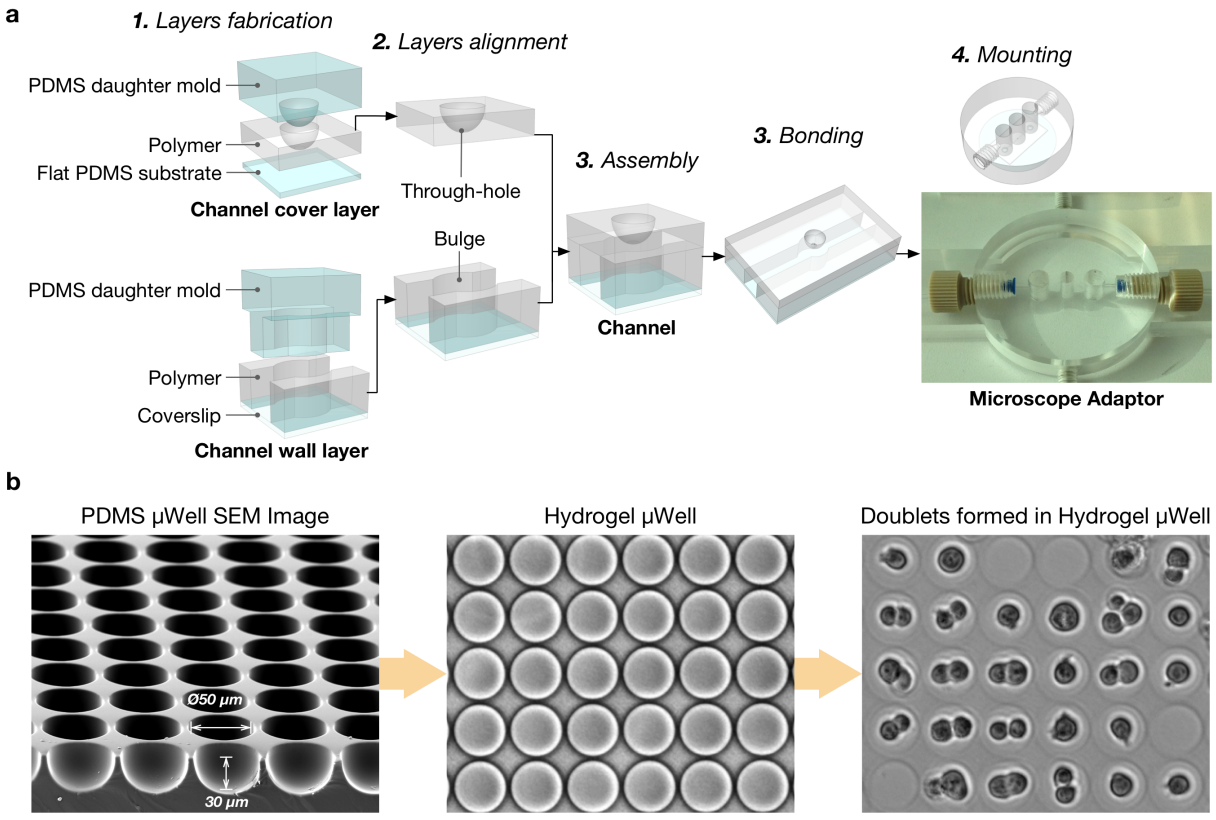
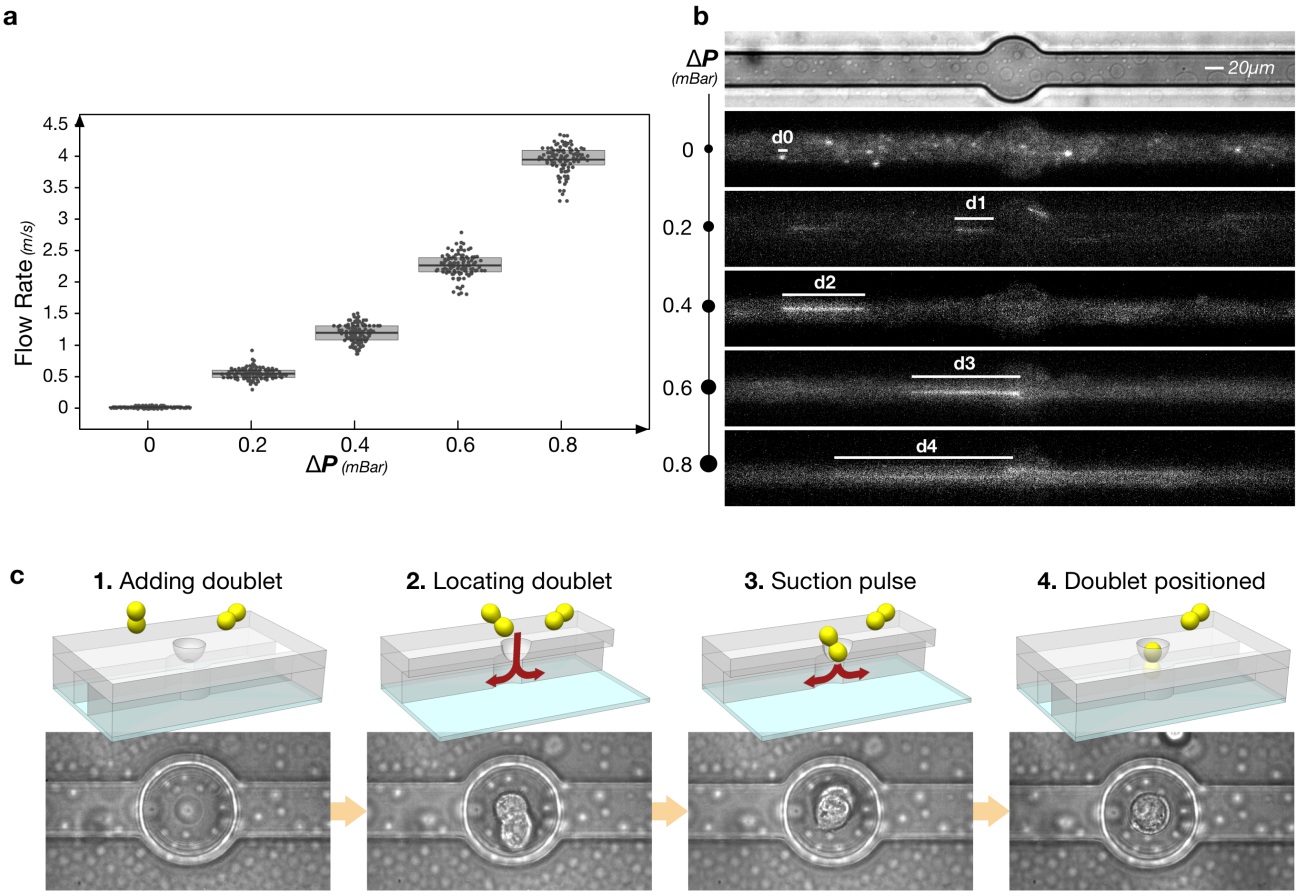
Title: Probing compression versus stretch activated recruitment of cortical actin and apical junction proteins using mechanical stimulations of suspended doublets.

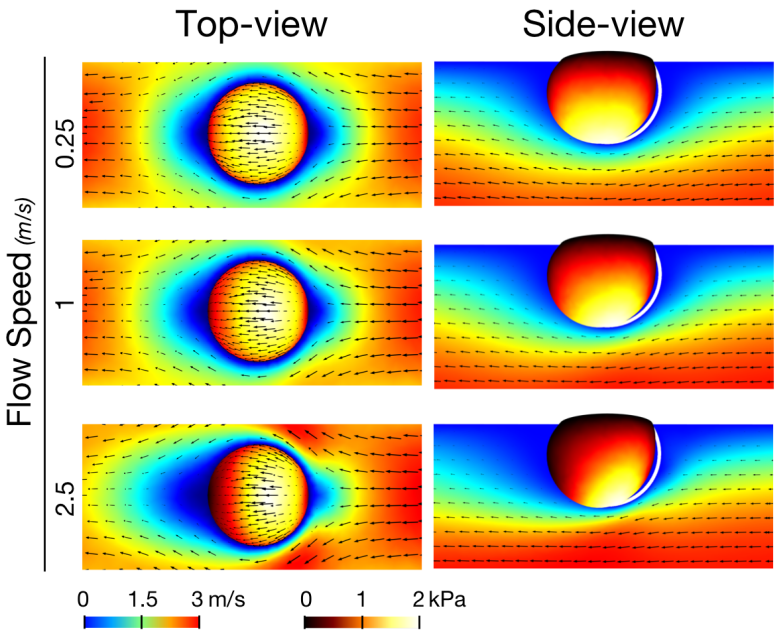
SUPPLEMENTARY FIGURES



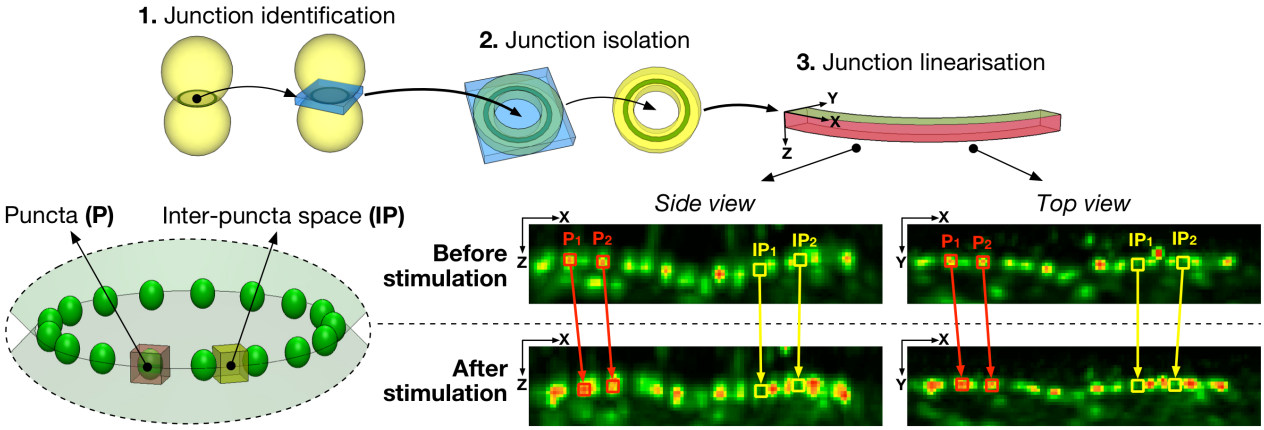
Supplementary Figure 1.
(a). Fabrication steps for SJS chip. First, channel cover layer and wall layer were molded into shape separately. They were then aligned and assembled properly to position the through-hole on top of the channel bulge, before mounted to the microscope adaptor. **(b).** Agarose-based hydrogel (0.8% v/v in PBS) micro-well arrays for cell doublets preparation. Round-bottom wells of 50μm in diameter and 30μm in depth were closely arranged to maximize the seeding efficiency.



Supplementary Figure 2.
(a). Correlation between the horizontal pressure difference across the channel system and the flow speed within. The pressure difference used in the all junction stimulations was adjusted to 0.5-0.8mBar, which corresponded to an averaged flow of 2-4m/s in the channel. Mean, 25th and 75th percentiles are indicated as boxed bar. **(b).** The flow speed in the channel induced by horizontal pressure difference was computed using fluorescent beads, based on the distance they traveled within 50ms (n=100-200 beads). **(c).** Positioning of doublet in SJS channel. Externally formed S180 cell doublets were loaded onto the stretcher from the outside. By applying the same pressure, either positive or negative, at both inlets simultaneously, we could create a net outward or inward flow across the through-hole. This flow helped to locate and select the desired doublet. Once the doublet was in close proximity to the through-hole, an inward flux triggered by a pulse of negative pressure might quickly capture and position the doublet. Through-hole size Ø10μm.



Supplementary Figure 3.
FE simulation of the doublet bottom cell in the flow. The cell was subjected to an uneven distribution of drag force over its surface. Such stress asymmetry increases with the flow speed. Cell size Ø15μm, contact size Ø10μm, channel width 40μm height 50μm.



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42 **Supplementary Figure 4.**
43 3D coordinates identification and tracking based on E-cadherin puncta
44 locations. The cell junction region was first identified and isolated according to
45 the characteristic E-cadherin junctional signal. It was then trimmed into a
46 donut-shape ring before linearized into a band. The 3D coordinates for each
47 distinct E-cadherin punctum was identified in this band. The coordinates for
48 the same punctum (or inter-puncta space) before and after the stimulation
49 were paired up. All coordinates, obtained from the E-cadherin image channel,
50 were then directly transferred onto the second image channels (i.e. actin, ZO1,
51 occludin, or plasma membrane marker), whereby their intensities in volumes
52 of 1μm x 1μm x 1μm (width, length, height) centered at these coordinates
53 were integrated and compared between before and after stimulation.

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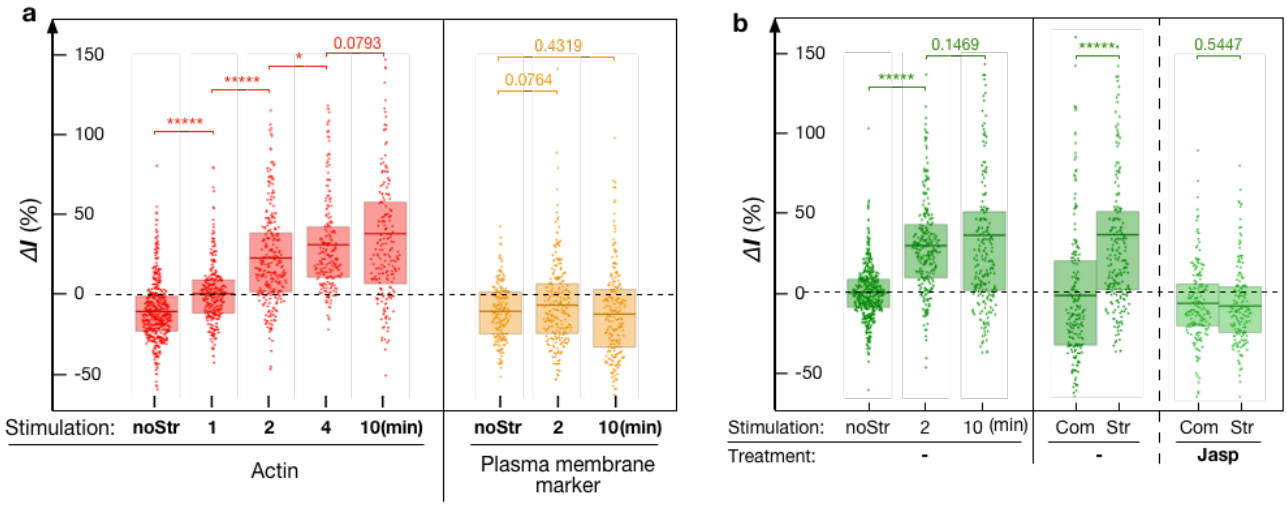
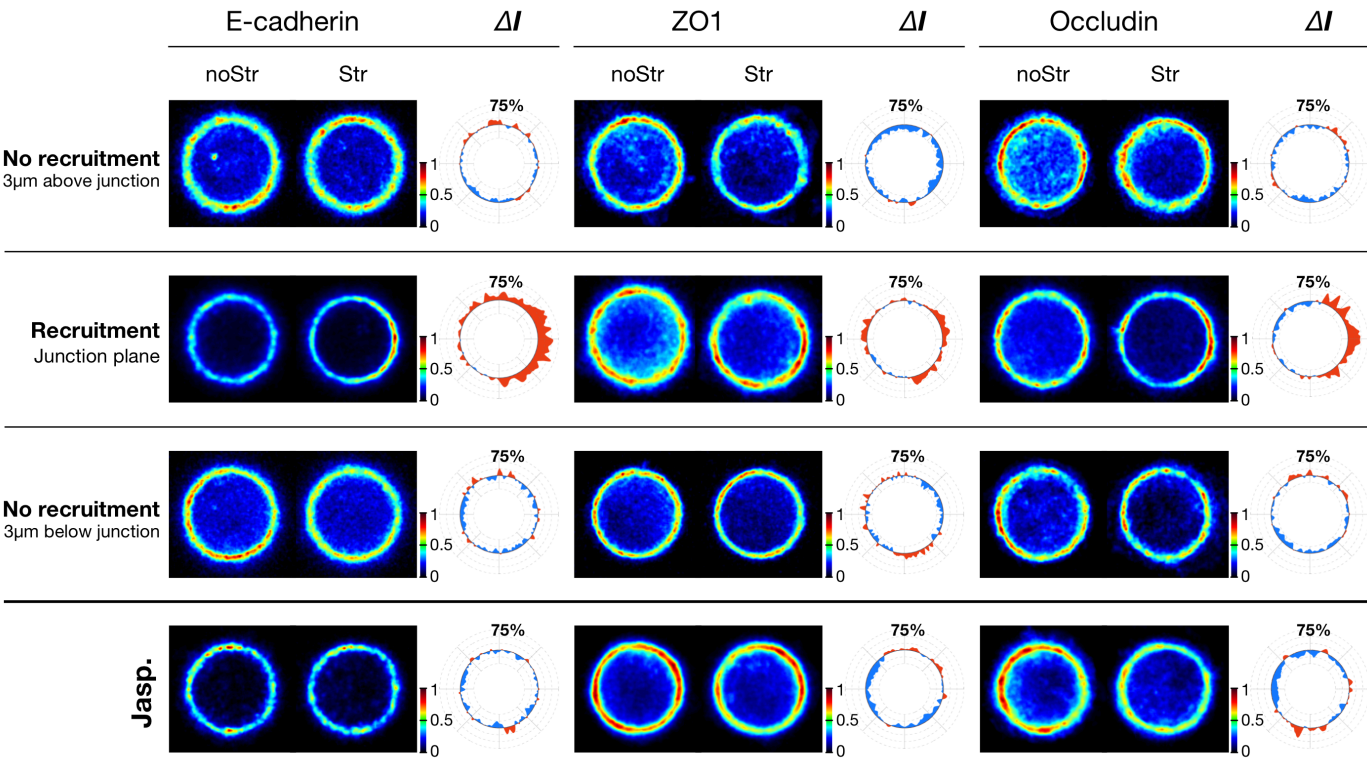
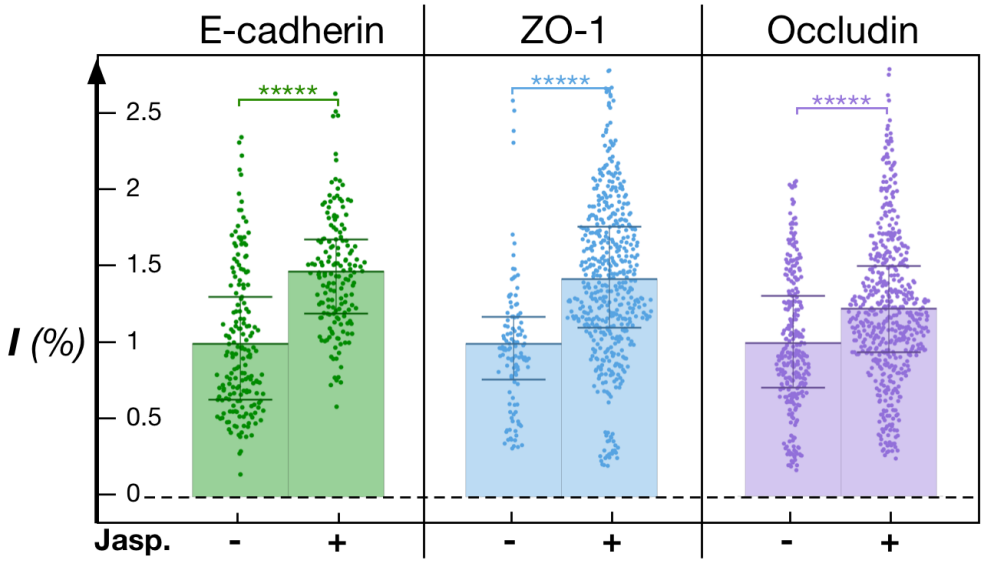


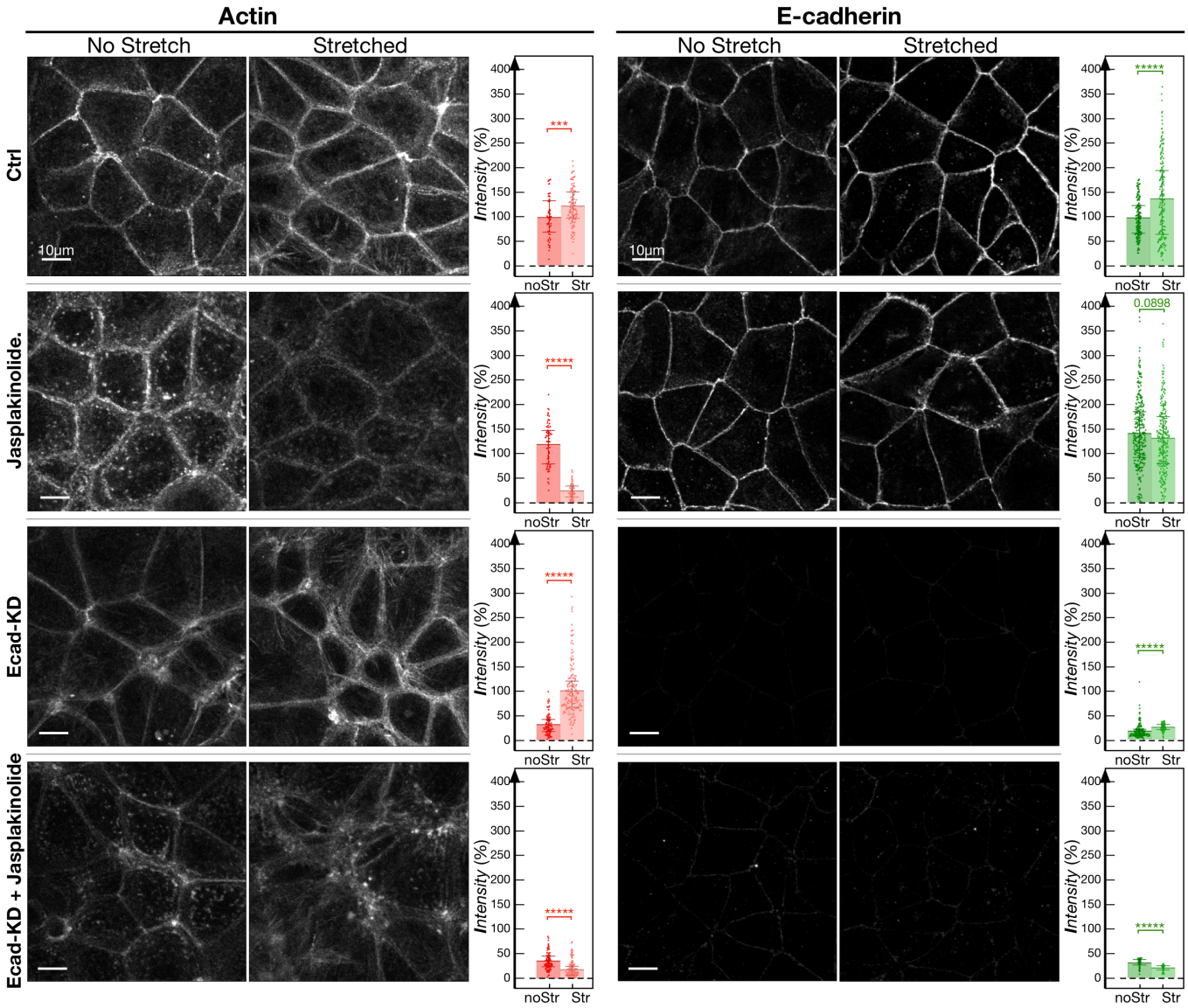
Supplementary Figure 5.
(a). Analysis of stretch-induced actin junctional recruitment at E-cadherin puncta regions as a function of stimulation time (n=250-450 puncta). Plasma membrane marker serves as a control (n=200-250 puncta). noStr: no stimulation (static) control. Decrease of signal in noStr case signifies the bleaching of mApple fluorescent tag. **(b).** Single puncta analysis (ΔI: relative recruitment in %) of the time dependence for E-cadherin (n=250-450 puncta). The recruitment occurs on the 2-minute time scale as for actin. In the case of a 1-sided stimulation (10 min) only the stretched side showed cadherin reinforcement (35.3±4.0%SE), which is abolished in presence of Jasplakinolide. Com: Compressed side; Str: stretched side; noStr: no stimulation case. Statistics has been performed via two-sample t-test with * for $p<0.05$, ** for $p<1x10^{-2}$, *** for $p<1x10^{-3}$, **** for $p<1x10^{-4}$, ***** for $p<1x10^{-5}$. Mean, 25th and 75th percentiles are indicated as boxed bar.



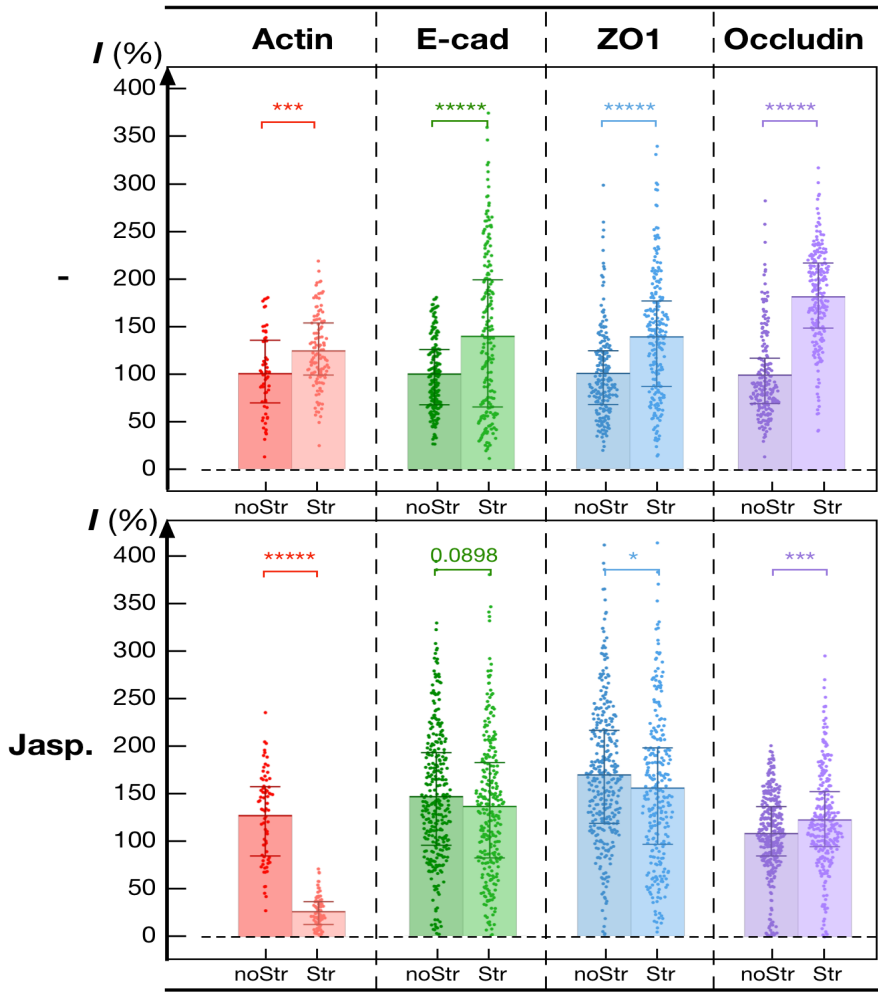
Supplementary Figure 6.
Junctional proteins mechanosensitivities in response to 10-min left-sided junction stimulation. E-cadherin (n=20 doublets), ZO-1 (n=8 doublets) and Occludin (n=11 doublets) were enriched at the cell contact region. Asymmetrical mode of stimulation resulted in directional recruitment of the proteins giving rise to a seemingly horizontally polarized junction. Treatment with Jasplakinolide (100nM, 1hr) abolished such polarized recruitment (n=10 doublets). Images were normalized separately as noStr vs. Str pairs for different focal planar locations.



Supplementary Figure 7.
Effect of Jasplakinolide on junction proteins. Treatment of Jasplakinolide (100nM, 60min) induced global increases in E-cadherin (47±3%s.e.m., n=200 punctate regions from 20 junctions), ZO-1 (42±2%s.e.m., n=150-400 punctate regions from 8 junctions) and Occludin (22±2%s.e.m., n=150-400 punctate regions from 11 junctions) along the entire junctions. Statistics has been performed via two-sample t-test with * for $p<0.05$, ** for $p<1x10^{-2}$, *** for $p<1x10^{-3}$, **** for $p<1x10^{-4}$, ***** for $p<1x10^{-5}$. Mean, 25th and 75th percentiles are indicated on the bar.

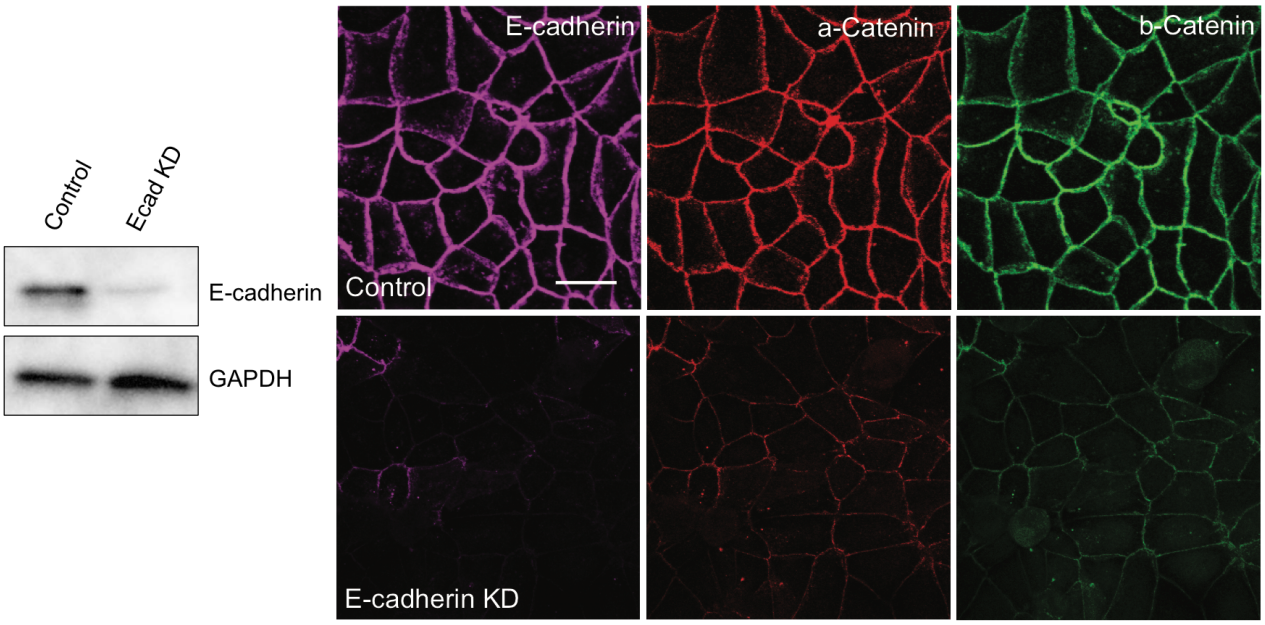


Supplementary Figure 8.
Effect of equiaxial stretching on junctional actin (n=60-150 junctions) and E-cadherin (n=70-300 junctions). The monolayers were immunostained for actin (AlexaFluor® 647) and E-cadherin (AlexaFluor® 546). Maximum projections (thickness 2μm) of representative image volumes containing apical junctions of Caco-2 monolayer were shown alongside the quantifications of junction intensity from linescan analysis (normalized to the No Stretch & Control condition). Statistics has been performed via two-sample t-test with * for $p<0.05$, ** for $p<1x10^{-2}$, *** for $p<1x10^{-3}$, **** for $p<1x10^{-4}$, ***** for $p<1x10^{-5}$. Mean, 25th and 75th percentiles are indicated on the bar.

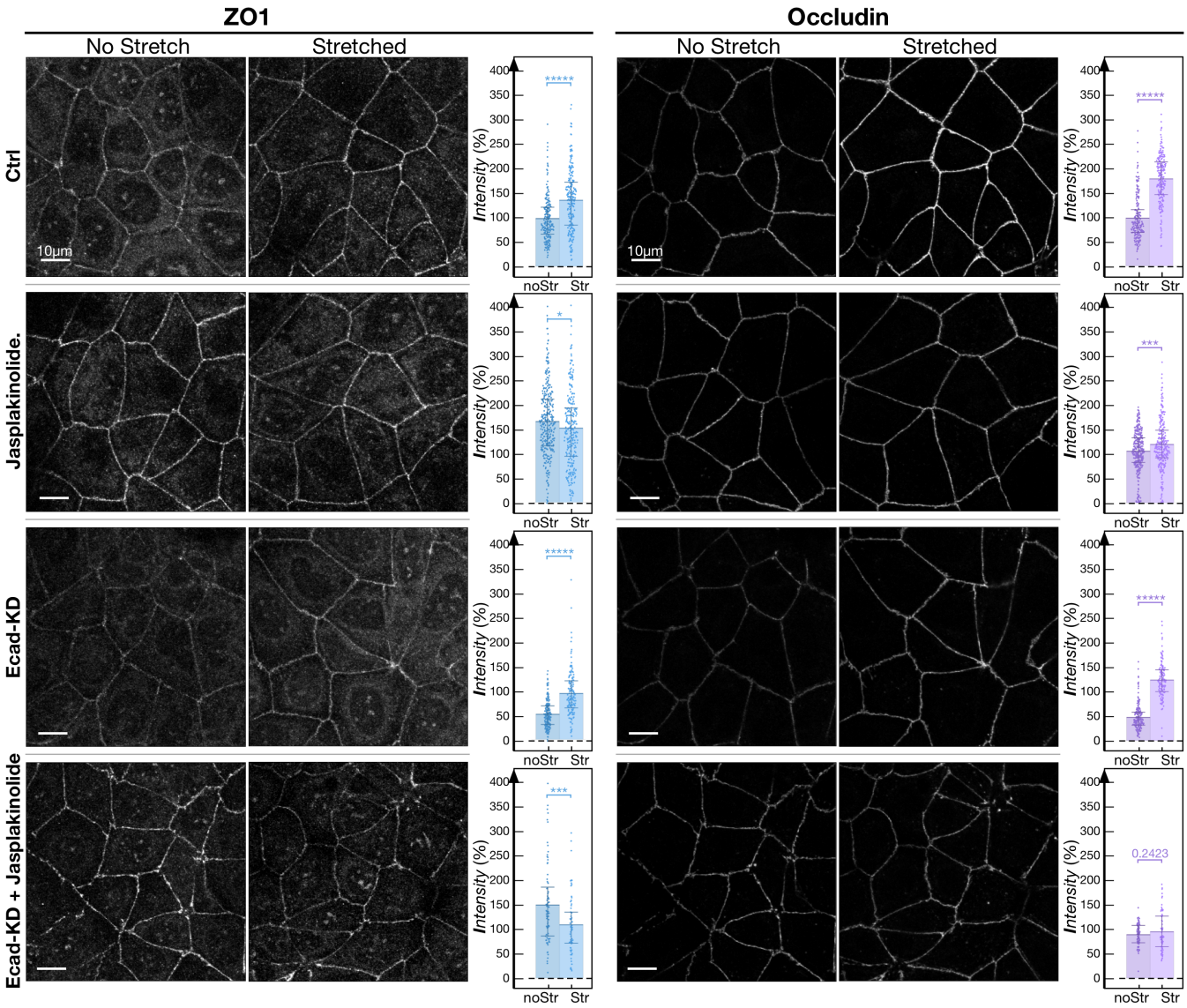


Supplementary Figure 9.: Mechanosensitive junctional response in monolayer.

Effect of equiaxial stretching on the recruitment of proteins at cell junctions in Caco-2 monolayer (n=60-200 junctions for actin, n=70-300 junctions for E-cadherin, ZO-1 and Occludin). The monlayer was stretched on a commercial biaxial strecther (Flexcell™) for 5 minutes at 10% constant strain. Control case (Ctrl), Jasplakinolide treatment (100nM, 60min) (Jasp.). Quantifications of junction intensity are based on linescan analysis (normalized to the No Stretch & Control condition). Str: stretched case; noStr: no stimulation case. Statistics has been performed via two-sample t-test with * for $p<0.05$, ** for $p<1x10^{-2}$, *** for $p<1x10^{-3}$, **** for $p<1x10^{-4}$, ***** for $p<1x10^{-5}$.Mean, 25th and 75th percentiles are indicated on the bar.



Supplementary Figure 10.
E-cadherin knockdown by RNAi was verified with western blotting. An overall reduction of ~80% in total protein level was detected. Immunofluorescent staining of E-cadherin, α-catenin and β-catenin was immunofluorescently stained to confirm the E-cadherin knockdown at cell junctions in monolayer. Scale bar 20μm.



Supplementary Figure 11.
Effect of equiaxial stretching on junctional ZO-1 (n=70-300 junctions) and occludin (n=70-300 junctions). The monolayers were immunostained ZO-1 (AlexaFluor® 488) and Occludin (AlexaFluor® 647). Maximum projections (thickness 2μm) of representative image volumes containing apical junctions of Caco-2 monolayer were shown alongside the quantifications of junction intensity from linescan analysis (normalized to the No Stretch & Control condition). Statistics has been performed via two-sample t-test with * for $p<0.05$, ** for $p<1x10^{-2}$, *** for $p<1x10^{-3}$, **** for $p<1x10^{-4}$, ***** for $p<1x10^{-5}$. Mean, 25th and 75th percentiles are indicated on the bar.