Supplemental Materials:

F–V Mapping of the Stiffness: For each PDL spot pattern, in the same region of the preliminary topographic imaging, a set of force-volume (F–V) data consisting of 50x50 individual force-distance curves (F-d) was acquired with the same cantilever probe. The approach and retract F–d cycle was driven at a frequency of 1 Hz and consisted of 2,048 data points. The 50x50 pixels sampling of the spatial area investigated in the F-V data set provides a reasonable trade-off between mapped area resolution and acquisition time (ca. 42 min). To avoid detrimental effects of thermal drift, the measurement was only started after that the drift rate of the cantilever deflection signal had decreased to a level below 0.05 V/min. The AFM software program provides automatic extraction of several quantities of interest from the F–V dataset, which allows for their direct mapping on the whole scanned area. In particular, the contact stiffness K could be mapped, that is, the slope of the F–d curve at the maximum load F_{max}, \( K=\frac{dF}{d\delta}|_{(\delta_{max},F_{max})} \), along the retract portion. In fact, we let our software calculate the differential slope \( \Delta F/\Delta \delta \) between the points of 80% and 95% of F_{max}.

Results:

To evaluate this difference, the contact stiffness K was mapped both on the PDL spots (K_{PDL}) and on the surrounding agarose (K_{agar}) for the case of thin agarose, and a stiffening coefficient S=K_{PDL}/K_{agar} was then determined as a function of the adhesion protein concentration (Fig.S1). The error bars in Fig.S1 (± 1 standard deviation) come from averaging over several measurements (n=4), and are relatively high (between 20 and 50%). However, the means show that for PDL of 0.1 mg/ml the stiffening is very close to the detection threshold; for the intermediate concentration of 0.3 mg/ml S increases rapidly - with almost doubled value - and it reaches a plateau for the highest concentrations (0.5 and 0.7 mg/ml). A sigmoid is plotted in Fig.S1 as a guide to the eye.
Supplementary Figure Legends

**Figure S1** Stiffness variation with PDL concentration. Stiffening coefficient $S=K_{PDL}/K_{agar}$ as a function of PDL concentration. For 0.1 mg/ml, the stiffening effect is very close to the detection limit; for the other tested concentrations (0.3, 0.5 and 0.7 mg/ml) $S$ is almost twofold and it reaches a plateau at the highest PDL concentrations. A dotted sigmoid in the figure is plotted as a guide to the eye.

**Figure S2** Characterization PDL spots on thick agarose layers. AFM characterization of the thick agarose layer with PDL spots. The topography panel (a) shows the presence of 500 nm deep wells at the site of PDL spots, as clearly indicated by the vertical cross section. In (b) the corresponding F/V mapping of the same area. The EFM characterization in air and the corresponding topography are shown in the bottom panel (d) and (c), respectively. Remarkably, in air the PDL spots have a reverse topography with respect to the topography measured in liquid, after swelling of the agarose.

**Fig. S3** Thick agarose layer impairs neural growth. Fluorescence microscopy image of neurons subject to immunostaining for neural specific marker beta-III tubulin (green), and counterstained for nuclear DNA with DAPI (blue), seeded on a PDL array printed onto a thick (200 nm in air) agarose coating. The contrast has been enhanced to evidence autofluorescence of PDL spots in the background. The array consisted in 10 x 30 spots, spaced by 40 µm in vertical direction and with increasing spacing in horizontal direction (ranging from 40 to 120 µm). The cross indicates the position of the first line and column of spots. Neurons adhered on some spots, but they did not survive and no neural network was formed at 14 DIV.

**Figure S4** Example of neural guidance: Fluorescence microscopy image of neurons subject to immunostaining for neural specific marker beta-III tubulin (green), and counterstained for nuclear DNA with DAPI (blue), Series of four adjacent Y-shaped arrays of discrete PDL spot patterns under neuronal culture condition, demonstrating selective neuronal growth only in the downward arm, with PDL spots separated by less than 50 µm, (inter-spot distance along the upward arm: 80 µm). Scheme of the Y-shaped pattern is illustrated on the array in the left

Supplementary Movie Legends

**M1** Time lapse imaging (0.33 frame/minute) of WT hippocampal neurons seeded on arrays made of PDL spots with lines with different pitches (i.e., 30-40-50-70 µm). The density of the neurons is 140 cells/mm². It shows in the first 22 hours after seeding (time length of the video) connections between neurons adhering on spots at 30 and 40 µm begin to form.