

SUPPLEMENTARY MATERIAL

The *Arabidopsis thaliana* poly(ADP-ribose) polymerases 1 and 2 modify DNA by ADP-ribosylating terminal phosphate residues

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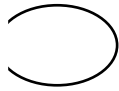

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Supplementary Material Table S1. Sequences of the oligonucleotides and their duplexes used in this study^a.

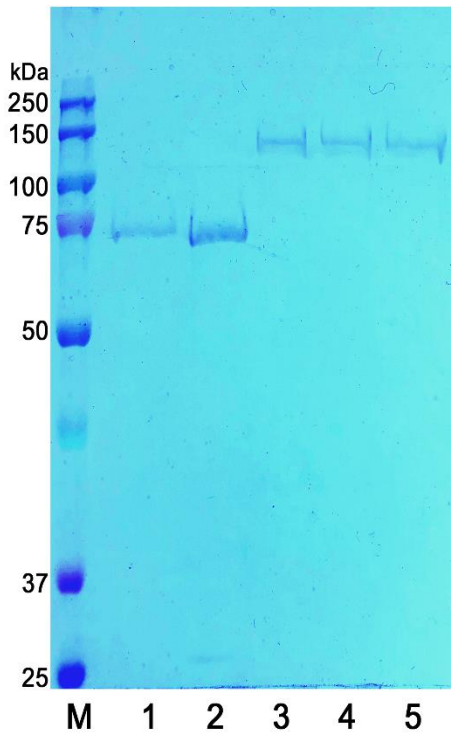
Name	Oligonucleotides sequences and structures
S1	$\begin{array}{c} \text{3'2P-} \\ \text{CACCGCGCCTCTGAATCTCTTTAAACCGCGCCCCTTAAGG } 5' \\ \text{RexT} \end{array}$
S2	$\begin{array}{c} \text{3'2P-} \\ \text{CACCGCGCCTCTGAATCTCTTTAAACCGTGCCCCTTAAGG } 5' \\ \text{Rex12T} \end{array}$
S3	$\begin{array}{c} \text{P-} \quad \text{ExoA} \\ \text{5' GTGGCGCGGAGACTTAGAGAA} \\ \text{CACCGCGCCTCTGAATCTCTTTAAACCGCGCCCCTTAAGG } 5' \\ \text{RexT} \quad \text{-3'2P} \end{array}$
S4	$\begin{array}{c} \text{P-} \quad \text{Exo20} \\ \text{5' GTGGCGCGGAGACTTAGAGA} \quad \text{dAM} \quad \text{3'2P} \\ \text{CACCGCGCCTCTGAATCTCTTTAAACCGCGCCCCTTAAGG } 5' \\ \text{RexT} \end{array}$
S5	$\begin{array}{c} \text{3'2P-} \quad \text{ExoA} \\ \text{5' GTGGCGCGGAGACTTAGAGAA} \\ \text{CACCGCGCCTCTGAATCTCTTTAAACCGCGCCCCTTAAGG } 5' \\ \text{RexT} \end{array}$
S6	$\begin{array}{c} \text{3'2P-} \quad \text{ExoA} \quad \text{P-} \\ \text{5' GTGGCGCGGAGACTTAGAGAA} \\ \text{CACCGCGCCTCTGAATCTCTTTAAACCGCGCCCCTTAAGG } 5' \\ \text{RexT} \end{array}$
S7	$\begin{array}{c} \text{3'2P-} \quad \text{Exo15} \\ \text{5' GTGGCGCGGAGACTT} \\ \text{CACCGCGCCTCTGAATCTCTTTAAACCGTGCCCCTTAAGG } 5' \\ \text{Rex12T} \end{array}$
S8	$\begin{array}{c} \text{3'2P-} \quad \text{ExoA} \quad \text{P-} \quad \text{Exo14} \\ \text{5' GTGGCGCGGAGACTTAGAGAA} \quad \text{GCGCGGGGAATTCC} \\ \text{CACCGCGCCTCTGAATCTCTTTAAACCGCGCCCCTTAAGG } 5' \\ \text{RexT} \end{array}$
S9	$\begin{array}{c} \text{3'2P-} \quad \text{ExoA} \quad \text{P-} \quad \text{Exo17} \\ \text{5' GTGGCGCGGAGACTTAGAGAA} \quad \text{TTGGCGCGGGGAATTCC} \\ \text{CACCGCGCCTCTGAATCTCTTTAAACCGCGCCCCTTAAGG } 5' \\ \text{RexT} \end{array}$
S10	$\begin{array}{c} \text{3'2P-} \quad \text{ExoA} \quad \text{P-} \quad \text{Exo18} \\ \text{5' GTGGCGCGGAGACTTAGAGAA} \quad \text{TTTGGCGCGGGGAATTCC} \\ \text{CACCGCGCCTCTGAATCTCTTTAAACCGCGCCCCTTAAGG } 5' \\ \text{RexT} \end{array}$

S11	<p>32P- ExoA Exo19</p> <p>5' GTGGCGCGGAGACTTAGAGAA ATTTGGCGCGGGGAATTCC CACCGCGCCTCTGAATCTCTT-TAAACCGCGCCCCTTAAGG 5'</p> <p>RexT</p>
S12	<p>32P- ExoA Exo19</p> <p>5' GTGGCGCGGAGACTTAGAGAA ATTTGGCGCGGGGAATTCC CACCGCGCCTCTGAATCTCTT-TAAACCGCGCCCCTTAAGG 5'</p> <p>RexT</p>
S13	<p>32P- ExoA Exo19</p> <p>5' GTGGCGCGGAGACTTAGAGAA ATTTGGCGCGGGGAATTCC CACCGCGCCTCTGAATCTCTT-TAAACCGCGCCCCTTAAGG 5'</p> <p>RexT</p>
S14	<p>32P- Exo15 Exo3'A-12-25</p> <p>5' GTGGCGCGGAGACTT AGAGAAATTTGGCACGGGGAATTCC CACCGCGCCTCTGAA-TCTCTTTAAACCGTGCCCCTTAAGG 5'</p> <p>Rex12T</p>
S15	<p>32P- Exo15 Exo19</p> <p>5' GTGGCGCGGAGACTT ATTTGGCGCGGGGAATTCC CGCCTCTGAATCTCTTTAAACCGCGCCCCTTAAGG 5'</p> <p>RexT-35</p>
S16	<p>P- ExoA Exo19</p> <p>5' GTGGCGCGGAGACTTAGAGAA ATTTGGCGCGGGGAATTCC CACCGCGCCTCTGAATCTCTT-TAAACCGTGCCCCTTAAGG 5'</p> <p>Rex12T</p>
S17	<p>P- Exo15 Exo3'A-12-25</p> <p>5' GTGGCGCGGAGACTT AGAGAAATTTGGCACGGGGAATTCC CACCGCGCCTCTGAA-TCTCTTTAAACCGTGCCCCTTAAGG 5'</p> <p>Rex12T</p>
S18	<p>32P- 10-RT P- T19-RT</p> <p>5' TGA CTGCATA TGCATGTAGACGATGTGCAT ACTGACGTAT-ACGTACATCTGCTACACGTA 5'</p> <p>RT-A</p>
S19	<p>32P- 7 13db P- 18</p> <p>5' GTCATTCGCCTGTGCCCTCAA CGAATTCACAAGCCTAGA  HEG linker 3' CGACACGGGAGTTGGCTTAAGTGTTCGGATCT ttt 32</p>
S20	<p>32P- 10db P- 22</p> <p>5' GCTGTGCCCT CAACCGAATTCACAAGCCTAGA  HEG linker 3' CGACACGGGA-GTTGGCTTAAGTGTTCGGATCT ttt 32</p>

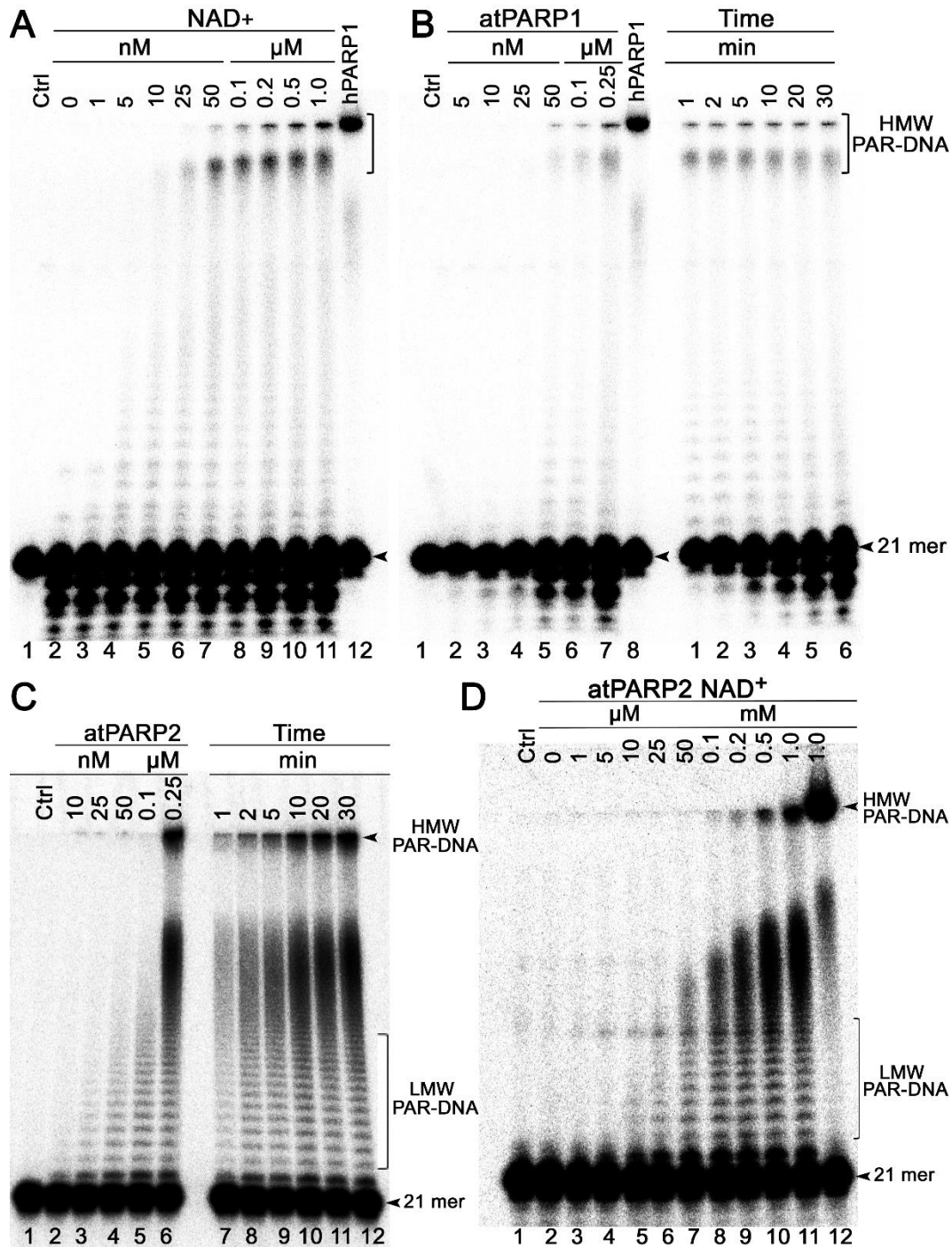
^aThe symbol “t” designates modified nucleotide containing a thiophosphate group; “HEG linker” denotes hexaethyleneglycol linker [(CH₂-CH₂-O)₆]; “Dbait” designates a long single-stranded hairpin

oligonucleotide in which complementary parts of DNA tethered with hexaethyleneglycol linker; “P” designates a phosphate group, “³²P” designates [γ -³²P]-ATP labeled oligonucleotide termini; “dAM³²P” designates [α -³²P]-3'-dATP (cordycepin 5'-triphosphate) labeled oligonucleotide termini; following abbreviations “ExoA”, “Exo20”, “Exo15”, “10RT”, “T19RT”, “10db”, “7-13db”, “Exo19”, “Exo18”, “Exo17”, “Exo14”, “Exo3'A-12-25”, “RexT”, “Rex12T”, “RexT-35”, “RT-A” “50-db”, “54-db” designate the single-stranded oligonucleotide fragments used to construct DNA structures. In DNA structure referred as “S19” the 7 nt 5' single-stranded overhang highlighted in red.

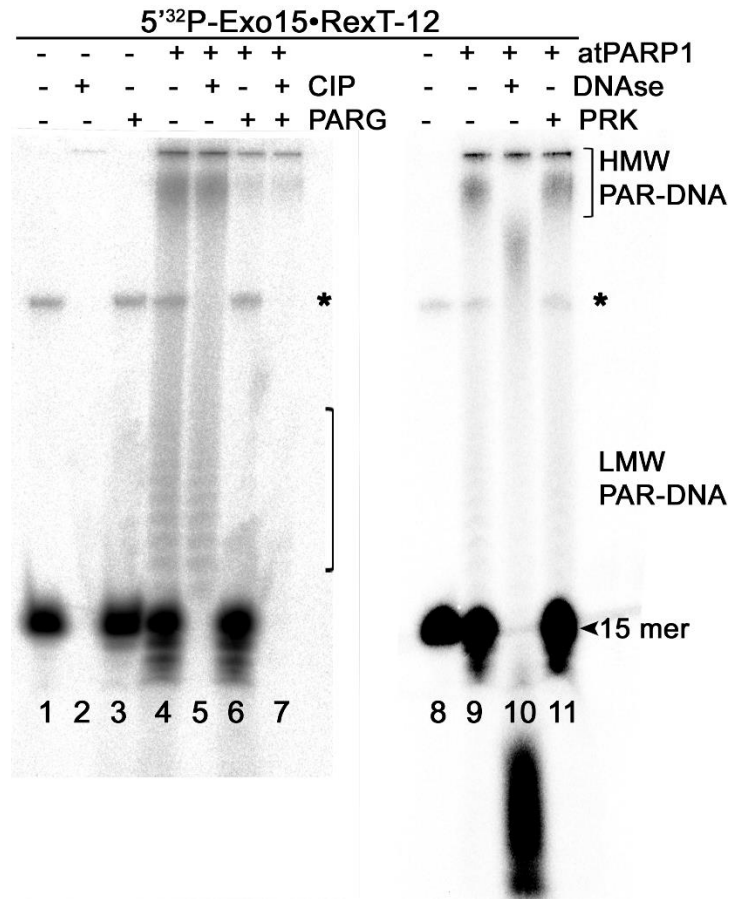
Supplementary Material Figures S1-S4.



Supplementary Figure S1. SDS-PAGE analysis of the purified wild type and mutant Arabidopsis atPARP2 and atPARP1 proteins. Lane M, protein size markers; lane 1, 1 μ g atPARP2-WT; lane 2, 1 μ g atPARP2-E614K mutant; lane 3, 1 μ g atPARP1-WT; lane 4, 1 μ g atPARP1-E960K mutant; lane 5, 1 μ g atPARP1 E960Q mutant. For details, see Materials and Methods.



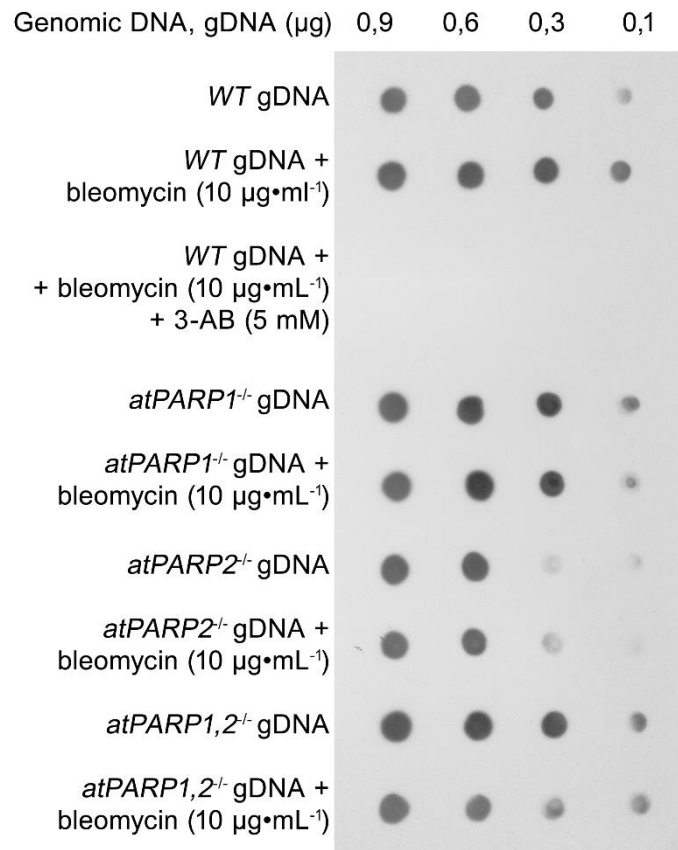
Supplementary Figure S2. The atPARP1- and atPARP2-catalysed DNA PARylation in the presence of varying concentrations of NAD⁺, protein and incubation time. **(A)** Protein concentration and time dependence of atPARP1-catalysed DNA PARylation. **(B)** NAD⁺ concentration dependence of atPARP1-catalysed DNA PARylation. **(C)** Protein concentration and time dependence of atPARP2-catalysed DNA PARylation. **(D)** NAD⁺ concentration dependence of atPARP2-catalysed DNA PARylation.



Supplementary Figure S3. Denaturing PAGE analysis of the products of digestion of PAR-DNA adducts by various enzymes. To generate PAR-DNA products 20 nM 5'-[³²P]-labelled Exo15•Rex12T^{rec} duplex was incubated with 250 nM atPARP1 in the presence of 1mM NAD⁺ for 30min at 37°C. After incubation, the reaction mixtures were heated for 20 min at 80°C and then incubated either in the presence of 50 pg•μL⁻¹ PARG (in ADPR buffer) for 60 min at 37°C, or 10 U CIP (in CIP buffer) or 10.5 U DNase I (in buffer with 0.5 mM CaCl₂) for 30 min at 37°C or 50 μg•mL⁻¹ proteinase K for 30 min at 50°C in the presence of 0.1% SDS. Arrows indicate HMW and LMW PAR-DNA products and free 15 mer oligonucleotide. Asterisk indicates a nonspecific ligation product produced by *E. coli* NAD⁺-dependent DNA ligase A. For more details, see Materials and Methods.



Supplementary Figure S4. Formation of the PAR-DNA products by atPARP2 for MALDI-TOF MS analysis. 25-250 nM atPARP2 was incubated with 20 nM 5'-[³²P]-labelled p10•RT-A^{Nick} oligonucleotide duplex (also referred as S18) in the presence of 1 mM NAD⁺ for 30 min at 37°C. The reaction products were analyzed by denaturing PAGE. Arrow indicates free 10 mer oligonucleotide. For more details, see Materials and Methods.



Supplementary Figure S5. Detection of PAR-DNA adducts in gDNA extracted from 14-days-old seedlings grown under either normal conditions or genotoxic stress. Different quantities of gDNA in TE buffer (10 mM Tris-Cl pH 8.0, 1 mM EDTA) were spotted onto a nylon membrane, followed by the pan-ADP-ribose reagent (MABE1016) dot blotting. For more details, see Materials and Methods.