Electronic Supplementary Material (ESI) for Integrative Biology.

Supplementary information

Figure S1

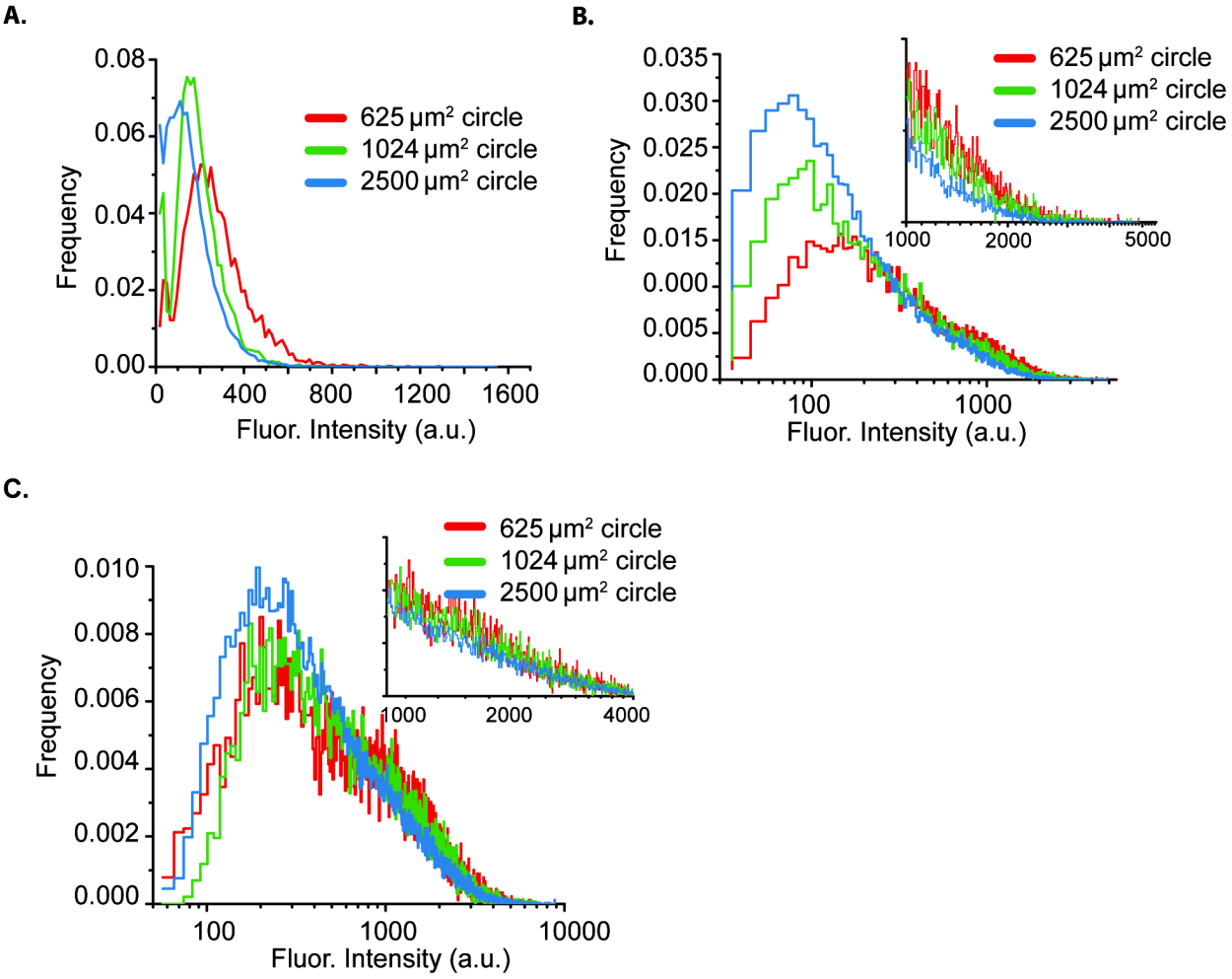

Figure S1. Intensity distribution of EGFP-clathrin light chain (EGFP-CLC) and immunostained adaptor protein 2 (AP2). A) EGFP-CLC structures were imaged by spinning disk confocal microscopy and detected in fixed cells using Gaussian mixture model fitting algorithm and plotted for their intensity distribution. We used Kolmogorov-Smirnov goodness-of-fit hypothesis test (KS test) to determine whether intensity distributions for three sized cells fall on the same distribution. KS test rejects the null hypothesis (intensity distributions for any two sized cells fall on the same distribution) at 0.00001 confidence level for any two distributions (625 μm^2 vs. 1024 μm^2, 625 μm^2 vs. 2500 μm^2 and 1024 μm^2 vs. 2500 μm^2 circular cells). N = 11, 11, 12 for 625 μm^2, 1024 μm^2, and 2500 μm^2 circular cells respectively. (B) Immunostaining of AP2 structures marked by AP6 antibodies were imaged by TIRF microscopy and detected using Gaussian mixture model fitting algorithm. Due to the large range of intensity, we used $\log_{10}$ scale for the x axis and plotted the large intensity range in the inset. KS test is performed to determine whether intensity distributions for the three sized cells fall on the same distribution. KS test rejects the null hypothesis (intensity distributions for any two sized cells fall on the same

distribution) at 0.00001 confidence level for any two distributions (625 μm^2 v.s. 1024 μm^2, 625 μm^2 v.s. 2500 μm^2 and 1024 μm^2 v.s. 2500 μm^2 circular cells). N = 17, 12, 10 for 625 μm^2, 1024 μm^2, and 2500 μm^2 circular cells respectively. C) mCherry-CLC structures were imaged by TIRF microscopy and detected in fixed cells using Gaussian mixture model fitting algorithm and plotted for their intensity distribution. KS test rejects the null hypothesis (intensity distributions for any two sized cells fall on the same distribution) at 0.00001 confidence level for any two distributions (625 μm^2 v.s. 1024 μm^2, 625 μm^2 v.s. 2500 μm^2 and 1024 μm^2 v.s. 2500 μm^2 circular cells). N = 17, 15, 16 for 625 μm^2, 1024 μm^2, and 2500 μm^2 circular cells respectively.

Figure S2

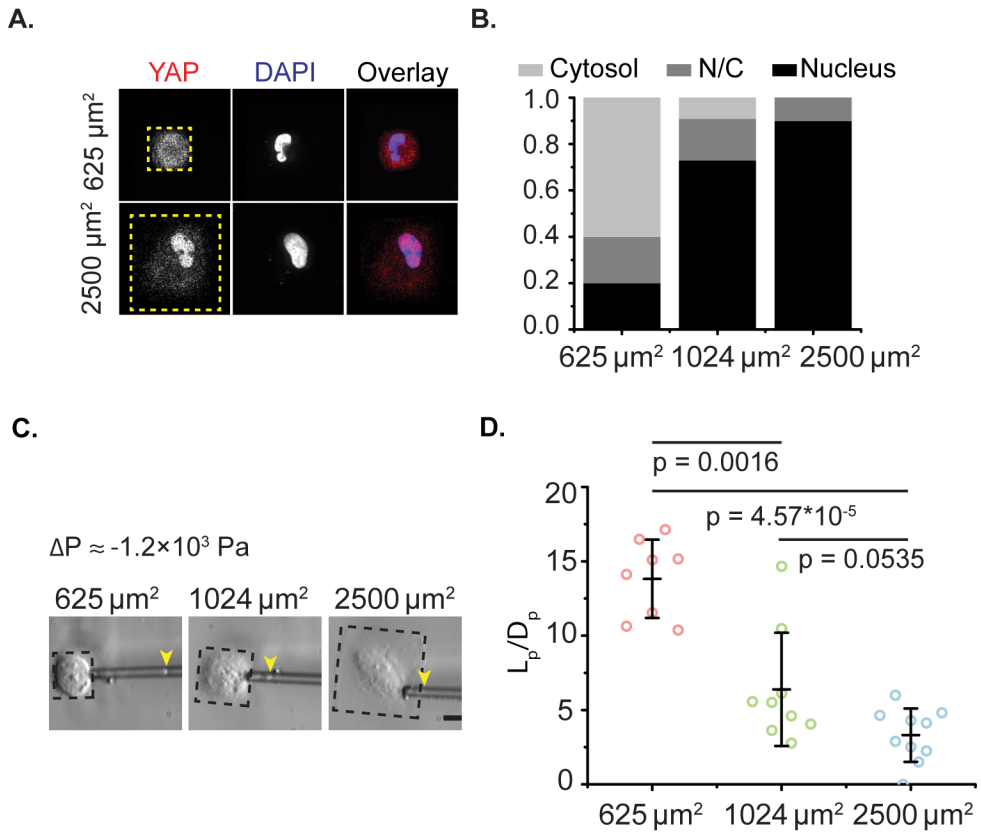

Figure S2. Tension increase with increasing cell spreading area. (A) Fluorescent images of immunostained YAP, nucleus (DAPI) for 625 μm^2 and 2500 μm^2 square cells (top). Yellow dotted lines show the outline of the cell. (B) Proportion of the cells displaying nuclear YAP localization (nucleus, black), even distribution of YAP/TAZ in nucleus and cytosol (N/C, dark grey), or cytoplasmic YAP (cytosol, light grey) for 625 μm^2, 1024 μm^2 and 2500 μm^2 square cells. Number of cells for each pattern = 10 - 13. (C) Brightfield images of micropipette aspirated cells on different cell spreading area with ~1.2 kPa suction pressure (left). Yellow arrows point to plasma membrane projection in the micropipette. (D) Graph of L_p/D_p for aspirated cells with an aspiration pressure of ~1.2 kPa (right). Each data set was represented as average +/- S.D.. *p < 0.05 by Wilcoxon rank sum test due to the non-normality of data of 1024 μm^2 cells.

Figure S3

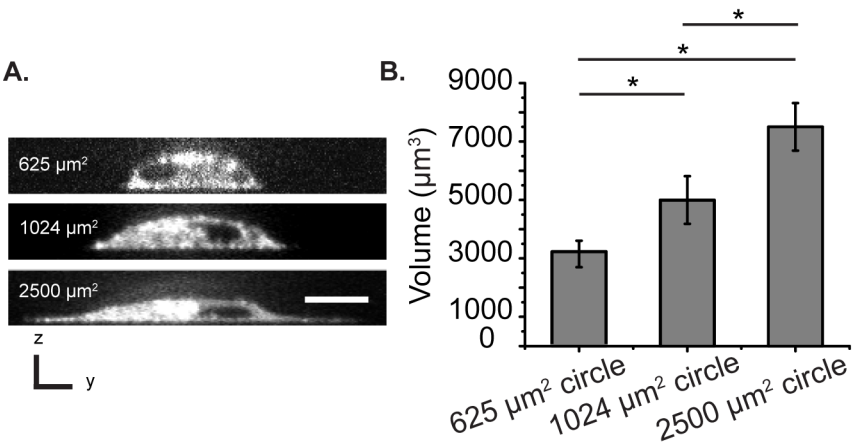

Figure S3. Cell volume increases with spreading area. (A) Side view of representative micropatterned cells. Scale bar, 10 μm. (B) Cell volume for 625 μm^2, 1024 μm^2, and 2500 μm^2 circular cells. Error bars, standard deviation. N = 11, 8, 7 for 625 μm^2, 1024 μm^2, and 2500 μm^2 circular cells respectively. *$p < 0.0002$ by Student's t test.

Supplementary methods

Quantification of static AP2 and clathrin light chain a intensity histograms. To quantify AP2 intensity histogram, alpha adaptin, a subunit of AP2, was immunostained and observed it using TIR-FM as described in Materials and Method. MDA-MB-231 cells expressing stable mCherry-clathrin light chain a (CLCa) were generated by retroviral transduction followed by fluorescence-activated cell sorting. Cells were fixed similarly to RPE cells and imaged by TIR-FM. To obtain CLCa intensity histogram, we fixed patterned EGFP-CLCa RPE cells with 4% PFA for 20 min, and then observed using an Olympus-IX81 microscope with a spinning disk confocal scanner unit. For both sets of images, the fluorescence structures were quantified using Gaussian mixture model fitting. The MATLAB program (MathWorks, Natick, Massachusetts) is part of single particle tracking software described previously [1]. The underlying algorithm is to iteratively fit each fluorescent structure with 2D Gaussian functions, until there is no statistical justification for adding another Gaussian function. The fluorescence intensity were extracted for all the detected structures in a group of cells with same size, and histograms with 500 bins were plotted for intensity of AP2 and 100 bins for intensity of EGFP-CLCa.

Micropipette aspiration and analysis. A homebuilt micropipette aspiration system with a graduated manometer was used for application of controlled suction pressure onto living cells. RPE cells were allowed to spread to patterned substrates onto 625 μm^2, 1024 μm^2, and 2500 μm^2 sized square patterns as described previously. Patterned substrates were integrated into a custom aspiration chamber and filled with culture medium. Glass micropipettes with inner diameters of 2-3 μm were filled with 0.2% bovine serum albumin (BSA) in 1X phosphate buffered saline (PBS) in order to allow smooth movement of the cell membrane inside the pipette. Negative pressure in the micropipette tip was generated by aspirating water from the manometer reservoir and increased gradually in -200 Pa increments. Nikon Advanced Modulation Contrast optics (NAMC) mounted on a Nikon Ti-S microscope and CoolSnap MYO

CCD camera (Photometrics, Tucson, AZ) were used to acquire live-cell brightfield images. ImageJ (http://rsb.info.nih.gov/ij/) software was used to manually measure the pipette diameters (D_p) and plasma membrane projection length within the pipette (L_p) at 1.2 kPa negative pressure for 8-10 cells per pattern size.

Cell volume calculation. Z-stacks of images for micropatterned cells was acquired using an Olympus-IX81 microscope with spinning disk confocal scanner unit (CSU-X1; Yokogawa), EMCCD camera (iXon X3; Andor), 60x objective (NA = 1.42). Depending on the height of the cell, 15 to 30 sections at 0.5 μm steps were obtained. 3D reconstruction was performed using Fiji (http://fiji.sc/Fiji) to generate the y-z projection of the cell. To calculate cell volume, the areas from all the sections of a cell were integrated by stack step.

Reference

1. K. Jaqaman, D. Loerke, M. Mettlen, H. Kuwata, S. Grinstein, S. L. Schmid and G. Danuser, *Nat Methods*, 2008, **5**, 695-702.