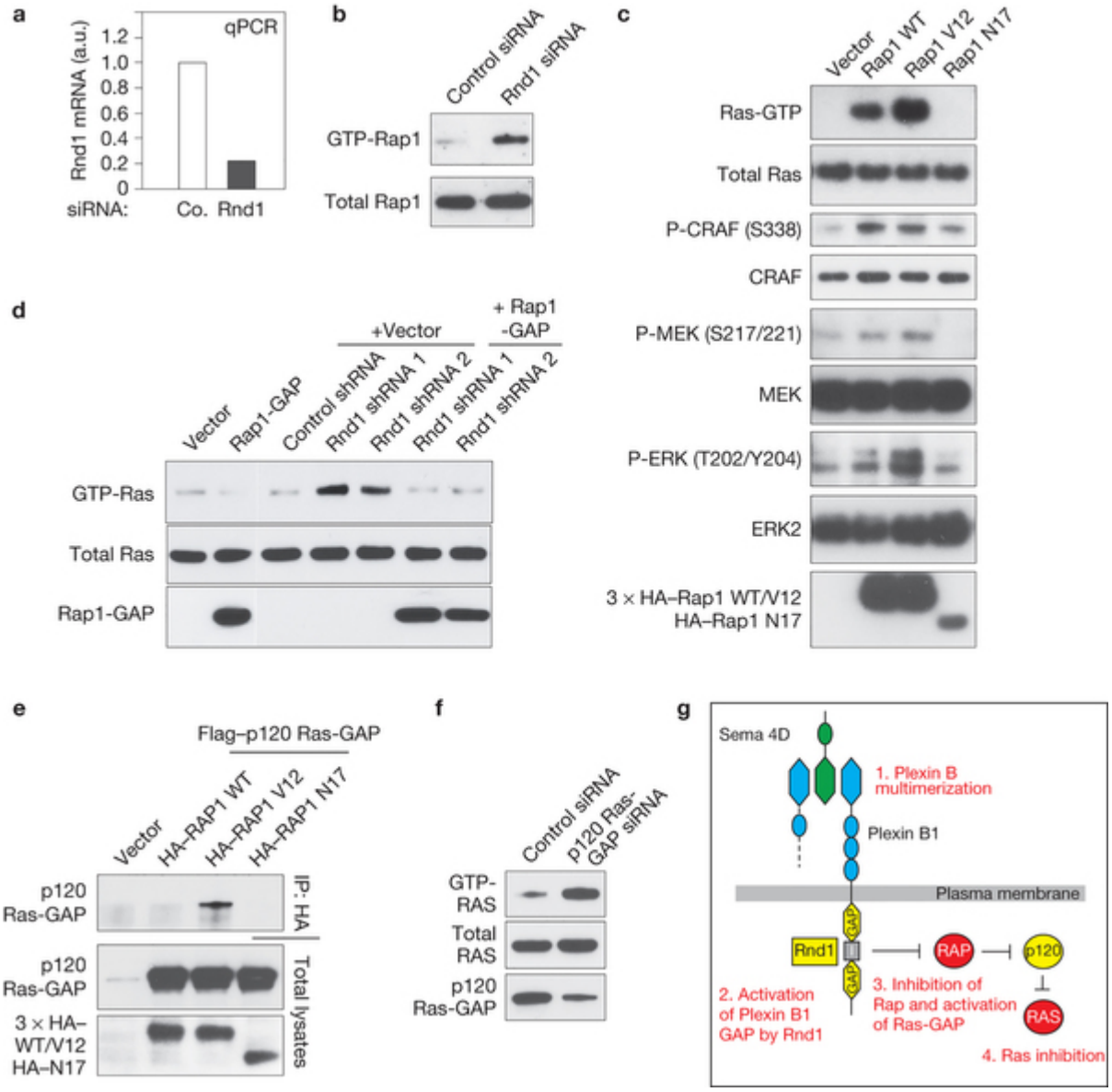
Figure 6: Loss of Rnd1 activates Rap1 and inhibits p120 Ras GTPase activity.



(**a**,**b**) MCF-10A cells were transfected with either control siRNA or Rnd1 specific siRNA, cultured for 3 days and subjected to qPCR. The experiment was repeated 2 times. (**a**) or pulldown assay using GST–RID (Ras-interacting domain of Ral-GDS) to detect activated Rap1 (**b**). (**c**) 293T cells were transfected with empty vector and plasmids encoding wild-type Rap1, constitutively active Rap1-V12 and dominant-negative Rap1-N17. Cell lysates were subjected to GST–RBD pulldown assay followed by immunoblotting with anti-Ras (top blot) or immunoblotted as indicated (bottom blots). (**d**) MCF-10A cells were infected with a control shRNA or 2 shRNAs targeting Rnd1 and, after 36 h, re-infected with an empty vector or one expressing Rap1-GAP. Cells were deprived of growth factors for 24 h and subjected to pulldown assay using GST–RBD to detect activated Ras. Control MCF-10A cells were infected with empty vector or one expressing Rap1-GAP and collected at 36 h post infection. (**e**) 293T cells were transfected with a Flag-tagged form of p120 Ras-GAP together with HA-tagged Rap1-WT, Rap1-V12 or Rap1-N17. Total lysates were subjected to immunoprecipitation with anti-HA followed by immunoblotting with anti-p120 Ras-GAP. Cells transfected with empty vector were used as control. (**f**) MCF-10A cells were transfected with control siRNA or siRNA targeting p120 Ras-GAP and total lysates were subjected to GST–RBD

pulldown assay to detect activated Ras. (**g**) Mechanistic model showing Rnd1-dependent regulation of Ras activation through Rap-mediated interaction of p120 Ras-GAP. **c**–**e** show one representative experiment out of three performed. The experiments in **b**,**f** were performed two times with similar results. For source data, see Supplementary Table 8. Uncropped images of blots are shown in Supplementary Fig. 9.