Supplementary information for

Forces and constraints controlling podosome assembly and disassembly

Nisha Mohd Rafiq[1,2], Gianluca Grenci[1,3], Cheng Kai Lim[1], Michael M. Kozlov[4], Gareth E. Jones[2], Virgile Viasnoff[1,5,6] and Alexander D. Bershadsky[1,7]

[1]Mechanobiology Institute, National University of Singapore, Singapore 117411, Singapore

[2]Randall Centre for Cell and Molecular Biophysics, King's College London, London SE1 1UL, UK

[3]Biomedical Engineering Department, National University of Singapore, 4 Engineering Drive 3, Singapore 117583, Singapore

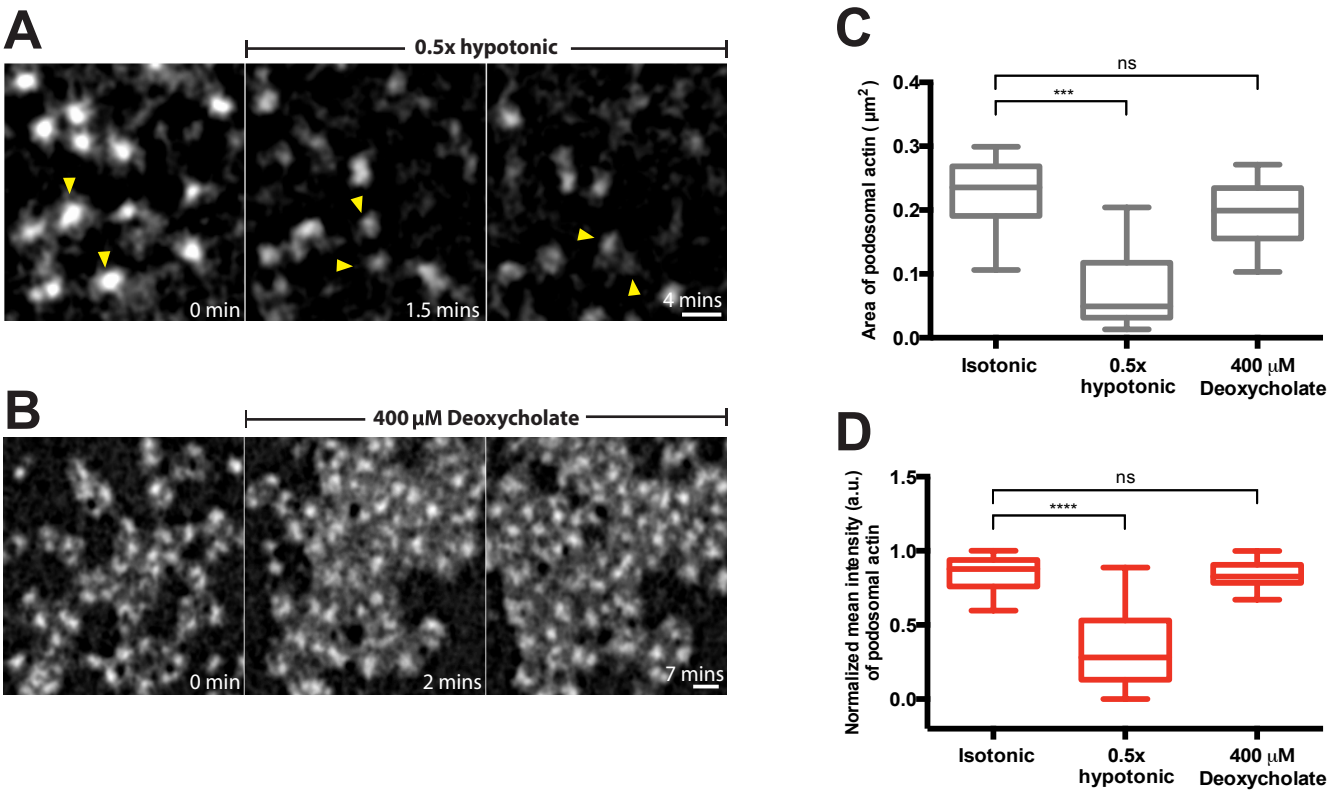
[4]Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel Aviv University, 69978 Tel Aviv, Israel

[5]CNRS UMI 3639, 5A Engineering Drive 1, 117411 Singapore, Singapore

[6]Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117543, Singapore

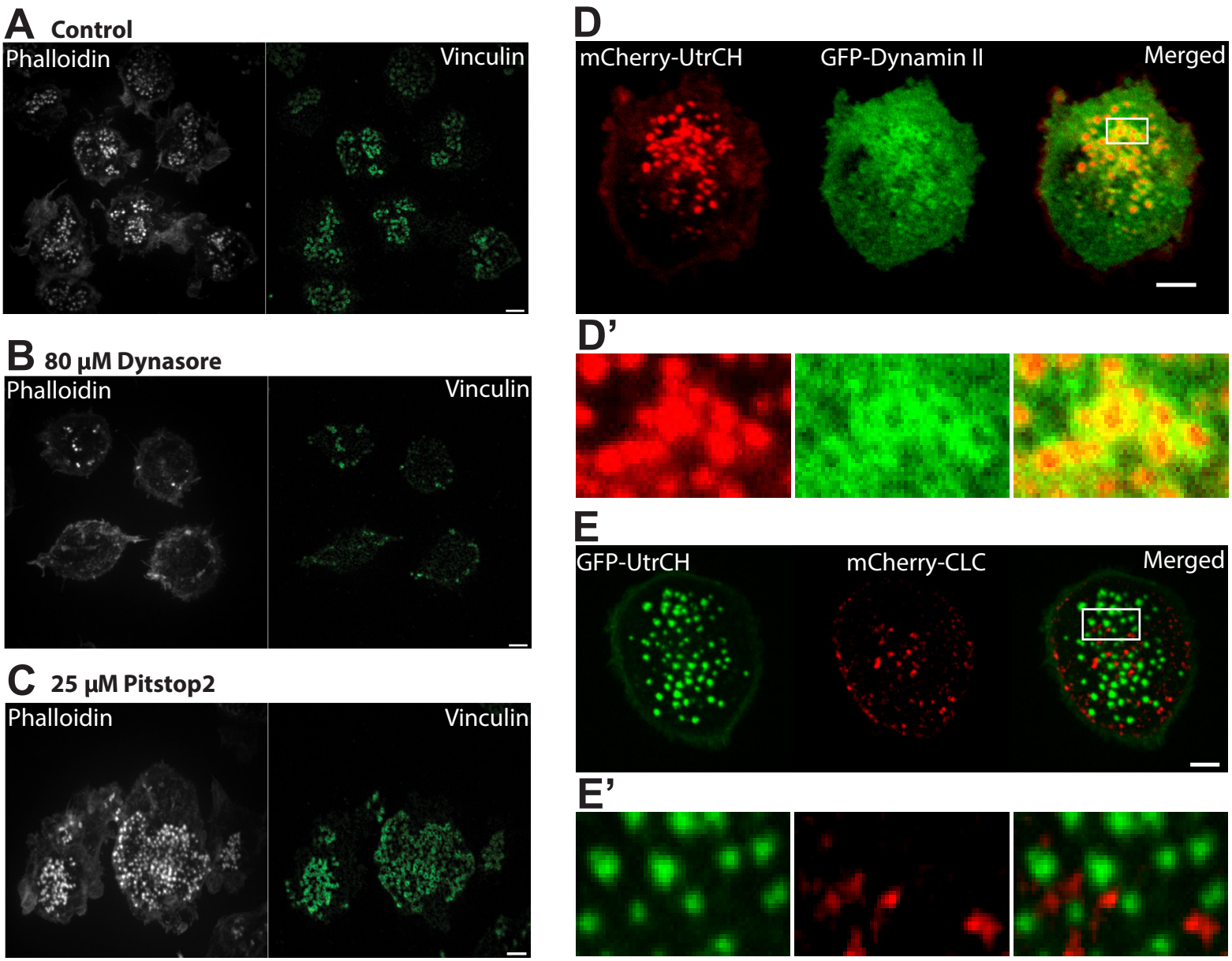
[7]Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot 76100, Israel

Table of Contents:



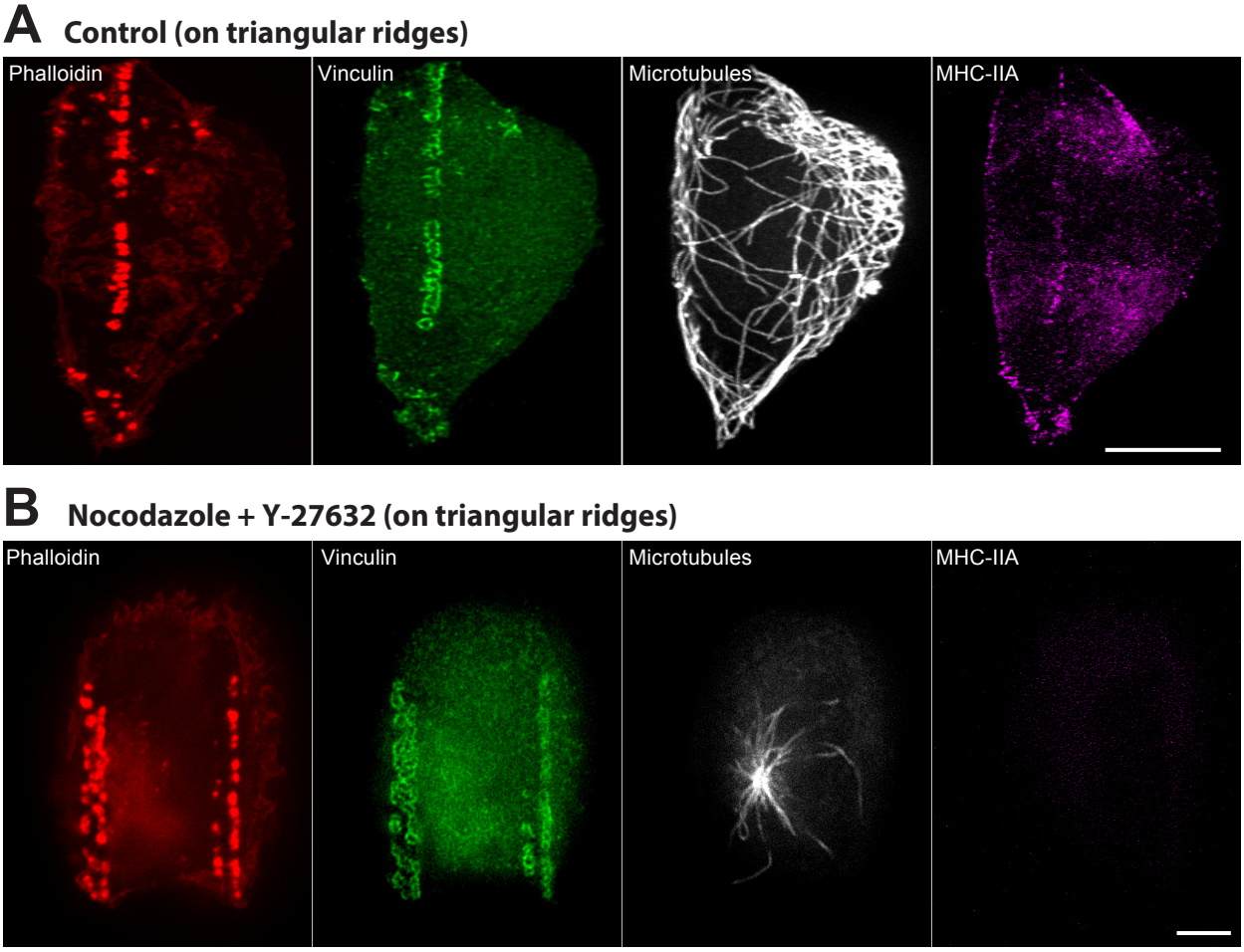
Supplementary Figure 1: Comparison of the effects of osmotic swelling and deoxycholate treatment on podosomes

(A and B) High magnification time-lapse sequences showing evolution of podosomes in cells transfected with GFP-β-actin incubated with 0.5x hypotonic medium (A) or 400 µM deoxycholate (B). The image sequences are taken from Movie 2 and 5, respectively. Two individual podosomes in (A) are indicated by yellow arrowheads to better follow their fate. Scale bars, 1 µm. Podosome area (C) and mean actin fluorescence intensity (D) in control cells, and in cells treated with 0.5x hypotonic medium and 400 µM deoxycholate. The data are presented as a box-and-whiskers plots. At least 20 podosomes per cell were analyzed for each group. The significance of the difference between groups was estimated by two-tailed Student's t-test, the range of p-values >0.05(non-significant), ≤ 0.05, ≤0.01, ≤0.001, ≤ 0.0001 are denoted by "ns", one, two, three and four asterisks (*), respectively. All images were taken using structured-illumination microscopy (SIM).



Supplementary Figure 2: Dynamin-II but not clathrin is required for podosome integrity

Images of cells treated with (A) 0.1% DMSO, (B) 80 µM dynasore, (C) 25 µM Clathrin inhibitor Pitstop®2 for 1 hour. Actin cores of podosomes were labeled with phalloidin (white) and podosome adhesive rings with antibody to vinculin (green). Inhibition of dynamin but not clathrin resulted in podosome disassembly. (D, E) Dynamin-II but not clathrin localized to podosomes. The cells were transfected (D) with mCherry-UtrCH (red) and GFP-Dynamin-II (green) or (E) with GFP-UtrCH (green) and mCherry-clathrin light chain (red). UtrCH labeles F-actin podosome cores. Note that dynamin-II surrounds the podosome cores (merged image in D), while clathrin patches did not colocalize with podosomes (merged image in E). Scale bars, 5 µm. High magnification images of the boxed areas in D and E are shown in D' and E', respectively. All images were taken using spinning disk confocal microscopy.



Supplementary Figure 3: Alignment of podosomes along the topographical cues does not depend on microtubules or myosin-II filaments

(A) Alignment of podosomes in control cell plated on the PDMS fibronectin-coated substrate with parallel triangular ridges. (B) Cell on the same substrate treated with 30μM Y-27632 for 30 minutes followed by addition of 1 μM nocodazole for additional 30 minutes. Note that podosomes are still aligned along the ridges in cell having few microtubules and lacking myosin-II filaments. Actin podosome cores were visualized by phalloidin (red); podosome rings (green), microtubules (white) and myosin-IIA filaments (purple) were visualized by immunofluorescence staining of vinculin, α-tubulin and myosin-IIA heavy chain, respectively. Scale bars, 5 μm. All images were taken using structured-illumination microscopy (SIM).

Supplementary Movie legends

Supplementary Movie 1

Disruption of podosomes labeled by RFP-lifeact (left, red) in THP1 cell plated on micropatterned circular fibronectin-coated islands (4 μm diameter) upon treatment with the RhoA activator CN03 (0.1 μg/ml). Local podosome disassembly was accompanied by a massive burst of myosin-II filament assembly in each individual islands, as visualized by GFP-MRLC (right, green). Structured-illumination microscopy (SIM) was used. Single plane close to the substrate is shown. The frames were recorded at 30-second intervals over a period of 15 minutes. Display rate is 10 frames/sec. The movie corresponds to the time-lapse series shown in Figure 1D. Scale bar, 5 μm.

Supplementary Movie 2

Dispersion and subsequent disassembly of podosomes labeled by GFP-β-actin in THP1 cell exposed to 0.5x hypotonic medium for 15 minutes. Single plane close to the substrate is shown. Structured-illumination microscopy (SIM) was used. The frames were recorded at 10-second intervals over a period of 15 minutes. Display rate is 10 frames/sec. The movie corresponds to the time-lapse series shown in Figure 2F. Scale bar, 5 μm.

Supplementary Movie 3

Inhibition of Rho kinase by 30 μM Y-27632 in THP1 cell did not prevent the disruptive effect of 0.5x hypotonic shock on podosomes labeled by GFP-β-actin. The imaging started at the 30th minute after addition of Y-27632 and was continued for another 30 minutes in the presence of both Y-27632 and 0.5x hypotonic medium. Structured-illumination microscopy (SIM) was used. Single plane close to the substrate is shown. The frames were recorded at 30-second intervals over a period of 30 minutes. Display rate is 10 frames/sec. The movie corresponds to the time-lapse series shown in Figure 2G. Scale bar, 5 μm.

Supplementary Movie 4

Incubation of mouse fibroblast in 0.5x hypotonic medium did not affect focal adhesions visualized by mCherry-vinculin. The frames were recorded at 1-minute intervals over a

period of 30 minutes using confocal microscopy. Single plane close to the substrate is shown. Display rate is 5 frames/sec. The movie corresponds to the time-lapse series shown in Figure 2H. Scale bar, 10 μm.

Supplementary Movie 5

Addition of 400 μM deoxycholate induced transient disassembly of podosomes and subsequent formation of podosome clusters in THP1 cell. Podosomes were labeled by GFP-β-actin. Single plane close to the substrate is shown. The frames were recorded at 30-second intervals over a period of 30 minutes using SIM. Display rate is 10 frames/sec. The movie corresponds to the time-lapse series shown in Figure 3F. Scale bar, 5 μm.

Supplementary Movie 6

The movie shows the recovery of uniform podosome distribution in cells incubated in 400 μM deoxycholate-containing medium. The cell was labeled by GFP-β-actin. The imaging started at the 30th minute after addition of deoxycholate and was continued for another 30 minutes in the presence of deoxycholate. Single plane close to the substrate is shown. The frames were recorded at 15-second intervals over a period of 30 minutes using SIM. Display rate is 10 frames/sec. The movie corresponds to the time-lapse series shown in Figure 3G. Scale bar, 5 μm.