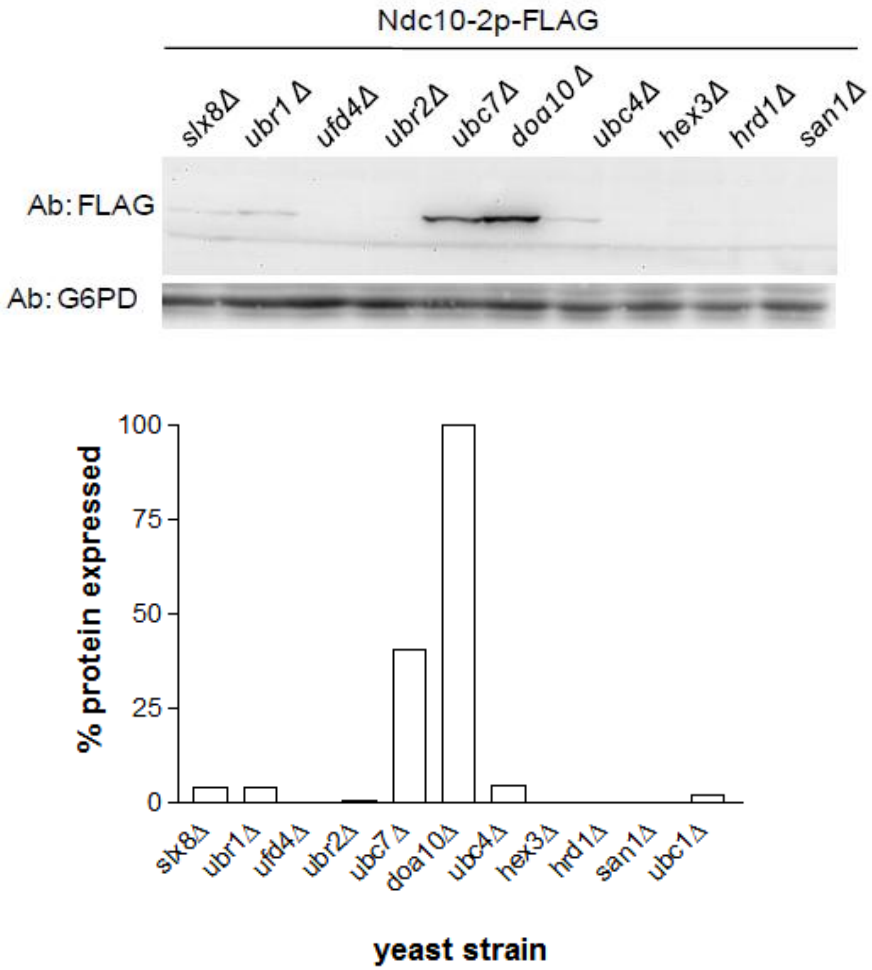
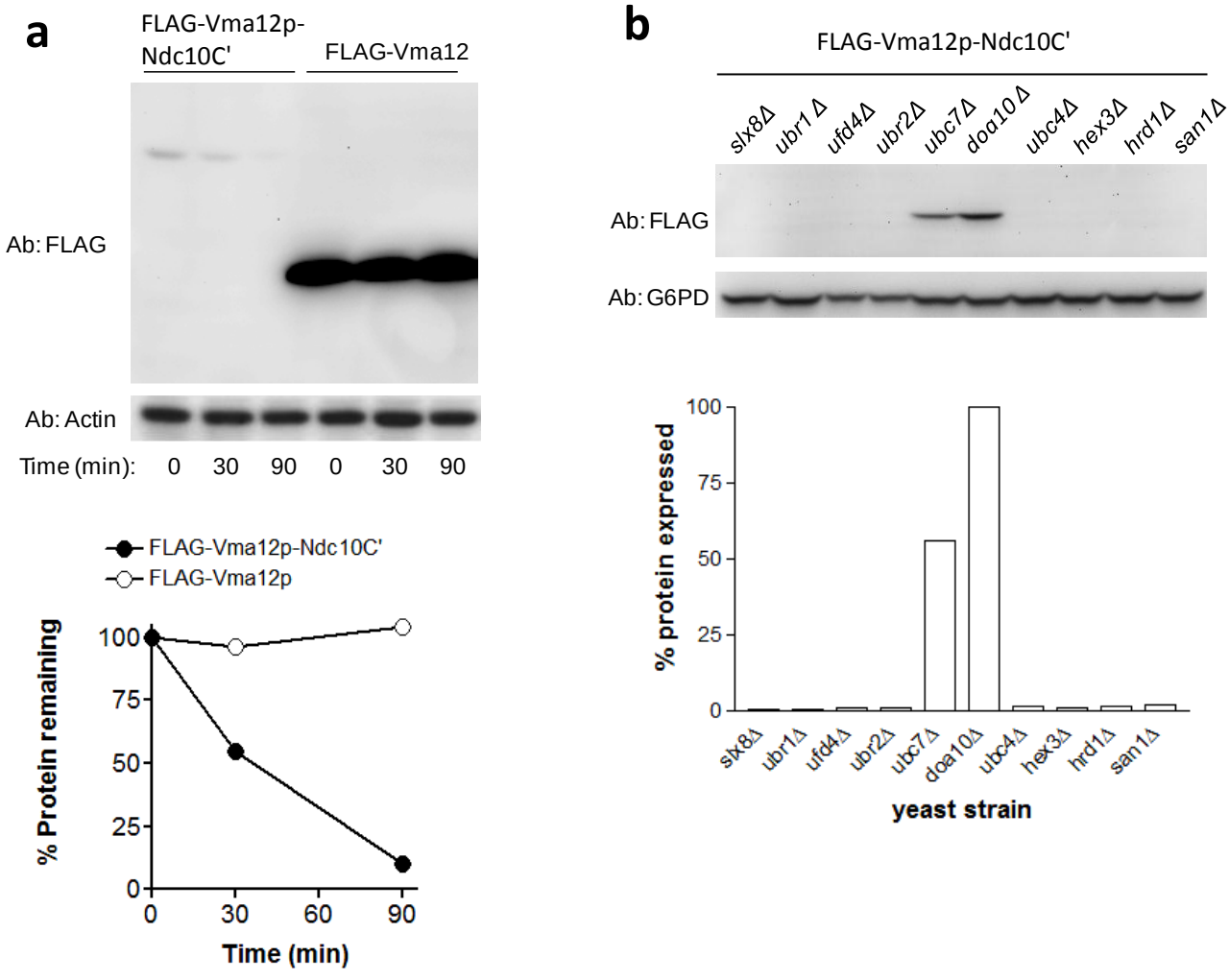
Supplementary Information

Supplemental Figures

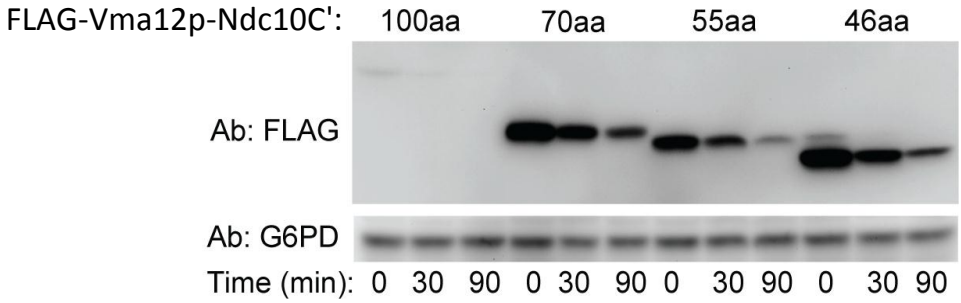
Supplementary Figure S1. The Doa10p complex is the major ubiquitylation system for Ndc10-2p degradation. *Upper panel:* Steady state levels of Ndc10-2p-FLAG in the indicated knockout strains were visualized by anti-FLAG immunobl otting. *Lower panel:* Quantitative analysis of the data in the upper panel. Ndc10-2p-FLAG levels were compared to that obtained for *doa10Δ* strain (100%).

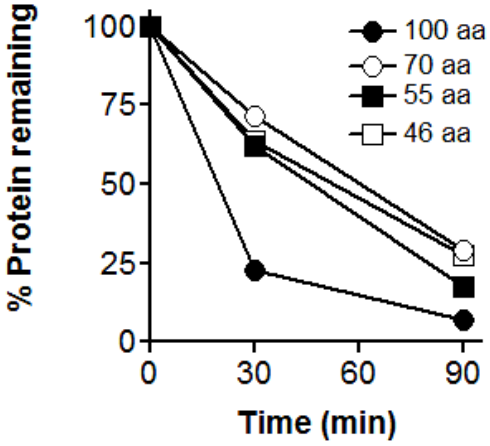


Supplementary Figure S2. FLAG-Vma12-Ndc10C' is a Doa10p substrate. (a) *Upper panel*: FLAG-Vma12-Ndc10C' is rapidly degraded while FLAG-Vma12p is a stable protein. Protein levels were measured after cycloheximide addition at the indicated time points. Proteins were subjected to immunoblotting after separation on SDS-PAGE using anti FLAG and anti actin antibodies. *Lower panel*: Quantitative analysis of the data. (b) *Upper panel:* Steady state levels of FLAG-Vma12p-Ndc10C' in different knockout strains visualized by anti-FLAG immunoblotting. *Lower panel:* Quantitative analysis of the data. Ndc10-2p-FLAG levels were compared to that obtained for *doa10Δ* strain (100%).

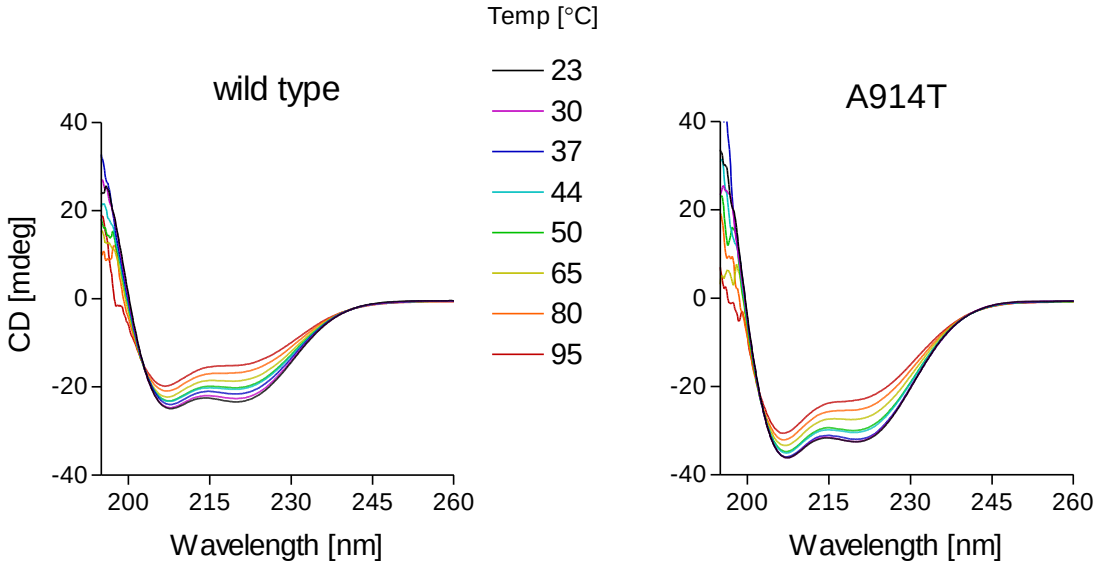


Supplementary Figure S3. Degradation kinetics of FLAG-Vma12-Ndc10C' truncation mutants. *Upper panel:* Systematic Ndc10C' N-terminal truncations in Vma12p-Ndc10C' were assayed for turnover rates using cycloheximide chase followed by anti-FLAG and anti-G6PD immunoblotting. *Lower panel:* Quantitative analysis of the data.

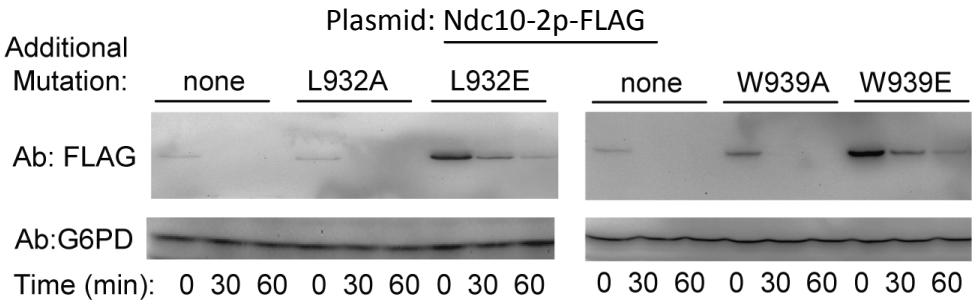




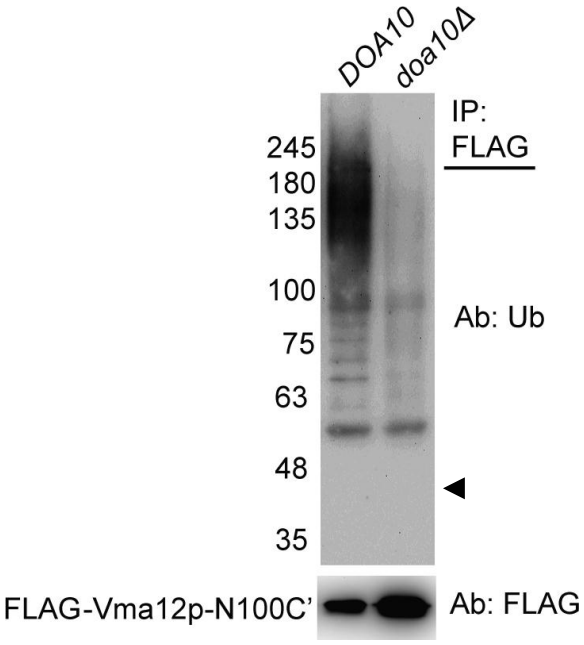
Supplementary Figure S4. Circular dichroism absorbance of Ndc10p- and Ndc10-2p (A914T)- derived peptides. Peptides derived from 910-942 aa of Ndc10p and Ndc10-2p were solubilized in 5% dodecylphosphocholine in phosphate buffer saline, and CD spectra (195-260 nm) were measured as a function of temperature elevation (23-95°C). Values for each measurement are an average of 5 consecutive repetitions.



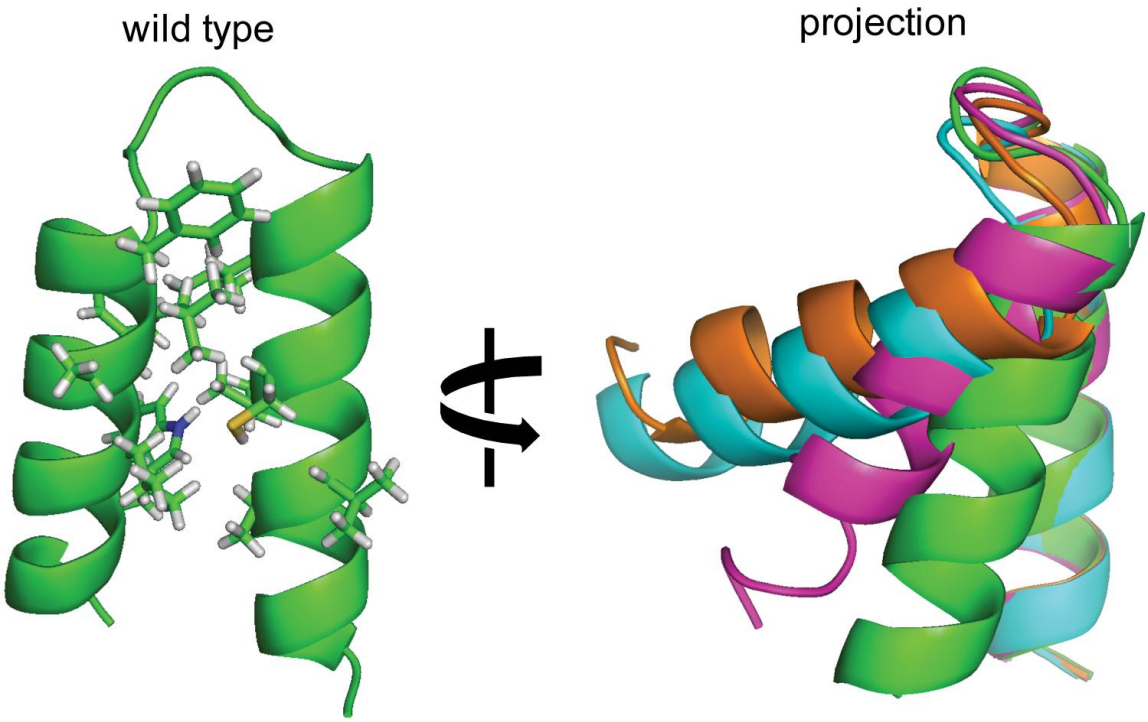
Supplementary Figure S5. Degradation kinetics of Ndc10-2p(A914T)-FLAG mutant proteins expressed in *ndc10Δ* strain. Protein degradation was assessed by cycloheximide chase followed by anti-FLAG and anti-G6PD immunoblotting.



Supplementary Figure S6. Ubiquitylation of FLAG-Vma12p-Ndc10C' extracted from microsomes. Microsome-enriched fractions were prepared from *DOA10* and *doa10Δ* cells, expressing FLAG-Vma12p-Ndc10C'. Proteins were extracted from the microsomes by boiling in SDS-sample buffer, followed by dilution and immunoprecipitation with anti-FLAG affinity gel. Ubiquitylated conjugates were visualized by anti Ub immunoblotting.



Supplementary Figure S7. Proposed model for the mechanism of misfolding and degradation of Ndc10-2p. 3D structure predictions of the helix-loop-helix region (908-943 aa) in Ndc10p C-terminus, calculated by the I-TASSER server (Roy et al., 2010). C-score: a confidence score for estimating the quality of predicted models by I-TASSER (Typical values are -5-2, where a C-score of -1.5 or higher signifies a model with a relatively high probability (Roy et al., 2010).



Color index		C-score
Green-	wild type	-1.18
Magenta-	A914T	-1.26
Cyan-	L921E	-1.33
Orange-	W939E	-1.13

Figure S8. Quantitation of Cycloheximide-chase experiments. Results are presented as % of protein remaining after cyxcloheximide addition. Value of 100% was given to protein levels in the steady state (time 0).

Figure 1

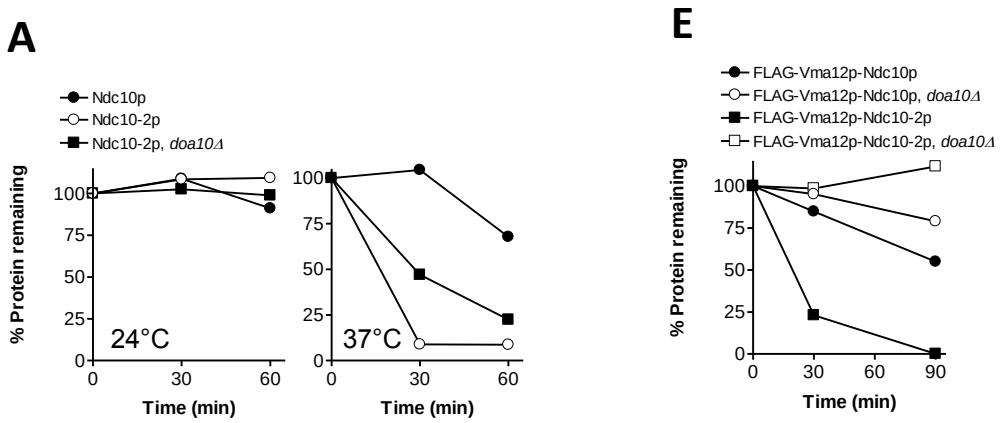


Figure 2

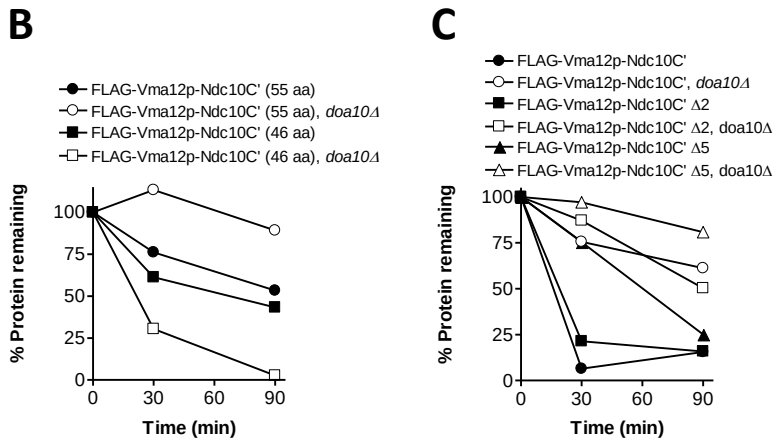


Figure 3

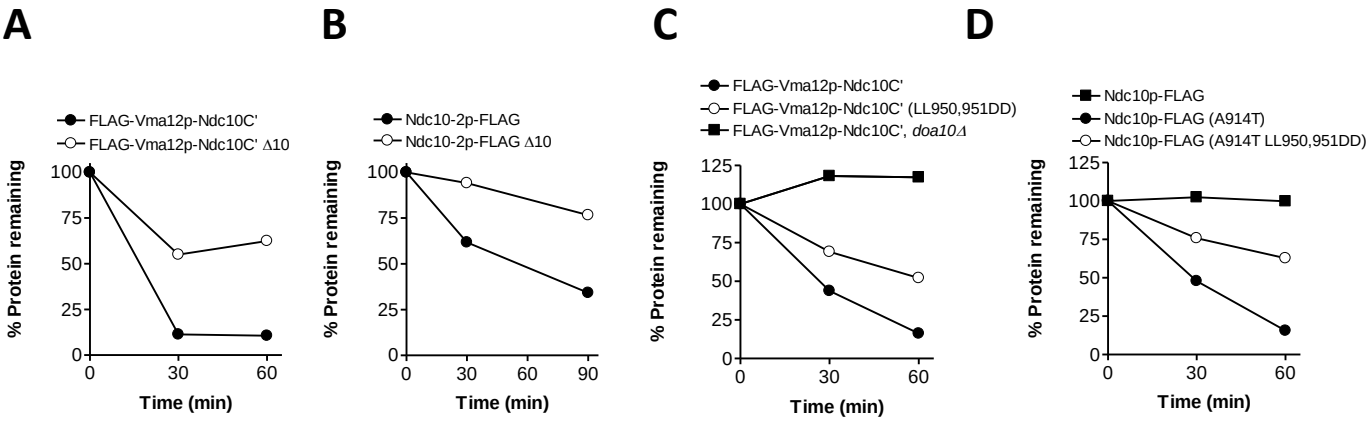


Figure 5

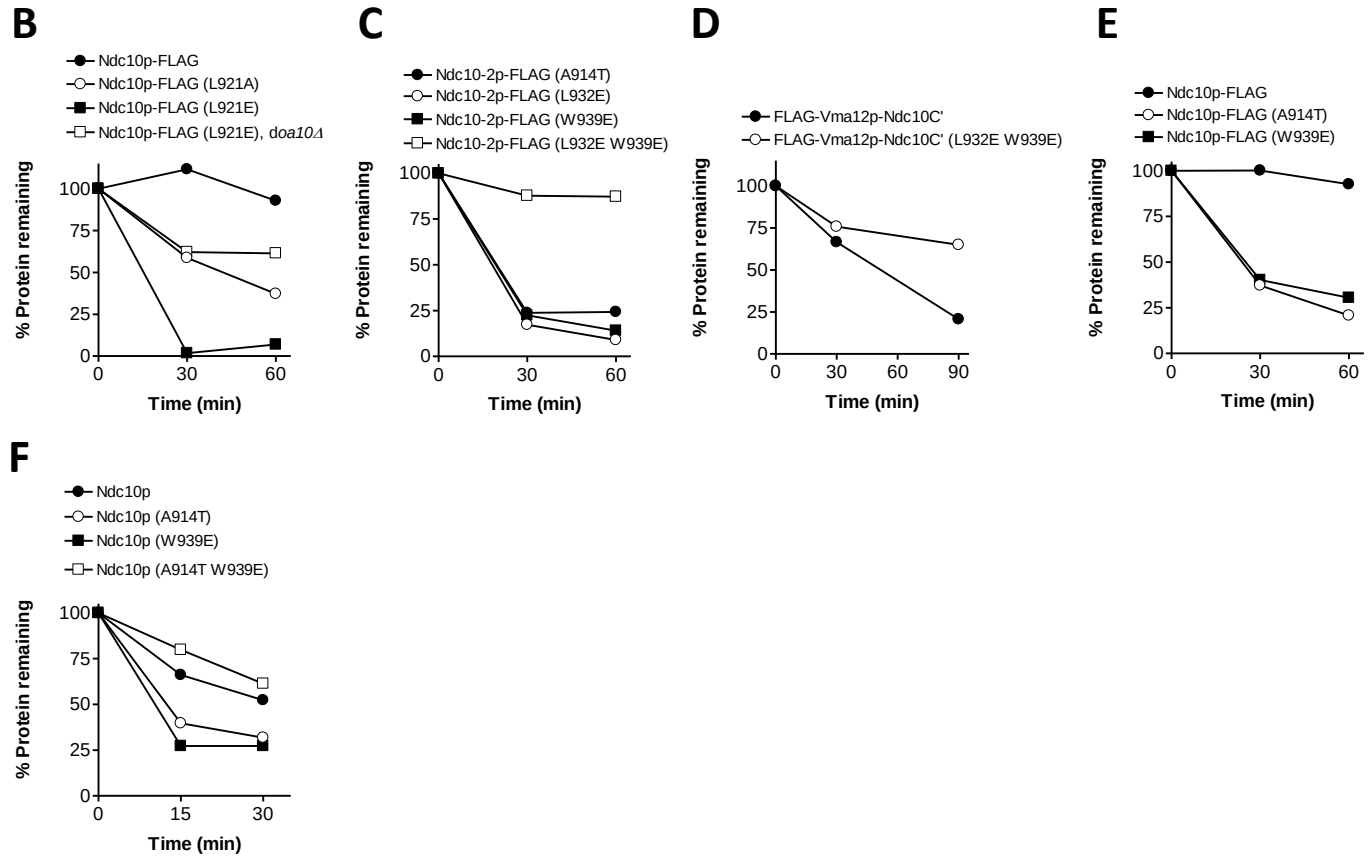


Figure 6