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

Supporting Information

Selective intracellular delivery and intracellular recordings combined on MEA biosensors

Andrea Cerea[1,*], Valeria Caprettini[1,*], Giulia Bruno[1,2], Laura Lovato[1], Giovanni Melle[1,2], Francesco Tantussi[1], Rosario Capozza[1], Fabio Moia[1], Michele Dipalo[1,*], Francesco De Angelis[1,*]

[1]Istituto Italiano di Tecnologia, 16163 Genova, Italy
[2]DIBRIS, Università degli Studi di Genova, 16145 Genova, Italy

Email: francesco.deangelis@iit.it, michele.dipalo@iit.it

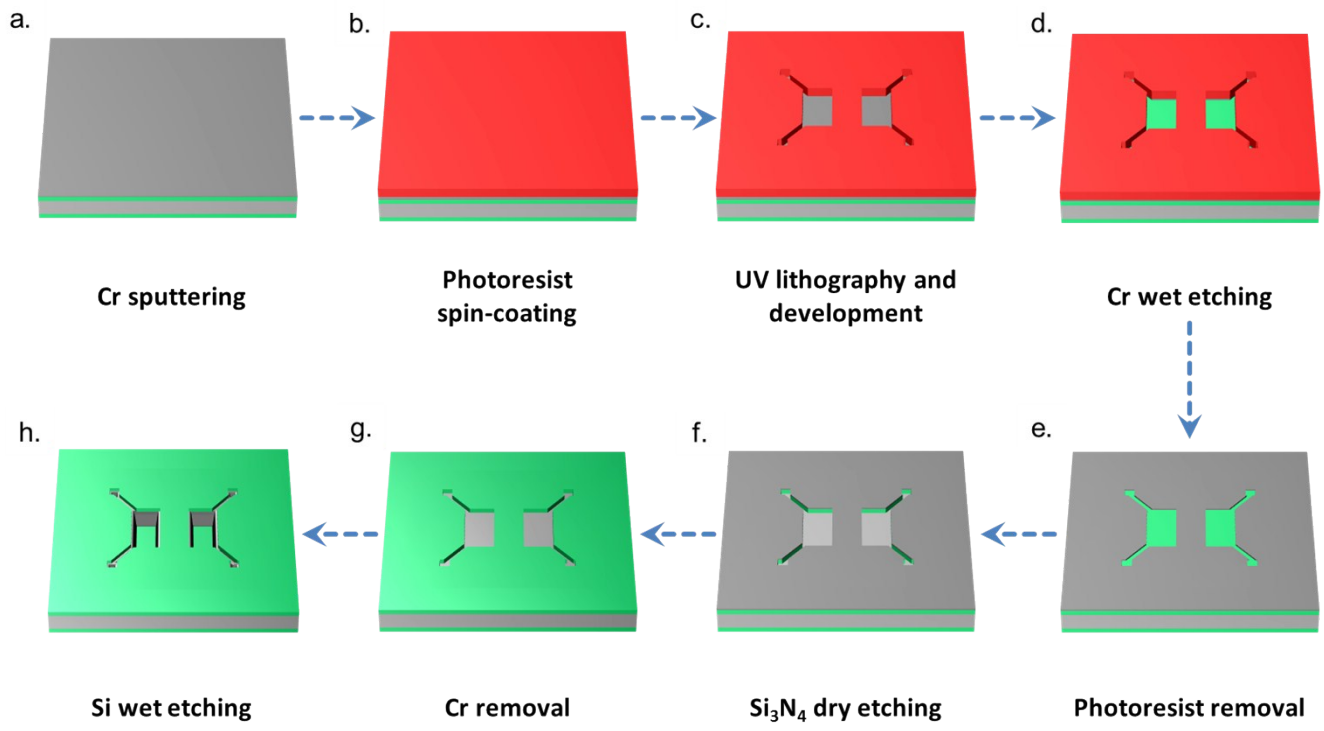

Figure S1. Schematic representation of membranes and micro-channels fabrication protocol.

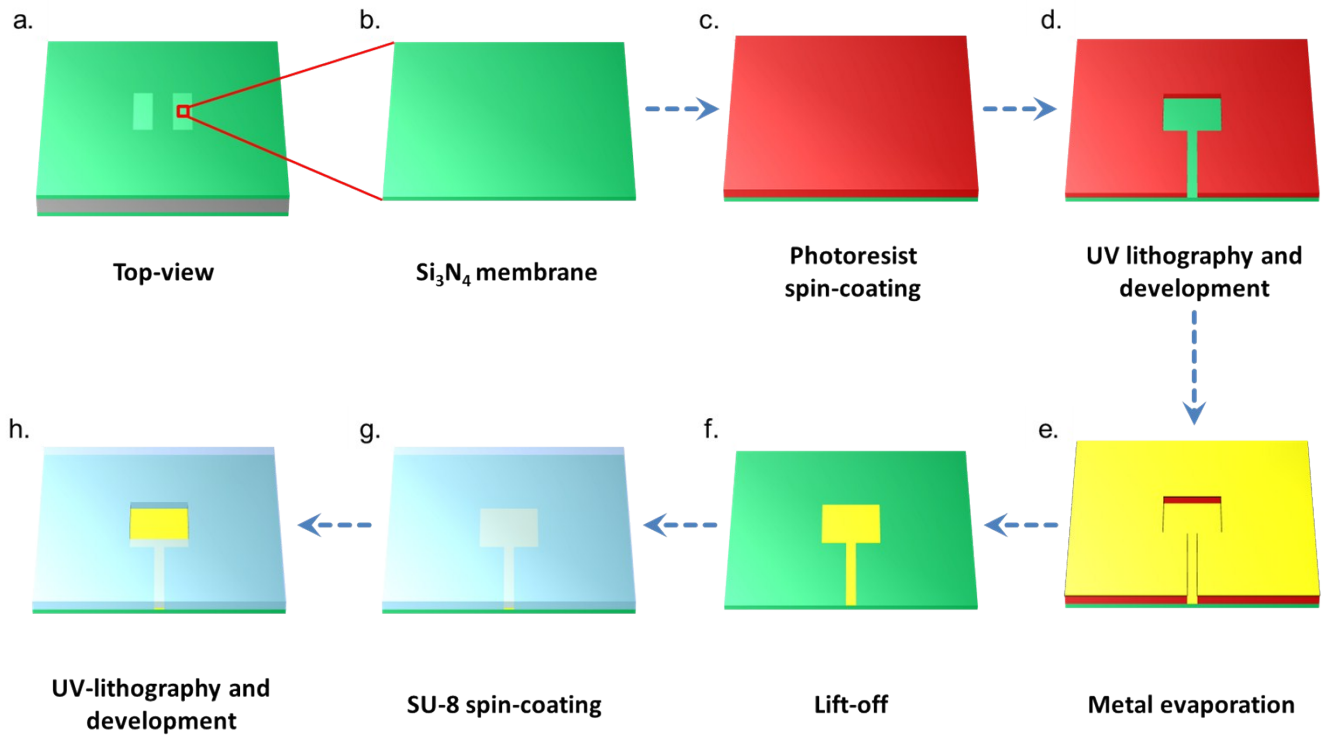

Figure S2. Schematic representation of the microfluidic MEA fabrication protocol.

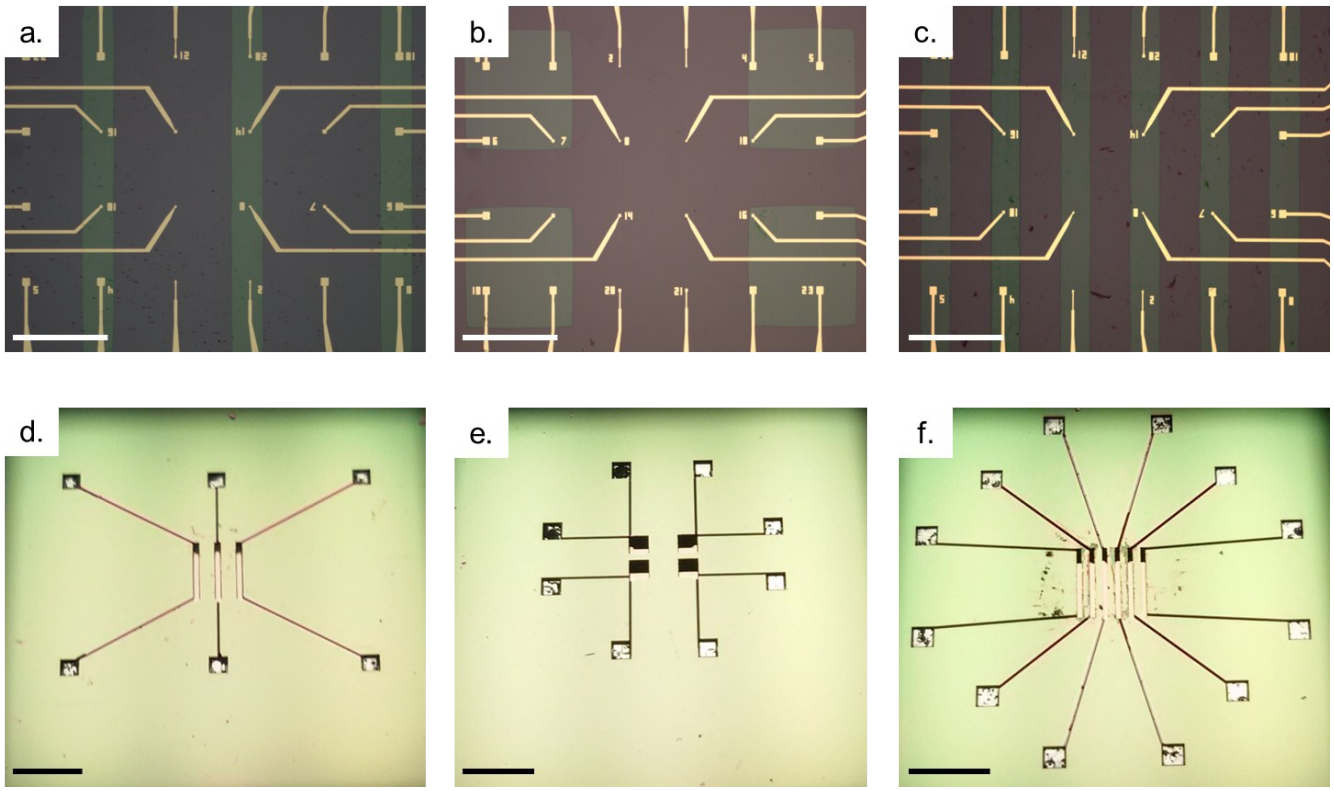

Figure S3. Different microfluidic arrangements. **a**)-**c**) Optical images of different microfluidic configuration, respectively with 3, 4 and 6 micro-channels, acquired from the recording side of the device. The scale bar is 500 μm. **d**)-**f**) Optical images of different microfluidic configuration, respectively with 3, 4 and 6 micro-channels, acquired from the delivery side of the device. The scale bar is 2.5 mm.

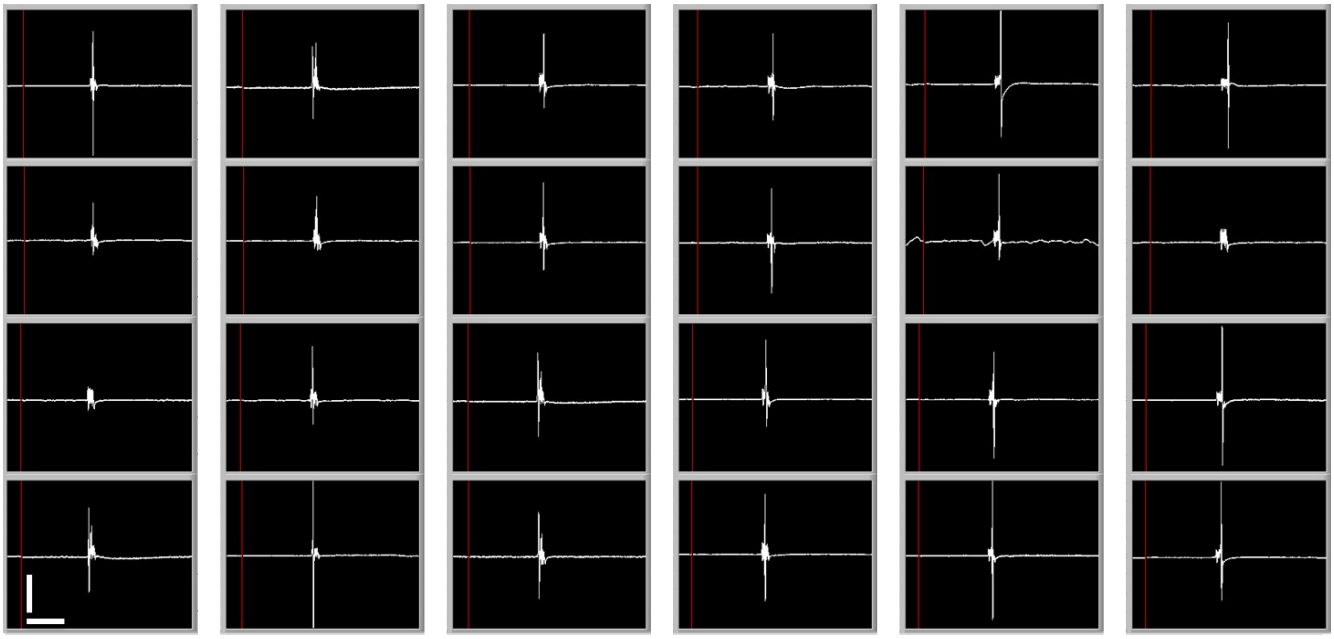

Figure S4. Whole MEA recording of hiPSC cardiomyocytes at 4 DIVS, showing a homogenous extracellular spontaneous firing activity over the 24 electrodes of the microfluidic-MEA. The scale bars at the bottom left apply to all the panels. The vertical scale bar is 1 mV, the horizontal one 200 ms.

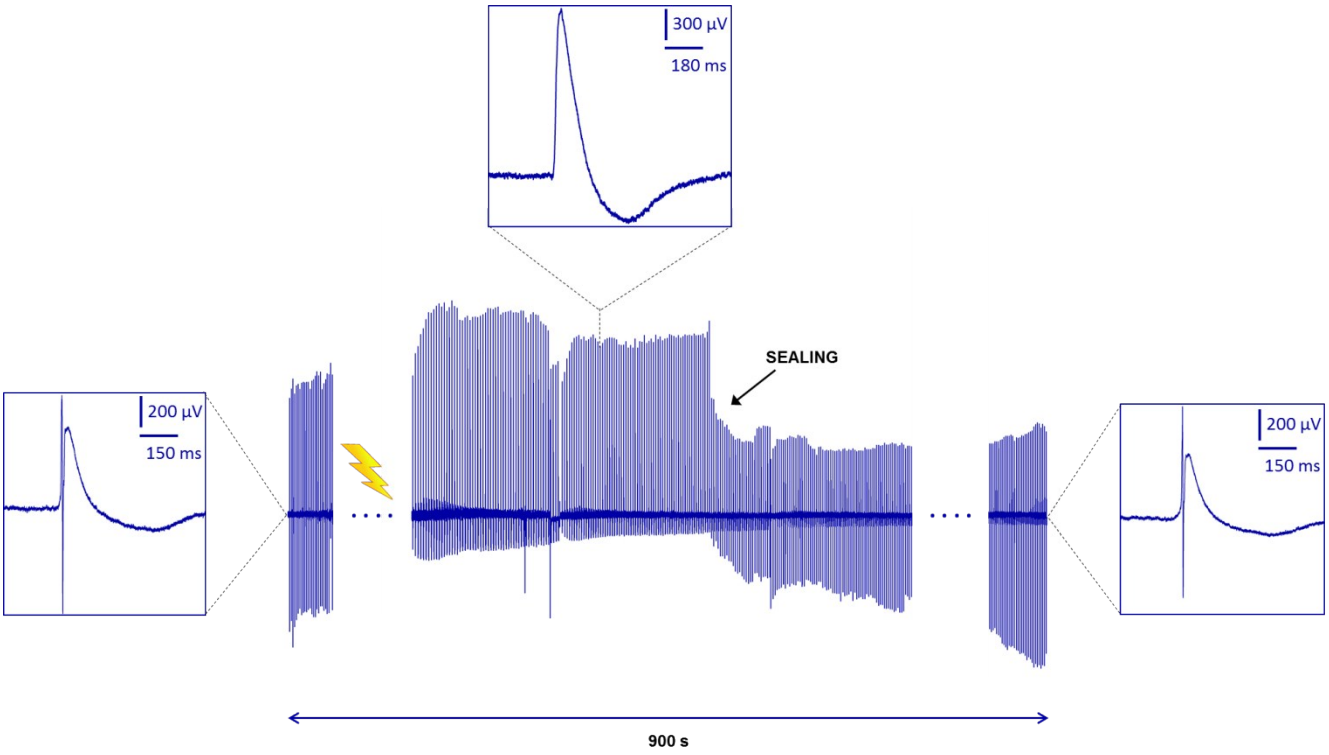

Figure S5. Long recording on hiPSC cardiomyocytes. After electroporation, the spontaneous electrophysiological spiking activity is modified into intracellular-like spikes. The spontaneous activity resumes extracellular features after 10 – 15 minutes upon resealing of the transient membrane pores.

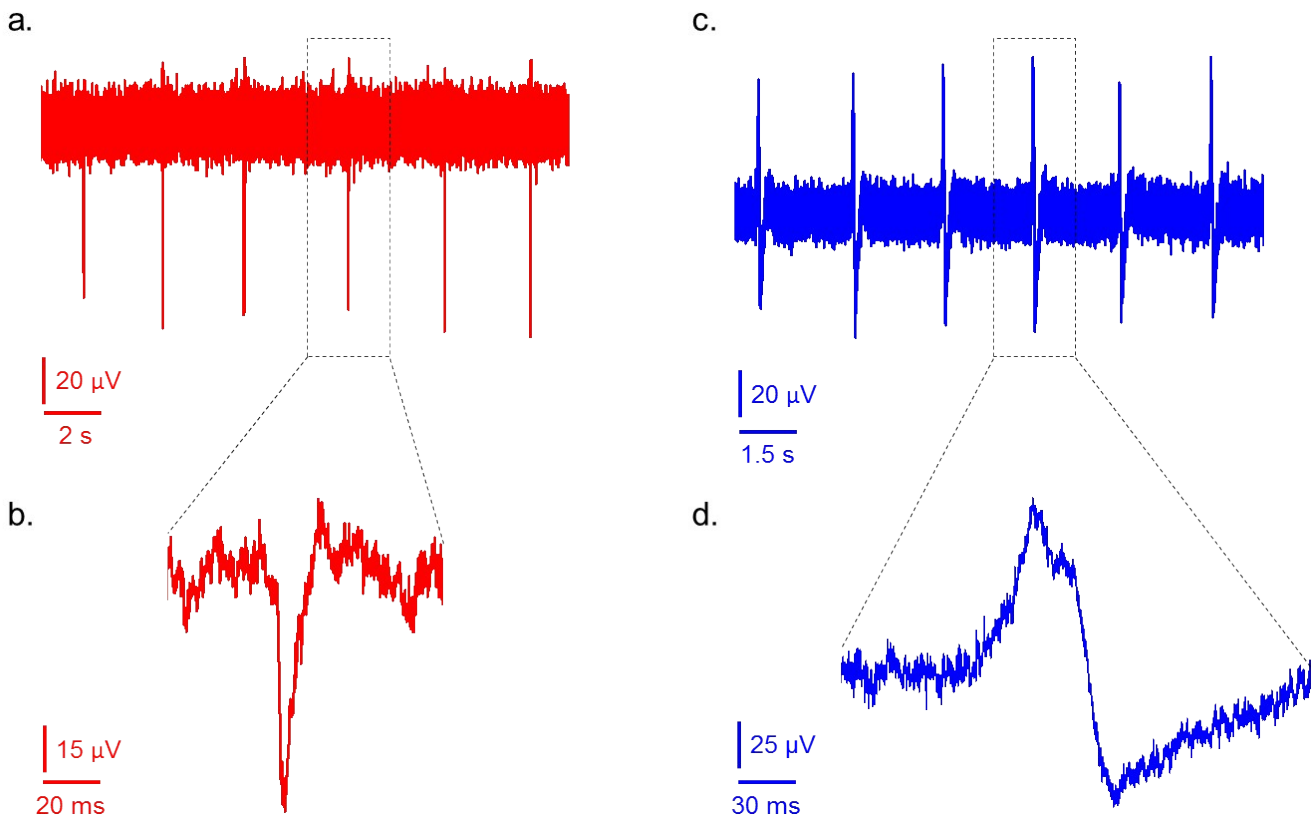

Figure S6. Extra and intra-cellular recording on HL-1 cardiomyocytes. **a**)-**b**) Spontaneous electrophysiological spiking activity of HL-1 cardiomyocytes from one of the 24 channels of a MF-MEA. **c**)-**d**) Intracellular-like activity of HL-1 cardiomyocytes acquired on the same electrode after electroporation.

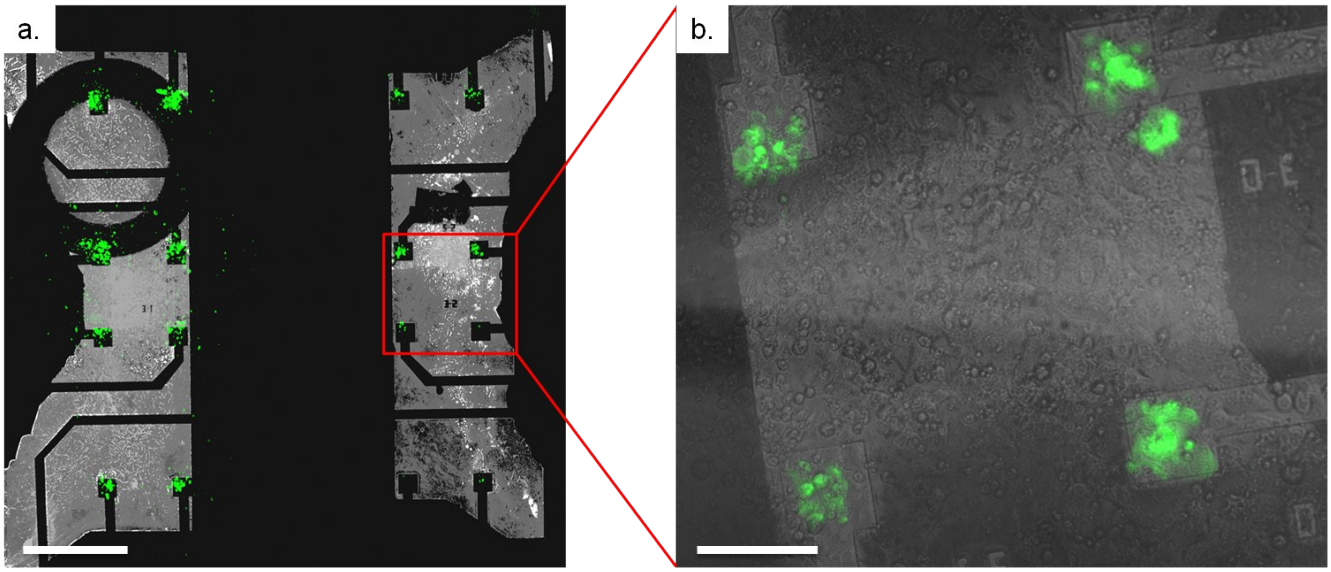

Figure S7. Selective calcein-AM delivery to HL-1 cells. **a**) Fluorescence image of intracellular delivery of calcein-AM on all the MF-MEA nanostructured electrodes. The scale bar is 500 μm. **b**) Zoomed detail of calcein-AM delivery onto four membrane electrodes. The scale bar is 100 μm.

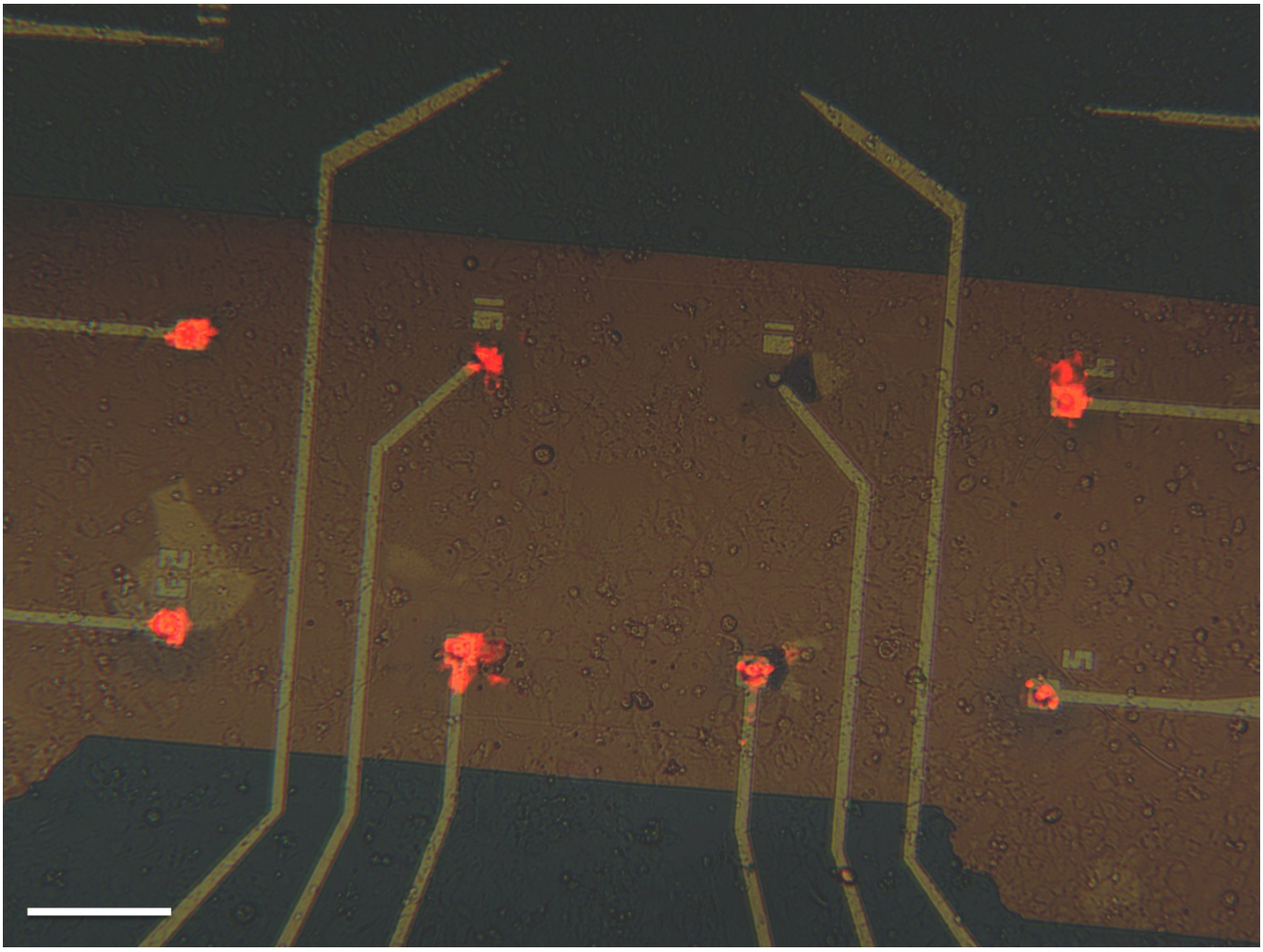

Figure S8. Fluorescence image of intracellular delivery of PrhD-1 into selectively electroporated cardiomyocytes. The scale bar is 200 μm.

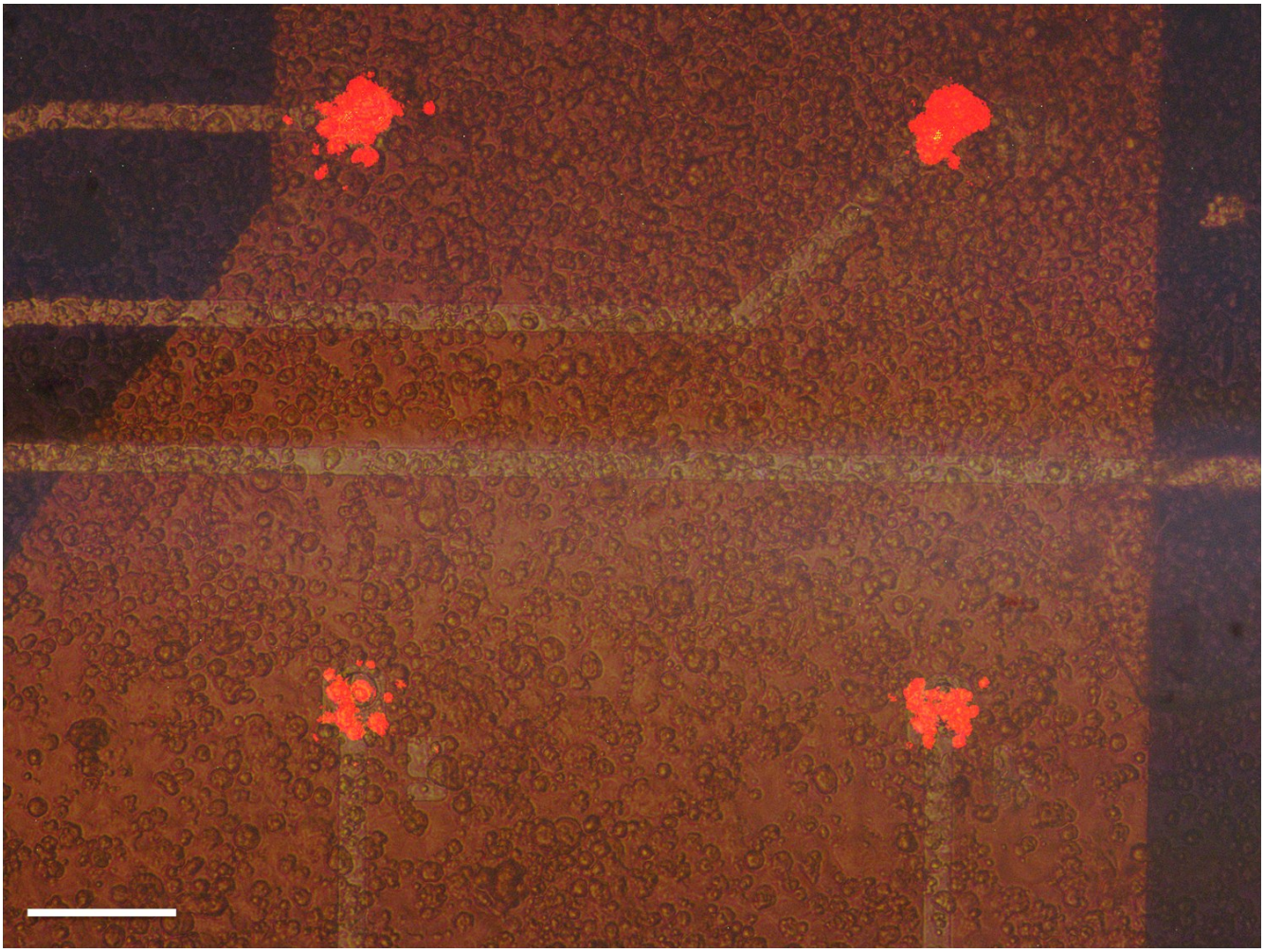

Figure S9. Fluorescence image of intracellular delivery of PrhD-1 into electroporated cardiomyocytes onto four membrane electrodes. The scale bar is 100 μm.

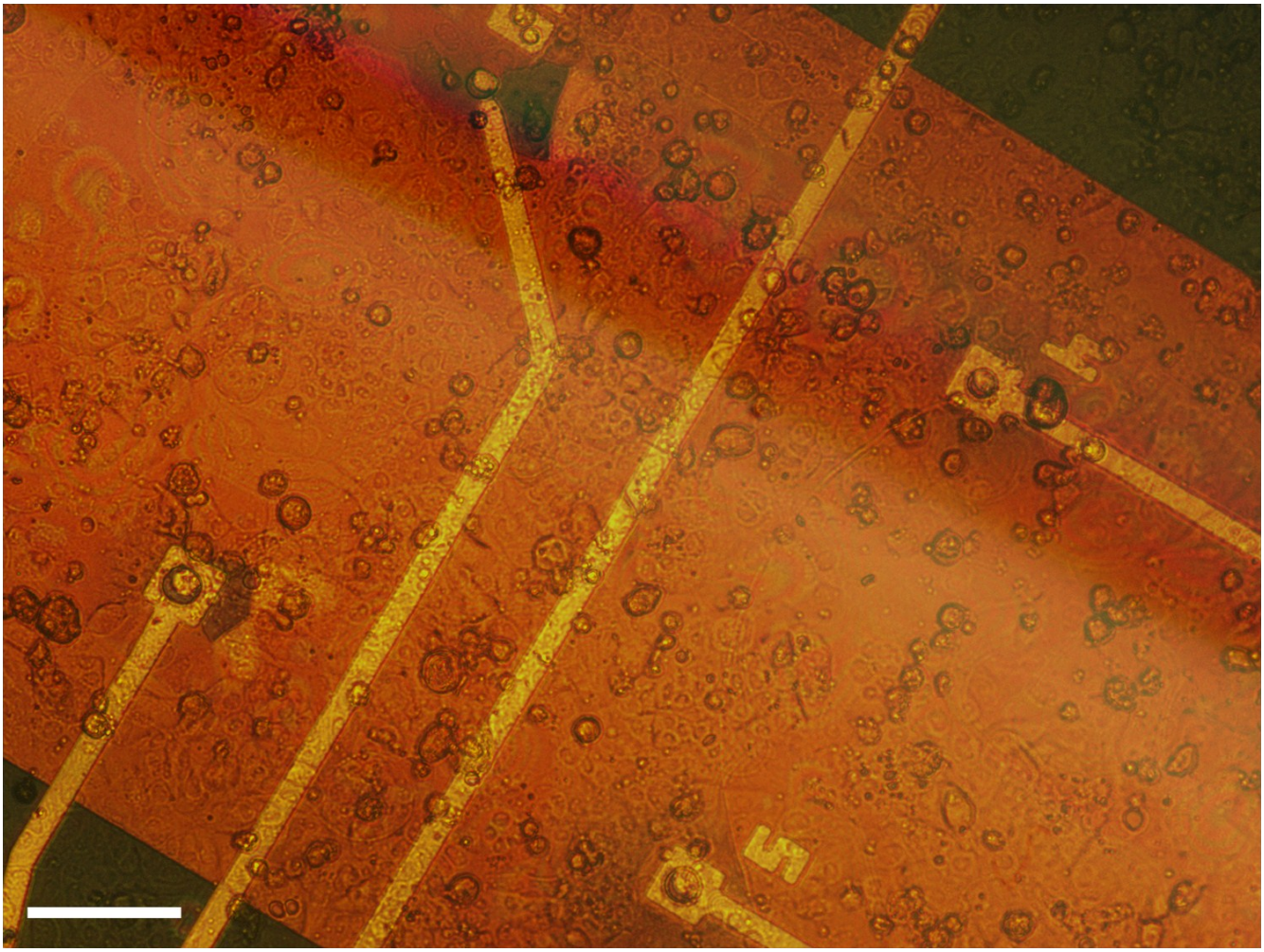

Figure S10. Bright-field optical image of four electrodes of a MF-MEA. The scale bar is 100 μm.

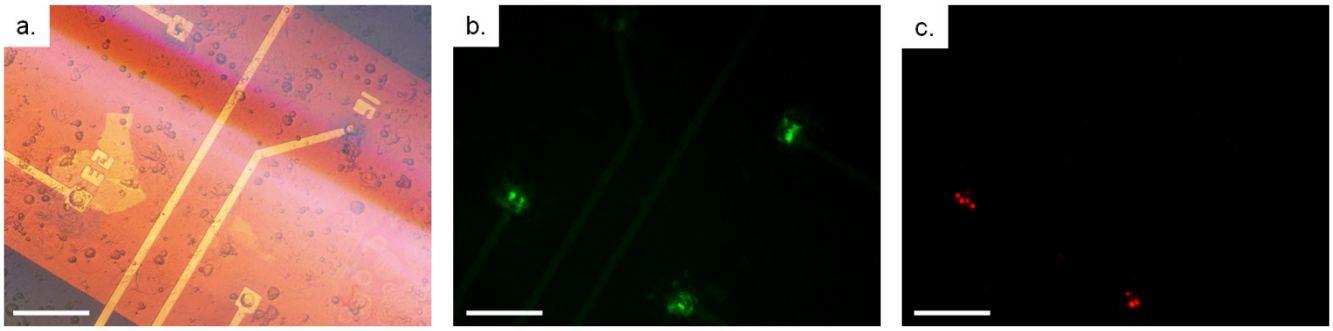

Figure S11. Selective intracellular drug delivery to HL-1 cells on MF-MEA. **a**) Bright-field optical image of four electrodes of a MF-MEA. The defect visible in correspondence of the bottom left electrode is a gold residue underneath the SU-8 passivation layer, remained after the lift-off process of the evaporated Ti/Au MEA electrodes. Being covered by the SU-8 passivation, the gold residue is excluded by the active area of the electrode and does not interact with the cells. The scale bar is 200 µm. **b**) The same 4 electrodes observed with a green fluorescence filter, showing calcein-AM delivery to the cells onto the 4 electrodes. The scale bar is 200 µm. **c**) The same four electrodes observed with a red fluorescence filter, showing PrhD-1 delivery to the cells onto the 2 electrodes where electroporation was performed. The scale bar is 200 µm.

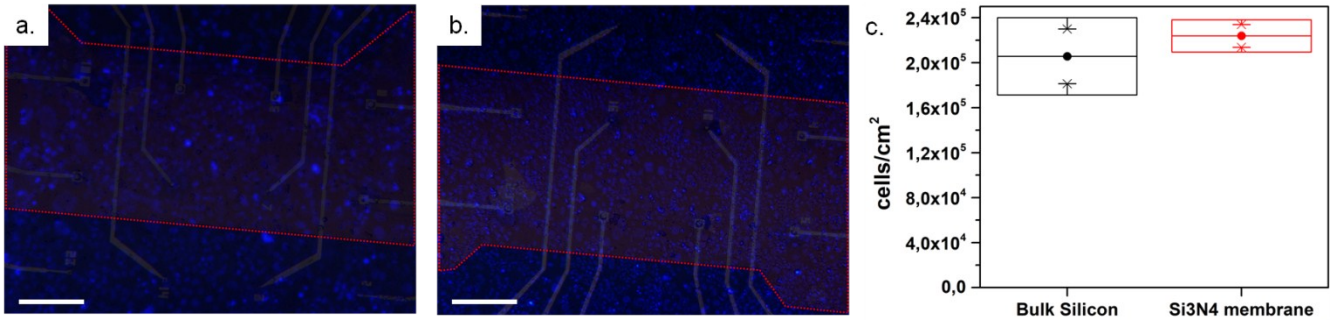

Figure S12. Cell density estimate after 4 DIV. **a)** Fluorescence image of HL-1 cardiomyocytes cultured on MF-MEA and stained with DAPI after 4 DIV. The red-bordered area delimits the nitride membrane. The scale bar is 300 µm. **b)** Fluorescence image, acquired on the other membrane region of the same device, of cardiomyocytes cultured on MF-MEA and stained with DAPI after 4 DIV. The red-bordered area delimits the nitride membrane. The scale bar is 300 µm. **c)** Box plot featuring the cell density distribution calculated for different areas (bulk silicon or nitride membranes) on the same device.

Delivery selectivity estimation

The fluorescence images acquired upon calcein-AM delivery were analyzed employing the open source image processing program ImageJ. The images were firstly superimposed onto their corresponding bright field counterparts in order to visualize both the stained cells and the Si_3N_4 membrane with the MF-MEA electrodes. By setting the optimal parameters of the built-in "analyze particle" tool, it was possible to calculate the total area of the membrane (A_{tot}) and the stained area ($A_{stained}$). From the stained area, we then subtracted the area of the electrodes (A_{el}), where cells are expected to be stained from the nanotubes. The remaining area ($A_{ext} = A_{stained} - A_{el}$) represents cells that have been stained, even though they were not interfaced with the nanotubes on the electrodes. Having defined all these parameters, the delivery selectivity of the device (in percentage) can be determined with the following formula:

$$S = 100\left(1 - \frac{A_{ext}}{(A_{tot} - A_{el})}\right)$$

The calculated selectivity values are summarized in the table below, together with the average value and its standard deviation.

Experiment (n°)	**S (%)**	**S_{mean} (%)**
1	99,2	98,6
2	99,1	
3	96,4	**σ_S (%)**
4	98,7	1,1
5	99,0	
6	98,9	

Table 1. Selectivity results obtained over 6 different experiments with both calcein-AM and PrHD-1.