## **Supplementary Information**

## DELTEX E3 ligases ubiquitylate ADP-ribosyl modification on nucleic acids

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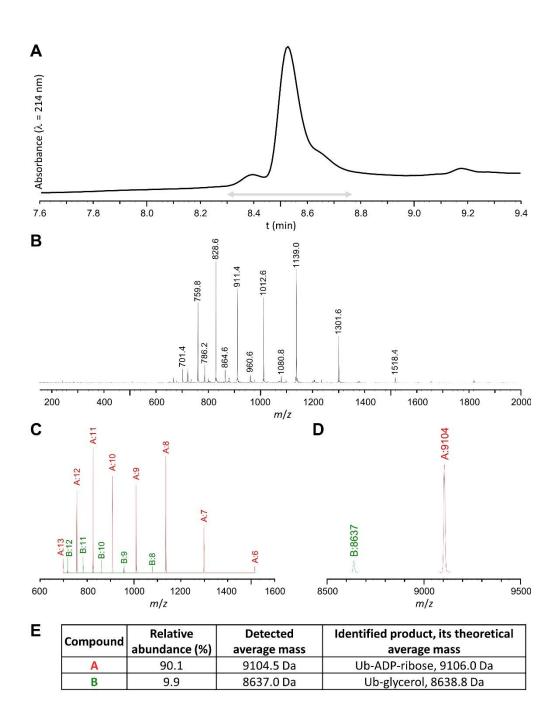


Figure S1 LCMS analysis of ADPr ubiquitylation mediated by DTX3L RING-DTC.
A. HPLC chromatogram; B. Experimental mass spectrum (sum of spectra, time window indicated as a grey arrow); C. Deconvoluted ions set, including charge state;
D. Deconvoluted spectrum; E. Identified compounds.

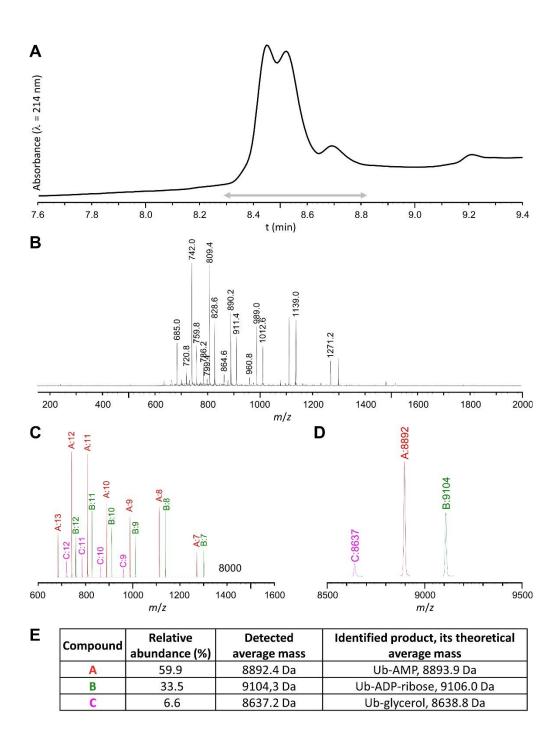


Figure S2 LCMS analysis of NUDT16-mediated cleavage of ubiquitylated ADPr obtained using DTX3L RING-DTC. A. HPLC chromatogram; B. Experimental mass spectrum (sum of spectra, time window indicated as a grey arrow); C. Deconvoluted ions set, including charge state; D. Deconvoluted spectrum; E. Identified compounds.

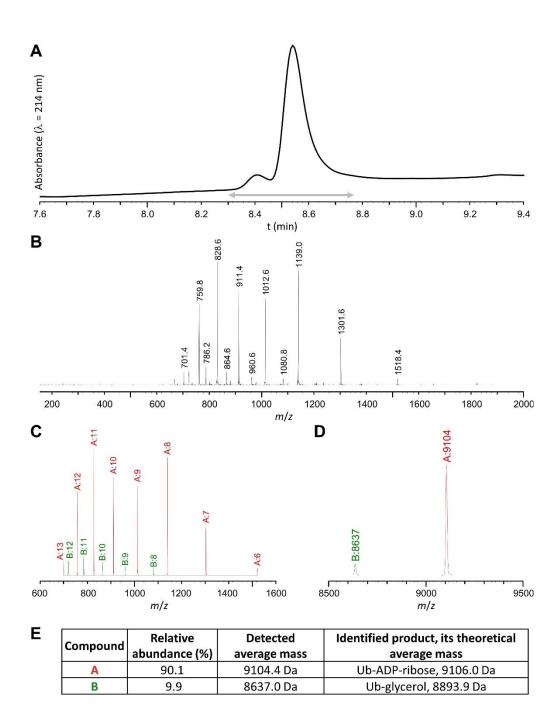


Figure S3 LCMS analysis of ADPr ubiquitylation mediated by PARP9:DTX3L. A. HPLC chromatogram; B. Experimental mass spectrum (sum of spectra, time window indicated as a grey arrow); C. Deconvoluted ions set, including charge state; D. Deconvoluted spectrum; E. Identified compounds.

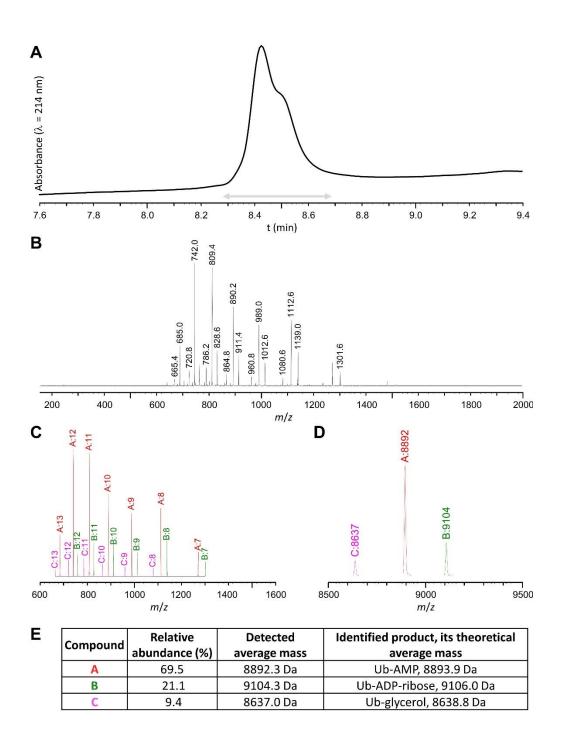
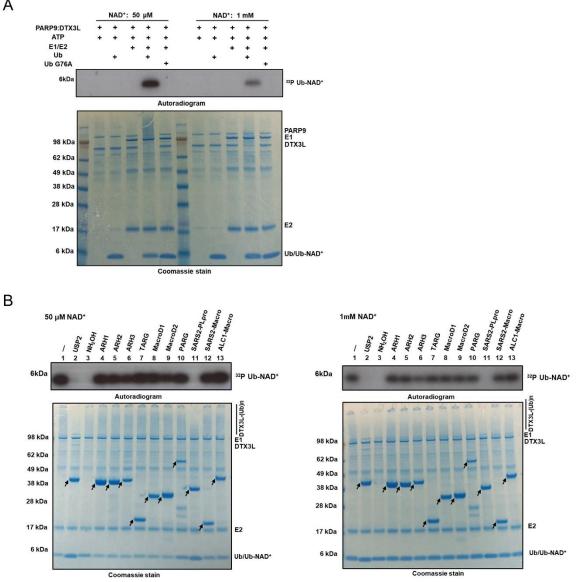


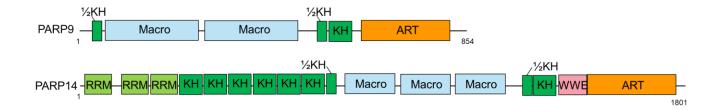
Figure S4 LCMS analysis of NUDT16-mediated cleavage of ubiquitylated ADPr obtained using PARP9:DTX3L. A. HPLC chromatogram; B. Experimental mass spectrum (sum of spectra, time window indicated as a grey arrow); C. Deconvoluted ions set, including charge state; D. Deconvoluted spectrum; E. Identified compounds.



## Figure S5. Hydrolase sensitivity of PARP9:DTX3L catalysed Ub-NAD<sup>+</sup>.

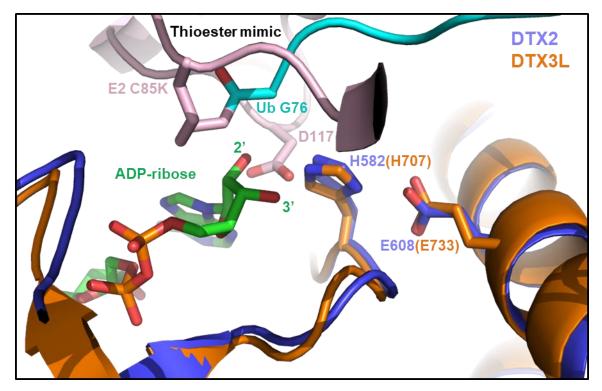
A. Biochemical reconstitution of the Ub-NAD<sup>+</sup>. <sup>32</sup>P Ub-NAD<sup>+</sup> was obtained by incubation of PARP9:DTX3L and E1, E2, ATP, Ub, and NAD<sup>+</sup> spiked with <sup>32</sup>P NAD<sup>+</sup>. Omitting any of these components blocked the <sup>32</sup>P Ub-NAD<sup>+</sup> generation. The reactions were analyzed on an SDS-PAGE gel and visualized by Coomassie staining and autoradiography. B. Hydrolysis of PARP9:DTX3L catalysed Ub-NAD+. Following NAD<sup>+</sup> ubiquitylation reaction performed with PARP9:DTX3L, the indicated ADPr hydrolases, DUBs or NH<sub>2</sub>OH were added and further incubated. The samples were analyzed on an SDS-PAGE gel, next visualized by Coomassie staining and autoradiography. The arrows indicate various hydrolases.

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## Figure S6. Domain organizations of PARP9 and PARP14. Multiple single-

stranded nucleic acids binding domains, such as KH and RRM domains, are present in PARP9 and PARP14.



**Figure S7 Comparison of the catalytic residues of DTX2 and DTX3L involved in ADP-ribose ubiquitylation.** DTX2 RING-DTC: ADP-ribose [Protein Data Bank (PDB): 6Y3J] was aligned with RNF38:UBCH5A~Ub (PDB: 4V3L) on the RING domain (RNF38 is not shown), and then DTX3L (AlphaFold: AF-Q8TDB6-F1) was aligned with DTX2 on the DTC domain. DTX3L's H707 and E733 have similar orientations as DTX2's H582 and E608, which are responsible for deprotonating the 3'-OH of proximal ribose of ADP-ribose to facilitate the ubiquitylation of ADP-ribose.

	610	620	630	640	650	660	670	680
DTX3L Human	GIOKGNOPEG.S	M. VETVSRDS	PGYESFGTIV	TTSMKAGI	OTEEHPNPGK	YP. GIORTA	LPDNKEGRK	VLKLLYRAFDQKLIFT
DTX3L Zebrafish	GKLEGNOPKG. T							VLALLORAFDOKLIFT
DTX3L Xenopus	GVVIGNOPEG.R	M. SHSAANFKI	PGH.NCGTIT	LNYNFPNGV	OGENHPNPGKI	YS. GTYRTA	LPDSKEGRE	VLOLLEKAFKOKLVFT
DTX3L_Mouse	GIQKGNQPEG.T	M.SYSTQKGSI	PGYEGCGTIV	INYEIKDGI	QTKEHPNPGKA	YH GTRRTAN	LPDNTEGRK	VLDLLHEAFKHRLTFT
DTX3L_Chicken	GLVQGDQPDG.R	MN.FKRTSLSI	PGYPNCGTIE	IEYVMQSGV	QTQSHPNPGK:	YYGITRKA	LPDNKEGQE	VLQLLRRAFNQKLIFT
DTX1_Zebrafish		M.EYHIIPHSI	PGHSDIKTIR	IVYDIPAGV	OTTEHPNPGKI	YSARGFPRHC	LPDNEKGRK	VLKLLITAWDRRLIFT
DTX1_Xenopus			PGFSDCKTIR	IVYDIPSGM	QGPEHPNPGKI	FTARGFPRHC	(LPDNDKGRK	VLRLLLAAWERR <mark>L</mark> IFA VLRLLITAWERRLIFT
DTX1_Human		M.EFHLIPHSI	PGFPDTQTIR	IVYDIPTGI	QGPEHPNPGKI	(FTARGFPRHC)	LPNNEKGRK	VLRLLITAWERRLIFT
DTX1_Mouse		M.EFHLIPHSI	PGFADTQTIR	IVYDIPTGI	QGPEHPNPGKI	(FTARGFPRHC)	(LPNNEKGRK	VLRLLITAWERRLIFT VLQLLRRAFNQKLIFT
DTX1_Chicken	GLVQ <mark>G</mark> D <b>QP</b> DG.R	MN.FKRTSLSI	PGYPNCGTIE	IEYVMQSGV	QTQSHPNPGK	TYY. GITRKA	LPDNKEGQE	VLQLLRRAFNQKLIFT
DTX2_Zebrafish	GEKTGTQPKG.K GEKTGTQPKG.N	M.EIYSIPQSI	PGHPDCGTIQ	IIYNIPPGI	QGPEHPNPGQI	Y T C R G F P R F C I	TLPDNDKGRK	VLELLKVAWTRRLIFT
DTX2_Xenopus	GEKTGTQPKG.N	M.EFSLINQSI	PGHQDCGTIH	IVYTIYPGT	QGPGHPNPGKI	YSARGFPRHC	(LPDNDKGRL	VLELLKLAWARRLIFT
DTX2_Chicken	GEKTGTQPKG.K	M.EVSTFPQSI M.EVFRFQMSI	PGHRDCGTIQ	IVYHISRGI	QGPEHPNPGM	YTARGEPRYC	LPDNEKGRK	VLELLKVAWNRRLIFT
DTX2_Mouse	GEKTGTQPWG.K	M. EVERFOMSI	PGHEDCGTLL	IVYNIPHGI	QGPEHPSPGK	FTARGEPROC	LPDSPQGRK	VLELLKVAWKRRLIFT VLELLKVAWKRRLIFT
DTX2_Human	GEKTGTQPQG.K GOLIGNOPANGS	M. EVLKFQMS	PGHEDCGILL	TTYCEPDOL	QGPEHPNPGK	T TAKGE PROC	TPDNAQGRK	VLELLKVAWKKRLIFI VLRMLRRAFEQRLIFT
DTX3_Zebrafish DTX3_Xenopus	GQLIGNQPANGS	MIVERDPDLE	PGHEGIGUIU	TOYVEDDCT	OCCEPHENEGVI	VD GTTBUA	TPDCPECNK	VLTLFKKAFDQRLTFT
DTX3_Human	GOLVGNOPONGR	MIVERDATIL	POIERIGITI	TOYVEDDOU	OCAPHPNPGVI	VD CTTRUA	TPDCPECNK	VI TI FREAFDORT TRT
DTX3_Mouse	GOLVGNOPONGR	MIVSKDATILI	PSYEKVCTTV	TOYVEPPCU	OCAEHPNPCVI	VP CTTRUA	TPDCPECNK	VLTLFRK <mark>A</mark> FDQRLTFT VLTLFRK <mark>A</mark> FDQRLTFT
DTX4 Zebrafish	GVKTGNOPAG.K	MEVHUTDHST	PCHPDCKTTP	TTYNTPDCT	OCDEHDNDAKI	FTARCEPRHC	TPDNFKCPK	VI.P.I.I.VAWDPRITEC
DTX4 Chicken	GVKTGTQPPG.K	MEYHTTPHAT	PGHSDCKTIR	TTYNTPPGV	OGPEHPNPGK	FTARGEPRHC	TPDSEKGRK	VLRLLLVAWDRRLIFS VLKLLLVAWDRRLIFA
DTX4_Mouse	GVKTGTOPPG.K	M. EYHLIPHSI	PGHPDCKTIR	IIYSIPPGI	OGPEHPNPGK	FSARGEPRHC	LPDSEKGRK	VLKLLLVAWDRRLIFA
DTX4 Human	GVKTGTOPPG. K	M.EYHLIPHSI	PGHPDCKTIR	IIYSIPPGI	OGPEHPNPGK	FSARGFPRHC	LPDSEKGRK	VLKLLLVAWDRRLIFA
	690 700	710	720	73	740			
	690 700	•	720	73	•			
DTX3L_Human	VGYSRVLGVSDV	ITWNDIHHKT	SRFGGPEMY <mark>GY</mark>	PDPSYLKR	KE <b>EL</b> KAK <b>G</b> IE			
DTX3L_Human DTX3L_Zebrafish	VGYSRVLGVSDV VGTSTTSGLENT	VTWNDIHHKT	SRFGGPEMY <mark>GY</mark> SQFGG <mark>AQ</mark> SY <mark>GY</mark>	PDPSYLKR PDPDYLKR	/KE <mark>EL</mark> KAK <mark>G</mark> IE /KD <mark>EL</mark> KAK <mark>G</mark> IE	<mark></mark> .		
DTX3L_Human DTX3L_Zebrafish DTX3L_Xenopus	VGYSRVLGVSDV VGTSTTSGLENT IGESRTTGAENT	TITWNDIHHKT VTWNDIHHKT VTWNDIHHKT	SRFGGPEMY <mark>GY</mark> SQFGGAQSY <mark>GY</mark> NTVGGPQEF <mark>GY</mark>	PDPSYLKR PDPDYLKR PDPDYLKR	, /KEELKAKGIE /KDELKAKGIE IREELKAKGIE	<mark></mark>		
DTX3L_Human DTX3L_Zebrafish DTX3L_Xenopus DTX3L_Mouse	VGYSRVLGVSDV VGTSTTSGLENT IGESRTTGAENT IGYSRATGVSDV	TTWNDIHHKT VTWNDIHHKT VTWNDIHHKT ITWNDIHHKT	SRFGGPEMY <mark>GY</mark> SQFGGAQSY <mark>GY</mark> NTVGGPQEF <mark>GY</mark> SKFGGPANF <mark>GY</mark>	PDPSYLKR PDPDYLKR PDPDYLKR PDPDYLKR	/KEELKAKGIE /KDELKAKGIE IREELKAKGIE /KEELKAKGIE			
DTX3L_Human DTX3L_Zebrafish DTX3L_Xenopus DTX3L_Mouse DTX3L_Chicken	VGYSRVLGVSV VGTSTTSGLENT IGESRTTGAENT IGYSRATGVSDV VGNSRTTGAEDV	ITWNDIHHKT VTWNDIHHKT VTWNDIHHKT ITWNDIHHKT ITWNDIHHKT	SRFGGPEMY <mark>GY</mark> SQFGGAQSY <mark>GY</mark> NTVGGPQEF <mark>GY</mark> SKFGGPANF <mark>GY</mark> SIYGGPMQF <b>GY</b>	PDPSYLKR PDPDYLKR PDPDYLKR PDPDYLKR PDPDYLKR PDPDYLKR	KEELKAKGIE /KDELKAKGIE REELKAKGIE /KEELKAKGIE /RSELKAKGIE	· · · · · · · · · · · · · · · · · · ·	:: ::	
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**Figure S8 Multiple sequence alignment of the DTC domains from the DELTEX family members.** Sequence alignment of DTC domains was generated using Clustal Omega and the figure was prepared using ESPript (http://espript.ibcp.fr). In this alignment, residues that are absolutely conserved and highly conserved are highlighted in dark and light red, respectively. The strictly conserved catalytic residues H707 and E733 (human DTX3L numbering) are marked with solid circles.