Supplemental Information

Microtubule Sliding within the Bridging Fiber
Pushes Kinetochore Fibers Apart
to Segregate Chromosomes

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SUPPLEMENTARY FIGURES AND LEGENDS

A. U2OS cell, CENP-A-GFP, mCherry-α-tubulin

B. Midpoint − line between displaced KCs (µm)

C. KC − midpoint distance (µm)

D. Detached KC-pole distance (µm)

E. \( \frac{I_b}{I_{bk}} \) before anaphase onset

F. Signal intensity (a.u.)

G. U2OS cells, CENP-A-GFP, α-tubulin-Alexa Fluor-485

H. U2OS cells, CENP-A-GFP, PRβ-mCherry

I. U2OS cell, CENP-A-GFP, mCherry-α-tubulin

J. KC−midpoint distance (µm)

\( x \) time

\( y \) signal intensity

\( \text{Position (µm)} \)

\( \text{Signal intensity (a.u.)} \)

\( \text{Bridging fiber} \)

\( \text{Bridging + k-fiber} \)

\( \text{Metaphase} \)

\( \text{Anaphase} \)

\( I_b \)

\( I_{bk} \)

\( \frac{I_b}{I_{bk}} \)

\( \text{U2OS cell, CENP-A-GFP, mCherry-α-tubulin} \)
Figure S1. Additional characterization of assay for dynamics of anaphase kinetochores lacking connection to one spindle pole, related to Figure 1

(A) Time-lapse images of the spindle (top row) in a U2OS cell expressing centromere protein CENP-A-GFP (magenta) and mCherry-α-tubulin (green), and enlargements of the boxed region (middle row). Schemes are shown under the images. K-fiber was cut 2.5 µm from the kinetochore in metaphase (yellow lightning sign). Time 0 is anaphase start. (B) Kymograph (consecutive maximum intensity projections onto the y axis) of the spindle from (A) showing merged channels (top), and traces of displaced kinetochores in magenta and control kinetochores in grey (bottom). (C) Time-lapse images of the spindle (top row) in a U2OS cell, and enlargements of the boxed region (bottom row). After the k-fiber cut 2.5 µm from the kinetochore (yellow lightning sign) in anaphase, the pair of sister kinetochores did not show displacement similar to that in metaphase (compare with Figures 1A and S1A). (D) Distance between spindle pole on the side of the ablation and detached kinetochore (see scheme, n=24 cells) over time, where time 0 is anaphase onset. Individual cells (thin lines), mean (thick line), s.d. (pink region). Note that the detached kinetochores are moving away from the spindle pole. Compare the movement of displaced kinetochores during anaphase with the movement of the control kinetochores in Figure S3A. (E) mCherry-α-tubulin signal intensity of bridging fiber, I_b (blue, measured along blue line on scheme), and the bundle consisting of bridging and k-fiber, I_bk (orange, measured along orange line), after cut in metaphase (left, n=13 cells) and during anaphase (right, n=13 cells). Horizontal lines mark background signal, vertical lines delimit area (grey) where signal was measured. (F) mCherry-α-tubulin signal intensity of the bundle consisting of bridging fiber and k-fiber, I_b/I_bk, in a displaced element in anaphase, as a function of I_b/I_bk in metaphase after the cut (n=13 cells). (G) Left panel: image of the fixed spindle after ablation (yellow lightning sign) 2.5 µm from kinetochore (top row) in a U2OS cell stably expressing centromere protein CENP-A-GFP (magenta) and immunostained for α-tubulin (Alexa Fluor-555 shown in green), and smoothed enlargements of the boxed region in both channels (middle row) and only green channel (bottom row). Right panel: image of the spindle after ablation (yellow lightning sign) 2.5 µm from kinetochore (top row) in a U2OS cell stably expressing centromere protein CENP-A-GFP (magenta) and stained with SiR-tubulin (green), and smoothed enlargements of the boxed region in both channels (middle row) and only green channel (bottom row). (H) Time-lapse images of the spindle (top row) in a U2OS cell expressing centromere protein CENP-A-GFP (magenta) and PRC1-mCherry (green), and smoothed enlargements of the boxed region in both channels (middle row) and only green channel (bottom row). K-fiber was cut 2.5 µm from the kinetochore in metaphase (yellow lightning sign). Time 0 is anaphase start. (I) Time-lapse images of the enlarged spindle region in a U2OS cell expressing centromere protein CENP-A-GFP (magenta) and mCherry-α-tubulin (green). Time 0 is anaphase start. White cross represents the midpoint between two displaced kinetochores at a beginning of anaphase. (J) Distance between line connecting displaced sister kinetochores and their midpoint (see scheme, n=24 cells) defined at the beginning of anaphase over time, where time 0 is anaphase onset (left panel). Average distance between detached (blue line) and attached (orange line) kinetochore from their midpoint (n=24 cells) over time, where time 0 is anaphase onset for attached displaced kinetochore (right panel). Note the delay in the anaphase onset for the detached displaced kinetochore.

Scale bars, 1 µm; time bars, 1 min; KC, kinetochore.
A. Cutting of 1 k-fiber and 2 bridging fiber

B. Midzone cut

C. Cutting of k-fiber 1 µm from KC

D. Astral cut

E. Graph showing KC-KC distance (µm) and Pole-pole distance (µm) over time for control (no ablation) and Midzone cut.

F. Graph showing KC-KC distance (µm) and Pole-pole distance (µm) over time for control (no ablation) and Midzone cut.
Figure S2. Additional characterization of disruption of the bridging fibers, related to Figure 2

(A) Time-lapse images of the spindle in a U2OS cell: before the k-fiber was cut 2.5 µm from the kinetochore (top left, yellow sign marks the cut), when the bridging fiber was cut (top center, time 0, yellow sign marks the cut) at the beginning of anaphase, and 120 s later (top right). Enlargements of the boxed region (middle row) and schemes (bottom; MTs, green; kinetochores, magenta).
(B) Kymograph of the spindle from (A) showing merged channels (top) and traces of displaced kinetochores in magenta and control kinetochores in grey (bottom).
(C) Time-lapse images of the spindle in a U2OS cell before the k-fiber was cut 1 µm from the kinetochore (top left, yellow sign marks the cut), at the beginning of anaphase (top center, time 0), and 45 s later (top right). Enlargements of the boxed region (middle row) and schemes (bottom).
(D) Kymograph of the time-lapse movie of the cell from (C) showing merged channels (top) and traces of displaced kinetochores in magenta and control kinetochores in grey (bottom).
(E) Distance between sister kinetochores (left panel) and spindle poles (right panel), in cells with midzone cut (top row, n=11 cells), astral cut (bottom row, n=29), over time, where time 0 is the beginning of cut and in control spindles (middle row, n=24 cells) over time normalized at an average duration from the beginning of anaphase until the midzone cut. Left panel: individual cells (thin lines), mean (thick line), s.d. (pink region); right panel: s.d. (green region). To test whether the continuous ablation had a nonspecific impact on kinetochore velocities, we performed continuous ablation between one spindle pole and the cell cortex with the same ablation parameters as in midzone cut experiment. The average kinetochore separation velocities obtained from that experiment were not different from control values (Figure 2I, Table 1), suggesting that the observed slower kinetochore and pole separation after severing of the midzone region is not a consequence of unspecific effects of ablation, but rather the result of the disruption of bridging fibers.
(F) Distance between kinetochores and the respective spindle poles, in anaphase spindles with midzone cut (top, n=11 cells) and control spindles (bottom, n=24 cells), over time, where time 0 is the beginning of midzone cut. Individual cells (thin lines), mean (thick line), s.d. (pink region).

Scale bars, 1 µm; time bars, 1 min; KC, kinetochore.
Figure S3. Additional characterization of the sliding of the bridging fiber in spindles with displaced kinetochores, related to Figure 3

(A) Distance between intact spindle pole and control kinetochores (see scheme, n=24 cells) over time, where time 0 is the anaphase onset. Individual cells (thin lines), mean (thick line), s.d. (shaded region). Compare with Figure 3C and S1D. Note that the control kinetochores are moving towards the spindle pole.

(B) \( L_{\text{stub}} \) (n=9 cells) during metaphase, measured as shown in Figure 3A, over time. Time 0 is anaphase onset. Legend as in (A). Note that the length of the stub remained constant during metaphase. The observed stable stub length in metaphase is contrary to some of the previously reported results (Maiato et al., 2004; Sikirzhytski et al., 2014), probably because in our experiments the newly created stub was completely free from the rest of the spindle, whereas this was not the case in the mentioned studies.

(C) Time-lapse images of displaced sister kinetochores and the stub in U2OS cell after k-fiber cut 2.5 \( \mu \)m from the kinetochore. The detached kinetochore remained linked to the plus end of the k-fiber stub throughout anaphase which excluded the possibility that motor proteins move the kinetochore along the stub.

(D) Photoactivation of the k-fiber stub tip. Spindle after k-fiber ablation (right, yellow lightning sign), smoothed enlargements of the boxed region in the channel showing CENP-A-GFP and PA-GFP-tubulin (green and magenta, top row) and mCherry-\( \alpha \)-tubulin channel (green, middle row) after photoactivation of the k-fiber stub at time 0. White arrowhead marks the photoactivated spot. Signal intensities of the PA-GFP-tubulin between the detached kinetochore and the stub tip in the respective frames above (bottom row). Vertical lines mark the signal intensity peaks, arrows show the approaching of the detached kinetochore to the photoactivated spot. Note that the photoactivated stub tip is stable during time while the stub length is decreasing. The observed stub shortening in anaphase is contrary to the previous observation of stable anaphase stubs (Sikirzhytski et al., 2014), yet this discrepancy may be due to a different imaging duration.

Scale bars, 1 \( \mu \)m; KC, kinetochore.

(E) Photoactivation of the bridging fiber at the start of the anaphase. Spindle after k-fiber ablation (left, yellow lightning sign), smoothed enlargements of the boxed region in the channel showing mCherry-\( \alpha \)-tubulin (green) and PA-GFP-tubulin (magenta) after photoactivation of the bridging fiber at time 0. White arrowheads mark the photoactivated spot, which splits into two spots.
Figure S4. Additional characterization of the photoactivation experiments in the intact spindle, related to Figure 4

(A) Photoactivation in intact spindles. Smoothed time-lapse images (top row) of the anaphase spindle after photoactivation of PA-GFP-tubulin at time 0 in the bridging fibers and k-fibers. Enlargements of the boxed region (middle row) and signal intensity of PA-GFP-tubulin (bottom row) measured along the gray line shown in inset. Vertical lines mark the signal intensity peaks; arrows show the distance between the photoactivated spots on the bridging and k-fiber.

(B) Smoothed time-lapse images (left panel) of the anaphase spindle after photoactivation of PA-GFP-tubulin at time of photoactivation (0s) and after photoactivation (60s) in the bridging fibers in STLC treated spindles (top row), KIF15 depleted spindles (middle row), Kif15 depleted and STLC treated spindles (bottom row). White arrowheads mark the photoactivated spot, which splits into two spots. Box plot (right panel) of sliding rates for control spindles and for the conditions from (B). n.s. non-significant.

(C) Smoothed time-lapse images (left panel) of the anaphase spindle after photoactivation of PA-GFP-tubulin at time of photoactivation (0s) and after photoactivation (60s) in the bridging fibers in MKLP1 depleted spindles (top row) and control spindles (bottom row). Signal intensity of PA-GFP-tubulin (right panel), measured across the midzone, at time of photoactivation (0s) and after photoactivation (60s) in the bridging fibers in MKLP1 depleted spindles (top row) and control spindles (bottom row).

(D) Box plot of signal intensity ratio (signal in the midzone 60s after photoactivation/signal in the midzone at time of photoactivation) for the conditions from (C). ***p<0.001

(E) Time-lapse images of the anaphase spindle in a U2OS cell treated with MKLP1 siRNA (top) and non-targeting siRNA (bottom). Kymographs in merged channels are shown at the right.

(F) Box plot of kinetochores and poles separation velocities for the conditions from (E). ****p<0.0001

Scale bars, 1 µm; time bars, 1 min; KC, kinetochore.
A

(i) Right bridging MT velocity (µm/min)

(ii) Right bridging MT velocity (µm/min)

(iii) Right pole velocity (µm/min)

(iv) Right kinetochore velocity (µm/min)

(v) Right kinetochore velocity (µm/min)

(vi) Right kinetochore velocity (µm/min)

B

Ablation of the bridge and k-fiber

C

No k-fiber–bridge crosslinks

No ablation

D

E

Detached KC–midpoint distance (µm)

Time (s)
Figure S5. Additional information regarding theoretical model of anaphase, related to Figure 5

(A) Parameter influence on the solutions of the theoretical model without ablation. Varied parameters are shown on the abscissa of the graphs and the corresponding curves are drawn in the same color. Dots represent parameter values in Figure 5B right. Other parameters are as in Figure 5B right; $x^+_k = \pm 2.5 \mu m$, $x^+_\text{kr} = \pm 4 \mu m$, $x^+_\text{br} = \pm 2 \mu m$ and $x^+_\rho = \pm 6 \mu m$.

(B) and (C) Above, scheme of the model. Below, positions of kinetochores, k-fibers, bridging fibers, and spindle poles are shown as a function of time for the parameters given in Figure 5B. Solid and dashed lines correspond to the upper and lower fiber respectively. (B) Solutions of the modified model in which the forces at the pole and also between the antiparallel MTs in the upper right fibers are set to zero at time $t = 0.5$ min. (C) Solutions of the modified model without the connection between k-fibers and bridging fibers.

(D) $L_{\text{Stub}}$ (see scheme, n=8 cells) over time, where time 0 is anaphase onset for cells containing the data in 0-180 s time interval. Individual kinetochores (colored lines), mean (black line), s.d. (shaded region).

(E) Distance between detached kinetochore (KC) and the midpoint (see scheme) over time, where time 0 is anaphase onset, for the same cells as in (D). Legend as in (D).