Rubenstein et al., http://www.jcb.org/cgi/content/full/jcb.201203061/DC1

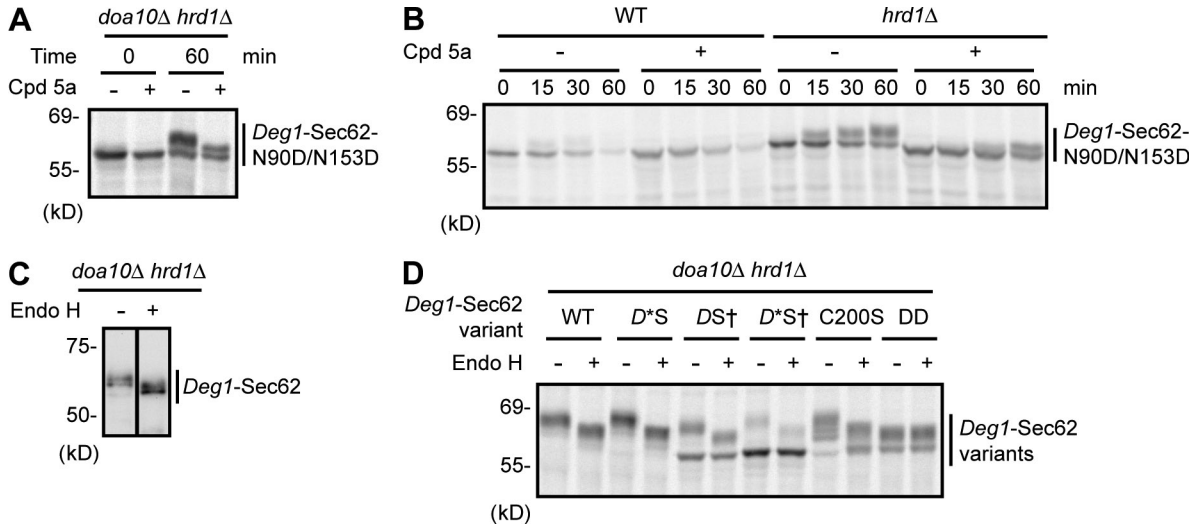

Figure S1. **PTM of *Deg1*-Sec62.** (A) *Deg1*-Sec62 is O-mannosylated. Pulse-chase analysis of *doa10*Δ *hrd1*Δ yeast expressing *Deg1*-Sec62-N90D/N153D cultured for 3 h in the presence 1 µM Compound 5a (a global inhibitor of O-mannosylation in yeast, see Materials and methods; Orchard et al., 2004) or DMSO at 30°C. Compound 5a (or DMSO) was maintained at the same concentrations throughout pulse labeling, washes, and chase in excess nonradioactive amino acids. Cycloheximide was included in the chase. *Deg1*-Sec62-N90D/N153D was precipitated with anti-Flag antibodies. (B) O-mannosylation is not necessary for Hrd1-mediated degradation. Pulse-chase analysis of *Deg1*-Sec62-N90D/N153D in the indicated yeast strains cultured for 3 h in the presence of 1 µM Compound 5a or DMSO. Compound 5a (or DMSO) was maintained at the same concentrations throughout pulse labeling, washes, and chase in excess nonradioactive amino acids. Cycloheximide was included in the chase. *Deg1*-Sec62-N90D/N153D was precipitated with anti-Flag antibodies. (C) A cell lysate of *doa10*Δ *hrd1*Δ cells expressing *Deg1*-Sec62 was incubated in the presence or absence of Endo H. Proteins were separated by SDS-PAGE, and *Deg1*-Sec62 was detected by immunoblotting with peroxidase anti-peroxidase antibody, which recognizes the Protein A tag. (D) N-glycosylation of *Deg1*-Sec62 variants. *doa10*Δ *hrd1*Δ yeast cells expressing "WT" *Deg1*-Sec62 or the indicated *Deg1*-Sec62 variants were pulse labeled for 10 min and lysed after 60 min in the presence of excess nonradioactive amino acids and cycloheximide. *Deg1* fusion proteins were precipitated with anti-Flag antibodies and incubated in the presence or absence of Endo H before being separated by SDS-PAGE and visualized by autoradiography. These results demonstrate that the modified subpopulations of the *Deg1*-Sec62 variants presented throughout this study are N-glycosylated, which indicates that they have each undergone the same manner of *Deg1*-stimulated topological rearrangement as "WT" *Deg1*-Sec62. The kinetics of attaining this rearrangement differ among the *Deg1*-Sec62 mutants (see main text). *D**S, *Deg1**-Sec62. *DS*†, *Deg1*-sec62†. *D**S†, *Deg1**-sec62†. C200S, *Deg1*-Sec62-C200S. DD, *Deg1**-Sec62-N90D/N153D. *Deg1**, F18S/I22T double mutant. sec62†, G127D of *Deg1*-Sec62, equivalent to G37D of untagged Sec62.

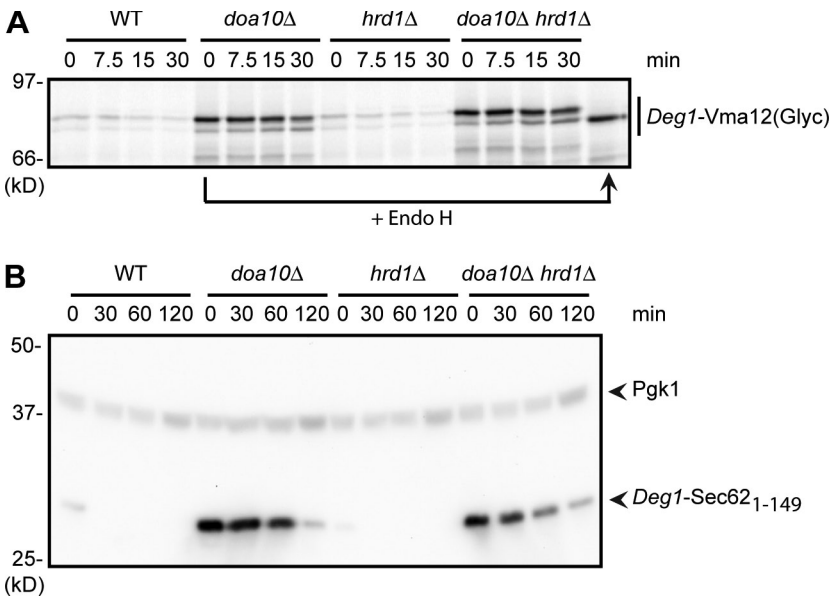

Figure S2. **Doa10-dependent degradation of *Deg1* fusion proteins.** (A) N-glycosylation does not inhibit Doa10-dependent degradation of *Deg1*-Vma12. Pulse-chase analysis of a *Deg1*-Vma12-KanMX6 derivative, which has a fragment of the Suc2 protein bearing two N-glycosylation sites inserted in its ER luminal loop, was performed in the indicated yeast strains. *Deg1*-Vma12(Glyc)-KanMX6 was precipitated with anti-*Deg1* antibodies. The indicated sample was treated with Endo H before separation by SDS-PAGE and autoradiography. (B) *Deg1*-Sec62$_{1–149}$ is a Doa10 substrate. Shown is a cycloheximide chase analysis of a *Deg1* fusion to the first 149 amino acids of Sec62 expressed from the *GAL1* promoter in the indicated yeast strains grown in SD medium containing 2% galactose. *Deg1*-Sec62$_{1–149}$ was detected by anti-Flag immunoblotting. Pgk1 serves as a loading control and was detected by anti-Pgk1 immunoblotting.

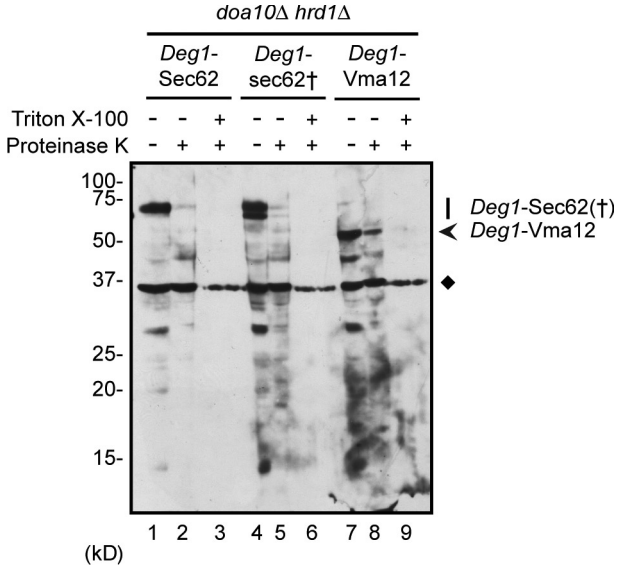

Figure S3. **Membrane topology of *Deg1*-Sec62, *Deg1*-sec62†, and *Deg1*-Vma12.** Intact microsomal membranes prepared from *doa10Δ hrd1Δ* cells expressing *Deg1*-Sec62, *Deg1*-sec62†, or *Deg1*-Vma12 were incubated with 5 µg/ml Proteinase K and 1% Triton X-100 as indicated. Samples were separated by SDS-PAGE and detected by anti-*Deg1* immunoblotting. The closed diamond denotes a nonspecific band. sec62†, G127D of *Deg1*-Sec62, equivalent to G37D of untagged Sec62.

Table S1. **Yeast strains used in this study**

Name	Genotype	Source
MHY496	MATa *his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 ubc6-Δ1::HIS3*	Sommer and Jentsch, 1993
MHY500	MATa *his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2*	Chen et al., 1993
MHY507	MATα *his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 ubc7::LEU2*	Jungmann et al., 1993
MHY552	MATα *his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 ubc6-Δ1::HIS3 ubc7::LEU2*	Chen et al., 1993
MHY1669	MATα *his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 hrd1Δ::LEU2*	Bays et al., 2001; Swanson et al., 2001
MHY1685	MATa *his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 doa10Δ::HIS3*	Huyer et al., 2004
MHY1702	MATa *his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 doa10Δ::HIS3 hrd1Δ::LEU2*	Huyer et al., 2004
MHY2822	MATa *his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 hrd1Δ::LEU2*	Huyer et al., 2004
MHY2972	MATa *his3Δ1 leu2Δ0 ura3Δ0 met15Δ0* (aka BY4741)	Tong et al., 2001
MHY3032	MATa *his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 hrd1Δ::kanMX4*	Tong et al., 2001
MHY3253	MATa *his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 der1Δ::kanMX4 dfm1Δ::kanMX4*	This study
MHY5306	MATa *his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 yos9Δ::kanMX4*	Tong et al., 2001
MHY6478	MATa *his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 usa1Δ::kanMX4*	Tong et al., 2001
MHY6701	MATa *his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 lhs1Δ::kanMX4 doa10Δ::HIS3 hrd1Δ::LEU2*	This study
MHY6703	MATa *his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 lhs1Δ::kanMX4*	This study
MHY6705	MATa *his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 lhs1Δ::kanMX4 hrd1Δ::LEU2*	This study
MHY6707	MATa *his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 lhs1Δ::kanMX4 doa10Δ::HIS3*	This study
MHY6792	MATa *his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 sec61Δ::HIS3* [pRS314-Sec61-C373S]	This study
MHY6794	MATa *his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 sec61Δ::HIS3 doa10Δ::hphMX4* [pRS314-Sec61-C373S]	This study
MHY6796	MATa *his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 sec61Δ::HIS3 hrd1Δ::kanMX4* [pRS314-Sec61-C373S]	This study
MHY6798	MATa *his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 sec61Δ::HIS3 doa10Δ::hphMX4 hrd1Δ::kanMX4* [pRS314-Sec61-C373S]	This study
MHY6893	MATα *his3-Δ200 leu2Δ1 ura3Δ99 trp1Δ99 ade2-101(ochre) sec63-201 doa10Δ::kanMX4* (derived from DNY65 and DNY234; Ng et al., 1996)	This study
MHY6894	MATa *his3-Δ200 leu2Δ1 ura3Δ99 trp1Δ99 ade2-101(ochre) sec63-201 hrd1Δ::kanMX4* (derived from DNY65 and DNY234; Ng et al., 1996)	This study
MHY6897	MATa *his3-Δ200 leu2Δ1 ura3Δ99 trp1Δ99 ade2-101(ochre) sec63-201* (derived from DNY65 and DNY234; Ng et al., 1996)	This study
MHY6899	MATa *his3-Δ200 leu2Δ1 ura3Δ99 trp1Δ99 ade2-101(ochre) sec63-201 hrd1Δ::kanMX4 doa10Δ::kanMX4* (derived from DNY65 and DNY234; Ng et al., 1996)	This study
MHY7120	MATa *his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 hrd3Δ::kanMX4*	Tong et al., 2001
MHY7321	MATa *his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 sec61Δ::HIS3* [pEM598/pRS315-sec61-L7B(ala)]	This study
MHY7323	MATa *his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 sec61Δ::HIS3 doa10Δ::hphMX4* [pEM598/pRS315-sec61-L7B(ala)]	This study
MHY7325	MATa *his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 sec61Δ::HIS3 hrd1Δ::kanMX4* [pEM598/pRS315-sec61-L7B(ala)]	This study
MHY7327	MATa *his3-Δ200 leu2-3,112 ura3-52 lys2-801 trp1-1 gal2 sec61Δ::HIS3 doa10Δ::hphMX4 hrd1Δ::kanXM4* [pEM598/pRS315-sec61-L7B(ala)]	This study

Table S2. **Plasmids used in this study (in order of first presentation)**

Name	Description	Source	Figures
pRS414-P_{MET25}-*Deg1*-Flag-Vma12-2xProtA	CEN, *TRP1*	Ravid et al., 2006	1 C and S3
pRS414-P_{MET25}-*Deg1*-Flag-Sec62-2xProtA	CEN, *TRP1*	Mayer et al., 1998	1 D, 1 F, and S3
pRS416-P_{MET25}-*Deg1**-Flag-Sec62-2xProtA	CEN, *TRP1* *Deg1** = F18S, I22T	This paper	1 H, 6 A, and S1 D
pRS416-P_{MET25}-*Deg1*-Flag-Sec62-2xProtA	CEN, *URA3*	This paper	2 A; 3, A and B; 4, A–C; 5, A, B, and D; and S1, C and D
pRS416-P_{MET25}-*Deg1*-Flag-Sec62-2xProtA-N90D	CEN, *URA3*	This paper	2 A
pRS416-P_{MET25}-*Deg1*-Flag-Sec62-2xProtA-N153D	CEN, *URA3*	This paper	2 A
pRS416-P_{MET25}-*Deg1*-Flag-Sec62-2xProtA-N90D/N153D	CEN, *URA3*	This paper	2, A and B; and S1, A, B, and D
pRS315-P_{PRC1}-CPY* (aka pMW319)	CEN, *LEU2* CPY* = G255R	Willer et al., 2008	3 A
pRS414-P_{MET25}-*Deg1*-Flag-sec62†-2xProtA	CEN, *TRP1* sec62† = sec62-1 = G127D	This paper	4 A and S3
pRS313	CEN, *HIS3*	Sikorski and Hieter, 1989	4 B
pRS313-Sec63 (aka pDN210)	CEN, *HIS3*	Ng and Walter, 1996	4 C
pRS414-P_{MET25}-*Deg1**-Flag-sec62†-2xProtA	CEN, *TRP1* *Deg1** = F18S, I22T sec62† = sec62-1 = G127D	This paper	4 D
pRS315-sec61-L7B(ala) (aka pEM598)	CEN, *LEU2* Q308A, I323A, W326A, L342A	Trueman et al., 2011	5 A
pRS416-P_{MET25}-Deg1-Flag-Sec62-2xProtA-C200S	CEN, *URA3*	This paper	5 C and S1 D
pRS314-Sec61-C373S	CEN, *TRP1*	Scott and Schekman, 2008	5 D
YCp50-P_{PRC1}-CPY*-HA (aka pDN431)	CEN, *URA3*	Ng et al., 2000	6 B
YCp50-$P_{GAL1/10}$-ApoB29-3HA (aka pSLW1-B29)	CEN, *URA3* Encodes 29% of human ApoB; Human pre-pro ApoB sequence replaced with pre-pro sequence from yeast pre-pro-alpha factor	Hrizo et al., 2007	7, A–C
pJJB20	CEN, *URA3* Vector encoding pre-pro sequence (amino acids 1–100) from yeast pre-pro-alpha factor	Hrizo et al., 2007	7 A
pRS416-P_{MET25}-*Deg1*-Flag-sec62†-2xProtA	CEN, *URA3* sec62† = sec62-1 = G127D	This paper	S1 D
pRS416-P_{MET25}-*Deg1**-Flag-sec62†-2xProtA	CEN, *URA3* *Deg1** = F18S, I22T sec62† = sec62-1 = G127D	This paper	S1 D
pRS414-P_{MET25}-*Deg1*-Flag-Vma12(Glyc)-KanMX6	CEN, *TRP1* Glyc = 2 glycosylation sites from Suc2 inserted in ER lumenal loop of Vma12	This paper	S2 A
pRS426-$P_{GAL1/10}$-*Deg1*-Flag-Sec62(1-149)	2μ, *URA3* Sec62 truncated at amino acid 149	Scott and Schekman, 2008	S2 B

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