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Supplemental Information

A Bistable Mechanism Mediated by Integrins Controls Mechanotaxis of Leukocytes

Alexander Hornung, Thomas Sbarrato, Nicolas Garcia-Seyda, Laurene Aoun, Xuan Luo, Martine Biarnes-Pelicot, Olivier Theodoly, and Marie-Pierre Valignat

Supplementary Information

Supplementary movies

Movie S1: T cell motility phenotype under flow (8 dyn.cm^{---2}) on ICAM-1 (left) and VCAM-1 (right) substrates. Bright-field images with magnification x10.

Movie S2: T cell motility phenotype under flow (8 dyn.cm^{-2}) on mixed ICAM-1/ VCAM-1 substrates. Transmission images with magnification x10.

Movie S3: T cell motility phenotype under flow (4 dyn.cm^{-2}) on mixed 50 % ICAM-1 / 50 % VCAM-1 substrate. Transmission (left) and RICM (right) images with magnification x63.

Movie S4: T cell orientation in a flow of changing direction by cell front focusing on ICAM-1 and uropod wind vane control on ICAM-1. VCAM-1 panel: The right cell is representative of cells displaying mostly crawling and the left cell of cells displaying a combination of crawling and rolling. ICAM-1 panel: Cells displaying representative crawling phenotype on ICAM-1. The yellow arrows indicate the direction of flow (8 dyn.cm^{-2}), which is from top to bottom in the beginning of the sequence and from right to left in the end. n > 20 cells/experiments. $n_{exp} = 3$.

Movie S5: T cell motility phenotype under flow (8 dyn.cm^{-2}) on ICAM-1 (left) and VCAM-1 (right) substrates. Top: Transmission images with magnification x63. Bottom: RICM images with magnification x63.

Movie S6: Flow triggers no calcium signaling. Relative intracellular calcium levels in crawling lymphocytes without and with flow, on ICAM-1 then VCAM-1 surfaces. Last part of the movie is the control experiment with ionomycin which increases calcium release by increasing the cell membrane permeability.

Movie S7: The level of integrins expression dictates orientation decision. First part of the movie (left) shows that on a mixed substrate 75% ICAM-1 – 25% VCAM-1, cells are mainly crawling upstream. By adding blocking antibody against LFA-1 (TS1/22) and decreasing the ratio LFA-1/ VLA-4, cells are mainly crawling downstream (right). Second part of the movie (left) shows that on a mixed substrate 25% ICAM-1 – 75% VCAM-1, cells are mainly crawling downstream. By adding blocking antibody against VLA-4 (Natalizumab) and decreasing the ratio LFA-1/ VLA-4, cells are mainly crawling upstream (right).

Supplementary Information 1: Quantification of ICAM-1 VCAM-1 amounts on substrates

Anti-human CD106-PE and anti-human CD54-PE (eBioscience by Thermo Fischer Scientific, Waltham, MA, USA) antibody were used for the quantification of substrates coatings with mixed ICAM-1 and VCAM-1. First we set up a bulk calibration curve by measuring the fluorescence intensity of 41 µm thick channels filled with antibody solutions at concentrations of 1.5, 3, 5 and 7 $\mu g.mL^{-1}$. Channels were pre-treated with 1% Pluronic F127 (Sigma-Aldrich, St Louis, MO) fluorescence to limit adsorption of antibodies on surfaces. Channels were nevertheless rinsed with PBS and the residual fluorescent intensity corresponding to adsorbed antibodies on the surface was measured and then subtracted to the previous measurements. Fig S1-a shows that the final values are proportional to the volume concentration of antibody. The molar weight of an antibody being of 150 kDA, then 1 $\mu g.mL^{-1}$ of antibody corresponds to 4 $molecules.\mu m^{-3}$ and assuming that the signal is given by the total number of molecules in the thin channel then the volume concentration can be turned in a surface concentration for a channel of height 41 µm (Fig S1-b). For each condition of substrate preparation with a given solution of mixed ICAM-1/VCAM-1, two samples were prepared and stained either with CD106-PE or with CD54-PE at 10 $\mu g.mL^{-1}$ overnight at 4°C. Fluorescent images were taken the next day. The fluorescent intensity on either ICAM-1 or VCAM-1 channel minus the fluorescent intensity measured on the Protein A channel was then converted into surface density by comparison with the calibration curves. Figure S1-c shows that, linear variations of Fc-ICAM-1 and Fc-VCAM-1 adsorbed on substrates were obtained versus respective ratio of Fc-ICAM-1 and Fc-VCAM-1 in solution.

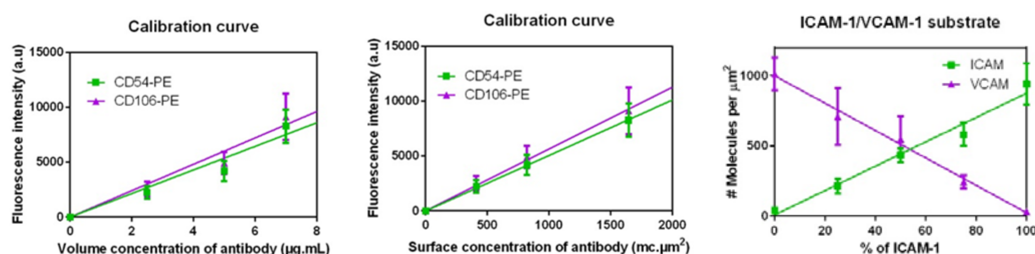

FIGURE S1 : Quantification of substrates with mixed ICAM-1/VCAM-1. (A) Bulk calibration data obtained by measuring the fluorescence intensity of 41 µm thick channels filled with antibody solutions. (B) Conversion of bulk calibration data into a calibration curve linking fluorescence intensity with surface concentration of antibody. (C) Quantification of substrates coated with mixed ICAM-1/VCAM-1 versus the percentage of ICAM-1 in mixed ICAM-1/VCAM-1 solutions used for incubations. All data are mean + s.d, n= 3 independent experiments

Supplementary Information 2: ***Quantification of LFA-1 and VLA-4 expression on effector T cells.***

Quantification LFA-1 and VLA-4 number per cell was performed by quantitative cytometry (FIGURE S2) and yielded an average number per cell of 25000 for LFA-1 and 13000 for VLA-4.

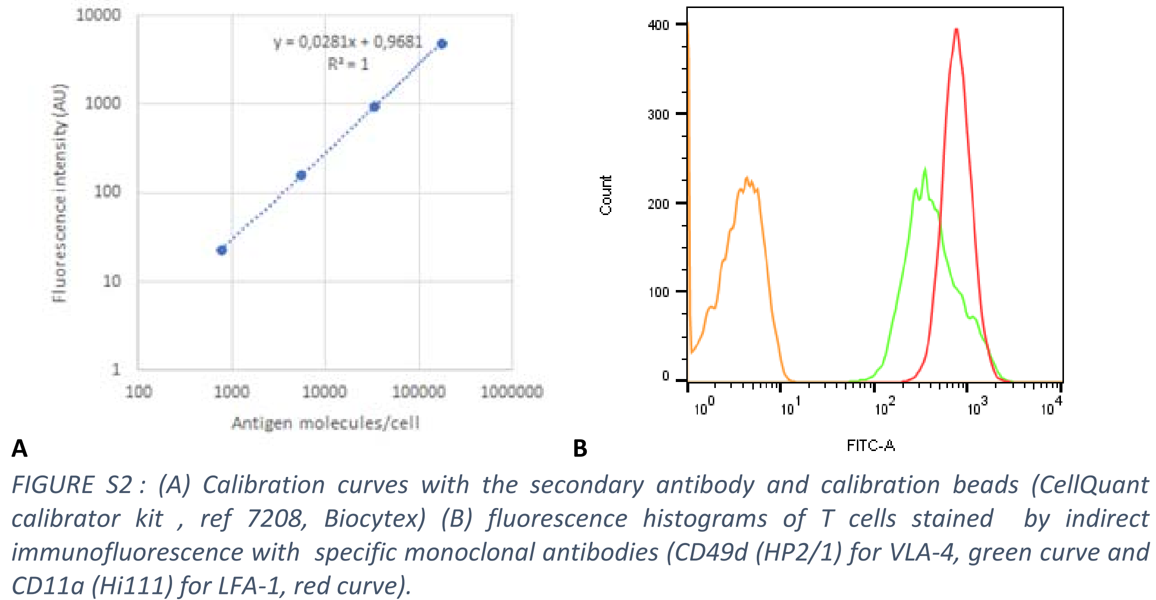

FIGURE S2 : (A) Calibration curves with the secondary antibody and calibration beads (CellQuant calibrator kit , ref 7208, Biocytex) (B) fluorescence histograms of T cells stained by indirect immunofluorescence with specific monoclonal antibodies (CD49d (HP2/1) for VLA-4, green curve and CD11a (Hi111) for LFA-1, red curve).

Supplementary Information 3: Distribution of step lengths for cells crawling aginst and with the flow

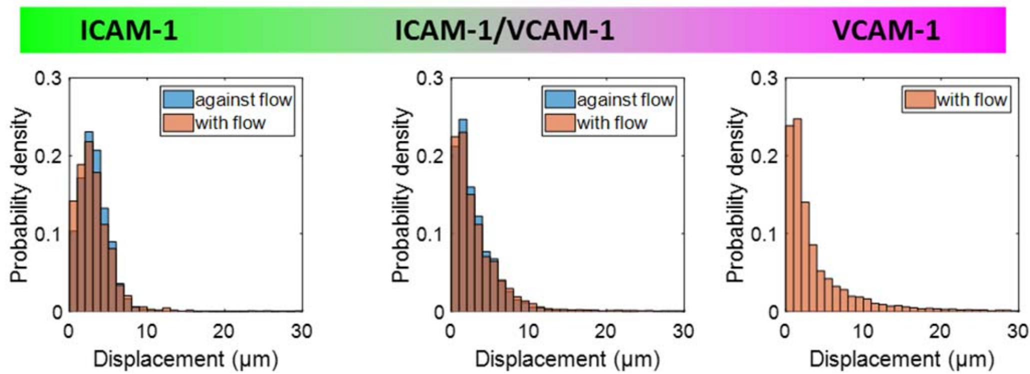

FIGURE S3: Distribution of step lengths for cells crawling against and with the flow. Histograms of displacement length of crawling cells for time intervals of 10s on substrates coated with ICAM-1 (left), mixed ICAM-1/VCAM-1 50/50 (middle) and VCAM-1 (right). Data are reported for usptream-bound (blue) and downstream bound (orange) cells. Ncells >200.

Supplementary Information 4: Calcium signaling

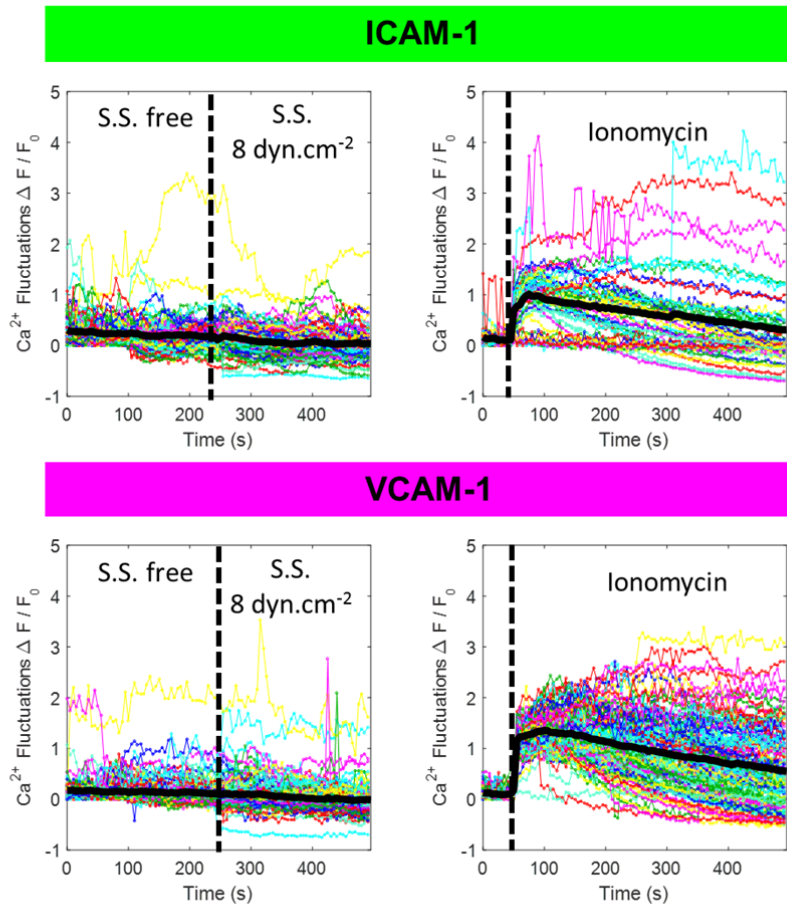

FIGURE S4: Absence of calcium signaling triggered by flow supports absence of mechanotransduction in flow mechanotaxis. Calcium signaling versus onset of flow (left) or addition of ionomycin (right) in cells loaded with Oregon Green 488 BAPTA-1 and crawling on ICAM-1 –top) or VCAM-1 (bottom) substrates.

Supplementary Information 5: Representative quantification of fluorescence intensity along cell axis for LFA-1 and VLA-4 in high affinity states versus substrates ligands densities.

Figure S5 supports the following trends: i-On VCAM-1 there is only VLA-4 and it is mostly in cell rear and central zone. Cell rear is attached, cell front is detached. ii-On ICAM-1 there is only LFA-1 and it is markedly in cell front. Cell rear is detached, cell front is attached. iii-For upstream cell on ICAM-1 25 % VCAM1 75 %, VLA-4 level is low and LFA-1 is markedly in cell front. Cell rear is detached, cell front is attached. iv-For downstream cells on ICAM-1 25 % VCAM1 75 %, LFA-1 level is low, VLA-4 is high in cell rear and central zone. Cell front (or extreme front) is detached, cell rear is attached.

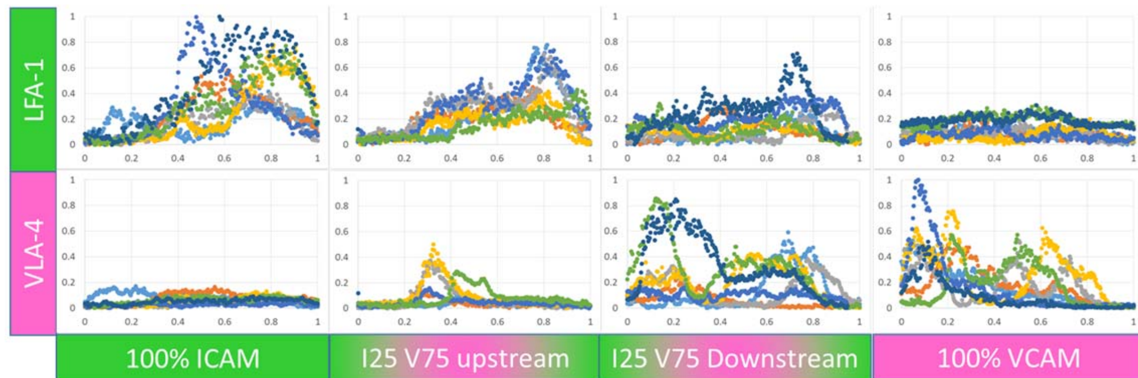

FIGURE S5: Fluorescence intensity profiles of high affinity LFA-1 (mAb 24) and VLA-4 (mAb B44) for different cells after normalization in intensity and units of cell length. Each color corresponds to a different cell, and the same color correspond to the same cell in a given column (i.e. for LFA-1 and VLA-4 data). The left part of each panel corresponds to the rear of each cell and the right part to the front.