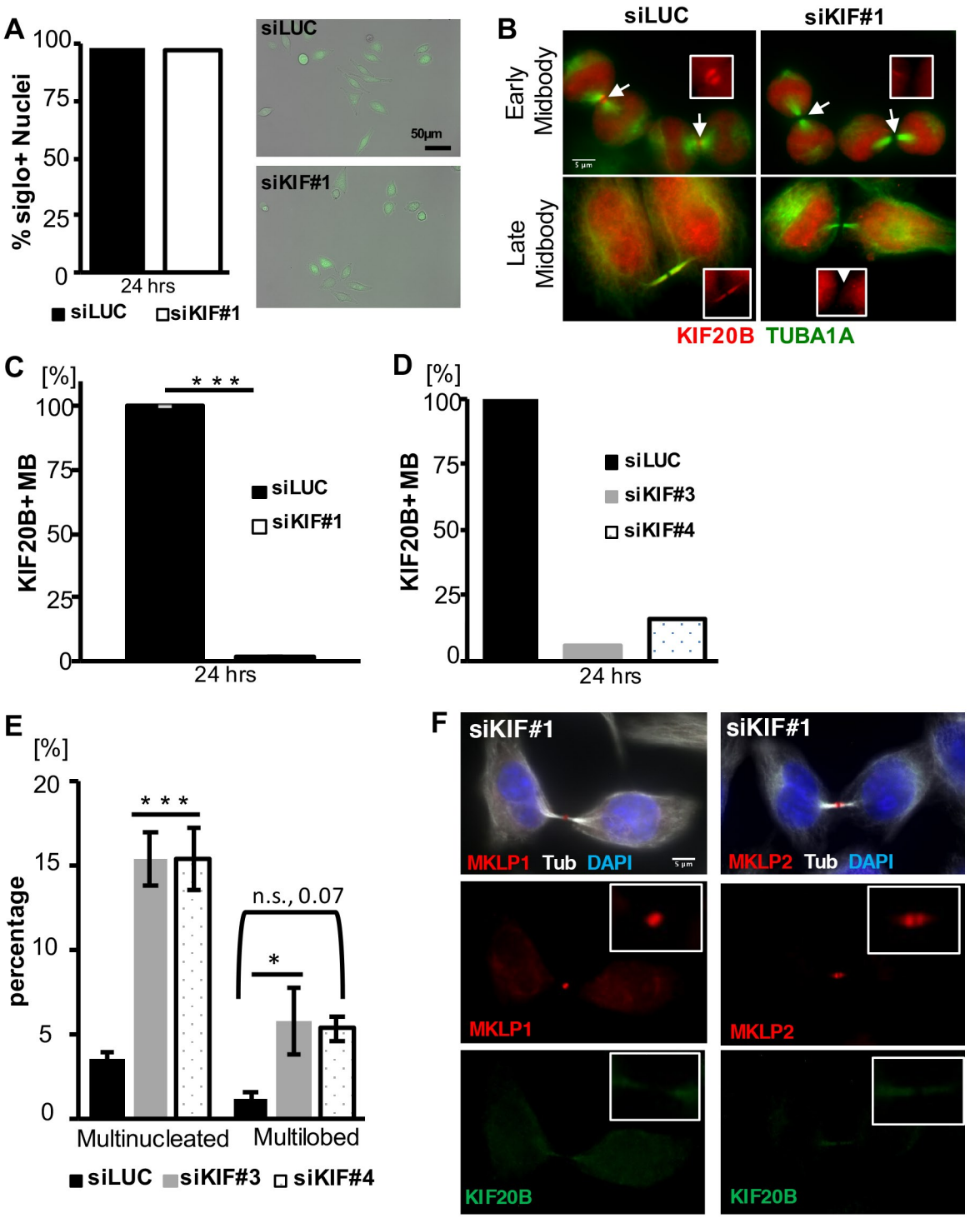
Supplemental Materials

Molecular Biology of the Cell

Janisch et al.



Supplemental Figure 1

Supplemental Figure 1. Knockdown efficacy of siRNAs to human KIF20B.

(A) Transfection efficiency of both control siRNA to the Luciferase gene (siLUC) and siRNA to KIF20b (siKIF#1 from (Abaza *et al.*, 2003) was > 98% as indicated by siGlo transfection indicator (Dharmacon). n = 118 siLUC cells, 125 siKIF cells from 1 coverslip/treatment each. Scale bar, 50 µm.

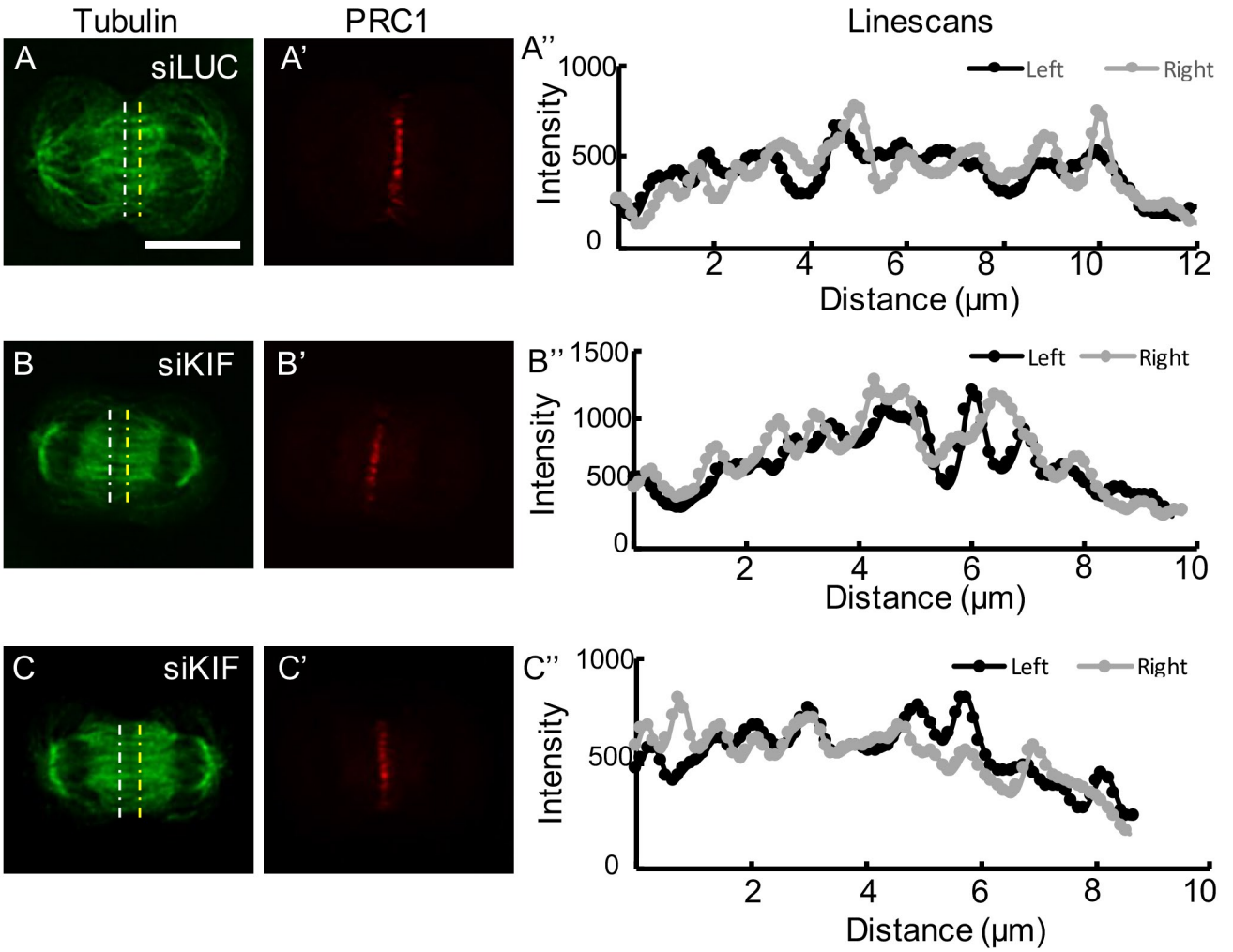
(B) Left, in cells treated with siLUC, endogenous KIF20B (polyclonal rabbit antibody) signal flanks the dark zone of early midbodies (white arrows) and is more spread out in the midbody at later stage. Right, in cells treated with siKIF#1, KIF20B is not detected in the midbodies at early (white arrows) or late (white arrow heads) stages. Insets show KIF20B staining only (red). Red signal in nucleus is background from the polyclonal rabbit antibody to Kif20b that is present in both control and knockdown human cells, and control or mouse mutant *Kif20b* cells. Scale bar, 5 µm.

(C) Quantification of siKIF#1 knockdown was nearly 100% depletion of detectable KIF20B in the midbody. If any KIF20B signal was detected at all in the midbody, it was counted as positive signal. n = 3 coverslips/treatment, 71 midbodies for siLUC and 60 midbodies for siKIF#1. ***, $p < 0.001$, Student's t-test.

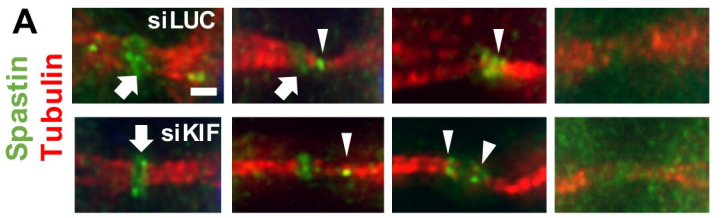
(D) Commercially available siRNA's to KIF20B (Life Technologies), siKIF#3 and siKIF#4, reduced KIF20B immunostaining signal to undetectable level in 94% and 84% of midbodies, respectively. n=50 midbodies each for siLUC, siKIF#3, and siKIF#4, 1 coverslip/treatment.

(E) Similar to results using siKIF#1 (Figure 2, D-H), the average percentages of multinucleate cells and multi-lobed nuclei out of total cells imaged were increased when KIF20B was depleted by either siKIF#3 or siKIF#4 for 24 hours before fixation. n = 4 coverslips/2 treatments; 2067 siLUC cells, 1885 siKIF#3 cells, 1777 siKif#4 cells. One- way ANOVA with Dunnet multi-comparison correction was used for comparison.

(F) siRNAs against KIF20B (#1, #3, and #4) did not knock down other Kinesin-6 family members MKLP1 and MKLP2. MeOH-fixed HeLa cells immunostained for endogenous KIF20B (mouse monoclonal antibody) and either MKLP1 or MKLP2 show that KIF20B is depleted to undetectable levels in the midbody while the other family members are present at normal levels and localization. siKif#3 and #4 data not shown. At least 20 cells were checked for each siRNA. * $p<0.05$, *** $p<0.001$. Scale bar, 5 µm.



Supplemental Figure 2. Example deconvolution images of normally organized central spindles in siLUC- (A) or siKIF-treated (B, C) HeLa cells in anaphase/early furrowing. Images are single z-planes taken from the center of each cell. (A', B', C') show normal PRC1 localization at the midzone of central spindles in siLUC- and siKIF-treated HeLa cells. (A'', B", C'') are linescans of tubulin signal from images A, B, and C, drawn perpendicular to the central spindle microtubule bundles on the left and right sides of the midzone (indicated by white and yellow dotted lines). Tubulin intensity peaks represent microtubule bundles that often show apparent symmetry on both halves of the central spindle. Scale bar 10µm.



B

					Total CS	Spas+ CS	Total MB	Spas+ MB
siLUC	8	5	2	1	7	7	16	15
siKIF	9	2	1	3	4	3	15	12

Supplemental Figure 3. Spastin recruitment in the midbody does not require KIF20B.
(A) Example deconvolution images of endogenous spastin (green) localization in the midbodies of siLUC- (top) and siKIF-treated (bottom) HeLa cells also stained for tubulin (red). Localization can be seen in the central bulge (arrows) or at the constriction sites (arrowheads), or both, or spastin may be diffuse rather than enriched at any particular site (right-most panels).
(B) Schematic representations of spastin localization as seen in examples in (A), with tallies underneath of midbodies observed with each localization pattern, and subtotals for constriction sites (CS) or midbodies (MB).