Supporting Information

Na *et al.* 10.1073/pnas.0711704105

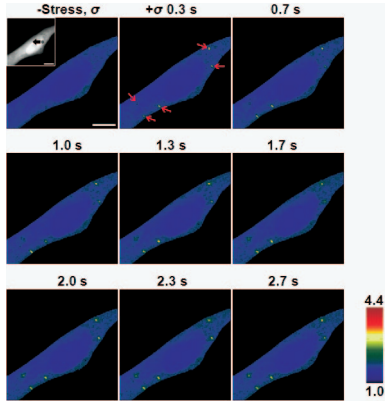

Fig. S1. Rapid Src activation at remote cytoplasmic sites in response to the local mechanical stress stimulation. A representative HASM cell (Fig. 1*A*) transfected with a cytosolic CFP-YFP Src reporter was subjected to a mechanical load (step function; stress = 17.5 Pa) via a RGD-coated magnetic bead. The stress application induced rapid (<0.3 s) Src activity at discrete, distant sites in the cytoplasm. The black arrow in the *Inset* of the first image indicates bead movement direction. Red arrows point to strong Src activation. $n = 12$ cells. (Scale bar, 10 μm.)

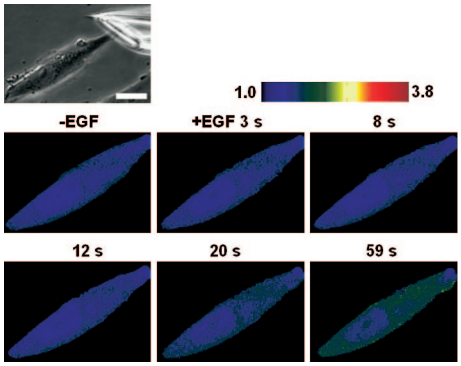

Fig. S2. The EGF-induced Src activation exhibits slower and different patterns than the stress-induced Src activation. EGF (50 ng/ml) backfilled in the micropipette was locally released 0.5–1 μm above the cell apical surface by using a micromanipulator (InjectMan NI2; Eppendorf) and a piston pump (CellTram vario; Eppendorf). Src is activated between 12 and 20 s. Note that Src activation by EGF appeared to originate at the plasma membrane and was uniformly distributed in the cytoplasm, quite different from the concentrated Src activation patterns induced by the localized mechanical stress (see Fig. S1 for comparison). $n = 8$ cells. (Scale bar, 20 μm.)

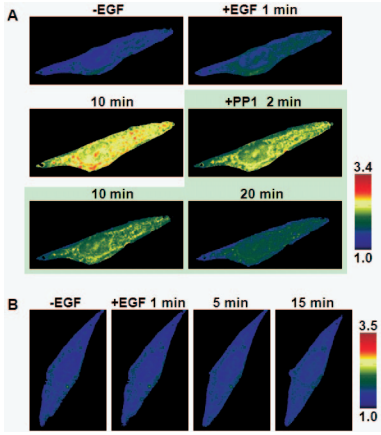

Fig. S3. The EGF-induced FRET response of the cytosolic Src reporter is inhibited by PP1. (*A*) A representative HASM cell transfected with cytosolic Src biosensor was first stimulated with EGF (50 ng/ml). At 10 min after EGF stimulation, PP1 (10 μM) was added. Src activation by EGF is significantly reduced after PP1 treatment. $n = 5$ cells. (*B*) A representative HASM cell transfected with cytosolic Src biosensor was pretreated with PP1 (20 μM) for 1 h before being subjected to EGF (50 ng/ml). PP1 effectively prevented EGF-induced Src activation. $n = 4$ cells.

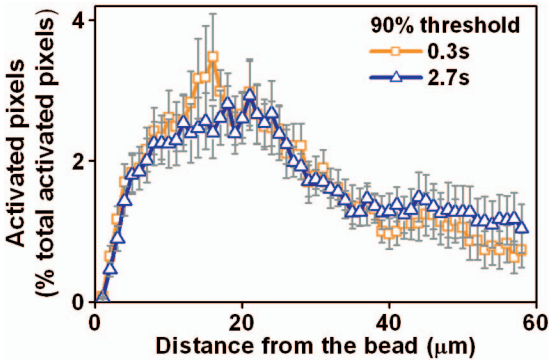

Fig. S4. Stress-induced Src activity at different cytoplasmic sites. The numbers of activated pixels (percentage of total activated pixels) at 0.3 and 2.7 s after mechanical stimulation are shown. Peak Src activation with a cut-off emission ratio threshold of 90% was observed at ≈15 μm away from the bead. $n = 8$ cells. Error bars represent SEM.

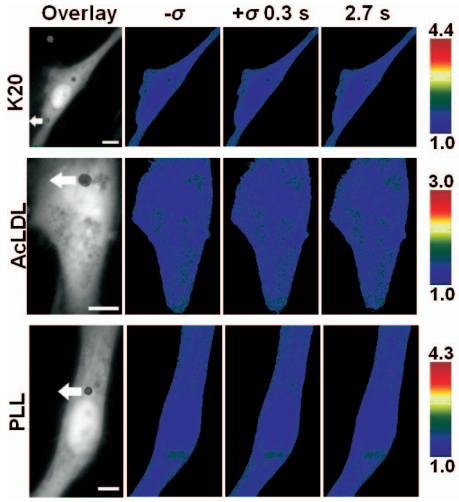

Fig. S5. Mechanical stresses do not elicit Src activation via nonactivated integrins or nonadhesion receptors. Representative cells were incubated with magnetic beads for 15 min, followed by the application of mechanical stress (17.5 Pa). Mechanical stresses applied via a magnetic bead coated with K20 (nonactivating integrin β1 antibody) (n = 3 cells), nonadhesion ligand acetylated low-density lipoprotein (AcLDL) (n = 4 cells) and nonspecific ligand poly-L-lysine (PLL) (n = 3 cells) did not activate Src. White arrows indicate bead movement direction. (Scale bar, 10 μm.)

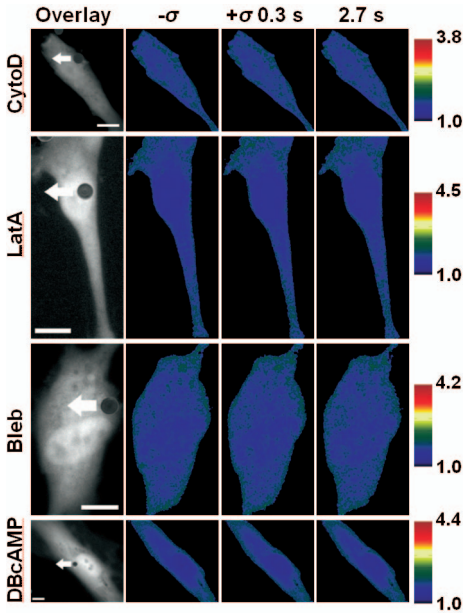

Fig. S6. F actin and prestress are necessary for rapid Src activation in the deep cytoplasm. Cells were pretreated with different specific cytoskeletal-disrupting drugs [cytochalasin D ($n = 5$ cells): 1 μg/ml for 15 min; latrunculin A ($n = 4$ cells): 1 μM for 15 min; blebbistatin ($n = 5$ cells): 50 μM for 20 min; dibutyryl adenosine 3′-5′ cyclic monophosphate (DBcAMP) ($n = 4$ cells): 1 mM for 15 min]. The cells were then bound with RGD-coated magnetic beads for 15 min, followed by the application of mechanical stress (17.5 Pa). Disrupting actin microfilaments with CytoD and LatA and inhibiting cytoskeletal prestress with Bleb and DBcAMP prevented stress-induced Src activation. White arrows indicate bead movement direction. (Scale bar, 10 μm.)

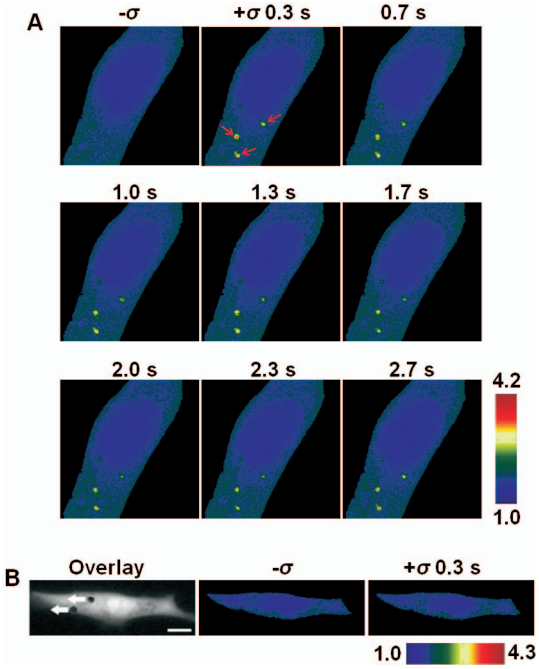

Fig. S7. Microtubules are essential for rapid, long-range Src activation in response to mechanical stresses. (*A*) Time-lapse images of Src acitivity in a representative cell (Fig. 3*A*) to show that strong Src activation sites colocalize with large deformation sites of microtubules in the same cell. $n = 4$ cells. (*B*) A representative cell was pretreated with colchicine (10 μM for 15 min) and then bound with a RGD-coated bead for 15 min. The cell was then subjected to mechanical stress (17.5 Pa). Disrupting microtubules with colchicine prevented stress-induced Src activation. White arrows indicate bead movement direction. $n = 6$ cells. (Scale bar, 10 μm.)

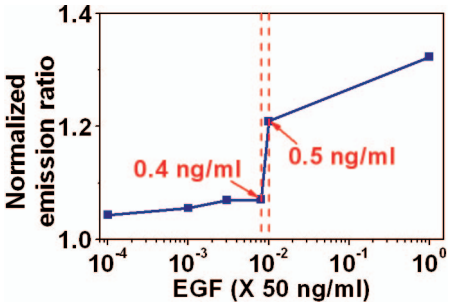

Fig. S8. Src activation depends on EGF concentration. Normalized CFP/YFP emission ratios are expressed as a function of EGF concentration. Each HASM cell was stimulated with different EGF concentrations (50 ng/ml: $n = 5$ cells; 0.5 ng/ml: $n = 4$ cells; 0.4 ng/ml: $n = 3$ cells; 0.17 ng/ml: $n = 3$ cells; 0.05 ng/ml: $n = 4$ cells; 0.005 ng/ml: $n = 3$ cells). Only mean emission ratios 15 min after EGF stimulation are shown for clarity. Strong Src activation is shown $>$0.4 ng/ml.

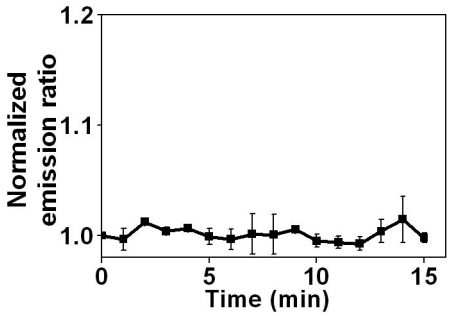

Fig. S9. RGD-coated magnetic bead binding does not induce local Src activation in the cytoplasm. RGD-coated magnetic beads were added directly to the medium containing the cell at 0 min, and CFP-YFP images were recorded continuously for 15 min. Most magnetic beads settled on the apical cell surface within 1 min. The size of the region containing each bead to obtain mean CFP/YFP emission ratio was 8 μm $\times$ 8 μm. $n = 6$ beads from three cells. Error bars represent SEM.