Suppl. Fig. 1

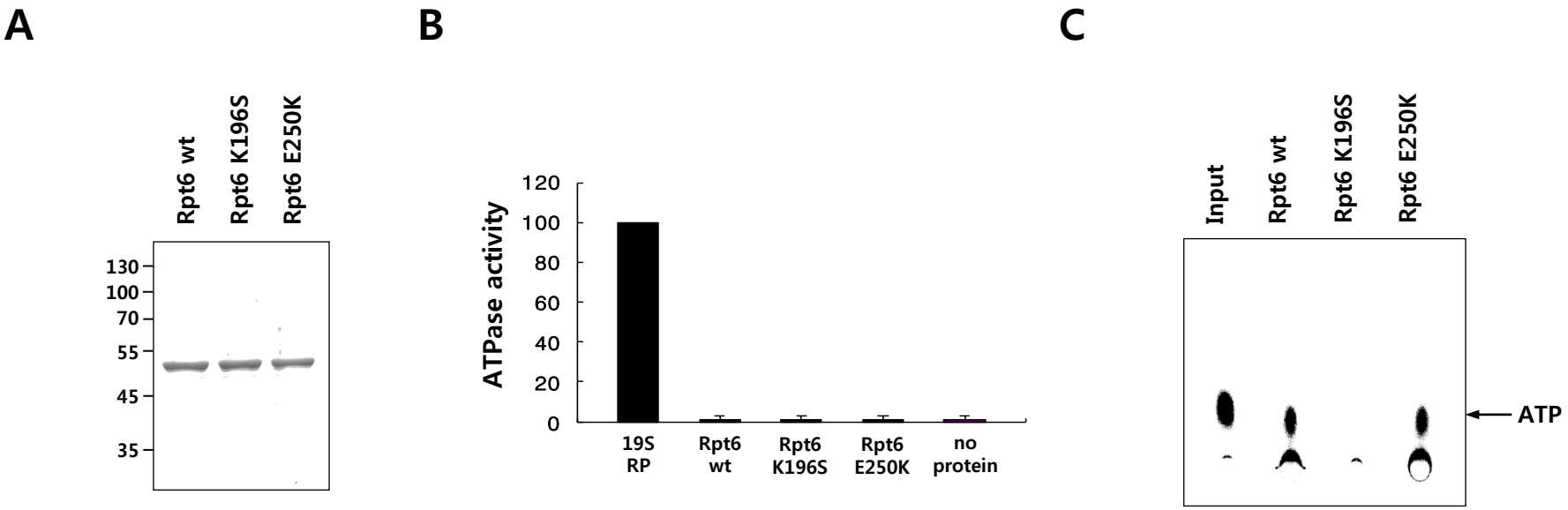

Fig. 1. ATP binding and hydrolysis activities of isolated wild-type and mutant Rpt6 proteins. *A*, SDS-PAGE analysis of purified Rpt6 proteins. HeLa cells were transfected with expression constructs of FLAG-tagged wild-type and mutant Rpt6. FLAG-Rpt6 were purified with anti-FLAG M2 affinity resin and free FLAG-Rpt6 was subsequently purified using a Superdex 200 size exclusion column. The purified fractions were analyzed by SDS-PAGE. *B*, ATPase activities of wild-type and mutant Rpt6 proteins. One μg of RP and 200 ng each Rpt6 proteins were assayed for ATPase activity as described under Experimental Procedures. *C*, ATP binding of isolated wild-type and mutant Rpt6 proteins. Two hundred ng each of purified Rpt6 proteins was incubated with 2 μCi of γ-^{32}P ATP (3000 Ci/mmole) at room temperature. ATP bound to Rpt6 was separated from the free nucleotide using a Sephadex G50 spin column. Ten μl each of the samples was spotted on a silica TLC plate and developed in a dioxane:NH_4OH: H_2O (6:2:9) solvent mixture. The radioactivity was visualized by phosphoimaging.