Supplementary Information

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

Miyako Shiraishi[1, 4, 5 #*], Sonoko Ishino[1], Matthew Heffernan[4], Isaac Cann[2, 3, 4, 5], and Yoshizumi Ishino[1, 4, 5*]

[1]Department of Bioscience and Biotechnology, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, 6-10-1 Hakozaki, Higashiku, Fukuoka 812-8581, Japan; [2]Department of Animal Science, [3]Department of Microbiology, [4]Institute for Universal Biology, and [5]Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

#Present address: Department of Materials Engineering Science, Graduate School of Engineering Science, Osaka University, 1-3 Machikaneyamacho, Toyonaka, Osaka 560-0043, Japan

*Correspondence to Miyako Shiraishi (m.shiraishi@chem.es.osaka-u.ac.jp) or Yoshizumi Ishino (ishino@agr.kyushu-u.ac.jp)

Table of Contents

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	GGGCATATGCCGGAAAATTATAACCTTA	MacExoIII (MA_RS10790)	Gene cloning	
MA_RS10790-R	CCCGCGGCCGCTCAAACCTGAAATTCGAGTTC			
MA_RS10790-E39A	TCGATGTCATCTGTGTCCAGGCAACCAAAGCCTCCCCGGAAAA			
MA_RS03380-F	CGCCATATGAAAGTCAATGCAGACCTCCATC	MacEndoQ (MA_RS03380)		
MA_RS03380-R	CGCGCGGCCGCTTAAAAATCGAAAAGGGATTTCTGCCC			
MA_RS03380-D192A	CTTACCTTCCTTACAAACTCCGCTGCCCATTCCCCTTACACCAAC			
MA_RS11760-F	CGCGCATATGAAGCCTGTTGAATACGTTAAATG	MacUDGlike (MA_RS11760)		
MA_RS11760-R	CCGGCGGCCGCTTACTTAATTAACTTCGCAGCTTC			
MA_RS18745-F	GGGCATATGAAGGAGCTAGAAATTGTGGC	MacUDG (MA_RS18745)		
MA_RS18745-R	GCGGCGGCCGCTTACTGCCATAGATCCCTGTTT			
MA_RS10790-RT-F	TGGGAATCGAGGAGTTTGAC	MacExoIII (MA_RS10790)	RT-PCR	101
MA_RS10790-RT-R	CTGAGAGGCTTTTCCGTTTG			
MA_RS03380-RT-F	CAGCGATTATGCAGACAGGA	MacEndoQ (MA_RS03380)		98
MA_RS03380-RT-R	ATTCCCTTGCCAGTTTGTTG			
MA_RS02415-RT-F	CGATTTCTGGAGGCTAGTCG	MacHDG (MA_RS02415)		118
MA_RS02415-RT-R	GGCTTTAAACACGTCCCAGA			
MA_RS11760-RT-F	TCCTGGATCACGGGTTCTAC	MacUDGlike (MA_RS11760)		76
MA_RS11760-RT-R	ACCGTATCTGCCGAAATCAC			
MA_RS18745-RT-F	AGAAGGCCAAAAGCAAGTGA	MacUDG (MA_RS18745)		143
MA_RS18745-RT-R	ACTCCCCGTTCCAGTTTTCT			
MA_RS04665-RT-F	GGGTCTAAAGGGTCCGTAGC	Mac16SrDNA (MA_RS04665)		80
MA_RS04665-RT-R	AGTATCCCCCGAAAGCCTAA			
MA_RS17460-RT-F	CTTGACTTCGTACGGGTGGT	MacGAPDH (MA_RS17460)		148
MA_RS17460-RT-R	TGATCGGGCCTTTCTTTATG			

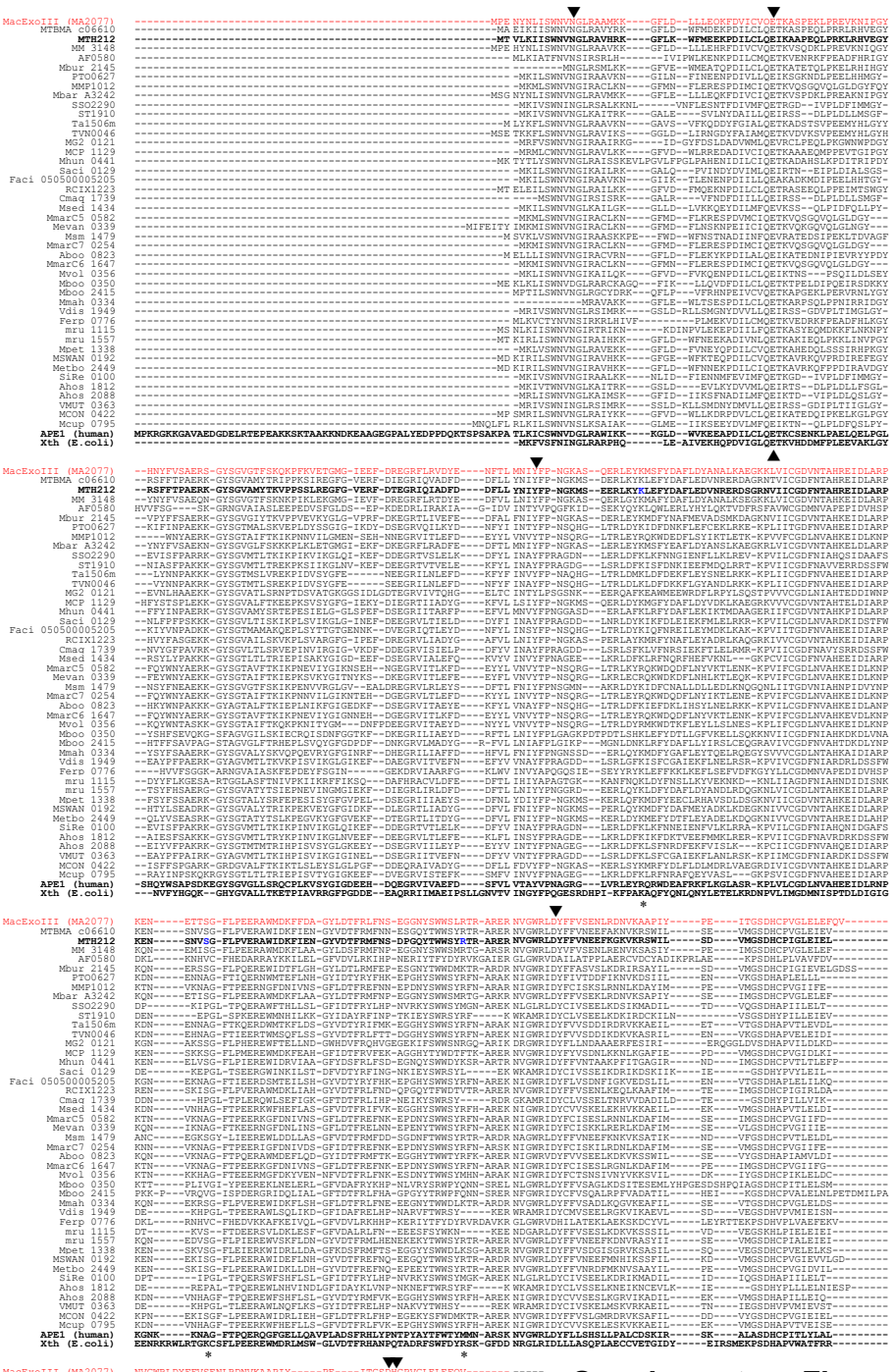
Supplementary Table S2. Oligonucleotides used for protein activity assays

#	Name	Sequence (5′-3′)	Description
1	23N	CGAACTGCCTGGAATCCTGACGA	DNA marker
2	24N	CGAACTGCCTGGAATCCTGACGAC	DNA marker
3	24N-3Phos	CGAACTGCCTGGAATCCTGACGAC-Pho	3′-phosphate ssDNA
4	32N	CGAACTGCCTGGAATCCTGACGACATGTAGCG	
5	45N	CGAACTGCCTGGAATCCTGACGACATGTAGCGAACGATCACCTCA	ssDNA (normal)
6	45-U24	CGAACTGCCTGGAATCCTGACGA**[dU]**ATGTAGCGAACGATCACCTCA	dU-ssDNA
7	45-I25	CGAACTGCCTGGAATCCTGACGAC**[dI]**TGTAGCGAACGATCACCTCA	dI-ssDNA
8	45-AP25	CGAACTGCCTGGAATCCTGACGAC**[AP]**TGTAGCGAACGATCACCTCA	AP-ssDNA
9	45-G25	CGAACTGCCTGGAATCCTGACGAC**[G]**TGTAGCGAACGATCACCTCA	
10	45-X27	CGAACTGCCTGGAATCCTGACGACAT**[X]**TAGCGAACGATCACCTCA	dX-ssDNA
11	21N-5Phos	5-Phos-ATGTAGCGAACGATCACCTCA	
12	32R	CGCTACATGTCGTCAGGATTCCAGGCAGTTCG	
13	45R	TGAGGTGATCGTTCGCTACATGTCGTCAGGATTCCAGGCAGTTCG	Complementary oligo of 45N for blunt-ended dsDNA
14	45R-3-overhang	GATCGTTCGCTACATGTCGTCAGGATTCCAGGCAGTTCGCTAGCC	Complementary oligo of 45N for 6 nt-3′-overhang dsDNA

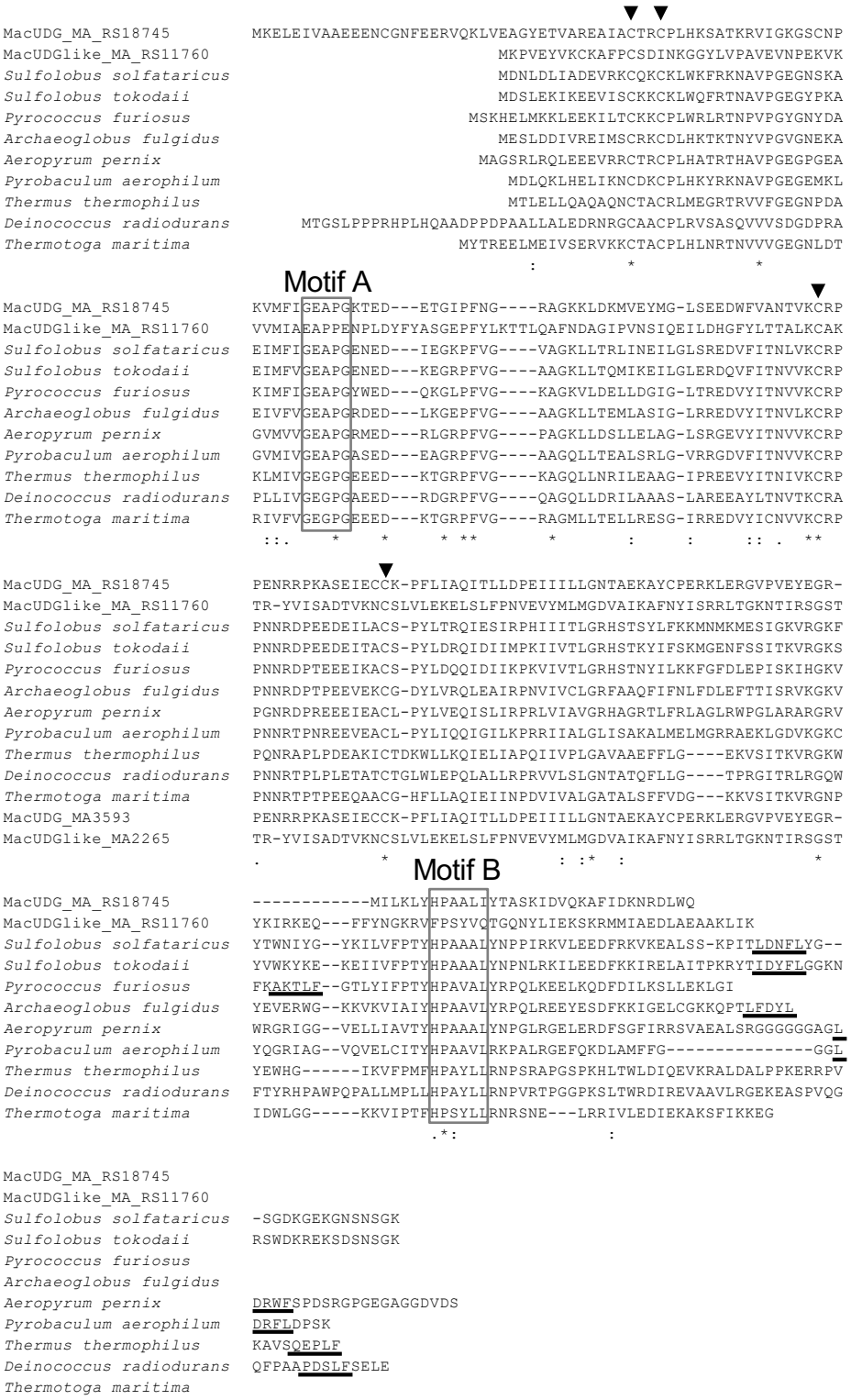
Supplementary Table S3. Preparation for dsDNA.

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 mismached 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

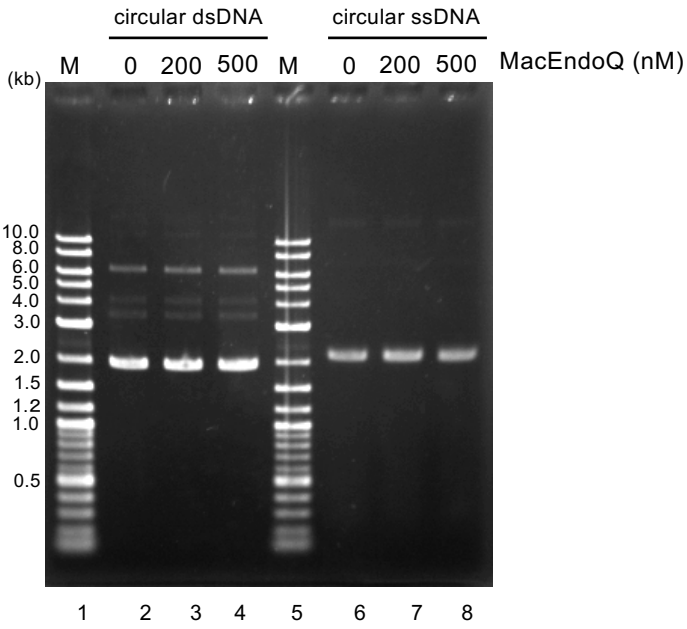
*The numbers on the combination column correspond to those in Supplementary table S2



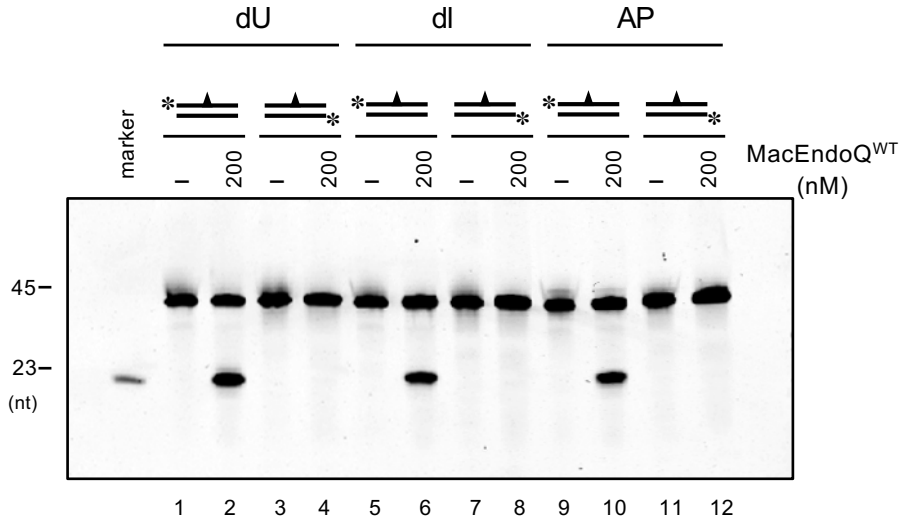
Supplementary Figure S1. Amino acid sequence alignment of ExoIII homologs. The 46 full-length amino acid sequences of archaeal homologs were aligned with human APE1, and *E. coli* Xth. The sequences from *E. coli*, *M. thermautotrophicus*, and *H. sapiens* are indicated with bold letters. MacExoIII is indicated with red letters. Catalytic residues are indicated with ▼ at the top. The putative residues in Mth212 contributing to the recognition of dU in DNA 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).



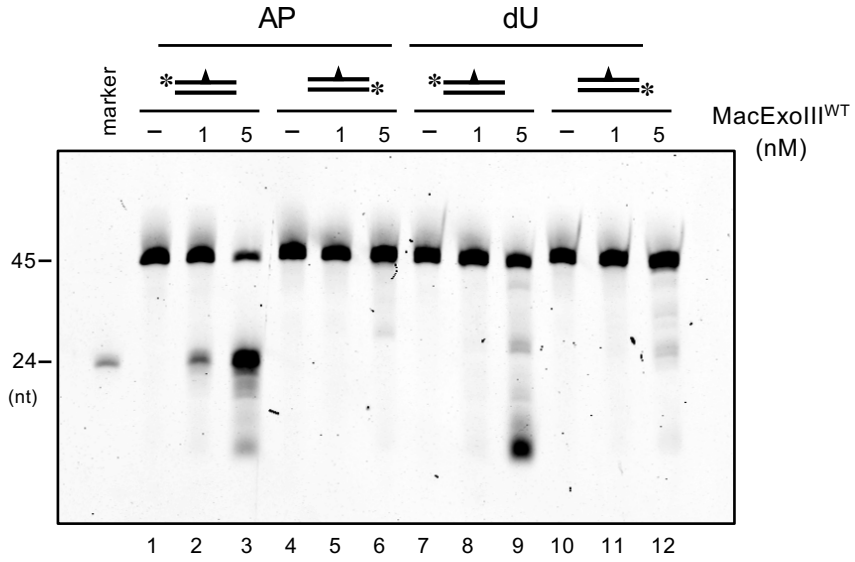
Supplementary Figure S2. Amino acid sequence alignment of the characterized family-4 UDG proteins, MacUDG, and MacUDG-like (MA_RS18745 and MA_RS11760). Motif A: GE(A/G)PG, Motif B: HPAAVL, Underlined peptides: (putative or characterized) PIP box/β-clamp motif, ▼: Cysteine residues involved in Fe-S clusters. 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.



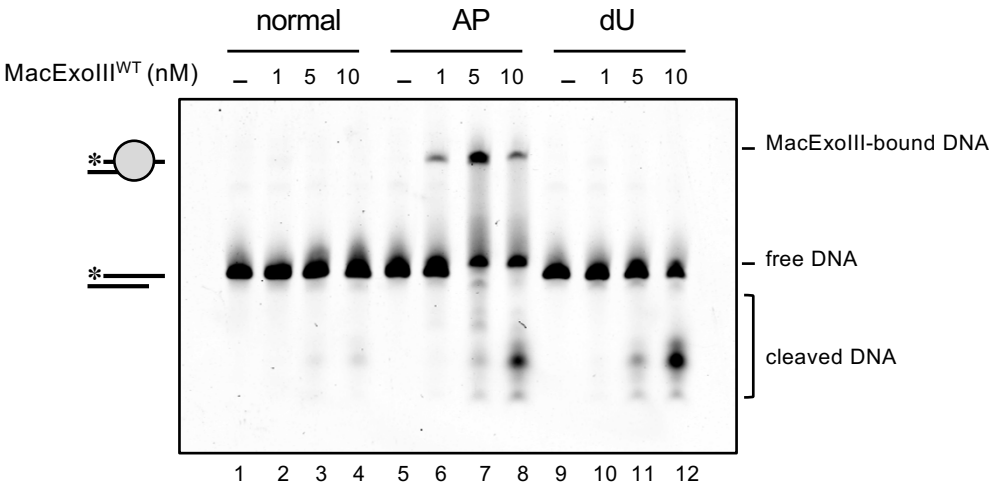
Supplementary Figure S3. Quality check of MacEndoQ sample. MacEndoQ (200 nM and 500 nM) was incubated with 20 ng/μl of supercoiled pBlueScript II SK(+) dsDNA (lanes 2–4) and pBlueScript II SK(+) ssDNA (lanes 6–8) at 37° C for 60 min in the reaction solution. Reaction products were analyzed by 0.8% agarose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



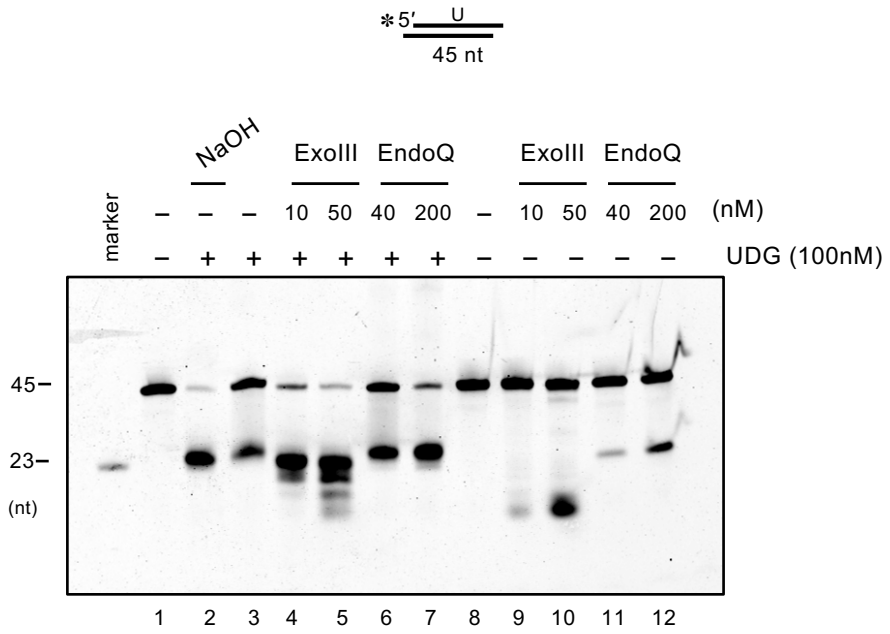
Supplementary Figure S4. Cleavage activity of MacEndoQ toward the opposite strand of a single lesion-containing strand. MacEndoQ cleavage reaction were performed at 37°C for 60 min using the single lesion-containing strand labeled DNA (lanes 1, 2, 5, 6, 9, 10) or the opposite (intact) strand labeled 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.



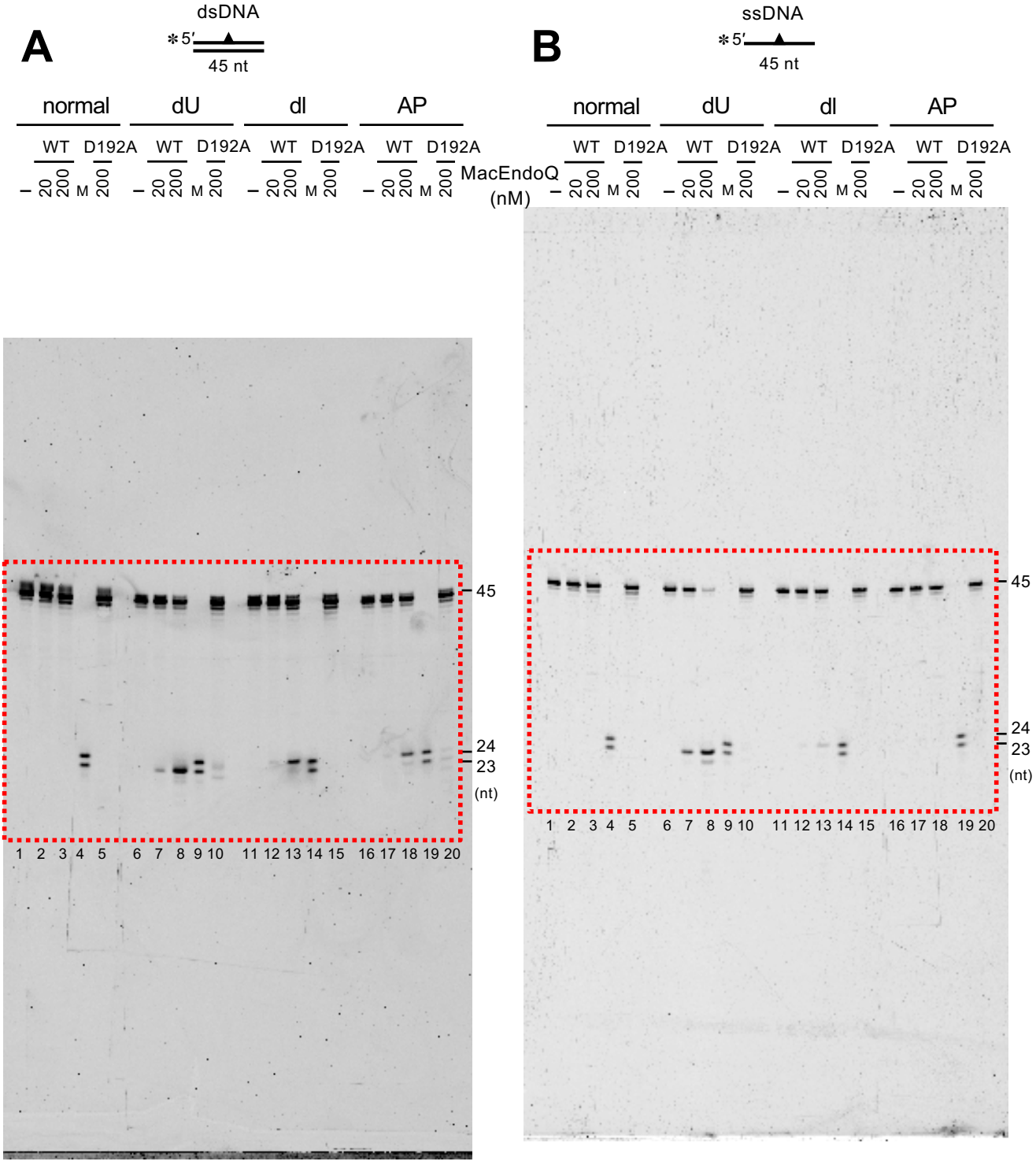
Supplementary Figure S5. Cleavage activity of MacExoIII toward the opposite strand of a single lesion-containing strand. MacExoIII cleavage reaction were performed at 37°C for 10 min using the (single lesion-containing) top strand labeled, blunt ended DNA (lanes 1–3, 7–9) or the bottom strand labeled, 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.



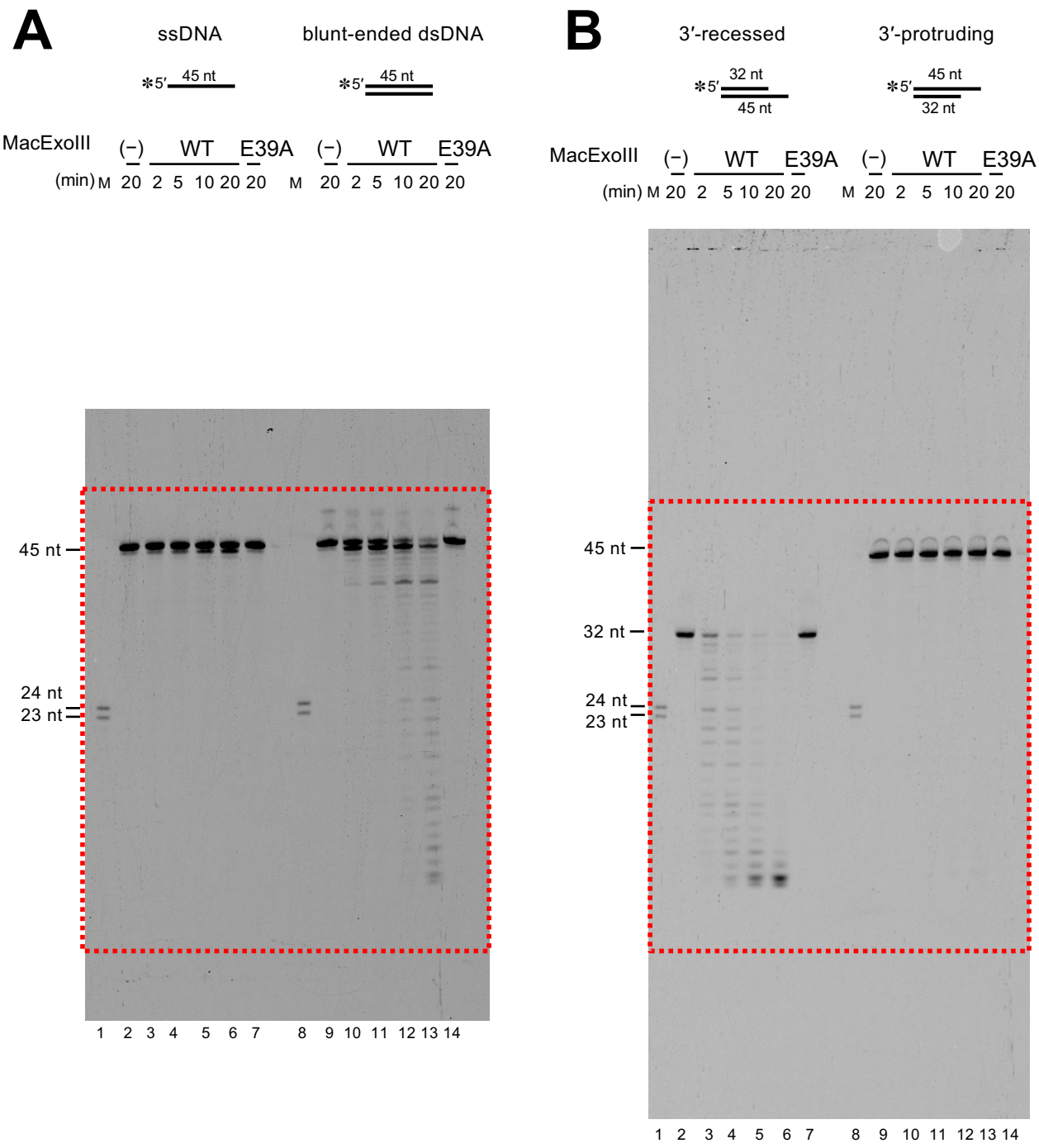
Supplementary Figure S6. Electrophoretic mobility shift assay of MacExoIII with normal/(AP/dU)-containing DNA. Various concentrations (0, 1, 5, and 10 nM) of MacExoIII^{WT} were incubated with 5 nM 5′-Cy5-labeled 6-nt 3′-overhang dsDNA (normal: lanes 1–4; AP site: lanes 5–8; dU: lanes 9–12), in reaction solution (50 mM Bis-Tris-HCl, pH 7.0, 1 mM DTT, 1 mM $MnCl_2$, 0.01% Tween 20; 20 µl) at 37°C for 10 min. After 5 µl 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 od the panel.



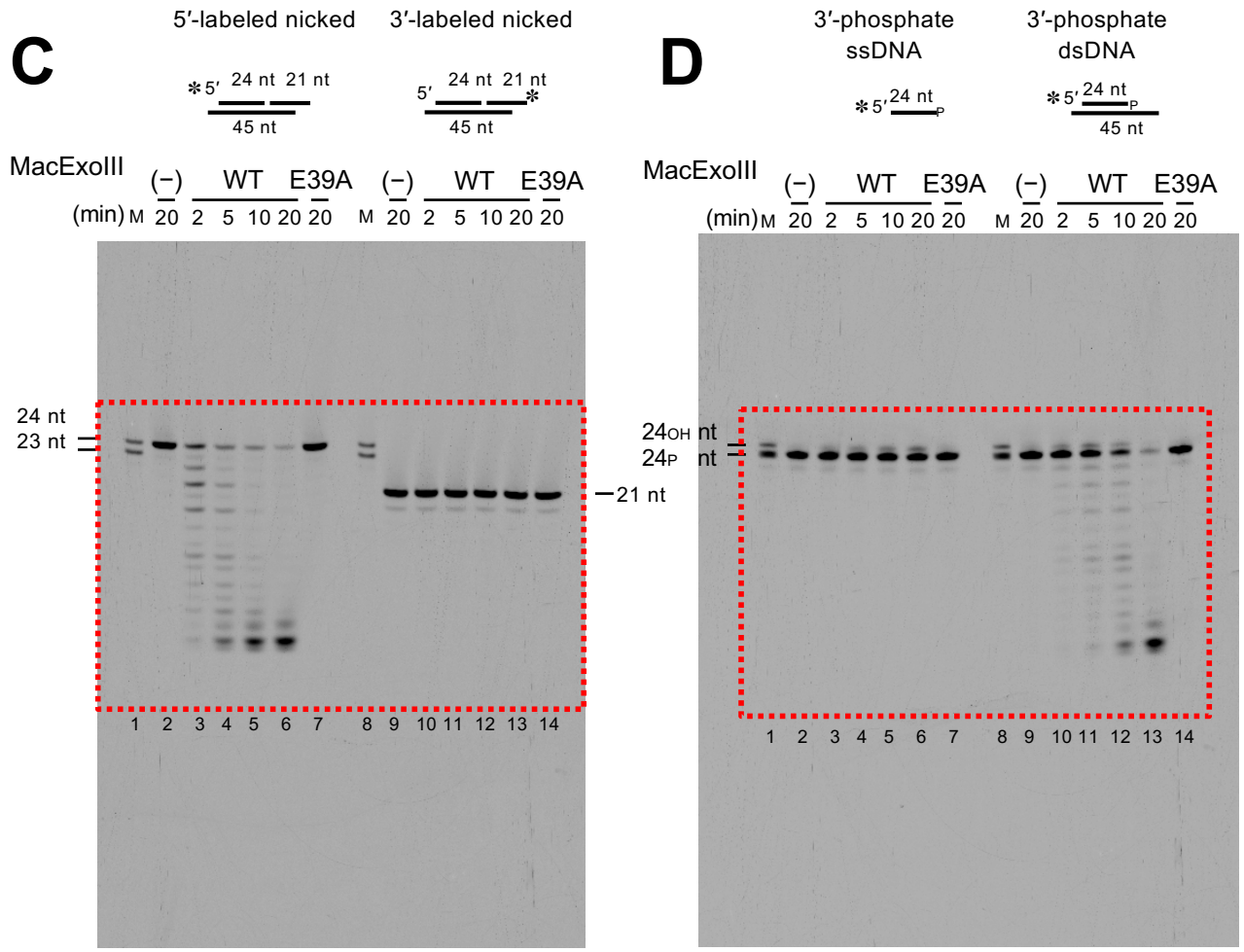
Supplementary Figure S7. Endonuclease activities by MacExoIII and MacExoQ toward MacUDG-catalyzed substrates. 5′-Cy5-labeled 6-nt 3′-overhanging, uracil-containing dsDNA were incubated with (100 nM; lanes 2–7) or without (lanes 1, 8–12) MacUDG in the 20 µl of reaction mixture (50 mM Tris-HCl, pH 8.0, 1 mM DTT, 0.1 µg/mL BSA) at 37°C for 10 min. After adding 1 µl of 20 mM $MnCl_2$ (lanes 1–5 and 8–10) or $MgCl_2$ (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 µl) 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.



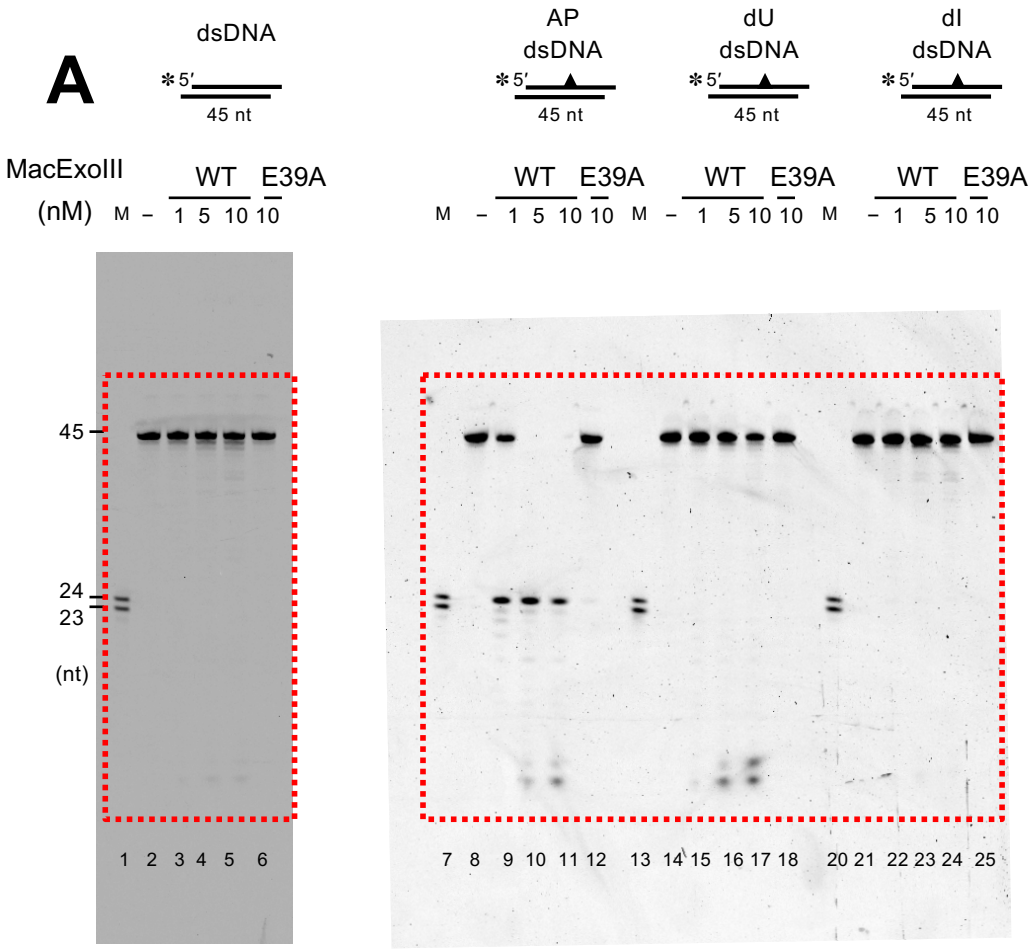
Supplementary Figure S8. Original gel images presented in Figure 2. Red dotted lines indicate the presented area in the main figure.



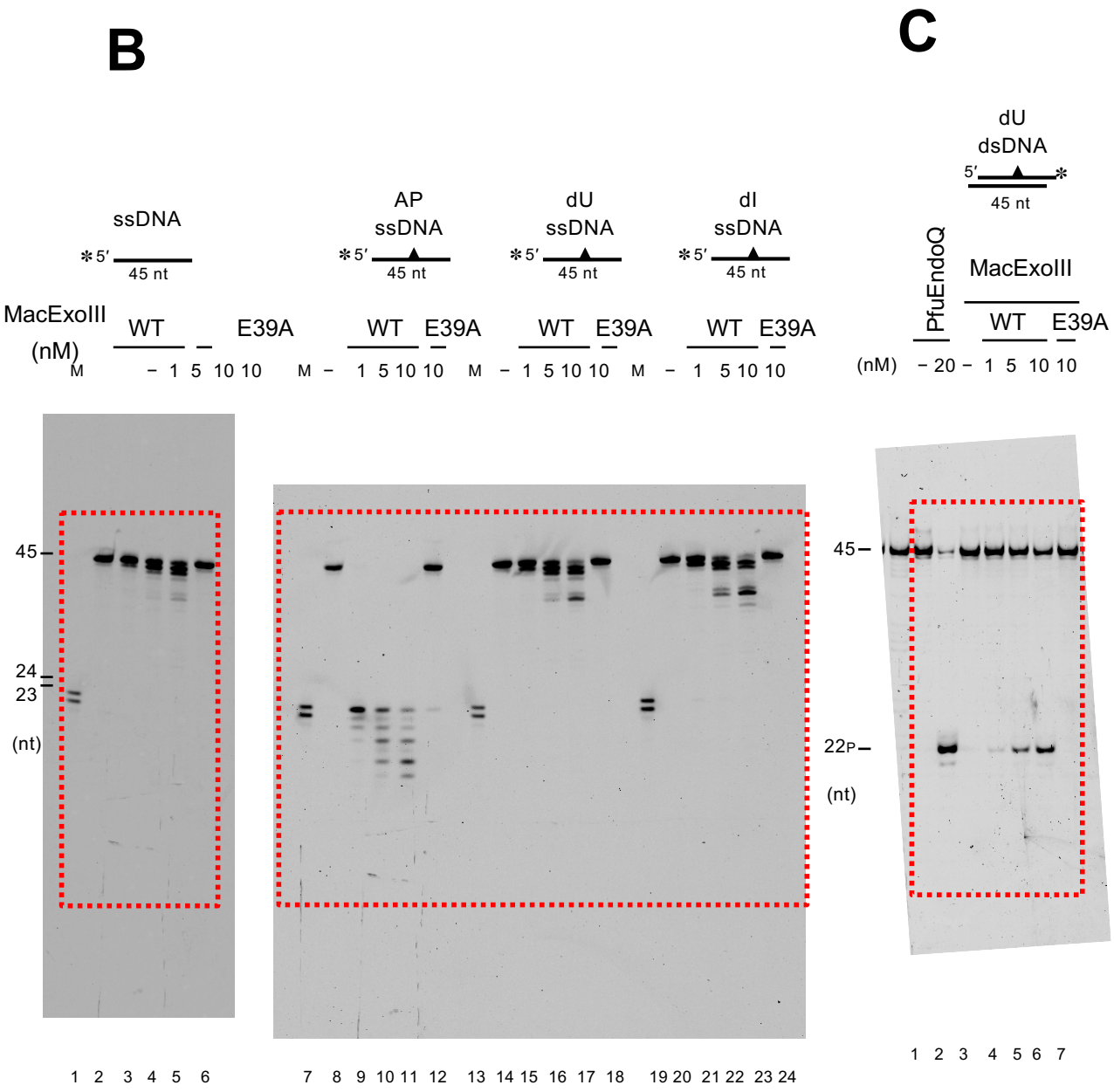
Supplementary Figure S9. Original gel images presented in Figure 3. Red dotted lines indicate the presented area in the main figure.



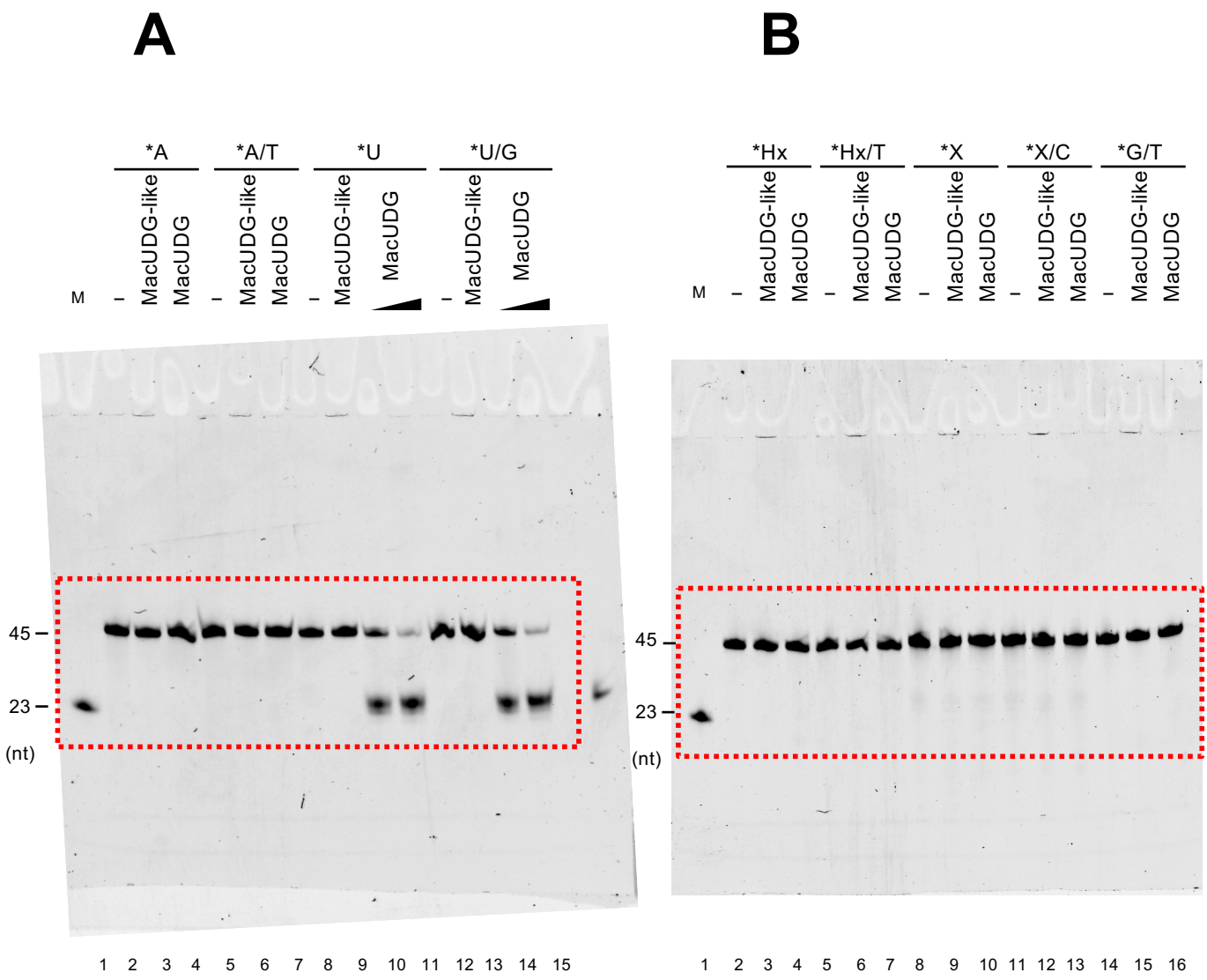
Supplementary Figure S9 (Continued). Original gel images presented in Figure 3. Red dotted lines indicate the presented area in the main figure.



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



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



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