Supplementary Data (ESI)

2.2.2.1 Appendix to Alamar Blue, Fluorescein diacetate Viability Assay and Confocal Microscopy

AB reagent was added directly to the culture medium at 10% volume of medium contained in each sample. After 3 h of incubation, 100 µl of AB containing medium were transferred in a 96 multi well plate for fluorescence measurement. We included a negative control (CTRL-) (only medium with 10% AB and without cells) and positive control (CTRL+) of 100% reduced AB reagent without cells. Given that AB reagent does not alter cellular viability, viability time course was evaluated on the same culture of astrocytes for each experimental trial.[16] Accordingly, it was calculated the proliferation rate analysis measured at different time points (3 DIV, 8 DIV). Data are plotted as the averaged percentages of reduced AB ± Standard Error (SE), normalized with respect to the data collected at 1 DIV; it confirmed an increase in the cell growth after 8 DIV both in random and aligned samples, however the proliferation rate is higher for cells seeded on aligned samples embedded with PnNs.

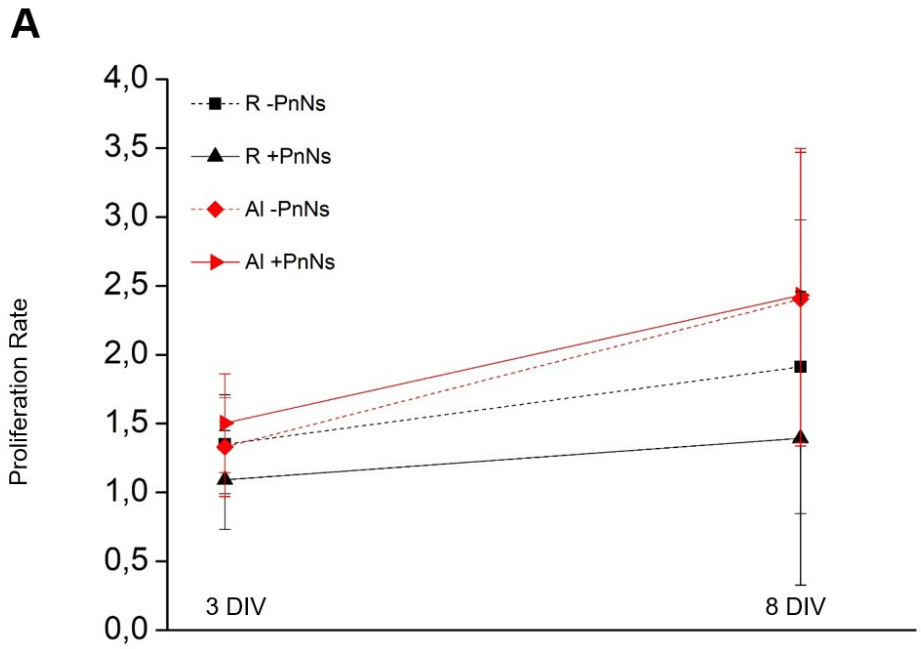

Figure S1 A) Proliferation rate of astrocytes seeded on random -PnNs (R –PnNs, dashed black line) and random +PnNs (R +PnNs, black line), aligned -PnNs (Al –PnNs, dashed red line) and aligned +PnNs (Al +PnNs, red line) substrates measured at different time points (3 DIV, 8 DIV). Data are plotted as the averaged percentages of reduced AB ± Standard Error (SE), normalized with respect to the data collected at 1 DIV; Student's t-test, pval> 0.05 for all the condition tested.

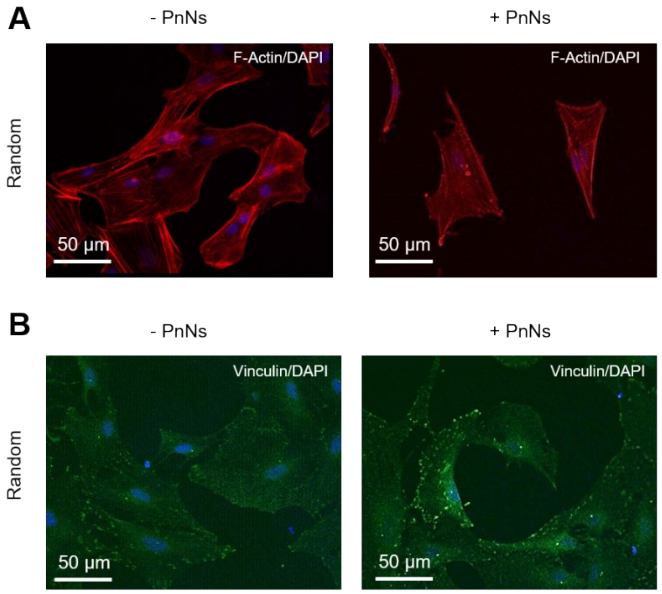

Figure S2: (A) Confocal images of Phalloidin-TRITC staining of the cytoskeleton (red) and with DAPI (blue) of astrocytes plated on random -PnNs, + PnNs substrates, collected after 3 DIV from the re-plating. (B) Confocal images of astrocytes plated on random -PnNs, +PnNs, substrates immune stained for Vinculin (green) and with DAPI (blue), collected after 3 DIV from the re-plating.

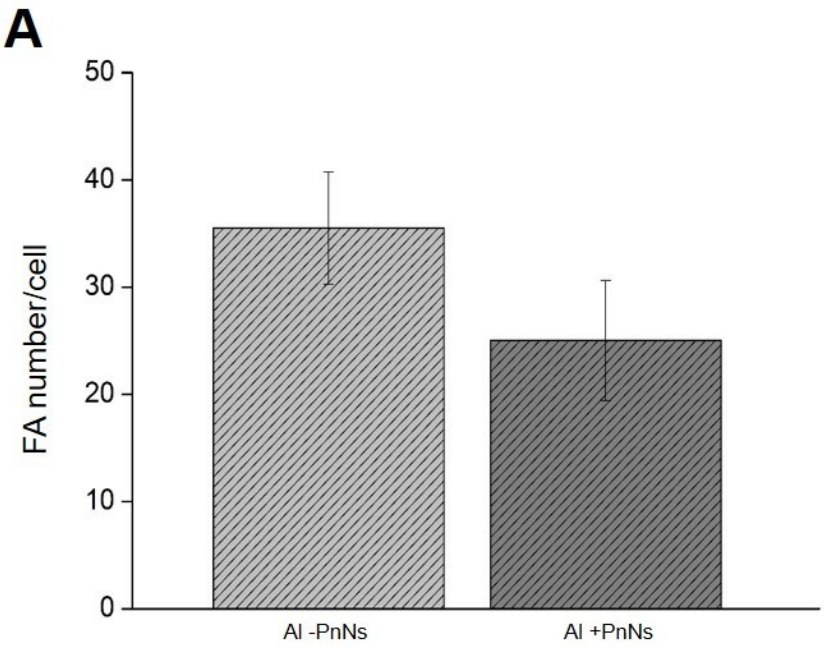

Figure S3: A) Bar plot shows the number of vinculin contacts per cell, counted after 3 DIV from the cells re-plating in cells plated on aligned -PnNs and +PnNs substrates. Data are expressed as means. S.E. (Al -PnNs n=138; Al +PnNs n=90). Student's t-test, * =pval<0.05, ** =pval<0.01, ***=pval<0.001.

2.2.3.1 Appendix to Electrophisiology and functional properties

Membrane currents were amplified, filtered at 2 kHz and acquired at a sample rate of 5 kHz by Axo patch 200B amplifier in voltage-clamp mode. Responses were amplified, low-pass filtered at 1 kHz, digitised at 20 kHz, stored and analysed with p CLAMP 10. Experiments were carried out at room temperature (22–24°C). Current amplitude was recorded, and values of the resting membrane potential (Vmem), input resistance (IR), specific conductance (SG) and capacitance (Cp) were calculated as described previously.[17] Patch pipettes were prepared from thin walled borosilicate capillaries (Harvard Apparatus) to have a tip resistance of 2-4 MΩ when filled with the standard internal solution.

	Cp (pF)	V_{mem} (mV)	SG (ns/pF)	IR (MΩ)	I (pA/pF) -120mV	I (pA/pF) +60mV
-PANi	64.9 ± 10.1	-49.7 ± 7.1	0.04 ± 0.01	812.3 ± 293.3	-4.4 ± 0.7	45.6 ±4.5
+PANi	38.0 ± 2.5*	-41.0 ± 3.3	0.05 ± 0.01	838.9 ± 137.9	-4.1 ± 0.5	48.9 ± 4.1

Table S1 Electrophysiological properties of astrocytes plated on aligned -PANi and +PANi substrates. Cp, membrane capacitance; Vmem, resting membrane potential; SG, mean specific conductance; IR, input resistance; I, current density. n=12 for -PANi and n= 16 for +PANi (ANOVA test, pval>0.05).

2.2.3.2 Solutions and chemicals

All salts and chemicals employed for the investigations were of the highest purity grade (Sigma). For electrophysiological experiments, the standard bath saline was (mM): 140 NaCl, 4 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 10 HEPES, 5 Glucose, pH 7.4 with NaOH and osmolarity adjusted to 315 mOsm with mannitol. The intracellular (pipette) solution was composed of (mM): 144 KCl, 2 $MgCl_2$, 5 EGTA, 10 HEPES, pH 7.2 with KOH and osmolarity 300 mOsm. When using external solutions with different ionic compositions, salts were replaced equimolarly.