SUPPLEMENTAL MATERIAL

Devi et al., http://www.jem.org/cgi/content/full/jem.20130056/DC1

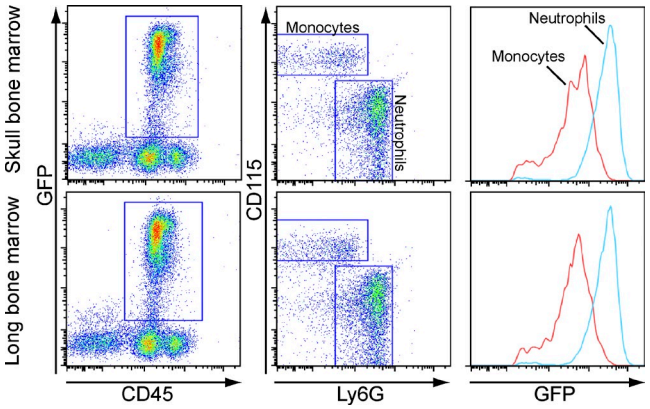
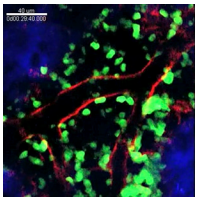
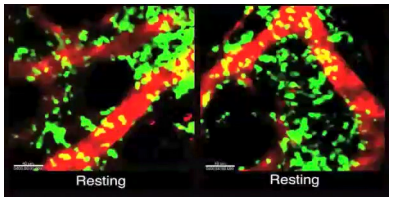


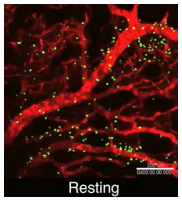
Figure S1. Assessment of leukocyte GFP expression in the BM of LysM-GFP mice. A representative flow cytometry plot showing the GFP expression of monocytes and neutrophils in the skull BM (top) and tibial BM (bottom) of LysM-GFP mice. The dot plots depict the gating strategy. Histograms show that monocytes ($CD115^+Ly6G^-$, red lines) have lower GFP intensity than neutrophils ($CD115^-Ly6G^+$, blue lines). Data were obtained from three independent experiments. As shown here, neutrophils constitute the major population of these GFP^+ cells (>70%), and they have the highest GFP expression. Consequently, neutrophils can be easily distinguished from other myelomonocytic cell types based on their high GFP intensity.



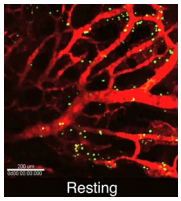
Video 1. Neutrophil behavior in the skull BM. A time-lapse image sequence of maximum intensity projection depicting the migratory behavior of GFP^+ neutrophils in the skull bone of chimeric mice (LysM-GFP BM into irradiated mT mice) under homeostatic conditions. White box indicates a region of interest, which shows GFP^+ neutrophils (arrows) migrating across sinusoid wall (red) to enter into circulation. Blue signals represent second harmonic generation from the bone collagen. Elapsed time is shown in hours:minutes:seconds.



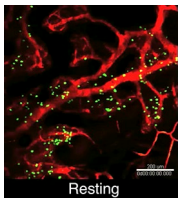
Video 2. Dynamic behavior of neutrophil in the BM in response to G-CSF or plerixafor treatment. Time-lapse image sequences of maximum intensity projection depicting the migratory behavior of GFP^+ neutrophils in response to G-CSF or plerixafor treatment. Sinusoids are labeled with intravenous injection of 155 kD TRITC-conjugated dextran. The first 10 min of video demonstrates the basal neutrophil activity. Left, within 30 min after G-CSF treatment, neutrophils could be seen to migrate toward the BM sinusoid and being released into the circulation. Right, plerixafor does not alter the motility pattern of GFP^+ neutrophils in the BM. White box indicates a region of interest. Elapsed time is shown in hours:minutes:seconds.



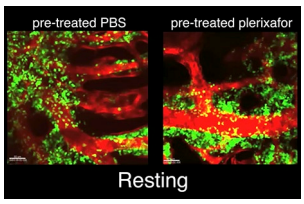
Video 3. G-CSF increases neutrophil egress from the BM. A time-lapse image sequence of maximum intensity projection depicting the migratory behavior of transferred GFP^+ neutrophils in response to G-CSF treatment. Sinusoids are labeled with intravenous injection of 155 kD TRITC-conjugated dextran. G-CSF treatment increases neutrophil motility and egress from the BM. White arrows indicates representative egressing neutrophils. Elapsed time is shown in hours:minutes:seconds.



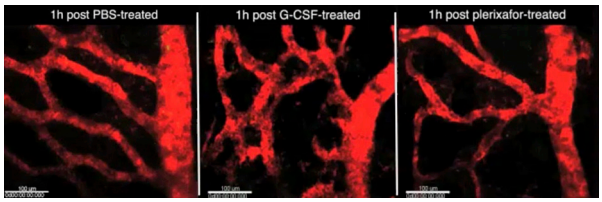
Video 4. Plerixafor does not increase the number of neutrophil egress from the BM. A time-lapse image sequence of maximum intensity projection depicting the migratory behavior of transferred GFP+ neutrophils in response to plerixafor treatment. Sinusoids are labeled with intravenous injection of 155 kD TRITC-conjugated dextran. Plerixafor treatment does not alter neutrophil egress from the BM. White arrows indicate egressing neutrophils. Elapsed time is shown in hours:minutes:seconds.



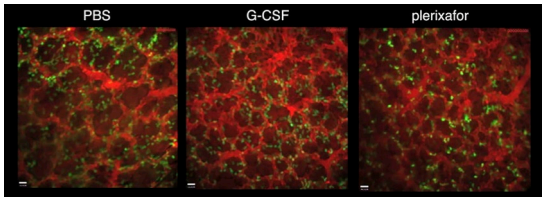
Video 5. Basal levels of neutrophil egress from the BM. A time-lapse image sequence of maximum intensity projection depicting the migratory behavior of transferred GFP+ neutrophils under resting state (PBS treated). Sinusoids are labeled with intravenous injection of 155 kD TRITC-conjugated dextran. White arrows indicate egressing neutrophils. Elapsed time shown is hours:minutes:seconds.



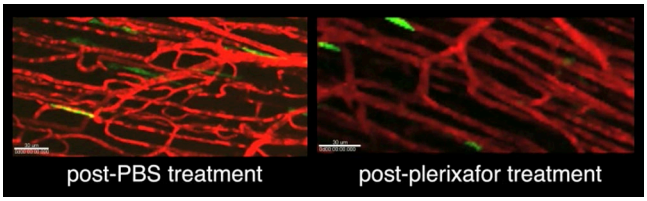
Video 6. Plerixafor pretreatment augments KC-induced neutrophil egress from the BM. A time-lapse image sequence of maximum intensity projection depicting the migratory behavior of GFP+ neutrophils in response to KC injection in a 1-h PBS- (control) or plerixafor-pretreated mouse. Sinusoids are labeled with intravenous injection of 155 kD TRITC-conjugated dextran. Elapsed time is shown in hours:minutes:seconds.



Video 7. Neutrophil trafficking to the BM. Time-lapse sequences of maximum intensity projection depicting the cellular behavior of transferred GFP+ neutrophils in the BM of a PBS-treated, G-CSF–, and plerixafor-treated mice. Sinusoids are labeled with intravenous injection of 155 kD TRITC-conjugated dextran. GFP+ transferred neutrophils (white arrows) can be seen to adhere to the luminal surface of BM endothelium followed by transmigration into the marrow space in PBS- and G-CSF–treated mice. Elapsed time shown is hours:minutes:seconds.



Video 8. Intravital imaging of neutrophils in the lung. Time-lapse image sequences depicting the cellular behavior of GFP$^+$ neutrophils in the lung of control (PBS-treated), GCSF-treated, or plerixafor-treated mice. Blood vessels are labeled with intravenous injection of Alexa Fluor 647–conjugated BSA. Images were captured over a period of 5 min.



Video 9. Intravital imaging of neutrophils in skeletal muscle. Time-lapse sequences of maximum intensity projection depicting the behavior of GFP$^+$ neutrophils in LysM-GFP mice in response to PBS (control) or plerixafor treatment. Vasculature is labeled with intravenous injection of Evan's blue. Elapsed time is shown in hours:minutes:seconds.