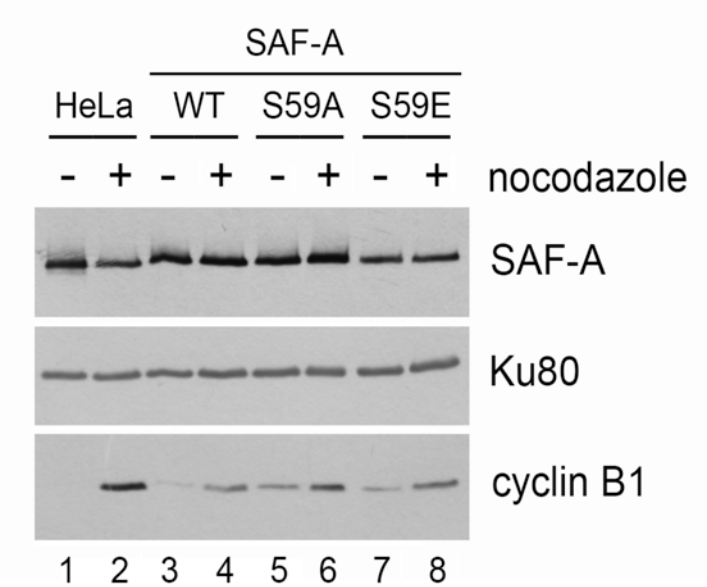
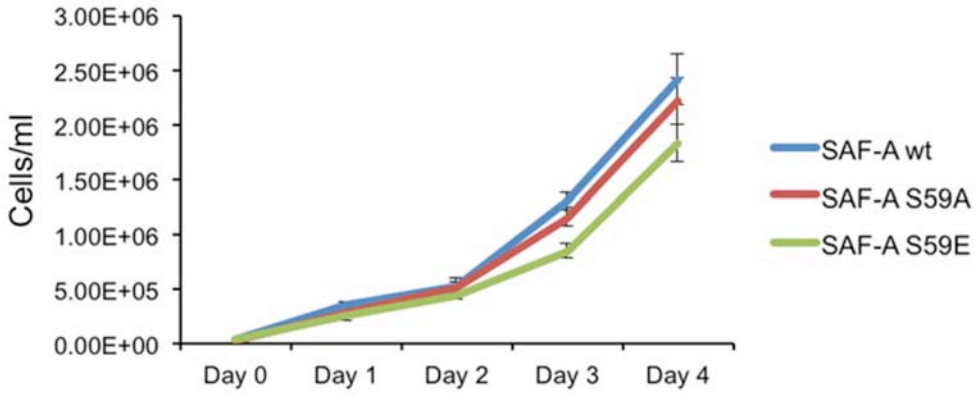
Supplementary Table 1. Summary of data for FACS analysis of MRC5-SV SAF-A WT, SAF-A S59A and SAF-A S59E cells shown in Figure 7. Results are average number of cells in each stage of the cell cycle (G1, S or G2) by propidium iodide staining or cells staining positive for histone H3 pS10 (n = 5), with standard deviation and statistical significance (Students T test). Values that are significant (p = < 0.05) are indicated by *. See text and Figure 7 for details.

		Ave	STD	T test	p	Significance
G1	**WT**	4.46	1.95	WT/S59A	0.112	
	S59A	2.22	0.79	WT/S59E	0.812	
	S59E	4.91	2.81	S59A/S59E	0.051	
S	**WT**	24.50	3.24	WT/S59A	0.138	
	S59A	20.26	6.68	WT/S59E	0.103	
	S59E	29.73	6.87	S59A/S59E	0.075	
G2	**WT**	71.03	5.00	WT/S59A	0.025	*
	S59A	77.51	6.08	WT/S59E	0.147	
	S59E	65.36	7.39	S59A/S59E	0.059	
H3 pS10	**WT**	28.70	1.83	WT/S59A	0.329	
	S59A	31.94	6.38	WT/S59E	0.0002	*
	S59E	18.04	2.90	S59A/S59E	0.0205	*

Supplementary Figure 1 A. SAF-A is expressed at equal levels in SAF-A WT, S59A and S59E cell lines. HeLa cells (lanes 1-2) or MRC-SV cells in which endogenous SAF-A had been depleted and replaced with FLAG-tagged WT (lanes 3-4), S59A (lanes 5-6) or S59E SAF-A (lanes 7-8) [see (Britton, Froment et al. 2009) for details] were grown under either asynchronous conditions (indicated by -), or treated with nocodazole (40 ng/ml, 16 hours) then harvested by mitotic shake off and allowed to recover in nocodazole-free media for 35 minutes (indicated by +) as described previously (Douglas, Ye et al. 2014). NETN extracts were prepared as described previously (Douglas, Zhong et al. 2010) and samples were analyzed by SDS PAGE and immunoblot with the antibodies indicated on the right. The antibody to total SAF-A was from Abcam (mouse monoclonal antibody, catalogue number 10297). The results show that SAF-A was expressed at similar levels in all MRC-SV cell lines and at similar levels to that of endogenous SAF-A in HeLa cells.

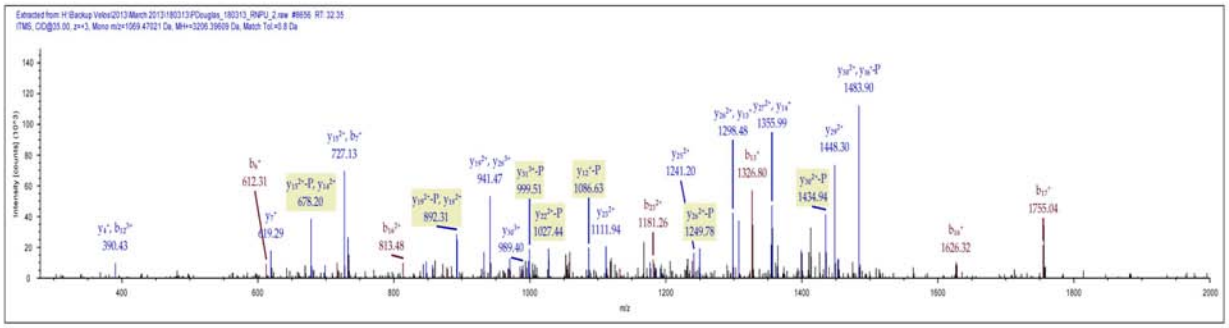


Supplementary Figure 1B. Growth curves of SAF-A WT, 59A and 59E cells. MRC5-SV stably expressing SAF-A WT, SAF-A S59A or SAF-A S59E cells were seeded at approximately 6000 cells/ml and an aliquot removed, stained with Trypan Blue 1, 2, 3 and 4 days later as indicated. Viable cells (exclusion of Trypan blue dye) were counted in triplicate using a haemocytometer. Results show the average of triplicate samples with STD.

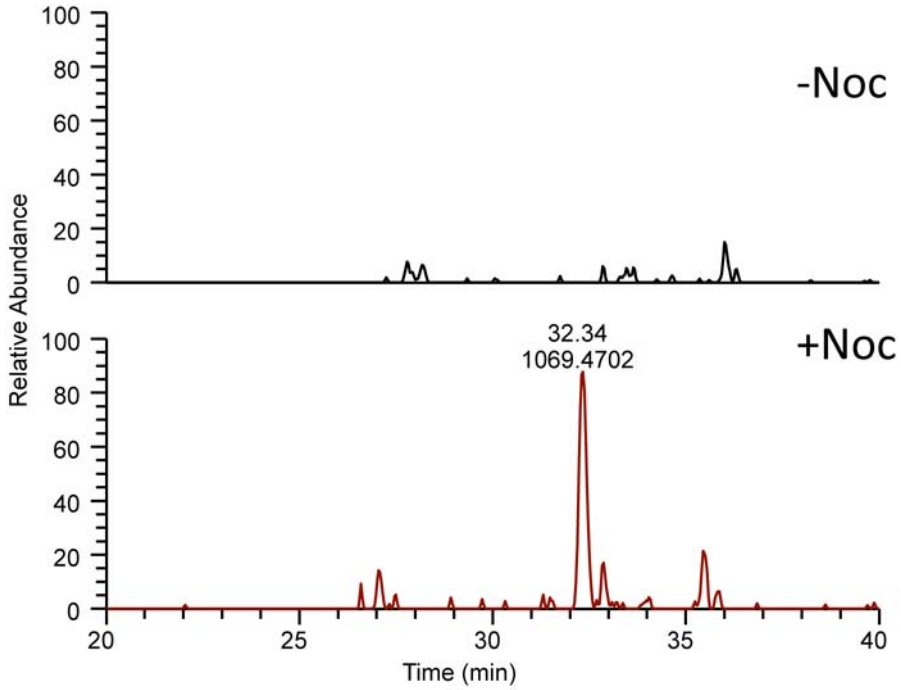


Supplementary Figure 2. Confirmation of SAFA-S59 phosphorylation in mitosis by mass spectrometry. MRC5-SV stably expressing wild type FLAG-tagged SAF-A (SAF-A WT) were grown under asynchronous conditions and harvested by trypsin EDTA or treated with nocodazole (40 ng/ml) for 16 hours then harvested by mitotic shake off. Nocodazole treated cells were left to recover in fresh media (minus nocodazle) for 35 minutes then SAF-A was immunoprecipitated as described previously (Douglas, Zhong et al. 2010, Douglas, Ye et al. 2014) with anti-FLAG antibodies and immunoprecipitates were resolved by SDS-PAGE, and stained with Bio-Safe Coomassie stain (Bio-Rad). Discrete gel slices were dissected from the gel. Trypsin digestion of the gel slices, mass spectrometry analysis and data base searching were performed as described in (Roget, Ben-Addi et al. 2012). Detailed spectra are available upon request.

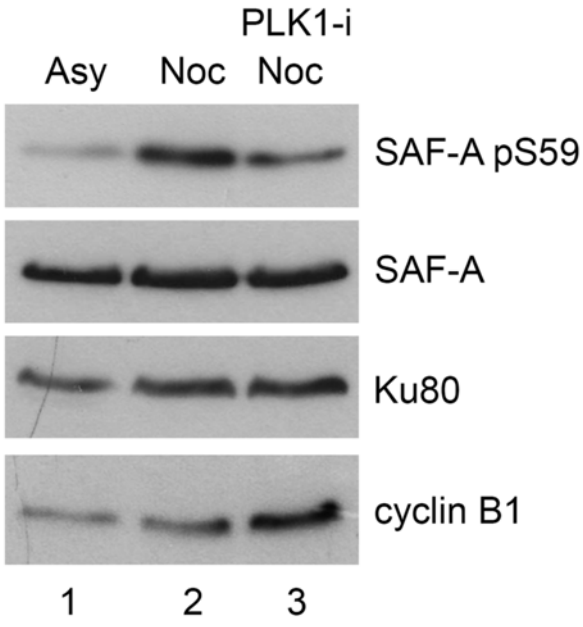
A:- MSMS spectrum of LQAALDDEEAGGRPAMEPGNGpSLDLGGDSAGR



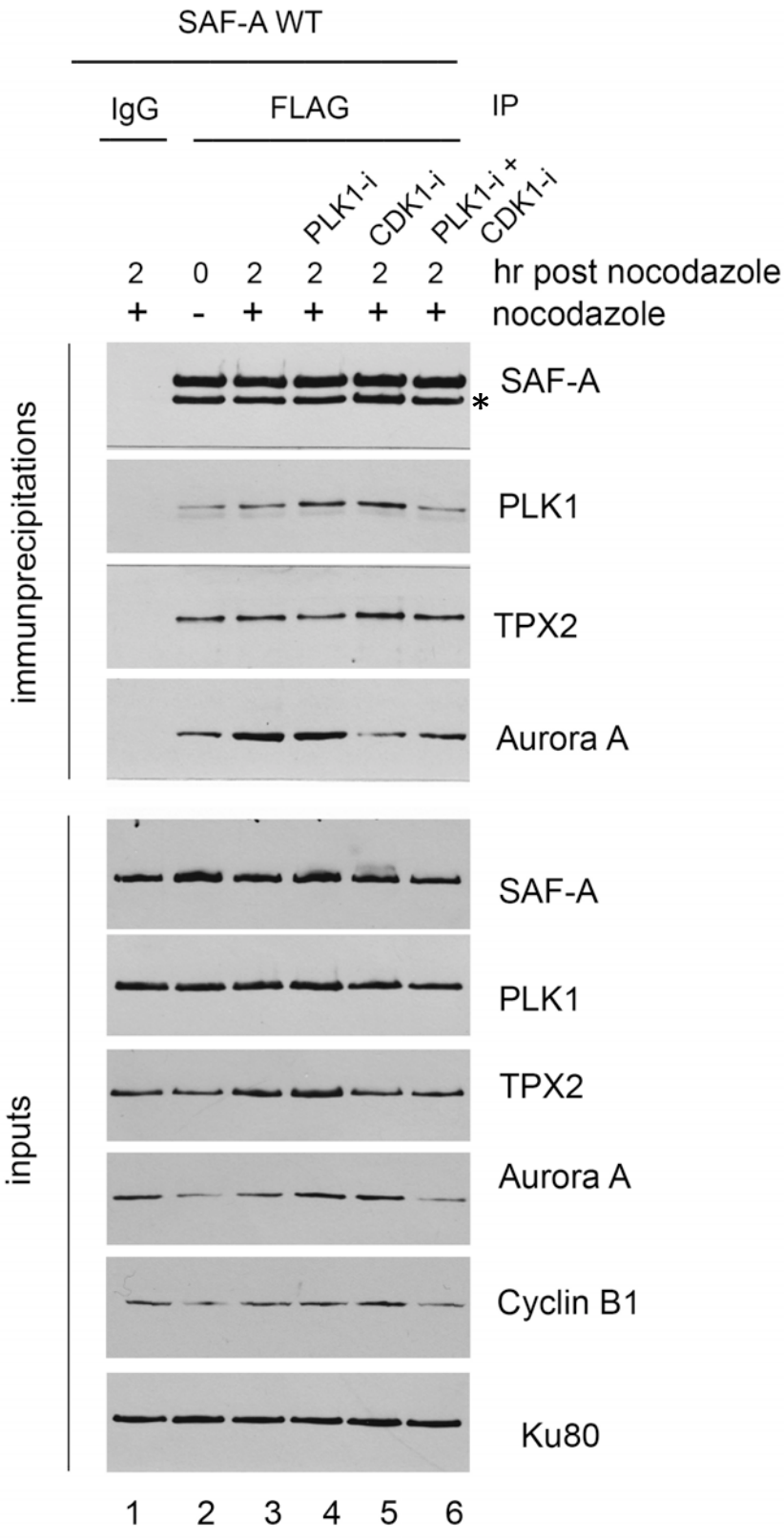
B:- Extracted ion chromatogram of LQAALDDEEAGGRPAMEPGNGpSLDLGGDSAGR (m/z 1069.4702 $^{3+}$) from the tryptic digest of SAF-A isolated from cells cultured in the absence or presence of 40ng/ml nocodozole.



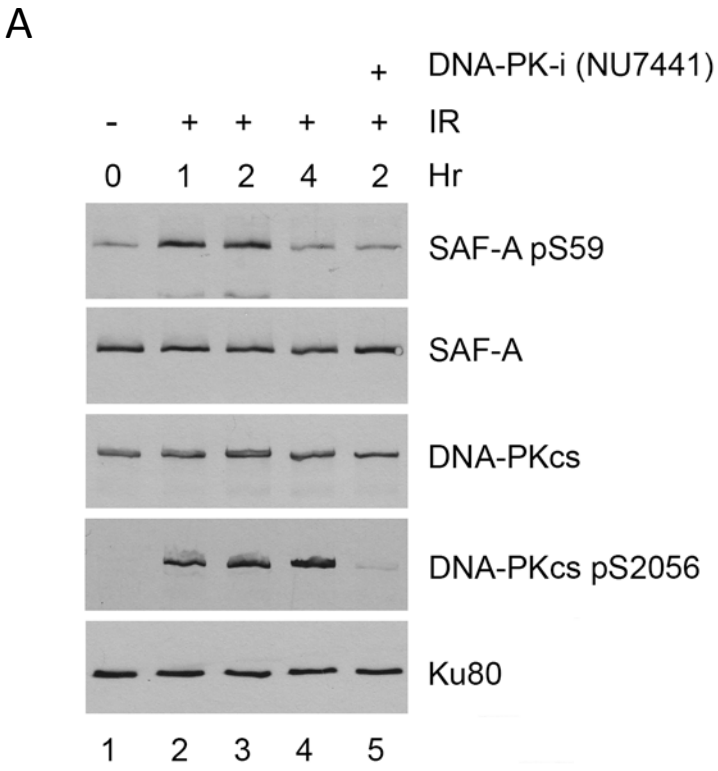
Supplementary Figure 3. Endogenous SAF-A is phosphorylated on S59 in nocodazole treated MCF7 cells. MCF-7 breast cancer cells were treated with nocodazole (40 ng/ml, 16 hours), collected by shake off and allowed to recover in nocodazole–free media for 35 minutes. Where indicated, the PLK1 inhibitor BI2536 was added (100 nM) for one hour before shake off and for 35 minutes after shake off, as described in the main text. Samples were run on SDS gels and probed with the in house generated rabbit polyclonal antibody to SAF-A phospho-S59, and antibodies to total SAF-A, Ku80 and cyclin B1 as indicated. The results show that endogenous SAF-A is phosphorylated on S59 in nocodazole-treated MCF7 cells and that PLK1 is , in part, responsible for SAF-A S59 phosphorylation in mitosis. The phosphospecific antibody to SAF-A pS59 was rabbit and the antibody to total SAF-A mouse.



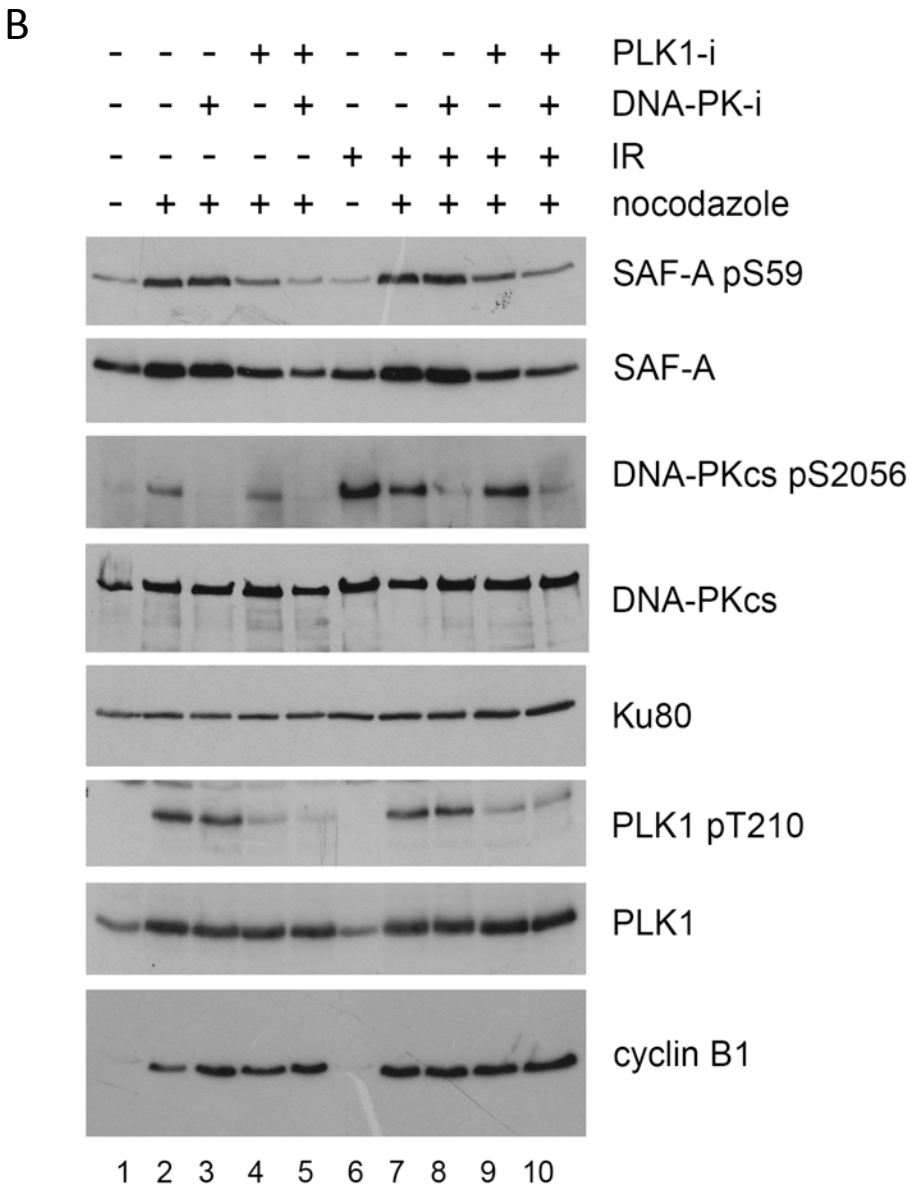
Supplementary Figure 4. Interaction of SAF-A with PLK1 is not disrupted by inhibition of PLK1 and/or CDK1. MRC5-SV cells expressing FLAG-tagged SAF-A WT were grown under asynchronous conditions (lane 2) or treated with nocodazole 40 ng/ml for 16 hours then harvested 2 hours after recovery in nocodazole-free media (lanes 1, 3-6). Where indicated, cells were incubated with the PLK1 inhibitor BI2536 (100 nM) (lane 4), the CDK1 inhibitor RO3366 (20 µM) (lane 5) or both inhibitors together (lane 6) for 1 hour before nocodazole removal and 2 hours post-nocodazole removal as indicated. Samples in lanes 2-6 were immunoprecipitated with anti-FLAG beads as in Figure 4. The sample in lane 1 was immunoprecipitated with IgG as a control. Immunoblots were probed with antibodies to SAF-A, PLK1 TPX2 and Aurora A as indicated. The lower panel shows western blots for 50 µg of protein for each immunoprecipitate. The antibody to total SAF-A in the immunoprecipitation panel was rabbit polyclonal (Abcam 20666), while that in the input panel was a mouse monoclonal (Abcam 10297). The asterisk marks a truncated form of SAF-A. See text for details.


SAF-A WT
IgG
FLAG
IP
PLK1-i
CDK1-i
PLK1-i + CDK1-i
2 0 2 2 2 2 hr post nocodazole
+ - + + + + nocodazole
immunprecipitations
SAF-A
*
PLK1
TPX2
Aurora A
inputs
SAF-A
PLK1
TPX2
Aurora A
Cyclin B1
Ku80
1 2 3 4 5 6

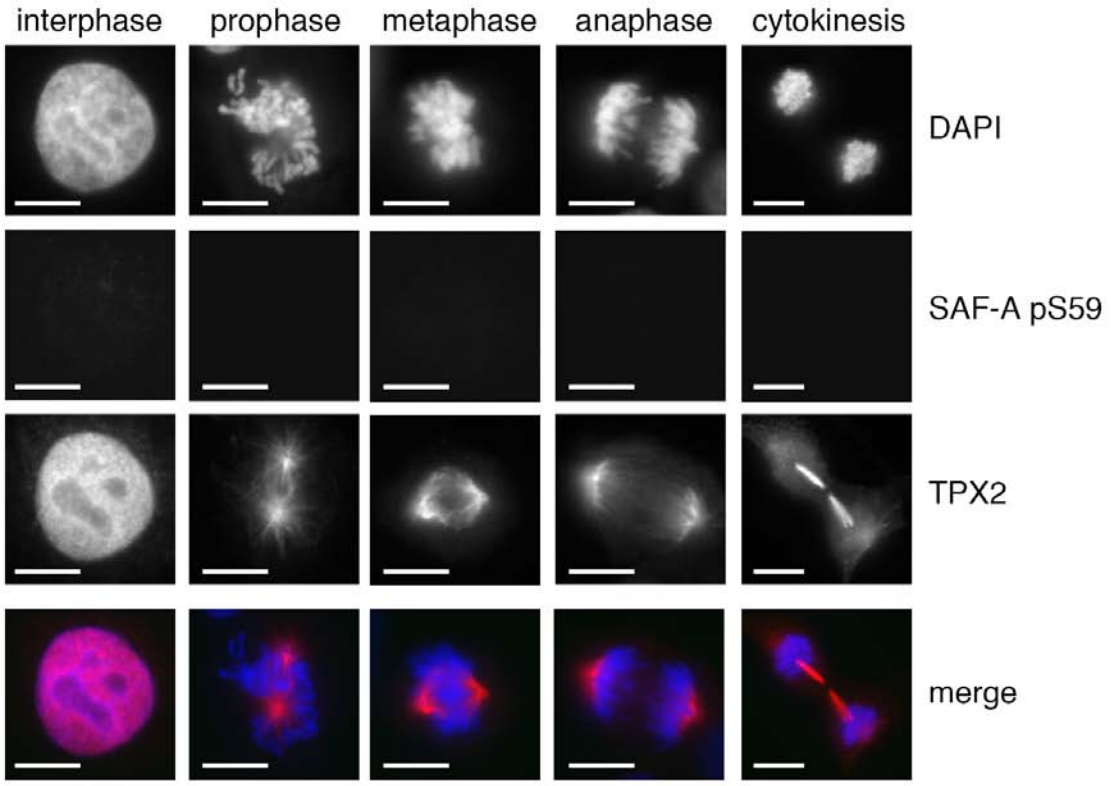
Supplementary Figure 5A. SAF-A S59 phosphorylation is partially DNA-PK-dependent after IR. HeLa cells were either left untreated (lane 1) or irradiated 10 Gy, and allowed to recover at 37°C for 1, 2 or 4 hours as indicated. In lane 5, cells were incubated with 8 µM NU7441 to inhibit DNA-PK for 1 hr prior to irradiation and 2 hr post-IR.



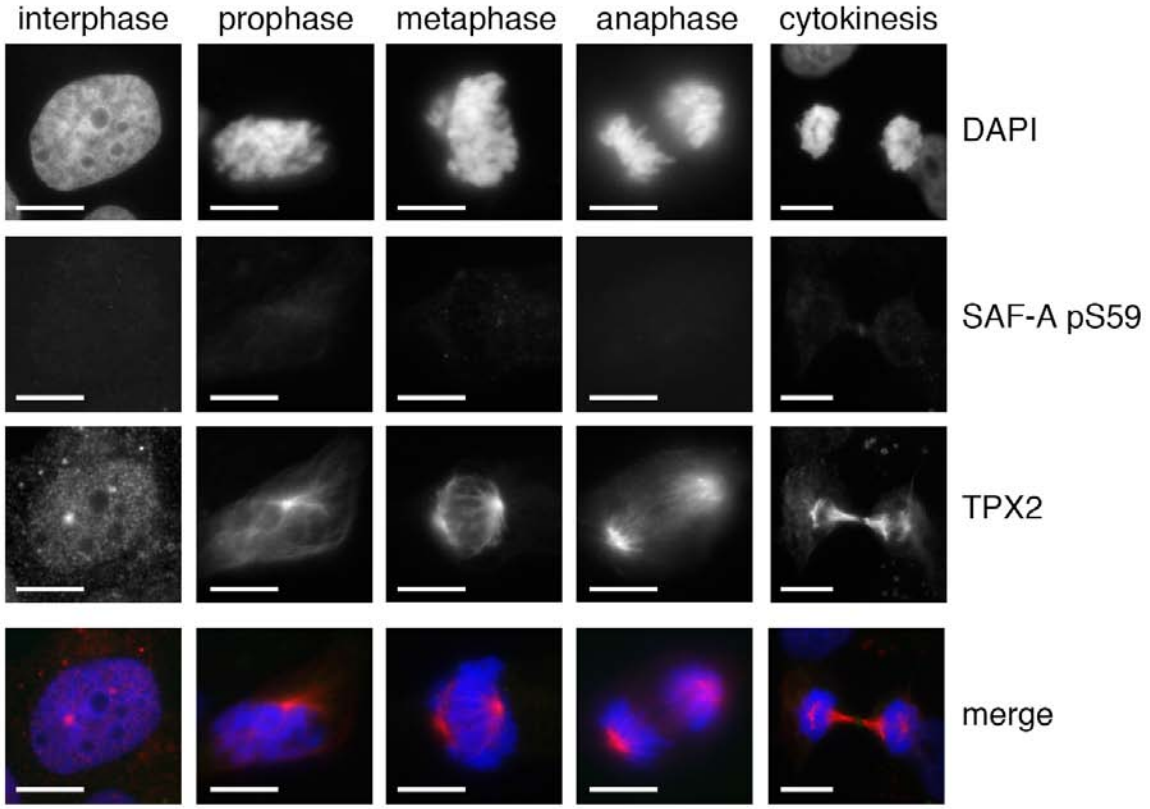
Supplementary Figure 5B. SAF-A S59 phosphorylation is largely PLK1-dependent when mitotic cells are exposed to IR. HeLa cells were either left untreated (lane 1 and 6) or treated with 40 ng/ml nocodazole for 16 hours (lanes 2-5 and 7-10). One hour prior to mitotic shake off, cells were treated with either 8 μM NU7441 (to inhibit DNA-PK, lanes 3, 5, 8 and 10) or 100 nM BI2536 (to inhibit PLK1, lanes 4, 5 9 and 10). Cells were harvested by mitotic shake off and either treated with 10 Gy IR (lanes 6-10) or not irradiated (lanes 1-5). All cells were left to recover in media minus nocodazole for 35 mins, with inhibitors added as indicated (lanes 3-5 and 8-10). NETN extracts were generated as described in Materials and Methods and 50 μg of lysate was run on SDS PAGE gels with various percentages of acrylamide and immunoblots were probed with various antibodies as indicated on the Figure.



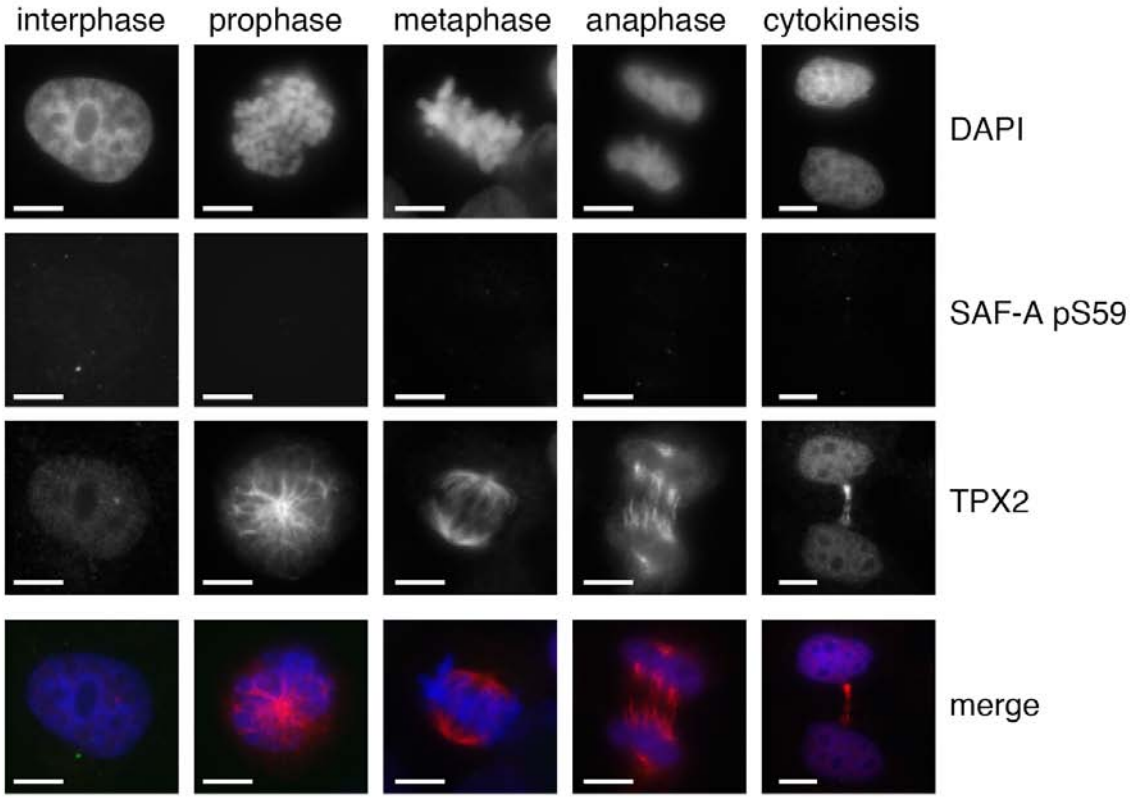
Supplementary Figure 6. **Incubation with a blocking peptide blocks SAF-A phospho-S59 staining in mitotic HeLa cells.** HeLa cells were grown on poly-L-lysine coated coverslips, then stained with DAPI (blue), a mouse monoclonal antibody to SAF-A phospho-S59 (green) in the presence of 10 µg/ml blocking peptide (EPGNG(pS)LDLGGC) and TPX2 (red) and processed for immunofluorescence as described in Materials and Methods. Representative cells in interphase, prophase, metaphase, anaphase and cytokinesis are shown. Panels for individual antibodies are shown in black and white for contrast and in colour in the merge, lower panel. Scale bars represent 10 µm.



Supplementary Figure 7. **Lack of signal for SAF-A pS59 in SAF-A S59A cells.** SAF-A S59A cells were grown on poly-L-lysine coated coverslips, then stained with DAPI (blue), a mouse monoclonal antibody to SAF-A phospho-S59 (green), and TPX2 (red) and processed for immunofluorescence as described in Materials and Methods. Representative cells in interphase, prophase, metaphase, anaphase and cytokinesis are shown as in Supplementary Figure 6. Scale bars represent 10 µm. The were taken at the same time and under the same conditions as the experiments shown in Figure 5.



Supplementary Figure 8. Incubation with the PLK1 inhibitor BI2536 blocks SAF-A S59 phosphorylation in vivo. HeLa cells were grown on poly-L-lysine coated coverslips, incubated with the PLK1 inhibitor BI2536 (100 nM, 2 hours), then stained with DAPI (blue), a mouse monoclonal antibody to SAF-A phospho-S59 (green), or TPX2 (red) and processed for immunofluorescence as described in Materials and Methods. Representative cells in interphase, prophase, metaphase, anaphase and cytokinesis are shown as in Supplementary Figure 6. Scale bars represent 10 µm.

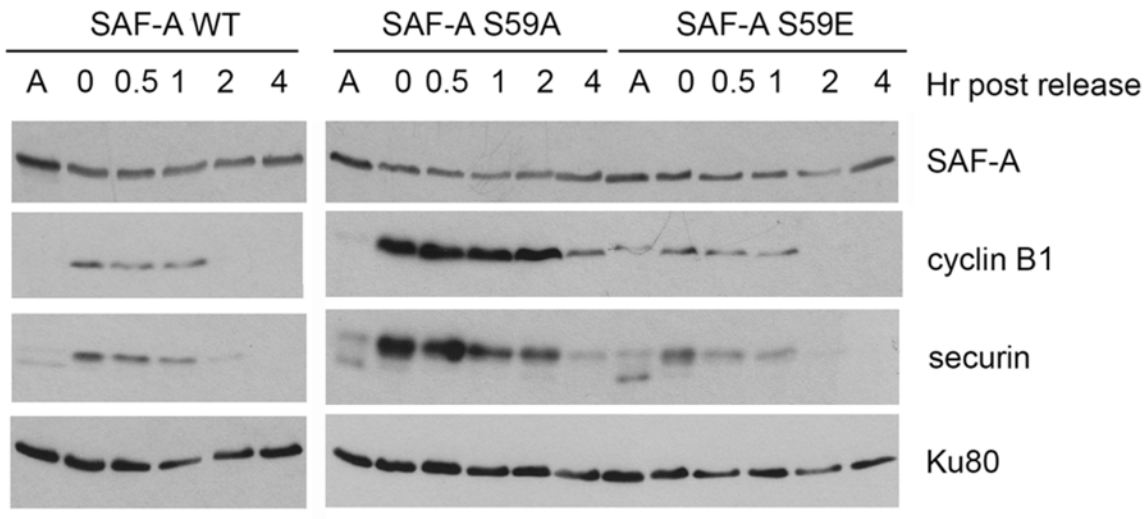


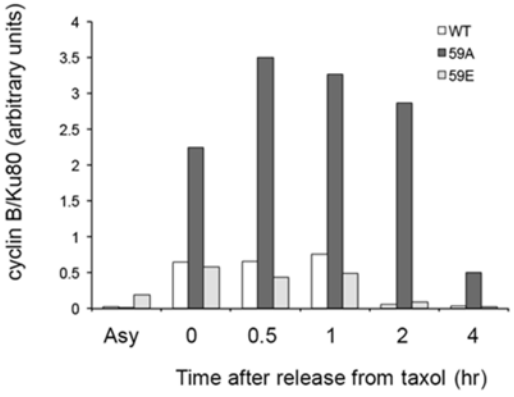
Supplementary Figure 9
Videos of live cell imaging results for SAF-A WT, SAF-A S59A and SAF-A S59E shown in Figure 8.

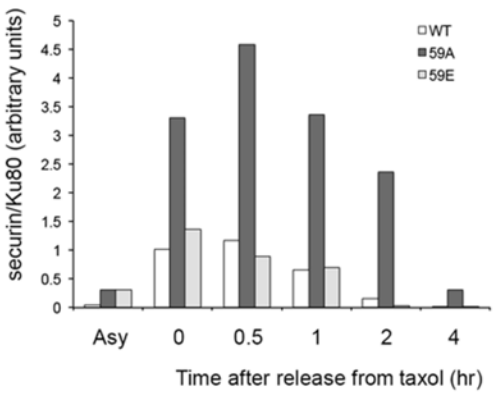
A: SAFAWT.mov
B: SAFA59A.mov
C: SAFA59E.mov

Additional videos of live cell imaging results are available upon request.

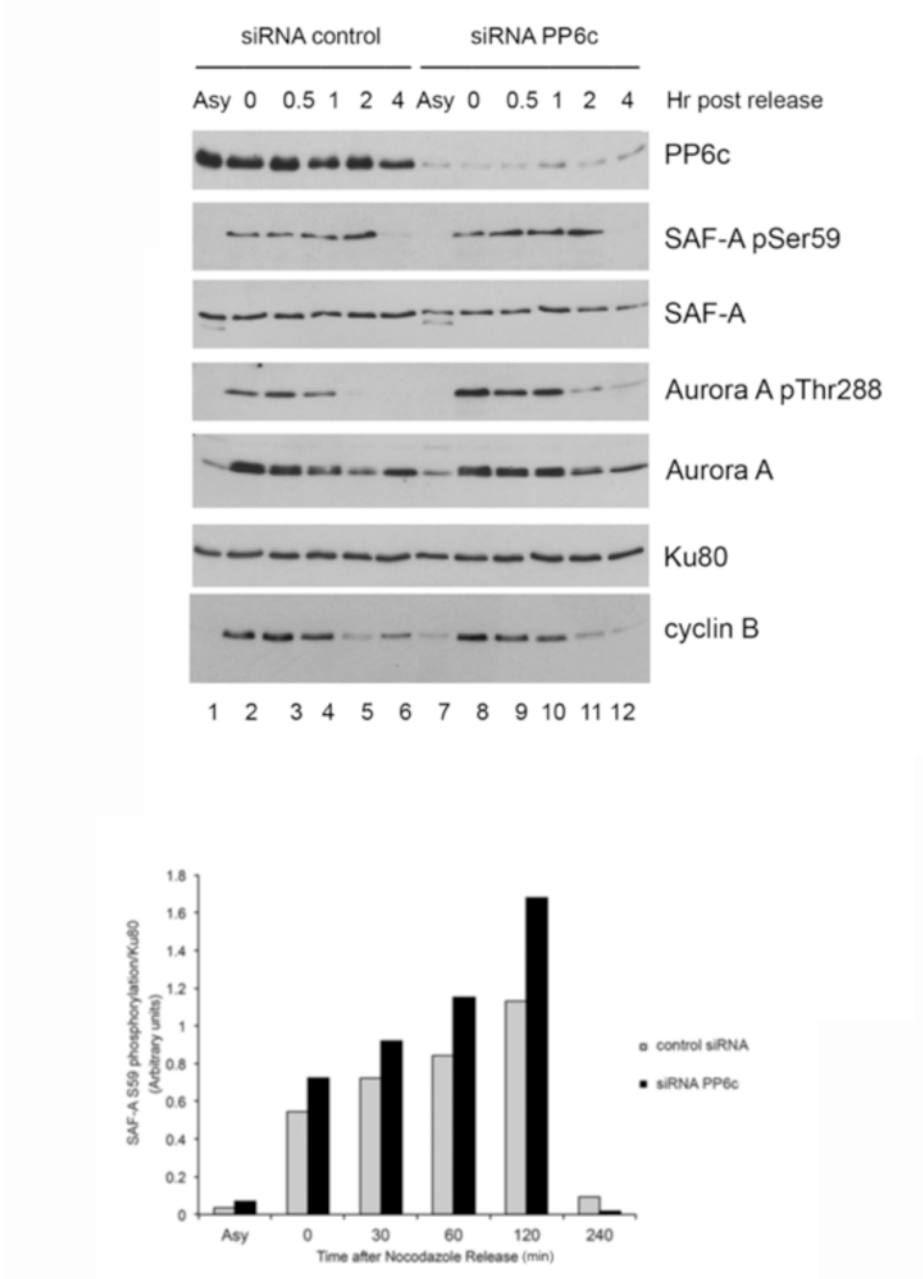
Supplementary Figure 10. SAF-A S59A cells have increased cyclin B1 and securin expression in response to taxol. SAF-A WT, SAF-A S59A and SAF-A S59E expressing MRC-SV cells were incubated with taxol (16 hours, 200 nM), collected by mitotic shake off and media was replaced with taxol free media and cells were harvested by NETN lysis either immediately (0), or after 0.5, 1, 2, or 4 hours recovery. Immunoblots were probed for total SAF-A, cyclin B1, securin and Ku80 as indicated. Bands were quantitated and normalized to Ku80 as a loading control. The lower panels show quantitation of cyclin B1 levels (left) and securin (right) in SAF-A WT, S59A and S59E cells.



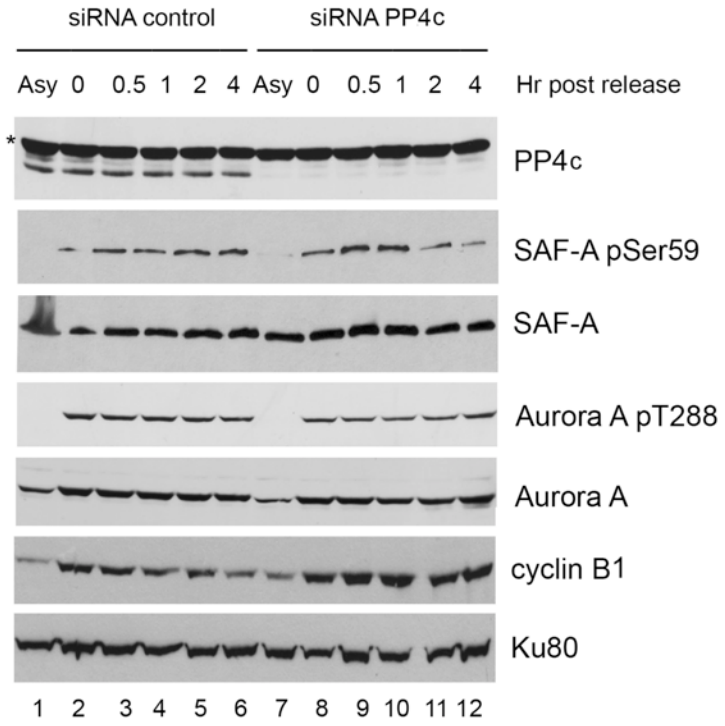


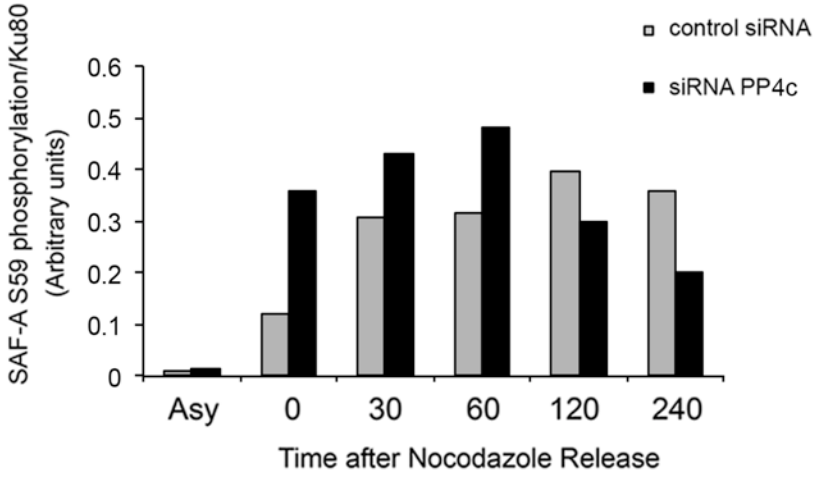


Supplementary Figure 11. PP6 does not dephosphorylate SAF-A S59 in mitosis. PP6c was depleted from HeLa cells using siRNA as described previously (Douglas, Zhong et al. 2010). Eighty hours after transfection, cells were incubated with nocodazole (40 ng/ml) for 16 hours, then cells were collected by mitotic shake off and incubated in nocodazole free media for the times indicated. NETN lysates were generated and 50 µg of sample was analyzed on SDS PAGE gels with immunoblot as described in the text. Blots were probed with antibodies to proteins indicated on the right of the figure. Immunoblots were quantitated using Image Quant software (GE Healthcare) and bands were normalized to Ku80. Relative phosphorylation of SAF-A on S59 in control and PP6c depleted cells is shown below.



Supplementary Figure 12. PP4c does not dephosphorylate SAF-A S59 in nocodazole treated mitotic cells. As in Supplementary Figure 11, but cells were depleted for PP4 using siRNA to PP4c. The asterisk indicated a non-specific band cross reacting with the PP4c antibody.





Supplementary References

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Douglas, P., R. Ye, L. Trinkle-Mulcahy, J. A. Neal, V. De Wever, N. A. Morrice, K. Meek and S. P. Lees-Miller (2014). "Polo-like kinase 1 (PLK1) and protein phosphatase 6 (PP6) regulate DNA-dependent protein kinase catalytic subunit (DNA-PKcs) phosphorylation in mitosis." Biosci Rep **34**(3): pii: e00113.

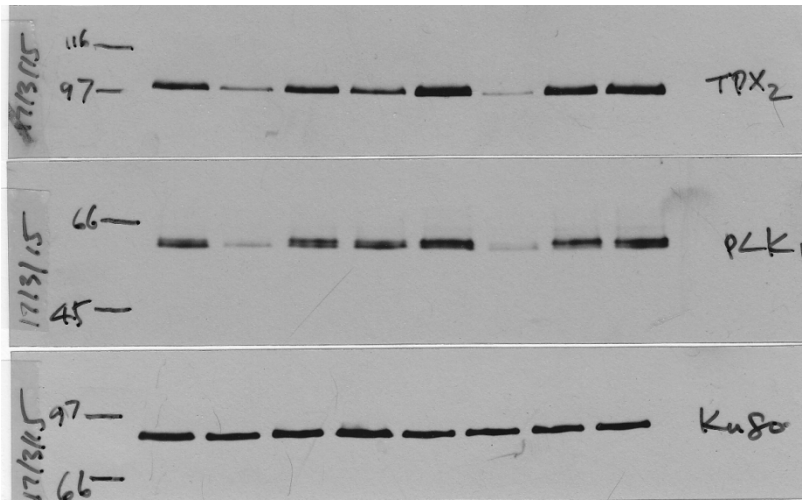
Douglas, P., J. Zhong, R. Ye, G. B. Moorhead, X. Xu and S. P. Lees-Miller (2010). "Protein phosphatase 6 interacts with the DNA-dependent protein kinase catalytic subunit and dephosphorylates gamma-H2AX." Mol Cell Biol **30**(6): 1368-1381.

Roget, K., A. Ben-Addi, A. Mambole-Dema, T. Gantke, H. T. Yang, J. Janzen, N. Morrice, D. Abbott and S. C. Ley (2012). "IkappaB kinase 2 regulates TPL-2 activation of extracellular signal-regulated kinases 1 and 2 by direct phosphorylation of TPL-2 serine 400." Mol Cell Biol **32**(22): 4684-4690.

Appendix

Representative western blots with molecular weight markers

Western blots: inputs: Figure 4

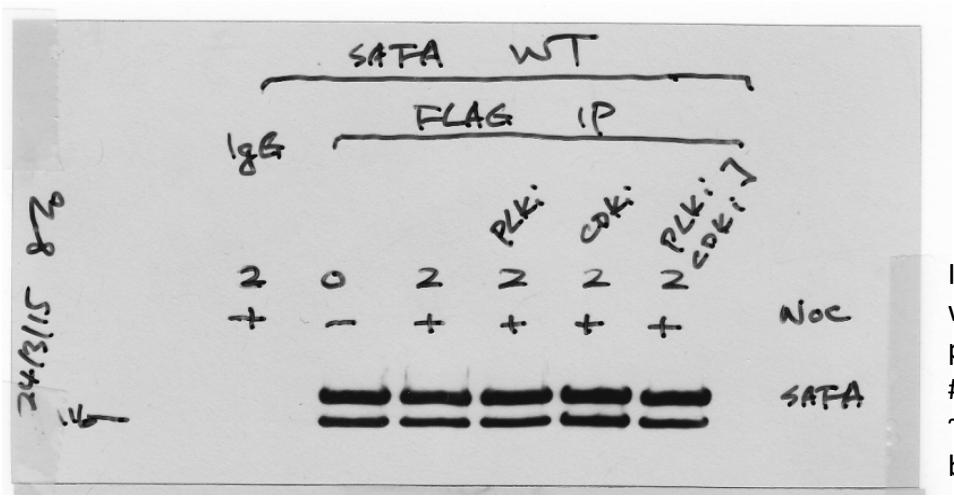


TPX2: ~ 97 kDa

PLK1 ~ 60 kDa

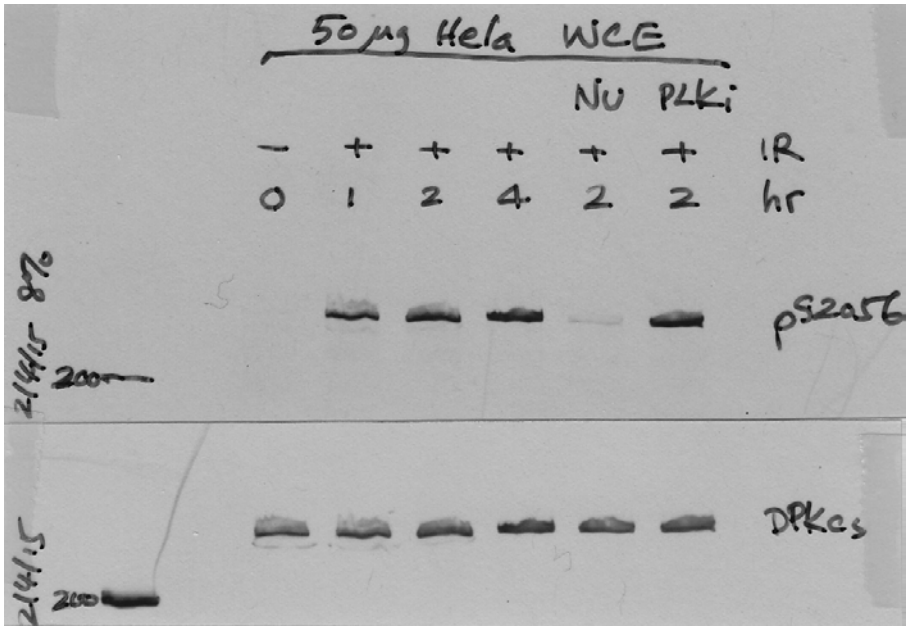
Ku80 ~ 86 kDa

Scans of western blots for SAF-A on 8% low bis SDS PAGE gels
From Supplementary Figure 4



IP: FLAG-SAF-A probed with SAF-A rabbit polyclonal Abcam #2066 showing ~125kDa and ~110 kda bands

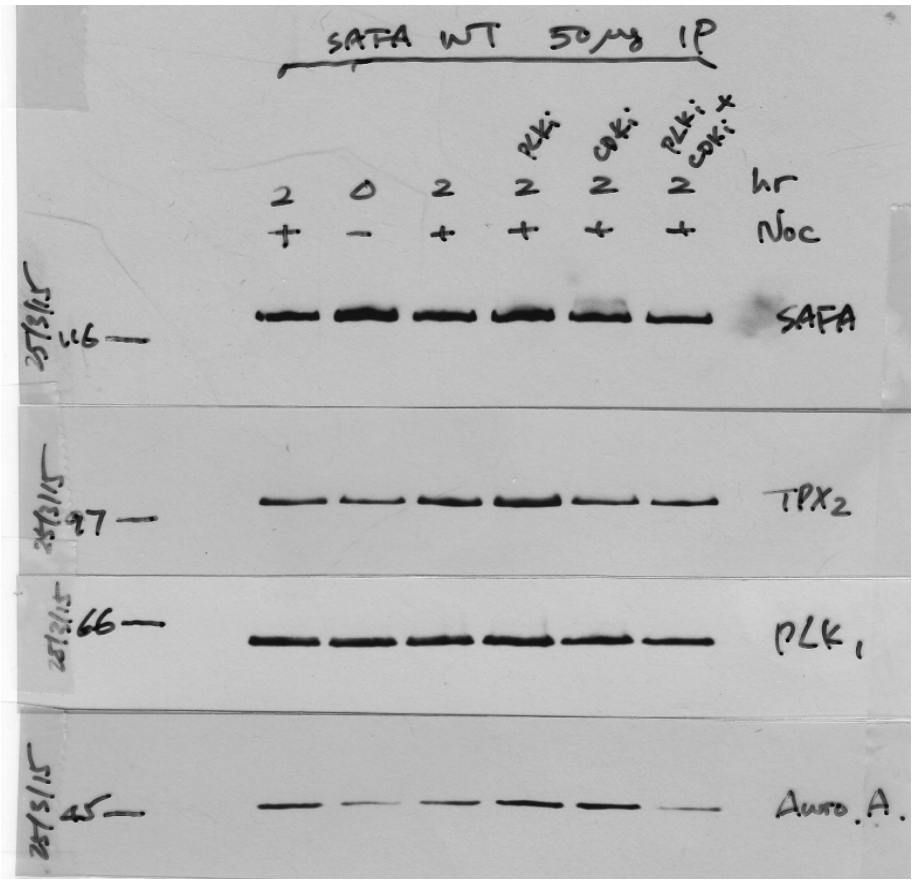
Western blots for DNA-PKcs, from Supplementary Figure 5



DNA-PKcs ~ 469 kDa

Scans of western blots for SAF-A and TPX2 on 10% SDS PAGE gels

Input samples, Supplementary Figure 4



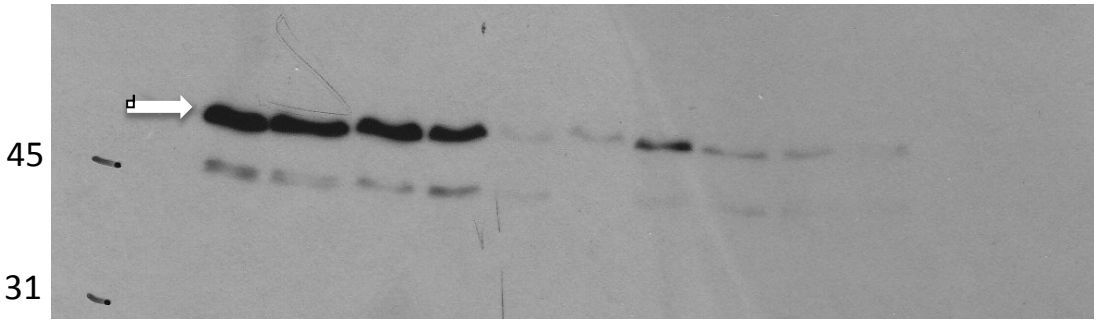
IP: FLAG-SAF-A probed with SAF-A mouse mono clonal Abcam #10297 showing ~110 kda band

TPX2: ~ 100 kDa

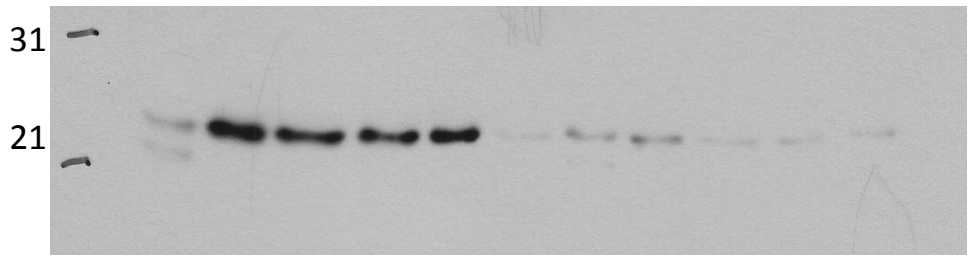
PLK1 ~ 60 kDa

Aurora A ~ 47 kDa

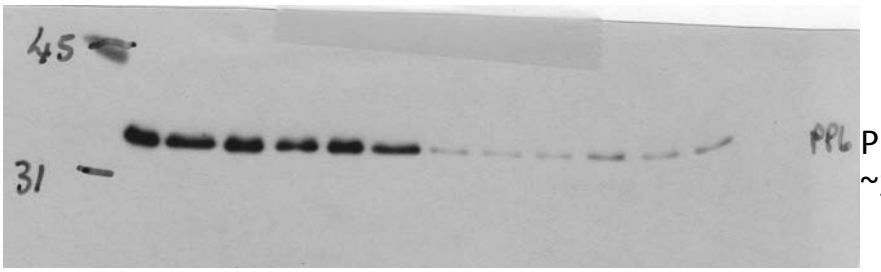
Representative western blots for cyclin B1 (approximately 55 kDa), securin (approximately 25 kDa) and PP6c (approximately 36 kDa). Positions of MWt markers are shown on the left hand side. Arrows indicate the appropriate bands, where there is more than one band on the blot.



Cyclin B1
~55 kDa



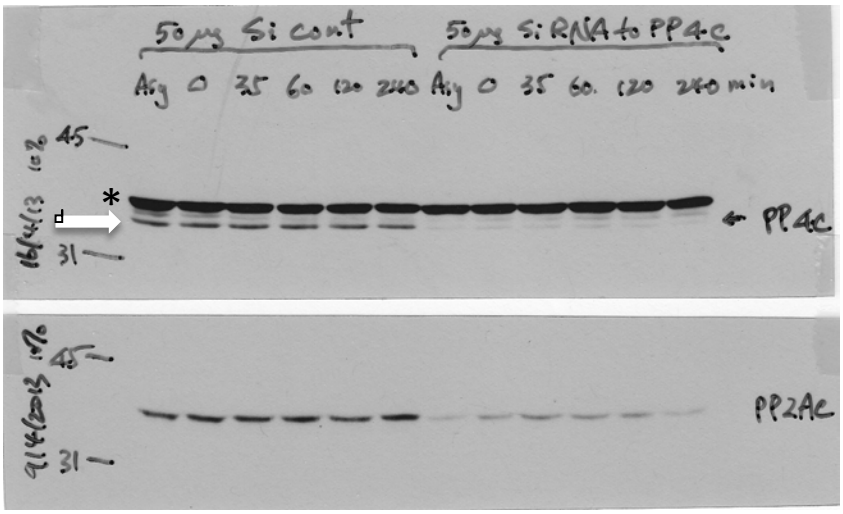
Securin
~25 kDa



PP6c
~36 kDa

From Supplementary Figure 12: Western blots for PP4c, ~ 35 kDa (arrow); the larger band marked by an asterisk is a cross-reacting band

From Figure 10: Western blots for PP2Ac ~ 35 kDa



PP4c
~ 36 kDa

PP2Ac
~ 36 kDa