SUPPLEMENTARY INFORMATION

Experimental Section

Fabrication of PDMS chips : The master molds for the PDMS chips were made by exposing spin-coated layers of SU-8 photoresist on a silicon wafer. The axonal structures (axon channels and obstacles) were imprinted in a SU-8 2005 layer with a height of 5 um, so as to exclude larger cell bodies. For the somato-dendritic channels, a layer of SU-8 3050 100 um high was used.

A mixture of PDMS base and curing agent (10 :1 ratio) was poured onto the master molds. After 1 hours in a 70°C oven, PDMS parts were turned out from the master molds. Holes were punched at the extremities of the macro-channels. Clean 50 mm coverslips and PDMS parts were treated 30 sec with oxygen plasma, and sealed together. Poly-D-Lysine solution (0.1% m/v ratio, Sigma) was injected in the macro channels and allowed to penetrate through the axon channels by establishing a hydrostatic pressure gradient in between the macro-channels. Poly-D-Lysine was incubated overnight to allow its homogeneous adsorption inside the chips, then rinsed twice with PBS.

Ethical statement : All animal experiments were performed in compliance with European Community guidelines on the care and use of laboratory animals: 86/609 and and 2010/63 /EEC. The purpose of the research and protocol were described in the Ethical Annex of ERCadg project CellO, which was approved and is regularly reviewed by the ERCEA. Institut Curie animal facility has received licence #C75-05-18, 24/04/2012, reporting to Comité d'Ethique en matière d'expérimentation animale Paris Centre et Sud (National registration number: #59)

Neuronal culture : Primary neurons were obtained from E17 embryos. The embryos were decapitated and their brains micro-dissected on ice in L-15 medium without phenol red (Life technologies) supplemented with 0.6% glucose and 0.2% gentamicin. Cortices were digested in papain solution (papain 100 units, DNAseI 1000 units, L-Cystein 2 mg, NaOH 1M 15 µL, EDTA 50mM 100 µL, $CaCl_2$ 100mM10 µL, dissection solution 10 mL) at 37°C for 20 minutes. After digestion, the supernatant was carefully removed and the cortices were rinsed with 10 mL of complete medium

(MEM without glutamine supplemented with 1% GlutaMAX, 1% sodium pyruvate, 10% horse serum and and 0.2% gentamicin) to eliminate the papain. Supernatant was removed, replaced with fresh complete medium with DNAseI 100 units/mL and tissues were triturated with a micropipette. Neurons were counted on a Malassez cell in 50% trypan blue, centrifuged 6min at 100g, and resuspended at 20 000 cells per microliter. The suspension was injected into the macro-channels inlets (5µL per channel), yielding a density of 2000 neurons/mm^2. The seeded chips were incubated 10 minutes in a humidified, 37°C and 5% CO_2 incubator to allow the attachment of neurons to the Poly-D-Lysine substrate. Complete medium was finally added in each reservoir (15 µL) and the cultures were placed back into the incubator. Medium was changed every two days.

Neurons were imaged with an inverted Nikon Eclipse Ti-E microscope controlled by the NIS Advance Research software (Nikon) using a 10 x and 20x long distance objectives. Images were digitized using a Coolsnap HQ2 camera (Roper Scientific).

Data analysis and Statistics: Axonal transmission data were obtained from each individual microchannel by measuring the intensity in their terminal section (in red, figure 3), substracting the background measured next to that microchannel region (in white), and normalizing it by the fluorescence intensity in the emiting chamber (in yellow). The resulting distributions of axonal transmission in the different conditions were compared using a two-tailed Student's t-test for independent samples.

The fluorescence intensity maps in figure 3 and 4 were obtained by aligning and averaging 24 regions containing the elementary pattern of each design (2 channels), using a home-made algorithm (MATLAB).

Movie S1. Time lapse video of axons growing in the forward (green) and reverse (red) direction. Neurons were extracted from Td Tomato and GFP-LifeAct E17 embryos respectively. Scale bar 100 microns.

Movie S2. Time lapse video of the axon undergoing lateral escape. Neurons were extracted from GFP mice.

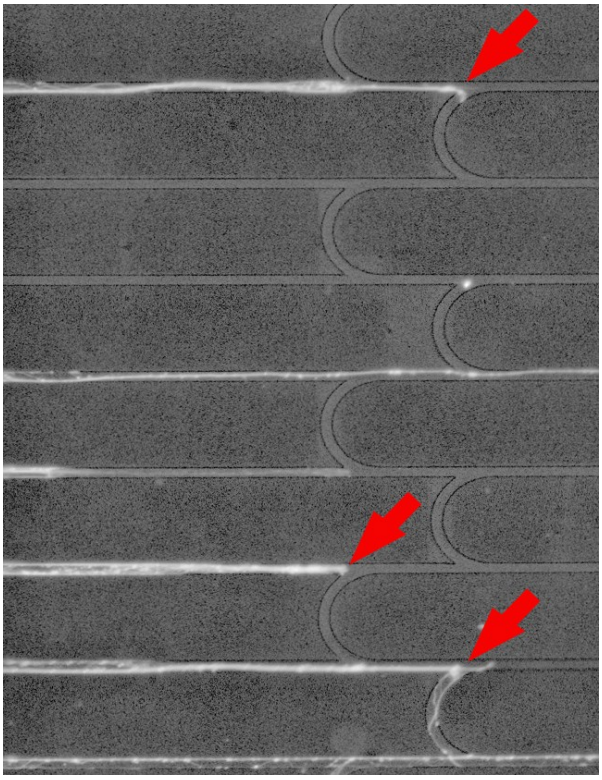

Figure S3. Axonal stalling at junctions. Image taken at DIV 2, showing growth cones localized at junctions (red arrows) as they grow in the forward direction. They are responsible for the intensity peaks in the profiles presented in figure 5 A.