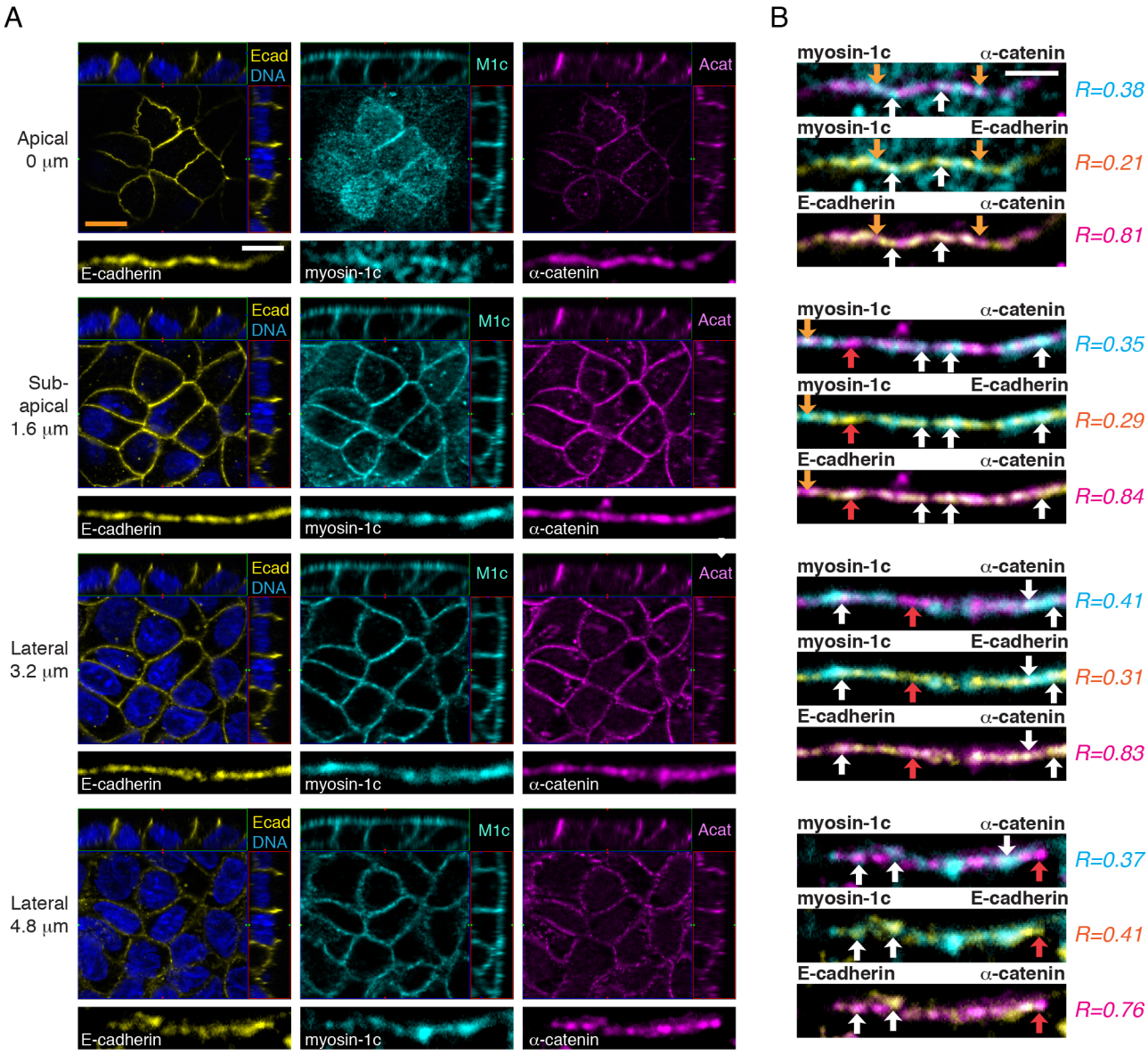
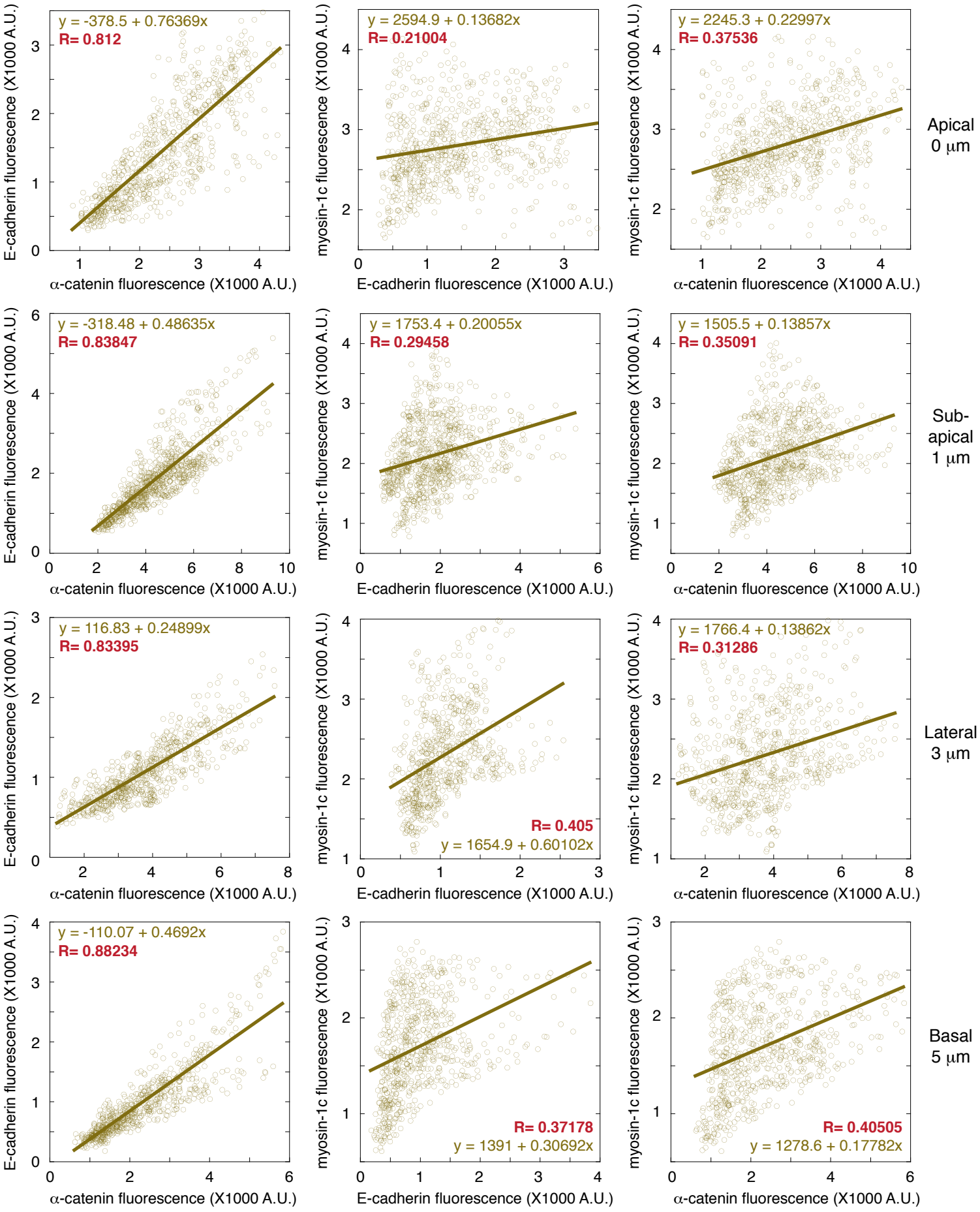
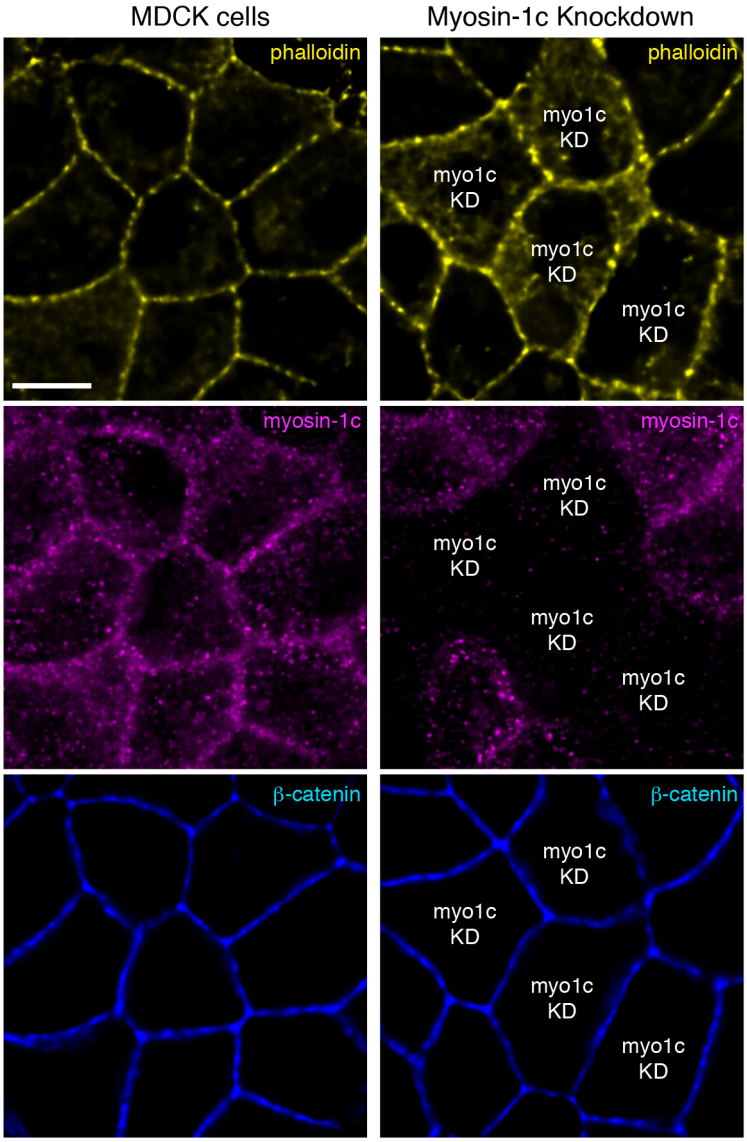


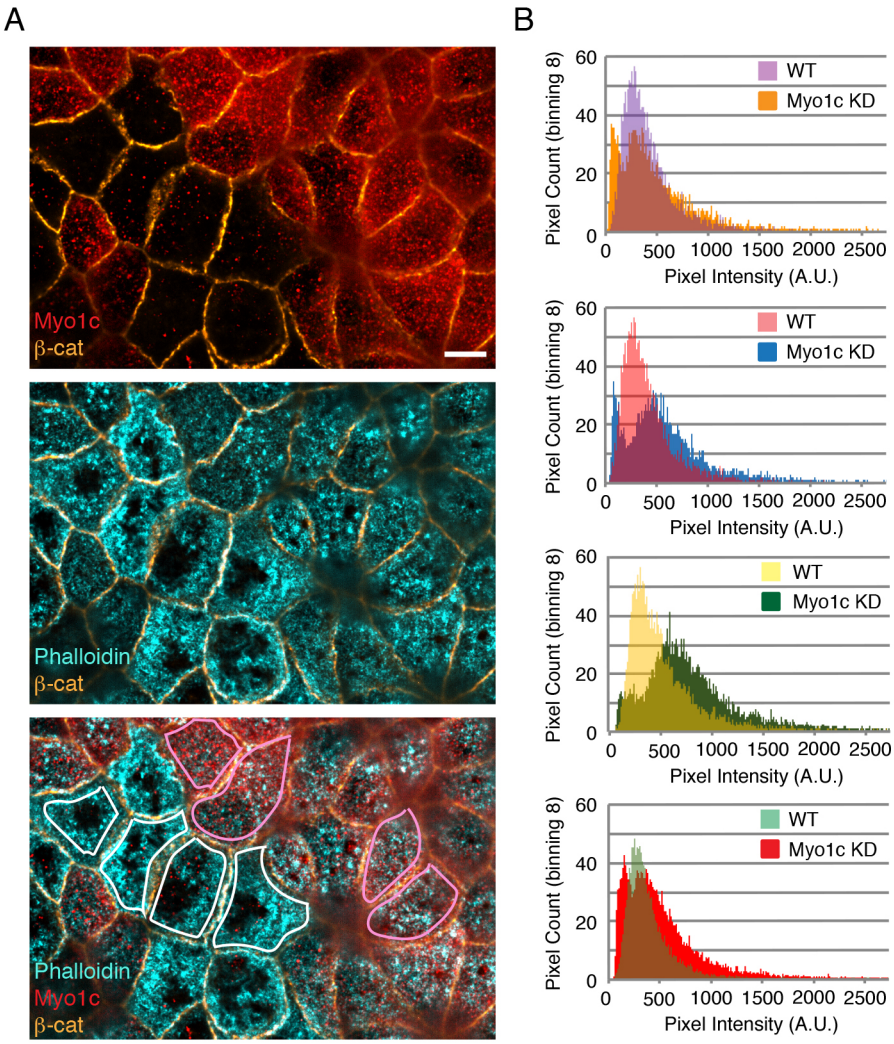
Supplementary Figure 1. Myosin-1c overlaps with α-catenin and E-cadherin at discreet regions along the lateral junction in polarized epithelial monolayer. (A) Optical sectioning structured illumination microscopy showing staining of myosin-1c (M1c), α-catenin (Acat), and E-cadherin (Ecad) at the apical (Z=0 μm), the sub-apical (Z=1.6 μm), and the lateral (Z=3.2 and 4.8 μm) junctions. For each Z-image, the top panel shows the X-Z, Y-Z, and X-Y images and the bottom panel shows a representative linear junction from the X-Y-image. Orange scale bar is 10 μm and white scale bar is 2 μm. Data is representative of 10 sets of images (N=10) from one experiment out of 3 independent experiments (N=3). (B) Merge images of the representative linear junction shown in A. White arrows point to colocalization of myosin-1c, α-catenin, and E-cadherin. White arrows point to colocalization of myosin-1c, α-catenin, and E-cadherin. Orange arrows point to colocalization of myosin-1c and α-catenin. Red arrows point to colocalization of α-catenin and E-cadherin. Pearson's correlation coefficient (R) between 2 proteins at the linear junction was calculated from 1000-1500 pixels per protein per z-image per one set of data (see Figure S8). Data set is representative of 6 sets of data (N=6) from one experiment out of 3 independent experiments (N=3). Scale bar is 2 μm.


y = -378.5 + 0.76369x
R= 0.812
E-cadherin fluorescence (X1000 A.U.)
α-catenin fluorescence (X1000 A.U.)
y = 2594.9 + 0.13682x
R= 0.21004
myosin-1c fluorescence (X1000 A.U.)
E-cadherin fluorescence (X1000 A.U.)
y = 2245.3 + 0.22997x
R= 0.37536
myosin-1c fluorescence (X1000 A.U.)
α-catenin fluorescence (X1000 A.U.)
Apical
0 μm
y = -318.48 + 0.48635x
R= 0.83847
E-cadherin fluorescence (X1000 A.U.)
α-catenin fluorescence (X1000 A.U.)
y = 1753.4 + 0.20055x
R= 0.29458
myosin-1c fluorescence (X1000 A.U.)
E-cadherin fluorescence (X1000 A.U.)
y = 1505.5 + 0.13857x
R= 0.35091
myosin-1c fluorescence (X1000 A.U.)
α-catenin fluorescence (X1000 A.U.)
Sub-
apical
1 μm
y = 116.83 + 0.24899x
R= 0.83395
E-cadherin fluorescence (X1000 A.U.)
α-catenin fluorescence (X1000 A.U.)
R= 0.405
y = 1654.9 + 0.60102x
myosin-1c fluorescence (X1000 A.U.)
E-cadherin fluorescence (X1000 A.U.)
y = 1766.4 + 0.13862x
R= 0.31286
myosin-1c fluorescence (X1000 A.U.)
α-catenin fluorescence (X1000 A.U.)
Lateral
3 μm
y = -110.07 + 0.4692x
R= 0.88234
E-cadherin fluorescence (X1000 A.U.)
α-catenin fluorescence (X1000 A.U.)
R= 0.37178
y = 1391 + 0.30692x
myosin-1c fluorescence (X1000 A.U.)
E-cadherin fluorescence (X1000 A.U.)
R= 0.40505
y = 1278.6 + 0.17782x
myosin-1c fluorescence (X1000 A.U.)
α-catenin fluorescence (X1000 A.U.)
Basal
5 μm

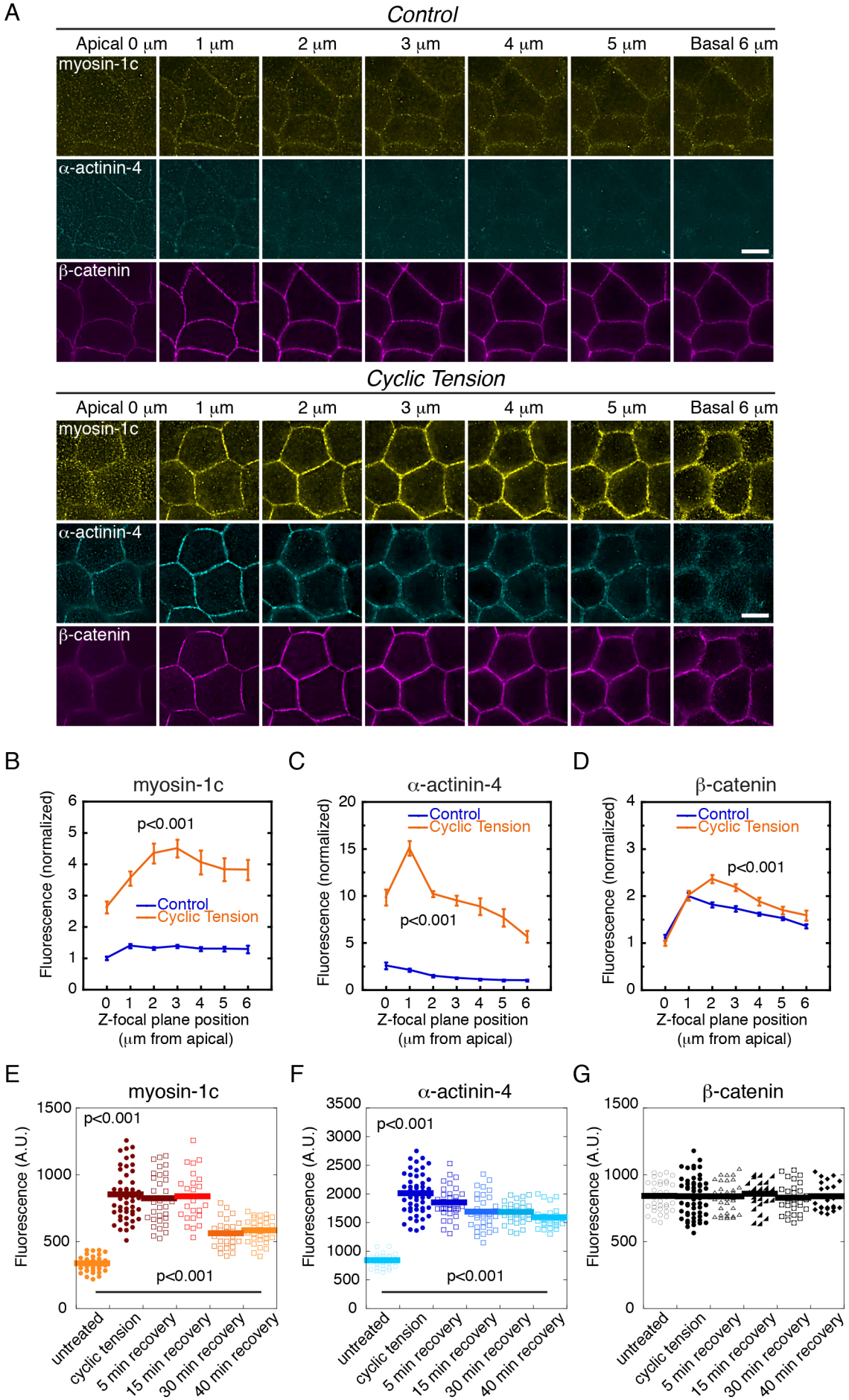
Supplementary Figure 2. Linear Regression analyses showing strong correlation between E-cadherin and α-catenin and modest correlation between myosin-1c and α-catenin on the lateral membrane in polarized MDCK monolayer. Linear regression analysis was performed for each protein pair. Pearson's correlation coefficient (R) between each protein-pair was calculated from 1000-1500 pixels per protein per z-image per one set of data. Data set is representative of 6 sets of data (N=6) from one experiment out of 3 independent experiments (N=3).



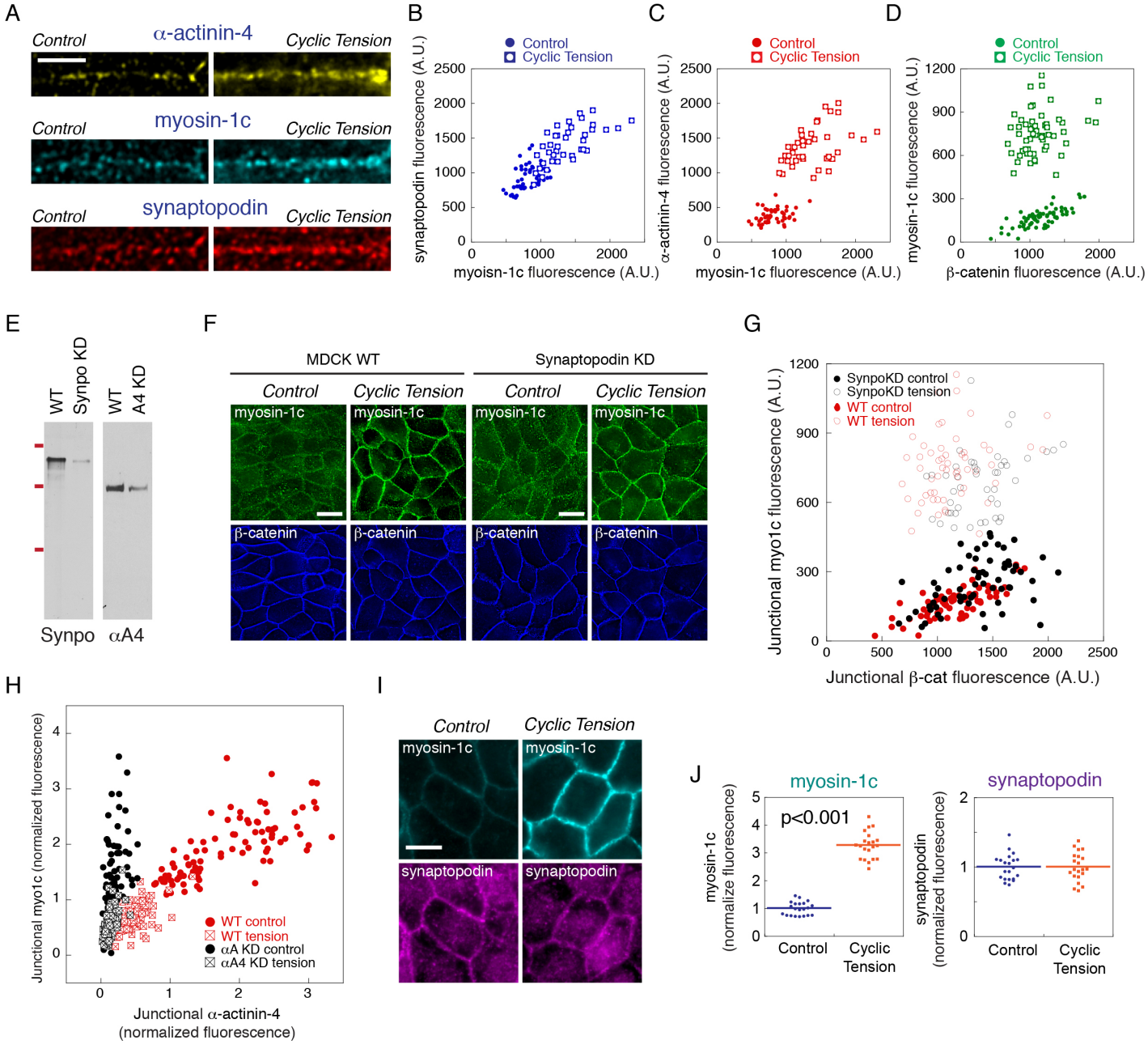
Supplementary Figure 3. Deconvolved images of myosin-1c, β-catenin, and actin staining in wild-type and heterogeneous myosin-1c knockdown cell monolayers. Myosin-1c knockdown (KD) cells have thicker cortical actin associated with the lateral cell-cell interface. Data set is representative of 8 sets of data (N=8) from one experiment out of 3 independent experiments (N=3). Scale bar is 10 μm.



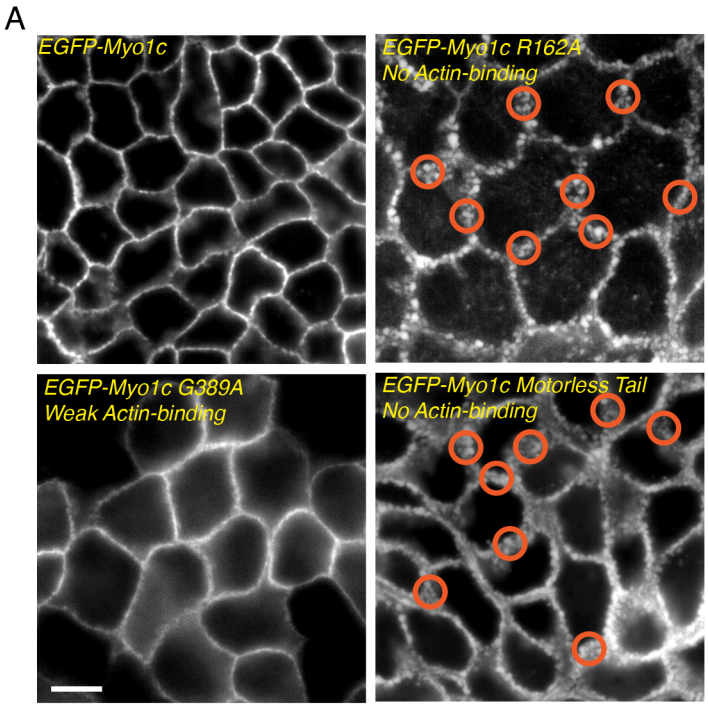
Supplementary Figure 4. Apical actin meshwork is disorganized in myosin-1c knockdown cells. (A) Fast iterative deconvolution microscopy showing myosin-1c (Myo1c), β-catenin (β-cat), and actin (Phalloidin) at the apical plane of polarized epithelial cell monolayer. Pink and white enclosures mark the apical regions of wild-type and myosin-1c knockdown cells, respectively. Data is representative of 12 sets of images (N=12) from one experiment out of 3 independent experiments (N=3). Scale bar is 10 µm. (B) Histograms of pixel intensity (binning 8) of myosin-1c and actin (phalloidin) from the pink and white enclosures in A. Data are representative of 8 sets of data (N=8) from one experiment out of 3 independent experiments (N=3).

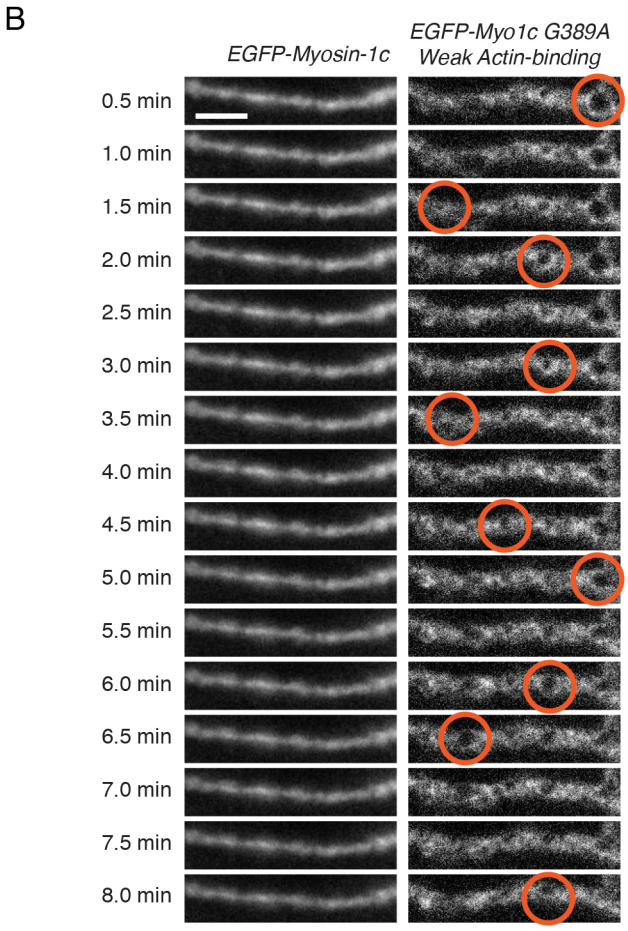


Supplementary Figure 5. Myosin-1c is recruited to the lateral membrane in a tension-sensitive manner. (A) Deconvolved z-slice images of myosin-1c, α-actinin-4, and β-catenin immunofluorescence in control or cyclic tension-treated polarized epithelial monolayers. Images were generated from constrained iterative deconvolution from 0.2 μm wide-field optical sections (from apical Z= 0 μm to basal Z= 6 μm). Data is representative of 12 sets of images (N=12) from one experiment out of 4 independent experiments (N=4). Scale bars are 10 μm. (B-D) Quantification of myosin-1c, α-actinin-4, and β-catenin intensities from apical (Z= 0 μm) to basal (Z= 6 μm) junctions in control and cyclic tension treated monolayers. Line graphs show the mean and standard errors of 24 junctions (N=24). $P<0.001$ for myosin-1c, α-actinin-4, β-catenin between control untreated and cyclic tension-treated. Data is representative of one experiment out of 5 independent experiments (N=5). (E-G) Quantification of myosin-1c, α-actinin-4, and β-catenin immunofluorescence at the apical junction in polarized cell monolayers before (untreated), immediately after (cyclic tension), and recovery from (5, 15, 30, 40 mins recovery) treatment with 60 min cyclic tension. $P<0.001$ for myosin-1c, and α-actinin-4 between untreated, cyclic tension-treated, and tension recovery. Data is representative measurements from one experiment out of 3 independent experiments (N=3).

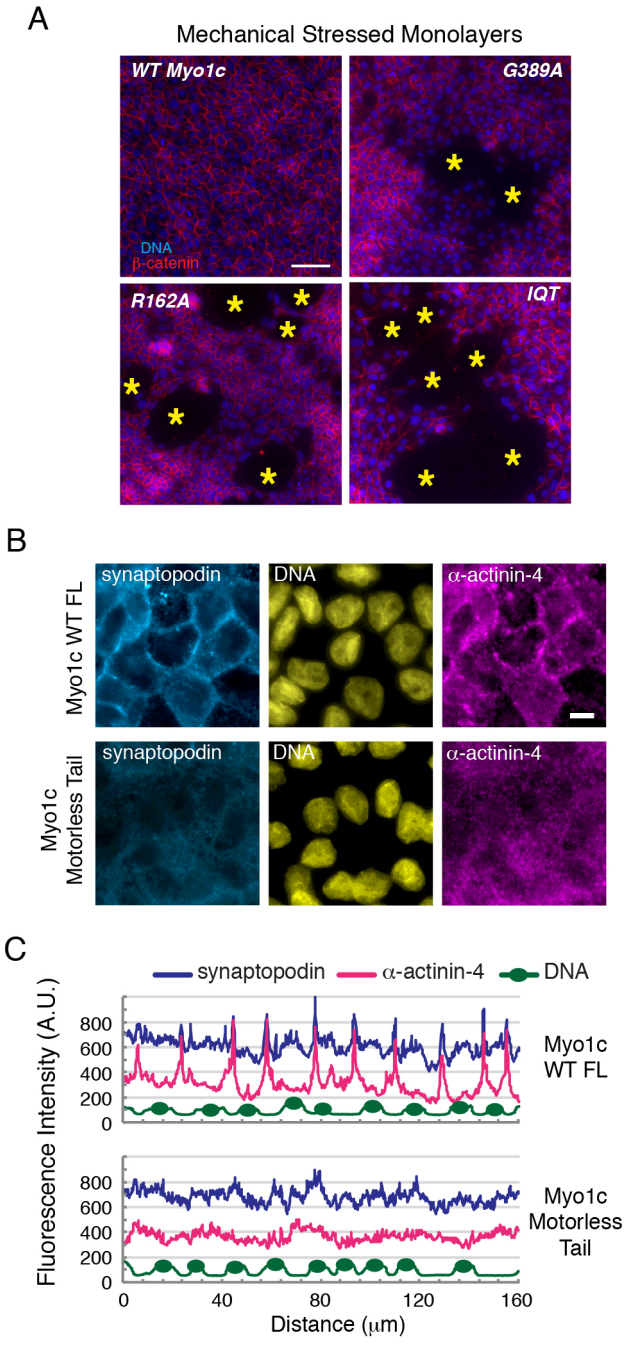

A
Control
α-actinin-4
Cyclic Tension
Control
myosin-1c
Cyclic Tension
Control
synaptopodin
Cyclic Tension
B
Control
Cyclic Tension
synaptopodin fluorescence (A.U.)
myoisn-1c fluorescence (A.U.)
C
Control
Cyclic Tension
α-actinin-4 fluorescence (A.U.)
myosin-1c fluorescence (A.U.)
D
Control
Cyclic Tension
myosin-1c fluorescence (A.U.)
β-catenin fluorescence (A.U.)
E
WT
Synpo KD
WT
A4 KD
Synpo
αA4
F
MDCK WT
Synaptopodin KD
Control
Cyclic Tension
Control
Cyclic Tension
myosin-1c
β-catenin
G
SynpoKD control
SynpoKD tension
WT control
WT tension
Junctional myo1c fluorescence (A.U.)
Junctional β-cat fluorescence (A.U.)
H
WT control
WT tension
αA KD control
αA4 KD tension
Junctional myo1c (normalized fluorescence)
Junctional α-actinin-4
(normalized fluorescence)
I
Control
Cyclic Tension
myosin-1c
synaptopodin
J
myosin-1c
p<0.001
myosin-1c
(normalize fluorescence)
Control
Cyclic
Tension
synaptopodin
synaptopodin
(normalized fluorescence)
Control
Cyclic
Tension

Supplementary Figure 6. Tension-induced junctional myosin-1c is independent of tension-sensitive synaptopodin and α-actinin-4. (A) Constrained iterative deconvolved images of myosin-1c, α-actinin-4, and synaptopodin in control and cyclic tension treated polarized monolayers. Data is representative of 6 sets of images (N=6) from one experiment out of 4 independent experiments (N=4). (B) Junctional synaptopodin is plotted against junctional myosin-1c. Data is representative of 6 sets of images (N=6) from one experiment out of 4 independent experiments (N=4). (C) Junctional α-actinin-4 is plotted against junctional myosin-1c. Data is representative of 6 sets of images (N=6) from one experiment out of 4 independent experiments (N=4). (D) Junctional myosin-1c is plotted against junctional β-catenin. Data is representative of 6 sets of images (N=6) from one experiment out of 4 independent experiments (N=4). (E) Western blots for synaptopodin (Synpo) and α-actinin-4 (αA4) of synaptopodin and α-actinin-4 knockdown (KD) total cell lysates. Data is representative of 6 independent experiments (N=6). (F) Constrained iterative deconvolved images of myosin-1c and β-catenin in control and cyclic tension-treated wild-type (WT) and synaptopodin knockdown (KD) cell monolayers. Data is representative of 12 sets of images (N=12) from one experiment out of 3 independent experiments (N=3). (G) Junctional myosin-1c (myo1c) is plotted against junctional β-catenin (β-cat) in control (control) and cyclic tension-treated (tension) wild-type (WT) and synaptopodin knockdown (Synpo KD) cell monolayers. Data is representative of 8 images (N=8) from one experiment out of 3 independent experiments (N=3). (H) Junctional myosin-1c (myo1c) is plotted against junctional α-actinin-4 in control (control) and cyclic tension-treated (tension) wild-type (WT) and α-actinin-4 knockdown (αA4 KD) cell monolayers. Data is representative of 8 images (N=8) from one experiment out of 3 independent experiments (N=3). (I) Constrained iterative deconvolved images of myosin-1c and synaptopodin in control and cyclic tension treated mature cell monolayers. Data is representative of 12 images (N=12) from one experiment out of 4 independent experiments (N=4). (J) Quantification of junctional myosin-1c and synaptopodin in control and cyclic tension-treated mature cell monolayers. Horizontal lines represent mean fluorescence intensities. $P<0.001$ for myosin-1c between control and cyclic tension-treated. Data is representative of 4 independent experiments (N=4).

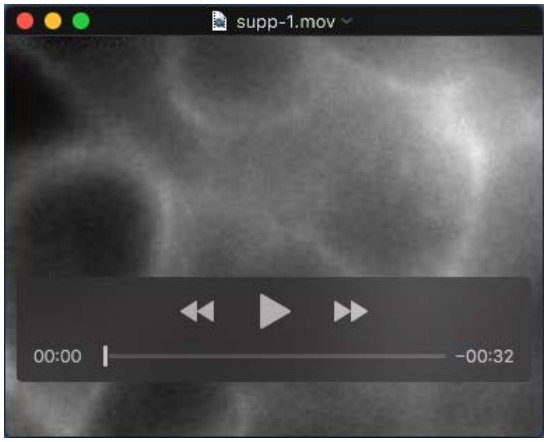




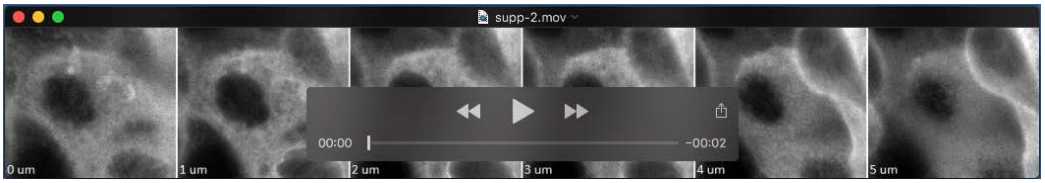
Supplementary Figure 7. Live-cell imaging of myosin-1c mutants showing bleb formation (orange circles) on the lateral membrane of polarized MDCK monolayers. (A) Live-cell images showing expression of EGFP-myosin-1c, EGFP-myosin-1c-G389A ATPase defective mutant with weak actin-binding, EGFP-myosin-1c-R162A ATPase defective mutant with no actin-binding, and EGFP-myosin-1c motorless mutant in polarized MDCK monolayers. Orange circles demarcate blebs along the lateral junction. Image set is representative of 8 sets of images (N=8) from one experiment out of 3 independent experiments (N=3). (B) Time-lapse OS-SIM images of EGFP-myosin-1c and EGFP-myosin-1c-G389A mutant showing blebbing along the lateral junctions in EGFP-myosin-1c-G389A mutant-expressing cells. Orange circles demarcate blebs along the lateral junction. Data is representative junction of 16 junctions (N=16) from of 6 sets of live-cell time-lapse movies (N=6). Scale bar is 2 μm.



Supplementary Figure 8. Actin-binding function of myosin-1c is required for epithelial cohesion and α-actinin-4 recruitment to cell junction. (A) Wide-field immunofluorescence images showing β-catenin and DNA (Hoechst 33528) in cell monolayers treated with cyclic tension. Cells expressing myosin-1c mutants (R162A, G389A, and IQT) were susceptible to mechanical stress, resulting in holes in the cell monolayers. Yellow asterisks mark damaged areas that are devoid of cell nuclei. Image set is representative of 12 sets of images (N=12) from one experiment out of 3 independent experiments (N=3). (B) Wide-field immunofluorescence of synaptopodin, α-actinin-4, and DNA (Hoechst 33528) in cell monolayers expressing the wild-type myosin-1c (Myo1c WT FL) or the motorless tail mutant (Myo1c Motorless Tail). Data is representative of 8 images (N=8) from one experiment out of 3 independent experiments (N=3). Scale bar is 5 μm. (C) Line scans showing the levels of synaptopodin (blue line), α-actinin-4 (pink line), and DNA (Hoechst 33528, green line) across several junctions and nuclei (green ovals) in cell monolayers expressing the wild-type myosin-1c (Myo1c WT FL) or the motorless tail mutant (Myo1c Motorless Tail). Data is representative of 12 line-scans (N=12) from one experiment out of 3 independent experiments (N=3).

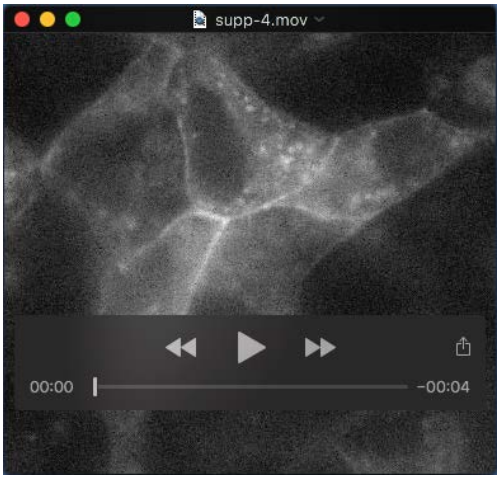


Movie 1. MDCK monolayer expressing venus-α-actinin at 5-day post-confluency showing repeated contractions and increase in junctional accumulation of venus-α-actinin.

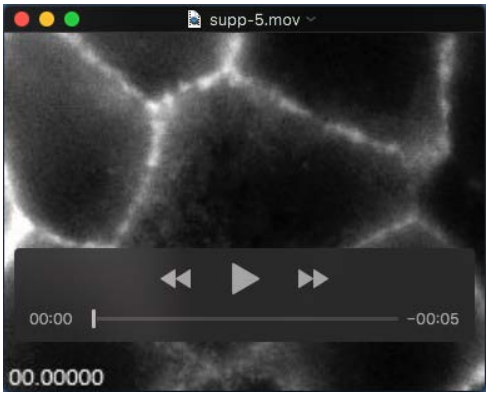


Movie 2. MDCK monolayer expressing venus-α-actinin at 1-day post-confluency showing one single whole cell contraction.

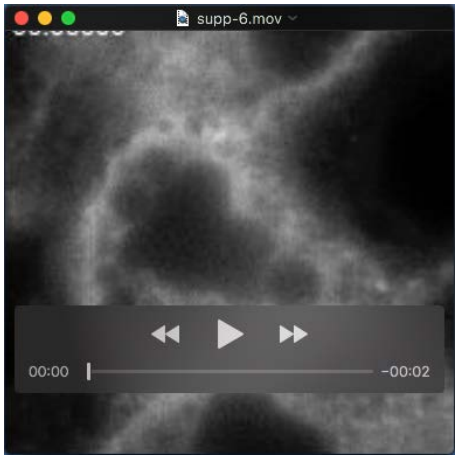
Movie 3. MDCK monolayer expressing lynD3cpV at 2-day post-confluency showing calcium spikes preceding cellular contractions.



Movie 4. MDCK monolayer expressing E-cadherin tension sensor at 5-day post-confluency showing a single contraction.

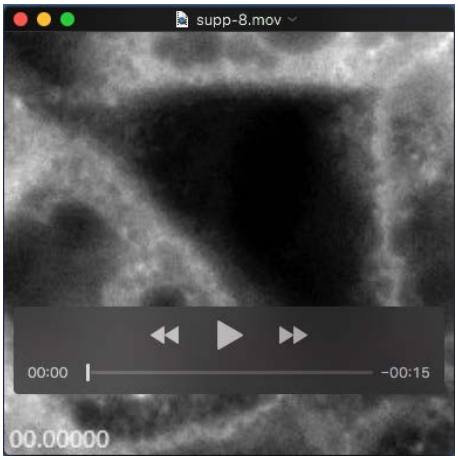


Movie 5. MDCK monolayer expressing EGFP-myosin-1c at 2-day post-confluency showing multiple short contractions.

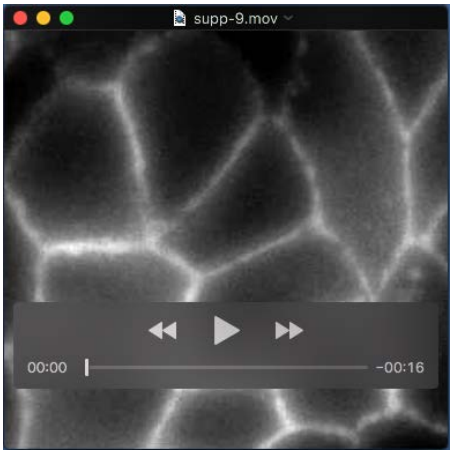


Movie 6. Myosin-1c knockdown cells expressing venus-α-actinin at 7-day post-confluency showing one single cell contraction and lack of venus-α-actinin accumulation.

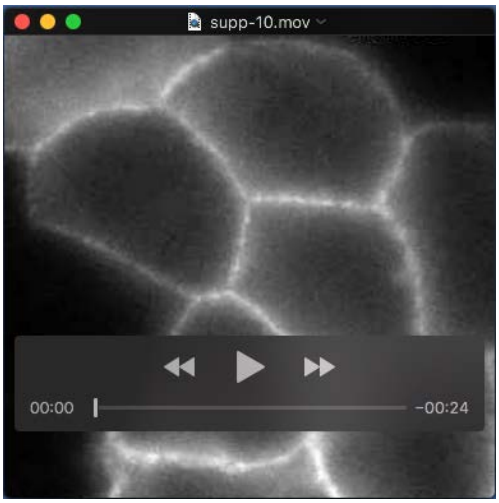
Movie 7. Myosin-1c knockdown cells expressing venus-α-actinin at 7-day post-confluency showing one single cell contraction and lack of venus-α-actinin accumulation.



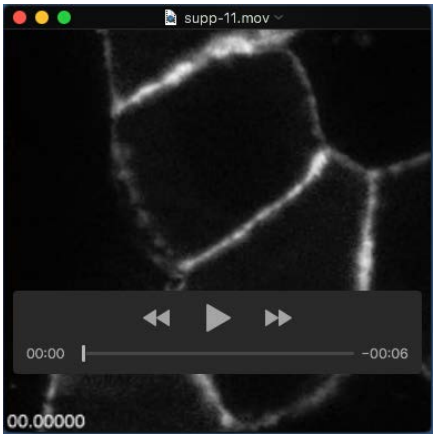
Movie 8. Myosin-1c knockdown cells expressing venus-α-actinin at 7-day post-confluency showing lack of venus-α-actinin accumulation at cell junction and constant formation of large blebs on the lateral membrane.



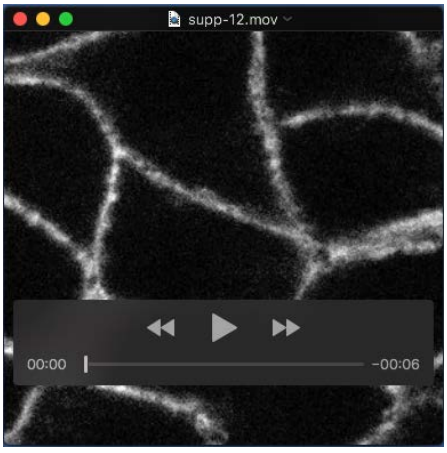
Movie 9. EGFP-Myosin-1c-expressing cells at 7-day post-confluency.



Movie 10. EGFP-Myosin-1c-G389A-expressing cells at 7-day post-confluency showing dynamic blebbing at cell-cell interface.



Movie 11. Live-OS-SIM pf EGFP-Myosin-1c-expressing cells at 7-day post-confluency.



Movie 12. Live-OS-SIM of EGFP-Myosin-1c-G389A-expressing cells at 7-day post-confluency showing dynamic blebbing at cell-cell interface.