SUPPLEMENTARY INFORMATIONS

The block of DNA polymerase δ strand displacement activity by an abasic site can be rescued by the concerted action of DNA polymerase β and flap endonuclease 1.

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Supplementary Experimental Procedures

Chemicals
Deoxynucleotides were purchased from GeneSpin (Milan, Italy). Labelled γ(^{32}P) ATP was purchased from GE Healthcare. Aphidicolin was purchased from Sigma. All the other reagents were of analytical grade and purchased from Merck or Fluka.

Oligonucleotide DNA substrates sequences
Template sequence (100mer):
5'TAAGGTAGTAGTATTATAAATTATGGAGGTTTTXAGTGGGAAATAAAATATAGTAAAGATTATTAGG
ATTTGAAATTATGTAATTGAAAGTAAATGTAGT-3'

Primers sequences

(29-mer): 5'-ACTACATTTACTTTCAATTACATAATTTC-3'

(30-mer): 5'-AATAATCTTTACTATATTTTATTTCCCACT-3'

(31-mer): 5'-AATAATCTTTACTATATTTTATTTCCCACT**A**-3'

Terminators sequences:

(33-mer): 5'-AAAACCTCCATAATTTATAATACTACTACCTTA-3'

(34-mer): 5'-**C**AAAACCTCCATAATTTATAATACTACTACCTTA-3'

(36-mer): 5'-**C**TCAAAACCTCCATAATTTATAATACTACTACCTTA-3'

Annealing positions:

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36-mer 3' ...TCCAAAACTC
34-mer 3' ...TCCAAAAC
33-mer 3' ...TCCAAAA
30-mer               TCACCCTTTATTTTATATCATTTCTAATAA5'
31-mer              ATCACCCTTTATTTTATATCATTTCTAATAA5'
29-mer                                              CTTTAA...5'
100-mer 5'...AGGTTTTXAGTGGGAAATAAAATATAGTAAAGATTATTAGGATTTGAAATT...3'
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X = AP site (THF) or G
bold = bases paired to AP site
underlined = template strand

Supplementary Figure Legends

Figure S1. PCNA and Fen-1 are required for efficient abasic site bypass by DNA pol β in the presence of salt. Reactions were performed as described in Materials and Methods. 30 nM human recombinant DNA pol β were tested on the 38 nt Gap AP template in the absence (lanes 1-4) or in the presence (Lanes 5-8) of 100 nM PCNA and 3 nM (Lanes 1, 2, 5, 6) or 1.5 nM (Lanes 3, 4, 7, 8) of human recombinant Fen-1, either in the absence (Lanes 1, 3, 5, 7) or in the presence (Lanes 2, 4, 6, 8) of 75 mM NaCl. Lane 9: mock reaction in the absence of DNA pol β.

Figure S2. DNA polymerase λ is less efficient in Fen-1 dependent abasic site bypass than DNA polymerase β. Reactions were performed as described in Materials and Methods. **A**. Human recombinant DNA pol λ was titrated in the presence of linear control (Lanes 1-3), linear AP (Lanes 4-6), 38 nt Gap control (Lanes 7-9) or 30 nt Gap AP (Lanes 10-12) templates. **B**. 100 nM DNA pol λ were tested on 38 nt Gap control (Lanes 1-4; 8-11) or 38 nt Gap AP (Lanes 5-7; 12-15) templates in the presence of RP-A alone (Lanes 1, 5), RP-A and PCNA (lanes 8, 12), RP-A and Fen-1 (Lanes 2-4; 6,7), RP-A, PCNA and Fen-1 (Lanes 9-11; 13-15).

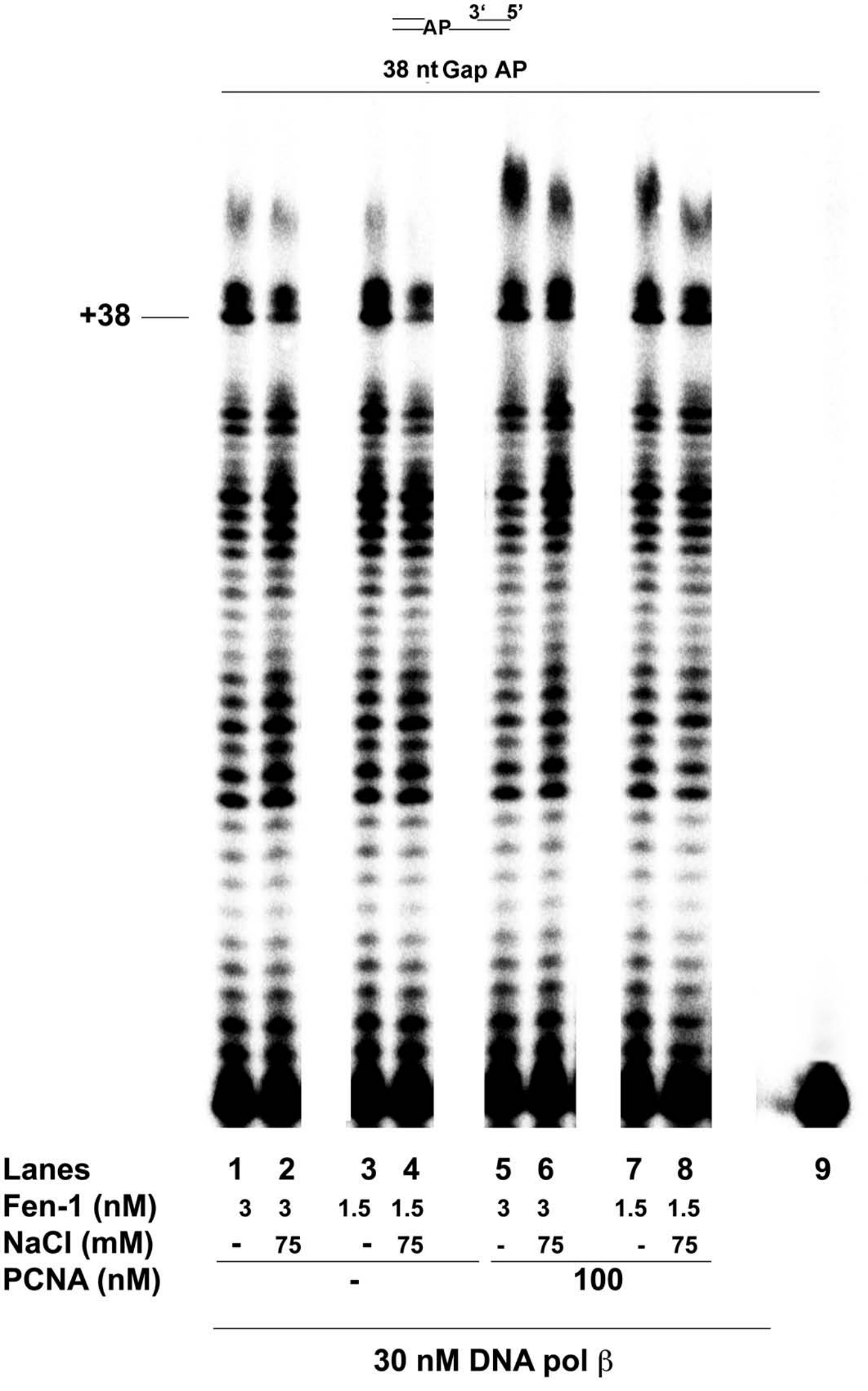
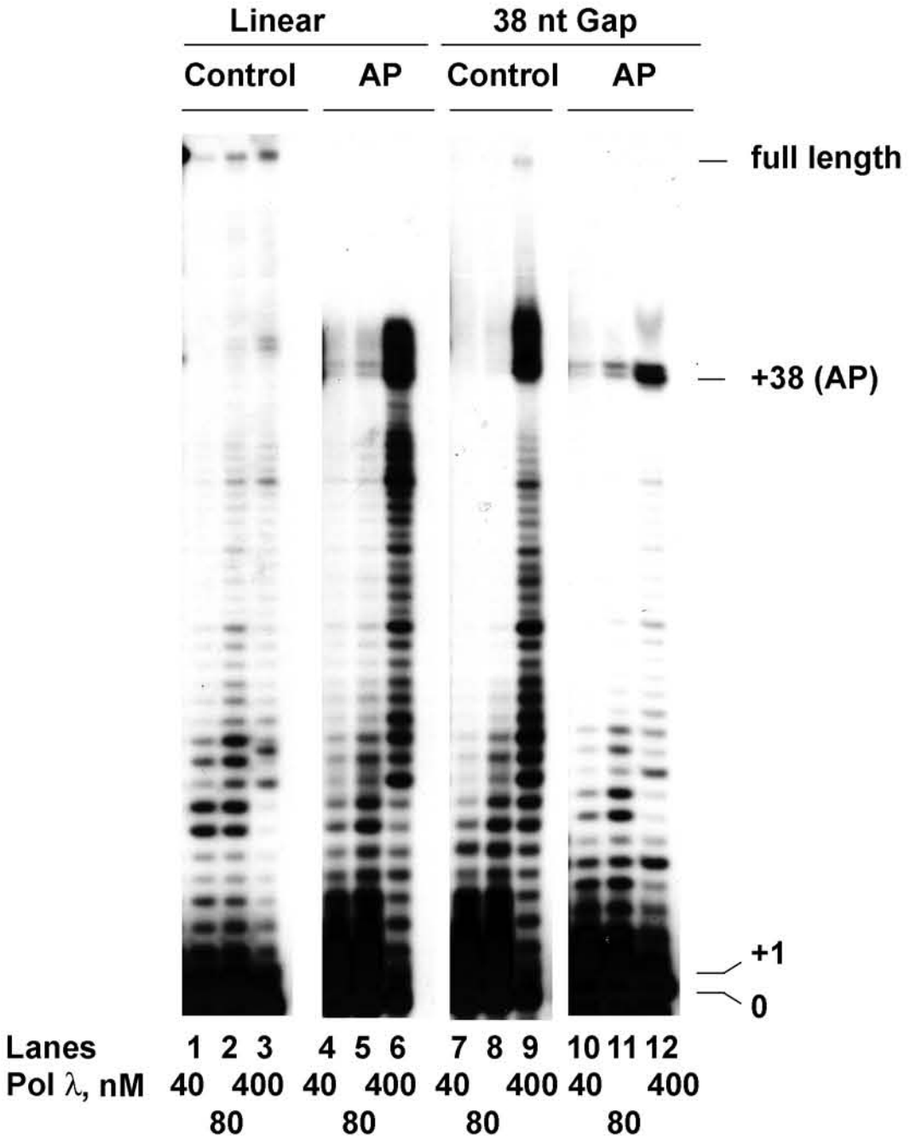

3' 5'
AP
38 nt Gap AP
+38
Lanes 1 2 3 4 5 6 7 8 9
Fen-1 (nM) 3 3 1.5 1.5 3 3 1.5 1.5
NaCl (mM) - 75 - 75 - 75 - 75
PCNA (nM) - 100
30 nM DNA pol β

Figure S1

A


Linear
38 nt Gap
Control
AP
Control
AP
full length
+38 (AP)
+1
0
Lanes 1 2 3 4 5 6 7 8 9 10 11 12
Pol λ, nM 40 400 40 400 40 400 40 400
80 80 80 80

B

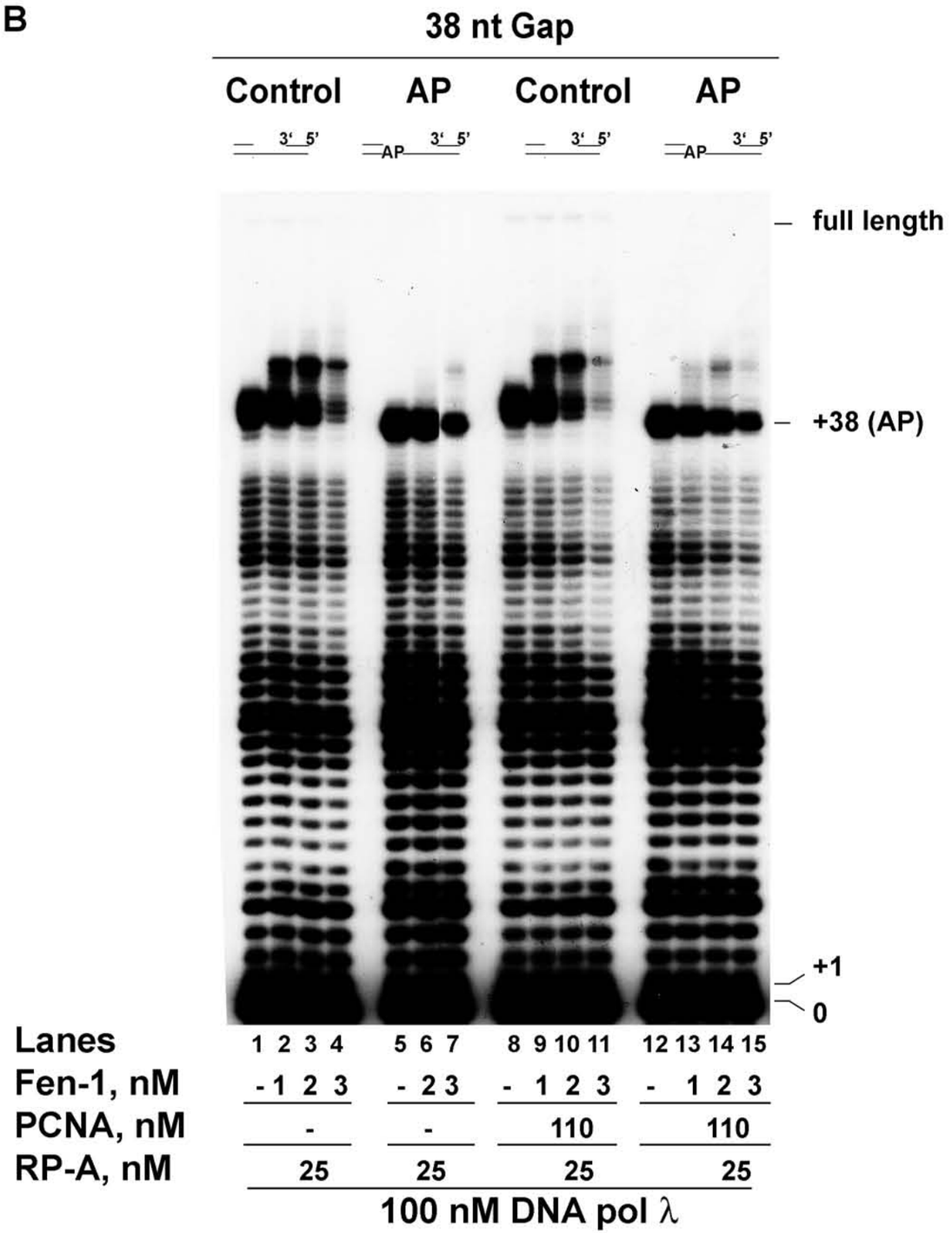

38 nt Gap
Control
AP
Control
AP
3' 5'
AP
full length
+38 (AP)
+1
0
Lanes 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Fen-1, nM - 1 2 3 - 2 3 - 1 2 3 - 1 2 3
PCNA, nM - - 110 110
RP-A, nM 25 25 25 25
100 nM DNA pol λ

Figure S2