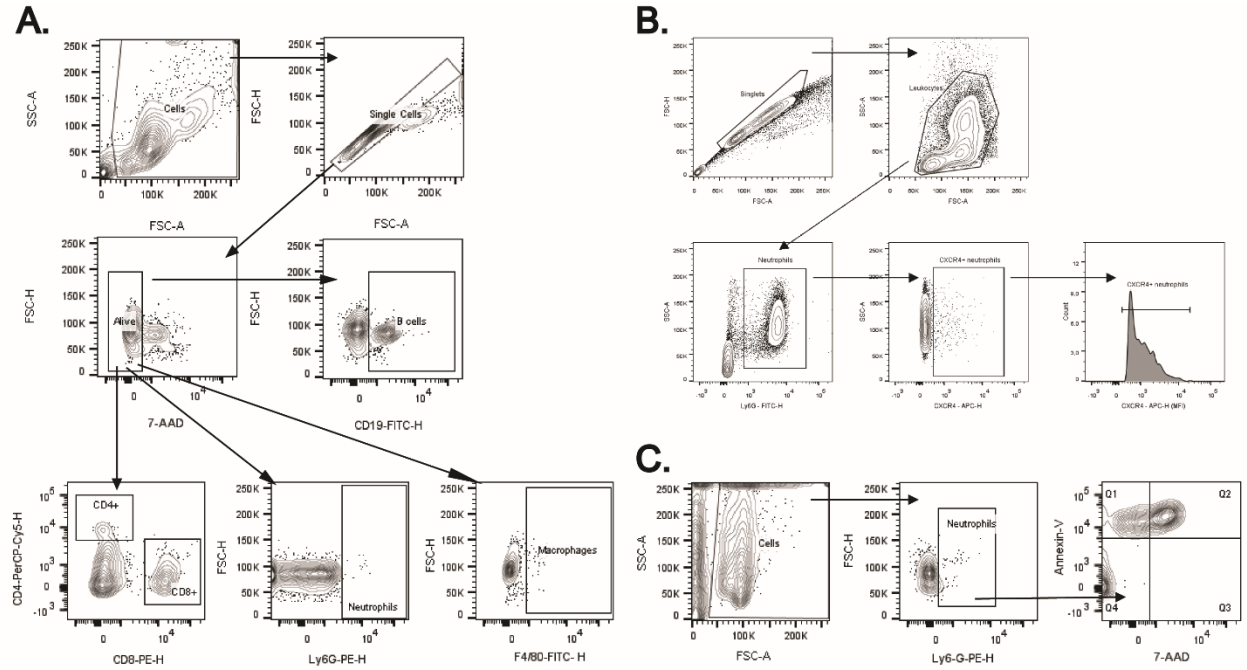
Supplementary figure 2. (A) Quantification of neutrophils in spleen of mice fed the different diets for 8 weeks. n=8. Quantification of neutrophils in liver of mice fed the different diets for (B) 4 weeks and (C) 8 weeks. n=5 for (B) and n=6 for (C). Quantification of the percentage of (D) neutrophils, (E) macrophages, (F) CD4 T cells, (G) CD8 T cells and (H) B cells in the spleen of mice fed with the different diets for 4 weeks. n=7. (I) Immunoperxodase staining of neutrophils in the spleen and liver of mice fed the different diets for 8 weeks. Scale bars indicate 100µm. Images representative of three independent experiments. Arrows point at the red pulp and asterisks mark the peri-marginal zone in the spleen. (J) Frequency of blood neutrophils in mice fed with HFD-P upon treatment with anti-G-CSF antibody or isotype control. n=10 mice per group. Data shown as mean + SEM. * p≤0.05, ** p≤0.01, *** p≤0.001, **** p≤0.0001.

Supplemental figure 3



Supplementary figure 3. HFD-P induced secretion of chemoattractant molecules to recruit neutrophils into the spleen. (A) Gene expression of CXCR2 in the spleen of mice fed LFD, HFD-S and HFD-P for 8 weeks, normalized to actin expression. n=4. Data shown as mean + SEM.

Supplemental figure 4



Supplementary figure 4. Gating strategies for flow cytometry experiments. (A) Gating strategy for the analysis of immune cells in the spleen. (B) Gating strategy for the analysis of CXCR4 expression in bone marrow neutrophils. The same strategy was used for the analysis of the CXCR2 expression in bone marrow neutrophils. (C) Gating strategy for the analysis of cell death of neutrophils in the spleen. Similar approach was used for the analysis of cell death of neutrophils in the blood.