Supplementary information

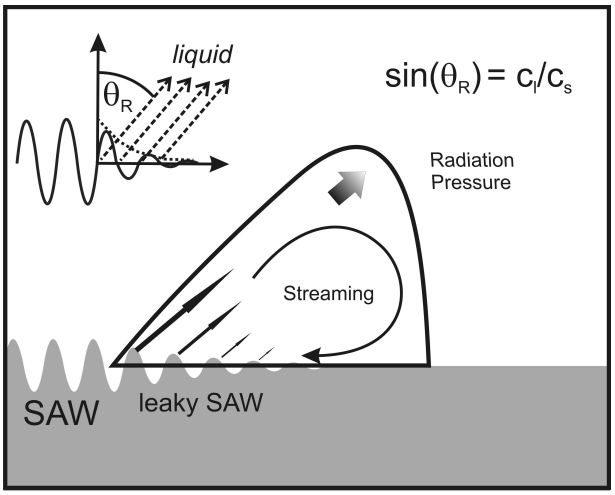

Fig. S1: A liquid drop, placed in the propagation path of a SAW, will be actuated as the SAW refracts within the drop, propagating within the fluid as a compressional wave. The speeds of sound on the surface, C_s, and in the liquid, C_l, are different and lead to a refraction of the waves at the Rayleigh angle, θ_R (see inset). The surface wave is damped by the liquid and a second-order flow develops in the droplet leading to acoustic streaming.

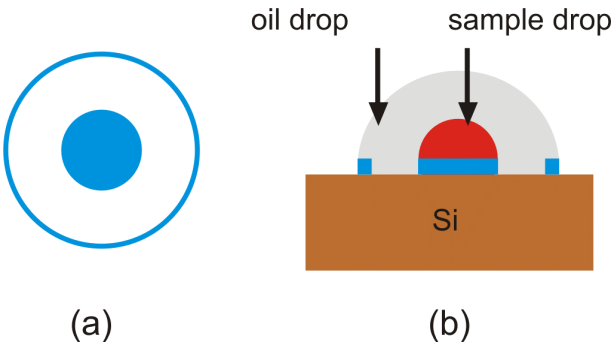

Fig. S2: Virtual Reaction Chamber: (a) the plan view of the hydrophilic disk and ring on a hydrophobic background. (b) The transverse view with a sample drop encapsulated within an oil droplet.

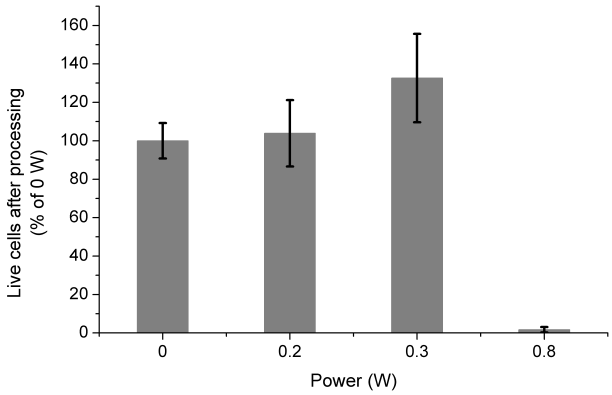

Fig. S3: The lysis of other cell types was confirmed by processing a mammalian cell line (HL60 cells – model for chronic myeloid leukaemia), which is non-adherent and mechanically closer to white blood cells than red blood cells. 15 µl of a solution containing 1 million HL60 cells per millilitre in PBS, were processed for 10 s. At the power of 0.2 and 0.3 W, the cells were only concentrated in the centre of the droplet, while they were lysed at a power of 0.8 W.

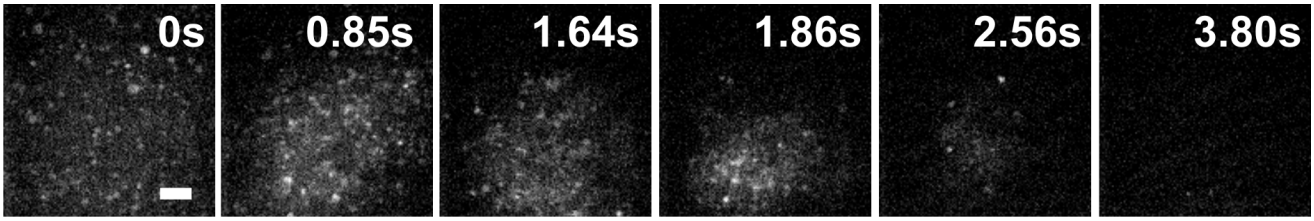

Fig. S4: SAW lysis of MCF7 cells: In order to better understand the dynamics of the process, a droplet of 10 µl of a suspension of actin-GFP MCF7 cells (2 million cells/ml) was lysed at 9.61 MHz. The power was increased from 0.06 W to 0.8 W while recording at 215 frames/s (Movie M2 in ESI). The fluorescent cells first concentrate in the centre of the drop near the surface of the device. As lysis begins (after ca. 1 sec), the large concentration of intracellular actin-GFP was released from the cells and is diluted throughout the drop (seen by the reduced fluorescence signal). The scale bar is 100 µm. The images were taken using epi-fluorescence on a confocal microscope (x10 objective, Zeiss, LSM 5 Live).

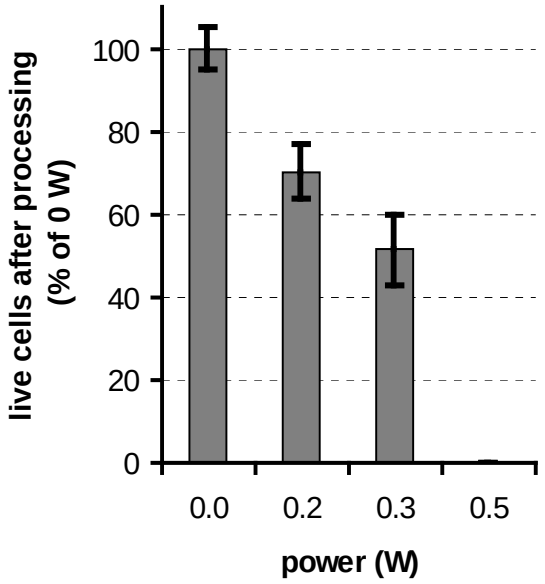

Fig. S5. The efficiency of lysis was evaluated of a third cell type, the trypansome, T. cyclops. The number of cell lysed (as the % of original contents) was determined for four different powers. 3 millions trypanosomes cyclops/ml were lysed for 10 s. At the power of 0.3 and 0.2 W, the cells were only concentrated in the centre of the droplet, while they were lysed at a power of 0.5 W (0.1 % ± 0.15 %, the point is not visible). Error bars are the standard deviation for 3 repeats. The trypanosomes are more fragile than the mammalian cells (Figure S3) and their numbers decrease with the power applied. The increased fragility of the trypanosome, compared to the mammalian cells may either be due to the membrane properties (being more susceptible to the shear forces generated by the vortices) and-or may be linked to the cell's elongated shape (resulting in the membrane experiencing a more pronounced exposure to shear). The results higher than 100 % for HL60 cells for low powers may be explained by a sampling variability.

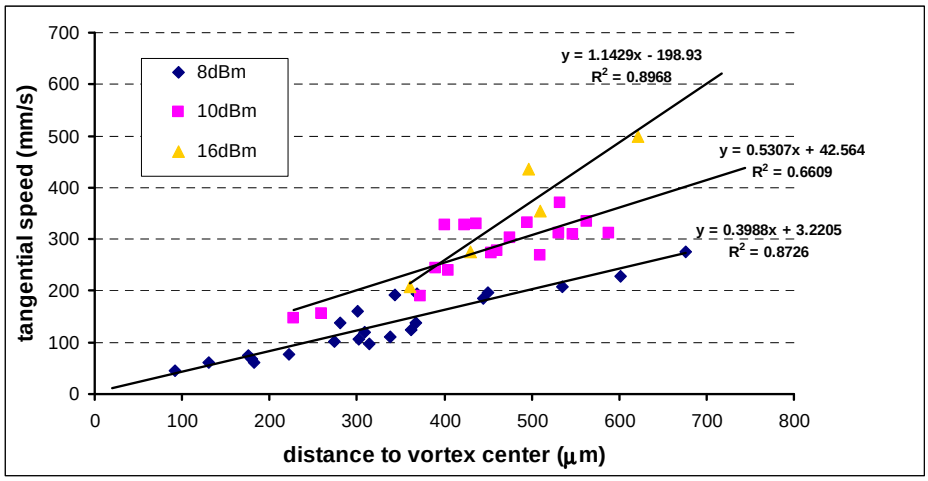

Fig. S6: The analysis of particle velocities in SAW actuated flows: The movement of 5µm polystyrene beads was used to characterise the distribution of the different flow velocities within the circulating droplet. The movement of the beads was observed on a fast confocal microscope (Zeiss, LSM 5 Live, OBJECTIVE 10x) at 550 fps at three different powers (without the amplifier), namely 8 dBm (6 mW, blue), 10 dBm (10 mW, purple) and 16 dBm (40 mW, yellow). The bead positions were recorded and their tangential velocity analysed, in a similar fashion as Raghavan et al (25) – see methods section below. The shear rate (in s^{-1}) was obtained using a linear regression algorithm.

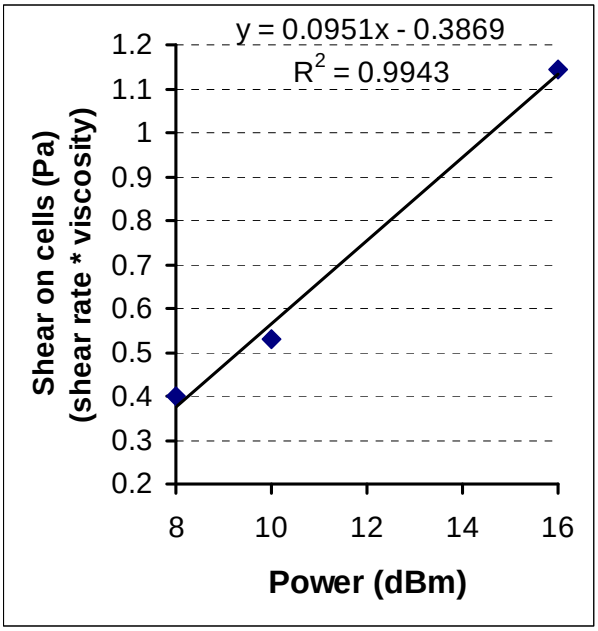

Fig. S7: Graph showing the shear that is exerted on cells, at three different powers (shear rate x viscosity - in Pa) using values extracted using the methods of Raghavan et al (25) using data presented in Figure S6 above.

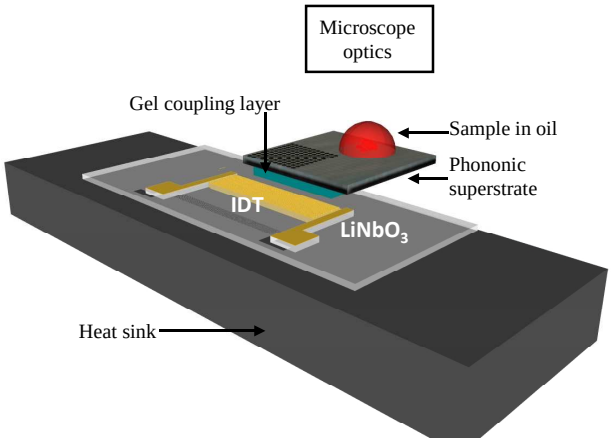

Fig. S8. Experimental set-up showing the $LiNbO_3$ piezoelectric wafer with IDT patterned, positioned on a metal heat sink, used to enable passive cooling during the PCR cycle. The sample drop was positioned on the phononic superstrate, which was coupled to the piezoelectric wafer using a gel layer. A microscope (Zeiss Scope A1) is used to observe the sample (x10 objective), while an IR camera (FLIR i60, FLIR Systems) records the temperature.

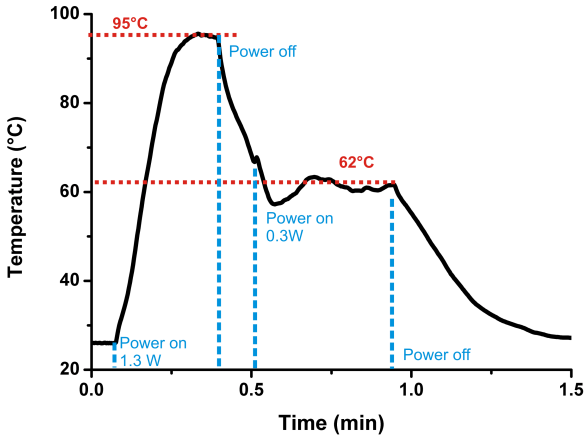

Fig S9. The temperature profile of the droplet during the PCR cycle. The temperature was recorded using a thermocouple (RS components, cat #6212164). The power was switched between 1.3 W and 0.3 W at 18 MHz to achieve the 2 temperatures of the cycle, at 95°C and 62°C respectively. A plateau at 95°C was achieved within 15 s.

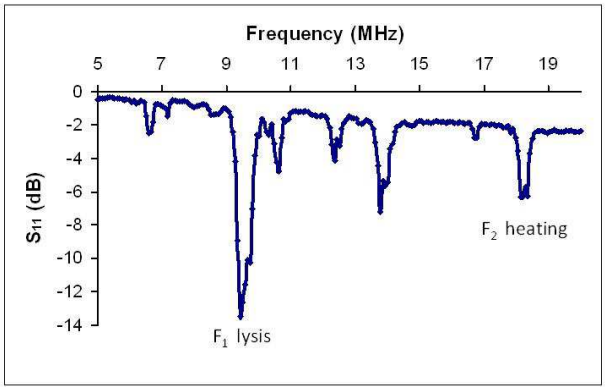

Fig S10. S11 parameter of the IDT, obtained using a network analyser (Agilent Technologies E5071C ENA series), showing excited acoustic waves at 9.5MHz and 18MHz, used for SAW lysis and heating respectively. Only one of the electrodes was driven, while the other one served as a reflector (1), which enabled us to generate the second harmonic (~18MHz).

Supplementary Methods

Phononic crystal design and fabrication:

The phononic superstrates comprise a square array (pitch 203 μm) of circular holes (radius 82 μm) in a 470 μm-thick [100] silicon wafer that scattered the SAW to obtain an asymmetry in the propagating waves. The surface holding the sample droplet was patterned with a hydrophilic spot of 3 μm in diameter, surrounded by a 200 μm wide annulus, 1 mm away (Figure S2). The rest of the surface was made hydrophobic by a silane (FOTS, Sigma), obtained by immersing the photoresist-patterned (AZ4562) wafer in a 1.6 mM silane solution in heptane (Sigma, H9629) for 10 min and dissolving the resist in acetone. This treatment resulted in a contact angle of 107º ± 0.2º (standard deviation) on silicon. The hydrophilic spot prevents the droplet from moving at higher powers, but is not essential for lysis.

Samples

EDTA-chelated human whole blood (O^+) was obtained from the Glasgow and West of Scotland Blood Transfusion Service and stored at 4°C until needed (always within 5 days of receipt); **HL60 cells** (ATCC CCL-240, acute promyelocytic leukemia) were maintained following the supplier's recommendations, in Dulbecco's RPMI media supplemented with 10% heat-inactivated fetal calf serum (FCS) and 5% penicillin-streptomycin, at 37°C (5% CO_2); ***Trypanosomes*** were maintained at 27 °C in Cunninghams media + 20 % FCS; ***MCF7 cells expressing GFP-actin*.** MCF7 cells were transfected with a mammalian expression vector encoded EGFP-actin and neomycin resistant sequences (kindly provided by the Beatson Institute for Cancer Research). 24 hours prior to transfection, MCF7 cells were seeded in 60 mm Petri dishes with density 500,000 cells per dish and incubated overnight at 37 °C in 5% CO_2. The cells were transfected with 20 μg of pEGFP-Actin plasmid DNA using calcium phosphate transfection kit (Invitrogen). To isolate stable transfectants, the transfected cells were trypsinized and replated in media containing 0.5 mg/ml Geneticin (G418). The media were changed every 3-4 days for several weeks to allow colonies of resistant cells to grow and to remove cell debris. Homemade cloning rings (made of plastic pipette tips) were used to select the desired colonies. The cloning rings were dipped in grease and placed over the marked colonies. The colonies in cloning ring were trypsinized and transferred to 96 well plates. The individual colonies were propagated for several weeks in selective media. Thereafter, the stable transfected MCF-7 cells were maintained in complete advanced DMEM containing 0.5 mg/ml of G418. The cells were harvested with trypsin-EDTA and resuspended in PBS at the desired concentration before the experiments. The cells were imaged using a fast confocal microscope (Zeiss, LSM 5 Live).

Estimation of shear rates.

Using the same experimental equipment, as described for the observation of MCF7 in Figure S4, 5 μm polystyrene beads exhibiting a green fluorescence (FluoroMax, Thermo Scientific, λ_{ex}=490nm, λ_{em}=510 nm) were centrifuged at low powers of up to 40 mW. Their positions were recorded at 550 fps (fast confocal microscope, x10 objective) in cylindrical coordinates and their instantaneous velocity averaged over at least 20 frames (36 ms). At higher powers, the high velocities prevent reliable position records. The shear rates were extracted from a linear fit (Figure S6) and extrapolated in a linear approximation (Figure S7) to higher powers. 16dBm represents a hundredth of the powers used for lysis, which leads to shears of 85 Pa. These are close to the conditions of shear required for mechanical effect on mammalian cells (100 Pa, ref 26 main text) and, combined with acoustic pressure and radiative forces lead to efficient lysis.

Quantitative PCR.

All samples were centrifuged at 13,000 rpm for 2 minutes to form a pellet of whole cells and cell debris. The DNA in the supernatant was quantified using qPCR. DNA in the whole blood samples was concentrated using the DNA Clean and Concentrator™-5 Kit (Zymo Research Corporation; D 4014). The purified DNA was eluted in 5 μL and a total aliquot of 1 μL was used in each qPCR reaction. For qPCR analysis of the ActB and GAPDH gene expression, in addition to 1 μL of the sample, each reaction contained 2.4 μM of the forward primer, 2.4 μM reverse primer, 0.15 μM of fluorescent probe and 1 X Brilliant III Ultra Fast qPCR Master Mix (Agilent Technologies; 600880).

All primers were designed with Primer3Plus and synthesized by Eurofins MWG (Germany), The nucleotide sequences of the primers used in each reaction are shown in Table S1 below. The DNA was analysed in a Lightcycler 480 (Roche Diagnostics) using the following amplification conditions: (i) for denaturation at 95°C for 10 minutes; (ii) 45 cycles of amplification at 95°C for 15 seconds then 52°C for 30 seconds after which the level of fluorescence in the sample was measured and finally, (iii) cooling to 40°C for 30 seconds.

Generation of standard curves for ActB and GAPDH. Linear dsDNA was used to generate standard curves for qPCR, after (2) highlighted the benefits of using linear DNA in preference to circular DNA, as follows. The PCR product of interest (ActB/GAPDH) was amplified from the plasmid DNA construct using the reagents and reaction conditions shown for the qPCR reactions. The PCR products were purified from a 1% Seakem LE Agarose gel (Lonza Biologics; 50004) containing 0.01% Sybr Safe DNA gel stain (Invitrogen; S33102) with a Zymoclean™ Gel DNA Recovery Kit (Zymo Research Corporation; D4007) and quantified on a Nanodrop spectrophotometer (Thermo Fisher Scientific). The DNA copy number was calculated based on the methods reported in (3). The DNA was diluted from 1 X 10^9 copies/μl to 0 copies/μl in a 10-fold dilution series. qPCR reactions were conducted on the DNA dilutions using the same reagents and reaction conditions reported for the qPCR reactions above. A standard curve was generated by plotting the Cq value against log of the copy number. The efficiency of the qPCR reaction (*E*) was calculated using the formula (Eq 1):

$$E = 10^{-\frac{1}{slope}} \qquad (1)$$

where the optimal efficiency of the qPCR reaction was 2. Primer pairs that have qPCR efficiencies of between 1.6 and 2.4 are typically used (4). The efficiency of the qPCR reactions and errors were calculated with the Lightcycler 480 qPCR software (Roche Diagnostics) and shown to be within acceptable ranges. The unknown samples were compared to the standard curve and the copy number of the unknown targets calculated.

***qPCR data analysis*.** Cq values obtained each qPCR reaction were converted to copy numbers using the appropriate ActB or GAPDH standard curves. The copy number for the gene of interest in untreated samples was normalized to 1.0 and the copy number for the samples treated with Triton or SAW was compared to the copy number in the control samples. Increases in the levels of DNA in the treated samples are expressed in the data herein. Each experiment involved three technical replicates and experiments were repeated in triplicate.

Table S1. Primer sequence for qPCR of human genomic DNA.

Primer	Gene target	Nucleotide sequence (5' → 3')
prMJ351	ActB F	CTCGGCCACATTGTGAACTT
prMJ352	ActB fluorescent probe	FAM-ATGCTCGCTCCAACCGAC-BHQ1
prMJ353	ActB R	AACGGTGAAGGTGACAGCA
prMJ510	GAPDH F	TGCACCACCAACTGCTTAG
prMJ511	GAPDH fluorescent probe	FAM-CCAAGGTCATCCATGACAACT-BHQ1
prMJ512	GAPDH R	TGTGGTCATGAGTCCTTCCA

Supplementary References:

1. Yen, K.H.; Lau, K.F.; Kagiwada, R.S., Second Harmonic SAW Resonators. *1978 Ultrasonics Symposium* **1978,** 442- 447,
2. Hou, Y.; Zhang, H.; Miranda, L.; Lin, S. Serious overestimation in quantitative PCR by circular (supercoiled) plasmid standard: microalgal *pcna* as the model gene. *PLoS ONE* **2010**, *5*, e9545
3. Whelan JA, Russell NB, Whelan MA: A method for using absolute quantification of cDNA using real-time PCR. *Journal of Immunological Methods* **2003**, *278*:261-269
4. Yun JJ, Heisler LE, Hwang IIL, Wilkins O, Lau SK, Hyrcza M, Jayabalasingham B, Jin J, McLaurin J, Tsao M-S, Der SD: Genomic DNA functions as a universal external standard in quantitative real-time PCR. *Nucleic Acids Research* **2006**, *34*:e85

Movie Legends

Movie M1: Lysis on phononic superstrates.
A droplet of 10 µL (3 mm wide) was positioned on the hydrophilic spot. The SAW was propagated at 9.5 MHz (with a power of 3.1 W). The square phononic lattice, machined into the superstrate using standard lithographic and etching methods, filtered the ultrasonic wave, creating lytic vortices within the drop. The bright rings observed on the droplet are due to reflections from the illumination source. The movie was recorded at 50 frames/s. The acoustic excitation was turned on after 1.26s, leading to agitation of the liquid and increased scattering, and resulted in the disappearance of the illumination rings. At the beginning of the process, the cells were concentrated towards the centre of the drop and then lysed, resulting in a translucent drop. The acoustic excitation was turned off after 3.76s, ending the actuation of the liquid surface, leading to the reappearance of the illumination rings.

Movie M2: Confocal analysis.
SAW lysis of MCF7 cells. A droplet of 10 µl of a suspension of actin-GFP MCF7 cells at a concentration of 2 million cells/ml was processed at 9.61 MHz. The power was increased from 0.06 W to 0.8 W while video recording was performed at 215 frames/s. The fluorescent cells first concentrate into the centre of the drop near the surface of the device, then disappeared as the actin-GFP was released from the cells following lysis. Due to the fast imaging, the image is only 65x65 pixels.