



Rapid viscoelastic changes are a hallmark of early leukocyte activation

Alexandra Zak, Sara Violeta Merino-Cortés, Anaïs Sadoun, Farah Mustapha, Avin Babataheri, Stéphanie Dogniaux, Sophie Dupre-Crochet, Elodie Hudik, Hai-Tao He, Abdul Barakat, et al.

► To cite this version:

Alexandra Zak, Sara Violeta Merino-Cortés, Anaïs Sadoun, Farah Mustapha, Avin Babataheri, et al.. Rapid viscoelastic changes are a hallmark of early leukocyte activation. *Biophysical Journal*, 2021, 120 (9), pp.1692-1704. <10.1016/j.bpj.2021.02.042>. <hal-03375058>

HAL Id: hal-03375058

<https://amu.hal.science/hal-03375058v1>

Submitted on 16 Oct 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

Rapid viscoelastic changes are a hallmark of early leukocyte activation.

Alexandra Zak^{1,2}, Sara Violeta Merino-Cortés³, Anaïs Sadoun^{4,5,6}, Farah Mustapha^{4,5,6,7}, Avin Babataheri¹, Stéphanie Dogniaux⁸, Sophie Dupré-Crochet², Elodie Hudik², Hai-Tao He⁹, Abdul I Barakat¹, Yolanda R Carrasco³, Yannick Hamon⁹, Pierre-Henri Puech^{4,5,6}, Claire Hivroz⁸, Oliver Nüsse², and Julien Husson^{1*}.

¹ LadHyX, CNRS, Ecole polytechnique, Institut Polytechnique de Paris, 91120, Palaiseau, France.

² Institut de Chimie Physique, CNRS UMR8000, Université Paris-Saclay, 91405, Orsay, France.

³ B cell Dynamics Laboratory, Department on Immunology and Oncology, Centro Nacional de Biotecnología (CNB)-CSIC, Madrid, Spain.

⁴ Aix Marseille University, LAI UM 61, Marseille, F-13288, France.

⁵ Inserm, UMR_S 1067, Marseille, F-13288, France.

⁶ CNRS, UMR 7333, Marseille, F-13288, France.

⁷ Centre Interdisciplinaire de Nanoscience de Marseille (CINaM), CNRS, Aix Marseille University, Marseille, F-13009 France.

⁸ Institut Curie, PSL Research University, INSERM U932, Integrative analysis of T cell activation team, 26 rue d'Ulm, 75248, Paris Cedex 05, France.

⁹ Aix Marseille University, CNRS, INSERM, CIML, Marseille, France.

* corresponding author, julien.husson@ladhyx.polytechnique.fr, <https://cellmechanics.jimdofree.com/>

Abstract

To accomplish their critical task of removing infected cells and fighting pathogens, leukocytes activate by forming specialized interfaces with other cells. The physics of this key immunological process are poorly understood, but it is important to understand them as leukocytes have been shown to react to their mechanical environment. Using an innovative micropipette rheometer, we show in three different types of leukocytes that, when stimulated by microbeads mimicking target cells, leukocytes become up to ten times stiffer and more viscous. These mechanical changes start within seconds after contact and evolve rapidly over minutes. Remarkably, leukocyte elastic and viscous properties evolve in parallel, preserving a well-defined ratio that constitutes a mechanical signature specific to each cell type. Our results indicate that simultaneously tracking both elastic and viscous properties during an active cell process provides a new way to investigate cell mechanical processes. Our findings also suggest that dynamic immuno-mechanical measurements can help discriminate between leukocyte subtypes during activation.

Significance

The mammalian immune response is largely based on direct interactions between white blood cells (leukocytes) and pathogens, inert particles or other host cells. Mechanical properties of leukocytes regulate their migration in the microcirculation to reach their targets, and their interaction with these targets. Yet, these mechanical properties and their evolution upon leukocyte activation remain poorly studied. We developed a micropipette-based rheometer to track cell viscous and elastic properties. We show that leukocytes become up to ten times stiffer and more viscous during their activation. Elastic and viscous properties evolve in parallel, preserving a ratio characteristic of the leukocyte subtype. These mechanical measurements set up a complete picture of the mechanics of leukocyte activation and provide a signature of cell function.

Introduction

The understanding of cell mechanics has progressed with the development of micromanipulation techniques such as micropipette aspiration (1), indentation techniques (2), and others (3). Microfluidics-based approaches such as real-time deformability cytometry now allow high-throughput mechanical measurements (4, 5). Yet, these techniques make it difficult to track mechanical changes in cells stimulated by soluble molecules (6, 7), and even more difficult when cells are stimulated by activating surfaces or partner cells (8).

Tracking these changes can bring a wealth of information on cell function in healthy conditions and diseases. Here we illustrate this by focusing on white blood cells (leukocytes). These cells, among other functions, fight infected cells or pathogens, remove dead cells, or identify antigens at the surface of other cells and eventually build an immune response against a detected threat. To do so, leukocytes activate by forming with other cells specialized interfaces called immunological synapses. Different leukocytes form different types of synapses, which share several molecular (9) but also mechanical features. One of these mechanical features is force generation, which is now well documented in different types of leukocytes. For instance, we have shown that a T lymphocyte generates forces when forming a synapse (10, 11). Others recently showed that B cells generate traction forces during activation (12, 13), and Evans *et al.* quantified decades ago contractile forces generated during phagocytosis (14). The role of these forces is still an open question. Their suggested functions, such as allowing a tight cell-cell contact or probing the mechanical properties of the opposing surface, place these forces at the core of leukocyte activation (15, 16). In this study, we focus on another mechanical feature: changes in leukocyte viscoelasticity during their activation. These changes have been shown to exist in T cells, phagocytes, and B cells. We have shown that during activation, T cells stiffen (17). Pioneering observations showed that during phagocytosis, the cortical tension of a phagocyte increases dramatically while this cell engulfs its prey (14, 18), and more recent observations confirmed mechanical stiffening of leukocytes during phagocytosis (19). We also recently quantified elastic changes in B cells during activation (20).

As for force generation, the function of the mechanical properties of leukocytes during activation is not yet understood and open to speculation (see Discussion). Yet, it is important to quantify and describe them for two reasons. First, we need a complete picture of the mechanics of leukocyte activation to properly incorporate mechanics into currently proposed models of the activation of the different types of leukocytes. Second, beside the fundamental interest of understanding these mechanical changes, they can have very important consequences in the context of disease. Indeed, mechanical properties of leukocyte dictate how easily leukocytes migrate in or out of tissue or flow in small blood vessels (21), so changes of these properties can perturb physiological processes. For instance, circulating neutrophils stimulated by proinflammatory molecules exhibit changes in their mechanical properties (22, 23), which contributes to their trapping in pulmonary vasculature in lung diseases such as acute respiratory distress syndrome (23, 24). Being trapped or slowed down while traveling in a capillary can be due to an increased cell stiffness, but also to an increased cell viscosity. Understanding cell viscous properties is thus also important, although viscous properties are much less explored than elastic ones due to inherent difficulties in quantifying them.

Most measurements of leukocyte mechanics, including the ones mentioned above, were focused on elastic and not viscous properties. However, it was recognized decades ago that leukocytes are viscoelastic and not only elastic (25–27), so in many situations we miss an important aspect of leukocyte mechanics. Fabry *et al.* (28) and others have shown that one can expect the viscous properties of leukocytes to be linked to their elastic properties. It was recognized that resting leukocytes such as neutrophils (27, 29) and macrophages (30) conserve a ratio of cortical tension to cell viscosity of about 0.2–0.3 $\mu\text{m/s}$, even though macrophages can be ten times more tensed than neutrophils. The ratio of viscous to elastic properties seems to be constrained within a rather narrow range of values, and many studies showed this peculiar aspect of biological matter in various cell types (28, 31, 32). Here, we asked if for a given cell type, elastic and viscous properties of leukocytes respected a relationship while they were both evolving over time. To do so, we quantified the evolution of both elastic

and viscous properties during the activation of three types of leukocytes. We used a micropipette rheometer to activate leukocytes with standardized activating antibody-covered microbeads (17, 33–35), and we further probed early mechanical changes occurring in leukocyte-target cell contacts by using Atomic Force Microscopy in Single Cell Force Spectroscopy mode (36).

Materials and Methods

Micropipette rheometer

The rheometer was build based on the evolution of our profile microindentation setup(37) and Micropipette Force Probe(17, 33, 38). Micropipettes were prepared as described previously(17, 33, 37, 38) by pulling borosilicate glass capillaries (Harvard Apparatus, Holliston, MA, USA) with a P-97 micropipette puller (Sutter Instruments, Novato, CA, USA), cutting them with an MF-200 microforge (World Precision Instruments, Sarasota, FL, USA) and bending them at a 45° angle with an MF-900 microforge (Narishige, Tokyo, Japan). Micropipettes were held by micropipette holders (IM-H1, Narishige) placed at a 45° angle relative to a horizontal plane, so that their tips were in the focal plane of an inverted microscope under brightfield or DIC illumination (T cells: TiE ; PLB cells: Ti2, Nikon Instruments, Tokyo, Japan) equipped with a 100× oil immersion, 1.3 NA objective (Nikon Instruments) and placed on an air suspension table (Newport). The flexible micropipette was linked to a non-motorized micropositioner (Thorlabs, Newton, NJ, USA) placed on top of a single-axis stage controlled with a closed loop piezo actuator (TPZ001; Thorlabs). The bending stiffness k of the flexible micropipette (about 0.2 nN/μm) was measured against a standard microindenter previously calibrated with a commercial force probe (model 406A; Aurora Scientific, Aurora, ON, Canada). Once the activating microbead was aspirated by the flexible micropipette, the cell held by the stiff micropipette was brought in adequate position using a motorized micromanipulator (MP-285; Sutter Instruments). Experiments were performed in glass-bottom Petri dishes (Fluorodish, WPI, Sarasota, FL, USA). Images were acquired using a Flash 4.0 CMOS camera (for T cells), or a SPARK camera (PLB cells), both from Hamamatsu Photonics, Hamamatsu City, Japan. In order to perform rheological experiments, the setup automatically detects the position of the bead at the tip of the force probe at a rate of 400-500 Hz and imposes the position of the base of the flexible micropipette by controlling the position of the piezo stage. The deflection of the force probe is the difference between the position of the bead and the position of the piezo stage. Thus, the force applied to the cell is the product of this deflection by the bending stiffness k . A retroaction implemented in Matlab (Mathworks) controlling both the camera via the Micromanager software (39) and the piezo stage moves the latter in reaction to the measurement of the bead position in order to keep a desired deflection of the cantilever: a controlled force is applied to the cell at any given time. Experiments were performed at room temperature to avoid thermal drift. Qualitative behavior of T cell and PLB cells is unchanged at 37°C (not shown). We quantified the fluid drag due to micropipette translation (Suppl. Mat. 1) and the potential effect of fast cell deformation on our rheological measurements (Suppl. Mat. 2). The accuracy when measuring the phase shift depends on the acquisition frequency of both force and cell deformation (x_{tip}), which is 400-500 Hz depending on the size of the region of interest chosen for the acquisition. This leads to a time resolution of 2-2.5 ms. The measured loss tangent had a typical value of $\eta = \frac{K''}{K'} = \tan(\varphi) \approx 0.3 - 0.5$, i.e. $\varphi \approx 0.3 - 0.5$. When expressed in seconds instead of radians, this phase lag represents a delay $\Delta t = \frac{\varphi}{2\pi} T$, where $T = 1$ s is the period of the oscillatory force modulation, which leads to $\Delta t = 46 - 74$ ms. This is reasonably long when compared to our time resolution so as to be accurately measured. Note that the uncertainty on the phase lag influenced more K'' than K' , because K'' is proportional to $\sin(\varphi)$ while K' is proportional to $\cos(\varphi)$. *Data analysis.* Rheological measurements were analyzed by post-processing using a custom-made Python code. During force modulation sinusoidal x_{tip} signal was fitted every 0.5-1 period interval over a window that was 1.5-2.5 periods long (i.e. every 0.5-1 second with a window of 1.5-2.5 seconds for a frequency of force modulation $f=1$ Hz) using a function corresponding to a linear trend with an added sinusoidal signal (two free parameters for the linear trend, two for the sinusoidal signal

144 when imposing the frequency $f=1$ Hz). Best fit was determined using classical squared error minimiza-
145 tion algorithm in Python.

146 **T cell experiments**

147 **Cells and reagents.** Mononuclear cells were isolated from peripheral blood of healthy donors on a Ficoll
148 density gradient. Buffy coats from healthy donors (both male and female donors) were obtained from
149 Etablissement Français du Sang (EFS, Paris, France) in accordance with INSERM ethical guidelines.
150 Human total CD4+ isolation kit (Miltenyi Biotech, 130-096-533) was used for the purification of T cells.
151 Isolated T-cells were suspended in FBS: DMSO (90%:10% vol/vol) and kept frozen in liquid nitrogen.
152 One to seven days before the experiment the cells were thawed. Experiments were performed in RPMI
153 1640 1x with GlutaMax, supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1%
154 penicillin-streptomycin (all from Life Technologies ThermoFisher Scientific, Waltham, MA), and filtered
155 with (0.22 μm diameter-pores, Merck Millipore).

156 **Beads.** Dynabeads® Human T-Activator CD3/CD28 for T Cell Expansion and Activation from Gibco,
157 were purchased from Thermo Fisher Scientific (ref. 11131D, Carlsbad, CA). These 4.5- μm , superpara-
158 magnetic polymer beads, coated with an optimized mixture of mouse anti-human monoclonal IgG an-
159 tibodies against the CD3 and CD28 cell surface molecules of human T cells, mimic stimulation by an
160 antigen presenting cell (APC). The CD3 antibody is specific for the epsilon chain of human CD3. The
161 CD28 antibody is specific for the human CD28 co-stimulatory molecule, which is the receptor for CD80
162 (B7-1) and CD86 (B7-2). These beads induce the phosphorylation of several signaling proteins (17, 40).
163 Control beads were non-coated 4.5- μm polystyrene beads.

164 **B cell experiments**

165 **B cells.** Primary B lymphocytes were isolated from spleens of adult C57BL/6J mice (10- to 20-week-
166 old), purified by negative selection using the MACS kit (130-090-862) in the total splenocytes. Animal
167 procedures were approved by the CNB-CSIC Bioethics Committee and conform to institutional, nation-
168 al and EU regulations. Cells were cultured in complete RPMI 1640-GlutaMax-I supplemented with 10%
169 FCS, 1% penicillin-streptomycin, 0.1% β -mercaptoethanol and 2% sodium pyruvate (denominated
170 hereafter as complete RPMI).

171 **Beads.** Silica beads (5×10^6 ; 5 μm diameter; Bangs Laboratories) were washed in distilled water
172 (5,000 rpm, 1 min, RT), and incubated with 20 μl 1,2-dioleoyl-PC (DOPC) liposomes containing GPI-
173 linked mouse ICAM-1 (200 molecules/ μm^2) and biotinylated lipids (1000 molecules/ μm^2) (10 min,
174 RT), and washed twice with beads-buffer (PBS supplemented with 0.5% FCS, 2 mM MgCl_2 , 0.5 mM
175 CaCl_2 , and 0.5 g/L D-Glucose). Then, lipid-coated beads were incubated with AF647-streptavidin (Mo-
176 lecular Probes) (20 min, RT) followed by biotinylated rat anti- κ light chain antibody (clone 187.1, BD
177 Biosciences) (20 min, RT), used as surrogate antigen (su-Ag) to stimulate the B cell receptor. Control
178 beads were coated with ICAM-1-containing lipids only (no su-Ag was added). The number of mole-
179 cules/ μm^2 of ICAM-1 and su-Ag/biotin-lipids was estimated by immunofluorometric assay using anti-
180 ICAM-1 or anti-rat-IgG antibodies, respectively; the standard values were obtained from microbeads
181 with different calibrated IgG-binding capacities (Bang Laboratories). The lipid-coated silica beads
182 were finally re-suspended in complete RPMI before use. Such a use of lipid-coated silica beads as
183 pseudo-APCs to study immune synapse formation, cell activation, proliferation and antigen extraction
184 by B cells has been previously set up and reported by us (20, 41). They provide the adhesion ligand
185 ICAM-1 for the integrin LFA-1, expressed at the B cell surface, and tethered BCR-stimulatory signal
186 (su-Ag) in a fluid phospholipid environment.

187 **PLB cell experiments**

188 **Cells and reagents.** The human acute myeloid leukemia cell line PLB-985 is a subline of HL-60 cells
189 based on STR fingerprinting (42, 43). Gene expression in the two lines is similar but not identical (44).
190 PLB-985 cells were cultured in RPMI 1640 1X GlutaMax (Gibco) supplemented with 10% sterile heat-
191 inactivated FBS and 1% sterile penicillin/streptomycin. Cells were passaged twice a week and differ-
192 entiated into a neutrophil-like phenotype by adding 1.25 % (v/v) of dimethyl sulfoxide (DMSO, Sigma-

193 Aldrich) to the cell suspension the first day after passage and, in a second time, three days after while
194 changing the culture media (45). Twenty four hours before experiments, 2000 U/ml IFN- γ (Immuno
195 Tools) were added into the cell culture flask, cells were centrifuged for 3 min at 300g and resuspended
196 in 0.22- μ m filtered HEPES medium (140 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 2 mM CaCl₂, 10 mM HEPES
197 pH 7.4, 1.8 mg/mL glucose, and 1% heat-inactivated FBS ; filters from Merck Millipore). Differentiated
198 PLB-985 cells stay in suspension and have neutrophil-like properties (Suppl. Mat. 14).

199 **Beads.** 20- μ m and 8- μ m diameter polystyrene microbeads at 10⁶ beads/mL (Sigma-Aldrich) were
200 washed three times by centrifugation at 16000 g for 3min and re-suspended in Dulbecco's Phosphate
201 Buffered Saline (DPBS, Gibco) filtered with 0.22 μ m diameter-pores (Merck Millipore). Beads were
202 then incubated overnight at room temperature with 5% (w/v) Bovine Serum Albumin (BSA, Sigma-
203 Aldrich) in DPBS. Beads were washed again three times by centrifugation at 16000 g during 3min, re-
204 suspended in DPBS, and incubated with 1:500 anti-BSA rabbit antibody (Sigma-Aldrich, ref. B1520) in
205 DPBS for an hour at room temperature. These IgG-coated beads were washed three times by centrifuga-
206 tion at 16000g for 3 min with DPBS and resuspended in DPBS at 10⁶ beads/mL before use. Control
207 beads were uncoated polystyrene beads, inducing very rare cases of adhesion with the cell but no
208 phagocytosis. PLB cells tried almost systematically to internalize 20- μ m activating beads by perform-
209 ing frustrated phagocytosis, and phagocytosed 8- μ m activating beads.

210 Atomic Force Microscope and Single Cell Force Spectroscopy

211 **Cells and reagents.** 3A9m T cells were obtained from D. Vignali (46) and cultured in RPMI completed
212 with 5 % FBS, 10 mM Hepes, 10mM Sodium Pyruvate in 5 % CO₂ atmosphere. COS-7 APCs were gen-
213 erated as previously described (47) by stably co-expressing the α and the β chains of the mouse MHC
214 class II I-Ak , cultured in DMEM (5 % FBS, 1mM Sodium Pyruvate, 10 mM Hepes, and geneticin
215 10 μ g/ml). Cells were passaged up to three times a week by treating them with either Trypsin/EDTA or
216 PBS 1X (w/o Ca²⁺/Mg²⁺), 0.53mM EDTA at 37°C for up to 5 min.

217 The anti CD45 antibodies used for this study were produced from the hybridoma collection of CIML,
218 Marseille, France (namely H193.16.3)(48). Briefly, cells were routinely grown in complete culture me-
219 dium (DMEM, 10% FBS, 1mM sodium pyruvate) prior to switching to the expansion and production
220 phase. Hybridoma were then cultured in DMEM with decreasing concentrations of low immunoglobu-
221 lin fetal bovine serum down to 0.5%. Cells were then maintained in culture for 5 additional days ena-
222 bling immunoglobulin secretion prior to supernatant collection and antibody purification according to
223 standard procedures.

224 The expression of TCR and CD45 molecules on T cells and MHC II molecules on COS-7 APC was as-
225 sessed once a week by flow cytometry (anti-TCR PE clone H57.597, anti-CD45 Alexa Fluor 647 clone
226 30F11 were purchased from BD Pharmingen). The count and the viability, with Trypan blue, were as-
227 sessed automatically twice by using Luna automated cell counter (Biozym Scientific). Mycoplasma test
228 was assessed once a month. Culture media and PBS were purchased from Gibco (Life Technologies).
229 PP2 (Lck inhibitor) was purchased from Calbiochem.

230 **Substrates.** Culture treated, sterile, glass bottom Petri dishes (World Precision Instruments
231 Fluorodish FD35-100) were incubated with 50 μ g/ml anti-CD45 antibody for 1 hour at room tempera-
232 ture. The surfaces were extensively washed with PBS 1X prior to a last wash with HBSS 1X 10 mM
233 Hepes. The surfaces were kept wet with HBSS 1X 10 mM Hepes before seeding T cells.

234 **T cell preparation.** T cells were counted, centrifuged and resuspended in HBSS 1X 10 mM Hepes and
235 were kept for one hour at 37 °C 5% CO₂ for recovery prior to seeding. The cells were seeded at room
236 temperature and after 30 minutes a gentle wash was done by using two 1mL micropipettes in order to
237 maintain, as much as possible, a constant volume in the Petri dish and hence avoid perturbations by
238 important flows. After one hour incubation the Petri dish was mounted in a Petri dish Heater system
239 (JPK Instruments) to maintain the temperature of the sample at ~ 37°C. 10 minutes before experi-
240 ments, 10 μ M (final concentration) of Lck kinase inhibitor, PP2 drug was added and homogenized in
241 the sample. We kept this drug present all along the experiments.

242 **COS-7 APC preparation.** The day before the experiment, COS-7 expressing MHCII cells were incubat-
243 ed with the peptide of interest with a final concentration of 10 μ M allowing 100% occupation of MHC II

244 molecules. Before the experiment the cells were detached from the cell culture plates by removing the
 245 cell medium, washed once with PBS 1X and then by a 0.53 mM EDTA treatment for 5 minutes at 37°C
 246 10% CO₂. The cells were resuspended in HBSS 1X 10mM Hepes and were allowed to recover before to
 247 be seeded into the sample. The peptide p46.61 (which is CD4 dependent) was purchased from Geno-
 248 sphere with a purity >95%.

249 **AFM setup.** The setup has been described in great detail elsewhere (49). Measurements were con-
 250 ducted with an AFM (Nanowizard I, JPK Instruments, Berlin) mounted on an inverted microscope
 251 (Zeiss Axiovert 200). The AFM head is equipped with a 15 μ m z-range linearized piezoelectric scanner
 252 and an infrared laser. The set-up was used in closed loop, constant height feedback mode (36). MLCT
 253 gold-less cantilevers (MLCT-UC or MLCT-Bio DC, Bruker) were used in this study. The sensitivity of the
 254 optical lever system was calibrated on the glass substrate and the cantilever spring constant by using
 255 the thermal noise method. Spring constant were determined using JPK SPM software routines *in situ* at
 256 the start of each experiment. The calibration procedure for each cantilever was repeated three times
 257 to rule out possible errors. Spring constants were found to be consistently close to the manufacturer's
 258 nominal values and the calibration was stable over the experiment duration. The inverted microscope
 259 was equipped with 10x, 20xNA0.8 and 40xNA0.75 lenses and a CoolSnap HQ2 camera (Photometrics).
 260 Bright field images were used to select cells and monitor their morphology during force measure-
 261 ments through either Zen software (Zeiss) or Micro-Manager (39).

262 **APC/T cell force measurements.** *Lever decoration.* To make the cantilevers strongly adhesive, we
 263 used a modified version of our previous protocols (50). Briefly, cantilevers were activated using a 10
 264 min residual air plasma exposure then dipped in a solution of 0.25mg/mL of Wheat Germ Agglutinin
 265 (WGA) or 0.5 mg/mL of Concanavalin A (ConA) in PBS 1x for at least one hour. They were extensively
 266 rinsed by shaking in 0.2 μ m filtered PBS 1x, stored in PBS 1x at 4°C. Before being mounted on the glass
 267 block lever holder, they were briefly dipped in MilliQ-H₂O in order to avoid the formation of salt crys-
 268 tals in case of drying and hence alteration of the reflection of the laser signal. *APC capture.* In separate
 269 experiments, we used side view and micropipette techniques, and we observed that (i) this allows the
 270 presented cell to be larger than the lever tip, excluding any unwanted contact of T cell with lectins and
 271 (ii) the binding is resistant enough to ensure that any rupture event recorded is coming from the
 272 cell/cell interface (Suppl. Mat. 3, Suppl. Fig. S2). After calibration, a lectin-decorated lever is pressed on
 273 a given COS7 cell for 20 to 60 seconds with a moderate force (typically 1-2nN), under continuous
 274 transmission observation. Then, the lever is retracted far from the surface and the cell is let to recover
 275 and adhere/spread for at least 5 minutes before starting the experiments (Suppl. Fig. S2). *SCFS exper-*
 276 *iment.* The surrogate APC is then positioned over a desired T cell. Because the lever is not coated with
 277 gold, hence almost transparent, one can finely position the probe over the target. Force curves are then
 278 recorded using the following parameters: max contact force 1nN, speeds 2 μ m/sec, acquisition fre-
 279 quency 2048 Hz, curve length 10 μ m and contact time 60 sec. At the end of the retraction, lateral mo-
 280 tion of the cells is made by hand by moving the AFM head in the (x,y) plane, relative to the Petri dish
 281 substrate, until no more force is recorded by the lever, signaling that full separation was achieved
 282 (Suppl. Fig. S2). One APC cell was used to obtain one force curve on at least three different T cells, and
 283 3 APCs at least were used for each condition. No apparent bias was detected in the data when carefully
 284 observing the succession of the measures for a given APC, for a given lever and for a set of T cells. *Data*
 285 *processing & Statistics.* Force curves were analyzed using JPK Data Processing software on a Linux 64
 286 bits machine. Each curve was evaluated manually, except for calculation and plotting of mean \pm SD for
 287 relaxation curves where *ad-hoc* python scripts were used. Data were processed using Graphpad Prism
 288 (v7) on a Windows 7 machine. On graphs, data are presented as scatter dot plots with median and in-
 289 terquartile range. A data point corresponds to a force curve obtained for a given couple T cell/APC,
 290 except for the small detachment events where one data point corresponds to one such event (all
 291 events were pooled). Significance was assessed using Mann-Whitney tests in GraphPad Prism v7 with
 292 p<0.05 *, <0.01 **, <0.001 ***, and **** below; not significant otherwise.

Results

Micropipette rheometer for monitoring rapid morphological and mechanical changes in non-adherent cells

We implemented a real-time feedback loop in our Micropipette Force Probe setup (17, 33)(Fig. 1a) allowing us to impose a controlled small oscillatory force modulation $\Delta F \cos(\omega t)$ (angular frequency $\omega = 2\pi f$, frequency $f = 1$ Hz) superimposed onto a constant force $\langle F \rangle$. A total force $F(t) = \langle F \rangle + \Delta F \cos(\omega t)$ is thus applied to the leukocyte during its activation following the contact with an activating microbead coated with cell-specific antibodies. The contact is ensured by pressing the bead against the cell with a force F_{comp} (0.12-0.36 nN). This initial compression, from which we extract the cell's effective Young's modulus E_{Young} (51, 52) (Fig. 1b, inset, bottom right, and Suppl. Mat. 4), is followed by an imposed force modulation regime, where we measure oscillations in the position of the tip of the flexible micropipette $x_{tip}(t) = \langle x_{tip} \rangle + \Delta x_{tip} \cos(\omega t - \varphi)$, of average value $\langle x_{tip} \rangle$, amplitude Δx_{tip} , (typically 100 nm, with a few-nanometer accuracy) and phase lag φ . Changes in $x_{tip}(t)$ reflect changes in cell length (Fig. 1b). As the cell is shorter when the force is higher, $x_{tip}(t)$ decreases when $F(t)$ increases, and corresponds to the lag between a maximum of the force and the following minimum of $x_{tip}(t)$ (Fig. 1b, inset, bottom right). From $x_{tip}(t)$ and $F(t)$, we deduce the complex cell stiffness $K^* = K' + iK''$, of elastic part $K' = \frac{\Delta F}{\Delta x_{tip}} \cos \varphi$ and viscous part $K'' = \frac{\Delta F}{\Delta x_{tip}} \sin \varphi$ (Suppl. Mat. 5, Suppl. Movie S1). We validated our setup by quantifying K^* of red blood cells (suppl. movie S2). We obtained values of K' very consistent with values predicted by existing models, and also obtained, as expected, very low values of K'' (Suppl. Mat. 6).

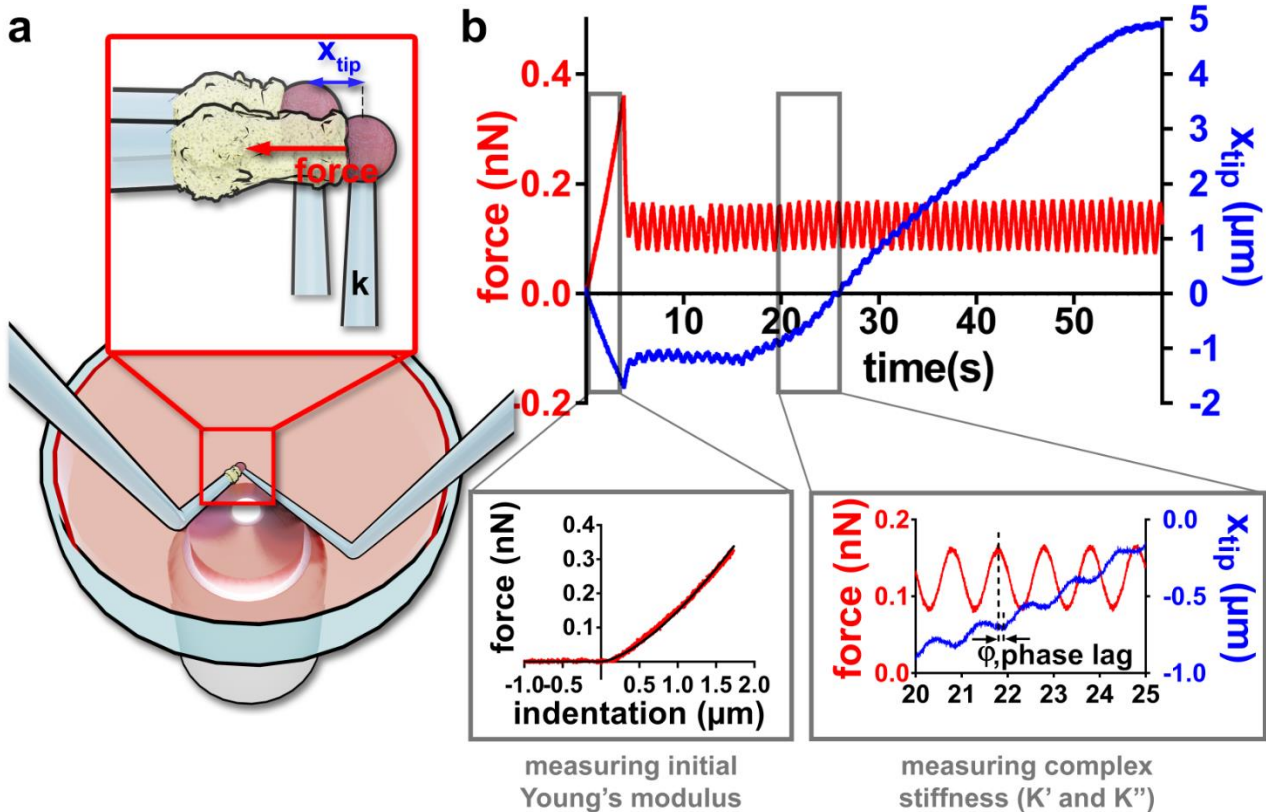
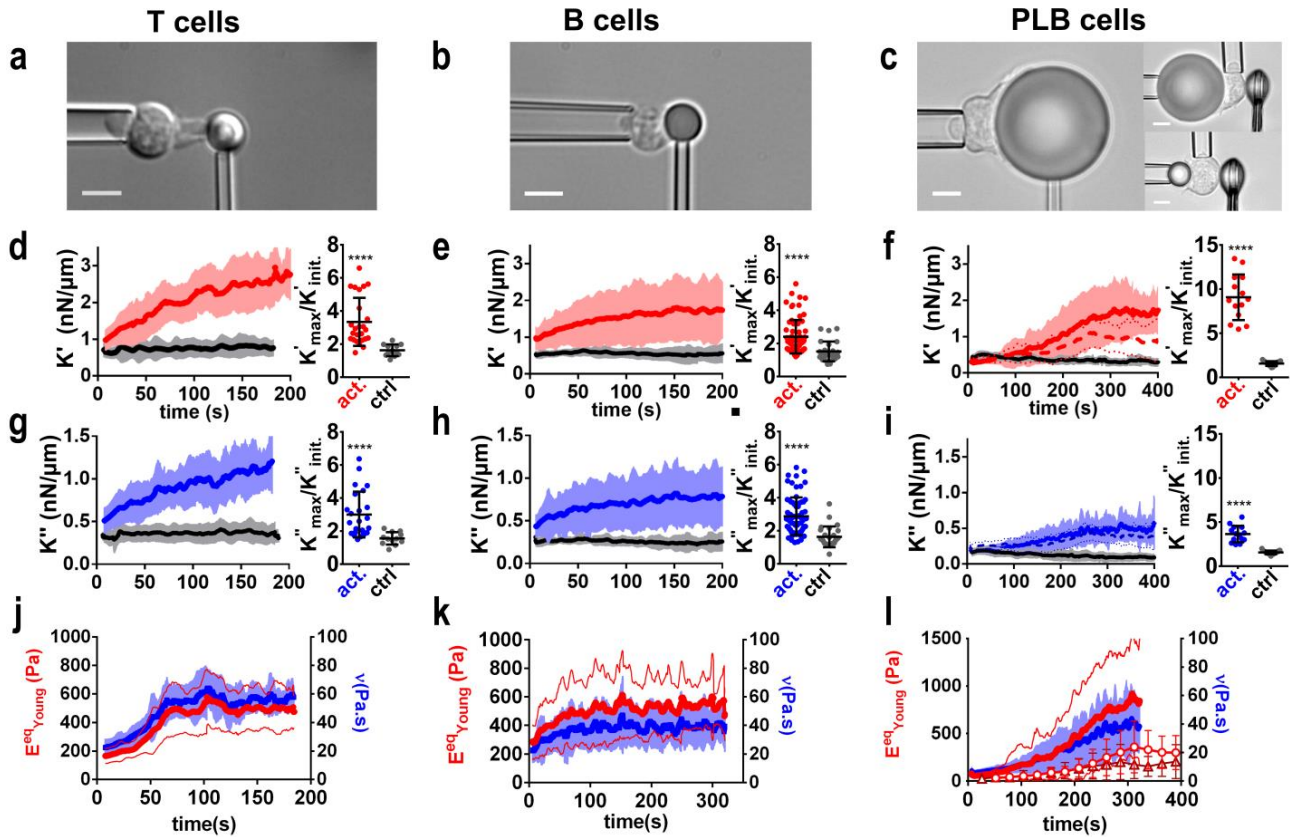


FIGURE 1. Rheological measurements during leukocyte activation. (a). Setup. Two micropipettes are plunged in a Petri dish. A flexible pipette (right, bending stiffness $k \approx 0.2$ nN/ μ m) holds an activating microbead firmly. A rigid micropipette (left) gently holds a leukocyte (aspiration pressure of 10-100 Pa). The base of the flexible micropipette is displaced to impose a desired force on the cell. (b) Force applied on the cell (in red) and the position x_{tip} of the tip of the flexible micropipette (in blue). The cell is first compressed with a force F_{comp} (here 0.36 nN) to measure the initial Young's modulus of the cell (inset, bottom left). Then an oscillatory force is applied to the cell,

321 leading to an oscillatory x_{tip} signal (inset, bottom right) superimposed to a slower variation of the average value
 322 $\langle x_{tip} \rangle$ due to the growth of a protrusion produced by the leukocyte.

323 Leukocytes become stiffer and more viscous during activation

324 We probed three different types of leukocytes during their specific activation and observed qualita-
 325 tively similar behavior: both K' and K'' increase and reach a maximum that is 3- to 8-fold larger than
 326 their initial value within few minutes (~ 2 minutes for T and B cells, ~ 5 minutes for neutrophils-like
 327 PLB985 cells) following the contact between the leukocyte and the relevant activating microbead (Fig.
 328 2, Suppl. Movie S1). The increase of K' and K'' is in clear contrast with the constant values obtained
 329 using control non-activating beads (black lines on Fig. 2). Yet it is worth mentioning that the initial
 330 value of K' and K'' is higher for activating beads than for control beads for T and B cells. We explain this
 331 by the fact that the initial time point for K' and K'' is actually already ~ 10 seconds after initial cell-bead
 332 contact, leaving some time for early mechanical changes to occur (see later regarding mechanical
 333 changes). We evaluated the effect of temperature in the case of T cells by performing experiments at
 334 37°C . T cells behaved qualitatively the same at 37°C , but reacted faster, and reached higher levels in K'
 335 and K'' (Suppl. Mat. 7).
 336



337
 338 **FIGURE 2.** Both K' and K'' increase during leukocyte activation. Each column corresponds to a different cell type.
 339 (a-c) Cell morphology. Scale bars are $5\ \mu\text{m}$. (d-f) Left: K' for activating (red lines) and non-activating control
 340 beads (gray lines). In f, solid red line is for $20\text{-}\mu\text{m}$ beads, dashed red line for $8\text{-}\mu\text{m}$ beads with indentation in the
 341 back (see Figure 3). Right: max/min ratio of K' values, for activating and non-activating control beads. (g-i) Left:
 342 K'' values corresponding to K' values in d-f. In i, solid blue line is for $20\text{-}\mu\text{m}$ beads, dashed blue line for $8\text{-}\mu\text{m}$
 343 beads with indentation in the back (see Figure 3). (j-l) Equivalent Young's modulus (red, left axis) and cell vis-
 344 cosity (blue, right axis). In l, dots are the Young's modulus measured in the back of the cell for $20\text{-}\mu\text{m}$ beads, tri-
 345 angles are for $8\text{-}\mu\text{m}$ beads. In all panels, leukocyte-bead contact time ($t=0$) is detected as a force increase, thick
 346 lines are mean \pm SD over at least 3 pooled experiments (T cells: 21 cells, B cells 71 cells, PLB cells: 14 cells). Mann-
 347 Whitney test, ****: $p<0.0001$.

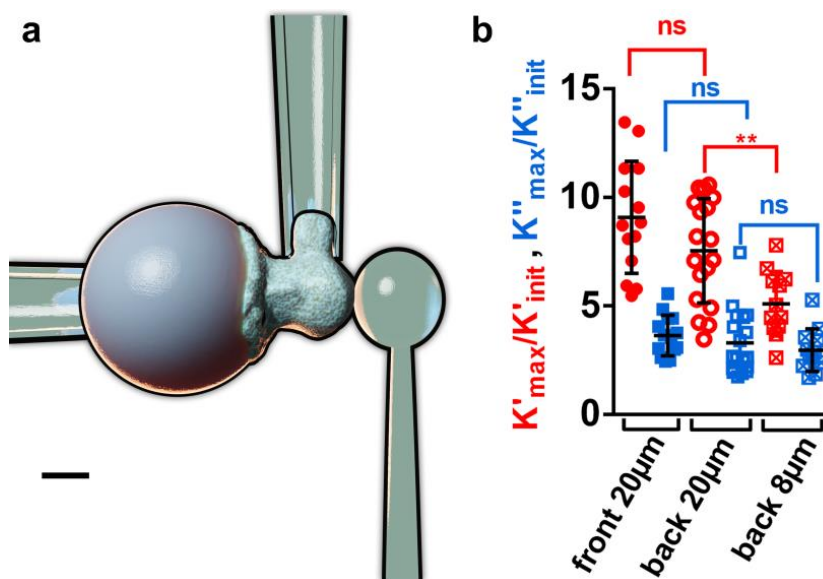
Changes in K' and K'' correspond to changes in intrinsic leukocyte mechanical properties

Both K' and K'' depend on cell geometry, so to exclude the possibility that changes in K' and K'' only reflect geometrical changes, cell intrinsic mechanical properties such as Young's modulus and viscosity have to be extracted from K' and K'' . To do so, we first convert K' and K'' into a storage modulus E' and a loss modulus E'' , respectively. In the case of T cells and B cells, we do so by modeling cell geometry (Suppl. Mat. 8).

For PLB cells, we use a modified setup where a non-adherent and non-activating glass bead indents the cell on its "back" (37) while the cell phagocytoses an activating bead at its "front" (Fig. 3a, Suppl. Movie S3) (37). This alternative setup leads to the same increase in K' and K'' as measured using the original setup with front indentation with an activating bead (Fig. 3b). Having a sphere-against-sphere geometry, we can use a linearized Hertz model to extract E' and E'' moduli (Suppl. Mat. 8). Lastly, we performed cyclic indentation experiments to measure directly the Young's modulus over time (with a limited time resolution of ~ 30 seconds, dotted curves in Fig. 2l): indentation occurs only for a short time at the beginning of each cycle, after which the indenter retracts from the cell, marking the end of a cycle, immediately followed by a new cycle (Suppl. Movie S4). In the case of PLB cells we tested the influence of bead size by performing indentation in the "back" of cells phagocytosing either 20- or 8- μm beads. For both bead sizes the cells' Young's modulus increased significantly during phagocytosis when comparing early ($t=52$ s) vs. late ($t=286$ s) times (Figure 2l, $E_{\text{Young}}(t=286 \text{ s}) > E_{\text{Young}}(t=52 \text{ s})$, at least 14 cells from at least 3 independent experiments for each condition, $p < 0.0001$ for 20- μm and 8- μm beads, Mann Whitney test).

To allow a simple interpretation of E' and E'' , which were obtained in a regime of oscillatory forces, we convert them respectively into an effective Young's modulus $E_{\text{Young}}^{\text{eq}}$ and an effective viscosity ν . To calculate $E_{\text{Young}}^{\text{eq}}$, we compare the Young's modulus measured by initial compression and E' measured right after, when force modulation begins (Suppl. Mat. 9; Suppl. Fig. S6). For PLB cells, we used again cyclic back-indentation as described above to measure both Young's modulus and E' not only at initial time but during the whole activation. This showed that E' and E_{Young} are proportional, with a phenomenological coefficient C such that $E' = CE_{\text{Young}}$. C is measured to be constant over time for PLB cells, and so for T and B cells we assume that C is also constant over time (Suppl. Mat. 9).

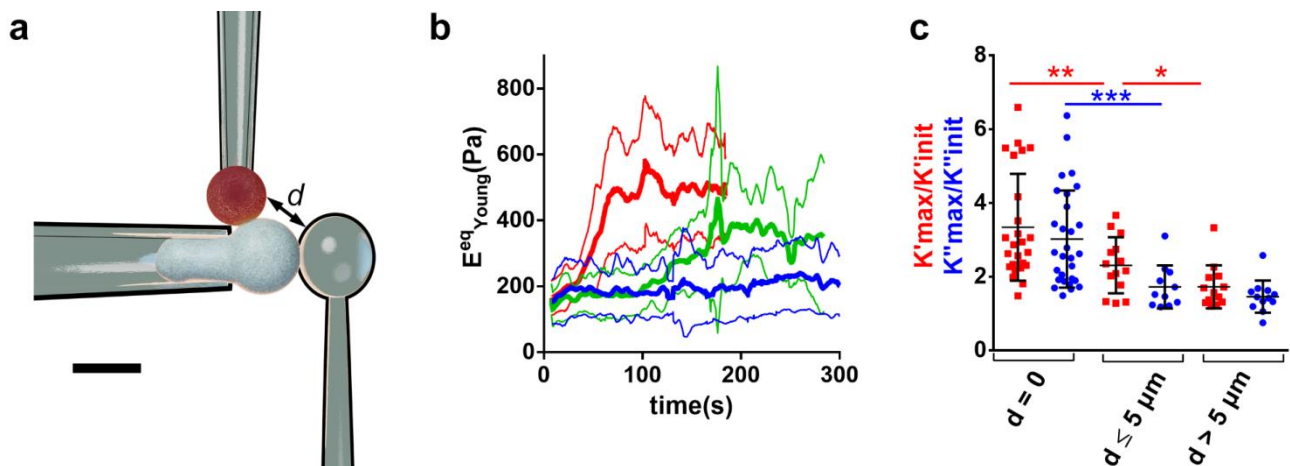
Using this approach, we measured the effective Young's modulus of the three cell types over time (Fig. 2j-l). To calculate the effective cell viscosity, we consider a simple model consistent with a Newtonian liquid and cortical tension model of a cell (2), where a Newtonian viscosity is introduced as $\nu = E''/\omega$. The resulting Young's modulus and viscosity increase until reaching a maximal value that is 2-3 fold higher than the baseline value for T and B cells, and about 11 fold higher for PLB cells (Fig. 2j-l).



383 FIGURE 3. Indenting the “back” of a phagocyte during activation. (a) Modified setup. A stiff pipette holds the activating microbead (left), an auxiliary (stiff) pipette holds the cell by its “side” (top) and a flexible pipette whose
384 tip consists of a non-adherent glass bead indents the cell on its “back” (right). Scale bar is 5 μm . (b) Max/min
385 ratios for K' and K'' obtained with both versions of the setup during PLB cell activation (ns: non-significant, two-
386 tailed unpaired t test). Using back indentation, in addition to the 20- μm beads used with the non-modified setup,
387 8 μm beads were tested.

389 Mechanical changes are localized close to the cell-bead interface

390 We asked if mechanical changes were localized close to the activation area in the leukocyte. In the case
391 of T cells, we used a modified setup similar to the one allowing to indent PLB cells on their back (Fig.
392 3a). In that case an auxiliary pipette brought the activating microbead in contact with the T cell on its
393 “side”: at the equatorial plane (distance between the activating and indenting beads smaller than 5
394 μm), or close to the tip of the holding pipette, at the farthest possible location from the activating bead
395 (distance between the activating and indenting beads larger than 5 μm , Fig. 4, Suppl. Movie S5). This
396 allowed us to quantify mechanical changes during activation depending on the distance to the cell-
397 bead contact area. We computed an equivalent Young’s modulus E^{eq}_{Young} as described in supplementary
398 material 9, which led to a good agreement with E_{Young} measured at the initial time. T cells exhibited a
399 different behavior depending on the location of the activating microbead: the closer the indenter was
400 to the activating bead, the larger the maximum value of E^{eq}_{Young} measured during activation. Cell stiff-
401 ening thus occurs with a different amplitude depending on the location on the cell relative to the con-
402 tact zone with an activating surface.



403 FIGURE 4. (a) Modified setup where an auxiliary (stiff) pipette holds the activating microbead (top, red) to bring
404 it in contact with the T cell at a chosen distance from a microindenter (right), which indents the cell on its “side”
405 while the T cell gets activated. Scale bar is 5 μm . (b) Equivalent Young’s modulus for three different ranges of
406 distance d between the activating bead and the microindenter (red: $d=0$; green: $0 < d \leq 5 \mu\text{m}$; blue: $d > 5 \mu\text{m}$). (c)
407 K'_{max}/K'_{init} (red) and K''_{max}/K''_{init} (blue) ratios for the same ranges of distance d as in panel b.

409 Mechanical changes in T cells start within seconds

410 In T cells, mechanical changes precede morphological ones: K' increases slowly within seconds after
411 cell-bead contact, before cell morphology starts changing (i.e. before the T cell produces a large pro-
412 trusion (17), Suppl. Movie S1). The onset of this growth is followed by a short period during which K'
413 stays relatively constant. This phase ends by a faster increase of K' during protrusion growth (Suppl.
414 Mat. 10, Suppl. Fig. S7). The start of the faster increase in K' starts when the tail of the cell in the hold-
415 ing pipette starts retracting (a sign that K' and cell tension are two equivalent ways to describe cell
416 stiffness, Suppl. Mat. 11). Of note, T cells have large membrane surface stores (38), so it is unlikely that
417 the acceleration in K' increase could be explained by reaching the limits of membrane stores (Suppl.
418 Mat. 12).

AFM measurements of early mechanical changes following T cell/APC contact

In order to confirm with an independent technique that T cell mechanics may be modulated within seconds when it encounters an antigen presenting cell (APC) before any large morphological change occurs, we used AFM and performed Single Cell Force Spectroscopy (SCFS) experiments (Fig. 5a, Suppl. Mat. 3)(36). As a model APC, we used COS-7 cells expressing MHCII molecules that can be loaded with peptides of desired affinity as previously shown (47, 48). We used murine 3A9m T cells and immobilized them on a coverslip by adhering them using anti-CD45 antibodies (53). To measure rapid mechanical changes using this technique, we prevented potential large active cell deformations by using the pan-Src kinase inhibitor PP2 (methods). Although PP2 inhibits TCR-mediated Lck- or Fyn- dependent intracellular phosphorylation events and downstream signaling cascades (54), it does not inhibit all molecular events such as migration arrest, kinapse or microcluster formation upon T cell activation (55–57). Wash-out experiment showed that PP2 in fact enables to pause signalization without abrogating the antigen dependence of early T cell activation (58, 59).

The SCFS experiments provide for each cell doublet the force-tip sample separation curve, which is the AFM equivalent of the force-indentation curve shown in Fig. 1b (inset, bottom left), with three parts : (i) pushing the cells together, (ii) maintaining the contact and (iii) detaching the cells by pulling them apart. The contact mechanics at initial time was quantified by measuring the slope of the force-tip sample separation curve (called contact slope herein), which reflects the stiffness of the cell doublet. This contact slope was not modified by the presence or absence on the APC of the HEL (Hen Egg Lysozyme, peptide p46.61)-derived peptide recognized by the murine 3A9m T cells (Suppl. Fig. S2d). However, in the separation part of the force curves, the presence of the peptide induced an increase in the force needed to fully detach the two cells, in parallel of an increase of the number of discrete separation events, indicating that the interaction was indeed peptide specific (Suppl. Fig. S2e-f).

The measurement of the contact slope corresponds to the very first instants of cell-cell contact (Fig. 5a, bottom). To measure cell mechanical changes following this contact, we quantified the force relaxation when the AFM lever motion was stopped and piezo position was kept constant after reaching a contact force of 1nN (Fig. 5b-c). We observed a striking difference in this force relaxation depending on the presence vs. absence of a saturating concentration of the antigenic peptide (Fig. 5b): the force relaxation was much slower in presence of the antigenic peptide, as quantified by the lower value of the ratio $\Delta F/F_0$ at different times following the start of the relaxation (Fig. 5c). This measurement reflects global viscoelastic properties of the cell-cell doublet, but does not disentangle elastic and viscous properties. Furthermore, having the two cells pressed against each other in series does not allow simply excluding a contribution of a mechanical change in the APC. However, mechanical properties of APC COS-7 cells were not modified by the presence of the peptide (Suppl. Fig. S3). As a consequence, we expect that the major contribution to the difference in force relaxation is indeed due to the T cell. Our SCFS experiments are thus consistent with our Micropipette Force Probe experiments, and show that specific recognition during T cell/APC contact may induce mechanical changes, within few seconds, and without any TCR signaling.

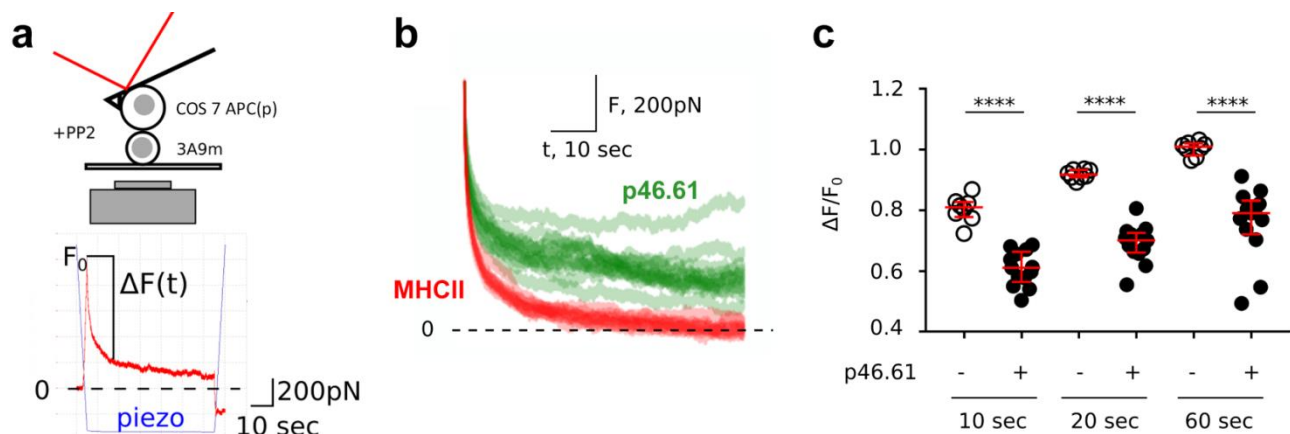
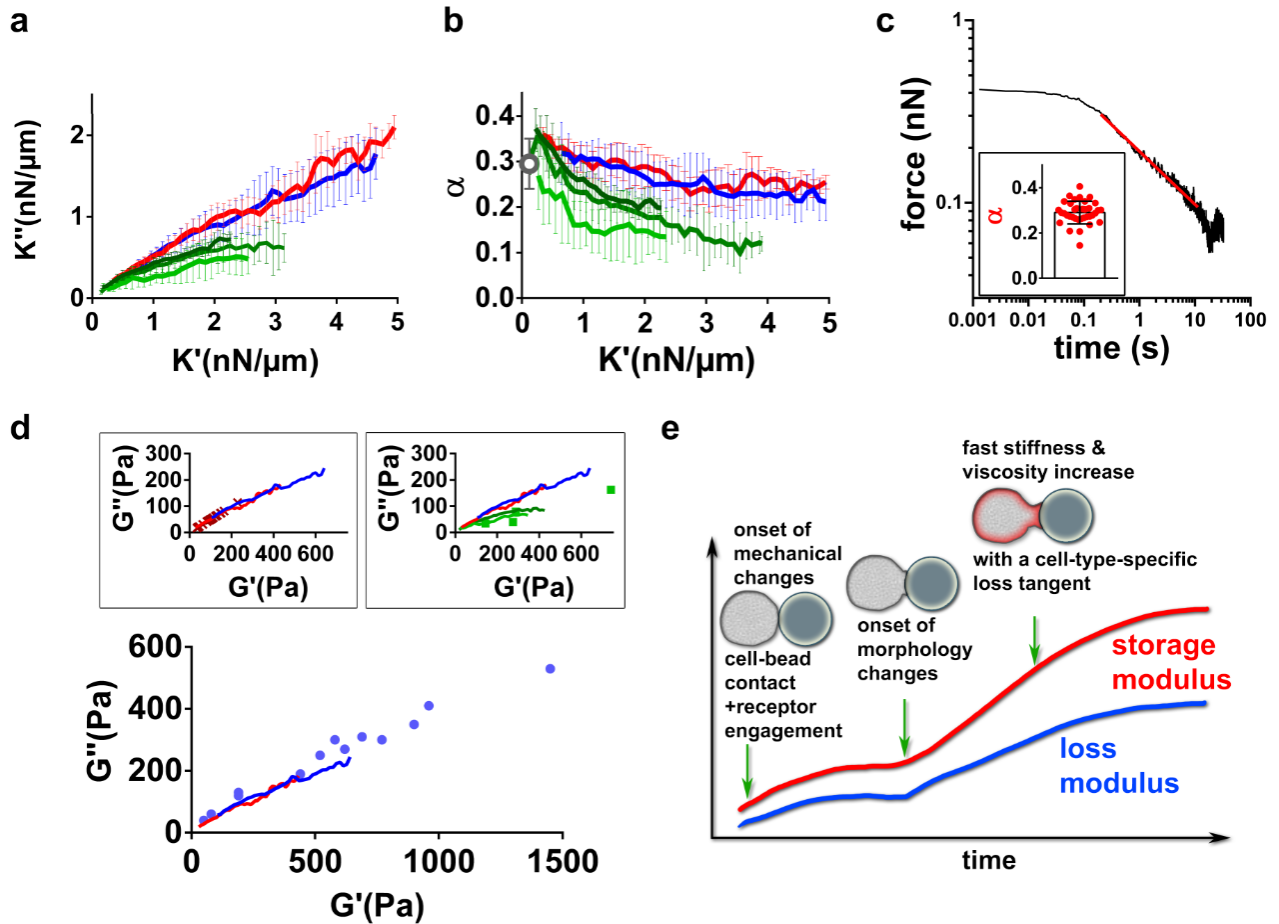


FIGURE 5. Cell-cell presentation induces early changes in force relaxation. (a) AFM SCFS Setup (top) and example of individual force (in red) vs. time curve (bottom). The piezo signal (in blue) reports the position of the base of the cantilever. (b) Relaxation part of force curves, for peptide (p46.61) vs. no-peptide (MHCII) cases. One curve corresponds to one cycle, ie. 1 APC/T cell couple. See Suppl. Fig. S2h for plot of mean \pm SD curves. (c) Quantification of the relaxation for three different time points, for MHCII (-) and MHCII/p46.61 (+); $\Delta F/F_0$ is the ratio of the drop in force ΔF and the force level F_0 imposed at initial time 0 (see a). This ratio is shown at three different time following cell-cell contact: 10, 20, and 60s, and shows a consistent lower ratio (hence a slower relaxation) in presence of the peptide (**** for $p < 0.001$).

Relationship between elastic and viscous properties during leukocyte activation

We then asked if variations in elastic and viscous properties were related to each other. Prior works show that in several cell types the loss modulus E'' and storage modulus E' of a cell are related to each other. In fact their ratio $\eta = \frac{E''}{E'}$, the loss tangent, lies within a narrow range of 0.1-0.3(6, 28, 31, 60-62). For a material such as a purely viscous liquid, the storage modulus vanishes so that η is arbitrarily large, while for a purely elastic material, the loss modulus vanishes and so does η . A finite and constant value of η for a cell implies that the stiffer the cell, the more viscous it is. This narrow range of loss tangent is consistent with the conserved ratio of cortical tension to cell viscosity in leukocytes (see introduction). In biological matter, similar to soft glassy materials (63, 64), stress relaxation over time often follows a power-law (with an exponent that we shall call α)(31, 32, 60, 65, 66). The loss tangent η is frequency-dependent, and is expected to be approximately equal to $\tan\left(\frac{\pi}{2}\alpha\right)$ at low frequencies (Suppl. Mat. 13). Therefore, by measuring the power-law exponent α independently, we should recover the value of $\eta = \frac{E''}{E'}$ (which should also be equal to $\frac{K''}{K'}$ as the contribution from geometry is eliminated in this ratio(7)). To test this prediction, we performed stress relaxation experiment on resting PLB cells (Fig. 6c) and obtained values for α that were very consistent with the predicted value of $\frac{2}{\pi} \arctan\left(\frac{K''}{K'}\right)$ (which is equivalent to comparing $\tan\left(\frac{\pi}{2}\alpha\right)$ and $\frac{K''}{K'}$, gray circle in Fig. 6b). We then compared K'' vs. K' curves obtained during activation of T cells, B cells, and PLB cells (Fig. 6a). In Figure 6b we plot the corresponding value of $\alpha = \frac{2}{\pi} \arctan \eta = \frac{2}{\pi} \arctan \frac{K''}{K'}$: these curves are remarkably consistent for T and B cells; for PLB cells, K'' - K' curves obtained by indentation in the front and in the back of the cell are very similar, and differ from the T and B cell common curve. Interestingly, Bufi *et al.* (67) measured storage and loss moduli of various leukocytes at resting state or after priming by different inflammatory signals. Their data lead to a loss tangent η consistent with our measurements (Fig. 6d) but their measurements were made at a single time point. Maksym *et al.* (6) tracked over time both the loss and storage moduli of human airway smooth muscle cells contracting due to administration of histamine (Fig. 6d, inset, top left). Their data are again very consistent with our measurements. Roca-Cusachs *et al.* (2) used AFM on neutrophils, and found a loss tangent very consistent with our measurements on PLB cells obtained with 20- μ m and 8- μ m beads (Fig. 6d, inset, top right). Based on our results, we propose a simple model of leukocyte mechanical changes during activation (Fig. 6e): mechanical changes begin seconds after receptor engagement at the leukocyte surface. Cell morphological changes start a few tens of seconds later, concomitant with stiffening and increase in viscosity. Viscous and elastic properties of the leukocyte evolve following a characteristic relationship defined by the loss tangent.



501

FIGURE 6. Relationship between elastic and viscous cell properties (a) K'' vs. K' during activation of T cells (red), B cells (blue) and PLB cells (front indentation: green (20-μm beads), back indentation: light green (20-μm beads) and dark green (8-μm beads)). Error bar are SDs. (b) $\alpha = \frac{2}{\pi} \arctan \left(\frac{K''}{K'} \right)$ corresponding to the same data as in a. The gray circle is the value of the power-law exponent in force relaxation experiments on resting PLB cells. (c) Example of force relaxation curve of a PLB cell. In the log-log plot, a power-law relaxation is identified by the straight line (red), whose slope is the exponent α . Inset: individual α values (1 dot per cell). (d) Loss modulus G'' vs. storage modulus G' obtained by Bufl *et al.* (67) (bottom, blue dots), Maksym *et al.* (6) (inset, top left, red crosses), and Roca-Cusachs *et al.* (2) (inset, top right, green dots). Our data from a (red and blue solid lines) are consistent with the above-mentioned published data. (e) Proposed model of mechanical changes during leukocyte activation.

512 Discussion

We showed that during activation, various types of leukocytes see their stiffness and viscosity increase significantly within few minutes. The initiation of mechanical changes upon contact with an activating surface starts within seconds and these changes precede morphological changes. By using the same technique – avoiding potential differences due to different techniques (3) – we show to our knowledge for the first time with ~1-s time resolution how both viscous and elastic properties evolve during immune cell activation. We further show that elastic and viscous properties follow a relationship that is cell-type dependent: T and B cells keep the same ratio between viscous and elastic properties, while neutrophils follow a different ratio, independently of the size of their target.

Most published measurements of cell viscosity were performed on leukocytes in a resting state. Some micropipette aspiration studies led to leukocyte viscosity in the range of 100-200 Pa.s (68–71), and are consistent with values obtained in fibroblasts (72). These values are higher than the viscosities of ~5-20 Pa.s that we measured in resting leukocytes. This discrepancy might be explained by model-dependence and by differences in the shear rate applied to cells. Our measurements are much

526 closer to results by other groups: Sung *et al.* (73) obtained a cytotoxic T cell viscosity of ~ 30 Pa.s,
527 Schmid-Schönbein *et al.* (25) measured a leukocyte viscosity of ~ 13 Pa.s, Hochmuth *et al.* (29) ex-
528 trapolated a value for neutrophil viscosity of ~ 60 Pa.s. Interestingly, Lipowsky *et al.* (74) did not re-
529 strict to baseline viscosity and studied neutrophils stimulated by the chemoattractant FMLP. The au-
530 thors reported a neutrophil viscosity increasing from ~ 5 Pa.s at resting state to ~ 70 Pa.s upon stimula-
531 tion.

532 Mechanical changes might not have a function *per se*, but might be concomitant to force gener-
533 ation by cells. Indeed, cell contractility and cell stiffness are correlated in muscle cells (75–79), and
534 single cells mirror mechanical and energetic features of muscle contraction: single myoblasts follow a
535 single-cell equivalent of a Hill relationship between force generation and rate of contraction (80, 81).
536 Furthermore, it also appears that single muscle cells become stiffer when they contract: Shroff *et al.*
537 (79) observed a roughly 2-fold increase in stiffness of a cardiomyocyte during its contraction. Alt-
538 hough leukocytes have a different organization of their actomyosin cytoskeleton, it is tempting to
539 speculate that all leukocytes share generic cytoskeletal-based properties requiring that cells stiffen to
540 generate force. This could explain the apparent contradiction that in leukocytes, the stiffening that we
541 have quantified occurs in situations requiring an efficient spreading. Indeed, phagocytes need to in-
542 crease their surface by spreading on their prey to engulf it, while T and B cells need to spread widely
543 on the cell they encounter to quickly scan a surface that is as large as possible on the cell they interact
544 with (18, 82). Alternatively, forming a stiff synapse might allow its stabilization (83, 84).

545 Although the molecular details leading to changes in viscoelastic properties are yet to be un-
546 derstood, cell-scale physical considerations allowed Evans *et al.* (71, 85) to conclude that viscous
547 properties of cells are conferred by bulk components of the cell, as opposed to surface viscosity due to
548 the actin cortex. As a result, we expect that the large increase in viscous properties of leukocytes that
549 we observed are largely due to intracellular reorganization, not only to cortical reorganizations. Inter-
550 estingly, Tsopoulidis *et al.* recently showed that T cell receptor engagement triggers the formation of a
551 nuclear actin network in T cells (86), which could contribute to these bulk mechanical changes.
552 Fritzsche *et al.* observed submembrane actin pattern reorganization that influenced the membrane
553 architecture of Hela cells, but not their mechanical properties (87). These observations may lift an
554 apparent contradiction in T cells, in which the formation of an immunological synapse requires actin to
555 depolymerize at the center of the synapse, including in cytotoxic T cells where actin gets actually de-
556 pleted across the synapse within 1 min (88). Other contributors must then explain the stiffening that
557 we observe, and in addition to the nuclear actin network (86), another possible contributor being a
558 secondary actin network. Indeed Fritzsche *et al.* (84) found in T cells that beside the dense cortical
559 network forming a rosette-shaped structure in the basal lamellipodium and devoid of actin at its cen-
560 ter up to 3 min after initial contact, another actin network assembles 150 to 300 nm above the cell-
561 substrate contact.

562 We expect inner reorganizations causing mechanical changes to impact inner cell dynamics,
563 such as organelle trafficking. This includes mitochondrial relocation that is known to happen in several
564 types of leukocytes upon activation (89, 90). Regarding cell surface, although we dismissed surface
565 effects as key contributors to cell viscosity, surface receptors are transmembraneous so that the for-
566 mation of diffusional barriers with slowed diffusion at the cell surface as observed during leukocyte
567 activation could be one manifestation of the increase in cell viscosity (91, 92). As an alternative to the
568 above considerations that mechanical changes might not have a specific function, these changes in
569 viscosity might help confining molecules of interest in the vicinity of the synapse. By sequestering
570 these molecules due to higher stiffness and viscosity (implicating an increased energetical cost to
571 move away from this zone), early mechanical changes might help integrating at the cellular scale the
572 information input incoming from the single-receptor scale at the synapse. Considering this efficient
573 processing from nano- to micro-scale is also worth integrating in mechanistic description of early im-
574 munological signaling, including models considering a frictional coupling of the TCR to the cytoskele-
575 ton (93, 94).

576 Our study highlights that variations of both elastic and viscous properties are relevant to un-
577 derstand immune cell mechanics during activation, as well as their relationship in the form of the loss

578 tangent η , and how the latter evolves over time. All the leukocytes that we tested show very large
579 changes in both elastic and viscous properties, but monitoring the time evolution of the loss tangent
580 exhibits a mechanical fingerprint specific to cell types. It is tempting to speculate that the evolution of
581 the loss tangent during activation can become a valuable tool to discriminate between healthy and
582 pathological leukocytes as already suggested by considering static values of η (95).

583 Finally, our observations suggest a possible generalization to cells other than leukocytes. In
584 line with what was observed by Thoumine et al. (96) in fibroblasts or Abidine et al. in epithelial cancer
585 cells (97), we propose that getting stiffer and more viscous while spreading is a common behavior
586 across cell types. The time evolution of the loss tangent during cell spreading remains to be explored.

587 **Author contributions**

588 P.-H.P., Y.H. and J.H. designed experiments; A.Z., S.V.M.C., A.S., F.M., Y.H., P.-H.P. and J.H. performed ex-
589 periments; A.Z., A.S., Y.H., P.-H.P. and J.H. analyzed the data; A.B., S.D., E.H., S.D.-C., H.-T.H., A.I.B., Y.R.C.,
590 Y.H., C.H. and O.N. contributed material; P.-H.P. and J.H. wrote the manuscript with helpful critical
591 reading by the other authors.

592 **Competing financial interests**

593 The authors declare no competing financial interests.

594 **Acknowledgements**

595 This work has benefited from the financial support of the Labex LaSIPS (ANR-10-LABX-0040-LaSIPS)
596 managed by the French National Research Agency under the "Investissements d'avenir" program
597 (n°ANR-11-IDEX-0003-02), from a CNRS PEPS grant, from Ecole Polytechnique, and from an endow-
598 ment in cardiovascular bioengineering from the AXA Research Fund. This work was supported by
599 funding from (a) Prise de Risques CNRS and ANR JCJC « DissecTion » (to PHP) ; (b) the Labex IN-FORM
600 (ANR-11-LABX-0054) and A*MIDEX project (n° ANR-11-IDEX-0001-02), sponsored by the Investisse-
601 ments d'Avenir project funded by the French Government, managed by the French National Research
602 Agency (ANR) (to LAI & CIML, and as PhD grant to AS) ; (c) the European Union's Horizon 2020 re-
603 search and innovation programme under the Marie Skłodowska-Curie grant agreement No713750,
604 with the financial support of the Regional Council of Provence- Alpes-Côte d'Azur and with of the
605 A*MIDEX (n° ANR- 11-IDEX-0001-02), funded by the Investissements d'Avenir project funded by the
606 French Government, managed by the French National Research Agency (ANR) (as PhD grant to FM).
607 Part of this work was also supported by institutional grants from INSERM, CNRS and Aix-Marseille
608 University to the LAI and CIML. Material or technical help: R. Balland (Sensome) for RBCs; M. Pélécot-
609 Biarnes (LAI); L. Borge (PCC cell culture facility); S. Mailfert / M. Fallet / PICSL imaging facility of the
610 CIML (ImagImm), member of the national infrastructure France-BioImaging supported by the French
611 National Research Agency (ANR-10-INBS-04); A. Formisano (He/Marguet lab). Discussion and support:
612 D. Gonzalez-Rodriguez (LCP-A2MC, Univ. De Lorraine), R Roncagalli (CIML); P. Recouvreux (IBDML); L.
613 Limozin, F. Rico (LAI). Companies: F. Eghiaian, T. Plake & JPK Instruments (Berlin, Germany), now part
614 of Bruker, for continuous support and generous help; Zeiss France for support. SVMC and YRC have
615 benefited from the financial support of an FPI contract (BES-2014-068006) and the grant BFU2013-
616 48828-P from the Spanish Ministry of Economy (MINECO).

617 **References**

- 618 1. Hochmuth, R.M. 2000. Micropipette aspiration of living cells. *J. Biomech.* 33: 15–22.
- 619 2. Roca-Cusachs, P., I. Almendros, R. Sunyer, N. Gavara, R. Farré, and D. Navajas. 2006. Rheology of
620 Passive and Adhesion-Activated Neutrophils Probed by Atomic Force Microscopy. *Biophys. J.* 91:
621 3508–3518.
- 622 3. Wu, P.-H., D.R.-B. Aroush, A. Asnacios, W.-C. Chen, M.E. Dokukin, B.L. Doss, P. Durand-Smet, A.
623 Ekpenyong, J. Guck, N. V. Guz, P.A. Janmey, J.S.H. Lee, N.M. Moore, A. Ott, Y.-C. Poh, R. Ros, M.
624 Sander, I. Sokolov, J.R. Staunton, N. Wang, G. Whyte, and D. Wirtz. 2018. A comparison of

methods to assess cell mechanical properties. *Nat. Methods*. 15: 491–498.

4. Toepfner, N., C. Herold, O. Otto, P. Rosendahl, A. Jacobi, M. Kräter, J. Stächele, L. Menschner, M. Herbig, L. Ciuffreda, L. Ranford-Cartwright, M. Grzybek, Ü. Coskun, E. Reithuber, G. Garriss, P. Mellroth, B. Henriques-Normark, N. Tregay, M. Suttorp, M. Bornhäuser, E.R. Chilvers, R. Berner, and J. Guck. 2018. Detection of human disease conditions by single-cell morpho-rheological phenotyping of blood. *Elife*. 7: e29213.
5. Guillou, L., J.B.J.B. Dahl, J.-M.G.J.-M.G. Lin, A.I.A.I. Barakat, J. Husson, S.J.S.J. Muller, and S. Kumar. 2016. Measuring Cell Viscoelastic Properties Using a Microfluidic Extensional Flow Device. *Biophys. J.* 111: 2039–2050.
6. Maksym, G.N., B. Fabry, J.P. Butler, D. Navajas, D.J. Tschumperlin, J.D. Laporte, and J.J. Fredberg. 2000. Mechanical properties of cultured human airway smooth muscle cells from 0.05 to 0.4 Hz. *J. Appl. Physiol.* 89: 1619–1632.
7. Smith, B.A., B. Tolloczko, J.G. Martin, and P. Grütter. 2005. Probing the Viscoelastic Behavior of Cultured Airway Smooth Muscle Cells with Atomic Force Microscopy: Stiffening Induced by Contractile Agonist. *Biophys. J.* 88: 2994–3007.
8. Dura, B., S.K. Dougan, M. Barisa, M.M. Hoehl, C.T. Lo, H.L. Ploegh, and J. Voldman. 2015. Profiling lymphocyte interactions at the single-cell level by microfluidic cell pairing. *Nat. Commun.* 6: 5940.
9. Niedergang, F., V. Di Bartolo, and A. Alcover. 2016. Comparative Anatomy of Phagocytic and Immunological Synapses. *Front. Immunol.* 7: 1–9.
10. Hivroz, C., and M. Saitakis. 2016. Biophysical Aspects of T Lymphocyte Activation at the Immune Synapse. *Front. Immunol.* 7: 1–12.
11. Huse, M. 2017. Mechanical forces in the immune system. *Nat. Rev. Immunol.* 17: 679–690.
12. Wang, J., F. Lin, Z. Wan, X. Sun, Y. Lu, J. Huang, F. Wang, Y. Zeng, Y.H. Chen, Y. Shi, W. Zheng, Z. Li, C. Xiong, and W. Liu. 2018. Profiling the origin, dynamics, and function of traction force in B cell activation. *Sci. Signal.* 11.
13. Kumari, A., J. Pineau, P.J. Sáez, M. Maurin, D. Lankar, M. San Roman, K. Hennig, V.F. Boura, R. Voituriez, M.C.I. Karlsson, M. Balland, A.M. Lennon Dumenil, and P. Pierobon. 2019. Actomyosin-driven force patterning controls endocytosis at the immune synapse. *Nat. Commun.* 10: 1–14.
14. Evans, E., A. Leung, and D. Zhelev. 1993. Synchrony of cell spreading and contraction force as phagocytes engulf large pathogens. *J. Cell Biol.* 122: 1295–300.
15. Kumari, S., M. Mak, Y. Poh, M. Tohme, N. Watson, M. Melo, E. Janssen, M. Dustin, R. Geha, and D.J. Irvine. 2020. Cytoskeletal tension actively sustains the migratory T-cell synaptic contact. *EMBO J.* : 1–18.
16. Masters, T.A., M.P. Sheetz, and N.C. Gauthier. 2016. F-actin waves, actin cortex disassembly and focal exocytosis driven by actin-phosphoinositide positive feedback. *Cytoskeleton*. 73: 180–196.
17. Sawicka, A., A. Babataheri, S. Dogniaux, A.I. Barakat, D. Gonzalez-Rodriguez, C. Hivroz, and J. Husson. 2017. Micropipette Force Probe to quantify single-cell force generation: application to T cell activation. *Mol. Biol. Cell*. 28: mbc.E17-06-0385.
18. Herant, M., V. Heinrich, and M. Dembo. 2005. Mechanics of neutrophil phagocytosis: behavior of the cortical tension. *J. Cell Sci.* 118: 1789–97.
19. Irmscher, M., A.M. De Jong, H. Kress, and M.W.J. Prins. 2013. A method for time-resolved measurements of the mechanics of phagocytic cups. *J. R. Soc. Interface*. 10.
20. Merino-Cortés, S. V., S.R. Gardeta, S. Roman-Garcia, A. Martínez-Riaño, J. Pineau, R. Liebana, I. Merida, A.-M.L. Dumenil, P. Pierobon, J. Husson, B. Alarcon, and Y.R. Carrasco. 2020. Diacylglycerol kinase ζ promotes actin cytoskeleton remodeling and mechanical forces at the B cell immune synapse. *Sci. Signal.* 13: eaaw8214.
21. Ekpenyong, A.E., N. Toepfner, C. Fiddler, M. Herbig, W. Li, G. Cojoc, C. Summers, J. Guck, and E.R. Chilvers. 2017. Mechanical deformation induces depolarization of neutrophils. *Sci. Adv.* 3: e1602536.
22. Worthen, G.S.S., B.I. Schwab, E.L. Elson, G.P.G.P.G.P. Downey, B. Schwab 3rd, E.L. Elson, and G.P.G.P.G.P. Downey. 1989. Mechanics of stimulated neutrophils: cell stiffening induces retention in capillaries. *Science*. 245: 183.
23. Bashant, K.R., A. Vassallo, C. Herold, R. Berner, L. Menschner, J. Subburayalu, M.J. Kaplan, C. Summers, J. Guck, E.R. Chilvers, and N. Toepfner. 2019. Real-time deformability cytometry reveals sequential contraction and expansion during neutrophil priming. *J. Leukoc. Biol.* 105:

- 1143–1153.
24. Preira, P., J.-M. Forel, P. Robert, P. Nègre, M. Biarnes-pelicot, F. Xeridat, P. Bongrand, L. Papazian, O. Theodoly, P. Negre, M. Biarnes-pelicot, F. Xeridat, P. Bongrand, L. Papazian, and O. Theodoly. 2016. The leukocyte-stiffening property of plasma in early acute respiratory distress syndrome (ARDS) revealed by a microfluidic single-cell study: the role of cytokines and protection with antibodies. *Crit. Care.* 20: 8.
 25. Schmid-Schönbein, G.G.W., K.K.L. Sung, H. Tözeren, R. Skalak, and S. Chien. 1981. Passive mechanical properties of human leukocytes. *Biophys. J.* 36: 243–256.
 26. Evans, E.A. 1984. Structural model for passive granulocyte behaviour based on mechanical deformation and recovery after deformation tests. *Kroc Found. Ser.* 16: 53–71.
 27. Tran-Son-Tay, R., D. Needham, A. Yeung, and R.M. Hochmuth. 1991. Time-dependent recovery of passive neutrophils after large deformation. *Biophys. J.* 60: 856–866.
 28. Fabry, B., G.N. Maksym, J.P. Butler, M. Glogauer, D. Navajas, and J.J. Fredberg. 2001. Scaling the Microrheology of Living Cells. *Phys. Rev. Lett.* 87: 148102.
 29. Hochmuth, R.M., H.P. Ting-Beall, B.B. Beaty, D. Needham, and R. Tran-Son-Tay. 1993. Viscosity of passive human neutrophils undergoing small deformations. *Biophys. J.* 64: 1596–601.
 30. Lam, J., M. Herant, M. Dembo, and V. Heinrich. 2009. Baseline mechanical characterization of J774 macrophages. *Biophys. J.* 96: 248–54.
 31. Alcaraz, J., L. Buscemi, M. Grabulosa, X. Trepas, B. Fabry, R. Farre, D. Navajas, R. Farré, and D. Navajas. 2003. Microrheology of Human Lung Epithelial Cells Measured by Atomic Force Microscopy. *Biophys. J.* 84: 2071–2079.
 32. Mahaffy, R.E.E., S. Park, E. Gerde, J. Käs, and C.K.K. Shih. 2004. Quantitative analysis of the viscoelastic properties of thin regions of fibroblasts using atomic force microscopy. *Biophys. J.* 86: 1777–93.
 33. Basu, R., B.M.B.M. Whitlock, J. Husson, A. Le Floch, W. Jin, A. Oyler-Yaniv, F. Dotiwala, G. Giannone, C. Hivroz, N. Biais, J. Lieberman, L.C.L.C. Kam, M. Huse, A. Le Floch, W. Jin, A. Oyler-Yaniv, F. Dotiwala, G. Giannone, C. Hivroz, N. Biais, J. Lieberman, L.C.L.C. Kam, and M. Huse. 2016. Cytotoxic T Cells Use Mechanical Force to Potentiate Target Cell Killing. *Cell.* 165: 100–110.
 34. Husson, J., K. Chemin, A. Bohineust, C. Hivroz, and N. Henry. 2011. Force Generation upon T Cell Receptor Engagement. *PLoS One.* 6: e19680.
 35. Zucchetti, A.E., N. Paillon, O. Markova, S. Dogniaux, C. Hivroz, and J. Husson. 2021. Influence of external forces on actin-dependent T cell protrusions during immune synapse formation. *Biol. Cell.* .
 36. Puech, P.H., K. Poole, D. Knebel, and D.J. Muller. 2006. A new technical approach to quantify cell-cell adhesion forces by AFM. *Ultramicroscopy.* .
 37. Guillou, L., A. Babataheri, P.-H. Puech, A.I. Barakat, and J. Husson. 2016. Dynamic monitoring of cell mechanical properties using profile microindentation. *Sci. Rep.* 6: 21529.
 38. Guillou, L., A. Babataheri, M. Saitakis, A. Bohineust, S. Dogniaux, C. Hivroz, A.I. Barakat, and J. Husson. 2016. T-lymphocyte passive deformation is controlled by unfolding of membrane surface reservoirs. *Mol. Biol. Cell.* 27: 3574–3582.
 39. Edelstein, A.D., M. a Tsuchida, N. Amodaj, H. Pinkard, R.D. Vale, and N. Stuurman. 2014. Advanced methods of microscope control using μ Manager software. *J. Biol. Methods.* 1: 10.
 40. Larghi, P., D.J. Williamson, J.-M. Carpiér, S. Dogniaux, K. Chemin, A. Bohineust, L. Danglot, K. Gaus, T. Galli, and C. Hivroz. 2013. VAMP7 controls T cell activation by regulating the recruitment and phosphorylation of vesicular Lat at TCR-activation sites. *Nat. Immunol.* 14: 723–731.
 41. Roman-Garcia, S., S. V. Merino-Cortes, S.R. Gardeta, M.J.W. de Bruijn, R.W. Hendriks, and Y.R. Carrasco. 2018. Distinct roles for Bruton’s Tyrosine Kinase in B cell immune synapse formation. *Front. Immunol.* .
 42. Drexler, H.G., W.G. Dirks, Y. Matsuo, and R.A.F. MacLeod. 2003. False leukemia-lymphoma cell lines: an update on over 500 cell lines. *Leukemia.* 17: 416–426.
 43. Romano, P., A. Manniello, O. Aresu, M. Armento, M. Cesaro, and B. Parodi. 2009. Cell Line Data Base: Structure and recent improvements towards molecular authentication of human cell lines. *Nucleic Acids Res.* .
 44. Rincón, E., B.L. Rocha-Gregg, and S.R. Collins. 2018. A map of gene expression in neutrophil-like cell lines. *BMC Genomics.* 19: 573.
 45. Pedruzzi, E., M. Fay, C. Elbim, M. Gaudry, and M.A. Gougerot-Pocidalo. 2002. Differentiation of

737 PLB-985 myeloid cells into mature neutrophils, shown by degranulation of terminally
738 differentiated compartments in response to N-formyl peptide and priming of superoxide anion
739 production by granulocyte-macrophage colony-stimulating fact. *Br. J. Haematol.* .
740 46. Vignali, D.A.A., and J.L. Strominger. 1994. Amino acid residues that flank core peptide epitopes
741 and the extracellular domains of cd4 modulate differential signaling through the t cell receptor.
742 *J. Exp. Med.* .
743 47. Salles, A., C. Billaudeau, A. Sergé, A.M. Bernard, M.C. Phélipot, N. Bertaux, M. Fallet, P. Grenot, D.
744 Marguet, H.T. He, and Y. Hamon. 2013. Barcoding T Cell Calcium Response Diversity with
745 Methods for Automated and Accurate Analysis of Cell Signals (MAAACS). *PLoS Comput.* 9:
746 24–26.
747 48. Hueber, A.O., M. Pierres, and H.T. He. 1992. Sulfated glycans directly interact with mouse Thy-1
748 and negatively regulate Thy-1-mediated adhesion of thymocytes to thymic epithelial cells. *J.*
749 *Immunol.* .
750 49. Cazaux, S., A. Sadoun, M. Biarnes-pelicot, M. Martinez, M. Pélicot-Biarnes, M. Martinez, S. Obeid,
751 P. Bongrand, L. Limozin, and P.H. Puech. 2016. Synchronizing atomic force microscopy force
752 mode and fluorescence microscopy in real time for immune cell stimulation and activation
753 studies. *Ultramicroscopy.* 160: 168–181.
754 50. Franz, C.M., A. Taubenberger, P.H. Puech, and D.J. Muller. 2007. Studying integrin-mediated cell
755 adhesion at the single-molecule level using AFM force spectroscopy. *Sci. STKE.* .
756 51. Johnson, K.L. 1985. Contact Mechanics. *J. Am. Chem. Soc.* 37: 1–17.
757 52. Rosenbluth, M.J., W.A. Lam, and D.A. Fletcher. 2006. Force microscopy of nonadherent cells: a
758 comparison of leukemia cell deformability. *Biophys. J.* 90: 2994–3003.
759 53. A. Sadoun , M. Biarnes-Pelicot , L. Ghesquiere-Dierickx A. Wu , O. Théodoly , L.Limozin, Y. Hamon,
760 P.-H.P. 2020. Controlling T cells shape, mechanics and activation by micropatterning. *bioRxiv.* :
761 1–29.
762 54. Hanke, J.H., J.P. Gardner, R.L. Dow, P.S. Changelian, W.H. Brissette, E.J. Weringer, B.A. Pollok, and
763 P.A. Connelly. 1996. Discovery of a novel, potent, and Src family-selective tyrosine kinase
764 inhibitor: Study of Lck- and FynT-dependent T cell activation. *J. Biol. Chem.* .
765 55. Moreau, H.D., F. Lemaître, K.R. Garrod, Z. Garcia, A.-M.M. Lennon-Duménil, P. Bousso, and M.D.
766 Cahalan. 2015. Signal strength regulates antigen-mediated T-cell deceleration by distinct
767 mechanisms to promote local exploration or arrest. *Proc. Natl. Acad. Sci. U. S. A.* 112: 12151–
768 12156.
769 56. Campi, G., R. Varma, and M.L. Dustin. 2005. Actin and agonist MHC-peptide complex-dependent
770 T cell receptor microclusters as scaffolds for signaling. *J. Exp. Med.* 202: 1031–1036.
771 57. Yokosuka, T., K. Sakata-Sogawa, W. Kobayashi, M. Hiroshima, A. Hashimoto-Tane, M. Tokunaga,
772 M.L. Dustin, and T. Saito. 2005. Newly generated T cell receptor microclusters initiate and
773 sustain T cell activation by recruitment of Zap70 and SLP-76. *Nat. Immunol.* 6: 1253–62.
774 58. Faroudi, M., R. Zaru, P. Paulet, S. Müller, and S. Valitutti. 2003. Cutting Edge: T Lymphocyte
775 Activation by Repeated Immunological Synapse Formation and Intermittent Signaling. *J.*
776 *Immunol.* .
777 59. Osborne, D.G., and S.A. Wetzel. 2012. Trogocytosis Results in Sustained Intracellular Signaling
778 in CD4 + T Cells . *J. Immunol.* .
779 60. Kollmannsberger, P., and B. Fabry. 2011. Linear and Nonlinear Rheology of Living Cells. *Annu.*
780 *Rev. Mater. Res.* 41: 75–97.
781 61. Balland, M., N. Desprat, D. Icard, S. Féréol, A. Asnacios, J. Browaeys, S. Hénon, and F. Gallet. 2006.
782 Power laws in microrheology experiments on living cells: Comparative analysis and modeling.
783 *Phys. Rev. E.* 74: 021911.
784 62. Bursac, P., G. Lenormand, B. Fabry, M. Oliver, D.A. Weitz, V. Viasnoff, J.P. Butler, and J.J. Fredberg.
785 2005. Cytoskeletal remodelling and slow dynamics in the living cell. *Nat. Mater.* 4: 557–561.
786 63. Sollich, P. 1997. Rheological constitutive equation for model of soft glassy materials. *Phys. Rev.*
787 *E - Stat. Physics, Plasmas, Fluids, Relat. Interdiscip. Top.* 58: 738–759.
788 64. Fabry, B., and J.J. Fredberg. 2003. Remodeling of the airway smooth muscle cell: are we built of
789 glass? *Respir. Physiol. Neurobiol.* 137: 109–124.
790 65. Fabry, B., G.N. Maksym, J.P. Butler, M. Glogauer, D. Navajas, N.A. Taback, E.J. Millet, and J.J.
791 Fredberg. 2003. Time scale and other invariants of integrative mechanical behavior in living
792 cells. *Phys. Rev. E - Stat. Physics, Plasmas, Fluids, Relat. Interdiscip. Top.* 68: 1–18.

66. Takahashi, R., and T. Okajima. 2015. Mapping power-law rheology of living cells using multi-frequency force modulation atomic force microscopy. *Appl. Phys. Lett.* 107.
67. Bufi, N., M. Saitakis, S. Dogniaux, O. Buschinger, A. Bohineust, A. Richert, M. Maurin, C. Hivroz, and A. Asnacios. 2015. Human primary immune cells exhibit distinct mechanical properties that are modified by inflammation. *Biophys. J.* 108: 2181–2190.
68. Tsai, M.A., R.E. Waugh, and P.C. Keng. 1998. Passive Mechanical Behavior of Human Neutrophils: Effects of Colchicine and Paclitaxel. *Biophys. J.* 74: 3282–3291.
69. Tsai, M.A., R.S. Frank, and R.E. Waugh. 1994. Passive mechanical behavior of human neutrophils: effect of cytochalasin B. *Biophys. J.* 66: 2166–2172.
70. Needham, D., and R.M.M. Hochmuth. 1992. A sensitive measure of surface stress in the resting neutrophil. *Biophys. J.* 61: 1664–1670.
71. Evans, E., and A. Yeung. 1989. Apparent viscosity and cortical tension of blood granulocytes determined by micropipet aspiration. *Biophys. J.* 56: 151–160.
72. Garcia, P.D., C.R. Guerrero, and R. Garcia. 2017. Time-resolved nanomechanics of a single cell under the depolymerization of the cytoskeleton. *Nanoscale.* 9: 12051–12059.
73. Sung, K.P., L.A. Sung, M. Crimmins, S.J. Burakoff, and S.H.U. Chien. 1986. Dynamic changes in viscoelastic properties in cytotoxic T-lymphocyte- mediated killing. *J. Cell Sci.* 91: 179–189.
74. Lipowsky, H.H., D. Riedel, and G.S. Shi. 1991. In vivo mechanical properties of leukocytes during adhesion to venular endothelium. *Biorheology.* 28: 53–64.
75. Wang, N., I.M. Tolić-Nørrelykke, J. Chen, S.M. Mijailovich, J.P. Butler, J.J. Fredberg, and D. Stamenović. 2002. Cell prestress. I. Stiffness and prestress are closely associated in adherent contractile cells. *Am. J. Physiol. Physiol.* 282: C606–C616.
76. MacKay, J.L., A.J. Keung, and S. Kumar. 2012. A genetic strategy for the dynamic and graded control of cell mechanics, motility, and matrix remodeling. *Biophys. J.* 102: 434–442.
77. Wang, N., K. Naruse, D. Stamenovic, J.J. Fredberg, S.M. Mijailovich, I.M. Tolic-Norrelykke, T. Polte, R. Mannix, D.E. Ingber, S.M. Mijailovic, G. Maksym, T. Polte, D.E. Ingber, D. Stamenović, S.M. Mijailovich, I.M. Tolić-Nørrelykke, and R. Mannix. 2001. Mechanical behavior in living cells consistent with the tensegrity model. *Proc. Natl. Acad. Sci.* 98: 7765–70.
78. Stamenović, D., Z. Liang, J. Chen, and N. Wang. 2015. Effect of the cytoskeletal prestress on the mechanical impedance of cultured airway smooth muscle cells. *J. Appl. Physiol.* 92: 1443–1450.
79. Shroff, S.G., D.R. Saner, and R. Lal. 2017. Dynamic micromechanical properties of cultured rat atrial myocytes measured by atomic force microscopy. *Am. J. Physiol. Physiol.* 269: C286–C292.
80. Étienne, J., J. Fouchard, D. Mitrossilis, N. Bufi, P. Durand-Smet, and A. Asnacios. 2015. Cells as liquid motors: Mechanosensitivity emerges from collective dynamics of actomyosin cortex. *Proc. Natl. Acad. Sci.* 112: 2740–2745.
81. Mitrossilis, D., J. Fouchard, A. Guirouy, N. Desprat, N. Rodriguez, B. Fabry, and A. Asnacios. 2009. Single-cell response to stiffness exhibits muscle-like behavior. *Proc. Natl. Acad. Sci. U. S. A.* 106: 18243–8.
82. Simon, S.I., and G.W. Schmid-Schönbein. 1988. Biophysical aspects of microsphere engulfment by human neutrophils. *Biophys. J.* 53: 163–173.
83. Lomakin, A.J., K.C. Lee, S.J. Han, D.A. Bui, M. Davidson, A. Mogilner, and G. Danuser. 2015. Competition for actin between two distinct F-actin networks defines a bistable switch for cell polarization. *Nat. Cell Biol.* 17: 1435–1445.
84. Fritzsche, M., R.A. Fernandes, V.T. Chang, H. Colin-York, M.P. Clausen, J.H. Felce, S. Galiani, C. Erlenkämper, A.M. Santos, J.M. Heddleston, I. Pedroza-Pacheco, D. Waithe, J.B. de la Serna, B.C. Lagerholm, T.L. Liu, T.-L.L. Chew, E. Betzig, S.J. Davis, C. Eggeling, B. Christoffer Lagerholm, T.L. Liu, T.-L.L. Chew, E. Betzig, S.J. Davis, and C. Eggeling. 2017. Cytoskeletal actin dynamics shape a ramifying actin network underpinning immunological synapse formation. *Sci. Adv.* 3: e1603032.
85. Yeung, a, and E. Evans. 1989. Cortical shell-liquid core model for passive flow of liquid-like spherical cells into micropipets. *Biophys. J.* 56: 139–149.
86. Tsopoulidis, N., S. Kaw, V. Laketa, S. Kutscheidt, C. Baarlink, B. Stolp, R. Grosse, and O.T. Fackler. 2019. T cell receptor-triggered nuclear actin network formation drives CD4+ T cell effector functions. *Sci. Immunol.* 4.
87. Fritzsche, M., D. Li, H. Colin-York, V.T. Chang, E. Moeendarbary, J.H. Felce, E. Sezgin, G. Charras, E. Betzig, and C. Eggeling. 2017. Self-organizing actin patterns shape membrane architecture but not cell mechanics. *Nat. Commun.* 8: 17–19.

849 88. Ritter, A.T., Y. Asano, J.C. Stinchcombe, N.M.G.G. Dieckmann, B.C. Chen, C. Gawden-Bone, S. van
850 Engelenburg, W. Legant, L. Gao, M.W. Davidson, E. Betzig, J. Lippincott-Schwartz, and G.M.
851 Griffiths. 2015. Actin Depletion Initiates Events Leading to Granule Secretion at the
852 Immunological Synapse. *Immunity*. 42: 864–876.

853 89. Quintana, A., C. Schwindling, A.S. Wenning, U. Becherer, J. Rettig, E.C. Schwarz, and M. Hoth.
854 2007. T cell activation requires mitochondrial translocation to the immunological synapse. *Proc.*
855 *Natl. Acad. Sci.* 104: 14418–14423.

856 90. Wu, Z., M. Su, C. Tong, M. Wu, and J. Liu. 2018. Membrane shape-mediated wave propagation of
857 cortical protein dynamics. *Nat. Commun.* 9: 1–12.

858 91. Freeman, S.A., A. Vega, M. Riedl, R.F. Collins, P.P. Ostrowski, E.C. Woods, C.R. Bertozzi, M.I.
859 Tammi, D.S. Lidke, P. Johnson, S. Mayor, K. Jaqaman, and S. Grinstein. 2018. Transmembrane
860 Pickets Connect Cyto- and Pericellular Skeletons Forming Barriers to Receptor Engagement.
861 *Cell*. 172: 305-317.e10.

862 92. Maxson, M.E., X. Naj, T.R. O’Meara, J.D. Plumb, L.E. Cowen, and S. Grinstein. 2018. Integrin-based
863 diffusion barrier separates membrane domains enabling the formation of microbiostatic
864 frustrated phagosomes. *Elife*. 7: 1–37.

865 93. Siokis, A., P.A. Robert, P. Demetriou, M.L. Dustin, and M. Meyer-Hermann. 2018. F-Actin-Driven
866 CD28-CD80 Localization in the Immune Synapse. *Cell Rep.* 24: 1151–1162.

867 94. DeMond, A.L., K.D. Mossman, T. Starr, M.L. Dustin, and J.T. Groves. 2008. T cell receptor
868 microcluster transport through molecular mazes reveals mechanism of translocation. *Biophys. J.*
869 94: 3286–3292.

870 95. Mey, I., A. Janshoff, J. Rother, H. No, H. Noding, I. Mey, and A. Janshoff. 2014. Atomic force
871 microscopy-based microrheology reveals significant differences in the viscoelastic response
872 between malign and benign cell lines. *Open Biol.* 4: 140046–140046.

873 96. Thoumine, O., O. Cardoso, and J.J. Meister. 1999. Changes in the mechanical properties of
874 fibroblasts during spreading: A micromanipulation study. *Eur. Biophys. J.* 28: 222–234.

875 97. Abidine, Y., A. Constantinescu, V.M. Laurent, V. Sundar Rajan, R. Michel, V. Laplaud, A. Duperray,
876 C. Verdier, R. Michel, V.M. Laurent, V. Sundar Rajan, C. Verdier, A. Duperray, Y. Abidine, A.
877 Constantinescu, V.M. Laurent, V. Sundar Rajan, R. Michel, V. Laplaud, A. Duperray, and C. Verdier.
878 2018. Mechanosensitivity of Cancer Cells in Contact with Soft Substrates Using AFM. *Biophys. J.*
879 114: 1165–1175.

880