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1 **Molecular basis of methyl salicylate-mediated plant airborne defense**

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21 **Abstract**

22 **Aphids transmit viruses and are destructive crop pests¹. Aphid-attacked plants**
23 **release volatile compounds to elicit airborne defense (AD) in neighboring plants²⁻**
24 **⁵. However, the mechanism underlying AD is unknown. Here, we reveal methyl-**
25 **salicylate (MeSA), salicylic-acid (SA)-binding protein-2 (SABP2), transcription**
26 **factor NAC2, and SA-carboxymethyltransferase-1 (SAMT1) form a signaling**
27 **circuit to mediate AD against aphids and viruses. Airborne MeSA is perceived and**
28 **converted to SA by SABP2 in neighboring plants. SA then cascades signal**
29 **transduction to activate the NAC2-SAMT1 module for MeSA biosynthesis to**
30 **induce plant anti-aphid immunity and reduce virus transmission. To counteract,**
31 **some aphid-transmitted viruses encode helicase-containing proteins to suppress**
32 **AD by interacting with NAC2 to subcellularly re-localize and destabilize NAC2.**
33 **Consequently, plants become less aphid-repellent, but more suitable for aphid**
34 **survival, infestation, and viral transmission. Our findings uncover mechanistic**
35 **basis of AD and a previously undefined aphid-virus co-evolutionary mutualism,**
36 **demonstrating AD as a potential bioinspired strategy to control aphids and**
37 **viruses.**

38 **Main**

39 Plants have evolved the capacity to sense environmental stimuli and induce changes of
40 metabolism to generate volatile organic compounds (VOCs) as intra/interplant
41 signals^{2,6}. VOCs, once released from ‘VOC emitter’ plants, can serve as aerial cues and
42 be perceived to elicit defenses in neighboring ‘receiver’ plants, a phenomenon called

43 airborne defense (AD)²⁻⁵. Such plant-plant communication (PPC) and its biological and
44 ecological significance have been observed in many species over decades^{2,3}. Very
45 recently, UDP-arabinoxyltransferase is found to be involved in airborne volatile
46 reception in tomato distal defense against cutworms⁷. However, molecular genetic
47 framework for the VOC-mediated PPC including AD is largely unknown². Moreover,
48 with the exception of the receptor for ethylene⁸, the receptors for mediating VOC-
49 sensing system in plants have remained unidentified.

50 Aphids are the most destructive agricultural and horticultural pests worldwide¹. They
51 are phloem-feeding and cause extensive destruction to crop production due to their
52 efficient transmission of numerous devastating viral pathogens¹. More than 40%
53 viruses depend on aphids for transmission to infect plants including many food, fruit,
54 and vegetable crops⁹. Aphid-attack induces plant emitting VOCs which are mainly
55 composed of MeSA¹⁰⁻¹³. MeSA has been implicated in plant defense against
56 herbivorous insects including aphids by repelling, attracting predators, or reducing
57 survival fitness of these insects¹⁴⁻¹⁶. MeSA, known as a within-plant and long-distance
58 mobile signal, is involved in inducing systemic acquired resistance (SAR) to microbial
59 pathogens and herbivorous insects^{17,18}. During SAR, SA accumulates in pathogen-
60 infected cells and is converted to MeSA by SAMT1¹⁹; MeSA then travels to distal
61 tissues via the phloem and is subsequently reconverted to SA by SABP2 for SAR in
62 systemic leaves²⁰. Although MeSA as a within-plant signal for SAR is known, how
63 MeSA serves as an interplant communication signal to activate anti-aphid defense in
64 neighboring 'receiver' plants has been a long-standing and unsolved question^{2,10,16}. For

65 instance, whether plants possess receptor systems to sense and perceive airborne MeSA
66 is unclear³. Mechanism for priming MeSA production during aphid infestation is also
67 not illuminated. Furthermore, it remains to be elucidated whether and how aphids and
68 viruses can regulate AD although MeSA is reported to mediate AD against *Tobacco*
69 *mosaic virus* (TMV, a Tobamovirus in the family *Virgaviridae*)⁴. In this study, we have
70 exploited a pathosystem comprising aphid, virus, VOC emitter, and receiver plants to
71 dissect AD. We establish the framework for MeSA mediating AD to suppress insect
72 infestation and virus transmission at molecular and genetic level, and identified an
73 odorant-binding protein (OBP)-like receptor for sensing and perceiving airborne MeSA.
74 Further, we found an unexpected viral counterdefense strategy to suppress plant AD,
75 suggesting an undefined ecological and co-evolutionary mutualism between aphids and
76 aphid-transmissible viruses. Moreover, our findings lay the groundbreaking work to
77 provide the detailed mechanism of VOC-triggered PPC and will pave the way for future
78 studies on PPC and plant adaptation to environment.

79 **Plant antiviral defense requires NAC2**

80 Following our work on *Cucumber mosaic virus* (CMV), a Cucumovirus in the family
81 *Bromoviridae*²¹, we investigated the role of CMV 1a protein (designated CMV1a
82 hereafter) in viral pathogenesis. Through conducting immuno-pulldowns coupled with
83 mass spectrometry, we identified *Nicotiana benthamiana* (*Nb*) transcription factor (TF)
84 NAC2 as a CMV1a-interactor ([Extended Data Fig. 1a](#)). NACs constitute a large plant-
85 specific TF family, some of which participate in regulation of within-plant immunity²².
86 We searched the *Nb* genome database and found two *NAC2* homologs *NAC2.1* and

87 *NAC2.2* that share 96.7% nucleotide identity and should represent two alleles of one
88 gene from different ancestry of allotetraploid *Nb*. We focused mainly on *NAC2.1*
89 (designated NAC2 hereafter) for further analyses. The CMV1a-NAC2 interaction was
90 verified by co-immunoprecipitation (Co-IP), bimolecular fluorescence (BiFC), and
91 luciferase complementation imaging (LCI) assay (Extended Data Fig. 1b-d). We
92 evaluated whether *NAC2* affects CMV infection in *NAC2.1/NAC2.2* double-knockout
93 (KO) mutants (*nac2*) which were generated by CRISPR-Cas9 gene editing (Extended
94 Data Fig. 2a) versus (vs) wild-type (WT) plants. CMV infection resulted in severer
95 symptom and higher accumulation of viral RNA and coat protein (CP) in *nac2* than WT
96 plants (Extended Data Fig. 1e-g). Similar results were also obtained in *nac2* vs WT
97 plants infected by GFP-tagged *Potato virus Y* (PVY-GFP, a Potyvirus in the family
98 *Potyviridae*) or GFP-tagged TMV (TMV-GFP) (Extended Data Fig. 1h-m). These data
99 suggest that *NAC2* is essential for plant antiviral defense.

100 ***NAC2* mediates AD against aphids via MeSA**

101 By serendipity we noticed that many more apterous green peach aphids colonized on
102 *nac2* than WT leaves. This unexpected discovery led us to examine the role of *NAC2*
103 in plant attractiveness to aphids. We performed circular-dish and Y-tube olfactometer
104 bioassays, and found that *nac2* plants attracted more aphids (*Myzus persicae*, Sulzer)
105 than WT plants, likely mediated by an airborne signal(s) (Extended Data Fig. 1n, o). To
106 determine which volatile was alterably generated, we used GC-MS assay to identify
107 volatiles emitted by aphid-attacked WT vs *nac2* plants. MeSA was the only constant
108 VOC differentially produced in aphid-attacked WT vs *nac2* plants, and more MeSA

109 was emitted by WT than *nac2* plants after aphid infestation (Extended Data Fig. 2e-h).
110 Consistently, MeSA is a well-documented major or even the only aphid-inducible VOC
111 in certain plant species¹⁰⁻¹³. To test whether the effect of *NAC2* on plant attractiveness
112 to aphid is attributed to MeSA emission, we measured the emission rate of aerial MeSA
113 from aphid-attacked WT plants by GC-MS and found that aerial MeSA was emitted at
114 approx. 34 ng/h (equivalent to 0.816 µg/day) per aphid-attacked WT plant (Extended
115 Data Fig. 2f). Furthermore, we found that the aerial MeSA concentrations in chambers
116 containing either 0.8 µg MeSA-trapped lanolin paste (MeSA/lanolin) or an aphid-
117 attacked WT plant was similar (Extended Data Fig. 2i, j). Thus, we used 0.8 µg
118 MeSA/lanolin per chamber, which is equal to at approx. 3 nM for the following
119 experiments. After smearing plants with MeSA/lanolin, *nac2* and WT plants showed
120 similar attractiveness to aphids (Extended Data Fig. 1p, q). However, when treated with
121 lanolin alone or lanolin with other volatiles such as 3,3-dimethyl-hexane, *nac2* plants
122 were more attractive to aphids than WT plants (Extended Data Fig. 1r, s). We also kept
123 *nac2* and WT plants under volatile MeSA for 24 hours followed by 2-hour
124 ventilation, and compared how gaseous MeSA would affect plants to attract aphids.
125 Under such conditions, WT plants were more repellent to aphids (Extended Data Fig.
126 1t, u). However, no obvious difference in aphid repellence was observed between *nac2*
127 plants that were given with and without volatile MeSA followed by ventilation
128 (Extended Data Fig. 1v, w). Further, like that without MeSA treatment, *nac2* remained
129 more attractive to aphids than WT plants after volatile MeSA treatment followed by
130 ventilation (Extended Data Fig. 1x, y).

131 To decipher reason(s) behind the intriguing phenomenon (Extended Data Fig. 1t-y), we
132 treated *nac2* or WT plants with volatile MeSA for 24 hours followed by ventilation, and
133 then quantified volatized MeSA emitted by MeSA-receiving plants (receivers). WT, but
134 not *nac2* plants emitted higher level of airborne MeSA (Fig. 1a, b). We next compared
135 aphid attractiveness in MeSA-receivers vs mock WT plants after smearing all plants
136 with MeSA/lanolin, but found that they showed no difference in aphid preference (Fig.
137 1c, d). Moreover, after smearing both *nac2* and WT receivers with MeSA/lanolin, these
138 MeSA-receivers also appeared equally to attract aphids (Fig. 1e, f). We then used aphid-
139 attacked plants as emitters to unravel the role of *NAC2* in plant AD under natural open-
140 air environment (Extended Data Fig. 3a). After infestation by aphid sap-sucking, WT
141 plants constantly emitted VOC MeSA (Fig. 1g, h). Intriguingly, *nac2* receivers released
142 less volatile MeSA, but exhibited higher attractiveness to aphids than WT receivers
143 when emitters were attacked by aphids (Fig. 1i-l). Besides, WT receivers neighboring
144 aphid-attacked emitters were more repellent to aphids than those neighboring mock
145 emitters, while no significant difference in aphid repellence was found between *nac2*
146 receivers neighboring mock- and aphid-attacked emitters (Fig. 1m, n). Furthermore,
147 aphid-feeding in emitters reduced aphid survival in WT but not *nac2* receivers after 24
148 hours feeding in receivers (Fig. 1o, p). These results suggest that once MeSA is
149 perceived, MeSA biosynthesis in neighboring receiver plants is regulated in a *NAC2*-
150 dependent manner to mediate AD against aphids.

151 ***NAC2* activates *SAMT1* transcription**

152 We next set up to dissect the molecular and genetic link between *NAC2* and MeSA

153 biosynthesis. As a TF (Extended Data Fig. 3b), NAC2 was found to localize in nucleus
154 (Extended Data Fig. 3c). We then performed RNA-seq and comparative transcriptome
155 analysis in WT and *nac2* plants with or without aphid feeding, and identified numerous
156 potential NAC2-regulated differentially expressed genes (Extended Data Fig. 4 and
157 Supplementary Table 1 and 2), of which *SAMT1* was of particular interest (Extended
158 Data Fig. 4e). *SAMT1* RNA level was lower in *nac2* than WT plants, no matter whether
159 these plants were attacked by aphids or not (Supplementary Table 1 and 2). Knowing
160 that SAMT1 converts SA into MeSA¹⁹, we quantified *SAMT1* transcripts in *nac2*, HA-
161 NAC2-overexpressing, and WT leaf tissues to assess whether NAC2 transcriptionally
162 modulates *SAMT1* expression. Primers used in qRT assays were listed in
163 Supplementary Table 3. *SAMT1* mRNA level was substantially reduced in *nac2* but
164 increased in HA-NAC2 overexpressing plants compared to WT plants (Extended Data
165 Fig. 3d, e). Moreover, Luciferase reporter assay showed that NAC2 enhanced
166 transcription of reporter genes under the control of the *SAMT1* promoter (*SAMT1_{pro}*) *in*
167 *vivo* (Extended Data Fig. 3f). CHIP-qPCR, yeast-one-hybrid, and electrophoretic
168 mobility shift assay (EMSA) all demonstrated that NAC2 bound to the *SAMT1*
169 promoter at the putative NAC TF-binding site²³ and activated reporter gene
170 transcription (Extended Data Fig. 3g-i). Moreover, transient *NAC2* over-expression
171 increased MeSA production in plants (Extended Data Fig. 3j). These results suggest
172 that a NAC2-SAMT1 module involves the regulation of the MeSA biosynthesis in
173 plants.

174 **SA-activated NAC2-SAMT1 module elicits AD**

175 To investigate if NAC2 affects MeSA production in receivers via activating *SAMT1*
176 transcription, we evaluated *NAC2* and *SAMT1* mRNA levels in *nac2*, *NahG*, and WT
177 plants with or without airborne volatized MeSA treatment followed by ventilation.
178 Exogenous MeSA dramatically increased *SAMT1* and *NAC2* mRNAs in WT plants, but
179 did not affect *SAMT1* mRNA level in *nac2* plants (Fig. 2a, b). Plants can sense MeSA
180 as a within-plant signal and convert intracellular MeSA into SA to elicit intracellular
181 defense^{17,20}. Considering that MeSA treatment did not change much expression of
182 *NAC2* and *SAMT1* as well as aphid repellence in *NahG* plants (Fig. 2a-c), we
183 hypothesized that SA might be the cue to initiate *NAC2* expression. To test this
184 hypothesis, we first examined the effect of aphid attack on *NAC2* expression and found
185 that *NAC2* transcription was upregulated in aphid-attacked plants (Fig. 2d). Then, we
186 examined the effect of aphid attack on SA and MeSA production as well as *SAMT1*
187 expression in WT vs *nac2* plants. In the absence of aphids, lower MeSA accumulated
188 in *nac2* than WT plants (Fig. 2f). Upon aphid infestation, SA level increased in both
189 WT and *nac2* plants to similar extents; however, increases in cellular MeSA and *SAMT1*
190 expression were only found in WT but not *nac2* plants (Fig. 2e-g). Similar results were
191 also found in WT receivers and *nac2* receivers neighboring with aphid-attacked plants
192 (Fig. 2h-j). These results suggest that NAC2 is required for aphid-directed induction or
193 aphid-mediated volatile priming of *SAMT1* expression and MeSA production.
194 Moreover, externally applied SA upregulated *NAC2* and *SAMT1* expression in WT
195 plants, whereas did not significantly alter *SAMT1* mRNA level in *nac2* (Fig. 2k, l).
196 Exogenous SA also induced stronger MeSA volatilization and aphid repellence in WT
197 than *nac2* plants (Fig. 2m-p), and increased the levels of *NAC2* and *SAMT1* transcripts
198 in *SAMT1*-KO (*samt1*) plants (Fig. 2q and Extended Data Fig. 2b, c). In addition, *samt1*
199 plants were more attractive to aphids whilst externally applied SA failed to induce aphid

200 repellence in *samt1* plants (Fig. 2r-t). Furthermore, volatile MeSA production was
201 compromised in *samt1* plants exposed to aphids-attack (Fig. 3a, b). In further aphid
202 behavior experiments, we uncovered that WT receivers neighboring aphid-attacked WT
203 emitters were more repellent to aphids than those with mock WT emitters, while no
204 significant difference in aphid repellence was found in WT receivers neighboring *nac2*
205 or *samt1* emitters despite whether these emitters were exposed to aphids or not (Fig.
206 3c-e). These results further confirm the role of MeSA as a PPC signal for interplant AD.
207 Collectively, our data demonstrate that SA can activate *NAC2-SAMT1* transcription to
208 increase MeSA volatilization in both emitter and receiver plants.

209 As an SA-binding protein, SABP2 can also bind to MeSA and is essential for the
210 conversion of intracellular MeSA into SA^{17,20}. Thus, SABP2 may act as an OBP-like
211 receptor that perceives and converts the volatized MeSA generated from emitters into
212 SA to trigger *NAC2*-mediated aphid resistance in receivers. To test this idea, we first
213 confirmed that SABP2 bound to SA (Fig. 3f), and then examined whether MeSA could
214 influence the specific SABP2 SA-binding activity in competition binding assays (Fig.
215 3g). We set the SABP2-[³H]SA (50 Ci/mmol) binding capacity in the absence of
216 competitor MeSA as 100%. However, under the same experimental conditions, the
217 binding activity of [³H]SA by SABP2 reduced to approx. 74% and 46% in the presence
218 of 3 nM and 15 nM MeSA, respectively (Fig. 3g). Thus, 3 nM MeSA was sufficient to
219 compete with [³H]SA for binding to SABP2, suggesting that MeSA can bind to SABP2
220 at physiological concentration. We next generated *SABP2*-KO lines (*sabp2*) and tested
221 aphid repellence in *sabp2* vs WT receivers with volatized MeSA treatment followed by
222 ventilation. Such volatile MeSA treatment increased aphid repellence and SA
223 biosynthesis in WT but not *sabp2* receivers (Fig. 3h-j and Extended Data Fig. 2d).
224 Furthermore, aphid-feeding in WT emitters increased aphid repellence in WT but not

225 *sabp2* receivers (Fig. 3k). In addition, after external applications of SA, no difference
226 in the volatized MeSA amount was found between WT and *sabp2* plants, indicating that
227 SABP2 is not required for MeSA emission (Fig. 3l, m). These results demonstrate that
228 SABP2 is indeed an OBP-like receptor that perceives and converts airborne MeSA into
229 SA to elicit NAC2-mediated aphid repellence in receiver plants.

230 SAMT1 is required for plant antiviral defense²⁴. To test if SAMT1 is a component in
231 NAC2-mediated plant antiviral defense. We knocked-down (KD) *NAC2* in *samt1* to
232 generate *nac2/samt1* double-mutant by *Tobacco rattle virus* (TRV)-based virus-induced
233 gene silencing (VIGS). Like *nac2* mutants, *NAC2*-KD plants (in WT *Nb* background)
234 showed normal growth and were susceptible to CMV and PVY (Extended Data Fig. 1e-
235 j and 5a-e, h-l), suggesting that *NAC2*-KD by VIGS mimics *NAC2*-KO. However,
236 *nac2/samt1* and *samt1* plants showed similar degrees of CMV or PVY infection
237 (Extended Data Fig. 5a-e, h-l). In addition, CMV infection enhanced the intracellular
238 MeSA level in plants (Extended Data Fig. 5f), while *NAC2*-KD, *samt1*, and *nac2/samt1*
239 produced similar amount of MeSA, but all lower than WT plants in systemic leaves
240 during virus infection (Extended Data Fig. 5g). Moreover, we investigated whether and
241 how MeSA are responsible for NAC2-mediated plant antiviral defense by treating *nac2*,
242 *sabp2*, *samt1*, and WT plants with external application of either MeSA or SA.

243 Compared to WT, *nac2*, *samt1*, and *sabp2* were more susceptible to CMV and PVY
244 (Fig. 3n, o). However, exogenous MeSA rescued virus hypersusceptibility phenotypes
245 of *nac2* or *samt1* but not *sabp2* plants (Fig. 3n, o), likely due to MeSA conversion into
246 SA in WT, *nac2* and *samt1* but not *sabp2*. On the other hand, external spray of SA
247 rescued virus hypersusceptibility phenotypes of *nac2*, *samt1*, and *sabp2* plants (Fig. 3n,
248 o), consistent with the fact that SA can suppress infection of plants by many viruses
249 including CMV, *Potato virus X* (PVX), and TMV²⁵.

250 Taken together, our results demonstrate that SA can trigger the NAC2-SAMT1 module
251 to increase *in vivo* MeSA production by activating the NAC2-driven transcription of
252 *SAMT1*. SA converted from perceived MeSA by SABP2 is the cue to elicit AD against
253 aphids and viruses in receiver plants.

254 **CMV1a destabilizes NAC2 to suppress AD**

255 Aphid-induced PPC and AD may impose some impacts on viral transmission by aphids.
256 To investigate this, we fed CMV-bearing aphids on WT-R/AE, i.e., WT receiver (WT-
257 R) plants neighboring virus-free aphid-damaged WT plants as emitters (AE) or WT-
258 R/mE, i.e., WT-R plants adjoining non-aphid damaged WT plants as mock emitters
259 (mE), and analyzed the aphid feeding behaviors on these plants by electrical penetration
260 graph (EPG). EPG showed that number of short intracellular punctures (potential drop,
261 pd) of individual aphids, which is responsible for non-persistent aphid-transmission of
262 CMV, was less in WT-R/AE than WT-R/mE plants (Fig. 4a), suggesting that AD
263 reduced aphids to transmit CMV in WT-R/AE plants. Furthermore, we fed WT-R/AE
264 or WT-R/mE plants with viruliferous aphids for 2 hours, removed all aphids from plants
265 afterwards, and tested viral accumulation in these plants 24 hours later. We found that
266 WT-R/AE plants accumulated less CMV than WT-R/mE plants, evidenced by reduction
267 of viral RNA or CP, respectively (Fig. 4b, c). In addition, fewer viruliferous aphids
268 survived in WT-R/AE than WT-R/mE plants that were fed with viruliferous aphids for
269 24 hours (Fig. 4d). These results suggest that PPC elicits AD against aphids (including
270 repellent and survival) and reduces their ability to transmit CMV. We also tested the
271 effect of using CMV-carrying aphids attacked plants as emitters (ACE) on AD, and

272 found that pd number of individual aphids, CMV accumulation, and proportion of
273 living aphids were similar in WT-R/ACE and WT-R/mE plants (Fig. 4e-h). These data
274 suggest that CMV infection can suppress interplant aphid-induced AD. However, the
275 short probing frequency of aphids differs in WT vs CMV-infected plants²⁶. By contrast,
276 no difference in short probes frequency is observed in aphid-fed WT vs CMV Δ 2b-
277 infected plants²⁶. Nevertheless, to assure that the effect of CMV infection on aphid-
278 induced AD was not caused by probing frequency difference between virus-free and
279 viruliferous aphids, we conducted EPG experiments and found that the pd number was
280 lower for virus-free than CMV-carrying aphids, but similar for virus-free and CMV Δ 2b-
281 carrying aphids (Fig. 4i), consistent with the previous report²⁶. We then fed WT-R/mE,
282 WT-R/AE, WT-R/ACE, and WT-R/AC Δ E (WT Receiver with WT plants attacked by
283 aphids carrying CMV Δ 2b as emitters) plants with viruliferous aphids for 2 hours, and
284 tested viral accumulation as well as aphid survival in these plants. We found that AD
285 against aphids and viruses was attenuated in WT-Rs that were positioned nearby either
286 ACE or AC Δ E (Fig. 4j-l). We next investigated if virus infection affects MeSA
287 production in plants. GC-MS analysis revealed that plants fed with CMV-carrying
288 aphids or CMV Δ 2b-carrying aphids emitted less MeSA than plants fed with virus-free
289 aphids (Fig. 4m, n). Taken together, these data suggest that CMV infection can suppress
290 aphid-induced AD for the benefit of aphid survival and virus transmission/infection,
291 likely via a CMV-mediated interference with MeSA production.

292 The CMV1a-NAC2 interplay (Extended Data Fig. 1a-d) suggests that CMV1a might
293 be involved in CMV-mediated suppression of AD. To test this, we first generated

294 transgenic *Nb* expressing CMV1a ([Extended Data Fig. 6a, b](#)) and evaluated the effect
295 of CMV1a on plant attractiveness to aphids and AD. Our circular-dish and Y-tube
296 olfactometer bioassays showed that CMV1a expression caused higher plant
297 attractiveness to aphids ([Extended Data Fig. 6c, d](#)). EPG assays revealed that the
298 number of pd of individual aphids was more in CMV1a vs WT plants ([Extended Data](#)
299 [Fig. 6e](#)). Further, when virus-free aphid-attacked CMV1a plants were used as emitter
300 (1a-AE), AD against aphids and viruses in WT-R was suppressed ([Extended Data Fig.](#)
301 [6f-h](#)). In addition, WT-R plants nearby non-aphid-attacked WT plants as mock emitters
302 (WT-mE) exhibited higher attractiveness to aphids than WT-Rs adjacent to virus-free
303 aphid-attacked WT plants as emitter (WT-AE), however WT-R plants exhibited similar
304 attractiveness to aphids when non-aphid-attacked or virus-free aphid-attacked
305 transgenic CMV1a plants were used as emitters (1a-mE or 1a-AE) in Y-tube
306 olfactometer bioassays ([Extended Data Fig. 6i, j](#)). Moreover, WT-R plants exhibited
307 higher attractiveness to aphids when the emitter was 1a-AE vs WT-AE ([Extended Data](#)
308 [Fig. 6k](#)). These data imply that CMV1a is involved in CMV-mediated suppression of
309 AD.

310 To understand the importance of CMV1a-NAC2 interaction in CMV-mediated AD
311 suppression, we identified a key amino-acid in CMV1a responsible for its interaction
312 with NAC2. CMV1a protein consists of an N-terminal methyltransferase and a C-
313 terminal ATP-dependent helicase domain (HD). We found that CMV1a HD is
314 responsible for the CMV1a-NAC2 interaction by LCI ([Extended Data Fig. 6l](#)). We also
315 modeled the structure of CMV1a-NAC2 complex by AlphaFold-Multimer²⁷ and

316 observed that Glycine (G) at position 983 in CMV1a has the nearest physical proximity
317 with NAC2, predicting that this residue might be essential for CMV1a to interact with
318 NAC2 (Extended Data Fig. 6m, n). Indeed, a G983D mutation in CMV1a HD or full-
319 length CMV1a dramatically impaired the CMV1a-NAC2 interaction in Co-IP or BiFC
320 assays (Extended Data Fig. 6o-q).

321 We next investigated subcellular localization of CMV1a-NAC2 interaction by BiFC
322 and found that CMV1a interacted with NAC2 in both nucleus and cytoplasm (Extended
323 Data Fig. 1c), which is different from NAC2 localization without CMV1a co-
324 expression (Extended Data Fig. 3c), suggesting that CMV1a can relocate some NAC2
325 from nucleus to cytoplasm. Consistently, CMV1a-MYC, but neither cLUC-MYC nor
326 CMV1a^{G983D}-MYC, partially caused cytoplasmic localization of RFP-NAC2 and less
327 RFP fluorescence in nucleus (Extended Data Fig. 7a). Notably, CMV1a-MYC did not
328 alter RFP nuclear localization, indicating that CMV1a-MYC-directed NAC2 re-
329 localization depends on the NAC2-CMV1a interaction (Extended Data Fig. 7b). Further,
330 we investigated the stability of cytoplasmic NAC2 by using nuclear export signal
331 (NES)-tagged NAC2. We found that NES-NAC2 was localized in cytoplasm and
332 subjected to 26S-proteasome system-mediated degradation than WT NAC2 (Extended
333 Data Fig. 7c, d). Moreover, transient CMV1a expression enhanced NAC2 degradation
334 by the 26S-proteasome system but did not affect RFP stability (Extended Data Fig. 7e-
335 i), while CMV1a^{G983D} failed to cause NAC2 degradation (Extended Data Fig. 7f-i).

336 Further transient expression assays showed that CMV1a but not CMV1a^{G983D}
337 suppressed NAC2-mediated activation of the *SAMT1* promoter (Extended Data Fig. 7j).

338 In addition, Y-tube olfactometer bioassays and GC-MS analysis in CMV1a^{G983D} or
339 CMV1a transgenic vs WT plants showed that CMV1a^{G983D} impaired CMV1a-mediated
340 plant attractiveness to aphids and suppression of MeSA volatilization ([Extended Data](#)
341 [Fig. 7k-m](#)). Moreover, when non-aphid-attacked or virus-free aphid-attacked transgenic
342 CMV1a^{G983D} plants were used as emitters (1a^{G983D}-mE or 1a^{G983D}-AE), WT-R plants
343 nearby 1a^{G983D}-mE exhibited higher attractiveness to aphids than WT-R plants adjacent
344 to 1a^{G983D}-AE ([Extended Data Fig. 7n](#)). These Y-tube olfactometer bioassays provide
345 additional evidence confirming that the amino acid residue G983 is essential for
346 CMV1a to suppress the interplant AD.

347 Taken together, our data suggest that CMV1a interferes with AD by affecting
348 subcellular localization and stability of NAC2 via its direct interaction with NAC2 to
349 impair NAC2-driven *SAMT1* transcription and MeSA production.

350 **Some aphid-borne viruses suppress AD**

351 CMV1a with methyltransferase and helicase activity, which forms part of the viral
352 replicase complex, interacts with NAC2 through its HD ([Extended Data Fig. 1a-d and](#)
353 [6l-q](#)). We aligned HDs of viral replicases and other HD-containing proteins of multiple
354 plant viruses transmitted by aphids or other vectors. Intriguingly, the HDs from many
355 aphid-transmitted viruses including *Potyvirus*, *Cucumovirus*, *Luteovirus*, and
356 *Alfamovirus* contain a conserved glycine at position corresponding to CMV1a G983
357 ([Extended Data Fig. 8, 9a](#)). We further confirmed that PVY infection affected plant
358 MeSA volatilization after aphid attack. We tested the effect of using virus-free aphids-

359 attacked plants as emitters (AE) on AD against PVY transmission, and found that WT-
360 R/AE plants accumulated less PVY than WT-R/mE plants, evidenced by reduction of
361 viral RNA and CP (Extended Data Fig. 9b, c). Further, GC-MS analysis revealed that
362 plants fed with PVY-carrying aphids emitted less MeSA than plants fed with virus-free
363 aphids (Extended Data Fig. 9d, e). We also tested the effect of using PVY-carrying
364 aphids attacked plants as emitters (APE) on AD, and found that PVY accumulation and
365 proportion of living aphids were similar between WT-R/APE and WT-R/mE plants
366 (Extended Data Fig. 9f-h). Moreover, we showed that two aphid-transmitted viruses
367 CMV and PVY enable to relocate NAC2 from nucleus to cytoplasm (Extended Data
368 Fig. 9i). Similarly, PVY CI, but not CI^{G347D} nor 126KD protein of non-aphid transmitted
369 virus TMV, interacted with NAC2 (Extended Data Fig. 9j, k) and partially impaired
370 nuclear localization of NAC2 (Extended Data Fig. 9l). These results reveal that some
371 aphid-transmitted viruses have evolved to deploy HD-containing proteins as a general
372 strategy to interfere with plant AD.

373 Discussion

374 Insect-attacked plants emit VOCs as interplant signals to elicit AD in non-attacked
375 neighboring receiver plants^{11,28-30}. However, it is completely unknown how receiver
376 plants perceive VOCs from neighboring stressed “emitter” plants and activate signal
377 transduction to elicit AD³. In this study, we reveal molecular genetic mechanism
378 underlying AD against aphids in plants.

379 Unlike chewing herbivore attacks, phloem-feeding insects such as aphids avoid or
380 suppress jasmonate (JA)-based defense response, instead, they elicit SA response and

381 increase SA level in attacked plants^{31,32}. As outlined in our model ([Extended Data Fig.](#)
382 [10a](#)), NAC2 can be activated by upregulated SA level and directly bind to *SAMT1*
383 promoter, activating its transcription ([Fig. 2k and Extended Data Fig. 3d-i](#)). SAMT1
384 then catalyzes SA to form MeSA^{19,24}. Consistently, MeSA is a predominant VOC
385 induced by aphid attack in numerous plant species such as bean, chilli, and peach, but
386 it is very low or absent in the VOC emissions induced by beetle attack or artificial
387 wounding^{10-13,33}. The OBP-like receptor for perceiving airborne MeSA in receiver
388 plants is yet unknown³. SABP2 is thought to be a potential receptor for airborne MeSA
389 because it binds to MeSA^{3,17,20}. However, it has been only reported to operate in the
390 same plants exposed to stress (i.e., within-plant signaling)³. We now show that SABP2
391 is an OBP-like receptor for perceiving airborne MeSA from emitter plants and
392 converting MeSA into SA in receiver plants (between-plant signaling) ([Fig. 3f-j](#)). Thus,
393 plants may possess an OBP-like receptor-mediated sense system to perceive diverse
394 airborne VOCs³. Because MeSA can mediate resistance to many insects including
395 aphids through attracting their predators or parasitoids in multiple plant species³⁴⁻³⁸,
396 MeSA-perceived plants are also likely to attract aphid parasitoids or predators via
397 volatile MeSA. MeSA-perceived plants repel aphids and reduce aphid survival fitness
398 ([Fig. 1m, o and 4d and Extended Data Fig. 1t, u](#)). Thus, NAC2-SAMT1-mediated
399 airborne PPC represents a multifunctional strategy to prevent aphid epidemic
400 infestation.

401 *Arabidopsis* SA methyltransferase *BSMT1* is strongly induced by JA and biotic stress-
402 induced MeSA production, therefore severely compromised in JA pathway mutants

403 while SA signaling mutants such as *npr1* or *pad4* do not show compromised MeSA
404 emission³⁹. This is different to the regulation of *NbSAMT1* by SA described in this study.
405 Nevertheless, our finding of *SAMT1* induction by exogenous SA, intracellular SA upon
406 aphid attack, or aphid-mediated volatile priming via NAC2 in *N. benthamiana* (Fig. 2a-
407 1 and Extended Data Fig. 3d-j) is consistent with that *SAMT1* can be induced by SA or
408 insect feeding in *Atropa belladonna* and rice^{40,41} as well as in *N. alata* and *N. sylvestris*⁴².
409 It is possible that diverse species may have evolved distinctive mechanisms to regulate
410 *BSMT1* vs *SAMT1* expression. MeSA biosynthesis can also respond to environmental
411 biotic and abiotic stresses in a plant species-specific manner.
412 MeSA has been extensively studied as a long-distance mobile signal and shown to be
413 involved in the induction of SAR to viruses and aphids, and herbivore-induced within
414 plants^{11,12,14,17}. In this study, NAC2-SAMT1 module participates in intra/interplant
415 defense against CMV, PVY, and TMV (Extended Data Fig. 1e-m and 5a-e, h-l). MeSA-
416 mediated AD induces aphid antixenosis and suppresses virus transmission by aphids in
417 neighboring receiver plants (Fig. 4a-d). Hence, the NAC2-SAMT1 module is important
418 not only for plant intra/intercellular antiviral defense but also for AD against aphids and
419 viral transmission among plants. Therefore, we have now unraveled a completely novel
420 role of MeSA in PPC and AD, which is in a NAC2-dependent manner.
421 Elucidation of the molecular insight into AD against aphids and viruses raises an
422 intriguing question on whether and how virus counteracts AD and affects aphid survival
423 in receiver plants. In some cases, virus infection benefits its insect vector in virus-
424 infected plants. For examples, TYLCCNV-encoded β C1 protein suppresses JA pathway

425 or reduces terpene synthase and alters glucosinolate profiles to increase whitefly
426 *Bemisia tabaci* attraction and performance in virus-infected plants^{43,44}. CMV 2b protein
427 targets plant JA pathway or alters emission of VOC (not MeSA) to induce attractiveness
428 of virus-infected plants to aphids^{45,46}. In addition, CMV1a, which influences viral
429 replicase complex assembly and viral systemic movement, prevents CMV 2b-induced
430 anti-aphid resistance (antibiosis), likely via limiting CMV 2b-Argonaute1 interaction⁴⁷.
431 Further, CMV 2a stimulates feeding deterrence against aphids⁴⁸. Moreover, NIa-Pro
432 encoded by PVY or TuMV increases ethylene production and inhibits callose
433 accumulation to benefit insects in virus-infected plants^{49,50}. However, there is no report
434 about mechanism underlying pathogen-induced regulation of MeSA-mediated plant
435 AD². We now reveal that some aphid-transmitted viruses can suppress MeSA emission
436 in aphid-attacked plants to impair AD against virus infection and aphid infestation in
437 adjacent plants (Fig. 4e-n and Extended Data Fig. 9b-h). This is achieved by the action
438 of the helicase domain-containing protein that suppresses AD through its interaction
439 with NAC2 to partially change the subcellular localization of NAC2 from nucleus to
440 cytoplasm, and to promote the NAC2 degradation via the 26S proteasome to block
441 *SAMT1* transcription and subsequent MeSA biosynthesis (Extended Data Fig. 10b).
442 Therefore, some aphid-transmitted viruses have evolved to use helicase domain-
443 containing proteins as a common means to suppress NAC2 transcription function and
444 counteract AD in plants. These findings further suggest that not only viruses can utilize
445 aphids as vector for transmission, but also aphids can in turn exploit viruses to facilitate
446 their surviving and thriving in plants. This unexpected countermeasure reveals an

447 undefined ecological and co-evolutionary mutualism between aphids and aphid-
448 transmissible viruses ([Extended Data Fig. 10](#)).

449 **References**

- 450 1. van Emden, H. & Harrington, R. Aphids as Crop Pests Second Edition Preface
451 to the Second Edition. *Aphids as Crop Pests, 2nd Edition*, Xxiii-Xxiv (2017).
- 452 2. Karban, R. Plant Communication. *Annual Review of Ecology, Evolution, and*
453 *Systematics*, **52**, 1-24 (2021).
- 454 3. Loreto, F. & D'Auria, S. How do plants sense volatiles sent by other plants?
455 *Trends in Plant Science* **27**, 29-38 (2022).
- 456 4. Shulaev, V., Silverman, P. & Raskin, I. Airborne signalling by methyl salicylate
457 in plant pathogen resistance. *Nature* **386**, 738-738 (1997).
- 458 5. Wenig, M. *et al.* Systemic acquired resistance networks amplify airborne
459 defense cues. *Nature Communications* **10**, 3813 (2019).
- 460 6. Pickett, J.A. & Khan, Z.R. Plant volatile-mediated signalling and its application
461 in agriculture: successes and challenges. *New Phytologist* **212**, 856-870 (2016).
- 462 7. Sugimoto, K. *et al.* Identification of a tomato UDP-arabinosyltransferase for
463 airborne volatile reception. *Nature Communications* **14**, 677 (2023).
- 464 8. Bleeker, A.B. & Schaller, G.E. The mechanism of ethylene perception. *Plant*
465 *Physiology* **111**, 653-660 (1996).
- 466 9. Fereres, A. & Moreno, A. Behavioural aspects influencing plant virus
467 transmission by homopteran insects. *Virus Research* **141**, 158-168 (2009).
- 468 10. Babikova, Z. *et al.* Underground signals carried through common mycelial
469 networks warn neighbouring plants of aphid attack. *Ecology Letters* **16**, 835-
470 843 (2013).
- 471 11. Moreira, X., Nell, C.S., Katsanis, A., Rasmann, S. & Mooney, K.A. Herbivore
472 specificity and the chemical basis of plant-plant communication in *Baccharis*
473 *salicifolia* (Asteraceae). *New Phytologist* **220**, 703-713 (2018).
- 474 12. Staudt, M. *et al.* Volatile organic compound emissions induced by the aphid
475 *Myzus persicae* differ among resistant and susceptible peach cultivars and a
476 wild relative. *Tree Physiology* **30**, 1320-1334 (2010).
- 477 13. Saad, K.A., Mohamad Roff, M.N., Hallett, R.H. & Idris, A.B. Aphid-induced
478 Defences in Chilli Affect Preferences of the Whitefly, *Bemisia tabaci*

- 479 (Hemiptera: Aleyrodidae). *Scientific Report* **5**, 13697 (2015).
- 480 14. Dong, Y.J. & Hwang, S.Y. Cucumber Plants Baited with Methyl Salicylate
481 Accelerates *Scymnus (Pullus) sodalis* (Coleoptera: Coccinellidae) Visiting to
482 Reduce Cotton Aphid (Hemiptera: Aphididae) Infestation. *Journal of Economic*
483 *Entomology* **110**, 2092-2099 (2017).
- 484 15. Mallinger, R.E., Hogg, D.B. & Gratton, C. Methyl Salicylate Attracts Natural
485 Enemies and Reduces Populations of Soybean Aphids (Hemiptera: Aphididae)
486 in Soybean Agroecosystems. *Journal of Economic Entomology* **104**, 115-124
487 (2011).
- 488 16. Ninkovic, V., Glinwood, R., Unlu, A.G., Ganji, S. & Unelius, C.R. Effects of
489 Methyl Salicylate on Host Plant Acceptance and Feeding by the Aphid
490 *Rhopalosiphum padi*. *Frontiers in Plant Science* **12**, 710268 (2021).
- 491 17. Park, S.W. Methyl salicylate is a critical mobile signal for plant systemic
492 acquired resistance. *Science* **321**, 342-342 (2008).
- 493 18. Vlot, A.C., Dempsey, D.A. & Klessig, D.F. Salicylic Acid, a Multifaceted
494 Hormone to Combat Disease. *Annual Review of Phytopathology* **47**, 177-206
495 (2009).
- 496 19. Dudareva, N., Raguso, R.A., Wang, J.H., Ross, E.J. & Pichersky, E. Floral scent
497 production in *Clarkia breweri* - III. Enzymatic synthesis and emission of
498 benzenoid esters. *Plant Physiology* **116**, 599-604 (1998).
- 499 20. Forouhar, F. *et al.* Structural and biochemical studies identify tobacco SABP2
500 as a methyl salicylate esterase and implicate it in plant innate immunity.
501 *Proceedings of the National Academy of Sciences of the United States of*
502 *America* **102**, 1773-1778 (2005).
- 503 21. Wang, Y.J. *et al.* A calmodulin-binding transcription factor links calcium
504 signaling to antiviral RNAi defense in plants. *Cell Host & Microbe* **29**, 1393-
505 1406 (2021).
- 506 22. Olsen, A.N., Ernst, H.A., Lo Leggio, L. & Skriver, K. NAC transcription factors:
507 structurally distinct, functionally diverse. *Trends in Plant Science* **10**, 79-87
508 (2005).
- 509 23. De Clercq, I. *et al.* The Membrane-Bound NAC Transcription Factor ANAC013
510 Functions in Mitochondrial Retrograde Regulation of the Oxidative Stress
511 Response in Arabidopsis. *Plant Cell* **25**, 3472-3490 (2013).
- 512 24. Zhu, F. *et al.* Salicylic acid and jasmonic acid are essential for systemic
513 resistance against tobacco mosaic virus in *Nicotiana benthamiana*. *Molecular*
514 *Plant-Microbe Interactions* **27**, 567-77 (2014).

- 515 25. Naylor, M., Murphy, A.M., Berry, J.O. & Carr, J.P. Salicylic acid can induce
516 resistance to plant virus movement. *Molecular Plant-Microbe Interactions* **11**,
517 860-868 (1998).
- 518 26. Guo, H.J. *et al.* Aphid-borne Viral Spread Is Enhanced by Virus-induced
519 Accumulation of Plant Reactive Oxygen Species. *Plant Physiology* **179**, 143-
520 155 (2019).
- 521 27. Evans, R. *et al.* Protein complex prediction with AlphaFold-Multimer. *bioRxiv*
522 <https://doi.org/10.1101/2021.10.04.463034> (2021).
- 523 28. Arimura, G. *et al.* Herbivory-induced volatiles elicit defence genes in lima bean
524 leaves. *Nature* **406**, 512-515 (2000).
- 525 29. Engelberth, J., Alborn, H.T., Schmelz, E.A. & Tumlinson, J.H. Airborne signals
526 prime plants against insect herbivore attack. *Proceedings of the National*
527 *Academy of Sciences of the United States of America* **101**, 1781-1785 (2004).
- 528 30. Karban, R., Yang, L.H. & Edwards, K.F. Volatile communication between plants
529 that affects herbivory: a meta-analysis. *Ecology Letters* **17**, 44-52 (2014).
- 530 31. Donovan, M.P., Nabity, P.D. & DeLucia, E.H. Salicylic acid-mediated
531 reductions in yield in *Nicotiana attenuata* challenged by aphid herbivory.
532 *Arthropod-Plant Interactions* **7**, 45-52 (2013).
- 533 32. Cao, H.H., Liu, H.R., Zhang, Z.F. & Liu, T.X. The green peach aphid *Myzus*
534 *persicae* perform better on pre-infested Chinese cabbage *Brassica pekinensis* by
535 enhancing host plant nutritional quality. *Scientific Report* **6**, 21954 (2016).
- 536 33. Blande, J.D., Korjus, M. & Holopainen, J.K. Foliar methyl salicylate emissions
537 indicate prolonged aphid infestation on silver birch and black alder. *Tree*
538 *Physiology* **30**, 404-416 (2010).
- 539 34. van Poecke, R.M.P. & Dicke, M. Indirect defence of plants against herbivores:
540 Using *Arabidopsis thaliana* as a model plant. *Plant Biology* **6**, 387-401 (2004).
- 541 35. James, D.G. Field evaluation of herbivore-induced plant volatiles as attractants
542 for beneficial insects: Methyl salicylate and the green lacewing, *Chrysopa*
543 *nigricornis*. *Journal of Chemical Ecology* **29**, 1601-1609 (2003).
- 544 36. Woods, J.L., James, D.G., Lee, J.C. & Gent, D.H. Evaluation of airborne methyl
545 salicylate for improved conservation biological control of two-spotted spider
546 mite and hop aphid in Oregon hop yards. *Experimental and Applied Acarology*
547 **55**, 401-416 (2011).
- 548 37. Rowen, E., Gutensohn, M., Dudareva, N. & Kaplan, I. Carnivore Attractant or
549 Plant Elicitor? Multifunctional Roles of Methyl Salicylate Lures in Tomato

- 550 Defense. *Journal of Chemical Ecology* **43**, 573-585 (2017).
- 551 38. Liu, J. *et al.* Herbivore-Induced Rice Volatiles Attract and Affect the Predation
552 Ability of the Wolf Spiders, *Pirata subpiraticus* and *Pardosa pseudoannulata*.
553 *Insects* **13**, 90 (2022).
- 554 39. Attaran, E., Zeier, T.E., Griebel, T. & Zeier, J. Methyl salicylate production and
555 jasmonate signaling are not essential for systemic acquired resistance in
556 *Arabidopsis*. *Plant Cell* **21**, 954-71 (2009).
- 557 40. Kwon, S. *et al.* Biotic and abiotic stresses induce AbSAMT1, encoding S-
558 adenosyl-L-methionine: salicylic acid carboxyl methyltransferase, in *Atropa*
559 *belladonna*. *Plant Biotechnology* **26**, 207-215 (2009).
- 560 41. Xu, R., Song, F. & Zheng, Z. OsBISAMT1, a gene encoding S-adenosyl-L-
561 methionine: salicylic acid carboxyl methyltransferase, is differentially
562 expressed in rice defense responses. *Molecular Biology Reports* **33**, 223-31
563 (2006).
- 564 42. Hippauf, F. *et al.* Enzymatic, expression and structural divergences among
565 carboxyl O-methyltransferases after gene duplication and speciation in
566 *Nicotiana*. *Plant Molecular Biology* **72**, 311-330 (2010).
- 567 43. Li, R. *et al.* Virulence Factors of Geminivirus Interact with MYC2 to Subvert
568 Plant Resistance and Promote Vector Performance. *Plant Cell* **26**, 4991-5008
569 (2014).
- 570 44. Zhao, P.Z. *et al.* Viruses mobilize plant immunity to deter nonvector insect
571 herbivores. *Science Advances* **5**, eaav9801 (2019).
- 572 45. Tungadi, T. *et al.* Cucumber mosaic virus and its 2b protein alter emission of
573 host volatile organic compounds but not aphid vector settling in tobacco.
574 *Virology Journal* **14**, 91 (2017).
- 575 46. Wu, D.W. *et al.* Viral effector protein manipulates host hormone signaling to
576 attract insect vectors. *Cell Research* **27**, 402-415 (2017).
- 577 47. Westwood, J.H. *et al.* A trio of viral proteins tunes aphid-plant interactions in
578 *Arabidopsis thaliana*. *PLoS One* **8**, e83066 (2013).
- 579 48. Rhee, S.J., Watt, L.G., Bravo, A.C., Murphy, A.M. & Carr, J.P. Effects of the
580 cucumber mosaic virus 2a protein on aphid-plant interactions in *Arabidopsis*
581 *thaliana*. *Molecular Plant Pathology* **21**, 1248-1254 (2020).
- 582 49. Casteel, C.L. *et al.* The NIa-Pro protein of Turnip mosaic virus improves growth
583 and reproduction of the aphid vector, *Myzus persicae* (green peach aphid). *Plant*
584 *Journal* **77**, 653-663 (2014).

585 50. Bak, A., Cheung, A.L., Yang, C.L., Whitham, S.A. & Casteel, C.L. A viral
586 protease relocalizes in the presence of the vector to promote vector performance.
587 *Nature Communications* **8**, 14493 (2017).

588 **Figure legends**

589 **Fig. 1 | *NAC2* regulates MeSA production to mediate PPC-induced aphid**
590 **resistance.**

591 **a, b**, GC-MS analysis. WT, but not *nac2* plants emitted higher level of airborne MeSA
592 after MeSA treatment followed by ventilation. **c, d**, After smearing plants with MeSA-
593 containing lanolin, WT and volatile MeSA-treated WT plants exhibited similar
594 attractiveness to aphids in circular-dish (**c**) or Y-tube olfactometer (**d**) bioassays. **e, f**,
595 Once smearing plants with MeSA-containing lanolin, volatile MeSA-treated WT and
596 *nac2* plants exhibited similar attractiveness to aphids in circular-dish (**e**) or Y-tube
597 olfactometer (**f**) bioassays. **g-j**, GC-MS analysis. Aphid sap-sucking caused the attacked
598 WT emitter plants to volatilize MeSA (**g, h**). When aphid-attacked WT plants were used
599 as emitters (AE), their WT receivers (WT-R) volatilize more MeSA than *nac2* receivers
600 (*nac2*-R) (**i, j**). **k, l**, *nac2*-R (AE) plants exhibited higher attractiveness to aphids than
601 WT-R (AE) plants in circular-dish (**k**) or Y-tube olfactometer (**l**) bioassays. **m, n**, WT-
602 R (AE) plants were more repelled by aphids than those neighboring mock emitter plants
603 (**m**), while no significant difference in aphid repellence between *nac2* receivers
604 neighboring mock- and aphid-attacked emitter plants (**n**) in Y-tube olfactometer
605 bioassays. **o, p**, Proportion of living aphids in WT-R (**o**) or *nac2*-R (**p**) plants with
606 aphid-attacked WT plants as emitters (AE) or non-aphid-attacked WT plants as mock
607 emitters (mE). Numerals shown inside each bar present number of choice-making
608 aphids. **b, h, j**, Two-sided Student's *t*-test, $n=3$ biologically independent samples. **o, p**,
609 One-way ANOVA with least significant difference (LSD), $n=4$ biologically
610 independent samples; letters A-B represent statistically different groups ($P < 0.05$).
611 Data are shown as mean \pm s.d.; n.s., no statistical significance. **c-f, k-n**, χ^2 test (df = 1).
612 All *P* values are shown in figure. Experiments were repeated at least three times with
613 similar results.

614 **Fig. 2 | NAC2 is required for SA-directed enhancement of MeSA volatilization.**
615 **a, b**, *NAC2* (or its deletion mutant) or *SAMT1* mRNA levels in WT, *nac2*, and *NahG*
616 plants with or without volatile MeSA treatment. **c**, Volatile MeSA treatment induced
617 aphid repellence in WT, but not *NahG* plants in Y-tube olfactometer bioassays. **d**, *NAC2*
618 expression was enhanced in aphid-attacked WT plants. **e-g**, Free SA (**e**), MeSA (**f**), and
619 *SAMT1* expression (**g**) in WT or *nac2* plants upon aphid attacks. **h-j**, Free SA (**h**), MeSA
620 (**i**), and *SAMT1* expression (**j**) in WT or *nac2* receivers neighboring aphid-attacked WT
621 emitters or mock WT emitters. **k, l**, *NAC2* or *SAMT1* expression in *nac2* and WT plants
622 with or without SA treatment. **m, n**, GC-MS analysis of volatized MeSA in WT or *nac2*
623 plants treated with SA for 24 hours. **o, p**, SA treatment induced aphid repellence in WT
624 (**o**), but not *nac2* (**p**) plants in Y-tube olfactometer bioassays. **q**, *NAC2* or *SAMT1*
625 expression in *samt1* plants with or without SA treatment. **r**, *samt1* plants exhibited
626 higher attractiveness to aphids than WT plants in Y-tube olfactometer bioassays. **s, t**,
627 SA treatment induced aphid repellence in WT (**s**), but not *samt1* (**t**) plants in Y-tube
628 olfactometer bioassays. **a, b, d, g, j-l, n, q**, Two-sided Student's *t*-test. **e, f, h, i**, One-
629 way ANOVA with Tukey's multiple comparisons test; letters A-C represent statistically
630 different groups ($P < 0.05$). **a, b, d-h, j-l, n, q**, $n=3$ biologically independent samples. **i**,
631 $n=4$ biologically independent samples. Data are shown as mean \pm s.d.; n.s., no statistical
632 significance. **c, o, p, r-t**, χ^2 test ($df = 1$). P values are shown in **a-d, g, j-l, n-t**; P values
633 for **e, f, h, i** are shown in the Source Data. Experiments were repeated at least three
634 times with similar results.

635 **Fig.3 | The conversion between MeSA and SA is required for NAC2-mediated AD**
636 **against aphids and plant defense against viruses.**

637 **a, b**, Volatile MeSA production was compromised in *samt1* plants exposed to aphids-
638 attack by GC-MS analysis. **c-e**, WT receivers neighboring aphid-attacked WT emitters
639 were more repellent to aphids than those with mock WT emitters (**c**), while no
640 significant difference in aphid repellence in WT receivers neighboring *nac2* (**d**) or
641 *samt1* (**e**) emitters exposed to aphids or not in Y-tube olfactometer bioassays. **f**, SA-
642 binding activity of purified SABP2 protein. **g**, SA competition binding assays with 0,

643 3, 15 nM MeSA. Binding activity of [³H]SA to SABP2 in the presence of 3 nM or 15
644 nM MeSA were calculated against the baselevel. **h**, *sabp2* showed no abnormal
645 developmental phenotypes. **i**, Volatile MeSA treatment induced aphid repellence in WT
646 plants, but not in *sabp2* plants in Y-tube olfactometer bioassays. **j**, Volatile MeSA
647 treatment increased free SA amount in WT, but not *sabp2* plants. **k**, WT receivers with
648 non-aphid-attacked WT plants as mock emitters (WT-mE) exhibited higher
649 attractiveness than those with virus-free aphid-attacked WT plants as emitters (WT-AE),
650 but not for *sabp2* receivers in Y-tube olfactometer bioassays. **l, m**, GC-MS assays. No
651 difference in the volatized MeSA amount was seen between WT and *sabp2* plants after
652 SA treatment. **n, o**, MeSA treatment rescued virus hypersusceptibility phenotypes of
653 *nac2* or *samt1* but not *sabp2* plants, whilst SA treatment rescued virus
654 hypersusceptibility phenotypes of *nac2*, *samt1* and *sabp2* plants. **b, g, j, m-o**, Two-
655 sided Student's *t*-test. **b, f, m-o**, *n*=3 biologically independent samples. **g, j**, *n*=4
656 biologically independent samples. Data are shown as mean ± s.d.; n.s., no statistical
657 significance. **c-e, i, k**, χ^2 test (*df* = 1). All *P* values are shown in figure. Experiments
658 were repeated at least three times with similar results.

659 **Fig. 4 | CMV1a suppresses AD.**

660 **a**, EPG analysis showed the pd number in aphid feeding was less in WT receivers (WT-
661 R) with virus-free aphid-attacked WT plants as emitters (AE) than WT receivers with
662 non-aphid-attacked WT plants as mock emitters (mE) when receivers were fed with
663 CMV-carrying aphids. *n*=21 individual aphids. **b-d**, CMV RNA (**b**), CP (**c**) or
664 proportion of living aphids (**d**) in WT-R (mE) and WT-R (AE) plants. **e**, The pd number
665 was similar between WT-R (mE) and WT-R plants with CMV-carrying aphids-attacked
666 plants as emitter (ACE) when these receivers were fed with CMV-carrying aphids. *n*=20
667 individual aphids. **f-h**, CMV RNA (**f**), CP (**g**) or proportion of living aphids (**h**) is
668 similar between WT-R (mE) and WT-R (ACE) plants. **i**, The pd number of virus-free
669 aphids was lower than that of CMV-carrying aphids, but similar to that of CMV Δ 2b-
670 carrying aphids. *n*=19 individual aphids. **j-l**, CMV RNA (**j**), CP (**k**) or proportion of
671 living aphids (**l**) in WT-R plants with mE, AE, ACE, or CMV Δ 2b-carrying aphid-

672 attacked WT plants as emitters (ACΔE). **m, n**, GC-MS analysis of volatized MeSA in
673 WT plants fed with virus-free aphids, CMV-carrying aphids, or CMVΔ2b-carrying
674 aphids for 3 days. **a, b, e, f, i, j, n**, Two-sided Student's *t*-test. **d, h**, One-way ANOVA
675 with LSD. **l**, One-way ANOVA with Tukey's multiple comparisons test; letters A-B
676 represent statistically different groups ($P < 0.05$). **b, d, f, h, j, l, n**, $n=3$ biologically
677 independent samples. Data are shown as mean \pm s.d.; n.s., no statistical significance. *P*
678 values are shown in **a, b, d-f, h-j, n**; *P* values for **l** are shown in the Source Data. In box
679 plots (**a, e, i**), the centre line represents the median, box edges delimit bottom and top
680 quartiles and whiskers show the highest and lowest data points. Experiments were
681 repeated at least three times with similar results.

682 **Methods**

683 **Plant Materials and Growth Conditions**

684 *Nicotiana benthamiana* (*Nb*) was used as wild-type (WT) plant. *NahG* lines were
685 transgenic *Nb* plants expressing bacterial *NahG* which encodes SA-degrading enzyme
686 salicylate hydroxylase⁵¹. Transgenic *CMV1a*, *CMV1a*^{G983D} lines are *Nb* lines
687 overexpressing *CMV1a* or *CMV1a*^{G983D} with C-terminal fusion to a MYC tag. The
688 *nac2*, *samt1*, and *sabp2* were *NbNAC2.1/2.2*, *NbSAMT1*, and *NbSABP2*-edited KO
689 mutant *Nb* lines, respectively. All seeds were surface-sterilized and cultivated on MS
690 medium (Murashige and Skoog medium, Sigma-Aldrich), grown at 26°C with a 16 h/8
691 h light/dark photoperiod. 10-day-old seedlings were then planted in soil and grown with
692 the same photoperiod.

693 **Generation of Transgenic Plants**

694 The MYC-tagged full-length *CMV1a* and *CMV1a*^{G983D} genes under the CaMV 35S
695 promoter were cloned into pCambia1300-based binary vector via the ligation-
696 independent cloning strategy. All constructs were verified by DNA sequencing and
697 transformed into *Agrobacterium* GV2260. To generate transgenic plants, leaf discs were

698 plated on MS medium in 100 mm petri dish (704001, NEST Biotechnology) with 0.1
699 mg/mL NAA (N8010, Solarbio), 1.0 mg/mL 6-BA (IB0100, Solarbio), 0.2 mg/mL
700 Timentin and 0.025 mg/mL Hygromycin B (400052, Merck Millipore) for callus, shoot,
701 and root regeneration. Western blot assays were used to select the T2 generation
702 transgenic lines of *35S:CMV1a-MYC* and *35S:CMV1a^{G983D}-MYC* plants, and selected
703 transgenic plants were then used for aphid attraction bioassays and GC-MS analysis.
704 The Cas9-edited knockout lines were generated as described⁵². Briefly, the fragment
705 containing single sgRNA (NAC2_LacZ/AtU3d-sgRNA, SABP2_LacZ/AtU3d-sgRNA)
706 or two sgRNAs (SAMT1_LacZ/AtU3d-sgRNA1-At3b-sgRNA2) were cloned into
707 pYLCRISPR/Cas9-DH to generate pYLCRISPR/Cas9-DH-NAC2,
708 pYLCRISPR/Cas9-DH-SABP2, and pYLCRISPR/Cas9-DH-SAMT1. The sgRNA
709 sequence is listed in [Supplementary Table 3](#). The editing target site of transgenic plant
710 DNA sequence was characterized by PCR sequencing to select homozygous transgenic
711 lines. PCR amplification was conducted using 2×T5 Super PCR Mix (TSE005, Beijing
712 Tsingke Biotech). Progenies of homozygous *nac2*, *sabp2*, and *samt1* lines showed no
713 developmental phenotype.

714 **MeSA supplement treatment – Lanolin smear assay**

715 0.8 µg pure MeSA chemical (M6752, Sigma-Aldrich) was dissolved in lanolin paste
716 (S5106, Selleck). Plants for MeSA supplement treatment were smeared with MeSA-
717 containing lanolin on the stems or leaf vein. MeSA-containing lanolin would release
718 quantitative volatile MeSA continuously and constantly^{53,54}.

719 **MeSA supplement treatment – Airborne assay**

720 0.8 µg pure MeSA was dissolved in lanolin paste. Lanolin containing 0.8 µg MeSA was
721 then smeared onto the surface of filter paper and the filter paper was placed in a 2 L

722 volume gas tight glass chamber. Plants for airborne MeSA supplement treatment were
723 placed in these chambers separately to perceive volatile MeSA (each chamber contains
724 one plant) for 24 hours, and then transferred from chambers to open-air area for
725 ventilation for 2 hours before further experiments as described⁵⁵.

726 **Exogenous SA supply treatment**

727 Solutions of 50 μ M SA (S5922, Sigma-Aldrich) were used for plant treatments. 4-week-
728 old seedlings were cultured in pots in a greenhouse and sprayed with the SA solutions.

729 **Aphid choice bioassay: Y-tube olfactometer assay**

730 Wingless *M. persicae* aphids were isolated from *Nb* plants and starved for 2 hours
731 before testing. Plants grown in soil for 4 weeks were used. The experimental procedure
732 and equipment were used as described⁴⁶. For pair-wise comparison of aphid
733 attractiveness, plants in different groups were separately placed into two chambers,
734 which were connected by the two arms of a glass Y-tube. The glass Y-tube was 1.0 cm
735 in inner diameter and 6 cm in arm length. A humidified continuous air flow purified by
736 activated charcoal flowed through the chambers at 200 mL/min, then flowed into the
737 arms of the Y-tube.

738 In each experiment, an individual aphid was placed at the end of the Y-tube stem.
739 Aphids walked upwind toward the arms and made its choice. Once the aphid entered
740 one arm and walked up to 3 cm away from the Y-junction, their choice would be marked.
741 To avoid any unknown asymmetry in the setup, the plant-containing chambers were
742 switched after every 10 tests, and the Y-tube and plants were replaced after 20 tests.
743 The used Y-tubes were cleaned by ethanol and ddH₂O before reuse. The same number

744 of aphids were used for each group in each experiment. All experiments were
745 independently repeated at least 3 times.

746 **Aphid choice bioassay: Petri disc assay**

747 Choice test for *M. persicae* was performed with detached leaves from 4 to 5-week-old
748 *Nb* in plastic petri dishes (15 cm diameter) following a previously described procedure⁵⁶.
749 Two leaves with similar leaf position and size from experimental group plants and
750 control group plants were detached and placed in petri dishes, with their petioles
751 inserted in moistened cotton swabs. Twenty wingless adult aphids were released at the
752 midpoint between pairs of leaves, and the aphids on each leaf were counted at 24 hours
753 after their release in the petri dishes. The same number of aphids were used for each
754 group in each experiment. All experiments were independently repeated at least three
755 times.

756 **Interplant communication assays**

757 Experimental plants were served as “emitters” or “receivers” respectively. The emitter
758 and receiver plants were placed on two trays at a distance of 30 cm from each other in
759 an open-air experimental set-up for interplant communication assays. Each emitter was
760 fed with fifty virus-free or viruliferous *M. persicae* aphids or no aphids, then the
761 emitters and receivers were incubated in same cage made by gauze. After 3 days, the
762 receiver plants were taken out for further experiments. Viruliferous *M. persicae* aphids
763 were obtained from aphids which were fed on CMV, CMVΔ2b, or PVY-GFP-infected
764 plants for 1 hour before the pre-acquisition starvation period (2 hours).

765 **Aphid survival and virus-transmission analysis**

766 To measure the effect of plant airborne defense on aphid fitness in neighboring receiver

767 plants, the experimental group receiver plants and mock receiver plants were fed with
768 the same number of wingless adult *M. persicae*. After 24 hours, numbers of alive aphids
769 on each host plant were counted. To measure the influence of airborne defense on aphid-
770 mediated virus-transmission in neighbor receiver plants, the experimental group
771 receiver plants and control receiver plants were fed with the same number of
772 viruliferous adult aphids (carrying CMV or PVY) for 2 hours as described⁵⁷, then aphids
773 were removed, 24 hours later, aphid-sucked leaves were taken to measure viral infection.
774 All experiments were conducted at least three times.

775 **EPG analysis of aphid feeding behavior**

776 Giga-8 EPG amplifier (Wageningen University, Wageningen, Netherlands) was used to
777 study feeding behavior of *M. persicae* on plants as described⁵⁸. The amplifier with eight
778 channels simultaneously recorded eight individual aphids on separate plants for 2 hours
779 in accordance with the inoculation access period (IAP) in viral transmission experiment.
780 The EPG waveforms of short intracellular punctures (potential drop, pd) were analyzed
781 during 2 hours of aphid feeding with the software “Stylet + a”.

782 **Measurement of plant endogenous free SA amount**

783 Free SA was extracted and measured as described⁵⁹. Briefly, 0.2 g leave tissue was
784 homogenized in 1 mL of ethyl acetate containing 10 ng of SA-d6, and vortexed for 10
785 min followed by centrifuging at 18000g at 4 °C for 20 min. Supernatants were collected
786 and evaporated to dry by a vacuum concentrator at 30 °C. 200 µL of MeOH: H₂O (70:30,
787 v/v) was added to resuspend the residues by centrifuging at 18000g for 10 min. The
788 collection of supernatants was analyzed by a high-performance liquid chromatography-

789 tandem mass spectrometry system (QTRAP 4500, AB Sciex).

790 **Measurement of plant endogenous MeSA**

791 MeSA was extracted and measured by Suzhou Michy Biomedical Technology Co., Ltd.

792 Briefly, frozen leaf tissues (150 mg) was homogenized with 200 μ L of extraction buffer

793 (water/1-propanol/HCl = 1:2:0.005). The homogenate was added 500 μ L of methylene

794 chloride and shaken thoroughly, then centrifuged at 18800g for phase separation.

795 Aliquots of the supernatants were separated by a gas chromatography (GC) system.

796 **Volatile MeSA measurement by GC-MS analysis**

797 VOCs emitted by plants were collected by a static-headspace sampling device with a

798 solid-phase microextraction (SPME) fibre (PDMS/DVB, 65 μ m) coated with

799 polydimethylsiloxane/divinylbenzene (57345-U, Supelco) as described¹³. Each sample

800 was enclosed in a 1.5-L glass container for 120 min, then the SPME fibre was extended

801 into the headspace to collect volatiles for 30 min. After collection of volatile substances,

802 the SPME fibre was inserted directly into a thermal desorption gas chromatograph-mass

803 spectrometer (Thermo Fisher Scientific, Bremen, Germany). The volatiles bound to the

804 fibre were desorbed for 2 min. A Thermo Scientific Trace 1300 gas chromatograph

805 equipped with a Thermo Scientific Q-Exactive Orbitrap mass spectrometer (GC-

806 Orbitrap-MS, Thermo Scientific, Bremen, Germany) was used for HRMS quantitative

807 analysis. The split-splitless injection port was held at 250°C for desorpting volatiles in

808 split mode at a split ratio of 1:10. The oven temperature program was set as: 60 °C held

809 for 2 min, then increased to 280°C at 20°C/min and held for 2 min.

810 **Mass Spectrometry Analysis**

811 Protein extracts were immunoprecipitated by MYC-Trap beads (M20012L, Abmart)

812 and the mixture was incubated at 4°C for 3 hours. The precipitated samples were
813 washed 5 times with Co-IP buffer, and immunoprecipitates were then separated by 10%
814 SDS-PAGE. The gel was silver stained and gel-embedded protein samples were
815 digested and LC-MS/MS analyzed.

816 **Yeast One Hybrid (Y1H) Assay**

817 The full-length cDNA sequence of *NAC2* was fused in frame with the GAL4 activation
818 domain in pGADT7-Rec2 (Clontech). The fusion construct was co-transformed with
819 the reporter vector (pHIS2-cis/promoter of *SAMT1*) into Y187 yeast cells (Clontech).
820 The empty vector pGADT7-Rec2 and the pHIS2-cis/35S promoter were co-
821 transformed as the negative controls. Co-transformed yeast strain Y187 were spotted
822 on selective media (minus (-) His, Leu and Trp) contained 0.5, 1, or 2 mM 3-AT. Images
823 were taken after 3 days incubation at 28°C. All experiments were repeated at least three
824 times.

825 **Bimolecular fluorescence complementation (BiFC) and firefly luciferase** 826 **complementation imaging (LCI) assays**

827 BiFC and LCI assays were conducted as described⁶⁰. For BiFC assay, the full-length
828 cDNA sequences of *NAC2*, *CMV1a*, *CMV1a^{G983D}*, *PVYCI*, *PVYCI^{G347D}*, and
829 *TMV126KD* were cloned into the binary cYFP or nYFP vector by ligation-independent
830 cloning technique. Primer pairs for making these constructs were made by Beijing
831 Tsingke Biotech Co., Ltd. and are listed in [Supplementary Table 3](#). These gene
832 sequences were PCR-amplified by M5 Magic High-Fidelity DNA Polymerase (MF740,
833 Mei5Bio) from plant cDNA or virus vector. The Mut Express II Fast Mutagenesis Kit
834 V2 (C214-01, Vazyme) was used to construct mutants. PCR products were purified by
835 HiPure Gel Pure DNA Mini Kit (D2111-02, Magen, China). The YFP fluorescence

836 signal for each combination was detected by an inverted confocal microscope (Leica
837 SP8) at 48 hpi. Confocal images were analyzed with Leica LAS X (3.3). Anti-GFP
838 (ab290, Abcam) or anti-Rabbit (HA1001, HUABIO) antibodies were used at a 1:3000
839 dilution for immunoblot analysis. For LCI assays, the full-length cDNA sequences of
840 *CMV1a*, *CMV1a-H*, *CMV1a-M*, and *NAC2* were cloned into nLUC vector and cLUC
841 vector by ligation-independent cloning assay. The luciferase substrate (luciferin) was
842 smeared onto the surface of leaves and the luciferase activity was captured with a
843 PlantView100 assay system (BLT PHOTON TECHNOLOGY). All experiments were
844 independently repeated at least three times.

845 **Co-immunoprecipitation (Co-IP) Assay**

846 The constructs were transiently co-expressed with indicated combinations in *Nb* leaves.
847 At 48 hpi, the leaves were taken for protein extraction. Protein extracts were
848 immunoprecipitated by RFP-Trap beads (rtma, ChromoTek) in Co-IP buffer [50 mM
849 Tris-HCl, pH 7.5, 150 mM NaCl, 10% glycerol, 0.5 mM EDTA, 0.5% NP-40, 5 mM
850 DTT, 1 mM PMSF and protease inhibitor cocktail (Roche)]. The mixture was incubated
851 for 3 hours at 4°C. The immunoprecipitates were then denatured by 2× protein loading
852 buffer contains β-mercaptoethanol (JS0150, Hong Kong JSENB International Trading
853 Co., Ltd) and separated in a 10% SDS-PAGE gel (M00664, GenScript) followed by
854 immunoblot analysis. Anti-MYC (M20002M, Abmart), anti-Mouse (AS003, ABclonal),
855 anti-RFP (5f8-100, ChromoTek), or anti-Rat (AS028, ABclonal) antibodies were used
856 at a 1:3000 dilution for immunoblot analysis. StarSignal Chemiluminescent Assay Kit
857 (E171-01, GenStar) was used for signal detection. All experiments were repeated three
858 times and generated similar results.

859 **RNA-seq Analysis**

860 RNAs extracted from 5-week-old WT and *nac2* plants (without any treatment) were
861 used for RNA-seq analysis. To examine the expression profiles of the WT and *nac2*
862 plants in response to aphids-attack, RNAs extracted from 3-week-old WT and *nac2*
863 plants (2 days post aphid-infestation) were used for RNA-seq analysis. RNA-seq was
864 performed by Biomarker Technologies (www.biomarker.com.cn). Raw data were
865 processed by Illumina NovaSeq 6000 sequencer (2 × 150 bp read length) and aligned
866 to *Nb* reference genome (<https://solgenomics.net/>). Differential expression genes
867 (DEGs) were identified according to the transcripts per million reads (TPM)
868 ($\log_2(\text{foldchange}) \geq 1$ or ≤ -1 and $P \leq 0.05$). Furthermore, Gene Ontology (GO,
869 <http://www.geneontology.org>) and Kyoto Encyclopedia of Genes and Genomes (KEGG,
870 <http://www.genome.jp/kegg/>) were performed to identify DEGs that were significantly
871 enriched in GO terms and KEGG pathways.

872 **Electrophoretic Mobility Shift Assay (EMSA)**

873 GST and GST-NAC2 proteins were purified by Glutathione Sepharose 4B (GE
874 Healthcare) affinity chromatography and eluted by 10 mM GSH followed by size
875 exclusion chromatography (Superdex 200 10/300, GE Healthcare). Oligonucleotide
876 probes were labeled with the biotin by RUIBIO Technology Company. DdH₂O was
877 purified by Milli Q (Merck) water purification system. Chemiluminescent EMSA Kit
878 (GS009, Beyotime) was used for EMSA. The detailed protocol was described²¹. EMSA
879 experiments were repeated at least three times.

880 **Quantitative Real-Time PCR (qRT-PCR)**

881 Total RNA was extracted with TransZol reagent (TransGen Biotech) and retained in 1.5

882 mL sterile centrifugal tube (HRK-150-TP, Beijing Huaruikang Technology Co., Ltd).
883 Then 5 µg of RNA was reverse-transcribed into cDNA with TransScript One-Step
884 gDNA Removal and cDNA Synthesis SuperMix (AT311-02, TransGen Biotech). qRT-
885 PCR was performed with Hieff[®] qPCR SYBR Green Master Mix (11201ES08,
886 YEASEN). Data was normalized to *ACTIN* expression by the cycle threshold (CT) 2-
887 $\Delta\Delta^{CT}$ method as described⁶¹ and analyzed by Prism9 software (Graphpad). All
888 experiments were repeated at least three times. Primers used for qRT-PCR are listed in
889 [Supplementary Table 3](#). Data are represented as mean \pm s.d.

890 **Virus infection and analysis of viral RNA and protein**

891 For testing the effect of *NAC2* on virus infection, *nac2* and *Nb* plants were grown in
892 soil for 3-4 weeks and infected with CMV⁶², PVY-GFP⁶³, or TMV-GFP through agro-
893 infiltration. Viral symptoms were photographed at 6 days post-inoculation (dpi) for
894 CMV and TMV-GFP, 7 dpi for PVY-GFP. For performing virus infection in silenced
895 plants, 20-day-old WT or *samt1* plants were first agro-infiltrated with TRV-*NAC2* or
896 TRV, and the upper leaves were then infected with CMV or PVY-GFP at 14 days after
897 VIGS treatment. Viral symptoms were photographically recorded at 7 dpi for CMV and
898 PVY-GFP. Anti-CMV (CMV21-A, Alpha diagnostic international), anti-PVY
899 (POTY11-A, Alpha diagnostic international), anti-TMV (TMV11-A, Alpha diagnostic
900 international), anti-Rabbit (HA1001, HUABIO), or anti-Goat (BE0103, EASYBIO)
901 antibodies were used at a 1:3000 dilution for immunoblot analysis.

902 For testing whether and how MeSA is responsible for *NAC2*-mediated plant antiviral
903 defense. *nac2*, *samt1*, *sabp2*, and *Nb* plants were grown in soil for 3-4 weeks, and plants
904 were sprayed with control or solutions of 500 µM SA or MeSA a daily for 3 days prior
905 to infection with CMV or PVY-GFP as described^{64,65}. Systemic leaves were taken for
906 viral infection analysis at 6 or 7 dpi.

907 **Chromatin immunoprecipitation-quantitative (ChIP-qPCR)**

908 ChIP-qPCR was performed following the published protocol with minor
909 modifications⁶⁶. *Nb* leaves over-expressing HA-NAC2 or HA-nLUC were collected
910 and chromatin isolated from 2 g of frozen leaf tissue was sonicated with a Bioruptor
911 sonicator for 6 min. SimpleChIP[®] Plus Kit (9005S, CST) was used to perform the
912 experiment. Enrichment of promoter DNA was measured using the % input method by
913 qRT-PCR analysis as described⁶⁷. Amplification of *ACTIN2* promoter sequence served
914 as the negative control. Primers for the ChIP-qPCR assays are listed in [Supplementary](#)
915 [Table 3](#). ChIP-qPCR experiments were repeated for three times and all showed similar
916 results.

917 **Semi-*In Vivo* and *In Vivo* Protein Degradation**

918 For chemical treatments, MG132 (C2211, Sigma), cycloheximide (CHX) (HY-12320,
919 MedChemExpress), and ATP (IA1310, Solarbio) were used. MG132 and CHX were
920 dissolved in DMSO and used at a concentration of 100 μ M. ATP was used at the
921 concentration of 10 mM.

922 For semi-*in vivo* protein degradation analysis, *Nb* leaves over-expressing CMV1a-
923 MYC, CMV1a^{G983D}-MYC, RFP-NAC2, or cLUC-MYC were collected after 2 days
924 post-infiltration. The proteins were respectively extracted with extraction buffer (50
925 mM Tris-MES pH 8.0, 0.5 M sucrose, 1 mM MgCl₂, 10 mM EDTA, 5 mM DTT,
926 protease inhibitor cocktail) as described⁶⁸. For analysis of NAC2 degradation by the
927 26S proteasome, the plant extract harboring RFP-NAC2 was mixed with chemicals
928 containing CHX, then the extract was split equally into two tubes, one tube was added
929 into ATP, the other was added equal volume of extraction buffer. Two tubes were
930 incubated in the Eppendorf Thermomixer at 25°C, and extracts were respectively
931 removed from two tubes as samples at different time points. For analysis of CMV1a-

932 mediated promotion of NAC2 protein degradation via the ubiquitin-proteasome
933 pathway, the RFP-NAC2 extract was mixed with CMV1a-MYC, CMV1a^{G983D}-MYC,
934 or cLUC-MYC extracts in a ratio of 1:1 before incubation. ATP and CHX together with
935 MG132 or DMSO were added to the prepared leaf extracts. The samples were collected
936 at different time points.

937 For *in vivo* analysis of NAC2 or NES-NAC2 protein degradation by the 26S proteasome,
938 RFP-NAC2 or RFP-NES-NAC2 were transiently expressed in *Nb* leaves for 60 h. Then,
939 the agro-inoculated leaves were treatment with MG132 or an equal volume of DMSO
940 control solution for 12 hours before sampling. For *in vivo* analysis of NAC2 protein
941 degradation promoted by CMV1a, RFP-NAC2 were co-expressed with CMV1a-MYC,
942 CMV1a^{G983D}-MYC, or cLUC-MYC respectively for 60 hours before MG132 treatment
943 for 12 hours.

944 **SA-MeSA competition binding assay**

945 [³H]SA binding of SABP2 was performed as described with modifications^{20,69}. The
946 GST-SABP2 proteins were expressed in *E. coli* and purified. The size exclusion
947 columns were prepared by adding 0.13 g of sephadex G-25 (GE healthcare) to QIAGEN
948 shredder columns. The columns were preequilibrated with reaction buffer (50 mM Tris-
949 HCl, 150 mM NaCl, pH 7.5) containing 0.1% Tween-20 overnight at 4°C, and excess
950 buffer was removed by spinning at 735 g for 2 min. The GST-SABP2 proteins were
951 respectively incubated with 10 nM, 20 nM, 50 nM, and 100 nM [³H]SA (50 Ci/mmol)
952 in reaction buffer on ice for 1 hour, and then loaded to the columns and centrifuged
953 immediately as above. The flow-through was collected and the radioactivity was
954 measured with 2 ml Ultima Gold™ AB Cocktails (PerkinElmer) by a scintillation
955 counter (MicroBeta2; PerkinElmer). For SA-MeSA competition binding experiments,
956 the GST-SABP2 proteins were incubated with 50 nM [³H]SA in the presence of 0 nM,

957 3 nM or 15 nM MeSA on ice for 1 hour. Binding activity of [³H]SA (50 Ci/mmol) by
958 SABP2 in the absence of competitor MeSA was set to 100%, and binding activity of
959 [³H]SA by SABP2 with 3 nM or 15 nM MeSA was calculated along with comparison.
960 GraphPad Prism 9.0 was used to construct nonlinear binding model of Michaelis-
961 Menten equation.

962 **Alignment of helicase domain**

963 Sequences of viral helicase domain-containing proteins were retrieved from Uniprot
964 (<https://www.uniprot.org/>). Multiple sequence alignments of proteins were done in
965 Jalview using Mafft (L-INS-i method) (<http://www.jalview.org/About>).

966 **Statistical analysis**

967 Statistical significance was determined by two-sided Student's *t*-test or one-way
968 ANOVA with Tukey's multiple comparisons test for multiple groups (≥ 3) of data. For
969 aphid attraction bioassays, data were statistically analyzed using the χ^2 test. For
970 analyzing aphid survival portion in two groups, data were statistically analyzed using
971 one-way ANOVA with least significant difference (LSD). Statistical analysis was
972 performed with GraphPad Prism 9.0 or IBM SPSS Statistics 26. Detailed statistical
973 analyses are explained in figure legends, and *P* values are indicated in figures or Source
974 Data. The chromatographic (TIC) data of each sample was exported by The Xcalibur
975 4.1 software. Graphics were drawn by GraphPad Prism 9.0 software. For quantification
976 analysis of blots, the intensities of bands were quantified with ImageJ software.

977 **Reporting summary**

978 Further information on research design is available in the Nature Research Reporting
979 Summary linked to this article.

980 **Data availability**

981 All data and materials needed to replicate the work are available. *NAC2.1*
982 (Niben101Scf01481g02006), *NAC2.2* (Niben101Scf07152g04032), *SAMT1*
983 (Niben101Scf05122g00005), and *SABP2* (Niben101Scf00034g00012) from *Nicotiana*
984 *benthamiana* genome database (<https://solgenomics.net/>). RNA-seq raw data have been
985 deposited in National Center for Biotechnology Information (NCBI) under Bioproject
986 PRJNA851626 (WT), PRJNA851854 (*nac2*), PRJNA955195 (WT-aphid), and
987 PRJNA955395 (*nac2*-aphid). Original data in graphs are shown in Source data files and
988 uncropped gel and immunoblotting images are shown in Supplementary Figure 1.
989 Source data are provided with this paper.

990 **References**

- 991 51. Ying, X.B. *et al.* RNA-Dependent RNA Polymerase 1 from *Nicotiana tabacum*
992 Suppresses RNA Silencing and Enhances Viral Infection in *Nicotiana*
993 *benthamiana*. *Plant Cell* **22**, 1358-1372 (2010).
- 994 52. Ma, X. *et al.* A Robust CRISPR/Cas9 System for Convenient, High-Efficiency
995 Multiplex Genome Editing in Monocot and Dicot Plants. *Molecular Plant* **8**,
996 1274-84 (2015).
- 997 53. Kessler, A. & Baldwin, I.T. Defensive function of herbivore-induced plant
998 volatile emissions in nature. *Science* **291**, 2141-2144 (2001).
- 999 54. Kost, C. & Heil, M. Herbivore-induced plant volatiles induce an indirect
1000 defence in neighbouring plants. *Journal of Ecology* **94**, 619-628 (2006).
- 1001 55. Ozawa, R., Arimura, G., Takabayashi, J., Shimoda, T. & Nishioka, T.
1002 Involvement of jasmonate- and salicylate-related signaling pathways for the
1003 production of specific herbivore-induced volatiles in plants. *Plant and Cell*
1004 *Physiology* **41**, 391-398 (2000).
- 1005 56. Feng, H. *et al.* Acylsugars protect *Nicotiana benthamiana* against insect
1006 herbivory and desiccation. *Plant Molecular Biology* **1-18**(2021).

- 1007 57. Fernandez-Calvino, L., Lopez-Abella, D., Lopez-Moya, J.J. & Fereres, A.
1008 Comparison of Potato virus Y and Plum Pox Virus transmission by two aphid
1009 species in relation to their probing behavior. *Phytoparasitica* **34**, 315-324
1010 (2006).
- 1011 58. Verdier, M., Chesnais, Q., Pirolles, E., Blanc, S. & Drucker, M. The cauliflower
1012 mosaic virus transmission helper protein P2 modifies directly the probing
1013 behavior of the aphid vector *Myzus persicae* to facilitate transmission. *PLoS*
1014 *Pathogens* **19**, e1011161 (2023).
- 1015 59. Xu, H.X. *et al.* A salivary effector enables whitefly to feed on host plants by
1016 eliciting salicylic acid-signaling pathway. *Proceedings of the National Academy*
1017 *of Sciences of the United States of America* **116**, 490-495 (2019).
- 1018 60. Wang, Y. *et al.* Geminiviral V2 Protein Suppresses Transcriptional Gene
1019 Silencing through Interaction with AGO4. *Journal of Virology* **93**, e01675-18
1020 (2019).
- 1021 61. Livak, K.J. & Schmittgen, T.D. Analysis of relative gene expression data using
1022 real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25**,
1023 402-8 (2001).
- 1024 62. Wang, X.B. *et al.* The 21-nucleotide, but not 22-nucleotide, viral secondary
1025 small interfering RNAs direct potent antiviral defense by two cooperative
1026 argonautes in *Arabidopsis thaliana*. *Plant Cell* **23**, 1625-38 (2011).
- 1027 63. Geng, C. *et al.* Tobacco vein banding mosaic virus 6K2 Protein Hijacks
1028 NbPsbO1 for Virus Replication. *Scientific Report* **7**, 43455 (2017).
- 1029 64. Mayers, C.N., Lee, K.C., Moore, C.A., Wong, S.M. & Carr, J.P. Salicylic acid-
1030 induced resistance to Cucumber mosaic virus in squash and *Arabidopsis*
1031 *thaliana*: Contrasting mechanisms of induction and antiviral action. *Molecular*
1032 *Plant-Microbe Interactions* **18**, 428-434 (2005).
- 1033 65. Bi, H.H., Zeng, R.S., Su, L.M., An, M. & Luo, S.M. Rice allelopathy induced
1034 by methyl jasmonate and methyl salicylate. *Journal of chemical ecology* **33**,
1035 1089-103 (2007).
- 1036 66. Saleh, A., Alvarez-Venegas, R. & Avramova, Z. An efficient chromatin
1037 immunoprecipitation (ChIP) protocol for studying histone modifications in
1038 *Arabidopsis* plants. *Nature Protocol* **3**, 1018-25 (2008).
- 1039 67. Yamaguchi, N. *et al.* PROTOCOLS: Chromatin Immunoprecipitation from
1040 *Arabidopsis* Tissues. *Arabidopsis Book* **12**, e0170 (2014).
- 1041 68. Liu, L. *et al.* An efficient system to detect protein ubiquitination by
1042 agroinfiltration in *Nicotiana benthamiana*. *Plant Journal* **61**, 893-903 (2010).

1043 69. Slaymaker, D.H. *et al.* The tobacco salicylic acid-binding protein 3 (SABP3) is
1044 the chloroplast carbonic anhydrase, which exhibits antioxidant activity and
1045 plays a role in the hypersensitive defense response. *Proceedings of the National*
1046 *Academy of Sciences of the United States of America* **99**, 11640-11645 (2002).

1047 **Acknowledgments**

1048 We thank Le Kang for his constructive suggestions, Xiangdong Li and Xianbing Wang
1049 for providing PVY-GFP and CMV (CMV-Fny and CMV Δ 2b, the latter is a CMV *2b*
1050 gene deletion mutant) infectious clones respectively, Pingzhi Zhao and Jian Ye for
1051 assistance in measurement of SA amount, De Li for her assistance in the radiolabeling
1052 experiments. We thank Facility Center of Metabolomics and Lipidomics of China
1053 National Center for Protein Sciences for GC-MS analysis. This work was supported in
1054 part by National Key R&D Program of China (2022YFD1400800 and
1055 2021YFD1400400), the National Natural Science Foundation of China (32130086,
1056 31920103013, and 31872636), the Young Elite Scientists Sponsorship Program by
1057 CAST (2022QNRC001), and Shuimu Tsinghua Scholar Program.

1058 **Author contributions**

1059 Y.L. conceived the research. Q.G., Y.J.W., and Y.L. designed all experiments. Q.G.
1060 and Y.J.W. performed all aphid choice bioassays, SA-MeSA competition binding
1061 experiments, semi-*in vivo* and *in vivo* protein degradation experiments, ChIP-qPCR,
1062 EMSA, GC-MS analysis experiments, virus infection and analysis of viral RNA and
1063 protein experiments, and aphid survival as well as virus-transmission analysis. Y.J.W.,
1064 L.H. and F.H. performed BiFC, Y1H and LCI experiments. Y.J.W., D.Z., Y.W., and
1065 X.W. generate transgenic plants. Q.G., L.L., and F.C. performed EPG analysis. Y.J.W.,

1066 H.M., and H.D. performed Mass Spectrometry analysis. Q.G., Y.J.W., Y.H., and Y.L.
1067 analyzed data, wrote paper with input from all authors.

1068 **Competing interests**

1069 The authors declare no competing interests.

1070 **Extended data are available for this paper**

1071 Extended Data Figs. 1-10

1072 **Additional information**

1073 Supplementary Table 1-3

1074 **Extended data figures**

1075 **Extended Data Fig. 1 | NAC2 interacts with CMV1a and is required for plant**
1076 **antiviral defense and MeSA mediated aphid repellence.**

1077 **a**, Representative LC-MS/MS spectrum of peptides in NAC2 protein. A peptide
1078 (**AGIAQDAFVLCR**) is shown. **b-d**, Confirmation of the CMV1a-NAC2 interaction.
1079 In Co-immunoprecipitation (Co-IP) assay (**b**), cLUC-MYC or CMV1a-MYC was co-
1080 expressed with RFP-NAC2 in *Nb* leaves and analyzed 2 days post-infiltration (dpi).
1081 Both Bimolecular fluorescence complementation (BiFC) (**c**) and firefly luciferase
1082 complementation imaging (LCI) assays (**d**) further confirmed CMV1a-NAC2
1083 interaction in *Nb* leaves. Scale bar = 25 μ m. **e-g**, Viral symptoms in WT and *nac2* plants
1084 infected with CMV at 6 dpi (**e**), and the relative accumulation of CMV RNA (**f**) or CP
1085 (**g**) in CMV-infected systemic leaves of WT or *nac2* plants. **h-j**, Viral symptoms in WT
1086 and *nac2* plants infected with PVY-GFP at 7 dpi (**h**), and the accumulation of PVY
1087 RNA (**i**) or CP (**j**) in PVY-GFP infected systemic leaves of WT or *nac2* plants. **k-m**,
1088 Viral symptoms in WT and *nac2* plants infected with TMV-GFP at 6 dpi (**k**), and the
1089 relative accumulation of TMV RNA (**l**) or CP (**m**) in TMV-GFP infected systemic
1090 leaves of WT or *nac2* plants. **n, o**, *nac2* plants exhibited higher attractiveness to aphids

1091 than WT plants in circular-dish (**n**) or Y-tube olfactometer bioassays (**o**). Numerals
1092 shown inside each bar present number of choice-making aphids. **p, q**, *nac2* plants
1093 smeared with MeSA containing lanolin exhibited similar attractiveness to aphids with
1094 WT plants under same treatment in circular-dish bioassay (**p**) or Y-tube olfactometer
1095 bioassays (**q**). **r, s**, *nac2* plants smeared with lanolin alone or with 3,3-dimethyl-hexane
1096 containing lanolin exhibited higher attractiveness to aphids than WT plants under same
1097 treatment in circular-dish (**r**) or Y-tube olfactometer bioassays (**s**). **t, u**, Volatile MeSA
1098 treatment caused WT plants more repellent to aphids in circular-dish bioassay (**t**) or Y-
1099 tube olfactometer bioassays (**u**). **v, w**, No significant difference in aphid repellence
1100 between *nac2* plants with and without volatile MeSA treatment in circular-dish (**v**) or
1101 Y-tube olfactometer (**w**) bioassays. **x, y**, After volatile MeSA treatment followed by
1102 ventilation, *nac2* plants showed higher attractiveness to aphids than WT plants in
1103 circular-dish (**x**) or Y-tube olfactometer (**y**) bioassays. **f, i, l**, Two-sided Student's *t*-test,
1104 $n=3$ biologically independent samples. Data are shown as mean \pm s.d.. **n-y**, χ^2 test (df=
1105 1). All *P* values are shown in figure. Experiments were repeated at least three times
1106 with similar results.

1107 **Extended Data Fig. 2 | GC-MS analysis of VOCs emitted from WT and *nac2***
1108 **plants.**

1109 **a-d**, Direct sequencing of PCR product containing targeted sites in CRISPR/Cas9-
1110 edited knockout *nac2*, *samt1*, and *sabp2* homozygous plants. The rectangular area
1111 indicates the start positions at or from which the mutations occurred. It is worth
1112 mentioning that there is only one *SAMT1* or *SABP2* copy in *N.benthamiana* genome
1113 although *Nb* is allotetraploid. **e**, GC-MS analysis of VOCs emitted from WT and *nac2*
1114 plants after 48 h aphid feeding. Wet weight per plant was 1.10 g on average.
1115 Identifications based on retention indices and GC-MS: (1) oxalic acid, allyl hexyl ester;
1116 (2) bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-; (3) benzene, 1,2,3-
1117 trimethyl-; (4) 6-methyl heptanoate; (5) butyl pyruvate; (6) benzene, 1,2,3,5-
1118 tetramethyl-; (7) methyl salicylate; (8) tridecane, 4-methyl-; (9) 3,3-dimethyl-hexane;
1119 (10) Cyclohexasiloxane, dodecamethyl-; (11) bicyclo[3.1.1]heptane, 6,6-dimethyl-3-

1120 methylene-; (12) heptane, 2,2,3,3,5,6,6-heptamethyl-; (13) heptane, 2,3,6-trimethyl-;
1121 (14) 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate; (15) phenol, 2,6-bis(1,1-
1122 dimethylethyl)-4-(1-methylpropyl)-. **f**, The mean amounts of volatile MeSA collected
1123 from the aphid-attacked plants in GC-MS analysis. **g, h**, Mass spectrum of putative
1124 MeSA produced by plants (**g**) and the authentic MeSA standard (**h**). **i, j**, Comparison of
1125 the aerial MeSA concentrations in chambers containing either MeSA dissolved lanolin
1126 paste or aphid attacked WT plants by GC-MS analysis. Experiments were repeated at
1127 least three times with similar results.

1128 **Extended Data Fig. 3 | NAC2 binds to *SAMT1* promoter to activate its**
1129 **transcription.**

1130 **a**, Emitter and receiver plants were placed on two trays at a distance of 30 cm from each
1131 other in an interplant communication assay set-up in open-air environment. Each
1132 emitter was fed with fifty virus-free or viruliferous *M. persicae* aphids or no aphids
1133 before the emitters and receivers were incubated in same cage (100 cm × 70 cm ×
1134 70 cm) made by gauze. After 3 days, the receiver plants were taken out for further
1135 experiments. **b**, NbNAC2 and AtNAC2 share similar conserved motifs. **c**, RFP-tagged
1136 NAC2 showed nuclear localization, scale bar = 20 μm. **d, e**, qRT-PCR quantification of
1137 relative *SAMT1* mRNA levels in *nac2* and WT plants (**d**) or leaves transiently over-
1138 expressing HA-NAC2 and HA-nLUC (**e**). **f**, Transient expression assays. *NAC2*
1139 activates luciferase reporter gene transcription under the control of the *SAMT1* promoter
1140 (*SAMT1pro*) in *Nb* leaves. Photograph was taken at 48 hours post-infiltration (hpi). **g**,
1141 *In planta* ChIP-qPCR. HA-NAC2, but not HA-nLUC, specifically binds to the *SAMT1*
1142 promoter DNA. Chromatin from plants expressing HA-nLUC or HA-NAC2 were
1143 immunoprecipitated and amplified with promoter-specific primers. **h**, Yeast one-hybrid
1144 assay. Yeast cells were co-transformed with an effector vector containing the *SAMT1pro*
1145 cloned into pHis2 vector and a prey vector encoding NAC2 cloned into pGADT7. **i**, *In*
1146 *vitro* EMSA. Hot probe is the biotin-labeled NAC2-binding motif DNA of *SAMT1pro*,
1147 cold probe or cold mutant probe is the unlabeled NAC2-binding motif DNA of
1148 *SAMT1pro* or its mutant DNA. **j**, Transient over-expression of *NAC2* increases MeSA

1149 production in plants, the samples were collected at 48 hpi. **d, e, g, j**, Two-sided
1150 Student's *t*-test, *n*=3 biologically independent samples. Data are shown as mean ± s.d.;
1151 n.s., no statistical significance. All *P* values are shown in figure. Experiments were
1152 repeated at least three times with similar results.

1153 **Extended Data Fig. 4 | RNA-seq transcriptome analysis of WT and *nac2* plants.**

1154 **a**, Comparison of WT and *nac2* plants (without any treatment) RNA-seq sequences on
1155 the reference genome. The Phred quality score Q20 (99% base call accuracy) and Q30
1156 (99.9% base call accuracy) were used to measure the quality of RNA sequencing. **b**,
1157 Hierarchical clustered heat map of 90 differential expressed genes (DEGs, 24-up genes
1158 and 66-down genes) based on the \log_2 (fold change) in transcript levels of WT and *nac2*
1159 plants. **c**, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of 90
1160 DEGs in (**b**), the module with the most enriched genes is “metabolism” (green color,
1161 marked by red font). **d**, Gene ontology (GO) analysis of 90 DEGs in (**b**), the “biological
1162 process” module (green color) with the most enriched genes is “metabolism process”
1163 (marked by red font). **e**, Phylogenetic analysis of SAMT1 proteins from various plant
1164 species. NtSAMT1, *Nicotiana tabacum* SAMT1 (GenBank ID: FJ015052); SISAMT1,
1165 *Solanum lycopersicum* SAMT1 (GenBank ID: NM_001247880); AtBSMT1,
1166 *Arabidopsis thaliana* BSMT1 (Tair ID: AT3G11480); AtGAMT1 (Tair ID:
1167 AT4G26420); AtIAMT1 (Tair ID: AT5G55250). **f**, Comparison of WT (aphid) and *nac2*
1168 (aphid) plants (under aphid attack) RNA-seq sequences on the reference genome. **g**,
1169 Hierarchical clustered heat map of 157 DEGs (100-up genes and 57-down genes) based
1170 on the \log_2 (fold change) in transcript levels of WT (aphid) and *nac2* (aphid) plants. **h**,
1171 KEGG pathway analysis of 157 DEGs in (**g**), the module with the most enriched genes
1172 is “metabolism” (green color, marked by red font). **i**, GO analysis of 157 DEGs in (**g**),
1173 the “biological process” module (green color) with the most enriched genes is
1174 “metabolism process” (marked by red font). All DEGs were identified according to the
1175 transcripts per million reads (TPM) (\log_2 (foldchange) ≥ 1 or ≤ -1 and $P \leq 0.05$).

1176 **Extended Data Fig. 5 | *NAC2* regulates *SAMT1* to fulfill anti-CMV and PVY**
1177 **function.**

1178 **a-e**, Analysis of antiviral role of *NAC2* and *SAMT1* during CMV infection in *nac2*,
1179 *samt1*, or *nac2/samt1* double mutants. Viral symptoms (**a**), relative *NAC2* mRNA levels
1180 in *NAC2*-silenced WT (**b**) or *NAC2*-silenced *samt1* plants (**c**), the accumulation of CMV
1181 RNA (**d**) or CP protein (**e**), and plant endogenous MeSA amount (**f**, **g**) of WT, *nac2*,
1182 *samt1*, or *nac2/samt1* double mutant plants infected with CMV. **h-l**, Analysis of
1183 antiviral role of *NAC2* and *SAMT1* during PVY-GFP infection in *nac2*, *samt1*, or
1184 *nac2/samt1* double mutants. Viral symptom (**h**), relative *NAC2* mRNA levels in *NAC2*-
1185 silenced WT (**i**) or *NAC2*-silenced *samt1* plants (**j**), PVY RNA accumulation (**k**) or PVY
1186 CP (**l**) of *nac2*, *samt1*, or *nac2/samt1* double mutant plants infected with PVY-GFP.
1187 Plants in panels (**a** and **h**) were photographed at 7 days post-inoculation (dpi). *NAC2*-
1188 knockdown (KD) was triggered by VIGS vector TRV-*NAC2* to mimic *nac2* mutant
1189 while TRV was used as negative control in these experiments. **b-d**, **f**, **g**, **i-k**, Two-sided
1190 Student's *t*-test, *n*=3 biologically independent samples. Data are shown as mean \pm s.d..
1191 All *P* values are shown in figure. Experiments were repeated at least three times with
1192 similar results.

1193 **Extended Data Fig. 6 | CMV1a suppresses MeSA-mediated AD, and CMV1a^{G983D}**
1194 **impairs its interaction with NAC2.**

1195 **a**, Transgenic *CMV1a-MYC* or *CMV1a^{G983D}-MYC* expression *Nb* plant showed normal
1196 growth and development. **b**, *CMV1a-MYC* and *CMV1a^{G983D}-MYC* were detected in
1197 transgenic plants by western blot. Rubisco was used as loading control. **c**, **d**, Transgenic
1198 *CMV1a* plants exhibited higher attractiveness to aphids than WT plants in circular-dish
1199 (**c**) or Y-tube olfactometer (**d**) bioassays. **e**, EPG analysis showed that the number of pd
1200 of individual aphids was more in *CMV1a* plants than WT plants. *n*=19 individual
1201 aphids. **f-h**, Accumulation of CMV RNA (**f**), CP (**g**) or proportion of living aphids (**h**)
1202 in CMV-carrying aphid-attacked leaves of WT receivers (WT-R) with non-aphid-
1203 attacked WT plants as mock emitters (WT-mE), virus-free aphid-attacked WT plants as
1204 emitters (WT-AE), non-aphid-attacked transgenic *CMV1a* plants as mock emitters (1a-
1205 mE), or virus-free aphid-attacked transgenic *CMV1a* plants as emitters (1a-AE). **i**, **j**,
1206 WT-R (WT-mE) plants exhibited higher attractiveness than WT-R (WT-AE) plants (**i**),

1207 but WT-R plants showed similar attractiveness to aphids when non-aphid-attacked or
1208 virus-free aphid-attacked CMV1a plants were used as emitters (1a-mE or 1a-AE) (**j**) in
1209 Y-tube olfactometer bioassays. **k**, WT-R plants nearby virus-free aphid-attacked
1210 CMV1a plants as emitters (1a-AE) exhibited higher attractiveness to WT-R plants close
1211 to virus-free aphid-attacked WT plants as emitters (WT-AE). **l**, LCI assay to show that
1212 CMV1a helicase domain (1a-H), but not methyltransferase domain (1a-M), interacts
1213 with NAC2. **m**, AlphaFold-Multimer predicted CMV1a-H-NAC2 interacting complex
1214 (parameters: “MMseqs2” and “AlphaFold2-Multimer-v2” pattern). Colours are given
1215 based on AlphaFold-Multimer-calculated prediction score: pLDDT. Protein structures
1216 with scores over 90 are represented in blue (very high confidence of prediction); scores
1217 between 70 and 90 in light blue (high confidence); scores between 50 and 70 in yellow
1218 (low), and anything below 50 in orange (very low confidence of prediction). **n**, Red and
1219 blue indicate the CMV1a-H and NAC2, respectively. The stick model represents the
1220 potential interacting site between 1a-H and NAC2, this region is predicted with a high
1221 confidence score. **o**, Co-IP assay to show that G983D mutation in CMV1a helicase
1222 domain impairs the 1a helicase domain-NAC2 interaction. **p**, **q**, Co-IP assay (**p**) and
1223 BiFC assay (**q**) to show that CMV1a^{G983D} failed to interact with NAC2 in *Nb*. Scale bar
1224 = 25 μ m. **e**, **f**, Two-sided Student’s *t*-test. **h**, One-way ANOVA with Tukey’s multiple
1225 comparisons test; letters A-B represent statistically different groups ($P < 0.05$). **f**, **h**, $n=3$
1226 biologically independent samples. Data are shown as mean \pm s.d.; n.s., no statistical
1227 significance. **c**, **d**, **i-k**, χ^2 test (df = 1). *P* values are shown in **c-f**, **i-k**; adjusted *P* values
1228 for **h** are shown in the Source Data. In box plot (**e**), the centre line represents the median,
1229 box edges delimit bottom and top quartiles and whiskers show the highest and lowest
1230 data points. Experiments were repeated at least three times with similar results.

1231 **Extended Data Fig. 7 | CMV1a re-localizes and degrades NAC2 by 26S-**
1232 **proteasome system, and CMV1a^{G983D} possesses a weakened aphid repellence.**

1233 **a**, CMV1a but not CMV1a^{G983D} partially changed NAC2 localization from nucleus to
1234 cytoplasm. **b**, CMV1a did not alter subcellular localization of RFP. **c**, **d**, Nuclear exit
1235 signal-tagged RFP-NAC2 (NES-NAC2) changed NAC2 localization to cytoplasm (**c**)

1236 and enhanced 26S-proteasome system-dependent degradation (**d**). Scale bar = 25 μ m
1237 in panels (**a-c**). **e**, Immunoblot assay of RFP protein levels. **f**, Immunoblots to show
1238 cLUC-MYC, CMV1a-MYC, and CMV1a^{G983D}-MYC protein levels with anti-MYC
1239 antibody. **g**, *In vivo* assay showing effects of MG132 and the CMV1a-NAC2 interaction
1240 on NAC2 protein stability. 100 μ M MG132 or an equal volume of DMSO (negative
1241 control) was infiltrated into leaves transiently co-expressing RFP-NAC2 with CMV1a-
1242 MYC, CMV1a^{G983D}-MYC, or cLUC-MYC for 12 hours before harvesting. **h**, *Semi-in*
1243 *vivo* assay to show that NAC2 protein stability is ATP-dependent. NAC2 protein levels
1244 were analyzed with anti-RFP antibody at different times following 100 μ M CHX
1245 treatment in the presence or absence of 10 mM ATP. **i**, *Semi-in vivo* assay to show that
1246 MG132 inhibits CMV1a-promoted NAC2 degradation. RFP-NAC2, cLUC-MYC,
1247 CMV1a-MYC, or CMV1a^{G983D}-MYC was transiently expressed in *Nb* leaves and
1248 extracted respectively. NAC2 degradation was performed as below: The RFP-NAC2
1249 protein extract was mixed with the cLUC-MYC, CMV1a-MYC, or CMV1a^{G983D}-MYC
1250 extracts in a 1:1 volume of 100 μ M CHX and 10 mM ATP, in the presence of 100 μ M
1251 MG132 or an equal volume of control DMSO. **j**, Effect of CMV1a on expression of
1252 luciferase reporter gene driven by the *SAMT1* promoter (*SAMT1pro*). Transient
1253 expression assays in *Nb* leaves to show that CMV1a but not CMV1a^{G983D} suppressed
1254 NAC2-mediated activation of the *SAMT1* promoter. Photographs were taken at 48 hpi.
1255 **k**, Transgenic plants expressing CMV1a, but not CMV1a^{G983D}, exhibited higher
1256 attractiveness to aphids than WT plants in Y-tube olfactometer bioassays. **l, m**, GC-MS
1257 analysis to show that transgenic plants expressing CMV1a, but not CMV1a^{G983D},
1258 emitted less volatized MeSA than WT plants once they were fed with virus-free aphids
1259 for 3 days. **n**, WT-R (WT-mE) plants exhibited higher attractiveness than WT-R (WT-
1260 AE) plants, WT-R plants showed similar attractiveness to aphids when non-aphid-
1261 attacked or virus-free aphid-attacked CMV1a-expressing plants were used as emitters
1262 (1a-mE or 1a-AE), whilst WT-R (1a^{G983D}-mE) plants exhibited higher attractiveness
1263 than WT-R (1a^{G983D}-AE) plants in Y-tube olfactometer bioassays. **m**, Two-sided
1264 Student's *t*-test, *n*=3 biologically independent samples. Data are shown as mean \pm s.d.;

1265 n.s., no statistical significance. **k, n**, χ^2 test (df = 1). All *P* values are shown in figure.
1266 Experiments were repeated at least three times with similar results.

1267 **Extended Data Fig. 8 | Alignment of helicase domain from aphid and non-aphid**
1268 **transmitted viruses.**

1269 BCMNV, *Bean common mosaic necrosis virus* (Uniprot ID: Q65399); BYMV, *Bean*
1270 *yellow mosaic virus* (Uniprot ID: P17765); MDMV, *Maize dwarf mosaic virus* (Uniprot
1271 ID: J7II85); PPV, *Plum pox potyvirus* (Uniprot ID: P13529); PRSV, *Papaya ringspot*
1272 *virus* (Uniprot ID: A0A1L2DBW1); PVY, *Potato virus Y* (Uniprot ID: A0A5J6BDG4);
1273 PeMV, *Pepper mottle virus* (Uniprot ID: Q01500); PVMV, *Pepper veinal mottle virus*
1274 (Uniprot ID: A0A6J4A295); SCMV, *Sugarcane mosaic virus* (Uniprot ID:
1275 A0A0K0Y0R3); SMV, *Soybean mosaic virus* (Uniprot ID: Q90069); TEV, *Tobacco*
1276 *etch virus* (Uniprot ID: P04517); TuMV, *Turnip mosaic virus* (Uniprot ID: Q9IC12);
1277 TVBMV, *Tobacco vein banding mosaic virus* (Uniprot ID: F5A3N8); ZYMV, *Zucchini*
1278 *yellow mosaic virus* (Uniprot ID: P18479); CLV, *Carnation latent virus* (Uniprot ID:
1279 A0A858Z687); CMV, *Cucumber mosaic virus* (Uniprot ID: P17769); PSV, *Peanut*
1280 *stunt virus* (Uniprot ID: P28726); TAV, *Tomato aspermy virus* (Uniprot ID: P28931);
1281 BYDV, *Barley yellow dwarf virus* (Uniprot ID: P29044); SbDV, *Soybean dwarf virus*
1282 (Uniprot ID: A0A6M8PRM6); AMV, *Alfalfa mosaic virus* (Uniprot ID: P03589); PEBV,
1283 *Pea early browning virus* (Uniprot ID: Q9WJD8); TRV, *Tobacco rattle virus* (Uniprot
1284 ID: Q9J942); PVX, *Potato virus X* (Uniprot ID: A0A7H1C8Y4); TMV, *Tobacco mosaic*
1285 *virus* (Uniprot ID: P03586); TYMV, *Turnip yellow mosaic virus* (Uniprot ID: P10358).

1286 **Extended Data Fig. 9 | PVY suppresses plant AD by CI-NAC2 interaction.**

1287 **a**, Alignment of helicase domain from multiple aphid- and non-aphid- transmitted
1288 viruses. Asterisk (*) indicates that the Glycine (G) amino acid residue is conserved
1289 among listed aphid-transmitted viruses. **b, c**, Accumulation of PVY RNA (**b**) or CP (**c**)
1290 in PVY-carrying aphid-attacked leaves of WT receivers (WT-R) with virus-free aphid-
1291 attacked WT plants as emitters (AE) or with non-aphid-attacked WT plants as mock
1292 emitters (mE) when these receiver plants were fed with PVY-containing aphids. **d, e**,

1293 GC–MS analysis of volatized MeSA in WT plants fed with virus-free aphids or PVY-
1294 carrying aphids for 3 days. **f-h**, Accumulation of PVY RNA (**f**), CP (**g**) or proportion of
1295 living aphids (**h**) in WT-R plants with PVY-containing aphid-attacked WT plants as
1296 emitters (APE) or WT-R (mE) plants when these receiver plants were fed with PVY-
1297 containing aphids. **i**, CMV and PVY infection changed NAC2 localization partially to
1298 cytoplasm. Scale bar = 25 μm . **j**, BiFC assay showing that PVY CI but not its mutant
1299 PVY CI^{G347D} or TMV 126KD, interacted with NAC2. Scale bar = 25 μm . **k**,
1300 Immunoblot assay of protein levels in BiFC assay. **l**, PVY CI but not its mutant PVY
1301 CI^{G347D} or TMV 126KD, changed NAC2 localization partially to cytoplasm, scale bar
1302 = 25 μm . **b, e, f**, Two-sided Student's *t*-test. **h**, One-way ANOVA with LSD. **b, e, f, h**,
1303 *n*=3 biologically independent samples. Data are shown as mean \pm s.d.; n.s., no statistical
1304 significance. The same letter A represents no statistical difference between samples (*P* >
1305 0.05). All *P* values are shown in figure. Experiments were repeated at least three times
1306 with similar results.

1307 **Extended Data Fig. 10 | Arms race among emitter and receiver plants, aphids,**
1308 **and viruses.**

1309 **a**, AD defends plants against aphids and viruses. When emitter plants are attacked by
1310 aphids, they can sense the aphid sap-sucking action and stimulate biosynthesis of SA
1311 that activates the NAC2-SAMT1 module to produce volatile MeSA, neighboring
1312 receiver plants perceive and convert volatile MeSA into SA by SABP2, which acts as
1313 the cue to trigger NAC2-SAMT1 module and elicit defense against aphids and viruses.
1314 **b**, Virus and aphid counterdefence. When emitter plants were attacked by viruliferous
1315 aphids, some aphid-transmitted viruses utilized their helicase-contained viral protein
1316 (for example, CMV1a and PVY CI) to subcellularly re-localize and destabilize NAC2,
1317 leading to suppression of NAC2-mediated plant airborne defense to facilitate aphid
1318 propagation and virus transmission. The graphical model was created with
1319 BioRender.com. In summary, we have exploited interplays among aphid, virus, VOC
1320 emitter, and receiver plants in a complexed pathosystem to dissect PPC and AD at
1321 genetic and molecular levels. Our study on deciphering AD also lays the

1322 groundbreaking work to empower VOCs as a novel bioinspired tool in defense of plants
1323 including agricultural and horticultural crops against insect infestation and virus
1324 epidemics.







