

## Supporting Information

**Cell culture.** C2C12 cells (ATCC CRL 1772) were cultured in 75 cm<sup>2</sup> tissue culture vessels (Corning Life Sciences, USA) at 37°C, 5% CO<sub>2</sub> in Dulbecco's Modified Eagle Medium (DMEM, Life Technologies, UK) supplemented with 10% fetal calf serum (FCS, Life Technologies), 2 mM Glutamine, Penicillin (10 U/mL<sup>-1</sup>), Streptomycin (100 µg/mL<sup>-1</sup>) and Sodium Pyruvate (all from Sigma-Aldrich, UK).

Schwann cells were isolated from neonatal rat sciatic nerves by positive selection with an anti-p75 antibody captured on MACS microbeads (Miltenyi Biotec Ltd., UK) according to the manufacturer's instructions. Briefly, sciatic nerve tissue was cut into ~1 mm pieces and digested overnight at 37°C using 250 U/mL Hyaluronidase Type I-S and 160 U/mL Collagenase Type I (Sigma-Aldrich) in culture medium (DMEM/F12 with 10% FCS), followed by trituration. Cells were plated on two poly-L-ornithine/laminin coated 25 cm<sup>2</sup> tissue culture flasks and cultivated for 24h at 37°C, 5% CO<sub>2</sub>.

**Live cell staining.** Cells were fluorescently labelled in three colors to aid subsequent imaging to verify the experimental results. For fluorescent labelling, cells were plated at ~40% confluency in 10 cm tissue culture dishes. The following day, prior to the patterning experiment, medium was replaced with 10 mL of DMEM containing Mitotracker Red FM (500 ng µl<sup>-1</sup>), MitoTracker Green FM (200 ng µl<sup>-1</sup>, both Life Technologies) or Hoechst 33342 (5 µg µl<sup>-1</sup>, Sigma-Aldrich, UK) for blue fluorescence. After 45 min incubation at 37°C, medium was removed, cells washed twice with HEPES saline solution (140 mM NaCl, 5 mM KCl, 5 mM D-Glucose, 0.001% Phenol Red Na-salt, 10 mM HEPES pH 7.2; all from Sigma-Aldrich), and detached from the culture dish using a 0.25% Trypsin solution (Life Technologies) in HEPES saline containing 0.5 mM

EDTA. After harvesting, cells were resuspended in 10 mL DMEM without Phenol Red (Life Technologies) to an approximate cell density of  $5 \times 10^5 \text{ mL}^{-1}$ .

For some experiments, CellTracker Orange Dye (Life Technologies) was used instead of MitoTracker Red FM. In these cases cells were incubated in working solution ( $10 \mu\text{mol l}^{-1}$  CellTracker Orange in serum-free DMEM) for 45 min before removing the dye-containing medium and replacing it with serum-containing DMEM for an additional 30 min. Cell harvest was conducted as described above.

**Magnetic Labelling and Separation of Schwann cells.** For magnetic labeling, cells were detached with Trypsin/EDTA, harvested by centrifugation and washed in 10 mL wash buffer (Phosphate buffered saline (PBS) pH7.2 + 2mM EDTA). Cells were resuspended in 100  $\mu\text{l}$  separation buffer (PBS pH7.2, 0.5% bovine serum albumin (BSA) + 2mM EDTA) containing 5  $\mu\text{l}$  mouse anti-NGFR (p75) antibody (clone 192-IgG, Millipore, UK) and incubated for 10 min at room temperature. After washing with 10 mL wash buffer and centrifugation, cells were resuspended in 80  $\mu\text{l}$  separation buffer and 20  $\mu\text{l}$  Rat anti-Mouse IgG1 MicroBeads (Miltenyi Biotec), followed by 15 min incubation at  $4^\circ\text{C}$ . Cells were washed with 10 mL wash buffer, centrifuged and resuspended in 500  $\mu\text{l}$  separation buffer.

For magnetic separation the cell suspension was applied to a MACS column (Miltenyi Biotec) equilibrated with 500  $\mu\text{l}$  separation buffer on a magnetic separator. Unlabelled cells were washed off by three consecutive washes with 500  $\mu\text{l}$  separation buffer. Bound cells were eluted by removing the column from the magnetic separator and flushing with 1 mL separation buffer. Eluted cells were collected by centrifugation and resuspended in 1 mL Schwann cell growth medium (SCGM – DMEM/F-12 with 10% FCS, 2mM L-glutamine (all from Life Technologies), 1.4 mM forskolin (Sigma-Aldrich) and 10 ng/mL

GGF (NRG1, Prospec, Israel) before plating on poly-L-ornithine/laminin coated culture flasks.

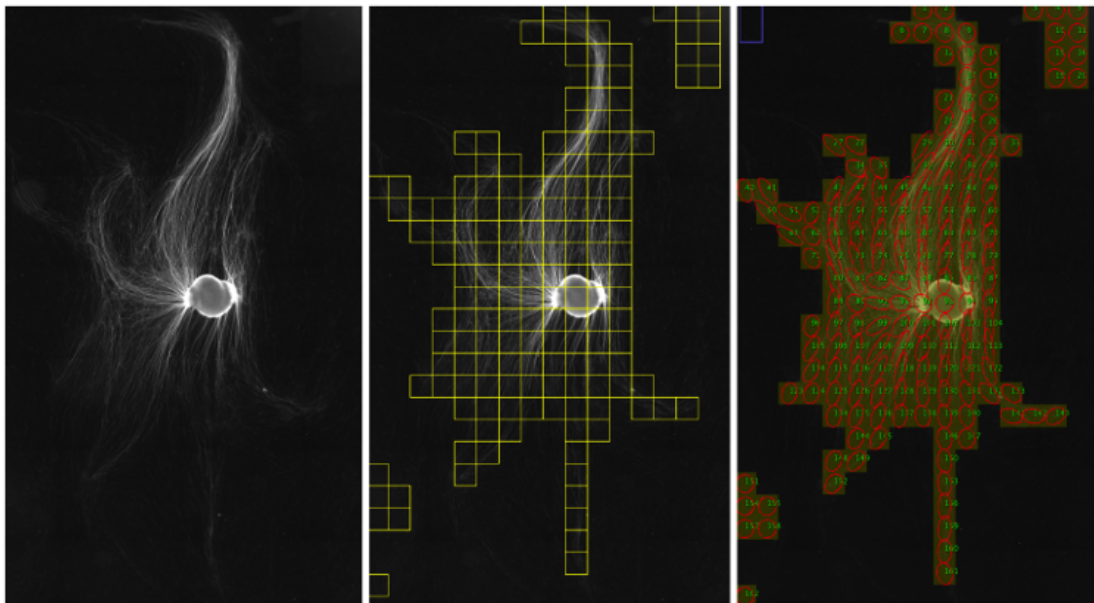
Immunodepletion of fibroblasts was performed, if required, by labeling cells with anti-rat CD90 antibody (AbD Serotec, UK) for 10 min at 37°C before addition of rabbit complement (Sigma-Aldrich, Poole, UK) followed by a 30 min incubation at 37°C. Immunodepleted cultures contained < 1% fibroblasts even after several weeks of culture.

**Image Analysis of DRG.** For the color representation of neurite outgrowth tiled images of  $\beta$ III-tubulin labeled neurons were analyzed with the *OrientationJ* Distribution plug-in with the following parameters: Structure tensor - Gaussian window  $\sigma$  1 pixel, Cubic Spline Gradient; Min Coherency 30%, Min Energy 1%. Higher threshold settings, especially for *Min Energy* resulted in a substantial reduction of pixels included in the output. Further reduction of this parameter on the other hand caused isotropic regions of the image to be included, distorting the analysis.

To account for variability in neurite densities and staining intensities, the histograms summarizing the distribution of pixel orientations were normalized against the maximum frequency of pixel orientations observed in each image (Fig 4C and F). For neurite outgrowth on patterned Schwann cells, the pixel orientations are relative to the direction of the underlying Schwann cell pattern (see below).

For quantitative analysis we used the *OrientationJ* Measure plug-in to determine the dominant growth direction in 250x250 pixel Regions of Interest (ROI). An *ImageJ* macro (see below) was programmed to divide the image into 250x250 pixel ROIs and, using a user-defined Integrated Density value, select those ROIs for analysis that contained  $\beta$ III-tubulin labeled neurites (Figure S1). Areas that contained background fluorescence only, the DRG itself and areas that were out of focus were manually excluded from the

analysis, as they provided no directional information. The calculated orientations (in degrees) were statistically analyzed using Microsoft *Excel* after correcting the values for rotation during handling of the cover slips. This correction was based on the position of the DRG after immunostaining relative to the initial position at the time of DRG seeding. The angle of rotation was determined by superimposing the two corresponding images (initial position at DRG seeding and after immunostain) and rotating one until the DRGs in both images were aligned. The corrected orientations were then normalized to the original angle of the Schwann cell pattern as determined at the time of DRG seeding with the *Angle* tool of *ImageJ*. For DRGs on randomly seeded Schwann cells the horizontal axis of the image was defined as zero degrees.



**Fig S1: Quantitative analysis using OrientationJ's *Measure* function**

The original image of the  $\beta$ III-tubulin stained neurite network (left panel) was subdivided into 250x250 pixel ROIs using a custom-written macro and those ROIs that exceeded a user-defined intensity threshold were passed on for analysis by OrientationJ's *Measure* function (middle panel). The graphical output (right panel) displays the dominant

direction within an ROI as an ellipse, with more slender ellipses indicating greater directional coherency.

Note that ROIs which were created due to high background (lower left and upper right corner in middle and right panel) as well as those that contained mainly the DRG (at the center) were omitted from subsequent analysis.

## **ImageJ Macro using OrientationJ's measure function, applying it to ROIs, and reading out local orientation maxima**

```
macro "Run OrientationJ measure" {
// This macro creates rectangular ROIs of user-specified dimensions
// across an entire image and runs OrientationJ Measure if integrated
// density exceeds a threshold

    // Dialog box - query dimension of selection
    Dialog.create("Define rectangular ROI");
    Dialog.addMessage("Enter height and width of ROI (in pixels)
        \n and threshold for analysis");
    Dialog.addNumber("Height", 250);
    Dialog.addNumber("Width", 250);
    Dialog.addNumber("Threshold", 250);
    Dialog.addCheckbox("Run analysis", true);
    Dialog.show();

    ROI_h = Dialog.getNumber(); //height of ROI (y-direction)
    ROI_w = Dialog.getNumber(); //Width of ROI (x-direction)
    ROI_T = Dialog.getNumber(); //Threshold (IntDen) for running analysis
    analyse = Dialog.getCheckbox();

    getDimensions(width, height, dummy, dummy, dummy); // get image dimensions

    x=0; // initialize starting coordinates (x,y)
    y=0; // start in upper left corner
    counter=1; // counter for overlays
    Overlay.remove; // clear existing overlays
    setFont("Sanserif", 48, "antialiased");

    while (y+ROI_h<=height) { // make sure ROI fits within image borders
        while (x+ROI_w<=width) { // make sure ROI fits within image borders
```

```

makeRectangle(x, y, ROI_w, ROI_h); // create rectangular ROI
List.setMeasurements;
IntDen = List.getValue("IntDen");
    // query Integrated Density of selection
if (IntDen>=ROI_T) {           // if IntDen>=Threshold, analyse!
    setColor("yellow");
    Overlay.drawRect(x, y, ROI_w, ROI_h);
        // draw selection rectangle if analysed
    setColor("green");
    Overlay.drawString("" +counter, x+50,y+50); // label rectangle

    counter++;
    if (analyse == true) run("OrientationJ Measure", "sigma=0.0");
}
x=x+ROI_w; // shift selection to right
}
y=y+ROI_h; // shift selection down when row complete
x=0; // reset x to zero for new row
}
Overlay.show;
}

```