

Supporting Information.

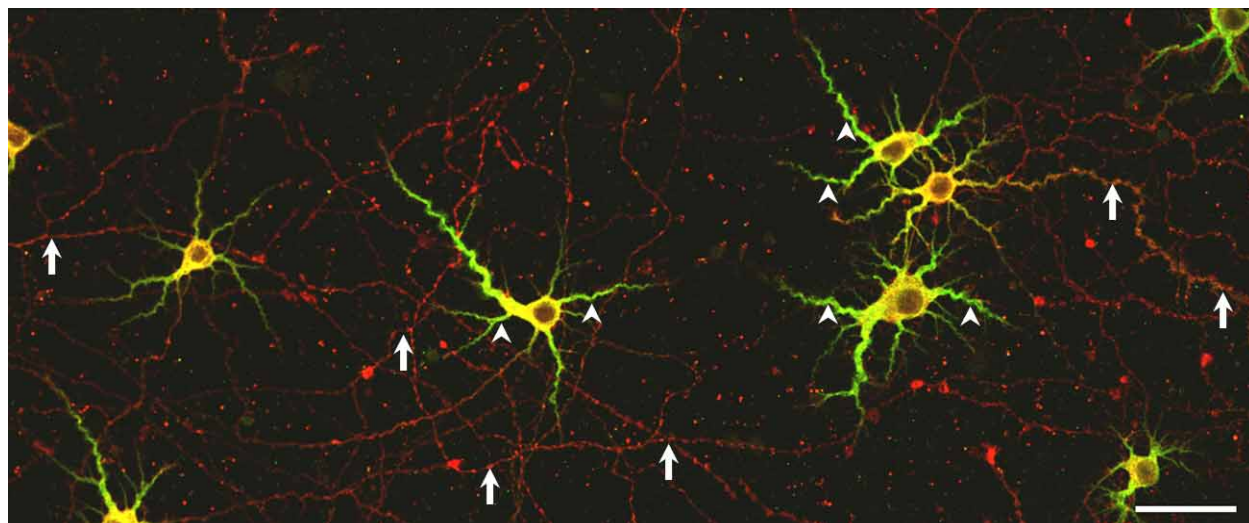


Fig. S1 Random meshwork of axons form on uniform, un-patterned substrates. Cultured postnatal hippocampal neurons quickly develop long branching processes that that can be distinguished as axons or dendrites based on the differential expression of cytoskeletal proteins, (*e.g.*, tau (an axonal marker) and microtubule-associated protein 2 (MAP2, a dendritic marker)). Freshly dissociated cells were plated at 100 cells mm⁻² and cultured for 7 DIV on PDL-coated coverslips. Following paraformaldehyde fixation, cultures were labeled with neuronal markers to distinguish axons (arrows), using antibodies against tau (Red; 1° antibody = sheep-anti tau (Pierce Endogen); 2° antibody = donkey anti-sheep Alexa 633 (Invitrogen)), and dendrites (arrowheads), using antibodies against MAP2 (Green; 1° antibody = rabbit anti-MAP2 (Chemicon); 2° antibody = donkey anti-rabbit Alexa 488 (Invitrogen)). Axons *in vitro* can easily reach millimeters in length. By 7-14 days, the long branching processes intermingle with numerous intersections from randomly migrating and branching axons (red), which makes neurite tracing difficult. To distinguish the individual response of neurons growing on patterned substrates, we collected our samples at 4 days *in vitro* (DIV), which is after axon differentiation and before axons undergo extensive development and branching. Scale bar = 50 μm.

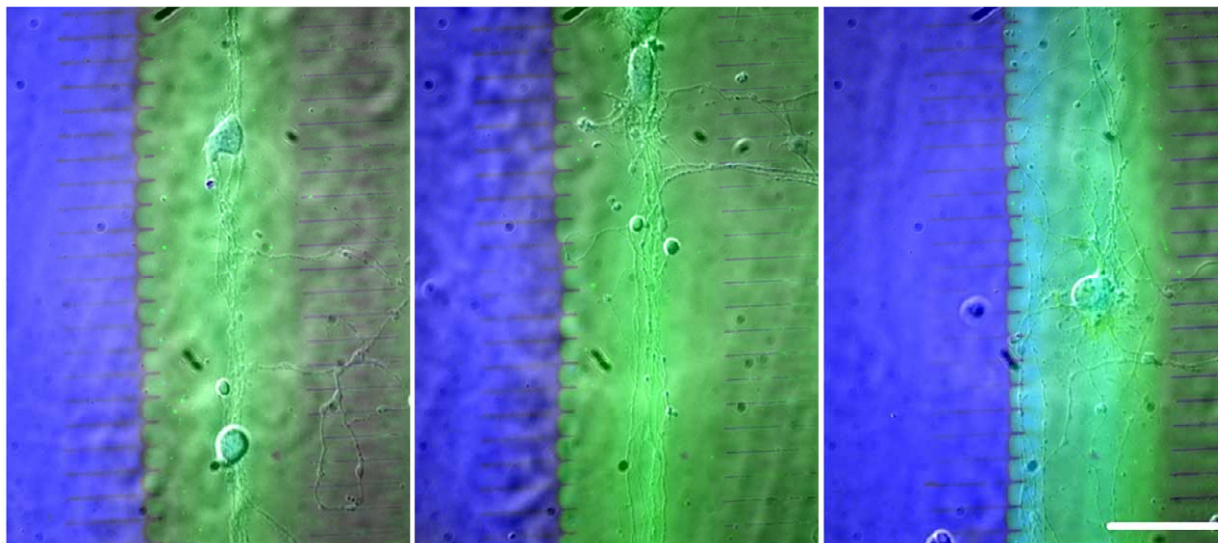


Fig. S2 Primary data images of neurons cultured on substrate gradients of LN and FITC-PLL. Neurons were cultured on fluorescently labeled substrate gradients and analyzed as described for Figure 6c-d and in the Methods section. These images display a range of neurite confinements from relatively few neurite bundles following a narrow center gradient (Left), to an individual neuron with more axonal bundles covering wider span (Middle), to a neuron with numerous processes spread over a wider area (Right). The smaller dendritic processes appear to emanate in all directions from the cell body. Scale bar = 50 μm .