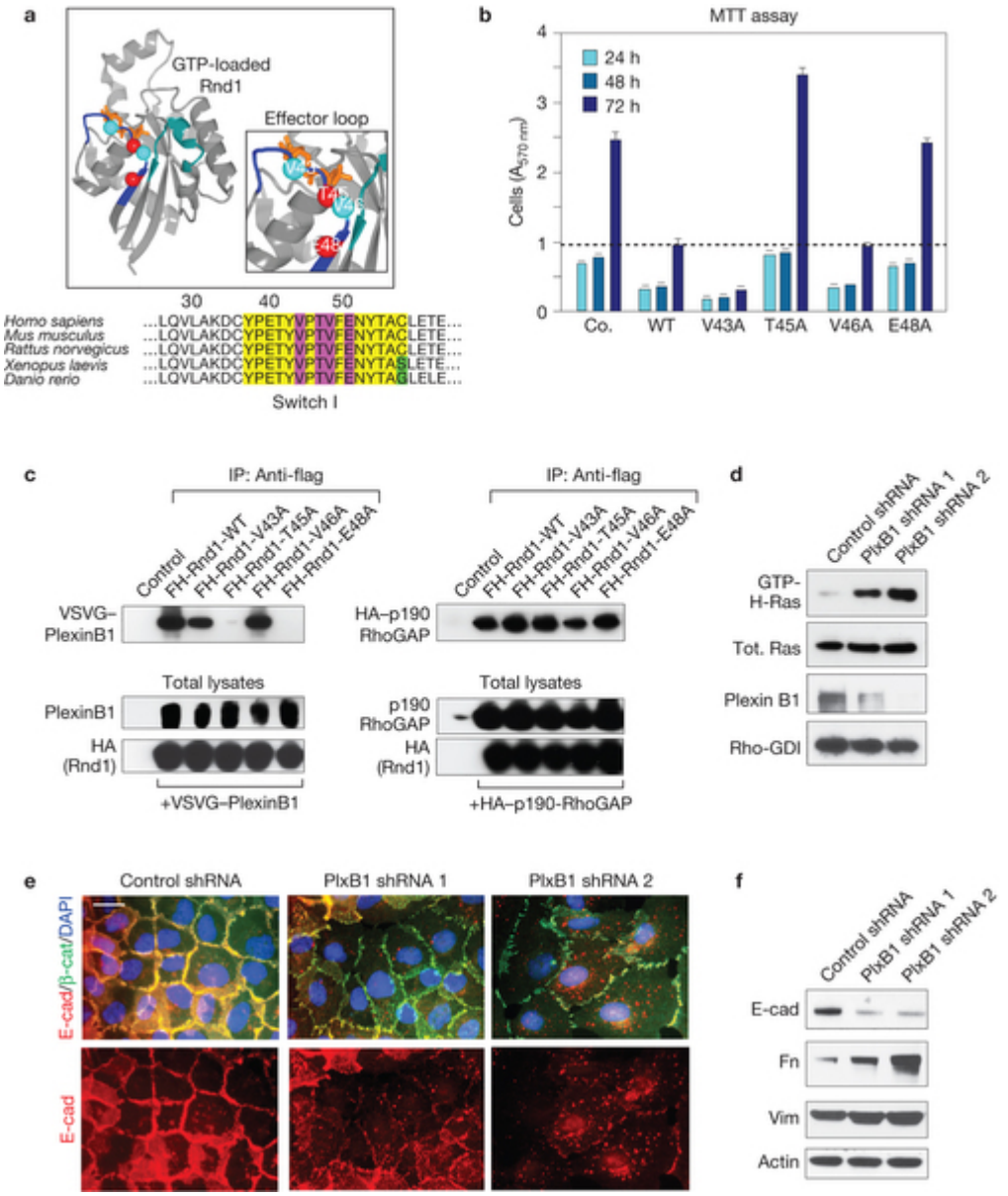
Figure 5: Rnd1 suppresses activation of Ras and EMT by binding to Plexin B1.



(a) Structure of GTP-loaded Rnd1. Switch I and II regions are depicted in blue and cyan, respectively. GTP is in orange. Mutated residues resulting in loss of function are shown as red balls, non-functional mutations as pale blue balls. (b) MCF-10A cells were infected with a retrovirus encoding HA-tagged wild-type or mutant Rnd1 or empty vector (Co.), plated under sparse conditions and subjected to MTT assay at the indicated times. Data are shown as averages and s.d. of n = 6 technical replicates (the experiment was repeated 2 times). (c) HEK293T cells were transfected with a vector encoding Flag–HA-tagged versions of wild-type or mutant RND1 or with empty vector together with a vector encoding VSVG–PlexinB1 or a vector encoding HA–p190-RhoGAP. Total lysates were subjected to immunoprecipitation with anti-Flag antibody followed by immunoblotting with VSVG–PlexinB1 (left) and HA–p190-RhoGAP (right). (d) MCF-10A cells were infected with lentiviruses carrying either a control shRNA (Co. shRNA) or 2 shRNA targeting PlexinB1 (PlxB1 shRNA 1 and 2). Total lysates were immunoblotted as indicated or subjected to pulldown assay using GST–RBD. Data are representative of two independent experiments. (e) PlexinB1 knockdown MCF-10A cells were subjected to immunofluorescent staining with antibodies as indicated followed by DAPI staining. Scale bar is 15 μM. (f) The above lysates were immunoblotted with antibodies against the indicated EMT markers. c–f show one representative experiment out of three performed independently. The biological repeat of b yielded similar results. For source data, see Supplementary Table8. Uncropped images of blots are shown in Supplementary Fig.9.