

1 **Characterization of pertussis-like toxin from *Salmonella* spp. that catalyzes ADP-**
2 **ribosylation of G proteins**

3
4 **Running title:** ADP-ribosyltransferase toxin of *Salmonella*

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A

<i>S. bongori</i> ATCC43975	1	VDFVYRVDSRPPDVI FRDGFSSHGNNRNLQQHIRGDSCAAGSRDSNYIATTS DINETYNI
<i>S. Typhimurium</i> DT104 U1	1	VDFVYRVDSRPPDVI FRDGFNSHGNNRNLQQHIRGDSCSAGSRDSNYIATTS DINETYNI
<i>S. Worthington</i> 182	1	VDFVYRVDSRPPDVI FRDGFSSHGNNRNLQQHIRGDSCSAGSRDSNYIATTS DINETYNI
<i>S. Agoueve</i> 213	1	VDFVYRVDSRPPDVI FRDGFSSHGNNRNLQQHIRGDSCSAGSRDSNYIATTS DINETYNI
<i>S. bongori</i> ATCC43975	61	ARVYYSRAATFSGRLYRIRADNSFYSLPPSVAYIESRGVQFNHFERVMMRLQSEYVAVN
<i>S. Typhimurium</i> DT104 U1	61	ARVYYSRTTTFSGRLYRIRADNSFYSLPPSVAYIESRGIQF SHFERVMMRLQSEYVAVN
<i>S. Worthington</i> 182	61	ARVYYSRTTTFSGRLYRIRADNSFYSLPPSVAYIESRGIQF SHFERVMMRLQSEYVAVN
<i>S. Agoueve</i> 213	61	ARVYYSRTTTFSGRLYRIRADNSFYSLPPSVAYIESRGIQF SHFERVMMRLQSEYVAVN
<i>S. bongori</i> ATCC43975	121	SIPIENIQEAVELVYDRNTSQVRDGP GTSNSRYLRVSTQSNPGVIPNLPVPQVSTRERIS
<i>S. Typhimurium</i> DT104 U1	121	SIPIENIQEAVELVYDRNTSQVRDGS GTSNSRYLRVSTQSNPGVIPNLPVPQVSTRERIS
<i>S. Worthington</i> 182	121	SIPIENIQEAVELVYDRNTSQVRDGS GTSNSRYLRVSTQSNPGVIPNLPVPQVSTRERIS
<i>S. Agoueve</i> 213	121	SIPIENIQEAVELVYDRNTSQVRDGS GTSNSRYLRVSTQSNPGVIPNLPVPQVSTRERIS
<i>S. bongori</i> ATCC43975	181	AFGTLISACFSMRGVRDDTRINSNYYEMEFYDARGVLTPELLK
<i>S. Typhimurium</i> DT104 U1	181	AFGTLISACFSMRGVRDDARSNYYEMEFYDARGVLTPELLD
<i>S. Worthington</i> 182	181	AFGTLISACFSMRGVRDDARSNYYEMEFYDARGVLTPELLN
<i>S. Agoueve</i> 213	181	AFGTLISACFSMRGVRDDARSNYYEMEFYDARGVLTPELLN

B

<i>S. bongori</i> ATCC43975	1	SNVYATVNNWYLKDTTKYENVKITNVFYADYV LHSPRICAYFTASS-GGSNV---TGCAV
<i>S. Typhimurium</i> DT104 U1	1	-----ADYNTYQSNVQINNLSYGVYRSGDKESQFFCVGLKRGSQVFNVHTLCKL
<i>S. Worthington</i> 182	1	-----ADYNTYQSNVQINNLSHGVIKSGGKDSQFFCIGLNNESQIPNANTMCKM
<i>S. Agoueve</i> 213	1	-----ADYNTYQSNVQINNLSHGVIKSGGKDSQFFCIGLNNESQIPNANTMCKM
<i>S. bongori</i> ATCC43975	56	ADNGYYQKNAGOTSPFMEIFD TVKYFYTTGEEKLSVYIRINAFS--HFDSSVSQNEIIVAG
<i>S. Typhimurium</i> DT104 U1	50	DV-----FGTHKQGF DNMLATARYYYATGEDVRIYYKENVWTD RNFTAAAFSGNELIAIT
<i>S. Worthington</i> 182	50	DV-----FGTHKQGF DNMLATARYYYTTGEEKVRIYYKENVWADRNF TAGFSGNELIAIT
<i>S. Agoueve</i> 213	50	DV-----FGTHKQGF DNMLATARYYYTTGEEKVRIYYKENVWADRNF TAGFSGNELIAIT
<i>S. bongori</i> ATCC43975	114	TCN--QWCFGEITK-
<i>S. Typhimurium</i> DT104 U1	104	TCN ¹ SSDYCMGPTLPN
<i>S. Worthington</i> 182	104	TCSSIDYCMGPTLPN
<i>S. Agoueve</i> 213	104	TCSSIDYCMGPTLPN

C

		Necessary for NAD binding		NAD-binding site		Catalytic glutamate
ArtA-DT104	2	DFVYRVDSR ... 45	SNYIATTS DINE ... 109	MMRLQSEYVAL		
ArtA-SW	2	DFVYRVDSR ... 45	SNYIATTS DINE ... 109	MMRLQSEYVAL		
ArtA-Sb	2	DFVYRVDSR ... 45	SNYIATTS DINE ... 109	MMRLQSEYVAL		
PltA	1	DFVYRVDST ... 35	SRYIATTS SVNQ ... 93	MMRLQREYVST		
MPN372	6	RFVYRVDLR ... 44	RSYFI STSETPT ... 126	SFAYQREWFTD		
Ptx	5	PATVRYDSR ... 46	NSAFV STSSRR ... 122	LATYQSEYLAH		
Ltx	3	DRLYRADS R ... 56	DDGYV STSISLR ... 106	PHPYEQEV SAL		
Ctx	3	DKLYRYVYE ... 56	DDGYV STSISLR ... 106	PHDPEQEV SAL		

Figure S1. Alignment of the amino acid (a.a.) sequences of ArtA (A) and ArtB (B) from *Salmonella* Typhimurium DT104 U1, *S. Agoueve* 213, *S. Worthington* 182, and *S. bongori* ATCC43975. The alignment was generated with ClustalW (<http://www.ebi.ac.uk/clustalw/>), and sequences were shaded using BoxShade (http://www.ch.embnet.org/software/BOX_form.html). Identical and similar regions are indicated by black and grey boxes, respectively. (C) Alignment of the residues conserved between ArtA and other ADP-ribosyltransferase toxins. PltA, *S. Typhi* pertussis-like toxin A; MPN372, *Mycoplasma pneumonia* toxin; Ptx, pertussis toxin; Ltx, *Escherichia coli* heat-labile enterotoxin; Ctx, cholera toxin. Conserved residues that are critical for Ptx function are shown in bold.

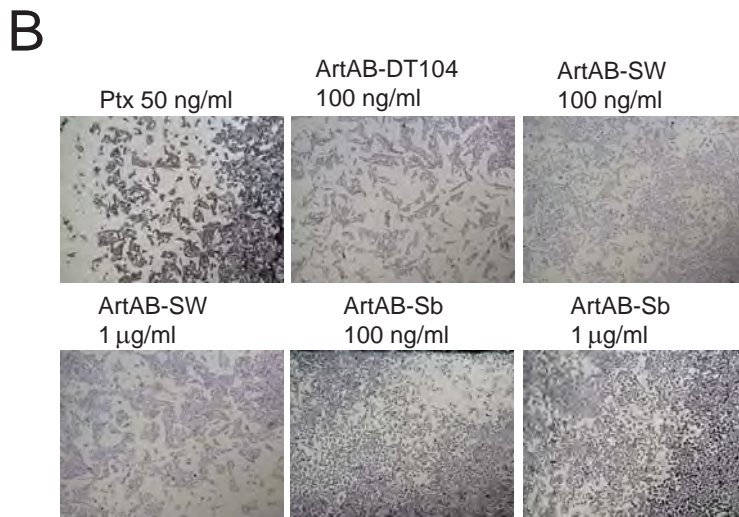
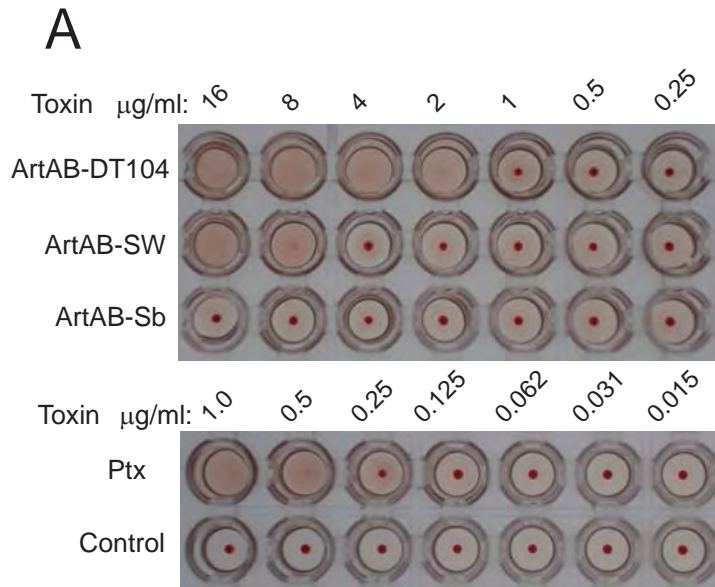
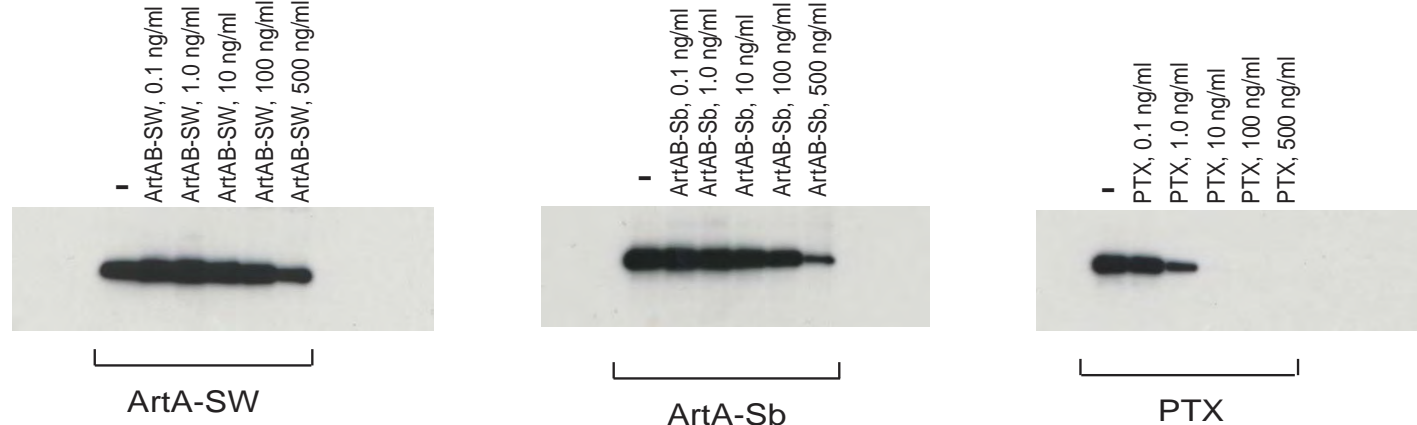


Figure S2. Hemagglutinin (HA) and CHO cell clustering activity of ArtABs and Ptx. (A) HA activity of ArtABs and Ptx. HA activity at different concentrations of sample (serial 2-fold dilutions) using chicken erythrocytes. (B) CHO cell clustering induced by ArtABs. Cells were exposed to Ptx (50 ng/well), ArtAB-DT104 (100 ng/well), ArtAB-SW (1 μg and 100 ng/well), or ArtAB-Sb (1 μg and 100 ng/well).

Toxin preincubated
with intact cells:



Toxin incubated with
membrane:

Figure S3. In vitro ADP-ribosylation of cell membrane proteins after pre-treatment of RAW 264.7 cells with ArtAB-SW, ArtAB-Sb, or PTX. RAW 264.7 cells were incubated with various concentrations of ArtAB-SW, ArtAB-Sb, or PTX, indicated in upper row; membranes were subsequently prepared. Membranes prepared from these pretreated cells were incubated with the in vitro expressed toxin indicated in bottom row (“Toxin with membrane”), along with biotinylated NAD, for an in vitro membrane labelling experiment. Samples were resolved by 12.5% SDS-PAGE, and ADP-ribosylated proteins were detected by western blotting using peroxidase-conjugated streptavidin, as described in the Materials and Methods.

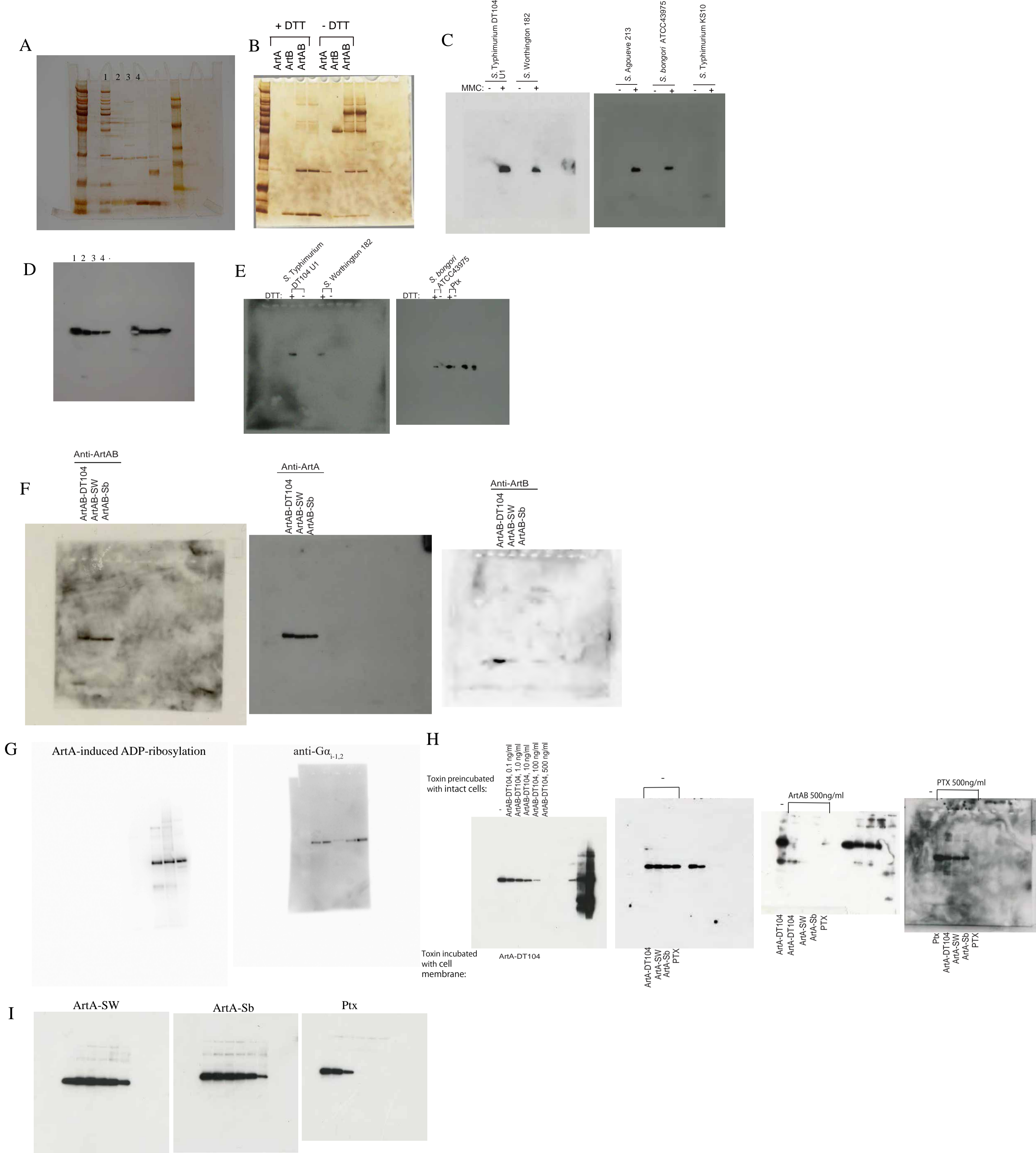


Figure S4. Full length gels and membrane. (A) Full length gel for Figure 1B. 1: Marker 2: *S. Typhimurium* DT104 U1 3: *S. Worthington* 182 4: *S. bongori* ATCC43975 (B) Full length gel for Figure 2B. (C) Full length membrane for Figure 1A. (D) Full length membrane for Figure 1C. 1: PTX 2: *S. Typhimurium* DT104 U1 3: *S. Worthington* 182 4: *S. bongori* ATCC43975 (E) Full length membrane for Figure 1D. (F) Full length membrane for Figure 1E. (G) Full length membrane for Figure 5A. (H) Full length membrane for Figure 5B-E. (I) Full length membrane for Fig.S3.