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Spatiotemporal control of micromechanics and microstructure in acoustically-responsive scaffolds using

acoustic droplet vaporization

**Supplemental Figures** 

A)







Figure S1. Confocal microscopy revealed local compaction of fibrin surrounding the bubbles generated by acoustic droplet vaporization (ADV). Images of acoustically-responsive scaffolds (ARSs) before (A) and 1-hour after (B) ADV. The fibrin matrix contained Alexa Flour 647-labeled fibrinogen (fibrinogen<sub>647</sub>, shown in red) while the phase-shift double emulsion contained Alexa Flour 488-labeled dextran (AF<sub>488</sub>, shown in green). The images were taken at the lowest power to minimize saturation for time dependent studies. Scale bar: 10  $\mu$ m.



Figure S2. Static compression was used to confirm the morphological features generated via acoustic droplet vaporization (ADV) in acoustically-responsive scaffolds (ARSs). ARSs (14mm in diameter) post-ADV were exposed to an overpressure of 35kPa for 60 seconds under static compression. (A) Macroscopic image of an ARS post ADV before (left) and after (right) compression. (B) 2D and (C) maximum intensity projection confocal images of the asymmetrically-collapsed features post compression. The shape and dimension of these macropores were similar before (see Fig. 6 (C)-(E)) and after static compression, indicating a non-

gaseous content. (D) 2D and (E) maximum intensity projection confocal images of the spherical gas-filled bubbles after compression (see Fig. 6(A) & (B) for before compression). Scale bar: 15µm.



Figure S3. The repeatability of atomic force microscopy for measuring micromechanical properties of acoustically-responsive scaffolds (ARSs) was confirmed. Sequential compression curves on the same location in (A) a fibrin-only gel and (B) ARS(+US) resulted in no permanent deformation, thus demonstrating the linear elastic regime.



Figure S4. Due to its terminal velocity, the phase-shift double emulsion in an acousticallyresponsive scaffold (ARS) began to settle during polymerization, resulting in a bubble free surface post acoustic droplet vaporization (ADV). A maximum intensity projection of confocal z-stacks, where zero is the top surface of the ARS, demonstrating the spatial distribution of bubbles generated via ADV.