Supplemental Data

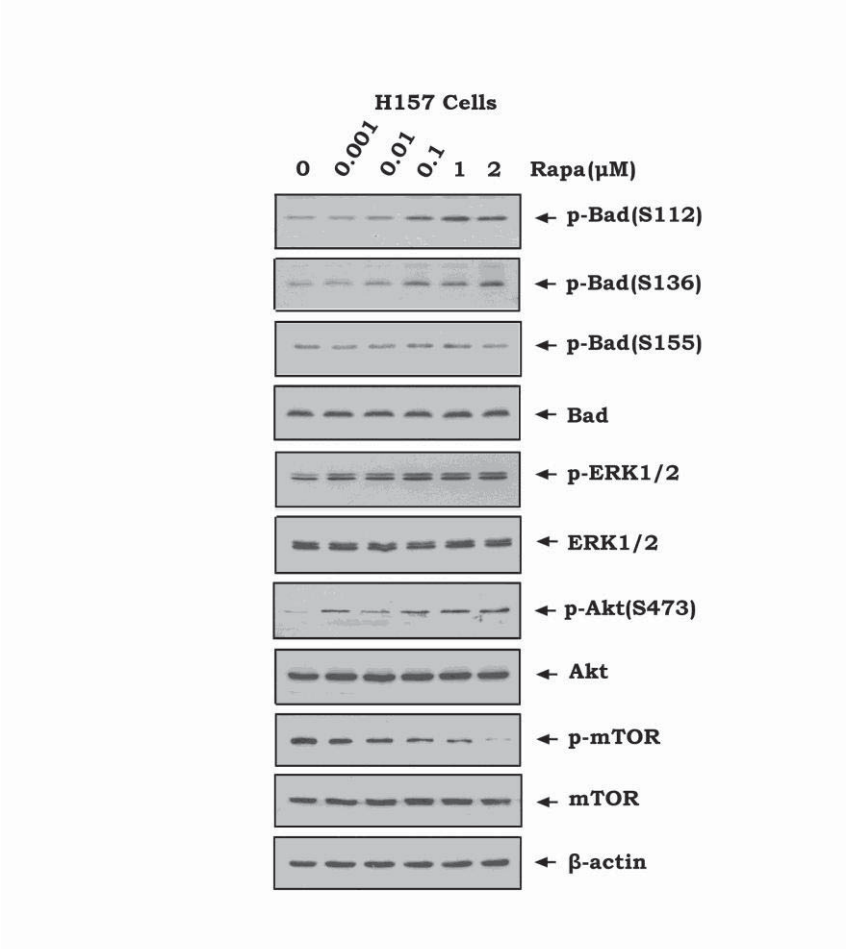

Figure S1. Inhibition of mTOR by rapamycin induces Bad phosphorylation in association with activation of Akt and ERK1/2 in H157 cells. H157 cells were treated with increasing concentrations of rapamycin (Rapa) for 45 min. Phosphorylation of Bad at S112, S136 or S155 was analyzed by Western blot using the phospho-specific S112, S136 or S155 Bad antibodies. Phosphorylation of ERK1/2, Akt, mTOR, p70S6K or 4EBP1 was also analyzed by Western blot.

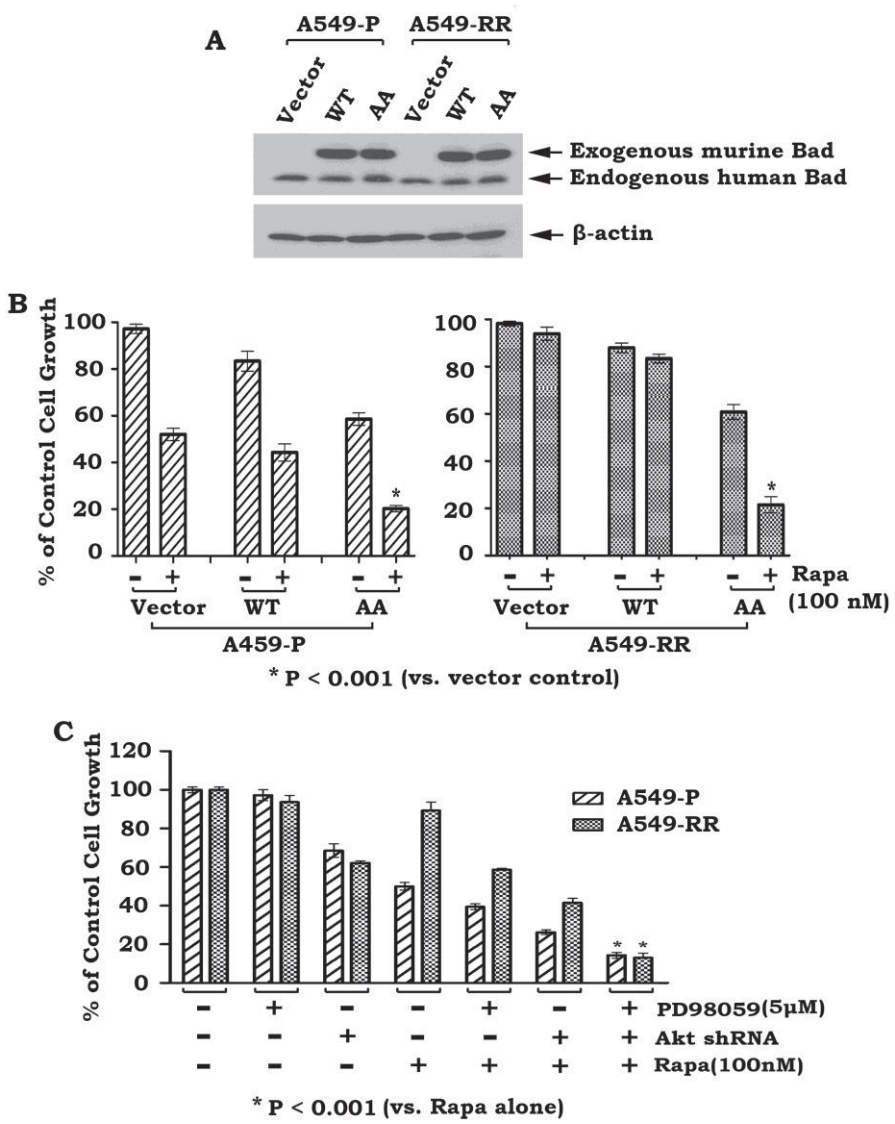

Figure S2. The non-phosphorylatable mutations of Bad at S112 and S136 or inhibition of Bad phosphorylation at these two sites by PD or Akt shRNA reverses rapamycin resistance. A, murine WT-Bad, S112A/S136A (AA) and vector-only control were transfected into A549 parental (A549-P) and rapamycin-resistant A549 cells (A549-RR). Bad expression was analyzed by Western blot using a Bad antibody. B, after transfection, cells were treated with rapamycin (100nM) for 48h. Cell growth was analyzed by SRB assay. C, A549-P or A549-RR cells expressing control shRNA or Akt shRNA were treated with rapamycin in the absence or presence of PD98059 for 48h. Cell growth inhibition was analyzed by SRB assay. Error bars represent ± S.D.

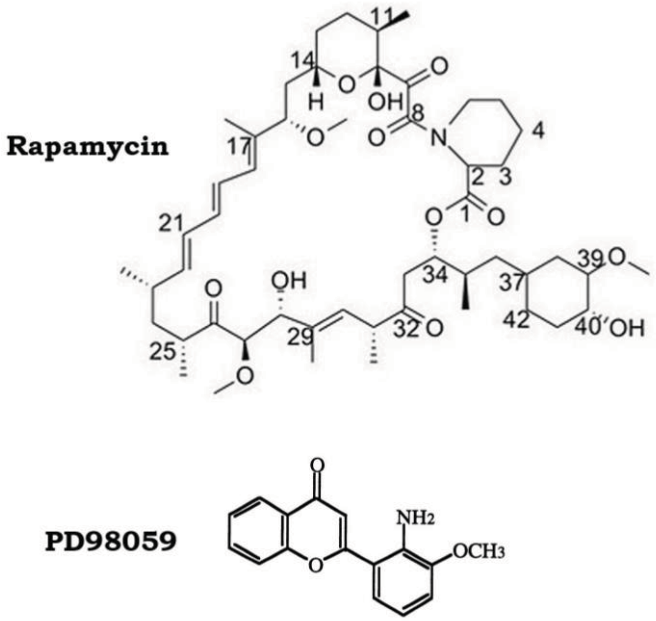

Figure S3. Structures of rapamycin and PD98059.

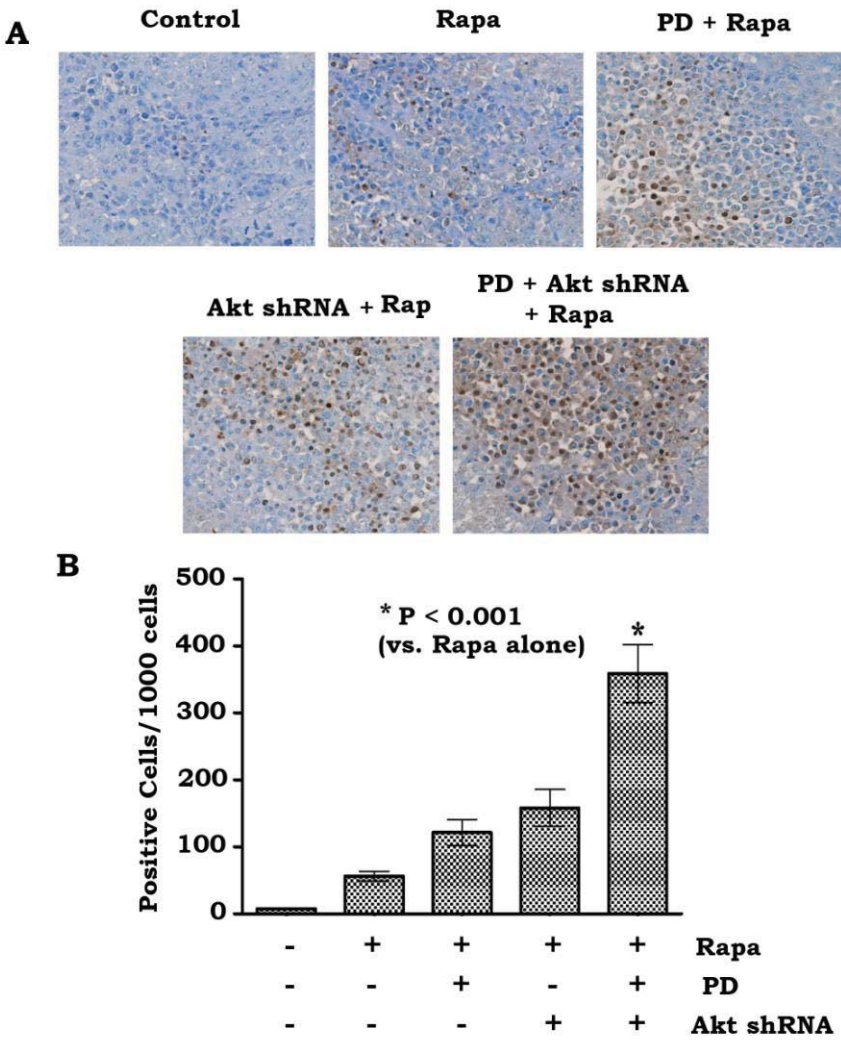

Figure S4. Apoptosis effects in tumor tissues following inhibition of rapamycin-induced Bad phosphorylation by PD98059 or silencing of Akt. A and B, Nu/Nu nude mice with H460 or Akt shRNA H460 xenografts were treated as indicated. Apoptosis in tumor tissues was analyzed by TUNEL assay using Tumor TACS^{TM} In Situ Apoptosis Detection Kit. TUNEL positive cells were counted for every 1000 cells from ten areas of each slide, and three different slides were analyzed for each sample. Data represents as mean ± S.D.

Supplemental Methods

TUNEL assay

To measure apoptosis in tumor tissues, TUNEL staining was performed using Tumor TACS™ In Situ Apoptosis Detection Kit following the protocol provided by manufacturer (Trevigen, Inc). After completion of all procedures, the TUNEL slides were visualized and counted under microscopy. TUNEL-positive cells were characterized by a dark brown nuclear staining (1). TUNEL positive cells were counted for every 1000 cells from ten areas of each slide, and three different slides were analyzed for each sample. Data represents as mean ± S.D. (2).

References

1. Garrity, M M., Burgart, L J, Riehle, D L, Hill, E M, Sebo, T J, and Witzig, T. Identifying and quantifying apoptosis: navigating technical pitfalls, Mod Pathol. 2003; 16: 389-394.
2. Miyake, I, Hakomori, Y, Misu, Y, Nakadate, H, Matsuura, N, Sakamoto, M, and Sakai, R. Domain-specific function of ShcC docking protein in neuroblastoma cells. Oncogene. 2005; 24: 3206-3215.