

## **Supplementary Information**

### **Dual impact of a benzimidazole resistant $\beta$ -tubulin on microtubule behavior in fission yeast**

**Mamika Minagawa, Minamo Shirato, Mika Toya and Masamitsu Sato**

Supplementary table S1

Supplementary figures S1 and S2

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

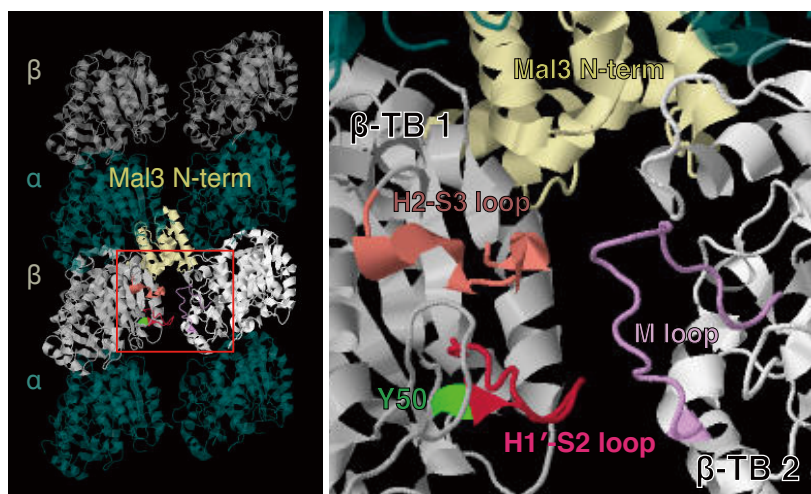
<b>Strains</b>	<b>Genotypes</b>	<b>Figures</b>
MJ1043	<i>h90 nda3-TB101 leu1-32 ura4-D18 ade6-M216</i>	1A, 4A
KK0274	<i>h90 nda3-TB101-bsd leu1-32 ura4-D18 ade6-M216</i>	1A, 7A
KK0275	<i>h90 nda3-TB101-bsd leu1-32 ura4-D18 ade6-M216</i>	1A
KK0285	<i>h90 nda3-bsd leu1-32 ura4-D18 ade6-M216</i>	1A
KK0286	<i>h90 nda3-bsd leu1-32 ura4-D18 ade6-M216</i>	1A
JY0878	<i>h90 leu1-32 ura4-D18 ade6-M216</i>	1A, 4A, 7A
KK0174	<i>h90 Z2-GFP-atb2-kan leu1-32 ura4-D18 ade6-M216</i>	3A-B, 5A, 6A-C, 7B, 7D, 8C, S2C
KK0337	<i>h90 nda3-TB101-bsd Z2-GFP-atb2-kan leu1-32 ura4-D18 ade6-M216</i>	3A-B, 5A-B, 6A-C, 7B, 7D
SAK836	<i>h90 ade6-M216 leu1-32 ura4-D18 caf5::bsd pap1-del pmd1-del mfs1-del bfr1-del dnf2-del erg5::ura4<sup>+</sup></i>	4A
MN0136	<i>h90 nda3-TB101 ade6-M216 leu1-32 ura4-D18 caf5::bsd pap1-del pmd1-del mfs1-del bfr1-del dnf2-del erg5::ura4<sup>+</sup></i>	4A
MM0090	<i>h90 nda3-TB101-bsd Z2-GFP-atb2-kan leu1-32 ura4-D18 ade6-M216</i>	4B, S2C
YS0021	<i>h<sup>-</sup> ade6-M216 leu1-32 ura4-D18 caf5::bsd pap1-del pmd1-del mfs1-del bfr1-del dnf2-del erg5::ura4<sup>+</sup> Z2-GFP-atb2-kan</i>	4B, 5A
MN0129	<i>h<sup>-</sup> nda3-TB101 ade6-M216 leu1-32 ura4-D18 caf5::bsd pap1-del pmd1-del mfs1-del bfr1-del dnf2-del erg5::ura4<sup>+</sup> Z2-GFP-atb2-kan</i>	4B, 5A-C
KK0306	<i>h90 alp14::ura4<sup>+</sup> leu1-32 ura4-D18 ade6-M216</i>	7A
KK0335	<i>h90 nda3-TB101-bsd alp14::ura4<sup>+</sup> leu1-32 ura4-D18 ade6-M216</i>	7A
KK0173	<i>h90 alp14::ura4<sup>+</sup> Z2-GFP-atb2-kan leu1-32 ura4-D18 ade6-M216</i>	7B-D
KK0333	<i>h90 nda3-TB101-bsd alp14::ura4<sup>+</sup> Z2-GFP-atb2-kan leu1-32 ura4-D18 ade6-M216</i>	7B-D
KK0392	<i>h90 klp6::ura4<sup>+</sup> Z2-GFP-atb2-kan leu1-32 ura4-D18 ade6-M216</i>	8A, 8C
MN0051	<i>h90 nda3-TB101-bsd klp6::ura4<sup>+</sup> Z2-GFP-atb2-kan leu1-32 ura4-D18 ade6-M216</i>	8A, 8C
MJ0009	<i>h<sup>+</sup> leu1-32 ura4-D18 his2 ade6-M216</i>	8B, S2D
MJ0073	<i>h90 klp6::ura4<sup>+</sup> leu1-32 ura4-D18 ade6-M216</i>	8B
MN0214	<i>h<sup>-</sup> nda3-TB101 leu1-32 ura4-D18 ade6-M216</i>	8B

KK0386	<i>h90 nda3-TB101-bsd klp6::ura4<sup>+</sup> leu1-32 ura4-D18 ade6-M216</i>	8B
KK0088	<i>h90 mal3-GFP-kan Z2-mCherry-atb2-hph leu1-32 ura4-D18 ade6-M216</i>	S2A
KK0317	<i>h90 nda3-TB101-bsd mal3-GFP-kan Z2-mCherry-atb2-hph leu1-32 ura4-D18 ade6-M216</i>	S2A
MM0282	<i>h90 alp7-3GFP-kan Z2-mCherry-atb2-hph leu1-32 ura4-D18 ade6-M216</i>	S2B
MM0283	<i>h90 nda3-TB101-bsd alp7-3GFP-kan Z2-mCherry-atb2-hph leu1-32 ura4-D18 ade6-M216</i>	S2B
MM0100	<i>h90 mal3::ura4<sup>+</sup> Z2-GFP-atb2-kan leu1-32 ura4-D18 ade6-M216</i>	S2C
MM0093	<i>h90 mal3::ura4<sup>+</sup> nda3-TB101-bsd Z2-GFP-atb2-kan leu1-32 ura4-D18 ade6-M216</i>	S2C
MM0095	<i>h90 nda3-TB101-bsd leu1-32 ura4-D18 ade6-M216</i>	S2D
MM0098	<i>h<sup>-</sup> mal3::ura4<sup>+</sup> leu1-32 ura4-D18 ade6-M216</i>	S2D
MM0099	<i>h<sup>-</sup> mal3::ura4<sup>+</sup> nda3-TB101-bsd leu1-32 ura4-D18 ade6-M216</i>	S2D

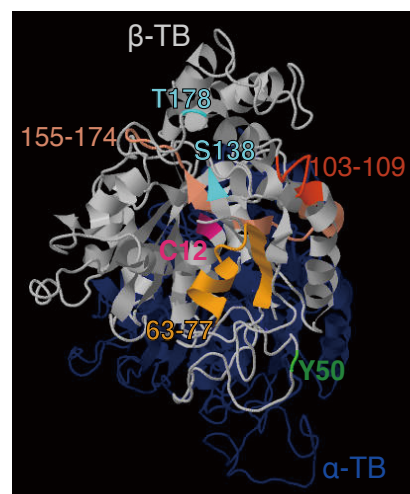
Origin of strains: the original *nda3-TB101* strain is a gift from M. Yamamoto [1]. SAK836 is a gift from S. Kawashima [2]. JY878 is our stock. Other strains were generated in this study.

## Supplementary figures

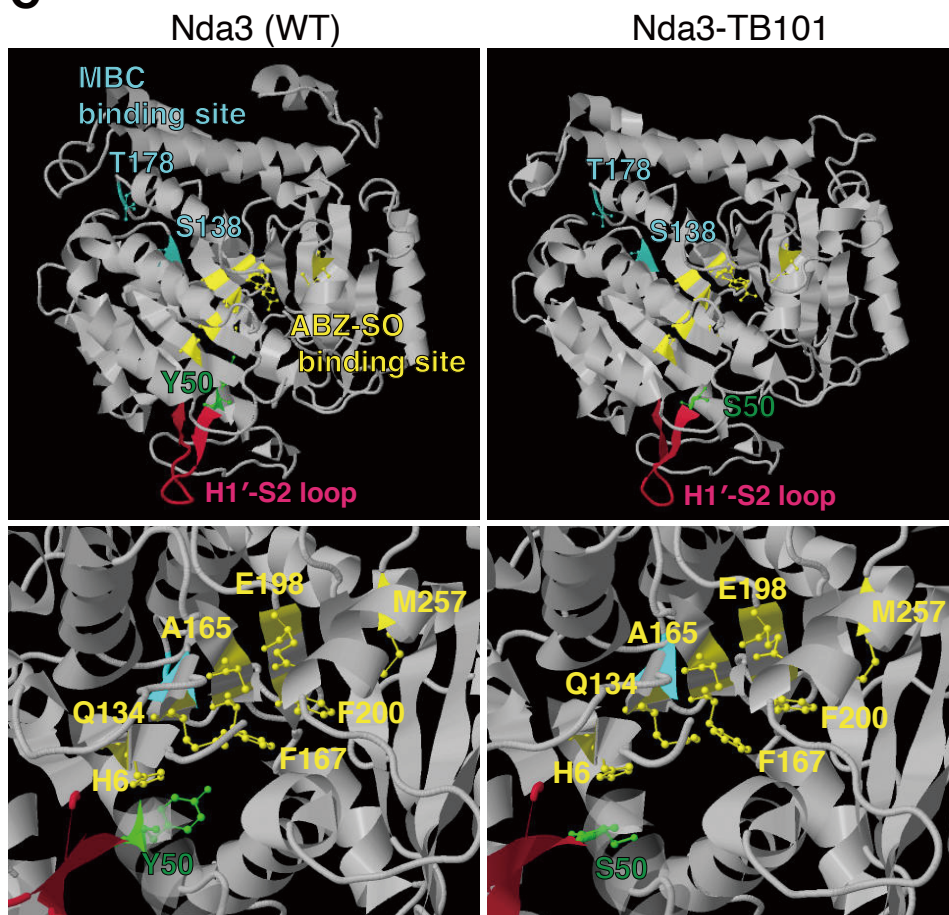
**A**



**B**



**C**



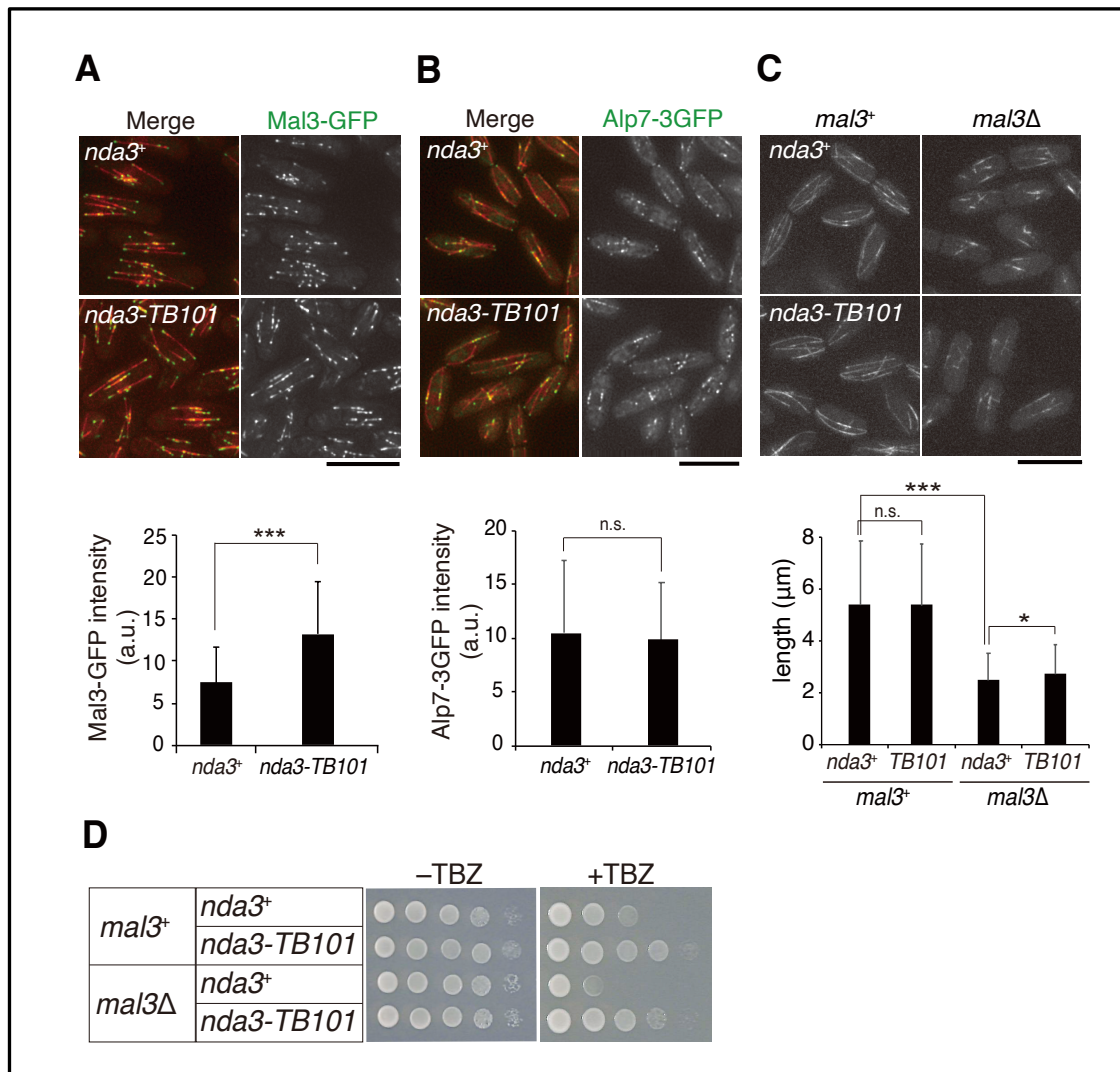


### **Supplementary figure 1. Relationship of Y50 and other major residues**

(A) Alignment of mammalian  $\alpha\beta$  dimers in the B-type lattice in a back view from the putative inside of the microtubule. Emerald,  $\alpha$ -tubulin ( $\alpha$ -TB); white,  $\beta$ -tubulin ( $\beta$ -TB 1 and 2), based on the model proposed in previous study [3]. N-terminal fragment of Mal3 (ivory) binds to the corner of a tetramer from outside of the microtubule. Inset (red) is magnified in the right. Representative loops are highlighted in colors: H1'-S2 loop (red), H2-S3 loop (coral) and M loop (purple). Y50 (green) is located close to the H1'-S2 loop.

(B) Regions for GTP-binding in  $\beta$ -tubulin, as summarized in [4,5]. Indigo,  $\alpha$ -tubulin ( $\alpha$ -TB); gray,  $\beta$ -tubulin ( $\beta$ -TB). C12 (magenta) and 63-77 (orange) are located by the guanine base of GTP, the region, 155-174 (coral) is by the ribose. 103-109 (red) is thought to affect the GTP hydrolysis. S138 and T178 (turquoise) are putative MBC binding sites that might affect GTP binding, according to discussion in the previous study [6].

(C) Geographical relationship between Y50 and other candidates of benzimidazole binding sites shown with ball-and-stick modeling. S138 and T178 (turquoise) are as in B. Residues H6, Q134, A165, F167, E198, F200 and M257 (yellow) and Y50 (green) are putative binding site of ABZ-SO [7]. left, Nda3 WT (green, Y50); right, Nda3-TB101 (green, S50).



**Supplementary figure 2. A structural model for  $\alpha\beta$ -tubulin in MT and positions of associating Mal3 and GTP**

(A) Localization of Mal3-GFP (green) to cytoplasmic microtubules (mCherry-Atb2, red) in WT and *nda3-TB101* cells. Fluorescence intensity of Mal3-GFP at plus-end of microtubules were quantified. *nda3<sup>+</sup>* (WT),  $n = 40$  tip signals; *nda3-TB101*,  $n = 39$ . \*\*\* $p = 7.3E-06 < 0.001$  (Welch's t-test); error bars, S.D.

(B) Localization of Alp7-3GFP (green) observed and quantified as in A. *nda3<sup>+</sup>* (WT),  $n = 40$  tip signals; *nda3-TB101*,  $n = 42$ . n.s., not significant (Student's t-test); error bars, S.D.

(C) Organization of cytoplasmic microtubules in *mal3Δ nda3-TB101* cells. Cytoplasmic microtubules were short in *mal3Δ* cells as shown previously [8]. This was almost similar in *mal3Δ nda3-TB101* cells. *mal3<sup>+</sup> nda3<sup>+</sup>*,  $n = 197$  bundles; *mal3<sup>+</sup> nda3-TB101*,  $n = 198$ ; *mal3Δ nda3<sup>+</sup>*,  $n = 166$ ; *mal3Δ nda3-TB101*,  $n = 163$ . n.s. not significant; \* $p = 0.036 < 0.05$  (Student's t-test); \*\*\* $p = 3.4E-38 < 0.001$  (Welch's t-test); error bars, S.D. Scale bars, 10 μm.

(D) Assays for TBZ sensitivity. The *mal3Δ* strain was sensitive to TBZ, but the *mal3Δ nda3-TB101* strain was resistant to TBZ. Ten-fold serial dilutions of indicated strains were spotted onto YE5S plates without and with 15 μg/ml TBZ for 3 days.

## References

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