

Supporting Information

FIGURE S1. Monitor the growth of DRG neurons in a microfluidic chamber by phase-contrast images. Boundaries of the compartments are visible as vertical thick black lines while microchannels appear as horizontal thin black lines. A) One day after plating, cell bodies of DRG neurons started to grow out axons. B) Extending axons grew across 500- μm -long microchannels by day 3. DRG neurons can be cultured in the microfluidic chamber for up to 6 weeks. C-F) show snapshots of the same neuronal culture 5, 7, 10, and 14 days after cell plating. As axons keep growing, cell bodies of DRG neurons were migrating away from the channel (compare location of blue circle in B) and C)). One week after the plating, axons started to bundle both before entering and after leaving the microchannels. Small dots in panels E and F are due to condensation of tiny water droplets on the bottom side of the glass coverslip. Dark area in the left and right part of E) and F) is due to the change of the chamber height. Scale bars on each panel represent 200 μm .

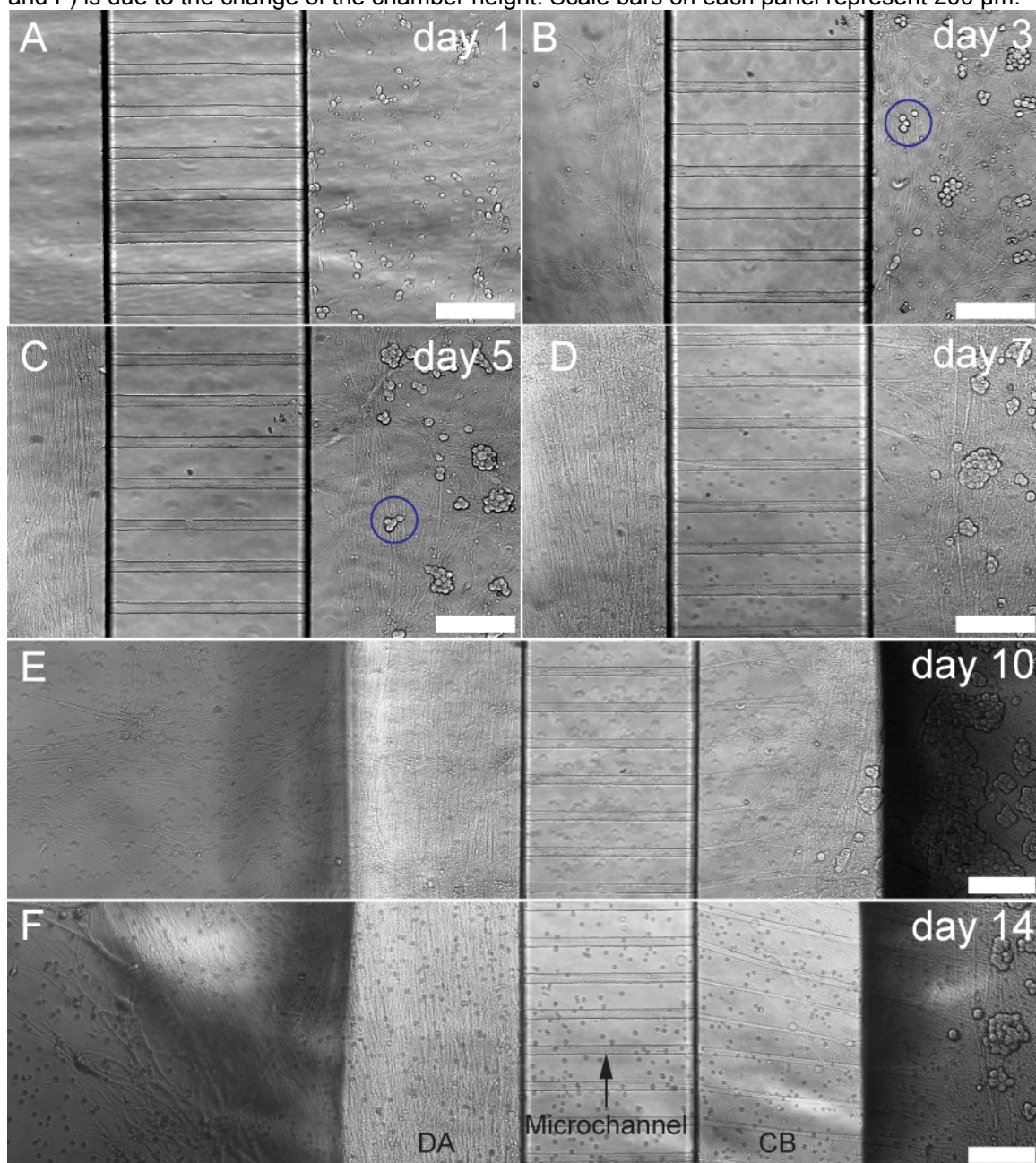


FIGURE S2. Comparing DRG neurons growing in microfluidic devices with (A) the previously reported four-well design (23) and (B) our two-well design. In four-well design, axons tend to form bundles along the wall that is perpendicular to the microchannels while they tend to grow more dispersed in the two-well microfluidic device.

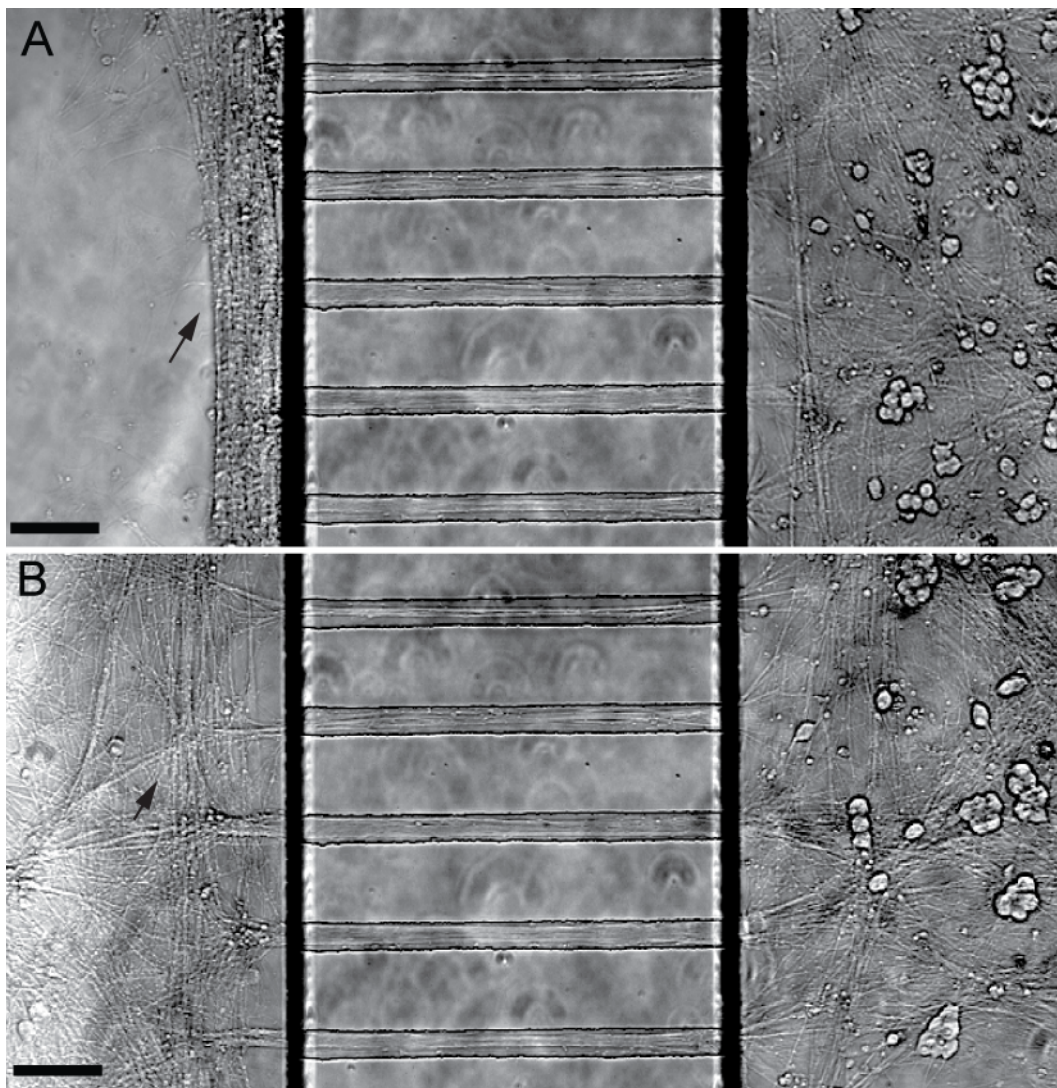


Figure S3. Comparison of Campenot chamber and microfluidic chamber for compartmentalized neuronal culture. (A) Image of Campenot chamber; (B) Image of a microfluidic chamber; (C) Axons in the distal axon compartment of the Campenot chamber. (D) Axons in the distal axon compartment of the microfluidic chamber.). The microfluidic chamber is easy to make once the master pattern is fabricated. The PDMS rubber is transparent (D) in comparison with the non-transparent Teflon divider (C), so it is more compatible with the optical microscopy.

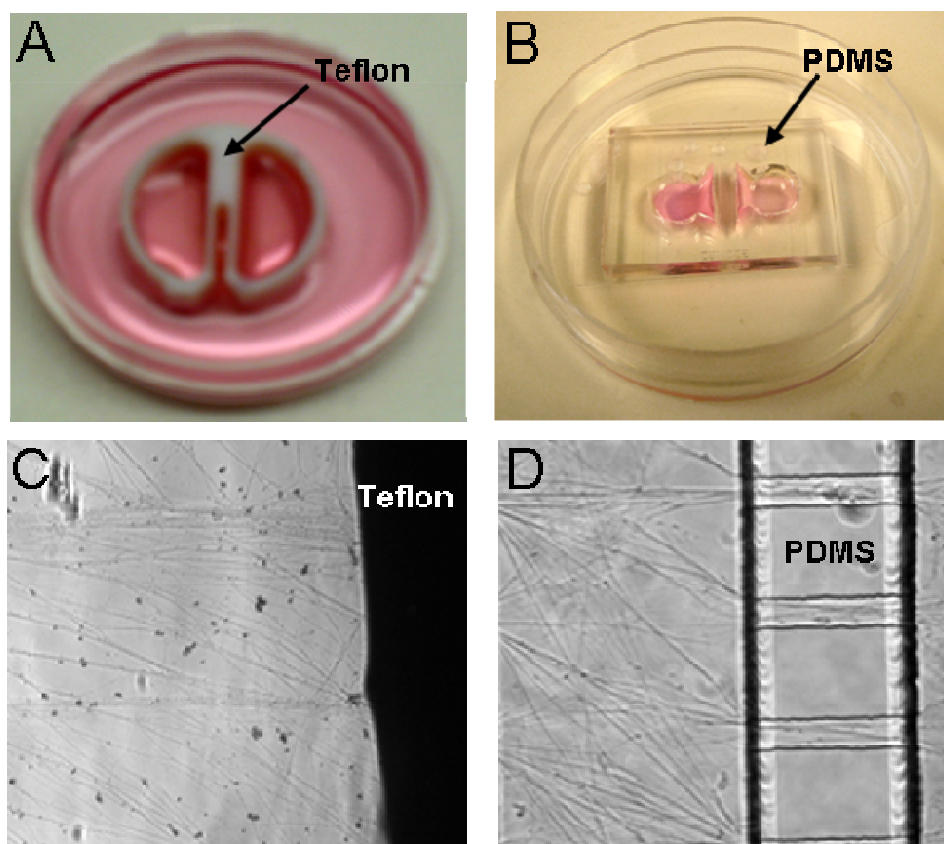


Figure S4: Single molecule imaging of Qdot-NGF transport in Campenot chamber (A, B) and the microfluidic chamber (C, D), before (A, C) and after (B, D) background subtraction. In (A), white arrows in point to Qdot signal while black arrows point to background fluorescence. White arrows in (C) points to the edges of the microfluidic channel. The raw data obtained using Campenot chamber had very strong background fluorescence - sometimes brighter than fluorescence signal from a single Qdot (A). We developed a powerful background-subtraction software to filter out the background noise (B). The movies shown in the previous report had been processed for background subtraction. In comparison, microfluidic chamber gave rise to much lower fluorescence background (C), and the background was almost completely removed after processed with the background-subtraction software (D). Corresponding movies are supplied in the supplementary materials.

