Supplementary information, Figure S1

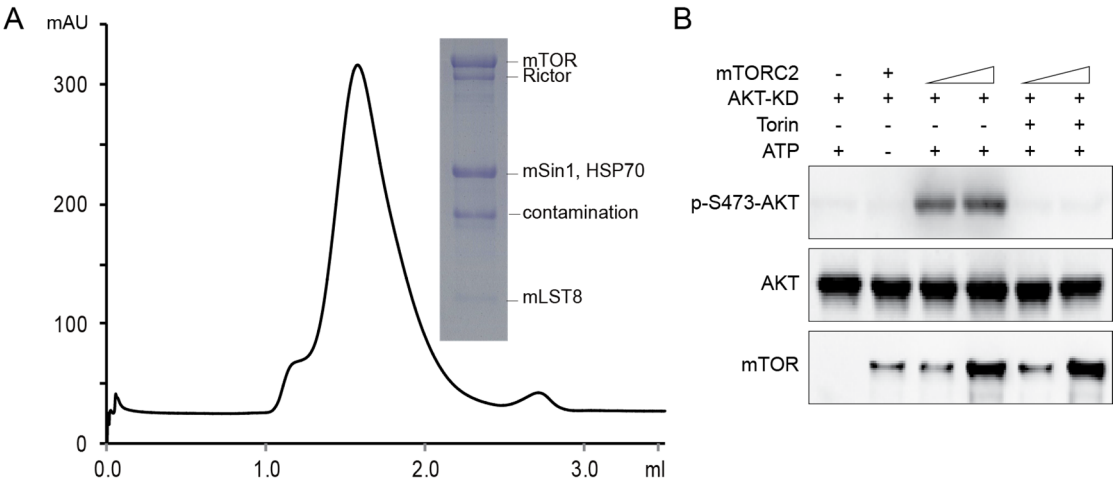

Figure S1 Purification and characterization of mTORC2 complex. **(A)** Size exclusion chromatography of the human mTORC2 complex after the gradient fixation (Grafix). The gel filtration was performed using a Superose 6 increase column (5/150 GL, GE Healthcare). The mTORC2 complex after the affinity purification was subjected to SDS-PAGE and stained by Coomassie blue. The mTORC2 complex would undergo degradation and Rictor dissociation during ion exchange and gel-filtration chromatography (data not shown). The existence of heat shock protein 70 (Hsp70) and other contaminated proteins did not affect further biochemical and structural analyses. **(B)** *In vitro* kinase assays. Phosphorylation of purified human AKT (kinase dead mutant) by increasing amount of mTORC2 in the presence or absence of Torin1, a well-characterized inhibitor of mTOR. The activity of mTORC2 was detected by immunoblotting with antibodies targeting phospho-Ser473 of AKT. The reaction products were subjected to SDS-PAGE followed by immunoblotting (13 μl reaction products). The amounts of mTORC2 and AKT used for the reactions were indicated by immunoblotting with antibodies targeting AKT and mTOR, respectively.