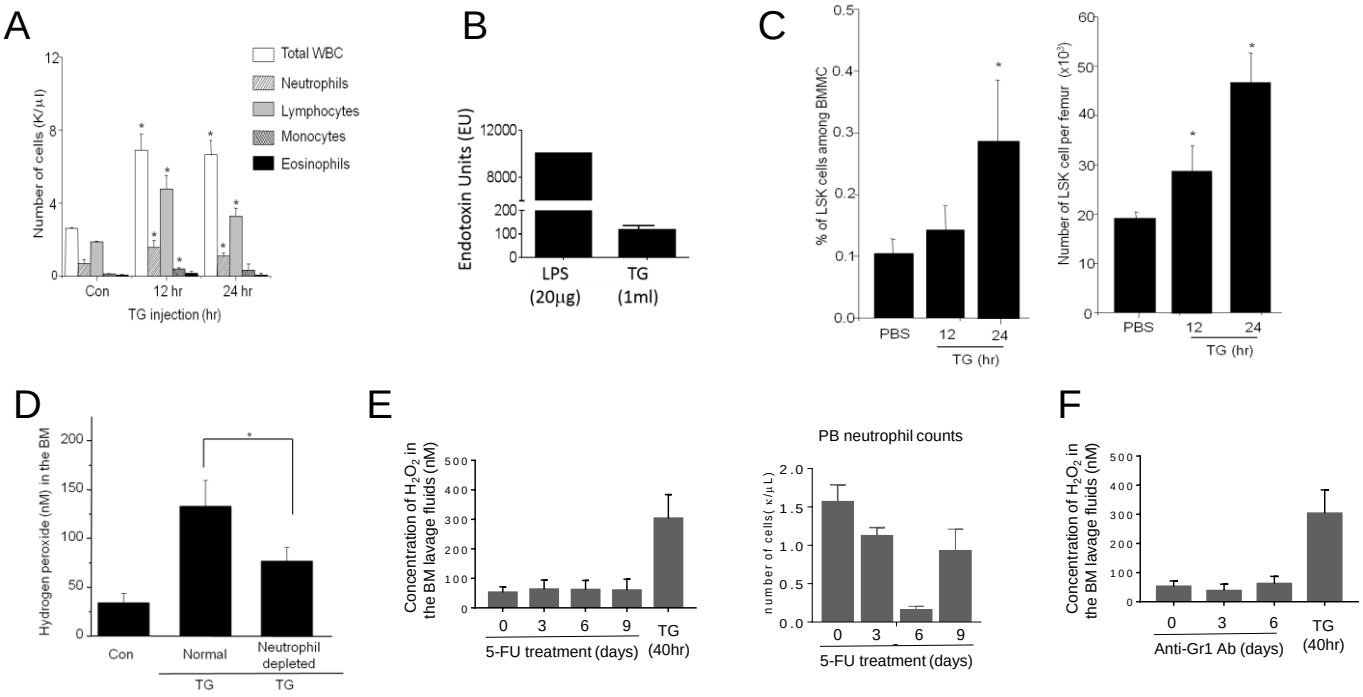


Figure S1.

(A) TG-induced sterile inflammation alters peripheral blood cell counts. The peritonitis was induced with TG as described in Figure 1. Peripheral blood was collected at 0, 12 and 24 hr after TG injection. Total cellularity and the differential leukocyte count were measured using a Hemavet-950FS Hematology system. Data shown are means ± SD of n=5 mice. **p*<0.01 versus time 0 (Con). **(B) Endotoxin levels in LPS and TG solution (3%)**. The endotoxin levels were measured using a ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit (GenScript). Sigma's lipopolysaccharides contain endotoxin levels of not less than 500 EU/μg. To induce detectable emergency granulopoiesis, we need to inject more than 20 μg LPS (10,000 EU) into one mouse. In current study, a sterile inflammation was induced by 1 ml 3% TG (Fluka). **(C) TG-elicited acute inflammation increases BM LSK cell number.** The experiment and the flow-cytometry based lineage analysis were conducted as described in Figure 1. Data shown are means ± SD of n=5 mice. **p*<0.01 versus mice injected with PBS. **(D) Depletion of neutrophils inhibits acute inflammation-elicited ROS production.** Neutrophils were depleted by a Gr-1 antibody in both peripheral blood and the bone marrow as previous described (Kwak et al., 2015). PB neutrophil count was reduced by more than 85% and BM neutrophil count was reduced by 70%. Two days after injection of anti-Gr1 antibody, peritonitis was induced by TG as described in Figure 1. The level of H_2O_2 in the BM was measured 24 hr after the TG injection as described in Figure 2A. Data shown are means ± SD of n=5 mice. **p*<0.01. **(E) BM extracellular ROS levels in 5-FU treated mice.** WT mice were treated with 5-FU (150mg/kg) for indicated time. BM extracellular ROS were measured using the Amplex® Red assay. Data shown are means ± SD of *n*=5 mice. 5-FU-induced neutropenia was confirmed by the drastic reduction of PB neutrophil count. **(F) BM extracellular ROS level after neutrophil depletion.** Neutrophils were depleted by a Gr-1 antibody as previous described (Kwak et al., 2015). At indicated time points, BM extracellular ROS were measured using the Amplex® Red assay. Data shown are means ± SD of *n*=5 mice.

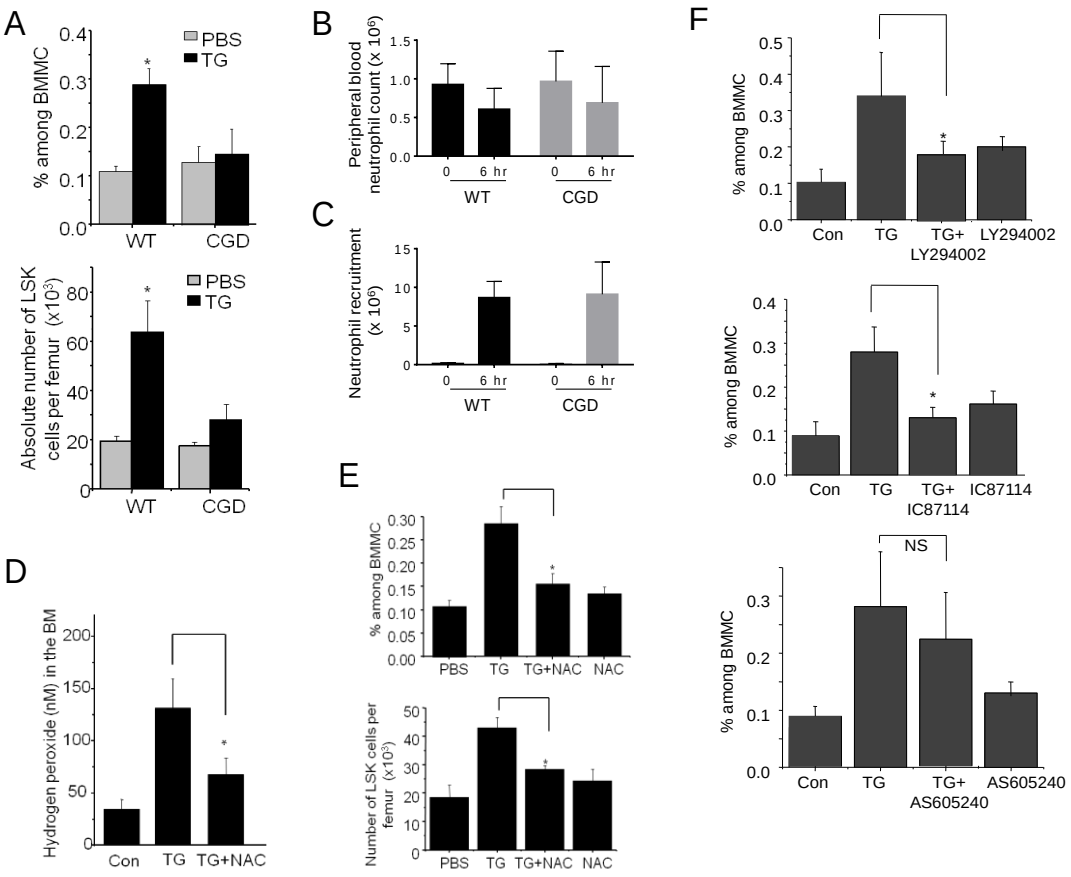


Figure S2.

(A) Disruption of phagocyte NADPH oxidase (NOX2) suppresses TG-elicited expansion of BM LSK cells. The experiment and the flow-cytometry based lineage analysis were conducted as described in Figure 3A-B. Data shown are means ± SD of n=5 mice. **p*<0.01 versus mice injected with PBS. **(B) Disruption of NOX2 does not affect the peripheral blood neutrophil count.** Peripheral blood was collected at 0 and 6 hr after TG injection. Data shown are means ± SD of n=5 mice. **(C) Disruption of NOX2 does not affect neutrophil recruitment during inflammation.** The peritonitis was induced with TG as described in Figure 1. The numbers of neutrophils in the inflamed peritoneal cavity were counts at 0 and 6 hr after TG injection. Data shown are means ± SD of n=5 mice. **(D) Treatment with antioxidant NAC suppresses TG-elicited ROS production in the BM**. NAC treatment and induction of peritonitis with TG were carried out as described in Figure 3E. The level of H_2O_2 in the BM was quantified as described in Figure 2A. Data shown are means ± SD of n=5 mice. **p*<0.01 versus untreated mice. **(E) Treatment with antioxidant NAC suppresses acute inflammation-elicited expansion of BM LSK cells.** The experiment and the flow-cytometry based lineage analysis were conducted as described in Figure 3E-F. Data shown are means ± SD of n=5 mice. *, *p*<0.01.

(F) The effect of PI3K inhibitors on TG-elicited expansion of BM LSK cells. The treatment with inhibitors and induction of peritonitis with TG were carried out as described in Figure 5E. The flow-cytometry based lineage analysis were conducted 24 hr after the TG injection as described in Figure 1B. Data shown are mean ± SD of n=5 mice. **p*<0.01 versus mice injected with TG alone. NS, p>0.05.

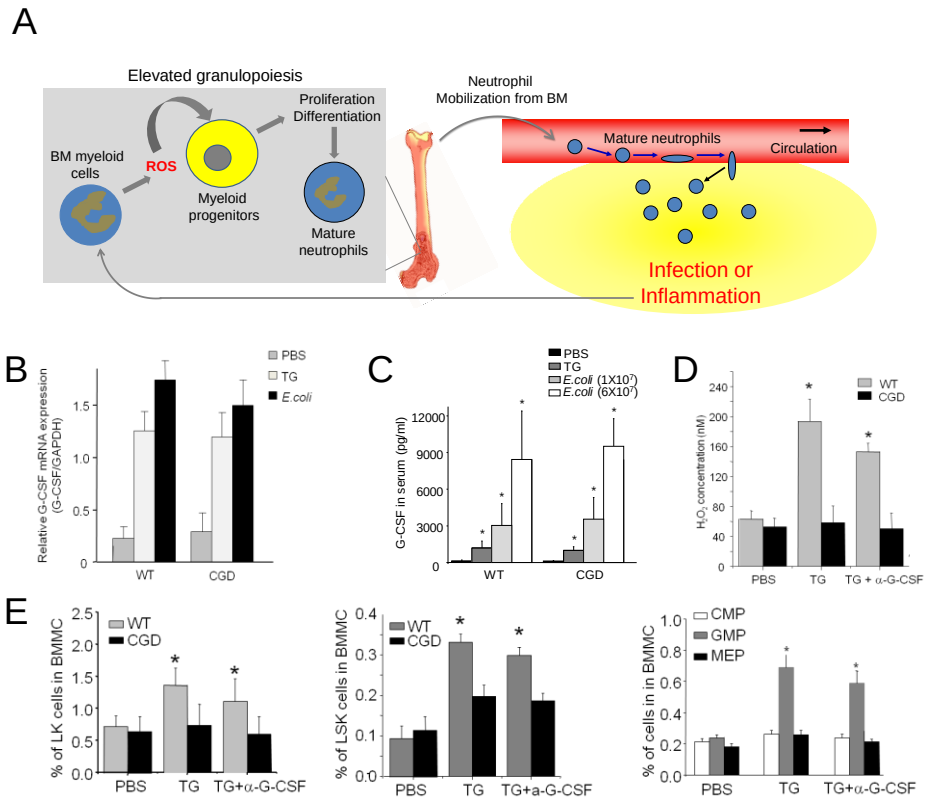
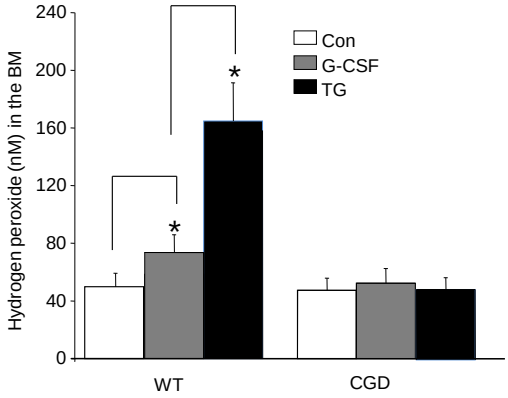


Figure S3.

(A) NADPH oxidase-dependent ROS production by BM myeloid cells is critical for proliferation of myeloid progenitors in both sterile inflammation-elicited reactive granulopoiesis and microbial infection-driven emergency granulopoiesis.

(B) Inhibition of NADPH oxidase-dependent ROS production does not alter sterile inflammation-elicited elevation of G-CSF expression in Gr1$^+$ myeloid cells. Induction of peritonitis in WT and CGD mice with TG or *E.coli* was carried out as described in Figure 1 and Figure S1, respectively. G-CSF mRNA expression was measured 24 hr after TG injection and 36 hr after *E.coli* injection by quantitative RT-PCR as described in Figure S2 and normalized to GAPDH. Data shown are means ± SD of n=3 mice. The results on *E.coli* were previously published (1). They are included here for comparison purpose. **(C) Disruption of phagocyte NADPH oxidase does not alter acute inflammation-elicited elevation of serum G-CSF level.** WT mice were intraperitoneally injected with PBS, TG or heat inactivated *E.coli*. The serum G-CSF level was measured 24 hr after the injection using an ELISA Kit following the protocol provided by the manufacturer (R&D Systems, Inc. Minneapolis, MN). Data shown are mean ± SD of n=5 mice. *p<0.01 versus mice injected with PBS. **(D) Inhibition of G-CSF does not inhibit acute inflammation-elicited ROS production.** The peritonitis was induced with TG as described in Figure 1. To neutralize G-CSF *in vivo*, mice were injected subcutaneously with 100 µg anti-mouse G-CSF antibody (R & D system, clone67604) 1 hr after the TG injection. The suppression of G-CSF effect by blocking antibody was confirmed by inhibition of G-CSF-elicited neutrophil mobilization from the BM and G-CSF-elicited expansion of myeloid progenitors (1). Hydrogen peroxide measurement was conducted 23 hr after the antibody administration as described in Figure 2A. Data shown are mean ± SD of n=5 mice. *p<0.01 versus mice injected with PBS. **(E) Inhibition of G-CSF does not inhibit TG-elicited emergency granulopoiesis.** The peritonitis was induced with TG as described in Figure 1. Hematopoietic cell lineage analysis was conducted 23 hr after the antibody administration as described in Figure 1B. Data shown are mean ± SD of n=5 mice. *, p<0.01 versus mice injected with PBS.

A



B

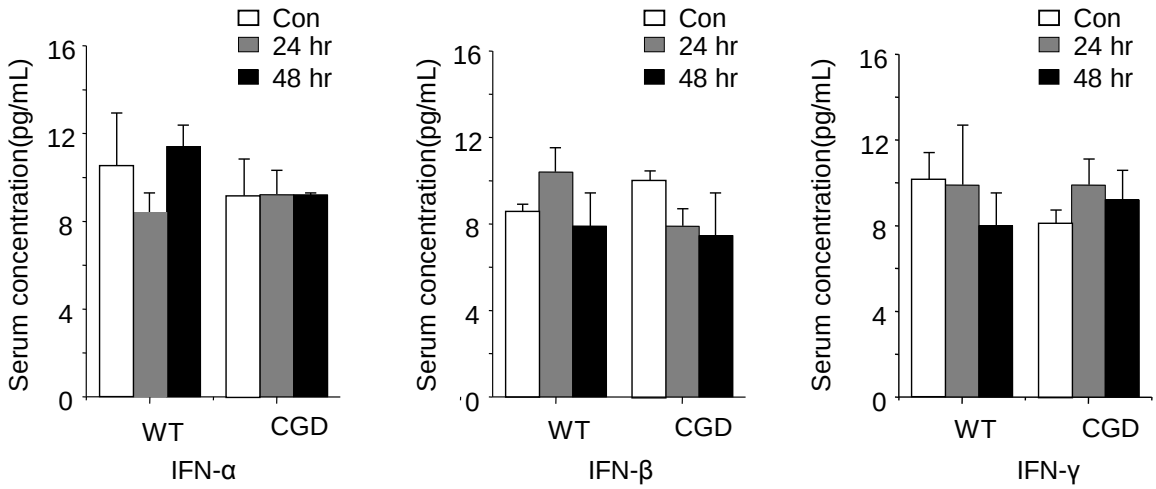


Figure S4.

(A) Sterile inflammation-elicited ROS production is not only mediated by G-CSF. The level of H_2O_2 in the BM was quantified 24 hr after the TG or G-CSF (250 μg/kg body weight) injection as described in Figure 2A. Data shown are mean ± SD of n=5 mice. *, $p<0.01$.

(B) Acute inflammation does not alter serum interferon levels. The induction of peritonitis in WT and CGD mice with TG was carried out as described in Figure 1. The levels of interferon were measured at each indicated time points with ELISA kits following the manufacturer's protocol (R&D Systems, Inc. Minneapolis, MN). Data shown are means ± SD of n=5 mice.