Fine et al., http://dx.doi.org/10.1083/jcb.201603109

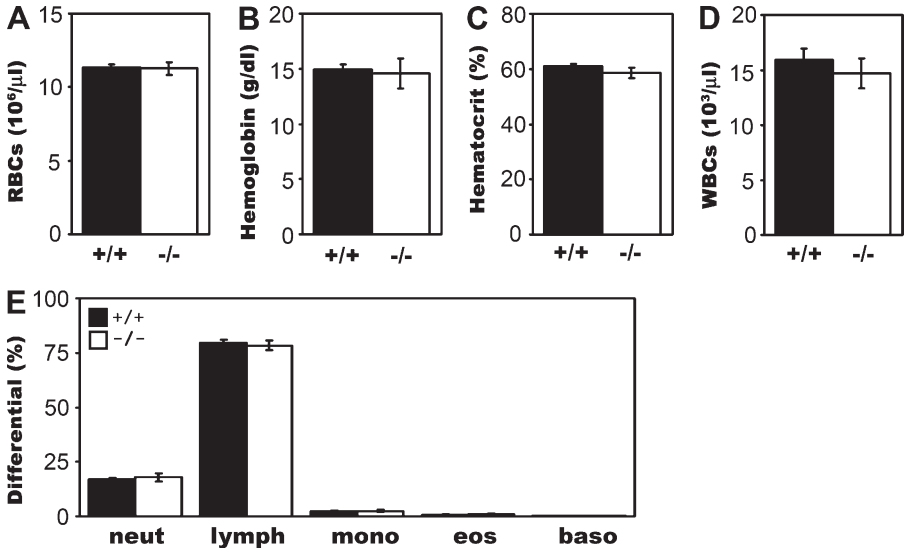


Figure S1. **Blood cell counts are normal in GEF-H1$^{-/-}$ mice.** Peripheral blood was collected from eight Lfc$^{+/+}$ and eight Lfc$^{-/-}$ mice in EDTA-coated capillary tubes (Drummond Scientific). Complete blood count analyses and differential analysis were performed using a Hemavet Hemoatology Analyzer (950FS; Drew Scientific Group) by standard protocols at the University Health Network mouse facility. (A–E). Red blood cell (RBC) counts (A), hemoglobin (B), hematocrit (C), and white blood cell (WBC) counts (D) are indicated. A differential analysis (E) indicates the percentages of several WBC subtypes, including neutrophils (neut), lymphocytes (lymph), monocytes (mono), eosinophils (eos), and basophils (baso). All values are expressed as mean ± SEM from GEF-H1$^{+/+}$ and GEF-H1$^{-/-}$ mice.

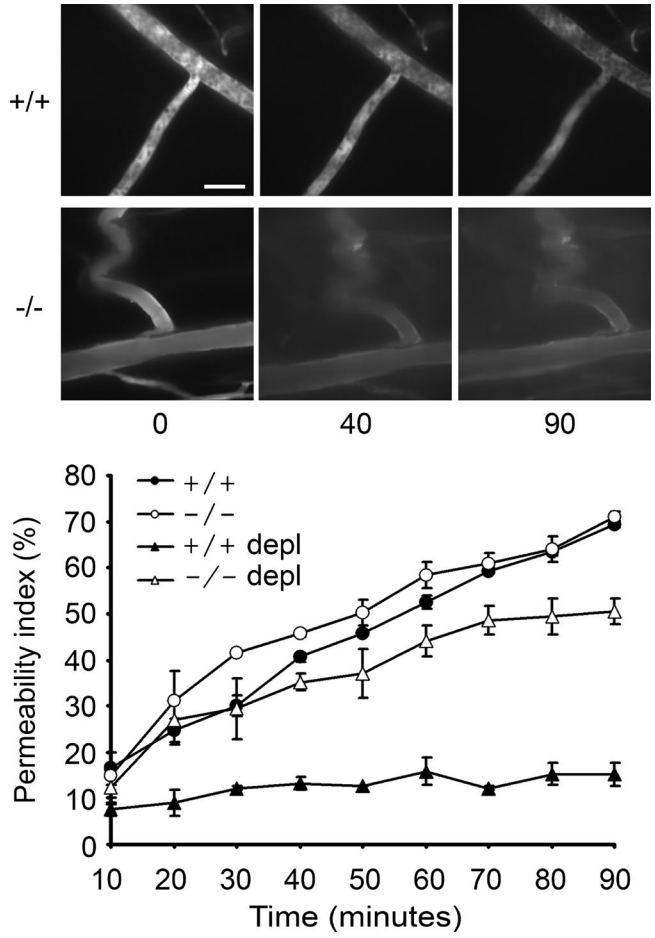


Figure S2. **Vascular leakage of FITC-labeled BSA was measured upon superfusion of cremasteric muscle preparations with *f*MLP, with or without prior depletion of neutrophils.** Representative images show fluorescence in the vasculature and, in the extravascular space, of neutrophil-depleted (depl) GEF-H1$^{+/+}$ and GEF-H1$^{-/-}$ mice at various time points after superfusion. Bar, 50 µm. The graph shows the time course of changes in the vascular permeability index of cremasteric venules in GEF-H1$^{+/+}$ and GEF-H1$^{-/-}$ mice, with or without prior depletion of neutrophils. Values are expressed as mean ± SEM from two mice in each group.

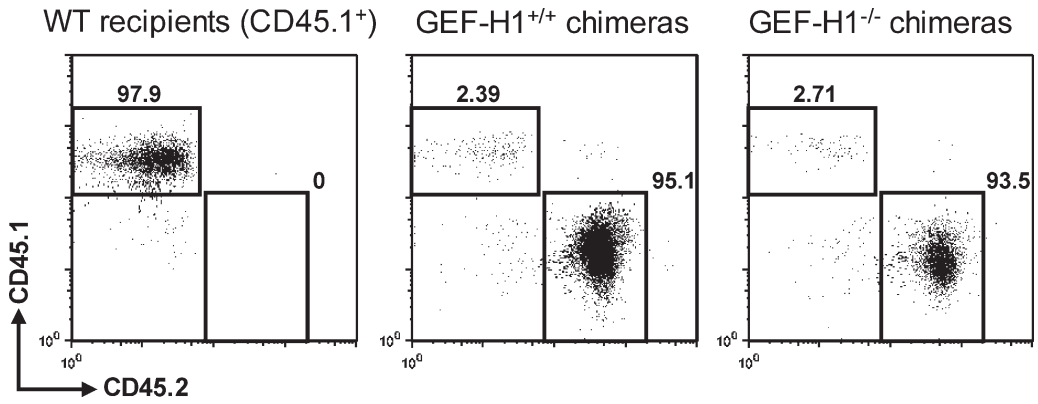


Figure S3. **Complete hematopoietic reconstitution was confirmed 10 wk after bone marrow transfer.** Wild-type (WT) recipients (CD45.1$^+$) were lethally irradiated and reconstituted the same day by intravenous injection of 10^7 bone marrow cells, purified using standard methods, from GEF-H1$^{+/+}$ or GEF-H1$^{-/-}$ mice (CD45.2$^+$), respectively. The chimeric mice were monitored after 10 wk for complete reconstitution by flow cytometry using anti-CD45.1 and anti-CD45.2 antibodies (eBioscience). Representative plots are shown.

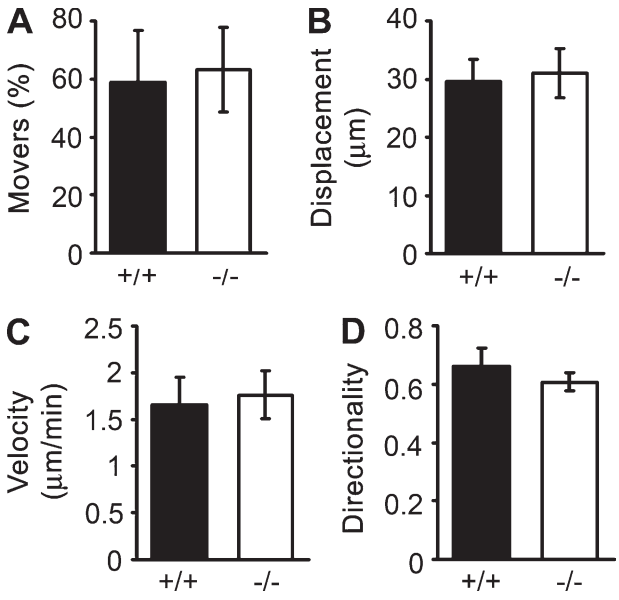


Figure S4. **GEF-H1 is not required for neutrophil adhesion or chemotaxis.** (A–D) Mean values ± SEM for percentage of migratory cells, displacement, velocity, and directionality were determined from two independent experiments. Analysis was performed in triplicate wells, and >100 cells were tracked per experiment.

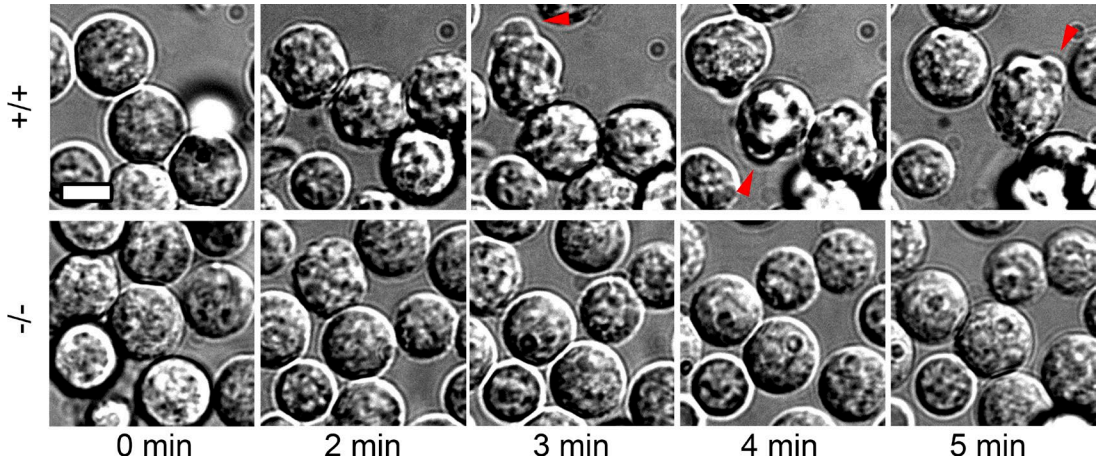
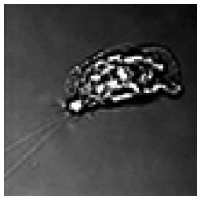
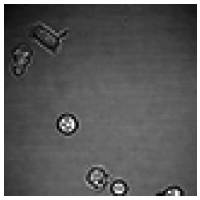


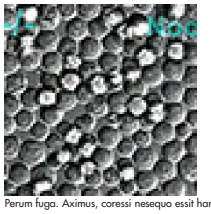
Figure S5. **MT depolymerization induced GEF-H1-dependent blebbing.** GEF-H1$^{+/+}$ and GEF-H1$^{-/-}$ neutrophils were treated with 10 µM nocodazole and imaged by phase-contrast microscopy in Labtekll chambered coverslips at 63× magnification. Membrane blebbing is indicated by arrowheads. Bar, 5 µm.



Video 1. **GEF-H1$^{+/+}$ neutrophils respond to shear stress.** GEF-H1$^{+/+}$ neutrophils were stimulated with 1 µM *f*MLP and allowed to settle on ICAM-1–coated surfaces for 5 min. Cells were then imaged for 5 min in the absence of shear stress and a further 5 min after exposure to 4 dynes/cm^2 constant shear stress. Images were acquired every 500 ms. The display rate is 33 frames per second.



Video 2. **GEF-H1$^{-/-}$ neutrophils do not respond to shear stress.** GEF-H1$^{-/-}$ neutrophils were stimulated with 1 µM *f*MLP and allowed to settle on ICAM-1–coated surfaces for 5 min. Cells were then imaged for 5 min in the absence of shear stress and a further 5 min after exposure to 4 dynes/cm^2 constant shear stress. Images were acquired every 500 ms. The display rate is 33 frames/second.



Video 3. **MT depolymerization induces cell contractility and uropod formation in GEF-H1$^{+/+}$, but not GEF-H1$^{-/-}$, neutrophils.** Primary GEF-H1$^{+/+}$ and GEF-H1$^{-/-}$ neutrophils are indicated. Neutrophils were imaged for 5 min, 25 min after stimulation with 10 µM nocodazole. Cells were imaged by phase-contrast microscopy at 63×. The display rate is 30 frames per second. Examples of contractile uropods are indicated with an asterisk.

Perum fuga. Aximus, coressi nesequo essit haritat.