Supplemental Data

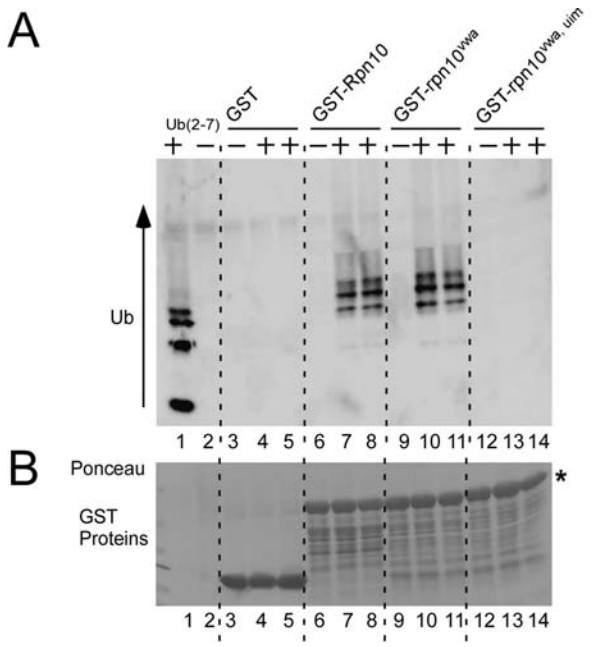

Fig. S1. Biochemical properties of rpn10 mutant proteins. GST-Rpn10, GST-rpn10^{vwa} and GST-rpn10$^{vwa,\ uim}$ were purified from *E. coli* and incubated with multiubiquitin chains (Ub_{2-7}). The bound proteins were released in SDS-containing electrophoresis buffer, and resolved in a 10% polyacrylamide gel. Binding was determined by immunoblotting using anti-ubiquitin antibodies (upper panel). As expected, no interaction was detected between GST and ubiquitin (lanes 4 and 5 represent duplicate reactions), and no spurious signal was generated with the affinity beads lacking ubiquitin chains (lane 3). In contrast, strong interactions between multiubiquitin chains and GST-Rpn10 and GST-rpn10^{vwa} were observed (lanes 7, 8 and 10, 11). Significantly, the interaction with Rpn10 and mono-ubiquitin (~ 9 kDa) and short chains (18 kDa, 27 kDa, etc) was negligible (see positions of the abundant mono- and short chain ubiquitins in lane 1), while binding to longer ubiquitin chains was evident even though their level in the preparation of mixed ubiquitin chains were markedly lower. Equal amount of all the GST-tagged proteins was detected on the glutathione-Sepharose affinity matrix (lower panel: Ponceau S staining).

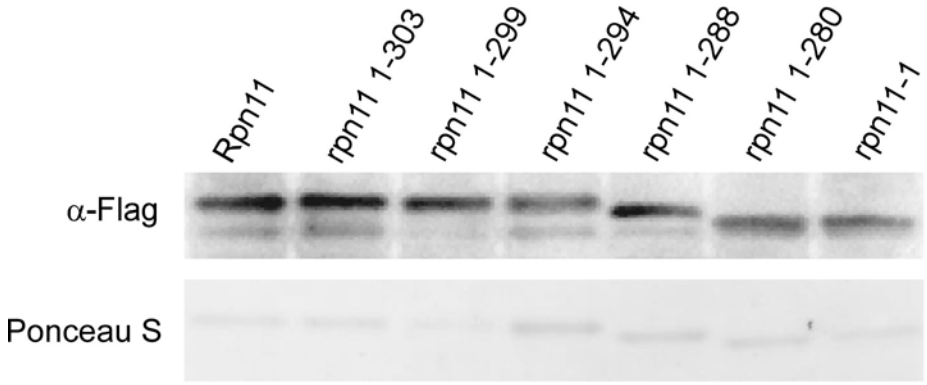

Fig. S2. Expression of carboxy-terminal truncations of Rpn11. Equal amounts of yeast extract were incubated with anti-FLAG affinity beads. The bound proteins were released by boiling in SDS-containing electrophoresis buffer, resolved by SDS/PAGE and examined. The levels and position of each mutant protein is shown by both, immunoblotting (upper panel) and Ponceau S staining (lower panel).

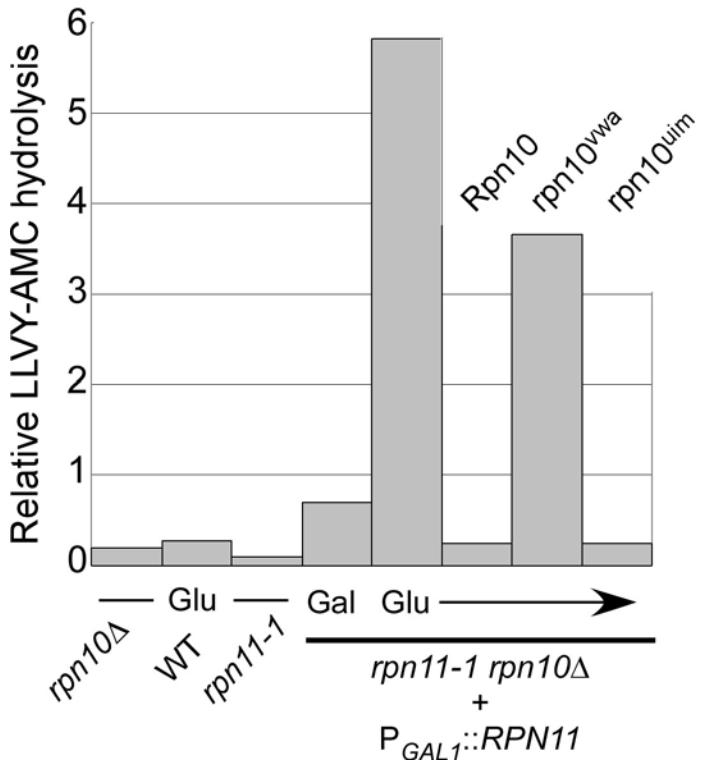

Fig. S3. Measurement of proteasome peptidase activity. ACY135 was transformed with plasmids expressing Rpn10 and rpn10 mutants. Protein lysates (5 μg) were combined with 200 ng of proteasome inhibitor epoxomicin or an equivalent volume of dimethyl sulfoxide (DMSO) lacking the inhibitor. Proteasome assay buffer (200 μl; 25 mM HEPES, pH 7.5, 0.5 mM EDTA) contained 40 μM LLVY-AMC. Reactions were incubated at 30 °C for 1 hr, and the fluorescence signal was measured using a Tecan Infinite F200. All measurements were collected in duplicate, and epoxomicin insensitive signal was subtracted and only the epoxomicin sensitive (proteasome specific) data were plotted.