Wandke et al., http://www.jcb.org/cgi/content/full/jcb.201110060/DC1

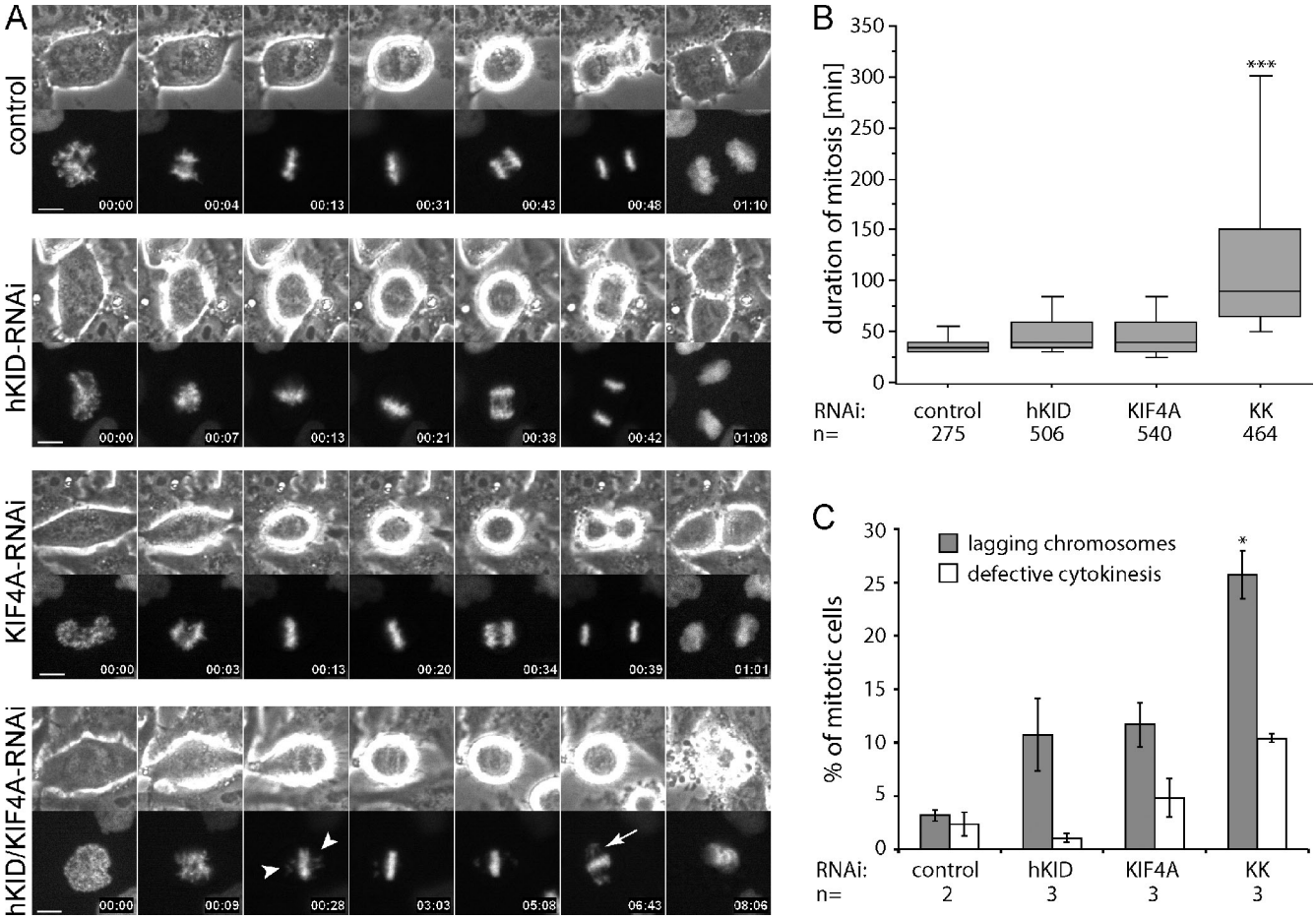


Figure S1. **Simultaneous knockdown of hKID and KIF4A impairs chromosome congression.** (A) Selected frames from live-cell time-lapse microscopy of HeLa H2B-GFP cells 30 h after different siRNA treatments. Time is min:s. Bar, 5 µm. Note that unlike in control cells, in hKID/KIF4A RNAi cells chromosome arms tend to direct toward the spindle poles (arrowheads). Some chromosomes (arrow) fail to congress to the metaphase plate, causing a prometaphase arrest. (B) Quantitative analysis of time-lapse movies of HeLa H2B-GFP cells after different siRNA treatments. Time-lapse data from at least three different experiments are represented as box-and-whisker plots. (C) Cells from the above-mentioned Videos were analyzed for lagging chromosomes and cytokinesis failures.

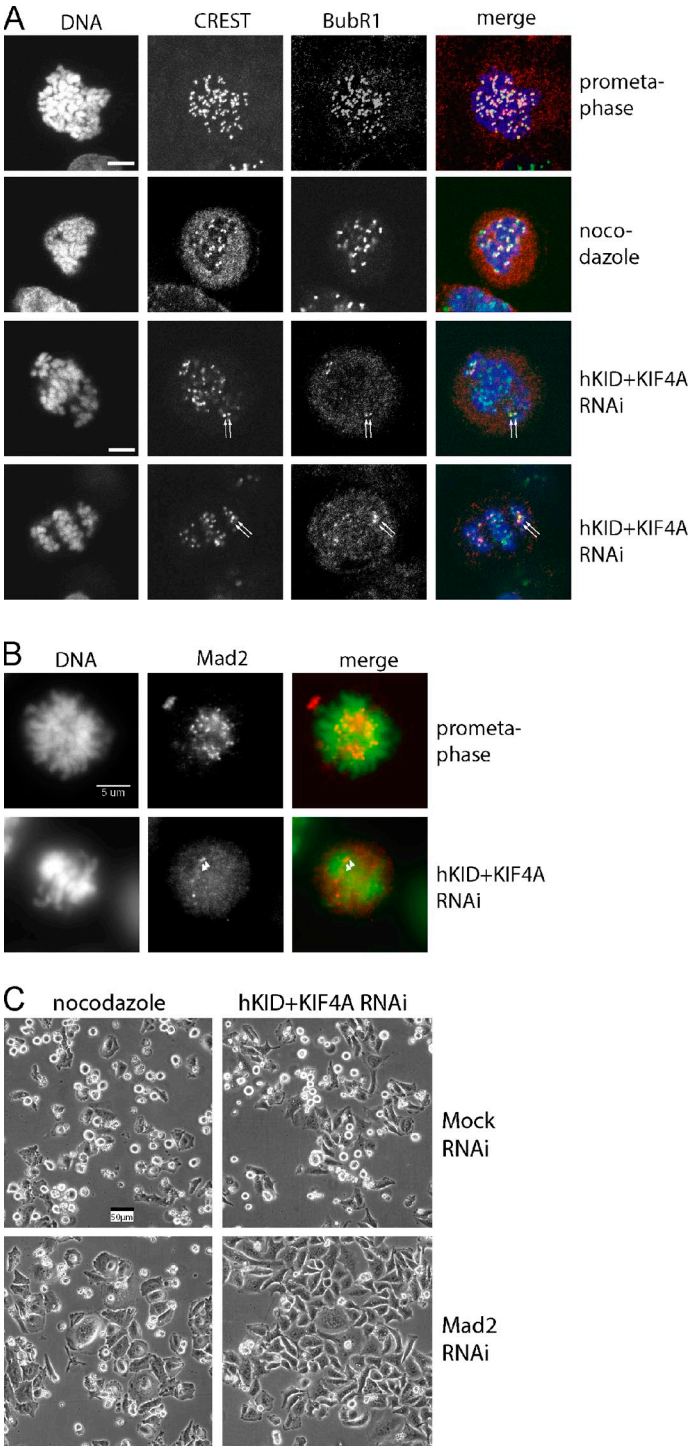


Figure S2. **The mitotic arrest upon chromokinesin knockdown depends on the mitotic checkpoint.** (A) HeLa cells stained for DNA (Hoechst), kinetochores (CREST serum), and BubR1 (green in the image overlay) reveal that BubR1 is only present at kinetochores of uncongressed chromosomes in hKID/KIF4A knockdown cells (arrows). Bar, 5 µm. (B) HeLa cells stained for DNA (Hoechst) and Mad2 (red) exhibit Mad2-positive kinetochores in hKID/KIF4A RNAi cells. (C) Phase-contrast images of HeLa cells after combined knockdown (kd) of hKID/KIF4A and Mad2 or mock, respectively. Depletion of Mad2 prevents the mitotic arrest caused by kd of hKID/KIF4A or nocodazole treatment. Due to the deficient spindle assembly checkpoint, cells undergo mitosis with frequent chromosome segregation errors causing micronuclei and aneuploid daughter cells. Bar, 50 µm.

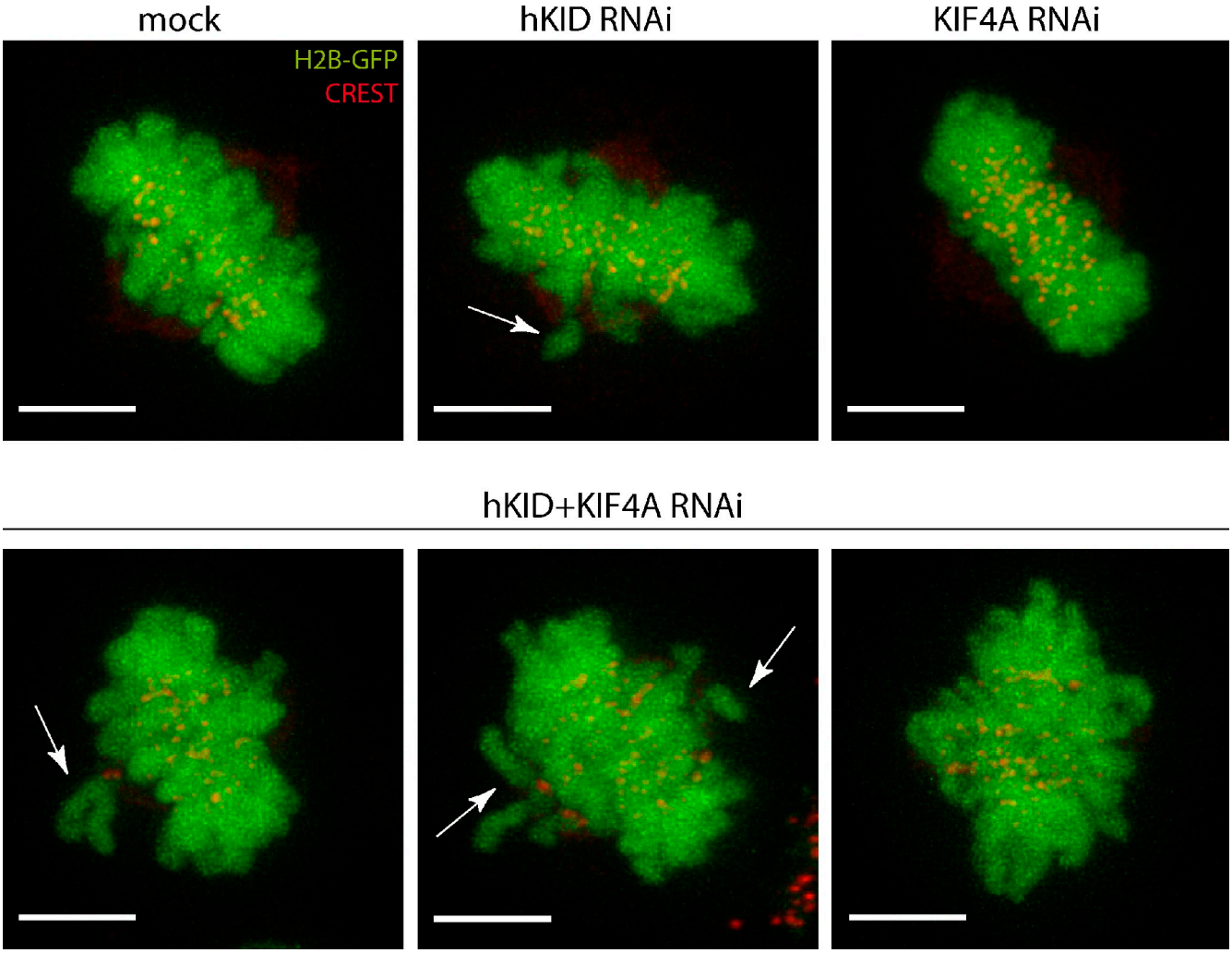


Figure S3. **Loss of hKID causes misorientation of long chromosome arms, which is strongly enhanced by additional loss of KIF4A.** Confocal z-stacks of kinetochore-stained (CREST, red) HeLa H2B-GFP cells after different siRNA treatments were processed by deconvolution and displayed as maximum intensity projections. Note that chromosomes are nicely aligned at the metaphase plate in control and KIF4A RNAi treated cells, whereas loss of hKID causes slight failures in chromosome arm orientation (arrows). The malorientation of long chromosome arms (arrows) is clearly visible in hKID/KIF4A-depleted cells. Bar, 5 μm.

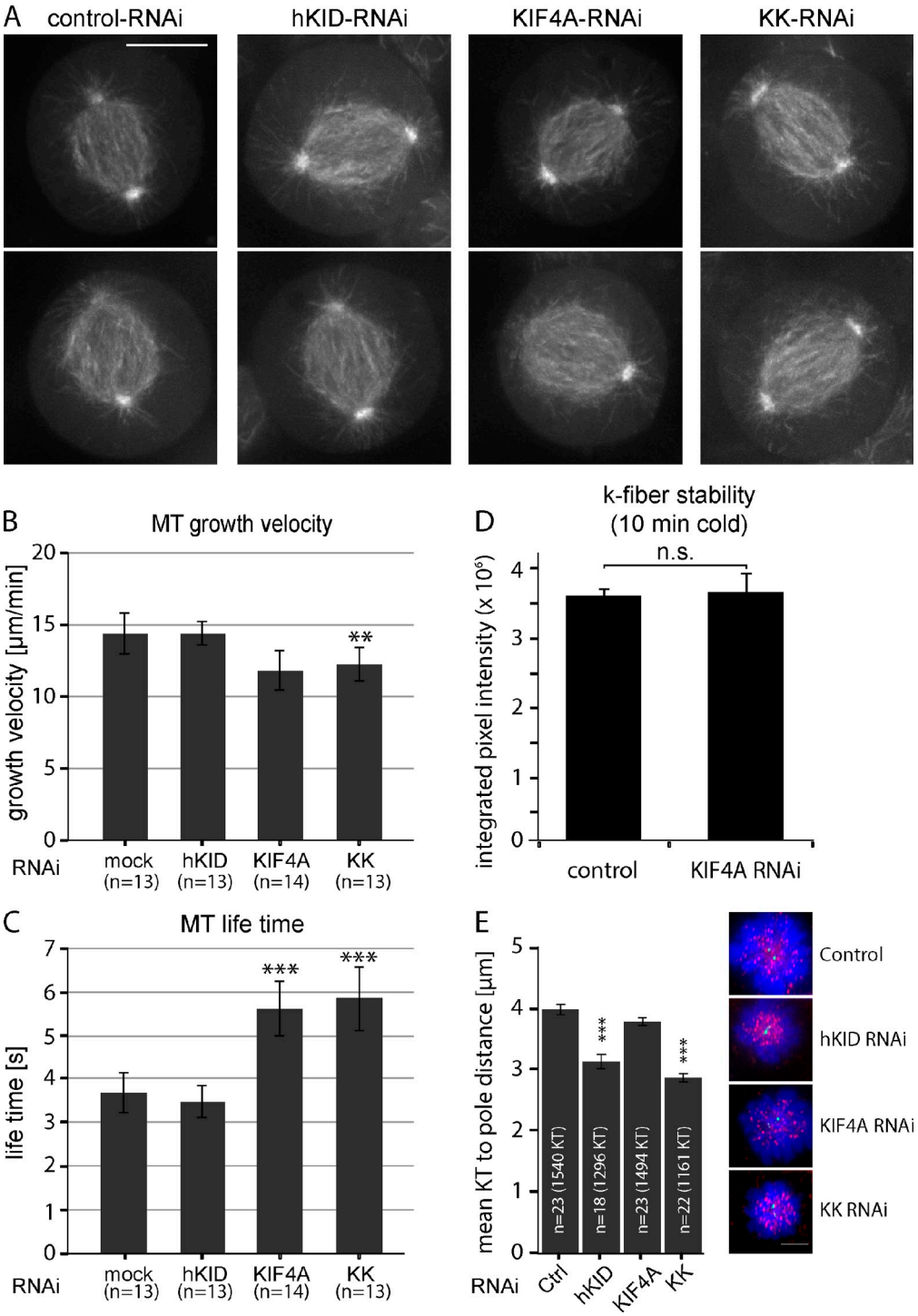


Figure S4. **Loss of KIF4A prolongs MT lifetime.** (A) HeLa EB3-YFP cells were transfected as in Fig. 1 and imaged on a spinning disk microscope (Axio Observer Z1; Carl Zeiss) with 400-ms frame-to-frame interval. Maximum intensity projections of 20-s time series. Bar, 10 µm. (B and C) EB3-YFP–labeled MT plus-ends were tracked using an ImageJ plugin for manual tracking (F.P. Cordelières). For each treatment, 13–14 cells (from two independent experiments) were analyzed (6–10 tracks per cell). Bars represent mean ± SD. MT growth speed (as shown in B) and lifetime/track length (C) were determined. (D) K-fiber stability after 10 min cold treatment and immunofluorescence staining was quantified as the integrated pixel intensity of α-tubulin signal and normalized against CREST staining. Two asterisks indicate Student's *t* test value $P < 0.01$; three asterisks $P < 0.001$. (E) HeLa cells expressing EGFP-centrin1 and α-tubulin–mRFP were treated with 100 µM monastrol for 3 h and stained for HEC1 (outer kinetochore marker) and DAPI (DNA marker). Bar, 5 µm. The mean distances between kinetochores and the closest centriole were measured in monopolar spindles. Mean ± SEM of cells from 2–3 independent experiments; *n* = 18–23 cells, >1,000 kinetochores. (***, $P < 0.001$).

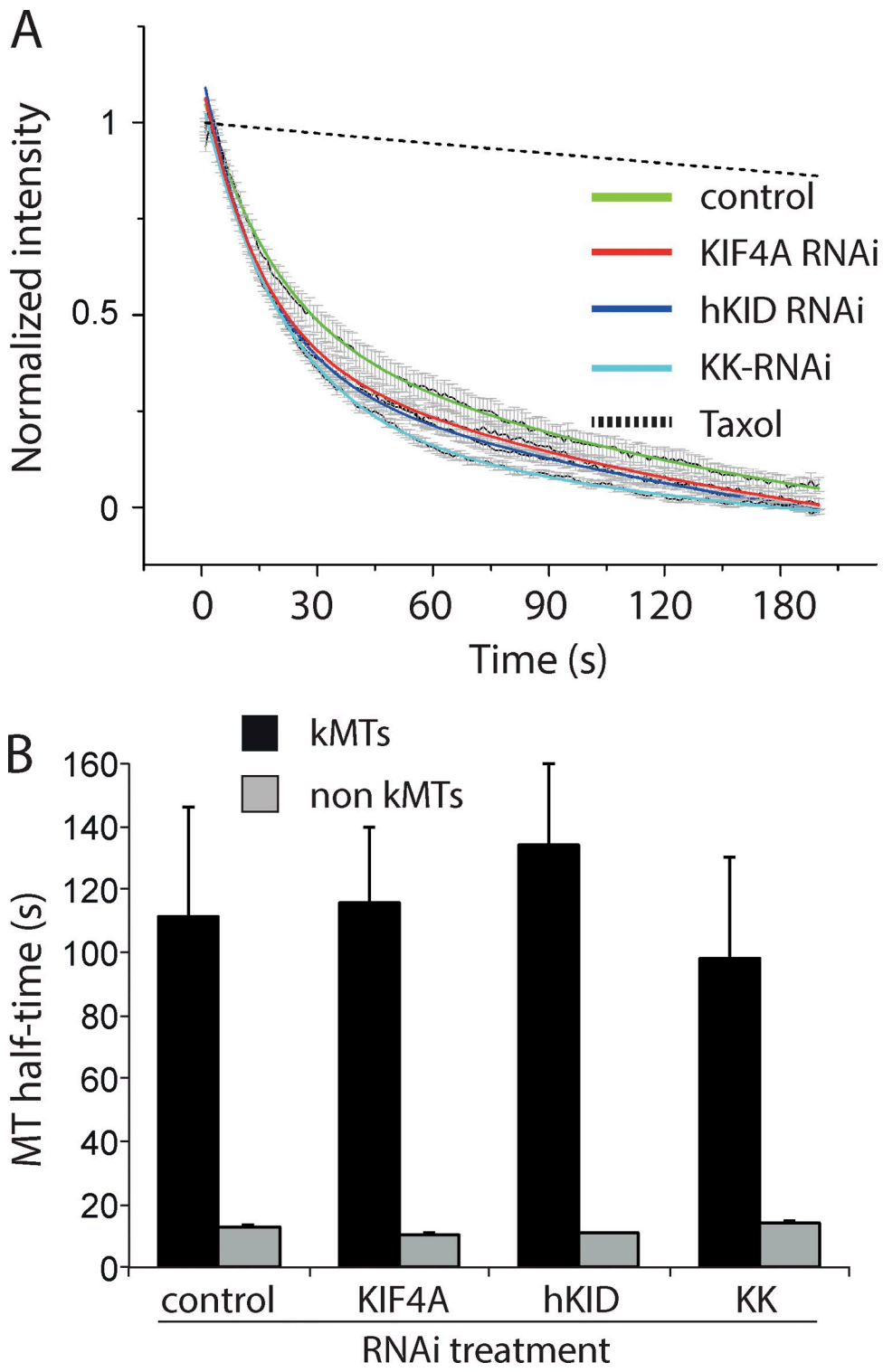
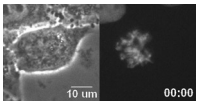
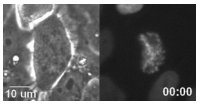


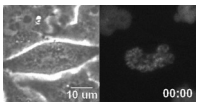
Figure S5. **KIF4A knockdown does not affect MT turnover.** (A) Normalized fluorescence intensity over time after photo-conversion of late prometaphase/metaphase U2OS-mEos-tubulin cells. Data points represent mean ± SEM, *n* = 11–17 cells. (B) MT half-lives of control, KIF4A, hKID, or KK double RNAi late prometaphase/metaphase U2OS-mEos-tubulin cells did not show any significant difference in the half-lives of kinetochore MTs (kMTs) or nonkinetochore MTs (non kMTs) according to *t* test ($P > 0.05$). Error bars represent SEM; *n* = 11–17 cells.



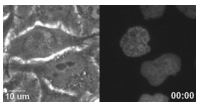
Video 1. **Control mitosis.** HeLa cells stably expressing histone H2B-GFP (green) were transfected with control siRNA (targeting Luciferase) and time-lapse movies were acquired 30–40 h after transfection. Prometaphase cells were selected (as judged by their histone H2B-GFP fluorescence) and imaged through mitosis. For each time point, phase-contrast and GFP images were acquired (Axiovert 200M; Carl Zeiss). A representative cell is shown. Images were taken every min over a period of 6–10 h. Time is h:min.



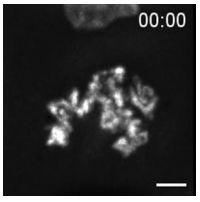
Video 2. **Mitosis in hKID RNAi cells.** HeLa cells stably expressing histone H2B-GFP (green) were transfected with hKID siRNA and time-lapse movies were acquired 30–40 h after transfection. Prometaphase cells were selected (as judged by their histone H2B-GFP fluorescence) and imaged through mitosis. For each time point, phase-contrast and GFP images were acquired (Axiovert 200M; Carl Zeiss). A representative cell is shown. Images were taken every min over a period of 6–10 h. Time is h:min.



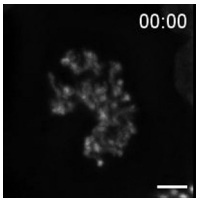
Video 3. **Mitosis in KIF4A RNAi cells.** HeLa cells stably expressing histone H2B-GFP (green) were transfected with KIF4A siRNA and time-lapse movies were acquired 30–40 h after transfection. Prometaphase cells were selected (as judged by their histone H2B-GFP fluorescence) and imaged through mitosis. For each time point, phase-contrast and GFP images were acquired (Axiovert 200M; Carl Zeiss). A representative cell is shown. Images were taken every min over a period of 6–10 h. Time is h:min.



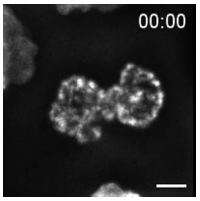
Video 4. **Mitosis in hKID/KIF4A double RNAi cells.** HeLa cells stably expressing histone H2B-GFP (green) were transfected with hKID/KIF4A siRNA and time-lapse movies were acquired 30–40 h after transfection. Prometaphase cells were selected (as judged by their histone H2B-GFP fluorescence) and imaged through mitosis. For each time point, phase-contrast and GFP images were acquired (Axiovert 200M; Carl Zeiss). A representative cell is shown. Images were taken every min over a period of 6–10 h. Time is h:min.



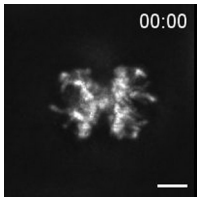
Video 5. **High resolution time-lapse imaging of mitosis in control cells.** HeLa cells stably expressing histone H2B-GFP (green) were transfected with control siRNA (targeting Luciferase) and time-lapse movies were acquired 30 h after transfection using a microscope (Axiovert 200M; Carl Zeiss). 30 z-stacks (step size 0.5 μm) were acquired every 15 s for a total time span of 30 min and processed by deconvolution using Huygens software (Scientific Volume Imaging). Maximum intensity projections were generated to visualize time-lapse recordings. Time is min:s, Bar, 5 μm.



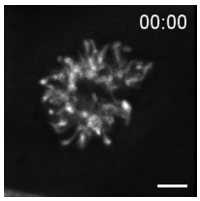
Video 6. **High resolution time-lapse imaging of mitosis in hKID RNAi cells.** HeLa cells stably expressing histone H2B-GFP (green) were transfected with hKID siRNA and time-lapse movies were acquired 30 h after transfection using a microscope (Axiovert 200M; Carl Zeiss). 30 z-stacks (step size 0.5 μm) were acquired every 15 s for a total time span of 30 min and processed by deconvolution using Huygens software (Scientific Volume Imaging). Maximum intensity projections were generated to visualize time-lapse recordings. Time is min:s. Bar, 5 μm.



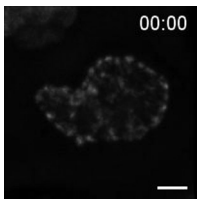
Video 7. **High resolution time-lapse imaging of mitosis in KIF4A RNAi cells.** HeLa cells stably expressing histone H2B-GFP (green) were transfected with KIF4A siRNA and time-lapse movies were acquired 30 h after transfection using a microscope (Axiovert 200M; Carl Zeiss). 30 z-stacks (step size 0.5 μm) were acquired every 15 s for a total time span of 30 min and processed by deconvolution using Huygens software (Scientific Volume Imaging). Maximum intensity projections were generated to visualize time-lapse recordings. Time is min:s. Bar, 5 μm.



Video 8. **High resolution time-lapse imaging of mitosis in hKID/KIF4A double RNAi cells.** HeLa cells stably expressing histone H2B-GFP (green) were transfected with hKID/KIF4A siRNA and time-lapse movies were acquired 30 h after transfection using a microscope (Axiovert 200M; Carl Zeiss). 30 z-stacks (step size 0.5 µm) were acquired every 15 s for a total time span of 30 min and processed by deconvolution using Huygens software (Scientific Volume Imaging). Maximum intensity projections were generated to visualize time-lapse recordings. Time is min:s. Bar, 5 µm.



Video 9. **High resolution time-lapse imaging of mitosis in hKID/KIF4A double RNAi cells.** HeLa cells stably expressing histone H2B-GFP (green) were transfected with hKID/KIF4A siRNA and time-lapse movies were acquired 30 h after transfection using a microscope (Axiovert 200M; Carl Zeiss). 30 z-stacks (step size 0.5 µm) were acquired every 15 s for a total time span of 30 min and processed by deconvolution using Huygens software (Scientific Volume Imaging). Maximum intensity projections were generated to visualize time-lapse recordings. Time is min:s. Bar, 5 µm.



Video 10. **High resolution time-lapse imaging of mitosis in hKID/KIF4A double RNAi cells.** HeLa cells stably expressing histone H2B-GFP (green) were transfected with hKID/KIF4A siRNA and time-lapse movies were acquired 30 h after transfection using a microscope (Axiovert 200M; Carl Zeiss). 30 z-stacks (step size 0.5 µm) were acquired every 15 s for a total time span of 30 min and processed by deconvolution using Huygens software (Scientific Volume Imaging). Maximum intensity projections were generated to visualize time-lapse recordings. Time is min:s. Bar, 5 µm.