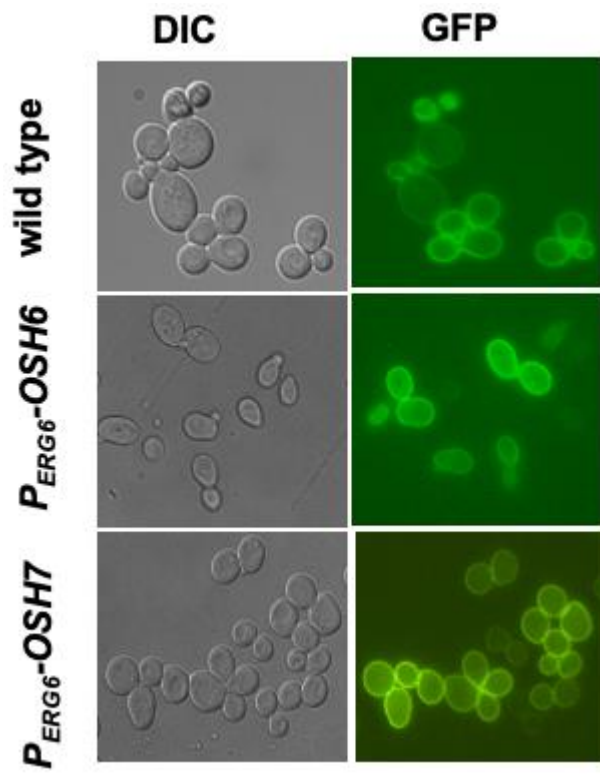
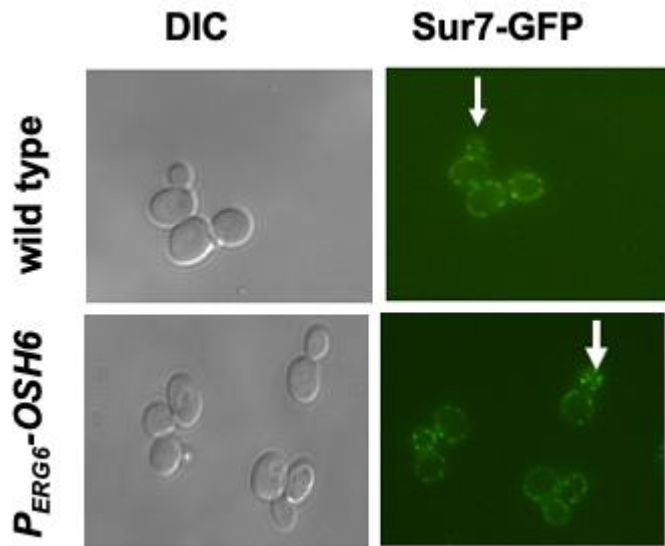


**FIGURE S1: *OSH6* differentially interacts with *DRS2* and *SAC1*.** (A) Vacuolar morphology of *drs2Δ* cells with a high copy *OSH6* (pCB237) or *OSH3* (pCB238) plasmid. (B) Growth of *sac1Δ* cells with the indicated plasmid on SC-URA with glucose or galactose media at 30°C for two days. (C) Vacuolar morphology of *sac1Δ* cells with the indicated plasmid.



**FIGURE S2: Localization of phosphatidylserine.** Cells of the indicated strains were transformed with the PS-labeling LactC2-GFP plasmid. The transformants were grown to early log phase and then photographed.



**FIGURE S3: Over-expression of *OSH6* does not alter *Sur7* secretion.** Cells of a wild type strain carrying *Sur7*-GFP (DLY046) and a  $P_{ERG6}$ -*OSH6* strain carrying the *Sur7*-GFP (FTY515) were grown in synthetic complete medium to early log phase and then photographed. The arrow points to a small bud of  $P_{ERG6}$ -*OSH6* cells with *Sur7* on the bud plasma membrane.

***sur4*Δ**

**FIGURE S4: Over-expression of *OSH6* promotes vacuole fusion in *sur4*Δ.** Cells of *sur4*Δ were transformed with the indicated plasmids, grown to early log phase, labeled by FM4-64 for one hour and chased for three hours at 30°C, and then photographed.

TABLE S1. Strains used in this study.

| Strain                          | Genotype                                                                                                                                                               | Source     | Used in                    |
|---------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|----------------------------|
| BY4742                          | MAT $\alpha$ <i>his3<math>\Delta</math>1 leu2<math>\Delta</math>0 lys2<math>\Delta</math>0 ura3<math>\Delta</math>0</i>                                                | Invitrogen | Figs. 2A, B, C, 5B, 6C, S2 |
| FTY536                          | BY4742 <i>URA3::KanMx-P<sub>ERG6</sub>-OSH6</i>                                                                                                                        | this study | Figs. 2A, B, C, S2         |
| FTY521                          | BY4742 <i>URA3::KanMx-P<sub>ERG6</sub>-OSH7</i>                                                                                                                        | this study | Figs. 2A, B, S2            |
| FTY624                          | BY4742 <i>URA3::SEC7-mCerry-P<sub>ERG6</sub>-OSH6</i>                                                                                                                  | this study | Fig. 2D                    |
| FTY625                          | BY4742 <i>URA3::SEC7-mCerry-P<sub>ERG6</sub>-OSH7</i>                                                                                                                  | this study | Fig. 2D                    |
| <i>drs2<math>\Delta</math></i>  | BY4742 <i>DRS2::KanMx</i>                                                                                                                                              | Invitrogen | Figs. 1, S1A               |
| <i>sac1<math>\Delta</math></i>  | BY4742 <i>SAC1::KanMx</i>                                                                                                                                              | Invitrogen | Fig. 2, S1B, C             |
| DLY046                          | BY4742 <i>SUR7-GFP::KanMX</i>                                                                                                                                          | [19]       | Fig. S3                    |
| FTY515                          | BY4742 <i>URA3-P<sub>ERG6</sub>-OSH6 SUR7-GFP-KanMx</i>                                                                                                                | this study | Fig. S3                    |
| QAY559                          | BY4742 <i>PMA1-mCherry-HIS3Mx</i>                                                                                                                                      | [19]       | Figs. 3A, 4A               |
| FTY518                          | BY4742 <i>URA3-P<sub>ERG6</sub>-OSH6 PMA1-mCherry-HIS3Mx</i>                                                                                                           | this study | Figs. 3A, 4B               |
| HHY103                          | BY4742 <i>PMA1-GFP-hphNT1</i>                                                                                                                                          | [12]       | Fig. 3B                    |
| FTY517                          | MAT $\alpha$ <i>his3<math>\Delta</math>1 leu2<math>\Delta</math>0 lys2<math>\Delta</math>0 ura3<math>\Delta</math>0 URA3-P<sub>ERG6</sub>-OSH6 PMA1-mCherry-HIS3Mx</i> | this study | Fig. 3B                    |
| <i>lag1<math>\Delta</math></i>  | BY4742 <i>LAG1::KanMx</i>                                                                                                                                              | Invitrogen | Fig. 6                     |
| FTY373                          | BY4742 <i>URA3-P<sub>ERG6</sub>-OSH6</i>                                                                                                                               | [5]        | Fig. 6C                    |
| FTY 527                         | BY4742 <i>URA3-P<sub>ERG6</sub>-OSH6 LAG1::KanMx</i>                                                                                                                   | this study | Fig. 6C                    |
| FTY 528                         | BY4742 <i>URA3-P<sub>ERG6</sub>-OSH6 PMA1-mCherry-HIS3Mx LAG1::KanMx</i>                                                                                               | This study | Fig 5A                     |
| <i>vps13<math>\Delta</math></i> | BY4742 <i>VPS13::KanMx</i>                                                                                                                                             | Invitrogen | Fig. 6C                    |
| FTY534                          | BY4742 <i>URA3-P<sub>ERG6</sub>-OSH6 VPS13::KanMx</i>                                                                                                                  | this study | Fig. 6C                    |
| FTY532                          | QAY559 <i>VPS13::KanMx</i>                                                                                                                                             | this study | Fig. 5                     |
| FTY541                          | BY4742 <i>URA3-P<sub>ERG6</sub>-OSH6 VPS13::KanMx PMA1-mCherry-HIS3</i>                                                                                                | this study | Fig. 5                     |
| <i>gga2<math>\Delta</math></i>  | BY4742 <i>GGA2::KanMx</i>                                                                                                                                              | Invitrogen | Fig. 5B                    |
| <i>sur4<math>\Delta</math></i>  | BY4742 <i>SUR4::KanMx</i>                                                                                                                                              | Invitrogen | Fig. S4                    |
| 560                             | BY4742 <i>GGA2::KanMx PMA1-mCherry-HIS3</i>                                                                                                                            | this study | Fig. 5B                    |
| 562                             | BY4742 <i>URA3-P<sub>ERG6</sub>-OSH6 GGA2::KanMx</i>                                                                                                                   | this study | Fig. 5B                    |
| 564                             | BY4742 <i>URA3-P<sub>ERG6</sub>-OSH6 GGA2::KanMx PMA1-mCherry-HIS3</i>                                                                                                 | this study | Fig. 5B                    |