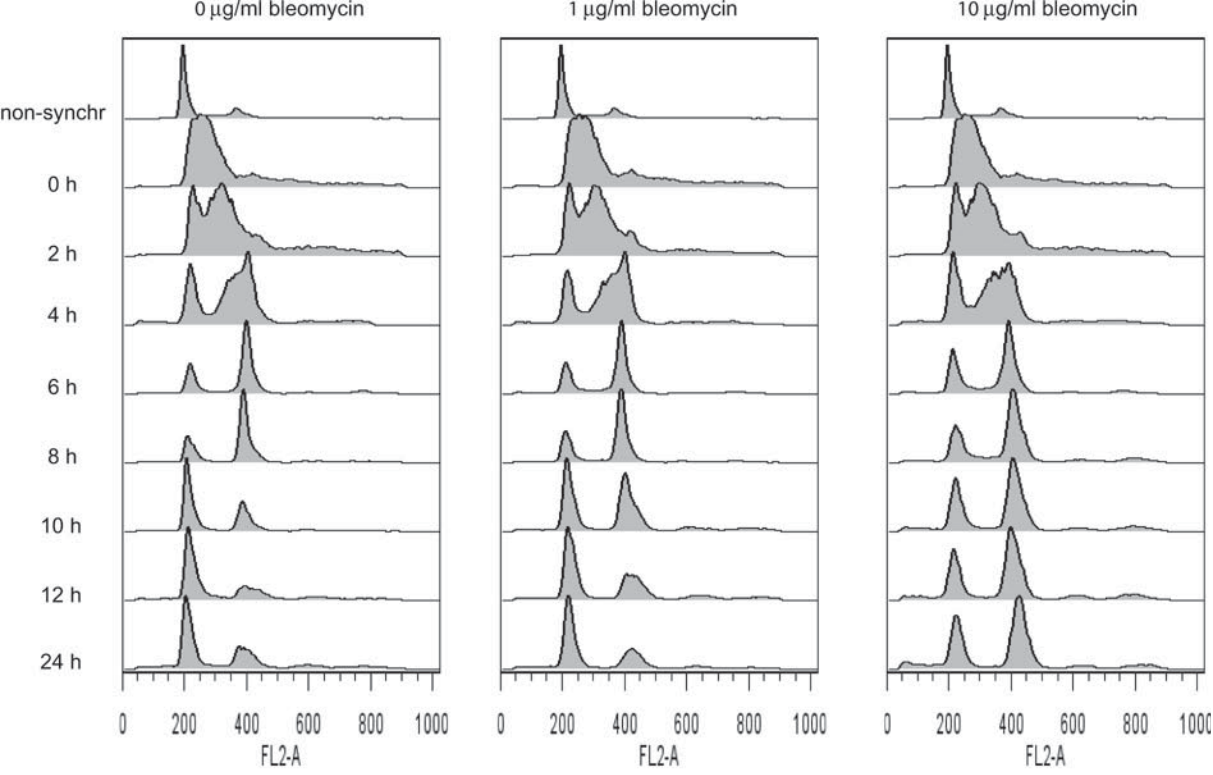
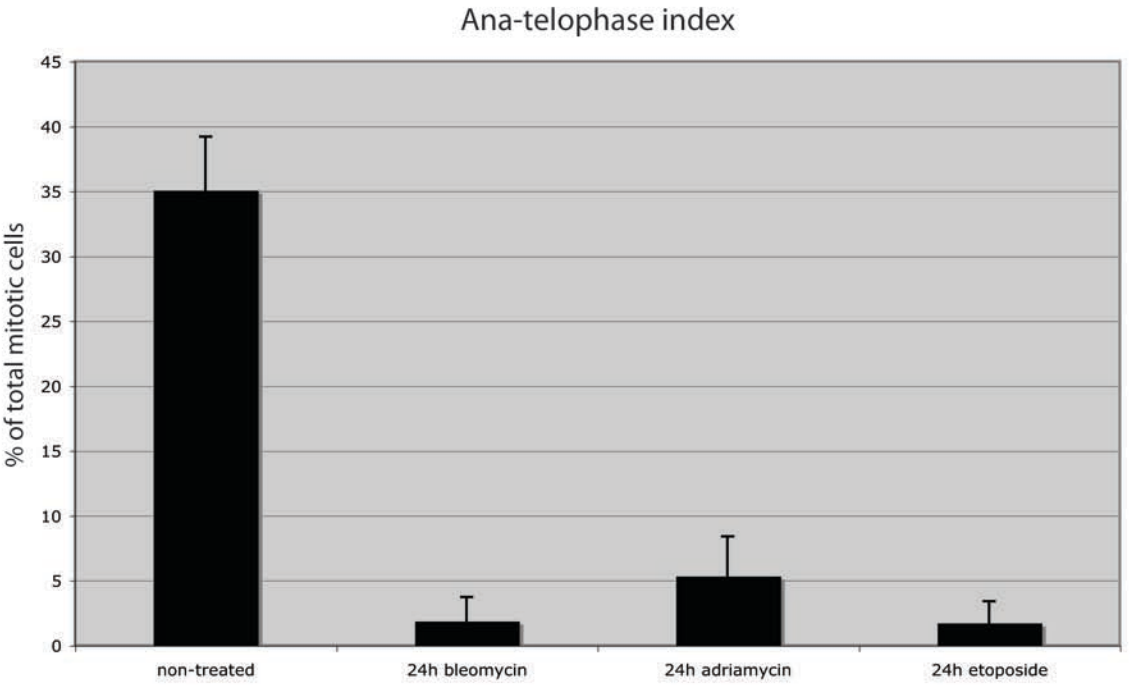


Supplementary figure 1

A



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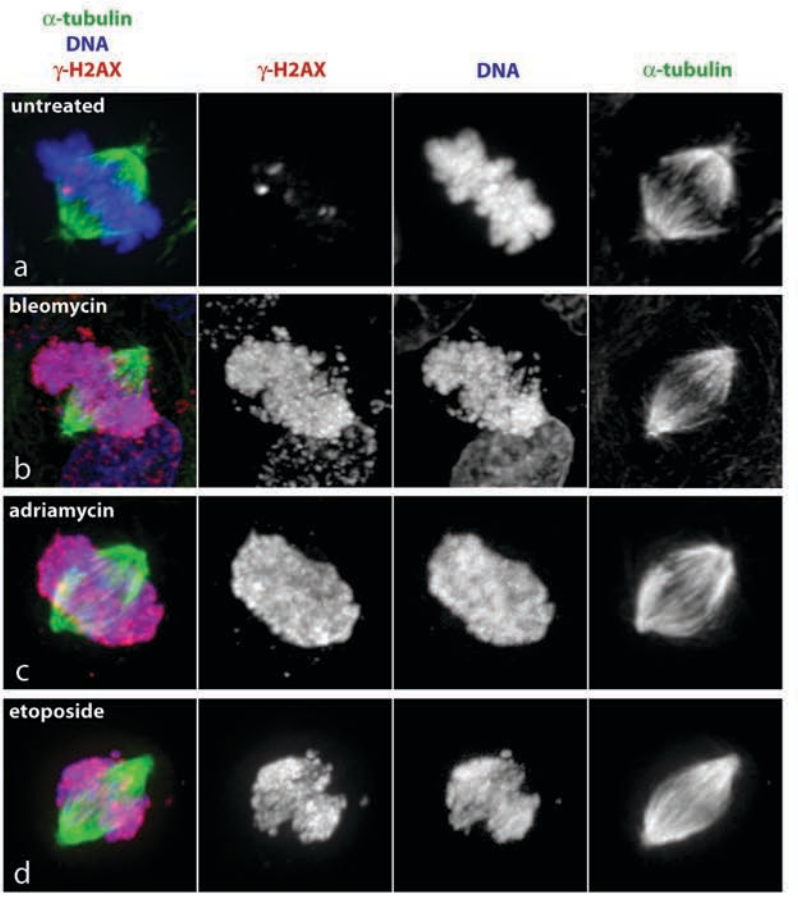
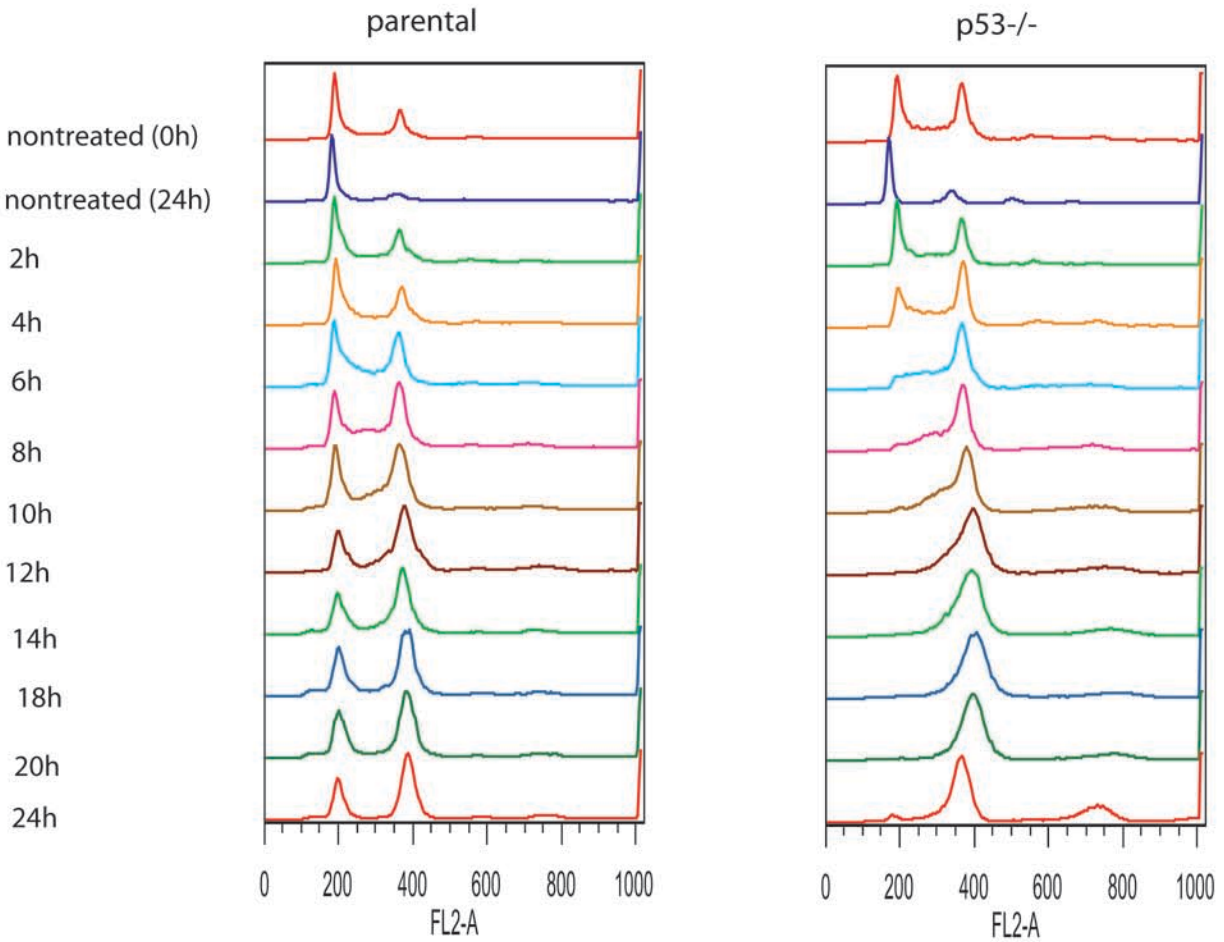


Supplementary figure 2

A



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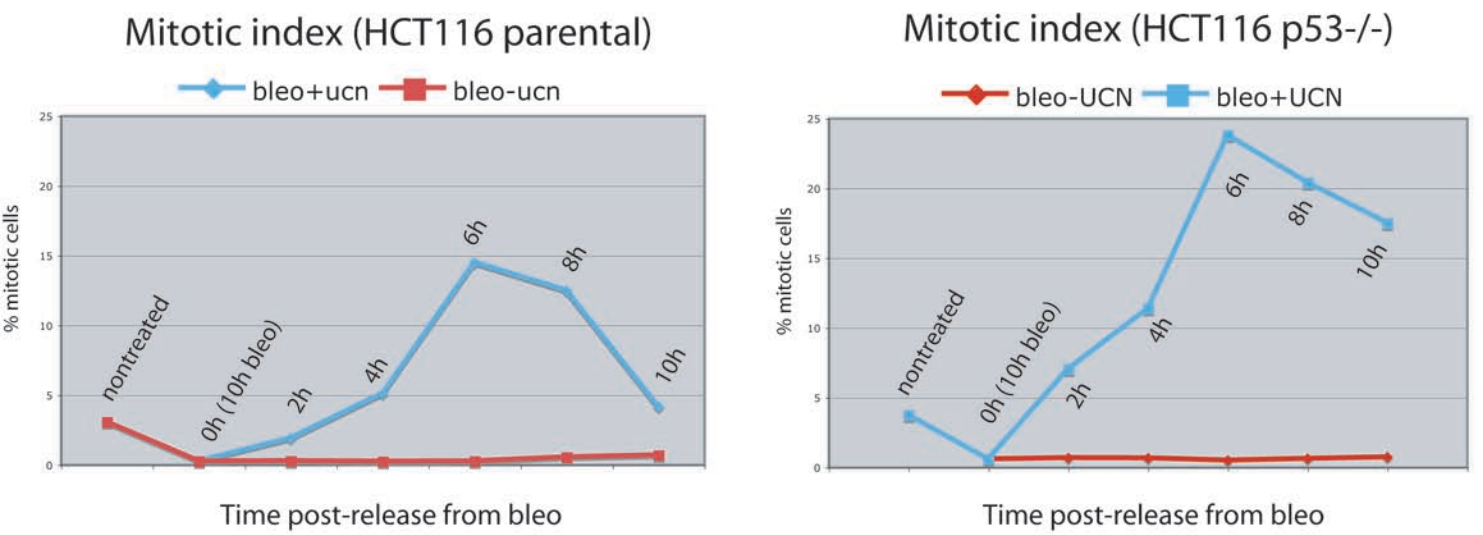
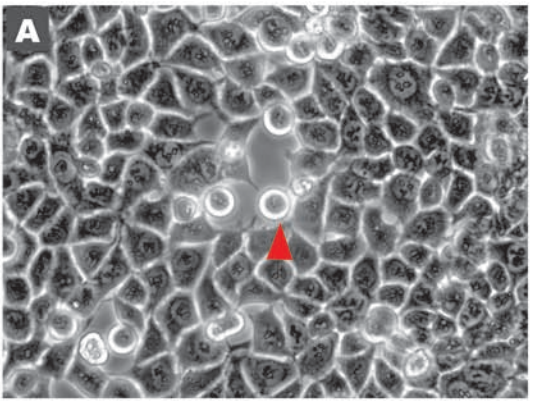
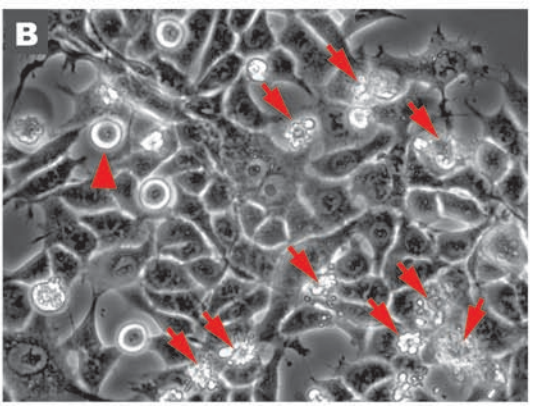


Supplementary figure 3

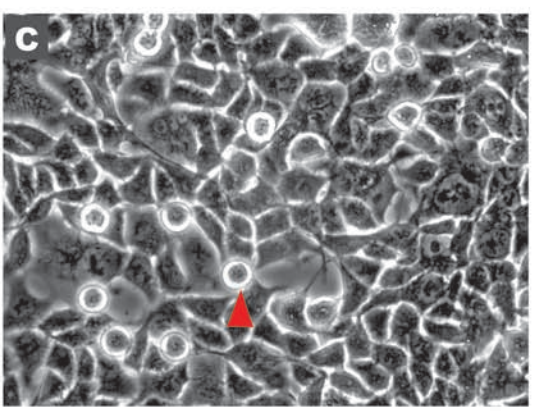
nontreated

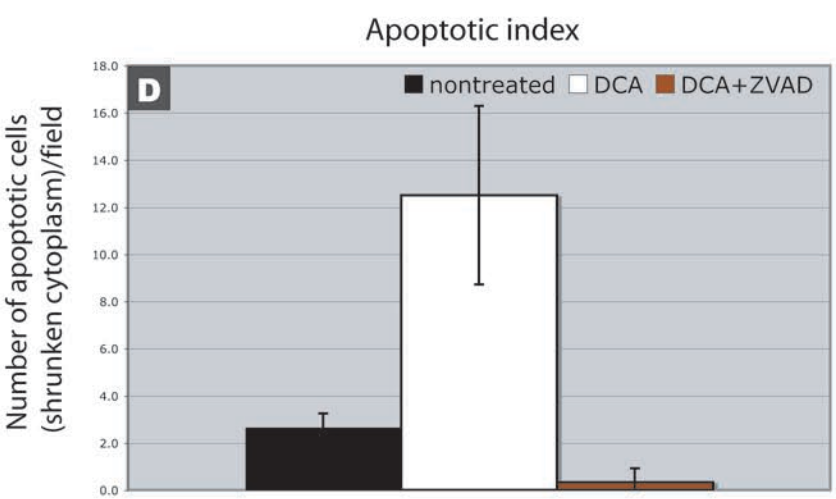


DCA

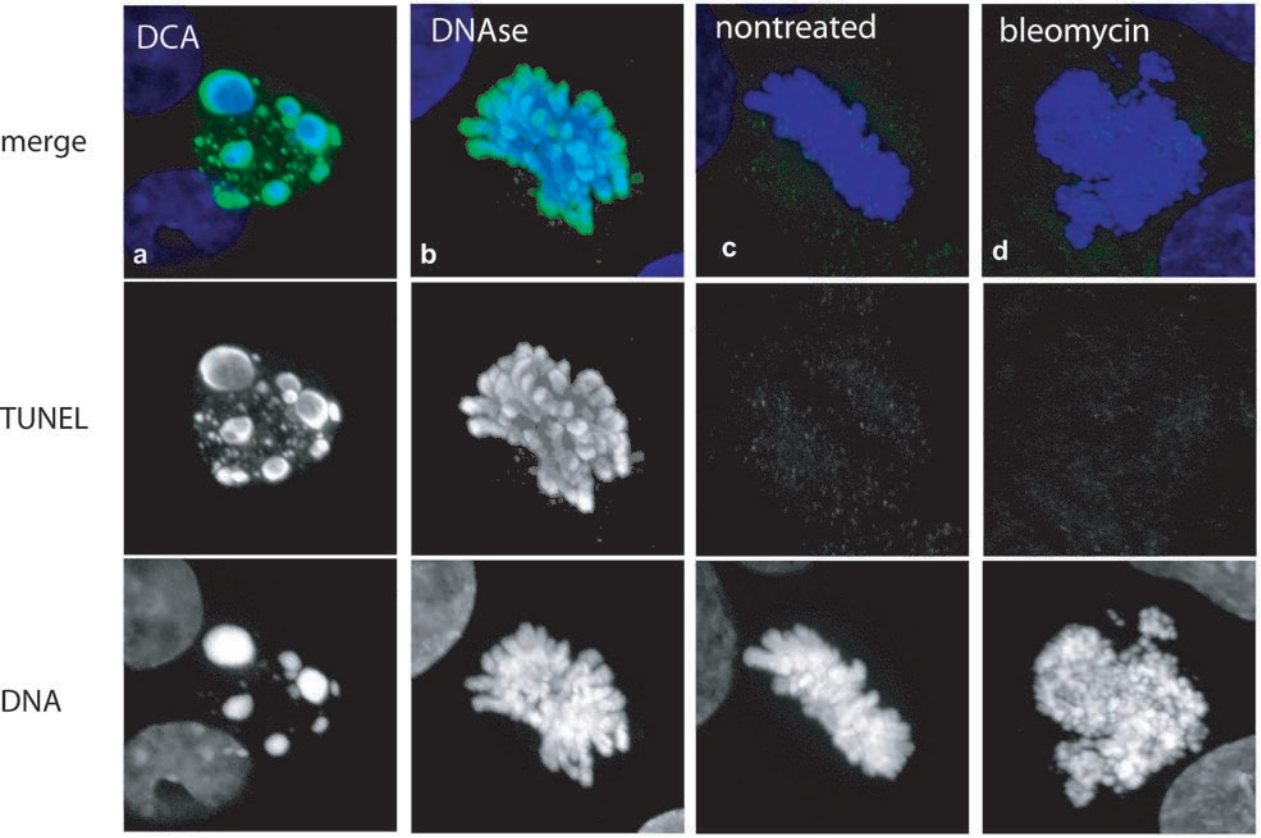


DCA +
Z-VAD-FMK

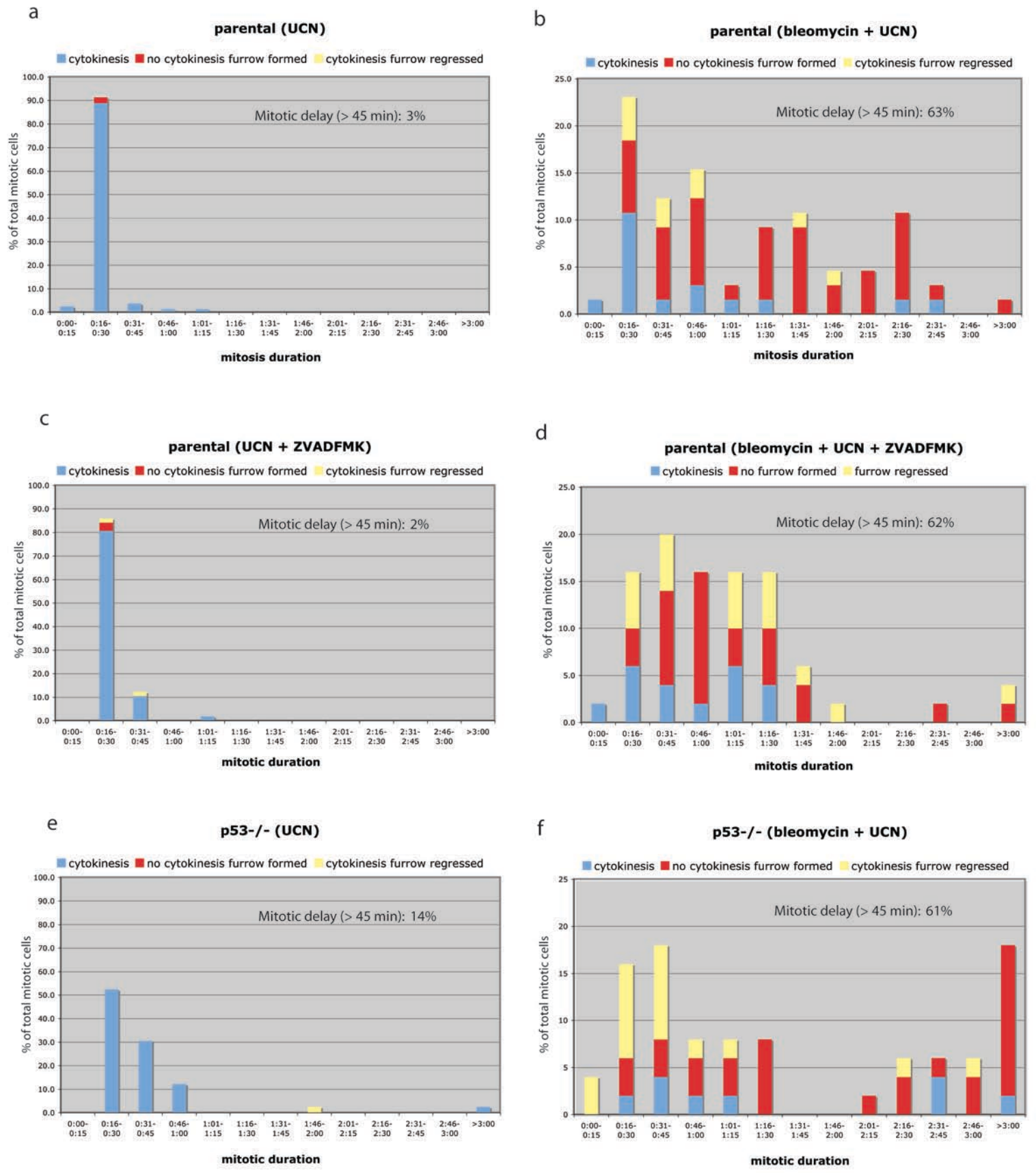




Supplementary figure 4



Supplementary figure 5



Supplementary figure 6

A

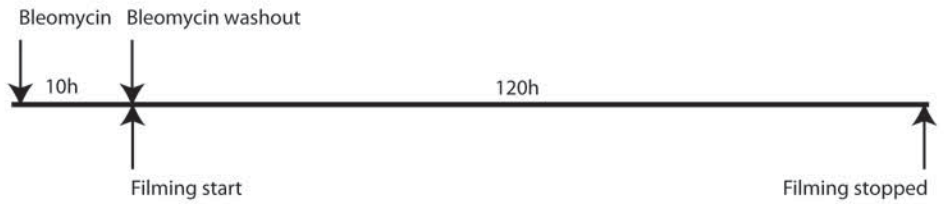
	Time of death (hh:mm)		
	Range	Average	n
Post-mitotic cells (time since mitotic exit)	5:17-70:16	26:24:00	23
Non-mitotic cells (time since start of acquisition)	3:25-55:59	25:48:00	10

B

	% death							
	parental		**parental + ZVADFMK**		**p53-/-**		**p53-/- + ZVADFMK**	
Daughters from failed cleavage	**47**	(n=15)	**30**	(n=23)	**26**	(n=35)	**33**	(n=36)
Daughters from cytokinesis	**94**	(n=17)	**53**	(n=15)	**43**	(n=7)	**67**	(n=6)

Supplementary figure 7

A



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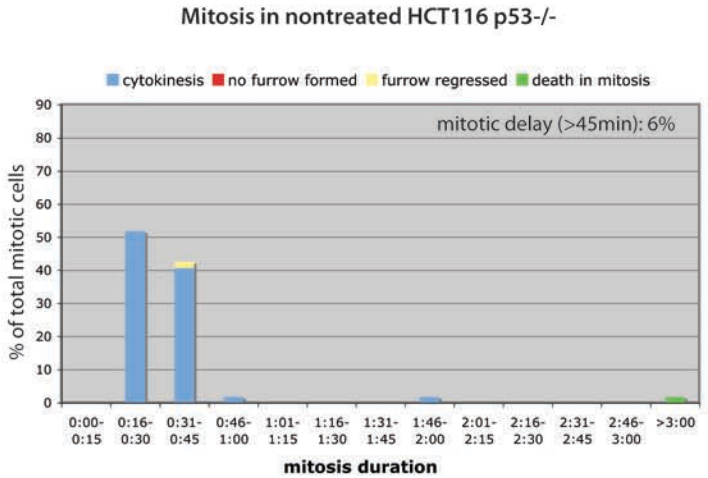

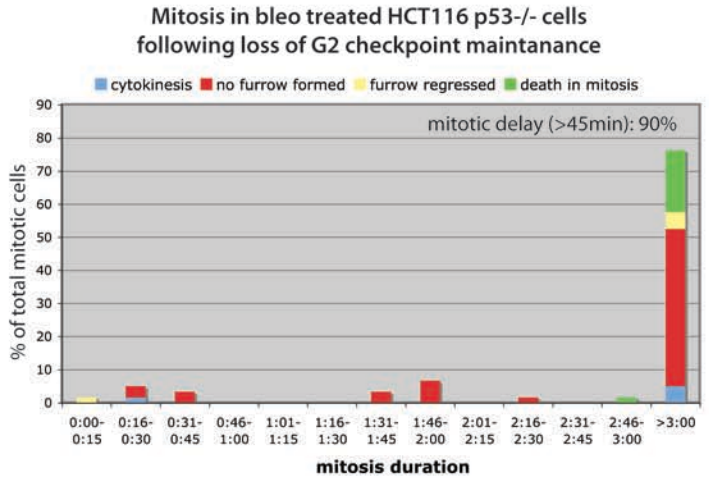

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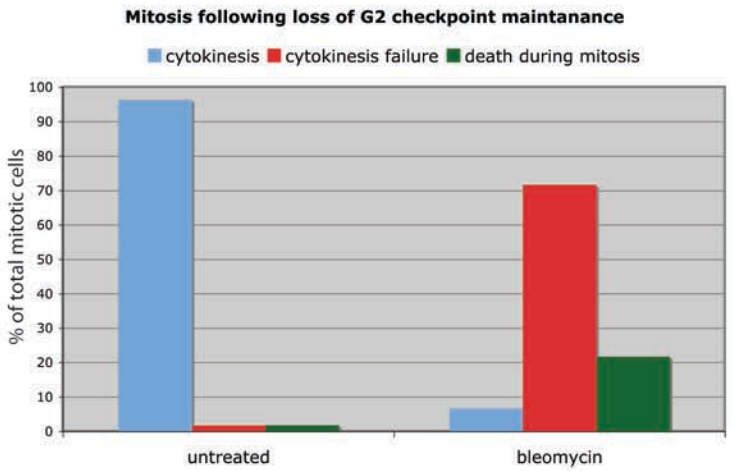
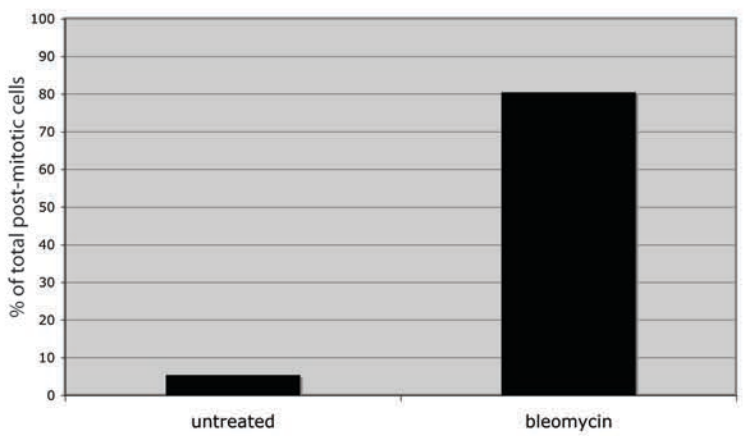
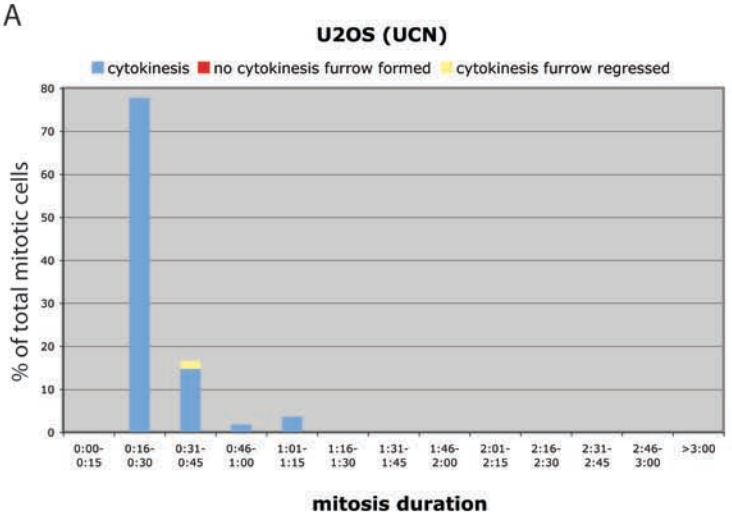


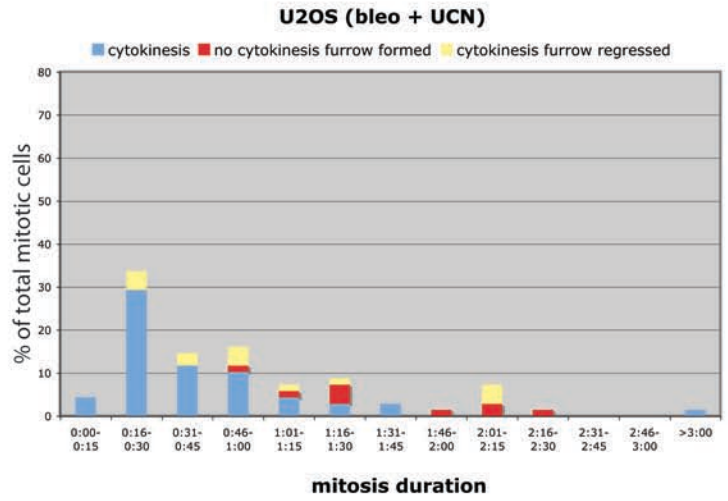
D



Supplementary figure 8

A





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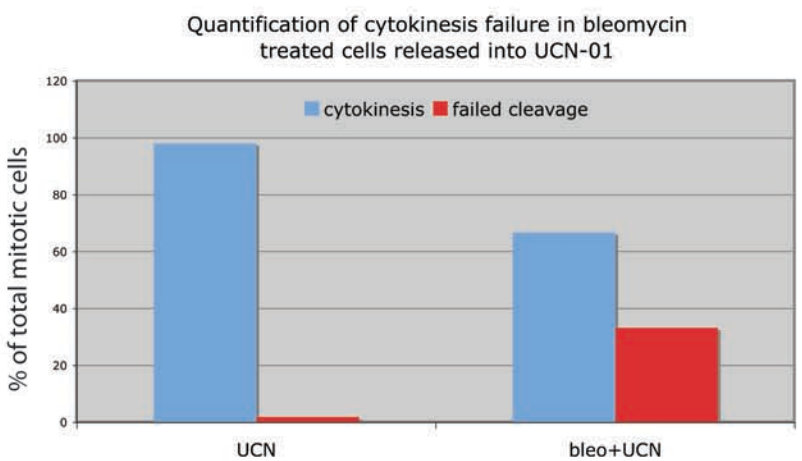

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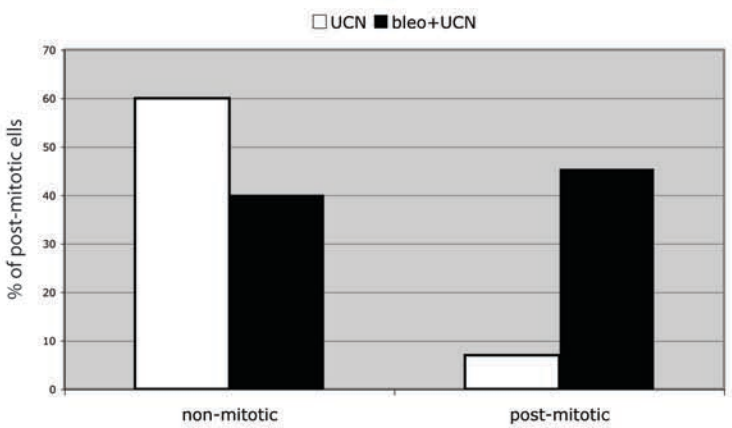

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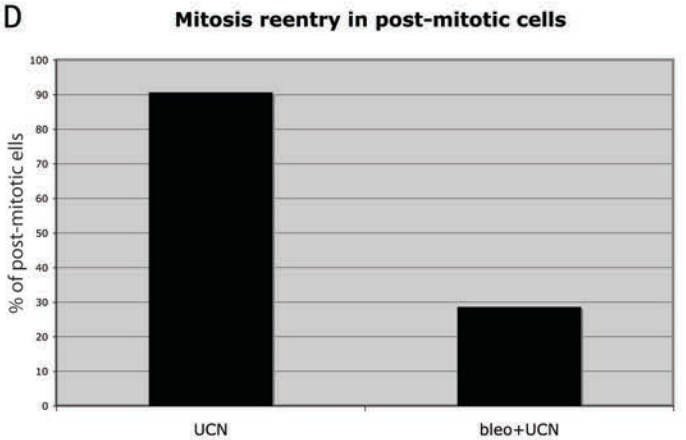

E

% death (bleo + UCN treated)	bleo+ucn	n
Among daughters from failed cleavage	61	18
Among daughters from cytokinesis	41	59

F

	Time of death (hh:mm)	Range	Average	n
Bleo+UCN	Post-mitotic cells (time since mitotic exit)	9:59-72:01	45:12:00	13
	Non-mitotic cells (time since start of acquisition)	8:17-54:59	34:55:00	17
UCN	Non-mitotic cells (time since start of acquisition)	9:03-69:07	38:46:00	15

Supplementary figure 9

Supplementary figure legends

Supplementary figure 1. Establishing the minimal bleomycin concentration sufficient for G2 arrest

HCT116 cells were synchronized with a double thymidine block and released into media with or without 1 or 10 μg/ml of bleomycin. Cells were then fixed at 2h time intervals and DNA content was measured by flow cytometry. Cells released from the double thymidine block into 1 μg/ml of bleomycin showed a delay in exit from the G2 and M phases, but did eventually cycle through to G1. Release into 10 μg/ml of bleomycin introduced a permanent G2/M arrest in the majority of the population.

Supplementary figure 2. Cells entering mitosis following adaptation to the G2 DNA damage checkpoint show mitotic chromatin defects and reduced ana-telophase index

Prior to fixation, HCT116 cells were grown for 24h in media with or without either 10 μg/ml bleomycin, 1 μg/ml adriamycin or 10 μg/ml etoposide.

A. In DNA damaged cells following adaptation to the G2 DNA damage checkpoint, the ana-telophase index was highly reduced. **B**. As demonstrated in figure 2A for a variety of assays based on bleomycin treatment and G2 checkpoint abrogation, global γH2AX label on mitotic chromatin was also observed in response to adaptation following treatment with bleomycin (b), adriamycin (c) and etoposide (d).

Supplementary figure 3. Maximum G2 accumulation is observed after 10h of bleomycin treatment

A. 10 μg/ml bleomycin was added to nonsynchronized HCT116 parental and p53-/- cells, which were fixed at 2h time intervals at 0-24h post drug addition. DNA content was analysed by flow cytometry. At 8-10h, the 4N population peaked, indicating that maximum G2 accumulation was reached. **B.** 10 μg/ml bleomycin was added to nonsynchronized HCT116, washed out after 10h and released into 300 nM UCN-01. Cells were fixed at 2h time intervals at 0-10h post UCN-01 addition and the mitotic index was analysed by flow cytometry. The mitotic index peaked after 6h of UCN-01 treatment, showing a 5-fold increase relative to the mitotic index of nontreated cells.

Supplementary figure 4. 100 μM of the poly-caspase inhibitor Z-VAD-FMK inhibits DCA induced apoptosis

HCT116 cells were incubated with 200 μM deoxycholic acid (DCA) for 24 h. To address caspase dependence, 100 μM Z-VAD-FMK was added 2h prior to addition of DCA and kept in the media throughout the experiment.

A-C. Phase contrast images of living cells 24h post-drug treatment. **Arrowheads** point to mitotic cells and **arrows** show apoptotic cells. In nontreated cultures, a monolayer of cells with several mitotic cells (arrowhead) was observed (A). Treatment with 200 nM deoxycholic acid (DCA) induced apoptotic cell death marked by cytoplasmic shrinkage and extensive membrane blebbing in a high fraction of the cells (B, arrows). Addition of 100 μM of the poly-caspase inhibitor Z-VAD-FMK inhibited the DCA induced apoptosis (C). **D**. Quantification of observations in panels A-C. Apoptotic index (number of

apoptotic cells per field) in nontreated (black), DCA treated (white) and DCA + Z-VAD-FMK treated (brown) HCT116 cells. While nontreated control cells have a very low background of apoptotic cells (black bar and panel A), DCA treatment induces high level of apoptosis (white bar and panel B, arrows), which is efficiently suppressed by 100 μM of the poly-caspase inhibitor Z-VAD-FMK (brown bar and panel C). Bars show average of 3 fields/drug condition.

Supplementary figure 5. Nucleosome level DNA fragmentation was not detected in bleomycin treated mitotic cells

Confocal projections of TUNEL labeled HCT116 cells counterstained for DNA. TUNEL label is shown in middle panel (green in merge) and DNA in the lower panel (blue in merge). Cells induced to undergo apoptosis with deoxycholic acid (DCA) showed a strong TUNEL signal (a). So did mitotic chromatin digested with DNase (b). Nontreated mitotic chromatin was TUNEL negative (c), and so was chromatin in bleomycin treated cells (d), suggesting that the chromatin in bleomycin treated mitotic cells was not highly fragmented.

Supplementary figure 6. Quantification of DNA damage induced mitotic delay in live analysis

HCT116 cells were treated as described in legend to figure 4. Mitotic duration (h:mm) is shown on x-axis. Mitotic fate is color-coded: blue = successful cytokinesis, red = no cytokinesis furrow formed, yellow = regression of cytokinesis furrow. Control treated parental cells generally completed mitosis in less than 45 min, and only 3% showing

mitosis longer than 45 min (a). Bleomycin treated parental cells driven into mitosis by UCN-01 had a highly variable mitotic timing, ranging from 15 min to more than 3 hours, and 63% of the mitotic cells showed delay (> 45min) (b). DNA damage-induced mitotic delay was also seen in parental bleomycin treated cells in the presence of poly-caspase inhibitor (62%) (d), and in p53 knockouts (61%) (f). Cleavage failure (red and yellow bars) did not correlate with mitotic delay (b, d, f). Number of cells scored for each condition: 84(a), 64(b), 57(c), 51(d), 85(e), 50(f).

Supplementary figure 7. DNA damage induced post-mitotic cell death occurs 5-70h after mitotic exit and does not require cleavage failure

HCT116 cells were treated as described in figure legend 5. Data of bleomycin and UCN-01 treated cells is shown.

A. Cell death in response to bleomycin occurred after a prolonged interphase arrest in both non-mitotic and post-mitotic cells. **B.** Table shows cell death in post-mitotic cells, separated into daughters resulting from a failed cleavage or successful cytokinesis. Post-mitotic cell death and cleavage failure does not correlate.

Supplementary figure 8. Progression through the G2/M transition with DNA damage, due to failed G2 checkpoint sustenance, leads to post-mitotic death

HCT116 p53-/- cells were treated with 10 μg/ml bleomycin for 10h, and following washout of the drug filmed for total of 120h (**A**).

B. Quantification of damage-induced mitotic delay in cells that enter mitosis due to failed G2 checkpoint sustenance. Blue = successful cytokinesis, red = no cytokinesis furrow

formed, yellow = regression of cytokinesis furrow, green = death during mitosis. **C**. Quantification of cytokinesis failure and death occurring during mitosis in bleomycin treated HCT116 p53-/- cells. **D**. Quantification of cell death in post-mitotic cells, which occurs following a prolonged interphase arrest. n=54 (control), n=59 (bleomycin).

Suppl figure 9. Mitosis and post-mitotic fate in bleomycin treated U2OS cells

U2OS cells were treated with 10 μg/ml bleomycin for 10h and following washout of the drug, released into 300 nM UCN-01. Filming was initiated shortly after UCN-01 addition and continued for a total of 72h. UCN-01 was washed out after 12-14h.

A. Quantification of damage-induced mitotic delay. Mitotic duration is shown on the x-axis (h:mm). Mitotic fate is colorcoded: Blue = successful cytokinesis, red = no cytokinesis furrow formed, yellow = regression of cytokinesis furrow. Control treated parental cells generally completed mitosis in 30-45 min, with only 6% (n=54) showing mitotic delay (> 45 min). Bleomycin treated cells driven into mitosis by UCN-01 had a highly variable mitotic timing, ranging from 15 min to more than 3 hours, and 38% (n=69) showed mitotic delay (> 45min). **B.** Quantification of cytokinesis failure in damaged mitotic U2OS cells. The vast majority of control cells (UCN-01 treated) exited mitosis following a successful cytokinesis (98%), and only 2% exited after a failed cleavage (n=54). In bleomycin and UCN-01 treated cells, 67% failed cytokinesis and exited mitosis without division (n= 69). **C.** In control (UCN-01 treated) cells, 60% of the cells non-mitotic cells died (n=30). The cells that escaped this UCN-01 induced interphase death progressed through mitosis and showed very little (7%) post-mitotic cell death (n=106). In contrast to control treated post-mitotic cells, 46% of the post-mitotic,

bleomycin and UCN-01 treated cells died (n=77). Thus, U2OS cells that progress through mitosis with DNA damage show high levels of post-mitotic death. Death in non-mitotic cells treated with bleomycin and UCN-01 was in a similar range as that observed in control treated cells (40%, n=44), suggesting that bleomycin did not increase interphase death above that induced by UCN-01. **D.** While 91% of post-mitotic control cells re-enter mitosis (n=44), that fraction was reduced to 29% in bleomycin and UCN-01 treated post-mitotic cells (n=77). **E.** Cell death in post-mitotic cells, separated into daughters resulting from a failed cleavage or successful cytokinesis. Post-mitotic cell death does not correlate with cleavage failure. **F**. Cell death in bleomycin treated cells occurred after a prolonged interphase arrest in both non-mitotic and post-mitotic cells. Time of death was scored as the first frame where a complete cytoplasmic shrinkage was observed.