

# Supplementary information

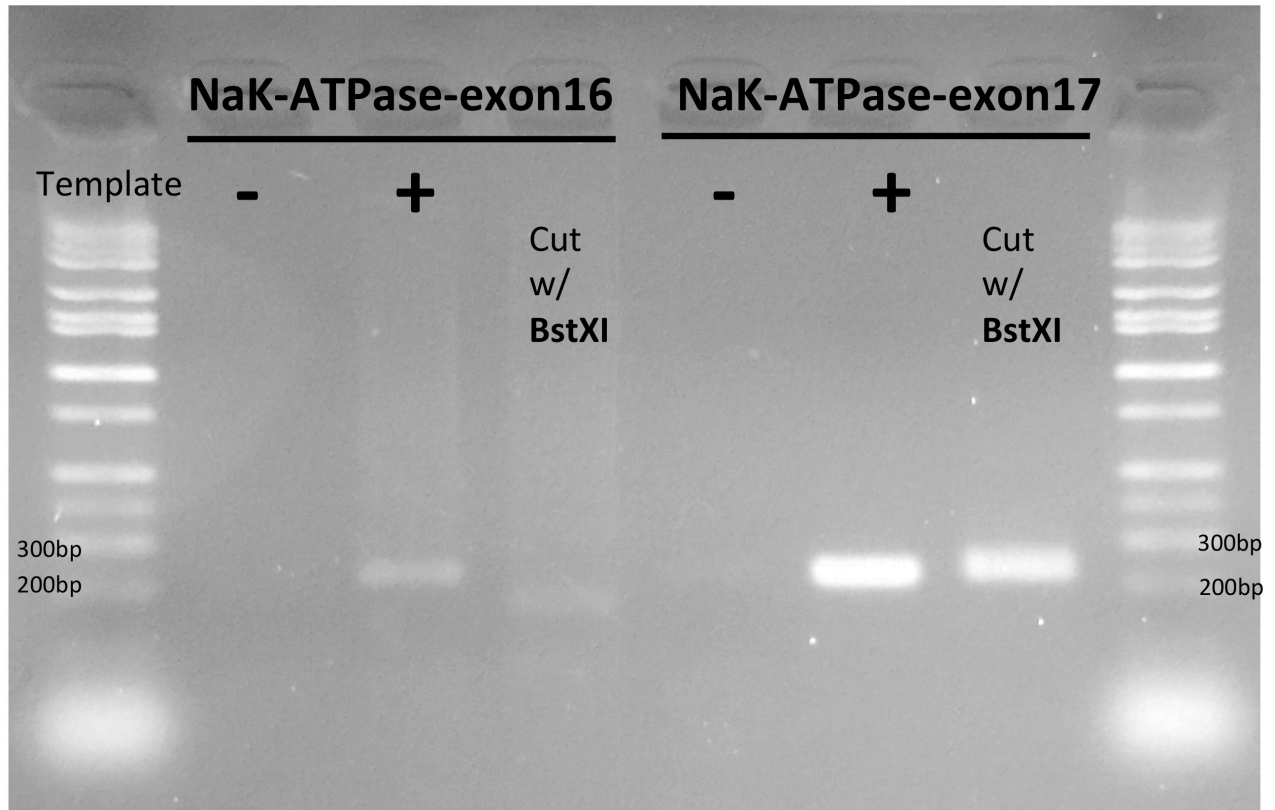
**Multiple functions of Na/K-ATPase in dopamine-induced salivation of the Blacklegged tick,  
*Ixodes scapularis***

Donghun Kim<sup>1</sup>, Joshua Urban<sup>1</sup>, Daniel L. Boyle<sup>2</sup>, and Yoonseong Park<sup>1\*</sup>

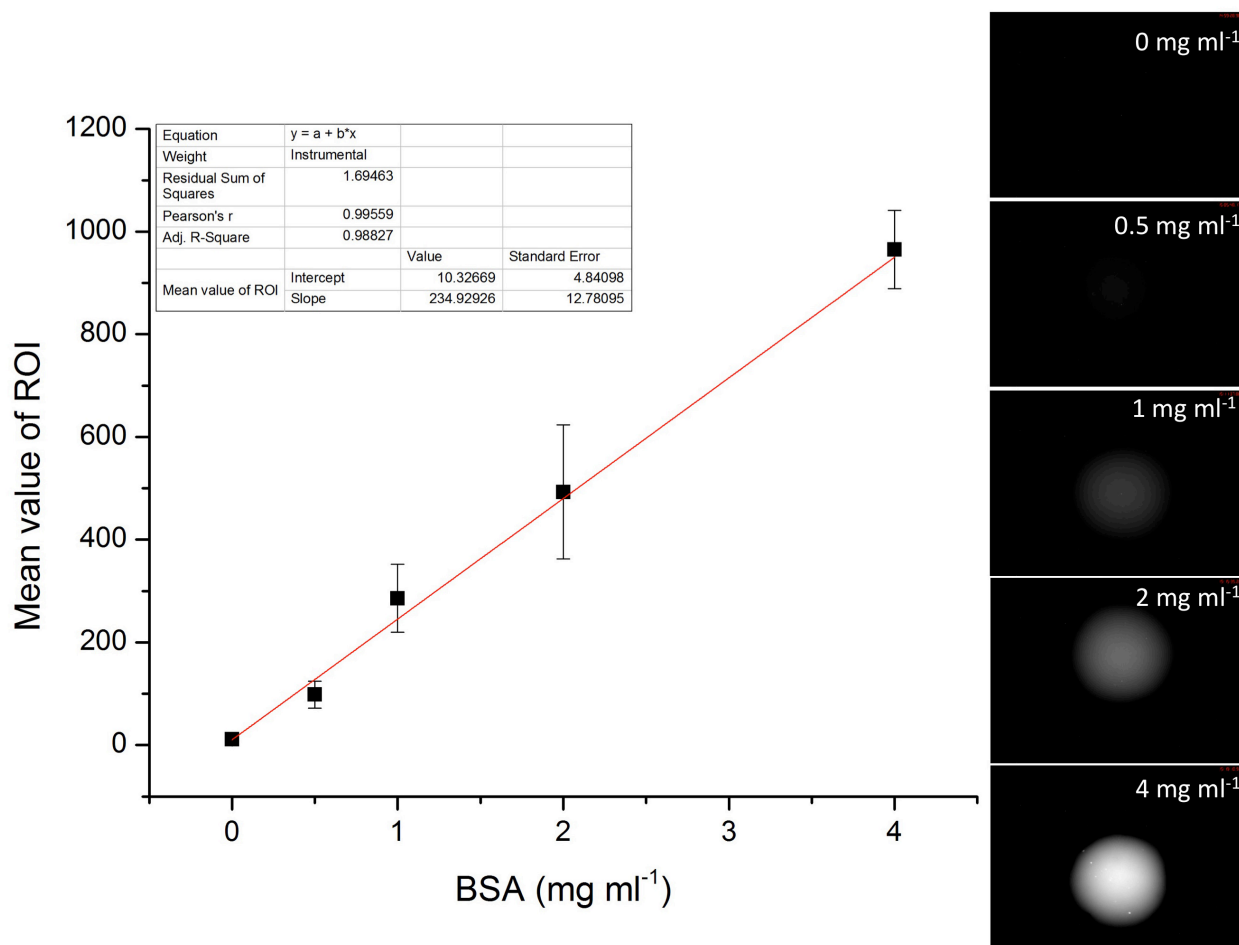
<sup>1</sup>Department of Entomology, Kansas State University, 123 Waters Hall, Manhattan, KS 66506, USA, <sup>2</sup>Division of Biology, Microscopy Facility, Kansas State University, Ackert Hall, Manhattan, Kansas 66506, USA

\*Corresponding author (ypark@ksu.edu)

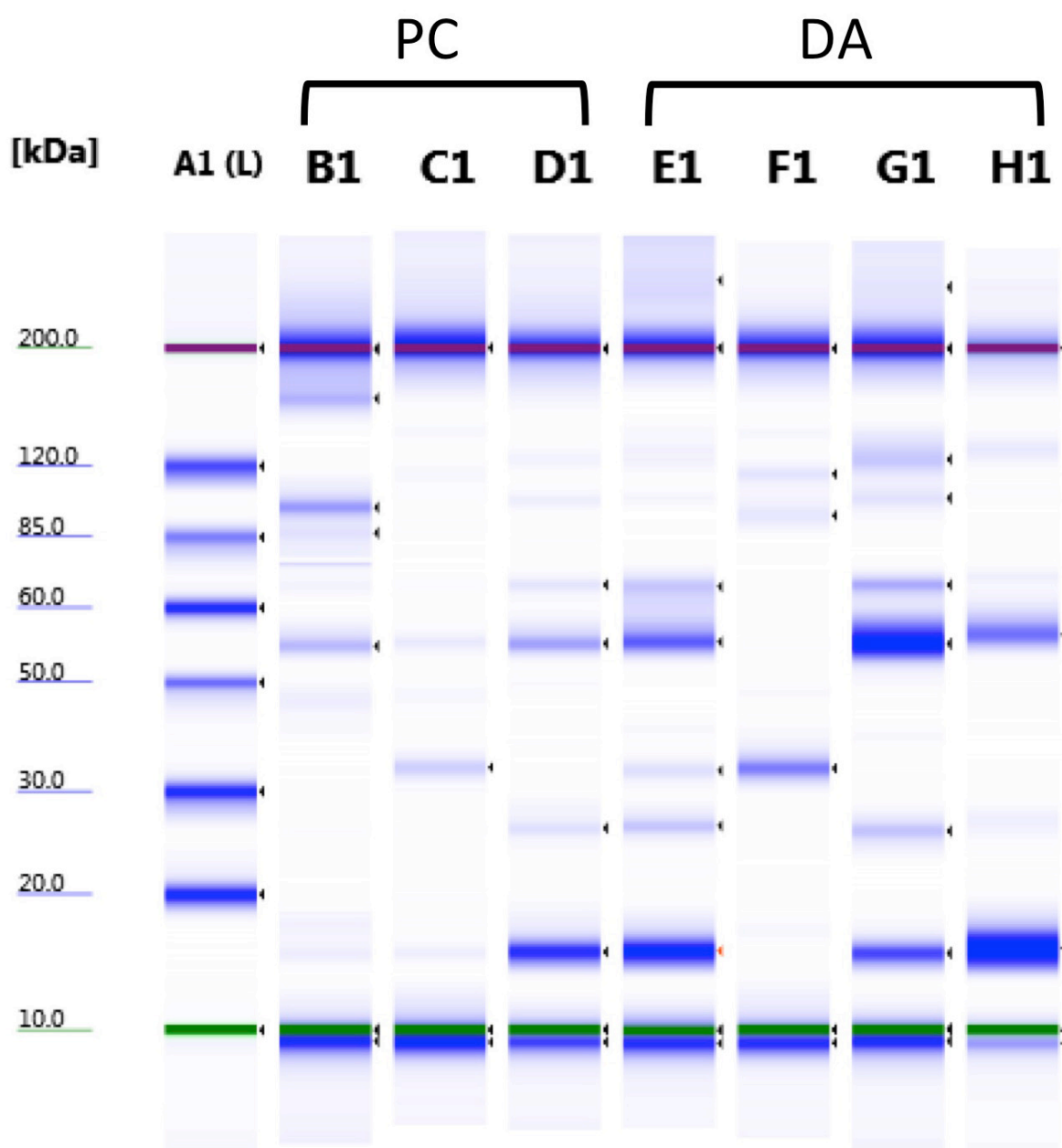
**Supplementary Figure S1.** Na/K-ATPase in the salivary glands utilizes mainly exon 17, while low levels of transcripts using exon 16 also exist. Only exon 16 carries the BstXI restriction motif and the BstXI-cut was confirmed by electrophoresis of the exon 16 PCR product and subsequent digestion.



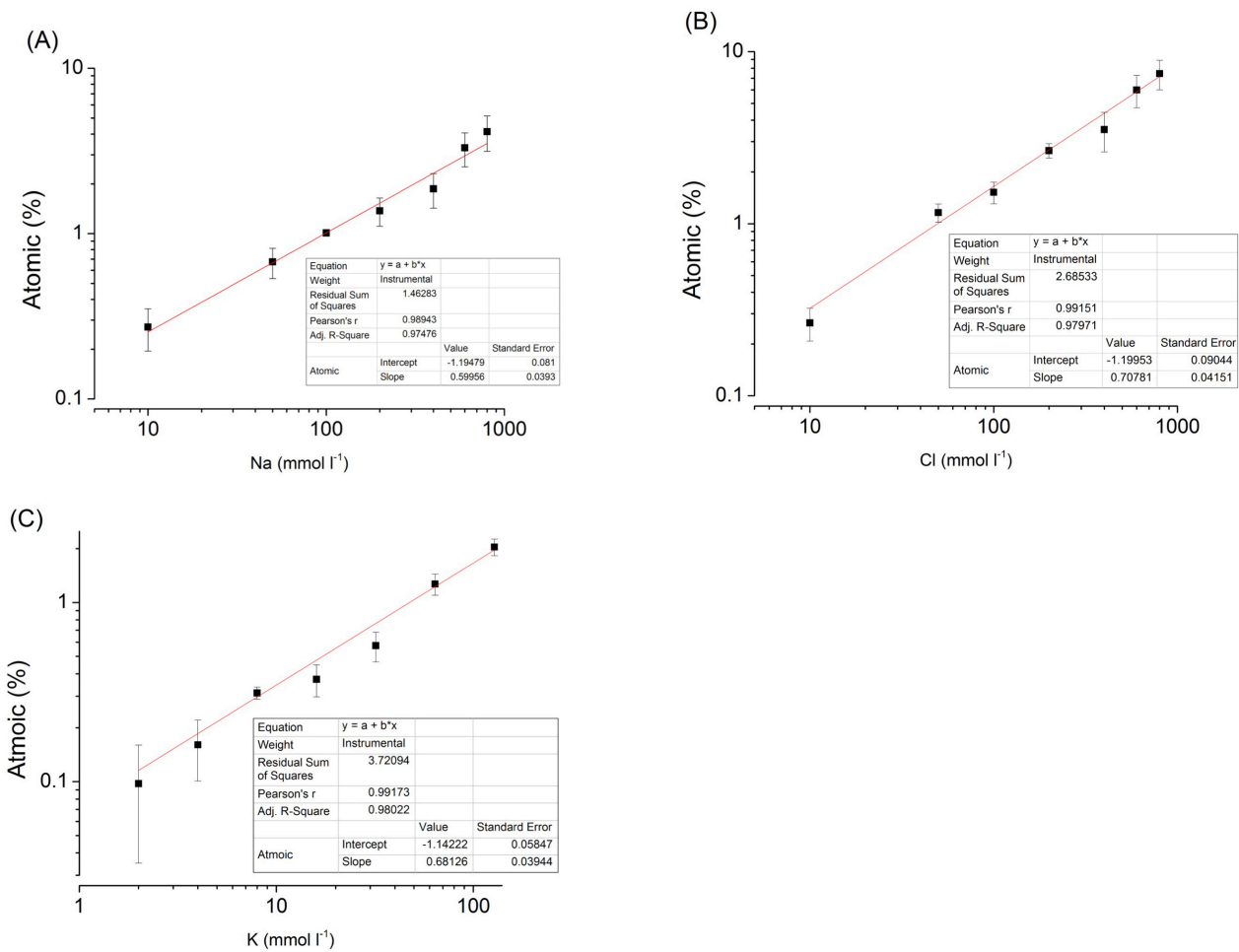
**Supplementary Figure S2.** Protein quantification using fluorescence-dye based method (CBQCA). Standard curve: bovine serum albumin (BSA, Serial 2-fold dilutions from 0 mg ml<sup>-1</sup> – 4 mg ml<sup>-1</sup>) and captured images of fluorescence from each concentration of BSA.



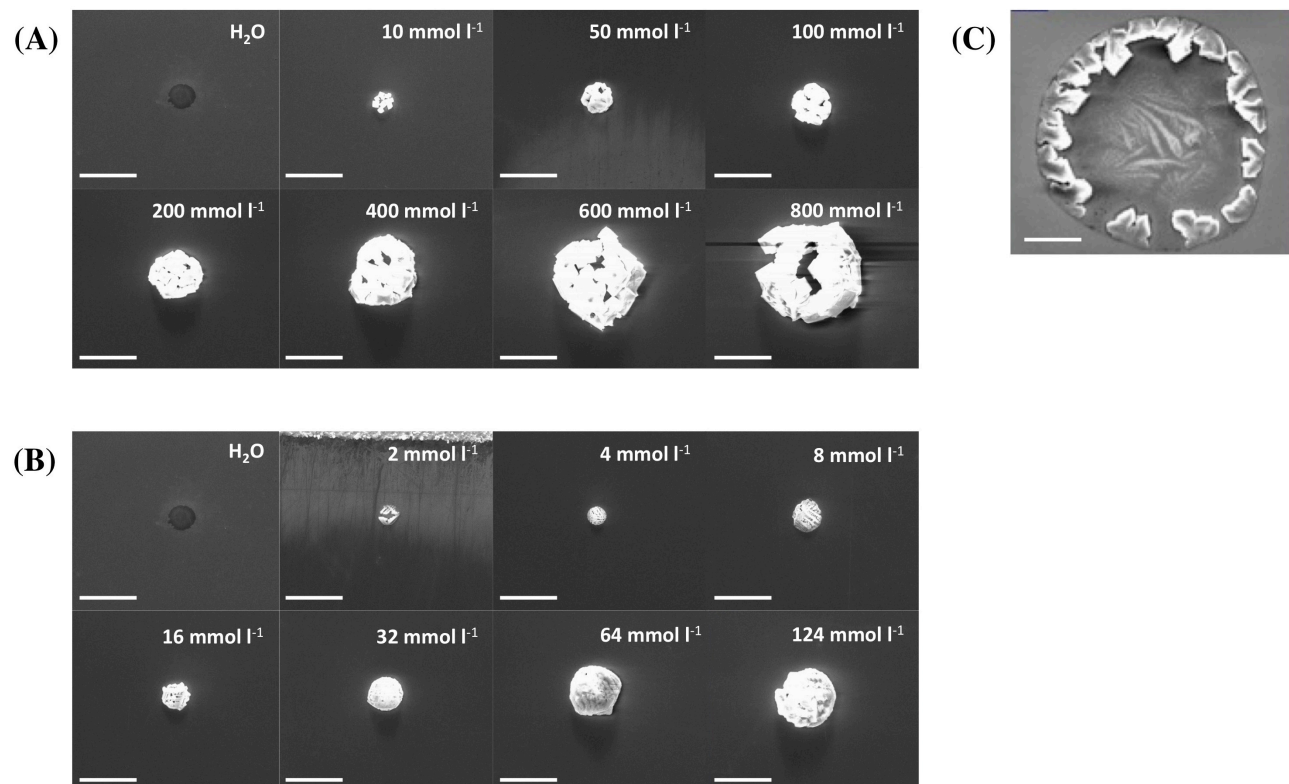
**Supplementary Figure S3.** Variations in the salivary proteins shown by Agilent 2200 TapeStation. Each lane represents pooled saliva collected from two individuals. A1: P200 ladder. B1-D1: saliva induced by injection of 2 $\mu$ l of pilocarpine (10mg/ml). E1-H1: saliva induced by injection of 2 $\mu$ l of dopamine (10 $\mu$ mol l<sup>-1</sup>). The band pattern indicated large variations among different individuals, but not between different methods of inducing salivation.



**Supplementary Figure S4.** Standard curves of major ions analyzed by SEM/EDS. (A) Na<sup>+</sup>; (B) Cl<sup>-</sup>; (C) K<sup>+</sup>.



**Supplementary Figure S5.** SEM images. A Nanoliter2000 (WPI) was used to place a drop (2 nL) of either standard solution or saliva onto the silicon grid. (A) NaCl standard (10mM to 800mM); (B) KCl standard (2mM to 124mM); (C) Representative image of a drop of saliva. The scale bar indicated is  $45\mu\text{m}$  in length.



**Supplementary Figure S6.** Fluorescence observation from water ingested (negative control) unfed tick. Auto-fluorescence was observed from the hindgut and rectal sac of a water ingested tick. (A) Overview of fluorescence from water ingested tick. (B) Lack of fluorescence from intact isolated salivary gland.

