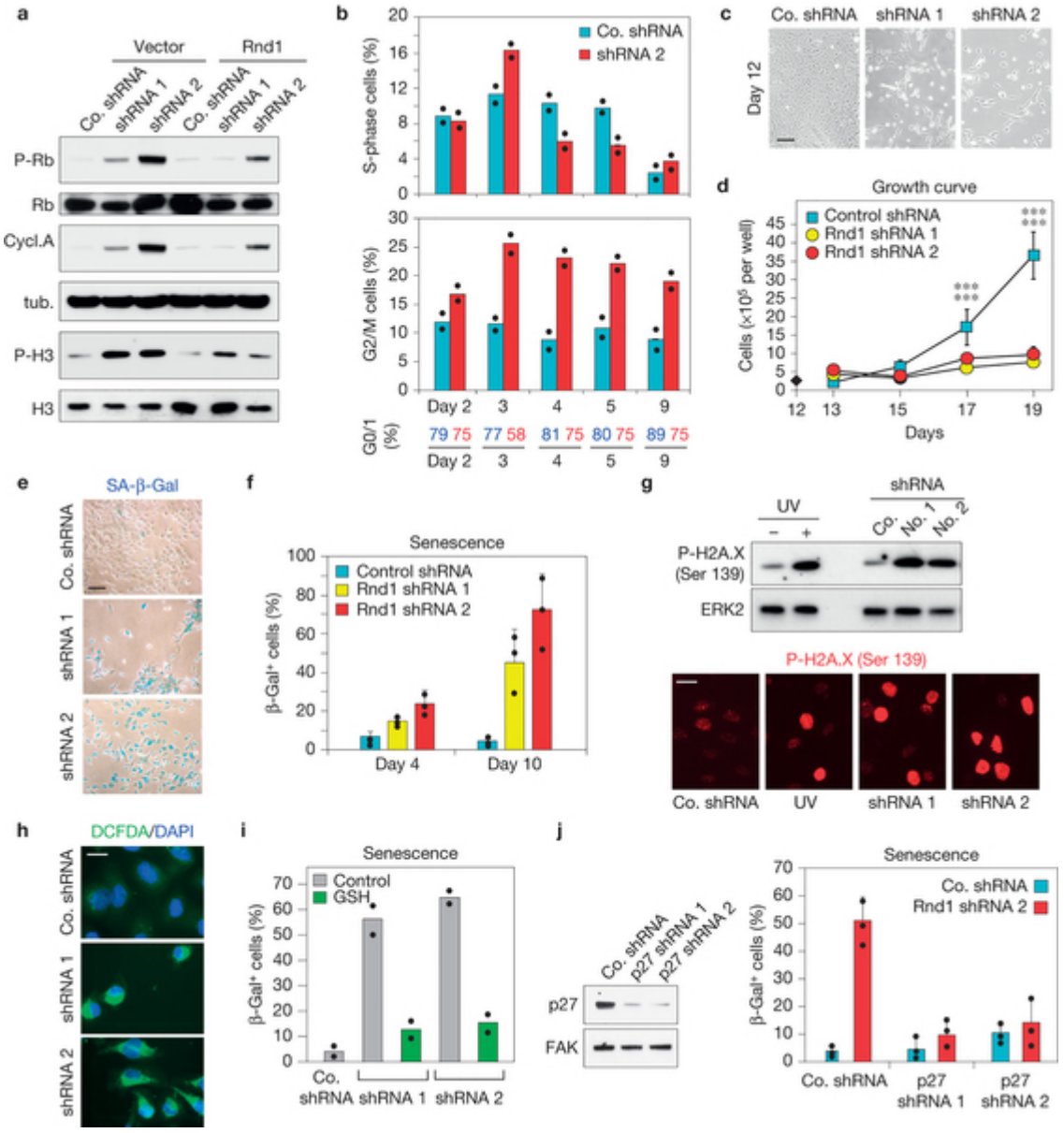
Figure 2: Depletion of Rnd1 causes hyperproliferative stress followed by premature senescence.



MCF-10A cells transduced with Rnd1 or control vector were infected with lentiviruses carrying either control shRNA or 2 shRNAs targeting Rnd1, and 2 days later subjected to immunoblotting with the indicated antibodies. (**b**) MCF-10A cells infected with either control shRNA or shRNA targeting Rnd1 (shRNA no. 2) were subjected to propidium iodide staining and FACS analysis. The graphs show the percentage of cells in S-phase (top) and G2/M-phase (bottom). The percentage of cells in G0/G1-phase is indicated below the graphs. Data from one experiment are shown as averages of two technical replicates (the experiment was repeated 2 times). (**c**) After transduction, control and Rnd1-silenced MCF-10A cells were passaged twice, cultured for 3 additional days, and photographed (total 12 days). Scale bar, 50 µm. (**d**) One day later, equal numbers of cells from **c** were plated and subjected to growth assay. The graph shows the average and s.d. from $n = 3$ biological replicates. *** represents $P < 0.001$ by the Student's *t*-test. (**e**) Control and Rnd1-silenced MCF-10A cells were subjected to senescence-associated (SA)-β-galactosidase staining. Scale bar, 50 µm. (**f**) The graph shows the percentage of SA-β-

galactosidase-positive cells at the indicated times. Individual data points, their average and s.d. are from one experiment and *n* = 3 technical replicates. The experiment was repeated 3 times. (**g**) MCF-10A cells were exposed to ultraviolet light (UV; 100 J cm^{-2}) or left untreated or they were transduced as indicated and cultured for 4 days before immunoblotting (top) or immunofluorescent staining (bottom). Scale bar, 15 μm. (**h**) Control and Rnd1-silenced MCF-10A cells were incubated with H_2DCFDA to detect ROS (green) and DAPI (blue). Scale bar, 15 μm. (**i**) MCF-10A cells infected as above were cultured with or without 0.5 mM reduced glutathione (GSH) for 24 h and subjected to staining for senescence-associated (SA)-β-galactosidase activity. Individual data points, their average and s.d. are from one experiment in which duplicate samples were assessed in parallel (the experiment was repeated 2 times). (**j**) MCF-10A cells were infected with control shRNA or shRNAs targeting p27 and subjected to immunoblotting (left). The p27-silenced cells were infected with control or Rnd1 shRNA (no. 2) and subjected to SA-β-galactosidase staining. The graph shows the percentage of SA-β-galactosidase-positive cells after 10 days (right). Individual data points, their average and s.d. are from one experiment and *n* = 3 technical replicates. The experiment was repeated 2 times. **a**,**g**,**h** show one representative experiment out of two, and **c**,**e** of three performed. The biological replicates of **b**,**f**,**i**,**j** yielded similar results. For source data see Supplementary Table 8. Uncropped blots are shown in Supplementary Fig. 9.