# Supporting Information

## Magnetic Control of Axon Navigation in Reprogrammed Neurons

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## Materials and Methods

***Synthesis and analysis of deleted in colorectal cancer (DCC) antibody-conjugated magnetic nanoparticles (DCC-MNP).*** Zinc-doped iron oxide MNPs (13 nm diameter) were prepared by the thermal decomposition of 1.765 g $Fe(acac)_3$ (Sigma-Aldrich, St. Louis, MO, USA) and 0.5 g $ZnCl_2$ (Sigma-Aldrich) in 20 mL trioctylamine (Sigma-Aldrich) containing 5 mL oleic acid (Sigma-Aldrich) and 15 mL oleylamine (Sigma-Aldrich) as previously reported.[29] The size and shape of MNPs were confirmed by imaging analysis using transmission electron microscopy (TEM, JEM-2100, JEOL, Japan). For bioconjugation, the surface of the nanoparticles was coated with carboxylated silica by reverse microemulsion method,[30] which was further used to conjugate with antibodies that specifically bind to the DCC receptor (anti-DCC-Ab, BS3232, Bioworld, St. Louis Park, MN, USA). Prior to conjugation of antibodies, 30 μg protein A (ProA, from staphylococcus aureus, P6031, Sigma-Aldrich) was covalently conjugated to 300 μg nanoparticles via EDC/NHS coupling. This ProA-MNP (300 μg) was mixed with the antibody (30 μg) in order to enable the Fc region of antibody to bind with the protein A. The mixture solution was then magnetically purified with 1× phosphate buffered saline (PBS) by MACS® columns (Miltenyl Biotech, Bergisch Gladbach, Germany) to remove unbound antibodies. To detect the attachment of nanoparticles on the cell surface, NHS-Fluorescein (46410, Thermo Fisher scientific, Waltham, MA USA) was conjugated to ProA-MNP before mixing with DCC antibody. Fluorescein conjugation was confirmed by photoluminescent analysis (Fluoromax-Plus-C, HORIBA Scientific, Kyoto, Japan). To confirm the bioconjugation, the hydrodynamic size of each nanoparticle conjugate was measured by using dynamic light scattering (DLS, Nano ZS, Malvern, UK).

***Mouse induced neuronal (iN) cell preparation and culture.*** iN cells were generated as previously reported.[23, 24] Primary mouse embryonic fibroblasts (pMEFs) were isolated from

ICR mouse embryo at E13.5 day (Orient Bio, Seongnam, Korea) and cultured in growth medium consisting of high glucose Dulbecco's Modified Eagle's Medium (DMEM) (#11995065, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% (v/v) fetal bovine serum (Thermo Fisher Scientific), 1% (v/v) penicillin/streptomycin (Thermo Fisher Scientific), and 1× non-essential amino acids (NEAA, Thermo Fisher Scientific). pMEFs (passage 1~2) were transfected with Brn2-, Ascl1-, and Myt1l-encoding plasmids (pCMV-Brn2, pCMV-Ascl1, pCMV-Myt1l, Addgene, Cambridge, MA, USA) by electroporation (2.4 μg per $10^5$ cells, Neon, Thermo Fisher Scientific), and then seeded onto the cover-glass coated serially with 0.02 mg/mL poly-L-lysine (PLL; Sigma-Aldrich) and 0.01 mg/mL laminin (Thermo Fisher Scientific). After 2 days of initial seeding, three transcriptional factor plasmids were re-delivered (1 μg per $10^5$ cells) by Lipofectamine 2000 (Thermo Fisher Scientific). The next day after the final transfection, pMEF growth medium was replaced by neuronal proliferation medium containing DMEM/F-12 (Thermo Fisher Scientific), 1% penicillin/streptomycin (Thermo Fisher Scientific), 1× NEAA (Thermo Fisher Scientific), N2 supplement (Thermo Fisher Scientific), 10 ng/mL basic fibroblast growth factor (R&D Systems, Minneapolis, MN, USA), and 10 ng/mL epidermal growth factor (Peprotech, Rocky Hill, NJ, USA). To generate iN cells, the cells were then cultured in a neuronal differentiation medium consisting of 3:1 DMEM/F-12/Neurobasal (Thermo Fisher Scientific), 1% penicillin/streptomycin (Thermo Fisher Scientific), N2 supplement (Thermo Fisher Scientific), B27 supplement (Thermo Fisher Scientific), 1× NEAA (Thermo Fisher Scientific), 1× GlutaMAX (Thermo Fisher Scientific), and 10 ng/mL brain-derived neurotrophic factor (Peprotech).

***Cell culture.*** Human induced pluripotent stem cells (hiPSCs) were kindly provided from Prof. Dong-Wook Kim at Yonsei University College of Medicine and the experiments were

conducted with the approval of the Institutional Review Board of Yonsei University (1040927-201510-BR-229-01E). The cells were maintained on feeder cell layers of mitomycin C-treated STO fibroblasts (American Type Culture Collection, Manassas, VA, USA) as previously described.[31] To induce differentiation, embryoid body (EB) was generated from hiPSCs and cultured in suspension. EBs were then attached onto Matrigel (Corning, Corning, NY, USA)-coated culture dish and further differentiated into neural progenitor cells (NPCs) for 4-5 days following the protocol of previous studies.[32, 33] hiPSC-derived NPCs were mechanically acquired from neural rosettes that appear in the center of attached EB colonies. Small rosette clumps were seeded on PLL (0.02 mg/mL) and fibronectin (0.01 mg/mL, Thermo Fisher Scientific)-coated dishes. Primary cortical neurons (pNeurons) were harvested from ICR mouse embryos (E14.5, Orient Bio, Sungnam, Korea), as previously described.[24] In brief, cortex tissues were dissected and digested using Trypsin/DNase I solution at 37°C for 20 minutes. Cells were then resuspended and cultured in the Neurobasal Medium supplemented with 1% penicillin/streptomycin (Thermo Fisher Scientific), 1× GlutaMAX (Thermo Fisher Scientific), and B27 supplement (Thermo Fisher Scientific). iN cells (passage 1) were seeded at a density of $2 \times 10^5$ cells/cm$^2$.

***Treatment of DCC-MNP and magnetic stimulation.*** DCC-MNP were suspended in neuronal differentiation medium at the concentration of 100 μg/mL. The cells were treated with the particle solutions and incubated for 1 hour at 37°C in humidified $CO_2$ incubator. Then, the cells were exposed to a magnetic field. As a permanent bulk magnet made of neodymium, the 1 cm cubic magnets were put aside the cell culture device to one or two sides. In addition, the cylindrical NdFeB magnet (diameter = 4 mm, length = 1 cm) with a sharp pointer steel (tip size, 100 μm) was used to induce a focused magnetic field. Simulation of magnetic field near

the magnet was carried out using Finite Element Method Magnetics (FEMM) software (http://www.femm.info/wiki/HomePage).

***Fabrication of micro-channel devices.*** All microfluidic devices were fabricated in poly(dimethylsiloxane) (PDMS) using the soft lithography technique. The master mold was fabricated on a 4-inch diameter silicon substrate using a two-layer photolithography process by sequentially patterning two layers of photosensitive epoxy (SU-8™, Microchem, Inc., Newton, MA, USA) with different thicknesses. For the three channel device, the first layer for the neurite guiding microgrooves was 4 µm in height and the second layer for the 3 channels was 100 µm in thickness. For the circular device, neurite grooves (4 µm height, 30-40 µm width, 350 µm length) radially surround the cylindrical cell chamber (somal compartment, 300 µm diameter, 100 µm height) at the center. Devices were replicated from the master by pouring PDMS pre-polymer (10:1 mixture, Sylgard® 184, Dow Corning, Inc., Midland, MI, USA), followed by curing at 70°C for 4 hours. The reservoirs to hold cell culture media in the three channel device were punched out using 3- and 5-mm diameter biopsy punches. For the circular device, a sharpened 26-gauge syringe needle was used to punch holes for cell loading in the middle of the circular cell chamber. For sterilization, coverslips were immersed in 70% ethanol and desiccated for 1 h, and coverslips and PDMS devices were autoclaved. To improve the bonding of PDMS devices onto the coverslips and to make the device hydrophilic for improved loading of culture medium, the PDMS devices and cover glasses were exposed to oxygen plasma for 40 s. After device bonding, 0.02 mg/mL PLL and 0.01 mg/mL laminin were serially coated onto the assembled devices.

***Immunocytochemistry.*** The immunocytochemical staining of the iN cells and hiPSC-NPCs was performed as previously described.[24] The following primary antibodies were used for the

staining overnight: rabbit anti-DCC (#BS3232P, dilution 1:200; Bioworld), mouse anti-Tuj1 (#4466, dilution 1:200; Cell Signaling Technology, MA, USA), rabbit anti-Tuj1 (#5568, dilution 1:200; Cell Signaling Technology), mouse anti-Tau1 (#MAB3420, dilution 1:500, EMD Millipore, MA, USA), rabbit anti-Synapsin I (#ab1543, dilution 1:500, EMD Millipore), and rabbit anti-PSD95 (#ab18258, dilution 1:500, Abcam, Cambridge, United Kingdom). After washing with PBS three times, secondary antibodies [Alexa Fluor-488 goat anti-mouse IgG (1:500) and Alexa Fluor-594 donkey anti-rabbit IgG (1:500); Thermo Fisher Scientific] were added and incubated with the cells for 1 hour. The cell nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI, TCI Co., Ltd, Tokyo, Japan). The fluorescence signals from the staining were observed under a confocal microscope (LSM 880, Carl Zeiss, Jena, Germany). The number of Tuj1 and Tau1-positive neurites and axons was manually counted in randomly selected areas ($0.7 \times 0.7$ mm$^2$) in the device from three independent experiments. The length of the axons was measured using ImageJ software (National Institutes of Health, Bethesda, MD, USA). The unit is expressed in percentage where the start point of microgroove near the main channel is 0 percent and the end of the microgroove (200 μm) is 100 percent.

***Quantitative real-time polymerase chain reaction (qPCR).*** Total RNA was extracted using RNA prep kit (Takara Bio Inc., Kusatsu, Shiga, Japan) as per the manufacturer's instructions. cDNA was prepared using first strand cDNA synthesis kit (Takara Bio Inc.) and PCR was performed using TaqMan Fast Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA). The gene expression profiles were quantified using TaqMan Gene Expression Assays (Applied Biosystems) for each target (mouse DCC: Mm00514509_m1, human DCC: Hs00180437_m1). The relative expression level of each target gene was determined using the comparative $C_t$ method in which the expression was normalized to that of a housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (mouse Gapdh: Mm99999915_g1, human Gapdh:

Hs02758991_g1).

***Western Blot.*** Western blot assay was performed using our previous protocol.[34] The primary antibodies were used as follow: rabbit polyclonal anti-phosphorylated ERK1/2 (pThr202/Tyr204, dilution 1:1000; Cell Signaling Technology), rabbit polyclonal anti-phosphorylated FAK (pTyr397, dilution 1:1000; Thermo Fisher Scientific), and rabbit polyclonal anti-β-actin (dilution 1:4000; Cell Signaling Technology). A Clarity Western ECL Substrate (Bio-Rad, Hercules, CA, USA) was used to detect the signal of the target proteins according to the manufacturer's instructions.

***Calcium imaging.*** Dual cell population models were chosen in the current study to mimic implantation of DCC-MNP-preconditioned iN cells into a host tissue. iN cells and pNeurons were incubated with the cell permeable calcium indicator 5 μM Fluo-4-AM (Thermo Fisher Scientific) for 30 minutes at 37°C. Time-lapse changes in $Ca^{2+}$ levels in live iN cells were imaged using a confocal microscope (LSM 880, Carl Zeiss) after adding 100 μM glutamate (Sigma-Aldrich) to the channel where the pNeurons were seeded. The treated cells were imaged every 0.93 seconds at room temperature. Zen software (Carl Zeiss) was used to determine the fluorescence intensity over a selected area as an indicator for the presence of cytosolic $Ca^{2+}$. The data were normalized to $\Delta F/F_0$ using the following equation: $y = (F_{\Delta\,\mathrm{sec}} - F_{0\,\mathrm{sec}})/F_{0\,\mathrm{sec}}$. To test the diffusion between the channels, 0.1 μg/mL Rhodamine B dye (Sigma-Aldrich) was added to the main channel where pNeurons were seeded. Time-lapse images were taken every 5 seconds and diffusion of the fluorescent dye was assessed over time.

***Statistical analysis.*** All results were expressed as mean ± standard deviation. The statistical significance of difference between groups was analyzed by t-tests using GraphPad Prism 5

software (GraphPad Software, San Diego, CA, USA). Statistical significance of the data was calculated at 95% ($p < 0.05$) and 99% ($p < 0.01$) confidence intervals.

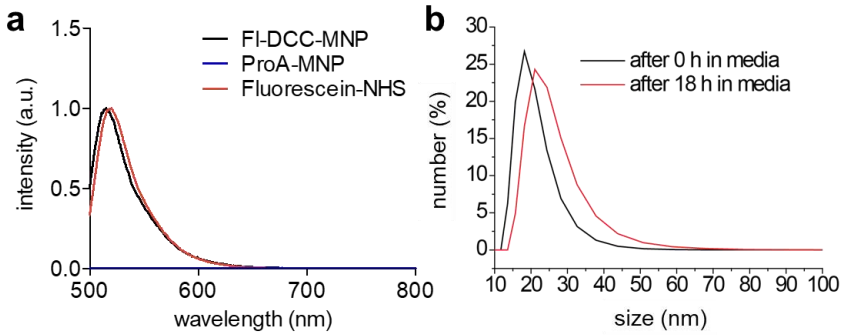


**Figure S1. Characterization of DCC-MNPs for fluorescent labeling and colloidal stability.**

(a) Measurement of the fluorescence intensity of Fl-DCC-MNPs. (b) Colloidal stability of DCC-MNPs in culture medium at 37°C.

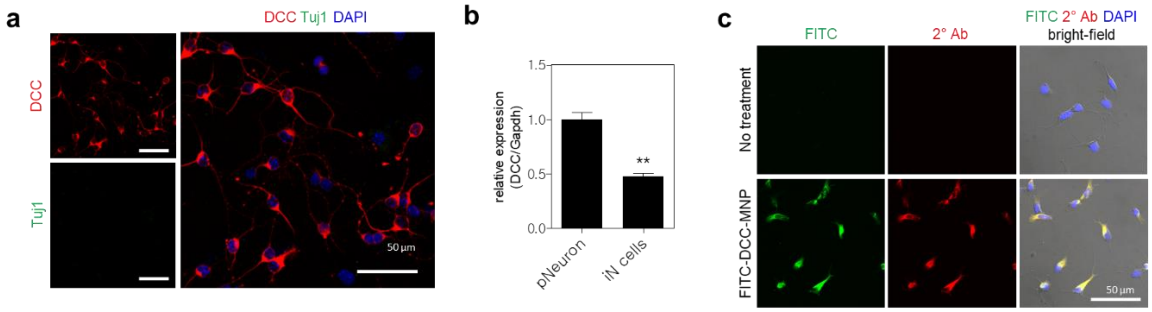


**Figure S2. Characterization of DCC through immunocytochemistry and mRNA-level investigation.** (a) Immunostaining of mouse induced neuronal (iN) cells for DCC and Tuj1 without cell membrane permeabilization (scale bar = 50 µm). (b) Comparison of DCC mRNA expression level in primary mouse neurons (pNeurons) and iN cells (**$P$ < 0.01 versus the pNeuron group). (c) Visualization of Fl-DCC-MNPs bound to iN cells and secondary antibody binding to the DCC antibody of DCC-MNPs bound to iN cells (scale bar = 50 µm).

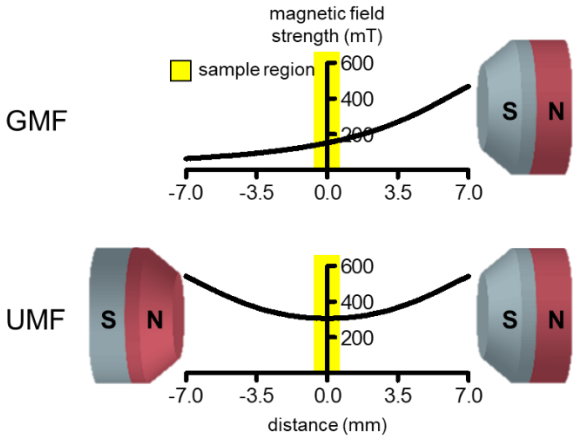


**Figure S3. Setup and simulation of the gradient magnetic field (GMF) and uniform magnetic field (UMF).** For the GMF setup, one permanent magnet was placed 7 mm from the sample. Otherwise, for the UMF setup, two permanent magnets were placed on opposite sides in parallel.

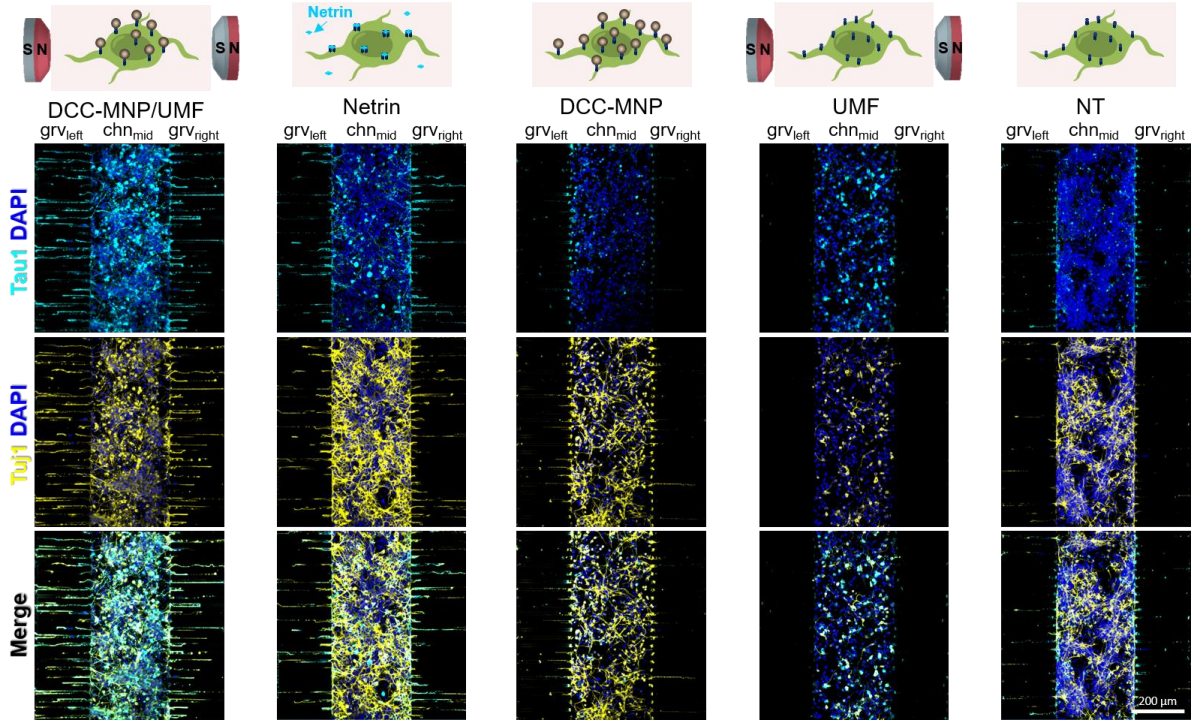


**Figure S4. Immunostaining analysis of iN cells for Tuj1 and Tau1 under the uniform magnetic field (UMF).** Fluorescence microscopy images of iN cells treated with DCC-MNPs and exposed to the UMF and of cells in the control groups (scale bar = 200 µm).

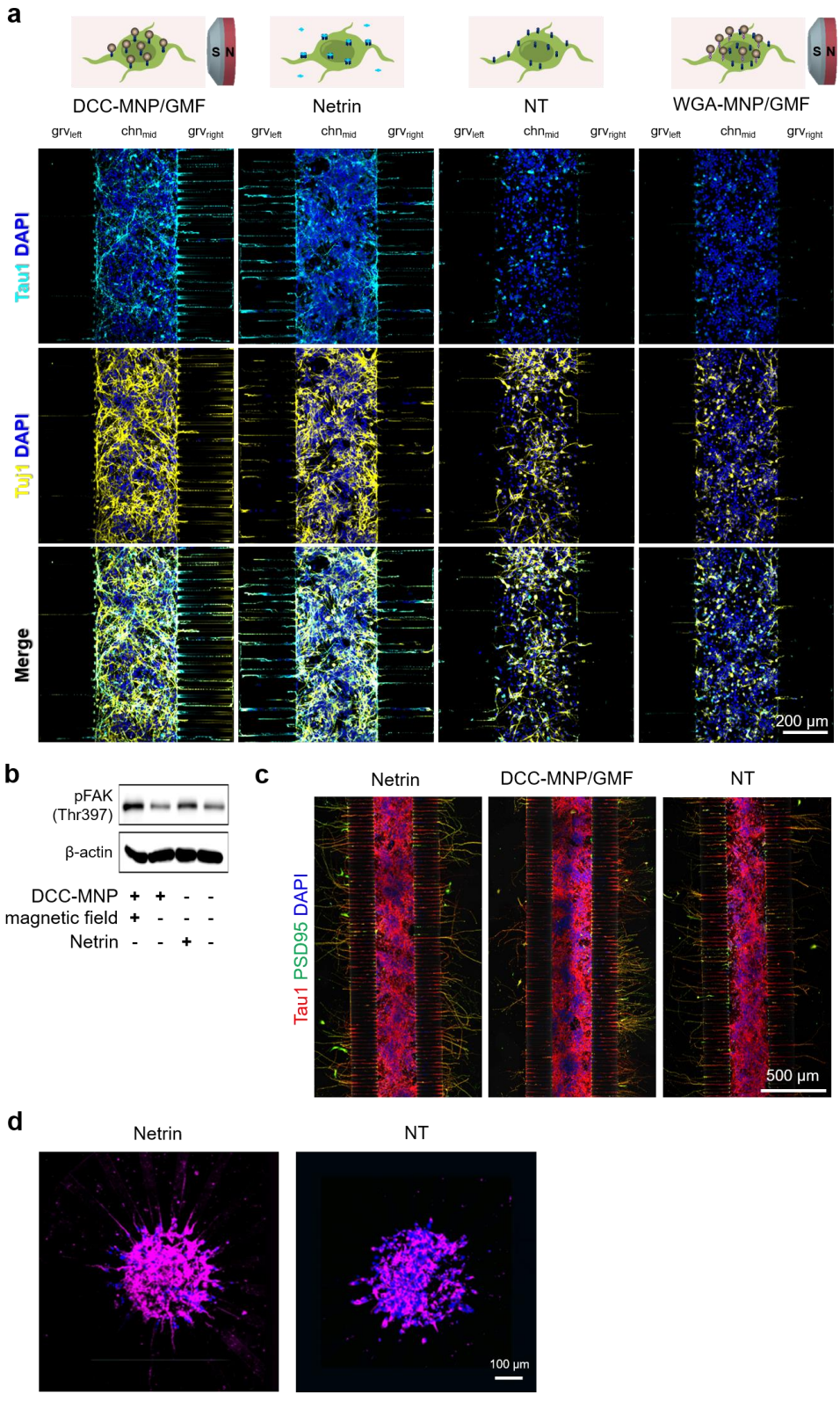


**Figure S5. Neurite guidance under the gradient magnetic field (GMF).** (a) Fluorescence microscopy images of iN cells treated with DCC-MNPs and exposed to the GMF and of cells in the control groups (scale bar = 200 µm). (b) Western blot analysis of phosphorylated focal

adhesion kinase at tyrosine 397 [(pFAK(Tyr397)] in the DCC-MNP/GMF, DCC-MNP, netrin, and NT groups. (c) Expression of the axonal marker Tau1 and the postsynaptic marker PSD95 in iN cells in the netrin, DCC-MNP/GMF, and NT groups (scale bar = 500 μm). (d) Neurite outgrowth from iN cells treated with or without netrin (scale bar = 100 μm).

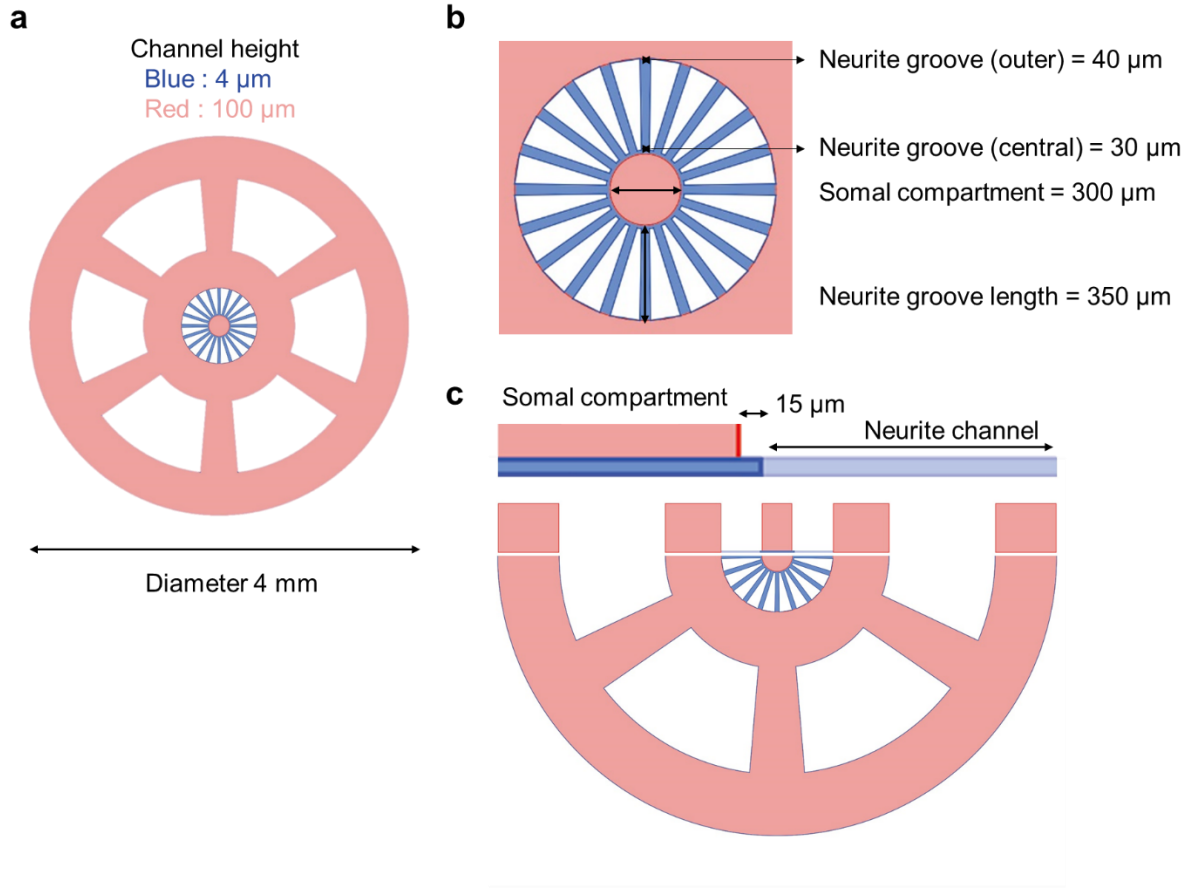


**Figure S6. Schematic design and dimensions of the circular microdevice used for the pin-point magnet setup.** (a) Top view of the circular device. (b) Top view and (c) side view of the cell seeding chamber (somal compartment) and neurite-guiding channels with dimensions.

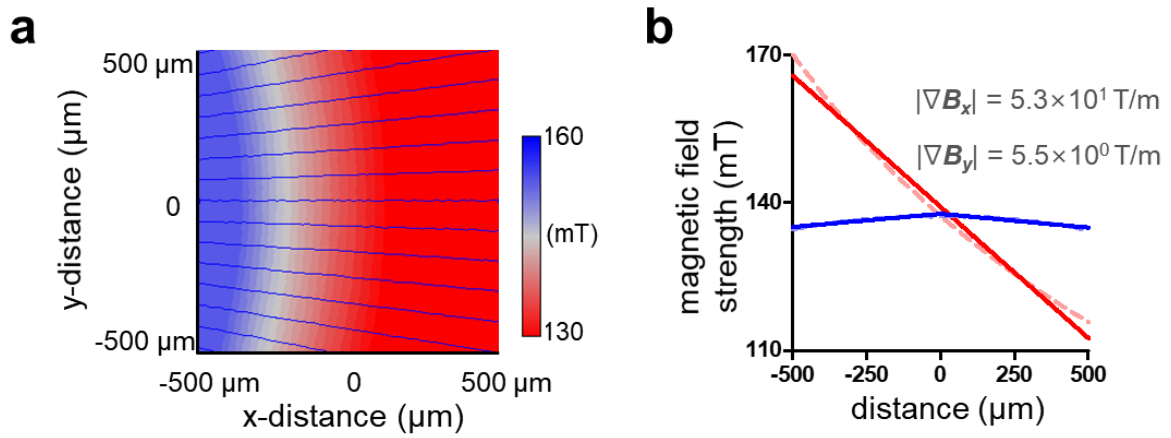


**Figure S7. Simulation of focal magnetic field by a pin-point magnet setup.** (a) Map of the magnetic field strength at the center of the device with the pin-point magnet setup. (b) Graph of the magnetic field strength (red lines, magnetic field strength along the x axis, $B_x$; blue lines, magnetic field strength along the y axis, $B_y$; dotted lines, simulation results along each axis; solid lines; linear fit for each magnetic field strength).

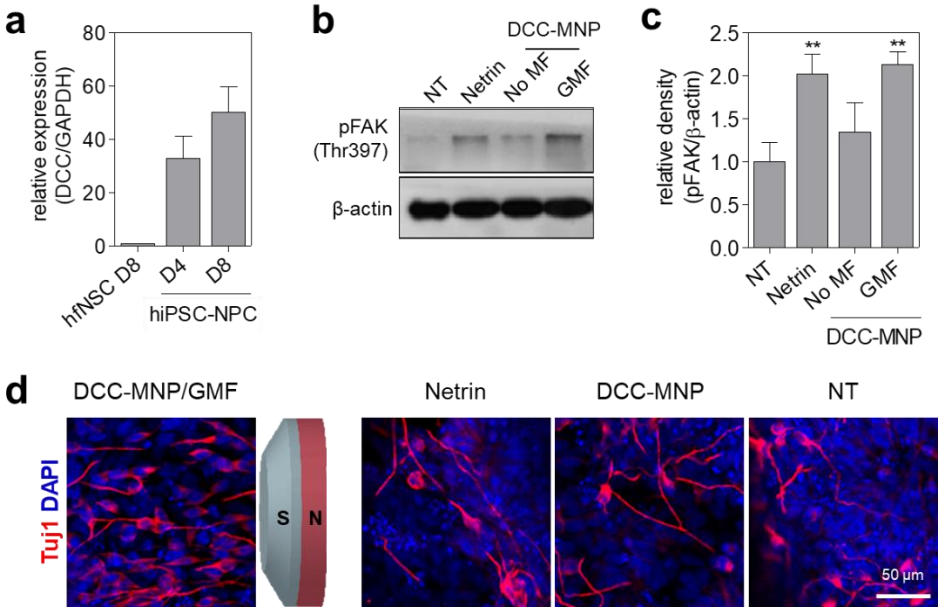


**Figure S8. Application of DCC-MNPs to human induced pluripotent stem cell-derived neural progenitor cells (hiPSC-NPCs).** (a) qPCR analysis was performed to compare the DCC expression levels of human fetal neural stem cells (hfNSCs) from the hippocampal region and hiPSC-NPCs differentiated for 4 and 8 days. (b) Representative Western blot images and (c) quantification of Western blot band intensity for pFAK in the no-treatment (NT), netrin, DCC-MNP and DCC-MNP/GMF groups. (d) NPCs showed directionality toward the magnetic field in only the DCC-MNP/GMF group (scale bar = 50 μm).

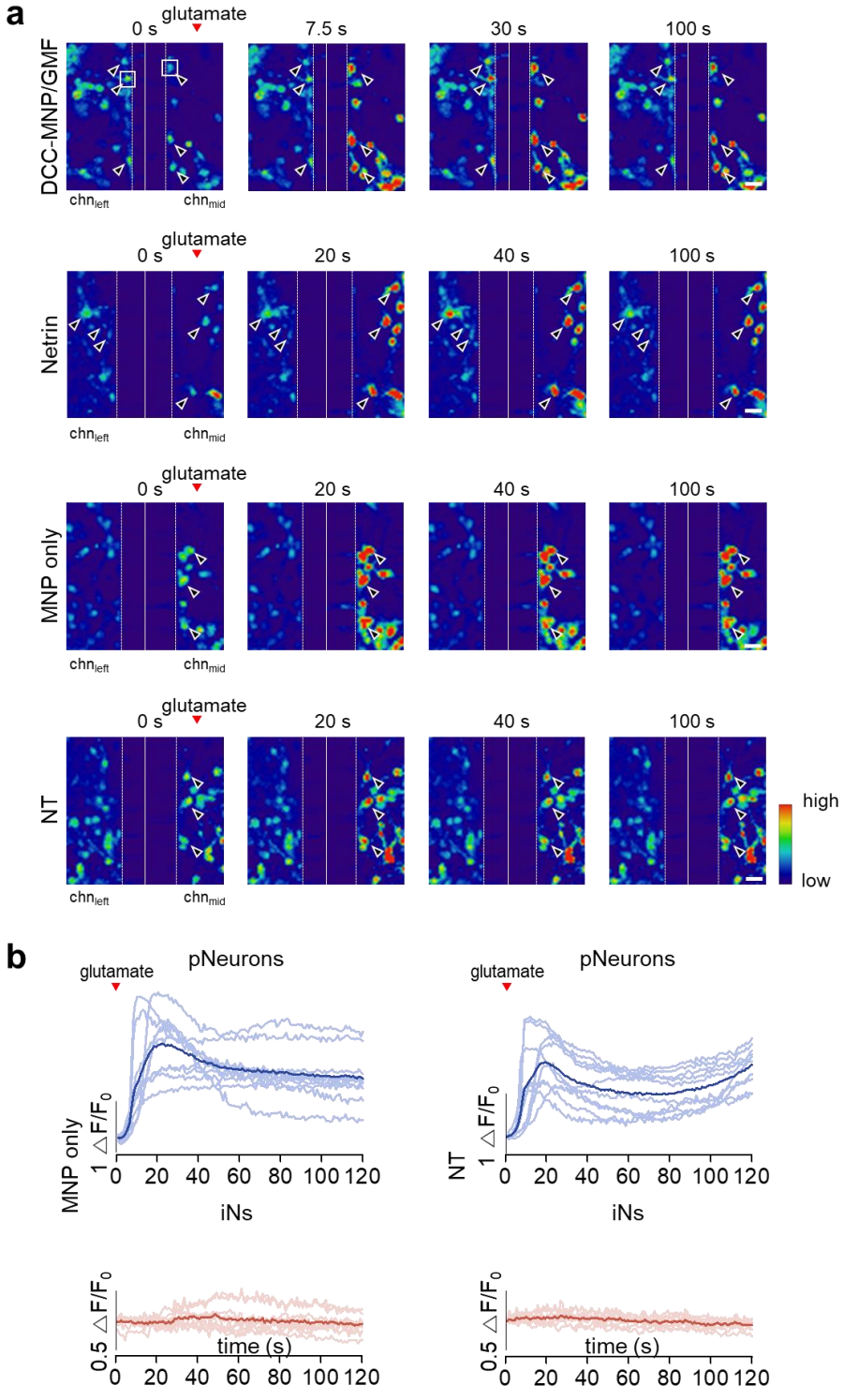


**Figure S9. Calcium signal changes in pNeuron and iN cells over time.** (a) Calcium imaging in various groups over time (scale bar = 20 μm). (b) Fluorescence signal change in control groups.

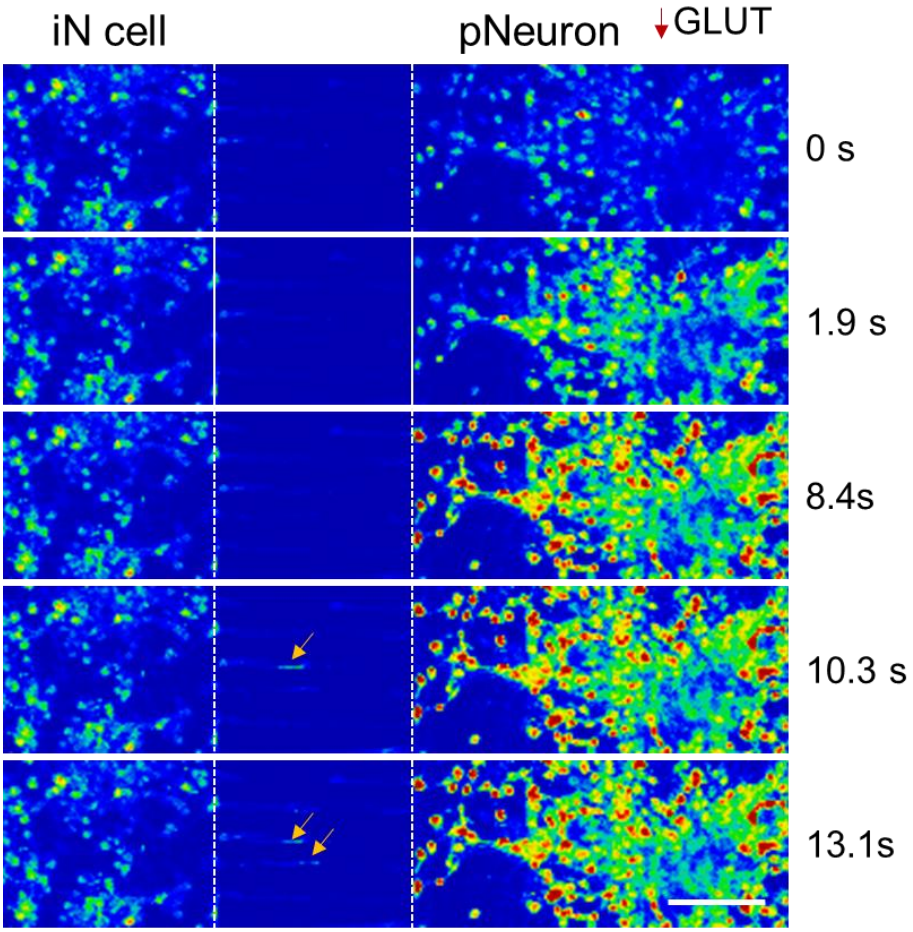


**Figure S10. Calcium imaging of iN cells and pNeurons after the addition of glutamate.** Radiometric images of calcium in Fluo-4-labeled cells and changes in the fluorescence intensity of calcium influx in neurites in grooves in the DCC-MNP/GMF group (yellow arrows) over time after the addition of glutamate to pNeurons (scale bar = 100 μm).

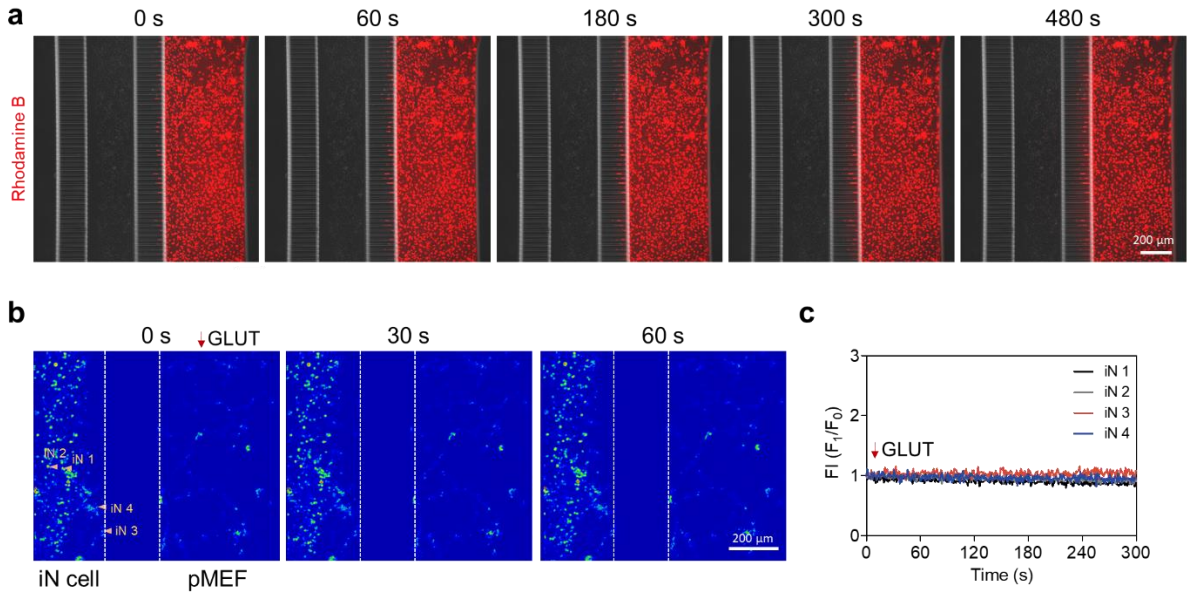


**Figure S11. Diffusion test of the main channels in the microfluidic device.** (a) Diffusion of Rhodamine B dye from the primary neuron-seeded channel (on the right-hand side) over time toward the adjacent channel where induced neuronal (iN) cells were seeded (scale bar = 200 µm). (b) Radiometric images of Fluo-4-AM-labeled primary mouse embryonic fibroblasts (pMEFs) and iN cells. Glutamate (100 µM) was added to the pMEFs (scale bar = 200 µm). (c) Changes in the cytoplasmic fluorescence intensity of Fluo-4 in the iN cells over time after the addition of glutamate to the pMEFs.