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Supplemental Information

Reconstitution of the Human Nigro-striatal Pathway on-a-Chip Reveals OPA1-Dependent Mitochondrial Defects and Loss of Dopaminergic Synapses

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Figure S1

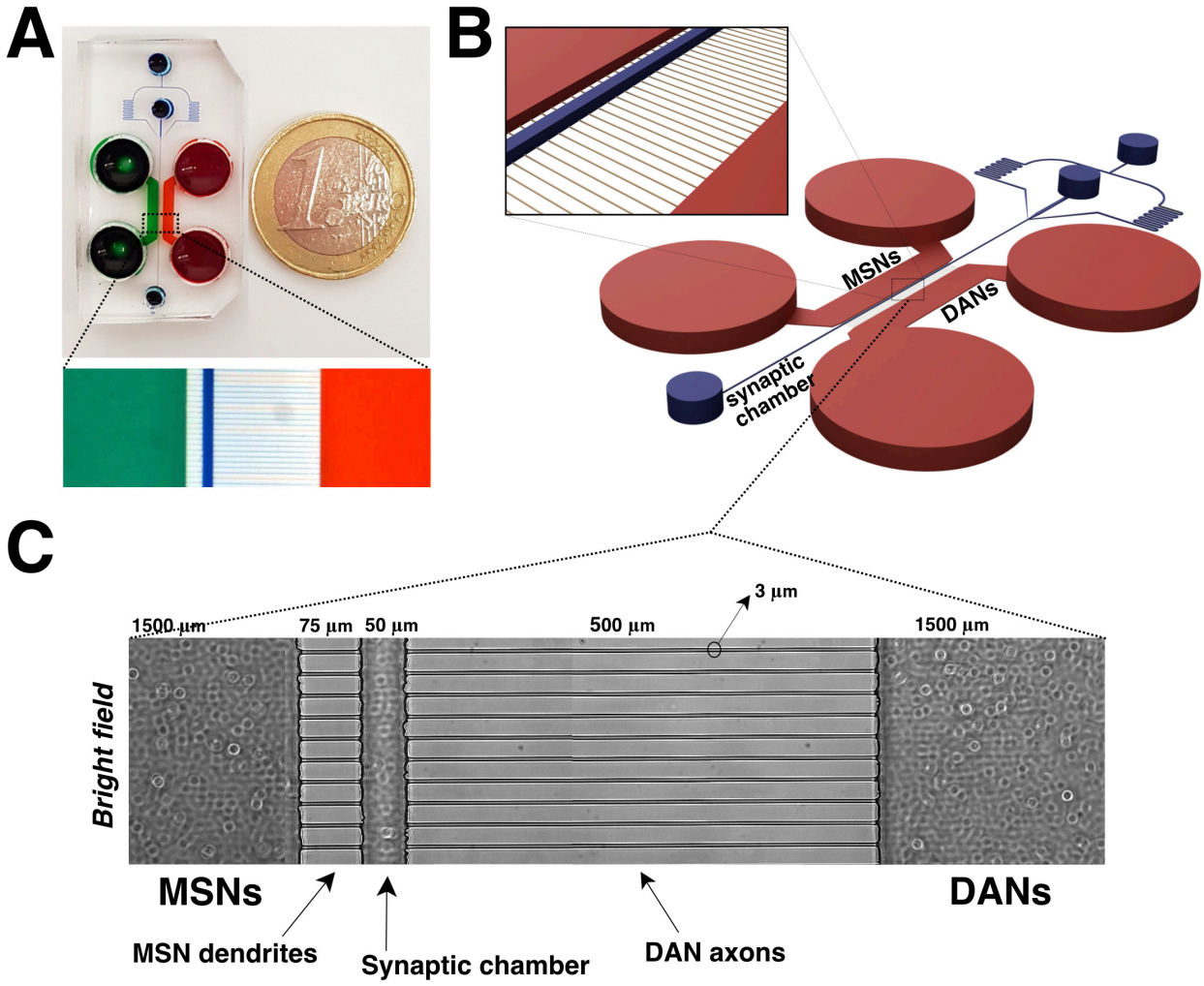

Figure S1. Schematic structure of the 3-compartment microfluidic device, Related to Figure 2.
(**A**) Overview of the microfluidic device with high magnification (bottom image) of the three chambers stained with different dyes to highlight their fluidic isolation. (**B**) 3D imaging reconstruction of the microfluidic device. This system includes the lateral striatal and dopaminergic chambers that are connected to each other through ~130 microgrooves. Between the two chambers there is an additional central space called synaptic channel to visualize and manipulate the synaptic terminals between MSNs and DANs. (**C**) High-power imaging of the principal microdevice elements with relative dimensions. Scale bar: 50 µm.

Figure S2

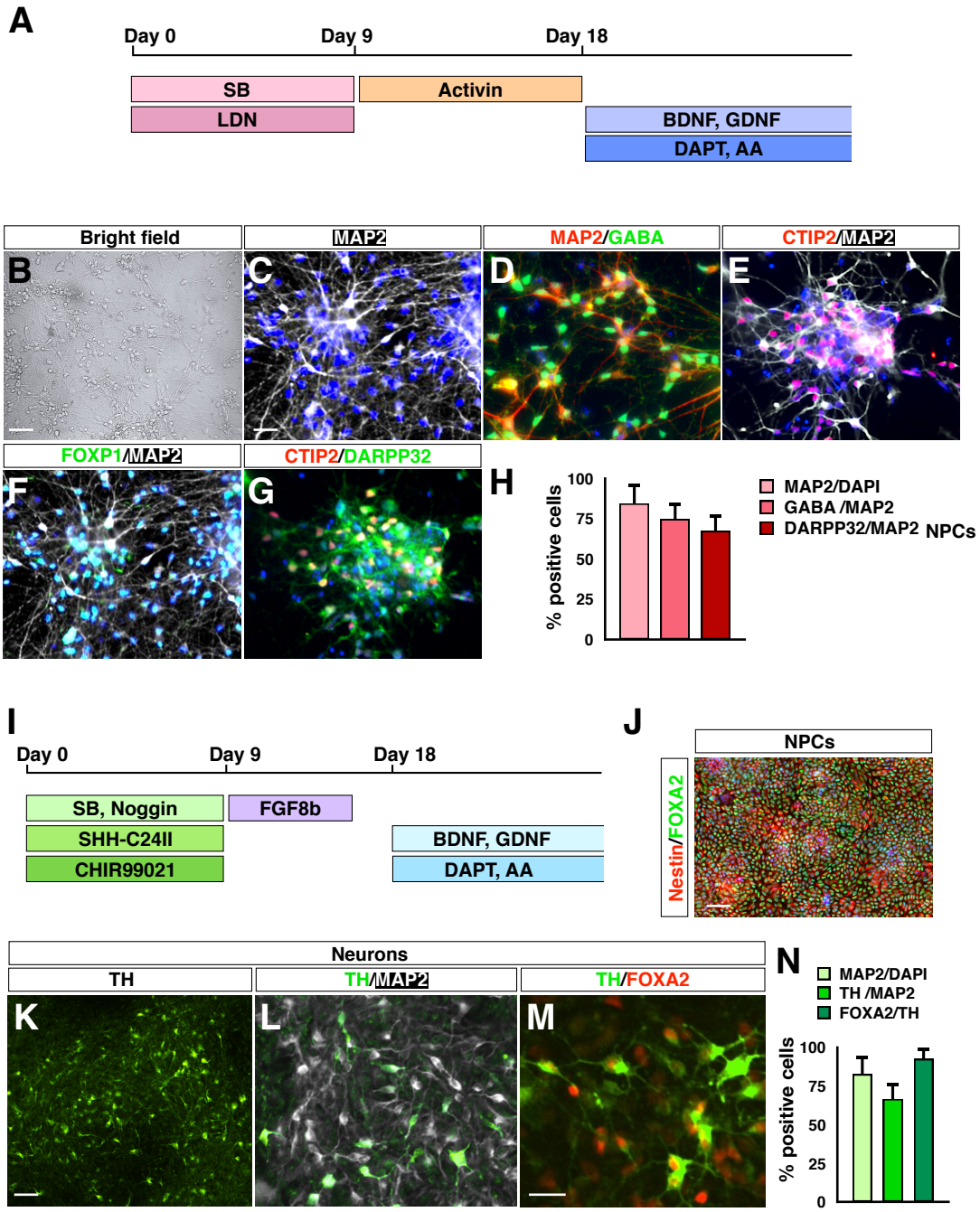

Figure S2. Experimental procedures to differentiate iPSCs into striatal medium spiny neurons (MSNs) and midbrain dopaminergic neurons (DANs), Related to Figure 2.

(**A**) Schematic representation of the culture conditions for generating MSNs**.** (**B-G**) Representative images of MSNs stained with the specific markers GABA (**D**), CTIP2 (**E**), FOXP1 (**F**) and DARPP32 (**G**). (**H**) Quantification of MAP2+, GABA+ and DARPP32+ neuronal cell populations. (**I**) Schematic representation of the culture conditions for generating DANs. (**J**) Nestin/FOXA2 double positive DA neural progenitors obtained as an intermediate step of the iPSC differentiation. (**K-M**) Representative pictures and quantitative analysis (**N,O**) of DANs expressing TH and FOXA2. Data are mean ± SEM, n = 3 independent experiments. Scale bar: 50 μm.

Figure S3

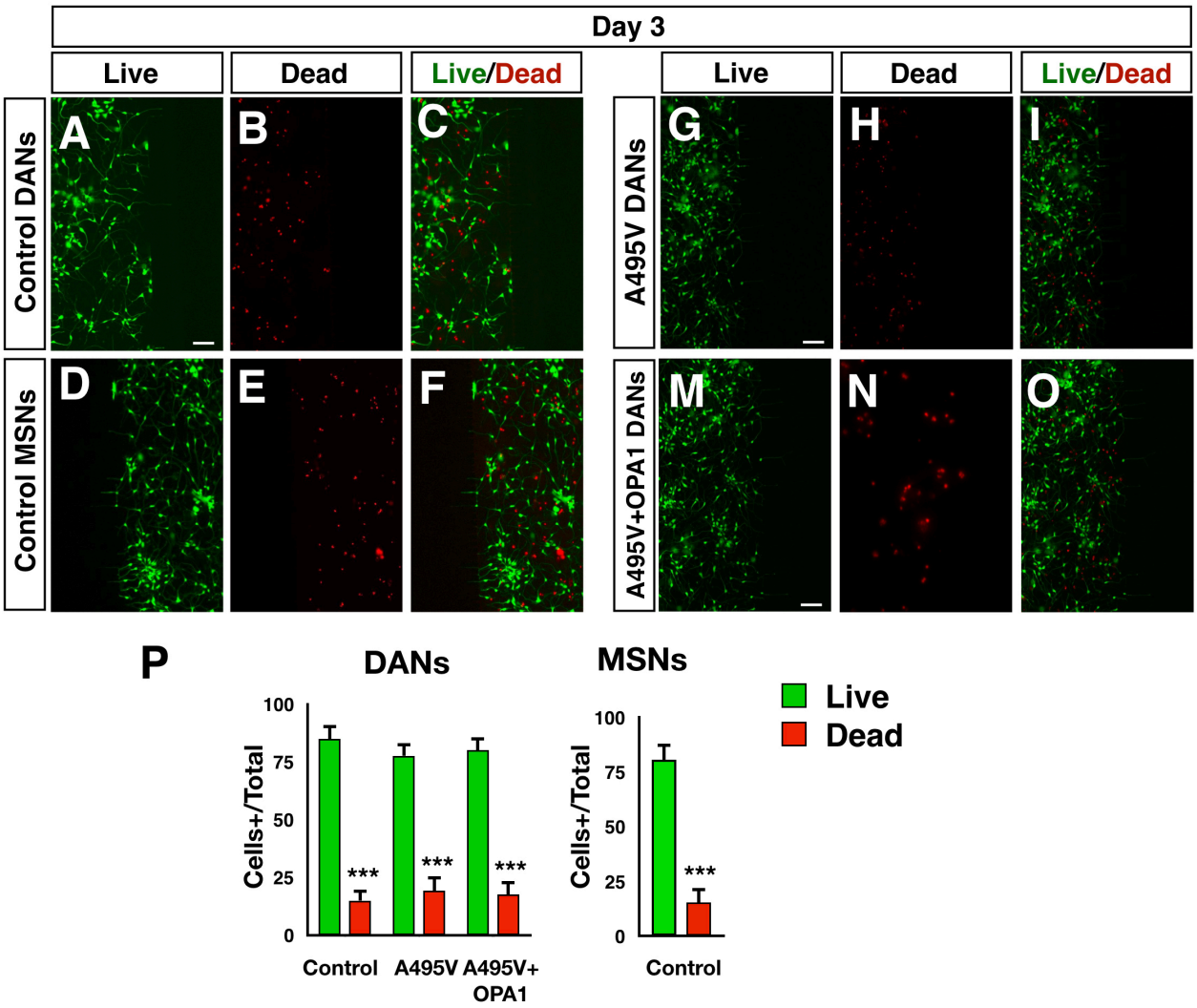

Figure S3. Survival efficiency of iPSC-derived neurons in the microfluidic environment, Related to Figure 2. (**A-O**) Representative images of survived (green) and dead (red) DANs of ND (**A-C**), PD-OPA1 (**G-I**), and gene-complemented patient (**M-O**) iPSC lines and MSNs of ND iPSCs (**D-F**) 3 days after plating into the microdevices. Seeded neurons were stained with the live/dead assay consisting of a green-fluorescent calcein-AM probe (green) to reveal the intracellular esterase activity and red-fluorescent ethidium homodimer-1 (red) to indicate loss of plasma membrane integrity. (**J**) Bar graphs illustrating the fraction of positive cells on the total cell population. Data are mean ± SEM, n = 3 independent experiments. ***P < 0,001; Statistical analysis is performed using Student's *t*-test. Scale bar: 100 µm.

Figure S4

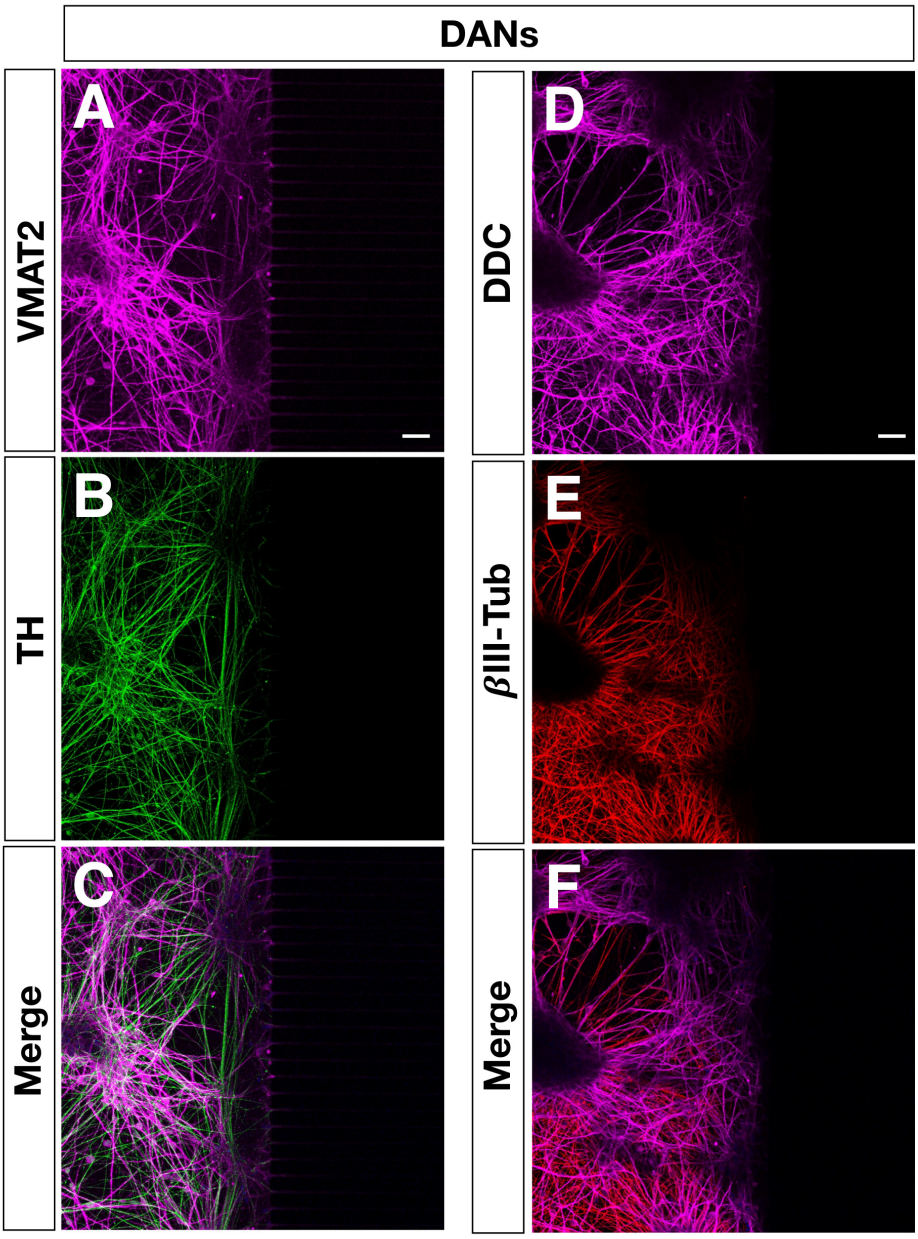

Figure S4. Characterization of DANs within the microfluidic device, Related to Figure 2.
(**A-F**) Representative images of DANs after 6 weeks of culture in the microfluidic device and stained for the key DAN markers VMAT2 (**A**), TH (**B**), DDC (**D**), and the general neuronal protein ßIII-Tubulin (ßIII-Tub) (**E**). Scale bars: 100 µm

Figure S5

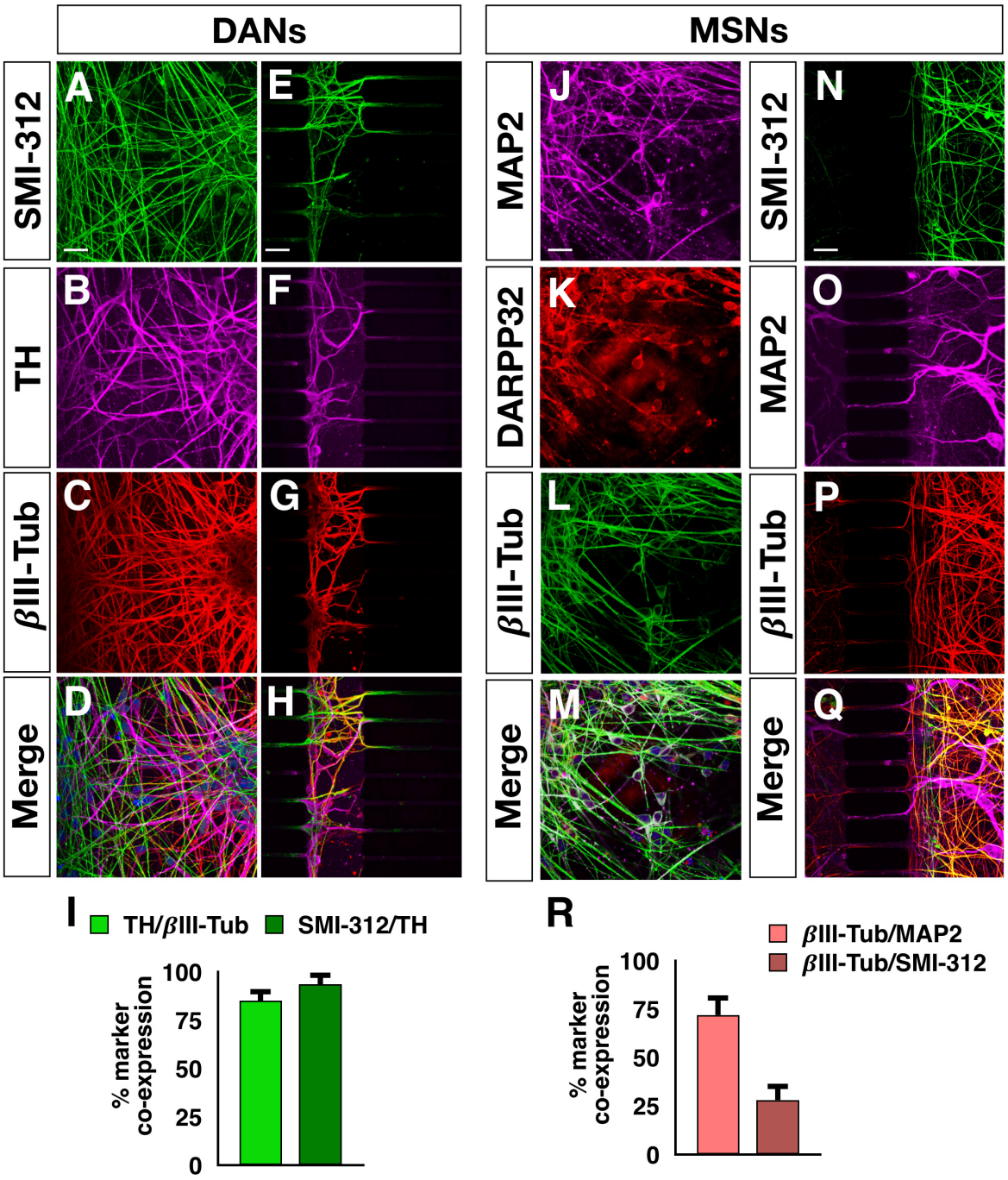

Figure S5. Assessment of the DAN and MSN axonal and dendritic projections within the channels of the microfluidic device, Related to Figure 3.

(A-H) Representative images of DAN axonal projections highlighted with SMI-312 (green) and TH (magenta) in the dopaminergic lateral chamber (**A-D**) and in the synaptic compartment (**E-H**). (**I**) Bar graphs showing the percentage of co-staining between TH and ßIII-Tubulin (ßIII-Tub) and SMI-312 in the synaptic compartment. (**J-M**) Representative images of MSNs expressing MAP2, DARPP32 and ßIII-Tubulin (ßIII-Tub) in the striatal lateral chamber. (**N-Q**) Visualization of the MSN axons (SMI-312) and dendrites (MAP2) over the total amount of ßIII-Tubulin (ßIII-Tub) positive neuronal projections in the microchannels connecting the striatal lateral chamber and the synaptic compartment. (**R**) Quantification of the striatal dendrites positive for either MAP2 or SMI-312. Data are mean ± SEM, n = 3 independent experiments. Scale bars: 50 µm