SUPPLEMENTAL METHODS

Intravital microscopy

Phase contrast intravital microscopy was utilized to measure leukocyte rolling/min as described previously.[1, 2] Mice were anesthetized with intraperitoneal injection of tribromoethanol (Fluka, 0.015 mg/g). Rhodamine6G (0.67 mg/kg)[3] was injected to illuminate circulating nucleated blood cells and was visualized using fluorescence intravital microscopy.[2] Venules with average diameter of 125 μm and an average shear rate of 150 s^{-1} were visualized using a Zeiss IM35 inverted microscope equipped with 10x/0.25 objective lenses (Zeiss). A doppler flow meter was employed to calculate shear rates. Video and images were recorded with a SVHS Panasonic AG-6720A video recorder (Matsushita Electric) and a CCD video camera (Hamamatsu Photonics Systems). Image processing was conducted with Adobe Premiere 6.0 (Adobe Systems).

The numbers of rolling leukocytes/min were quantified by counting the leukocytes passing through a perpendicular plane in 1 min. An average of four counts per venule were taken for one or two venules per mouse. Leukocyte velocity was calculated as the distance (μm) traveled per second. The leukocyte velocity for 12 or more leukocytes per venule was calculated and data from several mice was pooled. The values are presented as the average +/- S.E.M.

In vivo detection of P-selectin expression on endothelial surface

Yellow/green (excitation/emission, 505 nm/515 nm) and red (excitation/emission, 580 nm/605 nm) carboxylate-modified microspheres (1.0 μm diameter) (Invitrogen) were covalently coupled to anti-P-selectin monoclonal antibody RB40.34 or control rat IgG1k (BD-Pharmingen), respectively, according to the manufacturer's instructions. Mice were injected intravenously with 0.2×10^{9} of anti-P-selectin conjugated-FITC beads or IgG conjugated (control)-beads and mesenteric veins were visualized immediately using fluorescent intravital microscopy as described above. Images of 1 to 3 venules per mouse were

captured and the density (number of beads/mm^2) of bound anti-P-selectin and control beads were quantified in Ad-sFlt1, Ad-sEng and Ad-null-expressing mice.

VWF ELISA

For measurement of plasma VWF, plates were coated overnight at 4°C with polyclonal rabbit anti–human VWF (15 mg/ml in 50 mM sodium carbonate buffer (pH 9.6)). Plasma (1:20 dilution in PBS) was incubated for 1 hr at 37°C. After washing, a polyclonal anti–human VWF coupled to peroxidase (1:1000) was added. Following 3,3,5,5-tetramethylbenzidine substrate color development, the reaction was terminated with 1N, H_2SO_4 and the absorbance was read at 450 nm.

1. Mayadas TN, Johnson RC, Rayburn H, Hynes RO, Wagner DD. Leukocyte rolling and extravasation are severely compromised in P selectin-deficient mice. *Cell.* 1993;74:541-554.
2. Frenette PS, Johnson RC, Hynes RO, Wagner DD. Platelets roll on stimulated endothelium in vivo: an interaction mediated by endothelial P-selectin. *Proc Natl Acad Sci U S A.* 1995;92:7450-7454.
3. Eriksson EE, Werr J, Guo Y, Thoren P, Lindbom L. Direct observations in vivo on the role of endothelial selectins and alpha(4) integrin in cytokine-induced leukocyte-endothelium interactions in the mouse aorta. *Circ Res.* 2000;86:526-533.