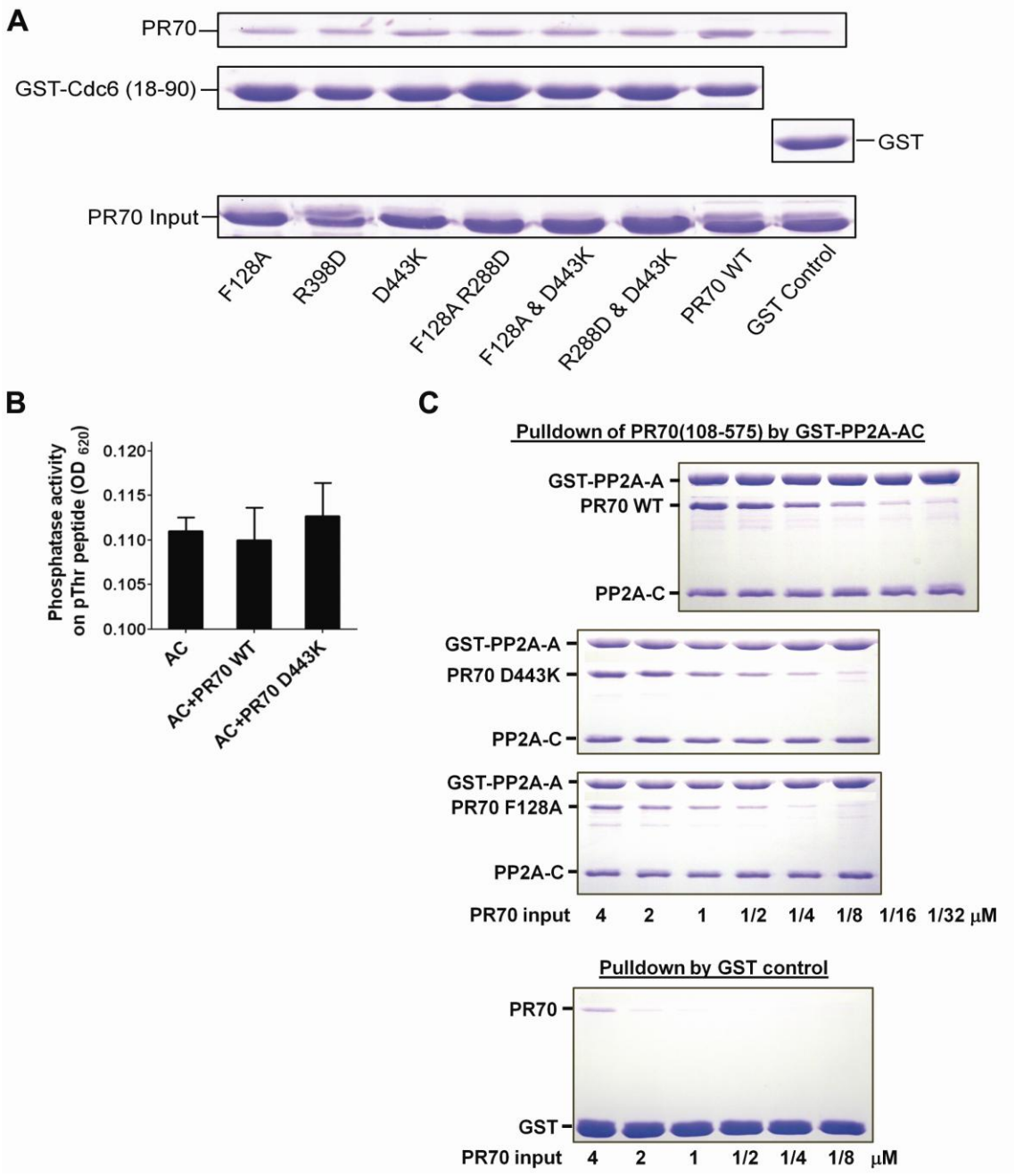


Figure S3. Related to Figure 6. (A) Pull-down of wild type and mutant PR70 (108-575) proteins by GST-Cdc6 (18-90). The PR70 proteins bound to GST-Cdc6 (18-90) immobilized on GS4B resins were examined on SDS-PAGE (upper and middle panels). GST-tag alone was used as control. The normalized input for the wild type and mutant PR70 proteins was shown by SDS-PAGE (lower panel). (B) Phosphatase activity of the PP2A core enzyme (AC heterodimer) and the holoenzymes involving PR70 (108-575) WT (AC+PR70 WT) and D443K mutation (AC+PR70 D443K) toward the phospho-

Thr peptide (K-R-pT-I-R-R), a universal substrate of all PP2A complexes. The experiments were performed in triplicate and repeated three times. Mean ± SEM were calculated. (C) Pull-down of wild type and mutant PR70 (108-575) with a series of titrated concentrations by GST-AC (PP2A core enzyme). The bound PR70 was examined by SDS-PAGE and normalized based on the signal of Coomassie blue staining. The experiments were performed three times; representative results are shown. Simulation of three independent experiments (Figure 6C) determined the estimated binding affinity ($K_d$) between PP2A core enzyme and WT and mutant PR70 (Figure 6D).