Supplementary Information

A novel microfluidic microelectrode chip for a significantly enhanced monitoring of NPY-receptor activation in live mode

Supplementary Materials and Methods

Fabrication of microelectrode arrays and microfluidic structures

Briefly, cleaned glass substrates (Borofloat 49/49/1.1 mm, Goettgens Industriearmaturen, Germany) were spin-coated with 3 µm negative resist (AR-N 4340, Allresist GmbH, Germany) and baked for 90 s at 95°C. Structures were passed on the substrate via photomask (10 s UV-light, 350 nm to 425 nm) and MA6 Mask Aligner (SÜSS MicroTec, Germany) followed by development in AR 300-475 (Allresist GmbH, Germany). Electrode structures were generated by sputtering (CREAMET 500, CREAVAC GmbH, Germany) of 50 nm ITO as adhesion layer followed by 350 nm gold onto the substrate. Afterwards, remaining photoresist and metal deposition were removed in acetone. A 1µm thick passivation layer was generated using negative photoresist SU8-2 (Micro Resist Technology, Germany). Finally the MEA was cleaned in ultrapure water, spin-dried and plasma-cleaned at 400 mA for 7 min into the vacuum chamber (CREAMET 500, CREAVAC GmbH).

Supplementary Figures

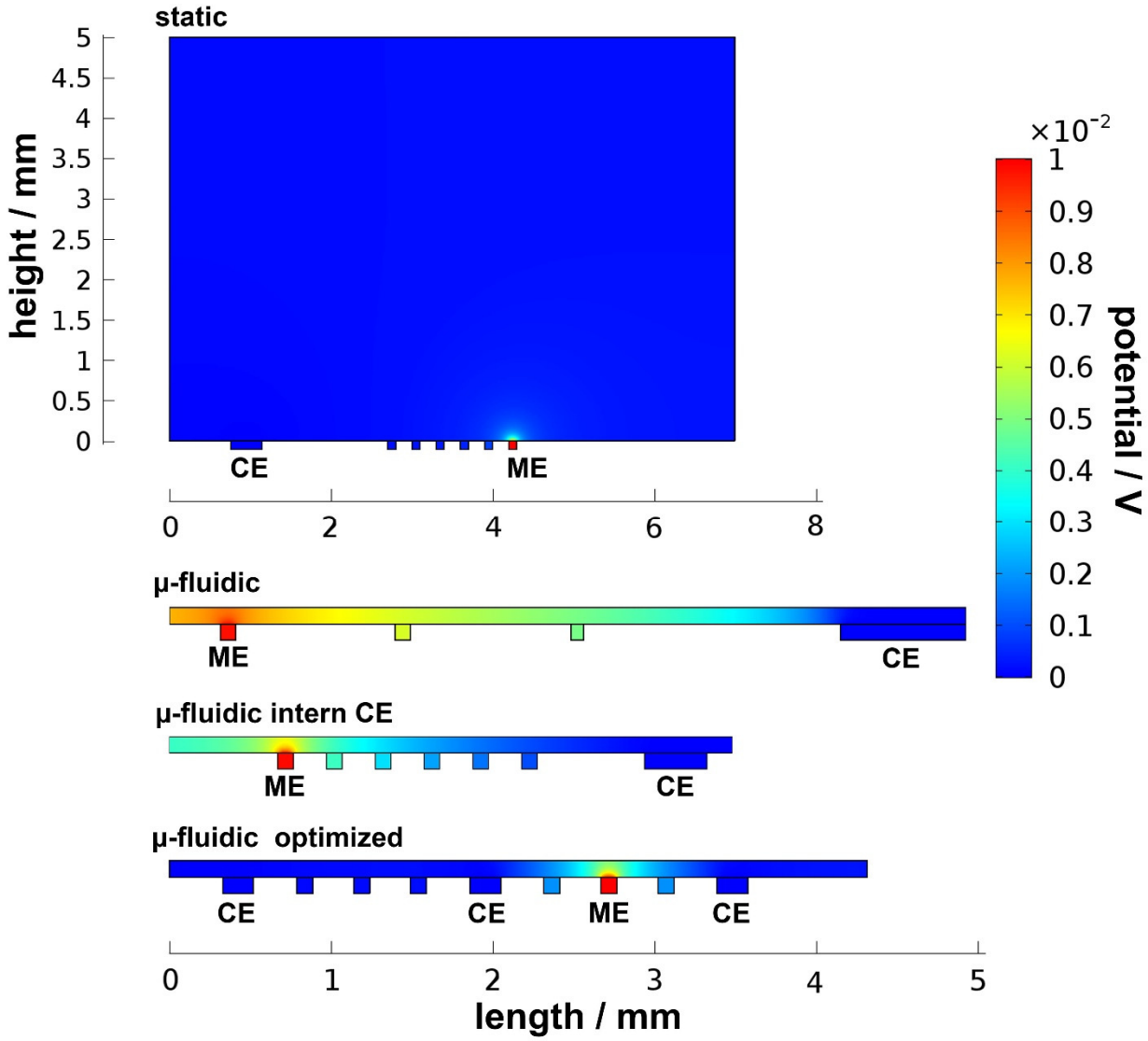

Figure S1. Cross-section of FEM simulation derived potential field. The cross section was done through the active measurement electrode (ME) with an applied potential of 10 mV and the counter electrode (CE) that is connected to the ground. The potential field is shown for 100 kHz. The height of the microfluidic devices is 100 µm.

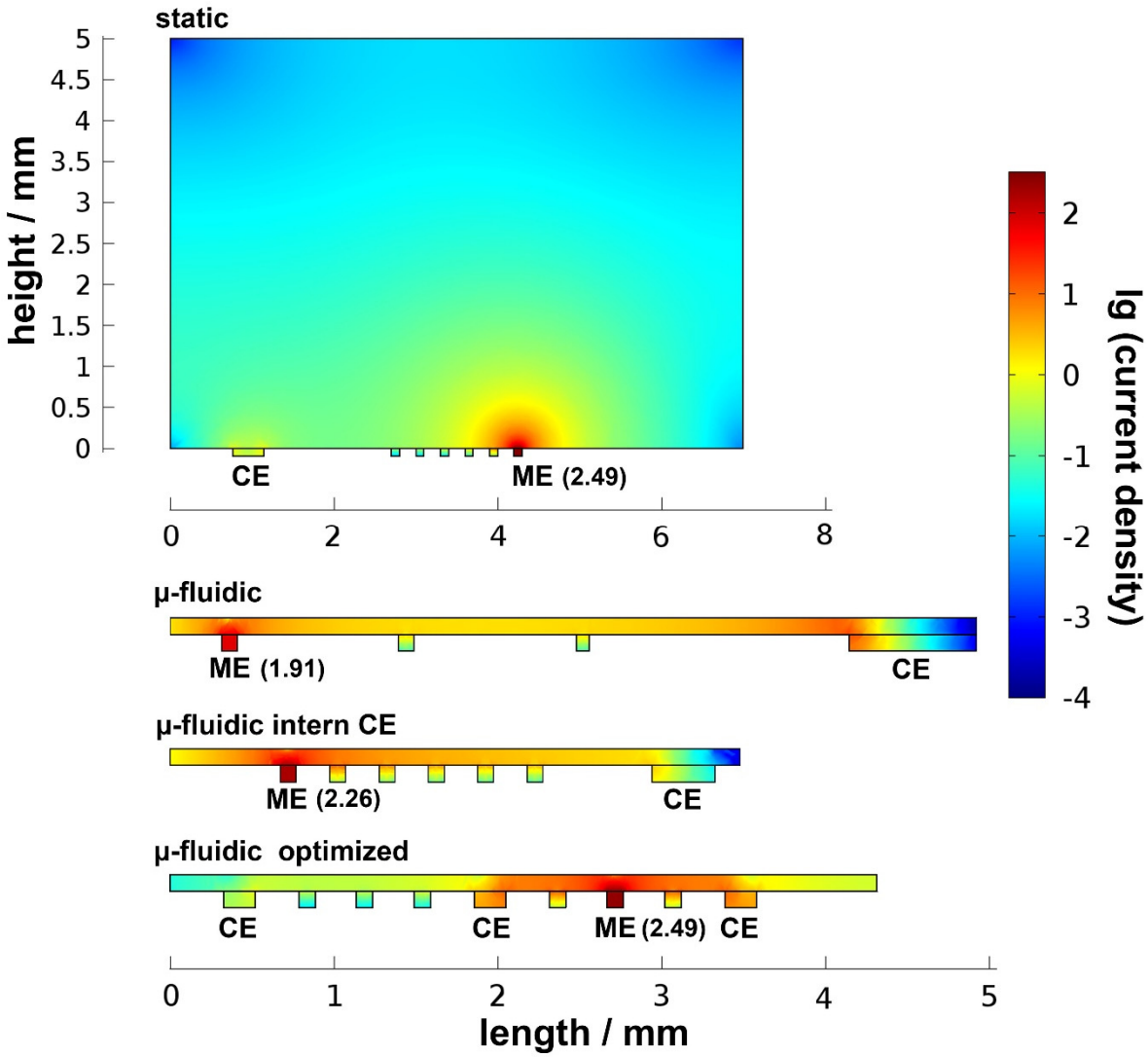

Figure S2. Cross-section of FEM simulation derived current density field. The cross section was done through the active measurement electrode (ME) with an applied potential of 10 mV at 100 kHz and the counter electrode (CE) that is connected to the ground. For better visualization, the current density is shown on a logarithmic scale and the maximum values at the measurement electrode is given in brackets. The height of the microfluidic devices is 100 µm.

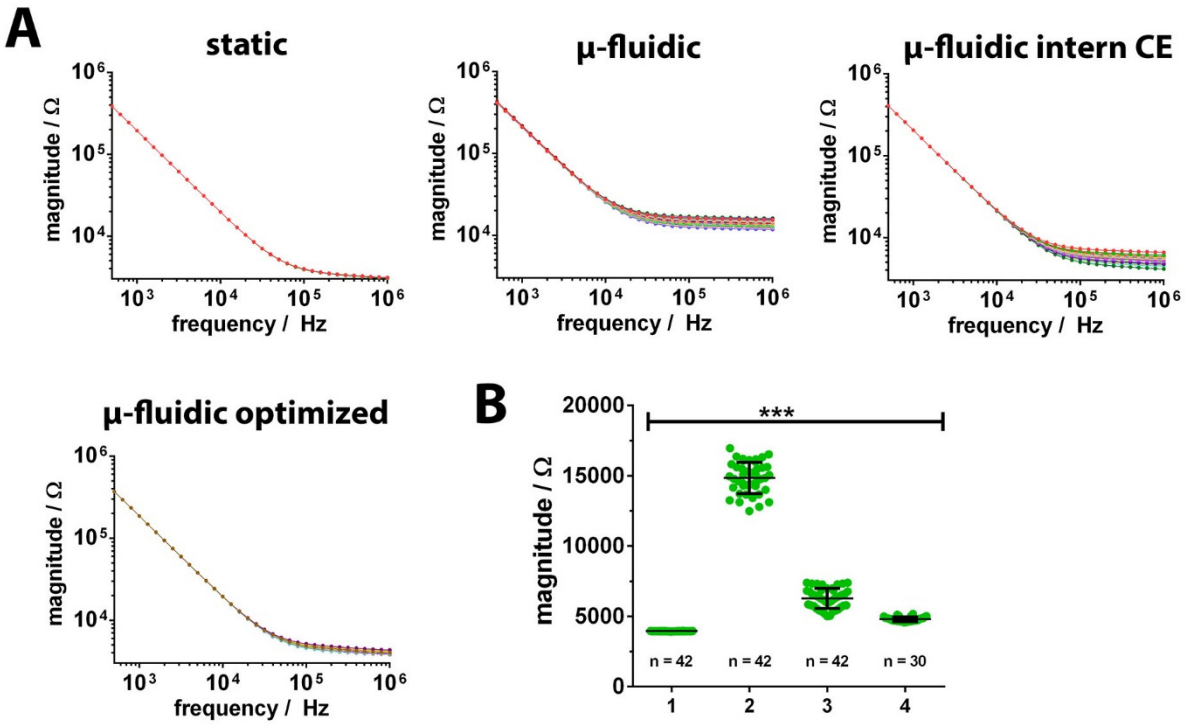

Figure S3. Influence of the microfluidic structure on the measurement-counter-electrode distance. (A) FEM simulations were performed for all measurement electrodes within the arrays with 42 electrodes for the static, µ-fluidic and µ-fluidic intern CE layout as well as 30 electrodes for the µ-fluidic optimized layout. The simulation derived impedance magnitude spectra are shown. (B) Statistical analysis for the impedance magnitude at 100 kHz (mean ± s.d.). The test of significance for the static (1), µ-fluidic (2), µ-fluidic intern CE (3) and µ-fluidic optimized (4) layout revealed an extremely significant difference for all groups against each other ($p < 0.001$).

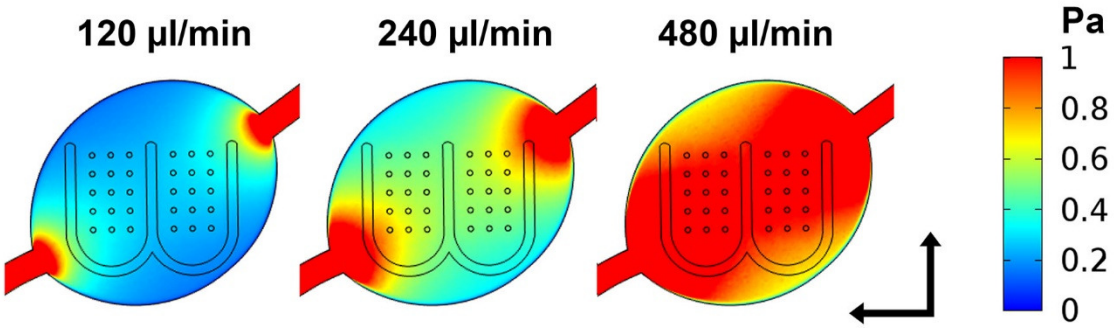

Figure S4. Influence of flow rate on shear stress. FEM simulation derived shear stress in Pascal dependent on flow rate (XY-scale = 1.5 mm).

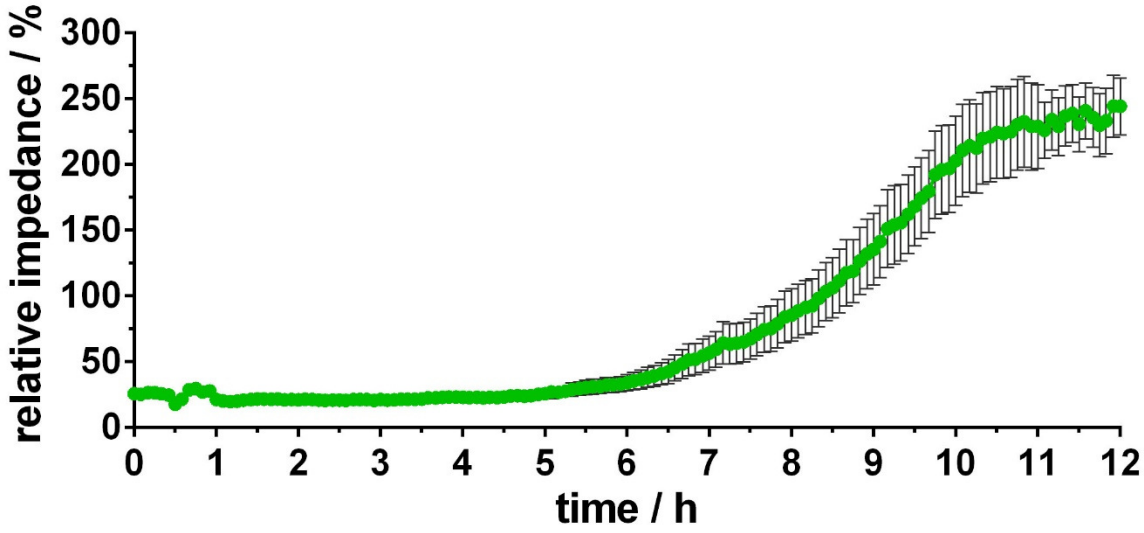

Figure S5. Relative impedance trace over time of HEK-Y1 cell cultured under microfluidic conditions. Culturing under microfluidic condition and recording of impedance spectra was started after initial 45 minutes of HEK-Y1 cell adherence, marked as time point 0 hours (n = 25 electrodes).

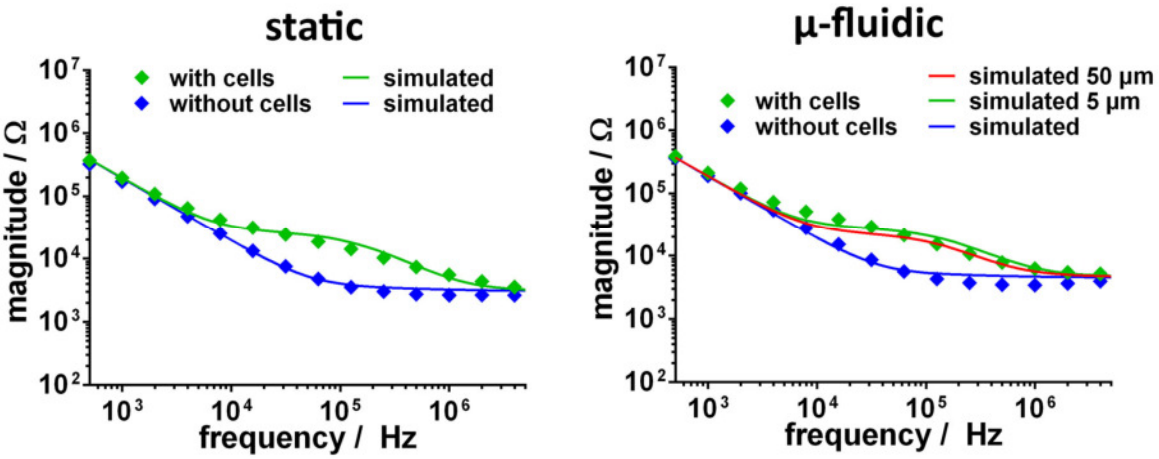

Figure S6. Influence of the cell height in the microfluidic structure on the impedance magnitude. The impedance spectra measured in the static setup were used to determine the cell capacitance (0.008 F/m²) and resistance (0.0002 Ohm x m²) by an equivalent circuit fitting. Therefore, a self-written LabView program was used. In the FEM simulation a cell layer represented by an electrode covering cylinder) with a height of 5 µm was introduced and covered with an contact impedance with the determined cell resistance and capacitance. This represents the impedance signal dominating cell membrane. Afterwards, the cell layer height was increased to 50 µm, which lead to a small cell impedance decrease but not to a basal impedance magnitude increase.

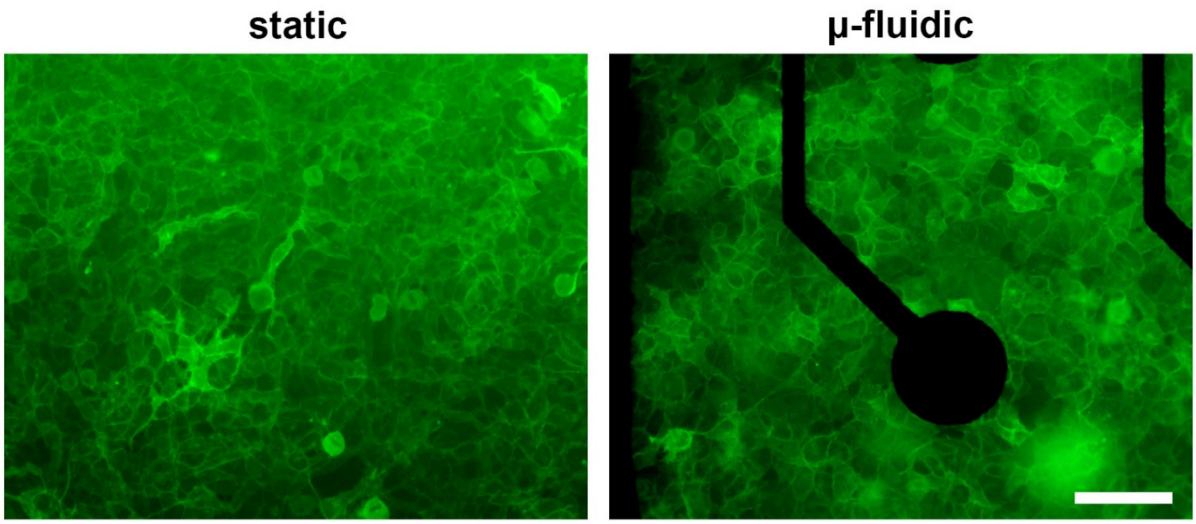

Figure S7. Recombinant Y1-receptor expression in HEKA cells under static and microfluidic conditions. (scale bar 100 µm).

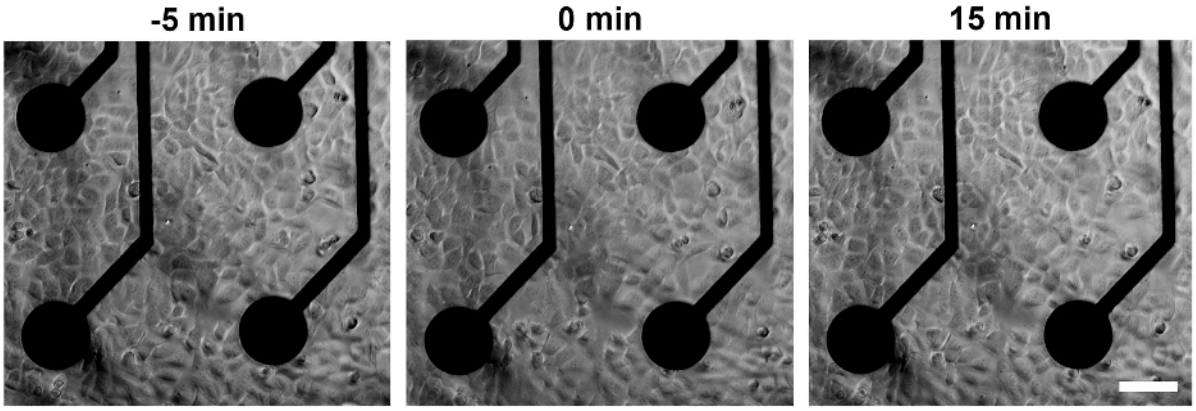

Figure S8. Microscopic images of NPY treated HEK-Y1 cells cultured under microfluidic conditions. HEK-Y1 cells were monitored by transmission light microscopy before (-5 min), at the time point of 100 nM NPY application (0 min) and 15 min after application. (scale bar 100 µm).

Supplementary Movie 1

Time lapse movie (12 hours) of HEK-Y1 cells that adhere and spread over the microelectrode array within the optimized microfluidic microelectrode array chip under microfluidic cultivation conditions. Flow direction is from lower right to upper left.