**Supplemental Figure S1.** Localization of Gip1 during sporulation. (A, B) TC544 (gip1Δ) cells were transformed with pRS424-GIP1-GFP and pRS316-MPC54-RFP (A) or pRS316-NOP1-mCherry (B), respectively, sporulated, and observed at 7 h. (C) H13 (sso1Δ) cells were transformed with pRS424-GIP1-GFP and pRS316-MPC54-RFP, sporulated, and observed at 11 h. (D) TC544 (gip1Δ) cells were transformed with pRS424-GIP1-GFP, sporulated, and observed at 7 h. Percentages of normally extended prospore membranes (left) and aberrantly over-extended prospore membranes (right) are shown. More than 200 cells were observed in three independent colonies. Bar, 5 μm.

**Supplemental Figure 2.** Analysis of Gip1-deletion mutants. AN120 (wild-type) and TC544 (gip1Δ) cells were transformed with pRS424-GIP-deletion-mutant-GFP coding for indicated mutants and pRS316-P_{TEF1}-mKate2-SPO20^{51–91}, sporulated and observed at 7 to 9 h. Representative cells are shown. In all images scale bars indicates 5 μm. Concerning wild-types cells of Gip1-N1 to N8 and gip1Δ cell of Gip1-N8, top images indicate extending prospore membranes, and bottom images indicate prospore membranes after rounding up. Concerning to Gip1-C1, top images indicate SPB patterns, middle image in wild-type cell and bottom image in gip1Δ cell indicate unclear cytosolic patterns, and bottom image of wild-type cell indicate a spore cytoplasmic pattern. Concerning to Gip1-C2, top images indicate SPB patterns, middle images indicate unclear cytosolic patterns, and bottom images indicate nuclear patterns. Concerning to Gip1-C3, top images indicate unclear cytosolic patterns, middle images indicate septin patterns, and bottom images indicate nuclear patterns. Bar, 5 μm.

**Supplemental Figure 3.** Analysis of two α-helices in Gip1 N-terminal region. (A-C) AN120 (wild-type) and TC544 (gip1Δ) cells were transformed with pRS424-GIP1-N1-mutant-GFP (A), pRS424-GIP1-N2-mutant-GFP (B), pRS424-GIP1-mutant-GFP (C), coding for indicated mutants, and pRS316-P_{TEF1}-mKate2-SPO20^{51–91}, sporulated and observed at 7 to 9 h. Concerning wild-types cells expressing Gip1-N2-HyM, HeM2, BM+HyM, and HeM1+HeM2, top images indicate extending prospore membranes, and bottom images indicate prospore membranes after rounding up. Concerning Gip1-HyM and Gip1-HeM2, top images indicate septin patterns, and bottom images indicate unclear cytosolic patterns. Bar, 5 μm.

**Supplemental Figure 4.** Analysis of Gip1 domains involved in its septin localization. TC544 (gip1Δ) cells were transformed with pRS424-GIP1-Δsep-GFP and pRS316-P_{TEF1}-mKate2-SPO20^{51–91}, sporulated and observed at 7 to 9 h. Alternative cells are shown. Bar, 5 μm.
**Movie S1.** Time-lapse video microscopy of prospore membranes, part 1. AN120 (wild-type) cells expressing GFP-Spo20\(^{51-91}\) were sporulated and analyzed by time-lapse fluorescence microscopy. The movie is shown at 5 frames/sec, and frames were taken at 2-min intervals. A z-stack of 12 images was collected at each time point. Each frame is a projection of the images. Time is indicated in minutes.

**Movie S2.** Time-lapse video microscopy of prospore membranes, part 2. TC544 (gip1\(\Delta\)) cells expressing GFP-Spo20\(^{51-91}\) were sporulated and analyzed by time-lapse fluorescence microscopy. The movie is shown at 5 frames/sec, and frames were taken at 1.5-min intervals. A z-stack of 12 images was collected at each time point. Each frame is a projection of the images. Time is indicated in minutes.

**Movie S3.** Time-lapse video microscopy of Gip1-GFP, part 1. TC544 (gip1\(\Delta\)) cells expressing Gip1-GFP were sporulated and analyzed by time-lapse fluorescence microscopy. The movie is shown at 5 frames/sec, and frames were taken at 2-min intervals. A z-stack of 12 images was collected at each time point and then deconvoluted. Each frame is a projection of the images. Time is indicated in minutes.

**Movie S4.** Time-lapse video microscopy of Gip1-GFP, part 2. TC544 (gip1\(\Delta\)) cells expressing Gip1-GFP were sporulated and analyzed by time-lapse fluorescence microscopy. The movie is shown at 5 frames/sec, and frames were taken at 2-min intervals. A z-stack of 12 images was collected at each time point and then deconvoluted. Each frame is a projection of the images. Time is indicated in minutes.

**Movie S5.** Time-lapse video microscopy of Gip1-GFP, part 3. TC544 (gip1\(\Delta\)) cells expressing Gip1-GFP were sporulated and analyzed by time-lapse fluorescence microscopy. The movie is shown at 5 frames/sec, and frames were taken at 2-min intervals. A z-stack of 12 images was collected at each time point. Each frame is a projection of the images. Time is indicated in minutes.

**Supplemental Table 1.** Yeast strains used in this study.

**Supplemental Table 2.** Oligonucleotides used in this study.

**Supplemental Table 3.** Plasmids used in this study.
Figure S4

Gip1-Δsep-GFP  mKate2-Spo20^{51-91}  Merge

gip1Δ
### Supplemental Table 1

**Yeast strains used in this study**

| Name      | Genotype                                                                 | Source                      |
|-----------|---------------------------------------------------------------------------|-----------------------------|
| AH109     | MATa trp1-901 leu2-3, 112 ura3-52 his3-200 gal4Δ gal80Δ                   | Clontech                    |
|           | LYS2::GAL1_SV-GAL1_SV-HIS3 GAL2_SV-GAL2_SV-ADE2                         |                             |
|           | URA3::MEL1_SV-MEL1_SV-lacZ                                               |                             |
| AN117-4B  | MATa his3ΔSK ura3 trp1::hisG leu2 arg4-NSp1 lys2 hoΔ::LYS2 rme1::LEU2    | Neiman et al. (2000)        |
| AN117-16D | MATa his3ΔSK ura3 trp1::hisG leu2 lys2 hoΔ::LYS2                         | Neiman et al. (2000)        |
| AN120     | MATa/MATa his3ΔSK his3ΔSK ura3/ura3 trp1::hisG/trp1::hisG                | Neiman et al. (2000)        |
|           | leu2/leu2 arg4-NSp1ARG4 lys2/lys2 hoΔ::LYS2 hoΔ::LYS2 rme1::LEU2 RME1   |                             |
| AN264     | MATa/MATa his3ΔSK his3ΔSK ura3/ura3 trp1::hisG/trp1::hisG                | Coluccio et al. (2004)      |
|           | leu2/leu2 arg4-NSp1ARG4 lys2/lys2 hoΔ::LYS2 hoΔ::LYS2 rme1::LEU2 RME1   |                             |
|           | dit1::HIS3MX6/dit1::HIS3MX6                                             |                             |
| HI3       | MATa/MATa his3ΔSK his3ΔSK ura3/ura3 trp1::hisG/trp1::hisG                | Nakanishi et al. (2006)     |
|           | leu2/leu2 arg4-NSp1ARG4 lys2/lys2 hoΔ::LYS2 hoΔ::LYS2 rme1::LEU2 RME1   |                             |
|           | sso1::HIS3MX6/sso1::HIS3MX6                                             |                             |
| HI29      | MATa/MATa his3ΔSK his3ΔSK ura3/ura3 trp1::hisG/trp1::hisG                | Nakanishi et al. (2007)     |
|           | leu2/leu2 arg4-NSp1ARG4 lys2/lys2 hoΔ::LYS2 hoΔ::LYS2 rme1::LEU2 RME1   |                             |
|           | vps13::HIS3MX6/vps13::HIS3MX6                                           |                             |
| HIY65     | MATa/MATa his3ΔSK his3ΔSK ura3/ura3 trp1::hisG/trp1::hisG                | This study                  |
|           | leu2/leu2 arg4-NSp1ARG4 lys2/lys2 hoΔ::LYS2 hoΔ::LYS2 rme1::LEU2 RME1   |                             |
|           | gip1::kanMX6/gip1::kanMX6 VPS3::GFP::HIS3MX6/VPS3::GFP::HIS3MX6          |                             |
| NY528     | MATa/MATa his3ΔSK his3ΔSK ura3/ura3 trp1::hisG/trp1::hisG                | Pablo-Hernando et al. (2008)|
|           | leu2/leu2 arg4-NSp1ARG4 lys2/lys2 hoΔ::LYS2 hoΔ::LYS2 rme1::LEU2 RME1   |                             |
|           | spr3::HIS3MX6/spr3::HIS3MX6                                             |                             |
| NY703     | MATa/MATa his3ΔSK his3ΔSK ura3/ura3 trp1::hisG/trp1::hisG                | Pablo-Hernando et al. (2008)|
|           | leu2/leu2 arg4-NSp1ARG4 lys2/lys2 hoΔ::LYS2 hoΔ::LYS2 rme1::LEU2 RME1   |                             |
|           | spr28::HIS3MX6/spr28::HIS3MX6                                           |                             |
| TC134     | MATa his3ΔSK ura3 trp1::hisG leu2 lys2 hoΔ::LYS2 GIP1::yEGFP::HIS3MX6    | This study                  |
| Name | Genotype | Source |
|------|----------|--------|
| TC544 | MATa/MATa his3ΔSK/ his3ΔSK ura3/ura3 trp1::hisG/trp1::hisG leu2/leu2 arg4-NspI/ARG4 lys2/lys2 hoΔ::LYS2/hoΔ::LYS2::RME1 gip1::kanMX6/gip1::kanMX6 | This study |
| TC545 | MATa/MATa his3ΔSK/ his3ΔSK ura3/ura3 trp1::hisG/trp1::hisG leu2/leu2 arg4-NspI/ARG4 lys2/lys2 hoΔ::LYS2/hoΔ::LYS2::RME1 spo73::kanMX6/spo73::kanMX6 | Okumura et al.. (2015) |
| TC555 | MATa/MATa his3ΔSK/ his3ΔSK ura3/ura3 trp1::hisG/trp1::hisG leu2/leu2 arg4-NspI/ARG4 lys2/lys2 hoΔ::LYS2/hoΔ::LYS2::RME1 spo73::HIS3MX6/spo73::HIS3MX6 | Okumura et al.. (2015) |
| TC564 | MATa/MATa his3ΔSK/ his3ΔSK ura3/ura3 trp1::hisG/trp1::hisG leu2/leu2 arg4-NspI/ARG4 lys2/lys2 hoΔ::LYS2/hoΔ::LYS2::RME1 gip1::kanMX6/gip1::kanMX6 spo73::HIS3MX6/spo73::HIS3MX6 | This study |
| TC572 | MATa/MATa his3ΔSK/ his3ΔSK ura3/ura3 trp1::hisG/trp1::hisG leu2/leu2 arg4-NspI/ARG4 lys2/lys2 hoΔ::LYS2/hoΔ::LYS2::RME1 vps13::kanMX6/vps13::kanMX6 | Okumura et al.. (2015) |
| TC581 | MATa/MATa his3ΔSK/ his3ΔSK ura3/ura3 trp1::hisG/trp1::hisG leu2/leu2 arg4-NspI/ARG4 lys2/lys2 hoΔ::LYS2/hoΔ::LYS2::RME1 spo71::kanMX6/spo71::kanMX6 | Okumura et al.. (2015) |
| TNY293 | MATa/MATa his3ΔSK::P_{PPA2}-mKateBFP2-SPO20{}^{5'-GC}::HIS3/ his3ΔSK ura3::MPC54-RFP::URA3/ura3 trp1::hisG/trp1::hisG leu2/leu2 arg4-NspI/ARG4 lys2/lys2 hoΔ::LYS2/hoΔ::LYS2::RME1 | This study |
| TNY294 | MATa/MATa his3ΔSK::P_{PPA2}-mKateBFP2-SPO20{}^{5'-GC}::HIS3/ his3ΔSK ura3::MPC54-RFP::URA3/ura3 trp1::hisG/trp1::hisG leu2/leu2 arg4-NspI/ARG4 lys2/lys2 hoΔ::LYS2/hoΔ::LYS2::RME1 gip1::kanMX6/gip1::kanMX6 | This study |
| TNY300 | MATa/MATa his3ΔSK::P_{PPA2}-mKateBFP2-SPO20{}^{5'-GC}::HIS3/ his3ΔSK ura3::SPR28-mKate2::URA3/ura3 trp1::hisG/trp1::hisG leu2/leu2 arg4-NspI/ARG4 lys2/lys2 hoΔ::LYS2/hoΔ::LYS2::RME1 gip1::kanMX6/gip1::kanMX6 | This study |
| TNY375 | MATa/MATa his3ΔSK::P_{PPA2}-mKate2-SPO20{}^{5'-GC}::HIS3/ his3ΔSK ura3/ura3 trp1::hisG/trp1::hisG leu2/leu2 arg4-NspI/ARG4 lys2/lys2 hoΔ::LYS2/hoΔ::LYS2::RME1 | This study |
| Name   | Genotype                                                                 | Source   |
|--------|---------------------------------------------------------------------------|----------|
| TNY411 | $\text{MAT}^a$/MATa $\text{his3}^+$/his3$^-$ $\text{SK}^+$/ura3 $\text{trp}1::\text{hisG}$/trp1::hisG $\text{leu2}$/leu2 $\text{arg4}$/NspI $\text{lys2}$/lys2 $\text{ho}^+$/$\text{LYS2}$/ho$^+$/$\text{LYS2}$ $\text{rme1}$::RME1 $\text{gip1}::\text{kanMX6}$/gip1::kanMX6 $\text{vps13}::\text{HIS3MX6}$/vps13::HIS3MX6 | This study |
## Supplemental Table 2

Oligonucleotides used in this study

| Name  | Sequence (5' → 3')                                      |
|-------|--------------------------------------------------------|
| HT66  | GAAGAACCGCGGAGATCTATATATACCTGGTTATCC                  |
| HT84  | GAAGAACTCGAGAAAGGTGTATTCGTTTCCCCCTTGG                 |
| HT281 | GTATAAGTACTCAAGGGCCAAAATTATATCGCTTGAGGAGGTTTTTT      |
|       | CGGATCCCGGTTAATTA                                      |
| HT282 | TTCACGTAACTACACGTACAGTTGTGGATCCTTATAGCTACAAAGTC      |
|       | GAATTCGAGCTGTGTTTAAA                                   |
| HT309 | AATCAAGTTCGAAATTTTTTGGGAAGAGCTATTAATATTAGCTAATT       |
|       | CGGATCCCGGTTAATTA                                      |
| HT315 | GAAGAACTCGAGTCAAAAAAACATCTCATCAAGC                    |
| HT480 | GCAGCTGCAAGATCGTGAAATTTGAAAAT                          |
| IC4   | ACATCGCCATTGGTGAGAATAAATAGTACTGTGA                    |
|       | AGCTATCTACCGATCCTCCCGGGTTAATTA                      |
| IC5   | TAAATCGATGAAATTATAGCTACATAGTGATACAAAAGCGGTATATACTT   |
|       | GAATTCGAGCTGTGTTAAAC                                   |
| IC7   | GAATCTTGGTCATGTAAT                                  |
| IC8   | TGTTCTGCATAATGTCACT                                  |
| TN25  | GAAGAAGAGCTCAATAGCTTCAAAATGTTCCTA               |
| TN26  | GAAGAAGAGCGGCGCCAAAATAACTTAGATAGTAGCTATGCTTCT       |
| TN27  | GAAGAAGAGCCGCGCCCTTTATGAGTAAGGAGAAGAAACT           |
| TN98  | GAAGAACTCGAGGTTAATAAAATAGCTAGAG                 |
| TN99  | GAAGAAGCTACAGATCTATATATACCGTGTTATC          |
| TN100 | GAAGAAGAGCTCCACACGACTCCTCAAGCAGATCCCTGGAACCTC       |
|       | CGGCGAGAACTAGTCTTAAGGCGTGCTAC                    |
| TN110 | GAAGAAGAGCTCGATATCAACGGATCGCTAC                   |
| TN175 | GAAGAAGAGCTCAAGCAATTAAAAGAATCAACAG                |
| TN176 | GAAGAAGAATTCGACCTTATTTTGAGAAGGAGAAGAACT          |
| TN194 | GAAGAAGAAGCGGCGCCAAAATACCCAAAAGGAATCTACTC       |
| TN211 | GAAGAAGCTCGAATGAGATATGCTTCCGTAC                  |
| TN357 | AAACCACAAGCGGCCCTTTATGAGGAGCGAGCTGATTAA         |
| TN358 | CAAATGTCCATTCTAGATCTGGCCCGAGTTTGCTAG            |
| TN360 | GAAGAAGCTCGAGTCAAAAGACATCTCATCAAGCG             |
| TN361 | GAAGAAGAGATCCGAGGAGGTTCAATATCTGGACAAACAG        |
| Name  | Sequence (5' → 3') |
|-------|-------------------|
| TN362 | GAAGAAGGATCCGCGAGGTAGTAATGATCGATCCACAGATGG |
| Name | Sequence (5’ → 3’) |
|------|------------------|
| YN5  | GAAGAAGCGGCCGCTCGATCCCCGGGTTAATTA |
| YN6  | GAAGAAGCGGCCGCTCTCCATATAATTAGCTAAAT |
| YN7  | GAAGAAGCGGCCGCAAGACCTTTTTTGTTTCTT |
| YN8  | GAAGAAGCGGCCGCTTCCATAATTAGCTAATAAT |
| YN9  | GAAGAAGCGGCCGCTCGAATGCCTGTCAG |
| YN10 | GAAGAAGCGGCCGCTCAGATAATCTGGACAAACC |
| YN11 | GAAGAAGCGGCCGCTGGATCTTTTAAAGGACAAAGCCCC |
| YN12 | GAAGAAGCGGCCGCTGAAATGGATACAGATCTTTG |
| YN14 | GAAGAAGCGGCCGCTTTATTCTCAGAAT |
| YN15 | GAAGAAGCGGCCGCTACATTGGATAAATGACAGAATAGA |
| YN16 | GAAGAAGCGGCCGCTGAAATGGATACAGATCTTTG |
| YN18 | GAAGAAGCGGCCGCTGAAATGGATACAGATCTTTG |
| YN38 | GAAGAAATCGATCGGATCCCCGGGTTAATTAAC |
| YN39 | GAAGAAATCGATCTCAGATCCGATTATTGGAATTC |
| YN68 | GAAGAAATCGATGCTGAAATCTCTGCAAAACAGG |
| YN69 | GAAGAATCGATGCTGAAATCTCTGCAAAACAGG |
| YN70 | CACGTGTGATGGAAGATCTGGAAGGACTGACAGG |
| YN72 | GCGAAAAGGGATTGAAAATACGTCATG |
| YN73 | ATTATCTCTAGCTACATAGAATTTTTCTTATATGCAACAGGTTT |
| YN78 | GAAGAAATCGATAATATATTTTTCATGGAAGGCAAG |
| YN79 | GAAGAAATCGATGCTGAAATCTCTGCAAAATCTC |
| YN82 | TCTTCTCAAAAGACTCAAATGGGTCAG |
| YN128 | GAAGAATCGATGCTGAAATCTCTGCAAAATGGGCAAAAC |
| YO255 | GGAAGGAGATGCTAAGTGGGAGTGACAGG |
| YO256 | GAGGAAGCTGACAGTTTCTTTCTTCATCAGC |
| YO322 | GAGGAAGGACCATAATGGAGATTCTGCAACAGGTTG |
| YO325 | GAGGAAGCTGACAGTTTCTTTCTTCATCAGC |
| YSM0017 | GAGGAAGCTGACAGTTTCTTTCTTCATCAGC |
| YSM0018 | GAGGAAGCTGACAGTTTCTTTCTTCATCAGC |
| YSO319 | GAGGAAGCTGACAGTTTCTTTCTTCATCAGC |

The table above lists various sequences along with their names.
### Supplemental Table 3

Plasmids used in this study

| Name                  | Protein Expressed/Description | Source               | Used Primer | Used Plasmid          |
|-----------------------|-------------------------------|----------------------|-------------|-----------------------|
| **Vectors**           |                               |                      |             |                       |
| pRS303                | Integration vector            | Sikorski and Hieter (1989) | -           | -                     |
| mod_pRS303            | Modified integration vector   | This study           | -           | FRP467                |
|                       |                               |                      |             | pRS303                |
| pRS306                | Integration vector            | Sikorski and Hieter (1989) | -           | -                     |
| pRS314                | Low-copy vector               | Sikorski and Hieter (1989) | -           | -                     |
| pRS314-TCYC1          | TCYC1                         | This study           | -           | pRS314                |
| pRS314-P_GFP-TCYC1    | P_GFP-TCYC1                   | This study           | TN431, TN432| pRS314-TCYC1          |
| pRS314-P_TEF1         | P_TEF1                        | This study           | TN25, TN26  | pRS314               |
| pRS314-P_TEF1-C-GFP-T_ADH1 | P_TEF1-C-GFP-T_ADH1         | This study           | -           | pRS314-P_TEF1         |
|                       |                               |                      |             | pRS424-C-GFP-T_ADH1   |
| pRS316                | Low-copy vector               | Sikorski and Hieter (1989) | -           | -                     |
| pRS316-P_TEF1         | P_TEF1                        | This study           | TN25, TN26  | pRS316               |
| pRS316-P_TEF1-C-mCherry-T_ADH1 | P_TEF1-C-mCherry          | This study           | TN98, TN99  | pRS316-P_TEF1        |
| pRS316-P_TEF1-C-mKate2-T_ADH1 | P_TEF1-C-mKate2            | This study           | TN380, TN381| pRS316-P_TEF1-C-mKate2-T_ADH1 |
| pRS424                | High-copy vector              | Christianson *et al.* (1992) | -           | -                     |
| pRS424-P_TEF1         | P_TEF1                        | This study           | TN25, TN26  | pRS424               |
| Name              | Protein Expressed/Description | Source          | Used Primer | Used Plasmid                      |
|-------------------|------------------------------|-----------------|-------------|-----------------------------------|
| pRS424-T<sub>CYC1</sub> | T<sub>CYC1</sub>              | This study      | -           | pRS424-P<sub>TEF1</sub>-GFP-SPO<sub>20</sub><sup>51-91</sup>
|                   |                              |                 |             | pRS424                            |
| pRS424-P<sub>SPR3</sub>-T<sub>CYC1</sub> | P<sub>SPR3</sub>-T<sub>CYC1</sub> | This study      | TN110, TN111 | pRS424-T<sub>CYC1</sub>           |
| pRS424-P<sub>SPR3</sub>-GFP-SPO<sub>20</sub><sup>51-91</sup>-T<sub>CYC1</sub> | P<sub>SPR3</sub>-GFP-SPO<sub>20</sub><sup>51-91</sup>-T<sub>CYC1</sub> | This study      | TN27, TN100 | pRS424-P<sub>SPR3</sub>-T<sub>CYC1</sub> |
| pRS424-C-GFP-T<sub>ADH1</sub> | C-GFP-T<sub>ADH1</sub>          | This study      | -           | -                                 |
| pRS424-P<sub>TEF1</sub>-C-GFP-T<sub>ADH1</sub> | P<sub>TEF1</sub>-C-GFP-T<sub>ADH1</sub> | This study      | YSMO0017,   | pRS424-P<sub>TEF1</sub>           |
|                   |                              |                 | YSMO0018    | pFA6a-GFP(S65T)-HIS3MX6          |
| pRS426            | High-copy vector             | Christianson et al. (1992) | -           | -                                 |

**Marker plasmids**

| Name              | Protein Expressed/Description | Source          | Used Primer | Used Plasmid                      |
|-------------------|------------------------------|-----------------|-------------|-----------------------------------|
| pRS424-P<sub>TEF1</sub>-GFP-SPO<sub>20</sub><sup>51-91</sup> | GFP-Spo<sub>20</sub><sup>51-91</sup> | Nakamishi et al. (2004) | -           | -                                 |
| pRS426-P<sub>TEF1</sub>-mRFP-SPO<sub>20</sub><sup>51-91</sup> | mRFP-Spo<sub>20</sub><sup>51-91</sup> | Suda et al. (2007) | -           | -                                 |
| pRS316-P<sub>TEF1</sub>-mKate2-SPO<sub>20</sub><sup>51-91</sup> | mKate2-Spo<sub>20</sub><sup>51-91</sup> | This study      | TN357, TN358 | pRS316-P<sub>TEF1</sub>           |
| mod_pRS303-P<sub>TEF1</sub> | mKate2-Spo<sub>20</sub><sup>51-91</sup> | This study      | -           | mod_pRS303                       |
| mKate2-SPO<sub>20</sub><sup>51-91</sup>     |                              |                 |             |                                   |
| mod_pRS303-P<sub>TEF1</sub> | mTagBFP2-Spo<sub>20</sub><sup>51-91</sup> | This study      | TN408, TN409 | mod_pRS303-P<sub>TEF1</sub>       |
| mTagBFP2-SPO<sub>20</sub><sup>51-91</sup>     |                              |                 |             | mKate2-SPO<sub>20</sub><sup>51-91</sup> |

*Note: Protein descriptions and source information are provided for each plasmid entry.*
| Name                 | Protein Expressed/Description | Source             | Used Primer   | Used Plasmid                                      |
|----------------------|------------------------------|--------------------|---------------|--------------------------------------------------|
| pRS316-SPR28-mKate2  | Spr28-mKate2                 | This study         | TN175, TN176  | pRS316-PrTEF1-C-mKate2-T_N                        |
| pRS306-SPR28-mKate2  | Spr28-mKate2                 | This study         | -             | pRS306                                           |
| pRS316-HTB2-mCherry  | Htb2-mCherry                 | Okumura et al. (2015) | -             | -                                                |
| pRS316-NOP1-mCherry  | Nop1-mCherry                 | This study         | TN503, TN525  | pRS316-PrTEF1-C-mCherry-T_N                       |
| Gip1 plasmid series  |                              |                    |               |                                                  |
| pRS316-GIP1-GFP      | Gip1-1-639 aa-yEGFP3         | This study         | HT281, HT282  | pRS316                                           |
|                      |                              |                    | HT66, HT84    |                                                  |
| pRS424-GIP1-GFP      | Gip1-1-639 aa-yEGFP3         | This study         | -             | pRS316-GIP1-GFP                                  |
|                      |                              |                    |               | pRS424                                           |
| Figure 3             |                              |                    |               |                                                  |
| pRS424-GIP1-N1-GFP   | Gip1-1-67 aa-yEGFP3          | This study         | YN5, YN16    | pRS424-GIP1-GFP                                  |
| pRS424-GIP1-N2-GFP   | Gip1-1-132 aa-yEGFP3         | This study         | YN5, YN7     | pRS424-GIP1-GFP                                  |
| pRS424-GIP1-N3-GFP   | Gip1-1-222 aa-yEGFP3         | This study         | YN38, YN39   | pRS424-GIP1-GFP                                  |
| pRS424-GIP1-N4-GFP   | Gip1-1-298 aa-yEGFP3         | This study         | YN5, YN18    | pRS424-GIP1-GFP                                  |
| pRS424-GIP1-N5-GFP   | Gip1-1-357 aa-yEGFP3         | This study         | YN5, YN9     | pRS424-GIP1-GFP                                  |
| pRS424-GIP1-N6-GFP   | Gip1-1-476 aa-yEGFP3         | This study         | YN5, YN11    | pRS424-GIP1-GFP                                  |
| pRS424-GIP1-N7-GFP   | Gip1-1-518 aa-yEGFP3         | This study         | YN5, YN14    | pRS424-GIP1-GFP                                  |
| pRS424-GIP1-N8-GFP   | Gip1-1-589 aa-yEGFP3         | This study         | YN5, YN15    | pRS424-GIP1-GFP                                  |
| pRS424-GIP1-C1-GFP   | Gip1-477-639 aa-yEGFP3       | This study         | YN6, YN12    | pRS424-GIP1-GFP                                  |
| Name                     | Protein Expressed/Description | Source       | Used Primer | Used Plasmid |
|--------------------------|------------------------------|--------------|-------------|--------------|
| pRS424-GIP1-C2-GFP       | Gip1-358-639 aa-yEGFP3       | This study   | YN6, YN10   | pRS424-GIP1-GFP |
| pRS424-GIP1-C3-GFP       | Gip1-133-639 aa-yEGFP3       | This study   | YN6, YN8    | pRS424-GIP1-GFP |
| pRS424-GIP1-N1-BM-GFP    | Gip1-1-67 aa-R17A, K18A-yEGFP3 | This study | TN445, TN446 | pRS424-GIP1-N1-GFP |
| pRS424-GIP1-N1-HeM1-GFP  | Gip1-1-67 aa-K18P-yEGFP3     | This study   | TN406, TN407 | pRS424-GIP1-N2-GFP |
| pRS424-GIP1-N2-HyM-GFP   | Gip1-1-132 aa-F98E, L102E-yEGFP3 | This study | TN70, TN72, YN73, YN73 | pRS424-GIP1-N2-GFP |
| pRS424-GIP1-N2-HeM2-GFP  | Gip1-1-132 aa-L102P-yEGFP3   | This study   | YN70, YN82  | pRS424-GIP1-N2-HeM2-GFP |
| pRS424-GIP1-N2-HeM1+HeM2-GFP | Gip1-1-132 aa-K18P, L102P-yEGFP3 | This study | TN406, TN407 | pRS424-GIP1-N2-HeM2-GFP |
| pRS424-GIP1-HyM-GFP      | Gip1-1-639 aa-F98E, L102E-yEGFP3 | This study | TN70, YN72, YN73 | pRS424-GIP1-GFP |
| pRS424-GIP1-HeM1-GFP     | Gip1-1-639 aa-K18P-yEGFP3    | This study   | TN70, YN82  | pRS424-GIP1-GFP |
| pRS424-GIP1-HeM2-GFP     | Gip1-1-639 aa-L102P-yEGFP3   | This study   | TN72, YN73  | pRS424-GIP1-GFP |
| Figure 4                 |                              |              |             |              |
| pRS424-GIP1-N2a-GFP      | Gip1-1-150 aa-yEGFP3         | This study   | YN38, YN68  | pRS424-GIP1-N3-GFP |
| pRS424-GIP1-N2b-GFP      | Gip1-1-177 aa-yEGFP3         | This study   | YN38, YN69  | pRS424-GIP1-N3-GFP |
| pRS424-GIP1-N2c-GFP      | Gip1-1-187 aa-yEGFP3         | This study   | YN38, YN78  | pRS424-GIP1-N3-GFP |
| pRS424-GIP1-N2d-GFP      | Gip1-1-209 aa-yEGFP3         | This study   | YN38, YN79  | pRS424-GIP1-N3-GFP |
| Figure 5                 |                              |              |             |              |
| Name                  | Protein Expressed/Description | Source   | Used Primer | Used Plasmid            |
|-----------------------|------------------------------|----------|-------------|-------------------------|
| pRS314-GIP1           | Gip1-1-639 aa                 | This study | -           | pRS424GIP1-GFP          |
|                       |                              |          |             | pRS424GIP1-GFP          |
|                       |                              |          |             | pRS306GIP1              |
|                       |                              |          |             | pRS314                  |
| pRS314-GIP1-\(\Delta\)sep | Gip1-1-177, 223-693 aa       | This study | YN69, YN128 | pRS314-GIP1             |
| pRS424-GIP1-\(\Delta\)sep-GFP | Gip1-1-177, 223-693 aa-\(\gamma\)EGFP3 | This study | YN69, YN128 | pRS424GIP1-GFP          |
| pRS314-GIP1-\(\Delta\)sep-GFP | Gip1-1-177, 223-693 aa-\(\gamma\)EGFP3 | This study | -           | pRS424-GIP1-GFP          |
|                       |                              |          |             | pRS424-GIP1-\(\Delta\)sep-GFP |
| pRS424-\(P_{TEF1}\)GIP1-SEP1-GFP | Gip1-178-222 aa-GFP         | This study | TN507, TN508 | pRS424-\(P_{TEF1}\)C-GFP-\(T_{ADH1}\) |
| pRS424-\(P_{TEF1}\)GIP1-SEP2-GFP | Gip1-133-222 aa-GFP         | This study | TN508, TN578 | pRS424-\(P_{TEF1}\)C-GFP-\(T_{ADH1}\) |
|                       |                              |          |             | Figure 6                |
| pRS424-GIP1-NLS1M-GFP | Gip1-1-639 aa-\(\gamma\)EGFP | This study | HT480, TN365 | pRS424-GIP1-GFP          |
|                       | K441A, K442A, K443A-\(\gamma\)EGFP |          |             |                         |
| pRS424-GIP1-BN1-GFP   | Gip1-1-639 aa-R17A, K18A, K441A, K442A, K443A-\(\gamma\)EGFP3 | This study | TN445, TN446 | pRS424-GIP1-NLS1M-GFP    |
| pRS424-GIP1-BN2-GFP   | Gip1-1-639 aa-R17A, K18A, K231A, R232A, K441A, K442A, K443A-\(\gamma\)EGFP3 | This study | TN494, TN495 | pRS424-GIP1-BN1-GFP      |
| Name                  | Protein Expressed/Description | Source                  | Used Primer    | Used Plasmid                  |
|-----------------------|------------------------------|-------------------------|----------------|------------------------------|
| pRS424-GIP1-BN2-GFP   | Gip1-1-639 aa-R17A, K18A, K231A, R232A, K441A, K442A, K443A-yEGFP3 | This study             | TN494, TN495   | pRS424-GIP1-BN1-GFP          |
| pRS424-GIP1-BN3-GFP   | Gip1-1-639 aa-R17A, K18A, K231A, R232A, K441A, K442A, K443A K563A, R564A-yEGFP3 | This study             | TN547, TN548   | pRS424-GIP1-BN2-GFP          |
| pRS424-GIP1-BN4-GFP   | Gip1-1-639 aa-R17A, K18A, K107A, K108A, K231A, K232A, K441A, K442A, K443A, K563A, R564A-yEGFP3 | This study             | TN589, TN590   | pRS424-GIP1-BN3-GFP          |
| Figure 7              |                              |                         |                |                              |
| pRS306-GIP1-C         | intermediate construct       | This study             | -              | pRS306-GIP1                  |
|                       |                              |                         |                | pRS306                       |
| pSB5                  | 3×HA-Gip1-1-621 aa           | Tachikawa et al. (2001)| -              | -                            |
| (pRS424-Psp20-3×HA-GIP1-1-621 aa) |                        |                         |                |                              |
| pRS426-Psp20-3×HA-GIP1| 3×HA-Gip1-1-639 aa           | This study             | -              | pRS306-Psp20-3×HA-GIP1       |
|                       |                              |                         |                | pRS426                       |
| pRS426-Psp20-3×HA-GIP1-G7M1 | 3×HA-Gip1-1-639 aa-V292A, F294A | This study             | TT201, TT202   | pRS426-Psp20-3×HA-GIP1       |
| pRS426-Psp20-3×HA-GIP1-G7M2 | 3×HA-Gip1-1-639 aa-V446A, F448A | This study             | TT203, TT204   | pRS426-Psp20-3×HA-GIP1       |
| pRS426-Psp20-3×HA-GIP1-G7M3 | 3×HA-Gip1-1-639 aa-V492A, F494A | This study             | TT205, TT206   | pRS426-Psp20-3×HA-GIP1       |
| Name                   | Protein Expressed/Description | Source          | Used Primer       | Used Plasmid         |
|------------------------|------------------------------|-----------------|-------------------|---------------------|
| pGADT7-GIP1            | GAD-HA-Gip1-1-639 aa         | This study      | HT315, YSO319     | pRS426-P<sub>spo20</sub>-3×HA-GIP1 |
| pGADT7-GIP1-G7M1       | GAD-HA-Gip1-1-639 aa-V292A, F294A | This study      | HT315, YSO319     | pRS426-P<sub>spo20</sub>-3×HA-GIP1-G7M1 |
| pGADT7-GIP1-G7M2       | GAD-HA-Gip1-1-639 aa-V446A, F448A | This study      | HT315, YSO319     | pRS426-P<sub>spo20</sub>-3×HA-GIP1-G7M2 |
| pGADT7-GIP1-G7M3       | GAD-HA-Gip1-1-639 aa-V492A, F494A | This study      | HT315, YSO319     | pRS426-P<sub>spo20</sub>-3×HA-GIP1-G7M3 |
| pRS424-GIP1-G7M3-GFP   | Gip1-1-639 aa-V492A, F494A-yEGFP3 | This study      | -                 | pRS424-GIP1-G1-GFP   |
|                        |                              |                 |                   | pRS424-GIP1-G7M3-GFP |
|                        |                              |                 |                   | pRS424-GIP1-G7M3-GFP |
|                        |                              |                 |                   | pRS424-GIP1-C1-GFP   |
|                        |                              |                 |                   | pRS424-GIP1-C1-GFP   |
|                        |                              |                 |                   | pRS424-GIP1-C1-GFP   |
| Figure 7               |                              |                 |                   | pRS424-GIP1-C1-GFP   |
|                        |                              |                 |                   | pRS424-GIP1-C1-GFP   |
| pRS424-P<sub>spo20</sub>-GFP-  | GFP-Spo20<sup>1-91</sup>-Gip1-477-639 aa | This study      | TN360, TN383     | pRS424-P<sub>spo20</sub>-GFP- |
| SPO20<sup>1-91</sup>-GIP1-C1 |                              |                 |                   | SPO20<sup>1-91</sup>-[SAGG]<sub>×4</sub>-T<sub>cyC1</sub> |
| pRS424-P<sub>spo20</sub>-GFP-  | GFP-Spo20<sup>1-91</sup>-Gip1-358-639 aa | This study      | TN360, TN362     | pRS424-P<sub>spo20</sub>-GFP- |
| SPO20<sup>1-91</sup>-GIP1-C2 |                              |                 |                   | SPO20<sup>1-91</sup>-[SAGG]<sub>×4</sub>-T<sub>cyC1</sub> |
| pRS424-P<sub>spo20</sub>-GFP-  | GFP-Spo20<sup>1-91</sup>-Gip1-133-639 aa | This study      | TN360, TN361     | pRS424-P<sub>spo20</sub>-GFP- |
| SPO20<sup>1-91</sup>-GIP1-C3 |                              |                 |                   | SPO20<sup>1-91</sup>-[SAGG]<sub>×4</sub>-T<sub>cyC1</sub> |
| Name                        | Protein Expressed/Description       | Source          | Used Primer | Used Plasmid                                      |
|-----------------------------|-------------------------------------|-----------------|-------------|--------------------------------------------------|
| pRS424-GIP1-mCherry         | Gip1-1-639 aa-mCherry               | This study      | -           | pFA6a-mCherry-HIS3MX6                            |
|                             |                                     |                 |             | pRS424-GIP1-GFP                                  |
| pRS426-GIP1-C1-mCherry      | Gip1-477-639 aa-mCherry             | This study      | -           | pRS424-GIP1-mCherry                              |
|                             |                                     |                 |             | pRS424-GIP1-C1-GFP                               |
|                             |                                     |                 |             | pRS426                                           |

**Other plasmids**

| Name                        | Protein Expressed/Description       | Source          | Used Primer | Used Plasmid                                      |
|-----------------------------|-------------------------------------|-----------------|-------------|--------------------------------------------------|
| pGBKT7-GLC7                 | GBD-Myc-Glc7                        | This study      | YO322, YO325| pGBKT7                                           |
| pRS424-P<sup>sprc</sup>-GFP-SPO20<sup>51-91</sup>-GLC7 | GFP-Spo20<sup>51-91</sup>-GLC7 | This study      | YO255, YO256| pRS424-P<sup>sprc</sup>-GFP-SPO20<sup>51-91</sup>-GLC7-T<sub>CYC1</sub> |
| pRS424-SPO71-GFP            | Spo71-yEGFP3                        | Park et al. (2013)| -         | -                                                |
| pRS424-P<sup>SPO20</sup>-GFP-SPO73 | GFP(S65T)-Spo73            | Okumura et al. (2015)| -         | -                                                |
| pRS424-VPS13-GFP            | Vps13-GFP(S65T)                    | This study      | TN194, TN211| pRS424-C-GFP-T<sub>Adh1</sub>                      |
| pFA6a-mCherry-HIS3MX6       | C-terminal tagging mCherry-HIS3MX6  | Gift from M. Onishi | -         | -                                                |
| pFA6a-kanMX6                | deletion with kanMX6               | Longtine et al. (1998) | -         | -                                                |
| pFA6a-GFP(S65T)-HIS3MX6     | C-terminal tagging GFP(S65T)-HIS3MX6| Longtine et al. (1998) | -         | -                                                |
| pFA6a-yEGFP-HIS3MX6         | C-terminal tagging yEGFP-HIS3MX6   | Nickas and Neiman (2002) | -         | -                                                |
| pGADT7                      | Yeast two-hybrid system vector     | Clontech        | -           | -                                                |
| pGBKT7                      | Yeast two-hybrid system vector     | Clontech        | -           | -                                                |
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