Supplementary text for:

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

Stretched CaCo Cells monolayers.

We extended our study to the examination of mature tight junctions formed between cells in a monolayer. As S180 cannot form dense monolayers we used Caco-2 cells, which are an extensively studied epithelial cell line that form well-defined polarized intercellular junctions with apical actin belt, mature adherens, and tight junctions. To assess the mature tight junctions we measured changes in the stretch-induced junctional proteins by quantitative immunofluorescence microscopy. As these cells cannot survive in suspension we could not use the previous system, and chose instead to grow the Caco-2 cells to confluence on stretchable membranes. These were then subjected to an equiaxial stretching with 10% constant strain for 5 min. The apical and tight junction regions of immunofluorescently labelled monolayers were then imaged using 100X confocal microscopy. Fluorescence of ZO-1 and Occludin peaked at these regions. We determined the level of each protein at individual apical junctions based on linescan analysis, based on the average intensity of a 1 μm-long central junction region for quantification (See Method section for detailed protocol). All treatment and stretch-induced protein recruitments were normalized against the unstretched controls.

In line with the established observations, **Supplementary Figure 9-11** shows that stretching the Caco-2 monolayer resulted in a significant reinforcement in junctional F-actin (24±3.5%s.e.m., n=120 junctions), E-cadherin (40±6%s.e.m., n=219

junctions), ZO-1 (39±4.5%s.e.m., n=219 junctions), and occludin (82±3%s.e.m., n=219 junctions). Despite the substantial increase in the baseline levels of each protein at the junction (22.5±5%s.e.m. for actin, n=72 junctions; and 44±4%s.e.m. for E-cadherin, 69±4%s.e.m. for ZO-1 and 8±2.5%s.e.m. for occludin, n=326 junctions), inhibition of actin dynamics using Jasplakinolide (100nM, 60min), abrogated their stretch-induced junctional recruitment. Of particular note, junctional F-actin levels showed a drastic decrease upon stretching in Jasplakinolide-treated cells (by 80±2%s.e.m., n=76 junctions). The actin structure across the junction also appeared disrupted. In this case it reorganized into a more diffuse pattern across the cell contacts. In contrast, E-cadherin, ZO-1 and occludin remained localized at the junctions in Jasplakinolide-treated cells after stretching. These results indicate that the recruitment of molecules to adherens and tight junctions is responsive to stretching, and strengthens junction reinforcement; an indispensable role played primarily by the dynamic actin network.

We further tested the role of mechanosensing by E-cadherin in the process. E-cadherin was reduced by RNAi in mature Caco-2 monolayers (>80% reduction by immunoblotting) (Supplementary Figure 10). To further minimize the potential influence of residual E-cadherin, only junctions with the lowest E-cadherin intensity (0-20%) were included in the quantification. Caco-2 has been reported to express predominantly E-cadherin [1]. The mislocalization of alpha-catenin and beta-catenin upon E-cadherin siRNA treatment indicates the absence of any compensatory mechanism by overexpression of P- or N-cadherins. Supplementary Figure 9 shows that E-cadherin depleted junctions preserved a reduced but substantial level of actin that was then increased by 225±5%s.e.m. (n=266 junctions) upon mechanical

stretching. This strongly suggests that the reinforcement of junctional actin did not arise exclusively from E-cadherin dependent mechanosensing.

Suplementary bibliography

1 M. Olander, J. R. Wisniewski, P. Matsson, P. Lundquist, and P. Artursson, J. Pharm. Sci. **105** (2), 817 (2016).