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SUPPLEMENTAL MATERIAL INVENTORY

Supplemental Figures:

Figure S1, related to Figure 2

Genes identified in our screen and listed in Figure 2 were also analyzed using Osprey to identify any known interactions either among the hits or between hits and known pheromone components. Figure S1 shows the interactions that were identified.

Table S1, related to Figure 2

We identified 92 essential genes required for pheromone signaling and listed these genes in Figure 2. However, we excluded an additional 97 genes due to their known role in cellular processes that would result in false-positives in our transcriptional reporter assay. These 97 genes are listed in Table S1.

Figure S2, related to Table 1

The high confidence hits from our screen listed in Table 1 were validated using a dual-reporter system. Figure S2 shows the immunoblots from this experiment.

Figure S3, related to Figure 3

We chose 6 essential genes to further characterize using transcriptional reporter assays and measurement of activation of the MAP kinases as shown in Figure 3. Figure S3 shows the measurement of total levels of the MAPK Fus3 and quantification of bands to demonstrate that phosphorylated Fus3 changes relative to total Fus3.

Figure S4, related to Figure 5

We tested the stability of two pheromone pathway components in an SCF-mutant strain as shown in Figure 5. As a control, we also tested the stability of 7 other pathway components and display the results in Figure S4. We show

that SCF^{Met30} cannot ubiquitinate Gpa1 *in vitro* (Figure 5). As a control, we show that Met30 can bind weakly to Gpa1, demonstrating it is functional. We also show that Gpa1 expressed in *E. coli* cannot be ubiquitinated by SCF^{Cdc4} *in vitro*.

Supplemental Methods

Table of PCR Primers

Supplemental References

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Figure S1, related to Figure 2

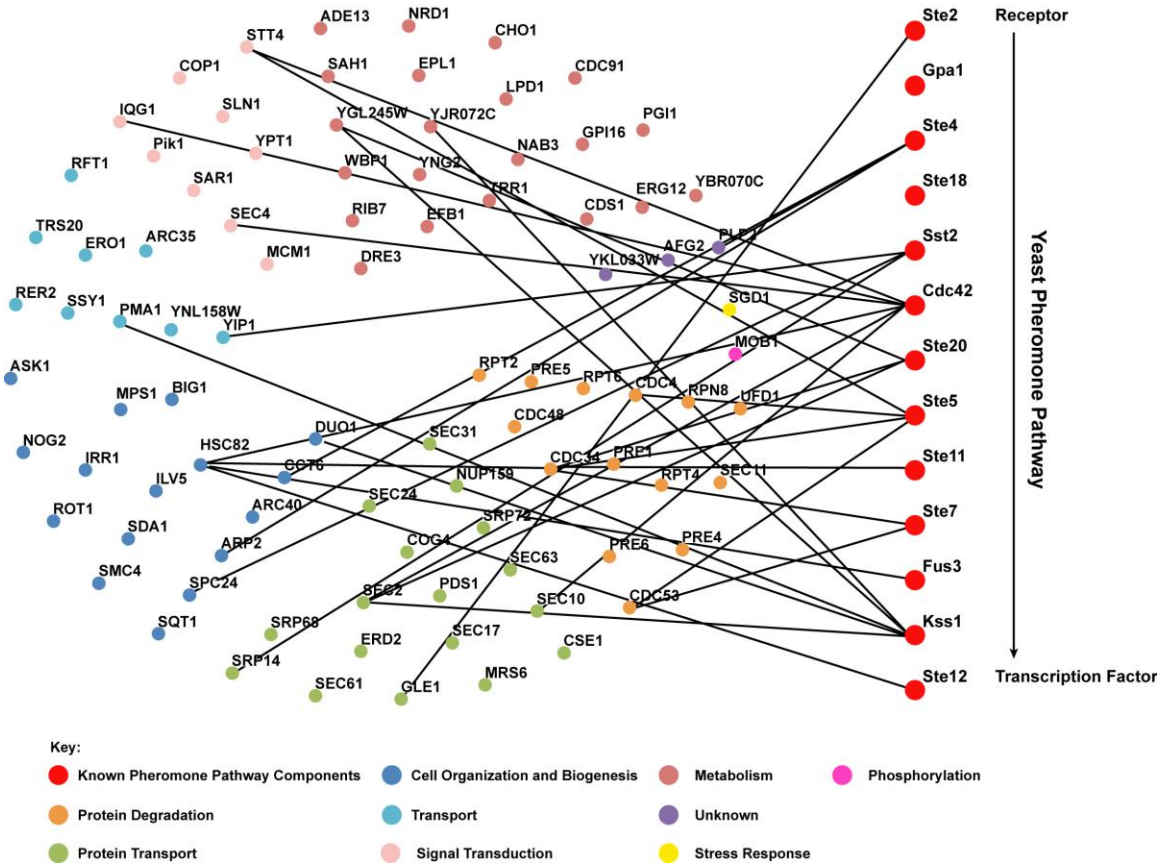

Figure S1. Many of the hits from the essential-gene screen have known interactions with pheromone pathway components, related to Figure 2.

The Osprey Network Visualization System software was used to generate an interaction network based on known genetic and physical interactions from BioGRID.

Figure S2, related to Table 1

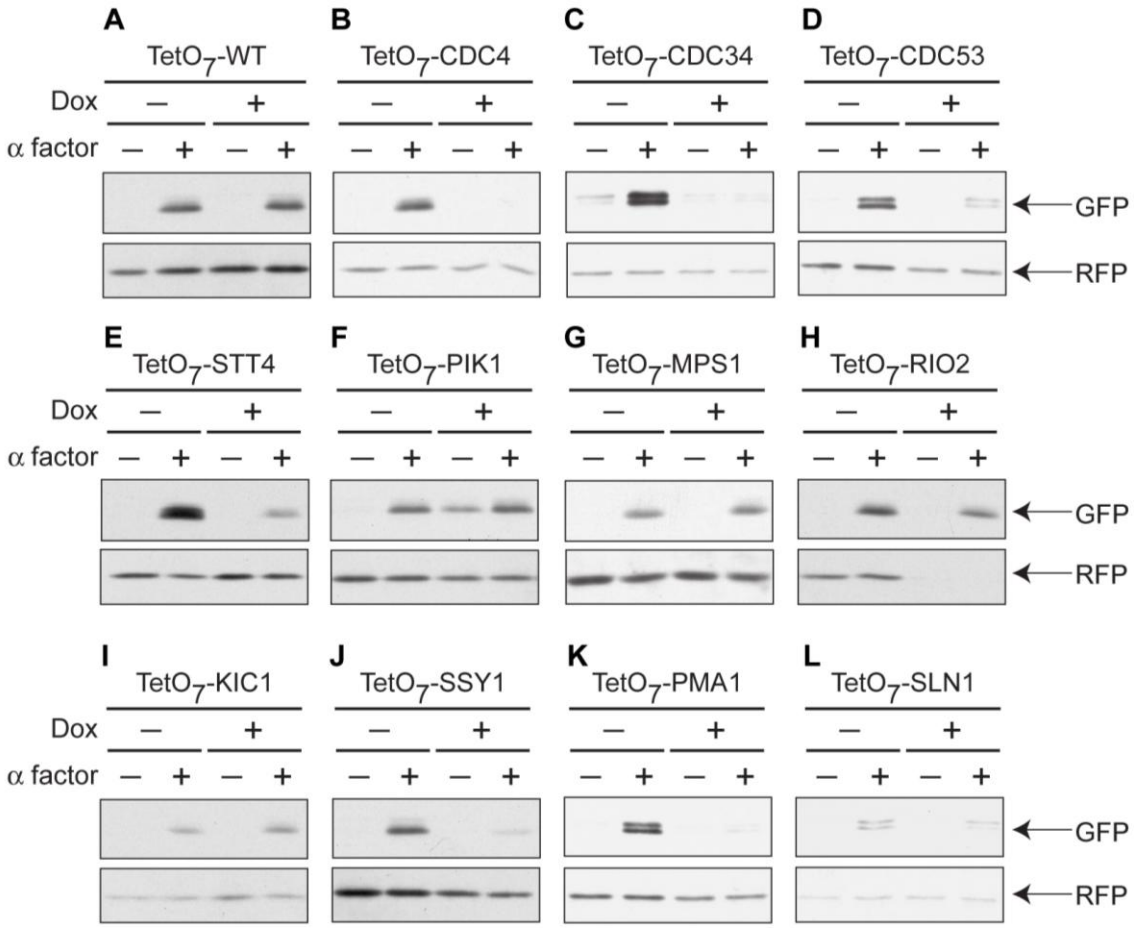

Figure S2. Hits from the essential-gene screen regulate G protein signaling upstream of translation, related to Table 1.

(A-L) The indicated $TetO_7$ strains expressing the pRS315 AR/FG dual reporter plasmid were treated with 10μg/mL doxycycline for 15hrs and 3μM α factor for 30min. Cell lysates were probed with GFP and RFP antibodies. Five additional strains, $TetO_7$-RIO2, $TetO_7$-KIC1, $TetO_7$-SSY1, $TetO_7$-PMA1 and $TetO_7$-SLN1, were also tested. Strains representing *RIO2*, *KIC1*, and *SSY1* exhibited changes in RFP expression. Strains representing *PMA1* and *SLN1* showed no changes in RFP expression but were not considered further.

Figure S3, related to Figure 3

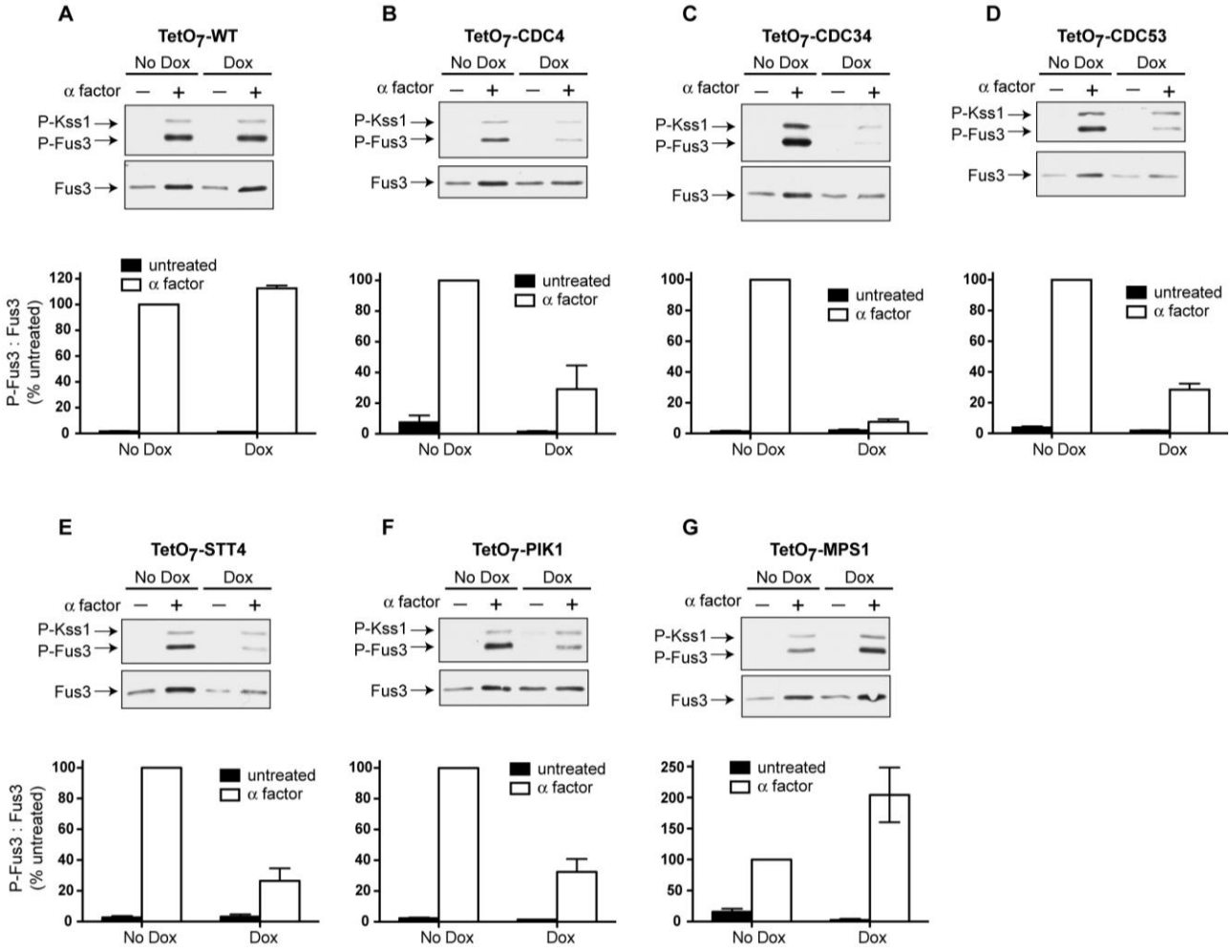

Figure S3. Knock-down of essential genes affects Fus3 activation.

(A-G) The indicated $TetO_7$ strains were treated with 10μg/mL doxycycline for 15hrs and 3μM α factor for 30min. Cell lysates were probed with phospho-p42/44 (P-Fus3, P-Kss1) and Fus3 antibodies. Bands were quantified by densitometry and the ratio of P-Fus3:Fus3 is shown below each immunoblot. Results are the mean ± S.E. (n=3).

Figure S4, related to Figure 5

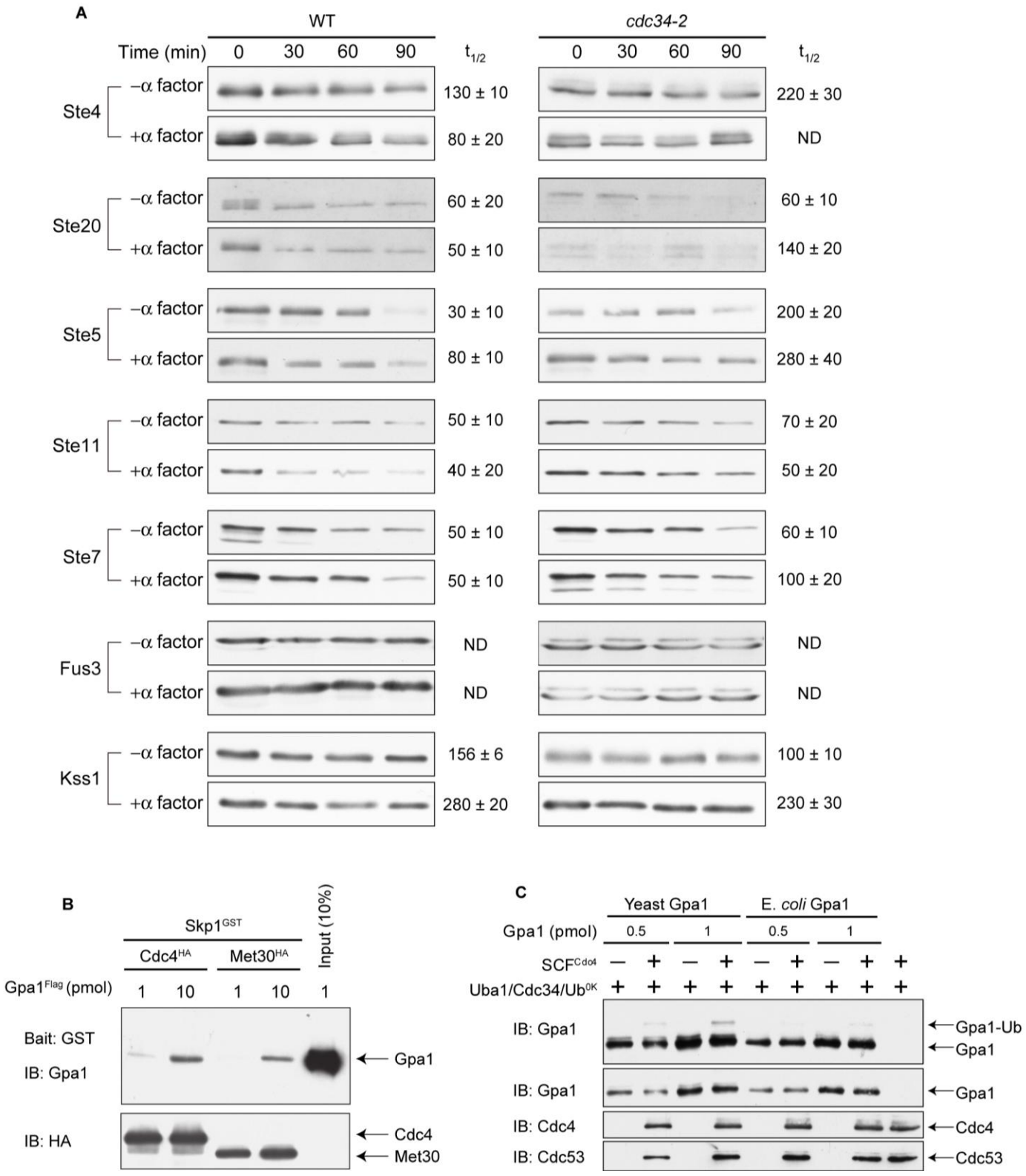

Figure S4. The SCF does not destabilize any positive regulators of G protein signaling and primarily functions to ubiquitinate Gpa1, related to Figure 5.

(A) The indicated TAP-fusion genes were integrated into wild-type and *cdc34-2* ts strains. Cycloheximide was administered at time zero and protein levels measured by immunoblotting with protein A antibodies.

(B) GST-Skp1/Cdc4-HA or Met30-HA complexes were immobilized on Glutathione Sepharose^{TM} resin and incubated with purified Gpa1-Flag, followed by washing and analysis of the bound proteins with Gpa1-antibodies to detect co-purification of Gpa1.

(C) *In vitro* ubiquitination of Gpa1 expressed in yeast and *E coli*, which lacks most post-translational modifications.

Table S1: Essential Gene Hits Involved in Global Gene Transcription and Translation, related to Figure 2

Systematic Name	Standard Name	Go Process	Systematic Name	Standard Name	Go Process
YAL043C	PTA1	RNA Processing	YKR062W	TFA2	Transcription
YBL014C	RRN6	Transcription	YKR086W	PRP16	RNA Processing
YBL035C	POL12	DNA Replication	YLL008W	DRS1	RNA Processing
YBL076C	ILS1	Protein Biosynthesis	YLL035W	GRC3	RNA Processing
YBR079C	RPG1	Protein Biosynthesis	YLR009W	RLP24	Protein Biosynthesis
YBR088C	POL30	DNA Replication	YLR033W	RSC58	Transcription
YBR193C	MED8	Transcription	YLR060W	FRS1	Protein Biosynthesis
YBR253W	SRB6	Transcription	YLR129W	DIP2	RNA Processing
YCR042C	TAF2	Transcription	YLR141W	RRN5	Transcription
YCR052W	RSC6	Transcription	YLR249W	YEF3	Protein Biosynthesis
YDL060W	TSR1	RNA Processing	YLR275W	SMD2	RNA Processing
YDL140C	RPO21	Transcription	YLR276C	DBP9	RNA Processing
YDL150W	RPC53	Transcription	YLR291C	GCD7	Protein Biosynthesis
YDL153C	SAS10	RNA Processing	YML046W	PRP39	RNA Processing
YDR045C	RPC11	Transcription	YML069W	POB3	DNA Replication
YDR091C	RLI1	Protein Biosynthesis	YML114C	TAF65	Transcription
YDR145W	TAF12	Transcription	YML127W	RSC9	Transcription
YDR167W	TAF10	Transcription	YMR005W	TAF4	Transcription
YDR228C	PCF11	RNA Processing	YMR061W	RNA14	RNA Processing
YDR341C	YDR341C	Protein Biosynthesis	YMR093W	YMR093W	RNA Processing
YDR398W	UTP5	RNA Processing	YMR235C	RNA1	RNA Processing
YDR460W	TFB3	Transcription	YMR236W	TAF9	Transcription
YDR489W	YDR489W	DNA Replication	YNL039W	TFC5	Transcription
YEL034W	YEL034W	Protein Biosynthesis	YNL113W	RPC19	Transcription
YER029C	SMB1	RNA processing	YNL151C	RPC31	Transcription
YER171W	RAD3	Transcription	YNL216W	RAP1	Transcription
YER172C	BRR2	RNA Processing	YNL221C	YNL221C	RNA Processing
YFR037C	RSC8	Transcription	YNL247W	YNL247W	Protein Biosynthesis
YGL122C	NAB2	RNA Processing	YOL005C	RPB11	Transcription
YGL207W	SPT16	Transcription	YOL021C	DIS3	RNA Processing
YGR005C	TFG2	Transcription	YOL077c	YOL077C	Protein Biosynthesis
YGR030C	POP6	RNA Processing	YOL139C	CDC33	Protein Biosynthesis
YGR094W	VAS1	Protein Biosynthesis	YOR116C	RPO31	Transcription
YGR158C	MTR3	RNA Processing	YOR145c	YOR145C	RNA Processing
YHR019C	DED81	Protein Biosynthesis	YOR151C	RPB2	Transcription
YHR062C	RPP1	RNA Processing	YOR159C	SME1	RNA Processing
YHR069C	RRP4	RNA Processing	YOR194C	TOA1	Transcription
YHR122W	YHR122W	Transcription	YOR204W	DED1	RNA Processing
YHR164C	DNA2	DNA replication	YOR224C	RPB8	Transcription
YHR170W	NMD3	Protein Biosynthesis	YOR340C	RPA43	Transcription
YJL011C	RPC17	Transcription	YPL016W	SWI1	Transcription
YJL069C	UTP18	RNA Processing	YPL043W	NOP4	RNA processing
YJL081C	ARP4	Transcription	YPL093W	NOG1	RNA Processing
YKL059C	YKL059C	RNA Processing	YPL126W	NAN1	RNA Processing
YKL125W	RRN3	Transcription	YPL228W	CET1	RNA Processing
YKL180W	RPL17	Protein Biosynthesis	YPL266W	DIM1	RNA Processing
YKL186C	MTR2	Ribosome Biogenesis	YPR016C	YPR016C	Protein Biosynthesis
YKR008W	RSC4	Transcription	YPR187W	RPO26	Transcription

SUPPLEMENTAL METHODS

Strains and Plasmids

Standard procedures for the growth, maintenance, and transformation of yeast and bacteria and for the manipulation of DNA were used throughout. Yeast *Saccharomyces cerevisiae* strains used in this study were BY4741 (*MAT***a** *leu2Δ met15Δ his3-1 ura3Δ*), MTY235 (*MAT***a** *ade2*-1 *his3-11,15 leu2-3,112 trp1*-1 *ura3-1 can1-100*), MTY670 (MTY235 *cdc34-2*), and MTY668 (MTY235 *cdc4-1*) (provided by Mike Tyers, Samuel Lunenfield Research Institute) (Tang et al., 2005), BY4741-derived strains containing a C-terminal tandem affinity purification (TAP)-tag (Yeast TAP-Fusion Library, Open Biosystems), and the BY4741-derived strain R1158 (*MAT***a** *URA3::CMV-tTA leu2Δ met15Δ his3-1 ura3Δ*) (Hughes et al., 2000). The tetracycline-repressible strains were purchased as the yeast Tet-promoter Hughes Collection (yTHC, Open Biosystems) (Mnaimneh et al., 2004).

Yeast shuttle plasmids used were pRS315 (CEN, amp^R, *LEU2*), and pRS316 (CEN, amp^R, *URA3*). Expression plasmids described previously were pRS423-FUS1-lacZ (Hoffman et al., 2002), pRS316-ADH1, pRS316-ADH1-GPA1 (Song et al., 1996), pFAGa-mRFP1-KanMX6 (Huh et al., 2003), pUG35 (provided by Johannes Hegemann, Heinrich-Heine-Universität), YCp50-STE11-4 (from George Sprague, University of Oregon) (Stevenson et al., 1992), and pRS315-GAL-STE4 (Dohlman et al., 1995). Plasmid pRS316-ADH-GPA1-Flag was constructed by PCR amplification of a 384 bp fragment of *GPA1* from pRS316-ADH-GPA1 using primers 1 and 2 (see Table of PCR primers below). Plasmid pRS316-ADH-GPA1$^{\Delta 128\text{-}236}$-Flag was constructed by QuikChange (Stratagene) using primers 3 and 4 to remove the 324 bp fragment corresponding to amino acids 128-236. Plasmid pRS315-STE11 was constructed by PCR amplification of *STE11* from BY4741 genomic DNA, followed by SacI and XmaI

digestion and ligation into the corresponding sites of pRS315. Plasmid pRS315-STE11-4 was constructed by engineering the single point mutation Thr-596-Ile (Stevenson et al., 1992) into pRS315-STE11 using QuikChange and primer 5 and its complement. Rescue plasmids for *CDC4*, *CDC34*, and *STT4* were constructed by PCR amplification of each gene from BY4741 genomic DNA, followed by SacI and XmaI digestion and ligation into the corresponding sites of pRS315. Rescue plasmids for *PIK1* and *MPS1* were made in a similar manner except that SacII was used in place of SacI.

The pRS316-ADH1-RFP/FUS1-GFP (AR/FG) dual reporter was constructed using the steps outlined below. The plasmid pRS316-ADH1-GFP was constructed by PCR amplification of *GFP* from the plasmid pUG35 using primers 6 and 7 including SacI sites, followed by SacI digestion and insertion into the corresponding site in pRS316-ADH1. The *ADH1* terminator sequence (*$ADH1_t$*) (from the stop codon to 600bp downstream) was PCR amplified from genomic DNA with primers 8 and 9 including XmaI and SalI sites. The resulting PCR product was digested with XmaI and SalI and inserted into the corresponding sites in pRS316-ADH1-GFP, resulting in pRS316-$ADH1_p$-$ADH1_t$-GFP. RFP was PCR amplified from pFA6a-mRFP1-KanMX6 using primers 10 and 11 with XmaI sites. This fragment was ligated into the corresponding XmaI sites of pRS316-$ADH1_p$-$ADH1_t$-GFP, resulting in the plasmid pRS316-$ADH1_p$-RFP-$ADH1_t$-GFP. The *FUS1* promoter (*FUS1p*) (600bp upstream of the start codon of *FUS1*) was PCR amplified from genomic DNA using primers 12 and 13 containing SalI sites. The resulting fragment was digested with SalI and inserted into the corresponding sites of pRS316-$ADH1_p$-RFP-$ADH1_t$-GFP resulting in the plasmid pRS316-$ADH1_p$-RFP-$ADH1_t$-FUS1-GFP (designated pRS316-AR/FG). pRS315-AR/FG was constructed by digestion of pRS316-AR/FG with PvuI and ligation into the corresponding sites in pRS315.

Bioinformatics

Physical and genetic interactions among the genes identified in the screen were analyzed using Osprey Network Visualization System (Breitkreutz et al., 2002) which incorporates published data from the Biological General Repository for Interaction Datasets (BioGRID) (Stark et al., 2006). Functional categories were assigned based on Gene Ontology annotations using Functional Clustering in Osprey. Genes involved in multiple GO processes were assigned a single GO term based on Osprey's hierarchical GO process order. Hierarchical clustering of $TetO_7$-Strain phenotypes was conducted with the open source software Cluster 3.0 (de Hoon et al., 2004) using uncentered correlation and centroid linkage. The generated clustering data was visualized with the open source software Java TreeView (v1.1.3) (Saldanha, 2004).

TAP-Fusion protein turnover screen

TAP-fusion genes were PCR amplified and integrated into MTY235 and *cdc34-2* cells. Cells were grown at room temperature to A_{600nm} ~0.25, shifted to 37^{o}C for 3hrs treated with 3 μM α factor for 1hr, and treated with cycloheximide for up to 90min. Protein extracts were resolved by 7.5% SDS-PAGE and immunoblotting with protein A (P3775, Sigma-Aldrich) antibodies at 1:50,000. Experiments were performed in triplicate, and bands were quantified by densitometry.

Cell-Extract Preparation and Immuno-Blot Analysis

The yeast $TetO_7$ strains were grown in selective medium to A_{600nm}~0.8 and re-inoculated at 1:80 into medium containing doxycycline at a final concentration of 10μg/ml and grown to A_{600nm}~0.8. Cell cultures were then divided in half, and either treated with 3μM α factor pheromone or left untreated at 30^{o}C for 30min. Protein extracts were produced by glass bead lysis in trichloroacetic acid (TCA) as previously described (Lee

and Dohlman, 2008). Protein extracts were resolved by 12% SDS-PAGE and immunoblotting with Phospho-p44/42 MAPK antibodies (9101L, Cell Signaling Technology) at 1:500, Fus3 antibodies (sc-6773, Santa Cruz Biotechnology, inc.) at 1:500, GFP antibodies (632375, BD Biosciences) at 1:500, dSRed antibodies (632496, Clontech) at 1:1000, and G6PDH antibodies (A9521, Sigma-Aldrich) at 1:100,000. Immunoreactive species were visualized by chemiluminescent detection (PerkinElmer Life Sciences LAS) of horseradish peroxidase-conjugated antibodies (170-5047 and 170-5046, Bio-Rad). Protein concentration was determined by Dc protein assay (Bio-Rad Laboratories). Where indicated, $TetO_7$ cells were transformed with pRS315-GAL-STE4, pRS315-STE11-4, pRS315-ADH-RFP/FUS1-GFP, or empty vector, and grown in selective medium containing 2% (w/v) dextrose or galactose to induce *STE4* expression.

Co-Immunoprecipitation Assay

Insect cell lysates containing GST-Skp1 and either Cdc4-HA or Met30-HA where mixed with Glutathione Sepharose^{TM} 4 Fast Flow resin (GE Healthcare) for 1hr rotating at 4^{o}C. The beads were then washed 3x with 50x bead-bed volume of binding buffer (50mM Tris-HCl, pH 7.5, 150mM KCl, 0.5% NP-40, 0.2mM dithiothreitol, 2mM $MgCl_2$, 20μM GDP, 10mM NaF, 10mM β-glycerol phosphate, 1mM sodium orthovanadate, and proteinase inhibitor tablets). Gpa1-Flag purified from yeast was added to the beads and incubated for 2hrs at 4^{o}C with rotating. The beads were washed 3x with 50x bead-bed volume of binding buffer for 5min each with rocking and 3x quick washes with 50x bead-volume of binding buffer. Bound protein was then eluted in 2x bead-bed volume of binding buffer supplemented with 20mM glutathione. Protein samples were resolved by 10% SDS-Page and immunoblotting with Gpa1 antibodies at 1:1000 and HA antibodies (3F10, Roche Applied Sciences) at 1:2000.

Preparation and Purification of Recombinant Proteins

BY4741 yeast cells were transformed with pRS316-ADH1-GPA1-Flag and pRS316-ADH1-GPA1$^{\Delta 128-236}$-Flag and grown to early log phase (A_{600nm} ~1.0) before harvesting by centrifugation. The cell pellet was frozen in liquid nitrogen and lysed by grinding cells blast-frozen in a 1:0.7 ratio of lysis buffer (50mM Tris-HCl, pH 7.5, 400mM KCl, 0.1% Triton, 0.2mM dithiothreitol) supplemented with 20μM GDP, 10mM NaF, 10mM β-glycerol phosphate, 1mM sodium orthovanadate, 1mM phenylmethylsulfonyl fluoride and 1 proteinase inhibitor tablet per 50mL (11873580001, Roche Applied Science). The cell lysate was thawed on ice, and centrifuged at 15,000xg for 30min at 4^{o}C. The supernatant was transferred and incubated with EZview anti-Flag M2 beads (Sigma-Aldrich) for 2hrs rotating at 4^{o}C. Beads were harvested by centrifugation and washed 3 times with 100x bead-bed volume of ubiquitination buffer (50mM Tris-HCl, pH 7.5, 50mM KCl, 0.2mM dithiothreitol, 2mM $MgCl_2$, 20μM GDP) supplemented with 5% glycerol, followed by elution with 2x bead-bed volume of the supplemented ubiquitination buffer containing 0.25mg/mL 3XFlag peptide (Sigma-Aldrich). Protein aliquots were frozen and stored at -80^{o}C.

Yeast ^{His}Cdc4 E2 was purified from *E. coli*, yeast ^{His}Uba1 E1 was purified from yeast, and yeast SCF E3 Complexes were purified from insect cells infected with the baculoviruses expressing yeast Flag-Skp1, Cdc53, Myc-Rbx1, and HA-Cdc4 or HA-Met30 for 40hrs as described previously (Skowyra et al., 1997). Cells were disrupted in NETN buffer (50mM Tris-HCl, pH7.5, 150mM KCl, 0.5% Nonidet P-40, 0.2mM dithiothreitol, 10mM NaF, 10mM β-glycerophosphate, 1mM phenylmethylsulfonyl fluoride) supplemented with proteinase inhibitor tablets and cleared by centrifugation at 15,000xg for 30min at 4^{o}C. Typically 3ml of NETN buffer was used per 0.5 x 10^{8} cells. For immunopurification 300μL of cell lysate was incubated with 10μL EZview anti-Flag

M2 beads with rotating for 1hr at 4°C. Beads were washed 3 times with 500μL of NETN buffer for 5min each with rocking and 3x quick washes with 500μL of ubiquitination buffer. Bound protein was eluted from the beads with 2x 10μL of ubiquitination buffer supplemented 0.25mg/mL 3XFlag peptide for 10min each. Eluted protein was added directly to ubiquitination reactions.

6xHIS-Gpa1 expression plasmid was described previously (Apanovitch et al., 1998) and transformed into BL21 (DE3) *E. coli*. Cells were grown from a single colony overnight at 37°C in Luria Broth (LB) supplemented with 100μg/mL carbenicillin and then diluted 1:100 into fresh media. Once cells grew to A_{600nm} ~0.7, 6xHIS-Gpa1 expression was induced by addition of 0.2mM isopropyl β-D-1-thiogalactopyranoside and incubation at room temperature for 5hr with shaking. Cells were harvested by centrifugation, resuspended in Buffer A (20mM Tris pH 8.0, 200mM NaCl, 5% glycerol, 20μM GDP, 2mM $MgCl_2$, 1mM DTT) supplemented with protease inhibitor tablets (Roche), and homogenized with an Emulsiflex-C5 Homogenizer (Avestin). Lysates were clarified by centrifugation at 12,000xg for 30min, and the resulting supernatent was mixed with Buffer A-equilibrated Ni-Sepharose™6 Fast Flow resin (GE Healthcare) for 2hrs rotating at 4°C. Resin was collected by centrifugation at 500xg for 5min and washed 3 times with Buffer A followed by elution with Buffer A supplemented with 250mM imidazole. The elution was mixed with His-tagged tobacco etch virus protease (to remove the N-terminal 6xHIS from Gpa1) and dializyed in 1L of Buffer B (20mM Tris, pH 8.0, 100mM NaCl,, 5% glycerol, 20μM GDP, 2mM $MgCl_2$, 1mM DTT) overnight. Sample was incubated with Ni-Sepharose resin for 1hr to remove tobacco etch virus protease and cleavage products. Flow-through from the Ni-Sepharose was concentrated using Vivaspin concentrators (Vivascience AG).

PCR Primers	
Name	**Primer Sequence**
1	5′-TAG GAT CCG TAG GAA ATA ATG GGG TGT AC-3′
2[a]	5′-TAA AGC TTT **GAT TAC AAG GAT GAC GAC GAT AAG** AGC CTT TAG CAG TAT
3	5′-GAA GGA TGA CGA CGA TAA GAA ACT TAT TCA CGA AGA CAT TGC-3′
4	5′-GCA ATG TCT TCG TGA ATA AGT TTC TTA TCG TCG TCA TCC TTC-3′
5	5′-GGT TGC GTA AAA ATT ATT GAT TTT GGT ATT TC-3′
6	5′-GAG CTC GCC ATG TCT AAA GGT GAA GAA TTA TTC ACT GGT G-3′
7	5′-GAG CTC GCC ATG TCT AAA GGT GAA GAA TTA TTC ACT GGT G-3′
8	5′-CTC GAG CCC GGG GCG AAT TTC TTA TGA TTT ATG ATT TTT ATT ATT AAA
9	5′-GAA TTC GGT ACC CAA TAG CAA TGG GGT TTT TTT CAG-3′
10	5′-CCC GGG AAC ATG GCC TCC TCC GAG GAC-3′
11	5′-CCC GGG GCC TTA GGC GCC GGT GG-3′
12	5′-GTC GAC CCA TGC AGA AGC TGT TGC GAA G-3′
13	5′-GTC GAC TTT GAT TTT CAG AAA CTT GAT GGC TTA TAT CCT GC-3′
Cdc4 F	5′-CAG TTA GAG CTC TGG TAT TCA TCA TCA AAA ACA GCC TTC CAG-3′
Cdc4 R	5′-CAG TTA CCC GGG AAA AAT CAG CAG AAT CAA CTT CTT AAC G-3′
Cdc34 F	5′-TAG AGC TCG GCA ATT ACT AAT GCG GGT TC-3′
Cdc34 R	5′-TAC CCG GGT CGC AAA GAT CGA CCT AAG AC-3′
Stt4 F	5′-GAG CTC AAA TTA GGC TGA ATA GAA CTG C-3′
Stt4 R	5′-CCC GGG AGA TTT TCC TTG TCC TCC CTT T-3′
Pik1 F	5′-CCG CGG CTA GAA GAT ATT GAC ATC GAT TCC G-3′
Pik1 R	5′-CCC GGG CGA TGT GCC ATA TAG TAA GCT GG-3′
Mps1 F	5′-CCG CGG ATC ACA ACA AAT GGT GAT TCT GG-3′
Mps1 R	5′-CCC GGG TCT AAG CAA GAA TGG CAA GAA AG-3′
Ste4 F	5′-CAG GTA CAC ATT ACG ATG GAA TTC CAT CAG ATG GAC TCG ATA ACG-3′
Ste4 R	5′-CGT TAT CGA GTC CAT CTG ATG GAA TTC CAT CGT AAT GTG TAC CTG-3′
Ste20 F	5′-AAG GAA AAA AGC GGC CGC CTC GCT TAT GGA TTG TGG GAT CTC ACC-3′
Ste20 R	5′-CCG CTC GAG CCT TTC CGT CAC TGT TCC ATG-3′
Ste5 F	5′-TCC CCG CGG GAC ATC AGC TGA TTT CTC ATA GAG-3′
Ste5 R	5′-AAG GAA AAA AGC GGC CGC GCT CAT TAT GTA ACC ATT CCG ATT GAC-3′
Ste11 F	5′-AAG GAA AAA AGC GGC CGC CTG CGC AGC TTT ATA CAA GTT AGC-3′
Ste11 R	5′-CCG CTC GAG ACG CTT CTA GAG GTA CAG GCG-3′
Ste7 F	5′-CGC GGA TCC TGG TAT CCT TCT TGG GAA TAT TCA ATG C-3′
Ste7 R	5′-CCG CTC GAG AGC GAG TAC AGT CAT TGT GTG CCA CC-3′
Fus3 F	5′-CGC GGA TCC GCA GCG ACT GCA CTA AAC TAG AGG-3′
Fus3 R	5′-CCG GAA TTC TCT AGT AAG ACG GTC TCT TGC TGC-3′
Kss1 F	5′-AAG GAA AAA AGC GGC CGC GAC AGA TTA CGA GAG TCC AGC C-3′
Kss1 R	5′-CCG CTC GAG CTT GGC TGG GTA TTA GTT GTG-3′
Ste12 F	5′-CGC GGA TCC TCT TCC CTT CCC AGA GAG AAA AAA GG-3′
Ste12 R	5′-CCG CTC GAG CTG CTT TTA TTA TTT GTT AGT GCC-3′

[a]FLAG sequence highlighted in bold

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