

Figure 1. Purification of Atroxlysin-III from *B. atrox* venom. **(A)** Crude venom of *B. atrox* was applied to Sephadryl 200 column. The column was equilibrated and eluted with 50 mM ammonium acetate buffer, pH 7.5, containing 0.3 M NaCl at a flow rate of 7.2 mL/h. The peak P1 (with proteolytic and hemorrhagic activity) was applied to a DEAE-ion exchange column. **(B)** The DEAE Sepharose CL-6B column was equilibrated with 50 mM Tris-HCl buffer, pH 8.0, and eluted with a linear salt gradient from 0–0.3 M NaCl at a flow rate of 13 mL/h. Peak C contained the hemorrhagic and proteolytic activity.

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Figure 2. The nucleotide and deduced amino acid sequence of Atr-III precursor from *B. atrox* venom. The predicted amino acid sequence is shown above the nucleotide sequence: sequence starts with the amino terminal amino acid of mature protein (glutamic acid). A signal peptide (MIHVLLVTICLAAFPYQG), a cysteine-switch motif (PKMCGV) are bold-underlined. The Zinc binding motif (HEGNHLGIHHD) and the methionine 168 of the Met-turn region are invariant and are highlighted in blue. Putative N-linked glycosylation site is bold and boxed in yellow. Disintegrin-like (ECD) sequences are highlighted in green. Termination codon and polyadenylation addition signal (aataa) are denoted. Accession number of Atroxlysin-III precursor in the GenBank database is KX821773.

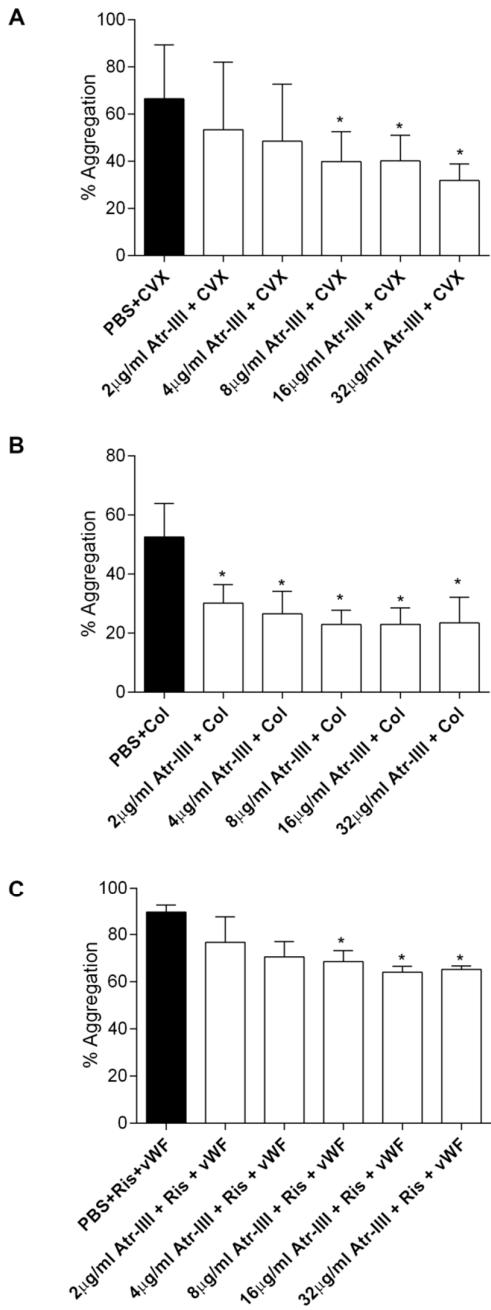


Figure S3: Washed human platelets (225 μ L, $2.5 \times 10^5/\mu$ L) were pre-incubated with different concentrations of Atr-III: 2 to 32 μ g/mL for 3 minutes and stirred (600 rpm) at 37 °C. After incubation, platelets were stimulated with different agonists: (A) 6 μ g/mL of convulxin (CVX), (B) 10 μ g/mL of collagen-I, (C) 5 μ g/mL of human vWF plus 0.5mg/mL of ristocetin. Black bars represent the positive control (platelets pre-incubated with 10 μ L PBS). *P < 0.05 compared with PBS plus agonist (1-way ANOVA followed by Tukey's test n = 4).