Supplemental Figure 1. RBC indices are altered in repeated CpG-treated mice; however, a single dose of CpG does not result in the full disease complex. A. Hemoglobin, mean corpuscular volume, and red cell distribution width for PBS- and repeated CpG-treated mice are shown. B. Mice were give a single injection of CpG (50 μg) and then were examined for pathology 24 hours later. Mice did demonstrate a leukopenia and thrombocytopenia. However, they did not develop an anemia, splenomegaly, hyperferritinemia, or histologic changes consistent with HLH/MAS.

Supplemental Figure 2. TLR9$^{-/-}$ mice to not develop CpG-mediated HLH/MAS-like syndrome. Wild type (WT) and TLR9$^{-/-}$ mice were treated as in Figure 1. TLR9-deficient mice do not develop cytopenia, splenomegaly, or elevated IFNγ.

Supplemental Figure 3. Bone marrow examination of CpG-treated mice does not reveal hemophagocytes but does show decreased cellularity with increased megakaryocytes. A. 200× micrograph of a representative section of bone marrow from a PBS-treated mouse (left), and CpG-treated mouse (right). Green arrows mark megakaryocyte clusters. B. Example of a physiologic hemophagocyte from a normal mouse. C. 500× micrograph of CD163 staining a hemophagocyte in the bone marrow of a LCMV-infected perforin-deficient mouse (left panel), and background staining in a CpG-treated bone marrow.

Supplemental Figure 4. CD8^{+} T cells show evidence of minimal activation after repeated CpG treatment. Mice were treated with CpG as in Figure 1. A. A minor but consistent increase in the number of CD62L^{lo}CD69^{hi}CD8^{+} T cells was seen in CpG-treated mice (upper left quadrant). Plots are representative of 3 experiments. B. CD8^{+} T cells show the greatest increase in the CD62L^{lo}CD69^{hi} population in CpG-treated mice. *

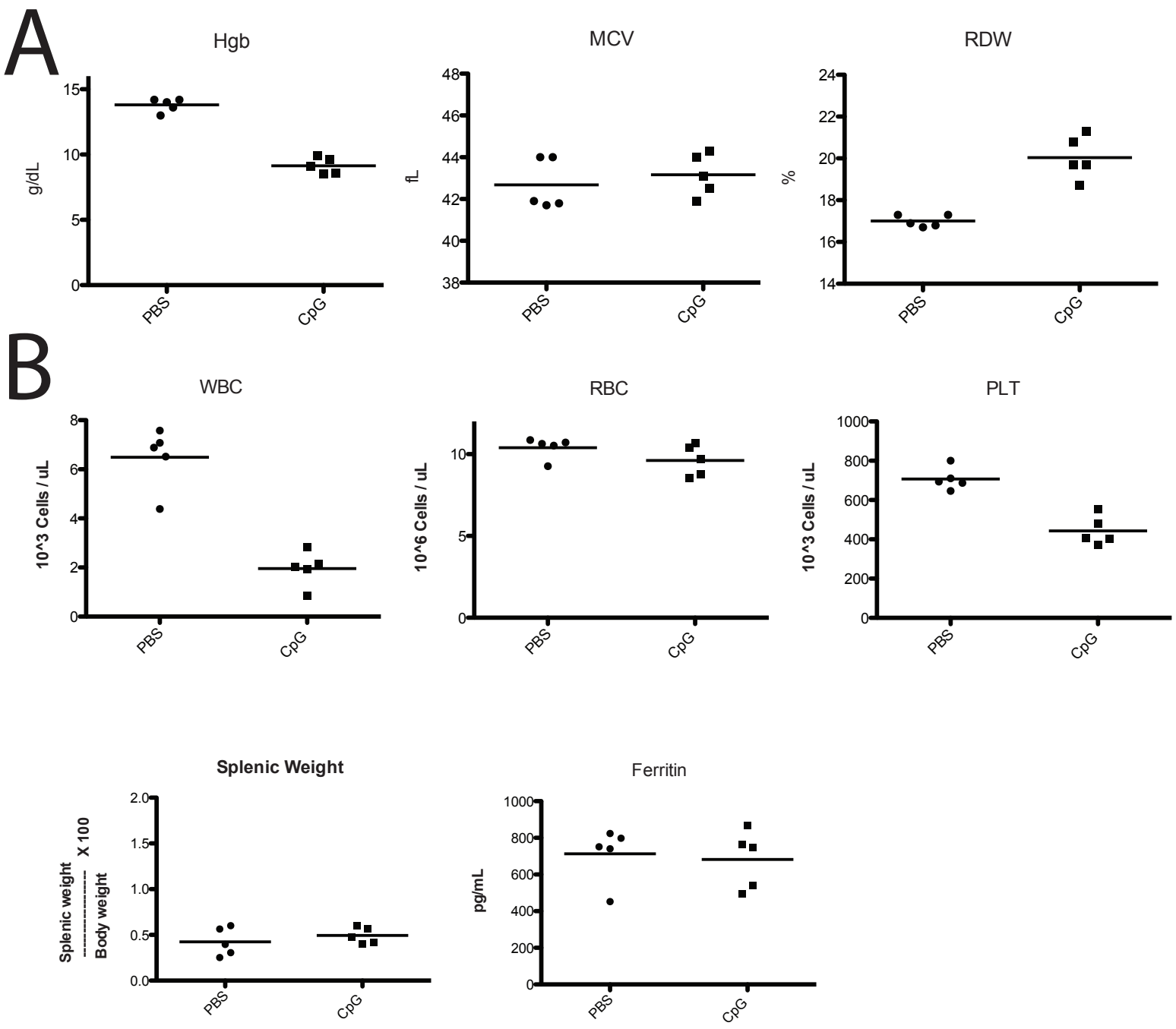
denotes $p < 0.05$ versus $CD8^+$ T cells. C. A subpopulation of splenic $CD8^+$ T cells but not $CD4^+$ T cells produces high levels of IFNγ in CpG-treated mice. Splenocytes from repeatedly treated mice were harvested and restimulated for 5 hours in PMA, ionomycin, and brefeldin A to determine maximal capacity for IFNγ production by intracellular flow cytometry. Dark lines represent a CpG-treated mouse, and the tinted histogram represents a PBS-treated mouse. Plots are representative of 3 experiments.

Supplemental Figure 5. Deletion of NK cells does not reduce the severity of CpG-induced HLH/MAS-like syndrome. A. Mice were treated with anti NK1.1 antibody (PK136, 100μg) or an irrelevant isotype antibody every day and were concurrently treated with either repeated PBS or CpG as in Figure 1. NK cells were measured by flow cytometry in peripheral blood on day 8 or in the liver at sacrifice on day 10. Spleen size was assessed at day 10 (B), and peripheral blood counts were assessed on day 8 (C).

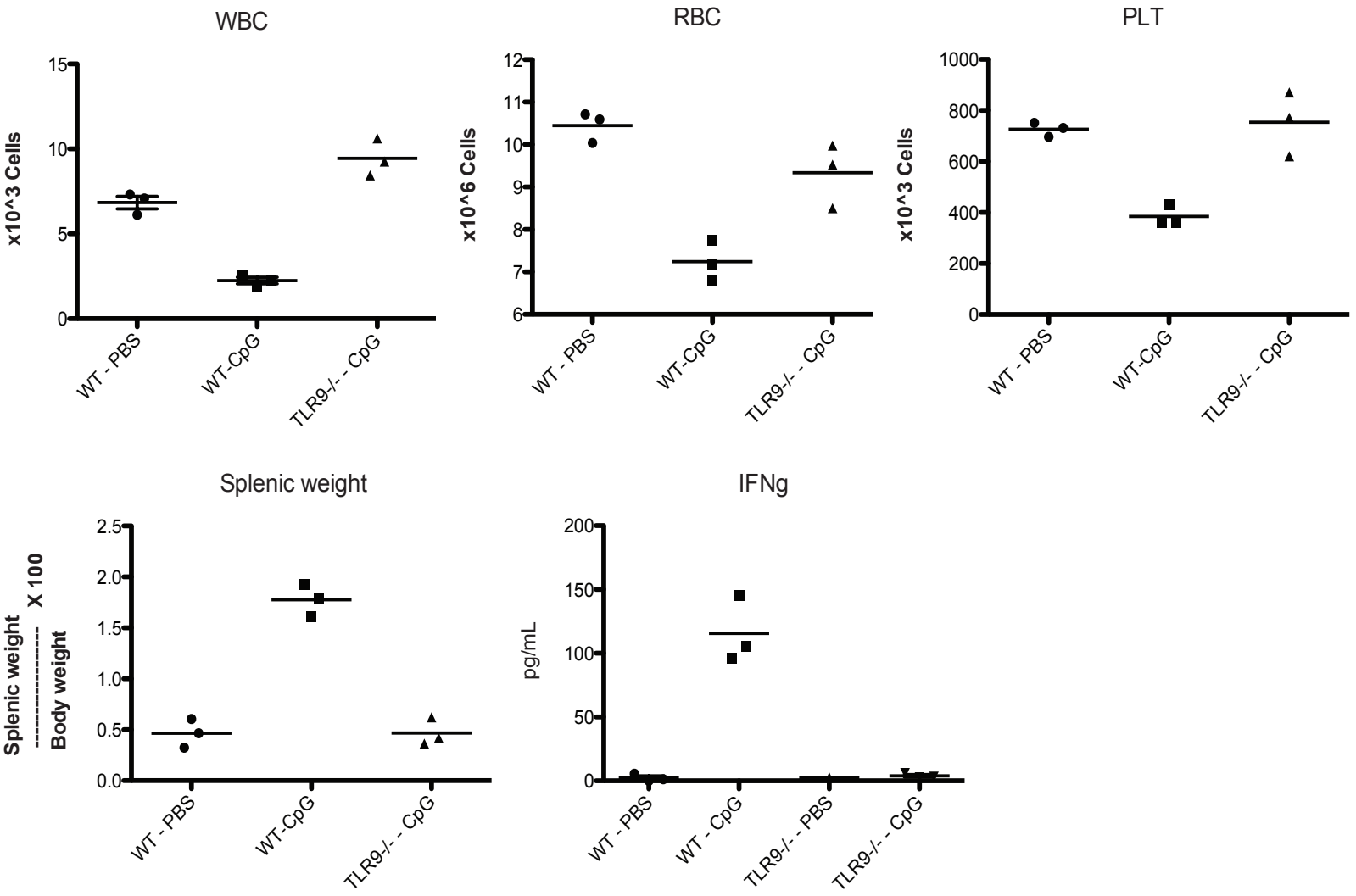
Supplemental Figure 6. IFNγ production shifts from early phase to late phase during CpG treatment. Yeti mice were injected with 4 doses of PBS (gray tinted histograms), one dose of CpG (green histogram, "early phase"), or 4 doses of CpG (green histogram, "late phase"). Mice were sacrificed, intrahepatic leukocytes were separated on a ficoll gradient, and splenocytes were stained to identify various cellular populations. These populations were analyzed for YFP florescence, which marks IFNγ transcription. The cellular populations were identified as follows: pDC – $NK1.1^-CD11c^{int}B220^+$, CD11b+DC – $NK1.1^-CD11c^+B220^-CD11b^+CD8\alpha^-$, CD8α+DC – $NK1.1^-CD11c^+B220^-CD11b^-CD8\alpha^+$, B-1 cells – $B220^+CD3^-CD5^+CD11c^-$, NKT cells – $CD3^+NK1.1^+CD1d$ $tetramer^+$, NK cells – $CD3^-NK1.1^+CD122^+DX5^+$. Macrophages ($CD11b^+CD11c^-NK1.1^-$)

did not show any YFP positivity (data not shown). Gating strategy is shown in A, and the YFP histograms for pDCs and $CD8a^{+}$DCs are shown in B.

Supplemental Figure 7. Depletion of DCs in CD11c-DTR chimeric mice. Wild type mice were lethally irradiated and reconstituted with either wild type or CD11c-DTR bone marrow. Mice were injected every other day with 100ng of DT, alternating with five doses of either CpG or PBS. Splenic DCs were identified by staining splenocytes for CD11c on day 10 of the experiment.

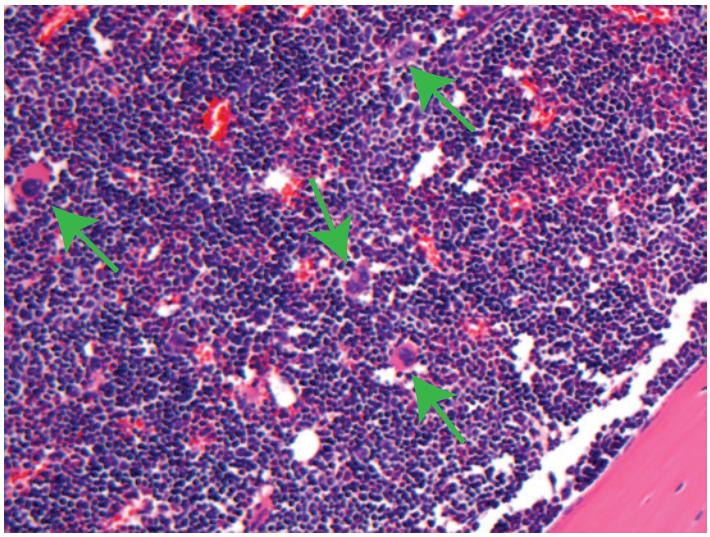


Supplememtal Figure 1

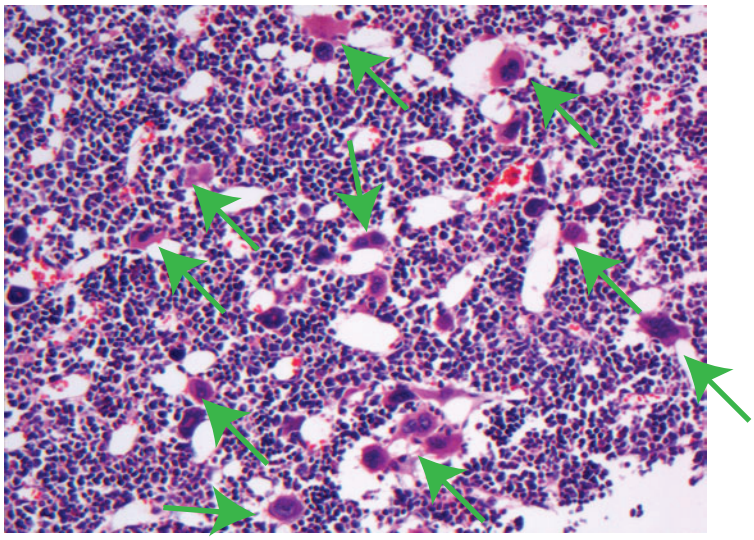


Supplemental Figure 2

A

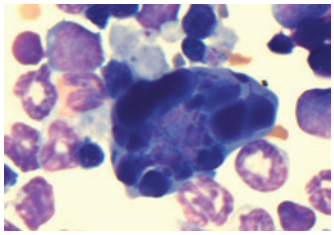


PBS treated bone marrow

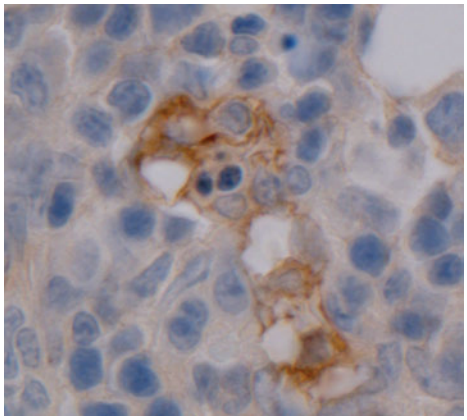


CpG treated bone marrow

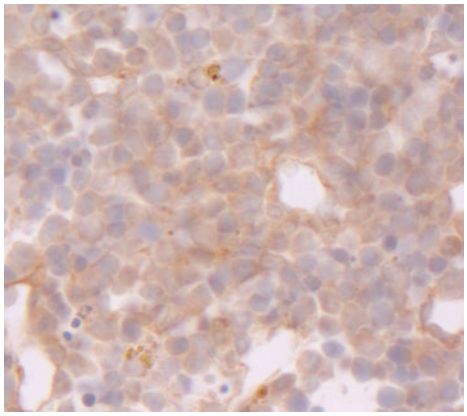
B



C

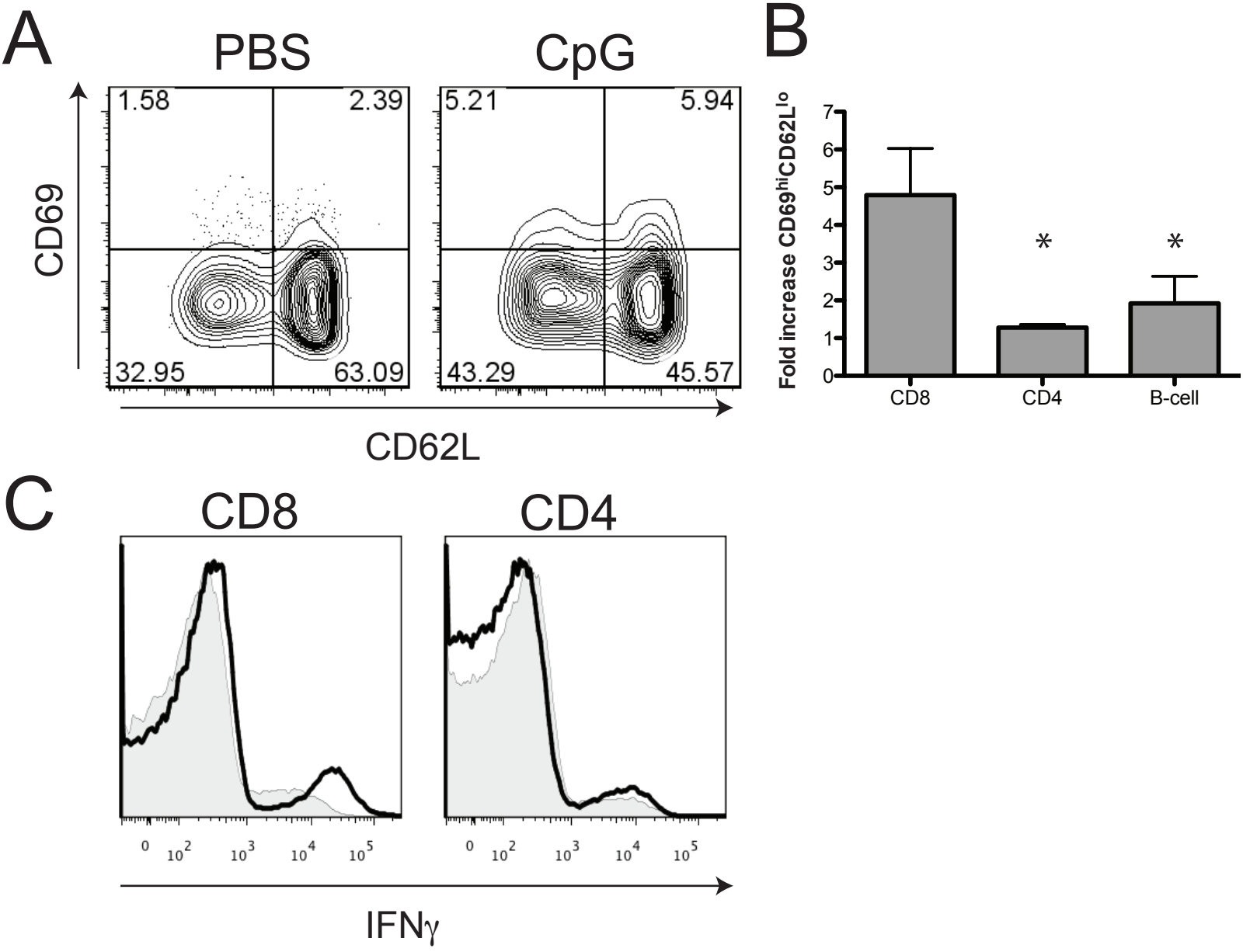


CD163
LCMV infected pfp-/-
bone marrow

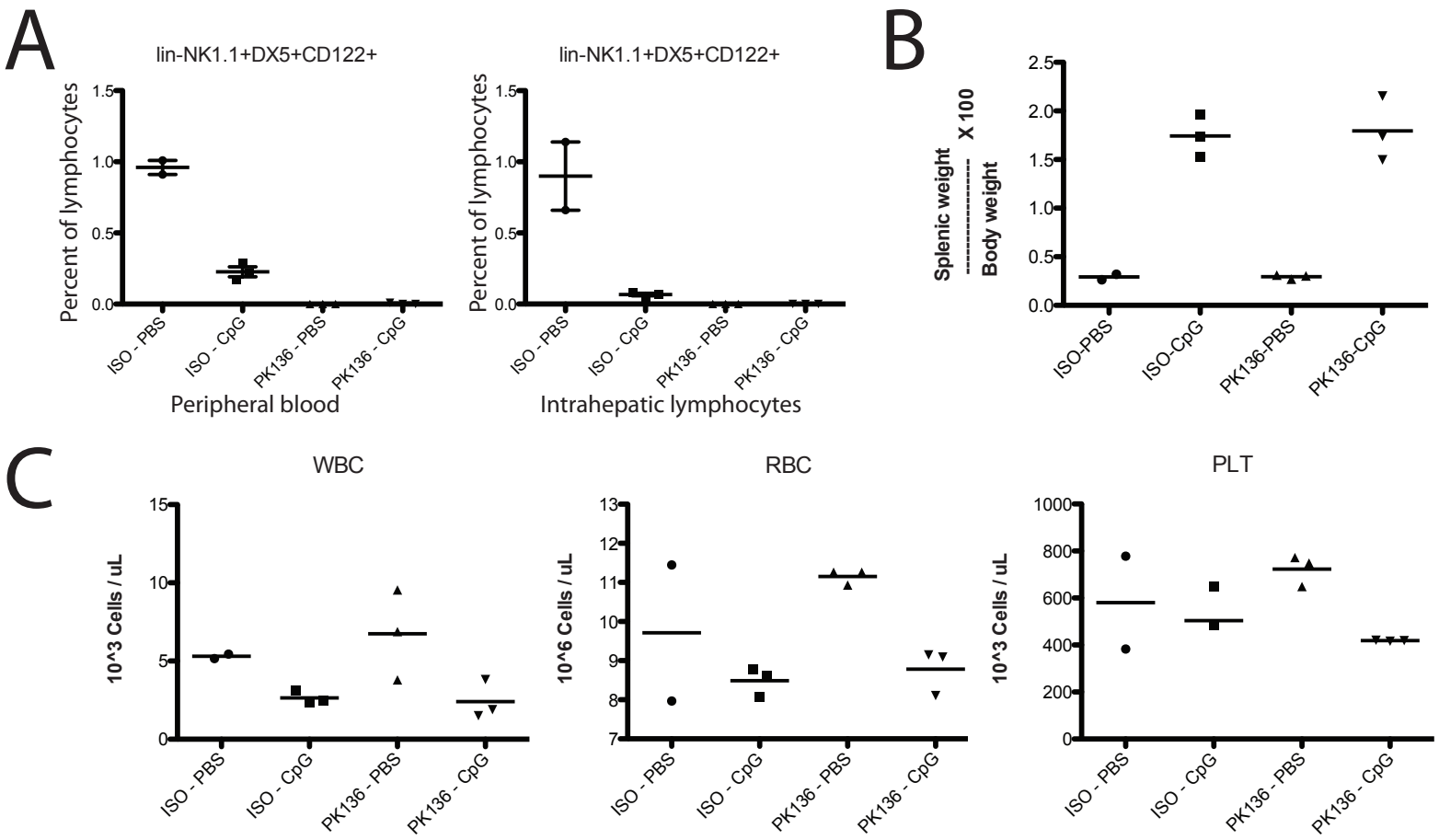


CD163
repeated CpG treated
bone marrow

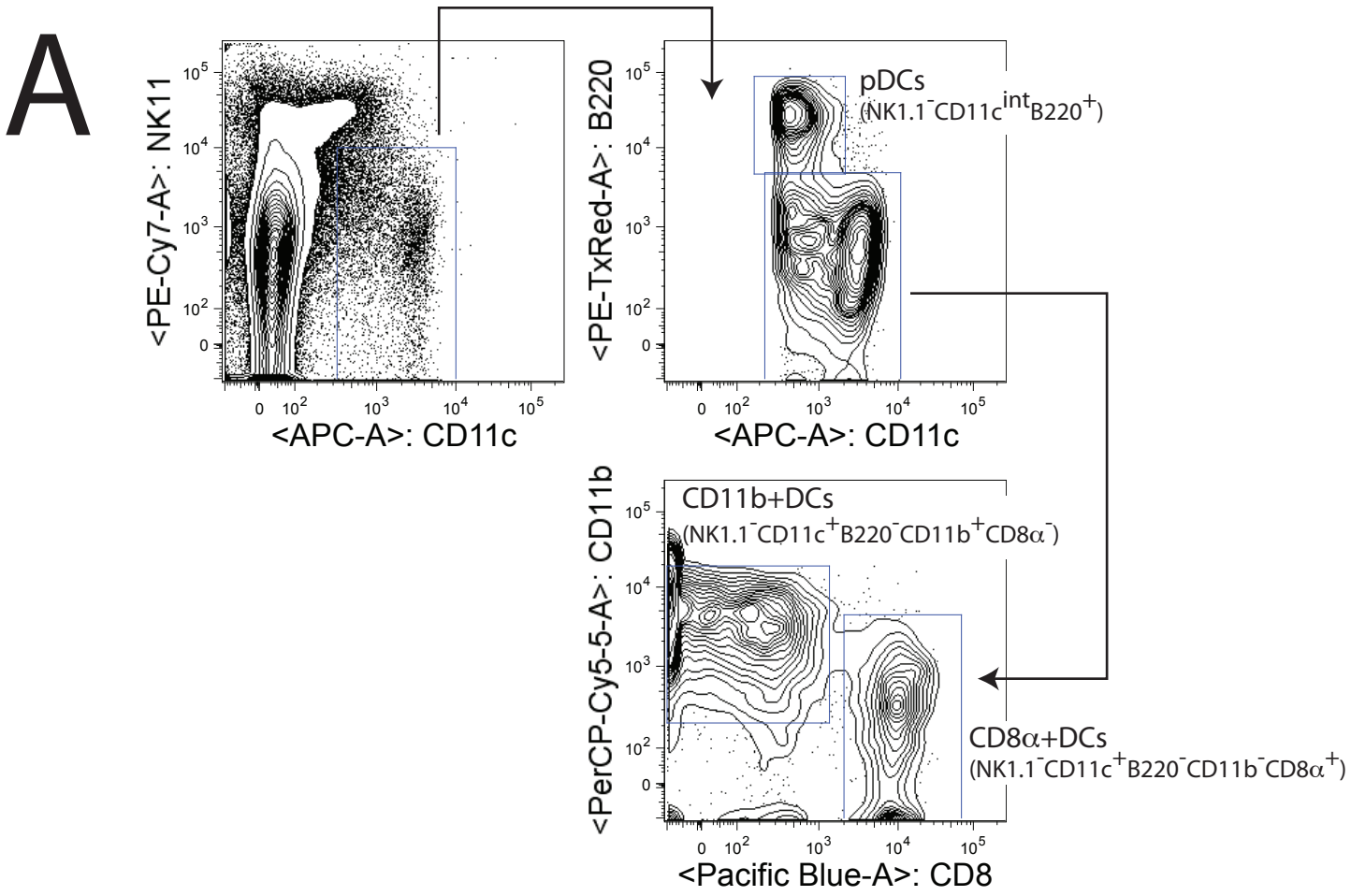
Supplemental Figure 3

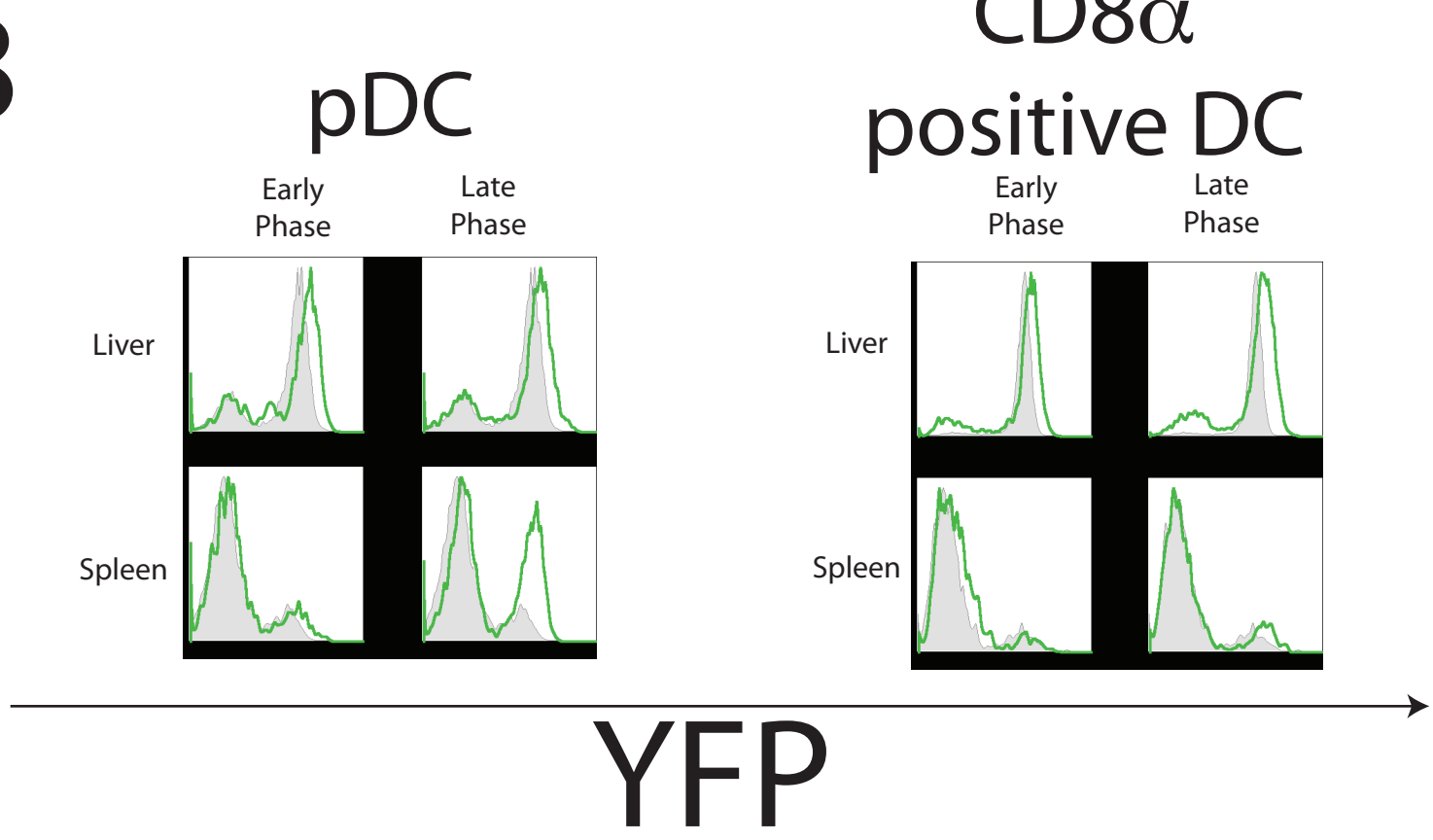


Supplemental Figure 4

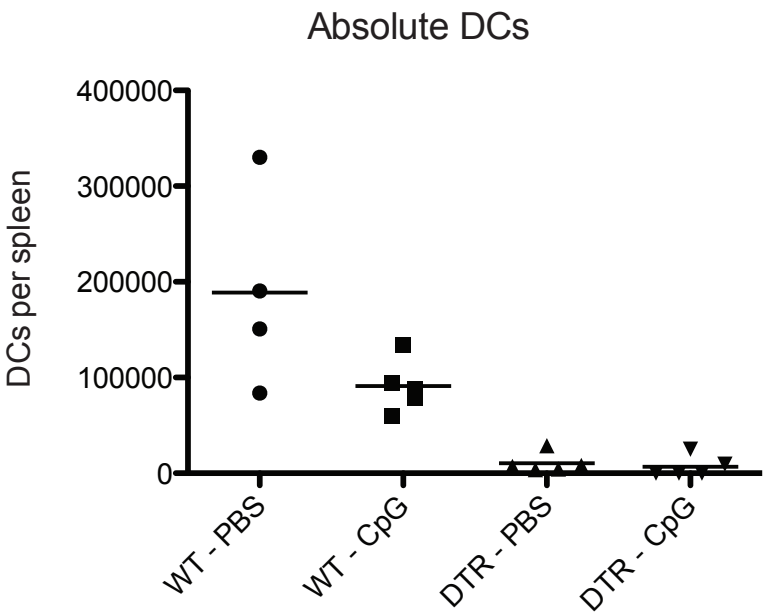


Supplemental Figure 5





Supplemental Figure 6



Supplemental Figure 7