

Supplementary Information

The mesophilic archaeon *Methanosarcina acetivorans* counteracts uracil in DNA with multiple enzymes: EndoQ, ExoIII, and UDG

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Table of Contents

TableS1	-----	S-1
Table S2	-----	S-2
Table S3	-----	S-3
Figure S1	-----	S-4
Figure S2	-----	S-5
Figure S3	-----	S-6
Figure S4	-----	S-7
Figure S5	-----	S-8
Figure S6	-----	S-9
Figure S7	-----	S-10
Figure S8	-----	S-11
Figure S9	-----	S-12,13
Figure S10	-----	S-14,15
Figure S11	-----	S-16

Supplementary Table S1. Oligonucleotides used for gene cloning and RT-PCR.

Name	Sequence (5'-3')	Target gene (Locus tag)	Usage	PCR products (bp)
MA_RS10790-F	GGGCATATGGCCGGAAAAATTAAACCTTA			
MA_RS10790-R	CCCGCGGCCCGCTCAAACCTGAAATTCGAGTTC	MacExoIII (MA_RS10790)		
MA_RS10790-E39A	TCGATGTCACTGTGTCTCCAGGCAACCAAGCCTCCCCGGAAAA			
MA_RS03380-F	CGCCATAATGAAAGTCAATGCAGACCTCCATC			
MA_RS03380-R	CCGCGGCCCGCTTAAAAAATCGAAAAAGGATTTCTGCCC	MacEndoQ (MA_RS03380)	Gene cloning	
MA_RS03380-D192A	CTTACCTTCCCTTACAAACTCCGCTGCCCAATCCCTTACACCAAC			
MA_RS11760-F	CGCCATAATGAAAGCCTGTTGAATACGTTAAATG	MacUDGlike (MA_RS11760)		
MA_RS11760-R	CCCGCGGCCCGCTTACTTAAATTAACCTTCGCAGCTTC			
MA_RS18745-F	GGGCATATGAAAGGAGCTAGAAAAATTGTGGC			
MA_RS18745-R	GCGCGGCCCGCTTACTGCCATAGATCCCTGTTT	MacUDG (MA_RS18745)		
MA_RS10790-RT-F	TGGGAAATCGAGGAGTTTGAC	MacExoIII (MA_RS10790)		101
MA_RS10790-RT-R	CTGAGAGGCTTTTCCGTTTG			
MA_RS03380-RT-F	CAGCGAATATGCAGACAGGA	MacEndoQ (MA_RS03380)		98
MA_RS03380-RT-R	ATTCCTTGGCCAGTTTGTG			
MA_RS02415-RT-F	CGATTTCTGGAGGCTAGTCTG			
MA_RS02415-RT-R	GGCTTAAACACGTCCACA	MacHDG (MA_RS02415)		118
MA_RS11760-RT-F	TCTGGATCACGGGTTCTAC			
MA_RS11760-RT-R	ACCGTATCTGCCGAAATCAC	MacUDGlike (MA_RS11760)	RT-PCR	76
MA_RS18745-RT-F	AGAAAGCCAAAAAGCAAGTGA			
MA_RS18745-RT-R	ACTCCCGTTCCAGTTTCT	MacUDG (MA_RS18745)		143
MA_RS04665-RT-F	GGGTC'AAAAGGGTCCGTAGC			
MA_RS04665-RT-R	AGTATCCCCCGAAAAGCCTAA	Mac16SrDNA (MA_RS04665)		80
MA_RS17460-RT-F	CTTGACTTCGTACGGGTGGT			
MA_RS17460-RT-R	TGATCGGGCCTTCTTTATG	MacGAPDH (MA_RS17460)		148

Supplementary Table S2. Oligonucleotides used for protein activity assays

#	Name	Sequence (5'-3')	Description
1	23N	CGAACTGCC'TGGAA'TCCTGACGA	DNA marker
2	24N	CGAACTGCC'TGGAA'TCCTGACGAC	DNA marker
3	24N-3Phos	CGAACTGCC'TGGAA'TCCTGACGAC-Pho	3'-phosphate ssDNA
4	32N	CGAACTGCC'TGGAA'TCCTGACGACATGTAGCG	
5	45N	CGAACTGCC'TGGAA'TCCTGACGACATGTAGCGAACGATCACCTCA	ssDNA (normal)
6	45-U24	CGAACTGCC'TGGAA'TCCTGACGAC[dU]ATGTAGCGAACGATCACCTCA	dU-ssDNA
7	45-I25	CGAACTGCC'TGGAA'TCCTGACGAC[I]TGTAGCGAACGATCACCTCA	dI-ssDNA
8	45-AP25	CGAACTGCC'TGGAA'TCCTGACGAC[AP]TGTAGCGAACGATCACCTCA	AP-ssDNA
9	45-G25	CGAACTGCC'TGGAA'TCCTGACGAC[G]TGTAGCGAACGATCACCTCA	
10	45-X27	CGAACTGCC'TGGAA'TCCTGACGAC[X]TGTAGCGAACGATCACCTCA	dX-ssDNA
11	21N+5Phos	5-Phos-ATGTAGCGAACGATCACCTCA	
12	32R	CGCTACATGTCAGGATTCAGGCAGTTTCG	
13	45R	TGAGGTGATCGTTCCGTACATGTCGTCAGGATTCAGGCAGTTTCG	Complementary oligo of 45N for blunt-ended dsDNA
14	45R-3-overhang	GATCGTTCCGTACATGTCGTCAGGATTCAGGCAGTTTCGCTAGCC	Complementary oligo of 45N for 6 nt-3'-overhang dsDNA

Supplementary Table S3. Preparation for dsDNA.

Name	Combination*
blunt-ended dsDNA (normal) or dA/dT	5 + 13
blunt-ended dU-dsDNA or dU/dG	6 + 13
blunt-ended dI-dsDNA or dI/dT	7 + 13
blunt-ended AP-dsDNA or AP/dT	8 + 13
blunt-ended G/T mismatched DNA or dG/dT	9 + 13
blunt-ended X-dsDNA or dX/dT	10 + 13
3'-recessed dsDNA	4 + 13
5'-protruding dsDNA	4 + 12
nicked dsDNA	2 + 11 + 14
3'-phosphate dsDNA	3 + 14
6 nt 3'-overhang dsDNA (normal)	5 + 14
6 nt 3'-overhang dU-dsDNA	6 + 14
6 nt 3'-overhang dI-dsDNA	7 + 14
6 nt 3'-overhang AP-dsDNA	8 + 14

*The numbers on the combination column correspond to those in Supplementary table S2

MacExoIII (Mac20771)	NTMBA 06610	Meth212	MM 3148	AF8080	Mbu 2145	Mmp1012	MS2 0121	S020290	Str1910	Tal506m	TVN046	M2 0121	MCF 1129	Mhu 0441	Sac1 0129	Fac1 0500005205	RC1X1223	Cma 1739	Msed 1434	M3acC 0582	M3evan 0339	M3em 1479	M3macC 0254	M3abo 0623	M3mco 0350	M3mo 2415	M3mah 0334	Vda 1949	Ferp 0776	Mhu 1115	M3nu 1557	M3met 1338	MSMAN 0192	M3etb 2449	Sire 0100	Ahos 1812	Ahos 1812	M3wut 0363	M3con 0422	M3cup 0795	AFE1 (human)	Xth (E. coli)													

Supplementary Figure S1. Amino acid sequence alignment of ExoIII homologs. The 46 full-length amino acid sequences of archaeal homologs were aligned with human APE1, and *E. coli* Xth. The sequences from *E. coli*, *M. thermautotrophicus*, and *H. sapiens* are indicated with bold letters. MacExoIII are indicated with red letters. Catalytic residues are indicated with ▼ at the top. The putative residues in Meth212 are indicated with cyan letters and their positions are indicated by asterisks at the bottom. ▲ indicates the mutated glutamic acid residue in this study (E39 in MacExoIII).

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MacUDG_MA_RS18745      MKELEIVAAEEENCNGFEERVQKLV EAGYETVAREIACTRCPLHKSATKRVIKGGSCNP
MacUDGlike_MA_RS11760  MKPVEVVKCKAFPCSDINKGGYLVPAVEVNPKEVK
Sulfolobus solfataricus MDNLDLIADEVKRCQKCKLWKFRKNAVPGEGNSKA
Sulfolobus tokodaii    MDSLEIKIKEEIVISCKKCKLWQFRTNVAVPGEGYPKA
Pyrococcus furiosus    MSKHLMKKLEEKILTCKKCPWLRLRTNVPVPGYGNDA
Archaeoglobus fulgidus MESLDDIVREIMSCRKCDLHKTKTNYVPGVNEKA
Aeropyrum pernix       MAGSRLRQLEEEVRRCTRCP LHATRTHAVPGEGGPEA
Pyrobaculum aerophilum MDLQKLHELKNCCKPLHKYRKNVAVPGEGEMKL
Thermus thermophilus   MTLELLQAQAQONCTACRLMEGRTRVVFEGGNPDA
Deinococcus radiodurans MTGSLPPRRHPLHQADPPDAALLALEDRNRGCAACPLRVASQVVDGDPDA
Thermotoga maritima    MYTREELMIEVSRVKKCTACPLHLNRTNVVVGEGNLD

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Motif A

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MacUDG_MA_RS18745      KVMFI▼GEAPGKTED---ETGIPFNG---RAGKKLDKMVEYMG-LSEEDWVAVNTVKCRP
MacUDGlike_MA_RS11760 VVMIAEAPPENPLDYFYASGEPFYLKTTLQAFNDAGIPVNSIQEILDHGTYLTALKCAK
Sulfolobus solfataricus EIMFI▼GEAPGENED---IEGRPFVVG---VAGKLLTRLINIEILGLSREDVFIITNLVKCRP
Sulfolobus tokodaii    EIMFV▼GEAPGENED---KEGRPFVVG---AAGKLLTQMIKEIILGLERDQVFITNVVKCRP
Pyrococcus furiosus    KIMFI▼GEAPGYWED---QKGLPFVVG---KAGKVLDELLEDGIG-LTREDVYITNVVKCRP
Archaeoglobus fulgidus EIVFV▼GEAPGRDED---LKGEPFVVG---AAGKLLTEMLASIG-LRREDVYITNVVKCRP
Aeropyrum pernix       GVMVVG▼GEAPGRMED---RLGRPFVVG---PAGKLLDLSLELAG-LSRGEVYITNVVKCRP
Pyrobaculum aerophilum GVMIV▼GEAPGASED---EAGRPFVVG---AAGQLLTEALSRLG-VRRGDVFIITNVVKCRP
Thermus thermophilus   KLMIV▼GEGPGEED---KTGRPFVVG---KAGQLLNRILEAAG-IPREEVYITNVVKCRP
Deinococcus radiodurans PLLIV▼GEGPQAEED---RDGRPFVVG---QAGQLLDRILAAAS-LAREEAYLTNVVKCRA
Thermotoga maritima    RIVFV▼GEGPGEED---KTGRPFVVG---RAGMLLTELLEESG-IRREDVYICNVVKCRP

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MacUDG_MA_RS18745      PENRRPKASEIEECK-PFLIAQITLLDPEIIILLGNTAEKAYCPEKRLERGVPEVEYGR-
MacUDGlike_MA_RS11760 TR-YVISADTVKNCSLVLEKELSLFPNVEVYMLMGDVAIKAFNYISRRLTGKNTIRSGST
Sulfolobus solfataricus PNNRDPEEDEILACS-PYLTRQIESIRPHIIITLGRHSTYLFKMMKMMESIGKVRGRKF
Sulfolobus tokodaii    PNNRDPEEDEITACS-PYLDQRIDIIMPKIIVTLGRHSTKYIFSKMGENFSSITKVRGKS
Pyrococcus furiosus    PNNRDPTEEIEKACS-PYLDQQIDI IKPKVIVTLGRHSTNYILKKGFDLEPIISKIHGKV
Archaeoglobus fulgidus PNNRDPTPEEVEKCG-DYLVQRLEAIRPNVIVCLGRFAAQFIFNLFDFLEFTTISRVKGV
Aeropyrum pernix       PGNRDPREEIEACL-PYLVEQISLIRPRLVIAVGRHAGRTLFRLAGLRWPLARARGRV
Pyrobaculum aerophilum PNNRTPNREEVEACL-PYLIQQIGILKPRRIIALGLISAKALMELMGRRAEKLGDKVKGKC
Thermus thermophilus   PQNRALPLDEAKICTDKWLLKQIELIAPQIIVPLGVAEAFFFLG---EKVSI▼TKVRGKW
Deinococcus radiodurans PNNRTPLEATCTGLWLEPQLALLRPRVLSLGNATATQFLLG---TPRGI▼TRLRGQW
Thermotoga maritima    PNNRTPPEEQAACG-HFLLAQIEIINPDVIVALGATALSFFVDG---KKVSI▼TKVRGNP
MacUDG_MA3593          PENRRPKASEIEECK-PFLIAQITLLDPEIIILLGNTAEKAYCPEKRLERGVPEVEYGR-
MacUDGlike_MA2265     TR-YVISADTVKNCSLVLEKELSLFPNVEVYMLMGDVAIKAFNYISRRLTGKNTIRSGST

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Motif B

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MacUDG_MA_RS18745      -----MILKLYHPAAALTYTASKIDVQKAFIDKNRDLWQ
MacUDGlike_MA_RS11760 YKIRKEQ---FFYNGKRVFSPYVCTGQNYLIEKSKRMMIAEDLAEAAKLIK
Sulfolobus solfataricus YTWNIYG--YKILVFPTYHPAAALYNPPIRKVLLEDFRKEALSS-KPITLDN▼FLYG--
Sulfolobus tokodaii    YVWKYKE--KEIIVFPTYHPAAALYNPNLRKI▼LEEDFKKIRELAITPKRYTIDYFLGGKN
Pyrococcus furiosus    FKA▼KTLE--GTYIFPTYHPAAALYR▼PQLKEELKQDFDILKSLLEKGLI
Archaeoglobus fulgidus YEVE▼RWG--KKVKVIAIYHPAAAVLYR▼PQLREEYESDFKKIGELCGKKQPTLFDY▼L
Aeropyrum pernix       WRGRIGG--VELLI▼AVTYHPAAALYNPGLRGELE▼RDPSGFI▼RRSVAEALS▼RGGGGGGAGL▼
Pyrobaculum aerophilum YQGR▼IAG--VQVELCITYHPAAAVIRK▼PALRGEFQKDLAMFFG-----GGL▼
Thermus thermophilus   YEWHG-----IKVFPMEHPAYLLRN▼PSRAPGSPKHL▼TWLDIQEVKRALDALPPKERRPV▼
Deinococcus radiodurans FTYRHPAWPQ▼PALLMPLLHPAYLLRN▼PVRTPGGPKSL▼TWRDIREVA▼AVL▼RGEKEAS▼SPVQ▼G
Thermotoga maritima    IDWLGG-----KKVIPT▼FHP▼SYLLRN▼RSNE---LRRIVLE▼DI▼EKAKS▼FIK▼KEG

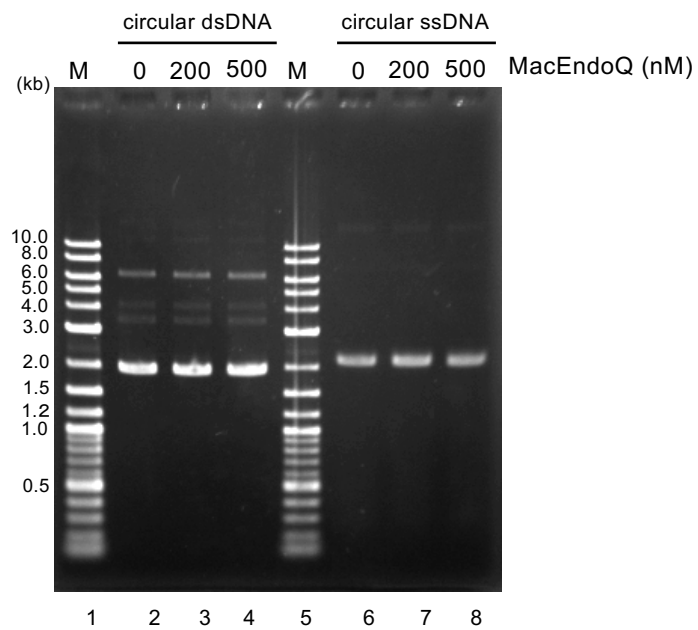
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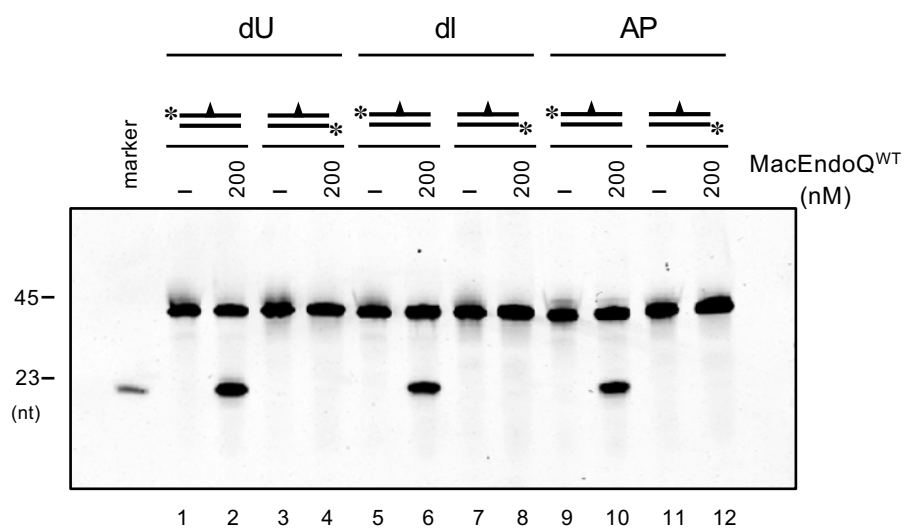
MacUDG_MA_RS18745      -SGDKGEKGSNSNGK
MacUDGlike_MA_RS11760 RSWDKREKSDSNSGK
Sulfolobus solfataricus DRWFSPDSRGPGEAGGDVDS
Sulfolobus tokodaii    DRELDPSK
Pyrococcus furiosus   KAVSQEPLF
Archaeoglobus fulgidus QFPAAPDSLFSELE
Aeropyrum pernix
Pyrobaculum aerophilum
Thermus thermophilus
Deinococcus radiodurans
Thermotoga maritima

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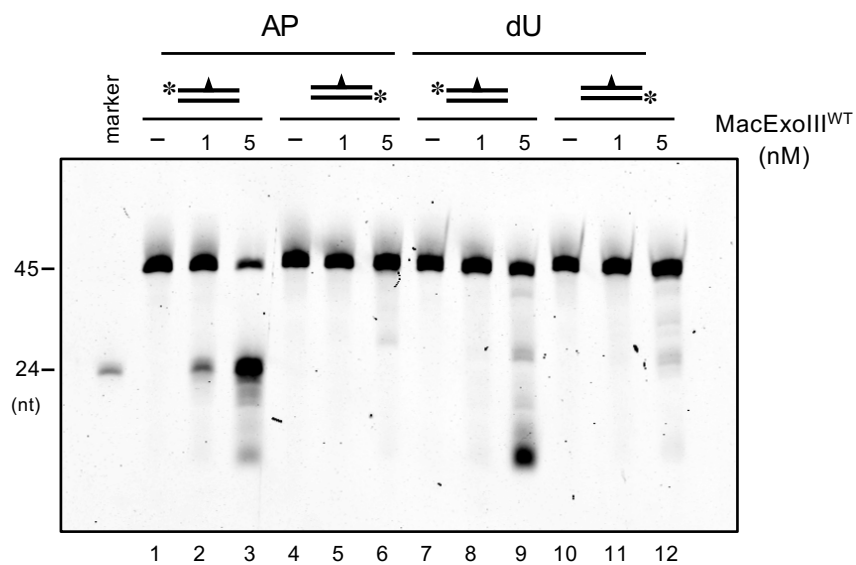
Supplementary Figure S2. Amino acid sequence alignment of the characterized family-4 UDG proteins, MacUDG, and MacUDG-like (MA_RS18745 and MA_RS11760). Motif A: GE(A/G)PG, Motif B: HPAAVL, Underlined peptides: (putative or characterized) PIP box/ β -clamp motif, \blacktriangledown : Cysteine residues involved in Fe-S clusters. Accession numbers: *Sulfolobus solfataricus*, WP_009991844.1; *Sulfolobus tokodaii*, WP_010980322.1; *Pyrococcus furiosus*, WP_011012532.1; *Archaeoglobus fulgidus*, WP_010879766.1; *Aeropyrum pernix*, BAA79385.2; *Pyrobaculum aerophilum*, AAL62921.1; *Thermus thermophilus*, WP_011228142.1; *Deinococcus radiodurans*, WP_010888386.1; *Thermotoga maritima*, WP_004081422.1.



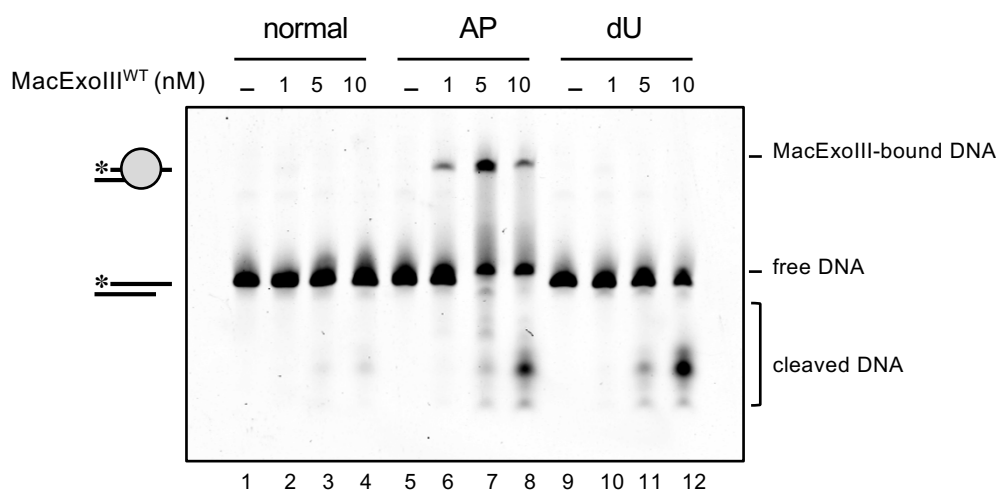
Supplementary Figure S3. Quality check of MacEndoQ sample. MacEndoQ (200 nM and 500 nM) was incubated with 20 ng/ μ l of supercoiled pBlueScript II SK(+) dsDNA (lanes 2–4) and pBlueScript II SK(+) ssDNA (lanes 6–8) at 37° C for 60 min in the reaction solution. Reaction products were analyzed by 0.8% agarose gel electrophoresis, followed by ethidium bromide staining. M, DNA marker (NEB, #3200).



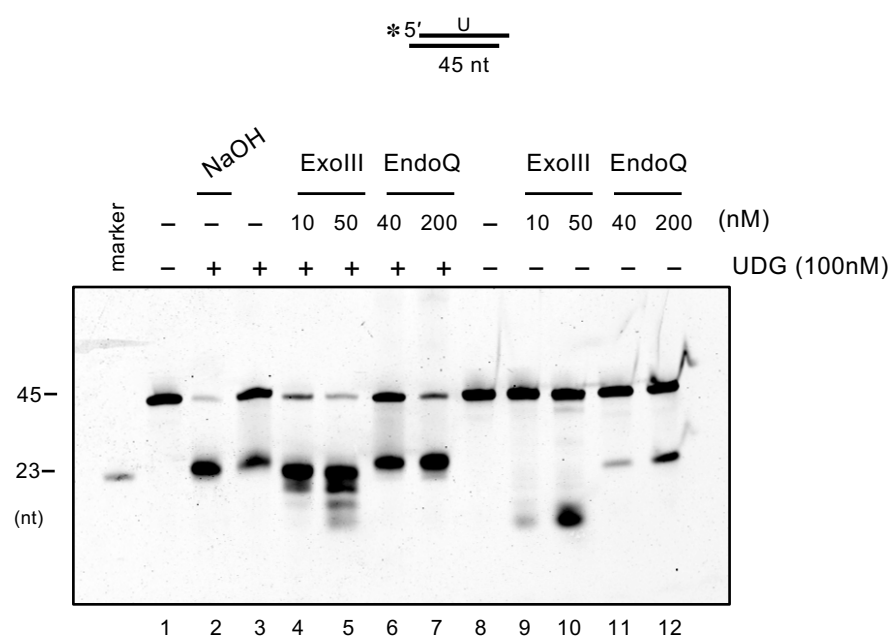
Supplementary Figure S4. Cleavage activity of MacEndoQ toward the opposite strand of a single lesion-containing strand. MacEndoQ cleavage reaction were performed at 37°C for 60 min using the single lesion-containing strand labeled DNA (lanes 1, 2, 5, 6, 9, 10) or the opposite (intact) strand labeled DNA (lanes 3, 4, 7, 8, 11, 12). For the detailed reaction conditions and the termination step, see the methods section in the main text. Cleavage products were separated by 8 M urea-12% PAGE.



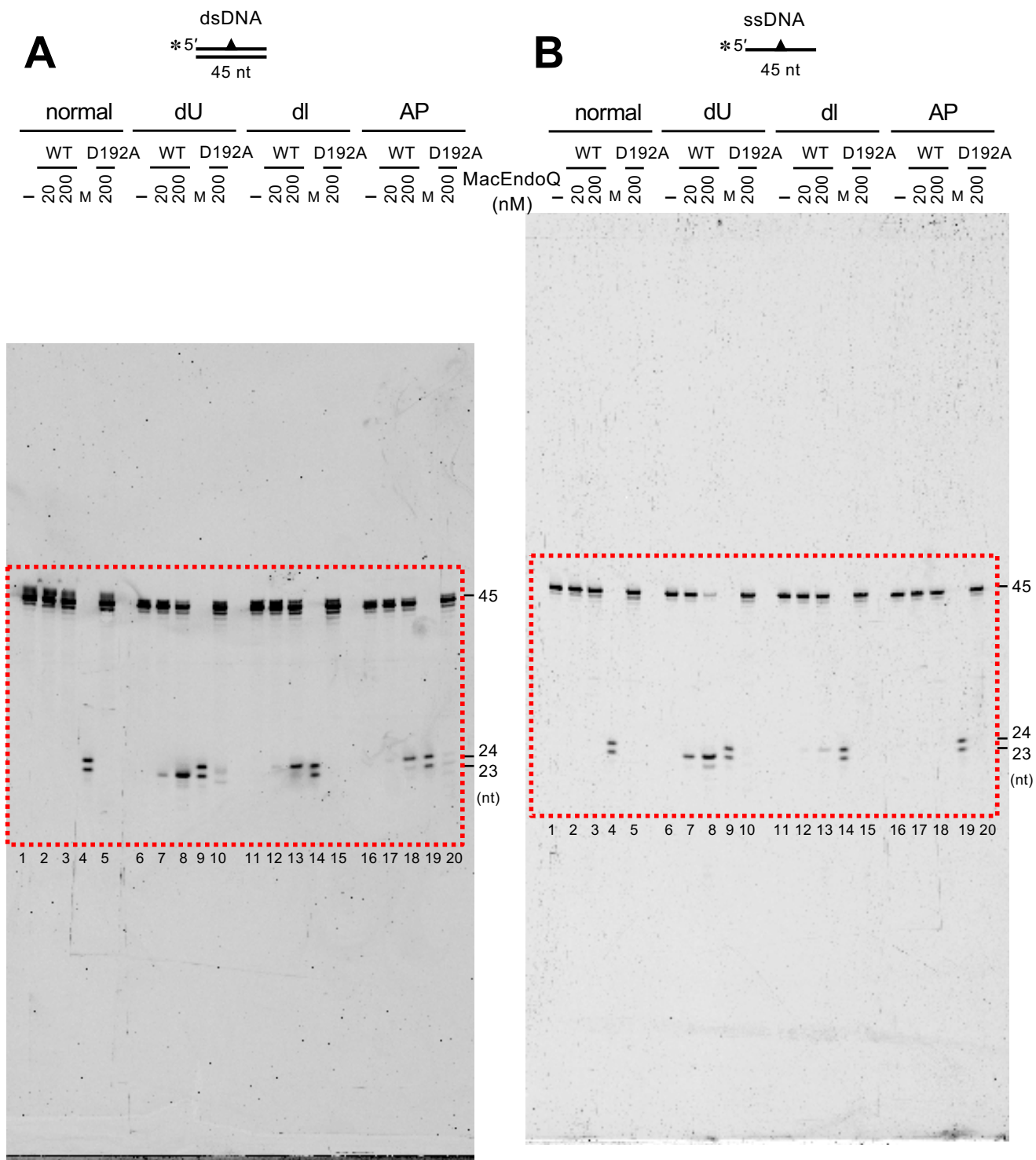
Supplementary Figure S5. Cleavage activity of MacExoIII toward the opposite strand of a single lesion-containing strand. MacExoIII cleavage reaction were performed at 37°C for 10 min using the (single lesion-containing) top strand labeled, blunt ended DNA (lanes 1–3, 7–9) or the bottom strand labeled, blunt ended DNA (lanes 4–6, 10–12). For the detailed reaction conditions and the termination step, see the methods section in the main text. Cleavage products were separated by 8 M urea-12% PAGE.



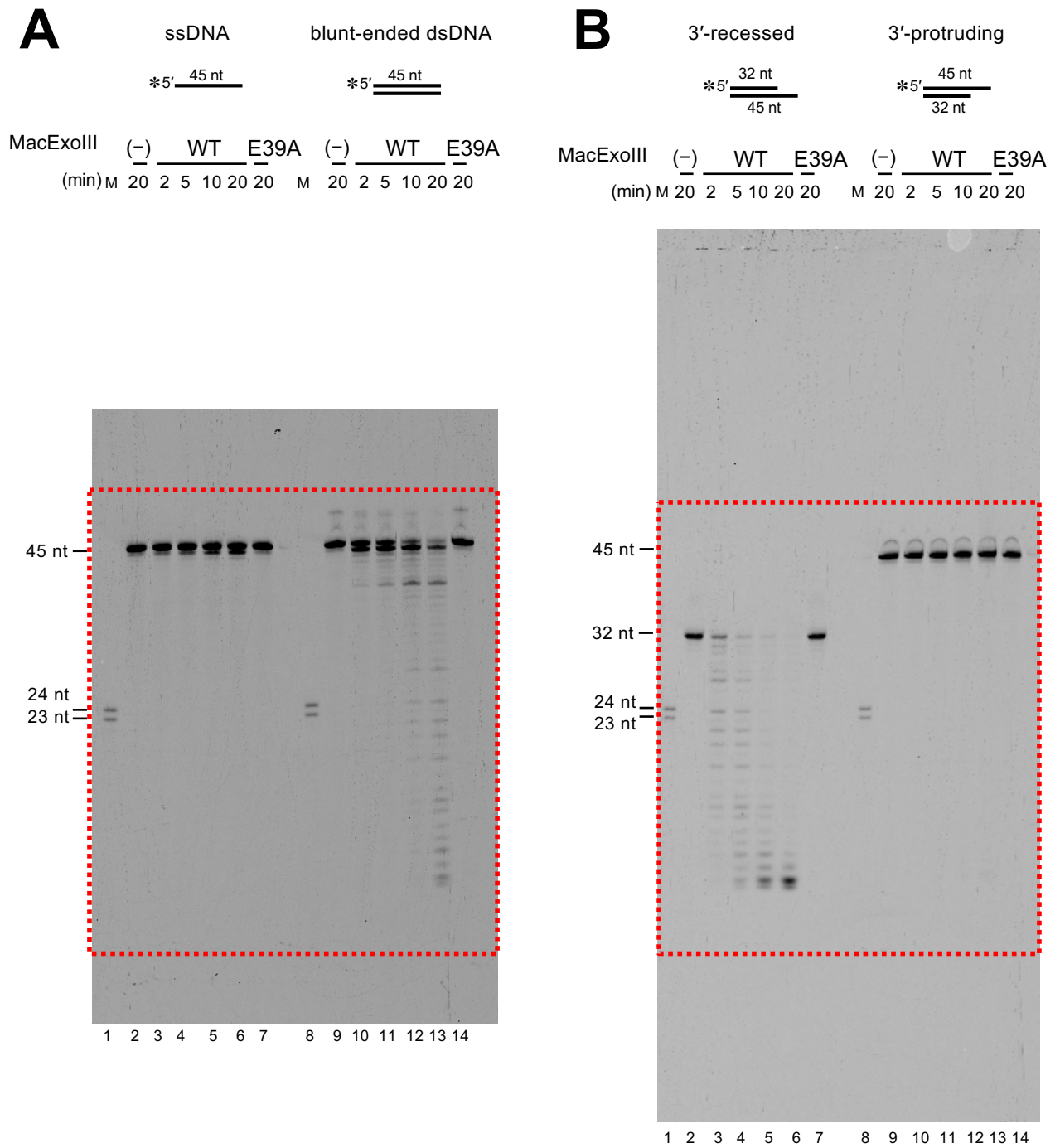
Supplementary Figure S6. Electrophoretic mobility shift assay of MacExoIII with normal/(AP/dU)-containing DNA. Various concentrations (0, 1, 5, and 10 nM) of MacExoIII^{WT} were incubated with 5 nM 5'-Cy5-labeled 6-nt 3'-overhang dsDNA (normal: lanes 1–4; AP site: lanes 5–8; dU: lanes 9–12), in reaction solution (50 mM Bis-Tris-HCl, pH 7.0, 1 mM DTT, 1 mM MnCl₂, 0.01% Tween 20; 20 μ l) at 37°C for 10 min. After 5 μ l of loading buffer (17% Ficoll, 10 mM Tris-HCl, pH 8.0 and 0.1% Orange G) was added, the samples were separated by 8% PAGE in TBE buffer and visualized by an image analyzer, Typhoon Trio+ (GE Healthcare). Assignments of the bands are shown on the left of the panel.



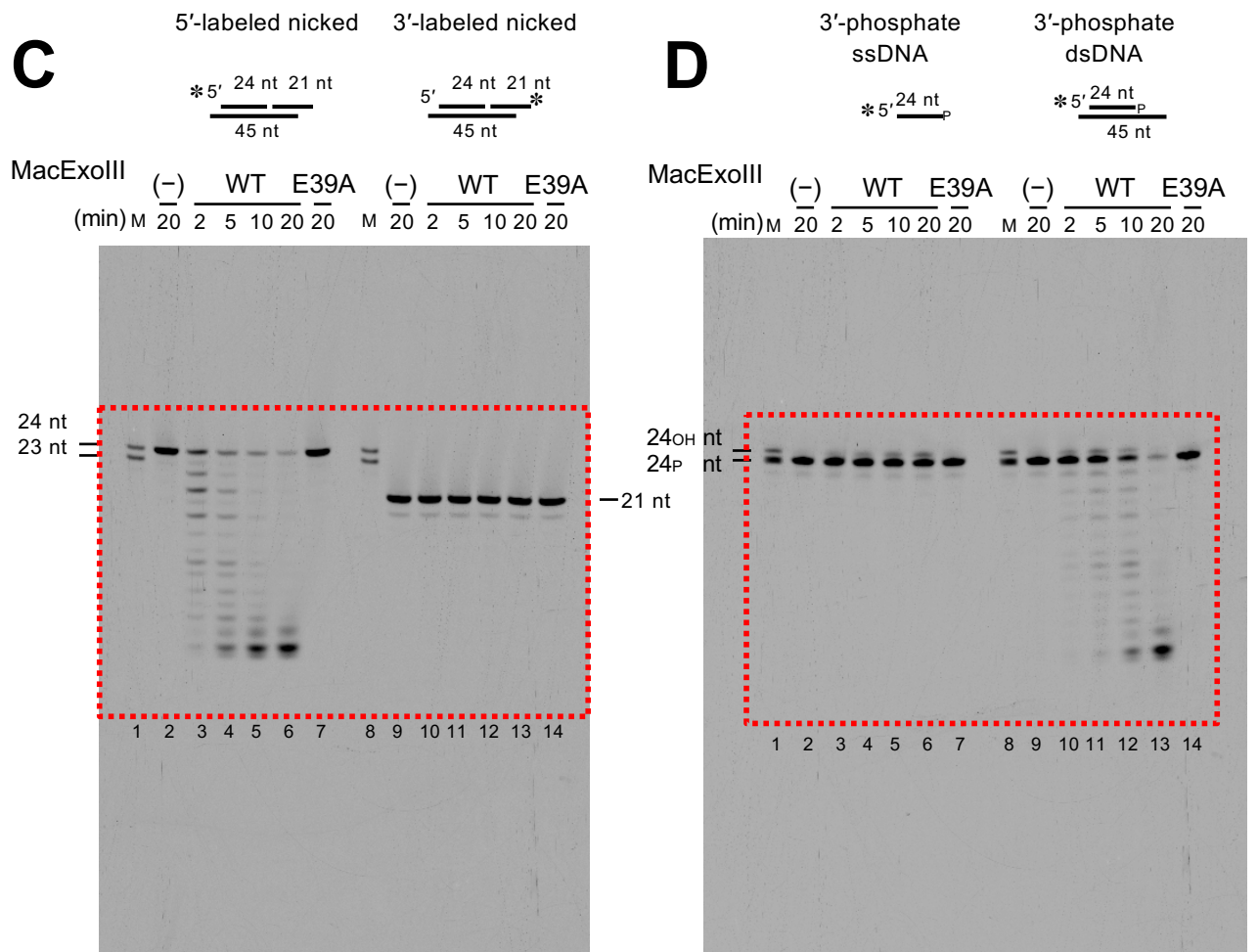
Supplementary Figure S7. Endonuclease activities by MacExoIII and MacExoQ toward MacUDG-catalyzed substrates. 5'-Cy5-labeled 6-nt 3'-overhanging, uracil-containing dsDNA were incubated with (100 nM; lanes 2–7) or without (lanes 1, 8–12) MacUDG in the 20 μ l of reaction mixture (50 mM Tris-HCl, pH 8.0, 1 mM DTT, 0.1 μ g/mL BSA) at 37°C for 10 min. After adding 1 μ l of 20 mM MnCl₂ (lanes 1–5 and 8–10) or MgCl₂ (6, 7, 11, and 12), the indicated concentrations of MacExoIII^{WT} (lanes 4, 5, 9, 10) or MacEndoQ^{WT} (lanes 6, 7, 11, 12) (2 μ l) were added to the reaction mixture and further incubated for 10 min. The lane 2 sample were treated with NaOH. For the details, see the methods section in the main text. Cleavage products were separated by 8 M urea-12% PAGE.



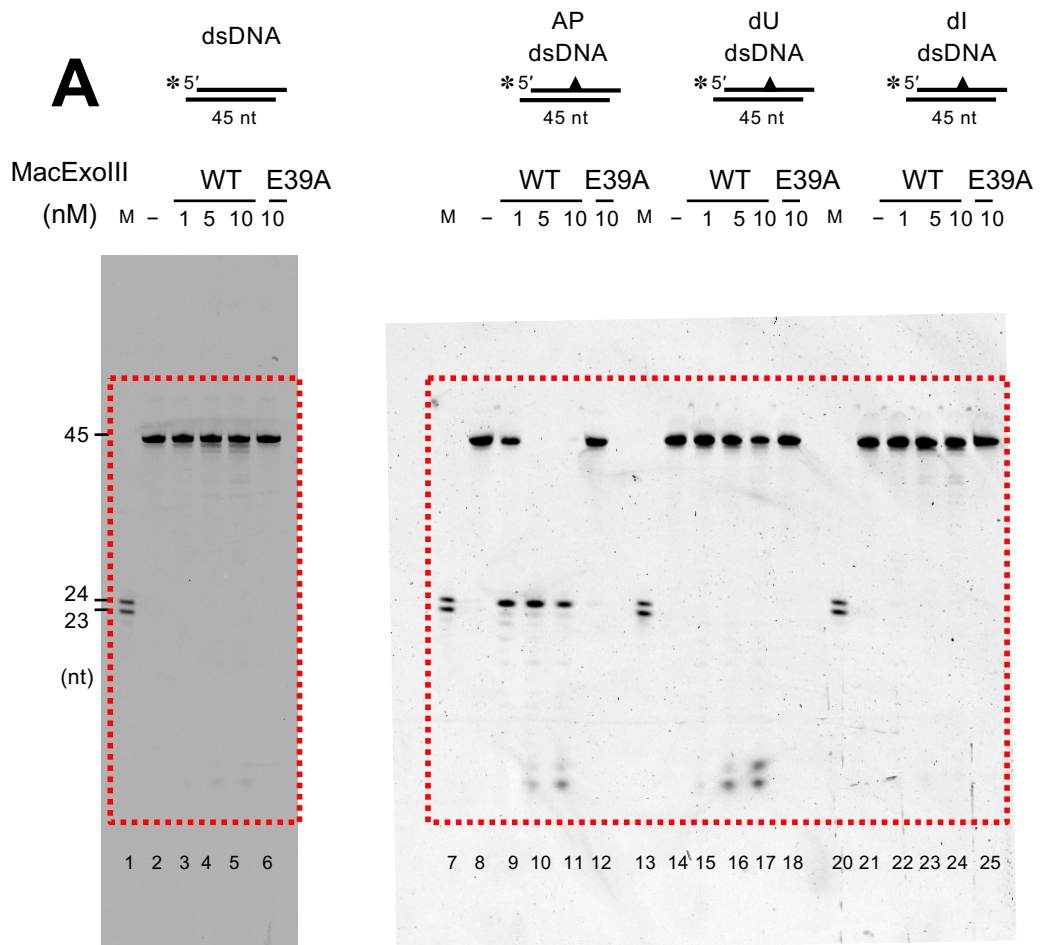
Supplementary Figure S8. Original gel images presented in Figure 2. Red dotted lines indicate the presented area in the main figure.



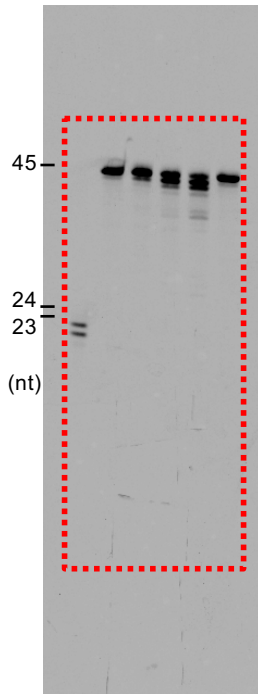
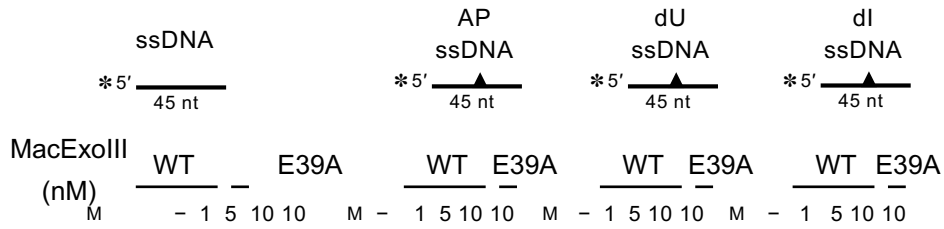
Supplementary Figure S9. Original gel images presented in Figure 3. Red dotted lines indicate the presented area in the main figure.



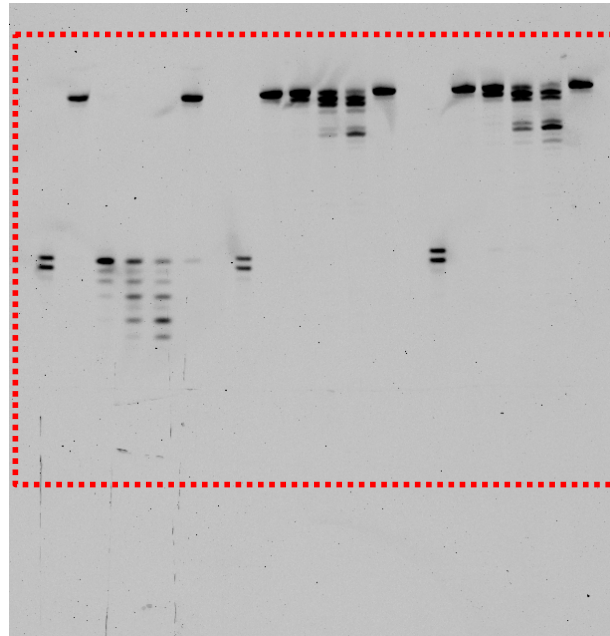
Supplementary Figure S9 (Continued). Original gel images presented in Figure 3. Red dotted lines indicate the presented area in the main figure.



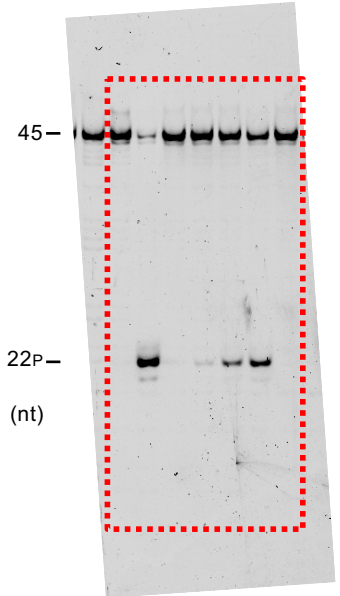
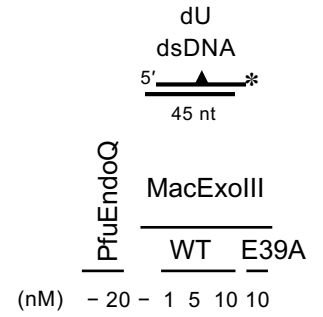
Supplementary Figure S10. Original gel images presented in Figure 4. Red dotted lines indicate the presented area in the main figure.

B

1 2 3 4 5 6



7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

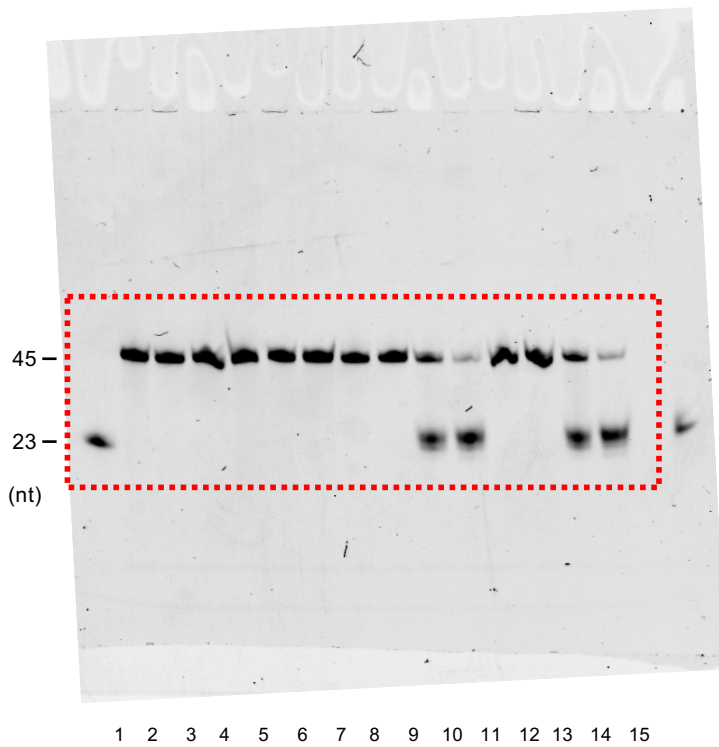
C

1 2 3 4 5 6 7

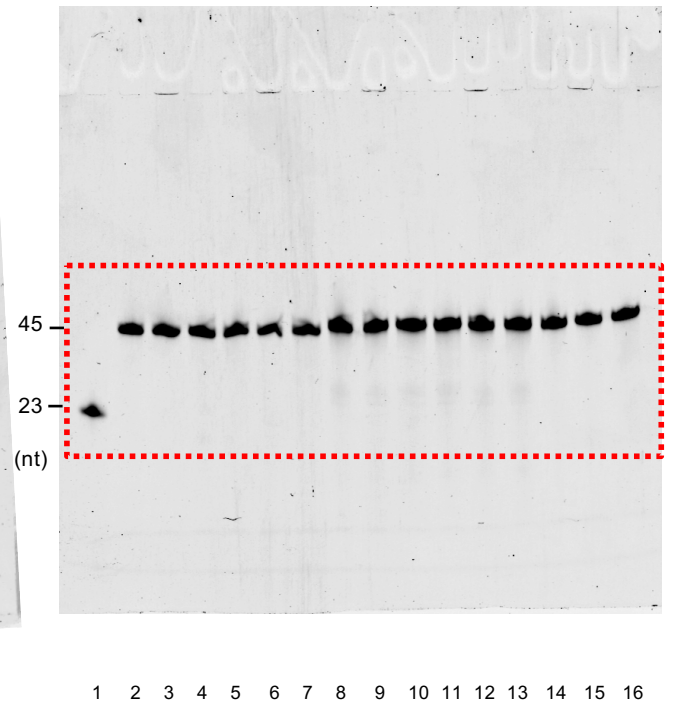
Supplementary Figure S10 (Continued). Original gel images presented in Figure 4. Red dotted lines indicate the presented area in the main figure.

A

	*A		*A/T		*U		*U/G	
M	-	MacUDG-like	-	MacUDG-like	-	MacUDG-like	-	MacUDG-like
		MacUDG		MacUDG		MacUDG		MacUDG

**B**

	*Hx		*Hx/T		*X		*X/C		*G/T	
M	-	MacUDG-like	-	MacUDG-like	-	MacUDG-like	-	MacUDG-like	-	MacUDG-like
		MacUDG		MacUDG		MacUDG		MacUDG		MacUDG



Supplementary Figure S11. Original gel images presented in Figure 5. Red dotted lines indicate the presented area in the main figure.