Supporting information

Culturing human iPSC-derived neural progenitor cells on nanowire arrays: Mapping the impact of nanowire length and array pitch on proliferation, viability, and membrane deformation

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Figure S1: Comparison of cell numbers, viability, and spreading of cells cultivated on glass control and planarly etched Si substrates. a-c) Exemplary epifluorescence microscopy images of smNPCs cultured on glass substrates after 0, 1, and 4 DIV. d-g) Bar charts of the determined quantities. $n \ge 20$.



Figure S2: Data from quantification that is not shown in the main manuscript.



Figure S3: Viable cells 1 DIV after plating. The cells cultured on the P5 L5 NW arrays maintain larger gaps between cells that are clustered in comparison to cells cultured on the control and the P1 L5 NW sample.



Figure S4: Collection of SEM images displaying smNPCs cultured on various NW arrays (tilt: 45°, corrected) and control samples (not shown in the main manuscript).



Figure S5: F-actin/cytoskeleton (green) on control substrate.



Figure S6: Exemplary images of anti-SOX2 stainings on all types of NW substrates and glass and Si control after 4 DIV. Hoechst: blue, SOX2: green.

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Figure S7: Exemplary images of anti-NES stainings on all types of NW substrates and glass and Si control after 4 DIV. Hoechst: blue, NES: green.

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Methods—Immunocytochemistry

Samples were rinsed once with PBS (Sigma-Aldrich), fixed in formaldehyde (4% in PBS, Sigma-Aldrich) for 10 min, and again rinsed three times with PBS. Covered with PBS, samples were stored until imaging at 4 °C. Fixed cells were permeabilized and blocked for 45 min with 3% BSA (bovine serum albumin, Carl Roth, Karlsruhe, Germany), 0.1% Tween (Tween 20, Sigma-Aldrich), and 0.1% Triton-X (Triton X 100, Carl Roth) in PBS prior to incubation with anti-SOX2 or anti-NES primary antibodies (0.1% BSA, anti-SOX2: 1:300 in PBS, mouse anti-SOX2, Santa Cruz Biotechnology, Cat. No. sc-365823, anti-NES: 1:1000 in PBS, mouse anti-NES, StemCell Technologies, Cat. No. 60091) overnight at 4 °C. The cells were washed twice with PBS and incubated with Alexa fluorophore-conjugated anti-mouse secondary antibodies (0.1% BSA, Alexa 488 1:1000 in PBS, goat anti-mouse IgG-Alexa Flour 488 polyclonal antibody, Invitrogen, Cat. No. A32723) for 1 h in the dark. Stained cells were kept in the dark and washed three times with Tween (0.05% in PBS) for 5 min. The second washing step contained Hoechst 33342 as counterstain. If not further noted, all steps were conducted at room temperature.