## Supplemental Figure 1



## Supplemental Figure 1. Neutrophils express IFN-γ prior to isolation from naïve or *T. gondii* infected WT and TLR11-/- mice.

C57BL/6 mice were infected i.p. with *T. gondii* and PECs were harvested day 5 post-infection. (A) PECs were incubated at 37C for 5 hours in media with GolgiPlug (Brefeldin A) or placed in media on ice for 5 hours. IFN- $\gamma$  expression in neutrophils, T cells, and NK cells was assessed by flow cytometry. (B) PECs were incubated for 5 hours in media with GolgiPlug with or without stimulation (PMA-ionomycin) and with increasing concentrations of Cycloheximide (CHX), a de novo protein synthesis inhibitor. The percentage of IFN- $\gamma$ -positive T cells or neutrophils were assessed by flow cytometry. (C) TLR11 KO mice were infected i.p. with *T. gondii* or were kept naïve and examined day 5 post-infection. PEC, blood, and bone marrow were examined for the presence of Ly6G+ neutrophils and IFN- $\gamma$  positivity as in Figure 1. (D) Thioglycollate elicited Ly6G+ neutrophils were examined for IFN- $\gamma$  positivity (blue). These data are representative of three independent experiments. \* p<0.05, ns - not significant.

## Supplemental Figure 2



## Supplemental Figure 2. Neutrophil developmental stage confirmation and earlier hematopoietic cells.

Bone marrow from C57BL/6 mice were harvested and examined for different stages of their development. (A) Neutrophil-specific lineage development was examined as in (Fig. 2A): promyelocytes (orange), myelocytes (purple), metamyelocytes (green), band/segmented neutrophils (blue). Relative expression of granule proteins, Proteinase 3, Lactoferrin, and Gelatinase, characteristic of different neutrophil precursor developmental stages. (B) Bone marrow from WT mice were harvested and examined for different stages of hematopoietic development. The neutrophil-specific lineage develops from the granulocyte-monocyte progenitor (GMP) and prior to that, the common-myeloid progenitor (CMP) and Lineage-negative Sca-1-positive c-kit-positive (LSKs). LSKs and GMPs were examined for IFN- $\gamma$  expression (black) and isotype control (gray) by flow cytometry. The data shown are representative of at least 3 independent experiments.