Non-homologous end joining repair in *Xenopus* egg extract

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Supplemental figure legends

Figure S1. The repair of non-compatible ends was suppressed by DNA-PK inhibition.

Xenopus egg extract was supplemented with or without DNA-PKcs inhibitor NU7026, and incubated with the 5'/3' NHEJ template. The repair activity (in relative to the control extract) was measured by colony numbers. A minimum of three experiments were carried out and the results are shown as the mean values and standard deviations. Statistical significance was analyzed using an unpaired 2-tailed Student's t-test. A p-value <0.05 was considered statistically significant.

Figure S2. Five types of NHEJ templates were generated using different restriction enzymes. Different restriction enzymes were utilized to generate five types of NHEJ templates for the repair assay.

Figure S3. Validation of the ATM and DNA-PK inhibitors.

(A & B) *Xenopus* egg extracts were treated with double-stranded oligonucleotides (dA-dT), and specific inhibitors as indicated. After incubation for 30 min at room temperature, samples were analyzed using specific antibodies as indicated.

Figure S4. The repair of non-compatible ends with DNA-PK inhibition.

Various NHEJ repair templates with non-compatible ends, as in Fig. S2, were incubated in *Xenopus* egg extracts, re-isolated, and transformed into bacteria cells. Final repair products were isolated and subjected to sequencing analysis. The NHEJ templates include: blunt/3'-overhang (A), blunt with 5'-overhang (B), 3'-overhang /5'-overhang (C), 3'-overhang /3'-overhang (D), and 5'-overhang /5'-overhang (E). The repair assay was performed with or without DNA-PK inhibitor (NU7441) as in Fig. 1E. In each reaction, approximately 10 final repair

products were sequenced and shown. Nucleotides deleted during DNA repair were indicated by empty triangles.

Figure S1

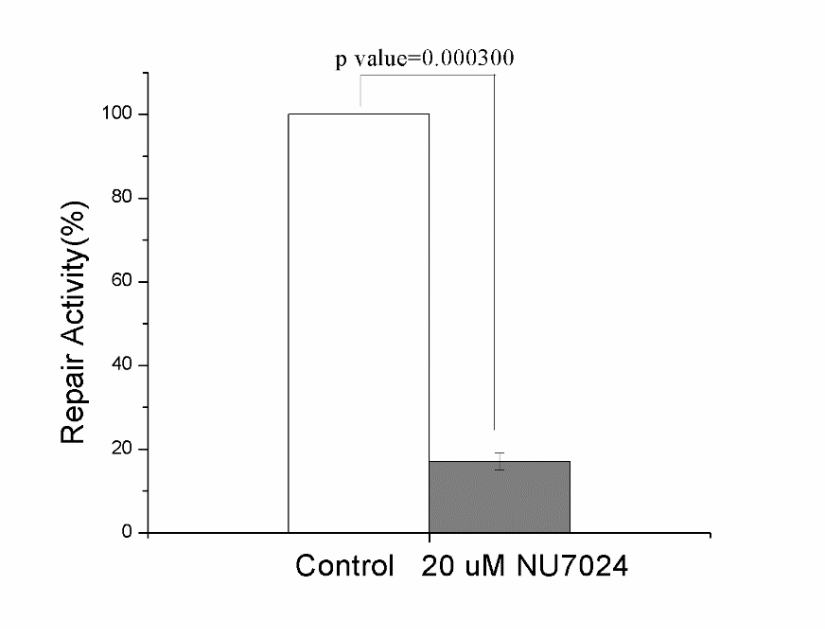
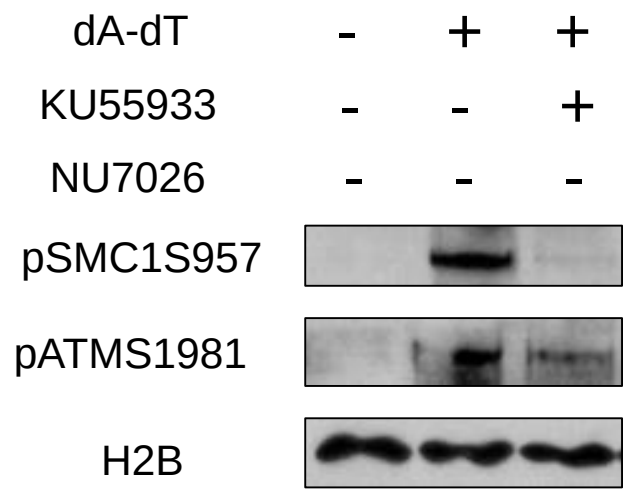

p value=0.000300
Repair Activity(%)
100
80
60
40
20
0
Control
20 uM NU7024

Figure S2

Enzymes	Terminus Configuration	Sequence
StuI / HindIII	Blunt / 5'overhang	AGG AGCTT TCC A
StuI / KpnI	Blunt / 3'overhang	AGG C TCC CATGG
XhoI / KpnI	5'overhang / 3'overhang	C C GAGCT CATGG
PstI / KpnI	3'overhang / 3'overhang	CTGCA C G CATGG
BamHI / HindIII	5'overhang / 5'overhang	G AGCTT CCTAG A

Figure S3

A


dA-dT - + +
KU55933 - - +
NU7026 - - -
pSMC1S957
pATMS1981
H2B

B

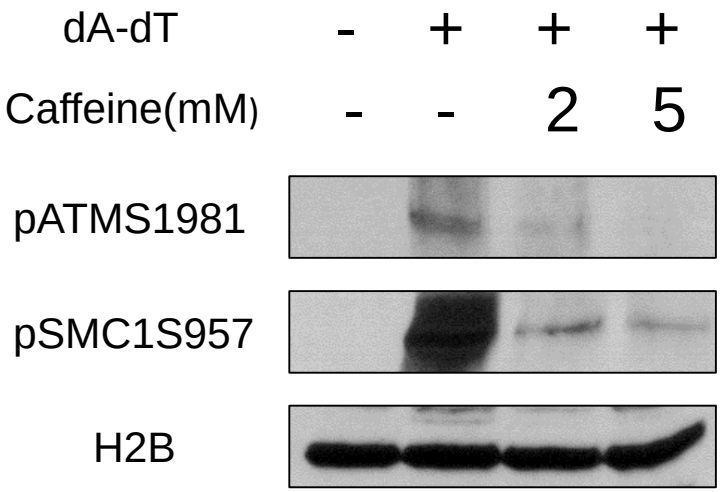

dA-dT - + + +
Caffeine(mM) - - 2 5
pATMS1981
pSMC1S957
H2B

Figure S4

A

StuI (AGG_^CCT) + KpnI (G_GTAC^C) Blunt + 3′overhang

```
5′ ATTCAA AGG
3′ TAAGTT TCC

   C AAGCTT 3′
CATGG TTCGAA 5′
```

		Number	Overall frequency
Control	ATTCAA**AGGGTACC**AAGCTT	10	100.00% **accurate**
	TAAGTT**TCCCATGG**TTCGAA		
+DNA-PK	ATTCAA**AGGGTACC**AAGCTT	10	100.00% **accurate**
Inhibitor	TAAGTT**TCCCATGG**TTCGAA		

B

StuI (AGG_^CCT) + HindIII (A^AGCT_T) Blunt + 5′overhang

```
5′ ATTCAA AGG
3′ TAAGTT TCC

AGCTT GGCACT 3′
   A CCGTGA 5′
```

		Number	Overall frequency
Control	ATTCAA**AGGAGCTT**GGCACT	9	90.00% **accurate**
	TAAGTT**TCCTCGAA**CCGTGA		
	ATTCAA**AGGΔΔCTT**GGCACT	1	10.00% **deletion**
	TAAGTT**TCCΔΔGAA**CCGTGA		
+DNA-PK	ATTCAA**AGGΔΔΔTT**GGCACT	8	88.89% **accurate**
Inhibitor	TAAGTT**TCCTCGAA**CCGTGA		
	ATTCAA**AGGΔΔΔTT**GGCACT	1	11.11% **deletion**
	TAAGTT**TCCΔΔΔAA**CCGTGA		

C

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

```
5′ TGCAGT C
3′ ACGTCA GAGCT

   C AAGCTT 3′
CATGG TTCGAA 5′
```

		Number	Overall frequency
Control	TGCAGT**CTCGAGTACC**AAGCTT	8	88.89% **accurate**
	ACGTCA**GAGCTCATGG**TTCGAA		
	TGCAGT**CTCGΔGTACC**AAGCTT	1	11.11% **deletion**
	ACGTCA**GAGCΔCATGG**TTCGAA		
+DNA-PK	TGCAGT**CTCGAGTACC**AAGCTT	11	100.00% **accurate**
Inhibitor	ACGTCA**GAGCTCATGG**TTCGAA		

D

PstI (C_TGCA^G) + KpnI (G_GTAC^C) 3′overhang + 3′overhang

```
5′ TAGAGC CTGCA
3′ ATCACG G

   C AAGCTT 3′
CATGG TTCGAA 5′
```

		Number	Overall frequency
Control	TAGAGC**CTGΔΔΔTACC**AAGCTT	7	63.64% **deletion**
	ATCACG**GACΔΔΔATGG**TTCGAA		
	TAGAGC**CTGCAΔΔΔCC**AAGCTT	3	27.27% **deletion**
	ATCACG**GACGTΔΔΔGG**TTCGAA		
+DNA-PK	TAGAGC**CTGΔΔΔTACC**AAGCTT	6	66.67% **deletion**
Inhibitor	ATCACG**GACΔΔΔATGG**TTCGAA		
	TAGAGC**CTGCAΔΔΔCC**AAGCTT	2	22.22% **deletion**
	ATCACG**GACGTΔΔΔGG**TTCGAA		
	TAGAGC**CTGCAΔΔΔΔC**AAGCTT	1	11.11% **deletion**
	ATCACG**GACGTΔΔΔΔG**TTCGAA		

E

BamHI (G^GATC_C) + HindIII (A^AGCT_T) 5′overhang + 5′overhang

```
5′ CGCCATG G
3′ GCGGTAC CCTAG

AGCTT GGCACT 3′
   A CCGTGA 5′
```

		Number	Overall frequency
Control	CGCCATG**GGATΔΔΔCTT**GGCACT	5	50.00% **deletion**
	GCGGTAC**CCTAΔΔΔGAA**CCGTGA		
	CGCCATG**GGAΔΔΔΔGCTT**GGCACT	3	30.00% **deletion**
	GCGGTAC**CCTΔΔΔCGAA**CCGTGA		
	CGCCATG**ΔΔΔΔΔΔGCTT**GGCACT	2	20.00% **deletion**
	GCGGTAC**ΔΔΔΔΔTCGAA**CCGTGA		
+DNA-PK	CGCCATG**GGATΔΔΔCTT**GGCACT	1	10.00% **deletion**
Inhibitor	GCGGTAC**CCTAΔΔΔGAA**CCGTGA		
	CGCCATG**GGAΔΔΔΔGCTT**GGCACT	1	10.00% **deletion**
	GCGGTAC**CCTΔΔΔCGAA**CCGTGA		
	CGCCATG**ΔΔΔΔΔΔGCTT**GGCACT	5	50.00% **deletion**
	GCGGTAC**ΔΔΔΔΔTCGAA**CCGTGA		
	CGCCATG**ΔΔΔΔΔΔΔCTT**GGCACT	3	30.00% **deletion**
	GCGGTAC**ΔΔΔΔΔΔΔGAA**CCGTGA		