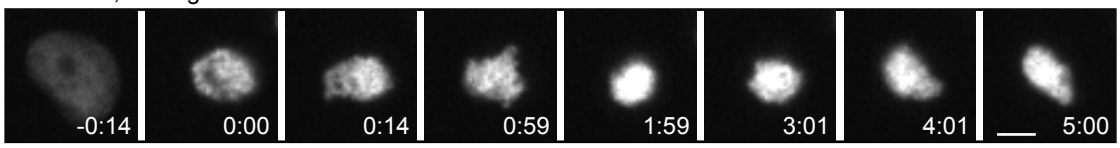
Dick & Gerlich, Supplementary Figure S1

a



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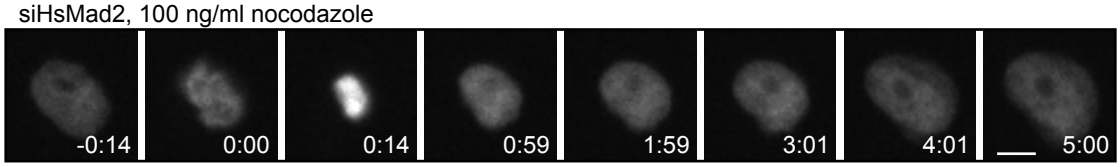
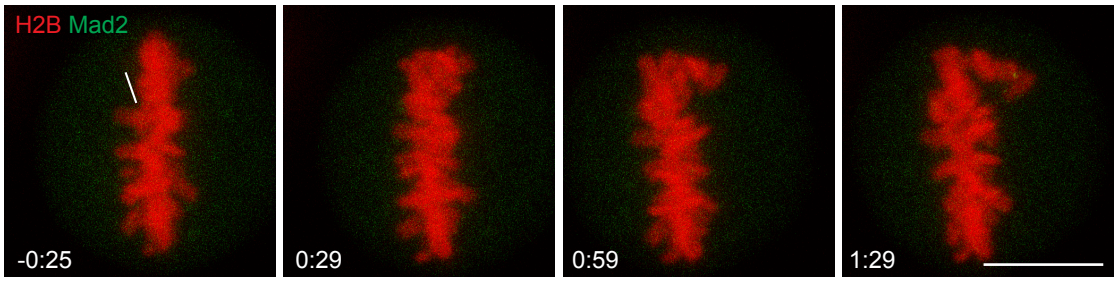


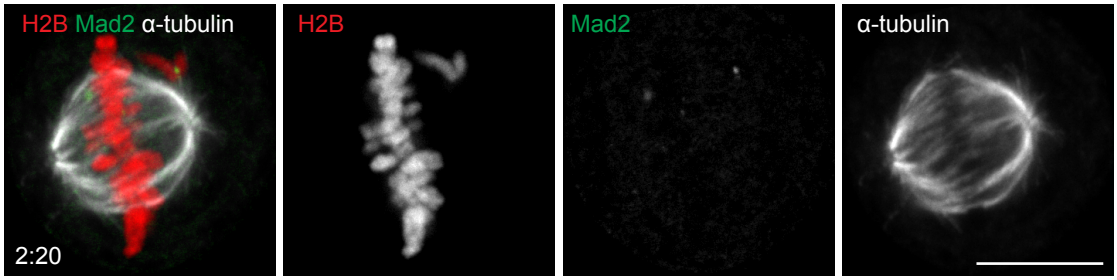
c

Cell line	RNAi: siControl	RNAi: siHsMad2
Securin-EGFP H2B-mCherry	0 %	93 %
EGFP-mmMad2 H2B-mCherry	0 %	0 %

d



e



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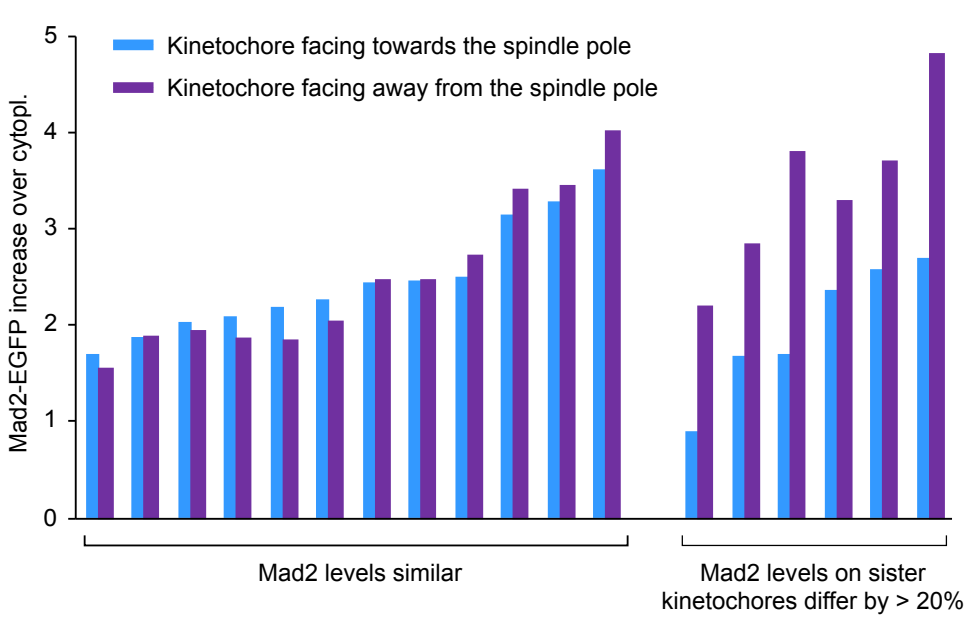
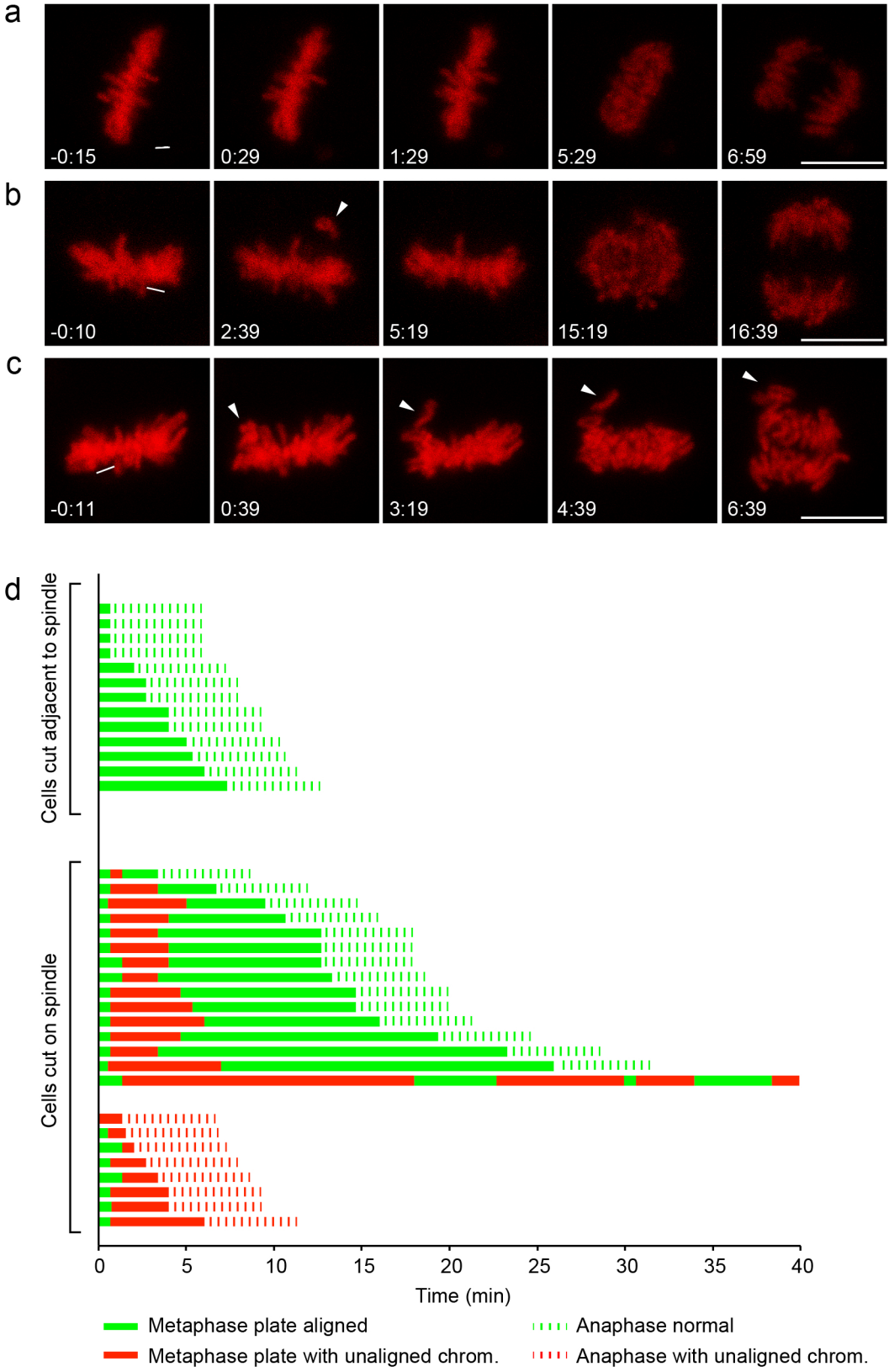
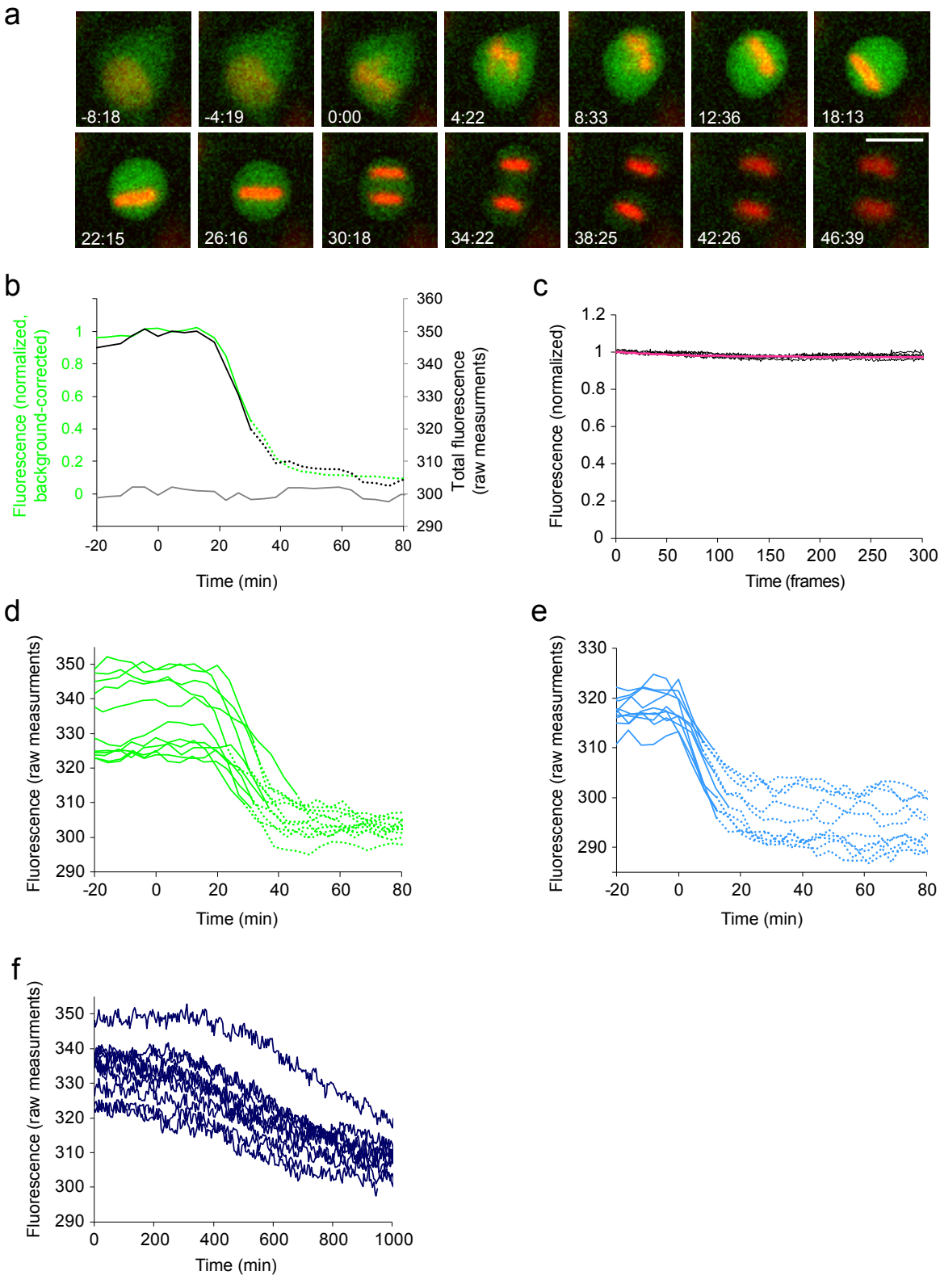


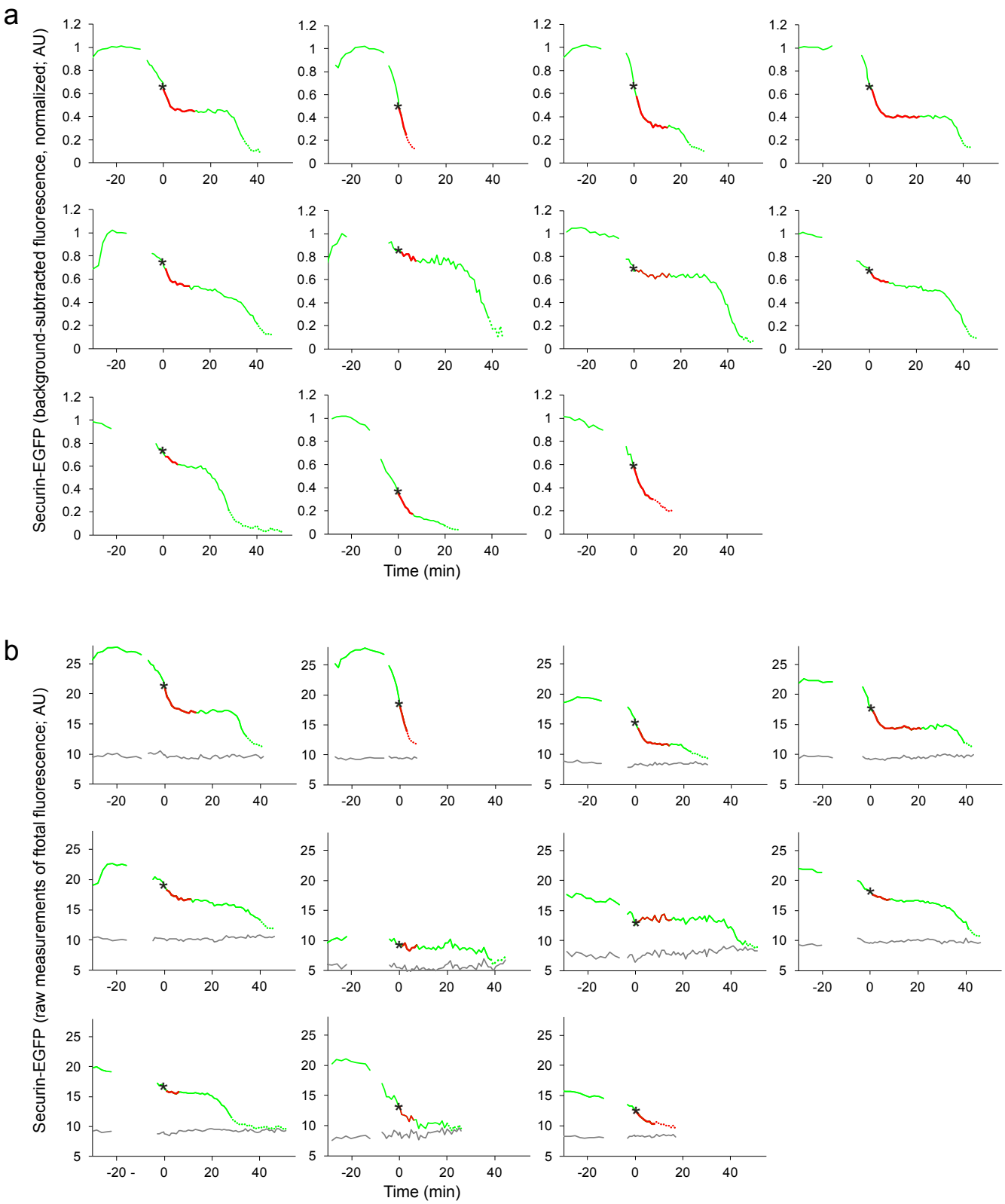
Dick & Gerlich, Supplementary Figure S2



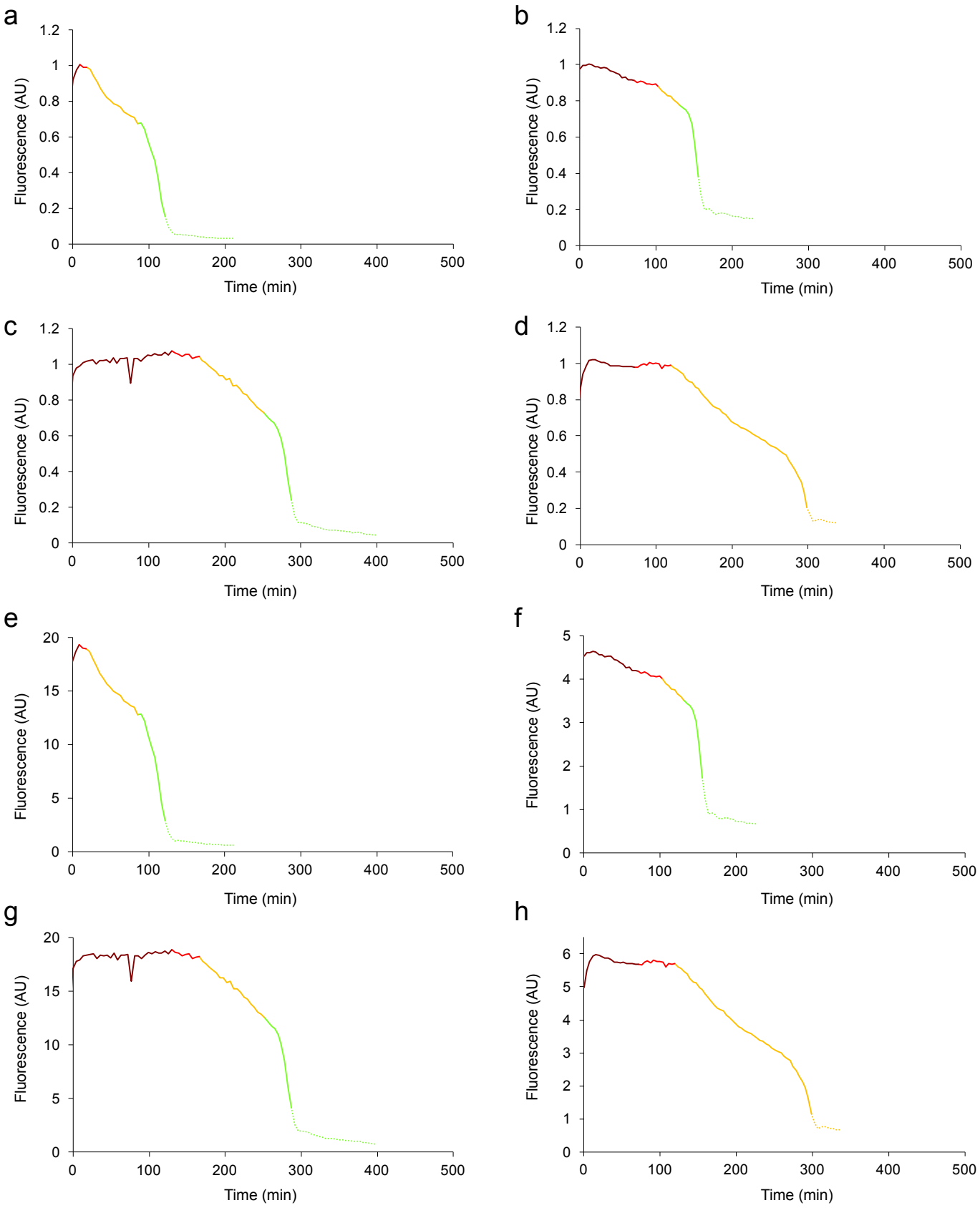
Dick & Gerlich, Supplementary Figure S3



Dick & Gerlich, Supplementary Figure S4



Dick & Gerlich, Supplementary Figure S5



Supplementary Figure Legends

Supplementary Figure S1.

(**a-c)** Validation of the mouse Mad2-EGFP-expressing cell line. (**a**) Prolonged mitosis in presence of 100 ng/ml nocodazole. HeLa cells stably expressing H2B-mCherry and securin-mEGFP were transfected with non-targeting control siRNA and imaged live after 24 h in presence of 100 ng/ml nocodazole. Time = 0:00 h:min at prometaphase onset. (**b**) Mitotic slippage after depletion of Mad2. Imaging was as in (a), but this cell was transfected with siRNA targeting human Mad2 (siHsMad2), 24 h prior to imaging. Bars 5 μm. (**c**) Mitotic slippage induced by transfection of siHsMad2 is suppressed in HeLa cells expressing mouse Mad2-EGFP. Imaging was as in (a, b) using cells stably expressing H2B-mCherry and securin-mEGFP, or H2B-mCherry and mouse-Mad2-EGFP. Mitotic slippage within 3 h after mitotic entry was scored based on the condensation status of H2B-mCherry (n = 30 for each condition). (**d-f**) Correlative laser microsurgery, time-lapse imaging, and immunofluorescence staining of the spindle as in Fig. 1b, c. (d) A live metaphase HeLa cell expressing H2B-mCherry and Mad2-EGFP was cut with a pulsed 915 nm laser at the area indicated by the white line, and imaged by 3D-confocal live-cell microscopy. (e) The cell shown in (d) was fixed 2:20 min:s after laser microsurgery and stained for α-tubulin. Bars: 10 μm. (f) Quantification of Mad2-EGFP levels on both sister kinetochores of 18 laser-displaced chromosomes with two Mad2 positive kinetochores.

Supplementary Figure S2. Fate of non-cancerous retinal pigmental epithelium cells, immortalized by stable overexpression of hTERT (hTERT-RPE1), after laser-induced chromosome detachment. hTERT-RPE1 cells stably expressing H2B-mCherry were cut with a pulsed 915 nm laser at the area indicated with the white lines and imaged by 3D-confocal live-cell microscopy for 40 min. Time = 0:00 min:s at the first image acquired immediately after laser microsurgery. (**a**) A representative control cell was cut in a cytoplasmic region away from the spindle so that no chromosome was detached. (**b**) A representative cell in which a single chromosome was displaced from the metaphase plate by laser microsurgery, which subsequently recongresses before anaphase onset. (**c**) As in (b), but this cell enters anaphase in presence of an unaligned chromosome. Bars: 10 μm. (**d**) Fate trajectories of 36 cells, of which 13 control cells were cut adjacent to the spindle without perturbing the metaphase plate, and 23 cells were cut on the spindle to displace one or few chromosomes from the metaphase plate.

Supplementary Figure S3. Analysis of securin-mEGFP degradation. (a) Original image frames of a HeLa cell stably expressing securin-mEGFP and H2B-mCherry. (b) Raw

measurements of total mEGFP-securin fluorescence (black curve; scale is indicated to the right of the plot), overlaid on background-subtracted and bleach-corrected curve normalized to prometaphase (green, scale on left y-axis). Solid line indicates pre-anaphase stages, dashed line indicates post-anaphase stages. Light gray line indicates background, measured in a region adjacent to the cell. (c) Acquisition photobleaching was measured in a mitotic cell treated with 200 ng/ml nocodazole, with fast time-lapse to minimize the effect of mitotic slippage degradation (time-lapse: 2.7 s/frame). An exponential function (pink curve) was fitted to 5 bleach measurements (black curves). This function was determined separately for each experimental condition to correct for acquisition photobleaching as indicated in the online methods. (d) Raw total fluorescence measurements for the data shown in Fig. 5a (control cells, non-targeting siRNA). (e) Raw total fluorescence measurements for the data shown in Fig. 5a (Mad2 RNAi). (f) Raw measurements for the data shown in Fig. 5b.

Supplementary Figure S4. Kinetics of APC/C inhibition after chromosome detachment for 11 cells, as shown for two selected examples in Figure 4c, d. HeLa cells expressing H2B-mCherry and securin-mEGFP were imaged by 3D-confocal microscopy from prophase until metaphase. At different time points during metaphase (indicated by black star), the spindle was cut by a pulsed 915 nm laser to detach individual chromosomes. Time = 0 min at the first image acquired immediately after laser microsurgery. Red indicates time points where individual chromosomes were dealigned from the metaphase plate. Dashed lines indicate anaphase, with (red) or without (green) unaligned chromosomes. The gaps in the curve indicate pauses during time-lapse imaging to reduce light exposure. (a) Background-subtracted total fluorescence was normalized to prometaphase as shown in Fig. 4c, d. The first two panels represent the cells shown in Fig. 4. (b) Raw measurements of total fluorescence intensity in mean intensity projections of z-stacks. Gray curves indicate measurements outside cells used for background subtraction.

Supplementary Figure S5. Four additional examples for securin-mEGFP degradation in correlation with unaligned chromosomes induced by low dose nocodazole. HeLa cells expressing H2B-mCherry and securin-mEGFP were imaged and analyzed as in Fig. 5f, g. Dark red indicates time intervals with >5 unaligned chromosomes, light red indicates time intervals with 2-5 unaligned chromosomes, orange indicates one unaligned chromosome, green indicates alignment of all chromosomes. Solid lines indicate pre-anaphase stages, dashed lines indicate anaphase stages. (a-d) Background-subtracted and bleach corrected total fluorescence normalized to prometaphase. (a) Cell treated with 6 ng/ml nocodazole. (b) 25 ng/ml nocodazole. (c, d) 12 ng/ml nocodazole. (e-h) Background-subtracted raw measurements of the cells shown in a-d.