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Supplementary Materials for

Biomolecular actuators for genetically selective acoustic manipulation of cells

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

Method S1. Calibration of the acoustofluidic channel.

The acoustofluidic channel is calibrated using a previously reported method based on single particle tracking(27). Briefly, the trajectory of polystyrene microbeads inside the acoustofluidic channel was recorded during ultrasound application. The acoustic energy density E_{ac} is determined by fitting the particle position over time, $x_p(t)$, to the equation:

$$x_p(t) = \frac{1}{k} tan^{-1}\left[\tan(x(0)k)\ \exp\left(\frac{4\phi(ka)^2 E_{ac} t}{3\eta}\right)\right] \quad [S1]$$

where ϕ is the particle acoustic contrast factor, k the wave number, η the solution viscosity, and a the particle radius.

The peak applied acoustic pressure p_{peak} is determined using the relationship:

$$p_{peak} = 2\sqrt{\rho_0 c_0 E_{ac}} \quad [S1]$$

where ρ_0 is the solution density, and c_0 the speed of sound.

Supplementary Figures

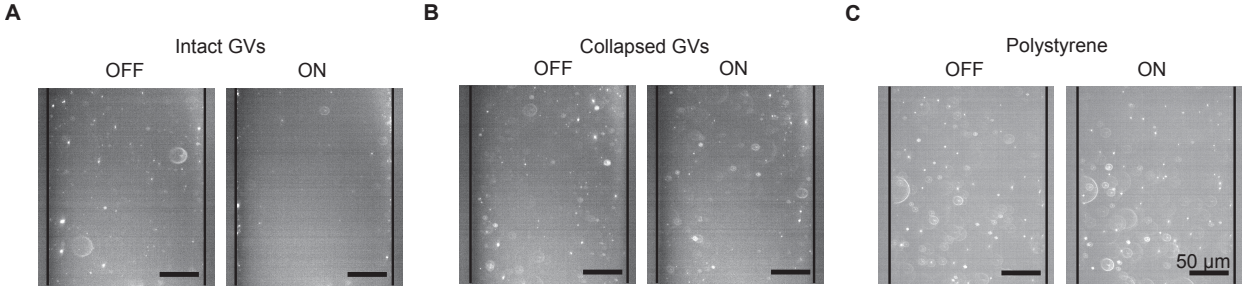

Fig. S1. Control particles do not experience substantial ARF. Fluorescence images of intact GVs (**A**) pressure-collapsed GVs (**B**), and polystyrene nanoparticles (**C**) inside the microfluidic channel before ultrasound (OFF) and 100 seconds after ultrasound has been turned on (ON). Device and acoustic conditions are as described in Fig. 2.

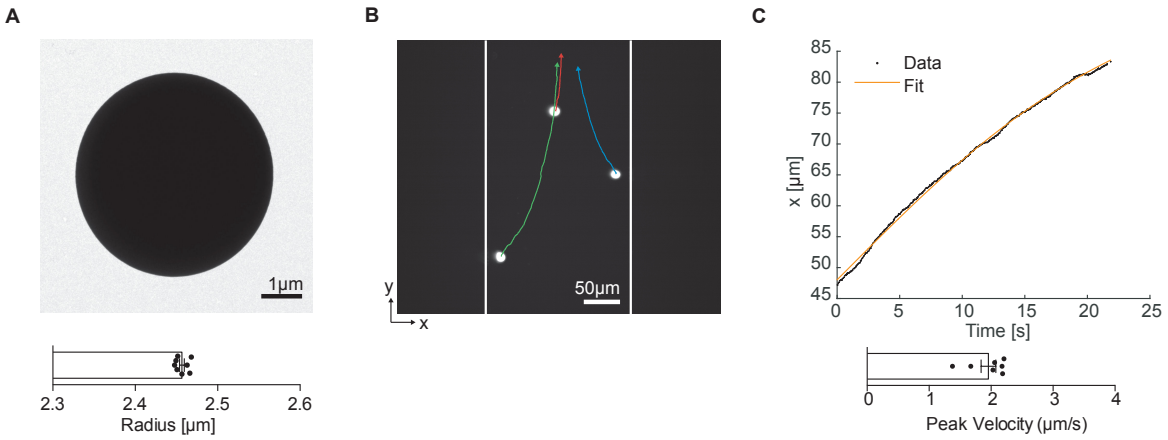

Fig. S2. Calibration of the acoustic energy inside the acoustofluidic channel. (**A**) Representative TEM image of a polystyrene particle (top) and quantification of the particle radius (bottom, 2.457±0.003 µm, mean±S.E.M., n=7). (**B**) Fluorescence image and overlaid acoustophoretic trajectory of polystyrene particles inside the acoustofluidic channel. The white lines demarcate the edges of the channel. Arrows indicated direction of particle movement. (**C**) Representative single-particle trajectory in the x-direction during ultrasound stimulation (top), and quantification of the peak particle velocity (bottom, 2.0±0.1 µm/s, mean±S.E.M., n=7). The acoustic energy is determined using the radius, the acoustic contrast factor and the position over time of polystyrene particles (Supplementary Method).

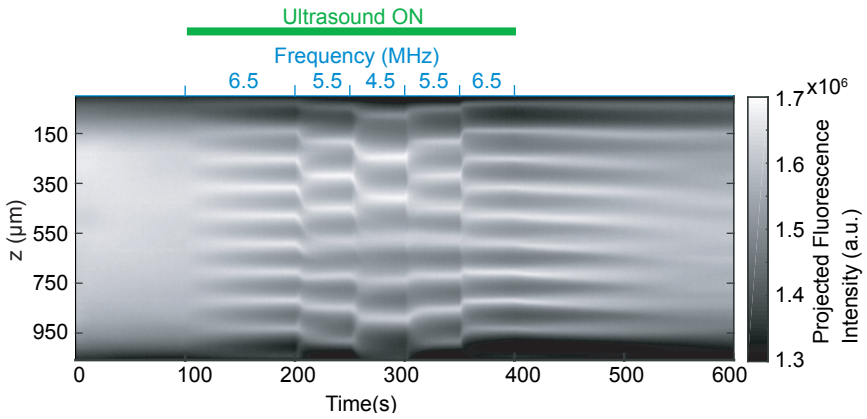

Fig. S3. Cell patterns can be reconfigured on the timescale of seconds. Kymograph of projected fluorescence signal from *bARG1*-expressing *E.coli* during the application of ultrasound at different ultrasound frequencies. Conditions are as described in Fig. 4, A-B.

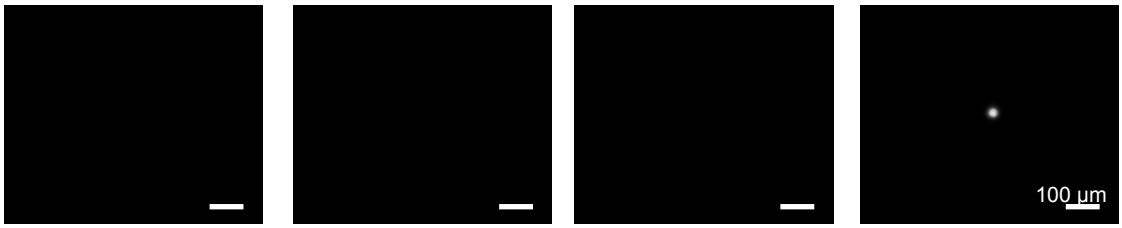

Fig. S4. Bacteria cluster formation requires intact intracellular GVs. Fluorescence images of *bARG1*-expressing *E.coli* with intact (+) and collapsed (-) intracellular GVs before and 40 seconds after ultrasound application.

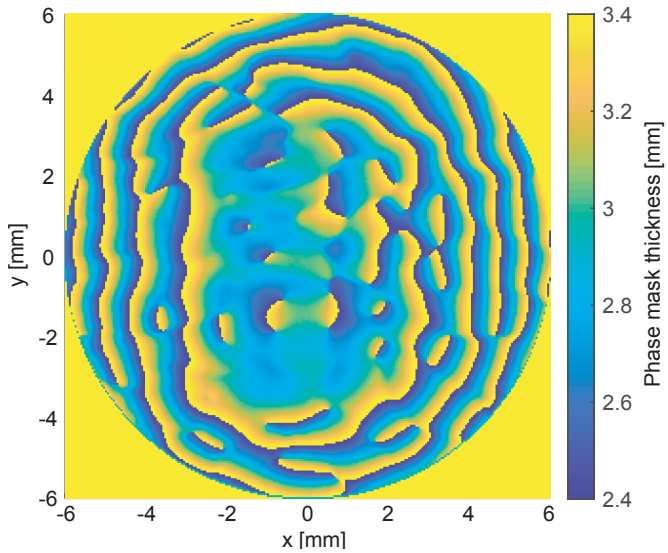

Fig. S5. Hologram phase mask. Thickness map of the 3D printed phase mask designed to produce an 'R'-shaped pressure profile.

Table S1. Estimated acoustic contrast factor of GV-expressing cells

Volume Fraction of GVs	Acoustic Contrast Factor	
	Bacteria	**Mammalian Cell**
0%	0.08	0.07
1%	-0.04	-0.05
3%	-0.3	-0.3
10%	-1.1	-1.1

This calculation assumes a cell volume-averaged density and compressibility according to $\rho_{cell} = (1-f) * \rho_{wildtype\ cell} + f * \rho_{GV}$ and $\beta_{cell} = (1-f) * \beta_{wildtype\ cell} + f * \beta_{GV}$, where f is the volume fraction of GVs. Values of $\rho_{wildtype\ cell}$, $\beta_{wildtype\ cell}$, ρ_{GV} and β_{GV} were obtained from literature. (*18, 19, 36, 65-67*)

Movie S1. Acoustic manipulation of engineered bacteria. *bARG1*-expressing bacteria are moved to pressure antinodes of a standing wave positioned at the walls of a microfluidic channel. Conditions are as described in Fig. 3C.

Movie S2. Dynamic acoustic patterning of engineered bacteria. *bARG1*-expressing bacteria are patterned dynamically in solution by different frequencies of an acoustic standing wave, followed by the disappearance of the pattern after ultrasound is turned off. Device and acoustic conditions are as described in Fig. 4, A-B.

Movie S3. Focal acoustic trapping of engineered bacteria. *bARG1*-expressing bacteria coalesce at the focal region of a focused-transducer. Device and acoustic conditions as described in Fig. 4, C-D.

Movie S4. Translation of acoustically trapped engineered bacteria. A cluster of acoustically trapped *bARG1*-expressing bacteria translated to different locations to form a spatiotemporal pattern writing out "CIT". Conditions are as described in Fig. 4, E-F.

Movie S5. ARF-silencing of GVs. Fluorescently labeled GVs experiencing an acoustic standing wave inside a microfluidic channel under continuous flow conditions. GVs in the center of the channel experience ARF towards the high-pressure regions at the channel walls; GVs in regions where the acoustic pressure is higher than the GVs' collapse pressure experience collapse and shut off their response to ARF. This results in a sharp material separation at the location of GV collapse. Acoustic conditions are as described in Fig. 8.

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