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

Technical details of operational components

The microfluidic cell culture system used in this report consists of the following components: (i) imaging system, (ii) cell culture unit and (iii) microfluidic control system (**Figure 1E**) as described by Kellog *et al.* [1] In the following section each of these operational components is specified in detail.

i) Imaging system

A Nikon Eclipse TI microscope equipped with an automated mechanical stage and a digital CMOS camera ORCA-Flash 4.0 (Hamamatsu, Japan) was used. Filters and light sources (Nikon LED and Lumencor SPECTRA X light engine) were automatically controlled by the supplier's software (NIS Elements). For setting up of microfluidic chips a Nikon Plan Fluor 4x objective (NA=0.1, WD=16500 μm) was used in bright field mode. For experiments Nikon Plan Fluor 10x (NA=0.3 WD=15200 μm) or Nikon Plan Fluor 20x (NA=0.5, WD=8200 μm) objectives were used in bright field and fluorescent mode. For time-lapse microscopy images were acquired using the supplier's software (NIS Elements) in conjunction with the microscopes add-on function "perfect focus system" (PFS).

ii) Cell culture unit

For culturing cells the microscope was equipped with a polystyrene temperature incubator (Life Cell Imaging Service GmbH, Basel, Switzerland) connected to a temperature control unit (Life Cell Imaging Service GmbH, Basel, Switzerland). For controlling CO_2 concentration (5%) and humidity (98%) a stage top incubator was connected to a humidifier and CO_2 mixer (Life Cell Imaging Service GmbH, Basel, Switzerland). For equilibration this culture unit was switched on 30 min before loading cells on chips.

iii) Microfluidic control system

A large-scale-integrated microfluidic system was developed and operated on the basis of previously described systems.[2,3] Specifically, the two-layer PDMS device was designed in a push-up configuration with a thin control layer for opening and closing of valves at the bottom and a thick flow layer for handling of experimental fluids and cells bonded to the top. For controlling microfluidic valves control channels were connected with metal pins (0.064

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[1] R. Kellogg, R. Gómez-Sjöberg, A.A. Leyart, S. Tay. "High-throughput microfluidic single-cell analysis pipeline for studies of signaling dynamics." *Nat. Prot.* **9**(7):1713-1726,(2014)
[2] M. Unger *et al.*,"Monolithic Microfabricated Valves and Pumps by Multilayer Soft Lithography." *Science.* **288**: 113-116,(2000)
[3] Gómez-Sjöberg et al. Versatile, fully automated, microfluidic cell culture system. *Anal. Chem.* **79,** 8557–8563 (2007).

cm OD, 0.33 ID, New England Small Tube Corporation, Litchfield, USA) and Tygon Tubing (Milian, Satigny, Switzerland) with miniature pneumatic solenoid valves (Festo, Dietikon, Switzerland). The tubes were filled with water or soybean oil and the device was operated with control pressure that was individually adjusted for each chip between 1.0 - 1.5 bar. Flow was generated by pressurizing the inlet vessel of the flow channel (0.1 bar) hereby moving fluid towards unpressurized outlets.

Solenoid valves were controlled by a costum-written MATLAB (Mathworks, Austin, USA) or LabVIEW (National Instruments, Austin, USA) graphical user interface (GUI) using a USB signal converter as described in detail elsewhere.[4] This allowed automated generation of gradients as described earlier.[5]

Image analysis algorithm

Images from time-lapsed microscopy were exported as TIFF or stored in Nikon's file format (.nd2). Raw imaging data were processed with Imaris (Bitplane AG, Zürich, Switzerland). Automatic object tracking via "Imaris Spots" was aided with manual corrections to retrieve cell spatial coordinates over time and the resulting data were analyzed in PRISM (Graphpad) and MATLAB (MathWorks).

Chip production

The microfluidic device was fabricated with polydimethylsiloxane (PDMS) using standard multilayer soft lithography. Two molds, a control mold and a flow mold, were patterned on silicon wafers with photolithography. For the control layer mold we used SU8-3025 as photoresist, while for flow layer mold we combined SU-8-3025 (Microchem, Westborough, USA) with AZ50XT (AZ Electronic Materials, Luxembourg) photoresist layers. The use of two layers of photoresist for flow layer mold allows generation of parabolic shaped channels at valve regions while non-valve regions have a rectangular structure.

Specifically, for fabrication of the flow layer mold a silicon wafer was cleaned with acetone, isopropanol and water. After spin-drying, the wafer was dry baked for 5 minutes at 200 ºC. Following this the wafer was treated for 5 minutes with hexamethyldisilazane (HMDS) for achieving surface hydrophobicity for prevention of photoresist delamination. Next, the wafer was placed on a spin coater, covered with AZ50XT and spun at 2700 rpm for 20 seconds to reach a height of 23 µm. This wafer was then cleaned with acetone on the back and

[4] https://sites.google.com/site/rafaelsmicrofluidicspage/valve-controllers/usb-based-controller

[5] Frank, T. & Tay, S. Flow-switching allows independently programmable, extremely stable, high-throughput diffusion-based gradients. *Lab Chip* **13,** 1273–1281 (2013).

rehydrated on a leveled plate at room temperature for 10 minutes and protected from light. Afterwards a soft bake at 115 ºC for 3 minutes was done before rehydrating again for 2 hours in the dark at room temperature. Photoresist was then exposed to UV light for 200 seconds using I-line filter of the mask aligner through a high-resolution positive mask (40 kdpi, Fine Line Imaging Inc., Colorado Springs, USA) containing valve-regions of the flow circuit. Light exposure was limited to 20-second intervals separated by 30 seconds breaks. Following light exposure, the wafer was developed with AZ400K (20 mL developer, 60 mL H_2O, AZ Electronic Materials, Luxembourg) for 6 minutes. Next, the wafer was rinsed with water and spin-dried. Following this the mold was baked 13 hours at 200 ºC to reflow the positive resist and create parabolic AZ50XT profile in valve-regions. The wafer with AZ-50XT structures was then retreated with HMDS, before spin-coating it with SU8-3025 at 3000 rpm for 30 seconds to reach a height of 25 µm. The coated wafer was then leveled for 5 minutes before being baked for 2 minutes at 65 ºC and 10 minutes at 95 ºC. Next, AZ-50XT structures of the wafer were aligned with a high-resolution positive mask of the flow circuit of non-valve regions and then exposed for 13 seconds to UV light using I-line filter. Following this a post exposure bake was performed by ramping the wafer on hot plates up to 95 ºC during 40 minutes, keep the temperature constant for 5 minutes before cooling down. The wafer was then developed in 40 mL of mrDEV-600 (micro resists technology GmbH, Berlin, Germany) for 3 minutes. Following rinsing with acetone, isopropanol and water the wafer was spin-dried. Finally, the mold was hard baked for 2 min at 65 ºC before ramping up 160 ºC for 2 hours.

The control wafer was produced in analogy to the SU8-3025 part of the flow-wafer production protocol.

For production of PDMS chips the molds were first treated with Chlorotrimethylsilane (TMCS; Sigma Aldrich, Buchs, Switzerland) for 20 minutes in a sealed box. In parallel a PDMS mixture of 10:1 (potting-agent:cross-linking agent) was mixed for 3 minutes with a mixing machine (Thinky ARE-250, Tokyo, Japan) with a degasing function (2 minutes degasing). Following this PDMS was degased for 15 minutes in a vacuum chamber. For fabrication of the flow layer PDMS (72g) was poured over a wafer, degased for 60 minutes, leveled for 15 minutes and cured for 1h at 80º C. For fabrication of the control layer PDMS (10g) was spin-coated (2300 rpm), leveled for 15 minutes and cured for 1 h at 80 º C.

The thicker PDMS block on the flow layer mold was peeled off, and holes were punched for fluidic inlets using a 22-gauge mechanical puncher (Syneo, Angleton, USA). The flow and the control layers were then exposed for 30s to oxygen plasma (40%, Diener FEMTO, Ebhausen, Germany), aligned under a stereoscope and bond for 2 hours at 80 º C. The two bonded layers were peeled off from the flow mold and holes for control line inlets were punched. A layer of PDMS was spun onto a glass slide at 1800 rpm for 60 s and baked for 1h

at 80 °C. The PDMS covered glass slide and the two-layer chip were exposed to oxygen plasma for 30s for bonding and were then baked at 80 º C for at least 12 hours. A similar protocol for the illustration of multilayer softlithography has been made available by the Stanford Microfluidics Foundry as a video.[6]

Chip set-up

To set up a chip for an experiment, the chip was taped on a slide holder (see figure 1E right bottom) and control lines were connected to the corresponding valves. Following placement under the microscope pressure in the control lines was gradually increased under optical control until all valves were closed. To this minimal closing pressure 0.5 bar were added to ensure tight closure of valves during fluid flow in flow channels. The waste outlets of the flow layer were the connected with waste containers via tygon tubing. Following this PBS was flushed into the control channels via one inlet until the entire chip was filled with PBS. For removing air bubbles outlet valves were closed while pressurizing the PDMS inlet which results in evaporation of air bubbles in the flow channels. Flow channels – but not the cell culture chamber – were then flushed with plurnoic acid (10 mg/mL; Millipore, Zug, Switzerland) for 3 minutes followed by extensive washing with PBS for 30 minutes. Cell culture chamber were then coated with fibronectin (c= 250 µg/mL, Millipore, Zug, Switzerland) for 1 hour followed by flushing of the entire chip with cell culture medium for 10 minutes.

[6] https://www.youtube.com/watch?v=xWdRczefirs