SUPPLEMENTAL INFORMATION

C-termini of proteasomal ATPases play non-equivalent roles in cellular assembly of mammalian 26S proteasome

Young-Chan Kim and George N. DeMartino

Figure S1.

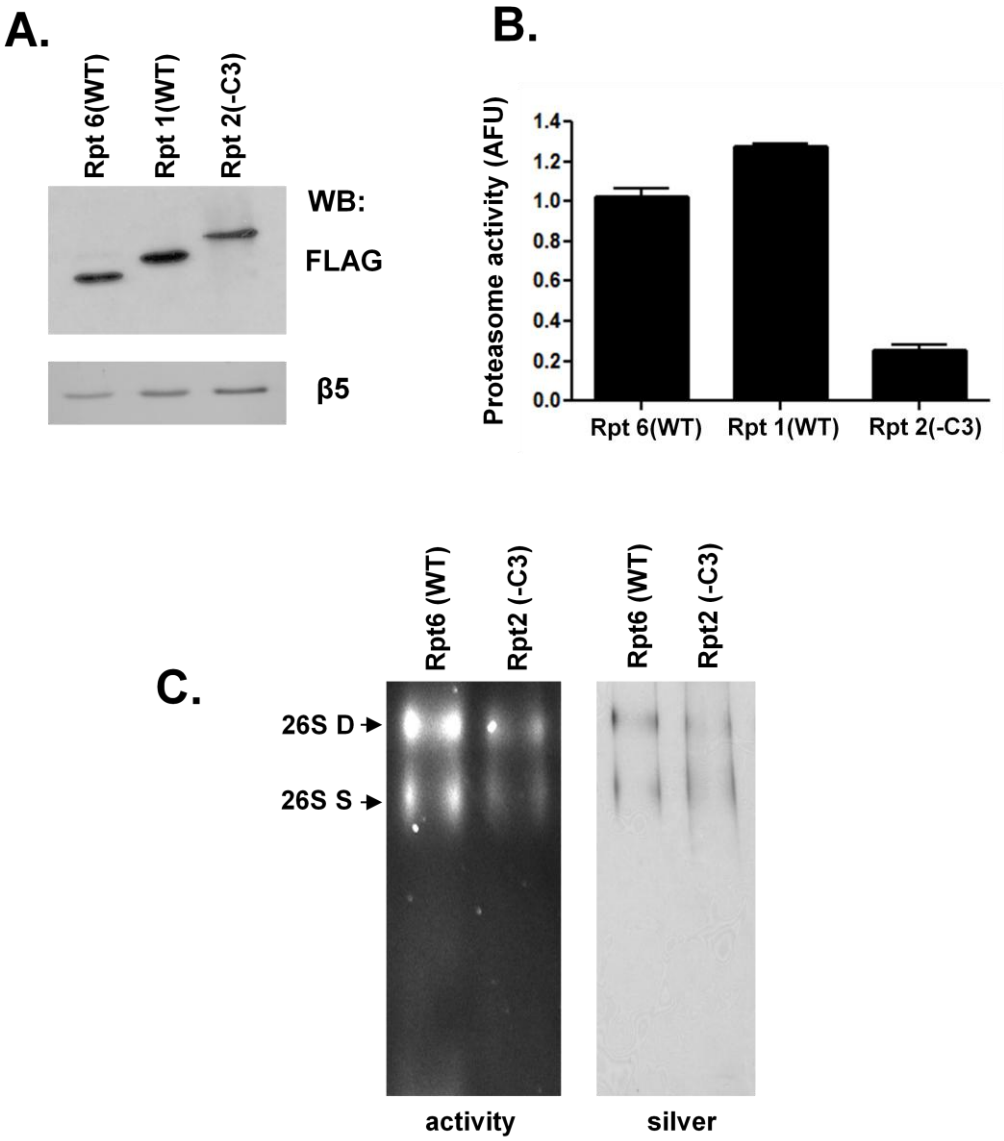

Figure S1. The C-terminal HbYX motif of Rpt2 is responsible for proteasome gating. *Panel A.* Anti-FLAG affinity purification was conducted with extracts from FLAG-Rpt6(WT), FLAG-Rpt1(WT) and FLAG-Rpt2(-C3) cells as described in the text. Affinity-purified samples were subjected to western blotting with antibodies against FLAG and the β5 subunits of 20S proteasome. The data show the equivalent levels of FLAG protein complexes contain similar levels of 20S proteasome. *Panel B.* Equivalent amounts of affinity-purified samples from Panel A were assayed for proteasome activity in solution using Suc-Leu-Leu-Val-Tyr-AMC substrate. *Panel C.* Equivalent amounts of affinity-purified samples from Panel A were subjected to native PAGE and either stained with silver (right) or assayed for proteasome activity with the peptide substrate overlay assay.

Figure S2.

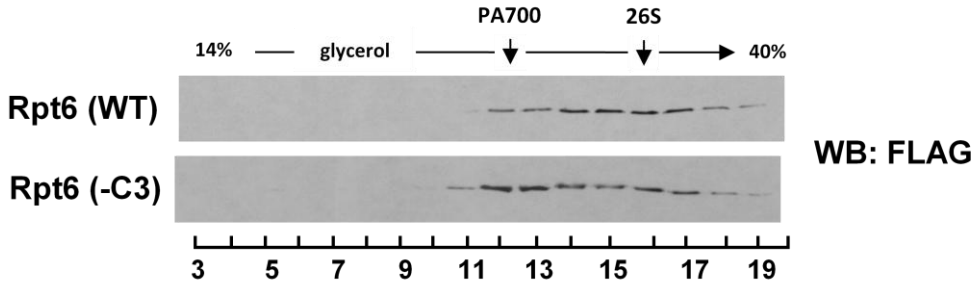

Figure S2. Deletion of C-terminal residues of Rpt6 destabilizes 26S proteasome structure. Extracts were prepared from cells expressing FLAG-Rpt6(WT) and FLAG-Rpt6(-C3) as described in Figure 7. The extracts were supplemented with NaCl to a final concentration of 150 mM and then centrifuged through glycerol density gradients containing 150 mM NaCl in gradient buffers. Gradient fractions were subjected to western blotting with antibodies against FLAG.

Figure S3.

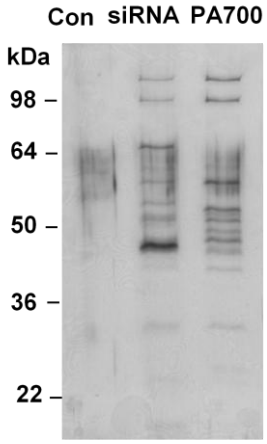

Figure S3. Detection of purified PA700 in siRNA-treated cell by SDS-PAGE. Combined fraction numbers 8-11 from each of the two glycerol gradients depicted in Figure 8F were subjected to SDS-PAGE and stained with silver. Purified bovine PA700 is presented as a standard. The data indicate that intact PA700 is detected in fractions from the siRNA gradients but not from Control gradients.

Figure S4.

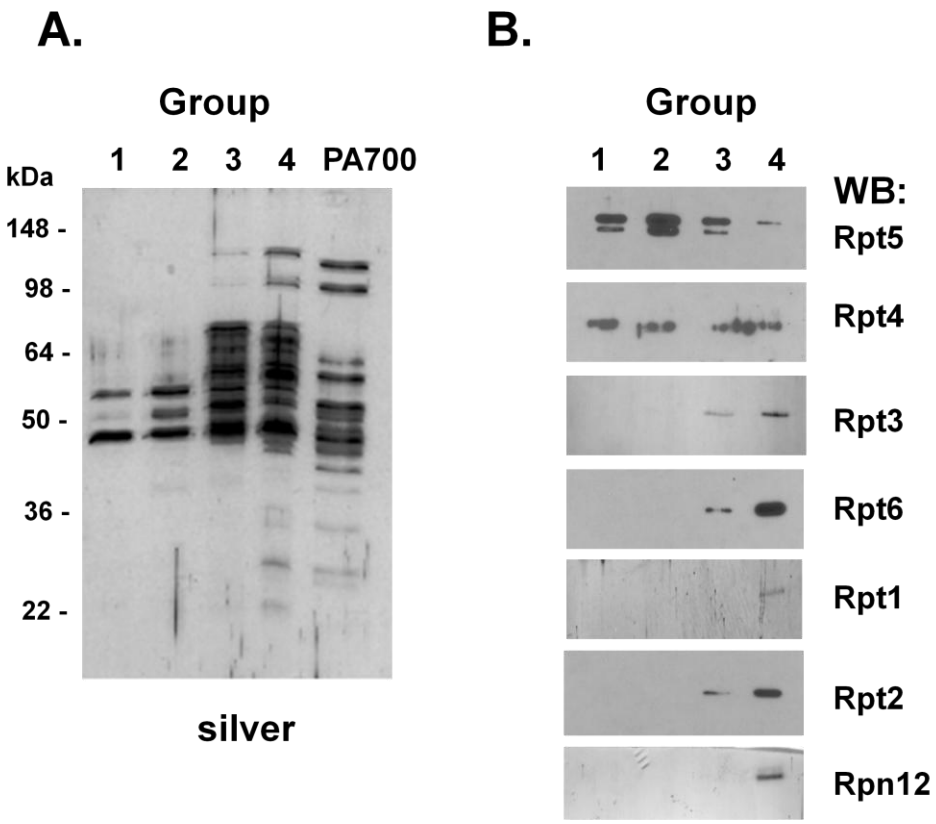

Figure S4. Detection of intermediate assembly intermediates of PA700 in cells expressing FLAG-Rpt5(-C3) . Approximately 15 µg of affinity-purified proteasome complexes from a FLAG-Rpt5(-C3) cell extract were fractionated by glycerol gradient centrifugation, as in Figure 2F. Gradient fractions 1-3, 4-6, 7-9, and 10-12 were combined and designated Group1, Group 2,Group 3, and Group 4, respectively. Each of the combined Groups was analyzed by SDS-PAGE with either silver staining (Panel A) or western blotting with antibodies against indicated subunits (Panel B). The slowest sedimenting Groups (1 and 2) contained Rpt5 and Rpt4, but not detectable levels of other PA700 subunits. Rpt5 and Rpt4 have been shown previously to associate in a PA700 assembly subcomplex. In contrast, the most rapidly sedimenting complex (Group 4) contains all other tested subunits of intact PA700 and has a subunit pattern similar to intact PA700.

Figure S5

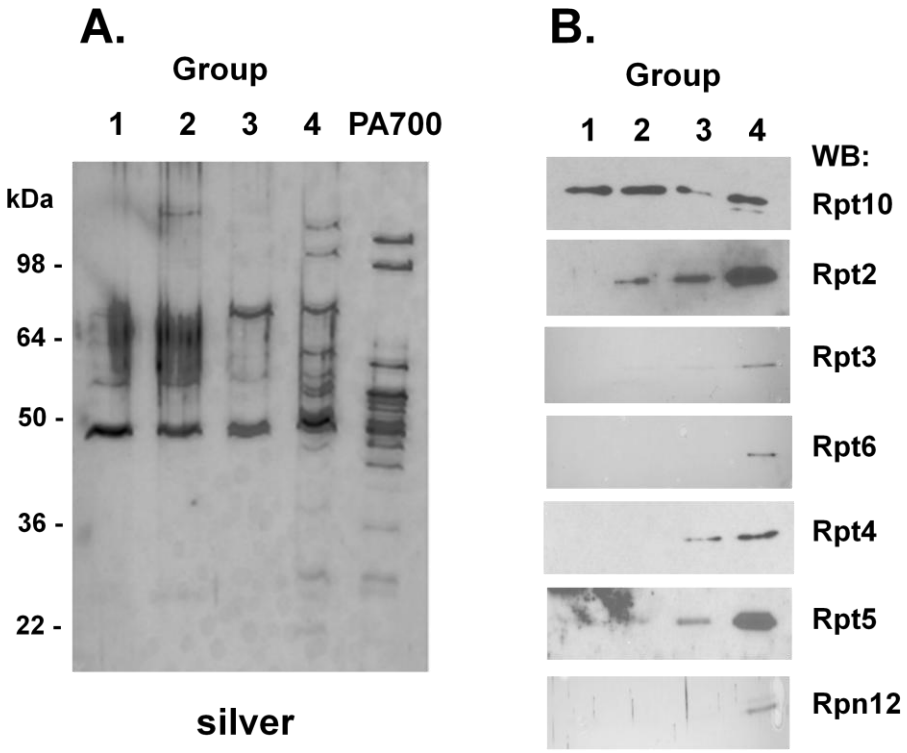

Figure S5. Detection of assembly intermediates of PA700 by affinity-purification followed by glycerol gradient analysis (FLAG-Rpt1). Approximately 15µg of affinity-purified proteasome complexes from FLAG-Rpt1(WT) cell extract were fractionated by glycerol gradient centrifugation. Gradient fractions 1-3, 4-6, 7-9, and 10-12 were combined and designated Group 1, Group 2, Group 3, and Group 4 respectively. Each of the fractions was analyzed by SDS-PAGE with either silver staining (Panel A) or western blotting with antibodies against indicated subunits (Panel B). Slowly sedimenting Group 2 fractions contained Rpt1 and Rpt2, but not most other detectable PA700 subunits. Rpt1 and Rpt2 have been shown previously to associate in a PA700 assembly subcomplex. In contrast, the most rapidly

sedimenting complex (Group 4) contains all other tested subunits of intact PA700 and has a subunit pattern similar to intact PA700.