Supporting Information

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SI Materials and Methods

SEM. For imaging cells grown on nanoparticle modified surfaces, cells were fixed in 4% paraformaldehyde (PFA) for 30 min, washed with PBS, and dehydrated in an increasing ethanol gradient followed by a drying phase in vacuum. Imaging was accomplished with a Quanta 250 FEG (FEI Inc.) equipped with the FEI xT software.

AFM. Scans were done in tapping mode with a Dimension V atomic force microscope (Bruker Ltd.) equipped with the Nanoscope software (V.7.3; Bruker Ltd.). Nanoparticle modified surfaces and AD samples were measured with a phosphorus-doped silica cantilever in air (k = 3N/m, f_0 = 74–90 kHz) at a scan rate of 0.9 Hz with 256 lines per image. Per batch of coated SNPs, three different substrates were analyzed and the root mean square roughness R_q calculated for three independent regions of each substrate. Amyloid-β plaque roughness was determined for a total of 100 areas (from two individual patients per condition) positively stained in the silver staining (as described below).

Cells were fixed in 4% PFA for 30 min, washed with PBS, and imaged in water with the help of a fluid cell. Scans were done with a silicone tip on nitride lever (k = 0.32 N/m, f_0 = 40–75 kHz) at 512 lines per image with a scan rate of 0.312 Hz. For astrocyte cell surface roughness measurements, 15 cells from three different animals were measured. For R_q calculations of the cell surface a total of 150 areas were analyzed.

Immunocytochemistry. Cells were fixed for 30 min with 4% PFA before antibody specific staining. Postmitotic neurons were visualized with an anti–MAP-2 antibody (Abcam), early-stage neurons with an anti-neuron specific class III β-tubulin antibody (TuJ-1; Abcam), and astrocytes with an anti-GFAP antibody (Dako). The stretch-activated ion channel Piezo-1 was identified with an anti-FAM38A antibody (Abcam Inc.).Visualization of the actin cytoskeleton was done with phallotoxin conjugated to Alexa Fluor488 (Invitrogen Life Technologies).

Cell Density/Composition Analysis. To analyze the cell density and composition of mixed neuron/astrocyte cultures on the different substrates at the end of the experiments, MAP-2$^+$, GFAP$^+$, and Tuj-1$^+$ cells were counted in four separately isolated cultures in 10 random image sections and on two different substrates of each isolated culture.

Morphological Analysis. Morphological changes in cells grown on SNP modified substrates were analyzed in biological and technical triplicates. Per condition and substrate 20 random pictures were used for evaluation of morphological changes. For PC-12 cells, only those not contacting other cells and only processes extending from the cell soma with a length bigger than the cell's diameter were used for analysis. The astrocyte form factor was analyzed with the help of ImageJ (Fiji V.1.47p; National Institutes of Health) and calculated according to the following formula:

$$\phi = \frac{4\pi A}{p^2},$$

where A is the cell's diameter and p the cell's perimeter. Values for the form factor can be between zero and one, zero almost being a line and one being a perfect circle.

siRNA Knockdown Experiment. For all transfection experiments, primary hippocampal neuron/astrocyte cultures were seeded as 7.5×10^4 cells/cm^2 and cultured in serum containing media. Rat FAM38A siRNA (On-Target Plus; Dharmacon), control siRNA (AllStars negative siRNA; Qiagen), and/or the transfection reagent DharmaFect 3 (Dharmacon) was added to the culture in varying concentrations after 24 h in serum-free Neurobasal media supplemented with B27 and glutamine and according to the manufacturer's instructions. The siRNA sequences targeting FAM38A were the following: 5′-GCACAAAGGCCUCCGACUU-3′, 5′-GGGUUGAAGAUUCGGGAGA-3′, 5′-CGGAAGAAUGGCAGCGCAU-3′, and 5′-CAGAUGAACAGUUGGGCGA-3′. Knockdown efficiency was assessed by quantitative real-time PCR.

Calcium-Sensitive Imaging. Intracellular calcium levels were measured with the cell-permeable probe FURA-2-acetoxymethyl ester (Invitrogen Life Technologies). Cells were exposed to5 μM FURA-2-AMin DMSO (final concentration of 0.2%) for 45 min in a humidified incubator at 37 °C. Increase in intracellular calcium levels following depolarization with 50 mM KCl was analyzed by the change in the absorption and emission spectra of FURA-2 upon Ca^{2+} binding using a custom-built perfusion chamber mounted to a Zeiss Observer Z1 and the ZEN blue software (Carl Zeiss AG). The rate of intracellular Ca^{2+} increase was calculated as the slope of the linear portion of the increase in FURA-2 intensity.

AChE Activity Measurement. PC-12 cells were detached from the substrate by trypsin treatment and washed twice with PBS. Cells were resuspended in 0.1 M Na-phosphate buffer (pH 8.0) containing 1% Triton X-100 and sonicated for 20 s. For enzyme activity measurement, 5 μL cell homogenate was mixed with 190 μL of a 10 mM dithiobisnitrobenzoic acid solution (DTNB; Sigma) and 5 μL were transferred into a 96-well plate. After addition of 5 μL acetylthiocholine iodide (final concentration 0.5 M; Sigma) the change of absorbance at 412 nm was followed for 10 min.

Immunohistochemistry of AD slices. Slices of patients diagnosed with AD and patients of the same age diagnosed negative for AD were provided by the University Hospital Freiburg and were obtained in accordance with institutional ethical guidelines. Paraffin-embedded samples were stained according to Bielschowsky's silver staining. Slices were deparaffinized and incubated in 10% silver nitrate solution for 15 min. After washing, samples were incubated for 30 min at 30 °C in an ammonium silver solution and treated with a developing solution followed by 1-min incubation in 1% ammonia hydroxide to stop the silver reaction. After washing, slices were imaged immediately with a Zeiss Observer A1 (Carl Zeiss AG) and with AFM as described above.

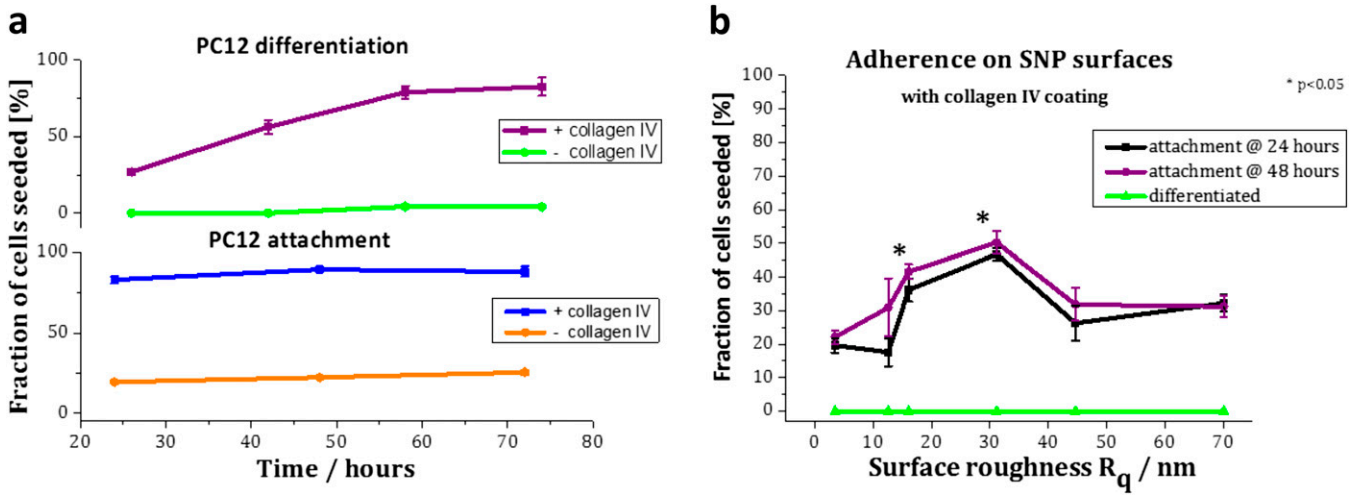

Fig. S1. Dependence of cell attachment and differentiation on collagen IV coating (on smooth glass substrates) (*A*). PC-12 cell attachment and differentiation on noncoated glass or SNP surfaces (*B*).

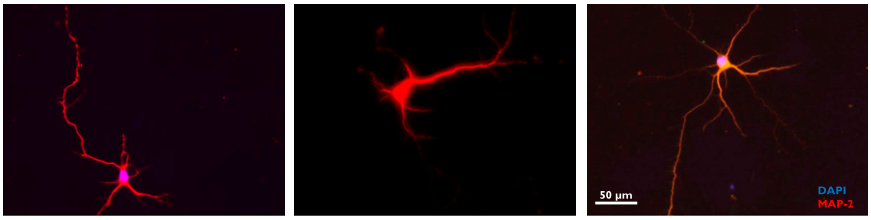

Fig. S2. Representative pictures of hippocampal neurons on surfaces with R_q of 32 nm showing an increased cell polarization with prominent, axon-like processes extending from the cell body.

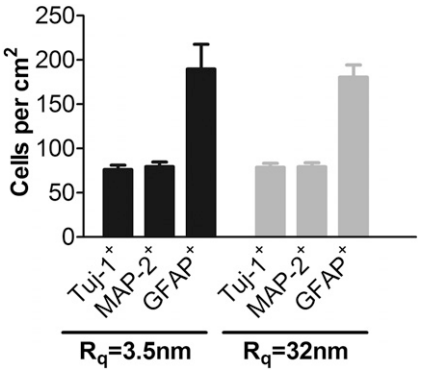

Fig. S3. Astrocyte/neuron density on surfaces with various R_qs. Astrocytes were visualized using GFAP and neurons were double-labeled for MAP-2 and Tuj-1.

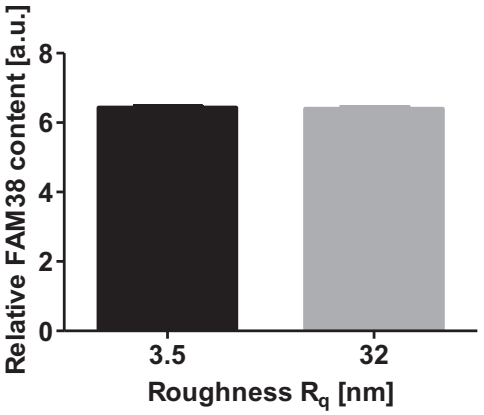

Fig. S4. Expression of FAM38A in differentiated PC-12 cells grown on smooth glass substrates (R_q = 3.5 nm) or R_q = 32 nm.

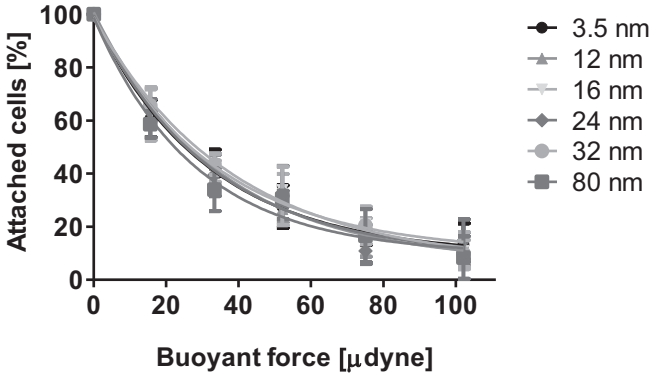

Fig. S5. Attachment force of PC-12 cells in dependence of the substrate they were grown on and on the buoyant force applied to the cells.

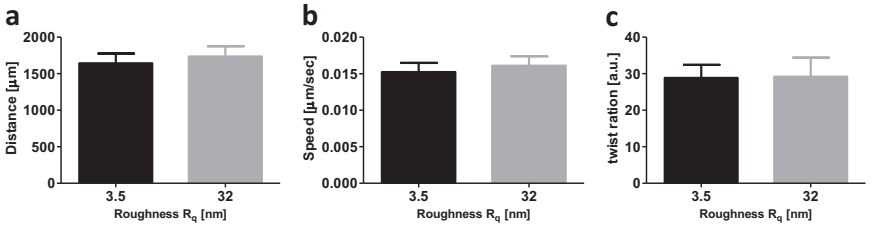

Fig. S6. PC-12 cell motility during differentiation. Distance of PC-12 cells traveled during differentiation (*A*), speed of cell movement during differentiation (*B*), and the twist ratio of the cells (*C*).

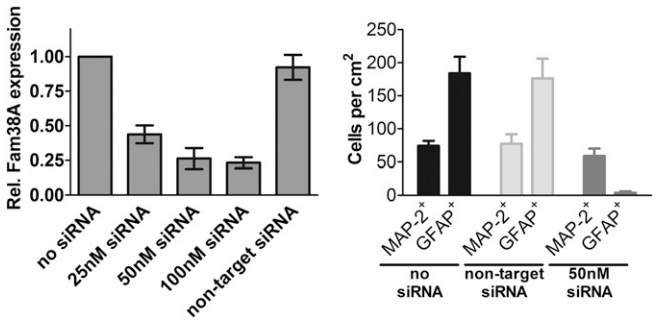

Fig. S7. siRNA knockdown of FAM38 in astrocyte/hippocampal neuron cocultures.

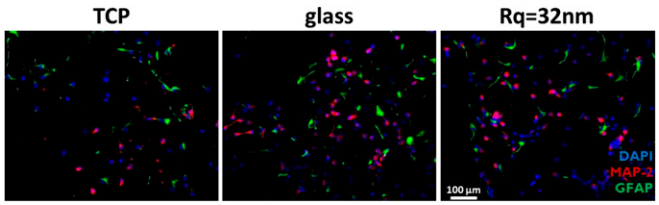

Fig. S8. Effects of BMP4 and Wnt3 on the differentiation of cortical neural stem cells. No differences in MAP-2 or GFAP expression could be observed indicating that this strong growth factor mixture overrides the topographical cues.

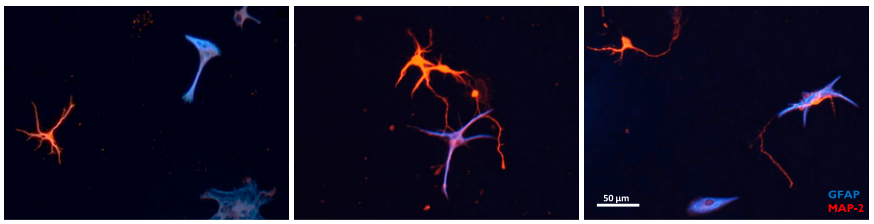

Fig. S9. Representative pictures of astrocytes showing a highly elongated, migratory phenotype (red, MAP-2; bBlue, GFAP) on surfaces with R_q of 32 nm.

Table S1. Surface characterization of SNP modified substrates

SNP particle size, nm	R_q, nm	Surface kurtosis
60	12	3.67
100	16	4.33
160	24	3.39
200	32	2.72
250	80	2.57