

The degradation of Rpn10-fusions is ubiquitin—independent, but proteasome dependent. The ubiquitin activating enzyme mutant, *uba1-2\**, and a proteasome mutant, *pre1-1*, *pre2-2\*\**, expressed the indicated fusion proteins. The *uba1-2* cells were incubated at 30° C, a temperature that elicits their mutant phenotypes. the *pre1-1*, *pre2-2* mutant was incubated at 37 °C for 2 hours before harvesting cells. Cell extracts were prepared and the level of

proteins was determined by Western blot analysis with anti-GFP. Rpt5 was immunoblotted as a loading control.

- \* MHY1409: *MAT* \_ *his3-200*, *leu2-3,112*, *lys2-801*, *trp1-1*, *ura3-52*, *gal2*, *uba1-2;mTn3* Swanson, R. and Hochstrasser, M. (2000) A viable ubiquitin-activating enzyme mutant for evaluating ubiquitin system function in Saccharomyces cerevisiae. FEBS Lett, 477, 193-198.
- \*\* WCG4-11/22a: *his3-11,15*, *leu2-3,112*, *ura3*, *pre1-1*, *pre2-2*, *MATa* Heinemeyer, W., Gruhler, A., Mohrle, V., Mahe, Y. and Wolf, D.H (1993). PRE2, highly homologous to the human major histocompatibility complex- linked RING10 gene, codes for a yeast proteasome subunit necessary for chrymotryptic activity and degradation of ubiquitinated proteins. J. Biol. Chem., 268: 5115 5120