SUPPLEMENTARY MATERIALS

SUPPLEMENTARY MATERIALS AND METHODS

Plasmid construction

pKK117 and pKK129, expression vectors for PrA*-HA and PrA*-Ab-HA, respectively. A 2.1-kb fragment was amplified from pES163 (Spear and Ng, 2005) by Vent polymerase-directed PCR (New England Biolabs, Beverly MA) using KKN71 and T3 primers, digested with *Xho*I, and purified. In parallel, a 0.5-kb fragment was amplified from pES163 using primers T7 and 5'-phosphorylated KKN72, digested with *Not*I and purified. These fragments were inserted into pRS316 (Sikorski and Hieter, 1989) create pKK117. Next, a N308Q mutation was introduced to eliminate the "B" glycosylation site. Using pKK117 as a template, the T3 and N537 primers amplified a 1.9 Kb fragment, digested with *Xho*I, and purified. A 0.7-kb fragment was amplified from pKK117 using primers T7 and N538, digested with *Not*I, and purified. These fragments were inserted into pRS316 to generate pKK129.

pKK247, an expression vector for PrA-HA. The *PEP4* gene was amplified from purified genomic DNA (strain W303a) by PCR with primers KKN274 and KKN275, digested with *Cla*I, and purified. This was used to replace the *Cla*I fragment in pKK117 to create plasmid pKK247.

pKK210, an expression vector for CPY*-abcD-Δ482-532. First, to create pAS68 carrying CPY*-ABCD-Δ482-532, A 2.2-kb fragment was amplified from pDN436 (pRS315-CPY*-ABCD-HA) using T7 and N572 primers, digested with *Eag*I, and purified. In parallel, 0.6-kb fragment was amplified from pDN436 using T3 and N573 primers, digested with *Sal*I, and purified. These fragments were inserted into pRS316 to generate plasmid pAS78, which expresses CPY*-

ABCD-Δ482-532. Next, a 2.0 kb *Sac*I-*Bgl*II fragment from pES147 (Spear and Ng, 2005) was used to replace the analogous fragment in pAS78 to produce pKK210.

The following plasmids were generated using site-directed mutagenesis of ERAD substrate clones (Sambrook et al., 1989). Substrate variant names are indicated in parentheses. Primer sequences are listed in *Supplementary Table S3*. All substrate genes are controlled by their endogenous promoters and encode the HA-epitope tag at their C-termini.

pKK156 (PrA*-Ab-Δ37-71). Using the KKN129 oligonucleotide, a segment encoding Glu37 through His71 was deleted from pKK129.

pKK157 (PrA*-Ab-Δ72-106). Using the KKN130 oligonucleotide, a segment encoding Pro72 to Val106 was deleted from pKK129.

pKK158 (PrA*-Ab-Δ110-146). Using the KKN131 oligonucleotide, a segment encoding Thr110 to Thr146 was deleted from pKK129.

pKK159 (PrA*-Ab-Δ147-183). Using the KKN132 oligonucleotide, a segment encoding Glu147 to Ser183 was deleted from pKK129.

pKK160 (PrA*-Ab-Δ184-220). Using the KKN133 oligonucleotide, a segment encoding Glu184 to Gln220 was deleted from pKK129.

pKK161 (PrA*-Ab-Δ221-257). Using the KKN134 oligonucleotide, a segment encoding Asp221 to Gly257 was deleted from pKK129.

pKK162 (PrA*-Ab-Δ258-294). Using the KKN135 oligonucleotide, a segment encoding Asp258 to Asp294 was deleted from pKK129.

pKK163 (PrA*-Ab-Δ295-331). Using the KKN136 oligonucleotide, a segment encoding Thr295 to Arg331 was deleted from pKK129.

pKK164 (PrA*-Ab-Δ332-368). Using the KKN137 oligonucleotide, a segment encoding Asp332 to Met368 was deleted from pKK129.

pKK216 (CPY*-abcD-Δ25-117). Using the JBN159 oligonucleotide, a segment encoding Arg25 to Lys117 was deleted from pES147.

pJB113 (CPY*-abcD-Δ116-206). Using the JBN160 oligonucleotide, a segment encoding Pro116 to Gln206 was deleted from pES147.

pJB114 (CPY-abcD-Δ207-297). Using the JBN161 oligonucleotide, a segment encoding Pro207 to Asn297 was deleted from pES147.

pKK218 (CPY*-abcD-Δ298-388). Using the JBN162 oligonucleotide, a segment encoding Tyr298 to Asp388 was deleted from pES147.

pKK228 (CPY*-abcD-Δ389-476). Using the KKN195oligonucleotide, a segment encoding Tyr389 to Lys476 was deleted from pES147.

pWX2 (PrA*-Δ155-405). The WXN2 primer was used to delete a segment encoding Lys105 to Ile405 from pKK129.

pWX75 (CPYΔ2). The WXN75 primer was used to delete a segment encoding Ile21 to Asp368 from pKK129.

pWX87 (CPY*). A 2.8 Kb *Not*I to *Xho*I fragment from pDN436 inserted into pRS316.

pWX92 (CPY-ABCD). This plasmid expresses wild-type CPY containing a C-terminal HA tag. Using primers WXN92 and WXN93, two *Bam*HI sites on pDN437 were destroyed while maintaining the native peptide sequence. This was done to facilitate the construction of more complex variants. The gene was sub-cloned into pRS316 vector digested with *Not*I and *Xho*I to generate pWX92.

pWX93 (CPY-abcd) was constructed similarly to pWX92 but the non-glycosylated CPY ORF was used as a template.

pWX141 (CPY-Δ112-123). The WXN141 primer was used to delete a segment encoding Lys112 to Pro123 from pWX92.

pWX142 (CPY-Δ127-138). The WXN142 primer was used to delete a segment encoding Gln127 to Asp138 from pWX92.

pWX143 (CPY-Δ185-196). The WXN143 primer was used to delete a segment encoding Asp185 to Asn196 from pWX92.

pWX144 (CPY-Δ201-212). The WXN144 primer was used to delete a segment encoding Val201 to Phe212 from pWX92.

pWX145 (CPY-Δ267-278). The WXN145 primer was used to delete a segment encoding Ala267 to Phe278 from pWX92.

pWX146 (CPY-Δ282-293). The WXN146 primer was used to delete a segment encoding Ser282 to Leu293 from pWX92.

pWX175 (CPY*-Abcd-Δ112-123). The WXN141 primer was used to delete a segment encoding Lys112 to Pro123 from pES149 and simultaneously restoring the A glycan site.

pWX176 (CPY*-Abcd-Δ127-138). The WXN142 primer was used to delete a segment encoding Gln127 to Asp138 from pES149 and simultaneously restoring the A glycan site.

pWX177 (CPY*-aBcd-Δ185-196). The WXN143 primer was used to delete a segment encoding Asp185 to Asn196 from pES149 and simultaneously restoring the B glycan site.

pWX178 (CPY*-aBcd-Δ201-212). The WXN144 primer was used to delete a segment encoding Val201 to Phe212 from pES149 and simultaneously restoring the B glycan site.

pWX163 (CPY*-abCd-Δ267-278). The WXN145 primer was used to delete a segment encoding Ala267 to Phe278 from pES149 and simultaneously restoring the C glycan site.

pWX164 (CPY*-abCd-Δ282-293). The WXN146 primer was used to delete a segment encoding Ser282 to Leu293 from pES149 and simultaneously restoring the C glycan site.

pWX99 (CPY-abcD-Δ25-117). A 1.6 kb fragment was amplified from pKK216 using primers WXN101 and kinased WXN100, digested with *Not*I and purified. In parallel, 1.3 kb fragment was amplified from pKK216 using primers WXN102 and kinased WXN99, digested with *Xho*I and purified. Then these two fragments were inserted into pRS316 digested with *Not*I and *Xho*I to generate pWX99.

pWX100 (CPY-abcD-Δ116-206), pWX101 (CPY-abcD-Δ298-388), pWX102 (CPY-abcD-Δ389-476), pWX103 (CPY-abcD-Δ482-532). These plasmids were constructed as described for pWX99 except that pJB113, pKK218, pKK228, and pKK210 were used as templates, respectively.

pWX94 (CPY-ABCD-C328P) and pWX95 (CPY-abcd-C328P) were generated by site directed mutagenesis with pWX92 and pWX93 as templates, respectively, using the WXN94 primer to convert Cys328 to Pro351.

pWX96 (CPY-ABCD-C351P) and pWX97 (CPY-abcd-C351P) were generated by site directed mutagenesis with pWX92 and pWX93 as templates, respectively, using the WXN96 primer to convert Cys351 to Pro351.

pWX113 (CPY-ABCD-S194K). A 1.4 kb fragment was amplified from pKK92 using WXN101 and kinased WXN113R primers, digested with *Not*I and purified. In parallel, a 1.5 kb fragment was amplified from pKK92 using primers WXN102 and kinased WXN113F, digested with *Xho*I

and purified. The fragments were inserted into pRS316 digested with *Not*I and *Xho*I to create pWX113.

pWX114 (CPY-ABCD-G227R), pWX116 (CPY-ABCD-I451R) and pWX120 (CPY-abcd-I451R) were constructed as described for pWX113 using the primer sets [WXN114F/WXN114R; WXN101/WXN102], [WXN116F/WXN116R; WXN101/WXN102], and [WXN116F/WXN116R; WXN101/WXN102], respectively.

pWX140 (CPY-abcD). A 2.0 kb *Not*I/*Bgl*II fragment from pWX92 and a 0.8 kb *Bgl*II/*Xho*I fragment from pWX93 were ligated into pRS316 digested with *Not*I and *Xho*I.

pWX138 (CPY-abcD-S194K). Site-directed mutagenesis on pWX140 using the WXN138 primer was performed to convert Ser194 to Lys194.

pWX121 (CPY-ABCd-S194K). A 2.0 kb *Not*I/*Bgl*II fragment from pWX113 and a 0.8 kb *Bgl*II/*Xho*I fragment from pWX93 were ligated into pRS316 digested with *Not*I and *Xho*I.

pWX107 (CPY-abcD-C328P). A 2.0 kb *Not*I/*Bgl*II fragment from pWX95 and a 0.8 kb *Bgl*II/*Xho*I fragment from pWX94 were inserted into pRS316 digested with *Not*I and *XhoI*.

pWX108 (CPY-ABCd-C328P). A 2.0 kb *Not*I/*Bgl*II fragment from pWX94 and a 0.8 kb *BglII*/*Xho*I fragment from pWX95 were ligated into pRS316 digested with *Not*I and *Xho*I.

pWX109 (CPY-abcD-C351P). A 2.0 kb *Not*I/*Bgl*II fragment from pWX97 and a 0.8 kb *BglII*/*Xho*I fragment from pWX96 were ligated into pRS316 digested with *Not*I and *Xho*I.

pWX110 (CPY-ABCd-C351P). A 2.0 kb *Not*I/*Bgl*II fragment from pWX96 and a 0.8 kb *BglII*/*Xho*I fragment from pWX97 were ligated into pRS316 digested with *Not*I and *Xho*I.

pWX157 (PrA-AB-N88D) was generated by site directed mutagenesis using the WXN157 primer on the pKK247 template to convert Asn88 to Asp88.

pWX158 (PrA-AB-G199K) and pWX161 (PrA-ab-G199K) were generated by site directed mutagenesis with pKK247 and pWX130 as templates, respectively, using the WXN158 primer to convert Gly199 to Lys199.

pWX159 (PrA-AB-G233K) was generated by site directed mutagenesis using the WXN159 primer on the pKK247 template to convert Gly233 to Lys233.

pWX160 (PrA-AB-I365R) and pWX162 (PrA-ab-G199K) were generated by site directed mutagenesis with pKK247 and pWX130 as templates, respectively, using the WXN160 primer to convert Ile365 to Arg365.

pWX166 (PrA-Ab-G199K). A 1.7 kb *Xho*I/*Nco*I fragment from pWX158 and a 0.9 kb *Nco*I/*Not*I fragment from pWX161 were inserted into pRS315 digested with *Not*I and *XhoI*.

pWX167 (PrA-aB-G199K). A 1.7 kb *Xho*I/*Nco*I fragment from pWX161 and a 0.9 kb *Nco*I/*Not*I fragment from pWX158 were inserted into pRS315 digested with *Not*I and *XhoI*.

pWX168 (PrA-Ab-I365R). A 1.7 kb *Xho*I/*Nco*I fragment from pWX160 and a 0.9 kb *Nco*I/*Not*I fragment from pWX162 were inserted into pRS315 digested with *Not*I and *XhoI*.

pWX169 (PrA-aB-I365R). A 1.7 kb *Xho*I/*Nco*I fragment from pWX162 and a 0.9 kb *Nco*I/*Not*I fragment from pWX160 were inserted into pRS315 digested with *Not*I and *XhoI*.

SUPPLEMENTARY TABLES

Table S1. is in a separate file in MS Excel format.

Table S2. Strains used in this study

Strain	Genotype	Source
W303a	*MAT***a**, *leu2-3, 112, his3-11, trp1-1, ura3-1, can1-100, ade2-1*	P. Walter
KKY62	*MAT***a**, pKK129, W303 background	This study
KKY126	*MAT***a**, pKK156, W303 background	This study
KKY127	*MAT***a**, pKK157, W303 background	This study
KKY128	*MAT***a**, pKK158, W303 background	This study
KKY129	*MAT***a**, pKK159, W303 background	This study
KKY130	*MAT***a**, pKK160, W303 background	This study
KKY131	*MAT***a**, pKK161, W303 background	This study
KKY132	*MAT***a**, pKK162, W303 background	This study
KKY133	*MAT***a**, pKK163, W303 background	This study
KKY134	*MAT***a**, pKK164, W303 background	This study
KKY201	*MAT***a**, pKK216, W303 background	This study
KKY202	*MAT***a**, pJB113, W303 background	This study
KKY203	*MAT***a**, pJB114, W303 background	This study
KKY204	*MAT***a**, pKK218, W303 background	This study
KKY273	*MAT***a**, pKK228, W303 background	This study
KKY206	*MAT***a**, pKK210, W303 background	This study
WXY3	*MAT***a**, pWX2, W303 background	This study
WXY183	*MAT***a**, *hrd1::KANMX,* pWX2, W303 background	This study
WXY9	*MAT***a**, *htm1::KANMX,* pWX2, W303 background	This study
WXY145	*MAT***a**, pWX75, W303 background	This study
WXY148	*MAT***a**, *hrd1::KANMX,* pWX75, W303 background	This study
WXY260	*MAT***a**, *htm1::KANMX,* pWX75, W303 background	This study
WXY206	*MAT***a**, *cue1::TRP1, pep4::HIS3, prc1::KANMX,* pDN437, W303 background	This study
WXY207	*MAT***a**, *cue1::TRP1, pep4::HIS3, prc1::KANMX,* pDN436, W303 background	This study
WXY208	*MAT***a**, *cue1::TRP1, pep4::HIS3, prc1::KANMX,* pES132, W303 background	This study
WXY185	*MAT***a**, *cue1::TRP1, pep4::HIS3, prc1::KANMX,* pWX87, W303 background	This study
WXY263	*MAT***a**, *cue1::TRP1, pep4::HIS3, prc1::KANMX,* pRS316, W303 background	This study
WXY264	*MAT***a**, *cue1::TRP1, pep4::HIS3, prc1::KANMX,* pES57, W303 background	This study
WXY265	*MAT***a**, *cue1::TRP1, pep4::HIS3, prc1::KANMX,* pWX75, W303 background	This study
WXY266	*MAT***a**, pWX141, W303 background	This study
WXY267	*MAT***a**, pWX142, W303 background	This study
WXY268	*MAT***a**, pWX143, W303 background	This study
WXY269	*MAT***a**, pWX144, W303 background	This study
WXY270	*MAT***a**, pWX145, W303 background	This study
WXY271	*MAT***a**, pWX146, W303 background	This study
WXY371	*MAT***a**, pWX175, W303 background	This study
WXY374	*MAT***a**, pWX176, W303 background	This study
WXY377	*MAT***a**, pWX177, W303 background	This study
WXY380	*MAT***a**, pWX178, W303 background	This study
WXY315	*MAT***a**, pWX163, W303 background	This study
WXY316	*MAT***a**, pWX164, W303 background	This study
WXY372	*MAT***a**, *htm1::KANMX,* pWX175, W303 background	This study
WXY375	*MAT***a**, *htm1::KANMX,* pWX176, W303 background	This study
WXY378	*MAT***a**, *htm1::KANMX,* pWX177, W303 background	This study
WXY381	*MAT***a**, *htm1::KANMX,* pWX178, W303 background	This study
WXY317	*MAT***a**, *htm1::KANMX,* pWX163, W303 background	This study
WXY318	*MAT***a**, *htm1::KANMX,* pWX164, W303 background	This study

WXY373	*MAT***a**, *hrd1::KANMX,* pWX175, W303 background	This study
WXY376	*MAT***a**, *hrd1::KANMX,* pWX176, W303 background	This study
WXY379	*MAT***a**, *hrd1::KANMX,* pWX177, W303 background	This study
WXY382	*MAT***a**, *hrd1::KANMX,* pWX178, W303 background	This study
WXY319	*MAT***a**, *hrd1::KANMX,* pWX163, W303 background	This study
WXY320	*MAT***a**, *hrd1::KANMX,* pWX164, W303 background	This study
WXY322	*MAT***a**, *htm1::KANMX,* pWX165, W303 background	This study
WXY323	*MAT***a**, *hrd1::KANMX,* pWX165, W303 background	This study
WXY196	*MAT***a**, pWX99, W303 background	This study
WXY197	*MAT***a**, pWX100, W303 background	This study
WXY198	*MAT***a**, pWX101, W303 background	This study
WXY199	*MAT***a**, pWX102, W303 background	This study
WXY200	*MAT***a**, pWX103, W303 background	This study
KKY631	*MAT***a**, *htm1::KANMX,* pWX99, W303 background	This study
KKY632	*MAT***a**, *htm1::KANMX,* pWX100, W303 background	This study
KKY633	*MAT***a**, *htm1::KANMX,* pJB114, W303 background	This study
KKY634	*MAT***a**, *htm1::KANMX,* pWX101, W303 background	This study
KKY635	*MAT***a**, *htm1::KANMX,* pWX102, W303 background	This study
KKY636	*MAT***a**, *htm1::KANMX,* pWX103, W303 background	This study
WXY201	*MAT***a**, *hrd1::KANMX,* pWX99, W303 background	This study
WXY202	*MAT***a**, *hrd1::KANMX,* pWX100, W303 background	This study
KKY613	*MAT***a**, *hrd1::KANMX,* pJB114, W303 background	This study
WXY203	*MAT***a**, *hrd1::KANMX,* pWX101, W303 background	This study
WXY204	*MAT***a**, *hrd1::KANMX,* pWX102, W303 background	This study
WXY205	*MAT***a**, *hrd1::KANMX,* pWX103, W303 background	This study
WXY220	*MAT***a**, pWX113, W303 background	This study
WXY221	*MAT***a**, pWX114, W303 background	This study
WXY222	*MAT***a**, pWX115, W303 background	This study
WXY223	*MAT***a**, pWX116, W303 background	This study
WXY192	*MAT***a**, *prc1::KANMX,* pWX94, W303 background	This study
WXY193	*MAT***a**, *prc1::KANMX,* pWX96, W303 background	This study
WXY295	*MAT***a**, *prc1::KANMX,* pWX114, W303 background	This study
WXY296	*MAT***a**, *prc1::KANMX,* pWX116, W303 background	This study
WXY294	*MAT***a**, *prc1::KANMX,* pWX140, W303 background	This study
WXY224	*MAT***a**, pWX107, W303 background	This study
WXY225	*MAT***a**, pWX108, W303 background	This study
WXY226	*MAT***a**, pWX109, W303 background	This study
WXY227	*MAT***a**, pWX110, W303 background	This study
WXY228	*MAT***a**, *hrd1::KANMX,* pWX107, W303 background	This study
WXY229	*MAT***a**, *hrd1::KANMX,* pWX108, W303 background	This study
WXY230	*MAT***a**, *hrd1::KANMX,* pWX109, W303 background	This study
WXY231	*MAT***a**, *hrd1::KANMX,* pWX110, W303 background	This study
WXY232	*MAT***a**, pWX121, W303 background	This study
WXY233	*MAT***a**, pWX138, W303 background	This study
WXY236	*MAT***a**, *hrd1::KANMX,* pWX121, W303 background	This study
WXY237	*MAT***a**, *hrd1::KANMX,* pWX138, W303 background	This study
WXY299	*MAT***a**, pWX157, W303 background	This study
WXY300	*MAT***a**, pWX158, W303 background	This study
WXY301	*MAT***a**, pWX159, W303 background	This study
WXY302	*MAT***a**, pWX160, W303 background	This study
WXY303	*MAT***a**, pWX166, W303 background	This study
WXY304	*MAT***a**, pWX167, W303 background	This study
WXY305	*MAT***a**, pWX168, W303 background	This study
WXY306	*MAT***a**, pWX169, W303 background	This study
WXY307	*MAT***a**, *htm1::KANMX,* pWX166, W303 background	This study
WXY308	*MAT***a**, *htm1::KANMX,* pWX167, W303 background	This study

WXY309	*MAT***a**, *htm1::KANMX,* pWX168, W303 background	This study
WXY310	*MAT***a**, *htm1::KANMX,* pWX169, W303 background	This study
WXY311	*MAT***a**, *hrd1::KANMX,* pWX166, W303 background	This study
WXY312	*MAT***a**, *hrd1::KANMX,* pWX167, W303 background	This study
WXY313	*MAT***a**, *hrd1::KANMX,* pWX168, W303 background	This study
WXY314	*MAT***a**, *hrd1::KANMX,* pWX169, W303 background	This study
WXY368	*MAT***a**, *alg3::HIS3*, W303 background	This study
ESY676	*MAT***a**, pES147, W303 background	Spear et al., 2005
ESY677	*MAT***a**, *htm1::KANMX*, pES147, W303 background	Spear et al., 2005
WXY383	*MAT***a**, *alg3::HIS3*, pES147, W303 background	This study
WXY384	*MAT***a**, *alg3::HIS3*, *htm1::KANMX*, pES147, W303 background	This study
WXY400	*MAT***a**, pES149, W303 background	This study
WXY401	*MAT***a**, *htm1::KANMX*, pES149, W303 background	This study
WXY402	*MAT***a**, *alg3::HIS3*, pES149, W303 background	This study
WXY403	*MAT***a**, *alg3::HIS3*, *htm1::KANMX*, pES149, W303 background	This study

Table S3. Oligonucleotide primers used in this study

Primer	Construct	Sequence (5' -> 3')
KKN129	PrA*-Ab-Δ37-71	ATAAACACGAGTTGTCCGATGATACTGGTTCTTCAAACCT
KKN130	PrA*-Ab-Δ72-106	AAAACTTCAAGGTTATTTTGAATGGTACTGAATTTGCCAT
KKN131	PrA*-Ab-Δ110-146	GCTACAAAGCTAATGGTACTGAGCCGGGCTTAACATTTGC
KKN132	PrA*-Ab-Δ147-183	ACTTCGCTGAGGCTACCAGCGATTTGTTGGACGAAAAGAG
KKN133	PrA*-Ab-Δ184-220	TTTACAACGCCATTCAACAAGATATCACTTGGTTACCTGT
KKN134	PrA*-Ab-Δ221-257	ACGAGTCTAAGTTCAAGGGCACTGGTACTTCTTTGATTAC
KKN135	PrA*-Ab-Δ258-294	GCCATGGTGCCGCCATCGATGACAATCTACCTGATCTAAT
KKN136	PrA*-Ab-Δ295-331	CTCTAGACTGTAACACCAGAGATTTCCCAGAACCTGTTGG
KKN137	PrA*-Ab-Δ332-368	TCTCTGCAATTACACCAATGTACCCATATGATGTTCCAGA
JBN159	CPY*-abcD-Δ25-117	CTAAGGCCATCTCATTGCAAATCCTGGGCATTGACCCACAG
JBN160	CPY*-abcD-Δ116-206	GTGTCAACAAGATTAAGGACCCTGTCAACGTTGGGTTCTC
JBN161	CPY-abcD-Δ207-297	CCGTGATCTTCCTTGACCAGTATTACGAACCAATGGCCTG
JBN162	CPY*-abcD-Δ298-388	GACCCATTGACTCAGTATAACTACTTAAACCAGGACTACGTC
KKN195	CPY*-abcD-Δ389-476	CGTTACAAGATATCGACGACGTACGTAACTGGACTGCTTC
N572	CPY*-abcD-Δ482-532	AGTCCAGTTACGTACTTT
N573	CPY*-abcD-Δ482-532	TACCCATATGATGTTCCA
WXN2	PrA*-Δ155-405	AATTTGCCATTCAATATGGTACTTACCCATATGATGTTCCAGATTACG
WXN75	CPYΔ2	CTGTCCACTACACTCGCTAAGGCCATCAGGAAGGATTGTGAAGGTGGC

WXN92	CPY-ABCD CPY-abcd	5’-CACCGCGGTGGCGGCCGCTCTAGAGCTGAGGCTAC CAGCGAGCCGGGCTTATTAGGTCTCTTATGGTAGTTTTTA-3’
WNX93	CPY-ABCD CPY-abcd	GCCTTAAGTATGGTTAACGAATGGATTCACGGTGGTTTCTCCTTATACCCA
WXN141	CPY-Δ112-123 CPY*-Abcd-Δ112-123	GAAAACTATCAGCTTCGTGTCAACAATGTCACACAGTACACGGGTTAC
WXN142	CPY-Δ127-138 CPY*-Abcd-Δ127-138	CTGGGCATTGACCCAAATGTCACAAAGCATTTCTTCTTTTGGACTTTT
WXN143	CPY-Δ185-196 CPY*-aBcd-Δ185-196	TTAGGACCCTCATCCATTGGACCTAGCAATGCCACCGTGATCTTCCTT
WXN144	CPY-Δ201-212 CPY*-aBcd-Δ201-212	TACTCTTGGAACAGCAATGCCACCTCGTATTCCGGGTCCTCAGGTGTT
WXN145	CPY-Δ267-278 CPY*-abCd-Δ267-278	GCCGGCCATTACATCCCTGTTTTTAACTTAACCTCCGTCTTGATCGGA
WXN146	CPY-Δ282-293 CPY*-abCd-Δ282-293	AAGGACAGAAACTTCAACTTAACCACTCAGTATAACTATTACGAACCA
WXN22	CPY*-abcB	ATCGTAAACGTTTCTGCCGGTACG
WXN23	CPY*-abcB	ATCAAAACGAAGAAAGACTGGGAC
WXN24	CPY*-abcB	5’-CCCCCTCTAGATTAAGCGTAATCTGGAACATCA TATGGGTAGAAATCTTGGCCCTTGTTGACGTA-3’
WXN165	CPY*-abcB-Δ482-534	AACCCTTACTCTTGGAACAGCAATGCCACCTACCCATATGATGTTCCAGAT
WXN99	CPY-abcD-Δ25-117 CPY-abcD-Δ116-206 CPY-abcD-Δ298-388 CPY-abcD-Δ389-476 CPY-abcD-Δ482-532	GGGGAATCCTACGCCGGCCATTACATC
WXN100	CPY-abcD-Δ25-117 CPY-abcD-Δ116-206 CPY-abcD-Δ298-388 CPY-abcD-Δ389-476 CPY-abcD-Δ482-532	AGCGATGTGGAAATCTTGGCCCTT
WXN101	CPY-abcD-Δ25-117 CPY-abcD-Δ116-206 CPY-abcD-Δ298-388 CPY-abcD-Δ389-476 CPY-abcD-Δ482-532 CPY*-abcB CPY-ABCD-S194K CPY-ABCD-G227R CPY-ABCD-I451R CPY-abcd-I451R	GGGGATGTGCTGCAAGGCGATTAA
WXN102	CPY-abcD-Δ25-117	ACACAGGAAACAGCTATGACCATG

	CPY-abcD-Δ116-206	
	CPY-abcD-Δ298-388	
	CPY-abcD-Δ389-476	
	CPY-abcD-Δ482-532	
	CPY-ABCD-S194K	
	CPY-ABCD-G227R	
	CPY-ABCD-I451R	
	CPY-abcd-I451R	
WXN94	CPY-ABCD-C328P	GCTATGGAAGACTCTTTGGAACGTCCTTTGGGCTTGATCGAGTCGTGCTAT
	CPY-abcd-C328P	
WXN96	CPY-ABCD-C351P	TCCTGTGTTCCAGCTACCATTTATCCTAATAACGCCCAATTGGCTCCTTAC
	CPY-abcd-C351P	
WXN113F	CPY-ABCD-S194K	AAGTGGAACAGCAATGCCACCGTGATC
WXN113R	CPY-ABCD-S194K	GTAAGGGTTCCCGATGGGTTTCAA
WXN114F	CPY-ABCD-G227R	AGGAAGGATGTCTATAACTTCTTGGAG
WXN114R	CPY-ABCD-G227R	AGCGGCGACAGTGTTGGAAACACC
WXN116F	CPY-ABCD-I451R	AGGTGTAACTGGTTGGGTAATAAGGCG
	CPY-abcd-I451R	
WXN116R	CPY-ABCD-I451R	GAAATCTTTATCGCCTGCATATAC
	CPY-abcd-I451R	
WXN138	CPY-abcD-S194K	GGGAACCCTTACAAGTGGAACAGCCAGGCCACCGTGATCTTCCTTGACCAG
KKN274	PrA-AB	GCCTATCGATTCGCAATCACAAATTGATCCTAGTAAGAAG
KKN275	PrA-AB	GGAGCCTGAAACTTCAAGCGTGTAATCGTA
WXN157	PrA-AB-N88D	GATGTTCCATTGACAAATTACTTGGACGCACAATATTACACTGACATTACT
WXN158	PrA-AB-G199K	TTTGGCAAGTTCGATGGTATTTTGAAGTTGGGTTACGATACCATTTCTGTT
	PrA-ab-G199K	
WXN159	PrA-AB-G233K	GAAAAGAGATTTGCCTTTTATTTGAAGGACACTTCAAAGGATACTGAAAAT
WXN160	PrA-AB-I365R	GTTTCAGGCTCCTGTATCTCTGCACGTACACCAATGGATTTCCCAGAACCT
	PrA-ab-I365R	

FIGURE AND TABLE LEGENDS TO SUPPLEMENTARY DATA

Figure S1. Intracellular processing of CPY and PrA point mutants. CPY and PrA fully glycosylated variants were analyzed by metabolic pulse-chase (10 min pulse) followed by immunoprecipitation and separation by SDS-PAGE. Although all variants carry C-terminal HA tags, it was necessary to express CPY variants in strains deleted of PRC1 (encodes CPY) and use anti-CPY antisera to visualize all recombinant forms because the CPY HA tag is proteolytically removed in the vacuole. (A and B) CPY variants. The positions of ER (p1), Golgi (p2), and mature vacuolar (m) forms are indicated. (C and D) PrA variants. The positions of ER (p1),

Golgi (p2), and mature vacuolar (m) forms are indicated. (A) *Δprc1* knockout cells expressing CPY-ABCD-G227R or CPY-ABCD-I451R. (B) Wild type cells expressing PrA-AB-N88D or PrA-AB-G233K. (C) Wild type cells expressing CPY-ABCD, CPY* or CPY-ABCD-S194K, and *Δprc1* knockout cells expressing CPY-ABCD-C328P or CPY-ABCD-C351P. (D) Wild type cells expressing PrA-AB-G199K or PrA-AB-I365R.

Table S1. Peptide analysis of CPYΔ2-associated protein. 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. M_r (expt) is the experimentally determined mass, and M_r (calc) is the calculated mass of the peptide. Delta represents the observed mass versus delta error. Miss indicates the number of missed trypsin cleavage sites, and the peptides identified column lists the actual sequence of the identified peptide. The ions score is -10*Log(P), where P is the probability that the observed match is a random event. Protein matches to NCBInr gi|6322426, *Saccharomyces cerevisiae* Kar2p. Nominal mass (Mr): 74480. Sequence coverage is 17%.

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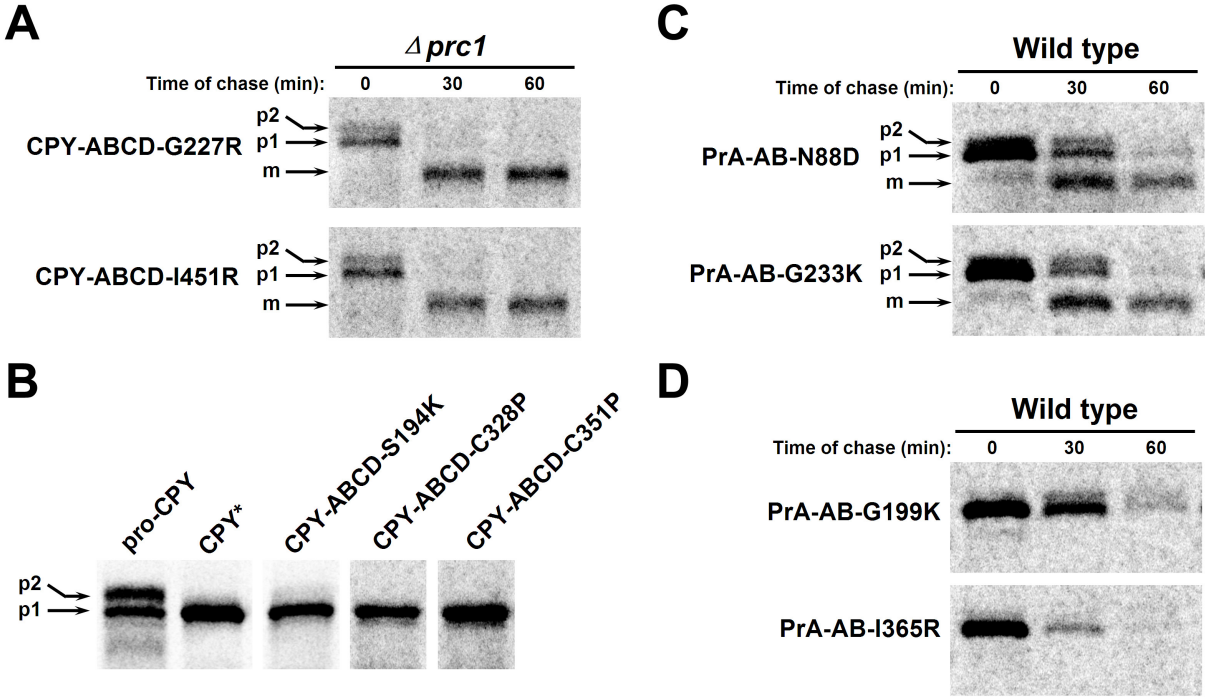

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