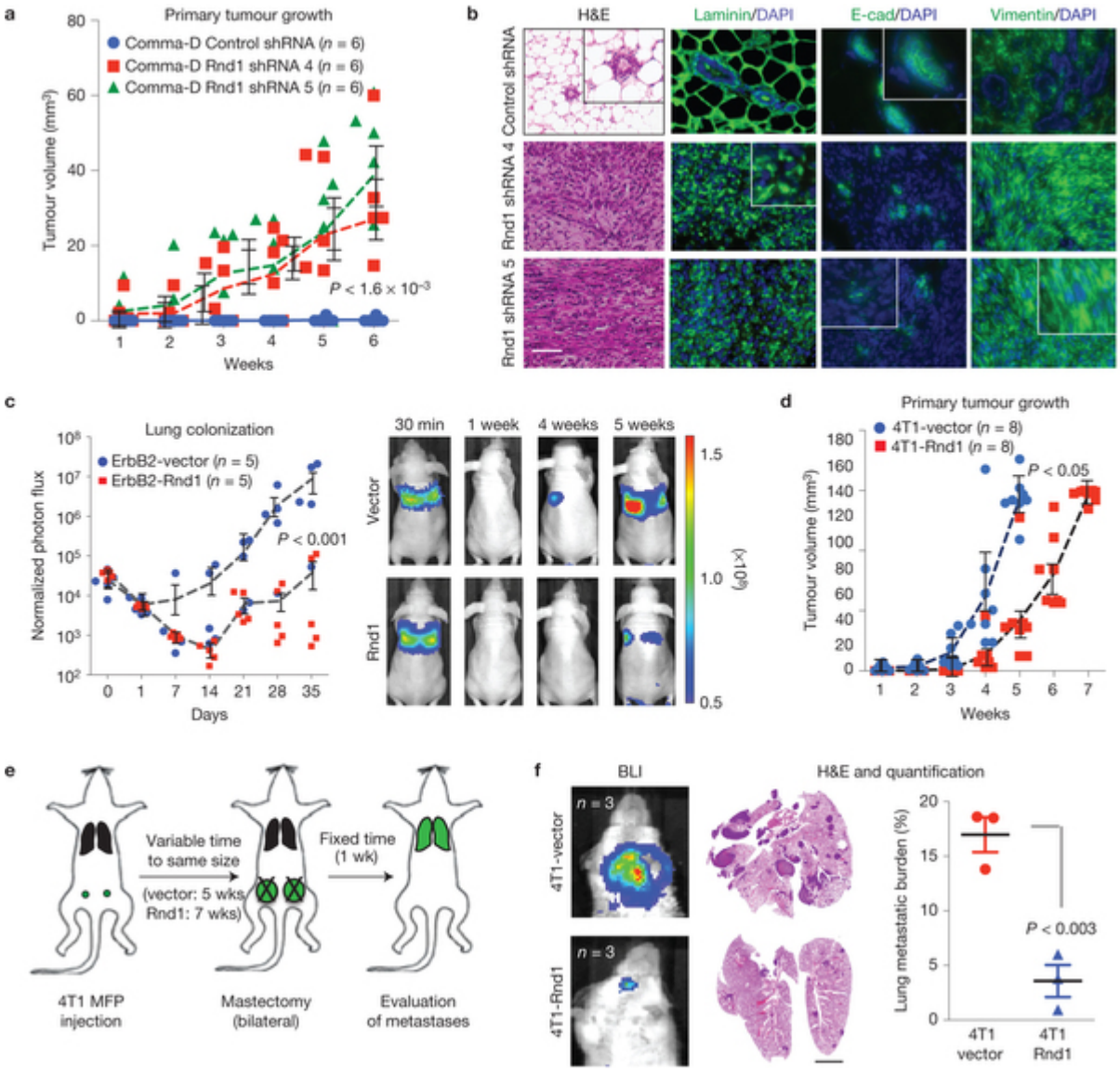
Figure 7: Loss of Rnd1 initiates mammary tumorigenesis.



(a) Comma-D cells were infected with lentiviruses carrying a control shRNA or two shRNAs targeting Rnd1. Cells (1×10^6) were injected into the fourth mammary fat pad of NOD-SCID-γ (NSG) mice. Tumour growth was measured at the indicated times using a calliper. The graph shows individual data points for tumour volumes, their average and s.e.m. from *n* = 6 tumours per group. (b) Comma-D-derived tumours carrying the indicated constructs were sectioned and subjected to immunofluorescent staining with antibodies against total laminin, E-cadherin or vimentin followed by DAPI. Scale bar, 50 µM. (c) ErbB2 transformed mammary tumour cells from MMTV-Neu (YD) mice infected with retroviral vector encoding HA–RND1 or a control vector and labelled with TGL were inoculated intravenously through the tail vein into nude mice (*n* = 5 mice per group). Lung colonization was measured by bioluminescent imaging (left). Data represent normalized photon flux at the indicated time points for each mouse. Representative images of one mouse are shown (right). (d) 4T1-TGL cells (1×10^3) were injected bilaterally into the fourth mammary fat pad of Balb/C mice and monitored for tumour growth. Data points represent tumour volumes in cubic millimetres at the indicated time points for each mouse in two cohorts of mice: one infected with retroviral vector encoding HA–RND1 (red squares) and the second with a control vector (blue circles). *n* = 8 tumours per group. (e) Schematic representation of the spontaneous metastasis experiment. (f) Representative images from the experiment showing lung bioluminescence at 1 week after mastectomy (left) and H&E-stained lung sections (middle). *n* = 3 lungs in each cohort. The graph shows the lung metastatic burden in mice injected with control and Rnd1-expressing 4T1 cells (right). Scale bar is 1 mm. The experiments in a,c,d were performed two times with similar results, whereas that

in f was performed once. Error bars are s.e.m. and *P* values were calculated by using the Student's *t*-test. For source data, see Supplementary Table 8.