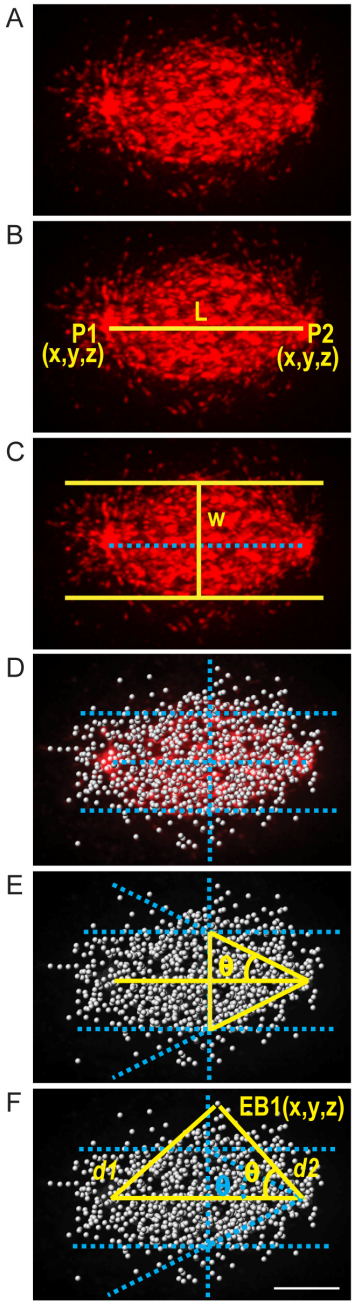
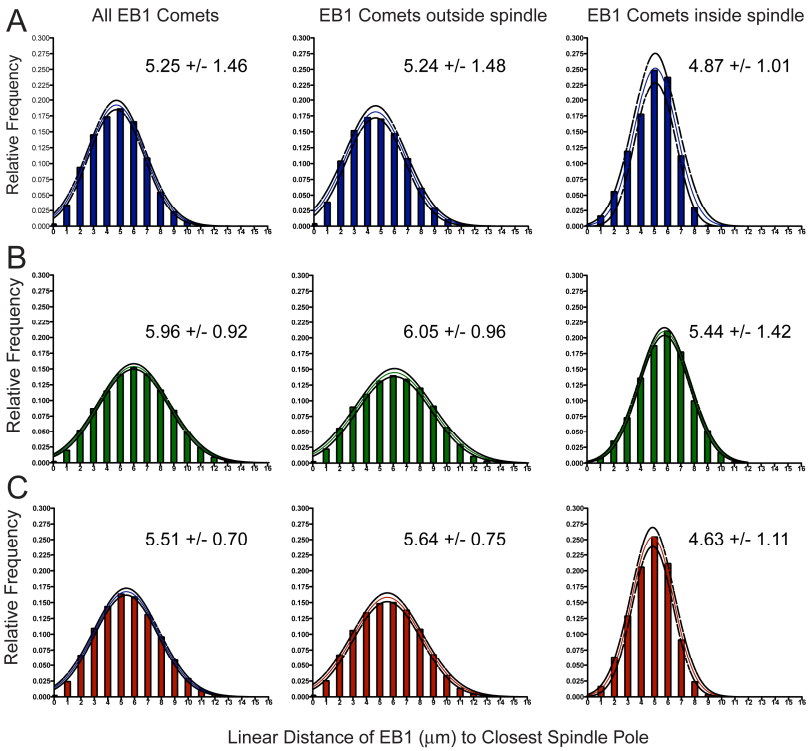
Stout et al.
Kif18B Interacts with EB1 and Controls Astral Microtubule Length during Mitosis

Supplemental Figure Legends

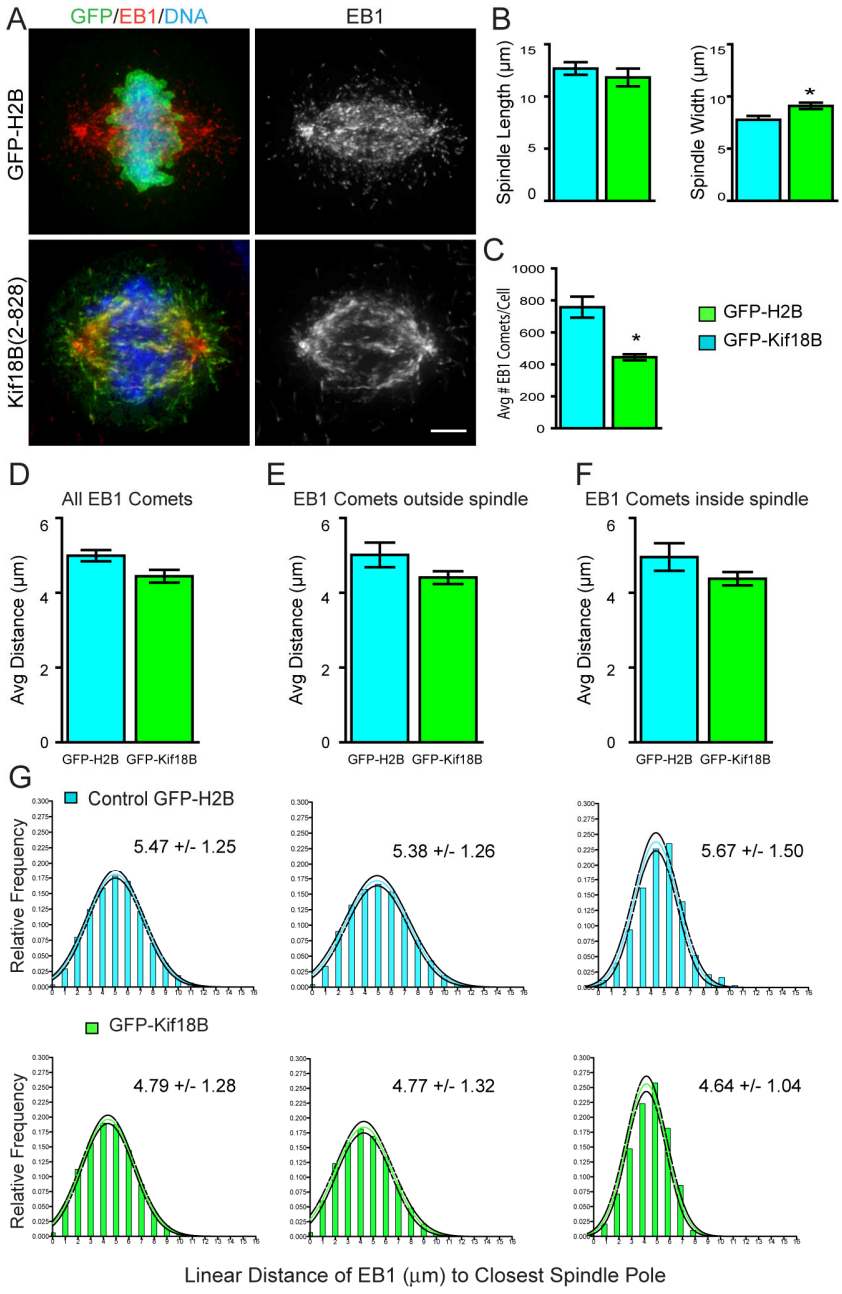


Supplemental FIGURE 1: Schematic representation of spindle measurements and EB1 comet identification. (A) Image of EB1 staining (red) in which the contrast was enhanced to clearly indicate the astral EB1 comets. Note that the enhancement of the intensity shown here does not

affect identification of the comets. (B) The spindle poles, P1 and P2, were defined as the center of the focal point of EB1 staining at each end of the spindle. The x,y,z-coordinates were used to calculated the spindle length (L; yellow line). (C) The width (w; vertical yellow line) was defined as the widest point of the dense EB1 staining within the spindle and demarcated by the two yellow lines tangent to this density and parallel to the spindle axis (dotted blue line). (D) Image of EB1 comets (represented as white spheres) identified using the spots function on IMARIS. (E) Angle (θ) of the spindle area is defined by the line drawn from the pole to the outer edge of the width of the spindle at its equator and the line of the spindle axis. Tangent θ = (spindle width/2)/(spindle length/2). (F) The xyz-coordinates of the EB1 spot and the 2 spindle poles can be used to calculate the distance (*d1*) of the EB1 spot to Pole 1 or the distance (*d2*) of the EB1 spot to Pole 2. The angle θ made by the line of the shortest distance (or *d2* in this representation) and the spindle pole axis is calculated using *d1* and *d2* and the cosine rule. In this representation, the angle is greater than the average control spindle angle as determined above, and therefore the EB1 spot is considered outside the spindle area and therefore representative of an astral MT.

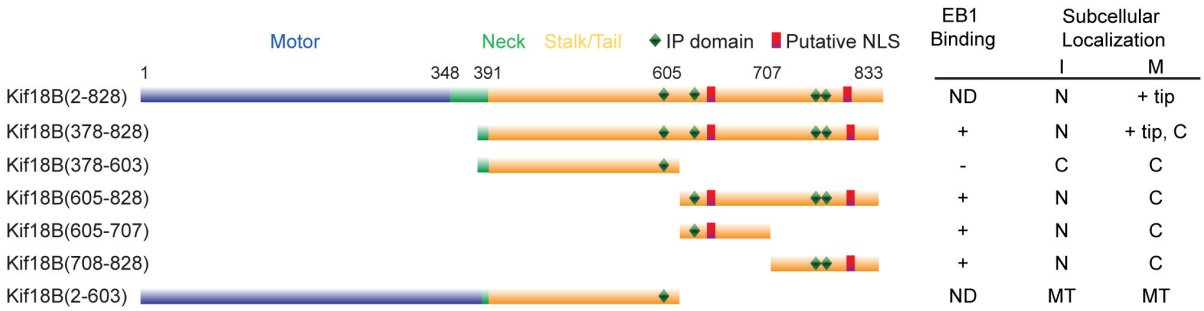


Supplemental FIGURE 2: As a representative measure of MT length, the distance between each EB1 comet spot to its nearest pole were calculated as detailed in the Materials and Methods, and then all were plotted as a relative frequency distribution for either control (A), Kif18B2 knockdown cells (B), or Kif18B4 knockdown cells (C) with the best-fit Gaussian curves. These data were then partitioned to represent either astral MTs (outside) or spindle MTs (inside the mean spindle size of control cells). Each histogram represents all MT lengths in 30 cells total per condition collected from three independent experiments. The means +/- SD are reported, and the best-fit Gaussian curves (solid line) +/- the 95% confidence interval (dotted line) are plotted.

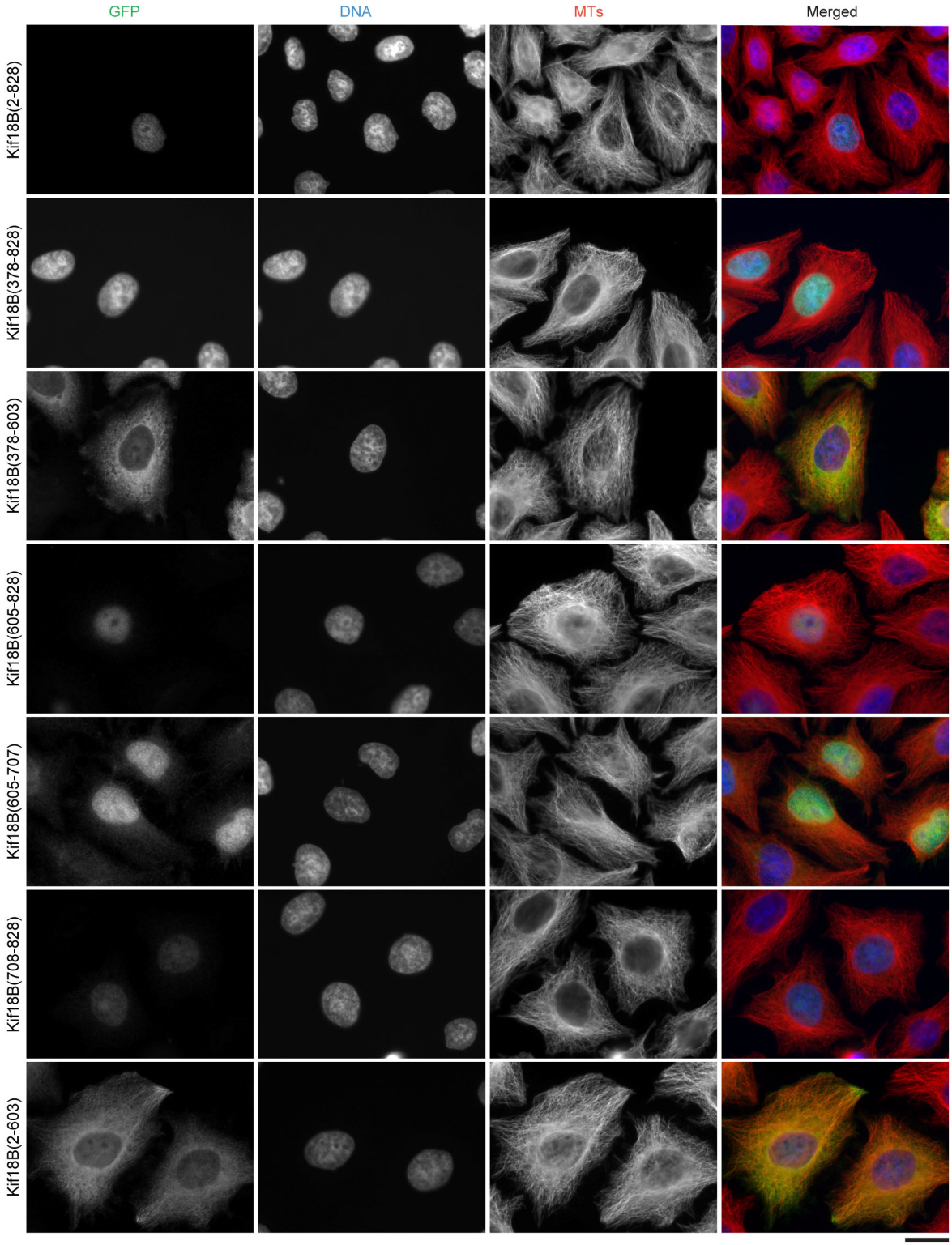


Supplemental FIGURE 3: Kif18B overexpression modulates the length of astral MTs. (A) HeLa cells were treated with negative control GFP-H2B (top) or GFP-Kif18B (bottom) and then stained for GFP (green), EB1 (red), and DNA (blue). Scale bar, 4 µm. (B) Quantification of spindle length (left) and spindle width (right) of control (GFP-H2B) vs. GFP-Kif18B

overexpression. Data represent the mean +/- SEM from three independent experiments in which at least 10 spindles were measured per experiment. (C) The average number of EB1 comets/cell is plotted for control and GFP-Kif18B overexpression and represents the mean +/- SEM from three independent experiments in which at least 10 spindles were analyzed per experiment. * indicates $p < 0.05$. (D-F) The average distance between the pole and the EB1 comets was calculated for all EB1 comets (D), those outside the spindle (E), and those within the spindle (F). Data represent mean +/- SEM from three independent experiments in which at least 10 spindles were analyzed per experiment. (G) The linear distance of the EB1 comet to the nearest pole was calculated as detailed in the Materials and Methods and plotted for either control (top) or GFP-Kif18B overexpression cells (bottom). The distribution of MT lengths was calculated for all EB1 comet spots, and then the data was partitioned to score the spots outside or inside the spindle. Each histogram represents the relative frequency of the binned MT lengths for each EB1 spot to its closest pole for 30 cells per condition from three independent experiments. The means +/- SD are reported, and the best-fit Gaussian curves (solid line) +/- the 95% confidence interval (dotted line) are plotted.



Supplemental FIGURE 4: Schematic summary of Kif18B localization data. NLS (nuclear localization signal), I (interphase), M (mitosis), N (nucleus), C (cytoplasm), ND (not determined).


GFP
DNA
MTs
Merged
Kif18B(2-828)
Kif18B(378-828)
Kif18B(378-603)
Kif18B(605-828)
Kif18B(605-707)
Kif18B(708-828)
Kif18B(2-603)

Supplemental FIGURE 5: Both NLS motifs are active for nuclear transport. HeLa cells were transfected with the indicated GFP-tagged fusion protein construct and then stained with anti-GFP (green), MTs (red), and DNA (blue). Scale bar, 20 μm.

Supplementary VIDEO 1: A prometaphase cell (from Figure 1A) showing MTs (white), EB1 (orange), Kif18B (green), and DNA (blue). The series shows a projection of 9.6 μm through the spindle. The image is then clipped to the center 2 μm so that the astral MTs above and below the spindle are removed. Note that the Kif18B staining is most prominent on the astral MT ends near the cortex.

Supplementary VIDEO 2: A monastrol treated cell showing MTs (white), EB1 (orange), Kif18B (green), and DNA (blue). The initial projection is 10.6 μm and then is clipped to the center 2 μm of the aster. Note that the Kif18B staining is most prominent on the astral MTs that extend away from the chromosomes toward the cell cortex.

Supplementary VIDEO 3: Time-lapse series of GFP-Kif18B expressing cell showing plus-tip tracking. Images are collected at 2 sec interval and played back at 30 frames/sec.