Electronic Supplementary Material (ESI) for Soft Matter.

Supporting Information

Neuron dynamics on directional surfaces

Joao Marcos Vensi Basso[1], Ilya Yurchenko[1], Matthew R. Wiens[1], Cristian Staii[1,*]

1. Department of Physics and Astronomy, Center for Nanoscopic Physics, Tufts University, Medford, Massachusetts 02155, *USA*

Fig S1

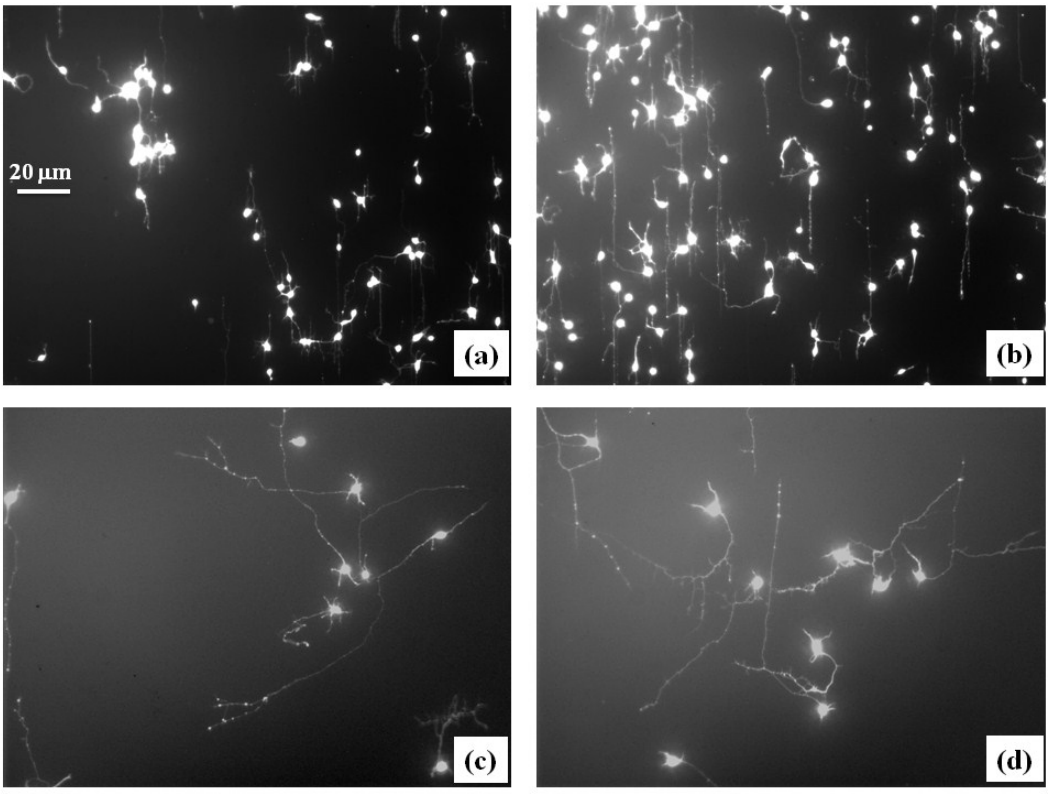

Fig S1 Additional examples of cultured cortical neurons on PDL coated PDMS surfaces with periodic micro-patterns. (a-b): Images for untreated neuronal cells cultured on surfaces with pattern spatial period $d = 1$ μm in (a) and $d = 5$ μm in (b). (c-d) Images for neurons treated with Blebbistatin cultured on surfaces with pattern spatial period $d = 1$ μm in (c) and $d = 5$ μm in (d). All images are captured 48 hrs after neuron plating.

Fig S2

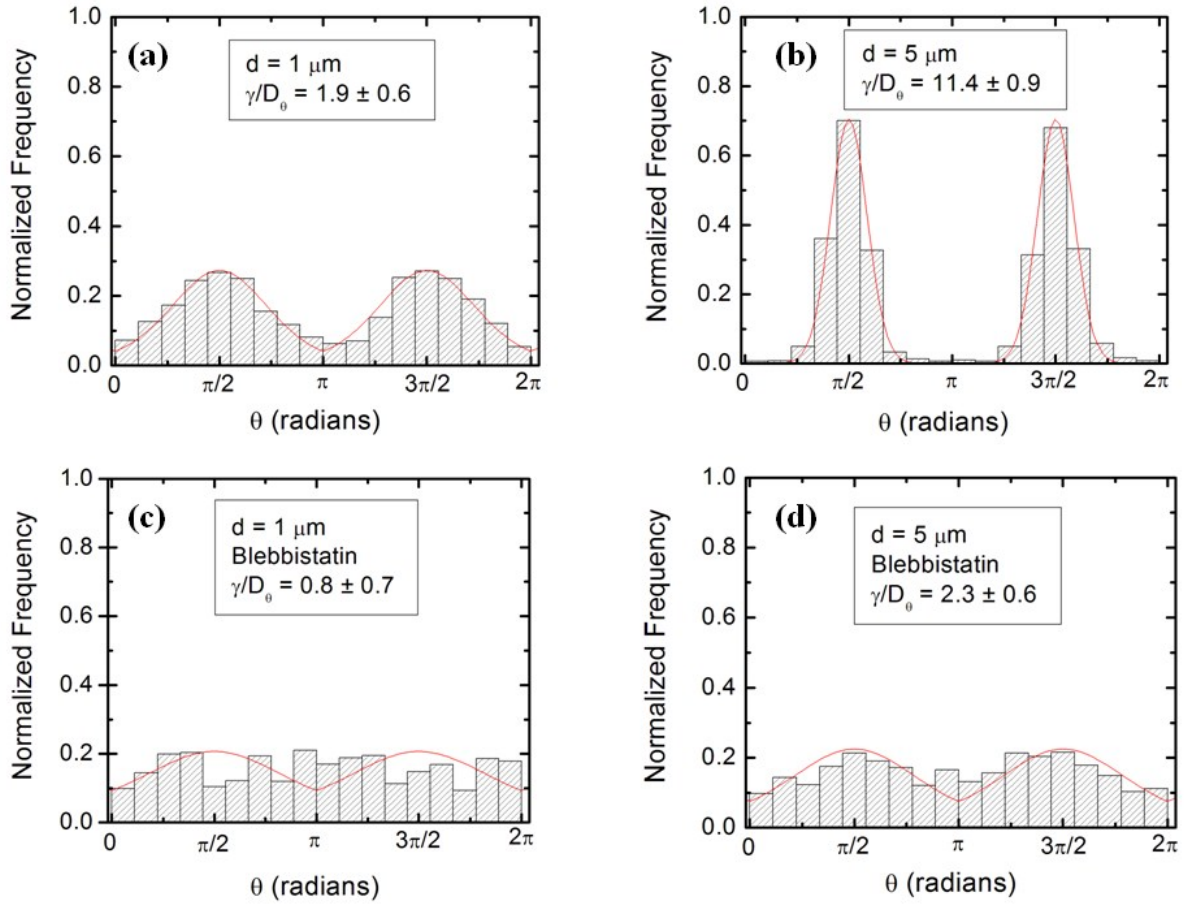

Fig S2 Additional examples of normalized experimental angular distributions for axonal growth. Each type of surface is characterized by the pattern spatial period *d***.** The vertical axis (labeled Normalized Frequency) represents the ratio between the number of axonal segments growing in a given direction and the total number N of axon segments measured on a given type of surface. Each axonal segment is of 20 μm in length (see Data Analysis section). (*a*) Data for N = 1089 different axon segments measured on substrates with d = 1 μm. (*b*) Data for N = 1781 different axon segments measured on substrates with d = 5 μm. (c) Data for N = 1210 different axon segments measured for neurons treated with Blebbistatin on substrates with d = 1 μm. Data for N = 1602 different axon segments measured for neurons treated with Blebbistatin on substrates with d = 5 μm The continuous red curves in each figure represent fit to the data points using Eqn. (6). The fit gives the ratio γ_θ / D_θ between the deterministic torque and the diffusion coefficient for the angular motion, for each type of surface (see text).

Fig S3

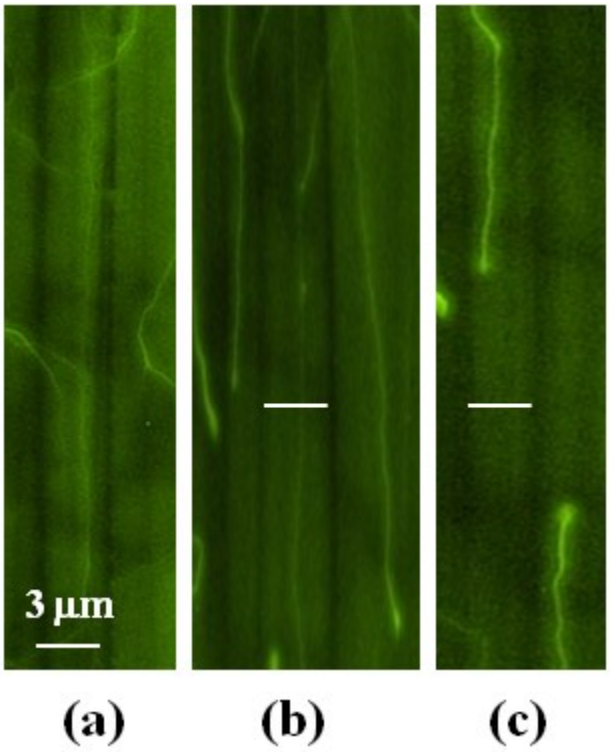

Fig S3. Examples of fluorescence images showing the position of axons with respect to the patterns. The images have been taken using the high magnification objective (60x) of the Nikon Eclipse Ti microscope, at different locations on 2 different substrates [33]. The images show the fluorescently labeled microtubules (green), i.e. the C domain (see reference [1] and reference [33]) inside the axons. The microtubules are labeled using Tubulin Tracker Green (see main text). The position of the micro-patterned troughs is shown by the vertical black lines. The 3µm white scale bar shows the distance between two adjacent troughs, and it has the same size for all images. The images show that the axons are located on the ridges of the patterns. The position of the ridges and troughs has been verified using AFM (images similar to the one shown in Fig. 2).

Fig S4

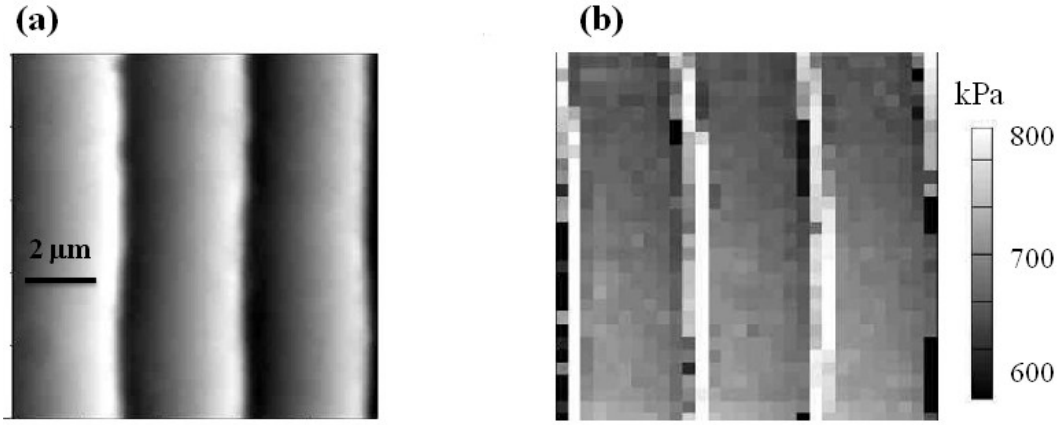

Fig S4 Examples of (a) AFM topographic image, and (b) AFM force map image measured on a micro-patterned PDMS surface coated with PDL. Each pixel corresponds to a value of the elastic modulus. The maps for the elastic modulus are measured following the procedure presented in detail in reference 6 and reference 21. The scale bar shown in (a) is the same for both images.

Fig S5

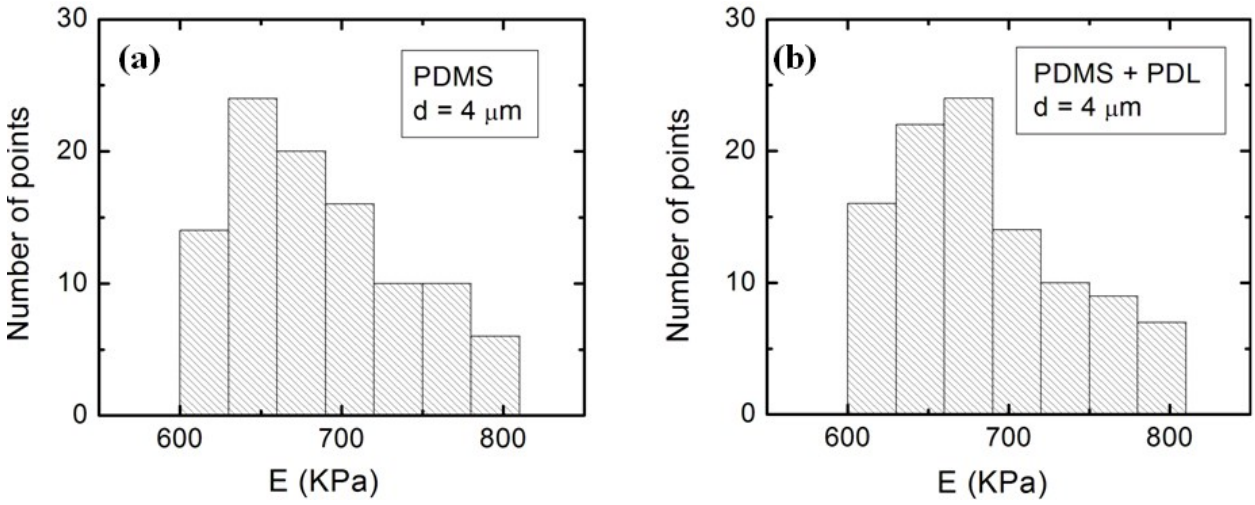

Fig S5 Examples of histograms for the substrate elastic modulus *E* measured from AFM force maps shown in Fig. S3. (a) Histogram for elastic modulus for a PDMS surface with d = 4 μm, measured before coating the surface with PDL. (b) Histogram for elastic modulus for the same PDMS surface shown in (a), measured after coating the surface with PDL. The two maps display similar ranges for *E*. The average elastic modulus between the two maps differs by only 5%. The data demonstrates that PDL coating does not change the elastic modulus of the PDMS substrate.

Fig S6

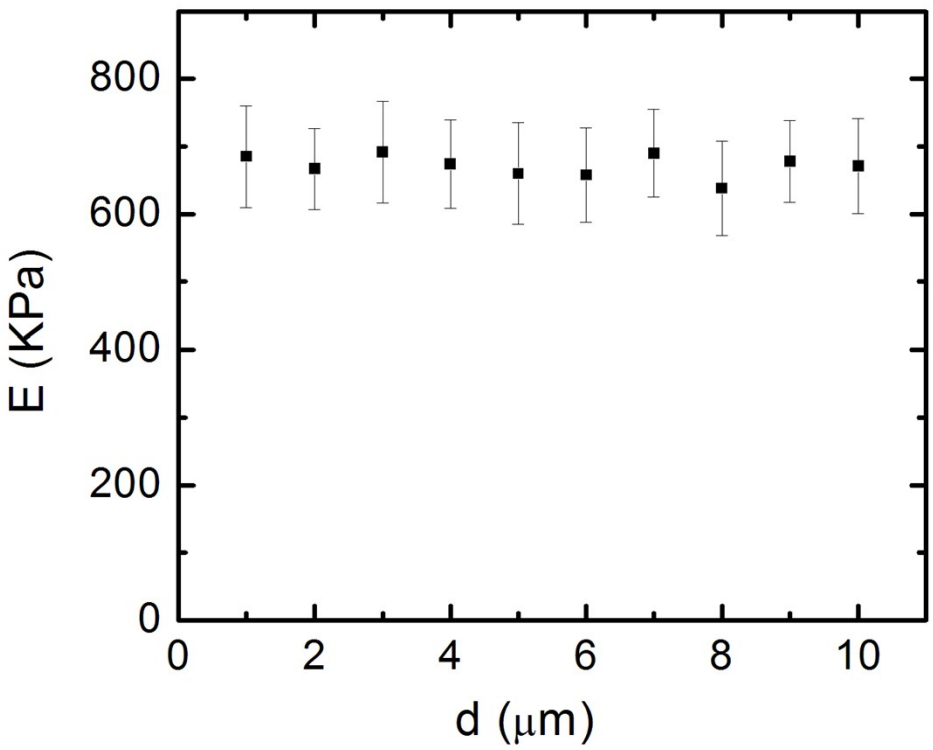

Fig S6 Average value of the elastic modulus measured on micro-patterned PDMS surfaces with spatial periods considered in this paper (*d* in the range 1 – 10 μm). The average value for the elastic modulus is obtained from elasticity maps similar to the ones shown in Fig. S3 and Fig. S4. The measurements are performed after the surface is coated with PDL. The error bars indicate the standard error of the mean. The data demonstrates that the elastic modulus does not vary significantly among the PDMS surfaces with different spatial periods *d*. The variation of the average elastic modulus among these substrates is less than 10%.