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Supplemental Material to:

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Targeting mitotic exit with hyperthermia or APC/C inhibition to increase paclitaxel efficacy

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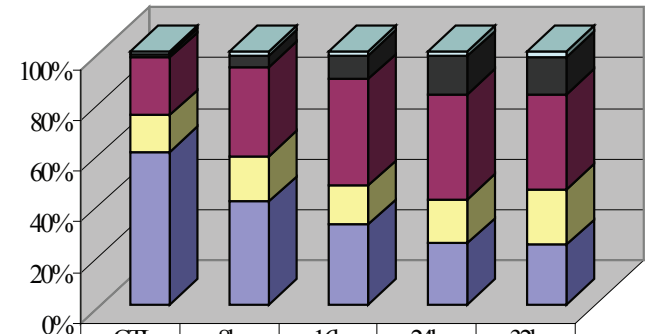
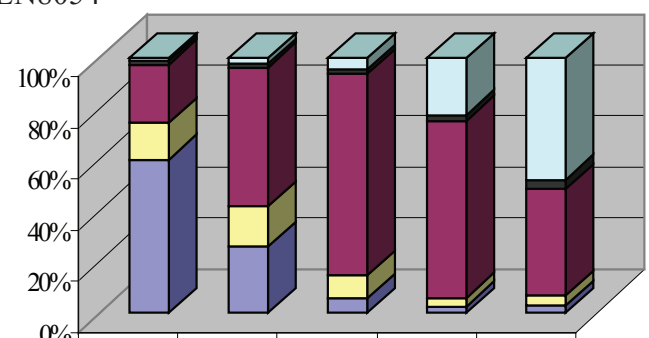
A

PTX



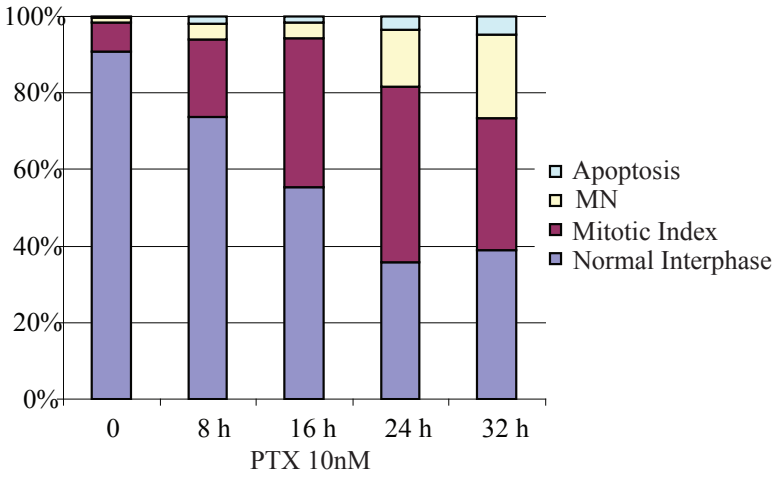
	CTL	8h	16h	24h	32h
>4N	1.1	1.6	1.5	1.8	2
Dead	1.2	3.8	8	12.9	12.6
G2/M	20	30.1	35.2	35.4	31.6
S	12.7	15.2	12.7	15	18.4
G1	53	34.9	26.6	20.7	20.1

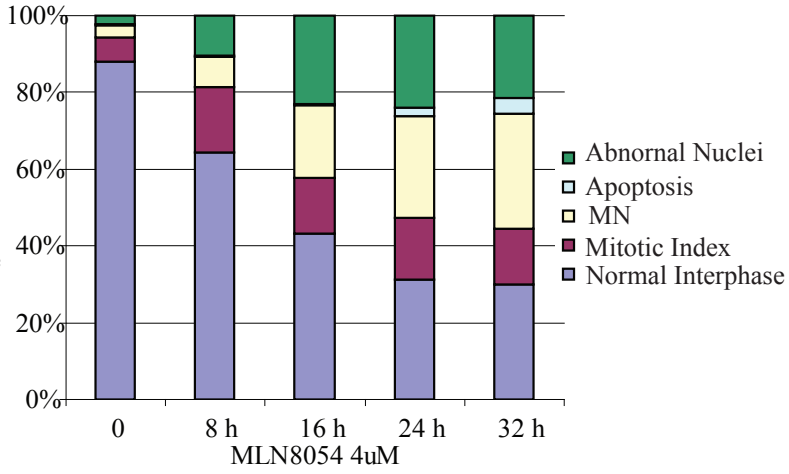
MLN8054



	CTL	8h	16h	24h	32h
>4N	1.1	2	4	17.4	35.6
Dead	1.2	1.3	1.3	1.7	2.6
G2/M	20	47.5	67.7	54	31.2
S	12.7	13.7	7.7	2.6	2.8
G1	53	22.7	5	2	2.2

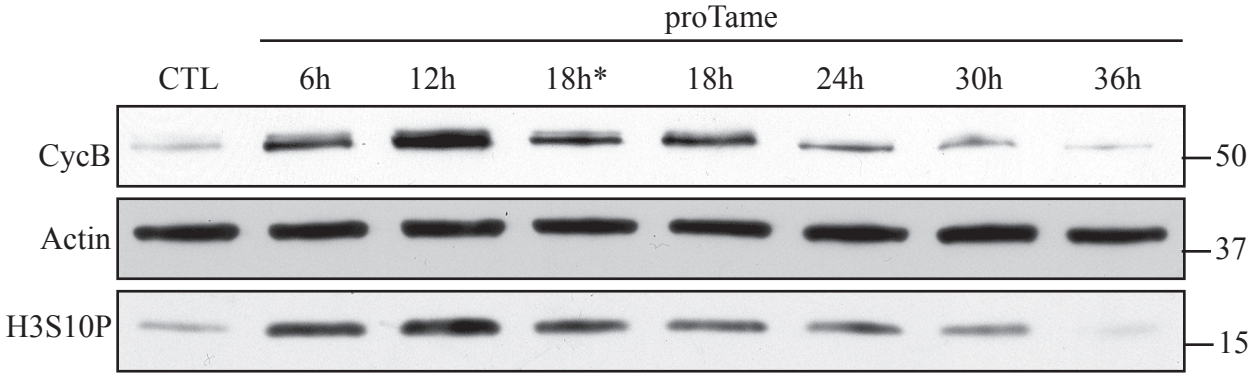
B





Giovinazzi et al Fig. S1

A



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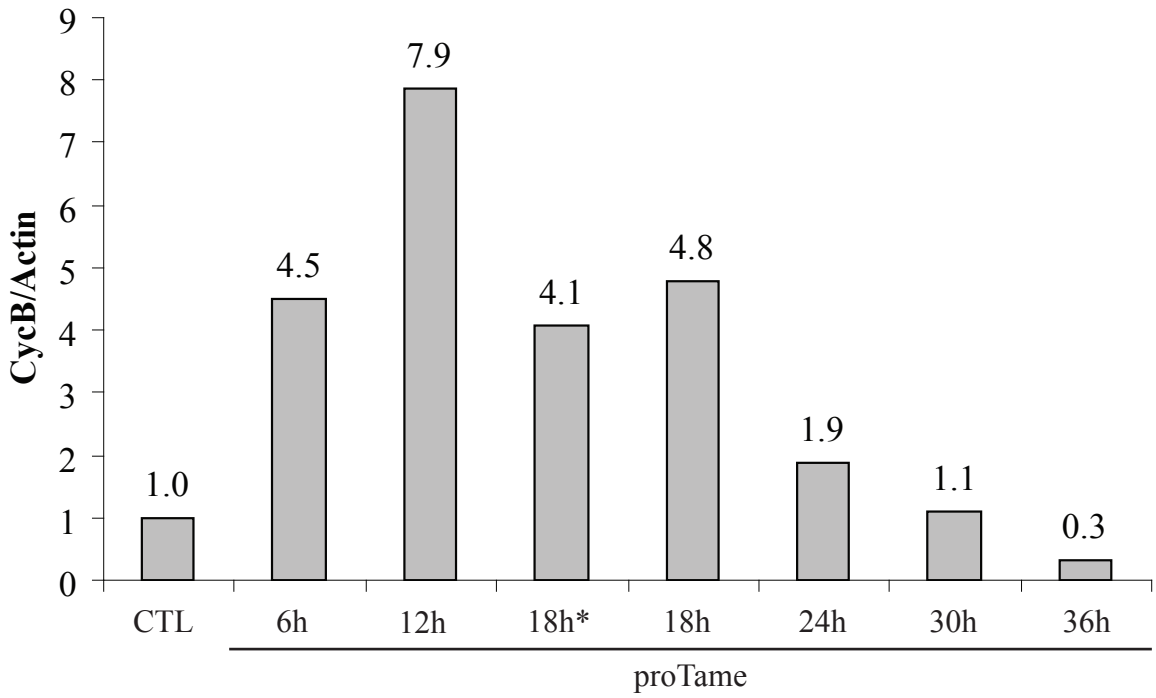
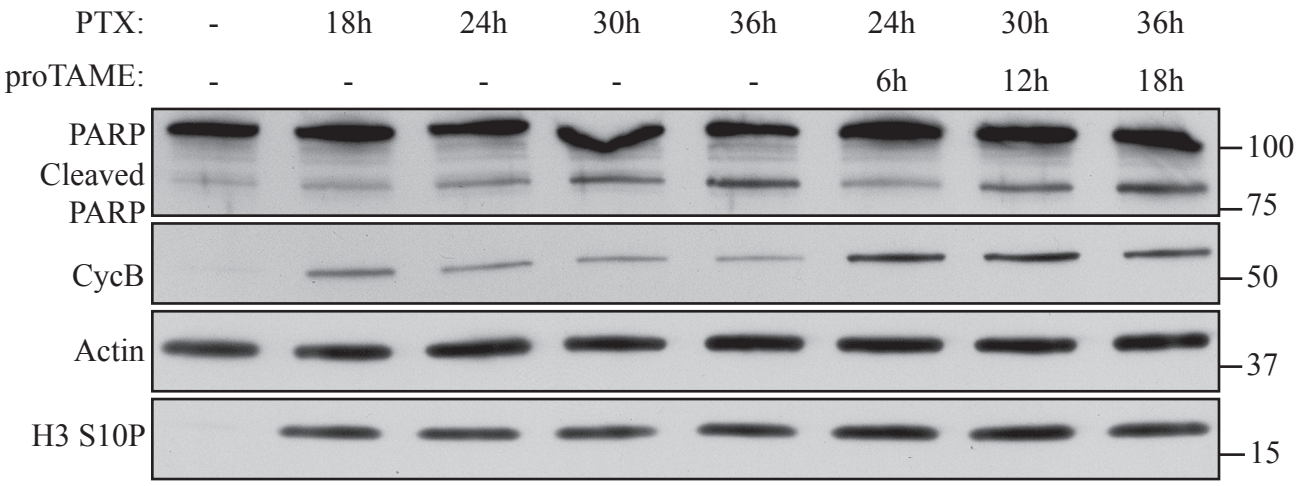


Giovinazzi et al Fig. S2

A



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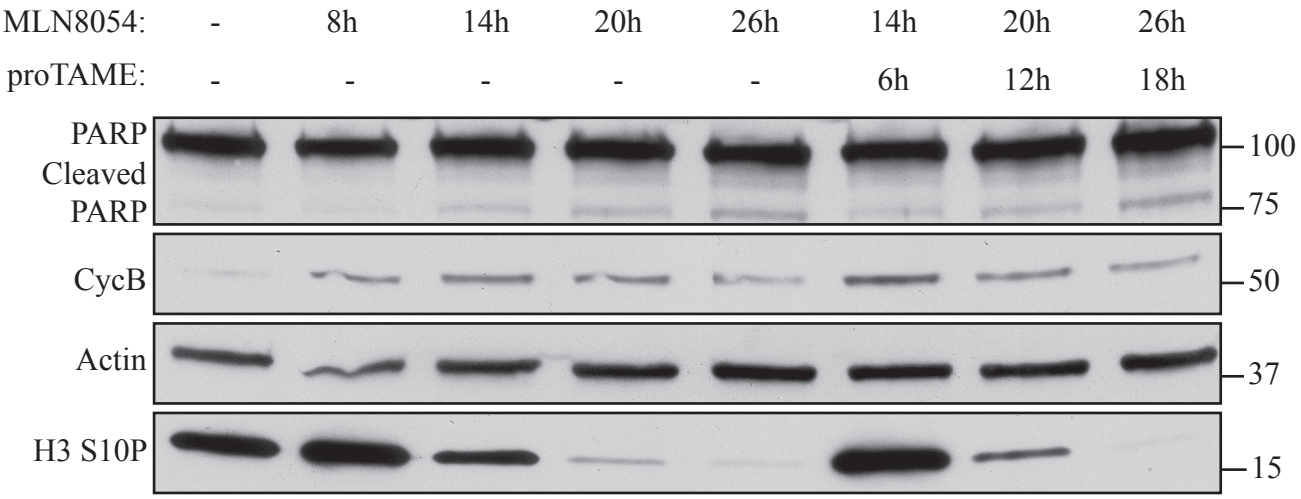

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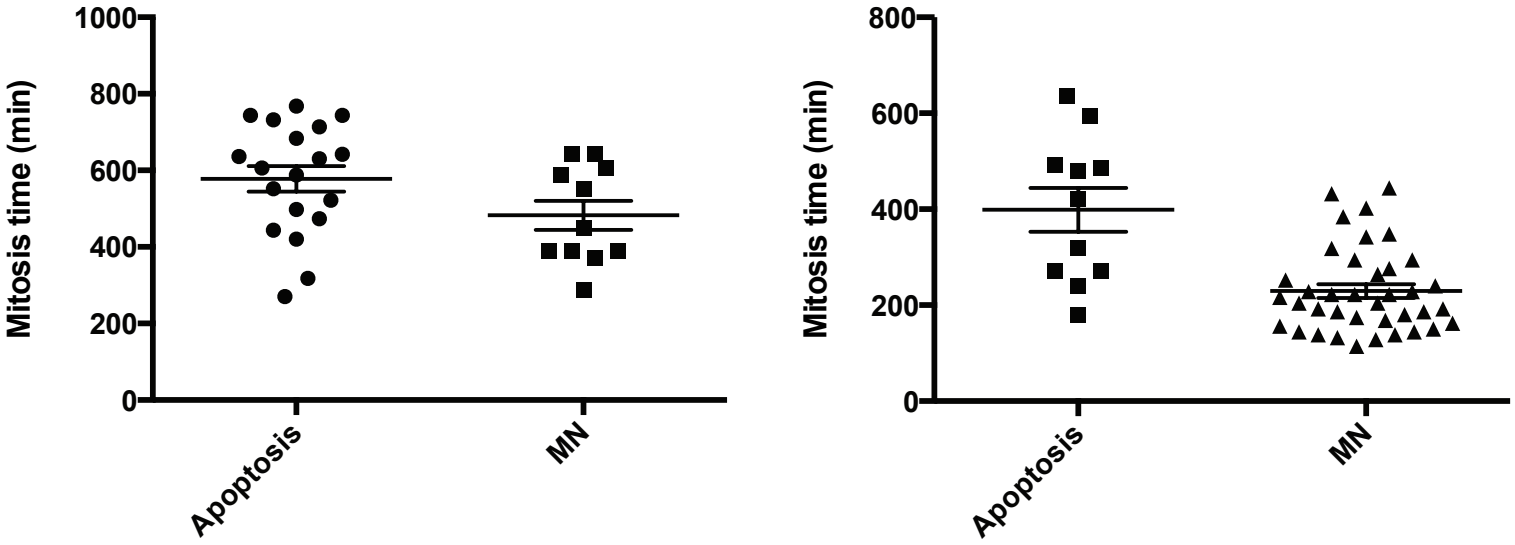
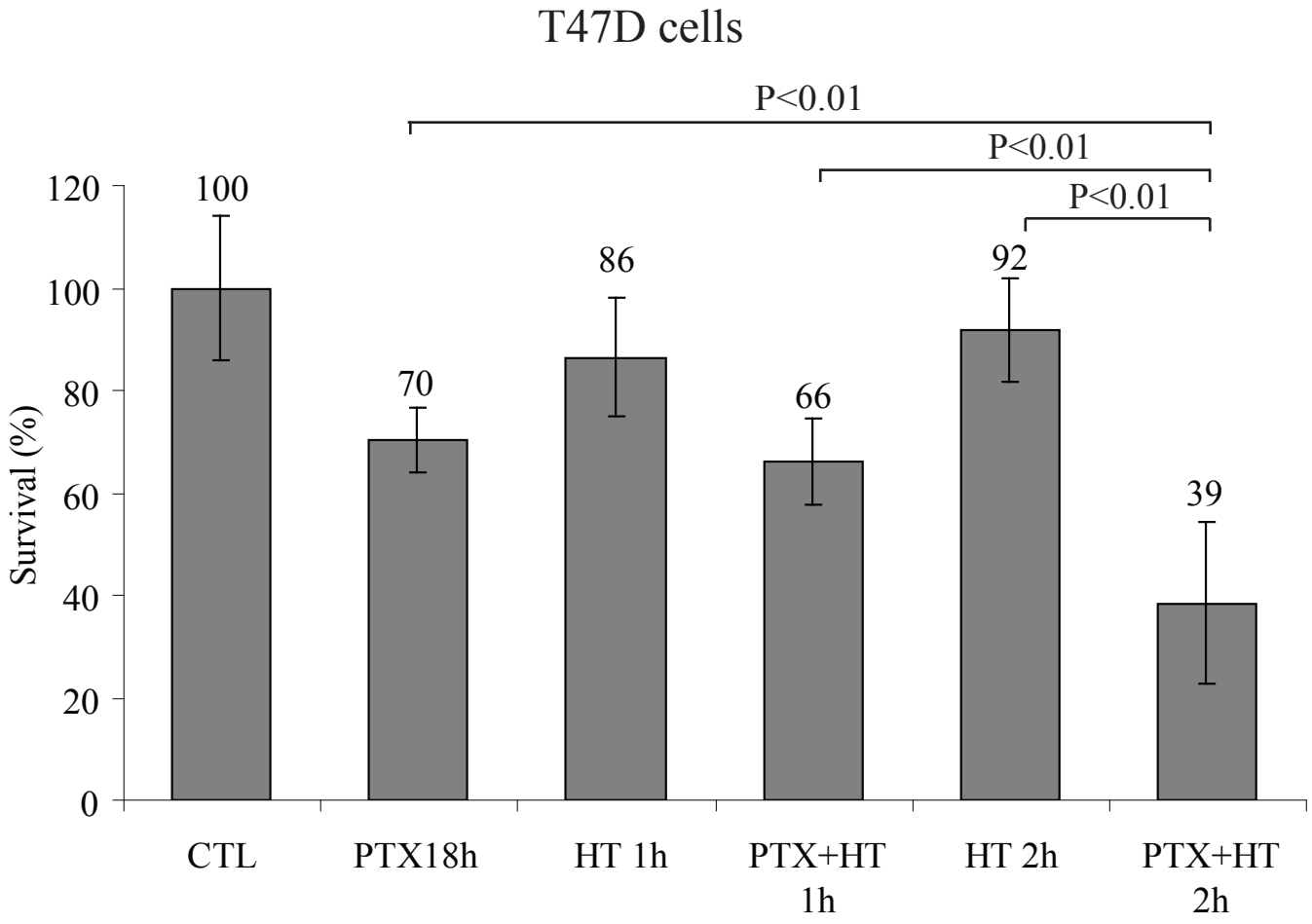
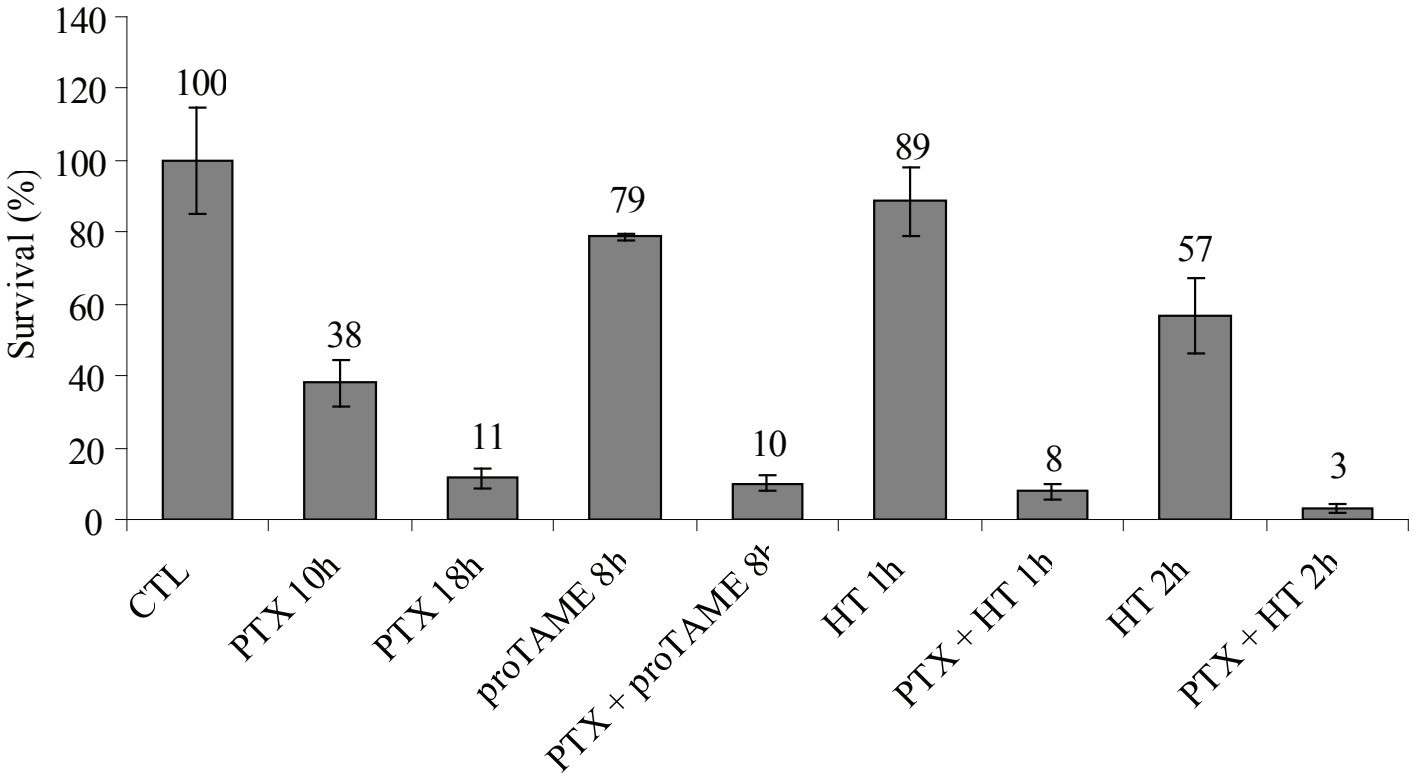


Giovinazzi et al. Fig. S3

A



B



Giovinazzi et al. Fig. S4

Supplementary Figures Description

Figure S1. Analysis of cell treated with anti-mitotic drugs. (A) Cell cycle analysis of HEp2 cells treated for the indicated times with 10 nM PTX or 4μM MLN8054. DNA content was evaluated to assess cell cycle stages, vitality and polyploidy. (B) The same conditions of treatment were adopted to evaluate in HEp2 cells mitotic index, apoptosis, micronuclei (MN) formation and, for MLN8054, the presence of abnormally shaped nuclei (enlarged, doughnut shaped or with protrusions). Microscopy analysis based on DNA morphology; for each experiment at least 100 cells were counted (SD±5). PTX maintains consistently mitotic arrest in cells and starts to accumulate MN after 24 hours of treatment. On the opposite, the Aurora A inhibitor MLN8054, as previously reported [55], blocked only transiently cells in mitosis, as indicated by lower mitotic index, and caused accumulation of cells in G2 and cells with ≥4N DNA content.

Figure S2. proTame transiently blocks cells in mitosis. (A) proTAME was added to HEp2 cells at 12 μM for the indicated time. proTAME causes maximal accumulation of cells in mitosis at 12 hours as demonstrated by cyclin B and H3 Ser10P western blots. (B) cyclin B levels were normalized towards the internal control Actin and protein levels in the control sample. The 18 hours timepoint: cells exposed to proTAME for the first 18 hours of treatment, while the 18* represents an internal control where proTAME was added to cells for the last 18 hours of treatment.

Figure S3. proTAME delays mitotic exit of cells treated with anti-mitotic drugs. Western Blots to evaluate cyclin B stability in HEp2 cells treated with 10 nM PTX (A) or 4 μM MLN8054 (B) with or without the addition of proTAME during the last 6, 12 or 18 hours of treatment. H3 Ser10P was used as positive control of accumulation of cells in mitosis and for MNL8054 activity and PARP to monitor induction of apoptosis. (C) Scatter plots representing mitotic timing of cells that commit either apoptosis or micronucleation (MN) monitored by time-lapse microscopy. 12 μM proTAME was added after a 12 hours pre-treatment with PTX (left, ± SEM) or for 8 hours with MLN8054 (right, pValue<0.001, ± SEM).

Figure S4. HT-induced mitotic exit kills efficiently cancer cells. (A) Survival assay of T47D cells (a cell line resistant to PTX, [42]) treated with 10 nM PTX for a total time of 18 hours with or without HT for 1 or 2 hours (SD±3). (B) Survival assay of HEp2 cells comparing the response of cells arrested in mitosis first by PTX and then treated with HT to trigger mitotic exit or with proTAME to prolong mitotic block. Cells were treated with PTX for a total time of 10 or 18 hours. HT was applied for the last 1 or 2 hours of PTX treatment. proTAME was added for the last 8 hours of PTX exposure (SD±3).

Table S1. proTAME prolongs mitotic arrest and can cause apoptosis. Table listing the mitotic timings of GFP-H2B HEp2 cells untreated, treated with PTX and MLN8054, with or without proTAME. Beside the time measurement for each event, the post-mitotic outcome is also reported. At the bottom of each column, the average mitotic timing, standard deviation and percentage of apoptotic cells (% of A) are shown.

Legend:
D = normal division
A = apoptosis
MC = mitotic catastrophe

Control	
Mitotic time (min)	Cell Fate
60	D
40	D
54	D
56	D
50	D
50	D
54	D
72	D
80	D
48	D
52	D
70	D
56	D
72	D
54	D
46	D
60	D
66	D
54	D
52	D
50	D
60	D
48	D
40	D
70	D
68	D
56	D
54	D
44	D
70	D
42	D
50	D
54	D
56	D
52	D
62	D
70	D
56	D
72	D
50	D
52	D
46	D
62	D
58	D
62	D
56	D
44	D
50	D
58	D
46	D
Average	56.1
St Dev	9.4
% of A	0

PTX	
Mitotic time (min)	Cell Fate
222	MC
246	MC
198	D
510	MC
480	MC
342	MC
174	MC
192	MC
126	MC
252	MC
300	MC
306	MC
588	MC
630	MC
444	A
426	MC
606	MC
234	MC
456	MC
516	MC
318	MC
624	MC
624	MC
488	A
312	MC
486	MC
114	MC
348	MC
306	MC
474	MC
Average	378.1
St Dev	157.6
% of A	6.7

PTX+proTAME	
Mitotic time (min)	Cell Fate
744	A
744	A
732	A
474	A
606	A
444	A
552	MC
588	A
420	A
588	MC
768	A
606	MC
390	MC
270	A
684	A
318	A
288	MC
642	MC
642	MC
552	A
390	MC
630	A
390	MC
714	A
642	A
450	MC
372	MC
636	A
498	A
522	A
Average	543.2
St Dev	144.4
% of A	63.3

MLN8054	
Mitotic time (min)	Cell Fate
576	MC
144	MC
102	MC
210	MC
132	MC
102	MC
222	MC
72	MC
144	MC
174	MC
204	MC
180	MC
348	MC
94	MC
144	MC
414	MC
126	MC
114	MC
216	MC
132	MC
204	MC
306	MC
408	MC
246	MC
120	MC
120	MC
234	MC
228	MC
330	MC
300	MC
354	MC
138	MC
174	MC
138	MC
270	MC
262	MC
294	MC
228	MC
90	MC
120	MC
210	MC
336	MC
156	MC
474	MC
210	MC
120	MC
234	MC
186	MC
318	MC
138	MC
Average	215.9
St Dev	107.4
% of A	0

MLN8054+proTAME	
Mitotic time (min)	Cell Fate
264	MC
276	MC
128	MC
204	MC
186	MC
240	A
270	A
240	MC
222	MC
186	MC
114	MC
348	MC
162	MC
144	MC
222	MC
138	MC
270	A
138	MC
636	A
372	MC
342	MC
420	A
486	A
144	MC
294	MC
216	MC
252	MC
294	MC
402	MC
492	A
480	A
384	MC
180	A
444	MC
174	MC
594	A
432	MC
318	A
222	MC
318	MC
228	MC
132	MC
180	MC
192	MC
156	MC
150	MC
204	MC
192	MC
168	MC
228	MC
Average	269.6
St Dev	107.4
% of A	22.0

Giovinazzi et al. Table S1