Electronic Supplementary Material (ESI) for Lab on a Chip.

Acoustically modulated biomechanical stimulation for human cartilage tissue engineering

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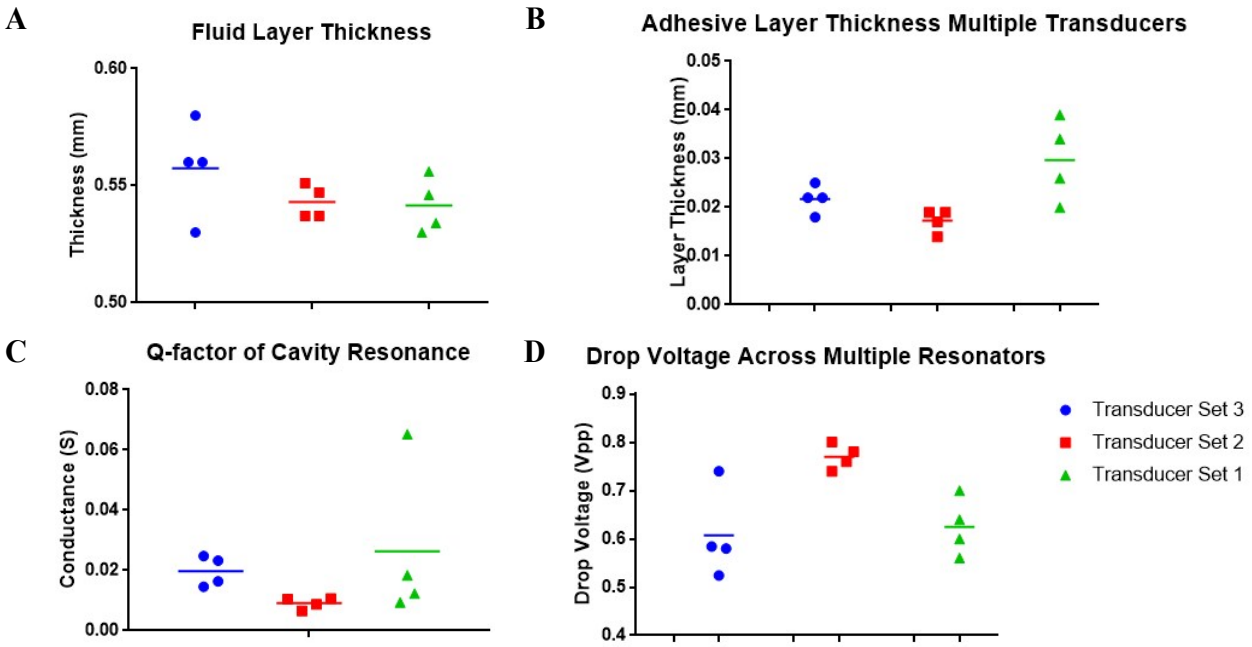
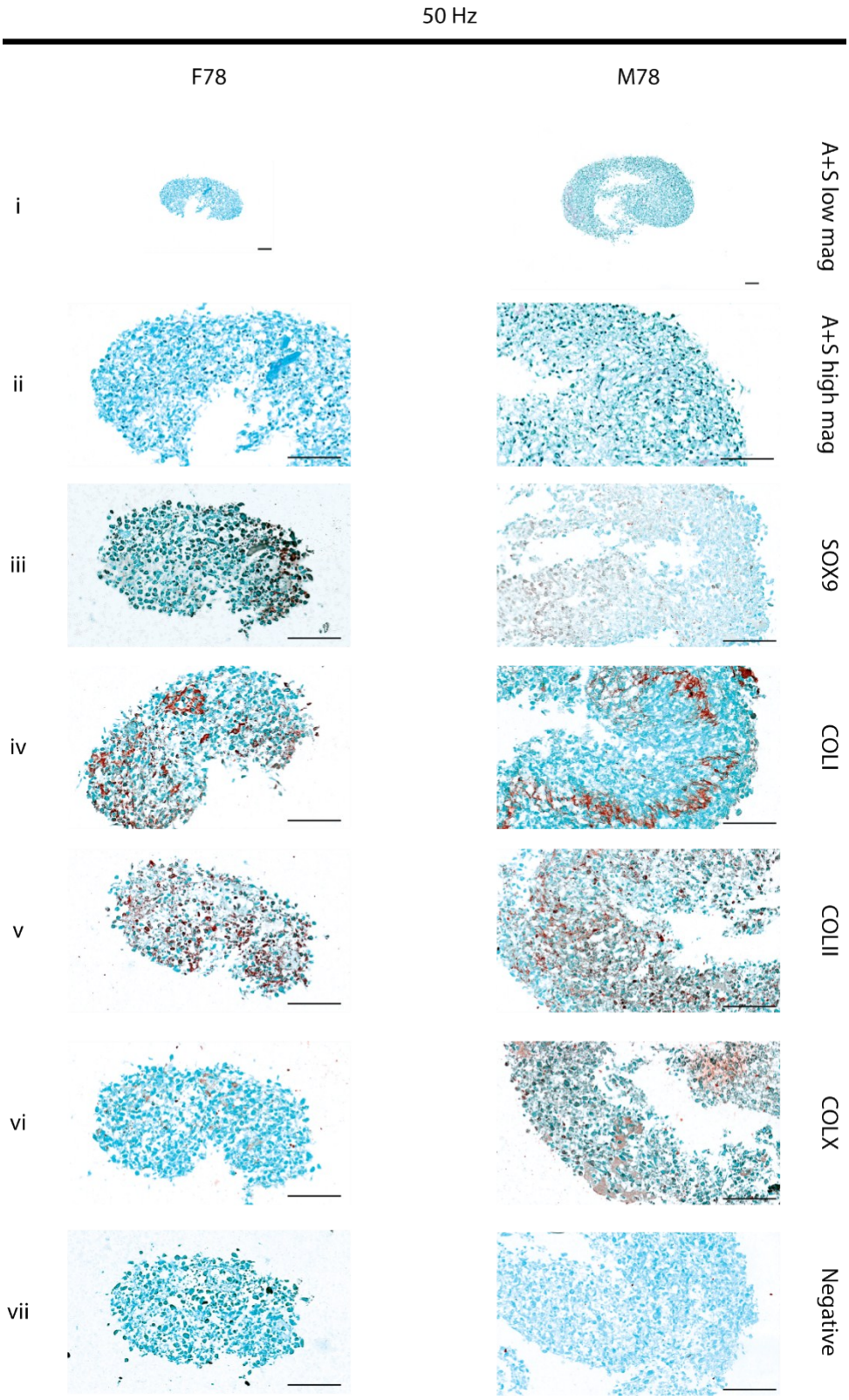
SUPPLEMENTARY INFORMATION

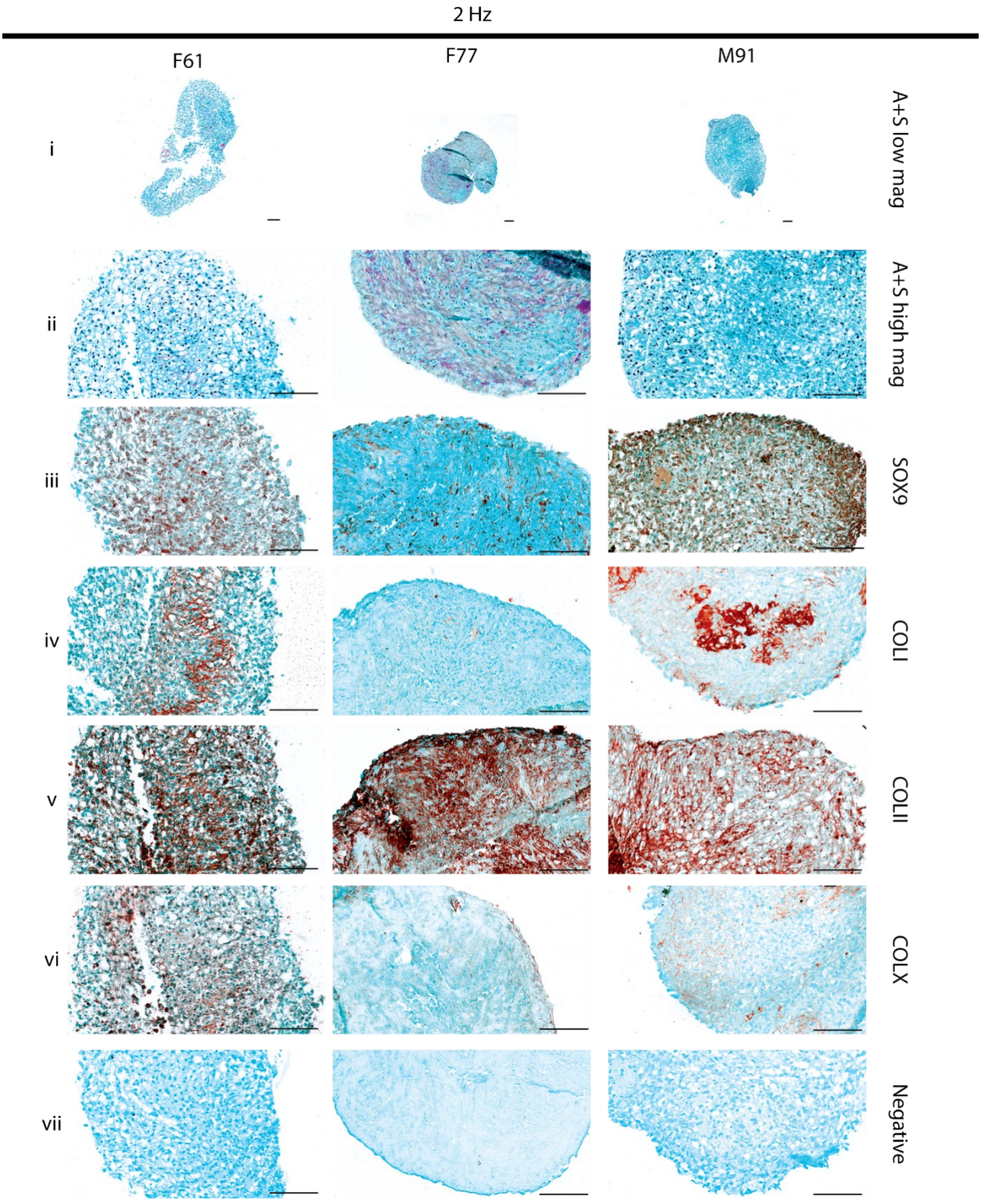


Supplementary Figure 1. Resonator characterization: Several plates were assessed for consistency by measuring (A) the fluid layer thickness and (B) coupling layer thickness. Variations in the physical dimensions of the resonators may explain the variability in (C) Q-factor of the cavity resonance peak and (D) the acoustic trap strength, determined through the drop voltage i.e. the minimum voltage to maintain a 10 µm particle in levitation.



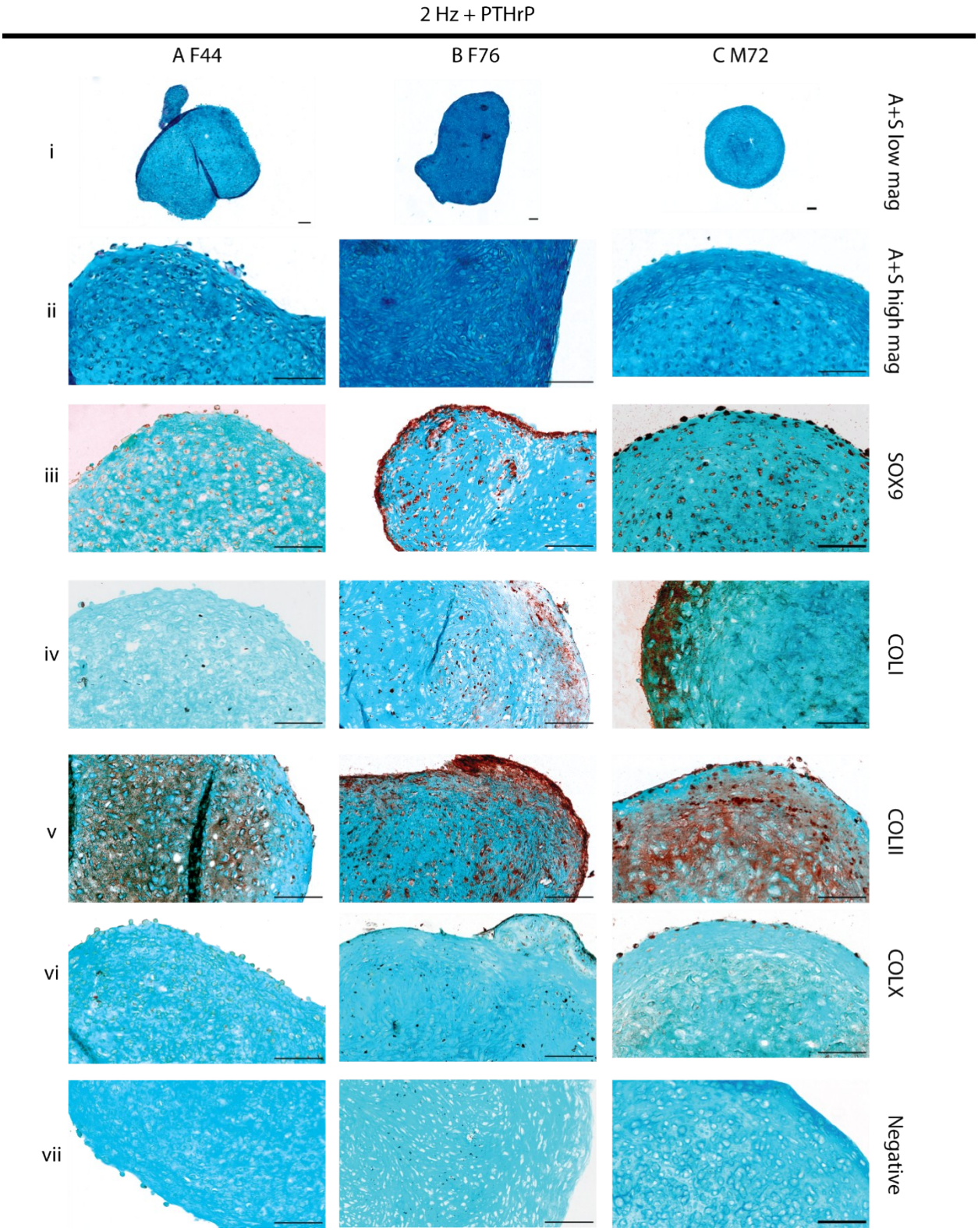
Supplementary Figure 2. Histological analyses of 21-day constructs generated in resonators at 50 Hz: Alcian blue + Sirius red (A+S) staining was performed to assess the histological structure of the constructs, while chondrogenic differentiation was assessed by immunostaining using antibodies against

SOX-9, Type II collagen (COLII), Type I collagen (COLI) and Type X collagen (COLX). No staining was observed in the negative controls (omission of the primary antisera) included in all immunostaining procedures. Scale bars = 100 μm.



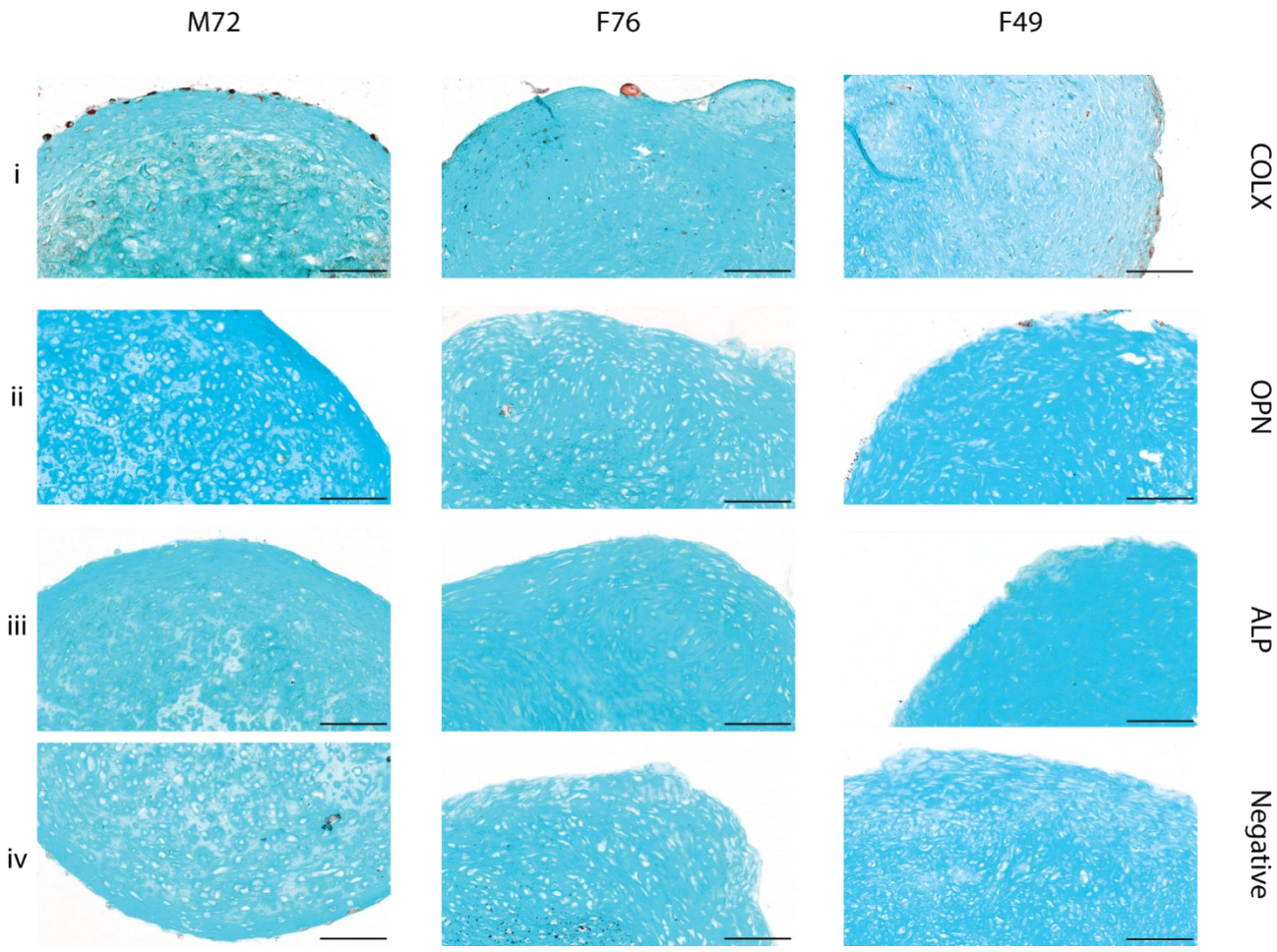
Supplementary Figure 3. Histological analyses of 21-day cartilage constructs engineered in resonators at 2 Hz: Alcian blue + Sirius red (A+S) staining was performed on sections of the cartilage constructs to assess the histological structure, while formation of hyaline-like cartilage was confirmed by robust immunostaining for SOX-9 and Type II collagen (COLII), and low-negligible immunostaining for

collagens Type I (COLI) and Type X (COLX). No staining was observed in the negative controls (omission of the primary antisera) included in all immunostaining procedures. Scale bars = 100 μm.

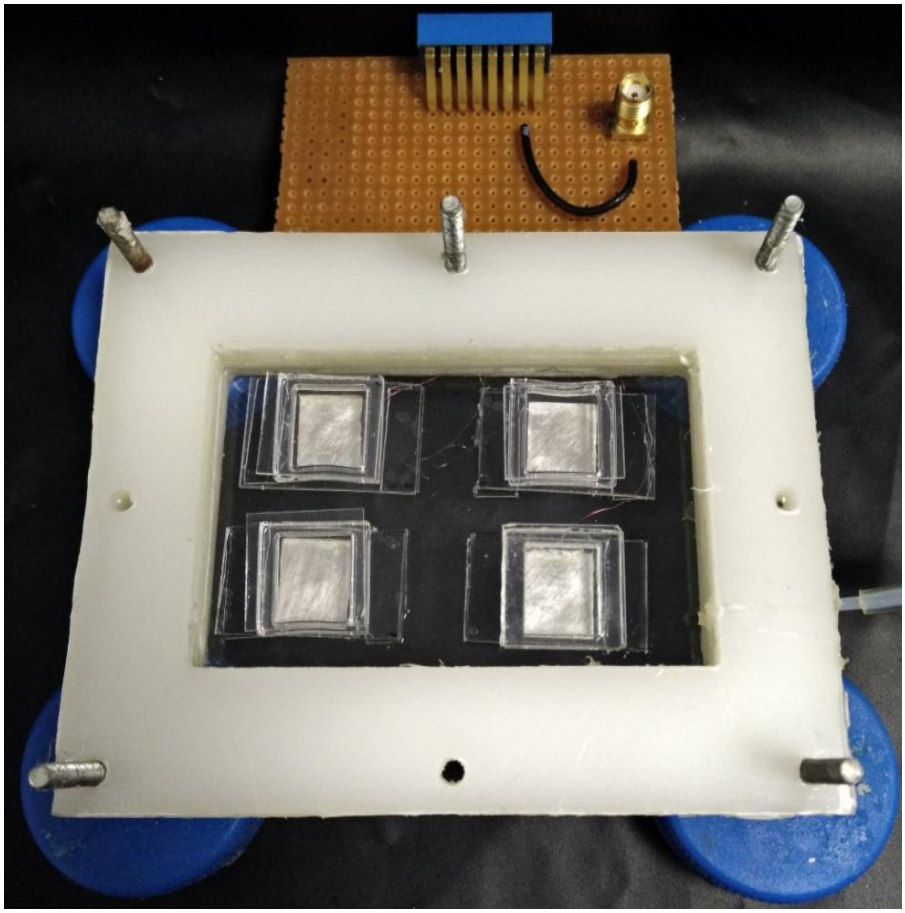


Supplementary Figure 4. Histological analyses of 21-day cartilage constructs engineered in resonators at 2 Hz in combination with 10 ng/ml PTHrP supplementation: Alcian blue + Sirius red (A+S) staining was performed on sections of the cartilage constructs to assess the histological structure, while formation of hyaline-like cartilage was confirmed by robust immunostaining for SOX-9 and Type II

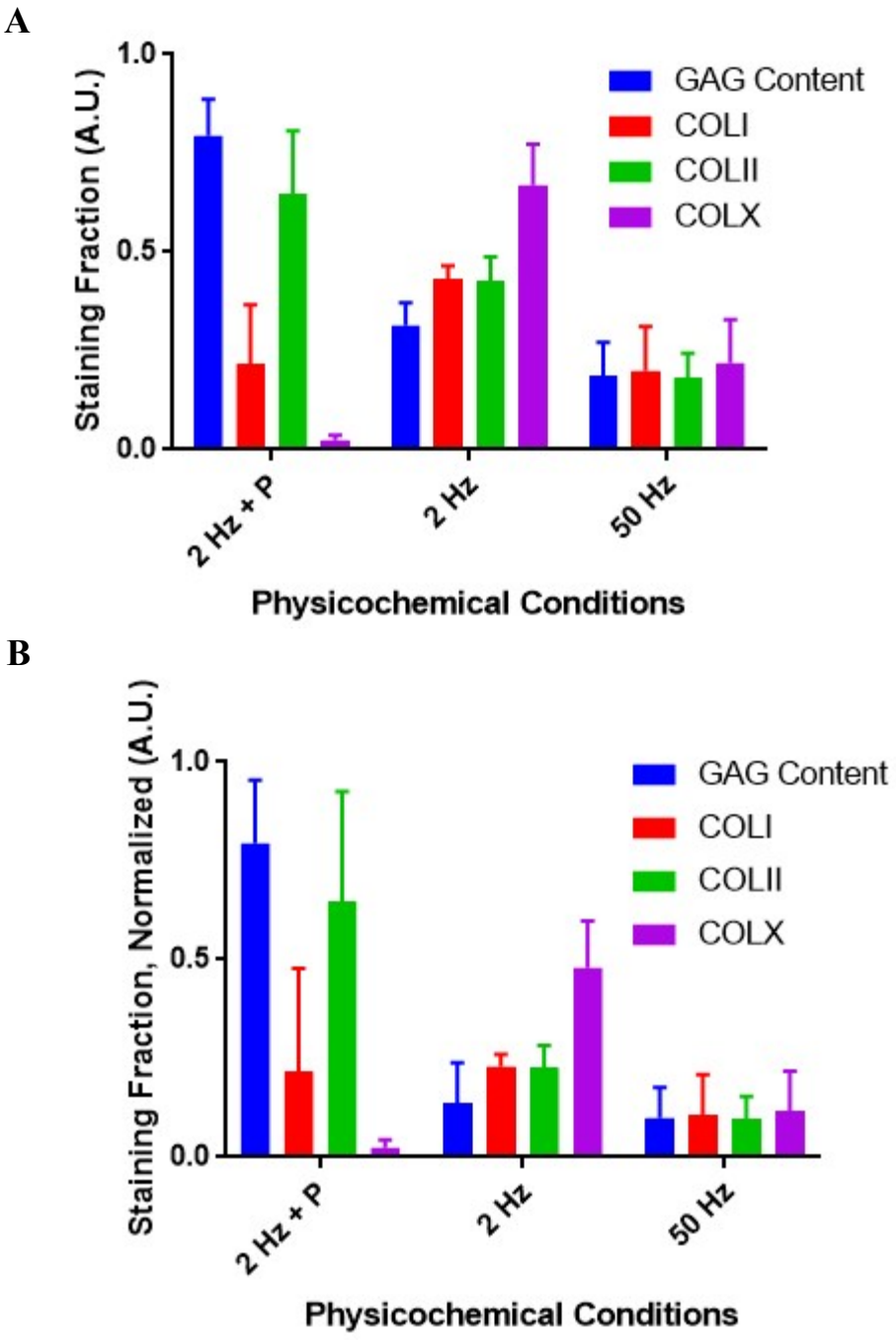
collagen (COLII), and negligible immunostaining for collagens Type I (COLI) and Type X (COLX). No staining was observed in the negative controls (omission of the primary antisera) included in all immunostaining procedures. Scale bars = 100 μm.



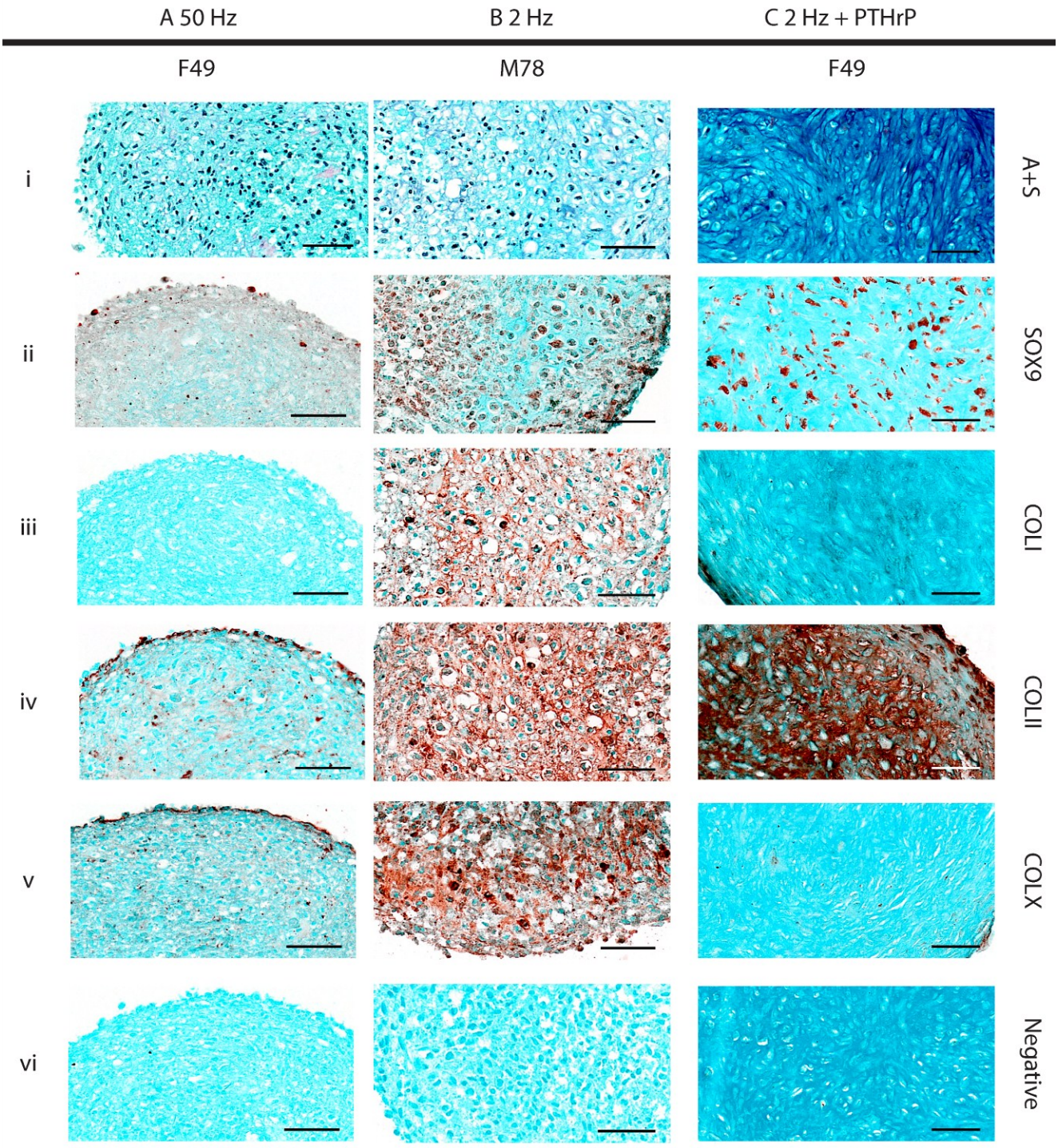
Supplementary Figure 5. Immunohistological analyses of expression of markers of chondrocyte hypertrophy in 21-day cartilage constructs engineered in resonators at 2 Hz in combination with 10 ng/ml PTHrP supplementation: Negligible immunostaining for hypertrophic markers, namely Type X collagen (COLX), Osteopontin (OPN), Alkaline phosphatase (ALP), was observed in the cartilage constructs. No staining was detected in negative controls (omission of primary antisera) included in all immunostaining procedures. Scale bars = 100 µm.



Supplementary Figure 6. Angled top view of assembled bioreactor plate in manifold: Angled top view of assembled bioreactor depicting the resonator plate fitted into the polypropylene manifold.



Supplementary Figure 7. Image segmentation of histological sections: (A) IHC and A/S stained tissue sections were semi-quantified by K-means cluster analysis to approximate the matrix composition of proteoglycans (GAG) and collagen (COLI, COLII, and COLX) in the engineered constructs following 21 days of culture under different acoustic and chemical culture conditions. (B) Considering the size variation noted in the tissue constructs, the stained area fractions were further normalized to the average cross sectional area of the 2 Hz + PTHrP tissue constructs to provide a better representation of the matrix composition of the tissues generated from the different culture conditions and relative to the average size of the constructs.



Supplementary Figure 8. High magnification imaging of cartilage constructs engineered under different physicochemical environments: 21-day cartilage constructs engineered in resonators at (A) 50 Hz, (B) 2 Hz and (C) 2 Hz in combination with 10 ng/ml PTHrP supplementation. Alcian blue + Sirius red (A+S) staining was performed on sections of the cartilage constructs to assess the histological structure, while formation of hyaline-like cartilage was confirmed by robust immunostaining for SOX-9 and Type II collagen (COLII), and negligible immunostaining for collagens Type I (COLI) and Type X (COLX). No staining was observed in the negative controls (omission of the primary antisera) included in

all immunostaining procedures Tissue sections were selected from a representative set of (A) three patients and (B, C) four patients. Scale bars = 50 μm.

S1 Resonator Characterization

To assess the reproducibility of the resonator assembly, the resonator dimensions were measured. The mean fluid layer thickness was measured to be 0.55±0.009 mm across twelve resonators (Supplementary Figure 1A), which is close to the designed fluid layer thickness of 0.55 mm and within tolerable variation. The coupling layer thickness between the PZT and carrier layer was measured (Supplementary Figure 1B) and the mean thickness was found to vary between resonators on both the same and different plates. This is also likely to have changed the resonance characteristics of the resonators, where it was observed that the Q factor of the resonators varied (Supplementary Figure 1C), suggesting discrepancies in the hand assembly process. The drop voltage was also evaluated as a means of determining variability in the acoustic pressure levels between devices (Supplementary Figure 1D). While it was noted that the mean drop voltage was similar between bioreactor plates, a wide variance exists for the tested resonators. This would suggest that some variability in fabrication process affected the measured drop voltage readings.

S2 Computational Method for Determining Fluid Shear

To determine the shear stress applied onto the cells, a 2D laminar flow based model was used to simulate the oscillatory motion of the cells and compute the magnitude of fluid shear stress on the cells. The model was based on the Stokes's oscillating boundary equation, whereby a moving boundary generates a fluid shear force against its surface in an arbitrarily long chamber. The experimentally derived velocity data was applied as a velocity boundary condition (after smoothing with a piecewise cubic function) to the moving wall to better simulate the aggregate movement within the fluid layer at different acoustic parameters.

Given the repeatable displacement path of the cells as they are stimulated, and that one full motion can be seen in a single period, the shear stress amplitude on the cells was quantified as the peak-to-peak. This was computed across resonators within the same plate as well as across multiple sweep repetition rates at a sweep range of 200 kHz.