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Supplemental Data

Microfluidic Local Perfusion Chambers for the Visualization and Manipulation of Synapses

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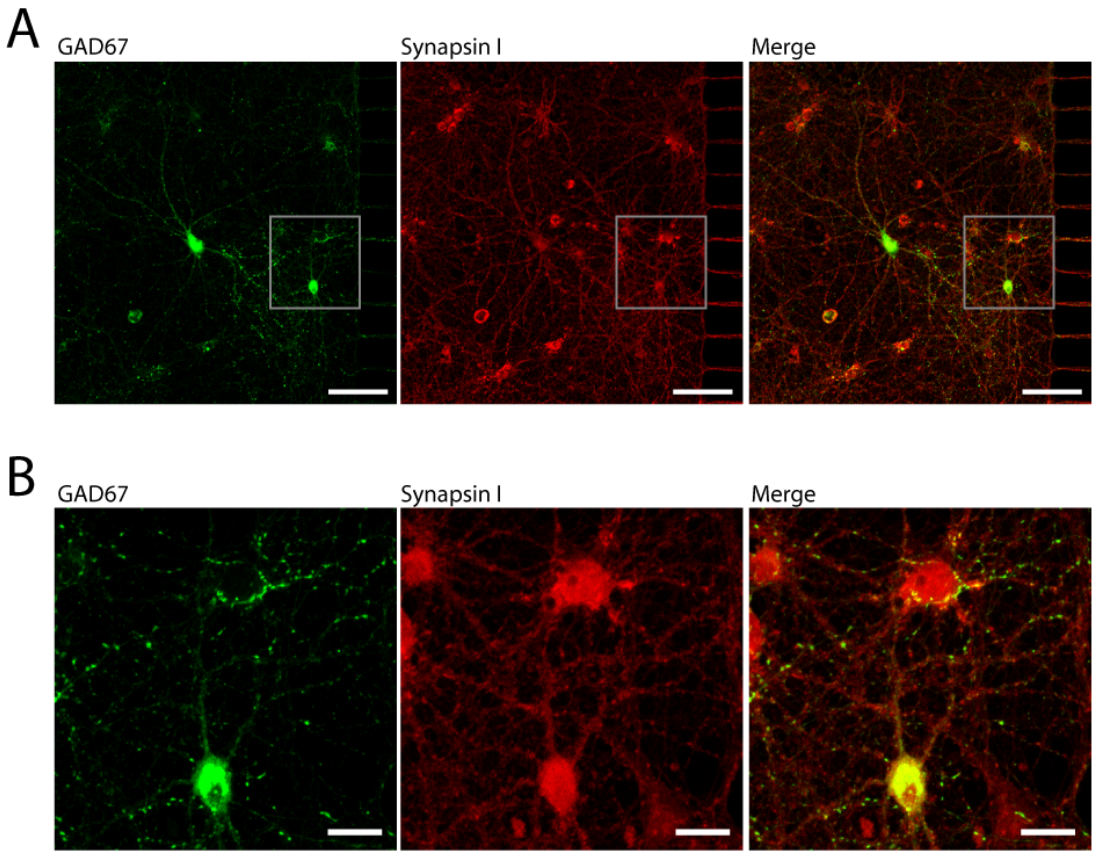

Figure S1, related to Figure 2: GABAergic neurons are present among neurons cultured in the microfluidic chambers. (A) Neurons were immunostained for glutamate decarboxylase 1 (GAD67, green), an enzyme required for GABA biosynthesis, and a presynaptic marker (Synapsin I, red). Scale bar = 100 μm. Two neurons have cell bodies positive for GAD67 immunoreactivity. (B) Enlarged image from boxed region in *(A)*. The cell body of the bottom neuron is GAD67 positive. Numerous GAD67 positive puncta decorate the cell body of the neuron at the top. Scale bar = 20 μm.

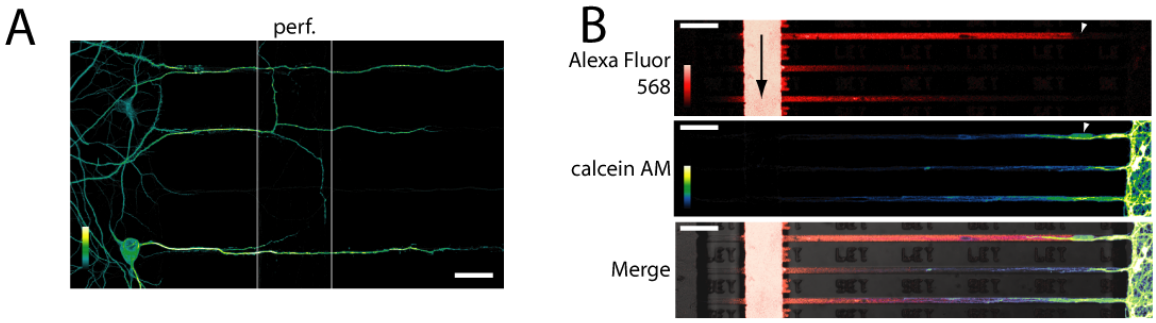

Figure S2, related to Figure 3: Neuronal growth and perfusion within the single inlet microfluidic perfusion chamber. (A) MAP2-labeled dendrites enter the local perfusion channel (21 days *in vitro*). Scale bar = 20 μm. (B) Perfusion of dye (Alexafluor 568, 1 μM, top) within the single inlet chamber. At 21 days *in vitro*, the dye diffuses into the microgrooves. This diffusion is caused by an accumulation of cellular material within the microgrooves (Calcein AM, middle) at advanced culture ages which prevents clear passage of fluid through the microgrooves into the perfusion channel. Flow within the microgrooves is necessary to prevent diffusion of the perfusate into the microgrooves. The white arrowhead shows a blockage in a microgroove. The three inlet design (described as the μLP chamber in the text) overcomes this issue. The bottom image is a merge of the top and middle images and the DIC image. Scale bar = 50 μm.

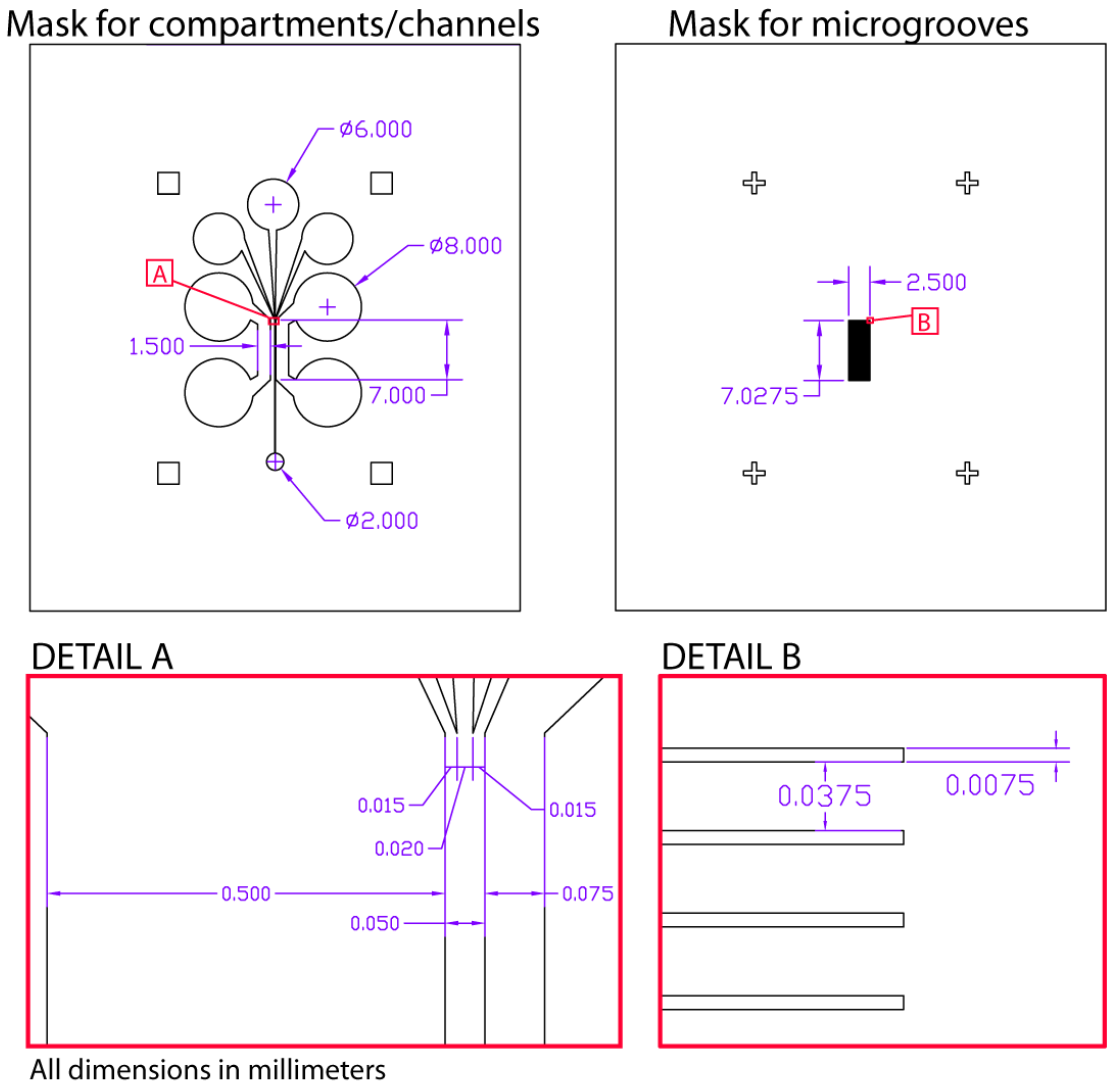

Figure S3, related to Figure 4: Feature dimensions for masks used to fabricate the μLP chamber masters.