Supporting Information Appendices

A salivary effector enables whitefly to feed on host plants by eliciting salicylic acid-signaling pathway

Hong-Xing Xu, Li-Xin Qian, Xing-Wei Wang, Ruo-Xuan Shao, Yue Hong, Shu-Sheng Liu and Xiao-Wei Wang

Materials and Methods

Insects and plants. The whitefly cryptic species used were MED1 (*mtCOI* GenBank: GQ371165), MEAM1 (*mtCOI* GenBank: GQ332577), Asia II 3 (*mtCOI* GenBank: DQ309076), Asia II 1 (*mtCOI* GenBank: AJ867557) and China 2 (*mtCOI* GenBank: AY686072). All whitefly cultures were reared on cotton plants in climate chambers at 27±1 °C, 14 h light: 10 h dark photoperiod and 70±10 % relative humidity. All insect-host plant experiments were conducted at this temperature and light regime. Whitefly adults within three days post-emergence were used in all bioassays.

Seeds of cotton (*Gossypium hirsutum* cv. Zhe-mian 1793) and tobacco (*Nicotiana tabacum* cv. NC89 and *N. benthamiana*) were from our lab. NahG (1) tobacco (*N. tabacum* var. Samsun NN, a transgenic plant that does not accumulate SA) seeds were kindly provided by Prof. Han-Song Dong (Nanjing Agricultural University, China). All the plants were cultivated singly in pots in a greenhouse under natural lighting supplemented with artificial illumination (light 05:00 – 18:00 h) and controlled temperature at 25 ± 3 °C. The tobacco plants used in this study were all at the five-to-six true-leaf stage unless otherwise specified.

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25 **Analysis of SA, JA, JA-Ile, ABA and IAA.** The phytohormones were analyzed as described
26 previously (2). Briefly, after different treatments, the third tobacco leaf from the top was
27 harvested and powdered in liquid nitrogen. Each treatment was replicated six to eight times.
28 Phytohormone extraction was performed by adding 1 mL of ethyl acetate containing 10 ng of
29 D4-SA, IAA-D2, ABA-D6 and D6-JA, and 1 ng JA-Ile-D6 to 150 mg of leaf powder. All
30 samples were then vortexed for 10 min and centrifuged at 14,000 rpm for 20 min at 4 ℃.
31 Supernatants were collected and evaporated using a vacuum concentrator to dry at 30 ℃.
32 Residues were resuspended in 250 μL of MeOH: H2O (70:30, v/v) and centrifuged at 13,000
33 rpm for 10 min. The supernatants were then collected and analyzed with a high-performance
34 liquid chromatography-tandem mass spectrometry system (TripleTOF 5600+, AB Sciex).
35
36 **Exogenous amplification of SA.** Tobacco plants were treated with SA solution and whitefly
37 performance assessed. For each plant, *ca* 5 ml of 5 μM SA (Sigma-Aldrich, purity > 99%)
38 solution was sprayed on tobacco leaves once a day. Control plants were treated with 5 ml
39 distilled water. After tobacco plants were sprayed for three days, tobacco plants were used for
40 defense-marker gene expression analyses and whitefly bioassays. Whitefly bioassays were
41 performed as described in the main text and each plant included 2 clip cages. Tobacco plants
42 were still sprayed with 5 μM SA or distilled water once a day during bioassays. Three days
43 after the release of whiteflies, the numbers of live adults and eggs deposited on the leaf within
44 clip cages were counted. Each clip cage was treated as a biological replicate; 16 replicates were
45 performed for each treatment.
46
47 **Quantitative real-time PCR (qRT-PCR) analysis.** To measure the expression levels of
48 marker genes in SA-JA pathways, total RNA was isolated from 100 mg tobacco leaves. cDNA

49 was synthesized using PrimeScript™ RT reagent kit with gDNA Eraser (Takara, Dalian,
50 China). qRT-PCRs were performed using the CFX96™ Real-Time PCR Detection System
51 (Bio-Rad, USA) with SYBR Premix Ex Tag II (Takara, China). Six to twelve biologically
52 independent replicates (plants) were conducted for each treatment and each gene was analyzed
53 in triplicate. The reference gene *GAPDH* was used for transcript normalization. Primers are
54 shown in Table S3.

55 For gene expression analyses of whitefly *Bt56* genes, total RNA was extracted from
56 different developmental stages (eggs, nymphs, pseudopupae, female and male adults) and
57 different tissues (salivary gland, midgut and ovary) of MED1. To detect the expression levels of
58 *Bt56* gene in different species of whiteflies, total RNA was extracted from whitefly adults two
59 days after emergence. All the experiments were repeated three times independently. qRT-PCR
60 was performed as described above. The primer pairs for *Bt56* and reference gene *TAF* (TATA
61 box binding protein associated factor) gene (3) are shown in Table S3.
62

63 **Clone and sequence analysis of Bt56 gene.** Total RNA was extracted from the adults of the
64 five species of whiteflies using SV total RNA isolation system (Promega, USA) and then
65 reverse transcribed into 1st-strand cDNA with Prime script^{TM} RT reagent kit for RT-PCR
66 (Takara, Dalian, China), following the manufacturer's protocol. The full-length *Bt56* gene was
67 obtained using a SMARTer RACE Kit (Takara, Dalian, China). Primers used for PCR
68 amplifying *Bt56* genes are listed in Table S3. The PCR products were cloned into a pMD18-T
69 vector (Takara) and sequenced. Sequences amplified from each species of whiteflies were
70 blasted against the non-redundant database at the NCBI website (http://www.ncbi.nlm.nih.gov/)
71 to search for orthologous genes. The predicted protein sequences were analyzed by SignalP 4.1
72 Server to predict the presence of signal peptides and cleavage sites

73 (http://www.cbs.dtu.dk/services/SignalP/). TMHMM Server v. 2.0
74 (http://www.cbs.dtu.dk/services/TMHMM/) was used for identifying transmembrane domains.
75 Protein sequence alignment was performed by Clustal Omega
76 (http://www.ebi.ac.uk/Tools/msa/clustalo/).
77
78 **Overexpression of *Bt56* gene in tobacco.** The sequence corresponding to the ORF encoding
79 the mature protein of Bt56 was amplified and ligated into binary vector pCABMIA1300-35S to
80 generate a 35S::Bt56 recombinant vector. The recombinant vector was validated by sequencing
81 and transformed into *Agrobacterium tumefaciens* strain GV3101. When recombinant *A.*
82 *tumefaciens* strains were grown in LB medium (50 mg/L Kan, 50 mg/L Rif) at 28 ℃ to an
83 OD_{600} of 0.6, the cultivated cells were collected by centrifuging and resuspended in infiltration
84 buffer (10 mM MES, 10 mM $MgCl_2$, 200 μM acetosyringone, pH5.7) to OD_{600} = 1. Three hour
85 after resuspension, leaves of tobacco at the four-to-five true-leaf stage were infiltrated with *A.*
86 *tumefaciens*. Tobacco leaves (the infiltrated area) were harvested at the time points shown in
87 Fig. S3*B* for RNA isolation and cDNA synthesis. The cDNA samples were subjected to PCR
88 reactions to detect the overexpression of *Bt56* gene. Primers used for *Bt56* detection are listed
89 in Table S3.
90 Whitefly bioassays were performed 48 h after infiltration. Clip cages were fixed on the
91 abaxial surface of the infiltrated leaf areas and five female adults were released into each clip
92 cage (4). Three days after the release of whiteflies, the number of live adults and eggs laid on
93 the leaf within clip cages were counted to assess whitefly performance. For whitefly life span
94 assays, five male and five female adults were released into each clip cage. The number of live
95 adults within cages was counted at 1, 3, 5, 7 and 9 days after release.

96 For phytohormones and defense-marker genes expression analysis, tobacco leaves (the
97 infiltrated area of the third leaf from the top) were harvested 48 h after infiltration and analyzed
98 as described in the main text.
99

100 **RNAi experiments.** To silence the *Bt56* gene, we fed whiteflies with artificial diet containing
101 *dsRNA* (15 % sucrose solution, 200 ng /μl *dsRNA*) using an artificial diet feeding device as
102 previously described (5). Two days after feeding *dsRNA*, the RNAi efficiency was analyzed by
103 qRT-PCR. Bioassays of *Bt56*-silenced whiteflies on uninfested control tobacco, *Bt56*
104 overexpressed tobacco and empty vector expressed tobacco were performed as described
105 above. For analyses of phytohormone levels and defense-marker gene expression, tobacco
106 leaves were harvested three days after they were infested by *Bt56*-silenced whiteflies and
107 analyzed as described in the main text.

108 To investigate the influence of *Bt56*-knockdown on whitefly life span on tobacco, cotton
109 and artificial diet, *Bt56*-silenced whiteflies were released into clip cages, 5 female and 5 male
110 adults in each cage. Every two days, the number of live whiteflies was counted. Six plants were
111 used for each treatment and each plant included two clip cages. Twelve replicates were
112 conducted for each treatment.
113

114 **Bt56 protein expression and antibody preparation.** The sequence encoding mature protein of
115 Bt56 was cloned into the pET-28a expression vector to express the protein fused with His tag.
116 The recombinant plasmid was transferred to *Escherichia coli* strain Rosetta 2. The fusion
117 protein was induced by 0.1 mM IPTG in 37 ℃ for 4 h. Cell cultures were collected and
118 disrupted by ultrasound. The soluble fusion protein was purified by Ni Sepharose 6 Fast Flow

119 (GE Healthcare, USA) following the manufacturer's protocol. Polyclonal antiserum was raised
120 by immunizing rabbits with the purified fusion protein and then polyclonal anti-Bt56 antibody
121 was purified on protein A agarose from antiserum.
122
123 **FISH and IHC.** To localize the transcript of *Bt56* in individual whitefly tissues, FISH was
124 performed using a short oligonucleotide probe for *Bt56* (5'- Cy3 -
125 AACGCGCTTGAAAGCTTCAGCA - 3'). PSG, midgut and ovary were dissected from
126 whitefly adults in phosphate-buffered saline (PBS) and used for FISH assays as described
127 previously (6). Short oligonucleotide probe for GFP (5'-Cy3-
128 ACAAGACCCGCGCCGAGGTG - 3') and no probe were used as controls. The location of
129 protein Bt56 in PSG, midgut and ovary was detected by immunohistochemical (IHC) staining
130 as previously described (7).
131
132 **Sample preparation for whitefly- infested plants, whitefly body parts and whitefly saliva.**
133 Approximately 5000 whiteflies were released to feed on each cotton and tobacco plant for 24 h
134 and then adults and eggs were removed carefully from each leaf. Whitefly-infested plant leaves
135 and whiteflies were harvested separately and homogenized in liquid nitrogen. Approximately
136 500 head and thorax parts and abdomen parts were dissected from whitefly adults and
137 homogenized in a FastPrep-24 Instrument (MP Biomedicals Inc.). The protein samples were
138 extracted using RIPA lysis buffer (Beyotime, China). After centrifuging at 12,000 g for 10 min
139 at 4 ℃, the supernatant was filtrated through a 0.45-μm membrane and then concentrated using
140 Amicon Ultra 3-kDa centrifugal filter device (Millipore).
141 To collect whitefly saliva, *ca* 40,000 whiteflies were transferred to the artificial diet
142 feeding device (5) for 24 h in climate chambers as described above. Resorcinol is a

143 neurostimulant that has been used successfully to enhance aphid salivation (8). In order to
144 collect more whitefly salivary proteins, 0.4% resorcinol diet was used (1 ml per device with 400
145 whitefly adults). Approximately 100 ml saliva-containing diet (from 100 feeding devices) was
146 collected after feeding, and concentrated to 30 μl using Amicon Ultra 3-kDa centrifugal filter
147 device. After adding 5×SDS loading buffer to the concentrated proteins, the protein samples
148 were analyzed by SDS-PAGE.
149
150 **Western blot.** To detect Bt56 protein in whitefly body parts, whitefly-infested plant leaves and
151 whitefly saliva, western blot was performed using purified anti-Bt56 polyclonal antibody. The
152 protein samples (*ca* 100 μg protein extracted from whitefly and plant, and 5 μg collected saliva
153 from 40,000 whiteflies) were isolated by 15% SDS-PAGE and transferred onto a PVDF
154 membrane. PVDF membrane was blocked with 5% instant nonfat dry milk for 2 h at room
155 temperature, and then incubated with purified polyclonal antibody (1:500 dilution) overnight.
156 The antigen-antibody complexes were visualized using a goat anti-rabbit IgG-conjugated
157 horseradish peroxidase (HRP) antibody (MultiSciences Biotech) at a 1:10,000 dilutions and
158 detect using a ECL Plus Detection System (Bio-Rad, Hercules, CA, USA) with the Molecular
159 Imager® ChemiDoc™ XRS System (Bio-Rad, Hercules, CA, USA). The monoclonal anti-Vg
160 antibody (5, 9) was used to detect whitefly vitellogenin protein and the goat anti-mouse IgG-
161 conjugated HRP was used as secondary antibody (MultiSciences Biotech).
162
163 **Analysis of whitefly feeding behaviors by electrical penetration graph (EPG).** The feeding
164 behaviors of *Bt56*-silenced whiteflies on cotton were monitored using an 8-channel DC-EPG
165 device (EPG systems, Wageningen University, The Netherlands) in an electrically grounded
166 Faraday cage in a temperature-controlled room (27±1 ℃). The *dsGFP*- and *dsBt56*-fed

167 whiteflies were released onto cotton for three days and then used for EPG assays. A gold wire,
168 2-cm long and 12.5-μm in diameter, was glued to the dorsum of the whitefly using a water-
169 soluble silver conductive paint (Colloidal Silver; Ted Pella, Inc.). The other end of the wire was
170 connected to the Giga-8 DC EPG amplifier through the EPG probe. Whiteflies glued to the gold
171 wire were carefully moved to the abaxial surface of cotton leaves. The plant electrode was a
172 hard copper wire inserted into the soil. The EPG waveforms were analyzed with PROBE 3.4
173 (Wageningen University, The Netherlands). A total of 76 *dsBt56*-fed whiteflies and 35 *dsGFP*-
174 fed whiteflies were used for EPG assays. Only the data from whiteflies achieving at least one
175 phloem ingestion period were used for parameter analysis (Table S1).
176
177 **Phylogenetic analysis.** The alignment of KNOX proteins was performed by Clustal Omega
178 (https://www.ebi.ac.uk/Tools/msa/clustalo) and phylogenetic trees were constructed by
179 MEGA5 software (neighbor-joining method) using bootstrap values performed on 1,000
180 replicates and the 50% value was accepted as an indicator of a well-supported branch. BLR
181 (accession no. AAP93641) and BEL1 (AAM62510) were used to create an out-group. The
182 KNOX proteins accession numbers were: STM (Q38874), KNAT1 (AAM03027), KNAT2
183 (P46640), KNAT3 (P48000), KNAT4 (P48001), KNAT5 (P48002), KNAT6 (NP_850951),
184 KNAT7 (NP_564805), PpKNOPE3 (DQ786755), MtKNOX3 (ABO33480), MtKNOX4
185 (ABO33481), MtKNOX5 (ABO33482), MdKNAP3 (Z71980), NTH20 (AB025714), NTH22
186 (AB025715), NtH23 (BAA25921), NtH201 (BAF95776), Osh45 (BAA08552), LeT12
187 (AAC49918).
188
189 **Laser capture microdissection (LCM) and phloem sap collection.** The samples were fixed
190 and embedded as described previously (10). Tobacco leaves were fixed in 4%

191 paraformaldehyde under reduced pressure overnight. The samples were then immersed in
192 progressively more concentrated sucrose solution (5%, 10%, 20%, 30%) for 1 h each at room
193 temperature for dehydration, and then immersed in 30% sucrose solution: OCT compound
194 (SAKURA) (1:1 v/v) for 1 h. Finally, the samples were embedded in OCT compound and
195 frozen in -80 °C for 30 min. Laser capture microdissection was performed using Arcturus^{XT}
196 Laser Capture Microdissection (ThermoFisher) system and RNA was extracted using PicoPure
197 RNA Isolation Kit (Arcturus) following the manufacturer's instructions. Phloem sap collection
198 was performed as previously described (11) and RNA was extracted using Trizol reagent. RNA
199 extracted from phloem cells were reverse transcribed to the 1st-strand cDNA. RT-PCR was
200 performed to detect *NTH202* gene. *Altered phloem development* (*APL*, NM_001326017) gene
201 and *thioredoxin H* (*TRXH*, XM_016612627.1) gene are known to be two phloem-specific
202 genes, and *ribulose bisphosphate carboxylase* (*Rubisco*, XM_016626460.1) gene has
203 previously been used as a marker of contamination by non-phloem tissue (12-14).
204
205 ***NTH202* promoter - GUS fusion, plant transformation and GUS staining.** To analyze the
206 *NTH202* promoter activity, a 1600-bp genome DNA sequence ahead of the *NTH202* first exon
207 was amplified using the primer (*NTH202* promoter F:
208 GGAATTCAGATGATAACCATAAATGCCCCGGTT and *NTH202* promoter R:
209 CATGCCATGGGGCTAATATGGCTACTATAGAGGAGT). *NTH202* promoter was cloned
210 in pCAMBIA1301 to generate a *NTH202* promotor::GUS recombinant vector. The recombinant
211 vector was validated by sequencing and transformed into *A. tumefaciens* strain GV3101. Two
212 days after *Agrobacterium*-mediated transient expression, the infiltrated leaves were collected
213 for detecting GUS activity as described (15).
214

215 **Protein-protein interaction assays.** For *N. tabacum* cDNA library construction, total RNA
216 was extracted from tobacco leaves infested by whiteflies at 0, 1, 2 and 3 days. The mixed RNA
217 samples were sent to TAKARA (Dalian, China) for library construction. Y2H screen was
218 performed using the MATCHMAKER Gold Yeast Two-Hybrid System (Clontech) following
219 the manufacturer's protocol. *In vitro* GST pull-down assay was performed with GST- and MBP-
220 fusion proteins as described (16). *In planta* protein interactions were investigated using BiFC
221 assays in a *N. benthamiana* transient expression system as described, and βC1 was expressed
222 with SAHH as a positive control (17).
223

224 **VIGS assays.** To silence *NTH202* gene in tobacco, VIGS assays were performed (18). Briefly,
225 a 192-bp fragment (1029-1321 of *NTH202* gene, GenBank accession no. KY986874) was
226 cloned into *Xba*I-*BamH*I-digested pBIN2mDNA1 plasmid to generate the gene-silencing vector
227 2mDNA1-*NTH202*. After the 2mDNA1-*NTH202* vector was sequenced to confirm the fidelity
228 of the inserts, it was transformed into *A. tumefaciens* strain EHA105 by electroporation.
229 Tobacco curly shoot virus (TbCSV) was used as a helper virus in the VIGS assay. Equal
230 volume *A. tumefaciens* (OD600 = 0.6) carrying TbCSV construct and *A. tumefaciens* carrying
231 2mDNA1-*NTH202* construct were mixed and co-infiltrated into the stem and leaves of each
232 plant at the one-to-two true-leaf stage (VIGS-silenced plants). Control plants were co-infiltrated
233 with *A. tumefaciens* carrying the empty-vector (pBIN2mDNA1) and TbCSV. All plants were
234 grown in a greenhouse under the same conditions as described above and cultivated to the five-
235 to-six true-leaf stage. Then, VIGS-silenced and empty-vector control plants were used for
236 whitefly bioassays and phytohormone determination as described above.
237

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278

279 **Tables**

280 **Table S1: Comparison of EPG parameters between *dsBt56*- and *dsGFP*-fed whiteflies.**

EPG parameters	***dsBt56*** **(n=16) (Mean ± SE)**	***dsGFP*** **(n=16) (Mean ± SE)**	***P*** *
% of whiteflies realizing at least 1 phloem ingestion phase **	21.1% (16/76)	45.7% (16/35)	
Time to 1st probe (sec)	2444.9 ± 834.6	1574.0 ± 442.9	0.364
Number of probes (#)	64.8 ± 10.6	64.8 ± 7.1	0.996
Total probing time (sec)	12405.9 ± 820.9	15316.8 ± 789.9	0.016
Number of C periods (#)	67.7 ± 10.4	68.2 ± 7.4	0.969
Average C duration (sec)	141.2 ± 18.3	162.4 ± 21.4	0.457
Total time of C (sec)	7669.2 ± 729.8	9363.0 ± 675.4	0.099
Time to the 1st E1 (sec)	14769.2 ± 1790.4	14830.8 ± 1674.7	0.980
Number of E1 periods (#)	3.1 ± 0.5	3.3 ± 0.5	0.771
Average E1 duration (sec)	20.7 ± 5.3	24.0 ± 6.1	0.677
Total time of E1 (sec)	53.3 ± 11.2	69.7 ± 18.0	0.446
Number of probes before 1st sustained E2	45.1 ± 13.0	39.8 ± 10.1	0.749
Number of E2 periods (#)	2.7 ±0.4	2.9 ± 0.4	0.661
Average E2 duration (sec)	440.2 ± 76.3	1066.3 ± 245.2	0.021
Total time of E2 periods (sec)	977.7 ± 149.0	2354.5 ± 420.6	0.004
Mean duration of a sustained E2 (sec)	646.5 ± 118.2	1625.5 ± 402.2	0.026
Number of E phases (#)	5.8 ± 0.8	6.2 ± 0.9	0.714
Total duration of E phases (sec)	1031.1 ± 147.3	2424.2 ± 421.3	0.004

281 * Statistical significance was evaluated using one-way ANOVA.
282 ** The number of whiteflies realizing at least 1 phloem ingestion phase divide by the total number of
283 whiteflies used to perform EPG analyses.
284

285 **Table S2: Y2H screened proteins from tobacco library using Bt56 as bait.**

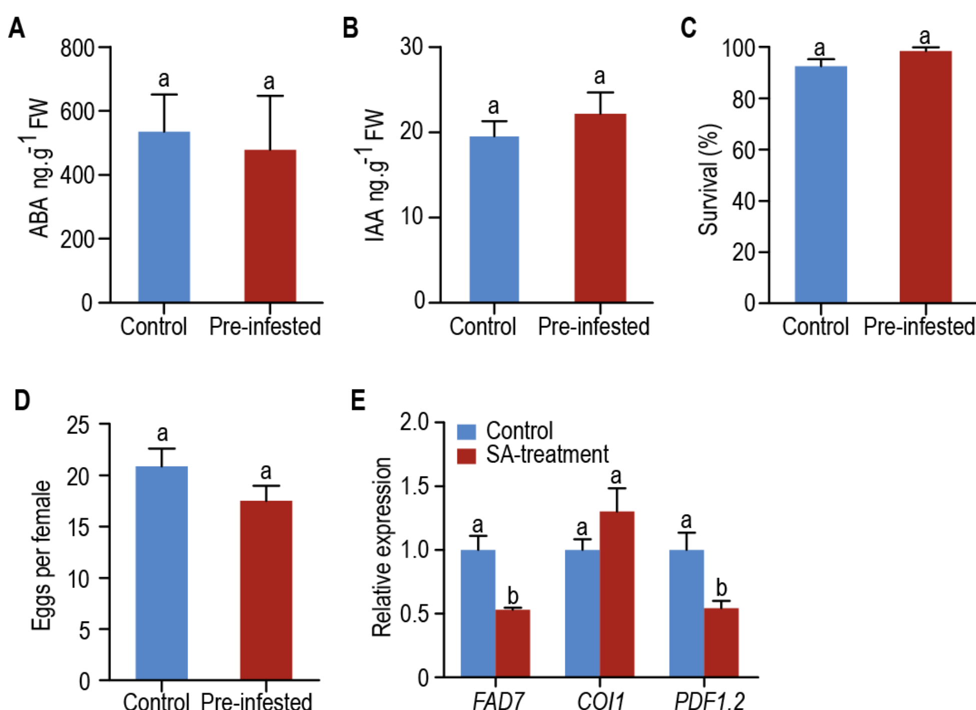
286

GenBank accession	Annotation
XM_016619856	Filament-like plant protein 3
XM_016642635	Protein NETWORKED 1D-like
XM_016592181	Protein FLX-like 2 (LOC107772699)
XM_016580083	Proline synthase co-transcribed bacterial homolog protein-like (LOC107761802)
XM_016626509	Myosin heavy chain, cardiac muscle isoform-like (LOC107802930),
KY986874	KNOTTED 1-like homeobox transcription factor

287 **Table S3: Primers used in this study.**

Primer	Gene/Application	Sequences (5'-3')	Modification
Bt56 F	Bt56/PCR	CCTCACTTTGCAGTTACGGTCCGAT	
Bt56 R	Bt56/PCR	AGGATTAGGTAATTTATTCACCGAT	
5’RACEGSP1	Bt56/5’RACE	CGGAGGCGGCATTCTTGATTGACTTGGC	
3’RACEGSP1	Bt56/3’RACE	TGCTGATCTACTGAGGGCACATGTTGAG	
MED1 Bt56 F	Bt56/ overexpression	GAGGATCCATGGGTGCTACAGAGAATC	BamH I
MED1 Bt56 R	Bt56/ overexpression	GAGAATTCATACAAATACATCGTAAATAAACAC	EcoR I
Asia II 3 Bt56 F	Bt56/ overexpression	GAGGATCCATGGGTGGTACAGAGATTCCT	BamH I
Asia II 3 Bt56 R	Bt56/ overexpression	GAGAATTCTTAGTTGGCCTTAAAGG	EcoR I
Bt56 T7 F	Bt56/ RNAi	TAATACGACTCACTATAGGGAGAAGAGATAGTAATT CACGAGCCT	T7 promoter
Bt56 T7 R	Bt56/ RNAi	TAATACGACTCACTATAGGGAGAAGTTGGCCTTAAA GGAAG	T7 promoter
GFP T7 F	GFP/ RNAi	TAATACGACTCACTATAGGCTCGTGACCACCCTGAC CTAC	T7 promoter
GFP T7 R	GFP/ RNAi	TAATACGACTCACTATAGGGTTCACCTTGATGCCGT TCTT	T7 promoter
Bt56 qRT F	qRT-PCR for MED1	TTGTCGGAGGTGCTACAGAG	
Bt56 qRT R		GTGCCCTCAGTAGATCAGCA	
qBt56 F	qRT-PCR for both	GGCGTCGTGGTGCTTGTAA	
qBt56 R	MED1 and Asia II 3	GGAATCCTCGGATCGTTTGA	
TAF qRT F	qRT-PCR	TGTGGGACACCCATTATCAG	
TAF qRT R	qRT-PCR	TGTGCAGCCAAGGAAATAAG	
q PAL F	qRT-PCR	AAGAAGCGTTCCGTGTTGCTG	
q PAL R	qRT-PCR	TCGGGCTTTCCATTCATCACC	
q NPR1 F	qRT-PCR	GCTGTAGCGTTCCTTGTTGA	
q NPR1 R	qRT-PCR	AGGCCTTATCAAGGGTTATG	
q PR1a F	qRT-PCR	GTGTAGAACCTTTGACCTGGGA	
q PR1a R	qRT-PCR	TTCGCCTCTATAATTACCTGGA	
q GAPDH F	qRT-PCR	GCAGTGAACGACCCATTTATCTC	
q GAPDH F	qRT-PCR	AACCTTCTTGGCACCACCCT	
Bt56-MED1 BK F	MED1 Bt56/ Y2H	GAGAATTCATGGGTGCTACAGAGAATC	EcoRI
Bt56-MEAM1 BK F	MEAM1 Bt56/ Y2H	GAGAATTCATGGGTGCTACAGAGATTCCT	EcoRI
Bt56-AsiaII1 BK F	AsiaII1 Bt56/ Y2H	GAGAATTCATGGGTGCTACAGAGATTCCT	EcoRI
Bt56-AsiaII3 BK F	AsiaII3 Bt56/ Y2H	GAGAATTCATGGGTGGTACAGAGATTCCT	EcoRI
Bt56-China2 BK F	China2 Bt56/ Y2H	GAGAATTCATGGAGATTCCTGACGCTGAT	EcoRI
Bt56-BK R	Bt56/ Y2H	GAGGATCCTTAGTTGGCCTTAAAGG	BamH I
GST-BT56 F	Bt56/ prokaryotic	GAGGATCCATGGGTGCTACAGAGAATC	BamH I
GST-BT56 R	expression	GAGAATTCTTAGTTGGCCTTAAAGG	EcoR I
MBP-NTH202F	NTH202/ prokaryotic	CGCGGATCCATGGCGTTTCAGGACCATTTTTCT	BamH I
MBP-NTH202R	expression	CCGGAATTCTCACTGCTTGATTTCACCTGCACT	EcoR I
Bt56-2Yc F	Bt56/ BiFC	CCCTTAATTAACATGGGTGCTACAGAGAATC	PacI
Bt56-2Yc R	Bt56/ BiFC	GGACTAGTTTAGTTGGCCTTAAAGG	SpeI
NTH202-2Yn F	NTH202/ BiFC	CCCTTAATTAACATGGCGTTTCAGGACCATTTT	PacI
NTH202-2Yn R	NTH202/ BiFC	GGACTAGTTCACTGCTTGATTTCACCT	SpeI
Bt56-GFP F	Bt56/ co-localization	GGGGTACCATGGGTGCTACAGAGAATCCT	KpnI
Bt56-GFP R		CGGGATCCGTTGGCCTTAAAGGAAGAGAAAG	BamHI
NTH202-CFP F	NTH202/ co-	GGGGTACCATGGCGTTTCAGGACCATTTTTC	KpnI
NTH202-CFP R	localization	CGCGGATCCCTGCTTGATTTCACCTGCA	BamHI
NTH202 VIGS F	KN1/ VIGS	GCTCTAGACTAAGTGGCCTTATCCAACT	XbaI
NTH202 VIGS R	KN1/ VIGS	CGGGATCCCCAAAAACTTAGTAGTTCAGT	BamH I
q NTH202 F	qRT-PCR	CGCGGATCCATGGCGTTTCAGGACCATTTTTCT	
q NTH202 F	qRT-PCR	CCGGAATTCTCACTGCTTGATTTCACCTGCACT	
APL F	PCR	ATGTTCCATGCCAAGAAACCTTCAACT	
APL R	PCR	TTACCCGAAAACAGAGTTTCGTCCACC	
Rubisco F	PCR	GTGGGCAACTATGCAATGACC	
Rubisco R	PCR	TAATTGGTGGCCACACCTGC	
TRXH F	PCR	ACTGAAGACTGTTGCGGAGG	
TRXH R	PCR	TGCACAAATCAGATTCCAAGCA	
NTH202 promoter F	Promoter	GGAATTCAGATGATAACCATAAATGCCCCGGTT	EcoRI
NTH202 promoter R	Promoter	CATGCCATGGGGCTAATATGGCTACTATAGAGGAGT	NcoI

288



289 **Fig. S1. The levels of ABA and IAA in whitefly-infested tobacco plants.** Three days after

290 whitefly infestation, the mean levels of ABA (*A*) and IAA (*B*) in control and pre-infested

291 tobacco plants were measured (n = 6). (*C-D*) Whitefly pre-infestation tests were performed

292 using NahG tobacco (a transgenic plant that does not accumulate SA). Whiteflies were allowed

293 to feed on whitefly pre-infested and uninfested control NahG tobacco plants for three days. The

294 survival (*C*) and fecundity (*D*) of whiteflies were examined. Each treatment included 6 plants

295 and each plant had 2 clip cages (n = 12). (*E*) Expression levels of defense-marker genes in JA-

296 signaling pathway of control and SA-treated tobacco plants (n = 6). Data shown are mean ±SE.

297 Letters above the bars indicate significant difference $P < 0.05$ (Nested ANOVA for whitefly

298 bioassays and one-way ANOVA for other experiments).

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1       AGCGATTAGATCGCCAAATAATCGTAGAATTATCGGAACTCAATTTCAAAAAATAGCAAT
61      TTTACTGTTGGAAAATTCTGCTTGGGTACTAGCCGTTATATTTTACCCAAAAAAAAATGC
121     ACAAAACAATTATTATGTATTAGACTTTAAAAACACACCAATTTCCATGTTTTTTTCGAG
181     CGCCCCTGAACACTTTTCTTTCTGCCGTCGCATCACCTGTATCTTTGCGTGATTCGATTT
241     AGTGCGCACTGATGTCATGCAATCGACAGTCCGAACTTAATCACTCCCCGCAGATTTTTT
301     AGTTCGCATGGAAAATACTATATTTCTGACGATATTGGCCACGCCAGACTTTTGAACGAG
361     CGTCATACAATATGCCTTGCAGACGAGTACCCATGTTTGCCCCAATCAGGAAGGGGGGGG
421     GGGGAAGAAATGCACGTCCTTGGATGATGTCAGCACGCAAGGAGCAAAATTGAATTTTAG
481     CAGAACATTGAATTGATACGCCTCACTTTGCAGTTACGGTCCGATGTTCCCTCGAAAAAT
                                                       M  F  P  R  K  M
541     GCATTCTAGCGGGTTGATTGCTTTTGGCGTCGTGGTGCTTGTAACAAGCGTTGTCGGAGG
         H  S  S  G  L  I  A  F  G  V  V  V  L  V  T  S  V  V  G  G
601     TGCTACAGAGAATCCTGACGCTGATAAAGAGATAGTAATTCACGAGCCTGCTGAAGCTTT
        ↑A  T  E  N  P  D  A  D  K  E  I  V  I  H  E  P  A  E  A  F
661     CAAGCGCGTTATTAACTATGCTGATCTACTGAGGGCACATGTTGAGAACCTGCGTCAAAC
         K  R  V  I  N  Y  A  D  L  L  R  A  H  V  E  N  L  R  Q  T
721     GATCCGAGGATTCCTGCAAAACAGCGTAACCGTAATGGACACGACCAGGAACATTTTGAG
         I  R  G  F  L  Q  N  S  V  T  V  M  D  T  T  R  N  I  L  R
781     AACCGAGAAAAAGCAAGCCAAGTCAATCAAGAATGCCGCCTCCGAGGCCATTAGGTCTTT
         T  E  K  K  Q  A  K  S  I  K  N  A  A  S  E  A  I  R  S  F
841     CTCTTCCTTTAAGGCCAACTAATAAAATTGCATGGAGGTGTACATGTTAACATCTAGAGA
         S  S  F  K  A  N  *
901     ATGGAAGTTATAATACTTATACCTACAGATTGTGTTTATTTACGATGTATTTGTATTTTA
961     AATCGGTGAATAAATTACCTAATCCTGAAACTAGAAAAAAAAAAAAAAAAAAAA
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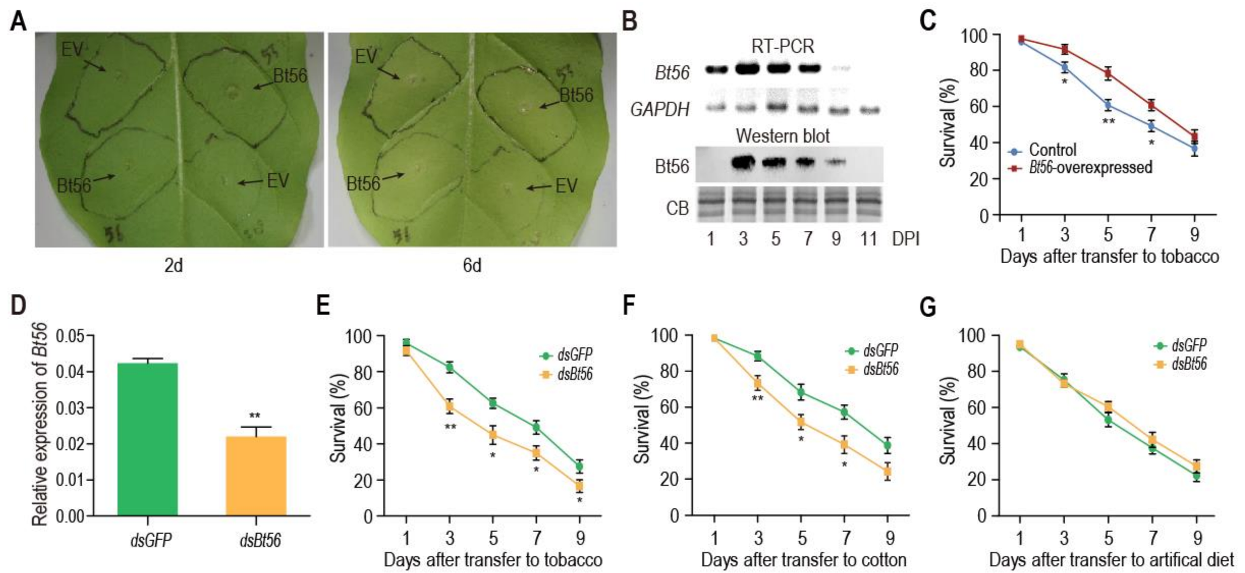
299 **Fig. S2.** Nucleotide sequence of transcript *Bt56* and deduced amino acid sequence of protein

300 Bt56. The underline shows the signal peptide predicted by SignalP-HMM and SignalP 4.1, and

301 the arrow shows the most likely signal peptide cleavage site predicted by SignalP-NN.



302 **Fig. S3.** Overexpression and silencing of *Bt56* gene. (*A*) Overexpression of *Bt56* by

303 agroinfiltration did not induce chlorosis or cell death in *N. tabacum* two and six days after

304 agroinfiltration. EV, empty vector. (*B*) Semi-quantitative RT-PCR on RNA and western blot on

305 proteins from *N. tabacum* transiently overexpressing *Bt56* at different time points. The plant

306 *GAPDH* gene was used as an internal control. Coomassie blue (CB) staining was used to

307 confirm equal loading. DPI, days post infiltration. (*C*) Survival ratio of whiteflies feeding on

308 *Bt56*-overexpressed tobacco for 9 days. Each treatment included 6 plants and each plant had 2

309 clip cages (n = 12). (*D*) The efficiency of RNAi by feeding *dsRNA*. After whiteflies had fed on

310 15% sucrose solution containing 200 ng *dsBt56* or *dsGFP* for 48 h, the transcript levels of *Bt56*

311 were examined by qRT-PCR (n = 3). (*E-G*) Survival ratio of whiteflies feeding on tobacco

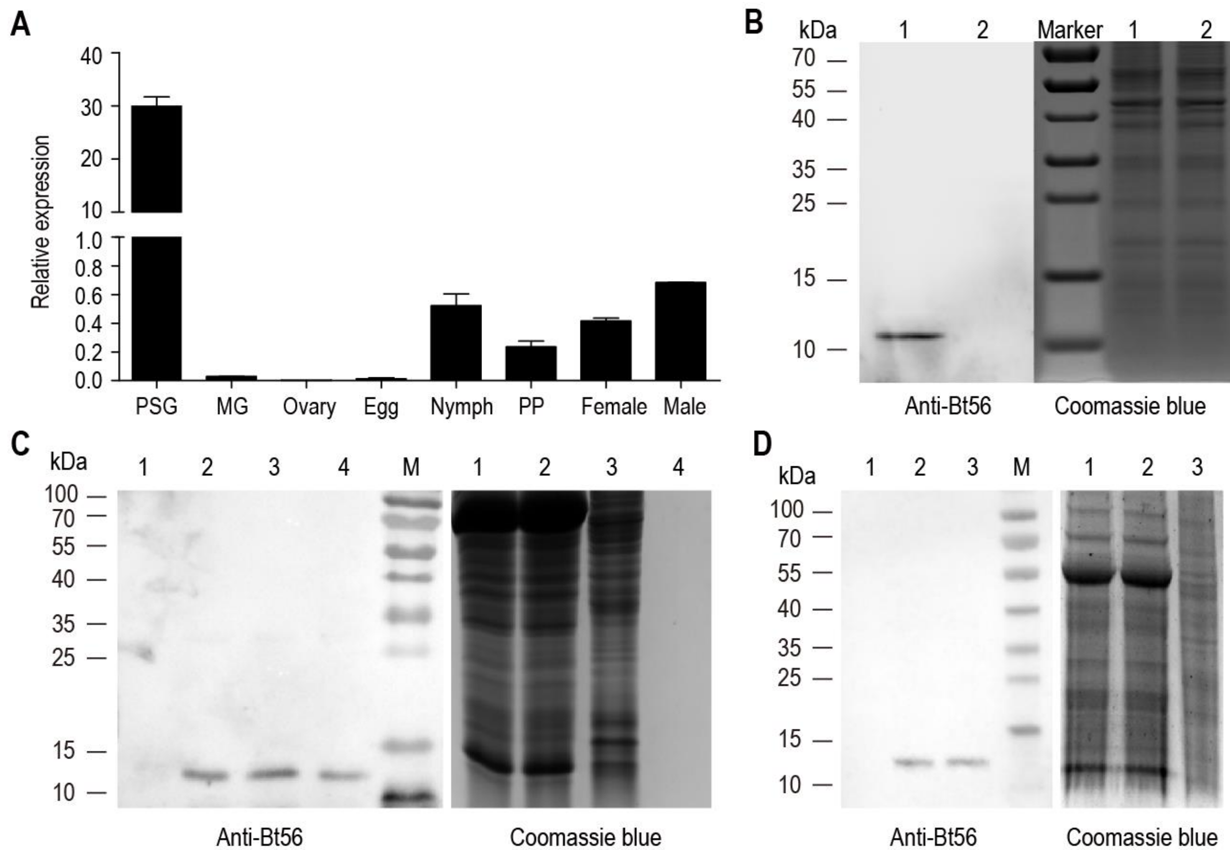
312 plants (*E*), cotton plants (*F*), and artificial diet (*G*) for 9 days after they started to feed on each

313 of the substrata; the artificial diet contained *dsGFP* or *dsBt56*. Each treatment included 6 plants

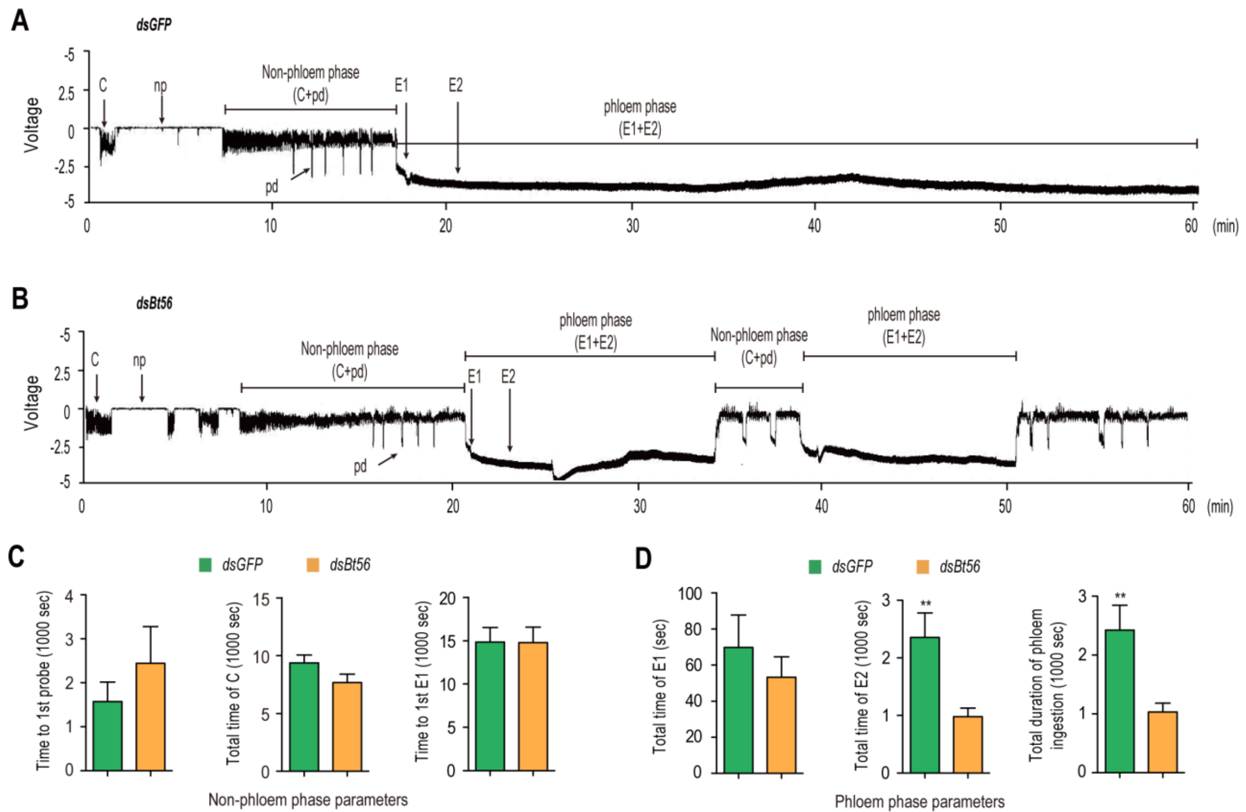
314 and each plant had 2 clip cages (n = 12). Values shown are mean ±SE, nested ANOVA was

315 performed for whitefly bioassays and one-way ANOVA for other experiments, LSD test, *$P <$

316 0.05, **$P < 0.01$.



317 **Fig. S4.** *Bt56* gene expression patterns and protein localization. (*A*) qRT-PCR analysis of *Bt56*
318 gene expression in different tissues and whiteflies of different developmental stages (n = 3).
319 Values shown are mean ± SE of three independent experiments. PSG, primary salivary gland;
320 MG, midgut; PP, pseudopupa are whiteflies in the late 4th instar with red eyes. (*B*) Polyclonal
321 anti-Bt56 antibody specifically recognizes Bt56 in whitefly. Western blot analyses were
322 performed to detect Bt56 protein using protein extracts form whitefly head and thorax (lane 1)
323 and abdomen (lane 2). (*C* and *D*) Bt56 is a saliva protein and can be secreted into the plant
324 during whitefly feeding. Western blot analyses were performed to detect Bt56 protein using
325 protein extracts from cotton (*C*) and tobacco (*D*). Lane 1, uninfested plant leaf; lane 2, whitefly-
326 infested plant leaf; lane 3, whitefly adults; lane 4, whitefly saliva. Coomassie blue staining was
327 used to confirm equal loading in lane 1 and lane 2.



328 **Fig. S5.** Silencing *Bt56* impairs whitefly feeding. Typical EPG waveforms of *dsGFP* (*A*) and

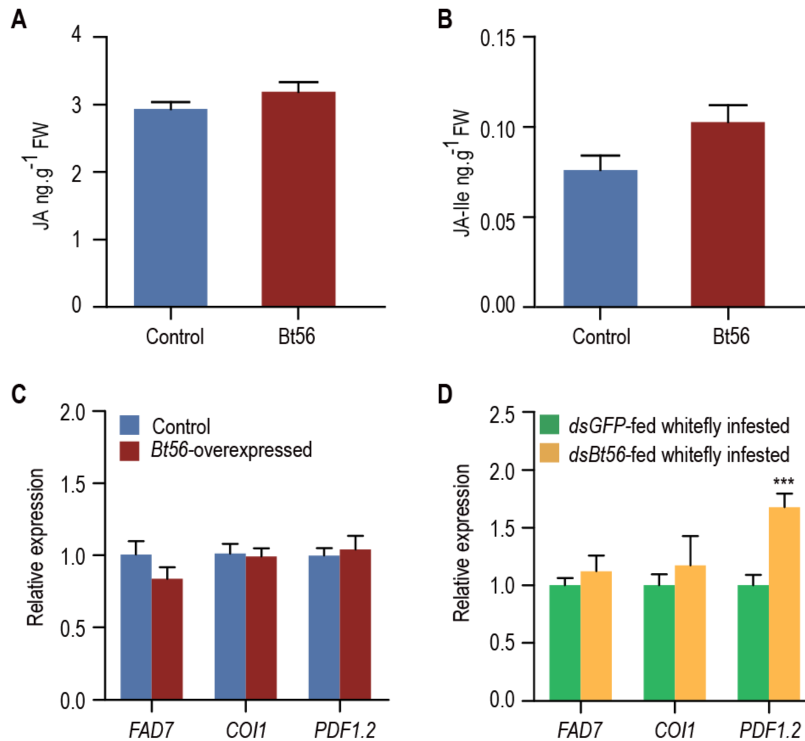
329 *dsBt56*-fed (*B*) whiteflies on cotton. C, pathway phase; np, nonprobing; pd, potential drop; E1,

330 watery salivation; E2, passive phloem ingestion. Comparison of EPG parameters from the non-

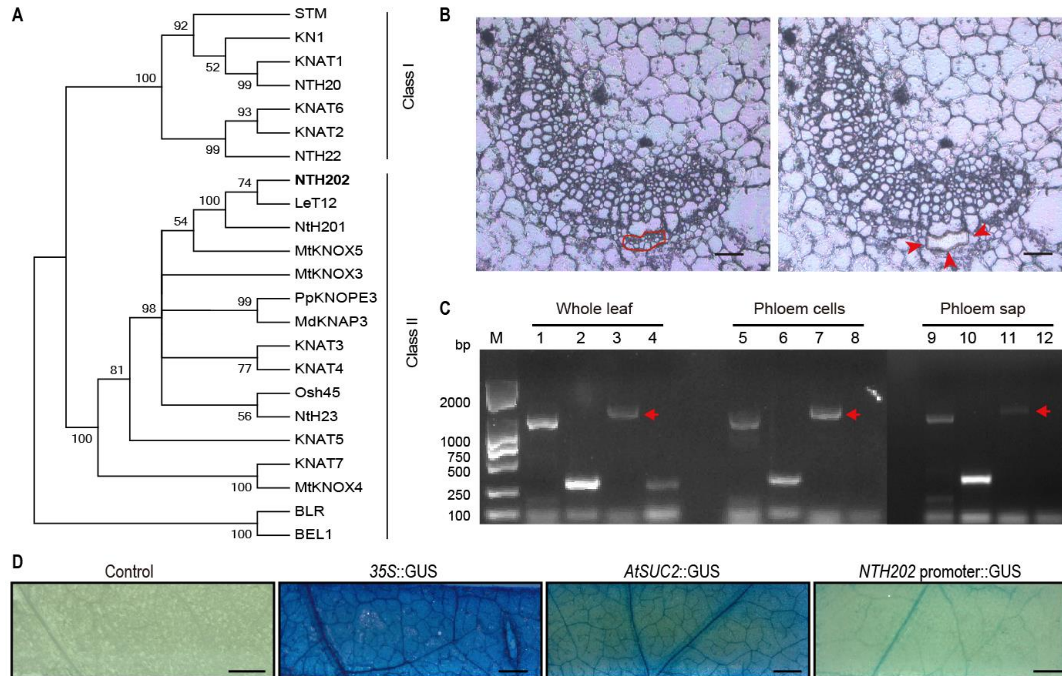
331 phloem phase (*C*) and phloem phase (*D*) in *dsGFP*- and *dsBt56*-fed whiteflies. Values are

332 reported as mean + SE, n = 16. All parameters are listed in Table S1. One-way ANOVA, LSD

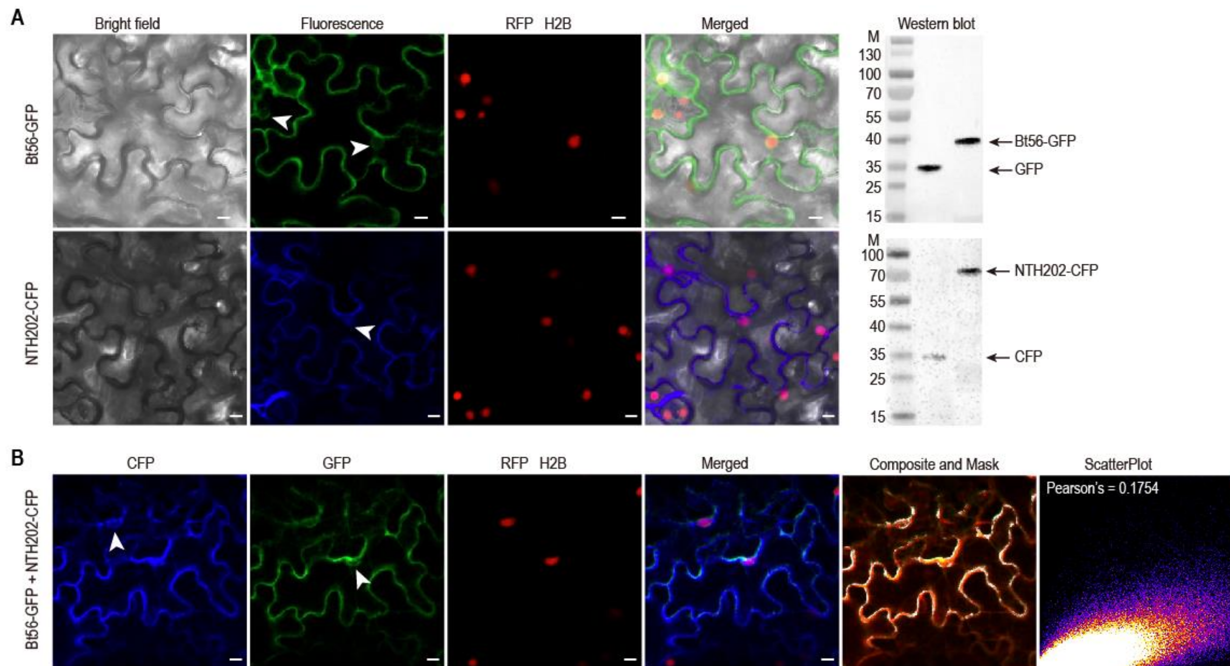
333 test, ** $P < 0.01$.



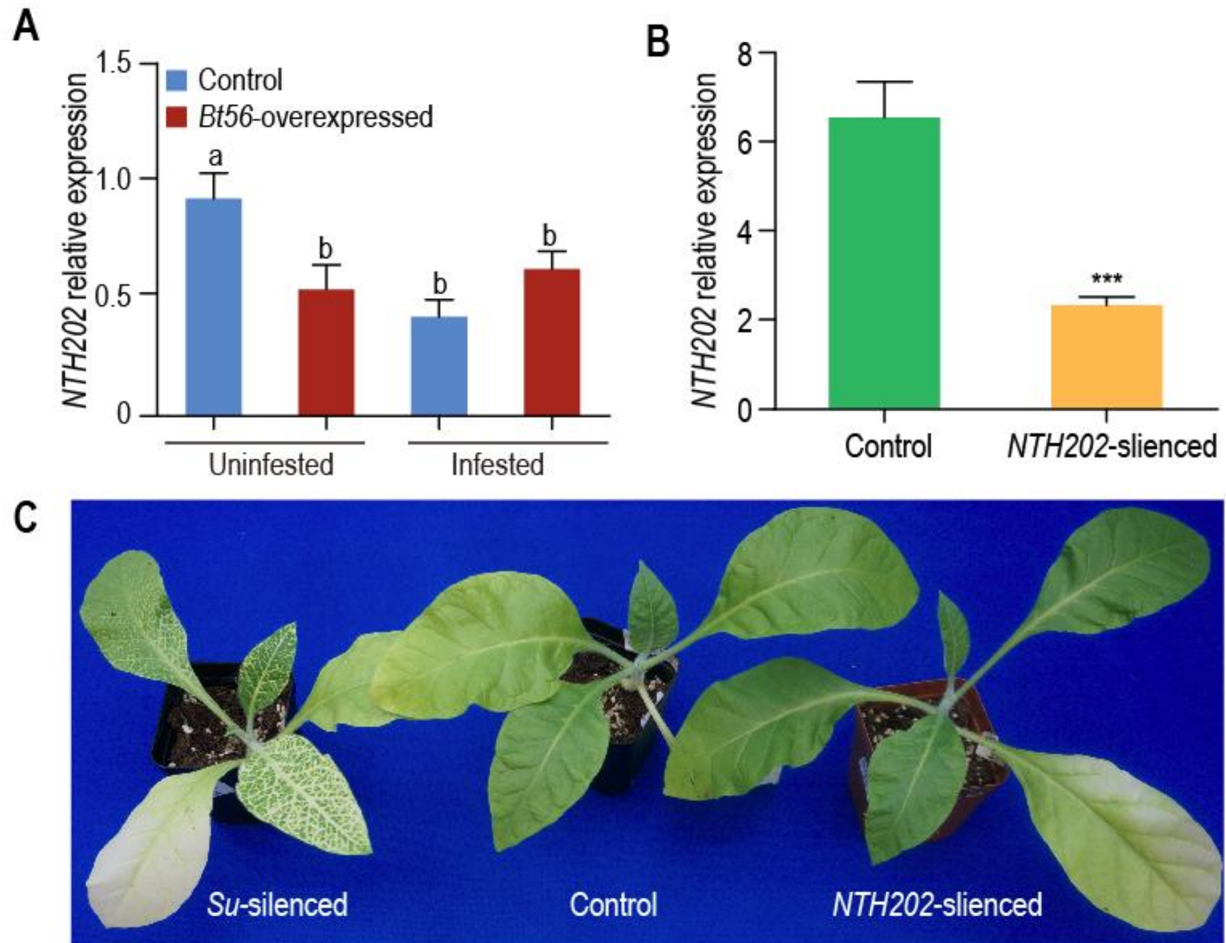
334 **Fig. S6. The effect of Bt56 on tobacco JA-signaling pathway.** (*A*-*B*) The effect of
335 overexpressing Bt56 on tobacco JA and JA-Ile levels. The levels of JA (*A*) and JA-Ile (*B*) were
336 measured 48 h after agroinfiltration. (*C*) The expression levels of JA defense-marker genes in
337 tobacco with overexpression of *Bt56* were measured 48 h after agroinfiltration. (*D*) The effect
338 of infestation by *Bt56*-silenced whiteflies on tobacco JA-signaling pathway. The expression
339 levels of JA defense-marker genes were measured three days after infestation by *dsGFP*- and
340 *dsBt56*-fed whiteflies. Data shown are mean ± SE, n = 12 for gene expression on *Bt56*
341 overexpressed tobacco and n = 8 for *dsRNA*-fed whitefly infested tobacco. One-way ANOVA,
342 LSD test, *** $P < 0.001$.



343 **Fig. S7. Phloem expression of *NTH202* in tobacco leaf.** (*A*) Phylogenetic tree of NTH202
344 protein sequences. Tobacco NTH202 protein in this study is shown in bold. (*B*) Laser capture
345 microdissection from tobacco leaf sections. Left, before microdissection; right, after
346 microdissection. Redline and arrowheads indicate the location of phloem cells captured. Bar,
347 100 μm. (*C*) RT-PCR products from RNA extracted from whole leaf, LCM-captured phloem
348 cells and phloem sap. Expression of the *NTH202* gene (lane 3, 7 and 11, red arrows indicate the
349 band) could be detected in whole leaf, phloem cells and phloem sap. *APL* (lane 1, 5 and 9) and
350 *TRXH* (lane 2, 6 and 10), the phloem-specific genes, are used as marker of phloem tissue, and
351 *Rubisco* (lane 4, 8 and 12) is used as a marker of contamination by non-phloem tissue (10-12).
352 (*D*) *NTH202* promoter activity assays using GUS as a reporter gene. The *NTH202*
353 promoter::GUS activity can be detected in the vascular tissues of tobacco leaves 2 days after
354 *Agrobacterium* mediated transient expression. Empty *Agrobacterium* used as negative control,
355 *CMV 35S* promoter and *Arabidopsis* phloem-specific promoter *AtSUC2* used as positive
356 control. Bars = 1 mm.



357 **Fig. S8. Co-localization of Bt56 and plant NTH202 transcription factors.** (*A*-*B*) Constructs
358 containing NTH202 fused with CFP and Bt56 fused with GFP driven by 35S promoter were
359 infiltrated into *N. benthamiana* leaf by agroinfiltration respectively (*A*) or simultaneously (*B*).
360 Two days after infiltration, excised leaves were observed under a confocal microscope. Western
361 blots show the presence of full-length fusion proteins. White arrowheads indicate nuclear
362 localization. The experiments were repeated three times and a total of 18 images were analyzed
363 for each treatment. The colocalization level was analyzed by ImageJ (http://imagej.net/). The
364 scattered plot represents the colocalization level in the composite mask. Co-localization
365 between Bt56-GFP and NTH202-CFP was quantified as 0.231 ± 0.017 (Pearson's coefficient,
366 mean ± SE. n = 18 images). Scale bar = 10 μm.



367 **Fig. S9. *NTH202* gene expression in tobacco plants of different treatments.** (*A*) Relative

368 gene expression of *NTH202* in control, *Bt56*-overexpressed, whitefly-infested control and

369 infested *Bt56*-overexpressed *N. tabacum* plants (n = 7). (*B*) Relative expression levels of

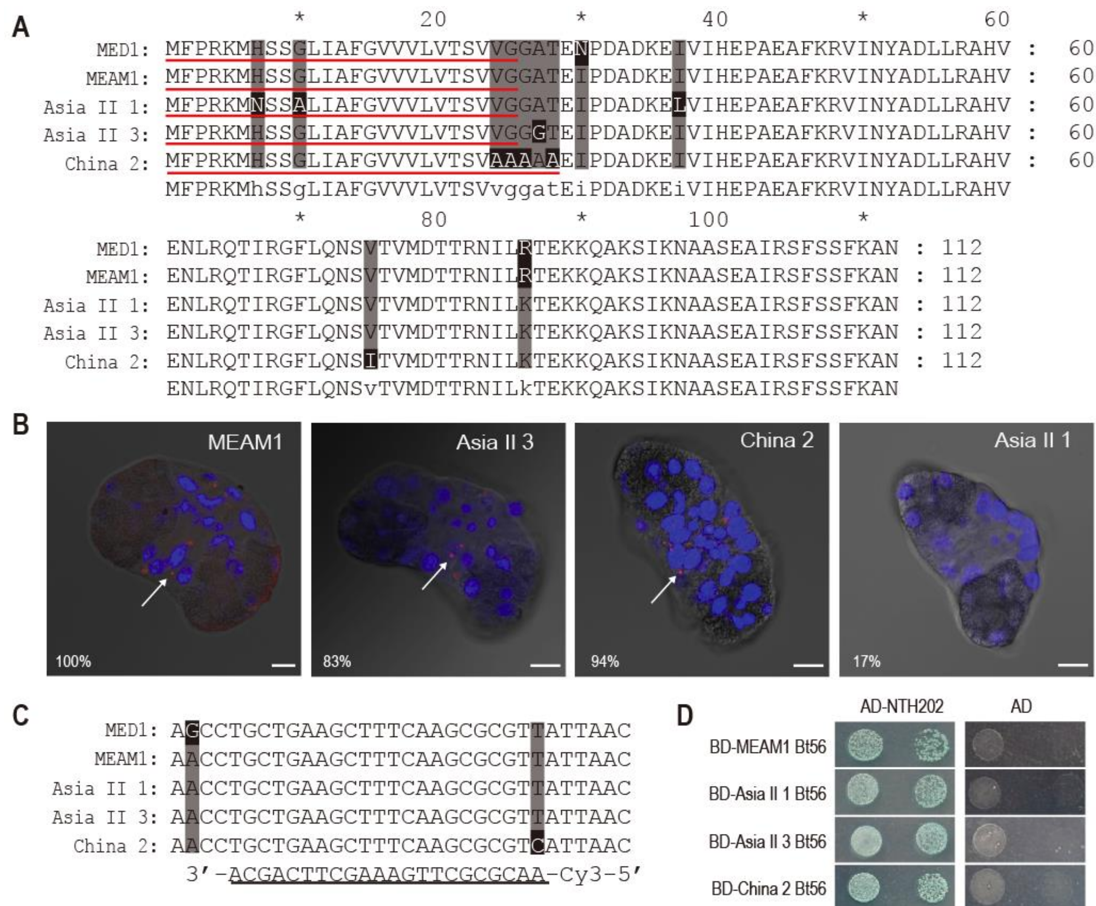
370 *NTH202* in control and *NTH202*-silenced *N. tabacum* plants (n = 6). Values shown are means ±

371 SE, One-way ANOVA followed by LSD test. Letters above the bars indicate significant

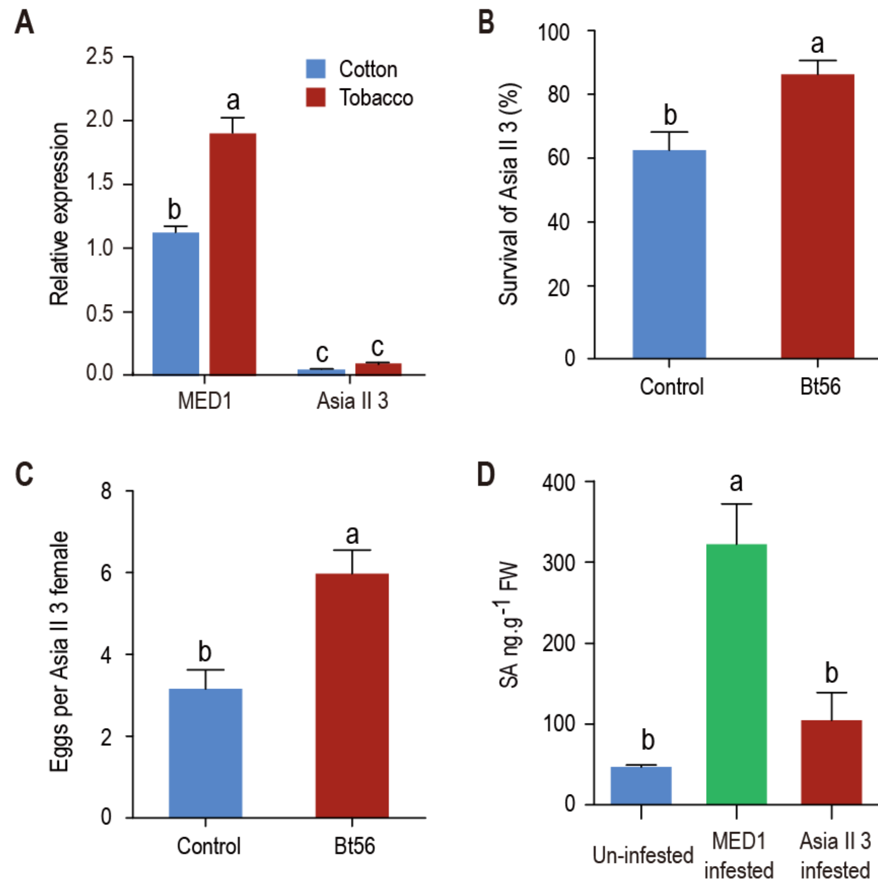
372 difference $P < 0.05$, *** $P < 0.001$. (*C*) VIGS to suppress *NTH202* gene in *N. tabacum*.

373 Tobacco endogenous gene *Su* (*Sulphur desaturase*) was silenced by VIGS as a positive control

374 to visualize the silencing process, and empty vector used as negative control.



375 **Fig. S10. Sequence analysis and location of *Bt56* genes in different whitefly species.** (*A*)
376 Alignment of protein sequence of Bt56 in MED1, MEAM1, Asia II 1, Asia II 3 and China 2
377 whiteflies. The predicted signal peptides are shown in red underline and the shaded areas
378 indicate the differential amino acids. (*B*) Fluorescence *in situ* hybridization (FISH) to detect the
379 transcripts of *Bt56* in different whitefly species. The percentages shown on the image represent
380 the ratios of positive signals from 18 dissected PSGs. Arrows indicate the fluorescence signals.
381 (*C*) Alignment of the probe sequence to the five *Bt56* genes. The underline shows the probe
382 sequence. The mismatches are highlighted in shading. (*D*) Bt56 from different whiteflies
383 interact with NTH202 in the yeast two-hybrid system. Yeast strain Y2H Gold co-transformed
384 with the indicated plasmids was spotted on quadruple dropout medium (SD/–Ade/–His/–Leu/–
385 Trp supplemented with X-α-Gal and Aureobasidin A).



386 **Fig. S11. Expression level of *Bt56* affects whitefly performance on host plants.** (*A*) The
387 expression patterns of *Bt56* in MED1 and Asia II 3. Whiteflies were transferred from cotton to
388 cotton and cotton to tobacco separately. After 24 h, the whiteflies were collected for gene
389 expression analysis by qRT-PCR (n = 3). (*B*-*C*) The effects of overexpression of Asia II 3 *Bt56*
390 gene in tobacco on the performance of Asia II 3. Each treatment included 8 plants and each
391 plant had 2 clip cages (n = 16). (*D*) When tobacco was infested by MED1 or Asia II 3
392 whiteflies for three days, the level of SA was measured in plants (n = 5-6). Data shown are
393 mean ± SE; letters above the bars indicate significant differences among different treatments at
394 $P < 0.05$ (Nested ANOVA for whitefly bioassays and one-way ANOVA followed by LSD test
395 for other experiments).