Strategy of Differentiation Therapy: Effect of Dual-Frequency

Ultrasound on the induction of Liver Cancer Stem-like Cells

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Figure S1. Schematics of the custom designed 1-to-N dual-frequency lowintensity ultrasound (LIUS) apparatus to allow concurrent ultrasound exposure to multiple cell culture wells. N = 4 was designed in this study. (A)

80 kPa, 20% duty cycle

80 kPa, 40% duty cycle



100 kPa, 20% duty cycle



100 kPa, 40% duty cycle



(**B**)

80 kPa, 20% duty cycle

80 kPa, 40% duty cycle



100 kPa, 20% duty cycle



100 kPa, 40% duty cycle



Figure S2. Effects of CSCs on $(PAH/HA)_6$ with LIUS stimulation in different intensities duty cycle treatment after (A) 3 days and (B) 5 days of LIUS treatment, respectively.



Figure S3. LDH assay of cells on $(PAH/HA)_6$ and LIUS treatment group after 3 and 5 days of LIUS exposure. Optical density of LDH was read at an absorbance of 490 nm with 630 nm reference wavelength.



Figure S4. Cell viability assay of cells on TCPS, $(PAH/HA)_6$ and $(PAH/HA)_6$ +LIUS after 3 and 5 days of LIUS stimulation. Asterisks denote significant differences in cell number (**p < 0.01) as determined by a Student's t test.