SUPPLEMENTAL DATA

Supplemental Figure Legends

Figure S1. Kif18A localizes dynamically to the plus-ends of kinetochore microtubules during mitosis.

(A) Mitotic HeLa cells in the indicated stages were fixed and stained with anti-Kif18A antibodies (green in overlay), anti-α-tubulin antibodies (red in overlay), and DAPI to visualize DNA (blue in overlay).

(B) Magnified view of the region indicated by the white box in the metaphase cell in (A). White arrows indicate kMTs with visible gradients of Kif18A.

Figure S2. Localization of Kif18A to kinetochores is dependent on microtubules

HeLa cells expressing EGFP-CENP-B (blue in overlay) were treated with the indicated drugs for 30 minutes prior to fixation and staining with anti-Kif18A antibodies (green in overlay), anti-α-tubulin antibodies (red in overlay) and DAPI to visualize DNA.

Figure S3. Treatment with Kif18A-specific siRNAs depletes Kif18A from spindles and delays cells in prometaphase.

(A and B) HeLa cells were treated with control or Kif18A-specific siRNA for 36 hours prior to fixation and staining with anti-Kif18A antibodies (green in overlay),

anti-α-tubulin antibodies (red in overlay in (A)), anti-Hec1 antibodies (red in overlay in (B)) and DAPI to visualize DNA. Scale bars are 5 μm.

(C) Western blot analysis of Kif18A in HeLa cell extracts after treatment with control or Kif18A-specific siRNAs for 24 or 48 hours as indicated. The numbers above each lane indicate the relative abundance of the GAPDH loading control.

(D) HeLa cells were transfected with control (black bars), Kif18A-specific (red bars), Mad2-specific (blue bars) or a combination of Kif18A and Mad2-specific siRNAs (gray bars) 24 hours prior to fixation or EGFP-Kif18A (green bars) 48 hours prior to fixation. The percentage of mitotic cells in prophase (Pro), prometaphase (Promet), metaphase (Meta), anaphase (Ana) and telophase (Telo) were then quantified for each population of cells using DNA and tubulin staining to determine mitotic stages. Percentages were averaged from two separate experiments. The total number of mitotic cells scored was 297 (control siRNA), 462 (Kif18A siRNA), 277 (Mad2 siRNA), 274 (Kif18A and Mad2 siRNA) and 260 (EGFP-Kif18A).

Figure S4. Deviation from Average Position (DAP) Measurements

Previous studies have established methods for quantifying mitotic kinetochore dynamics that involve measuring velocities and switch frequencies during intervals of poleward and away-from-pole movement. While these methods provide detailed information about kinetochore movements, their use is limited to cases where reversals in direction can be unambiguously scored. In some cases (e.g. Kif18A overexpression) directional switches are not readily apparent,

precluding this type of analysis. Therefore we developed an alternative method for quantifying the magnitude of oscillatory movements, based on the deviations that a kinetochore makes from its average position. This method has the advantage that it does not require hand-selection of directional changes. Therefore it avoids subjectivity and can be applied in cases where turnarounds are not apparent.

To define the average position for a kinetochore, a regression line (dashed black line) was fit to a position versus time plot of the kinetochore's movement relative to spindle pole position (red line and points). The distance between the kinetochore and the fit-line was measured at each time point and the standard deviation of those distances was defined as the deviation from average position (DAP). In cases where experimental treatments do not change the basic shape of the distance versus time plots (for example by significantly increasing pauses in movement), DAP provides a linear measurement of oscillation amplitude that is sensitive to changes in kinetochore oscillation frequency and velocity. In both control and Kif18A-depleted HeLa cells, DAP values are approximately 38% of the average oscillation amplitude.

Supplemental Movie 1. Kinetochore oscillations in a control-depleted HeLa cell.

The movie shows a HeLa cell expressing mRFP-CENP-B and Venus-centrin (not shown) imaged 36 hours after transfection with control siRNA. Images were

collected every 2 seconds. The movie is a projection of five optical sections played back at 25 frames per second (50X real-time).

Supplemental Movie 2. Kinetochore oscillations in a Kif18A-depleted HeLa cell.

The movie shows a HeLa cell expressing EGFP-CENP-B and Venus-centrin imaged 36 hours after transfection with Kif18A-specific siRNAs. Images were collected every 5 seconds. The movie is a projection of five optical sections played back at 10 frames per second (50X real-time).

Supplemental Movie 3. Kinetochore oscillations in a HeLa cell expressing EGFP-Kif18A.

The movie shows a HeLa cell expressing EGFP-Kif18A (not shown) and mRFP-CENP-B imaged 48 hours after DNA transfection. Images were collected every 2 seconds. The movie is a projection of five optical sections played back at 25 frames per second (50X real-time).

Supplemental Movie 4. Anaphase segregation of kinetochores in a Mad2-depleted HeLa cell.

The movie shows a HeLa cell expressing EGFP-CENP-B and Venus-centrin imaged 36 hours after transfection with Mad2 siRNAs. Images were collected every 5 seconds. The movie is a projection of five optical sections played back at 20 frames per second (100X real-time).

Supplemental Movie 5. Anaphase segregation of kinetochores in a Kif18A/Mad2 co-depleted HeLa cell.

The movies shows a HeLa cell expressing EGFP-CENP-B and Venus-centrin imaged 36 hours after transfection with a combination of Kif18A and Mad2 siRNAs. Images were collected every 5 seconds. The movie is a projection of five optical sections played back at 20 frames per second (100X real-time).

Supplemental Movie 6. Anaphase segregation of kinetochores in a control-depleted HeLa cell.

The movie shows a HeLa cell expressing EGFP-CENP-B and Venus-centrin imaged 36 hours after transfection with control siRNAs. Images were collected every 2 seconds. The movie is a projection of five optical sections played back at 50 frames per second (100X real-time).

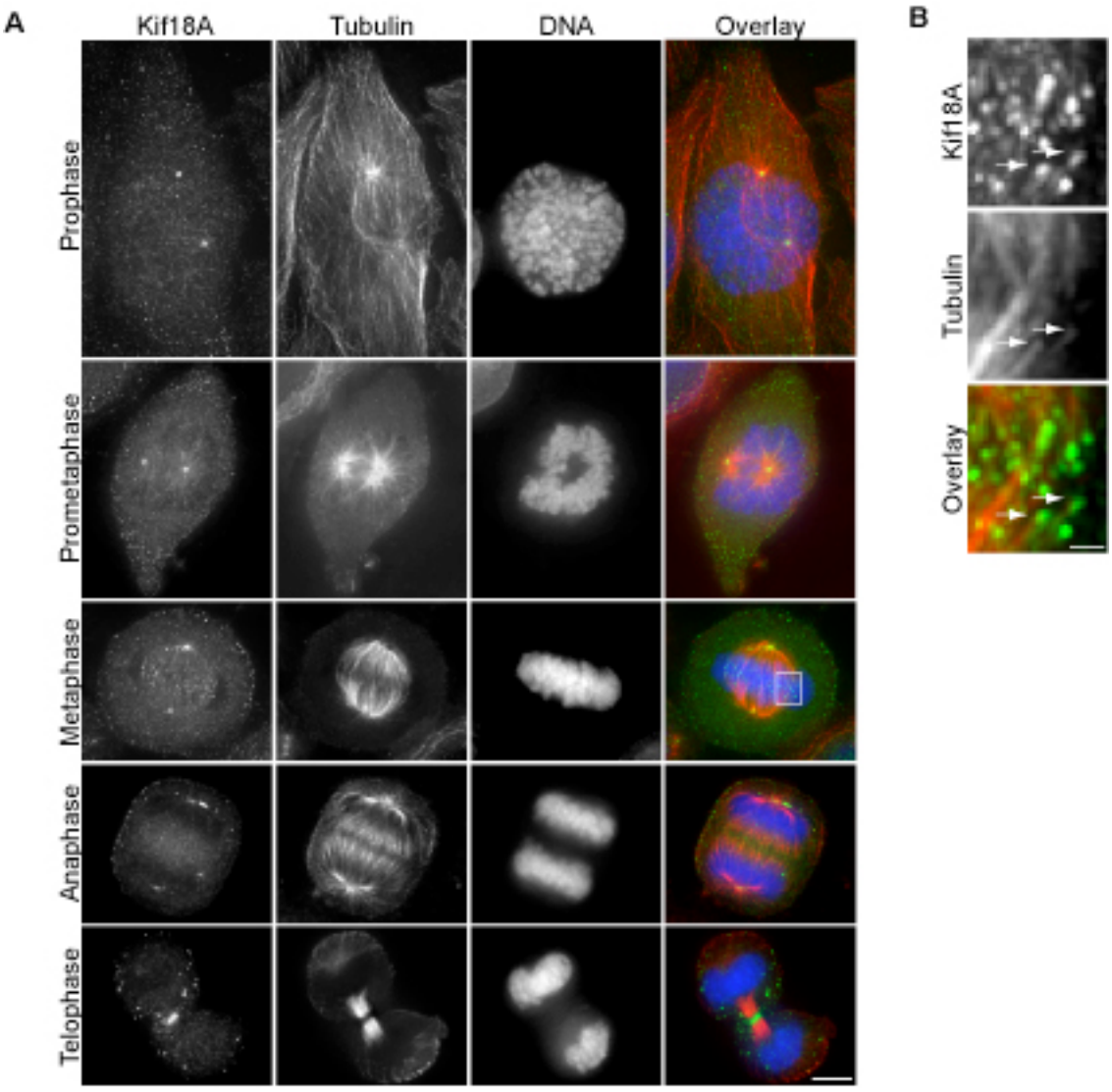
Supplemental Movie 7. Anaphase segregation of kinetochores in a Kif18A-depleted HeLa cell.

The movie shows a HeLa cell expressing EGFP-CENP-B and Venus-centrin imaged 36 hours after transfection with Kif18A siRNAs. Images were collected every 5 seconds. The movie is a projection of five optical sections played back at 20 frames per second (100X real-time).

Supplemental Movie 8. Anaphase segregation of kinetochores in a HeLa cell expressing EGFP-Kif18A.

The movie shows a HeLa cell expressing EGFP-Kif18A (green) and mRFP-CENP-B (red) imaged 48 hours after DNA transfection. Images were collected every 20 seconds. The movie is a projection of five optical sections played back at 5 frames per second (100X real-time).

Figure S1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Metaphase
Anaphase
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B
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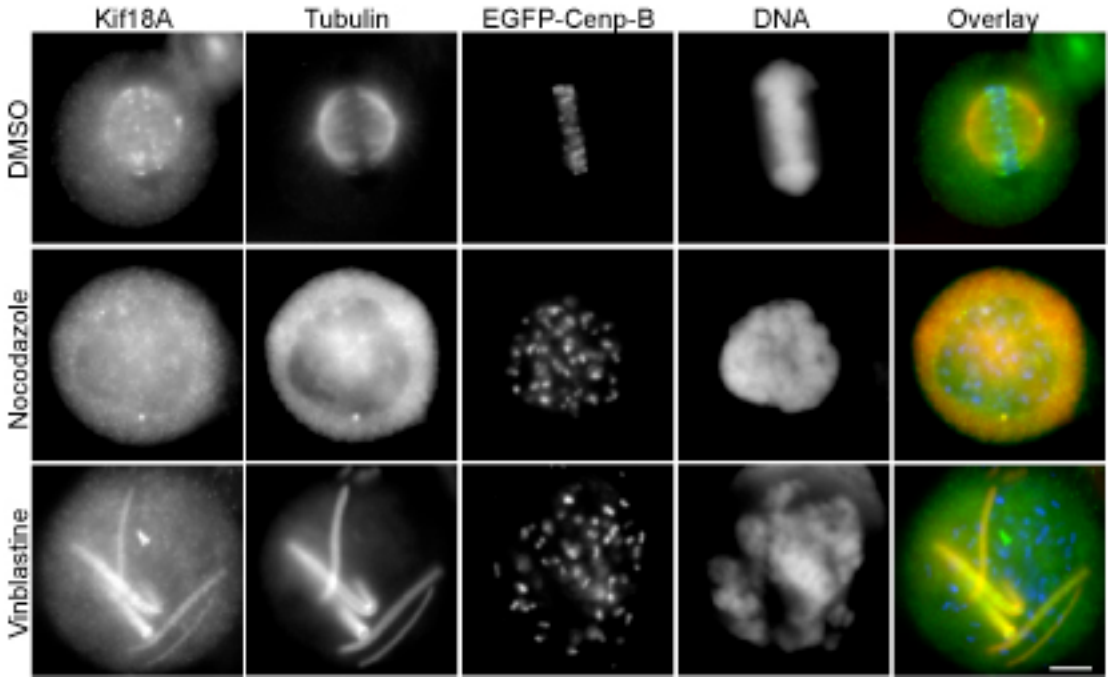
Tubulin
Overlay

Figure S2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Tubulin
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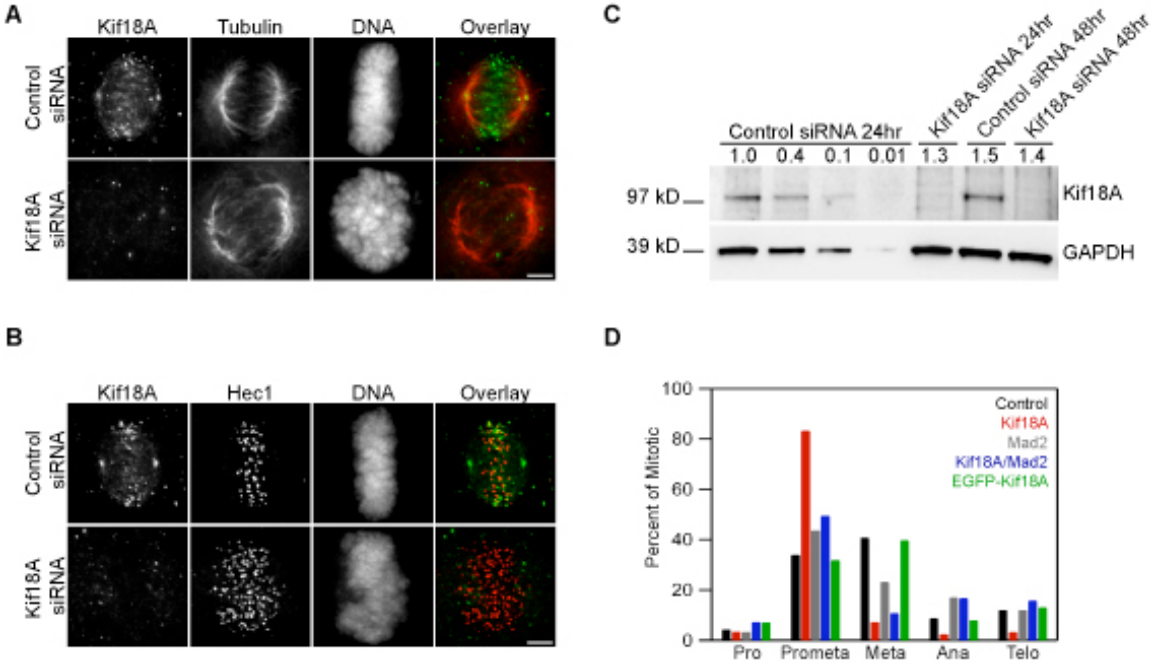
Figure S3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Tubulin
DNA
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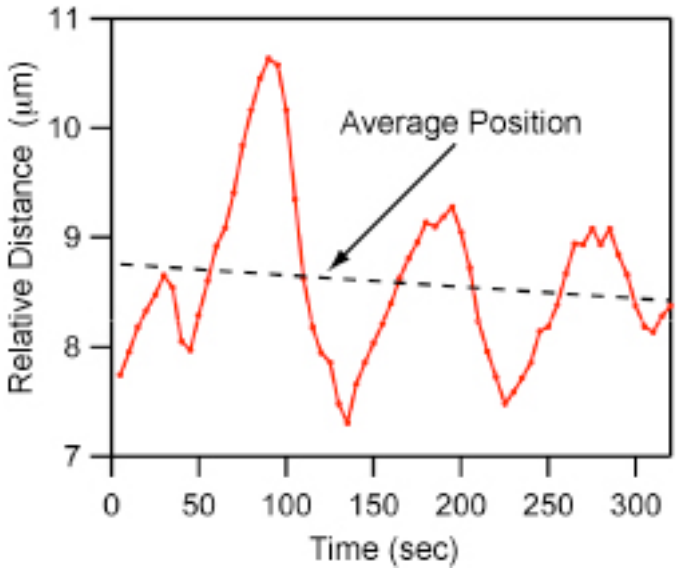
C
Control siRNA 24hr
Kif18A siRNA 24hr
Control siRNA 48hr
Kif18A siRNA 48hr
1.0 0.4 0.1 0.01 1.3 1.5 1.4
97 kD
39 kD
Kif18A
GAPDH
D
Percent of Mitotic
100
80
60
40
20
0
Pro
Prometa
Meta
Ana
Telo
Control
Kif18A
Mad2
Kif18A/Mad2
EGFP-Kif18A

Figure S4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10
9
8
7
Average Position
0
50
100
150
200
250
300
Time (sec)