LETTER TO THE EDITOR

Extramedullary hematopoiesis in the spleen contributes to natural killer cell development during infection and inflammation

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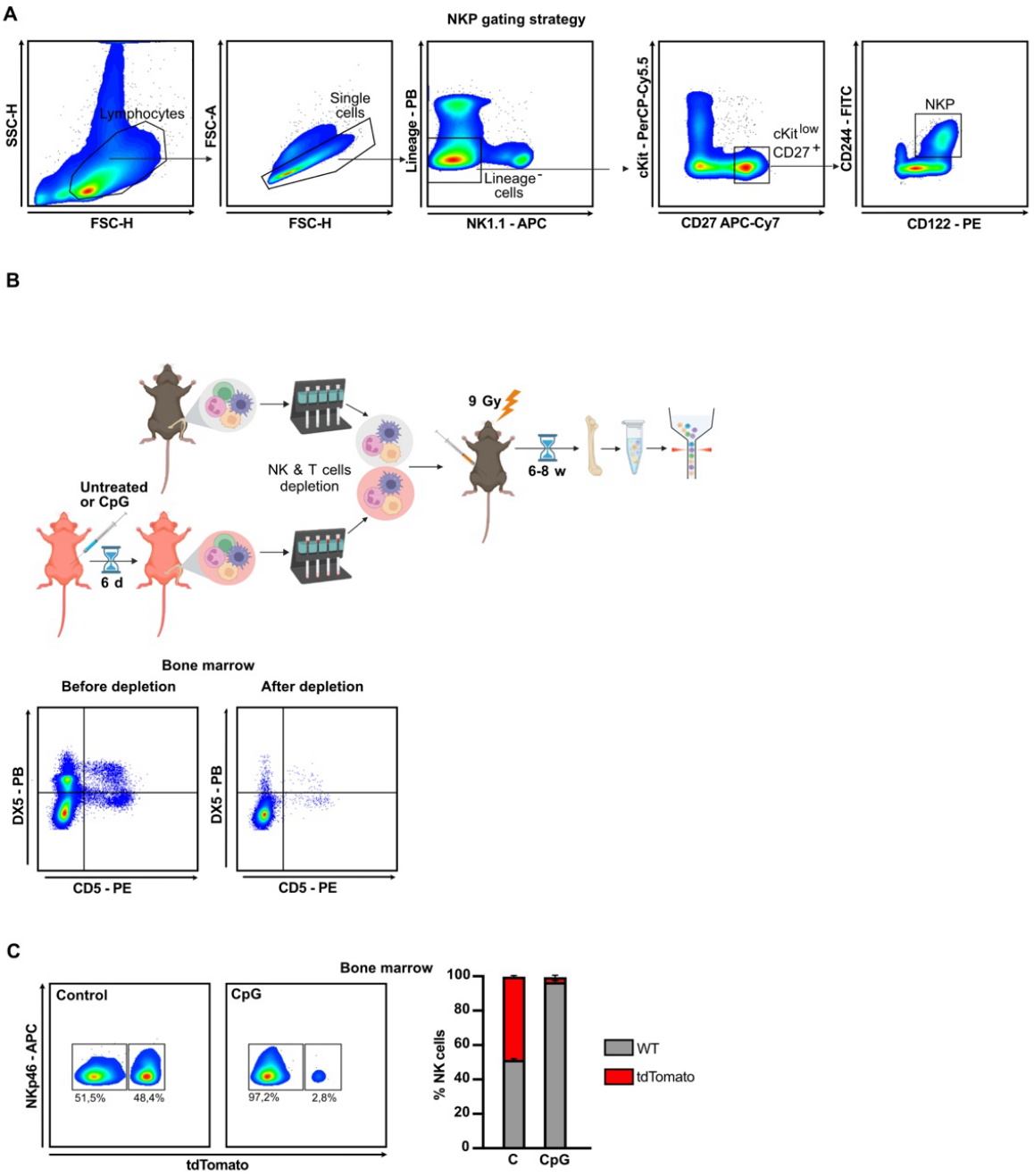
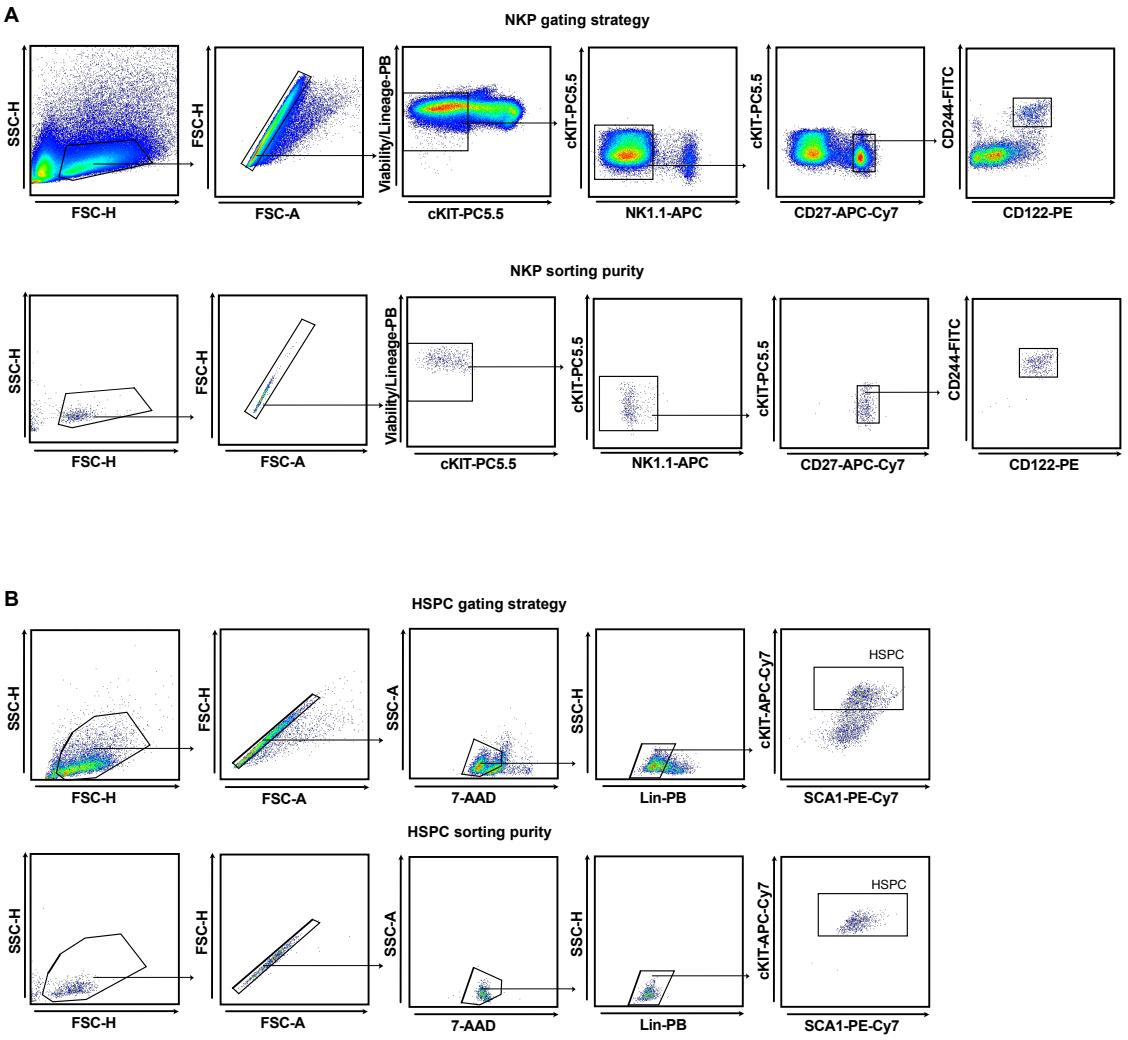
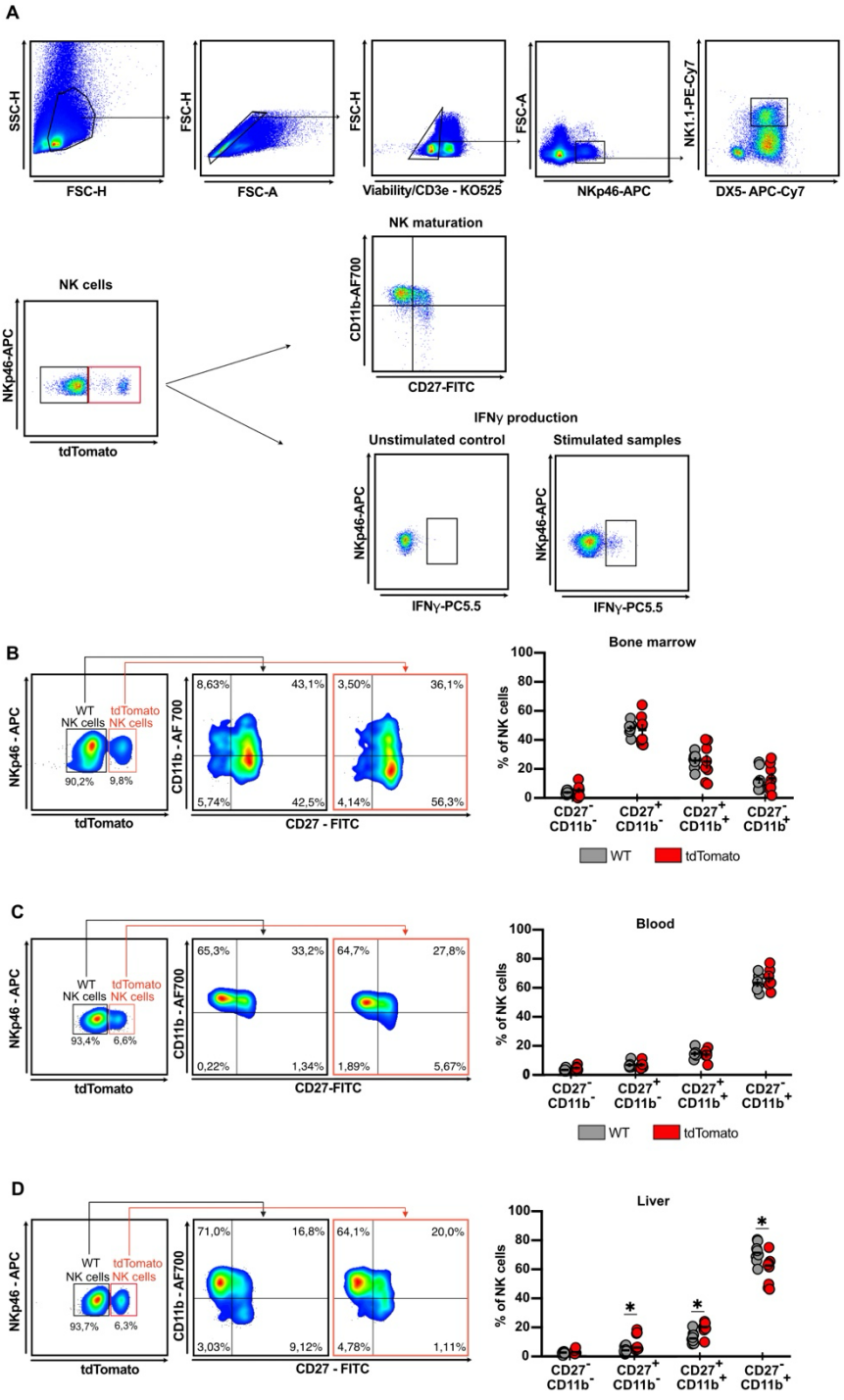


Supplementary Figure 1. **Acute inflammation reduces the BM's capacity to generate NK cells.** (A) Gating strategy for the analysis of NKPs. After exclusion of doublets, dead cells, lineage positive cells (CD19, CD3e, Ter 119, CD11b, Ly6G/Ly6C, F4/80) and NK cells (NK 1.1), cKit$^{low/-}$ CD27^{+} cells were gated. Out of this population, NKPs were defined as CD244^{+} and CD122^{+}. (B) Scheme of the experimental set-up and quality control for BM depletion. TdTomato mice were i.p. injected with 10 nM CpG-ODN. Six days later, femurs were flushed to obtain bone marrow cells. Mature

NK cells and T cells were depleted using anti-CD49b (DX5) and anti-CD5 labelled MACS beads (Miltenyi Biotech), then mixed 1:1 with wild-type (WT) BM cells and intravenously injected into WT recipient mice that had been lethally irradiated (9 Gy, 6 MV photons, Elekta Infinity linear accelerator) 24 h earlier under anaesthesia. Efficient depletion of $DX5^+$ and $CD5^+$ cells (i.e. mature NK cells and T cells) was confirmed by flow cytometry. Six to eight weeks later, NK cells in the BM of recipient mice were analysed. Created in BioRender. Strobl, B. (2025) https://BioRender.com/hf1sswc. (C) Percentage of NK cells in BM six to eight weeks after BM transplantation with representative flow cytometry plots (n=2, N=1). n, biological replicates; N, experimental repetitions. C, PBS treated controls.



Supplementary Figure 2. **Sorting strategy for NKPs and HSPCs.** (A) Gating strategy and post-sorting purity of NKPs used in the transplantation experiments. After exclusion of doublets, dead cells, lineage positive cells, and NK cells (NK 1.1), cKIT$^{low/-}$ CD27^{+} cells were gated. Out of this population, NKPs were sorted as CD244^{+} and CD122^{+}. (B) Gating strategy and post-sorting purity of HSPCs used in the transplantation experiments. After exclusion of doublets, dead cells, and lineage-positive cells, cKIT^{+} SCA1$^{-/+}$ cells were sorted.



Supplementary Figure 3. **Maturation of NK cells from transplanted HSPCs from CpG-ODN-challenged mice is fully intact in the BM and blood, but slightly impaired in the liver.** (A) Gating strategy for NK cell analysis. After exclusion of doublets, dead cells, and CD3$^+$ cells, classical NK cells were defined as NK1.1$^+$, NKp46$^+$, and DX5$^+$. Within this population, we distinguished tdTomato$^-$ and tdTomato$^+$ cells. For each subset, we quantified NK cell maturation using CD27 and CD11b

expression, as well as IFN-γ production. Unstimulated samples were used as controls to determine non-specific antibody binding. (B-D) NK-cell abundance and maturation with representative flow cytometry plots in (B) BM, (C) blood and (D) liver six to eight weeks after transplantation (n=4-5, N=2). Results are given as mean ± standard error of the mean (SEM). Statistical analysis was done with square root-transformed data. Statistical analysis was performed in GraphPad Prism 10 (Student's t-test). For clarity, statistical analysis is only shown for the comparison between WT and tdTomato NK cells. n, biological replicates; N, experimental repetitions.