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

Supplementary Data

Table 1. Parameters used for wafer substrate fabrication: photolithography process.

Thickness	Resin used	Spin coater parameter	Soft Bake	Insolation	Post Exposure Bake
5 µm	SU8 2005	Speed 500 rpm, acceleration 200, 10s Speed 2500 rpm, acceleration 500, 30s	2 min, 95°C	110 mJ	3 min, 95°C
9 µm	the process for 5 µm was repeated twice				
30 µm	SU8 3050	Speed 500 rpm, acceleration 1, 10s Speed 4000 rpm, acceleration 3, 30s	10 min, 95°C	170 mJ	1 min, 65°C and 5 min, 95°C

Figure SI 1. Pictures of the different components of the soft confiner system and their names disassembled (top) and assembled (bottom).
The clamping systems consist of two stainless steel parts (#1 and #2) to hold a glass coverslip (#3, not represented). Two silicone gaskets (#6) ensure water tightness. The Polycarbonate holder (#4) enable to maintain the molded agarose gel. It is placed onto a glass coverslip previously seeded with cells inside the system by a clamping washer (#5) using a specific clamping tool (#7).

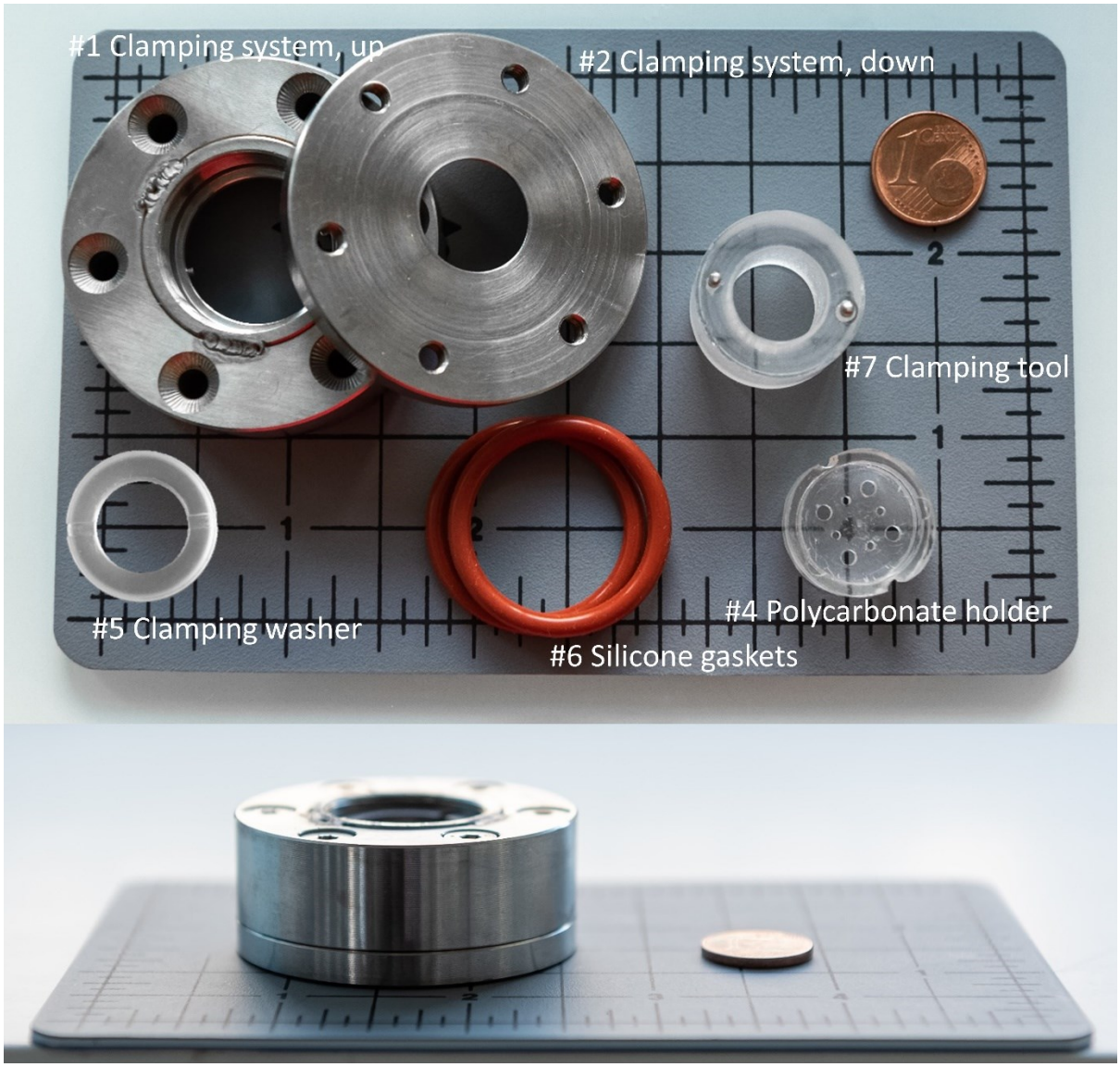


Figure SI 2. Details on the Polycarbonate holder (Element #4 in Fig. SI 1)
(A) Technical drawing of the holder (upper and side views). The upper part (B) will be the reserve of culture medium, while the lower part (C) allows to maintain the agarose gel. Holes are drilled to allow the connection between the two and external notches allow a good positioning during molding and to maintain its position in x,y during tightening.

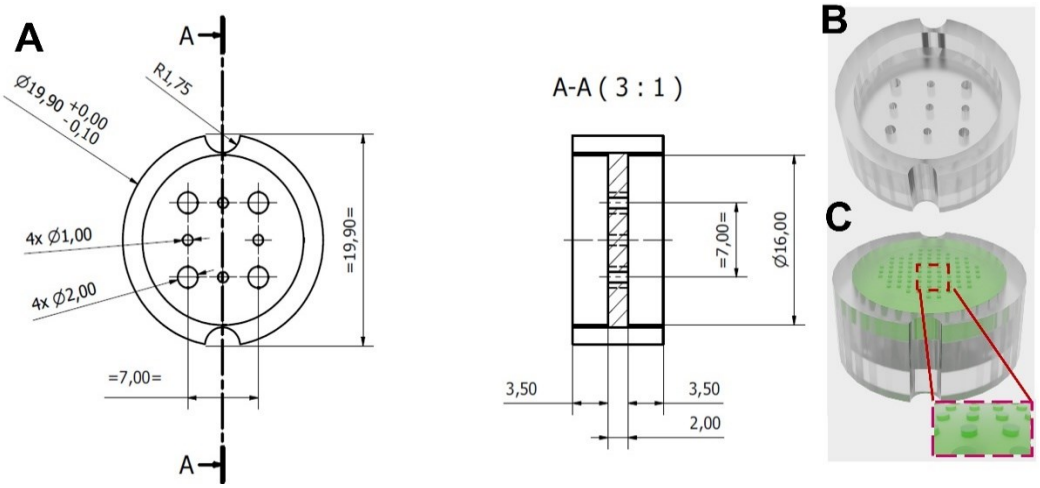


Figure SI 3. Technical drawing (upper and side views) of the lower part of the soft confiner (Element #2 in fig. SI 1). An opening is left for microscopic viewing.

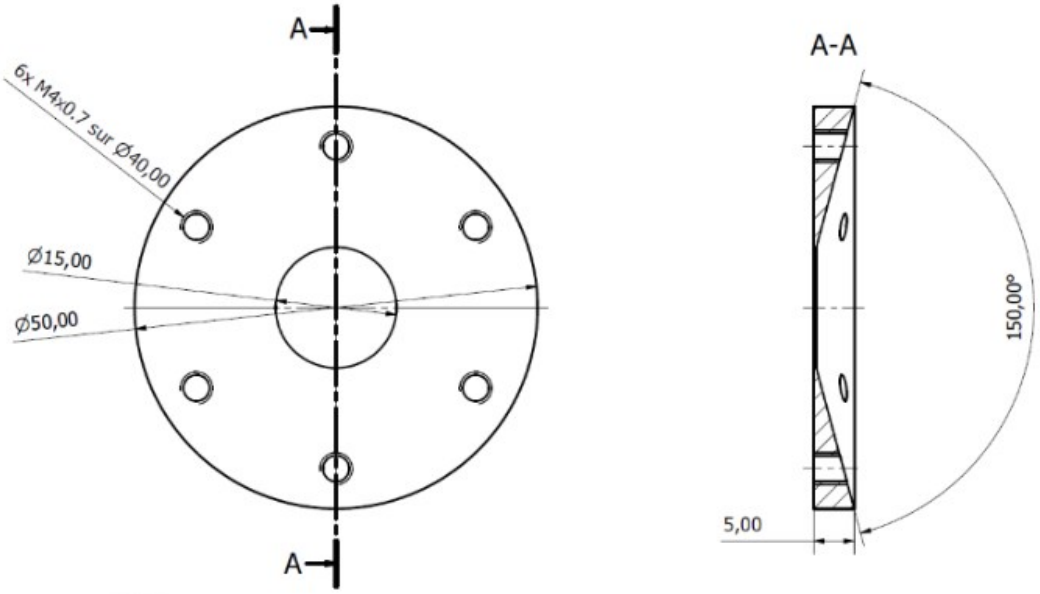


Figure SI 4: Rheological characterization of the different agarose used

Storage (G', circles) and loss (G", triangles) modulus of 4% (w/v) standard agarose (blue circle G', and blue triangle G") and 2% (w/v) ultra low agarose (red circle G', and red triangle G") measured as a function of strain for a normal force of 0.15N.

Viscoelastic curves present a plateau at small strains (0.1% for standard Agar, and 5% for Ultra low agarose), i.e., in the linear regime. On these plateaus, the elastic moduli is more than 10 times larger than the loss modulus, showing an elastic behavior. The value of the storage modulus given in the main text corresponds to the value of the G' on the plateau. **For Ultra Low agarose, it is 1,2 ± 0.06 kPa and 144± 7 kPa for standard agarose.**

For each type of agarose, rheological measurements were performed for at least 3 samples.

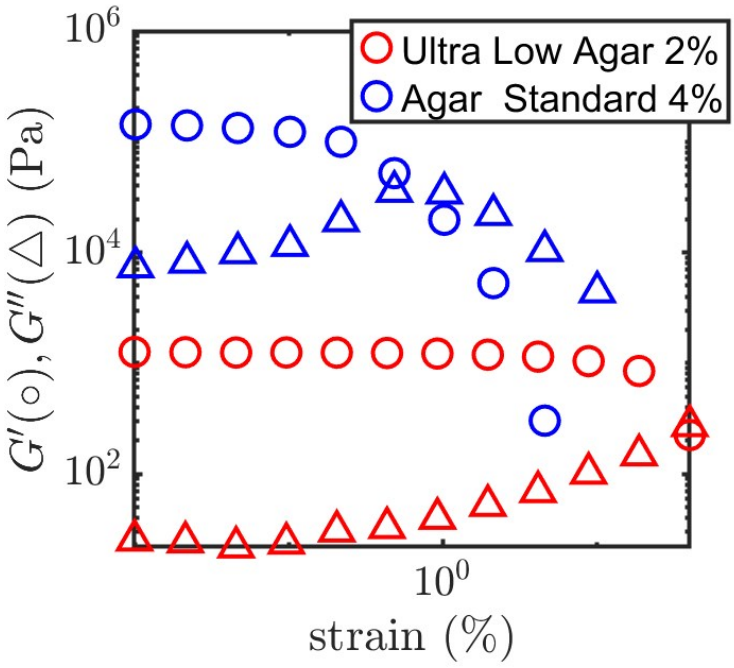


Figure SI 5: Medium diffusion inside the soft cell confiner for FITC (fluorescein isothiocyanate dissolved in PBS). Characteristic time-course of diffusion = 450 min (7 h 30 min).

Briefly, the soft cell confiner was mechanically sealed and loaded with PBS. A solution of fluoresceine Isothiocyanate (0.05 µM) dissolved in PBS was then added on the open upper part of the microsystem. The fluorescence intensity in the lower part of the system was monitored every 10 min using an inverted microscope. The intensity was then measured over time and normalized against the maximum intensity.

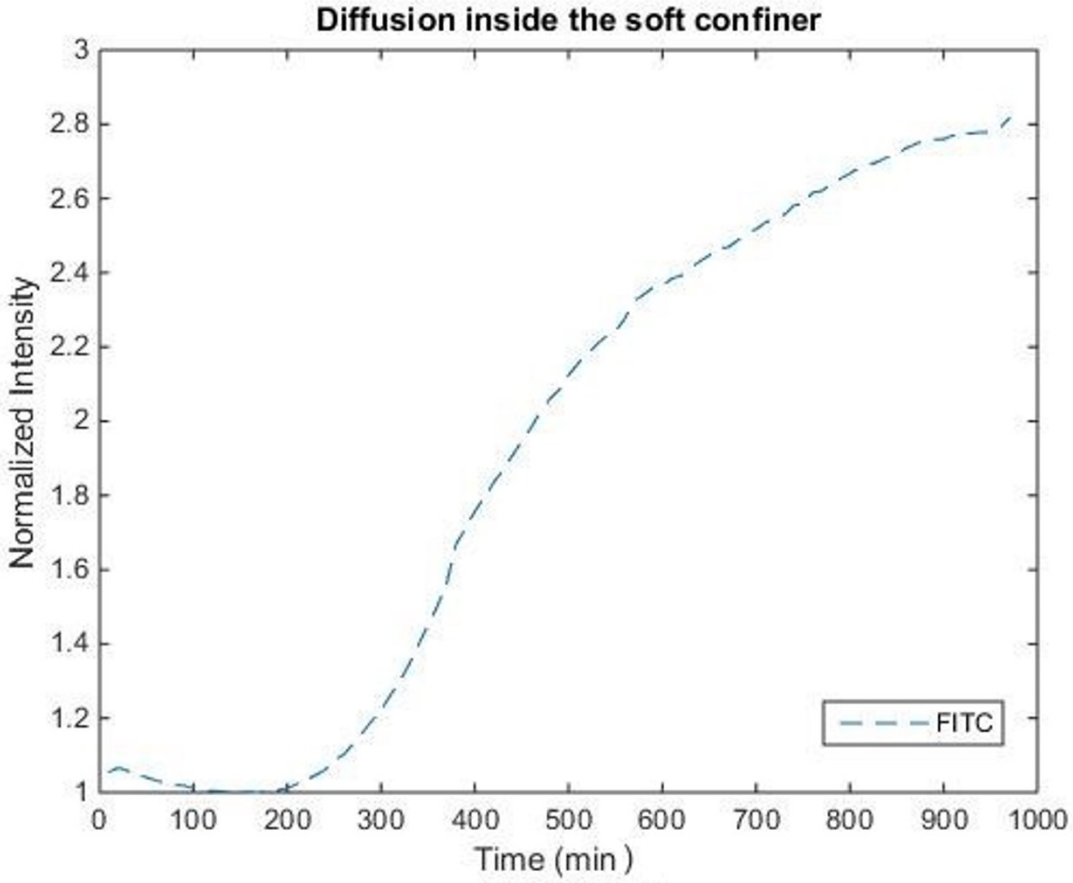


Figure SI 6: Cell response to drugs under confinement.

Resistance of TF1-BA cells to the tyrosine kinase inhibitor Imatinib (also known as, Gleevec or STI-571) was assed under confined (5 µm, bottom) or control (30 µm, up) conditions, in the presence (right) or absence (left) of 2µM Imatinib. After 3 days, in the presence of Imatinib, proliferation is reduced in both conditions. Bar = 50 µm

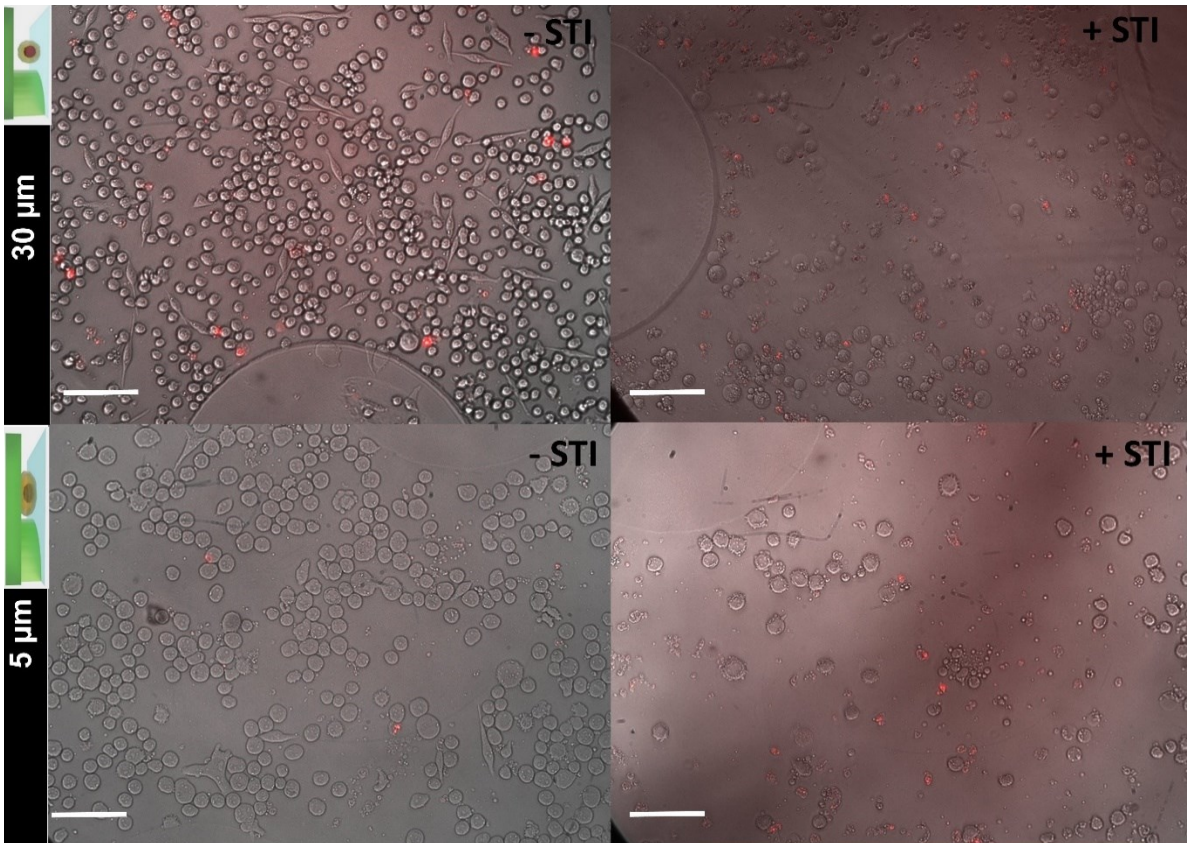


Figure SI 7: Quantification of the reproducibility and stability of the height of the agarose pillars
(A) Assessment of reproducibility: heights for three different systems, n=7 for each.

(B) Stability evaluation: heights on day 0, day 1 and day 2 of the same system kept in culture. n=7.

The differences on the average value are non-significant.

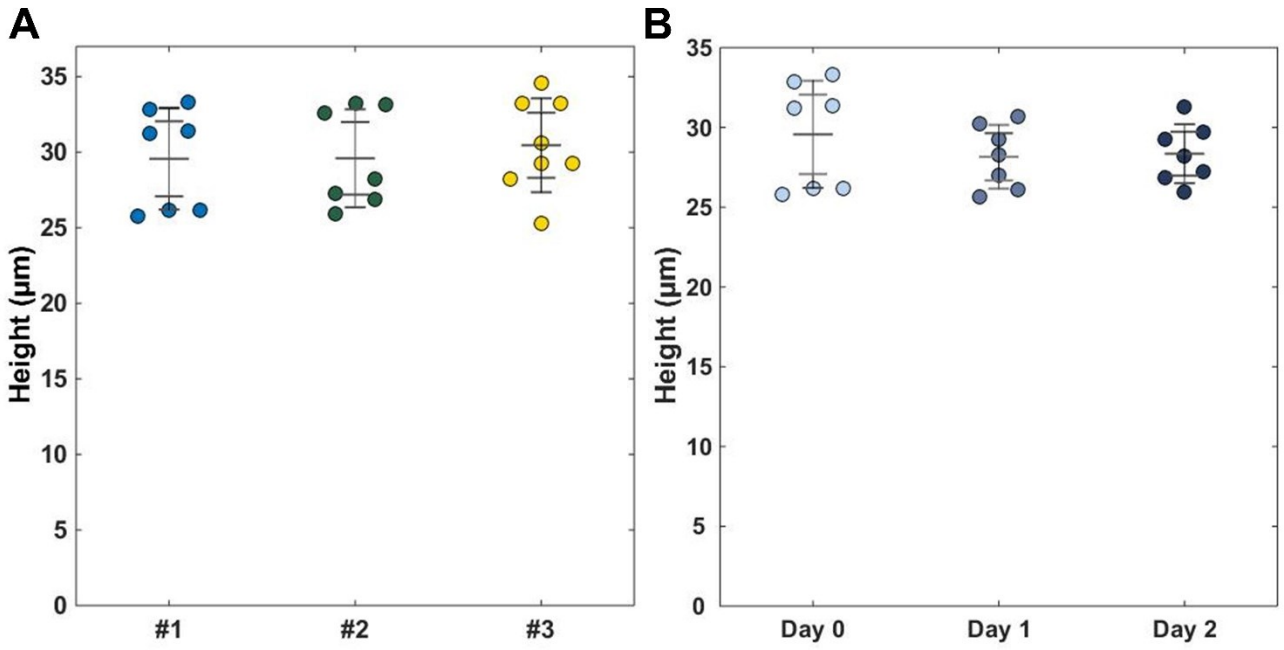


Figure SI 8. Proliferation of TF1-GFP, TF1-BA and HS27A cells over 3 days in a PDMS-based confiner. In preliminary experiments, the effects of 3 days confinement on hematopoietic cell lines and their stromal cell supports were investigated using a PDMS-based confiner similar to [55], leading to distinct behavioral changes between cells. Proliferation of the HS27A cell line was impacted by the system itself, since both the 5-µm confinement and the 30-µm control significantly decreased cell proliferation. We thus hypothesized that this effect was likely reflecting the lack of control of medium renewal, and/or hypoxic conditions induced by the presence of the glass coverslip supporting the PDMS pillars. These preliminary results were difficult to interpret as we could not discriminate if the cell response was due to mechanical stress and/or nutrient or oxygen deprivation (due to the glass coverslip used to maintain the PDMS pillars).

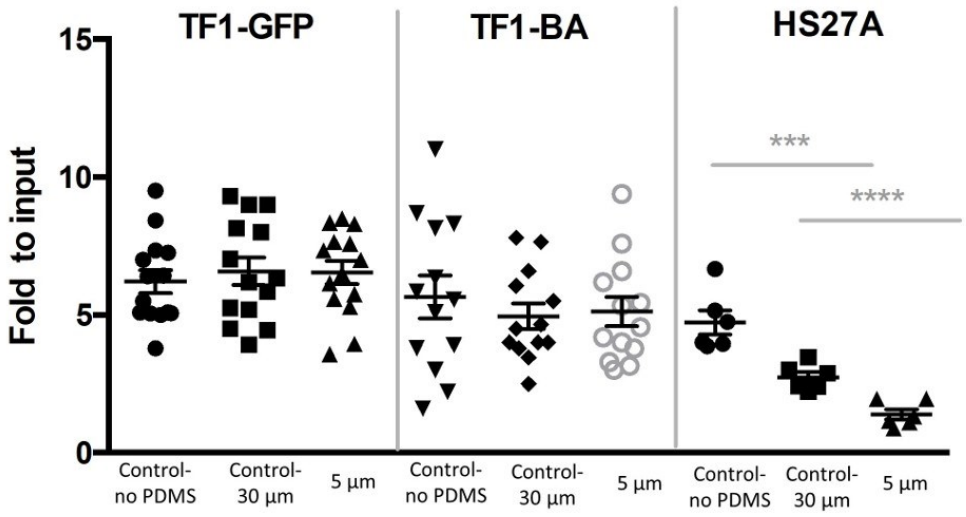
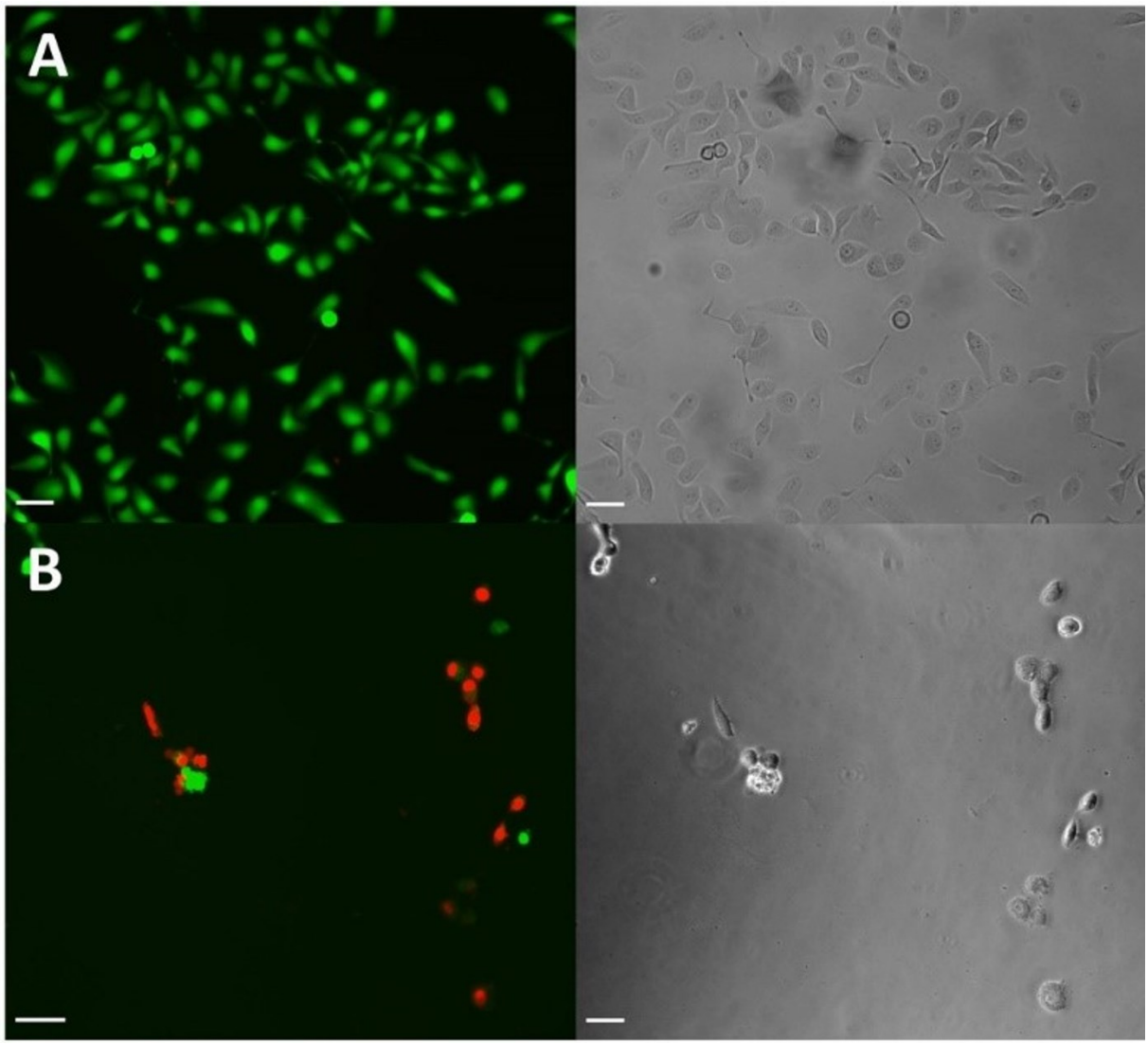


Figure SI 9: Control of live and dead MCF10A cells on glass coverslips after 1 day of incubation.
(A) Control live and (B) control dead cells obtained by adding 70% ethanol for 30 min prior to imaging. Calcein AM is represented in green and propidium iodide in red, scale bar = 100 µm.



Movie 1:
Assembly of the soft cell confiner. Step 1: the polycarbonate holder with agarose mold installation, step 2: polycarbonate clamping washer positioning, step 3: clamping washer tightening, step 4: adding medium.

Movie 2:
Time-lapse of phase-contrast images of immature TF1-BA hematopoietic cells over 1 day in the soft cell confiner with agarose gel presenting pillars much larger than their height (30 μm) on the left and under confinement (5 μm) on the right. Interval between images = 2 h, scale bar = 200 μm.

Movie 3:
Time-lapse of phase-contrast images of MCF10A epithelial cells over 1 day in the confiner with agarose gel presenting pillars much larger than their height (30 μm) on the left and under confinement (5 μm) on the right. Interval between images = 1 h, scale bar = 200 μm.