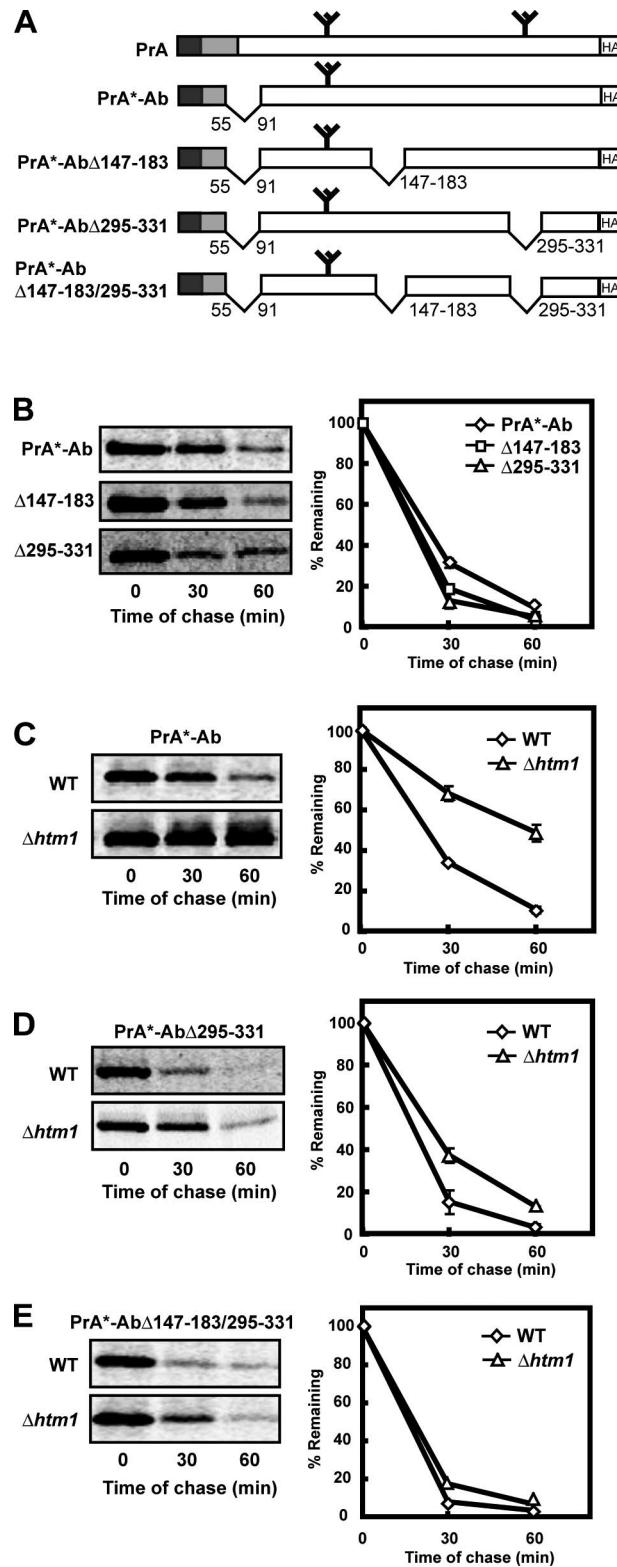


Figure S1. Specific PrA* variants bypass the Htm1p requirement for degradation. (A) Schematic representation of PrA variants with positions of deletions under each diagram. Carbohydrates are represented by branched symbols, signal sequences are shown in dark gray, prodomains are colored light gray, and the positions of HA epitope tags are shown. PrA* differs from PrA by lacking sequences Leu55 through Tyr91. (B) Wild-type cells expressing PrA*-Ab, PrA*-Ab Δ 147–183, or PrA*-Ab Δ 295–331 were pulse labeled for 10 min with [35 S]methionine/cysteine followed by a cold chase for the indicated times. Immunoprecipitations of substrate proteins were performed using anti-HA antibody and resolved by SDS-PAGE. Decay rates were quantified by phosphorimager analysis. (C) Wild-type (WT) and Δ htm1 cells expressing PrA*-Ab were analyzed by pulse-chase analysis as described in B. (D) Wild-type and Δ htm1 cells expressing PrA*-Ab Δ 295–331 were analyzed by pulse-chase analysis as described in B. (E) Wild-type and Δ htm1 cells expressing PrA*-Ab Δ 147–183/295–331 were analyzed by metabolic pulse chase as described in B. (B–E) The plotted data reflect three independent experiments with the SEM indicated by error bars. Representative gel images are shown to the left of each plot.



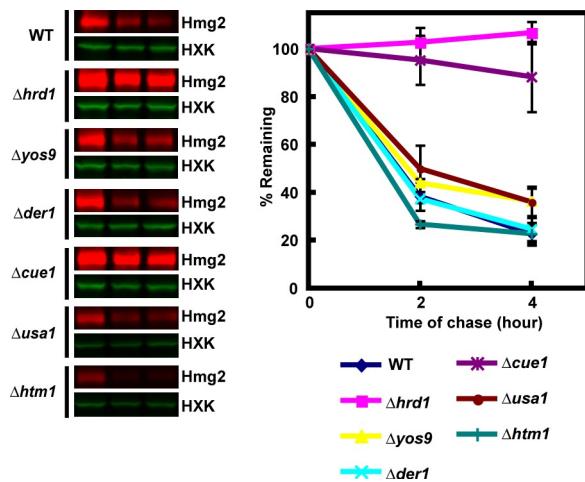


Figure S2. *DER1*, *USA1*, *YOS9*, and *HTM1* are dispensable for the degradation of the ERAD-M substrate Hmg2p. Wild-type (WT), $\Delta hrd1$, $\Delta yos9$, $\Delta der1$, $\Delta cue1$, $\Delta usa1$, and $\Delta htm1$ cells expressing Myc-tagged Hmg2p were grown to log phase, and cycloheximide was added to begin chase for the indicated times. Whole cell lysates were prepared, and proteins were resolved by SDS-PAGE. Hmg2p was detected on immunoblots using the anti-Myc mAb. Endogenous hexokinase (HXK) was detected on the same blots as a loading control. Antibody–protein complexes were visualized and quantified using an infrared imaging system (Odyssey). The data plotted represent three independent experiments with the SEM indicated by error bars.

Table S1. Peptide analysis of ngPrAΔ295–331-binding protein

Start–End	M_r (expt)	M_r (calc)	Δ	Miss	Sequence	Ion score
135–143	1,066.61	1,066.59	0.02	0	HLPFNVVNK	17
135–143	1,066.61	1,066.59	0.02	0	HLPFNVVNK	ND
502–512	1,196.67	1,196.66	0.01	0	FELTGIPPAPR	21
502–512	1,196.67	1,196.66	0.01	0	FELTGIPPAPR	ND
206–217	1,198.68	1,198.67	0.01	0	DAGTIAGLNVLR	ND
347–356	1,266.67	1,266.65	0.02	0	FEELNLDLFK	ND
375–387	1,344.7	1,344.69	0.01	0	DVDDIVLVGGSTR	25
375–387	1,344.7	1,344.69	0.01	0	DVDDIVLVGGSTR	ND
391–402	1,425.75	1,425.71	0.04	0	VQQLESYFDGK	ND
345–356	1,465.8	1,465.78	0.02	1	AKFEELNLDLFK	16
345–356	1,465.8	1,465.78	0.02	1	AKFEELNLDLFK	–β
83–95	1,512.74	1,512.71	0.03	0	ITPSYVAFTDDER	17
83–95	1,512.74	1,512.71	0.03	0	ITPSYVAFTDDER	ND
391–403	1,553.83	1,553.81	0.02	1	VQQLESYFDGKK	19
391–403	1,553.83	1,553.81	0.02	1	VQQLESYFDGKK	ND
218–233	1,644.89	1,644.87	0.02	0	IVNEPTAAAIAYGLDK	32
218–233	1,644.89	1,644.87	0.02	0	IVNEPTAAAIAYGLDK	ND
158–172	1,663.92	1,663.89	0.03	1	KVFTPEEISGMILGK	ND
103–117	1,671.89	1,671.86	0.03	0	NQVAANPQNTIFDIK	24
103–117	1,671.89	1,671.86	0.03	0	NQVAANPQNTIFDIK	ND
103–118	1,827.96	1,827.96	0	1	NQVAANPQNTIFDIK	ND
553–567	1,834.89	1,834.86	0.03	1	LTQEEIDRMVEEAEK	ND
185–201	1,887.01	1,886.96	0.04	0	VTHAVVTVPAYFNDAQR	26
185–201	1,887.01	1,886.96	0.04	0	VTHAVVTVPAYFNDAQR	ND
468–484	1,893.99	1,893.95	0.04	0	SQIFSTAVDNQPTVMIK	ND
327–344	2,022.06	2,022.02	0.05	0	IEIDSFVDGIDLSETLTR	31
467–484	2,022.06	2,022.05	0.02	1	KSQIFSTAVDNQPTVMIK	ND

M_r (expt), experimentally determined mass; M_r (calc), calculated mass of the peptide. The peptide sequences of Kar2p identified by mass spectrometry are summarized. Peptide position indicates the start and end point of the identified peptide within the sequence of Kar2p with the translation initiator methionine as position 1. Δ represents the observed mass versus Δ error. Miss indicates the number of missed trypsin cleavage sites, and the peptides identified column lists the actual sequence of the identified peptide. The ion score is $-10 \cdot \log(P)$, where P is the probability that the observed match is a random event.

Table S2. Yeast strains used in this study

Strain	Genotype	Source
W303a	MAT _a , <i>leu2-3, 112, his3-11, trp1-1, ura3-1, can1-100, ade2-1</i>	P. Walter ^a
MS10	MAT _a , <i>leu2-3, 112, ura3-52, ade2-101</i>	J. Brodsky ^b
KKY62	MAT _a , pKK129, W303 background	Xie et al., 2009
KKY129	MAT _a , pKK129, W303 background	Xie et al., 2009
KKY133	MAT _a , pKK163, W303 background	Xie et al., 2009
KKY63	MAT _a , <i>htm1::KANMX, pKK129, W303 background</i>	This study
KKY143	MAT _a , <i>htm1::KANMX, pKK163, W303 background</i>	This study
KKY191	MAT _a , pKK214, W303 background	This study
KKY192	MAT _a , <i>htm1::KANMX, pKK214, W303 background</i>	This study
KKY354	MAT _a , pKK232, W303 background	This study
KKY355	MAT _a , <i>htm1::KANMX, pKK232, W303 background</i>	This study
KKY357	MAT _a , <i>hrd1::KANMX, pKK232, W303 background</i>	This study
KKY595	MAT _a , pKK249, W303 background	This study
KKY596	MAT _a , <i>htm1::KANMX, pKK249, W303 background</i>	This study
KKY597	MAT _a , <i>hrd1::KANMX, pKK249, W303 background</i>	This study
KKY604	MAT _a , pKK252, W303 background	This study
KKY606	MAT _a , <i>hrd1::KANMX, pKK252, W303 background</i>	This study
KKY756	MAT _a , <i>usa1::KANMX, pKK252, W303 background</i>	This study
KKY699	MAT _a , <i>der1::KANMX, pKK252, W303 background</i>	This study
KKY697	MAT _a , <i>yos9::KANMX, pKK252, W303 background</i>	This study
KKY605	MAT _a , <i>htm1::KANMX, pKK252, W303 background</i>	This study
KKY622	MAT _a , pKK261, W303 background	This study
KKY623	MAT _a , <i>htm1::KANMX, pKK261, W303 background</i>	This study
KKY624	MAT _a , <i>hrd1::KANMX, pKK261, W303 background</i>	This study
KKY993	MAT _a , <i>cdc48-1, pKK261, W303 background</i>	This study
KKY992	MAT _a , <i>cue1::KANMX, pKK261, W303 background</i>	This study
KKY704	MAT _a , <i>der1::KANMX, pKK261, W303 background</i>	This study
KKY755	MAT _a , <i>usa1::KANMX, pKK261, W303 background</i>	This study
KKY678	MAT _a , <i>yos9::KANMX, pKK261, W303 background</i>	This study
KKY708	MAT _a , <i>scj1::KANMX, jem1::KANMX pKK261, W303 background</i>	This study
KKY88	MAT _a , <i>yos9::KANMX, pKK129, W303 background</i>	This study
KKY666	MAT _a , <i>yos9::KANMX, pKK129, pKK278, W303 background</i>	This study
KKY720	MAT _a , <i>yos9::KANMX, pKK129, pKK284, W303 background</i>	This study
KKY667	MAT _a , <i>yos9::KANMX, pKK261, pKK278, W303 background</i>	This study
KKY721	MAT _a , <i>yos9::KANMX, pKK261, pKK284, W303 background</i>	This study
KKY655	MAT _a , <i>pep4::HIS3, pKK261, W303 background</i>	This study
KKY745	MAT _a , pKK129, pRS315, W303 background	This study
KKY739	MAT _a , pKK129, pKK286, W303 background	This study
KKY746	MAT _a , pKK261, pRS315, W303 background	This study
KKY740	MAT _a , pKK261, pKK286, W303 background	This study
KKY931	MAT _a , pRH244, W303 background	This study
KKY932	MAT _a , <i>hrd1::KANMX, pRH244, W303 background</i>	This study
KKY933	MAT _a , <i>yos9::KANMX, pRH244, W303 background</i>	This study
KKY934	MAT _a , <i>der1::KANMX, pRH244, W303 background</i>	This study
KKY976	MAT _a , <i>cue1::KANMX, pRH244, W303 background</i>	This study
KKY977	MAT _a , <i>htm1::KANMX, pRH244, W303 background</i>	This study
KKY978	MAT _a , <i>usa1::KANMX, pRH244, W303 background</i>	This study
WXY81	MAT _a , pWX41, W303 background	This study
WXY56	MAT _a , pWX29, W303 background	This study
WXY57	MAT _a , pWX30, W303 background	This study
WXY58	MAT _a , pWX31, W303 background	This study
WXY103	MAT _a , pWX51, W303 background	This study
WXY104	MAT _a , <i>htm1::KANMX, pWX51, W303 background</i>	This study
WXY437	MAT _a , pKK261, MS10 background	This study
WXY438	MAT _a , <i>kar2-1, pKK261, MS10 background</i>	This study
WXY443	MAT _a , pSM1083, pKK286, W303 background	This study
WXY444	MAT _a , pSM1083, pRS315, W303 background	This study

^aUniversity of California, San Francisco, San Francisco, CA.^bUniversity of Pittsburgh, Pittsburgh, PA.

Table S3. Plasmids used in this study

Plasmid	Encoded protein	Primers used	Vector	Source
pKK129	PrA*-Ab-HA	NA	pRS316	Xie et al., 2009
pKK159	PrA*-Ab Δ 147–183-HA	NA	pRS316	Xie et al., 2009
pKK163	PrA*-Ab Δ 295–331-HA	NA	pRS316	Xie et al., 2009
pKK214	PrA*-Ab Δ 147–183, 295–331-HA	KKN136	pRS316	This study
pKK232	PrA*-GI	KKN228	pRS316	This study
pKK252	PrA-CTD	KKN279	pRS316	This study
pKK249	ngPrA* Δ 295–331-HA	NA	pRS316	This study
pKK261	ngPrA Δ 295–331-HA	NA	pRS316	Xie et al., 2009
pKK278	Yos9p	KKN303	pRS313	This study
pKK284	Yos9R200A	KKN306	pRS313	This study
pKK286	CPY*	KKN307	pRS315	This study
pWX41	PrA*-GI Δ 1	WZN41	pRS316	This study
pWX29	PrA*-GI Δ 2	WZN29	pRS316	This study
pWX30	PrA*-GI Δ 3	WZN30	pRS316	This study
pWX31	PrA*-GI Δ 4	WZN31	pRS316	This study
pWX51	PrA*-GI Δ 4CHO	WZN31	pRS316	This study
pSM1083	NA	NA	NA	Loayza et al., 1998
pRH244	Hmg2p	NA	NA	R. Hampton ^a

NA, not applicable.

^aUniversity of California, San Diego, La Jolla, CA.

Table S4. Oligonucleotide primers used in this study

Primer	Sequence
KKN303	5'-TGTTTCATCGAACATGATGAGCTTAGGGCATGGCCTACCCATATGATG-3'
KKN306	5'-TTCGATGTGACGGGGCTGAAGCAATGGTAAATACAATATGTCG-3'
KKN307	5'-ATGGATCCACGGTGGTTCTCTTATAGTCTAGATAATCTGCTTTGT-3'
KKN136	5'-CTCTAGACTGTAACACCAAGAGATTCCCAGAACCTGTTGG-3'
KKN228	5'-GAAGCTTCATCAAGCTACAAAGCTCAGGGTACTGAATTGCCATTCAATAT-3'
KKN279	5'-AGTGCTGCAAAAGTCCACAAGGCTAGCCATGGTGCCGCCATGATACTG-3'
WZN29	5'-ACCTCAAAACTTCAAGGTATTGGAGGCTACCAGCGATTGGACG-3'
WZN30	5'-GACCATCCAAAACAAGACTCGCTAGCCATGGGCCGCATCGATACTG-3'
WZN31	5'-AGGCAGCAGAGTACGCCGAATTGGAGTACCCATATGATGTTCCAGATTACG-3'
WZN41	5'-GGTCAGGCCAACCAAGTTGCTGCAGATACTGGTCTCAAACCTTGGG-3'

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