Clostridium perfringens α-Toxin Impairs Innate Immunity via Inhibition of Neutrophil Differentiation

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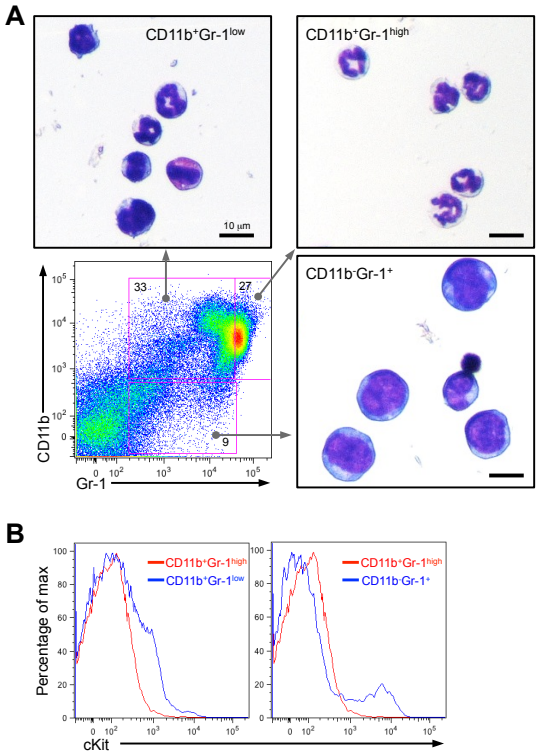

Figure S1. Expression of CD11b and Gr-1 represents the stages of neutrophil maturation. Bone marrow cells (BMCs) from C57BL/6 mice were labeled with specific antibodies against CD11b, Gr-1, and cKit. The cells were analyzed or sorted using a FACS Aria II. Three distinct populations were identified, and Giemsa staining of sorted $CD11b^{+}Gr\text{-}1^{high}$, $CD11b^{+}Gr\text{-}1^{low}$, and $CD11b^{-}Gr\text{-}1^{+}$ cells was performed (A). The expression of cKit in the $CD11b^{+}Gr\text{-}1^{low}$ and $CD11b^{-}Gr\text{-}1^{+}$ cell populations (blue) was compared with that of the $CD11b^{+}Gr\text{-}1^{high}$ cell population (red) (B).

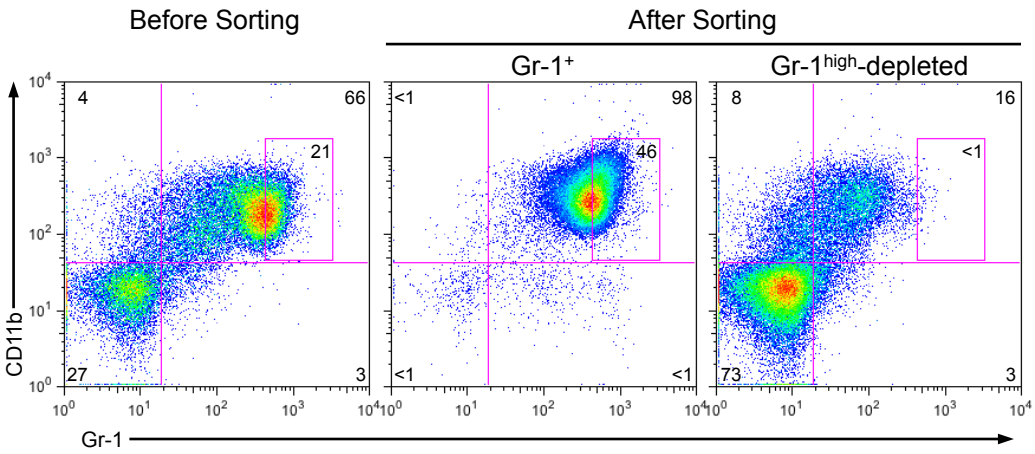

Figure S2. Preparation of Gr-1^{+} cells and CD11b^{+}Gr-1^{high} cell-depleted bone marrow cells. Preparation of Gr-1^{+} cells (Gr-1^{+}) and CD11b^{+}Gr-1^{high} cell-depleted Bone marrow cells (BMCs) (Gr-1^{high}-depleted) was performed using the EasySep system, as described in the Materials and Methods. Isolated cells were labeled with a specific antibody against CD11b, and flow cytometry analysis was performed using a Guava easyCyte. Almost all of the isolated Gr-1^{+} cells co-expressed CD11b, which means that the isolated cells were CD11b^{+}Gr-1^{+} cells. CD11b^{+}Gr-1^{high} cell-depleted BMCs contained less than 1% of CD11b^{+}Gr-1^{high} cells.

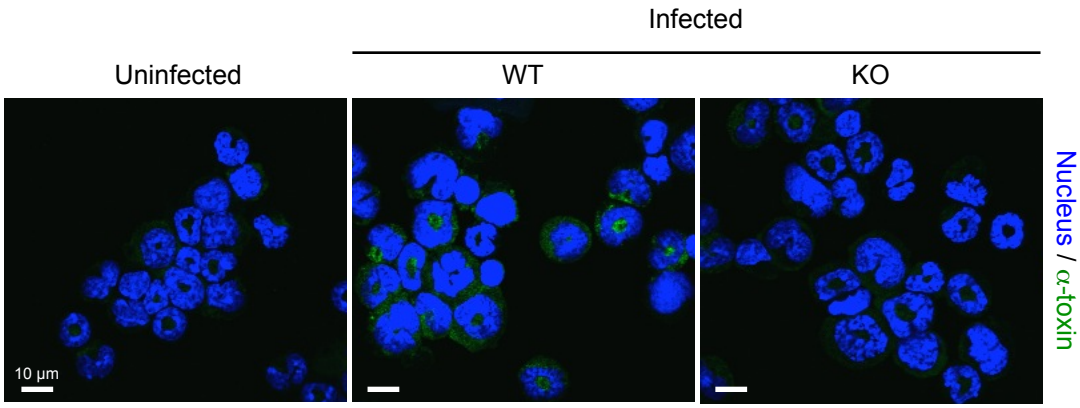

Figure S3. α-Toxin binds to bone marrow neutrophils in *Clostridium perfringens*-infected mice. Mice were intramuscularly injected with 1×10^7 CFU of *C. perfringens* Strain 13 (WT), PLC-KO (KO), or TGY medium as a control (uninfected), and bone marrow cells (BMCs) were isolated from the mice after 24 hours. Magnetically isolated Gr-1^+ cells from BMCs were incubated with an antibody against α-toxin. After incubation with a secondary antibody, the cells were inspected by confocal laser scanning microscopy.