ONLINE SUPPLEMENTARY DATA

Mammalian Polo-like Kinase 1-dependent Regulation of the PBIP1-CENP-Q Complex at Kinetochores

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SUPPLEMENTARY FIGURE LEGENDS

Fig. S1. PBIP1 does not significantly form a homomeric complex. *A–B*, HeLa cells transfected with the indicated constructs were subjected to immunoprecipitation with anti-Flag antibody, and then immunoblotting analyses. *Asterisks*, cross-reacting proteins. Note that, in comparison to the PBIP1-CENP-Q interaction (lane 2 in *A*), only a small fraction (but probably insignificant amount when compared with the 2% input) of EGFP-PBIP1 or the CENP-Q binding-incompetent PBIP1(L337P L344P) mutant was co-precipitated with Flag-PBIP1.

Fig. S2. Subcellular localization of various PBIP1 truncations in PBIP1 RNAi cells. HeLa cells depleted of endogenous PBIP1 (si-PBIP1) were infected with adenoviruses expressing the indicated EGFP-fused PBIP1 full-length or truncations (T1–T9). As soon as green fluorescence signals were detectable under a fluorescence microscope (to keep the expression of exogenous proteins at minimum levels), cells were harvested for immunostaining (*A*) and immunoblotting (*B*) analyses. Since the anti-PBIP1 antibody used in *B* was raised against the residues 1–199, the C-terminal T6–T9 fragments were not detectable in the α-PBIP1 panel. Note that PBIP1 full-length, T5, T6, and T7 fragments not only efficiently localize to centromeres (*A*) but also substantially stabilize CENP-Q (*B*, α-CENP-Q panel). Given that these four constructs bind to CENP-Q efficiently (Figure 2*C*), the results provided here strongly suggest that stabilization of CENP-Q via the PBIP1-CENP-Q interaction is critical for proper localization of PBIP1 to centromeres. Dots in *B*, full-length and various PBIP1 truncations expressed. Asterisks in *B*, cross-reacting proteins.

Fig. S3. Summary of the interaction domains of PBIP1. The N-terminal half of PBIP1 is critical for the interaction with Plk1, whereas the C-terminal half is required not only to interact with CENP-Q but also to localize to centromeres/kinetochores. Numbers indicate amino acid residues. NLS, putative nuclear localization signal; LZ, putative leucine zipper domain. See Figure *1A–B* for details.

Fig. S4. Impaired localization of CENP-Q and Plk1 to the kinetochores of cells expressing a nuclear localization-defective PBIP1(K308A K316A) mutant. *A–D*, HeLa cells expressing control vector or the indicated PBIP1 constructs were depleted of control luciferase (si-Luc) or endogenous PBIP1 (si-PBIP1), and then infected with lentivirus expressing EGFP-CENP-Q. The resulting cells were subjected to immunoblotting **(***A***)** and immunostaining **(***B***)** analyses. For the examination of the localization patterns of Plk1 and PBIP1, cells prior to EGFP-CENP-Q virus infection were co-immunostained **(***C***)** and quantified **(***D***)**. Bars in *D*, the averages of signal intensities with standard error of the mean obtained from greater than 14 cells per each sample. Note that, in addition to kinetochores, this particular batch of anti-PBIP1 antibody also generates si-PBIP1-insensitive (see the undiminished centrosomal signals in the si-PBIP1 panel), cross-reactive centrosomal signals (*asterisks*). These non-specific centrosomal signals serve as convenient markers to determine the relative levels of PBIP1-specific kinetochore signals in comparison to those of the si-PBIP1-insensitive, non-specific signals. In the cells depleted of PBIP1 (si-PBIP1) or the si-PBIP1 cells expressing the nuclear localization-defective

PBIP1(K308A K316A), CENP-Q fluorescence signals were markedly diminished due to the unstable nature of this protein in the absence of nuclear-localized PBIP1. In addition, the reduced level of the PBIP1(K308A K316A) mutant in *A* suggests that cytosolic PBIP1 is unstable.

Fig. S5. Plk1-dependent phosphorylation of CENP-Q *in vivo*. *A*, HeLa cells were depleted of either control luciferase (si-Luc), CENP-Q (si-CENP-Q), or PBIP1 (si-PBIP1) for 3 days, or treated with nocodazole (Noc) for 18 h. Where indicated, cells were additionally treated with a Plk1 inhibitor, BI 2536 (BI), for 4 h prior to harvest. Samples were separated by 10% SDS-PAGE and immunoblotted. *Asterisk*, a cross-reacting protein. Note that depletion of either CENP-Q or PBIP1 destabilizes the other (compare the first three lanes). Treatment of cells with BI 2536, which inhibits Plk1-dependent PBIP1 phosphorylation (Note the stacked PBIP1 bands in the bottom panel) (1), significantly diminishes the level of phosphorylated, slow-migrating CENP-Q. *B*, HeLa cells either growing asynchronously (Asyn) or depleted of control luciferase (si-Luc) or Plk1 (si-Plk1) were treated with nocodazole for 20 h before harvest. Samples were separated by SDS-PAGE and immunoblotted. Note that, unlike the loss of T78-dependent PBIP1 function in Figure *4D–E*, which decreases the level of phosphorylation on CENP-Q but not on others, depletion of Plk1 (si-Plk1) diminishes the levels of phosphorylated, slow-migrating forms of various Plk1 substrates, such as CENP-Q, Bub1, BubR1, and Cdc25C.

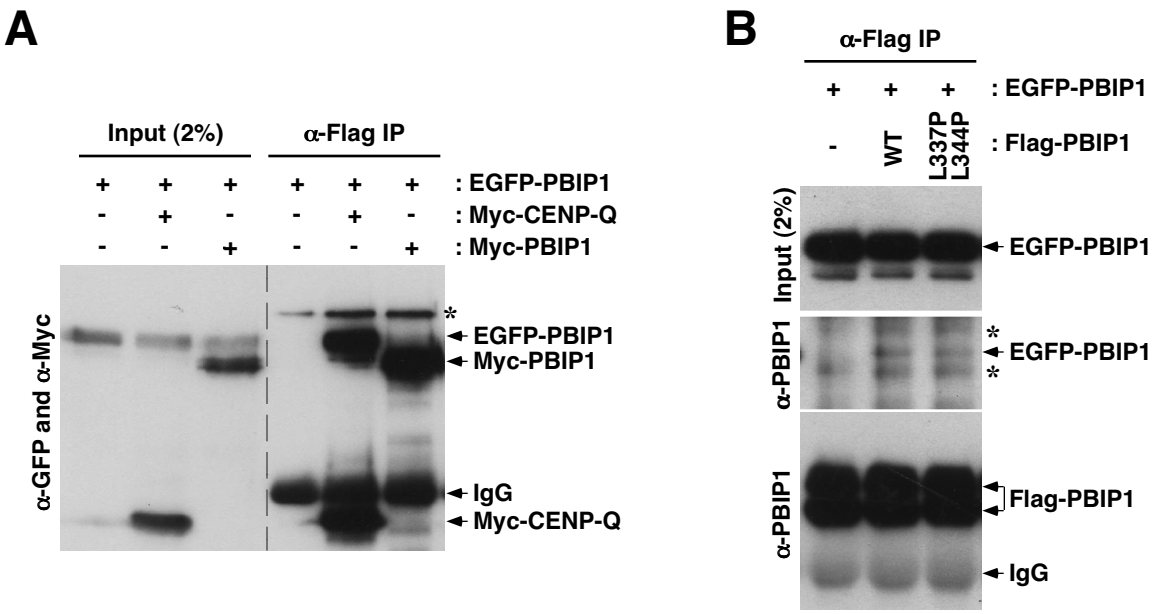
Fig. S6. Examples of purification of recombinant His-PBIP1 and His-MBP-CENP-Q proteins using the pETDuet-1 co-expression system and enhancement of protein stability by the formation of the PBIP1-CENP-Q complex. *A*, His-MBP-CENP-Q was expressed either alone or together with His-PBIP1 in *E. coli* and purified using an Ni-affinity column. Samples were separated by SDS-PAGE and stained with Coomassie (CBB). Note that in the absence of PBIP1, CENP-Q generates multiple cleavage products (dots). However, in the presence of His-PBIP1, which was co-purified with His-MBP-CENP-Q, CENP-Q becomes substantially stabilized. *B*, His-MBP-CENP-Q was co-expressed with either His-PBIP1, the His-PBIP1(T78A) mutant, or the PBIP1(L337P L344P) double mutant in *E. coli*. Proteins purified from Ni-beads were subjected to SDS-PAGE and stained with Coomassie (CBB). Dots indicate the putative PBIP1 fragments generated due to the disruption of the PBIP1-CENP-Q interaction by the L337P L344P mutations. Similarly, expression of PBIP1 WT alone also resulted in severe protein degradation (data not shown).

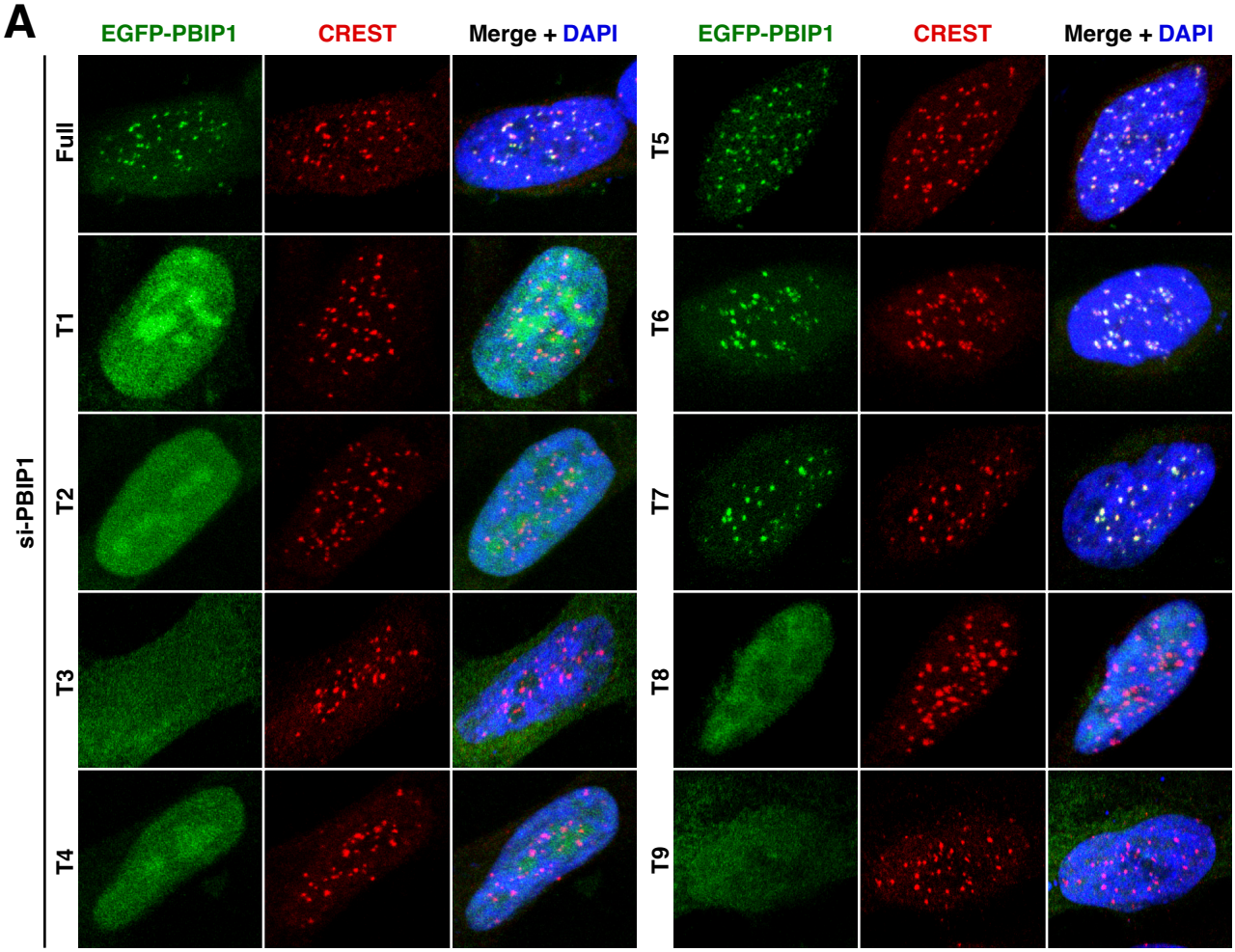
Fig. S7. Prolonged localization of PBIP1(T78A) and its primary binding target, CENP-Q, at metaphase kinetochores. *A–B*, HeLa cells expressing the indicated RNAi-insensitive PBIP1 constructs were depleted of control luciferase (si-Luc) or endogenous PBIP1 (si-PBIP1). The resulting cells were immunostained (*A*) to quantify the levels of fluorescence signal intensities (see Figure 6*C* for quantified results). The same cells were immunoblotted to determine the levels of protein expression (*B*). Asterisks in *A* indicate cross-reacting centrosomal signals (in PBIP1 panels) and centrosome-localized Plk1 signals (in Plk1 panels). Note that depletion of PBIP1 in control vector-expressing cells (*A*, second row from top) eliminates not only PBIP1 signals but also CENP-Q signals from kinetochores, even though CENP-Q is not completely co-depleted (*B*, second lane) under these conditions. Asterisks in *B*, cross-reacting proteins.

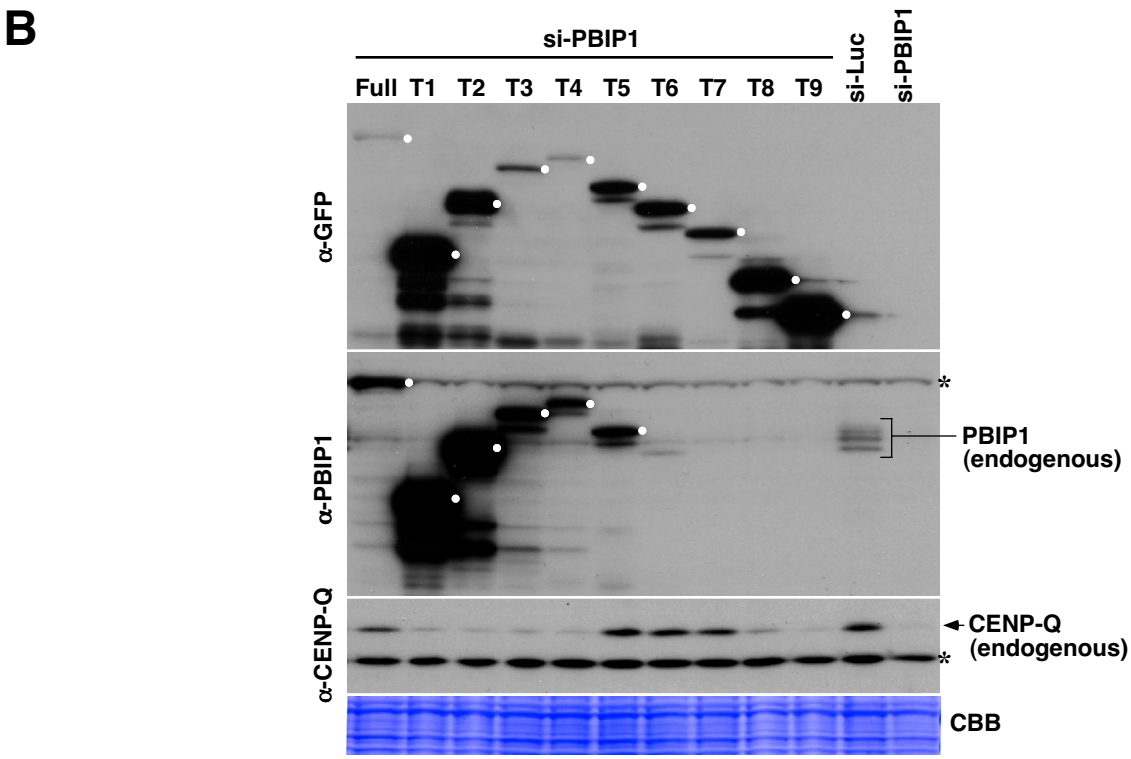
SUPPLEMENTARY REFERENCE

1. Lee, K. S., Park, J. E., Kang, Y. H., Zimmerman, W., Soung, N. K., Seong, Y. S., Kwak, S. J., and Erikson, R. L. (2008) *Cell Cycle* **7**, 141-145

Kang et al., Fig. S1




A
EGFP-PBIP1
CREST
Merge + DAPI
EGFP-PBIP1
CREST
Merge + DAPI
si-PBIP1
Full
T1
T2
T3
T4
T5
T6
T7
T8
T9


B
si-PBIP1
si-Luc
si-PBIP1
Full T1 T2 T3 T4 T5 T6 T7 T8 T9
α-GFP
α-PBIP1
α-CENP-Q
PBIP1
(endogenous)
CENP-Q
(endogenous)
CBB

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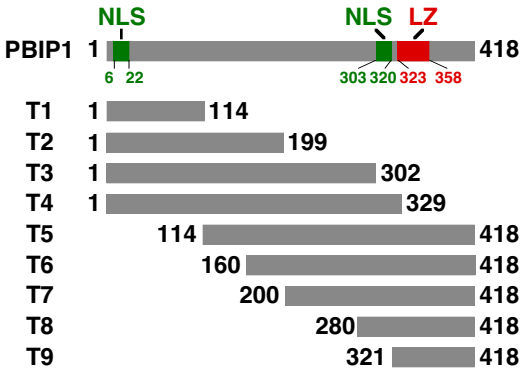
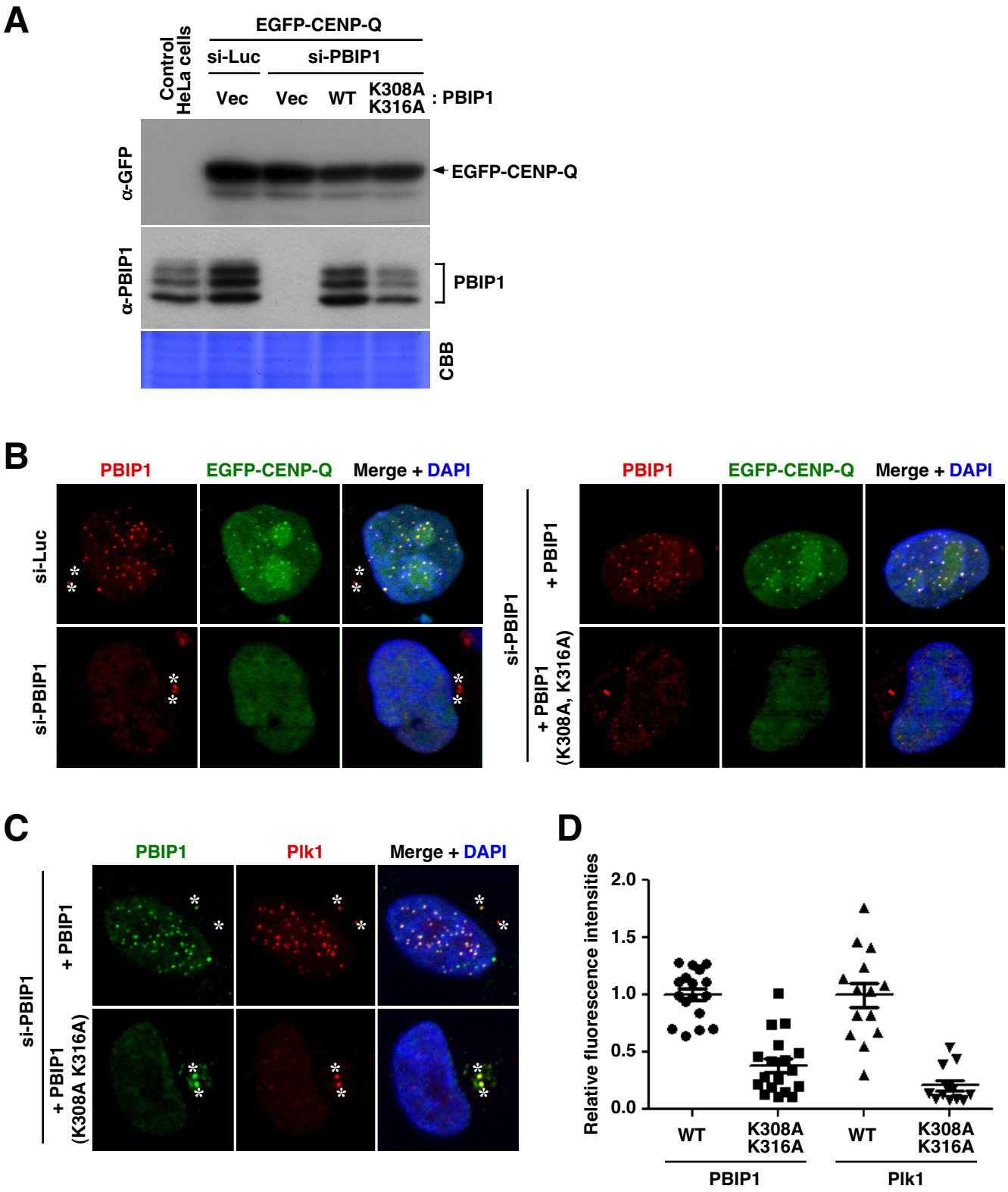
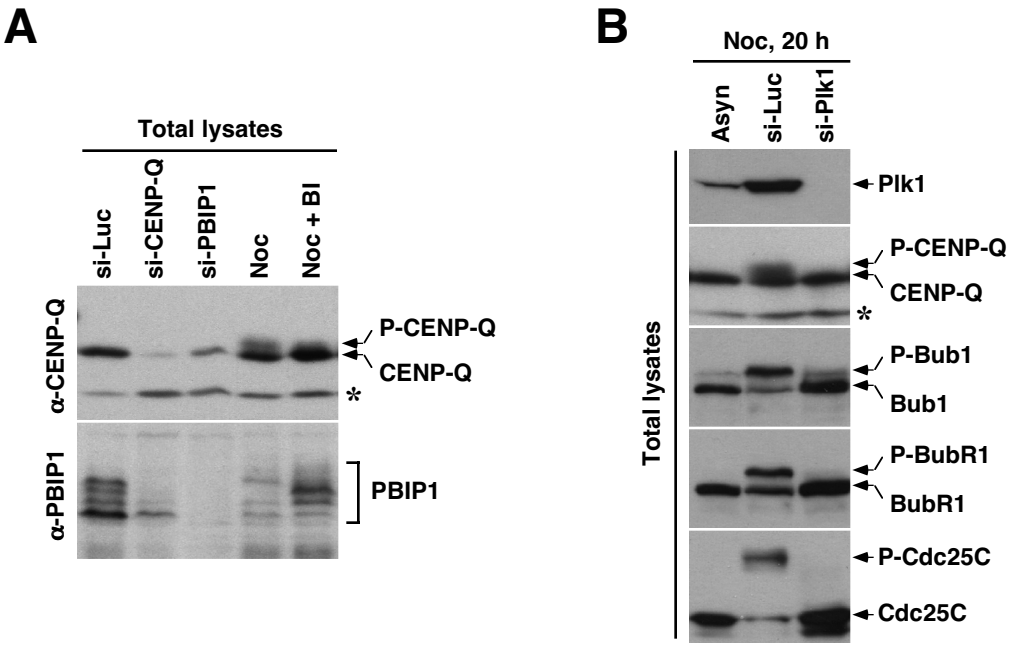


PBIP1	Centromere loc'n	CENP-Q binding	Plk1 binding
Full	+	+	+
T1	-	-	+
T2	-	-	+
T3	-	-	+
T4	-	-	+
T5	+	+	-
T6	+	+	-
T7	+	+	-
T8	-	-	-
T9	-	-	-


A
Control HeLa cells
EGFP-CENP-Q
si-Luc
si-PBIP1
Vec
Vec
WT
K308A K316A
: PBIP1
α-GFP
EGFP-CENP-Q
α-PBIP1
PBIP1
CBB
B
PBIP1
EGFP-CENP-Q
Merge + DAPI
si-Luc
si-PBIP1
si-PBIP1
+ PBIP1
+ PBIP1 (K308A, K316A)
C
PBIP1
Plk1
Merge + DAPI
si-PBIP1
+ PBIP1
+ PBIP1 (K308A K316A)
D
Relative fluorescence intensities
2.0
1.5
1.0
0.5
0.0
WT
K308A K316A
WT
K308A K316A
PBIP1
Plk1


A
Total lysates
si-Luc
si-CENP-Q
si-PBIP1
Noc
Noc + BI
α-CENP-Q
P-CENP-Q
CENP-Q
*
α-PBIP1
PBIP1
B
Noc, 20 h
Asyn
si-Luc
si-Plk1
Total lysates
Plk1
P-CENP-Q
CENP-Q
*
P-Bub1
Bub1
P-BubR1
BubR1
P-Cdc25C
Cdc25C

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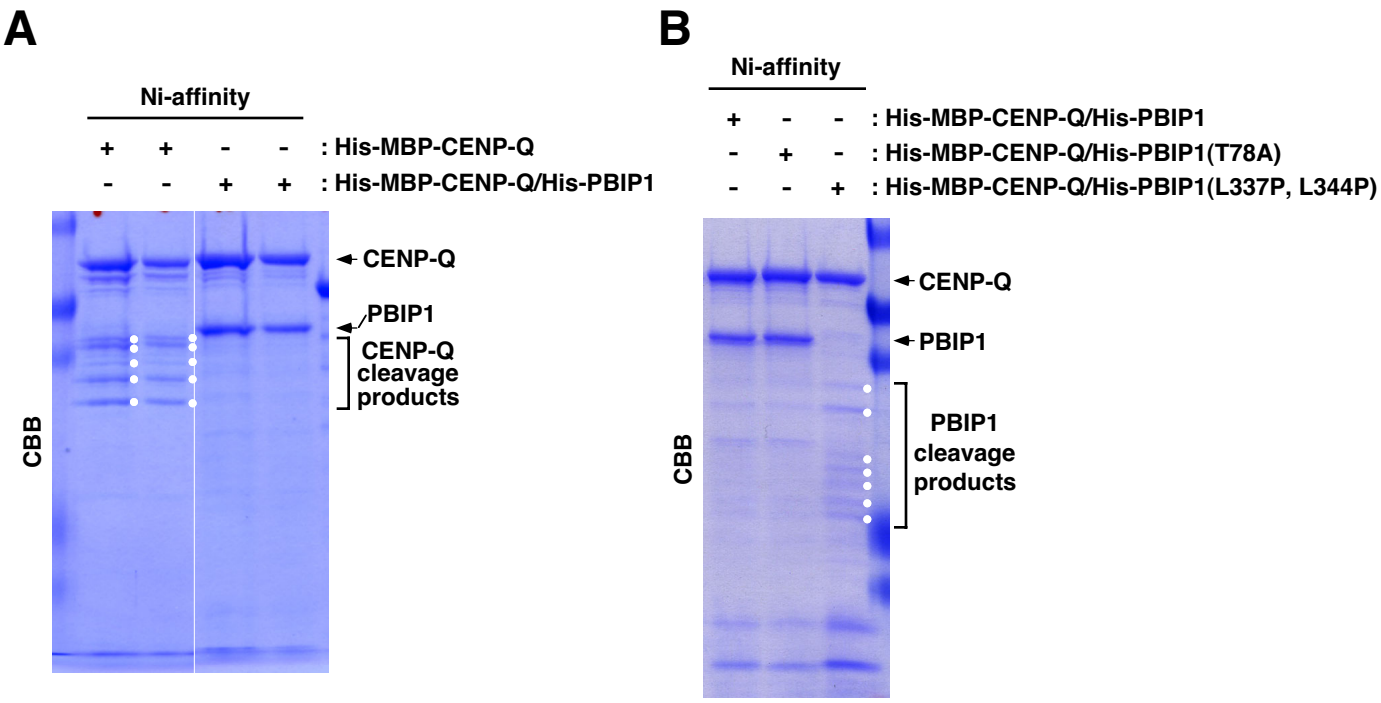

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