## *Supplementary data*

# Characterisation of DNA ADP-ribosyltransferase activities of PARP2 and PARP3: new insights into DNA ADP-ribosylation

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

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| N° 1 (5′-[$^{32}$P]labelled ExoA•RexT$^{nick}$) | $^{32}$P– ExoA / p– pExo19f<br>5′ GTGGCGCGGAGACTTAGAGAA ATTTGGCGCGGGGAATTCC<br>CACCGCGCCTCTGAATCTCTT-TAAACCGCGCCCCTTAAGG 5′<br>RexT |
| N° 2 | $^{32}$P– ExoA / p– pExo18f<br>5′ GTGGCGCGGAGACTTAGAGAA TTTGGCGCGGGGAATTCC<br>CACCGCGCCTCTGAATCTCTTTAAACCGCGCCCCTTAAGG<br>RexT |
| N° 3 | $^{32}$P– ExoA / p– pExo17f<br>5′ GTGGCGCGGAGACTTAGAGAA TTGGCGCGGGGAATTCC<br>CACCGCGCCTCTGAATCTCTTTAAACCGCGCCCCTTAAGG<br>RexT |
| N° 4 | $^{32}$P– ExoA / p– pExo14f<br>5′ GTGGCGCGGAGACTTAGAGAA GCGCGGGGAATTCC<br>CACCGCGCCTCTGAATCTCTTTAAACCGCGCCCCTTAAGG<br>RexT |
| N° 5 | $^{32}$P– ExoA<br>5′ GTGGCGCGGAGACTTAGAGAA<br>CACCGCGCCTCTGAATCTCTTTAAACCGCGCCCCTTAAGG<br>RexT |
| N° 6 | p–CTTAGT–<br>$^{32}$P– ExoA<br>5′ GTGGCGCGGAGACTTAGAGAA ATTTGGCGCGGGGAATTCC<br>CACCGCGCCTCTGAATCTCTT-TAAACCGCGCCCCTTAAGG<br>RexT |
| N° 7 | $^{32}$P– ExoA / Exo19f<br>5′ GTGGCGCGGAGACTTAGAGAA ATTTGGCGCGGGGAATTCC<br>CACCGCGCCTCTGAATCTCTT-TAAACCGCGCCCCTTAAGG<br>RexT |

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| N° 8 | $^{32}P$– ExoA Exo19f<br>5′ GTGGCGCGGAGACTTAGAGAA ATTTGGCGCGGGGAATTCC<br>CACCGCGCCTCTGAATCTCTT-TAAACCGCGCCCCTTAAGG-P<br>RexT-p |
| N° 9 | $^{32}P$– Exo10<br>5′ GTGGCGCGGA<br>CACCGCGCCTCTGAATCTCTTTAAACCGCGCCCCTTAAGG<br>RexT |
| N° 10 | $^{32}P$– Exo10 P– pExo30f<br>5′ GTGGCGCGGA GACTTAGAGAAATTTGGCGCGGGGAATTCC<br>CACCGCGCCT-CTGAATCTCTTTAAACCGCGCCCCTTAAGG<br>RexT |
| N° 11 | $^{32}P$– Exo30<br>5′ GTGGCGCGGAGACTTAGAGAAATTTGGCGC<br>CACCGCGCCTCTGAATCTCTTTAAACCGCGCCCCTTAAGG<br>RexT |
| N° 12 | $^{32}P$– Exo30 P– pExo10f<br>5′ GTGGCGCGGAGACTTAGAGAAATTTGGCGC GGGGAATTCC<br>CACCGCGCCTCTGAATCTCTTTAAACCGCG-CCCCTTAAGG<br>RexT |
| N° 13 | $^{32}P$– ExoA P– pExo19f<br>5′ GTGGCGCGGAGACTTAGAGAA ATTTGGCGCGGGGAATTCC<br>ACCGCGCCTCTGAATCTCTT-TAAACCGCGCCCCTTAAGG<br>Rex39 |
| N° 14 | $^{32}P$– ExoA P– pExo19f<br>5′ GTGGCGCGGAGACTTAGAGAA ATTTGGCGCGGGGAATTCC<br>CGCCTCTGAATCTCTT-TAAACCGCGCCCCTTAAGG<br>Rex35 |
| N° 15 | $^{32}P$– ExoA P– pExo19f<br>5′ GTGGCGCGGAGACTTAGAGAA ATTTGGCGCGGGGAATTCC<br>CTGAATCTCTT-TAAACCGCGCCCCTTAAGG<br>Rex30 |
| N° 16 | $^{32}P$– ExoA P– pExo19f<br>5′ GTGGCGCGGAGACTTAGAGAA ATTTGGCGCGGGGAATTCC<br>TCACCGCGCCTCTGAATCTCTT-TAAACCGCGCCCCTTAAGG<br>RexT-T |
| N° 17 | $^{32}P$– ExoA P– pExo19f P– p10RT<br>5′GTGGCGCGGAGACTTAGAGAA ATTTGGCGCGGGGAATTCC TGACTGCATA<br>CACCGCGCCTCTGAATCTCTT-TAAACCGCGCCCCTTAAGG-ACTGACGTAT<br>Re50m |

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| N° 18 | p- pExoA $^{32}$P- Exo19f p- p10RT<br>5'GTGGCGCGGAGACTTAGAGAA ATTTGGCGCGGGGAATTCC TGACTGCATA<br>CACCGCGCCTCTGAATCTCTT-TAAACCGCGCCCCTTAAGG-ACTGACGTAT<br>Re50m |
| N° 19 | p- ExoA p- pExo19f $^{32}$P- 10RT<br>5'GTGGCGCGGAGACTTAGAGAA ATTTGGCGCGGGGAATTCC TGACTGCATA<br>CACCGCGCCTCTGAATCTCTT-TAAACCGCGCCCCTTAAGG-ACTGACGTAT<br>Re50m |
| N° 20 | $^{32}$P- 10RT p- pExoA p- pExo19f<br>5'TGACTGCATA GTGGCGCGGAGACTTAGAGAA ATTTGGCGCGGGGAATTCC<br>ACTGACGTAT-CACCGCGCCTCTGAATCTCTT-TAAACCGCGCCCCTTAAGG<br>Re50 |
| N° 21 | p- p10RT $^{32}$P- ExoA p- pExo19f<br>5'TGACTGCATA GTGGCGCGGAGACTTAGAGAA ATTTGGCGCGGGGAATTCC<br>ACTGACGTAT-CACCGCGCCTCTGAATCTCTT-TAAACCGCGCCCCTTAAGG<br>Re50 |
| N° 22 | p- p10RT p- pExoA $^{32}$P- Exo19f<br>5'TGACTGCATA GTGGCGCGGAGACTTAGAGAA ATTTGGCGCGGGGAATTCC<br>ACTGACGTAT-CACCGCGCCTCTGAATCTCTT-TAAACCGCGCCCCTTAAGG<br>Re50 |
| N° 23 | $^{32}$P- p10RT p- pExo40f<br>5'TGACTGCATA GTGGCGCGGAGACTTAGAGA-AATTTGGCGCGGGGAATTCC<br>ACTGACGTAT-CACCGCGCCTCTGAATCTCT TTAAACCGCGCCCCTTAAGG<br>-p<br>Re50 |
| N° 24 | $^{32}$P- Exo19f<br>5' ATTTGGCGCGGGGAATTCC<br>CACCGCGCCTCTGAATCTCTTTAAACCGCGCCCCTTAAGG<br>RexT |
| N° 25 | ExoA $^{32}$P- Exo19f<br>5' GTGGCGCGGAGACTTAGAGAA ATTTGGCGCGGGGAATTCC<br>CACCGCGCCTCTGAATCTCTT-TAAACCGCGCCCCTTAAGG<br>RexT |
| N° 26 | p- ExoA $^{32}$P- Exo19f<br>5' GTGGCGCGGAGACTTAGAGAA ATTTGGCGCGGGGAATTCC<br>CACCGCGCCTCTGAATCTCTT-TAAACCGCGCCCCTTAAGG<br>RexT |
| N° 27 | ExoA $^{32}$P- Exo19f<br>5'GTGGCGCGGAGACTTAGAGAA ATTTGGCGCGGGGAATTCC<br>CACCGCGCCTCTGAATCTCTT-TAAACCGCGCCCCTTAAGG-p<br>RexT-p |

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| N° 28 | $^{32}$P- pExo40f<br>5' GTGGCGCGGAGACTTAGAGAAATTTGGCGCGGGGAATTCC<br>CACCGCGCCTCTGAATCTCTTTAAACCGCGCCCCTTAAGG<br>RexT |
| N° 29 | $^{32}$P- pExo40f<br>5'GTGGCGCGGAGACTTAGAGAAATTTGGCGCGGGGAATTCC<br>CACCGCGCCTCTGAATCTCTTTAAACCGCGCCCCTTAAGG-P<br>RexT-p |
| N° 30 | $^{32}$P- 10RT<br>5' TGACTGCATA<br>ACTGACGTATTCGTACATCTGCTACACGTA<br>RT-T |
| N° 31 | $^{32}$P- 10RT P- p19RT<br>5' TGACTGCATA GCATGTAGACGATGTGCAT<br>ACTGACGTATTCGTACATCTGCTACACGTA<br>RT-T |
| N° 32 | $^{32}$P- pExo19f P- ExoA<br>5' ATTTGGCGCGGGGAATTCC GTGGCGCGGAGACTTAGAGAA<br>TAAACCGCGCCCCTTAAGG-CACCGCGCCTCTGAATCTCTT<br>TxeR |
| N° 33 | P- pExo19f $^{32}$P- ExoA<br>5' ATTTGGCGCGGGGAATTCC GTGGCGCGGAGACTTAGAGAA<br>TAAACCGCGCCCCTTAAGG-CACCGCGCCTCTGAATCTCTT<br>TxeR |
| N° 34 | $^{32}$P- pExo30f<br>5' GACTTAGAGAAATTTGGCGCGGGGAATTCC<br>CACCGCGCCTCTGAATCTCTTTAAACCGCGCCCCTTAAGG<br>RexT |
| N° 35 | P- Exo10 $^{32}$P- pExo30f<br>5' GTGGCGCGGA GACTTAGAGAAATTTGGCGCGGGGAATTCC<br>CACCGCGCCT-CTGAATCTCTTTAAACCGCGCCCCTTAAGG<br>RexT |
| N° 36 | $^{32}$P- Exo 10f<br>5' GGGGAATTCC<br>CACCGCGCCTCTGAATCTCTTTAAACCGCGCCCCTTAAGG<br>RexT |
| N° 37 | P- Exo30 $^{32}$P- Exo 10f<br>5' GTGGCGCGGAGACTTAGAGAAATTTGGCGC GGGGAATTCC<br>CACCGCGCCTCTGAATCTCTTTAAACCGCG-CCCCTTAAGG<br>RexT |
| N° 38 | $^{32}$P- Exo10 P- pExo19f<br>5' GTGGCGCGGA ATTTGGCGCGGGGAATTCC<br>CACCGCGCCTCTGAATCTCTTTAAACCGCGCCCCTTAAGG<br>RexT |

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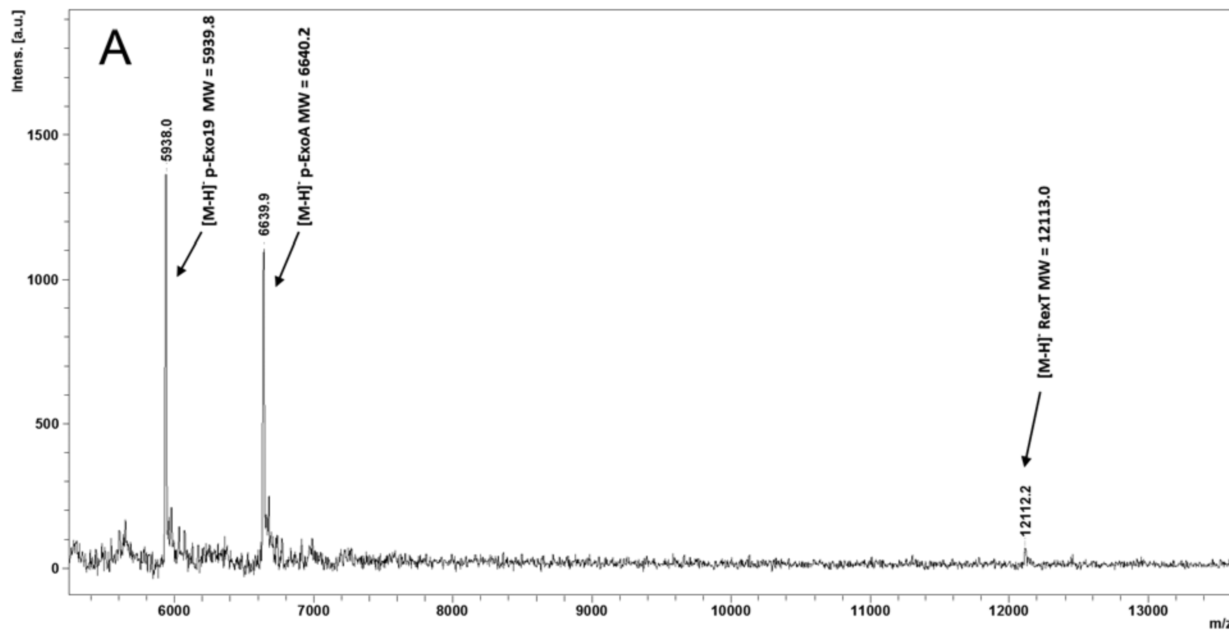
| N° 39 | P- Exo10 $^{32}$P- pExo19f<br>5' GTGGCGCGGA ATTTGGCGCGGGGAATTCC<br>CACCGCGCCTCTGAATCTCTTTAAACCGCGCCCCTTAAGG<br>RexT |
| N° 40 | $^{32}$P- pExo40f<br>5' GTGGCGCGGAGACTTAGAGA-AATTTGGCGCGGGGAATTCC<br>CACCGCGCCTCTGAATCTCT TTAAACCGCGCCCCTTAAGG<br>-P<br>RexT |
| N° 41 | $^{32}$P- pExo20<br>5' GTGGCGCGGAGACTTAGAGA<br>CACCGCGCCTCTGAATCTCT<br>Re20 |
| N° 42 | P- Exo10 $^{32}$P- pExo40f<br>5' TGACTGCATA GTGGCGCGGAGACTTAGAGA-AATTTGGCGCGGGGAATTCC<br>ACTGACGTAT-CACCGCGCCTCTGAATCTCT TTAAACCGCGCCCCTTAAGG<br>-P<br>Re50 |
| N° 43 | $^{32}$P- ExoA P- pExo19f<br>5' **G**TGGCGCGGAGACTTAGAGAA ATTTGGCGCGGGGAATTCC<br>**T**ACCGCGCCTCTGAATCTCTT-TAAACCGCGCCCCTTAAGG<br>Re40 (mm G:T 1) |
| N° 44 | $^{32}$P- ExoA P- pExo19f<br>5' GTG**G**CGCGGAGACTTAGAGAA ATTTGGCGCGGGGAATTCC<br>CAC**T**GCGCCTCTGAATCTCTT-TAAACCGCGCCCCTTAAGG<br>Re40 (mm G:T 4) |
| N° 45 | $^{32}$P- ExoA P- pExo19f<br>5' GTGGCGCGGAGACTTAGAGAA **A**TTTGGCGCGGGGAATTCC<br>CACCGCGCCTCTGAATCTCTT-**G**AAACCGCGCCCCTTAAGG<br>Re40 (mm A:G 22) |
| N° 46 | $^{32}$P- ExoA P- pExo19f<br>5' GTGGCGCGGAGACTTAGAGA**A** ATTTGGCGCGGGGAATTCC<br>CACCGCGCCTCTGAATCTCT**G**-TAAACCGCGCCCCTTAAGG<br>Re40 (mm A:G 21) |



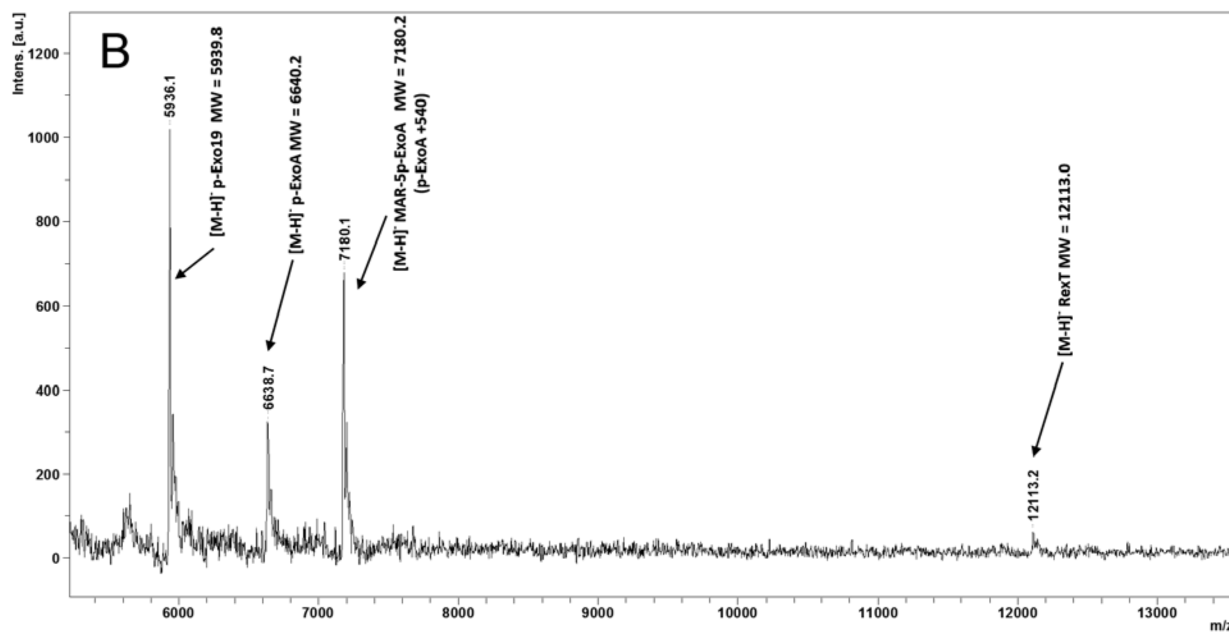




**Figure S1. MALDI-TOF mass spectrometric analysis of the mixture of oligonucleotides resulting from the incubation of the nicked oligonucleotide duplex with PARP3.** The 5′P-ExoA•RexT$^{nick}$ duplex at 10 μM was incubated with 2.8 μM PARP3 in the presence of 1 mM $NAD^+$ for 10 min at 37°C. After that, the reaction products were precipitated with 2% lithium perchlorate in acetone, then dissolved in water and subjected to the MALDI-TOF mass spectrometry. MALDI mass spectra were acquired in negative mode on a time-of-flight Microflex mass spectrometer (Bruker, Wissembourg, France), equipped with a 337-nm nitrogen laser and pulsed delay source extraction. The matrix was prepared by dissolving 3-hydroxypicolinic acid in 10 mM ammonium citrate buffer with a small amount of the Dowex-50W 50x8-200 cation exchange resin (Sigma). The matrix (1 μL) was added to the sample (1 μL) on the target plate and allowed to dry. The spectra were calibrated using reference oligonucleotides of known masses. (**A**) A MALDI TOF mass spectrum of the control untreated 5′P-ExoA•RexT$^{nick}$ duplex; (**B**) A MALDI TOF mass spectrum of the reaction products after incubation of 5′P-ExoA•RexT$^{nick}$ with PARP3.

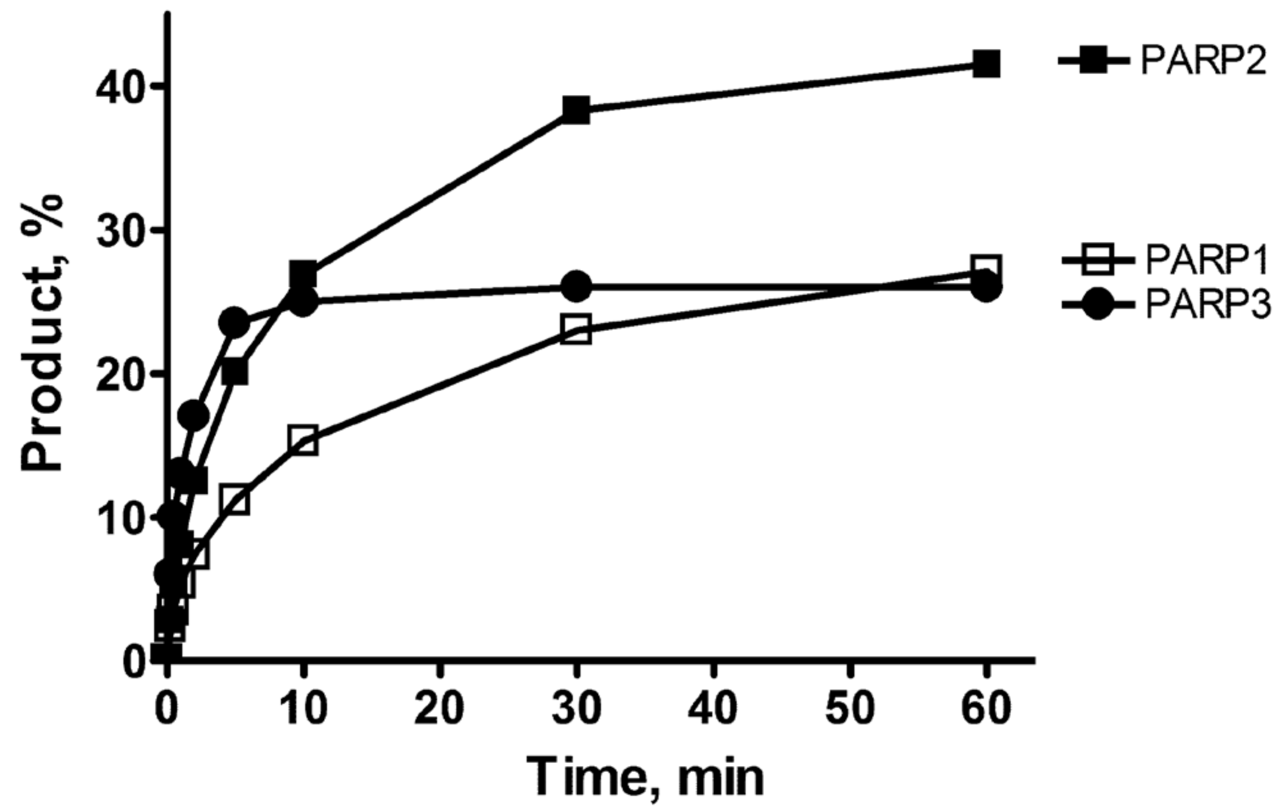



**Figure S2. Time dependence of PARP-catalysed DNA ADP-ribosylation.** Twenty nM PARP2 or PARP3 was incubated with 50 nM 5′-[$^{32}$P]labelled ExoA•RexT$^{nick}$ duplex in the presence of 1 mM NAD$^{+}$ at 37°C. Fifty nM PARP1 was incubated with 50 nM 5′-[$^{32}$P]labelled RexT•ExoA recessed duplex (DNA substrate N° 5, but 5′-[$^{32}$P]label was on RexT and not on ExoA oligonucleotide) in the presence of 1 mM NAD$^{+}$ at 37°C. The graph represents three independent experiments; error bars are too small to be seen at the scale used.

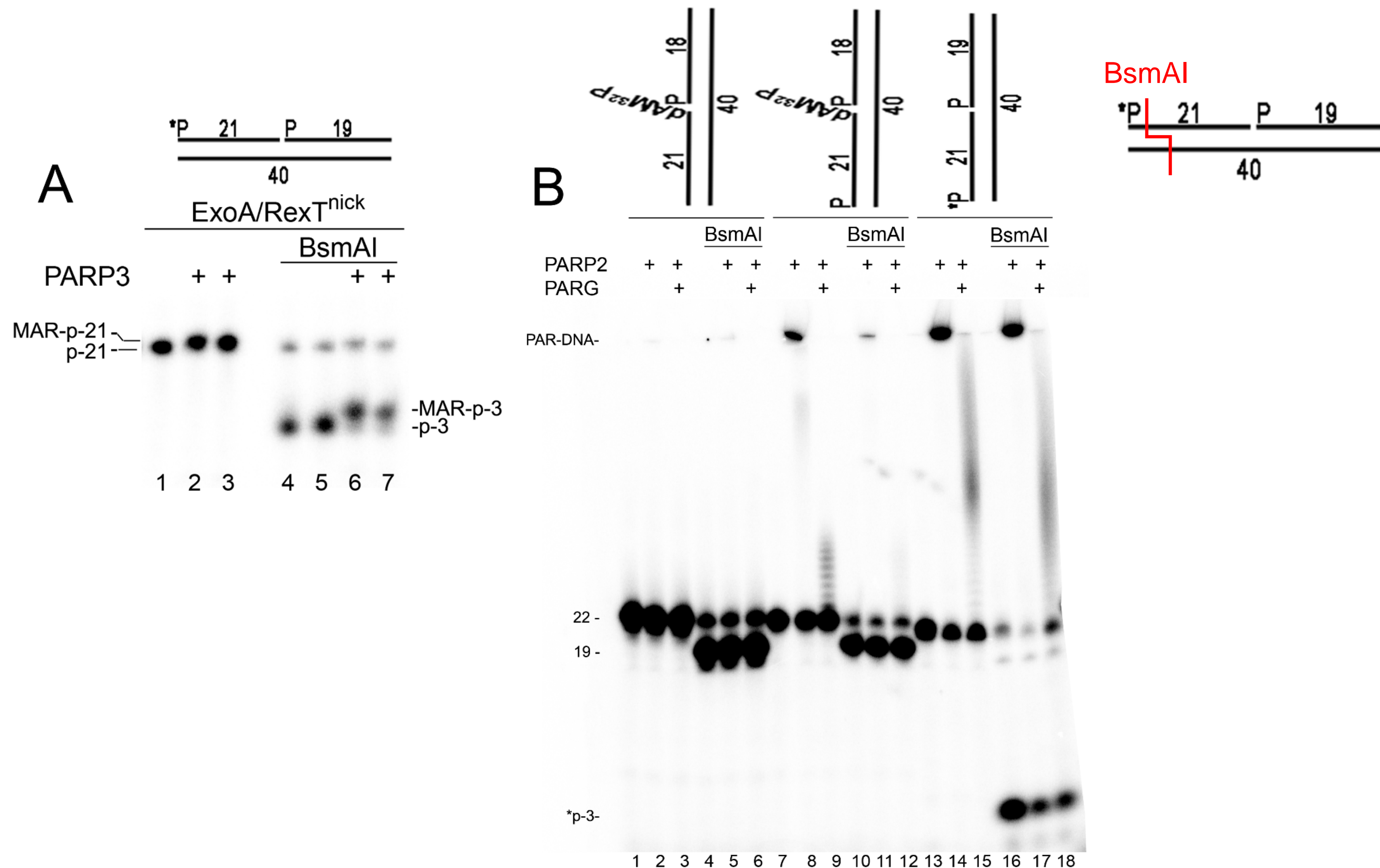



**Figure S3. Action of the *BsmA*I restriction enzyme on the PARP3- or PARP2-generated ADP-ribosylated oligonucleotides.** (**A**) The 5′-[$^{32}$P]labelled ExoA•RexT$^{nick}$ duplex (40 nM) was incubated with PARP3 (500 nM) under standard reaction conditions. After that, the samples were heated at 80°C for 20 min, supplemented with $MgCl_2$ up to 10 mM and incubated with 5 U of *BsmA*I (New England Biolabs, France) for 1 h at 55°C. Lanes 3 and 7 show repeats of the experiments in lanes 2 and 6, respectively. (**B**) The 3′-[3′dAM$^{32}$P]labelled ExoA•RexT$^{nick}$ duplex (20 nM) with or without 5′ terminal phosphate, as well as 5′-[$^{32}$P]labelled ExoA•RexT$^{nick}$ duplex (20 nM) were incubated with PARP2 (100 nM) under standard reaction conditions. After 20-min incubation at 80°C, the samples were treated with 20 nM PARG for 1 h at 37°C, and then incubated with 2.5 U of *BsmA*I for 1 h at 55°C. Products of the digestion reactions were analysed as describes in *Materials and Methods*.

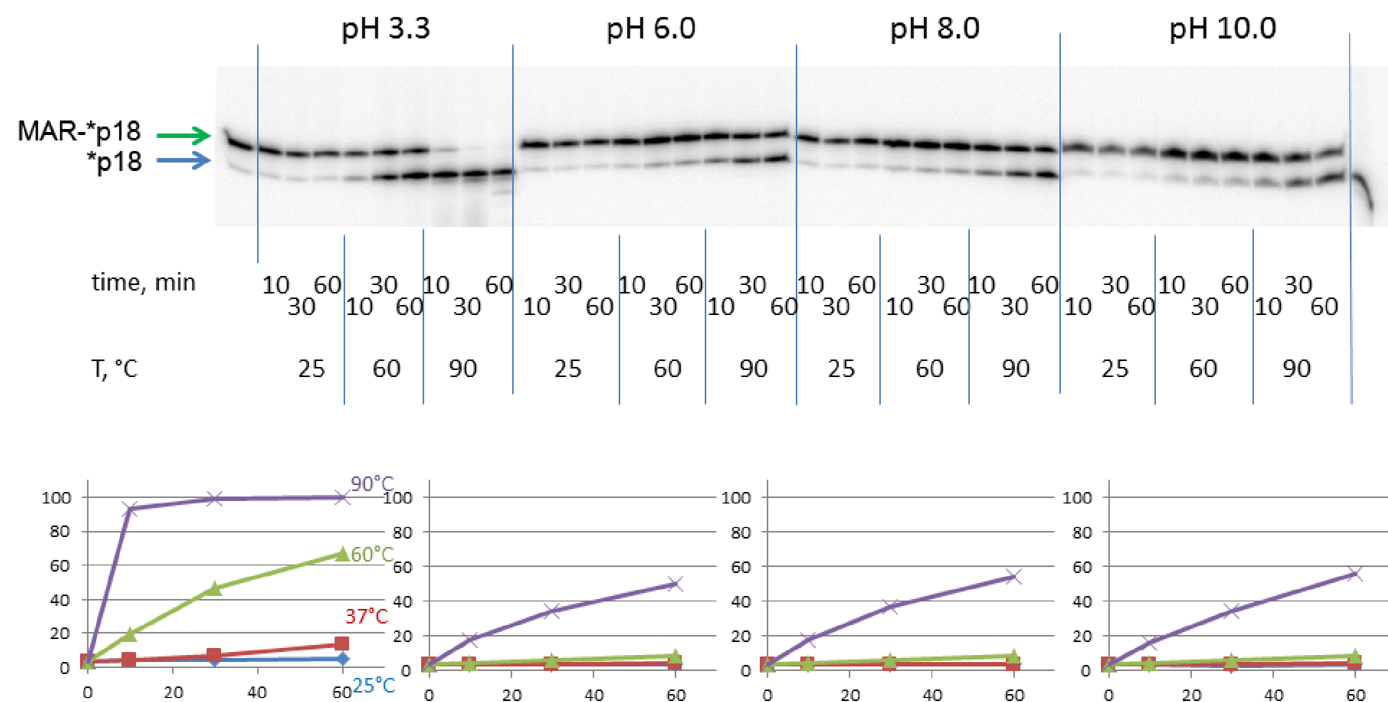



**Figure S4. Effects of pH and temperature on the stability of the linkage between the 5′ terminal phosphate and ADP-ribose in the DNA–ADP-ribose adduct.** To produce a MARylated oligonucleotide, 5 µM PARP3 was incubated with 1 µM 5′-[$^{32}$P]labelled KB34$^{nick}$ duplex, formed by annealing of oligonucleotides GGCTTCATCGTTGTCTCAGACCTGGTGGATACCG (34mer), 5′-[$^{32}$P]labelled CGGTATCCACCAGGTCTG (18mer) and 5′ phosphorylated AGACAACGATGAAGCC (16mer) (5'→3'), in 0.4 mL of a buffer consisting of 20 mM HEPES-KOH pH 8.6, 0.25 mg/mL BSA, 0.5 mM DTT, 2 mM $MgCl_2$ and 1 mM $NAD^+$ at 37ºC for 45 min. The reaction mixture was heated at 85ºC for 2 min and the MARylated [$^{32}$P]labelled oligonucleotide was purified on denaturing 20% (w/v) polyacrylamide gels containing 7 M urea and 10% formamide in TBE buffer. After passive elution from the corresponding gel band at 4°C for a few hours in 1 mL of 150 mM NaCl, the MARylated [$^{32}$P]labelled oligonucleotide was precipitated by 4% $LiClO_4$ in acetone and dissolved in water. The stability of the ADP-ribose–DNA adduct was examined at different pH levels and temperatures. The reaction mixtures (final volume 10 µL) containing 20 nM MARylated [$^{32}$P]labelled oligonucleotide in different buffers (100 mM sodium acetate, pH 3.3; 100 mM sodium acetate, pH 6.0; 100 mM Tris-HCl, pH 8.0; 100 mM $NH_4OH$-HCl, pH 10.0) were incubated at 25°C, 37°C, 60°C or 90°C for 10, 30 and 60 min.

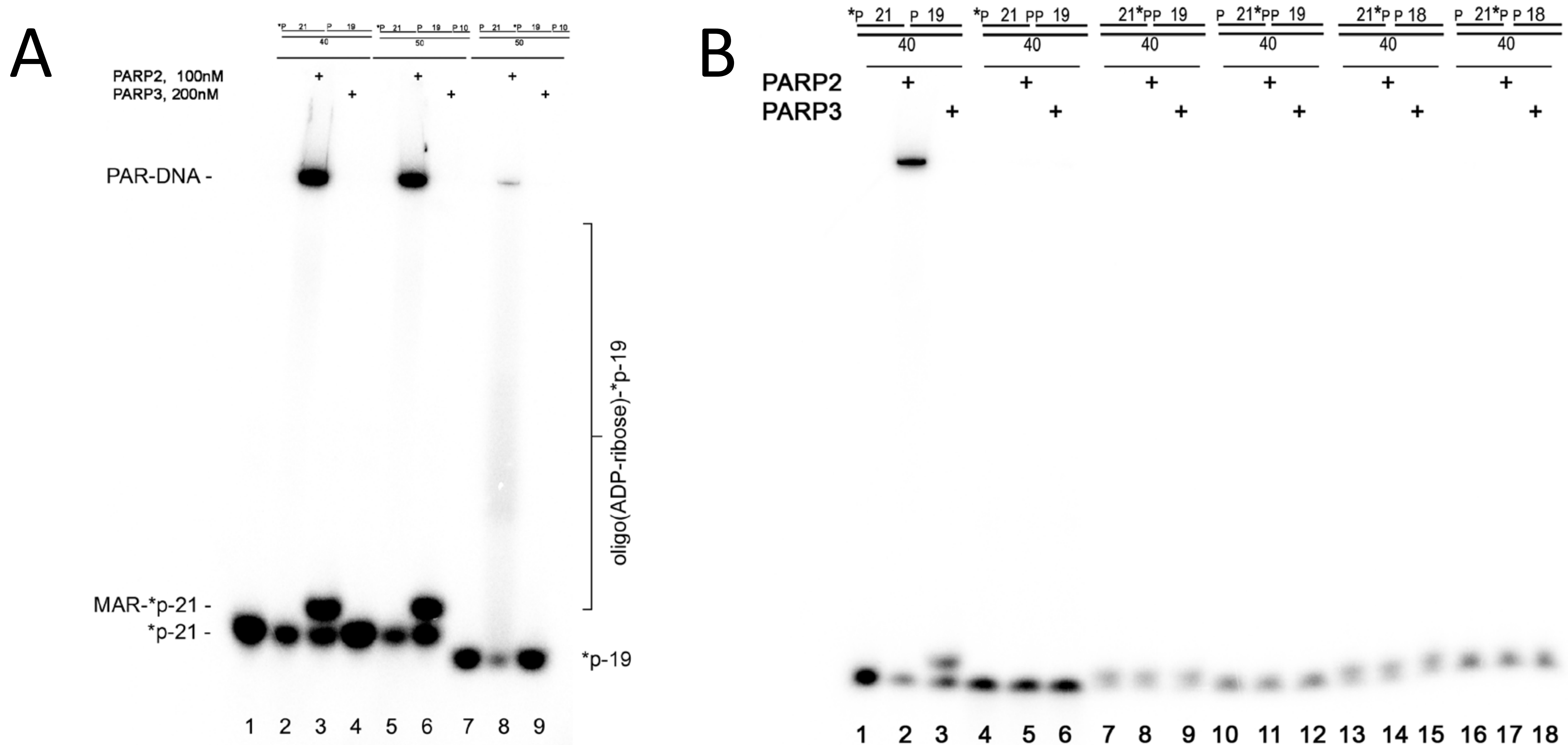



**Figure S5. Effects of a second nick or a 3′ phosphate at the site of the nick on the PARP3- or PARP2-catalysed formation of MAR- and PAR–DNA adducts, respectively.** (**A**) DNA substrates containing a radioactive phosphate ($^{32}P$) at the 5′ DSB terminus (substrates N° 1 [lanes 1–3] and N° 17 [lanes 4–6]) or at the 5′ end of the nick (substrate N° 18 [lanes 7–9]) were incubated with PARP3 or PARP2 under standard reaction conditions. (**B**) PARP3 (100 nM) or PARP2 (50 nM) activity towards DNA substrates (20 nM) containing a terminal phosphate at 3′ end of the nick (lanes 4–18). To generate a DNA duplex containing a 3′-terminal $^{32}P$ residue (lanes 7–18), the 3′-[3′dAM$^{32}$P]labelled ExoA•RexT recessed duplex was incubated with 100 nM tyrosyl-DNA phosphodiesterase 1 (Tdp1) in a buffer consisting of 50 mM Tris-HCl pH 7.5 and 25 mM KCl for 2 h at 25°C. After 20 min incubation at 80°C, the recessed duplex was annealed with Exo19f to generate the 3′-[3′dAM$^{32}$P]labelled ExoA•RexT$^{nick}$ substrate.

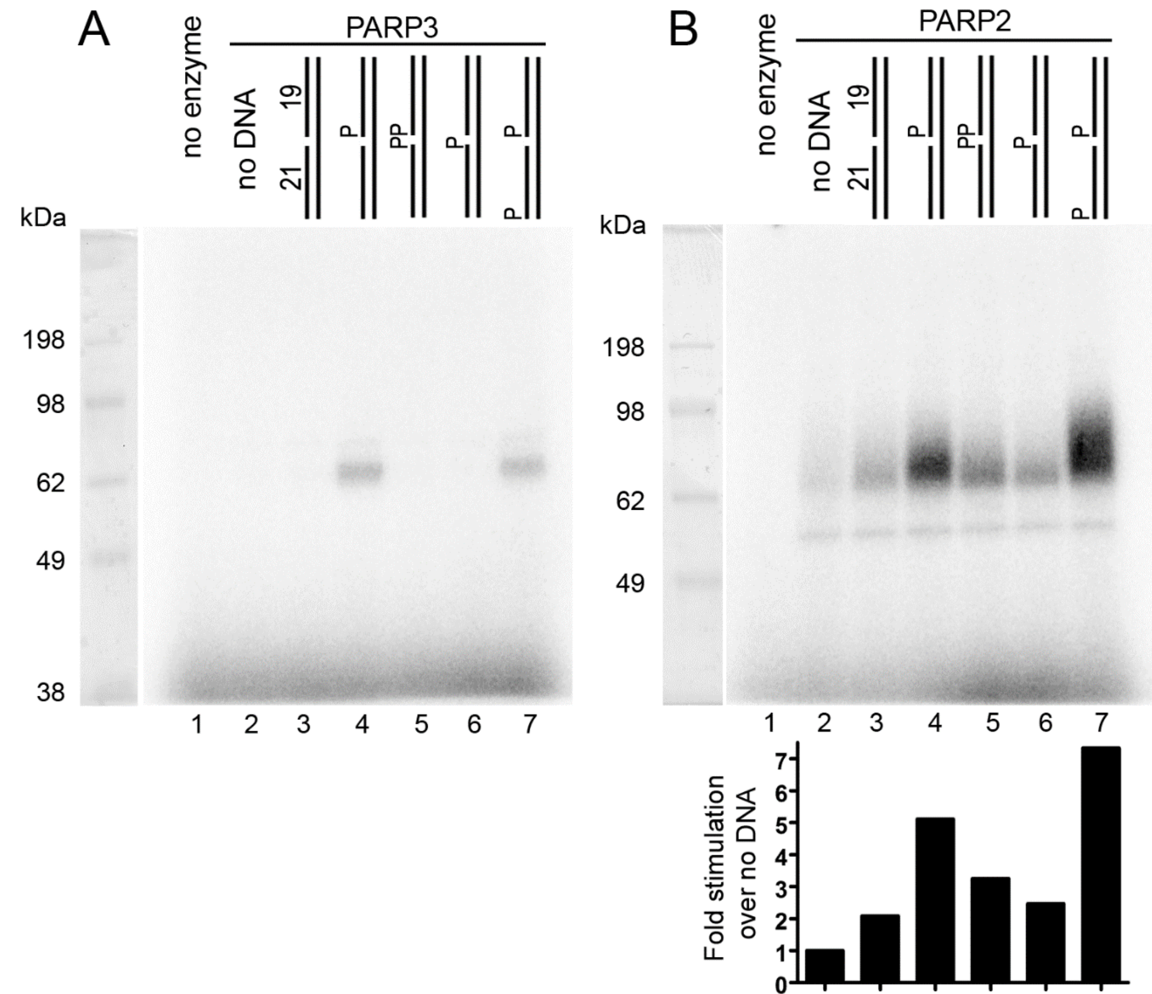



**Figure S6. Influence of the 3′-terminal phosphate residue at the nick site of DNA oligonucleotides on PARP3- or PARP2-catalysed auto-ADP-ribosylation.** One μM PARP3 (**A**) or PARP2 (**B**) was incubated with 1 μM cold ExoA•RexT$^{nick}$-based oligonucleotide duplexes in the presence of 1.5 μM [adenylate-$^{32}$P]NAD$^{+}$ in standard ADPR buffer for 30 min at 37°C. Oligonucleotide duplexes contained: no terminal phosphate (lane 3); terminal phosphate at 5′ end of the nick (lane 4); terminal phosphates at 3′ and 5′ ends of the nick (lane 5); terminal phosphate at 3′ end of the nick (lane 6); terminal phosphates at 5′ ends of the nick and the DSB terminus (lane 7). After heating for 1 min at 95°C in 1X LDS sample buffer (Invitrogen), the products of the reaction were separated on precast 10% SDS-PAGE gels (Invitrogen).

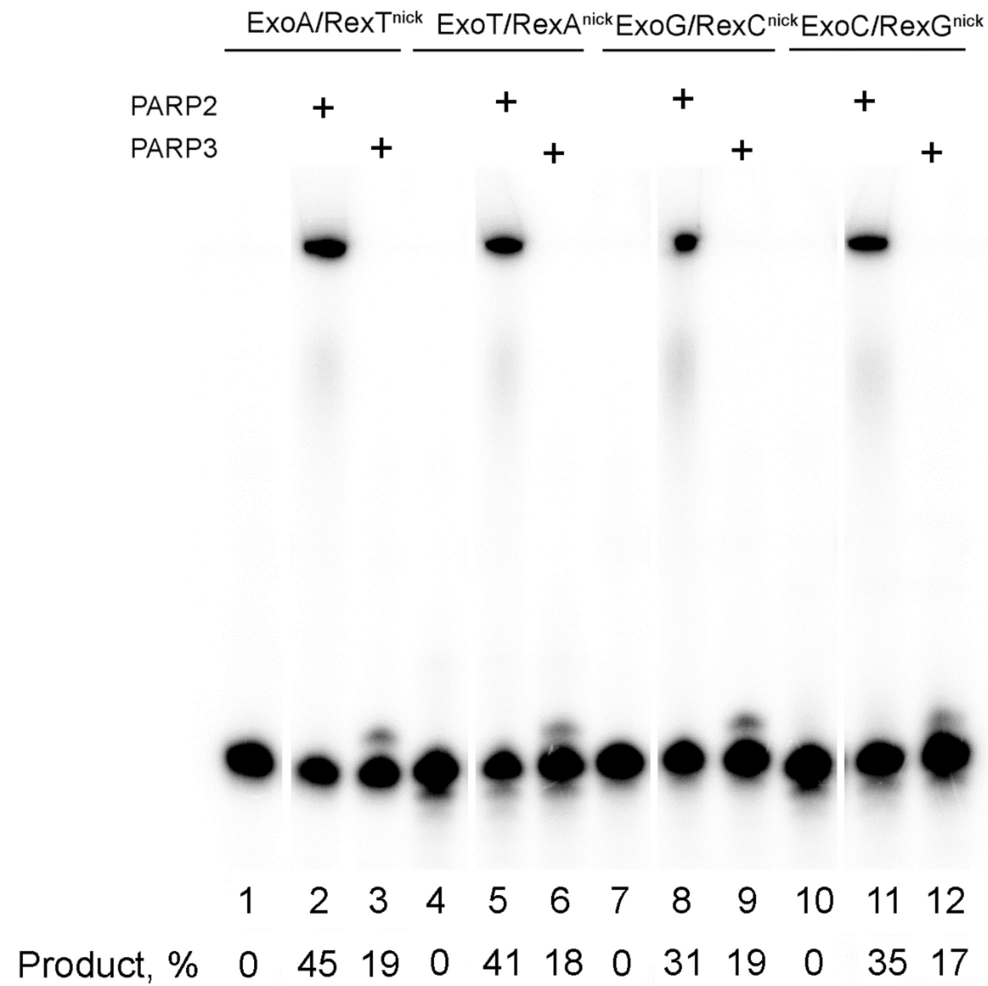



**Figure S7.** Effects of the nature of the DNA base at the 3′OH end of the nick. Twenty nM PARP2 or PARP3 was incubated with 40 nM 5′-[$^{32}$P]labelled DNA duplexes at standard reaction conditions. In Exo“X”•Rex“Y”$^{nick}$ duplexes “X” and “Y” indicate the nature of the 3′ terminal nucleotide in ExoA and the opposite one in complementary RexT oligonucleotide, respectively.

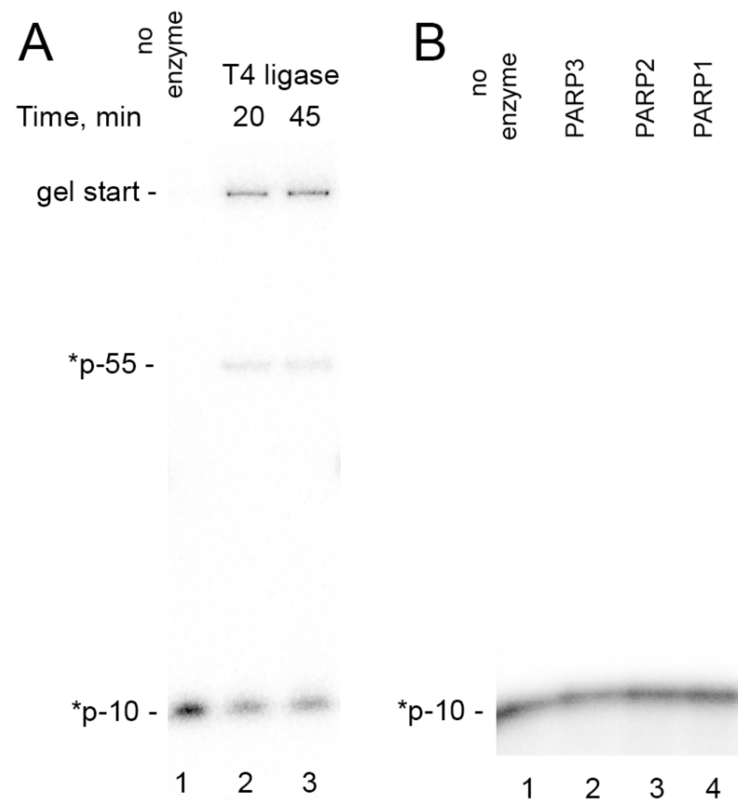



**Figure S8. A**) Presence of non-hybridised 5′-[$^{32}$P]labelled GAAACAACAG oligonucleotide in 5′-[$^{32}$P]labelled pML2 circular plasmid substrate containing a 1 nt gap placed upstream of two nicks. Two nM plasmid was incubated with 0.2 U/μL of T4 DNA ligase in T4 DNA Ligase Buffer (New England Biolabs, France) at 37°C for 20 or 45 min. **B**) Absence of PARPs DNA ADP-ribosylation activity on 5′-[$^{32}$P]labelled single-stranded GAAACAACAG oligonucleotide (*p-10 mer). Fifty nM PARPs were incubated with 1 nM 5′-[$^{32}$P]labelled GAAACAACAG oligonucleotide under the standard reaction conditions.

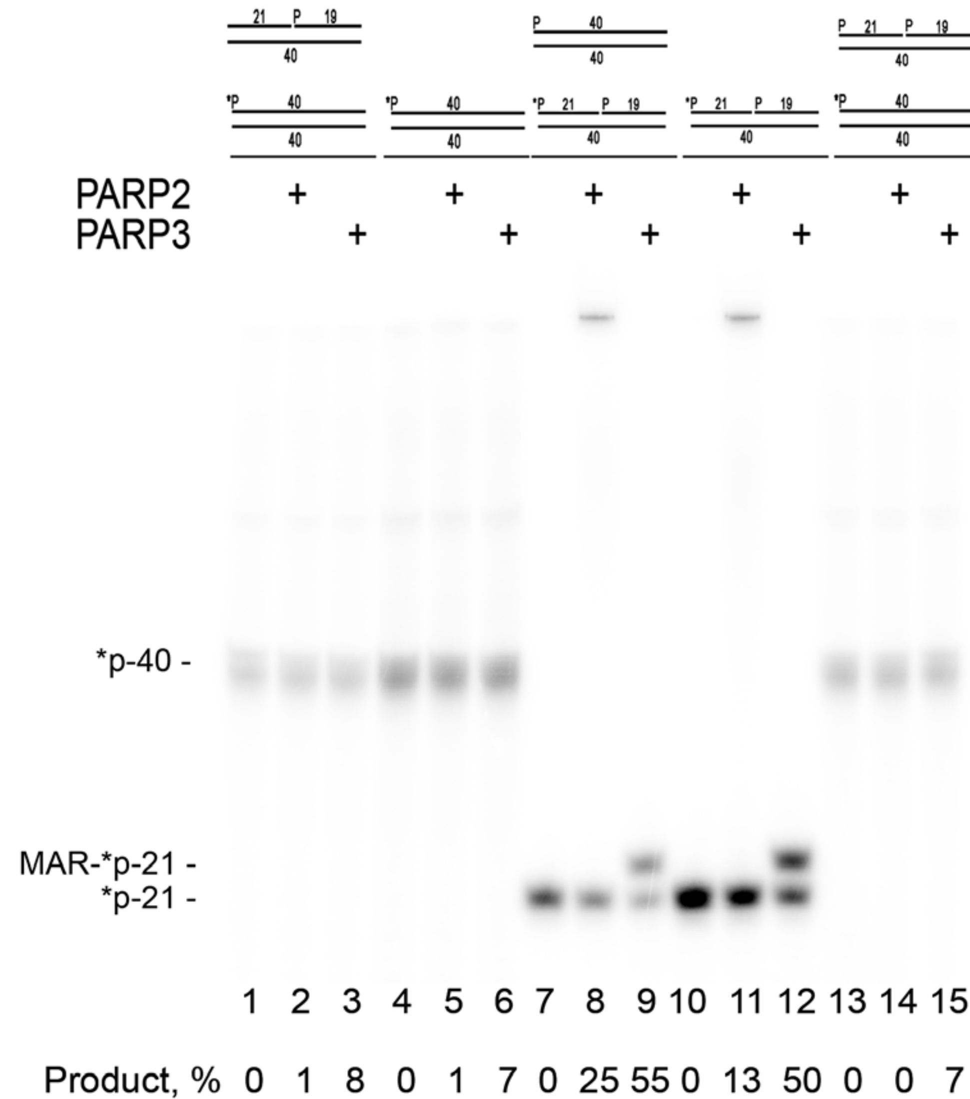



**Figure S9. DNA ADP-ribosylation activity of PARP3 and PARP2 towards DNA substrate mixtures.** The total concentration of ExoA•RexT$^{nick}$-based and 5′P-Exo40f•RexT oligonucleotide duplexes (substrate N° 27) was maintained at 40 nM. Cold oligonucleotide duplexes were mixed with an equimolar concentration (20 nM) of 5′-[$^{32}$P]labelled oligonucleotide duplexes (lanes 1–3, 7–9 and 13–15) prior to addition of a PARP. *P: [$^{32}$P] label.

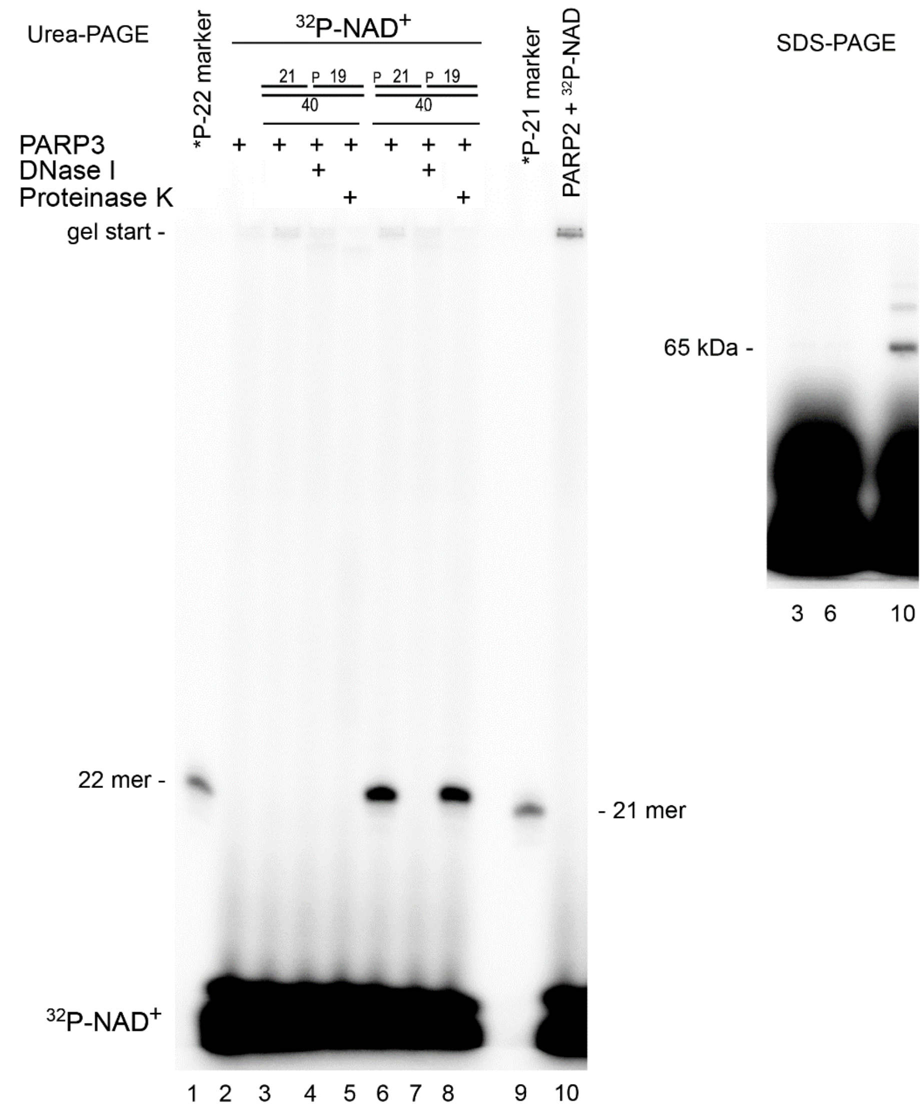



**Figure S10. Control experiments for comparison of efficiency of PARP3- or PARP2-catalysed auto- and DNA ADP-ribosylation with increased SDS concentrations during PAGE.** One µM PARP3 or PARP2 was incubated with 1 µM cold oligonucleotide duplexes in the presence of 0.5 µM [adenylate-$^{32}$P]NAD$^+$ for 30 min at 37°C under the standard reaction conditions. The reactions were terminated by the addition of a stop solution (7.5 M Urea, 2% SDS, 10 mM EDTA, bromophenol blue) at 1:1 (v/v), heated at 95°C for 2 min, and the products of the reactions were analysed by denaturing urea-PAGE but with 0.1% SDS in TBEx0.5 upper buffer and in 20% polyacrylamide. In parallel, several reaction samples (lane 3, 6 and 10) were separated on precast 10% SDS-PAGE gels (Invitrogen).

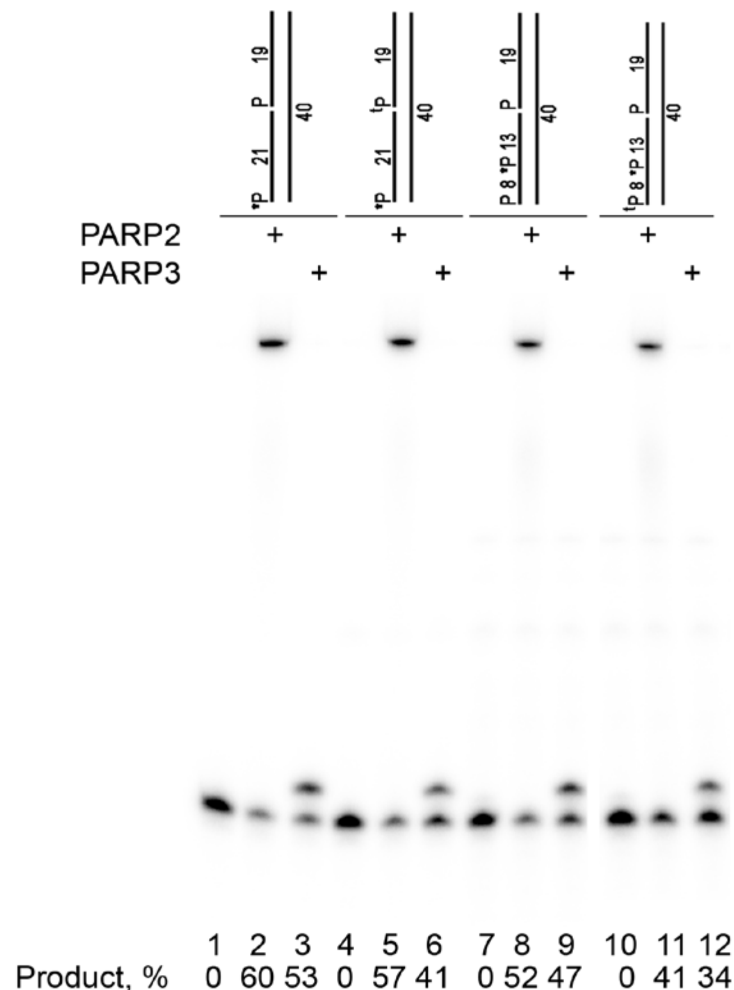



**Figure S11. Effects of terminal thiophosphate residues on PARP3- or PARP2- catalysed DNA ADP-ribosylation.** ExoA•RexT$^{nick}$-based oligonucleotide duplexes (32 nM) containing either a phosphate or a thiophosphate at the 5′ DSB termini or at the 5′ end of the nick were incubated with PARP3 or PARP2 under standard reaction conditions. DNA substrates containing an internal $^{32}$P label (*P) within the ExoA 21mer oligonucleotide sequence (lanes 7–12) were constructed by ligating two oligonucleotides: a cold 8mer oligonucleotide containing either a phosphate or a thiophosphate group at the 5′ end and a [$^{32}$P]labelled hot 13mer oligonucleotide that was pre-hybridised to the complementary 40mer RexT oligonucleotide. The ligation step was performed by means of 5 U of T4 DNA ligase (Thermo Fisher Scientific) at 37°C for 1 h in 1× T4 DNA ligase buffer (Thermo Fisher Scientific). After incubation at 80°C for 20 min, recessed duplexes were hybridised with the 5′-phosphorylated 19mer oligonucleotide to create the corresponding nicked duplexes.

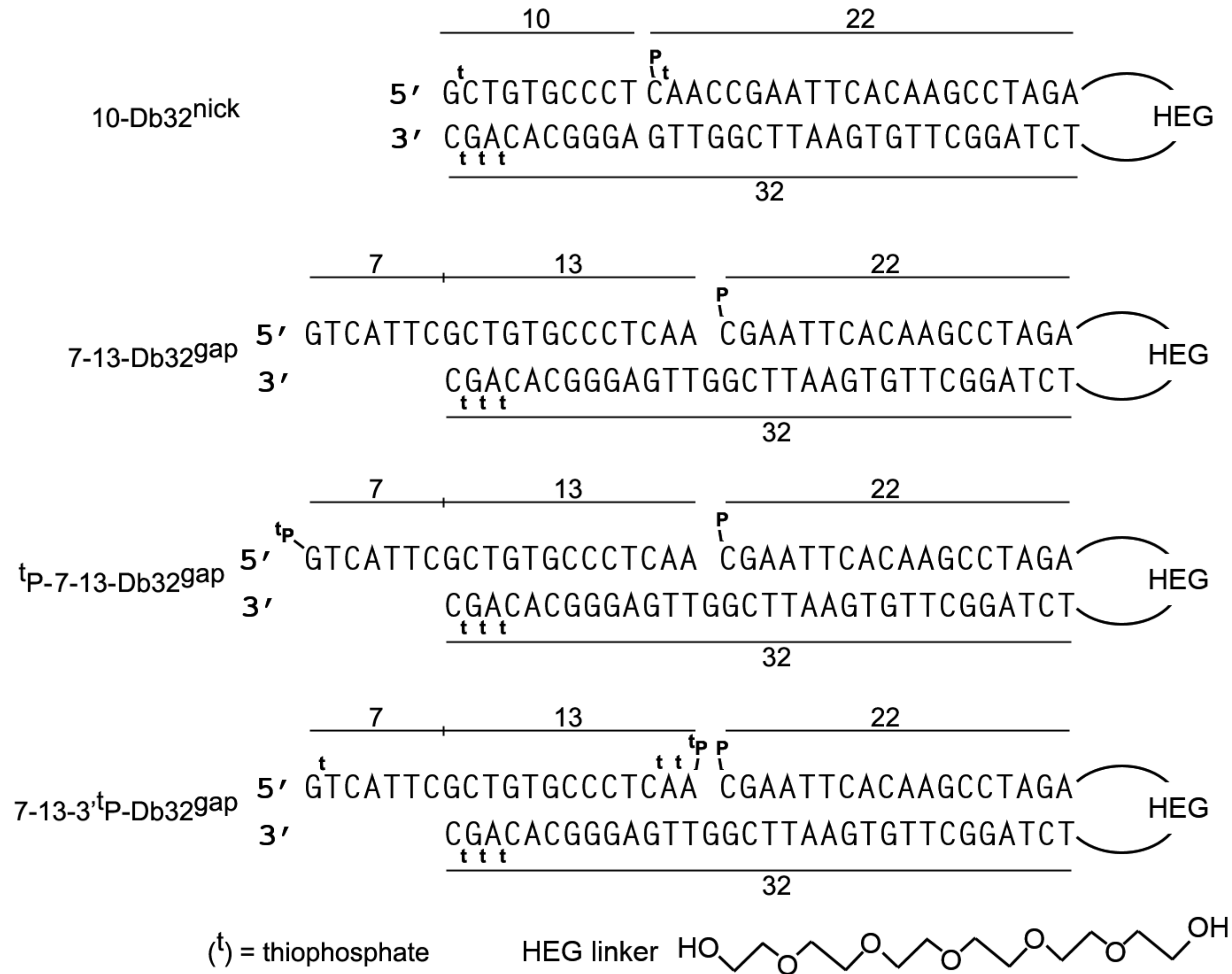



**Figure S12. Structures and sequences of the Dbait molecules used in this study.** Opposite strands of the duplexes were tethered together via a hexaethyleneglycol loop (HEG) at one DSB end and are protected against exonucleases by thiophosphate (ᵗP) groups.

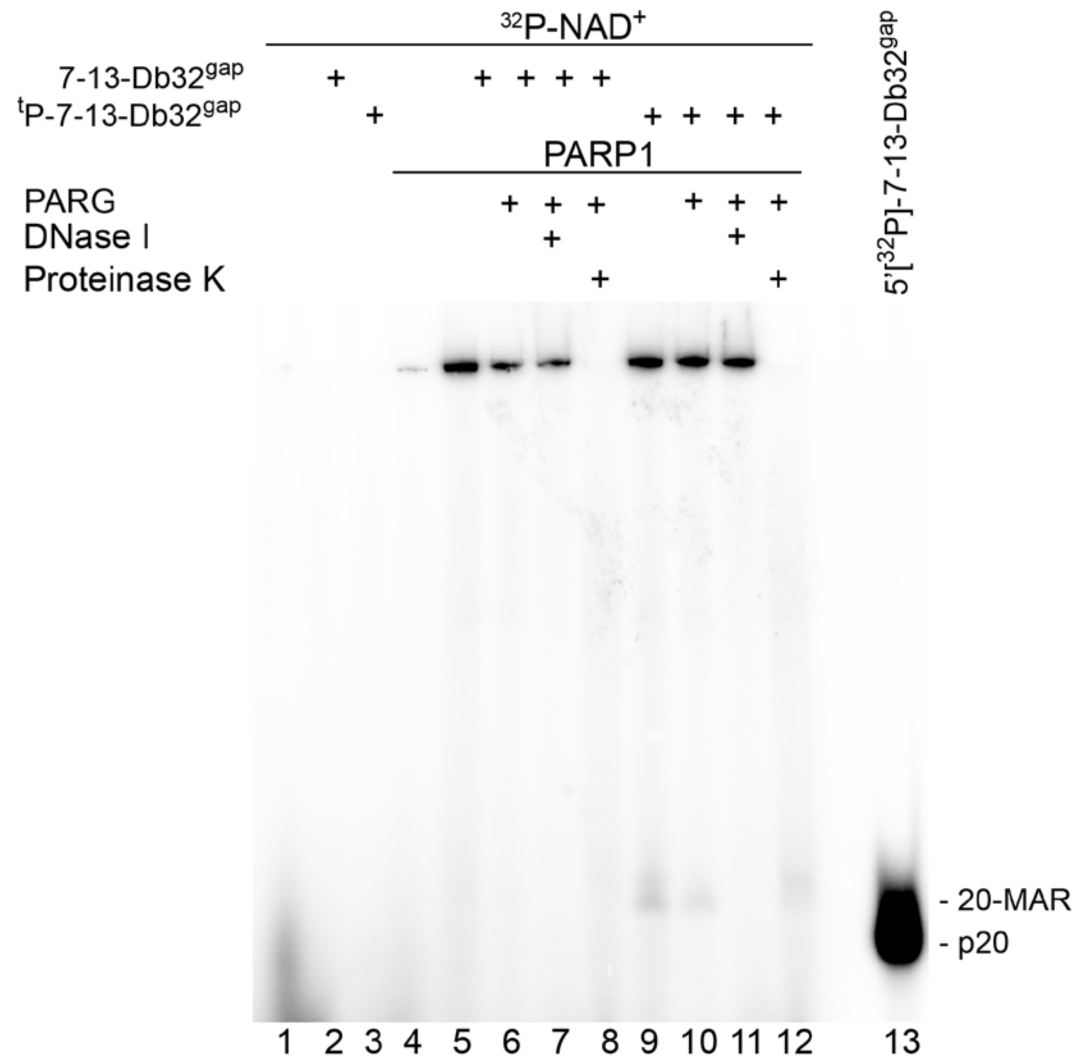



**Figure S13. Comparison of the efficiency of PARP1-catalysed auto- and DNA ADP-ribosylation.** The assay was performed as described for PARP2 (see Materials and Methods for details), except that the Dbait molecules $^t$P-7-13-Db32$^{gap}$ and 7-13-3′$^t$P-Db32$^{gap}$ served as substrates. The reaction products were analysed as described in *Materials and Methods*.

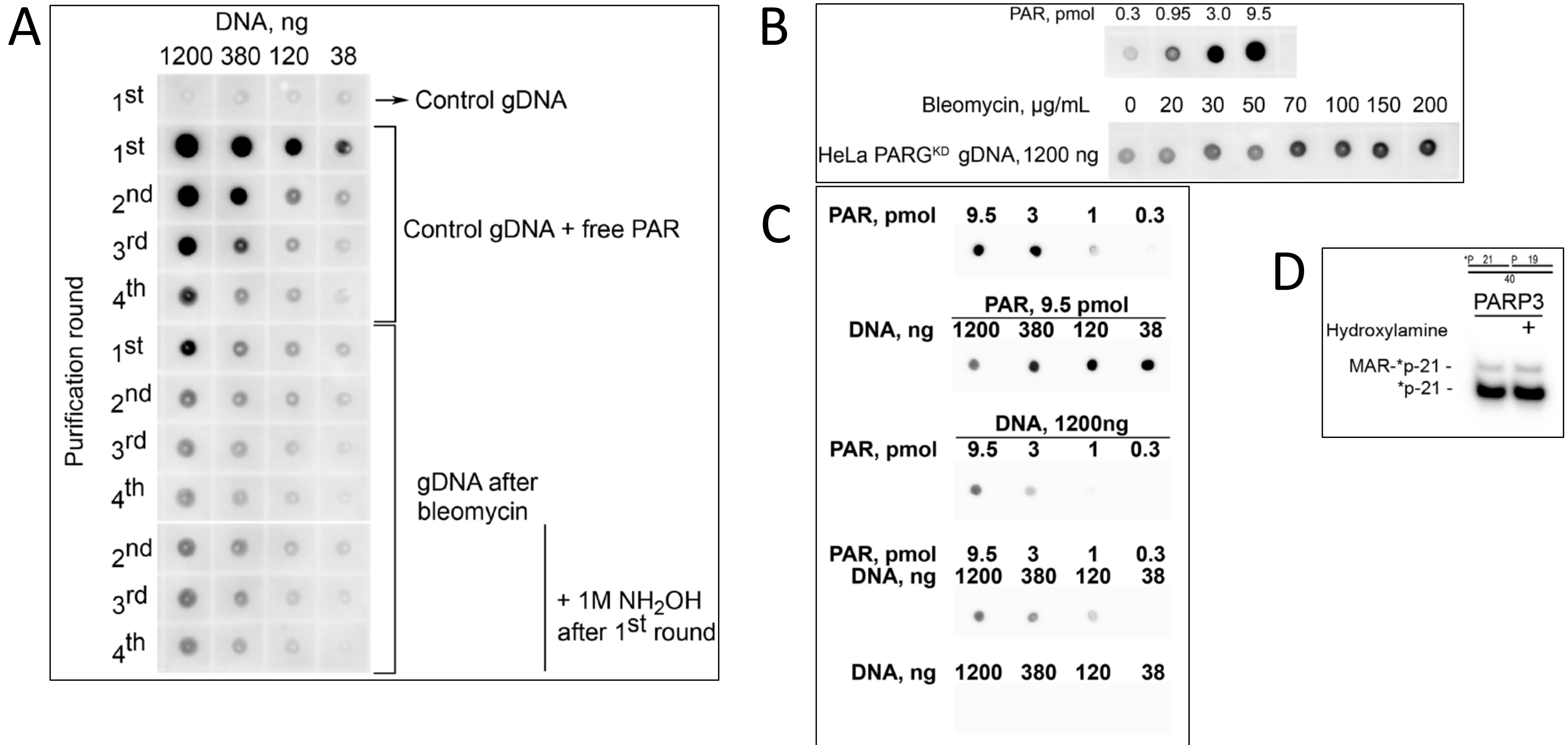



**Figure S14. Detection of PAR–DNA adducts in gDNA from HeLa PARG$^{KD}$ cells after bleomycin treatments.** (**A**) A representative image of anti-PAR dot blotting experiments conducted as described in *Materials and Methods*. (**B**) Dot blot images of free PAR and gDNA from HeLa PARG$^{KD}$ cells incubated with different doses of bleomycin after the first round of purification. (**C**) Dependence of the PAR signal in dot blotting experiments on the DNA presence. Different quantities of free PAR were mixed with different quantities of control gDNA in TE buffer (10 mM Tris-Cl pH 8.0, 1 mM EDTA) and loaded onto a nylon membrane, followed by anti-PAR dot blotting. (**D**) Hydroxylamine treatment of a MAR–DNA adduct. 5′[$^{32}$P]-ExoA•RexT$^{nick}$ was partially MARylated by PARP3, heat inactivated (20 min at 80°C) and incubated with 1 M hydroxylamine (pH 8.0) for 2 h at room temperature.