Liu et al., https://doi.org/10.1083/jcb.201606059

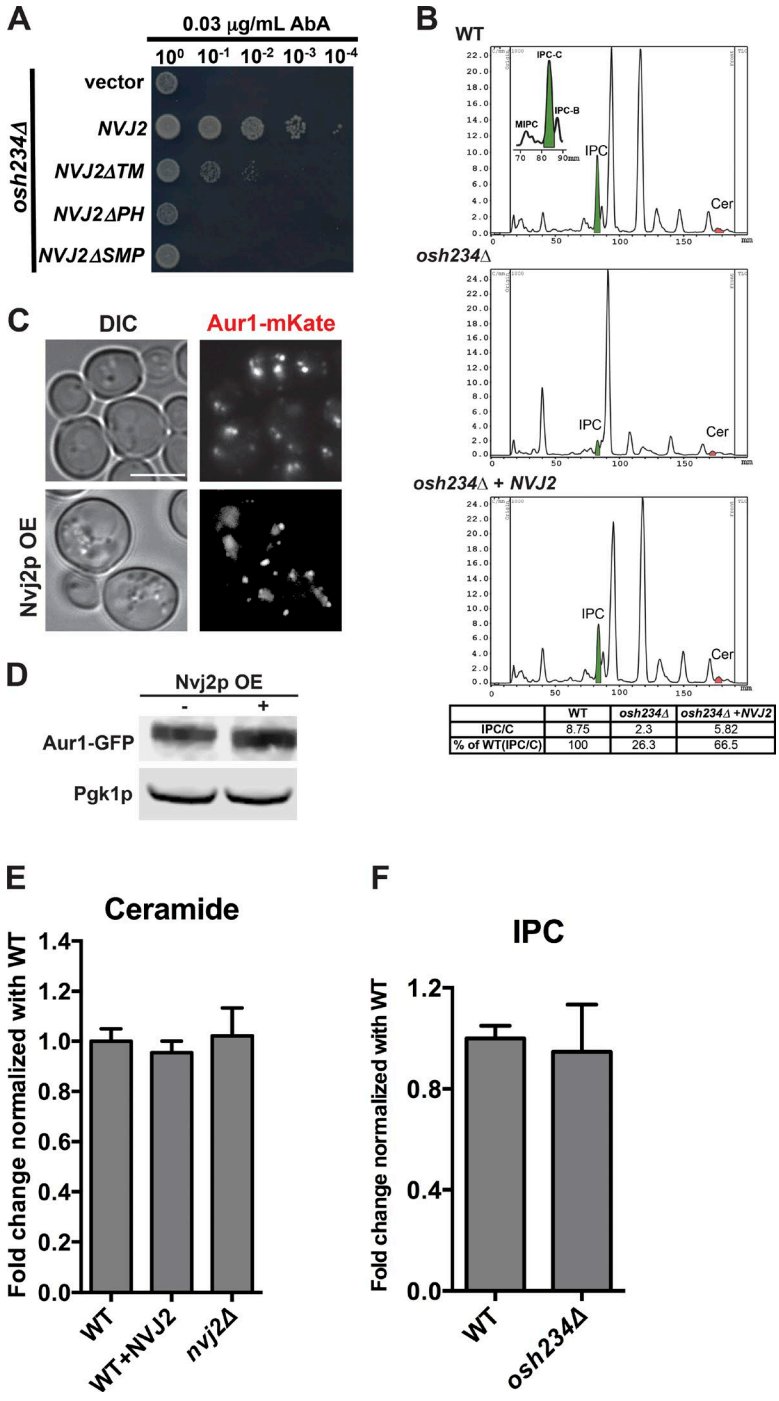

Figure S1. **Additional data related to Fig. 1.** (A) All three domains of Nvj2p are required for it to confer resistance to AbA in *osh234Δ* cells. Serial dilutions of *osh234Δ* cells with the indicated plasmids on plates containing 0.03 µg/ml AbA. (B) Representative scans of TLCs used to separate and quantitate ceramide and other sphingolipids for the results shown in Fig. 1 C. Table shows IPC/C of the indicated strains. (C) The localization of Aur1p does not change in cells overexpressing Nvj2p. Wild-type (WT) cells expressing endogenous Aur1-mKate with or without overexpression of Nvj2p (Nvj2p OE) were visualized live. Bar, 5 µm. (D) The expression of Aur1p does not change in cells overexpressing Nvj2p. Cells expressing endogenous Aur1-GFP with or without Nvj2p OE were grown to mid-logarithmic growth phase in SC medium. Cells were collected and immunoblotted with antibodies against GFP and Pgk1p. Aur1-GFP = 72 kD and Pgk1 = 45 kD. (E) Lysates from the indicated strains produce similar amounts of [^{3}H]ceramide when incubated with [^{3}H]DHS (control for experiments in Fig. 1, H–J). Lysates were incubated with [^{3}H]DHS for 2 h as described in Materials and methods, and the amount of radiolabeled ceramide formed was quantitated. Mean ± SD; *n* = 3 independent experiments. (F) No defect in in vitro ceramide transport in *osh234Δ* cells. Lysates from wild-type and *osh234Δ* cells were incubated for [^{3}H]DHS for 2 h as in Fig. 1, H and I and the amount of radiolabeled IPC formed quantitated. Mean ± SD; *n* = 3 independent experiments. Cer, ceramide; DIC, differential interference contrast.

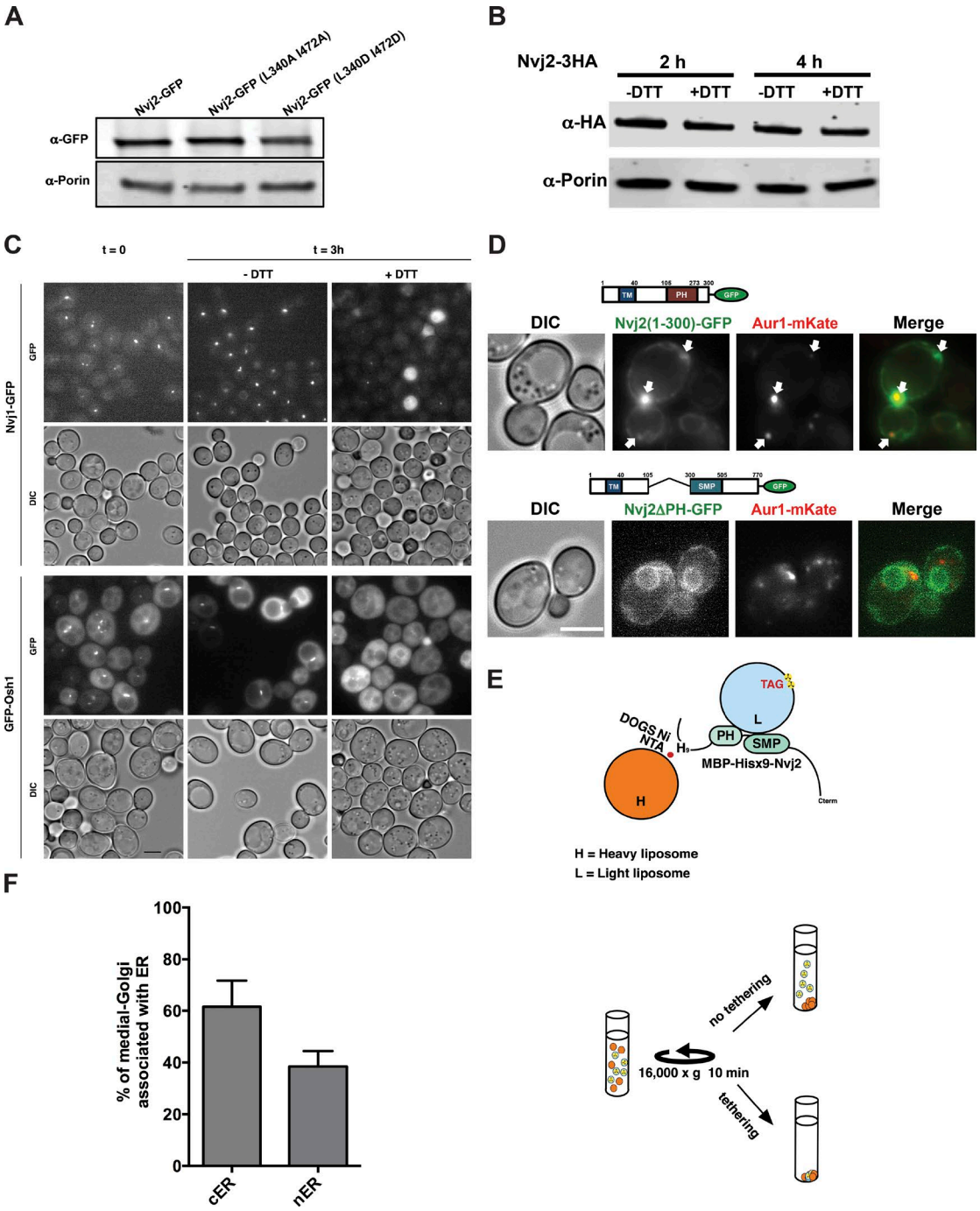

Figure S2. **Additional data related to Figs. 2, 3, and 4.** (A) Cells containing plasmids expressing Nvj2-GFP, Nvj2-GFP (L340A I472A), or Nvj2-GFP(L340D I472D) were grown to mid-logarithmic growth phase and immunoblotted with antibodies against GFP or the mitochondrial protein Porin. The Nvj2-GFP mutants were expressed at levels similar to Nvj2-GFP. Nvj2-GFP = 114 kD and Porin = 30.5 kD. (B) The expression of Nvj2p does not change when cells are treated with DTT. Cells expressing endogenous 3× HA-tag Nvj2 (Nvj2-3HA) were grown to mid-logarithmic growth phase in SC medium, then switched to fresh SC medium with or without 10 mM DTT, and incubated at 30°C for the indicated times. Cells were collected and immunoblotted with antibodies against HA and Porin. Nvj2-3HA = 90 kD. (C) Cells expressing Nvj1-GFP or GFP-Osh1 from plasmids were grown to mid-logarithmic growth phase in SC medium (t = 0) and then treated with or without DTT for an additional 3 h (t = 3 h). The cells were visualized live. Bar, 5 µm. (D) The PH domain of Nvj2p is necessary to target Nvj2p to Golgi membranes. Cells expressing the indicated GFP fusions from plasmids and endogenous Aur1-mKate are shown. White arrows indicate areas of Aur1-mKate and Nvj2(1–300)-GFP colocalization. Bar, 5 µm. (E) Scheme of tethering assay shown in Fig. 3 D. The N-terminal 40 residues of Nvj2p, which contain the TM domain, were replaced with MBP followed by 9 histidines. The resulting protein, MBP-His_{x9}-Nvj2, was purified from yeast and incubated with liposomes containing sucrose (heavy) and that contain the lipid DGS-NTA(Ni). This lipid is bound by the His_{x9} in MBP-His_{x9}-Nvj2. The heavy liposomes were mixed together with liposomes that do not contain sucrose (light) and that contain trace amounts of radiolabeled triacylglycerol (TAG). Heavy liposomes will pellet at 16,000 *g*, but light liposomes will not unless they are tethered to the heavy liposomes. After centrifugation, the percent of light liposomes in the pellet was determined using a scintillation counter. (F) Cells expressing endogenously tagged Sec61-GFP and Aur1-mKate were grown in media with DTT for 4 h as described in Fig. 4 (A and B). The percentage of Aur1-mKate vesicles associated with cortical ER (cER) or perinuclear ER (nER) was determined (vesicles not associated with the ER were not included in the totals). Mean ± SD of three independent experiments; n = 300 cells. DIC, differential interference contrast.

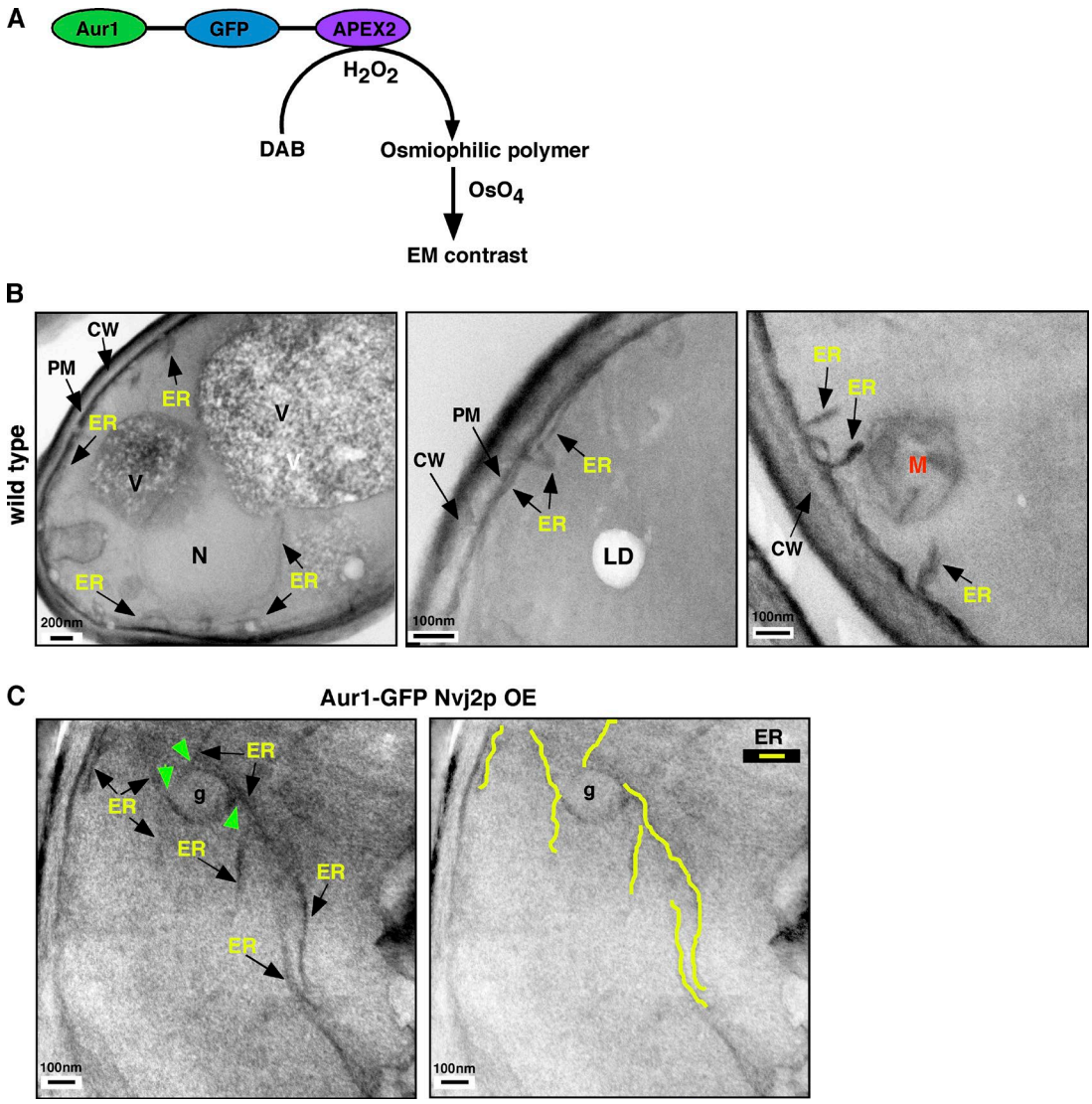

Figure S3. **Additional data related to Fig. 5.** (A) Scheme of precipitate formation by APEX2. Aur1-GFP-APEX2 localizes to medial-Golgi vesicles. Upon treatment of cells with DAB/H_2O_2 solution, APEX2 catalyzes the conversion of DAB into an insoluble precipitate. Subsequent treatment of the DAB polymer with OsO_4 generates EM contrast. (B) DAB staining does not produce contrast in cells that do not express APEX2. Wild-type cells not expressing APEX2 were fixed and subjected to DAB staining, and no precipitate was observed. Three examples are shown. (C) Nvj2p overexpression increases contact between the ER and vesicles that are likely medial-Golgi. Cells expressing endogenous Aur1-GFP and containing a plasmid overexpressing (OE) Nvj2p were chemically fixed and visualized by EM. In cells overexpressing Nvj2p, the ER frequently contacts Golgi-like vesicles. Green arrowheads denote close contacts between ER and Golgi-like vesicles. Right panel shows trace of ER in yellow of image in left panel. CW, cell wall; g, Golgi-like; LD, lipid droplet; M, mitochondria; N, nucleus; PM, plasma membrane; V, vacuole.

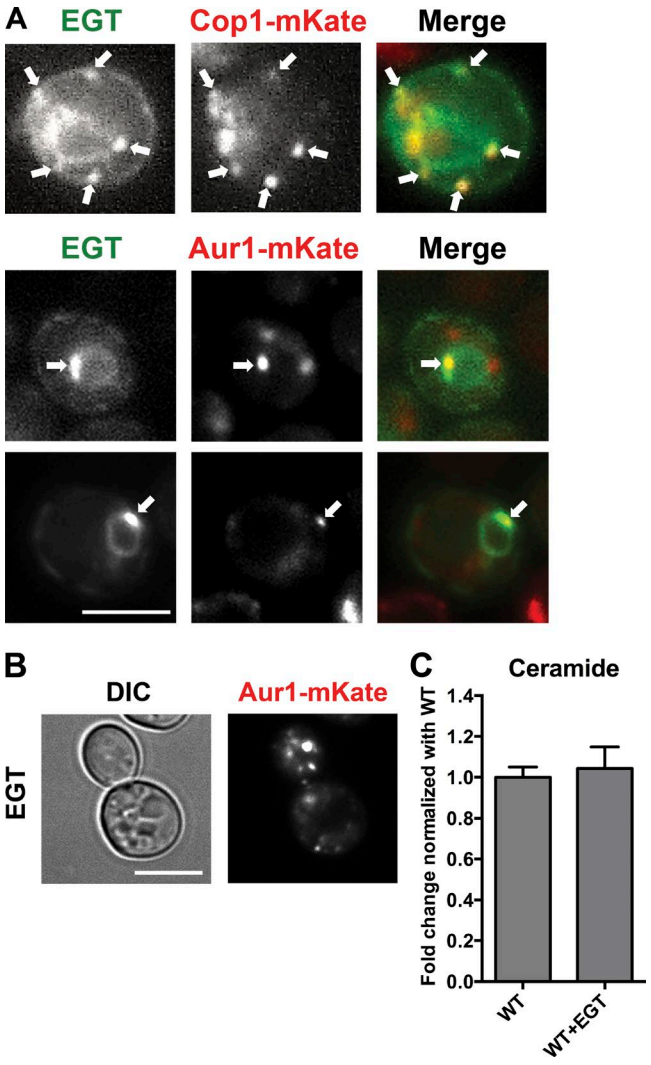

Figure S4. **Additional data related to Fig. 6.** (A) EGT colocalizes with cis- and medial-Golgi. Cells expressing EGT and the cis-Golgi marker Cop1-mKate (top panels) or EGT and the medial-Golgi marker Aur1-mKate (bottom panels). White arrows indicate the regions with colocalization signals. (B) Aur1-mKate localization is not altered by EGT expression. (C) Lysates from cells either with or without EGT produce similar amounts of [^{3}H]ceramide when incubated with [^{3}H]DHS (control for experiments in Fig. 4 E). Lysates were incubated with [^{3}H]DHS for 2 h as described in Materials and methods, and the amount of radiolabeled ceramide formed was quantitated. Mean ± SD; n = 3 independent experiments. DIC, differential interference contrast. Bars, 5 µm.

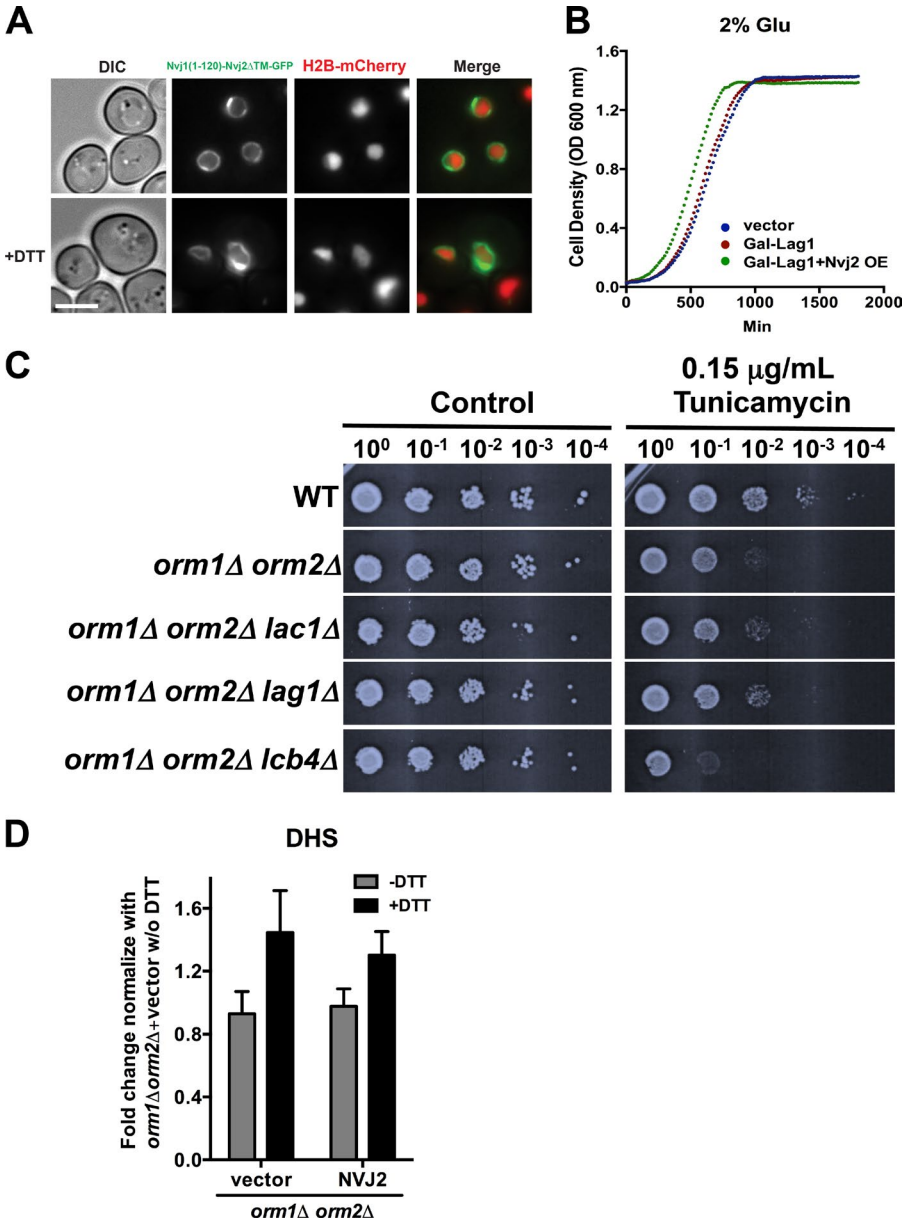

Figure S5. **Additional data related to Figs. 7 and 8.** (A) The localization of *Nvj1(1–120)-Nvj2ΔTM-GFP* does not change in cells treated with DTT. Cells expressing *Nvj1(1–120)-Nvj2ΔTM-GFP* (green) and histone H2B-mCherry (red) were visualized live. Where indicated, cells were treated with 10 mM DTT for 4 h before visualization. Bar, 5 µm. (B) Growth curve of wild-type (WT) cells with indicated plasmids in SC medium. The strains were grown to mid-logarithmic growth phase in SC with raffinose instead of glucose, washed, and resuspended in SC at an OD_{600nm} of 0.02. Growth was the followed over time. Mean ± SD; *n* = 3 independent experiments. (C) Serial dilutions of the indicated strains on SC plates with or without 0.15 µg/ml tunicamycin. (D) Nvj2p is probably not a negative regulator of serine palmitoyltransferase. Cells were grown for 4 h either with or without 10 mM DTT and labeled with [^{3}H] serine, and the amount of radiolabeled DHS formed was determined. Mean ± SD; *n* = 3 independent experiments. DIC, differential interference contrast.

Table S1. **Plasmids obtained from a high-copy library that allow *osh234Δ* cells to grow in the presence of AbA**

Suppressors	Gene	Hits
No. 1	**BET1**	
	CFD1	
	YIL002W-A	
	INP51	
No. 2	**UFE1**	
	SKI7	
	RTS2	
No. 3	YFR045W	
	CNN1	
	BNA6	
No. 4	**AUS1**	
	YOR012W	
No. 5	**GUP2**	
	POS5	
	MF(ALPHA)1	
	UIP4	
No. 6	YDL007C-A	
	RPT2	
	PTC1	
	PMP3	
	MTH1	
No. 7	UTP13	
	IFH1	
No. 8	YOR338W	
	UBC11	
	RPA43	
	RPA190	
No. 9	EAF7	
	FPR1	
	YNL134C	
	NAM9	
No. 10	**WHI2**	
	CUE5	
	GLO4	
No. 11	**OSH3**	
	QNS1	
No. 12	**PDR1**	2
	PUF4	
No. 13	RGA1	2
	ADE2	
	AFI1	
	ORT1	
No. 14	**IZH3**	2
	SDO1	
	IRC25	
	YEH2	
No. 15	CIN4	
	SIP5	
	RIM11	
No. 16	YIL108W	
	PFK26	
	SEC24	
No. 17	TRI1	
	FUS2	
	RNH1	
	RNA1	
No. 18	ECM11	
	YDR444W	
	SSN2	
No. 19	YCG1	
	UTP4	

Table S1. **Plasmids obtained from a high-copy library that allow *osh234Δ* cells to grow in the presence of AbA** *(Continued)*

Suppressors	Gene	Hits
	YSP2	
No. 20	SMF1	
No. 21	RRG1	
	RTR2	
	DOS2	
	DOA4	
	OCA6	
No. 22	RTA1	4
No. 23	RSM27	
	RPS0A	
	GPI1	
	SLI1	
No. 24	PDE1	
	BRR6	
	ZIP2	
No. 25	**PDR3**	
	LDB7	
No. 26	**IZH3**	2
	UBR2	
No. 27	VMA5	2
	TEF4	
	RRP14	
	HOT13	
No. 28	RIP1	
	YEL023C	
	GEA2	
	YEL025C	
No. 29	**OSH7**	
	QCR10	
	HSE1	
	RPL14B	
No. 30	UBR1	
	TIM13	
	QCR9	
	RNR4	
	OKP1	
No. 31	EHT1	
	ECM31	
	SWD3	
	UMP1	
	FZO1	
	SMY2	
No. 32	MYO5	
	HFD1	
No. 33	AKR2	
	SHE4	
	YOR034C-A	
	EXO1	
No. 34	FKS1	
	YLR342W-A	
	GAS2	
No. 35	INP53	
	RGS2	
	VAM3	
	LEU9	
No. 36	**OSH6**	
	PAP1	
No. 37	RPN13	
	URA4	
	YLR419W	
	DCK1	

Table S1. **Plasmids obtained from a high-copy library that allow *osh234Δ* cells to grow in the presence of AbA** *(Continued)*

Suppressors	Gene	Hits
No. 38	**SHR3**	
	NOP6	
	YDL211C	
No. 39	PGI1	
	MSI1	
	AIM4	
	MED8	
	RIM2	
	RPL21A	
No. 40	SSP1	
	PFS1	
	GND1	
No. 41	**DRS2**	
	MAK16	
	LTE1	
No. 42	**EMP65**	2
	COX15	
	RTR1	
	YER138W-A	
	UBP5	
	MAG1	
No. 43	RTR1	2
	EMP65	
No. 44	YDR338C	
	MRPS28	
	YDR336W	
	FCF1	
No. 45	**NVJ2**	2
	RDS3	
	ASR1	
	YPR089W	
No. 46	**OSH2**	
	ERP3	
No. 47	UTP18	
	YJL068C	
	YJL070C	
	ARG2	
	PSF2	
No. 48	FET5	
	YFL040W	
No. 49	YPS3	
	YLR125W	
No. 50	FDC1	
	PAD1	
	STL1	
No. 51	STP2	
	ERG11	
No. 52	RCK1	
	ARI1	
	AMS1	
No. 53	UIP3	
	YAR023C	
	YAR028W	
No. 54	ENT5	
	CTH1	
	GIR2	
	NUM1	
No. 55	CTF18	
	VPS20	
No. 56	**SLO1**	
	FAU1	

Table S1. **Plasmids obtained from a high-copy library that allow *osh234Δ* cells to grow in the presence of AbA** *(Continued)*

Suppressors	Gene	Hits
	FMP10	
No. 57	**TGL1**	
	SDH3	
	MRP8	
No. 58	NOC3	
	CMS1	
	YLR001C	
No.59	ARO10	
	YRA1	
	YDR381C-A	
	YDR379C-A	
No. 60	**OSH2**	
No. 61	SRP14	
	UBX3	
	RAM1	
No. 62	**ATR1**	
	NAB6	
	NGL3	
No. 63	**RHO4**	
	DYN1	
No. 64	**BCY1**	
	CAP2	
	CKA1	
No. 65	STP2	
	ERG11	
No.66	**VPS33**	
	AFG2	
	SKI2	
No. 67	**SEC24**	
	HPM1	
	COX5B	
No. 68	**VPS30**	
	DBP1	
	MEI5	
	IDI1	
	BEM3	
No. 69	WTM2	
	WTM1	
	MCP1	

The genes in bold indicate the functional genes we considered as rescuing the impaired growth of osh234Δ in the presence of AbA. The hits indicate how many times the plasmids are screened out from the selection.

Table S2. **Plasmids used in this study**

Plasmids	Description	Source
LKE132	EGT; express Svp26TM-GFP-Ubc6 under GPD promoter (*(LEU2/CEN*)	This study
pMCZ-Y	Express lacZ under the KAR2 promoter *(URA3/CEN)*	Y. Ye[a]
LKE219	Express Lag1 under GAL promoter *(URA3/2 μ)*	This study
LKE260	Express Nvj2 under Nvj2 promoter *(LEU2/2 μ)*	This study
pSH16	Express Orm1 under Orm1 promoter *(LEU2/2 μ)*	A. Chang[b]
LKE285	Express *Nvj2ΔTM* under Nvj2 promoter *(LEU2/2 μ)*	This study
LKE286	Express *Nvj2ΔPH* under Nvj2 promoter *(LEU2/2 μ)*	This study
LKE287	Express *Nvj2ΔSMP* under Nvj2 promoter *(LEU2/2 μ)*	This study
LKE307	Express *Nvj1(1–120)-Nvj2ΔTM* chimera under Nvj2 promoter *(LEU2/2 μ)*	This study
LKE309	Express *Nvj1(1–120)-Nvj2ΔTM-GFP* chimera under Nvj2 promoter *(LEU2/2 μ)*	This study
LKE311	Express *Nvj2-GFP* under Nvj2 promoter *(LEU2/2 μ)*	This study
LKE315	Express *Sec63-Nvj2ΔTM* chimera under Nvj2 promoter *(LEU2/2 μ)*	This study
LKE314	Express *Sec63-Nvj2ΔTM-GFP* chimera under Nvj2 promoter *(LEU2/2 μ)*	This study
LKE327	Express *Aur1-GFP-Apex2* under Aur1 promoter *(URA3/CEN)*	This study
LKE340	Express Nvj2 (L340A I472A) mutant under Nvj2 promoter *(LEU2/2 μ)*	This study
LKE342	Express Nvj2 (L340D I472D) mutant under Nvj2 promoter *(LEU2/2 μ)*	This study
pAT286	Express *MBP* under GAL promoter *(URA3/CEN)*	This study
pAT836	Express *MBP-(His)x9-Nvj2ΔTM* under GAL promoter *(URA3/CEN)*	This study
LKE345	Express *MBP-(His)x9-Nvj2ΔTM(L340D I472D)* under GAL promoter *(URA3/CEN)*	This study
LKE327	Express *Aur1-GFP-Apex2* under Aur1 promoter *(URA3/CEN)*	This study
pAT155	Express Nvj2 under GPD promoter *(URA3/CEN)*	This study
pAT165	Express Plant Nvj2 (*AT1G73200*) under GPD promoter *(URA3/CEN)*	This study
pAT177	Express Human Nvj2 (*HT008*) under GPD promoter *(URA3/CEN)*	This study

[a]Y. Ye, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD.
[b]A. Chang, University of Michigan, Ann Arbor, MI.

Table S3. **Strains used in this study**

Strains	Relevant genotype	Source
LKY001	BY4741 mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	This study
LKY002	BY4742 mat α his3Δ1 leu2Δ0 lys2Δ5 ura3Δ0	This study
LKY077	BY4742 mat a his3Δ1 leu2Δ0 lys2Δ5 ura3Δ0 *H2B-mCherry::URA3*	This study
LKY297	mat α *sec18-1* ura3-52 his4-619	This study
LKY348	mat a leu2-3,112 ura3-52 his3Δ200 lys2-801 trp1Δ901 suc2Δ9 *osh2::URA3 osh3::LYS2 osh4::HIS3*	C. Beh[a]
LKY374	mat a leu2-3,112 ura3-52 his3Δ200 lys2-801 trp1Δ901 suc2Δ9 *osh2::KanMX6 osh3::LYS2 osh4::HIS3*	This study
LKY392	BY 4741 mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 *nvj1::KanMX6*	This study
LKY393	BY 4741 mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 *nvj2::KanMX6*	This study
LKY394	mat a leu2-3,112 ura3-52 his3Δ200 lys2-801 trp1Δ901 suc2Δ9 *osh2::URA3 osh3::LYS2 osh4::HIS3 nvj1::KanMX6*	This study
LKY395	mat a leu2-3,112 ura3-52 his3Δ200 lys2-801 trp1Δ901 suc2Δ9 *osh2::URA3 osh3::LYS2 osh4::HIS3 nvj2::KanMX6*	This study
LKY398	mat α *sec18-1* ura3-52 his4-619 *nvj2::KanMX6*	This study
LKY404	BY4742 mat a his3Δ1 leu2Δ0 lys2Δ5 ura3Δ0 *Cop1-mKate::URA3*	C. Burd[b]
LKY405	BY4742 mat α his3Δ1 leu2Δ0 lys2Δ5 ura3Δ0 *Aur1-mKate::URA3*	C. Burd
LKY411	BY4742 mat α his3Δ1 leu2Δ0 lys2Δ5 ura3Δ0 *Aur1-mKate::URA3 nvj1::KanMX6*	This study
LKY414	ATCC 201388: mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 *Nvj2-GFP::HIS3*	Huh et al., 2003
LKY415	ATCC 201388: mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 *Nvj2-GFP::HIS3 nvj1::KanMX6*	This study
LKY416	BY4742 mat α his3Δ1 leu2Δ0 lys2Δ5 ura3Δ0 *nvj2::HIS3*	This study
LKY417	BY4742 mat α his3Δ1 leu2Δ0 lys2Δ5 ura3Δ0 *orm1::clonNAT orm2::KanMX6*	A. Chang
LKY419	BY4742 mat α his3Δ1 leu2Δ0 lys2Δ5 ura3Δ0 *orm1::clonNAT nvj2::HIS3*	This study
LKY420	BY4742 mat α his3Δ1 leu2Δ0 lys2Δ5 ura3Δ0 *orm2::KanMX6 nvj2::HIS3*	This study
LKY421	BY4742 mat α his3Δ1 leu2Δ0 lys2Δ5 ura3Δ0 *orm1::clonNAT orm2::KanMX6 nvj2::HIS3*	This study
LKY429	BY 4741 mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 *lag1::HIS3*	This study
LKY443	BY4742 mat α his3Δ1 leu2Δ0 lys2Δ5 ura3Δ0 *orm1::clonNAT orm2::KanMX6 lac1::HIS3*	This study
LKY445	BY4742 mat α his3Δ1 leu2Δ0 lys2Δ5 ura3Δ0 *orm1::clonNAT orm2::KanMX6 lag1::HIS3*	This study
LKY447	BY4742 mat α his3Δ1 leu2Δ0 lys2Δ5 ura3Δ0 *orm1::clonNAT orm2::KanMX6 lcb4::HIS3*	This study
LKY452	BY4742 mat α his3Δ1 leu2Δ0 lys2Δ5 ura3Δ0 *Nvj2-3HA::KanMX6*	This study
LKY454	BY4742 mat α his3Δ1 leu2Δ0 lys2Δ5 ura3Δ0 *dga1::KanMX6 lro1::lox-HIS3-lox*	This study
LKY456	BY4742 mat α his3Δ1 leu2Δ0 lys2Δ5 ura3Δ0 *dga1::KanMX6 lro1::lox-HIS3-lox nvj2::HIS3*	This study
LKY457	BY4742 mat α his3Δ1 leu2Δ0 lys2Δ5 ura3Δ0 *orm1::clonNAT orm2::KanMX6 nvj1::HIS3*	This study
LKY458	BY4742 mat α his3Δ1 leu2Δ0 lys2Δ5 ura3Δ0 *orm1::clonNAT orm2::KanMX6 Nvj2-GFP::HIS3*	This study
LKY463	BY4742 mat α his3Δ1 leu2Δ0 lys2Δ5 ura3Δ0 *Aur1-mKate::URA3 Nvj2-GFP::HIS3*	This study
LKY464	BY4742 mat α his3Δ1 leu2Δ0 lys2Δ5 ura3Δ0 *Aur1-mKate::URA3 Nvj2-GFP::HIS3 nvj1::KanMX6*	This study
LKY473	BY4742 mat α his3Δ1 leu2Δ0 lys2Δ5 ura3Δ0 *Aur1-mKate::URA3 Sec61-GFP::HIS3*	This study
LKY481	ATCC 201388: mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 *Aur1-GFP::HIS3*	Huh et al., 2003
LKY482	BY4742 mat α his3Δ1 leu2Δ0 lys2Δ5 ura3Δ0 *Aur1-mKate::URA3 Sec61-GFP::HIS3 nvj1::KanMX6*	This study
LKY484	ATCC 201388: mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 *Aur1-GFP::HIS3 nvj2::KanMX6*	This study
LKY485	BY 4741 mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 *arv1::KanMX6*	This study
LKY487	BY4742 mat α his3Δ1 leu2Δ0 lys2Δ5 ura3Δ0 *Aur1-mKate::URA3 Sec61-GFP::HIS3 nvj2::KanMX6*	This study
ATY989	BY 4741 mat a his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 *sec18-1::LEU2 Nvj2-GFP::HIS3*	This study

[a]C. Beh, Simon Fraser University, Burnaby, Canada.
[b]C. Burd, Yale School of Medicine, New Haven, CT.

Reference

Huh, W.K., J.V. Falvo, L.C. Gerke, A.S. Carroll, R.W. Howson, J.S. Weissman, and E.K. O'Shea. 2003. Global analysis of protein localization in budding yeast. *Nature*. 425:686–691. http://dx.doi.org/10.1038/nature02026