



## **Figure 1– figure supplement 2**

### **Differential lamellipodium phenotypes of neurons grown on hydrogels**

**A, B.** Phalloidin intensity profiling of neurons on soft ( $E=0.1$  kPa; **A**) and stiff ( $E=20$  kPa; **B**) hydrogels stained with phalloidin (Red) for F-actin filaments and antibodies against Tuj-1 (Green), as indicated. Lamellipodium occupancy (LO) of phalloidin-positive segments (“S”) along the cell periphery was calculated according to the formula shown in the lower panel. Cells exhibiting typical morphologies of the segmented lamellipodium (“SL”, A) or broad lamellipodium (“BL”, B) are shown.

**C, D.** Time-lapse bright-field images of newly plated neurons on 0.1 kPa (C) or 20 kPa (D) gels. Note that neurite initiation sites (arrowheads at time-point=15:10:00) are correlated with the positions of initial segmented lamellipodia (delimited by the dashed line in panel C; individual LO < 0.33). Broad lamellipodia of a neuron grown on a 20 kPa gel are delimited by solid lines in panel D. Scale bar = 20  $\mu\text{m}$ .

**E, F.** Distribution of individual lamellipodium occupancy (E) and total lamellipodium occupancy (F) from the same sets of experiments shown in main Figure 1A3. Simplified drawings above histograms illustrate the typical segmented lamellipodium and broad lamellipodium phenotypes of hippocampal neurons grown on soft ( $E = 0.1$  kPa or 1 kPa) and stiff ( $E = 20$  kPa) PA hydrogels.