SUPPLEMENTAL INFORMATION

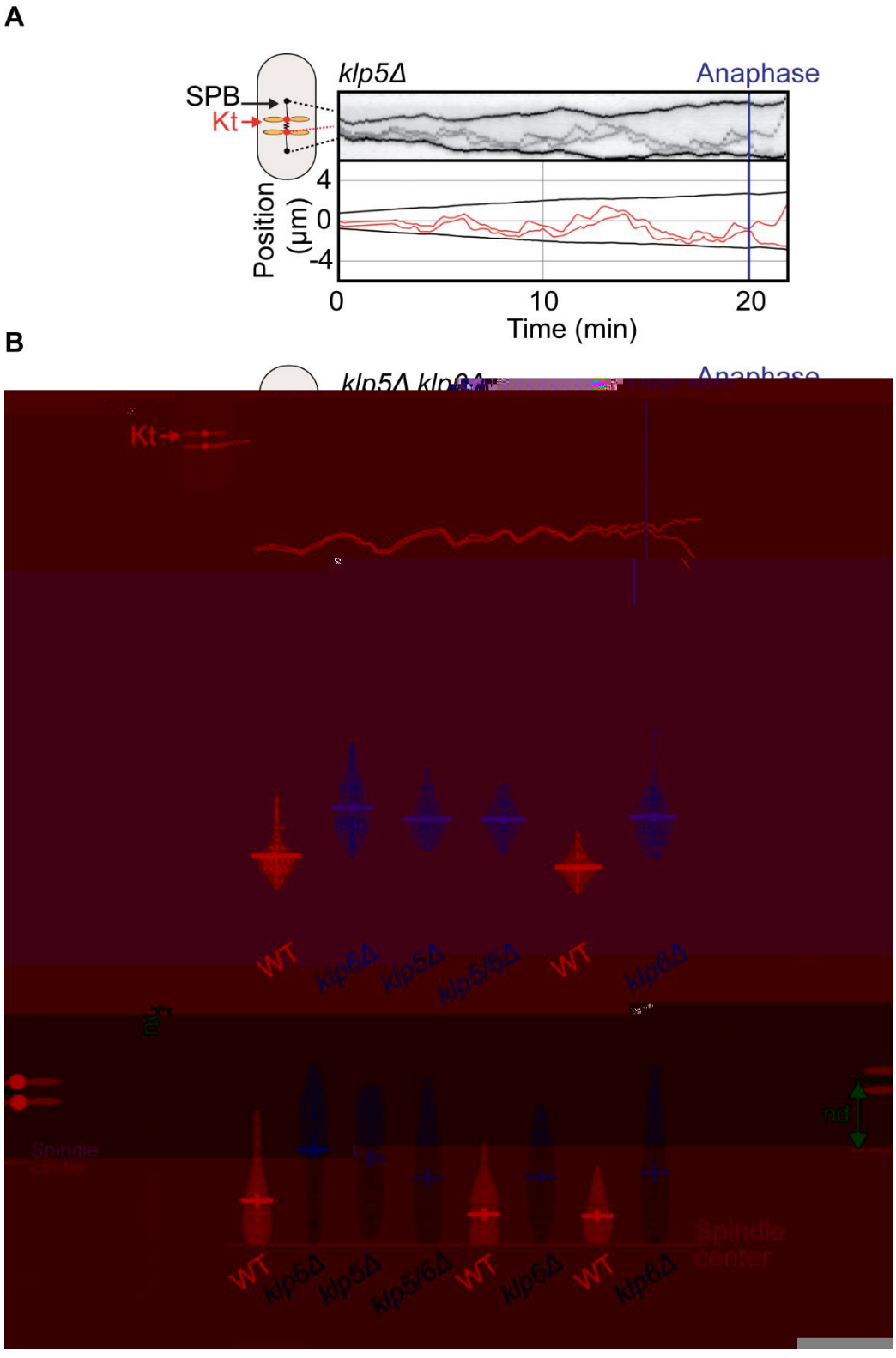

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Figure S1. *klp5* or double mutant *klp5*Δ *klp6* are deficient for chromosome centering.

(A-B) Typical time-lapse fluorescent images of *klp5*Δ or *klp5*Δ *klp6*! double mutant expressing Cen2-gfp (centromeric region of chromosome 2) and Cdc11-gfp (SPBs) during metaphase and anaphase. !t =10 s. The lower panel is showing the corresponding trajectories of Cen2 (red) and SPBs (black) projected on a 1-D axis whose origin is the spindle center. **(C)** Spindle size at anaphase onset for various cell type: wild type (n=52, with TBZ n=21), *klp6Δ* (n=63, with TBZ, n=36), *klp5Δ* (n=30), *klp5/6Δ* (n=21). **(D)** Global distribution of the relative distances between Cen2 to the spindle center at anaphase onset in wild type (n=52), *klp6Δ* cells (n=63), *klp5Δ* cells (n=30), *klp5/6Δ* cells (n=21), wild type cells with low dose of TBZ (n=21), *klp6Δ* cells with low dose of TBZ (n=36), *cdc25-22* cells (n=19) and *cdc25-22 klp6*Δ *cells* (n=26). Each distance d between sister kinetochores to the spindle center is normalized according to spindle size so that sister kinetochore position varies between 0 (spindle center) to 0.5 (poles).

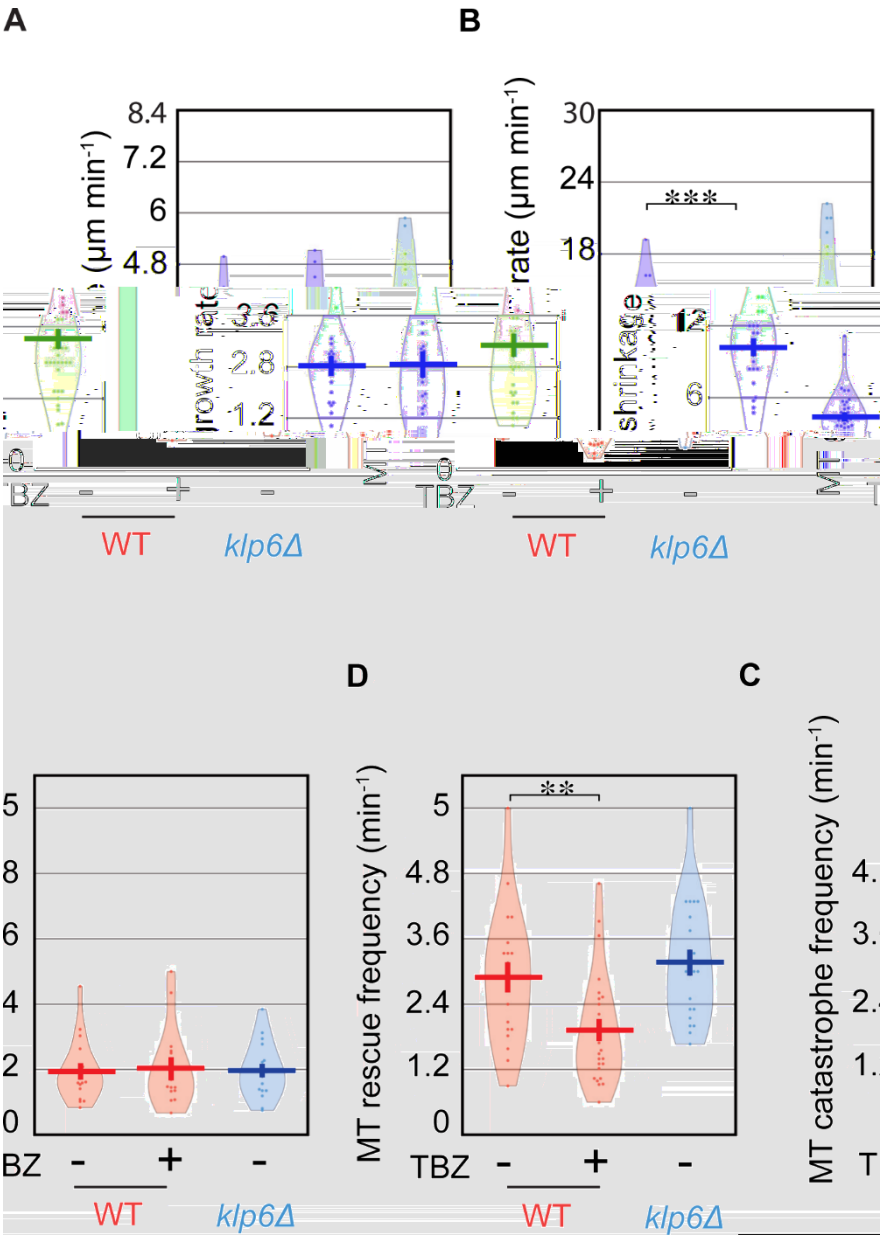


Figure S2. Analysis of microtubule dynamics in the presence or absence of TBZ.

(A). Microtubule growth rate for wild type (n=22, with TBZ n=19) and *klp6Δ* (n=27) cells. **(B)**. Microtubule shrinkage rate for wild type (n=29, with TBZ n=33) and *klp6Δ* (n=37) cells. **(C)**. Microtubule catastrophe frequency for wild type (n=19, with TBZ n=15) and *klp6Δ* (n=17) cells. **(D)**. Microtubule rescue frequency for wild type (n=20, with TBZ n=26) and *klp6Δ* (n=21) cells.

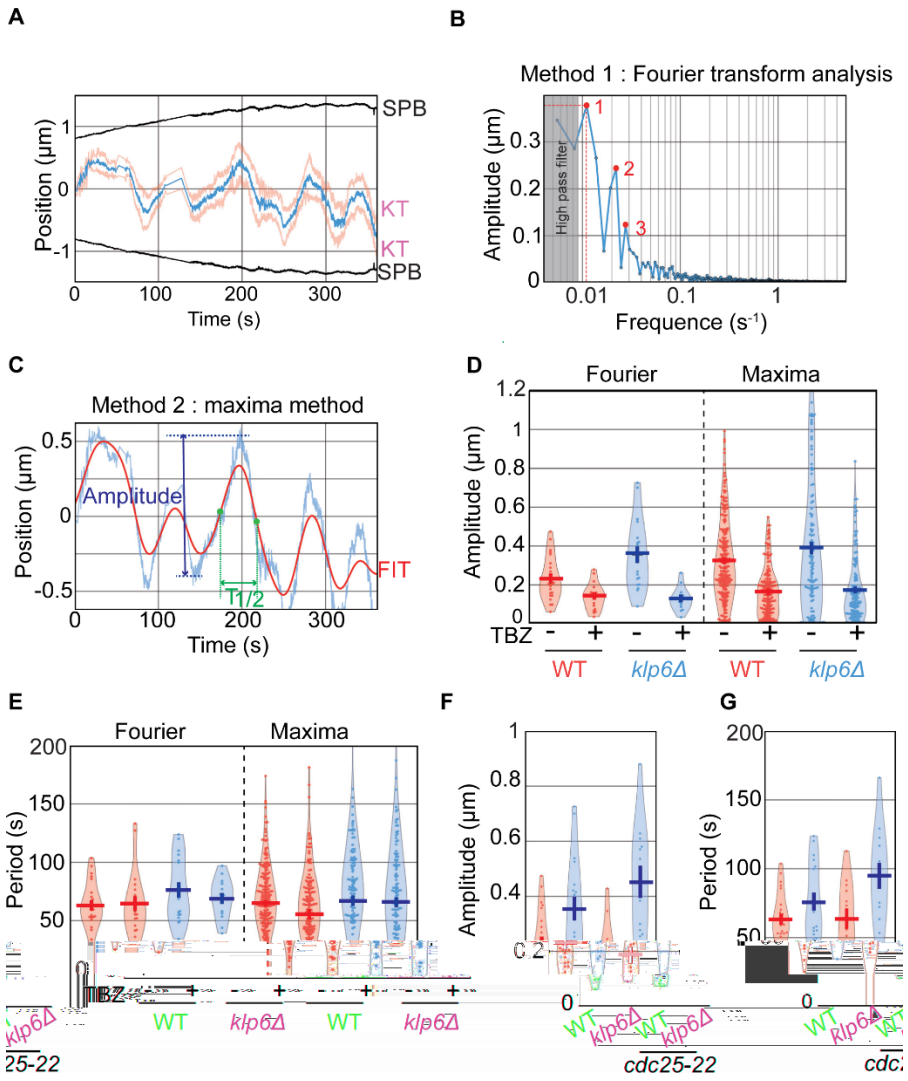
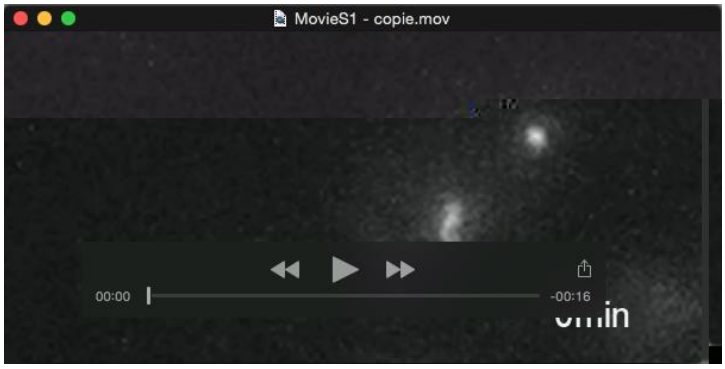


Figure S3. Characterization of kinetochore oscillation periods and amplitudes.

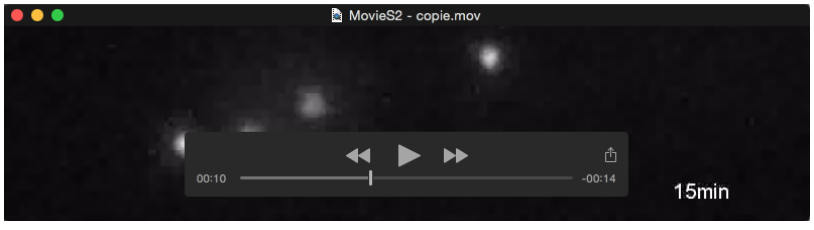
(A). Kinetochore (Cen2) trajectories used to illustrate oscillation analysis (frames were taken every 0.1 s). The blue line represents the middle of the Cen2 spots (shown in red). **(B)** Fourier transform analysis of kinetochore trajectories. The peak of highest amplitude is identified in the Fourier spectrum of each kinetochore trajectories obtained from high frame rate experiments. The corresponding amplitudes are reported as a function of the half periods of oscillations. **(C)** Position of the middle of Cen2 spots according to time (blue). This trajectory is interpolated by a spline function (red). The local maxima of the interpolated curve are identified and used to determine half periods ($T_{1/2}$, horizontal arrow) and amplitudes (A, vertical arrow) in kinetochore trajectories. **(D-E)**. Amplitude and period comparison between the two methods (see B and C) in various cell lines (wild type and *klp6Δ*) and different conditions (presence or absence of low doses of TBZ). Note that the two methods reproduce qualitatively but not quantitatively the differences between wild type or mutant cells. **(F-G)**. Amplitudes and periods in wild type and *klp6Δ* cells in control or *cdc25-22* background. Amplitudes and periods are not significantly different when cells are elongated in a *cdc25-22* background. Amplitude: wild type (n=24, *cdc25-22* n=13), *klp6Δ* (n=18, *cdc25-22* n=13). Period: wild type (n=24, *cdc25-22* n=13), *klp6Δ* (n=18, *cdc25-22* n=13).

α		
α	μ	
β		
	μ	
	μ	
	μ	

	cdc11-gfp::kr cen2(D107)-kanR-ura4+-lacO his7+-lacI-gfp-gfp	
	cdc11-gfp::kr cen2(D107)-kanR-ura4+-lacO his7+-lacI-gfp-gfp klp5::ura4	
	cdc11-gfp-kr cen2(D107)-kanr-ura4+-lacO_his7+-lacI-gfp-gfp cdc25-22	
	cdc11-gfp-kr cen2(D107)-kanr-ura4+-lacO_his7+-lacI-gfp-gfp cdc25-22 klp6::his3	
	h- klp5-GFP::kr leu1-32 ura4-D18 pPT77 nmt1-ura4-mRFP-atb2	
	klp5-GFP cdc25-22 ndc80-CFP-kr cdc11-CFP-kr	
	cdc11-gfp-kr cen2(D107)-kanR-ura4+-lacO _ his7+-lacI-gfp-gfp klp6::his3 mad2mcherry:CLONAT	
	ndc80-GFP:kr cdc11-CPF:kr	
	ndc80-GFP:kr cdc11-CPF:kr klp6::his3	
	cdc11-gfp-kr cen2(D107)-kanR-ura4+-lacO _ his7+-lacI-gfp-gfp klp5::ura4	
	cdc11-gfp-kr cen2(D107)-kanR-ura4+-lacO _ his7+-lacI-gfp-gfp klp5::ura4 klp6:his3	



Movie 1. Fluorescent time-lapse imaging of centromere 2 (Cen2-gfp) and spindle pole (Cdc11-gfp) dynamics in a wild type fission yeast cell. Frames were taken every 10 s.



Movie 2. Fluorescent time-lapse imaging of centromere 2 (Cen2-gfp) and spindle pole (Cdc11-gfp) dynamics in a *klp6Δ* cell. Frames were taken every 10 s.

Movie 3. Fluorescent time-lapse imaging of tubulin (Atb2-rfp) and Kinesin-8 (Klp5-gfp) during metaphase showing Klp5 accumulation at the tip of an intranuclear microtubule bundle. Frames were taken every 5 s.

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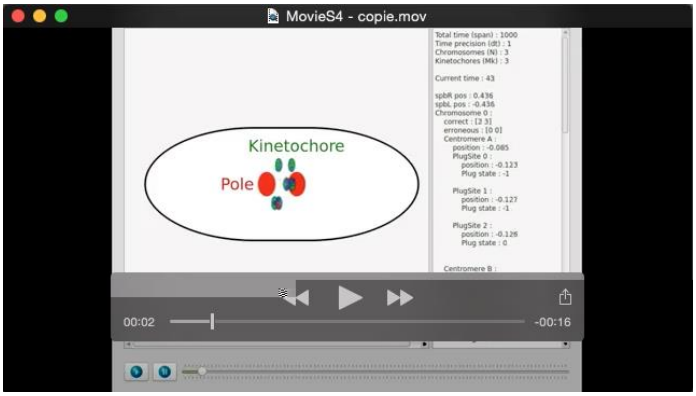

Movie 4. Cartoon recapitulating a typical simulation calculated with the force balance model of the mitotic spindle. The position of spindle poles and kinetochores are shown, as well as the attachment state of each attachment site (green = correct attachment, red = incorrect attachment, blue = unattached).