Figure. S1

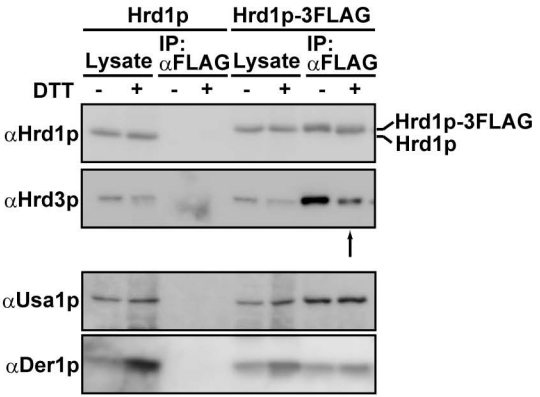
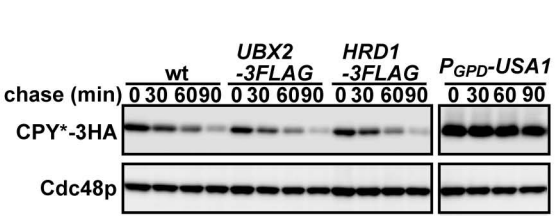


Figure. S1 Hrd3p inefficiently binds to Hrd1p upon treatment of cells with DTT

Membrane fractions were prepared from KNY140 (*HRD1*) and KNY220 (*HRD1-3FLAG*) cells that were treated with 5 mM DTT for 1 hr. Membranes were solubilized with 1% digitonin and Hrd1p-3FLAG was immunoprecipitated with anti-FLAG antibody. The immunoprecipitates were analyzed by western blotting with the indicated antibodies. The arrow indicates that a reduced amount of Hrd3p co-precipitated with Hrd1p when cells were treated with DTT.

Figure. S2



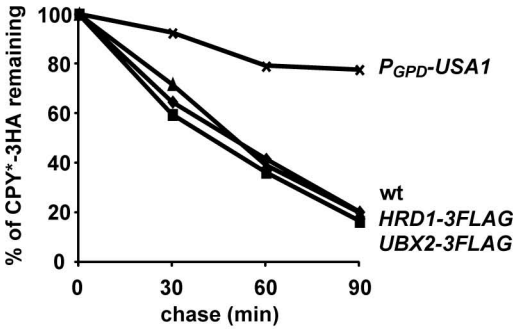


Figure. S2. Triply FLAG tagged forms of Ubx2p and Hrd1p are functional

Yeast strains KNY140 (wt), KNY218 (*UBX2-3FLAG*), KNY220 (*HRD1-3FLAG*), and KNY269 (P_{GPD}-*USA1*) expressing CPY*-3HA were treated with cycloheximide and collected at the indicated time points. Total cell lysates were prepared and the degradation of CPY* was analyzed by western blot analysis. The Cdc48p blot served as a loading control. Overexpression of Usa1p was previously shown to stabilize CPY* (Carvalho *et al.*, 2010) and was used as a positive control. The results were quantified and are shown in the accompanying graph. The signal at the 0 time point was set to 100%.

Figure. S3

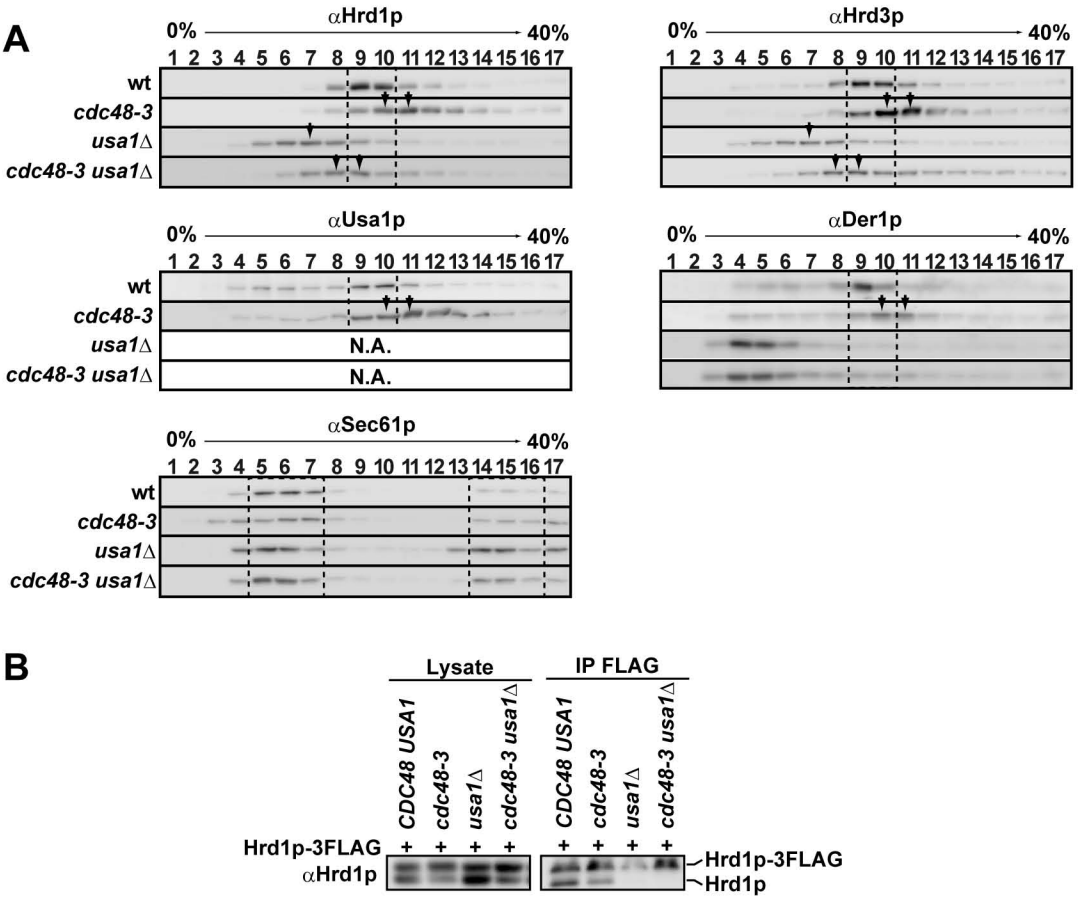


Figure. S3 Analysis of the Hrd1 complex in *cdc48-3usa1*Δ cells

(A) Digitonin-solubilized membrane fractions were prepared from the indicated cells and subjected to sucrose density gradient analysis as in Fig. 1. The Hrd1p and Hrd3p peaks in *cdc48-3usa1*Δ cells shifted to more dense/heavier molecular weight fractions (#8-9) than those for *usa1*Δ cells (#7), suggesting the Usa1p-independent remodeling of Hrd1p-Hrd3p interaction in *cdc48-3* cells.

(B) Inactivation of Cdc48p does not facilitate the oligomeric formation of Hrd1p in *usa1*Δ cells. Digitonin-solubilized membrane fractions were prepared from the indicated wild type and mutant cells expressing 3xFLAG-tagged Hrd1 from a chromosome-integrated and non-tagged form of Hrd1p in a low copy number plasmid. Hrd1p-3FLAG was immunoprecipitated with anti-FLAG antibody and blotted with anti-Hrd1 antibody after SDS-PAGE.

Figure. S4

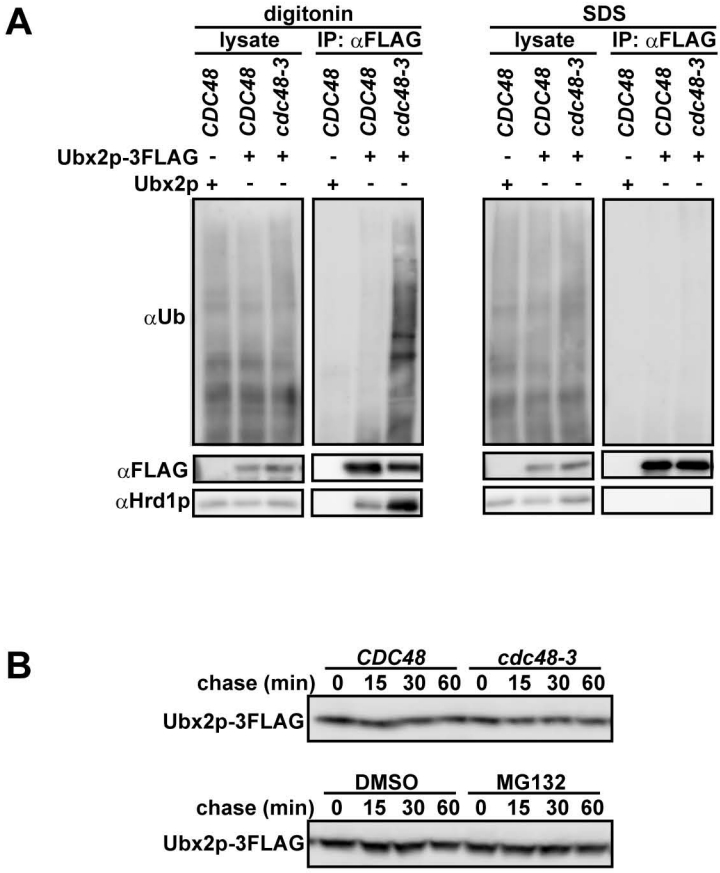


Figure. S4 Ubx2p is not ubiquitinated and is stable

(a) Wild type or *cdc48-3* cells expressing Ubx2p-3FLAG from a chromosome-integrated gene were disrupted with glass beads and membrane fractions were prepared. Membranes were solubilized in 1% digitonin or 1.8% SDS before Ubx2p-3FLAG was immunoprecipitated. The concentration of SDS was lowered to 0.18% with 0.6% TritonX-100 before immunoprecipitation. Proteins in the precipitated complex was separated by SDS-PAGE. Ubiquitinated species and Hrd1p bound to Ubx2p-3FLAG were detected by western blotting.

(b) Cells expressing Ubx2p-3FLAG as above were treated with cycloheximide (200 μg/ml) and aliquots were removed at the indicated times. Ubx2p-3FLAG was detected with anti-FLAG antibody. *CDC48* (KNY218) and *cdc48-3* cells (KNY219) were grown at 25˚C and shifted to 37˚C for 1 hr. MG132 (100 μM) was added to wild type cells (KNY218) 1 hr before the chase started.

Figure. S5

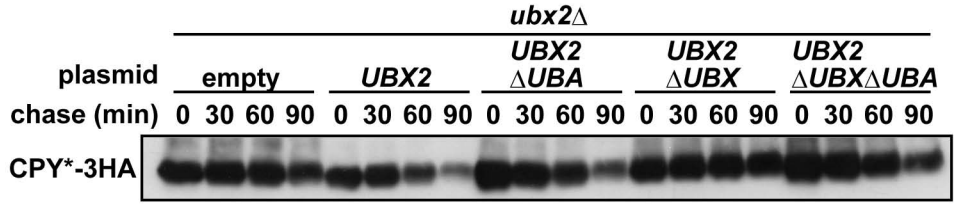


Figure. S5 The degradation of CPY*, a typical model ERAD substrate, is not slowed even when the *UBA* domain is deleted from Ubx2p.

Cycloheximide chase experiment was performed for *ubx2*Δ strain (KNY186) expressing CPY*-3HA and *UBX2* or the indicated mutants lacking either the *UBA* and/or *UBX* domain(s) from low copy plasmids. Samples were taken at the indicated times and the degradation of CPY* was analyzed by western blot analysis with anti-HA antibody.

Figure. S6

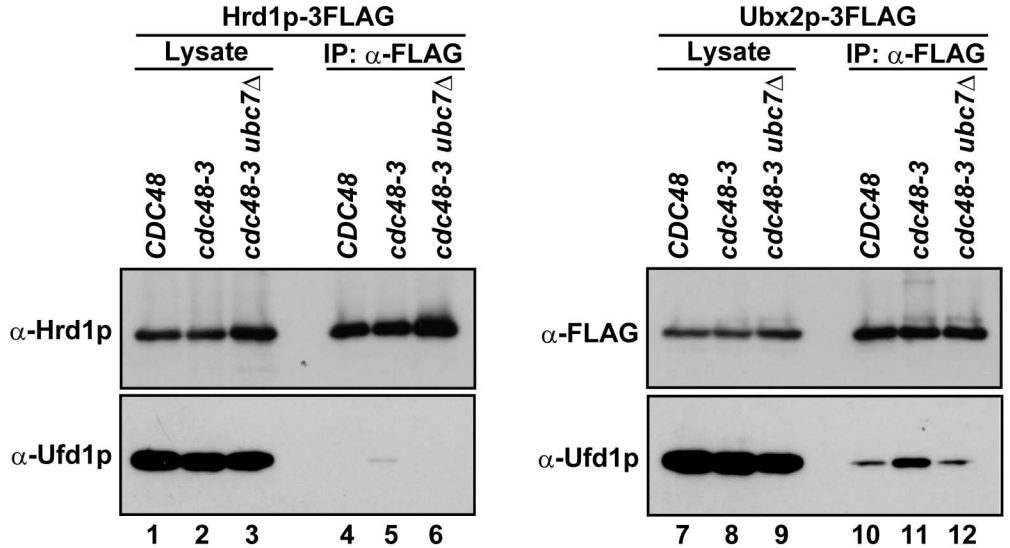


Figure. S6 Increased amount of Ufd1p is co-immunoprecipitated with Hrd1p or Ubx2p from *cdc48-3* cells.

The indicated wild-type or mutant cells expressing triply-FLAG tagged Hrd1p or Ubx2p were immunoprecipitated from TritonX-100 solubilized lysate. The immunoprecipitates were separated by SDS-PAGE and analyzed with anti-Ufd1p antibody. More Ufd1p was precipitated with Hrd1p or Ubx2p when *cdc48* was mutated (lanes 5 and 11) in Ubc7-dependent manner (lanes 6 and 12).

Table S1: Strains used in this study

KNY140	W303-1a, *pdr5*Δ::*HPH*, *pep4*::*LEU2*	This study
KNY169	KNY140, *hrd3*Δ::*CgHIS*	This study
KNY170	KNY140, *usa1*Δ::*CgHIS*	This study
KNY186	KNY140, *ubx2*Δ::*CgHIS*	This study
KNY208	KNY140, *cdc48-3*	This study
KNY218	KNY140, *UBX2-3FLAG-KanMX*	This study
KNY219	KNY140, *UBX2-3FLAG-KanMX*, *cdc48-3*	This study
KNY220	KNY140, *HRD1-3FLAG-KanMX*	This study
KNY222	KNY140, *HRD1-3FLAG-KanMX*, *cdc48-3*	This study
KNY232	KNY140, *UBX2-3FLAG-KanMX*, *ubc7*Δ::*CgTRP*	This study
KNY233	KNY140, *UBX2-3FLAG-KanMX*, *ubc7*Δ::*CgTRP*, *cdc48-3*	This study
KNY236	KNY140, *HRD1-3FLAG-KanMX*, *ubc7*Δ::*CgTRP*	This study
KNY238	KNY140, *HRD1-3FLAG-KanMX*, *ubc7*Δ::*CgTRP*, *cdc48-3*	This study
KNY254	KNY140, *HRD1-3FLAG-KanMX*, *usa1*Δ::*CgHIS*	This study
KNY255	KNY140, *HRD1-3FLAG-KanMX*, *usa1*Δ::*CgHIS*, *cdc48-3*	This study
KNY262	KNY140, *hac1*Δ::*CgHIS*	This study
KNY269	KNY140, *HIS3MX6-*P_{GPD}*-USA1*	This study
KNY279	KNY140, *RPT5-3FLAG-KanMX*	This study
KNY280	KNY140, *RPT5-3FLAG-KanMX*, *cdc48-3*	This study
KNY282	KNY140, *RPT5-3FLAG-KanMX*, *cdc48-3*, *usa1*Δ::*CgTRP*	This study
KNY288	KNY140, *RPT5-3FLAG-KanMX*, *cdc48-3*, *ubc7*Δ::*CgHIS*	This study
KNY292	KNY140, *usa1*Δ::*CgHIS*, *yos9*Δ::*CgTRP*	This study
KNY293	KNY140, *usa1*Δ::*CgHIS*, *hrd3*Δ::*CgTRP*	This study
KNY294	KNY140, *UBX2-3FLAG-KanMX*, *DOA10-13myc-HIS3MX6*, *cdc48-3*, *usa1*Δ::*CgTRP*	This study
W303-1a	*MAT***a** *can1-100*, *leu2-3*,*-112*, *his3-11*,*-15*, *trp1-1*, *ura3-1*, *ade2-1*	Lab stock

Table S2: Plasmids used in this study

Name	Description	
pFA6a-3FLAG-KanMX	Tagging at the C-terminus of an endogenous gene	Y. Tamura, and H. Sesaki, Johns Hopkins University
pUC18-CgTRP1	Gene deletion cassette	NBRP of the MEXT, Japan
pUC19-CgHIS3	Gene deletion cassette	NBRP of the MEXT, Japan
pKN41	*HIS3MX6-*P_{GPD} cassette	This study
pKN72	*CEN/ARS, TRP1,* P_{UBX2}*-UBX2-3FLAG*	This study
pKN73	*CEN/ARS, TRP1,* P_{UBX2}*-UBX2* (*UBA*Δ)*-3FLAG* (lacking 9-61 a.a. of Ubx2p)	This study
pKN75	*CEN/ARS, TRP1,* P_{UBX2}*-UBX2* (*UBA*Δ*UBX*Δ)*-3FLAG* (lacking both 9-61 a.a. and 421-584 a.a. of Ubx2p)	This study
pKN81	*CEN/ARS, TRP1,* P_{UBX2}*-UBX2* (*UBX*Δ)*-3FLAG* (lacking 418-584 a.a. of Ubx2p)	This study
pKN107	*CEN/ARS, URA3,* P_{GAL1}*-HAC1*(*i*)*-*T_{CYC1}	This study
12-22	*CEN/ARS, URA3, CPY*-3HA*	J. Weissman, UCSF