



*Supplementary Materials*

# The Putative RNA-Binding Protein Dri1 Promotes the Loading of Kinesin-14/Klp2 to the Mitotic Spindle and Is Sequestered into Heat-Induced Protein Aggregates in Fission Yeast

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Supplementary Figure S1: Suppression of the *ts* phenotype of *cut7* by *dri1Δ* is allele-specific.

Supplementary Figure S2: Various truncated/mutated Dri1 proteins are expressed normally like the full-length protein.

Supplementary Figure S3: Dri1 is dispensable for the Mal3 localization on the spindle microtubules.

Supplementary Figure S4: Dri1 is exported from the nucleus in a Rae1-dependent manner.

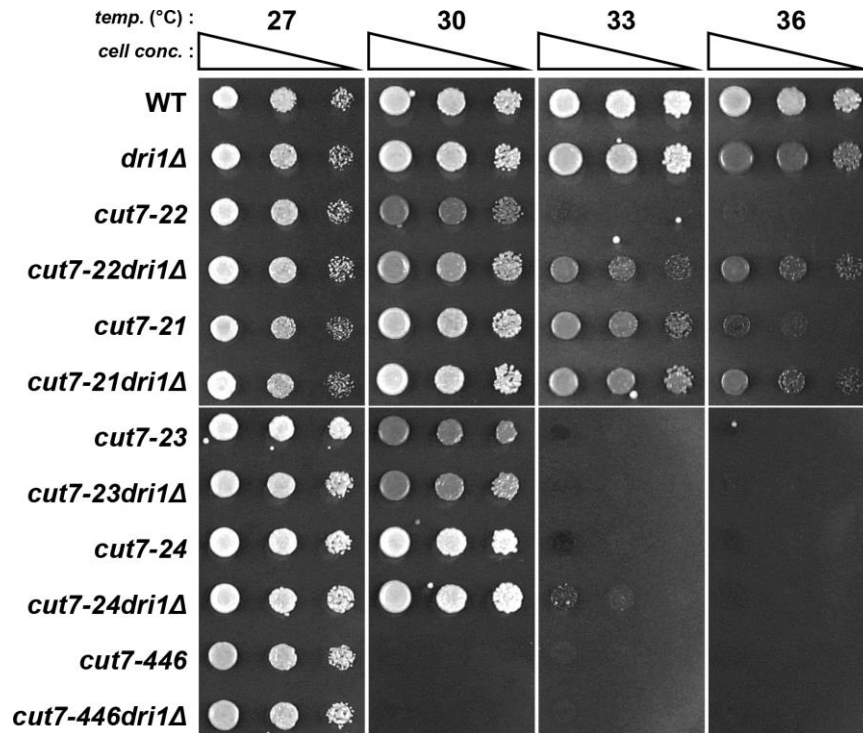
Supplementary Figure S5: A portion of the Dri1 protein co-localizes with other PAC components upon mild temperature shift-up.

Supplementary Figure S6: Characteristics of cell division in wild-type and *dri1Δ* cells grown at 39°C.

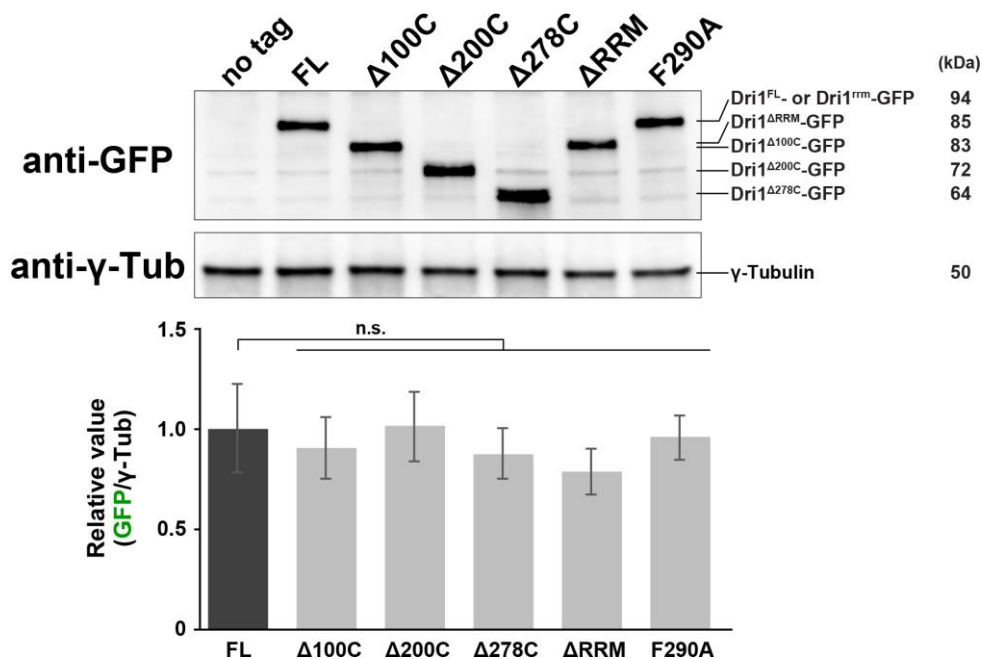
Supplementary Figure S7: Sensitivity/resistance of the *dri1* mutants is not altered under various stress conditions besides high temperature.

Supplementary Figure S8: Full scans of original images.

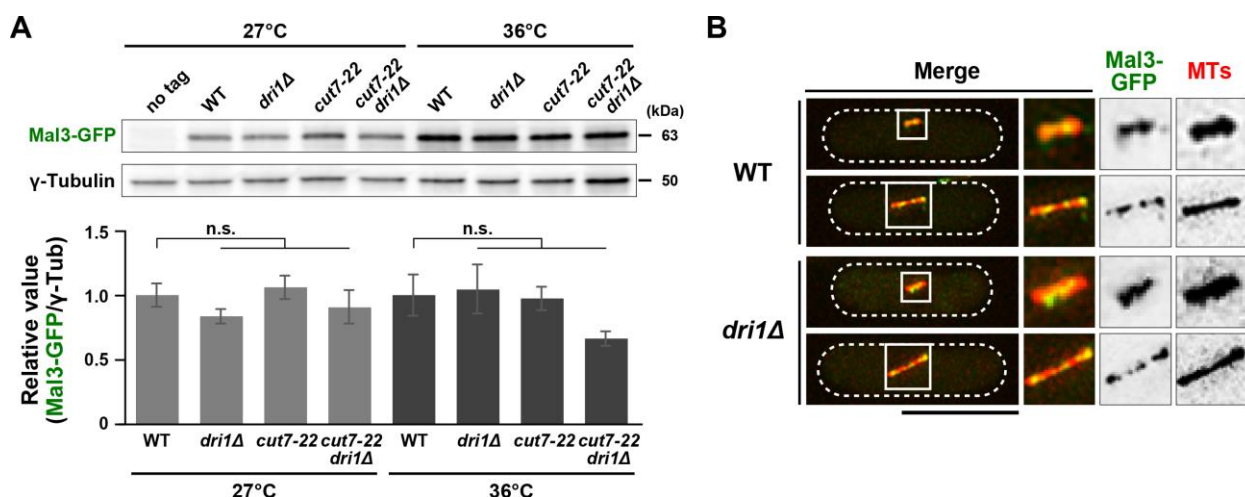
Supplementary Table S1: Fission yeast strains used in this study.



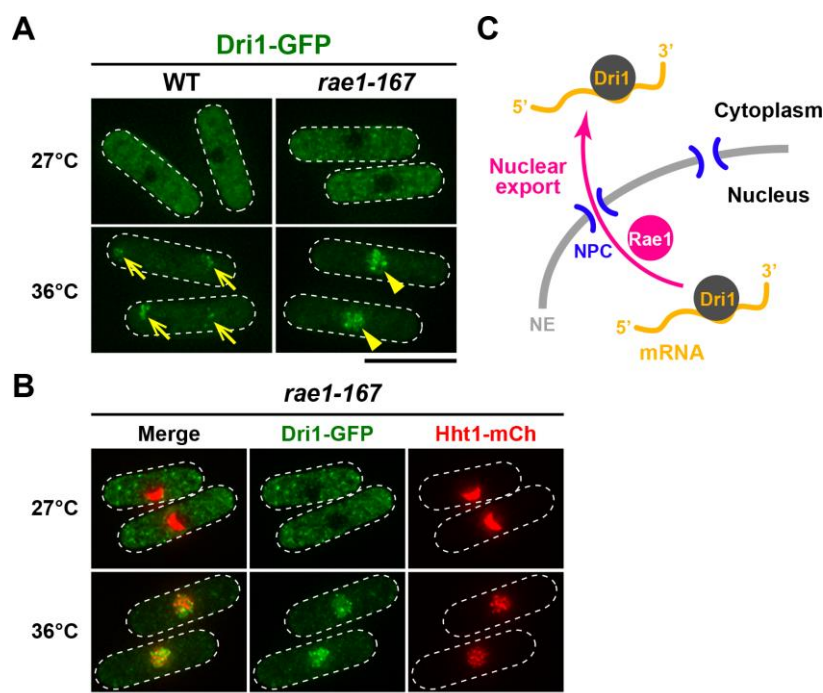
**Supplementary Figure S1.** Suppression of the ts phenotype of *cut7* by *dri1Δ* is allele-specific. Indicated strains were spotted onto rich YE5S agar plates and incubated at various temperatures for 2–3 days. 10-fold serial dilutions were performed in each spot.



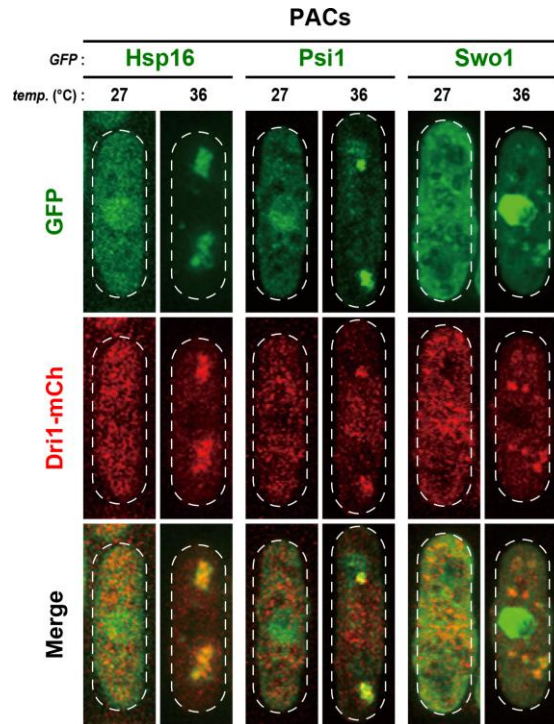
**Supplementary Figure S2.** Various truncated/mutated Dri1 proteins are expressed normally like the full-length protein. Quantification of the total levels of the full-length and various truncated/mutated Dri1-GFP proteins in whole-cell extracts was performed. Indicated strains containing Dri1-GFP and a control non-tagged strain were grown at 27 °C and protein extracts were prepared, followed by immunoblotting with anti-GFP and anti- $\gamma$ -tubulin antibodies. Relative amounts of each Dri1-GFP protein were normalized using those of  $\gamma$ -tubulin as a control. Results are given as the means  $\pm$  SE (n = 4). The relative amounts obtained from wild-type cells incubated at 27 °C were set as 1 and compared to those from other strains incubated at the same temperature. Data sets were compared with a two-tailed unpaired Student's t-tests (n.s., not significant).



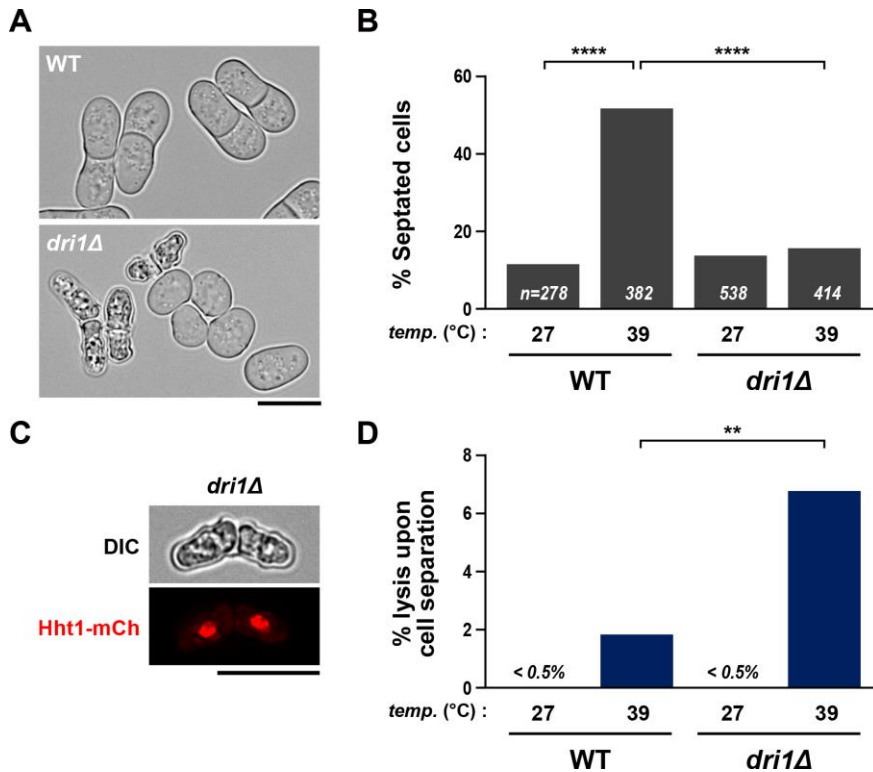
**Supplementary Figure S3.** Dri1 is dispensable for the Mal3 localization on the spindle microtubules. **(A)** Quantification of the total levels of Mal3-GFP protein in whole-cell extracts. Indicated strains containing Mal3-GFP and a control non-tagged strain were grown at 27 °C or 36 °C for 2h, and protein extracts were prepared, followed by immunoblotting with anti-GFP and anti- $\gamma$ -tubulin antibodies. Relative amounts of each Mal3-GFP protein were normalized using those of  $\gamma$ -tubulin as a control. Results are given as the means  $\pm$  SE ( $n = 2$ ). The relative amounts obtained from wild-type cells incubated at 27 °C and 36 °C were set as 1 and compared to those from other strains incubated at the same temperature. Data sets were compared with a two-tailed unpaired Student's *t*-tests (n.s., not significant). **(B)** Representative images showing mitotic localization of Mal3-GFP on the spindle microtubule are presented in wild-type and *dri1Δ* cells. Both strains contain Mal3-GFP and mCherry-Atb2. Cells were incubated at 27 °C. The cell peripheries are outlined with dotted lines and areas containing spindle microtubules (squares) are enlarged in the three panels on the right-hand side. Scale bars, 10  $\mu$ m (bottom left) and 1  $\mu$ m (right).



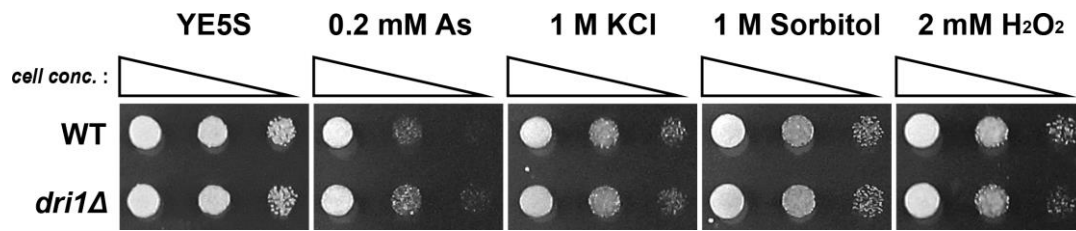
**Supplementary Figure S4.** Dri1 is exported directly or indirectly from the nucleus in a Rae1-dependent manner. **(A)** Wild-type and *rae1-167* cells containing Dri1-GFP were grown at 27 °C and then shifted to 36 °C for 2 h. The cytoplasmic aggregates formed at 36 °C in wild-type cells are pointed with yellow arrows, while the nuclear foci formed in *rae1-167* cells are shown with yellow arrowheads. **(B)** *rae1-167* cells containing Dri1-GFP and the chromatin marker Hht1-mCherry (Histone H3 h3.1) were grown at 27 °C and then shifted to 36 °C for 2 h. **(C)** A schematic illustration representing the export of Dri1 from the nucleus to the cytoplasm through the Rae1-dependent mRNA export pathway. The cell peripheries are indicated with dotted lines. Scale bars, 10  $\mu$ m.



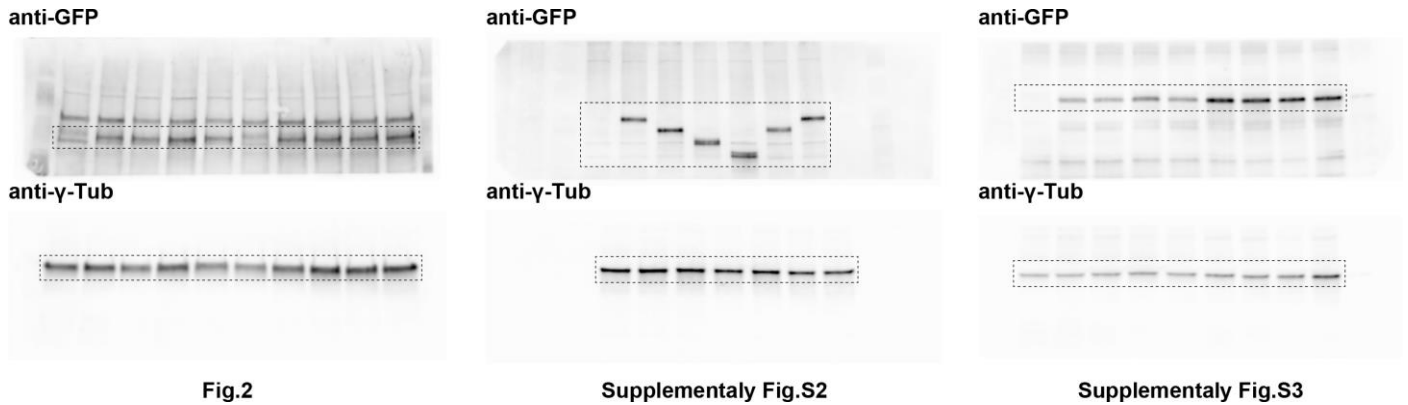
**Supplementary Figure S5.** A portion of the Dri1 protein co-localizes with other PACs components upon mild-temperature shift-up. Co-localization of Dri1-mCherry and different GFP-tagged chaperones (Hsp16, Psi1/Hsp40 and Swo1/Hsp90) after incubation at 36 °C for 2 h. The cell peripheries are indicated with dotted lines. Scale bar, 10  $\mu$ m.



**Supplementary Figure S6.** Characteristics of cell division in wild-type and *dri1Δ* cells grown at 39 °C. (A) Wild-type and *dri1Δ* cells grown at 27 °C were shifted to 39 °C and incubated for 12 h. Representative images of cell morphologies are shown (top, wild-type; bottom, *dri1Δ*). (B) The percentage of cells containing septa in wild-type and *dri1Δ* cells grown at 27 °C or 39 °C. The sample size (n) is shown on the bottom of each column. (C) Representative images of *dri1Δ* cells displaying lysis upon cell separation at 39 °C. *dri1Δ* cells containing Hht1-mCherry (Histone H3 h3.1) grown at 39 °C were imaged (top, DIC; bottom Hht1-mCherry). (D) The percentage of cells displaying the cell lysis phenotype upon cell separation. Cells used in (C) were also counted in this figure. All *p*-values were obtained from the two-tailed  $\chi^2$  test. \*\*, *p* < 0.01, \*\*\*\*, *p* < 0.0001, Scale bars, 10  $\mu$ m.



**Supplementary Figure S7.** Sensitivity/resistance of the *dri1* mutants is not altered under various stress conditions besides high temperature. Indicated strains were serially (10-fold) diluted, spotted onto rich YE5S plates that contains various reagents and incubated at various temperatures for 2–3 d.



**Supplementary Figure S8.** Full scans of original images.

**Supplementary Table S1.** Fission yeast strains used in this study

Strains	Genotypes	Figures used	Derivations
513	<i>h<sup>-</sup> leu1 ura4</i>	1C, 2D, 4D, 6C, S1, S2, S3A, S7	Our lab stock
YY146	<i>h<sup>+</sup> cut7-22 leu1ura4 his2</i>	1C, 4D, S1	Our lab stock
YY50	<i>h<sup>+</sup> cut7-22 dri1-W46X leu1ura4 his2</i>	1C	This study
MY1479	<i>h<sup>+</sup> cut7-22 dri1::kanR leu1 ura4 his2</i>	1C, S1	This study
MY2087	<i>h<sup>-</sup> cut7-22 dri1Δ100C-GFP-kanR leu1 ura4</i>	1C	This study
MY2106	<i>h<sup>-</sup> cut7-22 dri1Δ200C-GFP-kanR leu1 ura4</i>	1C	This study
MY2108	<i>h<sup>-</sup> cut7-22 dri1Δ278C-GFP-kanR leu1 ura4</i>	1C	This study
MY2089	<i>h<sup>-</sup> cut7-22 dri1ΔRRM-GFP-kanR leu1 ura4</i>	1C	This study
MY2135	<i>h<sup>-</sup> cut7-22 dri1-F290A-GFP-kanR leu1 ura4</i>	1C	This study
MY1744	<i>h<sup>-</sup> kanR-Palp4-GFP-klp2 aur1::aur1R-Pnda3-mCherry-atb2 leu1 ura4</i>	2A-D, 5C	This study
MY1746	<i>h<sup>+</sup> cut7-22 kanR-Palp4-GFP-klp2 aur1::aur1R-Pnda3-mCherry-atb2 leu1 ura4 his2</i>	2A-D	This study
MY1747	<i>h<sup>+</sup> cut7-22 dri1::kanR kanR-Palp4-GFP-klp2 aur1::aur1R-Pnda3-mCherry-atb2 leu1 ura4 his2</i>	2A-D	This study
MY1745	<i>h<sup>-</sup> dri1::kanR kanR-Palp4-GFP-klp2 aur1::aur1R-Pnda3-mCherry-atb2 leu1 ura4</i>	2B-D	This study
MiO222	<i>h<sup>+</sup> dri1-GFP-KanR sid4-mRFP-natR aur1::aur1R-Pnda3-mCherry-atb2 leu1 ura4</i>	3A	This study
MY2170	<i>h<sup>-</sup> dri1-GFP-kanR hht1-mCherry-kanR leu1 ura4</i>	3B-C	This study
MY2171	<i>h<sup>+</sup> dri1Δ100C-GFP-kanR hht1-mCherry-kanR leu1 ura4</i>	3B-C	This study
MY2174	<i>h<sup>+</sup> dri1Δ200C-GFP-kanR hht1-mCherry-kanR leu1 ura4 his2</i>	3B-C	This study
MY2175	<i>h<sup>+</sup> dri1Δ278C-GFP-kanR hht1-mCherry-kanR leu1 ura4 his2</i>	3B-C	This study
MY2177	<i>h<sup>+</sup> dri1ΔRRM-GFP-kanR hht1-mCherry-kanR leu1 ura4 his2</i>	3B-C	This study
MY2178	<i>h<sup>-</sup> dri1-F290A-GFP-kanR hht1-mCherry-kanR leu1 ura4</i>	3B-C	This study
MY2467	<i>h<sup>-</sup> dri1-mCherry-hphR hsp104-GFP-ura4<sup>+</sup> leu1? ura4?</i>	4A	This study
MY2477	<i>h<sup>-</sup> dri1-mCherry-hphR ssa1-GFP-natR leu1? ura4?</i>	4A	This study
MY2434	<i>h<sup>+</sup> dri1-mCherry-hphR sty1'-GFP-ssa2-leu1+ leu1 ura4</i>	4A	This study
MY2491	<i>h<sup>+</sup> dri1-mCherry-hphR sty1'-GFP-mas5-leu1<sup>+</sup> leu1? ura4? his2</i>	4A	This study



MY2473	<i>h<sup>-</sup> dri1-mCherry-hphR eIF4E-GFP-natR leu1? ura4?</i>	4A	This study
MY1799	<i>h<sup>-</sup> dri1-mNeonGreen-hphR leu1 ura4</i>	4B	This study
MY2520	<i>h<sup>-</sup> dri1-mNeonGreen-hphR hsp104::kanR leu1 ura4</i>	4B	This study
MY2526	<i>h<sup>-</sup> dri1-mNeonGreen-hphR psi1::ura4<sup>+</sup> leu1 ura4</i>	4B	This study
MY2536	<i>h<sup>-</sup> dri1-mNeonGreen-hphR mas5::kanR leu1 ura4</i>	4B	This study
MY2532	<i>h<sup>-</sup> hsp104-GFP-ura4<sup>+</sup> dri1::kanR leu1? ura4?</i>	4C	This study
MY2528	<i>h<sup>-</sup> sty1'-GFP-mas5-leu1<sup>+</sup> dri1::kanR leu1 ura4?</i>	4C	This study
MY2413	<i>h<sup>+</sup> hsp104::kanR ura4 leu1 his2</i>	4D	This study
MY2425	<i>h<sup>-</sup> psi1::ura4<sup>+</sup> ura4? leu1</i>	4D	This study
MY2544	<i>h<sup>+</sup> mas5::kanR leu1? ura4 his2</i>	4D	This study
MY2415	<i>h<sup>+</sup> cut7-22 hsp104::kanR leu1 his2</i>	4D	This study
MY2427	<i>h<sup>+</sup> cut7-22 psi1::ura4<sup>+</sup> ura4? leu1 his2</i>	4D	This study
MY2551	<i>h<sup>+</sup> cut7-22 mas5::kanR leu1? ura4 his2</i>	4D	This study
MY2698	<i>h<sup>+</sup> cut7-22 dri1-GBP-6HIS-mCherry-hphR sty1'-GFP-ssa2-leu1<sup>+</sup> leu1? ura4?</i>	5A-B	This study
MY2669	<i>h<sup>-</sup> dri1-GBP-6HIS-mCherry-hphR leu1? ura4?</i>	5B	This study
MY2623	<i>h<sup>+</sup> cut7-22 dri1-GBP-6HIS-mCherry-hphR leu1 ura4 his2</i>	5B	This study
MY2743	<i>h<sup>+</sup> cut7-22 sty1'-GFP-ssa2-leu1<sup>+</sup> aur1::aur1R-Pnda3-mCherry-atb2 leu1? ura4?</i>	5C	This study
MY2744	<i>h<sup>+</sup> cut7-22 dri1-GBP-6HIS-mCherry-hphR aur1::aur1R-Pnda3-mCherry-atb2 leu1? ura4?</i>	5C	This study
MY2746	<i>h<sup>+</sup> cut7-22 dri1-GBP-6HIS-mCherry-hphR aur1::aur1R-Pnda3-mCherry-atb2 leu1? ura4?</i>	5C	This study
MiO99	<i>h<sup>-</sup> dri1-GFP-kanR leu1 ura4</i>	6A, S2, S4A	This study
MY1978	<i>h<sup>-</sup> dri1-mNeonGreen-hphR pabp-mRFP-hphR leu1 ura4</i>	6B	This study
MiO233	<i>h<sup>+</sup> dri1-mNeonGreen-hphR dcp1-mCherry-natR leu1 ura4 his2</i>	6B	This study
MiO77	<i>h<sup>+</sup> dri1::kanR leu1 ura4 his2</i>	6C, S1, S7	This study
MY2064	<i>h<sup>-</sup> dri1Δ100C-GFP-kanR leu1 ura4</i>	6C, S2	This study
MY2066	<i>h<sup>-</sup> dri1Δ200C-GFP-kanR leu1 ura4</i>	6C, S2	This study
MY2068	<i>h<sup>-</sup> dri1Δ278C-GFP-kanR leu1 ura4</i>	6C, S2	This study
MY2691	<i>h<sup>-</sup> dri1ΔRRM-GFP-kanR leu1 ura4</i>	6C, S2	This study
MY2120	<i>h<sup>+</sup> dri1-F290A-GFP-kanR leu1 ura4 his2</i>	6C, S2	This study
MY1008	<i>h<sup>-</sup> cut7-21 leu1 ura4</i>	S1	Our lab stock
MiO107	<i>h<sup>-</sup> cut7-21 dri1::kanR leu1 ura4</i>	S1	This study
MA2-3D	<i>h<sup>-</sup> cut7-23 leu1</i>	S1	Our lab stock
MiO108	<i>h<sup>-</sup> cut7-23 dri1::kanR leu1 ura4</i>	S1	This study
IH136	<i>h<sup>-</sup> cut7-24 leu1</i>	S1	Our lab stock
MiO109	<i>h<sup>-</sup> cut7-24 dri1::kanR leu1 ura4</i>	S1	This study
NK193	<i>h<sup>+</sup> cut7-446 leu1 his2</i>	S1	Our lab stock
MiO110	<i>h<sup>+</sup> cut7-446 dri1::kanR leu1 ura4 his2</i>	S1	This study
MA145	<i>h<sup>-</sup> mal3-GFP-kanR leu1 ura4</i>	S3A-B	Our lab stock
MY2247	<i>h<sup>-</sup> mal3-GFP-kanR dri1::natR leu1 ura4</i>	S3A-B	This study
MY2264	<i>h<sup>-</sup> cut7-22 mal3-GFP-kanR leu1 ura4</i>	S3A	This study
MY2266	<i>h<sup>-</sup> cut7-22 dri1::natR mal3-GFP-kanR leu1 ura4</i>	S3A	This study
MiO190	<i>h<sup>-</sup> rae1-167 dri1-GFP-kanR leu1? ura4</i>	S4A	This study
MY2741	<i>h<sup>-</sup> rae1-167 dri1-GFP-kanR hht1-mCherry-kanR leu1? ura4</i>	S4B	This study
MY2475	<i>h<sup>-</sup> dri1-mCherry-hphR hsp16-GFP-natR leu1? ura4?</i>	S5	This study
MY2471	<i>h<sup>-</sup> dri1-mCherry-hphR sty1'-GFP-psi1-leu1<sup>+</sup> ura4?</i>	S5	This study
MY2469	<i>h<sup>+</sup> dri1-mCherry-hphR swo1-GFP-natR leu1? ura4? his2</i>	S5	This study
MY1281	<i>h<sup>-</sup> cut12-GFP-ura4<sup>+</sup> aur1::aur1R-Pnda3-mCherry-atb2 leu1 ura4</i>	S6A-B, S6D	This study
MY2757	<i>h<sup>-</sup> dri1::kanR cut12-GFP-ura4<sup>+</sup> aur1::aur1R-Pnda3-mCherry-atb2 leu1 ura4</i>	S6A-B, S6D	This study
MY2759	<i>h<sup>-</sup> dri1::kanR hht1-mCherry-kanR rlc1-GFP-ura4<sup>+</sup> leu1 ura4</i>	S6C	This study

\*Strains were developed for this study unless otherwise specified. *his2*=*his2*-245; *leu1*=*leu1*-32; *ura4*=*ura4*-D18.



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