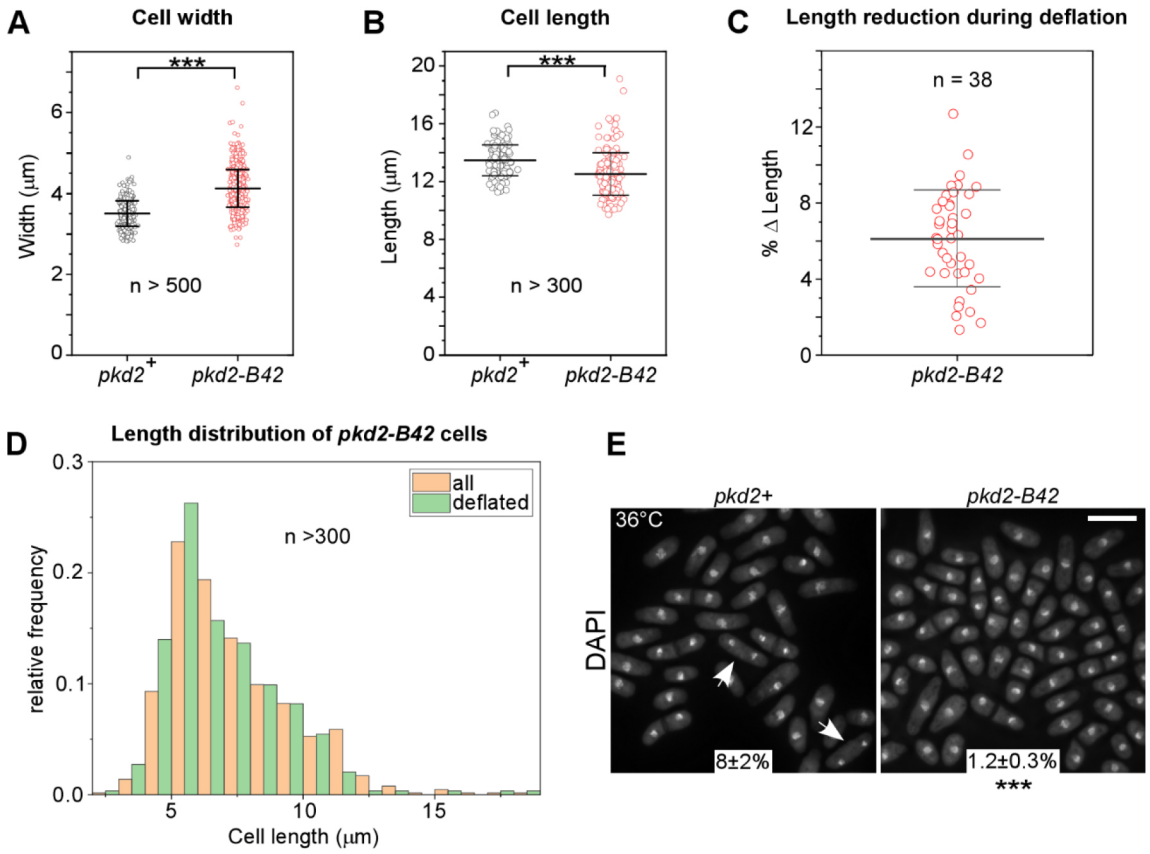


Fig. S1. Morphogenesis of the temperature-sensitive mutant *pkd2-B42*. Related to Figure 1 and 2.

(A–B) Dot plots of the width **(A)** and length **(B)** of *pkd2-B42* cells at 36°C, compared to the wild-type (*pkd2+*) n>500 and n>300 respectively. Lines and error bar represents average ± standard deviations **(C)** Dot plot showing reduction in cell length during deflation of *pkd2-B42* cells n=38. **(D)** Histogram showing cell length distribution of *pkd2-B42* cells. Green: deflated *pkd2* mutant cells. Orange: all *pkd2-b42* cells. n>300. **(E)** Fluorescence micrographs of either wild-type (left) and *pkd2-B42* (right) cells at 36°C, fixed and stained with DAPI. Arrow: mitotic cell with condensed nuclei. Number: percentage of mitotic cells (average ± standard deviations, n>500). The data were pooled from at least two independent biological repeats. Scale bar represents 10 μm. ***: P<0.001(Two-tailed student t-test).

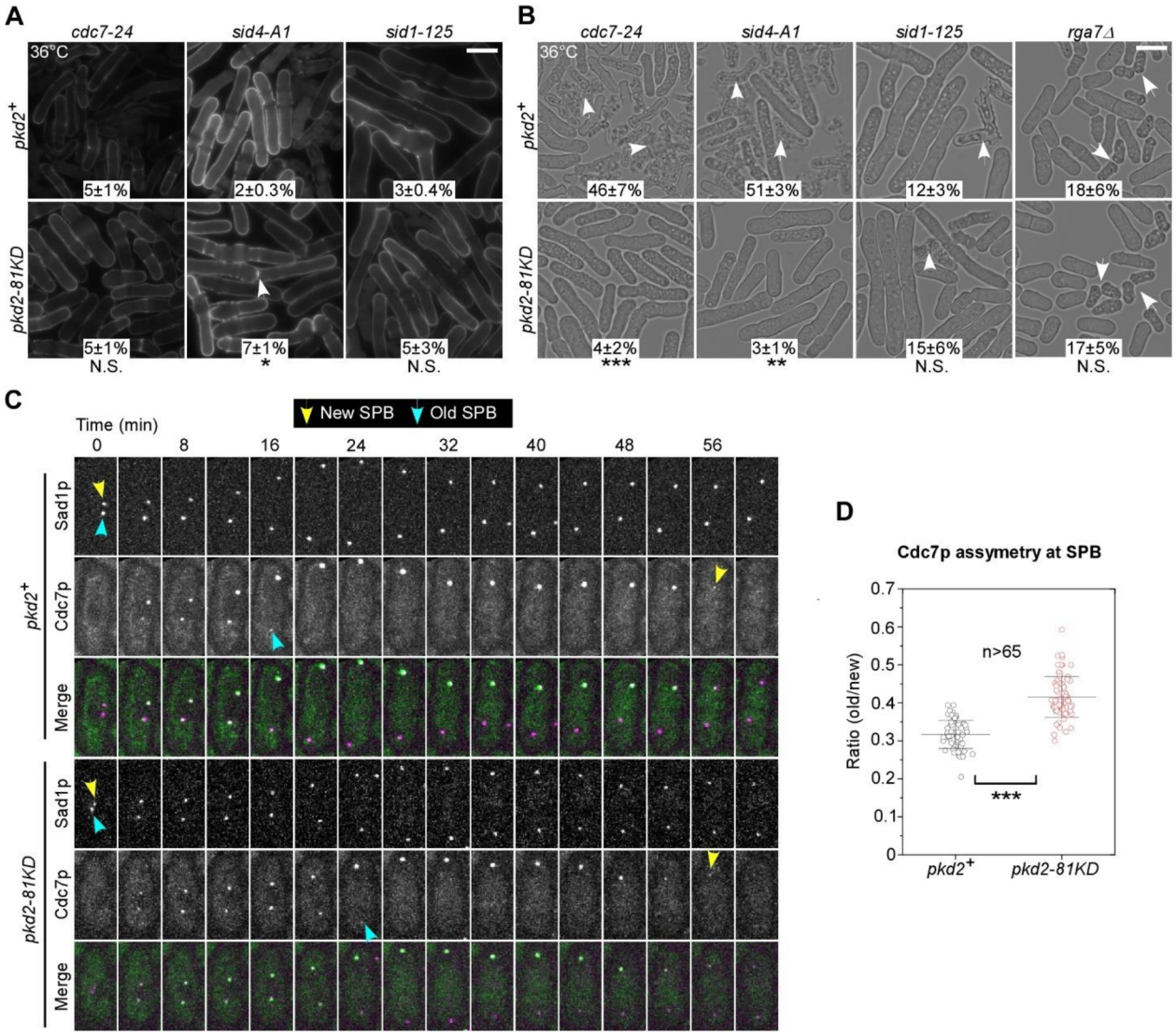


Fig. S2. SIN activities in *pkd2* mutant cells. Related to Figure 6

(A–B) Micrographs of fixed SIN (top), SIN *pkd2-81KD* (bottom), *rga7Δ* (top) *rga7Δ pkd2-81KD* (bottom) cells at 36°C. **(A)** Calcofluor stained cells. Arrow: septated cell. **(B)** Arrow: lysed cell. Number: percentages of either septated **(A)** or lysed cells **(B) (**average ± standard deviation, n > 400). (**C–D)** Asymmetric distribution of Cdc7p-GFP at two SPBs during cell division. **(C)** Time-lapse micrographs of either a wild-type or a *pkd2-81KD* expressing both Cdc7p-GFP and SPB marker Sad1-mCherry. Number represents time in mins after the SPB separation. Arrow: new (yellow) or old (blue) SPB. **(D)** Dot plots of ratio (old:new) of the dwelling time of Cdc7p-GFP at the two SPBs, n > 65. Lines and error bar represents average ± standard. The data were pooled from at least two independent biological repeats. Scale bar represents 10 μm. *:P<0.05, **:P<0.01, ***: P<0.001, NS: Not significant (Two-tailed student t-test).

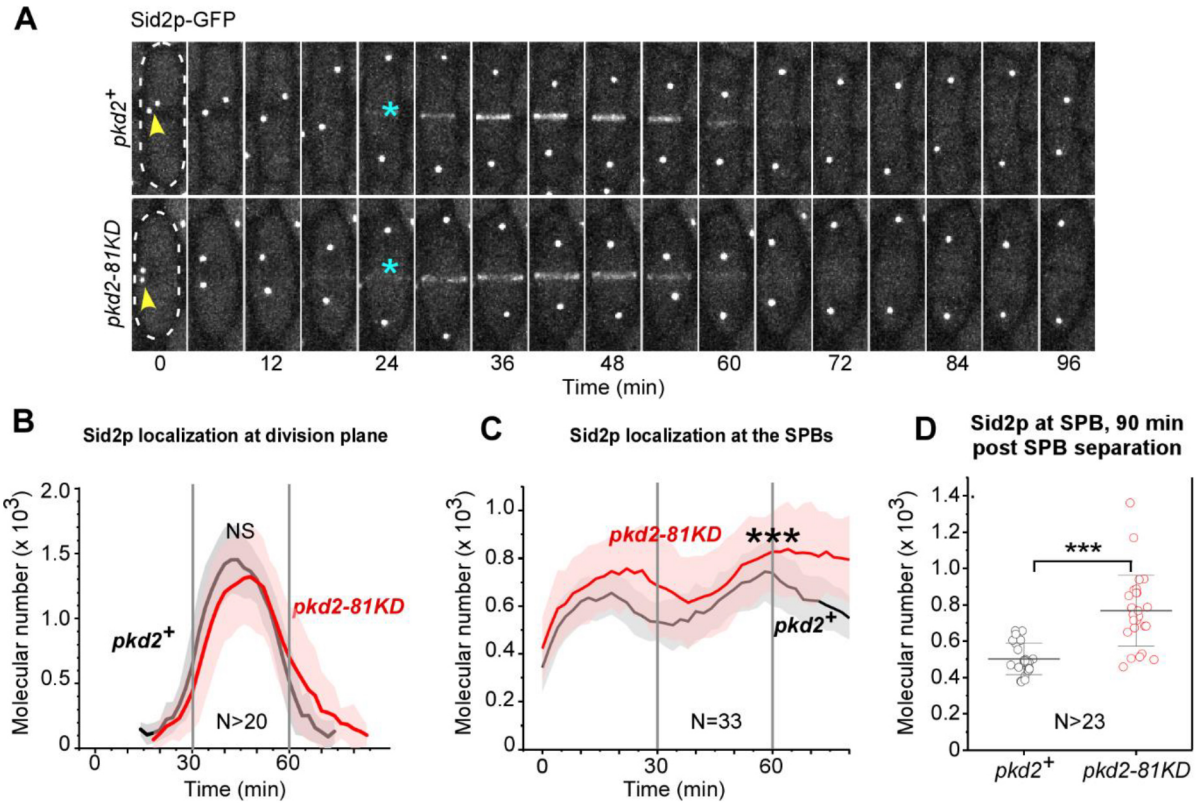


Fig. S3. SIN protein localization in *pkd2-81KD* cells during cytokinesis. Related to Figure 7

(A) Time-lapse micrographs of either a wild-type (top) or a *pkd2* mutant (bottom) cell expressing Sid2p-GFP. Number: time in mins after SPB separation. Interval = 6 min. Arrowhead: SPB. Asterisk: appearance of Sid2p-GFP at the division plane. **(B–C)** Average time-courses of the number of Sid2p-GFP molecules in the **(B)** division plane or **(C)** SPBs of either wild-type (black line) or the *pkd2-81KD* cells (red line), n>20 and n=33 for **(B)** and **(C)** respectively. Cloud represents standard deviation. **(D)** Dot plot showing number of Sid2 molecules at 90 min after SPB separation in wild-type or the *pkd2-81KD* cells (red line), n>23. Lines and error bar represents average ± standard The data was pooled from two independent biological repeats. ***: P < 0.001, NS: Not significant (Two-tailed student t-test).

Table S1. List of the missense mutations identified in *pkd2-B42*

Residue	Position in the transmembrane helices	Mutation	Amino acid change
335	3rd	F → S	Hydrophobic to Polar Uncharged
408	5th	S → P	Polar Uncharged to Polar Uncharged
521	Between 7th and 8th	N → D	Polar Uncharged to Negatively Charged
530	8th	M → V	Hydrophobic to Hydrophobic
534	8th	S → G	Polar Uncharged to Hydrophobic
543	8th	Q → E	Polar Uncharged to Negatively Charged
555	9th	I → T	Hydrophobic to Polar Uncharged
571	9th	I → T	Hydrophobic to Polar Uncharged

Table S2. Young's Elasticity Modulus of cellular turgor pressure from Hertz modeling

	26°C			37°C		
	pkd2+	*pkd2-81KD*	*pkd2-B42*	*pkd2+*	*pkd2-B42 (ND)*	*pkd2-B42 (D)*
Young's Elasticity Modulus (MPa)	1.3 ± 0.4	0.6 ± 0.3	0.6 ± 0.3	1.2 ± 0.4	0.6 ± 0.3	0.3 ± 0.2

Note: n > 5 cells evaluated for each condition

Abbreviations: MPa, Megapascal. ND, not deflated. D, deflated.

Table S3. Genetic interactions between *pkd2-81KD* and other cytokinetic mutants

Gene	Mutant	Genetic interactions
ace2	*ace2Δ*	-
agn1	*agn1Δ*	*No*
ags1	*mok1-664*	*No*
bgs4	*cwg1-2*	*No*
cdc11	*cdc11-123*	*No*
cdc14	*cdc14-118*	++
cdc16	*cdc16-116*	--
cdc42	*cdc42-1625*	*No*
cdc7	*cdc7-24*	*No*
eng1	*eng1Δ*	*No*
exo70	*exo70Δ*	-
mid1	*mid1Δ*	*No*
mid2	*mid2Δ*	*No*
mor2	*mor-282*	--
myp2	*myp2Δ*	*No*
orb6	*orb6-25*	--
rga7	*rga7Δ*	*No*
rgf1	*rgf1Δ*	*No*
rgf2	*rgf2Δ*	*No*
sid1	*sid1-125*	*No*
sid4	*sid4-A1*	+
spg1	*spg1-106*	++
sty1	*sty1Δ*	*No*

“++” or “+”: strong or weak positive interactions. “--" or “- “: strong or weak negative interactions. No: no interactions. Genetic interactions with the other 11 cytokinesis mutants were published in an earlier study (Morris et al., 2019).

Table S4. List of the primers used in the screen for *pkd2-B42*

Number	Sequence
QC-P596	*AGAGTCGAATTTTATTGATG*
QC-P597	*ATGCCGCATAGTTAAGCCAG*
QC-P598	*GTCGTTCTTTTCCTGACATA*
QC-P599	*TATGTCAGGAAAAGAACGAC*
QC-P600	*CTGAAGTCCCAAGCACGAAG*
QC-P601	*CTTCGTGCTTGGGACTTCAG*
QC-P602	*ACGTTTATTAACATTTTATTGAAACAATCTATAGACACCGGTAAGAATAAATCCATAAGCCA TTCCCAAA-GGAAATAGTAAGGCTAGTAG*
QC-P603	*GAATATATGCCTATTCGCAATCTAGAATTCCTTTGAATACACCCAATTACAAGCTTAAACGA TTCGGTAT-TAAAAATAGGCGTATCACGAGG*
QC-P604	*AGCATATTTGTTGGATGTGC*
QC-P605	*AA-CGATCG-ATGAGGCTTTGGAGAAGCCCAC*
QC-P606	*AA-GGATCC-TTTGGGAATGGCTTATGGATTTATTC*
QC-P607	*GACGAAGCTCTTTCTAGAAGCGTAGT*

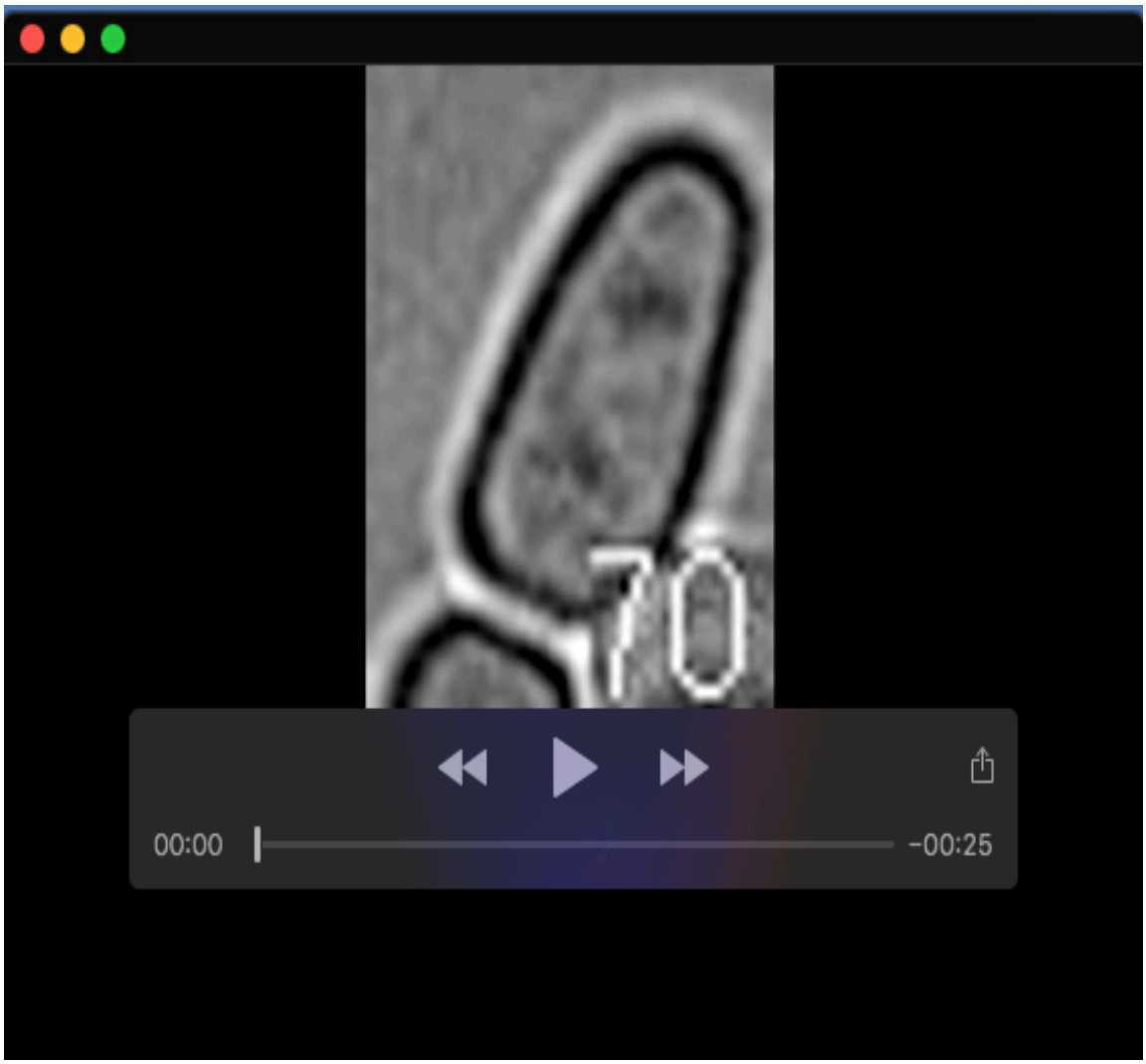
Table S5. List of fission yeast strains

Strains number	Genotype		Source
FY13846	*h-*	*cps212-282 or mor2-282*	(CP28-2) NBRP Japan
FY38515	*h-*	*his5+:pact1:CRIB[gic2aa1-181]-3mCherry:bsdMX*	(AV2324) NBRP Japan
FY527	*h-*	*leu1-32 ura4-D18 his3-D1 ade6-M216*	Lab stock
FY528	*h+*	*leu1-32 ura4-D18 his3-D1 ade6-M210*	Lab stock
JW7551	*h?*	*cwg1-2 leu1-32*	Jian Wu
JW7572	*h-*	*mok1-664 leu1-32 ura4-D18*	Jian Wu
MBY6218	*h-*	*ade5D ade7::Ade5 his5D leu1-32 ura4D-18*	Mohan Balasubramanian
PPG5660	*h+*	*leu1-32 ura4-D18 cdc42-1425::kanMX6*	Pilar Perez
QC-Y569	*h-*	*cdc11-123 ura4-D18*	Lab stock
QC-Y570	*h-*	*sid4-A1 ura4-D18 leu1-32*	Lab stock
QC-Y571	*h+*	*spg1-106 ura4-D18 leu1-32 ade-210*	Lab stock
QC-Y573	*h+*	*cdc16-116 ura4-D18 leu1-32 ade-M210*	Lab stock
QC-Y574	*h-*	*cdc7-24 ura4-D18 leu1-32 ade-M21X*	Lab stock
QC-Y576	*h+*	*sid2-250 ura4-D18 ade-M21X leu1-32*	Lab stock
QC-Y577	*h-*	*mob1-R4 leu1+ ura4-D18*	Lab stock
QC-Y624	*h+*	*cdc14-118 ura4-D18 ade-216*	Lab stock
QC-Y670	*h-*	*myp2::kanMX6 leu1-32 ura4-D18*	Lab stock
QC-Y675	*h-*	*mob1-mEGFP-kanMX6*	Lab stock
QC-Y676	*h-*	*sid2-mEGFP-kanMX6*	Lab stock
QC-Y723	*h+*	*sid1-125 ura4-D18 leu1-32 ade6-210*	Lab stock
QC-Y725	*h+*	*ace2::kanR ura4-D18 ade6-216 leu1-32 h+*	Lab stock
QC-Y743	*h+*	*far8::kanMX6 ade-M216 ura4-D18 leu1-32*	Bioneer
QC-Y803	*h-*	*orb6-25 leu1-32 ade-M21X*	Lab stock
QC-Y810	*h-*	*KanMX6-P81nmt1-pkd2 leu1-32 ura4-D18 his3-D1 ade6-M21X*	Lab stock
QC-Y817	*h+*	*kanMX6-81xnmt1-pkd2 leu1-32 ura4-D18 his3-D1 ade6-M21X*	Lab stock
QC-Y825	*h-*	*kanMX6-81xnmt1-Pkd2 sid2-250 ura4-D18 ade-M21X leu1-32*	Lab stock
QC-Y874	*h-*	*sty1::kanMX6*	Lab stock
QC-Y930	*h?*	*kanMX6-81xnmt1-Pkd2 sid2-mEGFP-kanMX6*	This study

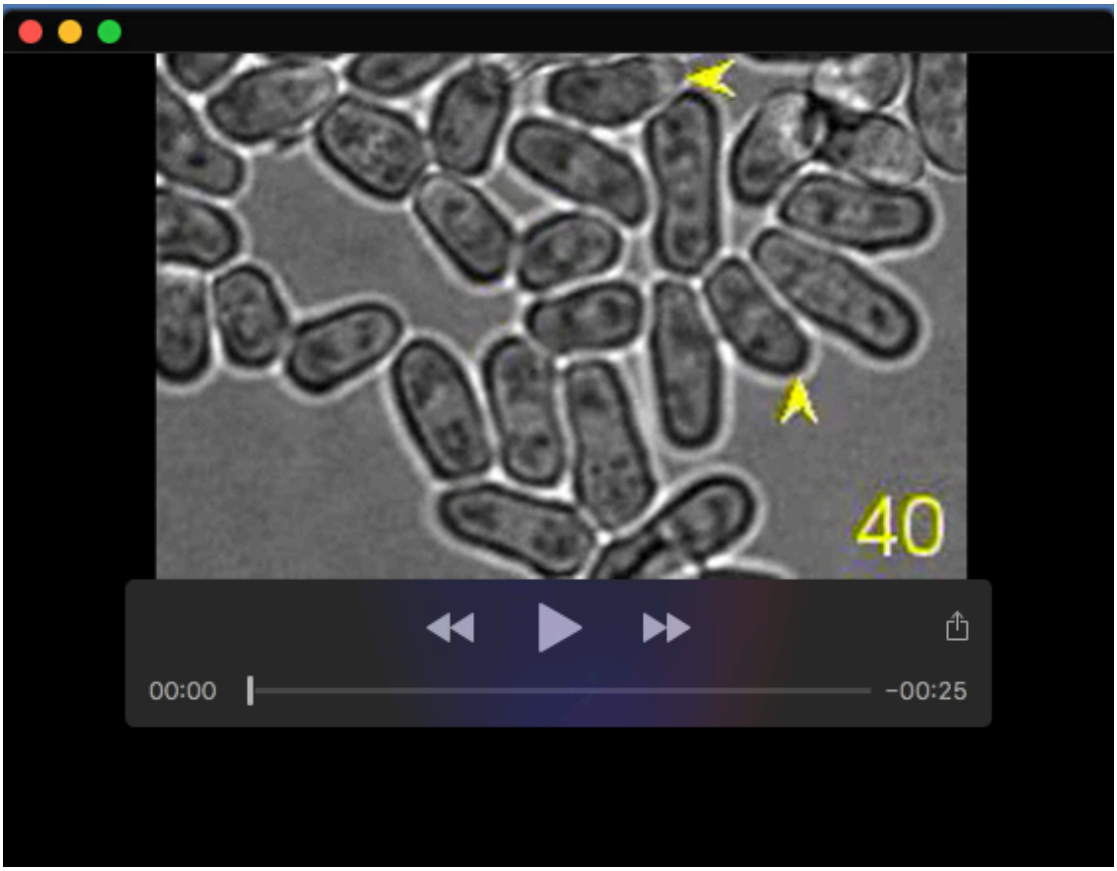
QC-Y933	*h?*	*kanMX6-81xnmt1-Pkd2 mob1-mEGFP-kanMX6*	This study
QC-Y976	*h?*	*kanMX6-81xnmt1-pkd2 cdc16-116 ura4-D18 leu1-32 his3-D1 ade-M210*	This study
QC-Y977	*h?*	*kanMX6-81xnmt1-pkd2 cdc14-118 ura4-D18 leu1-32 his3-D1 ade-216*	This study
QC-Y978	*h?*	*kanMX6-81xnmt1-pkd2 cdc11-123 ura4-D18*	This study
QC-Y979	*h?*	*kanMX6-81xnmt1-pkd2 spg1-106 ura4-D18 leu1-32*	This study
QC-Y980	*h?*	*kanMX6-81xnmt1-pkd2 sid4-A1 ura4-D18 leu1-32*	This study
QC-Y983	*h?*	*kanMX6-81xnmt1-pkd2 cdc7-24 ura4-D18 leu1-32 ade-M21X*	This study
QC-Y999	*h-*	*pkd2-3'UTR-his5CterD-ura4*	This study
QC-Y1010	*h+*	*mid1::kanMX6 ade-M216 ura4-D18 leu1-32*	Bioneer
QC-Y1011	*h+*	*mid2::kanMX6 ade-M216 ura4-D18 leu1-32*	Bioneer
QC-Y1012	*h+*	*rga7::kanMX6 ade-M216 ura4-D18 leu1-32*	Bioneer
QC-Y1013	*h+*	*rgf1::kanMX6 ade-M216 ura4-D18 leu1-32*	Bioneer
QC-Y1017	*h?*	*kanMX6-81xnmt1-pkd2 myp2::kanMX6 ade-M21X ura4-D18 leu1-32*	This study
QC-Y1022	*h?*	*kanMX6-81xnmt1-pkd2 mid1::kanMX6 ade-M21X ura4-D18 leu1-32*	This study
QC-Y1023	*h?*	*kanMX6-81xnmt1-pkd2 mid2::kanMX6 ade-M21X ura4-D18 leu1-32*	This study
QC-Y1024	*h?*	*kanMX6-81xnmt1-pkd2 rga7::kanMX6 ade-M21X ura4-D18 leu1-32*	This study
QC-Y1025	*h?*	*kanMX6-81xnmt1-pkd2 rgf1::kanMX6 ade-M21X ura4-D18 leu1-32*	This study
QC-Y1031	*h-*	*pkd2::pkd2-B42-ura4+-his5+ leu1-32*	This study
QC-Y1032	*h+*	*pkd2::pkd2-B42-ura4+-hist5+ leu1-32*	This study
QC-Y1032	*h+*	*pkd2::pkd2-B42-ura4+-hist5+ leu1-32*	This study
QC-Y1036	*h?*	*pkd2::pkd2-B42-ura4+-his5+ leu1-32 ade-M21X sid2-250*	This study
QC-Y1037	*h?*	*pkd2::pkd2-B42-ura4+-his5+ mob1-R4 leu1+*	This study
QC-Y1061	*h+*	*eng1:: KanMx6 ade-M216 ura4-D18 leu1-32*	Bioneer
QC-Y1062	*h+*	*rgf2::kanMX6 ade-M21X ura4-D18 leu1-32*	Bioneer
QC-Y1070	*h-*	*ura4::Pact-sfGFfP-ura4 leu1-32 his3-D1 ade6-M216*	This study
QC-Y1072	*h?*	*pkd2::pkd2-B42-ura4+-his5+ leu1-32 far8::kanMX6*	This study
QC-Y1073	*h?*	*kanMX6-81xnmt1-pkd2 far8::kanMX6 ade-M216 ura4-D18 leu1-32*	This study
QC-Y1081	*h?*	*eng1:: KanMx6 pkd2::pkd2-B42-ura4+-his5+ leu1-32*	This study
QC-Y1082	*h?*	*rgf2:: KanMx6 pkd2::pkd2-B42-ura4+-his5+ leu1-32*	This study
QC-Y1085	*h?*	*eng1:: KanMx6 kanMX6-81xnmt1-pkd2 ade-M21X ura4-D18 leu1-32*	This study

QC-Y1093	*h?*	*pkd2::pkd2-B42-ura4+-his5+ leu1-32 mok1-664*	This study
QC-Y1096	*h?*	*mok1-664 KanMX6-81xnmt1-pkd2 leu1-32 ura4-D18*	This study
QC-Y1108	*h?*	*cwg1-2 pkd2::pkd2-B42-ura4+-his5+ leu1-32*	This study
QC-Y1109	*h?*	*cwg1-2 leu1-32 kanMX6-81xnmt1-pkd2*	This study
QC-Y1162	*h+*	*agn1::kanMX6 ade-M216 ura4-D18 leu1-32*	Bioneer
QC-Y1168	*h?*	*KanMX6-81xnmt1-pkd2 sid1-125 ura4-D18 leu1-32 ade6-210*	This study
QC-Y1178	*h?*	*KanMX6-81xnmt1-pkd2 ace2::kanR*	This study
QC-Y1181	*h?*	*agn1::kanMX6 pkd2::pkd2-B42-ura4+-his5+ leu1-32*	This study
QC-Y1190	*h?*	*agn1::kanMX6 kanMX6-81xnmt1 pkd2 ura4-D18 leu1-32 ade6-210*	This study
QC-Y1198	*h?*	*orb6-25 pkd2::pkd2-B42-ura4+-his5+ leu1-32 ade-M21X*	This study
QC-Y1227	*h?*	*pkd2::pkd2-B42-ura4+-his5+ leu1-32 his5+:pact1:CRIB[gic2aa1-181]-3mCherry:bsdMX*	This study
QC-Y1233	h?	*ura4::Pact1-pkd2-sfGFP-ura4+ orb6-25 leu1-32 ade-M21X*	This study
QC-Y1250	*h+*	*mor2-282 (cps12-282) pkd2::pkd2-B42-ura4+-his5+ leu1-32*	This study
QC-Y1254	*h?*	*exo70Δ::kanMX6*	This study
QC-Y1256	*h?*	*pkd2::pkd2-B42-ura4+-his5+ leu1-32 exo70Δ::kanMX6*	This study
QC-Y1274	*h?*	*pkd2::pkd2-B42-ura4+-his5+ leu1-32 ade-M21X spg1-106*	This study
Qc-Y1307	*h?*	*cdc42-1625 pkd2::pkd2-B42-ura4+-his5+ leu1-32*	This study
QC-Y1317	h?	*pkd2::pkd2-B42-ura4+-his5+ leu1-32 ura4::Pact-sfGFfP-ura4*	This study
QC-Y1352	*h?*	*sty1::kanMX6 pkd2::pkd2-B42-ura4+-hist5+ leu1-32*	This study
QC-Y1408	h?	*cdc7-GFP::ura4 Sad1-mCherry-NatMX6*	This study
QC-Y1409	h?	*kanMX6-81xnmt1-pkd2 leu1-32 cdc7-GFP::ura4 Sad1-mCherry-NatMX6*	This study

Movie 1. Time-lapse series of a expanding wild-type cell at 37°C. Number represents time in minutes after the preceding cell separation**.**



Movie 2. Time-lapse series of a expanding *pkd2-B42* mutant cell at 37°C. Number represents time in minutes after the preceding cell separation.



Movie 3. Time-lapse series of *pkd2-B42* cells at 37°C. Arrow: The deflating cells. Number represents time in minutes.