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Supplemental Information

Micronuclei from misaligned chromosomes that satisfy the spindle assembly checkpoint in cancer cells

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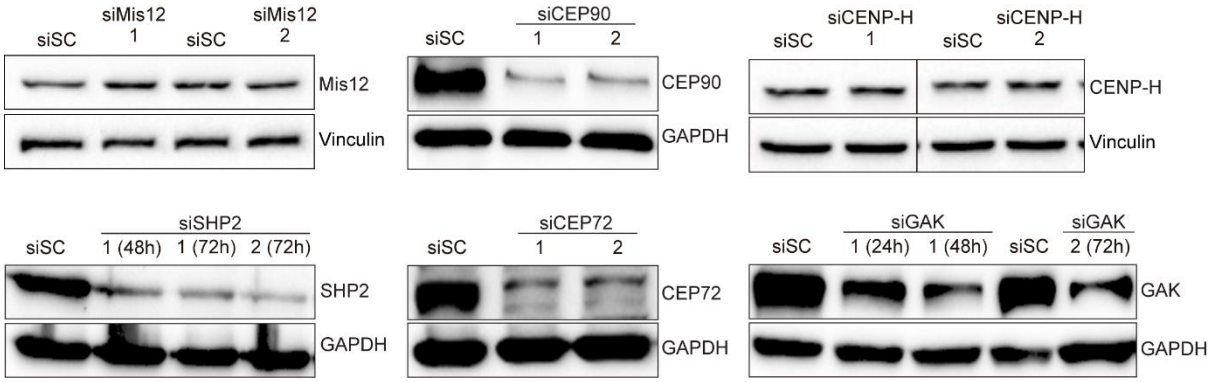
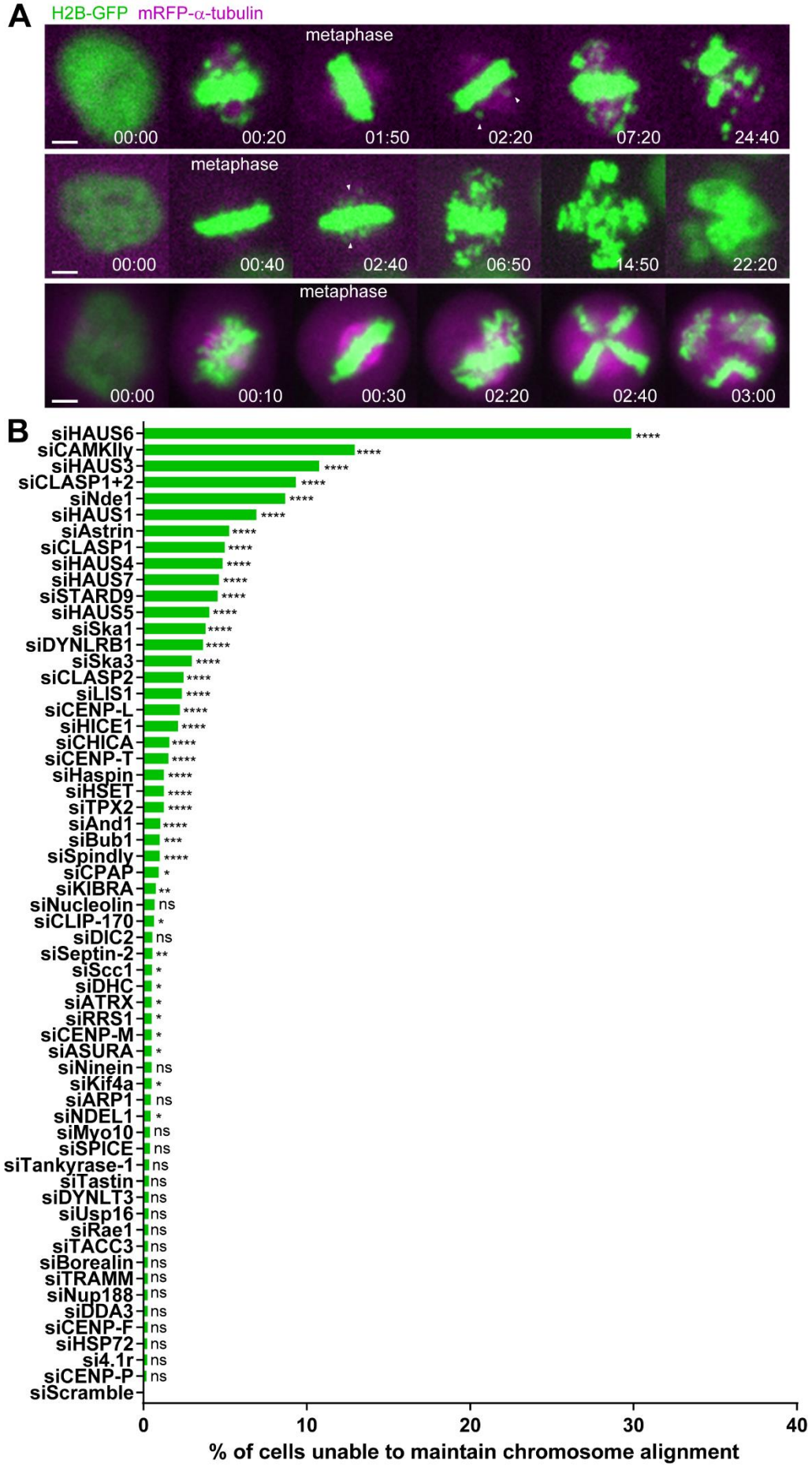


Figure S1. Identification of off-targets, related to Figure 1. Protein lysates obtained after RNAi treatment were immunoblotted with an antibody specific for each protein of interest (Mis12, CEP90, CENP-H, SHP2, CEP72 and GAK, upper bands). The bottom band corresponds to antibody detection of GAPDH or Vinculin, which were used as loading controls.


A
H2B-GFP mRFP-α-tubulin
metaphase
00:00
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B
siHAUS6 ****
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siHAUS5 ****
siSka1 ****
siDYNLRB1 ****
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siCLASP2 ****
siLIS1 ****
siCENP-L ****
siHICE1 ****
siCHICA ****
siCENP-T ****
siHaspin ****
siHSET ****
siTPX2 ****
siAnd1 ****
siBub1 ***
siSpindly ****
siCPAP *
siKIBRA **
siNucleolin ns
siCLIP-170 *
siDIC2 ns
siSeptin-2 **
siScc1 *
siDHC *
siATRX *
siRRS1 *
siCENP-M *
siASURA *
siNinein ns
siKif4a *
siARP1 ns
siNDEL1 *
siMyo10 ns
siSPICE ns
siTankyrase-1 ns
siTastin ns
siDYNLT3 ns
siUsp16 ns
siRae1 ns
siTACC3 ns
siBorealin ns
siTRAMM ns
siNup188 ns
siDDA3 ns
siCENP-F ns
siHSP72 ns
si4.1r ns
siCENP-P ns
siScramble
0
10
20
30
40
% of cells unable to maintain chromosome alignment

Figure S2. In addition to chromosome alignment defects, some genetic conditions also compromise the maintenance of chromosome alignment at the metaphase plate, related to Figure 2. a) Examples of time-lapse sequences illustrating the three main mitotic phenotypes of chromosome alignment maintenance defects observed: 1) cells showed a prolonged delay in chromosome alignment but eventually completed congression, after which chromosomes/chromatids underwent gradual scattering from the metaphase plate; 2) chromosomes aligned normally at the metaphase plate, but then underwent gradual scattering; 3) chromosomes aligned normally at the metaphase plate, followed by spindle pole fragmentation and chromosome scattering. Arrows indicate scattered chromosomes/chromatids in cells that were unable to maintain chromosome alignment at the metaphase plate. Scale bar = 5 µm. Time: h:min, from nuclear envelope breakdown (NEB) to cell death or mitotic exit. **b)** Frequency of cells exhibiting problems in the maintenance of chromosome alignment at the metaphase plate. Only the conditions exhibiting problems in the maintenance of chromosome alignment were included. At least 2 independent experiments were analyzed. The total number of cells analyzed for each condition is indicated in Table S1. (*p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001, ns corresponds to not significantly different from control, Fisher´s exact two-tailed test).

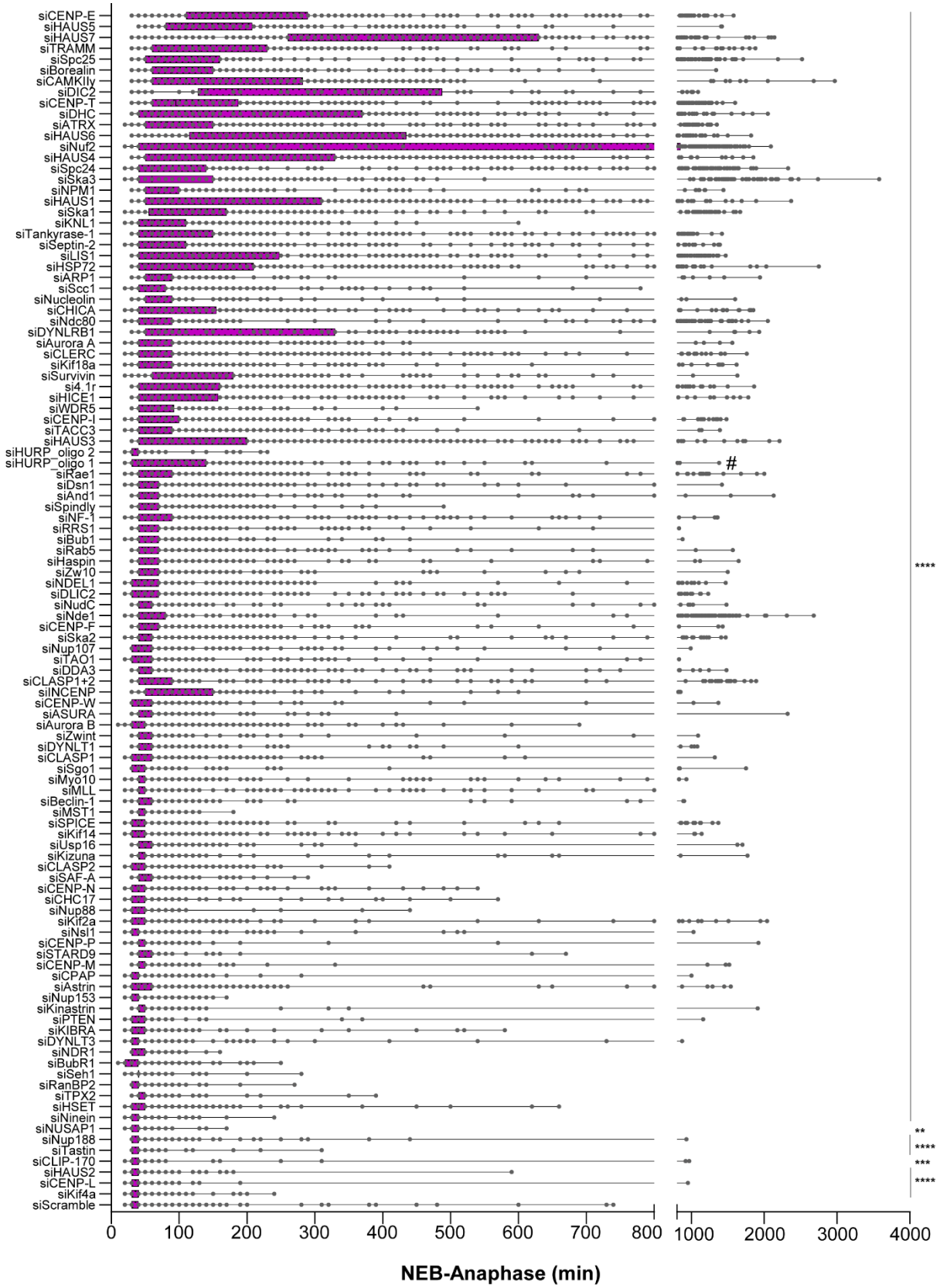

Figure S3. Mitotic duration upon gene-specific RNAi-mediated depletion, related to Figure 3. HeLa cells stably expressing H2B-GFP and α-tubulin-mRFP were

acquired every 10 minutes. Mitotic duration was determined by measuring the time between nuclear envelope breakdown (NEB) and anaphase onset, shown in minutes. Data was presented as box-and-whiskers and each point corresponds to one cell. The difference between mean values of each RNAi condition was statistically significant from the control mean values. At least 2 independent experiments per condition were performed. (*p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001, ns corresponds to not significantly different from control, Mann-Whitney test).

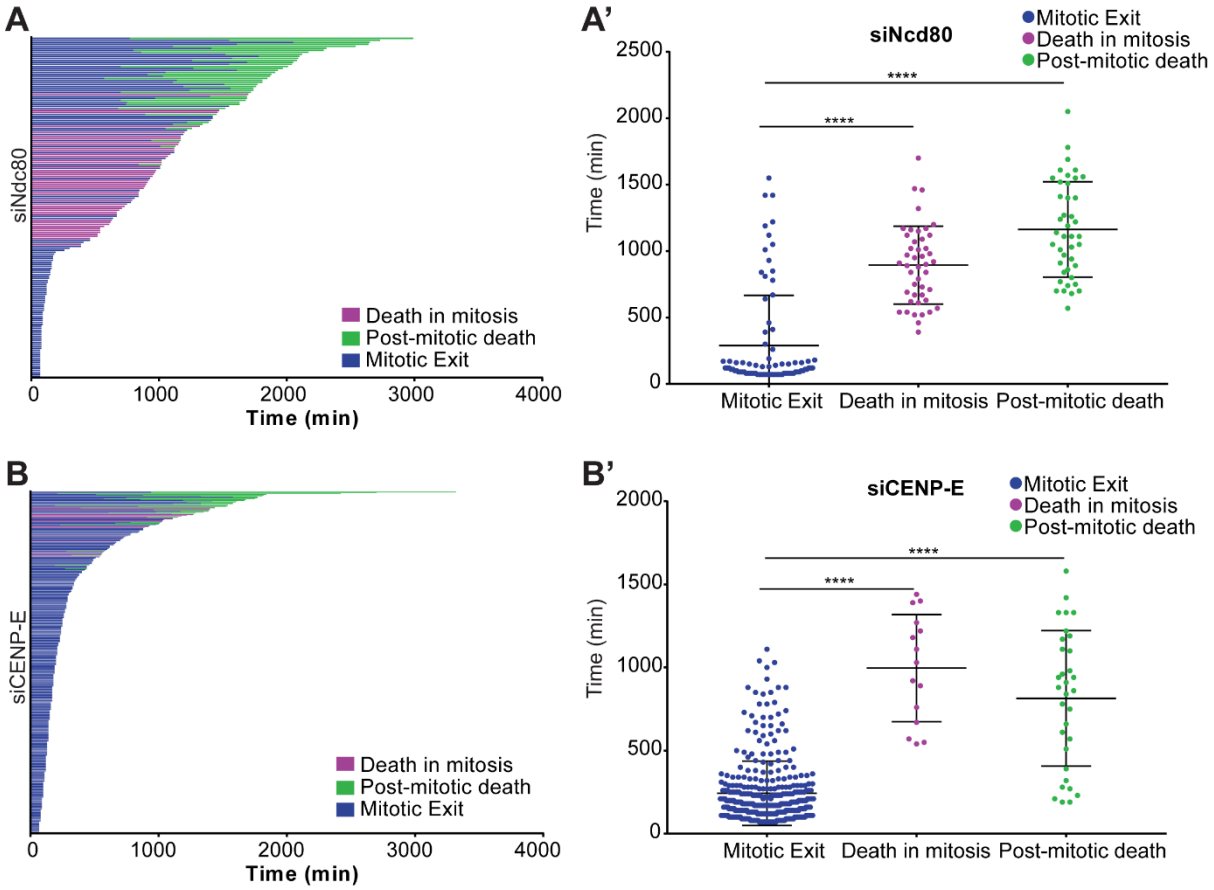

Figure S4. Cell fate upon induction of chromosome alignment defects of distinct molecular nature, related to Figure 3. a) Cell fate profiles of HeLa cells with delayed or failed chromosome alignment after Ndc80 depletion. Each line indicates a single cell and respective outcome. **a´)** Time that cells spent until mitotic exit (blue), death in mitosis (purple) or in subsequent interphase (green) after Ndc80 depletion. Each dot represents a single cell. The horizontal line indicates the mean of all quantified cells and the error bars represent the standard deviation from a pool of four independent experiments (Mitotic exit, 289±377 min, n=83; Death in mitosis, 894±292 min, n=45; Post-mitotic death 1162±359 min, n=42; ****p≤0.0001 relative to control, analyzed using a Mann-Whitney Test). **b)** Cell fate profiles of HeLa cells with delayed or failed chromosome alignment after CENP-E depletion. **b´)** Time that cells spent until mitotic exit (blue), death in mitosis (purple) or in the subsequent interphase (green) after

CENP-E depletion. Each dot represents a single cell. The horizontal line indicates the mean of all quantified cells and the error bars represent the standard deviation from a pool of five independent experiments (Mitotic exit, 243±193 min, n=358; Death in mitosis 996±322 min, n=15; Post-mitotic death 814±408 min, n=32; ****p≤0.0001 relative to control, analyzed using a Mann-Whitney Test).

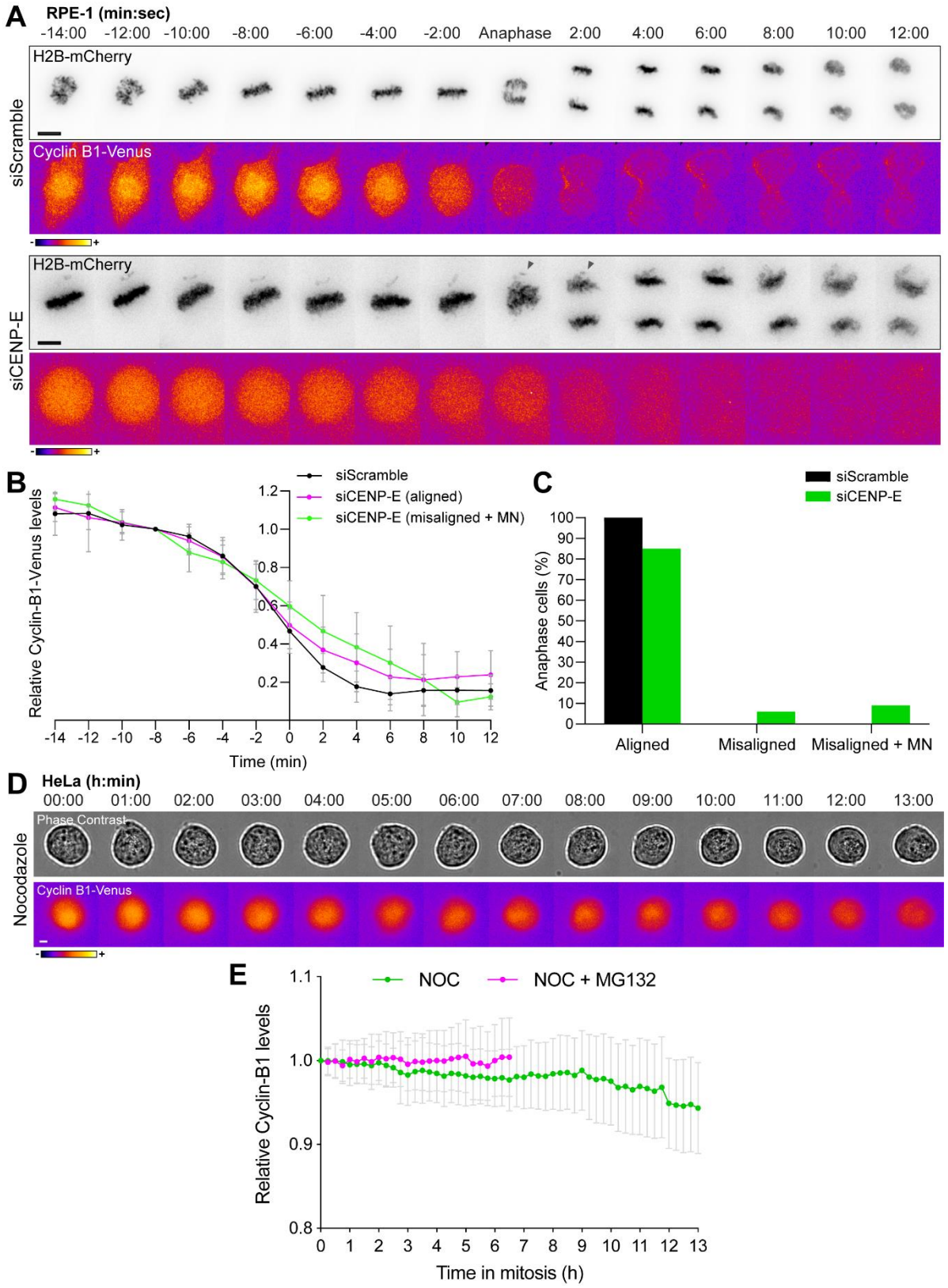

Figure S5. Further characterization of Cyclin B1 degradation profiles in RPE-1 and HeLa cells, related to Figure 5. a) Selective time frames from live-cell

microscopy of RPE-1 cells stably expressing H2B-mRFP and Cyclin B1-Venus in control and siCENP-E. Images were acquired every 2 min. Time = min:sec. Time 00:00 = anaphase onset. Black arrowheads point to misaligned chromosomes that remain upon anaphase onset. **b)** Cyclin B1 degradation profile for control, CENP-E- depleted cells that properly align their chromosomes at the metaphase plate and CENP-E-depleted cells that exit mitosis with misaligned chromosomes give rise to micronuclei. Fluorescence intensities were normalized to the levels at time = -8. The curves represent mean Cyclin B1-Venus fluorescence intensity from all analyzed cells and errors bars represent the standard derivation from a pool of three independent experiments (siScramble n=30; siCENP-E (aligned) n=30; siCENP-E (misaligned+micronuclei) n=5). **c)** Frequency of anaphase cells with aligned chromosomes, misaligned chromosomes and misaligned chromosomes that result in micronuclei in control (black bars) and CENP-E-depleted cells (green bars). **d)** Selected time frames from phase-contrast and fluorescence microscopy of Cyclin B1-Venus HeLa cells treated with nocodazole with or without MG132. Images were acquired every 15 min. Scale bar = 5 µm. Time = h:min. **e)** Cyclin B1 degradation profiles of nocodazole-treated Cyclin B1-Venus HeLa cells in the presence or absence of MG132. Fluorescence intensities were normalized to the levels at time = 0. The curves depict mean Cyclin B1-Venus fluorescence intensity from all analyzed cells per condition (nocodazole n=12; nocodazole + MG132 n=10), and error bars represent the standard deviation. Note that acquisition in the presence of MG132 was terminated earlier relative to acquisition without MG132 due to cell death.

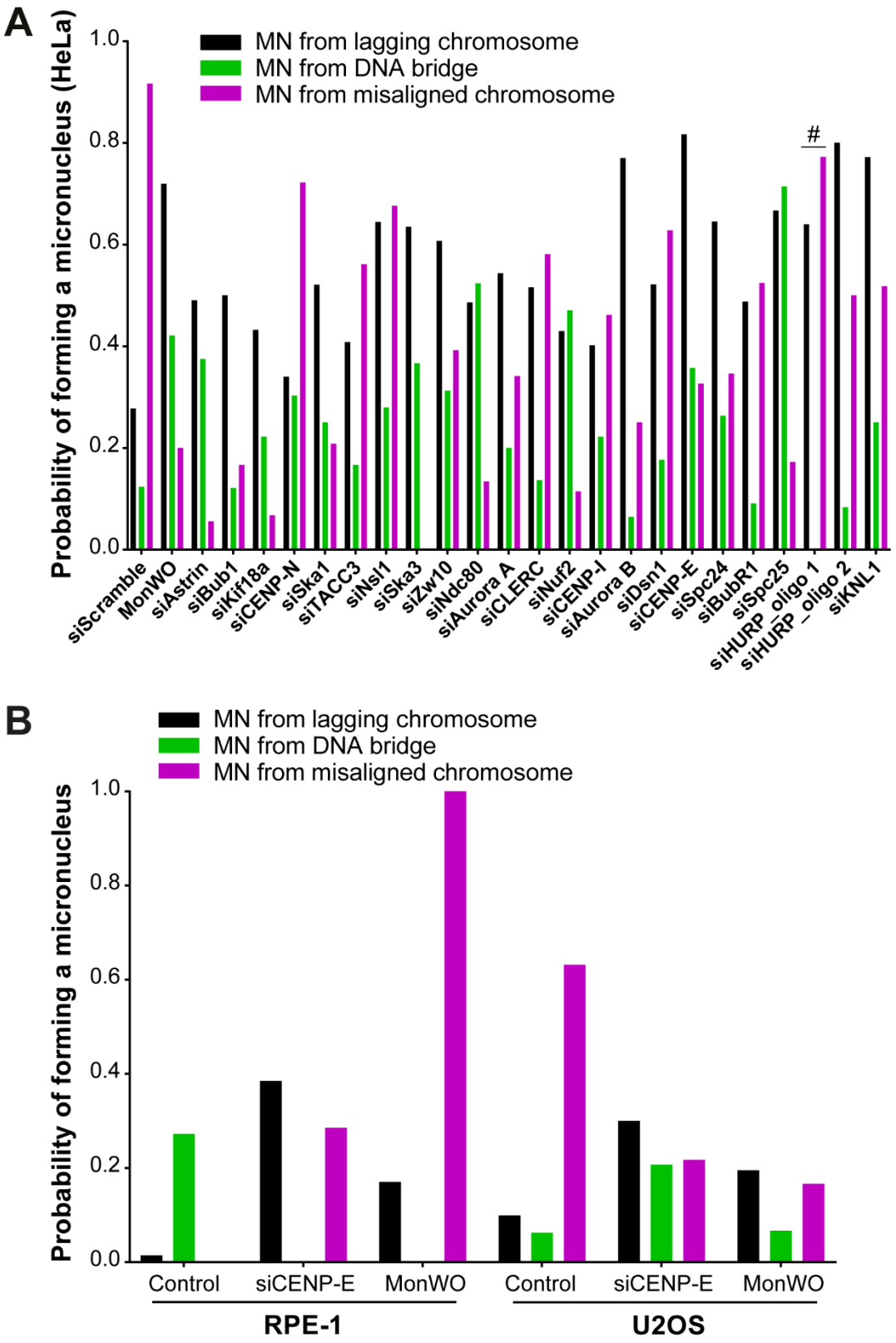

Figure S6. Absolute probabilities of forming a micronucleus from misaligned chromosomes, DNA bridges and lagging chromosomes in the different cell lines used, related to Figure 6 and Figure 7. a) Absolute probabilities of forming a

micronucleus of different origins in unperturbed HeLa cells and after molecular perturbations that weaken kinetochore-microtubule attachments or promote the formation of anaphase lagging chromosomes after monastrol treatment and washout (MonWO). [siScramble n=1700, MonWO n=327, siAstrin n=423, siBub1 n=457, siKif18a n= 540, siCENP-N n= 422, siSka1 n=395, siTACC3 n=485, siNsl1 n=400, siSka3 n= 383, siZw10 n= 404, siNdc80 n=440, siAurora A n= 388, siCLERC n=263, siNuf2 n=428, siCENP-I n= 389, siAurora B n=499, siDsn1 n=688, siCENP-E n= 346, siSpc24 n=418, siBubR1 n=387, siSpc25 n= 425, siHURP_oligo1 n=296, siHURP_oligo2 n=200, siKNL1 n=413; pool of 2 independent experiments for each siRNAi oligonucleotide per condition, with the exception of Aurora A and CLERC in which only 1 experiment for the second siRNAi oligonucleotide was performed. All independent experiments were pooled]. **b)** Absolute probabilities of forming a micronucleus of different origins in unperturbed RPE-1 and U2OS cells and after CENP-E depletion (siCENP-E) or monastrol treatment and washout. [RPE-1 cells: control, n=163; siCENP-E, n=95; MonWO, n=105]. [U2OS cells: control, n=250; siCENP-E, n=81; MonWO, n=49].

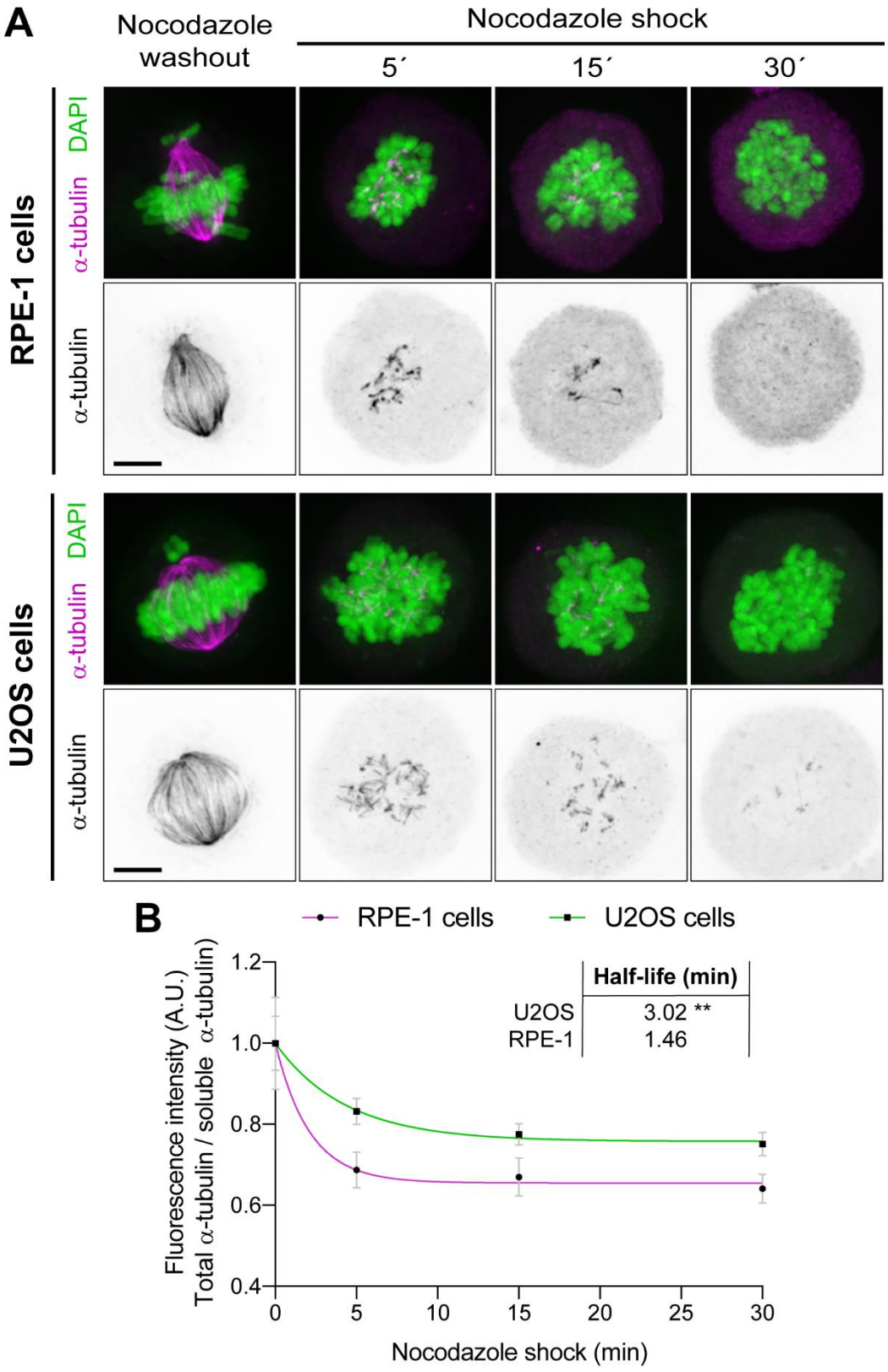

Figure S7. Misaligned chromosomes in chromosomally unstable cancer cells have hyper-stabilized kinetochore-microtubule attachments, related to Figure 7.

a) Representative immunofluorescence images of RPE-1 and U2OS cells stained for

DNA (green) and α-tubulin (magenta). RPE-1 and U2OS cells upon nocodazole treatment and washout to generate misaligned chromosomes were processed for immunofluorescence microscopy after a subsequent nocodazole shock 5, 15 and 30 min after drug addition. Representative immunofluorescence images of the mitotic spindle at each stage are shown. Images are maximum intensity projections of deconvolved z-stacks. Scale bar = 5 µm. **b)** Normalized α-tubulin fluorescence intensity at indicated time points in RPE-1 and U2OS cells after nocodazole shock. Fluorescence intensities were normalized to the levels at time = 0. Data represent mean ± s.d., U2OS n=22 cells, RPE-1 n=22 cells, from 2 independent experiments. Whole lines show single exponential fitting curve (**p≤0.01, extra sum-of-squares F test).

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