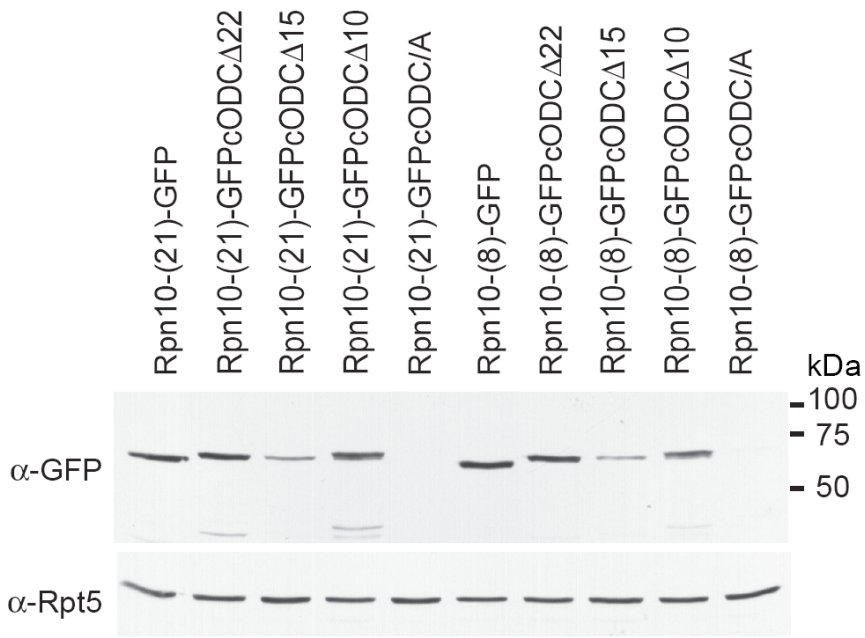
Supplemental figure 3.



Interaction between size of Rpn10-GFP linker and C terminal extension.

Does the total span of Rpn10-GFP-extension proteins rather than length of the terminal extension determine stability? We tested this directly by altering the length of the linker connecting Rpn10 and GFP. Our paradigmatic fusion proteins include a 21 residue linker connecting Rpn10 to GFP. Using this linker, Rpn10-GFP with no C-terminal extension was stable, a 15 residue extension did not destabilize, but a 22 residue extension was destabilizing and longer extensions destabilized further. If the 22-mer was marginally functional because it could barely reach a proteasome site needed for degradation and the 15-mer just falls short, then shrinking the linker from 21 to 8 residues should have changed this outcome, causing the 22-mer to now fall short. But this was not the outcome observed: stability was determined by the length of the C terminal extension of GFP and not influenced by whether 8 or 21 residues were positioned between Rpn10 and GFP. We conclude that it is the length of the GFP extension, not its reach, which determines whether the extension is functional.

In addition to the above-described direct experimental test, functional and structural considerations also support the same conclusion. Native Rpn10 is a polyubiquitin receptor. Its deletion impairs the degradation of multiple cellular proteins (Mayor et al., 2005). The UIM domain of Rpn10 engages ubiquitinated proteins (Deveraux et al., 1994),

thereby serving as a receptor (Elsasser et al., 2004; Verma et al., 2004). The von Willebrand factor type A domain at the N terminus of Rpn10 stabilizes the proteasome 19S complex when subjected to high salt concentrations (Glickman et al., 1998) and provides anchorage of Rpn10 to the proteasome. The UIM must therefore project sufficiently from the 19S complex to gain access to polyubiquitinated proteins. S5a is a human homolog of Rpn10 that contains two UIMs rather than the one present in Rpn10. The structure of a portion of S5a containing both UIMs in association with monoubiquitins was recently described (Wang et al., 2005). UIM1 of S5a, corresponding to the homologous domain of Rpn10, is composed of nine helical turns flanked by unstructured regions of 18 and 11 residues. This provides a span of about (18x3 + 9x5.4 + 11x3) Å = 135 Å. To this may be added the further distance potentially spanned by the 21 residue linker present in the paradigmatic constructs described here, which lies between Rpn10 and GFP (21x3 Å) plus the span between the N and C termini of GFP, ~25 Å, for a total of 135 + 63 + 25 = ~220 Å. This total distance approximates the maximum distance across the 19S complex (Walz et al., 1998). As Rpn10 is centrally placed within the complex, at the junction of base and lid, it is unlikely that the C terminal point of GFP in Rpn10-GFP is tethered at a distance short of that needed to reach a proteasome 19S engagement site.