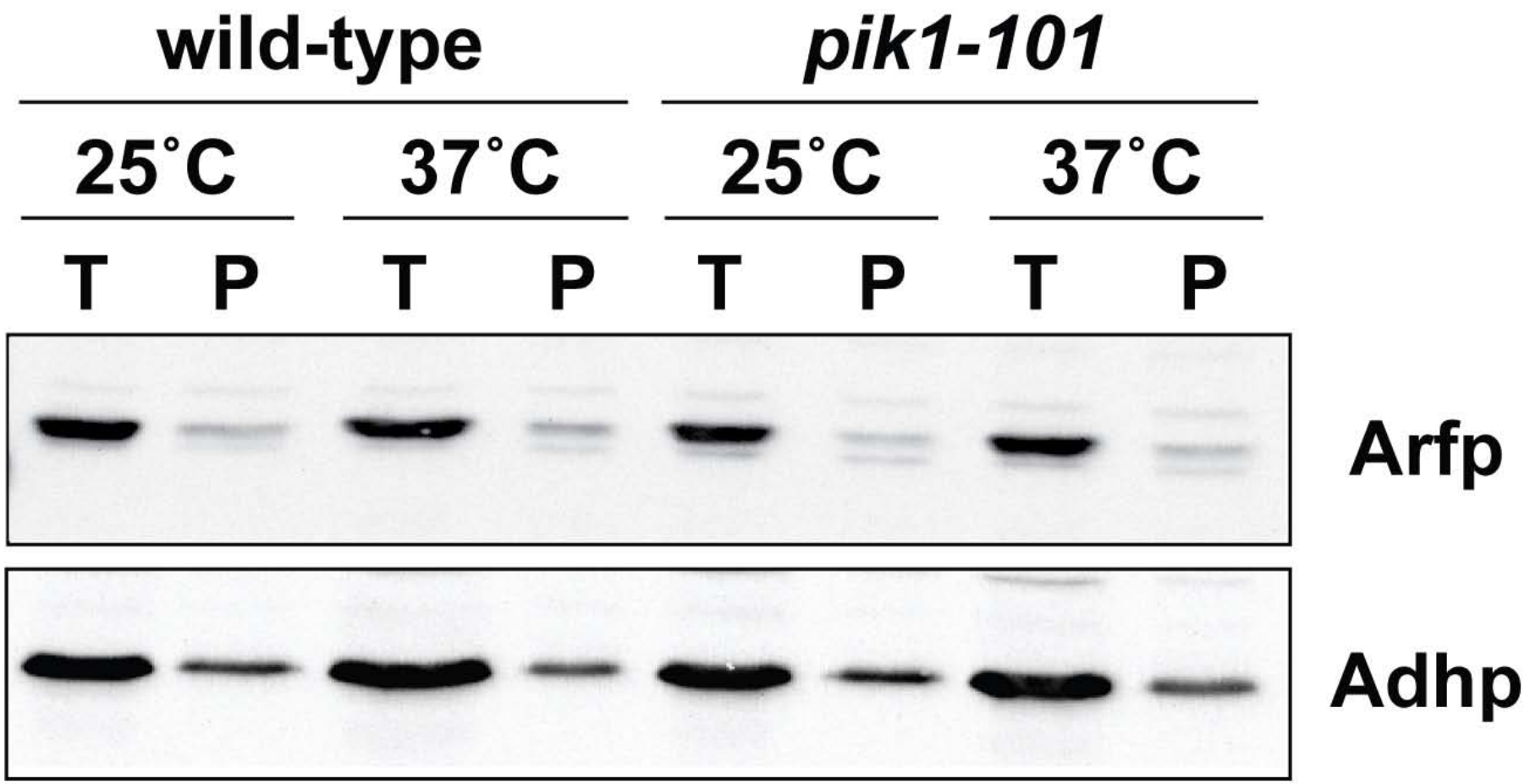
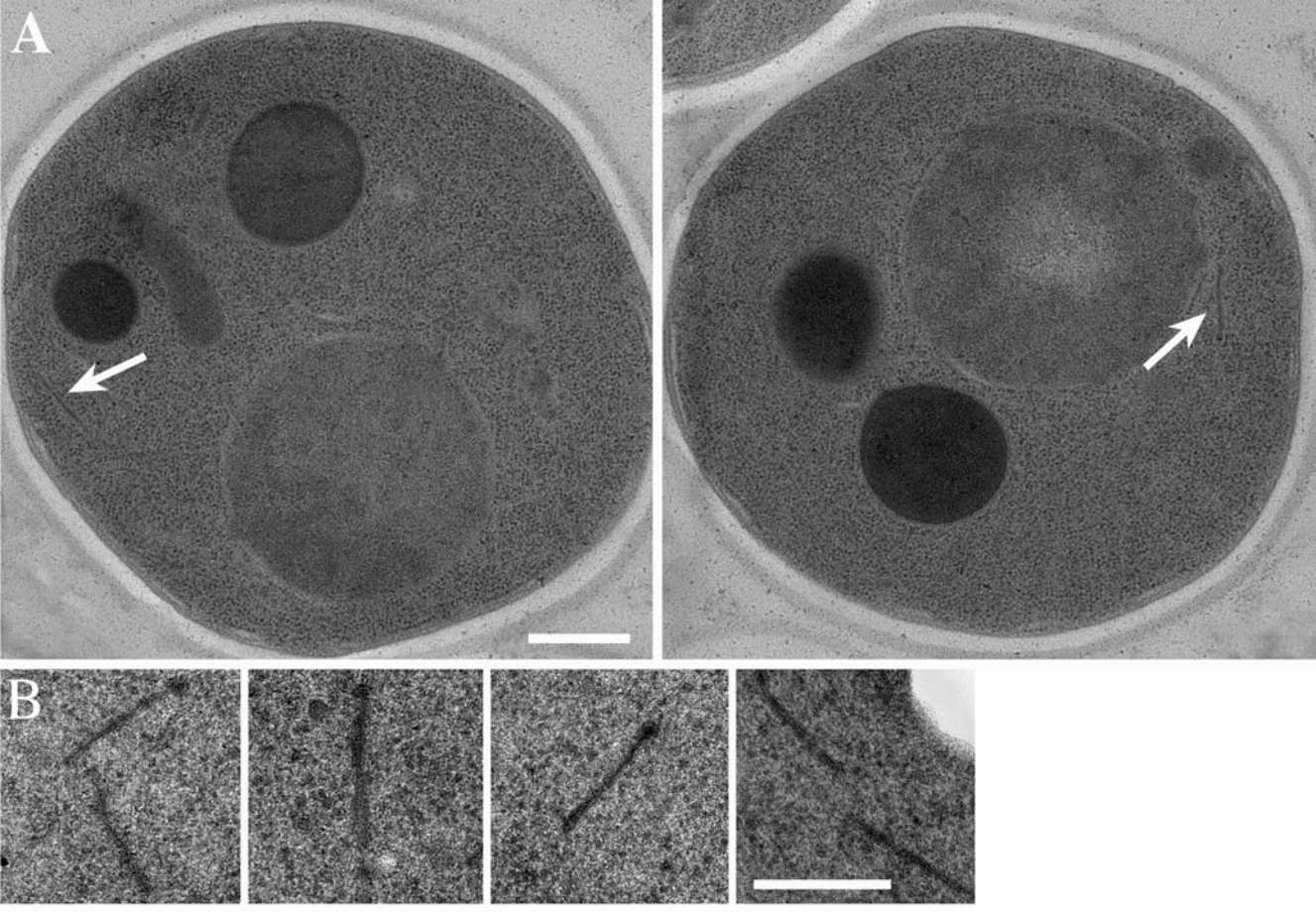
Supplemental Figure Legends

Supplemental Figure 1. Subcellular distribution of Arfp is not affected by overexpression of Sec7p-DsRed in wild-type cells used for microscopy in Figure 5A. Distribution of endogenous Arfp was evaluated by subcellular fractionation from wild-type (CSY349) and *pik1-101*(CSY906) cells used in Figure 5A. Strains were transformed with plasmid pCS136 and grown to mid-log phase in selective medium. Cells were then homogenized and soluble and membrane fractions were separated by centrifugation of a postnuclear supernatant at 100,000xg. A temperature shift to 37ºC was performed for 1 h prior to fractionation when indicated. Equal volumes of fractions were analyzed by SDS-PAGE and immunoblotting. Note that the distribution of Arf is the same in wild-type cells expressing an additional copy of Sec7p-DsRed under the control of a strong promoter and *pik1-101* cells containing genomically tagged Sec7p-DsRed.

Supplemental Figure 2. Ultrastructural analysis of *gga1Δ* cells. Yeast cells (CSY914) were grown to log phase, cryo-immoblilized, processed for freeze-substitution at –90°C for 2d in acetone with 1% osmium tetroxide and 0.1% uranyl acetate and viewed in a TECNAI 12 (FEI) transmission electron microscope. Elongated structures typical for this mutant are indicated with arrowheads. Scale bars represent (A) 500 nm (B) 250 nm.



Supplemental Figure 01



Supplemental Figure 02

Supplementary Methods

Genetic and DNA manipulations

The *pik1-101* query mutation was linked to a *URA3* selectable marker. A subgroup of identified mutants was confirmed by tetrad analysis. The query strain CSY544 was generated in several steps. First pMB108 was constructed by cloning a fragment containing the *PIK1* ORF±500 bp into the BLUESCRIPT II (KS+) vector digested with *Bam*HI and *Not*I. pMB196 and pMB197 were cloned by insertion of the *PIK1* ORF±500 bp or the *pik1-101* mutant ORF±500 bp respectively into pT3T7BM digested with *Bam*HI and *Not*I. A 3.7-kb fragment containing the *PIK1* open reading frame (ORF) and 500 bp upstream sequence was removed from pMB108 by first digesting with *Bsp*EI, blunting with Klenow fragment and afterwards digesting with *Hin*dIII leaving a 3.3-kb fragment of pMB108 containing the 500 bp downstream sequence of the *PIK1* open reading frame. This fragment was ligated to a 1.1-kb fragment containing the *URA3* ORF isolated from YEp24 by digestion with *Sma*I and *Hin*dIII generating pMB265. A 1.5-kb fragment containing the *URA3* ORF and the 500 bp *PIK1* downstream sequence was isolated from pMB265 by digesting with *Hin*dIII, blunting with Klenow-fragment and digesting with *Not*I. This 1.5-kb fragment was inserted into pMB197 previously digested with *Pac*I, blunted with Klenow polymerase and then cut with *Not*I. The resulting construct pMG2 was digested with *Bam*HI and *Not*I generating a fragment containing 500 bp *PIK1* upstream sequence, the *pik1-101* open reading frame, 178 bp *PIK1* downstream sequence containing the *PIK1* terminator region, the *URA3* ORF and 500 bp *PIK1* downstream sequence. This fragment was transformed into Y3656. Transformants were selected for growth on SC-URA medium (integration of *URA3* ORF) and tested for temperature-sensitive growth on YPD at 37°C (*pik1-101* mutation). Correct integration of the fragment was verified by PCR using isolated genomic DNA from

positive transformants as the template. Synthetic genetic array (SGA) analysis was performed in parallel using a query strain with the *PIK1* wild-type allele as reference (CSY549). This reference strain was generated as described for CSY544, with the difference that the 1.5-kb fragment isolated from pMB265 was inserted into pMB196 instead of pMB197 generating pMG3.

Subcellular fractionation

For subcellular fractionation, 35 ODu of washed cells were resuspended in 1 ml 50 mM K-phosphate pH 7.5, 1.4 M sorbitol, 0.4% (v/v) β-mercaptoethanol, 0.1 mg/ml zymolase-100T, and incubated for 45 min at 37˚C. The sample was cooled down on ice and centrifuged through a sorbitol cushion (50 mM K-phosphate pH 7.5, 1.7 M sorbitol, Complete) in a 15 ml Falcon tube (12 min, 3,000xg). The spheroplasts were resuspended in 1 ml lysis buffer containing 20 mM TEA pH 7.2, 0.8 M sorbitol, 125 mM KAc, 2.5 mM MgAc, 1 mM DTT, complete, 1 mM PMSF, 5 μM pepstatin, 1 mM ATP, 1 mM GTP, 5 mM creatine phosphate, 10 μg/ml creatine kinase, homogenized by 50 strokes in a glass-teflon homogenizer (Braun-Melsungen) and cleared by two centrifugation steps of 1,000xg for 3 min. 20 μl of the lysate were kept as 'input sample' and 500 μl of lysate were spun for 45 min at 100,000xg in a Beckman Optima Ultracentrifuge (TLA120.2 rotor).

Liposome recruitment assay

For liposome recruitment assays, PC, PE, PS and cholesterol were mixed in a molar ratio of 44:33:11:11 (in chloroform/ methanol solution) and phosphoinositides were added to 1% molar ratio (in chloroform/ methanol solution). The solvent was evaporated to dryness under a stream of nitrogen and resuspended in 'liposome

buffer' (15.4 mM citric acid, 69.2 mM dibasic sodium phosphate, pH 6.4). The unilamellar liposomes were adjusted to a size of 400 nm.

The liposome recruitment assay was performed in a total of 200 µl in 'recruitment buffer' (25 mM HEPES-KOH pH 7.25, 125 mM potassium acetate, 2.5 mM magnesium acetate, 1 mM DTT, protease inhibitors, 3 mM di-sodium hydrogenphosphate, 5 mM glycerol-2-phosphate). 25 µl liposomes (stock: 1.25 µmol) were incubated at 37˚C for 30 min with recombinant myristoylated human hARF1 (30 µg/l) {Franco, 1995 #114} and 0.5 µg GST or 1.5 µg GST-Gga2p/ GST-Gga2p$^{VHS-GAT}$, respectively. When indicated, GTPγS was added to a final concentration of 0.1 mM. Liposomes were spun down at 13,000xg for 30 min at 4˚C, washed once with ice-cold 'recruitment buffer' and again recovered by centrifugation. Membrane pellets were analyzed by SDS-PAGE and immunoblotting.

Table S1. Genes showing synthetic sick/lethal interaction with *pik1-101*

ORF[a]	gene[b]	function[c]		
VPS1	*YKR001C*	membrane trafficking	confirmed[d]	SL[e]
VPS9	*YML097C*	membrane trafficking	confirmed	SS
RIC1	*YLR039C*	membrane trafficking	confirmed	SS/SL
YPT6	*YLR261C*[f]	membrane trafficking	confirmed	SS
VPS51	*YKR020W*	membrane trafficking	confirmed	SL
VPS54	*YDR027C*	membrane trafficking	confirmed	SL
GGA2	*YHR108W*	membrane trafficking	confirmed	SS
YPT31	*YER031C*	membrane trafficking	confirmed	SL
SAC6	*YDR129C*	cytoskeleton organization	confirmed	SS/SL
CLA4	*YNL298W*	cytoskeleton organization	confirmed	SS
RRD1	*YIL153W*	cytoskeleton organization	not confirmed	SS
CIK1	*YMR198W*	cytoskeleton organization	not confirmed	SS
PFD1	*YGL179W*	cytoskeleton organization	not confirmed	SS
URM1	*YIL008W*	urmylation	confirmed	SS
UBA4[g]	*YHR111W*	urmylation	confirmed	SS
NCS2	*YNL119W*	urmylation	confirmed	SS
ELP2	*YGR200C*	urmylation	confirmed	SS
YNL120C	*YNL120C*	urmylation[h]	confirmed	SS
LIP5	*YOR196C*	lipid metabolism	not confirmed	SS
SPE1	*YKL184W*	lipid metabolism	not confirmed	SS
POR1	*YNL055C*	mitochondrial function	not confirmed	SS
ODC1	*YPL134C*	mitochondrial function	not confirmed	SS
OCT1	*YKL134C*	mitochondrial function	not confirmed	SS
MRP10	*YDL045W-A*	mitochondrial function	not confirmed	SS
MRP20	*YDR405W*	mitochondrial function	not confirmed	SS

MRPL10	*YNL284C*	mitochondrial function	not confirmed	SS
MRPL38	*YKL170W*	mitochondrial function	not confirmed	SS
ATP11	*YNL315C*	mitochondrial function	not confirmed	SS
ATP12	*YJL180C*	mitochondrial function	not confirmed	SS
MTF1	*YMR228W*	mitochondrial function	not confirmed	SS
SWF5	*YOR333C*	mitochondrial function	not confirmed	SS
ASC1	*YMR116C*	ribosomal protein	not confirmed	SS
RPS11A	*YDR025W*	ribosomal protein	not confirmed	SS
RPS16B	*YDL083C*	ribosomal protein	not confirmed	SS
RPL12A	*YEL054C*	ribosomal protein	not confirmed	SS
RPL19A	*YBR084C-A*	ribosomal protein	not confirmed	SS
RPL24A	*YGL031C*	ribosomal protein	not confirmed	SS
RPL40A	*YIL148W*	ribosomal protein	not confirmed	SS
RPL1B	*YGL135W*	ribosomal protein	not confirmed	SS
RPL29	*YFR032C-A*	ribosomal protein	not confirmed	SS
VMA2	*YBR127C*	vacuolar H+-ATPase subunit	not confirmed	SS
VMA7	*YGR020C*	vacuolar H+-ATPase subunit	not confirmed	SS
VMA13	*YPR036W*	vacuolar H+-ATPase subunit	confirmed	SL
VPH2	*YKL119C*	vacuolar H+-ATPase assembly protein	not confirmed	SS
YOL138C	*YOL138C*	carbohydrate metabolism	not confirmed	SS
TPS1	*YBR126C*	carbohydrate metabolism	not confirmed	SS
PFK1	*YGR240C*	carbohydrate metabolism	not confirmed	SS
GID8	*YMR135C*	carbohydrate metabolism	not confirmed	SS
LPD1	*YFL018C*	carbohydrate metabolism	not confirmed	SS
PKR1	*YMR123W*	unknown	confirmed	SS
SOV1	*YMR066W*	unknown	not confirmed	SS
MEH1	*YKR007W*	unknown	not confirmed	SS
PSP2	*YML017W*	unknown	not confirmed	SS
FYV1	*YDR024W*	unknown	not confirmed	SS
YEL048C	*YEL048C*	unknown	confirmed	SS
YMR010W	*YMR010W*	unknown	confirmed	SS

YMR119W-A	*YMR119W-A*	unknown	not confirmed	SS
YNL296W	*YNL296W*	unknown	not confirmed	SS
YNL235C	*YNL235C*	unknown	not confirmed	SS
YOR318C	*YOR318C*	unknown	not confirmed	SS
YHL005C	*YHL005C*	unknown	not confirmed	SS
YFL006W	*YFL006W*	unknown	not confirmed	SS
YKL118W	*YKL118W*	unknown	not confirmed	SS
GDA1	*YEL042W*	other[i]	confirmed	SS
SMI1	*YGR229C*	other	confirmed	SS
LYP1	*YNL268W*	other	not confirmed	SS
DEG1	*YFL001W*	other	not confirmed	SS
SWI4	*YER111C*	other	not confirmed	SS
CNB1	*YKL190W*	other	not confirmed	SS
LSM1	*YGL124C*	other	not confirmed	SS
TRM9	*YML014W*	other	not confirmed	SS
LSM7	*YNL147W*	other	not confirmed	SS
SRP40	*YKR092C*	other	not confirmed	SS
SLX8	*YER116C*	other	not confirmed	SS
SMF1	*YOL122C*	other	not confirmed	SS
TIP41	*YPR040W*	other	not confirmed	SS
CNE1	*YAL058W*	other	not confirmed	SS
DFG16	*YOR030W*	other	not confirmed	SS
HAT2	*YEL056W*	other	not confirmed	SS
SRB8	*YCR081W*	other	not confirmed	SS
URA1	*YKL216W*	other	not confirmed	SS
ZRT3	*YKL175W*	other	not confirmed	SS
ALR2	*YFL050C*	other	not confirmed	SS
SGF73	*YGL066W*	other	not confirmed	SS
RIM9	*YMR063W*	other	not confirmed	SS
MSC1	*YML128C*	other	not confirmed	SS

This table shows all genes isolated by SGA analysis whose deletion results in sickness or lethality of double mutants with *pik1-101*

[a] Selected ORF name according to SGD (www.yeastgenome.org) annotation.

[b] Systematic gene name according to SGD (www.yeastgenome.org) annotation.

[c] The functional grouping of genes is based on description in YPD (www.incyte.com)/SGD (www.yeastgenome.org) and/or published data.

[d] Selected genetic interactions were confirmed by tetrad analysis.

[e] growth phenotype of double mutants: SL-synthetic lethal; SS-synthetic sick.

[f] *VPS63* ORF overlaps to 98% with *YPT6* ORF.

[g] *UBA4* was not originally identified by SGA analysis but was found to exhibit synthetic sick interaction with *pik1-101* in the further course of this study.

[h] *YNL120C* overlaps with *NCS2*.

[i] Gene function annotated by YPD (www.incyte.com)/ SGD (www.yeastgenome.org) and/or published data does not fall into any of the above categories.

Table S2. Ultrastructural analysis of membranes accumulating in *pik1-101* and *gga2Δ* mutants

Structures	*pik1-101*[a]	*gga2Δ*[a]	*gga1Δ*[a]
A/F. Rings/Berkeley bodies[b]	60	24	4
B. Double rings	8	-	-
C. Tubular structures	54	22	74
D. Multilayered structures	<5	-	-
E. MVB[c] like structures	<5	-	-
F. Fragmented vacuoles	yes	yes	-
A. Aberrant vacuoles	28	2	-

[a]Counts represent number of cells of a total of 100 containing the corresponding structure
[b]Tlg1p positive structures
[c]Multivesicular bodies
pik1-101 (CSY712), *gga2Δ* (CSY909), *gga1Δ* (CSY914)

Table S3. GenBank accession numbers

ORF	GenBank accession number
HsGga1	NP_037497.1
XtGga1	NP_001016968.1
DrGga1	NP_001004000.1
DmGga1	NP_572571.1
HsGga2	NP_055859.1
XtGga2	NP_001016968.1
SsGga2	DY704086.1
HsGga3	NP_619525.1
XtGga3	CX928298.1
DrGga3	XP_698317.1
SpSPBC25H2.16c	NP_596351.1
SpSPAC1F3.05	NP_593008.1
ScGga1	NP_010645.1
ScGga2	NP_011976.1
HsTOM1	NP_005479.1
XtTOM1	NP_001016770.1
DrTOM1	AAH56566.1
DmTOM1	NP_648315.1
ENTH Epsin	1H0A
ANTH CALM	1HFA

Table S4. *S.cerevisiae* strains used in this study

Strain	Genotype	Reference
BY4741	MAT**a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Euroscarf [a]
	MAT**a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 orfΔ::kanMX4*	Euroscarf
CSY209	MAT**a** *ura3Δ0 leu2Δ0 his3Δ1 met15Δ0*	Euroscarf
CSY210	MATα *ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0*	Euroscarf
CSY349	MAT**a** *ura3-52 leu2-3,112 trp1-289::TRP-SEC7-DsRED.T4*	This study
CSY391	CSY544 *vps10Δ::VPS10-3xHA-kanMX6*	This study
CSY392	CSY210 *vps10Δ::VPS10-3xHA-kanMX6*	This study
CSY398	CSY544 *gga2Δ::kanMX4 VPS10-3xHA-HIS3*	
CSY399	CSY544 *vps28Δ::kanMX4 VPS10-3xHA-HIS3*	Euroscarf
CSY544	MATα *can1Δ::MFA1pr-HIS3-MFα1pr-LEU2 PIK1::pik1-101-URA3 ura3Δ0 his3Δ0 leu2Δ0 lys2Δ0*	This study
CSY545	MATα *PIK1::pik1-101-URA gga2Δ::kanMX4 ura3Δ0 his3Δ0 leu2Δ0 lys2Δ0*	This study
CSY549	MATα *can1Δ::MFA1pr-HIS3-MFα1pr-LEU2 PIK1-URA3 ura3Δ0 his3Δ0 leu2Δ0 lys2Δ0*	This study
CSY561	CSY544 *chs6Δ::kanMX4*	This study
CSY566	BY4741 *chs6Δ::kanMX4*	Euroscarf
CSY567	BY4741 *gga2Δ::kanMX4*	Euroscarf
CSY704	BY4741 *arf1Δ::kanMX4*	Euroscarf
CSY712	MATα *pik1-101 ura3-52 leu2-3,112*	This study
CSY900	CSY210 *vps28Δ::kanMX4 VPS10-3xHA-HIS3*	This study
CSY902	CSY210 *vps28Δ::kanMX4*	Euroscarf
CSY901	NY1211 *sec7Δ::Sec7-DsRED-kanMX6*	This study
CSY904	BY4741 *sst1Δ::kanMX4*	Euroscarf
CSY906	CSY712 *sec7Δ::Sec7-DsRED-kanMX6*	This study
CSY908	CSY210 *alp4Δ::kanMX4*	Euroscarf
CSY909	MATα *ura3-52 leu2-3,112 gga2Δ::kanMX4*	This study
CSY911	BY4741 *vps34::kanMX4*	
CSY914	MATα *ura3-52 leu2-3,112 his3Δ200 gga1Δ::HIS3*	This study
NY1175	MAT**a** *ura3-52 leu2-3,112 trp1-289*	Novick lab
NY1211	MATα *ura3-52 leu2-3,112 his3Δ200*	Novick lab
NY1295	MAT**a** *sec6-4 ura3-52 leu2-3,112*	Novick lab
NY604	MATα *ura3-52 leu2-3,112*	Novick lab

NY778	MATα *sec6-4 ura3-52 leu2-3,112*	Novick lab
Y3656	MATα *can1Δ::MFA1pr-HIS3-MFα1pr-LEU2 ura3Δ0 his3Δ1 leu2Δ0 lys2Δ0*	C. Boone
YAB200897	MATα *ade 2Δ:: hisG his3Δ200 leu2Δ0 met15Δ0 ura3Δ0 trp1Δ63*	A. Boman
YAB531	MATα *ade2Δ::hisG his 3Δ200 leu2Δ0 met15Δ0 trp1Δ63 ura3Δ0 gga1Δ::His3*	A. Boman
YAB532	MATα *ade2Δ::hisG his 3Δ200 leu2Δ0 met15Δ0 trp1Δ63 ura3Δ0 gga2Δ::His3*	A. Boman
YAB538	MATα *ade2Δ::hisG his 3Δ200 leu2Δ0 met15Δ0 trp1Δ63 ura3Δ0 gga1Δ::TRP1 gga2Δ::His3*	A. Boman

[a] European *Saccharomyces cerevisiae* Archives for Functional analysis, http://web.uni-frankfurt.de/fb15/mikro/euroscarf/index.html