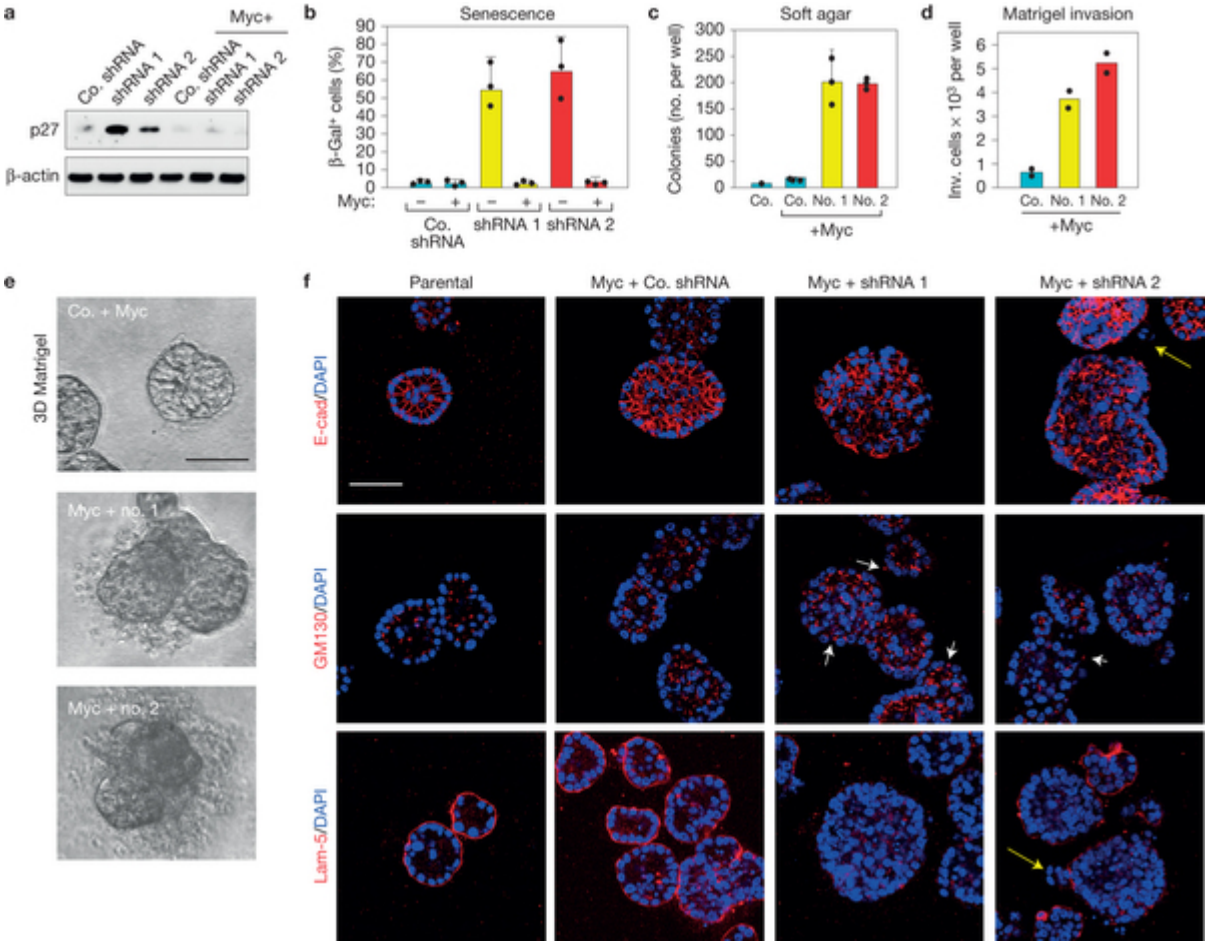
Figure 3: Inactivation of Rnd1 induces hyperproliferation and invasion in 3D Matrigel.



(a,b) MCF-10A cells were transduced or not with Myc, infected with either control shRNA or two shRNAs targeting Rnd1, cultured for 9 days and subjected to immunoblotting with antibodies against p27 and β-actin (a) or cultured for 4 additional days and subjected to SA-β-galactosidase staining (b). The graph shows the individual data points, their average, and s.d. from one experiment and *n* = 3 technical replicates. The experiment was repeated 2 times. See representative images in Supplementary Fig. 3e. (c) MCF-10A cells were transduced or not with Myc, infected with either control shRNA or two shRNAs targeting Rnd1, cultured for 12 days, and subjected to soft agar assay. The graph shows the individual data points, their average, and s.d. from one experiment and *n* = 3 technical replicates. The experiment was repeated 3 times. (d) The indicated cells were subjected to Matrigel invasion assay and the graph indicates the number of cells invaded per well in each group. Data are from one representative experiment shown as averages of two technical repeats (the experiment was repeated 3 times). (e) MCF-10A cell expressing Myc were infected with control shRNA or two shRNAs targeting Rnd1 and cultured in 3D Matrigel for 8 days. The pictures show representative phase-contrast images of pseudoacinar structures. Scale bars, 50 μm. (f) Parental MCF-10A cells and MCF-10A cells expressing Myc were infected as indicated above, cultured in 3D Matrigel for 8 days, and subjected to immunofluorescent staining with the indicated antibodies followed by DAPI. The long yellow arrows in the top and bottom panel of the right column show a group of cells invading through a discontinuity of the basement membrane. The small white arrows in the two panels of the central column show cells that have lost their apical positioning of the Golgi apparatus. Scale bar, 50 μM. a,e,f show the results of one representative experiment out of three performed independently. The biological repeats of b–d yielded similar results. For source data, see Supplementary Table 8. Uncropped blots are shown in Supplementary Fig. 9.