

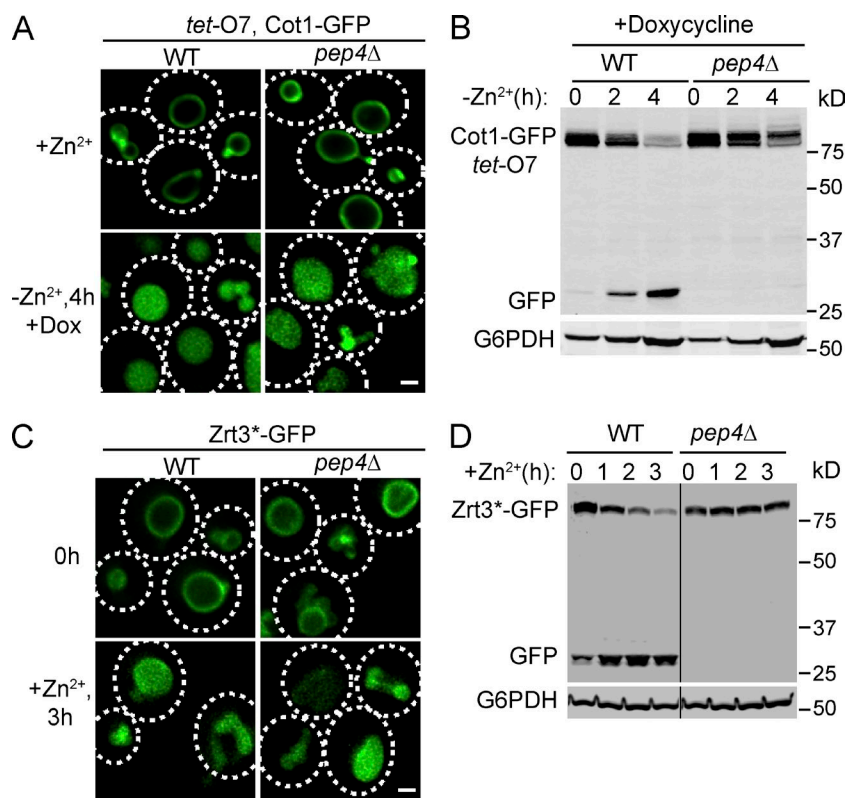
Li et al., <http://www.jcb.org/cgi/content/full/jcb.201505062/DC1>

Figure S1. **Degradation of both Cot1-GFP and Zrt3*-GFP happens in the vacuole lumen.** (A) Localization of *tet-O7 Cot1-GFP* in both WT and *pep4Δ* strains, before and after Zn²⁺ withdrawal from the YNB media. (B) Degradation kinetics for *tet-O7 Cot1-GFP* in both WT and *pep4Δ* strains. G6PDH was used as a loading control. Same volume of cells was loaded, with 1 OD₆₀₀ cells loaded at 0 h. (C) Localization of *Zrt3*-GFP* in wildtype and *pep4Δ* strains, before and after the addition of 2 mM ZnCl₂ in YNB media. Cells were pretreated with YNB without Zn²⁺ for 3 h (0 h). (D) Degradation kinetics for *Zrt3*-GFP* in WT and *pep4Δ* strains. G6PDH was used as a loading control. 1 OD₆₀₀ cells were loaded in each lane. Bar, 1 μm.

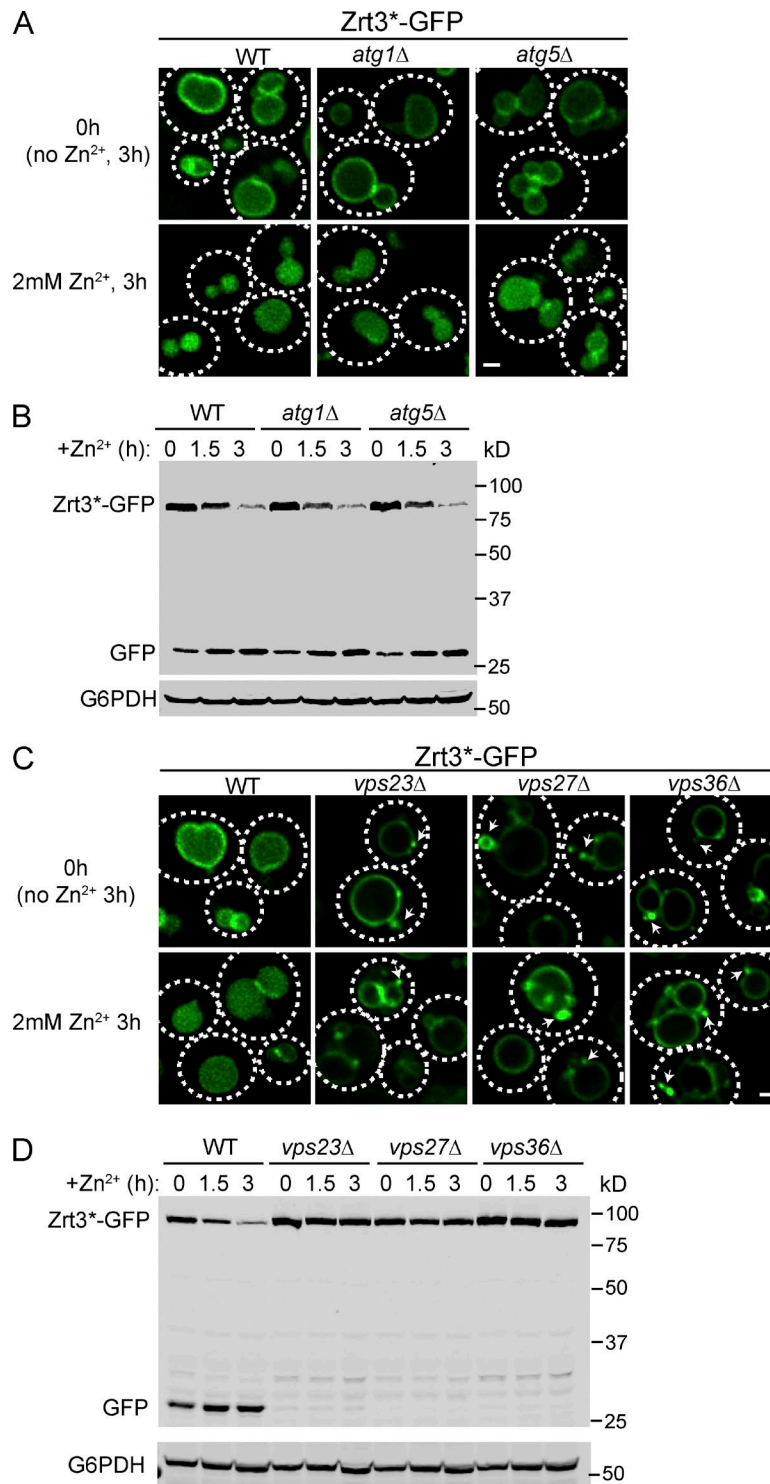


Figure S2. **ESCRTs, but not the autophagy machinery, are required for the degradation of Zrt3*-GFP.** (A) Localization of Zrt3*-GFP in WT and autophagy mutants before and after 2 mM ZnCl₂ treatment in YNB media. Cells were pretreated with YNB media without Zn²⁺ for 3 h. (B) Degradation kinetics for Zrt3*-GFP in WT and autophagy mutants. G6PDH was used as a loading control. Same volume of cells was loaded, with 1 OD₆₀₀ cells loaded at 0 h. (C) Localization of Zrt3*-GFP in WT and ESCRT mutants before and after 2 mM ZnCl₂ treatment in YNB media. Cells were pretreated with YNB media without Zn²⁺ for 3 h. Arrows highlight the aberrant endosomes. (D) Degradation kinetics for Zrt3*-GFP in WT and ESCRT mutants. G6PDH was used as a loading control. Same volume of cells was loaded, with 1 OD₆₀₀ cells loaded at 0 h. Bar, 1 μm.

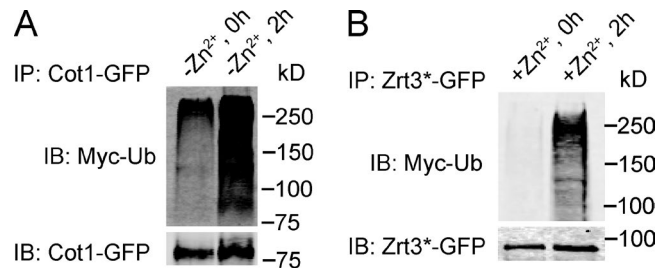


Figure S3. **Cot1-GFP and Zrt3*-GFP are poly-ubiquitinated during their degradation.** (A) Zn^{2+} withdrawal from the YNB media triggered the poly-ubiquitination of Cot1-GFP. (B) Addition of 2 mM $ZnCl_2$ to the YNB media triggered the poly-ubiquitination of Zrt3*-GFP. Cells were pretreated with YNB media without Zn^{2+} for 3 h.

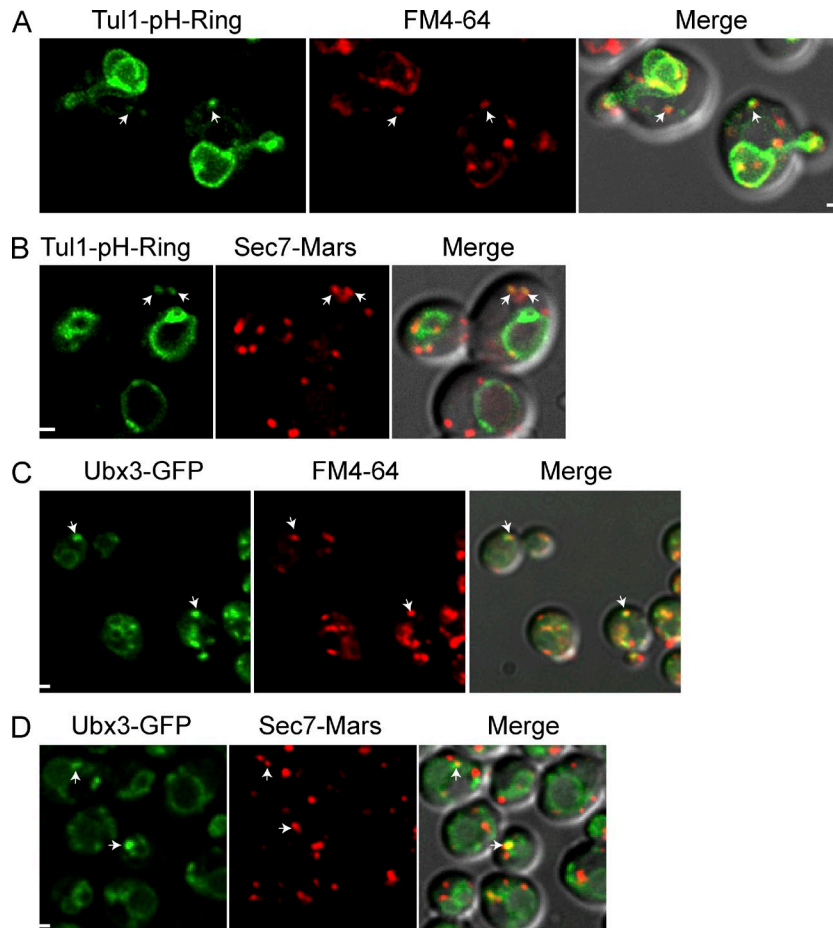


Figure S4. **A fraction of the Dsc complex localizes to Golgi and endosomes.** (A) A fraction of Tul1-pH-Ring colocalizes with FM4-64 labeled endosomes. The cells were stained with FM4-64 for 5 min before imaging. Arrows highlight the colocalization. (B) A fraction of Tul1-pH-Ring colocalizes with Sec7-Mars labeled Golgi. Arrows highlight the colocalization. (C) A fraction of Ubx3-GFP colocalizes with FM4-64-labeled endosomes. The cells were stained with FM4-64 for 5 min before imaging. Arrows highlight the colocalization. (D) A fraction of Ubx3-GFP colocalizes with Sec7-Mars labeled Golgi. Arrows highlight the colocalization. Bar, 1 μ m.

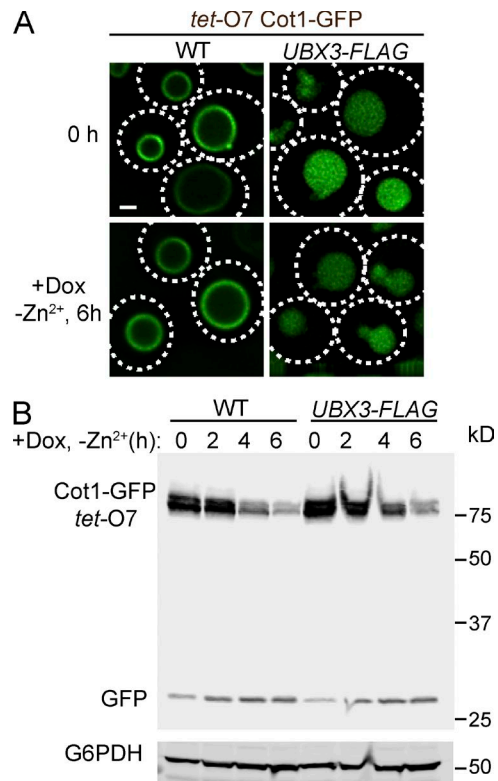


Figure S5. **Ubx3-Flag is functional.** (A) Localization of Cot1-GFP in WT and *UBX3-FLAG* strains, before and 6 h after Zn²⁺ withdrawal from the YNB media. (B) Degradation kinetics for *tet-O7* Cot1-GFP in both WT and *UBX3-FLAG* strains. G6PDH was used as a loading control. 1 OD₆₀₀ cells were loaded in each lane. Bar, 1 μm.

Table S1. Yeast strains and plasmids used in this study

Strain	Name	Genotype	Reference/source
<i>Saccharomyces cerevisiae</i> strains			
SEY6210	Wild type	<i>Matx, leu1-3, 112 ura3-52 his3-200, trp1-901 lys2-801 suc2-D9</i>	Robinson et al., 1988
SEY6210.1	Wild type	<i>Mata, leu1-3, 112 ura3-52 his3-200, trp1-901 lys2-801 suc2-D9</i>	Robinson et al., 1988
YML51	YCF1-GFP	6210, YCF1-GFP::HIS3	This study
YML52	ZRC1-GFP	6210, ZRC1-GFP::HIS3	This study
YML324	COT1-GFP	6210, COT1-GFP::KAN	This study
YML319	ZRT3*-GFP	6210, ZRT3*-GFP::KAN	This study
YML106	FET5-GFP	6210, FET5-GFP::HIS3	This study
YML108	FTH1-GFP	6210, FTH1-GFP::HIS3	This study
YYR1	NCR1-GFP	6210, NCR1-GFP::HIS3	This study
YML216	GFP-VCX1	6210.1, GFP-VCX::KAN	This study
YML577	VPH1-mCherry	6210.1, VPH1-mCherry::HIS3	This study
YML100	pep4Δ	6210, pep4Δ::LEU2	This study
YML228	pep4Δ, ZRT3*-GFP	6210, ZRT3*-GFP::KAN, pep4Δ::LEU2	This study
YML260	atg1Δ, COT1-GFP	6210.1, Cot1-GFP::KAN, atg1Δ::TRP1	This study
YML455	atg5 Δ, COT1-GFP	6210.1, Cot1-GFP::KAN, atg5Δ::TRP1	This study
YML456	atg7Δ COT1-GFP	6210.1, Cot1-GFP::KAN, atg7Δ::TRP1	This study
YML278	atg1 Δ ZRT3*-GFP	6210.1, ZRT3* -GFP::KAN, atg1Δ::TRP1	This study
YML279	atg5 Δ ZRT3*-GFP	6210.1, ZRT3*-GFP::KAN, atg5 Δ::TRP1	This study
YML445	vps23 Δ, COT1-GFP	6210.1, COT1-GFP::KAN, vps23 Δ::TRP1	This study
YML446	vps27 Δ, COT1-GFP	6210.1, COT1-GFP::KAN, vps27Δ::TRP1	This study
YML447	vps36 Δ, COT1-GFP	6210.1, COT1-GFP::KAN, vps36Δ::TRP1	This study
YML448	snf7 Δ, COT1-GFP	6210.1, COT1-GFP::KAN, snf7Δ::TRP1	This study
YML325	vps4 Δ, COT1-GFP	6210.1, COT1-GFP::KAN, Vps4Δ::TRP1	This study
YML441	vps23 Δ ZRT3*-GFP	6210.1, ZRT3*-GFP::KAN, vps23 Δ::TRP1	This study
YML442	vps27 Δ ZRT3*-GFP	6210.1, ZRT3*-GFP::KAN, vps27 Δ::TRP1	This study
YML443	vps36 Δ ZRT3*-GFP	6210.1, ZRT3*-GFP::KAN, vps36 Δ::TRP1	This study
YML354	ssh4 Δ	6210, ssh4 Δ::TRP1	This study
YML165	rsp5-1	6210, rsp5-1	Li et al., 2015
YML489	tul1Δ	6210.1, tul1Δ::TRP1	This study
YML802	ssh4Δ ZRT3*-GFP	6210.1, ZRT3*-GFP::KAN, ssh4 Δ::TRP1	This study
YML803	tul1Δ, rsp5-1 ZRT3*-GFP	6210, ZRT3*-GFP::KAN, rsp5-1, tul1Δ::TRP1	This study
YML329	tul1Δ, ZRT3*-GFP	6210.1, ZRT3*-GFP::KAN, tul1Δ::TRP1	This study
YML232	rsp5-1, ZRT3*-GFP	6210, ZRT3*-GFP::KAN, rsp5-1	This study
YML477	dsc2Δ	6210.1, dsc2Δ::TRP1	This study
YML479	dsc3Δ	6210.1, dsc3Δ::TRP1	This study
YML622	dsc5Δ	6210.1, dsc5Δ::HIS3	This study
YML515	dsc5Δ COT1-GFP	6210.1, COT1-GFP::KAN, dsc5Δ::TRP1	This study
YML478	dsc2Δ COT1-GFP	6210.1, COT1-GFP::KAN, dsc2Δ::TRP1	This study
YML480	dsc3Δ COT1-GFP	6210.1, COT1-GFP::KAN, dsc3Δ::TRP1	This study
YML519	cdc48 ^{E315K}	6210.1,cdc48Δ::TRP1, pRS415 cdc48 ^{E315K}	This study
YML234	YPQ2-GFP	6210.1,YPQ2-GFP::TRP1	This study
YML169	VPH1-GFP	6210.1, VPH1-GFP::KAN	This study
YYR6	YPQ1-GFP	6210, YPQ1-GFP::TRP1	Li et al., 2015
YML235	VBA4-GFP	6210, VBA4-GFP::TRP1	Li et al., 2015
YML321	YPL162C-GFP	6210.1, YPL162C-GFP::TRP1	This study
YML417	tul1Δ, COT1-Mars	6210.1, COT1-Mars::TRP1, tul1Δ::HIS3	This study
YML486	UBX3-GFP	6210.1, UBX3-GFP::TRP1	This study
YML856	UBX3-FLAG	6210.1, UBX3-3xFLAG::HIS3	This study
YML799	UBX3-GFP, Mars-SEC7	6210.1, UBX3-GFP::HIS3 Mars-SEC7::TRP1	This study
YML225	Mars-SEC7	6210.1, Mars-SEC7::TRP1	This study
YML499	pep4Δ UBX3-3xFLAG	6210, pep4Δ::LEU2 UBX3-3xFLAG::HIS3	This study
YML853	doa4Δ, estro-GAL4	6210.1, doa4Δ::HIS3, PADH1-GAL4BD-her-VP16::TRP1	This study
<i>S. cerevisiae</i> expression plasmids			
pCM189	COT1-GFP	Tet-off vector, tetO7 promoter C-terminal GFP	This study
pRS415	cdc48 ^{E315K}	Native promoter and terminator of CDC48, E315 mutated to K	This study
pCM189	TUL1	Tet-off vector, tetO7 promoter, for Tul1 overexpression	This study

Table S1. Yeast strains and plasmids used in this study (Continued)

Strain	Name	Genotype	Reference/source
pCM189	<i>SSH4</i>	Tet-off vector, <i>tet-O7</i> promoter, for Ssh4 overexpression	This study
pCM189	<i>TUL1-GFP</i>	Tet-off vector, <i>tet-O7</i> promoter C-terminal GFP, for Tul1-GFP overexpression	This study
pCM189	<i>TUL1-pH-RING</i>	Tet-off vector, <i>tet-O7</i> promoter pHluorin inserted between G689 and G690, for Tul1-pH-RING overexpression	This study
pRS425	<i>pGal1-MYC-UB</i>	GAL1 promoter, used together with YML853 for overexpression of Myc-Ub by addition of 100 nM β -estradiol	This study
pRS416	<i>Zrt3*-GFP</i>	Endogenous promoter	This study

Table S2. Tested vacuolar membrane transporters

Name	Localization of GFP-tagged protein	Function
<i>AVT1</i>	No signal	Imports large neutral amino acids; related to vesicular GABA-glycine transporters
<i>AVT2</i>	No signal	Imports large neutral amino acids; related to vesicular GABA-glycine transporters
<i>AVT3</i>	Stuck in ER	Imports large neutral amino acids; related to vesicular GABA-glycine transporters
<i>AVT4</i>	No signal	Imports large neutral amino acids; related to vesicular GABA-glycine transporters
<i>AVT5</i>	No signal	Imports large neutral amino acids; related to vesicular GABA-glycine transporters
<i>AVT6</i>	No signal	Aspartate and glutamate exporter; related to vesicular GABA-glycine transporters
<i>AVT7</i>	Stuck in ER	Imports large neutral amino acids; related to vesicular GABA-glycine transporters
<i>CCC1</i>	Vacuole lumen	Putative vacuolar Fe^{2+}/Mn^{2+} transporter
<i>COT1</i>	Vacuole membrane	Zinc transport into the vacuole
<i>CTR2</i>	Stuck in ER	Putative low-affinity copper transporter of the vacuolar membrane
<i>ERS1</i>	No signal	Efflux of cystine
<i>FET5</i>	Vacuole membrane	Multicopper oxidase; may have a role in iron transport
<i>FTH1</i>	Vacuole membrane	Putative high-affinity iron transporter; involved in transport of intravacuolar stores of iron
<i>NCR1</i>	Vacuole membrane	Involved in ergosterol transport and sphingolipid metabolism, homologue of mammalian NPC1
<i>OPT2</i>	No signal	Oligopeptide transporter
<i>PHO91</i>	Stuck in ER	Low-affinity phosphate transporter of the vacuolar membrane
<i>VBA1</i>	No signal	Permease for cationic amino acids
<i>VBA2</i>	No signal	Permease for cationic amino acids
<i>VBA3</i>	No signal	Permease for cationic amino acids
<i>VBA4^a</i>	Vacuole membrane	Permease for cationic amino acids
<i>VCX1</i>	N-terminal GFP, vacuole membrane	H/ Ca^{2+} antiporter
<i>VMA16</i>	Stuck in ER	Subunit C of the vacuolar ATPase
<i>VMR1</i>	No signal	ABC transporter involved in multidrug transport
<i>VTC1</i>	Stuck in ER	Subunit of the vacuolar transporter chaperone (VTC) complex
<i>VTC2</i>	Stuck in ER	Subunit of the vacuolar transporter chaperone (VTC) complex
<i>VTC3</i>	No signal	Subunit of the vacuolar transporter chaperone (VTC) complex
<i>VTC4</i>	Stuck in ER	Involved in synthesis and transfer of polyP to the vacuole
<i>YCF1</i>	Vacuole membrane	ABC transporter and glutathione S-conjugate transporter; also transports unconjugated bilirubin
<i>YPL162C^b</i>	Vacuole membrane	Protein of unknown function
<i>YPQ2^a</i>	Vacuole membrane	Vacuolar membrane transporter for cationic amino acids
<i>YVC1</i>	Stuck in ER	Cation channel, mediates release of Ca^{2+}
<i>ZRC1</i>	Vacuole membrane	Zinc transporter; transports zinc from the cytosol into the vacuole for storage
<i>ZRT3*</i>	Vacuole membrane	Zinc transporter; transports zinc from vacuole to the cytoplasm

^aTested in a previous study (Li et al., 2015).

^bNot tested because the function is unknown.

Table S3. **Tested E3 ubiquitin ligases**

Gene deleted	Note	Blocked Cot1-GFP degradation?
<i>RKR1</i>	RING finger protein YMR247C	No
<i>UBR2</i>	E3 ubiquitin-protein ligase UBR2	No
<i>HRT3</i>	F-box protein HRT3	No
<i>UFO1</i>	Ubiquitin ligase complex F-box protein UFO1	No
<i>HRD1</i>	ERAD-associated E3 ubiquitin-protein ligase HRD1	No
<i>DIA2</i>	Protein DIA2	No
<i>SKP2</i>	F-box protein YNL311C	No
<i>SAN1</i>	Protein SAN1	No
<i>YDR131C</i>	F-box protein YDR131C	No
<i>MOT2</i>	General negative regulator of transcription subunit 4	No
<i>UBR1</i>	E3 ubiquitin-protein ligase UBR1	No
<i>UFD4</i>	Ubiquitin fusion degradation protein 4	No
<i>TUL1</i>	Transmembrane E3 ubiquitin-protein ligase 1	Yes
<i>YLR224W</i>	F-box protein YLR224W	No
<i>MDM30</i>	Mitochondrial distribution and morphology protein 30	No
<i>RCY1</i>	Recyclin-1	No
<i>YJL149W</i>	F-box protein	No
<i>YLR352W</i>	F-box protein	No
<i>ELA1</i>	Elongin-A	No
<i>AMA1</i>	Meiosis-specific APC/C activator protein	No
<i>CDH1</i>	APC/C activator protein	No
<i>PIB1</i>	E3 ubiquitin-protein ligase	A subtle defect observed on Western blot
<i>YDR306C</i>	F-box protein	No
<i>ASI1</i>	Amino acid sensor-independent protein 1	No
<i>RAD5</i>	DNA repair protein RAD5	No
<i>UFD2</i>	Ubiquitin conjugation factor E4	No
<i>RAD7</i>	DNA repair protein	No
<i>HUL4</i>	Probable E3 ubiquitin-protein ligase	No
<i>RAD18</i>	Postreplication repair E3 ubiquitin-protein ligase	No
<i>SAF1</i>	F-box protein	No
<i>ASI3</i>	Amino acid sensor-independent protein 3	No
<i>TOM1</i>	E3 ubiquitin-protein ligase	No
<i>SSM4</i>	E3 ubiquitin-protein ligase	No
<i>HUL5</i>	Probable E3 ubiquitin-protein ligase	No
<i>BRE1</i>	E3 ubiquitin-protein ligase	No
<i>GRR1</i>	SCF E3 ubiquitin ligase complex F-box protein	No
<i>MET30</i>	F-box protein	No
<i>CDC4</i>	Cell division control protein 4	No

References

- Li, M., Y. Rong, Y.S. Chuang, D. Peng, and S.D. Emr. 2015. Ubiquitin-dependent lysosomal membrane protein sorting and degradation. *Mol. Cell.* 57:467–478. <http://dx.doi.org/10.1016/j.molcel.2014.12.012>
- Robinson, J.S., D.J. Klionsky, L.M. Banta, and S.D. Emr. 1988. Protein sorting in *Saccharomyces cerevisiae*: isolation of mutants defective in the delivery and processing of multiple vacuolar hydrolases. *Mol. Cell. Biol.* 8:4936–4948. <http://dx.doi.org/10.1128/MCB.8.11.4936>