Inventory of Supplemental Information

Supplemental Figures:

Figure S1, related to Figure 1.
Figure S2, related to Figure 2.
Figure S3, related to Figure 3.
Figure S4, related to Figure 3.
Figure S5, related to Figure 4.
Figure S6, related to Figure 5.
Figure S7, related to Figure 6.

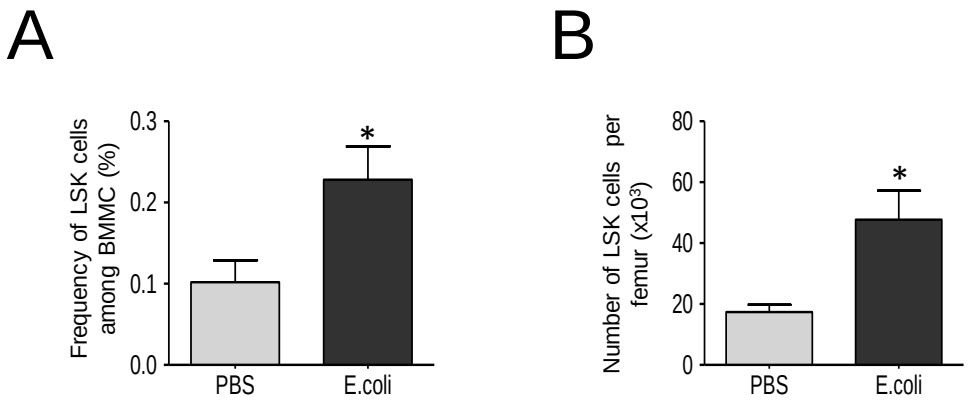

Figure S1, Related to Figure 1. *E.coli*-elicited acute inflammation increases BM LSK cell number. The experiment and the flow-cytometry based lineage analysis were conducted as described in Figure 1. **(A)** The percentage of each cell population among BM-derived mononuclear cells (BMMCs). **(B)** The absolute cell number per femur. Data shown are means ± SD of n=5 mice. * p<0.01 versus control (PBS treated mice)**.**

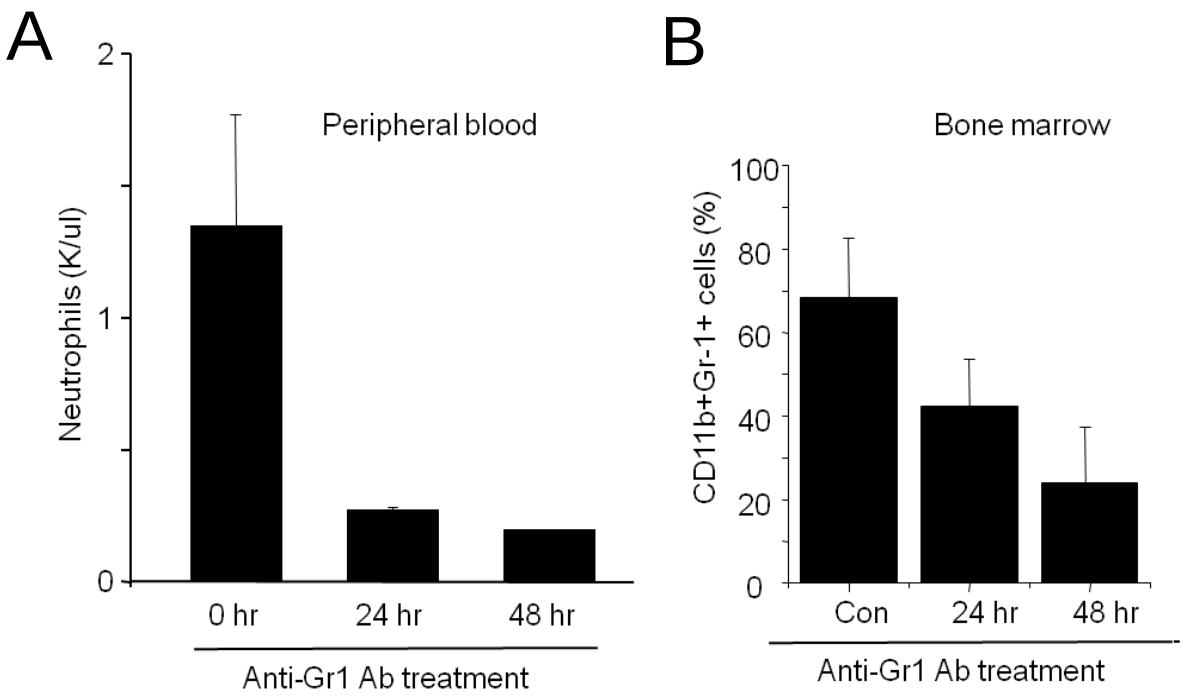

Figure S2, Related to Figure 2. Depletion of neutrophils by Gr1 antibody. (A) The total amount of granulocytes in the BM was assessed by Wright-Giemsa staining of cytospin cells. **(B)** The differential leukocyte count of peripheral blood was measured using a Hemavet-950FS Hematology system. Data shown are means ± SD of n=5 mice. *p<0.01.

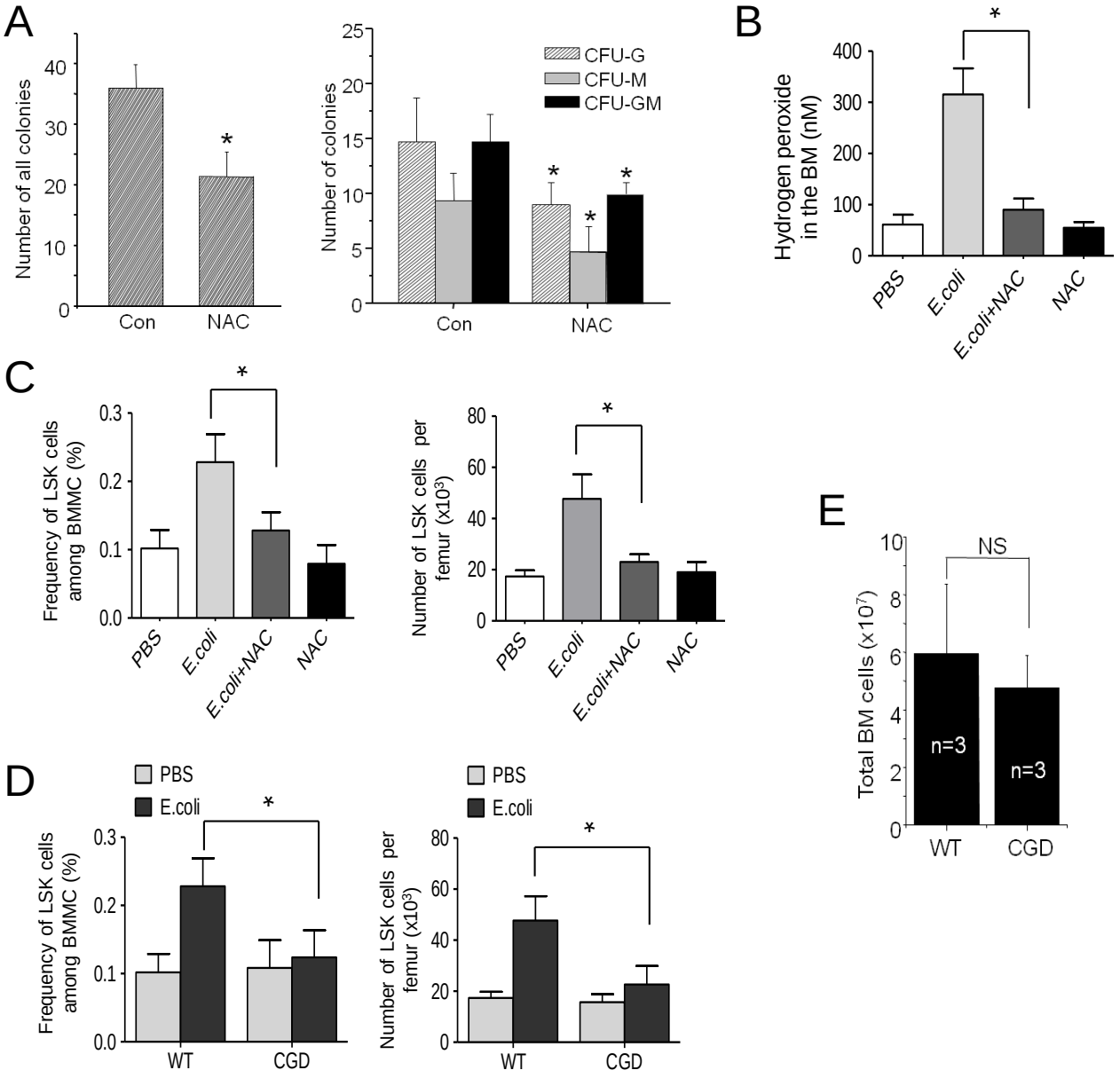

Figure S3, Related to Figure 3. (A) Treatment with antioxidant reduces the colony forming capability of myeloid progenitor cells. The assays were conducted in the presence or absence of NAC (10 mM) as described in Figure 1H. Shown are colony numbers from 20,000 BMMCs. *p<0.01 versus untreated sample. **(B) Treatment with antioxidant NAC suppresses acute inflammation-elicited ROS production in the BM**. NAC treatment and induction of peritonitis with *E.coli* were carried out as described in Figure 3A. The level of H_2O_2 in the BM was quantified as described in Figure 2. Data shown are means ± SD of n=5 mice. *p<0.01 versus untreated mice. **(C) 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 3A-B. Data shown are means ± SD of n=5 mice. *, p<0.01. **(D) Disruption of phagocyte NADPH oxidase (NOX2) suppresses acute inflammation-elicited expansion of BM LSK cells.** The experiment and the flow-cytometry based lineage analysis were conducted as described in Figure 3D-E. Data shown are means ± SD of n=5 mice. *p<0.01 versus mice injected with PBS. **(E) Phagocyte NADPH-oxidase (NOX2) deficiency does not alter bone marrow cellularity.** Shown are the total number of cells in 2 femurs. NS, p>0.05.

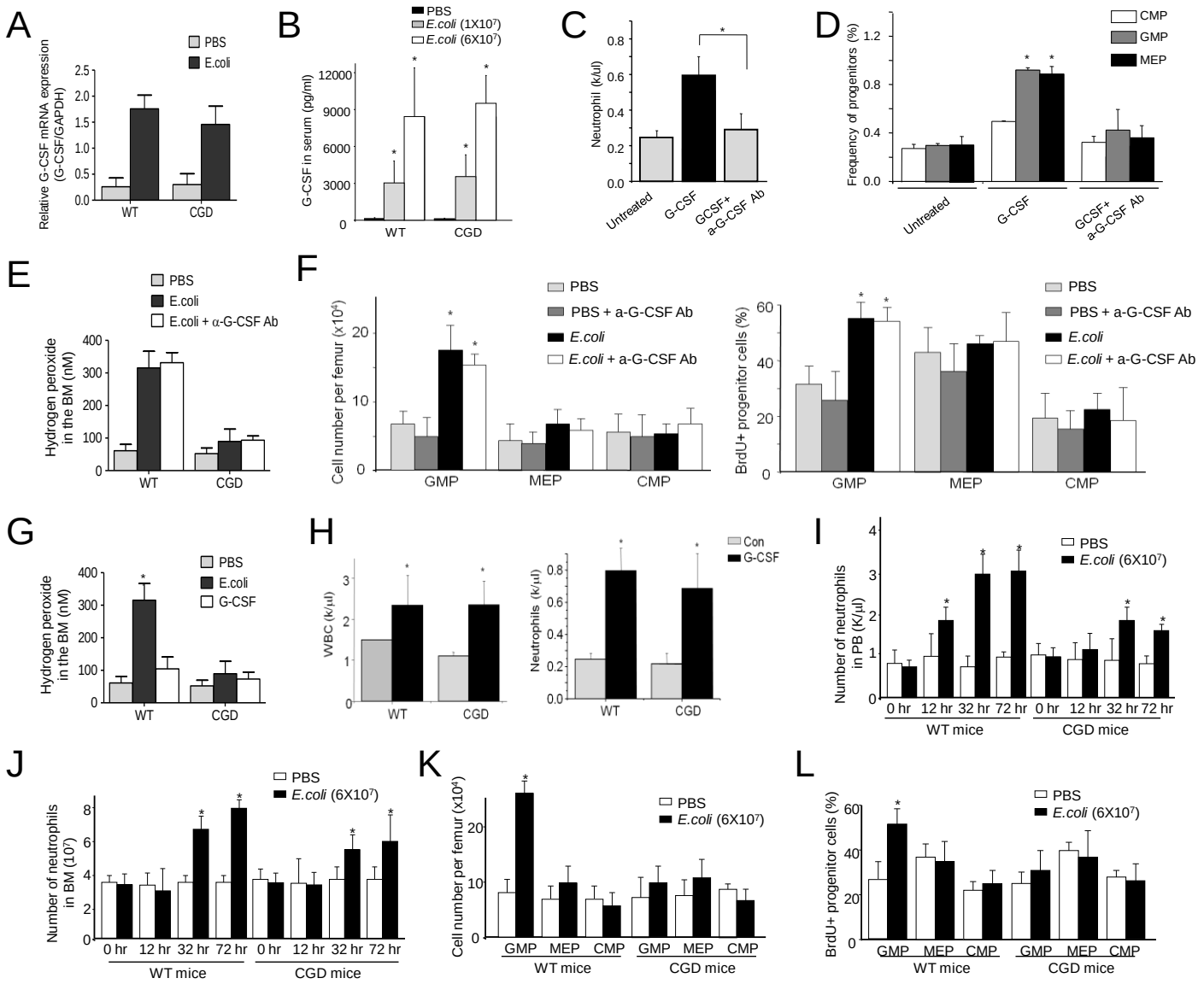

Figure S4, Related to Figure 3. (A) Inhibition of NADPH oxidase-dependent ROS production does not alter acute inflammation-elicited elevation of G-CSF expression in Gr1$^+$ myeloid cells. Induction of peritonitis in WT and CGD mice with *E.coli* was carried out as described in Figure 1. G-CSF mRNA expression was measured 36 hr after *E.coli* injection by quantitative RT-PCR and normalized to GAPDH. Data shown are means ± SD of n=3 mice. **(B) Disruption of phagocyte NADPH oxidase does not alter acute inflammation-elicited elevation of serum G-CSF level.** WT mice were intraperitoneally injected with PBS or heat inactivated *E.coli*. The serum G-CSF level was measured 36 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. **(C) Suppression of G-CSF effect by blocking antibody was confirmed by inhibition of G-CSF-elicited neutrophil mobilization from the BM.** G-CSF was administered by subcutaneous injection (200 μg/kg body weight or about 4μg/mouse). To neutralize G-CSF *in vivo*, mice were injected subcutaneously with 200 μg anti-mouse G-CSF antibody (R & D system, clone67604) 5 min before the G-CSF injection. **(D) Suppression of G-CSF effect by blocking antibody was confirmed by inhibition of G-CSF-elicited expansion of myeloid progenitors.** The flow-cytometry based lineage analysis were conducted 36 hr after the G-CSF injection. **(E) Inhibition of G-CSF does not block acute inflammation-elicited ROS production.** The peritonitis was induced with *E.coli* as described in Figure 1. To neutralize G-CSF *in vivo*, mice were injected subcutaneously with 100 μg anti-mouse G-CSF antibody 1 hr after the *E.coli* injection. Hydrogen peroxide measurement was conducted 35 hr after the antibody administration as described in Figure 2. Data shown are mean ± SD of n=5 mice. **p*<0.01 versus mice injected with PBS. **(F) Inhibition of**

G-CSF does not inhibit *E.coli*-elicited proliferation of BM progenitor cells. The peritonitis was induced with *E.coli* as described in Figure 1. Hematopoietic cell lineage analysis and proliferation assay were conducted 35 hr after the antibody administration. Data shown are mean ± SD of n=3 mice. *, $p<0.01$ versus mice injected with PBS. **(G) Treatment with G-CSF does not significantly elevate ROS level in the BM.** The level of H_2O_2 in the BM was quantified 36 hr after the *E.coli* or G-CSF (250 μg/kg body weight) injection as described in Figure 2. Data shown are mean ± SD of n=3 mice (5 for CGD mice). *, $p<0.01$ versus mice injected with PBS. **(H) G-CSF-elicited neutrophilia is independent of NADPH oxidase-mediated ROS production.** G-CSF was administered by subcutaneous injection (250 μg/kg body weight). Peripheral blood were collected 24 hr following the G-CSF administration. The differential leukocyte count were conducted as described in Figure 1. Data shown are mean ± SD of n=5 mice. *$p<0.01$ versus untreated mice.
(I-L). Emergency granulopoiesis induced by a higher amount of *E.coli*. WT or CGD mice were intraperitoneally injected with PBS or $6x10^7$ heat inactivated *E.coli*. The experiments were conducted essentially as described in Figure 1C-G. **(I)** The number of neutrophils in PB was measured as described in Figure 1A. Data shown are means ± SD of n=5 mice. *$p<0.01$ versus control (time "0"). **(J)** The number of neutrophils in the BM was measured as described in Figure 1B. Data shown are means ± SD of $n=5$ mice. * $p<0.01$. **(K)** Flow cytometry-based lineage analysis of the BM cells. Data shown are means ± SD of $n=3$ mice. * $p<0.01$ versus control (PBS treated mice). **(L)** Measurement of cycling cells in each progenitor population by incorporation BrdU. The percentages of $BrdU^+$ cells in each progenitor compartment are shown. Data shown are means ± SD of $n=3$ mice. * $p<0.01$ versus control (PBS treated mice).

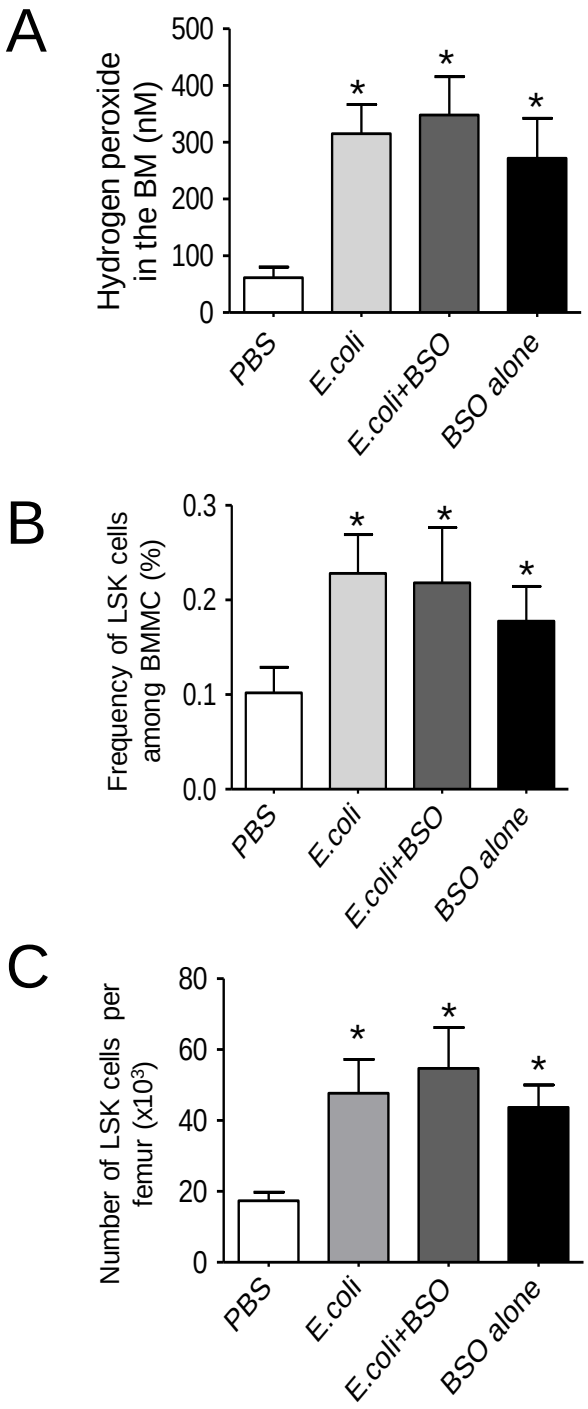

Figure S5, Related to Figure 4. (A) Treatment with BSO elevates ROS level in the BM. BSO treatment and induction of peritonitis with *E.coli* were carried out as described in Figure 4. The level of H_2O_2 in the BM was quantified 36 hr after the *E.coli* injection as described in Figure 2. Data shown are means ± SD of n=5 mice. *p<0.01 versus mice injected with PBS alone. **(B-C) Treatment with BSO leads to expansion of BM LSK cells.** BSO treatment and induction of peritonitis with *E.coli* were carried out as described in Figure 4. The flow-cytometry based lineage analysis were conducted 36 hr after *E.coli* injection as described in Figure 1. **(B)** The percentage of each cell population among BM-derived mononuclear cells (BMMCs). **(C)** The absolute cell number per femur. Data shown are means ± SD of n=5 mice. * p<0.01 versus control (PBS treated mice)**.**

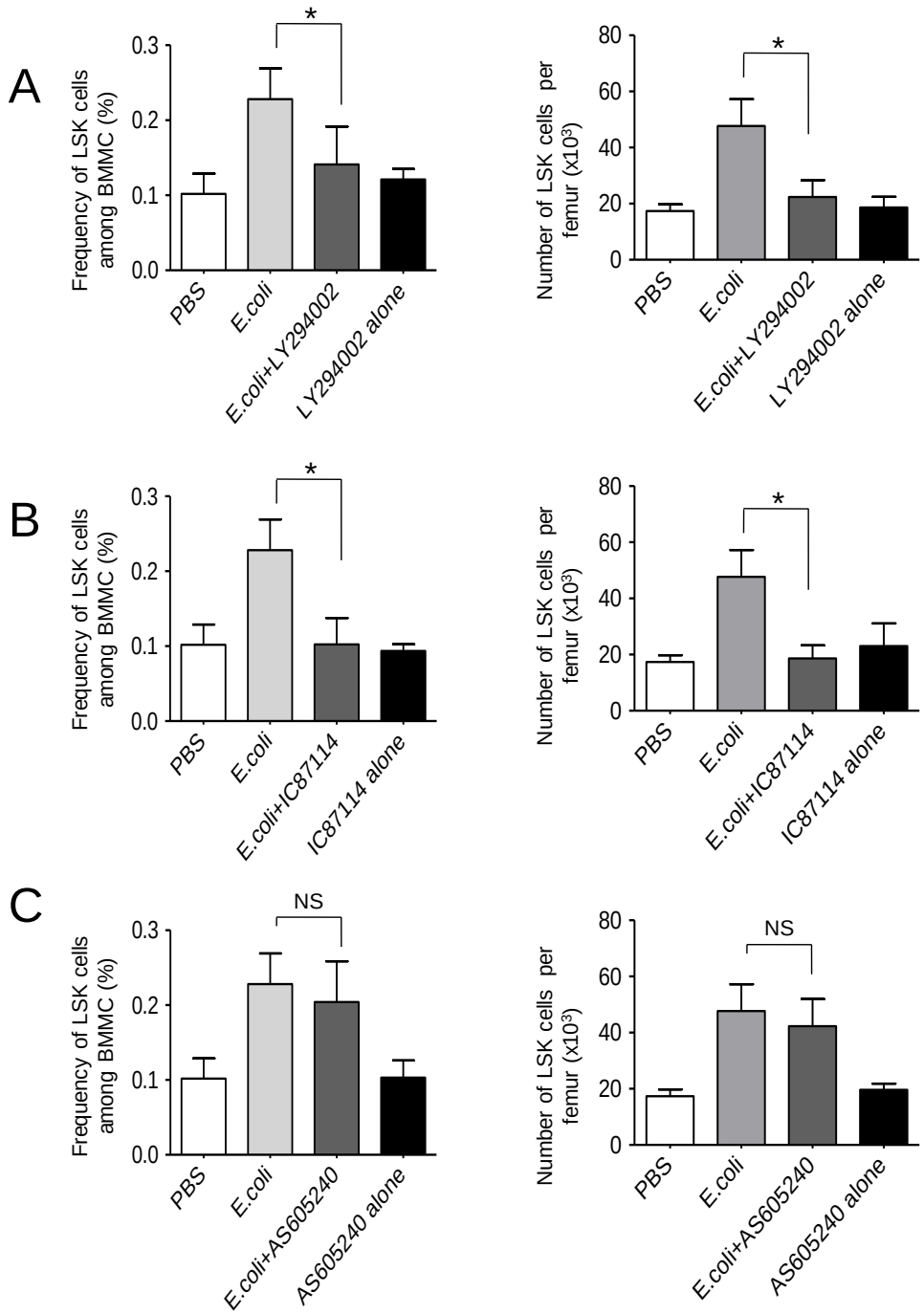

Figure S6, Related to Figure 5. The effect of PI3K inhibitors on acute inflammation-elicited expansion of BM LSK cells. The treatment with inhibitors and induction of peritonitis with *E.coli* were carried out as described in Figure 5F. The flow-cytometry based lineage analysis were conducted 36 hr after the *E.coli* injection as described in Figure 1D-E. Data shown are mean ± SD of n=5 mice. **p*<0.01 versus mice injected with *E.coli* alone. NS, p>0.05.

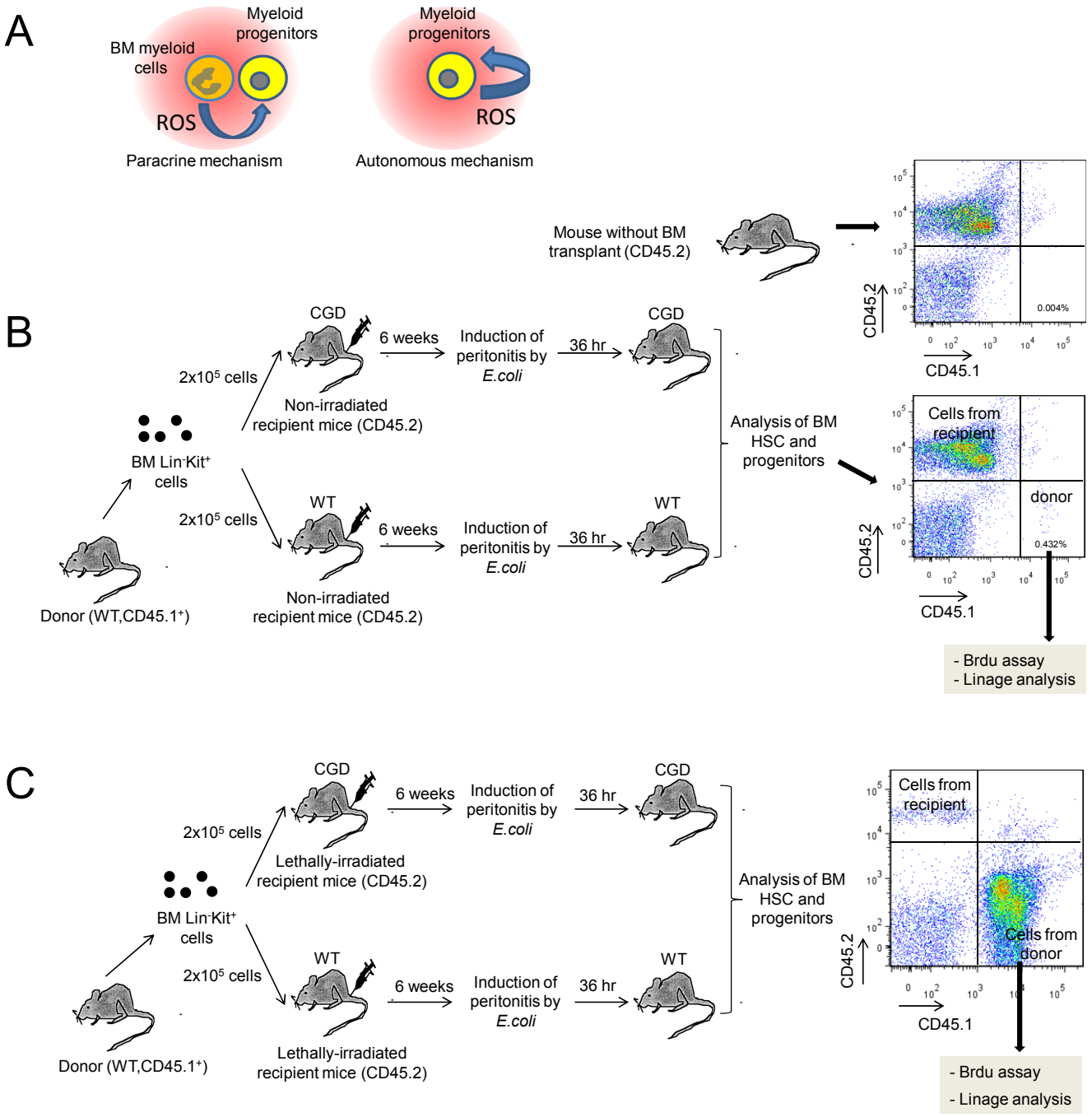

Figure S7, Related to Figure 6. (A) ROS may regulate emergency granulopoiesis both autonomously and non-autonomously. **(B)** The schematic of the BM transplantation experiment (related to Figure 6A-B). To investigate the proliferation of transplanted WT progenitors in CGD recipient mice during acute inflammation, the sorted LK cells of CD45.1 WT mice were transplanted into non-irradiated CD45.2 CGD mice or WT mice (as a positive control). **(C)** The schematic of the BM transplantation experiment (related to Figure 6C-D). Eight week-old WT and CGD mice were lethally irradiated with a dose of 10.2 Gy (two split doses of 5.1 Gy 4 hr apart) using a Cesium-137 Gamma animal irradiator. BM $Lin^{-}Kit^{+}$ cells were obtained by flow cytometry sorting, and were injected into the lethally irradiated recipients intravenously. Bone marrow reconstitution was confirmed 6 weeks after the BM transplantation.

Supplemental Table:

Table S1, Related to Figure 3.

Age	Mice	Peripheral blood parameters (K/ul)					
		WBC	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
8 wks	WT	2.390±0.551	0.775±0.134	1.315±0.445	0.205±0.176	0.085±0.049	0.010±0.014
	CGD	3.421±1.131	0.612±0.269	2.496±0.635	0.283±0.113	0.075±0.037	0.010±0.012
11 wks	WT	2.782±0.648	0.681±0.174	1.634±0.369	0.330±0.217	0.123±0.064	0.020±0.031
	CGD	2.364±0.735	1.103±0.521	0.727±0.262	0.110±0.043	0.122±0.072	0.010±0.023

Table S1, Related to Figure 3. Phagocyte NADPH-oxidase deficiency does not lead to abnormal peripheral blood counts. The differential leukocyte count was measured using a Hemavet-950FS Hematology system. Data shown are means ± SD of n=5 mice.

Supplemental Experimental Procedures

Mice

X-linked CGD mice (on C57BL/6 background) (Pollock et al., 1995) that contain disrupted alleles of the gene encoding gp91phox were purchased from Jackson Laboratories. In all of the experiments performed with the CGD mice, we used C57BL/6 mice of the same age as WT controls. Every donor and recipient mouse used in the transplantation experiments was carefully genotyped for its CD45.1 and/or CD45.2 expression before the actual experiment. The numbers of mice analyzed per group for these transplant experiments are indicated in the figures; the data presented are a combination of three sets of separate experiments. Mice conditionally expressing EGFP (eGFP loxP/loxP) and the myeloid-specific Cre mice were purchased from Jackson Laboratories. Myeloid specific EGFP gene expression was achieved by breeding EGFP mice with myeloid specific Cre mice (LyzM-EGFP mice). All animal manipulations were conducted in accordance with the Animal Welfare Guidelines of the Children's Hospital Boston. All procedures were approved and monitored by the Children's Hospital Animal Care and Use Committee.

Hematologic analysis

Adult wild type and CGD mice were anesthetized and immediately bled retro-orbitally into an EDTA-coated tube. Complete blood counts were performed using an automated hematology analyzer (Hemavet 850; Drew Scientific, Oxford, CT). For BM cells, the total cell counts were determined using a hemacytometer, and the differential cell counts were conducted by microscopic analysis of Wright-Giemsa–stained cytospin or FACS analysis using CANTOII flow cytometer and FACSDiva software (BD Biosciences). The absolute number of neutrophils was then determined based on the cytospin or FACS analysis.

Flow cytometry and antibodies

Mice used for analysis were between 7 and 9 weeks old males. Single-cell suspensions of BM were obtained by crushing both tibias and femurs using a mortar and pestle and filtering through 40-μm cell strainers. Erythrocytes in the sample were lysed with an ACK lysis buffer (GibcoBRL). The cells were washed with a buffer containing 2% FCS in PBS. The antibodies used for flow cytometry included the following: APC-conjugated lineage markers specific for CD3e (145-2C110), CD4 (RM4-5), CD8a (53-6.7), CD11b(M1/70), B220 (RA3-6B2), GR-1 (RB6-8C5), and Ter119 (TER119) from eBioscience, Biolegend or BD pharmingen. Other antibodies included PC-Cy7- or FITC-conjugated Sca-1 (D7), APC-Cy7-conjugated c-kit (2B8), APC-

conjugated CD45.2 (104), PE-Cy5-conjugated CD3e (145-2C11), PE-conjugated CD45.1 (A20), PE-conjugated CD16/32 (93), FITC-conjugated CD34 (RAM34). Unstained cells were used as negative control to establish the flow cytometer voltage setting, and single-color positive controls were used for adjustment of the compensation. The flow cytometric data were acquired using FACScalibar, and raw data were analyzed with Flowjo software (Treestar Inc). Hematopoietic stem cell population (HSC) was defined as $Lin^{-}IL\text{-}7R\alpha^{-}cKit^{+}Sca1^{+}$ (LSK) cells; hematopoietic progenitor cell (HPC) population was defined as $Lin^{-}IL\text{-}7R\alpha^{-}cKit^{+}Sca1^{-}$ (LK) cells; common lymphoid progenitor (CLP) population was defined as Lin^{-} $IL\text{-}7R\alpha^{+}cKit^{int}Sca1^{int}$ cells; common myeloid progenitor (CMP) population was defined as $Lin^{-}IL\text{-}7R\alpha^{-}$ $c\text{-}Kit^{+}Sca\text{-}1^{-}Fc\gamma II/III\ R^{Int}CD34^{+}$ cells; granulocyte and monocyte progenitor (GMP) population was defined as $Lin^{-}IL\text{-}7R\alpha^{-}c\text{-}Kit^{+}Sca\text{-}1^{-}Fc\gamma II/III\ R^{+}CD34^{+}$ cells; and Megakaryocyte erythroid progenitor (MEP) was defined as $Fc\gamma II/III\ R^{low}CD34^{low}c\text{-}Kit^{+}Sca\text{-}1^{-}IL\text{-}7R\alpha^{-}$ cells.

HSC and HPC sorting

Bone marrow cells were resuspended in 3 ml IMDM buffer and loaded on the top of Histopaque 1083 (Sigma-aldrich) to prepare low density bone marrow cells. Briefly, cells were centrifuged for 25 min at 1700 rpm with the brake off. The intermediate cell layer was removed and transferred to a 50 ml tube. Cell suspension was then centrifuged for 5 min at 1500 rpm and cell pellet was resuspended in 1 ml PBS with 2% PBS. For sorting, cells were stained with the APC-conjugated lineage-specific markers, PE-conjugated c-kit (2B8) and FITC-conjugated Sca-1 (D7). Cells were then sorted through a Lin-c-kit+Sca-1+ for LSK or Lin-c-kit+Sca-1- for LK cells, using FACS AriaII equipped with FACSDiva software (BD Bioscience).

Granulocyte/Monocyte Colony forming Unit (CFU-GM) assays

Bone marrow cells ($2x10^{4}$) from WT or CGD mice were seeded in semisolid Methocult GF M3534 medium containing rmSCF, rmIL-3 and rhIL-6 for detection of CFU-GM (Stem Cell Technologies). Number of colonies that contained more than 50 cells was counted on day 7. The size of the colonies were also measured on day 7. Colony morphology was scored on the basis of Stemcell Technologies criteria. L-butionine-sulfoxamine (BSO, Sigma-Aldrich) was added to methylcellulose media at the indicated concentrations at the time of plating.

Detection of hydrogen peroxide using Amplex Red

ROS accumulation in the bone marrow during acute inflammation was measured in freshly isolated bone marrow using an Amplex red Hydrogen Peroxide assay Kit. Amplex Red (Invitrogen), a H_2O_2-sensitive fluorescent probe, was prepared according to the manufacturer's instructions. WT and CGD mice were

intraperitoneally injected with 1 ml of 3% Thioglycollate or heat inactivated *E.coli* ($1x10^7$) in PBS. At the indicated times, mice were euthanized and BM were prepared by spinning femurs and tibias with 100 μl Krebs-Ringer phosphate buffer containing 5.5 mM glucose (pH 7.35). After further centrifugation (180*g* for 5 minutes) of the collected BM samples, the BM supernatant (extracellular ROS) was harvested and 50 μl was assayed (in duplicated) in 96-well fluorescent assay plates (Thermo Fisher Scientific) containing 50 μl/well Amplex Red solution with 0.2 U HRP. Fluorescence was recorded using a fluorometer (excitation, 540 nm; emission, 590 nm). The concentration of H_2O_2 was determined using a standard curve.

Analysis of *in vivo* cell proliferation by BrdU incorporation

Cell proliferation was determined using a BrdU labeling kit (BD bioscience). Twenty four hours before sacrifice, BrdU was administrated by intraperitoneal injection (2 mg/mouse in 200 μl PBS) as a single dose. At indicated time points, LSK, GMP, CMP, MEP cells were sorted from BM BMMC. Sorted cells were fixed in 70% ethanol overnight at –20°C, denatured in 2N HCl/0.5% Triton X-100 for 20 minutes at room temperature, neutralized with 0.1 M sodium borate for 5 minutes, and stained with anti-BrdU antibody (BD Biosciences, MD USA) for 30 minutes at room temperature in PBS/0.5% BSA/0.5% Tween 20. Cells were then resuspended in 500 μl PBS containing 10 μg RNase A and 5 μg Propidium Iodide (PI), incubated for 30 minutes, and immediately analyzed by flow cytometry. The mean frequencies of BrdU+ cells in the HSC and each progenitor populations were measured.

Neutrophil depletion with Gr-1 antibody

Neutrophil depletion was achieved by intraperitoneal injection of anti-Gr1 mAb RB6-8C5 (200 μg/kg). The antibody was administered i.p. to obtain a sustained depletion over the first 48 hours of the experiment. Differential white blood cell count using Wright-Giemsa staining was performed to confirm that the neutrophil depletion was successful.

G-CSF treatment and neutralization by anti-G-CSF antibody

Recombinant G-CSF (Amgen) was diluted in sterile PBS. G-CSF was administered by subcutaneous injection (250 μg/kg body weight). Bone marrow cells and peripheral blood were collected 24 hours following the G-CSF administration. Hydrogen peroxide production in the BM was measured as described above. To neutralize G-CSF *in vivo*, mice were injected subcutaneously with 100 μg anti-mouse G-CSF antibody (R & D system, clone67604). Hematopoietic cell lineage analysis and hydrogen peroxide measurement were conducted 24 hours following the antibody administration.

PTEN oxidation and Akt activation in the progenitor cells analyzed by western blotting

Wild-type and CGD mice were treated with 1 x 10^6 *E.coli* (PBS as control) intraperitoneally for 24 hrs. LK cells were sorted using a FACS AriaII cell sorter. To obtain enough materials for western blotting, three mice were used for one data point in one single experiment. The sorted LK cells from each of the 3 mice were put together and half million LK cells were used for the assay. The cell pellets were lysed with 1x lysis buffer (30% 4x Invitrogen Nu-Page LDS buffer, 6% b-mercaptoethanol and 8% protease inhibitor cocktail in PBS, 95C). Cell extracts were resolved on Nu-Page 4-12% Bis-Tris gels, transferred onto PVDF membranes and then immunoblotted against S473P-Akt antibody (1:1000) or Akt antibody (1:5000). Densitometry of the blots was performed using the ImageJ software Gel Analyzer plugin. Phospho-Akt levels were then normalized based on total Akt levels(Subramanian et al., 2007). For PTEN oxidation analysis, the cell pellets were lysed with non-reducing LDS loading buffer. Cell extracts were then resolved on non-reducing SDS-PAGE . Both reduced and oxidized PTEN could be detected using a specific PTEN antibody(Silva et al., 2008).

Two-photon intravital microscopy

Mice were anesthetized with ketamine hydrochloride (100 mg/kg intraperitoneally) and xylazine (10 mg/kg intraperitoneally) and frontoparietal skull bone was exposed and prepared following previously established protocols (Mazo et al., 1998). Two-photon microscopy on the calvarium bone marrow was performed using an Olympus BX50WI fluorescence microscope equipped with a 20×, 0.95 numerical aperture objective (Olympus) and a Bio-Rad Radiance 2000MP Multiphoton system, controlled by Lasersharp software (Bio-Rad). For two-photon excitation and second harmonic generation, a MAiTai Ti:sapphire laser was tuned to a range of wavelengths from 800 nm to 875 nm. The blood vesicles were labeled with Tetramethylrhodamine-dextran (Invitrogen, 2,000,000 MW). Myeloid cells in the mice expressing EGFP (LyzM-EGFP) were detected via eGFP-fluorescence and bone was visualized by its second harmonic generation signal. To detect H2O2 in the BM, PO1 (0.1mM in 100 µl PBS, a generous gift from Cris Chang, UC Berkley) was injected intravenously. Images were recorded every 30-40 sec for 10 minutes. The generated sequences of image stacks were transformed into volume-rendered four-dimensional movies using Volocity® (Perkin Elmer/Improvision) which was also used for tracking of cell motility in three dimensions.

Immunofluorescent staining and laser scanning cytometry of BM sections.

Mice were perfused post-mortem with 10 ml paraformaldehyde-lysine-periodate (PLP) fixative through the vena cava to achieve rapid *in situ* fixation and optimal preservation of the bone marrow tissue. Femoral bones

were isolated, fixed in PLP for 4-8 hours, rehydrated in 30% sucrose/PBS for 48 hours and snap frozen in OCT (TissueTek). Cryosections of non-decalcified whole longitudinal femoral bones were obtained using a Leica Cryostat and the Cryojane tape transfer system (Leica Microsystems). Bone marrow sections were stained with the indicated antibodies. DAPI (Invitrogen) staining was used for nuclear detection and sections were mounted with Vectashield mounting medium for immunofluoresence (Vector Labs). High resolution images of whole longitudinal immunostained femoral sections were obtained with a iCys Research Imaging Cytometer (Compucyte Corporation) equipped with four laser lines (405, 488, 561 and 633 nm) and four PMT detectors with bandpass emission filters at 450/,40, 521/15, 575/50 and 650LP.

Expression of gp91phox (NOX2) in hematopoietic and non-hematopoietic cells.

Bone marrow $CD45^+$ hematopoietic cells, $CD45^-$ nonhematopoietic cells, $Gr1^+$ myeloid cells, endothelial cells ($Sca\text{-}1^+CD31^+CD45^-Ter119^-$), CXCL12-abundant reticular (CAR) cells ($PDGFR\text{-}\beta^+Sca\text{-}1^-CD31^-CD45^-Ter119^-$)(Omatsu et al., 2014; Omatsu et al., 2010), and PαS multipotent stromal cells ($CD45^-Ter119^-CD31^-PDGFR\alpha^+Sca\text{-}1^+$) (Morikawa et al., 2009) were obtained by flow cytometry sorting using specific antibodies. Total RNA was extracted with the TRIzol reagent (Invitrogen) according to the manufacturer's protocol, digested by RNase-free DNase, dissolved in diethyl pyrocarbonate-treated water, and stored at −80°C prior to use. For quality control, RNA purity and integrity were evaluated by agarose gel electrophoresis and the OD260/OD280 ratio. Quantitative real-time RT-PCR was performed using a SYBR Green Quantitative RT–PCR Kit (Sigma) on a CFX96 Thermal Cycler. The sequence of murine gp91 primers were as follows: forward, 5' - AGGAGTGCCCAGTA - 3'; reverse, 5' - CACTAACATCACCAC - 3'. The qRT-PCR reactions were performed in a volume of 50 μl and contained 1 μg of total RNA, 320 nM forward and reverse primers. Reactions were incubated at 94°C for 3 min, and then amplified for 36 cycles, each cycle comprised of an incubation step at 94°C for 30 sec followed by 58°C for 1 min and 72°C for 1 min; and the reactions were completed with a step at 72°C for 2 min. The level of gene mRNA was evaluated in automated analysis by MxPro QPCR Software. The relative gene expression level was calculated using GAPDH as a control.

Supplemental References

Mazo, I.B., Gutierrez-Ramos, J.C., Frenette, P.S., Hynes, R.O., Wagner, D.D., and von Andrian, U.H. (1998). Hematopoietic progenitor cell rolling in bone marrow microvessels: parallel contributions by endothelial selectins and vascular cell adhesion molecule 1. J Exp Med *188*, 465-474.

Pollock, J.D., Williams, D.A., Gifford, M.A., Li, L.L., Du, X., Fisherman, J., Orkin, S.H., Doerschuk, C.M., and Dinauer, M.C. (1995). Mouse model of X-linked chronic granulomatous disease, an inherited defect in phagocyte superoxide production. Nat Genet *9*, 202-209.

Silva, A., Yunes, J.A., Cardoso, B.A., Martins, L.R., Jotta, P.Y., Abecasis, M., Nowill, A.E., Leslie, N.R., Cardoso, A.A., and Barata, J.T. (2008). PTEN posttranslational inactivation and hyperactivation of the PI3K/Akt pathway sustain primary T cell leukemia viability. J Clin Invest *118*, 3762-3774.

Subramanian, K.K., Jia, Y., Zhu, D., Simms, B.T., Jo, H., Hattori, H., You, J., Mizgerd, J.P., and Luo, H.R. (2007). Tumor suppressor PTEN is a physiologic suppressor of chemoattractant-mediated neutrophil functions. Blood *109*, 4028-4037.