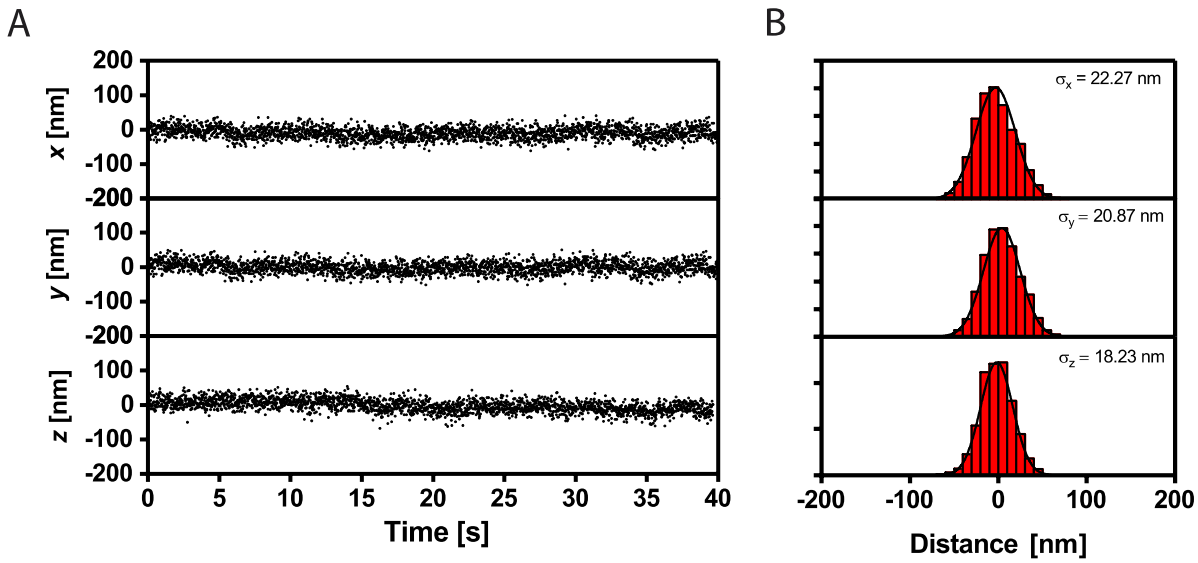
Supplemental Materials

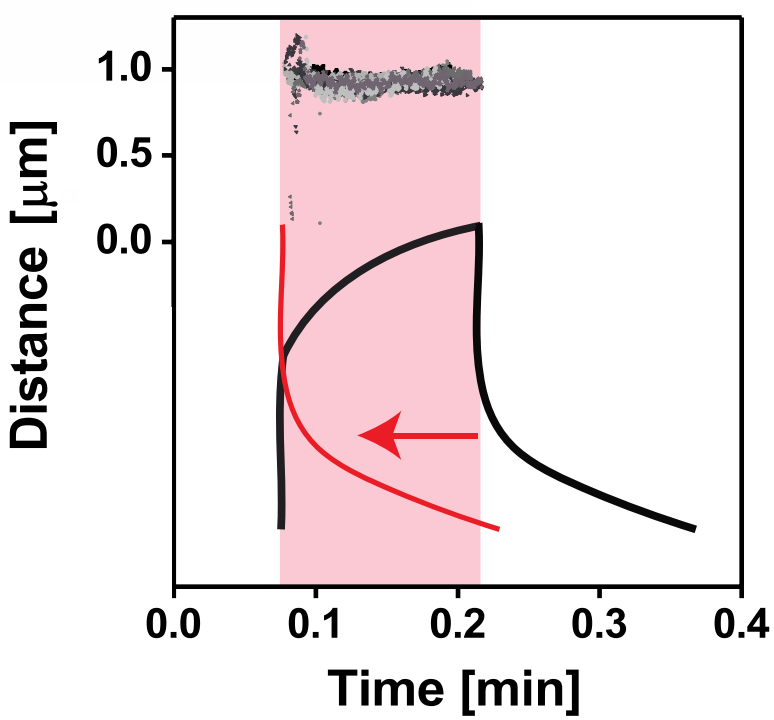
Molecular Biology of the Cell

Sorkin et al.

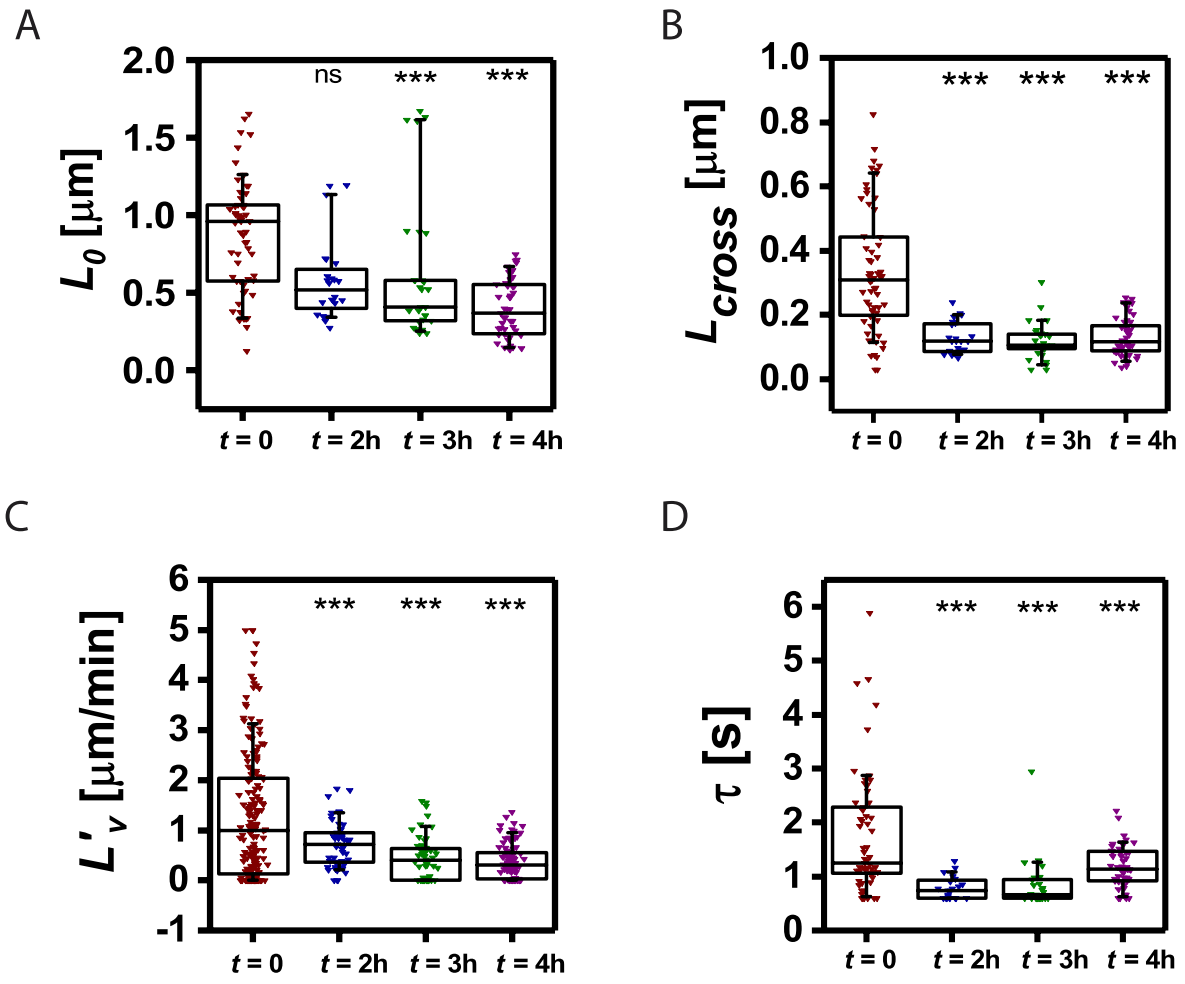
Supplementary material



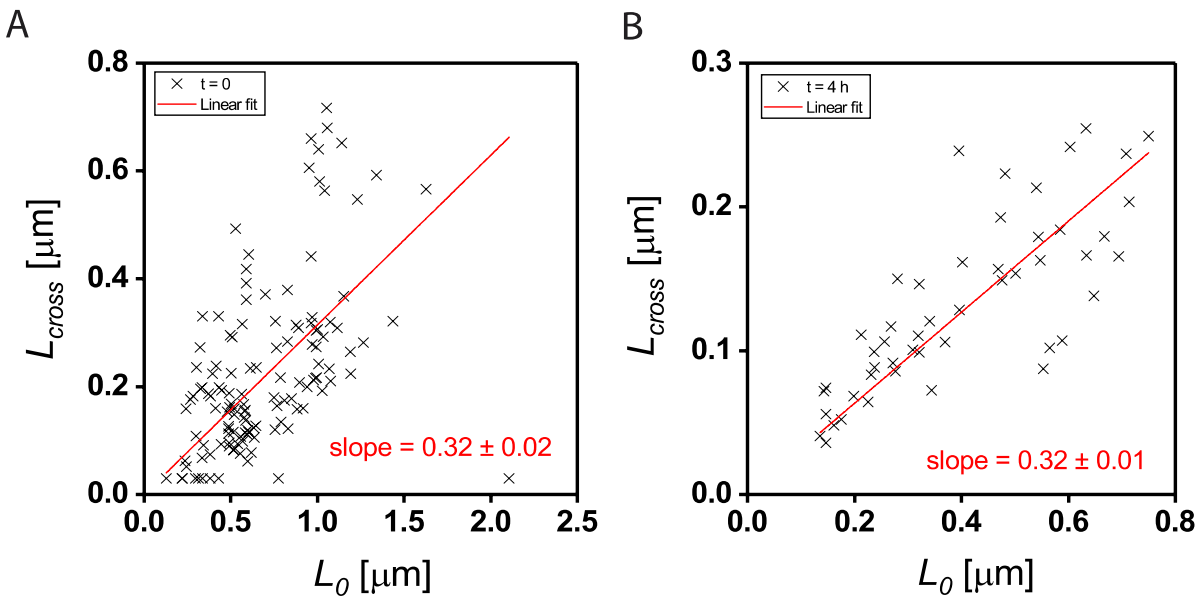
S1: Tracking accuracy of 6.8 µm diameter silica microsphere on top of RBCs. (A) Position of x, y and z recorded for 40 seconds at 60 Hz and no acoustic force is applied. As determined from the standard deviation of Gaussian fits (B) to the traces, the tracking accuracy is 22.3 nm, 20.9 nm and 18.2 nm for x, y and z, respectively.



S2: Cell response linearity check. To test whether the overall deformation cells undergo is linear, we sum the creep and relaxation responses, illustrated by the red and black curves in the pink shaded area. This sum is roughly constant, as can be seen in the grey plots. Ten different cells that were randomly picked from our data were analysed, depicted by different shades of grey in the figure. The roughly constant distance in time is indicating that the deformation is linear, and hence the use of a linear viscoelastic model is justified.

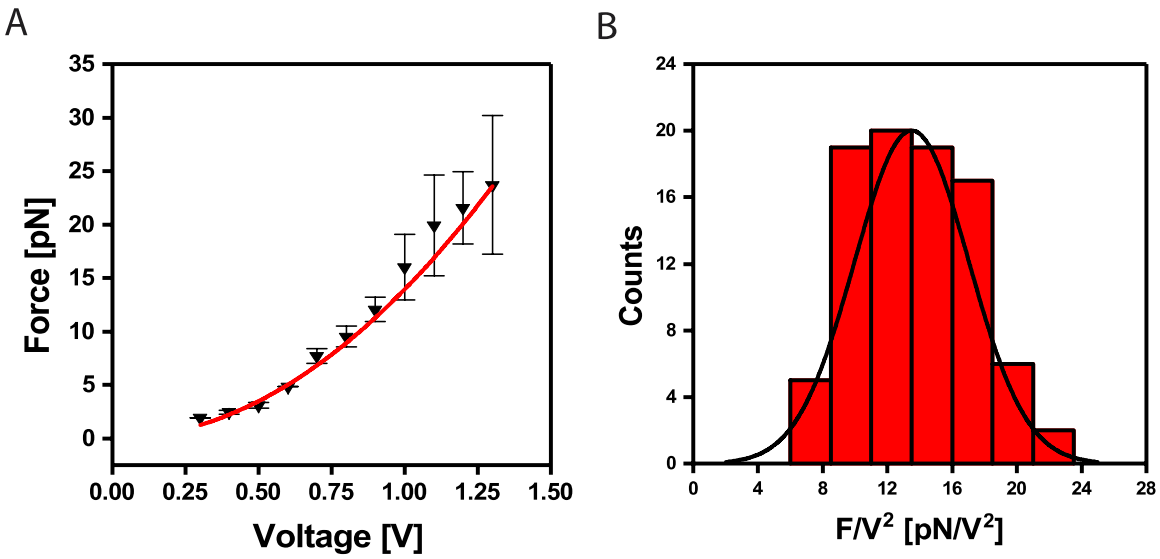


S3: Time effect on RBCs measurements. Healthy cells were measured at t = 0, 2 h, 3 h and 4 h respectively. The distribution of fitting parameters L_0 (A), L_{cross} (B), τ (C) and L'_v (D) for the different times are shown. Cells experience a decrease in elasticity after the first 2 hours, determining a progressively lower elongation (A), while the viscous parameters L_{cross}, τ and L'_v decrease considerably within 2 hours from the start of the experiment. *** corresponds to a $P < 0.005$ compared to t=0 as determined with KS test (see Methods).

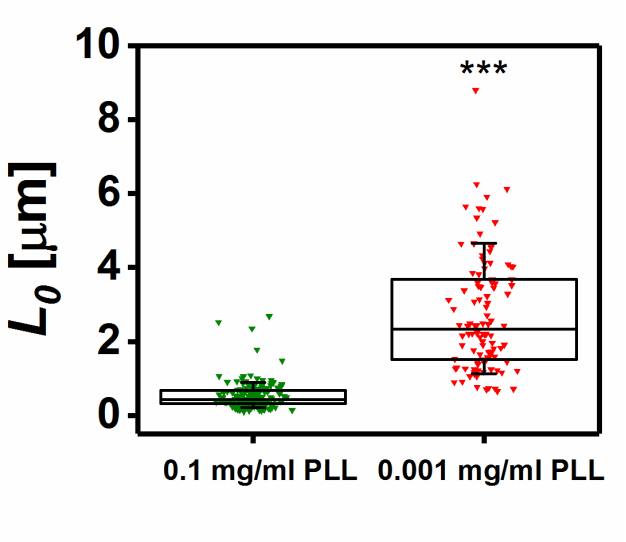


S4: Correlations between fitting parameters in time. (A) Correlation between L_0 and L_{cross} at t=0 h for healthy RBCs. Linear fit (red) yields y = 0.315 (± 0.016) x. (B) Correlation between L_0 and L*cross* at t=4 h for healthy RBCs. Linear fit (red) yields y = 0.317 (± 0.013) x. Note that the correlation coefficient (slope) seems to be constant in time. No correlations were found between the other parameters.

“Shooting beads” calibration:

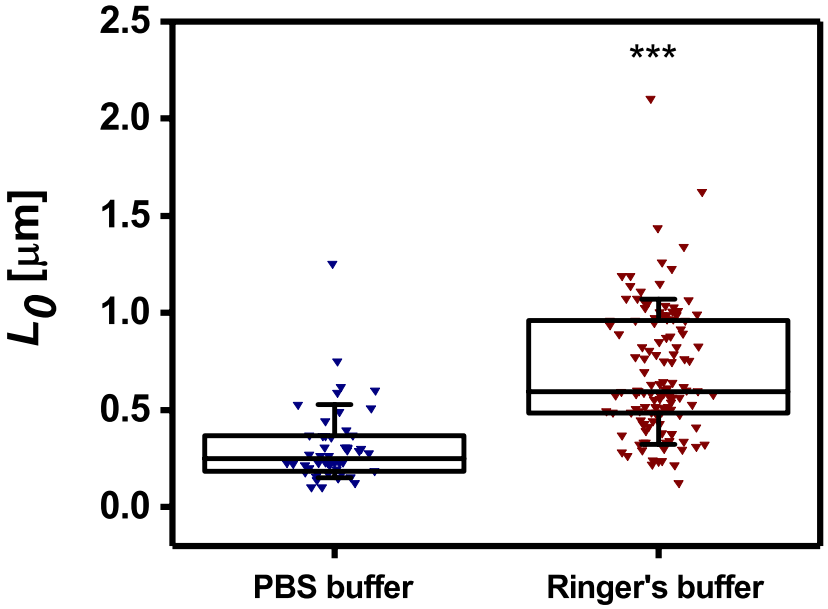


S5: Result of force calibration measurements. (A) The acoustic force profile within the fluid chamber is mapped using free suspended 6.84 µm silica microspheres (“shooting beads”). The measured calibration curve (N_{beads} = 10) follows a parabolic force profile, which scales quadratically with the voltage. A conversion factor between acoustic force F and applied voltage V is extracted from the fitting, F/V^2 = 13.9 ± 0.6 pN/V^2. (B) Histogram and Gaussian distribution of conversion factors, when computing per-bead (N_{beads} = 88) calibration (F/V^2 = 13.50 ± 3.53 pN/V^2).

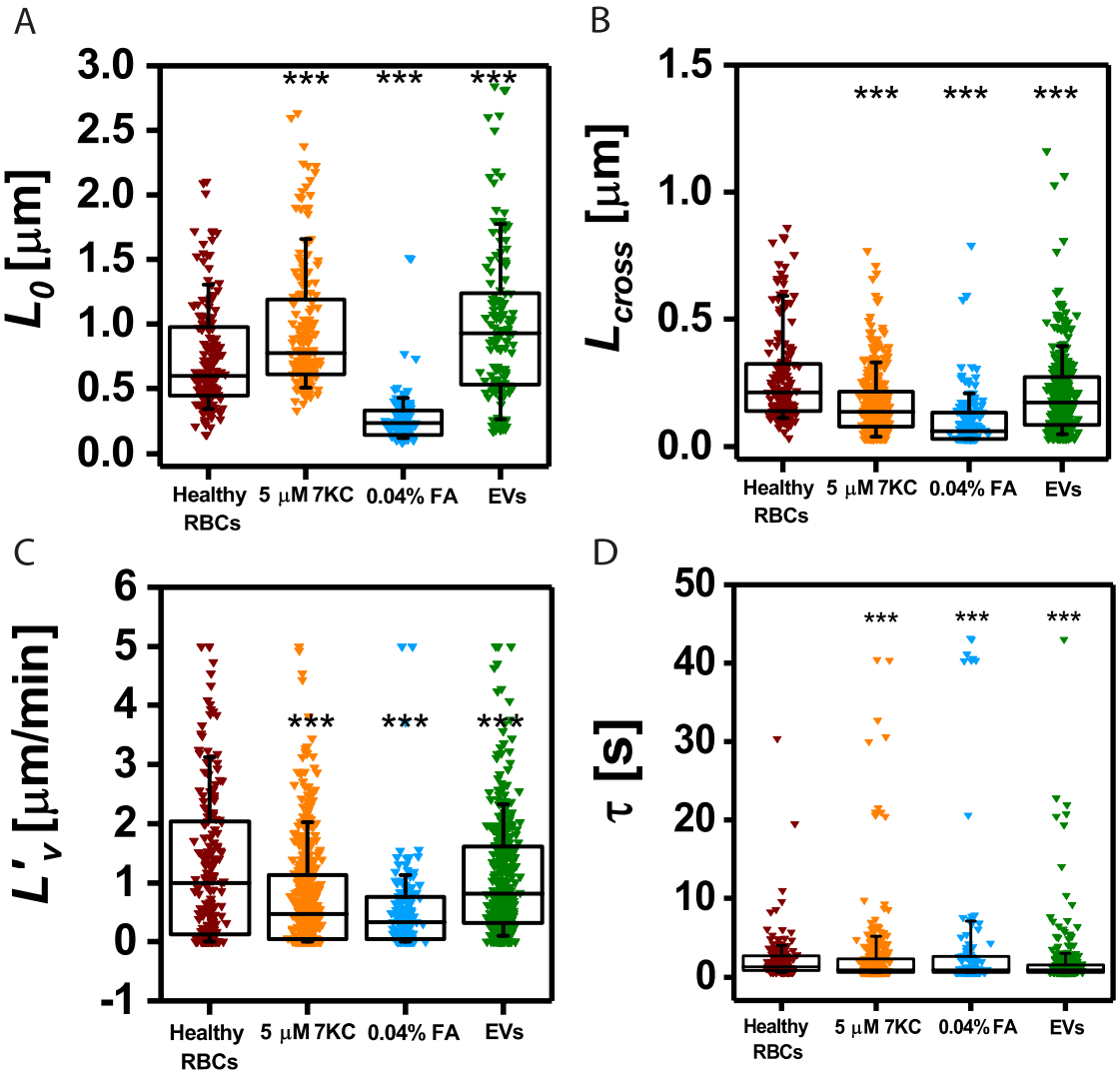


S6: Dependence of instantaneous elastic elongation (L_0) on Poly-L-lysine concentration.

Distribution of L_0 for RBCs attached to the surface with 0.1 mg/ml (green) and 0.001 mg/ml (red) Poly-L-lysine. Medians were found to be 0.60 µm (90% [CI 0.34-1.3]) and 2.34 µm (90% [CI 1.13-4.65]), for 0.1 and 0.001 mg/ml, respectively. Note the increase in stiffness due to higher PLL concentrations. $P < 0.005$ (***).



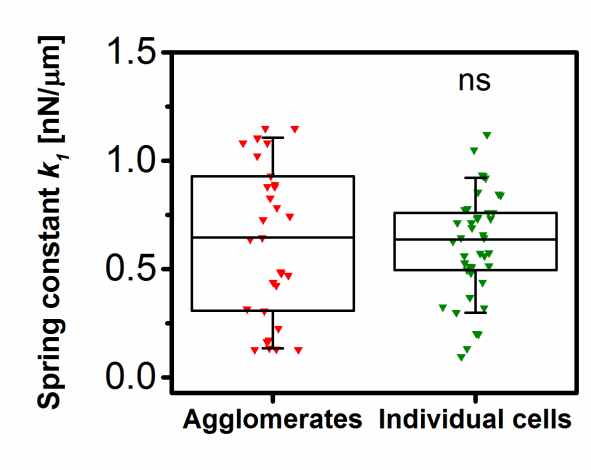
S7: Effect of buffer composition on RBC mechanics. Measurements on cells in PBS (pH = 7.4) show a lower instantaneous extension L_0 (0.25 µm, 90% [CI 0.15-0.53]) compared to cells in Ringers Buffer (0.60 µm, 90% [0.32-1.07]). Thus, cells measured in a buffer containing glucose (ATP) determine a softer response in pulling experiments. $P < 0.05$ (indicated with ***).



S8: Distribution of fitting parameters upon chemicals /vesicles treatment. The distribution of fitting parameters L_0 (A), L_{cross} (B), L'_v (C) and τ (D) for the different treatments are shown. *** corresponds to a $P < 0.005$ compared to healthy RBCs as determined with KS test (see Methods).

Effect of neighbouring Cells:

Cells are sandwiched between a surface and a bead, and in some cases (~15%) cells were in contact with a neighbouring cell. To demonstrate that these cells respond similarly to isolated cells, we compare a total of 31 health RBCs with a neighbouring cell to 42 RBCs without neighbouring cells and find no significant difference in the spring constant (k_1) between the populations.



S9: Comparison of spring constant (k_1) of agglomerates and individual cells. Distribution of k_1 for agglomerates (red) and individual RBCs (green).Medians were found to be $k_{agglomerates}$ = 0.65 nN/µm (90% confidence interval (CI) [0.14 – 1.1 nN/µm]), $k_{individual}$ = 0.64 nN/µm (90% confidence interval (CI) [0.3 – 0.92 nN/µm]). The two are not statistically significant (P = 0.23, at α = 0.005).