Supplementary Figure legends

Supplementary Fig. S1. Immunohistochemistry of FGLamide related allatostatin (FGLa/AST) neuropeptide in an *Ixodes scapularis* unfed female synganglion. Both dorsal (A) and ventral sides (B) are shown. For the naming of peptidergic neurons a nomenclature system previously used for the hard tick *Rhipicephalus appendiculatus* (Šimo et al., 2009) has been followed. The first two letters of each name refer to the position of each neuron in a specific lobe of the synganglion, specifically cheliceral (Ch), protocerebral (Pc), pedal 1-4 (Pd1-4), opisthosomal (Os), or postesophageal (Po). The letters that follow the first two letters of each name refer to the anatomical location of the neuron, specifically dorsal (D), ventral (V), anterior (A), posterior (P), medial (M) or lateral (L). Neurons that innervate the hindgut were labeled as HG. An unidentified number of four pairs of dorsal opistosomal neurons ($OsDM_{1-4}$) in A (red square) is/are likely the source (OsHG) of the rectal sac innervation. The specific location of three pairs of ventral neurons in B (red square) has not been determined. These neurons may be located in stomodeal or first pedal lobe. Scale bar = 50 µm.

Reference

Šimo, L., Slovak, M., Park, Y., Žitňan, D., 2009a. Identification of a complex peptidergic neuroendocrine network in the hard tick, *Rhipicephalus appendiculatus*. Cell Tissue Res 335, 639 - 655.

Supplementary Fig. S2. Amino acid sequences of four different classes of neuropeptides (FGLa/AST, FGLamide related allatostatin; MIP, myoinhibitory peptide; SIFamide; orcokinin) from *Ixodes scapularis*. Each group is encoded by a single gene. The underlined peptides

were found in the current MALDI analyses. The “a” at the C-termini are for putative amidation; double dot on the N- terminus indicates an unknown cleavage site. For the full neuropeptide precursor sequences see Christie (2008), Donohue et al. (2010) and Šimo et al. (2009b).

References

Christie, A.E., 2008. Neuropeptide discovery in Ixodoidea: an in silico investigation using publicly accessible expressed sequence tags. Gen Comp Endocrinol 157, 174 - 185.

Donohue, K.V., Khalil, S.M., Ross, E., Grozinger, C.M., Sonenshine, D.E., Michael Roe, R., 2010. Neuropeptide signaling sequences identified by pyrosequencing of the American dog tick synganglion transcriptome during blood feeding and reproduction. Insect Biochem Mol Biol 40, 79 - 90.

Šimo, L., Slovak, M., Park, Y., Žitňan, D., 2009a. Identification of a complex peptidergic neuroendocrine network in the hard tick, *Rhipicephalus appendiculatus*. Cell Tissue Res 335, 639 - 655.

Supplementary Fig. S3. Alignments of the predicted translations of the four *Ixodes scapularis* FGLamide related allatostatin receptors (FGLa/AST-R1 - 4) with other FGLa/AST-R related sequences from *Periplaneta americana* (GenBank **AAK52473.1)** and *Bombyx mori* (GenBank **AF303368.1**). The letters highlighted with a gray background are similar, and the letters highlighted with a black background are identical amino acids in a 50% majority rule. Seven conserved transmembrane segments (TM1-7) of putative FGLa/AST-R1 - 4 (red letters) were predicted using the HMMTOP Server v2.0 software (http://www.enzim.hu/hmmtop/).

Supplementary Fig. S4. Phylogenetic relationship of *Ixodes scapularis* FGLamide related allatostatin receptors 1 – 4 (FGLa/AST-R 1 - 4) with FGLA/AST-Rs of various insect species. The tree was constructed using a neighbor-joining method. The number at each node indicates the percentage of support from 500 bootstrap replicates. The *Drosophila melanogaster* neuropeptide F receptor (NPF-R) sequence (**AF364400.1)** was used as the out-group for the analysis. GenBank Accession numbers are: *I. scapularis* FGLa/AST-R1 **XP_002433372.1**; *I. scapularis* FGLa/AST-R2 **XP_002403852.1**; *I. scapularis* FGLa/AST-R3 **XP_002433373.1**; *I. scapularis* **XP_002414997.1**; *Drosophila melanogaster* FGLa/AST-R **AAF05299.1**; *Periplaneta americana* FGLa/AST-R **AAK52473.1**. ,.

Supplementary Fig. S5. Potentiation of spontaneous hindgut movement by SIFamide. Note that only a small number showed spontaneous movement among the high number of tested hindguts when incubated in dissection buffer. In those hindguts, SIFamide clearly potentiated the frequency of spontaneous hindgut movement.

Supplementary Movie S1. Selected video sequence of SIFamide–stimulated *Ixodes scapularis* hindgut motility, when applied at 1 µM concentration.