Supplementary Material

Sarah Young et al. doi: 10.1242/bio.201410363



Fig. S1. Regulators of microtubule ends influence spindle metaphase length

differentially. (A) Images of U2OS cells depleted with indicated siRNA and treated with STLC. (B) Bar graph showing the quantified average microtubule length for microtubules radiating from the centrosome for depletions in panel A (mean ± SD). (C) Average spindle length and SD at steady-state of U2OS cells expressing mCherry-tubulin treated with the indicated siRNA. (D) Table representing the number of cells and experiments performed monitoring spindle elongation. (E) Western blot showing levels of Kid and Clasp2 after Kid/ Clasp2 co-depletion for 48 hours. (F) Graph representing the average spindle length and SD during elongation for each indicated condition.



Fig. S2. Pairwise comparison of the dynamics of spindle assembly. Pairwise comparison graphs representing the average spindle length (A,C,E,G,I) and width (B,D,F,H,J) and the corresponding SD, of U2OS cells expressing mCherry-tubulin during spindle elongation after STLC washout. Cells were treated with the indicated siRNA for 48 hours before imaging. (K,L) Representative graphs for the individual spindle elongation profiles for each cell, aligned with respect to the initiation of spindle elongation for Clasp2 and Clasp2/Nuf2 depleted cells.



Fig. S3. Dynamics of spindle contraction for CLASP depletions. Graph representing the average spindle length and the corresponding SD as a function of time of Clasp1, Clasp2 and Clasp1+2 depleted U2OS cells expressing mCherry-tubulin during spindle elongation after STLC washout. Individual spindle elongation profiles were aligned with respect to the time at which spindles reached their maximum length.



Fig. S4. Photoactivation analysis process of a representative cell depleted with Control siRNA for 48 hours. (A,B) Raw intensity profile computed by a Radon transform alongside the centrosome-centrosome axis at times (A) t=0 s and (B) t=290 s. Blue line corresponds to the Radon transform of the background. (C) Heat map of the background subtracted intensity profile as a function of time. (D) Overlay of the background-subtracted intensity profiles as a function of the centrosome-centrosome distance for all measured times. (E) Fit of the background subtracted intensity profile by a 1D Gaussian mixture model at t=0 s and t=290 s. The blue Gaussian fit corresponds to the photoactivated spindle. (F) Evolution of the relative distance of the center of the photoactivated band to the centrosome position as a function of time. Red and blue lines show the output of a linear regression over the whole time range and half time range respectively. (G) Evolution of the background spindle corrected (black points) and fully corrected (red points) photoactivated intensity as a function of time. Dashed line indicates the output of the non-linear regression using a double exponential model.

Table S1. Parameters of the microtubule turnover fit for thephotoactivation data

	pf	tf (s)	ps	ts (s)						
Control RNAi										
Average fit	0.770281	20.6079	0.229719	178.58						
Average fit SE	0.0398843	1.67735	0.0191718	113.999						
Clasp2 RNAi										
Average fit	0.850469	17.583	0.149531	297.475						
Average fit SE	0.0235605	0.937045	0.0778402	411.734						
Kid RNAi										
Average fit	0.960666	16.773	0.0393339	143.484						
Average fit SE	0.0299124	0.893069	0.0155161	317.791						
Clasp2+Kid RNAi										
Average fit	0.944515	15.6438	0.0554848	86.1712						
Average fit SE	0.0305378	0.723418	0.0262876	70.6967						

Table S2. P-values calculated from an ANOVA Tukey's test for the differences between spindle lengths for different depletion conditions at steady-state metaphase length

RNAi	control	clasp1	clasp1+2	clasp1+kid	clasp2	clasp2+kid	clasp2+nuf2	kid
control								
clasp1	7.00E-07							
clasp1+2	7.00E-07	7.00E-07						
clasp1+kid	1	7.00E-07	7.00E-07					
clasp2	7.00E-07	7.01E–07	7.00E-07	7.00E-07				
clasp2+kid	0.781214	7.00E-07	7.00E-07	0.94895	7.00E-07			
clasp2+nuf2	1.05E-05	7.00E-07	7.00E-07	0.0116724	7.00E-07	7.04E-07		
kid	0.849049	7.00E-07	7.00E-07	0.921082	7.14E–07	1	9.46E-07	
nuf2	7.00E-07	7.00E–07	7.00E-07	7.00E-07	7.00E–07	7.00E-07	1.05E-06	7.00E-07