## **Supporting information**

## **Biofunctionalized 3D Nanopillar Arrays Fostering Cell Guidance and Promoting Synapse Stability and Neuronal Activity in Networks**

Hayder Amin<sup>1</sup>\*, Michele Dipalo<sup>2</sup>, Francesco De Angelis<sup>2</sup> and Luca Berdondini<sup>1</sup>

\*Correspondence to hayder.amin@iit.it

<sup>1</sup>Nets<sup>3</sup> Laboratory, Department of Neuroscience and Brain Technologies (NBT), and <sup>2</sup>Department of Plasmon Nanotechnologies, Fondazione Istituto Italiano di Tecnologia (IIT), Via Morego 30, 16163 Genoa, Italy.



Figure S1. Fluorescence images displaying neuronal morphological arrangement on planar Au surface pre-coated with biochemical adhesion molecules. (A) Hippocampal neurons grown on planar Au coated with PLO and stained with MAP-2. The Intensity profile of neuronal MAP-2 fluorescence signal detected in neurons shows a random morphological arrangement. (B) As in (A) but for random neuronal morphological arrangement and intensity profile of neurons grown on PLL/planar Au. (C) As in (A,B) but for random neuronal morphological arrangement and intensity profile of neurons grown on PDL/planar Au. Scale bars represent 50  $\mu$ m for all images on the *left* and (40  $\mu$ m x 40  $\mu$ m) for the intensity profiles on the *right*.



Figure S2. Quantification of combinatorial effects of nanotopographical and biochemical cues on synaptic maturation and stability. (A) Confocal micrographs displaying neuronal PSD-95 expression on planar surfaces of glass and Au functionalized with PDLO and PDL. All scale bars represent 30  $\mu$ m in images on the *left* and 10  $\mu$ m in images on the *right*. (B) Quantification of PSD-95 protein expression is performed by processing the images with the granulometric filter method. Magenta and green cross-sections indicate the fluorescence and the corresponding filtered intensities, respectively. The starting and the ending positions of the cross sections are read from the *left* (0  $\mu$ m) to the *right* (40  $\mu$ m). The position of the PSD-95 puncta was defined above an arbitrary offset on the filtered fluorescence scale, that is, 30 indicated by black dotted lines.



**Figure S3. Quantification of combinatorial effects of nanotopographical and biochemical cues on cellular activity.** Confocal micrographs showing the c-fos and MAP-2 immunofluorescence of neurons grown on planar glass and Au surface functionalized with PDLO and PDL. Scale bars represent 30 µm.