Supplementary figures

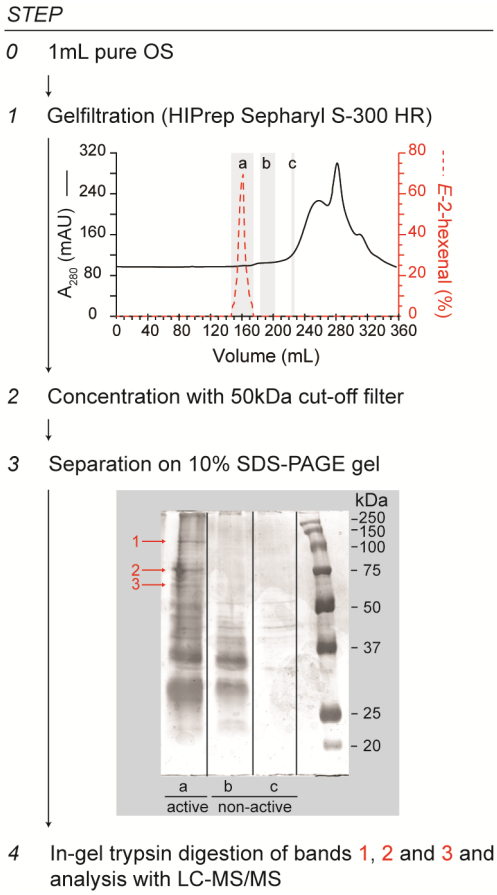

Fig. S1. Partial purification of (3*Z*):(2*E*)-hexenal isomerase from *M. sexta* OS. 1 ml of pure *M. sexta* OS was diluted 1:1 (v/v) with Tris buffer (100mM NaSO4, 40mM Tris pH 9.0), applied to a HIPrep Sepharyl S-300 column and proteins were eluted isocratically (**Step 1**). Protein elution was continuously monitored by UV absorption at 280nm and is given in milli absorbance units (mAU, black line). The activity of each fraction was monitored using the SPME-guided *in vitro* assay and (3*Z*):(2*E*)-hexenal isomerase activity is given in % *E*-2-hexenal (red, dotted line). The pooled active fractions (grey box, **a**) and two pools of non-active fractions (grey boxes, **b** and **c**) were first each concentrated with centrifugal filters; proteins were subsequently precipitated with 80% acetone and re-suspended in MiliQ-water (**Step 2**). Samples were separated on a 10% SDS-PAGE gel and proteins were visualized by silver-staining (**Step 3**). The three most prominent bands that were solely present in the active sample (lane a, red arrows 1, 2 and 3) and absent in non-active samples (b and c) were cut-out from the gel; proteins were digested and extracted prior to mass spectrometric analysis by liquid chromatography-tandem mass spectrometry (LC/MS/MS) (**Step 4**).

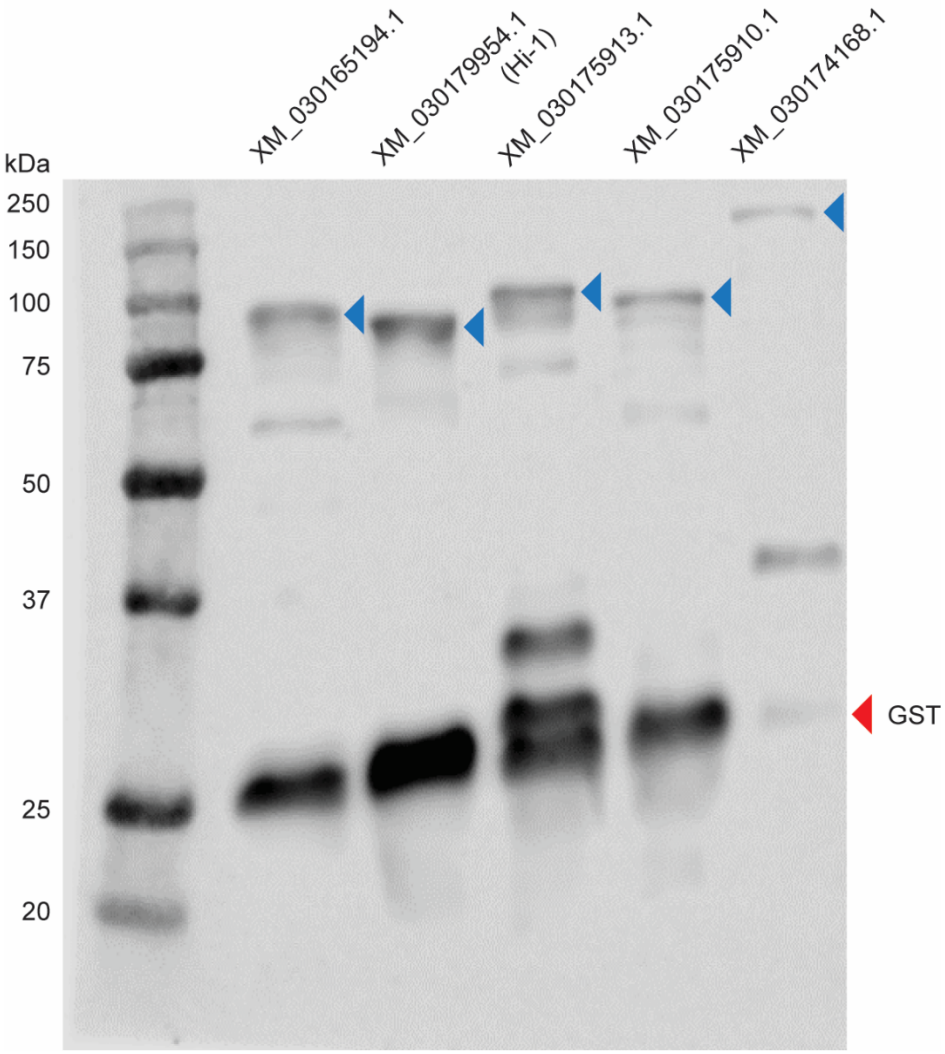

Figure S2. Full image of western blot shown in figure 2A. Purified GST-tagged recombinant protein of five candidate proteins (XM_030165194.1, XM_030179954.1, XM_030175913.1, XM_030175910.1 and XM_030174168.1) were visualized by western blot. Blue arrows indicate the position of each recombinant protein. Red arrow indicates position of GST.

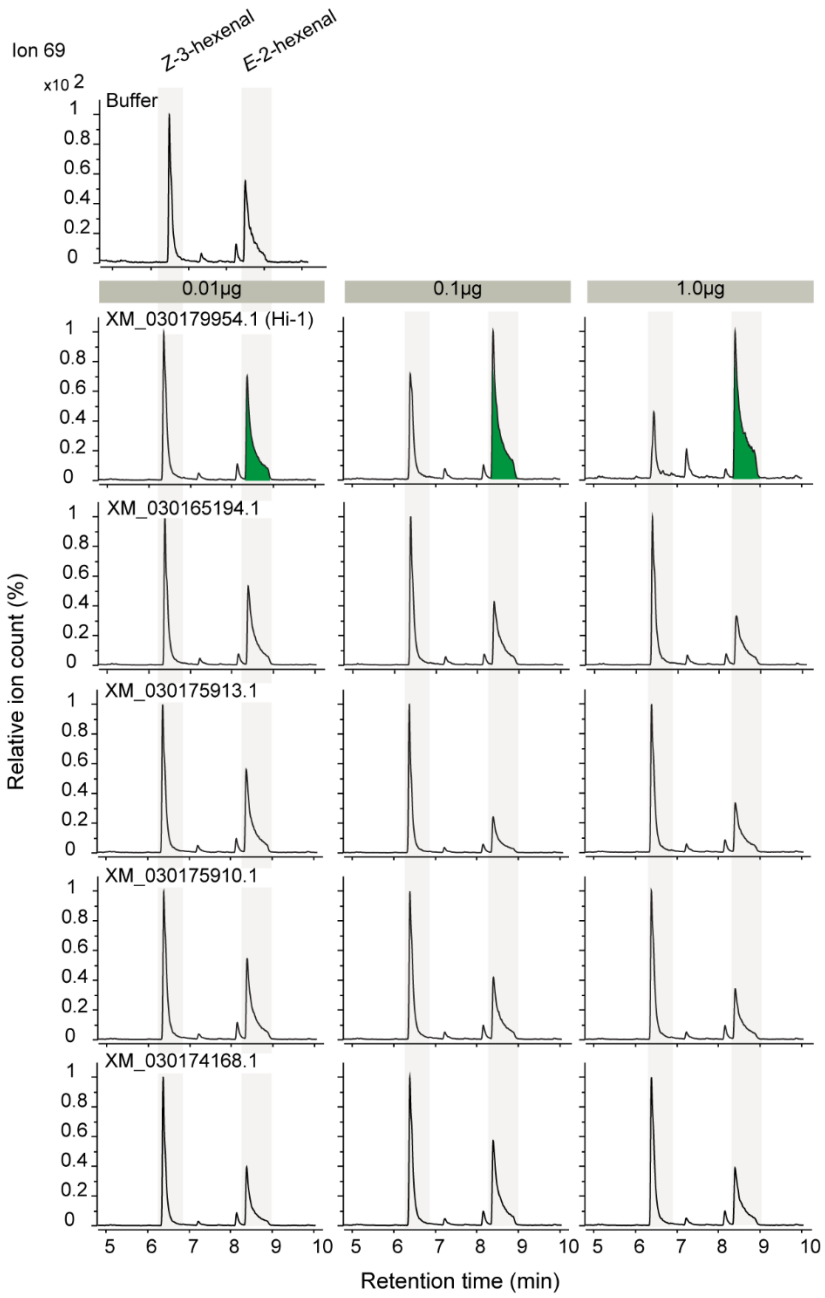

Figure S3. Representative extracted ion chromatograms (ion 69) of figure 2B. Hexenal isomerase activity of five candidate recombinant proteins with three different protein quantities (0.01, 0.1 or 1 μg) were determined by SPME-guided in vitro assay. Green highlight in Hi-1 indicates clear isomerization converting Z-3-hexenal to E-2-hexenal.

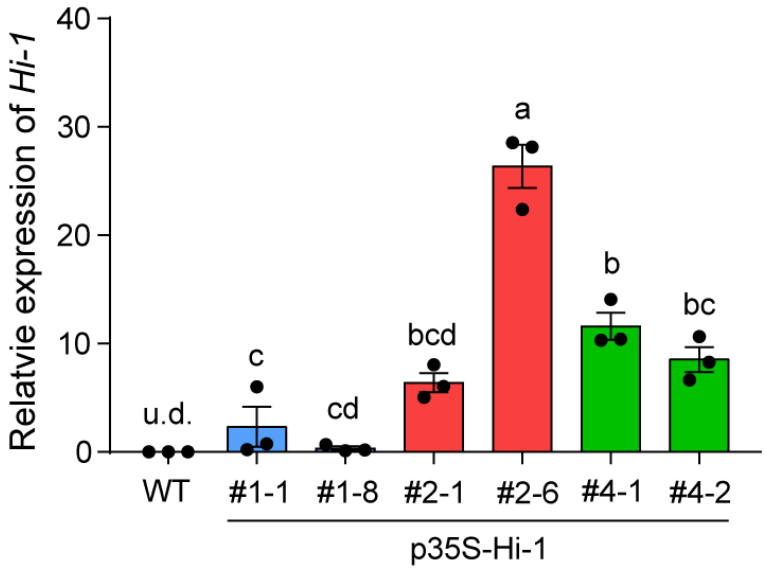

Figure S4. Transcript levels of *Manduca sexta*'s hexenal isomerase-1 (*Hi-1*) in stable expression lines of *Arabidopsis thaliana*. Measurement of *Hi-1* expression in transgenic *Arabidopsis* (Co1-0) T3-lines by qPCR. Whole shoot of 5-week-old plants were harvested. Expression level was determined by normalizing the *Hi-1* expression level to reference gene *sand* ($2^{\triangle CT}$ method). Different letters indicate significant differences (p <0.05) between groups (ANOVA followed by Tukey HSD post-hoc analysis, $F_{5,12}$= 47.04, p <0.0001, n = 3). Abbreviations: undetectable (u.d.), wildtype *Arabidopsis* (WT).

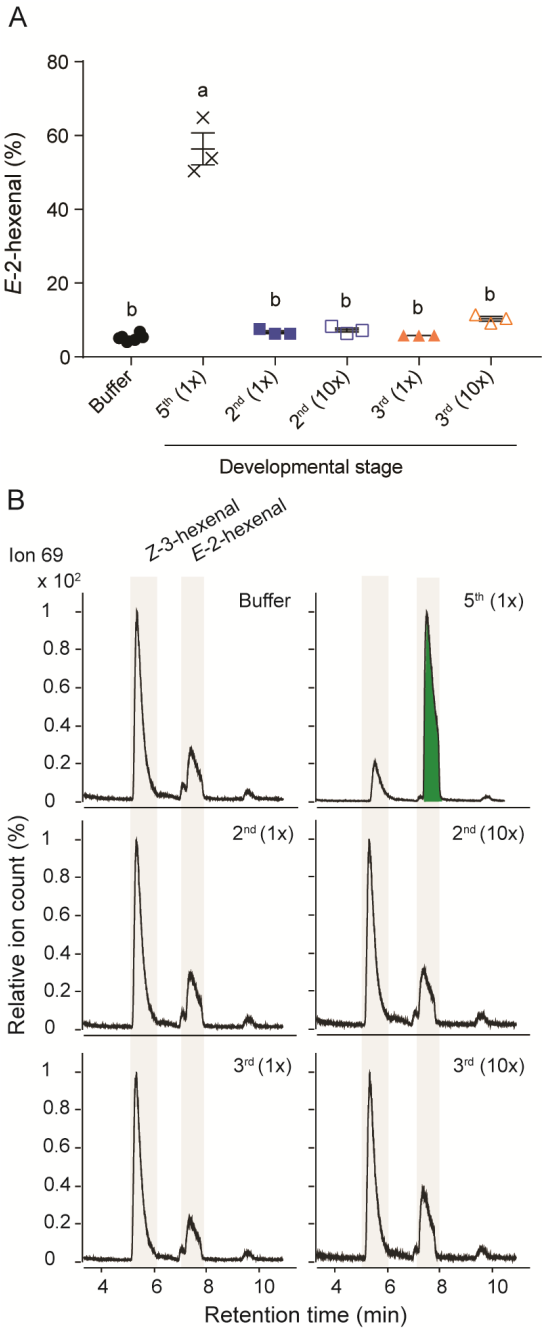

Figure S5. Hexenal isomerase activity of OS from 2nd and 3rd instar larvae with tenfold increased concentration. (A) 1X OS (0.5 µg total protein) or 10X OS (5 µg total protein) were incubated with Z-3-hexenal (200 mM), hexenal isomerase activity were determined by SPME-guided in vitro assay. The proportion of E-2-hexenal in total aldehyde (Z-3-hexenal + E-2-hexenal) was calculated. Different letters indicate significant differences (p <0.05) between tissues (ANOVA followed by Tukey HSD post-hoc analysis, $F_{5,15}$ = 155.3, p <0.0001, n = 3-6). **(B)** Representative extracted ion chromatograms (ion 69) as output of the SPME-guided assay. Green highlight in E-2-hexenal peak area indicates clear isomerization converting Z-3-hexenal to E-2-hexenal.

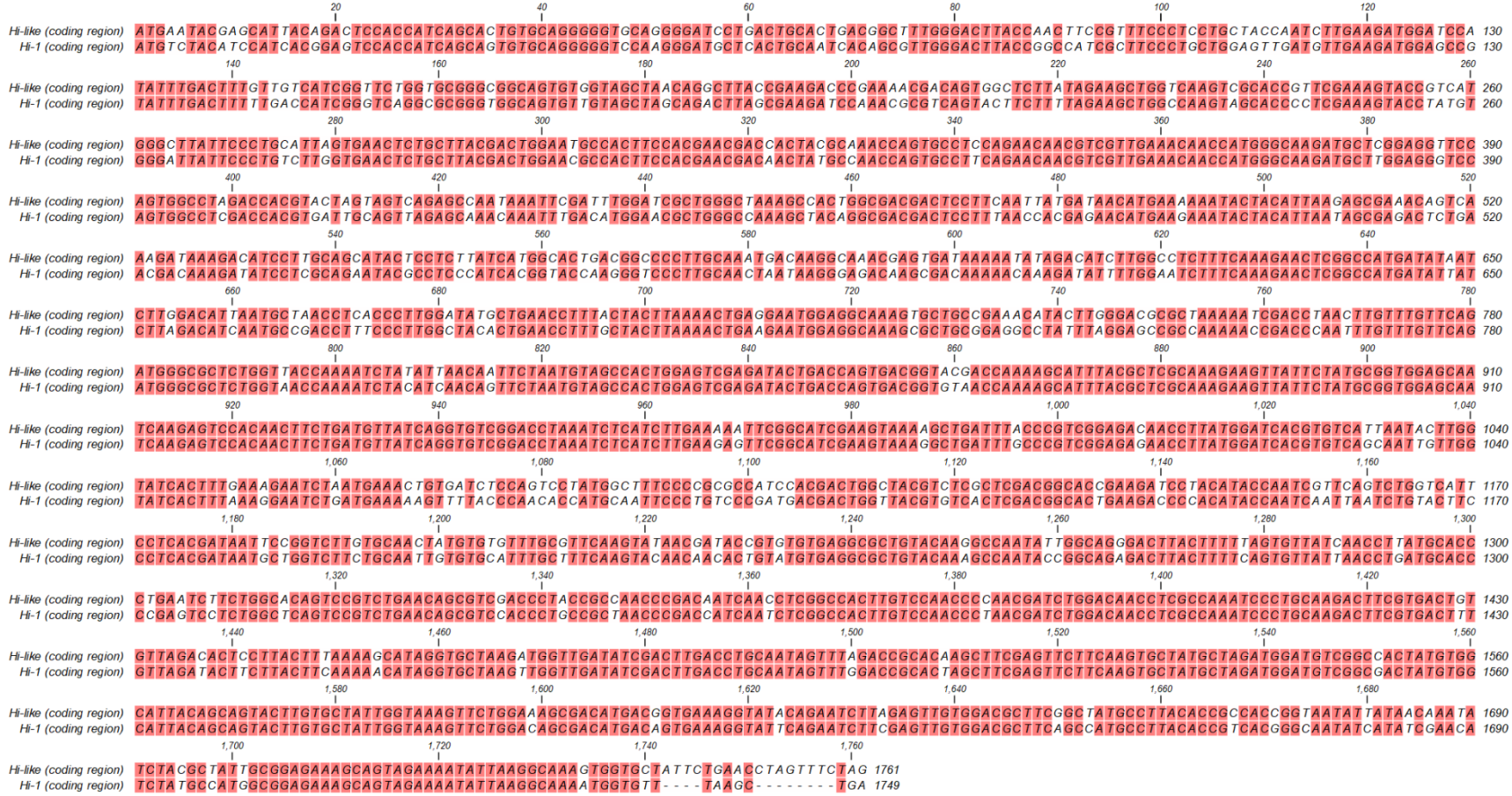

Figure S6. Alignment and sequence identity of *Hi-1* and *Hi-like* coding sequences. The coding sequences of Hi-1 (NCBI XM_030179954) and Hi-like (XM_030179956) were aligned by using CLC Main Workbench. The conserved nucleotides were highlighted by red color. Identity scores was analyzed by using NCBI BLAST2; sequence identities:1479/1733(85%), Gaps:10/1733(0%).

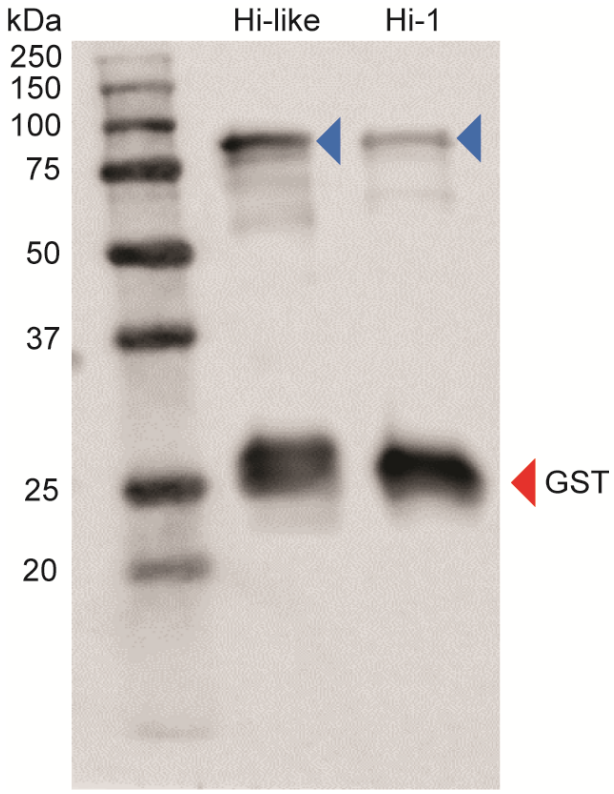

Figure S7. Full image of western blot shown in figure 5A. Purified GST-tagged recombinant proteins of Hi-like and Hi-1 were visualized on western blot. Blue arrows indicate the position of each recombinant protein. Red arrow indicates the position of GST.

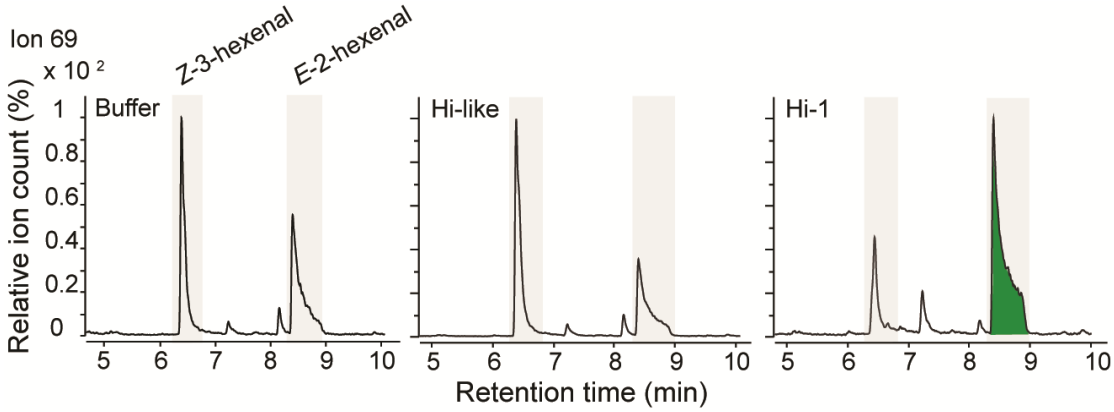

Figure S8. Representative extracted ion chromatograms (ion 69) of figure 5B as output of the SPME-guided assay. Green highlight in Hi-1 indicates clear isomerization converting Z-3-hexenal to E-2-hexenal.

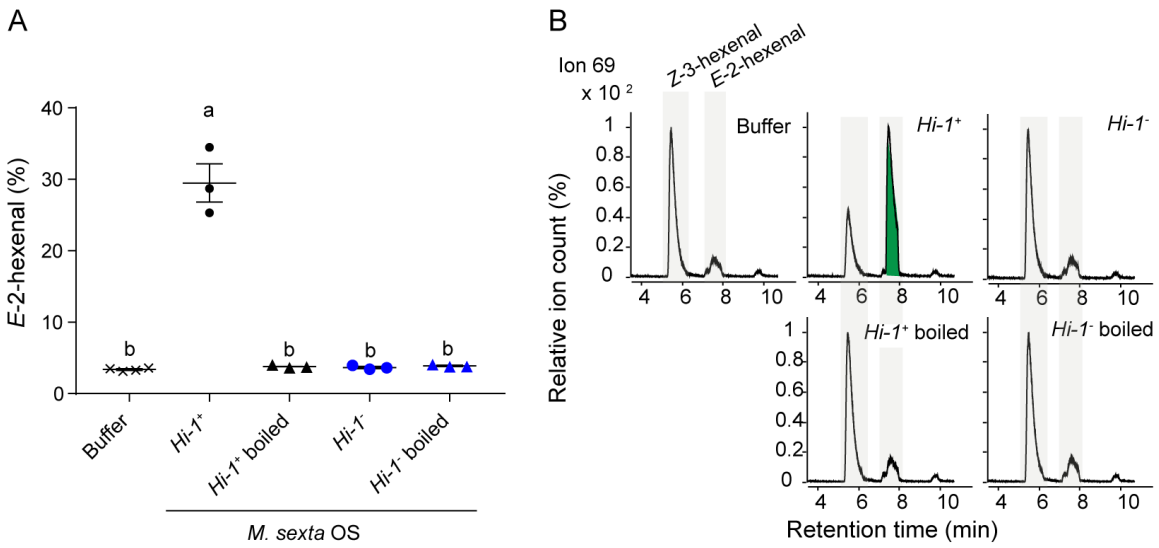

Figure S9. Hexenal isomerase activity of OS after heat treatment. **(A)** The OS samples (0.33 µg total protein) from wildtype (*Hi-1*$^{+}$ boiled) or homozygous mutant (*Hi-1*$^{-}$ boiled) were incubated at 95 ℃ for 5 min, hexenal isomerase activity were determined by SPME-guided in vitro assay with 200 mM of Z-3-hexenal subtract. The proportion of E-2-hexenal in total aldehyde (Z-3-hexenal + E-2-hexenal) was calculated. Different letters indicate significant differences ($P < 0.05$) between tissues (ANOVA followed by Tukey HSD post-hoc analysis, $F_{4,11} = 103.4$, $p < 0.0001$, n = 3-4). **(B)** Representative extracted ion chromatograms (ion 69) as output of the SPME-guided assay. Green highlight in E-2-hexenal peak area indicates clear isomerization converting Z-3-hexenal to E-2-hexenal.

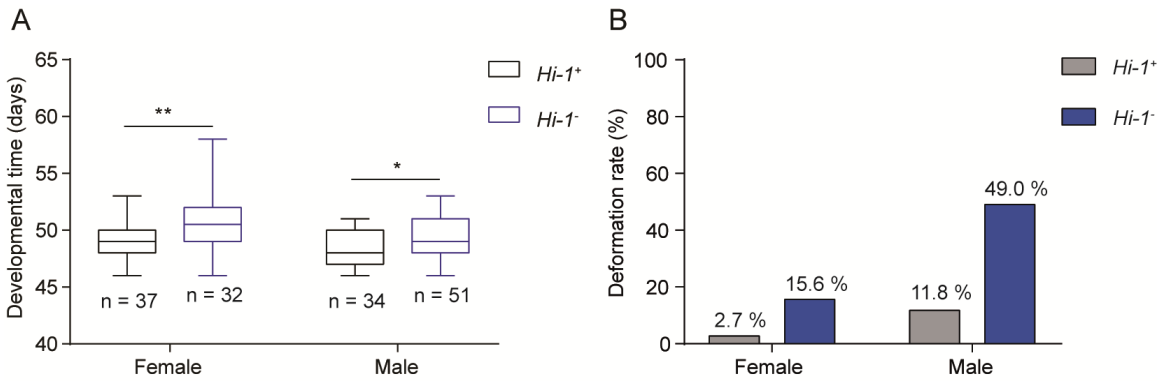

Figure S10. *Hi-1* mutates phenotypes under lower rearing temperature (24 ℃). **(A)** Developmental times was recorded from neonate till newly emerged adult stage. Two-tailed t-test was performance comparing between wildtype (*Hi-1*$^{+}$) and mutant (*Hi-1*$^{-}$) female ($t = 3.34$, $df = 67$, $p < 0.01$) or male ($t = 2.27$, $df = 83$, $p < 0.05$). **(B)** Proportions of deformed adults were recorded from one generation. The numbers of recorded individuals were presented as "n =" in figure S10A.

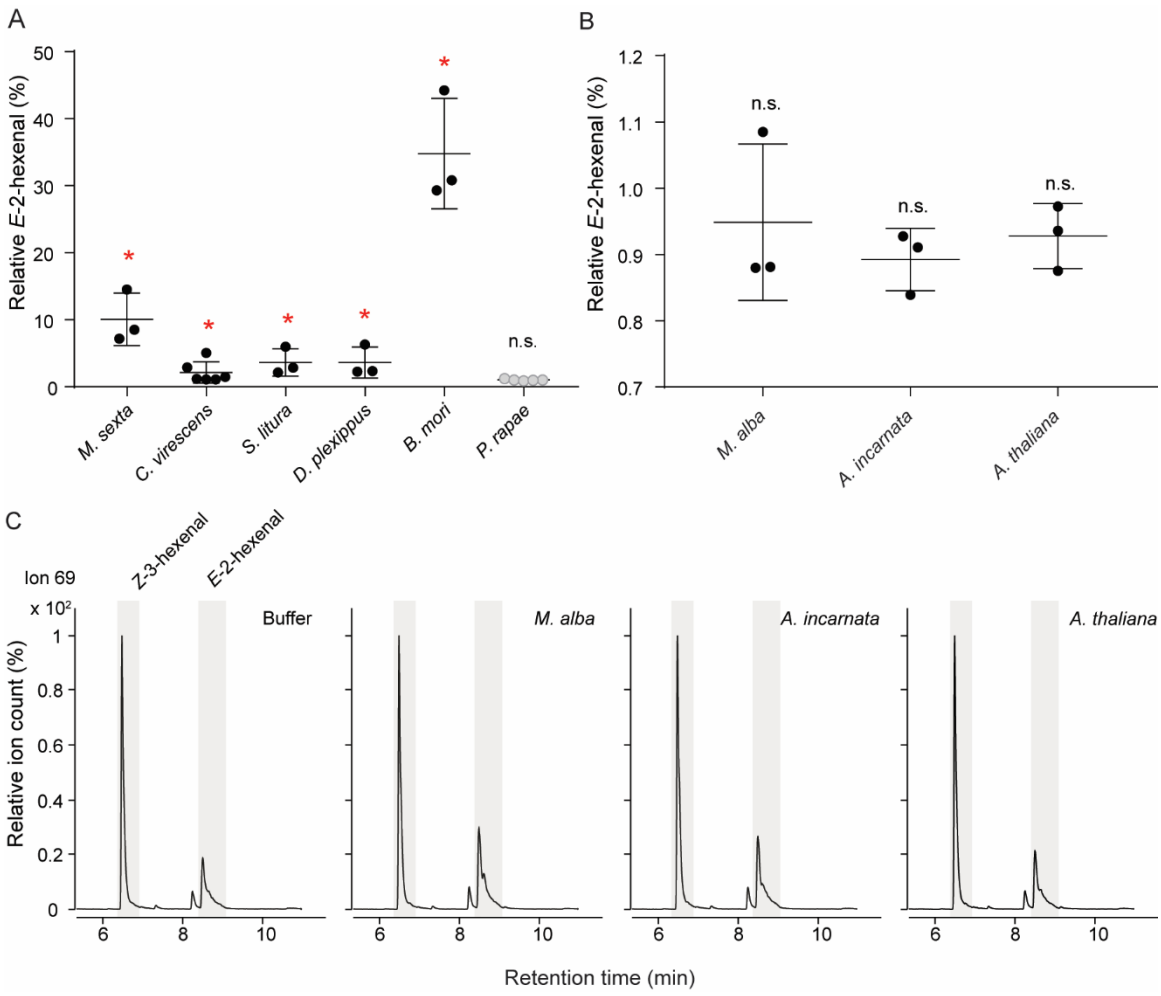

Figure S11. Hexenal isomerase activity in OS from six different lepidopterans and crude protein extract of their host plants. **(A)** OS (4 µg total protein) collected from 4th to 5th instar larvae, or **(B)** crude protein extract (4 µg total proteins) collected from leaves of three host plants were incubated with Z-3-hexenal (40 mM), hexenal isomerase activity were determined by SPME-guided in vitro assay. The proportion of E-2-hexenal in total aldehyde (Z-3-hexenal + E-2-hexenal) was calculated, and value was further normalized to buffer control. Asterisk mark indicates significant differences between buffer control and OS or plant extract (Mann–Whitney U test, *p <0.05, n.s. not significant, n = 3-6). (C) Representative extracted ion chromatograms (ion 69) as output of the SPME-guided assay from the leaves crude protein extracts with ten-fold concentration (40 µg total proteins).

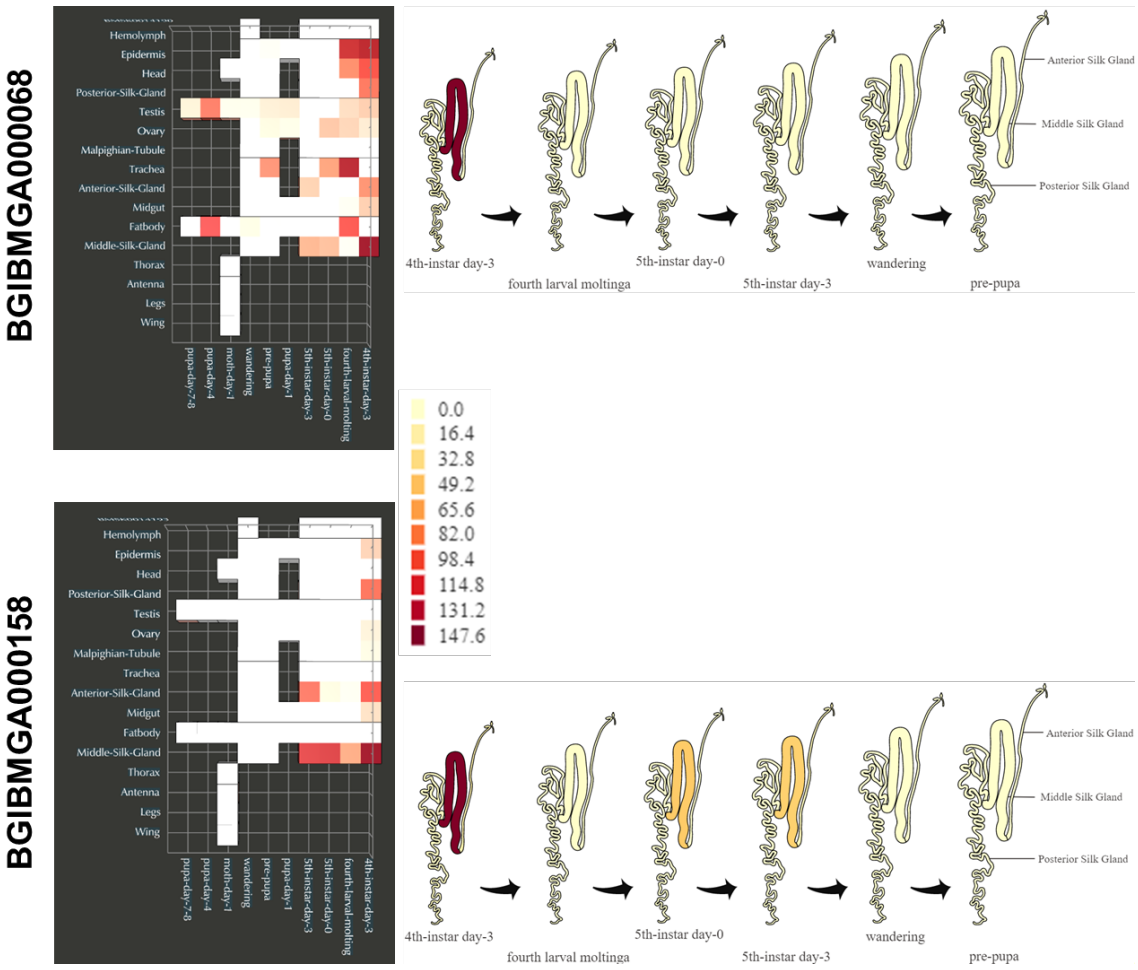

Figure S12. Spatial expression profiles of two *Bombyx mori* genes, BGIBMGA000068 and BGIBMGA000158. The data analysis and illustration are done by online tool, SilkDB 3.0.