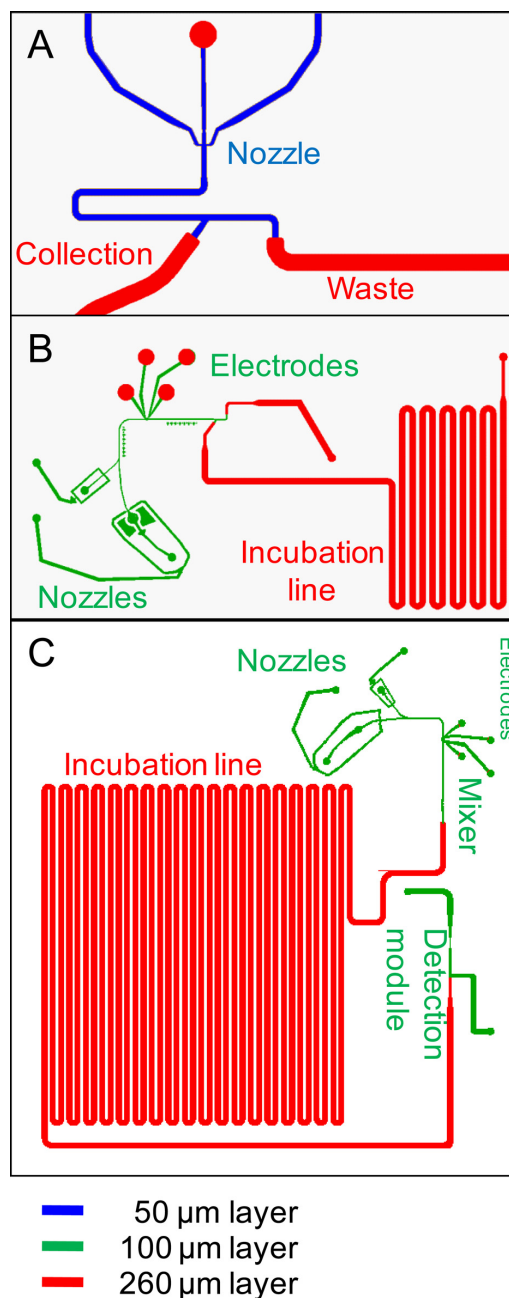
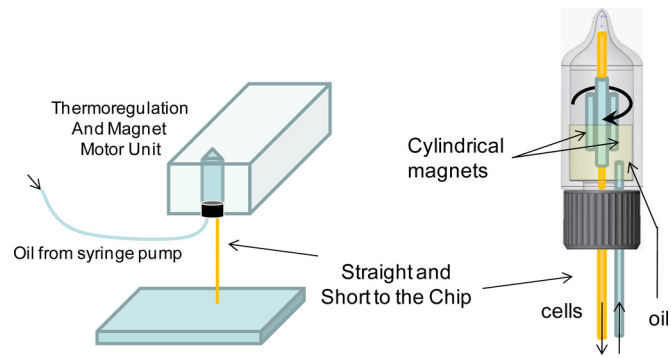


# Supporting Information

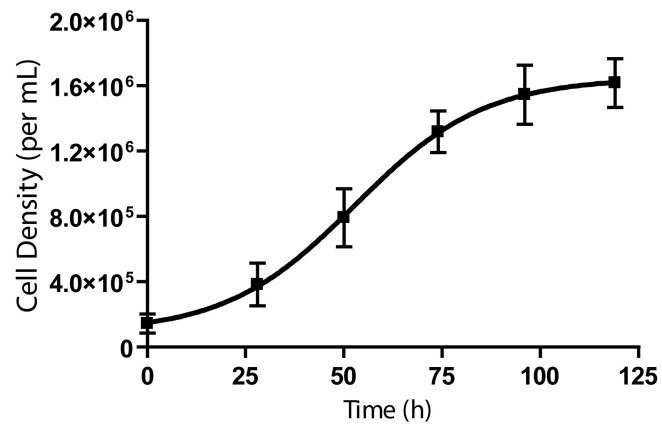
Brouzes et al. 10.1073/pnas.0903542106



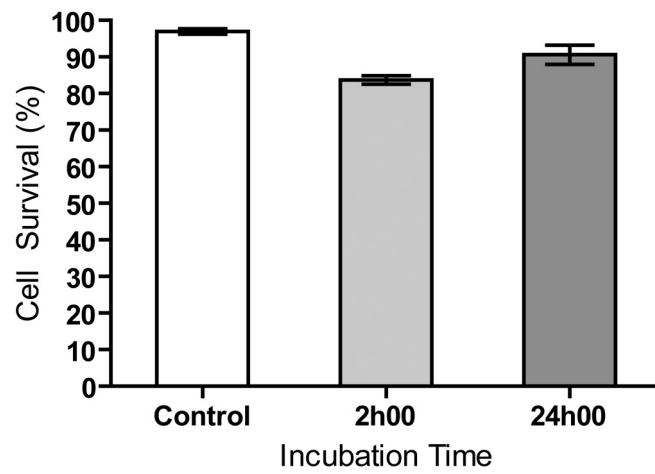
**Fig. S1.** Microfluidics chips layouts. (A) Cell library device. The design consisted of a simple nozzle with 2 outlets, 1 for waste and 1 for collection. At the beginning of encapsulation, the droplets were directed to the waste outlet. After allowing flows to stabilize, droplets were collected by forcing the flow out a second outlet by running a syringe pump in withdrawal mode. (B) Cell and library combination. The layout comprised a set of 2 nozzles in the bottom left corner. The left nozzle was used to space the library droplets, the right one was used to encapsulate cells. At the confluence of the streams the droplets interdigitated. The surfactant stabilized them against coalescence until they entered the region of the electric field and merged (see electrodes). The droplets were then incubated on-chip for a short period to ensure that the droplet interface was thoroughly populated with the surfactant to achieve optimal emulsion stability. (C) Cytotoxicity Chip. The operation of this chip was described in details in the main text. On the upper right corner 2 nozzles were used to respace the cell emulsion (Left) and to encapsulate the dye solution (Right). The droplet pairs were then merged with an electric field. The merged droplets were mixed before entering the incubation line. Finally, the droplets were interrogated at the detection module.



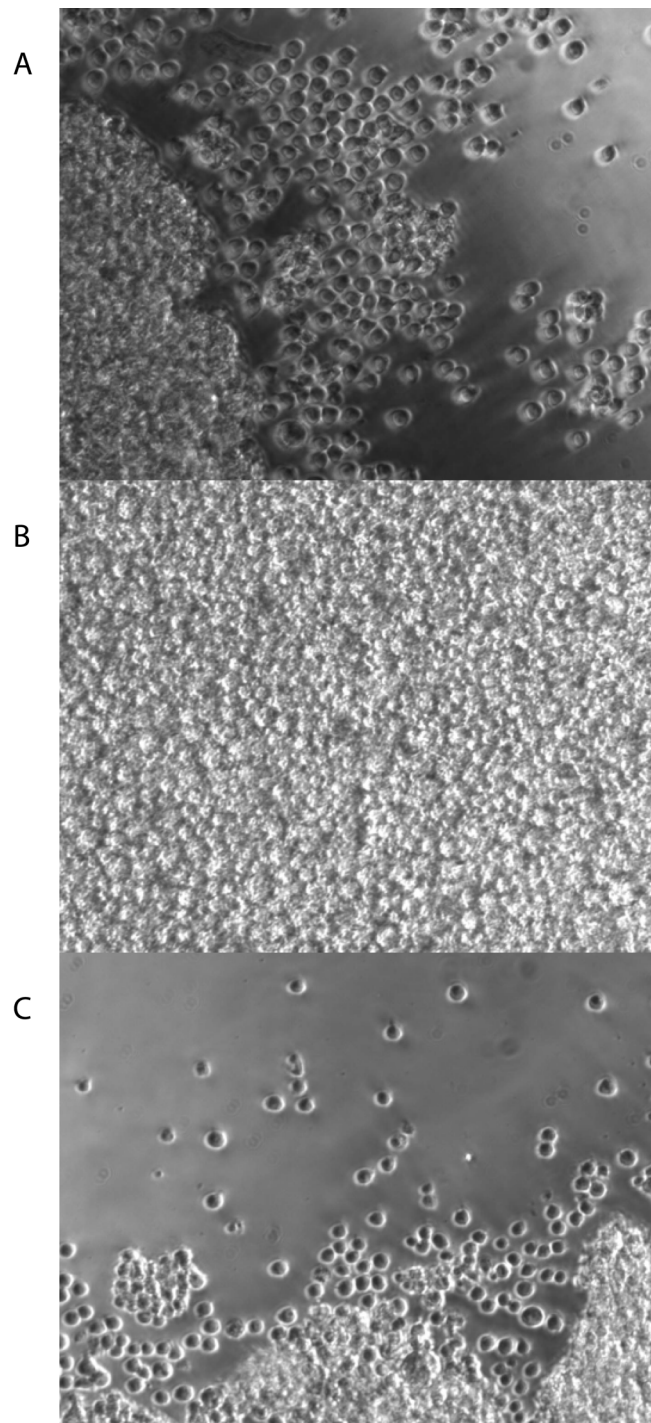
**Fig. S2.** Schematic of the cell injection module. We developed an injection module to minimize shear-stress experienced by the cells and to improve the cell flow consistency. The injection module consisted of a vial with conical tip, a magnetic stirrer that freely rotated around a central tubing used to eject cells to the chip and an additional lateral tubing to inject fluorinated oil. The system was used downward as depicted in the picture to avoid any curvature of the tube carrying the cells out to the chip. The vial was preloaded with the cell solution. Upon fluorinated oil injection, the cell solution was displaced upward and deflected back into the central tubing. The central tubing was made of FEP and had an inner diameter of 400  $\mu\text{m}$ .



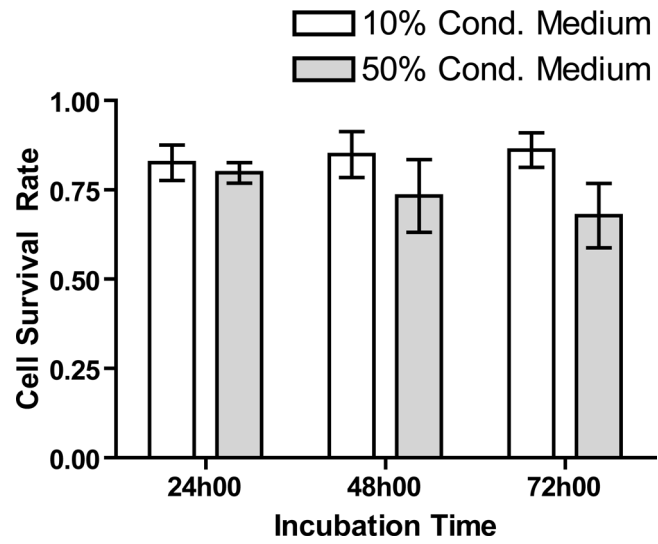
**Fig. S3.** Monocytic U937 cell growth in bulk. The equivalent cell density of a cell encapsulated in a 700  $\mu$ L droplet is  $\approx 1.4$  million cells/mL. The cell growth curve showed that this density is close to the plateau of the U937 growth curve. This may explain why cell growth of encapsulated cells was slow and limited to the first 24 h.



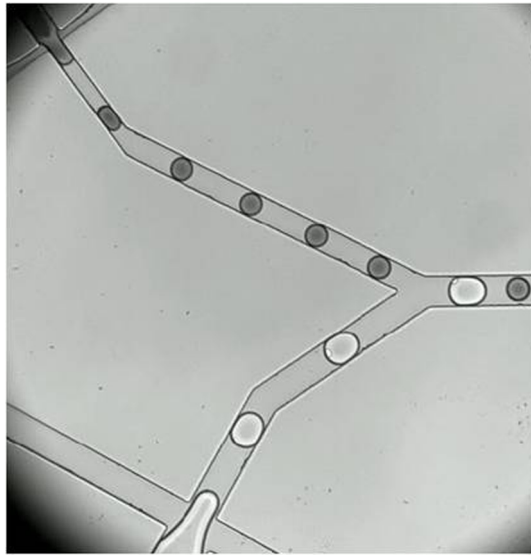
**Fig. S4.** Short term effect of cell encapsulation on cell survival. To assess the cytotoxic effect of cell encapsulation, encapsulated cells were assayed using the integrated live-dead assay device after short period of incubation. The results showed that the encapsulation process induced some cell death, likely because of shear-stress and/or cell-fluorosurfactant interaction. This loss was compensated within 24 h by the limited cell growth that occurred during this period.



**Fig. S5.** Effect of the Pluronic F68 additive on cell-interface interactions after 24 h incubation as assessed by overlay experiments. (A) U937 cells were seeded on a layer of fluorinated oil only in a microtiter plate. (B) Cells were seeded on a layer of fluorinated oil containing 2% weight of the ammonium salt of carboxy-PFPE surfactant, resulting the next day in a gel-like mixture. (C) Cells premixed with Pluronic F68 were seeded on the oil-surfactant formulation, resulting in morphologies similar to the F-oil formulation.

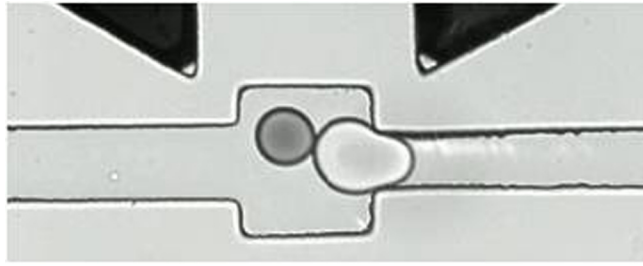


**Fig. S6.** Effect of conditioned medium on cell survival. To mimic cell overcrowding we encapsulated cells at the same density but with 50% conditioned medium. The conditioned medium had a negative impact on cell survival starting after 2 days, but clearly pronounced after 3 days of incubation.



**Movie S1.** Nozzle modules and the fork that collated both streams in an alternate fashion. For clarity reasons the dyes have been replaced by cyanol blue for droplets to show up as dark droplets. The movie was captured at a rate of 2,222 frames/s and is played at a rate of 10 frames/s (slowed down 222 times).

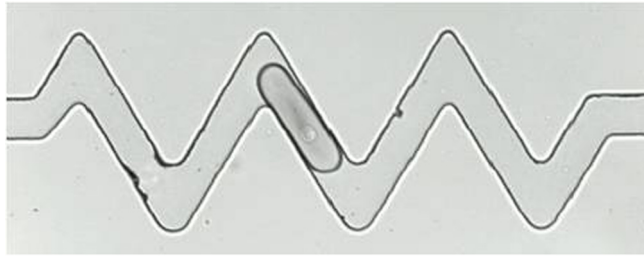
[Movie S1 \(MOV\)](#)



**Movie S2.** Merge module. The droplets entered the module as pairs, because the smaller droplets were faster than the bigger droplets, the speed differential assured that they caught up before the module. The fusion was induced by a high voltage- high frequency electric field delivered by 2 solder-injected electrodes. The shape of the merge box improved the merge efficiency by both slowing down the droplets and by bringing them closer together. The movie was captured at a rate of 8,620 frames/s and is played at a rate of 10 frames/s (slowed down 862 times).

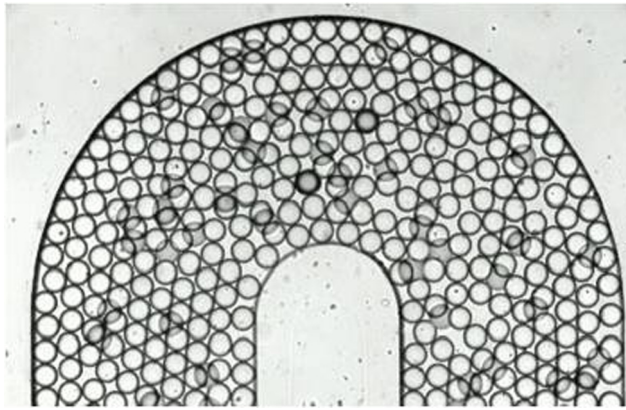
[Movie S2 \(MOV\)](#)





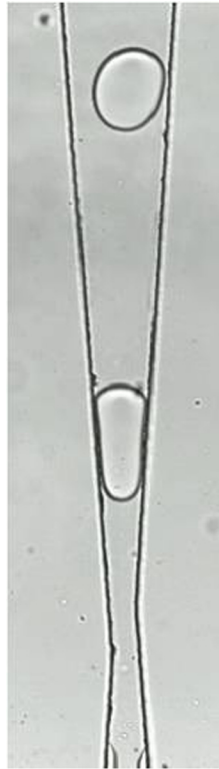
**Movie S3.** Mixing module serpentine that both squeezes each droplet to increase internal recirculation flows and alternates the droplet direction to enhance mixing. The movie was captured at a rate of 8,620 frames/s and is played at a rate of 5 frames/s (slowed down 1,724 times).

[Movie S3 \(MOV\)](#)



**Movie 54.** Delay-line for on-chip incubation. The incubation line relied on increasing the cross-section and the length of the channel so that the flow swept a large volume. This design resulted in a significant residence time on the chip, the incubation here was  $\approx 15$  min. The movie was captured at a rate of 100 frames/s and is played at a rate of 12 frames/s (slowed down 8 times).

[Movie 54 \(MOV\)](#)



**Movie S5.** Detection module. Droplets flowing through the detection module that confined droplets laterally and vertically assuring that each cell went consistently through the laser slit excitation. The movie was captured at a rate of 4,200 frames/s and is played at a rate of 10 frames/s (slowed down 420 times).

[Movie S5 \(MOV\)](#)