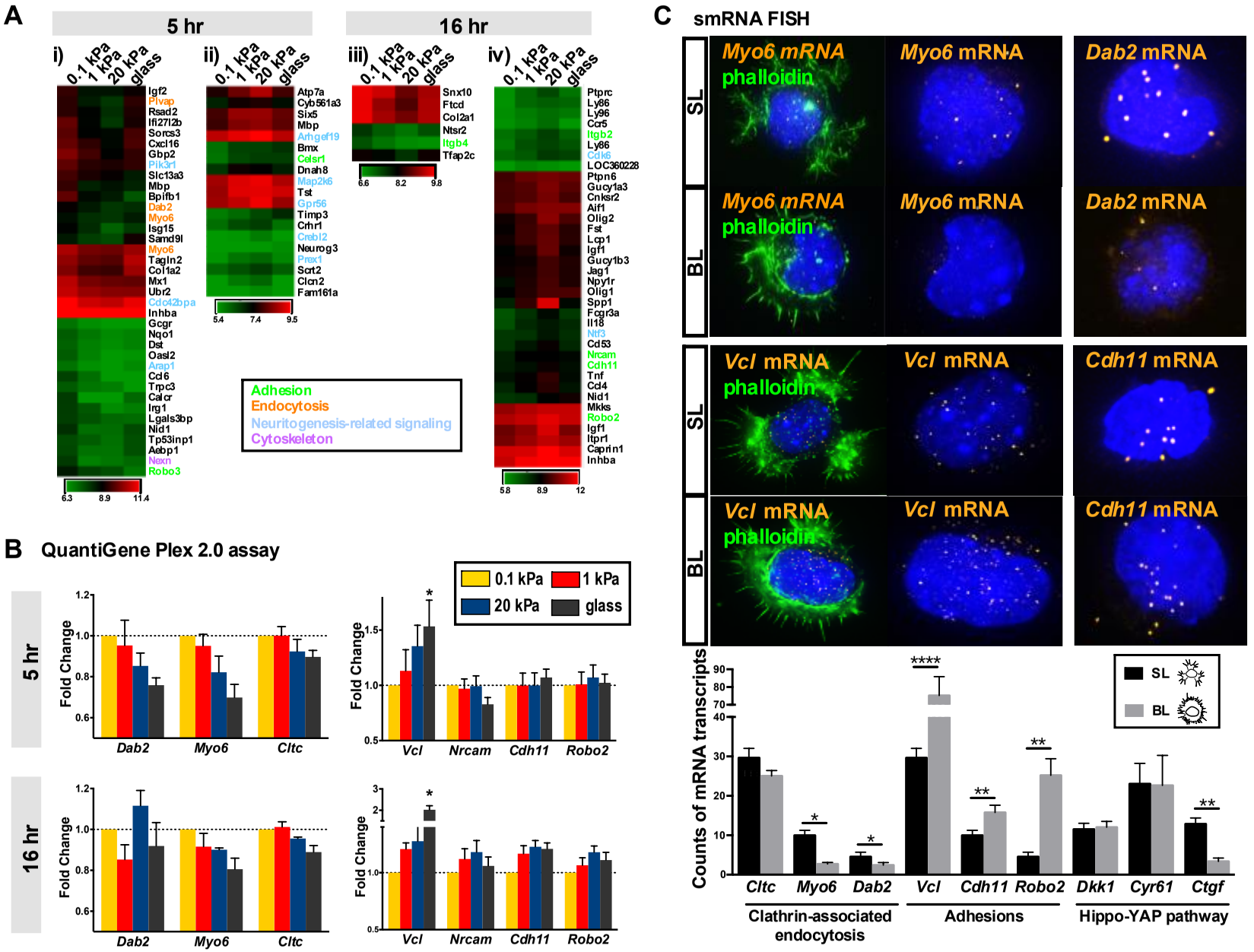
A
5 hr
16 hr
i)
ii)
iii)
iv)
0.1 kPa
1 kPa
20 kPa
glass
Igf2
Plvap
Rsad2
Ifi27l2b
Sorcs3
Cxcl16
Gbp2
Pik3r1
Slc13a3
Mbp
Bpifb1
Dab2
Myo6
Isg15
Samd9l
Myo6
Tagln2
Col1a2
Mx1
Ubr2
Cdc42bpa
Inhba
Gcgr
Nqo1
Dst
Oasl2
Arap1
Ccl6
Trpc3
Calcr
Irg1
Lgals3bp
Nid1
Tp53inp1
Aebp1
Nexn
Robo3
6.3 8.9 11.4
Atp7a
Cyb561a3
Six5
Mbp
Arhgef19
Bmx
Celsr1
Dnah8
Map2k6
Tst
Gpr56
Timp3
Crhr1
Crebl2
Neurog3
Prex1
Scrt2
Clcn2
Fam161a
5.4 7.4 9.5
Snx10
Ftcd
Col2a1
Ntsr2
Itgb4
Tfap2c
6.6 8.2 9.8
Ptprc
Ly86
Ly96
Ccr5
Itgb2
Ly86
Cdk6
LOC360228
Ptpn6
Gucy1a3
Cnksr2
Aif1
Olig2
Fst
Lcp1
Igf1
Gucy1b3
Jag1
Ngy1r
Olig1
Spp1
Fcgr3a
Il18
Ntf3
Cd53
Nrcam
Cdh11
Tnf
Ccl4
Nid1
Mkks
Robo2
Igf1
Itpr1
Caprin1
Inhba
5.8 8.9 12
Adhesion
Endocytosis
Neuritogenesis-related signaling
Cytoskeleton
B
QuantiGene Plex 2.0 assay
5 hr
16 hr
0.1 kPa
1 kPa
20 kPa
glass
Fold Change
Dab2
Myo6
Cltc
Vcl
Nrcam
Cdh11
Robo2
C
smRNA FISH
SL
BL
Myo6 mRNA
phalloidin
Myo6 mRNA
Dab2 mRNA
Vcl mRNA
phalloidin
Vcl mRNA
Cdh11 mRNA
Counts of mRNA transcripts
SL
BL
Cltc
Myo6
Dab2
Vcl
Cdh11
Robo2
Dkk1
Cyr61
Ctgf
Clathrin-associated endocytosis
Adhesions
Hippo-YAP pathway

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 (±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 (±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.