

Insight into DNA substrate specificity of PARP1 catalyzed DNA poly(ADP-ribosylation)

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Table S1. Sequences of the oligonucleotides and their duplexes used in this study.

Name	Oligonucleotides sequences and structures
S0 ⁿ	$ \begin{array}{l} \text{5' } \overset{32\text{P}}{\text{TCATAGGCTTAGTCGTCATTC}} \overset{n}{\text{GCTGTGCCCTCAACCGAATTCACAAGCCTAGA}} \\ \text{3' } \text{CGACACGGGAGTT GCTTAAGTGTTCCGGATCT} \end{array} $
S1 ⁿ	$ \begin{array}{l} \text{5' } \overset{32\text{P}}{\text{TCATAGGCTTAGTCGTCATTC}} \overset{n}{\text{GCTGTGCCCTCAA}} \overset{P}{\text{CGAATTCACAAGCCTAGA}} \\ \text{3' } \text{CGACACGGGAGTTGGCTTAAGTGTTCCGGATCT} \end{array} $
S2 ⁿ	$ \begin{array}{l} \text{5' } \text{GCTGTGCCCTCAA} \overset{32\text{P}}{\text{CGAATTCACAAGCCTAGA}} \\ \text{3' } \overset{P}{\text{CTTACTGCTGATTCGGATACT}} \text{CGACACGGGAGTTGGCTTAAGTGTTCCGGATCT} \end{array} $
S2 (S2 ⁰)	$ \begin{array}{l} \text{5' } \text{GCTGTGCCCTCAA} \overset{32\text{P}}{\text{CGAATTCACAAGCCTAGA}} \\ \text{3' } \overset{P}{\text{CGACACGGGAGTTGGCTTAAGTGTTCCGGATCT}} \end{array} $
S2 ⁻¹	$ \begin{array}{l} \text{5' } \text{GCTGTGCCCTCAA} \overset{32\text{P}}{\text{CGAATTCACAAGCCTAGA}} \\ \text{3' } \overset{P}{\text{GACACGGGAGTTGGCTTAAGTGTTCCGGATCT}} \end{array} $
S2 ⁻³	$ \begin{array}{l} \text{5' } \text{GCTGTGCCCTCAA} \overset{32\text{P}}{\text{CGAATTCACAAGCCTAGA}} \\ \text{3' } \overset{P}{\text{CACGGGAGTTGGCTTAAGTGTTCCGGATCT}} \end{array} $
S3 ⁿ	$ \begin{array}{l} \text{5' } \text{GCTGTGCCCTCAACCGAATTCACAAGCCTAGA} \\ \text{3' } \overset{P}{\text{CTTACTGCTGATTCGGATACT}} \text{CGACACGGGAGTT GCTTAAGTGTTCCGGATCT} \end{array} $
S4	$ \begin{array}{l} \text{5' } \overset{32\text{P}}{\text{GCTGTGCCCTCAACCGAATTCACAAGCCTAGA}} \\ \text{3' } \text{CGACACGGGAGTTGGCTTAAGTGTTCCGGATCT} \end{array} $
S5	$ \begin{array}{l} \text{5' } \text{GCTGTGCCCTCAA} \overset{32\text{P}}{\text{CGAATTCACAAGCCTAGA}} \\ \text{3' } \text{CGACACGGGAGTTGGCTTAAGTGTTCCGGATCT} \end{array} $
S6	$ \begin{array}{l} \text{5' } \text{GCTGTGCCCT} \overset{32\text{P}}{\text{AAGCGAATTCACAAGCCTAGA}} \\ \text{3' } \text{CGACACGGGAGTTGGCTTAAGTGTTCCGGATCT} \end{array} $
S7	$ \begin{array}{l} \text{5' } \text{GCTGTGCCCTCAAC} \overset{32\text{P}}{\text{CGAATTCACAAGCCTAGA}} \\ \text{3' } \overset{P}{\text{CGACACGGGAGTTG-GCTTAAGTGTTCCGGATCT}} \end{array} $
S8	$ \begin{array}{l} \text{5' } \text{GCTGTGCCCTCAACC} \overset{32\text{P}}{\text{AATTCACAAGCCTAGA}} \\ \text{3' } \text{CGACACGGGAGTTGGCTTAAGTGTTCCGGATCT} \end{array} $
S9	$ \begin{array}{l} \text{5' } \text{GCTGTGCCCTCAACCGAATT} \overset{32\text{P}}{\text{ACAAGCCTAGA}} \\ \text{3' } \text{CGACACGGGAGTTGGCTTAAGTGTTCCGGATCT} \end{array} $
S10	$ \begin{array}{l} \text{5' } \text{GCTGTGCCCTCAACCGAATTCAC} \overset{32\text{P}}{\text{AGCCTAGA}} \\ \text{3' } \text{CGACACGGGAGTTGGCTTAAGTGTTCCGGATCT} \end{array} $
S11	$ \begin{array}{l} \text{5' } \text{GCTGTGCCCTCAA} \overset{32\text{P}}{\text{AATTCACAAGCCTAGA}} \\ \text{3' } \text{CGACACGGGAGTTGGCTTAAGTGTTCCGGATCT} \end{array} $
S12	$ \begin{array}{l} \text{5' } \text{GCTGTGCCCTCAA} \overset{32\text{P}}{\text{CACAAGCCTAGA}} \\ \text{3' } \text{CGACACGGGAGTTGGCTTAAGTGTTCCGGATCT} \end{array} $
S13	$ \begin{array}{l} \text{5' } \text{GCTGTGCCCTCAA} \overset{32\text{P}}{\text{AGCCTAGA}} \\ \text{3' } \text{CGACACGGGAGTTGGCTTAAGTGTTCCGGATCT} \end{array} $
S14	$ \begin{array}{l} \text{5' } \text{GCTGTGCCCTCAACCGAA} \overset{P}{\text{TCACAAGCCTAGA}} \\ \text{3' } \text{CGACACGGGAGTTGGCTTAAGTGTTCCGGATCT} \end{array} $
S15	$ \begin{array}{l} \text{5' } \text{GCTGTGCCCTCAA} \overset{32\text{P}}{\text{CGAATTCACAAGCCTAGAGTTACATTAGCAGATACG}} \\ \text{3' } \text{CGACACGGGAGTTGGCTTAAGTGTTCCGGATCTCAATGTAATCGTCTATGC} \end{array} $
S16	$ \begin{array}{l} \text{5' } \text{GCTGTGCCCTCAACCGAAT} \overset{32\text{P}}{\text{CACAAGCCTAGAGTTACATTAGCAGATACG}} \\ \text{3' } \text{CGACACGGGAGTTGGCTTAAGTGTTCCGGATCTCAATGTAATCGTCTATGC} \end{array} $
S17	$ \begin{array}{l} \text{5' } \text{GCTGTGCCCTCAACCGAATT} \overset{32\text{P}}{\text{CACAAGCCTAGAGTTACATTAGCAGATACG}} \\ \text{3' } \text{CGACACGGGAGTTGGCTTAA-GTGTTCGGATCTCAATGTAATCGTCTATGC} \end{array} $
S18	$ \begin{array}{l} \text{5' } \text{GCTGTGCCCTCAACCGAATTCAC} \overset{32\text{P}}{\text{AGCCTAGAGTTACATTAGCAGATACG}} \\ \text{3' } \text{CGACACGGGAGTTGGCTTAAGTGTTCCGGATCTCAATGTAATCGTCTATGC} \end{array} $
S19	$ \begin{array}{l} \text{5' } \overset{32\text{P}}{\text{GCTGTGCCCTCAACCGAATTCACAAGCCTAGA}} \\ \text{3' } \text{CGACACGGGAGTTGGCTTAAGTGTTCCGGATCT} \\ \text{tt} \end{array} $
S20	$ \begin{array}{l} \text{5' } \overset{tt}{\text{GCTGTGCCCTCAA}} \overset{32\text{P}}{\text{CGAATTCACAAGCCTAGA}} \overset{t}{\text{}} \\ \text{3' } \text{CGACACGGGAGTTGGCTTAAGTGTTCCGGATCT} \\ \text{P-} \end{array} $
S21	$ \begin{array}{l} \text{5' } \overset{32\text{P}}{\text{TCATAGGCTTAGTCGTCATTCGCTGTGCCCTCAACCGAATTCACAAGCCTAGA}} \\ \text{3' } \text{CGACACGGGAGTTGGCTTAAGTGTTCCGGATCT} \end{array} $
S22	$ \begin{array}{l} \text{5' } \text{GCTGTGCCCTCAACCGAATTCACAAGCCTAGA} \\ \text{3' } \overset{P}{\text{CTTACTGCTGATTCGGATACTCGACACGGGAGTTGGCTTAAGTGTTCCGGATCT}} \end{array} $

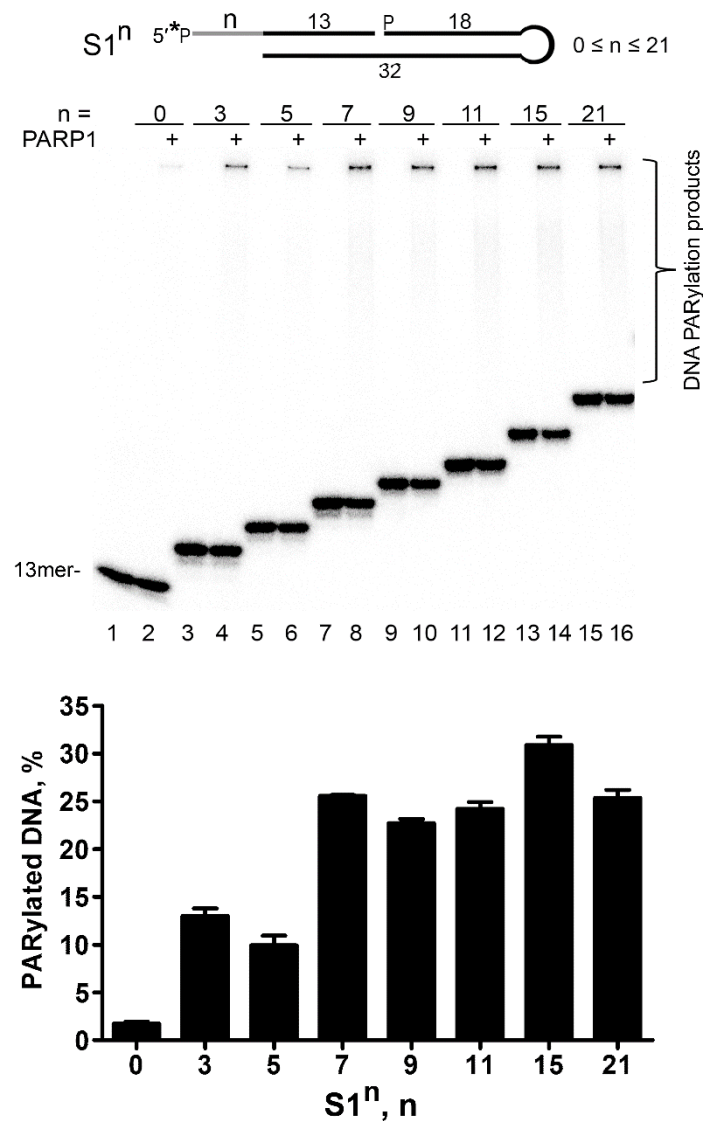


Figure S1. ADP-ribosylation of S1ⁿ DNA duplexes containing 5'-otherhangs by PARP1 at a non-saturating concentration of NAD⁺. [³²P]labelled S1ⁿ DNA duplexes (20 nM) were combined with 50 nM PARP1 in the presence of 50 μM NAD⁺ for 15 min at 37°C under standard reaction conditions. The data on PARP-catalysed formation of DNA ADP-ribosylation products are presented as mean ± SD from three independent experiments.

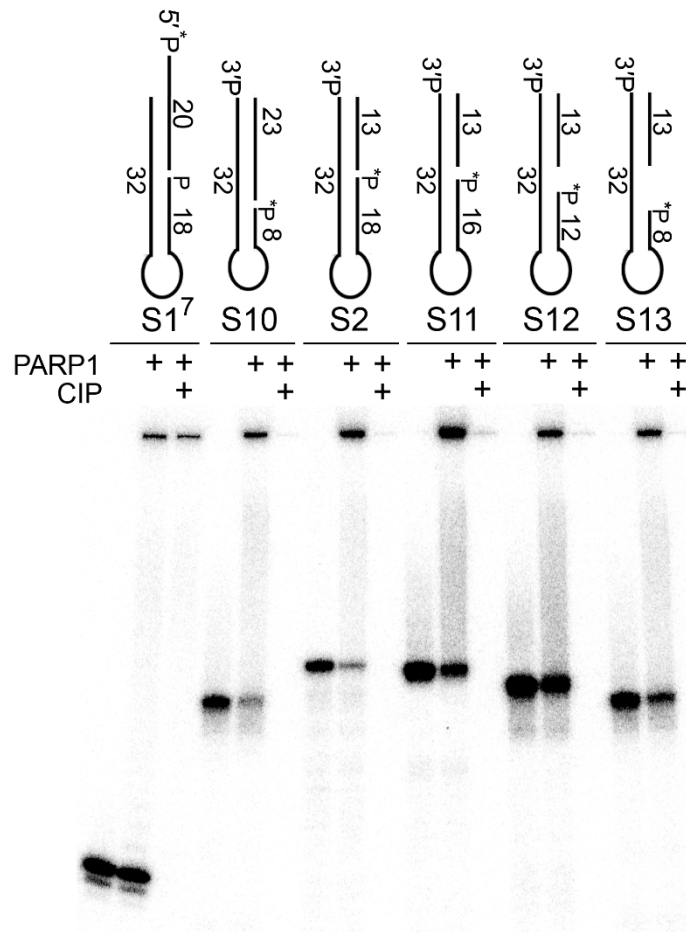


Figure S3. Validation of PARP1-dependent PARylation of 3'-phosphorylated DSB termini of DNA substrates S2 and S10-13 by CIP-induced dephosphorylation. After incubation with PARP1, the 5'-[³²P]labelled DNA samples were heated for 10 min at 85°C, and the resulting [³²P]labelled DNA PARylation products were further incubated with 10 U of CIP for 30 min at 37°C.

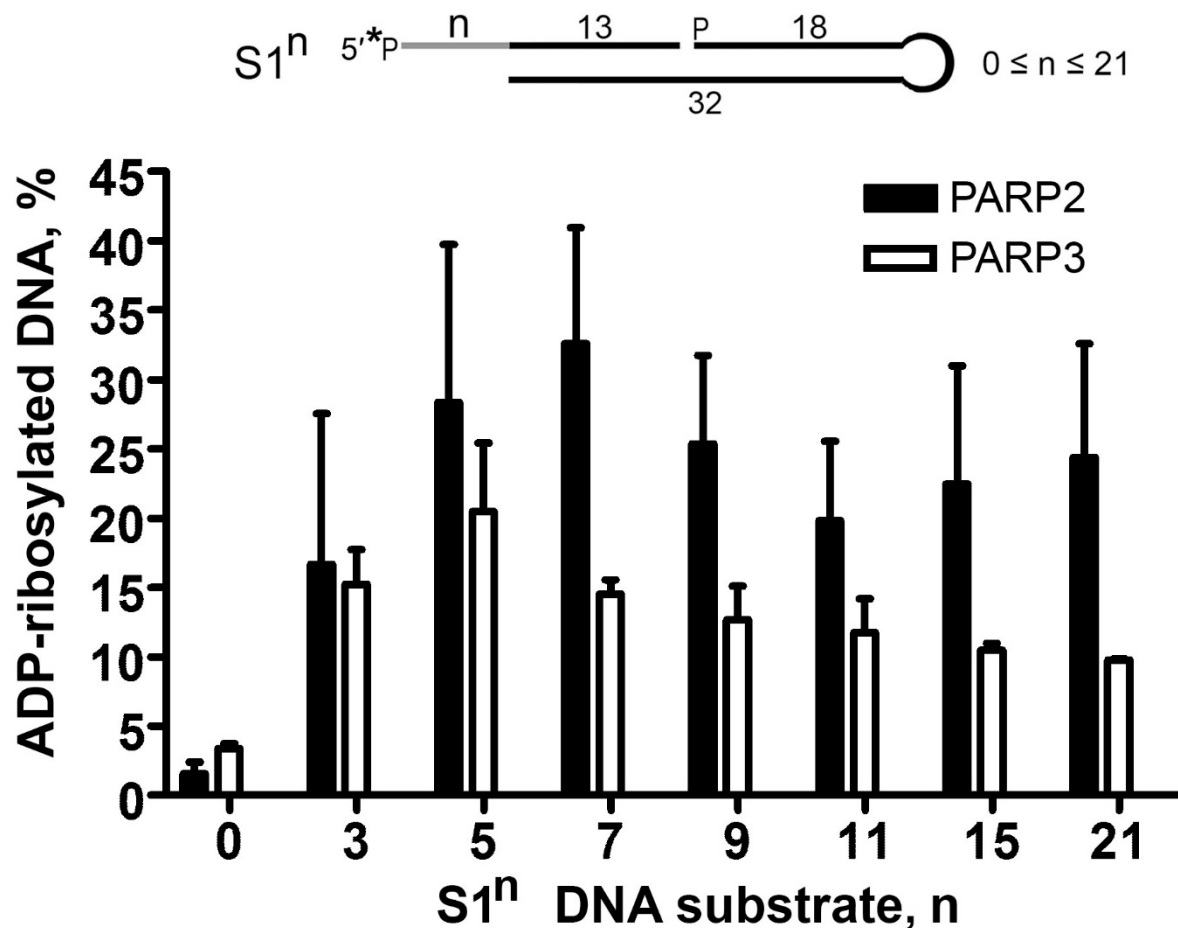


Figure S4. ADP-ribosylation of S1ⁿ DNA duplexes containing 5'-otherhangs by PARP2 and PARP3. [³²P]labelled S1ⁿ DNA duplexes (20 nM) were combined with 50 nM PARP3 or PARP2 in the presence of 1 mM NAD⁺ in ADPR buffer (20 mM HEPES-KOH pH 7.6, 50 mM KCl, 5 mM MgCl₂, 1 mM DTT and 100 μg/mL BSA). The mixture was incubated for 10 min (PARP3) or 30 min (PARP2) at 37°C. The data on PARP-catalysed formation of DNA ADP-ribosylation products are presented as mean ± SD from three independent experiments.