

Legends to Supplementary Figures

Figure S1. Design of the synthetic gene array screen for identifying components required for Psd2 activity in vivo. Schematic illustration of the screen aimed to find non-essential genes whose functional ablation, when combined with the *psd1Δ* allele, retards growth on Etn-free media. A *psd1Δ* allele was recombined into the yeast gene deletion collection using a standard automated mating, sporulation and selection protocol. Colony sizes of yeast containing both the query library gene deletion and *psd1Δ* (double mutants, DM) were analyzed by relating colony sizes achieved on plates lacking Etn relative to those on Etn-replete medium. To validate that the observed growth deficit was $\Delta psd1$ -dependent, and not simply a result of the library background, the same analysis was performed on yeast that were only selected for the mutant allele from the library (single mutants, SM). Normalized growth deficits were calculated and DM strains that exhibited a growth score < 1 standard deviation from the mean (< 0.47) were considered as potential hits.

Figure S2. Phenotypic assays of *stt4* mutants and Stt4 subunit over-expressing cells.

(A) Isogenic *stt4^{ts}* yeast transformed with YCp(*URA3*) or derivatives driving individual expression of *STT4*, *stt4^{G1782D}* or *stt4^{D1752A}* from the natural *STT4* promoter were spotted in 10-fold dilution series on uracil-free media and incubated for 48 hours at the permissive- and restrictive temperatures of 30°C and 37°C. (B) Isogenic *psd1Δ* *stt4^{G1782D}* yeast transformed with YCp(*URA3*) or derivatives driving individual expression of *STT4*, *stt4^{G1782D}* or *stt4^{D1752A}* genes from the natural *STT4* promoter were spotted in 10-fold dilution series on uracil-free media +/- 2 mM Etn and incubated at 30°C for 48 hours. (C) Isogenic yeast strains of the designated genotype were spotted in 10-fold dilution series on synthetic complete (SC) media +/- 2 mM Etn

and incubated at 30°C for 48 hours. **(D)** Isogenic *psd1Δ* or *psd1Δ stt4^{G1782D}* yeast transformed with multicopy YEp vectors driving *YPP1*, *EFR3* or *SFK1* over-expression (from their respective natural promoters) were spotted in 10-fold dilution series on uracil-free media +/- 2 mM Etn and incubated at 30°C for 48 hours.

Figure S3. Sfh4::PtdIns homology model and the PtdIns-binding motif. **(A)** Isogenic *psd1Δ sfh4Δ* yeast transformed with yeast episomal *URA3* plasmids driving high levels of expression of each individual member of the yeast Sec14-like PITP family (identified at left), from the *PMA1* promoter, were spotted in a 10-fold dilution series on uracil-free selection media with or without Etn as indicated at top. Plates were incubated at 30°C for 48 hours. **(B)** Figure illustrates homology model of Sfh4::PtdIns complex (gray ribbon) threaded onto the Sfh3 crystal structure (PDB i.d 4J7Q) template (orange ribbon). **(C)** Sfh4::PtdIns homology model (gray ribbon) is superimposed onto the Sfh1 structure (PDB i.d 3B7N, brown ribbon). Figure shows conservation of structural fold and of the PtdIns binding pose. **(D)** Homology model of the Sfh4::PtdIns complex is rendered as gray ribbon. Enlarged panel highlights the conserved Sec14-like PITP PtdIns headgroup coordination motif, and illustrate conserved residues T₂₆₆ and K₂₆₉ interacting with PtdIns head group phosphate via a network of hydrogen bonds. **(E)** Fluorescence dequenching PtdIns-transfer assay for purified recombinant Sfh4 and Sfh4^{266D,K269A}. Fluorescence intensity of pyrene-PtdIns is plotted as a function of time. Black arrows identify points at which a 1 ug increment of the indicated protein was added. After protein addition, the observed increase in fluorescence intensity is directly proportional to the relative transfer efficiency of the protein being assayed. Values represent averages from two independent experiments plotted as mean ± standard deviation.

Figure S4. PtdIn4P phosphatase and Sfh4/Stt4 requirement for Psd2 function. (A)

Yeast growth assay showing that *sac1Δ* gene failed to restore Psd2 pathway activity in mutants ablated for Sfh4 function and compromised for Stt4 activity. Isogenic *psd1Δ*, *psd1Δ stt4^{G1782D}*, *psd1Δ stt4^{G1782D} sac1Δ*, *psd1Δ sfh4Δ*, *psd1Δ sfh4Δ sac1Δ* yeast were spotted in 10-fold dilution series on SC media +/- 2 mM Etn and incubated at 30°C for 48 hours. **(B)** Yeast growth assay showing transplacement of *inp51Δ*, *inp52Δ*, *inp53Δ*, *inp54Δ* alleles into a *psd1Δ sfh4Δ* strain failed to rescue Psd2 activity. Isogenic yeast strains with the designated genotype were spotted in 10-fold dilution series on SC media +/- 2 mM Etn and incubated at 30°C for 48 hours.

Figure S5. The mutagenic PCR screen and the fluorescence dequenching PtdIns-transfer assay of *sfh4* mutants. (A) Design of the mutagenic PCR screen for isolation of Sfh4 mutants

specifically defective in Psd2 activity. A PCR-mutagenized *SFH4* fragment library was co-transformed with a gapped YCp(*URA3*) plasmid into the *ura3 sfh4Δ psd1Δ sec14^{ts}* recipient strain. Transformants displaying the unselected parental Etn auxotrophy and the ability to grow at 37°C were identified. **(B)** Fluorescence dequenching PtdIns-transfer assay for purified recombinant Sfh4, Sfh4^{F175L}, Sfh4^{F175A}, Sfh4^{F175R} and Sfh4^{F175E} proteins. Fluorescence intensity of pyrene-PtdIns is plotted as a function of time. Black arrows identify points at which a 1 ug increment of the indicated protein was added. After protein addition, the observed increase in fluorescence intensity is directly proportional to the relative transfer efficiency of the protein being assayed. Values represent averages from two independent experiments plotted as mean ± standard deviation. These mutant Sfh4 data were obtained in the same experiments as the data reported in Suppl. Figure S3E, so the same Sfh4 control data are represented in both Figures.

Figure S6. Co-Precipitation of Stt4 with Psd2. **(A)** Wild-type yeast cells, where *STT4-TAP* or *SFH3-TAP* cassettes were transplaced into the corresponding gene loci, were transformed with a multicopy YEp plasmid driving Psd2-HA expression. Transformants were cultured to mid-log phase and cell-free lysates prepared. Co-precipitation experiments were performed as described in the legend to Figure 5A. **(B)** Wild-type or *psd2Δ* yeast cells with *STT4-TAP*, *stt4^{G1782D}-TAP* or *PIK1-TAP* cassettes transplaced for the endogenous genes were transformed with a YEp yeast episomal plasmid expressing Psd2-HA, were cultured to mid-log phase and cell-free lysates prepared. Co-IP experiment was performed as described in the legend to Figure 5A.

Figure S7. Relationship between PtdSer decarboxylation pathways and de novo PtdCho biosynthesis. Isogenic yeast strains with the designated genotype were spotted in 10-fold dilution series on SC media +2 mM Etn, +10 uM choline, +100 uM choline or without Etn or choline and incubated at 30°C for 48 hours.

Figure S1

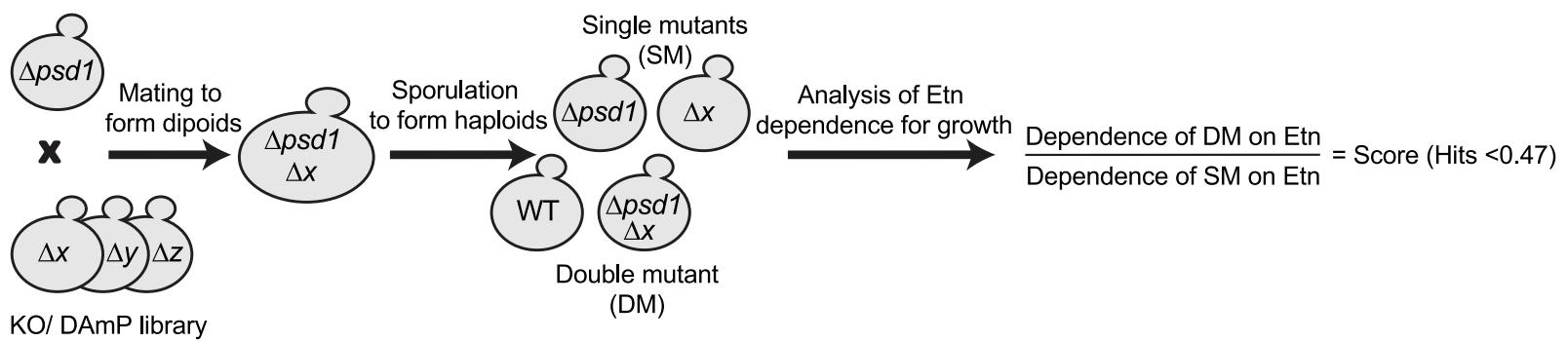
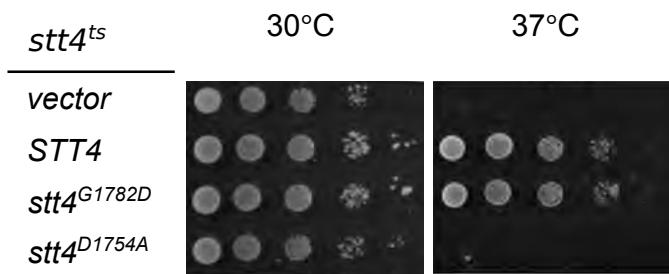
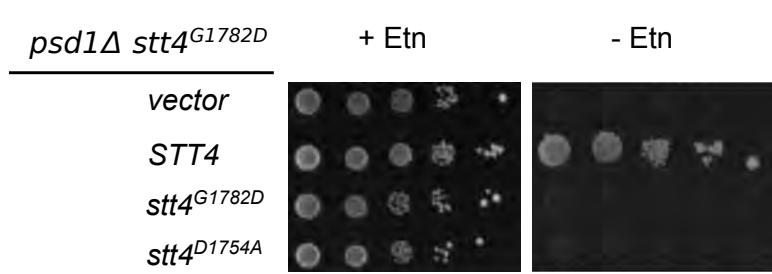


Figure S2

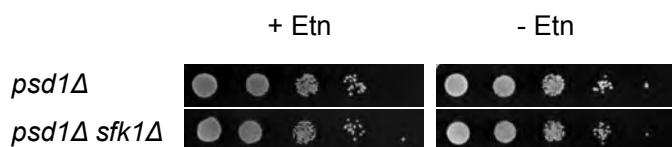
A



B



C



D

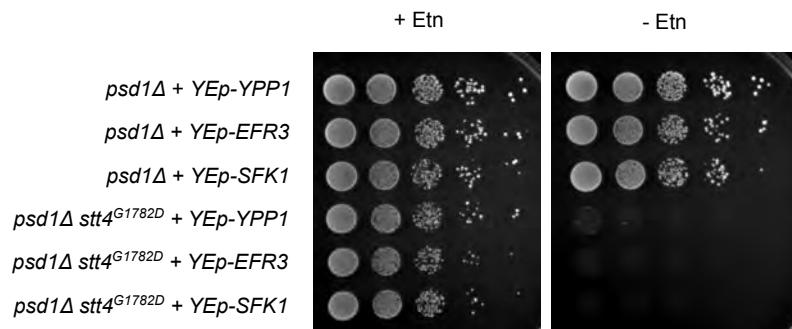
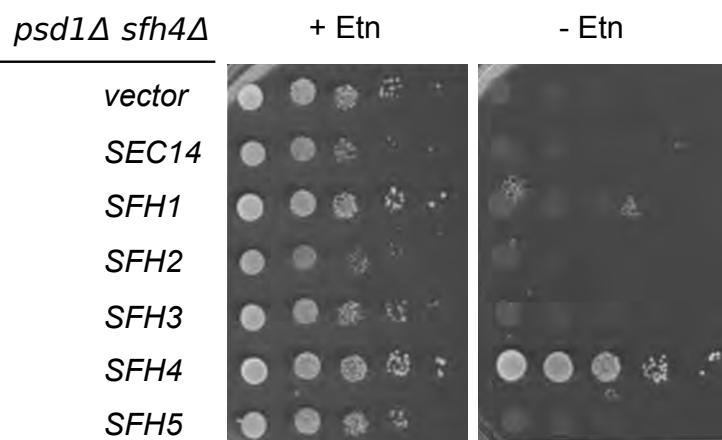
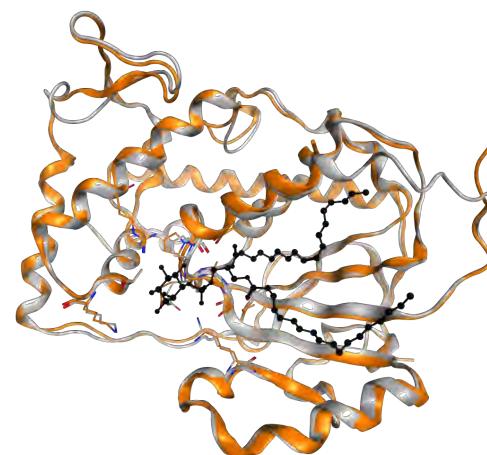


Figure S3

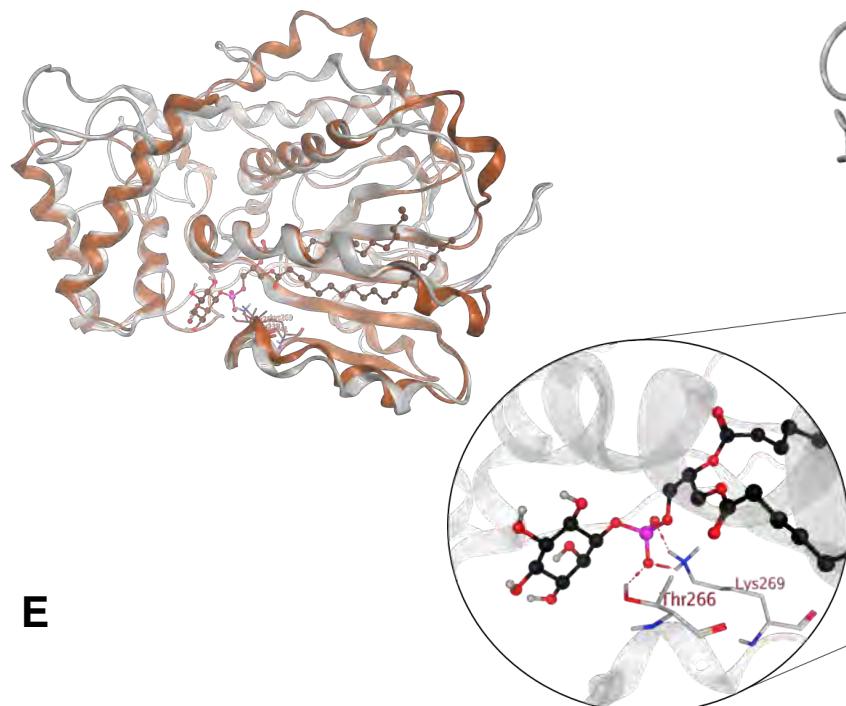
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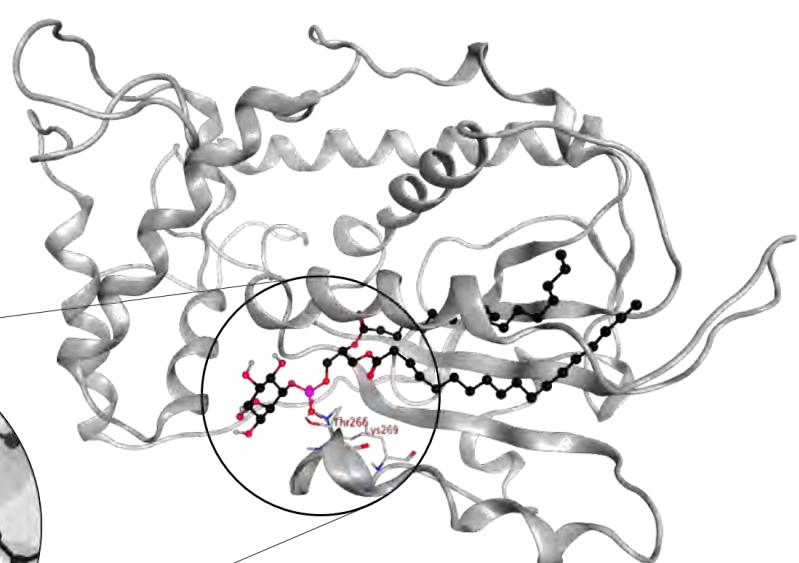
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C



D



E

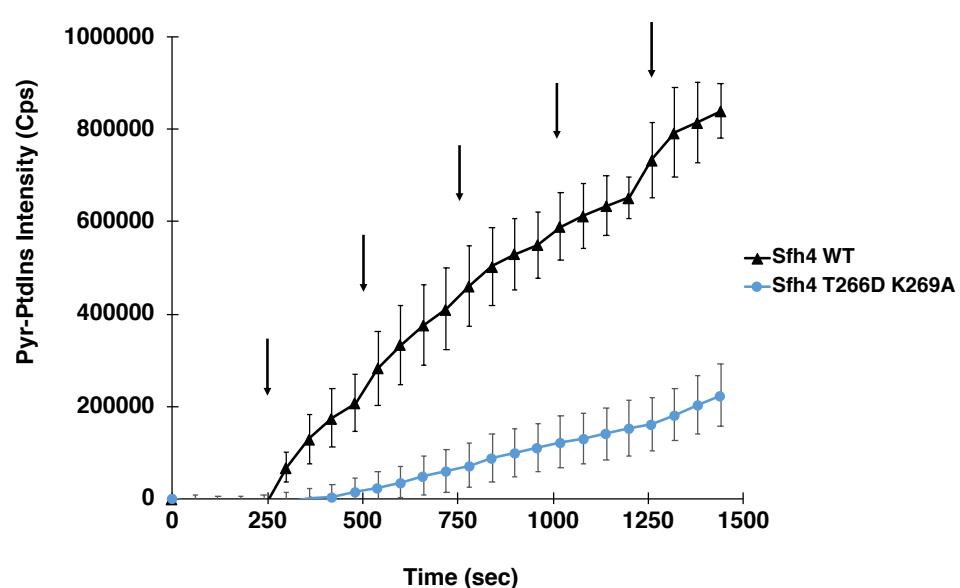
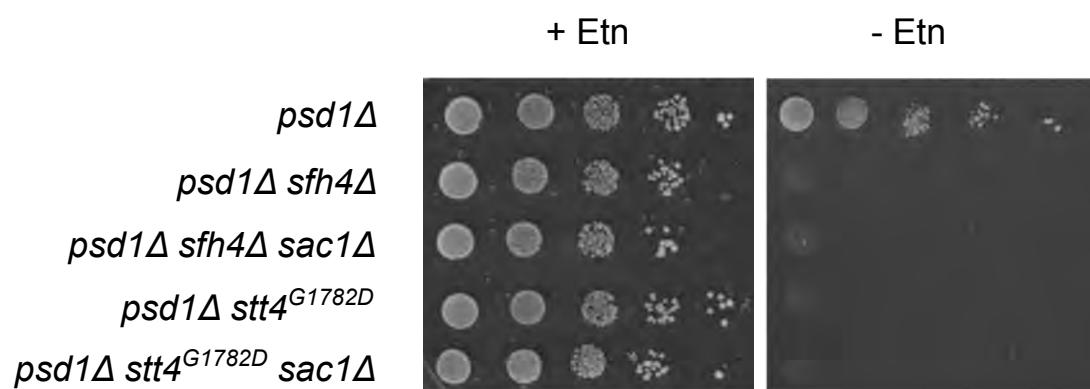


Figure S4

A



B

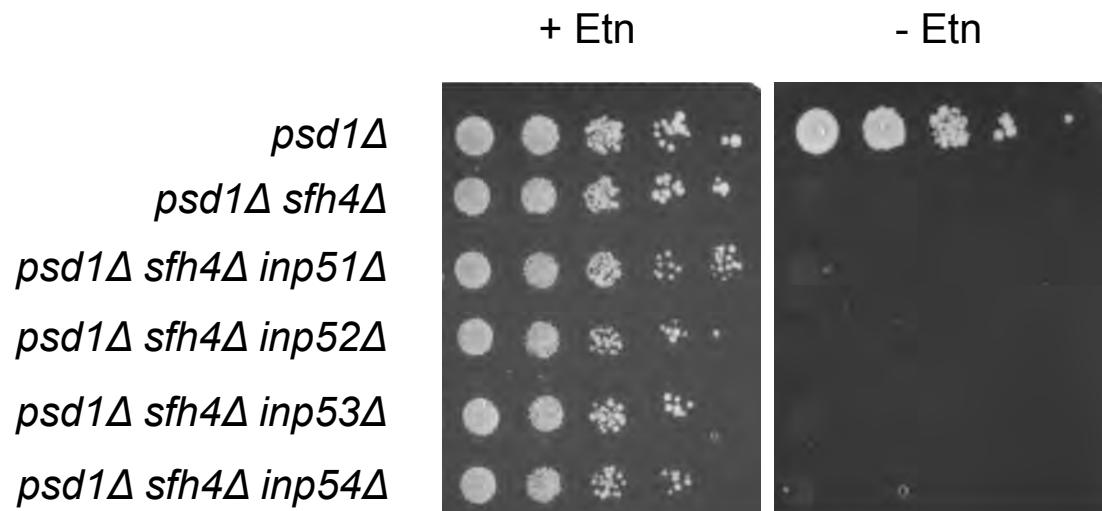
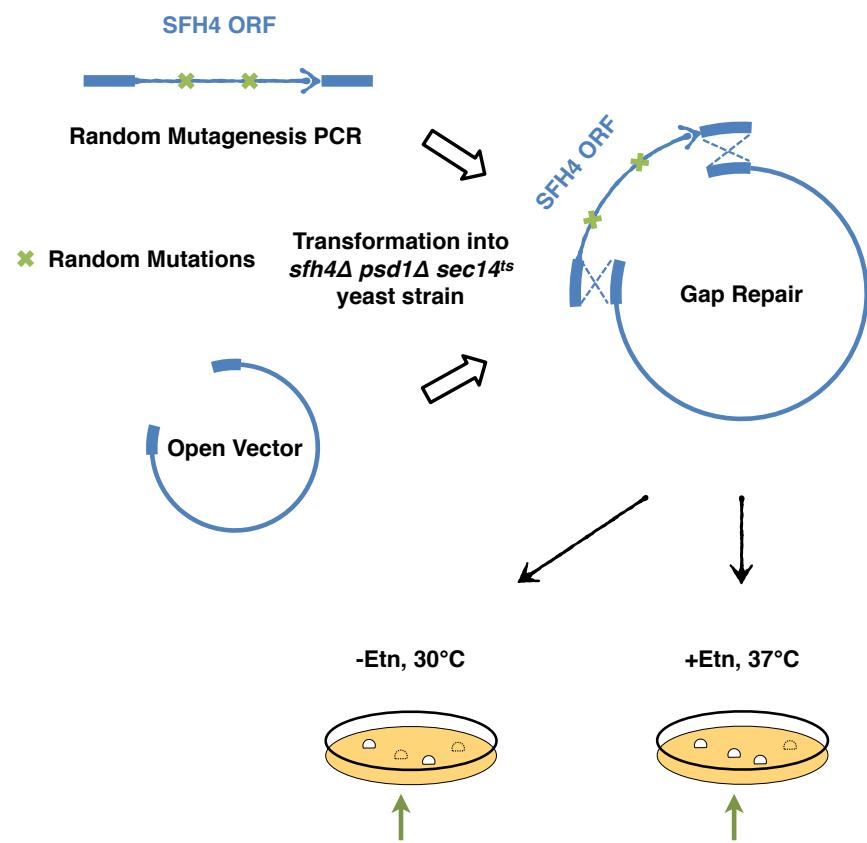


Figure S5

A



B

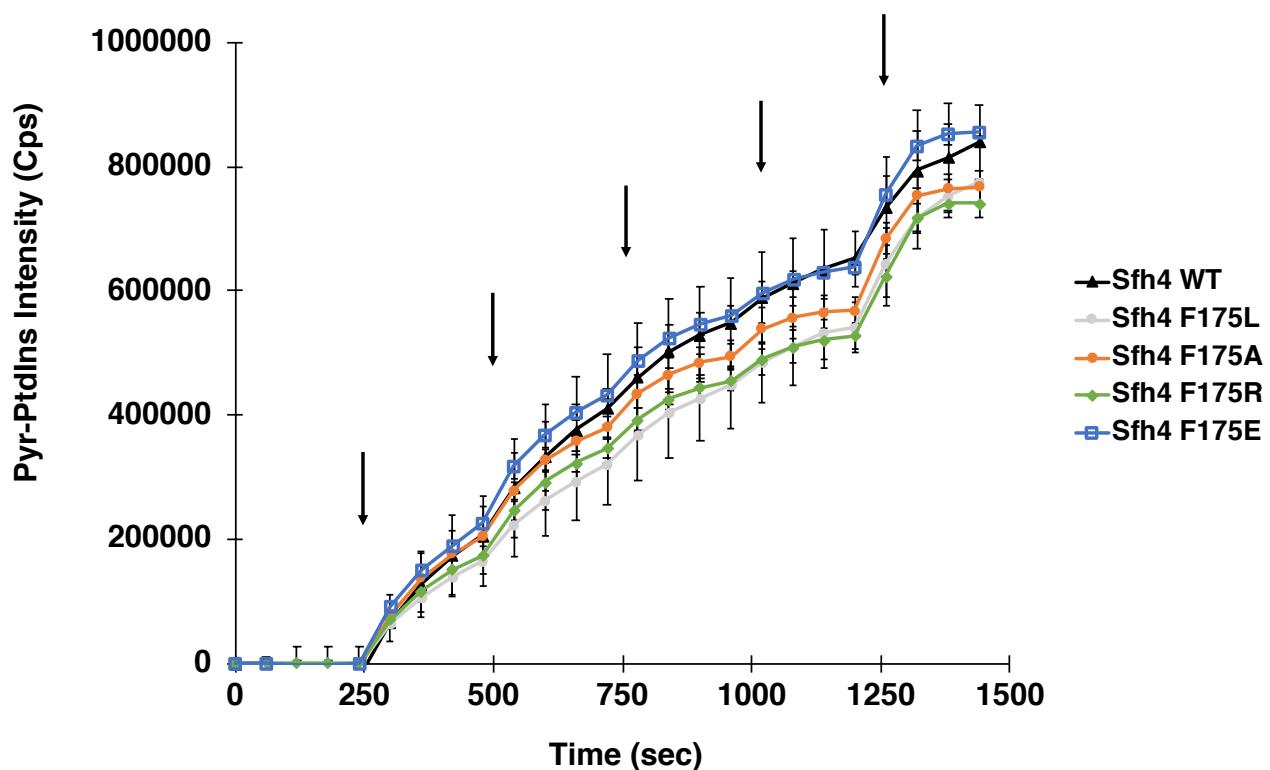


Figure S6

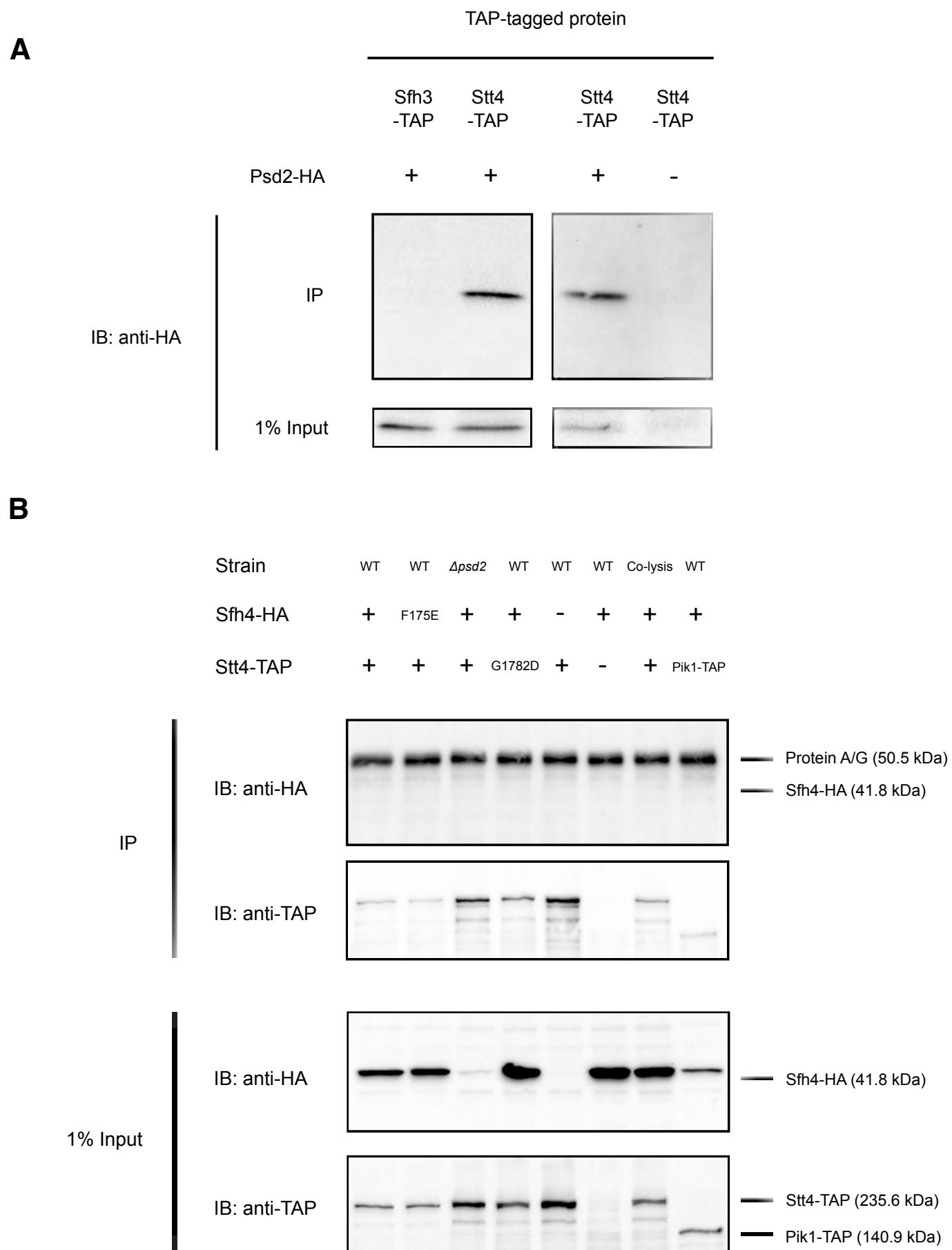
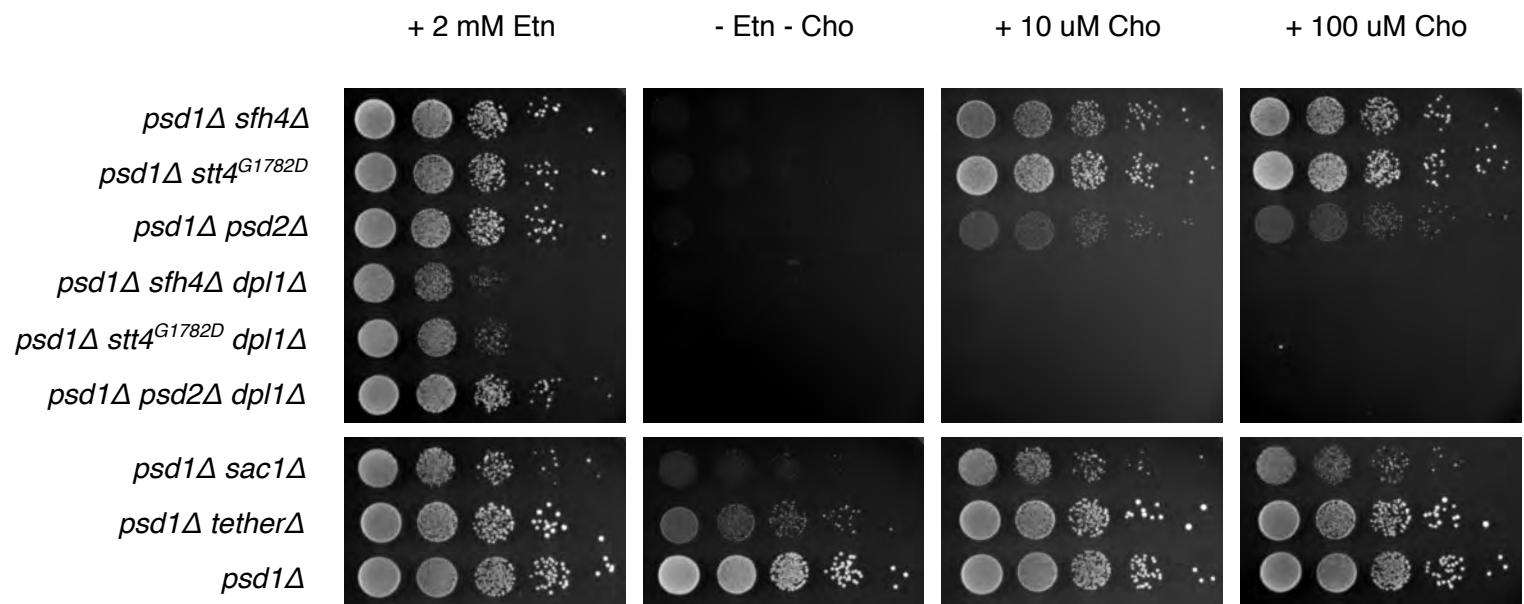


Figure S7



Legends to Tables

Table S1. A Synthetic Genetic Arrays (SGA) screen interrogating yeast genome for the loss-of-function (LOF) of any of yeast non-essential gene(s) which deactivates Psd2 pathway. The list of hits from the screen performed to identify non-essential genes whose deletion, in combination with a *psd1Δ* allele, resulted in reduced colony size when cultured on Etn-free media (see Suppl. Figure S1). SM= Single mutants, DM= Double mutants. Strains with colony size smaller than 50, when grown on Etn-replete medium, were considered as background and were omitted.

Supplementary Table S1. Genes whose deletion/hypomorphic allele in combination with a *psd1* Δ allele resulted in a growth delay on media without ethanolamine (Etn)

ORF	Gene	Description	Area SM+Etn	Area SM-Etn	Area DM+Etn	Area DM-Etn	DM-Etn/DM+Etn	SM-Etn/SM+Etn	(DM-Etn/DM+Etn)/(SM-Etn/SM+Etn)
YNL264C	SFH4	Phosphatidylinositol transfer protein (PITP); downregulates Plb1p-mediated vesicle formation	185	125	75	0	0.00	0.68	0.00
YGR170W	PSD2	Phosphatidylerine decarboxylase of the Golgi and vacuolar membranes	160	120	146	18	0.12	0.75	0.16
YDL006W	PTC1	Type 2C protein phosphatase (PP2C); dephosphorylates Hog1p, inactivating it	160	325	208	118	0.57	2.03	0.28
YDR322C	TIM11	Subunit e of mitochondrial F1FO-ATPase; ATPase is a large, evolutionarily conserved protein	164	181	81	25	0.31	1.10	0.28
YIL123W	SIM1	Protein of the SUN family (Simp1, Uth1p, Nca3p, Sun4p); may participate in nuclear envelope structure	171	150	371	93	0.25	0.88	0.29
YIL162W	SUC2	Invertase; sucrose hydrolyzing enzyme; a secreted, glycosylated form is involved in yeast mating	189	386	210	125	0.60	2.04	0.29
YNL124W	NAF1	RNA-binding protein required for the assembly of box H/ACA snoRNPs; involved in rRNA processing	211	141	277	55	0.20	0.67	0.30
YLR087C	CSF1	Protein required for fermentation at low temperature; the authentic, non-tagged protein	94	86	97	27	0.28	0.91	0.30
YIL121W	QDR2	Plasma membrane transporter of the major facilitator superfamily; mem	164	127	365	88	0.24	0.77	0.31
YNL050C	YNL050C	Putative protein of unknown function; YNL050c is not an essential gene	215	473	167	117	0.70	2.20	0.32
YBR137W	YBR137W	Protein of unknown function; localized to the cytoplasm; binds to Replicator RNA	197	152	387	97	0.25	0.77	0.32
YDR394W	RPT3	ATPase of the 19S regulatory particle of the 26S proteasome; one of ATPases involved in protein degradation	194	135	301	69	0.23	0.70	0.33
YDL095W	PMT1	Protein O-mannosyltransferase of the ER membrane; transfers mannose to proteins	163	135	310	90	0.29	0.83	0.35
YLR262C	TMA7	Protein of unknown that associates with ribosomes; null mutant exhibits growth delay on media without ethanolamine	139	106	62	17	0.27	0.76	0.36
YIL040W	APQ12	Protein required for nuclear envelope morphology; nuclear pore complex component	79	111	75	38	0.51	1.41	0.36
YDR438W	THI74	Mitochondrial transporter repressible by thiamine; THI74 has a paralog, YDR439W	170	138	353	104	0.29	0.81	0.36
YBR058C	TSC3	Protein that stimulates the activity of serine palmitoyltransferase; involved in sphingolipid metabolism	139	148	87	34	0.39	1.06	0.37
YNL059C	ARP5	Nuclear actin-related protein involved in chromatin remodeling; component of the nucleolus	82	97	69	30	0.43	1.18	0.37
YFL031W	HAC1	Basic leucine zipper (bZIP) transcription factor (ATF/CREB1 homolog); required for growth on media without ethanolamine	146	164	115	49	0.43	1.12	0.38
YBR138C	YBR138C	Cytoplasmic protein of unknown function; potentially phosphorylated by Cdk1p	155	184	181	82	0.45	1.19	0.38
YDR202C	RAV2	Subunit of RAVE complex (Rav1p, Rav2p, Skp1p); the RAVE complex associates with the 26S proteasome	161	130	254	79	0.31	0.81	0.39
YDR080W	VPS41	Vacuolar membrane protein that is a subunit of the HOPS complex; essential for protein transport to the vacuole	108	64	83	19	0.23	0.59	0.39
YNR012W	URK1	Uridine/cytidine kinase; component of the pyrimidine ribonucleotide salvage pathway	161	346	143	120	0.84	2.15	0.39
YKL028W	TFA1	TFIIE large subunit; involved in recruitment of RNA polymerase II to the promoter	173	139	282	89	0.32	0.80	0.39
YBR029C	CDS1	Phosphatidate cytidyltransferase (CDP-diglyceride synthetase); an enzyme involved in sphingolipid metabolism	179	155	295	102	0.35	0.87	0.40
YFL031W	HAC1	Basic leucine zipper (bZIP) transcription factor (ATF/CREB1 homolog); required for growth on media without ethanolamine	144	160	81	36	0.44	1.11	0.40
YHR079C	IRE1	Serine-threonine kinase and endoribonuclease; transmembrane protein involved in unfolded protein response	132	116	125	44	0.35	0.88	0.40
YKL212W	SAC1	Phosphatidylinositol phosphate (PtdInsP) phosphatase; involved in hydronium ion homeostasis	114	99	66	23	0.35	0.87	0.40
YLR276W	DBP9	DEAD-box protein required for 27S rRNA processing; exhibits DNA, RNA binding properties	213	133	327	83	0.25	0.62	0.41
YNL041C	COG6	Component of the conserved oligomeric Golgi complex; a cytosolic tethether	161	106	183	49	0.27	0.66	0.41
YDR444W	YDR444W	Putative hydrolase acting on ester bonds	175	242	142	81	0.57	1.38	0.41
YGL140C	YGL140C	Putative protein of unknown function; non-essential gene; contains multiple open reading frames	203	220	205	92	0.45	1.08	0.41
YKR031C	SPO14	Phospholipase D; catalyzes the hydrolysis of phosphatidylcholine, producing two fatty acids and choline	118	182	97	62	0.64	1.54	0.41
YGR24W	ADE3	Cyttoplasmic trifunctional enzyme C1-tetrahydrofolate synthase; involved in folate biosynthesis	179	184	248	106	0.43	1.03	0.42
YPR079W	MRL1	Membrane protein; has similarity to mammalian mannose-6-phosphate isomerase	201	314	175	114	0.65	1.56	0.42
YML049C	RSE1	Protein involved in pre-mRNA splicing; component of the pre-spliceosome	203	122	330	83	0.25	0.60	0.42
YML085C	TUB1	Alpha-tubulin; associates with beta-tubulin (Tub2p) to form tubulin dimers	199	139	372	109	0.29	0.70	0.42
YEI036C	ANP1	Subunit of the alpha-1,6-mannosidase complex; type II membrane protein	202	184	278	107	0.38	0.91	0.42
YNL191W	DUG3	Component of glutamine amidotransferase (GATase III); forms a complex with Dug1p and Dug2p	158	171	48	22	0.46	1.08	0.42
YJR039W	YJR039W	Putative protein of unknown function; the authentic, non-tagged protein	200	209	201	89	0.44	1.05	0.42
YBR298C	MAL31	Maltose permease; high-affinity maltose transporter (alpha-glucosidase transporter)	193	354	137	107	0.78	1.83	0.43
YNL098C	RAS2	GTP-binding protein; regulates nitrogen starvation response, sporulation	124	240	99	84	0.85	1.94	0.44
YER183C	FAU1	5,10-methenyltetrahydrofolate synthetase; involved in folic acid biosynthesis	206	214	173	79	0.46	1.04	0.44
YNL154C	YCK2	Palmitoylated plasma membrane-bound casein kinase I isoform; shares a common substrate with Yck1p	147	140	57	24	0.42	0.95	0.44
YDR475C	JIP4	Protein of unknown function; previously annotated as two separate ORFs	184	305	166	123	0.74	1.66	0.45
YPR133W	TOM5	Component of the TOM (translocase of outer membrane) complex; responsible for import of mitochondrial proteins	166	155	213	89	0.42	0.93	0.45
YBL071C	YBL071C	Putative protein of unknown function; identified by gene-trapping, microarray analysis, and mass spectrometry	163	147	265	107	0.40	0.90	0.45
YNL230C	ELA1	Elongin A; F-box protein that forms a heterodimer with Elc1p and is required for growth on media without ethanolamine	101	195	52	45	0.87	1.93	0.45
YDL085W	NDE2	Mitochondrial external NADH dehydrogenase; catalyzes the oxidation of NADH to NAD ⁺	166	344	126	118	0.94	2.07	0.45
YBR133C	HSL7	Protein arginine N-methyltransferase; exhibits septin and Hsl1p-dependent activity	162	130	281	102	0.36	0.80	0.45
YML071C	COG8	Component of the conserved oligomeric Golgi complex; a cytosolic tethether	167	125	162	55	0.34	0.75	0.45
YCR060W	TAH1	Component of conserved R2TP complex (Rvb1p-Rvb2p-Tah1p-Phf1p); R2TP complex	189	191	200	92	0.46	1.01	0.46
YGR252W	GCNS	Catalytic subunit of ADA and SAGA histone acetyltransferase complexes; involved in transcriptional regulation	206	188	187	78	0.42	0.91	0.46
YGL201C	MCM2	Protein involved in DNA replication; component of the Mcm2-7 hexamer	190	131	269	85	0.32	0.69	0.46
YBR172C	SMY2	Protein of unknown function involved in COPII vesicle formation; interacts with Sec23p and Sec24p	159	236	166	113	0.68	1.48	0.46
YIL179W	PFD1	Subunit of heterohexameric prefoldin; prefoldin binds cytosolic chaperone proteins	167	154	81	35	0.43	0.92	0.47

Supplementary Table S2. Yeast Strains.

Identifier	Genotype	Source
CTY182	<i>MAT_a ura3-52 lys2-801 his3-Δ200</i>	(Bankaitis, Malehorn et al., 1989)
CTY1-1A	<i>MAT_a ura3-52 lys2-801 his3-Δ200 sec14-1ts</i>	(Bankaitis et al., 1989)
PYY19	<i>MAT_a ura3-52 lys2-801 his3-Δ200 sfh4Δ::Ura psd1Δ::KanMX</i>	This study
PYY23	<i>MAT_a ura3-52 lys2-801 his3-Δ200 psd1Δ::KanMX</i>	This study
PYY29	<i>MAT_a ura3-52 lys2-801 his3-Δ200 psd2Δ::KanMX</i>	This study
PYY30	<i>MAT_a ura3-52 lys2-801 his3-Δ200 sfh4Δ::HIS psd1Δ::KanMX</i>	This study
PYY31	<i>MAT_a ura3-52 lys2-801 his3-Δ200 psd1Δ::KanMX scs2Δ::HIS3</i>	This study
PYY32	<i>MAT_a ura3-52 lys2-801 his3-Δ200 psd1Δ::KanMX scs22Δ::HIS3</i>	This study
YWY200	<i>MAT_a ura3-52 lys2-801 his3-Δ200 psd1Δ::KanMX pbi1Δ::HIS3</i>	This study
PYY36	<i>MAT_a ura3-52 lys2-801 his3-Δ200 sfh4Δ::HIS3</i>	This study
PYY40	<i>MAT_a ura3-52 lys2-801 his3-Δ200 sfh4Δ::KanMX</i>	This study
PYY49	<i>MAT_a ura3 met14 lys2 trp1 psd1::TRP1 or psd1Δ-1::TRP1 pstB1</i>	(Trotter, Wu et al., 1998)
PYY52	<i>MAT_a ura3-52 lys2-801 his3-Δ200 psd1Δ::KanMX scs2Δ::HIS3 scs22Δ::NatMX</i>	This study
PYY53	<i>CTY1-1A sac1-26 sfh4Δ::KanMX</i>	This study

PYY54	PYY49 <i>sac1Δ::NatMX</i>	This study
PYY55	PYY30 <i>sac1Δ::NatMX</i>	This study
PYY84	<i>MATa ura3-52 lys2-801 his3-Δ200 sfh4Δ::HIS psd1Δ::KanMX</i> <i>inp51Δ::NatMX</i>	This study
PYY85	<i>MATa ura3-52 lys2-801 his3-Δ200 sfh4Δ::HIS psd1Δ::KanMX</i> <i>inp52Δ::NatMX</i>	This study
PYY86	<i>MATa ura3-52 lys2-801 his3-Δ200 sfh4Δ::HIS psd1Δ::KanMX</i> <i>inp53Δ::NatMX</i>	This study
PYY87	<i>MATa ura3-52 lys2-801 his3-Δ200 sfh4Δ::HIS psd1Δ::KanMX</i> <i>inp54Δ::NatMX</i>	This study
PYY97	<i>MATa ura3-52 lys2-801 his3-Δ200 sac1-354::HIS3 psd1Δ::KanMX</i>	This study
YWY34	<i>MATa ura3 met14 lys2 trp1 psd1::TRP1 or psd1Δ-1::TRP1 pstB1</i> <i>scs2Δ::KanMX</i>	This study
YWY35	<i>MATa ura3-52 lys2-801 his3-Δ200 sfh4Δ::Ura psd1Δ::KanMX</i> <i>scs2Δ::HIS3</i>	This study
YWY36	<i>MATa ura3-52 lys2-801 his3-Δ200 sfh4Δ::Ura psd1Δ::KanMX</i> <i>pbi1Δ::HIS3</i>	This study
YWY37	<i>MATa ura3-52 lys2-801 his3-Δ200 psd1Δ::KanMX</i> <i>scs2Δ::His scs22Δ::NatMX</i> <i>sfh4Δ::URA3</i>	This study
YWY38	<i>MATa ura3 met14 lys2 trp1 psd1::TRP1 or psd1Δ-1::TRP1 pstB1</i> <i>pbi1Δ::URA3</i>	This study
YWY40	<i>MATa ura3-52 lys2-801 his3-Δ200 sfh4Δ::HIS psd1Δ::KanMX</i> <i>pbi1Δ::URA3</i>	This study
YWY46	<i>MATa ura3-52 lys2-801 his3-Δ200 sfh4Δ::HIS psd1Δ::KanMX</i> <i>scs22Δ::URA3</i>	This study
YWY47	<i>MATa ura3 met14 lys2 trp1 psd1::TRP1 or psd1Δ-1::TRP1 pstB1</i> <i>scs22Δ::URA3</i>	This study
YWY48	<i>MATa ura3-52 lys2-801 his3-Δ200 sfh4Δ::Ura psd1Δ::KanMX</i> <i>scs2Δ::HIS3 scs22Δ::NatMX</i>	This study

YWY49	<i>MAT_a ura3 met14 lys2 trp1 psd1::TRP1 or psd1Δ-1::TRP1 pstB1 scs2Δ::NEO scs22Δ::NatMX</i>	This study
YWY60	<i>MAT_a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SFH3-TAP::HIS3MX6</i>	Dharmacon
YWY64	<i>MAT_a ura3-52 lys2-801 his3-Δ200 SFH4-TAP::HIS3</i>	This study
YWY65	<i>MAT_a ura3-52 lys2-801 his3-Δ200 sfh4^{F175L}-TAP::HIS3</i>	This study
YWY66	<i>MAT_a ura3-52 lys2-801 his3-Δ200 sfh4^{F175A}-TAP::HIS3</i>	This study
YWY69	<i>MAT_a ura3-52 lys2-801 his3-Δ200 stt4^{G1782D}::NatMX4</i>	This study
YWY70	<i>MAT_a ura3-52 lys2-801 his3-Δ200 psd1Δ::KanMX stt4^{G1782D}::NatMX4</i>	This study
YWY71	<i>MAT_a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SFH3-TAP::HIS3MX6 stt4^{G1782D}::NatMX4</i>	This study
YWY72	<i>MAT_a ura3-52 lys2-801 his3-Δ200 SFH4-TAP::HIS3MX6 stt4^{G1782D}::NatMX4</i>	This study
YWY73	<i>MAT_a ura3-52 lys2-801 his3-Δ200 sfh4F175L-TAP::HIS3MX6 stt4^{G1782D}::NatMX4</i>	This study
YWY74	<i>MAT_a ura3-52 lys2-801 his3-Δ200 sfh4F175A-TAP::HIS3MX6 stt4^{G1782D}::NatMX4</i>	This study
YWY75	<i>MAT_a ura3-52 lys2-801 his3-Δ200 Δpsd1::KanMX STT4::NatMX4</i>	This study
YWY76	<i>MAT_a ura3-52 lys2-801 his3-Δ200 sfh4^{T266D,K269A}-TAP::HIS3</i>	This study
YWY104	<i>MAT_a ura3-52 lys2-801 his3-Δ200 dpl1Δ::KanMX4</i>	This study
YWY106	<i>MAT_a ura3-52 lys2-801 his3-Δ200 psd1Δ::HIS3</i>	This study
YWY108	<i>MAT_a ura3-52 lys2-801 his3-Δ200 Δect1::KanMX Δpsd1::HIS3</i>	This study

YWy110	<i>MAT_a ura3-52 lys2-801 his3-Δ200 ect1Δ::KanMX psd2Δ::HIS3</i>	This study
YWy111	<i>MAT_a ura3-52 lys2-801 his3-Δ200 stt4G1782D::NatMX4 psd2Δ::HIS3</i>	This study
YWy112	<i>MAT_a ura3-52 lys2-801 his3-Δ200 psd1Δ::NatMX6</i>	This study
PYy90	<i>MAT_a ura3-52 lys2-801 his3-Δ200 psd1Δ::KanMX osh6Δ::NatMX</i>	This study
YWy128	<i>MAT_a ura3-52 lys2-801 his3-Δ200 sfh4Δ::HIS psd1Δ::KanMX osh6Δ::URA3</i>	This study
YWy129	<i>MAT_a ura3 met14 lys2 trp1 psd1::TRP1 or psd1Δ-1::TRP1 pstB1 osh6Δ::URA3</i>	This study
PYy91	<i>MAT_a ura3-52 lys2-801 his3-Δ200 psd1Δ::KanMX osh7Δ::NatMX</i>	This study
YWy131	<i>MAT_a ura3-52 lys2-801 his3-Δ200 Δsfh4::HIS psd1Δ::KanMX osh7Δ::URA3</i>	This study
YWy132	<i>MAT_a ura3 met14 lys2 trp1 psd1::TRP1 or psd1Δ-1::TRP1 pstB1 osh7Δ::URA3</i>	This study
YWy136	<i>MAT_a ura3-52 lys2-801 his3-Δ200 psd1Δ::KanMX osh1Δ::URA3</i>	This study
YWy137	<i>MAT_a ura3 met14 lys2 trp1 psd1::TRP1 or psd1Δ-1::TRP1 pstB1 osh1Δ::URA3</i>	This study
YWy138	<i>MAT_a ura3-52 lys2-801 his3-Δ200 psd1Δ::KanMX osh2Δ::URA3</i>	This study
YWy139	<i>MAT_a ura3 met14 lys2 trp1 psd1::TRP1 or psd1Δ-1::TRP1 pstB1 osh2Δ::URA3</i>	This study
YWy140	<i>MAT_a ura3-52 lys2-801 his3-Δ200 psd1Δ::KanMX osh3Δ::URA3</i>	This study
YWy141	<i>MAT_a ura3 met14 lys2 trp1 psd1::TRP1 or psd1Δ-1::TRP1 pstB1 osh3Δ::URA3</i>	This study
YWy142	<i>MAT_a ura3-52 lys2-801 his3-Δ200 psd1Δ::KanMX osh4Δ::URA3</i>	This study

YWY143	<i>MATa ura3 met14 lys2 trp1 psd1::TRP1 or psd1Δ-1::TRP1 pstB1 osh4Δ::KanMX4</i>	This study
YWY144	<i>MATa ura3-52 lys2-801 his3-Δ200 psd1Δ::KanMX osh5Δ::URA3</i>	This study
YWY145	<i>MATa ura3 met14 lys2 trp1 psd1::TRP1 or psd1Δ-1::TRP1 pstB1 osh5Δ::URA3</i>	This study
YWY146	<i>MATa ura3-52 lys2-801 his3-Δ200 STT4-TAP::HIS3MX6</i>	This study
YWY147	<i>MATa ura3-52 lys2-801 his3-Δ200 psd2Δ::KanMX STT4-TAP::HIS3MX6</i>	This study
YWY148	<i>MATa ura3-52 lys2-801 his3-Δ200 sfh4Δ::KanMX STT4-TAP::HIS3MX6</i>	This study
YWY149	<i>MATa ura3-52 lys2-801 his3-Δ200 stt4^{G1782D}-TAP::HIS3MX6</i>	This study
YWY152	<i>MATa ura3-52 lys2-801 his3-Δ200 sfh4Δ::HIS psd1Δ::KanMX osh1Δ::URA3</i>	This study
YWY153	<i>MATa ura3-52 lys2-801 his3-Δ200 sfh4Δ::HIS psd1Δ::KanMX osh2Δ::URA3</i>	This study
YWY154	<i>MATa ura3-52 lys2-801 his3-Δ200 sfh4Δ::HIS psd1Δ::KanMX osh3Δ::URA3</i>	This study
YWY155	<i>MATa ura3-52 lys2-801 his3-Δ200 sfh4Δ::HIS psd1Δ::KanMX osh4Δ::URA3</i>	This study
YWY156	<i>MATa ura3-52 lys2-801 his3-Δ200 sfh4Δ::HIS psd1Δ::KanMX osh5Δ::URA3</i>	This study
SEY6210.1	<i>MATa leu2-3,112 ura3-52 his3-Δ200 trp1-Δ901 lys2-801 suc2-Δ9</i>	(Stefan, Audhya et al., 2002)
ANDY198	<i>SEY6210.1 ist2Δ::HISMX6 scs2Δ::TRP1 scs22Δ::HISMX6 tcb1Δ::KANMX6 tcb2Δ::KANMX6 tcb3Δ::HISMX6</i>	(Manford, Stefan et al., 2012)
YWY167	<i>SEY6210.1 psd1Δ::NatMX6</i>	This study
YWY168	<i>ANDY198 psd1Δ::NatMX6</i>	This study

YWY169	<i>SEY6210.1 psd2Δ::NatMX</i>	This study
YWY170	<i>ANDY198 psd2Δ::NatMX</i>	This study
YWY182	<i>MATa ura3-52 lys2-801 his3-Δ200 psd1Δ::HIS3 vps13Δ::KanMX4</i>	This study
YWY193	<i>MAT<u>a</u> ura3-52 lys2-801 his3-Δ200 psd1Δ::HIS3 lam5Δ::KanMX4</i>	This study
YWY197	<i>MAT<u>a</u> ura3-52 lys2-801 his3-Δ200 psd1Δ::HIS3 lam6Δ::KanMX4</i>	This study

Supplementary Table 3. Plasmid List.

Identifier	Recombinant DNA	Source
pYW18	<i>pET28b SFH4</i>	This study
pPY126	<i>pET28b sfh4^{T266D K269A}</i>	This study
pPY127	<i>pET28b sfh4^{T266W K269A}</i>	This study
pPY130	<i>pRS316 STT4</i>	This study
pPY131	<i>pRS316 stt4^{G1782D}</i>	This study
pPY132	<i>pRS316 stt4^{D1754A}</i>	This study
pPY195	<i>pET28b sfh4^{F175L}</i>	This study
pYW13	<i>YEp352 PSD2-HA</i>	(Gulshan, Shahi et al., 2010)
pYW31	<i>pRS316 SFH4</i>	This study
pYW32	<i>pRSII426 SFH4</i>	This study
pYW35	<i>pRS316 SFH4^{F175L}</i>	This study
pYW36	<i>pRS316 SFH4^{F175A}</i>	This study
pYW37	<i>pRS316 SFH4^{F175I}</i>	This study
pYW38	<i>pRS316 SFH4^{F175R}</i>	This study
pYW39	<i>pRS316 SFH4^{F175E}</i>	This study
pYW40	<i>pRS316 SFH4^{F175W}</i>	This study
pYW41	<i>pRS316 SFH4^{F175H}</i>	This study
pYW42	<i>pRS316 SFH4^{F175Y}</i>	This study
pYW43	<i>pRSII426 SFH4^{F175L}</i>	This study
pYW44	<i>pRSII426 SFH4^{F175A}</i>	This study
pYW46	<i>pRSII426 SFH4^{F175R}</i>	This study
pYW47	<i>pRSII426 SFH4^{F175E}</i>	This study
pYW58	<i>pRS316 PGK1_{Pro}-SFH4^{F175L}</i>	This study

pYW59	<i>pRS316 PGK1_{Pro}-SFH4</i> ^{F175A}	This study
pYW61	<i>pRS316 PGK1_{Pro}-SFH4</i> ^{F175R}	This study
pYW62	<i>pRS316 PGK1_{Pro}-SFH4</i> ^{F175E}	This study
pYW66	<i>pET28b sfh4</i> ^{F175A}	This study
pYW67	<i>pET28b sfh4</i> ^{F175R}	This study
pYW68	<i>pET28b sfh4</i> ^{F175E}	This study
pYW85	<i>pBluescript II SK(+) SFH4-TAP::HIS3</i>	This study
pYW86	<i>pBluescript II SK(+) SFH4-TAP::HIS3</i>	This study
pYW88	<i>pBluescript II SK(+) sfh4</i> ^{F175L} -TAP::HIS3	This study
pYW89	<i>pBluescript II SK(+) sfh4</i> ^{F175A} -TAP::HIS3	This study
pYW90	<i>pBluescript II SK(+) sfh4</i> ^{F175R} -TAP::HIS3	This study
pYW91	<i>pBluescript II SK(+) sfh4</i> ^{F175E} -TAP::HIS3	This study
pYW93	<i>pBluescript II SK(+) stt4G1782D::NatMX</i>	This study
pYW97	<i>pRS316-sfh4</i> ^{T266D,K269A}	This study
pYW109	<i>pBluescript II SK(+) ~ sfh4</i> ^{T266D,K269A} -TAP::HIS3	This study
pYW127	<i>pBluescript II SK(+) ~ STT4-TAP::HIS3</i>	This study
pYW128	<i>pBluescript II SK(+) ~ stt4</i> ^{G1782D} -TAP::HIS3	This study
pYW134	<i>YCplac111 PACT1-GFP-Myc-HMH-RitC</i>	(Quon, Sere et al., 2018)
pYW142	<i>pRSII426 YPP1</i>	This study
pYW143	<i>pRSII426 EFR3</i>	This study
pYW144	<i>pRSII426 SFK1</i>	This study
pYW145	<i>pDR195 OSH6</i>	This study
pYW146	<i>pDR195 OSH7</i>	This study

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