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Supplemental information

Distinct timing of neutrophil spreading and stiffening during phagocytosis

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Distinct timing of neutrophil spreading and stiffening during phagocytosis

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SUPPLEMENTAL INFORMATION

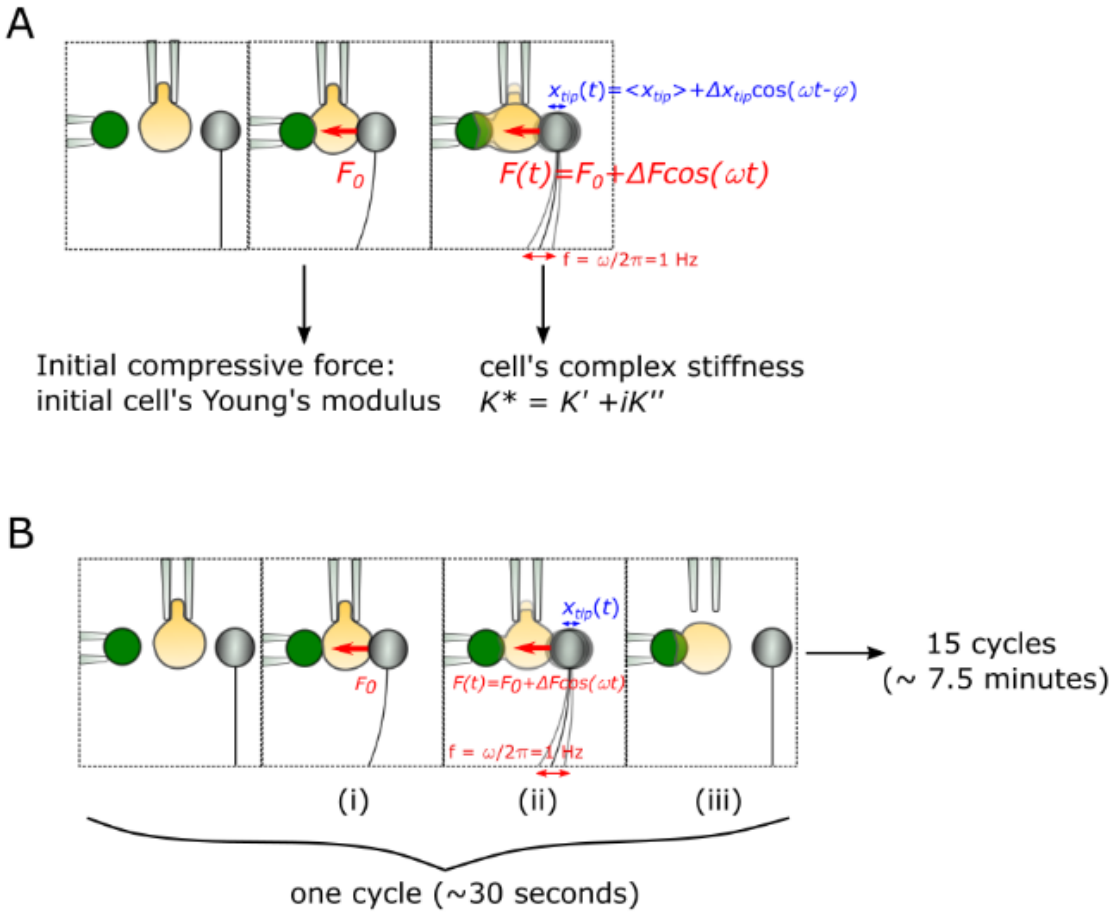

Figure S1. Measurement of the cell Young's modulus and complex stiffness using indentation in the back (A) and cyclic indentation in the back (B). (*A*) A phagocyte (yellow) is first compressed with an increasing force until a threshold of F_0= 180 pN is reached. The compression is performed by a glass bead at the tip a flexible micropipette (grey) whose base is translated towards the cell. On the opposite side, the cell is pressed against an activating bead (green) held by a stiff micropipette. This first compression phase allows extracting the Young's modulus of the cell at the beginning of the experiment. Once the force threshold F_0 is reached, the applied force is modulated by an added oscillatory force of amplitude ΔF and frequency 1 Hz. This force modulation leads to an oscillatory modulation of the cell length of amplitude ΔX_{tip}, of same frequency 1 Hz, but with a phase lag φ. This oscillations allows extracting K' and K'' respectively relative to the cell stiffness and viscosity (see Material and

Methods in main text. (B) Cyclic indentation. The procedure is initially the same as in (A): the cell is indented with the same initial compressive force threshold F_0 (i) and the Young's modulus is measured, then the force is modulated, leading to the quantification of both *K'* and *K''* (ii). After few seconds of oscillation, the flexible micropipette comes back to its initial position losing contact with the cell (iii), while the cell keeps on phagocyting the activating bead. This sequence of events describes a cycle and lasts 30 seconds. Fifteen cycles are repeated in a row for a single cell making the experiment lasts around 7.5 minutes.

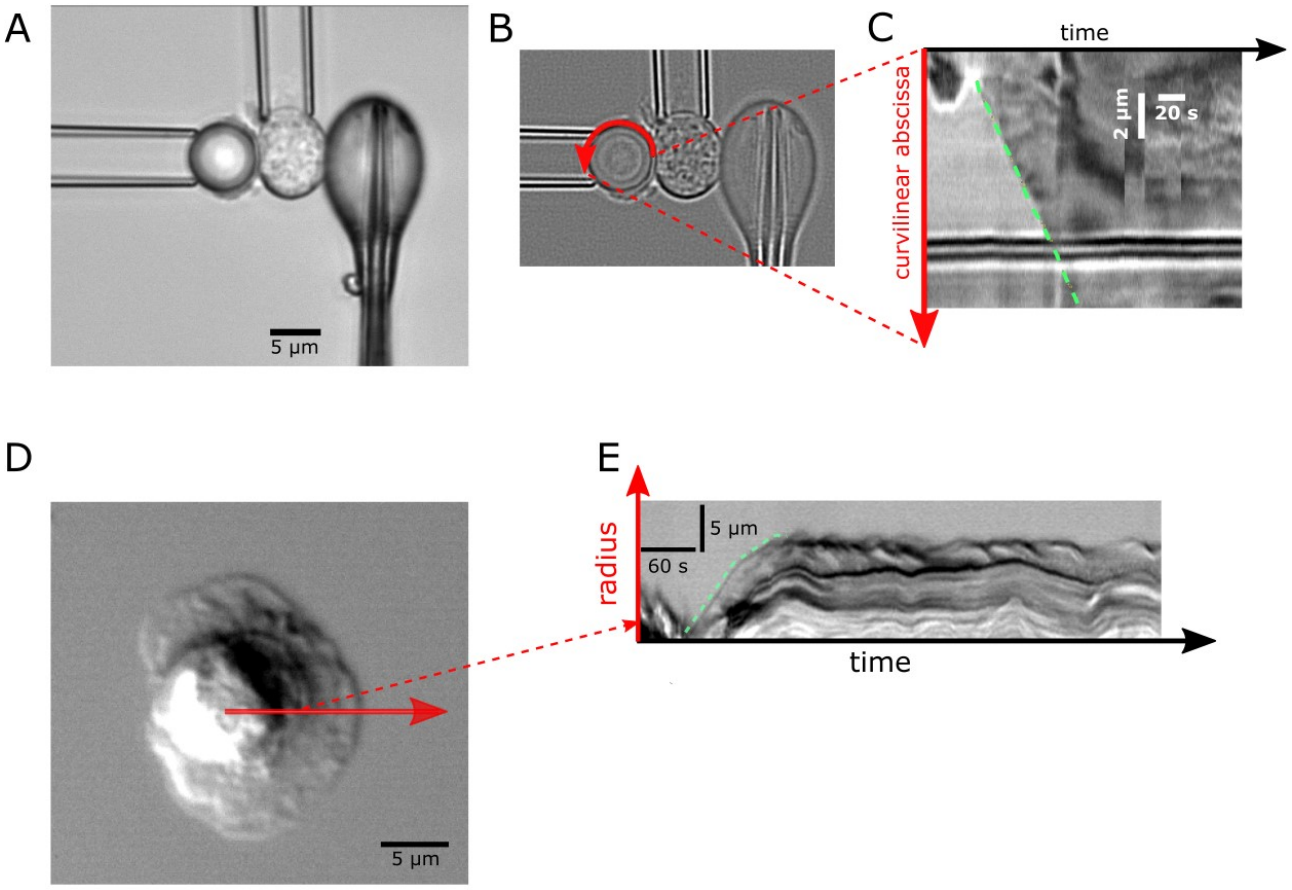

Figure S2. Spreading speed and area measurements. (*A*) Back indentation of a primary neutrophil phagocytosing an 8-µm IgG-coated polystyrene bead. (*B*) Image post-processing: using ImageJ, a mean-filtered image was subtracted from the original one (leading to an effect comparable with a high-pass filtering) in order to visualize more easily the progression of the phagocytic cup on the activating microbead. The position of the cell front is monitored along the curved arrow. (C) Kymograph used to measure curvilinear abscissa along the curved arrow in B. (D) Top view of a frustrated phagocytosis by a typical PLB cell on an IgG-coated flat surface. The red arrow is used to trace a kymograph (E) used to quantify the cell radius as a function of time during frustrated phagocytosis allowing to calculate the total cell area spreading on a flat surface.

Supplementary material 1. Predicted dA/dt for a constant linear speed v_0 during spreading

Experimentally the velocity $\frac{ds}{dt}$ is quite constant from the beginning of the cup formation up to the slowing down (Figure 2E). This slowing down happens at the same curvilinear coordinate $s = s_{slowdown} \approx 7\ \mu m$ on both large and small beads (8-µm beads: $s_{slowdown}$ = 6.8+/-1.3 µm; 20-µm beads:

$s_{slowdown}$ = 6.4+/-1.6 µm, mean+/- SD, p=0.22 two-tailed Mann Whitney test). At this transition, we measure a slower $\frac{dA}{dt}$ on smaller beads. This would be natural on smaller beads given a constant linear velocity. To quantify we calculate the expected ratio of spreading speeds on both beads at this slowing down point:

$A(s) = 2\pi R^2\left(1-\cos\frac{s}{R}\right)$, $\frac{dA}{dt} = 2\pi R \sin\left(\frac{s}{R}\right)\frac{ds}{dt}$ so at $s = s_{slowdown} \approx 7\ \mu m$, the ratio of $\frac{dA}{dt}$ for the beads of radius R_1 = 10 µm and R_2 = 4 µm is, considering that $\frac{ds}{dt}$ is constant and roughly equal to 0.14 µm/s on both beads:

$$\left(\frac{dA}{dt}\right)_1 \Big/ \left(\frac{dA}{dt}\right)_2 = R_1 \sin\left(\frac{S_{slowdown}}{R_1}\right) \Big/ R_2 \sin\left(\frac{S_{slowdown}}{R_2}\right) \approx \frac{10\sin\left(\frac{7}{10}\right)}{4\sin\left(\frac{7}{4}\right)} \approx 1.7$$

Experimentally we find $\left(\frac{dA}{dt}\right)_1 \Big/ \left(\frac{dA}{dt}\right)_2 \approx \frac{10\sin\left(\frac{7}{10}\right)}{4\sin\left(\frac{7}{4}\right)} \approx 1.6$, in good agreement with the predicted value.

Note that in the above calculation $\frac{dA}{dt}$ is taken as a maximum of $\frac{dA}{dt}$ at $= s_{slowdown}$, so this value is expected it to be larger that the average $\langle\frac{dA}{dt}\rangle = \frac{A_{max}}{\Delta t_{spreading}}$. Nevertheless, the ratio of average spreading speed is very close to the one computed above: $\langle\frac{dA}{dt}\rangle_1 / \langle\frac{dA}{dt}\rangle_2 \approx 1.6$. This suggests that the dependence on relative bead radius is robust and shows that the area increase is set by the velocity of the cell front independently of the local target curvature.

Supplementary material 2. Maximal stiffness and value 60 s post maximum

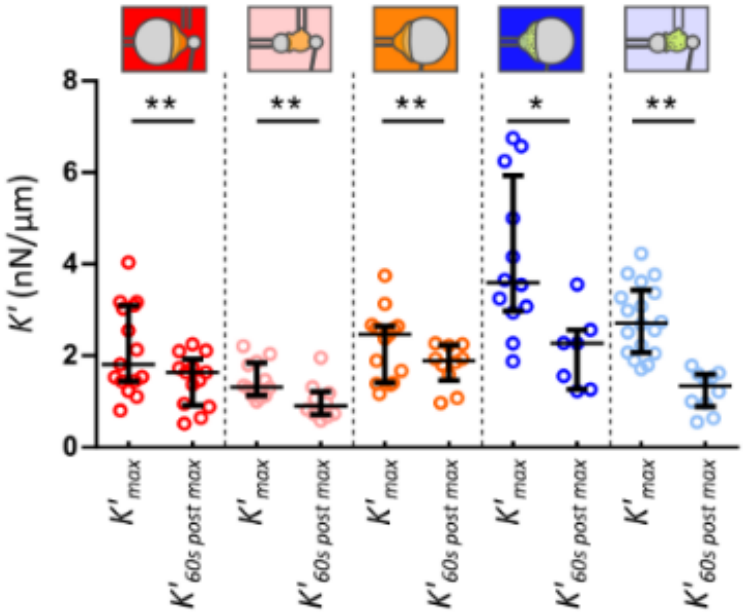

Figure S3. Maximal value of cell stiffness, K'_{max}, and value of K' sixty seconds after the maximum was reached, $K'_{60s\ post\ max}$. K' has decreased significantly 60 seconds after reaching its maximum in all cases (front and back indentation, 20-µm and 8-µm beads, PLB cells and primary neutrophils). Error bars are interquartile ranges. Statistical test: Wilcoxon matched-pairs signed rank test (* p<0.05; ** p<0.01).

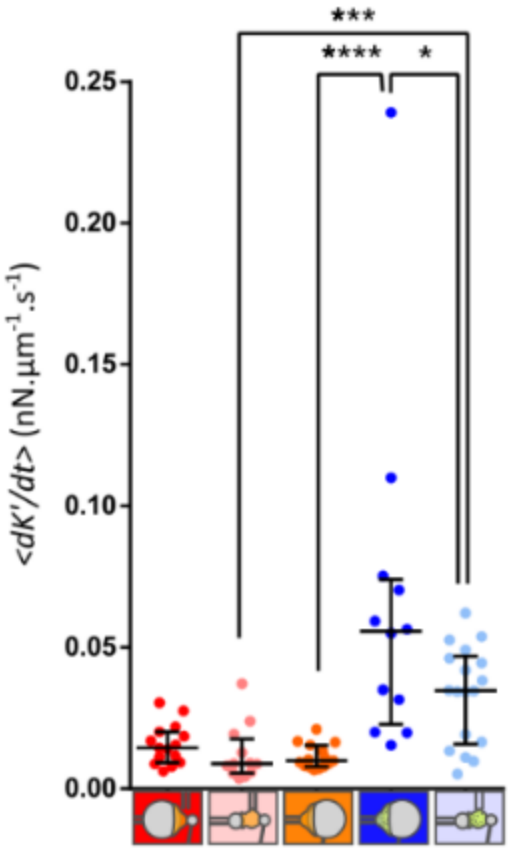

Figure S4. Average rate of increase of the cell stiffness, $\langle \frac{dK\prime}{dt} \rangle = \frac{K'_{max} - K'_{start}}{t_{K\prime max} - t_{K\prime start}}$. Error bars are inter-quartile ranges. Statistical test: two-tailed Mann-Withney (* p<0.05; *** p<0.001; **** p<0.0001).

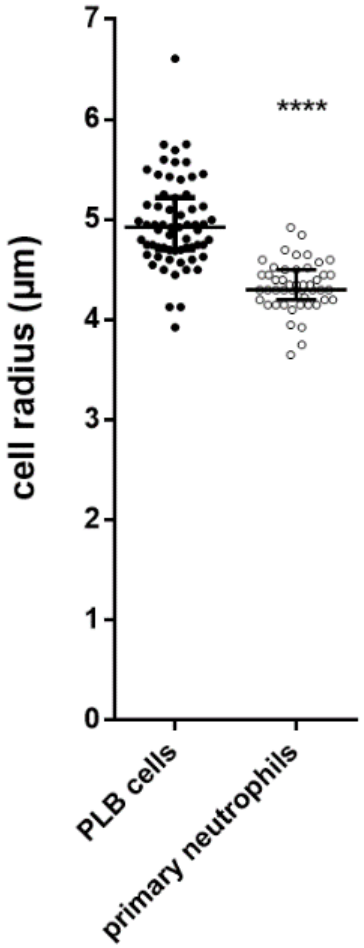

Figure S5. Initial radius of PLB cells (4.96 ± 0.45 µm, mean ± SD) and primary neutrophils (4.34 ± 0.25 µm, mean ± SD). Each dot represents a cell. Kolmogorov-Smirnov test (****, $p<0.0001$).

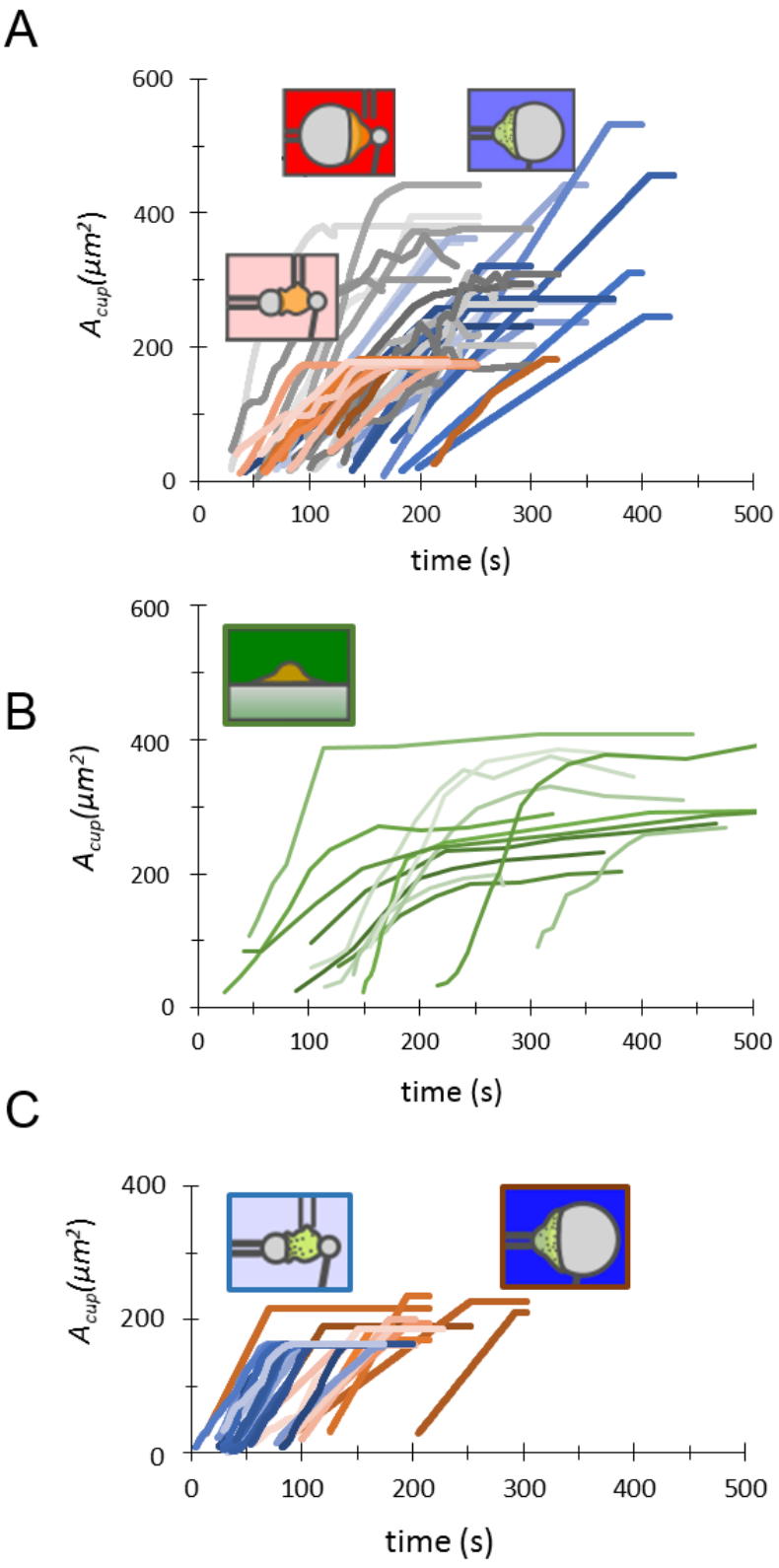

Figure S6. (A) Phagocytic cup area as a function of time following cell- bead contact for PLB cells (pink curves: back indentation with 8-µm beads; gray curves: back indentation with 20-µm beads) and for neutrophils (blue curves: back indentation and 8-µm beads; orange curves: front indentation and 20-µm beads). All individual curves are shown (each curve represents a different cell) and correspond to median values shown in Figure 2A and 3A. (B) Phagocytic cup area as a function of time following cell-substrate contact for PLB cells during frustrated phagocytosis on flat surfaces. Raw data are shown (each curve represents a different cell) and correspond to median values shown in

Figure 2A and 3A. (C) Phagocytic cup area as a function of time following cell-bead contact for neutrophils cells during back indentation on 8-µm beads (blue curves) and during front indentation on 20-µm beads (orange curves). Raw data are shown (each curve represents a different cell) and correspond to median values shown in Figure 2A and 3A.

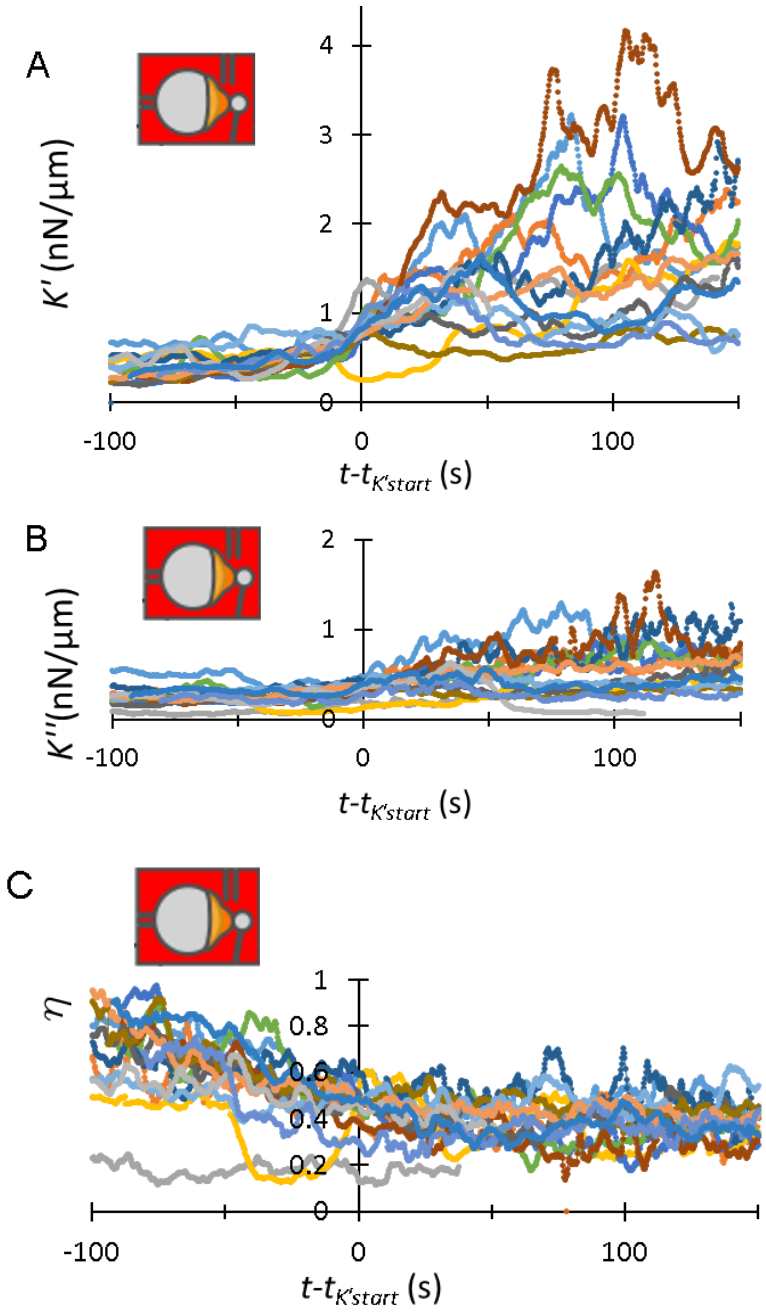

Figure S7. Viscoelastic behavior during phagocytosis of 20-µm beads by PLB cells indented in the back. Individual curves are shown, for which time was shifted so that time origin corresponds to the moment at which *K'* reaches a level of 0.5 nN/µm above the initial level ($t = t_{K'start}$). In A-C, individual curves are shown (each curve represents a different cell), which correspond to median values shown in Figure 4B,D,F. (*A*) *K' vs t-* $t_{K'start}$. (*B*) *K" vs t-t* $t_{K'start}$. (*C*) Loss tangent $\eta = \frac{K''}{K'}$ *vs t-t* $t_{K'start}$.

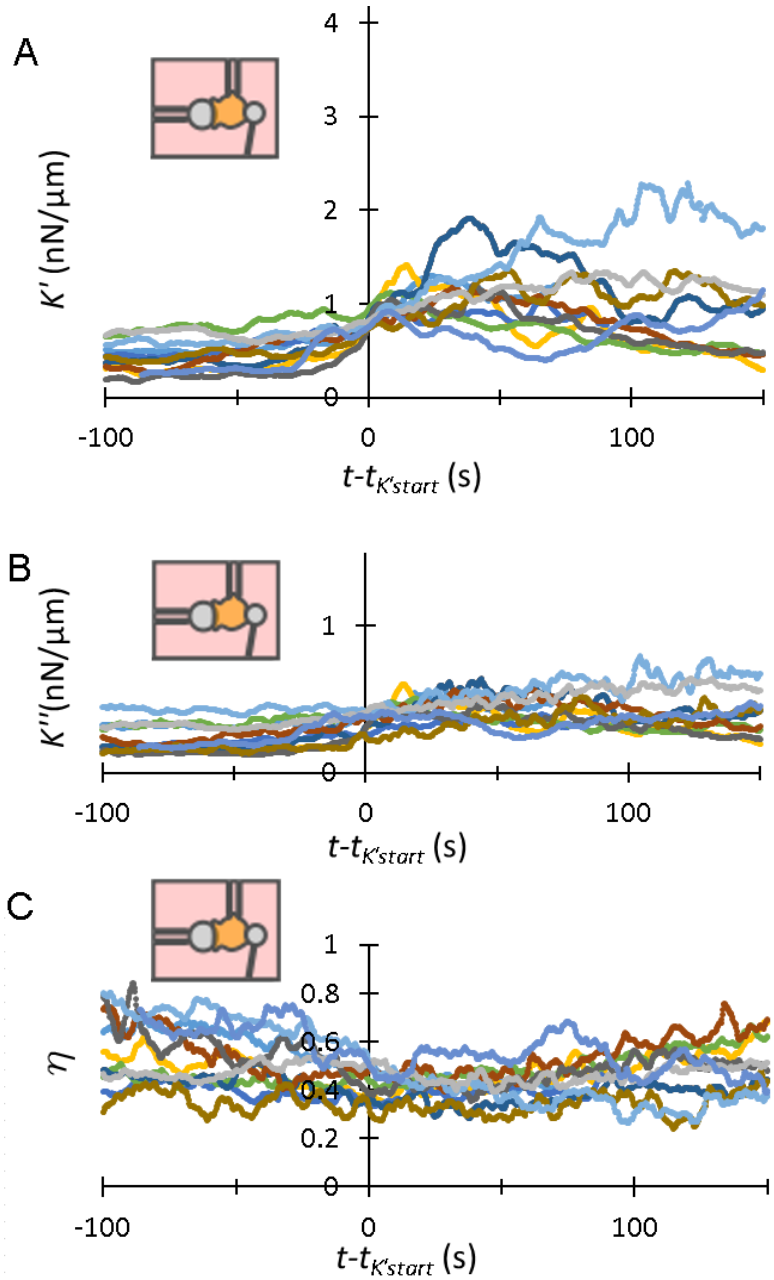

Figure S8. Viscoelastic behavior during phagocytosis of 8-µm beads by PLB cells indented in the back. Individual curves are shown, for which time was shifted so that time origin corresponds to the moment at which *K'* reaches a level of 0.5 nN/µm above the initial level ($t=t_{K'start}$). These data correspond to median values shown in Figure 4B,D,F. (*A*) *K' vs t-* $t_{K'start}$. (*B*) *K" vs t-t* $t_{K'start}$. (*C*) Loss tangent $\eta = \frac{K''}{K'}$ *vs t-t* $t_{K'start}$.

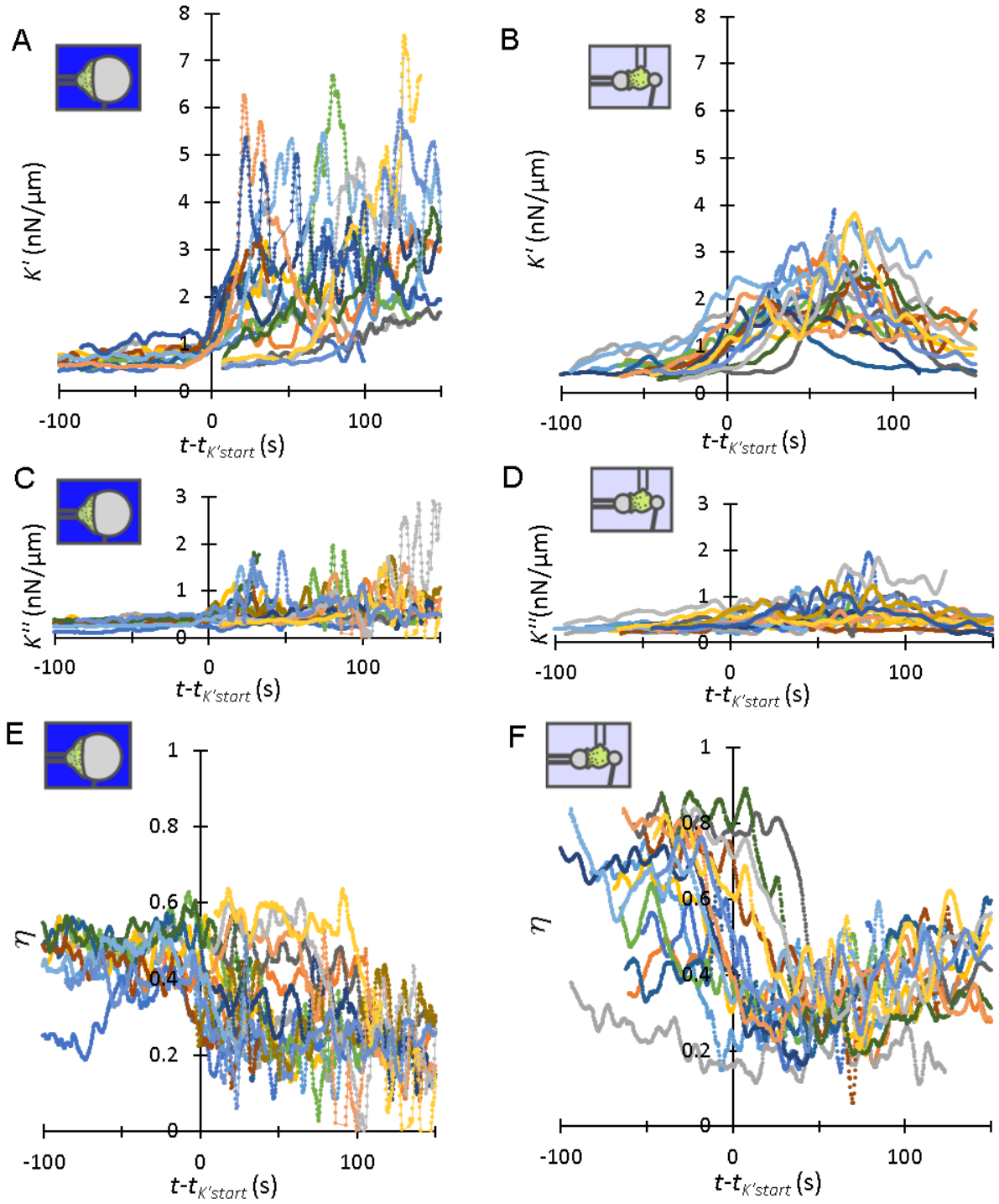

Figure S9. Viscoelastic behavior during phagocytosis by neutrophils of 20-µm beads while being indented in the front (*A,C,E*) and of 8-µm beads while being indented in the back (*B,D,F*). Individual curves are shown, for which time was shifted so that time origin corresponds to the moment at which *K'* reaches a level of 0.5 nN/µm above the initial level ($t=t_{K'start}$). These data correspond to median values shown in Figure 3C and Figure 4C,E,G. (*A-B*) *K' vs t-* $t_{K'start}$. (*C-D*) *K'' vs t-t* $t_{K'start}$. (*E-F*) Loss tangent $\eta = \frac{K''}{K'}$ *vs t-t* $t_{K'start}$.

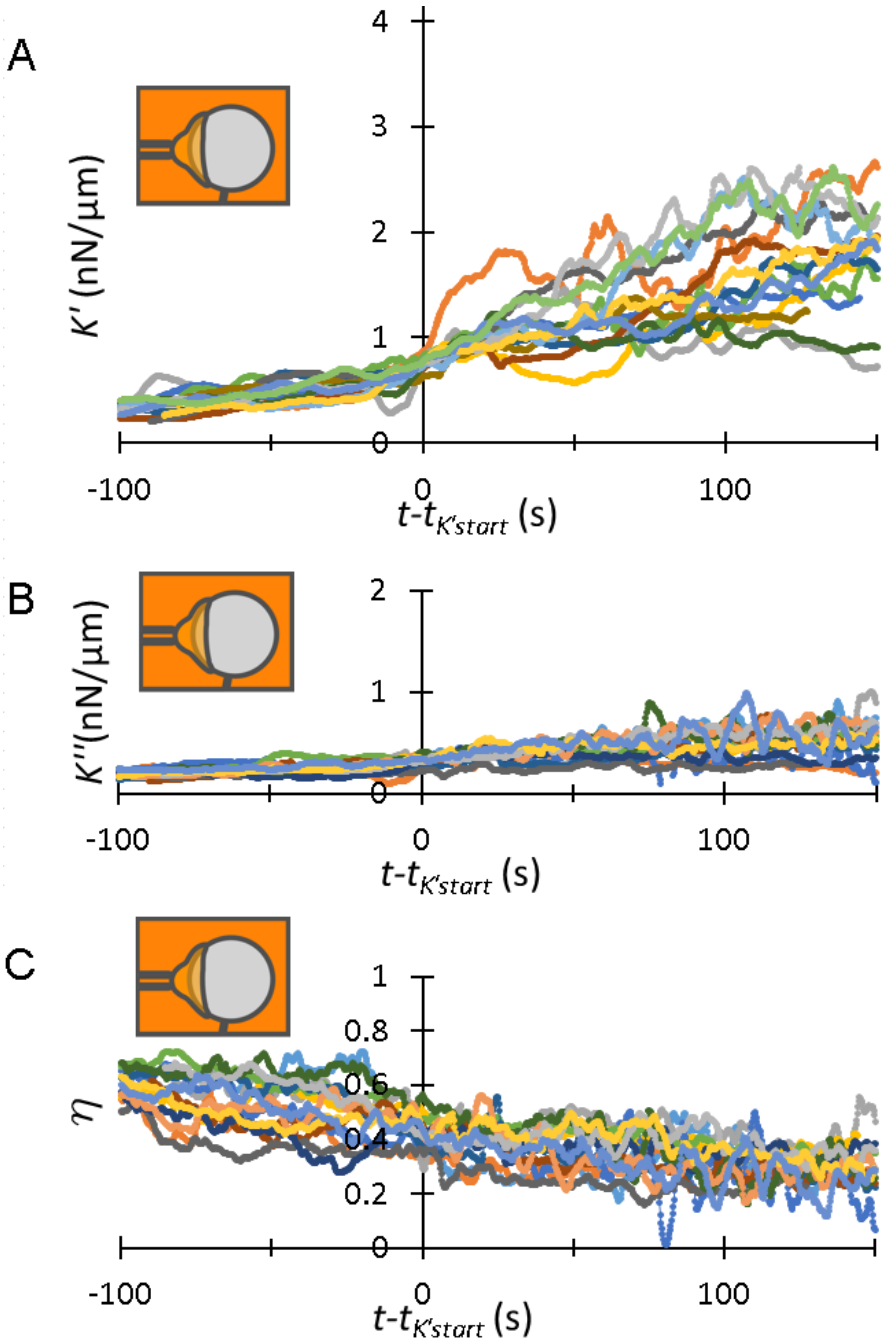

Figure S10. Viscoelastic behavior during phagocytosis of 20-μm beads by PLB cells while being indented in the front. Individual curves are shown, for which time was shifted so that time origin corresponds to the moment at which *K'* reaches a level of 0.5 nN/μm above the initial level (t=$t_{K'start}$). These data correspond to median values shown in Figure 3*C* and Figure 4*B,D,F*. (*A*) *K' vs t-* $t_{K'start}$. (*B*) *K" vs t-t* $t_{K'start}$. (*C*) Loss tangent $\eta = \frac{K''}{K'}$ *vs t-t* $t_{K'start}$.

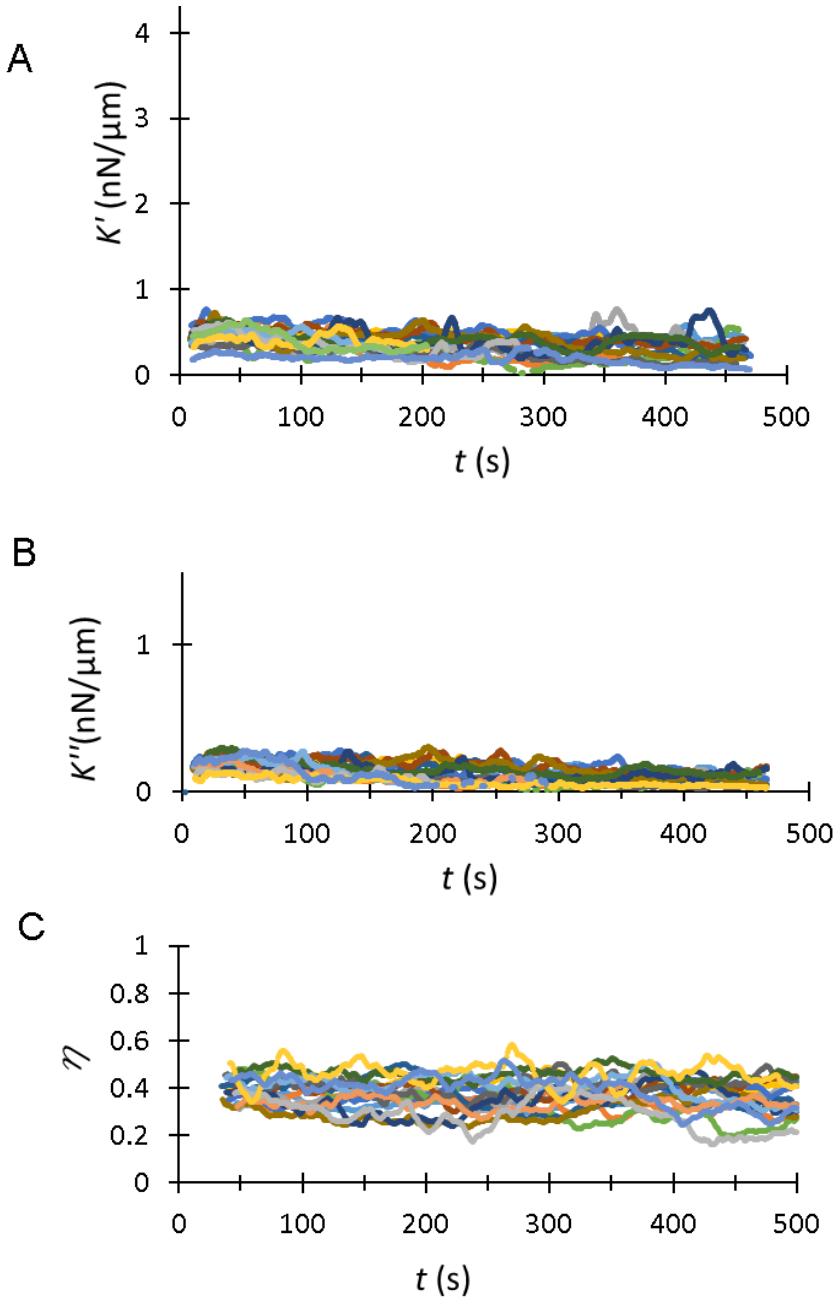

Figure S11. Viscoelastic behavior of PLB cells in contact with control beads not coated with activating antibodies. Individual curves are shown, time *t*=0 corresponds to cell-bead contact. These data correspond to median values shown in Figure 4*B,D,F*. (*A*) *K′ vs t.* (*B*) *K″ vs t*. (*C*) Loss tangent $\eta = \frac{K''}{K'}$ *vs t.*