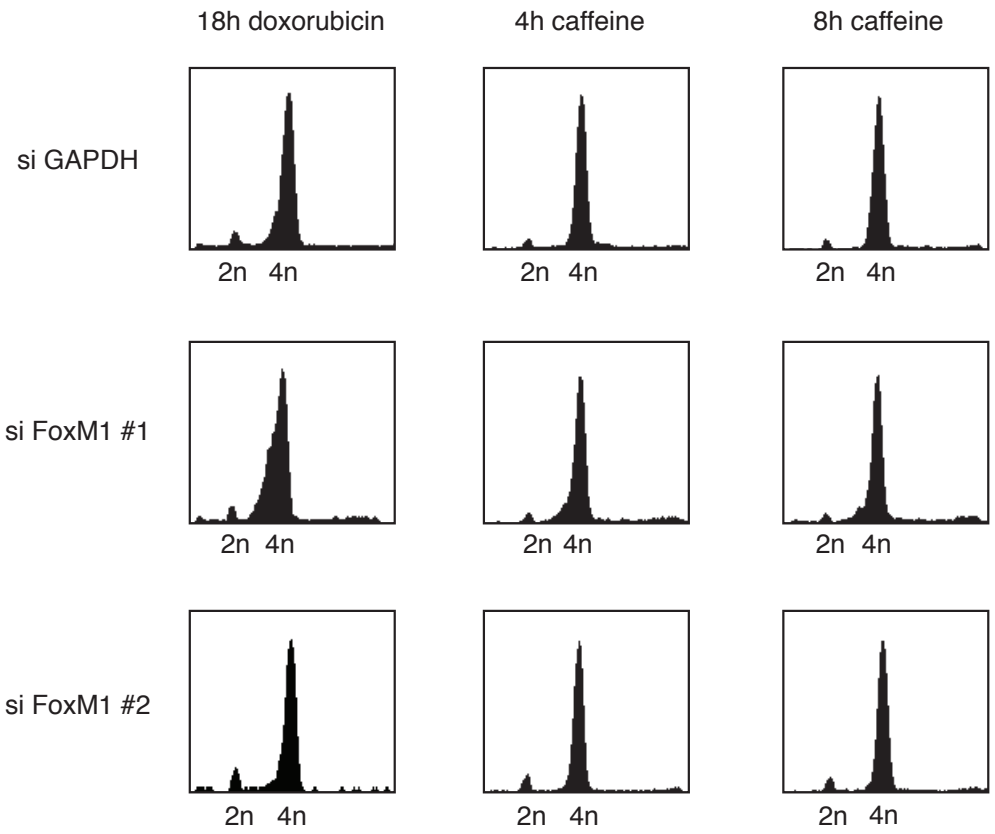
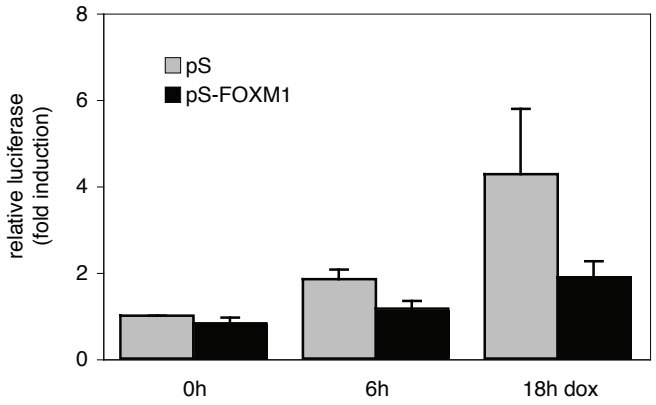
Supplemental Figure S1

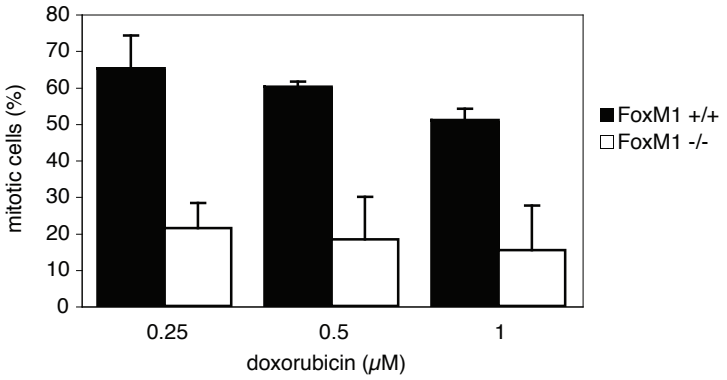


Supplemental Figure S2



Supplemental Figure S3

A



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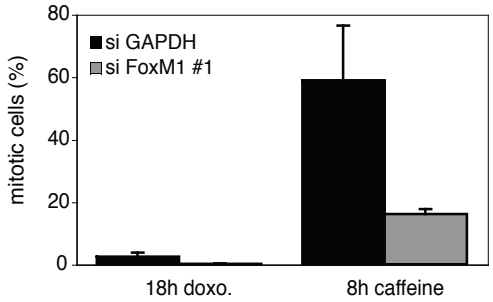
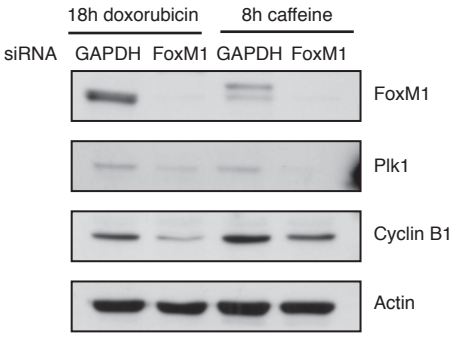
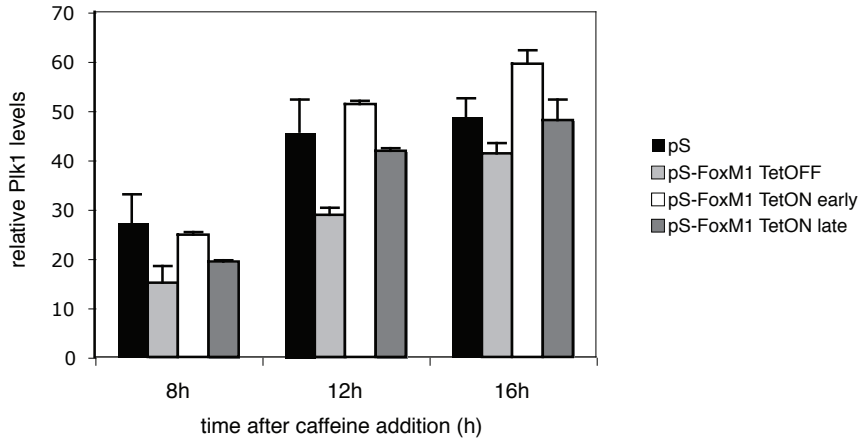




Supplemental Figure S4

A



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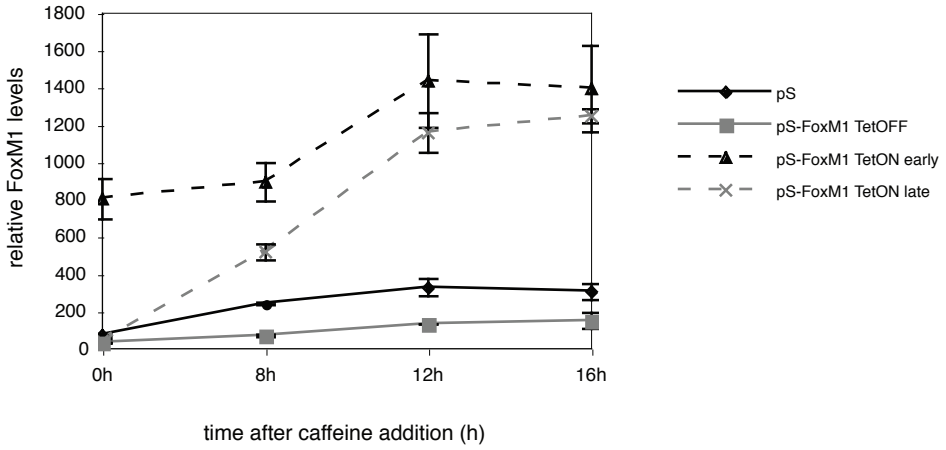
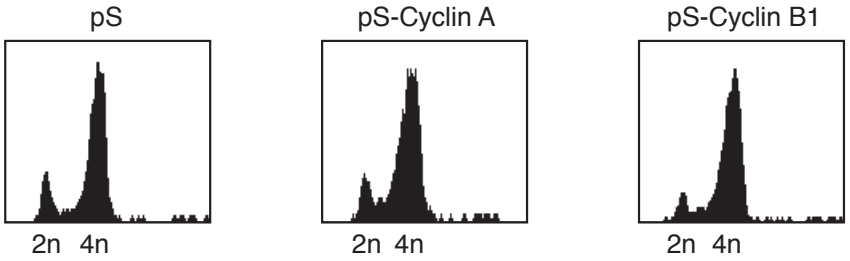


Supplemental Figure S5

A



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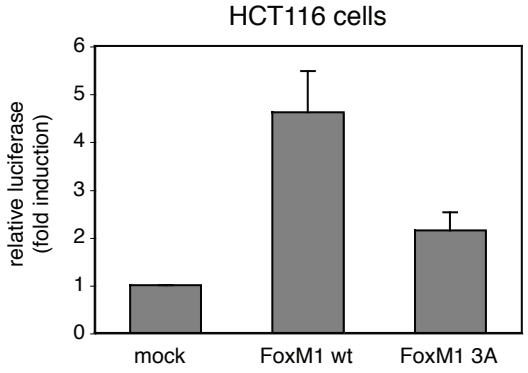
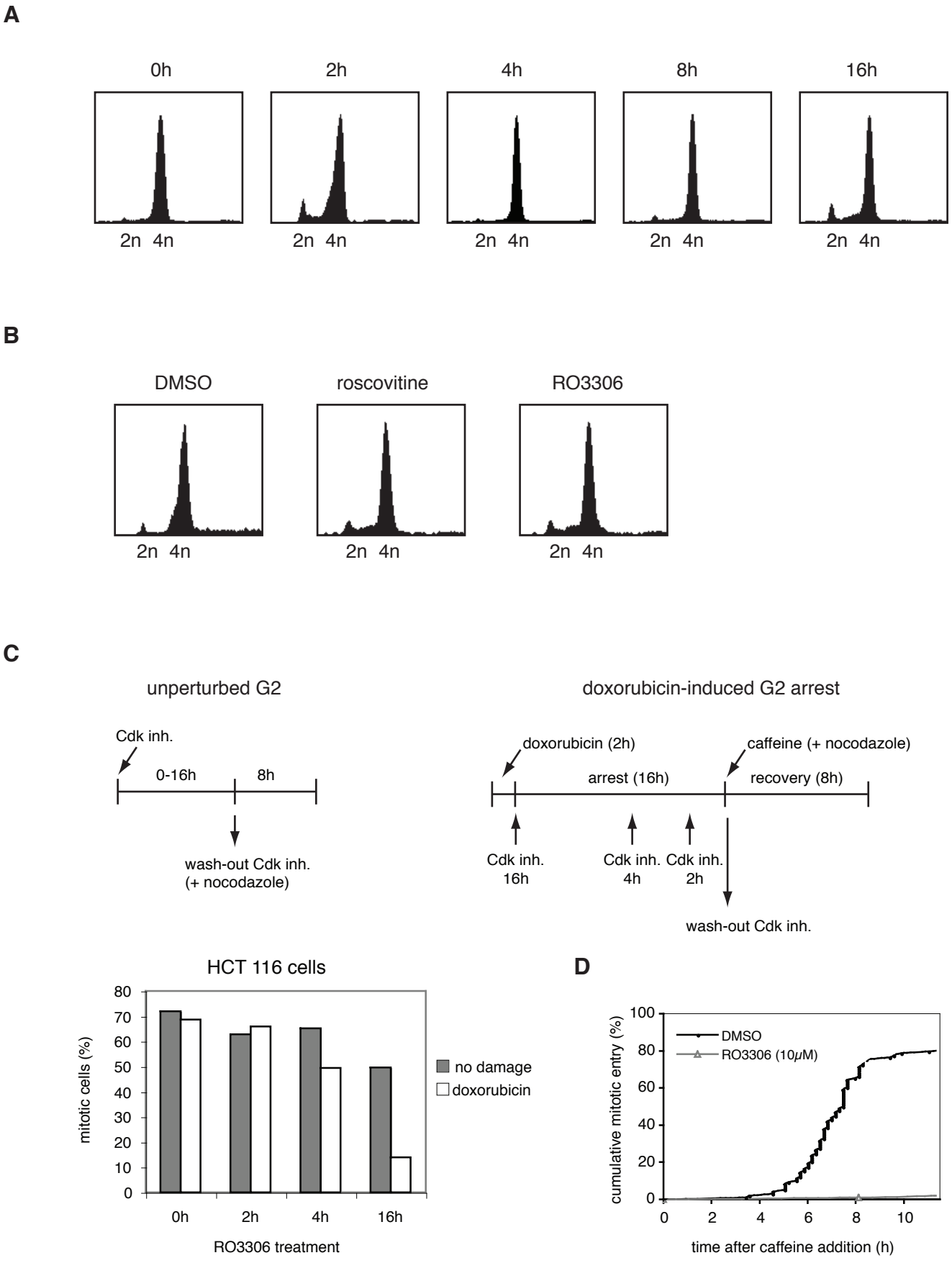


Supplemental Figure S6

	detected phosphosites on FOXM1	
AA. positions	**G2/M**	**Damage**
S35	√ (52)	√ (68)
S191	√ (55)	√ (52)
S290	√ (46)	
S331	√ (67)	
S451	√ (50)	
S481	√ (25)	√ (80)
S501	√ (50)	√ (35)
S505	√ (27)	
S506	√ (28)	√ (23)
S508	√ (35)	√ (23)
S516	√ (33)	
S518	√ (26)	
S522	√ (28)	√ (35)
T600	√ (47)	√ (35)
T611	√ (38)	
T620	√ (29)	√ (27)
T627	√ (31)	√ (34)
S717	√ (70)	
T719		√ (74)
S730	√ (70)	√ (82)
S732	√ (48)	

note: mascot score inside the brackets
Cdk-consensus sites in bold

Supplemental Figure S7



Supplementary Figure Legends

Fig. S1. FACS profiles of U2OS cells transfected with siRNAs against FoxM1 and treated with doxorubicin and caffeine corresponding to experiment shown in Figure 1B.

Fig. S2. U2OS cells were transfected with empty pSuper (pS) or pS-FoxM1, together with the luciferase reporter plasmid pBP-1. Cells were treated with thymidine for 24h, release for 6h and treated with doxorubicin for 1h. Relative luciferase activity was determined in cells in G1/S (0h), 6h after thymidine release and 18h after doxorubicin treatment (18h dox).

Fig. S3. **(A)** Immortalized mouse embryonic fibroblasts (MEFs) were treated with the indicated doses of doxorubicin for 1h and, 18h later, caffeine was added in the presence of paclitaxel. The amount of recovering mitotic cells was determined by pH3 positivity by FACS. **(B)** HCT116 cells were transfected with siRNAs against FoxM1 and GAPDH, and treated with doxorubicin (1 μM, 2h). Caffeine was added, in the presence of nocodazole, 18h after doxorubicin treatment. Cells were collected at the indicated time points and mitotic index was determined by FACS analysis. Efficiency of FoxM1 depletion and levels of FoxM1 targets were assessed by Western-blot.

Fig. S4. U2TR cells stably expressing inducible RNAi-insensitive FoxM1wt were co-transfected with GFP-spectrin and empty pS (pS) or pS-FoxM1. Cells were synchronized in early G2 by release 6h from a thymidine block and damaged with doxorubicin for 1h. Caffeine was added, 18h later, in the presence of nocodazole. Cells were fixed at the indicated time points and stained with antibodies against Plk1 **(A)** and FoxM1 **(B)** and fluorescence intensity of GFP positive cells was measured as described in Methods. At

least 200 cells were counted per well. Measurements were performed in triplicate and error bars represent standard deviation.

Fig. S5. (A) FACS profiles of U2OS cells arrested in G2 after depletion of Cyclin A or Cyclin B and doxorubicin treatment, corresponding to experiment in Figure 3A. **(B)** HCT116 cells were transfected with FoxM1wt and FoxM1 3A and treated with doxorubicin (1 μM) for 2 h. Transactivation of pBP1 reporter by FoxM1 was determined 18h after doxorubicin washout. The graph shows the relative luciferase activity.

Fig. S6. Overview of the phosphorylated residues of FoxM1 identified by mass-spectrometry analysis. FoxM1 was immunoprecipitated from U2OS cells stably expressing YFP-FoxM1 and arrested in G2 18h after doxorubicin treatment or synchronized in G2/M by nocodazole (16h). Gel bands containing FOXM1 were sliced-out from the gels and subjected to in-gel digestion as described previously (1). Protein reduction and alkylation was performed with 10 mM DTT (60 °C, 1 hour) and 55 mM Iodoacetamide (dark, RT, 30min), respectively. Digestion was performed with Trypsin and V8 (Glu-C) overnight at 37 °C. Peptides were extracted with 10 % FA. The extracted peptides were subjected to nanoscale liquid chromatography tandem mass spectrometry (nanoLC-MS/MS) analysis, performed on an Agilent 1100 HPLC system (Agilent technologies) connected to an LTQ Linear Ion Trap Mass Spectrometer combined with an Orbitrap (ThermoFisher, Waltham, MA) as previously described (2). Raw MS data were converted to peak lists using DTASuperCharge version 1.27. Spectra were searched against the IPI (International Protein Index) Human database version 3.37 (69164 sequences; 29064824 residues) using Mascot software version 2.2.0 (www.matrixscience.com), with Trypsin and V8 set as enzymes. The database search was

made with the following parameters set to consider a peptide tolerance of ± 50 ppm, a fragment tolerance of ± 0.9 Da, allowing 1 and 6 missed cleavages for Trypsin and V8 respectively, Carbamidomethyl (C) as fixed modification, Oxidation (M), N-acetylation (protein N terminal) and Phosphorylation (S,T,Y) as variable modification. All phosphorylated peptides were also manually inspected.

References:

1. Shevchenko A, Tomas H, Havlis J, Olsen JV, Mann M. 2006. In-gel digestion for mass spectrometric characterization of proteins and proteomes. Nat Protoc. 1(6):2856-60.
2. Pinkse MW, Mohammed S, Gouw JW, van Breukelen B, Vos HR, Heck AJ. 2008. Highly robust, automated, and sensitive online TiO2-based phosphoproteomics applied to study endogenous phosphorylation in Drosophila melanogaster. J Proteome Res. 7(2):687-97.

Fig. S7. (A) Cell cycle profiles of U2OS cells arrested in G2 after different periods of roscovitine treatment corresponding to experiment in Figure 4A. **(B)** Cell cycle profiles of U2OS cells arrested in G2 after doxorubicin and Cdk inhibitors treatment corresponding to experiment in Figure 4B **(C)** HCT116 cells were treated with the Cdk inhibitor RO3306 (10 μM) alone or after doxorubicin treatment for the indicated period of times, as described in the scheme of the experimental setting. Percentage of mitotic cells was determined by FACS analysis 8h after washing out the inhibitor, in the absence or presence of caffeine, respectively. **(D)** HCT116 cells were treated with doxorubicin

(1μM) for 2h. After doxorubicin washout, RO3306 was added for 16h. Mitotic entry after washing-out the inhibitor and caffeine addition was monitored by time-lapse microscopy.