Supplemental Materials

Molecular Biology of the Cell

Sawicka et al.
SUPPLEMENTAL MATERIAL

Micropipette Force Probe to quantify single-cell force generation: application to T cell activation.

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This file contains Supplemental Figures S1-S9 (pages 2-10) and the description of Videos 1-6 (page 11).
SUPPLEMENTAL FIGURE S1. Experimental chamber with a glass roof reduces the bead micropipette vibrations. (A) Design of the chamber made of two glass coverslips (50 mm diameter at the bottom, 8x24 mm on top, Thermo Scientific Menzel) spaced with two pieces of adhesive paste (patafix, UHU France, Courbevoie, France). The space between the two cover slips is filled with medium; micropipettes are inserted through the open part at the front. (B) Timetrace of the position of the tip of a bead micropipette of bending stiffness $k=0.51$ nN/µm, placed in an open Petri dish filled with medium (left) and in the experimental chamber (right). Individual data points acquired at a frequency of 400 Hz are shown in black, a window average over 50 data points is
shown as a yellow line. Vibrations were measured at room temperature. Root mean square value (RMS) is reported for both datasets.
SUPPLEMENTAL FIGURE S2. Comparison between the Micropipette Force Probe (MFP) and the Biomembrane Force Probe (BFP). The pulling loading rate was measured for the activation of human primary CD4+ T cells with anti-CD3/anti-CD28 beads (MFP) and anti-CD3 beads (BFP). The BFP data was published before (Husson, J., Chemin, K., Bohineust, A., Hivroz, C., and Henry, N. (2011). Force Generation upon T Cell Receptor Engagement. PLoS One 6, e19680.), the MFP data are in Figure 3C. Each data point shows mean±s.d. over one day of experiments, representing 3 to 10 cells (N=7±2, mean±s.d.).
SUPPLEMENTAL FIGURE S3. Scanning Electron Microscopy images of T cell activation. Human primary CD4+ T cells were incubated on glass slides coated with poly-L-lysine for 20 minutes. Then, anti-CD3/anti-CD28 beads were added, and cells were fixed 5 minutes later. (A) Pushing protrusion (punch) grows at a sharp angle from the cell body. This image was taken with the sample table tilted at 45° angle. (B) Images used for the insets in Figure 4. Frames with dashed line show the parts that were cropped. The magnification used is marked in each image; beads have the diameter of 4.5 \( \mu \text{m} \).
SUPPLEMENTAL FIGURE S4. Examples of the punch morphology during buckling. Scanning Electron Microscopy images (left) and brightfield images chosen based on similarity (right, scale bar is 10 µm). In both cases the T cells were activated with anti-CD3/anti-CD28 beads (4.5 µm diameter). The SEM images were cropped from large views (1000x magnification, insets zoomed 2x, top and middle, and 7000x magnification, bottom image) and show human primary CD4+ T cells. The brightfield images show a human primary CD4+ T cell (top), a human pre-activated CD4+ T cell (T lymphoblast) that picked the bead out of the bead micropipette (middle), and a human primary CD4+ T cell during the experiment with the cell micropipette retracted (bottom, see Fig. 1D). Brightfield images show both designs of the bead micropipette (see Supplemental Figure S6).
SUPPLEMENTAL FIGURE S5. Pushing speed after the buckling of the punch is the same as before the buckling. Pushing speed measured before and after buckling (see Figure 3A) for resting human primary CD4+ T cells activated with anti-CD3/anti-CD28 beads (N=34). Red thick line shows median, whiskers span the interquartile range.
SUPPLEMENTAL FIGURE S6. Two designs for the bead micropipette. Drawings of cell and bead micropipette during an experiment (top) with corresponding brightfield images (bottom). Scale bar is 5 µm in both images. The part of the micropipettes that is vertical in the drawing bends when a cell exerts forces, leading in both cases to the bead movement along the x axis.
SUPPLEMENTAL FIGURE S7. SiR-actin staining of activated T cells. Human primary CD4+ T cells were incubated with 1 µM SiR-actin and 10 µM verapamil for 1 h at 37°C, then spun down (300g, 3 min, room temperature) and imaged in a Petri dish with complete medium and beads. The figure shows two different fields of view during the same experiment. Brightfield images (left), actin filaments in green (middle) and overlay (right). During the activation, the SiR-actin staining moves gradually towards the back of the cell (the side opposite to the bead), and in the end stains the crescent-shape back of the flytrap cells (the cell in the top left corner) and the uropods of the nepenthes cells. See also Video 6. Scale bar is 50 µm.
SUPPLEMENTAL FIGURE S8. Calibration of the bead micropipette. (A) Drawing of the calibration setup. The bead micropipette is placed on the micropositioner with a piezoelectric controller along with a strain gauge reader (Thorlabs, Newton, NJ, USA), which enables the controlled movement of the base of the micropipette. The standard microindenter, of known stiffness, $k_s$, is held by a second micropositioner, with the tip placed in the microscope field of view. Inset: brightfield image of a micropipette and a microindenter before calibration, scale bar is 20 µm. (B) Calculation of the bending stiffness of the micropipette, $k_p$. During calibration the micropipette is first brought in contact with the tip of the indenter (brightfield image on the left). Then the base of the micropipette is moved by a distance $z$, set on the piezoelectric controller. The tip of the micropipette moves together with the tip of the indenter by a smaller distance $x$, tracked in the microscopy image. The same force causes the bending of the indenter by a distance $d_{\text{standard}} = x$ and the bending of the micropipette by a distance $d_{\text{pipette}} = z - x$. We obtain $k_p$ from the slope of the linear regression of the $x(z)$ plot (right).
SUPPLEMENTAL FIGURE S9. ML-7 treatment of cells inhibits phosphorylation of the myosin light chain (MLC). Human primary CD4+ T cells were pre-incubated for 15 minutes at 37°C with 30 μM ML-7, the MLCK inhibitor, or the vehicle (DMSO) alone. Cells were then activated for 10 to 30 minutes with anti-CD3/anti-CD28 beads in the presence of ML-7. Phosphorylation of the MLC was measured by Western blot analysis. The gp96 protein in each lane is shown as a loading control. Compared to control (DMSO), the cells incubated with ML-7 show less phosphorylation of MLC demonstrating that ML-7 is inhibiting MLCK in our conditions.