Supplemental material

JCB

Yang et al., http://www.jcb.org/cgi/content/full/jcb.200906150/DC1

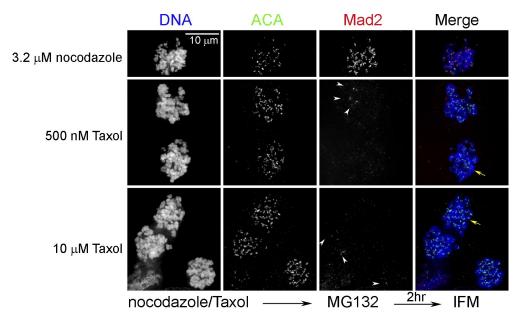


Figure S1. In the presence of Taxol, Mad2 is progressively depleted from kinetochores in RPE1 cells. Each row depicts mitotic RPE1 cell(s) in cultures that were exposed for 8–12 h to 3.2 µM nocodazole (top), 500 nM Taxol (middle) or 10 µM Taxol (bottom). During the last 2 h the cultures were exposed to 10 µM MG132 to prevent exit from mitosis, after which they were fixed and stained for centromeres (ACA) and Mad2. Note that one cell in the 500-nM and 10-µM panels (yellow arrows) entirely lack kinetochore-associated Mad2 staining. White arrowheads note kinetochore Mad2 staining.

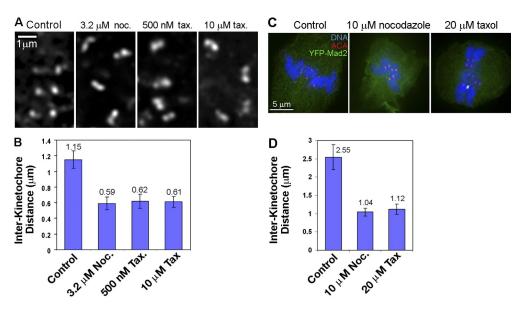


Figure S2. Taxol-treated RPE1 and PtK2 cells satisfy the mitotic checkpoint in the absence of interkinetochore tension. (A) The distance between sister kinetochores on ACA-stained centromeres was measured in nontreated metaphase cells, in nocodazole-treated cells lacking MTs, and in cells treated with 500 nM or 10 μM Taxol. (B) As for cells lacking MTs, there was no significant tension between sister kinetochores in either Taxol concentration. (C) Individual PtK2 cells were followed until they reached metaphase, after which they were either fixed (control) or treated for 5 min before fixation with 10 μM nocodazole or 20 μM Taxol. (D) Both nocodazole and Taxol rapidly eliminate the tension between sister kinetochores.

Table S1. The duration of mitosis (M) under various experimental conditions

Cell Type	Treatment	n	Duration of M	\sim Duration of M	Survival
			min	h	%
RPE1	None ^a	33	18 ± 3	0.3	100
	5-nM Taxol°	171	161 ± 85	2.5	99
	50-nM Taxol	132	510 ± 290	8.5	95
	500-nM Taxol ^a	112	729 ± 396	12	73
	5 μM Taxol	200	312 ± 165	5	99
	10 μM Taxol	200	224 ± 106	3.5	99
	5 nM Epothilone B	193	154 ± 97	2.5	100
	500 nM Epothilone B	70	800 ± 472	13	67
	2.5 μM Epothilone B	158	263 ± 156	4.5	100
	3.2 μM nocodazole ^a	25	1140 ± 732	19	71
	3.2 μM nocodazole + 5 μM Taxol	158	564 ± 476	9	73
	10 μM vinblastine + 5 μM Taxol	35	1688 ± 405	28	23
	100 nM Hesperadin	108	20 ± 4	0.3	100
	3.2 µM nocodazole + 100 nM Hesperadin	66	1024 ± 228	17	
	500 nM Taxol + 100 nM Hesperadin	150	78 ± 26	1.3	100
	10-µM Taxol + 100 nM Hesperadin	140	89 ± 29	1.5	100
ВЈ	5 nM Taxol	110	165 ± 55	2.5	
	500 nM Taxol	106	1057 ± 459	18	
	5 μM Taxol	33	497 ± 333	8	
	10 μM Taxol	95	320 ± 224	5	
HeLa	5 nM Taxol	100	842 ± 321	14	
	500 nM Taxol	110	1483 ± 400	25	
	5 μM Taxol	80	997 ± 392	16.5	
U2OS	5 nM Taxol	120	63 ± 31	1	
	500 nM Taxol	132	591 ± 206	10	
	5 μM Taxol	120	483 ± 267	8	

All data are from ≥ 2 live-cell video records obtained on different days. The duration of M is as defined in Brito et al., 2008; n = number of cells. °From Brito et al., 2008.

References

Brito, D.A., Z. Yang, and C.L. Rieder. 2008. Microtubules do not promote mitotic slippage when the spindle assembly checkpoint cannot be satisfied. *J. Cell Biol.* 182:623–629.