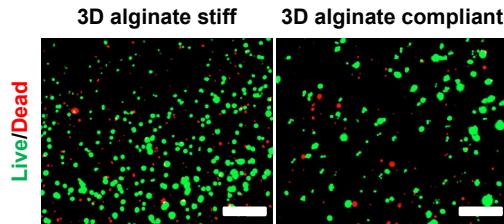


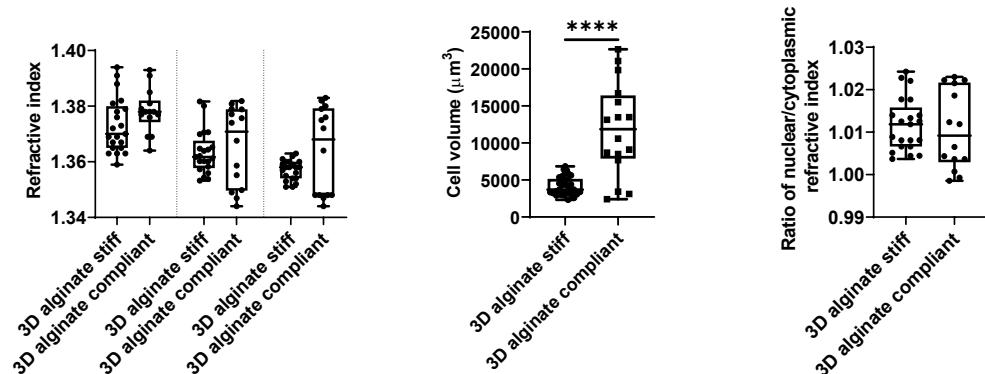
## Supplementary information

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

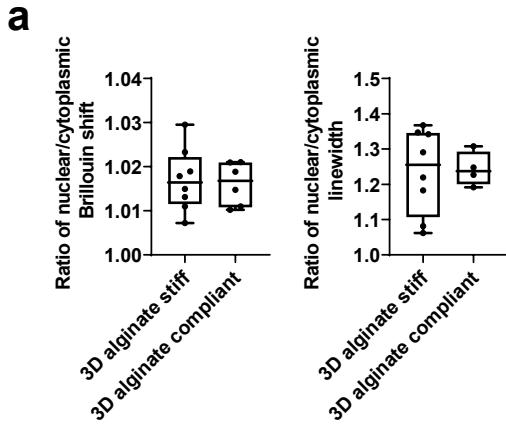
Sadra Bakhshandeh<sup>a</sup>, Hubert M. Taïeb<sup>a</sup>, Raimund Schlüßler<sup>b</sup>, Kyoohyun Kim<sup>b,c</sup>, Timon Beck<sup>b,c</sup>, Anna Taubenberger<sup>b</sup>, Jochen Guck<sup>\*b,c</sup> and Amaia Cipitria<sup>\*a</sup>



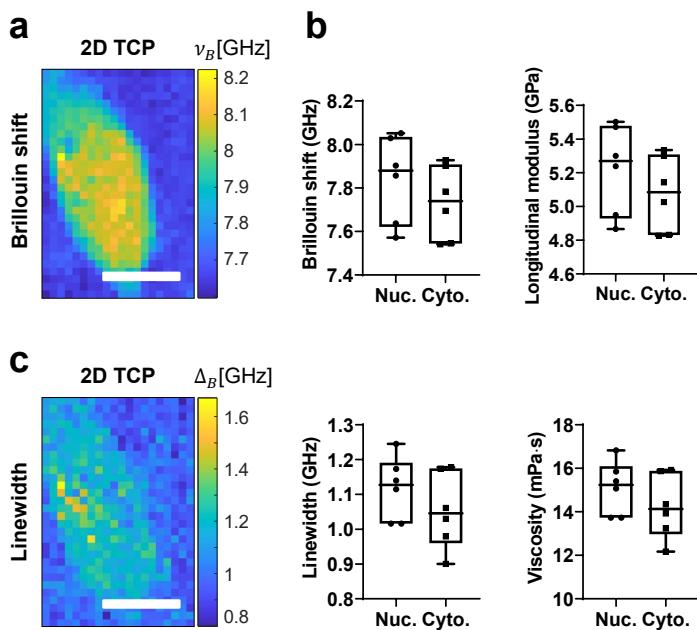
**Fig. S1 MDA-MB-231 retain high viability after 5 days of encapsulation.** Live/Dead images of MDA-MB-231 cells encapsulated in 3D alginate stiff (left image) and 3D alginate compliant (right image) after 5 days. Green (calcein) and red (ethidium homodimer-1) correspond to live and dead cells respectively.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



**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 ( $v_B$ ) 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 ( $v_B$ ) 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  $\mu$ m.