

Supplementary data

**Dusky-like Is Critical for Morphogenesis of the Cellular
Protuberances and Formation of the Cuticle in
*Henosepilachna vigintioctopunctata***

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Table S1. A list of primers used for RT-PCR, dsRNA synthesis and qRT-PCR

Fragment name	Forward primer (5'-3')	Reverse primer (5'-3')
RT-PCR		
<i>HvDusky-like</i>	ATACTGGTACAGCAAACAAA	CTCTGCATTGTCGTAACCT
dsRNA synthesis		
<i>dsHvDusky-like</i> -F	TAATACGACTCACTATAGGGAT	TAATACGACTCACTATAGGGACT
1R1	ACAAGTGGGCAAAGG	ACGGAAGCAGATGG
<i>dsHvDusky-like</i> -F	TAATACGACTCACTATAGGGGC	TAATACGACTCACTATAGGGCCA
2R2	CCCTTGCTACACCAGATA	CCATAACTAGCGGCATT
<i>dsegfp</i>	TAATACGACTCACTATAGGGAA	TAATACGACTCACTATAGGGCAC
	G TTCAGCGTGTCCG	CTTGATGCCGTTC
qRT-PCR		
<i>qHvDusky-like</i>	TTCAGCAGCAGATCCAGAGA	CCTCTAGCATGGACGAAAGC
<i>qHvRPS18</i>	CGCAATCAAAGGTGTTGGAAG	GCCTAGGGTTGGCCATAATAG
<i>qHvRPL13</i>	AGCATCCTTCGCTCGTTTAG	TTCGACAACCTGCCATTAGG

HvDyl

ATGACAAGGTCACCTTCATATGTTGAGCATCGCTATACTTGCAGTGTTATTTTAAAGGAACACACGC
TGTGATGCACCAATACCAGACTCTGCGTCTCTTCCATTAGAACACAGAGGAATATACGGACCACCA
CTTTCAGGTCCTGCTTATGCTCCTCCGTCCGCTCTCATACAGAGCGGAGGTGGTGGTGGAGGTTCA
GATGATCCATGGCCCCCTTGCTACACCAGATATGCCCCAAATCAAACATTTGCAAGTACAGTGTGAA
AAAACACATATGAGGGTGAATATTGAATTCGACCGCCCTTTCTATGGTATGATATTCTCCAAGGGT
TTCTACAGTGATCACCCTGTGTACATTTGAAACCTGGTACTGGTCACTTGAGCGCCACATTTGAA
ATATTCTTGAACAGTTGCGGCATGTCTTCATCGGCCAATCATAATGCCGCTAGTTATGGTGGACCA
ACACCTAGTGGAAGCTATGTAGAGAACACAATTATAATTCAGTACGATCCATTTCGTACAAGAAGTT
TGGGATCAAGCAAGAAAACCTACGTTGTACCTGGTATGATTTCTATGAGAAGGCTGTTACTTTCAGA
CCATTCCAAGTAGATATGCTACATGCAGTTACTGCTAACTTCTTGGGCGATAATTTACAATGCTGG
ATGCAAAATACAAGTGGGCAAAGGACCCTGGGCTTCTGAAGTATCTGGAATAGTGAATAATTGGACAA
ACAATGACAATGGTACTCGCTATAAAAGATGATGAAAATAAGTTTCGATATGTTGGTCAGAACTGT
GTGGCTCATGATGGAAGAGAGCACCAATTCAATTGGTTGATCAGTATGGTTGCGTTGTTAGACCT
AAAATTATGAGTAAATTCCAGAAGATAAAGAACTTCGGACCATCTGCTTCCGTAGTGTTCATTTCGCT
TATTTCCAAGCATTCAAATCCCAGATTCAATGAATGTTCAATTTCCAATGTGTAATTCAAGTCTGC
AGATACAATTGTCCAGAACCTAAATGCGGGCATGGTCTTGGATTGTGAATGAGTATGGCGTTCCT
GCATTAGGTGGCAGCGGACATGGAGGTAATTTGCATGCTGAATATGGACCTCCACCACTTCCAGAA
TACAACGGAGTTTACCTGCATTCCCAGACCCAGACACCCTTCAGGCCCAACTGGAGCTTATTCT
GAACCGAATCCTGATGTTGTTCTGCTCCACAAGCTCAAACATCTTCTCATCGACTTCGAATCCG
TCAAATCTTTCTTCTTAGCACCGCAAAGTAACTCTCAGACAGGAAACGATTTGCATTTACCTCCA
CCACCTCCACCTGGACACCACGGTCAATACAACACTGTGAAGAGGAAGAGCGATATCGAAGGAGAA
TTCGAAAACAACCTAGCTACCTTAGGTGGACGTCCACGGACTTTGGAAAATCCTGCACAACCTCCAG
GGTGTGAGACGTAGGCGCAGTCCTGACACATTCAACATTGTGGTTAGTCACGTCTATAAAAGAGAA
GCTCAAGAAATGACTGACGTTAATACTAGTAGGATCATTCAAGTGGTTGCACCTGGAGATGTTAAC
TTTGCTCTAAATTCTGCTCAAAACAACGAAACCGTAGTTATTCAATCAGCTTCAGCAGCAGATCCA
GAGACAATTTGTATGTCCATCCCTAGTTTTGTGCTGGACTTGTGCTGCTCCTTTTAGTGCTTATC
GTGTCGAGTTTAGTGGCTGCCCTTCTGTTTCGTGAGAGTACGCGCCATTGATAGGAAAGGAGCAGCA
CCAGCTTTCGTCCATGCTAGAGGTTACGACAATGCAGAGTTCGTCAAAGTACCAAATTAG

Figure S1. The positions of the primary and secondary dsRNA on the mRNA. The green represents the position of primary dsRNA (dsHvDusky-like-F1R1), The yellow represents the position of secondary dsRNA (dsHvDusky-like-F2R2).

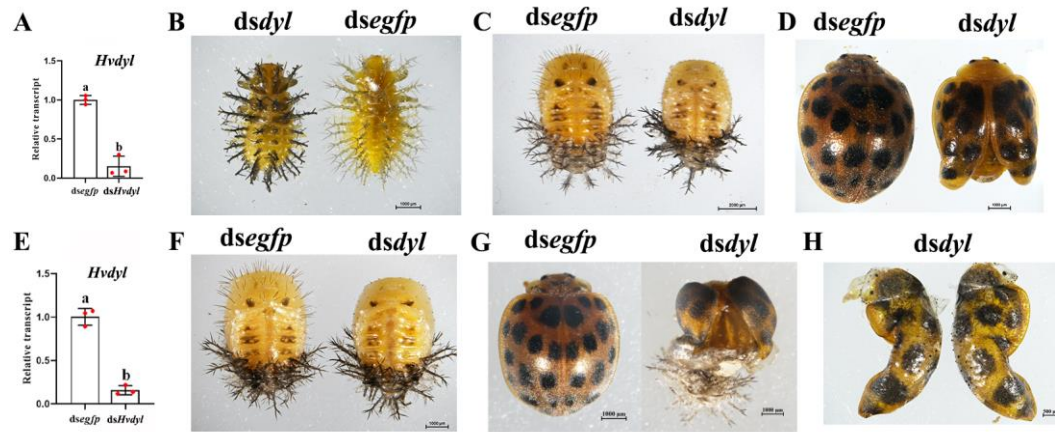


Figure S2. Knockdown of *Hvdyl* with secondary dsRNA at the third- and fourth-instar larval stage impairs growth of the cellular protuberances and the formation of cuticle in *Henosepilachna vigintioctopunctata*. The newly molted third- and fourth-instar larvae were injected with 300 ng *dsegfp* or *dsdyl* and then transferred to potato foliage. Two days after third-instar larvae injection, transcript levels of *Hvdyl* were measured (A). The phenotypes of fourth-instar larvae (B), pupae (C) and adults (D) were observed and imaged (third-instar larvae injection). Two days after fourth-instar larvae injection, transcript levels of *Hvdyl* were measured (E). Relative transcript levels are the ratios of relative copy number in treated individuals to that of *dsegfp*-injected controls, which was set as 1. The values represent means (\pm SE). Different letters indicate significant differences at $P < 0.05$ using t test. The three biological replicates are marked with red dots. The phenotypes of pupae (F), adults (G) and elytra (H) were observed and imaged (fourth-instar larvae injection).