Supplemental Table I: Plasmids used in this study.

Plasmid	Reference
pSLW1	This study
pSLW1-B29HA	This study
p414TEF	Shaner *et al*., 2004
p414TEF*SSE1*	Shaner *et al*., 2004
p414TEF*SSE1*K69Q	Shaner *et al*., 2004
p414TEF*SSE1*G205D	Shaner *et al*., 2004
p414TEF*SSE1*G233D	Shaner *et al*., 2004
p414TEF*SSE1*-CTD(394-693)	Shaner *et al*., 2004
pRS426-CFTR-HA	Zhang, *et al*., 2001
pSM36-ppαfΔG-HA	Kim, *et al*., 2005
pRS315-CPY*-HA	Ng, *et al*., 2000
pSM1082-Ste6p*-HA	Loayza, *et al*., 1998

Supplemental Table II: Antibodies used in this study.

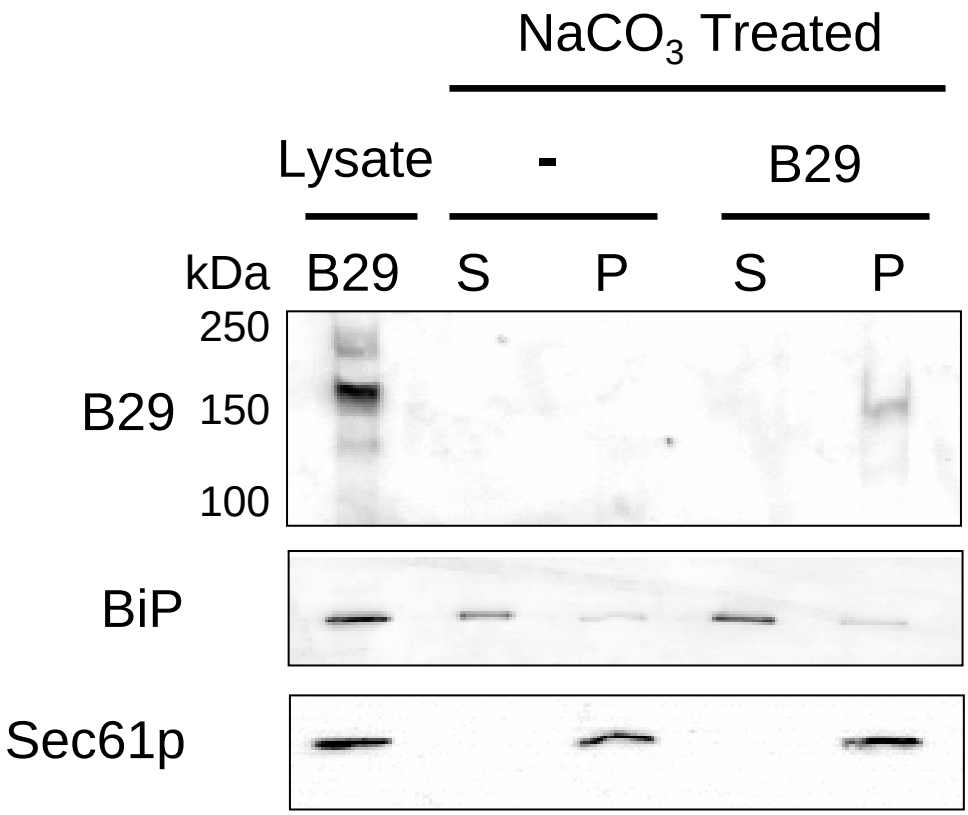
Antibody		Source
anti-ApoB (1D1)	Mouse monoclonal	R. Milne
anti-Albumin	Sheep polyclonal	Bethyl Laboratories
anti-BiP	Rabbit polyclonal	R. Schekman
anti-HA	Mouse monoclonal	Roche
anti-Hsp82p	Rabbit polyclonal	A. Caplan
anti-L3	Mouse monoclonal	J. Warner
anti-mammalian Hsp110	Rabbit polyclonal	Stressgen
anti-Met19p	Rabbit polyclonal	Sigma
anti-PKC1p	Rabbit polyclonal	Santa Cruz Biotechnology
anti-Sba1p	Rabbit polyclonal	A. Caplan
anti-Sec61p	Rabbit polyclonal	R. Schekman
anti-Sec63p	Rabbit polyclonal	D. Feldheim
anti-Ssa1p	Rabbit polyclonal	J. Brodsky
anti-Ssb1p	Rabbit polyclonal	E. Craig
anti-Sse1p	Rabbit polyclonal	J. Brodsky
anti-Sti1p	Mouse monoclonal	D. Toft
anti-Tim23p	Rabbit polyclonal	T. Endo
anti-Ydj1p	Rabbit polyclonal	A. Caplan

Supplemental Figure 1: ApoB29 is carbonate inextractable. A total of 40μg of cell extract from yeast transformed with pSLW1 or pSLW1-B29 were treated with 100 mM sodium carbonate, pH 11.5. The pellet and supernatant from a 230,000*g* centrifugation of the cell extracts were analyzed by western blotting. The ER-lumenal Hsp70 chaperone, BiP, was used as a control and was found in the supernatant, as predicted. In contrast, Sec61p was exclusively in the membrane fraction.

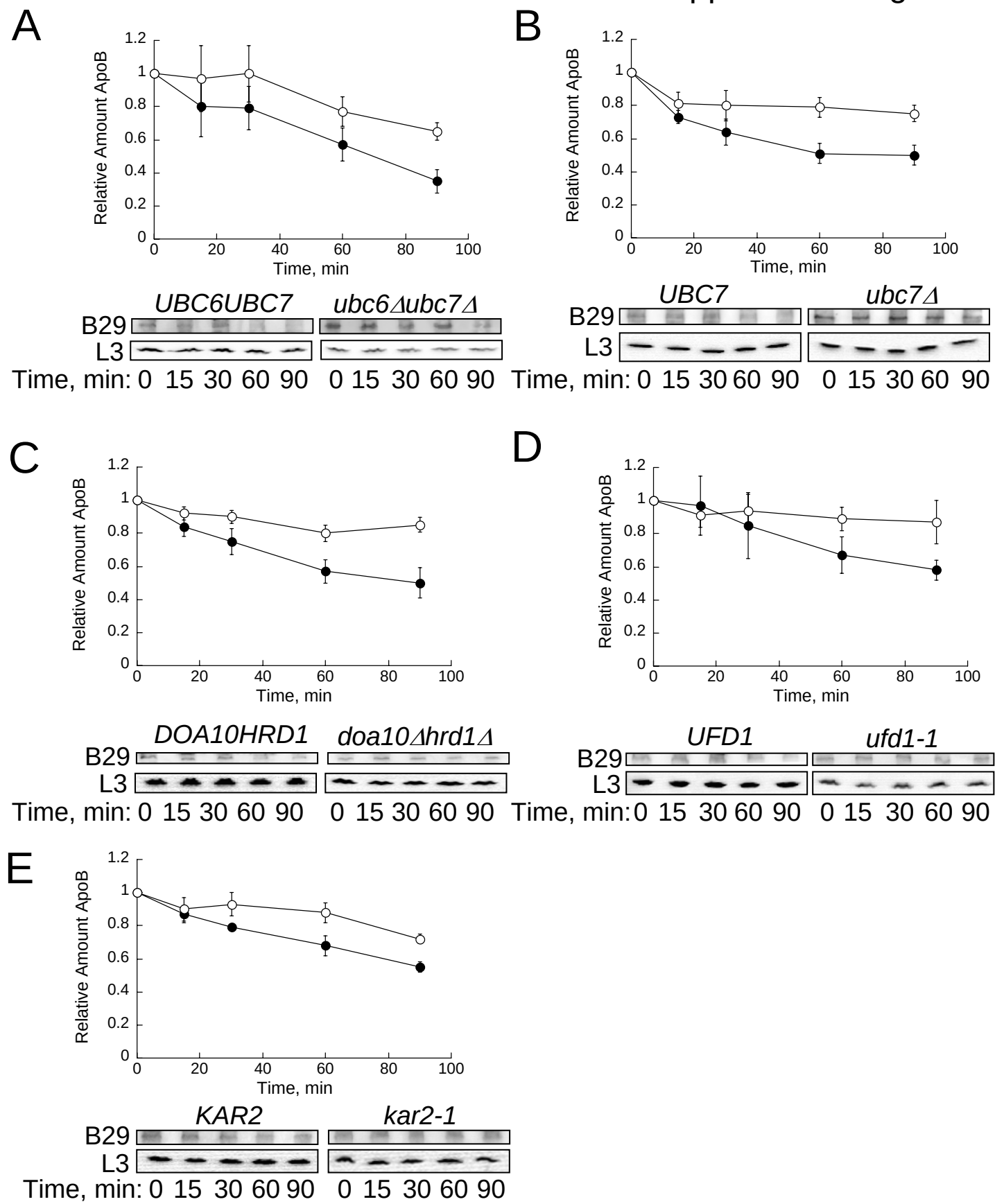
Supplemental Figure 2: ApoB degradation requires both cytosolic and ER lumenal factors in yeast. (A) A cycloheximide chase was performed in *UBC6UBC7* (●) or *ubc6Δubc7Δ* cells (○) transformed with pSLW1-B29. ApoB29 was detected using anti-HA antibodies and L3 was used as a loading control. For all cycloheximide chase analyses, each time point was normalized to the L3 loading control and the relative amount of apoB was calculated by dividing the values at each time by the value at t = 0. Data represent the means from 6 independent experiments ± SE of the means: 90 min, $p < 0.005$ (B) A cycloheximide chase was performed in *UBC7* (●) or *ubc7Δ* cells (○) transformed with pSLW1-B29. ApoB29 was detected using anti-HA antibodies and Sec61p was used as a loading control. Data represent the means from 7 independent experiments ± SE of the means: 60 min, $p < 0.02$; 90 min, $p < 0.02$ (C) A cycloheximide chase was performed in *DOA10HRD1* (●) or *doa10Δhrd1Δ* cells (○) transformed with pSLW1-B29. Data represent the means from 5 independent experiments ± SE of the means: 60 min, $p<0.03$; 90 min, $p<0.002$ (D) A cycloheximide chase was performed in *UFD1* (●) or *ufd1-1* cells (○) transformed with pSLW1-B29. Data represent the means from 4 independent experiments ± SE of the means: 90 min, $p<0.05$ (E): A cycloheximide chase was performed in *KAR2* cells (●) and *kar2-1* cells (○) transformed with pSLW1-B29. Data represent the means from 5 independent experiments ± SE of the means: 60 min, $p<0.03$; 90 min, $p<0.005$

Supplemental Figure 3: Sse1p differentially impacts the ERAD of diverse substrates. (A) A cycloheximide chase was performed in *SSE1* (●) or *sse1Δ (E0020)* cells (○) transformed with pRS426-CFTR-HA. CFTR was detected using anti-HA antibodies and Sec61p was used as a loading control. For all cycloheximide chase analysis, each time point was normalized to the Sec61p loading control and the relative amount of CFTR was calculated by dividing the values at each time by the value at t = 0. Data represent the means from 6 independent experiments ± SE of the means. All experiments were performed at 30°C. (B) A cycloheximide chase was performed in *SSE1* (●) or *sse1Δ (E0020)* cells (○) transformed with pSM1082-Ste6p*-HA. Ste6p* was detected using anti-HA antibodies and Sec61p was used as a loading control, as above. Data represent the means from 6 independent experiments ± SE of the means: 60 min, $p<0.006$; 90 min, $p<0.007$ (C) A pulse chase was performed on ^{35}S methionine labeled *SSE1* (●) or *sse1Δ (E0020)* cells (○) transformed with pSM36-ppαfΔG-HA. Pαf was immunoprecipitated from the cell extracts using anti-HA antibodies. Data represent the means from 4 independent experiments ± SE of the means. (D) A pulse chase was performed on ^{35}S methionine labeled *SSE1* (●) or *sse1Δ (E0020)* cells (○) transformed with pRS315-CPY*-HA. CPY* was immunoprecipitated from the cell extracts using anti-HA antibodies. Data represent the means from 4 independent experiments ± SE of the means.

Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3