

**Figure 1: Identification of *RND1* as a candidate breast tumour suppressor.**

(a) Kaplan–Meier analysis of the correlation between the level of the mRNA encoding each Rho-family GTPase and metastasis-free survival in the MSKCC data set. The graph shows the hazard ratio (top) and the log-rank *P* value (bottom) associated with expression of each Rho GTPase. (b) MCF-10A cells were transduced with lentiviral vectors encoding two shRNAs targeting *Rnd1* or a control shRNA and subjected to qPCR for *Rnd1* (left) or infected with a retrovirus encoding wild-type *Rnd1* followed by lentiviruses carrying two shRNAs targeting *Rnd1* or a control shRNA and subjected to immunoblotting with affinity-purified antibody against *Rnd1* (right). See Methods for the antibodies. The graph shows the average and s.d. (*n* = 3 biological replicates). (c–e) Control and *Rnd1*-silenced MCF-10A cells were cultured for 5 days and photographed; scale bar, 50  $\mu$ m (c) or they were subjected to immunofluorescent staining for  $\beta$ -catenin ( $\beta$ -cat, green), E-cadherin (E-Cad, red) and DAPI (d) or anti-E-cadherin (E-cad, Red), phalloidin (actin, green) and DAPI (e). Pictures show areas of similar cell density. Scale bars, 15  $\mu$ m. (f) Control and *Rnd1*-silenced MCF-10A cells were subjected to immunoblotting with the indicated antibodies. Arrows point to the mesenchymal (1) and epithelial (3) splice isoform of p120 catenin. The graph shows a densitometric quantification of the abundance of isoforms as fold change over control values. (g) Control and *Rnd1*-silenced MCF-10A cells were subjected to qPCR for the indicated genes. Data are from one experiment in which triplicate samples were assessed in parallel, the experiment was repeated twice. (h) Correlation between normalized *RND1* mRNA levels and oestrogen receptor (ER) status (left) and transcriptomic tumour subtypes (middle and right) in breast cancer DNA microarray data sets available from Oncomine (1: ref. 62; *n* = 47, 2: ref. 63; *n* = 118, 3: ref. 13; *n* = 121, 4: ref. 64; *n* = 129, 5: ref. 65; *n* = 286, 6: ref. 66; *n* = 55, 7: ref. 67; *n* = 49, 8: ref. 68; *n* = 133). *n* number of patients. Box represents median values. TN, triple-negative. (i) Kaplan–Meier analysis of relapse-free survival for all patients (*n* = 2,324), ER-negative patients (*n* = 494), or ER-positive patients (*n* = 1,830) using the open source KM Plotter (<http://kmplot.com/analysis>)<sup>18</sup>. c–f show one of three representative experiment. *P* values by the Student’s *t*-test are: \*\**P* < 0.01; \*\*\**P* < 0.001. The biological replicates yielded similar results. For source data, see Supplementary Table 8. Uncropped blots are shown in Supplementary Fig. 9.

