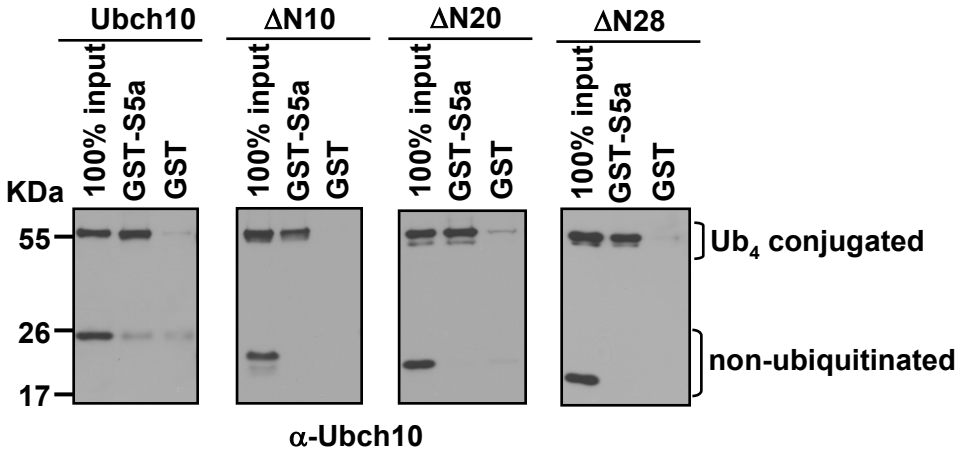

Figure S1 K48 Ub_4-conjugated UbcH10 or N-terminally truncated mutants (ΔN10-, ΔN20- and ΔN28-UbcH10) efficiently bind the S5a subunit of the 26S proteasome.

60 pmol recombinant GST or GST-S5a was preincubated with 6 μL of glutathione Sepharose 4B beads for 2 hours at 4 ^{0}C with rotation in the binding buffer containing 20 mM Tris, pH 7.8, 50 mM NaCl, 2 mM DTT. After washing, the beads were resuspended in 50 μL of binding buffer supplemented with 0.2 mg/mL BSA and 300 nM Ub_4-conjugated UbcH10 or UbcH10 mutants. The mixtures were rotated for 2 hours at room temperature and then washed by binding buffer supplemented with 0.1% NP-40. The bound proteins were eluted by mixing beads with 60 μL of 1.5X SDS sample buffer containing 3 M urea, separated by SDS-PAGE and evaluated by immunoblotting.

Supplementary figure 1

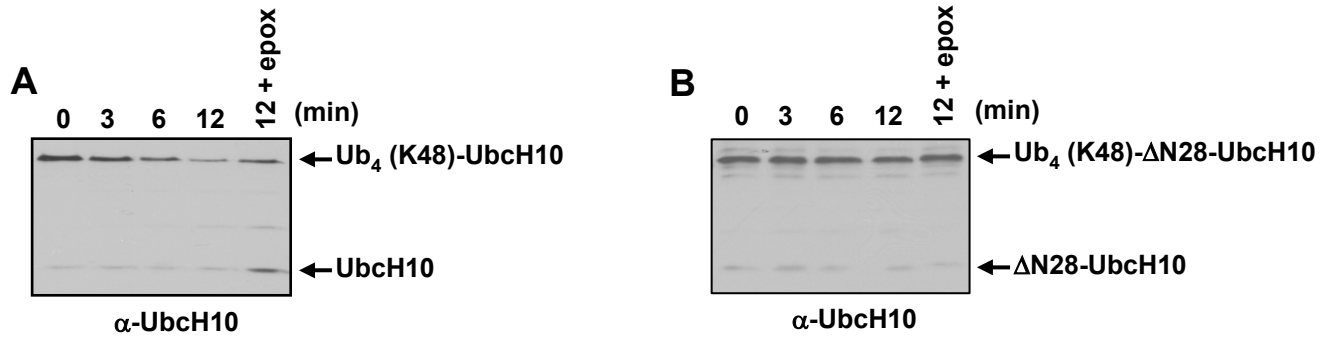

Figure S2 Ub_4 (K48) targets C-terminally His_6-tagged UbcH10, but not ΔN28-UbcH10, for 26S proteasomal degradation.
C-terminally His_6-tagged UbcH10 or ΔN28-UbcH10 was used for *in vitro* ubiquitination reaction. Degradation assays were analogous to Fig. 2C.

Supplementary figure 2

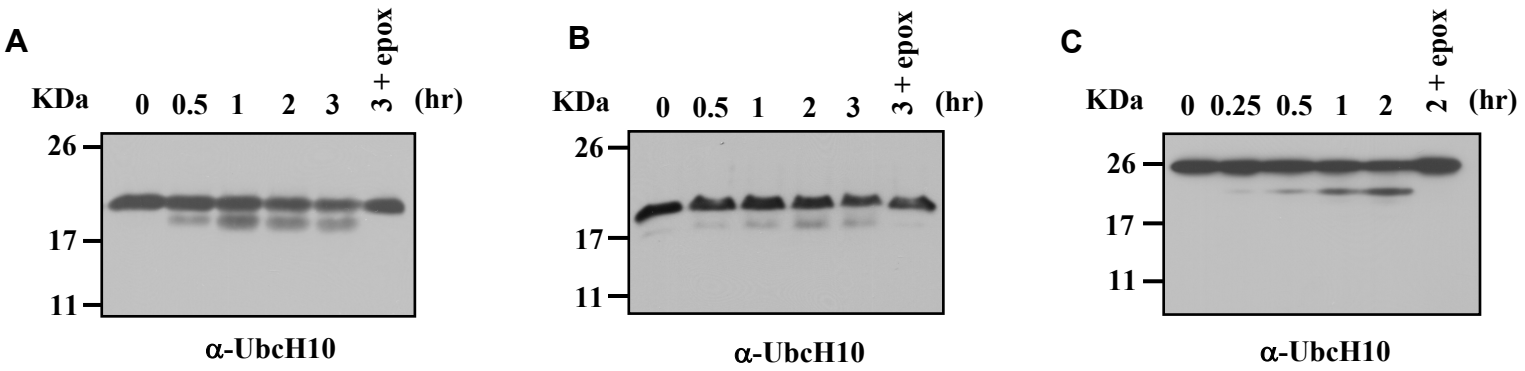

Figure S3 The unstructured N-terminal His_6 tag or the PEST domain of ODC can initiate partial degradation of ΔN28-UbcH10. Degradation of 200 nM His_6-ΔN28-UbcH10-His_6 (A), ΔN10-$UbcH10_{24}$ (B) or PEST-ΔN28-UbcH10-His_6 (C) by 13.5 nM doubly-capped 26S proteasome was evaluated by immunoblotting.

Supplementary figure 3

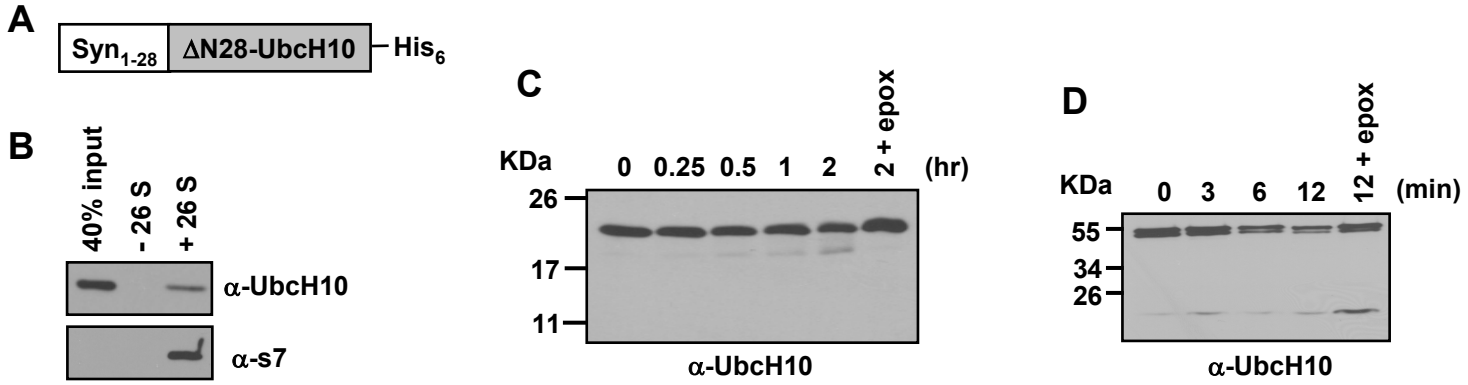

Figure S4 The unstructured N-terminal region of alpha-synuclein promotes Ub-dependent ΔN28-UbcH10 degradation.

(A) Schematic representation of the construct with the N-terminal 28 amino acids of alpha-synuclein (Syn) fused at the N-terminus of △N28-UbcH10.

(B) Syn_{1-28}-△N28-UbcH10 binds the 26 S proteasome. The binding assay was performed by using the Bio-Spin 30 spin column as described in the Experimental Procedures.

(C) The unstructured Syn_{1-28} region in Syn_{1-28}-△N28-UbcH10 can be slowly cleaved by the 26S proteasome. Degradation assays were analogous to Fig. 1E and Fig. S3.

(D) The Syn_{1-28} region promotes Ub-dependent degradation of △N28-UbcH10. Degradation assays were analogous to Fig. 2C.