

## Supplemental Information for:

### ***secCl* is a cys-loop ion channel necessary for the chloride conductance that mediates hormone-induced fluid secretion in *Drosophila*.**

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Running Title: a cys-loop channel mediates hormone-induced diuresis.

secC1	1	-MNLHLSEIFWITLILVLSIWTSVGVKAEEEE-----CPSMADAGS-----LQQTQ	45
glc-1	1	MATWIVGKLI IASLILGIQAQQARTKSQDIFEDDNDNGTTTLESLARLTSPIHIPIEQPQ	60
secC1	46	LIQR--LTHVCR--YDRLERPFEYDSDGKRLPIVVKTRIYVYFLQNLNSDLLQFKMHALL	101
glc-1	61	TSDSKILAHLFTSGYDFRVRPPTDN--GG--PVVSVNMLLRTISKIDVVNMEYSAQLTL	116
secC1	102	QLRFQDKRLAYKAFNRSDNILGQKHLSERLWLPHIFFANERESSILGTDEKDVLTSLSP	161
glc-1	117	RESWIDKRLSYGVKGDGQPDFVILTVGHQIWPDTFFPNKQAYKHTIDKPNVLIRIHND	176
secC1	162	GNV I ISTRMQASLYCWMNFQKFPFDQQFCSTVLESWMYNTSDLILEWEPHTPI SFDPEMR	221
glc-1	177	GTVLYSVRISLVLS <span style="border: 1px solid red;">CPMYLQYYPMDVQCC</span> SIDLASYAYTTKDIEYLWKEHSPLQLKVGLS	236
		M1	
secC1	222	LTEYNMAQFWHNTTIVQSDGDNLRHGAFAGNYSSLSFTVNLKREIGFYLLDYLLPMMIV	281
glc-1	237	SSLPSFQLTNTSTTYCTSVTN-----TGIYSCLRTTIQLKREFSFYLLQLYIPSCMLV	289
		M2	
secC1	282	AISWVSFWLQADASPPRIMLGTSTMLSFITLSSSQSKNLPKVSYIKVSEVWFLGCTFFIF	341
glc-1	290	IVSWVSFWFDRTAIPARVTLGVTLLTMTAQAGINSQLPPVSYIKAIDVWIGACMTFFIF	349
		M3	
secC1	342	GSLVEFAFVNTIWRKKEINIELKKNVSKYIIKSTLTPRPARRQIGGSLSNESRARS CSSLD	401
glc-1	350	CALLEFALVNHIAN-KQGVVERKARTER-----EKAEIPLLQNLHNDVP-----T---	392
secC1	402	NIVSSTESVRNNGTGNQGFNNYLTVHPNLP IIRTECAEADTVSICSARTNNDHIVDVDK	461
glc-1	393	KVFNQEEKVR-----TVPLNR-----RQMNSFLNLL-	419
		M4	
secC1	462	DKKDTPTFTTMTTPQEIAMWIDRRSRFLFPAMFLAFNALYWTFVY---VL-	508
glc-1	420	-----T-KTEWNDISKRVDLISRALFPVLFVFNILYWSRFGQONVLF	461

**Supplemental Figure S1: secC1 encodes a protein that contains the features of a typical cys-loop pentameric ligand-gated ion channel (pLGIC) subunit.** Amino acid alignment of *secC1* with the *Caenorhabditis elegans* pLGIC subunit *glc-1*. The two cysteines that form the cys-loop are boxed in red. The alpha-helical transmembrane domains M1-M4 are marked with solid green lines. Shading indicates degree of conservation (darker is more conserved) as scored by BLOSUM26 substitution matrix (Henikoff, S. & Henikoff, J.G. Amino Acid Substitution Matrices from Protein Blocks. *Proc. Natl. Acad. Sci. U. S. A.* **89**, 10915–10919 (1992)). Sequences aligned using CLUSTAL W (Thompson, J.D., Higgins, D.G. & Gibson, T.J. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Res*, **22**,4673-4680 (1994).)

## Supplemental Data S2

Primers for Amplification of *secCl* cDNA from mRNA:

5'GCGGGGACAAGTTTGTACAAAAAAGCAGGCTCGATATCATGAATCTGCATCTGAG  
TGA3'

5'GGGGACCACTTTGTACAAGAAAGCTGGGTGAGCTCTAGATTACAAAACATAGACG  
AGGT3'.

Primers for amplification of *secCl* genomic rescuing fragment:

5'CACCAATCGACGCAGGTAAATGGAC 3' and

5'AGGATCGAGGATAGACGCATGGTT3'.

Primers for amplification of *secCl* promoter for *secCl*-promoter::GFP fusion:

5'-CACCAATCGACGCAGGTAAATGGAC -3' and

5'-TCTTCCTCGGCTTTGACCCTA-3'

The primers used to introduce site specific mutations to remove extraneous start codons from the promoter:

5'-GCAGATTCCTCAGcTGGcTTGATTC-3' and 5'-TTGGATCACGCTGATCTTGGTC-3'

where lower case letters refer to the nucleotide bases that were mutated.

Primers for amplification of *secCl* intracellular loop for antibody production:

5'TAAGGCCTCTGTCGACCAGTTTGCCTTTGTCAACAC3'

5'CAGAAATTCGCAAGCTTCTACGGCGTGCCTTTTTATCCT-3'

Nucleotides 1-16 of both primers correspond to homologous portions of the pEcoli- Nterm 6xHN vector.