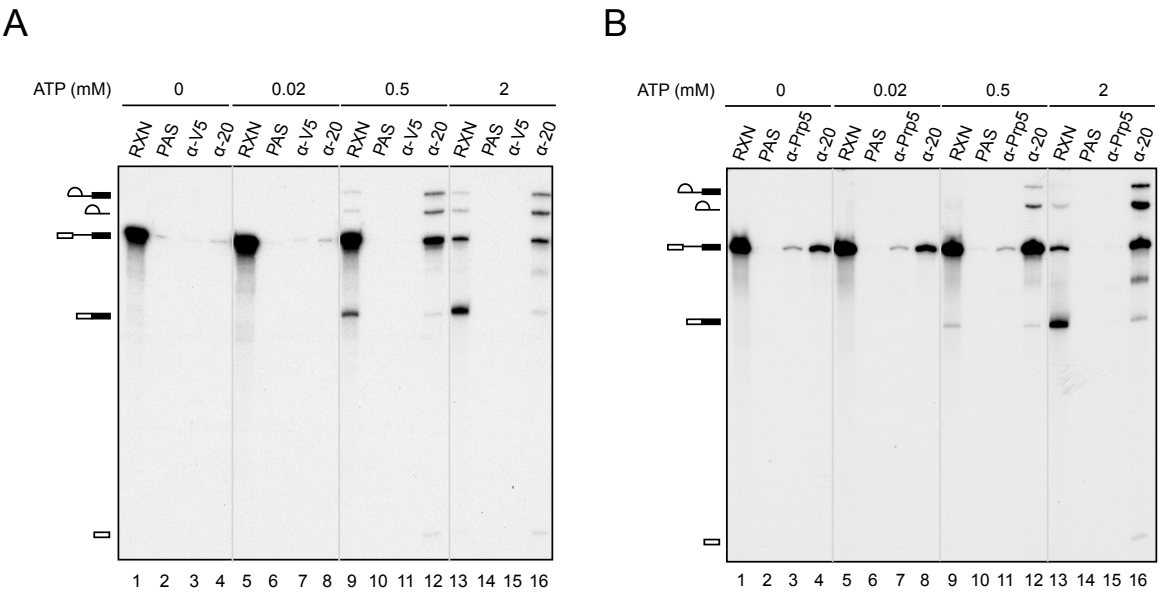

Figure S1. Prp5 is not stably associated with the spliceosome. Splicing reactions were carried out in the presence of 0 mM (lanes 1 to 4), 0.02 mM (lanes 5 to 8), 0.5 mM (lanes 9 to 12) or 2 mM (lanes 13 to 16) ATP (A) in Prp5-V5 extracts, and the reaction mixtures were precipitated with anti-Ntc20 or anti-V5 antibody, or (B) in regular extracts, and the reaction mixtures were precipitated with anti-Ntc20 or anti-Prp5 antibody. RXN, 1/10 of the reaction mixture; PAS, protein A-Sepharose; α-20, anti-Ntc20 antibody.

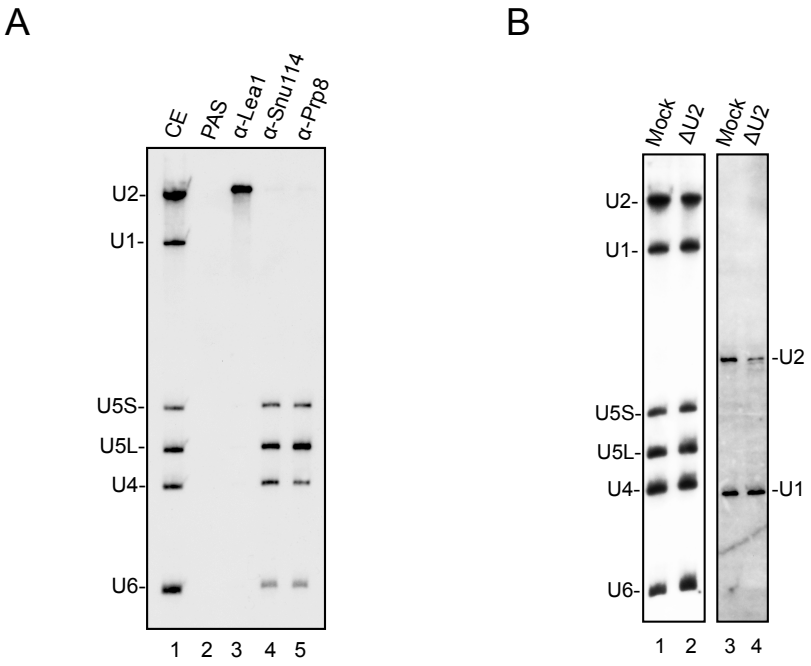

Figure S2. Ananlysis of U2 snRNA. (A) Anti-Lea1 antibody coprecipitates U2 snRNA. Splicing extracts were precipitated with antibodies against Lea1, Snu114 or Prp8, and probed with five snRNAs. CE, cell extract; PAS, protein A-Sepharose. (B) Depletion of U2 snRNA by oligo-directed RNase H cleavage. Splicing extracts were treated with U2-specific oligonucleatide U2-C, and total RNA was isolated for Northern blotting probed with five snRNAs. Lanes 3 and 4 contained the same samples as lanes 1 and 2, but with longer electrophoresis time. ΔU2, U2-C treated.

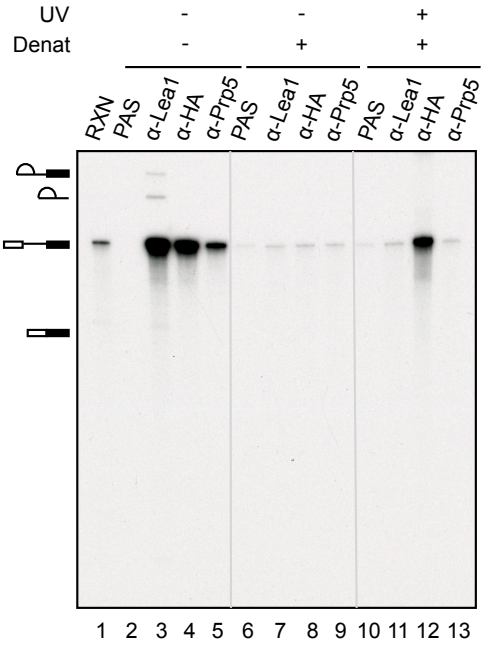

Figure S3. Prp5 does not directly interact with the pre-mRNA. Splicing reactions were carried out with U257C pre-mRNA in Hsh155-HA extracts, and the reaction mixtures were separated into three aliquots. One aliquot was not further treated (lanes 2-5), another was subjected to denaturation (lanes 6 to 9), and the third was irradiated with $UV_{254\ nm}$ followed by denaturation (lanes 10 to 13). The reaction mixtures were then precipitated with anti-Lea1, anti-HA or anti-Prp5 antibody. Denat, denaturation.

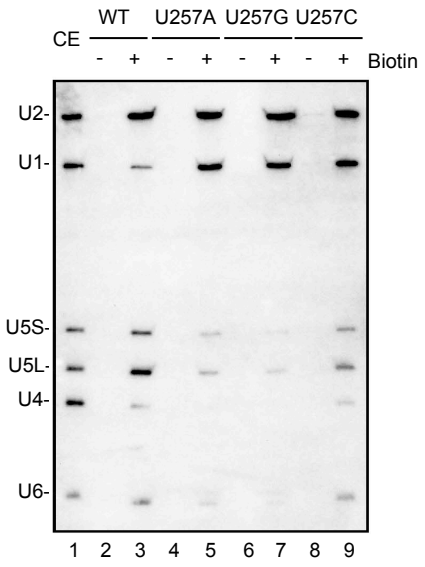

Figure S4. Spliceosomes assembled on U257 mutant pre-mRNAs do not contain tri-snRNP. Spliceosomes assembled on wild-type, U257A, U257G and U257C biotinylated pre-mRNAs were pulled down with streptavidin Sepharose, and the components were analyzed by Northern blotting probed with five snRNAs.

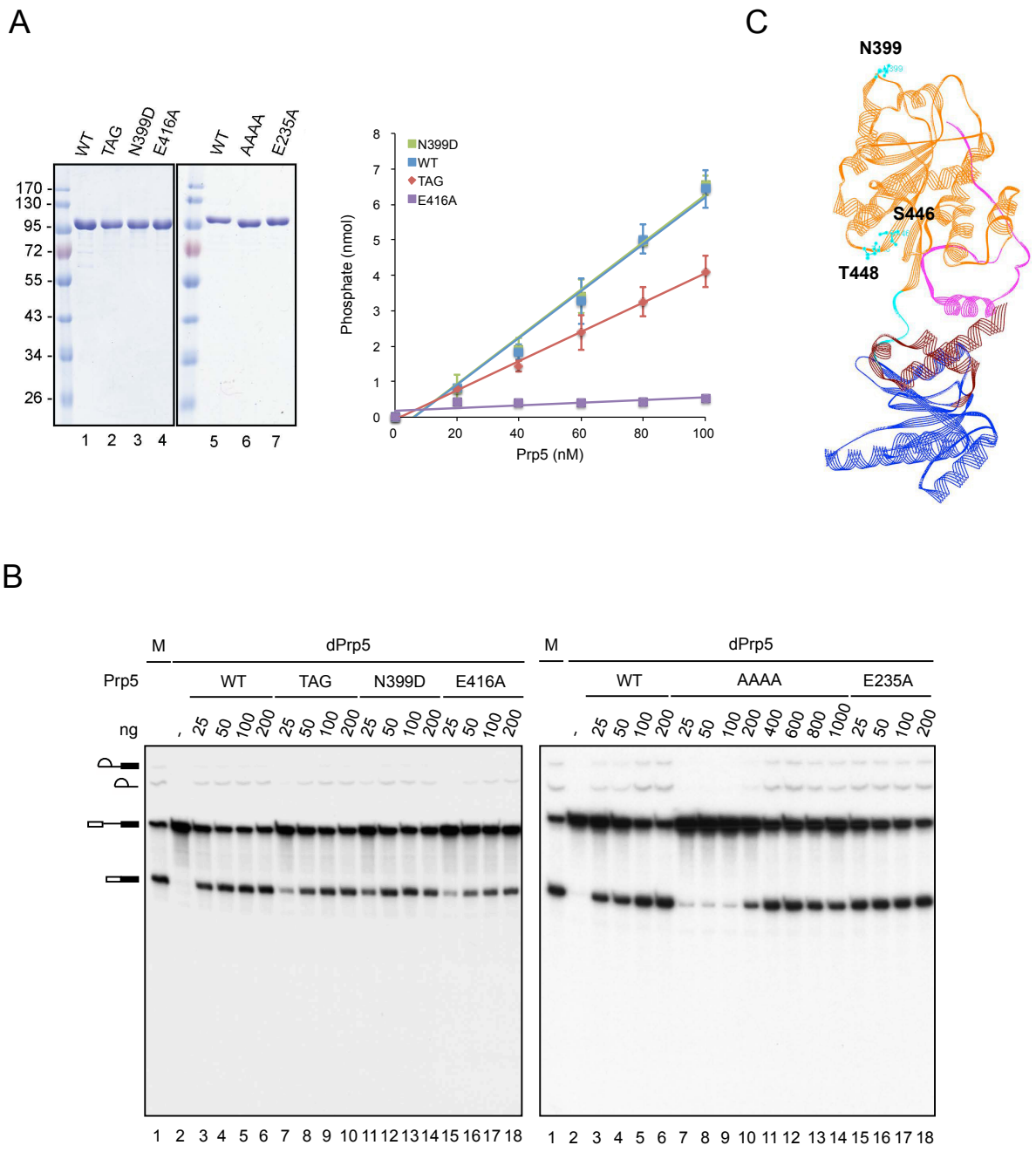

Figure S5. Analysis of recombinant Prp5 mutant proteins and the location of the mutations. (A) Purified recombinant wild-type and mutant Prp5-V5 proteins proteins stained with Coomassie blue, and ATPase assays of wild-type, N399D and TAG mutants of Prp5. (B) Complementation assays of recombinant wild-type and mutant Prp5 proteins. Purified recombinant Prp5 proteins were added in various amounts to Prp5-depleted cus2Δ extracts for splicing complementation. (C) Crystal structure of a Prp5 fragment from residue 206 to 699, with N399 and SAT motif marked. (Zhang et al., 2013).