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Supplementary Materials for

DNA-dependent protein kinase promotes DNA end processing by MRN and CtIP

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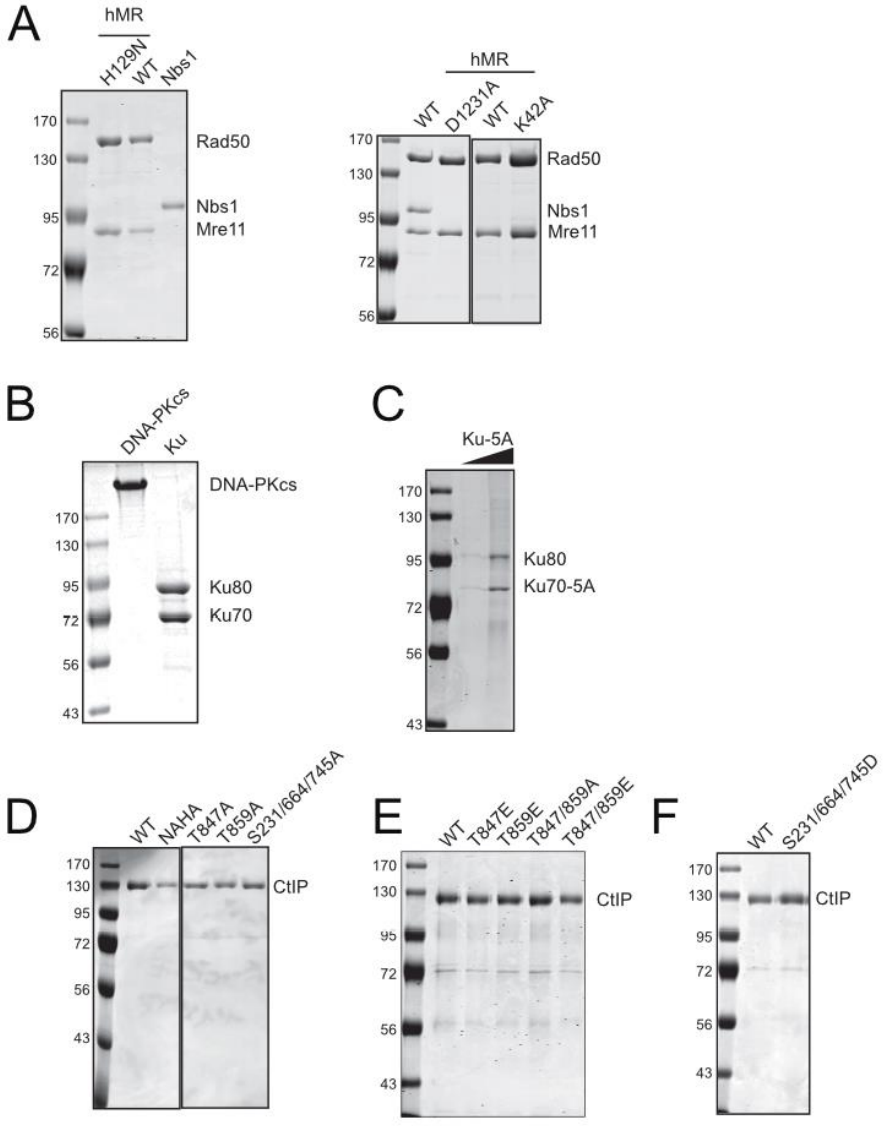

Fig. S1. Purified recombinant proteins used in this study. Human recombinant Mre11/Rad50 (hMR) and Nbs1 and hMR(K42A) and hMR(D1231A) mutants (**A**), Ku70/80 wild-type and DNA-PKcs (**B**), Ku70(5A)/Ku80 (**C**), and CtIP proteins (**D**, **E**, **F**) were separated by SDS-PAGE and stained with Coomassie blue. All proteins shown here were purified from insect cells (See materials and methods for details) except DNA-PKcs which is native protein purified from human cells.

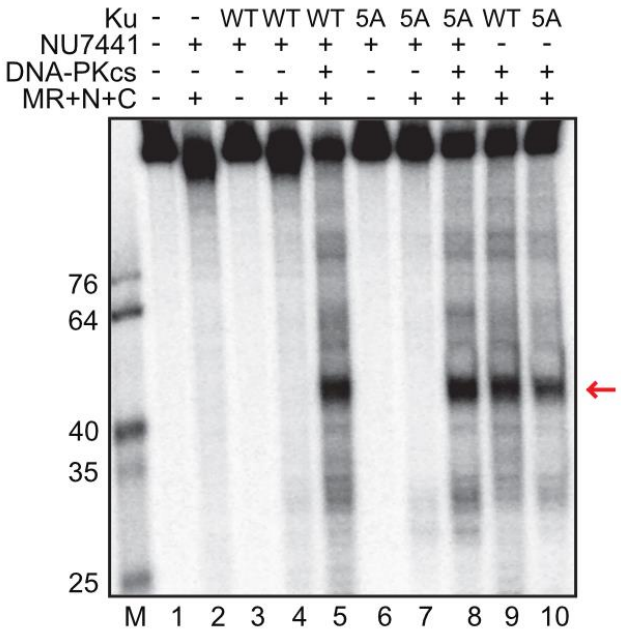

Fig. S2. Ku(5A) promotes MRN-dependent endonuclease activity. Nuclease assays were performed as in Fig. 1 in the presence of both magnesium and manganese with wild-type MRN, CtIP, DNA-PKcs, NU7441, and with either wild-type (WT) or 5A mutant (5A) versions of Ku as indicated. Red arrow indicates predominant DNA-PK-dependent MRN product.

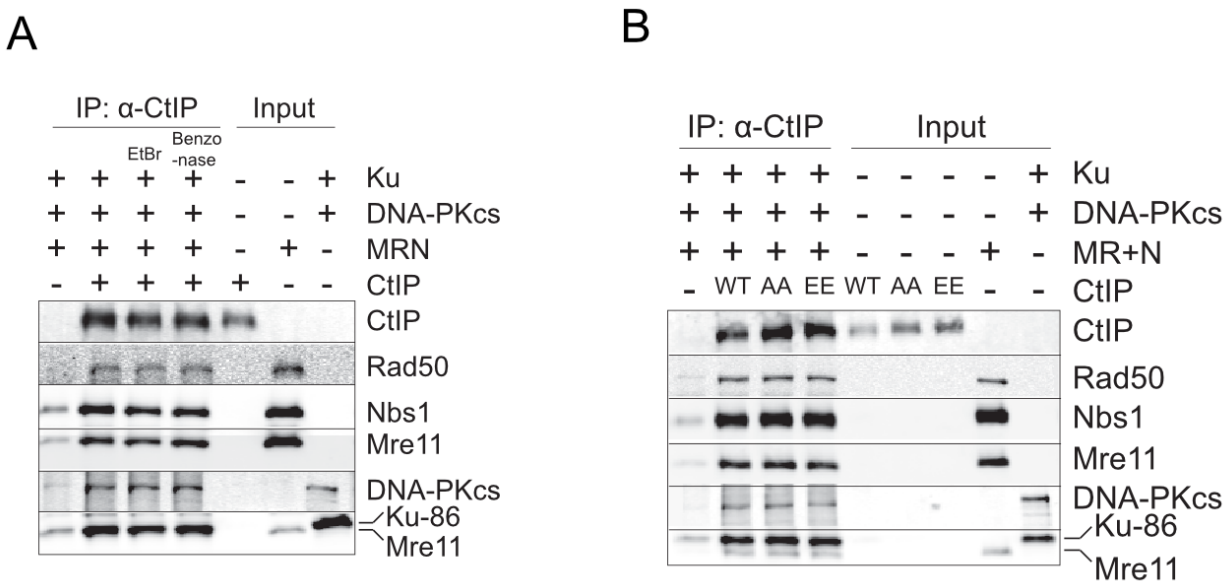

Fig. S3. Interactions between CtIP, DNA-PKcs, Ku, and MRN do not depend on DNA or CtIP phosphorylation. (**A**) Ethidium bromide (5 μg/ml) or Benzonase (1.25 kU) was added to the reactions as indicated. Recombinant proteins were incubated and CtIP isolated by immunoprecipitation as in Fig. 1D. Bound factors were monitored by western blot as indicated. (**B**) Binding reactions were performed with MRN, DNA-PKcs, Ku, and CtIP wild-type (WT) or mutant T847A/T859A (AA) or T847E/T859E (EE) proteins as in (A).

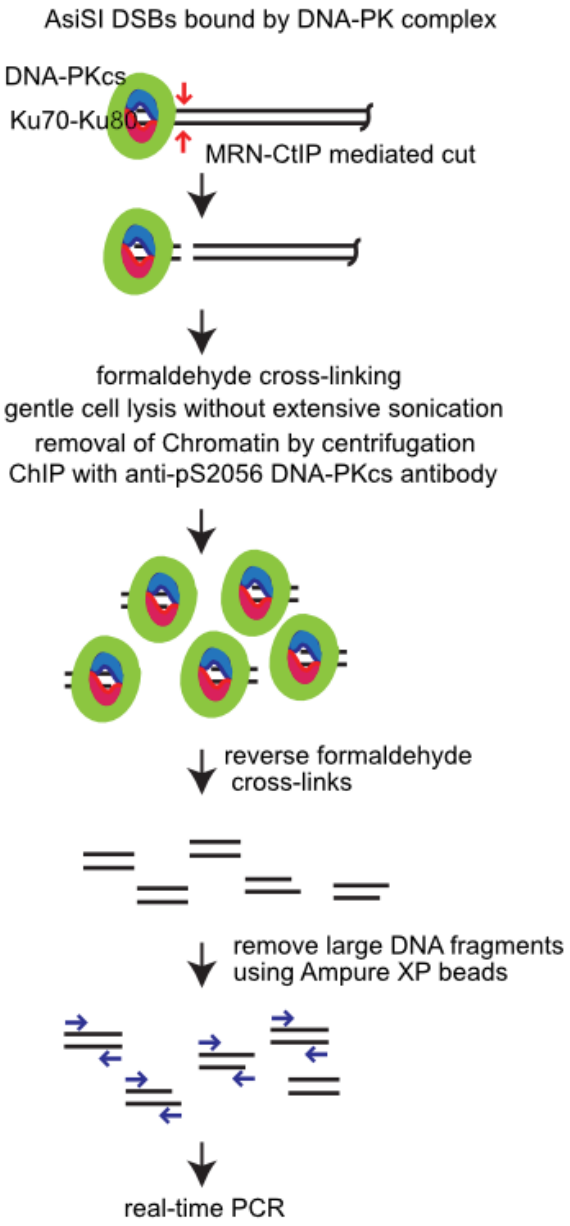

Fig. S4. Schematic diagram of GLASS-ChIP protocol. See Materials and Methods for detailed procedure. AsiSI DSBs created in U2OS cells are bound by DNA-PK. Small DNA fragments bound by DNA-PK are generated through endonucleolytic cleavage by MRN with stimulation by CtIP. These protein-bound fragments were stabilized by standard formaldehyde cross-linking but gentle cell lysis was used without excessive sonication in order to avoid fragmentation of genomic DNA. After removal of bulk chromatin, ChIP was performed using anti-DNA-PKcs-pS2056 antibody. After reversal of cross-links and size selection with Ampure XP beads, these DNA fragments were quantified using real-time PCR.

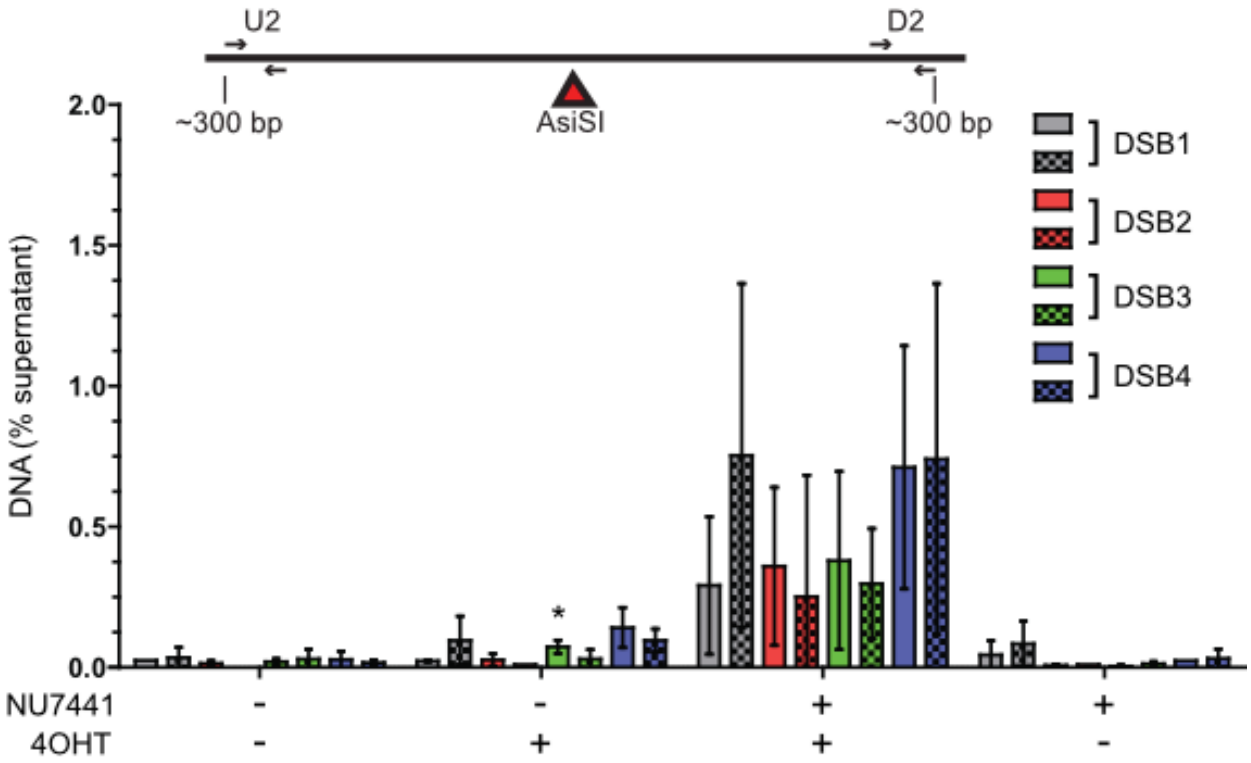

Fig. S5. Quantification of DNA immunoprecipitated with anti–DNA-PKcs pS2056 using primers 300 bp away from AsiSI DSB. Small dsDNA products resulting from nucleolytic cleavage of DNA-PK bound AsiSI-generated DNA ends (dashed line box in Figure 4E) were isolated from U2OS cells using the GLASS-ChIP protocol as described in Fig. S4 and quantified by qPCR using primers located ~300 nt from the AsiSI cut site. Primer set U2 (solid) is upstream whereas D2 (checkered) is downstream of the AsiSI cut sites. The DNA quantitated from U2OS cells in the presence or absence of 4OHT to induce AsiSI and DNA-PKcs inhibitor (NU7441) is shown for 4 AsiSI sites as described in main text. Results shown are from 3 independent biological replicates with student 2-tailed T test performed; * indicates $p < 0.05$ in comparison to equivalent samples without 4OHT.

Table S1. Primers used for qPCR in Fig. 5 and fig. S5.

AsiSI site	Location as in hg18	Primer	Forward	Reverse	Amplicon size (bp)
DSB1	chr18:7556705	U1	TCGGGGCCAGCGGCGTGTA	CGCCAGCCCGCTCCC	52
		D1	CGCGGGGCTCGGCGC	GGGAGATGGCGCGGGAGC	40
		U2	GTGCTGGCTCAATGTGCTTATT	ACGATTTTGGGTCTGAGTGAA	132
		D2	CGCAGCCTCTTCCACAGTCA	GCCACTACCGCCGCCGAA	139
DSB2	chr21: 32167382	U1	GGGAGCGGCCGCCCAG	GCTCCTAGCCGTGCGCT	40
		D1	CGGGAGCCCGACCCAA	CGCCGTCTGGCCCGCA	40
		U2	CGAAAGGTCCAGAAAACCCAA	GAAGCCACCTGAGCGCCAGA	132
		D2	TTGTCTACGCGCCTCGCT	CGGCTTCCCCGGCTTCT	119
DSB3	chr9:129732985	U1	GACTGCGGCTGCATCCAA	CGCCAGCGCCTCCCGC	41
		D1	CGCCTGCGGGTCCCGC	CTGAAGGATGCTGCAGCCGT	40
		U2	CCGCACTGGATGAGAGCTT	CCTGGCGGATATCCCTCAA	112
		D2	GGACATCCATTCATTGAACACA	GATCACGCGGGCAGCTGA	113
DSB4	chr22:37194040	U1	CCCGGCCAAGAGTGCGT	CGCACCCGCGCGCCG	40
		D1	CGCGGAGCTGTGAGGC	GTCTCTAGGTGCCCCAGA	45
		U2	AAGATGAGGACAATAGCAGGAA	AAGCCCCAATCTCTGCCTCA	125
		D2	CAGGGCGCTCCAGGTGT	GGGTCCTCCTCCTCTGAT	118

DSB sites from Aymard et al, 2014