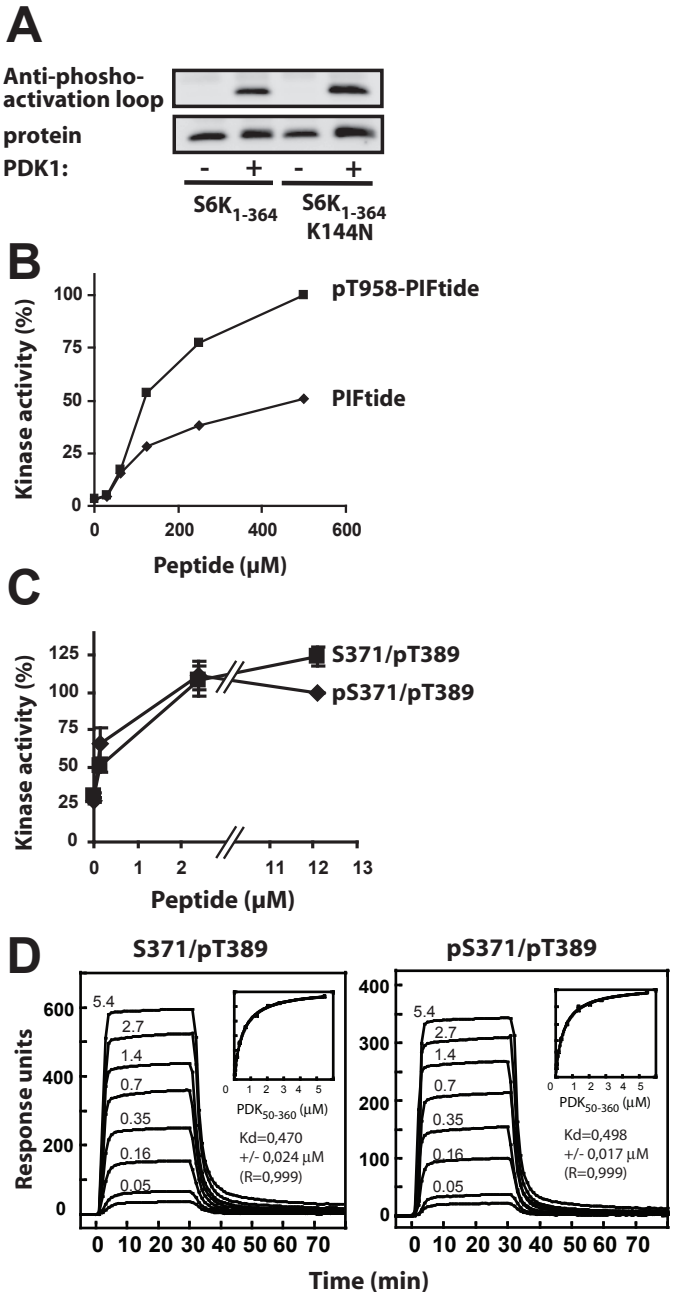
Supplementary Fig. 6



Supplementary Fig. 6 (**A**) Aliquots of S6K1 protein from bar 1, 2, 5 and 6 in Fig. 5B were subjected to immunoblotting with phosphorylation site specific Ab against the activation loop or anti-HA Ab. **(B)** The kinase activity of PKBα143-479, prephosphorylated by PDK1 in the activation loop, was determined in the presence of increasing concentrations of synthetic PRK2 tail peptides encompassing the HM, which were either non-phosphorylated (PIFtide) or phosphorylated at the tail site (pT958-PIFtide). The figure shows a representative experiment with kinase activity expressed as per cent. **(C)** The kinase activity of purified PDK1 was determined in the presence of increasing concentrations of synthetic S6K tail peptide (residues 366-395) that was either phosphorylated at T389 (S371/pT389) or phosphorylated at both S389 and S371 (pS371/pT389). Kinase activity is expressed as per cent, and data are means +/- SD of 3 independent experiments. **(D)** Synthetic S371/pT389 S6K tail peptide and pS371/pT389 S6K tail peptide were biotinylated and used to coat streptavidin Sensor Chips. $PDK1_{50-360}$ was injected at different concentrations (0.05-5.4mM) onto the peptide coated chips. In the inserts, the kinetic constants were obtained by fitting the data to a hyperbola using kaleidagraph software. Representative results from one of several experiments are shown.