**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.