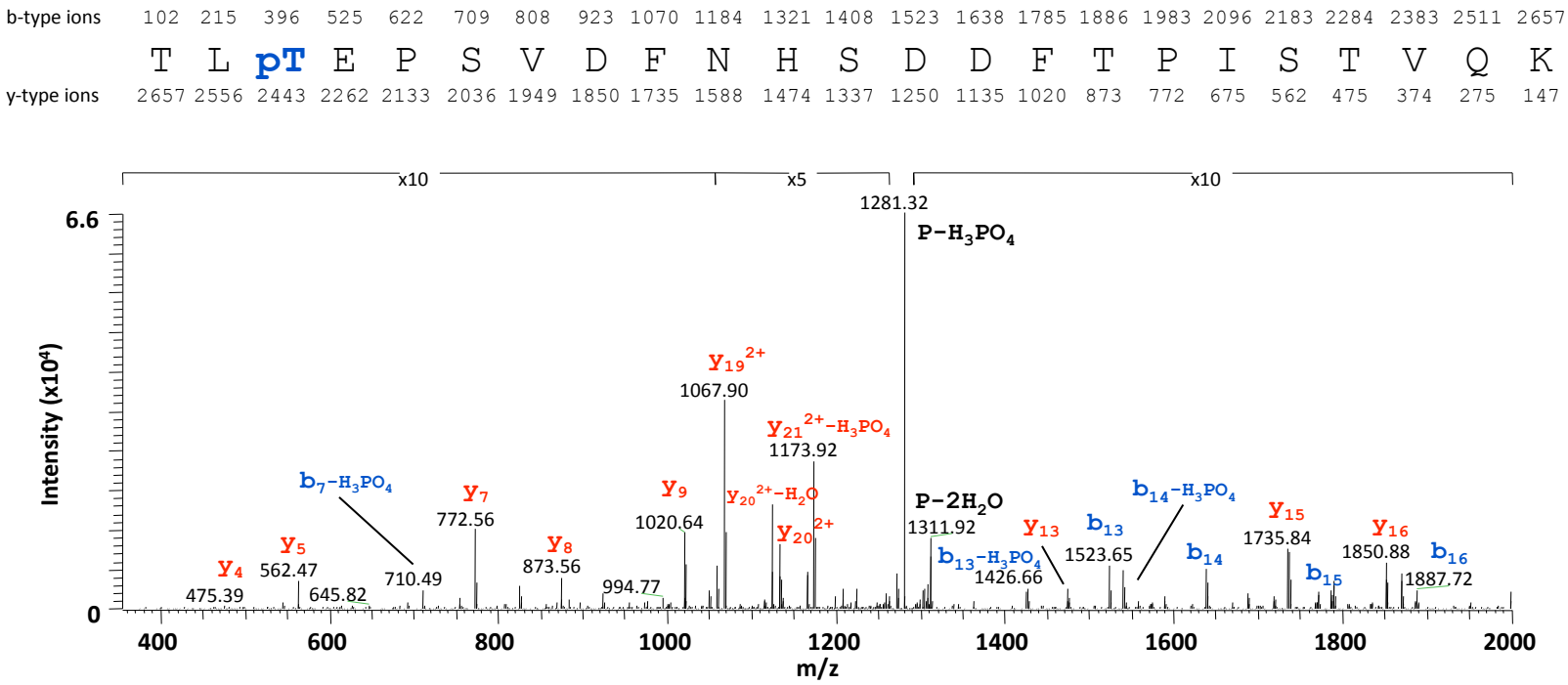
Supplemental data

Supplemental Fig. 1 Identification of the phospho-rictor Thr-1135 and Ser-1177 peptides by mass spectrometry. MS was performed on the rictor protein purified by immunoprecipitation from Hela cells cultured in 10% serum. Ten rictor immunoprecipitations were combined and coomassie-stained rictor band was sliced and analyzed. The MS/MS spectrums recorded on the doubly charged ions as shown at *m/z* 1329.5 (*A,* indicates the phospho-Thr-1135 rictor peptide) and at *m/z* 946.4 (*B,* indicates the phospho-Ser-1177 rictor peptide). Spectrums were acquired on a LTQ ion trap mass spectrometer in a data dependent fashion and all spectra were searched against a human non-redundant database using Spectrum Mill Proteomics Workbench (See Methods). Predicted nominal masses for all b-type and y-type ions are shown above and below the sequences of the matched peptides. Both spectrums are annotated showing the observed b-type and y-type ions.

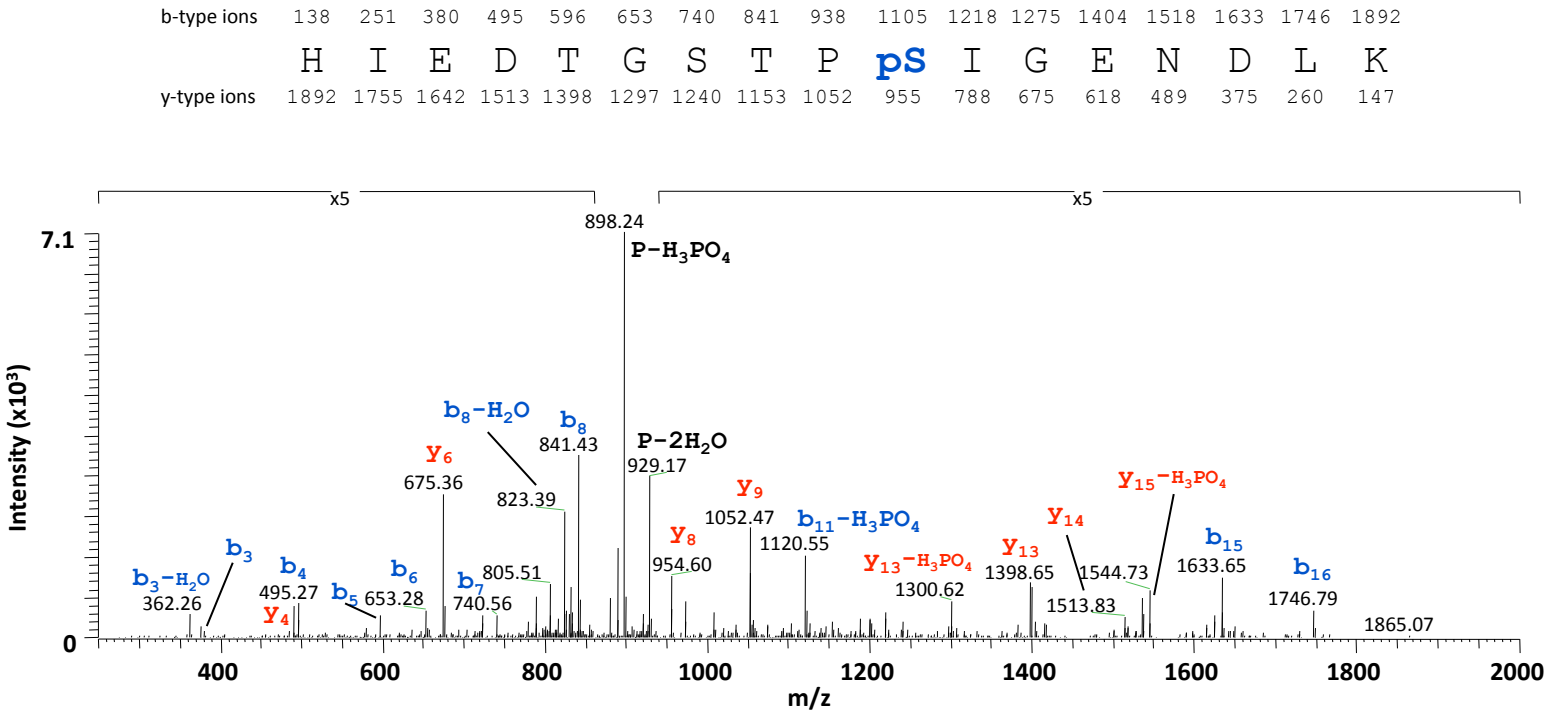
Supplemental Fig. 2. Validation of the phospho-specific antibodies and characterization of the rictor Thr-1177 phosphorylation. *A.* The cross-validation of the phospho-specific rictor mutants and phospho-rictor antibodies. The Xpress tagged wild type or phosphospecific rictor recombinant proteins were expressed in HEK 293T cells and immunopurified using Xpress antibody. The immunopurified wild type and phospho-mutants rictor proteins were analyzed by western blotting with the indicated phospho-rictor and Xpress antibodies. *B.* The functional study of the rictor T1177 phosphorylation site. Reconstitution of the mTORC2 signaling in rictor null MEFs by expressing the wild type and phospho-rictor mutants (T1177A and T1177D). Following 48 hrs after transfection cells were lysed and cell extracts analyzed by immunoblotting for the indicated proteins and phosphorylation state of Akt on the Ser-473 site.

Supplemental Fig. 3. Detection of the IGF-I dependent phosphorylation site of mTOR. Upper panel: Rictor but not raptor is co-purified with mTOR phosphorylated on the Ser-2481 site. The mTOR complexes were purified by the rictor (right panel) or raptor (left panel) immunoprecipitations from the serum starved MDA-MB-435 cells with or without the IGFI stimulation and were analyzed by immunoblotting for the indicated proteins and phosphorylation state of mTOR. Lower panel: The IGFI-dependent phosphorylation of the mTOR substrates S6K1 and Akt in the total cellular lysates.

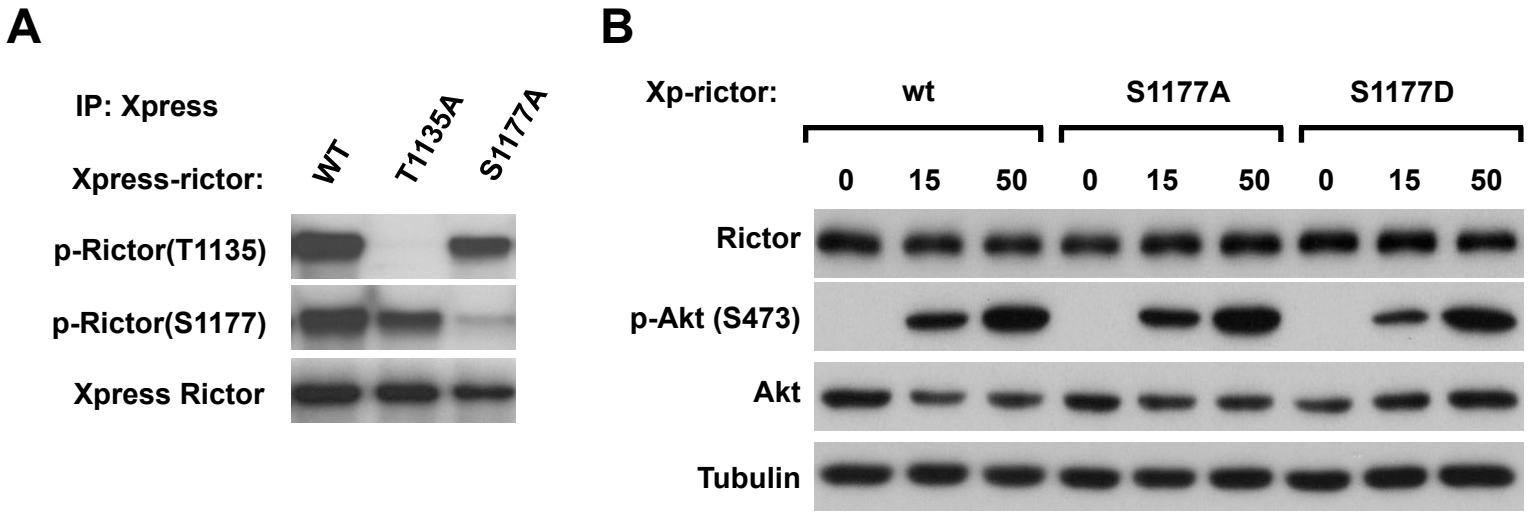
Supplemental Data, Figure 1A



Supplemental Data, Figure 1B



Supplemental Data, Figure 2



Supplemental Data, Figure 3