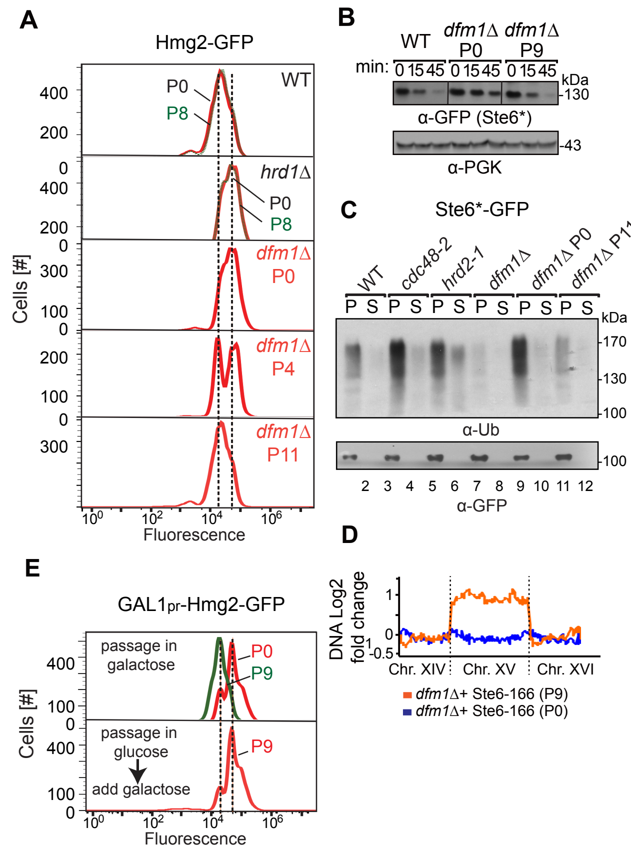
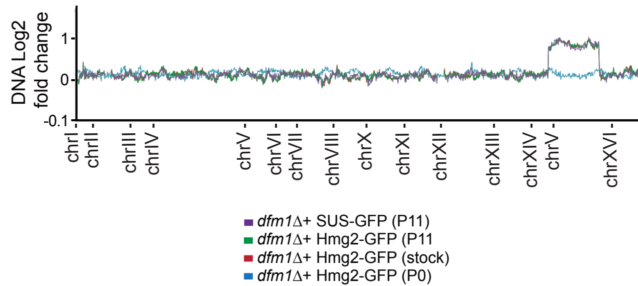


Figure S1. Related to Figure 6. *dfm1*Δ suppression is substrate induced. (A) Same as Fig. 6A, except *dfm1*Δ cells overexpressing Hmg2-GFP were passaged to suppression. Cells were passaged at the indicated number of times into fresh minimal media (P0, P4, and P9). Hmg2-GFP levels were analyzed by flow cytometry. WT and *hrd1*Δ cells over expressing Hmg2-GFP were also passaged as controls. Histograms of 10,000 cells are shown, with the number of cells *versus* GFP fluorescence. (B) Ste6*-GFP is degraded to WT levels in *dfm1*Δ suppressed cells. Same as Fig. 6B except degradation of Ste6*-GFP was measured by CHX-chase assay in WT, *dfm1*Δ P0 and *dfm1*Δ P9 cells. (C) Ste6*-GFP retrotranslocation was completely restored in substrate induced *dfm1*Δ suppressed cells. Lysates were ultracentrifuged to discern ubiquitinated Ste6*-GFP that either has been retrotranslocated into the soluble fraction (S) or remained in the membrane (P). Following fractionation, Ste6*-GFP was immunoprecipitated from both fractions, and immunoblotted with α-GFP and α- Ubi. (D) ChrXV duplication is triggered by Ste6*-GFP expression in *dfm1*Δ cells. Chromosome profiles of whole genome sequencing data mapped across ChrXV. (E) *dfm1*Δ suppression is induced by Hmg2-GFP overexpression. Same as Fig. 6A, except *dfm1*Δ cells overexpressing GAL driven Hmg2-GFP were passaged to suppression. *dfm1*Δ cells were grown either in the presence of galactose to turn on Hmg2-GFP expression (upper panel) or glucose to turn off Hmg2-GFP expression and passaged at the indicated number of times into fresh minimal media (P0 and P9). *dfm1*Δ cells passaged in glucose were replaced with galactose to trigger Hmg2-GFP expression. Flow cytometry was used to assess Hmg2-GFP levels. Histograms of 10,000 cells are shown, with the number of cells *versus* GFP fluorescence.

A



B

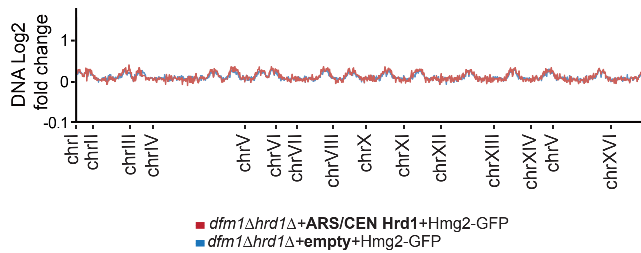


Figure S2. Refer to Figure 6. Entire ChrXV is duplicated in substrate-induced *dfm1*Δ suppressed cells. (A) Chromosome profiles of whole genome sequencing data mapped across whole yeast genome. Genomic levels of entire ChrXV are twice as high in suppressed *dfm1*Δ cells expressing Hmg2-GFP or SUS-GFP with respect to passaged *dfm1*Δ cells containing empty vector. (B) ChrXV is not duplicated with addition of ARS/CEN plasmid containing Hrd1. Same as (A) except chromosome profiles were analyzed in *dfm1*Δ*hrd1*Δ cells containing the native promoter driven Hrd1 on an ARS/CEN plasmid or an empty vector ARS/CEN plasmid.

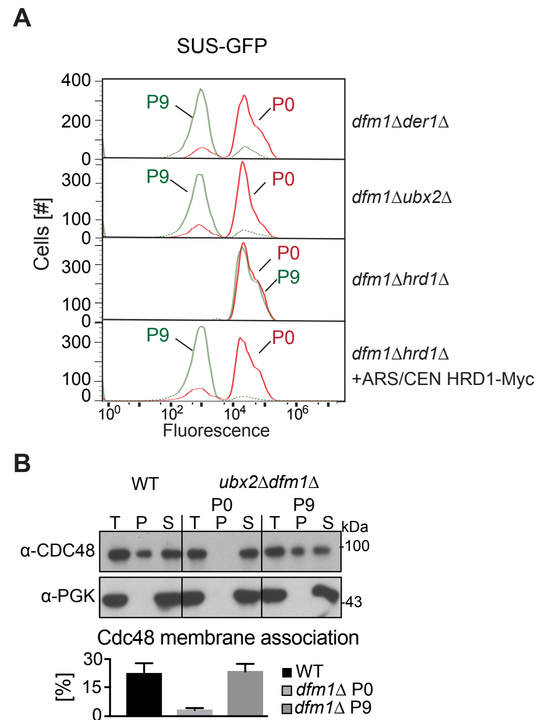


Figure S3. Related to Figure 6. Hrd1 is required for *dfm1*Δ suppression. (A) The indicated strains overexpressing SUS-GFP were passaged to suppression. Cells were passaged and SUS-GFP levels were analyzed by flow cytometry. Histograms of 10,000 cells are shown, with the number of cells versus GFP fluorescence. (B) Ubx2 does not restore Cdc48 recruitment to microsomes in *dfm1*Δ suppressed cells. Total cell lysate (T) from the indicated strains were separated into soluble cytosolic fraction (S) and pellet microsomal fraction (P) upon centrifugation at 14,000 x g. Each fraction was analyzed by SDS-PAGE and immunoblotted with Cdc48 with α-CDC48 and PGK1 with α-PGK1. The graph shows the quantification of Cdc48 in the pellet fractions of the respective cells as measured from ImageJ. Data is represented as percentage of Cdc48 that is bound to pellet fraction and is shown as mean ± SEM from three independent experiments.

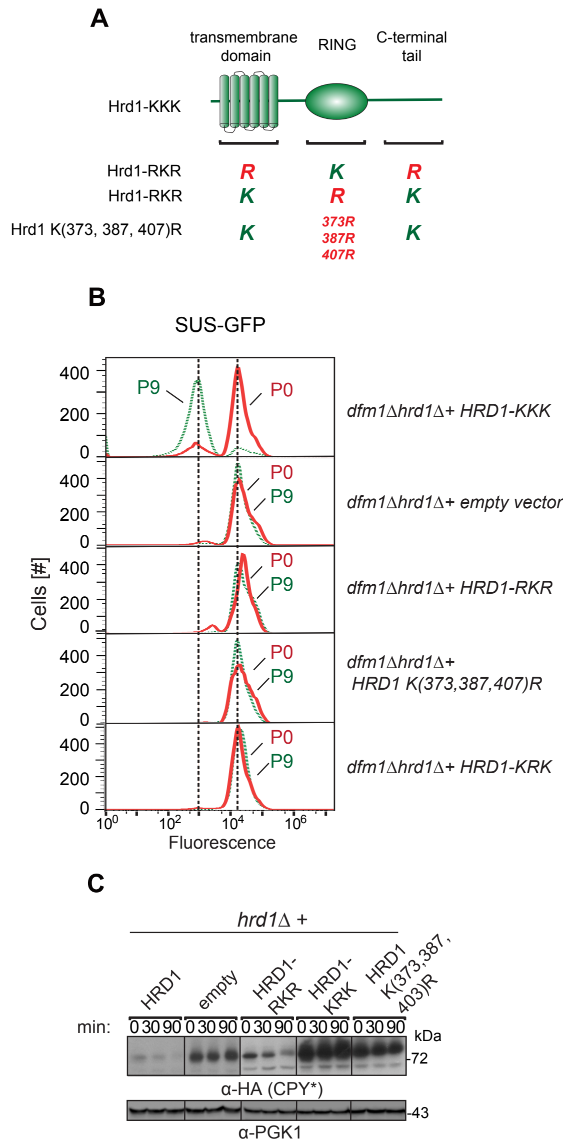


Figure S4. Related to Figure 6. Autoubiquitination of Hrd1 RING domain is not required for *dfm1*Δ suppression. (A) Depiction of Hrd1, Hrd1-RKR, Hrd1-KRK, Hrd1 K(373, 387, 404)R. (B) The indicated strains overexpressing SUS-GFP and containing Hrd1 variants with lysine to arginine mutations were passaged to suppression. Cells were passaged and SUS-GFP levels were analyzed by flow cytometry. Histograms of 10,000 cells are shown, with the number of cells versus GFP fluorescence. (C) Hrd1 autoubiquitination on RING domain affects ERAD of CPY*. In the indicated strains, degradation of CPY*-HA was measured by CHX-chase assay. Cells were analyzed by SDS-PAGE and immunoblotted for CPY*-HA with α-HA.

Table S1: Plasmids used in this study, Related to STAR Methods

Plasmid	Gene			Reference
pRH 2071	YIp	URA3	pTDH3-SUS	Garza et al., 2009
pRH 2900	YIp	TRP1	pTDH3-SUS-GFP	This study
pRH 2901	YIp	URA3	pTDH3-SUS-GFP	This study
pRH 469	YIp	URA3	pTDH3-HMG2-GFP	Garza et al., 2009
pRH 1960	YCp	URA3	pCAU-KWW-3HA	Vashistha et al., 2016
pRH 1958	YCp	URA3	pCAU-KHN-3HA	Vashistha et al., 2016
pRH 2497	YIp	TRP1	pHRD1-5xMYC	Vashistha et al., 2016
pRH 2058	2μ	URA3	pPGK-STE6-166-3HA-GFP	Vashistha et al., 2016
pRH 2013	YCp	LEU2	pDFM1-3HA	Sato et al., 2006
pRH 2889	YCp	LEU2	pDFM1-5Ashp-3HA	Sato et al., 2006
pRH 2890	YCp	LEU2	pDER1-SHP-3HA	Sato et al., 2006
pRH 2826	YCp	LEU2	pDFM1-AA-3HA	This study
pRH 2812	YCp	LEU2	pDFM1-Ax$_3$A-3HA	This study
pRH 1120	YCp	URA3	pGAL-HMG2-GFP	Federovitch et al., 2008
pRH 2571	YCp	HIS3	pHRD1	Baldridge et al., 2016
pRH 2841	YCp	HIS3	pHRD1-KRK	Baldridge et al., 2016
pRH 2843	YCp	HIS3	pHRD1-RKR	Baldridge et al., 2016
pRH 2844	YCp	HIS3	pHRD1 -(373, 387, 404)R	Baldridge et al., 2016