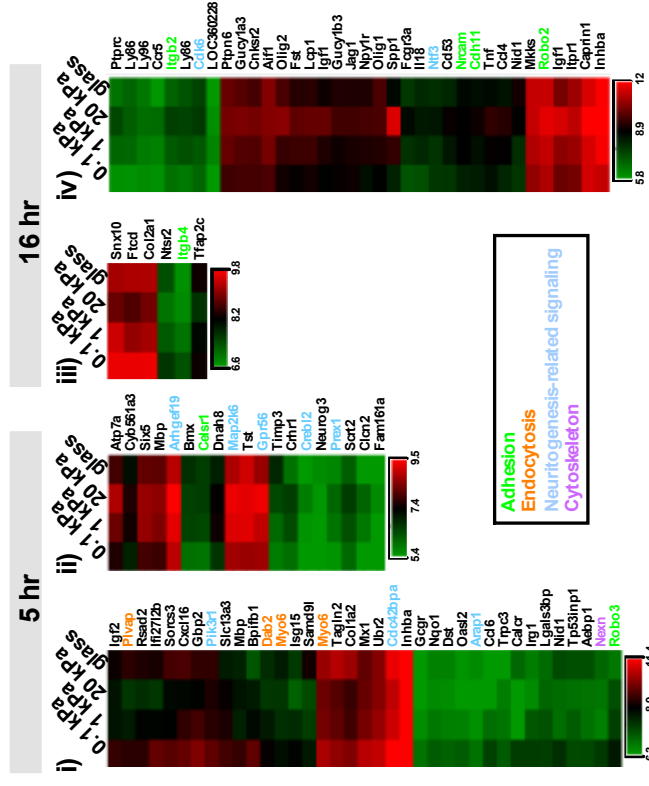
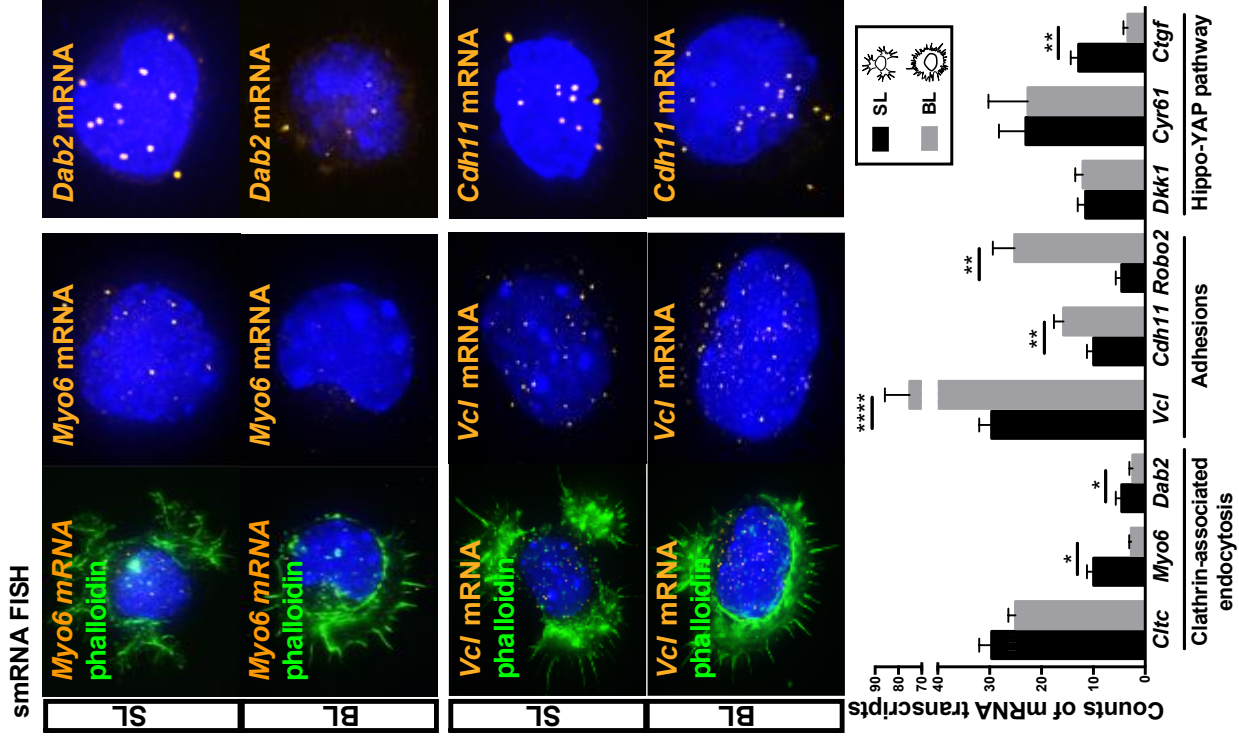


A



C



B

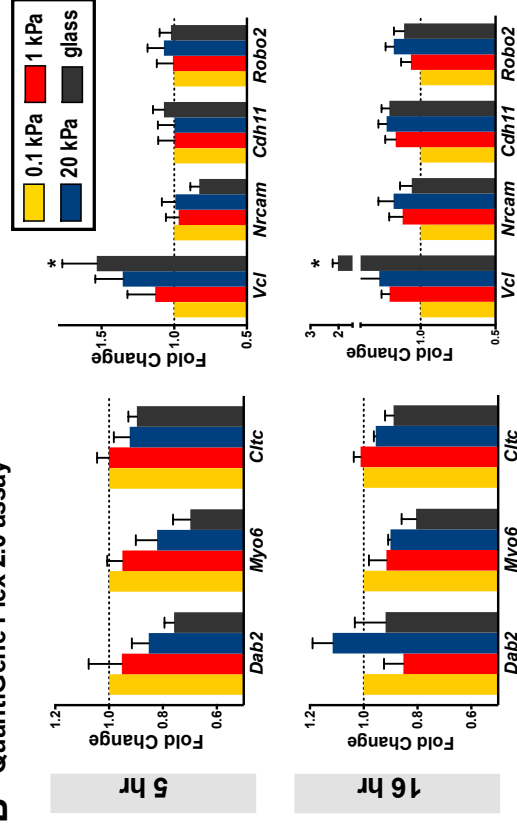


Figure 1– figure supplement 3

Differential gene expression pattern of neurons grown on hydrogels

A. Up-regulation of clathrin-associated endocytosis genes in 0.1 kPa cultures. Gene expression patterns emerging from microarray analysis were grouped into four modules: expression increased with substrate softness in 5 hr (i) or 16 hr (iii) cultures, or increased with substrate stiffness in 5 hr (ii) or 16 hr (iv) cultures (cut off: >1.5-fold change, $p < 0.05$, $n = 3$ independent experiments). Normalized expression levels are represented by a color-coded heatmap.

B. A QuantiGene Plex branched-chain DNA amplification assay validated the relatively increased levels of mRNAs encoding the clathrin-associated endocytosis factors Dab2, Myo6, and Cltc in neurons grown on 0.1 kPa gels. Data represent fold changes in RNA levels (\pm SEM; $n = 4$ -5 independent experiments; relative to that of 0.1 kPa culture; “*”, $p < 0.05$; t test).

C. Representative single-molecule RNA fluorescence in situ hybridization (smRNA FISH) images of mRNA encoding Myo6, Dab2, Chd11, Vcl, or Cry61 on segmented lamellipodium (SL) or broad lamellipodium (BL) neurons in 5 hr cultures, as indicated. Histograms showing average number (\pm SEM; $n = 42$ -52 cells; “*”, $p < 0.05$; “***”, $p < 0.01$; “*****”, $p < 0.0001$; t test) of smRNA FISH puncta for each gene per single neurons of the SL and BL cells.