Supplementary information

Optical quantification of intracellular mass density and cell mechanics in 3D mechanical confinement

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**Fig. S1** MDA-MB-231 retain high viability after 5 days of encapsulation. Live/Dead images of MDA-MB-231 cells encapsulated in 3D alginate stiff (left image) and 3D alginate compliant (right image) after 5 days. Green (calcein) and red (ethidium homodimer-1) correspond to live and dead cells respectively. Scale bar equals 200 µm.

**Fig. S2** Comparison of RI, cell volume and ratio of nuclear to cytoplasmic RI among cells encapsulated in stiff and compliant 3D alginate hydrogels.

- **a)** Comparison of nuclear, perinuclear and cytoplasmic RI among cells encapsulated in stiff and compliant 3D alginate hydrogels.
- **b)** Comparison of volume of cells encapsulated in stiff and compliant 3D alginate hydrogels.
- **c)** Ratio of nuclear to cytoplasmic RI for cells in 3D alginate stiff and compliant hydrogels. Only statistically significant differences are marked.
Fig. S3 Nuclear to cytoplasmic ratio of Brillouin shift and linewidth among cells encapsulated in stiff and compliant 3D alginate hydrogels. Ratio of nuclear to cytoplasmic a) Brillouin shift and b) linewidth for cells in 3D alginate stiff and compliant hydrogels. Only statistically significant differences are marked.

Fig. S4 Brillouin measurements of cells seeded on 2D TCP. a) Brillouin shift ($\nu_B$) images of MDA-MB-231 cells seeded on 2D TCP. Segmentation of cytoplasm and nucleus was performed using brightfield and epifluorescence images, respectively. b) Brillouin shift ($\nu_B$) and corresponding longitudinal modulus ($M$) quantification for cells seeded on 2D TCP. c) Linewidth images ($\Delta_B$) of MDA-MB-231 cells seeded on 2D TCP. d) Linewidth ($\Delta_B$) and corresponding viscosity ($\eta$) quantification for cells seeded on 2D TCP. Only statistically significant differences are marked. Scale bar equals 10 µm.