Supplemental Materials

Molecular Biology of the Cell

Mackay et al.

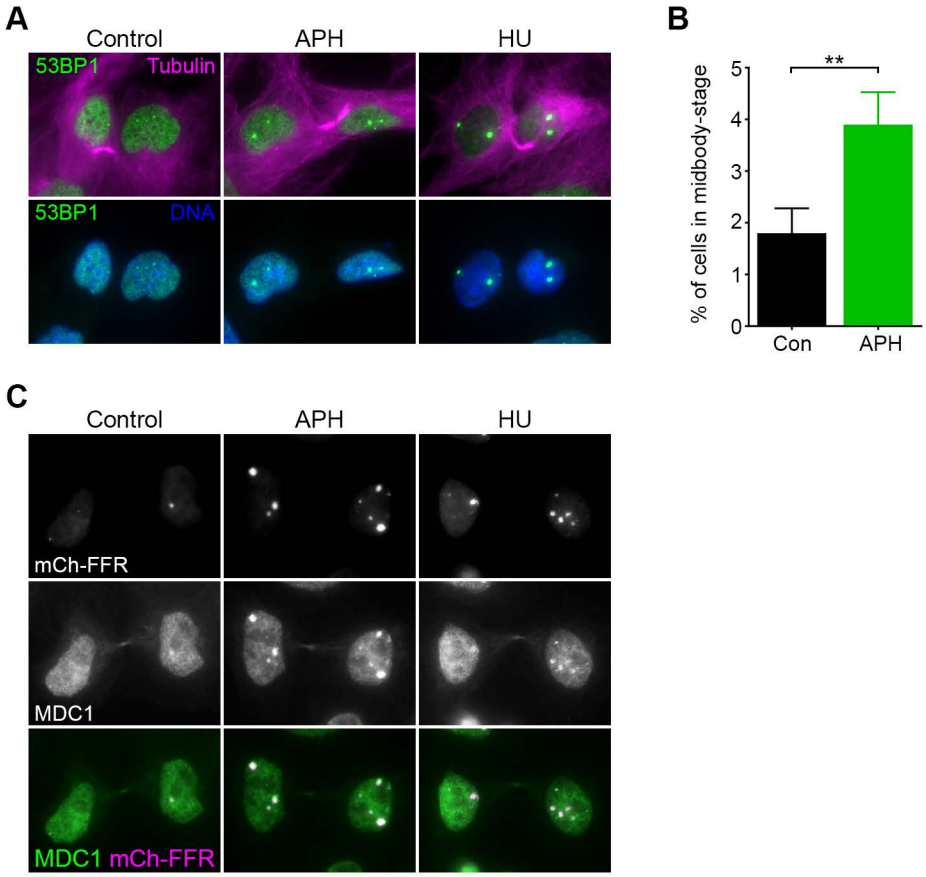

Figure S1. Additional characterization of postmitotic genome surveillance. (A) 53BP1 foci are present in nuclei of BJ-Tert cells prior to abscission. Cells were treated with APH or HU for 24 hours and analyzed for the presence of nuclear foci containing 53BP1 (green), as described in Figure 1. (B) Quantification of the number of midbody-stage cells (BJ-Tert) after the indicated treatments. Error bars are mean and SD from 4 experiments. ***p*=0.002 (Mann-Whitney test). (C) Nuclear foci detected in the mCherry-53BP1-FFR / GFP-tubulin cell line also contain MDC1 (green). Midbody-stage cells were identified by residual GFP-tubulin signal after immunofluroescence staining.

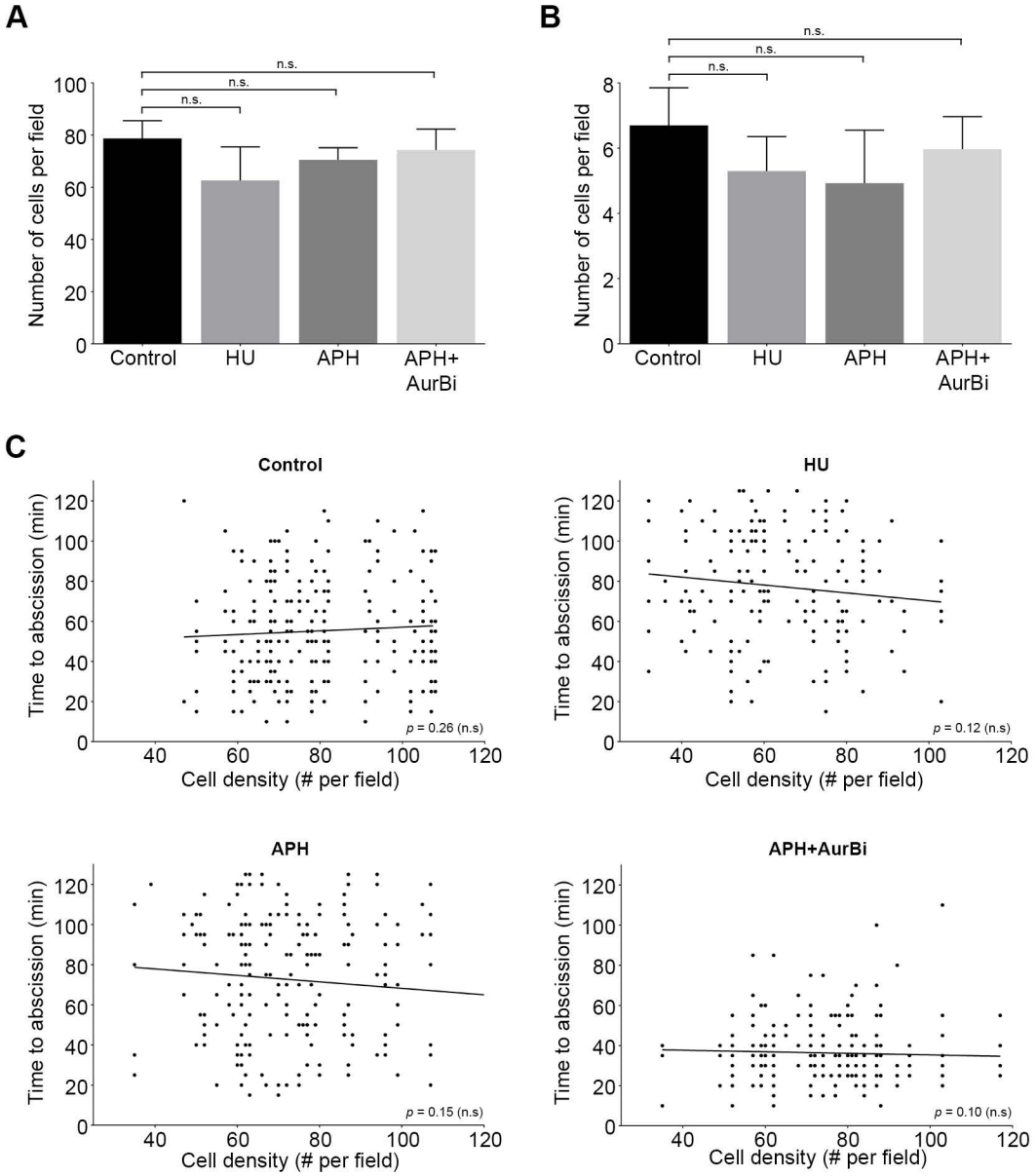

Figure S2. Differences in cell density are not a factor in abscission timing in this study. (A and B) Graphs representing total cell density (number of cells per field) and midbody density per field. Error bars are the mean and SD of at least 3 experiments in which 13 fields were analyzed; n.s., not significant (Mann-Whitney test). Note that the cell density used throughout this study likely corresponds to medium density used by Lafaurie-Janvore et al. (2013). (C) Correlation plots indicate that within the cell density range used here, there was no significant correlation between cell density and abscission timing among the different treatments. *p*-values were determined using the Spearman correlation test.

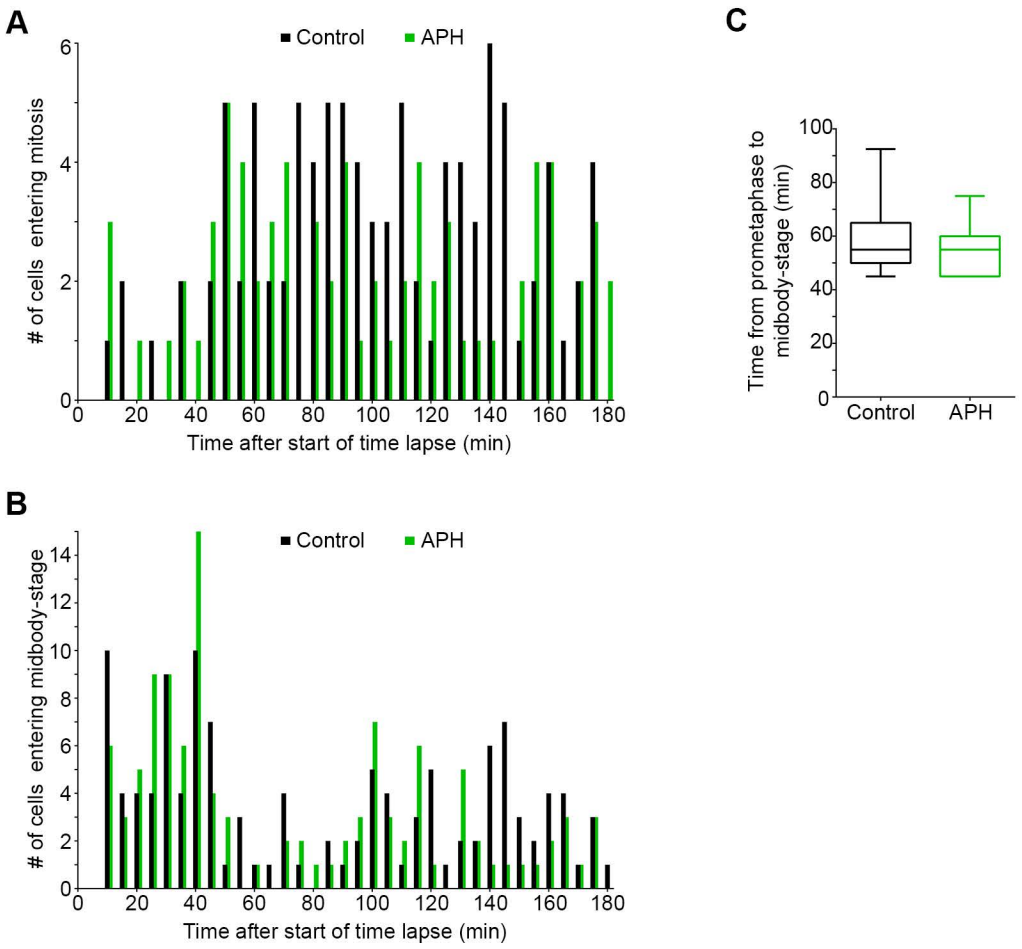

Figure S3. Replication stress conditions used throughout this study do not result in cell cycle synchronization. (A and B) HeLa cells expressing GFP-tubulin and H2B-mCherry were treated for 24 hours with APH or DMSO (Control) and then subjected to time-lapse imaging every 5 minutes for 3 hours. The frame in which cells either entered mitosis (A) or midbody-stage (B) was recorded. Shown is the number of cells entering each stage at the indicated time during the time-lapse imaging. (C) There is no difference in the median time in mitosis (defined as the time from onset of mitosis to midbody formation) between Control and APH-treated cells (55 min).

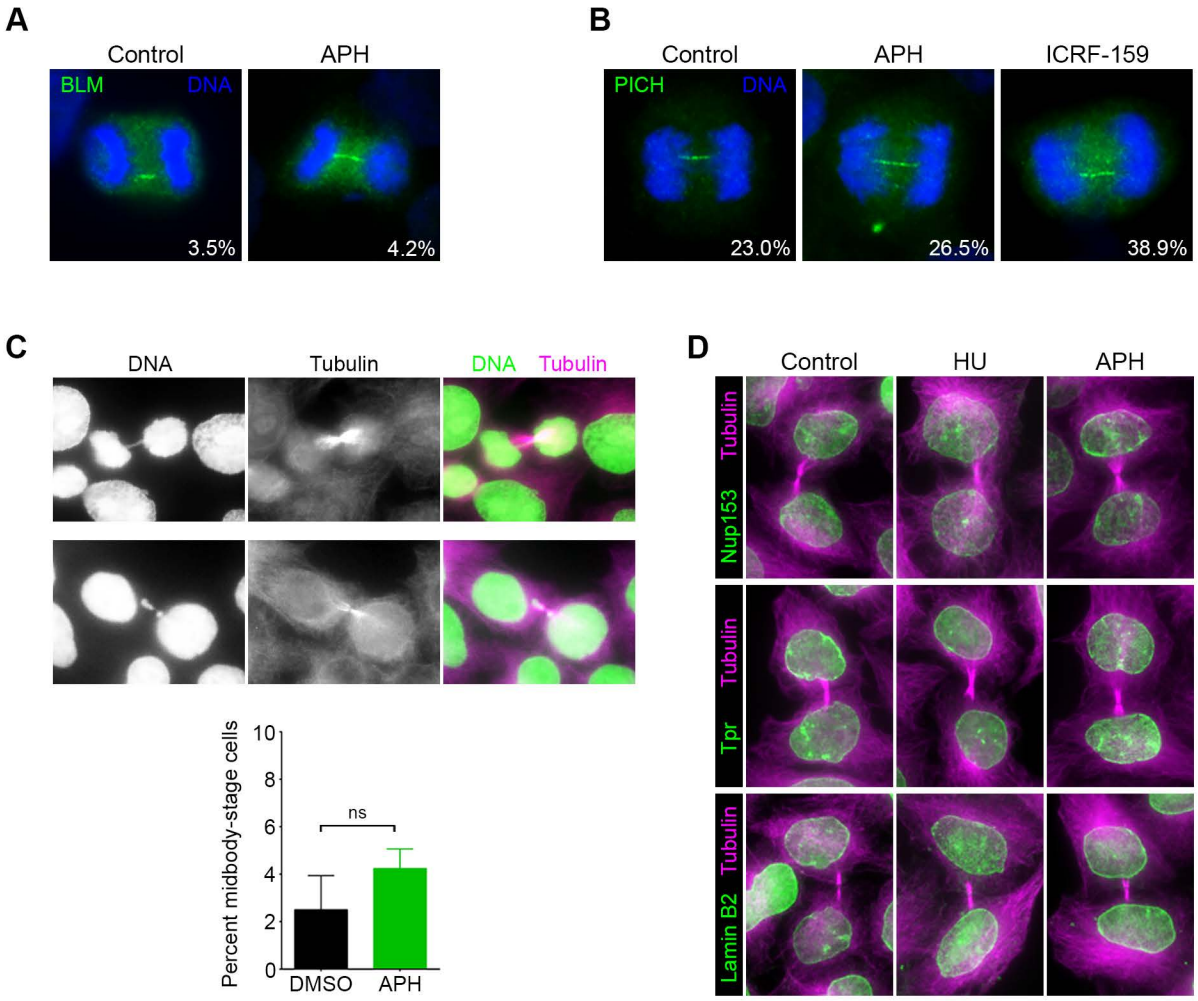

Figure S4. Increased duration of the midbody-stage of cell division following replication stress is not attributable to ultrafine bridges, chromatin bridges, or altered nuclear architecture. (A and B) HeLa cells were cultured for 24 hours in the presence of DMSO (control), 0.4μM aphidicolin (APH), or 10μM ICRF-159 and analyzed for the presence of ultrafine DNA bridges using antibodies directed against either BLM or PICH (green in A and B, respectively). Anaphase / telophase cells were identified by DNA morphology (DAPI, blue) and the extent of cleavage furrow ingression. The percentage of anaphase cells with either BLM- or PICH-positive ultrafine bridges is indicated. The difference in total numbers between the two markers may be due to sensitivity of detection. BLM- or PICH-positive bridges were never observed in later stage cells. (C) HeLa cells were treated as in (A) and analyzed for the presence of chromatin bridges in midbody-stage cells using Sytox Green (DNA, green). Midbody-stage cells were identified with an antibody directed against α-tubulin (magenta). Shown are two representative examples of what was scored as a chromatin bridge, detected by high exposure in the DNA channel. Quantification of the percentage of midbody-stage cells displaying chromatin bridges after control (DMSO) or APH treatment illustrates a small, but not statistically significant, increase in chromatin bridges after APH treatment. Data represents the combined results (mean and SD) from 3 independent experiments where >100 midbody- stage cells were analyzed per experiment. *p*-values determined using the Mann-Whitney test; ns, not significant. (D) Replication stress does not result in abnormalities in nuclear pore or nuclear envelope assembly, as assessed by immuno-localization of the nucleoporins Nup153 and Tpr, as well as the nuclear lamina marker Lamin B2.

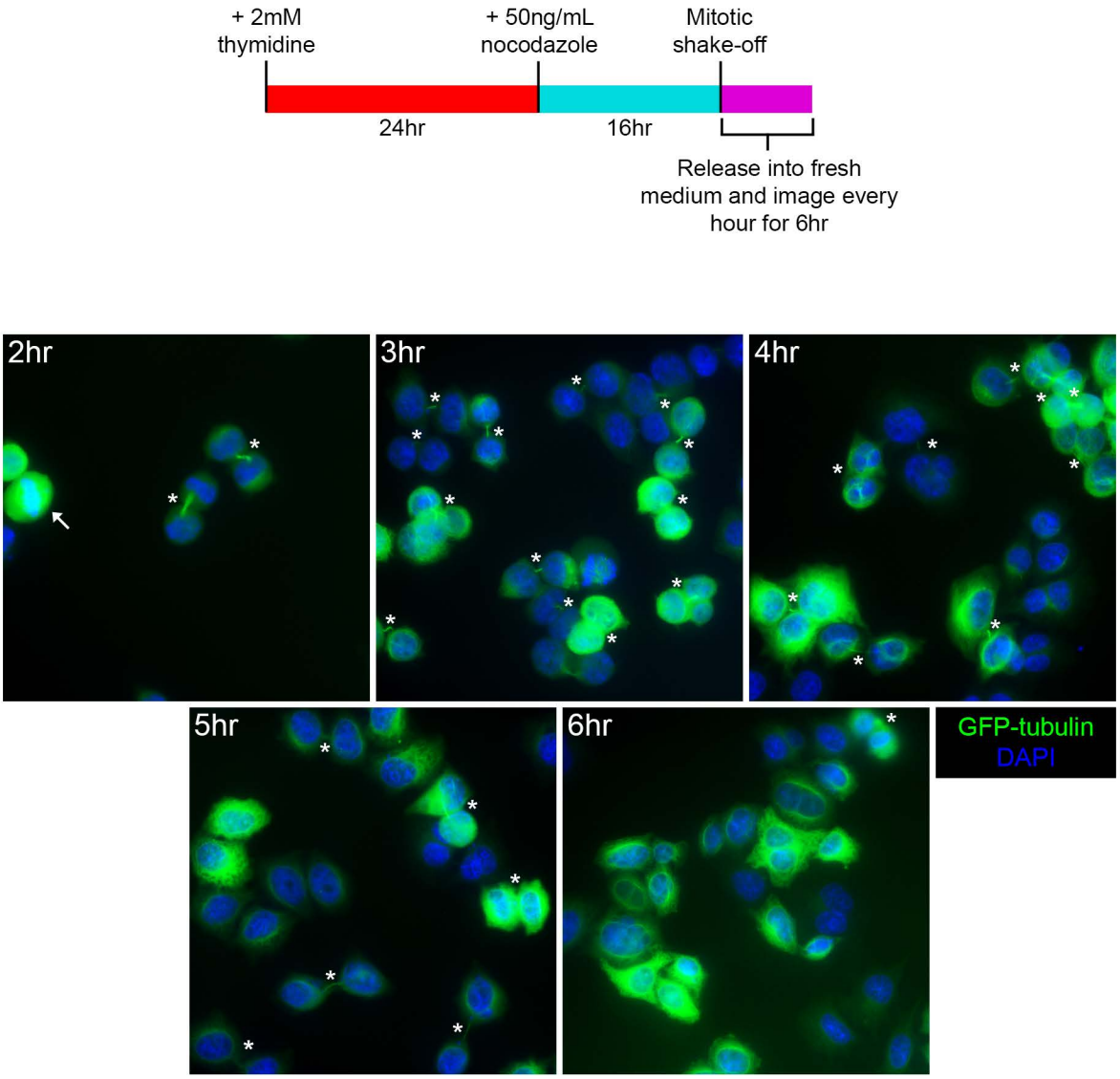

Figure S5. Thymidine/nocodazole synchronization protocol enriches for cells in midbody-stage. Cells were synchronized by treating with 2mM thymidine for 24hr, then washed several times with PBS and released into medium containing 50ng/mL nocodazaole for an additional 16hr. Mitotic cells were isolated by mitotic shake-off, cultured in fresh medium, and harvested for analysis at 1hr time points. Shown above are GFP-tubulin-expressing cells analyzed at the indicated time points. Note the enrichment of midbody-stage cells (*) at 3-5hr post-release. While there were some midbody-stage cells present at 2hr, a majority of cells were still in mitosis (arrow), most of which did not attach to the coverslips analyzed.

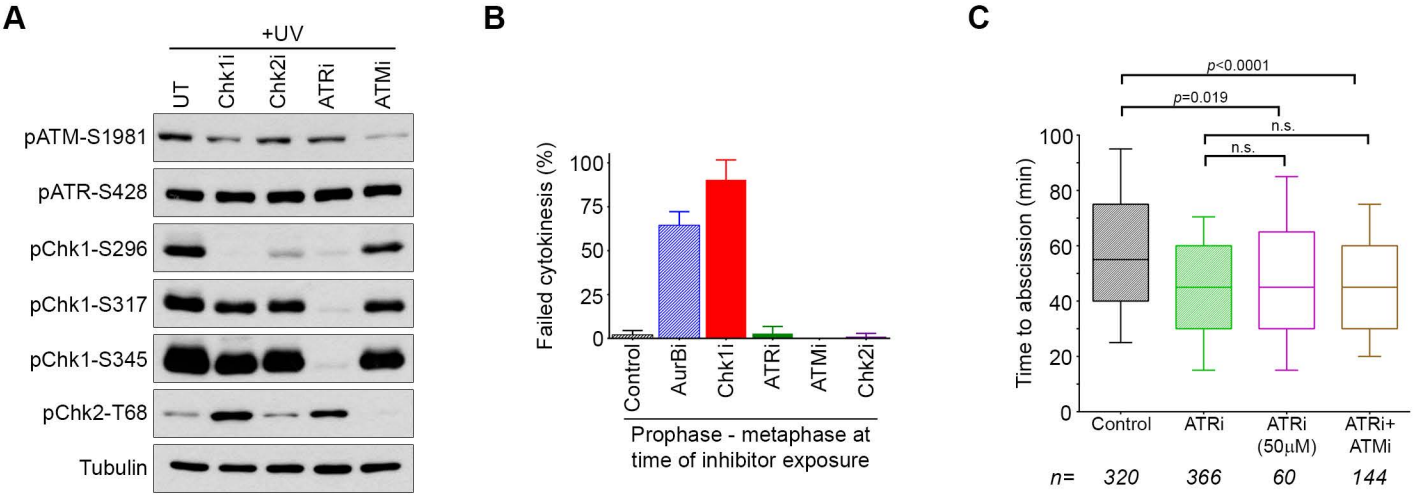

Figure S6. Validation and further characterization of inhibitors used throughout this study. (A) Inhibitors used effectively inhibit their targets. Cells were incubated with the indicated compounds for 30 min before exposure to UV (1.2x105μJ/cm2) to induce DNA damage, followed by additional 2hr incubation before harvesting and analysis by western blot (Chk1i, 2μM; ATRi, 10μM; ATMi, 10μM; Chk2i, 10μM). pChk1-S317 and pChk1-S345 indicate ATR activity, pChk1-S296 indicates Chk1 activity, and pChk2-T68 indicates ATM activity. ATR and ATM phosphorylation states shown here are induced upon DNA damage, but do not necessarily reflect catalytic activity. (B) Inhibition of Chk1 while cells are in mitosis leads to cytokinesis failure. Percent of mitotic cells exposed to the indicated inhibitors that exhibit failed cytokinesis within 2 hours. Control and AurBi data from Figure 2 are included for comparison. (C) The modest effect of ATR inhibition on abscission timing is not augmented by either increasing the concentration of ATRi or by treatment with both ATRi and ATMi. Data represents quantification of abscission timing using the assay described in Figure 2. Control and ATRi data from Figure 5 are included here for comparison. n.s., not significant (Mann-Whitney test).