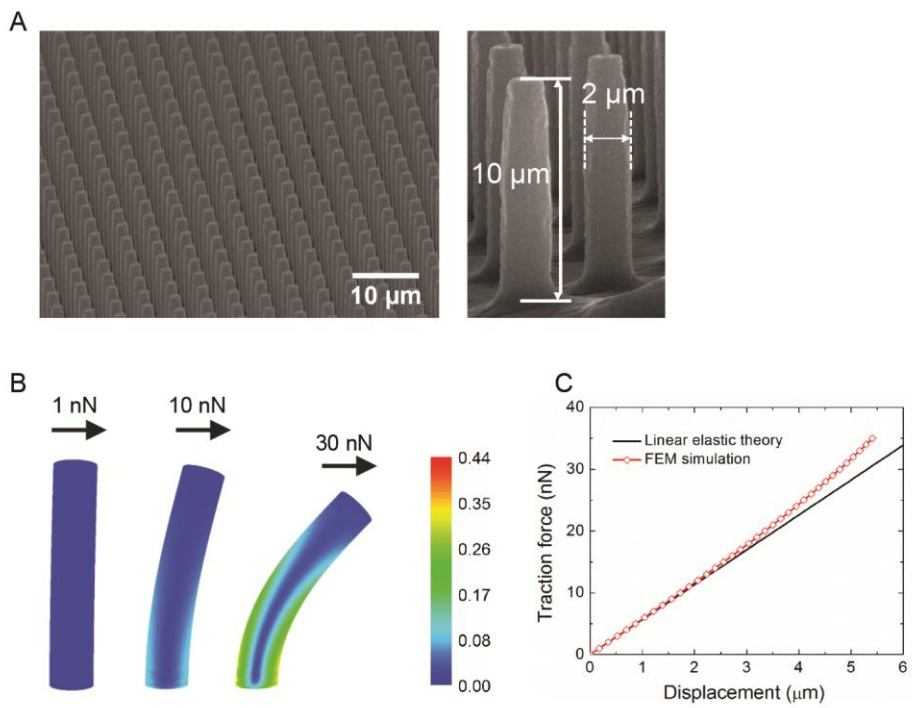
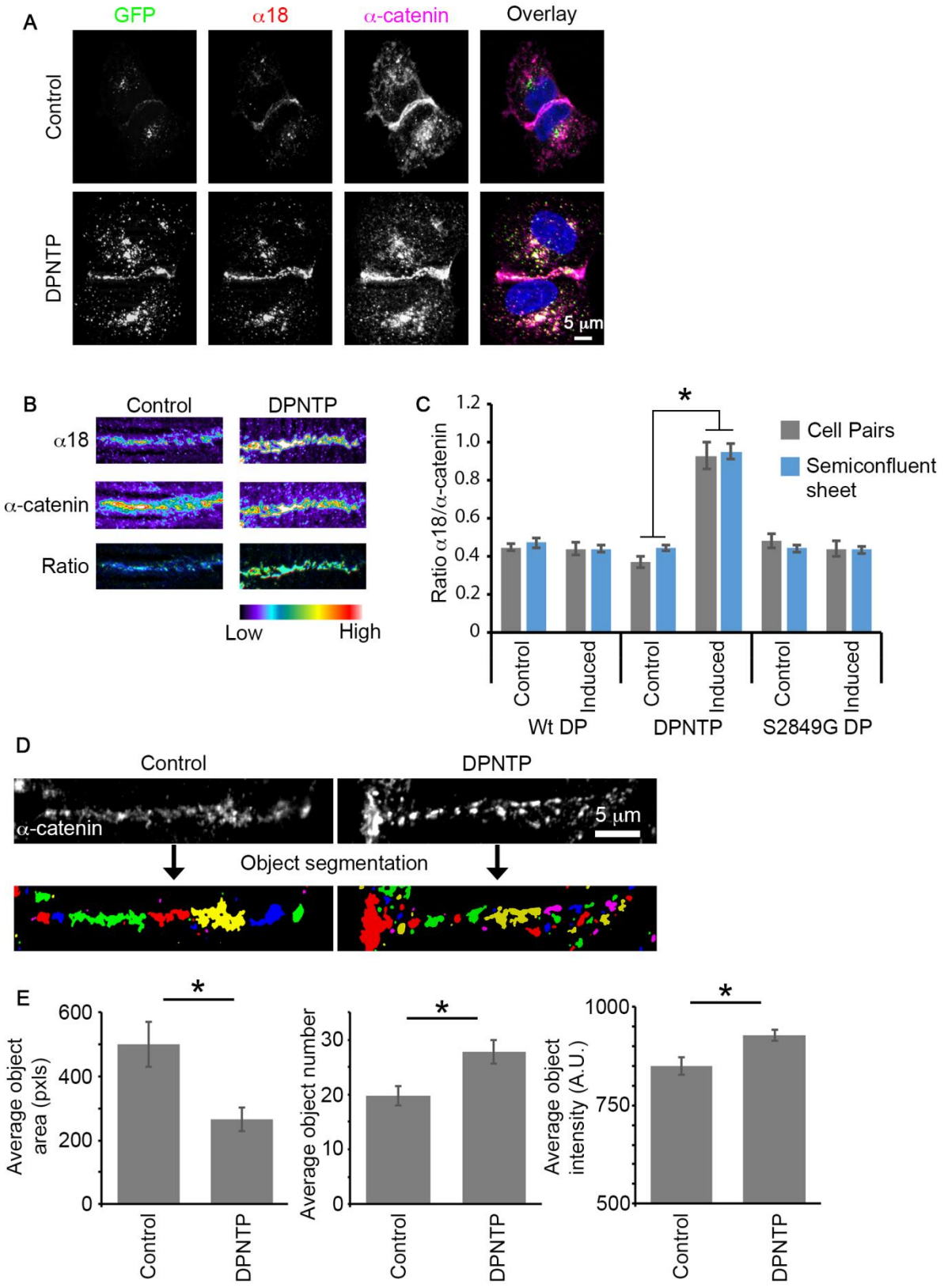
Supplemental Materials

Molecular Biology of the Cell

Broussard et al.

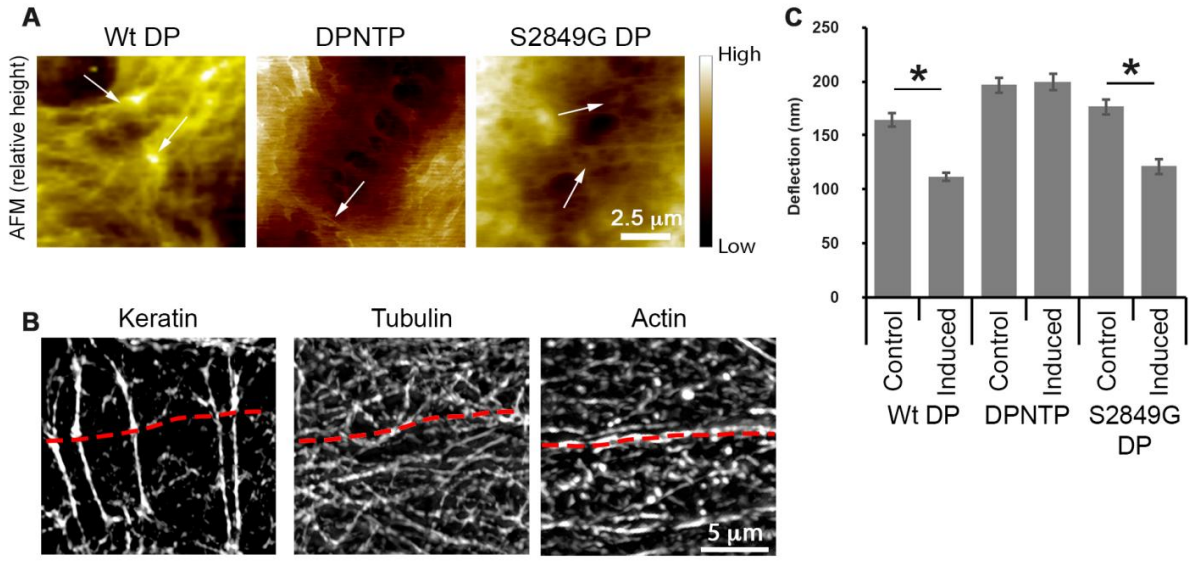


Supplemental Figure 1. (A) Representative SEM images of the MA. Pillars are orthogonally patterned with a height of 10 µm and a diameter of 2 µm. (B) Finite element simulations of pillar deflection under various traction levels. (C) The traction-displacement relationship begins to deviate from linear elastic theory as pillars undergo large deformation (>2.5 µm).

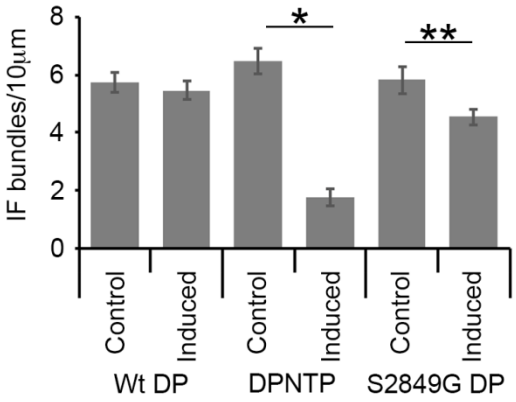


Supplemental Figure 2. (A) Apotome micrographs of a control (uninduced) and induced A431 cell pair expressing GFP-DPNTP and stained with the tension sensitive α-catenin (α18) antibody and total α-catenin are shown. Dapi indicates nuclei in blue. (B) Cell-cell border staining of α18 and total α-catenin as well as ratio images of α18 to total α-catenin in a control (uninduced) and induced A431

cell pair expressing GFP-DPNTP are shown in a pseudocolor scale in which cool colors represent relatively low values while warm colors represent relatively high values. (C) Quantification of the average cell-cell border intensity ratio of α18 to total α-catenin for control (uninduced) and induced cell pairs and semiconfluent (80%) cell sheets expressing the indicated DP variants are shown. Error bars represent the standard error of the mean from at least 14 cell pairs and 43 cell-cell junctions in the semiconfluent cell sheet from 3 independent experiments. *p<0.0001. (D) Upper, representative apotome micrographs of the cell-cell interface of control (uninduced) and induced A431 cells expressing GFP-DPNTP and immunostained with an α-catenin antibody are shown. Lower, images were thresholded and object segmentation was performed using MetaMorph Software. Colors represent individual objects segmented from the above fluorescence images. (E) The average object area, number of objects per cell-cell interface, and average α-catenin intensity per segmented object are shown for control (uninduced) and induced A431 cells expressing GFP-DPNTP. Error bars represent the standard error of the mean from at least 32 cells from 3 independent experiments. *p≤0.004.



Supplemental Figure 3. (A) Representative AFM height images at cell-cell junctions of pairs of induced A431 cells expressing the indicated DP variants are shown. Images were contrasted to highlight the cytoskeletal bundles attached at cell-cell contacts (arrows). (B) Representative super-resolution images at the cell-cell interface (red dashed line) of control cells immunostained for the indicated cytoskeletal networks (keratin 18, Keratin; α-tubulin, Tubulin; and phalloidin, Actin) are shown. (C) Quantification of the average deflection of individual cytoskeletal bundles at cell junctions in control (uninduced) or induced A431 cells expressing the indicated DP variants as determined using AFM with a load up to 0.5 nN is shown. Error bars represent the standard error of the mean from at least 10 cells from 3 independent experiments. *p<0.05.



Supplemental Figure 4. Quantification of the number of IF bundles detected by immunofluorescence analysis of keratin staining entering sites of cell-cell contact in uninduced (control) and induced semiconfluent sheets of A431 cell lines expressing the indicated DP variants is shown. Error bars represent the standard error of the mean from at least 26 cells from 3 independent experiments. *$p<0.0001$, **$p=0.008$.