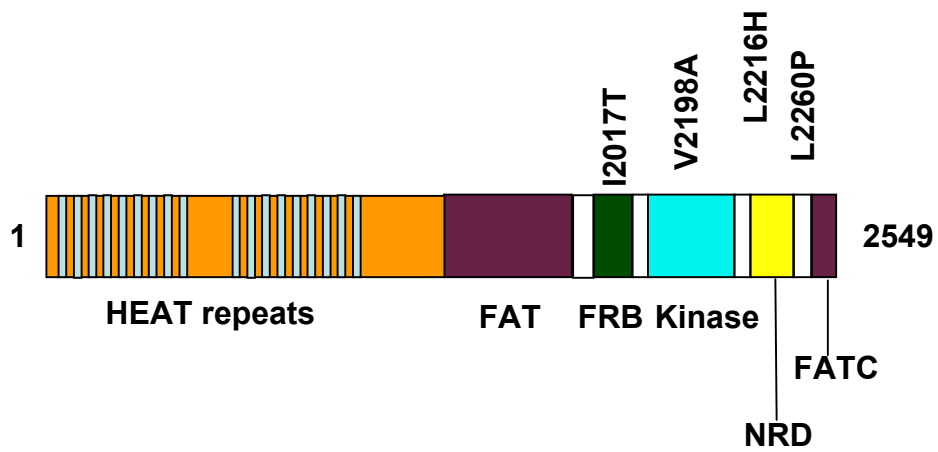
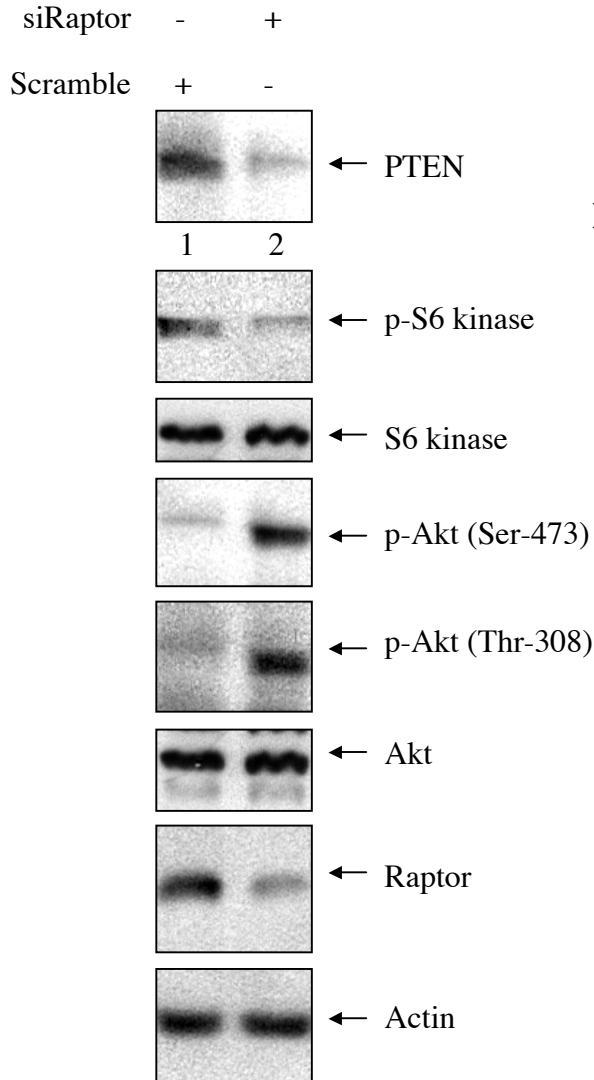
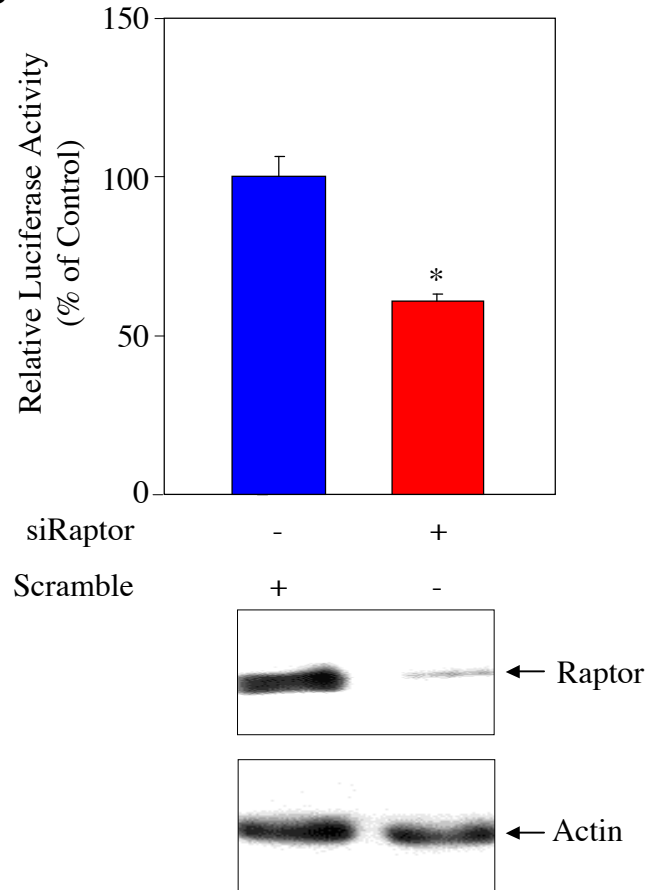


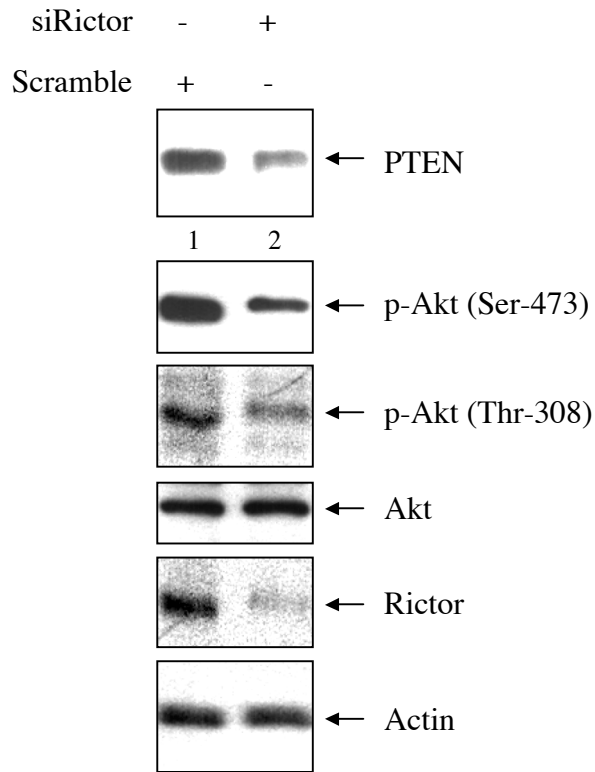
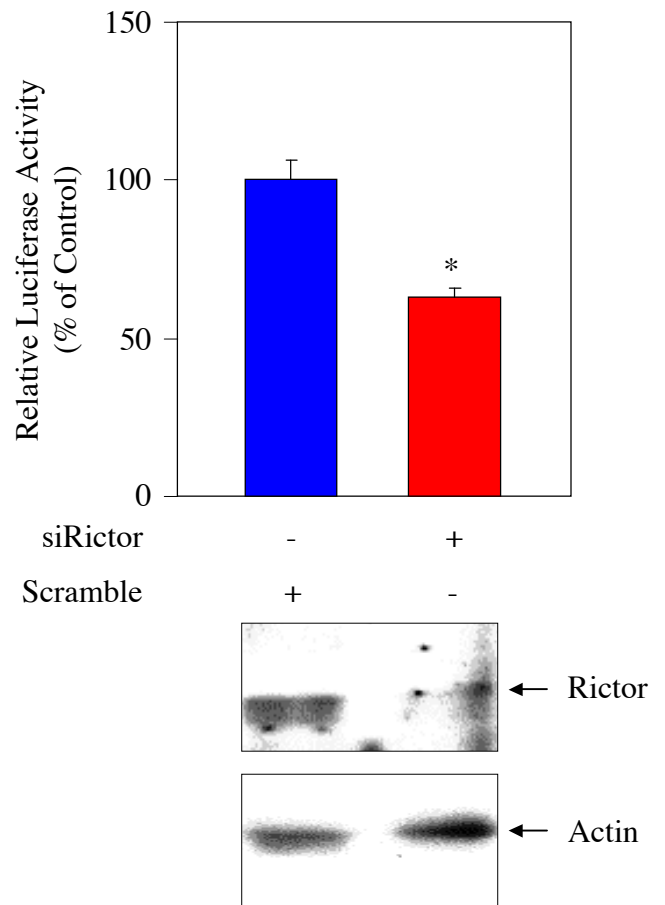
Supplementary Fig S1. TSC2 deficient murine embryonic fibroblasts exhibit increased PTEN protein concomitant with enhanced TORC1 activity. Lysates of TSC2^{+/+} and TSC2^{-/-} murine embryonic fibroblasts were immunoblotted with the indicated antibodies.



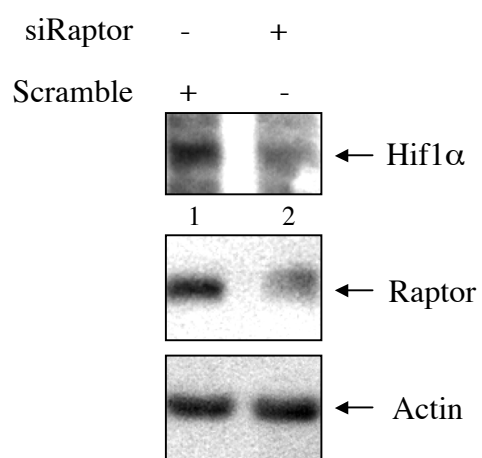
Supplementary Fig S2. Structure of the constitutively active mutant of mTOR. The amino acid changes are shown at the top. The protein domains are described at the bottom. HEAT = Huntington, EF3, A subunit of PP2A, TOR1; FAT = FRAP, ATM, TRAP; FRB = FKBP12-Rapamycin Binding; NRD = Negative Regulatory Domain. FATC = FAT c-terminal domain.

A**B**

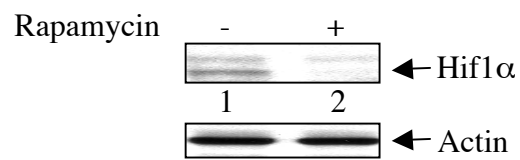
Supplementary Fig S3. Downregulation of raptor decreases PTEN expression in 293 cells. (A) 293 cells were transfected with a pool of siRNA targeting raptor. The cell lysates were immunoblotted with indicated antibodies. (B) 293 cells were cotransfected with PTEN-Luc and pool of siRNAs targeting raptor. The luciferase activity was determined in the cell lysates. Mean \pm SE of 6 measurements is shown. * $p = 0.01$ vs control. Bottom panel shows the expression of raptor in a representative lysate.

A**B**

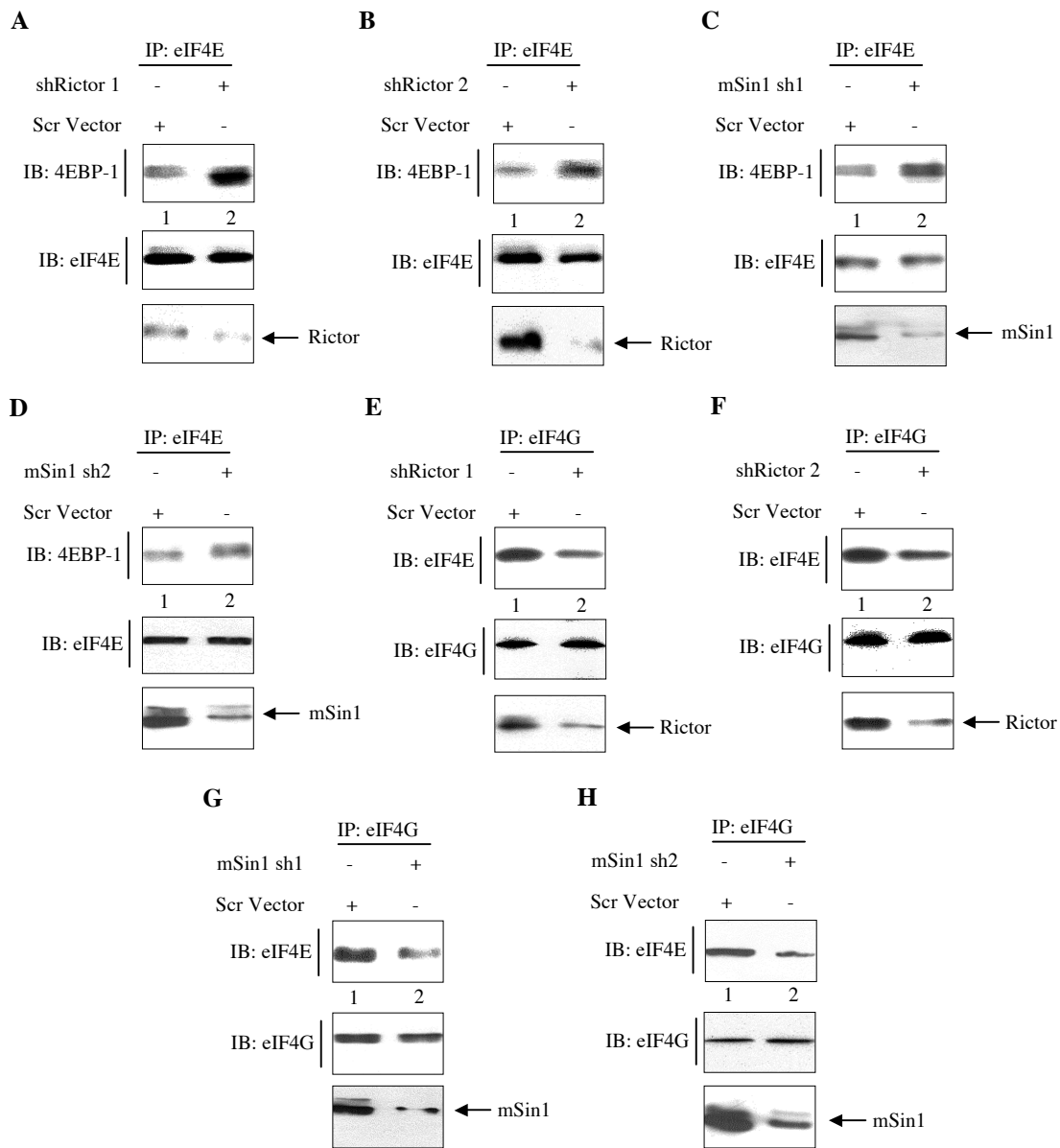
Supplementary Fig S4. Downregulation of rictor decreases PTEN expression in 293 cells. (A) 293 cells were transfected with a pool of siRNA targeting rictor. The cell lysates were immunoblotted with indicated antibodies. (B) 293 cells were cotransfected with PTEN-Luc and pool of siRNAs targeting rictor. The luciferase activity was determined in the cell lysates. Mean \pm SE of 6 measurements is shown. *p = 0.01 vs control. Bottom panel shows the expression of rictor in a representative lysate.



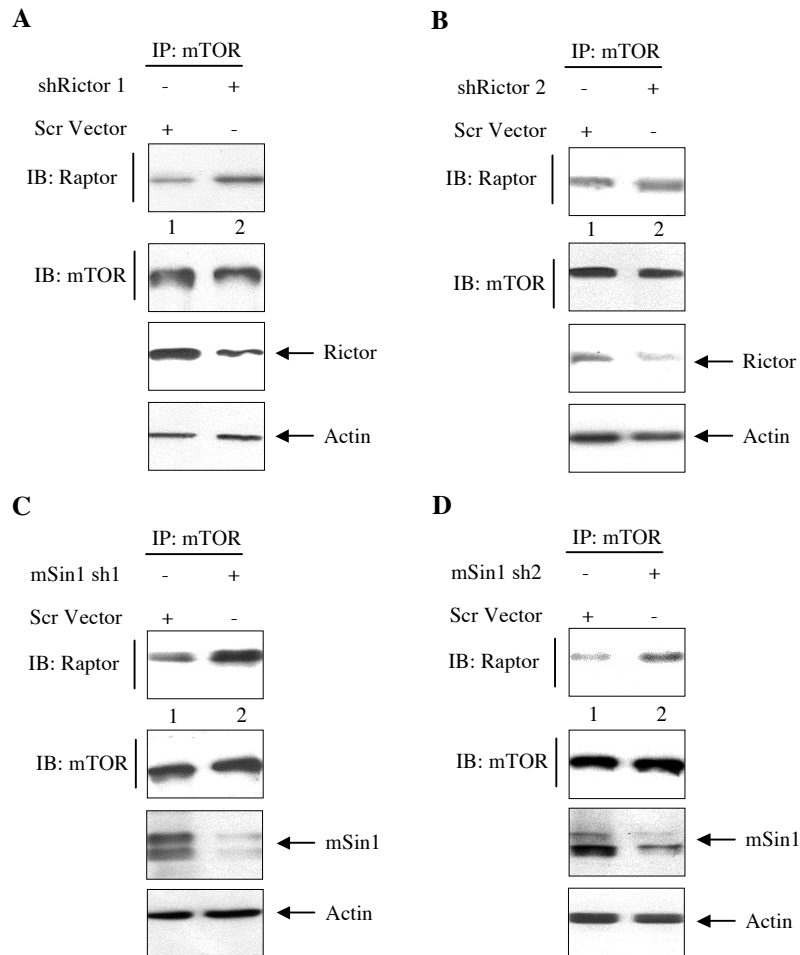
Supplementary Fig S5. Downregulation of raptor decreases Hif1 α expression in 293 cells. 293 cells were transfected with a pool of siRNA targeting raptor. The cell lysates were immunoblotted with indicated antibodies.



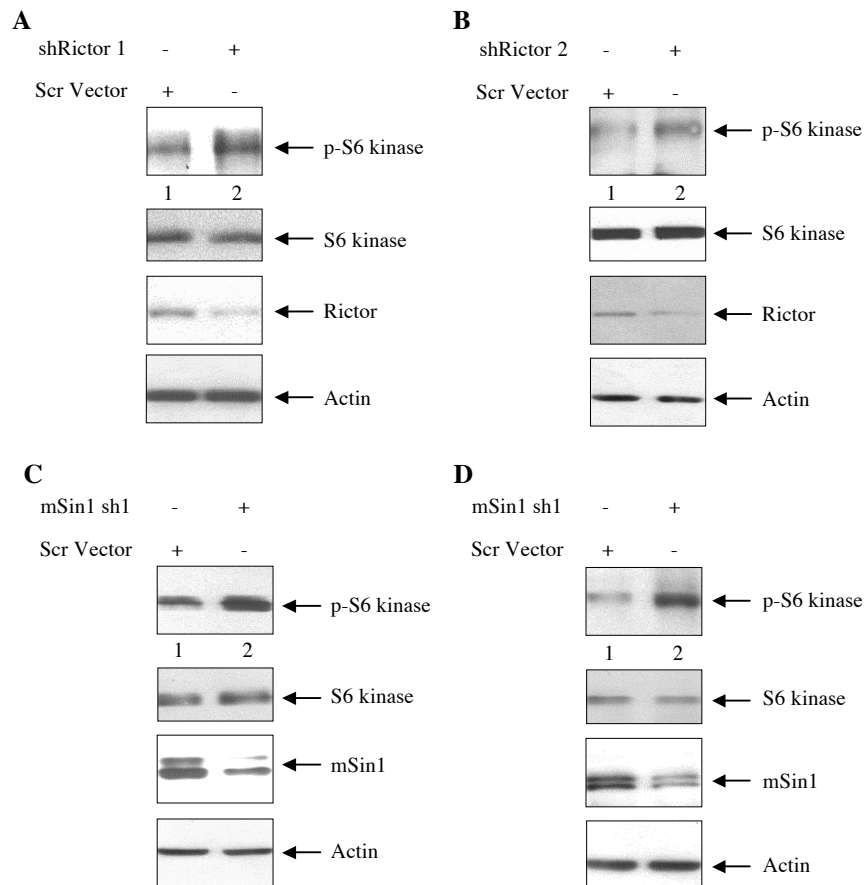
Supplementary Fig S6. Rapamycin inhibits Hif1 α expression in TSC2^{-/-} murine embryonic fibroblasts. TSC2 null murine embryonic fibroblasts were serum starved for 24 hours prior to incubation with 25 nM rapamycin for 24 hours. The cell lysates were immunoblotted with indicated antibodies.



Supplementary Fig S7. TORC2 regulates dissociation of eIF4E-4EBP1 complex to form eIF4E-eIF4G complex. 293 cells were transfected with either shRictor 1 (panels A and E) or shRictor 2 (panels B and F) or mSin1 sh1 (panels C and G) or mSin1 sh2 (panels D and H) expression vectors. (Panels A - D) The cell lysates were immunoprecipitated with eIF4E antibody followed by immunoblotting with 4EBP-1 and eIF4E antibodies. (Panels E - H) The cell lysates were immunoprecipitated with eIF4G antibody followed by immunoblotting with eIF4E and eIF4G antibodies.



Supplementary Fig S8. Downregulation of TORC2 increases association of mTOR with raptor. 293 cells were transfected with either shRictor 1 (panel A) or shRictor 2 (panel B) or mSin1 sh1 (panel C) or mSin1 sh2 (panel D) expression vectors. The cell lysates were immunoprecipitated with mTOR antibody followed by immunoblotting with raptor and mTOR antibodies. The bottom panels show expression of Sin1 and actin.



Supplementary Fig S9. Downregulation of rictor and Sin1 increases TORC1 activity. 293 cells were transfected with either shRictor 1 (panel A) or shRictor 2 (panel B) or mSin1 sh1 (panel C) or mSin1 sh2 (panel D) expression vectors. The cell lysates were immunoprecipitated with phospho-S6 kinase (Thr-389), S6 kinase, rictor, mSin 1 and actin antibodies as indicated.