

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


Fig. 2. HAI PP2Cs are responsible for ABA-mediated MAPK inactivation. (A) Seedlings of Col, hai1, ha1 hai2 hai3 (hai1/2/3) were pre-treated with ABA ( $10 \mu \mathrm{M}$ ) for 6 h , followed by flg22 treatment ( $1 \mu \mathrm{M}$ ) for 10 min . Proteins were detected by immunoblotting using the indicated antibodies. Ponceau S-stained RuBisCO was 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 $N$. benthamiana by Agrobacterium infiltration. ABI2 was used as a negative control. The pictures were taken at 3 days after infiltration. (Bars: $50 \mu \mathrm{~m}$ ) (C) HAl1 dephosphorylates MPK3 and MPK6 in vitro. Recombinant MAPKs were phosphorylated by GST-MKK4DD. Phosphorylated MAPKs ( 500 ng ) were mixed with GST or GST-HAl1 ( $2 \mu \mathrm{~g}$ ) and incubated at $30{ }^{\circ} \mathrm{C}$ for 1 h . Proteins were detected by immunoblotting using the indicated antibodies.


Fig. 3. Pto DC3000 induces HAl1 through coronatine-mediated activation of JA signaling. (A and B) Leaves of 4 to 5 -week-old Col plants were infiltrated with mock (water), Pto DC3000, Pto AvrRpt2 or Pto AvrRpm1 $\left(\mathrm{OD}_{600}=0.001\right)$ and harvested at 6 and 24 h after infiltration (A). Leaves of 4 to 5 -week-old Col or coi1 plants were infiltrated with mock (water), Pto DC3000 or COR-deficient Pto DC3118 $\left(\mathrm{OD}_{600}=0.001\right.$ ) and harvested at 6 h after infiltration (B). The expression levels of HAII, 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 points ( ${ }^{*} P<0.05$; ${ }^{* *} P<0.01$, two-tailed t -tests). (C) Seedlings of Col, $m y c 2$, and myc2 myc3 myc4 (myc2/3/4) were treated with mock (DMSO) or COR ( $5 \mu \mathrm{M}$ ) for the indicated time periods. The expression level of HAl1 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 correcting multiple hypothesis testing. Two groups not sharing any letters show statistically significant differences (Adjusted $P<0.05$ ). (D) ChIP-qPCR was performed using the p35S::MYC2GFP line. The G box motif located 193 bp upstream of the transcription start site of HA/1 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 the YFP-HA plants ( $P<0.05$, two-tailed t-tests).
A
Col
hai1

| Mock | COR |
| :---: | :---: |
| flg22 | flg22 |
|  | $\begin{aligned} & \text { 言 } \\ & \text { L } \\ & \text { ले } \\ & \text { ले } \end{aligned}$ |



| Anti－p44／42 MAPK | こニニニニ ニニニ二コ |  |
| :---: | :---: | :---: |
| Anti－MPK6 |  |  |
| Anti－MPK3 | － |  |
| PonceauS | $4-6-5-5-6$ |  |

B


Fig．4．HAl1 is required for coronatine－mediated MAPK inactivation and immune suppression．（A）Seedlings of Col and hai1 were pretreated with $5 \mu \mathrm{M}$ COR for 6 h ， followed by treatment with $1 \mu \mathrm{M}$ flg22 for the indicated time periods．Proteins were detected by immunoblotting using the indicated antibodies．Ponceau S－stained RuBisCo was shown as a loading control．（B）Leaves of Col，mpk3，mpk6 and hait were infiltrated with Pto DC3000 or Pto DC3118 COR $\left(\mathrm{OD}_{600}=0.0002\right)$ ．The bacterial titers at 0 or 2 dpi were measured．Bars represent means and standard errors of three independent experiments with at least 4 or 12 biological replicates for 0 dpi or 2 dpi in each experiment，respectively．The Benjamini－Hochberg method was used to adjust $p$－ values for correcting multiple hypothesis testing．Statistically significant differences are indicated by different letters（Adjusted $P<0.05$ ）．


Fig. 5. Effector-triggered immunity counteracts the HAl1-dependent coronatine virulence. (A) Seedlings of the estradiol-inducible AvrRpt2 transgenic line in Col background or in rps2 or sid2 mutant backgrounds were treated with estradiol ( $10 \mu \mathrm{M}$ ) for 6 h , followed by COR treatment ( $5 \mu \mathrm{M}$ ) for 1 h . The expression levels of HAl1 and VSP2 were determined by RT-qPCR. Bars represent means and standard errors of the $\log _{2}$ 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 0 or 2 dpi were measured. Bars represent means and standard errors of three independent experiments with at least 4 or 12 biological replicates for 0 dpi or 2 dpi in each experiment, respectively. The Benjamini-Hochberg method was used to adjust $p$-values for correcting multiple hypothesis testing. Statistically significant differences are indicated by different letters (Adjusted $P<0.05$ ).


Fig. S1. ABA-mediated MAPK inactivation depends on HAI PP2Cs. (A) Wild-type seedlings were treated with mock ( $0.1 \% \mathrm{EtOH}$ ) or ABA ( $10 \mu \mathrm{M}$ ) for the indicated time periods. The expression levels of HAI1, HAI2 and HAI3 were measured by RT-qPCR. Bars represent means and standard errors of the $\log _{2}$ expression levels relative to Actin2 calculated from two independent experiments using mixed linear models. Asterisks indicate statistically significant differences from the corresponding controls ( ${ }^{*} P<0.01$, two-tailed t-tests). (B-D) Seedlings of Col, hai1/2/3 and abi2 (loss-of-function abi2-2 mutant) were pre-treated with mock ( $0.1 \% \mathrm{EtOH}$ ) or ABA ( $10 \mu \mathrm{M}$ ) for 6 hours, followed by flg22 treatment $(1 \mu \mathrm{M})$ for the indicated time periods. Phosphorylated MAPKs were detected by immunoblotting. Ponceau S-stained RuBisCo is shown as a loading control. Results of three independent experiments are shown (B-D).


Fig. S2. HAI PP2Cs co-localize with MAPKs. (A-F) Venus-fused MAPKs and tagRFP-fused HAI PP2Cs were expressed together with p19 silencing suppressor in $N$. benthamiana by Agrobacterium infiltration. The pictures were taken at 3 days after infiltration. Bars: $50 \mu \mathrm{~m}$. (A) MPK3 and HAI1. (B) MPK6 and HAI1. (C) MPK3 and HAI2. (D) MPK6 and HAI2. (E) MPK3 and HAI3. (F) MPK6 and HAI3. (G-H) Expression of nYFP-fused HAI1, HAI2, HAl3 and ABI2 and cYFP-fused MPK3 and MPK6 in the BiFC experiment shown in Fig. 2B were confirmed by immunoblotting using specific antibodies. Ponceau S -stained RuBisCo is shown as a loading control.

A ロmock ■ABA



C
D


Fig. S3. ABA and the Pto DC3000 effectors AvrPto/AvrPtoB suppress immunity though HAI PP2Cs. (A) Leaves of Col plants were infiltrated with Pto DC3000 or Pto $\triangle$ AvrPto 0 AvrPtoB ( $O D 600=0.0002$ ) together with EtOH $(0.1 \%)$ or $A B A(10 \mu \mathrm{M})$. (B) Leaves of Col, hai1 hai2 hai3 and aba2 plants were infiltrated with Pto DC3000 ( $\mathrm{OD} 600=0.0002$ ). ( A and B ) The bacterial titers at 2 dpi were measured. Bars represent means and standard errors of two independent experiments with 16 biological replicates in each experiment. The Benjamini-Hochberg method was used to adjust $p$-values for correcting 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). ( C and D ) Leaves of 4 to 5 -week-old Col plants were infiltrated with Pto DC3000 or Pto $\triangle$ AvrPto $\Delta$ AvrPtoB ( $O D 600=0.01$ ) and harvested at the indicated time points. Immunoblot analysis was performed to detect phosphorylated MAPKs, MPK3 or MPK6. Ponceau S-stained RuBisCo is shown as a loading control. Results of two independent experiments are shown.

HAl1
HAl2
HAl3
$\square$ Mock $\square$ COR




C


HAI2 םMock ■MeJA


HAl3
■Mock ■MeJA


D
HAl1


E



Fig. S4. COR and JA induces HAl1 directly through MYC2. (A and C) Seedlings of Col were treated with COR ( $5 \mu \mathrm{M}$ ) (A) or MeJA (100 $\mu \mathrm{M})(\mathrm{C})$ for the indicated time periods. Mock samples were also harvested. The expression levels of HAl1, HAI2 and HAl3 were 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 mixed linear models. Asterisks indicate statistically significant differences compared to mock at each time points (* $P<$ 0.05 ; ** $P<0.01$, two-tailed t-tests). (B and E) ChIP-qPCR was performed using the p35S::MYC2-GFP seedlings treated with COR ( $5 \mu \mathrm{M}$ ) (B) or MeJA $(100 \mu \mathrm{M})(\mathrm{E})$ for 3 h . Mock treatment was also performed. The G box motif located 193 bp upstream of the transcription start site of HAl1 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 the wildtype plants set to 1 , calculated from two independent experiments. Asterisks indicate statistically significant differences from the wildtype plants ( $P<0.05$, two-tailed t-tests). ( D ) Seedlings of Col, myc2, and myc2/3/4 were treated with mock (water) or MeJA ( $100 \mu \mathrm{M}$ ) for the indicated time periods. The expression level of HAl1 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 correcting multiple hypothesis testing. Two groups not sharing any letters show statistically significant differences at each time point (Adjusted $P<0.05$ ).


Fig. S5. Differential requirements of AREB/ABF and MYC transcription factors for ABA- and JA-mediated transcriptional induction of HAI1, HAI2 and HAI3. (A-C) Seedlings of Col, areb1 areb2 abf3, myc2, myc2/3/4 were treated with mock ( $0.1 \% \mathrm{EtOH}$ ) or ABA ( $10 \mu \mathrm{~m}$ ) for the indicated time periods. The expression levels of HAl1, HAl2 and HAl3 were measured by RT-qPCR. (D) Seedlings of Col, areb1 areb2 abf3, myc2, myc2/3/4 were treated with mock (water) or MeJA ( $100 \mu \mathrm{M}$ ) for the indicated time periods. The expression level of HA/1 was measured by RT-qPCR. (A-D) Bars represent means and standard errors of the $\log _{2}$ expression levels relative to Actin2 calculated from two independent experiments using mixed linear models. The Benjamini-Hochberg method was used to adjust $p$-values for correcting multiple hypothesis testing. Two groups not sharing any letters show statistically significant differences at each time point (Adjusted $P<$ $0.05)$.


Fig. S6. HAl1 is required for coronatine-mediated MAPK inactivation. Seedlings of Col and hai1 were pre-treated with mock ( $0.1 \%$ DMSO) or COR $(5 \mu \mathrm{M})$ for 6 hours, followed by flg22 treatment ( $1 \mu \mathrm{M}$ ) for the indicated time periods. Phosphorylated MAPKs were detected by immunoblotting. Ponceau S-stained RuBisCo is shown as a loading control. Results of three independent experiments with different time-courses are shown.


PonceauS




B


Fig. S7. Coronatine suppresses MAPK activation through HAl1 during Pto DC3000 infection. (A) Leaves of 4 to 5 -week-old Col or hai1 were infiltrated with Pto DC3000 $\left(\mathrm{OD}_{600}=0.01\right)$ and harvested at the indicated time points. (B) Leaves of 4 to 5 -week-old Col were infiltrated with Pto DC3000 or COR-deficient Pto DC3118 $\left(\mathrm{OD}_{600}=0.01\right)$ and harvested at the indicated time points. Immunoblot analysis was performed using antibodies specific to phosphorylated MAPKs, MPK3 or MPK6. Ponceau S-stained RuBisCo is shown as a loading control. Results of two independent experiments with different time courses are shown.
-1h Mock -1h DC3000 ■3h Mock ■3h DC3000


Fig. S8. HAl1 is not essential for stomatal reopening by Pto DC3000. Leaf epidermis was treated with mock or Pto DC3000 ${\left(O_{6}\right.}^{600}$ $=0.2$ ). Stomatal aperture was measured by taking ratio of width and length of approx. 20 stomata 1 h and 3 h after treatments. Bars represent means and standard errors calculated from three independent experiments using a mixed linear model. The BenjaminiHochberg method was used to adjust $p$-values for correcting multiple hypothesis testing. Statistically significant differences are indicated by different letters in each genotype (Adjusted $P<0.01$ ).


ANAC019

ANAC055


ANAC072


Fig. S9. ETI activation counteracts induction of COR-responsive NAC transcription factors by Pto DC3000. Leaves of 4 to 5-week-old Col plants were infiltrated with mock (water), Pto DC3000, Pto AvrRpt2 or Pto AvrRpm1 $\left(\mathrm{OD}_{600}=0.001\right)$ and harvested at 6 h after infiltration. The expression levels of ANAC019, ANAC055 and ANAC072 were 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. Asterisks indicate statistically significant differences compared to mock ( ${ }^{*} P<0.05$, two-tailed t -tests).


Fig. S10. Phylogenetic analysis of clade A PP2C orthologues. The proteins belonging to the same group as A. thaliana ABI1, ABI2, HAB1, HAB2, AHG1, AHG3, HAI1, HAI2 and HAl3 (highlighted with red circles) were identified by OrthoMCL. A maximum likelihood phylogenetic tree was constructed based on the amino acid sequences using the MEGA6 software.

CrHAl1


B


C


D


CrHAI2


EsHAI2
$\square$ Mock $\square$ ABA


CrHAl2
-Mock $\square$ MeJA


EsHAl2
םMock ■MeJA


CrHAI3
$\square$ Mock $\square$ ABA


EsHAl3
$\square$ Mock $\square$ ABA


CrHAl3
-Mock ■MeJA


EsHAl3
-Mock ■MeJA


Fig. S11. Conservation of transcriptional induction of HAI PP2Cs by ABA and JA in Brassicaceae. (A and B) Seedlings of Capsella rubella and Eutrema salsugineum were treated with mock ( $0.1 \% \mathrm{EtOH}$ ) or ABA ( $10 \mu \mathrm{M}$ ) for the indicated time periods. (C and D) Seedlings of C. rubella and E. salsugineum were treated with mock (water) or MeJA ( $100 \mu \mathrm{M}$ ) for the indicated time periods. The expression levels of HAI1, HAI2 and HAI3 were measured by RT-qPCR. Bars represent means and standard errors of the $\log _{2}$ expression levels relative to Actin2 calculated from two independent experiments using mixed linear models. Asterisks indicate statistically significant differences compared to mock at each time point ( ${ }^{*} P<0.01$, two-tailed t-tests).

| I sug ineum |  |
| :---: | :---: |
| B. rapa |  |
| C. grandiflora | ggattgetattagaatit-C |
| C. rubella |  |
| A. thal iana |  |
| A. lyrata |  |
| E. sal sug ineum |  |
| B. rapa |  |
| C. grandiflora |  |
| C. rubella |  |
| A. thal iana |  |
| A. Iyrata |  |
| E. sal sug ineum |  |
| B. rapa |  |
| C. grandiflora |  |
| C. rubel la | GTCAAGATTATTTCGTCCAGATAGAGGGGGATAGGTCAGGCCATTCACCAAGCTTCCTCTCTCTCTCTCTCTCTCTCTTCTCTTCTTCTTCATTCTTTCTTTCATTTTGATTTTTTTGAGTTTTTGGTGATCTGCCCGTTT-AAAGTTTGGGGCAAGACCTGCCGGTAAC-AtAAAGA |
| A. thal iana |  |
| A. Iyrata |  |
| E. sal sug ineum |  |
| B. rapa |  |
| C. grandiflora |  |
| C. rubella | тGAtTTTTTATTTTATTTATATCTTCTGGTGAAGAAGCTAATAT-AAAGCTTCCATGGTAATCTTGTTTAAGCTTCTCTTCTTCTGTTCTCTCTTTCTCTCTCTCTCTCCTGTGTCTCGTTCAGTAGTTTTTTTTTTATATTTATTTTTGGTCGGG |
| A. thal iana | tGA-ttatati-tattiatatcttctagtaangagctantat- |
| A. Iyrata | tGA---tttat-tatttatatctict--------Gctaatat-- |

## B

| C. grandiflora <br> C. rubella |  |
| :---: | :---: |
|  |  |
| $\begin{aligned} & \text { B. rapa } \\ & \text { E. sal sug ineum } \end{aligned}$ |  |
|  |  |
| A. thal iana |  |
| A. Iyrata |  |
| C. grandiflora |  |
| C. rubella |  |
| B. rapa |  |
| E. sal sugineum |  |
| A. thal iana |  |
| A. Iyrata |  |
| C. grandiflora |  |
| C. rubella |  |
| B. rapa |  |
| E. sal sug ineum |  |
| A. thal iana |  |
| A. lyrata |  |
| C. grandiflora |  |
| C. rubella |  |
| B. rapa | ggG-gtataatgaccccctacctigtaagtet-acgatgata-tttattcaatca-- |
| E. sal sug ineum |  |
| A. thal iana | ggatatataAt-gggcacg-ctttgtahcgtgt-Aagat- |
| A. Iyrata | ggatatataAatgggcaca-ctttgtahgetgt-Aagat- |
| C. grandiflora |  |
|  |  |
| B. rapa |  |
| E. sal sug ineum A. thaliana | aACGGGatGgagttittattittattteggggatcagatattcag |
|  |  |
|  |  |

## C

B. rapa E. sal sug ineum C. grandiflor C. rubella A. thal iana A. I lyrata
B. rapa
E. sal sug ineum
C. grandiflora
C. rubel la
A. thaliana
A. Iyrata
B. rap
E. sal sug ineum
C. grandiflora
C. rubel la
A. thal iana
A. Iyrata
B. rapa
E. sal sug ineum
C. grand iflora
C. rubella
A. thal iana
A. lyrata
B. rapa
E. sal sug ineum
C. grandiflora
C. rubella

A thal ian
A. Iyrata


Fig. S12. Alignment of HAI PP2C promoters of Brassicaceae species. The 500 bp upstream of the transcription start sites were aligned using MUSCLE. The CACGTG G box motif is shown in bold red letters ,and the ACGTG core ABRE motif is highlighted in yellow. (A) HAl1 promoter. (B) HAl2 promoter. (C) HAl3 promoter.

