1 **Supporting Information for**

2 **Endocytosis-mediated entry of a caterpillar effector into plants is countered by**
3 **Jasmonate**

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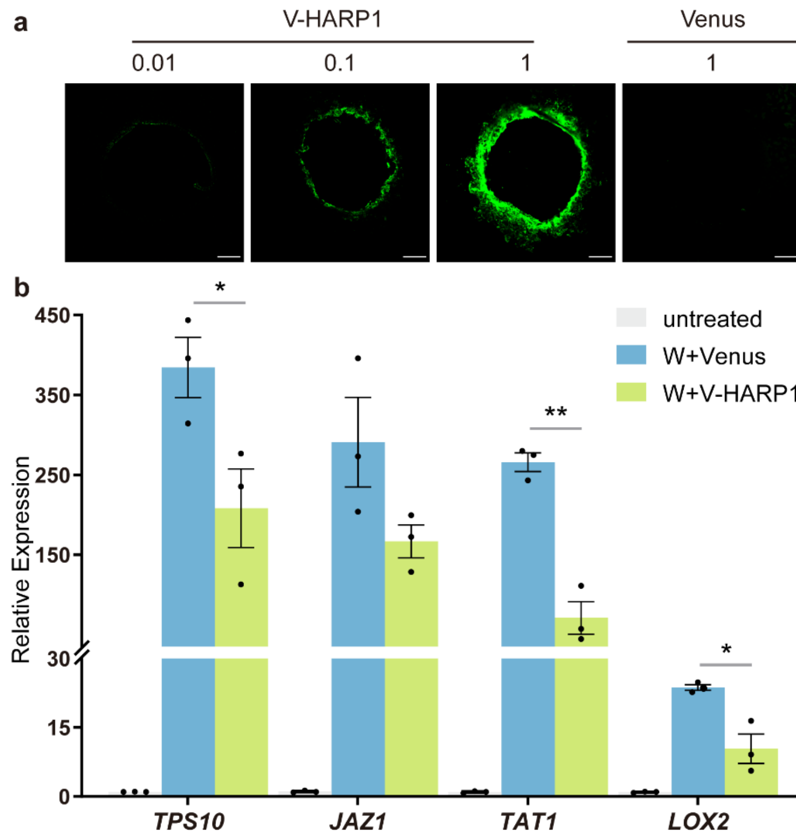
21 ## Supplementary Figures



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23 **Supplementary Figure 1. Weakened plant wounding response by V-HARP1**
24 **application. (a)** Confocal microscopy observation of V-HARP1 around the wounding
25 site of *Arabidopsis* leaves. The wounded leaves were incubated with V-HARP1 (0.01,
26 0.1 and 1 mg/ml) and Venus (1 mg/ml) for four hours, respectively. Scale bar, 200 μm.
27 **(b)** *Arabidopsis* leaves were wounded and painted with 1 mg/ml Venus (W+Venus,
28 blue indicated) or V-HARP1 (W+V-HARP1, green indicated) on the wounding sites
29 quickly. The expressions of the indicated genes were detected 4 hours post treatments
30 by qRT-PCR. The gene expressions in the unwounded plants (untreated, gray
31 indicated) were set to 1. Data are means ± SEM (n = 3 biological replicates) and
32 analyzed by two-sided Student's t test (*P < 0.05, **P < 0.01). Source data are
33 provided as a Source Data file.

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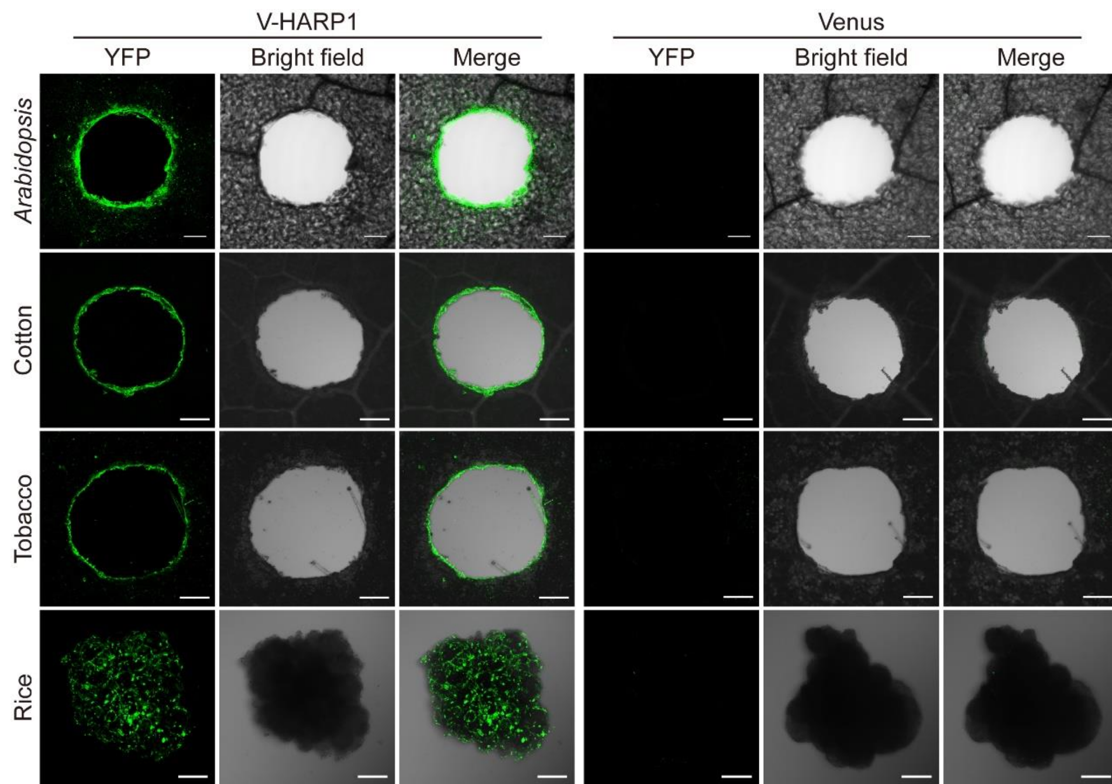

35 **Supplementary Figure 2. Entry of V-HARP1 into *Arabidopsis*, cotton, tobacco,**
36 **and rice callus.** The wounded *Arabidopsis*, cotton and tobacco leaves as well as rice
37 callus were incubated with V-HARP1 or Venus, respectively and then washed with
38 PBS containing 0.08% BSA for 6 times. Confocal microscopy was used for detection.
39 YFP indicates the fluorescence signal of Venus and V-HARP1. Scale bar, 200 μm.

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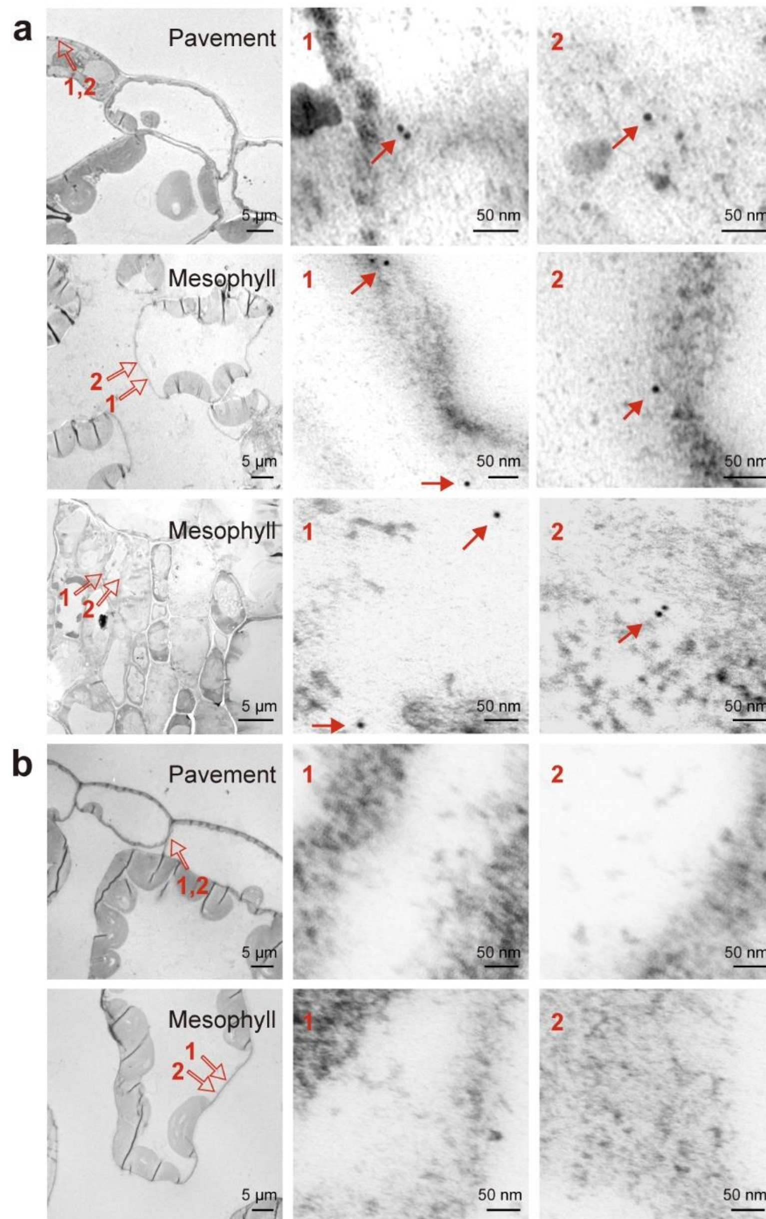

41 **Supplementary Figure 3. It was V-HARP1 not Venus that was found in**
42 **pavement and mesophyll cells under transmission electron microscope (TEM)**
43 **observation.** Ultrathin sections from V-HARP1 **(a)** and Venus **(b)** treated
44 *Arabidopsis* leaves were immune-gold labeled with anti-GFP antibody. The enlarged
45 view of the hollow arrow indicated regions (1 and 2) were shown independently. The
46 solid arrows indicate the immune-gold labeled V-HARP1. Scale bars were indicated
47 in each image.

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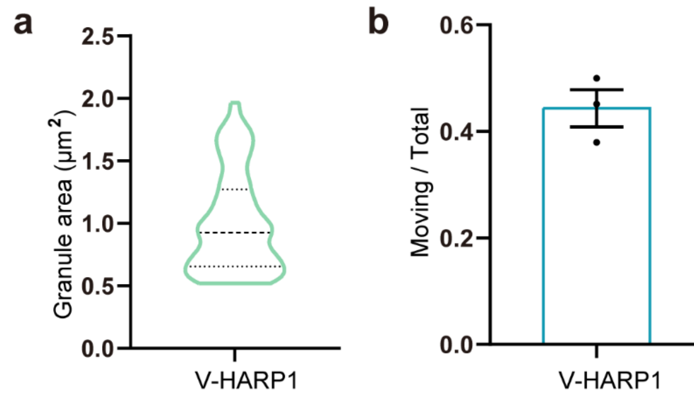

49 **Supplementary Figure 4. Quantification of moving V-HARP1 granules. (a)** Size
50 quantification of V-HARP1 granules. The areas of about 108 granules from six
51 independent sights were calculated in ImageJ. Data are means ± SD (n = 108). **(b)**
52 The ratio of moving to total V-HARP1 granules. The moving V-HARP1 was tracked
53 and counted by Olympus cellSens software. Three independent sights with total of
54 119 V-HARP1 granules were used for calculation. Data are means ± SEM (n = 3
55 biological replicates). Source data are provided as a Source Data file.

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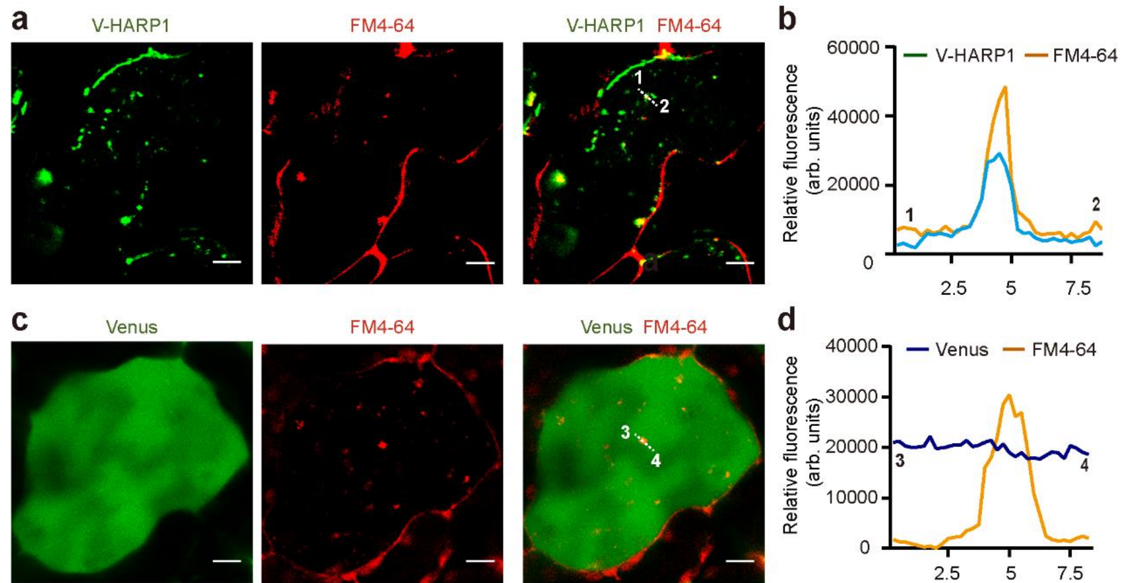

57 **Supplementary Figure 5. V-HARP1 not Venus was observed as granules in plant**
58 **leaves.** *Arabidopsis* leaves were injected with V-HARP1 and Venus at the
59 concentration of 0.2 μg/ml, respectively. And four hours post injection, the leaves
60 were stained with FM4-64 for 30 min to mark membrane and endosomes. Scale bar,
61 10 μm. **(a)** Injected V-HARP1 in *Arabidopsis* leaves. **(b)** Fluorescence intensity (in
62 arbitrary units, arb. units) of the cross sections [dotted line in (**a**)]. Blueand orange
63 lines indicate V-HARP1 and FM4-64 fluorescence signals, respectively. **(c)** Injected
64 Venus in *Arabidopsis* leaves. **(d)** Fluorescence intensity (in arbitrary units, arb. units)
65 of the cross sections [dotted line in (**c**)]. Blue and orange lines indicate Venus and
66 FM4-64 fluorescence signals, respectively.

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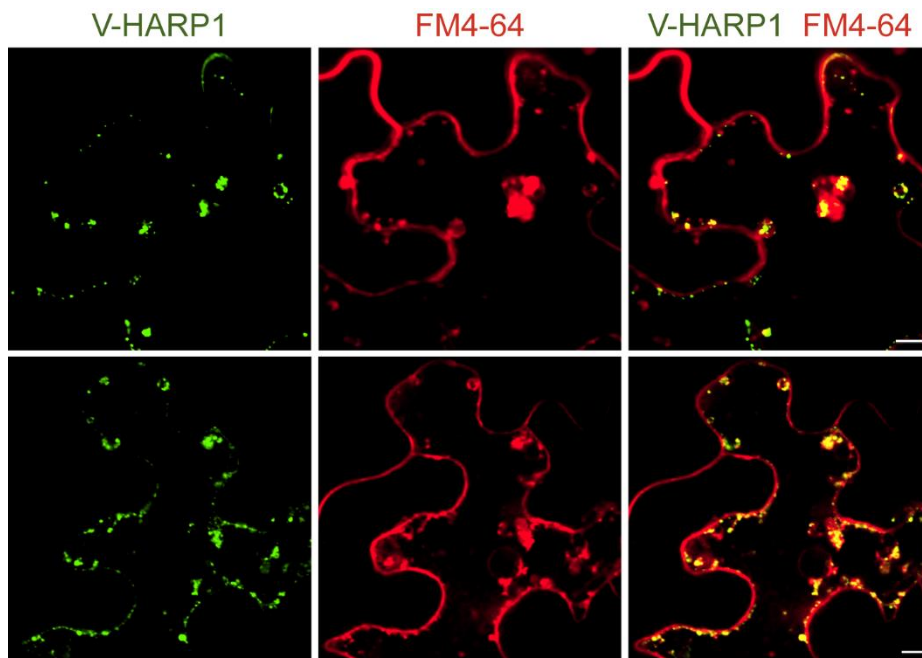

68 **Supplementary Figure 6. V-HARP1 locates in internalized endosomes.** The
69 wounded *Arabidopsis* leaves were incubated with V-HARP1 and further stained with
70 FM4-64 to trace internalized endosomes. Scale bar, 5 μm.

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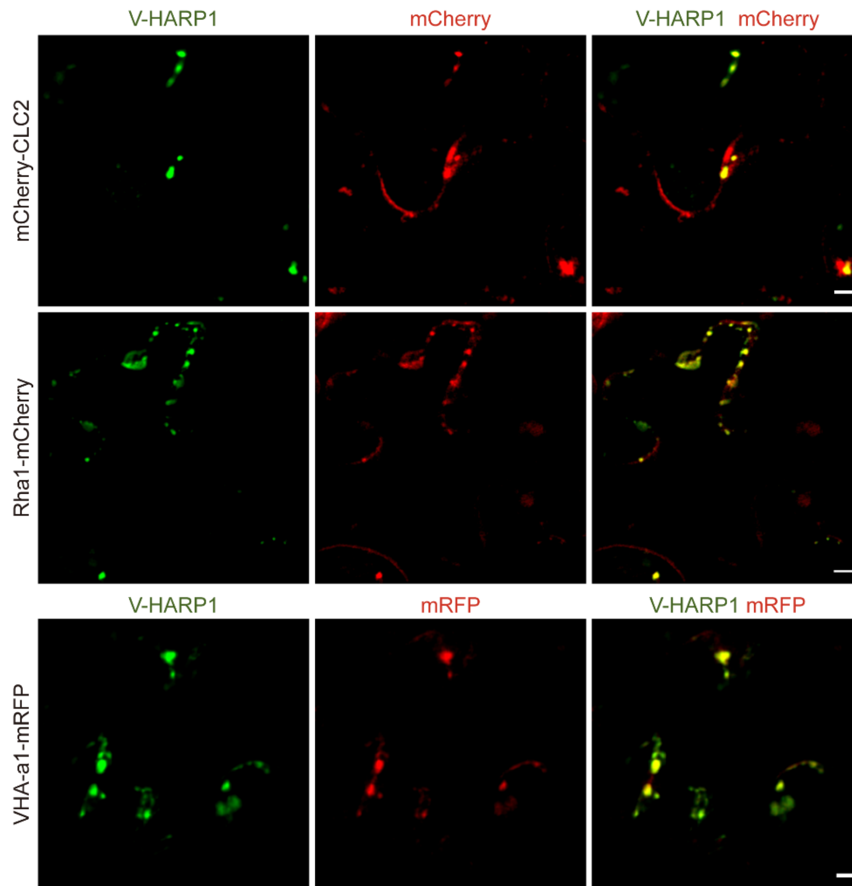

72 **Supplementary Figure 7. V-HARP1 colocalizes with mCherry-CLC2, Rha1-**
73 **mCherry, and VHA-a1-mRFP marked endosomes.** The wounded leaves of
74 indicated *Arabidopsis* plants were incubated with V-HARP1, respectively. mCherry-
75 CLC2 and VHA-a1-mRFP indicate clathrin-coated vesicles and secretory vesicles of
76 early endosomes, respectively. Rha1-mCherry indicates prevacuolar
77 compartment/multivesicular body/late endosomes. Scale bar, 5 μm.

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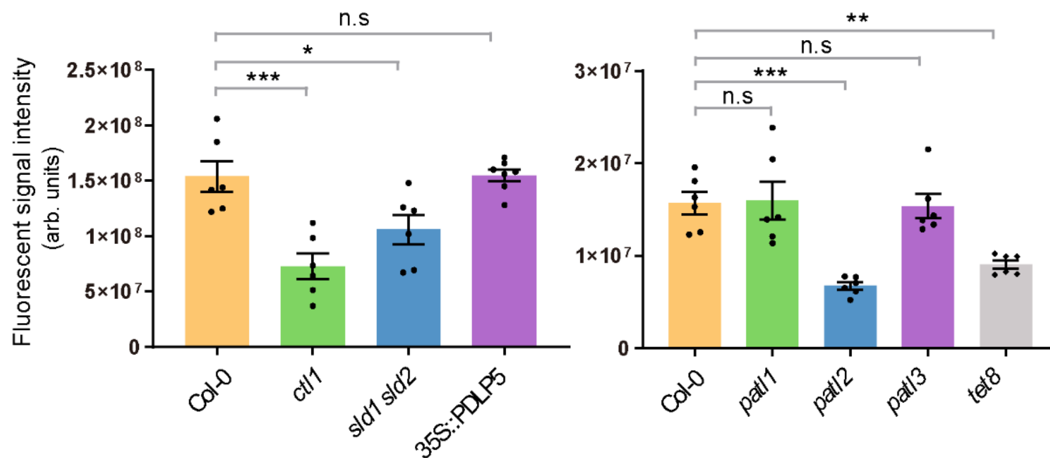

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80 **Supplementary Figure 8. Quantifications of V-HARP1 signals in Fig. 3a and 3b.**

81 The indicated *Arabidopsis* leaves were wounded and incubated with V-HARP1. 6

82 random sights were selected for calculation. Integrated intensity of fluorescence(in

83 arbitrary units, arb. units) was measured in the whole sight selected. Data are means ±

84 SEM (n = 6 biological replicates) and analyzed by one-way ANOVA with two-sided

85 Dunnett's post hoc test (n.s: no significance, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

86 Source data are provided as a Source Data file.

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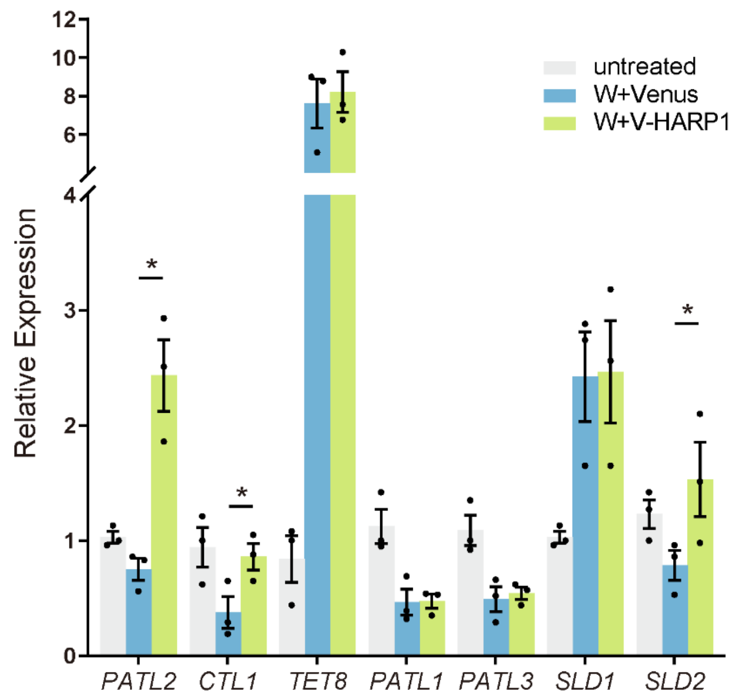

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89 **Supplementary Figure 9. V-HARP1 induces the expressions of *PATL2*, *CTL1* and**
90 ***SLD2* in wounding response.** *Arabidopsis* leaves were wounded and immediately
91 painted with 1 mg/ml Venus (W+Venus, blue indicated) or V-HARP1 (W+V-HARP1,
92 green indicated) on the wounding sites. Gene expressions four hours post treatments
93 were detected by qRT-PCR. The expressions in the untreated (gray indicated) plants
94 were set to 1. Data are means ± SEM (n = 3 biological replicates) and analyzed by
95 two-sided Student's t test (*$P < 0.05$). Source data are provided as a Source Data file.

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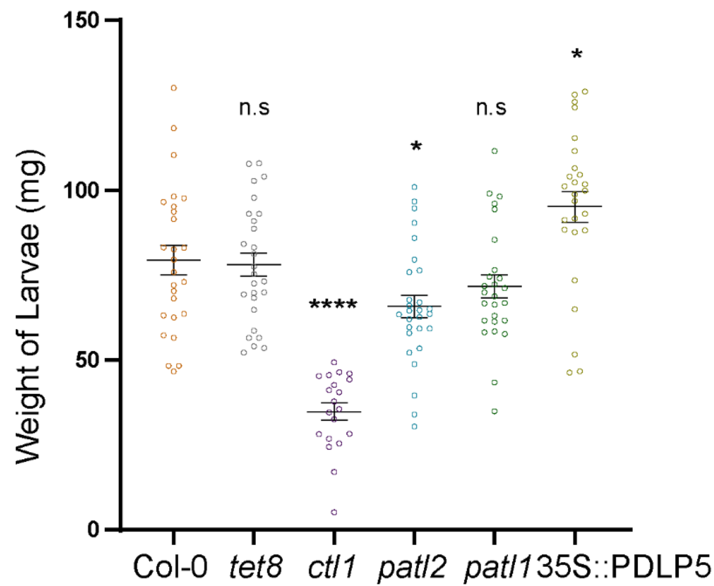

97 **Supplementary Figure 10. The growth of *H. armigera* larvae fed on *ctl1* or *patl2***
98 **plants were inhibited.** The synchronous third-instar larvae were fed on wild type
99 (Col-0) and the indicated mutants for 4 days and subsequently weighted. Data are
100 means ± SEM (n = 20-28) and analyzed by two-sided Student's t test (n.s: no
101 significance, *$p < 0.05$, ****$p < 0.0001$). Source data are provided as a Source Data
102 file.

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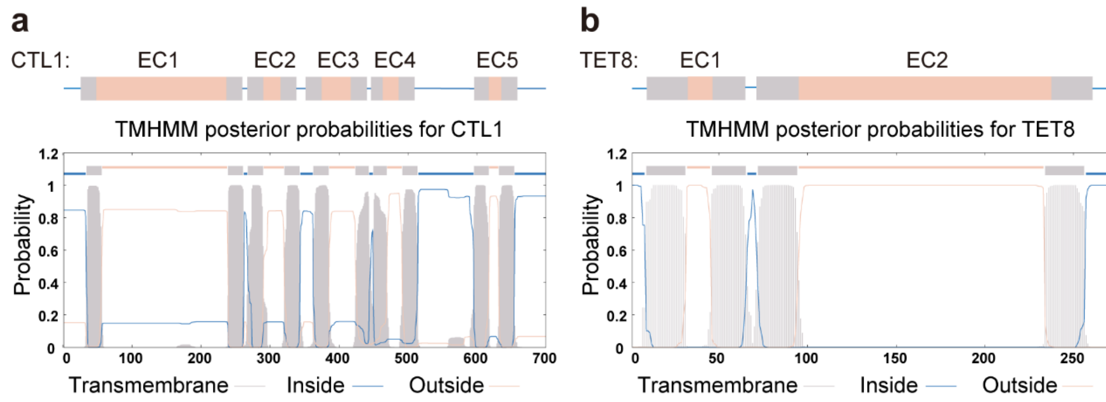

104 **Supplementary Figure 11. Predicted extracellular loops (ECs, pink) and**
105 **transmembrane helices (gray) of CTL1 and TET8.** The transmembrane helices in
106 CTL1 **(a)** and TET8 **(b)** were predicted by TMHMM
107 (http://www.cbs.dtu.dk/services/TMHMM/).

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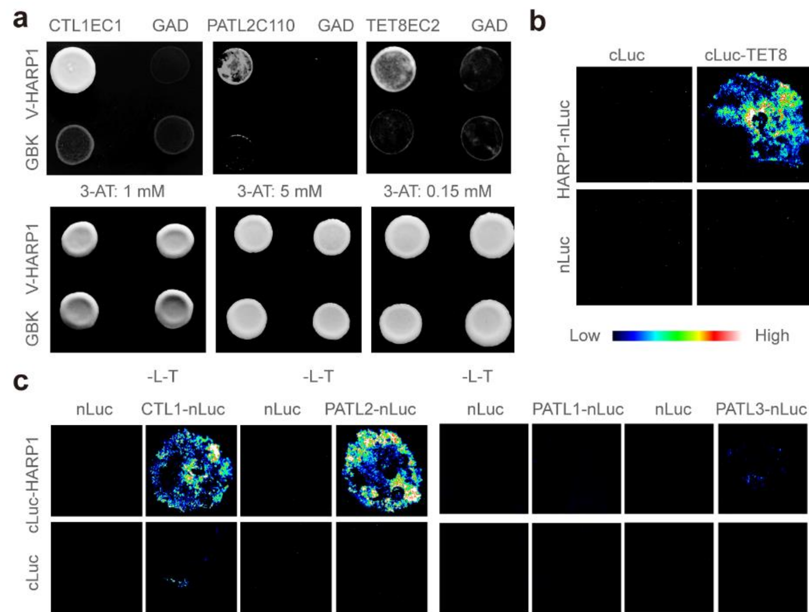

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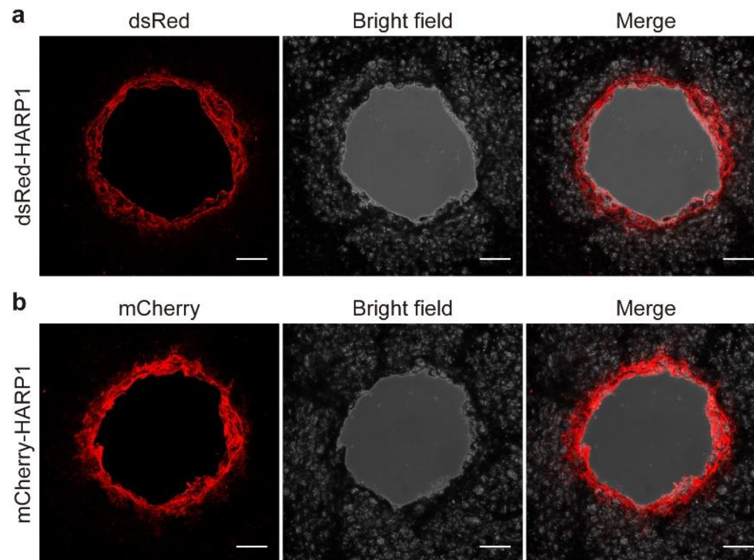
110 **Supplementary Figure 12. HARP1 interacts with CTL1, PATL2 and TET8. (a)**
111 Yeast two-hybrid assays of CTL1EC1, PATL2C110 and TET8EC2 interaction with
112 V-HARP1. V-HARP1 was fused to GAL4 DNA-binding domain (BD), CTL1EC1,
113 PATL2C110 and TET8EC2 were fused to GAL4 activation domain (AD),
114 respectively. To examine the interaction with CTL1EC1 and TET8EC2, yeast cells
115 were grown on medium (-L-T-H) with 1 mM and 0.15 mM 3-AT, respectively. To
116 examine the interaction with PATL2C110, yeast cells were grown on medium (-A-L-
117 T-H) with 5 mM 3-AT. **(b-c)** Detection of HARP1 interaction with TET8 **(b)** as well
118 as with PATLs (PATL1, 2, 3) and CTL1 **(c)** by BiLC assay. The colors ranged from
119 white, red, green to blue indicate a strong to weak interaction. **(b)** TET8 was fused to
120 the carboxyl-terminal half of LUCIFERASE (cLuc) and HARP1 was fused to the
121 amino-terminal half of LUCIFERASE (nLuc). **(c)** CTL1, PATL1, PATL2 and PATL3
122 were fused to nLuc and HARP1 was fused to cLuc.



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124 **Supplementary Figure 13. Imports of dsRed-HARP1 and mCherry-HARP1 into**
125 ***Arabidopsis* leaf tissues.** The wounded *Arabidopsis* leaves were incubated with
126 dsRed-HARP1 **(a)** or mCherry-HARP1 **(b)**, respectively and observed under
127 fluorescence microscope. Scale bar, 200 μm.

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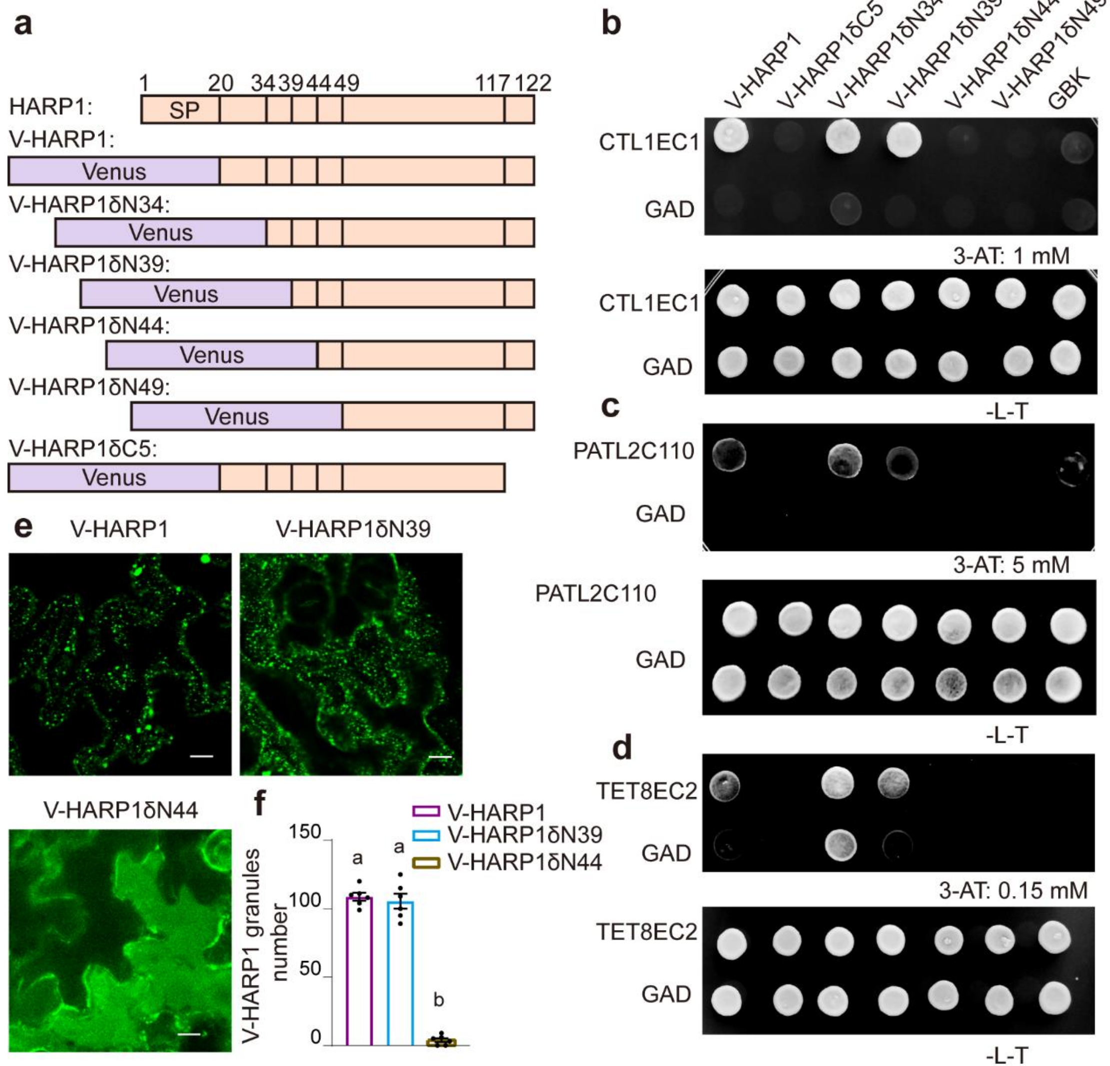

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130 **Supplementary Figure 14. The interactions of truncated V-HARP1 with**
131 **CTL1EC1, PATL2C110 and TET8EC2. (a)** Schematic diagram of truncated V-
132 HARP1. **(b-d)** Yeast two-hybrid assay to examine the interactions of full length and
133 truncated V-HARP1 with CTL1EC1 **(b)**, PATL2C110 **(c)** and TET8EC2 **(d)**. V-
134 HARP1 and its indicated truncated forms were fused to GAL4 DNA-binding domain
135 (BD), CTL1EC1, PATL2C110 and TET8EC2 were fused to GAL4 activation domain
136 (AD), respectively. Yeast cells were examined as described in Supplementary Figure
137 12. **(e)** Confocal image of V-HARP1, V-HARP1δN39 and HARP1δN44 which were
138 delivered into Arabidopsis leaves by injection. Scale bar, 5 μm. **(f)** Quantification of
139 the V-HARP1 granules number in (**e**). 6 random sights were selected to count the

140 granule numbers of V-HARP1 (purple indicated), V-HARP1δN39 (blue indicated)
141 and HARP1δN44 (brown indicated). Data are means ± SEM (*n* =6 biological
142 replicates) and analyzed by one-way ANOVA with two-sided Dunnett's post hoc test.
143 Different letters indicate significant differences ($P < 0.05$). Source data are provided
144 as a Source Data file.

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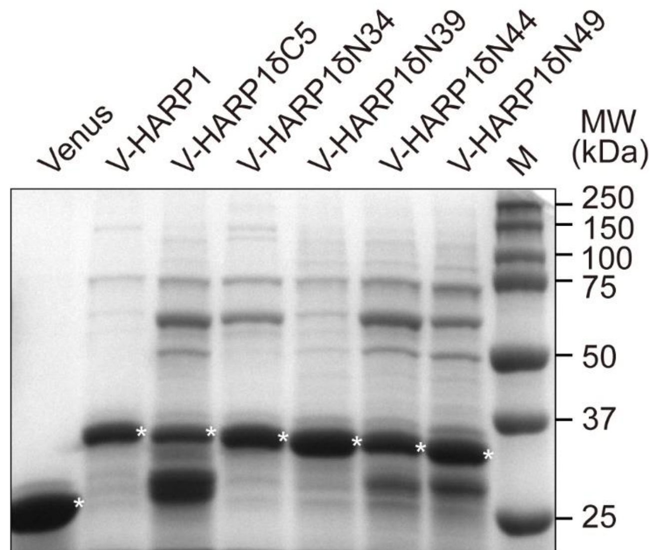

146 **Supplementary Figure 15.** Coomassie blue staining of Venus and indicated
147 truncated V-HARP1s mentioned in Fig. 4f. The procaryotically expressed proteins
148 were purified and subjected to SDS agarose gel electrophoresis. * Stands for the target
149 band of the indicated protein.

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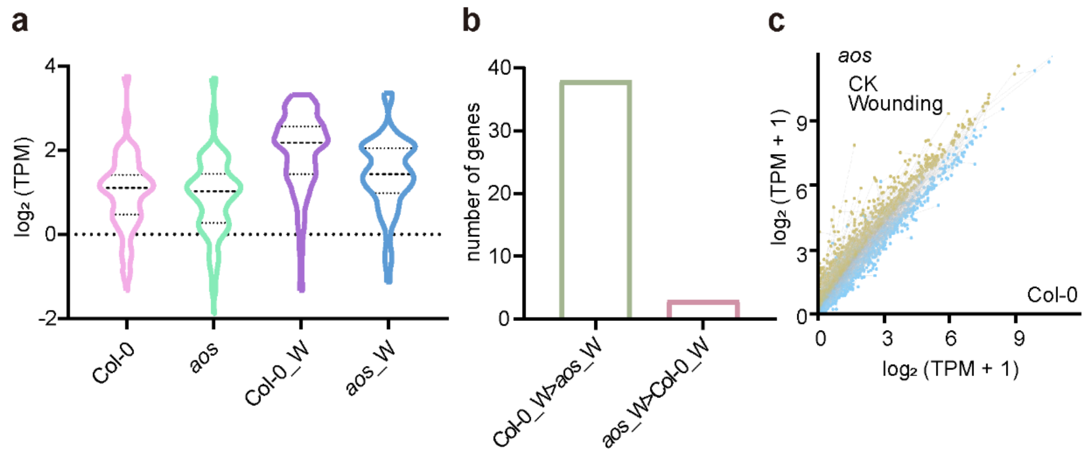

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152 **Supplementary Figure 16. Transcriptomic analysis of gene expressions in Col-0**
153 **and *aos*. (a)** Expression of 92 genes annotated as response to JA which were detected
154 in our transcriptome data (Supplementary Data file2). CK stands for untreated plants
155 and W stands for plants two hours post wounding. **(b)** The expressions of most of
156 genes as described in **(a)** were higher in Col-0 than in *aos* after wounding. **(c)** Scatter
157 plot analysis of the 1121 genes with higher expression in *aos* than in Col-0 after
158 wounding (*aos*_W / Col-0_W > 2, FDR < 0.05).

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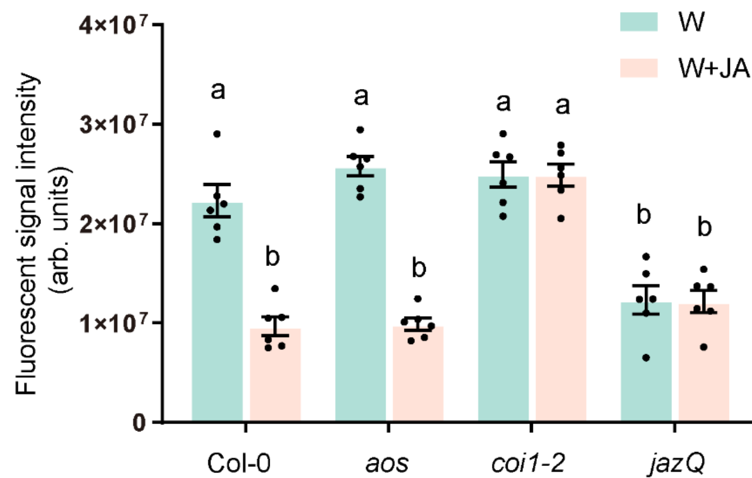

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161 **Supplementary Figure 17. Quantification of V-HARP1 signals in Fig. 6a.** The
162 fluorescent signal intensity (in arbitrary units, arb. units) of V-HARP1 in leaves of
163 Col-0, *aos*, *coi1-2* and *jazQ* were measured. Light blue and light pink box indicate
164 Ethanol (W) or 50 μM MeJA (W+JA) treatment, respectively.Data are means ± SEM
165 (n = 6 biological replicates) and analyzed by two-way ANOVA followed by multiple
166 comparisons with Fisher's LSD test. Different letters indicate significant differences
167 ($P < 0.05$). Source data are provided as a Source Data file.

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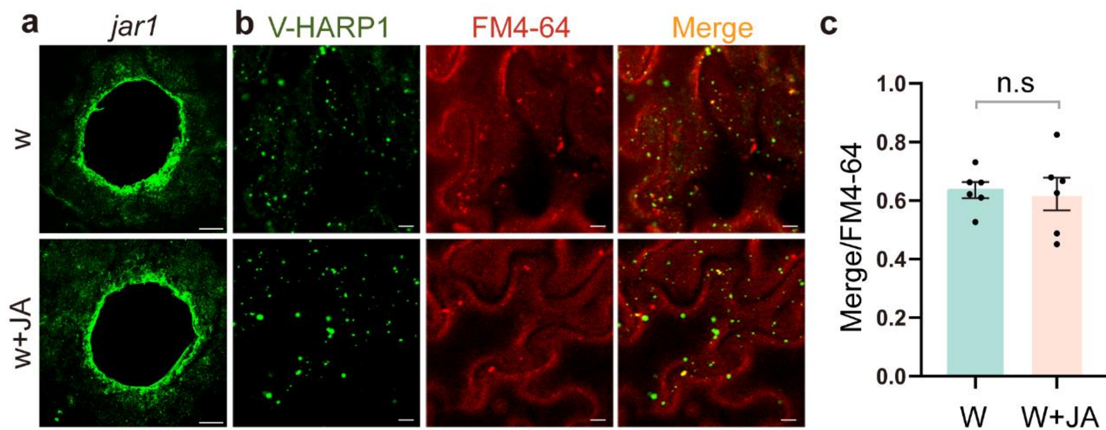

169 **Supplementary Figure 18**. **JA has no detectible impacts on HARP1 import into**
170 **jar1.** (**a)** Confocal image of the V-HARP1 signals around the wounding sites of the
171 *jar1* leaves. Leaves of *jar1* were pretreated with Ethanol (W) or 50 µM MeJA
172 (W+JA) for two hours and subsequently incubated with V-HARP1. Scale bar: 200
173 µm. (**b)** V-HARP1-loaded endosomes in the plants. Plants were treated as described
174 in (**a**). The internalized endosomes were traced by FM4-64. Scale bar: 5 µm. (**c)**
175 Quantification of endosomes from (**b**). 6 random sights were selected to count the
176 number of endosomes. Data are means ± SEM (n = 6 biological replicates) and
177 analyzed by two-way ANOVA followed by multiple comparisons with Fisher's LSD
178 test (n.s: no significance). Source data are provided as a Source Data file.

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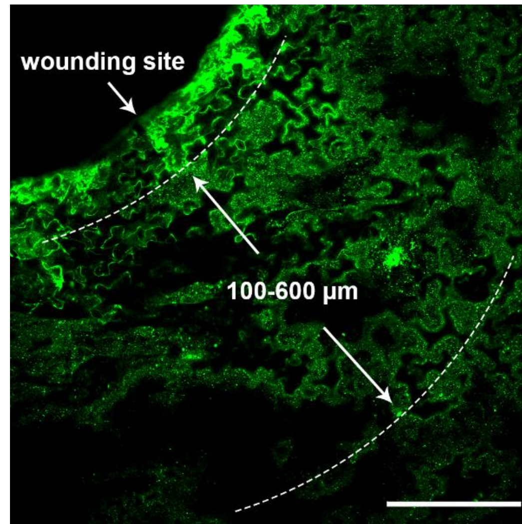

180 **Supplementary Figure 19. Regions used for observing the subcellular**
181 **localization and movement of HARP1.** The wounded *Arabidopsis* leaves were
182 incubated with V-HARP1 for four hours. Samples were washed with PBS containing
183 0.08% BSA for at least 5 times (20 minutes for each time) and then were detected
184 under confocal microscopy. The dotted lines indicated the area where the subcellular
185 localization and movement of HARP1 were observed. Scale bar, 200 μm.

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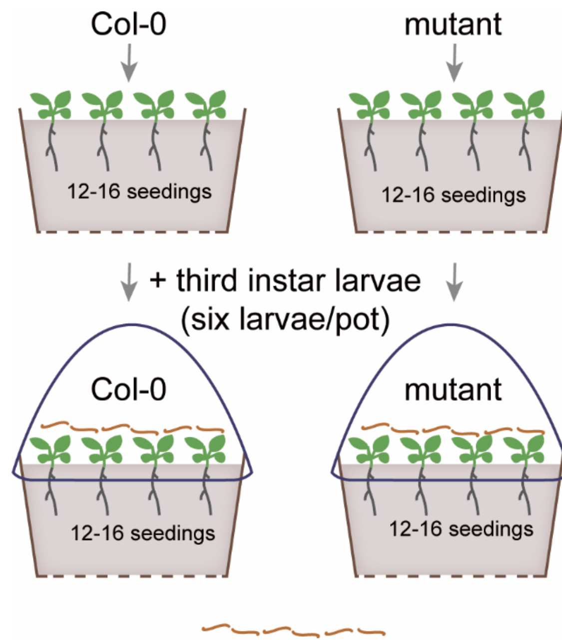

187 **Supplementary Figure 20. Diagram of cotton bollworm feeding test.** About 12-16
188 seedlings of *Arabidopsis* were planted in one pot. 5-6 pots of three-week-old plants of
189 wild type (Col-0), *tet8*, *ctl1*, and *patl2* were used for insect feeding test. About 5-6 3rd
190 instar larvae were put into one pot and covered with a plastic cover. After feeding for
191 3-4 days, larvae were weighted individually.