Supplemental Figures and tables

Polo-Like Kinase 1 phosphorylates and stabilizes KLF4 to promote tumorigenesis in nasopharyngeal carcinoma

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Supplemental Figure 1

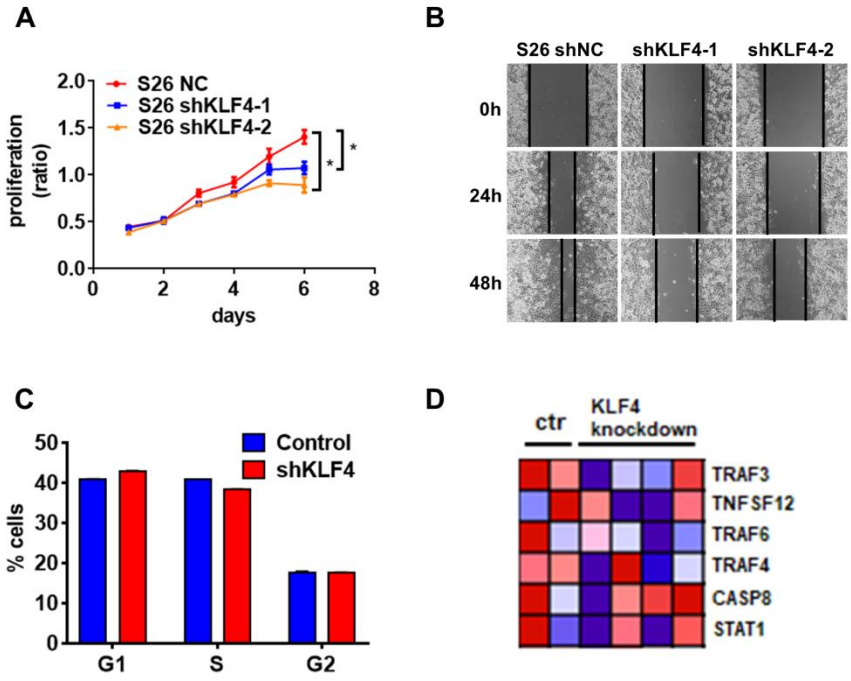

Figure S1 The characteristics of KLF4 knockdown cells.

(A) Cell growth assays of S26 cells with indicated genotypes.

(B) The cell scratch was monitored after 24h and 48h in S26 shNC, S26 shKLF4-1 and S26 shKLF4-2 cells.

(C) The percentage of cells in cell cycle phases.

(D) Heatmap showed TRAF6 differentially expressed in KLF4-deficient tumor cells.

Supplemental Figure 2

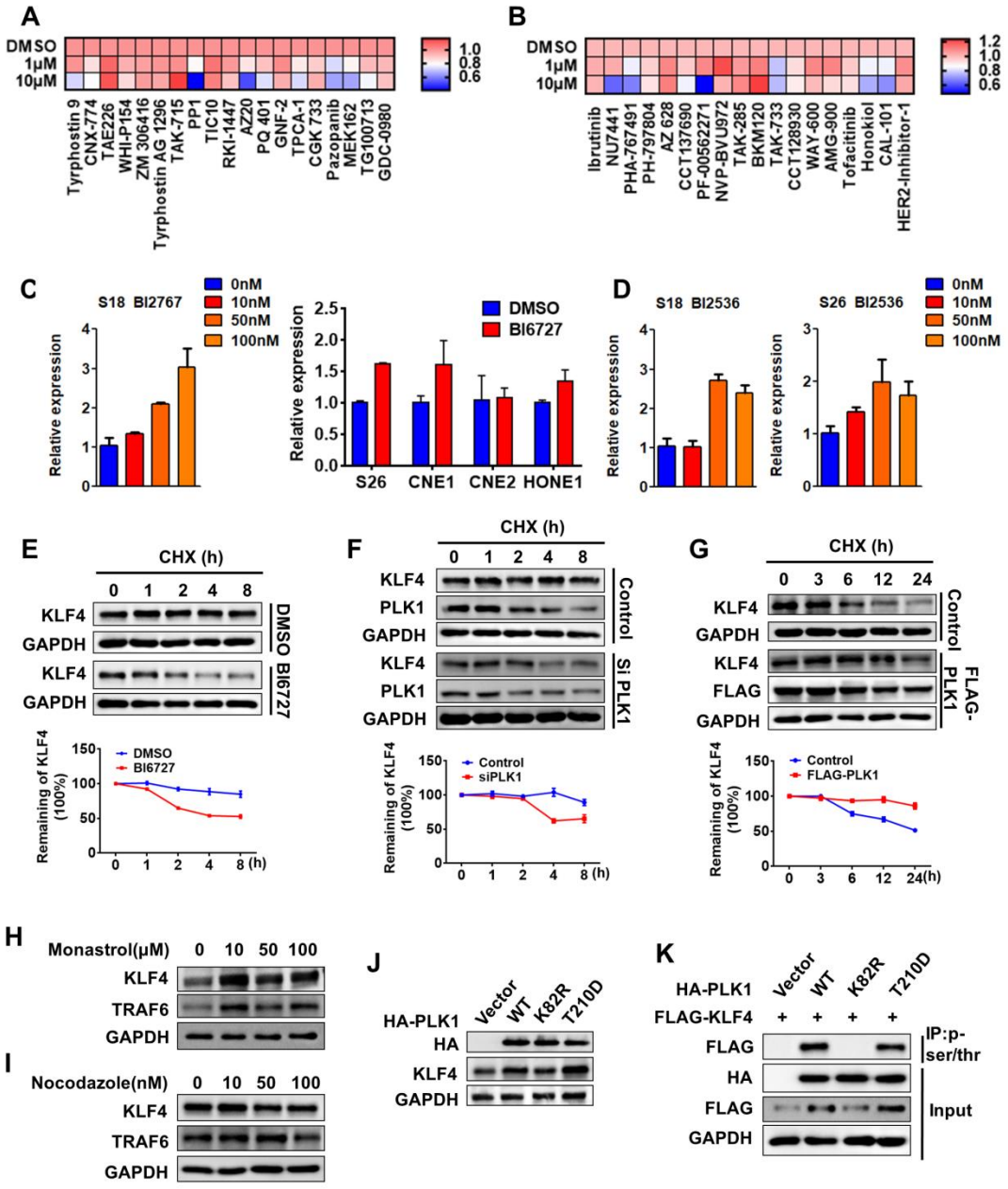

Figure S2 KLF4 protein stability is mediated by PLK1.

(A-B) Heatmap shows changes of KLF4 protein level in treated by different kinase inhibitors by western blot analysis. Calculate gray value with image J.

(C-D) Relative KLF4 mRNA levels were quantitated by real-time qPCR. Data shown represent the means (±SEM) of triplicates.

(E) CNE2 cells were treated with BI6727 following by the treatment with 20μM CHX. Cells were collected at the indicated time for immunoblotting using antibodies against

KLF4.

(F) CNE2 cells were transfected with PLK1 siRNA or control siRNA following by the treatment with 20μM CHX. Cells were collected at the indicated time for immunoblotting using antibodies against KLF4 and PLK1.

(G) 293T cells were transfected with FLAG-PLK1 or control plasmid following by the treatment with 20μM CHX. Cells were collected at the indicated time for immunoblotting using antibodies against KLF4 and PLK1.

(H) CNE2 cells were treated with monasrol for 24H and cell lysates analyzed for the level of KLF4 and TRAF6.

(I) CNE2 cells were treated with nocodazole for 24H and cell lysates analyzed for the level of KLF4 and TRAF6.

(J) 293T cells were transfected with vector, HA-WT-PLK1, HA-K82R-PLK1 and HA-T210D-PLK1 as indicated.

(K) 293T cells were co-transfected FLAG-KLF4 with vector, HA-WT-PLK1, HA-K82R-PLK1 or HA-T210D-PLK1 as indicated.

Supplemental Figure 3

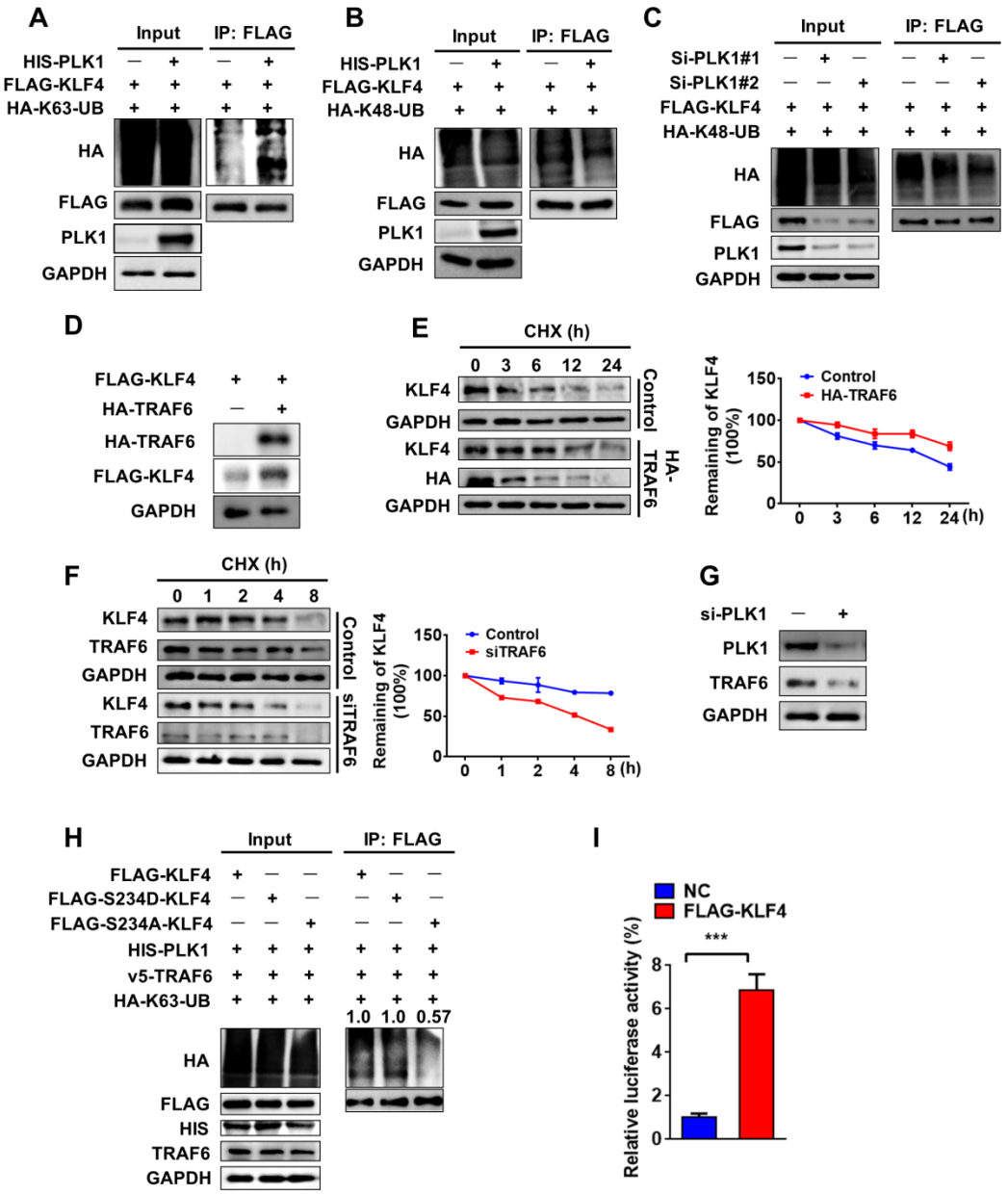

Figure S3 PLK1 Recruits TRAF6 to induce K63-Linked Ubiquitination of KLF4

(A) PLK1 can upregulate KLF4 Lys-63-linked ubiquitination. FLAG-KLF4 and HA-K63-UB were transfected into 293T cells together with HIS-PLK1 or vector. Protein extracts were immunoprecipitated (IP) using anti-FLAG antibody.

(B) FLAG-KLF4 and HA-K48-UB were transfected into 293T cells together with HIS-PLK1 or vector. Protein extracts were immunoprecipitated (IP) using anti-FLAG antibody.

(C) FLAG-KLF4 and HA-K48-UB were transfected into 293T cells together with

PLK1 siRNA or control. Protein extracts were immunoprecipitated (IP) using anti-FLAG beads.

(D) 293T cells were transfected with FLAG-KLF4 and HA-TRAF6 as indicated.

(E) 293T cells were transfected with HA-TRAF6 or control plasmid following by the treatment with 20μM CHX. Cells were collected at the indicated time for immunoblotting using antibodies against KLF4 and TRAF6.

(F) CNE2 cells were transfected with TRAF6 siRNA or control siRNA following by the treatment with 20μM CHX. Cells were collected at the indicated time for immunoblotting using antibodies against KLF4 and TRAF6.

(G) PLK1 depletion by specific siRNA in CNE2 cells. PLK1 and TRAF6 protein levels were analyzed by immunoblot, with GAPDH as a loading control.

(H) 293T cells were transfected with FLAG-KLF4 (WT, S234A, S234D), HA-K63-UB, HIS-PLK1 and V5-TRAF6 as indicated.

(I) 293T cells were co-transfected with FLAG-KLF4 or vector with the TRAF6-Luc reporter as indicated for 48H and then subjected to a luciferase activity assay.

Supplemental Figure 4

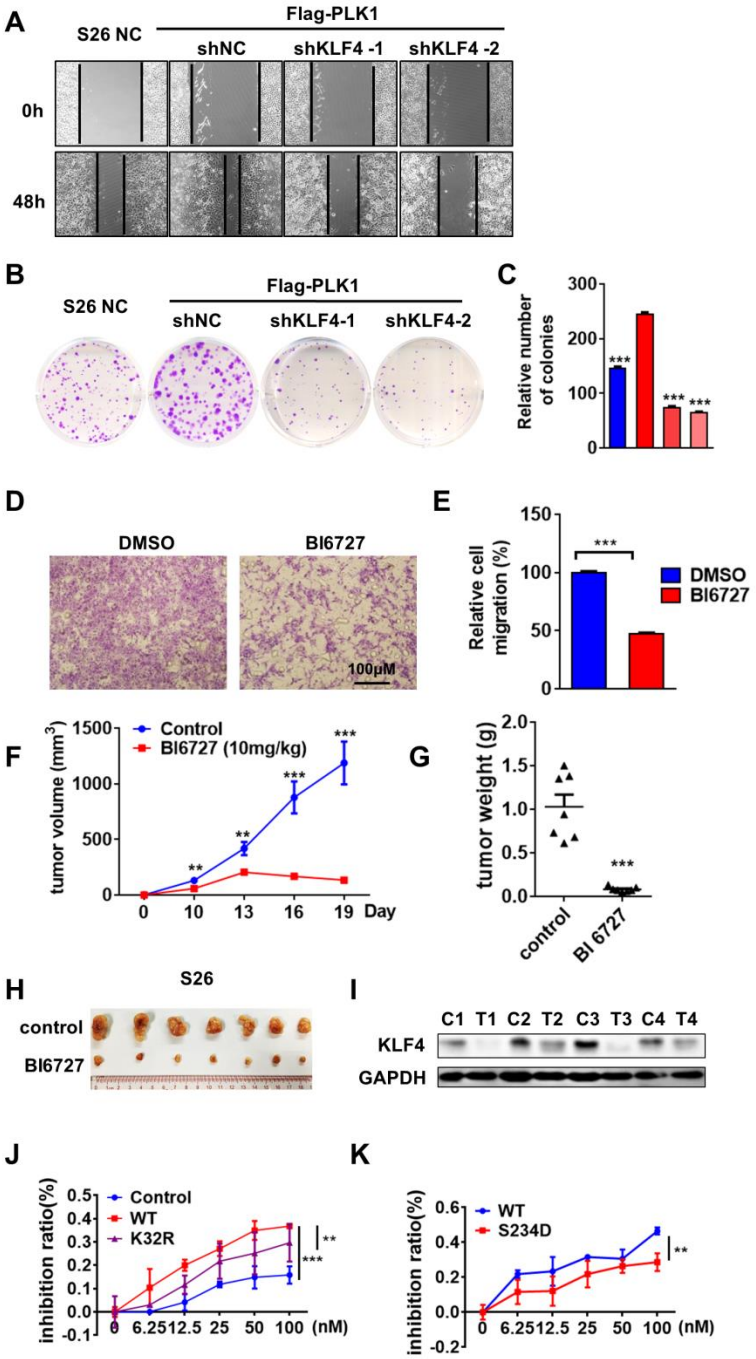

Figure S4 Polo-Like Kinase 1 Inhibitor is an Effective Therapeutics for Nasopharyngeal Cancer.

(A) The cell scratch was monitored after 48h in cell lines as indicated.

(B and C) Colony formation assay using cell lines as indicated for 10 days. Crystal violet was used to stain the formed colonies (B). The colony numbers were calculated as mean ± SD (n=3). ***p<0.001, student's t-test (C).

(D and E) The migratory ability of S18 cells was assayed using an uncoated transwell assay with or without BI6727 treated. Crystal violet was used to stain the cells (D). The migratory cell numbers were calculated as mean ± SD (n=3). ***p<0.001, student's test (E).

(F-H) Representative tumors from S26 allografts mice treated with BI6727, 10mg/kg/day. Tumor volumes and tumor weight shows in figure. (n=7 for each group).

(I) S26 allografts were taken off from the mouse, and then extract the protein to do western blot. The expression of KLF4 was show in the picture (C, control; T, treated).

(J) Inhibition ratio of indicated cell lines were examined by MTT assay after 48H treatment with BI6727. Data shown represent the means (±SEM) of triplicates.

(K) Inhibition ratio of indicated cell lines were examined by MTT assay after 48H treatment with BI6727. Data shown represent the means (±SEM) of triplicates.

Table S1 Clinical Characteristics of Nasopharyngeal Carcinoma patients according to the KLF4 expression.

Characteristic	$KLF4^{low}$ (n=83)	$KLF4^{high}$ (n=69)	*p*-value
Sex			
Male	69	54	0.535
Female	14	15	
Age			
< 45	42	31	0.517
⩾45	41	38	
T stage			
Ⅰ-Ⅱ	9	14	0.117
Ⅲ-Ⅳ	24	55	
N stage			
−	44	28	0.144
+	39	41	
TNM stage			
Ⅰ-Ⅱ	5	4	1
Ⅲ-Ⅳ	78	65	

Table S2 Univariate and multivariate cox regression analysis of KLF4 expression level and local relapse-free survival (LRFS).

Variable	Univariate analysis			Multivariate analysis		
	HR	95%CI	*p*-value	HR	95%CI	*p*-value
Sex (Male *vs* Female)	0.036	0-7.378	0.22	0	0-5.6*10^288	0.969
Age (< 45 *vs* ⩾45 years)	0.622	0.226-1.712	0.358	0.433	0.145-1.294	0.134
T stage (Ⅰ-Ⅱ *vs* Ⅲ-Ⅳ)	0.563	0.16-1.977	0.37	0.496	0.102-2.168	0.333
N stage (- *vs* +)	0.457	0.104-2.013	0.301	0.053	0.006-0.494	0.01
TNM stage Ⅰ-Ⅱ *vs* Ⅲ-Ⅳ)	0.949	0.125-7.2	0.96	12.62	0.578-275.236	0.107
KLF4 (Low *vs* High)	3.1	1.079-8.968	0.036	4.255	1.3-13.929	0.017

Table S3 List of 56 small molecule inhibitor of kinase

NO	CatalogNo.	NAME	Targets	NO	CatalogNo.	NAME	Targets
1	S2235	BI6727	PLK	29	S7195	RKI-1447	ROCK
2	S2406	Chrysophanic Acid	mTOR,EGFR	30	S7050	AZ20	ATM/ATR
3	S2626	LY2603618	Chk	31	S8003	PQ 401	IGF-1R
4	S2671	AS-252424	PI3K	32	S2899	GNF-2	Bcr-Abl
5	S2686	NVP-BSK805 2HCl	JAK	33	S2824	TPCA-1	IκB/IKK
6	S2703	GSK1838705A	IGF-1R,ALK	34	S7136	CGK 733	ATM/ATR
7	S2729	SB415286	GSK-3	35	S3012	Pazopanib	PDGFR,c-Kit,VEGFR
8	S2745	CCT137690	Aurora Kinase	36	S7007	MEK162	MEK
9	S2808	GDC-0068	Akt	37	S2870	TG100713	PI3K
10	S2542	Phenformin HCl	AMPK	38	S2696	GDC-0980	mTOR,PI3K
11	S2723	ZM 336372	Raf	39	S2680	Ibrutinib	BTK
12	S2391	Quercetin	PKC,Src,PI3K,Sirtuin	40	S2638	NU7441	DNA-PK,PI3K
13	S2661	WYE-125132	mTOR	41	S2742	PHA-767491	CDK
14	S2682	CAY10505	PI3K	42	S2726	PH-797804	p38 MAPK
15	S2699	CH5132799	mTOR,PI3K	43	S2746	AZ 628	Raf
16	S7435	AR-A014418	GSK-3	44	S2744	CCT137690	Aurora Kinase
17	S8050	ETP-46464	mTOR,ATM/ATR	45	S2672	PF-00562271	FAK
18	S2922	Icotinib	EGFR	46	S2762	NVP-BVU972	c-Met
19	S6005	VX-702	p38 MAPK	47	S2788	TAK-285	EGFR,HER2
20	S2895	Tyrphostin 9	EGFR	48	S2247	BKM120	PI3K
21	S7257	CNX-774	BTK	49	S2617	TAK-733	MEK
22	S2823	TAE226	FAK	50	S2635	CCT128930	Akt
23	S2867	WHI-P154	JAK,EGFR	51	S2689	WAY-600	mTOR
24	S2897	ZM 306416	VEGFR	52	S2719	AMG-900	Aurora Kinase
25	S8024	Tyrphostin AG 1296	FGFR,c-Kit,PDGFR	53	S2789	Tofacitinib	JAK
26	S2928	TAK-715	p38 MAPK	54	S2310	Honokiol	MEK,Akt
27	S7060	PP1	Src	55	S2226	CAL-101	PI3K
28	S7127	TIC10	Akt	56	S2752	HER2-Inhibitor-1	HER2,EGFR

Table S4 List of 33 putative sites that can be phosphorylated by PLK1

position	code	peptide	score
13	S	GESDMAVSDALLPSF	5.302
22	T	ALLPSFSTFASGPAG	5.333
33	T	GPAGREKTLRQAGAP	5.302
49	S	NRWREELSHMKRLPP	6.721
69	T	PYDLAAATVATDLES	5.64
72	T	LAAATVATDLESGGA	6.88
76	S	TVATDLESGGAGAAC	7.6
86	S	AGAACGGSNLAPLPR	7.233
119	S	SLTHPPESVAATVSS	5.826
125	S	ESVAATVSSSASASS	3.833
127	S	VAATVSSSASASSSS	6.333
129	S	ATVSSSASASSSSSP	7.222
131	S	VSSSASASSSSSPSS	6.667
132	S	SSSASASSSSSPSSS	11.188
133	S	SSASASSSSSPSSSG	6
134	S	SASASSSSSPSSSGP	8.208
135	S	ASASSSSSPSSSGPA	5.945
137	S	ASSSSSPSSSGPASA	6.512
138	S	SSSSSPSSSGPASAP	9.604
139	S	SSSSPSSSGPASAPS	9.279
149	S	ASAPSTCSFTYPIRA	8.636
167	T	PGVAPGGTGGGLLYG	5.24
194	S	LADINDVSPSGGFVA	7.147
234	S	GKFVLKASLSAPGSE	8.61
240	S	ASLSAPGSEYGSPSV	6.04
249	S	YGSPSVISVSKGSPD	7.04
258	S	SKGSPDGSHPVVVAP	12.532
283	S	KIKQEAVSSCTHLGA	4.222
284	S	IKQEAVSSCTHLGAG	4.333
326	S	LGLEEVLSSRDCHPA	3.5
387	S	KPKRGRRSWPRKRTA	5.419
393	T	RSWPRKRTATHTCDY	6.722
395	T	WPRKRTATHTCDYAG	6.667

Table S5 List of 12 putative sites that can be phosphorylated by PLK1

position	code	peptide
13	S	GESDMAVSDALLPSF
19	S	VSDALLPSFSTFASG
20	S	DALLPSFSTFASGPA
21	T	ALLPSFSTFASGPAG
49	S	RWREELSHMKRLPP
76	S	TVATDLESGGAGAAC
86	S	GAACGGSNLAPLPR
234	S	GKFVLKASLSAPGSE
242	Y	LSAPGSEYGSPSVIS
246	S	GSEYGSPSVISVSKG
315	T	GRQLPSRTTPTLGLE
444	T	FARSDELTRHYRKHT