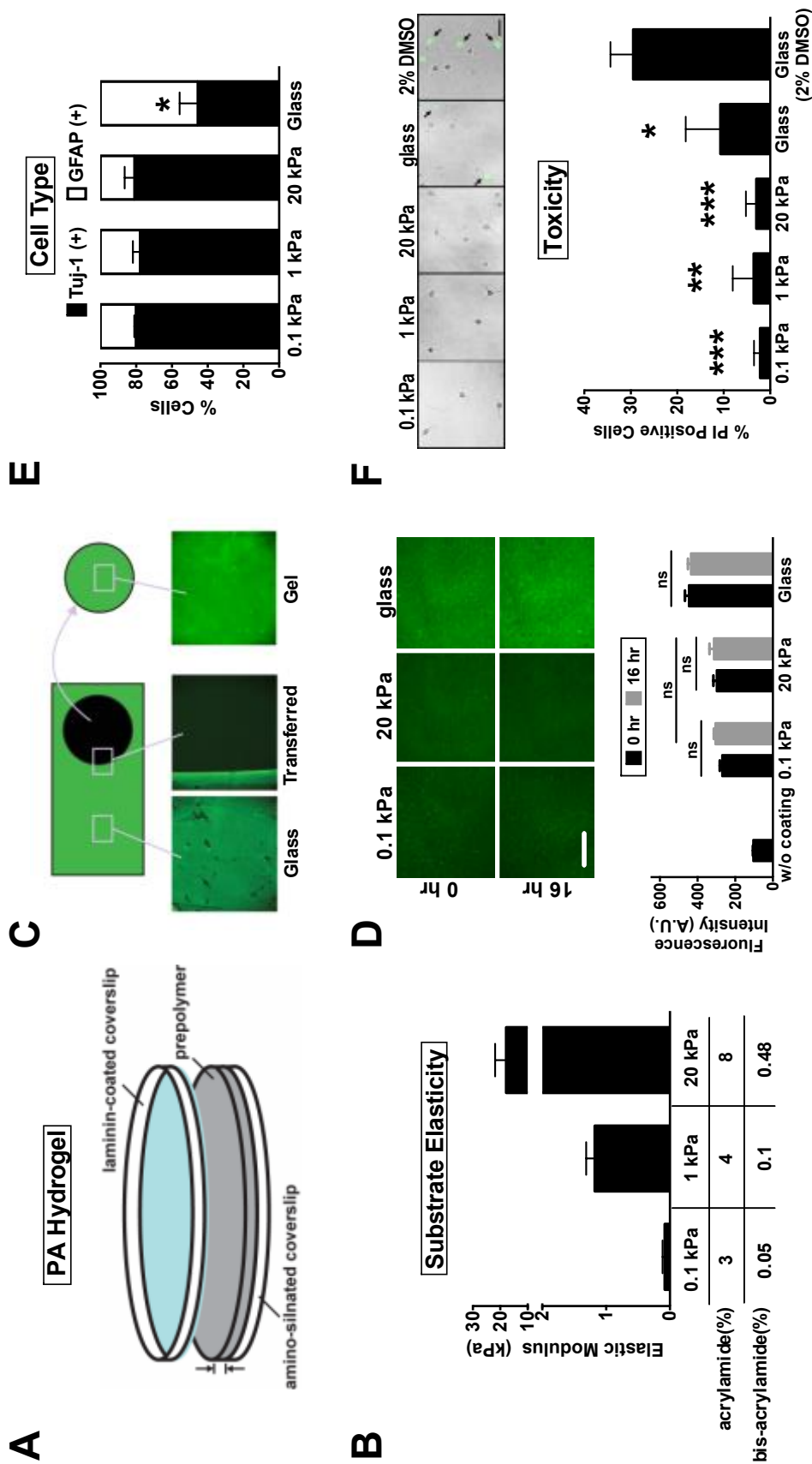


Figure 1-figure supplement 1



## **Figure 1-figure supplement 1**

### **Hippocampal neurons cultured on polyacrylamide hydrogels of varying stiffness**

**A.** Schematic of the three-layer assembly of polyacrylamide (PA) hydrogels (diameter=1.8 mm, thickness=200  $\mu$ m). Light blue, the poly-L-Lysine/laminin-coated side of the coverslip.

**B.** Atomic force microscopy measurement of elastic modulus ( $\pm$  SEM,  $n=3$ ) of PA gels. Table shows the ratio of acrylamide and bis-acrylamide used to determine the crosslink density and the stiffness (0.1 kPa, 1 kPa, and 20 kPa) of the polymers.

**C, D.** Transfer printing of laminin on hydrogels. (C) Representative images of PA hydrogel peeled from laminin-coated coverslips, both immunostained with antibodies against laminin (shown in green). Note that laminin was completely transferred from the laminin-coated coverslip to the gel surface. (D) Quantification of laminin fluorescence intensity ( $\pm$  SEM,  $n>3$ ; “ns”, no significance; *t*-test) on the gel surface before and after incubation for 16 hr with culture medium. Acid-washed coverslips were pre-coated with laminin (a stock concentration of 1.62 mg/ml) in a 1:50 dilution. Note that the staining intensity of surface laminin was comparable between 0.1 kPa and 20 kPa gels.

**E.** Cell type enrichment analysis for hippocampal cultures. Cultured neurons plated on substrates were immunostained with antibodies against the neuron-specific marker Tuj-1 and the astroglial marker glial fibrillary acidic protein (GFAP). Histogram showing percentages ( $\pm$  SEM;  $n>200$  cells for each group from more than three independent experiments; “\*”,  $p < 0.05$ ; relative to that of 0.1 kPa culture, *t*-test) of Tuj-1- or GFAP-positive cells in hippocampal cultures at 3 days in vitro (DIV).

**F.** Cell viability analysis for hippocampal neurons cultured on PA gels and glass. DIV3 cultures were subjected to propidium iodide (PI) staining. Arrow, apoptotic cells with fragmented nuclei (PI positive). Dimethyl sulfoxide (2%) was used as a positive control of maximum cytotoxicity. Data represent mean  $\pm$ SEM ( $n>200$  cells for each group from more than three independent experiments; “\*”,  $p < 0.05$ ; “\*\*\*\*”,  $p<0.001$ ; relative to that of glass culture, *t*-test).