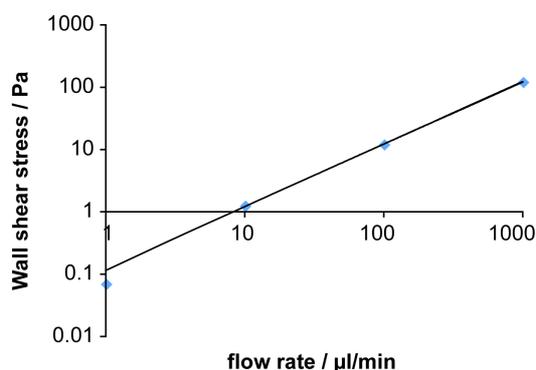


## Optimization of microfluidic single cell trapping for long-term on-chip culture

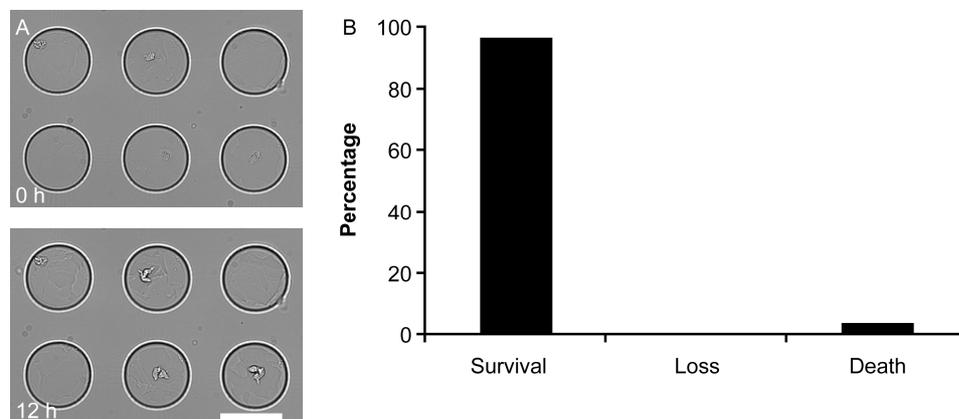
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### Supplementary information

SI Movie 1 and 2: The trapping of single cells in a 1-mm and a 4-mm device, respectively. Trapping yields were assessed as number of trapped cells per number of cells passing a trap. In order to record a sufficient number of trapping events, trapped cells were removed from the trap by reversing the flow, which was sufficient to generate an unbiased population. Because the ratio of the fluxes through the trap and through the main channel was only about 50% in the 1-mm, many cells (65% in average) miss the first trap. Increasing the main channel length to 4 mm also increases the ratio of fluxes to about 80% and the trapping efficiency to 97%.



SI Figure 1: The simulated wall shear stresses in the main channel of a 2-mm design for different flow rates used to calculate the shear stresses in the microfluidic experiments.



SI Figure 2: Single cell survival in poly(ethylene glycol) (PEG)  $\mu$ -well arrays. (A) Two images of single EG7 cells cultured statically on PEG  $\mu$ -well arrays, at the beginning and at the end of the time-lapse experiment. (B) Quantification of the single cell survival on PEG micro-well arrays. Images were acquired every 30 min. The arrays were fabricated according to a recently published protocol (see reference 8 for details).

SI Table 1: Estimates of the shear stresses in the main channel for the flow rates used for the on-chip cell culture. The shear stresses were extrapolated from shear stresses in SI Figure 1.

| Flow rate  | Shear stress            |
|------------|-------------------------|
| 20 nl/min  | $2.4 \times 10^{-3}$ Pa |
| 100 nl/min | $1.2 \times 10^{-2}$ Pa |
| 500 nl/min | $6.0 \times 10^{-2}$ Pa |

