

Supplementary Table S1: Fission yeast strains used in this study

Strains	Genotypes	Figures used	Derivation*
513	<i>h⁻ leu1 ura4</i>	1A-C, S1A	Our lab stock
YY153	<i>h⁻ klp2::hphR leu1 ura4</i>	1A-C	This study
MY1008	<i>h⁻ cut7-21 leu1 ura4</i>	1A, S1A-B	Our lab stock
MY784	<i>h⁻ cut7-21 pk11::natR leu1 ura4</i>	1A	This study
YY161	<i>h⁻ cut7-21 klp2::hphR leu1 ura4</i>	1A	This study
YY180	<i>h⁻ cut7-21 pk11::natR klp2::hphR leu1 ura4</i>	1A	This study
MY637	<i>h⁻ cut7-22 ade6-210</i>	1B, S1A-B	Our lab stock
YY147	<i>h⁻ cut7-22 pk11::natR leu1 ura4</i>	1B	This study
YY163	<i>h⁻ cut7-22 klp2::hphR leu1 ura4</i>	1B	This study
YY165	<i>h⁻ cut7-22 pk11::natR klp2::hphR leu1 ura4</i>	1B	This study
NK193	<i>h⁺ cut7-446 leu1 his2</i>	1C, S1A-B	Our lab stock
YY169	<i>h⁻ cut7-446 pk11::natR leu1 ura4?</i>	1C	This study
YY171	<i>h⁻ cut7-446 klp2::hphR leu1 ura4?</i>	1C	This study
YY184	<i>h⁻ cut7-446 pk11::natR klp2::hphR leu1</i>	1C	This study
YY186	<i>h⁻ cut7::bleR pk11::natR klp2::hphR leu1 ura4</i>	1D	This study
YY156	<i>h⁺ cut7-GFP-kanR klp2::hphR leu1 ura4 his2</i>	1D	This study
MY844	<i>h⁻ kanR-GFP-<i>alp4</i> cut11-GFP-<i>ura4⁺</i> aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4</i>	1E, 3A-D, 4A-B	This study
YY204	<i>h⁺ cut7::bleR pk11::natR kanR-GFP-<i>alp4</i> cut11-GFP-<i>ura4⁺</i> aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4 his2</i>	1E, 3A-D	This study
YY203	<i>h⁻ cut7::bleR pk11::natR klp2::hphR kanR-GFP-<i>alp4</i> cut11-GFP-<i>ura4⁺</i> aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4</i>	1E, 3A-D	This study
YY209	<i>h⁺ klp2::hphR kanR-GFP-<i>alp4</i> cut11-GFP-<i>ura4⁺</i> aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4 his2</i>	1E, 4A-B	This study
YY207	<i>h⁻ pk11::natR kanR-GFP-<i>alp4</i> cut11-GFP-<i>ura4⁺</i> aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4</i>	1E, 4A-C	This study
YY205	<i>h⁻ pk11::natR klp2::hphR kanR-GFP-<i>alp4</i> cut11-GFP-<i>ura4⁺</i> aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4</i>	1E, 4A-C	This study
YY190	<i>h⁻ kanR-GFP-<i>alp4</i> aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4</i>	2A-E	This study
YY236	<i>h⁻ cut7-22 pk11::natR kanR-GFP-<i>alp4</i> aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4 his2</i>	2A-E	This study
YY238	<i>h⁻ cut7-22 klp2::hphR kanR-GFP-<i>alp4</i> aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4 his2</i>	2A-E	This study
YY240	<i>h⁻ cut7-22 pk11::natR klp2::hphR kanR-GFP-<i>alp4</i> aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4 his2</i>	2A-E	This study
MY858	<i>h⁻ kanR-Palp4-GFP-<i>pk11</i> aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4</i>	5A	This study
MY1030	<i>h⁻ kanR-Palp4-GFP-<i>klp2</i> aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4</i>	5B	This study
MY1028	<i>h⁻ kanR-GBP-6HIS-mCherry-<i>alp4</i> kanR-Palp4-GFP-<i>klp2</i> aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4</i>	5C-D	This study
MY1032	<i>h⁻ kanR-GBP-6HIS-mCherry-<i>alp4</i> kanR-Palp4-GFP-<i>klp2</i> pk11::natR aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4</i>	5C-E	This study
MY1040	<i>h⁻ kanR-Palp4-GFP-<i>klp2</i> cut11-GFP-<i>ura4⁺</i> aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4</i>	5D	This study
MY1042	<i>h⁻ kanR-Palp4-GFP-<i>klp2</i> pk11::natR cut11-GFP-<i>ura4⁺</i> aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4</i>	5D	This study
TK221	<i>h⁻ kanR-GBP-6HIS-mCherry-<i>alp4</i> pk11::natR cut7::bleR aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4 his2</i>	5E	This study
MY1317	<i>h⁻ pcp1-CFP-hphR aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4 [pREP41-GFP-HSET]</i>	6A, 6E-F	This study
MY1379	<i>h⁻ cut7-22 pk11::natR kanR-GFP-<i>alp4</i> aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4 [pREP41-GFP]</i>	6B	This study
MY1383	<i>h⁻ cut7-22 pk11::natR kanR-GFP-<i>alp4</i> aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4 [pREP41-GFP-HSET]</i>	6B	This study
MY1385	<i>h⁻ cut7-22 klp2::hphR kanR-GFP-<i>alp4</i> aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4 [pREP41-GFP]</i>	6B	This study
MY1389	<i>h⁻ cut7-22 klp2::hphR kanR-GFP-<i>alp4</i> aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4 [pREP41-GFP-HSET]</i>	6B	This study
TY170	<i>h⁺ lys1⁻-Pnmt41-GFP-HSET kanR-GBP-6HIS-mCherry-<i>alp4</i> aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4 his2</i>	6C-D	This study
TY174	<i>h⁺ lys1⁻-Pnmt41-GFP-HSET kanR-GBP-6HIS-mCherry-<i>alp4</i> pk11::natR aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4 his2</i>	6C-D	This study
TY168	<i>h⁺ lys1⁻-Pnmt41-GFP-HSET cut11-GFP-<i>ura4⁺</i> aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4 his2</i>	6D	This study
TY158	<i>h⁻ lys1⁻-Pnmt41-GFP-HSET pk11::natR cut11-GFP-<i>ura4⁺</i> aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4</i>	6D	This study
MY1313	<i>h⁻ pcp1-CFP-hphR aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4 [pREP41-GFP]</i>	6E-F	This study
MY1315	<i>h⁻ pcp1-CFP-hphR aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4 [pREP41-GFP-<i>pk11</i>]</i>	6E-F	This study
MY1377	<i>h⁻ pcp1-CFP-hphR aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4 [pREP1-<i>klp2</i>]</i>	6E-F	This study
MA2-3D	<i>h⁻ cut7-23 leu1</i>	S1A-B	Our lab stock
IH136	<i>h⁻ cut7-24 leu1</i>	S1A-B	Our lab stock
MY1403	<i>h⁺ cut7-GFP-kanR cut11-GFP-<i>ura4⁺</i> aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4 his2</i>	S2	This study
MY1405	<i>h⁺ cut7-GFP-kanR cut11-GFP-<i>ura4⁺</i> aur1R-Pnda3-mCherry-<i>atb2</i> pk11::natR leu1 ura4 his2</i>	S2	This study
MY1399	<i>h⁻ cut7-GFP-kanR kanR-GBP-6HIS-mCherry-<i>alp4</i> aur1R-Pnda3-mCherry-<i>atb2</i> leu1 ura4</i>	S2	This study
MY1401	<i>h⁻ cut7-GFP-kanR kanR-GBP-6HIS-mCherry-<i>alp4</i> aur1R-Pnda3-mCherry-<i>atb2</i> pk11::natR leu1 ura4</i>	S2	This study

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

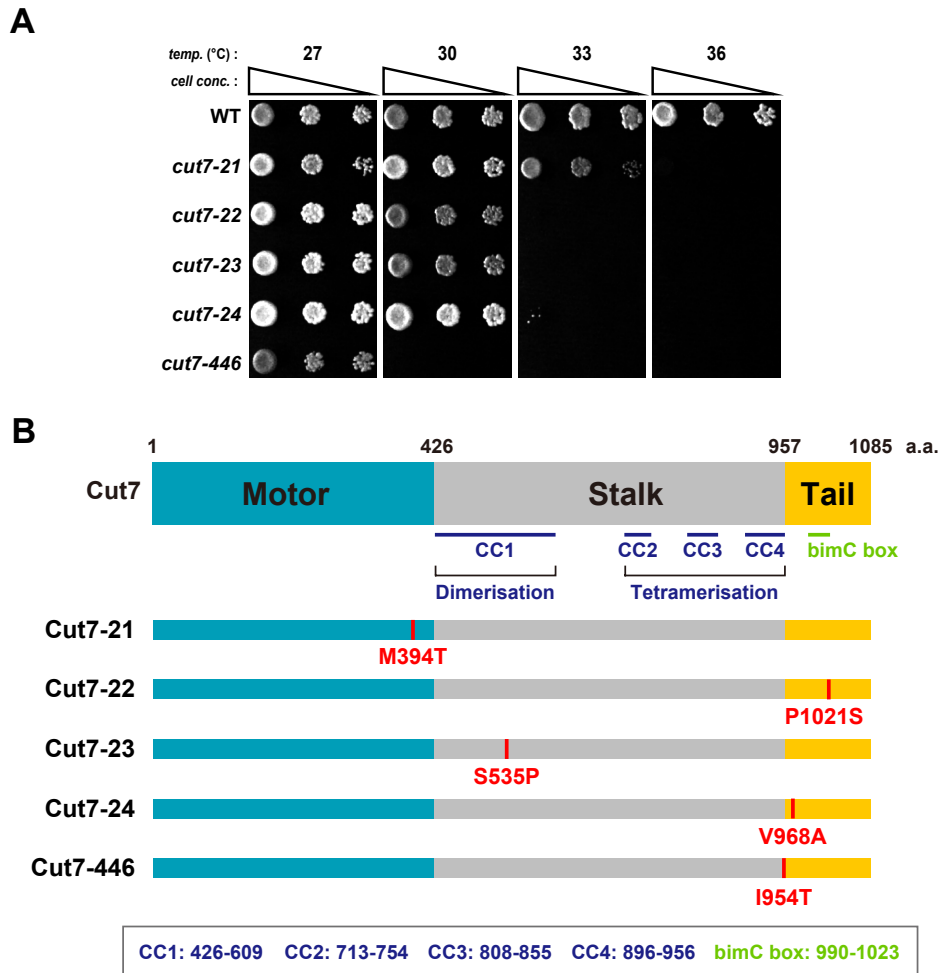


Figure S1. Growth properties of various *cut7* ts strains and their mutation sites

(A) Spot tests. Wild type (top) and indicated *cut7* ts strains were serially (10-fold) diluted, spotted onto rich YE5S plates and incubated at indicated temperatures for 2 d. *cell conc.*, cell concentration, *temp.*, temperature.

(B) Mutation sites in individual *cut7* ts mutants. Overall domain structure of Cut7 is shown at the top. CC, coiled-coil.

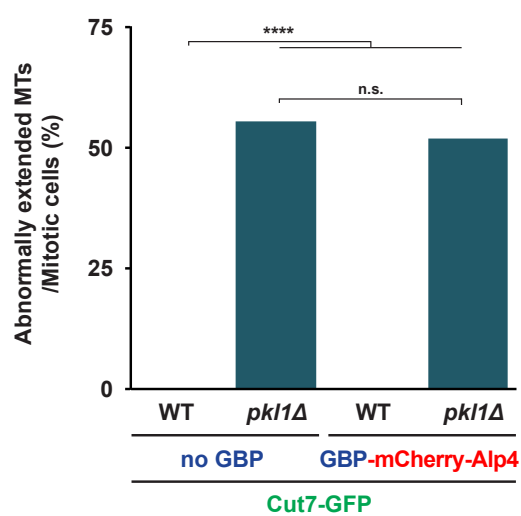


Figure S2. Tethering Cut7 to the SPB in *pk11* deleted cells

Cut7-GFP was tethered to the SPB using GBP-mCherry-Alp4 in *pk11Δ* cells, and spindle anchorage was inspected. >25 mitotic cells were observed in each strain. All p-values are derived from a two-tailed χ^2 test (****, $P < 0.0001$; n.s., not significant).