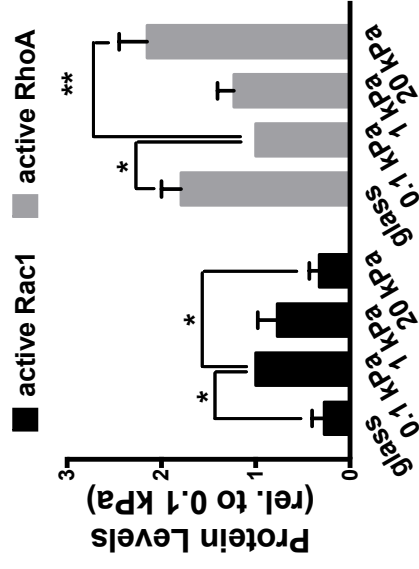
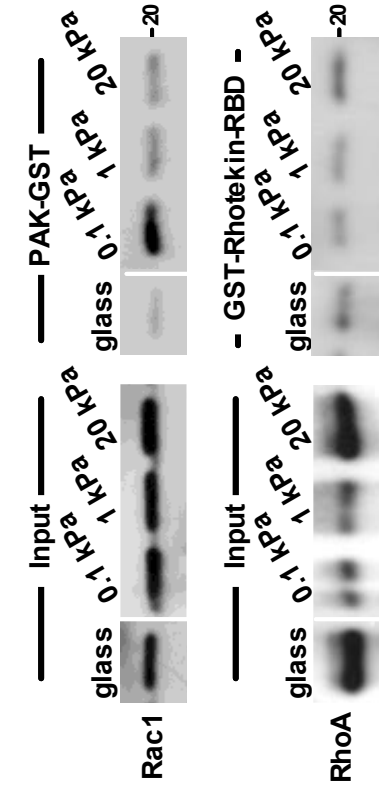


A



B

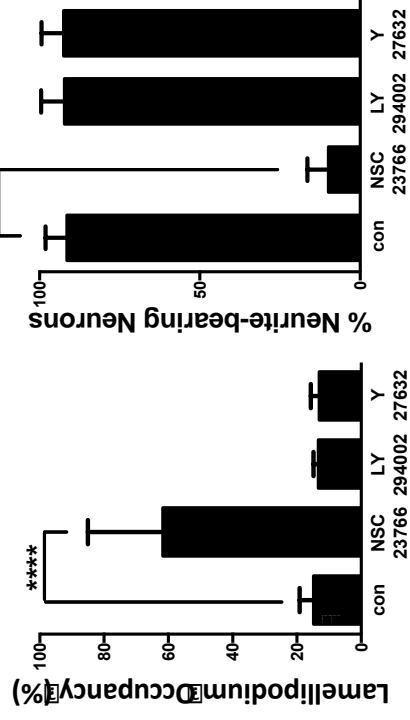
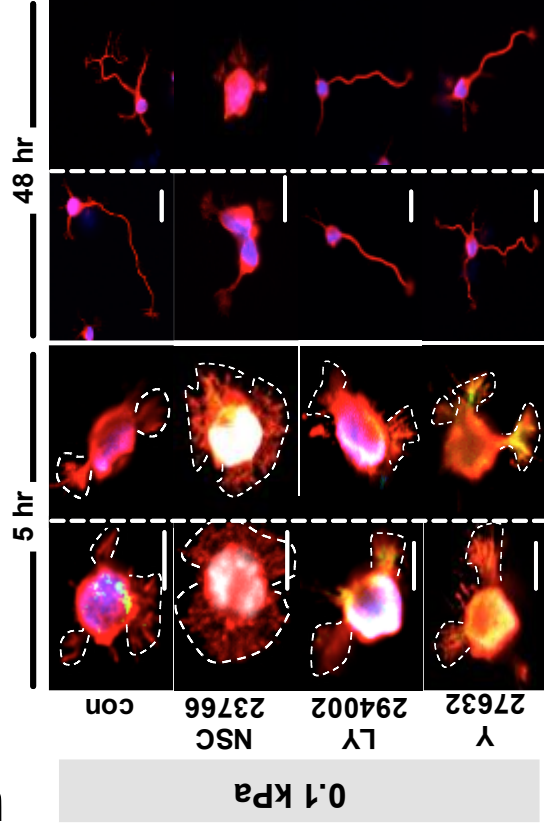


Figure 2– figure supplement 1

Increased Rac1 activity in neurons grown on 0.1 kPa gels

A. Active Rac1 and active RhoA pull-down assay of neurons grown on 0.1 kPa or 20 kPa hydrogels. Cell lysates obtained from cortical neurons grown on substrates (5 hr of culture) were subjected to a GST resin pull-down assay using PAK-GST (upper panel) or GST-Rhotekin-RBD (lower panel), as indicated. The precipitants were analyzed by Western blotting with antibodies specific against Rac1 or RhoA. Histograms reflect quantitative measurement of band intensities (\pm SEM, $n=3$; normalized to the corresponding protein input and relative to that of 0.1 kPa culture; “*”, $p<0.05$; “***”, $p<0.001$, t test) of the active GTP-bound forms of Rac1 or RhoA.

B. Pharmacological inhibition of Rac1 activation significantly increases the lamellipodium occupancy in 5 hr neuronal cultures on 0.1 kPa gels. Right panel shows representative images of neuronal cultures on 0.1 kPa gels, in the absence and presence of the selective Rac1-GEF inhibitor NSC-23766 (1 μ M; 1 hr treatment), the PI3K inhibitor LY-294002 (10 μ M; 1 hr treatment), or the ROCK inhibitor Y-27632 (25 μ M; 1 hr treatment), stained with phalloidin (red) at the 5 hr or 48 hr time-points after cell plating, as indicated. Dashed line delimits lamellipodium area. Bar, 20 μ m. Histograms summarize average lamellipodium occupancy in 5 hr neurons and percentages of neurite-bearing neurons in 16 hr cultures (\pm SEM; $n=300$ total cells for each group from three independent experiments; “*****”, $p<0.0001$, unpaired t test) from all experiments similar to that shown in the right panel.