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

Compartmentalized microfluidic chambers enable long-term maintenance and communication between human pluripotent stem cell-derived forebrain and midbrain neurons

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Supplementary Figure 1. Schematic diagram of NeuroChip fabrication procedure.

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Supplementary Figure 3. SEM and bright field microscopy characterizations of NeuroChip.

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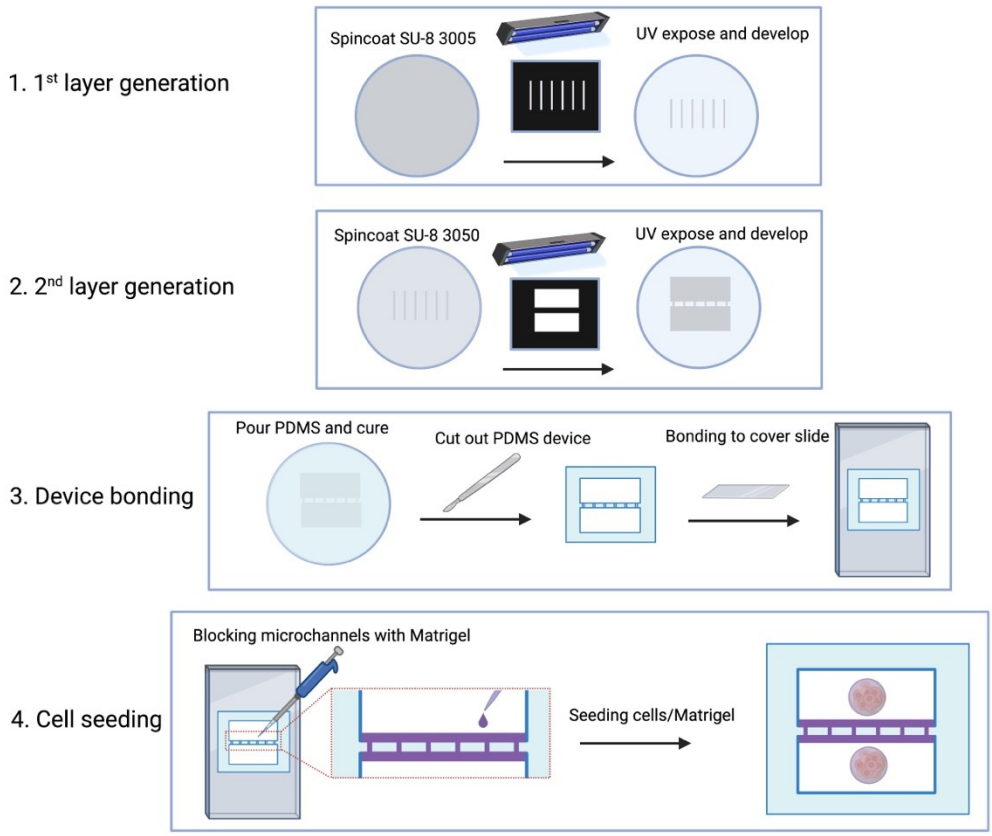
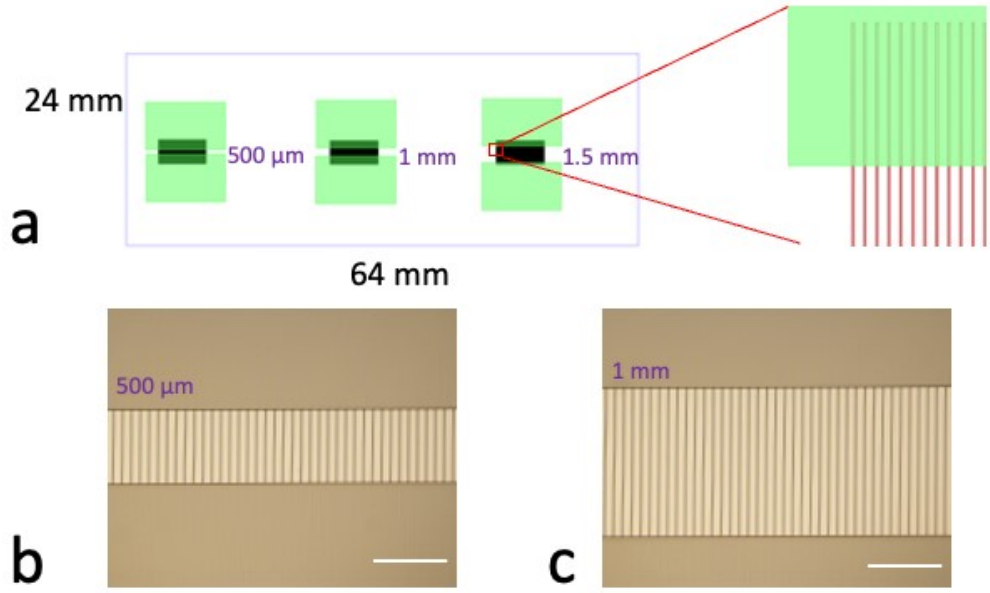
Supplementary Video 1. Immunolabeling of neuronal clusters inside Matrigel matrix. Neuronal culture was stained with phalloidin (red) and DAPI for nuclei (rendered in green).

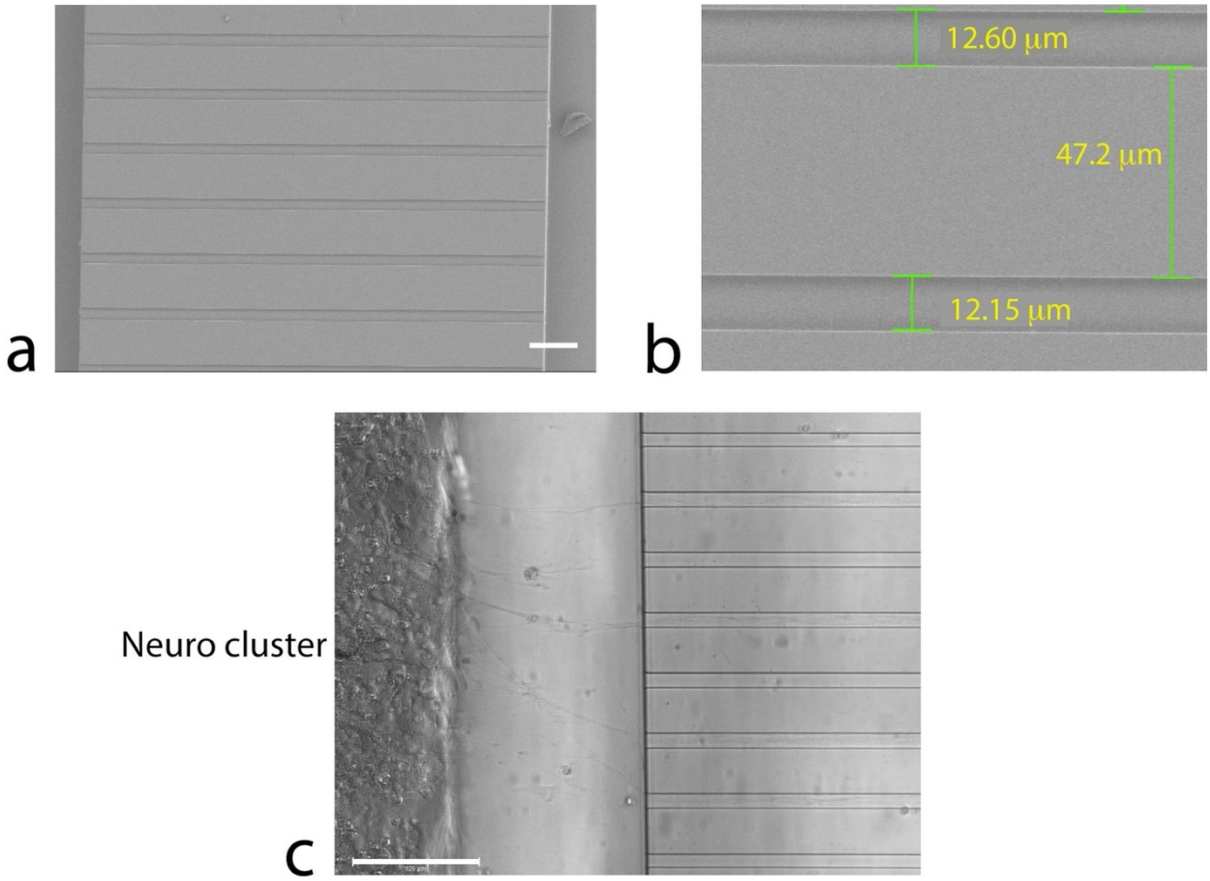
Supplementary Video 2. Spontaneous and stimulant-induced calcium activities of the hESCs in NeuroChips.



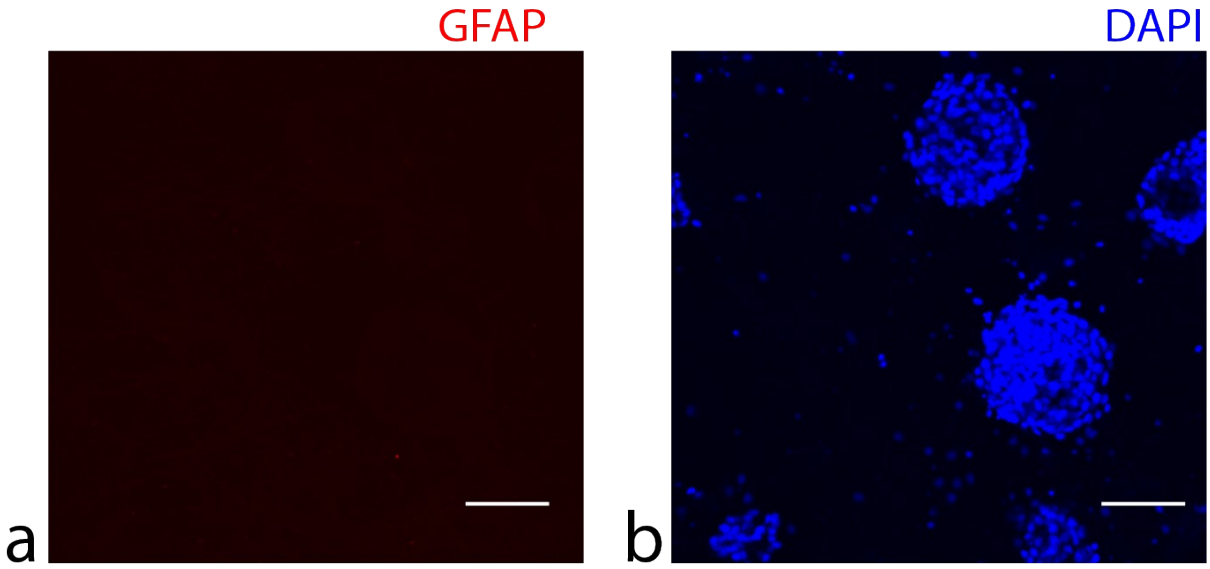
Supplementary Figure 1. Schematic diagram of NeuroChip fabrication procedure. The process includes four main steps: 1. The generation of first layer of SU-8 3005; 2. The generation of second layer of SU-8 3050; 3. Device bonding where the PDMS mixture was poured and cured on the silicon wafer prior to removal and bonding the PDMS to the glass cover slide; 4. The microchannels were filled with Matrigel before seeding neuronal cells embedded inside Matrigel into two chambers. *This figure is created with BioRender.com.*



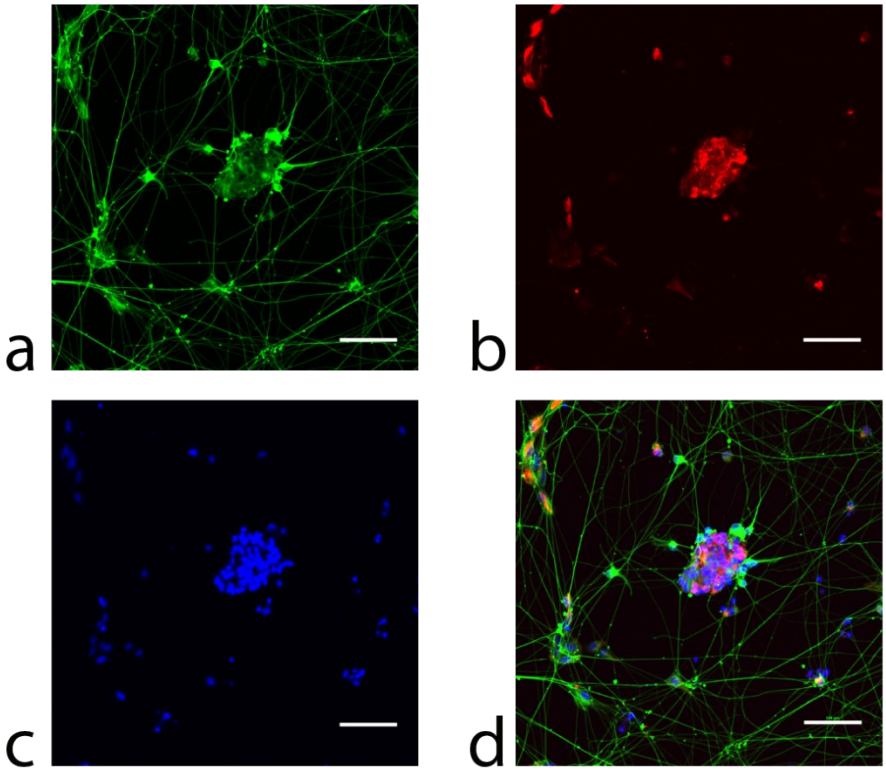
Supplementary Figure 2. NeuroChip design with different microchannel lengths. Panel (a) shows a schematic depiction of NeuroChip with 3 different microchannel lengths, i.e., 500 µm, 1 mm, and 1.5 mm. Red lines represent microchannels, green shading represents the chambers. Bright field microscope images of the microchannels at 500 µm (b) and 1mm (c) in length. Scale bars: b-c, 500 µm.



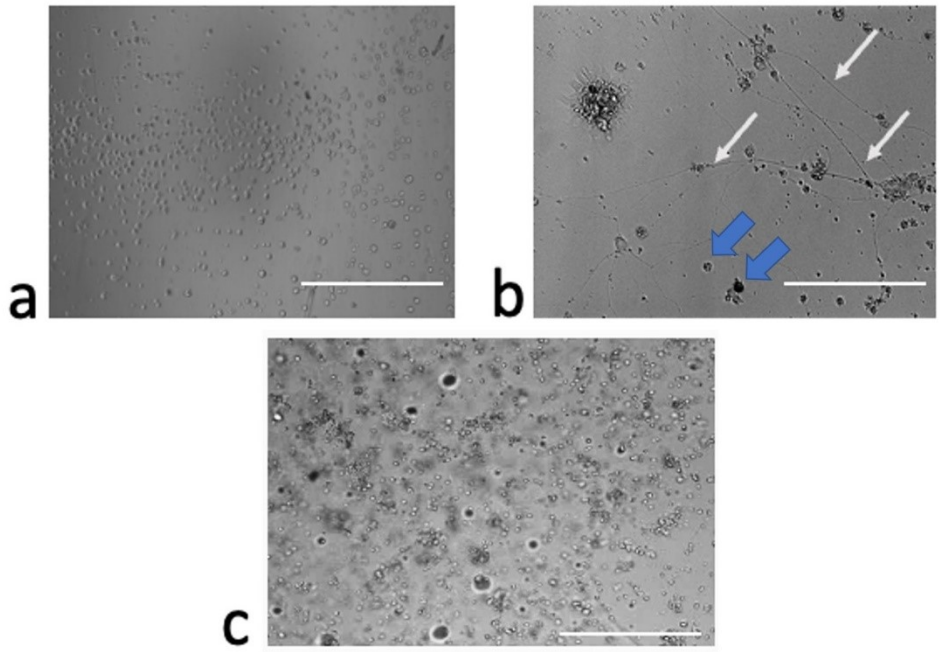
Supplementary Figure 3. SEM and bright field microscopy characterizations of NeuroChip. Panels (a) and (b) show SEM images at low (a) and high (b) magnifications of the microchannels between the chambers. (c) Bright field microscopy image of a cluster of neurons with axons starting to cross the microchannels (after 14 days on the NeuroChips). Scale bars: a, 50 µm; c, 125 µm.



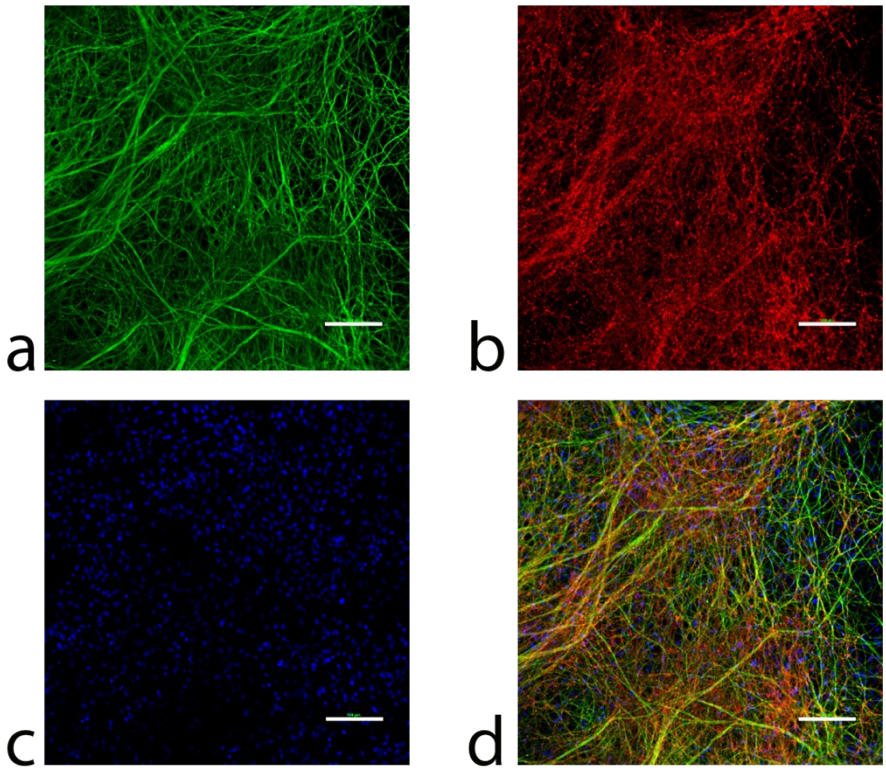
Supplementary Figure 4. Immunocytochemistry does not show the presence of the mature/reactive astrocyte marker glial fibrillary acidic protein (GFAP). Panel (a) shows the absence of the reactive mature/astrocyte marker GFAP. Panel (b) shows the corresponding cell nuclei labeling (DAPI). Scale bars: a-b, 100 μm.



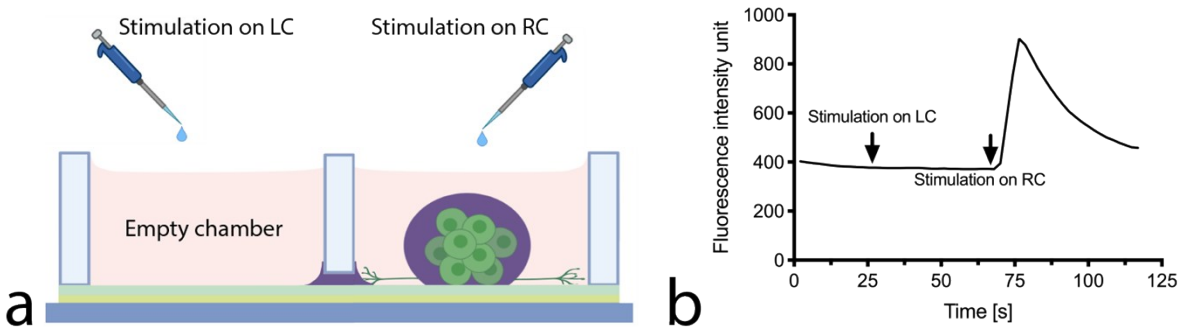
Supplementary Figure 5. Differentiating cultures (40 days) show the presence of both neurons and astrocytes. Panel (a) Shows the neuronal marker β-III-tubulin. Panel (b) shows the presence of the early astrocyte marker, S100 calcium binding protein B (S100β). Panel (c) shows cell nuclei stained with Hoechst 33258. Panel (d) shows composite images of all three channels merged. Scale bars: a-d, 100 μm.



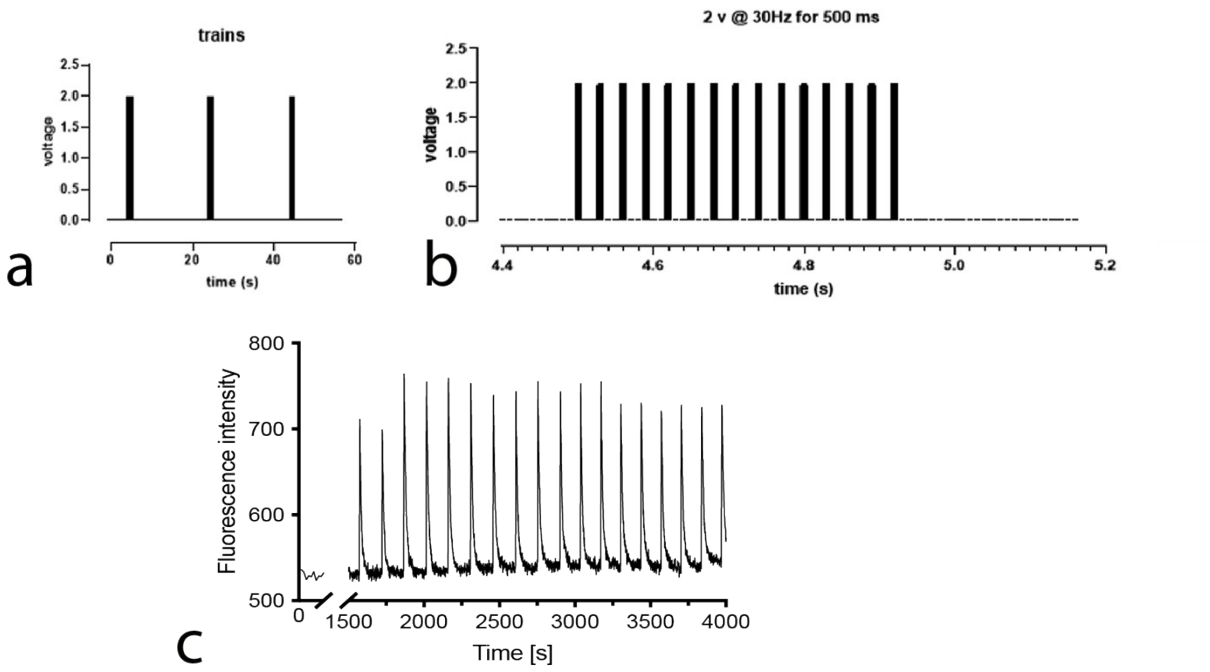
Supplementary Figure 6. Cell coating plays a role in regulating attachment. Panel (a) shows neural progenitors initially seeded in poly-L-ornithine and laminin/fibronectin coated NeuroChips. Panel (b) shows axonal projections (white arrows) after 7 days in culture (note the sparsity of cells and the high number of dead cells, blue arrows). Panel (c) shows initial neural progenitor seeding in Matrigel (note how the density per unit area can be increased in comparison with panel (a)). Scale bars: a - c, 200 μm.



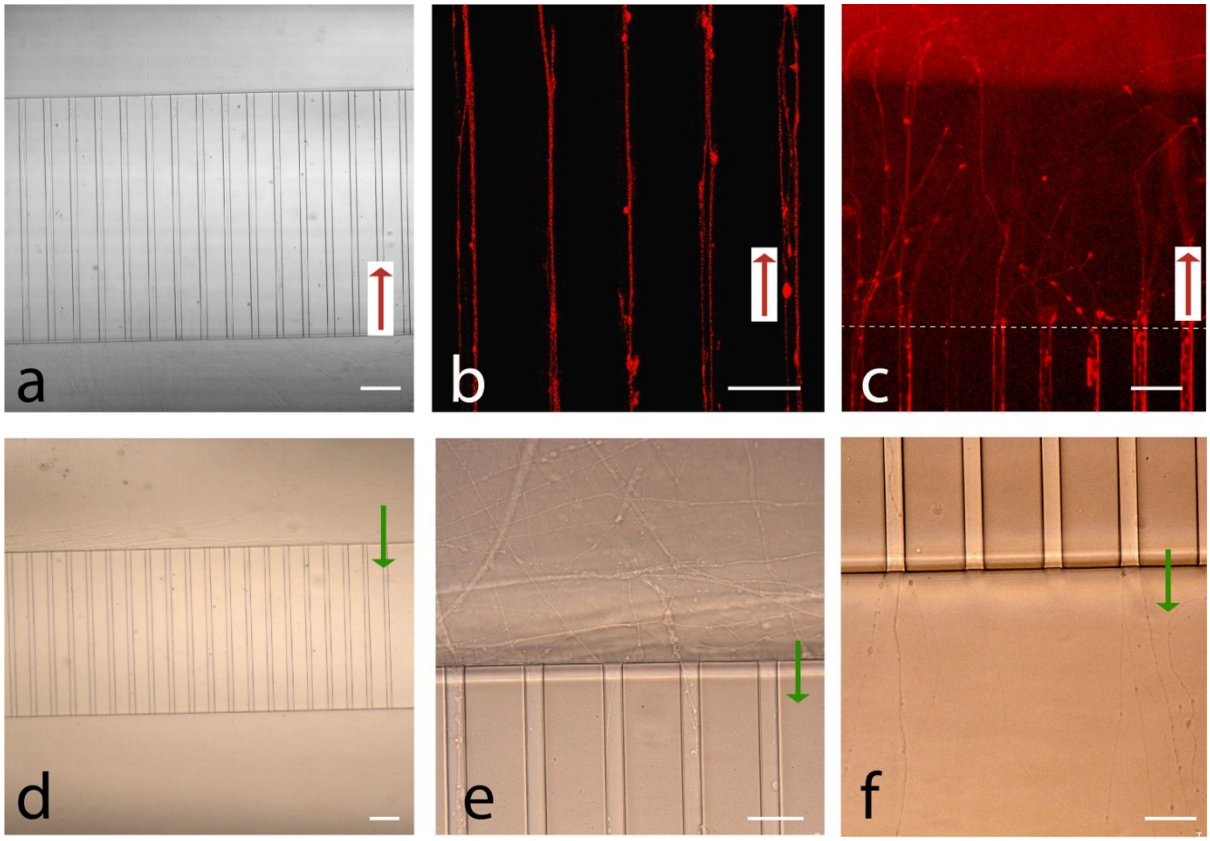
Supplementary Figure 7. Immunocytochemical analysis indicates the presence of synapses in human embryonic stem cell (hESC) derived forebrain neurons (day 60 in culture). Panel (a) shows the neuronal marker β-III-tubulin, panel (b) the synaptic marker, synaptophysin, and panel (c) shows cell nuclei stained with Hoechst 33258. (d) Shows the composite of all three channels. Images were taken at 20X magnification. Scale bars: a-d, 100 μm.



Supplementary Figure 8. KCl does not readily flow across microchannels. The schematic panel (a) shows that KCl (100 mM) applied to the left chamber (LC, cell free) does not increase fluorescence intensity in the cell containing right chamber (RC). In contrast KCl applied on the right chamber promotes an immediate elevation of intracellular calcium (panel (b)). Panel *(a) is created with BioRender.com.*



Supplementary Figure 9. Electrical stimulation parameters used for NeuroChip stimulation. Panel (a) shows a typical series of 500 ms trains of square pulses at 20 s intervals. Panel (b) shows that each train comprised a series of pulses (right panel). In this example, 15 pulses at 2 V. Panel (c) shows hESCs-derived forebrain neural culture (day 120) responding to direct electrical stimulation with 500 ms pulses of 2 V at 30 Hz applied every 2 min without loss of response over 40 min.



Supplementary Figure 10. Single neural populations (hESCs-derived forebrain and midbrain neurons) seeded separately on NeuroChips. (a-c) GAPtrap-tomato cells were seeded on the lower chamber. (a) Low magnification brightfield image indicating the direction of axon projection. (b) Fluorescence image of axons inside microchannels connecting the chambers. (c) Axons exiting the microchannels. (d-f) LMX1A-eGFP cells were seeded on the upper chamber. Low magnification brightfield images with arrows indicating the direction of axon growth. Axons were shown to enter (e) and exit (f) the channels after 2 weeks in culture. Scale bars: a, 100 μm; b-c, 50 μm; d, 100 μm; e-f, 50 μm.