Supporting Information

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SI Materials and Methods

Paw Edema Model. Paw volume was measured by using a hydroplethismometer specially modified for small volumes (Ugo Basile, Milan, Italy). Immediately before the subplantar injection and 2, 4, 6, 24, 48, 72, 96, 120, 144, and 168 h thereafter. The increase in paw volume was calculated by subtracting the initial paw volume (basal) to the paw volume measured at each time point. In some experiments, paws were fixed in paraformaldehyde, and frozen sections (6 μm) were stained with hematoxylin and eosin to examine the leukocyte infiltration or with CD68, a monocyte/macrophage differentiation marker, to evaluate the mononuclear cells 72 h after carrageenan injection.

Carrageenan Air Pouch. The exudates were centrifuged at $180 \times g$ for 10 min at room temperature. Cell-free exudates were kept at −80 °C until analysis of soluble inflammatory mediators or NO_2^- content, and migrated leukocytes, mainly neutrophils, were counted by trypan blue. Western blot analysis for inducible nitric oxide synthase and COX-2 was performed on air-pouch-recruited leukocytes 24 h after carrageenan injection, as previously described (7).

In Vitro Migration Assay. Chemotaxis of bone marrow neutrophils in response to N-formyl-Met-Leu-Phe (fMLP) and human IL-8 was assayed by using the ChemoTx System (Neuro Probe) according to the manufacturer's protocol with a slight modification. Briefly, fMLP (0.1, 0.3, and 1 μM) and IL-8 (1, 10, and 50 ng/mL) were added (29 μL per well) to the lower chamber of the ChemoTx System and served as chemoattractants. Bone marrow neutrophils were resuspended in Hepes (without Ca^{2+}), 1×10^5 cells per mL and were added (24 μL per well) to the upper chamber of the ChemoTx System (3-μm pore size filter) and incubated for 2 h at 37 °C, 5% CO_2. The cells migrated into the bottom chamber and were counted by trypan blue.

Primary Culture of Bone-Marrow-Derived Monocytes. Bone marrow cells were flushed from femurs and tibias of 8- to 9-week-old female mice. After depletion of red blood cells, the pellet was resuspended in complete medium consisting of RPMI medium 1640 (Gibco) supplemented with 1% penicillin–streptomycin (Gibco), 10% heat-inactivated FBS (Gibco), and 10% L929 cell-conditioned medium as a source of macrophage-colony-stimulating factor. The latter was prepared by plating 8×10^6 L929 cells in a T75 flask containing 40 mL of RPMI medium 1640 supplemented with 10% FBS. After 1 week at 37 °C in a 5% CO_2 humidified atmosphere, the medium was harvested, filtered, and stored at −20 °C until use. Bone marrow cells were seeded at a density of 0.1×10^6 cells per mL onto 100-mm diameter tissue culture dishes (Falcon). Every other day, one-third volume of complete medium was added to the culture. After 6 days at 37 °C in a 5% CO_2 humidified atmosphere, nonadherent cell clusters were gently flushed and centrifuged at $110 \times g$. The cell pellet was resuspended in a mixture containing RPMI medium 1640, 1% penicillin–streptomycin, 10% FBS, and 20% L929 cell-conditioned medium (incomplete medium). Cells were seeded in six-well plates at a density of 1×10^6 cells per well. Cells were cultured overnight at 37 °C before being stimulated by medium supplemented by exudate (diluted 1:10) from the 24 h carrageenan air pouch.

Flow Cytometry Analysis. Bone-marrow-derived monocytes were grown in six-well plates. At confluence, the cells were detached from the culture dish by PBS supplemented with EDTA (1 mM) and washed twice with Dulbecco's PBS–1% BSA. The cells were incubated with rat anti-mouse CD16/CD32 to block these epitopes on the extracellular domains of the mouse FcgIII and FcgII receptors for 10 min at room temperature. The cells were washed and incubated with fluorochrome-coniugated monoclonal antibodies, FITC-conjugated rat anti-CD11a (PharmMingen), FITC-coniugated rat anti-CD11b (BioLegend), Phycoerythrin-Cy5 (PE-Cy5)-conjugated rat anti-F4/80 (eBioscience), and PE-conjugated mouse anti-PGSL-1 (BD Biosciences), for 45 min on ice, washed twice, and followed by analysis with FACS Calibur (BD Biosciences). Mouse lung endothelial cells (MLECs) were grown in 6-multiwell plates. At confluence, the endothelial cells were incubated with murine TNF-α (Genzyme) at a final concentration of 10 ng/mL for 6 h. MLECs cultured in complete media alone served as controls (designated as 0 h). For FACS analysis, MLECs were detached and incubated with PE-conjiugated mouse anti-E-selectin for 45 min on ice, followed by analysis with FACS Calibur (BD Biosciences).

Fig. S1. Loss of Akt1 does not influence N-formyl-Met-Leu-Phe (fMLP)- and IL-8-induced bone marrow neutrophil (BMN) migration and bone-marrow-derived monocyte (BMDM) activation in vitro. (*A*) Bone marrow neutrophils were isolated from three bone marrow of 8- to 12-week-old mice. Chemotaxis of BMNs to (*A*) fMLP and (*B*) IL-8 was measured with the ChemoTx System (Neuro Probe), as described in *SI Materials and Methods*. The data shown represent the mean ± SEM of cell counts taken from three independent experiments (three replicates from each experiment). (*D*) Representative histograms of adhesion and lineage molecule expression, CD11a, CD11b, PSGL-1, and F4/80, on WT, *Akt1*$^{-/-}$, and *Akt2*$^{-/-}$ BMDMs (gray filled) and isotype-matched control antibody (black line). (*E*) Relative quantitative PCR in WT, *Akt1*$^{-/-}$, and *Akt2*$^{-/-}$ BMDMs stimulated for 6 h with exudate (diluted 10 times in media) recovered from mouse air pouch 24 h after carrageenan injection. (*F*) Western blot for COX-2 expression in WT and *Akt1*$^{-/-}$ BMDMs 24 h after exudate stimulation. Data are expressed as the mean ± SEM of three individual experiments.

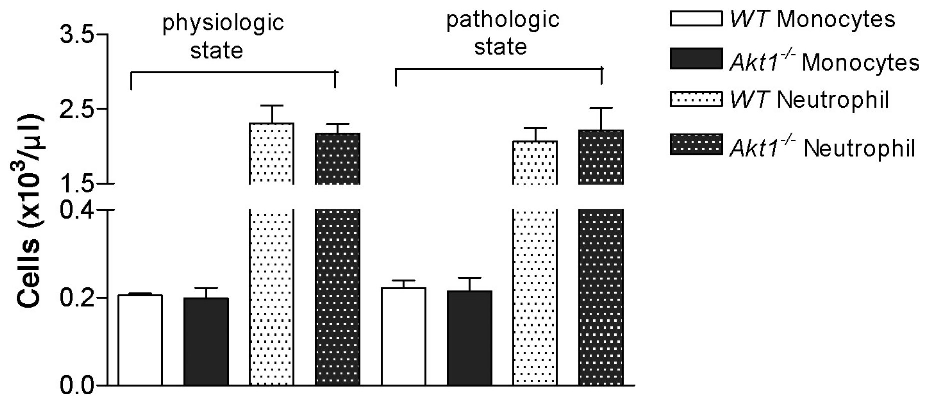

Fig. S2. Blood neutrophil and monocyte number in WT and *Akt1*$^{-/-}$ mice in physiological conditions and 72 h after carrageenan injection (pathological state), determined by using an automated cell counter (Hemavet Multispecies Hematology System; Drew Scientific). (n = 5 mice per group)

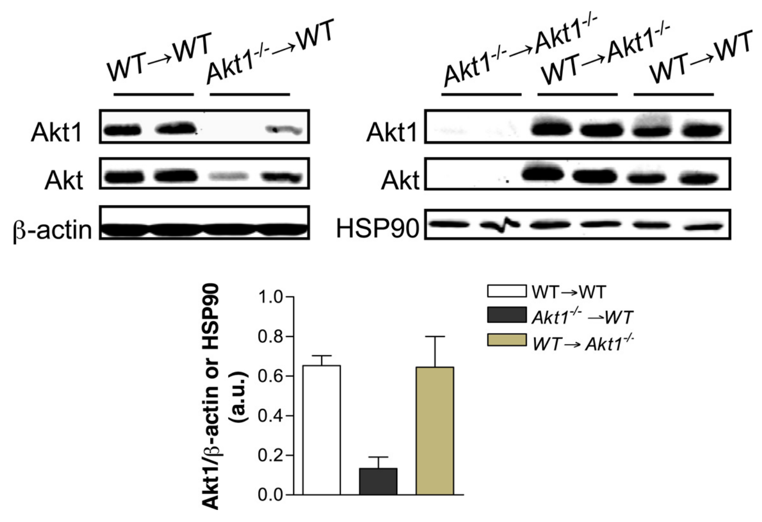

Fig. S3. Evidence for chimerism in transplanted mice. The levels of Akt1 and total Akt were assessed in neutrophils recovered from the pouches of transplanted mice. The bottom graph depicts the relative levels of Akt1 in bone marrow transplantation experiments.

Fig. S4. E-selectin expression analysis by flow cytometry in endothelial cells isolated from WT or *Akt1*$^{-/-}$ mice that were treated with TNF-α for 16 h. These data are representative of three separate experiments that yielded similar results.