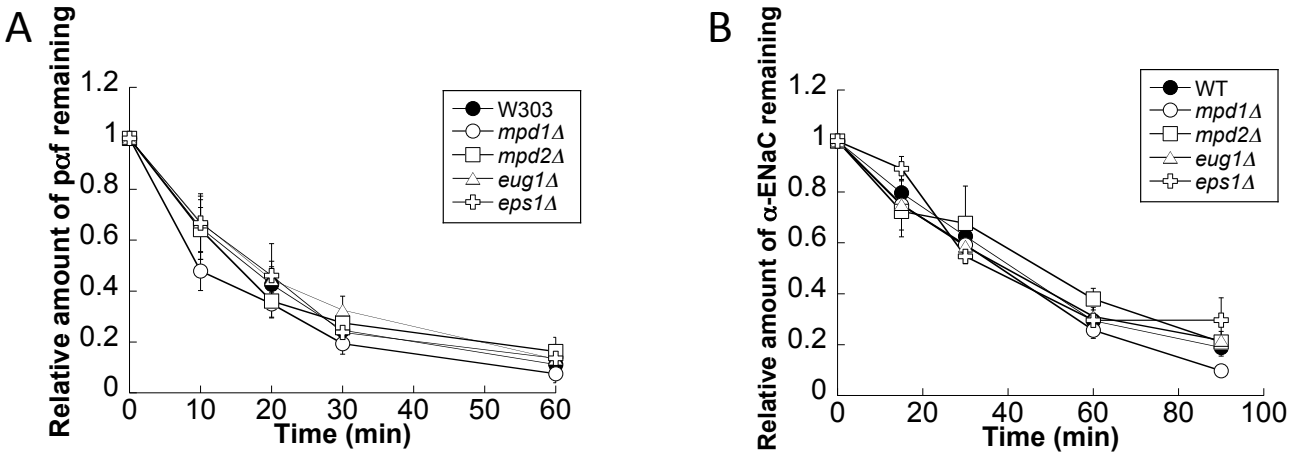
Supplemental Table 1 Yeast Strains

Strain	Genotype	Source
W303	*MATα, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1*	This lab
mpd1Δ	*MATα, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, mpd1::KANMX*	This study
mpd2Δ	*MATα, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, mpd2::KANMX*	This study
eug1Δ	*MATα, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, eug1::KANMX*	This study
eps1Δ	*MATα, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, eps1::KANMX*	This study
M4492	*MATα pdi1::HIS3 Δeps1 Δeug1 Δmpd1 Δmpd2::G418 ura3 trp1 his3 [pBH1800 (MPD1 CEN TRP1)]*	Norgaard et al., 2001
SRH01	*MATα pdi1::HIS3 Δeps1 Δeug1 Δmpd1 Δmpd2::G418 ura3 trp1 his3* [pSG01]	This study
pdi1Δ[$PDI1_{CGHC\text{-}CGHC}$]	*MATα, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, pdi1::HIS3,* [pBH1464]	Luz and Lennarz, 1998
pdi1Δ[$PDI1_{SGHS\text{-}CGHC}$]	*MATα, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, pdi1::HIS3,* [pBH1852]	Luz and Lennarz, 1998
pdi1Δ[$PDI1_{CGHC\text{-}SGHS}$]	*MATα, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, pdi1::HIS3,* [pBH1630]	Luz and Lennarz, 1998
pdi1Δ[$PDI1_{222\text{-}302\Delta}$*]*	*MATa, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1, pdi1::HIS3,* [pRS-Δ222-302]	Gillece et al., 1999
M4130	*MATα, pdi1::HIS3, ade2, can1, ura3, leu2, trp1, his3,* [pCT37]	Norgaard et al., 2001
ire1Δ	*Matα, lys2, his3, leu2, ura3, ire1::KANMX*	This lab
SEY6210	*MATα, ura3-52, leu2-3,112, trp1-Δ901, his3-Δ200, lys2-801, suc2-Δ9*	Nakatsukasa et al., 2001
htm1Δ	*MATα, ura3-52, leu2-3,112, trp1-Δ901, his3-Δ200, lys2-801, suc2-Δ9, htm1::HIS3*	Nakatsukasa et al., 2001
W303	*MATa, ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1*	Gauss et al., 2011
KKY415	*MATa, pdi1-1, his3-11::HIS3-UPRE LacZ, trp1-1, his3-11,15, ura3-1 , can1-100, ade2-1 , leu2-3,112*	Gauss et al., 2011

Supplemental Table 2. Plasmids

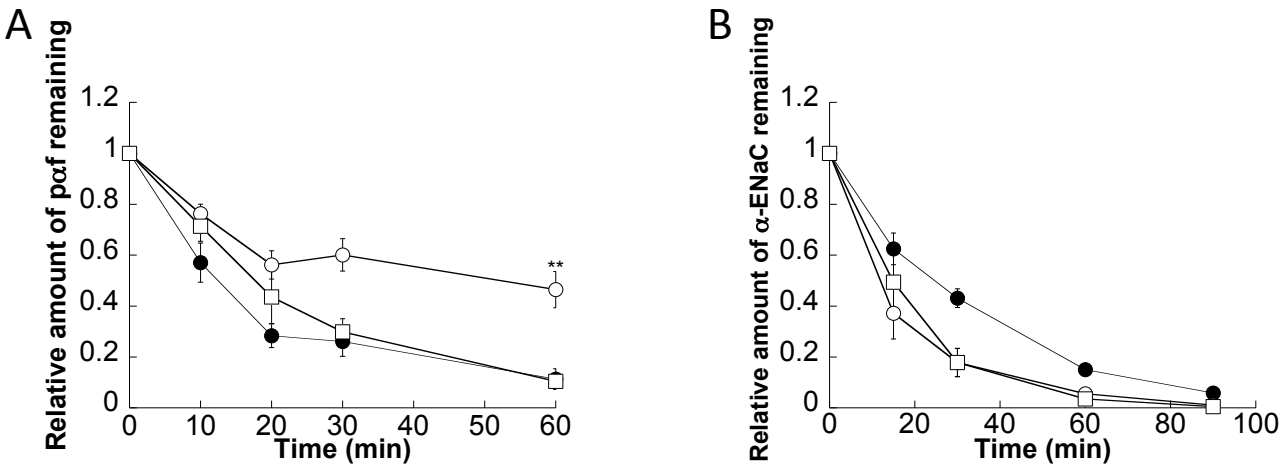
Plasmid Name	Notes	Selectable Marker	Reference
pRS316CPY*-3HA	CPY* expression	*URA3*	Bhamidipati *et al.,* 2005
pSLW1-B29	ApoB29 expression	*URA3*	Hrizo *et al.,* 2007
pBH1800	CEN *MPD1*	*TRP1*	Norgaard *et al.,* 2001
pBH1464	*CEN PDI1*$_{CGHC\text{-}CGHC}$	*TRP1*	Holst *et al.,* 1997
pBH1852	*CEN PDI1*$_{SGHS\text{-}CGHC}$	*TRP1*	Holst *et al.,* 1997
pBH1630	*CEN PDI1*$_{CGHC\text{-}SGHS}$	*TRP1*	Holst *et al.,* 1997
pRS-Δ222-302	*CEN PDI1*$_{222\text{-}302\Delta}$	*TRP1*	Gillece et al., 1999
pCT37	Galactose inducible *PDI1*	*URA3*	Norgaard *et al.,* 2001
pFA6a-KanMX6	KanMX	*AMP*^{R}	Longtine *et al.,* 1998
pSG01	*CEN PDI1*$_{CGHC\text{-}CGHC}$	*LEU2*	This Study
pRS426GPD ENaC-HA	ENaC alpha subunit expression	*URA3*	Buck *et al*., 2010
pRS426MET25 ENaC-HA	Methionine repressible ENaC alpha subunit expression	*URA3*	This Study
pSM36-ppαfΔG-HA	pαf expression	*URA3*	Kim *et al.* 2005
pKK223	PrA*-Ab expression	*LEU2*	Kanehara *et al*. 2010
pJJB20	Vector control corresponding to ApoB29	*URA3*	Hrizo *et al.,* 2007
pRS316	Vector control corresponding to CPY*	*URA3*	Bhamidipati *et al.,* 2005
pcDNA3.1	Vector control corresponding to PDI, ERp57, and ERp72	Neomycin^R	Invitrogen, Iowa City, IA
pcDNA3.1-hPDI	Human PDI expression	Neomycin^R	This Study
pcDNA3.1-hERp57	Human ERp57 expression	Neomycin^R	This Study
pcDNA3.1-hERp72	Human ERp72 expression	Neomycin^R	This Study

Supplemental Figure 1



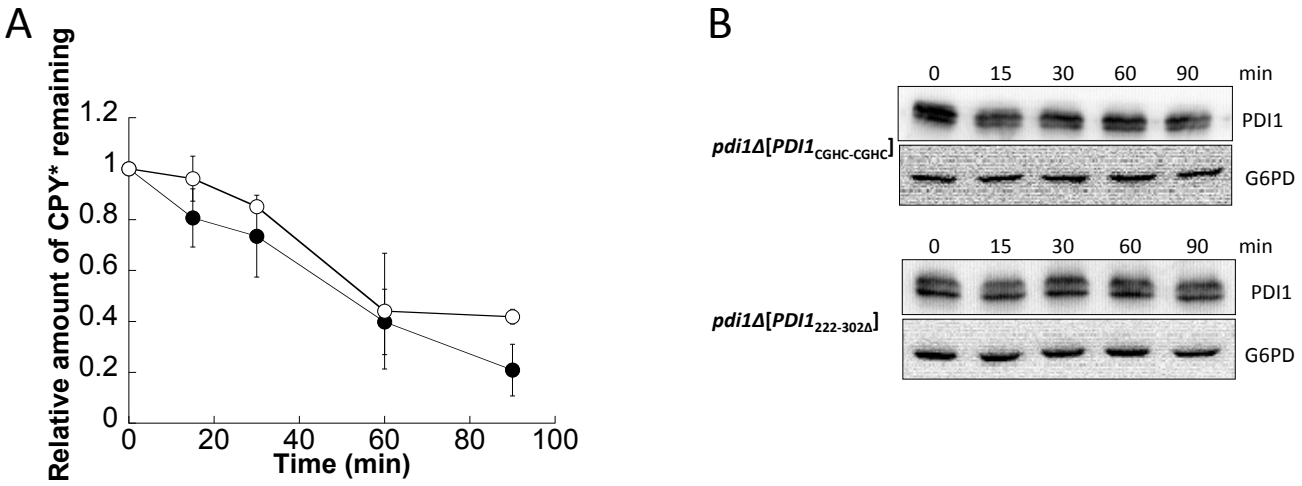
Supplemental Figure 1. The non-essential PDIs do not contribute to the ERAD of pαf or α-ENaC. Cycloheximide chase reactions were performed as described in the Materials and Methods in wild type (●), *mpd1Δ* (○), *mpd2Δ* (□), *eug1Δ* (△), and *eps1Δ* (✞) yeast strains expressing pαf (A) from the pSM36-ppαfΔG-HA plasmid or α-ENaC (B) from the pRS426GPD ENaC-HA plasmid. Chase reactions were performed at 30°C, and lysates were immunoblotted with anti-HA antibody. Anti-G6PD antiserum was used as a loading control. Data represent the means of 4-6 experiments, ± SEM. The lack of visible error bars indicates that the SEM is less than the size of the symbol.

Supplemental Figure 2



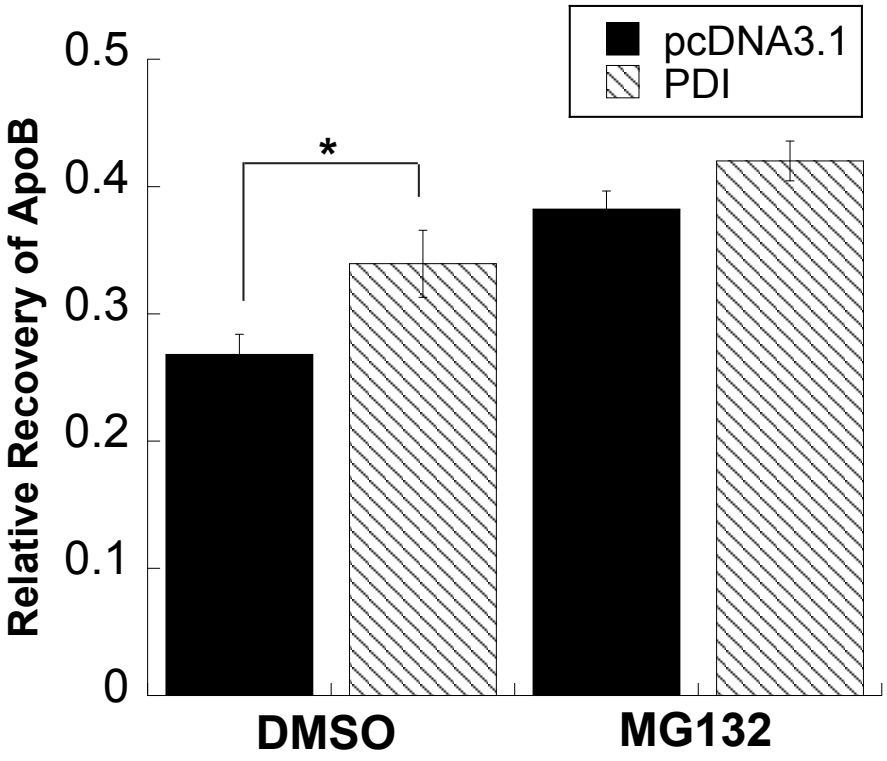
Supplemental Figure 2. Pdi1 is necessary for the degradation of pαf, but not α-ENaC. Cycloheximide chase reactions were performed as described in the Materials and Methods in wild type (●), M4492 (○), or SRH01 (□) yeast strains expressing pαf (A) from the pSM36-ppαfΔG-HA plasmid or α-ENaC (B) from the pRS426MET25 ENaC-HA plasmid. For expression of α-ENaC, strains were grown overnight at 26°C in selective medium supplemented with 2mM methionine to repress the expression of α-ENaC. The cells were then harvested and resuspended in selective medium without methionine for 90 min to induce the expression of α-ENaC . Chase reactions were subsequently performed at 30°C, and lysates were immunoblotted with anti-HA antibody. Anti-G6PD antiserum was used as a loading control. Data represent the means of 4-6 experiments, ± SEM. The lack of visible error bars indicates that the SEM is less than the size of the symbol. Where indicated (**) $p<0.01$.

Supplemental Figure 3



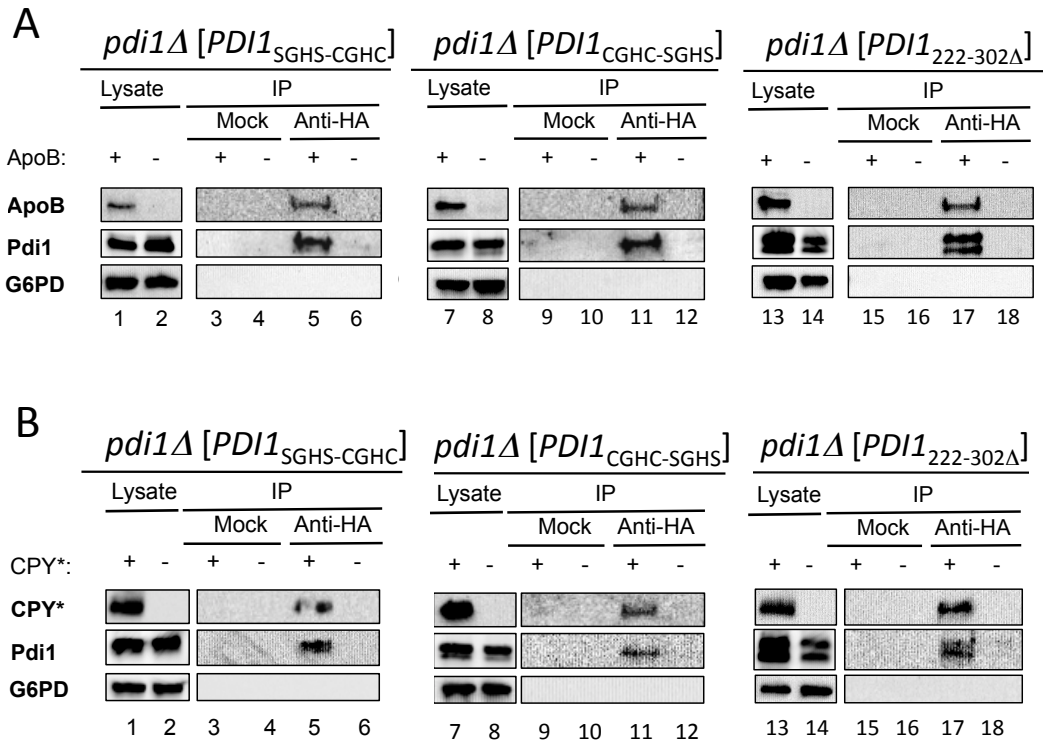
Supplemental Figure 3. The chaperone activity of Pdi1 is not necessary for the ERAD of CPY*. Cycloheximide chase reactions were performed as described in the Materials and Methods in *pdi1Δ* [$PDI1_{CGHC\text{-}CGHC}$] (●) and *pdi1Δ* [$PDI1_{222\text{-}302\Delta}$] (○) yeast strains expressing CPY* (A). Chase reactions were performed at 30°C, and lysates were immunoblotted with anti-HA antibody. Anti-G6PD antiserum was used as a loading control. Data represent the means of 4-6 experiments, ± SEM. The lack of visible error bars indicates that the SEM is less than the size of the symbol. Lysates were also immunoblotted with anti-Pdi1 antiserum and representative images are shown (B).

Supplemental Figure 4



Supplemental Figure 4. PDI promotes ApoB secretion independent of ERAD activity. Following a metabolic labeling reaction in the presence of either DMSO or the proteasome inhibitor MG132, as indicated, a 90 min chase was performed as described in the Materials and Methods in McArdle-RH7777 cells transfected with a vector control (pcDNA3.1 lacking an insert), or containing the PDI gene in pcDNA3.1. The "Relative Recovery of ApoB" indicates the amount of ApoB-precipitable material recovered from cell lysates and secreted into the medium at the completion of the chase divided by the amount of ApoB-precipitable material recovered after 30 min of chase. Data represent the means of 3 independent experiments, ± SEM. Where indicated (*) $p < 0.05$.

Supplemental Figure 5



Supplemental Figure 5. Mutant forms of Pdi1 are able to co-precipitate with ApoB29 and CPY*. Native immunoprecipitation reactions were performed using anti-HA resin, or unconjugated Sepharose ("Mock"), using lysates from *pdi1Δ* yeast strains expressing active site mutant (lanes 1-6 and 7-12) or chaperone mutant (lanes 13-18) forms of *PDI1* on a plasmid. As indicated, each strain also expressed (A) ApoB29 ("ApoB"), (B) CPY* ("CPY*"), or harbored an empty vector control ("-"). A total of 1% of the input for the precipitation was also examined ("Lysate"). After precipitation and SDS-PAGE, the indicated proteins were examined by immunoblot analysis. The doublet observed for Pdi1 in some panels is due to differential glycosylation.