Supplemental Material

Suppressor analysis uncovers that MAPs and microtubule dynamics balance with the Cut7/Kinesin-5 motor for mitotic spindle assembly in *Schizosaccharomyces pombe*

Masashi Yukawa, Yusuke Yamada and Takashi Toda

**Supplemental Table S1:** Fission yeast strains used in this study

**Supplemental Figure S1:** The positions of intragenic mutations and mutated tubulin genes that suppress *cut7-22*

**Supplemental Figure S2:** Proper localization of spindle midzone markers in *mal3* or *alp16* deleted cells

**Supplemental Figure S3:** Fluorescence intensities of GFP-Pkl1 in individual strains

**Supplemental Figure S4:** Fluorescence intensities of spindle microtubules and the γ-tubulin complex in *cut7-22* cells

**Supplemental Figure S5:** Fluorescence intensities of GFP-Klp2 and spindle microtubules in Pkl1-overproduced cells

**Supplemental Figure S6:** Fluorescence intensities of Msd1-GFP and Wdr8-GFP in *cut7-22* ts cells

**Supplemental Figure S7:** A microtubule-depolymerizing drug, MBC, renders *cut7Δ* cells viable

**Supplemental Figure S8:** TBZ ameliorates spindle structures in Pkl1-overproduced cells
# Supplemental Table S1: Fission yeast strains used in this study

| Strains | Genotypes | Figures used | Derivations |
|---------|------------|--------------|-------------|
| 513     | h<sup>-</sup> leu1 ura4 | 1A, 1C-D, 4A, 5A-B, S1C, S7 | Our lab stock |
| MY638   | h<sup>-</sup> cut7-22 ade6-210 | 1A, 1C-D, 4A | This study |
| YY60    | h<sup>-</sup> cut7-22 skf1-7 (pk11-E726D) ade6-210 | 1A | This study |
| YY03    | h<sup>-</sup> cut7-22 skf2-5 (wdr8-W399R) ade6-210 | 1A | This study |
| MY1515  | h<sup>-</sup> cut7-22 skf3-2 (msd1-L217FfsX4) ade6-210 | 1A | This study |
| MY1472  | h<sup>-</sup> cut7-22 skf4-1 kanR (nda3-G56D-kanR) ade6-210 | 1A | This study |
| YY66    | h<sup>-</sup> cut7-22 skf5-1 (atb2-G410A) ade6-210 | 1A | This study |
| YY61    | h<sup>-</sup> cut7-22 skf6-1 (mal3-K47KfsX4) ade6-210 | 1A | This study |
| YY147   | h<sup>-</sup> cut7-22 pkl1::natR leu1 ura4 | 1C | This study |
| MY942   | h<sup>-</sup> cut7-22 wdr8::natR ade6-210 | 1C | This study |
| MY598   | h<sup>-</sup> cut7-22 mds1::hphR leu1 ura4 ade6-210 | 1C | This study |
| YY183   | h<sup>-</sup> cut7-22 ath2::ura4 leu1 ura4 his2 | 1C | This study |
| YY214   | h<sup>-</sup> cut7-22 mat3::ura4 ura4 | 1C | This study |
| YY251   | h<sup>-</sup> cut7-22 alp16::ura4 leu1 ura4 | 1D | This study |
| YY163   | h<sup>-</sup> cut7-22 klp2::hphR leu1 ura4 | 1D | This study |
| YY177   | h<sup>-</sup> cut7-22 alp7::hphR leu1 ura4 | 1D | This study |
| MY1528  | h<sup>-</sup> cut7-22 alp14::kanR leu1 ura4 | 1D | This study |
| YY231   | h<sup>-</sup> cut7-22 dis1::hphR leu1 ura4 | 1D | This study |
| MY331   | h<sup>-</sup> kanR-GFP-alp4 aur1R-Pnda3-mCherry-atb2 leu1 ura4 | 1E-F, S4C-D | This study |
| MY652   | h<sup>-</sup> cut7-22 kanR-GFP-alp4 aur1R-Pnda3-mCherry-atb2 leu1 ura4 | 1E-F, 3A-C, S4A-D | This study |
| YY241   | h<sup>-</sup> cut7-22 mal3::kanR-GFP-alp4 aur1R-Pnda3-mCherry-atb2 leu1 ura4 | 1E-F, 3C | This study |
| YY271   | h<sup>-</sup> cut7-22 alp16::ura4 kanR-GFP-alp4 aur1R-Pnda3-mCherry-atb2 leu1 ura4 | 1E-F, 3C | This study |
| YY236   | h<sup>-</sup> cut7-22 pkl1::natR kanR-GFP-alp4 aur1R-Pnda3-mCherry-atb2 leu1 ura4 his2 | 1F, 3C | This study |
| MY1334  | h<sup>-</sup> cut7-22 wdr8::natR KanR-GFP-alp4 aur1R-Pnda3-mCherry-atb2 leu1 ura4 | 1F | This study |
| MY728   | h<sup>-</sup> cut7-22 mds1::natR KanR-GFP-alp4 aur1R-Pnda3-mCherry-atb2 leu1 ura4 ade6-210 | 1F | This study |
| MY1777  | h<sup>-</sup> cut7-22 nda3-G56D-kanR KanR-GFP-alp4 aur1R-Pnda3-mCherry-atb2 leu1 ura4 | 1F | This study |
| YY238   | h<sup>-</sup> cut7-22 klp2::hphR KanR-GFP-alp4 aur1R-Pnda3-mCherry-atb2 leu1 ura4 his2 | 1F, 3C | This study |
| MO51    | h<sup>-</sup> cut7-22 alp7::hphR KanR-GFP-alp4 aur1R-Pnda3-mCherry-atb2 leu1 ura4 | 1F | This study |
| MY1789  | h<sup>-</sup> cut7-22 alp14::hphR KanR-GFP-alp4 aur1R-Pnda3-mCherry-atb2 leu1 ura4 his2 | 1F | This study |
| MY1780  | h<sup>-</sup> cut7-22 dis1::hphR KanR-GFP-alp4 aur1R-Pnda3-mCherry-atb2 leu1 ura4 | 1F | This study |
| Reference | Description | Source |
|-----------|-------------|--------|
| MY1030    | $h^-$ kanR-Palp4-GFP-klp2 aur1R-Pnda3-mCherry-atb2 leu1 ura4 | 2A-B, 4B-C, This study |
| MY1439    | $h^-$ mal3::ura4+ kanR-Palp4-GFP-klp2 aur1R-Pnda3-mCherry-atb2 leu1 ura4 | 2A-B, This study |
| MY1458    | $h^-$ cut7-22 mal3::ura4+ kanR-Palp4-GFP-klp2 aur1R-Pnda3-mCherry-atb2 leu1 ura4 | 2B, 4B-C, This study |
| MY1490    | $h^-$ cut7-22 mal3::ura4+ kanR-Palp4-GFP-klp2 aur1R-Pnda3-mCherry-atb2 leu1 ura4 | 2B, This study |
| MY1492    | $h^-$ cut7-22 mal3::ura4+ kanR-Palp4-GFP-klp2 aur1R-Pnda3-mCherry-atb2 leu1 ura4 | 2B, This study |
| MY1695    | $h^-$ leu1 ura4 [pREP41-GFP] | 2C, 4E, This study |
| MY1697    | $h^-$ leu1 ura4 [pREP41-GFP-klp2] | 2C, 4E, This study |
| MY1699    | $h^-$ cut7-22 leu1 ura4 his2 [pREP41-GFP] | 2C, 4E, This study |
| MY1701    | $h^-$ cut7-22 leu1 ura4 his2 [pREP41-GFP-klp2] | 2C, 4E, This study |
| MY1703    | $h^-$ cut7-22 mal3::ura4+ leu1 ura4 his2 [pREP41-GFP] | 2C, This study |
| MY1705    | $h^-$ cut7-22 mal3::ura4+ leu1 ura4 his2 [pREP41-GFP-klp2] | 2C, This study |
| MY1707    | $h^-$ cut7-22 alp16::ura4+ leu1 ura4 [pREP41-GFP] | 2C, This study |
| MY1709    | $h^-$ cut7-22 alp16::ura4+ leu1 ura4 [pREP41-GFP-klp2] | 2C, This study |
| MY844     | $h^-$ cut11-GFP-ura4+ kanR-GFP-klp4 aur1R-Pnda3-mCherry-atb2 leu1 ura4 | 3A-C, S4A-B, This study |
| MY1008    | $h^-$ cut7-21 leu1 ura4 | 4A, Our lab stock |
| MA2-3D    | $h^-$ cut7-23 leu1 | 4A, Our lab stock |
| I1136     | $h^-$ cut7-24 leu1 | 4A, Our lab stock |
| NK193     | $h^-$ cut7-446 leu1 his2 | 4A, Our lab stock |
| MY858     | $h^-$ kanR-Palp4-GFP-klp1 aur1R-Pnda3-mCherry-atb2 leu1 ura4 | 4D, S3A-B, This study |
| MY1482    | $h^-$ cut7-22 kanR-Palp4-GFP-klp1 aur1R-Pnda3-mCherry-atb2 leu1 ura4 | 4D, S3B, This study |
| YY305     | $h^-$ leu1 ura4 [pREP41-GFP-klp1] | 4E, This study |
| YY308     | $h^-$ cut7-22 leu1 ura4 his2 [pREP41-GFP-klp1] | 4E, This study |
| MY990     | $h^-$ cut7::bleR pk11::natR leu1 ura4 his2 | 5A, This study |
| MY1660    | $h^-$ cut7::bleR leu1 ura4? ura4? his2? | 5B, S7, This study |
| MY899     | $h^-$ cut7::bleR pk11::natR leu1 ura4 | 6A, This study |
| CH61      | $h^-$ wdr8::kanR leu1 ura4 | 6A, Our lab stock |
| MY1616    | $h^-$ skf4-1-kanR (nda3-G56D-kanR) cut7-GFP-HPHR leu1 ura4? ade6-210? | 6A, This study |
| MY1618    | $h^-$ skf4-2-kanR (nda3-Q334R-kanR) cut7-GFP-HPHR leu1 ura4? ade6-210? | 6A, This study |
| YY173     | $h^-$ atb2::ura4+ cut7-GFP-kanR leu1 ura4 | 6A, This study |
| YY201     | $h^-$ mal3::ura4+ cut7-GFP-kanR leu1 ura4 | 6A, This study |
| iHR2239   | $h^-$ alp16::kanR leu1 ura4 | 6A, Our lab stock |
| MY986     | $h^-$ alp7::ura4+ leu1 ura4 | 6A, This study |
| MY988   | \( h^{-} \) alp14::kanR leu1 ura4                              | 6A   | This study |
| MY1006  | \( h^{-} \) dis1::hphR leu1 ura4                              | 6A   | This study |
| YY68    | \( h^{-} \) cut7-22, 68 ade6-210                             | S1A  | This study |
| YY70    | \( h^{-} \) cut7-22, 70 ade6-210                             | S1A  | This study |
| YY71    | \( h^{-} \) cut7-22, 71 ade6-210                             | S1A  | This study |
| MT31    | \( h^{-} \) mal3::kanR leu1 ura4                             | S1C  | This study |
| MY1785  | \( h^{-} \) skf4-1-kanR (nda3-G356D-kanR) leu1 ade6-210?      | S1C  | This study |
| MY1840  | \( h^{-} \) skf5-1 (atb2-G410A) leu1 ade6-210                | S1C  | This study |
| MY1462  | \( h^{-} \) klp9-GFP-kanR aur1R-Pnda3-mCherry-ath2 leu1 ura4 | S2A, S2C | This study |
| MY1464  | \( h^{-} \) mal3::ura4\(^{-}\) klp9-GFP-kanR aur1R-Pnda3-mCherry-ath2 leu1 ura4 | S2A, S2C | This study |
| MY1792  | \( h^{-} \) alp16::ura4\(^{-}\) klp9-GFP-kanR aur1R-Pnda3-mCherry-ath2 leu1 ura4 | S2A, S2C | This study |
| MY1466  | \( h^{-} \) asel1-GFP-kanR aur1R-Pnda3-mCherry-ath2 leu1 ura4 his2 | S2B, S2D | This study |
| MY1469  | \( h^{-} \) mal3::ura4\(^{-}\) asel1-GFP-kanR aur1R-Pnda3-mCherry-ath2 leu1 ura4 his2 | S2B, S2D | This study |
| MY1796  | \( h^{-} \) alp16::ura4\(^{-}\) asel1-GFP-kanR aur1R-Pnda3-mCherry-ath2 leu1 ura4 | S2B, S2D | This study |
| MY1508  | \( h^{-} \) alp16::ura4\(^{-}\) kanR-Palp4-GFP-pkl1 aur1R-Pnda3-mCherry-ath2 leu1 ura4 | S3A-B | This study |
| MY1485  | \( h^{-} \) cut7-22 mal3::ura4\(^{-}\) kanR-Palp4-GFP-pkl1 aur1R-Pnda3-mCherry-ath2 leu1 ura4 | S3B | This study |
| MY1487  | \( h^{-} \) cut7-22 alp16::ura4\(^{-}\) kanR-Palp4-GFP-pkl1 aur1R-Pnda3-mCherry-ath2 leu1 ura4 | S3B | This study |
| MY1807  | \( h^{-} \) msd1-GFP-kanR aur1R-Pnda3-mCherry-ath2 leu1 ura4 | S3C, S6A | This study |
| MY1831  | \( h^{-} \) alp16::ura4\(^{-}\) msd1-GFP-kanR aur1R-Pnda3-mCherry-ath2 leu1 ura4 | S3C | This study |
| MY1809  | \( h^{-} \) cut7-22 msd1-GFP-kanR aur1R-Pnda3-mCherry-ath2 leu1 ura4 | S3C, S6A | This study |
| MY1834  | \( h^{-} \) cut7-22 alp16::ura4\(^{-}\) msd1-GFP-kanR aur1R-Pnda3-mCherry-ath2 leu1 ura4 | S3C | This study |
| MY195   | \( h^{-} \) wdr8-GFP-kanR aur1R-Pnda3-mCherry-ath2 leu1 ura4 | S3D, S6B | This study |
| MY1837  | \( h^{-} \) alp16::ura4\(^{-}\) wdr8-GFP-kanR aur1::aur1R-Pnda3-mCherry-ath2 leu1 ura4 | S3D | This study |
| MY1774  | \( h^{-} \) cut7-22 wdr8-GFP-kanR aur1::aur1R-Pnda3-mCherry-ath2 leu1 ura4 | S3D, S6B | This study |
| MY1828  | \( h^{-} \) cut7-22 alp16::ura4\(^{-}\) wdr8-GFP-kanR aur1::aur1R-Pnda3-mCherry-ath2 leu1 ura4 [pREP41] | S3D | This study |
| MY1816  | \( h^{-} \) kanR-Palp4-GFP-klp2 aur1R-Pnda3-mCherry-ath2 leu1 ura4 [pREP41] | S3D | This study |
| MY1819  | \( h^{-} \) kanR-Palp4-GFP-klp2 aur1R-Pnda3-mCherry-ath2 leu1 ura4 [pREP41-pkl1] | S3D | This study |
| Strain | Genotype | Source |
|--------|----------|--------|
| YY309  | h⁻ klp2::hphR cut12-GFP-ura4⁺ aur1R-Pnda3-mCherry-atb2 leu1 ura4 [pREP41-GFP] | S8 This study |
| YY311  | h⁻ klp2::hphR cut12-GFP-ura4⁺ aur1R-Pnda3-mCherry-atb2 leu1 ura4 [pREP41-GFP-pk11] | S8 This study |

*Strains were developed for this study unless otherwise specified.

his2=his2-245; leu1=leu1-32; ura4=ura4-D18.
Figure S1. Yukawa et al.

A

| Genes       | Groups | Alleles | Mutation sites         |
|-------------|--------|---------|------------------------|
| phlf        | I      | skf1-1  | I36NfsX3               |
|             |        | skf1-2  | H46X                   |
|             |        | skf1-3  | K468NfsX8              |
|             |        | skf1-4  | K477N                  |
|             |        | skf1-5  | S562R                  |
|             |        | skf1-6  | H695Q                  |
|             |        | skf1-7  | E726D                  |
| wdr8        | I      | skf2-1  | C75F                   |
|             |        | skf2-2  | W127X                  |
|             |        | skf2-3  | S254R                  |
|             |        | skf2-4  | T356I                  |
|             |        | skf2-5  | W399R                  |
| msd1        | I      | skf3-1  | L358                   |
|             |        | skf3-2  | L217F/5xX4             |
| nda3        | II     | skf4-1  | G56D                   |
|             |        | skf4-2  | Q334R                  |
| atb2        | II     | skf5-1  | Q410A                  |
|             |        | skf5-2  | K477fsX4               |

B

C

D

E
Figure S1. The positions of intragenic mutations and mutated tubulin genes that suppress *cut7-22*

(A) Mutation sites in intragenic suppressors of the *cut7-22* ts mutant. Overall domain structure of Cut7 are shown on the top: the N-terminal motor domain (blue), the medial stalk domain (gray) including multiple coiled-coil regions (CC) and the C-terminal region (yellow) containing the characteristic BimC. The positions of 3 intragenic mutations are also shown. (B) A summary table of *skf* mutant alleles and corresponding genes. Group I (*skf1-skf3*) shown in green consists of genes encoding the MWP complex, while Group II (*skf4-skf6*) shown in orange is composed of those encoding *nda3*, *atb2* and *mal3*. Mutation sites in individual alleles are also provided on the far right corner. X and fs stand for termination codons and frame-shift mutations, respectively. (C) Spot test. Indicated strains were spotted onto rich YE5S agar plates in the absence or presence of TBZ (15 μg/ml or 20 μg/ml) and incubated at 30°C for 3 d. 10-fold serial dilutions were performed in each spot. cell conc., cell concentration. (D) Mutation sites in *nda3/skf4* and *atb2/skf5*. Alignments of amino acid sequences corresponding to the regions surrounding mutated amino acid residues (marked with green columns) are also shown on the bottom. (E) 3D-simulation of the α-/β-tubulin heterodimer. The positions of the mutated amino acid residues in *nda3/skf4* and *atb2/skf5* are indicated.
Figure S2. Proper localization of spindle midzone markers in mal3 or alp16 deleted cells

(A, B) Representative images of Klp9-GFP (A) or Ase1-GFP (B). Wild-type, mal3Δ or alp16Δ cells containing mCherry-Atb2 and Klp9-GFP (A) or mCherry-Atb2 and Ase1-GFP (B) were grown at 27°C, and images of mitotic cells were taken. (C, D) Quantification of Klp9-GFP (C) or Ase1-GFP signal intensities (D). Fluorescence intensities of Klp9-GFP (C) or Ase1-GFP (D) on the spindle microtubule were measured. All p-values were obtained from the two-tailed unpaired Student’s t test. Data are presented as the means ± SE (≥ 21 cells). n.s., not significant.
Figure S3. Fluorescence intensities of GFP-Pkl1 in individual strains

(A) Representative images showing mitotic localization of GFP-Pkl1 at the SPB are presented in indicated cells. All strains contain GFP-Pkl1 and mCherry-Atb2. Cells were incubated at 27°C. Scale bar, 10 μm. (B-D) Quantification of GFP-Pkl1 (B), Msd1-GFP (C) or Wdr8-GFP (D) intensities at the mitotic SPB. Each cell was incubated at 27°C (B) or 36°C for 2 h (B-D), and the total values of GFP fluorescence intensities at the SPB were measured. The values of wild-type cells were set as 100% (27°C and 36°C each) and compared to those from other strains under the same condition. All p-values were obtained from the two-tailed unpaired Student’s t test. Data are presented as the means ± SE (n≥12). *, P < 0.05, ***, P < 0.001, n.s., not significant.
Figure S4. Yukawa et al.
Figure S4. Fluorescence intensities of spindle microtubules and the γ-tubulin complex in cut7-22 cells

(A) Comparison of intensities of preanaphase spindle microtubules (< 3 μm) between wild-type and cut7-22 cells in the same field. These two strains were mixed in the same culture, grown at 27°C, shifted to 36°C and further incubated for 2 h. While wild-type cells contain mCherry-Atb2 (MTs), Cut11-GFP (SPB/NE) and GFP-Alp4 (SPB), cut7-22 cells contain mCherry-Atb2 (MTs) and GFP-Alp4 (SPB). Images of mitotic cells were captured in the same field. Note that SPB signals are brighter in a wild-type cell (lower) than those in a cut7-22 cell (upper), as the former cell contains Cut11-GFP in addition to GFP-Alp4, thereby wild-type and cut7-22 cells precisely being assigned. Scale bar, 10 μm. (B) Quantification. Fluorescence intensities of spindle microtubules obtained in (A) were measured in each strain and plotted in relation to the spindle length. A vertical dotted line represents the spindle length (3 μm) at metaphase. Note that only cells that displayed bipolar (not monopolar) spindles were taken into account. (C) Distribution of GFP-Alp4 intensities. Wild-type or cut7-22 cells containing mCherry-Atb2 and GFP-Alp4 were grown at 27°C. A half of the cultures was shifted to 36°C, while the other half was kept at 27°C. After 2 h incubation, fluorescence intensities of GFP-Alp4 were measured in each strain and plotted in relation to the spindle length (dark- and light-gray circles, wild-type cells at 27°C or 36°C respectively; dark- and light-green circles, cut7-22 cells at 27°C or 36°C respectively). A vertical dotted line represents the spindle length (3 μm) at metaphase. Note that only cells that displayed bipolar (not monopolar) spindles were taken into account. (D) Quantification. Fluorescence intensities of GFP-Alp4 were measured in wild-type or cut7-22 cells that were incubated at either 27°C or 36°C. The values of wild-type cells incubated at 27°C or 36°C were each set as 100%, and compared to those of cut7-22 cells under the same condition. Data are presented as the means ± SE (n>30).
Figure S5. Fluorescence intensities of GFP-Klp2 and spindle microtubules in Pkl1-overproduced cells (A, B) Wild type cells containing GFP-Klp2 and mCherry-Atb2 were transformed with vector plasmids (vec.) or plasmids carrying the thiamine-repressible nmt41-pkl1" gene (pkl1"OE) and grown in the liquid minimal medium in the absence (ON) or presence (OFF) of thiamine for 16 h at 30°C. Under this condition, ~50% of cells contained monopolar spindles. Signal intensities of GFP-Klp2 on the spindles (A) and mCherry-Atb2 (spindle microtubules, B) were quantified in cells containing bipolar spindles. The levels of spindle microtubule were plotted against the spindle length in individual mitotic cells (B). All p-values were obtained from the two-tailed unpaired Student’s t test. Data are presented as the means ± SD (≥20 cells). **, P < 0.01, n.s., not significant.
Figure S6. Fluorescence intensities of Msd1-GFP and Wdr8-GFP in cut7-22 ts cells
(A, B) Fluorescence intensities of Msd1-GFP (A) or Wdr8-GFP (B) at the mitotic SPB were measured in wild-type and cut7-22 cells that were incubated at 27°C for 12-16 h in the absence or presence of 20 μg/ml TBZ. All p-values were obtained from the two-tailed unpaired Student’s t test. Data are presented as the means ± SE (n≥16). ***, P < 0.001, ****, P < 0.0001. n.s., not significant.
**Figure S7.** Yukawa et al.

A microtubule-depolymerizing drug, MBC, renders cut7Δ cells viable

Spot test. One of wild-type or cut7Δ colonies obtained from tetrad dissection shown in Figure 5A were spotted onto YE5S plates in the absence or presence of various concentrations of MBC, and incubated at 27°C for 3 d.

*cell conc.*, cell concentration.
Figure S8. TBZ ameliorates spindle structures in Pkl1-overproduced cells

*klp2Δ* cells containing GFP-Klp2 and mCherry-Atb2 were transformed with vector plasmids (vec.) or plasmids carrying the thiamine-repressible *nmt41-GFP-pkl1* gene (*pkl1*OE) and grown in the liquid minimal medium containing thiamine for 24 h at 30°C in the absence or presence of 20 μg/ml TBZ. The morphology of mitotic spindle microtubules was observed and classified into bipolar (green) or monopolar spindles (magenta). All *p*-values were obtained from the two-tailed χ² test. Data are presented as the means ± SE (≥42 cells). *, *P* < 0.05, ****, *P* < 0.0001.