Supporting Information

Bashour et al. 10.1073/pnas.1315606111

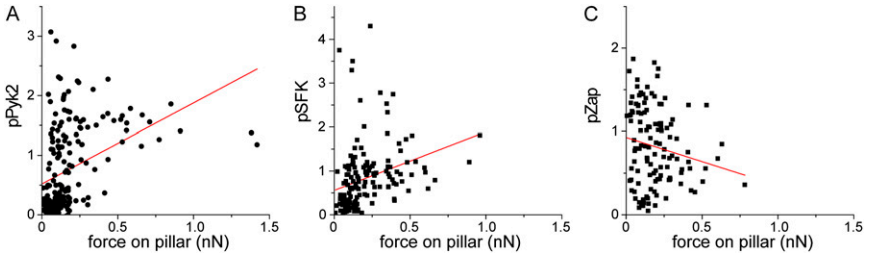
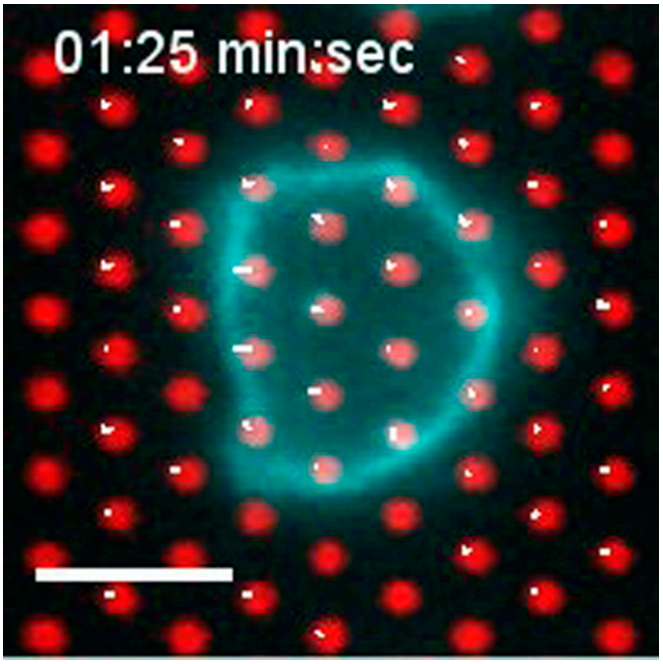
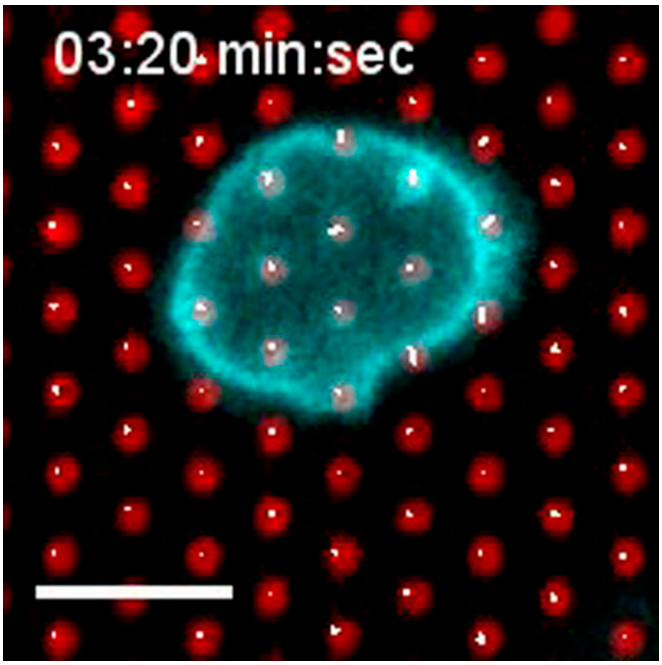


Fig. S1. Activation of signaling proteins correlates with force generation. The role of specific signaling molecules in modulating traction force was assayed by comparing staining for the phosphorylated, active versions of each protein and pillar deflection. Primary cells were seeded onto arrays coated with OKT3 + CD28.6, then fixed for staining and analysis 30 min later. For each protein, the average intensity within a 1.1-μm diameter region covering each pillar top was normalized against the average signal measured across the cell–array interface; this normalized intensity is compared as a function of applied force in the graphs. Staining for (*A*) pPyk2, collected from 10 cells across three independent experiments, and (*B*) pSFK, representing 9 cells, exhibited a moderate degree of correlation between concentration and applied force (R_s = 0.49 and 0.50, respectively). In contrast, no correlation was observed for (*C*) pZap70, collected from 11 cells (R_s = −0.17). Red lines indicate the best linear fit between enrichment and force.



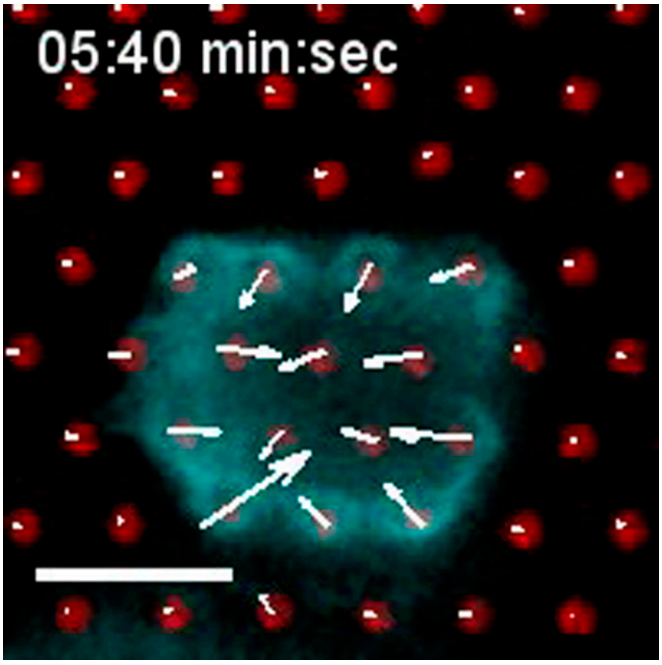
Movie S1. Initial contact and spreading of T cells on pillar arrays. Traction force dynamics during initial contact with an array coated with OKT3 + CD28.6 antibodies, which target CD3 and CD28, respectively. Timestamps indicate minutes:seconds, with negative numbers indicating time before initial contact. Cells (cyan) were visualized using Fab fragments to CD45, whereas pillars (red) included a small fraction of labeled OKT3. Scale bar: 5 μm and 1 nN (for forces indicated as arrows). This scaling between distance and force is for presentation only and does not correspond to a physical relation between pillar displacement and cell interaction.

Movie S1



Movie S2. Interaction of T cells on arrays coated with α-CD3 alone. Traction forces for T cells (cyan) during initial contact with an array (red) coated with OKT3. Timestamps indicate minutes:seconds, with negative numbers indicating time before initial contact. Scale bar: 5 μm and 1 nN.

Movie S2



Movie S3. Progression of T cell into a stable, contractile phase. Traction forces for T cells (cyan) in contact with an array (red) coated with OKT3 + CD28.6. Timestamps indicate minutes:seconds since time of initial contact. Scale bar: 5 μm and 1 nN.

Movie S3