Figure S1, related to Figure 1.

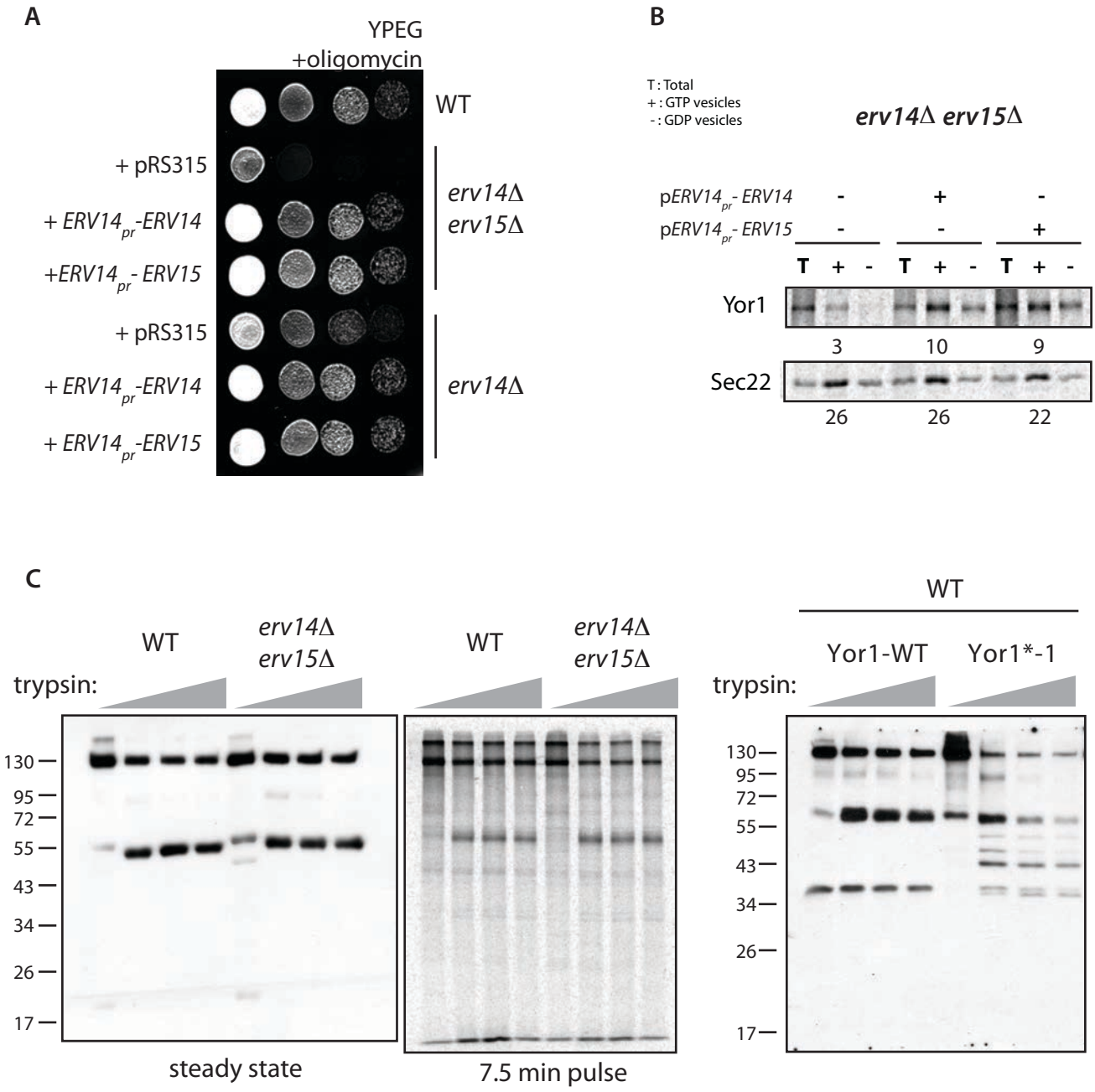


Figure S2, related to Figure 2 and Figure 6.

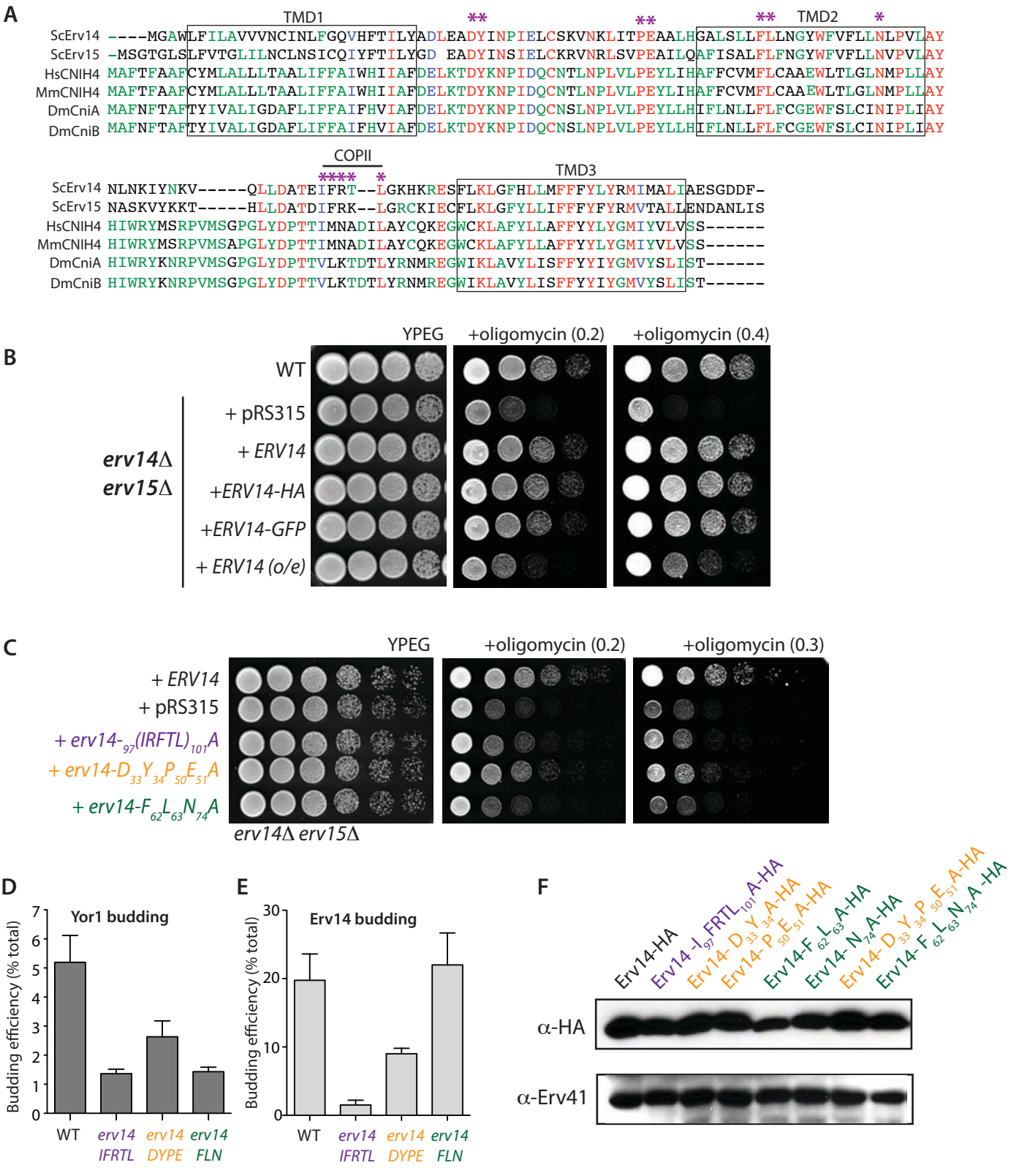


Figure S3, related to Figure 2.

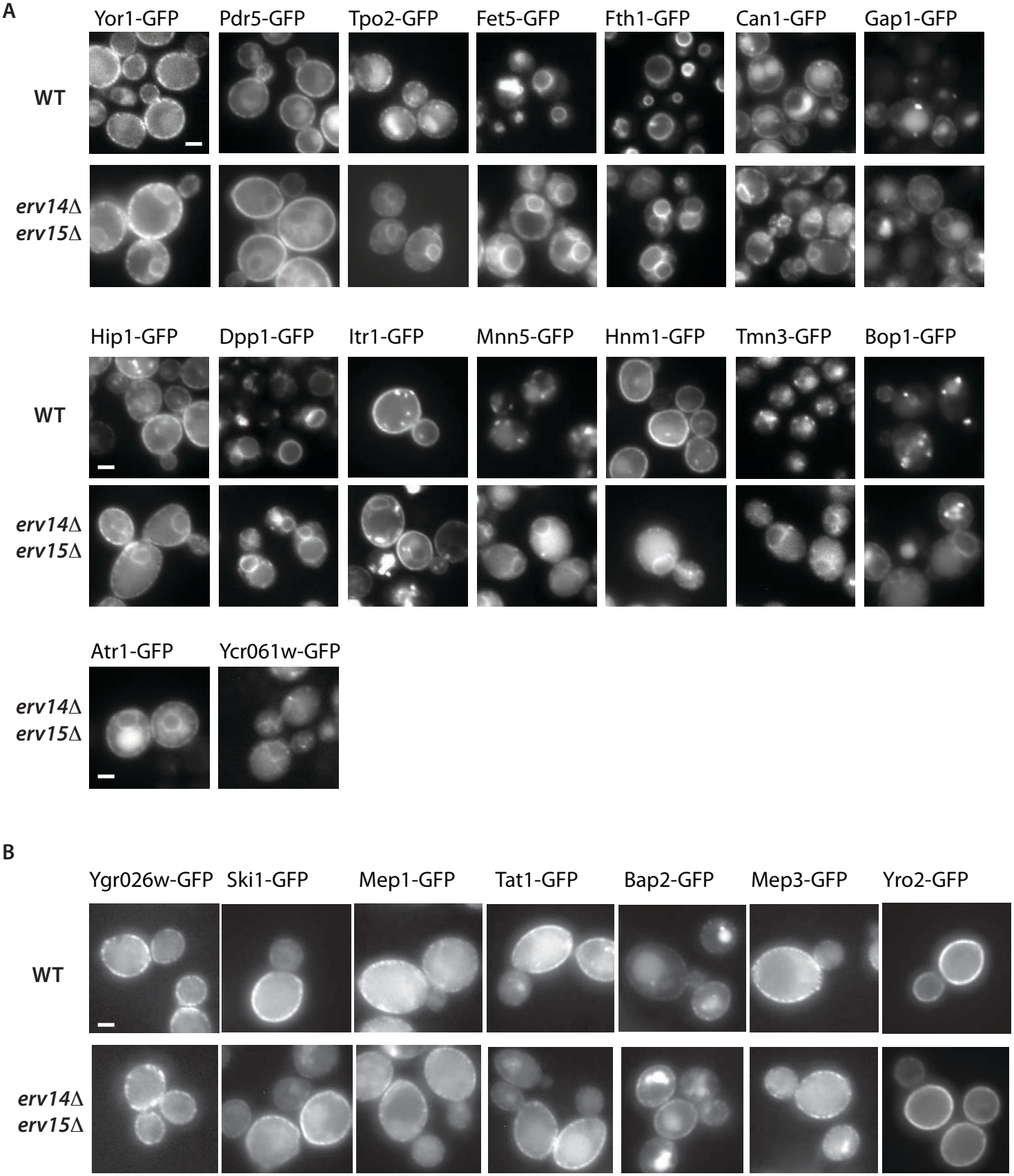


Figure S4, related to Figure 3.

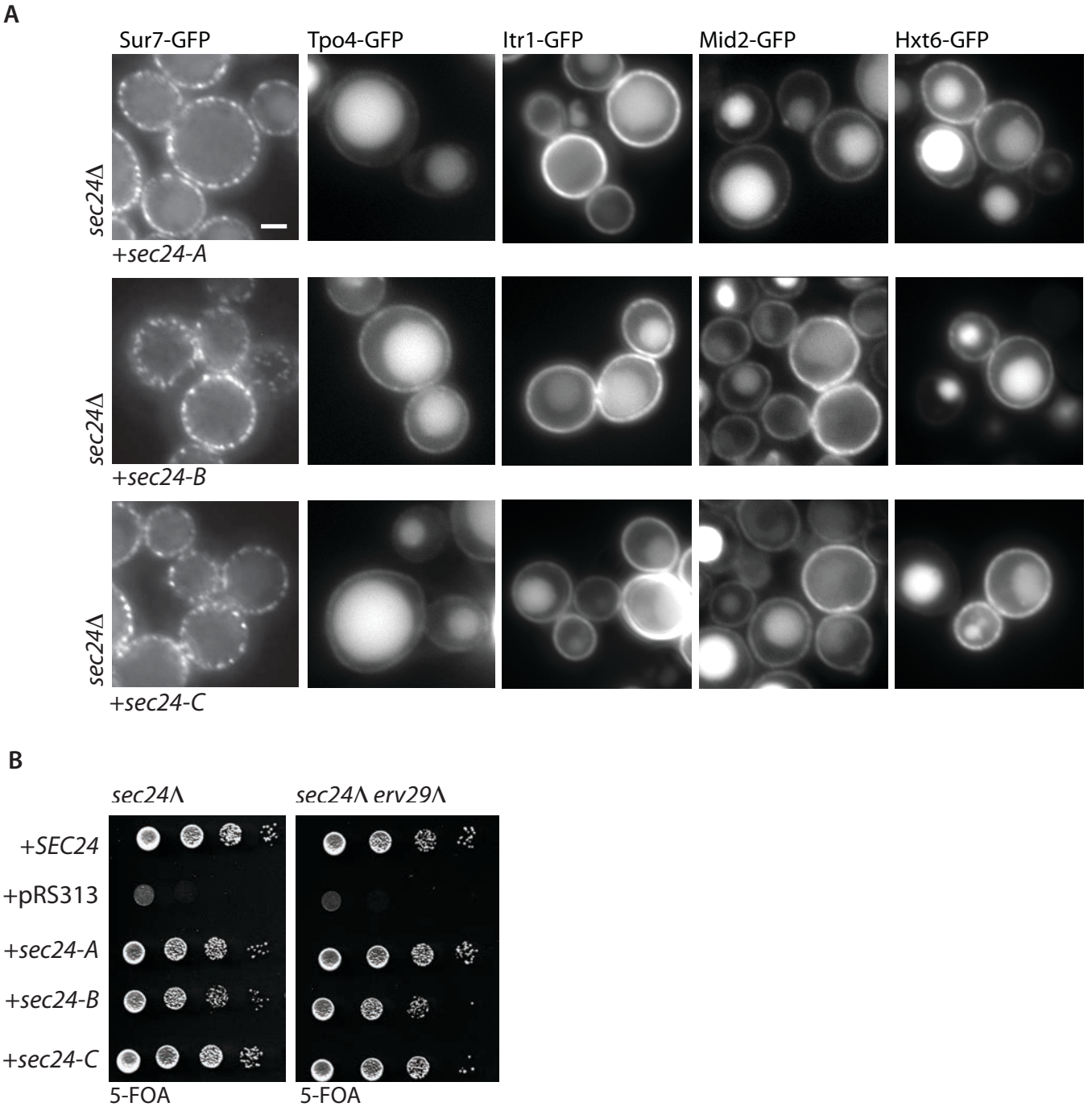


Figure S5, related to Figure 4.

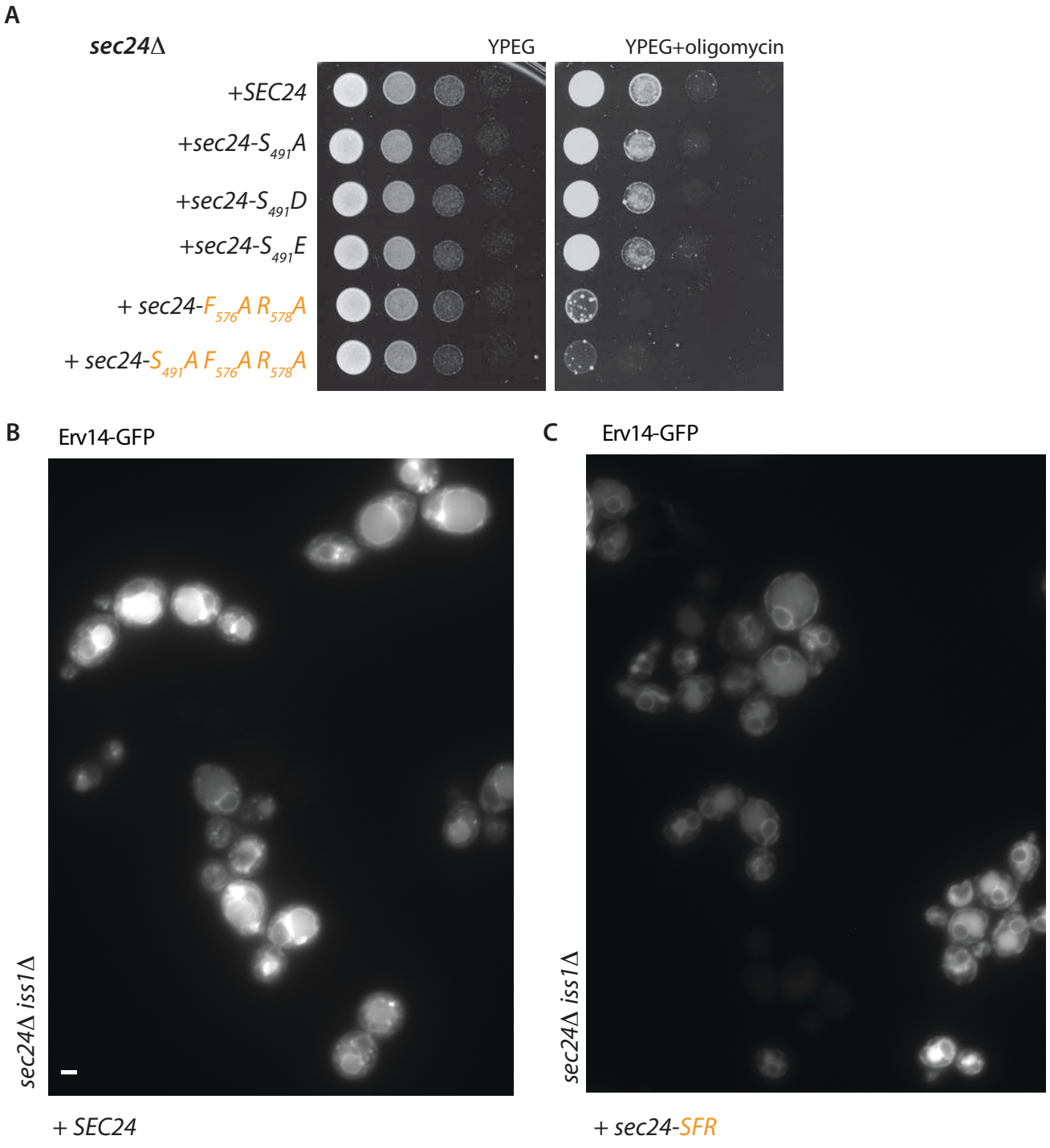
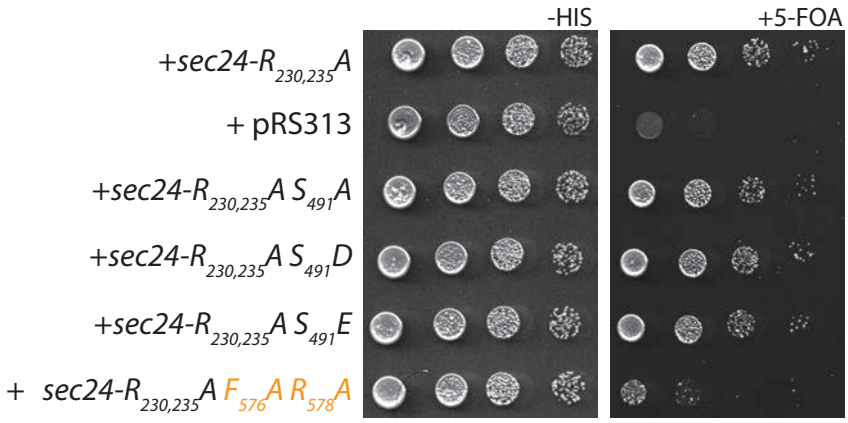
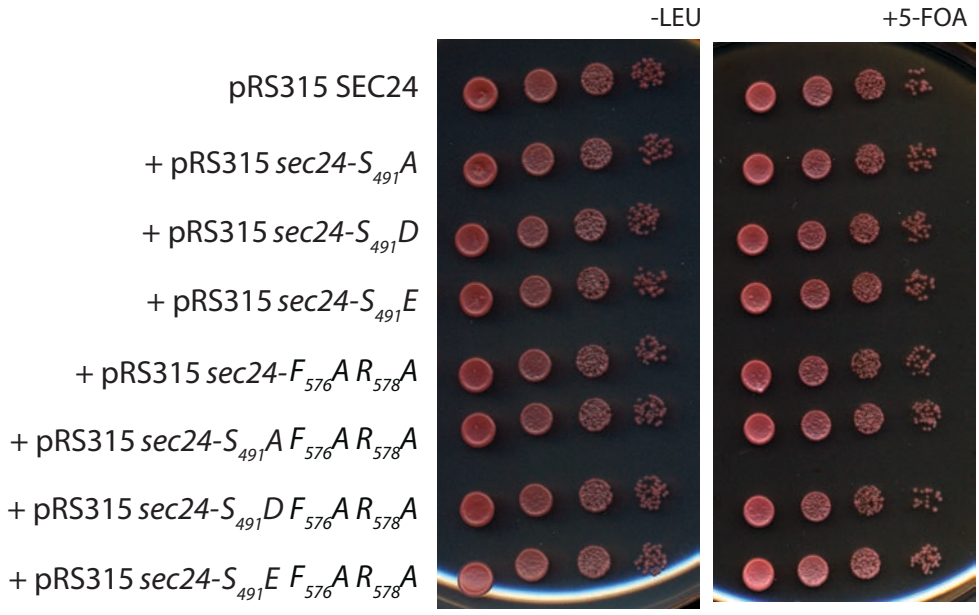


Figure S6, related to Figure 5.

A ***sec24*Δ**



B ***sec24*Δ + pRS313 *sec24-R*$_{230,235}$*A***



Supplemental Figure Legends.

Figure S1 (related to Figure 1)**. Both Erv14 and Erv15 are necessary for Yor1 trafficking but do not contribute to Yor1 folding**. (A) The phenotype of *erv14Δ* or *erv14Δ erv15Δ* mutants expressing *ERV14* or *ERV15* under the control of the *ERV14* promoter was tested by serial dilutions onto YPEG + oligomycin (0.4 µg/ml). (B) Incorporation of Yor1 into COPII vesicles was measured from membranes isolated from *erv14Δ erv15Δ* cells that express either *ERV14* or *ERV15* under the control of the *ERV14* promoter as indicated. (C) Microsomal membranes (left panel) or radiolabelled semi intact cells (center panel) isolated from wild-type or *erv14Δ erv15Δ* cells expressing wild-type Yor1-HA were treated with increasing amounts of trypsin followed by SDS-PAGE and detection of HA-tagged proteolytic fragments. Misfolding of Yor1 results in the appearance of multiple lower molecular weight bands, as observed for a steady-state experiment using a misfolded allele of Yor1-HA (Yor1*-HA; right panel).

Figure S2 (related to Figure 2 and Figure 6)**. Structure-function analysis of Erv14** (A) Multiple sequence alignment between Erv14 and Erv15 from *Saccharomyces cerevisiae* (Sc) and their Cornichon orthologs in human (Hs), mouse (Mm) and *Drosophilia* (Dm), generated by Multalin (http://npsapbil.ibcp.fr/cgibin/npsa_automat.pl?page=/NPSA/npsa_multalin.html). Conserved and similar residues are indicated in red and green, respectively. Boxes delimit the positions of three TMDs previously defined by Powers et al. (2002). The purple stars specify the residues analyzed in this study. (B) Ten-fold serial dilutions of *erv14Δ erv15Δ* cells expressing untagged, HA- or GFP-tagged versions of *ERV14* from a *CEN* plasmid or untagged *ERV14* from a 2µ plasmid (o/e) were tested for sensitivity to the indicated concentrations of oligomycin. Addition of HA and GFP epitopes at the C-terminus of Erv14 did not influence complementation of the oligomycin sensitivity displayed by the *erv14Δ erv15Δ* strain. Over-expression of *ERV14* accentuated drug sensitivity. (C) Cultures of *erv14Δ erv15Δ* cells bearing the plasmids indicated were

spotted onto media containing increasing concentrations of oligomycin (0, 0.2 µg/ml and 0.3 µg/ml). Neither the cytosolic mutant (*erv14-IFTRL*) nor the TMD2 mutant (*erv14-FLN*) could complement oligomycin sensitivity. The lumenal mutant (*erv14-DYPE*) showed an intermediate phenotype. (D) Incorporation of Yor1-HA into COPII vesicles was assayed as described in Fig1 from membranes isolated from the strains indicated. Quantification (right panel; n=3; error bars represent SD) showed that the capture of both Yor1-HA (D) and Erv14-HA (E) into the vesicle fraction was inhibited by mutation of the Erv14 *IFTRL* signal. Conversely, the abundance of Yor1-HA in COPII vesicles was perturbed in the context of *erv14-FLN* whereas packaging of *erv14-FLN-HA* itself was unaffected compared to wild-type *ERV14-HA*. Membranes expressing *erv14-DYPE* were partially affected both for Yor1 capture and Erv14 capture. (E) Anti-HA immunoblots of whole cell lysates expressing the indicated Erv14 variants.

Figure S3 (related to Figure 2)**. Erv14 traffics a subset of plasma membrane proteins.** (A and B) Wide-field fluorescent micrographs of the indicated cargo proteins tagged with GFP, expressed in wild-type or *erv14Δ erv15Δ* cells (Scale bar =2µm). (A) Novel Erv14 clients identified in this study. (B) Plasma membrane proteins whose trafficking is independent of Erv14.

Figure S4 (related to Figure 3)**. Most Erv14 clients do not use known Sec24 binding sites.** (A) Localization of GFP-tagged versions of the indicated Erv14 clients were examined by epifluorescence microscopy in *sec24Δ* strains expressing the cargo-binding site *sec24* mutants indicated. None of the Sec24 mutants impaired cell surface localization of Sur7-GFP, Tpo4-GFP, Itr1-GFP, Mid2-GFP and Hxt6-GFP (Scale bar =2µm). (B) The indicated variants of *SEC24* were introduced into *sec24Δ* or *sec24Δ erv29Δ* cells expressing wild-type *SEC24* from a *URA3*-marked plasmid and serial dilutions of the transformants were spotted onto medium containing 5-FOA, which counterselects for the *URA3*-marked plasmid. All *SEC24* mutants were viable in both *sec24Δ* and *sec24Δ erv29Δ* strains.

Figure S5 (related to Figure 4)**. Characterization of the Erv14 binding site (Ser_{491}, Phe_{576}, Arg_{578}) on Sec24.** (A) Strains expressing the indicated variants of *SEC24* as the sole copy were spotted on media containing oligomycin (0.4 µg/ml) to test for Yor1 function at the plasma membrane. (B and C) Erv14-GFP localization was monitored by epifluorescence microscopy in *sec24Δ iss1Δ* strains expressing (B) wild-type *SEC24* or (C) the *sec24-SFR* mutant (Scale bar =2µm).

Figure S6 (related to Figure 5)**. Phenotypic characterization of the Erv14 binding site (Ser_{491}, Phe_{576}, Arg_{578}) on Sec24.** (A) A *sec24Δ* strain expressing wild-type *SEC24* from a *URA3*-marked plasmid was transformed with the indicated variants of *SEC24* and serial dilutions were spotted onto medium containing 5-FOA, which counterselects for the *URA3*-marked wild-type version. A variant of Sec24 bearing mutations that affect both the B-site (Arg_{230} and Arg_{235}) and the Erv14-binding site (Phe_{578} and Arg_{578}) was unable to sustain growth at 30ºC. (B) A *sec24Δ* strain co-expressing wild-type *SEC24* on a *URA3*-marked plasmid and the B-site mutant *sec24*-R_{230}A R_{235}A on a *HIS3*-marked plasmid was transformed with the indicated variants of *SEC24* on a *LEU2*-marked plasmid and viability was tested after counterselection of the *URA3*-marked wild-type in presence of 5-FOA. No growth defect could be observed upon co-expression of Sec24 mutants independently affected for the B-site or for the Erv14-binding site.

Table S2 (related to Figure 6) – Oligomycin phenotypes of *ERV14* mutants

Plasmid number	Mutation	Oligomycin Sensitivity
LMB773	WT	-
pRS315	Empty plasmid	+++
QC1889	I97A F98A	++
QC1890	R99A T100A L101A	-
QC1888	I97A F98A R99A T100A L101A	+++
QC1891	D33A Y34A	+
QC1892	C41A	-
QC1893	P50AE51A	-
QC1913	D33A Y34A P50A E51A	++
QC1894	F62A L63A	++
QC1895	W68A	-
QC1896	N74A	++
QC1908	F62D L63A N74A	+++
QC1897	P76A	-
QC1898	Y80A	-
QC1899	E107A	-
QC1900	K111A	-
QC1901	F119A	-
QC1902	F120A	-
QC1903	Y124A	-

An *erv14Δ erv15Δ* strain (SPY36) was transformed with the plasmids listed in the first column. Each transformant was spotted as 10-fold serial dilutions onto YPEG plates supplemented with oligomycin (0.2 µg/ml) to test for their sensitivity to the drug (third column). Sensitivity was scored relative to wild type and empty vector controls.

Table S3 (related to Figure 6)– Mass spectrometry of Erv14-HA co-IPs

Putative client	Erv14-$_{97}$IFRTL$_{101}$A emPAI score	Erv14-$_{97}$IFRTL$_{101}$A F$_{62}$AL$_{63}$A emPAI score
Bpt1†	0.0666	0.0147
Chs2†	0.106	(0)
Cps1	2.64	0.833
Dnf1	0.345	0.158
Dnf2	0.487	0.292
Drs2	0.204	(0)
Fks1†	1.26	0.808
Hoc1	0.237	0.126
Hxt2	1.43	2.04
Hxt3	0.627	0.782
Itr1	0.0843	0.0888
Ktr3†	0.177	(0)
Lem3†	0.302	0.475
Mnn10†	0.34	0.227
Mnn11	0.127	(0)
Mnn5	0.111	(0)
Mnn5	0.111	(0)
Mnn9†	0.369	0.0595
Mns1†	0.335	0.0854
Pdr5	0.181	0.223
Pho89†	0.301	0.103
Rsn1	2.12	1.89
Snq2	0.338	0.414
Sur7	0.742	0.395
Svp26†	0.183	0.208*
Tcb2	0.155	0.067
Tcb3†	0.165	(0)
Tna1	0.608	0.581
Vph1†	0.0992	0.14
Yor1	0.0747	0.0622*

† Candidate clients identified by mass spectrometry only

* emPAI scores were equivalent between “wt” and “TM2” Erv14 variants, but spectral counts were lower for the TM2 co-IPs, suggesting a decreased recovery in the TM2 IPs.

Supplemental Experimental Procedures

Yeast Strains and Strain Construction.

S. cerevisiae strains used in this study are listed below with a complete list of primers used for stain construction. Unless otherwise stated, strains harboring a deletion in a specific ORF were taken from the yeast deletion library [S1]. Strains harboring an ORF endogenously tagged with GFP at the C-terminus were taken from the yeast GFP library [S2]. *ERV14* and/or *ERV15* were deleted in various strain backgrounds (SPY34, SPY36, SPY81, and SPY82) by transformation with a polymerase chain reaction (PCR) product composed of the *erv14Δ::NatMX* or *erv15Δ::KanMX* disruption cassettes, generated by PCR amplification from the pAG25 plasmid or pFA6a-KanMX6 respectively [S3]. All allelic replacements were confirmed by PCR. SPY80 (*Matα can1Δ::STE2pr-URA3*) was created as a query strain compatible with secretome techniques [S4] with the yeast GFP library, which is marked with the *HIS3* marker. The coding sequence of the *URA3* gene was introduced downstream of the *STE2* promoter by transformation in BY4741 [Mata] of the PCR product generated with the primers olm4779 and 4780. The *STE2pr-URA3* cassette was then re-amplified by PCR with the primers olm4779 and olm4780 and was integrated by transformation at the *CAN1* locus of the BY4742 strain [Matα]. Genomic DNA of transformants showing resistance to canavanine was tested for the correct insertion of *can1Δ::STE2pr-URA3* by sequencing the PCR product generated with the primers olm4783 and olm4796. For confirmation of Erv14 independence, GFP fusions, marked by *HIS5*, were integrated at the 3' end of various Erv14 non-clients by transformation of SPY37 (*erv14Δ::KanMX erv15::NatMX*) with a PCR product generated by amplification from a GFP(S65T)-*HIS5* plasmid. All GFP integrations were confirmed by genomic PCR. The split ubiquitin analysis was conducted in the THY AP4 strain background [S5]. The CYT (Cub-(YFP)LexA-VP16) tag, marked by the KanMX marker, was integrated at the 3' end of various Erv14 clients by transformation with a PCR product generated by amplification from the pCYT-L3 plasmid. All CYT insertions were confirmed by PCR of the genomic locus. For analysis of Sec24-dependence, GFP fusions, marked by

TRP1, were integrated at the 3' end of various Erv14 clients by transformation of YTB1 (*sec24Δ::LEU2* with wild-type *SEC24* borne on a *URA*-containing plasmid) with a PCR product generated by amplification from a GFP(S65T)-*TRP* plasmid. All GFP integrations were confirmed by genomic PCR and cell imaging. Strains bearing mutant forms of *SEC24* as the sole copy of this essential gene were created by transforming YTB1 or its GFP-containing client derivatives with either wild-type or mutant forms of *SEC24* on *HIS*-marked plasmids. Cells were cured of the wild-type *SEC24::URA* plasmid by growth on 5-fluoroorotic acid (0.1% final concentration), leaving the plasmid-borne copy as the sole copy of *SEC24*.

Strains used in this study

Strain	Genotype	Source
BY4741	*MAT***a** *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	Open Biosystems
BY4742	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	Open Biosystems
LMY829	MATα *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 can1Δ::STE2pr-spHIS5*	[S6]
LMY1017	*erv14Δ::KanMX* in BY4741	Open Biosystems
SPY34	*erv14Δ::NatMX* in LMY829	This study
SPY35	*erv15Δ::KanMX* in BY4741	Open Biosystems
SPY36	*erv14Δ::NatMX erv15Δ::KanMX* in LMY829	This Study
SPY37	*erv14Δ::KanMX erv15Δ::NatMX* in BY4741	This Study
SPY80	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 can1Δ::STE2pr-URA3*	This Study
SPY81	SPY80 *erv14Δ::NatMX*	This Study
SPY82	SPY80 *erv14Δ::NatMX erv15Δ::KanMX*	This Study
THY AP4	*MATa ura3 leu2 lexA*::*lacZ*::*trp1 lexA*::*HIS3 lexA*::*ADE2*	[S5]
YOR1-CYT	THY AP4 YOR1-CYT*::KanMX*	[S7]
SUR7-CYT	THY AP4 SUR7-CYT*::KanMX*	This Study
QDR2-CYT	THY AP4 QDR2-CYT*::KanMX*	This Study
LYP1-CYT	THY AP4 LYP1-CYT*::KanMX*	This Study
ITR1-CYT	THY AP4 ITR1-CYT*::KanMX*	This Study
HXT3-CYT	THY AP4 HXT3-CYT*::KanMX*	This Study
YTB1	*MATα can1-100 leu2-2,112 his3-11,15 trp1-1 ura3-1 ade2 sec24::LEU2* carrying pLM22 (*CEN SEC24-URA3*)	[S8]
SPY100	YTB1 MID2-sfGFP::*TRP1*	This Study
SPY101	YTB1 DPP1-sfGFP::*TRP1*	This Study
SPY102	YTB1 HIP1-sfGFP::*TRP1*	This Study
SPY103	YTB1 MEP2-sfGFP::*TRP1*	This Study
SPY104	YTB1 HXT6-sfGFP::*TRP1*	This Study
SPY105	YTB1 ITR1-sfGFP::*TRP1*	This Study
SPY106	YTB1 TPO4-sfGFP::*TRP1*	This Study

SPY107	YTB1 MNN5-sfGFP::*TRP1*	This Study
SPY108	YTB1 DRS2-sfGFP::*TRP1*	This Study
SPY109	YTB1 SUR7-sfGFP::*TRP1*	This Study
SPY110	YTB1 PDR5-sfGFP::*TRP1*	This Study
SPY111	YTB1 FTH1-sfGFP::*TRP1*	This Study
SPY112	SPY37 TAT1-sfGFP::*HIS5*	This Study
SPY113	SPY37 BAP2-sfGFP::*HIS5*	This Study
SPY114	SPY37 MEP1-sfGFP::*HIS5*	This Study
SPY115	SPY37 MEP3-sfGFP::*HIS5*	This Study
SPY116	SPY37 YR02-sfGFP::*HIS5*	This Study
SPY117	SPY37 SKI1-sfGFP::*HIS5*	This Study
SPY118	SPY37 Ygr026w-sfGFP::*HIS5*	This Study
RSY620	*MATα leu2-3,112 ura3-52 ade2-1 trp1-1 his3-11,15 pep4::TRP1*	Schekman lab

PCR Primers used in this study

Primer Name	Sequence	Purpose	Template
olm2257	CTTTGAACTGCAATTAAAGTAAAGTAAAAAAATTAAGAATAAAAAGA AAACAGCTGAAGCTTCGTACGC	*ERV14::NatMX* deletion	pAG25
olm2258	CTATTATTCTTGGCCCTTCAGTCTTCTTTGGATTTCAATGTCTTGTTGGA GCATAGGCCACTAGTGGATCTG		
olm4441	AGACATTAATACCTTTATTCATATAAGCACTTTCATTATCATTTTTTACT AAAGATAAAACTAACTGCAGCAGCTGAAGCTTCGTACGC	*ERV15::KanMX* deletion	pFA6a-KanMX6
olm4442	ACTTCCATACTAAGGAAGTTGTGTTATAAGGTCATTTTTATGTGGTAG ACATACTTCATAGATATGCACAGCATAGGCCACTAGTGGATCTG		
olm4196	CTATAGGGCGAATTGGAGCTCCACCGCGGTGGCGGCCGCTCTAGAAC TAGTGGAATTTTGAGATAATTGTTGG	Cloning (gap repair) of *ERV14* into pRS31X	Genome
olm4197	CTCGAGGTCGACGGTATCGATAAGCTTGATATCGAATTCCTGCAGCCC GGGTTTCATTTAGATTCAGATTTGG		
olm4445	TACTTAACAAAGACATTTACCTTTGAACTGCAATTAAAGTAAAGTAAA AAAATTAAGAATAAAAAGAAAAATGTCAGGAACCGGATTATCG	Replacing (gap repair) *ERV14* ORF with *ERV15* ORF	Genome
olm4446	GGGCGGCCATTAAGTATGTCTATTATTCTTGGCCCTTCAGTCTTCTTTG GATTTCAATGTCTTGTTGGATTAGCTTATCAAATTTGCATC		
olm4097	AATACGAAGGAGAGACCTGG	Checking *ERV14* locus	Genome
olm4098	TTATTCTTGGCCCTTCAGTC		
olm4447	GTAGACATACTTCATAGATATGC	Checking *ERV15* locus	Genome
olm4448	GACATTAATACCTTTATTCATATAAGC		
olm4047	CAGAATGATCATGGCTTTGATTGCTGAAAGTGGTGATGACTTCCGGA TCCCCGGGTTAATTAA	C-term tagging of *ERV14*	pFA6a-GFP or pFA6a-3xHA
olm4048	GGCCCTTCAGTCTTCTTTGGATTTCAATGTCTTGTTGGAGTTTAAACGA GCTCG AATTC		
oLM2539	AGATTACGCTGGATCCAAGCAGTGGTATCAACGCAGAGTGGCCATTA CGGATGGGTGCTTGGTTATTTATC	Cloning (gap repair) of *ERV14* into pDSL-Nx	Genome
oLM2540	CGACGGTATCGATAAGCTTGATATCGAATTCTCGAGAGGCCGAGGCG GCCTTAGAAGTCATCACCACTTT		
olm4779	CCTGCTCTGGCTATAATTATAATTGGTTACTTAAAAATGCACCGTTAA GAACCATATCCAAGAATCAAAAATGTCGAAAGCTACATATAAGG	Insertion of *URA3* ORF	pRS306

Primer Name	Sequence	Purpose	Template
olm4780	AGCACCTGAATCTAGTAGTAACCTTATACCGAAGGTCACGAAATTACT TTTTCAAAGCCGTAAATTTTGATTAGTTTTGCTGGCCGCATC	downstream of *STE2* promotor.	
olm4781	ATCCAATATCACCTGACCTTC	Checking insertion of *STE2pr-URA3*	Genome
olm4783	TTAGTTTTGCTGGCCGCATC		
olm4793	CGAAAGTTTATTTCAGAGTTCTTCAGACTTCTTAACTCCTGTAAAAACA AAAAAAAAAAAAGGCATAGCAATCCAATATCACCTGACCTTC	Insertion of *STE2pr-URA3* cassette at the *CAN1* locus	Genome
olm4795	GTGTATGACTTATGAGGGTGAGAATGCGAAATGGCGTGGAAATGTG ATCAAAGGTAATAAAACGTCATATTTAGTTTTGCTGGCCGCATC		
olm4796	CTTCAGACTTCTTAACTCCTG	Checking insertion of *STE2pr-URA3* at the *CAN1* locus	Genome
olm4783	TTAGTTTTGCTGGCCGCATC		
olm4948	CTCTTATTTATTCTCTTGAGAAAAGGTAAAGAATTAGCCTTTAAGAGG AAAAAGCAAGAACTGGGAGTAAATATGTCGGGGGGGATCCCTCC	CYT tagging *QDR2* C-terminus	pCYT-L3
olm4949	ATTACTTTCTATTTGAGAAGACTTATCACACCAATTCCTCTTTCTCGGT AGTGGAGCGATCAAAAGGAACATTTTCCTTACTATAGGGAGACCGGC AG		
olm4956	CAACCTAAAGGTATTAACTTCTTCACTATAAGAAAATCACACGAGCGC CCGGACGATGTCTCTGTTATGTCGGGGGGGATCCCTCC	CYT tagging *SUR7* C-terminus	pCYT-L3
olm4957	AAATATAGGATATTATTAATATTAGAATTAAACTATTATATTGCAGGG GAGAGAAGAAAGGGGTATAAACTATAGGGAGACCGGCAG		
olm5006	GGTGCTAACTACGATGCTGATGCATTGATGCATGATGACCAGCCATTC TACAAGAAAATGTTCGGCAAGAAAATGTCGGGGGGGATCCCTCC	CYT tagging *HXT3* C-terminus	pCYT-L3
olm5007	ATATAAAGTCATAAATCTTGTGTATTATTTATCATTATTGACTAGCACA TCGAATCTTAAAATACACTATTAACTATAGGGAGACCGGCAG		
olm5012	AAGAGAAAACAGCAAGTTGCCAGAGTTCATGAATTGAAATATGAACC AACTCAAGAGATTATAGAGGATATA ATGTCGGGGGGGATCCCTCC	CYT tagging *ITR1* C-terminus	pCYT-L3
olm5013	TACAATGAACTTTTTTCTATCTTGTAATTTATTTTTATTTATTTTCTACTA TGTATTTGAATATTCAATTGCGT ACTATAGGGAGACCGGCAG		
olm5014	GAAGAAATGAGGATTGAGCGAGAAACATTAGCAAAAAGATCCTTCGT AACAAGATTTTTACATTTCTGGTGTATGTCGGGGGGGATCCCTCC	CYT tagging *HIP1* C-terminus	pCYT-L3
olm5015	CAGTAATATACACAGATTCCCGCGGACGTGGGAAGGAAAAAATTAGA TAACAAAATCTGAGTGATATGGAAATTCCGACTATAGGGAGACCGGC AG		
olm4926	GATGCAATGTGTGAGTATTTGACCAATCATGTTAATTATCTGGAGAGC ACACACAAAGAAGCTATGGGGGAGAAAAACCGGATCCCCGGGTTAA TTAA	GFP tagging *MNN5* C-terminus	pFA6a-GFP
olm4927	TTTTCTAAGATGTATATATATCCGTGCACATATATGCTACATTATATTA TACAAGGTCGGATGGCGAGTTATTATTGAATTCGAGCTCGTTTAAAC		
olm4936	GATGCAATGTGTGAGTATTTGACCAATCATGTTAATTATCTGGAGAGC ACACACAAAGAACTATGGGGGAGAAAAACCGGATCCCCGGGTTAATT AA	GFP tagging *DRS2* C-terminus	pFA6a-GFP
olm4937	TATGGTAAATGTACATATTTCAGTAATGGTAATAATGACTTTTCTTTTT ATCTTATTTTTATTTTTGTATTTTGAATTCGAGCTCGTTTAAAC		
olm4938	CAACCTAAAGGTATTAACTTCTTCACTATAAGAAAATCACACGAGCGC CCGGACGATGTC TCTGTTCGGATCCCCGGGTTAATTAA	GFP tagging *SUR7* C-	pFA6a-GFP

Primer Name	Sequence	Purpose	Template
olm4939	AAATATAGGATATTATTAATATTAGAATTAAACTATTATATTGCAGGG GAGAGAAGAAAGGGGTATAAGAATTCGAGCTCGTTTAAAC	terminus	
olm4940	CACCCTGGAGGAAGCAGTCACCCATCAGAGGATCCCGGATGAGGAA TTACATCCTTTGTCCGATGAAGGTATGCGGATCCCCGGGTTAATTAA	GFP tagging *DPP1* C-terminus	pFA6a-GFP
olm4941	AGAAGTAACATGTCATCTTTATGTAATCGCTGTTATTCCATACAGAAC AATAAATACGTATATTGAATTCGAGCTCGTTTAAAC		
MID2-GFPf	TTGCGTCAAATGACATAATAGAAGAAAAATTCTATGATGAACAAGGT AACGAATTATCACCACGAAATTATCGGATCCCCGGGTTAATTAA	GFP tagging *MID2* C-terminus	pFA6a-GFP
MID2-GFPr	TCCACCTACTCAATATCAGAAATATAATTAAGATGGTCAATTTACAATA ATTGAGGAATGAAAAGTAGCCGAATTCGAGCTCGTTTAAAC		
ITR1-GFPf	AAGAGAAAACAGCAAGTTGCCAGAGTTCATGAATTGAAATATGAACC AACTCAAGAGATTATAGAGGATATA CGGATCCCCGGGTTAATTAA	GFP tagging *ITR1* C-terminus	pFA6a-GFP
ITR1-GFPr	TACAATGAACTTTTTTCTATCTTGTAATTTATTTTTATTTATTTTCTACTA TGTATTTGAATATTCAATTGCGTGAATTCGAGCTCGTTTAAAC		
HIP1-GFPf	GAAGAAATGAGGATTGAGCGAGAAACATTAGCAAAAAGATCCTTCGT AACAAGATTTTTACATTTCTGGTGT CGGATCCCCGGGTTAATTAA	GFP tagging *HIP1* C-terminus	pFA6a-GFP
HIP1-GFPr	CAGTAATATACACAGATTCCCGCGGACGTGGGAAGGAAAAAATTAGA TAACAAAATCTGAGTGATATGGAAATTCCGGAATTCGAGCTCGTTTAA AC		
FTH1-GFPf	TCAGAGCCAGACAGTCAAAGACGGTCCAAAGATAGTTCTGTGCCCCT AATAATTGATAGCAGTGGTTCAGCAAAT CGGATCCCCGGGTTAATTAA	GFP tagging *FTH1* C-terminus	pFA6a-GFP
FTH1-GFPr	GCTATTTTTAATGACGAATATATATGTACATAGTATTTACTGTAAAATG TATGAAAAGAAGCTTAAAATTATTAGAATTCGAGCTCGTTTAAAC		
PDR5-GFPf	ATTATATCGCTGGTGTCTTTTTCTACTGGTTAGCAAGAGTGCCTAAAA AGAACGGTAAACTCTCCAAGAAACGGATCCCCGGGTTAATTAA	GFP tagging *PDR5* C-terminus	pFA6a-GFP
PDR5-GFPr	TGAAATGTAGAAAGCTCGCTGAATTAAGAAAAAAAAAAATAAAAACTT ATTATTACGCACCTATATGTAGTGAATTCGAGCTCGTTTAAAC		
TPO4-GFPf	CAGCTATCAAGGATAATGAAGACGGTTATTCGTATACGGAAATGGCC ACCGATGCTTCCGCCAGAATGGTTCGGATCCCCGGGTTAATTAA	GFP tagging *TPO4* C-terminus	pFA6a-GFP
TPO4-GFPr	TTGTATGAAACTTCTATAAATTTAAATTGGTGAATTTTATCCAGCTATC CCCCAATTTACTATTTGAATATATGAATTCGAGCTCGTTTAAAC		
HXT6-GFPf	GAGGTGCCAACTACGACGCTGAAGAAATGGCTCACGATGATAAGCCA TTGTACAAGAGAATGTTCAGCACCAAACGGATCCCCGGGTTAATTAA	GFP tagging *HXT6* C-terminus	pFA6a-GFP
HXT6-GFPr	TAAATCGTAAGGGTTCATTAAAAATATAAAATCAGAATTAGAGTGCAT TTCAAATGCACAAATTAGAGCGGAATTCGAGCTCGTTTAAAC		
MEP2-GFPf	GAAAAGAATTCTACGCCTTCCGACGCTTCTTCTACTAAGAACACTGAC CATATAGTACGGATCCCCGGGTTAATTAA	GFP tagging *MEP2* C-terminus	pFA6a-GFP
MEP2-GFPr	TATCAAAAAAAGAAAATATATCAAATATTAAAAATAAAATTATTAAAT GAAGCGTTACATAAAGATTAAACATAAGAATTCGAGCTCGTTTAAAC		

Plasmid construction.

Plasmids used in this study are listed bellow. LMB769 and LMB770 were generated by in-vivo gap repair of a PCR-generated product that comprises the promoter, the ORF and the 3' UTR of *ERV14* into the gapped plasmid pRS315 or pRS316 respectively.

Transformants were first selected on –LEU or –URA media respectively. Plasmids were recovered and sequenced. LMB772 and LMB773 were created as follows: the 3HA-KanMX or GFP(S65T)-KanMX sequences were amplified from the corresponding pFA6a plasmids and co-transformed into yeast with the wild-type *ERV14* plasmid, LMB769. Transformants were first selected on media supplemented with G418, then expression of Erv14-GFP or Erv14-3HA was tested by Western blotting. Plasmids were recovered and sequenced to confirm the integration. A PCR-generated product corresponding to the ORF of *ERV14* was introduced by in-vivo gap repair into the linearized plasmid pDSL-Nx to create the *NUB-ERV14* plasmid (LMB718) used as bait for the split ubiquitin analysis. These plasmids were the basis for site-directed mutagenesis by using QuikChange mutagenesis (Stratagene) to obtain various HA-, GFP- or NUB-tagged Erv14 mutants. The plasmid pJW1512-*SEC24* [S9] was the basis for site-directed mutagenesis by using QuikChange mutagenesis (Stratagene) to create the various A-, B- and C-site mutants of *SEC24* under the control of the CUP1 promoter. LMB856 and LMB857 were generated by in-vivo gap repair of a PCR-generated product that comprises the promoter, ORF and 3' UTR of *MEP1* or *MEP3* respectively into the gapped plasmid pRS316. LMB858 and LMB858 were created by in-vivo integration of the GFP(S65T) cassette into the *MEP1* plasmid LMB856 or the *MEP3* plasmid LMB857, respectively. Plasmids were recovered and sequenced to confirm the integration.

Plasmids used in this study

Plasmid	Description	Source
LMB769	ERV14 in pRS315	This Study
LMB770	ERV14 in pRS316	This Study
LMB772	ERV14-3HA*::KanMX* in pRS315	This Study
LMB773	ERV14-sfGFP*::KanMX* in pRS315	This Study
LMB776	*ERV14pr*-ERV15 in pRS315	This Study
LMB718	ERV14 in pDSL-Nx	This Study
LMB856	MEP1 in pRS316	This Study
LMB857	MEP3 in pRS316	This Study
LMB858	MEP1-sfGFP*::TRP1* in pRS316	This Study
LMB859	MEP3-sfGFP*::HIS5* in pRS316	This Study
spQC1885	(I97A, F98A, R99A, T100A, L101A) mutation in LMB772	This Study
spQC2012	(F62A, L63A, N74A) mutation in LMB772	This Study
spQC2007	(D33A, Y34A, P50A, E51A) mutation in LMB772	This Study

spQC1888	(I97A, F98A, R99A, T100A, L101A) mutation in LMB773	This Study
spQC1908	(F62A, L63A, N74A) mutation in LMB773	This Study
spQC1913	(D33A, Y34A, P50A, E51A) mutation in LMB773	This Study
spQC1891	(D33A, Y34A) mutation in LMB773	This Study
spQC1892	(C41A) mutation in LMB773	This Study
spQC1893	(P50A, E51A) mutation in LMB773	This Study
spQC1894	(F62A, L63A) mutation in LMB773	This Study
spQC1895	(W68A) mutation in LMB773	This Study
spQC1896	(N74A) mutation in LMB773	This Study
spQC1897	(P76A) mutation in LMB773	This Study
spQC1898	(Y80A) mutation in LMB773	This Study
spQC1899	(E107A) mutation in LMB773	This Study
spQC1900	(K111A) mutation in LMB773	This Study
spQC1901	(F119A) mutation in LMB773	This Study
spQC1902	(F120A) mutation in LMB773	This Study
spQC1903	(Y124A) mutation in LMB773	This Study
spQC2182	(I97A, F98A, R99A, T100A, L101A) mutation in LMB718	This Study
spQC1982	(D33A, Y34A, P50A, E51A) mutation in LMB718	This Study
spQC2241	(L63A) mutation in LMB718	This Study
spQC2242	(F62A, L63A) mutation in LMB718	This Study
spQC2243	(F62A) mutation in LMB718	This Study
pLM22	4.2kb XhoI-SpeI fragment containing 6xHis-tagged *SEC24* in pRS316	[S8]
pLM23	4.2kb XhoI-SpeI fragment containing 6xHis-tagged *SEC24* in pRS313	[S8]
pLM134	SEC24-B1: (R230A, R235A) mutation in pLM23	[S8]
pLM251	SEC24-A: (W897A) mutation in pLM23	[S8]
pLM174	SEC24-C: (R342A) mutation in pLM23	[S8])
pTKY9	SEC23 in p426GAL1	[S10]
pLM129	SEC24 in p425GAL1	[S8]
pLM125	SEC24-B1: (R230A, R235A) mutation in pLM129	[S8]
pLM253	SEC24-A: (W897A) mutation in pLM129	[S8]
pLM208	SEC24-C: (R342A) mutation in pLM129	[S8]
pJW1512	*CUP1pr CEN LEU3*	Rothstein Lab
pJW1512-SEC24	*CUP1pr-SEC24* in pJW1512	[S8]
pJW1512-sec24-B	SEC24-B1: (R230A, R235A) mutation in pJW1512-SEC24	This study
pJW1512-sec24-A	SEC24-A: (W897A) mutation in pJW1512-SEC24	This study
pJW1512-sec24-C	SEC24-C: (R342A) mutation in pJW1512-SEC24	This study
spQC2553	(S491D, F576A, R578A) mutation in pLM129	This study
spQC2554	(S491E, F576A, R578A) mutation in pLM129	This study
spQC2555	(S491A, F576A, R578A) mutation in pLM129	This study
spQC2508	(S491A) mutation in pLM23	This study
spQC2509	(S491D) mutation in pLM23	This study
spQC2510	(S491E) mutation in pLM23	This study
spQC2512	(F576A, R578A) mutation in pLM23	This study
spQC2518	(S491A) mutation in pLM134	This study
spQC2519	(S491D) mutation in pLM134	This study
spQC2520	(S491E) mutation in pLM134	This study
spQC2522	(F576A, R578A) mutation in pLM134	This study
pEAE83	YOR1-HA in pRS316	[S11]
pLM308	D71E73A (Yor1D71A,E73A) mutation in pEAE83	[S10]
pEAE93	YOR1-GFP in pRS316	[S11]

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