Supplement Material

Supplemental Methods

RT PCR

Primers sets were designed against mouse VEGF-A (5'-GAGGATGTCCTCACTC GGATG-3 and' 5'-GTCGTGTTTCTGGAAGTGAGCAA-3') and β-actin (5'-GGGAAATCGTGCG TGACATCAAAG-3' and 5'-CATACCCAAGAAGGAAGGCTGGAA-3'). For accuracy of comparison, all primer sets were designed to amplify across exon boundaries, confirmed to produce a single product, and to have amplification efficiencies roughly equal to that of the reference set.

TNF−α ELISA

TNF-a levels in air-pouch exudates were measured using the mouse TNF-a ELISA Ready-Set-Go assay from eBioscience (San Diego, CA) according to the manufacturer's protocol. Results were quantified by comparison to a standard curve.

NFκB reporter assay

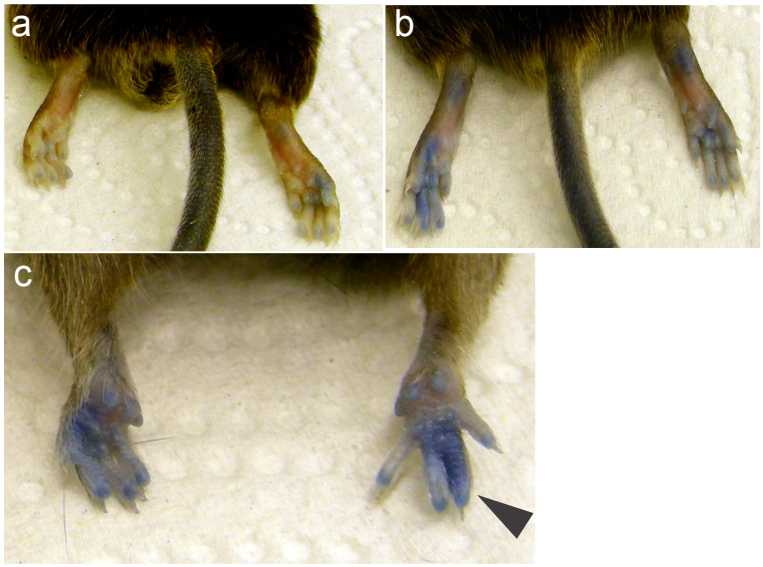
Reporter assays were performed using the Dual-Glo luciferase assay system (Promega, Madison, WI) according to the manufacturer's instructions. Firefly luciferase activity produced by the NFκB-dependent firefly luciferase reporter plasmid pNFκB-Luc (Stratagene, La Jolla, CA) was measured using a Synergy H4 hybrid plate reader (Biotek, Winooski, VT). All transfections included the constitutive Renilla luciferase plasmid pRL-TK, which was used to normalize for transfection efficiency. As appropriate, cells were treated with 25ng/mL TNF−α for 4 hours prior to luciferase detection.

FACS analysis

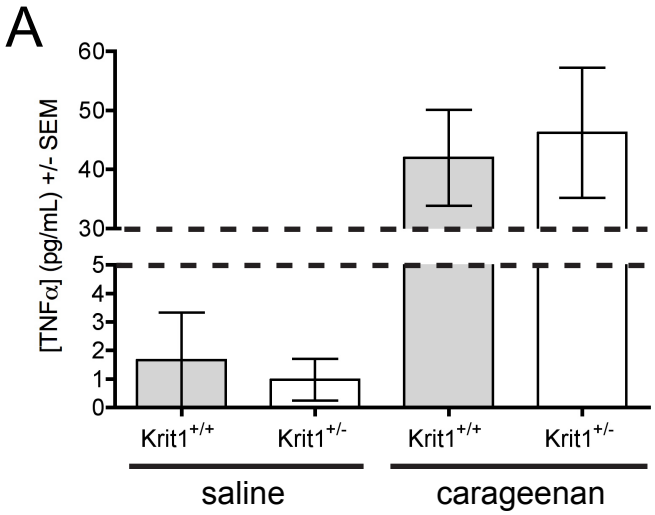
Cell surface expression of 6 hematopoetic cell markers was measured by antibody labeling, followed by FACS analysis. Briefly, peripheral blood and bone marrow cells were isolated using standard protocols. Cells were then aliquoted into tubes and spun down in buffer containing 10mM HEPES, 10mM glucose, 1.2 mM CaCl2, 135mM NaCL, 1.2 mM MgCl2, and 0.05% BSA (pH 7.4), and then labeled with the appropriate antibody for 1hr on ice. The cells were washed, and incubated with secondary antibody for 30 min on ice. The cells were washed again, and fluorescent labeling was detected using a FACScan flow cytometer and analyzed using FlowJo software. All antibodies were obtained from BD Biosciences. Data were analyzed using 2-way ANOVA with Bonferroni post-hoc testing.

Cytokine/chemokine array

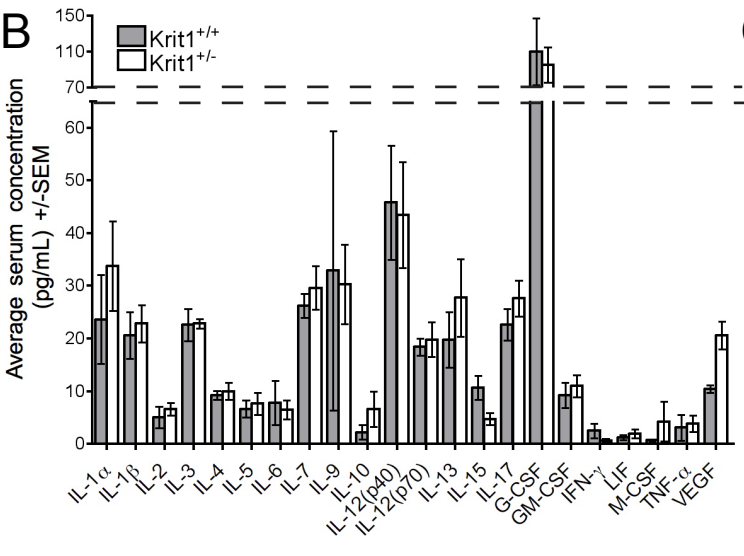
Serum levels of inflammatory cytokines/chemokines were measured in duplicate using a multiplex bead assay (Eve Technologies, Calgary). Results were quantified according to a standard curve. Data were analyzed using 2-way ANOVA.

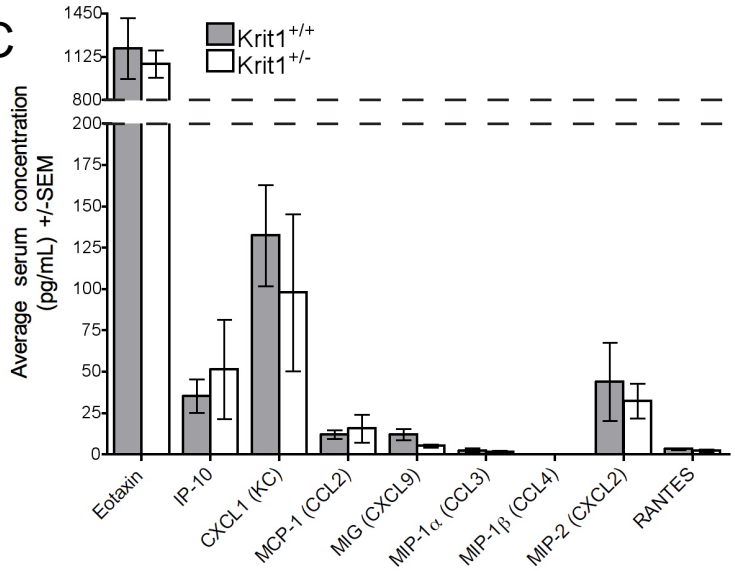


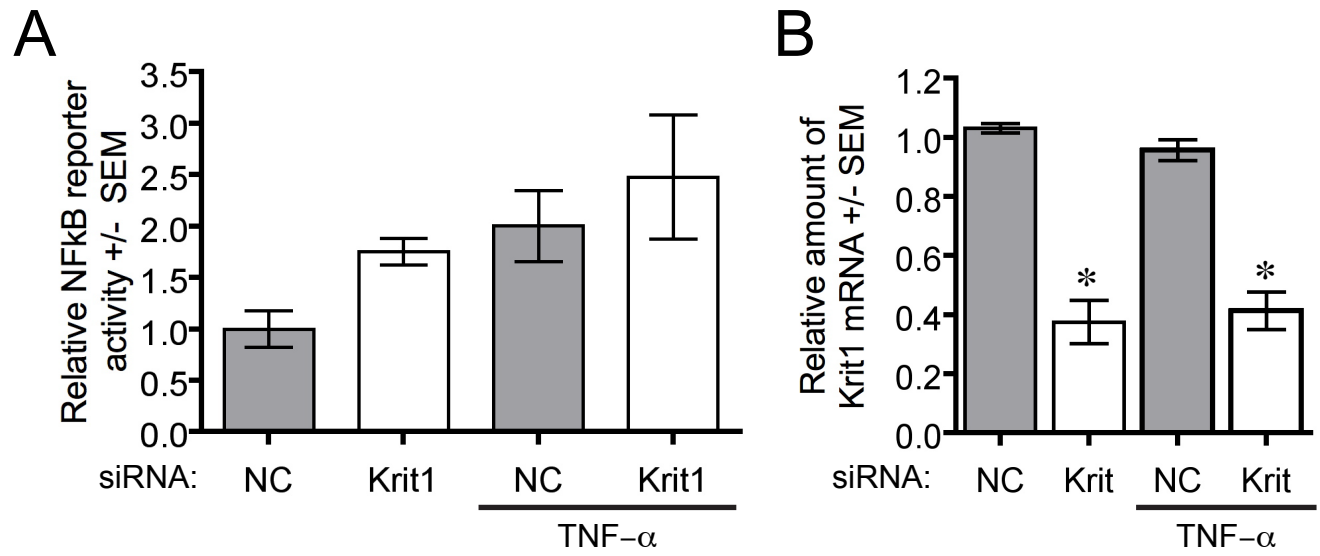
Supplemental Figure I. Visualization of edema formation using Evans Blue dye. A) Representative images of K/BxN inflamed mouse paws following Evans Blue injection. a) Hind limbs of *Krit1*$^{+/+}$ mouse, b) hind limbs of *Krit1*$^{+/-}$ mouse, c) closer view of *Krit1*$^{+/-}$ mouse demonstrating swollen digit (arrowhead) with increased dye extravasation.



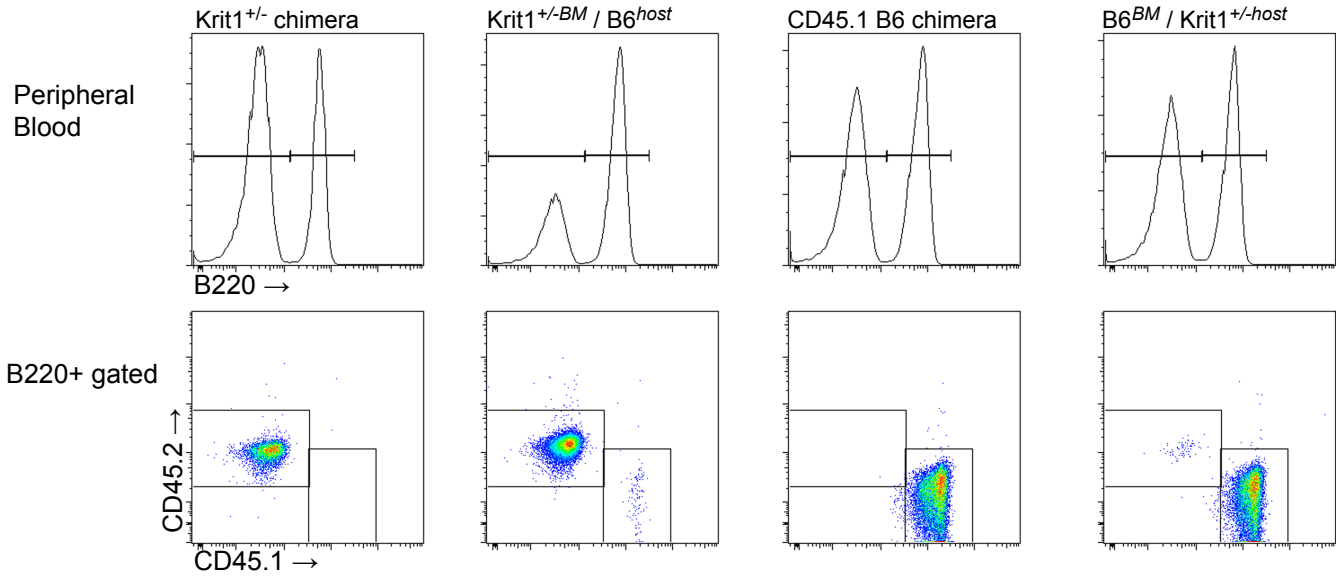
Supplemental Figure II. Krit1 heterozygous mice do not exhibit significant changes in cytokine or chemokine expression, but do exhibit increased VEGF expression. A) TNF-α levels in air pouch exudate of saline and carageenan treated *Krit1*$^{+/+}$ and *Krit1*$^{+/-}$ mice as measured by ELISA. B) Expression of cytokines, etc. in *Krit1*$^{+/+}$ and *Krit1*$^{+/-}$ mice. *p<0.01, no other differences are statistically significant. n=6 mice/group. C) Expression of chemokines in *Krit1*$^{+/+}$ and *Krit1*$^{+/-}$mice. n=6 mice/group.



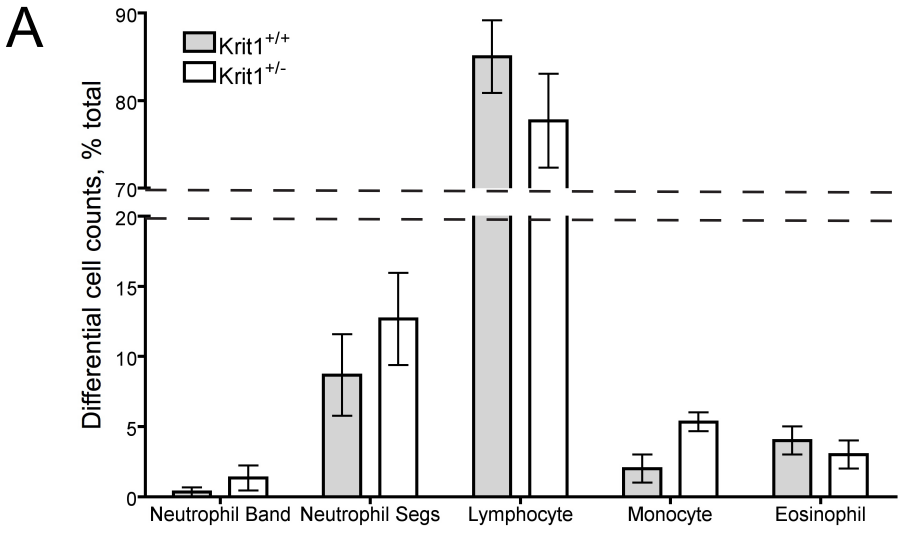


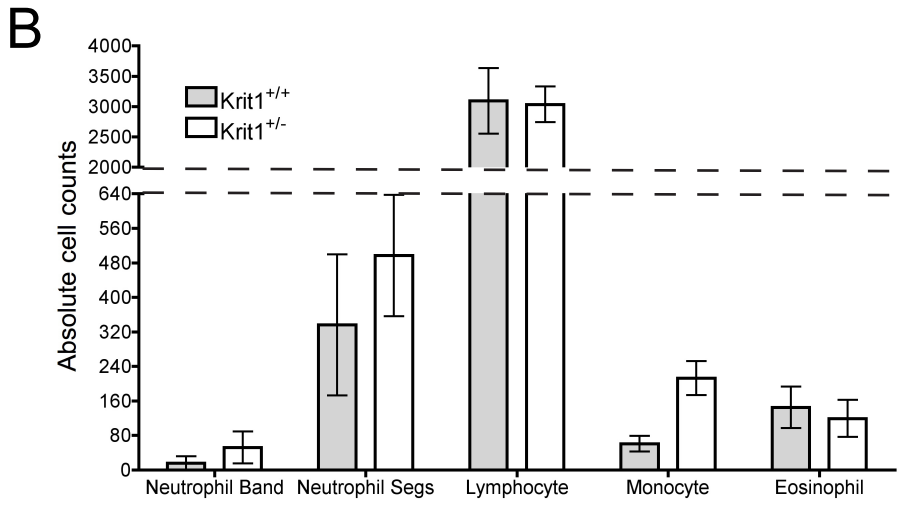


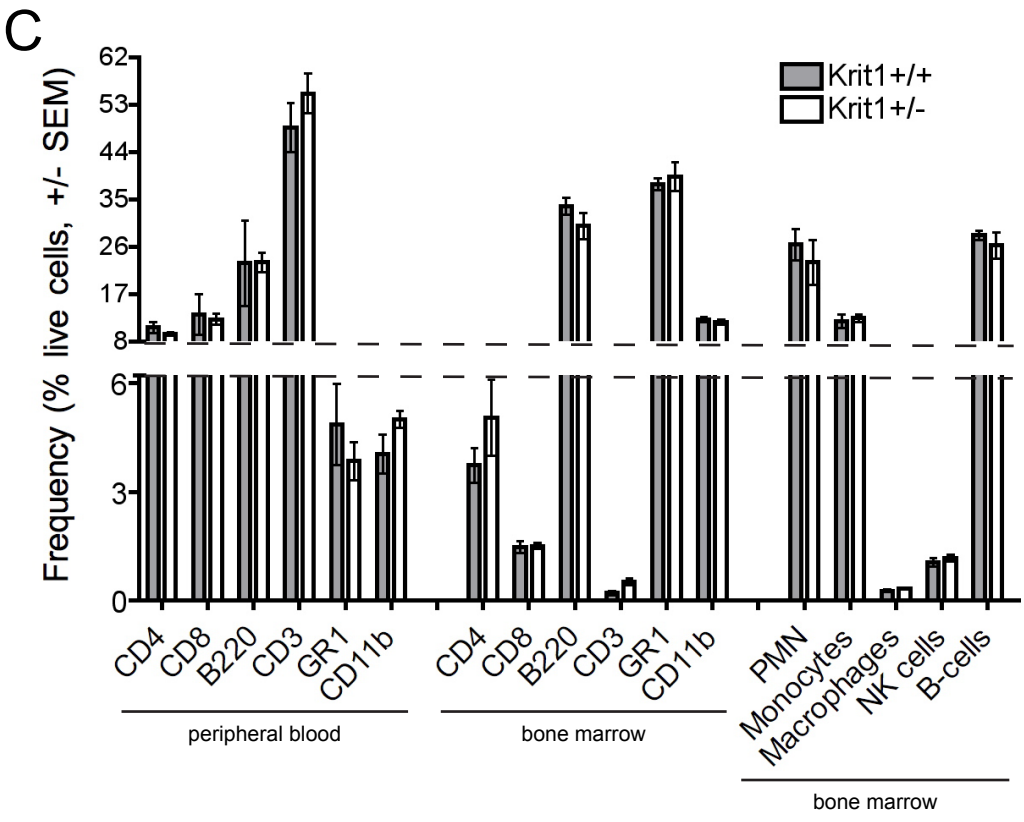
Supplemental Figure III. TNF−α dependent NFκB activity is not affected by loss of KRIT1. A) NFκB reporter activity in negative control (NC) and anti-Krit1 siRNA (Krit1) transfected human microvascular endothelial cells (MVEC) with or without 4hr TNF−α treatment. Data shown is relative to untreated negative control. Differences are not significant by ANOVA, n=3. B) Relative amount of Krit1 mRNA in NC and Krit1 siRNA transfected MVEC used in A. *p<0.01 vs. NC, n=3.



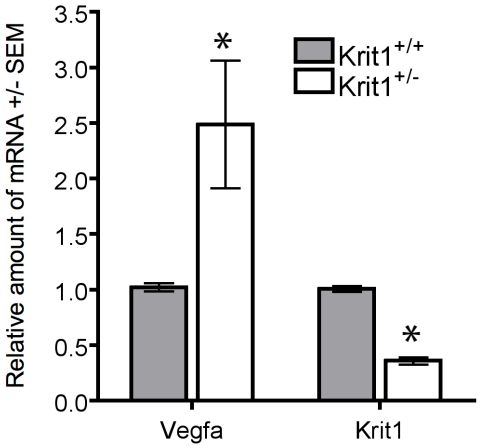
Supplemental Figure IV. Representative peripheral blood stains following adoptive transfer of *Krit1*$^{+/-}$ bone marrow. Live peripheral blood cells were stained for B220, CD45.1 and CD45.2 expression. Data shown is relative CD45.2 and CD45.1 expression in B220 positive cells. Quantitation of relative CD45.1 expression is shown in Supplemental Table 1.







Supplemental Figure V. Differential cell counts from peripheral blood of *Krit1*$^{+/+}$ and *Krit1*$^{+/-}$ mice. n=3 mice/group. A) Cell counts as percent total, +/- SEM. B) Absolute cell counts, +/- SEM. All values fall within the normal range for the C57BL/6J background stain. No strain differences are statistically significant as calculated by 2-way ANOVA. C) FACS analysis of hematopoetic cell markers from peripheral blood and bone marrow of *Krit1*$^{+/+}$ and *Krit1*$^{+/-}$ mice. Individual markers are shown, as well as frequency of neutrophils (PMN, Ly6c hi, Ly6g hi), monocytes (Ly6c hi, Ly6g low), macrophages (CD11b+, F4-80+), natural killer cells (NK, CD3-, Nk1.1+), and B-cells (CD3-, Nk1.1-, CD19+). n=6 mice/group.



Supplemental Figure VI. mRNA levels of VEGF (Vegfa) and Krit1 in bone marrow derived cells of *Krit1*$^{+/+}$ and *Krit1*$^{+/-}$mice. n=6 mice/group *p<0.01.

Genotype Donor/Host	% CD45.1 positive, ± SD	% CD45.2 positive, ± SD
Krit1$^{+/-}$ / *Krit1*$^{+/-}$	0.011 ± 0.009	99.73 ± 0.071
Krit1$^{+/-}$ / *CD45.1 B6*	2.874 ± 2.810	96.85 ± 2.78
CD45.1 B6 / CD45.1 B6	99.28 ± 0.575	0.146 ± 0.17
CD45.1 B6 / Krit1$^{+/-}$	97.44 ± 1.334	2.081 ± 1.199

Supplemental Table I. Reconstitution of reciprocal bone marrow chimeras.