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Supplemental Information

Murine Myeloid Progenitors Attenuate Immune Dysfunction Induced by Hemorrhagic Shock

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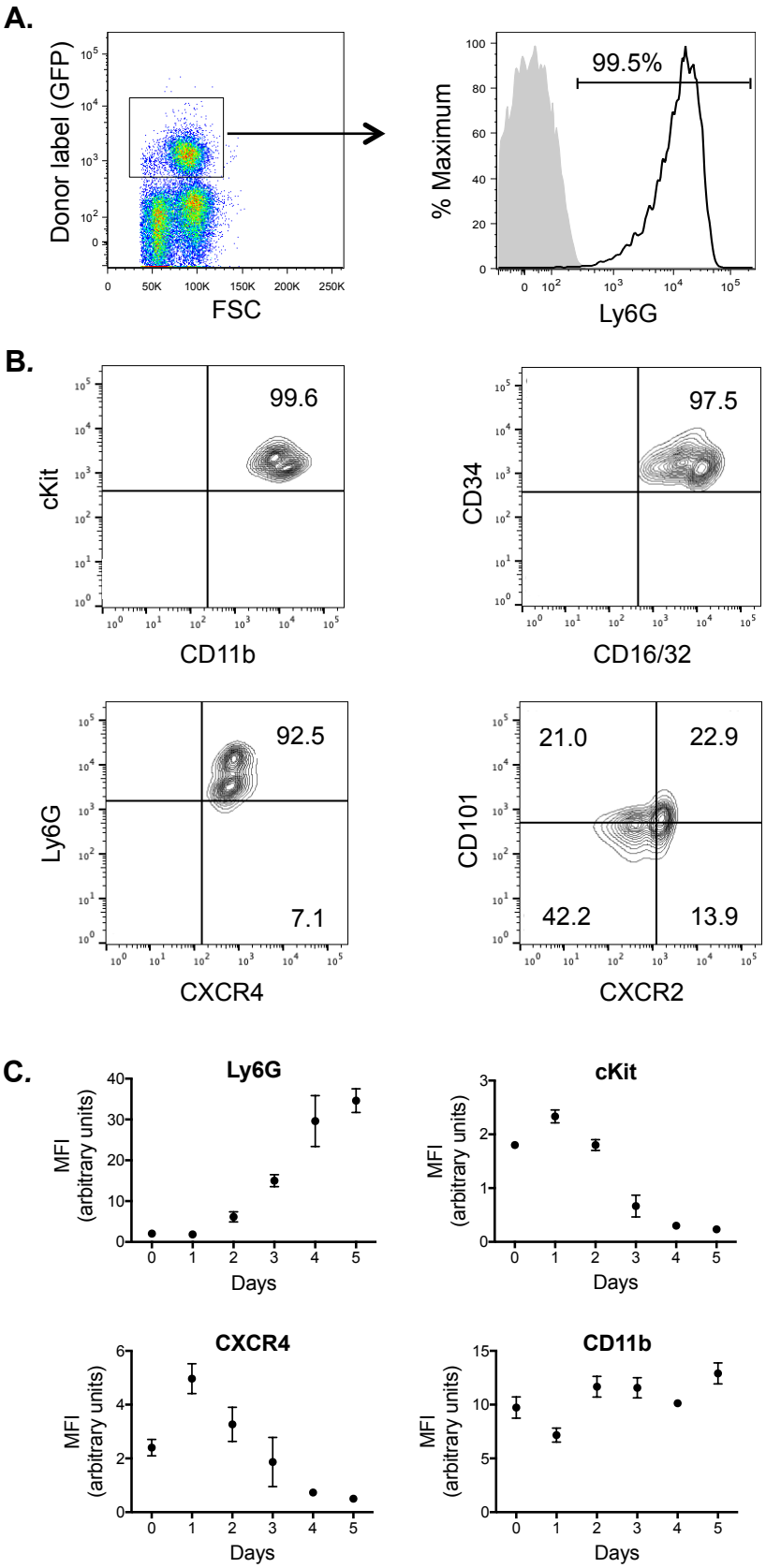
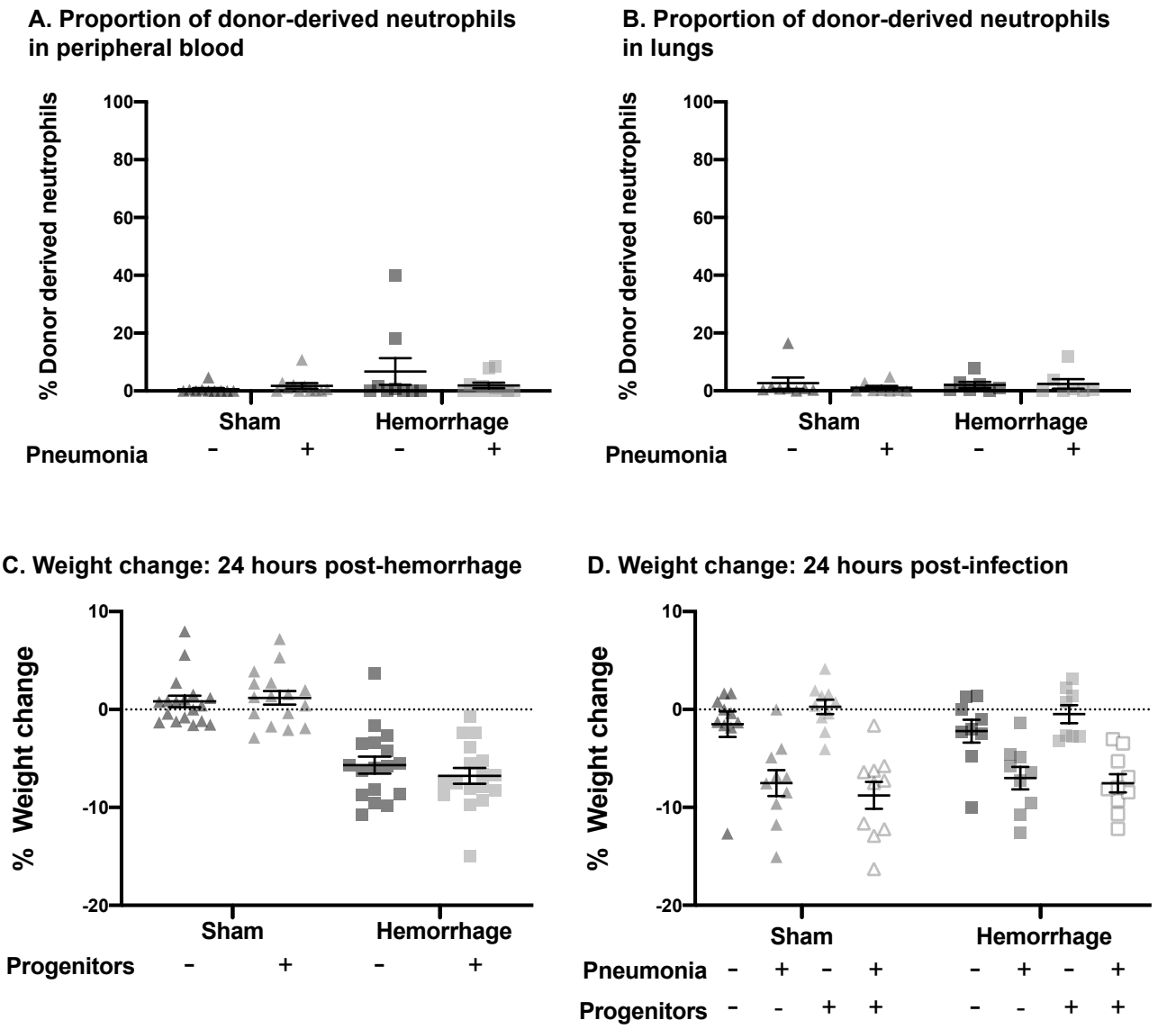
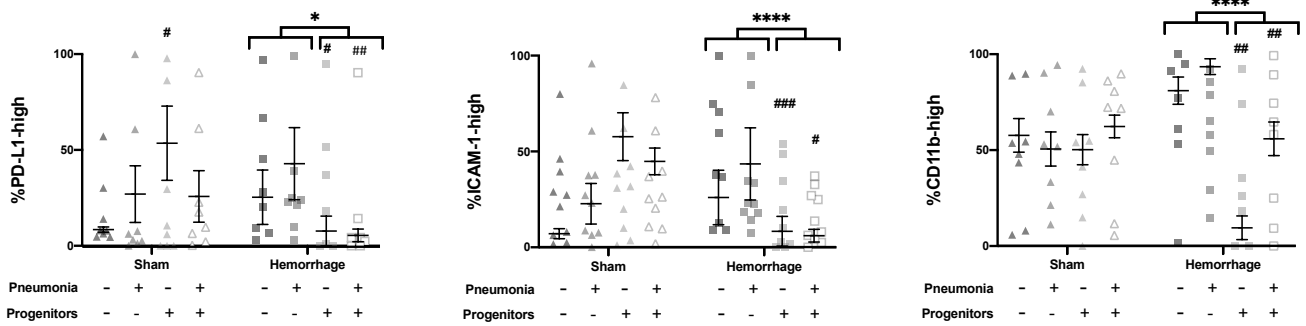


Supplementary Figure 1. HOXB8 conditionally immortalized myeloid derived neutrophil progenitors are most similar to a PreNeu. (A) Representative flow cytometry data analyses of the Ly6G expression of all donor-derived cells (gated by GFP expression) in the peripheral blood 7 days after NP transplantation. (B) Flow cytometry analysis of cKit, CD11b, CD34, CD16/32, CXCR4, Ly6G, CD101, and CXCR2 to determine cell maturity state. Data are expressed as MFI and gates shown are based on unlabeled controls. Plots shown are representative of three independent experiments. (C) Flow cytometry quantification of expression changes in Ly6G, cKit, CXCR4, and CD11b during *in vitro* differentiation in the presence of G-CSF.

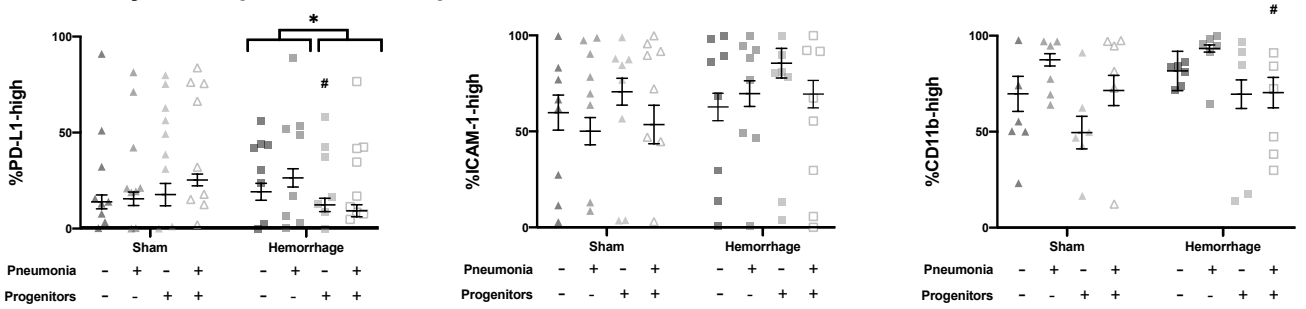


Supplementary Figure 2. Microchimerism and mouse response to hemorrhage/infection. (A-B) The degree of neutrophil michrochimerism in the (A) peripheral circulation (n = 10 mice for sham, 11 mice for sham/pneumonia, 9 mice for hemorrhage, and 11 mice for hemorrhage/pneumonia) and (B) lung homogenate (n = 8 mice for sham conditions and 7 mice for hemorrhage conditions) at 24 hours post-infection was determined by flow cytometry 24 hours after infection in mice that had received prior hemorrhagic shock or sham hemorrhage, with or without adoptive transfer of neutrophil progenitors. Neutrophils were identified by Ly6G expression. Donor-derived neutrophils were distinguished from host neutrophils by GFP expression. Data are expressed as mean percent donor derived neutrophils ± SEM. Data were fit to a binomial distribution, analyzed by generalized linear models, and the Holms test was used to adjust for multiple comparisons. (C-D) Mice were subjected to hemorrhagic shock or sham hemorrhage, with or without adoptive transfer of neutrophil progenitors, followed by induction of *S. aureus* pneumonia 48 hours after hemorrhage. Animal weight was measured prior to hemorrhage, 24 hours post-hemorrhage, prior to infection, and 24 hours post-infection. (C) Animal weight prior to hemorrhage and 24 hours post-hemorrhage were compared, as were (D) animal weight prior to infection and 24 hours post-infection. Weight change data represents a repeated measure of the same mice 24 hours post-hemorrhage and then 24 hours post-infection. Data are expressed as mean percent weight change ± SEM. Data were fit to a lognormal distribution, analyzed by generalized linear models, and the Holms test was used to adjust for multiple comparisons.

A. Circulating Neutrophils: 24 hours post-infection

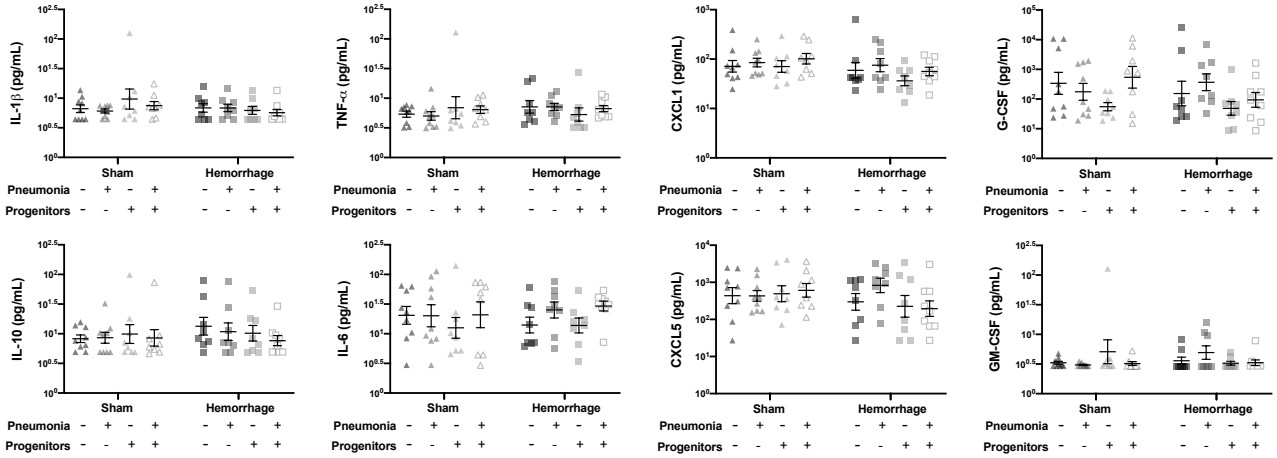


B. Pulmonary Neutrophils: 24 hours post-infection

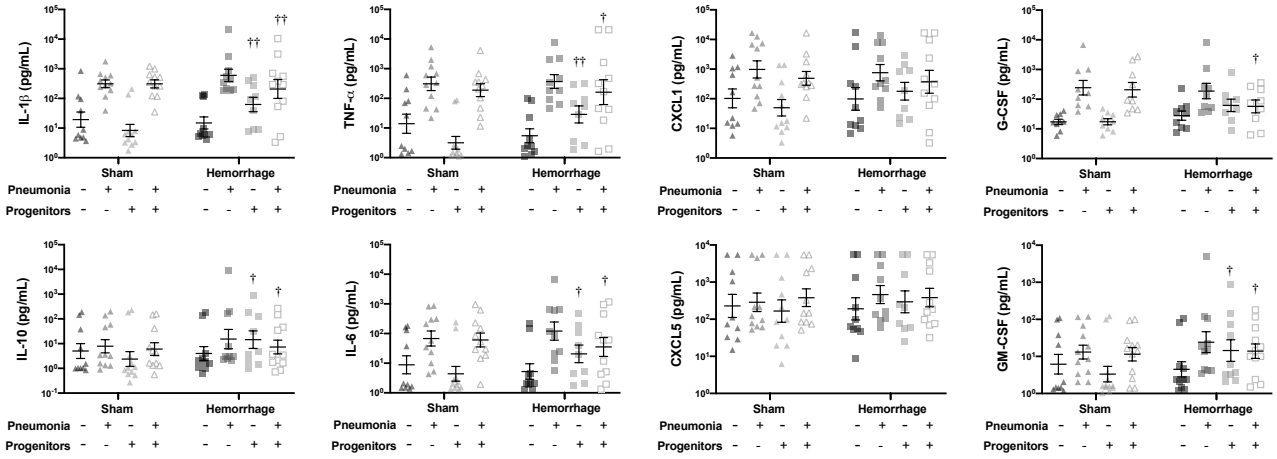


Supplementary Figure 3. The proportion of suppressive, primed, and activated neutrophils is decreased by transplantation of neutrophil progenitors independent of pneumonia. Flow cytometry analysis of PD-L1, ICAM-1, and CD11b expression by peripheral blood and pulmonary neutrophils. Mice were subjected to hemorrhagic shock or sham hemorrhage with or without adoptive transfer of progenitor cells followed by induction of *S. aureus* pneumonia 48 hours after hemorrhage. (A) Mean proportion of peripheral blood neutrophils expressing PD-L1 (n=8 mice per condition except for hemorrhage with progenitors without pneumonia where n=7 mice), ICAM-1 (n=10 mice per condition except for hemorrhage conditions without pneumonia where n=9 mice), and CD11b (n=8 mice per condition except for hemorrhage conditions without pneumonia where n=7 mice) 24 hours post-infection. Each distinct surface marker represents a repeated measure of the same sample. (B) Mean proportion of pulmonary neutrophils expressing PD-L1 (n=9 mice per sham condition and hemorrhage with progenitors with pneumonia, n=7 mice for hemorrhage with progenitors without pneumonia, n=8 mice per condition for remaining conditions), ICAM-1 (n=8 mice per condition except hemorrhage with progenitors without pneumonia where n=7 mice), and CD11b (n=6 mice per condition except hemorrhage with progenitors without pneumonia where n=5 mice) 24 hours post-infection. Each distinct surface marker represents a repeated measure of the same sample. Data are presented as mean percentage of neutrophils expressing a given surface protein ± SEM. Data were fit to a binomial distribution, analyzed by generalized linear models, and the Holms test was used to adjust for multiple comparisons. The hemorrhaged mice presented represent the same experiments that are presented in Figure 2, but are included to demonstrate statistical comparisons and for the reader clarity. *: $P<0.05$, ****: $P<0.0001$ comparing the effect of progenitors on hemorrhage independent of pneumonia; #: $P<0.05$, ##: $P<0.01$, ### $P<0.001$ compared with matched group that received no progenitor cells.

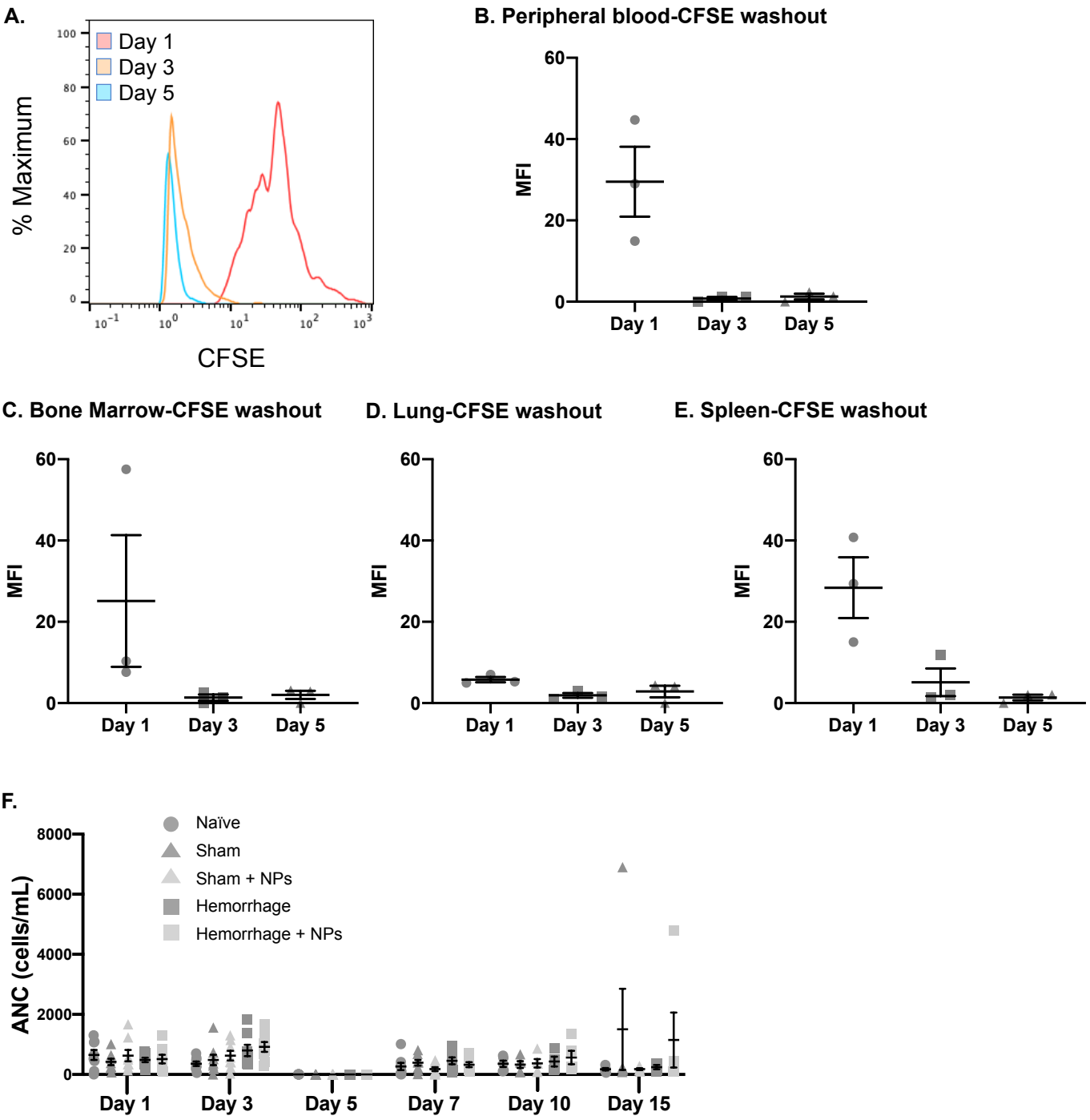
A. Serum cytokine levels: 24 hours post-infection



B. Lung cytokine levels: 24 hours post-infection



Supplementary Figure 4. Neutrophil progenitors do not influence the serum cytokine profile 24 hours after hemorrhage or pneumonia but do modulate the pulmonary microenvironment. Concentration of IL-6, CXCL1, TNFa, IL-1bIL-10, G-CSF, GM-CSF, and CXCL5 were analyzed by cytometric bead array in (A) the serum 24 hours following induction of pneumonia (n=9 mice for hemorrhage with progenitors with pneumonia and sham conditions without pneumonia, n=8 mice for the remaining conditions, each distinct cytokine represents a repeated measure of the same sample) and (B) Lung homogenate 24 hours following induction of pneumonia (n=9 mice for hemorrhage with progenitors without pneumonia, n=10 mice for hemorrhage conditions without progenitors and the sham condition without progenitors or pneumonia, n=11 for the remaining conditions, each distinct cytokine represents a repeated measure of the same sample) were measured by cytometric bead array 24 hours post-infection. The hemorrhaged mice presented represent the same experiments that are presented in Figure 3, but are included to demonstrate statistical comparisons and for reader clarity. Data are expressed as means ± SEM. Data were fit to a lognormal distribution, analyzed by generalized linear models, and the Holms test was used to adjust for multiple comparisons. *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$, ****: $P<0.0001$ comparing the effect of progenitors on hemorrhage independent of pneumonia; #: $P<0.05$, ##: $P<0.01$ compared with matched sham hemorrhage controls. †: P<0.05, ††: P<0.01 compared with matched group that received no neutrophil progenitors.



Supplementary Figure 5. Transplanted neutrophil progenitors proliferate *in vivo*. CFSE-labeled neutrophil progenitors were adoptively transferred into naïve mice. Proliferation of progenitors was determined by CFSE dilution at days 1, 3, and 5 after transplant. (A) Representative plots of expression levels by mean fluorescence intensity (MFI) over the course of 3 serial time points in bone marrow. MFI for (B) peripheral blood, (C) bone marrow, (D) lungs, and (E) spleen at days 1, 3, and 5 (n=3 independent experiments). CFSE wash-out data were taken from distinct samples. Data are expressed as mean MFI ± SEM. (F) Mice were subjected to either hemorrhagic shock or sham hemorrhage and then received transplant with or without NPs. Absolute neutrophil count (ANC) was determined by the number of Ly6G-positive cells per mL in the peripheral blood (n = 6 for hemorrhage day 5, n = 7 for sham day 5, n = 5 mice per condition for days 10 and 15, n = 8 for the remaining conditions). Data are expressed as mean cells/mL ± SEM. Data were fit to a lognormal distribution, analyzed by generalized linear models, and the Holms test was used to adjust for multiple comparisons.