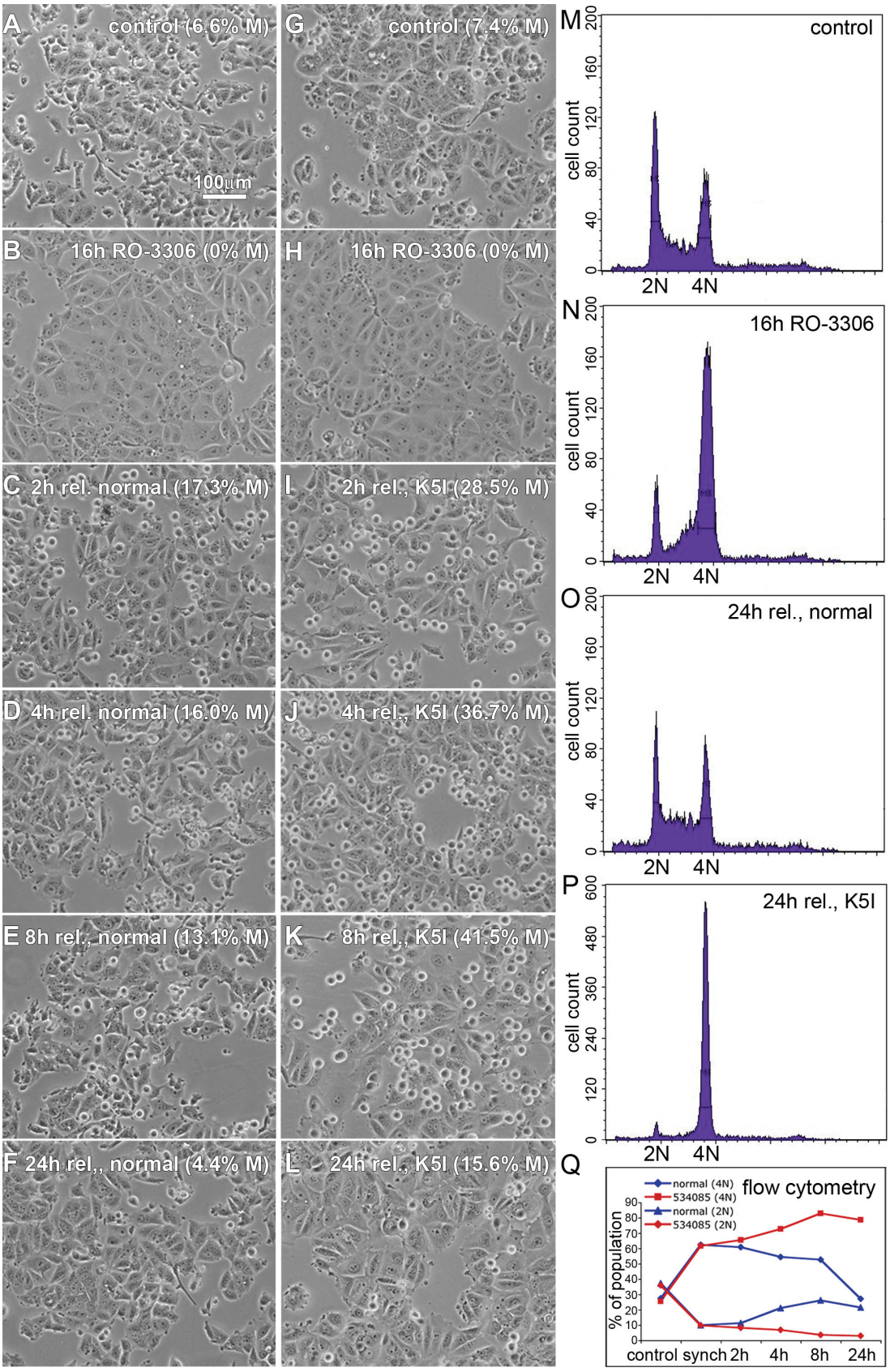
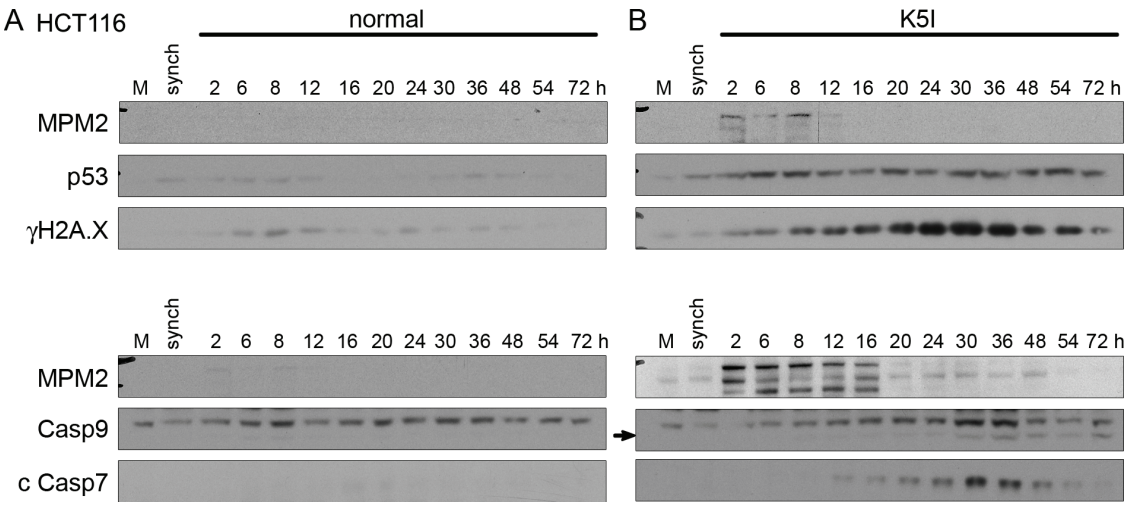
Supplemental Figure legends:

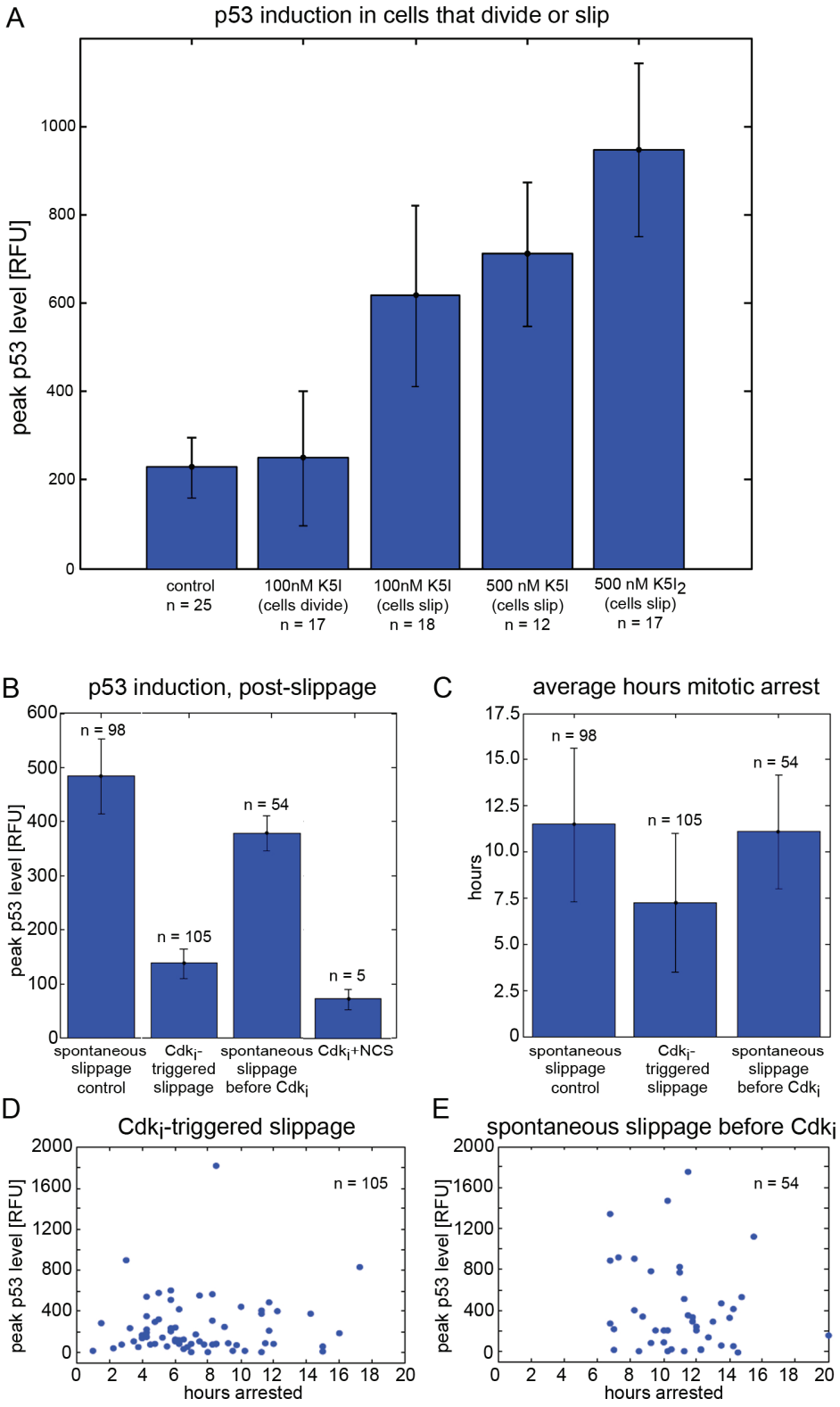


Supplemental Figure 1. G2-phase synchrony and release. Growing MCF7 cells at 50-60% confluency were treated with 10µM of Cdk1-selective inhibitor RO-3306 (diluted into

growth medium) for 16 hours. Cells were then washed thoroughly in normal medium, before replacing with either normal medium of medium containing 500nM K5I. Cells were then imaged using phase-contrast microscopy and/or harvested for immunoblotting or flow cytometry using standard propidium iodide staining. (A-L) Representative images of pre-synchronized (control), synchronized (16h RO-3306), and 2, 4, 8 and 24 hour release into normal medium or K5I-medium. The mitotic index determined by visual scoring of the rounded-up mitotic phenotype is provided (e.g. 6.6% M). (A, G) Control cells showed 6-7% mitotic index. (B, H) After 16 hours RO-3306 there were essentially no mitotic cells. (C-F) Upon release into normal medium, the mitotic index initially increased, and then returned to similar levels as pre-synchrony control by 24 hours (approximately one cell cycle time for these cells). The mitotic index did not continue to increase, as normal mitosis is transient, lasting only 1 hour. These images agree well with immunoblotting showing the mitotic index (phosphorylated serine-10 histone-H3) is initially elevated and returns to control levels after ~8 hours release. (I-L) In contrast, cells released into K5I show increasing mitotic index initially as cells enter mitotic arrest and remain arrested. By 24 hours, the mitotic index is decreased compared to 8 hours, indicating mitotic slippage, in good agreement with immunoblotting, but remaining slightly higher than release into normal medium, which also agrees with immunoblotting data. (M-Q) Flow cytometry of cell synchrony. (M, Q) Pre-synchronized controls show approximately 37% 2N and 27% 4N cells. (N, Q) After 16 hours RO-3306, most cells are now 4N (65%) but are not mitotic (see panels B, H) and the 2N population has decreased to only 10%. (O, Q) Upon release into normal medium for 24 hours, the 2N and 4N populations return to levels very similar to pre-synchrony levels. Compare panels M, N and O. (P, Q) In contrast, release into K5I results in a gradual increase and maintenance of the 4N population and decrease of any remaining 2N population as most of the cells after 16 hours RO-3306 (N) progressed into and through mitotic arrest. Compare panels M, N and P.

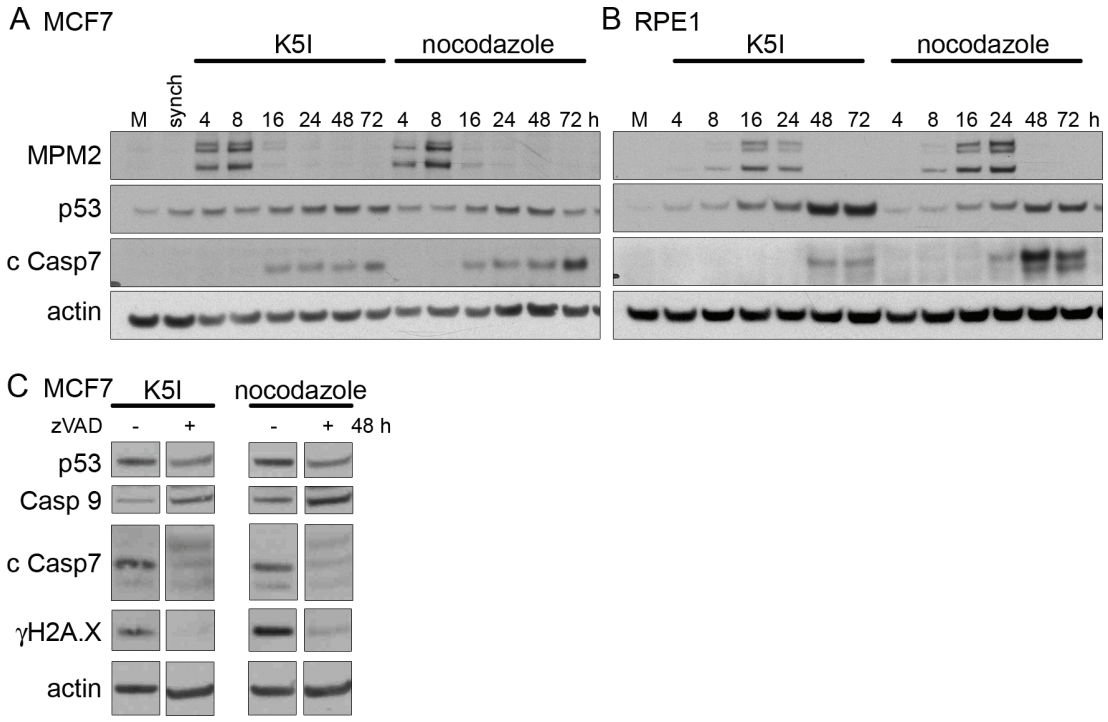


Supplemental Figure 2. P53 induction, DNA damage and caspase activation after K5I treatment of HCT116 cells. (A) Release after G2 synchrony (synch) into normal medium did not result in significant, persist p53 induction, or γH2A.X increase. Pro-caspase 9 (Casp9) and cleaved caspase 7 (c Casp7) levels were unchanged. Please note that cells released from G2 synchrony into normal medium had largely passed through mitosis by 2 hours, resulting in very low MPM2 signal. (B) Cells released into K5I show high-level, persistent induction of p53 and significant γH2A.X 6 hours after release. Note: HCT116 have a strong apoptotic response compared to MCF7, contributing to the high levels of γH2A.X. Pro-caspase 9 and caspase 7 are activated only after release into K5I and not normal medium.

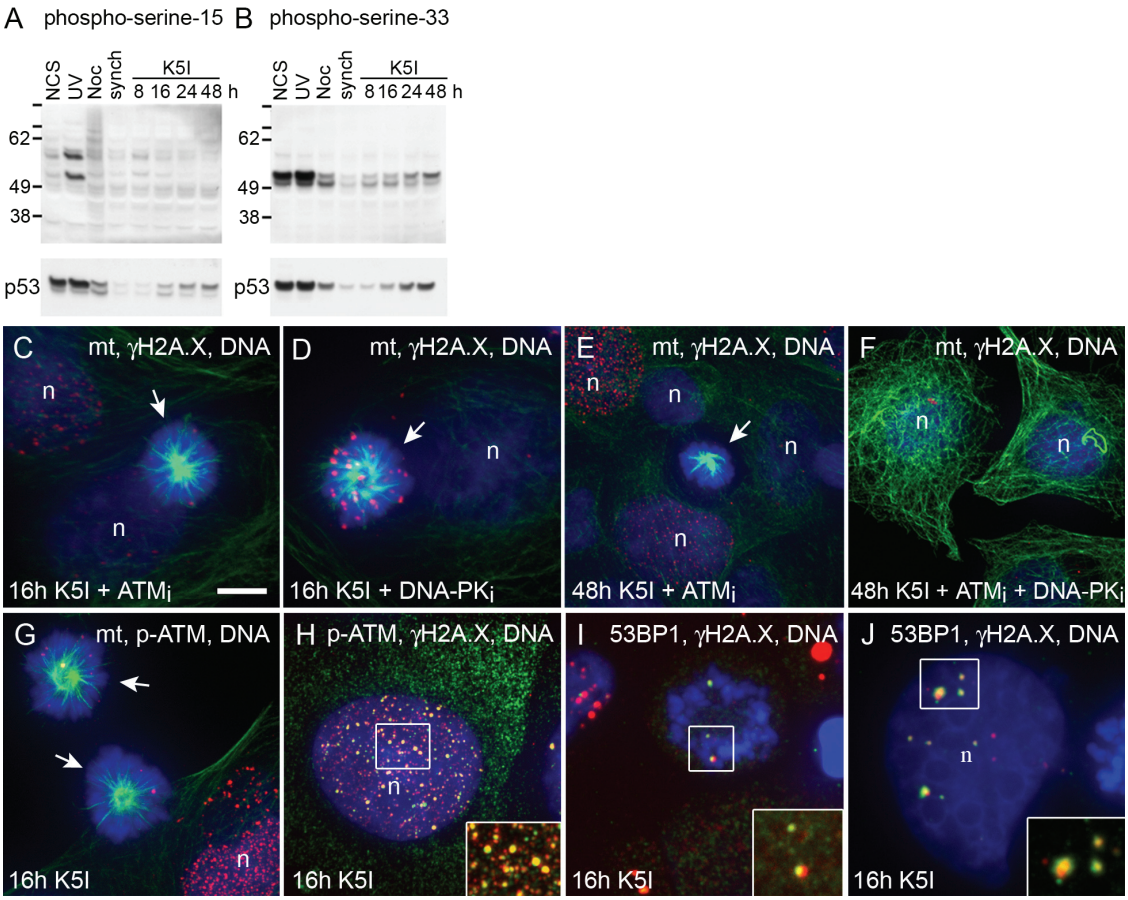


Supplemental Figure 3. Post-slippage p53 induction depends on prolonged mitotic arrest but tetraploidy and mitotic slippage are not directly responsible for p53 induction. For (A, B), the peak p53 level is the median (±std. error) obtained from the highest fluorescence intensity value for each cell in the population (sustained or pulsatile induction) during the

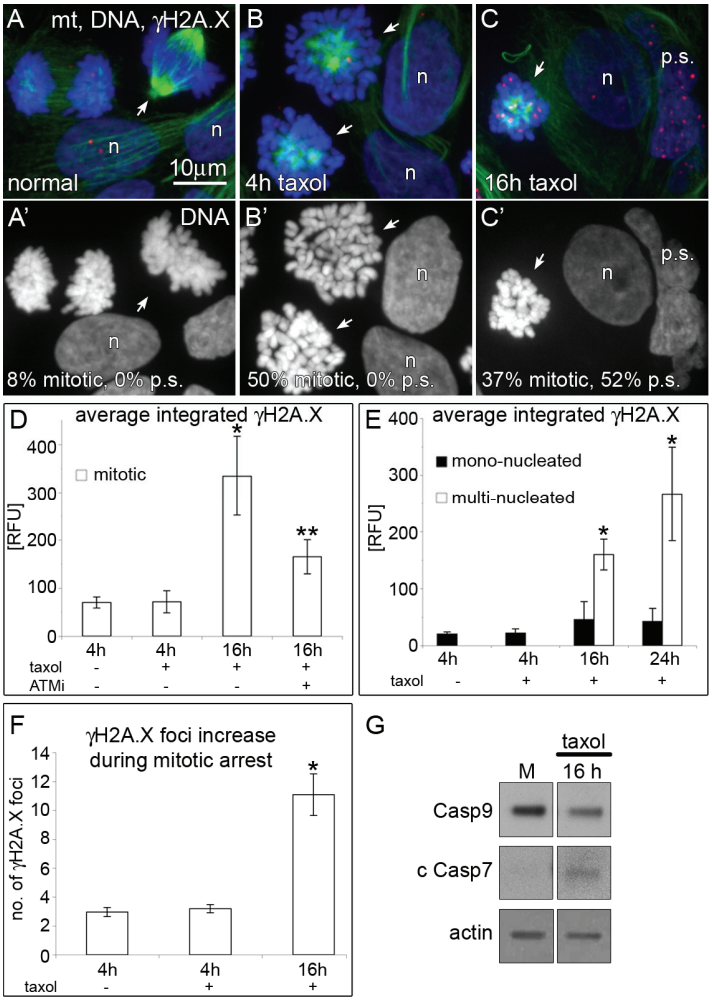
imaging period. Sample sizes are noted in the plots. (A) p53 induction in normal daughter cells and daughter cells dividing at sub-saturating K5I (100nM) after a short delay is low, indicating the presence of K5I during mitosis does not directly cause p53 induction and that prolonged mitotic arrest and/or mitotic slippage is required for induction. Cells that arrested and slipped in 100nM K5I showed the same high p53 induction as cells treated with two different K5Is at saturation (500nM). (B-E) Cells were treated with K5I and followed through mitotic arrest and slippage, or, after 24 hours of microscopy in K5I, Cdk1 inhibitor RO-3306 (Cdk_i) was used to trigger slippage from mitotic arrest. p53 induction was determined in all post-mitotic cells. (B) Spontaneous slippage control and cells that slipped spontaneously before Cdk_i showed the same p53 induction. Cells with Cdk_i triggered slippage showed dramatically less p53 induction indicating tetraploidy does not induce p53. Cdk_i itself did not prevent p53 induction as low level pulses were still induced by the DNA double-strand break inducer, neocarzinostatin (NCS, see also Results). (C) Average mitotic arrest times for K5I alone, Cdk_i-triggered slippage, and cells that slipped before Cdk1 inhibitor. (D, E) Correlation plots of time arrested in mitosis and p53 induction for cells Cdk_i triggered slippage and spontaneous slippage before Cdk_i addition in the same population. Cells with triggered slippage showed lower p53 induction than cells that spontaneously slipped before Cdk_i, regardless of arrest time.



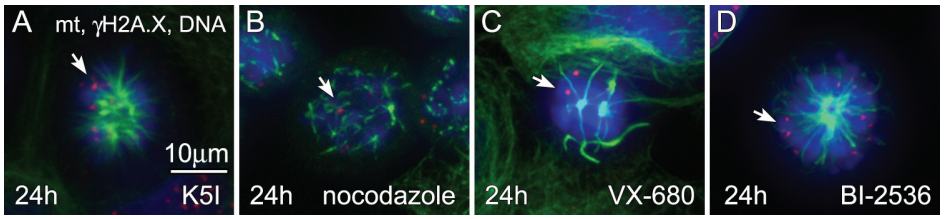
Supplemental Figure 4. P53 induction and role of caspases after nocodazole in MCF7 and RPE1 cells. (A) Immunoblotting after synchrony (synch) and release into nocodazole show p53 indcution correlating with late mitotic arrest/early slippage (MPM2 blot) like for K5I. Caspase 7 is also activated (c Casp7) after nocodazole. Actin is loading control. (B) RPE1 cells treated with nocodazole also gave results like K5I and like MCF7 cells. (C) Nocodazole-based p53 induction, activation of caspases 9 and 7 and γH2A.X are partially blocked by zVAD-fmk like for K5I.



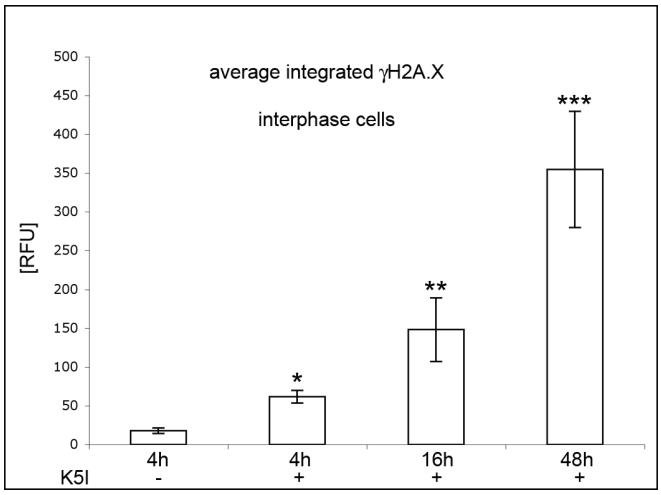
Supplemental Figure 5. p53 phosphorylation after release into K5I suggests DNA damage and the role of DNA damage response kinases, ATM and DNA-PK, and DNA break site marker 53BP1. (A) Phospho-serine-15 and phospho-serine-33 antibodies were used. UV resulted in high serine-15 phosphorylation, while all other conditions showed low-levels. Phospho-serine-15 was modest but detectable during K5I treatment when total p53 levels are also low. (B) NCS, UV and nocodazole for 24 hours each resulted in serine-33 phosphorylation. Synchronized cells released into K5I showed initially low levels of phosphorylation when total p53 levels were low, that increased over time concomitant with p53 increase. The p53 doublet is characteristic of this polyclonal antibody. (C) ATM inhibition blocks γH2A.X foci during mitotic arrest. (D) DNA-PK inhibition does not block γH2A.X foci during mitotic arrest. (E) ATM inhibition results in decreased γH2A.X foci in 48 hour post-slippage cells. (F) ATM plus DNA-PK inhibition decreased γH2A.X foci in 48 hour post-slippage cells more than ATM inhibition alone. (G, H) Phosphorylated ATM (p-ATM) localizes to foci in 16 hour mitotic arrest cells and co-localizes with γH2A.X foci in the nucleus after K5I treatment. (I, J) The DNA double-strand break marker 53BP1 co-localizes with γH2A.X foci in 16 hour mitotic arrest cells and in the nucleus after K5I treatment. n, nucleus. Bar, 15μm for C-G, I and 5μm for H, J.



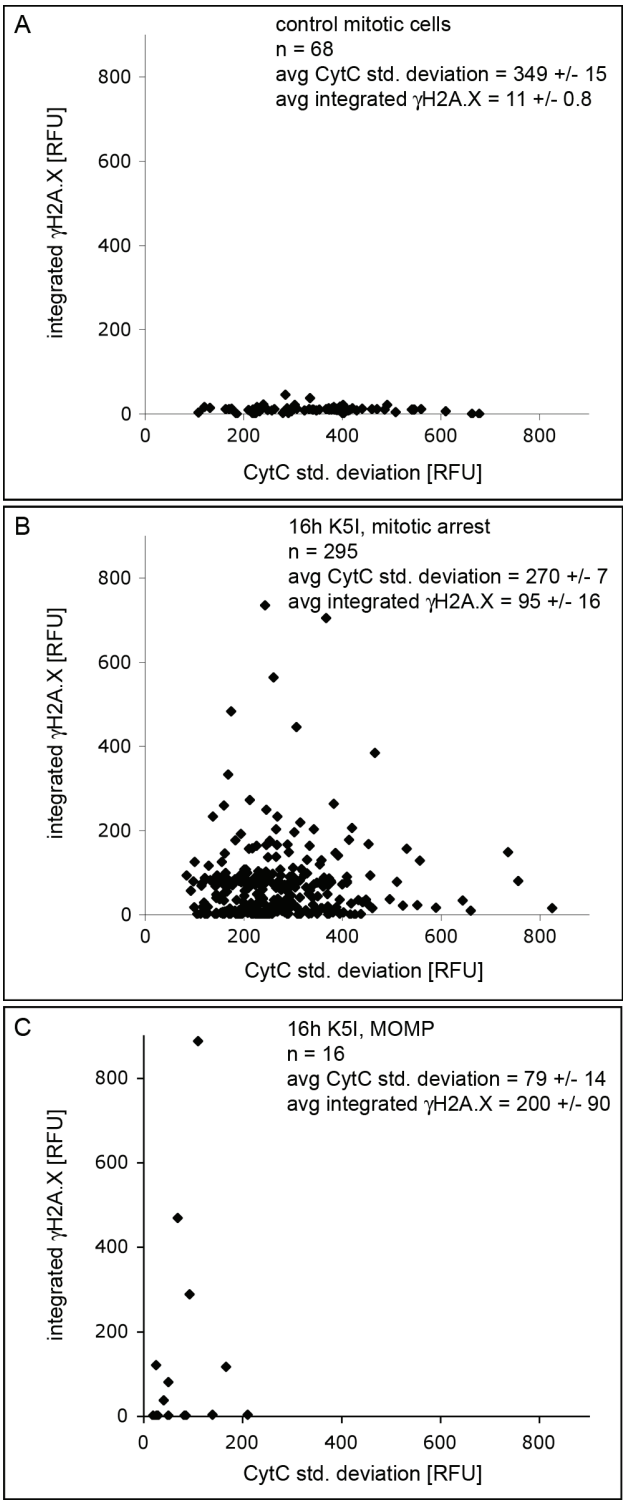
Supplemental Figure 6. Taxol treatment results in DNA damage during mitotic arrest through ATM and in post-slippage cells. Cells were fixed and co-stained for microtubules (mt, green), γH2A.X (red) and DNA (blue). (A-D) Normal mitotic and 4 hour taxol treated, mitotic arrested cells have low levels of γH2A.X; this increases significantly in 16 hour arrested cells. Arrows point to mitotic cells. n = nucleus. p.s. = post-slippage. Bar is 10 μm for all images. (E) In p.s. cells, the average γH2A.X tends to increase from 16 to 24 hours, while mono-nucleated cells in the same population remain relatively constant. Multinucleated cells at 16 hours represent early post-slippage (a few to ~8 hours); at 24 hours they represent a few to 16 hours post-slippage. Timing is based on separate time-lapse data monitoring the duration of mitotic arrest. The percent in mitotic arrest or post-slippage is also indicated. (F) Like for K5I, after 16 hours release into taxol, caspase 9 and 7 are activated. (D, E) Average values ± std. errors are reported. * = $p<0.05$ against respective controls. n = >50 cells each condition.



Supplemental Figure 7. Mitotic arrested cells after 24 hour treatment with different anti-mitotic drugs contain γH2A.X. Non-synchronized cells were treated with respective drugs; K5I = 500nM, nocodazole = 500nM, VX-680 = 250nM, BI-2536 = 250nM. Cells were fixed and co-stained for microtubules (mt, green), γH2A.X (red) and DNA (blue). Arrows point to foci. Bar = 10 μm for all images.

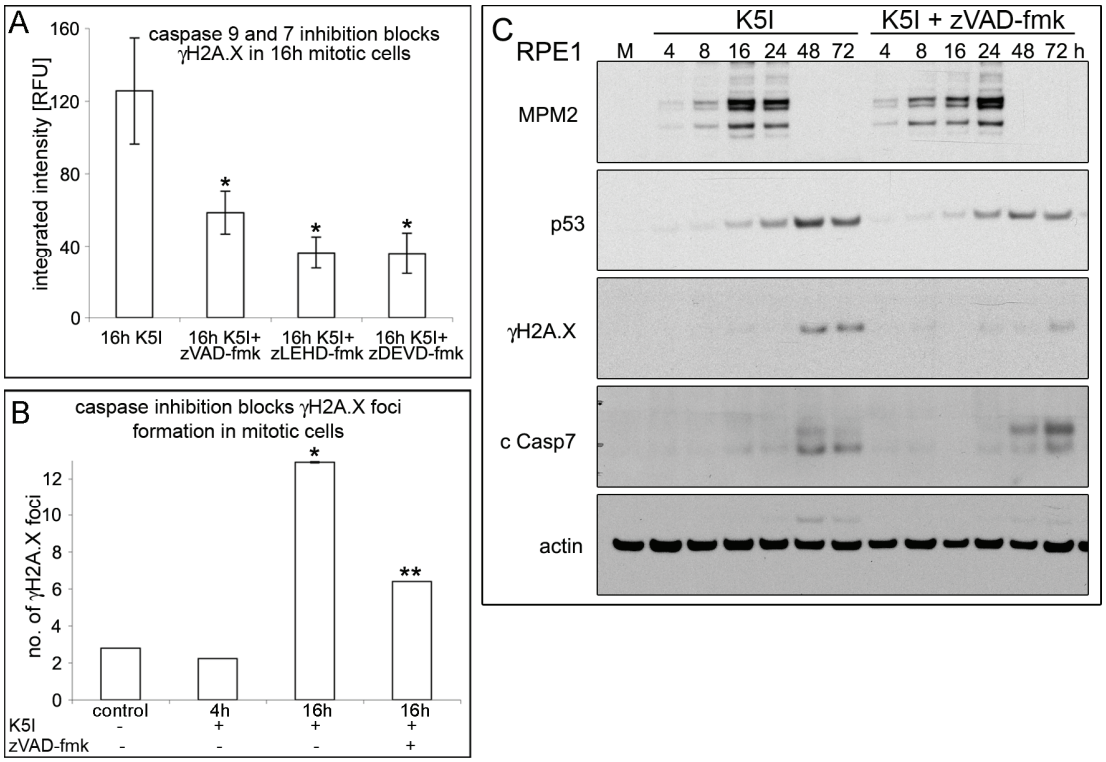


Supplemental Figure 8. DNA damage in interphase nuclei of K5I-treated cells. Average integrated γH2A.X levels (see Figure 2) of single interphase nuclei show that the level of damage is increased at 16 hours, and further still at 48 hours. Nuclei are 4 hours are nearly all pre-mitotic, with a rare number representing very early (≤1 hour) post-slippage cells. Nuclei at 16 hours represent some pre-mitotic and early post-slippage (a few to ~8 hours) cells, whereas most nuclei at 48 hours represent cells many hours post-slippage (16-40 hours). These data suggest DNA can continue after mitotic slippage. Timing is based on separate time-lapse data monitoring the duration of mitotic arrest. Average values ± std. errors are reported. * = $p<0.05$ against no K5I control. n = >50 cells each condition.

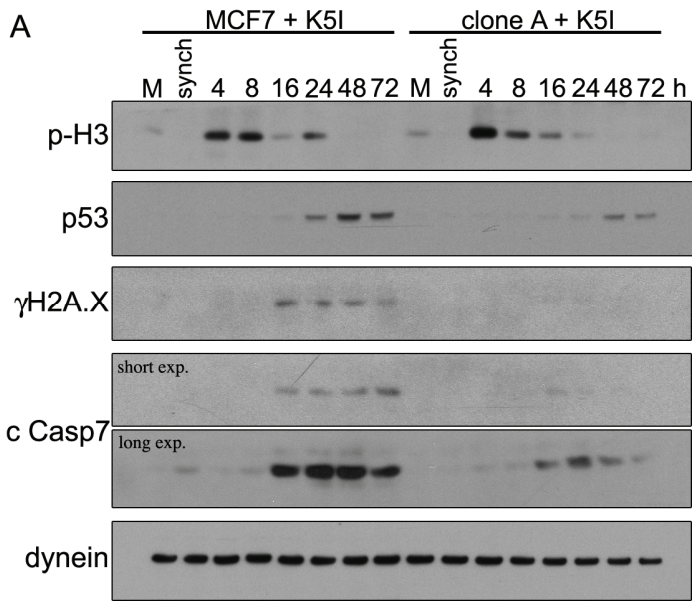


Supplemental Figure 9. Correlation plots of integrated γH2A.X fluorescence vs. CytC pixel-to-pixel fluorescence standard deviation in normal mitotic, 16 hour K5I mitotic arrested and momp cells at 16 hour K5I reveal three separable populations. The average values and sample size are indicated on each graph. (A) Normal mitotic cells show very

low integrated γH2A.X and some cell-to-cell variability in the CytC standard deviation. The CytC std. deviation is significantly different than for 16 hour mitotic and momp cells (see Figure 3). (B) 16 hour K5I mitotic arrested cells have increased γH2A.X and a decreased CytC std. deviation (shifted to the left) compared to normal mitotic cells. The integrated γH2A.X and CytC std. deviation are significantly different compared to normal mitotic cells and the CytC std. deviation is significantly different from momp cells (see Figure 3). (C) Momp cells are rare, resulting in a small sample size. Because the sample size is small the integrated γH2.AX, while trending higher than 16 hour arrested mitotics, is not significantly different (see Figure 3). The CytC std. deviation is much lower than both normal and 16 hour arrested mitotic cells (see Figure 3 for p values).



Supplemental Figure 10. Selective Caspase-9 and -7 inhibition blocks DNA damage in late mitotic-arrested cells and caspase-7 dependent DNA damage and p53 induction also occurs in normal, diploid RPE1 cells. (A) Three separate caspase inhibitors; zVAD-fmk, zLEHD-fmk (caspase-9), and zDEVD-fmk (caspase-7) each blocked the increase of integrated γH2A.X (average ± std. error) in individual mitotic-arrested cells at 16 hour K5I treatment. n = >150 each condition, 2 experiments. * $p<0.05$ against 16 hour K5I. (B) Scoring of individual γH2A.X foci shows the number of break sites increases in late mitotic arrest and that these can be considerably blocked by inhibiting caspase activity. n = >40 each condition, 2 experiments. * $p<0.05$ against control and 4h mitotics, ** $p<0.05$ against 16 hour K5I. (C) RPE1 are normal, diploid, telomerase immortalized cells. RPE1 cells are not synchronized. RPE1 cells were treated with K5I or K5I + zVAD-fmk and immunoblotted. Inhibition of caspases results in less activation/cleavage of caspase-7 (see increased upper band), and decreased γH2A.X and p53induction. MPM2 indicates several bands during mitosis. Actin is loading control. All blots in each panel are from the same gels and/or samples.



Supplemental Figure 11. Mitotic DNA damage requires ICAD cleavage. G2-synchronized MCF7 and clone A non-cleavable ICAD expressing cells, were released into K5I. ICAD cleavage is required for p53 induction and DNA damage (γH2A.X) as mutant ICAD blocks these events. Caspase-7, shown to be required for the DNA damage and p53 induction (Fig. 4), and a known protease for ICAD cleavage, remains activated after K5I. Dynein is loading control. All blots in each panel are from the same gels and/or samples.