Supplementary Information

PLK4 consensus phosphorylation motif

A consensus sequence obtained by the peptide library screen is consistent with the PLK4 phosphorylation site and is represented here as a one letter code:

Λ/Ψ/B(M/S) – X – [(L)] – S/T - Λ[E] – Φ/Λ(L) – X where Φ (Phi), Ψ (Psi), Λ (Lambda), B and E (Epsilon) represent aromatic, hydrophobic, aliphatic, basic and charged residues, respectively. In brackets are unfavoured residue functions and in parentheses, information on the steric nature of residues is mentioned; where S, M and L denote small, medium and large residues, respectively. X corresponds to any residue. As a reference, the consensus phosphorylation motif contains the phosphorylated serine (S) or threonine (T) at position 0 (depicted S/T). Medium to small aliphatic or basic residues are found at position –3 such as Ile, Pro, Val, Met, Gly, Arg and Lys. Large residues are unfavoured at position –1 such as His, Phe, Tyr and Trp. The *in-vitro* screen analysis suggests that residues at positions +1 and +2 correspond mainly to aliphatic residues, moreover large aromatic residues are mainly allowed at position +2. Interestingly, those residues found at positions +1 and +2 are mainly turn breakers and helical formers. The autophosphorylation PLK4 sequence Ser-Ile-Ser-Gly-Ser-Leu-Phe-Asp-Lys-Arg-Arg-Leu-Leu is in agreement with the optimal consensus sequence where Ile-Ser-Gly-Ser-Leu-Phe corresponds to positions -3 -2 -1 0 +1 +2 respectively and therefore the second Ser is the residue phosphorylated by the kinase.

Supplementary Table 1: List of peptides screened in array

peptide number	Biotin-Ttds-------Glycinamide	derived from:	site	mutation
1	GQRDSSYYWEIE	c-Raf (positive control)		
2	GQRDSAYYWEIE	c-Raf S339A		
3	GQRDASYYWEIE	c-Raf S338A		
4	NLLGKGSFAGVYR	PLK4 kin domain	Ser22	
5	QLKHPSILELYNY	PLK4 kin domain	Ser71	
6	LYNYFEDSNYVYL	PLK4 kin domain	Ser82	
7	ILHRDLTLSNLLL	PLK4 kin domain	Thr138 Ser140	
8	ILHRDLALSNLLL	PLK4 kin domain	Thr138 Ser140	T138A
9	ILHRDLTLANLLL	PLK4 kin domain	Thr138 Ser140	S140A
10	SAHGLESDVWSLG	PLK4 kin domain	Ser192	
11	DYEMPSFLSIEAK	PLK4 kin domain	Ser232 Ser235	
12	DYEMPAFLSIEAK	PLK4 kin domain	Ser232 Ser235	S232A
13	DYEMPSFLAIEAK	PLK4 kin domain	Ser232 Ser235	S235A
14	DYEMPTFLSIEAK	PLK4 kin domain	Ser232 Ser235	S235T (polymorphism)
15	LRRNPADRLSLSS	PLK4 kin domain	Ser255	
16	LRRNPADRLALSS	PLK4 kin domain	Ser255	S255A
17	DRLSLSSVLDHPF	PLK4 kin domain	Ser257-258	
18	DRLSLASVLDHPF	PLK4 kin domain	Ser257-258	S257A
19	DRLSLSAVLDHPF	PLK4 kin domain	Ser257-258	S258A
20	SISGSLFDKRRLL	PEST sequence 1	Ser303 Ser305	
21	SISGALFDKRRLL	PEST sequence 1	Ser303 Ser305	S305A
22	SIAGSLFDKRRLL	PEST sequence 1	Ser303 Ser305	S303A
23	PLPNKMTVFPKNK	after destruction box	Thr323	
24	DGNSFYTQWGNQ	Linker region	Ser342 T345	

25	DGNAFYTQWGNQ	Linker region	Ser342 T345	S342A
26	DGNSFYAQWGNQ	Linker region	Ser342 T345	T345A
27	NEERYSPTDNNAN	Linker region	Ser421	
28	NEERYAPTDNNAN	Linker region	Ser421	S421A
29	SSSGSFERPDNNQ	Linker region	Ser443	
30	SSSGAFERPDNNQ	Linker region	Ser443	S443A
31	AAAGSFERPDNNQ	Linker region	Ser443	S439-441A
32	EYDSISPNRDFQG	Linker region	Ser497	
33	EYDAISPNRDFQG	Linker region	Ser497	
34	EYDSIAPNRDFQG	Linker region	Ser497	
35	SDGNTITIYYPNG	Crypto-Polo Box	Thr 645 647	
36	SDGNAITIYYPNG	Crypto-Polo Box	Thr 645 647	T645A
37	SDGNTIAIYYPNG	Crypto-Polo Box	Thr 645 647	T647A
38	DNISRYSFDNL*PE*	Crypto-Polo Box	Ser674	
39	DNISRYAFDNL*PE*	Crypto-Polo Box	Ser674	S674A
40	DNIARYSFDNL*PE*	Crypto-Polo Box	Ser674	S671A
41	KSPKITYFTRYAK	Crypto-Polo Box	Thr 704 707	
42	KSPKIAYFTRYAK	Crypto-Polo Box	Thr 704 707	T704A
43	KSPKITYFARYAK	Crypto-Polo Box	Thr 704 707	T707A
44	KIHKTEDFIQVIE	Crypto-Polo Box	Thr736	
45	QVIEKTGKSYTLK	Crypto-Polo Box	Ser749	
46	QVIEKAGKSYTLK	Crypto-Polo Box	Ser749	T746A
47	QVIEKTGKAYTLK	Crypto-Polo Box	Ser749	S749A
48	QVIEKTGKSYALK	Crypto-Polo Box	Ser749	T751A
49	PPSVDSNYPTRDR	Crypto-Polo Box	Ser824	
50	PPSVDANYPTRDR	Crypto-Polo Box	Ser824	S824A
51	PPSVDSNYPARDR	Crypto-Polo Box	Ser824	T828A
52	RDRASFNRMVMH	Crypto-Polo Box	Ser833	

53	GTDISSNSLKDC	Crypto-Polo Box	Ser873 874	
54	GTDIASNSLKDC	Crypto-Polo Box	Ser873 874	S873A
55	GTDISANSLKDC	Crypto-Polo Box	Ser873 874	S874A
56	GTDISANALKDC	Crypto-Polo Box	Ser873 874	S874A S876A
57	AQLLKSVFVKNVG	PEST sequence 3	Ser890	
58	VGWATQLTSGAVW	PEST seq 3 + Polo Box	Thr899 Ser904	
59	VGWAAQLTSGAVW	PEST seq 3 + Polo Box	Thr899 Ser904	T899A
60	VGWATQLASGAVW	PEST seq 3 + Polo Box	Thr899 Ser904	T903A
61	VGWATQLTAGAVW	PEST seq 3 + Polo Box	Thr899 Ser904	S904A
62	VQFNDGSQLVVQA	PEST seq 3 + Polo Box	Ser915	
63	GVSSISYTSPNGQ	Polo Box	Ser927	
64	GVSSIAYTSPNGQ	Polo Box	Ser927	S927A
65	GVASISYTSPNGQ	Polo Box	Ser927	S924A
66	GVSAISYTSPNGQ	Polo Box	Ser927	S925A
67	QKLQCLSSILLMF	Polo Box	Ser956-Ser957	
68	QKLQCLASILLMF	Polo Box	Ser956-Ser957	S956A
69	QKLQCLSAILLMF	Polo Box	Ser956-Ser957	S957A
+	GQRDSSYYWEIE	c-Raf (positive control)	Raf duplo	
71	RGQRDSSYYWE	c-Raf (positive control)	shorter	
72	RGQRDASYYWE	c-Raf S339A	shorter	
73	RGQRDSAYYWE	c-Raf S338A	shorter	

Supplementary Figure S1. Specificity of anti-PLK4 polyclonal antibodies

(**A**) Western blotting detection of endogenous PLK4 from KE37 purified centrosomes using antibodies raised against the kinase and crypto Polo-box domains of PLK4. Both of the antibodies predominantly recognized a protein migrating at 107 kD, which corresponded well with the predicted molecular of PLK4 of 109 kD. (**B**) RPE1 cells transiently transfected with an EGFP-PLK4 expression construct were fixed and stained with the KD and CPB antibodies. The labelling obtained with the KD and CPB antibodies co-localizes with the overexpressed PLK4 demonstrating that the antibodies recognize their target antigen. (**C**) HeLa GFP-centrin-1 (green) cells were treated with siRNA against PLK4 or lamin A/C, as a control, for 48 hours and stained with DAPI (blue) to label DNA and PLK4 KD antibody (red). The lamin A/C control siRNA-treated cell has a duplicating centrosome (two centrioles and two procentrioles) and robust PLK4 staining. Two images of PLK4 siRNA-treated cells are shown with diminished PLK KD antibody staining and a single focus of GFP-centrin 1 labelling, suggesting that only a single centriole is present. These data indicate that PLK4 has been successfully depleted as only one centriole was present in each cell and that the PLK4 KD antibody is specific. Scale bar 10 μm; inset 1μm.

Supplementary Figure S2. PLK4 pS305 antibody specifically recognizes S305 phosphorylated PLK4

(**A**) Western blotting of KE37 purified centrosomes with the PLK4 pS305 antibody in the presence or absence of competing non-phosphorylated and phosphorylated peptides corresponding to the sequence used for immunization. A reduction in signal was observed upon incubation of the PLK4 pS305 antibody with the phosphorylated competing peptide, but not with the non-phosphorylated peptide. (**B**) RPE1 cells were transfected with EGFP alone, EGFP-PLK4, -PLK4 K41M, and -PLK4 S305A and treated with 5 μM MG115 for 16 hours to induce the accumulation of S305 phosphorylated PLK4. Western blotting was carried out on whole cell lysates using the PLK4 KD and PLK4 pS305 antibodies.

The PLK4 KD antibody showed that over-expressed EGFP-PLK4 was present in the appropriate samples. Western blotting with the PLK4 pS305 antibody showed that it recognized only the active kinase and no detectable signal was present in the PLK4 K41M and PLK4 S305A mutant samples. (**C**) Images of mitotic cells after PLK4 or lamin A/C RNAi. HeLa GFP-centrin-1 (green) cells were treated with siRNA against PLK4 or lamin A/C for 48 hours and stained with DAPI (blue) to label DNA and PLK4 pS305 (red) antibody. Scale bar 10 μm; inset 1μm. The mitotic PLK4-depleted cell has a single centriole that is labelled extremely weakly by the PLK4 pS305 antibody. Together these data demonstrate that the PLK4 pS305 antibody specifically recognizes S305 phosphorylated PLK4.

Supplementary Figure S3. Inhibition of proteasome results in the accumulation of S305 phosphorylated PLK4.

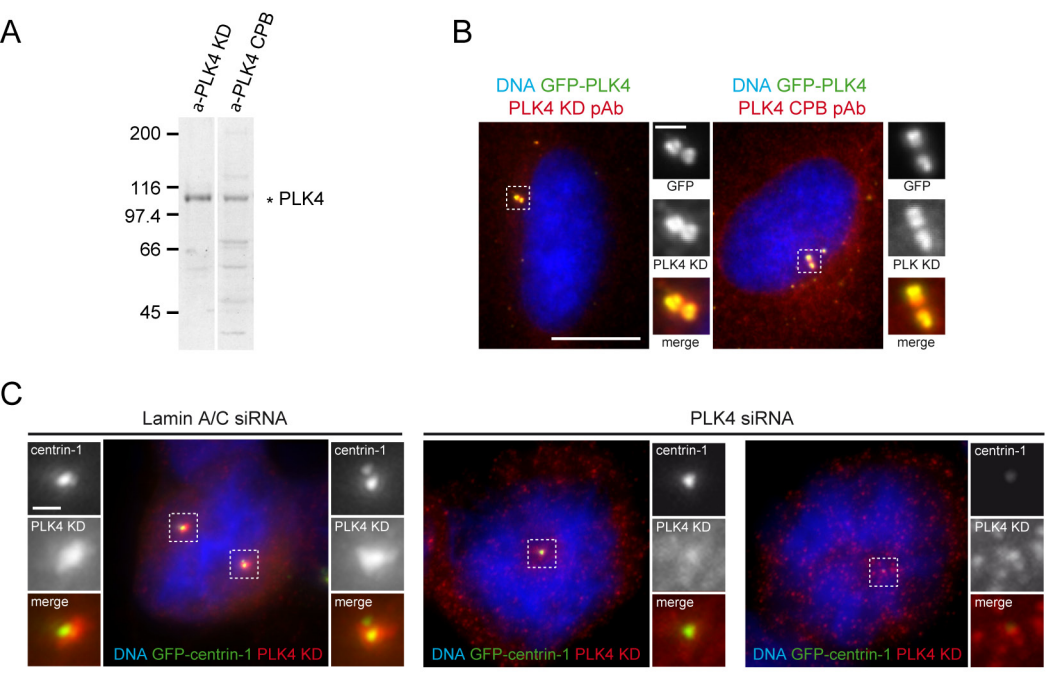
(**A**) RPE1 cells were transfected with wild-type EGFP-PLK4 and then treated 6 hours later with the proteasome inhibitors MG115 (5 μM), MG132 (1 μM) or AdaAhx$_3$L$_3$VS (1 μM) for a total of 16 hours. Western blotting of transfected cell lysates was carried out using the PLK4 KD and PLK4 pS305 antibodies and shows that all of the proteasome inhibitors promoted the accumulation of S305 phosphorylated PLK4. The inhibitor AdaAhx$_3$L$_3$VS caused an increase in the total amount of EGFP-PLK4 as well.

Supplementary Figure S4. PLK4 S305 mutants are catalytically active

(**A**) PC3 cells were transfected with EGFP-PLK4-3xFLAG expression constructs, treated 24 hours later with 1 μM MG115 for 3 hours and the exogenously expressed PLK4 immunoprecipitated using an anti-FLAG antibody. Eighty percent of the immunoprecipitated material was used to carry out an *in vitro* kinase assay (upper panel), while the remainder was subjected to western blotting with the PLK4 KD antibody (lower panel). The results show that both PLK4 S305A and PLK4 S305E mutants are catalytically active, whereas PLK4 K41M and PLK4 K41M ND are not.

Supplementary Figure S5. PLK4 S305 autophosphorylation enhances centriole amplification in RPE1 cells.

(**A**) Images of RPE1 cells transfected with EGFP-PLK4 constructs (green), fixed and stained with DAPI (blue) to label DNA and anti-HsSAS-6 antibody (red). All of the catalytically active forms of PLK4 were able to trigger centriole amplification albeit to varying efficiencies. Scale bar 10 μm; inset 1μm. (**B**) Quantification of EGFP-PLK4 transfected cells possessing supernumerary centrioles. The results represent four independent experiments counting 300 transfected cells in each group.



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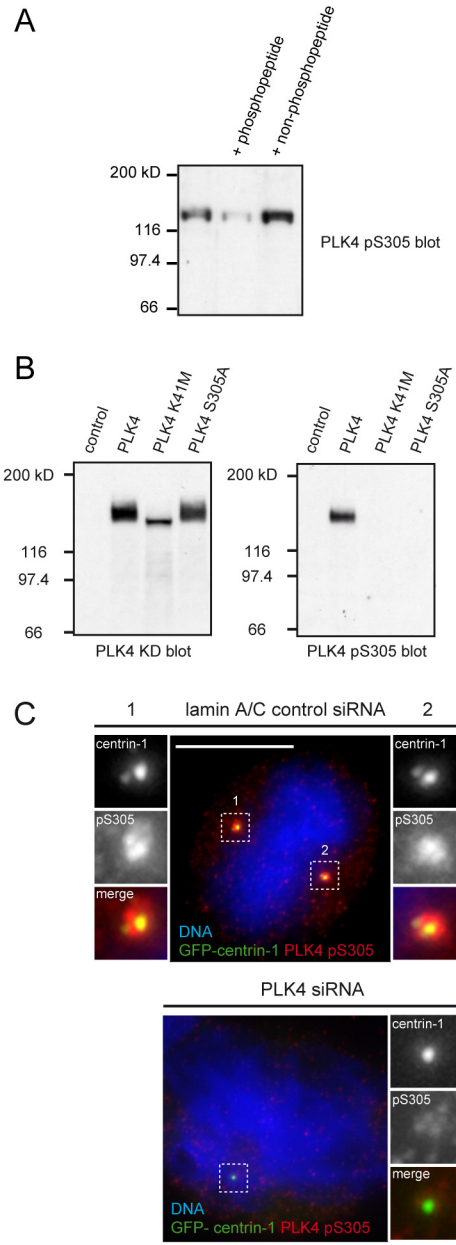

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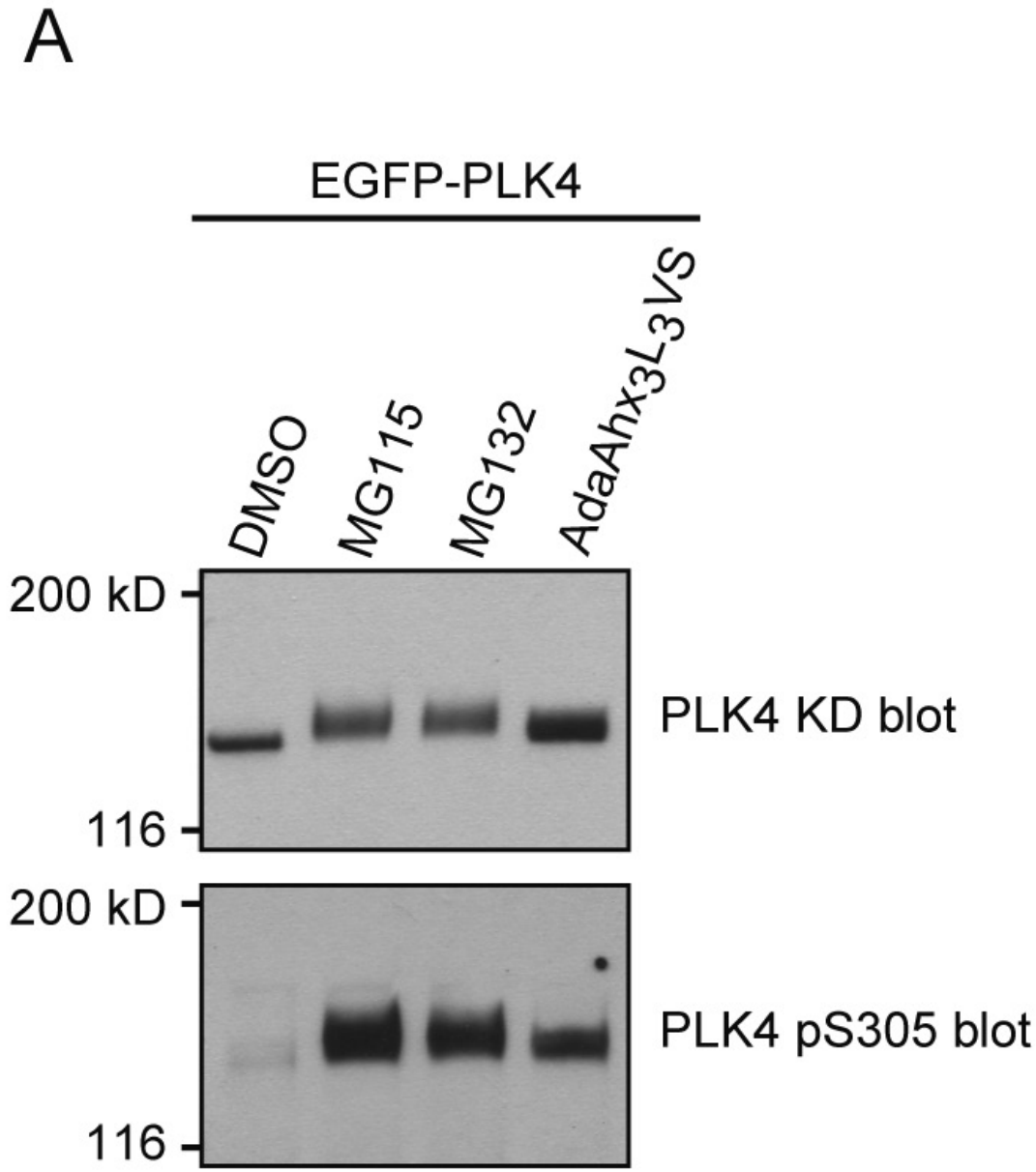

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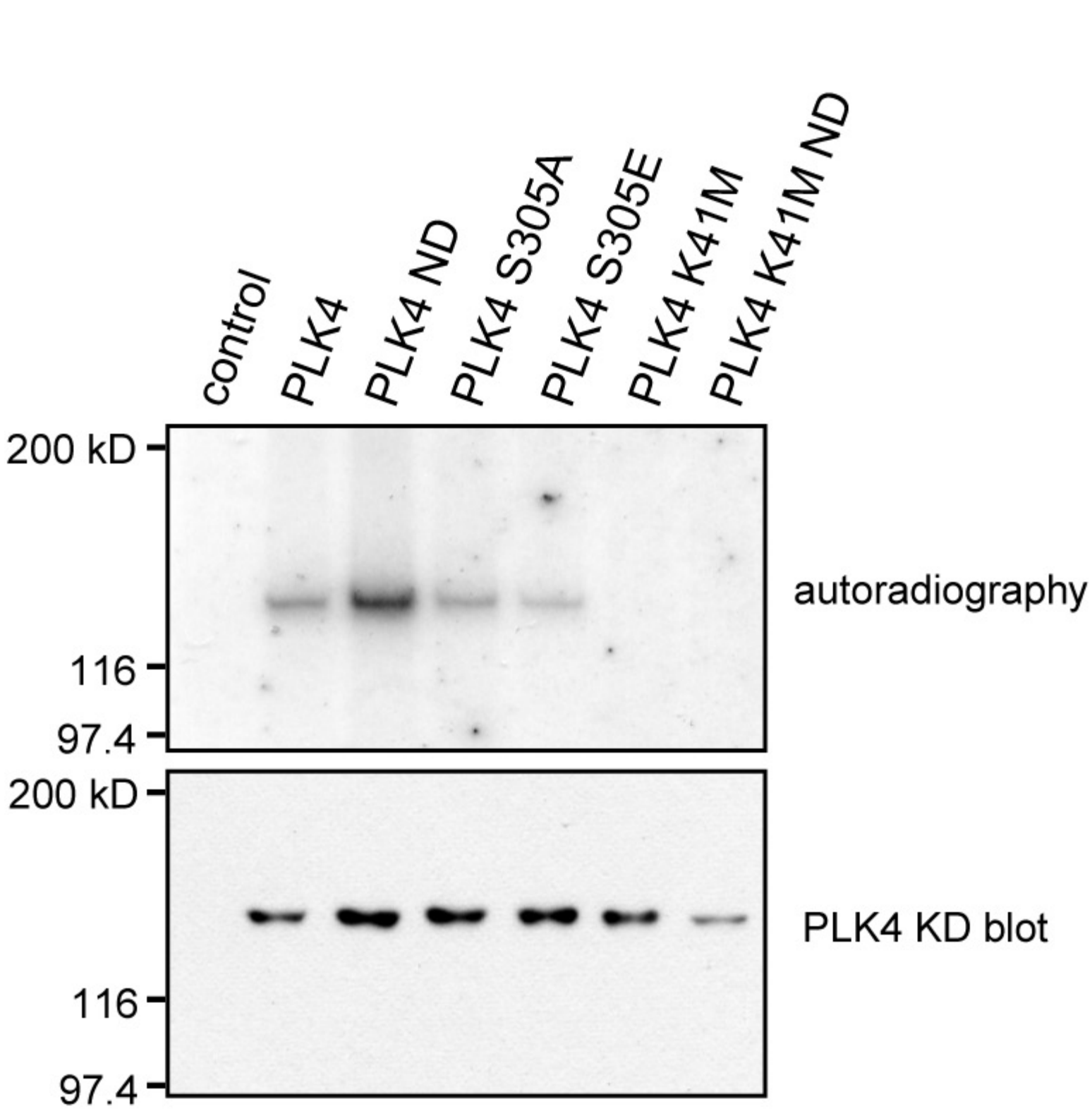

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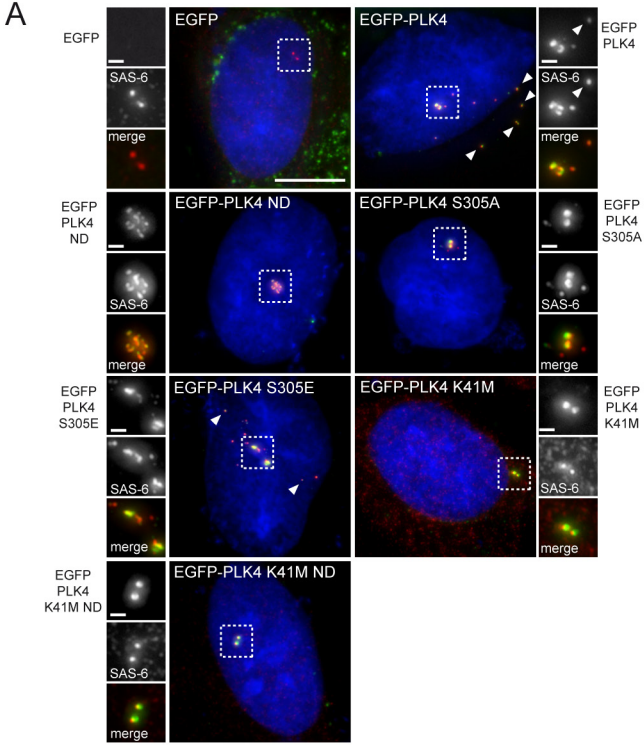

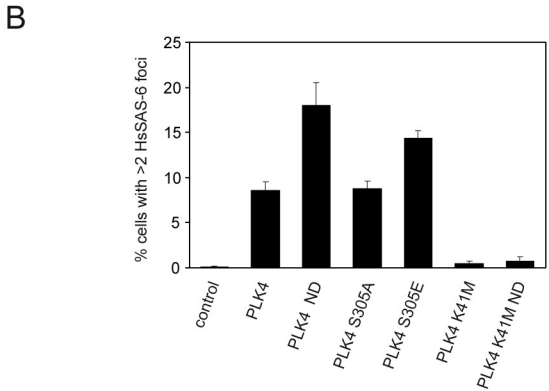

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