Supplementary Table 1

Table S1

Yeast strains used in this study

Strain	Genotype	Reference
BY4742	*MATα, his3Δ1, leu2Δ0, ura3Δ0, lys2Δ0, MET15*	Open Biosystems
vms1Δ::HIS3	*MATα, his3Δ1, leu2Δ0, ura3Δ0, lys2Δ0, MET15, vms1Δ::HIS3*	This study
vms1Δ::KanMX	*MATα, his3Δ1, leu2Δ0, ura3Δ0, lys2Δ0, MET15, vms1Δ::KanMX*	Open Biosystems
vms1Δ	*MATα/A, his3Δ1, leu2Δ0, ura3Δ0, lys2Δ0, MET15, vms1Δ::KanMX, vms1Δ::HIS3*	This study
ufd2Δ::KanMX	*MATα, his3Δ1, leu2Δ0, ura3Δ0, lys2Δ0, MET15, ufd2Δ::KanMX*	Open Biosystems
vms1Δufd2Δ	*MATα, his3Δ1, leu2Δ0, ura3Δ0, lys2Δ0, MET15, vms1Δ::HIS3, ufd2Δ::KanMX*	This study
ubx1Δ	*MATα, his3Δ1, leu2Δ0, ura3Δ0, lys2Δ0, MET15, ubx1Δ::KanMX*	Open Biosystems
vms1Δubx1Δ	*MATα, his3Δ1, leu2Δ0, ura3Δ0, lys2Δ0, MET15, vms1Δ::HIS3, ubx1Δ::KanMX*	This study
ubx2Δ	*MATα, his3Δ1, leu2Δ0, ura3Δ0, lys2Δ0, MET15, ubx2Δ::KanMX*	Open Biosystems
vms1Δubx2Δ	*MATα, his3Δ1, leu2Δ0, ura3Δ0, lys2Δ0, MET15, vms1Δ::HIS3, ubx2Δ::KanMX*	This study
ubx3Δ	*MATα, his3Δ1, leu2Δ0, ura3Δ0, lys2Δ0, MET15, ubx3Δ::KanMX*	Open Biosystems
vms1Δubx3Δ	*MATα, his3Δ1, leu2Δ0, ura3Δ0, lys2Δ0, MET15, vms1Δ::HIS3, ubx3Δ::KanMX*	This study
ubx4Δ	*MATα, his3Δ1, leu2Δ0, ura3Δ0, lys2Δ0, MET15, ubx4Δ::KanMX*	Open Biosystems
vms1Δubx4Δ	*MATα, his3Δ1, leu2Δ0, ura3Δ0, lys2Δ0, MET15, vms1Δ::HIS3, ubx4Δ::KanMX*	This study
ubx5Δ	*MATα, his3Δ1, leu2Δ0, ura3Δ0, lys2Δ0, MET15, ubx5Δ::KanMX*	Open Biosystems
vms1Δubx5Δ	*MATα, his3Δ1, leu2Δ0, ura3Δ0, lys2Δ0, MET15, vms1Δ::HIS3, ubx5Δ::KanMX*	This study
ubx6Δ	*MATα, his3Δ1, leu2Δ0, ura3Δ0, lys2Δ0, MET15, ubx6Δ::KanMX*	Open Biosystems
vms1Δubx6Δ	*MATα, his3Δ1, leu2Δ0, ura3Δ0, lys2Δ0, MET15, vms1Δ::HIS3, ubx6Δ::KanMX*	This study
ubx7Δ	*MATα, his3Δ1, leu2Δ0, ura3Δ0, lys2Δ0, MET15, ubx7Δ::KanMX*	Open Biosystems
vms1Δubx7Δ	*MATα, his3Δ1, leu2Δ0, ura3Δ0, lys2Δ0, MET15, vms1Δ::HIS3, ubx7Δ::KanMX*	This study
npl4Δ	*MATα, his3Δ1, leu2Δ0, ura3Δ0, lys2Δ0, MET15, npl4Δ::KanMX*	Open Biosystems
vms1Δnpl4Δ	*MATα, his3Δ1, leu2Δ0, ura3Δ0, lys2Δ0, MET15, vms1Δ::HIS3, npl4Δ::KanMX*	This study
cdc48-3	*MATα, his3Δ1, leu2, ura3, lys2Δ0, MET15, cdc48-3*	This study
vms1Δcdc48-3	*MATα, his3Δ1, leu2, ura3, lys2Δ0, MET15, cdc48-3, vms1Δ::KanMX*	This study
pdr5Δ	*MATα, his3Δ1, leu2Δ0, ura3Δ0, lys2Δ0, MET15, pdr5Δ::KanMX*	Open Biosystems
vms1Δpdr5Δ	*MATα, his3Δ1, leu2Δ0, ura3Δ0, lys2Δ0, MET15, vms1Δ::HIS3, pdr5Δ::KanMX*	This study

Supplementary Table 1. List of strains used in this study. All strains were in the BY4742 background.

Supplementary Table 2

Table S2

Oligos used in this study

Oligo	Sequence
*VMS1*Δ::*KanMX*-F	ggattttcaaaagatctgcacgcctgttgacaagcttccaatagcatctgtgcggtatttcacaccg
*VMS1*Δ::*KanMX*-R	gcaaatgctaagaaaaatcctaaaaatttgaatatgagatattccagattgtactgagagtgcac
*VMS1*Δ::*HIS3*-F	ggattttcaaaagatctgcacgcctgttgacaagcttccaatagcatcggatccccgggttaattaa
*VMS1*Δ::*HIS3*-R	gcaaatgctaagaaaaatcctaaaaatttgaatatgagatattccgaattcgagctcgtttaaac
VMS1 screen-F	ttcttggaggagtgccacag
VMS1 screen-R	ggcgtcattttcgcgttgag
ufd2 Δ::His3-F	ccaatagaaaggtaaagttgaccacaagttgtttaaggggaaaagttaactttgaaagtagaaccctcattccatagatccggatccccgggttaattaa
ufd2 Δ::His3-R	aaatataagacacattgagcgatgaaataagccttatttgattagggtcaattttgcaatttattctatcacttattcatgaattcgagctcgtttaaac
UFD2 screen-F	ccagtttcgagaatctagtgctg
UFD2 screen-R	gaagcaaatcgctttcccacaa
UBX1 screen-F	gtagtgacaaacatgcctctggat
UBX1 screen-R	gcagcagttattcatgatgctggt
UBX2 screen-F	tggctgaggattgccgccaagctg
UBX2 screen-R	actataaaggtaggccccagctcc
UBX3 screen-F	agaccgcctaattggatcatcg
UBX3 screen-R	aaactgatgcacgtgacactt
UBX4 screen-F	aagatagcgggcgcctcaaccgct
UBX4 screen-R	gtacaagttacggaaggcggagct
UBX5 screen-F	ctcgatgtctctgcagaagcga
UBX5 screen-R	caacagcggcagatgcatcgct
UBX6 screen-F	ggatttacctctagcgcgtcaacc
UBX6 screen-R	aaccaggatttgcacgagcca
UBX7 screen-F	gtgctgcccatatacagcaactt
UBX7 screen-R	gctgagttcttttgcggtgat
CDC48-NotI-F1	ttgcggccgcggtggccagcccaagaaacgga
CDC48-XhoI-R1	agctcgagacgaccgaggtcctacagcct
CDC48-Cterm-BamHI-R1	acggatccactatacaaatcatcatcttcc
CDC48-Myc-BamHI-F1	acggatccGAACAAAAACTCATCTCAGAAGAGGATCTGtagtagttatatgccaggtatatttttattttaaatcg
CDC48-HA-BamHI-F1	acggatccTACCCATACGACGTCCCAGACTACGCTtagtagttatatgccaggtatatttttattttaaatcg
VMS1-NotI-F	ctgcggccgcttcttggaggagtgccacag
VMS1-SalI-R	tcggtcgacggcgtcattttcgcgttgag
VMS1-Cterm-BamHI-R1	acggatccgtatttcttttttcatcctttcttctgcg
VMS1-Myc-BamHI-F1	acggatccGAACAAAAACTCATCTCAGAAGAGGATCTGtgatgaggaatatctcatattcaaatttttagg
VMS1-HA-BamHI-F1	acggatccTACCCATACGACGTCCCAGACTACGCTtgatgaggaatatctcatattcaaatttttagg

Supplementary Table 2. List of oligonucleotide primers used in this study. Restriction enzyme recognition sites are underlined and epitope tags are capitalized.

Supplementary Table 3

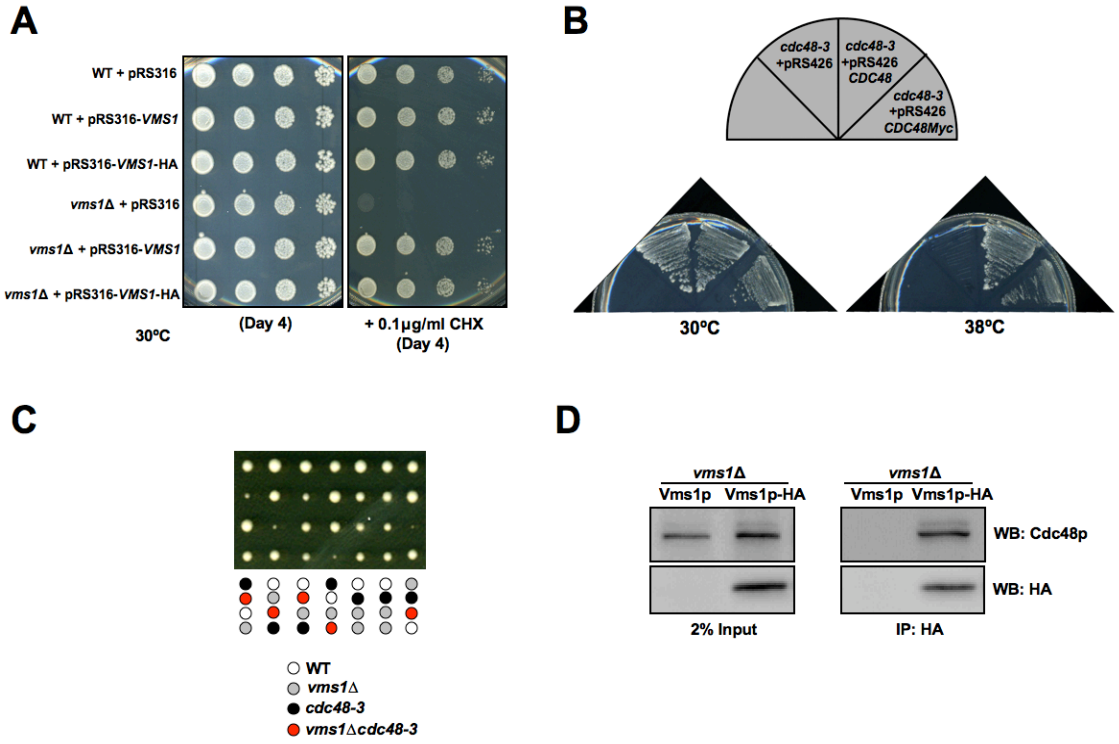
Table S3

Plasmids used in this study

Plasmid name	Description	Reference
pSM1152	PGK1 promoter, CFTR-HA expression plasmid, 2 micron	Zhang, et al., 2002
pSM1911	PGK1 promoter, Ste6p*-HA expression plasmid, 2 micron	Huyer, et al., 2006
pUB23-Ub-Pro	*GAL* promoter, Ubiquitin-Proline β galactosidase, 2 micron	Bachmair, et al., 1986
CPY*-3xHA	Endogenous promoter, CPY* 3xHA expression plasmid, CEN	Bhamidipati, et al., 2005
pRS316-*CDC48*	Endogenous promoter, untagged *CDC48*, CEN	This study
pRS426-*CDC48*	Endogenous promoter, untagged *CDC48*, 2 micron	This study
pRS316-*CDC48*Myc	Endogenous promoter, C-terminal 1xmyc tagged *CDC48*, CEN	This study
pRS316-*CDC48*HA	Endogenous promoter, C-terminal 1xHA tagged *CDC48*, CEN	This study
pRS426-*CDC48*Myc	Endogenous promoter, C-terminal 1xmyc tagged *CDC48*, 2 micron	This study
pRS426-*VMS1*	Endogenous promoter, untagged *VMS1*, CEN	This study
pRS315-*VMS1*HA	Endogenous promoter, C-terminal 1xHA tagged *VMS1*, CEN	This study
pRS316-*VMS1*HA	Endogenous promoter, C-terminal 1xHA tagged *VMS1*, CEN	This study
pRS426-*VMS1*HA	Endogenous promoter, C-terminal 1xHA tagged *VMS1*, 2 micron	This study

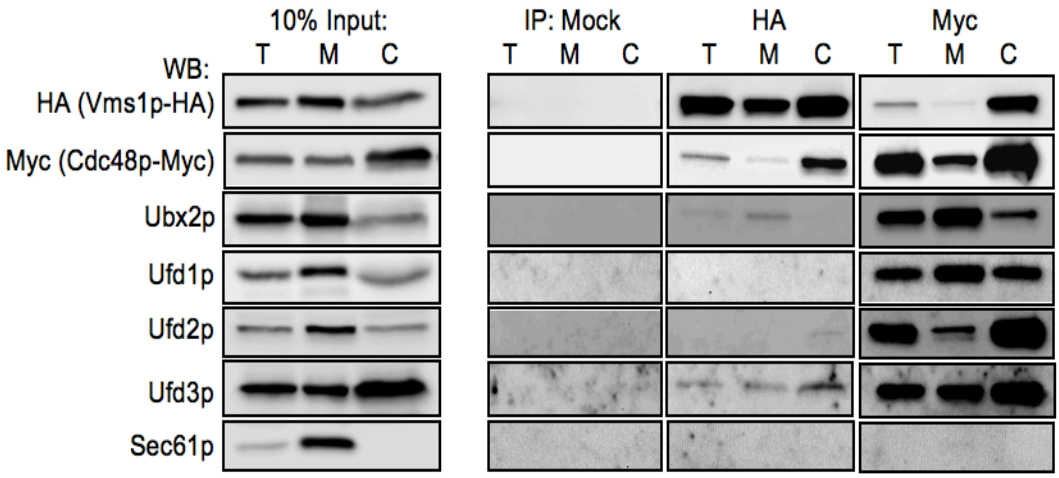
Supplementary Table 3. Plasmids used in the study. Unless referenced, all plasmids were constructed by PCR amplification and cloning as detailed in the Experimental Procedures section.

Supplementary Figure S1



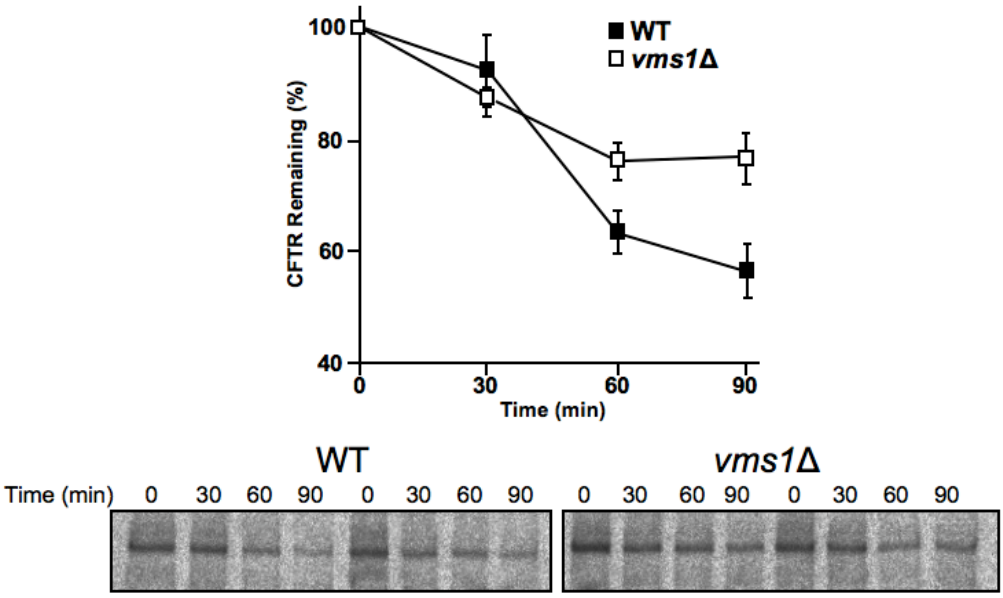
Supplementary Figure S1. *A*. An epitope-tagged form of Vms1p is functional. WT BY4742 and *vms1*Δ strains were transformed with an empty vector or a CEN plasmid containing either the untagged or HA-tagged form of Vms1p. Serial dilutions were spot plated on selective media lacking or containing 0.1µg/ml cycloheximide. *B*. An epitope-tagged form of Cdc48p is functional. The indicated strains were transformed with an empty vector or untagged or Myc-tagged forms of Cdc48p. Serial dilutions of the transformants were spot plated on selective media and then incubated at 30ºC or 38ºC. *C*. *VMS1* genetically interacts with a mutant allele of *CDC48*. A temperature sensitive mutant allele of *CDC48*, *cdc48-3*, was genetically crossed with strains lacking the *VMS1* gene. Tetrads were sporulated, dissected, and grown at 30ºC. *D*. Cdc48p coimmunoprecipitates with Vms1p from ER-enriched fractions. ER-membranes were prepared as described in the Experimental Procedures, solubilized and immunoprecipitated with anti-HA agarose. Immunoprecipitates were resolved by SDS-PAGE and immunoblotted for anti-HA and Anti-Cdc48p.

Supplementary Figure S2



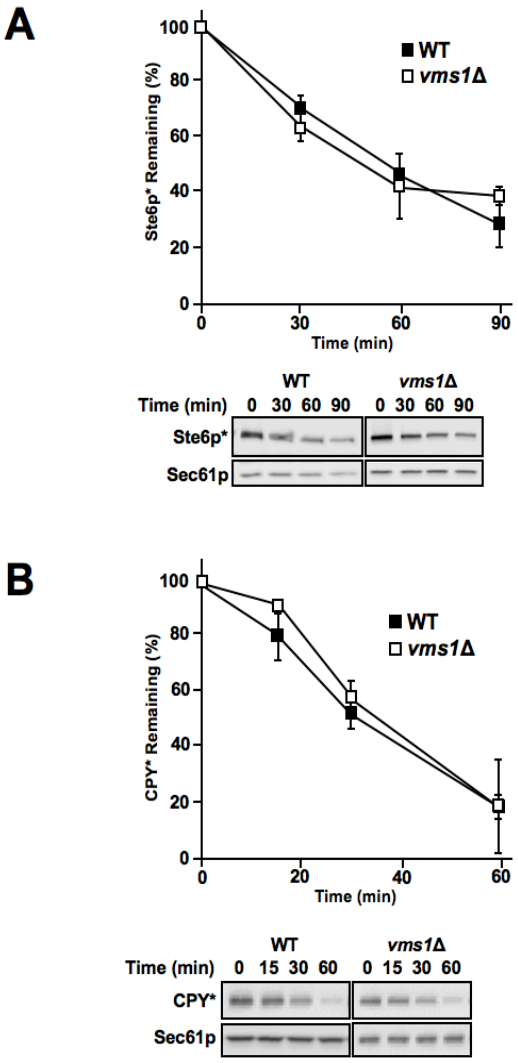
Supplementary Figure S2. Vms1p physically associates with other members of the Cdc48p complex. Total lysate (T), membrane (M), and cytosolic (C), fractions were prepared from cells expressing Vms1p-HA from a 2μ plasmid the under control of its endogenous promoter. Vms1p-HA was immunoprecipitated with anti-HA agarose and Cdc48p-Myc was immunoprecipitated with anti-Myc agarose as described in the Experimental Procedures. Immunoprecipitated material was resolved by SDS-PAGE followed by immunoblot analysis with the indicated antibodies.

Supplementary Figure S3



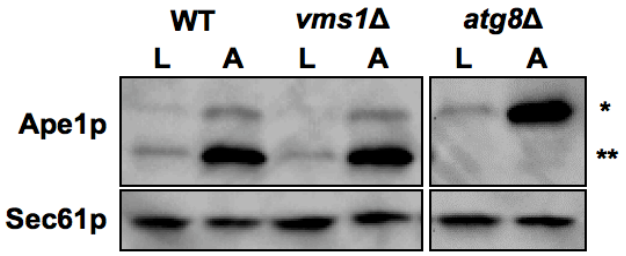
Supplementary Figure S3. Loss of *VMS1* affects the ERAD of CFTR as assessed by pulse-chase analysis. Wild type and *vms1*Δ cells expressing CFTR-HA were radio-labeled for 1 hour and chased with cold methionine and cysteine. The indicated time points were taken, the cells were lysed and CFTR-HA was immunoprecipitated with anti-HA agarose. The immunoprecipitate was resolved on a 10% SDS-polyacrylamide gel and subject to radiography. Data were quantitated relative to the zero time point. N = 10, +/- SEM. Wild-type (WT) cells are denoted by the filled squares and *vms1*Δ cells are represented by the unfilled squares.

Supplementary Figure S4



Supplementary Figure S4. Loss of *VMS1* has no effect on the degradation of two other model ERAD substrates, (*A*.) Ste6p* and (*B*.) CPY*, as assessed by cycloheximide chase. For both (*A*.) and (*B*.), wild-type is represented by filled squares and *vms1*Δ is represented by open squares.

Supplementary Figure S5

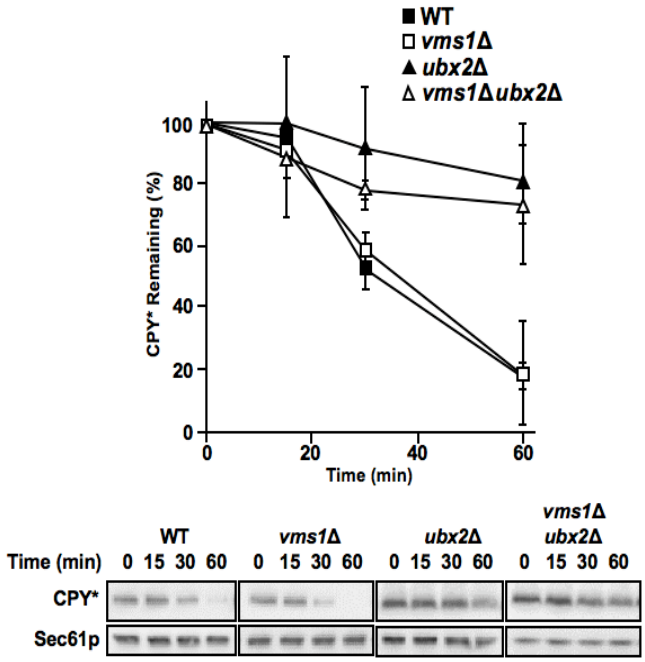


L: Log-phase
A: Nitrogen starvation/Autophagic induction

*** Unprocessed Ape1p**
**** Processed Ape1p**

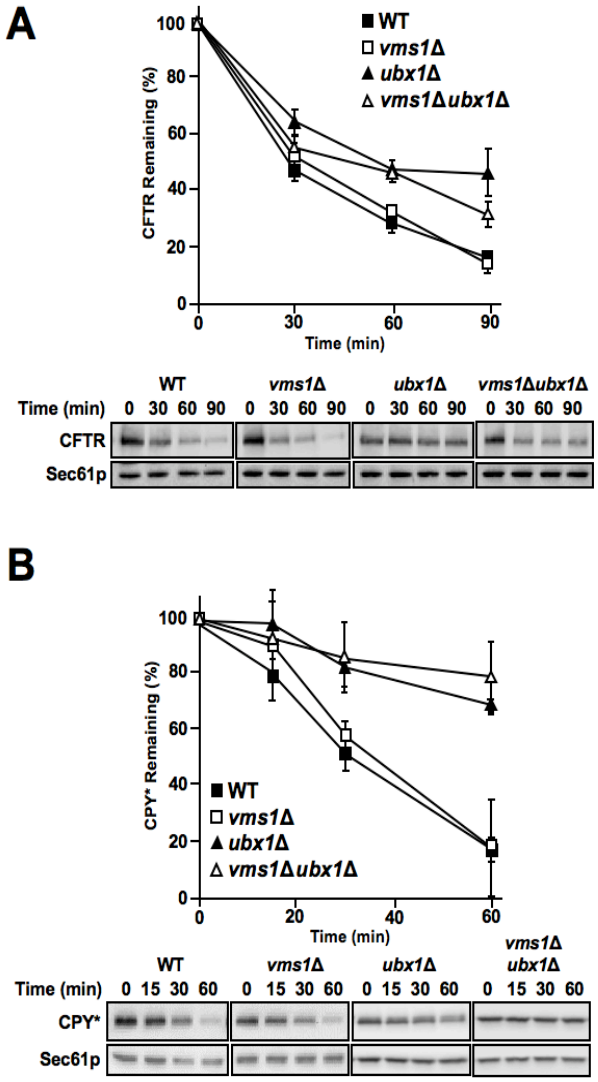
Supplementary Figure S5. Strains lacking *VMS1* do not exhibit a defect in the Cytoplasmic-to-Vacuole Transport (CVT) pathway. Wild-type (WT), *vms1*Δ, and *atg8*Δ cells were grown in rich medium or in nitrogen-poor medium. Total lysates were prepared, and equal amounts of lysate were separated by SDS-PAGE for immunoblotting with a marker of CVT activity, Ape1p. Sec61p was analyzed as a loading control.

Supplementary Figure S6



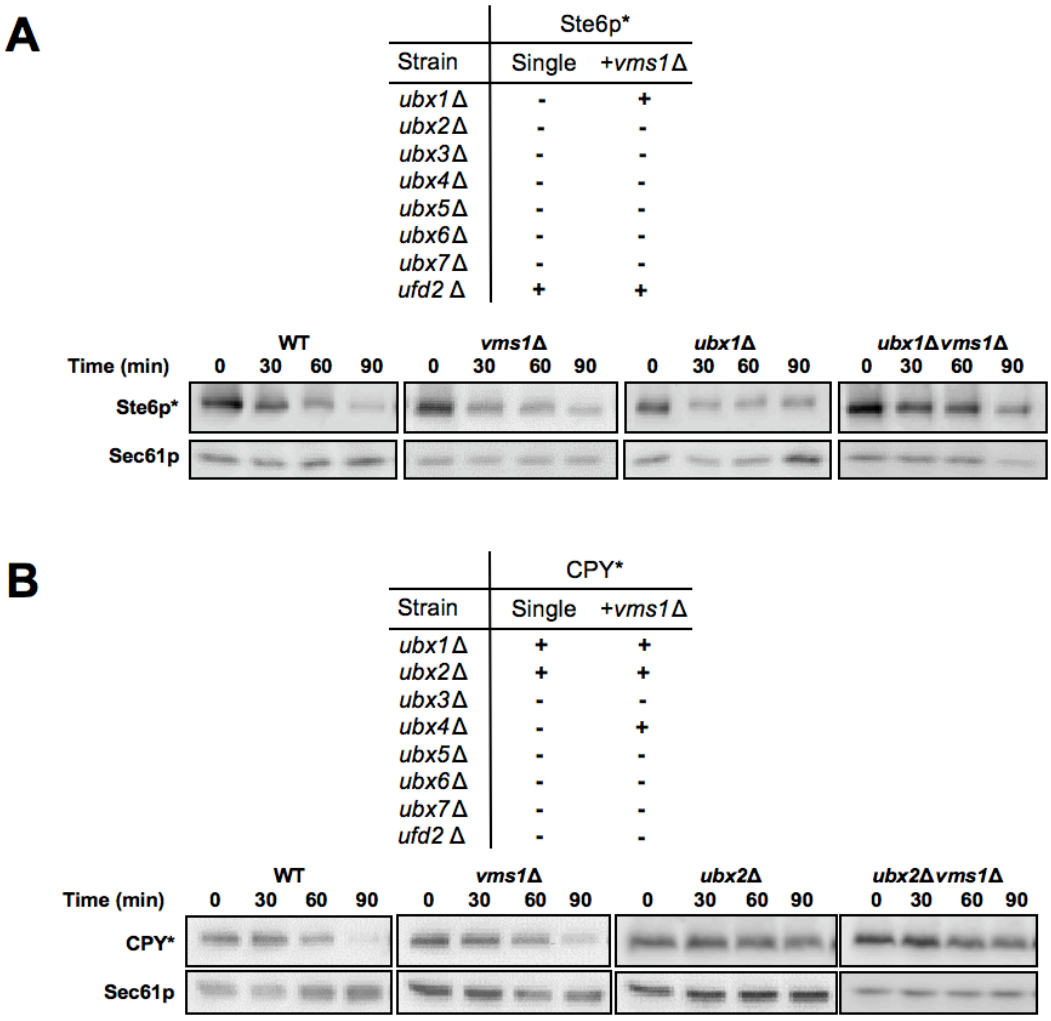
Supplementary Figure S6. The simultaneous loss of *UBX2* and *VMS1* does not result in a synthetic ERAD defect for CPY*. Wild-type is denoted by filled squares, *vms1*Δ by open squares, *ubx2*Δ by filled triangles, and *vms1*Δ*ubx2*Δ by open triangles.

Supplementary Figure S7



Supplementary Figure S7. The loss of *UBX1* results in an ERAD defect for CFTR and CPY*. For (*A*.) and (*B*.), wild-type is denoted by filled squares, *vms1*Δ by open squares, *ubx1*Δ by filled triangles, and *vms1*Δ*ubx1*Δ by open triangles.

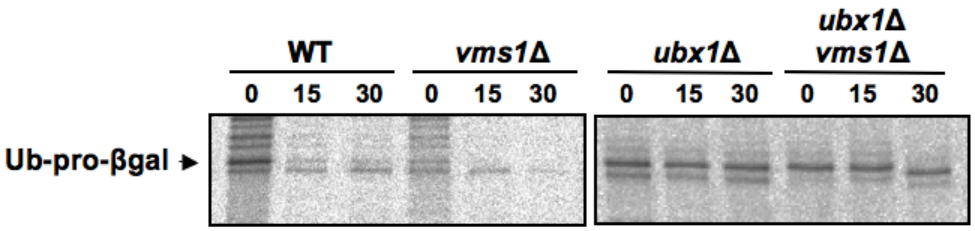
Supplementary Figure S8



Supplementary Figure S8. A summary of degradation assays for the ERAD substrates Ste6p* *(A)* and CPY* *(B)*. Strains listed in the tables in *(A)* and *(B)* were transformed with a plasmid engineered to express Ste6p*-HA or the CPY*-HA, respectively. Cycloheximide chase analyses were performed and data were quantitated relative to the zero time point. Beneath each table are representative images from select experiments.

Supplementary Figure S9

Strain	Ub-Pro-β-gal	
	Single	+*vms1*Δ
*ubx1*Δ	+	+
*ubx2*Δ	+	+
*ubx3*Δ	-	-
*ubx4*Δ	-	-
*ubx5*Δ	-	-
*ubx6*Δ	-	-
*ubx7*Δ	-	-
*ufd2*Δ	+	+



Supplementary Figure S9. A summary of degradation assays for the N-end rule substrate, Ub-Pro-βgal. A radio-labeling pulse cycloheximide chase was performed and data were quantitated relative to the zero time point. A representative image corresponding to a select experiment is shown.