

Supporting Materials

The Cytoskeleton Regulates Cell Attachment Strength

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Figure S1

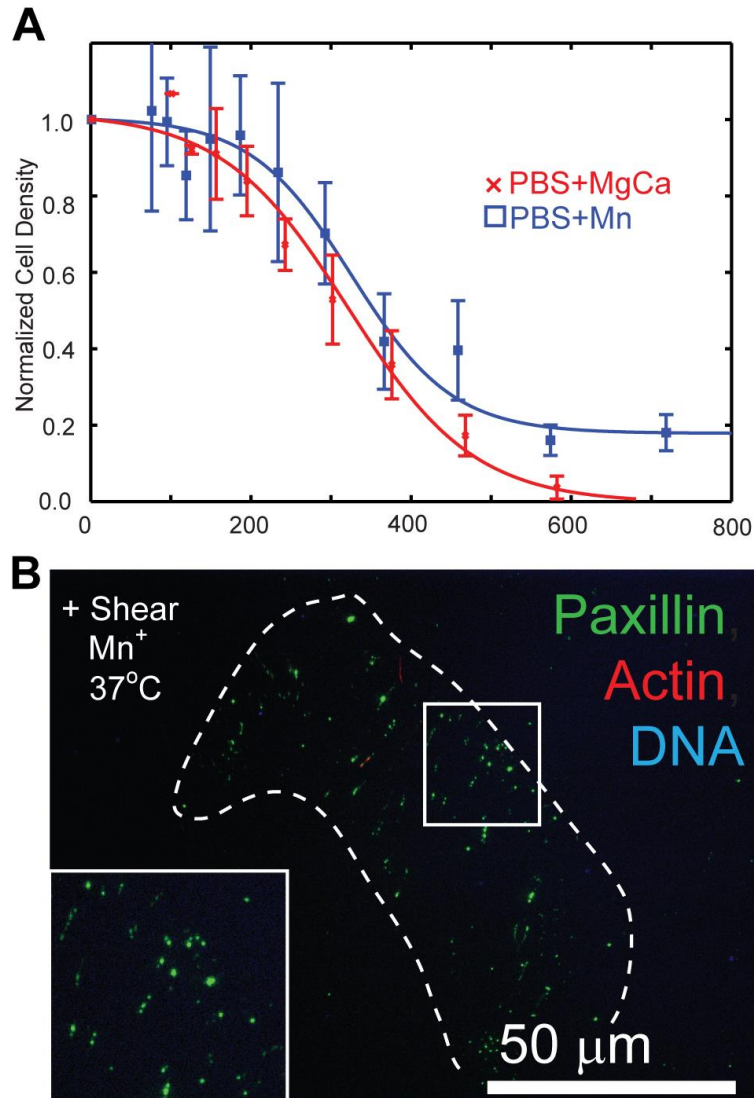


Figure S1: Effect of Manganese (Mn²⁺) on cell adhesion strength.

(A) Attachment strength of HT1080 in presence of 0.5mM Mn²⁺ compared to PBS+MgCa. (B) Cellular footprint of a detached cell in PBS+Mn condition at high shear (>600 dynes/cm²).

Figure S2

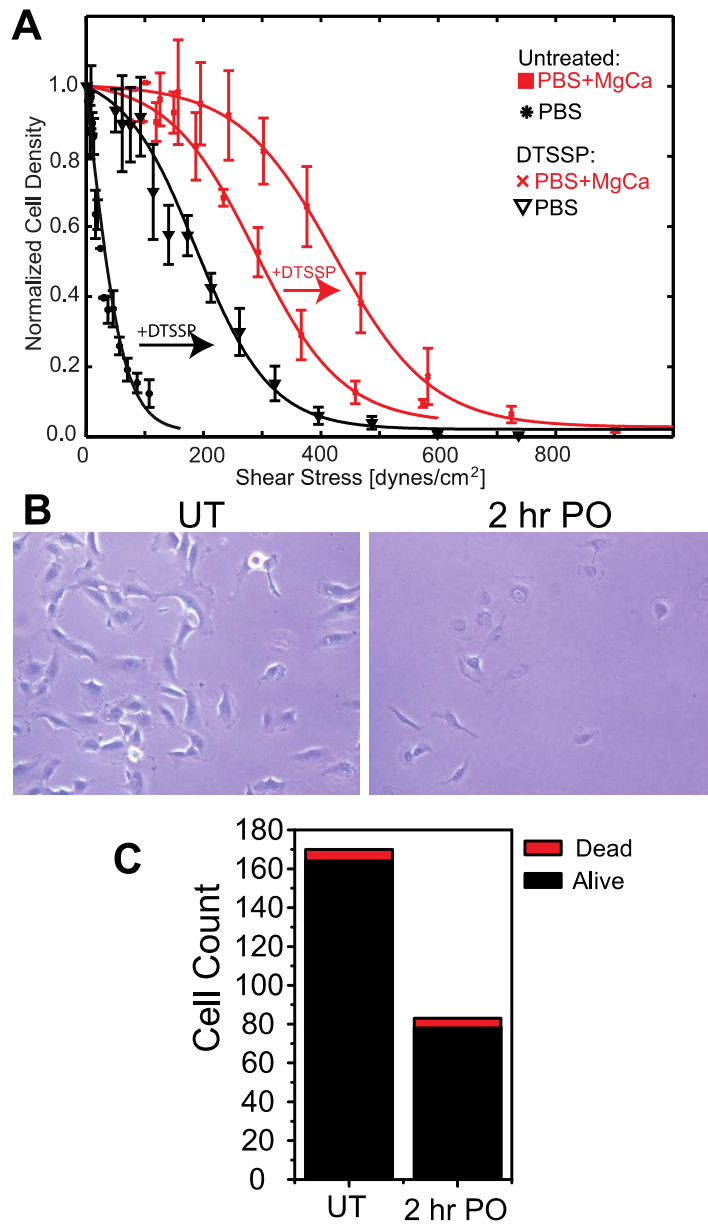


Figure S2: Function test of DTSSP treatment and viability test of PO treated cells. (A) DTSSP treatment increases attachment strength ~40% in presence of cations (red). In absence of cations (black), DTSSP treated cell's attachment strength is slightly reduced (~30%) but nearly one order of magnitude higher compared to untreated cells. (B) Representative 10x brightfield images of 3T3 fibroblasts. Cells were seeded at same density and then either treated for 2 hours with PO (right) or untreated (left). No visual differences between treatments but PO treated cell density is visually smaller. (C) Quantification of cell numbers after 24hrs and live-dead assay with Trypan Blue. Untreated 3T3 cell number doubled within 24hrs but PO treated cells did not. No significant difference in Trypan Blue positive cell ratio of PO treated cells.

Figure S3

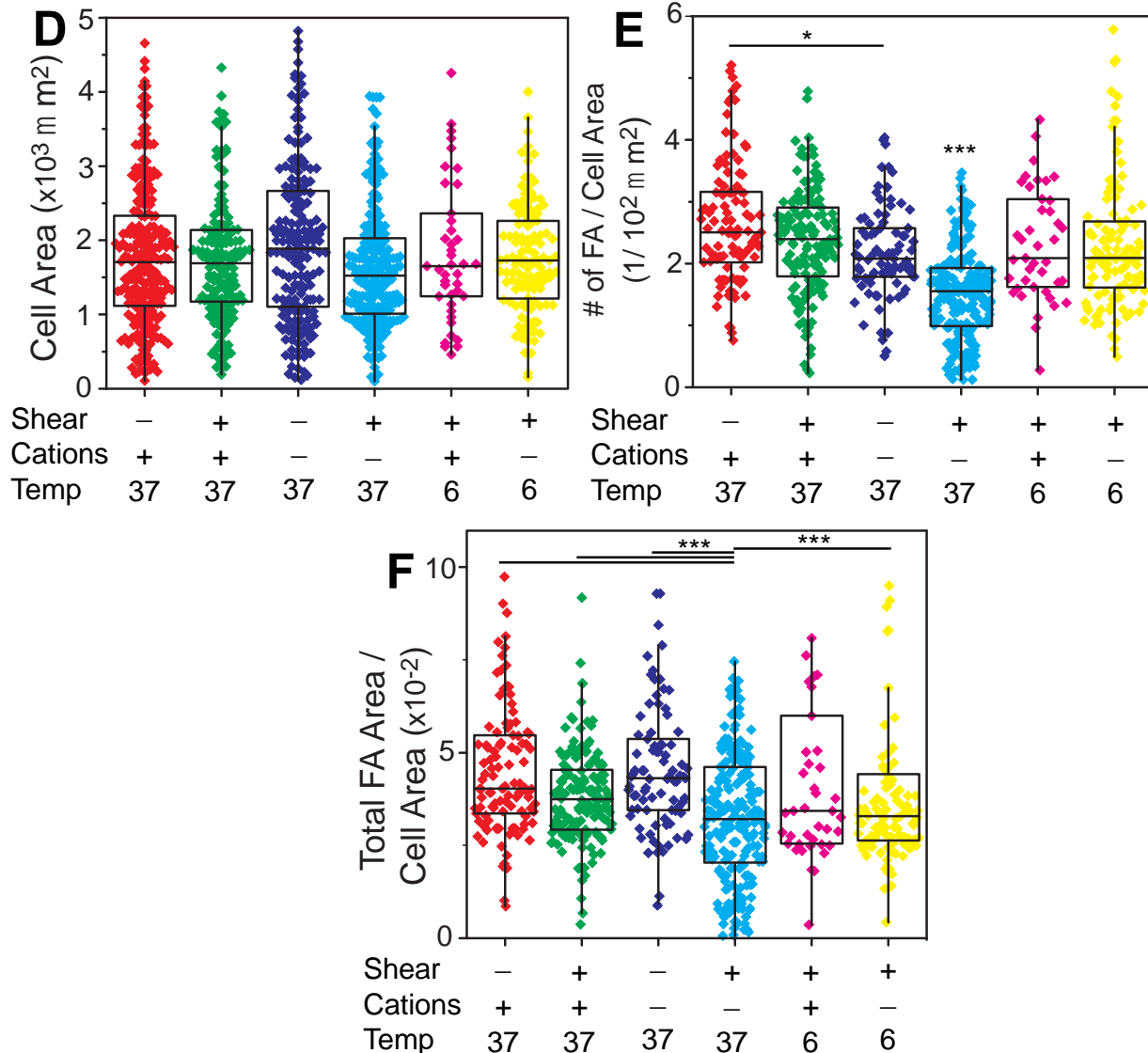
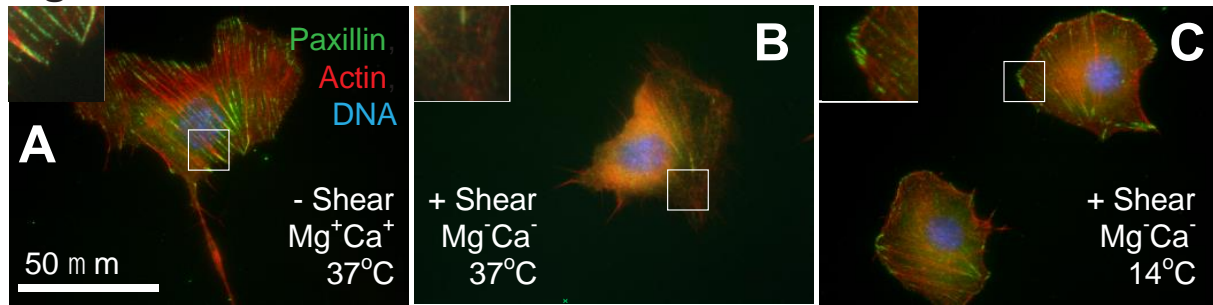


Figure S3: Fibroblast adhesion metrics measured as a function of shear, cations, and temperature. (A-C) Images of fibroblasts stained for paxillin (green), actin (red), and nuclei (blue). Cells were cultured in identical media and temperature conditions prior to 5-minute application of shear and/or change in the indicated culture conditions. Scale bar is 50 microns. (D-F) Quantification of indicated cell parameters as a function

of the applied shear, cation concentration in the buffer, and temperature of the buffer during 5-minute application of shear and/or change in the indicated culture conditions.

Figure S4

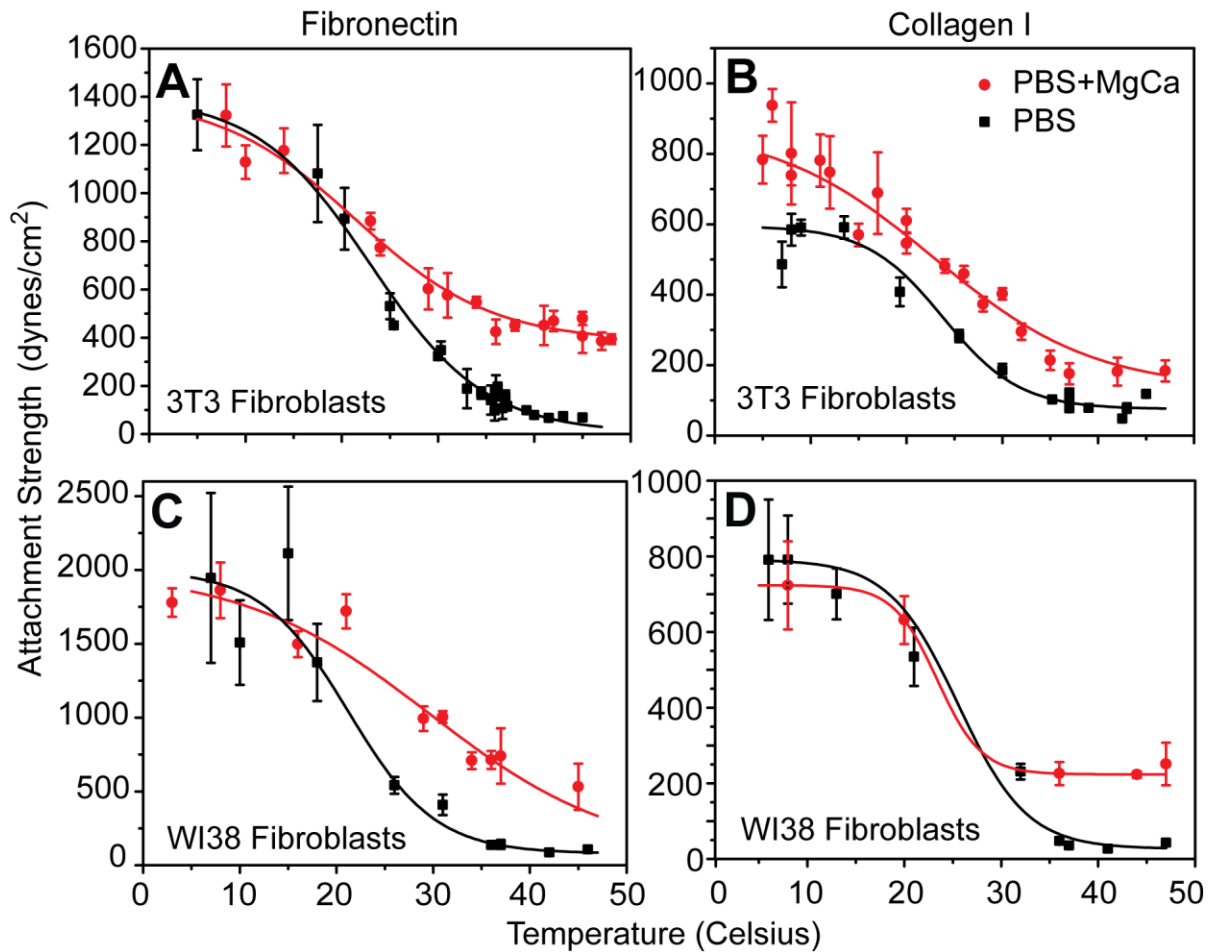


Figure S4: Attachment strength of 3T3 and WI38 cells monotonically increases with decreasing temperature independent of substrate. Attachment strength of 3T3 (top) and WI38 (bottom) cells in dependence of temperature with cations (red) or without cations (black) on fibronectin (A and C) or Collagen type 1 (B and D).