Supplementary materials

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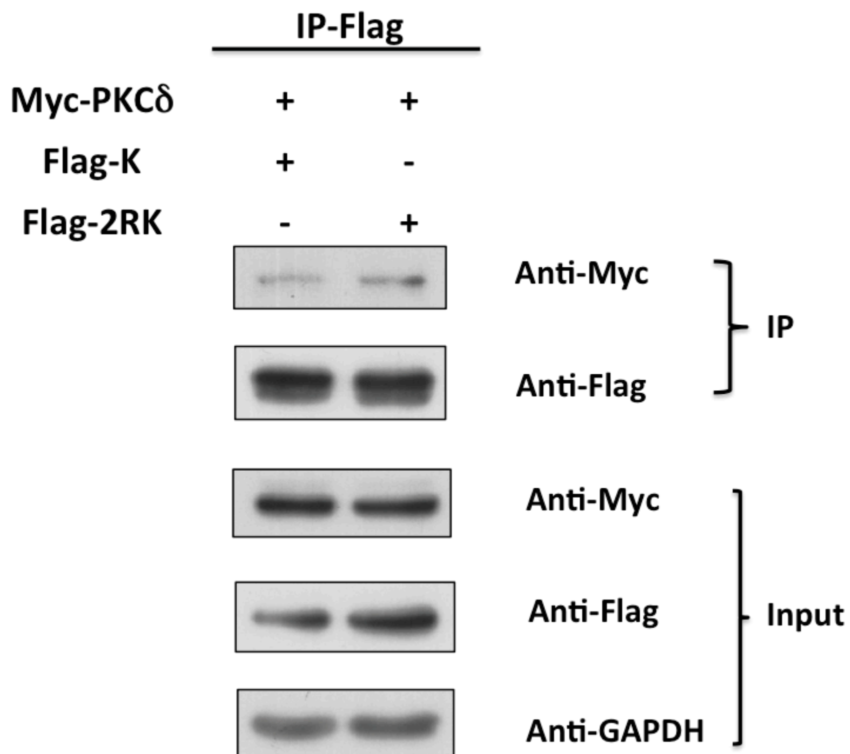
Supplementary Table 1. A list of hnRNPK mutants with the indicted arginine-to lysine mutations

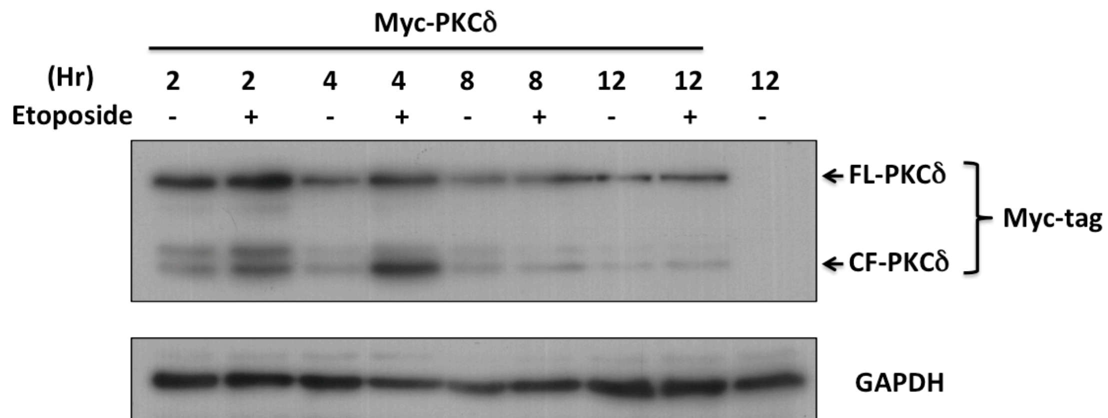
	256	258	268	296	299
WT	R	R	R	R	R
2RK	R	R	R	K	K
3RK-1	R	R	K	K	K
3RK-2	K	K	K	R	R
4RK-1	R	K	K	K	K
4RK-2	K	R	K	K	K
4RK-3	K	K	R	K	K
4RK-4	K	K	K	R	K
4RK-5	K	K	K	K	R
5RK	K	K	K	K	K

Supplementary Table 2. List of proteins exhibiting functional interplay between methylation and nearby phosphorylation.

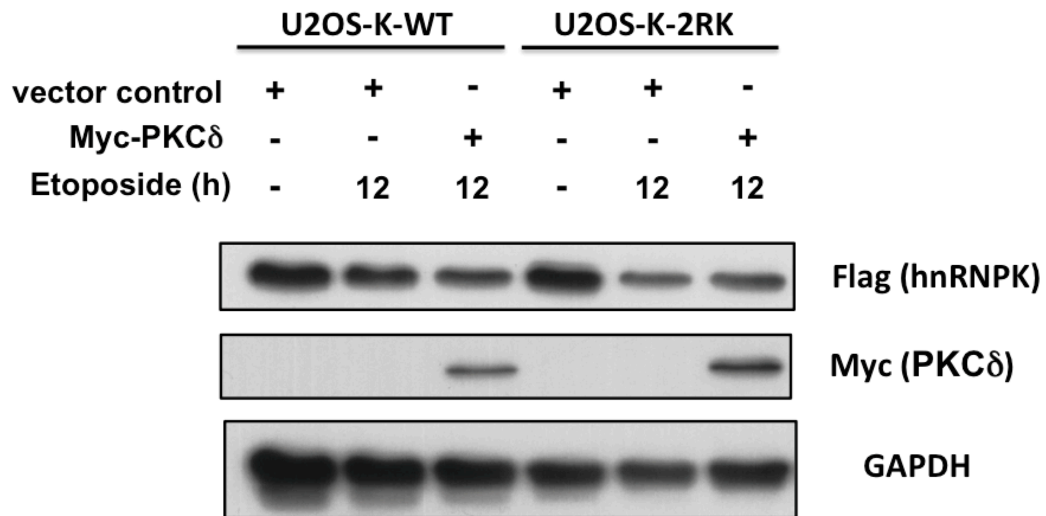
Target Protein	Methyl Transferase	Kinase	Sequence	References
FOXO	PRMT1	Akt	SPRRRAASMD	Molecular Cell, 2008, 32:221-231.
BAD	PRMT1	Akt	PFRGRSRSAP	PNAS, 2011, 108:6085-90.
FEN1	PRMT5	Cdc2-CycE	LTFGSPVLMRHL	Nature chemical biology, 2010, 6:776-773.
EGFR	PRMT5	EGFR	GSTAENAEYLR	Nature cell biology, 2011, 13:174-181.



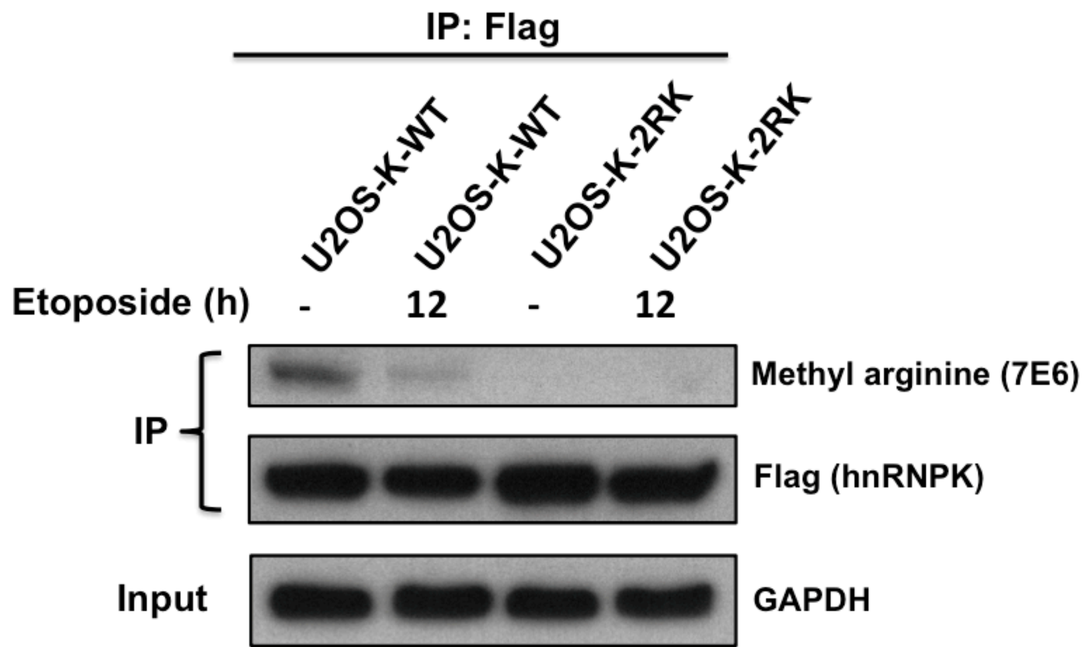
Supplementary Figure 1. Arginine methylation of hnRNPK suppresses its binding to PKCδ. U2OS cells were transfected with Myc-PKCδ and either Flag-WT or Flag-2RK hnRNPK for 24 h, followed by etoposide treatment. Flag-hnRNPKs were subsequently immunoprecipitated from U2OS lysates, followed by the Western blot analysis to determine the degree of interaction between Flag-hnRNPKs and PKCδ.



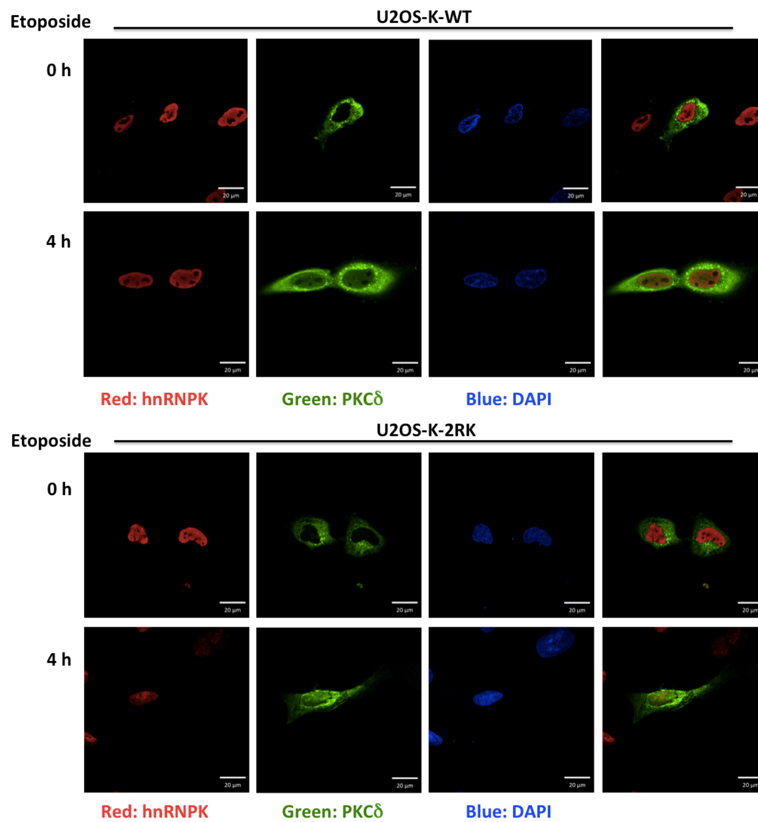
Supplementary Figure 2. Determination of etoposide-induced activation of PKCδ in U2OS cells. U2OS cells were transfected with Myc-PKCδ, followed by etoposide treatment. The levels of catalytic fragment (CF) and full-length (FL) PKCδ were measured through Western blot analysis at the indicated time points to determine the maximal effect of PKCδ activation (cleaved CF-PKCδ) induced through etoposide treatment.



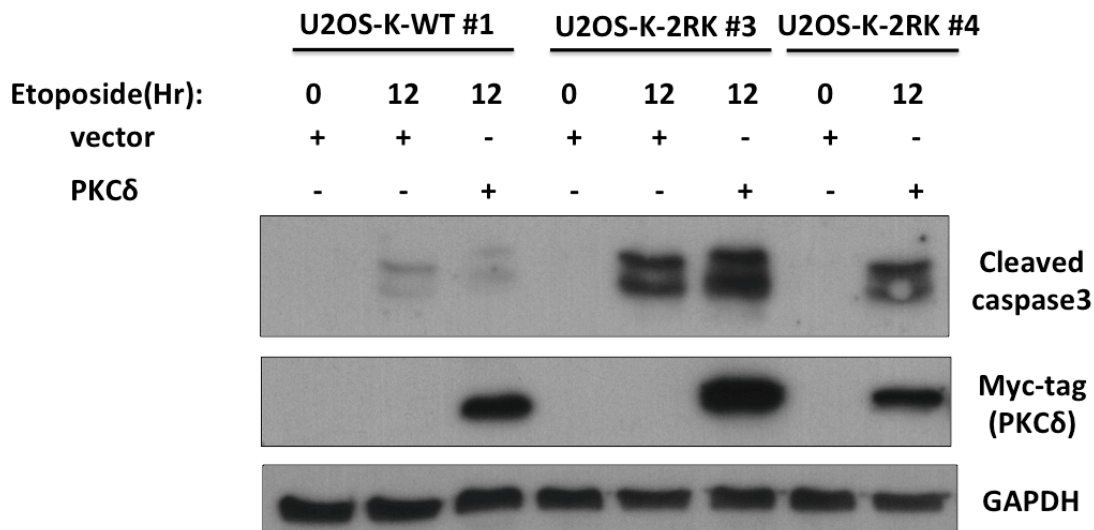
Supplementary Figure 3. Reduction of total hnRNPK levels upon etoposide treatment in U2OS cells. The U2OS-K-WT and U2OS-K-2RK cells were respectively induced for DNA damage through transfection of Myc-PKCδ or etoposide treatment or both. Expression levels of the total hnRNPK, Myc-PKCδ and GAPDH were detected using specific antibodies.



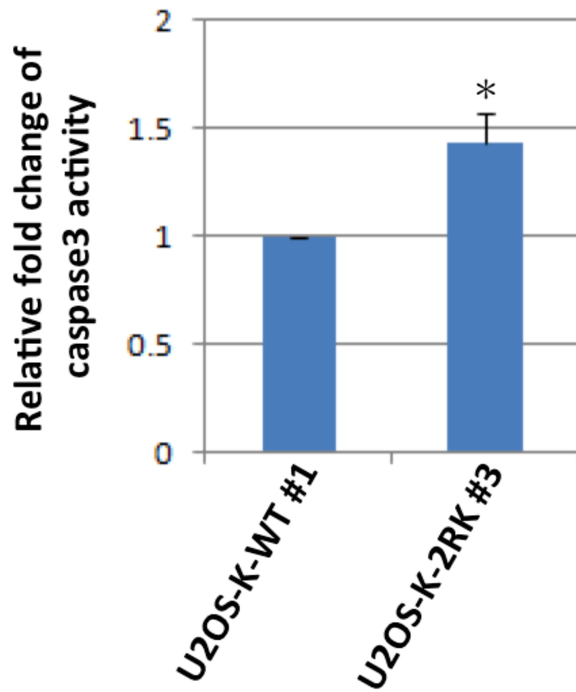
Supplementary Figure 4. Arginine methylation of hnRNPK is suppressed upon etoposide treatment. The U2OS-K-WT and U2OS-K-2RK cells were respectively treated with etoposide, and followed by immunoprecipitation of hnRNPK. Subsequent methylation detection and expression of the precipitated hnRNPK was carried out using pan-asymmetric methyl-arginine antibody and Flag antibody.



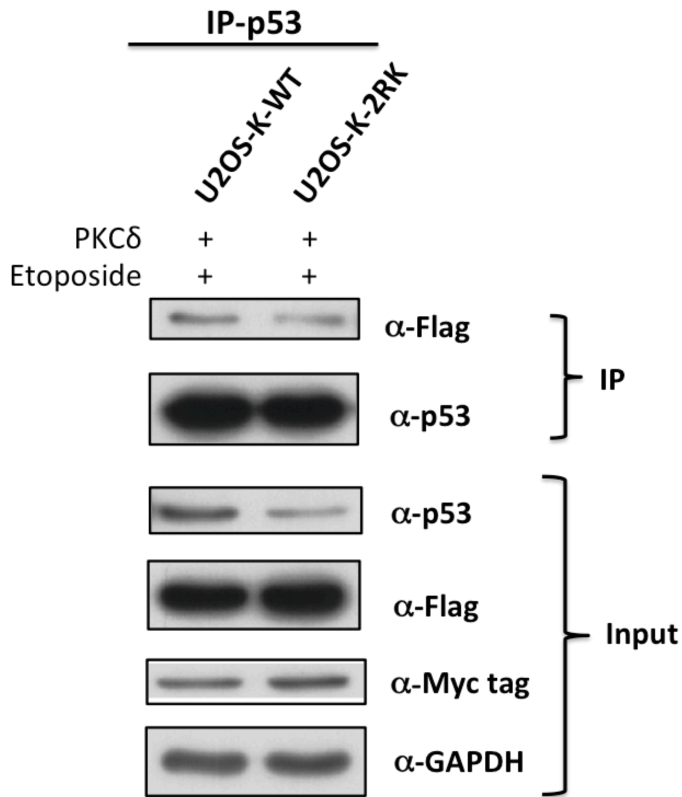
Supplementary Figure 5. HnRNPK and PKCδ co-localizes in nucleus upon etoposide treatment. Both U2OS-K-WT and U2OS-K-2RK cells were transfected with PKCδ and treated with etoposide for 4 h. Detection of immunofluorescence of the resulting cells was carried out using specific antibody and a laser scanning confocal microscope.



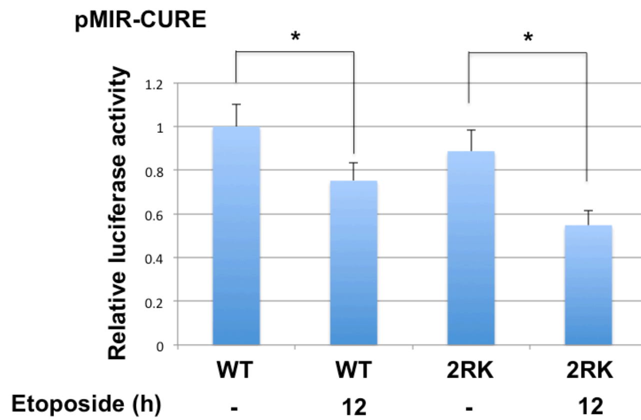
Supplementary Figure 6. The loss of Arg299 and Arg296 methylation in hnRNPK promotes caspase 3 activity and apoptosis in different methylation-defective clones treated with etoposide. The U2OS-K-WT, U2OS-K-2RK #3 and U2OS-K-2RK #4 cells were induced for apoptosis through the transfection of Myc-PKCδ or etoposide treatment or both. The expression levels of the Myc-PKCδ, GAPDH and cleaved caspase 3 were detected using specific antibodies.



Supplementary Figure 7. Measurement of caspase 3 activity in U2OS-K-WT and U2OS-K-2RK cells upon PKCδ activation and DNA damage. U2OS-K-WT and U2OS-K-2RK cells were transfected with Myc-PKCδ for 24 h, followed by etoposide treatment. The activity of caspase 3 in these two cells after treatment was determined by measuring the fluorescence levels of the caspase-3 fluorogenic substrate.



Supplementary Figure 8. Interaction of hnRNPK and p53 during DNA damage is not affected by the arginine methylation of hnRNPK. U2OS-K-WT and U2OS-K-2RK cells were transfected with Myc-PKCδ for 24 h, followed by etoposide treatment for 12 hr. Flag-hnRNPKs were immunoprecipitated from cell lysates, followed by Western blot analysis to determine the interaction of Flag-hnRNPKs and p53.



Supplementary Figure 9. Mutation of arginine methylation of hnRNPK does not significantly affect its RNA binding capacity. Plasmid of a firefly luciferase conjugated with a 3'-untranslated region of thymidine phosphorylase (pMIR-CURE) was transfected into U2OS-K-WT or U2OS-K-2RK cells for 24 h, and then treated with etoposide for 12 h. In addition, the β-galactosidase vector was also co-transfected into these cells at the same time to serve as an internal control. After collection of cell lystes, luciferase activities were measured according to the recommended procedures by manufacture (Promega, WI, USA) using an AutoLumat LB953 (Berthold Technologies, Bad Wildbad, Germany) instrument.