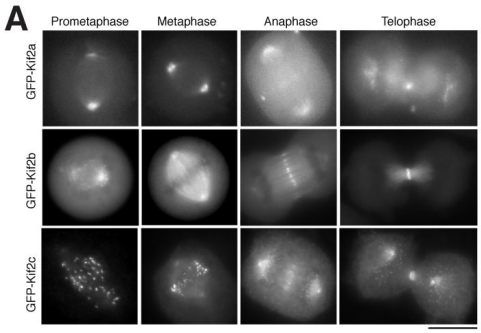
Supplemental Figure Legends

Supplemental Figure 1



B

	Mitosis		Interphase		
	Spindle	Centrosome	Kinetochores	Microtubules	Plus ends
NaMaCa	x	x	x (weakly)	x	
NaMbCb	x	x		x	
NaMcCc	x	x	x (weakly)	x	
NbMbCb	x			x	
NbMaCa	x	x	x	x	
NbMcCc	x	x	x	x	
NcMcCc	x	x	x		x
NcMaCa	x	x	x		x
NcMbCb	x			x	
Na (ΔMaCa)		x			
Nb (ΔMbCb)					
Nc (ΔMcCc)		x	x		
MaCa (ΔNa)	x				
MbCb (ΔNb)	x				
McCc (ΔNc)	x				

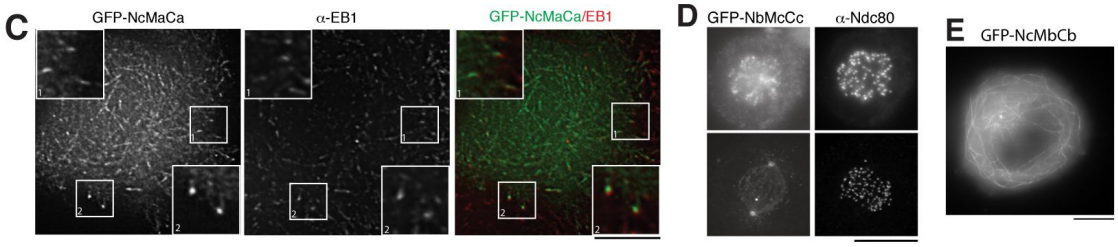


Figure S1. Summary of the localization behavior for the kinesin-13 domains and chimeras. (*A*) Images of clonal cell lines stably expressing GFP-tagged Kif2a, Kif2b, or Kif2c at different stages of mitosis. (*B*) Table summarizing the localization of each kinesin-13 chimera and of the N-terminus and Motor/C-terminus alone of each kinesin-13 family member. (*C*) Images of HeLa cells in interphase transfected with GFP-$N_CM_AC_A$ and stained with EB1. GFP-$N_CM_AC_A$ and EB1 are in green and red respectively. (*D*) Immunofluorescence images of HeLa cells in mitosis transfected with GFP-$N_BM_CC_C$ and stained with Ndc80. (*E*) Images of HeLa cells in interphase transfected with GFP-$N_CM_BC_B$, showing binding to the length of microtubules.

Supplemental Figure 2

A

Protein	MW (kDa)	% Sequence Coverage Kifc3	Kif2a	DDa3	Kif2c
Kifc3	92.8	67.7			
Cep170	161.4	27.6			
Kif2a	84.1		46.2		
DDa3	32.8			66.9	
Kif2c	81.3				53.8

B

% sequence coverage for EB3		
	untreated	Size
Protein	(IP)	(Kda)
EB3	40.2	32
EB1	36.9	29.9
NdeI	23.6	37.7
EB2	9.8	37

C

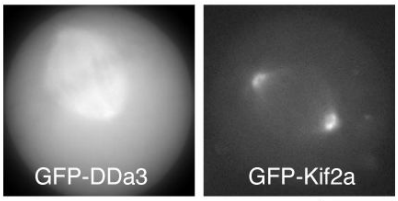


Figure S2. Proteomic analysis of kinesin-13 purifications from human cells. (A) GFP^{LAP} tagged fusions were used to isolate Kif2a, Kif2c, Kifc3 and DDa3 from stable cell lines using one step IPs. Percent sequence coverage from the mass spectrometric analysis is shown for these samples. (*B*) One step purification of GFP^{LAP}-EB3. Percent sequence coverage from the mass spectrometric analysis and molecular weight of each protein is shown. (*C*) Images of HeLa cells transiently transfected with GFP-DDa3 or GFP-Kif2a. Scale bars, 10 μm.

Supplemental Figure 3

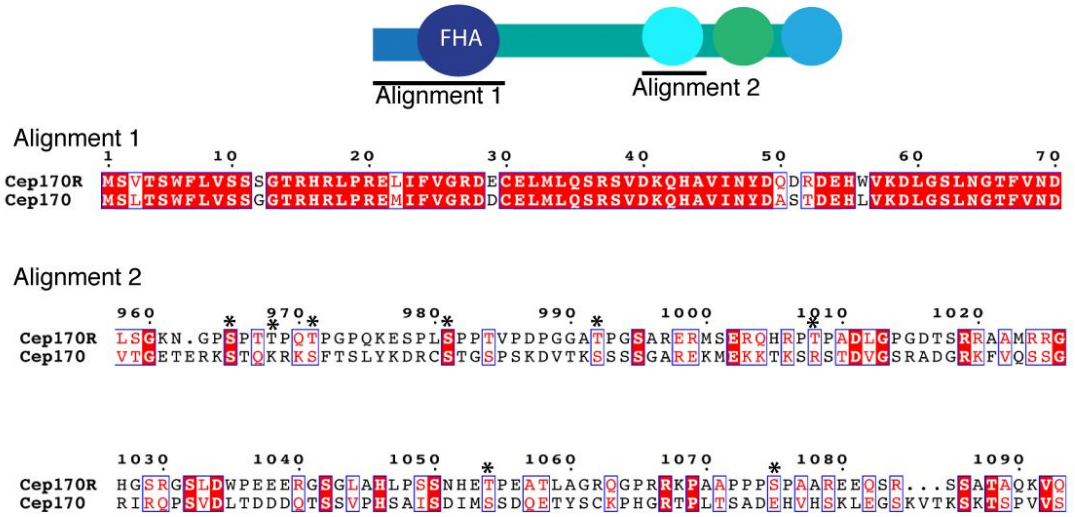


Figure S3. Sequence alignment of Cep170 and Cep170R. Cep170 and Cep170R show strong sequence conservation. Top, schematic diagram of the domain structure of Cep170. Bottom, the sequences from two domains of Cep170 (1-70, 1094-1229) were aligned to Cep170R using Clustalw and formatted with ESPRIPT (Gouet et al., 1999). Non-conserved CDK sites present in Cep170R are indicated with a star (*).

Supplemental Figure 4

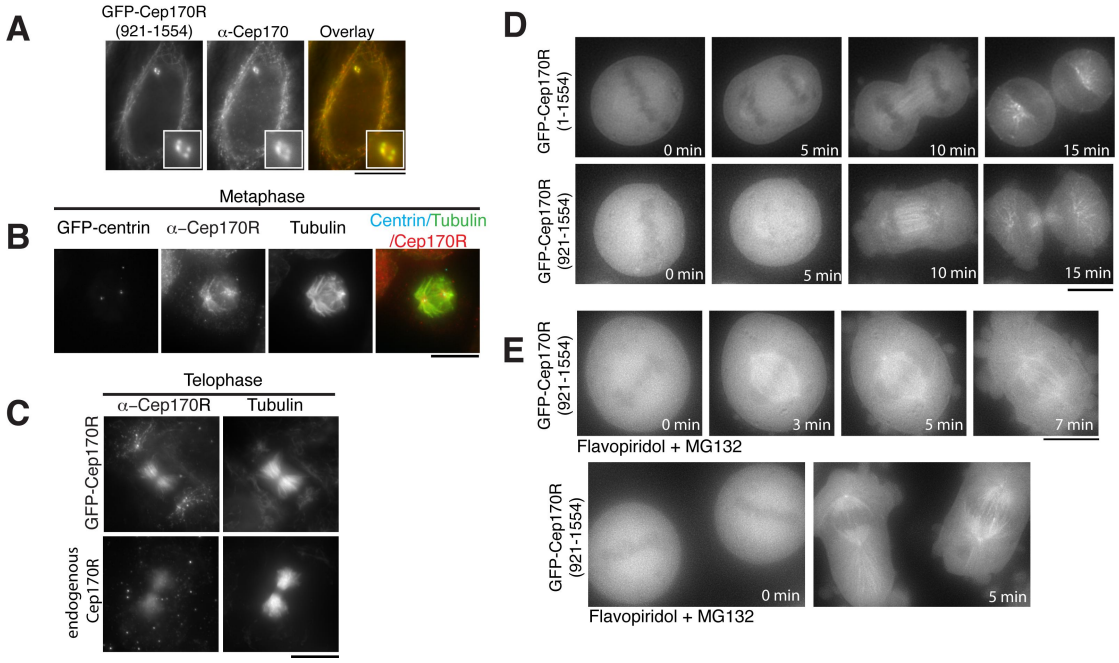


Figure S4. Cep170 and Cep170R localize to microtubules. (*A*) Immunofluorescence images showing the localization of Cep170 and GFP-Cep170R (C-terminus). The inset shows the centrioles. Overlay, Cep170R and Cep170 are shown green and red, respectively. (*B*) Immunofluorescence images of metaphase cells expressing GFP-Centrin stained for Cep170R and microtubules. (*C*) Immunofluorescence images showing control HeLa cells and Hela cells expressing GFP-Cep170R in telophase stained for Cep170R and tubulin. (*D*) Images of live cells expressing GFP-Cep170R, and GFP-Cep170R-CT at the indicated time points throughout mitosis. Cep170R accumulates on microtubules at anaphase onset. (*E*) Images of live cells expressing GFP-Cep170R-CT. Cells were arrested in mitosis with MG132 and visualized shortly after the addition of the CDK inhibitor flavopiridol (at t =0). Scale bars, 10 μm.

Supplemental Figure 5

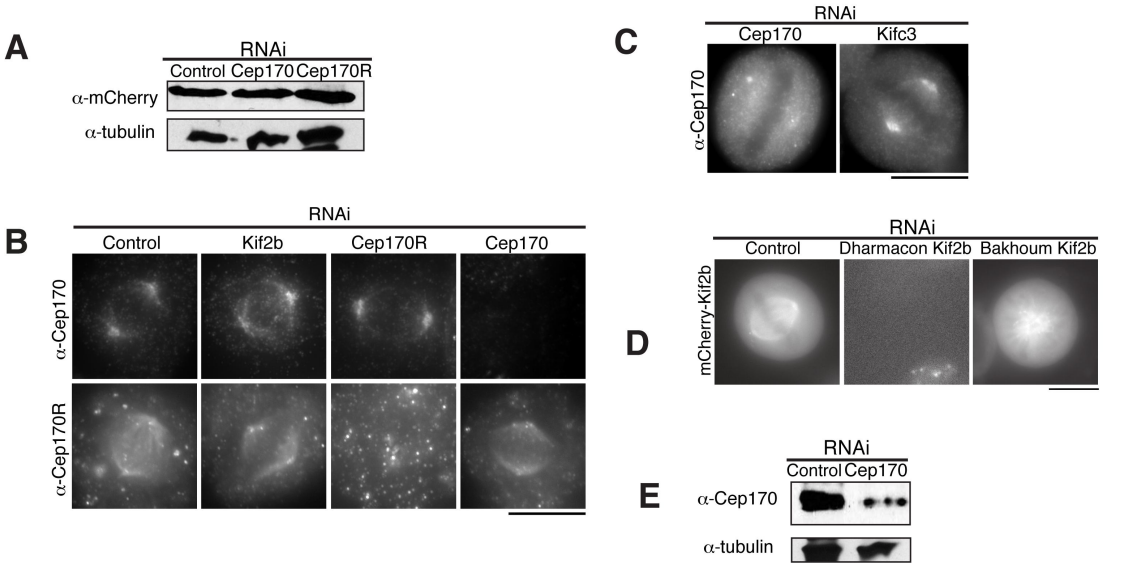


Figure S5. Kifc3 and Kif2b do not affect Cep170 and Cep170R localization. (*A*) Western blot showing the levels of mCherry-Kif2b upon depletion of Cep170 and Cep170R using Cep170 and Cep170R RNAi. The Western blot was probed with anti-mCherry antibodies. Tubulin was used as a loading control. (*B*) Immunofluorescence images acquired using the indicated antibodies against Cep170 and Cep170R in control cells or following depletion of Kif2b, Cep170 and Cep170R by RNAi (*C*) Immunofluorescence images stained for Cep170 following 24 hr of RNAi-based depletion of Cep170 or Kifc3. (*D*) Images of live mitotic cells expressing mCherry-Kif2b under control conditions or following depletion of Kif2b using a pool of pre-designed siRNAs obtained from Dharmacon or siRNAs described previously by Bakhoum et al, 2009. For these experiments, the mCherry-Kif2b was mutated to make it resistant to the Bakhoum siRNAs, but not the Dharmacon pool. These mutations prevent depletion when the Bakhoum siRNAs are used, but do not rescue the observed monopolar spindle phenotype. (*E*) Western blot showing the depletion of Cep170 using Cep170 RNAi. The Western blots were probed with anti-Cep170 and anti-tubulin antibodies. Bars, 10 μm.

Supplemental Figure 6

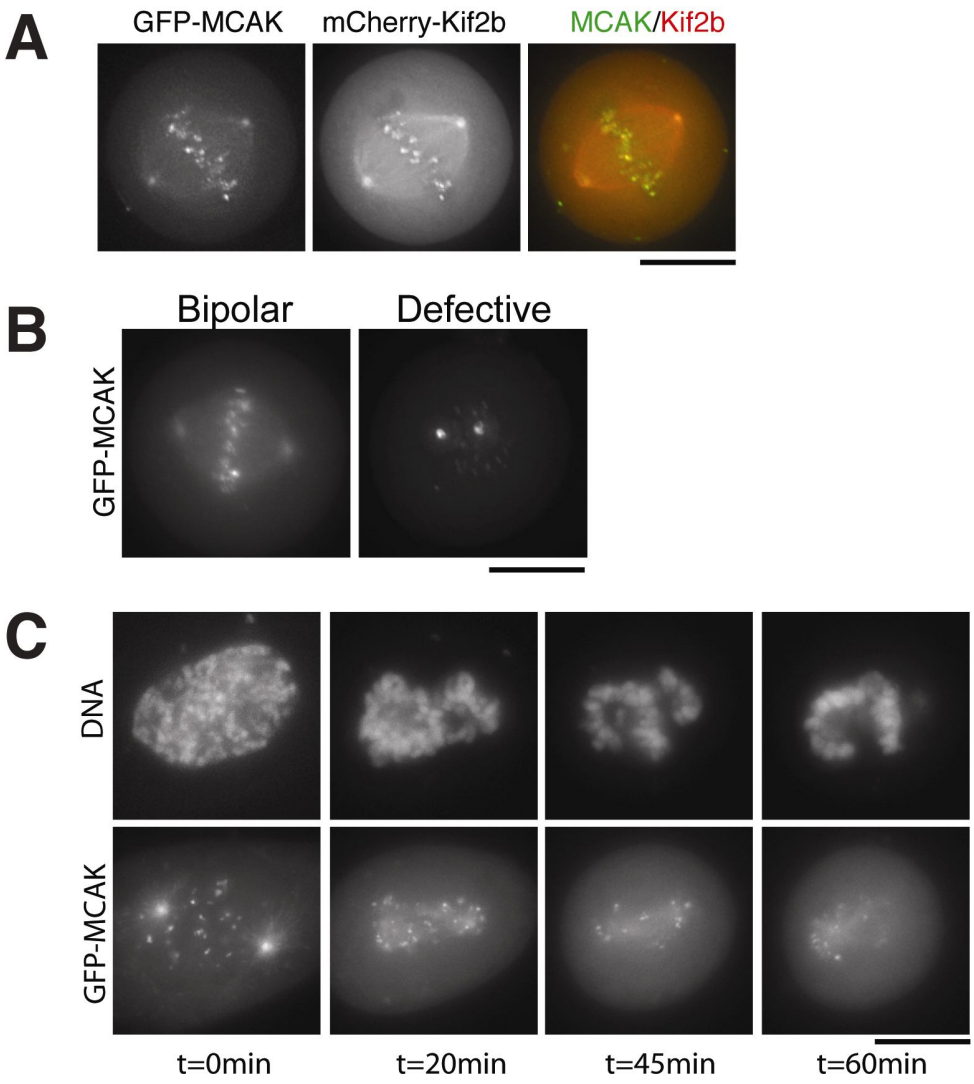


Figure S6. MCAK overexpression shows spindle defects similar to Kif2b overexpression. (*A*) Images of HeLa cells transiently expressing mCherry-Kif2b in a GFP-MCAK cell line, showing that both localize to the kinetochore in metaphase. (*B, C*) Images and a time-lapse series of HeLa cells transiently overexpressing GFP-MCAK, showing that MCAK overexpression can cause spindle defects and monopolar spindles. Scale bars, 10 μm.