C-terminal UBA Domains Protect Ubiquitin Receptors by Preventing Initiation of Protein Degradation

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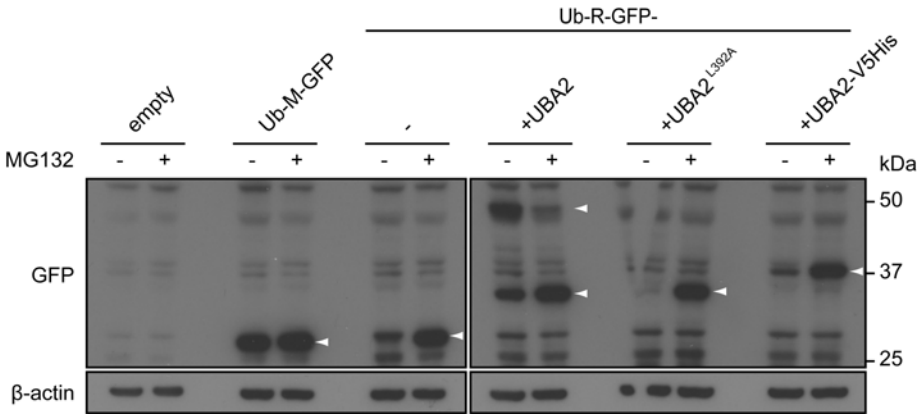
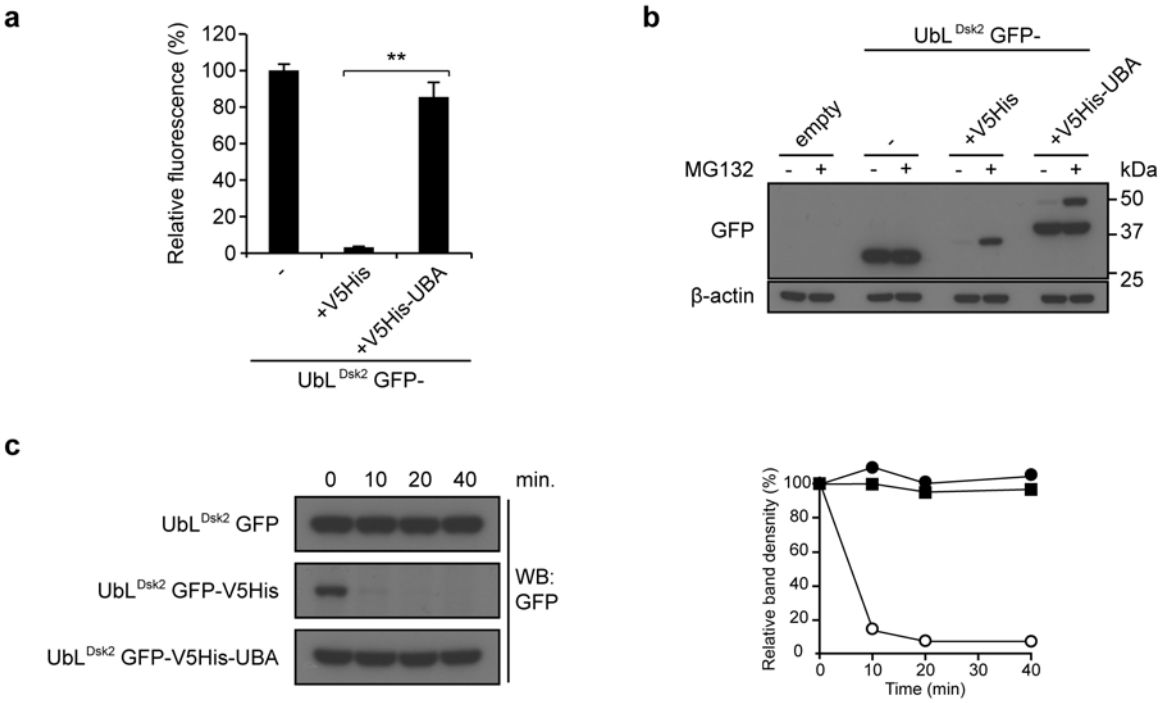
The Supplementary information contains:

Supplementary Figures S1 and S2



Supplementary Figure S1. C-terminal extensions abrogate the protective effect of the UBA2 domain. Steady-state levels of Ub-M-GFP, Ub-R-GFP, Ub-R-GFP-UBA2, Ub-R-GFP-UBA2^{L392A}, and Ub-R-GFP-UBA2-V5His in the absence of presence of the proteasome inhibitor MG132. Fusions were detected by Western blotting with a GFP-specific antibody. Note that the upper band in the Ub-R-GFP-UBA2 corresponds with diubiquitylated Ub-R-GFP-UBA2^{Rad23}. β-actin is shown as loading control. Molecular weight markers are indicated. Specific bands are indicated with arrowheads.



Supplementary Figure S2. Dsk2's C-terminal UBA domains can shield an internal unstructured polypeptide. **a)** Flow cytometric quantification of the mean fluorescence intensities of yeast expressing UbL^{Dsk2}GFP, UbL^{Dsk2}GFP-V5His, and UbL^{Dsk2}GFP-V5His-UBA. UbL^{Dsk2}GFP was standardized as 100%. Values are means and standard deviations (n=3). *P<0.05, **P<0.01 (Student's *t* test). **b)** Steady-state levels of UbL^{Dsk2}GFP, UbL^{Dsk2}GFP-V5His, and UbL^{Dsk2}GFP-V5His-UBA in yeast expressed under the control of the GAL1 promoter without and with proteasome inhibitor MG132. β-actin is shown as loading control. Molecular weight markers are indicated. **c)** Turnover of UbL^{Dsk2}GFP (closed circles), UbL^{Dsk2}GFP-V5His (open circles), and UbL^{Dsk2}GFP-V5His-UBA (closed squares). Samples were collected at the indicated time points and detected by Western blotting with a GFP-specific antibody. Densitometric quantification of the blot is shown to the right.