Protein Phosphatase 1 and Phosphatase 1 Nuclear Targeting Subunit-dependent Regulation of DNA-dependent Protein Kinase and Non-homologous End Joining

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Figure S1. PP1 depletion does not significantly alter the mode of end processing.

The plasmid-based NHEJ assay was performed in *Xenopus* egg extracts with or without PP1 depletion, as in Fig. 2A. 24 repair products were isolated and subjected to sequencing analysis. Repair products with large-fragment deletion (200 bp-2 kb) were determined by agarose gel electrophoresis (data not shown).

Figure S2. PP1 is required for NHEJ in human cells.

As described in Materials and Methods, HeLa cells were treated with control or PP1γ siRNA. NHEJ assay was performed using a GFP-expressing template linearized with Nhe1, as described in Material and Methods. The repair efficiency, measured by GFP expression, is shown. A

minimum of three experiments were carried out and the results are shown as the mean values and standard deviations.

Figure S3. The repair of the chromosomal, I-SceI-induced DSB is dependent on Ku70.

As described in Materials and Methods, the chromosome-integrated, I-SceI-induced NHEJ reporter cell line was characterized in a previous study (36), The cells were transfected with control or Ku70 siRNA. The repair efficiency, measured by GFP expression, is shown. The cell lysates were analyzed by immunoblotting for Ku70 and β-actin. A minimum of three experiments were carried out and the results are shown as the mean values and standard deviations.

Figure S4. PP1 functions in NHEJ via regulation of DNA-PKcs.

An alternative PP1γ siRNA, as described in Materials and Methods, was transfected into HeLa cells. The cells were treated with 10 Gy IR, incubated as indicated, and analyzed by immunoblotting.

Figure S5. PP1 associates with multiple regions of DNA-PKcs.

Five segments of DNA-PKcs (N, JK, PQR, ABCDE and C) were purified as in Fig. 3F. Pull-down experiments were performed using these segments in *Xenopus* egg extracts, and analyzed by immunoblotting. A mock pull-down using empty amylose beads was performed as control (Ctr).

Figure S6. PNUTS is required for efficient DSB repair.

As described in Materials and Methods, HeLa cells were mock treated with control, depleted of PNUTS with PNUTS siRNA, or PNUTS-depleted and then reconstituted with siRNA resistant

GFP-PNUTS. After doxorubicin treatment for 4h, the cell lysates were analyzed by immunoblotting for PNUTS, γ-H2AX and β-actin.

Figure S7. PNUTS is required for the efficient DNA repair via NHEJ.

Immunodepletion of PNUTS was performed in *Xenopus* egg extracts as described in Materials and Methods. Extracts were mock treated with beads alone, depleted of PNUTS (Dep), or PNUTS-depleted and then reconstituted with purified recombinant MBP-PNUTS (Add-back). These extracts were analyzed by immunoblotting for MBP, PNUTS and H2B (lower panels). The NHEJ assay was carried out in these extracts, as in Fig. 2A, and the repair activity was measured by colony formation. A minimum of three experiments were carried out and the results are shown as the mean values and standard deviations.

Figure S8. The PNUTS and Ku70 association is not mediated via DNA.

(A) HeLa cell lysates were treated with or without 50 μg/ml ethidium bromide (EtBr) on ice for 30 min. Ku70 IP was performed in these lysates. The lysate input, control IP, and Ku70 IP products were analyzed by immunoblotting for PNUTS and Ku70. (B) HeLa cell lysates were treated with 100 μg/ml DNase I for 20 min at 37°C. PNUTS IP was performed in these lysates. The lysate input, control IP, and PNUTS IP products were analyzed by immunoblotting for Ku70 and PNUTS. (C) GST-Ku70 and MBP-PNUTS were purified as described in Materials and Methods, and incubated with each other. GST-Ku70 or control beads pull-down were performed, washed, and analyzed by immunoblotting for PNUTS and Ku70. (D) GST-PNUTS and MBP-Ku70 were purified as described in Materials and Methods, and incubated with each other. GST-PNUTS or control beads pull-down were performed, washed, and analyzed by immunoblotting for PNUTS and Ku70.

Figure S9. PP1 and PNUTS do not indirectly influence NHEJ by modulating the expression of core NHEJ factors.

(A) PP1 depletion was performed in *Xenopus* egg extracts. The levels of known NHEJ repair proteins, including Artemis, DNAL IV, Ku70/Ku80, XRCC4 and DNA-PKcs, were analyzed by immunoblotting. The level of PP1γ was monitored by immunoblotting, and that of histone H2B served as a loading control. (B) PP1γ knockdown was performed in HeLa cells using siRNA. The levels of PP1γ, H2B, Artemis, DNAL IV, Ku70/Ku80, XRCC4 and DNA-PKcs, were analyzed by immunoblotting. (C) PNUTS knockdown was performed in HeLa cells using siRNA. The levels of PNUTS, β-actin, DNAL IV, Artemis, Ku70/Ku80, XRCC4 and DNA-PKcs, were analyzed by immunoblotting.

Figure S1

XhoI(C^TCGA_G) + KpnI (G_GTAC^C) 5’overhang + 3’overhang

		Number	Overall frequency
Control	TGCAGT**CTCGAGTACC**AAGCTT	13	92.86% **accurate**
	ACGTCA**GAGCTCATGG**TTCGAA		
	TGCAGT**CTCG∆GTACC**AAGCTT	1	7.14% **deletion**
	ACGTCA**GAGC∆CATGG**TTCGAA		
	Deletion (large deletion)	10 of 60	16.67% **deletion**
PP1 Depletion	TGCAGT**CTCGAGTACC**AAGCTT	10	100.00% **accurate**
	ACGTCA**GAGCTCATGG**TTCGAA		
	Deletion (large deletion)	4 of 36	11.11% **deletion**

Figure S2.

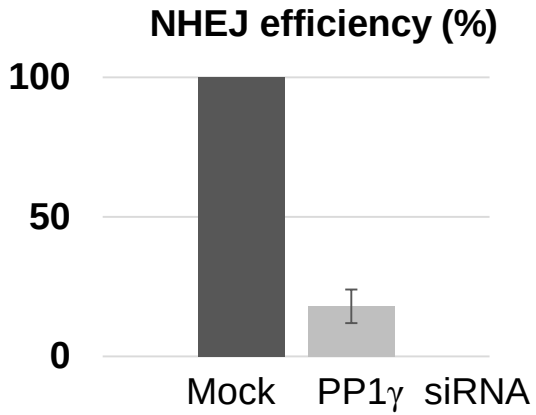


Figure S3

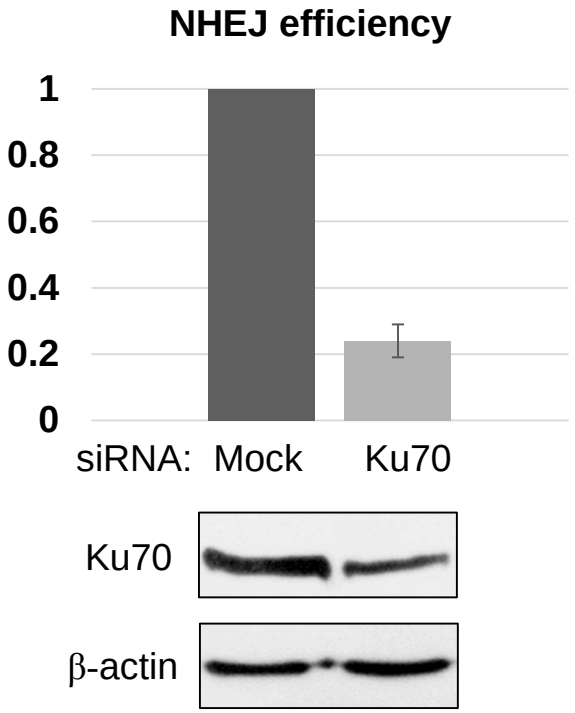


Figure S4

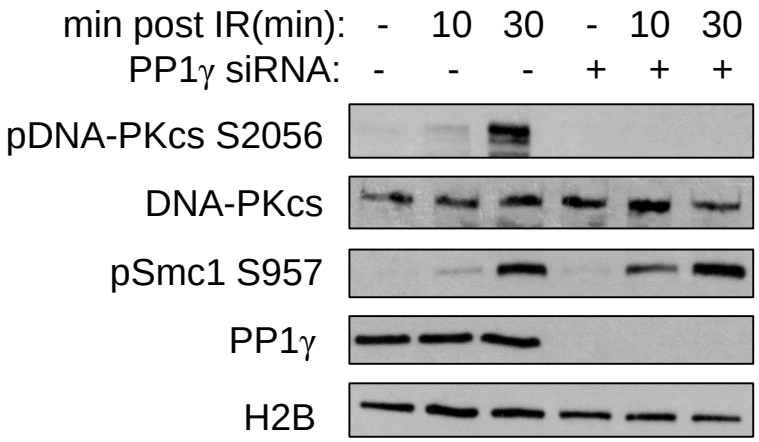

min post IR(min): - 10 30 - 10 30
PP1γ siRNA: - - - + + +
pDNA-PKcs S2056
DNA-PKcs
pSmc1 S957
PP1γ
H2B

Figure S5

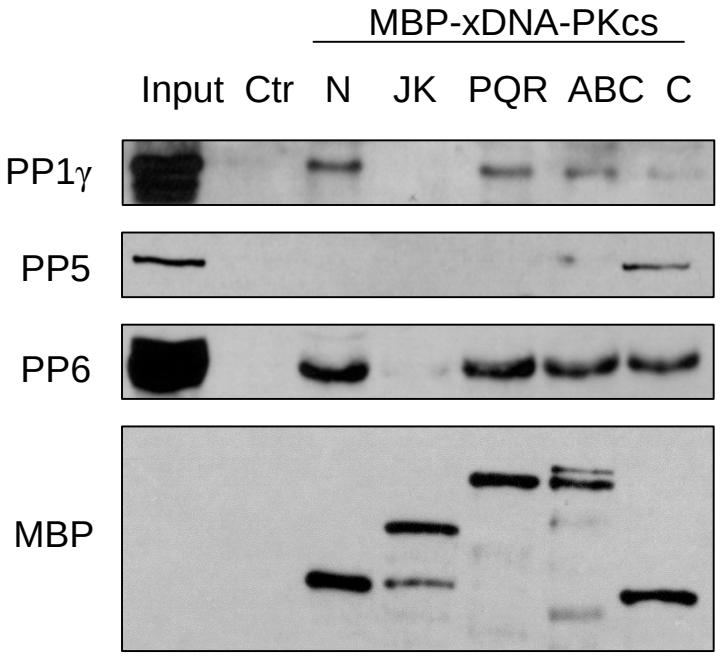


Figure S6

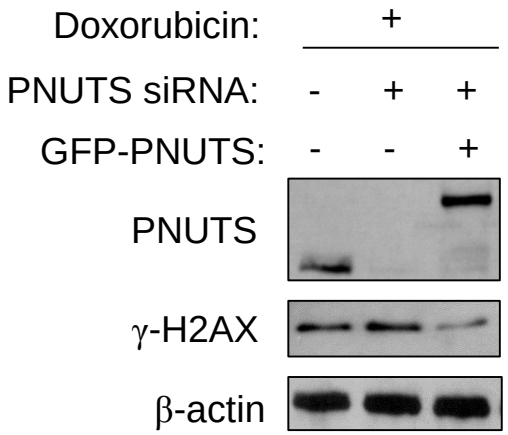


Figure S7

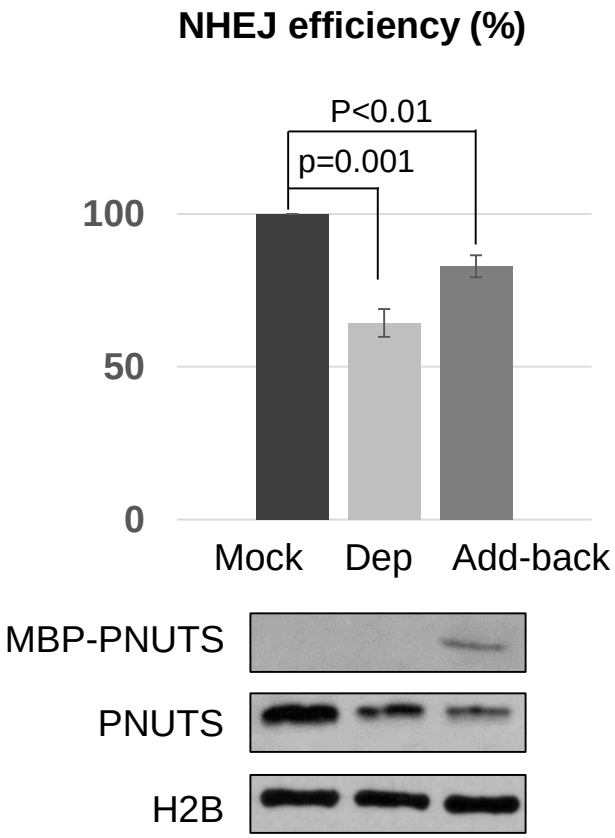

NHEJ efficiency (%)
P<0.01
p=0.001
100
50
0
Mock
Dep
Add-back
MBP-PNUTS
PNUTS
H2B

Figure S8

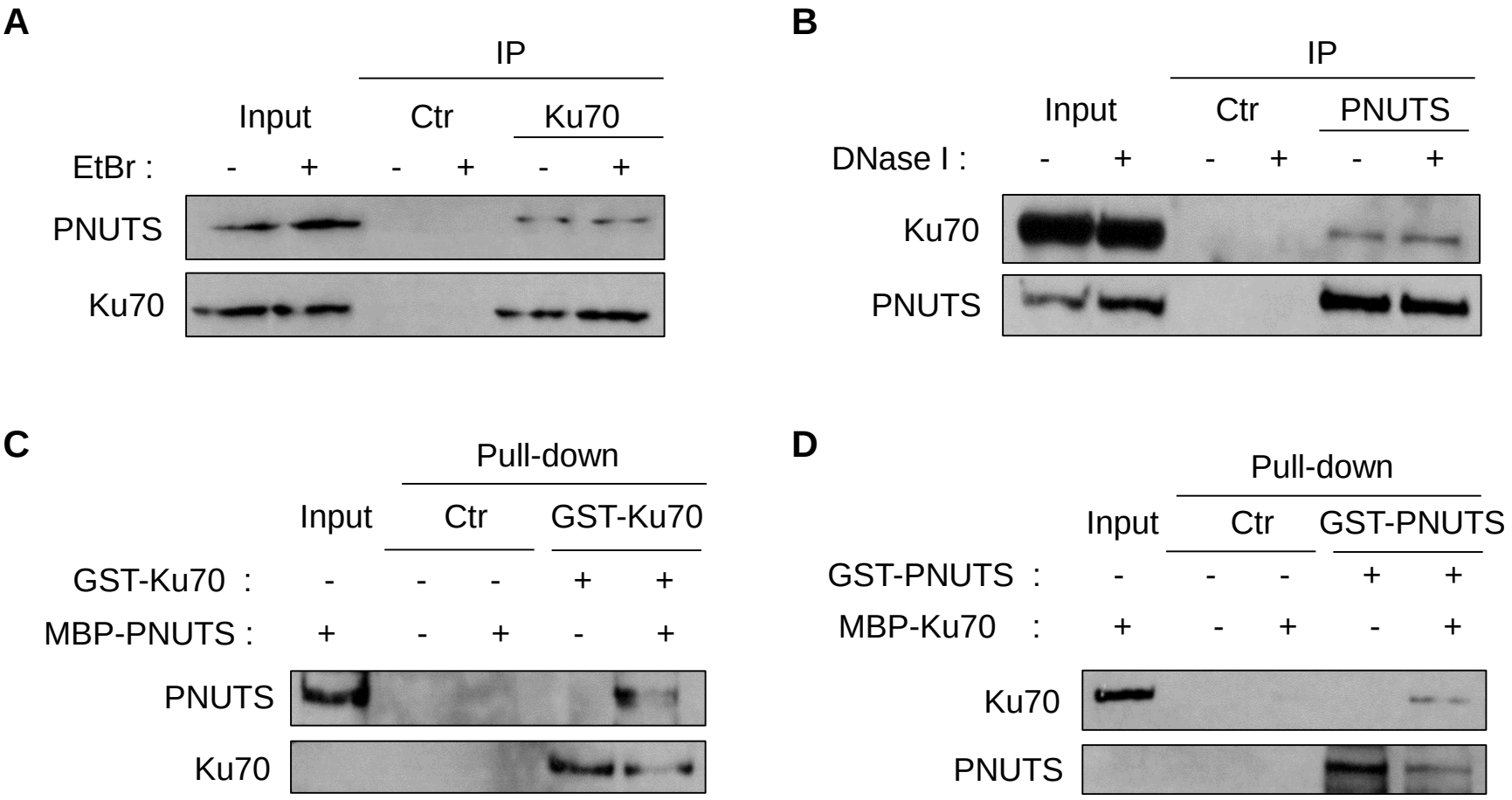
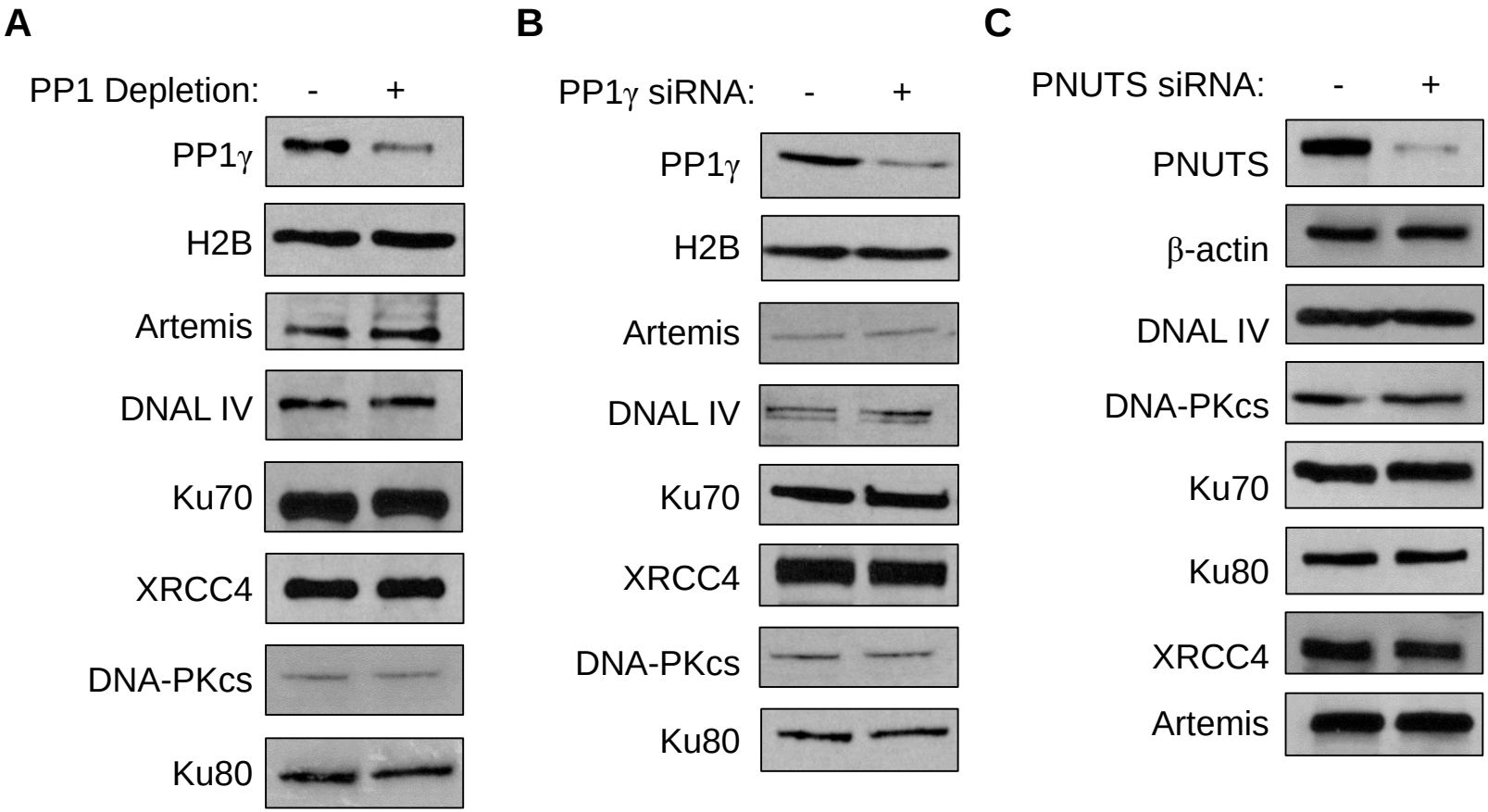

A
IP
Input
Ctr
Ku70
EtBr :
- + - + - +
PNUTS
Ku70
B
IP
Input
Ctr
PNUTS
DNase I :
- + - + - +
Ku70
PNUTS
C
Pull-down
Input
Ctr
GST-Ku70
GST-Ku70 :
- - - + +
MBP-PNUTS :
+ - + - +
PNUTS
Ku70
D
Pull-down
Input
Ctr
GST-PNUTS
GST-PNUTS :
- - - + +
MBP-Ku70 :
+ - + - +
Ku70
PNUTS

Figure S9


A
PP1 Depletion: - +
PP1γ
H2B
Artemis
DNAL IV
Ku70
XRCC4
DNA-PKcs
Ku80
B
PP1γ siRNA: - +
PP1γ
H2B
Artemis
DNAL IV
Ku70
XRCC4
DNA-PKcs
Ku80
C
PNUTS siRNA: - +
PNUTS
β-actin
DNAL IV
DNA-PKcs
Ku70
Ku80
XRCC4
Artemis