Supplementary Information for:

Receptor Characterization and Functional Activity of Pyrokinins on the Hindgut in the Adult Mosquito, *Aedes aegypti*.

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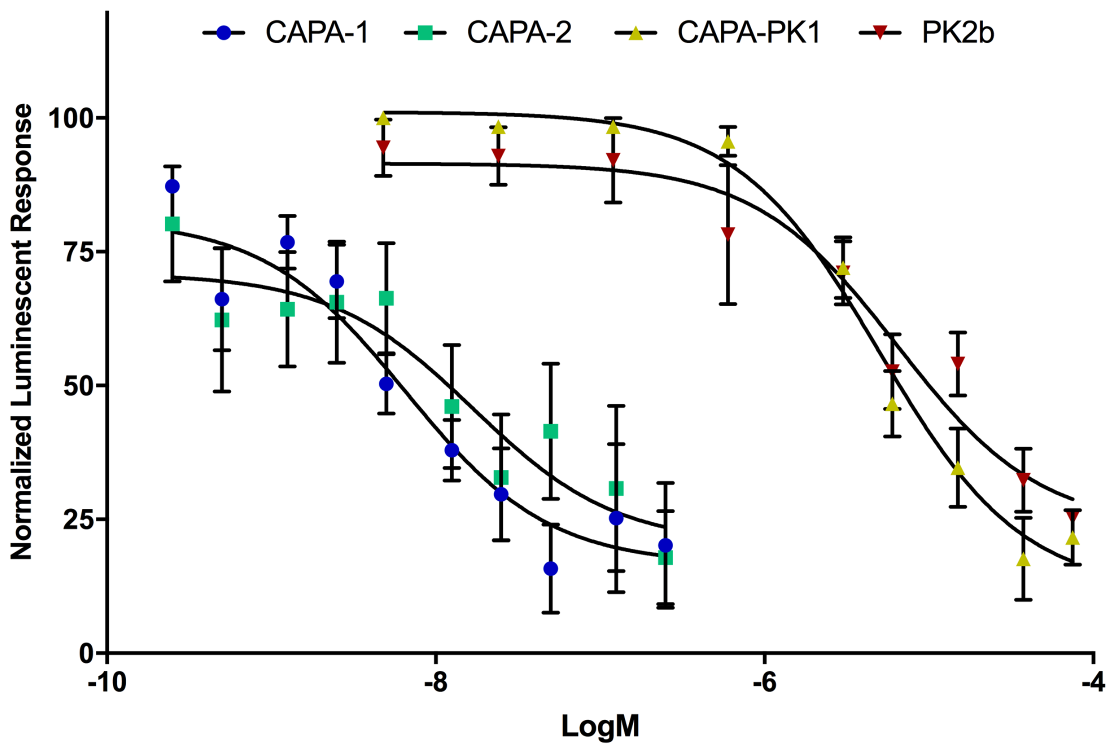
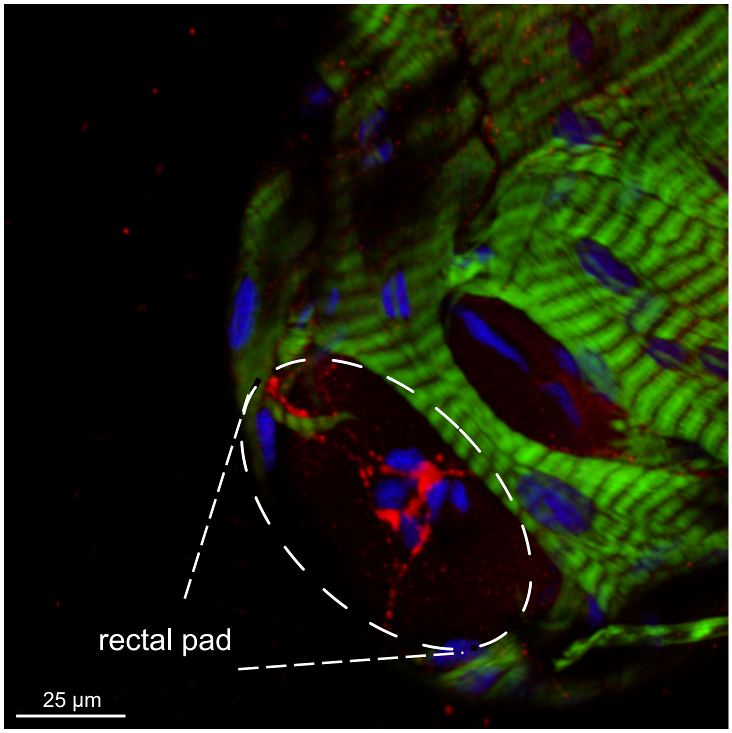
Supplementary Table S1. Sequence characteristics of the various peptides used in the study including endogenous *A. aegypti* neuropeptides and homologous peptides from other insects. Peptides in black font were used in this study whereas peptides in blue font are *A. aegypti* peptides listed for sequence comparison. The family-specific C-terminal motifs and conserved residues required for peptide bioactivity are denoted in red font.

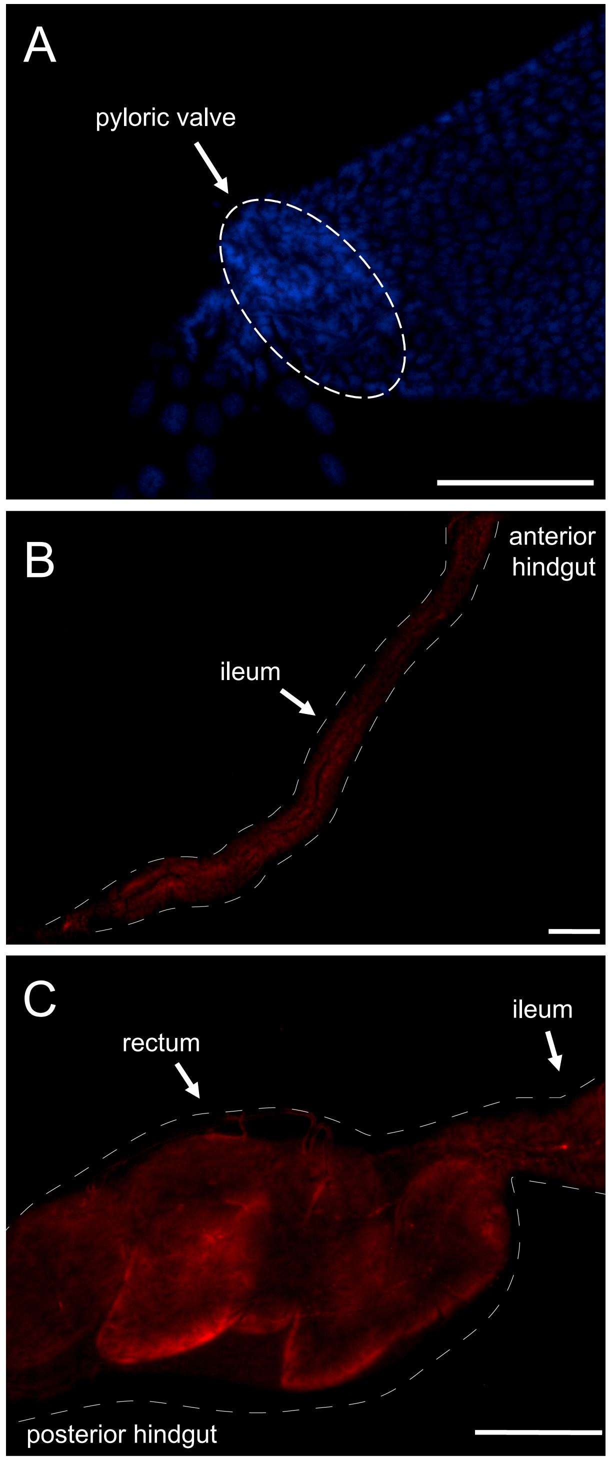
Peptide	Sequence	Reference
*Aedae*CAPA-PK1	AGNSGANSGMWFGPRL-NH_2	Predel et al., 2010
*Aedae*PK1	AAAMWFGPRL-NH_2	Predel et al., 2010
*Aedae*PK2	DASSSNENNSRPPFAPRL-NH_2	Predel et al., 2010
*Aedae*PK3	NLPFSPRL-NH_2	Predel et al., 2010
*Rhopr*PK2a	NTVNFSPRL-NH_2	Paluzzi and O'Donnell, 2012
*Rhopr*PK2b	SPPFAPRL-NH_2	Paluzzi and O'Donnell, 2012
*Aedae*CAPA-1	GPTVGLFAFPRV-NH_2	Predel et al., 2010
*Aedae*CAPA-2	pQGLVPFPRV-NH_2	Predel et al., 2010
Droso-leucokinin	NSVVLGKKQRFHSWG-NH_2	Zandawala et al., 2018
Aedae-leucokinin 1	NSKYVSKQKFYSWG-NH_2	Veenstra, 1994
Aedae-leucokinin 2	NPFHAWG-NH_2	Veenstra, 1994
Aedae-leucokinin 3	NNPNVFYPWG-NH_2	Veenstra, 1994
*Rhopr*MIP-7	AWNSLHGGW-NH_2	Paluzzi et al., 2015
*Aedae*MIP1	TWKNLQGGW-NH_2	Kim et al., 2010
*Aedae*MIP2	AWNKINGGW-NH_2	Kim et al., 2010
*Aedae*MIP3	VNAGPAQWNKFRGSW-NH_2	Kim et al., 2010
*Aedae*MIP4	EPGWNNLKGLW-NH_2	Kim et al., 2010
*Aedae*MIP5	SEKWNKLSSSW-NH_2	Kim et al., 2010



Supplementary Figure S1. Competitive ELISA used to confirm cross-reactivity and binding affinity of CAPA2-targeted antibody to other structurally related peptides. The custom-synthesized antibody designed to target the antigen sequence EGGFISFPRV-NH_2 selectively targets *Aedae*CAPA-1 (IC_{50} = 6.57nM) and *Aedae*CAPA-2 (IC_{50} = 16.93nM), but was also able to recognize and bind pyrokinins, including *Aedae*CAPA-PK1 (IC_{50} = 4.99μM) and *Rhopr*PK2b (IC_{50} = 6.43μM). Normalized responses represent mean ± SEM (n = 4).



Supplementary Figure S2. PRXa-like immunoreactive processes (red) terminating in close association to cells within the rectal pad (one of six shown) of adult female mosquitoes. No co-localization with phalloidin-stained F-actin (green) was detected.



Supplementary Figure S3. Pre-incubation of primary antibody with 5 μM *Aedae*CAPA-PK1 abolishes immunoreactivity along the pyloric valve (**A**), ileum (**B**) and rectum (**C**). All microscope acquisition exposures are equalized to settings used for experimental treatments. Scale bars, 100 μm.

Videos:

Supplementary Video S1. Sample recording of mosquito ileal motility in response to saline (vehicle control), *Rhopr*PK2b and 5-HT (stimulatory control). While *Rhopr*PK2b decreased contraction frequency, 5-HT reversed this effect and notably increased activity above baseline levels. All video speeds are increased to 300%.

Supplementary Video S2. Sample recording of mosquito ileal motility in response to saline (vehicle control), *Rhopr*PK2b and *Rhopr*MIP-7 (inhibitory control). Both *Rhopr*PK2b and *Rhopr*MIP-7 treatments reduced contractile activity of dissected ilea. All video speeds are increased to 300%.

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