

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 was shown as a loading control.

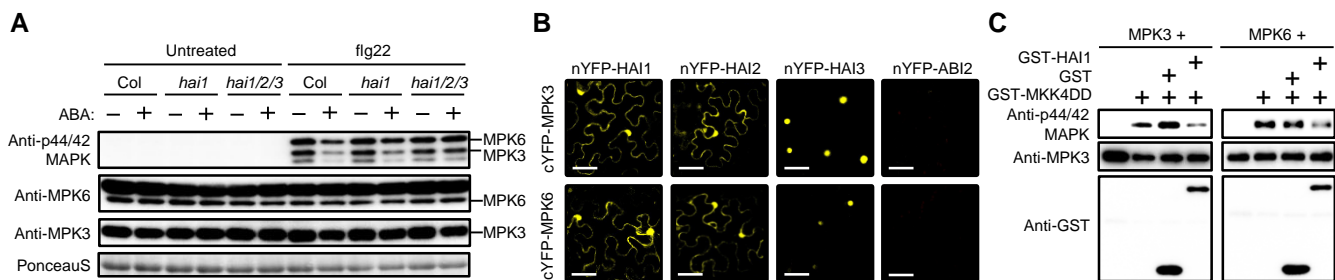


Fig. 2. HAI PP2Cs are responsible for ABA-mediated MAPK inactivation. (A) Seedlings of Col, *hai1*, *hai1 hai2 hai3* (*hai1/2/3*) were pre-treated with ABA (10 μ M) for 6 h, followed by flg22 treatment (1 μ M) for 10 min. Proteins were detected by immunoblotting using the indicated antibodies. Ponceau S-stained RuBisCO 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 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 $^{\circ}$ C for 1 h. Proteins were detected by immunoblotting using the indicated antibodies.

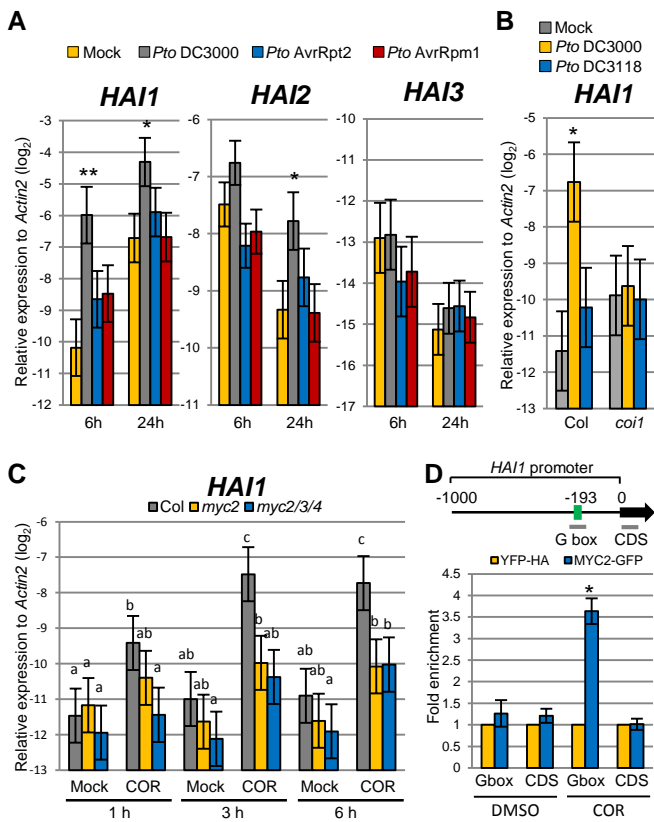


Fig. 3. *Pto* DC3000 induces *HAI1* through coronatine-mediated activation of JA signaling. (A and B) Leaves of 4 to 5-week-old Col plants were infiltrated with mock (water), *Pto* DC3000, *Pto* AvrRpt2 or *Pto* AvrRpm1 ($OD_{600} = 0.001$) and harvested at 6 and 24 h after infiltration (A). Leaves of 4 to 5-week-old Col or *coi1* 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). 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 points ($*P < 0.05$; $**P < 0.01$, 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 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::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 the YFP-HA plants ($P < 0.05$, two-tailed t-tests).

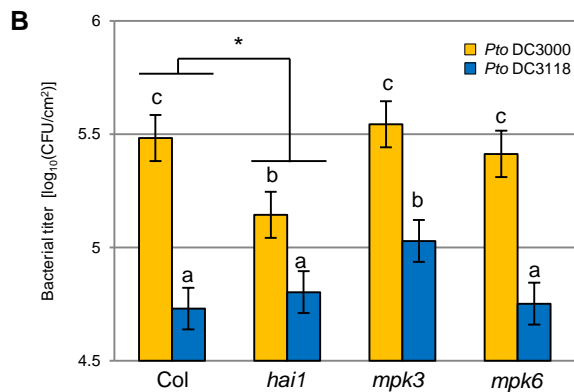
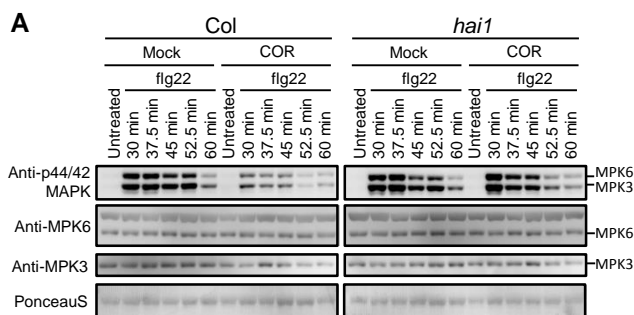


Fig. 4. HAI1 is required for coronatine-mediated MAPK inactivation and immune suppression. (A) Seedlings of Col and *hai1* were pretreated with 5 μ M COR for 6 h, followed by treatment with 1 μ M flg22 for the indicated time periods. Proteins were detected by immunoblotting using the indicated antibodies. Ponceau S-stained RuBisCo was shown as a loading control. (B) Leaves of Col, *mpk3*, *mpk6* and *hai1* were infiltrated with *Pto* DC3000 or *Pto* DC3118 COR⁺ ($OD_{600} = 0.0002$). 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).

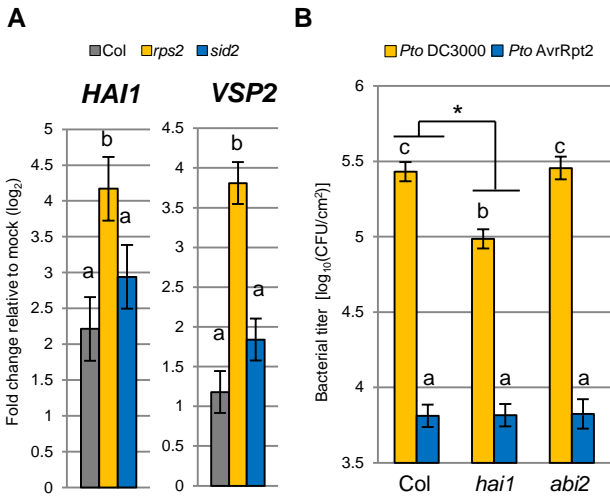


Fig. 5. Effector-triggered immunity counteracts the HAI1-dependent coronatine virulence. (A) Seedlings of the estradiol-inducible AvrRpt2 transgenic line in Col background or in *rps2* or *sid2* mutant backgrounds were treated with estradiol (10 μ M) for 6 h, followed by COR treatment (5 μ M) for 1 h. 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 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 $P < 0.05$).

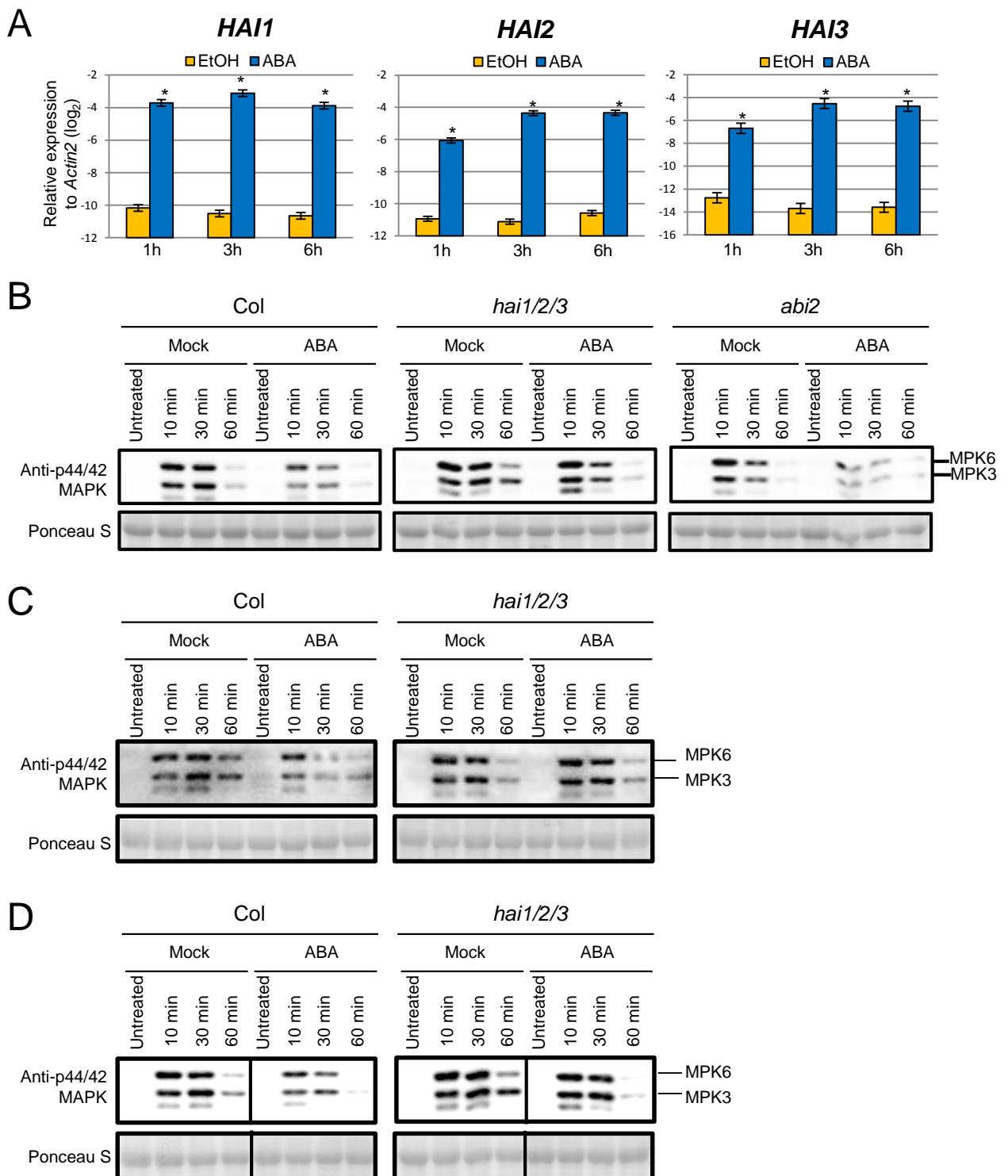


Fig. S1. ABA-mediated MAPK inactivation depends on HAI PP2Cs. (A) Wild-type seedlings were treated with mock (0.1% EtOH) or ABA (10 μ M) for the indicated time periods. The expression levels of *HAI1*, *HAI2* and *HAI3* were measured by RT-qPCR. Bars represent means and standard errors of the \log_2 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% EtOH) or ABA (10 μ M) for 6 hours, followed by flg22 treatment (1 μ 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).

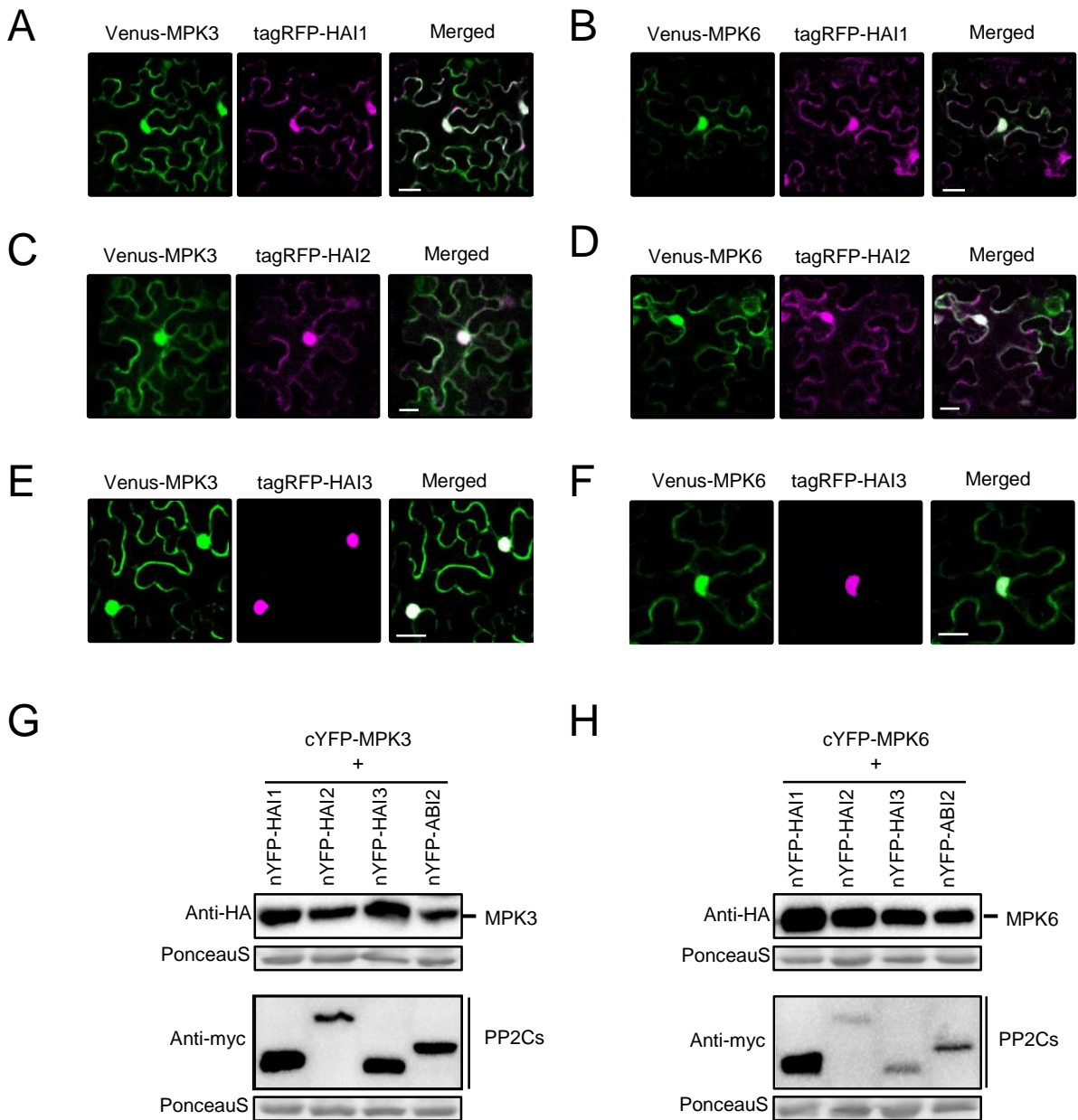


Fig. S2. HAI PP2Cs co-localize with MAPKs. (A-F) Venus-fused MAPKs and tagRFP-fused HAI PP2Cs were expressed together with p19 silencing suppressor in *N. benthamiana* by *Agrobacterium* infiltration. The pictures were taken at 3 days after infiltration. Bars: 50 μ m. (A) MPK3 and HAI1. (B) MPK6 and 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, HAI3 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.

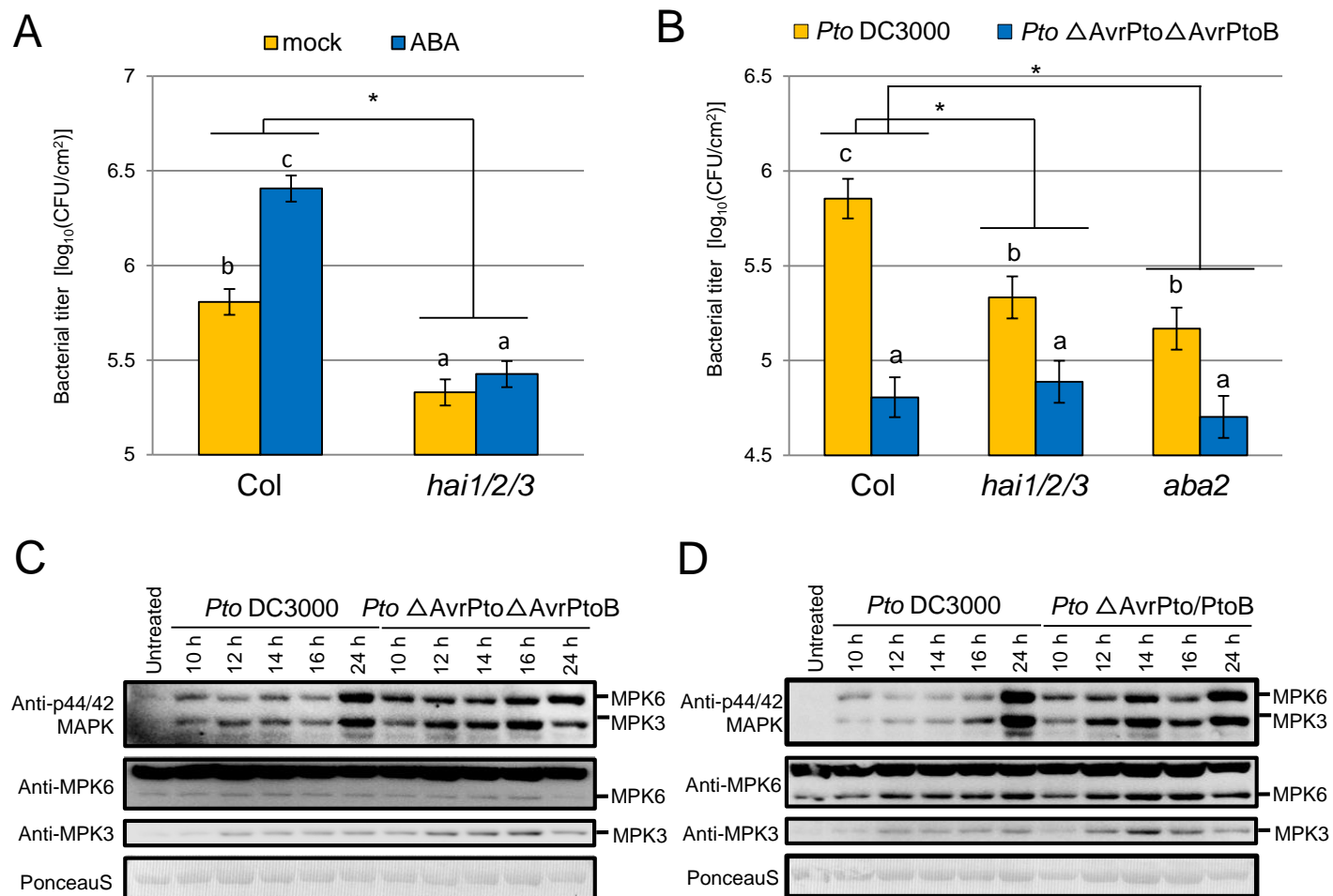


Fig. S3. ABA and the *Pto* DC3000 effectors *AvrPto/AvrPtoB* suppress immunity through HAI PP2Cs. (A) Leaves of Col plants were infiltrated with *Pto* DC3000 or *Pto* Δ*AvrPto*Δ*AvrPtoB* (OD₆₀₀ = 0.0002) together with EtOH (0.1%) or ABA (10 μM). (B) Leaves of Col, *hai1 hai2 hai3* and *aba2* plants were infiltrated with *Pto* DC3000 (OD₆₀₀ = 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* Δ*AvrPto*Δ*AvrPtoB* (OD₆₀₀ = 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.

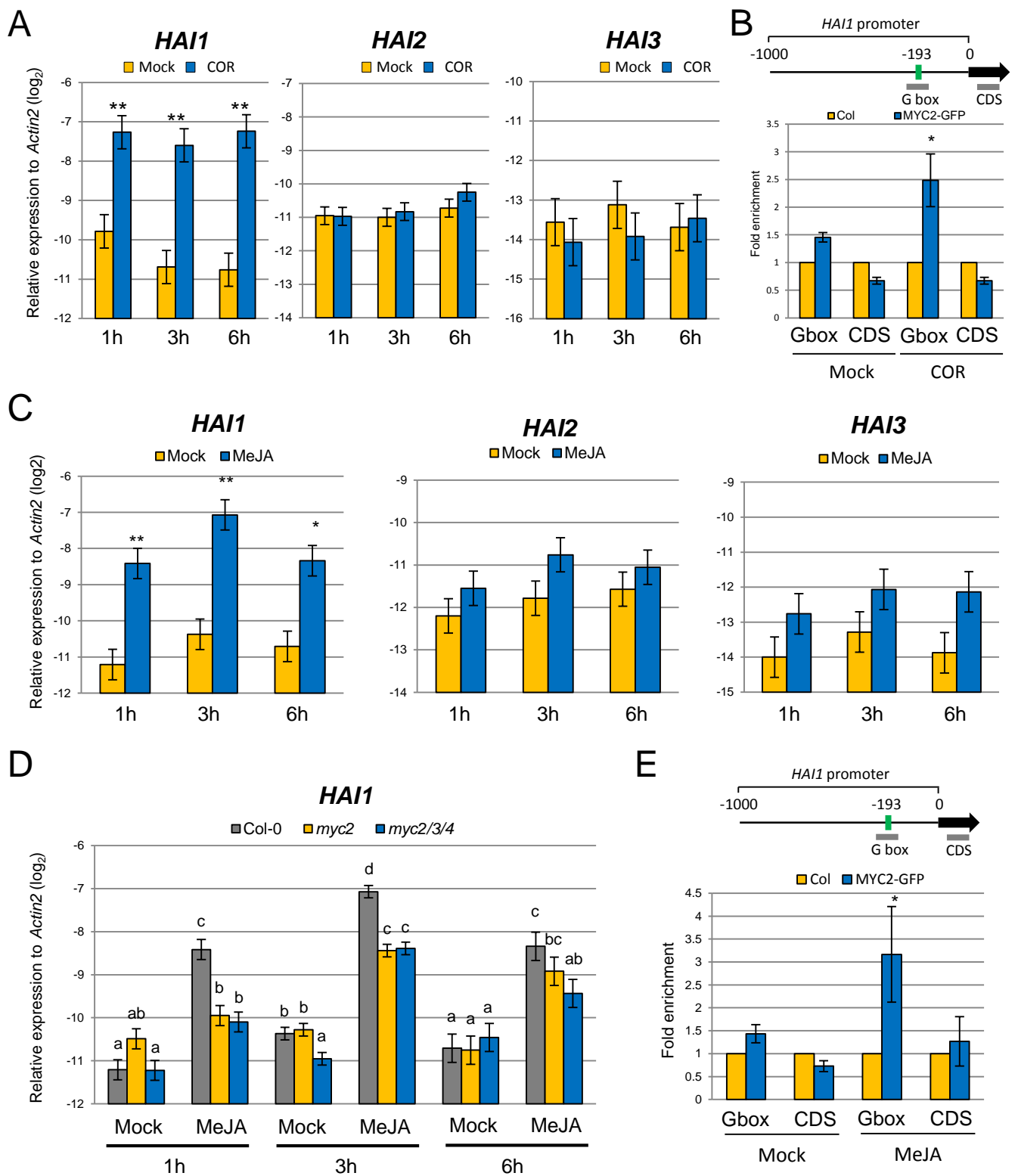


Fig. S4. COR and JA induces *HAI1* directly through MYC2. (A and C) Seedlings of Col were treated with COR (5 μ M) (A) or MeJA (100 μ M) (C) for the indicated time periods. Mock samples were also harvested. The expression levels of *HAI1*, *HAI2* and *HAI3* were determined by RT-qPCR. Bars represent means and standard errors of the \log_2 expression levels relative to *Actin2* calculated from 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 μ M) (B) or MeJA (100 μ M) (E) for 3 h. Mock treatment was also performed. 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 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 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 correcting multiple hypothesis testing. Two groups not sharing any letters show statistically significant differences at each time point (Adjusted $P < 0.05$).

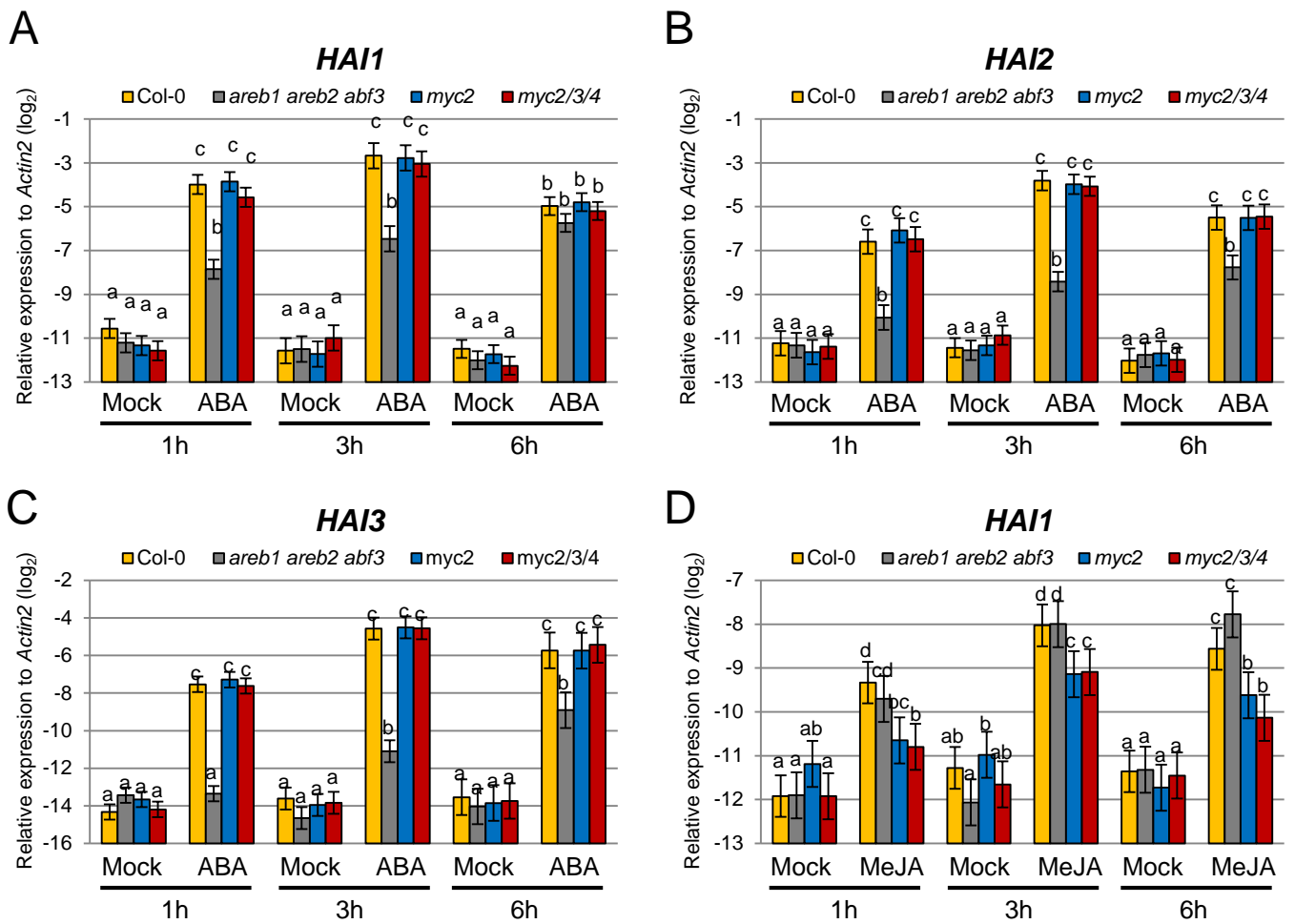


Fig. S5. Differential requirements of AREB/ABF and MYC transcription factors for ABA- and JA-mediated transcriptional induction of *HAI1*, *HAI2* and *HAI3*. (A-C) Seedlings of Col, *areb1 areb2 abf3*, *myc2*, *myc2/3/4* were treated with mock (0.1% EtOH) or ABA (10 μ M) for the indicated time periods. The expression levels of *HAI1*, *HAI2* and *HAI3* were measured by RT-qPCR. (D) Seedlings of Col, *areb1 areb2 abf3*, *myc2*, *myc2/3/4* were treated with mock (water) or MeJA (100 μ M) for the indicated time periods. The expression level of *HAI1* 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 $P < 0.05$).

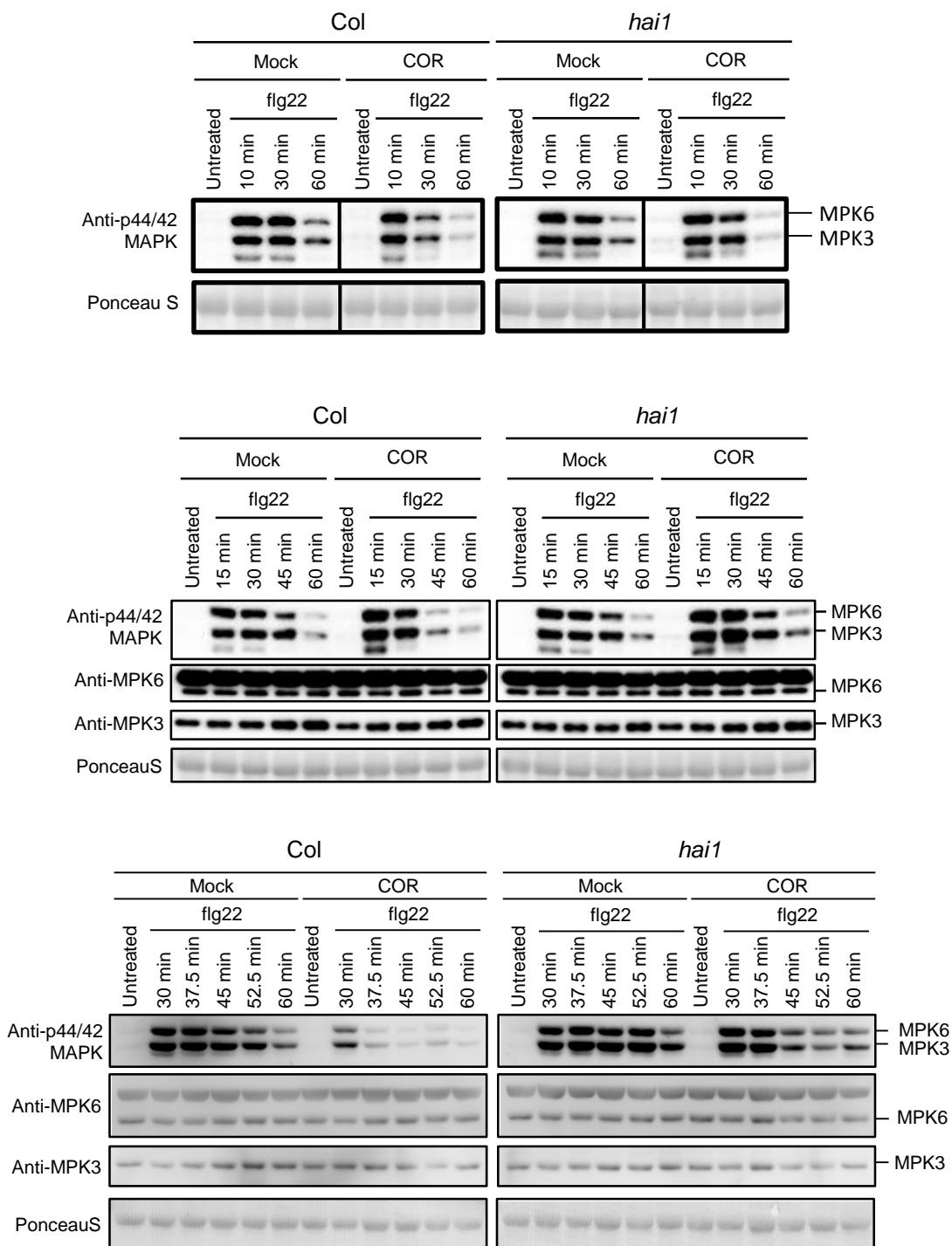
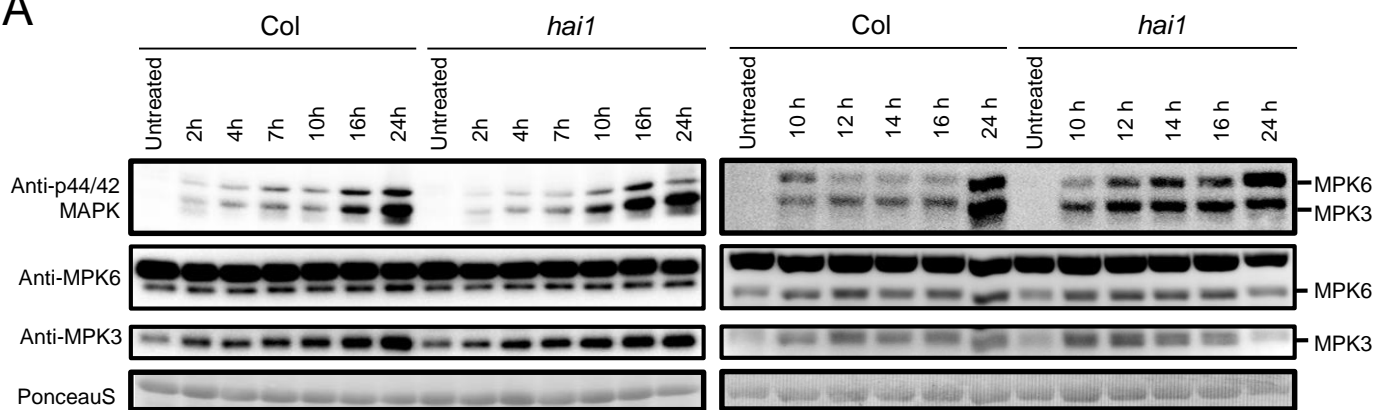


Fig. S6. HAI1 is required for coronatine-mediated MAPK inactivation. Seedlings of Col and *hai1* were pre-treated with mock (0.1% DMSO) or COR (5 μ M) for 6 hours, followed by flg22 treatment (1 μ M) for the indicated time periods. Phosphorylated MAPKs were detected by immunoblotting. Ponceau S-stained RuBisCo is shown as a loading control. Results of three independent experiments with different time-courses are shown.

A



B

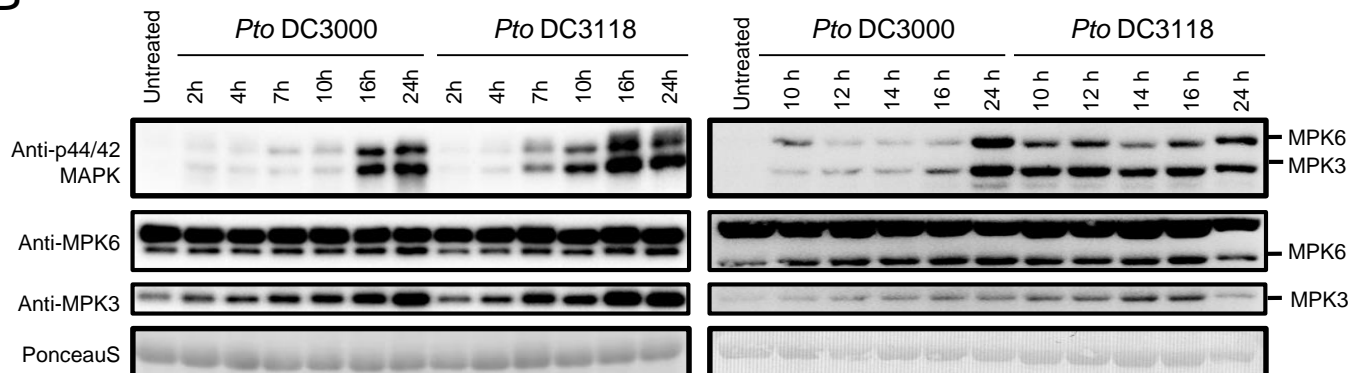


Fig. S7. Coronatine suppresses MAPK activation through HAI1 during *Pto* DC3000 infection. (A) Leaves of 4 to 5-week-old *Col* or *hai1* were infiltrated with *Pto* DC3000 ($OD_{600} = 0.01$) and harvested at the indicated time points. (B) Leaves of 4 to 5-week-old *Col* were infiltrated with *Pto* DC3000 or COR-deficient *Pto* DC3118 ($OD_{600} = 0.01$) 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.

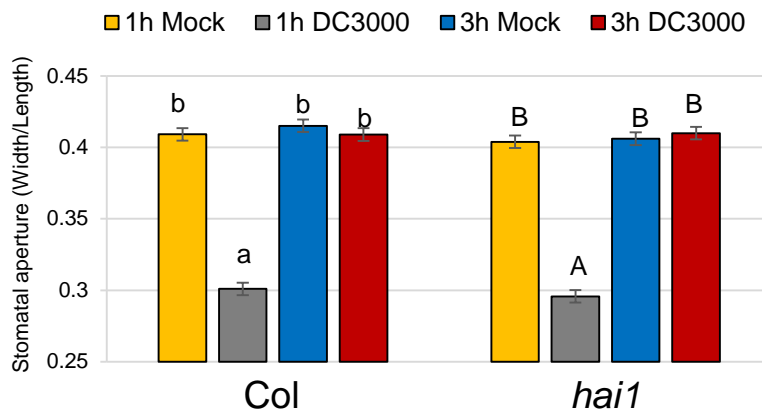


Fig. S8. HAI1 is not essential for stomatal reopening by *Pto* DC3000. Leaf epidermis was treated with mock or *Pto* DC3000 ($OD_{600} = 0.2$). Stomatal aperture was measured by taking ratio of width and length of approx. 20 stomata 1 h and 3 h after treatments. Bars represent means and standard errors calculated from three independent experiments using a mixed linear model. The Benjamini-Hochberg method was used to adjust p -values for correcting multiple hypothesis testing. Statistically significant differences are indicated by different letters in each genotype (Adjusted $P < 0.01$).

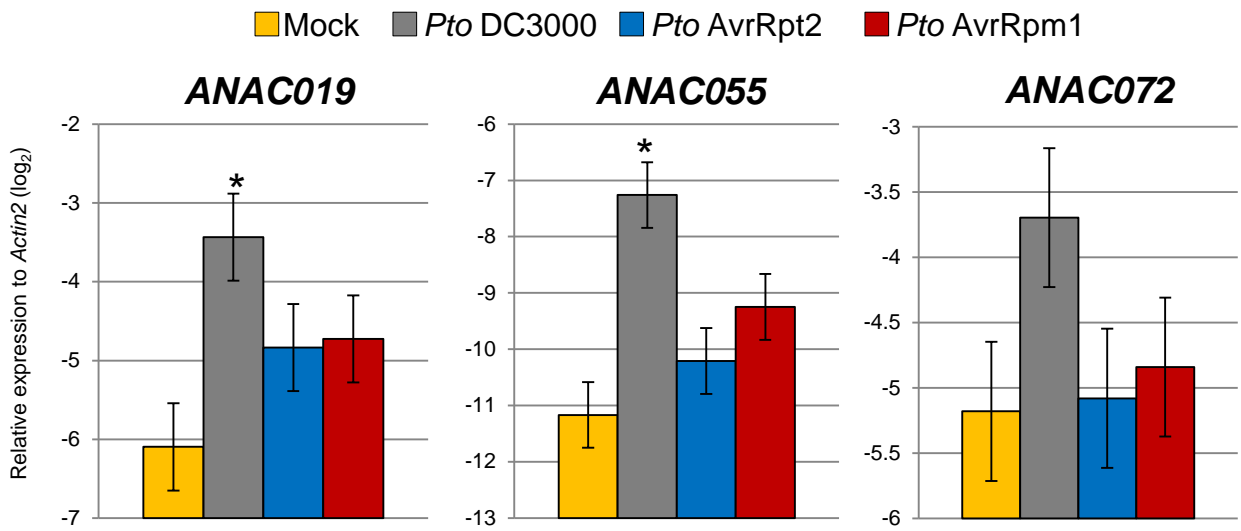


Fig. S9. ETI activation counteracts induction of COR-responsive NAC transcription factors by *Pto* DC3000. Leaves of 4 to 5-week-old Col plants were infiltrated with mock (water), *Pto* DC3000, *Pto* AvrRpt2 or *Pto* AvrRpm1 ($OD_{600} = 0.001$) and harvested at 6 h after infiltration. The expression 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).

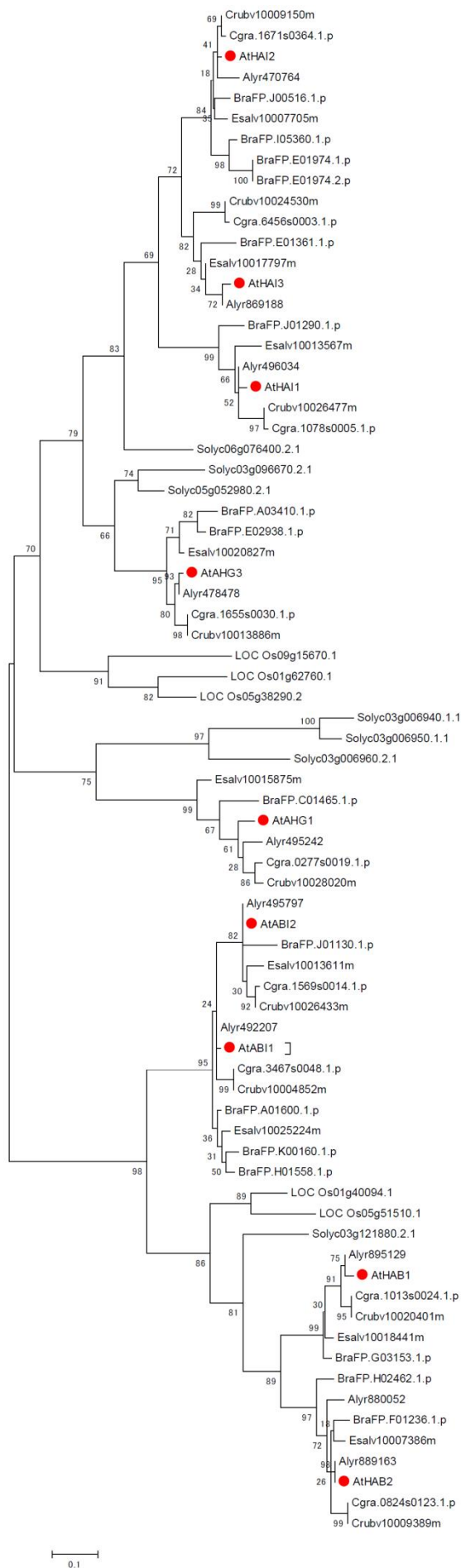


Fig. S10. Phylogenetic analysis of clade A PP2C orthologues. The proteins belonging to the same group as *A. thaliana* ABI1, ABI2, HAB1, HAB2, AHG1, AHG3, HAI1, HAI2 and HAI3 (highlighted with red circles) were identified by OrthoMCL. A maximum likelihood phylogenetic tree was constructed based on the amino acid sequences using the MEGA6 software.

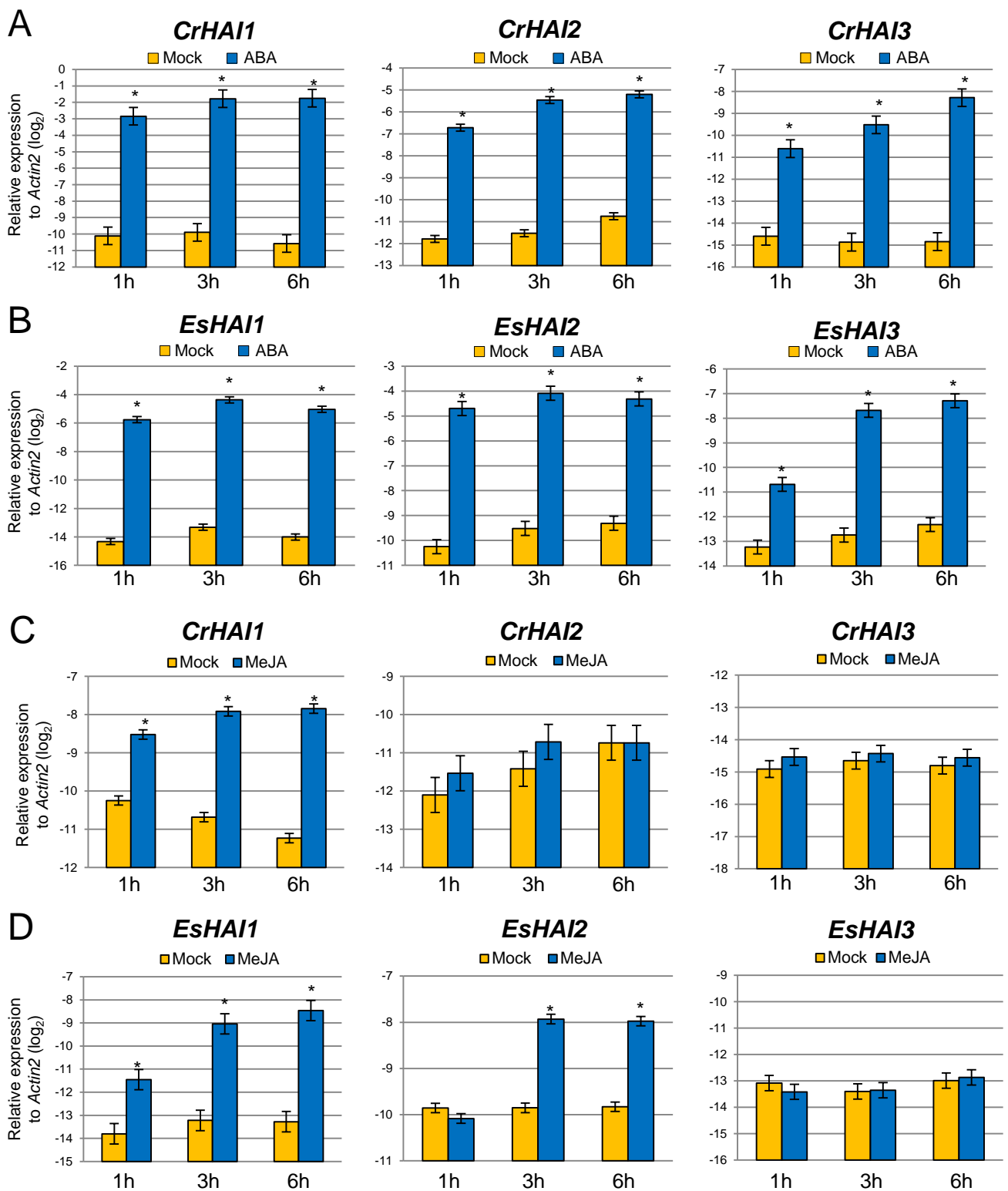


Fig. S11. Conservation of transcriptional induction of HAI PP2Cs by ABA and JA in Brassicaceae. (A and B) Seedlings of *Capsella rubella* and *Eutrema salsugineum* were treated with mock (0.1% EtOH) or ABA (10 μ M) for the indicated time periods. (C and D) Seedlings of *C. rubella* and *E. salsugineum* were treated with mock (water) or MeJA (100 μ 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 log₂ 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).

