Supplementary Information

An uncharacterized FMAG_01619 protein from *Fusobacterium mortiferum* ATCC 9817 demonstrates that some bacterial macrodomains can also act as poly-ADP-ribosylhydrolases

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UniProt code	Superkingdom	Class	UniProt code	Superkingdom	Class	UniProt code	Superkingdom	Class
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A0A0B7A2D5	Eukaryota	Gastropoda	A0A218UDT5	Eukaryota	Aves	H3FC23	Eukaryota	Chromadorea
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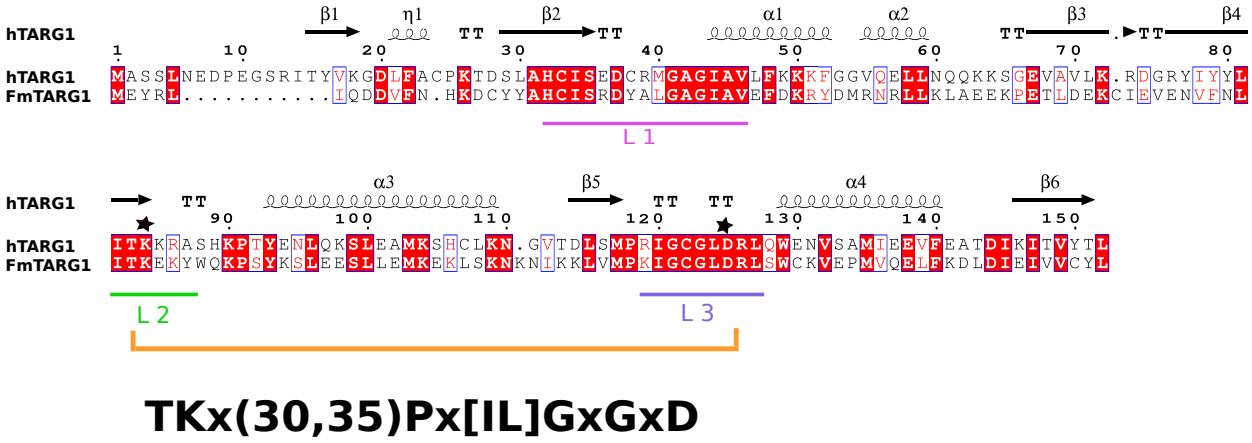
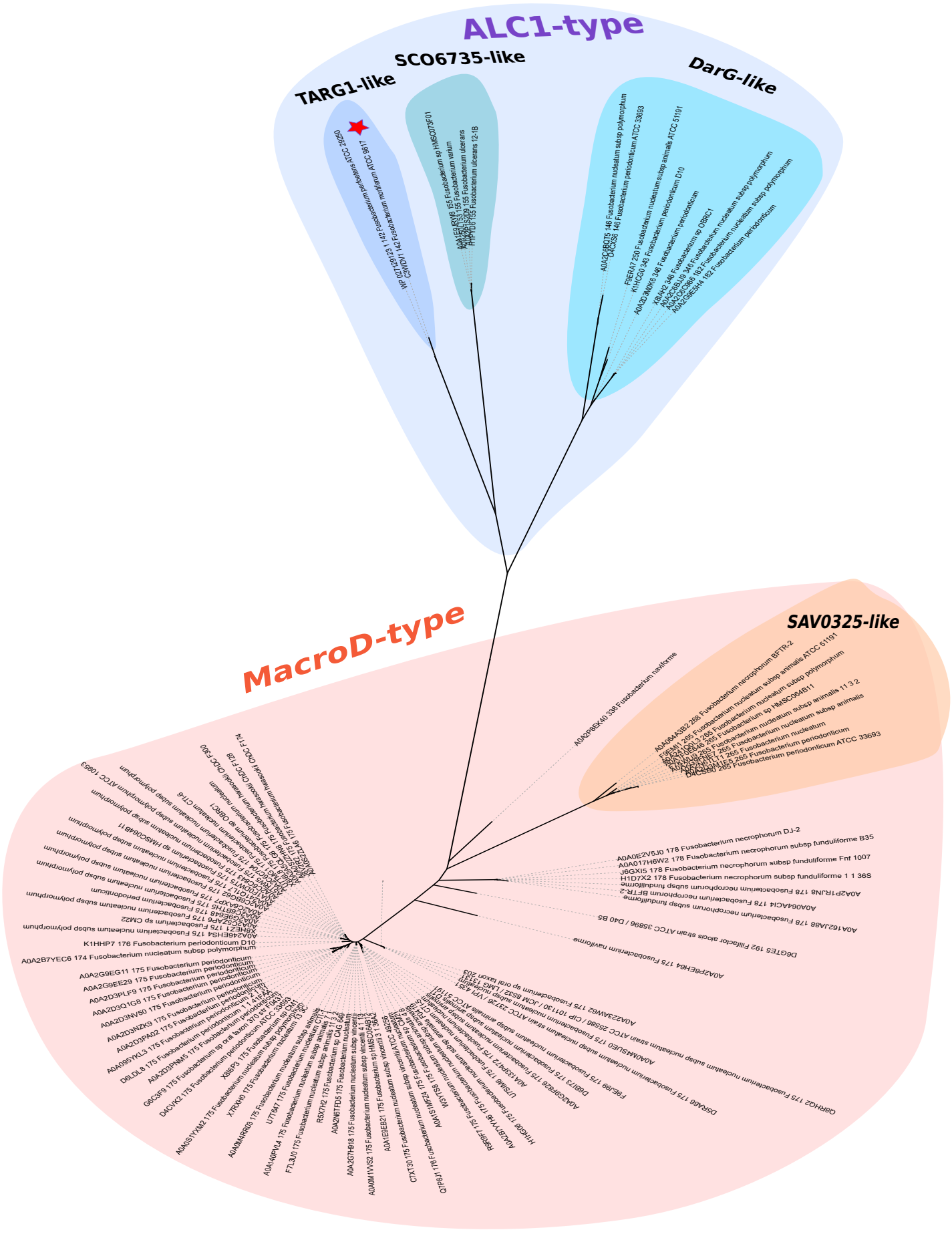
Supplementary Table S1. Eukaryotic TARG1-like sequences found in NCBI non-redundant (NR) and UniProt databases. These sequences were used in the phylogenetic analysis of Fig. 2. Sequences in italics represent the taxonomic level of Order.

UniProt code	Superkingdom	Class	species
A0A089HU69	Bacteria	Bacilli	*Paenibacillus durus*
A0A089IBX6	Bacteria	Bacilli	*Paenibacillus sp.* FSL H7-0357
A0A089J1F3	Bacteria	Bacilli	*Paenibacillus durus*
A0A089LZ90	Bacteria	Bacilli	*Paenibacillus stellifer*
A0A0A2UAG7	Bacteria	Bacilli	*Paenibacillus sp.* MAEPY2
A0A0B5AXM2	Bacteria	Bacilli	*Jeotgalibacillus malaysiensis*
A0A0G0CQ63	Bacteria	Candidatus	*candidate division* TM6 bacterium GW2011_GWF2_33_332
A0A0Q7T4C6	Bacteria	Bacilli	*Paenibacillus sp.* Root52
A0A0R1XH32	Bacteria	Bacilli	*Lactobacillus harbinensis* DSM 16991
A0A1B2DGN8	Bacteria	Bacilli	*Paenibacillus sp.* BIHB4019
A0A1C5N978	Bacteria	Clostridia	*uncultured Clostridium sp.*
A0A1G5EWD0	Bacteria	Clostridia	*Ruminococcus bromii*
A0A1G5FHU0	Bacteria	Bacilli	*Paenibacillus polysaccharolyticus*
A0A1G6RQD2	Bacteria	Bacilli	*Paenibacillus sp.* CF095
A0A1H3S2B1	Bacteria	Bacilli	*Thermoactinomyces sp.* DSM 45892
A0A1H8B9R9	Bacteria	Bacilli	*Paenibacillus sp.* OK076
A0A1R1FDX5	Bacteria	Bacilli	*Paenibacillus sp.* FSL R5-0765
A0A1S2L2Y3	Bacteria	Bacilli	*Anaerobacillus sp.* NB2006
A0A1V4G637	Bacteria	Bacilli	*Paenibacillus sp.* VT-16-81
A0A1V5SB14	Bacteria	Bacteroidetes	*Bacteroidetes bacterium* ADurb.Bin302
A0A209DKE5	Bacteria	Bacilli	*Saccharibacillus sp.* O16
A0A264DW85	Bacteria	Bacilli	*Paenibacillus taichungensis*
A0A268AZ29	Bacteria	Bacilli	*Paenibacillus sp.* 7523-1
A0A268SI35	Bacteria	Bacilli	*Paenibacillus sp.* 7516
A0A2E3BYV5	Bacteria	Alphaproteobacteria	*Magnetococcales bacterium*
A0A2G7LZ77	Bacteria	Bacilli	*Paenibacillus sp.* LK1
C3WDV1	Bacteria	Fusobacteriia	*Fusobacterium mortiferum* ATCC 9817
N1ZQV1	Bacteria	Clostridia	*Eubacterium plexicaudatum* ASF492
R9KG45	Bacteria	Clostridia	*Lachnospiraceae bacterium* A2
R9KYR0	Bacteria	Clostridia	*Lachnospiraceae bacterium* COE1
U4TVH3	Bacteria	Bacilli	*Lactobacillus shenzhenensis* LY-73
W4ANJ1	Bacteria	Bacilli	*Paenibacillus sp.* FSL R5-192
WP_027129123	Bacteria	Fusobacteriia	*Fusobacterium perfoetens*

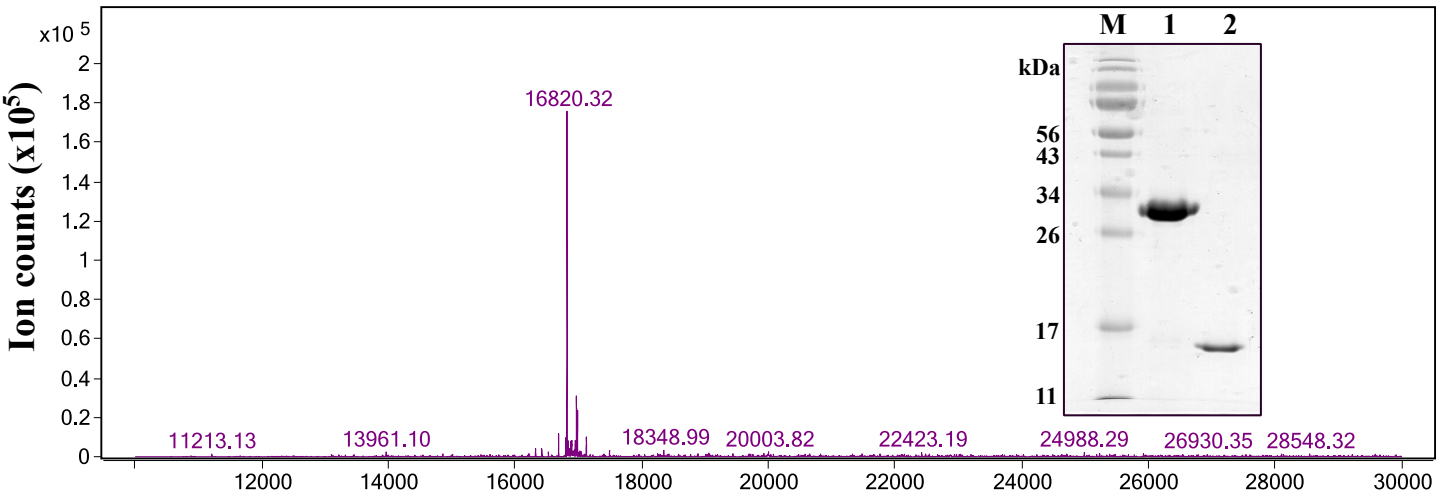
Supplementary Table S2. Bacterial TARG1-like sequences found in NCBI non-redundant (NR) and UniProt databases. These sequences were used in the phylogenetic analysis of Fig. 3. Sequences underlined represent not fully classified bacteria.



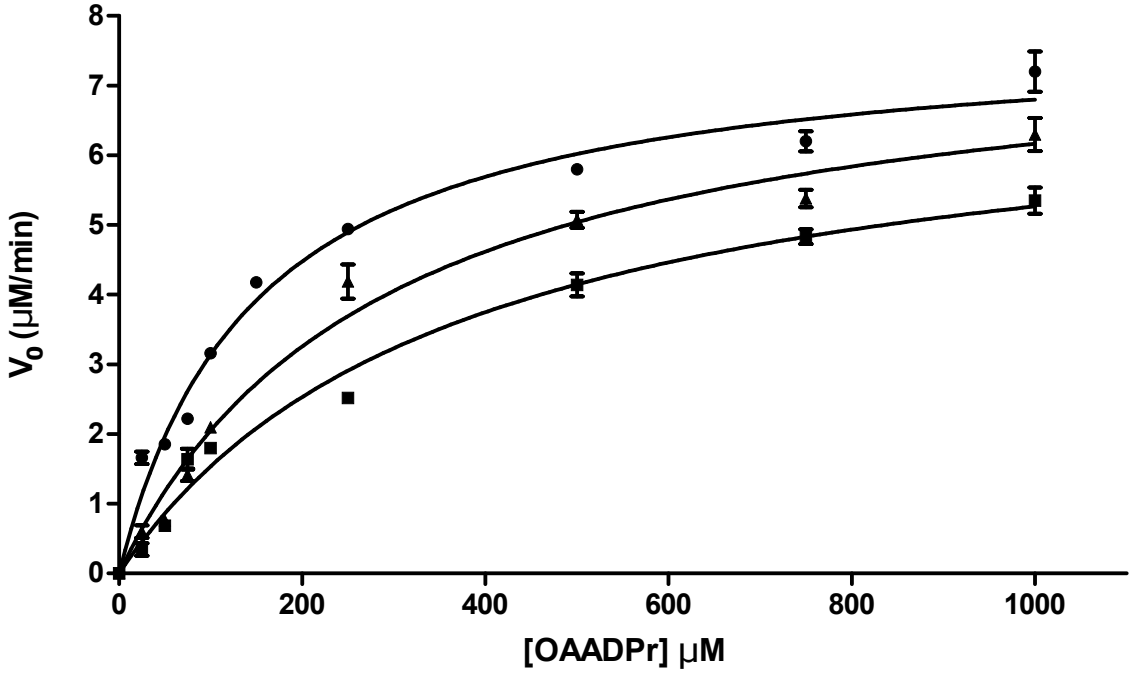
Supplementary Figure S1. The structure-based sequence alignment of FmTARG1 and hTARG1 sequences. The symbols and legends are the same as in Fig. 1.



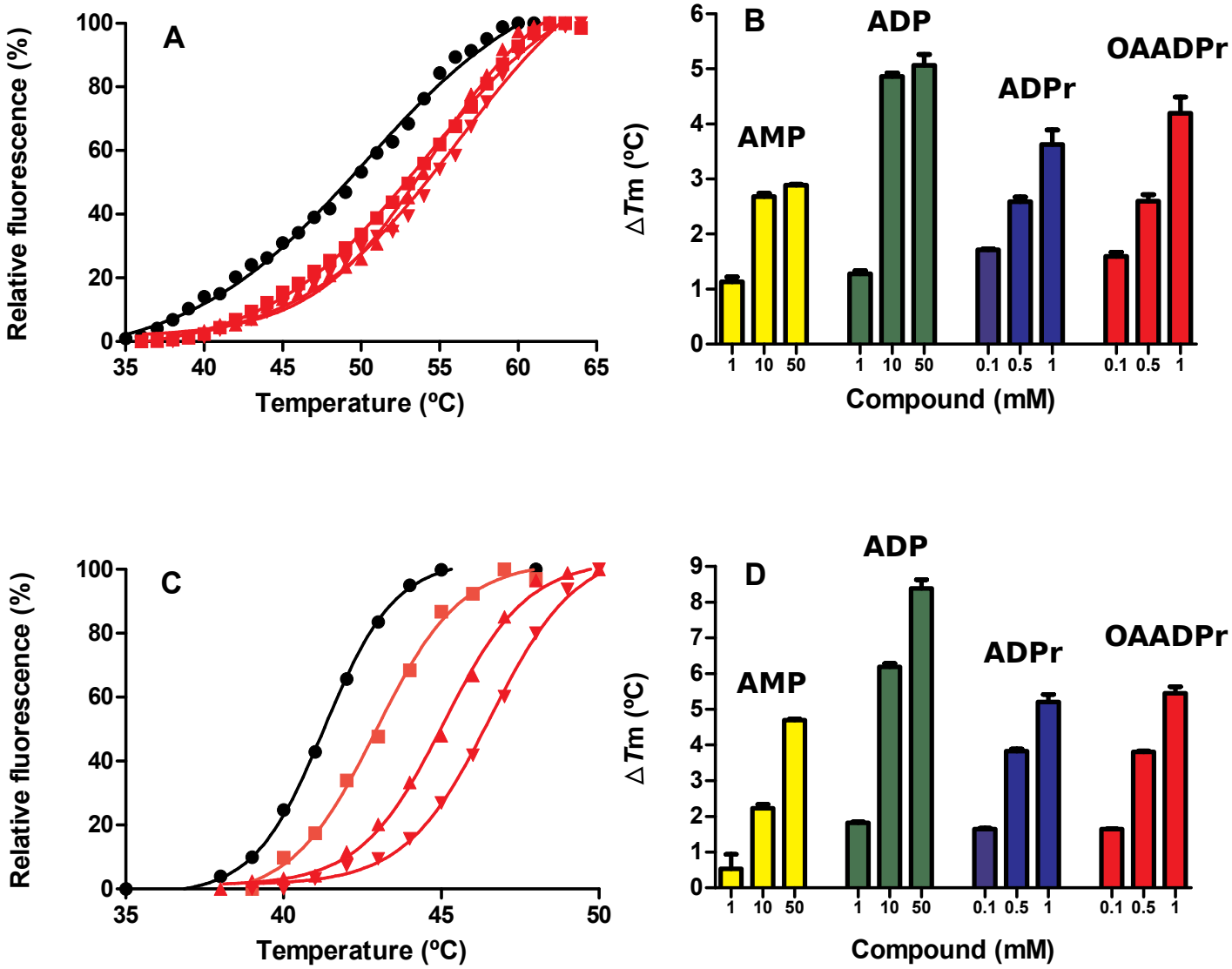
Supplementary Figure S2. Phylogenic tree of *Fusobacterium* macrodomains. The neihbour-joining tree with the 94 sequences found in UniProt and NCBI databases was constructed using 1000 replicates. MacroD-type macrodomains (red) are most abundant, although ALC1-type macrodomain (blue) sequences are also found, including TARG1-, SCO6735- and DarG-like macrodomains, respectively. The *F. mortiferum* ATCC 9817 TARG1 macrodomain is marked with a red star.



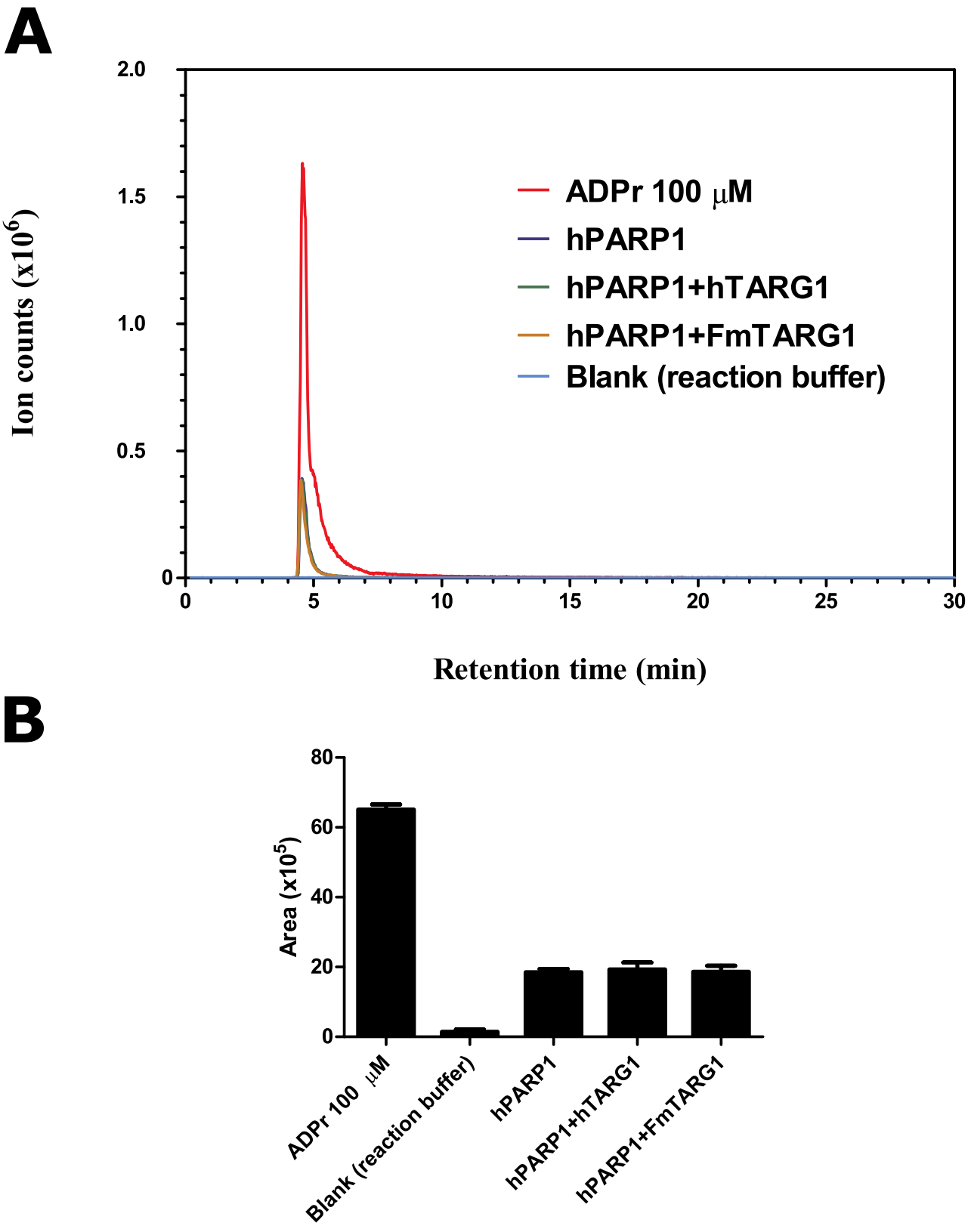
Supplementary Figure S3. Purification of FmTARG1. Mass spectra of purified FmTARG1 showed a molecular mass of 16820.32 Da. Insert. SDS-PAGE (15 %) gel of FmTARG1 purification process. Molecular mass standards (M) (kDa) are indicated in the left margin (EZ-Run™ Pre-Stained *Rec* Protein ladder, Fisher). AFV-FmTARG1 and FmTARG1 proteins are shown as a band of about 30 kDa (lane 1) and 16 kDa (lane 2), respectively.



Supplementary Figure S4. FmTARG1 activity towards O-acetyl-ADP-ribose. The steady-state kinetic of FmTARG1 (●) was also compared with hTARG1 (■) at a fixed enzyme concentration (0.2 μM). The inhibitory effect by ADPr on the deacetylation reaction of OAADPr was studied at 200 μM (▲).



Supplementary Figure S5. Thermal shift assay of FmTARG1 and hTARG1. (**A**) Melting temperature curves of FmTARG1 in 50 mM sodium phosphate buffer pH 7.0 (●) and in the presence of different OAADPr concentrations: 0.1 mM (■), 0.5 mM (▲) 1 mM (▼). (**B**) Increment in melting temperature (Δ *T*m) of FmTARG1 in the presence of different ADPr analogues. The compound used were AMP and ADP at 1-50 mM, and ADPr and OAADPr at 0.1-1 mM. (**C**) Melting temperature curves of hTARG1. (**D**) Increment in melting temperature (Δ *T*m) of hTARG1 in the presence of different ADPr analogues. The conditions and concentrations used for hTARG1 were the same as for FmTARG1.



Supplementary Figure S6. Analysis of the reaction products of hTARG1 and FmTARG1 on poly(ADP-ribosyl)ated PARP1 by mass spectrometry. (**A**) Ion chromatograms of the monoisotopic mass of the ADP-ribose [M + H]+ adduct (560 m/z) obtained after the de-PARylation reactions catalyzed by FmTARG1 and hTARG1. (**B**) Area of mass 560.0 found in the different de-PARylation reactions. Error bars represent the standard deviation of three replicates. hPARP1 represents auto-PARylated hPARP1, whereas hPARP1+hTARG1 and hPARP1+FmTARG1 represent the de-PARylation reactions.