

Supplementary Table 1

Gene Name	Ratio H/L Normalized	PEP	Significance(A)	Significance(B)	Variability [%]	PCU
HBT1	6.366	1.77E-56	1.66E-141	4.50E-70	103.16	6
FMP45	5.723	9.45E-06	6.16E-126	1.47E-62	10.26	4
YJR015W	5.179	2.02E-02	3.43E-112	6.42E-56	2.78	2
RTN2	4.335	3.16E-95	1.07E-89	3.14E-93	48.11	16
PFA4	2.864	2.09E-03	3.07E-47	2.07E-24	154.88	4
PRC1	2.495	3.59E-112	3.50E-36	1.88E-40	34.23	14
ERG3	2.296	2.89E-275	2.95E-30	3.13E-37	12.65	22
ERG25	2.253	2.75E-124	5.84E-29	1.33E-35	12.22	14
HSP12	1.870	3.83E-104	5.34E-18	6.33E-19	13.67	10
MSC1	1.697	2.52E-244	2.22E-13	4.10E-16	16.08	48
DER1	1.542	4.53E-09	1.56E-09	5.15E-07	26.12	6
ERG5	1.434	9.45E-158	4.00E-07	1.29E-08	12.29	42
ALG14	1.410	1.67E-118	1.27E-06	4.46E-05	6.67	8
YJL171C	1.377	1.57E-80	5.93E-06	1.79E-06	22.91	8
GWT1	1.370	8.86E-14	8.01E-06	7.46E-04	2.17	4
BIG1	1.314	7.53E-19	9.23E-05	4.52E-05	15.10	12
GTT1	1.309	7.39E-212	1.10E-04	2.43E-05	8.07	22
CSG2	1.302	2.61E-27	1.50E-04	1.13E-03	0.97	4
KRE5	1.301	2.68E-44	1.55E-04	7.83E-05	19.85	24
ERP5	1.283	5.41E-30	3.16E-04	5.27E-03	16.36	6
ERG4	1.252	7.18E-20	1.03E-03	3.71E-04	12.91	24
APE1	1.243	9.41E-139	1.44E-03	5.62E-04	15.86	40
OST5	1.242	3.45E-11	1.45E-03	8.33E-04	15.97	6
SEC11	1.242	1.63E-60	1.48E-03	8.52E-04	26.98	8
YIP4	1.232	1.21E-12	2.12E-03	7.07E-03	32.71	4
SPC3	1.227	3.33E-101	2.52E-03	1.11E-03	9.69	16
USA1	1.223	7.67E-31	2.85E-03	1.71E-03	12.41	20
YLL055W	1.221	6.58E-102	3.06E-03	9.15E-03	9.92	6
ENO1	1.211	0.00E+00	4.32E-03	2.74E-03	27.63	26
ERP6	1.208	9.97E-06	4.75E-03	1.25E-02	4.99	6
ALG8	1.208	6.52E-06	4.80E-03	1.25E-02	17.13	8
ERV46	1.205	1.99E-173	5.14E-03	2.62E-03	14.61	20
ERG2	1.198	1.32E-236	6.45E-03	4.53E-03	9.82	10
ERV25	1.197	2.77E-292	6.84E-03	4.88E-03	69.02	16
ALG13	1.196	1.94E-79	6.88E-03	4.68E-03	11.42	14
YLR050C	1.193	2.41E-09	7.71E-03	1.76E-02	7.12	6
GDH1	1.192	0.00E+00	7.99E-03	5.93E-03	36.45	58;20
CWP1	1.190	4.71E-05	8.54E-03	3.25E-02	3.10	4
SOP4	1.189	6.20E-53	8.64E-03	6.03E-03	58.16	8
YOR152C	1.187	8.81E-36	9.19E-03	5.95E-03	12.80	16
HXT7	1.186	3.39E-145	9.56E-03	5.52E-03	7.67	10;10;2
YOS9	1.185	2.89E-15	9.76E-03	2.08E-02	13.35	6
GPI12	1.184	4.45E-13	1.02E-02	5.95E-03	9.63	8
SUS1	1.180	2.21E-09	1.16E-02	6.95E-03	43.56	10
RAX2	1.179	2.36E-05	1.17E-02	3.89E-02	8.89	4
HRD3	1.175	3.38E-44	1.32E-02	9.65E-03	32.07	22
ALG1	1.173	3.34E-62	1.40E-02	9.35E-03	18.18	10
UIP3	1.173	1.68E-04	1.40E-02	4.32E-02	16.14	4
PST1	1.170	7.28E-09	1.57E-02	4.60E-02	29.67	4
YNL024C-A	1.169	3.00E-10	1.59E-02	2.95E-02	16.86	8
YNL058C	1.168	2.28E-51	1.62E-02	2.99E-02	24.31	4
SNA3	1.168	3.60E-05	1.63E-02	4.72E-02	12.49	4

EMP24	1.164	6.00E-168	1.85E-02	1.68E-02	17.40	20
NCP1	1.163	0.00E+00	1.89E-02	1.72E-02	17.52	62
OST3	1.162	1.38E-81	1.94E-02	1.29E-02	6.53	24
STT3	1.161	1.58E-194	2.03E-02	1.88E-02	17.13	50
YDR367W	1.160	6.09E-19	2.08E-02	3.59E-02	12.48	4
SEC61	1.159	1.65E-155	2.13E-02	1.99E-02	23.35	26
FLC3	1.159	4.85E-20	2.13E-02	3.65E-02	17.05	2
SCJ1	1.157	1.15E-293	2.24E-02	1.73E-02	11.73	24
AKL1	1.157	1.08E-20	2.27E-02	1.57E-02	45.44	12
RBD2	1.156	3.33E-05	2.34E-02	1.62E-02	24.27	8
ERV29	1.155	3.06E-210	2.35E-02	1.62E-02	15.83	14
DAP2	1.155	1.16E-99	2.36E-02	1.83E-02	17.90	22
PPN1	1.155	7.13E-27	2.40E-02	3.97E-02	9.38	10
YMR134W	1.151	2.29E-55	2.62E-02	2.05E-02	19.21	18
ALG2	1.151	5.32E-136	2.66E-02	2.09E-02	11.98	22
YFR006W	1.150	5.68E-109	2.73E-02	2.15E-02	18.59	22
PMT1	1.149	0.00E+00	2.78E-02	2.76E-02	7.95	50
FMP52	1.149	2.14E-223	2.82E-02	2.80E-02	13.16	26
YBL029C-A	1.148	2.11E-28	2.90E-02	4.55E-02	6.91	4
SPC2	1.146	1.80E-145	3.03E-02	2.18E-02	5.61	16
SIR3	1.144	2.24E-13	3.17E-02	4.87E-02	5.95	4
ROT2	1.144	6.63E-62	3.21E-02	2.58E-02	25.24	24
NDC1	1.143	1.34E-27	3.31E-02	2.34E-02	97.24	12
KTR1	1.139	1.23E-234	3.62E-02	3.79E-02	10.21	38
MET17	1.139	0.00E+00	3.63E-02	3.80E-02	14.91	52
DPM1	1.138	0.00E+00	3.74E-02	3.94E-02	9.07	36
MNR2	1.137	3.14E-06	3.82E-02	2.74E-02	16.26	8
ALG12	1.136	8.05E-125	3.94E-02	2.98E-02	17.79	24
ERP1	1.133	8.81E-158	4.29E-02	3.30E-02	6.75	14
NTE1	1.130	0.00E+00	4.62E-02	3.60E-02	25.24	66
PMT2	1.127	6.24E-290	4.94E-02	3.90E-02	11.11	38
KRE6	1.127	2.30E-249	4.94E-02	3.90E-02	25.19	24;2
YPR091C	1.127	3.34E-48	4.98E-02	4.17E-02	12.22	24
YLR414C	0.869	1.13E-72	4.08E-02	3.42E-02	8.65	4
ADH3	0.868	1.66E-159	3.95E-02	2.48E-03	13.85	30
TPO4	0.861	1.75E-11	3.22E-02	2.60E-02	17.58	8
HMG1	0.858	2.84E-100	2.87E-02	3.51E-03	19.18	18
PLB1	0.854	4.03E-14	2.51E-02	1.95E-02	12.87	8
FET5	0.854	2.87E-109	2.48E-02	2.68E-03	14.42	22
CHO1	0.853	1.70E-93	2.40E-02	5.66E-04	13.99	14
UBC6	0.852	7.51E-94	2.38E-02	2.47E-03	8.07	14
TSC10	0.847	1.09E-45	1.96E-02	1.73E-03	9.38	20
PDR16	0.844	2.09E-135	1.75E-02	2.67E-04	10.43	30
OPI1	0.842	1.59E-53	1.65E-02	1.25E-03	16.03	16
LEU2	0.840	0.00E+00	1.54E-02	3.86E-04	23.60	40
TRL1	0.837	7.37E-51	1.35E-02	3.70E-02	98.00	8
YRO2	0.829	6.28E-30	9.84E-03	6.50E-03	12.32	2
GTR2	0.829	1.41E-55	9.72E-03	6.41E-03	24.42	10
GAS1	0.827	0.00E+00	9.18E-03	1.40E-04	23.70	22
YEL048C	0.826	1.28E-44	8.90E-03	2.69E-02	6.75	4
GAS3	0.817	1.88E-215	6.04E-03	1.89E-04	74.84	18
YOR1	0.808	5.18E-222	3.95E-03	8.45E-05	13.91	34
LDB19	0.804	2.11E-04	3.35E-03	4.04E-02	44.39	4
PDR12	0.801	4.66E-287	2.83E-03	3.33E-06	58.86	48

YND1	0.796	2.75E-10	2.33E-03	9.65E-03	13.27	10
STE13	0.795	9.72E-18	2.14E-03	9.04E-03	13.69	8
ZEO1	0.785	8.02E-86	1.32E-03	5.18E-07	16.91	18
VTC3	0.784	1.93E-116	1.24E-03	4.44E-07	22.79	40
TDH1	0.777	0.00E+00	8.34E-04	1.68E-07	8.43	22
HXT1	0.774	4.72E-46	7.22E-04	3.30E-06	12.11	14
VAP1	0.766	2.22E-39	4.67E-04	1.74E-04	5.87	6
MTR2	0.765	3.00E-28	4.19E-04	1.41E-02	9.55	4
PEM1	0.762	5.91E-161	3.62E-04	2.45E-07	52.73	54
PBI2	0.759	3.89E-25	2.91E-04	1.17E-02	4.43	6
DKA1	0.727	5.35E-04	3.67E-05	3.98E-04	8.07	6
ITR1	0.727	3.35E-93	3.57E-05	7.14E-11	20.71	12
MRH1	0.725	3.54E-104	3.15E-05	2.05E-09	9.88	8
STE18	0.718	9.10E-24	1.83E-05	2.32E-04	9.84	4
PEM2	0.715	1.15E-182	1.47E-05	7.91E-12	8.41	12
HXT2	0.714	1.93E-20	1.36E-05	1.85E-04	39.19	4
YNL146W	0.690	1.15E-03	1.90E-06	9.36E-04	10.92	2
YDL124W	0.672	0.00E+00	3.66E-07	3.48E-13	18.96	42
PDR5	0.650	0.00E+00	4.17E-08	5.10E-15	17.59	58;2
GPA1	0.648	1.27E-163	3.10E-08	1.71E-18	13.44	28
FTH1	0.646	1.58E-04	2.67E-08	1.11E-04	11.81	4
SCW10	0.634	1.64E-03	7.06E-09	5.69E-05	12.37	4
MNN1	0.621	6.89E-221	1.39E-09	7.30E-22	20.62	42
FLC1	0.610	1.83E-04	3.50E-10	1.27E-05	14.02	2
ATF2	0.524	5.69E-82	3.85E-16	1.15E-12	19.50	10
SAG1	0.041	1.07E-03	0.00E+00	5.88E-177	18.50	4

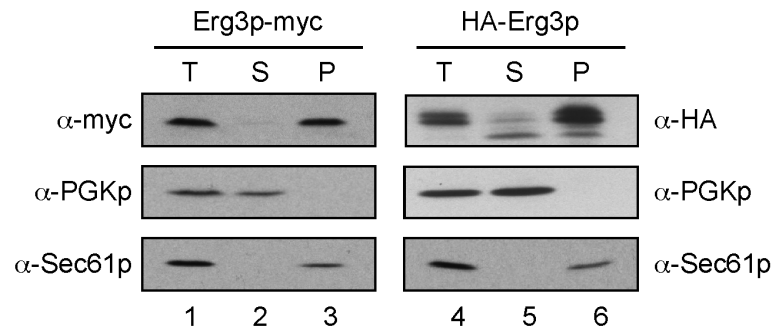
Supplementary Table 2

Strain	Genotype	Reference
YCH012	a Δ htm1::HIS3, prc1.1, trp1, his3, ura3, lys2, leu2	this work
YCH066	a Δ vos9::HIS3, prc1.1, trp1, his3, ura3, lys2, leu2	this work
YCH452	a prc1.1-0000, trp1, his3, ura3, lys2, leu2	this work
YCH480	a YOS9-R200A, prc1.1-0000, trp1, his3, ura3, lys2, leu2	this work
YCH485	a Δ vos9::TRP1, prc1.1-0000, trp1, his3, ura3, lys2, leu2	this work
YCH490	a Hrd1 Δ 347::Ura, prc1.1-0000, trp1, his3, ura3, lys2, leu2	this work
YCH552	a 6xHA-Hrd3, ERG3-13xmyc-N45Q(TRP1), trp1, his3, ura3, lys2, leu2	this work
YCH553	a 6xHA-Hrd3, YOS9-R200A, ERG3-13xmyc-N45Q(TRP1), trp1, his3, ura3, lys2, leu2	this work
YCH555	a 6xHA-Hrd3, Δ vos9::HIS3, ERG3-13xmyc(TRP1), trp1, his3, ura3, lys2, leu2	this work
YCH556	a 6xHA-Hrd3, Δ vos9::HIS3, ERG3-13xmyc-N45Q(TRP1), trp1, his3, ura3, lys2, leu2	this work
YCH557	a 6xHA-Hrd3, Δ hrd1::NatNT2, ERG3-13xmyc(TRP1), trp1, his3, ura3, lys2, leu2	this work
YCH558	a 6xHA-Hrd3, Δ hrd1::NatNT2, ERG3-13xmyc-N45Q(TRP1), trp1, his3, ura3, lys2, leu2	this work
YCH482	a 6xHA-Hrd3, Δ prc1::LEU2	this work
YCH483	a 6xHA-Hrd3 YOS9-R200A, ERG3-13xmyc(TRP1), Δ prc1::LEU2, trp1, his3, ura3, lys2, leu2	this work
YCH484	a 6xHA-Hrd3, Δ vos9::HIS3, ERG3-13xmyc(TRP1), Δ prc1::LEU2, trp1, his3, ura3, lys2, leu2	this work
YCH485	a 6xHA-Hrd3, Δ hrd1::NatNT2, ERG3-13xmyc(TRP1), Δ prc1::LEU2, trp1, his3, ura3, lys2, leu2	this work
YHB016	alpha Δ arg4::KAN, Δ pro3::TRP1, Δ ire1::LEU2, prc1.1, trp1, his3, ura3, lys2, leu2	this work
YHB017	alpha Δ arg4::KAN, Δ pro3::TRP1, Δ ire1::LEU2, Δ doa10::his, prc1.1, trp1, his3, ura3, lys2, leu2	this work
YHB018	a Δ arg4::KAN, Δ pro3::HIS3, Δ ire1::LEU2, Δ hrd1::trp, prc1.1, trp1, his3, ura3, lys2, leu2	this work
YJU037	a prc1.1, Δ hrd1::TRP1, trp1, his3, ura3, lys2, leu2	Friedlander et. al., 2000
YLJ001	a Δ ire1::URA3, ERG3-13xmyc(HIS3), trp1, his3, ura3, lys2, leu2	this work
YLJ002	a ERG3-13xmyc:(TRP1), prc1.1, trp1, his3, ura3, lys2, leu2	this work
YLJ004	a Δ ire1::LEU2, Δ doa10:HIS3, ERG3-13xmyc(TRP1), prc1.1, trp1, his3, ura3, lys2, leu2	this work
YLJ005	a Δ doa10::HIS3, Δ hrd1::TRP1, Δ ire1::LEU2, ERG3-13xmyc:(kanMX), prc1.1, trp1, his3, ura3, lys2, leu2	this work
YLJ006	a Δ hrd1::TRP1, Δ ire::LEU2, ERG3-13xmyc:(HIS3), trp1, his3, ura3, lys2	this work

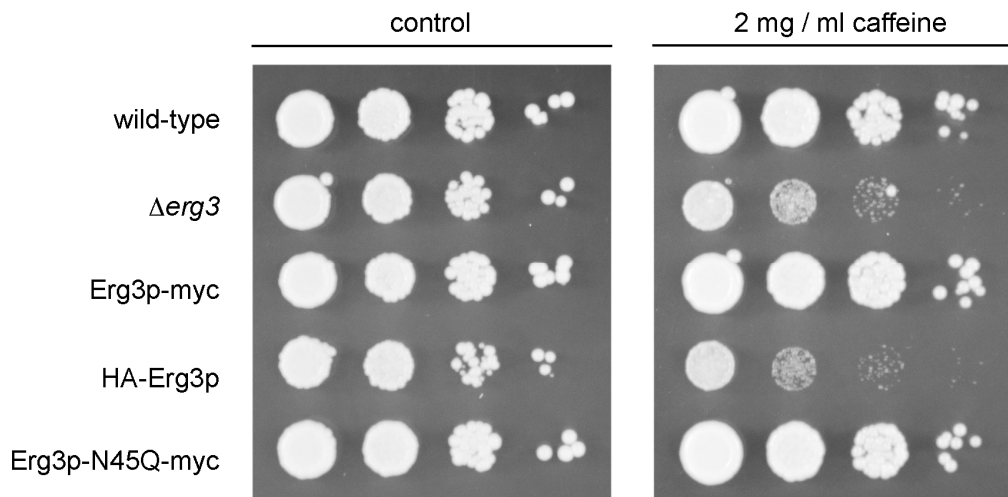
YLJ008	a	Δ der1::HIS3, prc1.1, ERG3-13xmyc(TRP1), trp1, his3, ura3, lys2, leu2	this work
YLJ010	a	Δ hrd1::TRP1, ERG3-13xmyc(HIS3), prc1.1, trp1, his3, ura3, lys2, leu2	this work
YLJ011	a	6xHA-HRD3, prc1.1, ERG3-13xmyc(TRP1), trp1, his3, ura3, lys2, leu2	this work
YLJ012	a	6xHA-HRD3, Δ ubc7::LEU2, prc1.1, ERG3-13xmyc(TRP1), trp1, his3, ura3, lys2, leu2	this work
YLJ015	a	Δ yos9::HIS3, ERG3-13xmyc(kanMX), prc1.1, trp1, his3, ura3, lys2, leu2	this work
YLJ022	a	Δ erg3::TRP1, prc1.1, trp1, his3, ura3, lys2, leu2	this work
YLJ041	a	Δ ubc7::LEU2, ERG3-13xmyc(TRP), trp1, his3, ura3, lys2, leu2	this work
YLJ042	a	6xHA-ERG3, prc1.1, trp1, his3, ura3, lys2, leu2	this work
YLJ048	a	Δ htm1::HIS3, Erg3p-myc(kanMx), prc1.1, trp1, his3, ura3, lys2, leu2	this work
YLJ052	a	Δ hrd1::TRP1, Δ erg3::kanMx, prc1.1, trp1, his3, ura3, lys2, leu2	this work
YLJ054	a	Δ yos9::HIS3, Δ erg3::kanMx, prc1.1, trp1, his3, ura3, lys2, leu2	this work
YLJ057	a	Δ erg3::TRP1, yos9R200A, prc1.1, trp1, his3, ura3, lys2, leu2	this work
yRF013	n.d.	Δ hrd1::TRP1, Δ ire::LEU2, trp1, his3, ura3, lys2	Friedlander et. al., 2000
YRG131	a	6xHA-HRD3, Δ ubc7::LEU2, prc1.1, trp1, his3, ura3, lys2, leu2	Gauss et. al., 2006
YTX140	a	prc1.1, trp1, his3, ura3, lys2, leu2	Biederer et al., 1996
YTX139	alpha	Δ ire1::URA3, trp1, his3, ura3, lys2, leu2	this work
YTX435	alpha	Δ ire1::LEU2, Δ doa10::HIS3, prc1.1, trp1, his3, ura3, lys2, leu2	this work
YTX436	a	Δ doa10::HIS3, Δ hrd1::TRP1, Δ ire1::LEU2, prc1.1, trp1, his3, ura3, lys2, leu2	this work

Supplementary figure 1.

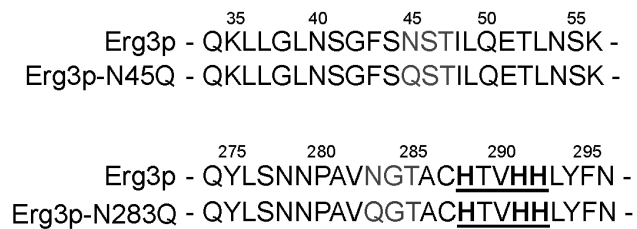
A



B



C



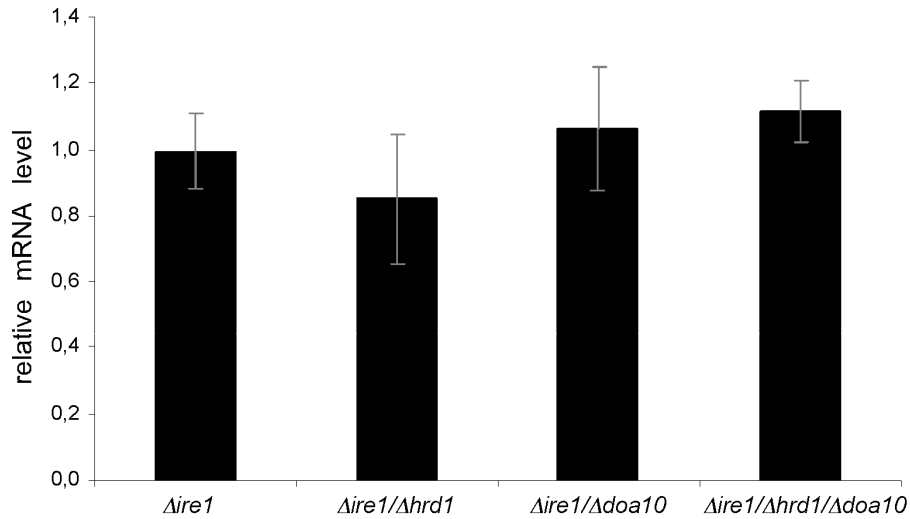
Supplementary figure 1. C-terminally tagged Erg3p is functional and integrates into the ER membrane.

(A) To determine if epitope-tagged Erg3p is associated with membranes, total cell extracts (T) were separated into pellet (P) and supernatant (S) fraction by high-speed centrifugation, separated by SDS-PAGE and analysed by immunoblotting with the indicated antibodies. Blotting against the integral membrane protein Sec61p and the cytosolic protein PGK showed no detectable cross-contamination.

(B) Ten-fold serial dilutions of the indicated yeast strains were spotted onto SD⁺ plates either in the absence (left panel, control) or the presence (right panel) of 2 mg/ml caffeine.

(C) Sequences surrounding the putative glycosylation sites that were mutated. Histidine residues that contribute to the enzymatic activity of Erg3p are in bold.

Supplementary figure 2.

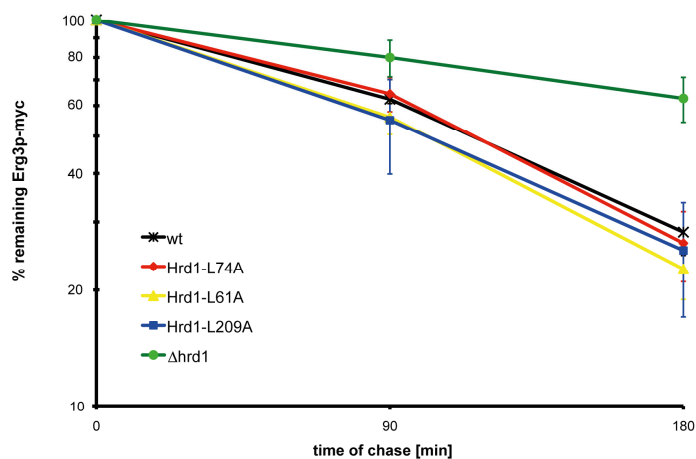


Supplementary figure 2. *ERG3* is not transcriptionally upregulated.

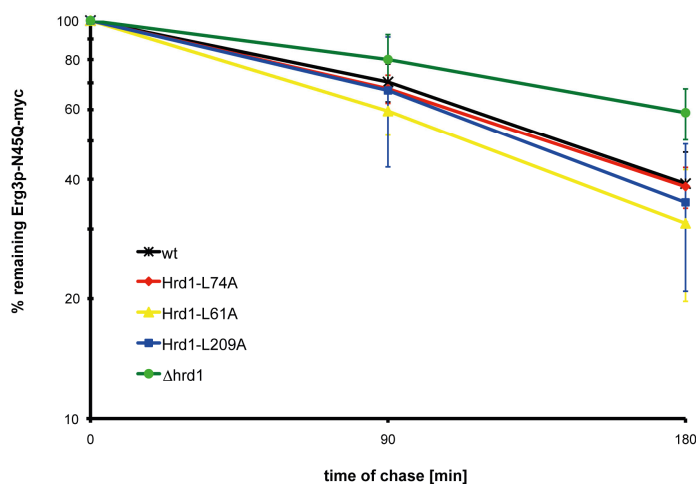
RNA was isolated from the indicated yeast strains. Expression of *ERG3* was determined by quantitative real-time PCR using *ACT1* as a standard. The relative mRNA expression of $\Delta ire1$ was set as 1. The relative mRNA expression levels of the other strains are in relation to the mRNA levels of the $\Delta ire1$ strain. Error bars indicate standard deviations of technical triplicates.

Supplementary Figure 3

A



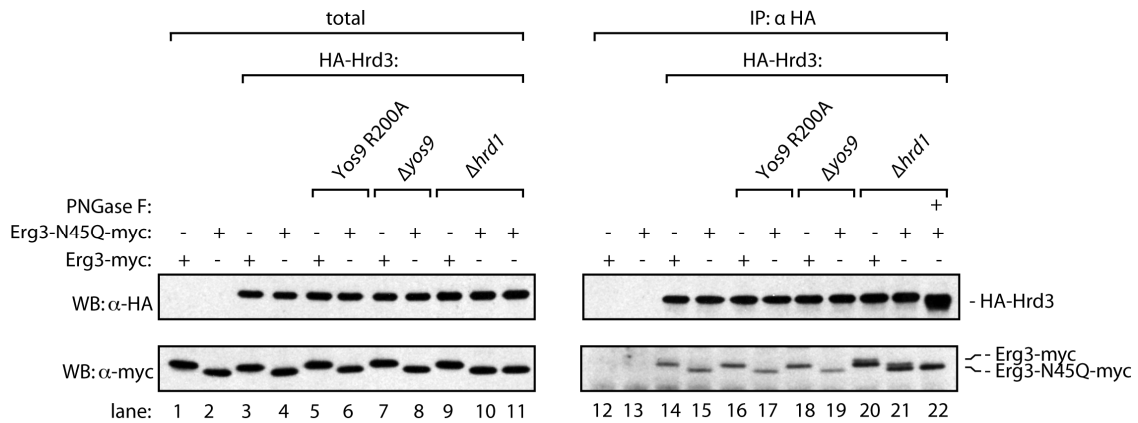
B



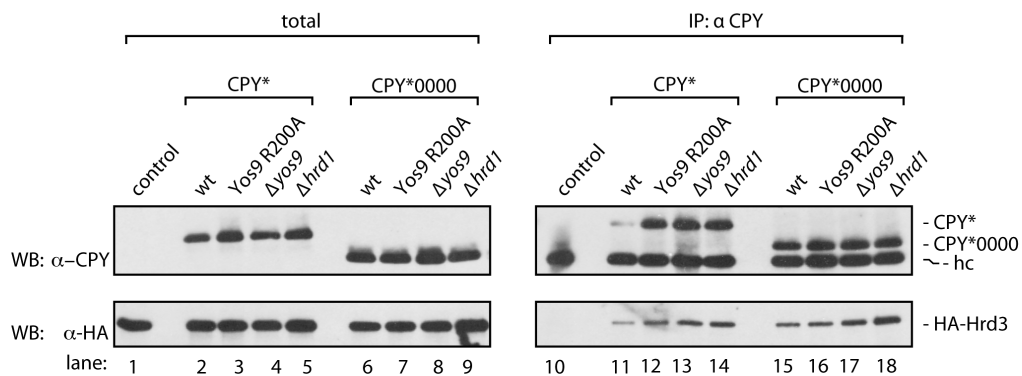
Supplementary figure 3. Hrd1p is not directly involved in the recognition of Erg3p
(A) Pulse-chase experiments were performed to analyse the stability of Erg3p-myc in the $\Delta hrd1$ cells carrying plasmids encoding the indicated Hrd1p mutants. Three independent experiments were quantified using a PhosphorImager and the results were averaged. The errors bars indicate the standard error of the experiments.
(B) Same as in (a), but Erg3p-N45Q was used as substrate.

Supplementary figure 4

A



B



Supplementary figure 4. The HRD ligase binds its substrates mainly via Hrd3p
(A) Microsomes isolated from cells expressing HA-Hrd3p and Erg3p-myc or Erg3p-N45Q in the indicated yeast strains were solubilised with NP40 lysis buffer and HA-Hrd3p was immunoprecipitated. Samples from the total lysates (left panel) and the precipitates (right panel) were separated by SDS-PAGE followed by immunoblotting using the indicated antibodies. Of note: Deletion of *HRD1* results in the appearance of a slower migrating, myc-reactive band in the immunoblot. This signal disappears upon treatment with PNGase F (compare lanes 21 and 22), indicating that this band represents

a hyperglycosylated form of Erg3p. Presumably, deletion of *HRDI* impairs the function of the ER and a fraction of Erg3p does not integrate correctly into the ER membrane, resulting in the glycosylation of Erg3p at N283.

(B) Interaction between CPY* or CPY*0000 and the HRD ligase in the indicated yeast strains was analysed by co-immunoprecipitation. Microsomes from the denoted strains were solubilised in NP40 lysis buffer and CPY* was immunoprecipitated. Samples from the total lysates (left panel) and the precipitates (right panel) were separated by SDS-PAGE followed by immunoblotting using the indicated antibodies. (hc) refers to the heavy chain of the a-CPY antibody used for precipitation.