

Supplementary Table S1 *S. pombe* strains used in this study

| Strain | Genotype | Source |
|---------|---------------------------------------------------------------------------------------------------------|-------------------------------|
| SPDK9 | <i>h⁹⁰ leu1-32 ura4DS/E ade6-M216 grc3::grc3-EGFP-kanMX6</i> | This study |
| SPDK12 | <i>h⁹⁰ leu1-32 ura4DS/E ade6-M216 grc3::grc3-5FLAG-kanMX6</i> | This study |
| SPDK53 | <i>h⁹⁰ leu1-32 ura4DS/E ade6-M216 grc3::grc3-TAP-kanMX6</i> | This study |
| SPDK55 | <i>h⁹⁰ leu1-32 ura4DS/E ade6-M216 grc3::grc3-13myc-kanMX6</i> | This study |
| SPDK59 | <i>h⁹⁰ leu1-32 ura4DS/E ade6-M216 crb3::crb3-5FLAG-kanMX6</i> | This study |
| SPDK63 | <i>h⁹⁰ leu1-32 ura4DS/E ade6-M216 las1::las1-5FLAG-kanMX6</i> | This study |
| SPDK67 | <i>h⁹⁰ leu1-32 ura4DS/E ade6-M216 grc3::grc3-TAP-Hyg^r crb3::crb3-5FLAG-kanMX6</i> | This study |
| SPDK72 | <i>h⁹⁰ leu1-32 ura4DS/E ade6-M216 grc3::grc3-TAP-Hyg^r las1::las1-5FLAG-kanMX6</i> | This study |
| SPDK84 | <i>h⁹⁰ leu1-32 ura4DS/E ade6-M216 crb3::crb3-EGFP-kanMX6</i> | This study |
| SPDK87 | <i>h⁹⁰ leu1-32 ura4DS/E ade6-M216 las1::las1-EGFP-kanMX6</i> | This study |
| SPDK128 | <i>h- leu1-32 ura4DS/E ade6-M210 grc3::grc3-EGFP-kanMX6 swi6::KOR-swi6-Hyg^r</i> | This study |
| SPEK8 | <i>h⁹⁰ leu1-32 ura4DS/E ade6-M216 ipi1::ipi1-EGFP-kanMX6</i> | This study |
| SPEK14 | <i>h⁹⁰ leu1-32 ura4DS/E ade6-M216 rix1::rix1-EGFP-kanMX6</i> | This study |
| SPEK32 | <i>h- leu1-32 ura4DS/E ade6-M210 crb3::crb3-EGFP-kanMX6 swi6::KOR-swi6-Hyg^r</i> | This study |
| SPEK40 | <i>h⁹⁰ leu1-32 ura4DS/E ade6-M216 las1::las1-EGFP-kanMX6 swi6::KOR-swi6-Hyg^r</i> | This study |
| SPEK45 | <i>h⁹⁰ leu1-32 ura4DS/E ade6-M216 ipi1::ipi1-EGFP-kanMX6 swi6::KOR-swi6-Hyg^r</i> | This study |
| SPEK49 | <i>h⁹⁰ leu1-32 ura4DS/E ade6-M216 rix1::rix1-EGFP-kanMX6 swi6::KOR-swi6-Hyg^r</i> | This study |
| SPEK55 | <i>h⁹⁰ leu1-32 ura4DS/E ade6-M210 las1::las1-EGFP-kanMX6 clr4Δ::ura4⁺</i> | This study |
| SPEK57 | <i>h⁹⁰ leu1-32 ura4DS/E ade6-M216 grc3::grc3-EGFP-kanMX6 clr4Δ::ura4⁺</i> | This study |
| SPEK64 | <i>h⁹⁰ leu1-32 ura4DS/E ade6-M210 crb3::crb3-EGFP-kanMX6 clr4Δ::ura4⁺</i> | This study |
| SPEK76 | <i>h⁹⁰ leu1-32 ura4DS/E ade6-M216 rix1::rix1-EGFP-kanMX6 clr4Δ::ura4⁺</i> | This study |
| SPEK80 | <i>h⁹⁰ leu1-32 ura4DS/E ade6-M216 ipi1::ipi1-EGFP-kanMX6 clr4Δ::ura4⁺</i> | This study |
| SPEK84 | <i>h⁹⁰ leu1-32 ura4DS/E ade6-M216 rix1::rix1-EGFP-kanMX6 swi6Δ::ura4⁺</i> | This study |
| SPEK87 | <i>h⁹⁰ leu1-32 ura4DS/E ade6-M216 ipi1::ipi1-EGFP-kanMX6 swi6Δ::ura4⁺</i> | This study |
| SPEK90 | <i>h⁹⁰ leu1-32 ura4DS/E ade6-M216 las1::las1-EGFP-kanMX6 swi6Δ::ura4⁺</i> | This study |
| SPEK94 | <i>h⁹⁰ leu1-32 ura4DS/E ade6-M216 crb3::crb3-EGFP-kanMX6 swi6Δ::ura4⁺</i> | This study |
| SPEK98 | <i>h⁹⁰ leu1-32 ura4DS/E ade6-M216 grc3::grc3-EGFP-kanMX6 swi6Δ::ura4⁺</i> | This study |
| SPEK121 | <i>h⁹⁰ leu1-32 ura4DS/E ade6-M216 grc3::grc3-5FLAG-kanMX6 swi6Δ::Hyg^r</i> | This study |
| SPEK167 | <i>h⁹⁰ leu1-32 ura4DS/E ade6-M216 grc3::grc3-5FLAG-kanMX6 clr4Δ::Hyg^r</i> | This study |
| SPM1467 | <i>h- leu1-32 ura4DS/E ade6-M210</i> | This study |
| SPM1617 | <i>h- leu1-32 ura4DS/E ade6-M210 grc3::grc3-ura4⁺ (grc3-3)</i> | This study |
| SPM1620 | <i>h- leu1-32 ura4DS/E ade6-M210 grc3::grc3-ura4⁺ (grc3-7)</i> | This study |
| SPM1623 | <i>h- leu1-32 ura4DS/E ade6-M210 grc3::grc3-ura4⁺ (grc3-8)</i> | This study |
| SPYB106 | <i>h⁹⁰ leu1-32 ura4DS/E ade6-M216 his2 Kint2::ura4⁺</i> | Sadaie <i>et al.</i> (2004) |
| FY648 | <i>h+ leu1-32 ura4DS/E ade6-M210 otr1R(SphI)::ura4⁺</i> | Allshire <i>et al.</i> (1994) |
| SPM2396 | <i>h+ leu1-32 ura4DS/E ade6-M210 otr1R(SphI)::ura4⁺ crb3::crb3-Hyg^r (crb3-3)</i> | This study |
| SPM2416 | <i>h+ leu1-32 ura4DS/E ade6-M210 otr1R(SphI)::ura4⁺ ipi1::ipi1-Hyg^r (ipi1-1)</i> | This study |
| SPM2421 | <i>h+ leu1-32 ura4DS/E ade6-M210 otr1R(SphI)::ura4⁺ las1::las1-Hyg^r (las1-1)</i> | This study |
| SPM2441 | <i>h+ leu1-32 ura4DS/E ade6-M210 otr1R(SphI)::ura4⁺ rix1::rix1-Hyg^r (rix1-2)</i> | This study |

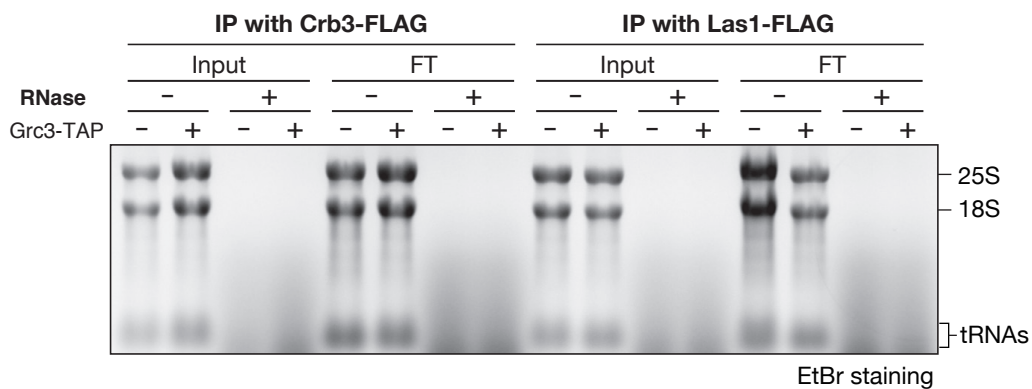
Supplementary Table S2 Primers used in this study

| Primer Name | Target | Sequence |
|-------------|--------------------|----------------------------------|
| act1_FW | <i>act1</i> | 5'-GAAGTACCCCATGAGCACGG-3' |
| act1_RV | <i>act1</i> | 5'-CAATTTACGTTTCGGCGGTAG-3' |
| B15E1-2490 | <i>E12 (tel)</i> | 5'-CGATGCTCTCGACAAAGCCGTTCT-3' |
| B15E1-3010 | <i>E12 (tel)</i> | 5'-CCATCTCAAACCTTCTGTTACATT-3' |
| dg223-FW | <i>dg223 (cen)</i> | 5'-TGGTAATACGTACTAGCTCTCG-3' |
| dg223-RV | <i>dg223 (cen)</i> | 5'-AACTAATTCATGGTGATTGATG-3' |
| K-R 17879 | <i>K-R (mat)</i> | 5'-CTCGCCTGCTTACATTTTAAGG-3' |
| K-R 11557 | <i>K-R (mat)</i> | 5'-GTATGTGGAACAAGAGAAG-3' |
| NB629_2 | <i>ITS1</i> | 5'-ATTCCCAAAAAGTTAAAAGATGGAAA-3' |
| NB1102_2 | <i>ITS1</i> | 5'-TTAGATATAATTAATTCAGACTTC-3' |
| rRNA_25S | 25S rRNA | 5'-CGCTTATTGATATGCTT-3' |
| rRNA_5.8S | 5.8S rRNA | 5'-CGCATTTTCGCTGCGTTCTTC-3' |
| rRNA_18S | 18S rRNA | 5'-CTTAGACATGCATGGCT-3' |
| M13_Fw | sequencing | 5'-GTAAAACGACGGCCAGT-3' |
| M13_Rv | sequencing | 5'-CAGGAAACAGCTATGAC-3' |
| act1-RT_Fw | <i>act1</i> | 5'-CGTGCCCCTGAAGCTCTTT-3' |
| act1-RT_Rv | <i>act1</i> | 5'-CTCATGAATACCGGCGTTTTTC-3' |
| dhII-RT_Fw3 | <i>dh</i> | 5'-CCCATGCTGTTGGATCAATG -3' |
| dhII-RT_Rv3 | <i>dh</i> | 5'-GCTCAAAAAGTGTGGCGCTATATC-3' |

Supplementary Table S3 Temperature-sensitive mutant alleles and mutated amino acids

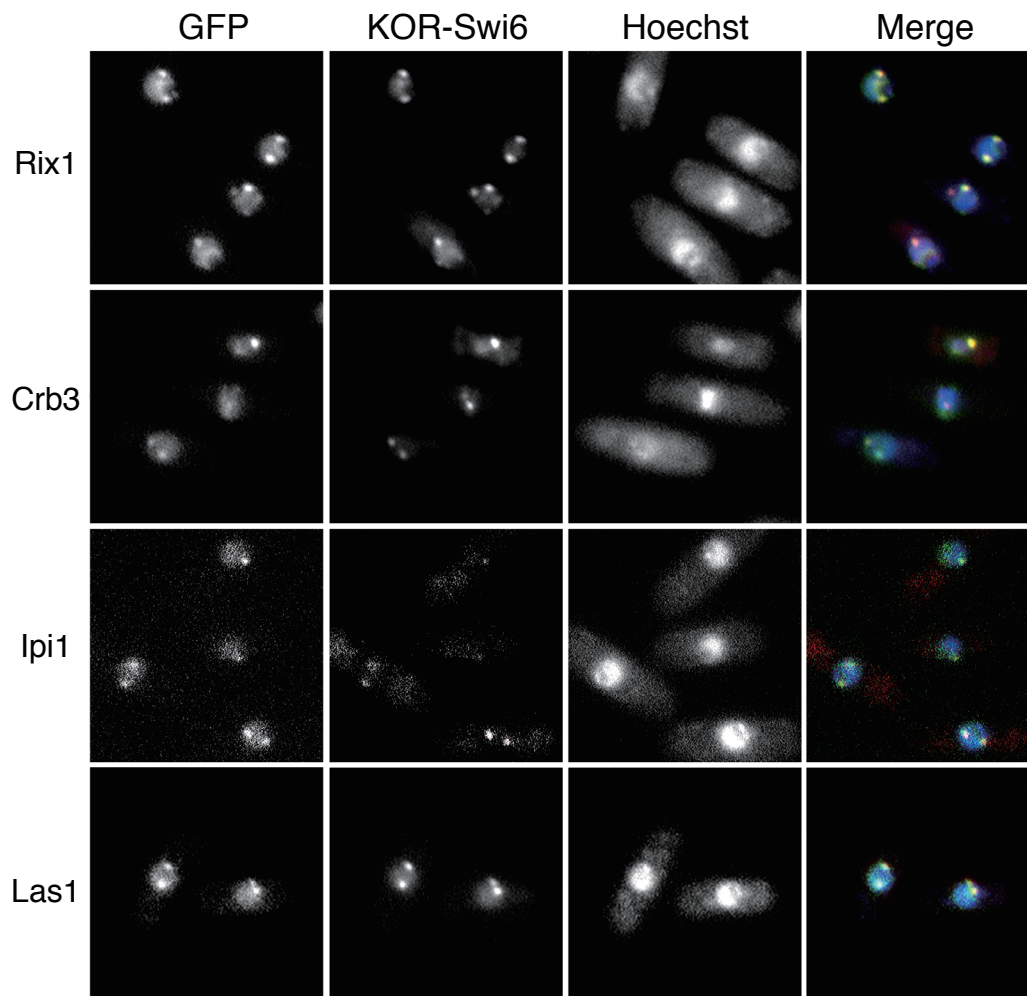
| Allelic No. | Amino acids change |
|----------------|---------------------|
| <i>grc3-3</i> | C423R, Y544H |
| <i>grc3-6</i> | S543P |
| <i>grc3-7</i> | S141P, S327T, G378R |
| <i>grc3-8</i> | Q390R, I467T, L654P |
| <i>grc3-10</i> | L689H, E704G |
| <i>grc3-11</i> | L427P |
| <i>grc3-12</i> | N305D, L513P, Q521R |
| <i>grc3-13</i> | D637N, L682I, L689P |
| <i>grc3-14</i> | I351V, S574P, I670T |
| <i>grc3-15</i> | N590S, R684G |
| <i>grc3-16</i> | F190L, L655P |
| <i>grc3-20</i> | I626M, K679E, Y702N |
| <i>crb3-2</i> | I381T |
| <i>crb3-3</i> | L389P |
| <i>crb3-5</i> | L396P, E414G |
| <i>crb3-10</i> | C380R |
| <i>crb3-11</i> | S309P |
| <i>ipil-1</i> | W189R, I364V |
| <i>ipil-1'</i> | W316R |
| <i>ipil-4'</i> | n.d.* |
| <i>ipil-7'</i> | M154K |
| <i>ipil-9'</i> | L172H, L280S |
| <i>las1-1</i> | S329P, S432P |
| <i>las1-2</i> | S432P |
| <i>las1-3</i> | S462P |
| <i>las1-4</i> | S432P |
| <i>las1-5</i> | I303T, S329P, M424V |
| <i>rix1-1</i> | L553P |
| <i>rix1-2</i> | L553P |
| <i>rix1-3</i> | L553P |
| <i>rix1-4</i> | S567P |
| <i>rix1-5</i> | L553P, T779A |

* No missense mutation was found within the suspected coding region.



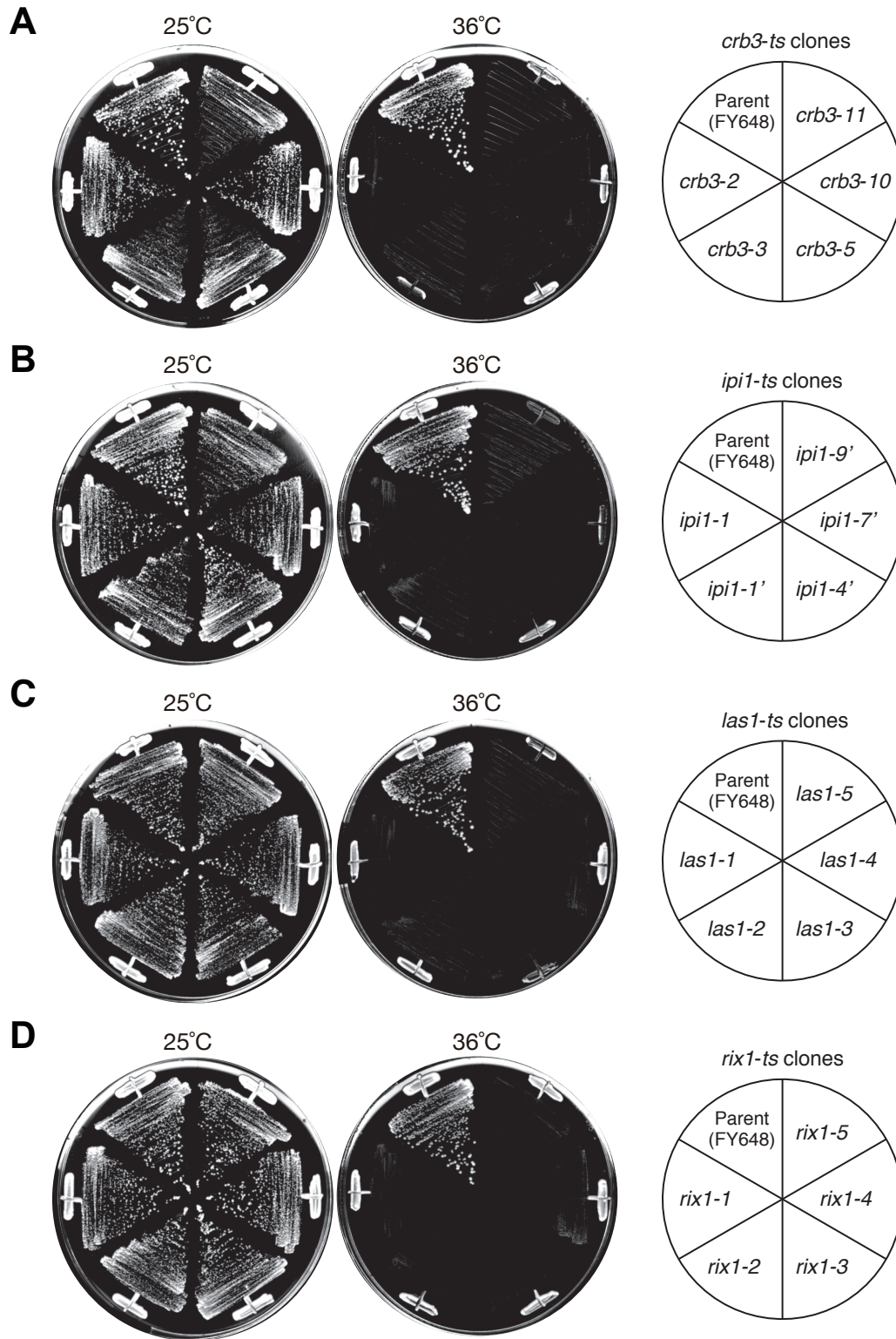
Supplementary Figure S1. Interaction between Grc3 and Crb3 or Las1 is independent of cellular RNAs.

Protein extracts prepared from cells expressing both Las1-FLAG and Grc3-TAP or Las1-FLAG and Grc3-TAP were subjected to immunoprecipitation using IgG Sepharose. The extracts were mock (-) or RNaseA-treated (+) prior to precipitation. Included RNAs were isolated, separated by electrophoresis on a 1.25% agarose gel containing 6.7% formaldehyde, and visualized by EtBr staining.



Supplementary Figure S2. Co-localization of Grc3-interacting proteins and Swi6.

Cells expressing Rix1-GFP, Crb3-GFP, Ipi1-GFP, or Las1-GFP, in conjunction with Kusabira orange (KOR)-fused Swi6 were analyzed by fluorescence microscopy. Three-dimensional optical section images were taken, and the acquired images were deconvoluted. A merged image of GFP-fused proteins (green), KOR-Swi6 (red), and DNA (blue) is shown at right.



Supplementary Figure S3. Temperature-sensitive mutants for Grc3-interacting factors.

Parental wild-type cells (FY648) and temperature-sensitive mutants for *crb3* (A), *ipi1* (B), *las1* (C), or *rix1* (D) were grown on YEA plate at 25°C or 36°C for 3-5 days.