

Table S2. Strains Used in This Study

| Strain | Genotype | Source | Figure |
|------------|--|------------------------|--|
| BY4741 | <i>MATa; his3Δ1, leu2Δ0, ura3Δ0, met15Δ0</i> | Euroscarf | 1A-D, S1A/B, S2, 3A/B/D/E, S3, Table 1 |
| Y7092 | [BY4741] <i>MATα, can1Δ::STE2pr-Sp_his5, lyp1Δ</i> | Boone lab | |
| YSB084 | [Y7092] <i>MATα, rim15Δ::natMX4</i> | This study | SGA screen |
| YFL033C | [BY4741] <i>MATa; rim15Δ::kanMX4</i> | Euroscarf | 1A/D, S1B, 2C, 3A/E, Table 1 |
| YSB147 | [BY4741] <i>MATa; rim15Δ::natMX4</i> | This study | 1B/C, S1A |
| CDV288-12A | [BY4741] <i>MATa; igo1Δ::kanMX4, igo2Δ::kanMX4</i> | (Talarek et al., 2010) | 2B, 3A, Table 1 |
| XL119-14A | [BY4741] <i>MATa; igo1Δ::natMX4, igo2Δ::hphMX4</i> | This study | 1A-D, S1B, 3E |
| YDL134C | [BY4741] <i>MATa; pph21Δ::kanMX4</i> | Euroscarf | 1A-D, S1B, S2, 3A/B, Table 1 |
| YSB148 | [BY4741] <i>MATa; cdc55Δ::kanMX6</i> | This study | 1A-D, S1B, S2, 2B/C, 3A/B |
| YDL188C | [BY4741] <i>MATa; pph22Δ::kanMX4</i> | Euroscarf | 1D, S1B |
| YOR014W | [BY4741] <i>MATa; rts1Δ::kanMX4</i> | Euroscarf | 1D, S1B |
| CDV319-1C | [BY4741] <i>MATa; rim15Δ::kanMX4, pph21Δ::kanMX4</i> | This study | 1A-D, S1B, 3A |
| CDV321-1D | [BY4741] <i>MATα; igo1Δ::kanMX4, igo2Δ::kanMX4, pph21Δ::kanMX4</i> | This study | 1A-D, S1B, 2B 3A |
| YSB150 | [BY4741] <i>MATa; rim15Δ::natMX4, cdc55Δ::kanMX6</i> | This study | 1A-D, S1B, 3A/E |
| YSB201 | [BY4741] <i>MATa; igo1Δ::natMX4, igo2Δ::hphMX4, cdc55Δ::kanMX6</i> | This study | 1A-D, S1B, 2B, 3A/E |
| YSB220 | [BY4741] <i>MATa; rim15Δ::natMX4, cdc55Δ::kanMX6, igo1Δ::natMX4, igo2Δ::hphMX4</i> | This study | 2B |
| YSB163 | [BY4741] <i>MATa; rim15Δ::natMX4, pph22Δ::kanMX4</i> | This study | 1D, S1B |
| YSB199 | [BY4741] <i>MATa; rim15Δ::natMX4, rts1Δ::kanMX4</i> | This study | 1D, S1B |
| YSB202 | [BY4741] <i>MATa; igo1Δ::natMX4, igo2Δ::hphMX4, rts1Δ::kanMX4</i> | This study | 1D, S1B |
| YSB229 | [BY4741] <i>MATa; rim15Δ::natMX4, pph21Δ::kanMX4, msn2Δ::kanMX4, msn4Δ::kanMX4, gis1Δ::kanMX4</i> | This study | 1D, S1B |
| YSB230 | [BY4741] <i>MATa; rim15Δ::natMX4, cdc55Δ::kanMX6, msn2Δ::kanMX4, msn4Δ::kanMX4, gis1Δ::kanMX4</i> | This study | 1D, S1B |
| YSB204 | [BY4741] <i>MATα; msn2Δ::kanMX4, msn4Δ::kanMX4, gis1Δ::kanMX4</i> | This study | 1D, S1B, 3D, S3 |
| YSB205 | [BY4741] <i>MATa; msn2Δ::kanMX4, msn4Δ::kanMX4, gis1Δ::kanMX4, pph21Δ::kanMX4</i> | This study | 1D, S1B, 3D, S3 |
| YSB217 | [BY4741] <i>MATa; msn2Δ::kanMX4, msn4Δ::kanMX4, gis1Δ::kanMX4, cdc55Δ::kanMX6</i> | This study | 1D, S1B, 3D, S3 |
| NMY51 | <i>MATa; his3Δ200, trp1-901, leu2-3,112, ade2, LYS::(lexAop)4-HIS3, ura3::(lexAop)8-lacZ, ade2::(lexAop)8-ADE2, GAL4</i> | Dual-systems | 2A |
| BZ001-5AL | [NMY51] <i>MATa; rim15Δ::natMX4</i> | This study | 2A |
| YSB222 | [W303] <i>MATa; ura3::TRP1::PDS-lacZ</i> | This study | 3C |
| YSB224 | [W303] <i>MATa; ura3::TRP1::PDS-lacZ, gis1Δ::kanMX2</i> | This study | 3C |

Table S3. Plasmids Used in This Study

| Plasmid | Description | Source | Figure |
|----------------|--|--------------------------|---------------|
| YEp351 | 2μ, LEU2 | (Hill et al., 1986) | |
| pSB001 | [YEp351] HSP26p-yEmRFP | This study | SGA screen |
| Yep352 | 2μ, URA3 | (Hill et al., 1986) | |
| pSB002 | [YEp352] HSP26p-yEmRFP | This study | 1A |
| YCplac33 | CEN, URA3 | (Gietz and Sugino, 1988) | |
| pCDV260 | [YCplac33] RIM15 | De Virgilio lab | SGA screen |
| pRS416 | CEN, URA3 | (Brachmann et al., 1998) | |
| pSB003 | [pRS416] ADH1p-CDC55-HA ₃ | This study | 2B/C, S2, 3B |
| pSB004 | [pRS416] ADH1p-CDC55 | This study | 2B, S2, 3B |
| pSB005 | [pRS416] ADH1p-HA ₃ -RPC53 | This study | 2B |
| pSB006 | [pRS416] ADH1p-HA ₃ -PPH21 | This study | 2B, S2, 3B |
| pSB011 | [pRS416] ADH1p-HA ₃ -NAP1 | This study | 3B |
| YCplac111 | CEN, LEU2 | (Gietz and Sugino, 1988) | 2B, 3B |
| p1432 | [YCplac111] IGO1-myc ₈ | De Virgilio lab | S1A, 2B, S2 |
| p1434 | [YCplac111] IGO1 ^{S64A} -myc ₈ | De Virgilio lab | S1A, 2B |
| pSB013 | [YCplac111] ADH1p-GIS1-myc ₁₃ | This study | 3B |
| pSB014 | [YCplac111] ADH1p-GIS1 ^{S425A} -myc ₁₃ | This study | 3B |
| YEplac195 | 2μ, URA3 | (Gietz and Sugino, 1988) | |
| pCDV487 | [YEplac195] GAL1p-GST-RIM15-HA ₃ | (Pedruzzi et al., 2003) | 2C |
| pHAC33 | CEN, HA ₃ , URA3 | (Soulard et al., 2010) | 3C/D, S3 |
| pSB008 | [pHAC33] GIS1-HA ₃ | This study | 3A, S3 |
| pSB009 | [pHAC33] ADH1p-GIS1-HA ₃ | This study | 3C-E, S3 |
| pSB010 | [pHAC33] ADH1p-GIS1 ^{S425A} -HA ₃ | This study | 3C-E, S3 |
| pDL2-Alg5 | 2μ, ADH1-ALG5-HA-NUBG, TRP1 | Dualsystems | 2A |
| pAI-Alg5 | 2μ, ADH1-ALG5-HA-NUBI, TRP1 | Dualsystems | 2A |
| pPR3-N | 2μ, CYC1-NUBG-HA, TRP1 | Dualsystems | |
| pNT059 | [pPR3-N] CYC1-NUBG-HA-PPH21 | This study | 2A |
| pNT061 | [pPR3-N] CYC1-NUBG-HA-CDC55 | This study | 2A |
| pCabWT | CEN, CYC1-CUB-LEXA, LEU2 | Dualsystems | |
| pNT015 | [pCabWT] CYC1-IGO1-CUB-LEXA | This study | 2A |
| pNT038 | [pCabWT] CYC1-IGO1 ^{S64A} -CUB-LEXA | This study | 2A |
| pET-24d | lac Operator, kan ^R | Novagen | |
| pNT051 | [pET-24d] IGO1-HIS ₆ | This study | 2C |
| pSB007 | [pET-24d] IGO ^{S64A} -HIS ₆ | This study | 2C |

Table S4. qPCR Oligonucleotides Used in This Study

| Name | Sequence | Figure |
|-------------|-----------------------------|-------------|
| RTN2qF1 | 5'-ATTGGATCGTCTGCCTGCGTTAGA | 1D, 3D |
| RTN2qR1 | 5'-AGGCCGTGCAAGAAATAGAGGGAA | 1D, 3D |
| SOL4qF1 | 5'-AGGATCGATGAACCAAGCGCTGTA | S1B |
| SOL4qR1 | 5'-AATTGGTTGCCCTGATGCACTAGC | S1B |
| TBP1qF1 | 5'-CGCTACATGCCCGTAATGCAGAAT | 1D, S1B, 3D |
| TBP1qR1 | 5'-TACTGGCCAGCTTGAGTCATCCT | 1D, S1B, 3D |
| STE3qF1 | 5'-CGTCAAGGACCTTGTGATTAGC | 3E |
| STE3qR1 | 5'-GCGCCCACAAATGACCATATAAGC | 3E |
| ProRTN2qF1 | 5'-GTTCAATTGCTTGTCTCAACTGCC | 3E |
| ProRTN2qR1b | 5'-CCGTCTTGTCTGATGACCTGCTT | 3E |