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Supplementary Information for:

A Novel Effector from Cotton Bollworm Oral Secretion Impairs Host Plant Defense Signaling

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SI Materials and Methods

Plant Materials and Treatments. The tobacco plants (*Nicotiana benthamiana*), the pair of glanded and glandless isogenic lines of cotton plants (*Gossypium hirsutum*) (1) and the *Arabidopsis thaliana* (ecotype Col-0) plants were grown at 22°C under long-day (LD, 16 h light/8 h dark) condition. *35S:JAZ3-HA*, *35S:JAZ3δN-HA*, *coi1-2* and *jazQ* are as described (2-4).

For overexpressing *HARP1* and *REPAT38* in *Arabidopsis*, the coding regions of *HARP1* and *REPAT38* were inserted into the binary vectors (pCAMBIA1300) behind the 35S promoter, respectively, with a 6×MYC N-terminal fusion. The dsHARP1-pBI121 were constructed as previously described (5). Briefly, a sense sequence of HARP1, a 120-nucleotide intron of *A. thaliana* RTM1 gene and the HARP1 in antisense orientation were constructed into pBI121 between the region of a 35S promoter and a NOS terminator. Then the constructed binary vectors were transferred into *Agrobacterium tumefaciens* strain GV3101 (pMP90) by the freeze–thaw method. A floral dip method (6) was used to generate transgenic *Arabidopsis* which further screened by 40 mg/L hygromycin or 50mg/L Kanamycin on half-strength MS plates. For transiently expressing HARP1, GFP in tobacco (*N. benthamiana*), the *A. tumefaciens* cells carrying *35S:6MYC-HARP1*, *35S:GFP-HARP1* or *35S:GFP* were resuspended in infiltration buffer at OD_{600}= 0.8 and injected into the *N. benthamiana* plant leaves of four weeks old. After 2-3 days, the leaves were used for assay.

The second pair of true leaves of *Arabidopsis* plants at the fast-expanding stage (about 16-day-old seedlings) and the first pair of true leaves of the *G. hirsutum* plants were used in wounding and MeJA treatments. For wounding assay, about 1/3 areas of the leaves were punched and the unwounded leaves were used as control. For oral secretion (OS) treatment, leaves were wounded and immediately painted

39 with different OS samples from 4th instar larvae that were reared on artificial diet (Fig. S1) or the 4th instar

40 larvae which had reared on wild-type and *35S: dsHARP1-4* plants for 4 days (Fig. 2*E*). For HARP1 and

41 REPAT38 treatments, recombinant proteins of HARP1, REPAT38 and Venus (used as control) were

42 purified and dissolved in 20 mM Tris-HCl buffer (pH 8.5) to a final concentration of 1 mg/ml. The wounded

43 leaves were painted with indicated purified protein solutions. For MeJA assay, MeJA (Aldrich) was

44 dissolved in ethanol to 50mM and was further diluted in a final concentration of 50 μM in double-distilled

45 water. Water solutions with 50 μM MeJA or with an equal volume of ethanol (mock) were sprayed to aerial

46 parts of the tested plants. All samples were harvested at the indicated time post-treatment and were used

47 for qRT-PCR or other analyses as indicated. The oligonucleotide primers used in this investigation are

48 given in *SI Appendix*, Table S5.

49 **Insect Culture and Feeding Test.** The cotton bollworm (*Helicoverpa armigera*), beet armyworm

50 (*Spodoptera exigua*) and Diamondback moth (*Plutella xylostella*) eggs were obtained from the Institute of

51 Zoology, Chinese Academy of Science. The larvae were reared in the laboratory at 25°C, 70% relative

52 humidity and a 14-h-light/10-h-dark cycle. The modified artificial diet as described (5) and *Arabidopsis*

53 leaves were used to feed the *H. armigera* and *P. xylostella* larvae, as indicated. For insect feeding test,

54 second-instar or third-instar larvae of *H. armigera* at synchronous later stage were weighed individually,

55 and for *P. xylostella*, every 5 third-instar larvae were weighted together. Larvae were divided into groups

56 and each group contained 15–30 individuals. When fed on plants, the individual *H. armigera* larva was

57 raised in separate container and fed with 1-2 *Arabidopsis* plants. The larva was transferred to fresh plants

58 once a day. For *P. xylostella* larvae, every 5 larvae were raised in one container and fed with 1-2

59 *Arabidopsis* plants or at least two tobacco leaves transiently expressed HARP1 or GFP, and the

60 plants/leaves were changed once a day. After fed on indicated diets for indicated days, weight increases

61 were recorded.

62 **OS Collection and Preparation.** For OS collection, the larva was gently fixed between fingers and thumb,

63 and was softly touched by a 0.1-10 μl pipette tip at the larval mouth cavity. Usually the larvae would spit

64 out OS under such stimuli (Movie S1). The OS sample was then collected into a tube. The samples were

65 centrifuged at 12,000× rpm for 10 min at 4℃ to get rid of the food debris and the supernatants were used

66 for further study.

67 **Yeast Two Hybrid.** HARP1, REPAT38 and PXHL1 were introduced into the pGBKT7 (Clontech). A series

68 of JAZs from *Arabidopsis* (JAZ1, JAZ2, JAZ4, JAZ5, JAZ7, JAZ9, JAZ10, JAZ11, JAZ12 and JAZ3 (JAZ3,

69 JAZ3δN, JAZ3δC, JAZ3δZIM)) (4), *G. hirsutum* (Gh_A12G2441, Gh_D12G2567, Gh_A06G0705,

70 Gh_D08G2564, Gh_A05G0260) and *N. benthamiana* (BAD04852.2) were introduced into the pGADT7

71 (Clontech). A LiCl-polyethylene glycol method was used to transfer the plasmid into yeast strain AH109

72 (Clontech). Transformants were screened on SD-Leu-Trp plates and the interactions were tested on SD -

73 Leu-Trp-His plates with 1mM 3-amino-1,2,4-triazole, incubating at 30°C for 3-4 days. At least 10 individual

74 clones for each construct were analyzed.

75 **Prokaryotic Expression and Purification of HIS Fusion Proteins.** For expression of HIS fusion proteins

76 (HIS-HARP1, HIS-REPAT38, HIS-JAZ3, HIS-JAZ3δN, HIS-Venus and HIS-Venus-HARP1),

77 the corresponding fragments were inserted into pET32a (Stratagene) with a HIS N-terminal fusion. The

78 recombinant protein was expressed in *Escherichia coli* strain BL21 (DE3) and purified by Ni affinity column

79 (Ni-NTA resin, Novagen). Briefly, Total proteins of *E. coli* were extracted by lysis buffer containing 50 mM

80 Tris-Cl, pH 8.0, 100 mM NaCl, 25 mM imidazole, 10% (v/v) glycerol, 0.1% (v/v) Tween 20, 1 mM

81 phenylmethanesulfonyl fluoride (PMSF), 10 μM MG-132 (Sigma-Aldrich) and Protease Inhibitor Cocktail,

82 followed by centrifugation at 14,000g for 10 min at 4°C. The supernatants were loaded onto a Ni affinity

83 column (Ni-NTA resin, Novagen), which was then washed with 3× volumes of lysis buffer, and finally eluted

84 with lysis buffer that contained 250 mM imidazole.

85 **Immunoblot and Pull-down Assays.** Total midgut proteins of fourth-instar larvae were extracted by 50

86 mM Tris-Hcl buffer (pH 9.0). The gut fluid, oral secretion and the total protein solutions of midgut were

87 extracted by chloroform in equal volume and the precipitate was washed by 75% ethanol and finally

88 dissolved in solution buffer (Tris-HCl, 10 mM, pH 8.5). Samples (10 μg proteins per lane) were loaded onto

89 a 15% SDS–polyacrylamide gel electrophoresisgel. Anti-HARP1 antibody (generated by Prof. Xia's lab in

90 Xiamen University, China; dilution, 1:3,000) was used for HARP1 detection. The specificity of Anti-HARP1

91 antibody was detected by immunoblot using the samples of wild-type and *35S:6MYC-HARP1* transgenetic

92 plants; the Anti-MYC antibody was used as positive control. The immunoblot with the Anti-HARP1 antibody

93 shows that only the samples of the transgenic plants expressing HARP1 have a main signal band and this

94 indicates that the Anti-HARP1 antibody is specific to bind to HARP1 proteins. (*SI Appendix*, Fig. S14).

95 To examine the indicated protein level in plants, total proteins of plant leaves were extracted by an

96 extraction buffer (50 mM HEPES, 10 mM EDTA, 50 mM NaCl, 10% glycerol, 1% polyvinylpolypyrrolidone,

97 2 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 10 mM MG-132, and 1×protease inhibitor cocktail, pH 7.5).

98 In each sample 75 μg proteins were used. Anti-HA-peroxidase antibody (Cat No. 12013819001, Roche;

99 dilution, 1:2,000), anti-COI1 antibody (7) (obtained from Prof. Xie, dilution, 1:3000), anti-MYC antibody

100 (Cat No. 30602ES60, Yeasen; dilution, 1:2,000) and anti-GFP antibody (Cat No. 31003ES50, Yeasen;

101 dilution, 1:2,000) were used to detect the HA fusion (JAZ3-HA, JAZ3δN-HA and JAZ3δC-HA) , COI1, MYC

102 fusion (6MYC-HARP1 and 6MYC-REPAT38) and GFP/GFP-HARP1 protein levels in plants, respectively.

103 To examine the interaction of JAZ3 and HARP1, total protein extracts (5 mg proteins) of *35S:JAZδN-HA*

104 and *35S:JAZδC-HA* plant leaves were mixed with 80 μg recombinant HIS-HARP1 in a total volume of 1

105 ml. Ni-NTA resin (Novagen) was used to bind HIS-HARP1. After incubation for one hour at 4°C, the Ni-

106 NTA resin was washed and eluted with imidazole. Samples were used to detect the truncated fusion

107 proteins of JAZ3-HA by immunoblotting using anti-HA, and the anti-HIS antibody was used to detect the

108 HIS-HARP1.To examine the expression of JAZs and HARP1 in yeast cells, the indicated co-transformed

109 yeast cells were cultured on medium lacking Leu and Trp and subsequently transferred into YPDA liquid-

110 medium overnight, then the total proteins were extracted. Samples were used to detect the JAZs and

111 HARP1 by immunoblotting. Since there was a MYC epitope tag followed the GAL4 DNA-binding domain

112 (BD) and a HA epitope tag followed the GAL4 activation domain (AD), we used Anti-HA and Anti-MYC

113 antibody to detect the JAZs-AD and HARP1-BD respectively.

114 To examine the effect of HARP1 on JAZ3-COI1 interaction, total protein extracts of wild-type and

115 *35S:6MYC-HARP1-1* plant leaves were mixed with 80 μg recombinant HIS-JAZ3 or HIS-JAZ3δN in 1 ml

116 and Coronatine was added to a final concentration of 50μM. Ni-NTA resin (Novagen) was used to bind

117 HIS-JAZ3 and HIS-JAZ3δN. The anti-COI1 antibody was used to detect the COI1 level in the samples

118 before (crude) or after pull-down. The anti-MYC antibody was used to detect 6MYC-HARP1 and the anti-

119 HIS antibody was used to detect the truncated JAZ3 proteins (HIS-JAZ3 and HIS-JAZ3δN).

120 The anti-HIS，anti-HA and anti-MYC antibody were directly conjugated with horseradish peroxidase (HRP)
121 and for anti-HARP1 and anti-COI1 antibody, a horseradish peroxidase (HRP) conjugated goat anti-mouse
122 IgG second antibody were used to form antibody-antigen complex. SuperSignal™ West Femto Maximum
123 Sensitivity Substrate (Thermo) (for weak signaling) and Pierce™ ECL Western Blotting Substrate (Thermo)
124 (for strong signaling) were used to detect the HRP enzyme activity.

125 **Whole Amount Immunohistochemistry.** The three-week old *Arabidopsis* were incubated with fourth-
126 instar larvae of *H. armigera*. The leaves after insect wounding damage were collected immediately and
127 transferred to the FAA-fixative solution for 4 hours. The mechanically wounded leaves were used as control.
128 Leaf samples were dehydrated through a series of graded alcohol solutions, followed by rehydration. After
129 incubation for 2 hours with blocking buffer (1×PBS containing 0.1% Tween 20 and 1% Albumin from bovine
130 serum BSA), samples were incubated with the primary antibody (anti-HARP1) in 4°C overnight. The
131 samples were washed by PBST (PBS containing 0.1% tween 20) for 4 times. The HARP1 signals were
132 visualized by Western Blue stabilized substrate for Alkaline Phosphatase (Promega) and observed under
133 an Olympus BX63 microscope equipped with a DP73 digital camera and differential interference contrast
134 modules.

135 **Sub-Cellular Localization of HARP1 in Plant Leaves.** To detect the translocation of prokaryotic
136 expressed Venus-HARP1 in plant cells, the second true leaves of *Arabidopsis* were punched and soaked
137 into the 50mM Tris-HCl buffer containing the purified HIS fusion protein of Venus-HARP1 or Venus (1
138 mg/ml) for one hour. And then the samples were washed with wash solutions (PBS containing 0.1% Tween
139 20 and 1% Albumin from bovine serum, BSA) for 3-4 times to removal the extra proteins adhering on the

140 leaves surface. 4,6-diamidino-2-phenylindole (DAPI) was applied for nuclear staining. Confocal laser
141 scanning microscopy (CLSM) was performed with an Olympus FV3000 microscope equipped with a 40x
142 dry objective (UPLSAPO40X2 NA 0.95) and 1.25x objective (PLAPON1.25X NA 0.04). Images were
143 sequentially recorded with excitation wavelengths of 405 and 514 nm with the corresponding dichroic
144 mirror and analyzed by Olympus cellSens (version Dimension 1.18) software.

145 For the subcellular localization of HARP1 in transgenic plants, GFP-HARP1 and GFP (used as control)
146 were transiently expressed in *N. benthamiana* leaf cells via *Agrobacterium* infiltration. Two days later the
147 leaf tissues were observed under a laser scanning confocal microscope (Olympus FV1000, Japan).

148 **Gene Expression Analyses.** Total RNAs from *Arabidopsis* plants or from *H. armigera* larvae were isolated
149 by Trizol reagent (Invitrogen). About 1 μg of total RNAs was treated with 1 μl of DNase I (1 unit per μl;
150 Fermentas) and used to prepare the first strand cDNA (Invitrogen). qRT-PCR was performed. *S18* in
151 *Arabidopsis* (At4g09800) and actA3b (GenBank No. X97615.1) in *H. armigera* were used as internal
152 standard for analysis of gene expressions in *Arabidopsis* and in insects, respectively. Biological triplicates
153 with technical duplicates were performed.

154 **RNA-Seq and Transcriptome Analysis.** The third and fourth true leaves of the 16 day-old *Arabidopsis*
155 and *35S:6MYC-HARP1* plants were mechanically wounded. After 4 h post-wounding, samples (W) are
156 collected and the unwounded leaf samples are used as control (CK). The total RNA was extracted by Trizol
157 reagent (Invitrogen), and library construction and sequencing were accomplished by Shanghai Personal
158 Biotechnology Co., Ltd. Three biological replicates were performed. Briefly, sequencing libraries were
159 prepared with the TruSeq RNA Sample Preparation Kit (Illumina) according to the manufacturer's

160 instructions. The libraries were pooled and sequenced on the Illumina Nextseq platform (High output, 2
161 runs) with read length of 150 nt in paired end configuration. All reads can be downloaded on NCBI
162 (SRR6668925, SRR6668926, SRR6668927, SRR6668928). Low quality sequencing reads (Q < 20, reads
163 of N > 5%, and adaptors) were removed before analysis. The clean reads were mapped to
164 the *Arabidopsis* genome (TAIR10) using TopHatt (8). Statistics of mapping was presented in Dataset S2.

165 HTSeq (9) was used to calculate the mapping results and DESeq (10) was used for differential gene
166 expression analysis (fold change > 2 or < 0.5; adjusted *p*-value < 0.05; baseMean >10 in at least one
167 sample). RPKM（Reads Per Kilo bases per Million reads）or baseMean (normalized by DESeq) was used
168 to measure gene expression levels. Gene ontology (GO) enrichment analysis was performed with AgriGO
169 (11). Hypergeometric tests with Yekutieli adjustment were used for the calculation of *p*-values and false
170 discovery rates (FDRs). Terms with FDR<0.05 were considered to be enriched. Scatter diagram of the 418
171 HARP1-affected genes was drawn by Perl package, in which gene expression levels were calculated as
172 RKPM values.

173 **Phylogenetic Analyses.** HARP1 protein sequences were screened within 11 insect species from five
174 families of Lepidoptera (*SI Appendix*, Table S4). The most similar protein sequences (*SI Appendix*, Table
175 S4) from these insect species were used for further analyses. Protein sequences were aligned using the
176 MAFFT with G-INS-i algorithm (12). Phylogenetic analysis was performed using Neighbour-Joining
177 methods using MEGA (version 5.03) (13) by sampling 1000 bootstrap replicates.

178 **Liquid Chromatography-Mass Spectrometry and Proteomic Analyses.** To examine the variations of
179 protein level in the OS of *H. armigera* larvae, the synchronous fourth-instar larvae of *H. armigera* were fed

180 on artificial diet and 3-week old *Arabidopsis* leaves, respectively, for one day. The OS samples were
181 collected and sent to Shanghai Applied Protein Technology Co. Ltd. for Label free LC-MS/MS analysis.
182 Three biological replicates were performed separately.

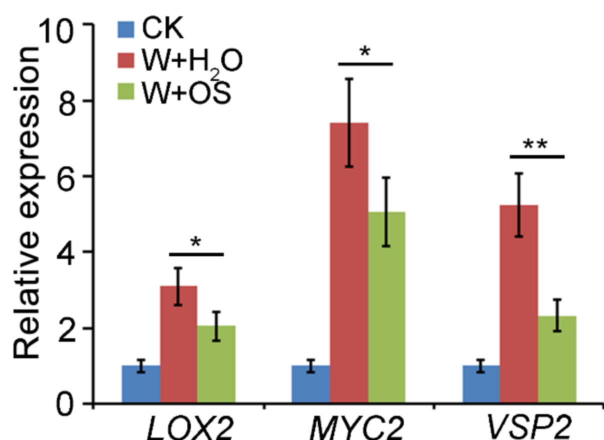
183 Trypsin (Promega) was used to digest the protein samples (100 μg) according to the FASP (filter-aided
184 sample preparation) procedure (14). The peptide samples were auto-loaded into the C18- reversed phase
185 trap column (Thermo Scientific Acclaim PepMap100, 100μm*2cm, nanoViper C18) and separated by the
186 C18- reversed phase analytical column (Thermo Scientific Easy Column, 10 cm long, 75 μm inner diameter,
187 3 μm resin) with buffer A (0.1% Formic acid) for balancing and buffer B (0.1% formic acid and 84%
188 acetonitrile) for separation. The peptides were separated at a flow rate of 300 nl/min controlled by
189 IntelliFlow technology with a liner gradient: 0 min, 0% B; 110 min, 55% B; 115 min, 100% B; 115 to 120
190 min, 100% B. The MS/MS experiment was performed on a Q Exactive mass spectrometer (Thermo
191 Scientific) coupled to Easy nLC (Thermo Fisher Scientific), The Q Exactive was operated in positive ion
192 mode for 120 min, based on a data-dependent top20 method for MS data collection which dynamically
193 chose the most abundant precursor ions from the survey scan (300–1800 m/z)(MS^1 scan) for HCD
194 fragmentation (MS^2 scan). The resolution for the survey scans and HCD spectra was 70,000 at m/z 200
195 and 17,500 at m/z 200, respectively.

196 MS/MS raw data were analyzed by Maxquant (version 1.3.0.5) for label free quantitative analysis (LFQ)
197 against with the insect protein database (including 22 insect genus) from NCBI (*SI Appendix*, Table S6)
198 and the *H. armigera* protein database derived from the transcriptome database (15). The search
199 parameters were: Missed cleavage, 2; enzyme, Trypsin; Fixed modification, Carbamidomethyl(C); Variable

200 modification, Oxidation(M), Acetyl (Protein N-term); Decoy database pattern, reverse; LFQ, TRUE; Peptide
201 FDR, 0.01; Protein FDR, 0.01. The Statistics of identified peptides and proteins as well as Label-free
202 quantitation results are listed in Dataset S1. The LFQ intensity >1.5-fold changes were defined as up-
203 regulated peptides and proteins.

204 **Statistical Analysis.** Data were presented as means± SD or SEM and analyzed by Student's *t* test or two-
205 way ANOVA performed with GraphPad Prism software, that were also described in figure legends. At least
206 three biological replicates were used to perform each of the experiments, and all the experiments were
207 repeated for multiple times and the results were consistent.
208

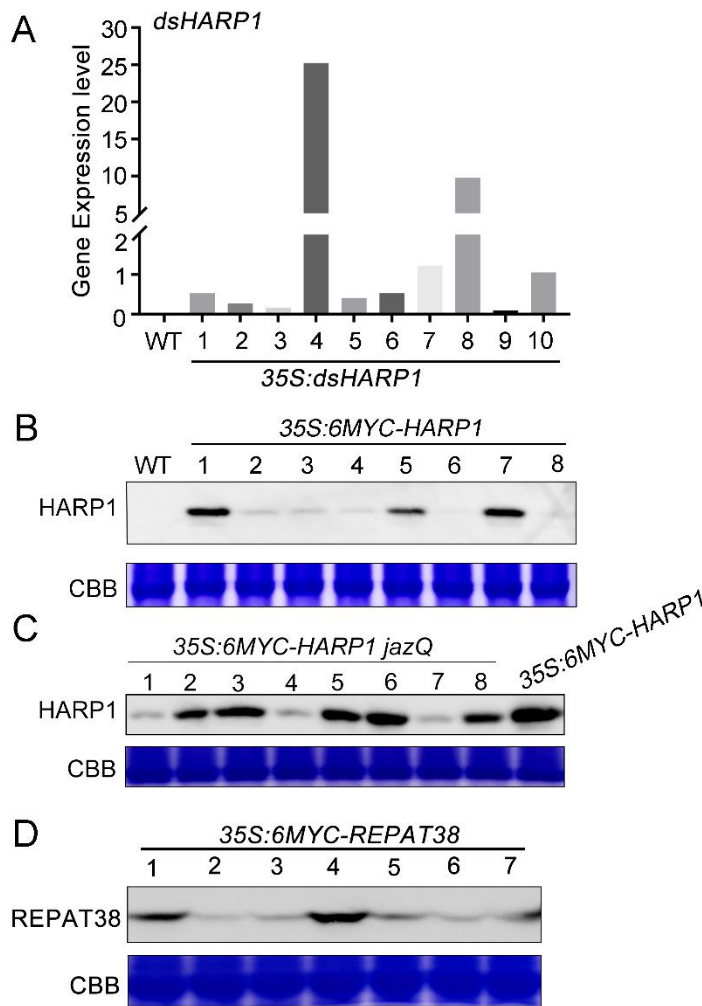
209 Supplementary Figures:



210

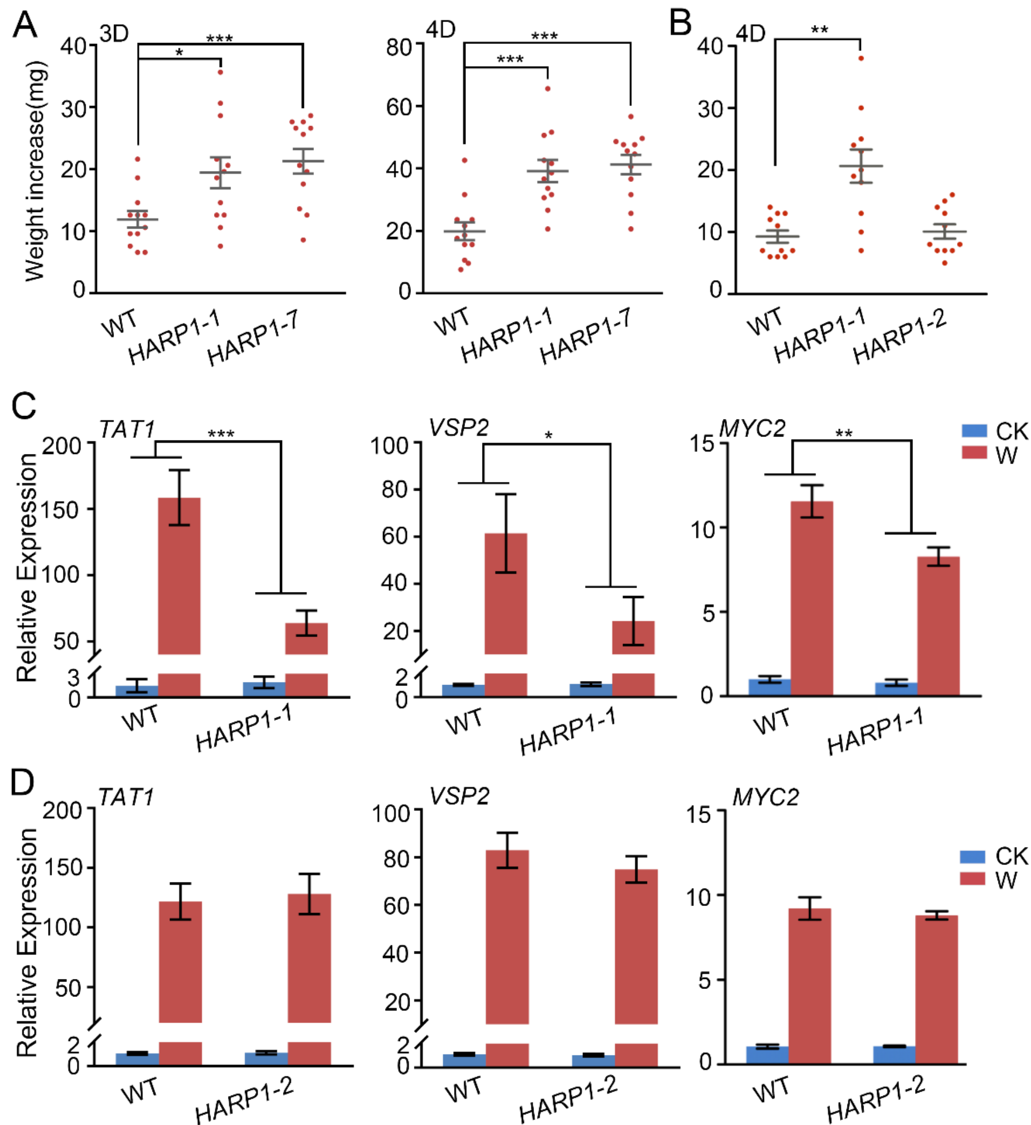
211 **Fig. S1.** HARP1 contributes to cotton bollworm OS on suppressing plant wounding response. The oral
212 secretion (OS) of *H. armigera* larvae attenuate the induction of genes upon wounding. *Arabidopsis* leaves
213 were wounded and painted with the double-distilled water (W+H_2O) or the OS of the fourth-instar larvae
214 (W+OS). Thirty minutes later, leaves were collected and qRT-PCR was used to detect transcripts of the
215 selected genes. The expression in the unwounded leaves (CK) was set to 1. **P*<0.05, ***P*<0.01. Error bars
216 represent ±SD (n=3 biological replicates)

217



218

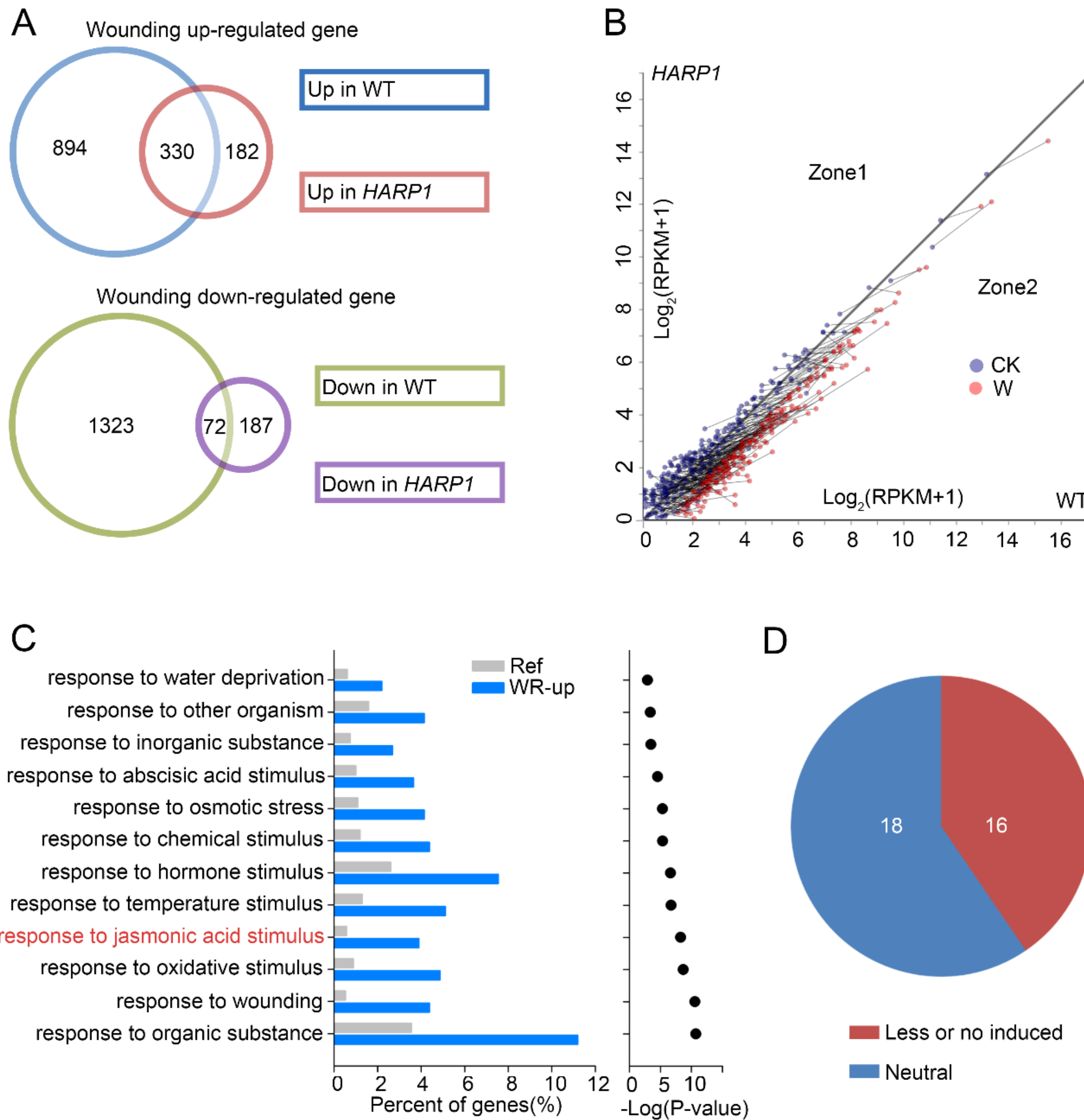
219 **Fig. S2.** The overexpression level detection of HARP1 and REPAT38 proteins as well as dsHARP1 in
220 transgenic *Arabidopsis*. (*A*) The expression level of dsHARP1 in transgenic plants were detected by qRT-
221 PCR. The *dsHARP1* expression level was normalized to the house-keeping gene S18 using $2^{-\Delta CT}$. (*B*-*D*)
222 Immunoblot detection of HARP1 and HARP1-like protein REPAT38 in transgenic plants. 6MYC-HARP1
223 (HARP1) in *35S:6MYC-HARP1* (*B*) and *35S:6MYC-HARP1 jazQ* (*C*), 6MYC-REPAT38 (REPAT38) in
224 *35S:6MYC-REPAT38* (*D*). The newly initiated leaves from wild-type (WT) or from T1 transgenic lines were
225 analyzed. 6MYC-HARP1 and 6MYC-REPAT38 in plants was detected by Anti-6MYC antibody. The amount
226 of total proteins in each loading was monitored with Coomassie Brilliant Blue (CBB) staining.



227

228 **Fig. S3.** Insect resistance and wounding responses were reduced in the plants with high expression level
229 of HARP1. (*A* and *B*) Cotton bollworm larvae grew faster when reared on plants highly expressing HARP1.
230 Third-instar larvae were fed on indicated plant leaves, Weight increases were recorded. Data were
231 analyzed by Student's *t*-test. ***P*<0.01. Error bars represent ±SEM (n=12). (*A*) Larvae were fed on WT and
232 two transgenic lines with high expression level of HARP1 (*35S:6MYC-HARP1-1*/*HARP1-1* and *35S:6MYC-*
233 *HARP1-7*/*HARP1-7*) for 3 (left) and 4 (right) days. (*B*) Larvae were fed on WT and one transgenic line with
234 low expression level of HARP1 (*35S:6MYC-HARP1-2/HARP1-2*) for 4 days. (*C* and *D*) The indicated gene
235 inductions were reduced in *35S:6MYC-HARP1-1* (*C*) but not in *35S:6MYC-HARP1-2* (*D*). Plant leaves
236 were collected 4 h after wounding (W), the unwounded leaves (CK) were used as control. The expression
237 in WT was set to 1. Data were analyzed by two-way ANOVA followed by multiple comparisons (Tukey test)
238 (**P*<0.05, ***P*<0.01, ****P*<0.001). Error bars represent ±SD (n=3 biological replicates). The experiments
239 were repeated three times and the results were consistent.

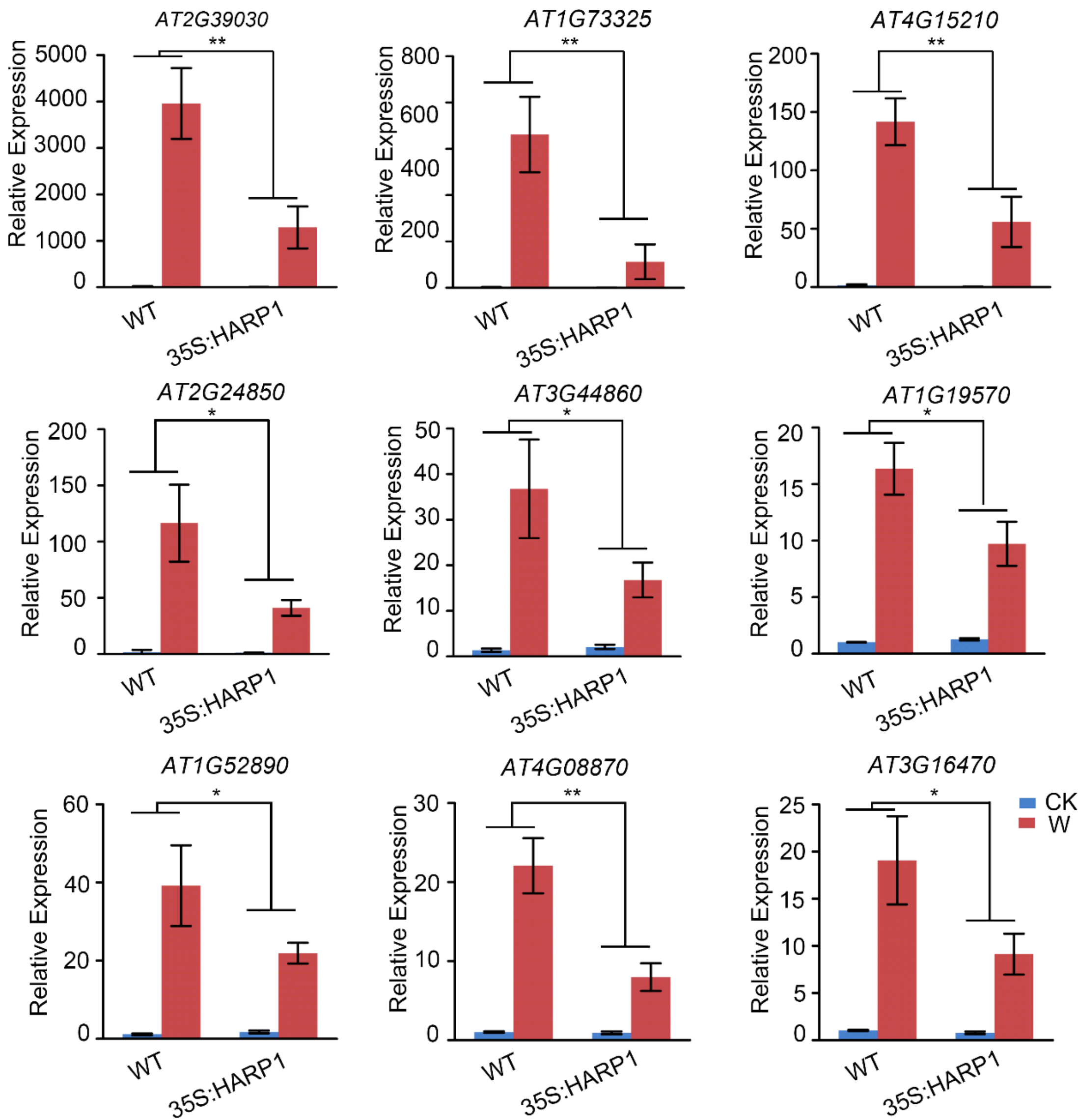
240



241

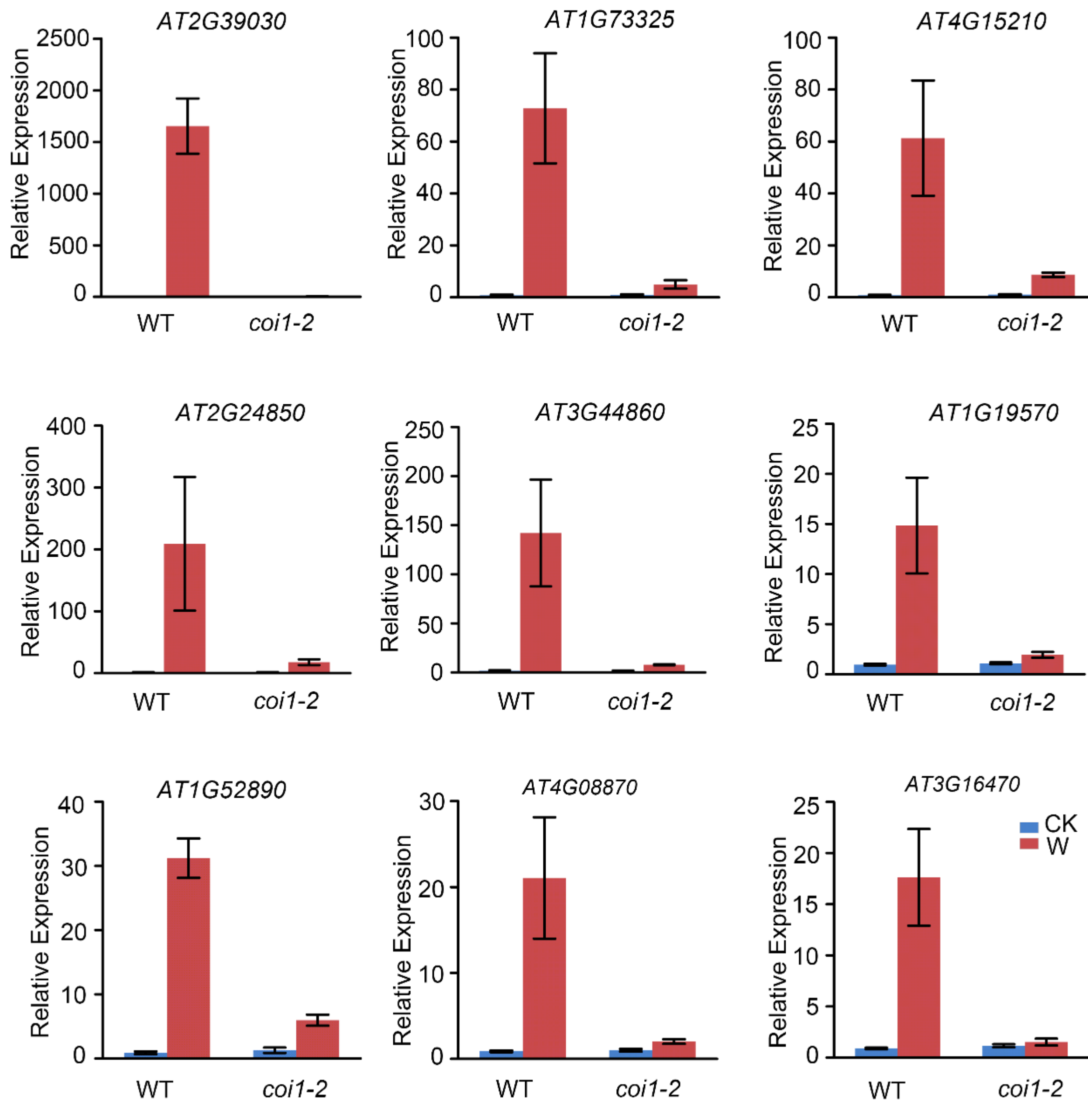
242 **Fig. S4.** RNA-seq analysis revealed reduced wounding response in *35S:6MYC-HARP1*. (*A*) The venn
243 diagrams of wounding up- and down- regulated genes in wild-type and *35S:6MYC-HARP1-1* (*HARP1*)
244 plants. The overlapped regions stand for genes up- or down-regulated both in WT and *35S:6MYC-HARP1-*
245 *1*. (*B*) Scatter plot analysis of the 418 genes up-regulated in wild-type but less or not induced in *35S:6MYC-*
246 *HARP1-1* upon wounding (WT-Wounding/ *35S:HARP1*-Wounding >2, *P*<0.05). The X- and Y-axis stands
247 for the gene expression (Log_2(RPKM+1)) in wild-type (WT) and *35S:6MYC-HARP1-1* (*HARP1*) leaves,
248 respectively. Blue and red spots represent the gene expressions in unwounded (CK) and wounded (W)
249 plants 4 h post-treatment, respectively. The quadrant diagonal line divides the first quadrant into two parts
250 equally. Spots in Zone2 indicate the less induced genes upon wounding in *35S:6MYC-HARP1*. (*C*)
251 Significantly enriched GO terms of the 418 genes in zone 2 as described in (*B*) are indicated by blue
252 columns. Gray columns indicate the percentage of enrichment across the whole genome. The spots
253 indicate -log (*P*-value) by hypergeometric test, which are adjusted by Yekutieli (FDR under dependency)
254 multi-test. (*D*) The induction of JA response genes was largely suppressed in *35S:6MYC-HARP1* upon
255 wounding. Pie diagram analysis revealed that totally 34 (18+16) genes clustered in JA response were

256 obviously induced by wounding treatment in wild-type, among them 16 genes (red part), about 47% (16/34),
257 were less or no induced in *35S:6MYC-HARP1* plant upon wounding. The rest 18 genes which had no
258 significant differences between WT and *HARP1* in wounding treatment were indicated in blue (Neutral).
259



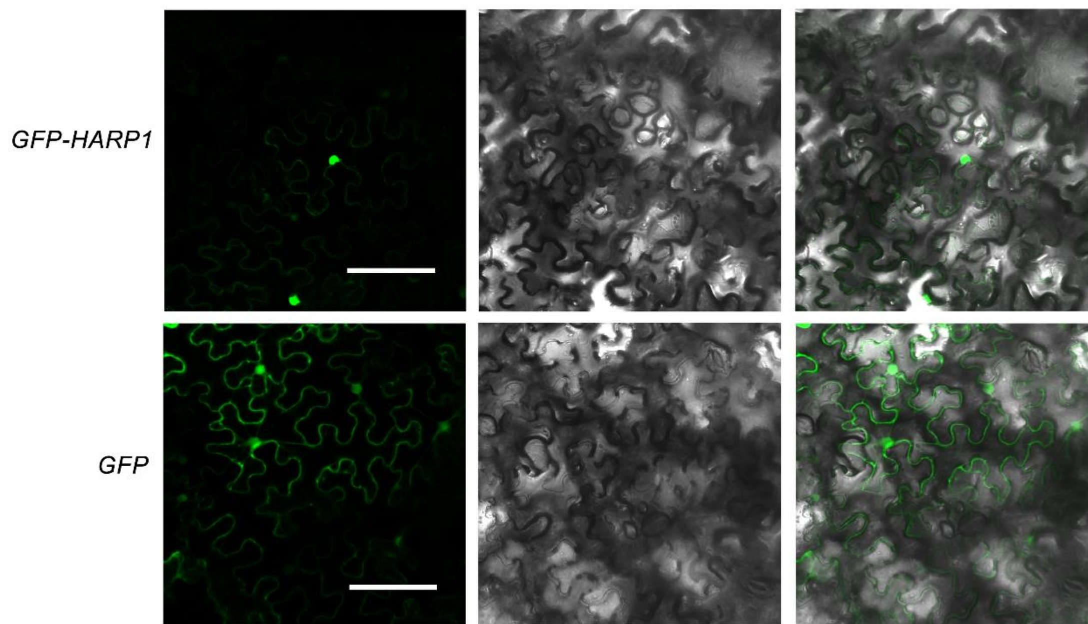
260

261 **Fig. S5.** Relative gene expressions of nine genes selected from the 418 wounding up-regulated genes as
262 described in Fig. 2. Wild-type (WT) and *35S:6MYC-HARP1-1* (*HARP1*) leaves were wounded; samples of
263 the unwounded (CK) and the wounded (W) leaves (4 H post-wounding) were investigated by qRT-PCR.
264 Data were analyzed by two-way ANOVA followed by multiple comparisons (Tukey test) (**P*<0.05, ***P*<0.01).
265 Error bars represent ±SD (n=3 biological replicates).



266

267 **Fig. S6.** Wounding induction of the selected genes shown in Fig. S4 is JA signal dependent. Leaves of the
268 wild-type (WT) and the JA insensitive mutant (*coi1-2*) plants were wounded (W) or unwounded (CK) and
269 samples were collected for gene expression analysis by qRT-PCR 4 H post-treatment. Error bars represent
270 ±SD (n=3 biological replicates).



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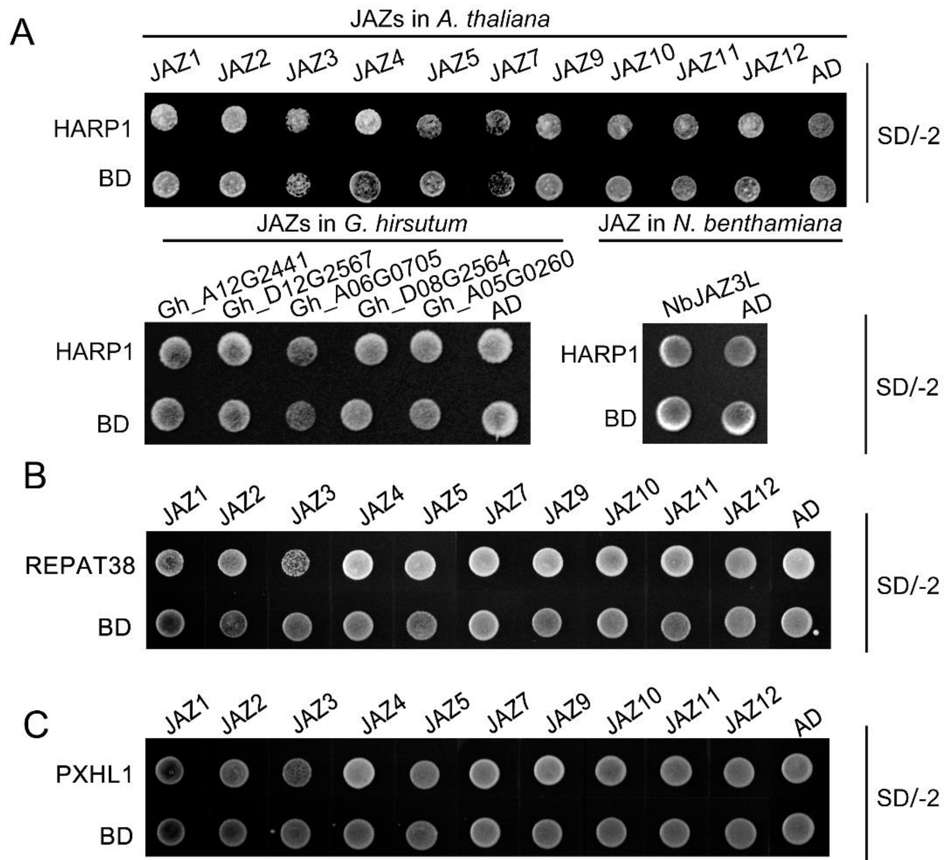
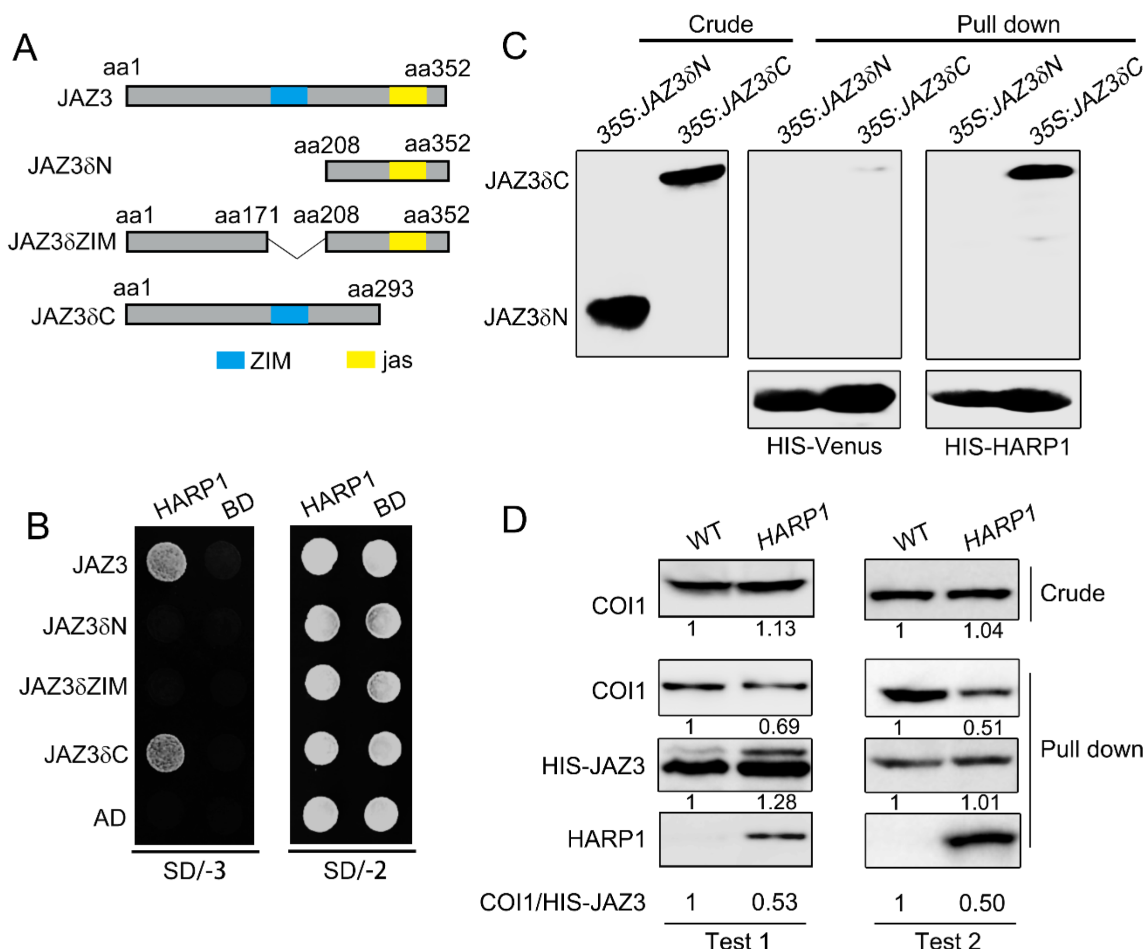
272 **Fig. S7.** HARP1 is mainly localized in the nucleus of *N. benthamiana* cell. *35S:GFP-HARP1* and *35S:GFP*
273 were transiently expressed in *N. benthamiana* leaves. Confocal laser scanning microscopy was used to
274 visualize the fluorescence. Scale bar, 100 μm.



275

276 **Fig. S8.** Additional information of yeast two-hybrid assay in Fig. 3A, Fig. 5B and Fig. 6A. (*A*) Control growth
277 of yeast cells transformed with HARP1-BD and indicated JAZs-AD in Y2H assay. HARP1 was fused to
278 GAL4 DNA-binding domain (BD), JAZ proteins of *Arabidopsis* (*A. thaliana*), cotton (*G. hirsutum*) and
279 tobacco (*N. benthamiana*) were fused to GAL4 activation domain (AD), respectively. Yeast cells co-
280 transformed with the indicated combinations were selected and subsequently cultured on medium lacking
281 Leu and Trp (SD/-2). (*B* and *C*) Control growth of yeast cells transformed with REPAT38 (*B*) or PXHL1 (*C*)
282 and indicated JAZs of *Arabidopsis* (*A. thaliana*) in Y2H assay. REPAT38, PXHL1 was fused to GAL4 DNA-
283 binding domain (BD), the indicated JAZ proteins were fused to GAL4 activation domain (AD), respectively.
284 Yeast cells co-transformed with the indicated combinations were selected and subsequently cultured on
285 medium lacking Leu and Trp (SD/-2). All the co-transformed yeast cells have good growth conditions when
286 cultured on SD/-2 medium.
287

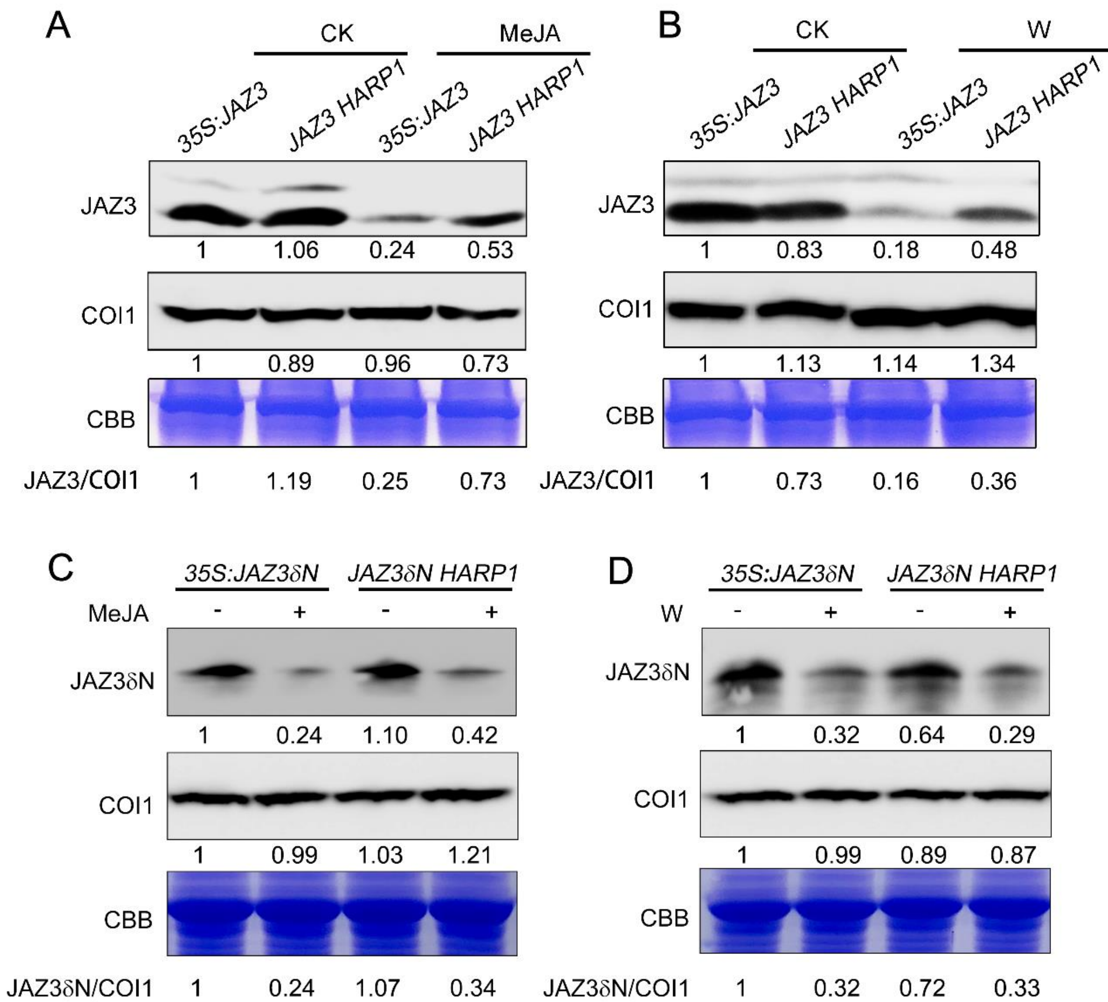


288

289 **Fig. S9.** JAZ3 N-terminal region containing ZIM domain required for interaction with HARP1. (*A*) Schematic
290 diagrams of truncated versions of JAZ3 used in Yeast two-hybrid assay. Blue and yellow box indicate ZIM
291 and jas domain, respectively. (*B*)Yeast two-hybrid assay. HARP1 was fused to GAL4 DNA-binding domain
292 (BD), different truncated versions of JAZ3 were fused to GAL4 activation domain (AD), respectively. Yeast
293 cells co-transformed with the indicated combinations were selected and subsequently cultured on medium
294 lacking Leu and Trp (SD/-2). Interactions were examined with yeast cells grown on medium lacking His,
295 Leu and Trp (SD/-3) with 1 mM 3-amino-1,2,4-triazole. (*C*) The pull-down assay of truncated version of
296 JAZ3 binding with HARP1. Recombinant HIS-HARP1 and HIS-Venus protein was incubated with total
297 proteins of the *35S:JAZ3δN-HA* (*35S:JAZ3δN*) and *35S:JAZ3δC-HA* (*35S:JAZ3δC*) plant leaves,
298 respectively. Anti-HA antibody was used to detect the truncated fusion proteins of JAZ3 before (Crude) or
299 after (Pull down) immunoprecipitation. Anti-HIS was used to detect the recombinant HIS-Venus and HIS-
300 HARP1 protein. Before pull down assay, JAZ3δN-HA (JAZ3δN) and JAZ3δC-HA (JAZ3δC) are both
301 detectable in total protein extractions of *35S:JAZ3δN-HA* and *35S:JAZ3δC-HA* plant leaves (Crude). After
302 pull down, only JAZ3δC-HA can be co-immunoprecipitated with HIS-HARP1 but not HIS-Venus, this means
303 that the ZIM domain containing N-terminal are required for JAZ3-HARP1 interaction. (*D*) HARP1 reduces
304 COI1-JAZ3 co-precipitation. Recombinant proteins of HIS-JAZ3 were incubated with total leaf proteins of
305 the wild-type (WT) and *35S:6MYC-HARP1-1* (*HARP1*) *Arabidopsis* in the presence of 50 μM Coronatine.
306 Anti-COI1 antibody was used to detect COI1 level before (Crude) or after (Pull down) immunoprecipitation.

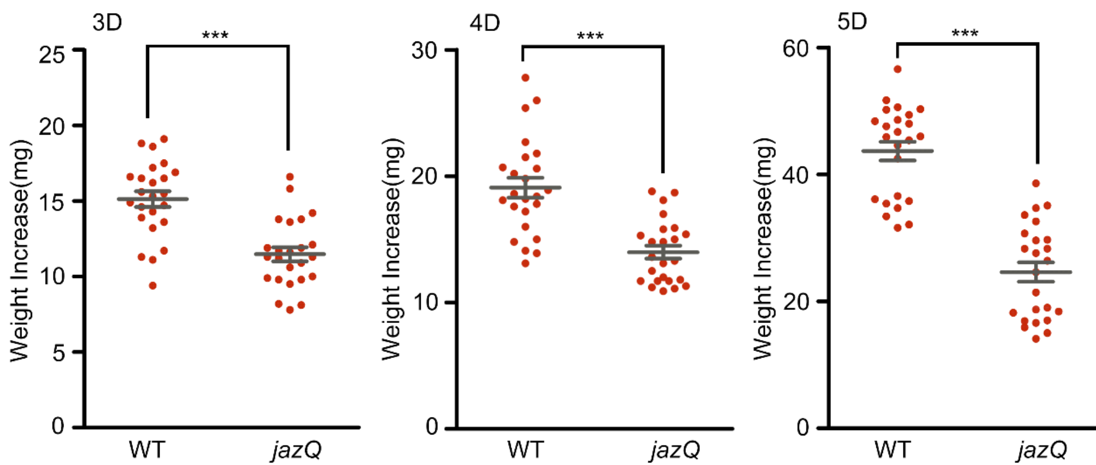
307 Anti-MYC antibody was used to detect the 6MYC-HARP1 (HARP1) and Anti-HIS antibody was used to
308 detect HIS-JAZ3. Band intensity was quantified by ImageJ and was shown under each blot. The intensity
309 of the wild-type (WT) sample was set to 1. The relative COI1/HIS-JAZ3 ratio was listed in the bottom.

310



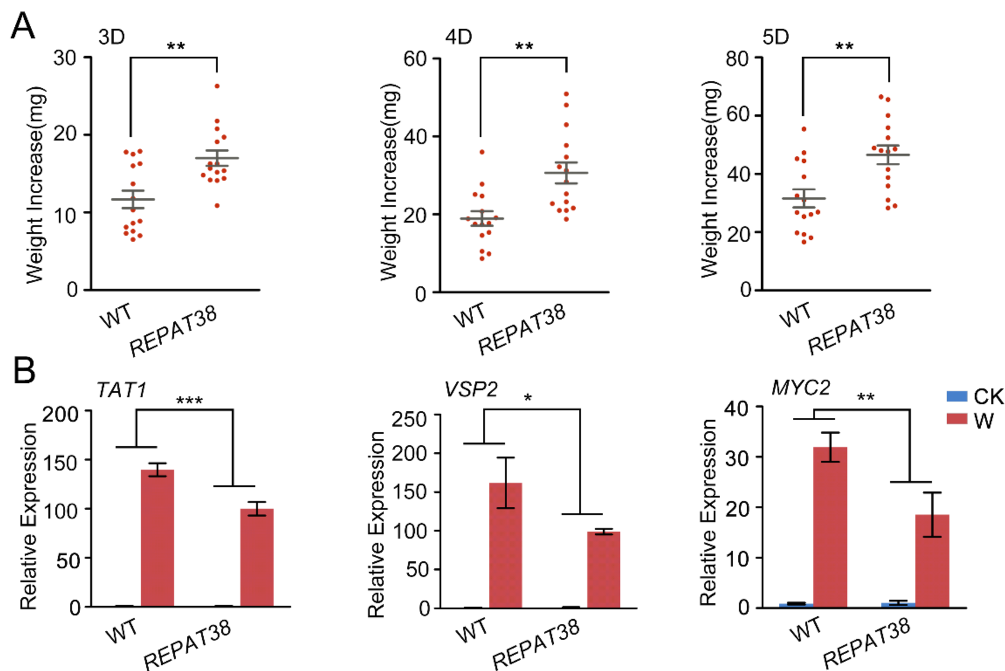
311

312 **Fig. S10.** The JAZ3-HA (JAZ3) but not JAZ3δN-HA (JAZ3δN) is more stable with the existence of HARP1
313 post MeJA or wounding treatment. (*A* and *B*) HARP1 increase JAZ3-HA (JAZ3) accumulation after 50 μM
314 MeJA or wounding treatment. The plant leaves were collected 45 min post-MeJA (MeJA) (*A*) or post-
315 wounding (W) (*B*) treatment, the unwounded plant leaves (CK) were used as control. Anti-HA antibody
316 was used to detect JAZ3-HA (JAZ3). Anti-COI1 antibody was used to detect COI1. (*C* and *D*) HARP1 had
317 no influence on the degradation of JAZ3δN. The plant leaves of *35S:JAZ3δN-HA* (*35S:JAZ3δN*) and
318 *35S:JAZ3δN-HA 35S:6MYC-HARP1* (*JAZ3δN HARP1*) were treated with MeJA (50 μM) (*C*) or with
319 wounding (*D*), samples were harvested 45 min post-treatment. Anti-HA and anti-COI1 antibody were used
320 to detect JAZ3δN-HA (JAZ3δN) and COI1 respectively in plants. The experiments in (*C*) and (*D*) were
321 repeated twice and the results were consistent. The band intensity was quantified by ImageJ and was
322 shown under each blot. The intensity of untreated *35S:JAZ3-HA* or *35S:JAZ3δN-HA* was set to 1. The
323 relative JAZ3/COI1 and JAZ3δN/COI1 ratios were listed in the bottom.

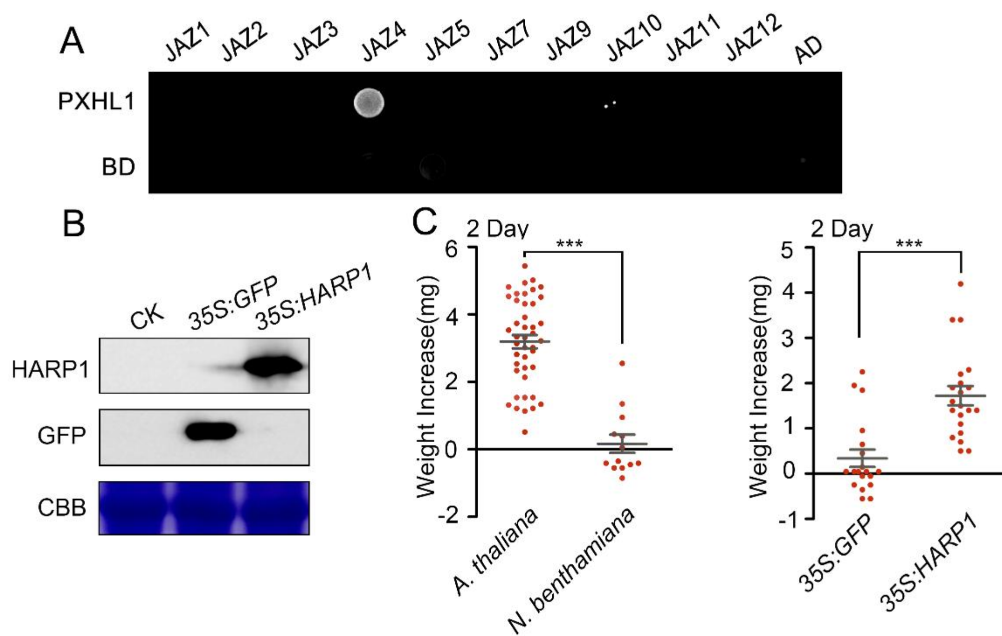


324

325 **Fig. S11.** The growth of *H. armigera* larvae fed with *jazQ* plants was inhibited compared to those fed with
326 wild-type. The third-instar larvae of *H. armigera* were fed with leaves of the wild-type (WT) and the *jazQ*
327 plants for 3, 4 and 5 days. The larval weight was recorded. Data were analyzed by Student's *t*-*t*est.
328 ****P*<0.001. Error bars represent ±SEM (n=24). The experiments were repeated for three times and the
329 results were consistent.

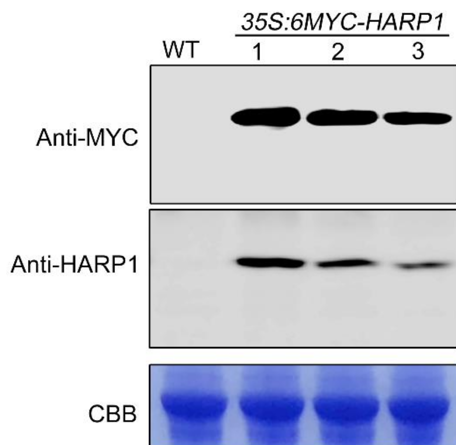


330
331 **Fig. S12.** HARP1-like proteins have similar function as effectors. (*A*) *H. armigera* larvae grew faster in
332 *35S:6MYC-REPAT38*. The later stage of second instar larvae were fed on wild-type (WT) and *35S:6MYC-*
333 *REPAT38* (*REPAT38*) leaves for indicated days, larval weight was recorded. Data were analyzed by
334 Student's *t*-test. ***P*<0.01. Error bars represent ±SEM (n=15). (*B*) Gene inductions were reduced in
335 *35S:6MYC-REPAT38* (*REPAT38*) than in wild-type (WT) leaves upon wounding. Gene expressions in the
336 unwounded (CK) and the wounded (W) plants were detected by qRT-PCR 2 h post-treatment (W). Data
337 were analyzed by two-way ANOVA followed by multiple comparisons (Tukey test). (**P*<0.05, ***P*<0.01,
338 ****P*<0.001). Error bars represent ±SD (n=3 biological replicates). All the experiments were repeated three
339 times and the results were consistent.



340

341 **Fig. S13.** HARP1 effector contributes to insect adaptation to plant host. (*A*) Yeast two-hybrid assay. PXHL1
342 was fused to GAL4 DNA-binding domain (BD) and the indicated JAZs were fused to GAL-activation domain
343 (AD), respectively. Interactions were examined with 1 mM 3-amino-1,2,4-triazole. PXHL1 did not interact
344 with JAZs in yeast except JAZ4. (*B*) Immunoblot assay of GFP and 6MYC-HARP1 (HARP1) transiently
345 expressed in *N. benthamiana* leaves driven by 35S promoter. Anti-GFP and anti-MYC were used to detect
346 GFP and HARP1, respectively. (*C*) *P. xylostella* larvae gained more weight increase on host (*A. thaliana*)
347 than on non-host (*N. benthamiana*) plants (left), and on HARP1-expressed than on GFP-expressed non-
348 host plants (right). HARP1 or GFP (as control) were transiently expressed, and leaves were used for
349 feeding. Data were analyzed by Student's *t*-test. ***P<0.001. Error bars represent ±SEM (n=20-50). The
350 experiments were repeated twice and the results were consistent.



351

352 **Fig. S14.** The specificity of Anti-HARP1 antibody was detected. The wild-type (WT) and the indicated lines
353 of *35S:6MYC-HARP1* plants were used. Proteins of MYC-HARP1 in plants were detected by Anti-MYC
354 antibody and Anti-HARP1 antibody respectively. The immunoblot with the Anti-HARP1 antibody shows
355 that only the samples of the transgenic plants expressing HARP1 have a main signal band and this
356 indicates that the Anti-HARP1 antibody is specific to bind to HARP1 proteins.

357

358 Supplementary Tables:

359 **Table S1.** The induced proteins (excludes digestive enzymes from *H. armigera* oral secretion when fed on

360 *Arabidopsis* compared to those fed on artificial diet.

Protein IDs	Annotation	Signals		Relative content (Col-0/AD)
		AD	Col-0	
comp83964_c0_seq5*	chitin binding PM protein	1083540000	7182233333	6.628489334
comp83964_c0_seq3*	chitin deacetylase 5b	122973333.3	791316666.7	6.434863929
comp86604_c0_seq2*	protein Skeletor	160175000	655916666.7	4.09500026
ADX96001.1	Small heat shock protein 19.9	27852000	67363333.33	2.418617454
ADV60539.1	Ala-tRNA synthetase	4465000	10671000	2.389921613
ABK29472.1	HMG176	3521400	8253400	2.343783722
AKD49099.1	Chitin deacetylase 17	33489000	73049666.67	2.181303314
comp82363_c0_seq11*	HMG176	2793800	5986700	2.142852029
comp87582_c0_seq1*	putative gram negative bacteria-binding protein	241293333.3	510170000	2.114314528
comp84499_c0_seq1*	ZZ-type zinc finger-containing protein 3-like	32458500	64800333.33	1.996405667
comp80008_c0_seq1*	probable salivary secreted peptide-like	65417000	123245333.3	1.883995495
comp89199_c0_seq3*	multiple epidermal growth factor-like domains protein	7142950	13273966.67	1.858331175
KPI95249.1	Talin-1	16028500	29485000	1.839535827
XP_013165576.1	glycerol-3-phosphate acyltransferase 1	58966000	104119000	1.765746362
comp84473_c1_seq1	chlorophyllide A binding protein	84705666.67	147770000	1.744511
comp84473_c1_seq3*	chlorophyllide A binding protein	107102333.3	175706666.7	1.640549382

361 The fourth-instar larvae were fed with artificial diet (AD) or *Arabidopsis* (Col-0) for one day, the OS were
362 collected and analyzed by LC-MS. HARP1 is shown in red. ***indicated that** the data were obtained from
363 Xiong et al. BMC Genomics (2015) 16: 321. https://doi.org/10.1186/s12864-015-1509-1 and the rest were
364 obtained from NCBI.

365 **Table S2.** HARP1 is similar to R-like/venom protein.

Species	Accession	Identities	Query cover
Nasonia vitripennis	NP_001155164.1	28%	79%
Trichomalopsis sarcophagae	OXU27876.1	30%	68%
Pristhesancus plagipennis	AQM58365.1	29%	96%

366

367 **Table S3.** Thirteen selected genes with less induction upon wounding based on RNA-seq data. qRT-PCR
368 analysis (Fig. S4) further conformed that nine of these genes obviously less induced in *35S:6MYC-HARP1-*
369 *1* than in wild-type (shown in red color).

Gene	Name	Annotation
AT1G19570	DHAR5	response to jasmonic acid， dehydroascorbate reductase
AT1G52890	ANAC19	a NAC transcription factor
AT1G73325	--	Kunitz family trypsin and protease inhibitor protein
AT2G24850	TAT3	biosynthetic process, cellular amino acid metabolic process, response to jasmonic acid, response to wounding
AT2G39030	NATA1	L-ornithine N5-acetyltransferase NATA1, response to jasmonic acid
AT3G16470	JAL35,	response to wounding, response to jasmonic acid
AT3G44860	FAMT	a farnesoic acid carboxyl-O-methyltransferase
AT4G08870	ARGAH2	Involved in the defence response to B.cinerea
AT4G15210	BMY1, RAM1	response to herbivore
AT4G30460	--	glycine-rich protein
AT5G05600	--	cellular response to toxic substance, flavonoid biosynthetic process, oxidation-reduction process,
AT5G20230	BCB	response to oxidative stress, response to wounding
AT5G47330	--	palmitoyl-(protein) hydrolase activity

370

371 **Table S4.** The HARP1-like proteins in Lepidoptera insects.

Species	Family	Database	Accession Number	Length (aa)	Score	Identity
Helicoverpa armigera	Noctuidae	ha_unigene *	comp80008_c0_seq1* (HARP1)	122	--	--
Heliothis virescens	Noctuidae	NCBI nr	PCG70847.1	121	206	92%
Agrotis ipsilon	Noctuidae	Obtained from Prof. Zhan (szhan@sibs.ac.cn)		125	155	67%
Spodoptera exigua	Noctuidae	NCBI nr	AFH57158.1 (REPAT38)	121	188	83%
Spodoptera frugiperda	Noctuidae	NCBI EST	FP350599.1	121	210	83%
Mamestra configurata	Noctuidae	NCBI nr	AEA76315.1	123	154	63%
Trichoplusia ni	Noctuidae	NCBI EST	FF375809.1	121	121	60%
Hyphantria cunea	Erebidae	Obtained from Prof. Zhan (szhan@sibs.ac.cn)		118	129	53%
Bombyx mori	Bombycidae	NCBI nr	XP_004925327.1	118	112	50%
Manduca sexta	Sphingidae	NCBI EST	GR919303.1	118	105	43%
Plutella xylostella	Plutellidae	NCBI EST	XP_011548876.1	116	83.2	41%

372 * The HARP1 sequence information was obtained from Xiong et al. BMC Genomics (2015) 16: 321.
373 https://doi.org/10.1186/s12864-015-1509-1.

374 **Table S5.** Primers used in this investigation.

Gene	Primer Sequence (5'-3')	Purpose
HARP1	CGAACGCAGTGCAATATCAG GGCTGATGTCAGTCTGATGG	qRT-PCR
actA3b	AAGTTGCTGCGCTGGTAGTAG AGTTCGTAGGACTTCTCCAGG	qRT-PCR
S18	CCAGCGATCGTTTATTGCTT AGTCTTTCCTCTGCGACCAG	qRT-PCR
TAT1	CCCTCAAAGACGTCAATGGT ACACGACACGACAAGTCCAA	qRT-PCR
VSP2	ACCCTCCTCTCTAGTATTCCC ACTTGTACACCACTTGCCTCA	qRT-PCR
MYC2	CAAGGAGGAGTGTTTGGGATGC GTCGAAAAATTAAGTTCTCGGGAG	qRT-PCR
LOX2	TTGGTGTGGTAACTACGATTGC CACCAGCTCCAGCTCTATTCTT	qRT-PCR
GhHIS3	CGGTGGTGTGAAGAAGCCCTAT AATTTCACGAACAAGCCTCTGGAA	qRT-PCR
AT2G39030	CCCCCTTTCTTGAGACGCAT CACCCAACTTCACTGCTTGC	qRT-PCR
AT1G73325	CCGGTTCAGTCCAATGTCCA CACACTTTGGAGTCAGGGCA	qRT-PCR
AT4G15210	GCGTTGATGGCGTTATGGTC GACATCGCGAACCCATTGTG	qRT-PCR
AT2G24850	CGGATGAGAATACCGTCGCA ATGGATCCGAGCGTGATCAC	qRT-PCR
AT3G44860	TTCACCGCGGTACAAACCTT	qRT-PCR

	TGTTCCACACAAGCGAGTCA	
AT1G19570	ATCGACGACAAGTGGGTGAC GGAAACTCTTTCTCCGGCGA	qRT-PCR
AT1G52890	ACCGAACAGAAAACCGGGTT TTTGAGCTAACTCGCCCGAG	qRT-PCR
AT4G08870	TCATGGAAGGTGGCTATGCG CTCCTGGTTCGAAGTGGGAC	qRT-PCR
AT3G16470	CCGTGTCGGGCTACTATGAC GGCGCAACATTGACTCCAAG	qRT-PCR
Gh_Sca005135G01	CCATCGTCCTTTTCGT ACCGTTGTTGTTTCGC	qRT-PCR
Gh_A10G2353	ATGAAAACCACAACAGTTTCGG AACATGAACTACTTGTTGAATC	qRT-PCR
Gh_D11G1335	GGCAAAGATGGAGAGA GTAGGGGGACGAACAA	qRT-PCR
HARP1	CGGGATCCATGAAGAGCCTTATCCTCG CGAGCTCTTATCGGCCCCAGATTTC	Pull down
JAZ3	CCGGAATTCATGGAGAGAGATTTTCTCGG ACGCGTCGACTTAGGTTGCAGAGCTGAGAG	Pull down
JAZ3δN	CCGGAATTCATGGGTTCCTCTATGCCTCAA ACGCGTCGACTTAGGTTGCAGAGCTGAGAG	Pull down
REPAT38	GGAATTCATGAAGAGTCTGATTCTGGTTGC CCCAAGCTTTTAACGACCCCAAATCTCAACAC	Prokaryotic expression
Venus	CGGGATCCATGGTAGATCTGACTAGTAAAG CGAGCTCTTATTTGTATAGTTCATCCATGC	Prokaryotic expression
Venus-HARP1	CGAGGCAGCTAGATCCACCATGAACCCTGCCTTCAGGGCC	Prokaryotic expression

	CCCAAGCTTTTATCGGCCCCAGATTTCGA	
HARP1	GGAATTCATGAACCCTGCCTTCAGGGCC CGGGATCCGATCATGTAGTAGTAGCCCC	Y2H
REPAT38	GGAATTCATGGCCTTCAGAGCTAACCTT CGGGATCCTTAACGACCCCAAATCTCAAC	Y2H
PXHL1	CGGAATTCATGGTGGTCAAGACCCCGCTCACCC ACGCGTCGACTCAGCGTCCGTAGATCACCACA	Y2H
JAZ5	CGGAATTCATGTCGTCGAGCAATGAAAATG CGGGATCCCTATAGCCTTAGATCGAGAT	Y2H
Gh_A12G2441	CGGAATTCATGGAGAGAGATTTTATCGGTT CGGGATCCTTAATTGATGGCTTGTAAAGGA	Y2H
Gh_D12G2567	CGGAATTCATGGAGAGAGATTTTCTCGGTTT CGGGATCCTTAATTGATGGCTTGTAAAGGA	Y2H
Gh_A06G0705	CGGAATTCATGAATATGTCGTGTTCACCGG CGGGATCCCTACGGAGATTGAGCAGCCAAA	Y2H
Gh_D08G2564	GGAATTCCATATGATGTTTGGTTCACCGGAGAAA TCCCCCGGGCTACTGCAGTGATTCAACAGCTA	Y2H
Gh_A05G0260	CGGAATTCATGTCGTCTTGCTCGGAATCTA CGGGATCCCTATGGTGATTGAGCAGCCAAA	Y2H
NbJAZ3L (BAD04852.2)	GGAATTCATGGAGAGAGATTTTATGGGTT TCCCCCGGGTTACGTCTCCTTGACCAAATTG	Y2H
HARP1	TCCCCCGGGATGAACCCTGCCTTCAGGGCC ACGCGTCGACTCGGCCCCAGATTTCGATCA	Transgene
REPAT38	CGGGATCCATGGCCTTCAGAGCTAACCTT ACGCGTCGACTTAACGACCCCAAATCTCA	Transgene
GFP-HARP1	CGGGATCCATGAACCCTGCCTTCAGGGCC ACGCGTCGACTTATCGGCCCCAGATTTC	Transgene

375 **Table S6.** The insect protein database information used in proteomic analyses.

Order	Family	Genus	Protein Number
Lepidoptera	Pieridae	Pieris	620
Lepidoptera	Noctuidae	Helicoverpa	39899
Lepidoptera	Papilionidae	Papilio	4614
Lepidoptera	Nymphalidae	Danaus	16409
Lepidoptera	Bombycidae	Bombyx	17430
Coleoptera	Tenebrionidae	Tribolium	21815
Coleoptera	Curculionidae	Dendroctonus	23845
Hymenoptera	Formicidae	Solenopsis	14754
Hymenoptera	Formicidae	Camponotus	15848
Hymenoptera	Formicidae	Acromyrmex	13982
Hymenoptera	Apidae	Apis	21088
Hymenoptera	Apidae	Bombus	20815
Hymenoptera	Pteromalidae	Nasonia	13861
Diptera	Culicidae	Aedes	19131
Diptera	Culicidae	Culex	22209
Diptera	Ceratopogonidae	Culicoides	690
Diptera	Psychodidae	Phlebotomus	747
Diptera	Tephritidae	Ceratitis	18958
Diptera	Drosophilidae	Drosophila	237741
Diptera	Culicidae	Anopheles	35530
Diptera	Muscidae	Musca	16771
Orthoptera	Acrididae	Locusta	647
Orthoptera	Acrididae	Schistocerca	238

376

377

378 **Other Supplementary Material for this manuscript includes the following:**

379 **Movie S1.** Oral secretion collection.

380 **Dataset S1.** Proteomic data of *H. armigera* oral secretion.

381 **Dataset S2.** RNA-seq analysis of differently expressed genes in the wild-type and *35S:6MYC-HARP1*
382 plant response to wounding.

383
384

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