

Supplementary Information (SI)

Supplemental figures

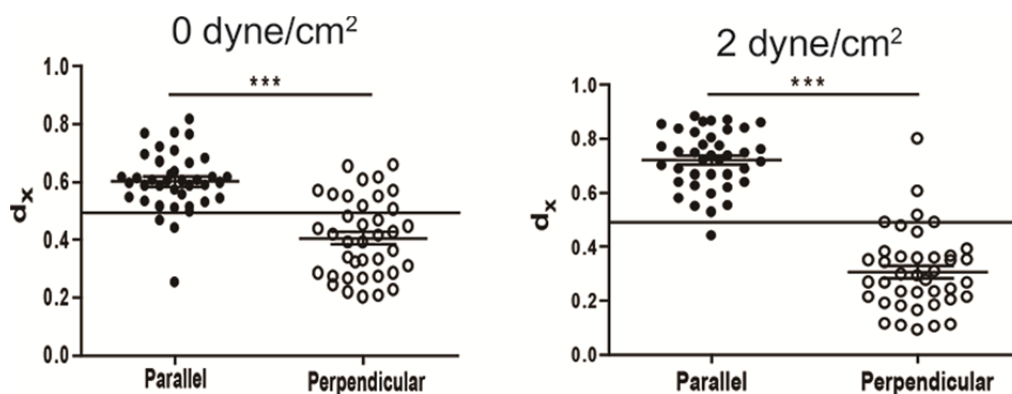


Figure S1. d_x values of T cells in the absence of SDF-1 α . (Mann-Whitney U-test, two-tailed, ***p < 0.001)

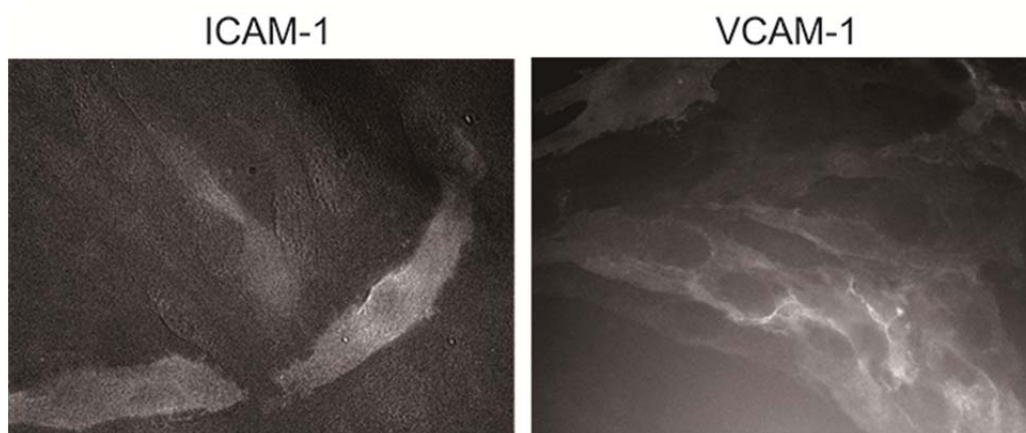


Figure S2 Distribution of adhesion molecules (ICAM-1, VCAM-1) on the surfaces of ECs. TNF- α -treated EC layers were fixed and stained with anti-ICAM-1-FITC or anti-VCAM-1-FITC and fluorescence images were acquired.

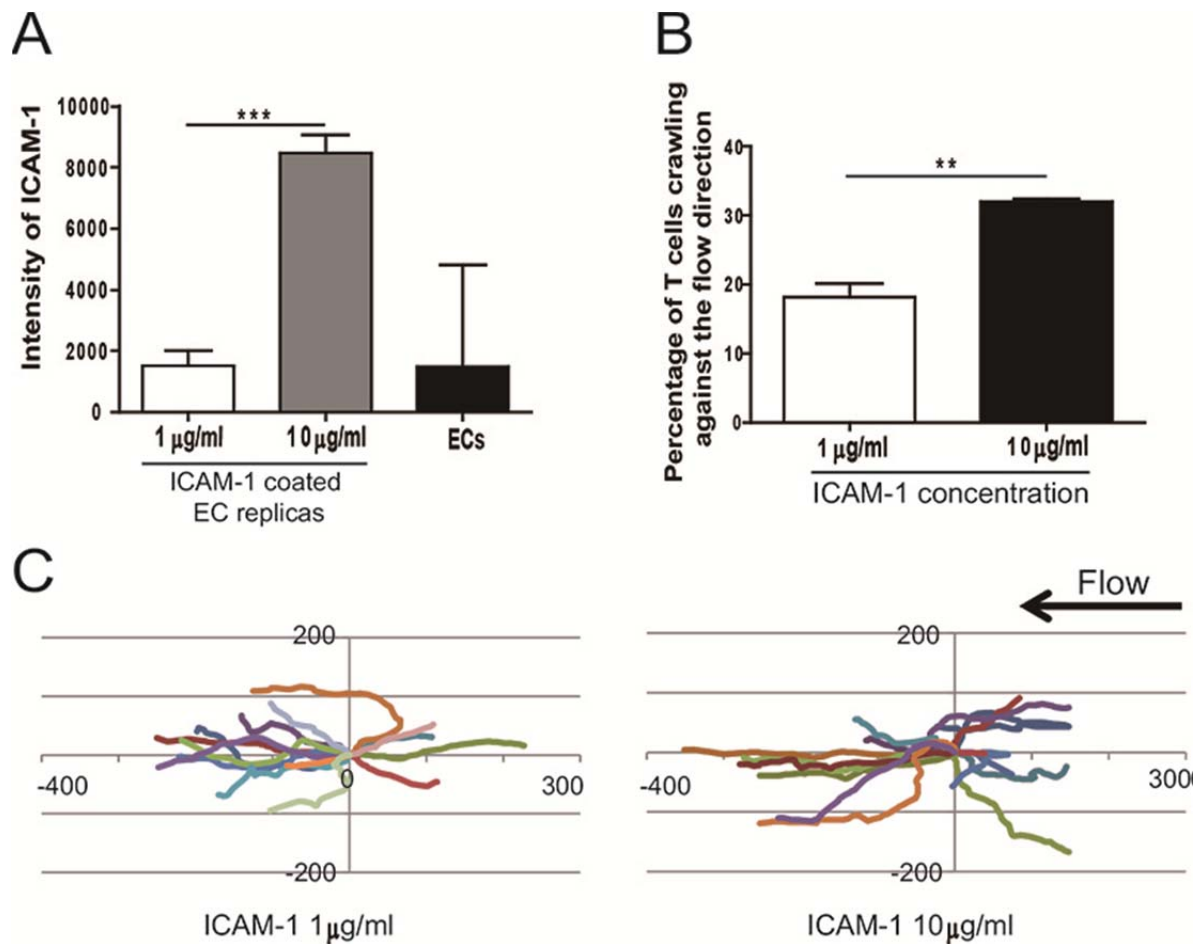


Figure S3 Effect of ICAM-1 concentration on T cell crawling. (A) Relative ICAM-1 surface density of ICAM-1-coated EC replicas and TNF- α -stimulated ECs measured by fluorescence intensity of anti-ICAM-1-FITC. EC replicas coated with ICAM-1 (1 $\mu\text{g/ml}$ or 10 $\mu\text{g/ml}$) and fixed EC layers were stained with anti-ICAM-1-FITC, and imaged with fluorescence microscope with identical settings; fluorescence intensities were measured and plotted after background subtraction. (B, C) Effect of ICAM-1 concentration on crawling direction of T cells with respect to the flow direction. EC replicas of well-aligned ECs were coated with ICAM-1 (1 $\mu\text{g/ml}$ or 10 $\mu\text{g/ml}$) and flow assays were performed with the parallel configuration. Percentages of T cells crawling against the flow direction were measured and plotted (B) and representative trajectories of T cells were drawn (C). Data are pulled from 5 independent experiments (Mann-Whitney U-test, two-tailed, $**p < 0.01$).

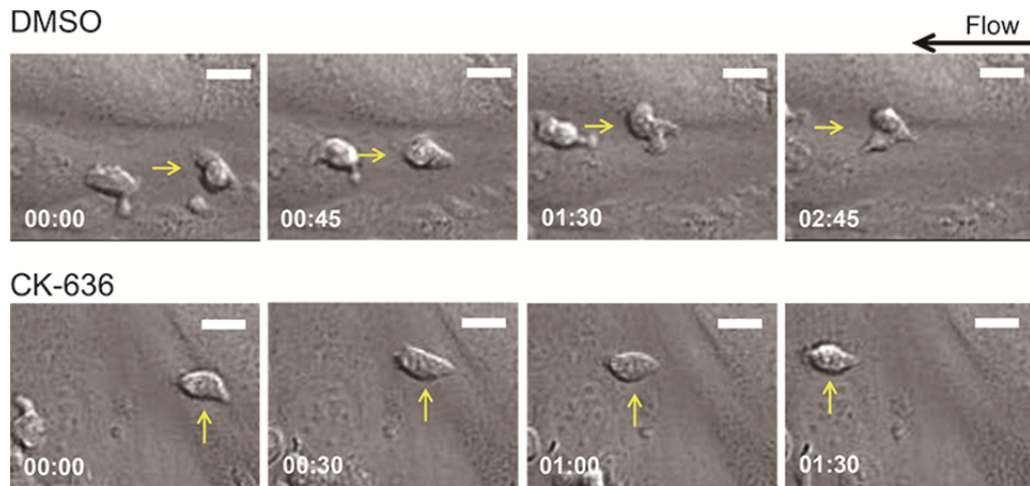


Figure S4 Time-lapse images of T cells treated with either DMSO or CK-636. Scale bar: 10 μm . Elapsed time: mm:ss.

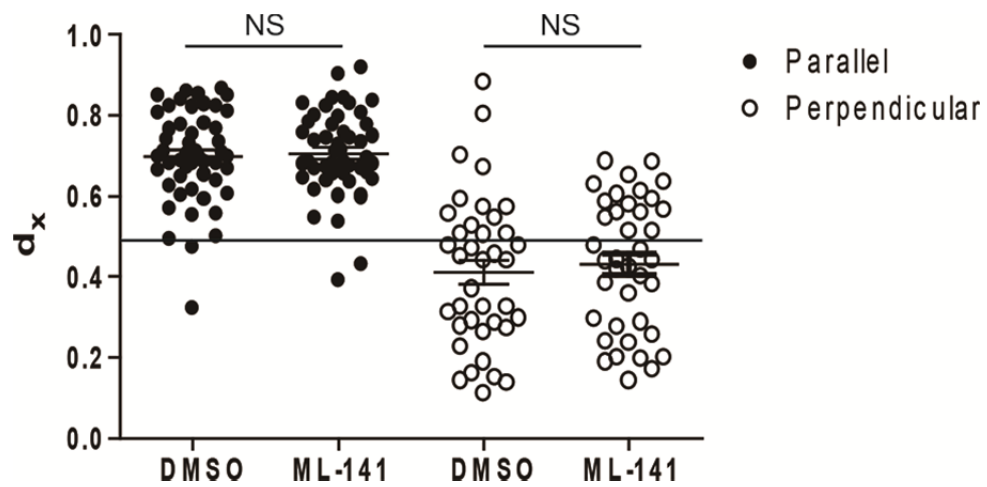


Figure S5 Effect of ML-141 treatment on d_x values of crawling T cells on ECs (Mann-Whitney U-test, two-tailed).

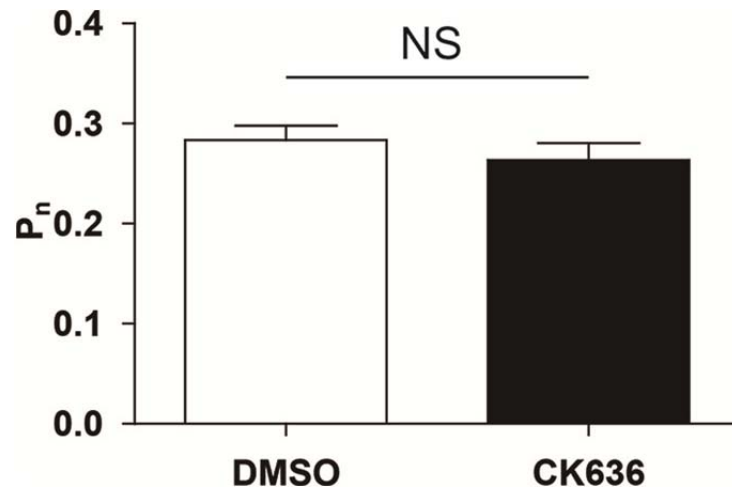


Figure S6 Effect of CK636 treatment on P_n values of crawling T cells on ECs. Data are pooled from 5 independent experiments (DMSO: $n = 116$, ML-141: $n = 110$).

Supplemental movie legends

Movie S1. A representative T cell crawling along junctions of endothelial cells (ECs). Red: T cell, green: VE-cadherin, and blue: nuclei of ECs.

Movie S2. A representative T cell avoiding nucleus of an EC. Blue: nuclei of ECs.

Movie S3. A representative DMSO-treated T cell crawling on ECs.

Movie S4. A representative CK636-treated T cell crawling on ECs.