Supporting information

This file includes: Figure S1 to S8

Fig. S1 shows the glutathionylation of α4 at different time points. Fig. S2 shows the effect of NEM on the binding between neutrophils and VCAM-1. Fig. S3 shows the expression of Grx1 in murine $Grx1^{-/-}$ neutrophils or Grx1 overexpression dHL60 cells. Fig. S4 shows the adhesion of murine neutrophils to HUVEC monolayer with or without Grx1 protein pre-treatment. Fig. S5 shows the effect of administrated Grx1 in mice. Fig. S6 shows the ratio of neutrophils to whole marrow cells after zaurategrast, Grx1 treatment, or Grx1 depletion. Fig. S7 shows staining of bone marrow venous sinusoidal endothelium. Fig. S8 shows glutathionylation of recombinant G-CSF, MIP-2 and KC.

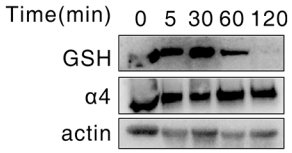

Figure S1. Related to Figure 1. Detection of the glutathionylation of α4 at different time points. dHL60 cells were treated with 10 μM fMLF for indicated time. Cells were harvested and subjected to immunoblotting for GSH, α4 and actin.

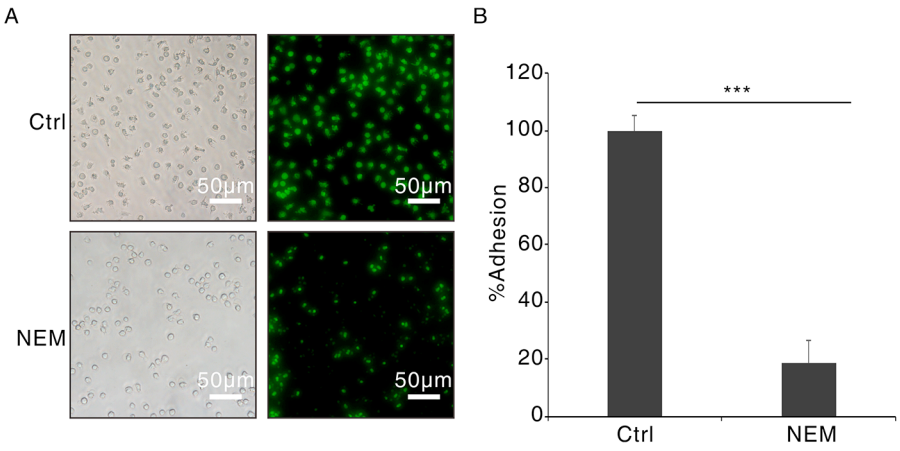

Figure S2. Related to Figure 2. NEM inhibited the binding between neutrophils and VCAM-1. CFSE-labeled murine neutrophils were left untreated or pretreated with NEM, and then adhered to immobilized VCAM-1 for 60 min at 37°C. After washing nonstick cells, marrow neutrophil counts were tested by fluorescence microscope observation(A).and Microplate Reader (B). *p<0.001 versus NEM untreatment.

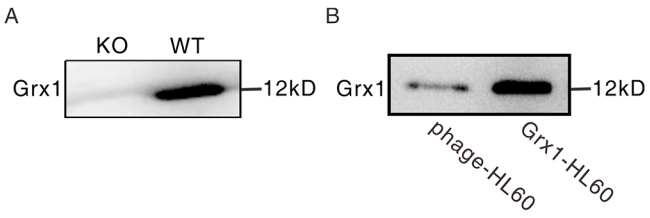

Figure S3. Related to Figure 3. Detection of the expression of Grx1. A.WT and Grx1$^{-/-}$ murine neutrophils were harvested and subjected to immunoblot analysis for Grx1.B. Grx1-dHL60 and Phage-dHL60 cells were harvested and subjected to immunoblot analysis for Grx1.

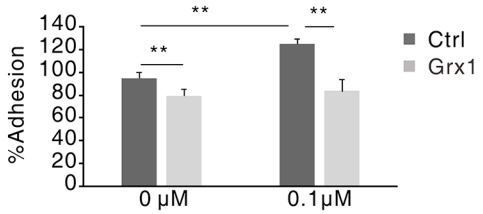

Figure S4. Related to Figure 5. CFSE-labeled murine neutrophils were preincubated with Grx1 protein (10 μg/mL) or not for 30min and then were stimulated with 0, 0.1μM H2O2 and allowed to adhere to HUVEC. The adhesion of cells without Grx1 incubation and stimulation was defined as 100%. **p<0.01, versus control.

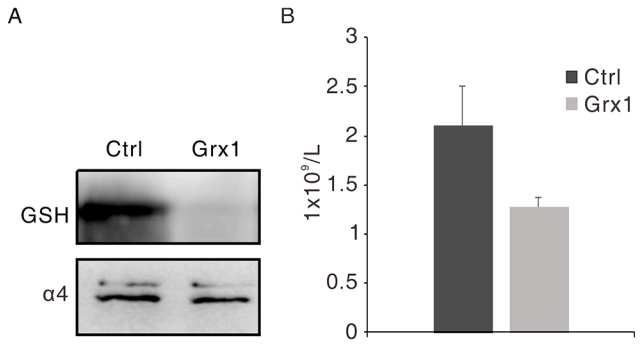

Figure S5. Related to Figure 6. The effect of administrated Grx1 in mice. Mice were pre-treated with Grx1 (intravenously injected, 1 μg /mouse) or PBS for 1h. (A). Murine neutrophils were purified at 1hr time point and subjected to immunoblot analysis for GSH and α4(n=3 per group). (B) Neutrophils in peripheral blood were counted 1 hr after challenge (n=3 per group). *$p<0.05$ versus PBS pre-administrated mice.

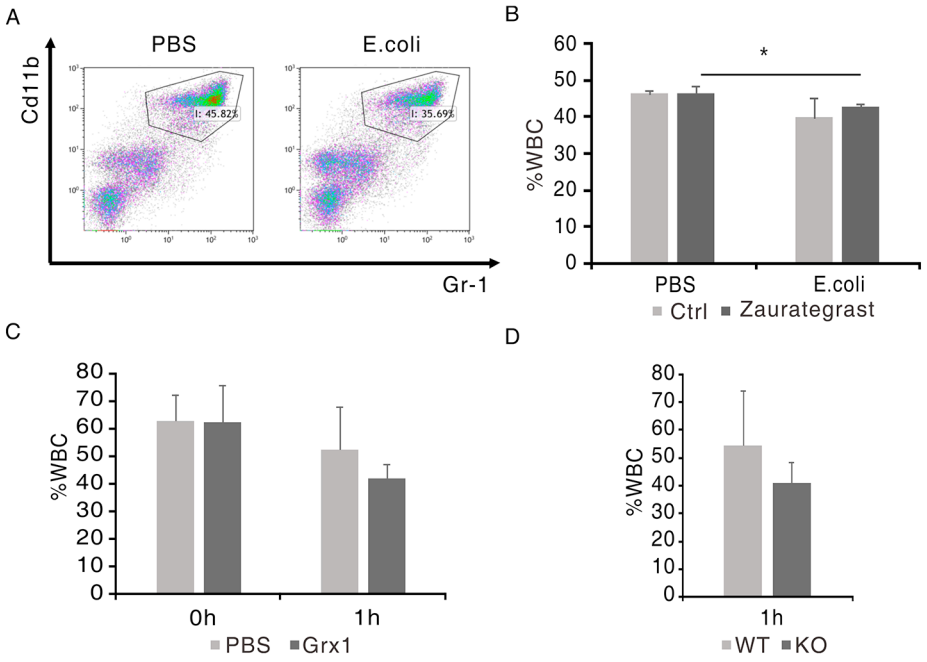

Figure S6. Related to Figure 6. The ratio of neutrophils to whole marrow cells (except red blood cells). A. Enclosed area ($CD11b^{+}Gr\text{-}1^{+}$) indicated neutrophils in bone marrow by cytometry. B. Mice were pre-treated with zauractegrast (intravenously injected, 1μg /mouse) or PBS for 4h. Mice were then challenged with E.coli (intraperitoneally injected, 1×107 /mouse). Marrow neutrophils counts were tested at 1 hr time points after challenge (n=3 per group). **p<0.05 versus PBS pre-administrated mice. C. Mice were pre-treated with Grx1 protein (intravenously injected, 1μg /mouse) or PBS for 1h. Mice were then challenged with E.coli (intraperitoneally injected, 1×10^{7} /mouse). Marrow neutrophils counts were tested at 1 hr time points after challenge by flow cytometry(n=3 per group). D. WT and $Grx1^{-/-}$ marrow neutrophil counts were tested at 1hr time point after intraperitoneally injected live E.coli (n=3 per group).

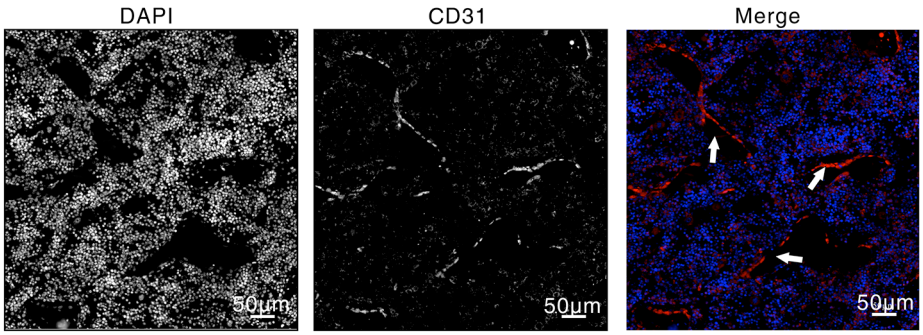

Figure S7. Related to Figure 6. Bone marrow vascular endothelium which expressed CD31 were red staining (white arrow). Blue staining (DAPI) represented cell nuclei. Scale bars represented 50 μm.

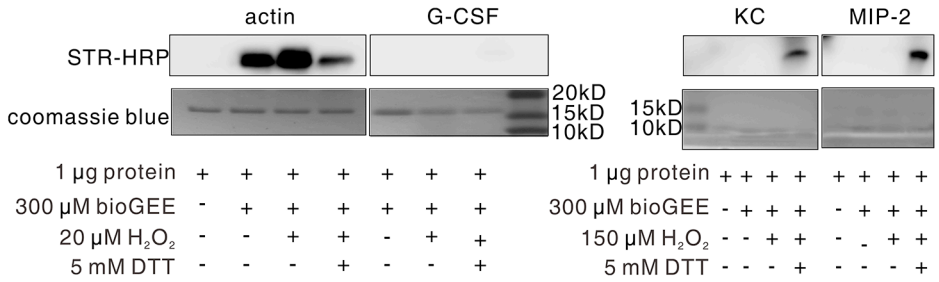

Figure S8. Related to Figure 6. S-glutathionylation of recombinant G-CSF, MIP-2 and KC. After treated with the indicated amounts of BioGEE, H_2O_2 and DTT as described, proteins were resolved on non-reducing PAGE gels and probed for the biotinylated-glutathione modification using streptavidin-HRP. Total protein loading was evaluated by coomassie blue dye. Actin was used as positive control.