## E08-04-0426 Bankaitis

## LEGENDS TO SUPPLEMENTAL FIGURES.

**Supplemental Figure S1.** Morphological manifestations of secretory pathway dysfunction in *sec14-1$^{ts}$* **(A)** and *sec6-4$^{ts}$* mutants **(B)** were analyzed by thin-section electron microscopy. Yeast were cultured to early logarithmic growth phase in YPD medium at 30°C. The cultures were then shifted to the 37°C for the indicated times. Cells were fixed, embedded in Spurr's resin, stained with uranyl acetate and imaged using a transmission electron microscope (20 kV). Representative images are shown (bar = 2μm). Berkeley bodies (defective TGN/endosomal structures) and undocked secretory vesicles are highlighted by arrows in images of the *sec14-1$^{ts}$* and *sec6-4$^{ts}$* mutants, respectively.

**Supplemental Figure S2.** CPY maturation in *sec14-1$^{ts}$ tlg2Δ* yeast. **(A)** Wildtype, *sec14-1$^{ts}$ , tlg2Δ,* and *sec14-1$^{ts}$ tlg2Δ* yeast were grown in minimal media at 30°C, and then shifted to 37°C or maintained at 30$^{o}$C for 2hrs, and radiolabeled with [$^{35}$S]-amino acids for 30 min. Chase (0, 15, 30 and 45 min) was initiated by introducing 2mM of both unlabeled methionine and cysteine, and reactions were terminated with ice-cold tricholoroacetic acid (final concentration 5%). CPY was immunoprecipitated with anti-CPY antibodies (1μl/OD600$_{nm}$). **(B)** Wild-type, *sec14-1$^{ts}$ , tlg2Δ,* and *sec14-1$^{ts}$ tlg2Δ* yeast were grown in minimal media at 30°C, and then shifted to 33.5°C or maintained at 30$^{o}$C for 2hrs, and radiolabeled with [$^{35}$S]-amino acids for 30 min. Chase (10 min) was initiated by introducing 2mM of both unlabeled methionine and cysteine, and reactions were terminated with ice-cold tricholoroacetic acid (final concentration 5%). CPY was immunoprecipitated with anti-CPY antibodies (1μl/OD600$_{nm}$). **(C)** Whole cell lysates were prepared from yeast strains of the indicated genotype, resolved by SDS-PAGE and blotted to

nitrocellulose. Blots were probed with either anti-alpha factor or anti-Sec61p polyclonal antibody. Where indicated strains were grown in the presence of 100μg/ml tunicamycin (Tm). The ER translocated, signal peptide cleaved, form of alpha-factor (Pαf) and the core glycosylated pαf modified on each of its three N-linked glycosylation sites (g3αf) are indicated at right. Wild-type yeast and *sec14-1$^{ts}$* mutants efficiently mature alpha-factor and those forms are not detectable in the SDS-PAGE system employed. Sec61 (indicated at right) was monitored as normalization control.

**Supplemental Figure S3.** *sec14-1$^{ts}$ tlg2Δ* yeast possess growth deficiencies consistent with defects in the UPR. **(A)** Wild-type, *sec14-1$^{ts}$, tlg2Δ,* and *sec14-1$^{ts}$ tlg2Δ* yeast were grown overnight at 30$^{o}$C in either inositol containing (dark grey boxes) or inositol free media (light grey boxes). The $A_{600nm}$ was recorded both before (white boxes) and after growth. **(B)** Wildtype, *sec14-1$^{ts}$, tlg2Δ,* and *sec14-1$^{ts}$ tlg2Δ* yeast were grown overnight at 30$^{o}$C in synthetic defined media in the absence (dark grey boxes) or presence (light grey boxes) of DTT (4mM). The $A_{600nm}$ was recorded both before (white boxes) and after growth.

**Supplemental Figure S4.** Hac1$^{I}$ stability is not compromised in *sec14-1$^{ts}$ tlg2Δ* cells. Wild-type and *sec14-1$^{ts}$ tlg2Δ* yeast harboring a yeast centromeric vector encoding for the expression of the constitutively active HA-Hac1$^{I}$ mutant were grown in minimal media at 30°C, shifted to 37°C or maintained at 30$^{o}$C for 2hrs and radiolabeled with [$^{35}$S]-amino acids for 30 min. HA-Hac1$^{I}$ was immunoprecipitated with anti-HA antibody **(A)** and the relative amount of HA-Hac1$^{I}$ isolated from each sample was determined by densitometry **(B)**.

**Supplemental Figure S4.** Effects of genetic ablation of Isc1 and Ppn1 function on ceramide mass. **(A)** Dihydroceramide and **(B)** phytoceramide mass (fmol ceramide/nmol Pi) are reduced in *sec14-1$^{ts}$ tlg2Δ isc1Δ* and *sec14-1$^{ts}$ tlg2Δ ppn1Δ* yeast relative to *sec14-1$^{ts}$ tlg2Δ* parental cells. Quantitative lipidomics were used to measure endogenous yeast ceramides. Lipids were extracted from 3 pooled cultures (30 $OD_{600nm}$) of each yeast strain grown overnight at 30$^{o}$C and shifted to 37$^{o}$C for 2 hours, prior to lipid extraction.

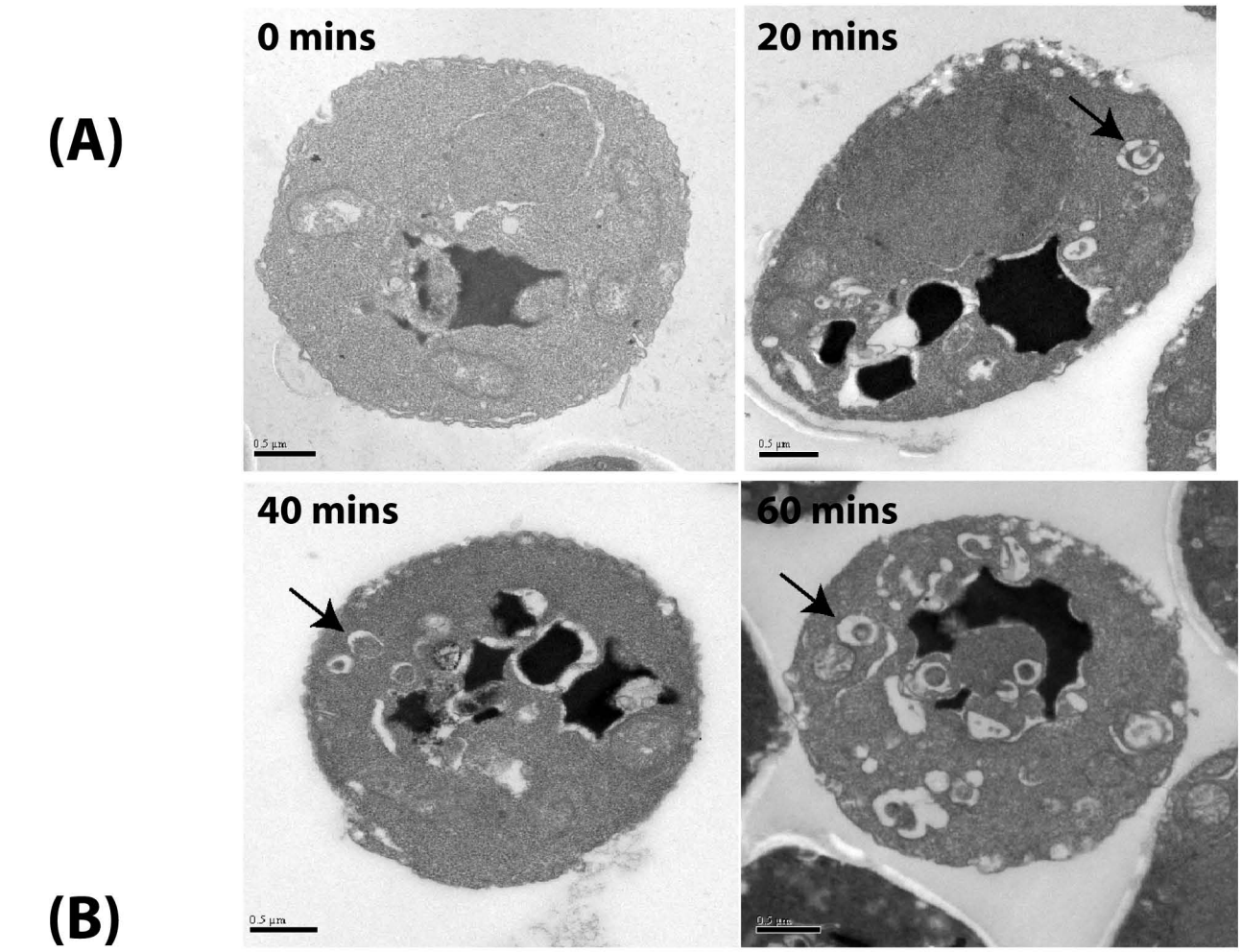
## LEGENDS TO SUPPLEMENTAL TABLES.

**Supplemental Table S1 –** Plasmids used in this study.

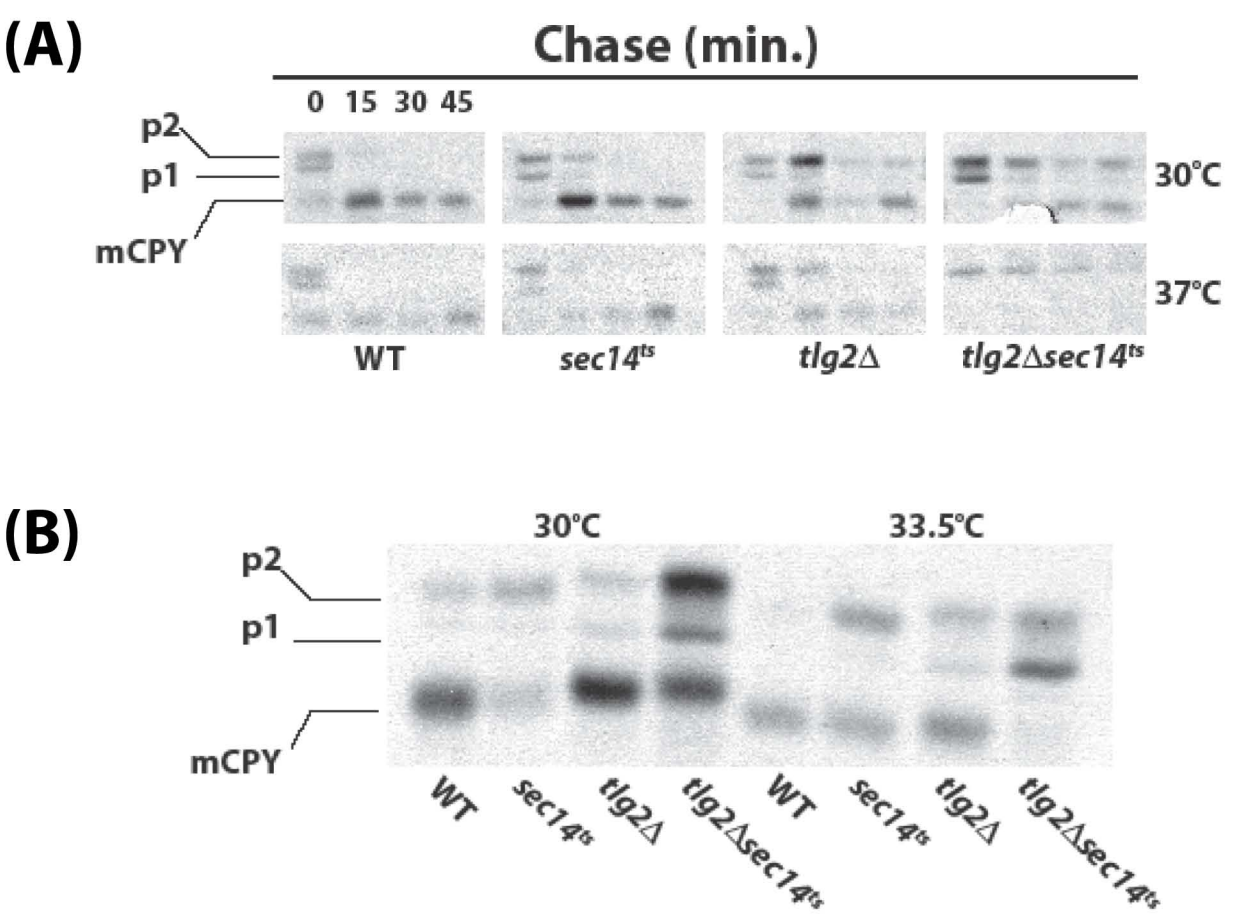
**Supplemental Table S2 –** A partial listing of genes identified in the *sec14-1$^{ts}$* SGA screen. These genes were consistently identified in three independent SGA experiments. The interactions with *arf1Δ*, *spo14Δ*, *sfh3Δ*, and *gcs1Δ* alleles confirm previous studies (see text), whereas the interactions with *snc2Δ*, *gsg1Δ* and *tlg2Δ* alleles are documented here. Otherwise, the listed genetic interactions have not been independently validated by meiotic analyses.

**Supplemental Table S3 - (A)** The 20 genes with the greatest magnitude of transcriptional down-regulation in *sec14-1$^{ts}$ tlg2Δ* mutants relative to wild-type. **(B)** The 20 genes with the greatest magnitude of transcriptional up-regulation in *sec14-1$^{ts}$ tlg2Δ* mutants relative to wild-type.

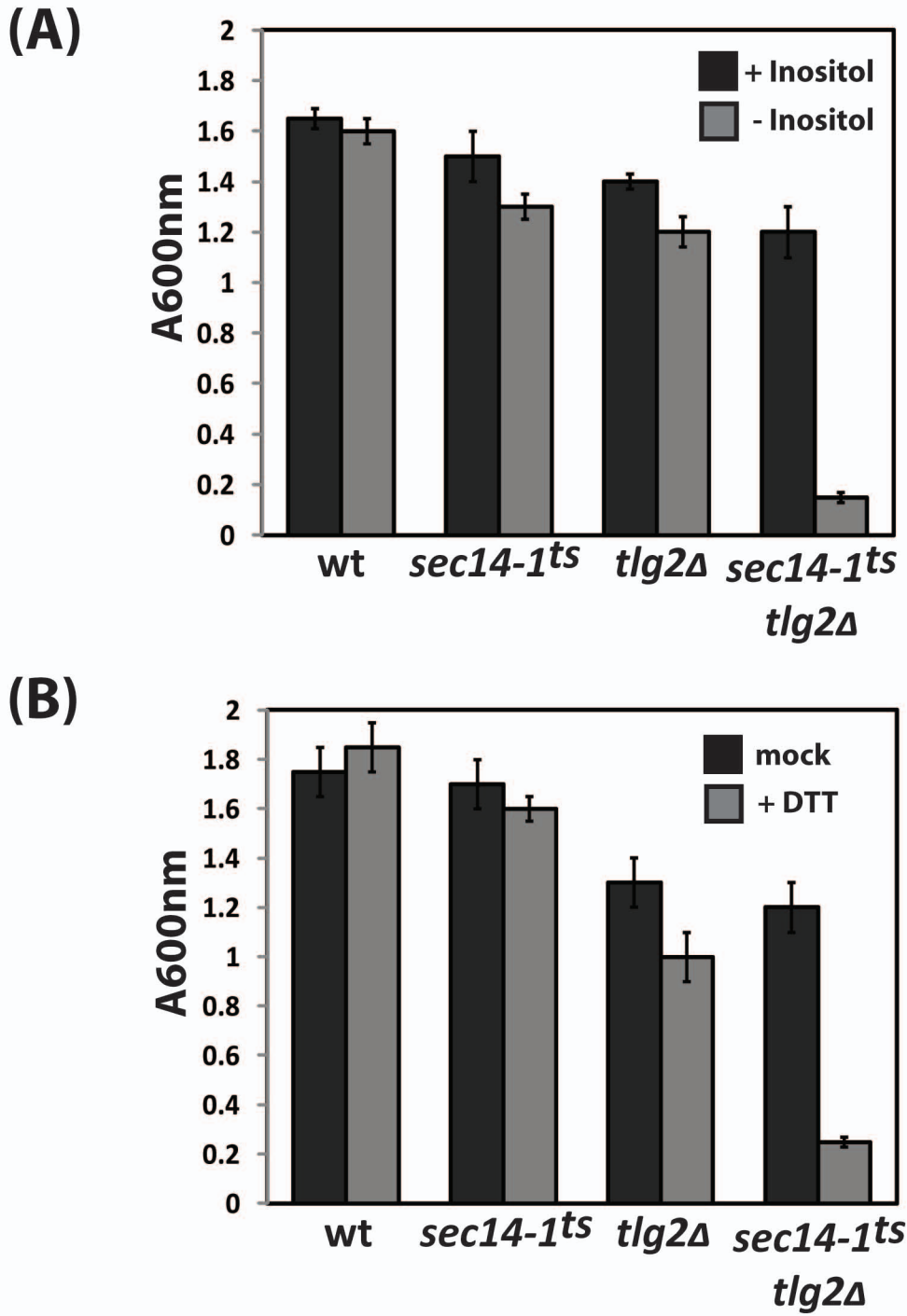
# Supplemental Figure S1



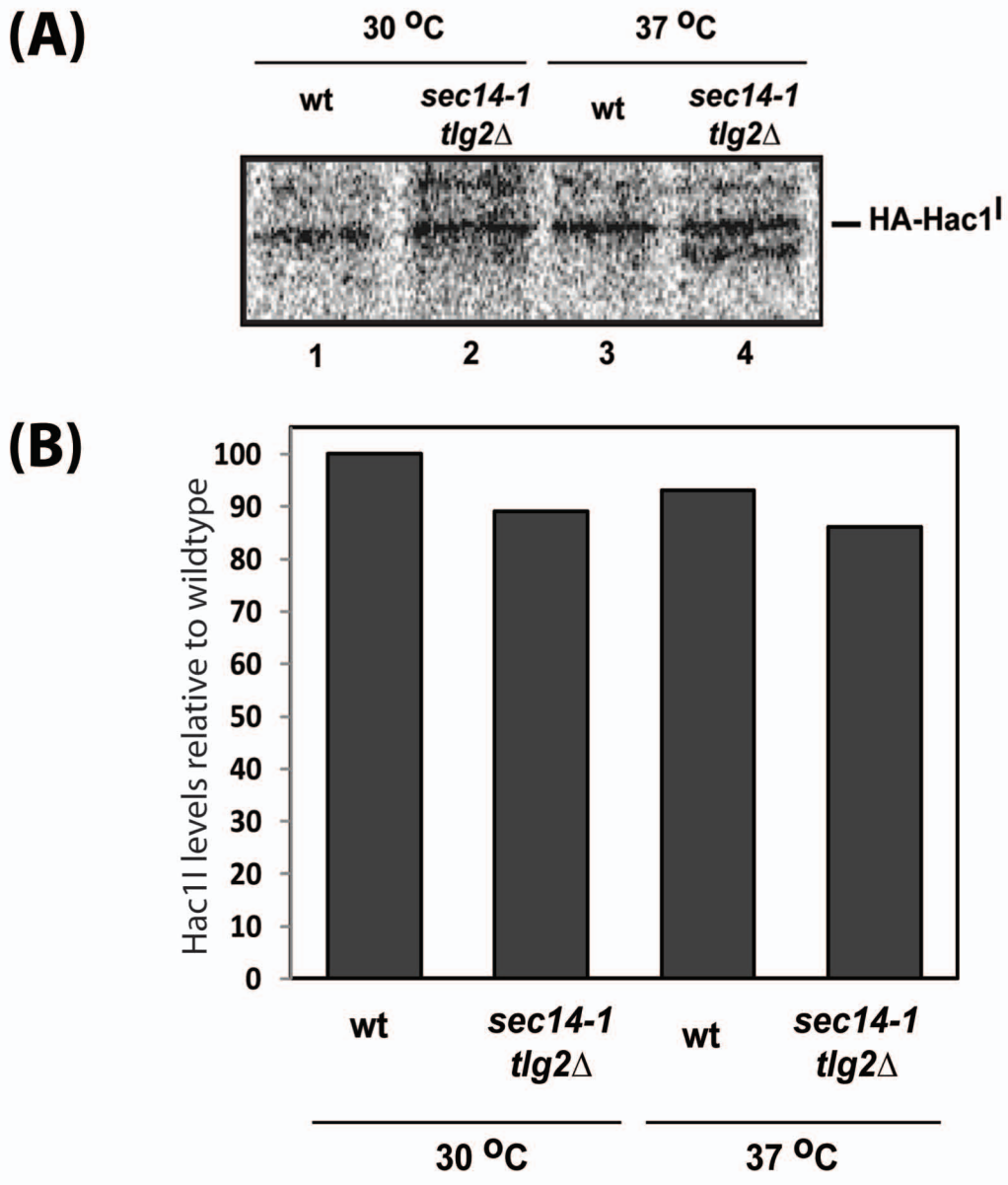
# Supplemental Figure S2



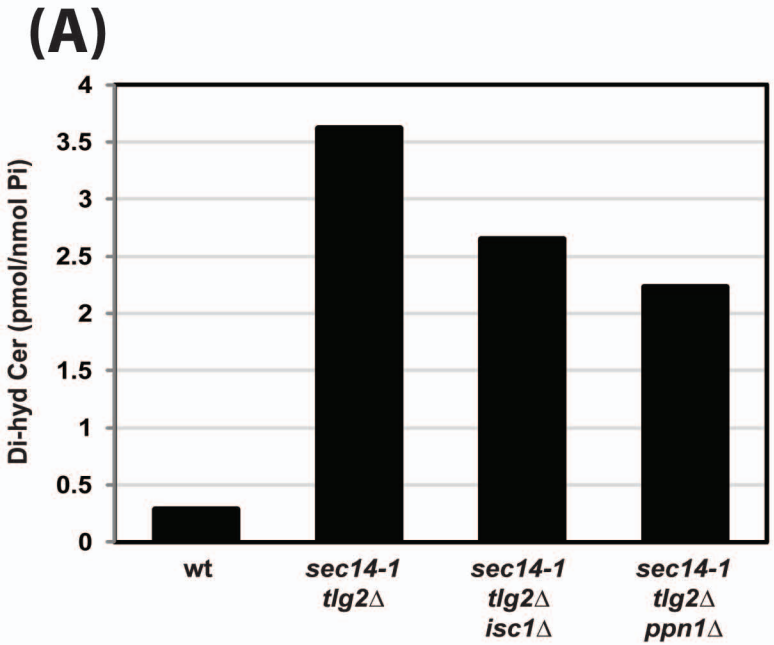
# Supplemental Figure S3

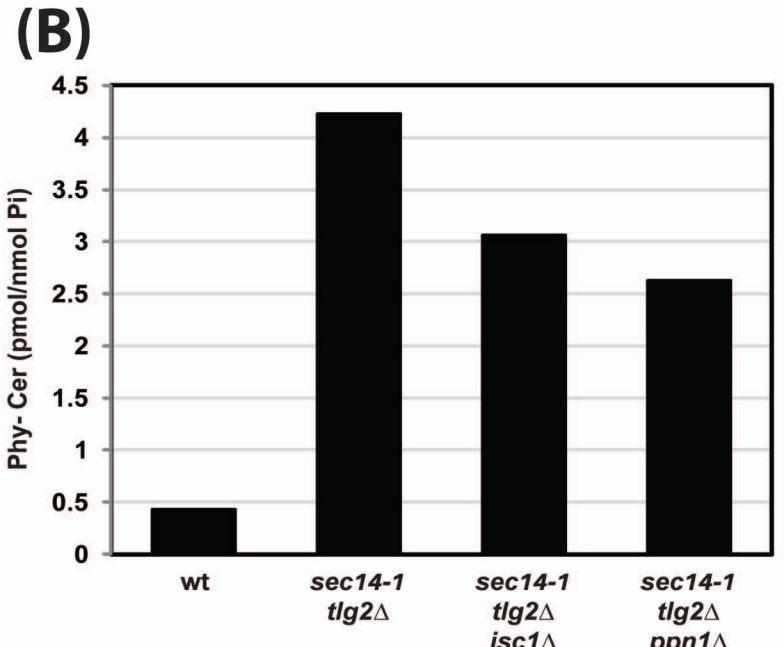


# Supplemental Figure S4



# Supplemental Figure S5



# Supplemental Table S1

| Plasmid | Description |
|---|---|
| pJT30 | YCp (*UPRE::LACZ, URA3*) |
| pRC43 | YCp (*HAC1$^{I}$, HIS3*) |
| pCTY468 | YCp (*sec14-1, URA3*) |
| p180 | YCp (*GCN4::LACZ, URA3*) |
| pCB506 | YCp (*GFP-SNC1, URA3*) |
| p DsRed-FYVE | YCp (*MET3::FYVE-dsRED, HIS3*) |
| pCTY468 | YCp (*sec14-1, URA3*) |
| pRE853 | Cloning intermediate for YIp *sec14-1::URA3* |
| pRE860 | Cloning intermediate for YIp *sec14-1::URA3* |
| pRE861 | YIp *sec14-1::URA3* |
| pCTY1501 | YCp (*TLG2, URA3*) |
| pCTY1502 | YCp (*tlg2$^{R13Q}$, URA3*) |
| pCTY1503 | YCp (*tlg2$^{Q278R}$, URA3*) |
| pCTY1504 | YEp *(YPC1, URA3)* |

# Supplemental Table S2

## *sec14*$^{ts}$ Synthetic Interactions: Synthetic Genomic Array Results

### PROTEIN TRAFFICKING

| ORF | Gene | Function |
|---|---|---|
| YJL204C | RCY1 | Endocytosis |
| YBR266C | SLM6 | Endocytosis |
| YOR327C | SNC2 | V-Snare Exocytosis |
| YDR108W | GSG1 | ER to Golgi Transport (TRAPP Subunit) |
| YDL192W | ARF1 | Exocytosis |
| YGR261C | APL6 | Golgi to Vacuole Transport |
| YER166W | DNF1 | Phospholipid Flippase |
| YPL051W | ARL3 | Endosome to TGN (ARF-Like) |
| YOL018C | TLG2 | Endosome to TGN T-SNARE |
| YNL154C | YCK2 | Casein Kinase I |
| YLL040C | VPS13/SOI1 | TGN Retention |
| YGL104C | VPS73 | Vacuolar Protein Sorting |
| YPR029C | APL4 | Clathrin Adaptor |
| YOR070C | GYP1 | YPT31/32 GAP |
| YPR051W | MAK3 | N-Acetyltransferase (ARL) |

### CELL WALL BIOGENESIS

| ORF | Gene | Function |
|---|---|---|
| YNL322C | KRE1 | Cell Wall Organization/Biogenesis |
| YLR436C | ECM30 | Cell Wall Organization/Biogenesis |
| YMR307W | GAS1 | Cell Wall Organization/Biogenesis |
| YGR059W | SPR3 | Cell Wall Organization/Biogenesis |
| YLR371W | ROM2 | Cell Wall Organization/Biogenesis |
| YGR229C | SMI1 | Cell Wall Organization/Biogenesis |
| YOR008C | SLG1 | Cell Wall Organization/Biogenesis |
| YCR089W | FIG2 | Cell Wall Organization/Biogenesis |

### CYTOSKELETON/POLARITY

| ORF | Gene | Function |
|---|---|---|
| YOR299W | BUD7 | Bud Site Selection |
| YDL151C | BUD30 | Bud Site Selection |
| YOR300W | HUF1 | Bud Site Selection |
| YCR047C | BUD23 | Bud Site Selection |
| YPL161C | BEM4 | Bud Site Selection |
| YLR429W | CRN1 | MT Binding/Actin Filament Organization |
| YDR150W | NUM1 | Tubulin Binding/NUC Migration |
| YNL153C | GIM3 | Tubulin Binding/Folding |
| YML094W | GIM5 | Tubulin Binding/Folding |
| YGR078C | PAC10 | Tubulin Binding/Folding |

### LIPID TRAFFICKING/METABOLISM

| ORF | Gene | Function |
|---|---|---|
| YER166W | DNF1 | Phospholipid Flippase |
| YKR031C | SPO14 | Phospholipase D |
| YMR015C | ERG5 | C-22 Sterol Desaturase |
| YLR056W | ERG3 | C-5 Sterol Desaturase |
| YLR450W | HMG2 | HMG CoA Reductase |
| YPL057C | SUR1 | MIPC Synthase |
| YBL011W | SCT1 | Glycerol-3-P O-Acyltransferase |
| YNL231C | SFH3 | Sec14-Like PITP |
| YPL087W | YDC1 | DI-Hydroceramidase |
| YLR372W | SUR4/ELO3 | Fatty Acid Elongase |
| YCR034W | FEN1/ELO2 | Fatty Acid Elongase |
| YOR011W | AUS1 | MDR-Like Sterol Transporter |
| YPL069C | BTS1 | Farnesyltransferase |

# Supplemental Table S3

**A**

**Top 20 genes, annotated, up regulated**

| | |
|---|---|
| *BTN2* | *TOS3* |
| *SPG1* | *SMP1* |
| *RPN4* | *CIT2* |
| *NRG1* | *BRR6* |
| *ICY2* | *NCE103* |
| *HSP42* | *ARO10* |
| *TIM18* | *ARL3* |
| *OSW1* | *UPC2* |
| *STI1* | *HSP82* |
| *RTS3* | *TPO4* |

**B**

**Top 20 genes, annotated, down regulated**

| | |
|---|---|
| *VPS20* | *PHO3* |
| *HMRA1* | *YHB1* |
| *COS7* | *RPL16B* |
| *URA3* | *RPL22A* |
| *COS12* | *RPL20B* |
| *ADE17* | *RPL14A* |
| *RPS22A* | *RPS18B* |
| *RPL20A* | *RPS11A* |
| *RPS7A* | *RPS10A* |
| *RPS4A* | *RPS11B* |