

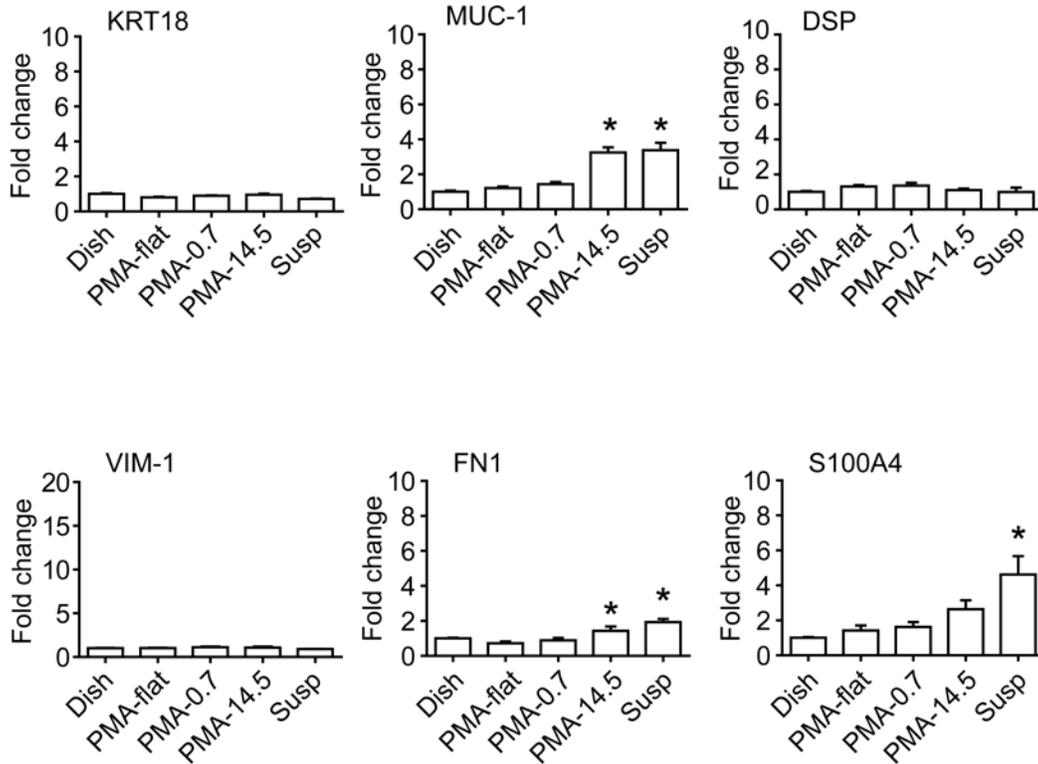
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Supplemental Information

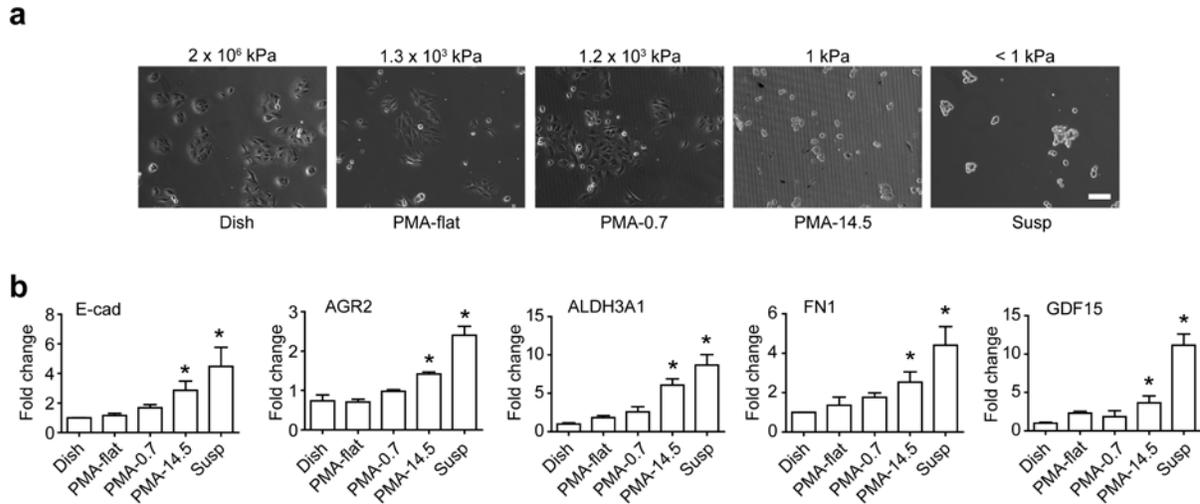
**Mechanotransduction-Induced Reversible Phenotypic Switching in
Prostate Cancer Cells**

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SUPPLEMENTARY FIGURES

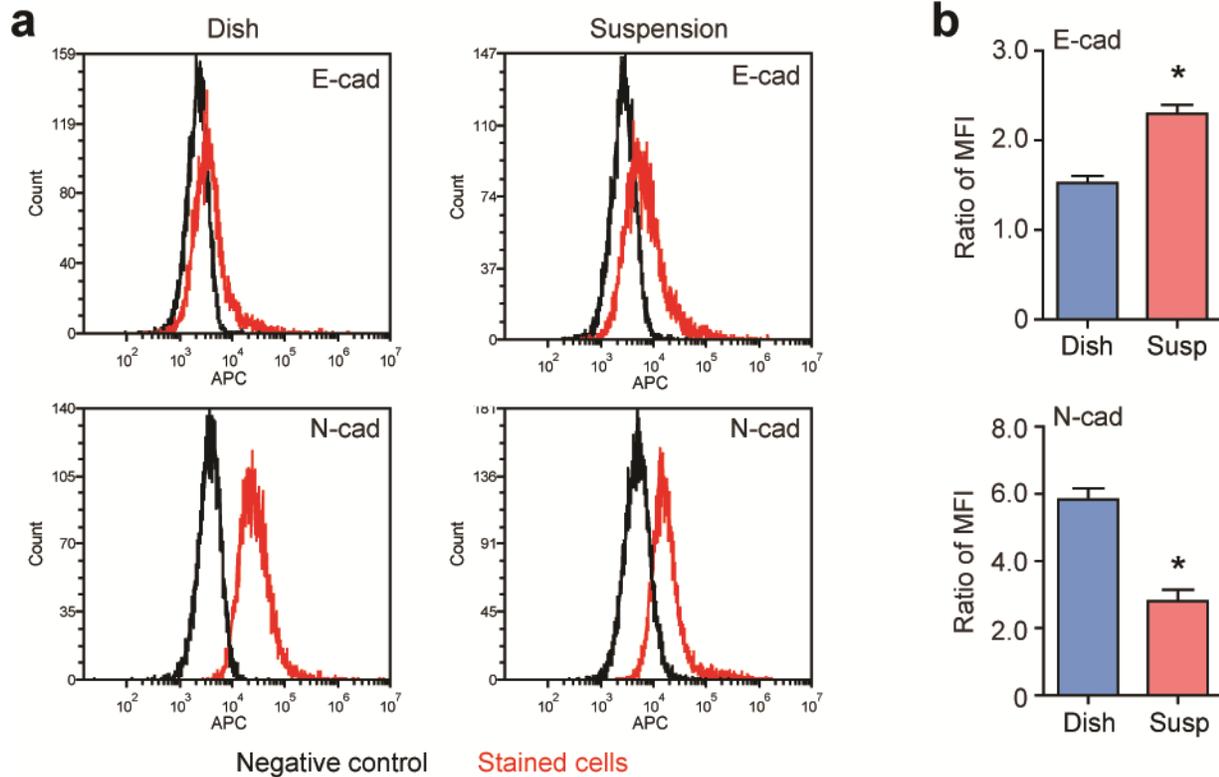


Supplementary Figure 1. Effect of mechanotransduction on gene expression associated with epithelial or mesenchymal phenotype. qRT-PCR analysis of cytokeratin 18 (KRT18), mucin (MUC-1), desmoplakin (DSP), vimentin (VIM), fibronectin (FN1), and fibroblast specific protein (FSP, S100A4) in PC3 cells grown on substrates with decreasing rigidities (tissue culture plastic dish (Dish), flat PDMS (PMA-flat), PDMS micropost arrays (PMAs) with post heights of 0.7 μm (PMA-0.7) and 14.5 μm (PMA-14.5)) or in suspension (Susp) for 72 hr as indicated. Data represents the mean \pm s.e.m with $n = 3$. P -values were calculated using student's t-test. *, $P < 0.05$.



Supplementary Figure 2. Mechanotransduction induced phenotypic transition in DU145

prostate cancer cells. a. Phase images of DU145 cells grown on substrates with decreasing rigidities (tissue culture plastic dish (Dish), flat PDMS (PMA-flat), PDMS micropost arrays (PMAs) with post heights of 0.7 μm (PMA-0.7) and 14.5 μm (PMA-14.5)) or in suspension (Susp) as indicated. Rigidity value associated with each condition was indicated. Scale bar, 100 μm . **b.** qRT-PCR analysis of gene expression of E-cad, AGR2, ALDH3A1, FN1, and GDF15 for DU145 cells grown on substrates with decreasing rigidities or in suspension for 72 hr as indicated. N-cad was undetectable in DU145. Data represents the mean \pm s.e.m with $n = 3$. P -values were calculated using student's t-test. *, $P < 0.05$.



Supplementary Figure 3. Phenotypic transition occurs throughout the whole cell

population of PC3 cells. a. Flow cytometry analysis of PC3 cells stained with E-cad-APC

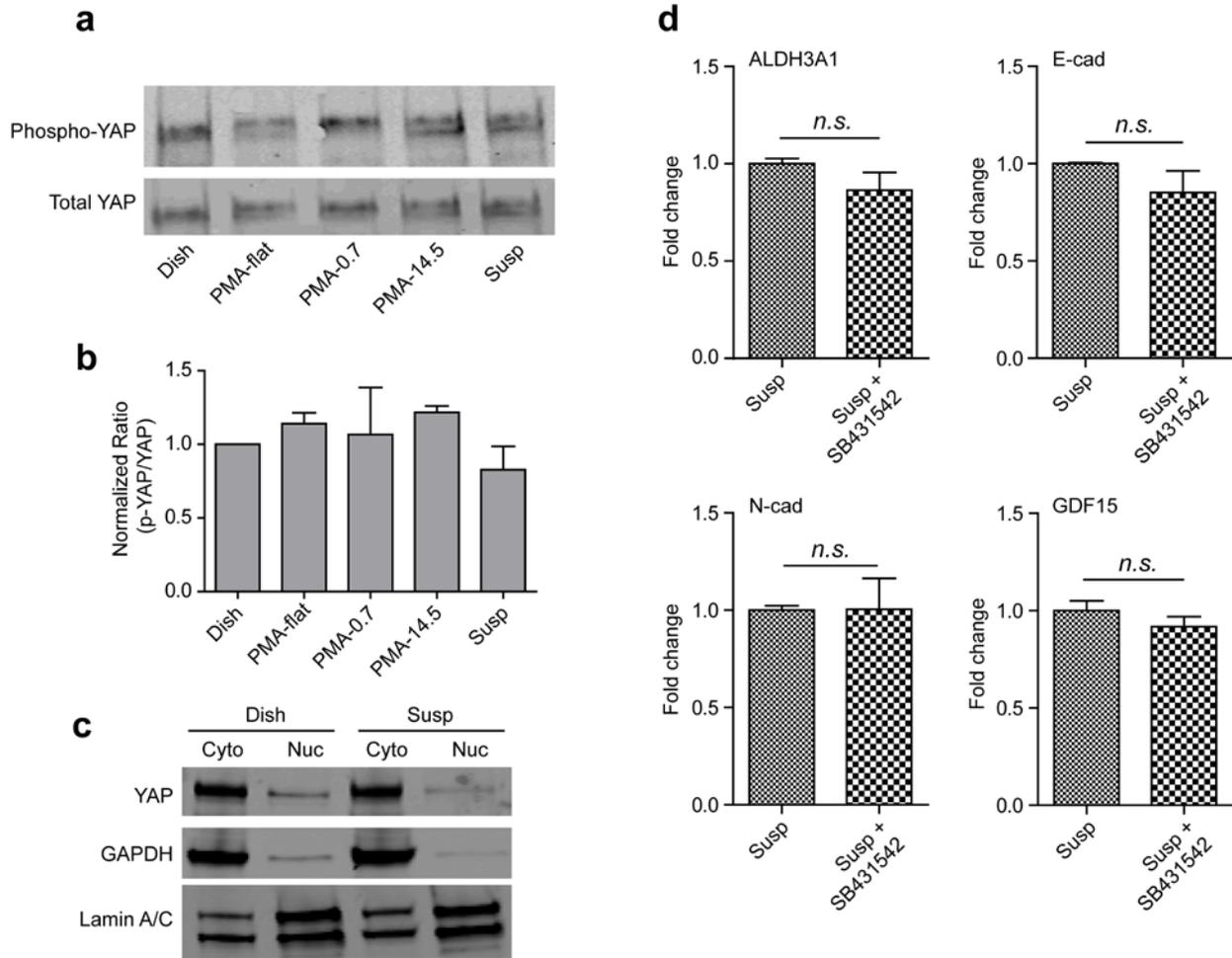
conjugated antibodies or N-cad-APC conjugated antibodies as indicated. PC3 cells were grown on tissue culture plastic dish or in suspension for 72 hr before flow cytometry analysis.

Unstained cells were used as negative control. **b.** Bar graph showing the ratio of median

fluorescence intensity (MFI) of stained over unstained PC3 cells grown on tissue culture plastic

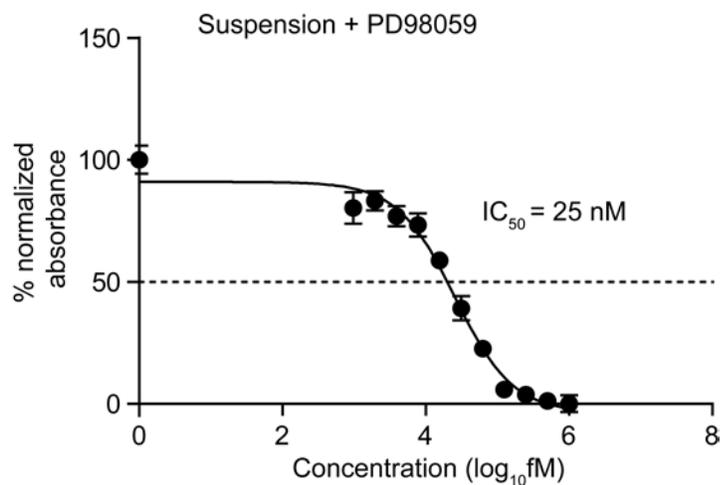
dish or in suspension as indicated. Data represents the mean \pm s.e.m with $n = 3$. P -values were

calculated using student's t -test. *, $P < 0.05$.



Supplementary Figure 4. YAP activity in mechanotransduction induced phenotypic transition of PC3 cells. **a.** Immunoblot assay for phosphorylated YAP (phospho-YAP) and total YAP expression in PC3 cells grown on substrates with decreasing rigidities (tissue culture plastic dish (Dish), flat PDMS (PMA-flat), PDMS micropost arrays (PMAs) with post heights of 0.7 μm (PMA-0.7) and 14.5 μm (PMA-14.5)) or in suspension (Susp) for 72 hr as indicated. Rigidity value associated with each condition was indicated. **b.** Quantification of the ratio of phospho-YAP over total YAP in PC3 cells grown on decreasing substrate rigidities as indicated. **c.** Immunoblot of YAP in nuclear (Nuc) and cytoplasmic (Cyto) protein fractions from PC3 cells grown on tissue culture plastic dish or in suspension as indicated. **d.** qRT-PCR analysis of ALDH3A1, E-cad, N-cad, and GDF15 for PC3 cells cultured in suspension treated with or

without Smad inhibitor SB431542 as indicated. Data represents the mean \pm s.e.m with $n = 3$. P -values were calculated using student's t-test. *n.s.*, statistically not significant with $P > 0.05$.



Supplementary Figure 5. Effects of pathway inhibitors on decreased paclitaxel sensitivity in cells grown in suspension. PC3 cells grown in suspension were treated with 10 μM PD98059 in conjunction with increasing amounts of paclitaxel. Cell viability was measured using WST-1 after 48 hrs of paclitaxel and inhibitor/s exposure and IC-50 calculated using non-linear regression.