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

Supplementary information for

Effect of capillary fluid flow on single cancer cell cycle dynamics, motility, volume, and morphology

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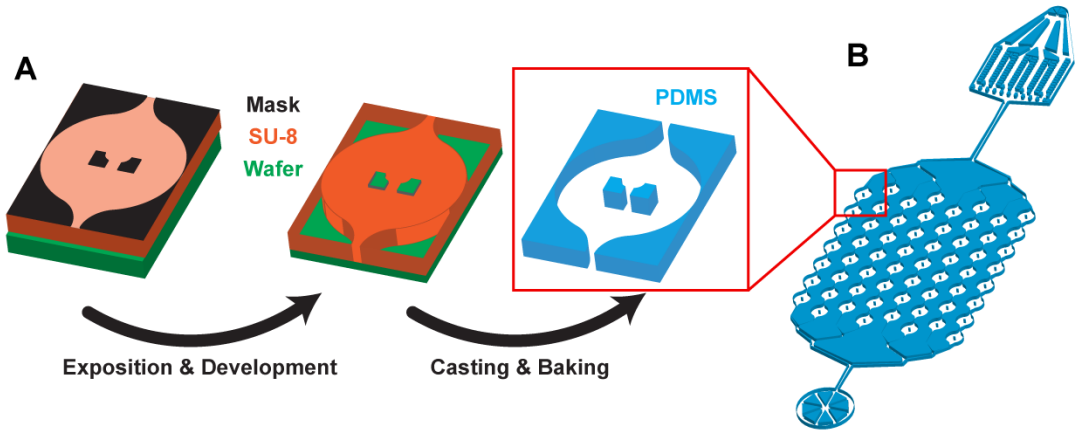


Fig. S1 Schematic of the wafer and microfluidic device fabrication process. (**A**) A silicon wafer was spin-coated with SU-8 to a 30 µm thickness, then exposed to UV light through a mask containing the design of the microfluidic device. After chemical development to remove non-cured SU-8, a wafer master mold was generated. Liquid PDMS was then poured into the wafer master mold and baked to create the microfluidic device. (**B**) Overview of one complete microfluidic device just showing PDMS in the region of the channels".

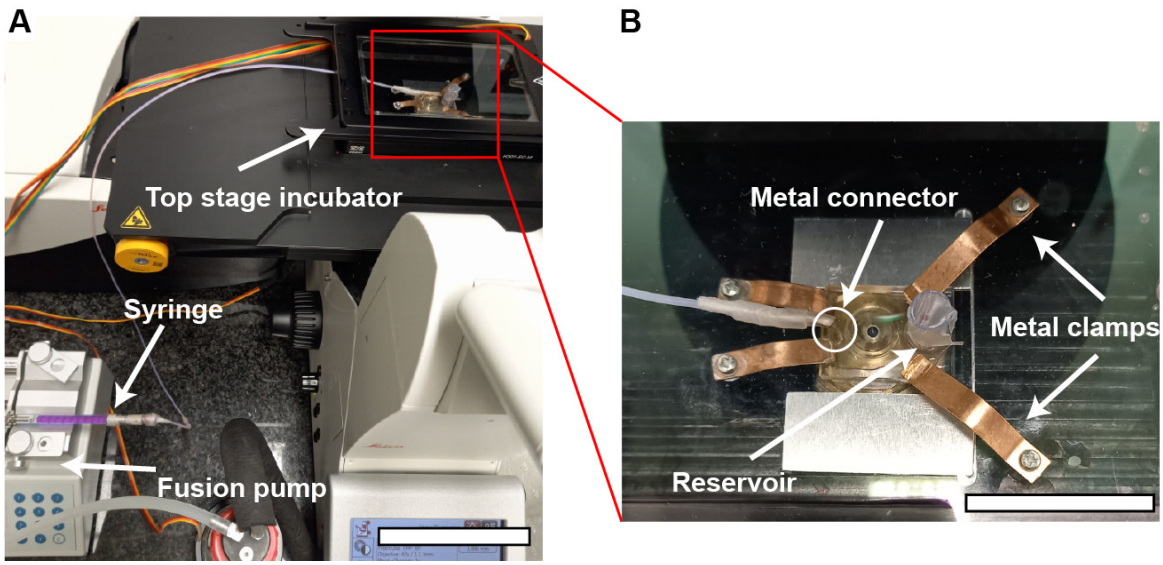


Fig. S2 Photographs of the complete microfluidic device setup with syringe and pump. (**A**) Top stage incubator mounted on a confocal SP8 microscope containing the microfluidic device. The microfluidic device is attached to a syringe mounted on a pump in withdraw mode to create the flow. Scale bar is 12 cm. (**B**) Close-up view of the inside of the top stage incubator. The microfluidic device is mounted on a custom-made holder with four metal clamps to prevent movement during time lapse. The outlet of the microfluidic device is attached to the syringe via a metal connector to ensure a tight connection and avoid formation of air bubbles. Scale bar is 4 cm.

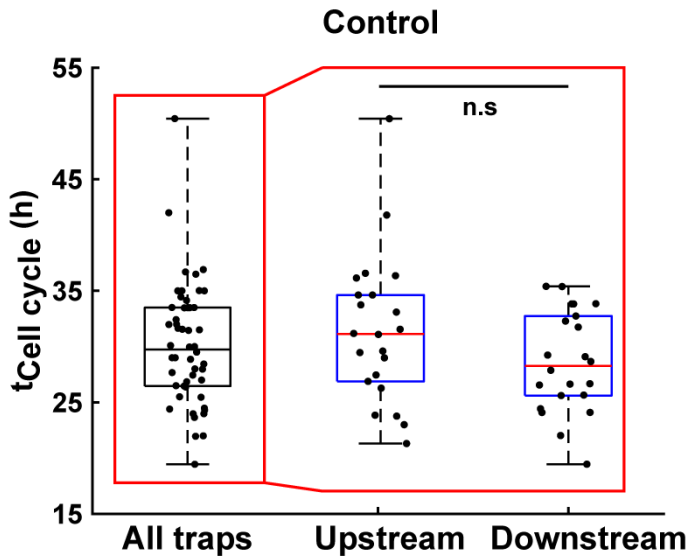


Fig. S3 Cell cycle time separated by cells found in trap upstream or downstream. Upstream cells were defined as cells found in the trap 3 traps closest to the inlet, and Downstream cells as cells found in the 3 traps the further away from the inlet.

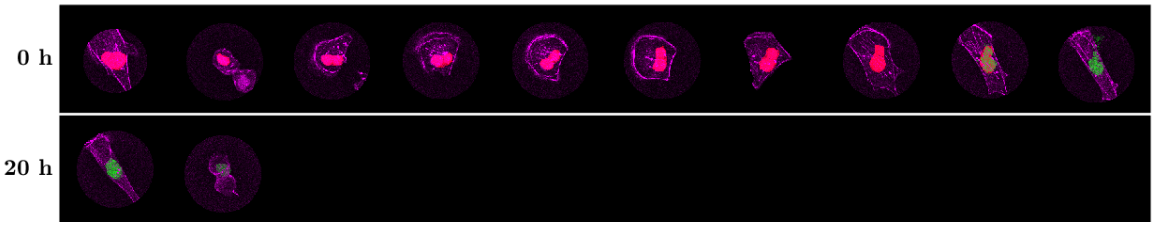


Fig. S4 Maximum projection of FUCCI2 + SiR-Actin of an exemplary cell with its varying shape over time (24 hours in 2 hours interval). The magenta corresponds to the F-Actin staining, the red and green corresponds to the FUCCI2 signals.

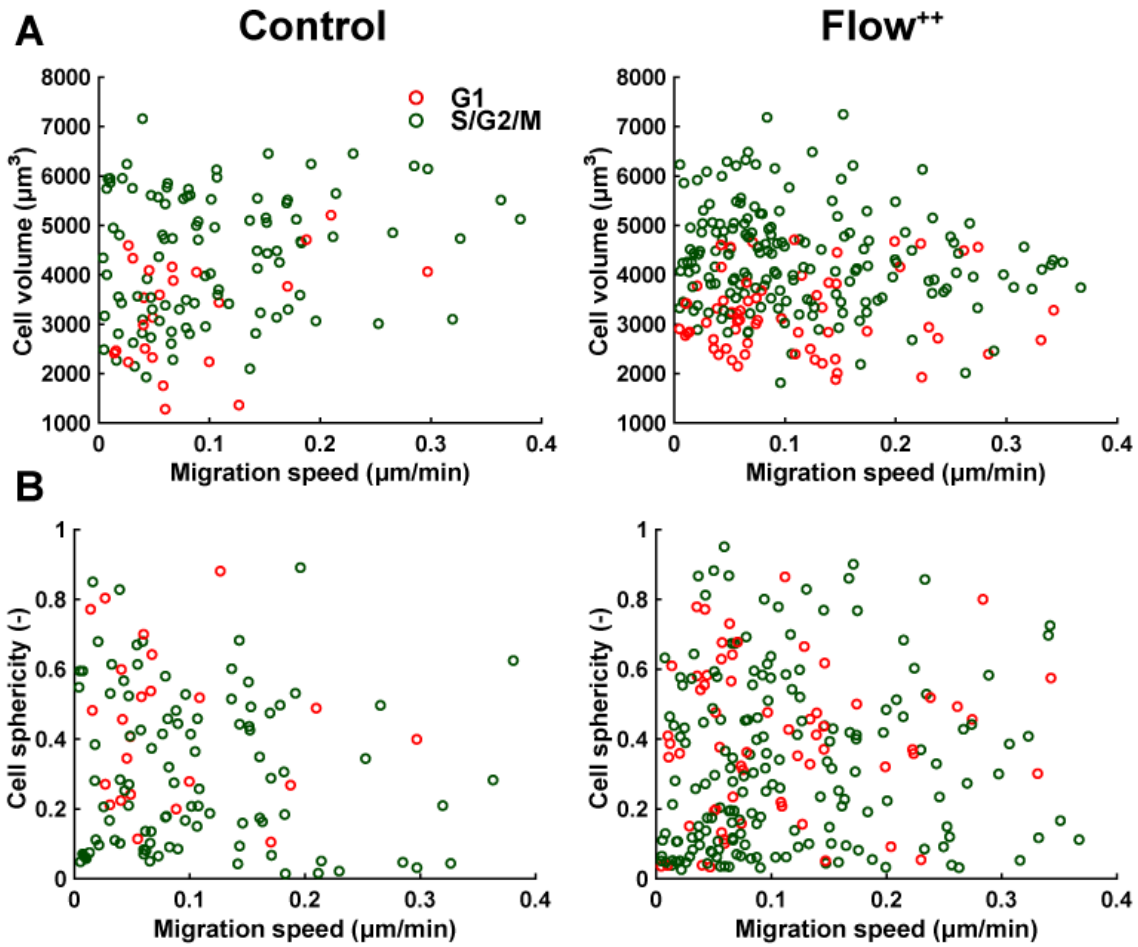


Fig. S5 Cell correlation between morphological features (volume and sphericity) and migration speed. (**A**) Cell volume and (**B**) sphericity as a function of migration speed for the control and Flow^{++} groups (two biological repeats, N= 8 and 15 cells, respectively).

Computational fluid dynamic simulation

In addition to experimental 3D time lapse imaging, computational fluid dynamic (CFD) simulation was performed to estimate the fluid velocity inside the microfluidic device and to compute the wall shear stress for each cell and for each timepoint automatically. The CFD simulations were performed using OpenFOAM (Open-Source Field Operation and Manipulation) and the results visualized with the open-source software ParaView.

3D Model and assumptions

In the CFD simulation, the goal was to solve the Navier-Stokes equation that governs fluid dynamics in the microfluidic device. There are several conditions that were assumed: (i) the fluid is Newtonian and (ii) incompressible. In addition, (iii) all variables (pressure and velocity) are independent of time (steady state), (iv) there is no leak and (v) there is an equal distribution of the flow between all channels of the microfluidic device. Gravitational forces were neglected and the flow was considered laminar (Re << 1). The base 3D model (without a living cell) corresponds to one trap with two posts in the middle, since all traps in the microfluidic device are identical. The flow at the inlet was obtained by assuming an equal repartition between the different channels of the microfluidic device and therefore dividing the flow of the syringe by eight (number of parallel channels).

A simulation was performed for each z-stack acquired by confocal microscopy and for each timepoint. The simulations were parametrized and interfaced with OpenFOAM automatically using a custom-made script written in Python 3.8.6: Automated Simulation On Cluster (ASOC).

From the SiR-Actin channels, each cell was 3D modelled by segmenting the z-stacks. The surface generation was done using the Amira software (“Generate surface” module) that uses the marching cube algorithm to create a mesh from 3D datasets.

The cell surface was modeled as a rigid surface as first approximation and a no-slip condition was imposed on the walls of the trap and on the cell boundary, meaning that the velocity at this interface is set to 0. Since the cell shape changes with time, the updated shape at each time point obtained from experiments was used to calculate the shear stress on the cell surface.

Choosing the right mesh

The models (the microfluidic chip trap and the cells inside, Fig. S6A) were meshed using the cfMesh library within OpenFOAM and were of cartesian type. The mesh was mostly composed of uniform elements with coarse size of 4 µm, locally refined to 2 µm around the central posts and to 1.5 µm around each cell boundary (Fig. S6B). Since the wall shear stress calculation depends on the element size close to the cell, combining coarse and fine meshing was found to maintain sufficient accuracy, while improving the simulation time by an order of magnitude, compared to using uniform elements of 1.5 µm everywhere.

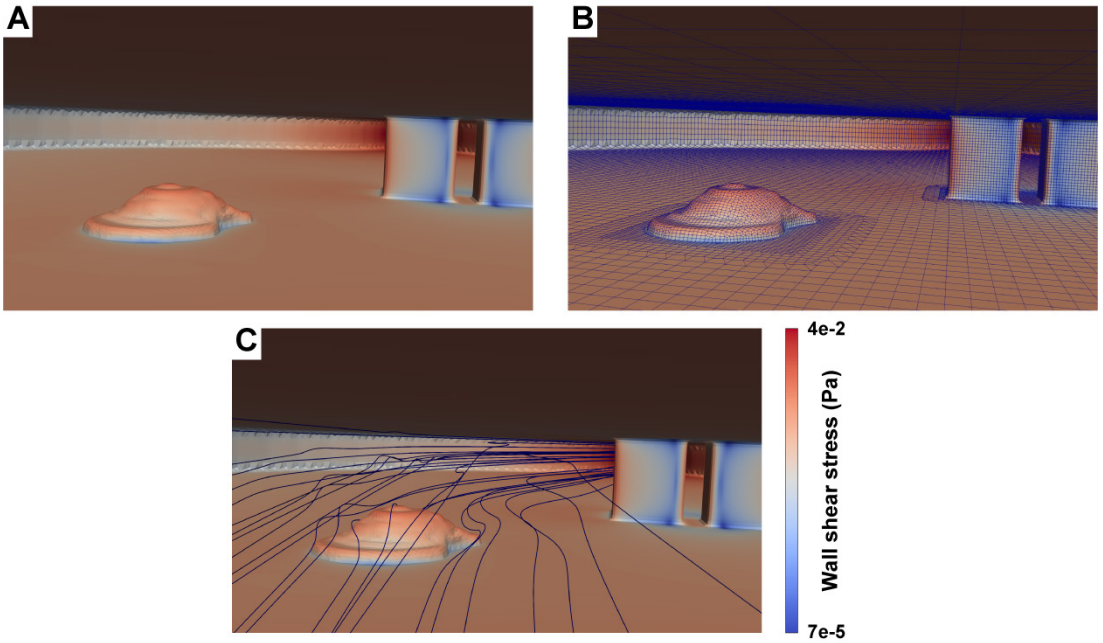


Fig. S6 CFD simulation on a real experimentally obtained cell volume reveals wall shear stress experience by a metastatic breast cancer cell. (**A**) View from within the 3D CFD simulation model containing a cell inside a trap with the color corresponding to the wall shear stress. (**B**) Corresponding view of the mesh at the surface used for the computation. The mesh is finer around the cell with 1.5 µm and around the trap with 2 µm, and coarse further away with 4 µm, in order to reduce computation time. (**C**) Overlay of the wall shear stress with the fluid streamlines around the cell.

Wall shear stress calculations by coupling 3D time-lapse live cell imaging experiments and CFD simulations

The wall shear stress was computed using OpenFOAM on a computer cluster of the Max Planck Institute. The SiR-Actin channel was used to approximate the cell membrane. A simulation was performed for each z-stack acquired by confocal microscopy, for each timepoint of the cell cycle (Fig. S7A). The simulations were parametrized and interfaced with OpenFOAM automatically using a custom-made script written in Python 3.8.6 (ASOC). The cells in the trap were 3D-modelled and meshed like previously described. The wall shear stress value was obtained by averaging every element of the mesh representing the cell (Fig. S7B). Combining 3D time-lapse confocal imaging of single cells and CFD simulations, we could calculate in a more accurate way the mechanical stress that each cell experiences under fluid flow. For this exemplary cell taken from the Flow^{++} group, the wall shear stress experienced was on average 6 ± 4 mPa or 0.06 ± 0.04 dyne/cm² throughout the cell cycle.

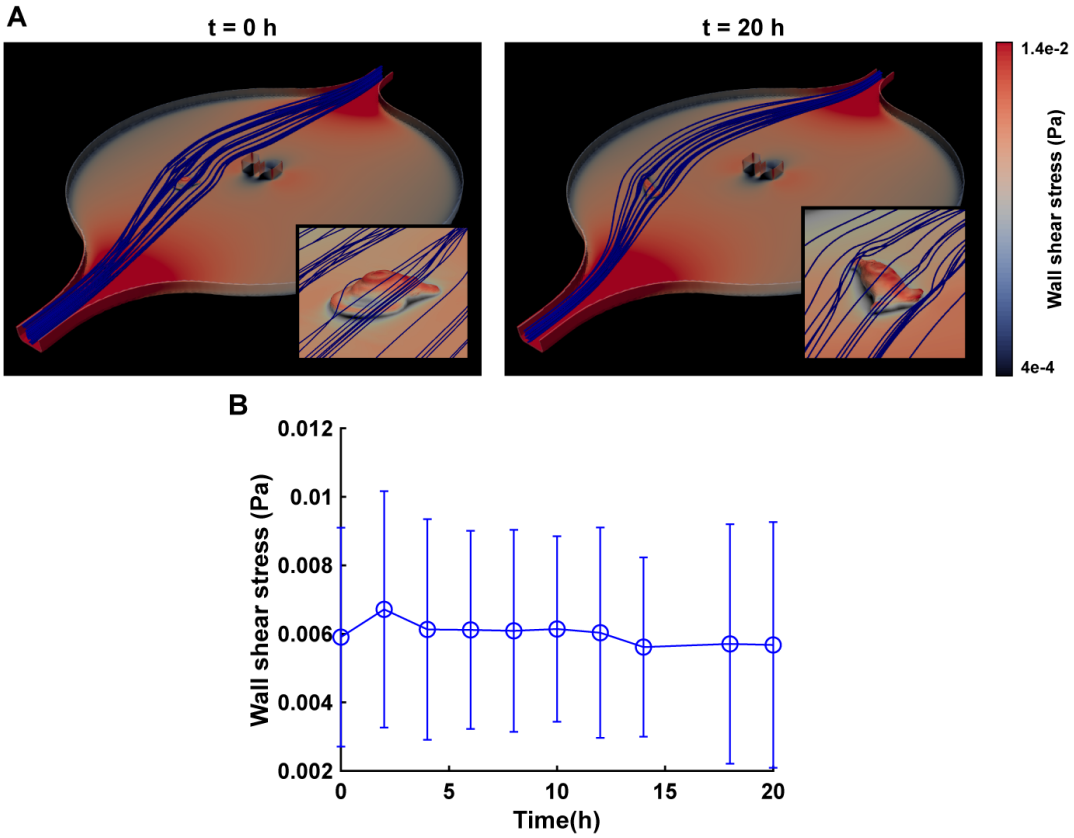
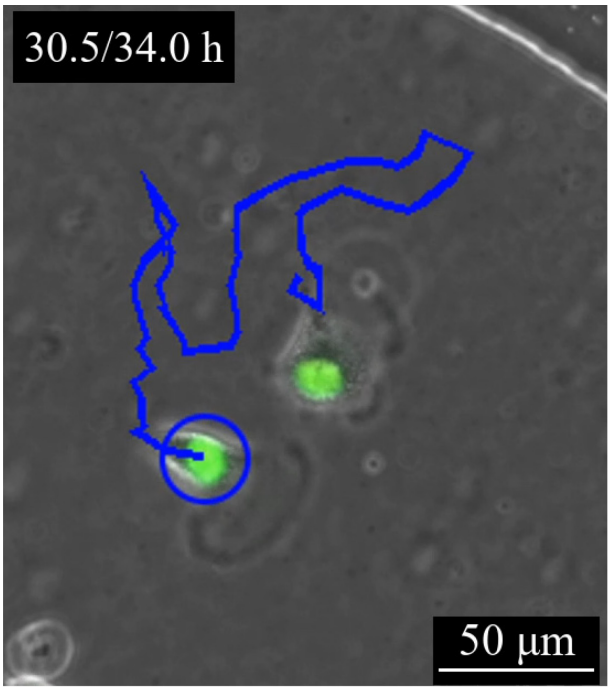
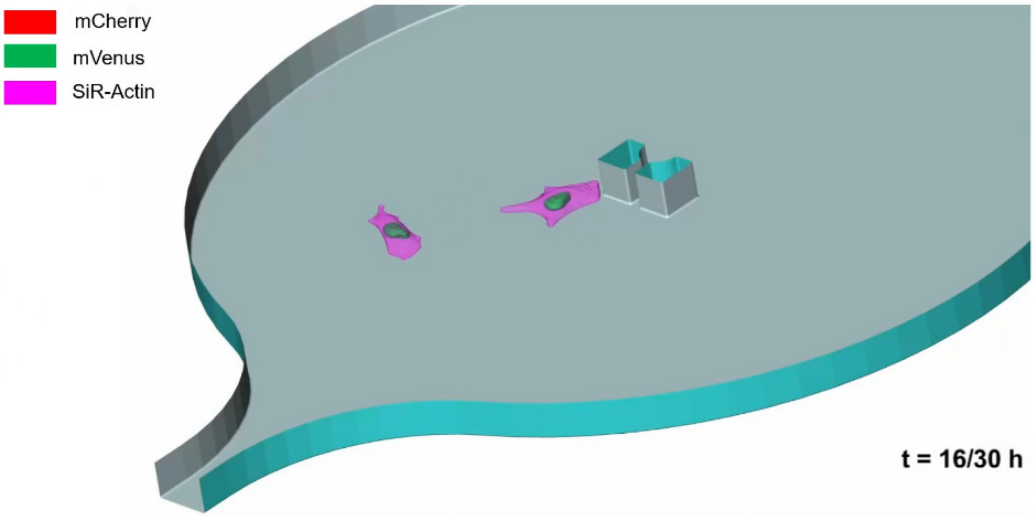


Fig. S7 Time-lapse wall shear stress calculation using CFD and 3D confocal imaging of single cells. (**A**) Finite element simulation of an exemplary real cell shape (obtained with SiR-Actin) inside a trap for the Flow^{++} group (streamlines around the cell indicated in blue) at time point 0 and 20 h. (**B**) Average wall shear stress over the surface of one exemplary MDA-FUCCI2 cell experienced during its cell cycle. The error bars correspond to the standard deviation.



Supplementary Movie 1 (Separate file) Phase contrast and fluorescence time-lapse imaging of single MDA-FUCCI2 cells trapped in microfluidics, with an overlay of their trajectory in blue. Time in hours.



Supplementary Movie 2 (Separate file) 3D rendering of confocal fluorescence time-lapse imaging of single MDA-FUCCI2 cells trapped in microfluidics. The FUCCI2 signals are depicted in red and green and the SiR-Actin in magenta. Time in hours.