Table S1. Primers used in RT-PCR, RACE, ORF verification, dsRNA synthesis, and qPCR.

Fragment name	Forward primer	Reverse primer
RT-PCR		
LdChSA-1	GTCAAAGGTGCGATGTTA	AGAAACGGAAACTGGAAT
LdChSA-2	CGCTACTCGGTTTCCTAC	ACACTGTTCCGATGGTTT
LdChSA-3	CTTGCCTCCTACCTAACC	TTCGATACTGCCTTCGTC
LdChSB-1	TGACTTAGCGGACGACTC	AAGAAACAGCGAAGAGGG
LdChSB-2	GCAGCCATAATGAGTGTT	CTGAGTATGCCTGGGAAG
RACE		
LdChSA 5'-GSP		TTCCCACCAGCCGACAGA
LdChSA 5'-NGSP		AAGTCTGCTCGCCCCTCC
LdChSB 5'-GSP		CGCTGCGTAATTGACGGATT
LdChSB 5'-NGSP		CATGCGTAGATGCGAGTA
LdChSA 3'-GSP	ACCAATGACGATGACGAAGG	
LdChSA 3'-NGSP	CCAACGAAACCATCGGAACA	
LdChSB 3'-GSP	GGACAAAATGGTGGTGAC	
LdChSB 3'-NGSP	AAACTGAGAAACCCAAGGCACC	
ORF verification		
LdChSA	GGCGAGTGGATTGCGACC	CGATTATTTGTTCCCATC
LdChSB	ATGCAGAGGCGATATCAGT	TCATAATCTGAGAGATGCAG
dsRNA synthesis		
ds*ChSA*-1	TTTCGACCAGACGAGATA	GTAGGTTAGGTAGGAGGC
ds*ChSA*-2	ATTCCTTATGTTGGTGGG	CCCAGGATACATTGTTTAGA
ds*ChSAa*	ATCAGTCTTTGCTTTCTT	ACTTTATGTGGAGGTTGT
ds*ChSAb*	GAACTGCGGAACAAGTCG	CTCGGAAGTCTCCTCAATAT
ds*ChSB*-1	AGGGTACTCTATTATTCATG	GTCAACATAGCTCCTTTT
ds*ChSB*-2	GTAGTGGCTGCCGTCTTG	GCATTTGGACCTTTGAGT
ds*egfp*	AAGTTCAGCGTGTCCG	CACCTTGATGCCGTTC
qPCR		
q*LdChSA*	TTGGAACCATAGCTCACATCTT	AATCTCCACTGCCTGCTTATC
q*LdChSAa*	TAAGGAAGAGAAGGCCCGTA	AAGGGCCACTTTATGTGGAG
q*LdChSAb*	ACGTTAAGTGGCCGTTAGGA	GACCAAGATGAGTGCGAAGA
q*LdChSB*	AGACTTCTGGTGTTGCTCTTC	GTAGGCGCATTCGTCCTTAT
q*LdRP4*	AAAGAAACGAGCATTGCCCTTCCG	TTGTCGCTGACACTGTAGGGTTGA
q*LdRP18*	TAGAATCCTCAAAGCAGGTGGCGA	AGCTGGACCAAAGTGTTTCACTGC
q*LdARF1*	CGGTGCTGGTAAAACGACAA	TGACCTCCCAAATCCCAAAC
q*LdARF4*	GTGCTCGTGAACCATGTGAA	AACCTCCAATCCCTCGTGAA

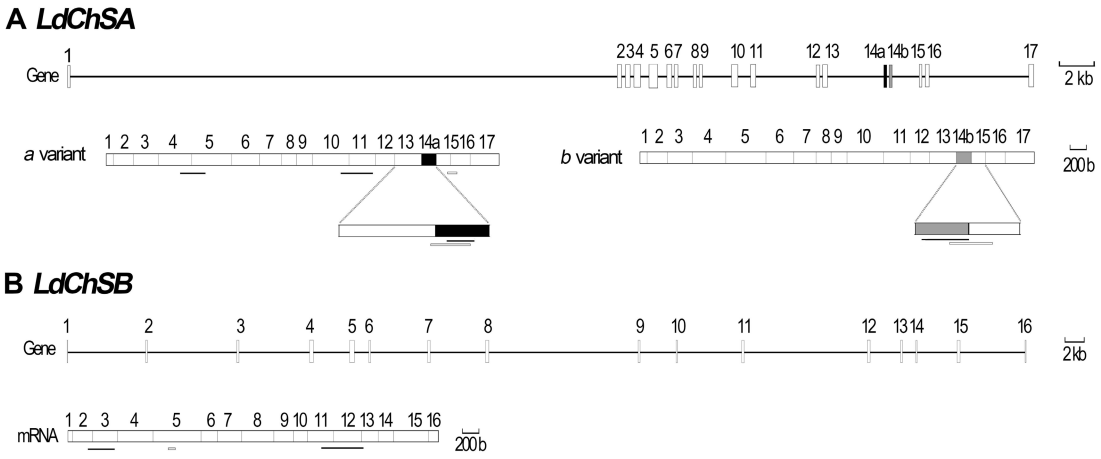

Figure S1. Exon/intron and mRNA structures of putative *ChS*s from *Leptinotarsa decemlineata*. Boxes mark exons. Lines mark introns. *LdChSA* and *LdChSB* genes respecitvely contains 17 and 16 exons, and 17 and 15 introns. For *LdChSA*, alternative splicing of exon 14a or 14b forms two splicing variants, *LdChSAa* and *LdChSBb*. The dsRNA and qRT-PCR sequences were marked with black and gray lines.

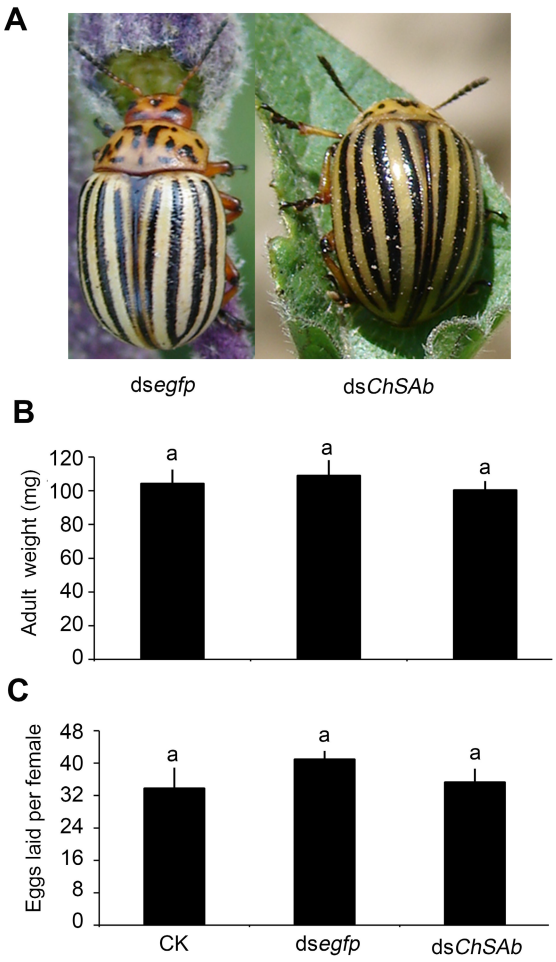

Figure S2. Effects of RNAi of *LdChSAb* in *L. decemlineata* second-instar larvae on adult performance. The resulting adults did not have obvious defective phenotypes and had similar size (A), weight (B) and fecundity (C) to control adults. The bars represent values (± SE). Different letters indicate significant difference at P value < 0.05.

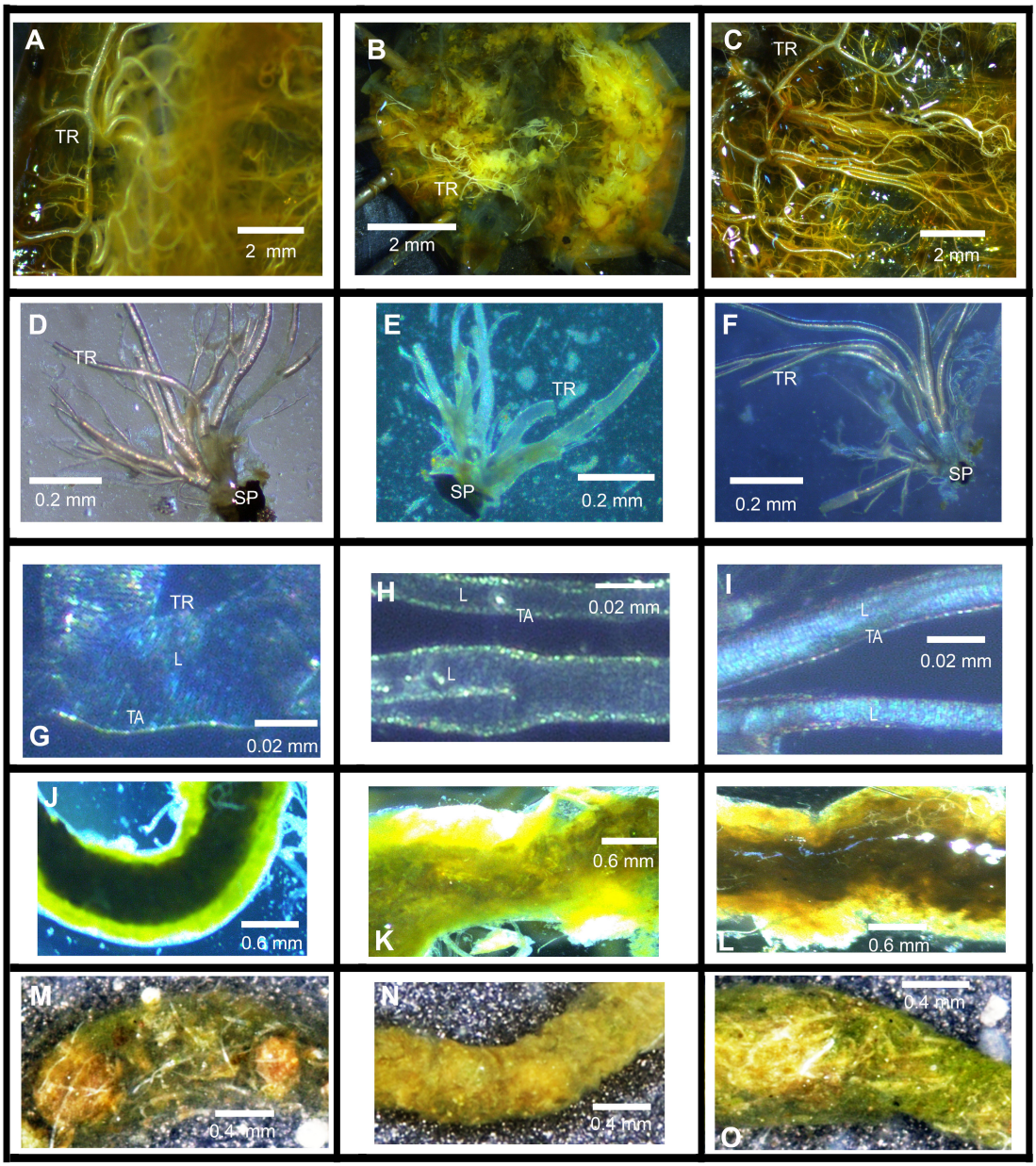

Figure S3. Knockdown of *LdChSAa* and *LdChSAb* on chitin-containing structures in *L. decemlineata*. The PBS (the left column)-, ds*ChSAa* (the middle column)-, and ds*ChSAb* (the right column)-ingested larvae are dissected and observed under a light microscope. The tracheae were directly seen (A-F), or are incubated with 10 M NaOH at 95 °C for 2 hrs (G-I). SP, spiracle; TR, tracheae; L, tracheal lumen; TA, taenidia. Larvae previously fed PBS and ds*ChSAb* have well developed tracheae (A, C, D, F), there are distinct taenidia in the tracheae (G, I). The taenidia run around the tracheal tube and form parallel transverse folds lining the lumen of the tracheae (G, I). In contrast, the ds*ChSAa*-fed larvae possess underdeveloped tracheae (B), the taenidia are thinned (E, H). Moreover, the larvae previously fed PBS, ds*ChSAa* and ds*ChSAb* have clear gut lumen, which was full of food (J, K, L). After removal of the midgut epithelia cells, integrate peritrophic matrix envelops food in these larvae (M, N, O).