SUPPLEMENTAL DATA FOR

THE DNA REPAIR PROTEIN XRCC1 FUNCTIONS IN THE PLANT DNA DEMETHYLATION PATHWAY BY STIMULATING 5-MEC EXCISION, GAP TAILORING AND DNA LIGATION

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Figure S1. Cell extracts from *xrcc1* $^{-/-}$ plants are not deficient in AP endonuclease activity. (A) Schematic diagram of molecules used as DNA substrates in the endonuclease assay. The 51-mer double-stranded oligonucleotides contained a synthetic AP site (THF) at position 29 of the labeled upper-strand. The alexa fluor-labeled 5´ end is denoted by a star. (B) DNA substrate (20 nM) was incubated with 35 μg of cell-free extract from WT, and *xrcc1* $^{-/-}$ mutant plants at 30ºC for 3 h. Reaction products were separated in a 12% denaturing polyacrylamide gel and detected by fluorescence scanning. The panel on the right shows the percentage of incision products from two independent experiments. Values are mean ± SE from two independent experiments.

Figure S2. Effect of anti-XRCC1 serum on mutant *xrcc1*$^{-/-}$ extracts. (A) Purified ROS1 (70 nM) was incubated at 30ºC for 8 h with a double-stranded oligonucleotide substrate (20 nM). Reaction products, were purified and incubated with 35 μg of cell-free extract from *xrcc1*$^{-/-}$ plants at 30ºC for 3 h in a reaction mixture that contained increasing amounts of anti-*Os*XRCC1 serum (0, 2.5 and 5 μl) and either no dNTPs or all four dNTPs, as indicated. Reactions were stopped, products were digested with *Hpa*II, and separated in a 12% denaturing polyacrylamide gel for detection with fluorescence scanning. (B) Percentage of fully-demethylated products. Values are mean ± SE from two independent experiments.

Figure S3. Effect of anti-Con7 serum on 3´P-end processing and ligation of DNA demethylation intermediates catalyzed by cell extracts. (A) Analysis of DNA incision products generated on the upper strand during DNA demethylation. Purified ROS1 (70 nM) was incubated at 30ºC for 8 h with a 51-mer double-stranded oligonucleotide substrate (20 nM) containing a single 5-meC:G pair. Reaction products, which contained a mixture of β- and β,δ-elimination products (lane 1), were purified and incubated with 35 μg of cell-free extract from WT plants at 30ºC for 3 h in a reaction mixture that contained increasing amounts of anti-Con7 serum (0, 2.5, 5 and 7 μl) and all four dNTPs, except when indicated. Reactions were stopped and products were separated in a 12% denaturing polyacrylamide gel and detected by fluorescence scanning. The lower panel shows the percentage of 3´-P not converted into 3´-OH in two independent experiments. (B) DNA ligation assay. DNA duplexes containing a nick in the upper, 5´-alexa fluor labeled strand were incubated with 35 μg of extract from WT plants at 30ºC for 3 h in a reaction mixture supplemented with increasing amounts of anti-Con7 serum (0, 2.5, 5 and 7 μl) and all four dNTPs, except when indicated. Reaction products were separated using a 12% denaturing polyacrylamide gel and detected by fluorescence scanning. The lower panel shows the percentage of fully-ligated products. Values are mean ± SE from two independent experiments.

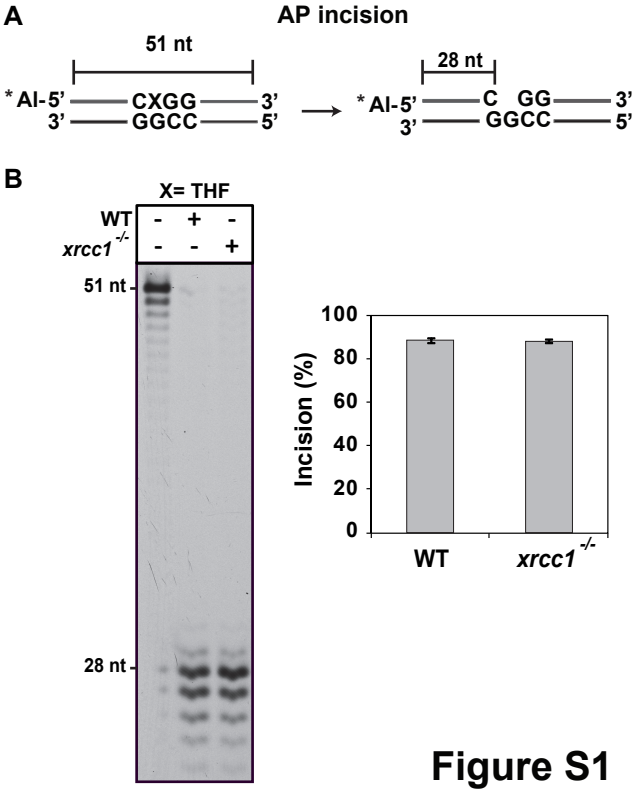
A
AP incision
51 nt
28 nt
*Al-5'
CXGG
3'
3'
GGCC
5'
*Al-5'
C
GG
3'
3'
GGCC
5'
B
X= THF
WT
xrcc1 -/-
- + -
- - +
51 nt
28 nt
Incision (%)
100
80
60
40
20
0
WT
xrcc1 -/-

Figure S1

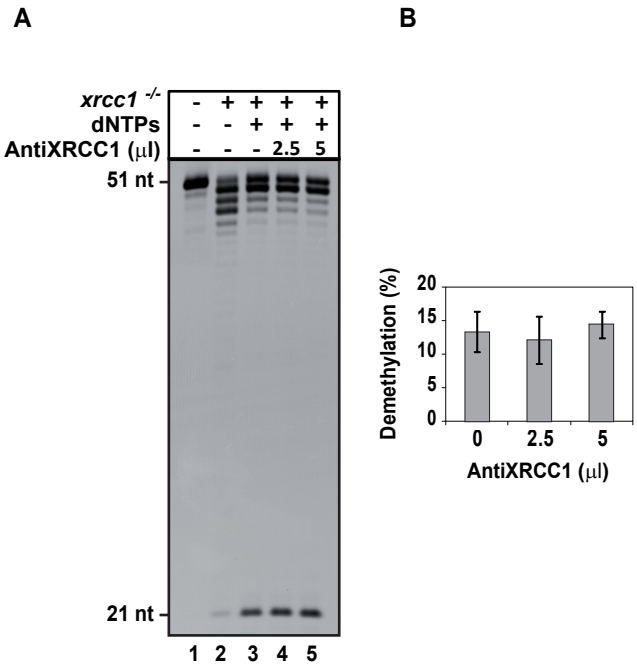
A
B
xrcc1 -/-
dNTPs
AntiXRCC1 (μl)
- + + + +
- - + + +
- - - 2.5 5
51 nt
21 nt
1 2 3 4 5
Demethylation (%)
20
15
10
5
0
0 2.5 5
AntiXRCC1 (μl)

Figure S2

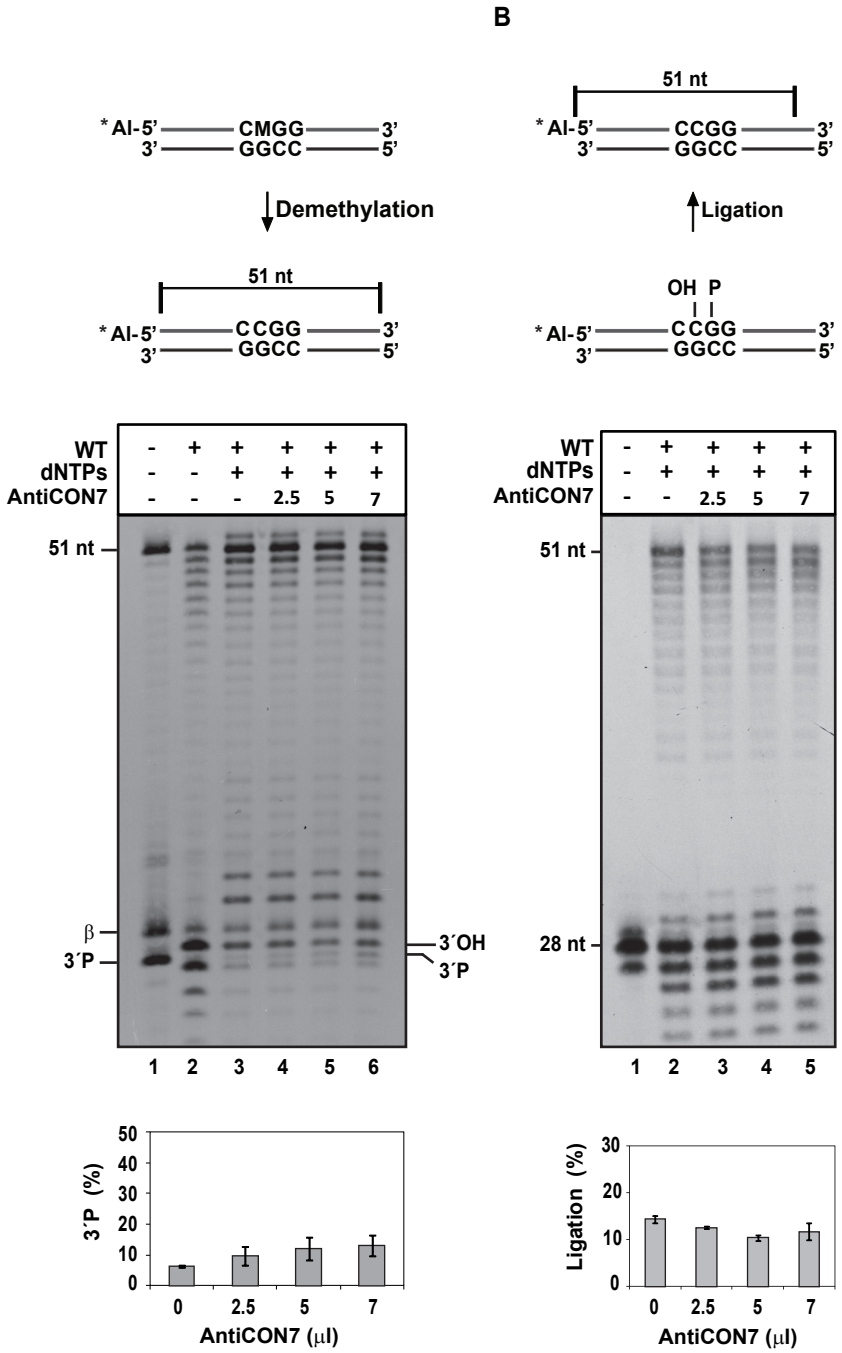
A
B
*Al-5' CMGG 3'
3' GGCC 5'
Demethylation
51 nt
*Al-5' CCGG 3'
3' GGCC 5'
Ligation
OH P
WT - + + + + +
dNTPs - - + + + +
AntiCON7 - - - 2.5 5 7
51 nt
β
3´P
3´OH
3´P
1 2 3 4 5 6
28 nt
1 2 3 4 5
3´P (%)
AntiCON7 (μl)
Ligation (%)
AntiCON7 (μl)

Figure S3