Supplemental Figure Legends

Figure S1

Specificity of the phospho-Bcl-xL(ser49) antibodies**.** Expression of phospho-HA-Bcl-xL(Ser49) in Namalwa cells expressing expressing wt HA-Bcl-xL and HA-Bcl-xL(Ser49Ala) mutant exposed to VP16 (10 μM for 30 min). HA-Bcl-xL expression is shown as control. In the right panels, antibodies were first incubated with excess phophorylated peptide (CTESEMETP(pS)AING) prior to Western blotting.

Figure S2

A) Co-localization of CDK1 with γ-tubulin and phospho-Bcl-xL(ser49) with γ-tubulin, the centrosome bio-markers during the G2 arrest induced by VP16.

B) *In vitro* CDK1 kinase activity in the presence of recombinant Bcl-xL(ΔTM)(Ser49Asp) protein. CDK1 kinase activity assays were conducted as described in Supplemental Table S3 and quantitation performed by densitometry analysis of autoradiograms (40). Symbols and bars represent the means ± s.e.m. of 6 independent experiments.

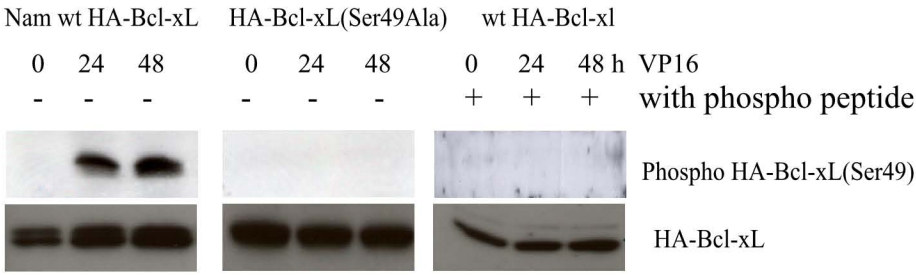
Figure S3

In vitro kinase assays. **A)** Enzyme activities were tested on control substrates with recombinant purified kinases. Velocities are expressed as nmole/min/mg. 2 independent experiments are reported.

B) BubR1, Bub1, Bub3 and Cdk1(cdc2) enzyme activities tested on histone H1 and myelin basic protein control substrates with kinases purified by immunoprecipitation. 2 independent immunoprecipitation assays are reported.

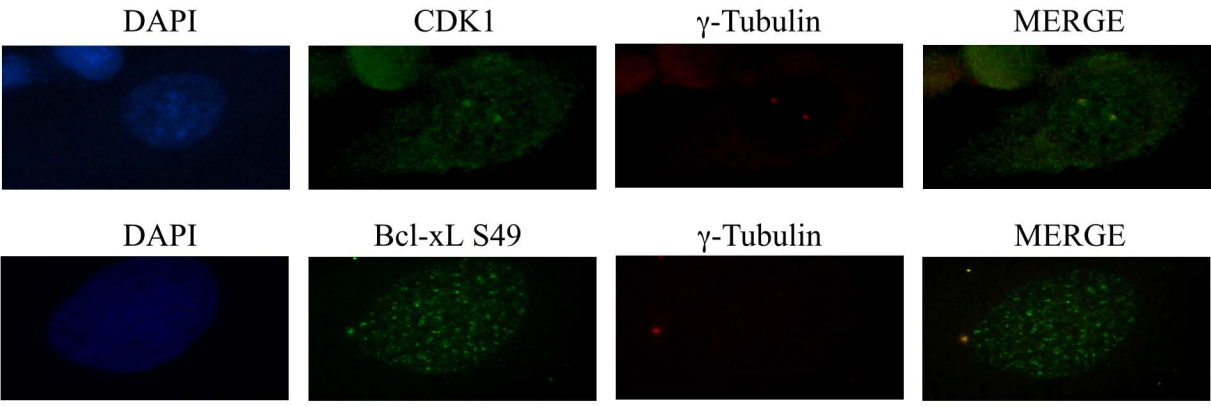
C) Tandem mass spectra of phospho(Ser49) analysed using Mascot (Matrix Science, London, UK; version Mascot). Mascot was searched with a fragment ion mass tolerance of 0.50 Da and a parent ion tolerance of 2.0 Da. Scaffold (version Scaffold_3_00_02, Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications. Peptide identifications were accepted if they could be established at greater than 95.0% probability. Validation of phosphate positions were conducted using Ascore. On that spectrum, Ascore score is of 42.65

Supplemental Figure S1

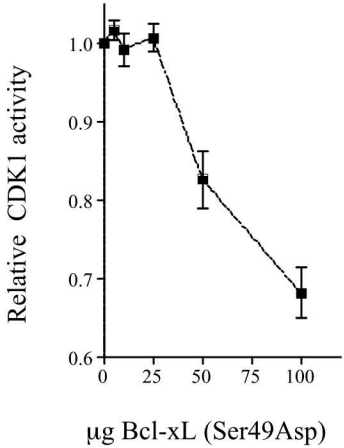


Supplemental Figure S2

A) Hela cells after 48 h VP16



B) In vitro CDK1 kinase activity assay

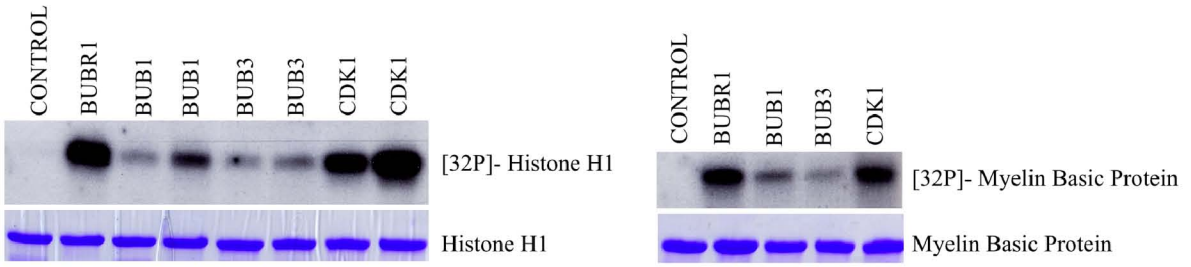


Supplemental Figure S3

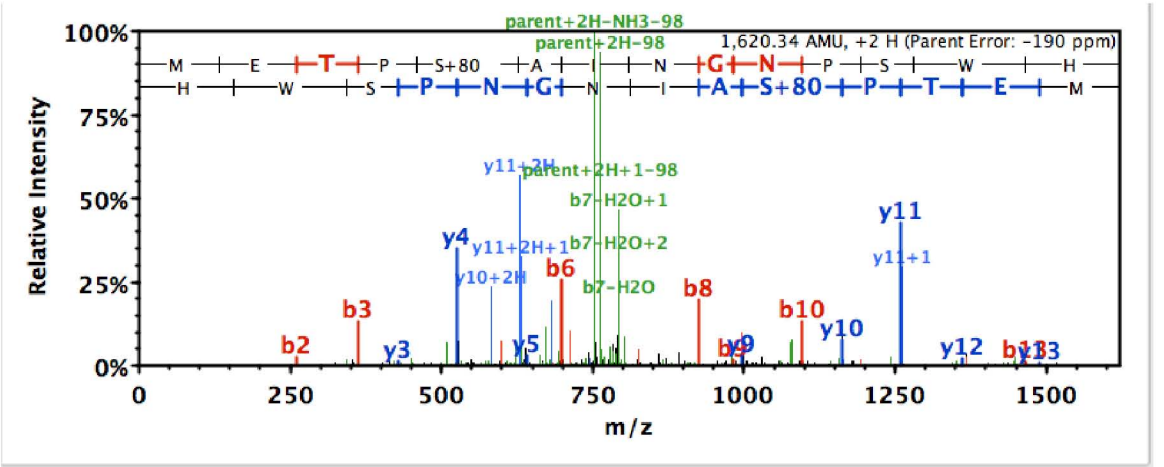
A) Recombinant Enzyme

	Specific activity	
	Assay #1	Assay #2
CHK1	7.7 nmole/min/mg	7.9 nmole/min/mg
CHK2	176 nmole/min/mg	268 nmole/min/mg
p38α /MAPK14	76 nmole/min/mg	129 nmole/min/mg
JNK1/MAPK8	39 nmole/min/mg	48 nmole/min/mg
JNK2/MAPK9	138 nmole/min/mg	263 nmole/min/mg
MAPKAPK2	541 nmole/min/mg	778 nmole/min/mg
PLK1	8.4 nmole/min/mg	14.3 nmole/min/mg
PLK3	13.9 nmole/min/mg	17.5 nmole/min/mg
Aurora A	14.5 nmole/min/mg	18 nmole/min/mg
Aurora B	508 nmole/min/mg	420 nmole/min/mg
NEK2	398 nmole/min/mg	530 nmole/min/mg
GSK3β	5.0 nmole/min/mg	8.6 nmole/min/mg

B) Immunoprecipitated enzyme



C) MS/MS Spectrum



SUPPLEMENTAL TABLE S1 Phase distribution *versus* labeling in synchronized human wt HeLa cells collected from 9 to 12 h after double thymidine block-release

Phospho Bcl-xL (S49)	PROMETAPHASE	METAPHASE	ANAPHASE	TELOPHASE	CYOKINESIS	TOTAL
+ CENPA	28/28 (-)	12/12 (-)	10/10 (-)	12/12 (-)	18/18 (-)	80
+ PLK1	41/41 (-)	32/32 (-)	30/30 (-)	25/31 (-)	23/23 (-)	157
+ γ-Tubulin	20/28 (-)	23/23 (-)	13/13 (-)	10/16 (-)	12/15 (-)	95
+ Clip-170	30/30 (-)	22/22 (-)	19/19 (-)	10/14 (-)	18/21 (-)	106
+ Dynein	35/35 (-)	21/21 (-)	11/11 (-)	10/10 (+)	08/11 (+)	88
+ HEC1	36/36 (-)	25/25 (-)	09/09 (-)	09/13 (-)	12/15 (-)	98

Supplemental TABLE S2 : Listing of antibodies

ANTIBODIES	ID	Species	Source
Cdc2/CDK1	# PC25	rabbit pAb	Calbiochem
Cdc2/CDK1	clone 1/cdk1	mouse mAb	BD Biosciences
Plk1	clone 208G4	rabbit mAb	Cell Signaling
Plk3	clone B37-2	mouse mAb	BD Biosciences
Aurora A	clone 1G4	rabbit mAb	Cell Signaling
Aurora B	# 3094	rabbit mAb	Cell Signaling
MAPKAPK2	# 3042	rabbit pAB	Cell Signaling
MAPK14/SAPK p38α	clone L53F8	mouse mAb	Cell Signaling
MAPK8/ JNK1	clone G151-333	mouse mAb	BD Biosciences
MAPK9/ JNK2	# 4672	rabbit pAb	Cell Signaling
MPS1/TKK	# 3255	rabbit pAb	Cell Signaling
MPS1/TKK	clone BC032858	mouse mAb	Abcam
Bub1	clone 14H5	mouse mAb	Upstate/ Millipore
BubR1	# A300-386A	rabbit pAb	Bethyl
BubR1	# 4116	rabbit pAb	Cell Signaling
BubR1	clone9/BubR1	mouse mAb	BD Transduction
Bub3	clone31/Bub3	mouse mAb	BD Transduction
Bub3	# 3049	rabbit pAb	Cell Signaling
Mad2	# A300-300A	rabbit pAb	Bethyl Lab
Mad2	clone 48/Mad2	mouse mAb	BD Transduction
cdc20	clone 41/p55cdc	mouse mAb	BD Transduction
cdc20	# A301-179A	rabbit pAb	Bethyl Lab
cdc20	# A301-180A	rabbit pAb	Bethyl Lab
CENP-A	# 07-574	rabbit pAb	Upstate/Millipore
CENP-A	clone 3-19	mouse mAb	Assay Designs
Dynein	clone 74.1	mouse mAb	Millipore
HEC1	clone 9G3	mouse mAb	Abcam
EB1	clone 5/ EB1	mouse mAb	BD Transduction
Clip-170	custom	from Niels Galjart,	Rotterdam
Bcl-xL	clone 2H12	mouse mAb	BD Biosciences
Phospho-BclxL Ser49	custom	rabbit pAb	GenScript
HA tag	clone 12CA5	mouse mAb	Roche Applied Sci
HA tag	# A00168	goat pAb	GenScript
γ-tubulin	clone GTU88	mouse mAb	Abcam
β-Actin	clone 4C40	mouse mAb	Sigma
Phospho H3(ser10)	# 06-570	rabbit pAb	Upstate/Millipore
Phospho H3 ser10 Alexa 488	# 9708	rabbit pAb	Cell Signaling
Anti-Mouse IgG Alexa 488	# A11001	goat pAb	InVitroGen
Anti-Rabbit IgG Alexa 488	# A11008	goat pAb	InVitroGen
Anti-Mouse IgG Alexa 594	# A11005	goat pAb	InVitroGen
Anti-Rabbit IgG Alexa 594	#A11012	goat pAb	InVitroGen
Anti-Mouse IgG HP-linked	# NA931V	sheep pAb	GE Healthcare
Anti-Rabbit IgG HP-linked	# NA934V	donkey pAb	GE Healthcare
Normal Rabbit IgG	# sc-2027	rabbit IgG	Santa Cruz Biotech
Normal Mouse IgG	# sc-2025	mouse IgG	Santa Cruz Biotech

Supplemental TABLE S3 : Listing of the protein kinase assays

Recombinant Enzyme	**Source**	**Control Substrate**	**Source**	**Buffer**
CHK1	Sigma-Aldrich	RXRX(L/A)S((R/F)	Cell Signaling	A
MAPK14/SAPK p38α	Cell Signaling	ATF-2 (19-96)	Cell Signaling	B
MAPK8/ JNK1	Cell Signaling	c-Jun (1-89)	Cell Signaling	B+
MAPK9/ JNK2	Cell Sciences	c-Jun (1-89)	Cell Signaling	A
MAPKAPK2	Cell Signaling	KKKLNRTLSVA	AnaSpec	A+
PLK1	Cell Signaling	RISDELMDATFADQEAK	AnaSpec	A
PLK3	Cell Signaling	RISDELMDATFADQEAK	AnaSpec	A
Aurora A	Cell Signaling	RRSLLE	Cell Signaling	A
Aurora B	Cell Signaling	LRRLSLGLRRLSLGLRRL SLGLRRLSLG	AnaSpec	A
NEK2	Cell Signaling	RFRRSRRMI	AnaSpec	A
GSK3α	Cell Signaling	RRAAEELDSRAGSPQL	AnaSpec	A
GSK3β	Sigma-Aldrich	GPHRSTPESRAAV	AnaSpec	A+
Mps1	Cell Signaling	Myelin basic protein	Sigma-Aldrich	B
Immunprecipitated Enzyme				
CDC2/CDK1	Namalwa cells	Histone H1/ MBP	Sigma-Aldrich	B+
Bub1	Namalwa cells	Histone H1/MBP	Sigma-Aldrich	B+
BubR1	Namalwa cells	Histone H1/MBP	Sigma-Aldrich	B+
Bub3	Namalwa cells	Histone H1/ MBP	Sigma-Aldrich	B+

Buffer A (5x):	25 mM MOPS pH 7.2 25 mM $MgCl_2$ 12.5 mM β-glycerol-2-phosphate 0.5 mM Na_3VO_4 5 mm EGTA 2 mM EDTA 0.25 mM dithiothreitol 500 μM ATP* * 0.05 μCi/μl [^{32}P]-γATP
Buffer A+ (5x):	buffer A + 50 μg/ ml BSA

Buffer B (5x):	125 mM TRIS pH 7.2 50 mM $MgCl_2$ 25 mM β-glycerol-2-phosphate 0.5 mM Na_3VO_4 10 mM dithiothreitol 500 μM ATP* * 0.05 μCi/μl [^{32}P]-γATP
Buffer B+ (5x):	buffer B + 50 μg/ ml BSA