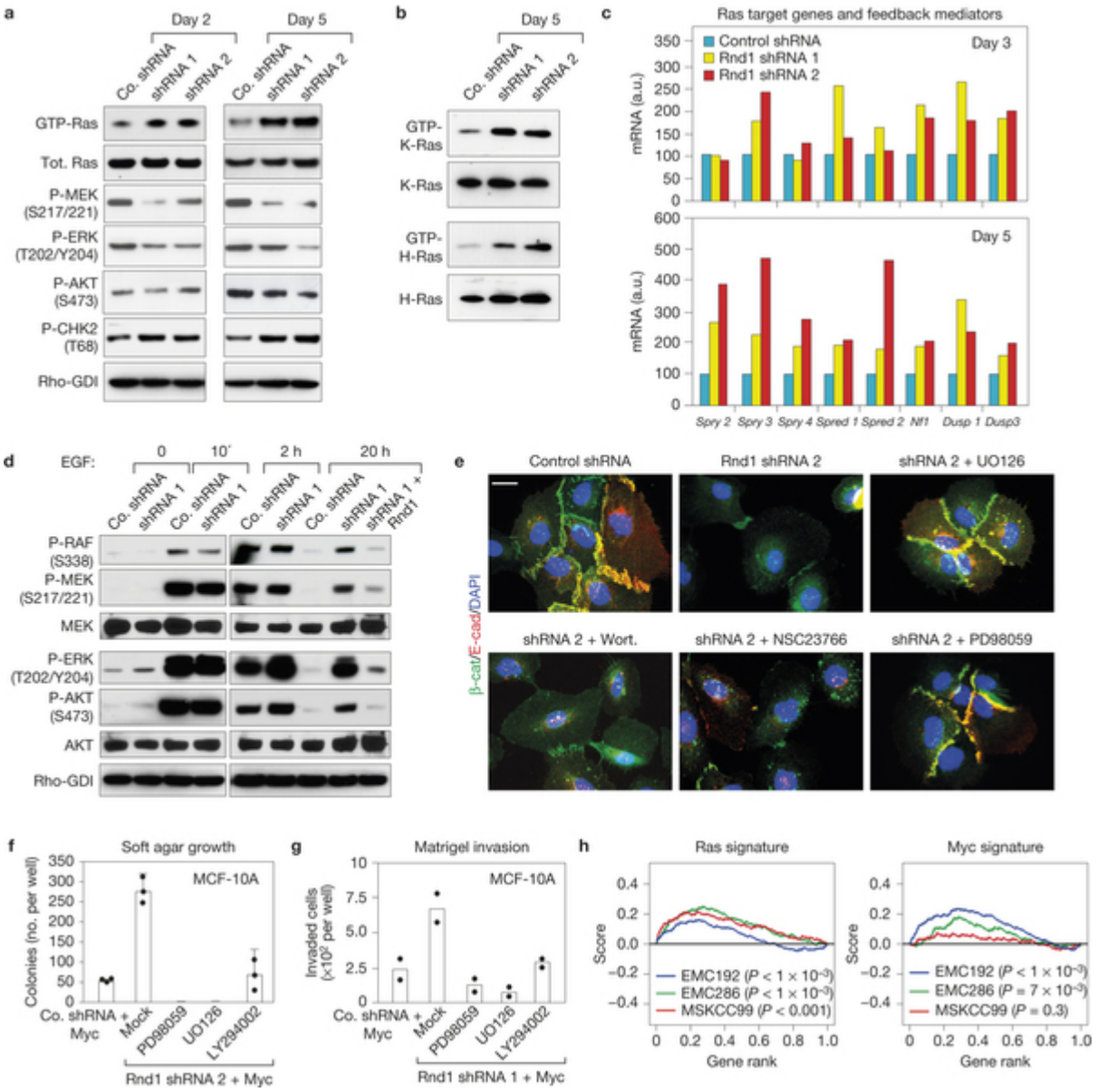
Figure 4: Loss of Rnd1 activates oncogenic Ras signalling.



(a) MCF-10A cells infected with a control or 2 shRNAs targeting Rnd1 were deprived of growth factors for 24 h at the indicated times after infection and subjected to pulldown assay using GST–RBD and immunoblotting with the indicated antibodies. (b) MCF-10A cells were infected as above, cultured for 5 days and subjected to pulldown assay using GST–RBD followed by immunoblotting with antibodies against K-Ras or H-Ras. (c) MCF-10A cells were infected as above, cultured for 3 (top) or 5 days (bottom) and subjected to qPCR for the indicated genes. Values represent fold change from one representative experiment (the experiment was repeated 2 times). (d) MCF-10A cells expressing a control shRNA (Co. shRNA) and spontaneously immortalized Rnd1-silenced MCF-10A cells (shRNA 1) were deprived of growth factors for 24 h, stimulated with EGF (5 ng ml^{-1}) for the indicated times and subjected to immunoblotting as indicated. As a control, spontaneously immortalized Rnd1-silenced cells were re-infected with a retroviral vector encoding Rnd1 (shRNA 1 + Rnd1), deprived of growth factors, stimulated with EGF for 20 h, and analysed as above. (e) Four days after infection with a control shRNA or two shRNAs targeting Rnd1, MCF-10A cells were cultured for 24 h with U0126 and PD98059 (MEK inhibitors), wortmannin (PI(3)K inhibitor) or NSC23766 (Rac inhibitor). Cells were subjected to immunofluorescent staining as indicated. Scale bar, 15 μM. (f,g) MCF-10A cells expressing Myc were infected with a control shRNA (Co. shRNA + Myc) or one targeting Rnd1 (Rnd1 shRNA + Myc) and subjected to soft agar assay (f) or Matrigel invasion assay (g) in the

presence of the indicated inhibitors. Data in f are from one experiment and are shown as averages and s.d. of *n* = 3 technical replicates. Data in g are from one experiment and are shown as averages of two technical replicates. The experiment in f and g were repeated 2 times. (h) Gene set enrichment analysis plots showing that low *RND1* mRNA levels are inversely correlated with the expression of a Ras and a Myc signature. See Methods for details. a,b,d,e show one representative experiment out of three performed independently. The biological replicates of c,f,g yielded similar results. For source data, see Supplementary Table 8. Uncropped blots are shown in Supplementary Fig. 9.