Figure S1: Effect of antibody labeling protocols on leukocyte function. Tissue was locally stimulated by superfusion of fMLP (10μM, for 10 minutes). Data were collected 40 minutes after the completion of fMLP application. Leukocyte adhesion (A) and TEM (B) in straight and converging venular regions were quantified in the absence of labeling antibodies (control conditions, using transilluminating light), or in the presence of the following combinations: anti-CD11a (M17/4, 3μg/mouse, i.v) + anti-PECAM-1 (ER-MP12, 10 μg/ml, local cannulation) or anti-CD11a (M17/4, 3μg/mouse, i.v) + anti-VE-Cadherin antibodies (BV13, 30 μg/ml, local cannulation). For each condition the labeling procedures did not significantly affect leukocyte behavior ($P>0.05$). (C) Anti-CD11a (M17/4) was able to block leukocyte adhesion and TEM at a higher concentration (30μg/mouse, i.v) than that used in panels A and B. All images were acquired using transilluminating light, in the absence of labeling antibodies. For all groups n=7-8 venules were observed in 3 mice.

Figure S2: Labeling procedures had no significant effect on leukocyte crawling behavior. Time lapsed microscopy (x90) was used to quantify leukocyte crawling fraction and crawling distances (A), crawling velocities (B) and direction of crawling (C) in straight and converging venular regions, either in the absence of labeling antibodies (control conditions, using transilluminating light), or after immunofluorescently labeling the endothelial junctions (anti-PECAM-1, ER-MP12, 10 μg/ml, local cannulation) and leukocytes (using anti-CD11a, M17/4, 3μg/mouse, i.v). The labeling procedures did not

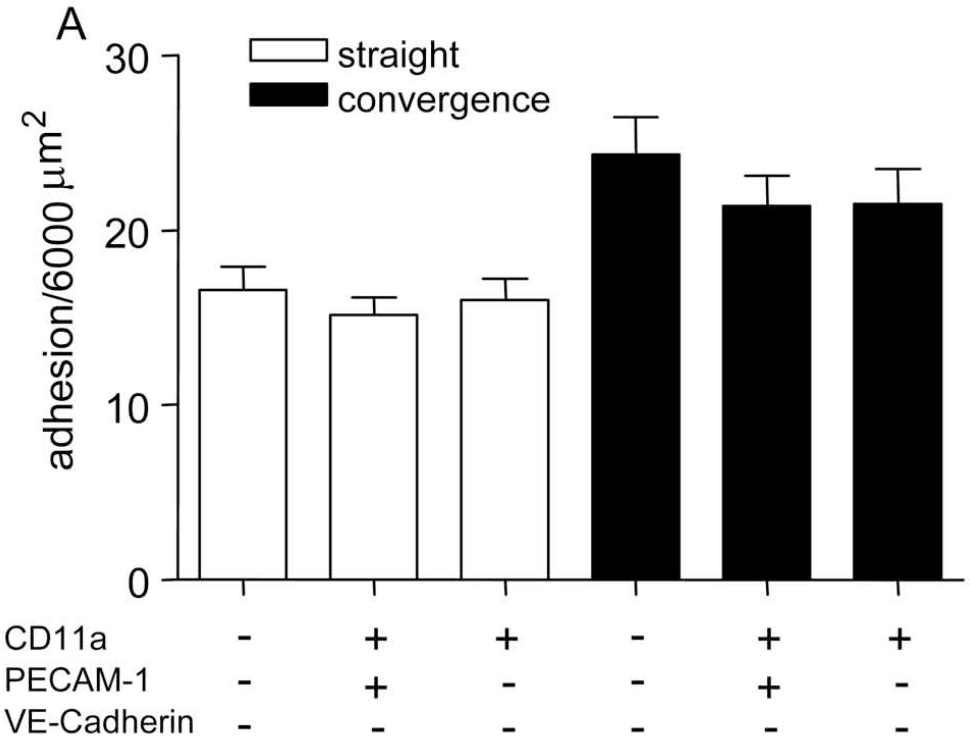
significantly affect leukocyte crawling behaviors (P>0.05). n=88 leukocytes were tracked in 7 venules in 3 mice.

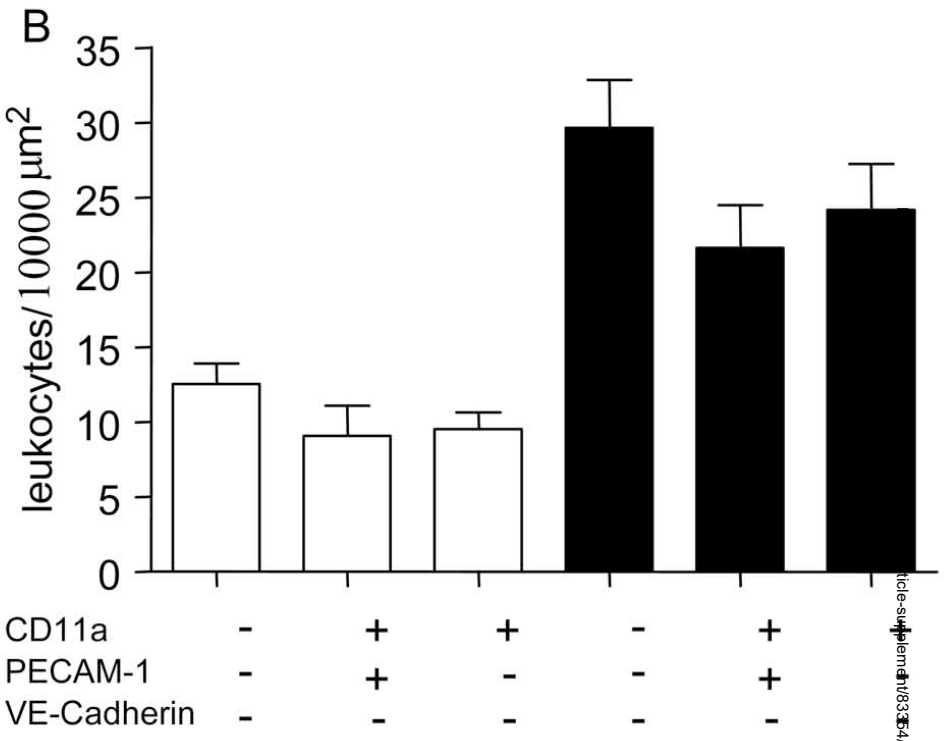
Figure S3: Endothelial cell junctions stained for VE-Cadherin. Unstimulated cremaster venules were locally stained for VE-Cadherin (BV13 antibody, 30 μg/ml) using local cannulation technique as described in the Methods section. The representative images, as shown, were obtained using intravital fluorescence confocal microscopy at 20 minutes (A) and 2hours (B) following the completion of perfusion. Continues antibody fluorescence at the EC-junctions was observed at both time points. For each group the observations were made in n=3 mice.

Figure S4: ICAM-1 is significantly upregulated after fMLP treatment. (A) The relative expression of ICAM-1 on venular ECs was quantified in either control or TNFα activated conditions (4 hours, 0.5μg/mouse intrascrotally), as well as 40 minutes post fMLP application (10μM, for 10 minutes, superfusion). Selected venules were locally stained for ICAM-1 (monoclonal rat anti-ICAM-1(YN-1) conjugated to Alexa 488, 50μg/ml) using local cannulation technique as described in the Methods. The relative expression was measured as a function of normalized fluorescence intensity as described in reference# 23. A significant increase in ICAM-1 expression was observed following both TNFα and fMPL treatment. (B) Using a similar protocol ICAM-1 expression in venular convergences (as defined in Methods) was compared to straight venular regions

40 minutes post fMLP application (10μM, for 10 minutes, superfusion). No significant difference was observed. * Significantly different from control group, $p<0.05$. ** Significantly different from control group, $p<0.01$. For each group n=3 mice, 7venules.

Figure S1





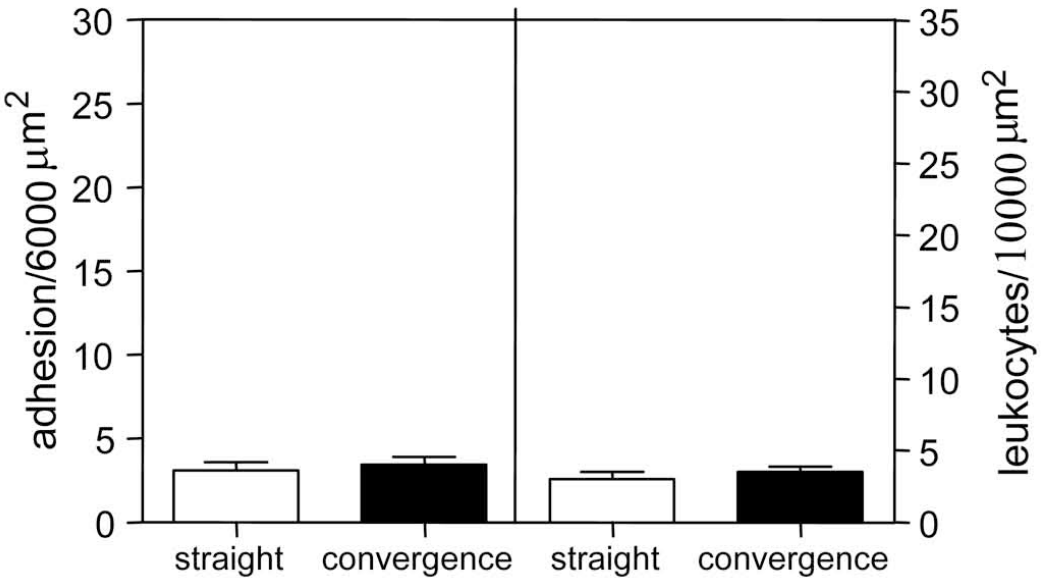
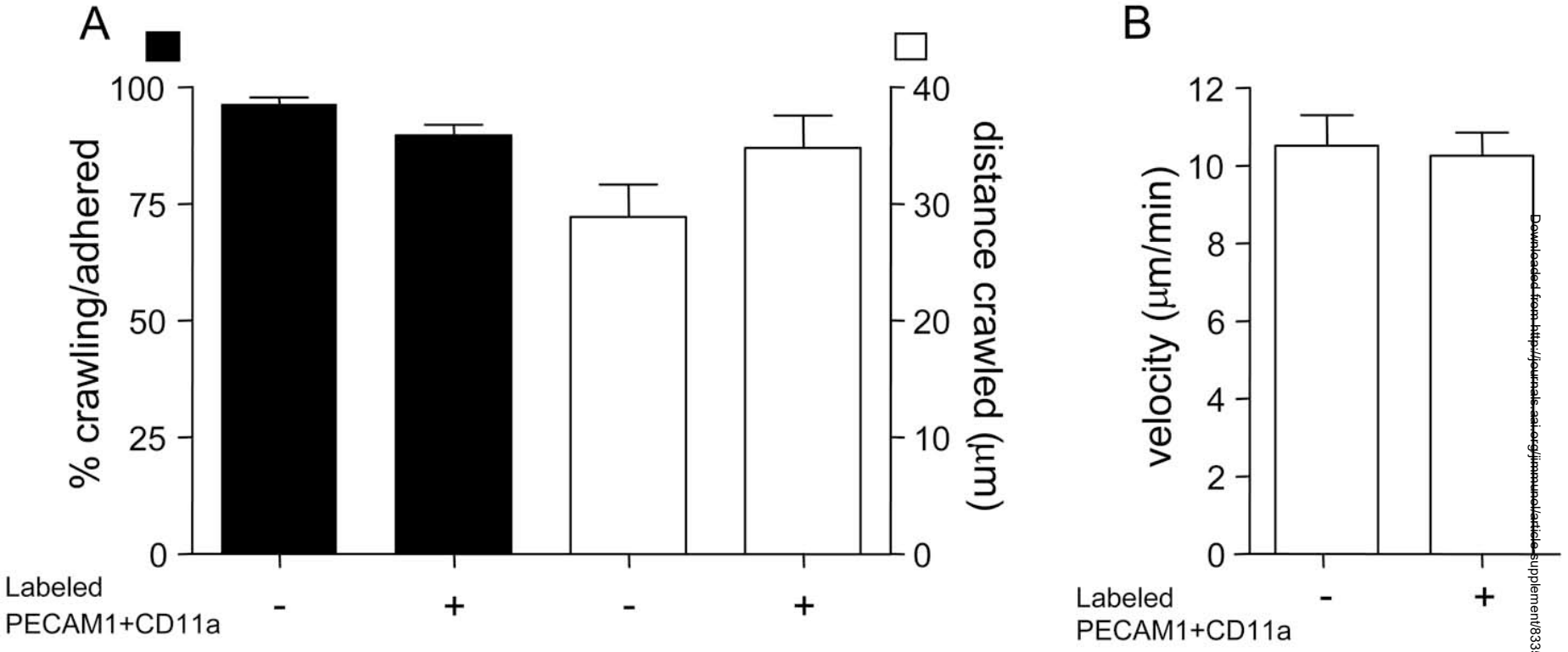


Figure S2



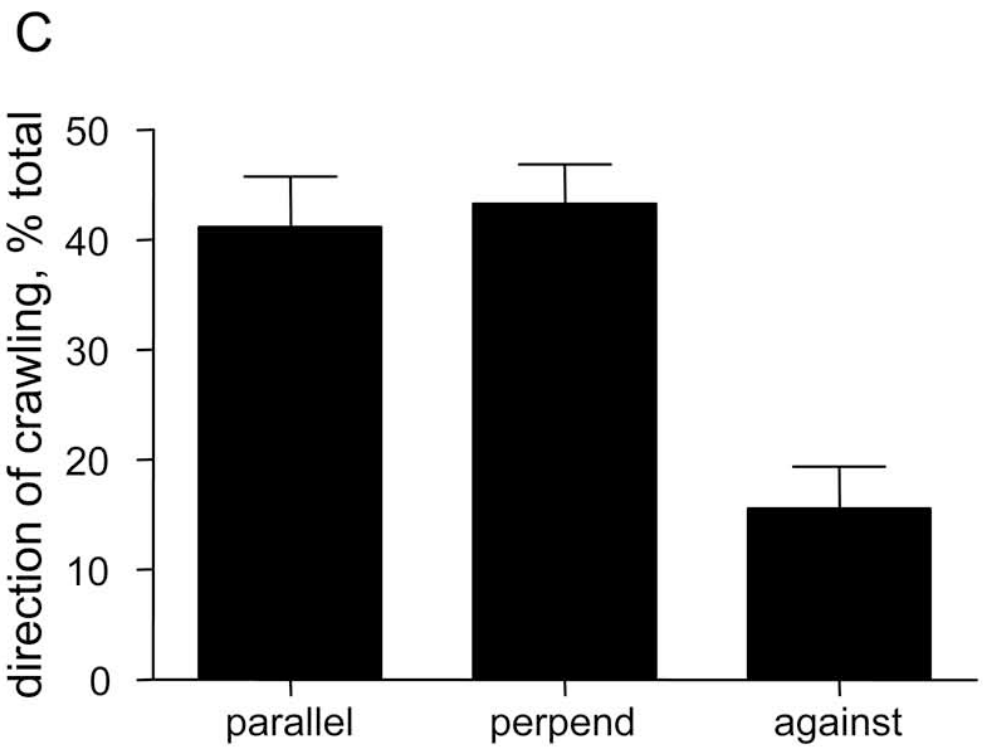


Figure S3

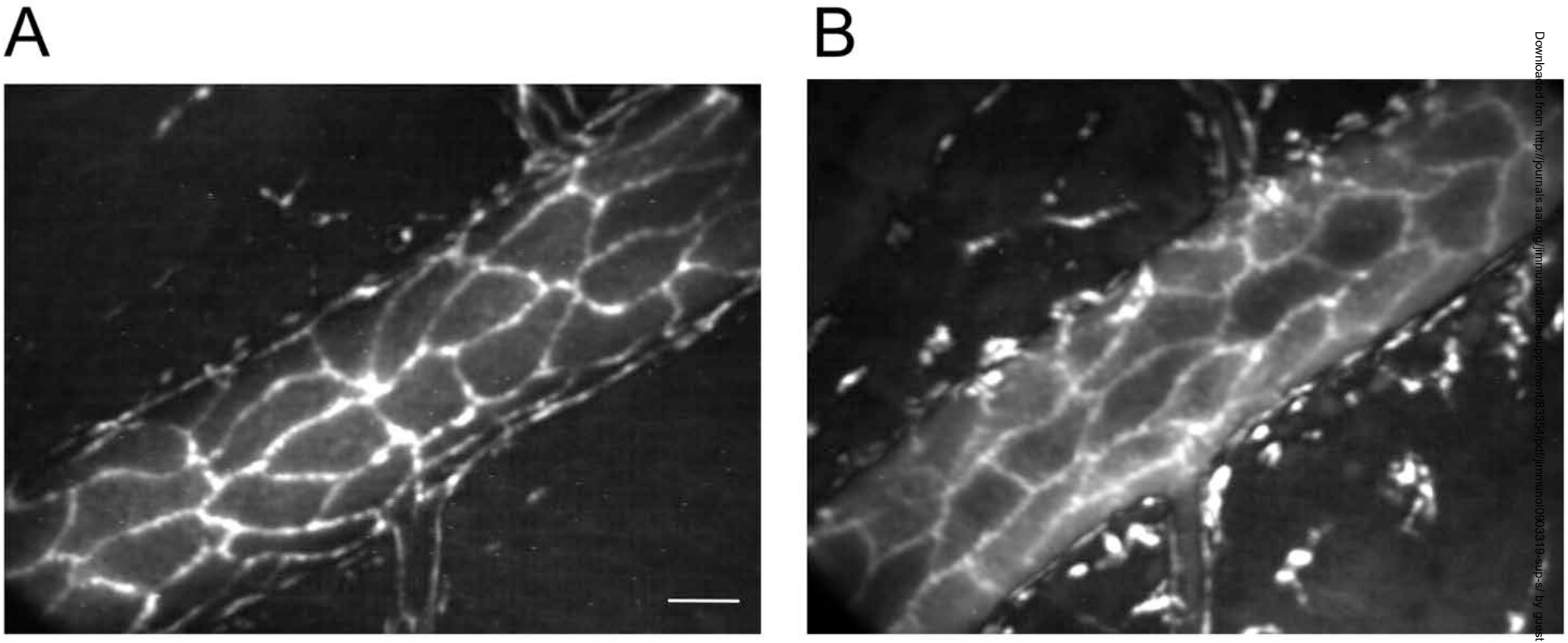


Figure S4