Figure S1

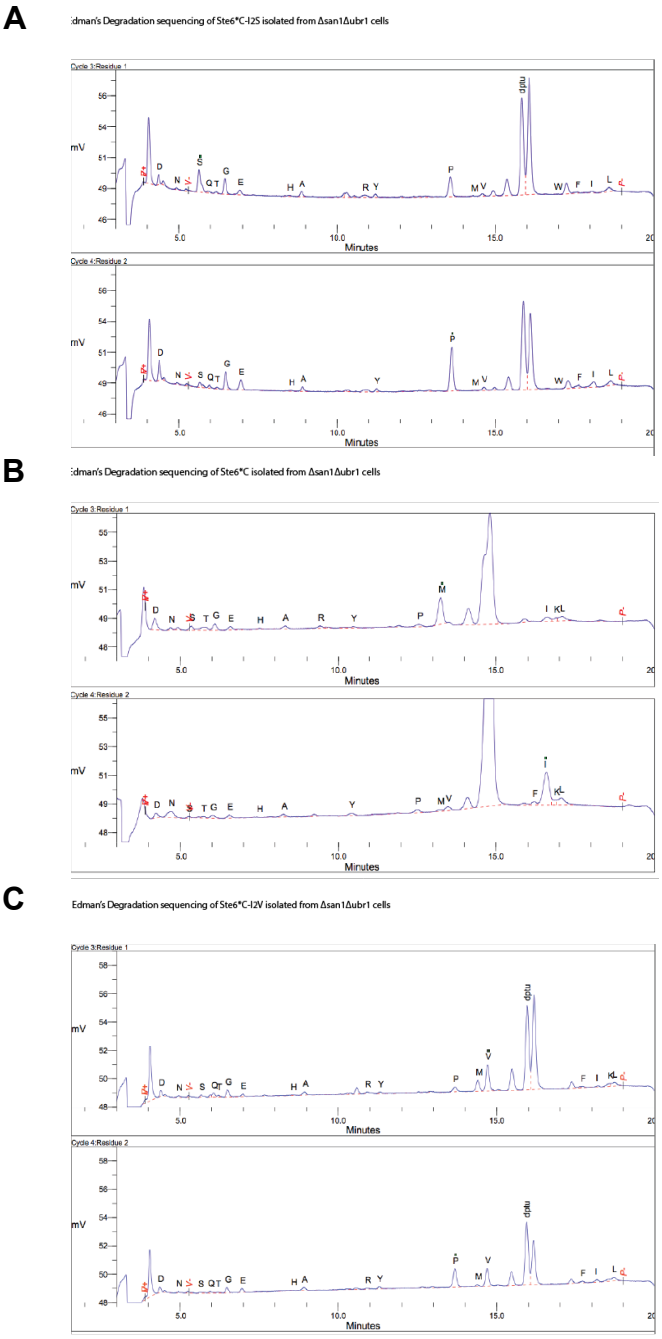

Fig. S1. N-terminal sequencing of Ste6*C constructs. (A) Lysate was prepared from Δsan1Δubr1 cells expressing Ste6*C-HA-I2S. Protein was immunoprecipitated from lysate with anti-HA affinity matrix (Roche), resolved by SDS-PAGE, and transferred to PVDF membrane. Protein band carrying Ste6*C-HA was excised from the membrane and sequenced via Edman degradation. (B) Lysate was prepared from Δsan1Δubr1 cells expressing Ste6*C-HA. Protein was immunoprecipitated from lysate and sequenced as described in Fig S1A. (C) Lysate was prepared from Δsan1Δubr1 cells expressing Ste6*C-HA-I2V. Protein was immunoprecipitated from lysate and sequenced as described in Fig S1A.

Figure S2

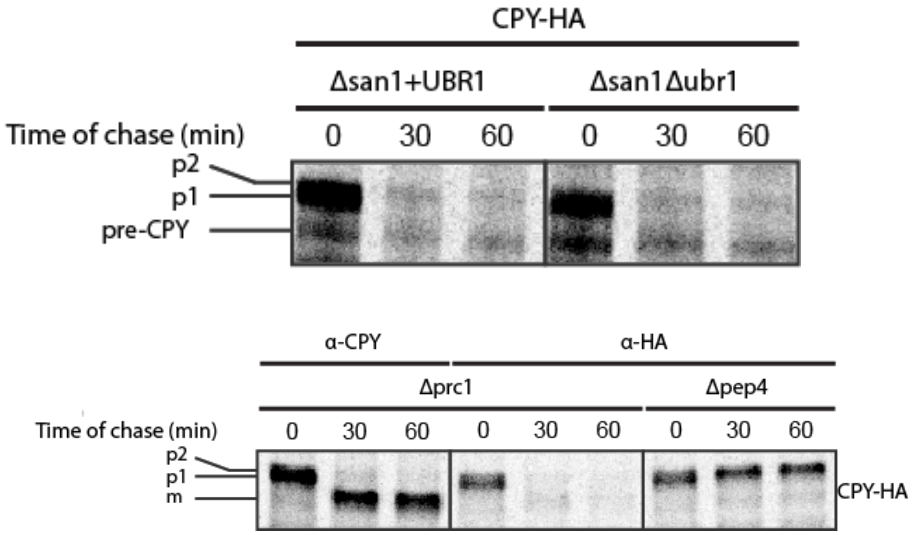

Fig S2. C-terminal HA-tag is removed from HA-tagged CPY in the vacuole. Turnover rate of CPY-HA was assessed in Δprc1, Δpep4, Δsan1+UBR1, and Δsan1Δubr1 cells by metabolic pulse-chase as described in Fig 1. CPY was probed with anti-CPY and anti-HA antibodies in Δprc1 cells, and anti-HA in Δpep4 cells.

Figure S3

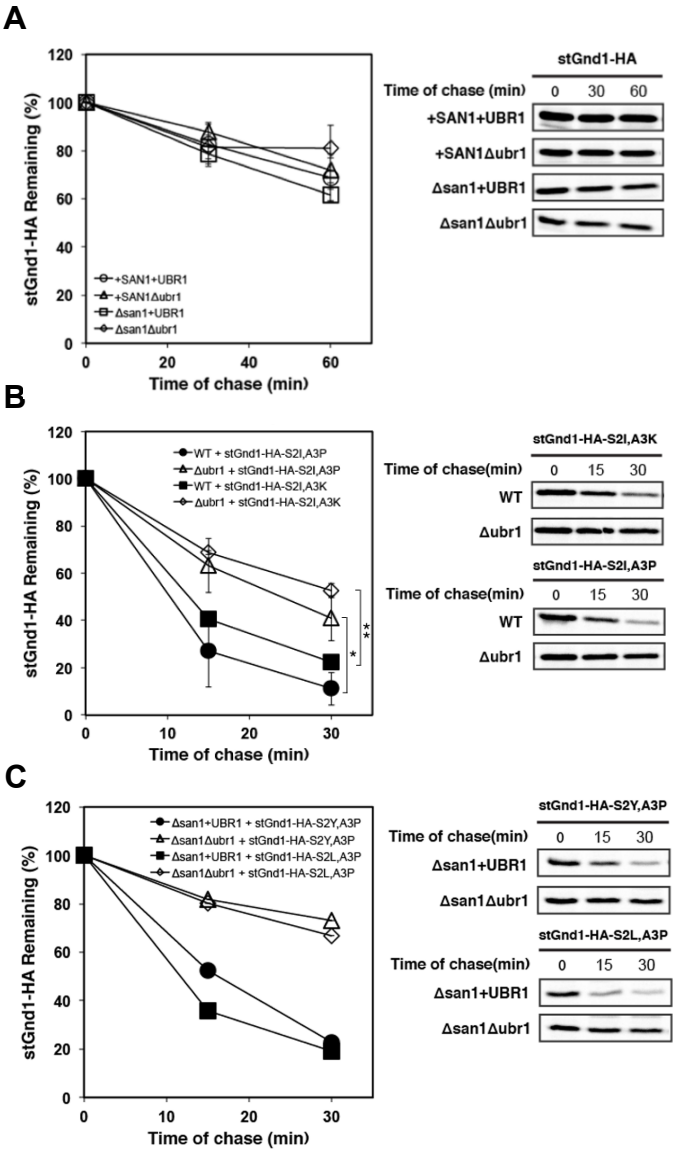

Fig S3. Ubr1-mediated degradation of stGnd1 is P2-residue/N-degron dependent. (A) Turnover rate of C-terminally HA-tagged stGnd1 (stGnd1-HA) expressed in +SAN1+UBR1 (wild-type), Δsan1+UBR1, +SAN1Δubr1, and Δsan1Δubr1 cells was assessed by metabolic pulse-chase. Error bars, mean +/- SD of two independent experiments (N=2, biological replicates). (B) Turnover rates of C-terminally HA-tagged stGnd1-HA-S2IA3K and stGnd1-HA-S2IA3P expressed in wild-type and Δubr1 cells were assessed by metabolic pulse-chase. Error bars, mean +/- SD of two or three independent experiments (N=2 for stGnd1-HA-S2IA3K; N=3 for stGnd1-HA-S2IA3P). (C) Turnover rates of C-terminally HA-tagged stGnd1-HA-S2YA3P and stGnd1-HA-S2LA3P expressed in Δsan1+UBR1 and Δsan1Δubr1 cells were assessed by metabolic pulse-chase (N=1, biological replicates). (A-C) Metabolic pulse-chase was performed as described in Fig 1.

Tables S1–S32 have been uploaded to the Dryad Digital Repository at https://doi.org/10.5061/dryad.r837vv7 (Tran, 2019).