



Supporting Information

for

Effects of substrate stiffness on the viscoelasticity and migration of prostate cancer cells examined by atomic force microscopy

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Additional figures

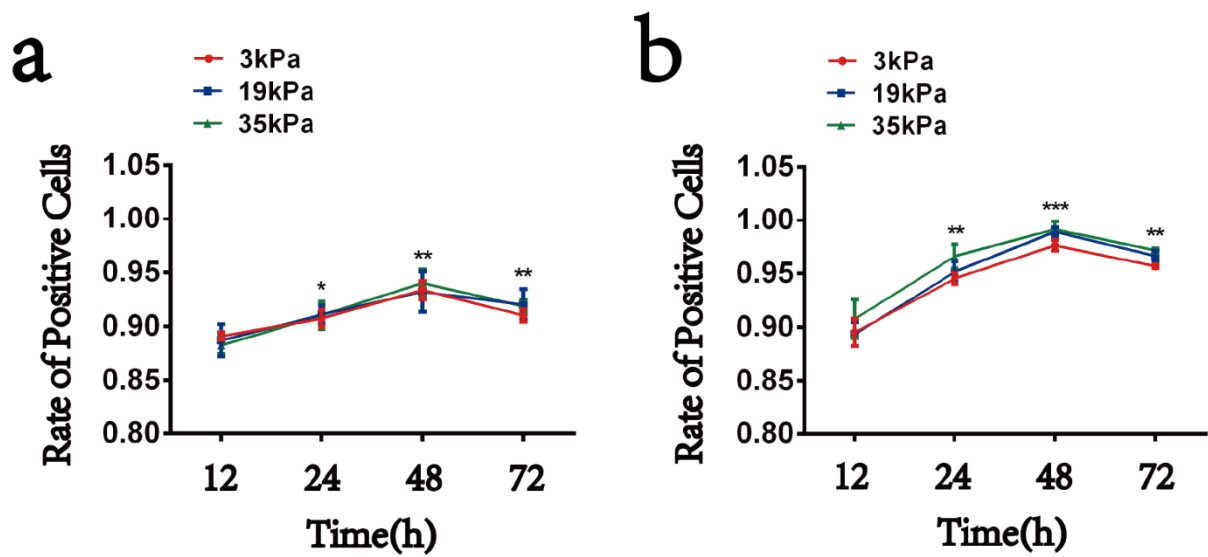


Figure S1: The toxic effect of polyacrylamide hydrogel substrates on prostate cancer cells. (a,b) Ratio of Calcein-AM/PI labelled HPV-PZ-7 and PC-3 cells, respectively.

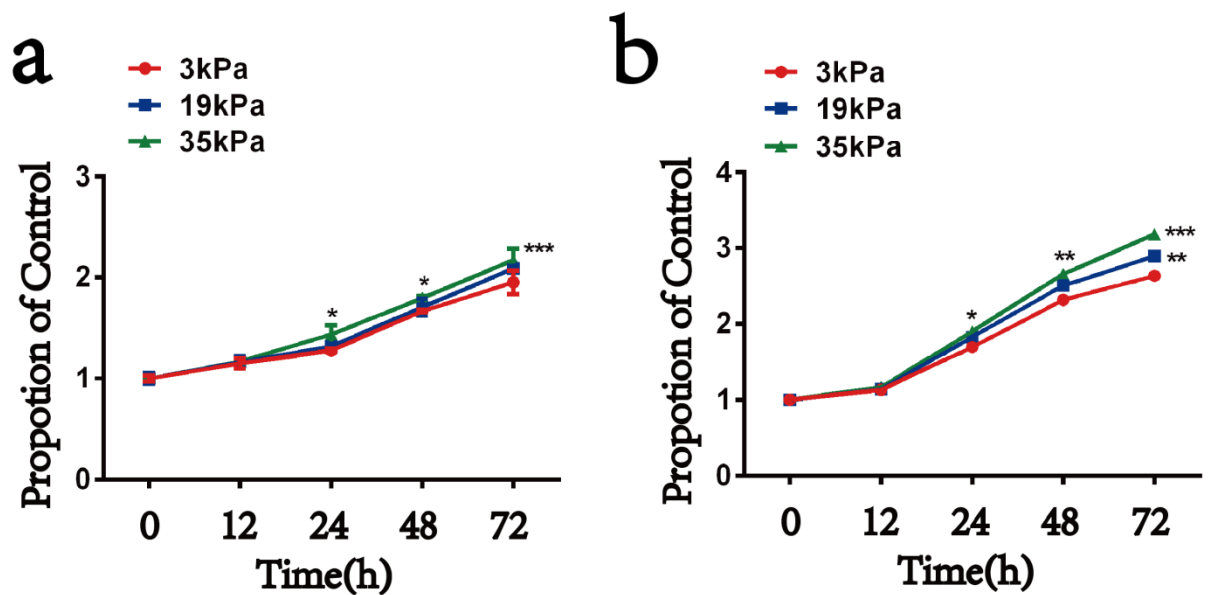


Figure S2: The effect of substrate stiffness on cell proliferation. (a,b) CCK-8 detects proliferation of HPV-PZ-7 and PC-3 cells. The proliferation rate of cells plated on 19 and 35 kPa was compared to that of cells plated on at 3 kPa substrates.

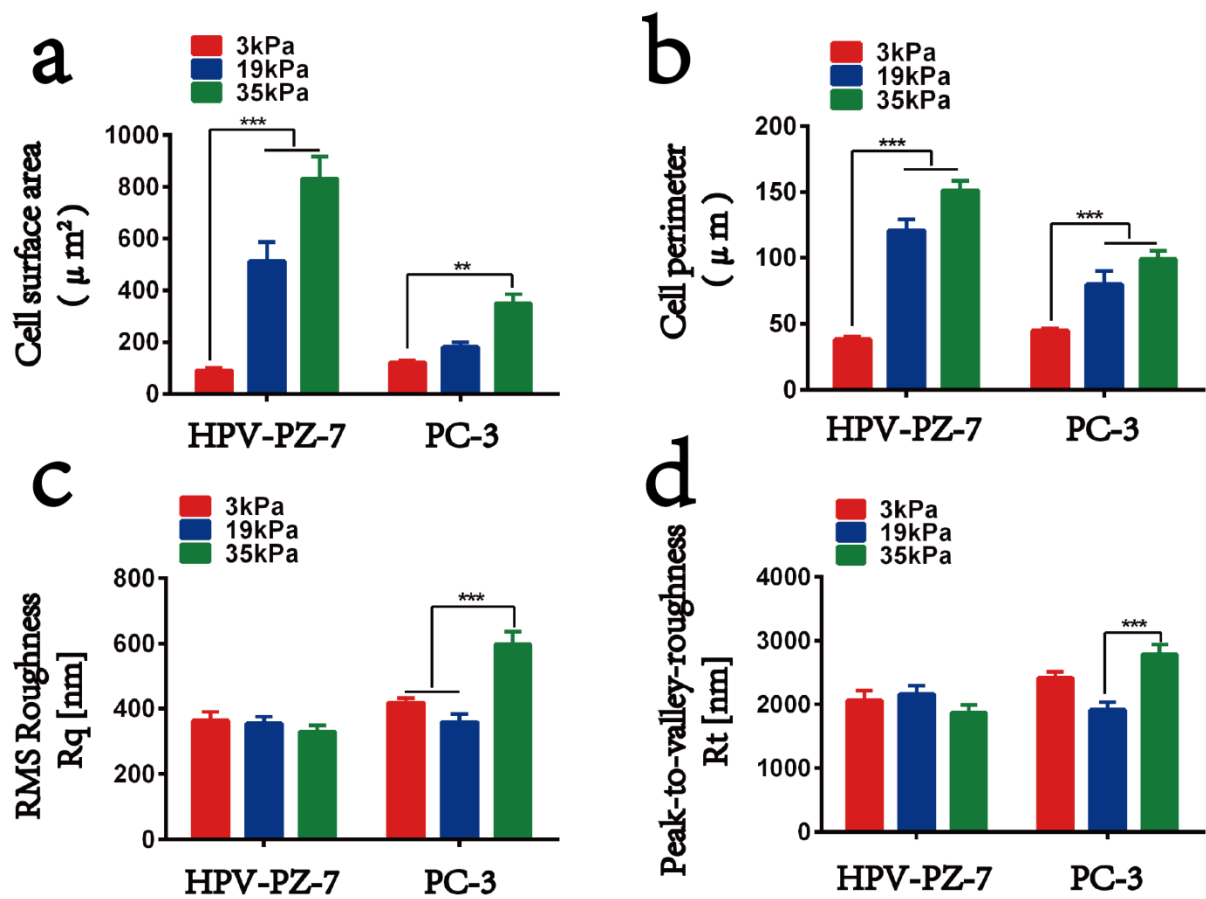


Figure S3: The effect of substrate stiffness on cell morphology. (a) Phase-contrast microscopy imaging of PZ-HPV-7 and PC-3 cells on different stiffness substrates were used to obtain measurements of cell area. (b) Measurements of cell perimeter. (c) Atomic force microscopy imaging of HPV-PZ-7 and PC-3 cells on substrates with different stiffness was used to obtain measurements of the root mean square surface roughness (Rq). (d) Measurements of peak-to-valley ratio roughness (Rt). ** $p < 0.01$, *** $p < 0.001$.

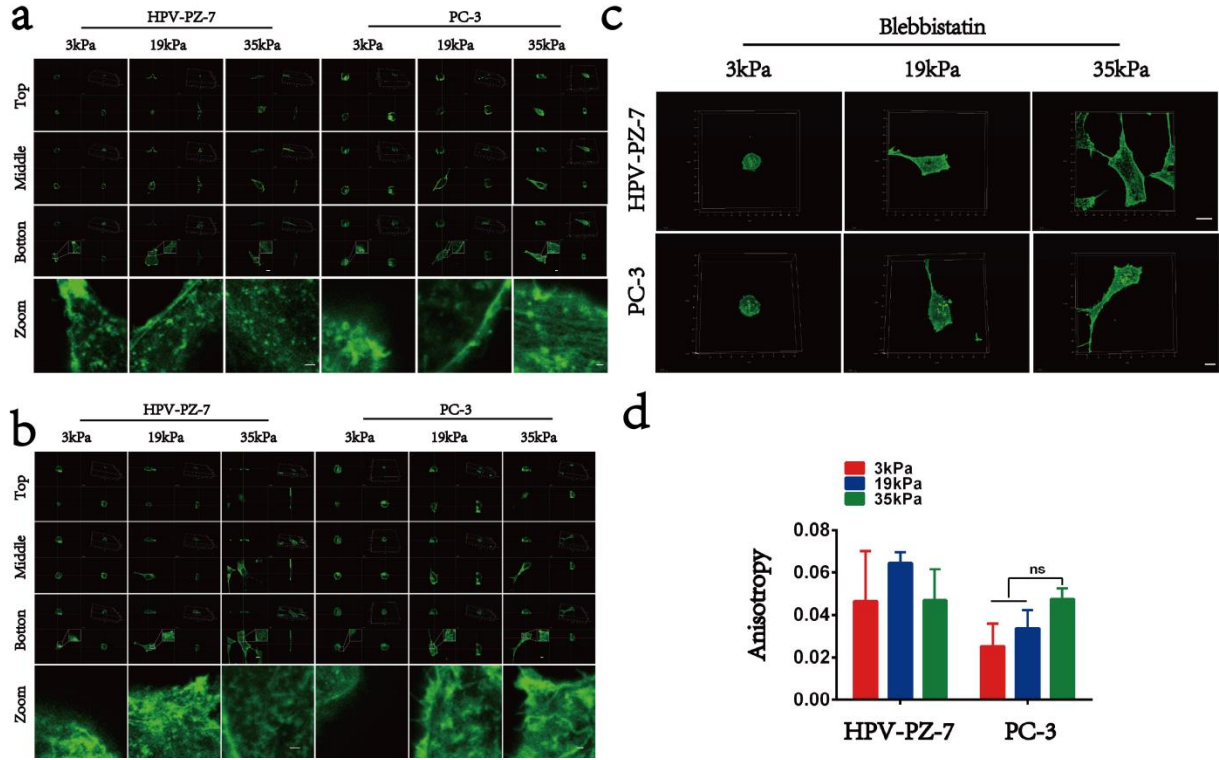


Figure S4: The effect of blebbistatin on PCa cytoskeleton microfilaments. (a) Three-dimensional fluorescence images of cytoskeletal microfilaments of HPV-PZ-7 and PC-3 cells on substrates with different stiffness. The zoomed image in the top right corner is a 2.5 \times enlargement of an area taken from the original image with a size of 2.2×2.2 . The scale bar of HPV-PZ-7 cells is 20 μm and the scale bar of PC-3 cells is 10 μm . (b) Three-dimensional fluorescence images of cytoskeletal microfilaments of HPV-PZ-7 and PC-3 cells on substrates with different stiffness after blebbistatin treatment. (c) The fluorescence images of HPV-PZ-7 and PC-3 cytoskeletal microfilaments on substrates with different stiffness after blebbistatin treatment; the scale bar of HPV-PZ-7 cells is 20 μm , and the scale bar of PC-3 cells is 10 μm . (d) Anisotropy quantification maps of the fibrillar structure of HPV-PZ-7 and PC-3 cells on substrates with different stiffness after blebbistatin treatment; "ns" means no difference.

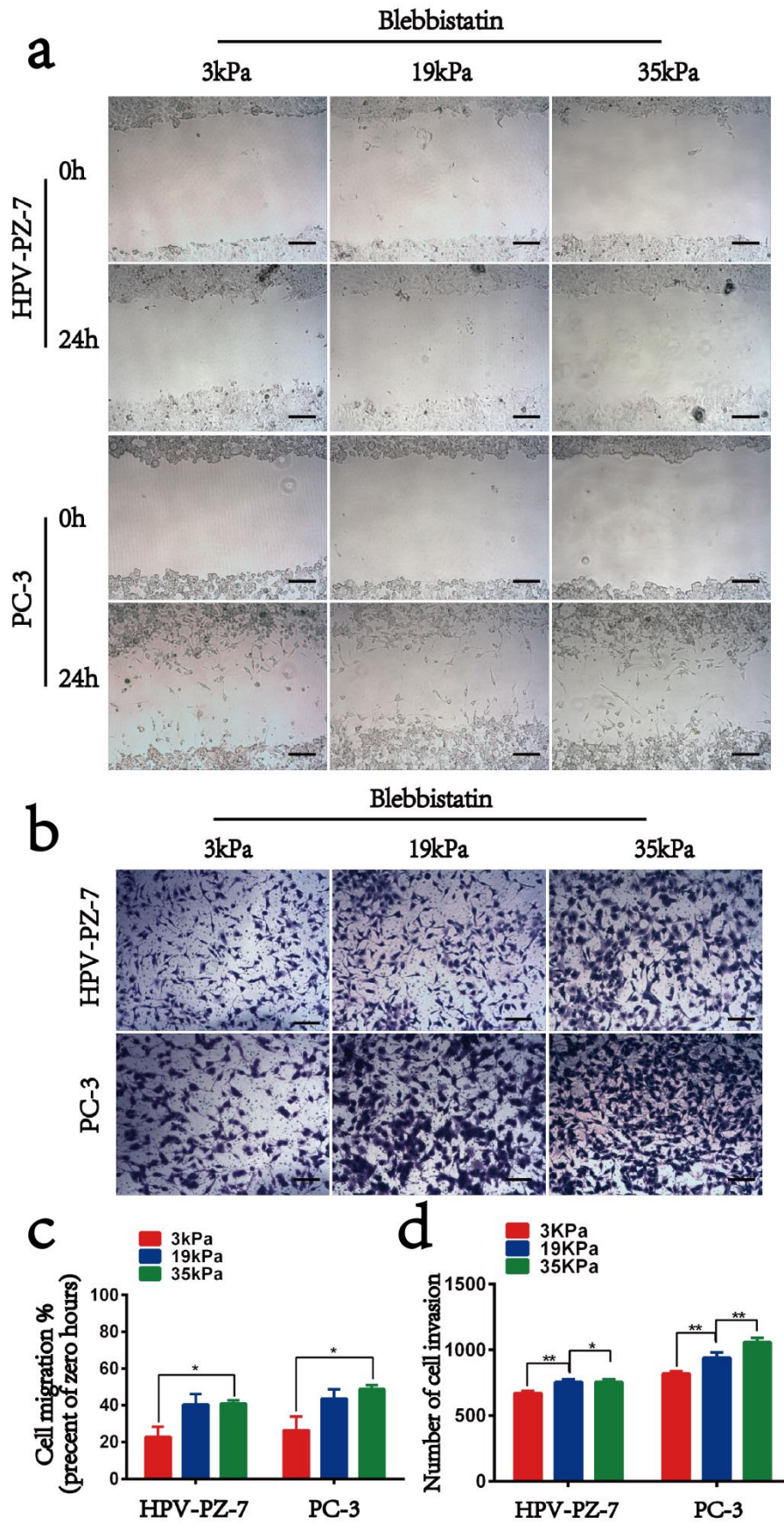


Figure S5: The effect of blebbistatin on PCa cell migration. (a) Cell wound healing analysis of the migration ability of HPV-PZ-7 and PC-3 cells on substrates with different

stiffness after blebbistatin treatment. (b) Analysis of transwell invasion ability of HPV-PZ-7 and PC-3 cells on substrates with different stiffness after blebbistatin treatment. (c) Quantitative analysis of the migration ability of HPV-PZ-7 and PC-3 cells on substrates with different stiffness after blebbistatin treatment. (d) Quantitative analysis of the invasion ability of HPV-PZ-7 and PC-3 cells on substrates with different stiffness after blebbistatin treatment. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

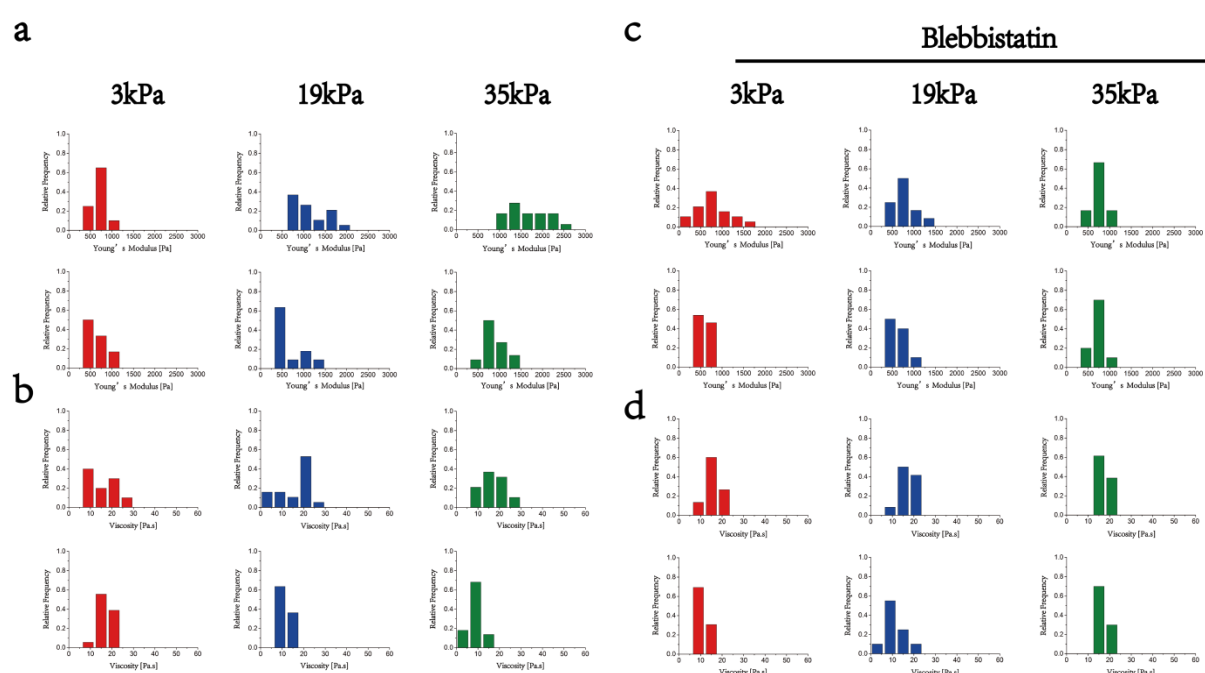


Figure S6: Mechanical properties respond to the effect of substrate stiffness on prostate cancer cells. (a) Histograms of the elastic frequency distribution of HPV-PZ-7 and PC-3 cells on substrates with different stiffness. (b) Histograms of the viscosity frequency distribution of HPV-PZ-7 and PC-3 cells on substrates with different stiffness. (c) Histogram of elastic frequency distribution of HPV-PZ-7 and PC-3 cells on substrates with different stiffness after blebbistatin treatment. (d) Histogram of the viscosity frequency distribution of HPV-PZ-7 and PC-3 cells on substrates with different stiffness after blebbistatin treatment.