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Supplemental Information

Metastatic State of Cancer Cells May Be Indicated by Adhesion Strength

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SUPPLEMENTAL FIGURES

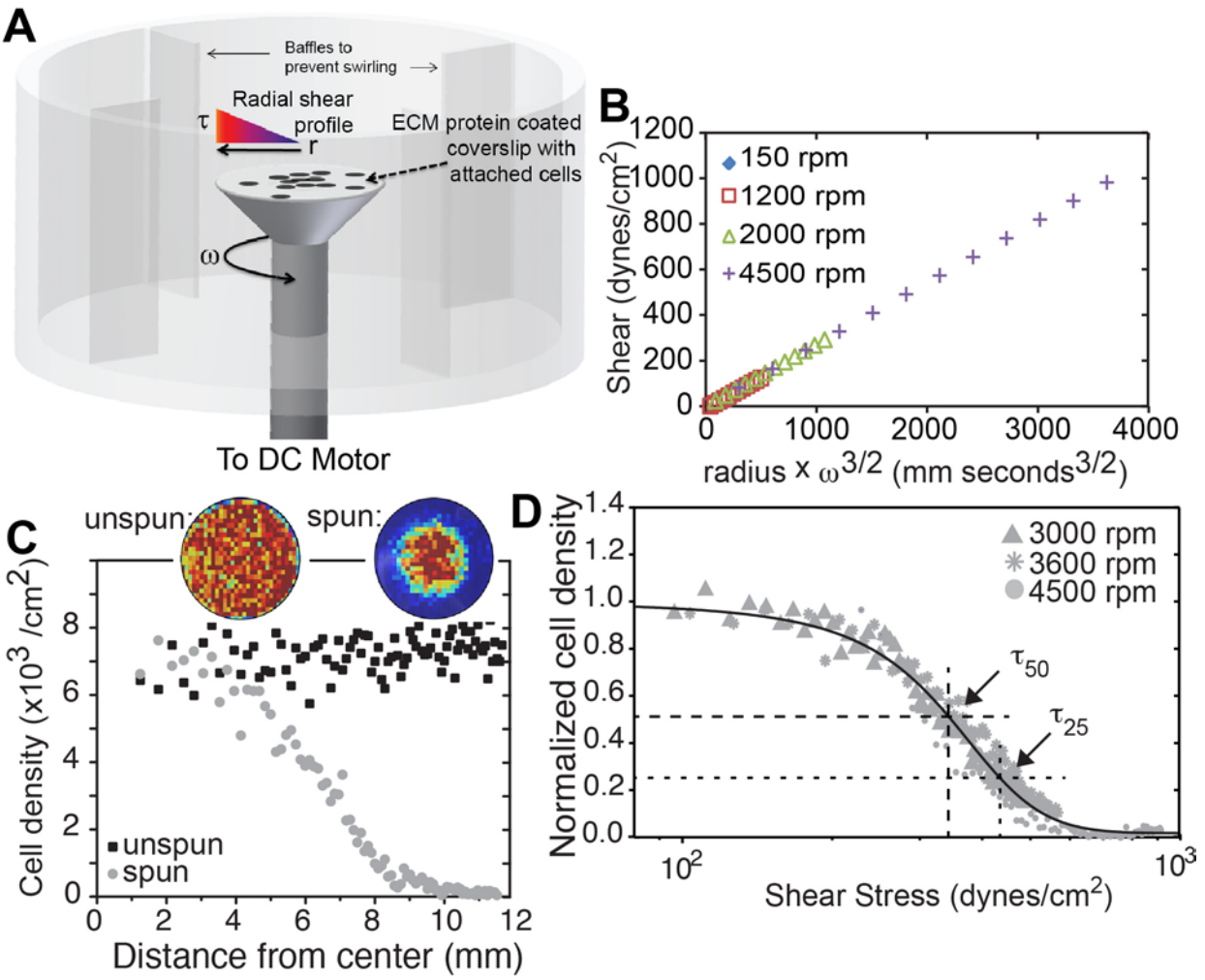

Figure S1: Spinning Disc Assay Creates a Radially-dependent Shear Profile. (A) The spinning disc device is illustrated with cells attached to an extracellular matrix protein-coated coverslip mounted and rotating on a spinning rod in buffer. The radially-dependent shear profile is highlighted showing that cells at the center only rotate in place while those at the edge move around at a high linear velocity. (B) The plot shows the relationship of radial position on the coverslip and angular velocity versus applied shear stress at a given point for the indicated velocities (in revolutions per minute; rpm). (C) Plot of the relationship between radial position and cell density. Inset images show heat maps of cell density. Warm (red) and cool (blue) colors indicate high and low densities, respectively. (D) Plot of cell density, normalized to the center of the coverslip, versus the applied shear. Data is plotted for the indicated velocities. τ_{25} and τ_{50}, i.e. the shear to detach 25 and 50% of cells, respectively, are indicated in the plot and are 438 and 346 dynes/cm^2, respectively.

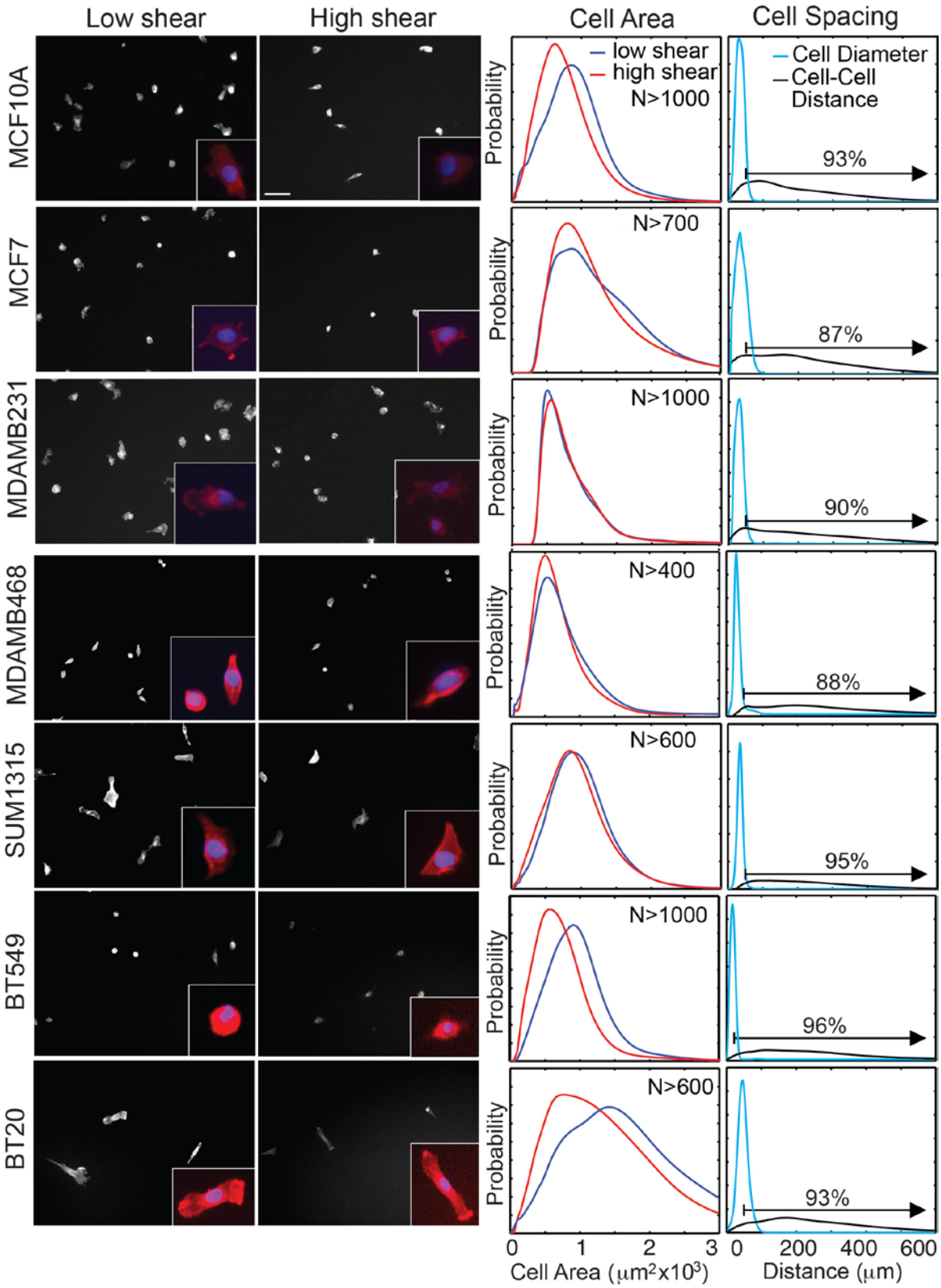

Figure S2: Cell Morphology and Distribution are Independent of Mammary Epithelial Cell Line. At the left are low magnification images of MCF10A, MCF7, MDAMB231, MDAMB468,

SUM1315, BT549, and BT20 cells at low and high shear, which were stained with Rhodamine-Phalloidin. Inset images at higher magnification were also stained with DAPI. At right are plots of cell area (blue and red lines indicating high and low shear) and cell-to-cell spacing frequency for the indicated number of cells (N). Indicated within the plots is the percentage of cells spaced further apart than the average diameter of each cell line.

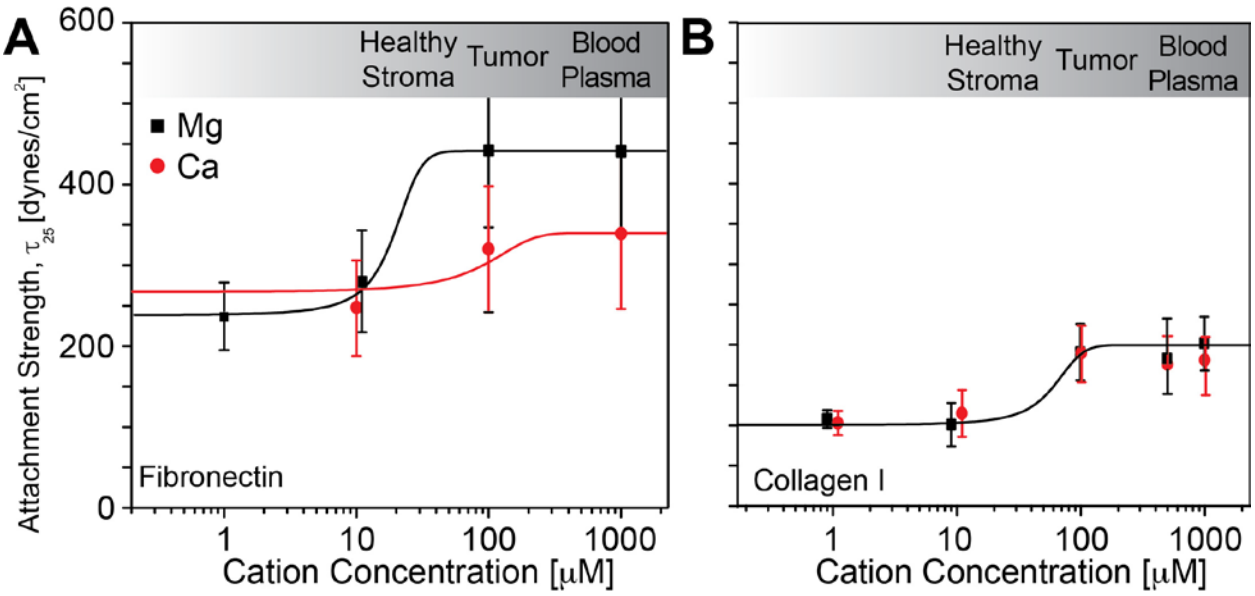

Figure S3: MCF10A Cells Exhibit Cation-Sensitive Change in Attachment Strength. For MCF10A, cells had homogeneous and strong attachment strengths, i.e. τ_{25}, as plotted versus cation concentration for Mg^{2+} (black squares) and Ca^{2+} (red circles) for cells bound to (A) collagen type I-coated and (B) fibronectin-bound coverslips. Cation concentration range for the indicated tissue is provided for reference. A sigmoidal fit for each cation is shown in panel A but they are combined in panel B.

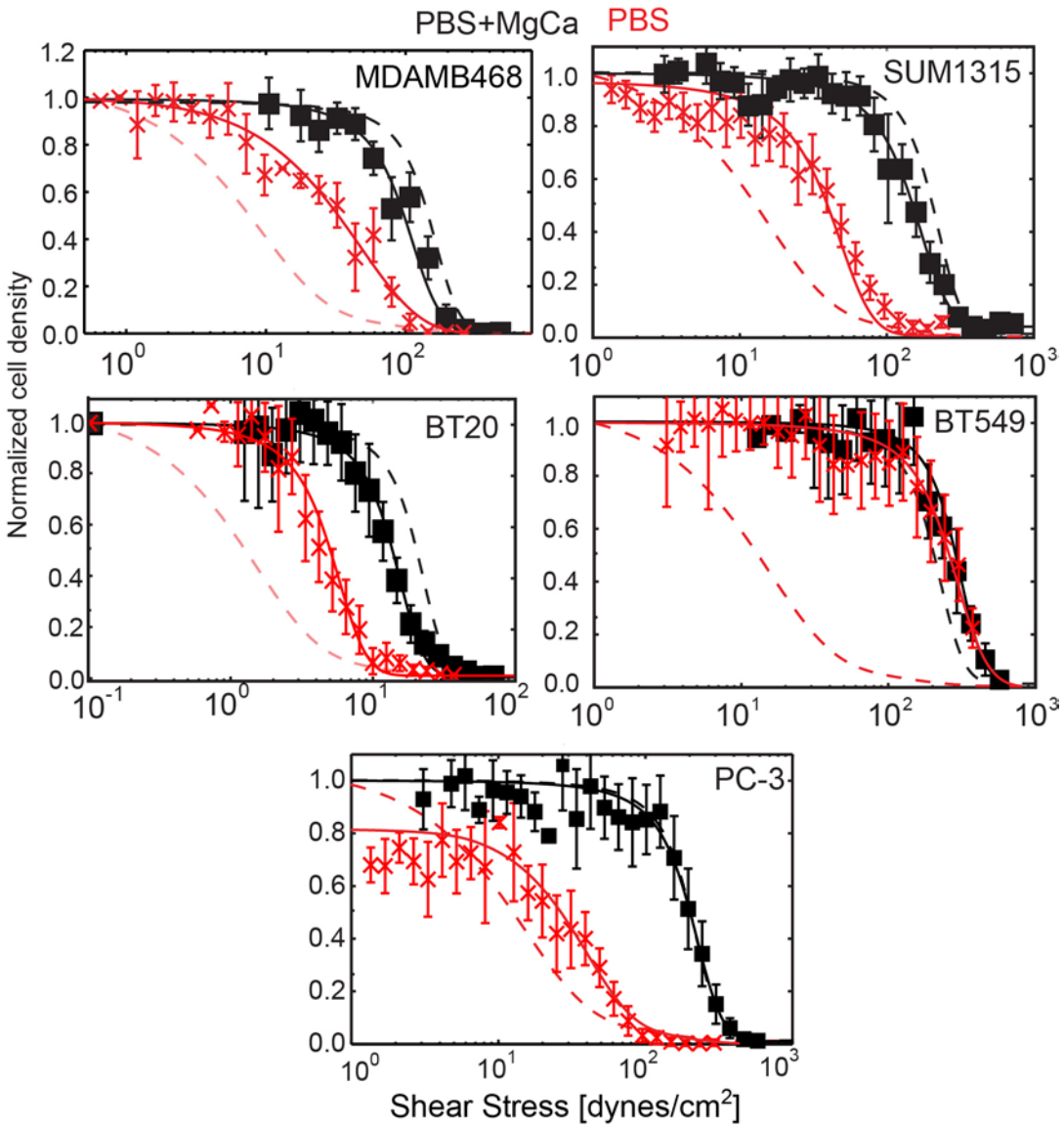

Figure S4: Attachment Strength is Heterogeneous for Additional Mammary Epithelial Cells and Prostate Cancer Cells in Stromal-like Niche. Normalized cell density is plotted versus shear for MDAMB468, SUM1315, BT20, BT549 and PC-3 cells. Cells were tested with (black) and without (red) media containing cations as defined in Figure 1. Dashed lines in each plot indicate the fits for MDAMB231 cells with (black) and without (red) media containing cations.

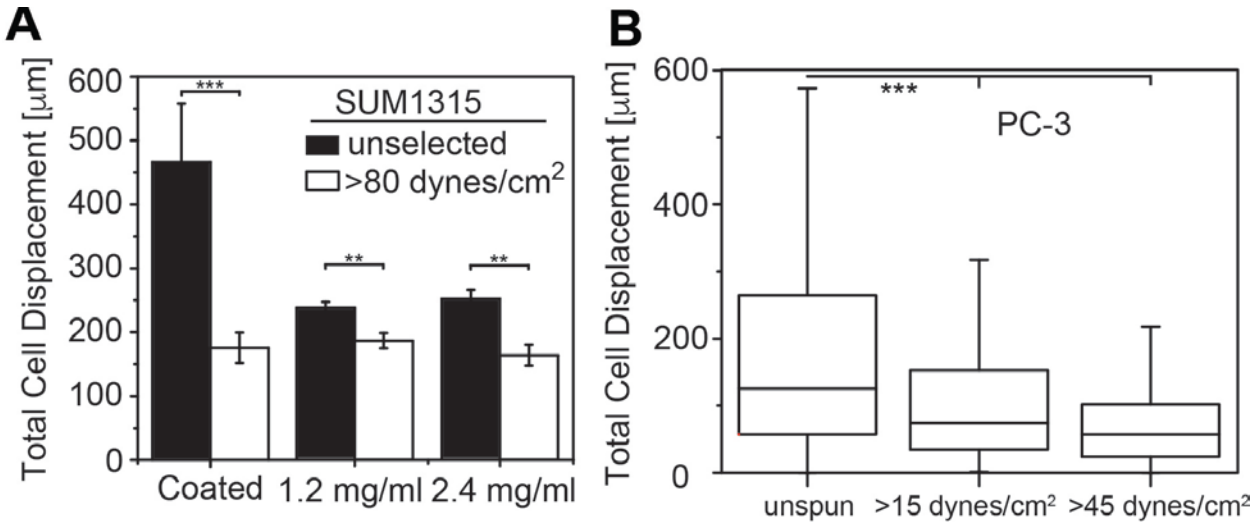

Figure S5: Migration for SUM1315 and PC-3. (A) SUM1315 cells, either unselected (blue) or selected with 80 dynes/cm^2 (orange), plated onto collagen-coated, planar substrates (left) and 1.2 mg/ml (center) and 2.4 mg/ml (right) collagen hydrogels were plotted for the total distance migrated over 24 hours post-plating. Note that the migration of many unselected cells on planar surfaces exceeded the viewable window of the microscope over 24 hours, and thus these data represent a minimum distance traveled. (B) Total cell displacement over 24 hours for PC3 cells are plotted for the indicated shear stress selection conditions on collagen-coated substrates. PC-3 cell migration is more heterogeneous and thus displayed in a box and whisker plot **p <0.01, ***p < 0.001.

SUPPLEMENTAL TABLE

Cell Line	Base Media	Serum	Antibiotics	Others
MCF10A, MCF10AT	DMEM/ F12	5% HS	100 units/ml Penicillin, 100 µg/ml Streptomycin	0.5 µg/ml Hydrocortisone, 20 ng/ml hEGF, 10 µg/ml Insulin, 100 ng/ml Cholera toxin
MCF7	DMEM	10% FBS	100 units/ml Penicillin, 100 µg/ml Streptomycin	10 µg/ml Insulin
MDAMB231, MDAMB468, BT20	DMEM	10% FBS	100 units/ml Penicillin, 100 µg/ml Streptomycin	
SUM1315	DMEM/ F12	5% FBS	100 units/ml Penicillin, 100 µg/ml Streptomycin	5µg/ml hEGF, 5 µg/ml Insulin
BT549	DMEM	10% FBS	100 units/ml Penicillin, 100 µg/ml Streptomycin	1 µg/ml Insulin
PC3	F-12K	10% FBS	100 units/ml Penicillin, 100 µg/ml Streptomycin	

Table S1: Media formulations for the indicated cell lines.