Supplementary Information

Acoustic tweezing cytometry for live-cell subcellular modulation of intracellular cytoskeleton contractility

Zhenzhen Fan[1,*], Yubing Sun[2,*], Di Chen[1,*], Donald Tay[1],
Weiqiang Chen[2], Cheri X. Deng[1], and Jianping Fu[1,2]

[1]Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI, 48109, USA;
[2]Department of Mechanical Engineering, University of Michigan, Ann Arbor, MI, 48109, USA.

* These authors contributed equally to this work.

Correspondence and requests for materials should be addressed to Cheri X. Deng (email: cxdeng@umich.edu) or Jianping Fu (email: jpfu@umich.edu).

Supplementary Information:

SUPPLEMENTARY FIGURE CAPTIONS

Supplementary Fig. S1. A schematic showing integration of acoustic tweezing cytometry with the PDMS micropost array. Lipid microbubbles were mixed with biotinylated RGD peptides before added to cell culture dish where single cells were seeded on the PDMS micropost array. The culture dish holding the PDMS micropost array was then flipped upside down to allow bubbles to float up towards cells to facilitate binding of microbubbles to cell membrane. Unbound bubbles were removed by a gentle wash with culture media.

Supplementary Fig. S2. Temporal evolution of total cytoskeleton contractile force (normalized to contractility before US applications) for individual NIH/3T3 fibroblasts (thin grey lines) and population means (colored lines with symbols). The cells were treated under different US conditions as indicated. For A & B, individual single NIH/3T3 fibroblasts was attached with one microbubble and was exposed to 10 sec (A) or 20 sec (B) US stimulations (A: $n = 31$; B: $n = 10$). For C & D, cells without microbubbles attached were treated with (C) or without (D) 10 sec US stimulations (C: $n = 8$; D: $n = 6$). US was applied at $t = 0$ min, with a peak negative pressure amplitude of 0.05 MPa, PRF of 1 Hz, and duty cycle of 5%. Data plotted in A-D using colored lines with symbols are means ± s.e.m.

Supplementary Fig. S3. (A) Brightfield and colorimetric images showing individual hMSCs and their subcellular cytoskeleton contractile force maps before ($t < 0$ min) and after 10 sec stimulation with acoustic tweezing cytometry. Scale bar, 10 μm. (B-D) Temporal evolution of total cytoskeleton contractile force (normalized to contractility before US applications) for individual hMSCs (thin grey lines) and population means (colored lines with symbols). hMSCs

were treated under different conditions as indicated. For B, individual hMSCs was attached with one microbubble and was exposed to 10 sec US stimulation (n = 8). Control groups without MBs attached and treated with (C; n = 3) or without (D; n = 3) US were included for comparison. US was applied at t = 0 min, with a peak negative pressure amplitude of 0.05 MPa, PRF of 1 Hz, and duty cycle of 5%. Data plotted in B-D using colored lines with symbols are means ± s.e.m.

Supplementary Fig. S4. Dependence of subcellular distribution of contractile force increase on MB attachment location. (A&B) Colorimetric maps showing summation of contractile force increase for individual NIH/3T3 fibroblasts, with MB on each cell aligned at the same location (black circle). For A (n = 15), MB was located within the outer 1/3 region to the cell periphery. For B (n = 16), MB was located within the cell interior region. (C) Temporal evolution of total cytoskeleton contractile force (normalized to contractility before US excitation) for the two groups of cells where MBs were located either close to cell periphery (*blue*) or within the cell interior region (*red*).

Supplementary Fig. S5. Dynamic regulation of cytoskeleton contractility by acoustic tweezing cytometry was ROCK dependent and required intact actin cytoskeleton and actomyosin activity. (A-C) Representative brightfield images (left) showing single NIH/3T3 fibroblasts with (bottom panel) or without (top panel) one MB (arrow head) attached to cell membrane, with the cells attached with MBs treated with 10 sec US stimulation at t = 0 min. Pharmacological drugs were added to culture media 30 min before US application (t = -30 min): Cytochalasin D (40 μM; A), Blebbistatin (100 μM; B), and Y-27632 (10 μM; C). Corresponding colorimetric maps of subcellular contractile force distribution right before drug treatments (t = -30 min), 1 min before

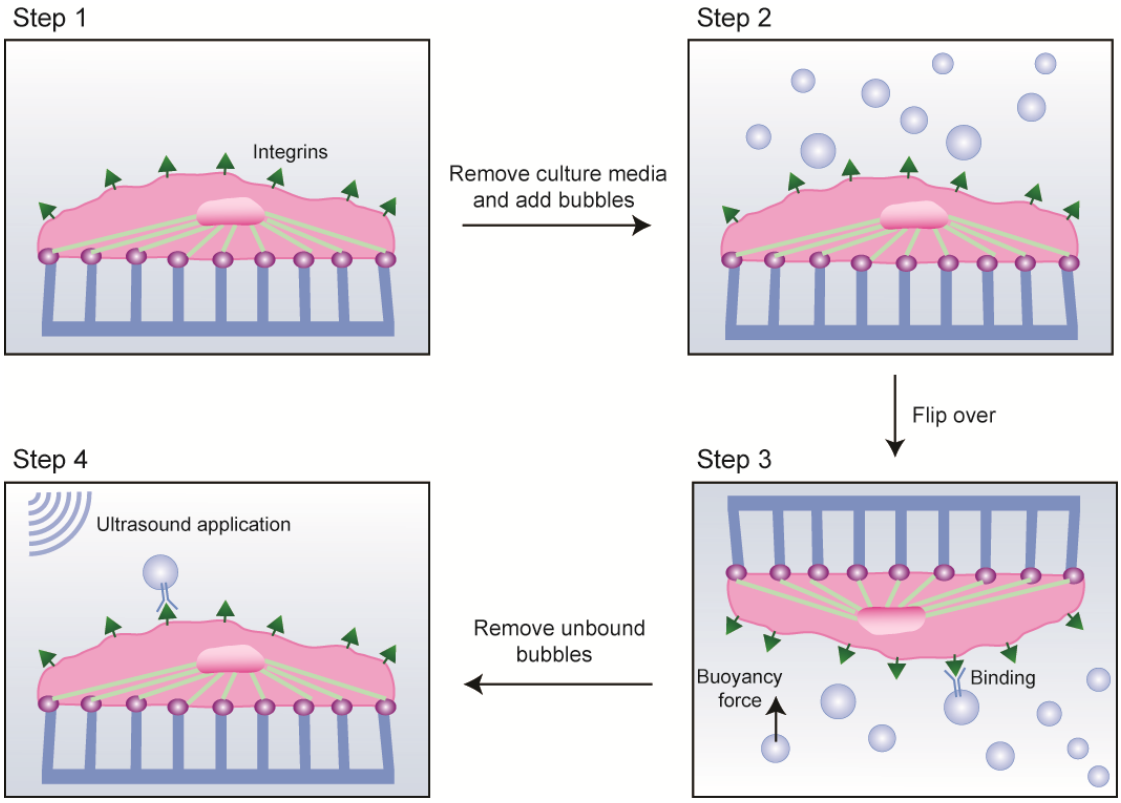
US application (t = -1 min), and 30 min after US application (t = 30 min) were included as indicated. The US pulses with a center frequency of 1.25 MHz had a peak negative pressure of 0.05 MPa, PRF of 1 Hz, and duty cycle of 5%. Scale bars in A-C, 10 μm. (D-F) Evolution of total cytoskeleton contractile force (normalized to contractility prior to drug treatments) for single NIH/3T3 fibroblasts before and after US treatments. Cells were treated with Cytochalasin D (D), Blebbistatin (E), and Y-27632 (F) at t = -30 min. US stimulation was applied at t = 0 min. Data in D-F are shown as mean ± s.e.m. ($n \geq 4$).

SUPPLEMENTARY VIDEO CAPTION

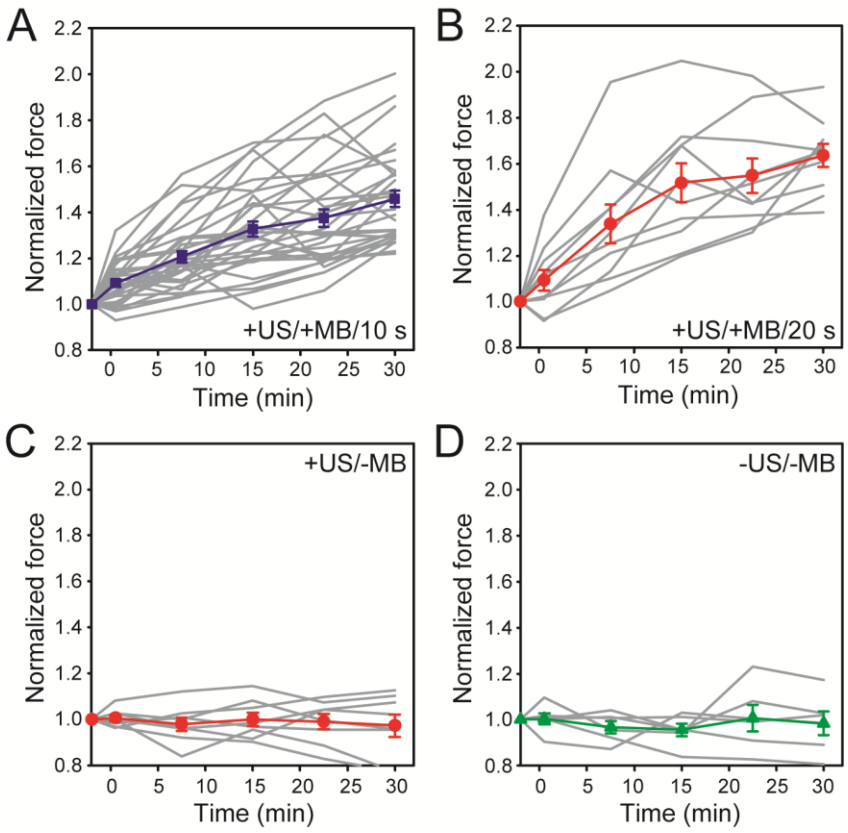
Supplementary Video 1. Video showing translational displacement of one MB attached to a single NIH/3T3 fibroblast during the 10-sec US stimulation. US was applied at t = 0 min, with a peak negative pressure amplitude of 0.05 MPa, PRF of 1 Hz, and duty cycle of 5%. Scale bars, 12 μm.

SUPPLEMENTARY FIGURES

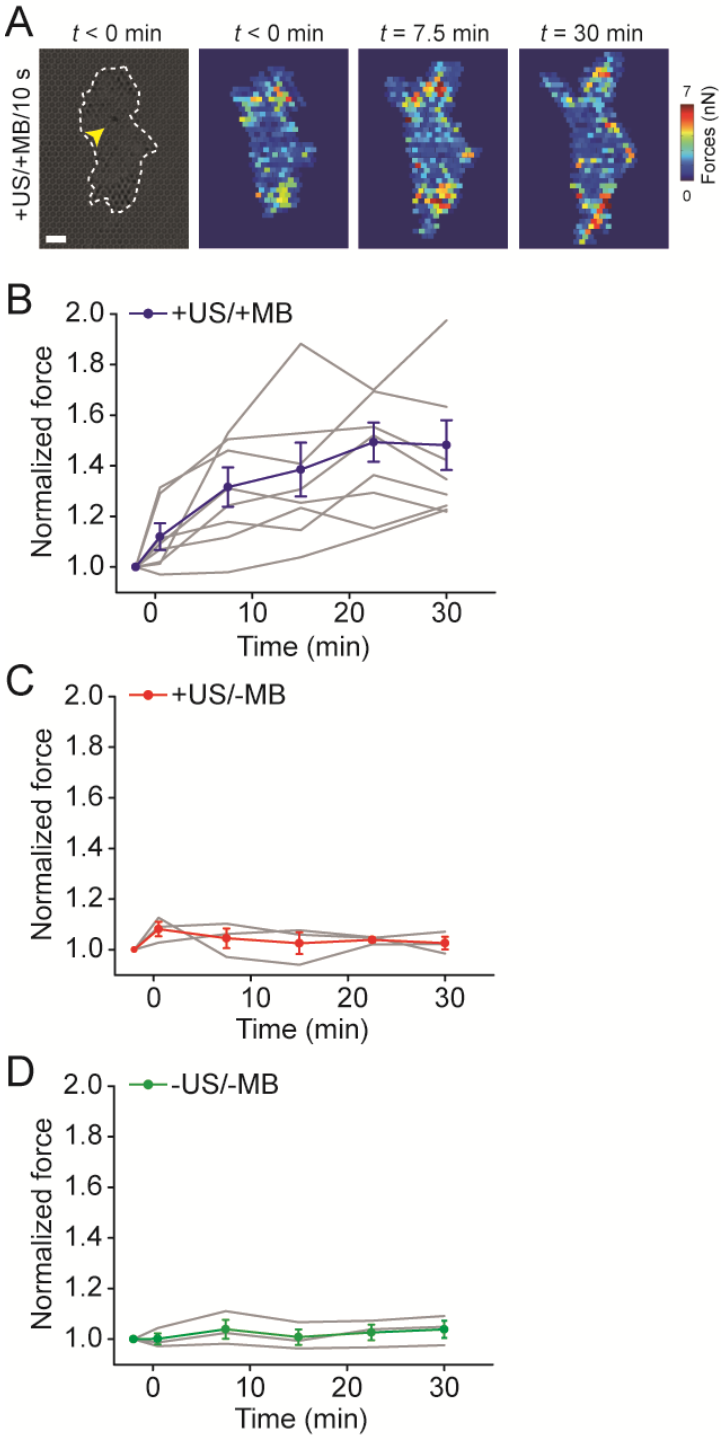
Supplementary Figure 1



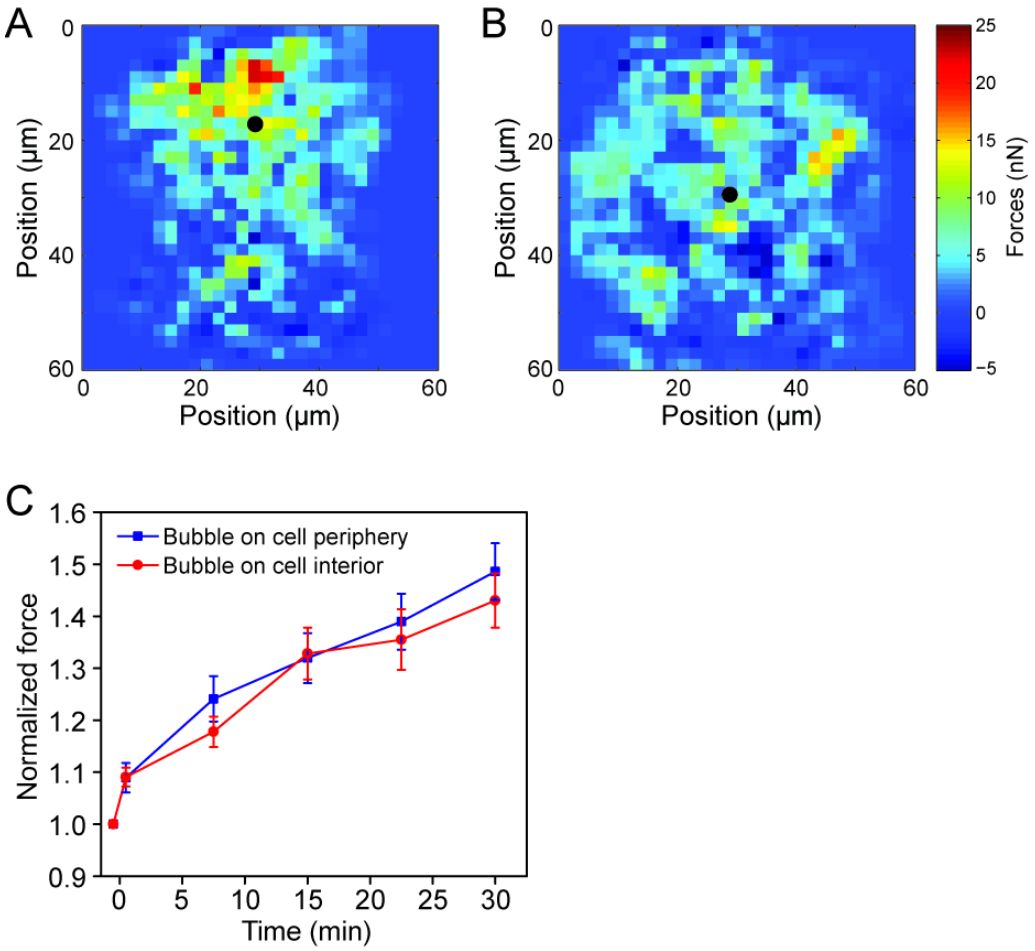
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5