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Supplementary Materials for

Extension of chemotactic pseudopods by nonadherent human neutrophils does not require or cause calcium bursts

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Legends for movies S1 to S4

Other Supplementary Material for this manuscript includes the following:
(available at www.sciencesignaling.org/cgi/content/full/11/521/eaal4289/DC1)

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Movie S2 (.mp4 format). Absence of Ca^{2+} bursts during complement-mediated, pure chemotaxis of a human neutrophil toward zymosan.
Movie S3 (.mp4 format). Absence of Ca^{2+} bursts during complement-mediated, pure chemotaxis of a human neutrophil toward β-glucan.
Movie S4 (.mp4 format). Supraphysiological concentrations of C5a or costimulation by shear flow can induce Ca^{2+} bursts in nonadherent human neutrophils.

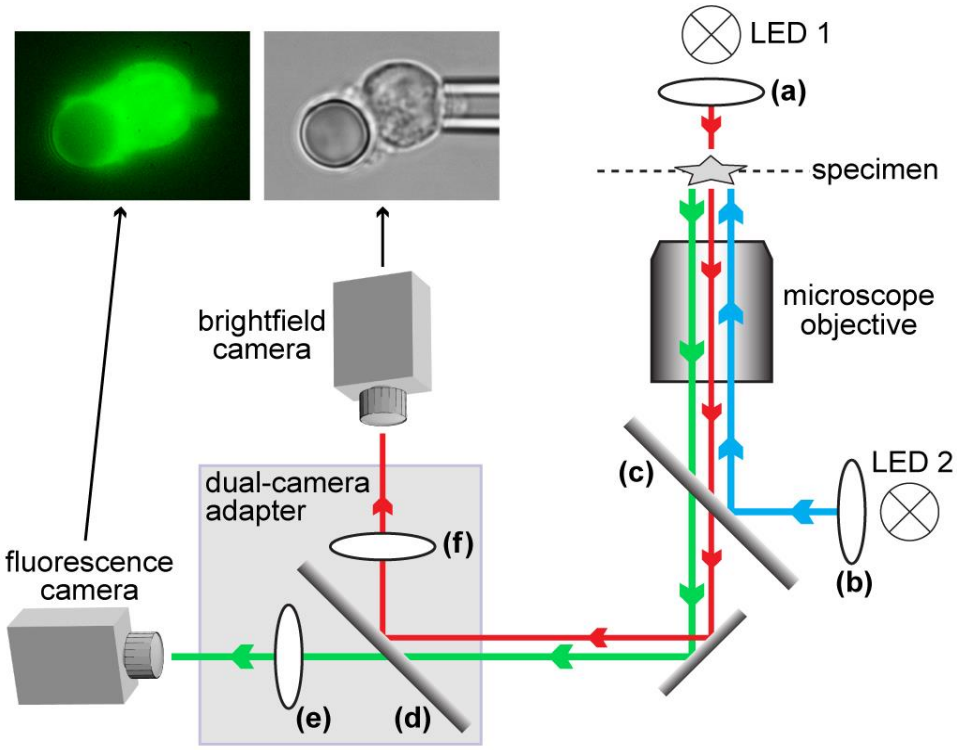

Fig. S1. Dual-camera setup for simultaneous recording of bright-field and fluorescence images. The LEDs emitted white (LED 1) or cyan (LED 2) light. The following filters and dichroic mirrors (Chroma Technology) were used: (a) ET630/75m; (b) ET480/40x; (c) T510lpxrxt; (d) 59001bs; (e) ET535/50m; (f) ET610/20. See Materials and Methods for further details.

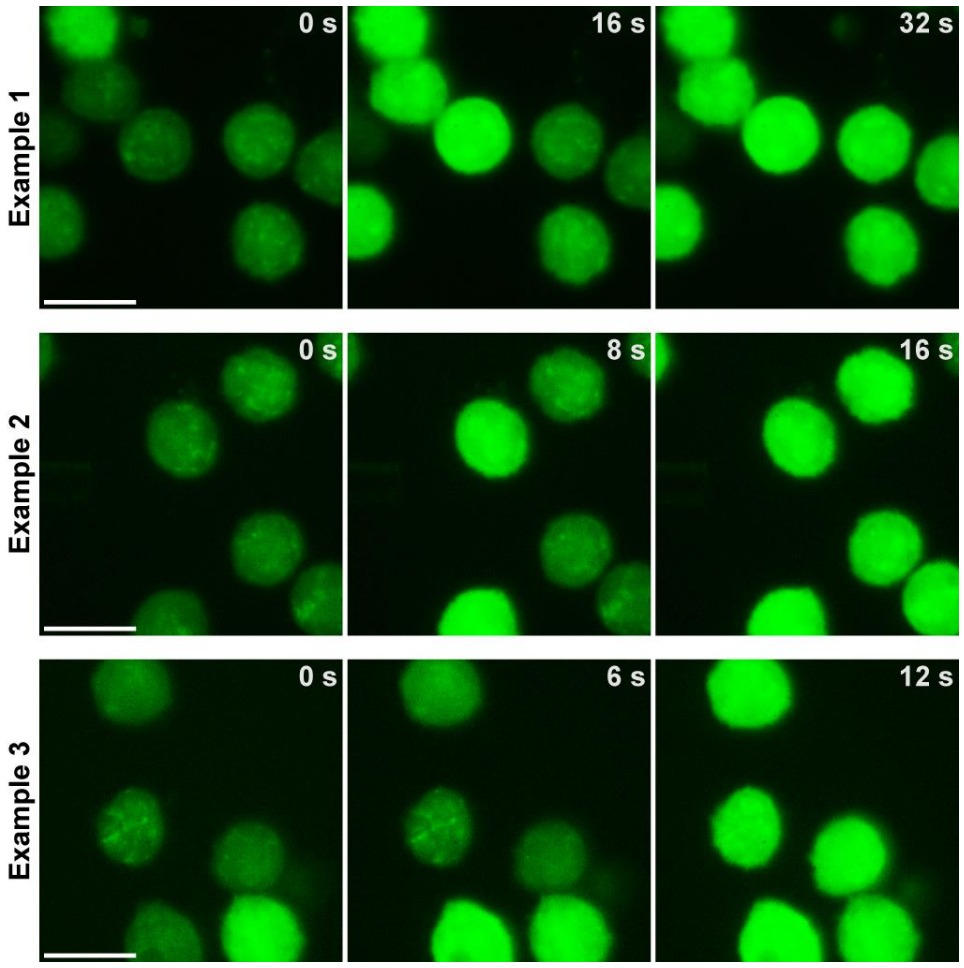

Fig. S2. Supraphysiological concentrations of C5a trigger Ca^{2+} bursts in resting human neutrophils without inducing chemotaxis. These examples (representing a total of $N = 67$ tested cells) illustrate the response of neutrophils pre-loaded with the calcium indicator Fluo-4 to jets of a 0.1 μM C5a solution applied from the left. The relative time at which each image was recorded is included. Scale bars, 10 μm.

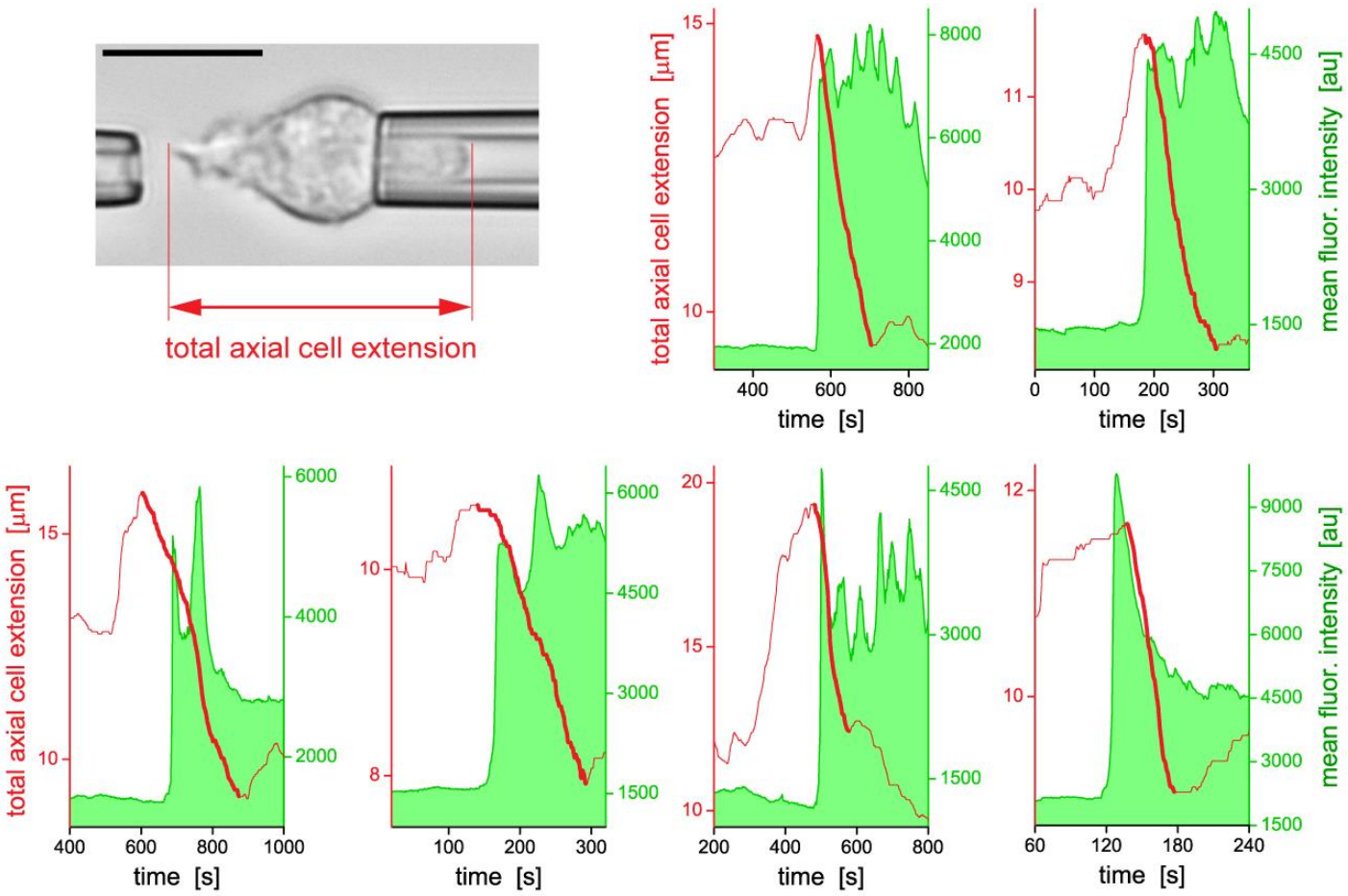

Fig. S3. Concurrence of Ca^{2+} bursts and cellular contraction. Six example graphs show the time course of the total axial cell extension (red line) of pipette-held neutrophils that exhibited Ca^{2+} bursts in response to jets of C5a solution. The included micrograph depicts a neutrophil extending a chemotactic pseudopod toward the pipette from which the C5a jet was ejected and illustrates our measurement of the total axial cell extension. The aspiration pressure in the cell-holding pipette was kept constant during the shown time windows. Prolonged periods of cellular contraction are emphasized in the graphs by thick line segments. Underlaid in each graph is the simultaneously measured time course of the mean fluorescence intensity of the Ca^{2+} indicator Fluo-4. We observed the shown behavior—Ca^{2+} bursts that were accompanied by a decrease or arrest of the total axial cell extension—in 24 out of $N = 26$ single-cell experiments. Scale bar, 10 µm.

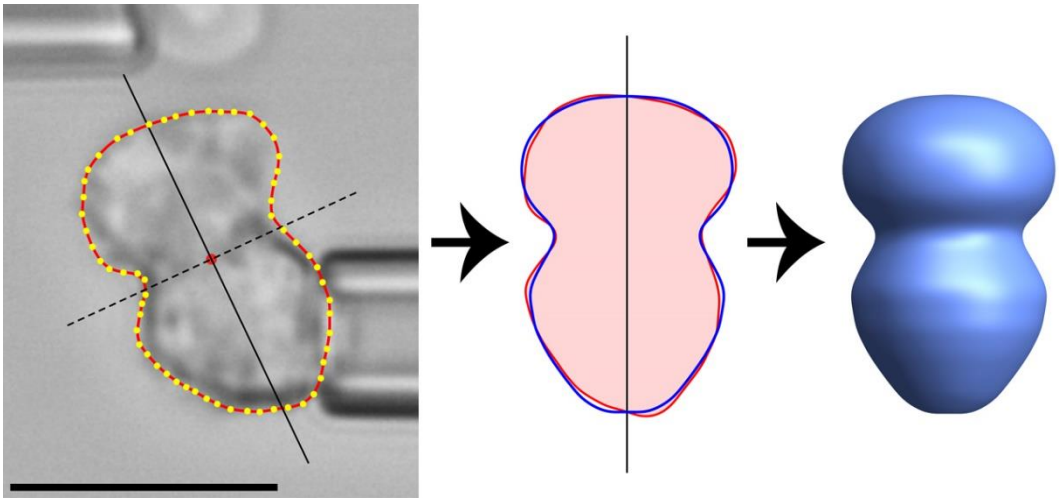

Fig. S4. Estimation of the cell surface area. The figure illustrates the workflow of our calculation of the surface area of the cell body outside the pipette. It is based on the assumption that the video image of the cell body shows a 2D cross-section of an axisymmetric 3D body. After finding a polygonal outline of the cell contour (polygonal vertices are shown as yellow dots), we computed a smooth and continuous mathematical representation of the contour (red line). We then determined the most likely axis of rotation (solid black line) and reconstructed the 3D body using a symmetric version of the contour (blue line). Our calculation takes into account that the total cell volume remains constant during the deformations considered here. Scale Bar, 10 μm.

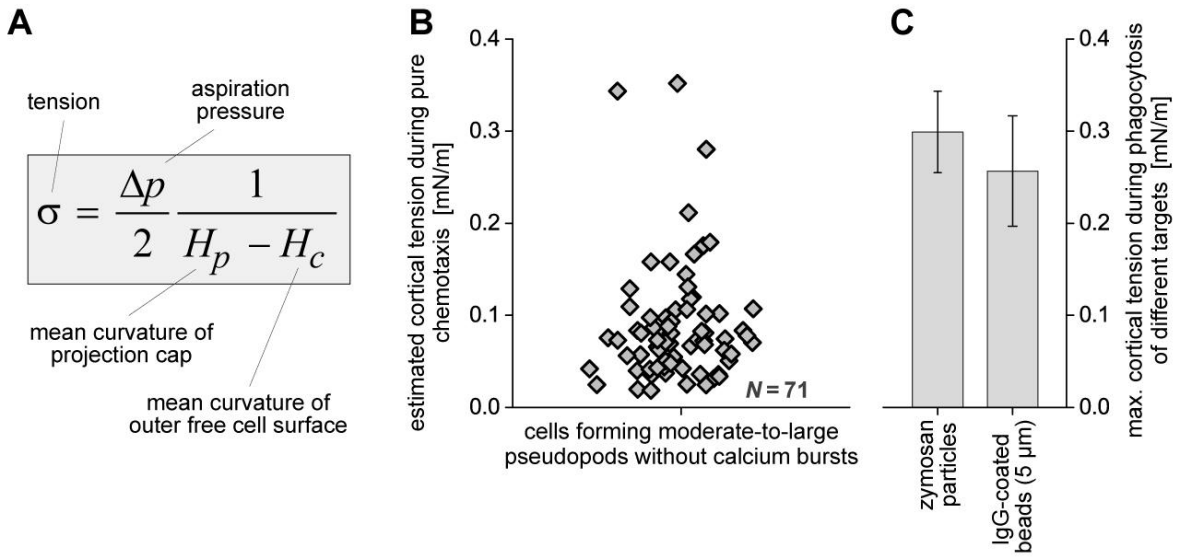

Fig. S5. Effective cortical tension during pure chemotaxis. (A) The equilibrium equation for converting the measured aspiration pressure Δp to the tension σ of a pipette-held fluid membrane capsule follows from Laplace's law. If the length of the cell projection into the pipette exceeds the pipette radius R_p, then the mean curvature of the cap of this projection is given by $H_p = 1/R_p$. For the mean curvature of the cell surface outside the pipette H_c, we use the inverse radius of a sphere that approximates the outer part of the cell. **(B)** This column-scatter plot presents a total of $N = 71$ individual measurements of the cortical tension of nonadherent neutrophils extending chemotactic pseudopods without Ca^{2+} bursts. Because of the dynamic nature of the cell shape and other uncertainties in the current experiments, these values are crude estimates. **(C)** For direct comparison with our current data, we here include previously measured mean values (error bars denote standard deviations) of the maximum cortical tension of human neutrophils phagocytosing zymosan particles or 5 µm antibody-coated beads (*58*).

Supplemental Movies

Movie S1. A Ca^{2+} burst in a human neutrophil. The video starts with a composite brightfield+fluorescence movie of a Ca^{2+} burst during phagocytosis of an antibody-coated bead by a human neutrophil that had been pre-loaded with the Ca^{2+} indicator Fluo-4. The second part presents a plot of the fluorescence intensity versus time for the same experiment.

Movie S2. Absence of Ca^{2+} bursts during complement-mediated, pure chemotaxis of a human neutrophil toward zymosan. The video combines simultaneously recorded brightfield and fluorescence movies of a human neutrophil that had been pre-loaded with the Ca^{2+} indicator Fluo-4 and presented with a zymosan particle made from yeast cell walls. At first, the zymosan particle is maneuvered to different sides of the pipette-held, nonadherent neutrophil. The neutrophil responds vigorously by extending chemotactic pseudopods toward the target particle. Eventually, the particle is handed over to the cell, resulting in its phagocytosis.

Movie S3. Absence of Ca^{2+} bursts during complement-mediated, pure chemotaxis of a human neutrophil toward β-glucan. The video combines simultaneously recorded brightfield and fluorescence movies of a human neutrophil that had been pre-loaded with the Ca^{2+} indicator Fluo-4 and presented with a cluster of β-glucan particles. At first, the β-glucan cluster is maneuvered to different sides of the pipette-held, nonadherent neutrophil. The neutrophil responds vigorously by extending chemotactic pseudopods toward the target cluster. Eventually, the cluster is handed over to the cell, resulting in its phagocytosis.

Movie S4. Supraphysiological concentrations of C5a or costimulation by shear flow can induce Ca^{2+} bursts in nonadherent human neutrophils. This video showcases three example experiments in which pipette-held neutrophils that had been pre-loaded with the Ca^{2+} indicator Fluo-4 are subjected to jets of different C5a solutions at varying intensity: a low-intensity jet of 0.1 nM C5a, a jet of 10 nM C5a that starts at low intensity and then is strengthened by raising the jet pressure 5-fold, and a low-intensity jet of 0.1 μM C5a.