SUPPLEMENTARY DATA

Ubr1 and Ubr2 function in a quality control pathway for degradation of unfolded cytosolic proteins

Nillegoda et al

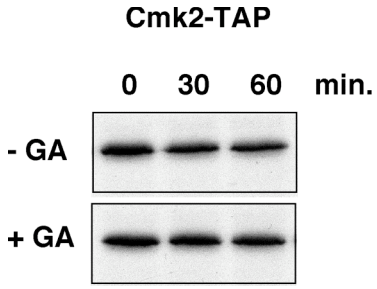


Figure S1A. **Cmk2 stability in a wild type strain treated with GA.** C-terminal TAP-tagged Cmk2 was immunoprecipitated from wild type cells after pulse chase analysis in the absence and the presence of 50μM geldanamycin.

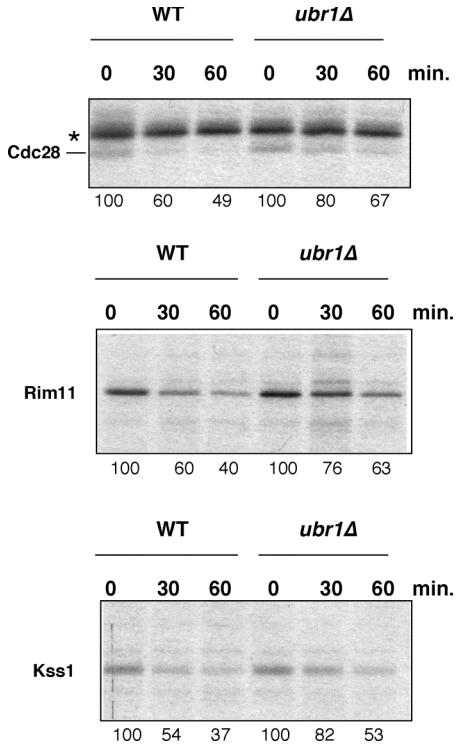


Figure S1B. **Protein kinase degradation in wild type and *ubr1*Δ strains**. Untagged Cdc28, and C-terminal TAP-tagged Rim11 and Kss1 were immunoprecipitated from wild type (WT) and *ubr1*Δ cells after pulse chase analysis in the presence of 50μM geldanamycin. Numbers below each panel refer to percentage of kinase remaining after pulse (0-time). n = 2.

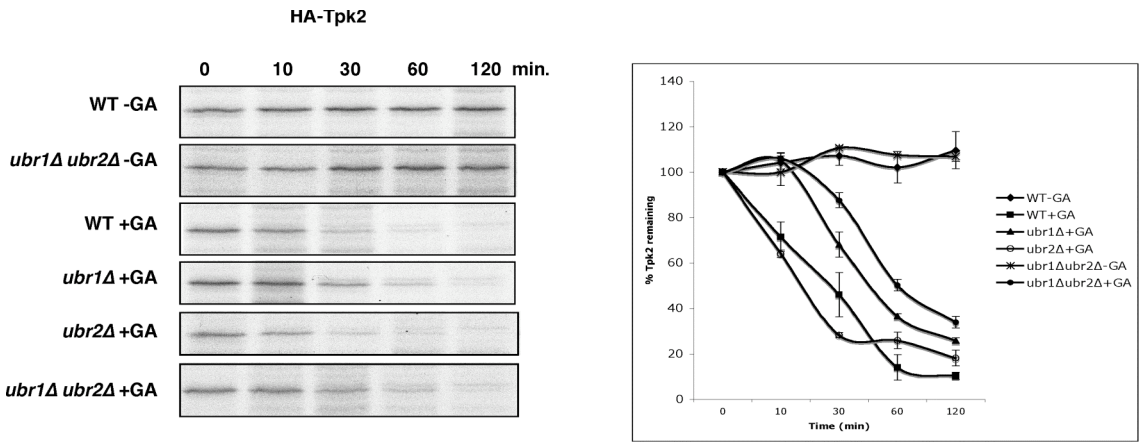


Figure S1C. **HA-Tpk2 degradation during a 2-hour chase period**. Wild type (WT), *ubr1Δ, ubr2*Δ and *ubr1Δ/ubr2*Δ strains were analyzed by pulse chase analysis. The chase period was over a two-hour period as indicated. The graph at right shows the combined analysis from three individual experiments. Note that only strains deleted for *UBR1* display defective degradation of HA-Tpk2 over the first ten minutes of chase. N = 3. Bars represent SEM.

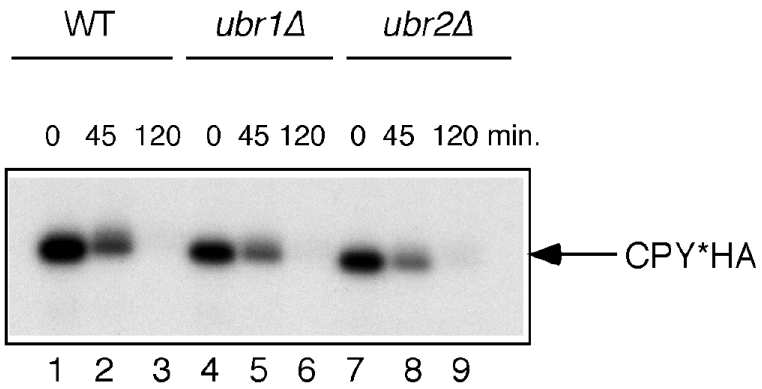


Figure S1D. **Neither Ubr1 nor Ubr2 function in ER associated degradation of CPY*.** Pulse chase analysis of CPY* in wild type cells (WT), *ubr1Δ* and *ubr2Δ* cells. The pulse was for 10 minutes and chase times as shown in the figure.

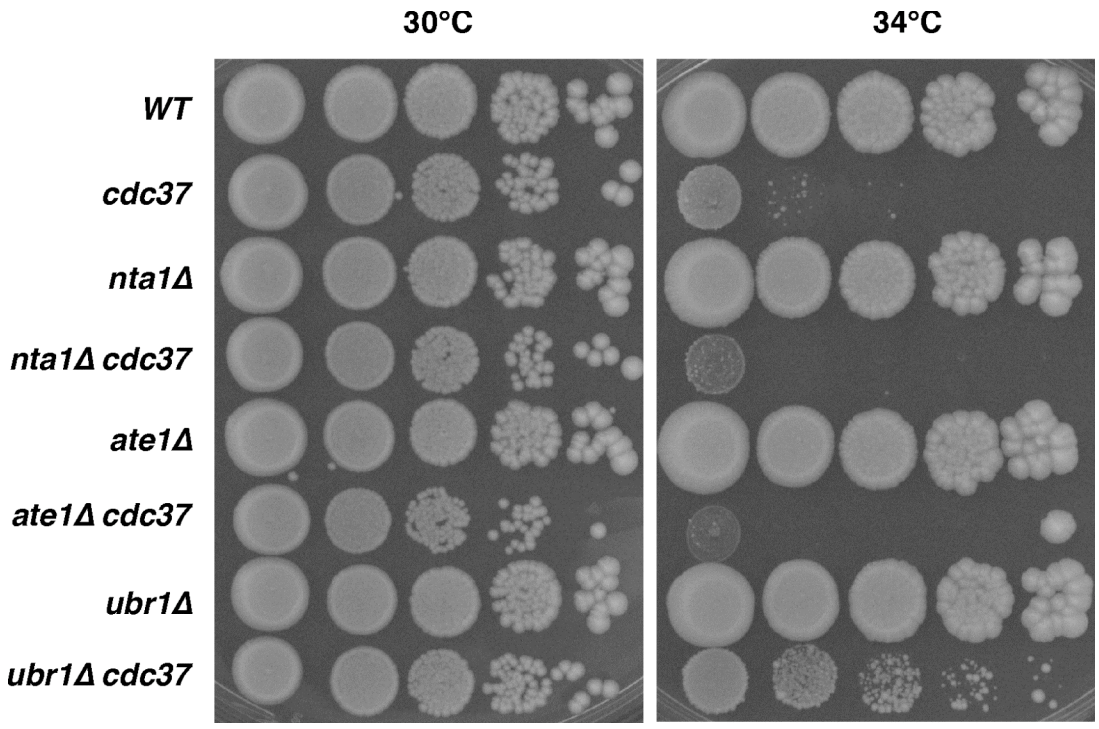


Figure S2A. **Growth of strains deleted for genes encoding N-end rule genes at 30°C and 34°C.** Yeast strains as indicated were incubated for 3 days at either temperature.

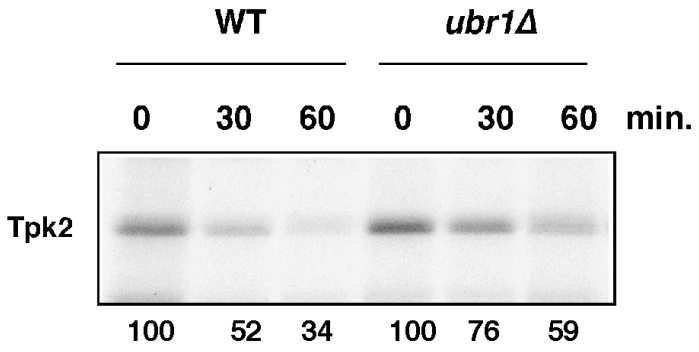


Figure S2B. **Degradation of C-terminal TAP-tagged Tpk2 in wild type and *ubr1Δ* mutant cells.** Wild type and *ubr1*Δ mutant cells were pulse labeled for 10' with ^{35}S-met followed by chase for times indicated in the figure in the presence of 50μM geldanamycin.

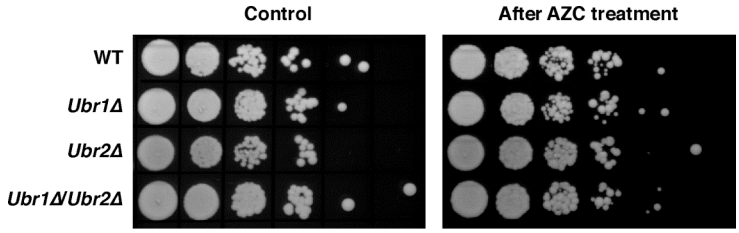


Figure S3A. **AZC has a similar effect on wild type and *ubr1/ubr2Δ* cell growth**. Wild type and *ubr1Δ, ubr2*Δ and *ubr1Δ/ubr2Δ* cells were incubated under pulse labeling conditions with 50 mM AZC, then plated in serial dilution. Cells were photographed after 3 days.

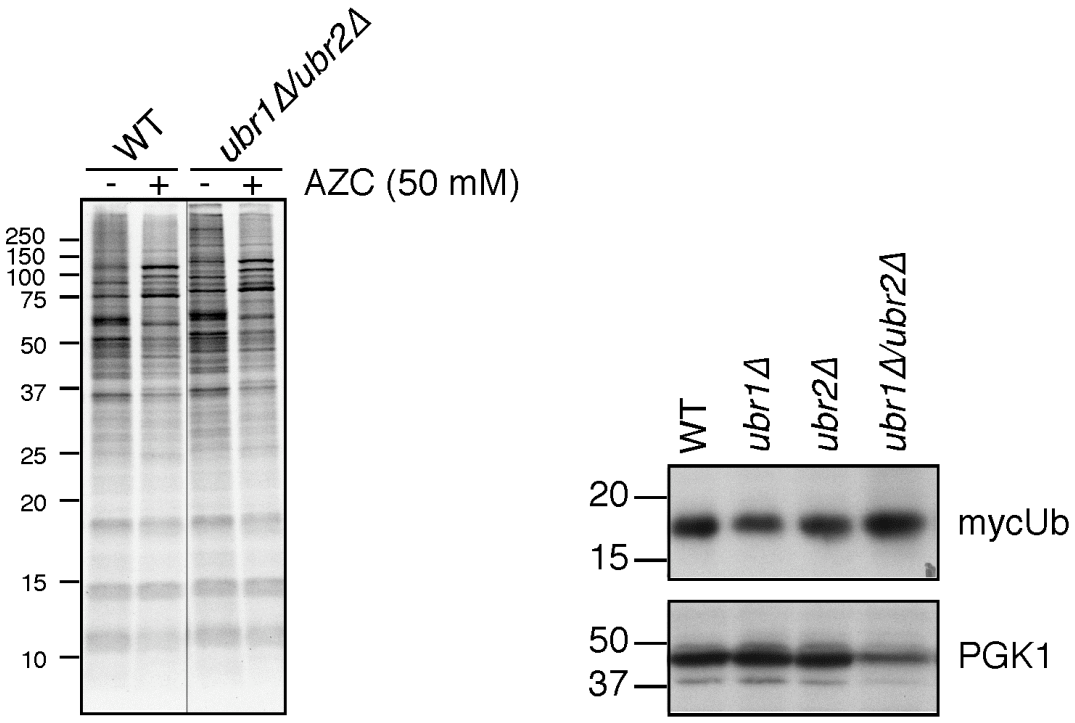


Figure S3B. **Profiles of proteins synthesized in the presence of AZC and expression of ubiquitin**. **Left panel.** Input of ^{35}S- labeled cell lysates in presence and absence of 50 mM AZC in WT and *ubr1Δ/ubr2Δ* yeast strains. 1% of total cell lysate was loaded in a 12% SDS-PAGE, gel was dried, and exposed for autoradiography. **Right panel.** Western blot of mycUb in yeast strains. myc tagged Ub was expressed from a copper inducible plasmid after 15 hrs of induction. Pgk1 serves as a loading control.

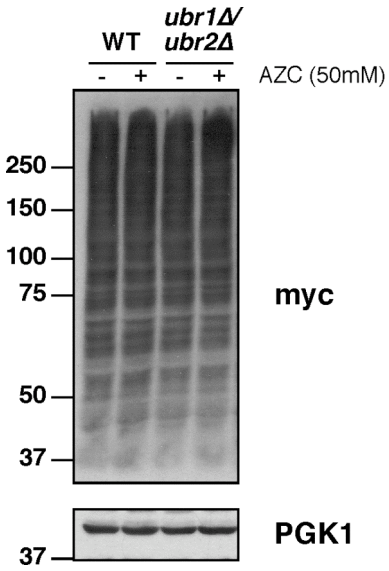
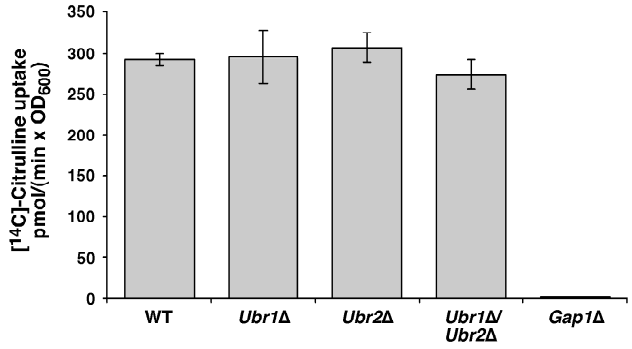


Figure S3C. **Western blot of anti-myc from extracts expressing Myc-Ubquitin in wild and ubr1Δ/ubr2Δ cells in the absence and presence of AZC**. PGK is shown as a loading control (bottom panel)



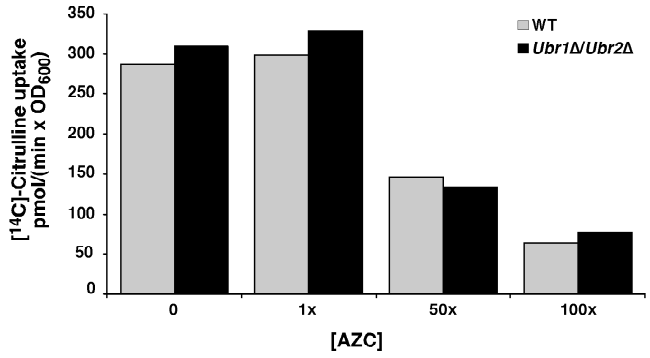


Figure S3D. **AZC uptake is independent of Ubr1 and Ubr2. Upper panel**. The activity of the Gap1 permease was determined in WT, *ubr1*Δ, *ubr2*Δ, *ubr1*Δ/*ubr2*Δ and *gap1*Δ strains by measuring the uptake of L-[^{14}C]-citrulline. The bars represent the standard error of three independent experiments. **Lower Panel.** A competition assay was performed by titrating AZC against 35.7μM of L-[^{14}C]-citrulline. The AZC concentration is given as fold excess. For the competition assay, AZC was used at the same time with L-[^{14}C] citrulline in concentrations up to 100 times more than L-[^{14}C] citrulline.

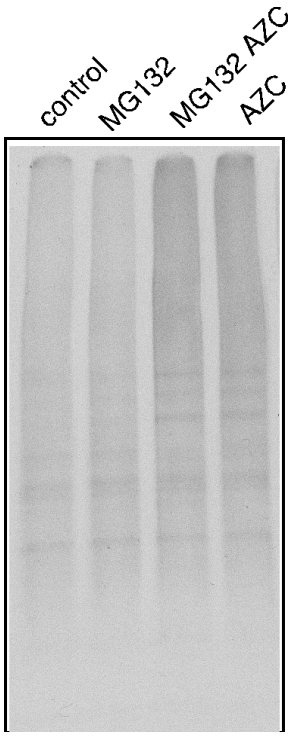


Figure S3E. **Comparison of the effects of MG132 and AZC on the profile of ubiquitinylated newly synthesized proteins**. Pulse labeling analysis was performed as per materials and methods with 50mM AZC and 100μM MG132 (preincubated for 45 and 30 minutes respectively prior to labeling) using a wild-type strain expressing myc-ubiquitin. Figure shows profile of newly synthesized proteins immunoprecipitated with anti-myc.

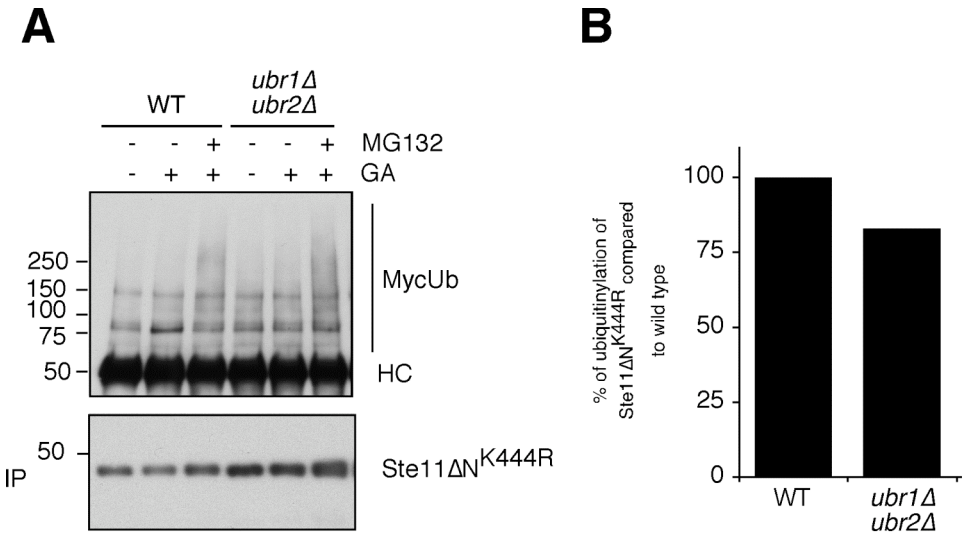


Figure S3F. **Ubiquitinylation of a protein kinase**. A. Ubiquitinylation assay of Ste11Δ^{NK444R} in the absence and presence of GA (25μM) and the proteasome inhibitor MG132 (100μM) for one hour. Ste11Δ^{NK444R} was expressed in wild type and *ubr1Δ/ubr2Δ* strains co-expressing myc-Ub. The Ste11Δ^{NK444R} was immunoprecipitated after treatment and a Western blot analysis performed with anti-myc (upper panel) and anti-His for Ste11ΔN^{K444R} (lower panel). The ubiquitinylation smear above the heavy chain (HC) was quantified and normalized to the levels of Ste11ΔN^{K444R} from the lower panel. The normalized data are shown in the bar graph in panel B (mean of two independent experiments). Method for immunoprecipitation is detailed in Mandal et al, Mol. Biol. Cell (2010) *accepted for publication March 17, 2010*.

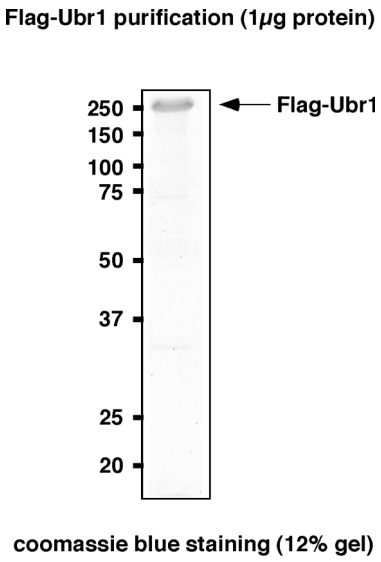


Figure S4A**. Coomassie Blue stained gel of FLAG affinity- purified Ubr1**.

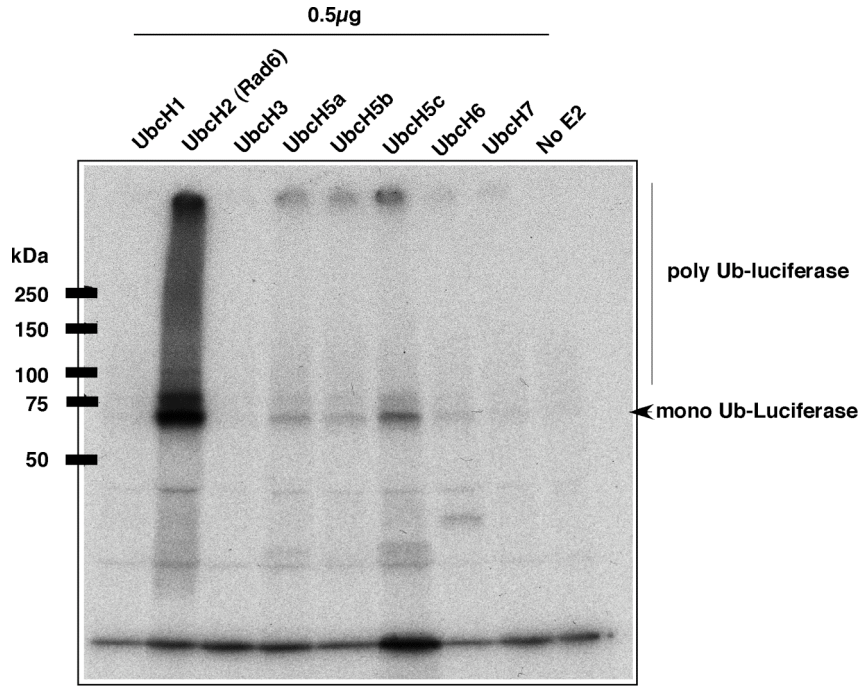


Figure S4B. **Specificity of E2 enzymes in Ubr1 activity.** Heat denatured luciferase was incubated with Ubr1, ATP and ^{32}P-labeled ubiquitin (see Materials and Methods in main paper) and different human E2 enzymes as noted in the figure. The major activity is from UbcH2, the human Rad6 ortholog.

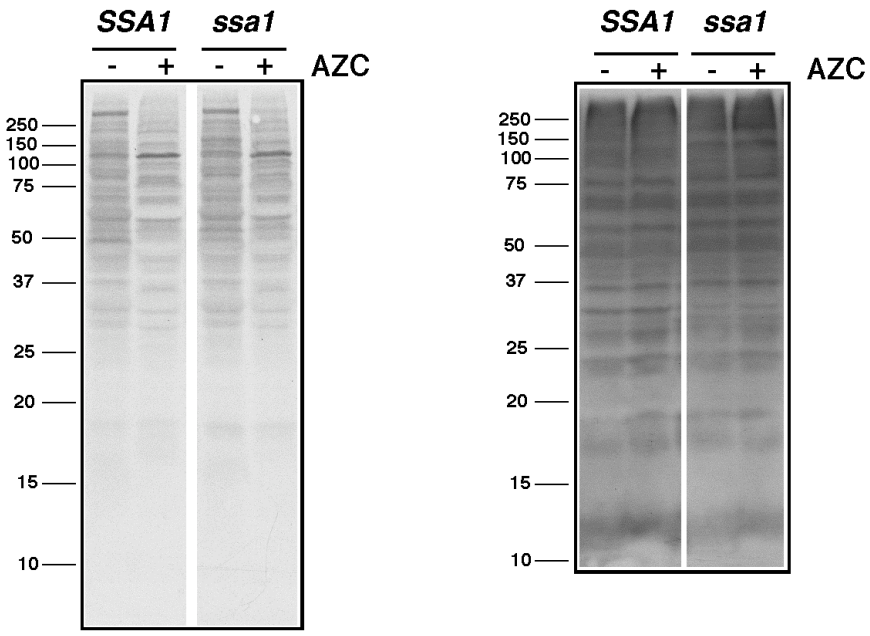


Figure S4C. **Analysis of protein levels and ubiquitin in *SSA1* and *ssa1* mutant strains**. A. Input for the experiment shown in Fig. 7A of the main paper. Input is 15% of sample used for immunoprecipitation. B. Western blot analysis with anti-myc performed on input samples to analyze for myc-Ubiquitin levels.

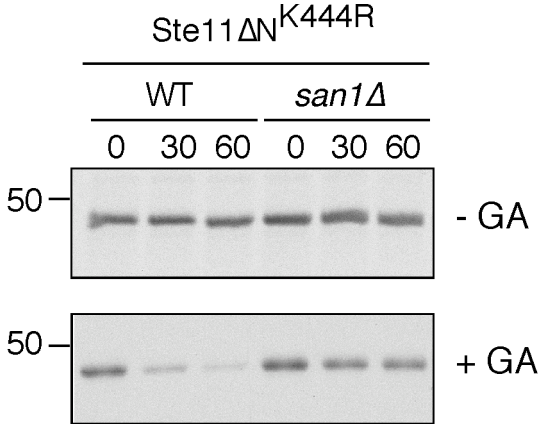


Figure S5. **Role for San1 in degradation of a protein kinase**. Pulse chase analysis of Ste11Δ^{NK444R} in the absence (top panel) and presence (lower panel) of 50μM GA. Time of chase is shown in minutes.