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

Supplementary Materials

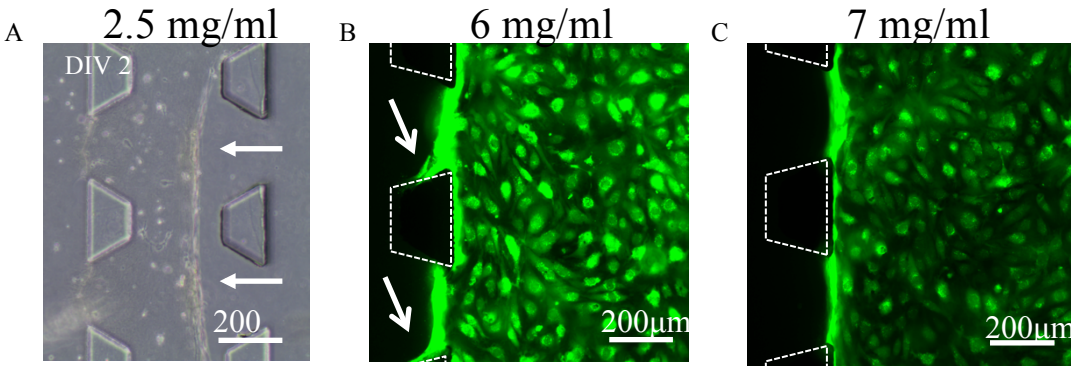

Figure S1. Optimization of the collagen gel concentration for the channel containing astrocytes. (A) Phase contrast image showing the pulling force of astrocytes on a 2.5 mg/ml collagen hydrogel. (B,C) Fluorescence image showing GFP-HUVEC seeded close to the collagen hydrogel with astrocytes with a concentration of 6 mg/ml (B) and 7 mg/ml (C).

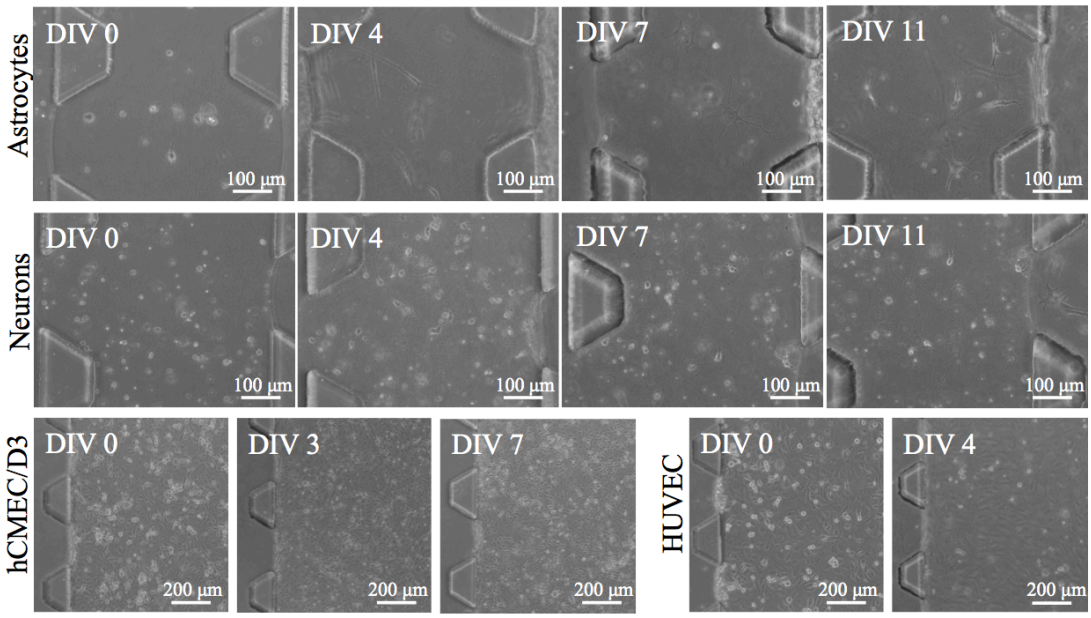

Figure S2. Phase contrast images showing growth of primary astrocytes, primary neurons and endothelial cells (HUVEC and hCMEC/D3), in their respective microfluidic channels at the indicated time points.

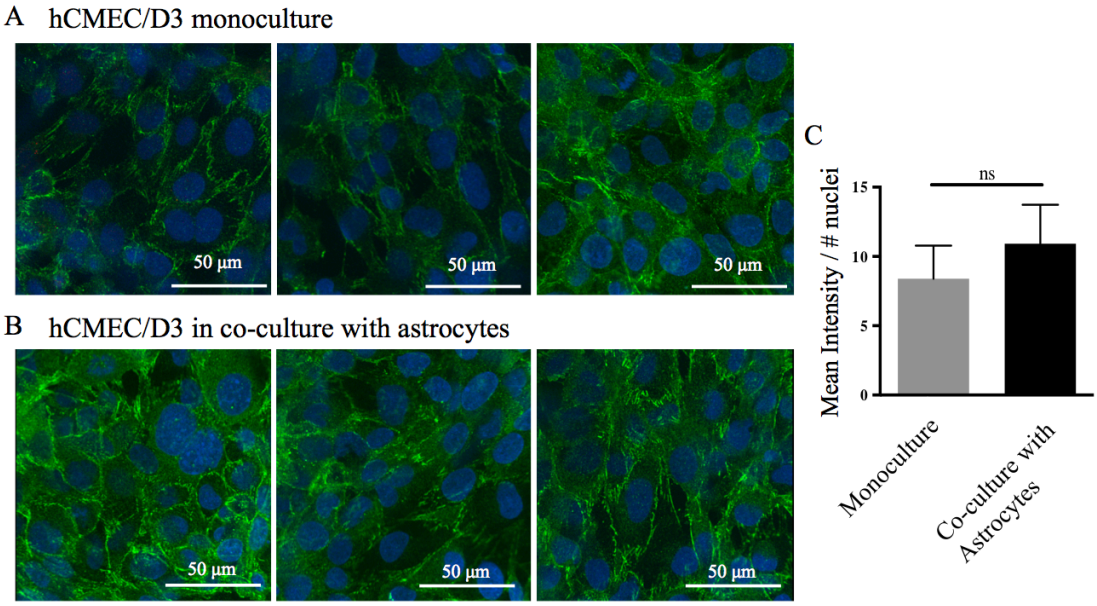

Figure S3. ZO-1 expression in hCMEC/D3 for monoculture (A) and co-culture with astrocytes (B) at 7 DIV after the endothelial cell seeding. (C) Quantification of the mean fluorescent intensity normalized with the total number of nuclei in each region of interest (ROI) (n= 9 randomly selected ROI from 2 NVC per condition).

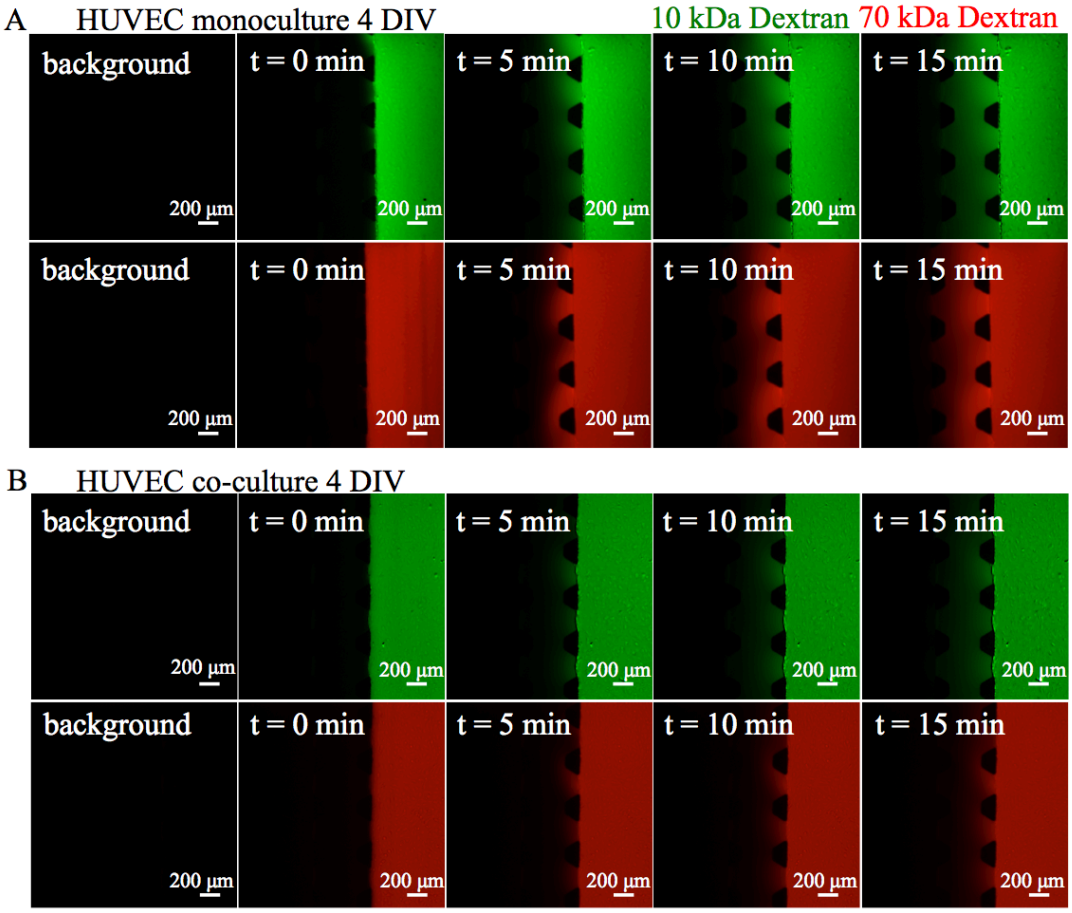

Figure S4. Representative images of the permeability of the endothelial barrier to 10 kDa (in green) and 70 kDa (in red) dextran at 0, 5, 10 or 15 min for HUVEC in monoculture (A) or in co-culture condition (B) at 4 days *in vitro* (DIV).

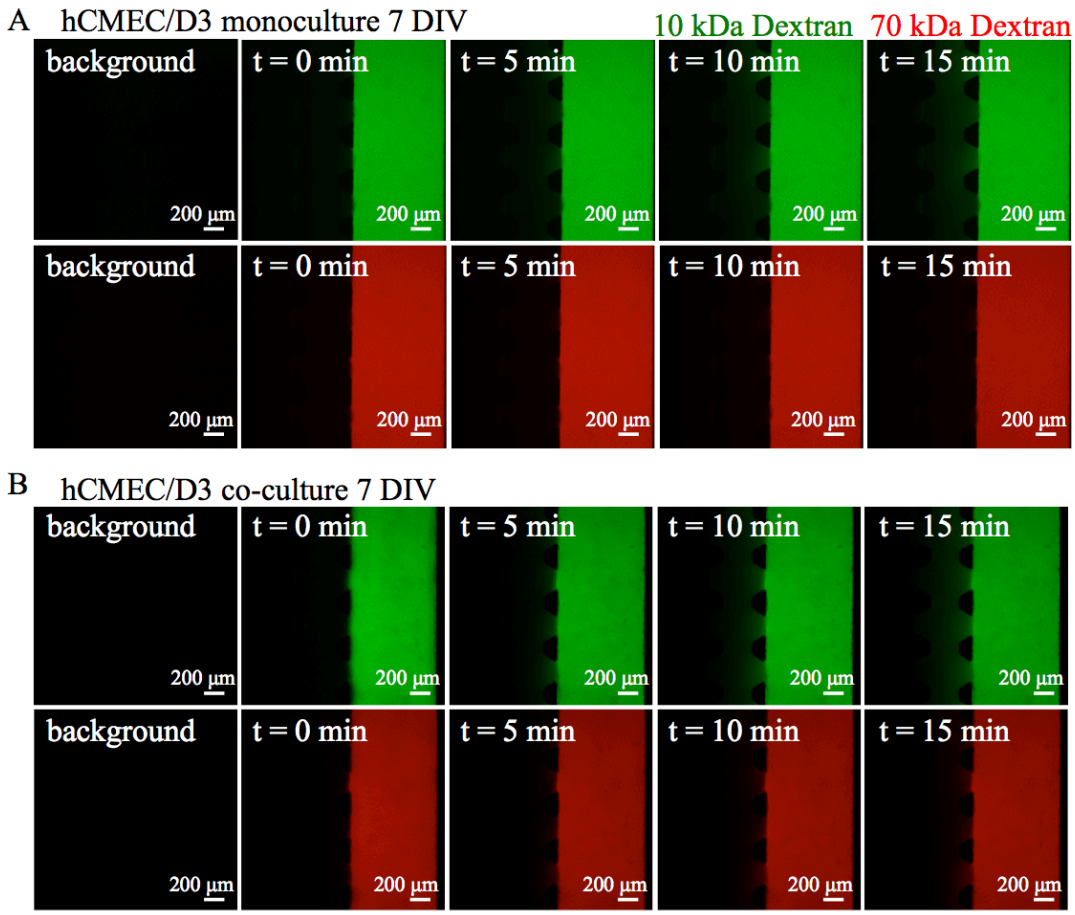

Figure S5. Representative images of the permeability of the endothelial barrier to 10 kDa (in green) and 70 kDa (in red) dextran at 0, 5, 10 or 15 min for hCMEC/D3 in monoculture (A) or in co-culture condition (B) at 7 days *in vitro* (DIV).

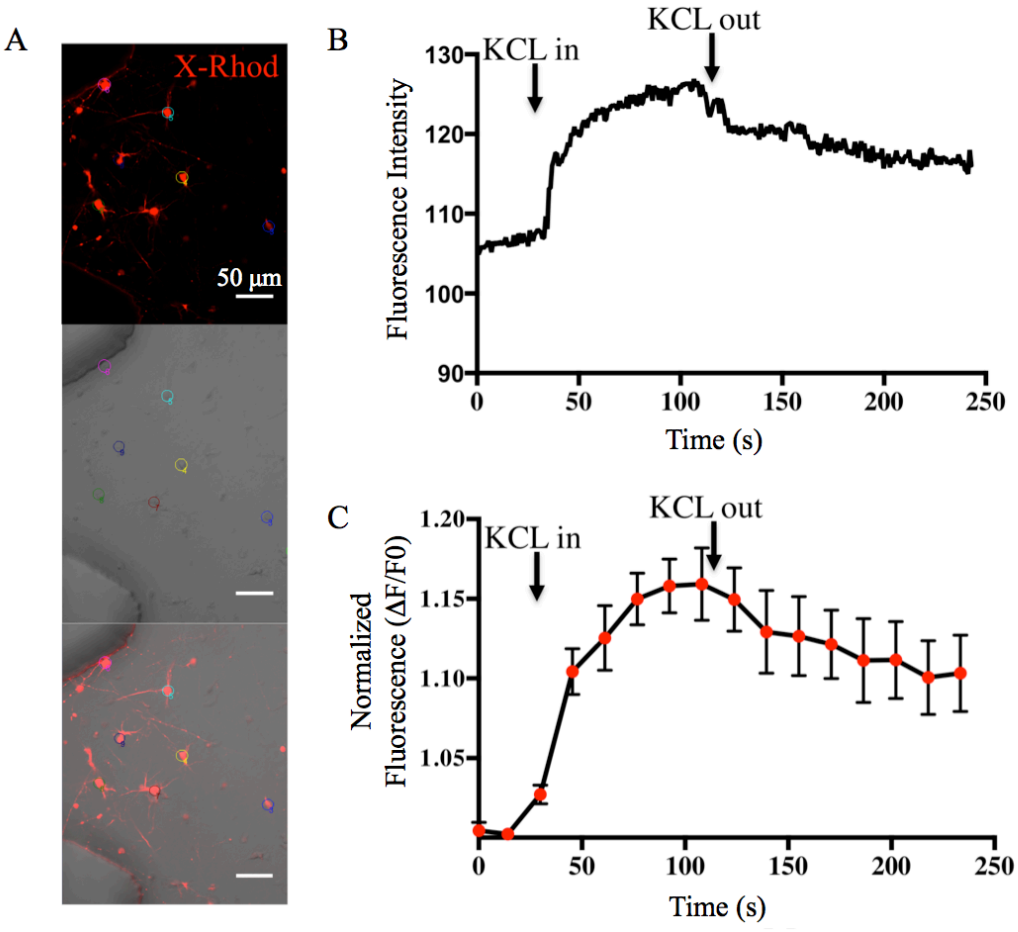

Figure S6. Functional characterization of neurons using calcium imaging. (A) Representative fluorescence and phase contrast images showing X-Rhod-1 (red) in neurons. Circles indicate the neurons in which calcium concentration was measured. (B) Representative fluorescence intensity from one neuron measured over time (250 s with images taken every 500 ms). (C) Graph showing average normalized fluorescence intensities from the 5 neurons circled in (A). Data show mean values and SEM.