SUPPLEMENTAL PROCEDURES

Strains, Plasmids, and Microbiological Techniques

Yeast strains are listed in Table S1. Yeast and bacterial strains were manipulated by standard methods [1, 2]. All yeast strains were grown in standard YEPD media for 16 h unless otherwise indicated. Cells were pelleted by centrifugation and supernatants were used for immunoblot analysis and to examine properties of shed Flo11p. All mat/biofilm assays were performed on YEP with glucose or galactose containing 0.3% agar unless specified [3]. Epitope (hemaglutinin [HA] and MYC) fusions were constructed as indicated [4]. Gene disruptions and *GAL1* promoter fusions were made by PCR-based methods [5, 6], including cassettes containing antibiotic resistant markers [7]. Integrations were confirmed by PCR analysis and phenotype. Flo11p-HA$^{GPI\Delta}$ was constructed by making a C-terminal deletion of Flo11p from 1340 to 1367 aa residues by homologous recombination. Flo11p-HA and Flo11p-MYC fusion proteins showed equivalent shedding. Flo11p-HA and Myc^{30}-Flo11p-HA showed wild type mat expansion and invasive growth, whereas Myc$^{\Delta 29\text{-}95}$-Flo11p-HA showed a defect in mat expansion and agar invasion equivalent to a *flo11* mutant.

The plate-washing assay was performed as published [8]. Plasmids overexpressing *YPS1* (pDK63) and *YPS2* (pDK62) have been described [9], and were provided by Dr. Damian Krysan (University of Rochester Medical Center). NCBI blast and sequence comparison algorithms showed that Flo11p did not contain an autocatalytic SEA domain, characteristic of a subset of transmembrane mucins [10, 11]. *Saccharomyces Genome Database* was used to compile information about gene process

and function [12, 13]. Assays to evaluate mat form growth were based on established procedures [3]. Fluid volumes were determined by tilting agar plates at a 45˚ angle and collecting fluid that was released. All mat expansion assays were performed in duplicate unless otherwise noted, and error bars represent the standard deviation between experiments.

Secretion Profile Analysis

An ordered collection of ~5,400 ORFs under the control of the *GAL1* inducible promoter was used [14] (Open Biosystems). Plasmid DNA was prepared from *Escherichia coli* in 96-well format. Colony transfer was performed using a 96- fixed pin pinning tool (V & P Scientific, 23 VP 408) and plate replication tool (V & P Scientific, VP 381). Sterilization was performed by sequential washes in 5% bleach, distilled water, 70% ethanol, and 95% ethanol. Plasmids were transformed into strains containing Flo11p-HA (PC2043) fusions by a high-throughput microtiter plate transformation protocol [15]. Transformants were pinned onto SD-URA and screened by pinning to S-GAL-URA on nitrocellulose membranes (Millipore, Billicera, CA) to induce plasmid-dependent overexpression of ORFs. Colonies were incubated for 48 h, at which point filters were washed in a stream of water to remove cells and probed by immunoblot analysis with antibodies that recognized the HA epitope (12CA5.16.4). Microscopic examination confirmed that cells were separated from filters. Cross-contamination was estimated at 0.8% based on growth in blank positions, and ~93% of the collection was examined. Aliquots of the collection were stored at -80˚C. Comparative profiling was performed in strains containing Msb2p-

HA (PC999) and Hkr1p-HA (PC2740). Overexpression plasmids in strains containing Flo11p-HA are frozen in aliquots in -80˚C and are available upon request.

Immunoblots and Protein Analysis

Immunoblots were performed as described [16]. Immunofluorescence was performed as described [17] using monoclonal antibodies against the HA epitope and Cy3-conjugated goat anti-mouse secondary antibodies (Jackson Immunoresearch). For immunoblots of epitope tagged proteins, proteins were separated by SDS-PAGE on 10% precast gels (Bio-Rad, Hercules CA) and transferred to nitrocellulose membranes (protran BA85, VWR International Inc. Bridgeport NJ). Membranes were incubated in 10ml of blocking buffer (5% nonfat dry milk, 10mM Tris-HCl pH8, 150mM NaCl and 0.05% Tween 20) for 1 h at 25˚C or 16h at 4˚C. Preblocked membranes were incubated in blocking buffer containing primary antibodies for 1h at 25˚C. Blots were washed 3 times for 5 min each in TBST (10mM Tris-HCl pH8, 150mM NaCl and 0.05% Tween 20). Blots were incubated in horseradish peroxidase (HRP) conjugated secondary antibodies for 1 h at room temperature, and washed as above. ECL Plus Immunoblotting reagent (GE Lifesciences) was used to detect proteins.

Microscopy

Differential-interference-contrast (DIC) and fluorescence microscopy using rhodamine filter sets were performed using an Axioplan 2 fluorescent microscope (Zeiss) with 5X, 10X, 20X, and 40X objectives, and a PLAN-APOCHROMAT 100X/1.4 (oil) objective (N.A. 0.17). Digital images were obtained with the Axiocam MRm camera (Zeiss).

Axiovision 4.4 software (Zeiss) was used for image acquisition. Intensity maps of the secretion profiles of Msb2p-HA and Flo11p-HA were generated using the ImageJ Plugin Interactive 3D Surface Plot (http://rsbweb.nih.gov/ij/plugins/surface-plot-3d.html).

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Flo11p-HA is uniformly released from the cell surface by washing. A) Wild-type cells expressing Flo11p-HA (PC2043) or control cells (PC538, No Tag) were grown for 16 h in YEP-GAL medium and transferred to YEP-GAL medium (Not Fixed) or YEP-GAL medium containing 3.9% formaldehyde (Fixed) for 1 h at 30˚C to irreversibly fix Flo11p-HA to the surface. Cells were harvested by centrifugation and washed in buffer containing 50 mM TRIS pH 7 for 3 h at 4˚C. Washed cells were incubated in Cy3-conjugated anti-HA antibodies for 1 h, washed 3 times in PBS and visualized by fluorescence microscopy using a 40X objective. Exposure times for each sample were 1 sec. DIC images of the cells in focus are shown in the upper panels. Bar, 50 microns. B) Quantitation of fluorescence intensity. Fluorescence intensity was determined using ImageJ. After background subtraction, total intensity values were divided by the number of cells. Two separate populations of cells were analyzed and the average value is shown. Error bars represent the standard deviation between samples.

Figure S2. Purification of shed Flo11p. A) Elution profile of precipitated mucins run on a sepharose CL-4B column. Peak "a" (fractions 4 and 5) corresponds to the elution of Flo11p in the void volume. Peaks b and c show the elution of smaller secreted proteins. B) Immunoblot showing supernatant (Sup Input) collected from cells overexpressing Flo11p that were grown for 48 h in S-GAL+AA medium. Flo11p-HA was precipitated from supernatants by addition of 8% polyethylene glycol (8% PEG) followed by centrifugation. Residual proteins that were not precipitated are also shown (Sup Final). C)

Silver stained blot on samples shown in Fig. S2B showing enrichment of Flo11p-HA following PEG precipitation. 'Pure' refers to the purified Flo11p-HA after elution and concentration.

Figure S3. Flo11p is shed from mats in complex patterns. A) Wild-type cells expressing Flo11p-HA (PC2043) were spotted onto YEPD media (0.3% agar) overlaid with a nitrocellulose filter and grown for 23 d. Bar, 1 cm. B) The mat of cells was washed off the nitrocellulose, which was probed by immunoblot using anti-HA antibodies. Bar, 1 cm. C) A region within the mat was selected (red square) which showed a complex zebra-striped pattern. D) Intensity map of Flo11p-HA shedding from the mat shown in panel C, using the ImageJ plugin Interactive 3D Surface Plot. All parameters were set to default values except smoothing, which was set to 1. At right, graph of intensity values (blue line) as compared to a control (grey line). Pixel Inspector was used to determine the intensity value (Y-axis) for each pixel in the selection (red line at left) over a given distance (100 pixels, 1 mm, X-axis). For the control intensity measurements, aliquots of purified Flo11p-HA were spotted onto nitrocellulose filters and analyzed by immunoblot analysis. Immunoblots were quantitated as above for variations in intensity for background fluctuations in image intensity. The patterns are likely the result of mucin expression, shedding, and stability differences and may reflect fine-tuning of adherence and lubrication functions in colonial microenvironments. *FLO11* expression is regulated by nutrient and pH levels [18, 19] both of which vary considerably in different parts of the biofilm [20]. E) A similar profile for Msb2p-HA secretion.

Figure S4. Analysis of candidate Flo11p proteases and phospholipases. A) Immunoblot analysis of S and P fractions for the indicated mutants. Cells were grown to mid-log phase in YEPD and harvested by centrifugation. Supernatant (S) and cell pellet (P) fractions were analyzed by SDS-PAGE followed by immunoblot analysis. WT, wild type (PC2043). B) Plate-washing assay of protease and phospholipase mutants. Equal concentrations of cells were spotted onto YEPD medium for 4 d at 30˚C. Plates were photographed, rinsed in a stream of water and photographed again. For the lower panel, overexpression of *YPS1* or *YPS2* partially suppresses the invasive growth defect of the *kex2*Δ mutant, in line with a previous report [21], which is consistent with the idea that the protease activity of Kex2p is required for Flo11p function. The *kex2*Δ mutant harboring control and *YPS* overexpression plasmids were grown on YEP-GAL for 36h. C) Mats of the indicated genoypes were spotted onto YEPD + 0.3% agar for 4 d at 30˚C. Bar, 1 cm.

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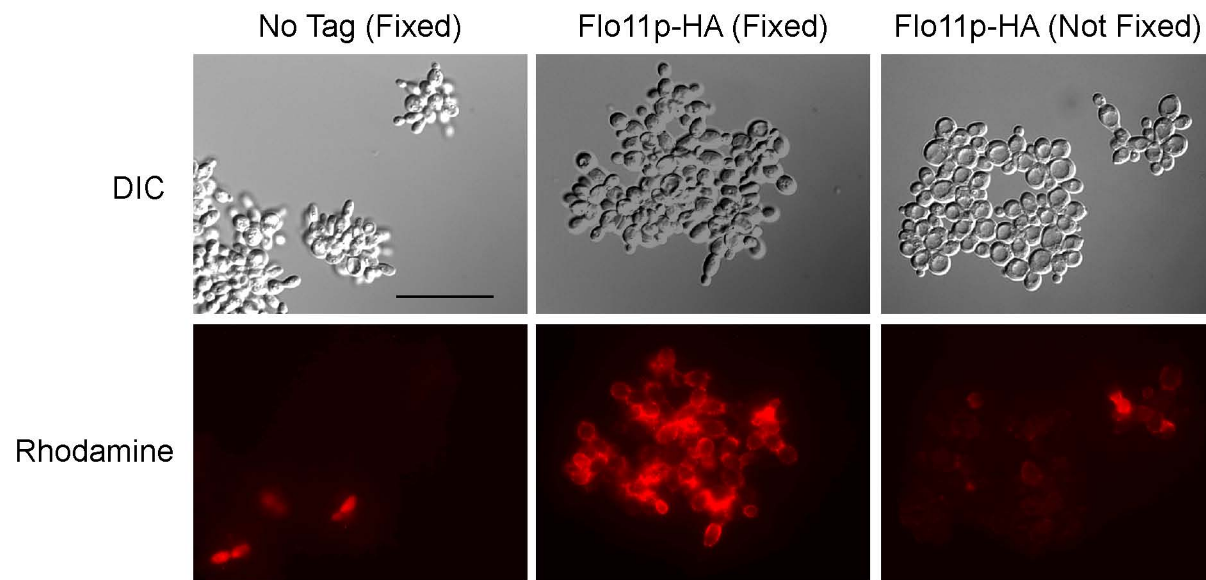

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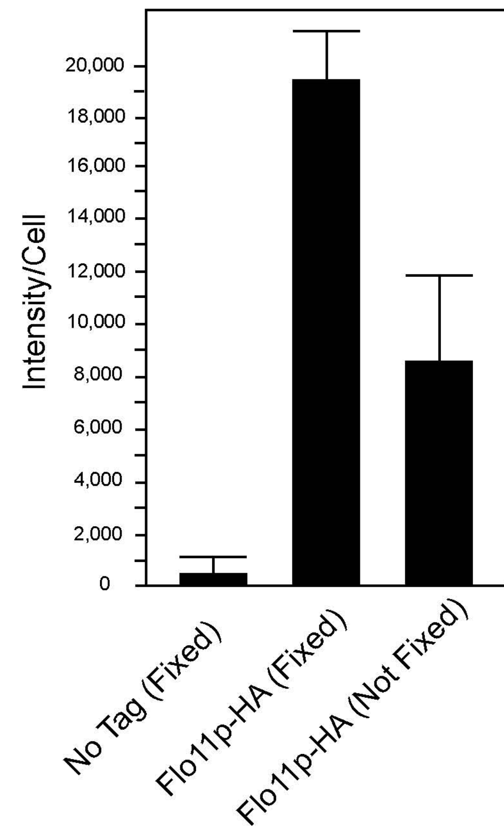
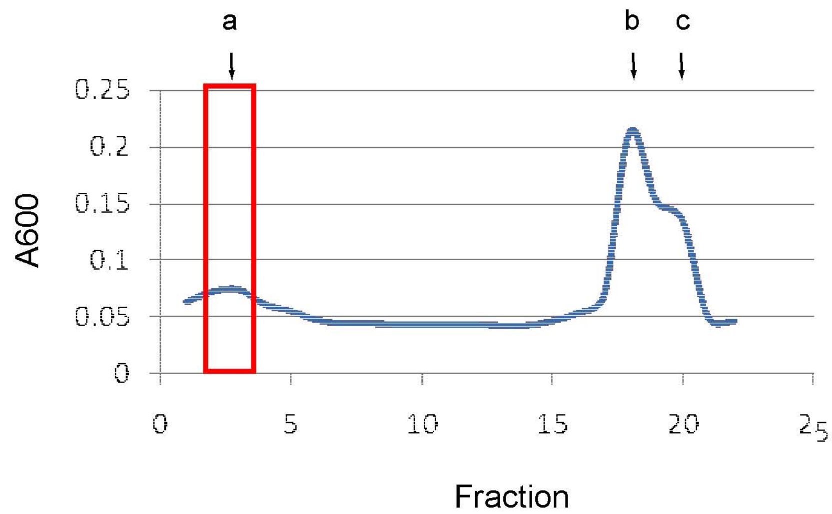
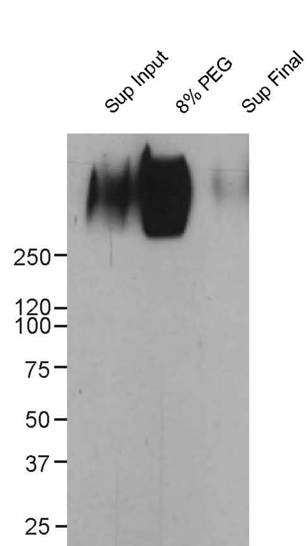


Figure S2





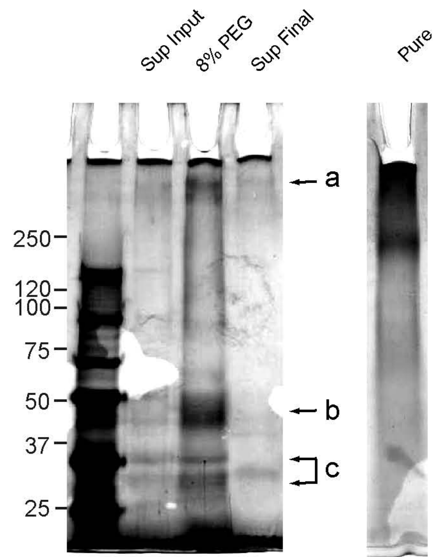


Figure S3

A

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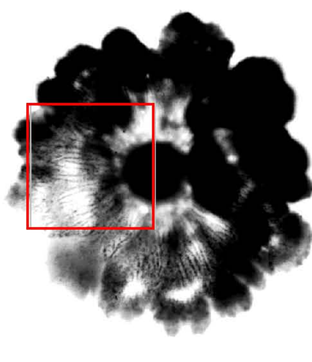

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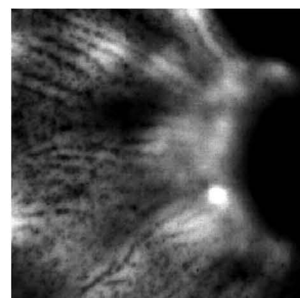

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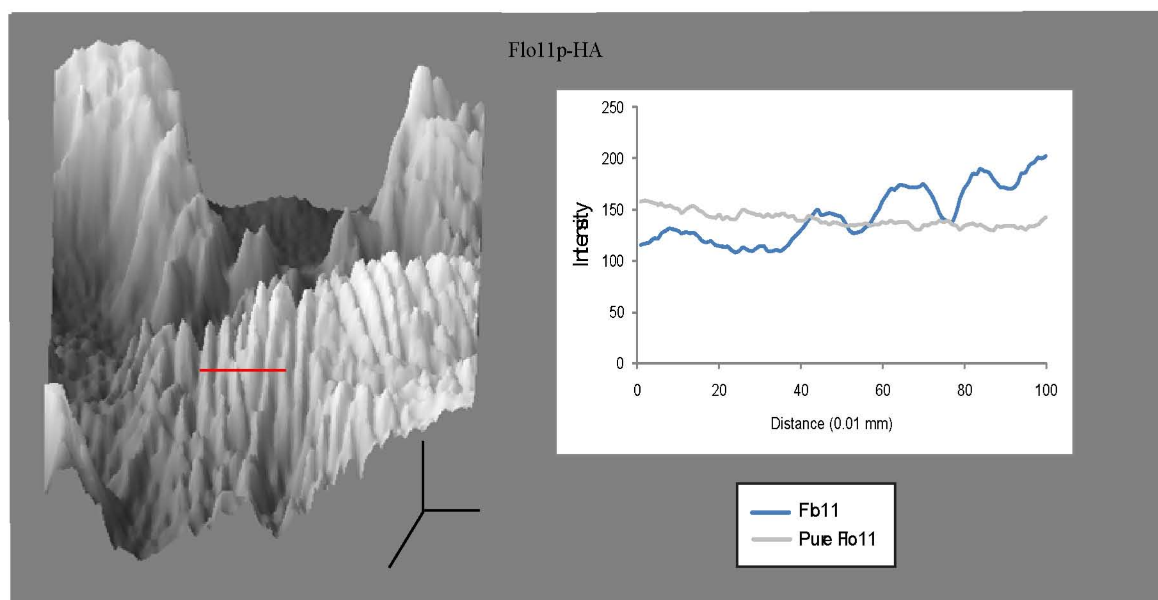

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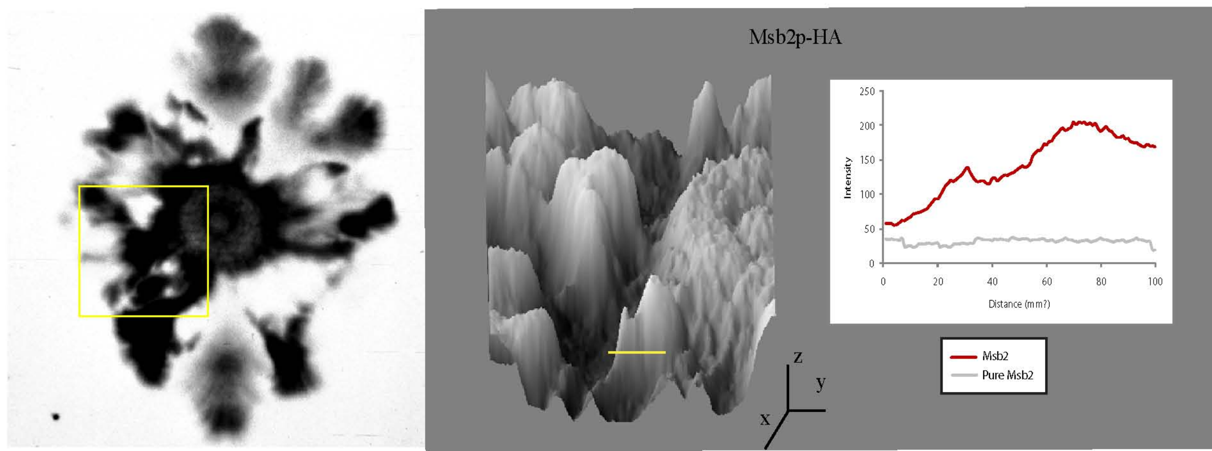
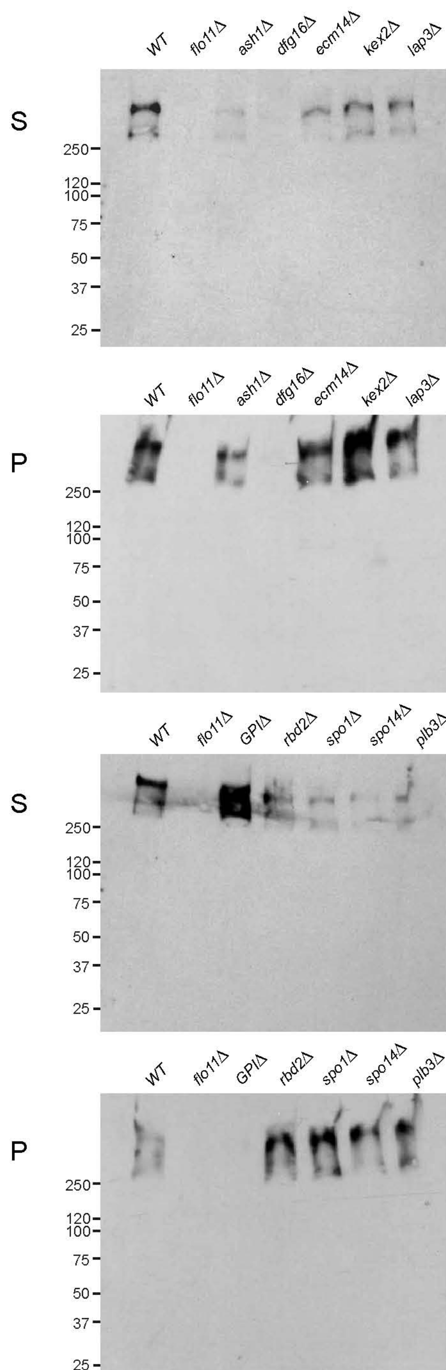
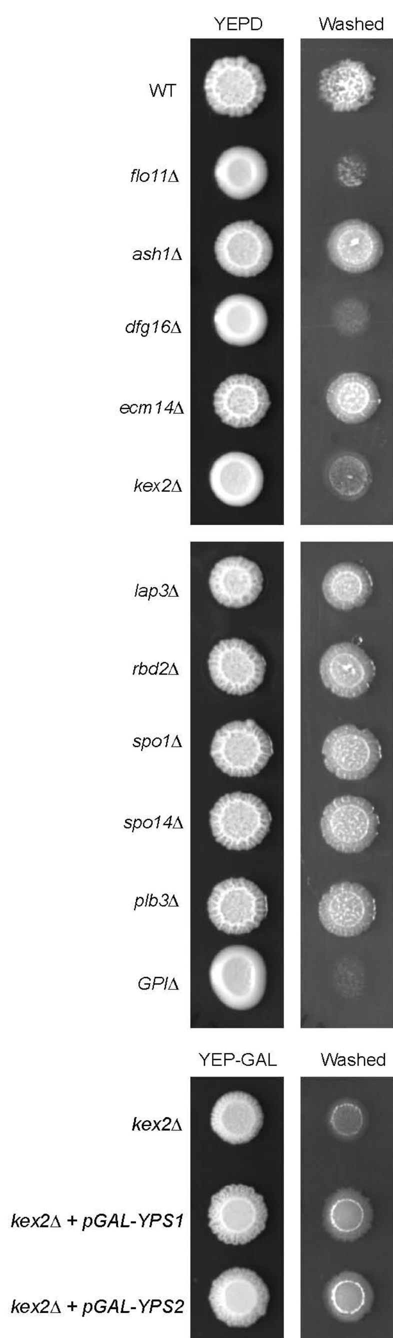


Figure S4





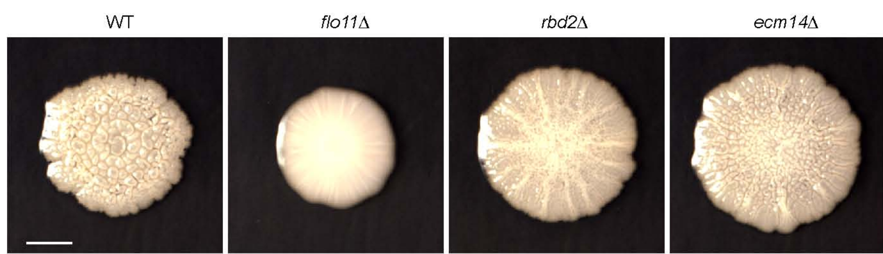


Table S1. Yeast Strains.

Strain	Genotype	Source
10560-2B	*MAT***a** ura3-52 *his3:hisG leu2:hisG*	[1]
PC313	*MATa ura3-52*	[2]
PC538[a]	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52*	[3]
PC999	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 MSB2-HA*	[3]
PC1519	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 pgu1::KanMX6*	[4]
PC1702	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 SHO1-HA::KanMx6*	[5]
PC2740	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 HKR1-HA*	[4]
PC1083	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 GAL-MSB2-HA*	[3]
PC2382	*MAT***a** *ura3-52 ste12::KanMX6*	This study
PC1029	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 flo11::KanMX6*	This study
PC2043	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 FLO11-HA*	This study
PC2090	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 FLO11-MYC*	This study
PC2712	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 GAL-FLO11::KanMX6*	This study
PC2713	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 GAL-FLO11-HA::KanMX6*	This study
PC2714	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 GAL-FLO11-HA::KanMX6 msb2::URA3*	This study
PC2716	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 GAL-MSB2-HA flo11::URA3*	This study
PC3415	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 FLO11-HA dfg16:URA3*	This study
PC3416	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 FLO11-HA rbd2:URA3*	This study
PC3417	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 FLO11-HA spo1:URA3*	This study
PC3418	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 FLO11-HA lap3:URA3*	This study
PC3419	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 FLO11-HA ash1:URA3*	This study
PC3420	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 FLO11-HA kex2:URA3*	This study
PC3421	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 FLO11-HA plb3:URA3*	This study
PC3422	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 FLO11-HA GPIΔ::KlURA3*	This study
PC3425	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 FLO11-HA FLO11-MYC@30 aa*	This study
PC3426	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 FLO11-HA FLO11-MYCΔ29-95*	This study
PC3513	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 FLO11-HA GPIΔ::ura3*$^-$ with p*GAL-SPO1*	This study
PC3515	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 FLO11-HA GPIΔ::ura3*$^-$ pGAL-YPS1	This study
PC3516	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 FLO11-HA GPIΔ::ura3*$^-$ pGAL-YPS2	This study
PC3700	*MAT***a** *ste4 FUS1-lacZ FUS1-HIS3 ura3-52 FLO11-HA FLO11-MYC@30 aa kex2:URA3*	This study

a. All strains are in the Σ1278b background unless otherwise indicated.

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Table S2. Comparative secretion profiling of yeast mucins.

P1	P2	P3	ORF	Gene	Flo11p secretion	[a]Secretion Level	[b]Msb2p Secretion	[c]Hkr1p secretion	Process
36	D	6	*YKL185W*	*ASH1*	Hypersecretor	++	Not Tested	Not Tested	Cell cycle
25	B	9	*YFL029C*	*CAK1*	Hypersecretor	‘++	W	W	Cell cycle
31	D	7	*YMR168C*	*CEP3*	Hypersecretor	+++	W	W	Cell cycle
25	H	3	*YBR135W*	*CKS1*	Hypersecretor	+	W	W	Cell cycle
38	D	6	*YGL197W*	*MDS3*	Hypersecretor	+	W	W	Cell cycle
42	A	7	*YKR077W*	*MSA2*	Hypersecretor	+++	W	W	Cell cycle
27	E	1	*YOR368W*	*RAD17*	Hypersecretor	+	W	W	Cell cycle
24	E	9	*YDL047W*	*SIT4*	Hypersecretor	+	W	W	Cell cycle
27	H	3	*YNL012W*	*SPO1*	Hypersecretor	+	W	W	Cell cycle
38	B	3	*YCL024W*	*KCC4*	Hypersecretor	+	Not Tested	Not Tested	Cell Polarity
50	H	5	*YNR035C*	*ARC35*	Hypersecretor	++	W	W	Cell Polarity
42	H	1	*YHR143W*	*DSE2*	Hypersecretor	+	W	W	Cell Wall
64	H	2	*YLR037C*	*DAN2*	Hypersecretor	+	W	W	Cell Wall
21	H	3	*YNL190W*	*YNL190W*	Hypersecretor	‘++	W	W	Cell Wall
33	E	12	*YNL059C*	*ARP5*	Hypersecretor	‘++	W	W	Chromatin Remodeling
43	E	2	*YNL031C*	*HHT2*	Hypersecretor	++	W	W	Chromatin Remodeling
66	F	6	*YEL044W*	*IES6*	Hypersecretor	+	W	W	Chromatin Remodeling
66	D	2	*YLR055C*	*SPT8*	Hypersecretor	+	W	W	Chromatin Remodeling
3	E	12	*YBR110W*	*ALG1*	Hypersecretor	+	W	W	Glycosylation
43	G	11	*YBR015C*	*MNN2*	Hypersecretor	++	W	W	Glycosylation
5	D	7	*YMR281W*	*GPI12*	Hypersecretor	+	W	W	GPI anchor biosynthesis
3	A	11	*YPL076W*	*GPI2*	Hypersecretor	++	W	W	GPI anchor biosynthesis
27	H	10	*YOR336W*	*KRE5*	Hypersecretor	++	Not Tested	W	Metabolism
46	D	2	*YGL055W*	*OLE1*	Undersecretor	-	W	W	Metabolism
27	G	3	*YJR134C*	*SGM1*	Hypersecretor	+	Undersecretor	W	Metabolism
21	H	11	*YLR027C*	*AAT2*	Hypersecretor	+	W	W	Metabolism
19	G	5	*YEL027W*	*CUP5*	Hypersecretor	+	W	W	Metabolism
42	F	1	*YHR100C*	*GEP4*	Hypersecretor	+	W	W	Metabolism
51	G	10	*YLR258W*	*GSY2*	Hypersecretor	+	W	W	Metabolism
24	F	8	*YBR159W*	*IFA38*	Hypersecretor	‘++	W	W	Metabolism
43	G	12	*YPR159W*	*KRE6*	Hypersecretor	+	W	W	Metabolism
25	E	10	*YJR073C*	*OPI3*	Hypersecretor	+	W	W	Metabolism
34	H	4	*YHR150W*	*PEX28*	Hypersecretor	+	W	W	Metabolism
52	G	1	*YMR123W*	*PKR1*	Hypersecretor	++	W	W	Metabolism
66	H	7	*YBR117C*	*TKL2*	Hypersecretor	+	W	W	Metabolism
47	D	6	*YBR127C*	*VMA2*	Hypersecretor	+	W	W	Metabolism
70	H	11	*YGR287C*	*YGR287C*	Hypersecretor	+	W	W	Metabolism
20	D	2	*YPL215W*	*CBP3*	Hypersecretor	+	W	W	Mitochondria
50	H	8	*YGR062C*	*COX18*	Hypersecretor	+	W	W	Mitochondria
56	D	2	*YNL185C*	*MRPL19*	Hypersecretor	+	W	W	Mitochondria
21	D	2	*YKR085C*	*MRPL20*	Hypersecretor	+++	W	Not Tested	Mitochondria
42	E	1	*YHR076W*	*PTC7*	Hypersecretor	+	W	W	Mitochondria
36	A	1	*YBR046C*	*ZTA1*	Undersecretor	-	W	Hypersecretor	Mitochondria
4	C	11	*YPR020W*	*ATP20*	Hypersecretor	+	W	W	Mitochondria
47	E	4	*YDR330W*	*UBX5*	Hypersecretor	+	W	W	Protein Degradation
43	D	2	*YPL186C*	*UIP4*	Hypersecretor	+	W	W	Protein Degradation
51	F	11	*YOL098C*	*YOL098C*	Hypersecretor	+	W	W	Protein Processing
59	F	2	*YNL238W*	*KEX2*[d]	Hypersecretor[e]	+	W	W	Protein Processing
58	C	11	*YPL246C*	*RBD2*[d]	Hypersecretor[e]	+	W	W	Protein Processing
50	A	11	*YHR132C*	*ECM14*[d]	Hypersecretor[e]	+	W	W	Protein Processing
			YNL012C	*SPO1*[d]	Hypersecretor[e]	+	W	W	Protein Processing
47	F	11	*YER017C*	*AFG3*	Hypersecretor	++	W	W	Protein Processing
30	F	3	*YLR163C*	*MAS1*	Hypersecretor	+	W	W	Protein Processing
44	H	3	*YOR197W*	*MCA1*	Hypersecretor	++	W	W	Protein Processing
14	A	12	*YJR062C*	*NTA1*	Hypersecretor	++	W	W	Protein Processing
37	G	7	*YCL057W*	*PRD1*	Hypersecretor	++	W	W	Protein Processing
22	A	12	*YMR274C*	*RCE1*	Hypersecretor	+	W	W	Protein Processing
32	B	5	*YJR117W*	*STE24*	Hypersecretor	++	W	W	Protein Processing
47	D	4	*YHR113W*	*YHR113W*	Hypersecretor	++	W	W	Protein Processing

49	F	1	*YIL108W*	*YIL108W*	Hypersecretor	+	W	W	Protein Processing
51	G	7	*YNL239W*	*LAP3*	Hypersecretor	+	W	W	Protein Processing - Peptidas
50	E	5	*YBR286W*	*APE3*	Hypersecretor	+++	W	W	Protein Processing - Peptidas
24	B	12	*YDR136C*	*VPS61*	Hypersecretor	+	W	W	Protein Sorting
5	D	12	*YOR016C*	*ERP4*	Hypersecretor	+	W	W	Protein Sorting
41	E	12	*YGR223C*	*HSV2*	Hypersecretor	+	W	Undersecretor	Protein Sorting
40	D	1	*YFL005W*	*SEC4*	Hypersecretor	+	W	W	Protein Sorting
34	H	7	*YJL192C*	*SOP4*	Hypersecretor	++	W	W	Protein Sorting
27	G	11	*YPL210C*	*SRP72*	Hypersecretor	+	W	W	Protein Sorting
34	H	5	*YIL039W*	*TED1*	Hypersecretor	++	W	W	Protein Sorting
24	B	5	*YKR001C*	*VPS1*	Hypersecretor	+	W	W	Protein Sorting
15	F	1	*YOL129W*	*VPS68*	Hypersecretor	+	W	W	Protein Sorting
50	G	9	*YDR304C*	*CPR5*	Hypersecretor	+++	W	W	Protein Transport
23	H	5	*YHR034C*	*NOP17*	Hypersecretor	+	Hypersecretor	W	RNA Modification
16	B	6	*YGL243W*	*TAD1*	Hypersecretor	+	W	W	RNA Modification
25	D	10	*YCR063W*	*BUD31*	Hypersecretor	+	Hypersecretor	W	RNA Modification
26	D	8	*YLR270W*	*DCS1*	Hypersecretor	+	W	W	RNA Modification
72	B	9	*YNL085W*	*MKT1*	Hypersecretor	++	W	W	RNA Modification
51	F	3	*YKR056W*	*TRM2*	Hypersecretor	+	W	Hypersecretor	RNA Modification
50	B	10	*YNL112W*	*DBP2*	Undersecretor	-	W	W	RNA Modification
38	A	5	*YJR022W*	*LSM8*	Undersecretor	-	W	Hypersecretor	RNA Modification
36	C	12	*YFR014C*	*CMK1*	Hypersecretor	+	W	W	Signal Transduction
64	F	2	*YFL053W*	*DAK2*	Hypersecretor	++	W	Not Tested	Signal Transduction
27	F	2	*YOR030W*	*DFG16*	Hypersecretor	+	W	W	Signal Transduction
26	B	4	*YBL016W*	*FUS3*	Hypersecretor	+	W	W	Signal Transduction
47	A	5	*YLL019C*	*KNS1*	Hypersecretor	+	W	W	Signal Transduction
52	H	1	*YNL279W*	*PRM1*	Hypersecretor	+	W	W	Signal Transduction
3	B	10	*YDL235C*	*YPD1*	Hypersecretor	'+++	W	W	Signal Transduction
16	A	7	*YPR056W*	*TFB4*	Hypersecretor	+	W	W	Transcription
16	C	12	*YHL009C*	*YAP3*	Hypersecretor	++	W	W	Transcription
32	B	4	*YOL148C*	*ADA5*	Undersecretor	-	W	Hypersecretor	Transcription
30	A	8	*YKL062W*	*MSN4*	Hypersecretor	+	W	W	Transcription
27	G	10	*YKL015W*	*PUT3*	Hypersecretor	+	Undersecretor	W	Transcription
51	H	2	*YPL042C*	*SSN3*	Hypersecretor	+	W	W	Transcription
64	F	12	*YDL048C*	*STP4*	Hypersecretor	++	W	W	Transcription
33	H	12	*YGL099W*	*KRE35*	Hypersecretor	++	Not Tested	W	Translation/Ribosome
14	H	6	*YKL021C*	*MAK11*	Undersecretor	-	W	W	Translation/Ribosome
29	H	11	*YJL010C*	*NOP9*	Hypersecretor	‘++	W	W	Translation/Ribosome
53	E	10	*YNL002C*	*RLP7*	Hypersecretor	+	W	Hypersecretor	Translation/Ribosome
29	G	8	*YNL236W*	*SIN4*	Hypersecretor	+++	W	W	Translation/Ribosome
4	H	4	*YKL014C*	*URB1*	Hypersecretor	+	W	W	Translation/Ribosome
4	F	10	*YPL195W*	*APL5*	Hypersecretor	+	W	W	Transport
50	H	4	*YCR010C*	*ATO1*	Hypersecretor	+	W	W	Transport
40	H	2	*YEL065W*	*SIT1*	Hypersecretor	+	W	Undersecretor	Transport
45	G	2	*YLR092W*	*SUL2*	Hypersecretor	+	W	W	Transport
16	B	12	*YAL064W*	*YAL064W*	Hypersecretor	+	W	W	Unknown
34	E	1	*YGR042W*	*YGR042W*	Hypersecretor	+	W	W	Unknown
26	D	6	*YLR063W*	*YLR063W*	Hypersecretor	+	W	W	Unknown
42	B	12	*YLR232W*	*YLR232W*	Undersecretor	-	Not Tested	Not Tested	Unknown
35	D	9	*YGR043C*	*NQM1*	Hypersecretor	+	W	W	Unknown
66	F	5	*YCR045C*	*RRT12*	Hypersecretor	+	W	W	Unknown
66	H	5	*YDL012C*	*YDL012C*	Hypersecretor	+	W	W	Unknown
24	H	10	*YDL180W*	*YDL180W*	Hypersecretor	+	W	W	Unknown
21	F	11	*YGR137W*	*YGR137W*	Hypersecretor	+	W	W	Unknown
3	H	7	*YGR273C*	*YGR273C*	Hypersecretor	+	W	W	Unknown
9	E	1	*YMR152W*	*YIM1*	Hypersecretor	+	W	W	Unknown
23	C	6	*YJR085C*	*YJR085C*	Hypersecretor	++	W	W	Unknown
42	G	5	*YKL171W*	*YKL171W*	Hypersecretor	+	W	Undersecretor	Unknown
42	G	7	*YLR050C*	*YLR050C*	Hypersecretor	++	W	W	Unknown
26	C	7	*YLR149C*	*YLR149C*	Hypersecretor	++	Not Tested	W	Unknown
26	E	8	*YLR302C*	*YLR302C*	Hypersecretor	+	W	W	Unknown

59	E	7	*YML083C*	*YML083C*	Hypersecretor	+	W	W	Unknown
58	C	7	*YMR148W*	*YMR148W*	Hypersecretor	+	W	W	Unknown
4	G	6	*YMR185W*	*YMR185W*	Hypersecretor	+	W	W	Unknown
25	H	8	*YNR014W*	*YNR014W*	Hypersecretor	+	W	W	Unknown
9	F	6	*YOL029C*	*YOL029C*	Hypersecretor	+	W	W	Unknown
33	E	9	*YOR214C*	*YOR214C*	Hypersecretor	++	W	W	Unknown
58	B	10	*YOR376W*	*YOR376W*	Hypersecretor	+	W	Not Tested	Unknown
41	H	3	*YPR114W*	*YPR114W*	Hypersecretor	+++	W	Undersecretor	Unknown
34	C	9	*YKL111C*	*YKL111C*	Undersecretor	-	W	Hypersecretor	Unknown
34	B	12	*YLR282C*	*YLR282C*	Undersecretor	-	W	Hypersecretor	Unknown
38	E	4	*YOL085C*	*YOL085C*	Undersecretor	-	W	W	Unknown

a. Flo11p-HA secretion was determined by colony immunoblot analysis. Each ORF was independently verified by retesting overexpressic plasmids from original E.coli stocks.

b. Secretion level was estimated by comparison to neighboring spots and visually compared to colony size. Densitometry was also used to determine relative levels of Flo11p-HA secretion.

c. Determined in strain PC999, which contains a functional Msb2p-HA fusion.

d. Identified by directed approaches. C-terminal epitope would be expected to influence the maturation of these proteins.

e. Phenotype of the gene deletion in PC2043.

Function
Zinc-finger inhibitor of HO transcription; mRNA is localized and translated in the distal tip of anaphase cells, resulting in accumulation of Ash1p in daughter cell
Cyclin-dependent kinase-activating kinase required for passage through the cell cycle, phosphorylates and activates Cdc28p; nucleotide-binding pocket differs sig
Essential kinetochore protein, component of the CBF3 complex that binds the CDEIII region of the centromere;
Cyclin-dependent protein kinase regulatory subunit and adaptor; modulates proteolysis of M-phase targets through interactions with the proteasome; role in trans
Protein with an N-terminal kelch-like domain, putative negative regulator of early meiotic gene expression; required, with Pmd1p, for growth under alkaline conc
Putative transcriptional activator, that interacts with G1-specific transcription factor, MBF and G1-specific promoters; ortholog of Msa2p, an MBF and SBF activ
Checkpoint protein, involved in the activation of the DNA damage and meiotic pachytene checkpoints; with Mec3p and Ddc1p, forms a clamp that is loaded onto
Type 2A-related serine-threonine phosphatase that functions in the G1/S transition of the mitotic cycle; cytoplasmic and nuclear protein that modulates functions
Meiosis-specific protein with similarity to phospholipase B, required for meiotic spindle pole body duplication and separation; required for spore formation
Protein kinase of the bud neck involved in the septin checkpoint, associates with septin proteins, negatively regulates Swe1p by phosphorylation, shows structura
Subunit of the ARP2/3 complex, which is required for the motility and integrity of cortical actin patches; required for cortical localization of calmodulin
Daughter cell-specific secreted protein with similarity to glucanases, degrades cell wall from the daughter side causing daughter to separate from mother; express
Cell wall mannoprotein with similarity to Tir1p, Tir2p, Tir3p, and Tir4p; expressed under anaerobic conditions, completely repressed during aerobic growth
Cell wall protein of unknown function; proposed role as a hydrophilin induced by osmotic stress; contains a putative GPI-attachment site
Nuclear actin-related protein involved in chromatin remodeling, component of chromatin-remodeling enzyme complexes
Histone H3, core histone protein required for chromatin assembly, part of heterochromatin-mediated telomeric and HM silencing; one of two identical histone H3
Protein that associates with the INO80 chromatin remodeling complex under low-salt conditions; human ortholog INO80C is a member of the human INO80 con
Subunit of the SAGA transcriptional regulatory complex but not present in SAGA-like complex SLIK/SALSA, required for SAGA-mediated inhibition at some p
Mannosyltransferase, involved in asparagine-linked glycosylation in the endoplasmic reticulum (ER); essential for viability, mutation is functionally complement
Alpha-1,2-mannosyltransferase, responsible for addition of the first alpha-1,2-linked mannose to form the branches on the mannan backbone of oligosaccharides,
ER membrane protein involved in the second step of glycosylphosphatidylinositol (GPI) anchor assembly, the de-N-acetylation of the N-acetylglucosaminylphos
Protein involved in the synthesis of N-acetylglucosaminyl phosphatidylinositol (GlcNAc-PI), the first intermediate in the synthesis of glycosylphosphatidylinosit
Protein required for beta-1,6 glucan biosynthesis; mutations result in aberrant morphology and severe growth defects
Delta(9) fatty acid desaturase, required for monounsaturated fatty acid synthesis and for normal distribution of mitochondria
Protein of unknown function, required for wild-type growth rate on galactose and mannose; localizes to COPI coated vesicles and the Golgi apparatu
Cytosolic aspartate aminotransferase, involved in nitrogen metabolism; localizes to peroxisomes in oleate-grown cells
Proteolipid subunit of the vacuolar H(+)-ATPase V0 sector (subunit c; dicyclohexylcarbodiimide binding subunit); required for vacuolar acidification and importa
Protein of unknown function required for respiratory growth; detected in highly purified mitochondria in high-throughput studies; null mutation confers sensitivit
Glycogen synthase, similar to Gsy1p; expression induced by glucose limitation, nitrogen starvation, heat shock, and stationary phase; activity regulated by cAMP
Microsomal beta-keto-reductase;
Protein required for beta-1,6 glucan biosynthesis; putative beta-glucan synthase; appears functionally redundant with Skn1p
Phospholipid methyltransferase (methylene-fatty-acyl-phospholipid synthase), catalyzes the last two steps in phosphatidylcholine biosynthesis
Peroxisomal integral membrane peroxin, involved in the regulation of peroxisomal size, number and distribution; genetic interactions suggest that Pex28p and Pe
V-ATPase assembly factor, functions with other V-ATPase assembly factors in the ER to efficiently assemble the V-ATPase membrane sector (V_0); overproductio
Transketolase, similar to Tkl1p; catalyzes conversion of xylulose-5-phosphate and ribose-5-phosphate to sedoheptulose-7-phosphate and glyceraldehyde-3-phosp
Subunit B of the eight-subunit V1 peripheral membrane domain of the vacuolar H+-ATPase (V-ATPase), an electrogenic proton pump found throughout the endo
Isomaltase (alpha-D-glucosidase); may interact with ribosomes, based on co-purification experiments; authentic, non-tagged protein detected in purified mitocho
Mitochondrial protein required for assembly of ubiquinol cytochrome-c reductase complex (cytochrome bc1 complex); interacts with Cbp4p and function is parti
Mitochondrial integral inner membrane protein required for membrane insertion of C-terminus of Cox2p; interacts genetically and physically with Mss2p and Pn
Mitochondrial ribosomal protein of the large subunit
Mitochondrial ribosomal protein of the large subunit
Mitochondrially localized type 2C protein phosphatase; expression induced by growth on ethanol and by sustained osmotic stress; possible role in carbon source
Zeta-crystallin homolog, found in the cytoplasm and nucleus; has similarity to E. coli quinone oxidoreductase and to human zeta-crystallin, which has quinone ox
Subunit g of the mitochondrial F1F0 ATP synthase; reversibly phosphorylated on two residues; unphosphorylated form is required for dimerization of the ATP sy
UBX (ubiquitin regulatory X) domain-containing protein that interacts with Cdc48p
Protein that interacts with Ulp1p, a Ubl (ubiquitin-like protein)-specific protease for Smt3p protein conjugates; detected in a phosphorylated state in the mitochor
Putative metalloprotease
Subtilisin-like protease (proprotein convertase), a calcium-dependent serine protease involved in the activation of proproteins of the secretory pathway
Possible rhomboid protease, has similarity to eukaryotic rhomboid proteases including Pcp1p
Putative metalloprotease with similarity to the zinc carboxypeptidase family, required for normal cell wall assembly
Meiosis-specific prospore protein; required for meiotic spindle pole body duplication and separation; required to produce bending force necessary for proper pros
Component, with Yta12p, of the mitochondrial inner membrane m-AAA protease that mediates degradation of misfolded or unassembled proteins and is also requ
Smaller subunit of the mitochondrial processing protease (MPP), essential processing enzyme that cleaves the N-terminal targeting sequences from mitochondria
Putative cysteine protease similar to mammalian caspases; involved in regulation of apoptosis upon hydrogen peroxide treatment; proposed to be involved in cell
Amidase, removes the amide group from N-terminal asparagine and glutamine residues to generate proteins with N-terminal aspartate and glutamate residues tha
Zinc metalloendopeptidase, found in the cytoplasm and intermembrane space of mitochondria; with Cym1p, involved in degradation of mitochondrial proteins ar
Type II CAAX prenyl protease involved in the proteolysis and maturation of Ras and the a-factor mating pheromone
Highly conserved zinc metalloprotease that functions in two steps of a-factor maturation, C-terminal CAAX proteolysis and the first step of N-terminal proteolyti
Cytoplasmic aspartyl aminopeptidase; cleaves unblocked N-terminal acidic amino acid residues from peptide substrates; forms a 12 subunit homo-oligomeric cor

Putative metalloprotease
Cysteine aminopeptidase with homocysteine-thiolactonase activity; protects cells against homocysteine toxicity; has bleomycin hydrolase activity in vitro; transc
Vacuolar aminopeptidase Y, processed to mature form by Prb1p
Dubious ORF
Protein with similarity to Emp24p and Erv25p, member of the p24 family involved in ER to Golgi transport
Phosphatidylinositol 3,5-bisphosphate-binding protein, plays a role in micronucleophagy; predicted to fold as a seven-bladed beta-propeller; displays punctate cy
Secretory vesicle-associated Rab GTPase essential for exocytosis; associates with the exocyst component Sec15p and may regulate polarized delivery of transpor
ER-membrane protein; suppressor of pma1-7, deletion of SOP4 slows down the export of wild-type Pma1p and Pma1-7 from the ER
Core component of the signal recognition particle (SRP) ribonucleoprotein (RNP) complex that functions in targeting nascent secretory proteins to the endoplasm
Conserved phosphoesterase domain-containing protein that acts together with Emp24p/Erv25p in cargo exit from the ER; deletion confers sensitivity to 4-(N-(S-
vacuolar sorting
Vacuolar membrane protein of unknown function involved in vacuolar protein sorting; also detected in the mitochondria
Peptidyl-prolyl cis-trans isomerase (cyclophilin) of the endoplasmic reticulum, catalyzes the cis-trans isomerization of peptide bonds N-terminal to proline residu
Protein of unresolved function; may function in protein folding and/or rRNA processing, interacts with a chaperone (Hsp82p), two chromatin remodeling factors
tRNA-specific adenosine deaminase, deaminates adenosine-37 to inosine in tRNA-Ala
Component of the SF3b subcomplex of the U2 snRNP; diploid mutants display a random budding pattern instead of the wild-type bipolar pattern
Non-essential hydrolase involved in mRNA decapping, may function in a feedback mechanism to regulate deadenylation, contains pyrophosphatase activity and
Protein that forms a complex with Pbp1p that may mediate posttranscriptional regulation of HO endonuclease; involved in propagation of M2 dsRNA satellite of
tRNA methyltransferase, 5-methylates the uridine residue at position 54 of tRNAs and may also have a role in tRNA stabilization or maturation; endo-exonucleas
Essential ATP-dependent RNA helicase of the DEAD-box protein family, involved in nonsense-mediated mRNA decay and rRNA processing
Lsm (Like Sm) protein; forms heteroheptameric complex (with Lsm2p, Lsm3p, Lsm4p, Lsm5p, Lsm6p, and Lsm7p) that is part of spliceosomal U6 snRNP and i
Calmodulin-dependent protein kinase; may play a role in stress response, many CA++/calmodulan dependent phosphorylation substrates demonstrated in vitro, a
Dihydroxyacetone kinase, required for detoxification of dihydroxyacetone (DHA); involved in stress adaptation
Probable multiple transmembrane protein, involved in diploid invasive and pseudohyphal growth upon nitrogen starvation; required for accumulation of processe
Mitogen-activated serine/threonine protein kinase involved in mating; phosphoactivated by Ste7p; substrates include Ste12p, Far1p, Bni1p, Sst2p; inhibits invasi
Nonessential putative protein kinase of unknown cellular role; member of the LAMMER family of protein kinases, which are serine/threonine kinases also capab
Pheromone-regulated multispanning membrane protein involved in membrane fusion during mating; predicted to have 5 transmembrane segments and a coiled co
Phosphorelay intermediate protein, phosphorylated by the plasma membrane sensor Sln1p in response to osmotic stress and then in turn phosphorylates the respo
Subunit of TFIIH complex, involved in transcription initiation, similar to 34 kDa subunit of human TFIIH; interacts with Ssl1p
Basic leucine zipper (bZIP) transcription factor
Subunit of the SAGA transcriptional regulatory complex, involved in maintaining the integrity of the complex
Transcriptional activator related to Msn2p; activated in stress conditions, which results in translocation from the cytoplasm to the nucleus; binds DNA at stress re
Transcriptional activator of proline utilization genes, constitutively binds PUT1 and PUT2 promoter sequences and undergoes a conformational change to form th
Cyclin-dependent protein kinase, component of RNA polymerase II holoenzyme; involved in phosphorylation of the RNA polymerase II C-terminal domain; invo
Protein containing a Kruppel-type zinc-finger domain; has similarity to Stp1p, Stp2p, and Stp3p
Putative GTPase involved in 60S ribosomal subunit biogenesis; required for the release of Nmd3p from 60S subunits in the cytoplasm
Protein involved in an early, nucleolar step of 60S ribosomal subunit biogenesis; essential for cell growth and replication of killer M1 dsRNA virus; contains four
Essential component of pre-40S ribosomes that is required for early cleavages of 35S pre-rRNA and hence formation of 18S rRNA; binds RNA in vitro and conta
Nucleolar protein with similarity to large ribosomal subunit L7 proteins; constituent of 66S pre-ribosomal particles; plays an essential role in processing of precur
Subunit of the RNA polymerase II mediator complex; associates with core polymerase subunits to form the RNA polymerase II holoenzyme; contributes to both
Nucleolar protein required for the normal accumulation of 25S and 5.8S rRNAs, associated with the 27SA2 pre-ribosomal particle; proposed to be involved in the
Delta adaptin-like subunit of the clathrin associated protein complex (AP-3); functions in transport of alkaline phosphatase to the vacuole via the alternate pathwa
Acetate transporter required for normal sporulation; phosphorylated in mitochondria
Ferrioxamine B transporter, member of the ARN family of transporters that specifically recognize siderophore-iron chelates; transcription is induced during iron
High affinity sulfate permease; sulfate uptake is mediated by specific sulfate transporters Sul1p and Sul2p, which control the concentration of endogenous activat
Protein of unknown function; may interact with ribosomes, based on co-purification experiments
Putative protein of unknown function; green fluorescent protein (GFP)-fusion protein localizes to both the cytoplasm and the nucleus
Putative protein of unknown function; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm; YLR063W is not an essential gene
Dubious open reading frame unlikely to encode a protein, based on available experimental and comparative sequence data; partially overlaps the verified gene BN
Transaldolase of unknown function; transcription is repressed by Mot1p and induced by alpha-factor and during diauxic shift
Putative protein of unknown function; non-essential gene identified in a screen for mutants with decreased levels of rDNA transcription
Plasma membrane protein of unknown function; may contribute to Non-homologous end-joining (NHEJ) based on double deletion with htz1; YDL012C is not an
Putative protein of unknown function; green fluorescent protein (GFP)-fusion protein localizes to the vacuole
Dubious open reading frame unlikely to encode a functional protein, based on available experimental and comparative sequence data
Putative protein of unknown function; deletion mutant has no readily detectable phenotype; expression downregulated by treatment with 8-methoxypsoralen plus
Protein of unknown function; null mutant displays sensitivity to DNA damaging agents; the authentic, non-tagged protein is detected in highly purified mitochon
Putative protein of unknown function; GFP-fusion protein is induced in response to the DNA-damaging agent MMS; the authentic, non-tagged protein is detecte
Putative protein of unknown function; predicted protein kinase; implicated in proteasome function; epitope-tagged protein localizes to the cytoplasm
Putative protein of unknown function; green fluorescent protein (GFP)-fusion protein localizes to the endoplasmic reticulum; YLR050C is not an essential gene
Putative protein of unknown function; YLR149C is not an essential gene
Dubious open reading frame unlikely to encode a functional protein, based on available experimental and comparative sequence data

Putative protein of unknown function; strong increase in transcript abundance during anaerobic growth compared to aerobic growth; cells deleted for YML083C
Putative protein of unknown function; predicted to contain a transmembrane domain; YMR148W is not an essential gene
Putative protein of unknown function; essential gene required for viability
Putative protein of unknown function; expression is cell-cycle regulated, Azf1p-dependent, and heat-inducible
Putative protein of unknown function; identified as interacting with Hsc82p and Hsp82p in high-throughput two-hybrid screens
Putative protein of unknown function; YOR214C is not an essential gene
Dubious open reading frame unlikely to encode a protein, based on available experimental and comparative sequence data; YOR376W is not an essential gene.
Putative protein of unknown function
Dubious open reading frame, unlikely to encode a protein; not conserved in closely related Saccharomyces species; partially overlaps the verified essential gene
Dubious open reading frame unlikely to encode a functional protein, based on available experimental and comparative sequence data; deletion mutation confers a
Dubious open reading frame unlikely to encode a protein, based on experimental and comparative sequence data; partially overlaps the dubious gene YOL085W-

on

ll nuclei and inhibition of HO expression; potential Cdc28p substrate
ignificantly from those of most other protein kinases

scriptional regulation, recruiting proteasomal subunits to target gene promoters
ıditions
vator that regulates G1-specific transcription and cell cycle initiation
:o partial duplex DNA; homolog of human and S. pombe Rad1 and U. maydis Rec1 proteins
; mediated by Pkc1p including cell wall and actin cytoskeleton organization

al homology to bud neck kinases Gin4p and Hsl1p

ısion is repressed by cAMP

3 proteins (see HHT1); regulated by acetylation, methylation, and phosphorylation
mplex; implicated in DNA repair based on genetic interactions with RAD52 epistasis genes
promoters
ıted by human ortholog
;, localizes to an early Golgi compartment
sphatidylinositol intermediate; functional homolog of human PIG-Lp
tol (GPI) anchors; homologous to the human PIG-C protein

tant for copper and iron metal ion homeostasis
ity to tunicamycin and DTT and decreased levels of phosphatidylethanol
P-dependent, Snf1p and Pho85p kinases as well as by the Gac1p-Glc7p phosphatase

ex29p act at steps upstream of those mediated by Pex30p, Pex31p, and Pex32p
ın confers resistance to Pichia farinosa killer toxin
phate in the pentose phosphate pathway; needed for synthesis of aromatic amino acids
omembrane system; contains nucleotide binding sites; also detected in the cytoplasm
ondria in high-throughput studies
tially redundant with that of Cbp4p
ıt1p; similar to S. cerevisiae Oxa1, N. crassa Oxa2p, and E. coli YidC

: utilization in low oxygen environments
ɔxidoreductase activity
ynthase complex

ındrial outer membrane; also detected in ER and nuclear envelope

ɔspore membrane assembly during sporulation; has similarity to phospholipase B
quired for correct assembly of mitochondrial enzyme complexes
ally imported proteins
ll cycle progression
at are targets of ubiquitin-mediated degradation
ınd of presequence peptides cleaved from imported proteins

tic processing; contains multiple transmembrane spans
ɔmplex; M18 metalloprotease family member; may interact with ribosomes

cription is regulated by galactose via Gal4p; orthologous to human BLMH

ytoplasmic localization
ırt vesicles to the exocyst at the plasma membrane

mic reticulum (ER) membrane
-glutathionylacetyl)amino) phenylarsenoxide (GSAO)

ues; transcriptionally induced in response to unfolded proteins in the ER
s (Rvb1p, Rvb2p) and two rRNA processing factors (Rrp43p, Nop58p)

a HIT (histidine triad) motif; interacts with neutral trehalase Nth1p
f L-A virus; contains a DTG signature typical of retroviral proteases
ıse with a role in DNA repair

is also implicated in processing of pre-tRNA, pre-snoRNA, and pre-rRNA
amino acid sequence similar to Cmk2p and mammalian Cam Kinase II

ed Rim101p
ive growth during mating by phosphorylating Tec1p, promoting its degradation
ble of phosphorylating tyrosine residues
coil domain; localizes to the shmoo tip; regulated by Ste12p
onse regulators Ssk1p in the cytosol and Skn7p in the nucleus

esponse elements of responsive genes, inducing gene expression
the active state; has a Zn(2)-Cys(6) binuclear cluster domain
volved in glucose repression

ır beta-transducin repeats
ains
ursors to the large ribosomal subunit RNAs

ıe biogenesis of the 60S ribosomal subunit
vay, suppressor of loss of casein kinase 1 function

deprivation and diauxic shift; potentially phosphorylated by Cdc28p
ated sulfate intermediates

ŝNA5

n essential gene

s UVA irradiation
ndria in high-throughput studies
ed in highly purified mitochondria in high-throughput studies

: do not exhibit growth defects in anerobic or anaerobic conditions

ABF1
an increase in Ty1 transposition
Y-A