Supplemental Materials

Molecular Biology of the Cell

Miller *et al.*

SUPPLEMENTAL FIGURE LEGENDS:

Figure S1: Skb1-Slf1-Psy1 nodes are stable after treatment with 1,6-hexanediol.
(A) WT cells expressing P-body marker Sts5-GFP grown to saturation and then treated with 10% 1,6-hexanediol or DMSO control. (B) *GFP-psy1 mCherry-slf1* cells were treated with 10% 1,6-hexanediol or DMSO control. Images (right) are close ups of the white boxed region showing intact Skb1-Slf1-Psy1 nodes. Maximum intensity projections were created from the top half of cell z-stack images that encompass one side of the cell membrane. Bars, 4μm.

Figure S2: Genetic interactions between node mutants and exocytosis mutants. (A) Table summarizing growth of indicated single or double mutants shown below. Red shaded rows indicate temperature sensitive growth with double mutants compared to single mutants. Green shaded rows indicate growth suppression with double mutants. (B) The indicated strains were spotted with 10× serial dilutions onto YE4S or YE4S + 0.5M KCl plates. Plates incubated for 2-4 days prior to imaging.

Figure S3: Additional analysis of Skb1-Slf1-Psy1 nodes with Rga4 and Rga6 GAPs. (A) Localization of Rga4-mNG (Left) or Rga6-GFP (Right) and mCherry-Slf1 in WT cells. Representative images with boxed region of close-up of mCherry and GFP node signals (Right panels) are shown.

Figure S4: Psy1 domain deletion analysis. (A) Diagram of Psy1 protein domains (top). Regions included in each psy1 domain mutant is shown (below). (B) Co-localization of mCherry-Slf1 with each GFP-tagged psy1 domain mutant as listed. Images are maximum projections from z-series except for brightfield images which are single middle z-plane images.

Figure S5: Additional characterization of GFP-tagged psy1 mutants. (A) Note the absence of nodes in GFP-psy1-Ha-m1 and GFP-psy1-Hc-m1 cells. psy1-Hc-m1 also localizes to internal puncta. Single middle z-slice images are shown. Bar, 7μm. (B) Normalized global intensity (mean ± SD) of GFP-Psy1 in indicates strains. *p < 0.05; ** p < 0.0003; and p***<0.0001 determined by ANOVA. n > 100 for each cell type.

Figure S6: Nodes remain intact in psy1-Ha-m1 mutant. (A) Co-localization of mCherry-Slf1 with GFP-tagged Psy1, psy1-Ha-m1, or psy1-Ha-m2. Images are maximum projections from z-series except for brightfield images which are single middle z-plane images. (B) Average number of mCherry-Slf1 nodes per cell of indicated cell type (mean ± SD). No significant difference among strains determined by ANOVA. n>75 for each cell type. (C) Average mCherry-Slf1 node intensity per cell of indicated strains. mean ± SD shown. No significant difference among strains determined by ANOVA. n>75 for each cell type.

Supplemental figure 1

A

Sts5-GFP

DMSO | 1,6-hexanediol

B

	GFP-Psy1	mCherry-Slf1	Merge	
DMSO				GFP / mCherry / Merge
1,6-hexanediol				GFP / mCherry / Merge
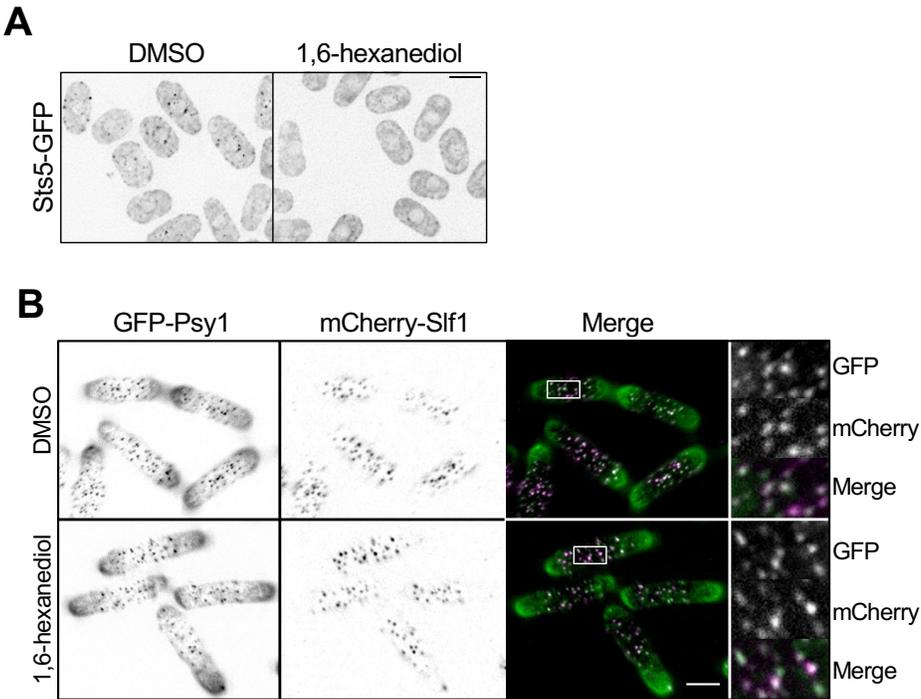
Supplemental figure 2

A

Growth on YE4S plates

|	Single mutant	with *skb1Δ*	with *slf1Δ*
exo70Δ	Δ	-	-
for3Δ	Δ	-	-
sec3-2	Δ	++	Δ
sec8-1	Δ	++	++
rho3Δ	Δ	++	++

Δ, typical growth of single mutant
++, enhanced growth
-, reduced growth

B
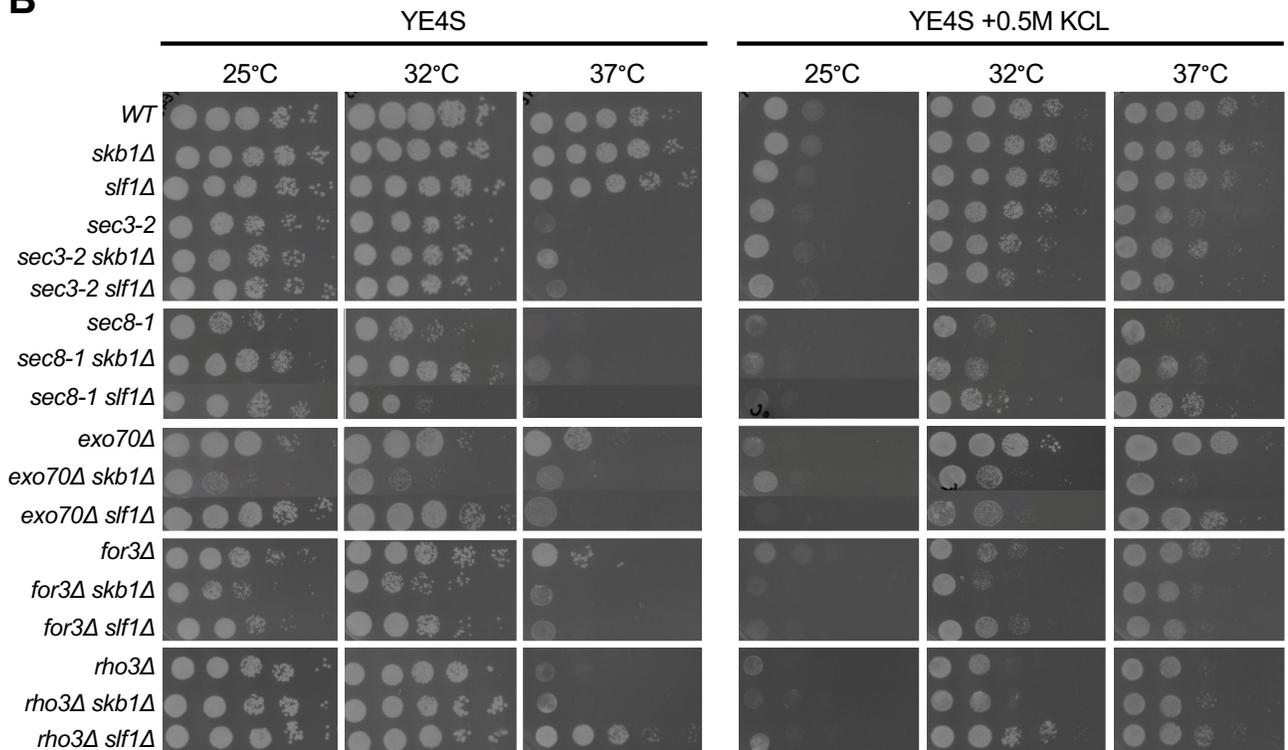
Supplemental figure 3

A

Rga4-mNG	
mCherry-Slf1	
Merge	

Rga6-GFP	
mCherry-Slf1	
Merge	

Supplemental figure 4

A

N-terminal extension
H_{abc} domain

SNARE
domain

Membrane
Anchor (MA)

| Ha | Hb | Hc | | **Psy1** |

$H_{abc}\Delta$

| Ha | Hb | Hc | **SNAREΔ** |

| Ha | Hb | Hc | **MAΔ** |

B

	Brightfield	GFP-Psy1	mCherry -Slf1
$H_{abc}\Delta$			
SNAREΔ			
MAΔ			

Supplemental figure 5

A

Middle Z plane images

B
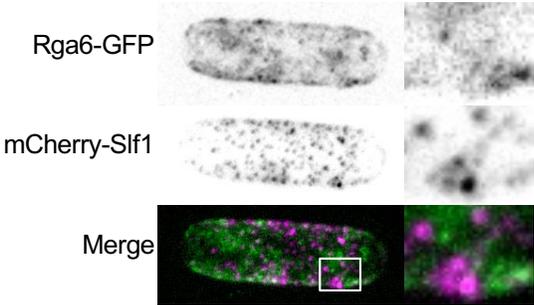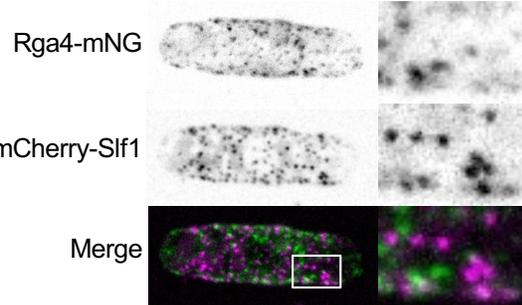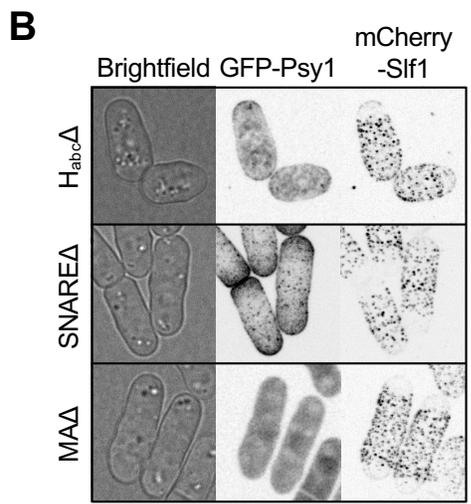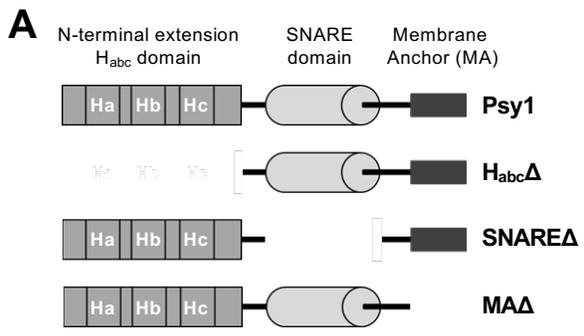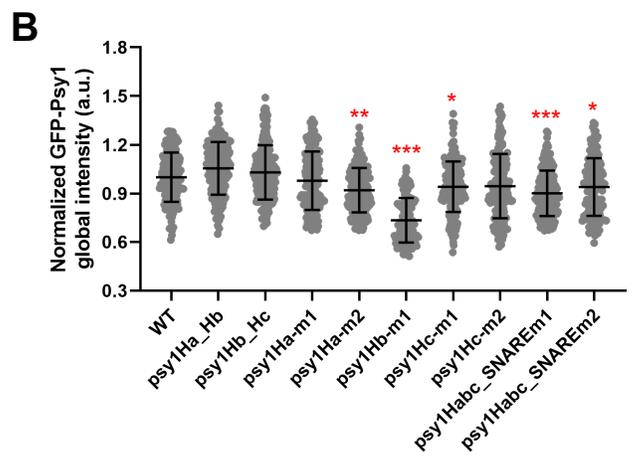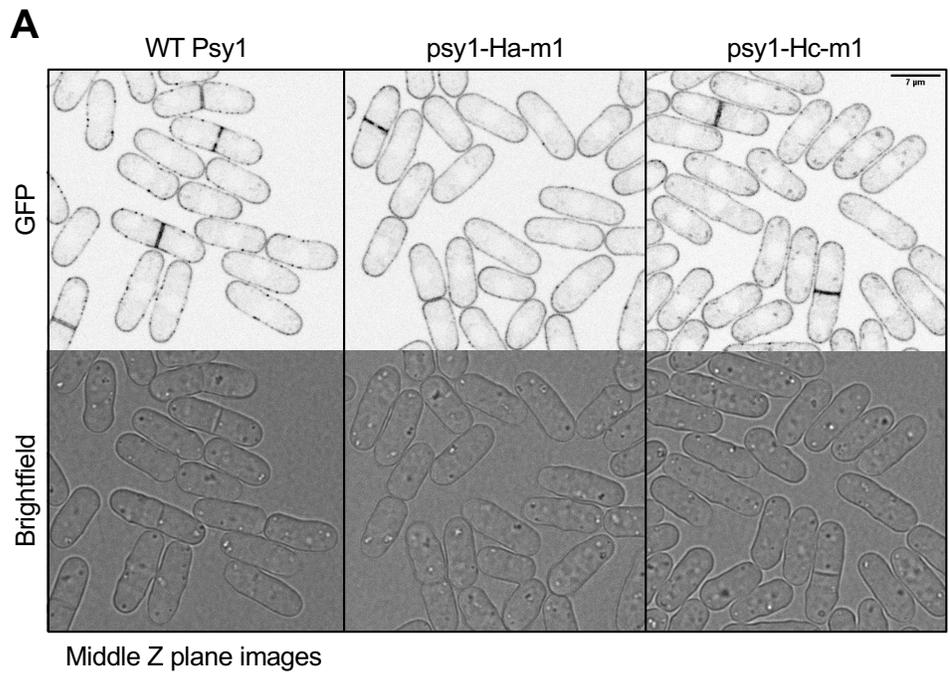
Supplemental figure 6

A

B

C
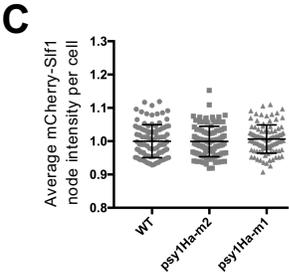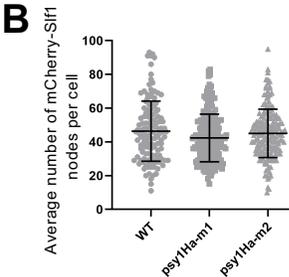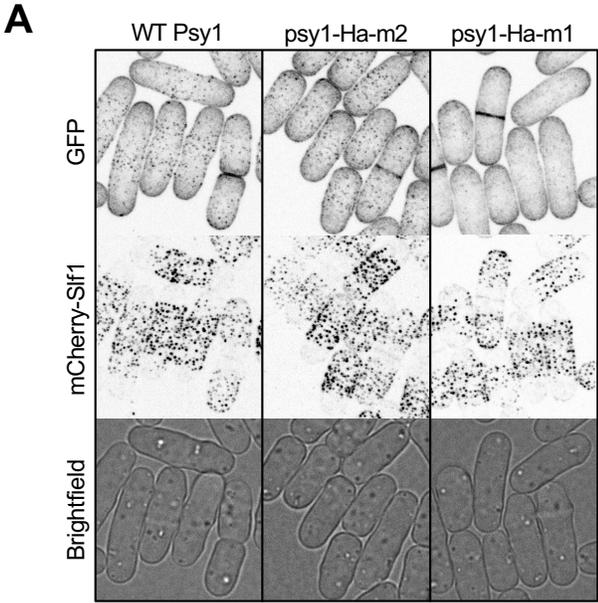
Table S1. Yeast strains used in this study

Strain	Genotype	Source
JM6543	*pAct1-Lifeact-mCherry::leu+ GFP-psy1::kanMX6*	This Study
JM4209	*GFP-psy1::kanMX6 mCherry-slf1::kanMX6*	This Study
JM4497	*GFP-psy1::kanMX6 mCherry-slf1::kanMX6 skb1Δ::natR*	This Study
JM4207	*GFP-Psy1::kanMX6 skb1Δ::natR*	Lab collection
JM4467	*GFP-psy1::kanMX6 slf1Δ::hphR*	This Study
JM4200	*GFP-psy1::kanMX6*	This Study
JM2325	*skb1-GFP::kanMX6 h-*	Lab collection
JM1801	*slf1-GFP::kanMX6 h+*	Lab collection
JM366	*972 h-*	Lab collection
JM906	*skb1Δ::kanMX6 h+*	Lab collection
JM909	*skb1Δ::ura4+ ura4-D18 leu1-32*	Lab collection
JM656	*sec8-1 ura4- D18 leu1-32 h+*	Lab collection
JM1449	*exo70::kanMX6 ura4- D18 leu1-32 ade6-M21X h+*	Lab collection
JM665	*for3Δ::kanMX6 ura4-D18 leu1-32 ade6-M21X h+*	Lab collection
JM6226	*sec3-2-his5+-ura+ ade6- leu1- ura4D-18 h-*	(Bendezú *et al.,* 2012)
JM6256	*rho3Δ::kanMX6 h-*	This Study
JM6057	*skb1Δ::kanMX6 exo70Δ::kanMX6 ura4-D18*	This Study
JM6262	*skb1Δ::kanMX6 for3Δ::kanMX6 ura4-D18 h-*	This Study
JM6113	*skb1Δ::kanMX6 sec8-1 ura4-D18 leu1-32 h-*	This Study
JM6313	*rho3Δ::kanMX6 skb1Δ::natR h+*	This Study
JM6374	*sec3-2-his5+-ura+ skb1Δ::natR ura4-D18 h-*	This Study
JM6112	*slf1Δ::hphR h-*	This Study
JM6127	*slf1Δ::hphR exo70Δ::kanMX6 h-*	This Study
JM6264	*slf1Δ::hphR for3Δ::kanMX6 h-*	This Study
JM6330	*slf1Δ::hphR rho3Δ::kanMX6 h-*	This Study
JM6357	*slf1Δ::hphR sec8-1 ura4-D18 leu1-32 h+*	This Study
JM6420	*slf1Δ::kanMX6 sec3-2-his5+-ura4+ ura4-D18 leu1-32*	This Study
JM6211	*bgs4Δ::ura4+ Pbgs1+::GFP-bgs4+:leu1+ skb1Δ::NAT h+*	This Study
JM4011	*leu1-32 ura4-18 his3-1 bgs4Δ::ura4+ Pbgs1+::GFP-bgs4+:leu1+ h-*	(Cortes *et al.,* 2005)
JM6210	*bgs4Δ::ura4+ Pbgs1+::GFP-bgs4+:leu1+ slf1Δ::kanMX6 h+*	This Study
JM6061	*slf1Δ::kanMX6 sec8-mNeonGreen::hphR h+*	This Study
JM6838	*slf1Δ::hphR for3Δ::kanMX6 sec8-mNeonGreen::hphR h+*	This Study
JM6802	*slf1Δ::hphR rga4Δ::kanMX6 rga6Δ::Kan sec8-mNeonGreen::hphR h-*	This Study

JM6087	*sec8-mNeonGreen::hphR h+*	This Study
JM6398	*skb1Δ::natR sec8-mNeonGreen::hphR*	This Study
JM6641	*skb1Δ::natR for3Δ::kanMX6 sec8-mNeonGreen::hphR h+*	This Study
JM6643	*for3Δ::kanMX6 sec8-mNeonGreen::hphR h+*	This Study
JM6669	*rga4Δ::kanMX6 rga6Δ::kanMX6 sec8-mNeonGreen::hphR h+*	This Study
JM6671	*skb1Δ::nat rga4Δ::kanMX6 rga6Δ::Kan sec8-mNeonGreen::hphR h+*	This Study
JM508	*skb1Δ::kanMX6 h-*	This Study
JM2139	*slf1Δ::hphR h+*	This Study
JM6224	*rga4Δ::kanMX6 rga6Δ::kanMX6 h-*	This Study
JM6225	*rga4Δ::kanMX6 rga6Δ::kanMX6 skb1Δ::natR*	This Study
JM6465	*slf1Δ::hphR rga4Δ::kanMX6 rga6Δ::kanMX6*	This Study
JM6114	*Pslf1-mCherry-slf1::kanMX6 rga4-mNeonGreen::hphR h-*	This Study
JM6124	*Pslf1-mCherry-slf1::kanMX6 rga6-GFP::kanMX6 h+*	This Study
JM7116	*psy1Δ::kanMX6 pDC99[Ppsy1-GFP-psy1-Tpsy1]::ura+*	This Study
JM7046	*mCherry-slf1::kanMX6 pDC99[Ppsy1-GFP-psy1ΔHabc-Tpsy1]::ura+ h+*	This Study
JM7044	*mCherry-slf1::kanMX6 pDC99[Ppsy1-GFP-psy1ΔSNARE-Tpsy1]::ura+ h-*	This Study
JM7045	*mCherry-slf1::kanMX6 pDC99[Ppsy1-GFP-psy1ΔMA-Tpsy1]::ura+ h-*	This Study
JM7276	*psy1Δ::kanMX6 pDC99[Ppsy1-GFP-psy1(51QEID54→51GGGG54)-Tpsy1]::ura+*	This Study
JM7359	*psy1Δ::kanMX6 pDC99[Ppsy1-GFP-psy1(91MQLPPD96→91GGGGSG96)-Tpsy1]::Ura+*	This Study
JM7328	*psy1Δ::kanMX6 pDC99[Ppsy1-GFP-psy1(E20A, E23A, D26A, H27A, D30A)-Tpsy1]::Ura+ h-*	This Study
JM7329	*psy1Δ::kanMX6 pDC99[Ppsy1-GFP-psy1(R33A, E36A, D37A, R41A, M44A)-Tpsy1]::Ura+*	This Study
JM7360	*psy1Δ::kanMX6 pDC99[Ppsy1-GFP-psy1(R145A, E148A, D149A, D150A, F151A)-Tpsy1]::Ura+*	This Study
JM7421	*psy1Δ::kanMX6 pDC99[Ppsy1-GFP-psy1(V163A, L168A, R173A)-Tpsy1]::Ura+*	This Study
JM7422	*psy1Δ::Kan pDC99[Ppsy1-GFP-psy1(D98A, K103A, K111A, K112A, D115A)-Tpsy1]::Ura+ h-*	This Study
JM7423	*psy1Δ::kanMX6 pDC99[Ppsy1-GFP-psy1(R63A, H64A, E66A, Y68A, D71A)-Tpsy1]::Ura+*	This Study
JM7424	*psy1Δ::kanMX6 pDC99[Ppsy1-GFP-psy1(R118A, H119A, L121A, K125A, R128A, R136A, R137A)-Tpsy1]::Ura+ h-*	This Study
JM6888	*sec8-tdTomato-kanMX6 h+*	This Study
JM7430	*sec8-tdTomato-kanMX6 psy1Δ::kanMX6 pDC99[Ppsy1-GFP-psy1(E20A, E23A, D26A, H27A, D30A)-Tpsy1]::Ura+*	This Study
JM7445	*sec8-tdTomato-kanMX6 rga4Δ::kanMX6 rga6Δ::kanMX6 psy1Δ::kanMX6 pDC99[Ppsy1-GFP-psy1(E20A, E23A, D26A, H27A, D30A)-Tpsy1]::Ura+*	This Study
JM7446	*sec8-tdTomato-kanMX6 rga4Δ::kanMX6 rga6Δ::kanMX6*	This Study
JM6025	*sts5-mNeonGreen::HphR ura4D-18 leu1-32*	This Study
JM7185	*psy1Δ::kanMX6 pDC99[Ppsy1-GFP-psy1-Tpsy1]::Ura+ Pslf1-mCherry-slf1::kanMX6*	This Study

JM7372	*psy1Δ::kanMX6 pDC99[Ppsy1-GFP-psy1(E20A, E23A, D26A, H27A, D30A)-Tpsy1]::Ura+ Pslf1-mCherry-slf1::kanMX6*	This Study
JM7373	*psy1Δ::kanMX6 pDC99[Ppsy1-GFP-psy1(R33A, E36A, D37A, R41A, M44A)-Tpsy1]::Ura+ Pslf1-mCherry-slf1::kanMX6*	This Study

Table S2. Plasmids used in this study

Strain	Genotype	Source
pJM210	pRep3x	Lab Collection
pJM482	pREP3x-6His-skb1	Deng *et al.,* 2013
pJM1551	pDC99-Ppsy1-GFP-psy1-Tpsy1	This Study
pJM1567	pDC99-Ppsy1-GFP-psy1ΔH$_{abc}$(Δ11-170aa)-Tpsy1	This Study
pJM1568	pDC99-Ppsy1-GFP-psy1ΔSNARE(Δ180-250aa)-Tpsy1	This Study
pJM1569	pDC99-Ppsy1-GFP-psy1ΔMA(Δ252-283aa)-Tpsy1	This Study
pJM1591	pDC99-Ppsy1-GFP-psy1(51QEID54 →51GGGG54)-Tpsy1 Ha_Hb mutant	This Study
pJM1599	pDC99-Ppsy1-GFP-psy1(91MQLPPD96 → 91GGGGSG96)-Tpsy1 Hb_Hc mutant	This Study
pJM1597	pDC99-Ppsy1-GFP-psy1(E20A, E23A, D26A, H27A, D30A)-Tpsy1 Ha-m1 mutant	This Study
pJM1598	pDC99-Ppsy1-GFP-psy1(R33A, E36A, D37A, R41A, M44A)-Tpsy1 Ha-m2 mutant	This Study
pJM1600	pDC99-Ppsy1-GFP-psy1(R145A, E148A, D149A, D150A, F151A)-Tpsy1 Habc_SNARE-m1 mutant	This Study
pJM1601	pDC99-Ppsy1-GFP-psy1(V163A, L168A, R173A)-Tpsy1 Habc_SNARE-m2 mutant	This Study
pJM1604	pDC99-Ppsy1-GFP-psy1(R63A, H64A, E66A, Y68A, D71A)-Tpsy1 Hb-m1 mutant	This Study
pJM1603	pDC99-Ppsy1-GFP-psy1(D98A, K103A, K111A, K112A, D115A)-Tpsy1 Hc-m1 mutant	This Study
pJM1605	pDC99-Ppsy1-GFP-psy1(R118A, H119A, L121A, K125A, R128A, R136A, R137A)-Tpsy1 Hc-m2 mutant	This Study