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Supplemental Information

Breast Cancer Cells Adapt Contractile Forces to Overcome Steric Hindrance

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Note 1: Correlation analysis between cell contractility and aspect ratio

We find a weak but not statistically significant ($p > 0.05$) negative correlation between contractility and aspect ratio of the cell contour in a collagen gel, indicating that contractility is only slightly lower in more elongated cells (Fig. S1). This holds true for all 3 conditions (MDA-control, MDA-lamA, MDA-beads).

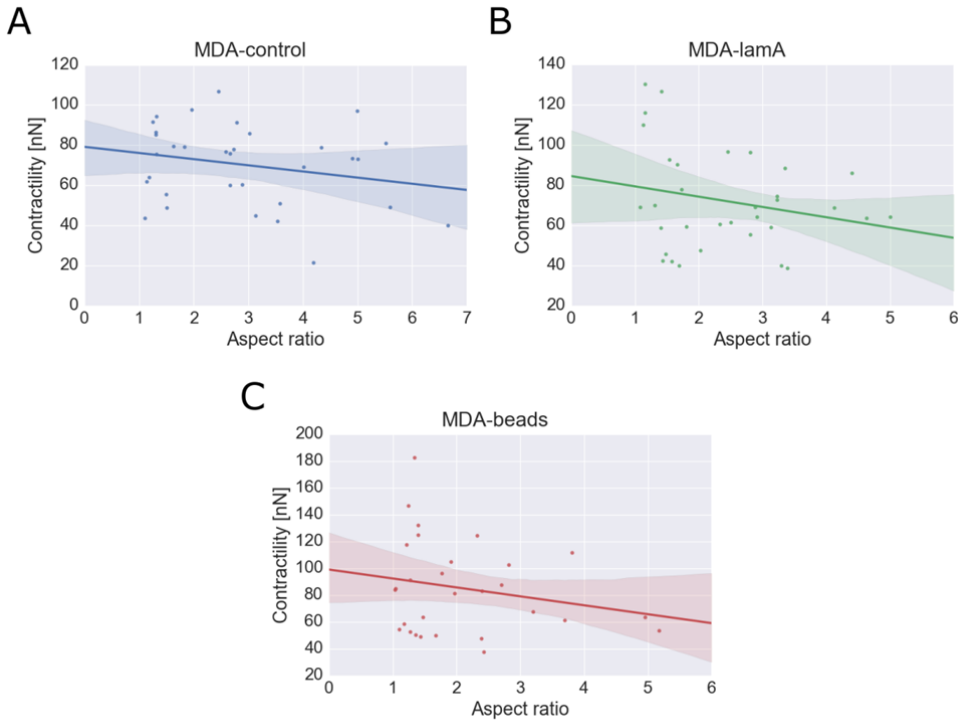

Figure S1. Correlation analysis between cell contractility and aspect ratio for: A) MDA-MD-231 control cells, B) MDA-MD-231 cells with lam-A overexpression and C) MDA-MD-231 cells with a 5-µm polystyrene bead.

Note 2: 3-Dimensional force field around individual MDA-MD 231 cells

Below, the individual 3D force fields around all measured MDA-MB-231 breast carcinoma cells (n = 33), MDA-MD-231 cells with lam-A overexpressed (n = 33) and MDA-MD-231 cells with 5-µm polystyrene beads (n = 30) in 1.2 mg/ml collagen gels are presented.

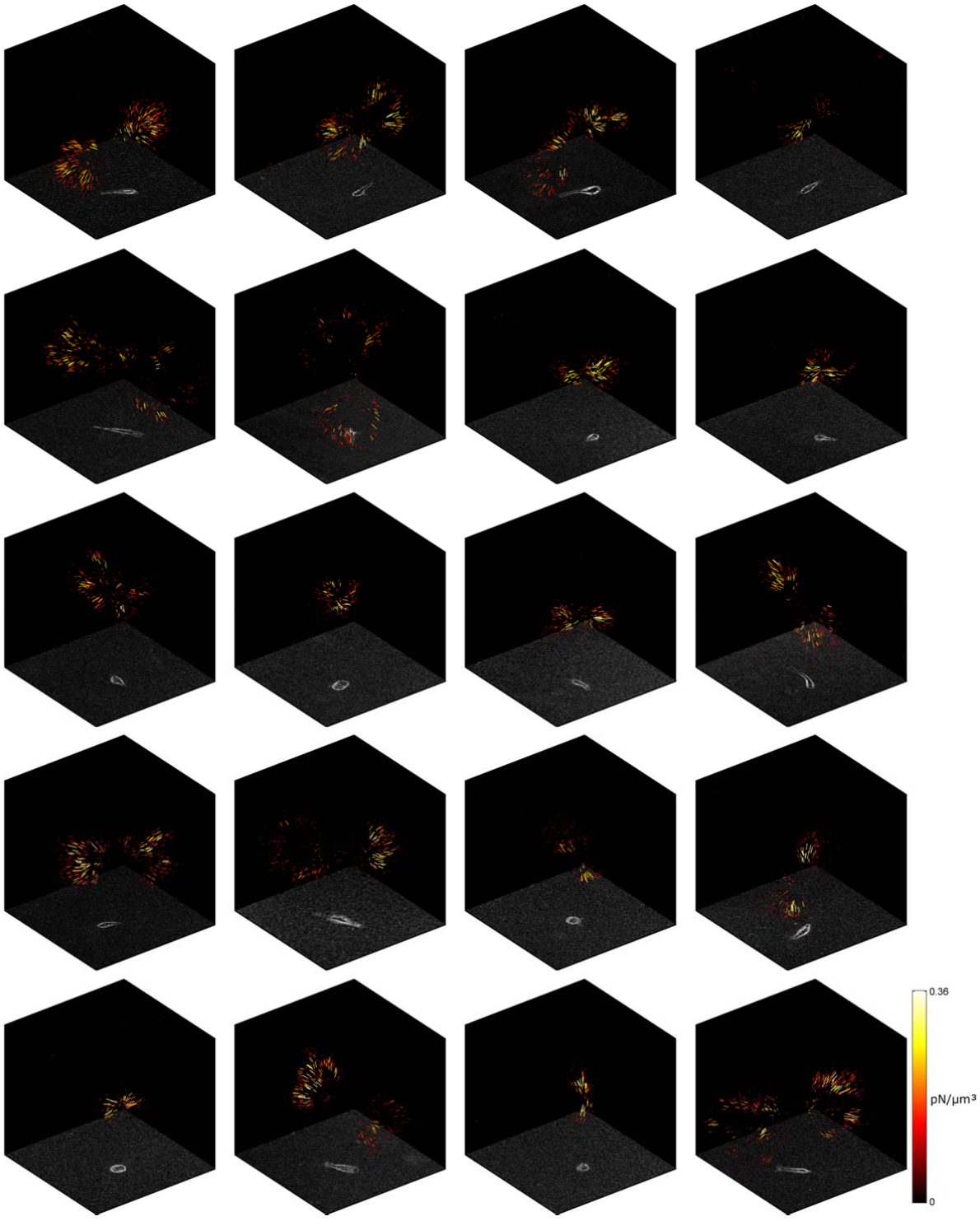

Figure S2: For caption see next figure.

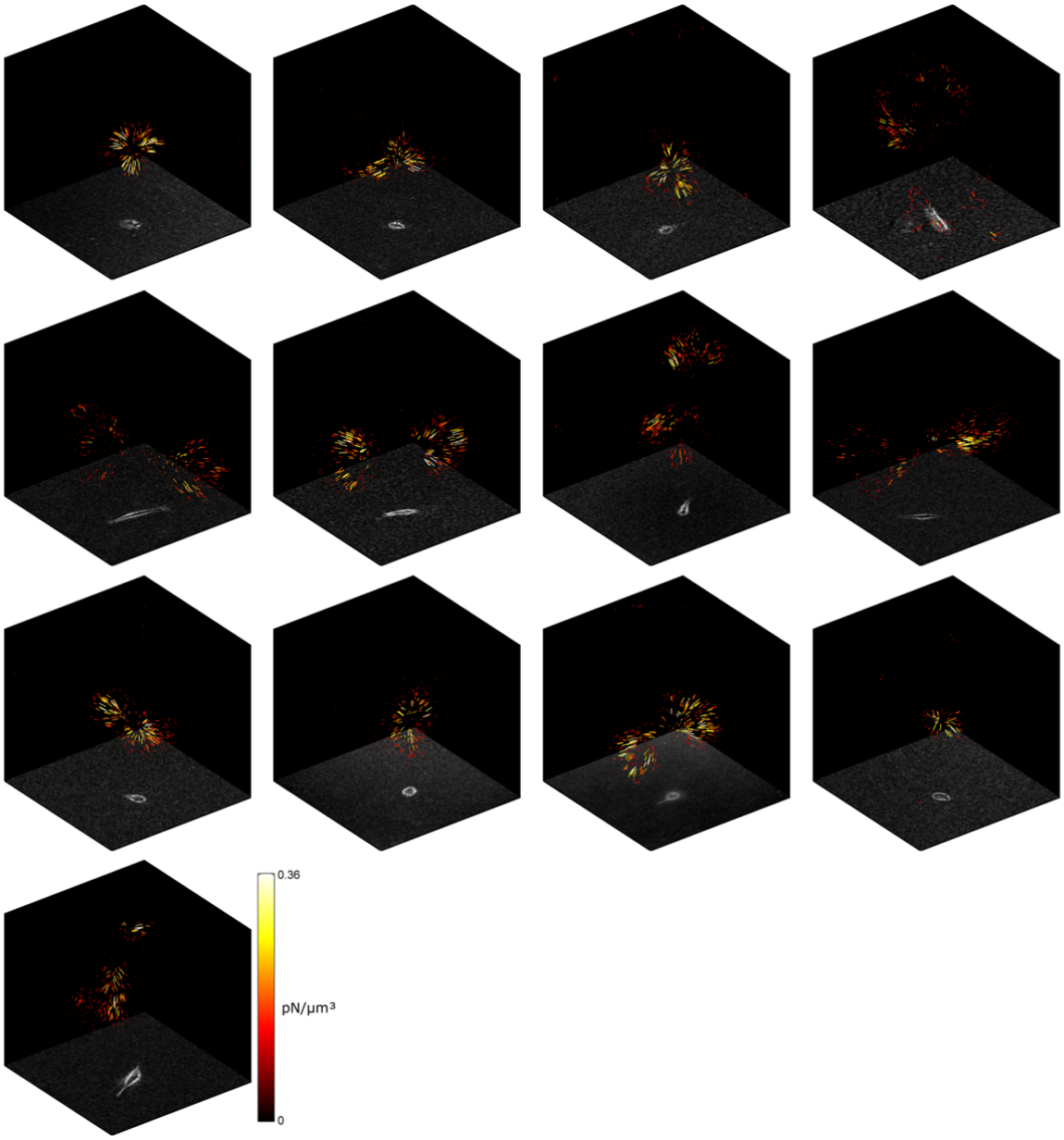

Figure S3. 3-D traction force density maps for all 33 control MDA-MB 231 breast carcinoma cells included in this study. Marker length and color indicate the magnitude of the force vectors. Edge length of the cubic box is 130µm. The bottom face shows a bright-field maximum intensity z-projection of gel volume.

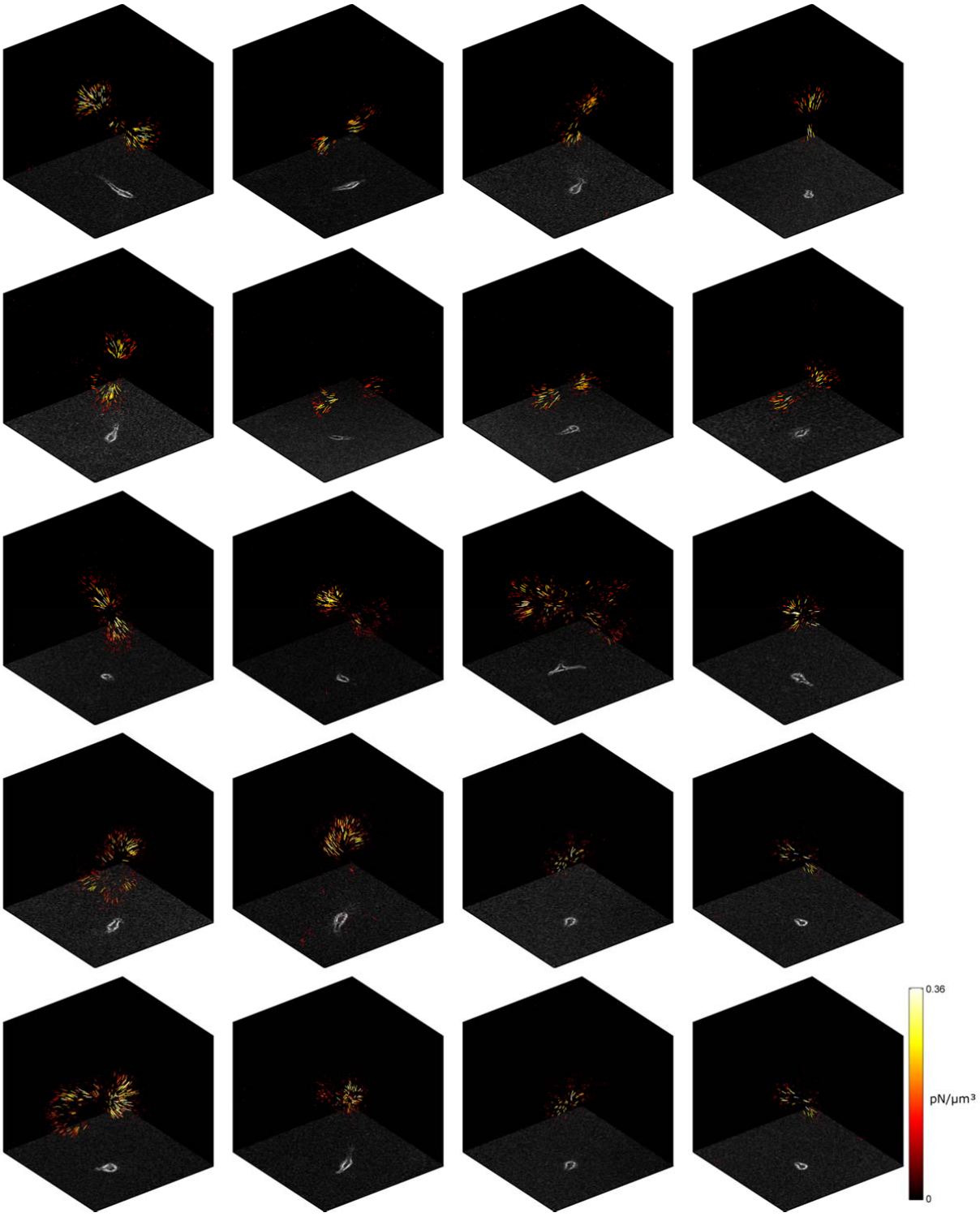

Figure S4: For caption see next figure.

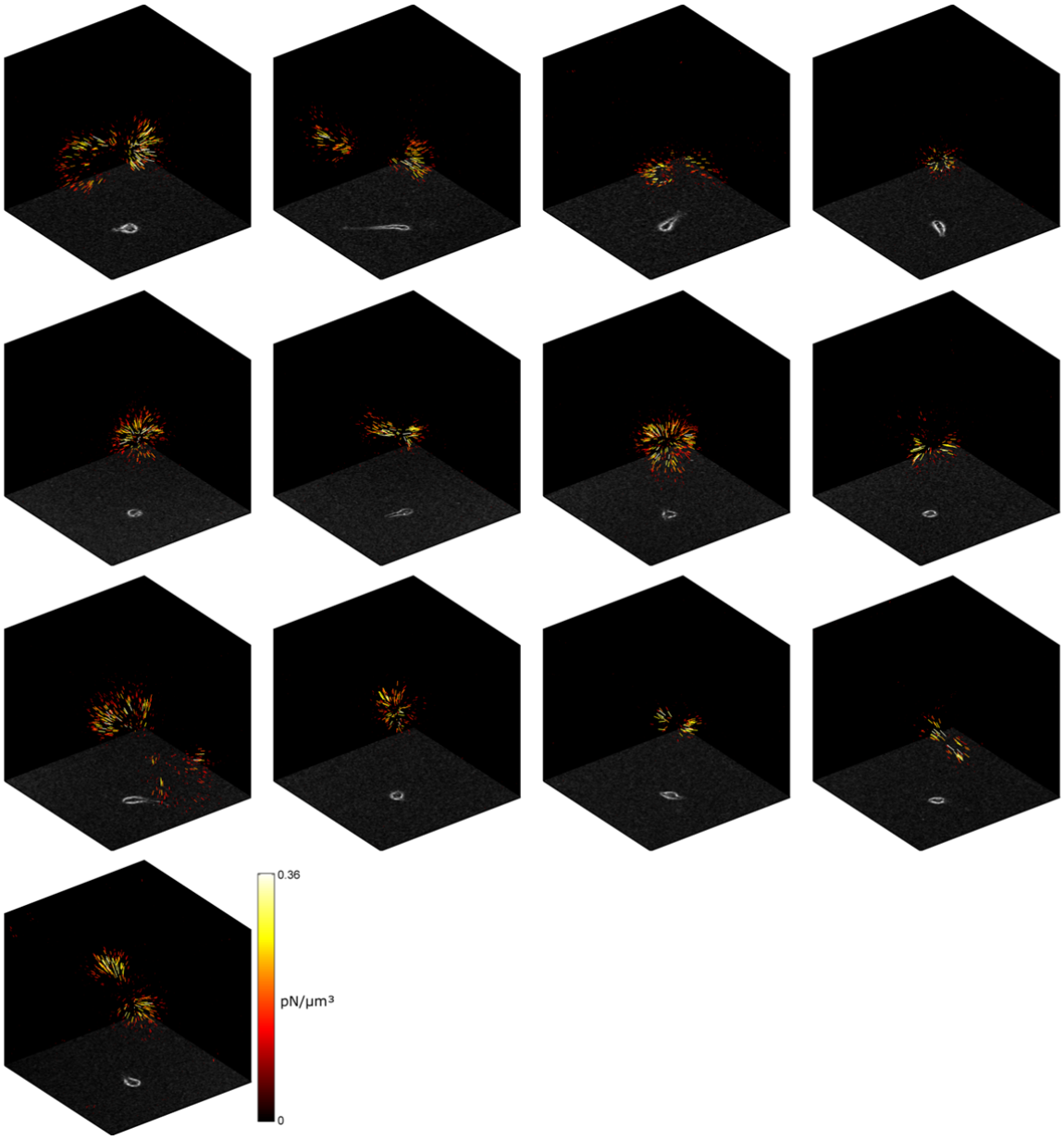

Figure S5: 3-D traction force density maps for all 33 MDA-MB 231 breast carcinoma cells with lamin A overexpression included in this study. Marker length and color indicate the magnitude of the force vectors. Edge length of the cubic box is 130 µm. The bottom face shows a bright-field maximum intensity z-projection of the gel volume.

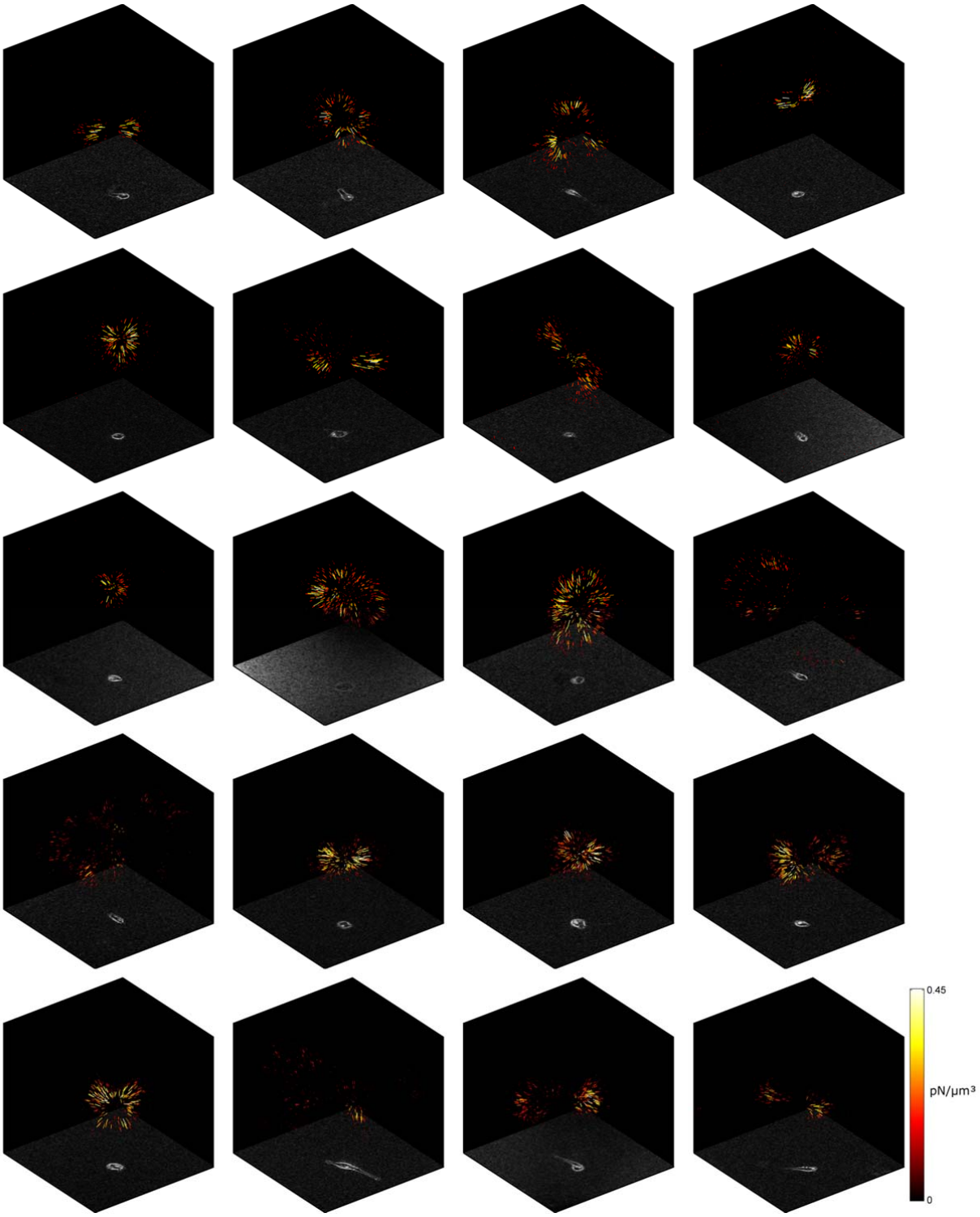

Figure S6: For caption see next figure.

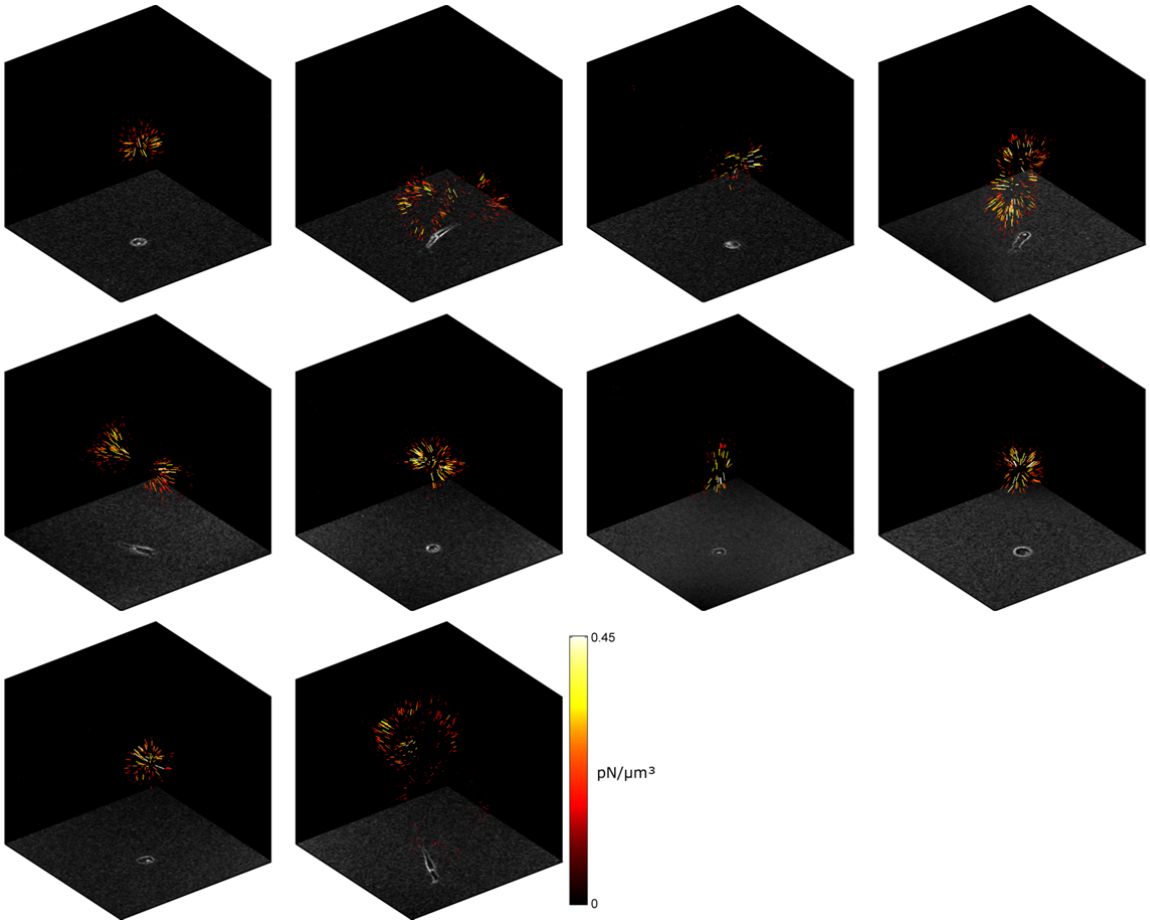

Figure S7: 3-D traction force density maps for all 30 MDA-MB 231 breast carcinoma cells with a 5-µm polystyrene bead. Marker length and color indicate the magnitude of the force vectors. Edge length of the cubic box is 130 µm. The bottom face shows a bright-field maximum intensity z-projection of the gel volume.

Note 3: Collagen matrix structure

Confocal reflection images of the collagen fibers show a denser structure in close proximity around the cells, suggesting that this is caused by fiber alignment and compaction due to contractile forces exerted by cells (1, 2). No apparent holes or tunnels can be observed in the collagen matrix for the 3 cases studied (Fig. S8)

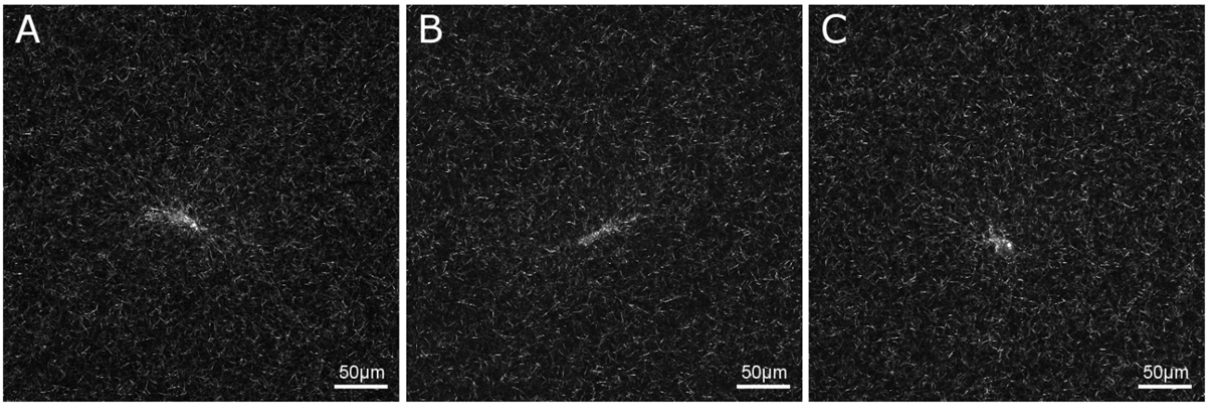

Figure S8. Collagen matrix structure around cells. Collagen fiber network imaged using confocal reflection microscopy around an embedded cell: A) MDA-MB-231 cell, B) MDA-MD-231 cell with lam-A overexpressed and C) MDA-MD-231 cell with a 5-µm polystyrene bead. Cells locally compact collagen fibers by a similar degree, as indicated by the increased brightness of the matrix around each cell.

Note 4: Mechanical characterization of collagen gels

Following (3), we assumed that the collagen gels deform in a non-affine and non-linear way. This behavior can be described with 4 parameters: a linear elastic stiffness K_0, a strain range L_S at which the matrix starts to stiffen, a strain scale d_S describing the exponential stiffening under stretch, and a strain scale d_0 describing the exponential softening under compression. To measure these 4 parameters, two different macrorheological experiments were performed. In the first experiment, the stress versus strain relationship of the matrix for simple shear deformation is measured in a cone-plate rheometer (Fig. S9A). In the second experiment, the vertical contraction of the matrix under horizontal uniaxial stretch is measured (Fig. S9B). For more information about these experiments, see Supplementary notes in (3).

The four material parameters used in this work to describe the behavior of the 1.2 mg/ml collagen gels were taken from (3): K_0=1645 Pa, d_0=0.00032, L_S=0.0075 and d_S=0.033 as estimated by fitting to macrorheological measurements (stress versus strain, and vertical contraction versus horizontal stretch) (Fig. S9).

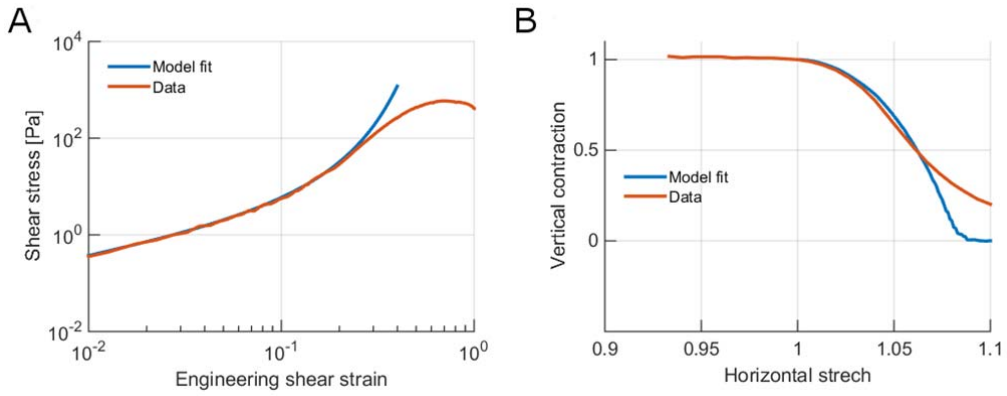

Figure S9. 1.2 mg/ml collagen mechanics. A) Material stress as a function of engineering shear strain predicted by the semi-affine model (blue), and the corresponding data (taken from Ref. (3)) measured with a cone-plate rheometer (red). B) Vertical contraction as a function of horizontal stretch predicted by the semi-affine model (blue), and the corresponding data (taken from Ref. (3)) measured in a uniaxial stretch experiment (red).

Note 5: Bead internalization

Fluorescent imaging of cells expressing td-Tomato-farnesyl (a live cell plasma membrane staining) is performed to check whether the beads were internalized. MDA-MD 231 cells are transfected with Lipofectamine 2000 (Life Technologies) according to the manufacturer's instructions (4).

Subsequently, cells (0.5×10^5) are seeded on fibronectin-coated coverslips (22 x 22 mm) and incubated overnight in complete cell culture medium (DMEM, Biochrom). To analyze the bead internalization, image stacks with a z-distance of 0.5 µm are taken using a motorized Leica 6000 inverse fluorescence microscope with a 63x 1.40 NA oil immersion objective (Fig. S.10). A total of 12 cells are imaged, and in all cases the cell membrane is covering the bead volume, confirming complete internalization of the bead.

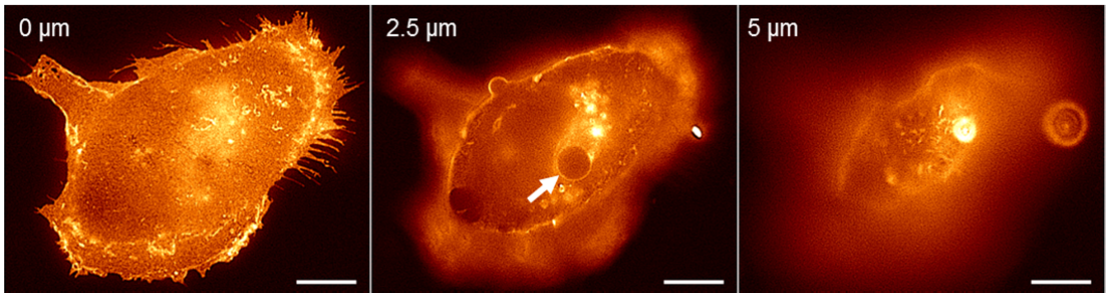

Figure S.10. Live cell membrane staining with td-Tomato-farnesyl. Fluorescence imaging of different z-sections of a representative MDA-MD 231 cell with an internalized polystyrene bead (arrow). Scale bar is 10 µm.

Supporting References

1. Lang, N. R., K. Skodzek, S. Hurst, A. Mainka, J. Steinwachs, J. Schneider, K. E. Aifantis, and B. Fabry. 2015. Biphasic response of cell invasion to matrix stiffness in three-dimensional biopolymer networks. Acta Biomaterialia 13:61-67.

2. Yang, Y. L., and L. J. Kaufman. 2009. Rheology and Confocal Reflectance Microscopy as Probes of Mechanical Properties and Structure during Collagen and Collagen/Hyaluronan Self-Assembly. Biophysical Journal 96(4):1566-1585.

3. Steinwachs, J., C. Metzner, K. Skodzek, N. Lang, I. Thievessen, C. Mark, S. Munster, K. E. Aifantis, and B. Fabry. 2016. Three-dimensional force microscopy of cells in biopolymer networks. Nature Methods 13(2):171-+.

4. Thievessen, I., N. Fakhri, J. Steinwachs, V. Kraus, R. S. McIsaac, L. Gao, B. C. Chen, M. A. Baird, M. W. Davidson, E. Betzig, R. Oldenbourg, C. M. Waterman, and B. Fabry. 2015. Vinculin is required for cell polarization, migration, and extracellular matrix remodeling in 3D collagen. Faseb Journal 29(11):4555-4567.