Supporting Information

Liu *et al*. 10.1073/pnas.0712306105

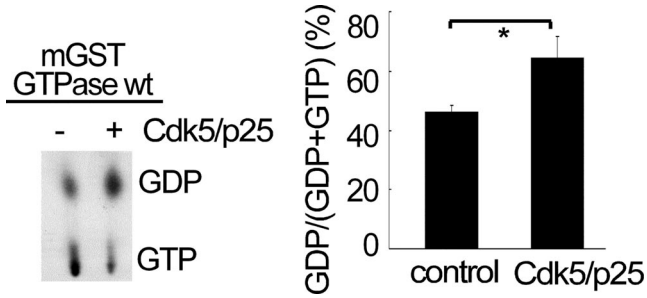

Fig. S1. Stimulation of PIKE-A GTPase activity by Cdk5 *in vitro*. mGST GTPase WT immunoprecipitated from HEK293 cells was phosphorylated by Cdk5/p25 complex *in vitro* with cold ATP and then analyzed for GTPase activity as described in Fig. 2.

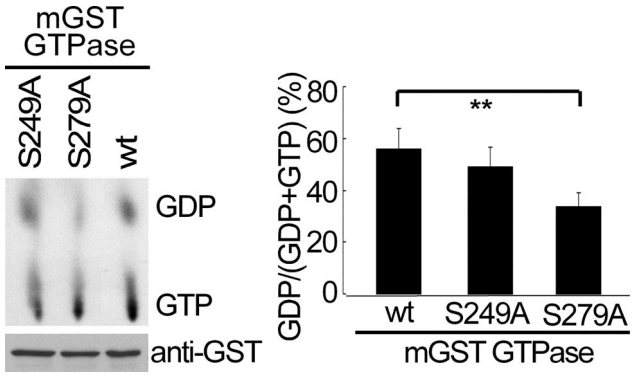

Fig. S2. Determination of GTPase activity in S249A and S279A mutants. GTPase WT, S249A, and S279A overexpressed in HEK293 cells were compared for their GTPase activity as described in Fig. 2.

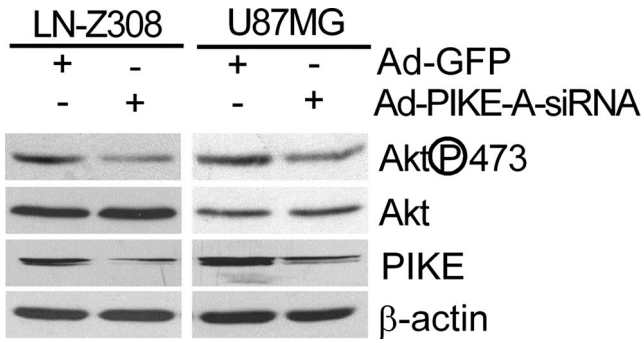

Fig. S3. The effect of knockdown of PIKE-A on phosphorylation of Akt. LN-Z308 and U87MG cells were infected with adenovirus expressing PIKE-A-siRNA or GFP control. Akt phosphorylation was determined by Western blot.

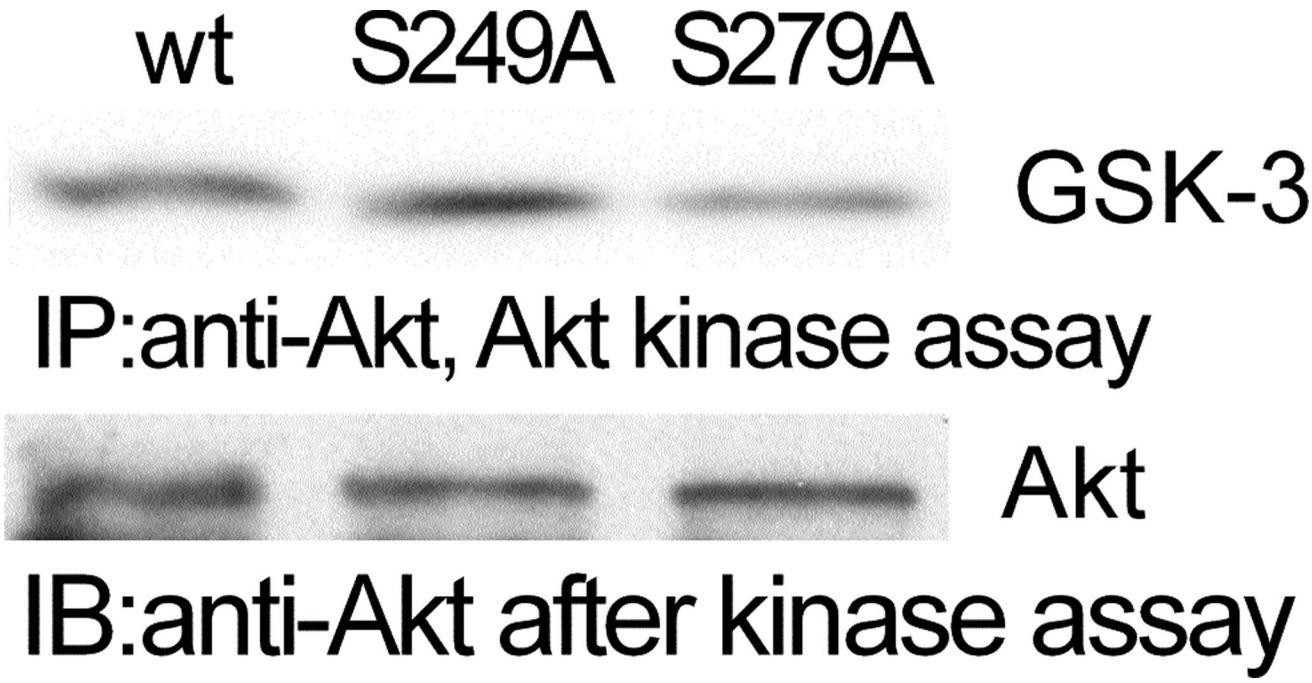

Fig. S4. The effects of S279A mutation on PIKE-A reduced Akt activity. After expression of indicated PIKE-A in HEK293, endogenous Akt activity was determined by an *in vitro* kinase assay.

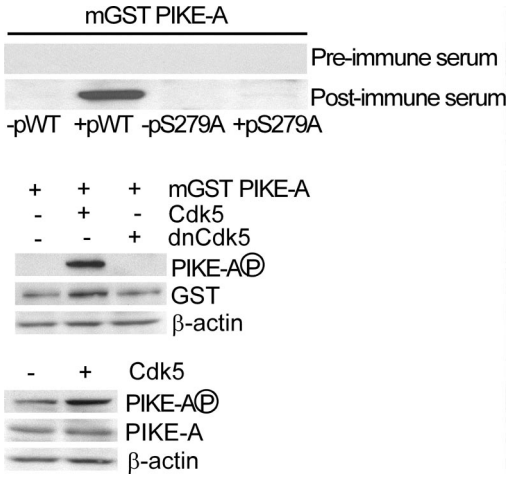

Fig. S5. Characterization of phospho S279 PIKE-A antibody *in vitro*. mGST PIKE-A or S279A mutant immunoprecipitated from HEK293 cells was phosphorylated by Cdk5 *in vitro* with cold ATP and analyzed by immunoblotting using preimmune or postimmune sera from a rabbit immunized with a peptide containing a phosphorylated serine residue corresponding to position 279. +p or −p, PIKE proteins either phosphorylated or unphosphorylated by Cdk5 *in vitro* (*Top*). (*Middle*) HEK293 cells were transfected with vectors as indicated. Phosphorylation of mGST PIKE-A was determined by phospho PIKE-A antibody. The membrane was reblotted for mGST PIKE-A and actin. (*Bottom*) Phosphorylation of endogenous PIKE-A was determined after overexpression of Cdk5/p25 in LN-Z308 cells.

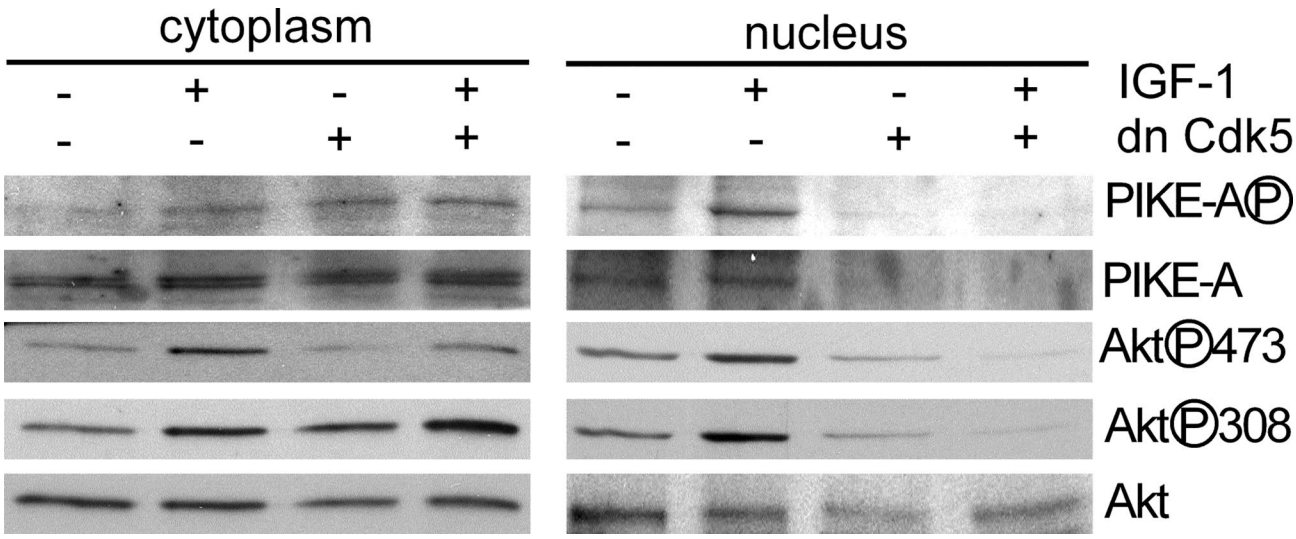

Fig. S6. Reduction of phospho PIKE-A and phospho Akt in the nucleus by dnCdk5. LN-Z308 cells were transfected with dnCdk5 and starved overnight and stimulated with IGF-1 as described in Fig. 4*C*. Cytoplasmic and nuclear fractions were prepared and assayed for various phospho signals and proteins.

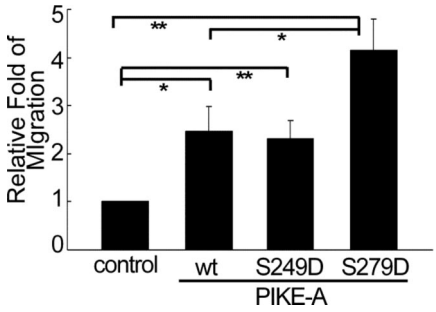

Fig. S7. The effect of PIKE-A phosphorylation status on U87MG cell migration. Equal number of U87MG cells transfected with construct for GFP-PIKE WT, GFP-S249D, or RFP-S279D, respectively, was plated into transwell chambers and scored for the migration of GFP or RFP positive cells. All of the data above were calculated as means (± SD) of three independent experiments (*, $P < 0.05$ and **, $P < 0.01$, Student's *t* test).