**Supplementary File 1.** *S. pombe* strains used in this study.

|  |  |  |
| --- | --- | --- |
| **Strain** | **Genotype** | **Source/Reference** |
|  | **Figure 1** |  |
| FPR645 | *h- rlc1::kanR ade6-M216 leu1-32 ura4.294* | This work |
| FPR961 | *h+ Pcp1-GFP:kanR rlc1::kanR Rlc1-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR965 | *h+ Pcp1-GFP:kanR rlc1::kanR Rlc1(S35A)-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR968 | *h- Pcp1-GFP:kanR rlc1::kanR Rlc1(S36A)-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR971 | *h? Pcp1-GFP:kanR rlc1::kanR Rlc1(S35AS36A)-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR1700 | *h? Pcp1-GFP:kanR rlc1::kanR Rlc1(S35D)-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR1698 | *h? Pcp1-GFP:kanR rlc1::kanR Rlc1(S35DS36D)-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR667 | *h? Pcp1-GFP:kanR Rlc1-GFP:kanR cdc2ASM17:bsdR ade6-M216 leu1-32*  | This work |
|  | **Figure 2** |  |
| MBY4489  | *h- pak1-2xMyc-GFP::ura4+* | Loo *et al.,* 2008 |
| MBY5064  | *h- pak1-M460G-2xMyc-GFP::ura4+* | Loo *et al.,* 2008 |
| FPR1621 | *h? pak1-2xMyc-GFP::ura4+ rlc1::kanR Rlc1-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR1635 | *h? pak1M460G-2xMyc-GFP::ura4+ rlc1::kanR Rlc1-GFP::ura4+ade6-M216 leu1-32* | This work |
| FPR1641 | *h? pak1-2xMyc-GFP::ura4+ rlc1::kanR shk2::kanR Rlc1-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR1668 | *h? pak1M460G-2xMyc-GFP::ura4+ rlc1::kanR shk2::kanR Rlc1-GFP::ura4+  ade6-M216 leu1-32* | This work |
| FPR1269 | *h? pak1-2xMyc-GFP::ura4+ Pcp1-mcherry::ura4+ Rlc1-mcherry::kanR ade6-M216 leu1-32 ura4.D18* | This work |
| FPR1281 | *h? pak1M460G-2xMyc-GFP::ura4+ Pcp1-mcherry::ura4+ Rlc1-mcherry::kanR ade6-M216 leu1-32 ura4.D18* | This work |
| FPR1272 | *h? pak1-2xMyc-GFP::ura4+ Pcp1-mcherry::ura4+ Rlc1-mcherry::kanR shk2::kanR ade6-M216 leu1-32 ura4.D18* | This work |
| FPR1282 | *h? pak1M460G-2xMyc-GFP::ura4+ Pcp1-mcherry::ura4+ Rlc1-mcherry::kanR shk2::kanR ade6-M216 leu1-32 ura4.D18* | This work |
| PPG4.69 | *h+ pak1-GFP::kanR leu1-32 ura4.D18* | Pilar Pérez |
| FPR1530 | *h- shk2::hphR prompak1+shk2-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR1460 | *h- shk2::hphR shk2-3xGFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR1554 | *h- shk2::hphR shk2-3xGFP::ura4+ ste11::natR ade6-M216 leu1-32* | This work |
| FPR1481 | *h- shk2::hphR promste11+A/G:shk2-3xGFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR1607 | *h? shk2::hphR shk2-3xGFP::ura4+ pka1::kanR ade6-M216 leu1-32* | This work |
| FPR1608 | *h? shk2::hphR shk2-3xGFP::ura4+ rst2::natR ade6-M216 leu1-32* | This work |
| FPR1611 | *h? shk2::hphR shk2-3xGFP::ura4+ pka1::kanR rst2::natR ade6-M216 leu1-32* | This work |
|  | **Figure 3** |  |
| E888 | *h+ for3-3xGFP::ura4+ sty1-HA6H::ura4+ ade6-M216 leu1-32 ura4-D18* | Gomez-Gil *et al.,* 2020 |
| FPR1441 | *h? for3DAD-2xGFP::kanR sty1-HA6H::ura4+ rlc1::kanR rlc1-GFP::ura4+ ade6-M216 leu1-32 ura4-D18* | This work |
| FPR1443 | *h? for3DAD-2xGFP::kanR sty1-HA6H::ura4+ rlc1::kanR rlc1(S35A)-GFP::ura4+ ade6-M216 leu1-32 ura4-D18* | This work |
| FPR961 | *h+ Pcp1-GFP:kanR rlc1::kanR Rlc1-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR965 | *h+ Pcp1-GFP:kanR rlc1::kanR Rlc1(S35A)-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR1292 | *h? Pcp1-GFP:kanR rlc1::kanR Rlc1-GFP::ura4+ for3DAD ade6-M216 leu1-32* | This work |
| FPR1296 | *h? Pcp1-GFP:kanR rlc1::kanR Rlc1(S35A)-GFP::ura4+ for3DAD ade6-M216 leu1-32* | This work |
| FPR1340 | *h? Pcp1-mcherry::ura4+ Rlc1-mcherry::kanR for3-3xGFP::ura4+ pak1-M460G-2xMyc-GFP::ura4+ shk2::kanR ade6-M216 leu1-32 ura4.D18* | This work |
| FPR1345 | *h? Pcp1-mcherry::ura4+ Rlc1-mcherry::kanR pak1-M460G-2xMyc-GFP::ura4+ for3DAD-2xGFP::kanR shk2::kanR ade6-M216 leu1-32 ura4.D18* | This work |
| FPR1089 | *h? for3-3xGFP::ura4+ rlc1::kanR rlc1-HA::ura4+ kanR ade6-M216 leu1-32* | This work |
| FPR1094 | *h? for3-3xGFP::ura4+ rlc1::kanR rlc1(S35A)-HA::ura4+ kanR ade6-M216 leu1-32* | This work |
| FPR1410 | *h? for3DAD-2xGFP::kanR rlc1::kanR rlc1-HA::ura4+ kanR ade6-M216 leu1-32* | This work |
| FPR1415 | *h? for3DAD-2xGFP::kanR rlc1::kanR rlc1(S35A)-HA::ura4+ kanR ade6-M216 leu1-32* | This work |
|  | **Figure 4** |  |
| FPR1000 | *h+ for3-3xGFP::ura4+ sty1-HA6H::ura4+ rlc1::kanR rlc1-GFP::ura4+ ade6-M216 leu1-32*  | This work |
| FPR1048 | *h+ for3-3xGFP::ura4+ sty1-HA6H::ura4+ rlc1::kanR rlc1(S35A)-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR1054 | *h? for3-3xGFP::ura4+ sty1-HA6H::ura4+ rlc1::kanR rlc1-GFP::ura4+ wis1::his ade6-M216 leu1-32* | This work |
| FPR1059 | *h? for3-3xGFP::ura4+ sty1-HA6H::ura4+ rlc1::kanR rlc1(S35A)-GFP::ura4+ wis1::his ade6-M216 leu1-32* | This work |
| FPR727 | *h- rlc1::kanR rlc1-HA::ura4+ ade6-M216 leu1-32*  | This work |
| FPR645 | *h- rlc1::kanR ade6-M216 leu1-32 ura4.294* | This work |
| FPR730 | *h- rlc1::kanR rlc1(S35A)-HA::ura4+ ade6-M216 leu1-32*  | This work |
| FPR1228 | *h- rlc1::kanR rlc1-HA::ura4+ wis1::his ade6-M216 leu1-32*  | This work |
| FPR1231 | *h- rlc1::kanR wis1::his ade6-M216 leu1-32* | This work |
| FPR1237 | *h- rlc1::kanR rlc1(S35A)-HA::ura4+ wis1::his ade6-M216 leu1-32* | This work |
| FPR961 | *h+ Pcp1-GFP:kanR rlc1::kanR Rlc1-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR965 | *h+ Pcp1-GFP:kanR rlc1::kanR Rlc1(S35A)-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR1233 | *h? Pcp1-GFP:kanR rlc1::kanR Rlc1-GFP::ura4+ wis1::his ade6-M216 leu1-32* | This work |
| FPR1235 | *h? Pcp1-GFP:kanR rlc1::kanR Rlc1(S35A)-GFP::ura4+ wis1::his ade6-M216 leu1-32* | This work |
| FPR1361 | *h? Pcp1-GFP:kanR rlc1::kanR Rlc1-GFP::ura4+ pyp1::kanR ade6-M216 leu1-32* | This work |
| FPR1406 | *h? Pcp1-GFP:kanR rlc1::kanR Rlc1(S35A)-GFP::ura4+ pyp1::hphR ade6-M216 leu1-32* | This work |
|  | **Figure 5** |  |
| MM1 | *h+ ade6-M216 leu1-32 ura4.D18* | Madrid *et al.,* 2006 |
| FPR876 | *h- myo2.E1 ade6-M216 leu1-32 ura4.D18* | Balasubramanian *et al.,* 1998 |
| FPR462 | *h+ myo3::kanR ade6-M216 leu1-32 ura4.D18* | Lab Stock |
| FPR441 | *h+ cdc4-8 ade6-M216 leu1-32 ura4.D18* | Lab Stock |
| FPR645 | *h- rlc1::kanR ade6-M216 leu1-32 ura4.294* | This work |
| FPR1461 | *h? Pcp1-GFP:kanR rlc1::kanR Rlc1-GFP::ura4+ myo2.E1 ade6-M216 leu1-32* | This work |
| FPR1498 | *h? Pcp1-GFP:kanR rlc1::kanR Rlc1-GFP::ura4+ myo2.E1 for3DAD ade6-M216 leu1-32* | This work |
|  | **Figure 6** |  |
| E888 | *h+ for3-3xGFP::ura4+ sty1-HA6H::ura4+ ade6-M216 leu1-32 ura4-D18* | Gomez-Gil *et al.,* 2020 |
| FPR961 | *h+ Pcp1-GFP:kanR rlc1::kanR Rlc1-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR965 | *h+ Pcp1-GFP:kanR rlc1::kanR Rlc1(S35A)-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR1461 | *h? Pcp1-GFP:kanR rlc1::kanR Rlc1-GFP::ura4+ myo2.E1 ade6-M216 leu1-32* | This work |
| FPR1233 | *h? Pcp1-GFP:kanR rlc1::kanR Rlc1-GFP::ura4+ wis1::his ade6-M216 leu1-32* | This work |
| FPR727 | *h- rlc1::kanR rlc1-HA::ura4+ ade6-M216 leu1-32*  | This work |
| FPR645 | *h- rlc1::kanR ade6-M216 leu1-32 ura4.294* | This work |
| FPR730 | *h- rlc1::kanR rlc1(S35A)-HA::ura4+ ade6-M216 leu1-32*  | This work |
| FPR1489 | *h? rlc1::kanR rlc1-HA::ura4+ myo2.E1 ade6-M216 leu1-32*  | This work |
| FPR1142 | *h? pak1M460G-2xMyc-GFP::ura4+ rlc1::kanR shk2::kanR rlc1-HA::ura4+  ade6-M216 leu1-32* | This work |
| FPR1228 | *h- rlc1::kanR rlc1-HA::ura4+ wis1::his ade6-M216 leu1-32*  | This work |
| FPR1130 | *h? rlc1::kanR rlc1-HA::ura4+ for3::natR ade6-M216 leu1-32*  | This work |
| FPR1135 | *h? rlc1::kanR rlc1(S35A)-HA::ura4+ for3::natR ade6-M216 leu1-32* | This work |
|  | **Figure 1 supplement 1** |  |
| MM1 | *h+ ade6-M216 leu1-32 ura4.D18* | Madrid *et al.,* 2006 |
| FPR645 | *h- rlc1::kanR ade6-M216 leu1-32 ura4.294* | This work |
| FPR961 | *h+ Pcp1-GFP:kanR rlc1::kanR Rlc1-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR965 | *h+ Pcp1-GFP:kanR rlc1::kanR Rlc1(S35A)-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR719 | *h- rlc1::kanR pZ3EVrlc1-HA::ura4+ adh1-Z3EV:leu1+ ade6-M216*  | This work |
| FPR722 | *h- rlc1::kanR pZ3EVrlc1-HA::ura4+ adh1-Z3EV:leu1+ ade6-M216* | This work |
| FPR520 | *h- rlc1:natR* | Pilar Pérez |
| FPR518 | *h- rlc1::natR* | Pilar Pérez |
| FPR521 | *h- rlc1(S35A):natR* | Pilar Pérez |
| FPR522 | *h- rlc1(S35D):natR* | Pilar Pérez |
|  | **Figure 1 supplement 2** |  |
| FPR961 | *h+ Pcp1-GFP:kanR rlc1::kanR Rlc1-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR965 | *h+ Pcp1-GFP:kanR rlc1::kanR Rlc1(S35A)-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR1656 | *h? Pcp1-GFP:kanR rlc1::kanR Rlc1-GFP::ura4+ myo51::ura4+ ade6-M216 leu1-32* | This work |
| FPR1671 | *h? Pcp1-GFP:kanR rlc1::kanR Rlc1(S35A)-GFP::ura4+ myo51::ura4+ ade6-M216 leu1-32* | This work |
|  | **Figure 2 supplement 1** |  |
| FPR1680 | *h? rlc1::kanR rlc1-GFP::ura4+ shk1::natR shk1(M460A)::hphR ade6-M216 leu1-32*  | This work |
| FPR961 | *h+ Pcp1-GFP:kanR rlc1::kanR Rlc1-GFP::ura4+ ade6-M216 leu1-32* | This work |
| PPG4.69 | *h+ pak1-GFP::kanR leu1-32 ura4.D18* | Pilar Pérez |
| FPR1530 | *h- shk2::hphR prompak1+shk2-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR1482 | *h? pak1-GFP::kanR shk2::hphR shk2-3xGFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR1559 | *h? shk2::hphR prompak1+shk2-GFP::ura4+ pcp1-mcherry::ura4+ Rlc1-mcherry::kanR ade6-M216 leu1-32* | This work |
| FPR1132 | *h? pak1-2xMyc-GFP::ura4+ rlc1::kanR rlc1-HA::ura4+  ade6-M216 leu1-32* | This work |
| FPR1141 | *h? pak1M460G-2xMyc-GFP::ura4+ rlc1::kanR rlc1-HA::ura4+  ade6-M216 leu1-32* | This work |
| FPR1145 | *h? pak1-2xMyc-GFP::ura4+ rlc1::kanR shk2::kanR rlc1-HA::ura4+  ade6-M216 leu1-32* | This work |
| FPR1142 | *h? pak1M460G-2xMyc-GFP::ura4+ rlc1::kanR shk2::kanR rlc1-HA::ura4+  ade6-M216 leu1-32* | This work |
|  | **Figure 3 supplement 1** |  |
| FPR961 | *h+ Pcp1-GFP:kanR rlc1::kanR Rlc1-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR1126 | *h? Pcp1-GFP:kanR rlc1::kanR Rlc1-GFP::ura4+ for3::natR ade6-M216 leu1-32* | This work |
| FPR727 | *h- rlc1::kanR rlc1-HA::ura4+ ade6-M216 leu1-32*  | This work |
| FPR1130 | *h? rlc1::kanR rlc1-HA::ura4+ for3::natR ade6-M216 leu1-32*  | This work |
| E880 | *h+ for3-3xGFP::ura4+ ade6-M216 leu1-32 ura4-D18* | Gomez-Gil *et al.,* 2020 |
| E998 | *h? for3-3xGFP::ura4+ mts3-1 ade6-M216 leu1-32 ura4-D18* | Gomez-Gil *et al.,* 2020 |
|  | **Figure 3 supplement 2** |  |
| E32 | *h- CRIB-GFP::ura4+ 1 ade6-M216 leu1-32*  | Lab Stock |
| E888 | *h+ for3-3xGFP::ura4+ sty1-HA6H::ura4+ ade6-M216 leu1-32 ura4-D18* | Gomez-Gil *et al.,* 2020 |
| FPR961 | *h+ Pcp1-GFP:kanR rlc1::kanR Rlc1-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR965 | *h+ Pcp1-GFP:kanR rlc1::kanR Rlc1(S35A)-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR1292 | *h? Pcp1-GFP:kanR rlc1::kanR Rlc1-GFP::ura4+ for3DAD ade6-M216 leu1-32* | This work |
| FPR1296 | *h? Pcp1-GFP:kanR rlc1::kanR Rlc1(S35A)-GFP::ura4+ for3DAD ade6-M216 leu1-32* | This work |
| FPR1340 | *h? Pcp1-mcherry::ura4+ Rlc1-mcherry::kanR for3-3xGFP::ura4+ pak1-M460G-2xMyc-GFP::ura4+ shk2::kanR ade6-M216 leu1-32 ura4.D18* | This work |
| FPR1345 | *h? Pcp1-mcherry::ura4+ Rlc1-mcherry::kanR pak1-M460G-2xMyc-GFP::ura4+ for3DAD-2xGFP::kanR shk2::kanR ade6-M216 leu1-32 ura4.D18* | This work |
|  | **Figure 4 supplement 1** |  |
| FPR961 | *h+ Pcp1-GFP:kanR rlc1::kanR Rlc1-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR965 | *h+ Pcp1-GFP:kanR rlc1::kanR Rlc1(S35A)-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR1233 | *h? Pcp1-GFP:kanR rlc1::kanR Rlc1-GFP::ura4+ wis1::his ade6-M216 leu1-32* | This work |
| FPR1235 | *h? Pcp1-GFP:kanR rlc1::kanR Rlc1(S35A)-GFP::ura4+ wis1::his ade6-M216 leu1-32* | This work |
| MM1 | *h+ ade6-M216 leu1-32 ura4.D18* | Madrid *et al.,* 2006 |
| FPR17 | *h- win1-1 sty1-HA6H:ura4+ ade6-M216 leu1-32 ura4.D18* | Lab stock |
| FPR29 | *h+ wak1::ura4+ sty1-HA6H:ura4+ ade6-M216 leu1-32 ura4.D18* | Lab stock |
| FPR36 | *h- wis1::his sty1-HA6H:ura4+ ade6-M216 leu1-32 ura4.D18* | Lab stock |
| FPR152 | *h- sty1::ura4+ ade6-M216 leu1-32 ura4.D18* | Lab stock |
| FPR232 | *h- atf1::ura4+ sty1-HA6H:ura4+ ade6-M216 leu1-32 ura4.D18* | Lab stock |
| FPR1000 | *h+ for3-3xGFP::ura4+ sty1-HA6H::ura4+ rlc1::kanR rlc1-GFP::ura4+ ade6-M216 leu1-32*  | This work |
| FPR1048 | *h+ for3-3xGFP::ura4+ sty1-HA6H::ura4+ rlc1::kanR rlc1(S35A)-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR1441 | *h? for3DAD-2xGFP::kanR sty1-HA6H::ura4+ rlc1::kanR rlc1-GFP::ura4+ ade6-M216 leu1-32 ura4-D18* | This work |
| FPR1443 | *h? for3DAD-2xGFP::kanR sty1-HA6H::ura4+ rlc1::kanR rlc1(S35A)-GFP::ura4+ ade6-M216 leu1-32 ura4-D18* | This work |
| FPR1361 | *h? Pcp1-GFP:kanR rlc1::kanR Rlc1-GFP::ura4+ pyp1::kanR ade6-M216 leu1-32* | This work |
| FPR1406 | *h? Pcp1-GFP:kanR rlc1::kanR Rlc1(S35A)-GFP::ura4+ pyp1::hphR ade6-M216 leu1-32* | This work |
|  | **Figure 5 supplement 1** |  |
| FPR1461 | *h? Pcp1-GFP:kanR rlc1::kanR Rlc1-GFP::ura4+ myo2.E1 ade6-M216 leu1-32* | This work |
| FPR1498 | *h? Pcp1-GFP:kanR rlc1::kanR Rlc1-GFP::ura4+ myo2.E1 for3DAD ade6-M216 leu1-32* | This work |
|  | **Figure 5 supplement 2** |  |
| FPR961 | *h+ Pcp1-GFP:kanR rlc1::kanR Rlc1-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR1547 | *h- Pcp1-GFP:kanR rlc1::kanR Rlc1-GFP::ura4+ myo3::kanR ade6-M216 leu1-32* | This work |
| FPR965 | *h+ Pcp1-GFP:kanR rlc1::kanR Rlc1(S35A)-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR1619 | *h? Pcp1-GFP:kanR rlc1::kanR Rlc1(S35A)-GFP::ura4+ myo3::kanR ade6-M216 leu1-32* | This work |
|  | **Figure 6 supplement 1** |  |
| FPR961 | *h+ Pcp1-GFP:kanR rlc1::kanR Rlc1-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR965 | *h+ Pcp1-GFP:kanR rlc1::kanR Rlc1(S35A)-GFP::ura4+ ade6-M216 leu1-32* | This work |
| FPR1461 | *h? Pcp1-GFP:kanR rlc1::kanR Rlc1-GFP::ura4+ myo2.E1 ade6-M216 leu1-32* | This work |