



1 *Supplementary Materials*

2 **The putative RNA-binding protein Nrp1 promotes the loading**
3 **of kinesin-14/Klp2 to the mitotic spindle and is sequestered into**
4 **heat-induced protein aggregates in fission yeast**

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7 **Supplementary Figure S1: Suppression of the *ts* phenotype of *cut7* by *nrp1Δ* is allele-specific.**

8 **Supplementary Figure S2: Various truncated/mutated Nrp1 proteins are expressed normally like the**
9 **full-length protein.**

10 **Supplementary Figure S3: Nrp1 is dispensable for the Mal3 localization on the spindle microtubules.**

11 **Supplementary Figure S4: A portion of the Nrp1 protein co-localizes with other PAC components upon**
12 **mild temperature shift-up.**

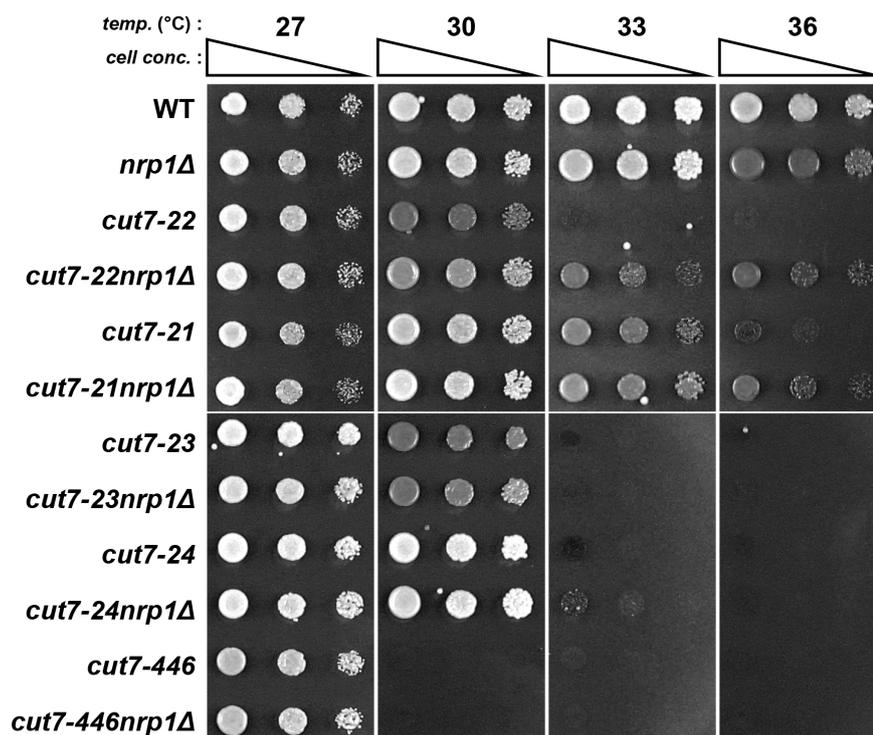
13 **Supplementary Figure S5: Sensitivity/resistance of the *nrp1* mutants is not altered under various stress**
14 **conditions besides high temperature.**

15 **Supplementary Figure S6: Full scans of original images.**

16 **Supplementary Table S1: Fission yeast strains used in this study**

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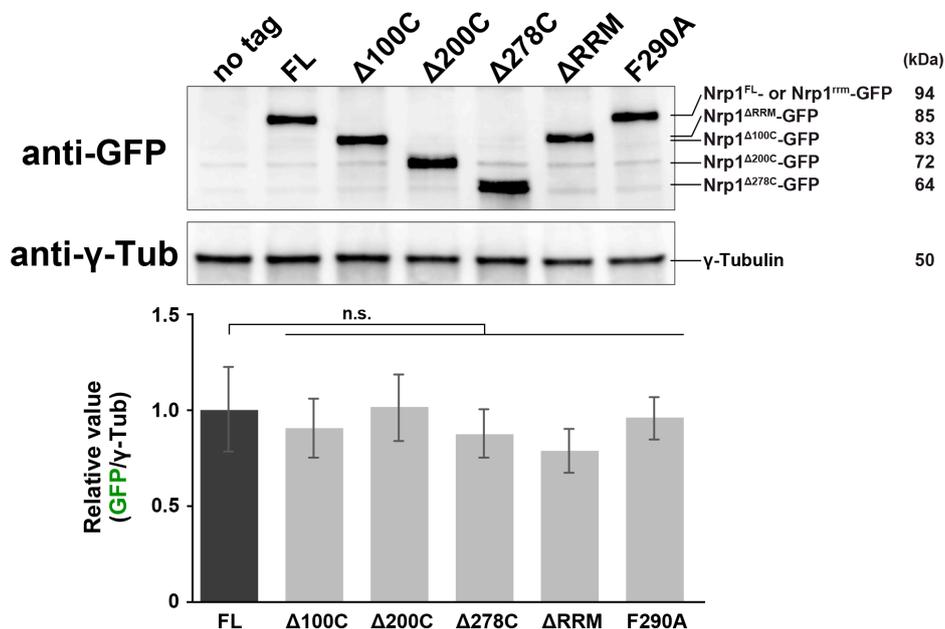
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Supplementary Figure S1. Suppression of the ts phenotype of *cut7* by *nrp1Δ* is allele-specific. (A) Spot tests. Indicated strains were spotted onto rich YE5S agar plates and incubated at various temperatures for 2-3 days. 10-fold serial dilutions were performed in each spot.



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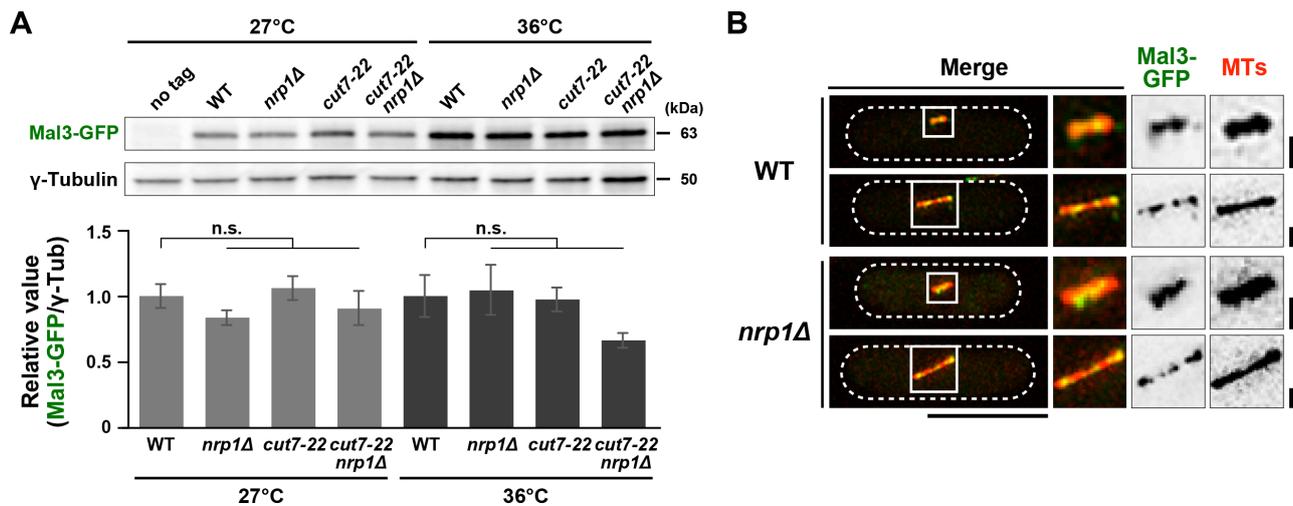
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Supplementary Figure S2. Various truncated/mutated Nrp1 proteins are expressed normally like the full-length protein. Quantification of the total levels of the full-length and various truncated/mutated Nrp1-GFP proteins in whole-cell extracts. Indicated strains containing Nrp1-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- γ -tubulin antibodies. Relative amounts of each Nrp1-GFP protein were normalized using those of γ -tubulin as a control. Results are given as the means \pm SD (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).



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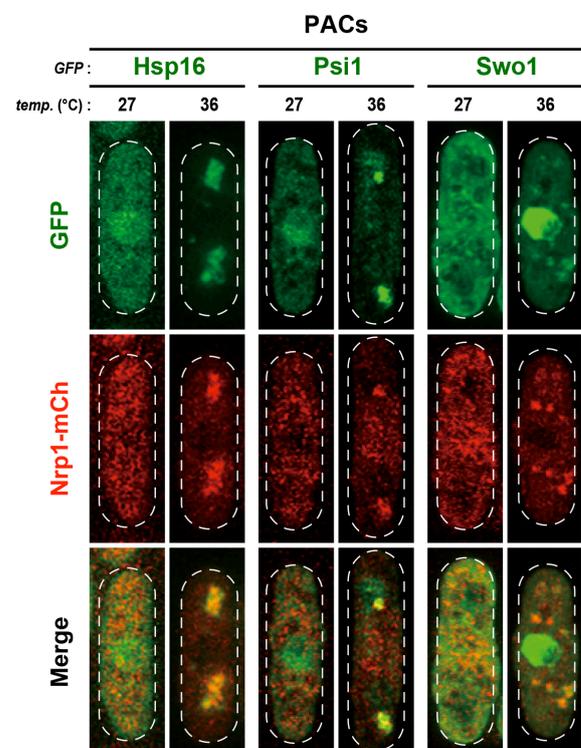
Supplementary Figure S3. Nrp1 is dispensable for the Mal3 localization on the spindle microtubules. (A) Quantification of the total levels of Mal3-GFP protein in whole-cell extracts. Indicated strains containing Mal3-GFP and a control non-tagged strain were grown at 27°C or 36°C for 2h, and protein extracts were prepared, followed by immunoblotting with anti-GFP and anti-γ-tubulin antibodies. Relative amounts of each Mal3-GFP protein were normalized using those of γ-tubulin as a control. Results are given as the means ± SD (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 *nrp1Δ* 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 μm (bottom left) and 1 μm (right).

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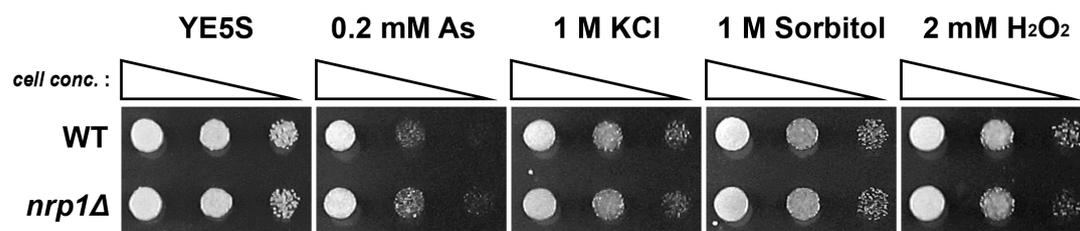
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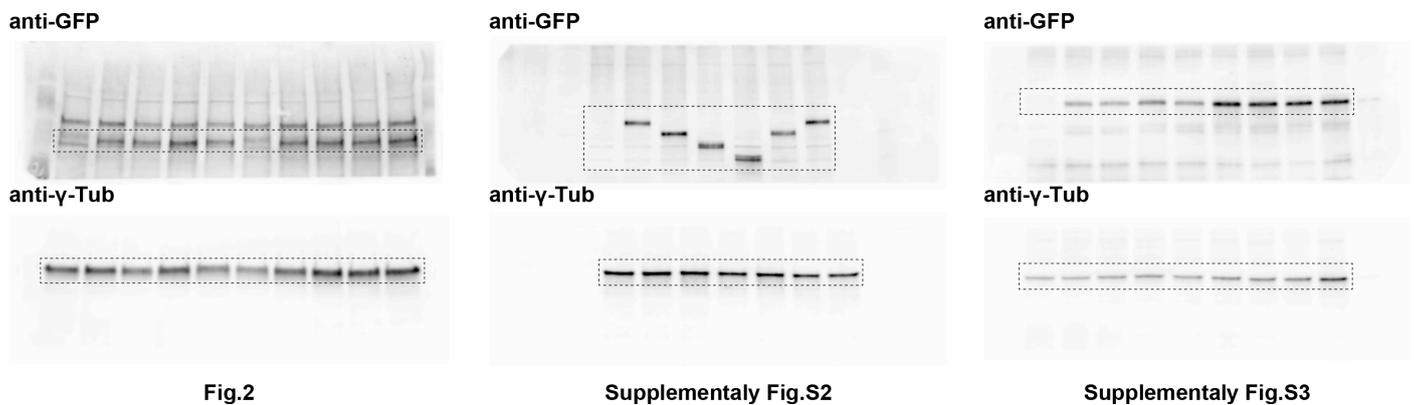


Supplementary Figure S4. A portion of the Nrp1 protein co-localizes with other PACs components upon mild-temperature shift-up. Co-localization of Nrp1-mCherry and different GFP-tagged chaperones (Hsp16, Psi1/Hsp40 and Swo1/Hsp90) after incubation at 36°C for 2 h. Scale bar, 10 μm.



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49 **Supplementary Figure S5.** Sensitivity/resistance of the *nrp1* mutants is not altered under various stress conditions be-
 50 sides high temperature. Indicated strains were serially (10-fold) diluted, spotted onto rich YE5S plates that contains var-
 51 ious reagents and incubated at various temperatures for 2-3 d.



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53 **Supplementary Figure S6.** Full scans of original images.

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

Strains	Genotypes	Figures used	Derivations
513	<i>h⁻ leu1 ura4</i>	1C, 4D, 6C, S1, S2, S3A, S5	Our lab stock
YY146	<i>h⁺ cut7-22 leu1ura4 his2</i>	1C, 4D, S1	Our lab stock
YY50	<i>h⁺ cut7-22 nrp1-W46X leu1ura4 his2</i>	1C	This study
MY1479	<i>h⁺ cut7-22 nrp1::kanR leu1 ura4 his2</i>	1C, S1	This study
MY2087	<i>h⁻ cut7-22 nrp1Δ100C-GFP-kanR leu1 ura4</i>	1C	This study
MY2106	<i>h⁻ cut7-22 nrp1Δ200C-GFP-kanR leu1 ura4</i>	1C	This study
MY2108	<i>h⁻ cut7-22 nrp1Δ278C-GFP-kanR leu1 ura4</i>	1C	This study
MY2089	<i>h⁻ cut7-22 nrp1ΔRRM-GFP-kanR leu1 ura4</i>	1C	This study
MY2135	<i>h⁻ cut7-22 nrp1-F290A-GFP-kanR leu1 ura4</i>	1C	This study
MY1744	<i>h⁻ kanR-Palp4-GFP-klp2 aur1::aur1R-Pnda3-mCherry-atb2 leu1 ura4</i>	2A-D	This study
MY1746	<i>h⁺ cut7-22 kanR-Palp4-GFP-klp2 aur1::aur1R-Pnda3-mCherry-atb2 leu1 ura4 his2</i>	2A-D	This study
MY1747	<i>h⁺ cut7-22 nrp1::kanR kanR-Palp4-GFP-klp2 aur1::aur1R-Pnda3-mCherry-atb2 leu1 ura4 his2</i>	2A-D	This study
MY1745	<i>h⁻ nrp1::kanR kanR-Palp4-GFP-klp2 aur1::aur1R-Pnda3-mCherry-atb2 leu1 ura4</i>	2B-D	This study
MiO222	<i>h⁺ nrp1-GFP-KanR sid4-mRFP-natR aur1::aur1R-Pnda3-mCherry-atb2 leu1 ura4</i>	3A	This study
MY2170	<i>h⁻ nrp1-GFP-kanR hht1-mCherry-kanR leu1 ura4</i>	3B-C	This study
MY2171	<i>h⁺ nrp1Δ100C-GFP-kanR hht1-mCherry-kanR leu1 ura4</i>	3B-C	This study
MY2174	<i>h⁺ nrp1Δ200C-GFP-kanR hht1-mCherry-kanR leu1 ura4 his2</i>	3B-C	This study
MY2175	<i>h⁺ nrp1Δ278C-GFP-kanR hht1-mCherry-kanR leu1 ura4 his2</i>	3B-C	This study
MY2177	<i>h⁺ nrp1ΔRRM-GFP-kanR hht1-mCherry-kanR leu1 ura4 his2</i>	3B-C	This study
MY2178	<i>h⁻ nrp1-F290A-GFP-kanR hht1-mCherry-kanR leu1 ura4</i>	3B-C	This study
MiO99	<i>h⁻ nrp1-GFP-kanR leu1 ura4</i>	3D, 6A, S2	This study

MiO190	<i>h⁻ rae1-167 nrp1-GFP-kanR leu1? ura4</i>	3D	This study
MY2467	<i>h⁻ nrp1-mCherry-hphR hsp104-GFP-ura4⁺ leu1? ura4?</i>	4A	This study
MY2477	<i>h⁻ nrp1-mCherry-hphR ssa1-GFP-natR leu1? ura4?</i>	4A	This study
MY2434	<i>h⁺ nrp1-mCherry-hphR sty1'-GFP-ssa2-leu1⁺ leu1 ura4</i>	4A	This study
MY2491	<i>h⁺ nrp1-mCherry-hphR sty1'-GFP-mas5-leu1⁺ leu1? ura4? his2</i>	4A	This study
MY2473	<i>h⁻ nrp1-mCherry-hphR eIF4E-GFP-natR leu1? ura4?</i>	4A	This study
MY1799	<i>h⁻ nrp1-mNeonGreen-hphR leu1 ura4</i>	4B	This study
MY2520	<i>h⁻ nrp1-mNeonGreen-hphR hsp104::kanR leu1 ura4</i>	4B	This study
MY2526	<i>h⁻ nrp1-mNeonGreen-hphR psi1::ura4⁺ leu1 ura4</i>	4B	This study
MY2536	<i>h⁻ nrp1-mNeonGreen-hphR mas5::kanR leu1 ura4</i>	4B	This study
MY2532	<i>h⁻ hsp104-GFP-ura4⁺ nrp1::kanR leu1? ura4?</i>	4C	This study
MY2528	<i>h⁻ sty1'-GFP-mas5-leu1⁺ nrp1::kanR leu1 ura4?</i>	4C	This study
MY2413	<i>h⁺ hsp104::kanR ura4 leu1 his2</i>	4D	This study
MY2425	<i>h⁻ psi1::ura4⁺ ura4? leu1</i>	4D	This study
MY2544	<i>h⁺ mas5::kanR leu1? ura4 his2</i>	4D	This study
MY2415	<i>h⁺ cut7-22 hsp104::kanR leu1 his2</i>	4D	This study
MY2427	<i>h⁺ cut7-22 psi1::ura4⁺ ura4? leu1 his2</i>	4D	This study
MY2551	<i>h⁺ cut7-22 mas5::kanR leu1? ura4 his2</i>	4D	This study
MY2698	<i>h⁺ cut7-22 nrp1-GFP-6HIS-mCherry-hphR sty1'-GFP-ssa2-leu1⁺ leu1? ura4?</i>	5A-B	This study
MY2669	<i>h⁻ nrp1-GFP-6HIS-mCherry-hphR leu1? ura4?</i>	5B	This study
MY2623	<i>h⁺ cut7-22 nrp1-GFP-6HIS-mCherry-hphR leu1 ura4 his2</i>	5B	This study
MY1978	<i>h⁻ nrp1-mNeonGreen-hphR pabp-mRFP-hphR leu1 ura4</i>	6B	This study
MiO233	<i>h⁻ nrp1-mNeonGreen-hphR dcp1-mCherry-natR leu1 ura4 his2</i>	6B	This study
MiO77	<i>h⁺ nrp1::kanR leu1 ura4 his2</i>	6C, S1, S5	This study
MY2064	<i>h⁻ nrp1Δ100C-GFP-kanR leu1 ura4</i>	6C, S2	This study
MY2066	<i>h⁻ nrp1Δ200C-GFP-kanR leu1 ura4</i>	6C, S2	This study
MY2068	<i>h⁻ nrp1Δ278C-GFP-kanR leu1 ura4</i>	6C, S2	This study
MY2691	<i>h⁻ nrp1ΔRRM-GFP-kanR leu1 ura4</i>	6C, S2	This study
MY2120	<i>h⁺ nrp1-F290A-GFP-kanR leu1 ura4 his2</i>	6C, S2	This study
MY1008	<i>h⁻ cut7-21 leu1 ura4</i>	S1	Our lab stock
MiO107	<i>h⁻ cut7-21 nrp1::kanR leu1 ura4</i>	S1	This study
MA2-3D	<i>h⁻ cut7-23 leu1</i>	S1	Our lab stock
MiO108	<i>h⁻ cut7-23 nrp1::kanR leu1 ura4</i>	S1	This study
IH136	<i>h⁻ cut7-24 leu1</i>	S1	Our lab stock
MiO109	<i>h⁻ cut7-24 nrp1::kanR leu1 ura4</i>	S1	This study
NK193	<i>h⁺ cut7-446 leu1 his2</i>	S1	Our lab stock
MiO110	<i>h⁺ cut7-446 nrp1::kanR leu1 ura4 his2</i>	S1	This study
MA145	<i>h⁻ mal3-GFP-kanR leu1 ura4</i>	S3A-B	Our lab stock
MY2247	<i>h⁻ mal3-GFP-kanR nrp1::natR leu1 ura4</i>	S3A-B	This study
MY2264	<i>h⁻ cut7-22 mal3-GFP-kanR leu1 ura4</i>	S3A	This study
MY2266	<i>h⁻ cut7-22 nrp1::natR mal3-GFP-kanR leu1 ura4</i>	S3A	This study
MY2475	<i>h⁻ nrp1-mCherry-hphR hsp16-GFP-natR leu1? ura4?</i>	S4	This study
MY2471	<i>h⁻ nrp1-mCherry-hphR sty1'-GFP-psi1-leu1⁺ ura4?</i>	S4	This study
MY2469	<i>h⁺ nrp1-mCherry-hphR swo1-GFP-natR leu1? ura4? his2</i>	S4	This study

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

