Supplemental Data

Self-Organized Podosomes Are Dynamic Mechanosensors

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Supplemental Experimental Procedures

Cell Culture and Reagents

Baby hamster kidney cells transformed by Rous sarcoma virus (BHK-RSV cells) were maintained in DMEM containing 10% fetal bovine serum, 0.3mg/ml L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C, 5% CO2 atmosphere. For transient transfections, BHK-RSV cells were plated in 12-well plates 24 h before Lipofectamine-mediated DNA transfer (Invitrogen, Carlsbad, Ca), according to the manufacturer's protocols, by using a total of 2 μg of mCherry-Actin DNA, 3 μg of GFP-Myosin Light Chain DNA, 3 μg of EGFP-Zyxin, or 3 μg of EGFP α-Actinin per well. The mCherry-actin probe is a gift from Dr. R. Tsien's lab. For live cell imaging, cells were plated on type I collagen-coated rigid dishes or type I collagen coated polyacrylamide gels 24 h prior to experiments in DMEM medium supplemented with 10% fetal bovine serum and 10 mM HEPES. The Blebbistatin (Toronto Research Chemical, North York, On), ML7 and Y27632 (Sigma, Saint Louis, Mo), and Jasplakinolide (Calbiochem, San Diego, Ca) are diluted in the serum containing media at respective final concentrations of 50 μM, 25 μM, 50 μM, and 500 nM.

Polyacrylamide Gel Substrates

Polyacrylamide gels were prepared as previously described [1]. 0.2μm yellow-green fluorescent microspheres (Molecular Probe, Carlsbad, Ca) were embedded in the gels for traction detection; the gels were coated with type I collagen. The elastic Young moduli of the polyacrylamide gels used were 2 kPa (5% acrylamide and 0.05% bis-acrylamide), 3.5 kPa (5% acrylamide and 0.1% bis-acrylamide), 5 kPa (5% acrylamide and 0.15% bis-acrylamide) and 6.5 kPa (5% acrylamide and 0.2% bis-acrylamide) [2].

Magnetic Twisting Cytometry and Microscopy

The technique of magnetic twisting cytometry has been previously described [3]. An RGD-coated ferromagnetic bead of 4.5 μm diameter bound to the apical surface of BHK cells was submitted to an oscillatory magnetic field of 25, 50 and 75 Gauss (8.7, 17.5 and 26.2 Pa stress respectively) at 0.8 Hz to cells of podosome α-Actinin rings or of 17.5 Pa at 0.3 Hz to cells of podosome actin rings. Three cycles of stresses were applied (~4 s) for each stress magnitude and there was <1 min interval between stress applications. Although the duration of each applied stress is only a few sec and thus the endogenous podosome dynamics could be minimal within each stress application, we cannot rule out the potential dynamic changes of podosomes between different magnitudes of stress applications, which could result from stress-induced structural and biochemical changes in podosomes. To acquire α-Actinin or actin displacement images, the synchronized movements of the α-Actinin or the actin cytoskeleton to the applied oscillatory mechanical stresses were quantified using the synchronous detection method [4, 5]. This sensitive approach can quantify stress-induced cytoskeletal deformation to the resolution of ~4 nm [5] while eliminating contributions from spontaneous endogenous movements or stage drifts. The images are acquired using a 63x 1.32NA oil immersion objective.

Image Analysis

The optical flow of podosome rings was computed with FlowJ [6] using the algorithm of Lucas and Kanade with 2 images difference gradient method. The temporal flow was then converted in an 8-bit green-red image to visualize the disappearing and appearing fluorescent regions. The tractions exerted by the podosome rings during a time-lapse sequence on the polyacrylamide gels were determined through the displacement field of the gel substrate [1, 7]. Student T-test was used for statistical analysis.

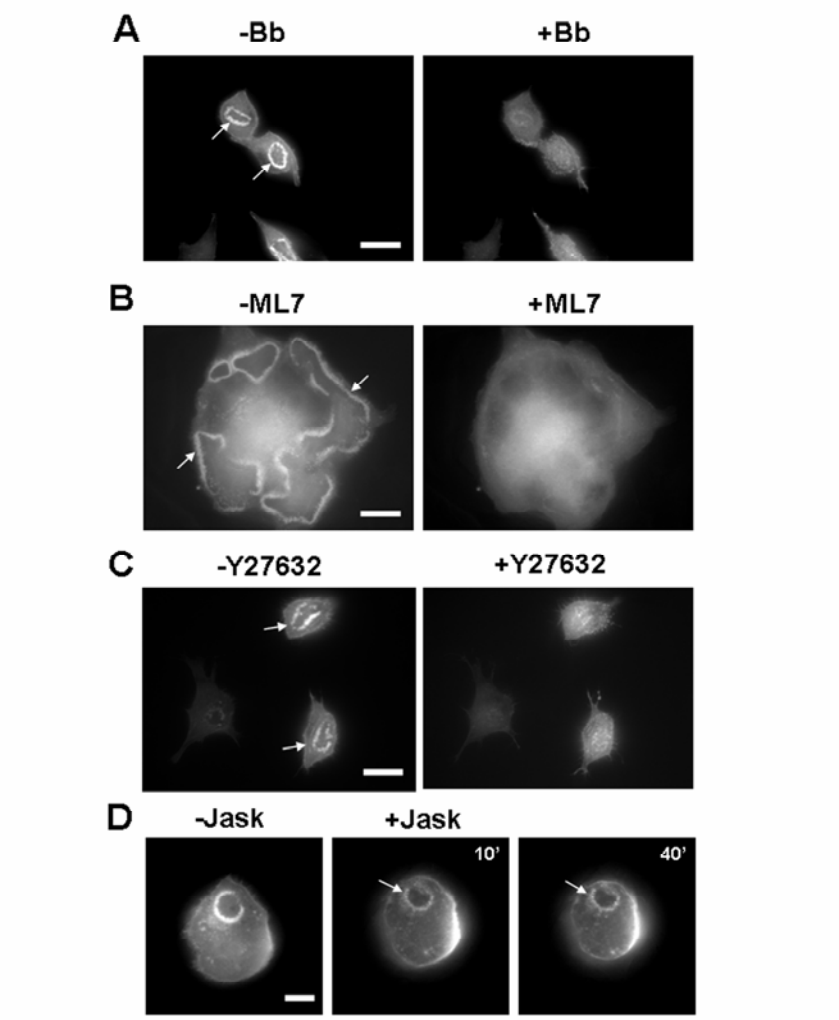

Figure S1. Myosins and Actin Dynamics Are Essential in Podosome Ring Formation

A representative BHK-RSV cell, transfected with mCherry-actin, exhibited either small podosome actin rings (see control image (-Bb) in (*A*), arrows) or larger bands of podosomes (see control image (-ML7) in (*B*), arrows) (Note that (*B*) is a relatively large cell). Inhibition of myosins by blebbistatin (+Bb, 50 μM for 8 min) (*A*) or myosin light chain kinase by ML7 (25 μM for 8 min) (*B*) or ROCK by Y27632 (50 μM for 20 min) (*C*) abolished pre-existing podosome rings and inhibited formation of new podosome rings. Three different other cells showed similar behaviors. Scale bars = 20 μm. (*D*) Stabilization of actin polymerization with Jasplakinolide (+Jask, 500 nM for up to 40 min) led to complete cessation of the actin ring dynamics (arrows). Two other cells showed similar effects. Scale bars = 10 μm.

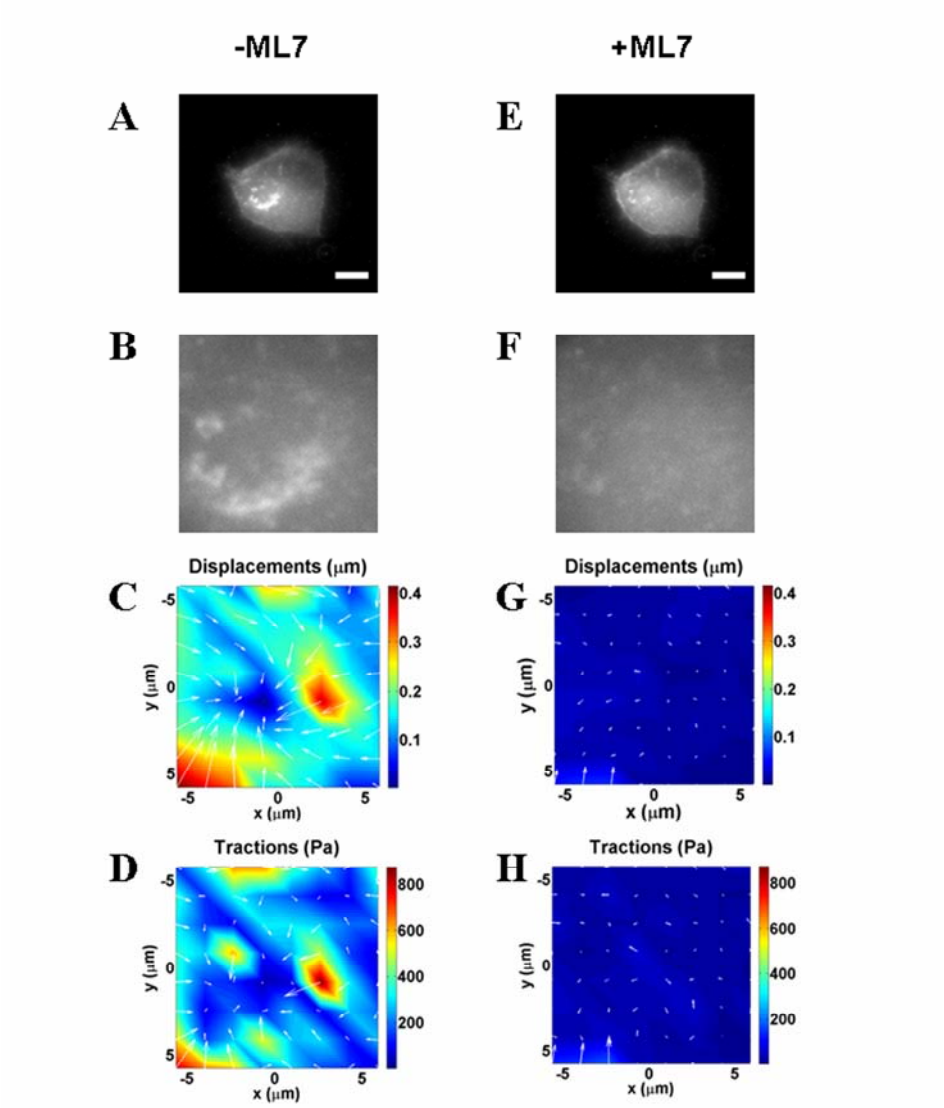

Figure S2. ML7 Dissipates the Podosome Ring and Inhibits Podosomes Tractions

A BHK Cell transfected with m-Cherry actin is plated on a 6.5 kPa polyacrylamide gel and displays a podosome ring (scale bar = 10 μm). (B) the cropped podosome ring exhibits a contraction during a 4-minute period that leads to substrate deformations up to 0.4 μm (C), and tractions up to 800 Pa (D). (E) Treating the same cell with ML7 (25μM) results in the disappearance of the podosome ring within 10 minutes (F). (G) The disappearance of the podosome ring corresponds to a dramatic decrease of the substrate deformation and (H) a disappearance of the tractions. Note the some large displacements and large tractions at the lower left are from contributions outside the podosome ring.

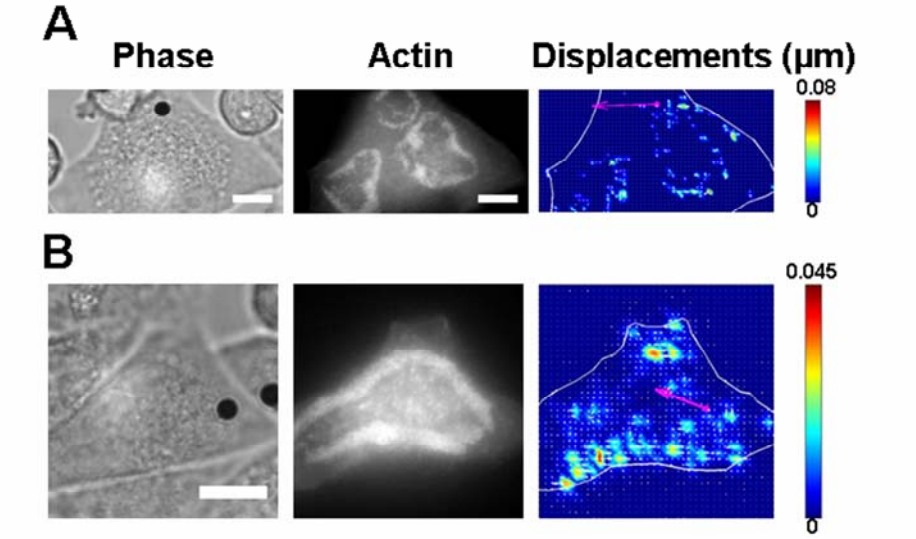

Figure S3. Applied Stresses Are Transmitted to Podosome Rings via the Cytoskeleton

(*A*) An RGD-coated 4.5µm ferromagnetic bead (black dot at top center) was bound to the apical surface of a mCherry-actin BHK cell. The cell exhibited 3 podosome rings at the basal face (Actin). Direct deformations of podosome rings were quantified (maximum up to 0.08 µm was observed) in response to a local stress of 17.5 Pa (at 0.3 Hz). (*B*) A cell displayed a large podosome band at its periphery. A local stress of 17.5 Pa (at 0.3 Hz) resulted in punctual deformations (up to 0.045 µm) at the external side of the podosome band. The pink arrow represents direction of the magnetic bead center displacement and white arrows represent induced podosome displacements. These podosome deformations were synchronized with the applied stress, and thus were not due to endogenous movements of the podosomes. Scale bar=20 µm.