

Pathogen exploitation of an abscisic acid- and jasmonate-inducible MAPK phosphatase and its interception by *Arabidopsis* immunity

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Abstract

Phytopathogens promote virulence by, for example, exploiting signaling pathways mediated by phytohormones such as abscisic acid (ABA) and jasmonate (JA). Some plants can counteract pathogen virulence by invoking a potent form of immunity called effector-triggered immunity (ETI). Here, we report that ABA and JA mediate inactivation of the immune-associated MAP kinases (MAPKs), MPK3 and MPK6, in *Arabidopsis thaliana*. ABA induced expression of genes encoding the protein phosphatases 2C (PP2Cs), *HAI1*, *HAI2* and *HAI3*, through ABF/AREB transcription factors. These three HAI PP2Cs interacted with MPK3 and MPK6 and were required for ABA-mediated MPK3/MPK6 inactivation and immune suppression. The bacterial pathogen *Pseudomonas syringae* pv. *tomato* (*Pto*) DC3000 activates ABA signaling and produces a JA-mimicking phytotoxin, coronatine (COR) that promotes virulence. We found that *Pto* DC3000 induces *HAI1* through COR-mediated activation of MYC2, a master transcription factor in JA signaling. *HAI1* dephosphorylated MPK3 and MPK6 *in vitro* and was necessary for COR-mediated suppression of MPK3/MPK6 activation and immunity. Intriguingly, upon ETI activation, *A. thaliana* plants overcame the *HAI1*-dependent virulence of COR by blocking JA signaling. Finally, we showed conservation of induction of *HAI* PP2Cs by ABA and JA in other *Brassicaceae* species. Taken together, these results suggest that ABA and JA signaling pathways, which are hijacked by the bacterial pathogen, converge on the *HAI* PP2Cs that suppress activation of the immune-associated MAPKs. Also, our data unveil interception of JA signaling activation as a host counter-strategy against the bacterial suppression of MAPKs during ETI.

Significance

Pathogens cause disease by deploying virulence effectors that interfere with various host targets, whereas plants counteract pathogen virulence when invoking a potent immunity known as effector-triggered immunity (ETI). Little is known about the mechanism underlying this molecular battle between plant immunity and pathogen virulence. We find that the phytohormones abscisic acid and jasmonate (JA), whose signaling pathways are often exploited by pathogens, transcriptionally activate a common family of protein phosphatases that suppress immune-associated MAP kinases. We demonstrate that a bacterial pathogen exploits the JA-mediated suppression of MAP kinases by using a JA-mimic, whereas ETI blocks JA signaling to counteract this bacterial virulence. Our results highlight suppression and protection of MAP kinases as a molecular battle between pathogens and plants.

¥body

Plants have an innate immune system consisting of pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) that restricts microbial invasions (1, 2). In PTI, cell surface-localized pattern recognition receptors detect conserved microbial molecules, termed microbe-associated molecular patterns (MAMPs), leading to a diverse array of immune responses such as a burst of reactive oxygen species, cellular Ca^{2+} spikes, activation of MAP kinases (MAPKs) and production of phytohormones (2, 3). Signaling pathways mediated by these immune components form intricate networks that orchestrate defense reactions (2, 4). Pathogens deliver virulence effectors into the plant cell that interfere with immune signaling components, resulting in PTI suppression (5). Recognition of specific effectors by intracellular nucleotide-binding/leucine-rich repeat (NLR) receptors activates ETI, which counteracts pathogen virulence and restricts pathogen growth (6).

The highly-interconnected network structure of plant immune signaling pathways provides versatile immune regulation, but is sometimes exploited by pathogens. For instance, various strains of the bacterial pathogen *Pseudomonas syringae*, including the model strain *P. syringae* pv. *tomato* (*Pto*) DC3000, produce the phytotoxin coronatine (COR) that activates jasmonate (JA) signaling and promotes virulence (7-9). COR is a structural mimic of the biologically active form of JA, (+)-7-iso-jasmonoyl-L-Ile (JA-Ile). Perception of COR or JA-Ile by COI1 triggers proteasome-dependent degradation of JAZ proteins. This releases JAZ-mediated repression of transcription factors, including the master transcription factor MYC2 and its homologues MYC3 and MYC4 (10). Activation of MYC2 by COR contributes to its virulence function of antagonizing salicylic acid (SA)-mediated immunity against *P. syringae* by transcriptionally activating the NAC transcription factors ANAC019, ANAC055 and ANAC072, which repress the SA biosynthesis gene *SID2* and induce the

SA catabolism gene *BSMT1* (8). However, COR appears to have another virulence mechanism(s) in addition to SA suppression (8).

The phytohormone abscisic acid (ABA) plays a pivotal role in abiotic stress responses (11). ABA signaling is repressed by clade A protein phosphatases 2C (PP2Cs), which inhibit SNF1-related kinases 2 (SnRK2s) via dephosphorylation. Upon perception, the PYR/PYL/RCAR ABA receptors bind to clade A PP2Cs to release inhibition of SnRK2s. This leads to SnRK2-mediated phosphorylation and activation of a group of AREB/ABF transcription factors, including AREB1, AREB2 and ABF3, thereby regulating genes containing ABA responsive elements (ABREs) in their promoters (12). Interestingly, *Pto* DC3000 activates ABA biosynthesis and signaling in infected tissues through the actions of proteinaceous effectors delivered by the type III secretion system (T3SS) and COR (13, 14). Activation of ABA signaling antagonizes SA-mediated immunity against the bacterial pathogen (15).

Upon activation by upstream MAPK kinases, MAPKs modulate functions of a number of substrates via phosphorylation, thereby regulating diverse biological processes including plant immunity (16). The *Arabidopsis thaliana* MAPKs, MPK3 and MPK6, are activated during PTI and ETI and regulate diverse immune responses (16) .

Here, we report unprecedented interconnections between MAPK and ABA/JA signaling. ABA induces expression of the clade A PP2Cs *HAI1*, *HAI2* and *HAI3* through the AREB/ABF transcription factors. These HAI PP2Cs interact with MPK3 and MPK6 and are required for ABA-mediated MAPK inactivation and immune suppression. We found that *Pto* DC3000 induces *HAI1* through COR-mediated activation of MYC2, inactivating MPK3/MPK6 and suppressing immunity. Interestingly, the COR-mediated *HAI1* induction and immune suppression is counteracted upon ETI activation. Finally, we show that the transcriptional effects of ABA and JA on the *HAI* PP2Cs are conserved in other *Brassicaceae* species. Our results indicate that ABA and JA signaling pathways

converge on the *HAI* PP2Cs, suppressing the immune-associated MAPKs in *A. thaliana* and possibly in other *Brassicaceae* species. Additionally, our results reveal that blocking JA signaling is a mechanism by which ETI counteracts the bacterial suppression of MAPKs.

Results

ABA Suppresses MAPK Activation

The phosphorylation status of MPK3 and MPK6 changes dynamically during pathogen infection (16-19). In search of a regulator of MAPK activation during immunity, we found that an ABA deficient mutant, *aba2*, shows enhanced activation of MPK3 and MPK6 after treatment with flg22, a bacterial MAMP (Fig. 1A). Conversely, exogenous ABA inhibited flg22-triggered MPK3/MPK6 activation (Fig. 1B). The protein levels of MPK3 and MPK6 did not explain the inhibitory effects of ABA on MPK3/MPK6 activation (Fig. 1A and B), indicating that ABA affects the phosphorylation status of MAPKs. To explore the point of MAPK inactivation by ABA, we utilized the transgenic *A. thaliana* line expressing a constitutively-active form of MKK4 (MKK4DD), which phosphorylates MPK3 and MPK6, under a dexamethasone (DEX)-inducible promoter (20). ABA strongly inhibited MPK3/MPK6 activation by MKK4DD without dramatic changes of protein levels of MKK4DD, MPK3 and MPK6 (Fig. 1C). Thus, ABA inhibits MPK3/MPK6 activation.

HAI PP2Cs Are Responsible for ABA-Mediated MAPK Inactivation and Immune Suppression

The clade A PP2C ABI1 was shown to specifically inhibit MPK6 activation (21). However, this does not explain our observation that ABA suppresses activation of both MPK3 and MPK6. Among the clade A PP2Cs, HAI1, HAI2 and HAI3 play a distinctive role in ABA-regulated physiological responses (22). It was therefore

proposed that the HAI PP2Cs may have as-yet-unidentified dephosphorylation substrates in addition to SnRK2s (22). Consistent with previous research (23), we found that ABA induces expression of all three *HAI*s (Fig. S1A). ABA-mediated MPK3/MPK6 inactivation at 10 min after flg22 treatment was observed in the wild type, *hai1* and *abi2*, but compromised in *hai1 hai2 hai3* (Fig. 2A and Fig. S1B-D). We then tested whether HAI PP2Cs interact with MAPKs using bimolecular fluorescent complementation (BiFC) assays. Interactions of HAI1 or HAI2 with MAPKs were detected in the cytoplasm and nucleus, whereas interactions between HAI3 and MAPKs were observed exclusively in the nucleus (Fig. 2B). The sites of interactions were consistent with our and previous observations of the subcellular localization patterns of HAI PP2Cs and MAPKs (Fig. S2A-F) (24-26). Interaction of another PP2C phosphatase, ABI2, with MAPKs was not detected (Fig. 2B). Consistently, ABI2 was previously shown not to target MAPKs for dephosphorylation (27, 28). Immunoblot analysis confirmed the accumulation of MAPKs and PP2Cs (Fig. S2G and H). These results suggest that HAI PP2Cs interact with MPK3 and MPK6 and contribute redundantly to ABA-mediated MPK3/MPK6 inactivation.

To test whether HAI PP2Cs can directly dephosphorylate MAPKs, we produced recombinant HAI PP2Cs in *E. coli*. As reported previously (24), the full-length HAI1 was insoluble but the PP2C domain of HAI1 was soluble. HAI2 and HAI3 full-length proteins and PP2C domains could not be solubilized. Therefore, only the PP2C domain of HAI1 was purified as a GST fusion protein (GST-HAI1) and tested for its phosphatase activity toward MPK3 and MPK6 *in vitro*. Kinase-inactive His-tagged MPK3 and MPK6 (His-KIMPK3/MPK6) (29) were used to avoid MAPK autophosphorylation, which could disturb dephosphorylation assays. GST-HAI1 but not GST reduced the amounts of MPK3/MPK6 phosphorylated by MKK4DD (Fig. 2C). Thus, HAI1 directly dephosphorylates MPK3 and MPK6.

ABA has a negative impact on immunity against *Pto* DC3000 (13, 14). We found that exogenous ABA promotes *Pto* DC3000 growth in the wild type but not in *hai1 hai2 hai3* (Fig. S3A). The T3SS effector AvrPtoB increases ABA accumulation (14). *Pto* Δ AvrPto Δ AvrPtoB, which lacks AvrPtoB and its functionally-redundant effector AvrPto (30), triggered stronger MAPK activation than *Pto* DC3000 during infection (Fig. S3C and D). Promotion of bacterial growth, mediated by AvrPto/AvrPtoB, was significantly reduced in *hai1 hai2 hai3* and *aba2* (Fig. S3B). These results suggest that ABA-mediated MAPK inactivation through HAI PP2Cs may be one of the virulence functions of AvrPto/AvrPtoB.

Coronatine Suppresses MAPK Activation and Immunity through HAI1

We investigated if *HAI1*, *HAI2* and *HAI3* are induced during infection by *Pto* DC3000. As shown in Fig 3A, *HAI1* was highly induced at 6 and 24 hours post infiltration (hpi). Only slight induction of *HAI2* was detected at 24 hpi. No induction was observed for *HAI3*. Since all *HAI* PP2Cs are highly responsive to ABA within one hour (Fig. S1A), we speculated that a signal(s) other than ABA may be responsible for the predominant induction of *HAI1* by *Pto* DC3000. To address this, we compared the promoter sequences of *HAI* PP2Cs using the online tool Athena (31), and found a MYC2-binding motif (G box; CACGTG) in the promoter of *HAI1* but not those of *HAI2* and *HAI3* (Fig. 3D and Fig. S12). This finding, together with the fact that MYC2 is a master transcription factor in JA signaling (32), led us to hypothesize that the JA-mimicking phytoalexin COR is involved in *HAI1* induction by *Pto* DC3000. Indeed, *HAI1* induction was not detected in the wild type infected with a COR-deficient *Pto* mutant (*Pto* DC3118) (33) or in a *coil* mutant infected with *Pto* DC3000 (Fig. 3B). Moreover, COR or methyl jasmonate (MeJA) treatment induced *HAI1* but not *HAI2* and *HAI3* (Fig. S4A and C). The *HAI1* induction by COR or MeJA was compromised similarly in *myc2* and *myc2 myc3 myc4* (Fig. 3C and Fig. S4D). We then tested whether

MYC2 directly binds to the G box in the *HAI1* promoter *in planta* by chromatin immunoprecipitation (ChIP) using a transgenic *A. thaliana* line expressing the MYC2-GFP fusion protein driven by the 35S promoter (34). The ratio of the immunoprecipitated DNAs from MYC2-GFP plants to those from the control YFP-HA or wild type plants was determined by qPCR. After treatment with COR or MeJA, we found a significant enrichment of the promoter region surrounding the G-box motif, but not of a DNA segment in the coding sequence (CDS) used as a negative control (Fig. 3D and Fig. S4B and E). Taken together, these results show that *Pto* DC3000 induces *HAI1* through COR-mediated activation of MYC2.

MYC2 was originally identified as a transcription factor for ABA-regulated gene expression (35). We therefore tested whether MYC transcription factors also play a role in induction of *HAI* PP2Cs by ABA. No significant effects of *myc2* or *myc2 myc3 myc4* mutations were observed (Fig. S5A-C). In contrast, expression of the *HAI* PP2Cs was highly compromised in the *areb1 areb2 abf3* triple mutant (Fig. S5A-C). Recently, a large-scale ChIP-seq analysis demonstrated binding of AREB2 and ABF3 to the promoters of *HAI1*, *HAI2* and *HAI3* (36). We also observed wild-type like expression of *HAI1* in the *areb1 areb2 abf3* mutant after MeJA treatment (Fig. S5D). Thus, ABA and JA signaling pathways converge onto *HAI1* through AREBs and MYC2, respectively.

The ability of COR to induce *HAI1* suggests the possibility that MAPKs are virulence targets of COR. To test this, flg22-triggered MAPK activation was monitored in wild type and *hai1* seedlings pretreated with COR or mock for 6 h. We observed faster MAPK inactivation in COR-treated wild type seedlings (Fig. 4A and Fig. S6). This effect of COR was much weaker in *hai1* (Fig. 4A and Fig. S6). We then monitored MAPK activation over time in the wild type or *hai1* infected with *Pto* DC3000 or COR-deficient *Pto* DC3118. Faster activation of MPK3 and MPK6 was seen in *hai1* compared to the wild type during *Pto* DC3000 infection (Fig. S7A) and during infection

by *Pto* DC3118 compared to *Pto* DC3000 in the wild type (Fig. S7B). Based on these data and the ability of HAI1 to dephosphorylate MPK3 and MPK6 *in vitro*, we concluded that COR suppresses MPK3/MPK6 activation through HAI1 during *Pto* DC3000 infection.

MPK3 and MPK6 contribute redundantly to stomatal closure in response to *Pto* DC3000, which is reversed by COR (8, 37, 38). We found that *Pto* DC3000 is able to reopen stomata both in the wild type and *hai1* (Fig. S8), suggesting that HAI1-mediated MAPK inactivation is not essential for the COR virulence function that overcomes stomatal immunity.

To test the impact of COR-mediated MAPK inactivation on apoplastic immunity, we syringe-infiltrated *Pto* DC3000 or *Pto* DC3118 into leaves of the wild type, *mpk3*, *mpk6* and *hai1*. COR-mediated bacterial growth promotion was significantly reduced in *hai1* (Fig. 4B). Interestingly, the growth of *Pto* DC3118 was enhanced in *mpk3* (Fig. 4B). Overall, these results support that COR suppresses apoplastic immunity through HAI1-mediated MAPK inactivation.

ETI Overcomes the HAI1-Dependent Coronatine Virulence Effect

AvrRpt2 and AvrRpm1 are *P. syringae* effectors whose virulence actions are recognized by the NLR receptors RPS2 and RPM1, respectively, triggering ETI in Col (1). We found no significant induction of *HAI1* and other COR-responsive genes, *ANAC019/055/072*, during infection by *Pto* DC3000 carrying AvrRpt2 or AvrRpm1 (Fig. 3A and Fig. S9). A possible explanation for this observation is that ETI blocks activation of JA signaling by COR. We tested this possibility using *A. thaliana* lines carrying an estradiol-inducible *AvrRpt2* transgene (*XVE-AvrRpt2*) (17). *XVE-AvrRpt2* seedlings were treated with estradiol for 6 h, followed by mock or COR treatment for 1 h. Induction of *HAI1* and another JA-responsive gene, *VSP2*, was significantly lower in the wild type than in the *rps2* mutant background but comparable between the wild type

and the SA-deficient *sid2* mutant background (Fig. 5A). Consistent with this finding, HAI1-dependent bacterial growth promotion was no longer seen for *Pto* DC3000 carrying AvrRpt2 (Fig. 5B). Thus, ETI triggered by AvrRpt2 blocks JA signaling in an SA-independent manner and counteracts the HAI1-dependent virulence effect of COR.

Conservation of ABA- and JA-mediated regulation of the *HAI* PP2Cs in *Brassicaceae*

Our data showed that ABA and JA activate expression of specific members of *HAI* PP2Cs that suppress MAPKs in *A. thaliana* (Figs S1A, S4C, and S5). The importance of these transcriptional effects could be reflected by evolutionary conservation in plants. To address this, we used the amino acid sequences of *A. thaliana* HAI1, HAI2, HAI3 and other clade A PP2Cs to search for their possible orthologues in some *Brassicaceae* species, tomato and rice plants whose genome sequences and gene annotations are available. Construction of a phylogenetic tree suggested that the HAI1, HAI2 and HAI3 clades are restricted to *Brassicaceae* (Fig. S10). We therefore tested whether *HAI1*, *HAI2* and *HAI3* are responsive to ABA and JA in *Capsella rubella*, a close relative of *A. thaliana*, and in *Eutrema salsugineum*, a relatively phylogenetically distant species from *A. thaliana*. ABA induced all the *HAI* PP2Cs in both *C. rubella* and *E. salsugineum* (Fig. S11A and B). We found that the core ABRE motif (ACGTG) is present in the promoters of the *HAI* PP2Cs not only in *A. thaliana* but also in other *Brassicaceae* species including *C. rubella* and *E. salsugineum* (Fig. S12), suggesting that *Brassicaceae* species may commonly employ AREB/ABF transcription factors for the transcriptional regulation of the *HAI* PP2Cs by ABA. After MeJA treatment, rapid *HAI1* induction was observed in both *C. rubella* and *E. salsugineum* (Fig. S11C and D). The CACGTG G box motif is present in the *HAI1* promoters of the *Brassicaceae* species analyzed (Fig. S12A). Thus, MYC2 may be a key transcription factor for JA-mediated *HAI1* induction in *Brassicaceae* species as it is in *A. thaliana*. Overall, the

evolutionary conservation of the transcriptional effects of ABA and JA on the *HAI* PP2Cs suggests their importance in ABA- and JA-regulated processes in *Brassicaceae*.

Discussion

In this study, we showed that ABA and JA signaling suppresses activation of the immune-associated MAPKs, MPK3 and MPK6, through the HAI PP2Cs. This provides a new framework for explaining the negative impacts of ABA and JA on plant immunity. We demonstrated that the bacterial pathogen *Pto* DC3000 exploits JA-mediated MAPK suppression by producing COR. Since MPK3 and MPK6 are immune signaling components shared by PTI and ETI (2), a fundamental question is how ETI prevents pathogen suppression of the MAPKs. Here, we showed that the transcriptional induction of *HAI1* and the HAI1-dependent immune suppression by COR are prevented upon ETI activation, demonstrating that ETI overcomes pathogen targeting of MAPKs. Interception of JA signaling during ETI may also be relevant to sustained MAPK activation (17), as well as to inhibition of COR-mediated stomatal reopening (37).

ABA negatively impacts plant immunity to diverse pathogens, which can be attributed to ABA-mediated suppression of defense activation by immune hormones including JA, ET and SA (15). In this study, we demonstrated that ABA promotes MPK3/MPK6 inactivation and susceptibility to *Pto* DC3000 through HAI PP2Cs (Figs. 2A, S1 and S3A). Interestingly, in rice, ABA was shown to mediate MPK6 inactivation thereby suppressing rice blast resistance (39), although phosphatases of a different type are involved. Thus, MAPK inactivation by ABA is a convergent evolution in monocots and eudicots.

COR is a JA-mimicking phytotoxin produced by various strains of *P. syringae* (7). A virulence function of COR involves suppression of SA accumulation mediated by

the MYC2-ANAC019/055/072 signaling module (8). However, this mechanism does not fully account for the ability of COR to promote *P. syringae* growth in the apoplast (8). Here, we showed that COR directly induces expression of *HAI1* through MYC2 (Fig. 3C and D). *HAI1* showed phosphatase activity toward MPK3 and MPK6 (Fig. 2C) and was necessary for MPK3/MPK6 inactivation by COR (Figs 4A and S6). Importantly, *HAI1* was required for the promotion of *Pto* DC3000 growth in a COR-dependent manner (Fig. 4B). Thus, our results unveiled a novel mechanism by which COR suppresses MAPK activation and immunity through *HAI1*. Although *HAI1* was specifically induced by COR at an early stage of *Pto* DC3000 infection, ABA-responsive *HAI2* was also induced at a late stage (Fig. 3A). It is thus possible that pathogen-induced ABA contributes to MAPK suppression, as the lack of ABA-inducing effector AvrPtoB together with AvrPto resulted in enhanced MAPK activation (Fig. S3C and D). COR and pathogen-induced ABA may compensate virulence of *Pto* DC3000 lacking a functional HopAII effector, which inactivates MAPKs (40, 41).

Although PTI and ETI share immune signaling components, ETI is effective against pathogens that dampen PTI (2, 6). While *HAI1* was induced by *Pto* D3000, this virulence mechanism was prevented upon ETI activation by AvrRpt2 or AvrRpm1 (Fig. 3A). Moreover, ETI triggered by the AvrRpt2 effector in the absence of pathogen infection was sufficient to inhibit *HAI1* induction by COR (Fig. 5A). These results suggest that ETI blocks activation of JA signaling by COR. Our experiments do not rule out the possibility that ETI also affects COR production in the bacterial cell and/or COR delivery into the plant cell. Recently, Liu et al reported that expression of some JA-responsive genes is activated during ETI (42). The discrepancy between our and their studies could be explained by selective activation or repression of JA-responsive genes during ETI. Consistent with the ability of ETI to block *HAI1* induction by COR, *HAI1*-dependent growth promotion was not observed for *Pto* DC3000 carrying AvrRpt2

(Fig. 5B). These results are analogous to the previous report that AtMIN7, an immune component required for both PTI and ETI, is protected from degradation by the *P. syringae* effector HopM1 during ETI (43). ETI activation also leads to interference with ABA signaling, often exploited by pathogens for virulence (14, 44, 45). Collectively, these findings support an emerging idea that ETI counteracts pathogen virulence actions that directly or indirectly interfere with immune components, thereby conferring pathogen resistance.

We showed that ABA and JA induce specific sets of *HAI1*, *HAI2* and *HAI3* not only in *A. thaliana* but also in *C. rubella* and *E. salsugineum* (Figs. S1A, S4C and S11), suggesting their importance in ABA- and JA-regulated processes in *Brassicaceae* species. Notably, MPK3 and/or MPK6 are activated by ABA and JA and are implicated in ABA- and JA-regulated developmental and stress responses in *A. thaliana* (46, 47). Thus, transcriptional activation of the *HAI* PP2Cs may provide fitness advantages as regulatory modules of ABA and JA signaling pathways for controlling intensity and timing of MPK3/MPK6 activation in *A. thaliana* and possibly in other *Brassicaceae* species and it is sometimes exploited by pathogens. As a countermeasure, ETI can overcome pathogen manipulation of ABA and JA signaling pathways.

Materials and methods

Details of the materials and methods used are described in SI materials and methods, including plant materials, cloning procedures, MAPK assays, confocal microscopic analysis, gene expression analysis, stomatal aperture measurement, bacterial growth assays, ChIP, and phylogenetic analysis.

Footnotes

Author Contributions: A.M. and K.T. designed research; A.M., M.L.B., T.N., S.A., K.F.,

T.M.W., A.T., and K.T. performed research; A.M. and D.B. contributed new reagents or analytical tools; A.M and K.T analyzed data and wrote the paper.

The authors declare no conflicts of interest

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Figure legends

Fig. 1. ABA suppresses MAPK activation. (A) Seedlings of Col and *aba2* were treated with flg22 (1 μ M) for the indicated time periods. (B) Seedlings of Col were pre-treated with mock (0.1% EtOH) or ABA (10 μ M) for 6 h, followed by flg22 treatment (1 μ M) for the indicated time periods. (C) Leaves of 4 to 5-week-old MKK4DD plants were infiltrated with DEX (2 μ M) together with mock (0.1% EtOH) or ABA (10 μ M) and harvested at the indicated time points. Proteins were detected by immunoblotting using the indicated antibodies. Ponceau S-stained RuBisCo is shown as a loading control.

Fig. 2. HAI PP2Cs are responsible for ABA-mediated MAPK inactivation. (A) Seedlings of Col, *hai1*, *hai1 hai2 hai3* (*hai1/2/3*) were pre-treated with ABA (10 μ M) or mock for 6 h, followed by flg22 treatment (1 μ M) for 10 min. Proteins were detected by immunoblotting using the indicated antibodies. Ponceau S-stained RuBisCo is shown as a loading control. (B) BiFC analysis of interactions between HAI PP2Cs and MAPKs. HAI PP2Cs fused to the N-terminal half of YFP and MAPKs fused to the C-terminal half of YFP were expressed in *Nicotiana benthamiana* by *Agrobacterium* infiltration. ABI2 was used as a negative control. The pictures were taken at 3 days after infiltration. (Bars: 50 μ m) (C) HAI1 dephosphorylates MPK3 and MPK6 *in vitro*. Recombinant MAPKs were phosphorylated by GST-MKK4DD. Phosphorylated MAPKs (500 ng) were mixed with GST or GST-HAI1 (2 μ g) and incubated at 30°C for 1 h. Proteins were detected by immunoblotting using the indicated antibodies.

Fig. 3. *Pto* DC3000 induces *HAI1* through coronatine-mediated activation of JA signaling. (A) Leaves of 4 to 5-week-old Col plants were infiltrated with mock

(water), *Pto* DC3000, *Pto* AvrRpt2 or *Pto* AvrRpm1 ($OD_{600} = 0.001$) and harvested at 6 and 24 h after infiltration. (B) Leaves of 4 to 5-week-old Col or *coil* plants were infiltrated with mock (water), *Pto* DC3000 or COR-deficient *Pto* DC3118 ($OD_{600} = 0.001$) and harvested at 6 h after infiltration (B). (A and B) The expression levels of *HAI1*, *HAI2* and *HAI3* were determined by RT-qPCR. Bars represent means and standard errors of the \log_2 expression levels relative to *Actin2* calculated from three independent experiments using mixed linear models. Asterisks indicate statistically significant differences compared to mock at each time point (* $P < 0.05$; ** $P < 0.01$, two-tailed t-tests). (C) Seedlings of Col, *myc2*, and *myc2 myc3 myc4* (*myc2/3/4*) were treated with mock (DMSO) or COR (5 μ M) for the indicated time periods. The expression level of *HAI1* was determined by RT-qPCR. Bars represent means and standard errors of the \log_2 expression levels relative to *Actin2* calculated from two independent experiments using a mixed linear model. The Benjamini-Hochberg method was used to adjust P -values for multiple hypothesis testing. Groups not sharing any letters show statistically significant differences (Adjusted $P < 0.05$). (D) ChIP-qPCR was performed using the p35S::MYC2-GFP line. The G box motif located 193 bp upstream of the transcription start site of *HAI1* is shown by a tick. Bold gray horizontal lines show the regions amplified by different qPCR primers. Bars represent means and standard errors of the fold enrichment relative to YFP-HA plants set to 1, calculated from two independent experiments. Asterisks indicate statistically significant differences from YFP-HA plants ($P < 0.01$, two-tailed t-tests).

Fig. 4. HAI1 is required for coronatine-mediated MAPK inactivation and immune suppression. (A) Seedlings of Col and *hai1* were pretreated with 5 μ M COR or mock for 6 h, followed by treatment with 1 μ M flg22 for the indicated time periods. Proteins were detected by immunoblotting using the indicated antibodies. Ponceau S-stained

RuBisCo is shown as a loading control. (B) Leaves of Col, *mpk3*, *mpk6* and *hai1* were infiltrated with *Pto* DC3000 or *Pto* DC3118 COR⁻ (OD₆₀₀ = 0.0002). The bacterial titers at 2 dpi were measured. Bars represent means and standard errors of three independent experiments with at least 12 biological replicates in each experiment. The Benjamini-Hochberg method was used to adjust *P*-values for multiple hypothesis testing. Statistically significant differences are indicated by different letters (Adjusted *P* < 0.05). Asterisks indicate statistically significant differences of the differences (*P* < 0.01, two-tailed t-tests).

Fig. 5. Effector-triggered immunity counteracts the HAI1-dependent coronatine virulence effect. (A) Seedlings of the estradiol-inducible AvrRpt2 transgenic line in Col background or in *rps2* or *sid2* mutant backgrounds were treated with estradiol (10 μM) for 6 h, followed by COR treatment (5 μM) for 1 h. The expression levels of *HAI1* and *VSP2* were determined by RT-qPCR. Bars represent means and standard errors of the log₂ fold change relative to mock, calculated from three independent experiments using mixed linear models. Statistically significant differences are indicated by different letters (*P* < 0.01, two-tailed t-tests). (B) Leaves of Col, *hai1* and *abi2* were infiltrated with *Pto* DC3000 or *Pto* AvrRpt2 (OD₆₀₀ = 0.0002). The bacterial titers at 2 dpi were measured. Bars represent means and standard errors of four independent experiments with at least 8 biological replicates in each experiment. The Benjamini-Hochberg method was used to adjust *P*-values for multiple hypothesis testing. Statistically significant differences are indicated by different letters (Adjusted *P* < 0.01). Asterisks indicate statistically significant differences of the differences (*P* < 0.01, two-tailed t-tests).