Expanded View Figures

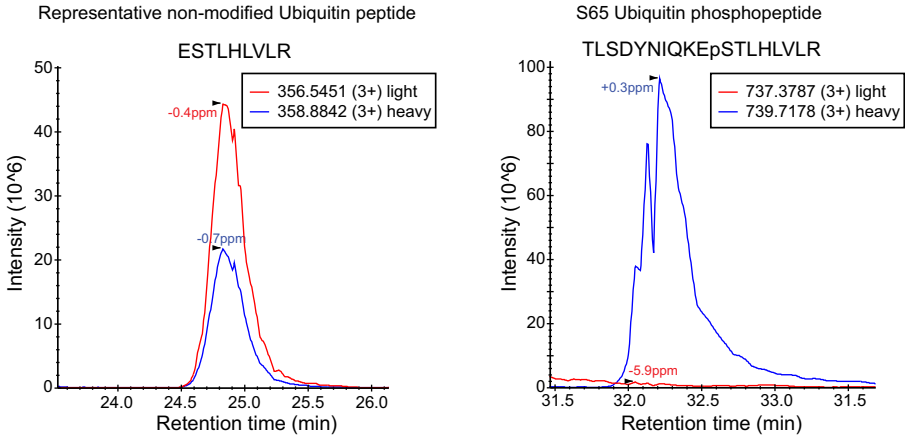


Figure EV1. Phosphorylation stoichiometry of ubiquitin Ser65.
Extracted ion chromatograms of a representative non-modified ubiquitin peptide (left) and S65 ubiquitin phosphopeptide (right). Red lines correspond to endogenous peptides, and blue lines are heavy-labeled AQUA peptides, which served as internal standards for quantification. S65 AQUA phosphopeptide was spiked in at 1% of the non-modified ubiquitin peptides prior to IMAC enrichment.

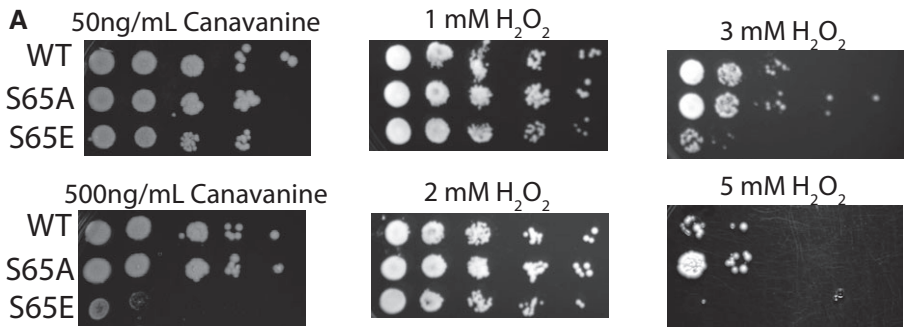
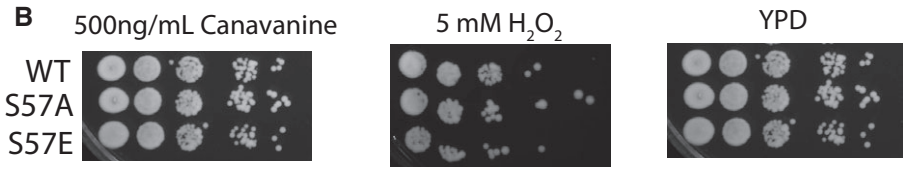


Figure EV2. The effect of H_2O_2 and canavanine on the cell viability of ubiquitin S65 mutant strains.
A Viability assay by serial dilution (1:10) of yeast strains exclusively expressing either WT or S65 mutant, ubiquitin. Strains were spotted on plates at different concentrations of the stressors hydrogen peroxide and canavanine (as indicated).
B For two conditions under which S65E strains were sensitive, the spotting assays were also performed with mutants to an alternate phosphorylation site, S57.



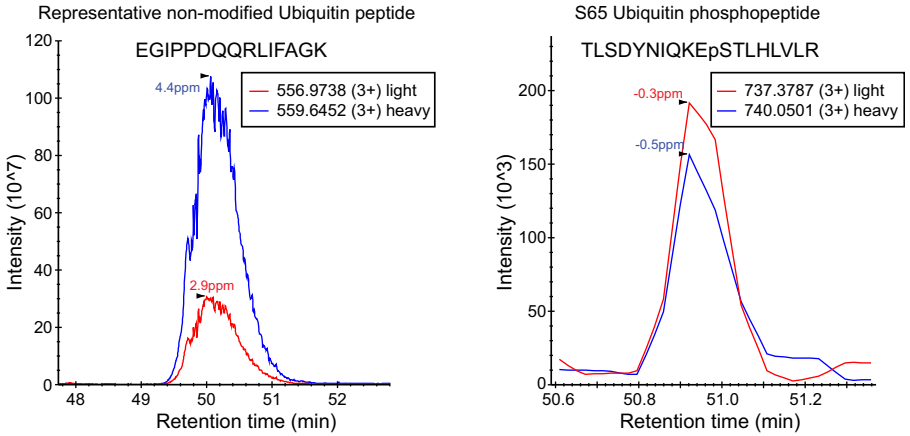


Figure EV3. Quantification of ubiquitin and S65 ubiquitin upon canavanine treatment.
Extracted ion chromatograms of a representative non-modified ubiquitin peptide (left) and S65 ubiquitin phosphopeptide (right). Blue lines correspond to heavy-labeled peptides from the canavanine treated culture, and red lines correspond to light peptides from the control culture.

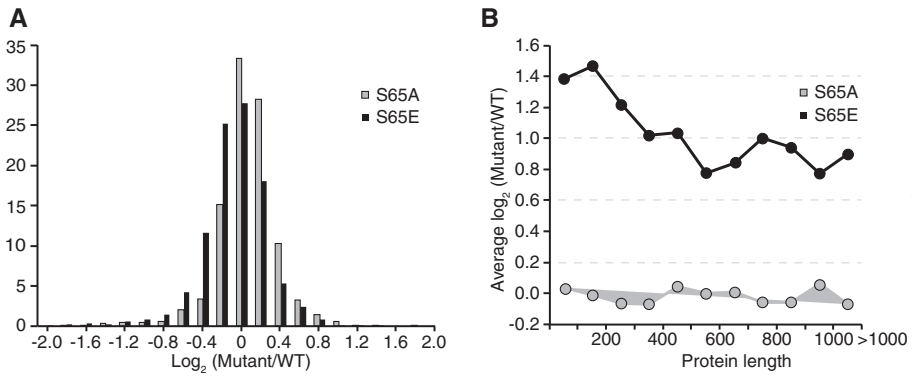


Figure EV4. Quantification of S65 ubiquitin mutant proteins.

A Log_2 distribution of protein abundance in S65A and S65E mutant strains relative to WT.

B Histogram displaying the average of the log_2 relative ubiquitylation site abundance (mutant/WT) in each protein length bin.

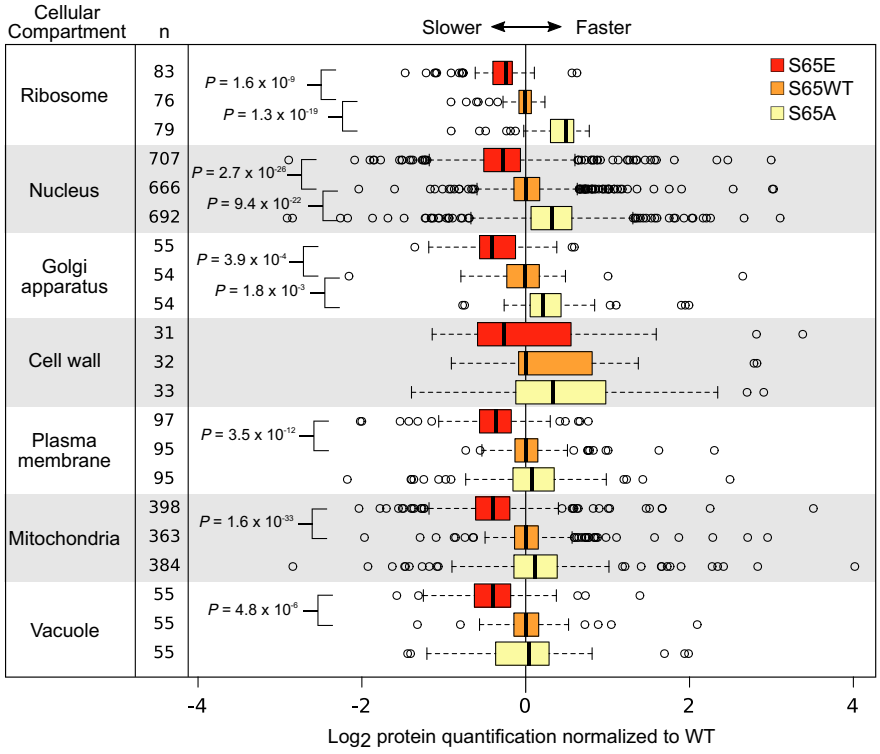


Figure EV5. Protein turnover rates in different cellular compartments.

Comparison of global turnover rates for different compartments, measured as log_2 (heavy/medium). In the box plot, center lines show the median of each distribution, and all distributions have been normalized such that the median of the WT sample is centered at zero. Strains with rates slower than WT have median values < 0, while those with faster rates have median values > 0. Box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, and outliers are represented by empty circles. *P*-values displayed are the result of a two-sided *t*-test assuming unequal variance. The number of proteins on each compartment is also provided (*n*).