The Plant Cell, Vol. 28: 1662–1681, July 2016, www.plantcell.org

Arabidopsis HOOKLESS1 Regulates Responses to Pathogens and Abscisic Acid through Interaction with MED18 and Acetylation of *WRKY33* and *ABI5* Chromatin

Chao-Jan Liao,[a] Zhibing Lai,[b] Sanghun Lee,[a] Dae-Jin Yun,[c] and Tesfaye Mengiste[a,1]

[a] Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907
[b] National Key Laboratory of Crop Genetics Improvement, Huazhong Agricultural University, Wuhan 430070, China
[c] Division of Applied Life Sciences (BK 21 Program), Gyeongsang National University, Jinju City 660-701, Korea

ORCID IDs: 0000-0001-6114-388X (Z.L.); 0000-0002-3638-6043 (D.-J.Y.)

***Arabidopsis thaliana HOOKLESS1* (*HLS1*) encodes a putative histone acetyltransferase with known functions in seedling growth. Here, we show that HLS1 regulates plant responses to pathogens and abscisic acid (ABA) through histone acetylation at chromatin of target loci. The *hls1* mutants show impaired responses to bacterial and fungal infection, accelerated senescence, and impaired responses to ABA. HLS1 modulates the expression of *WRKY33* and *ABA INSENSITIVE5* (*ABI5*), known regulators of pathogen and ABA responses, respectively, through direct association with these loci. Histone 3 acetylation (H3Ac), a positive mark of transcription, at *WRKY33* and *ABI5* requires HLS1 function. ABA treatment and pathogen infection enhance HLS1 recruitment and H3Ac at *WRKY33.* HLS1 associates with Mediator, a eukaryotic transcription coregulatory complex, through direct interaction with mediator subunit 18 (MED18), with which it shares multiple functions. HLS1 recruits MED18 to the *WRKY33* promoter, boosting *WKRY33* expression, suggesting the synergetic action of HLS1 and MED18. By contrast, MED18 recruitment to *ABI5* and transcriptional activation are independent of HLS1. ABA-mediated priming of resistance to fungal infection was abrogated in *hls1* and *wrky33* mutants but correlated with ABA-induced HLS1 accumulation. In sum, HLS1 provides a regulatory node in pathogen and hormone response pathways through interaction with the Mediator complex and important transcription factors.**

INTRODUCTION

Plants fend off infection by deploying multiple immune responses that correspond to the various pathogen virulence strategies. Pathogen-associated molecular patterns (PAMPs), such as components of fungal cell walls, chitin, or flagellin protein from bacterial flagella by pattern recognition receptors, stimulate PAMP-triggered immunity. A more specialized resistance, commonly known as effector-triggered immunity, is activated upon recognition of effectors by intracellular resistance (R) proteins (Jones and Dangl, 2006). Downstream of pathogen recognition, a cascade of regulatory hierarchy activates an array of molecules that counteract pathogens. Transcriptional regulations of genes encoding diverse molecules are widely recognized to be important in plant immune responses. Posttranslational modifications of histone tails are prominent mechanisms that modulate gene expression in plant responses to pathogens as well as regulate other plant functions. Posttranslational modifications of histone tails alter interactions between DNA and histones, resulting in chromatin structure that is permissive or repressive to transcription and other DNA metabolic processes (Chen and Tian, 2007). The open or permissive state of chromatin allows the recruitment of transcriptional complexes and enzymes, including DNA binding proteins, cofactors, and RNA polymerase II (RNAPII), to enhance transcription.

The acetylation of lysine residues on histones is dynamically regulated by histone acetyltransferases (HATs) and histone deacetylase that ultimately alter gene expression patterns. HATs are divided into four groups: GENERAL CONTROL OF NON-DEREPRESSIBLE 5-RELATED N-ACETYLTRANSFERASE; CREB binding protein (CBP)/p300 super families; members of MOZ-YBF2-SAS2-TIP60 (MYST); and TBP-associated factor 1, all of which have conserved catalytic domains (Pandey et al., 2002). GCN5 in *Arabidopsis thaliana* is implicated in plant developmental functions, such as floral meristem formation or root meristem differentiation (Bertrand et al., 2003; Kornet and Scheres, 2009), as well as responses to cold, light, or iron homeostasis (Vlachonasios et al., 2003; Benhamed et al., 2006; Xing et al., 2015). Histone acetyltransferases also play vital roles in plant resistance to the bacterial pathogen *Pseudomonas syringae* pv *tomato* DC3000 (*Pst* DC3000) (Defraia et al., 2013; Wang et al., 2013; Singh et al., 2014).

Mediator is a conserved multisubunit protein complex that functions as a cofactor between transcription factors and RNAPII (Reeves and Hahn, 2003). Through interaction with histone acetylation complexes, mediator also stimulates transcription (Liu et al., 2008). Mediator activates or represses transcription through interaction with transcription factors (An and Mou, 2013). Among 34 Mediator subunits identified in Arabidopsis, six are implicated in plant immunity functions through jasmonic acid/ethylene (JA/ET)- or salicylic acid (SA)-dependent defense pathways. For example, by interacting with MYC2, MEDIATOR25 (MED25) induces JA signaling genes to enhance resistance to fungal pathogens

[1] Address correspondence to mengiste@purdue.edu.
The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantcell.org) is: Tesfaye Mengiste (mengiste@purdue.edu).
www.plantcell.org/cgi/doi/10.1105/tpc.16.00105

(Chen et al., 2012). MED16 regulates both JA/ET and SA pathways and contributes to resistance to *Botrytis cinerea* (Zhang et al., 2012). Recent data show that MED21 interacts with the chromatin-modifying enzyme, HISTONE MONOUBIQUITINATION1 (HUB1), affecting plant resistance to fungal infection (Dhawan et al., 2009). MED18 functions as a coactivator or repressor modulating abscisic acid (ABA) responses, flowering time, and plant defense through interactions with different transcription factors (Lai et al., 2014). Interestingly, MED18 also modulates specific gene expression and histone methylation of genes that function in pathogen and hormone responses (Lai et al., 2014). MED16 interacts with MED25 or nonmediator proteins to form a complex that regulates EIN3/EIL1-mediated pathway in iron homeostasis (Yang et al., 2014). Together, mediator subunits form a complex with RNAPII and different transcription factors or cofactors to regulate gene expression in hormone or defense signaling.

Arabidopsis HOOKLESS1 (HLS1) was previously implicated in the regulation of seedling growth responses to ethylene. However, despite its high sequence similarity to histone acetyltransferases, its molecular, biochemical, and physiological functions are still poorly understood. Here, we describe the functions of HLS1 in plant defense and the underlying molecular mechanisms. The *hls1* mutant displayed enhanced disease symptoms in response to fungal and bacterial infections, accelerated senescence, and impaired responses to the plant hormone ABA, suggesting the critical role of HLS1 in regulating these processes. The *hls1* mutant expressed enhanced bacterial disease symptoms regardless of the *Pst* strain. By contrast, the *hls1* mutant supported increased bacterial growth when inoculated with *Pst* DC3000 (*avrRpm1*) with no increase in bacterial growth after inoculation with virulent and nonpathogenic strains. Ectopic expression of HLS1 enhanced resistance to *B. cinerea*, which was associated with *B. cinerea*- or ABA-induced accumulation of HLS1 protein. In the absence of HLS1, ABA promotes senescence and enhanced susceptibility to *B. cinerea*. In addition, HLS1 and MED18, which are both required for seedling apical hook formation, responses to ABA, and resistance to *B. cinerea*, physically interact. HLS1 recruits MED18 to the *WRKY33* locus, which then enhances transcriptional activation of WRKY33. Interestingly, HLS1 modulates histone H3 acetylation at hormone and pathogen response regulatory loci *ABI5* and *WRKY33*. ABA and *B. cinerea* enhance HLS1 transcriptional and posttranslational regulation and HLS1-mediated histone H3 acetylation at target loci. Our results shed light on molecular mechanisms underlying the multiple biological functions of HLS1 in plant hormone and defense responses.

RESULTS

Arabidopsis HLS1 Mediates Responses to Fungal and Bacterial Pathogens

We recently described the Arabidopsis *med18* mutant, which displays enhanced susceptibility to the necrotrophic fungal plant pathogen *B. cinerea* and *hookless* phenotype in seedlings (Lai et al., 2014). The *hookless* phenotype in the *med18* mutant involves altered seedling apical hook formation in response to ethylene and is associated with impaired defense responses in adult plants. Due to the potential link between defense and the hookless phenotype, the Arabidopsis *hls1* mutant was tested for altered defense responses. Two Arabidopsis T-DNA insertion alleles of *HLS1* (*hls1-1*, SALK-136528C; *hls1-2*, SLAK-009473) that lacked any *HLS1* transcript (Supplemental Figure 1) displayed increased susceptibility to *B. cinerea* with larger disease lesions, enhanced necrosis, and chlorosis compared with the wild-type Col-0 plants (Figures 1A to 1C). More fungal biomass accumulated in the *hls1* mutant as measured by quantitative PCR amplification of *B. cinerea ActinA* DNA, confirming the role of *HLS1* in suppressing fungal growth in infected plants (Figure 1D).

To test the role of HLS1 in bacterial resistance, *hls1* mutant plants were infiltrated with different *Pst* DC3000 strains. Regardless of the strain, *hls1* mutants displayed enhanced disease symptoms, composed primarily of chlorotic lesions (Figure 1E). Response of *hls1* mutants to the nonpathogenic strain of *Pst* DC3000 *hrcC*$^-$ was comparable to wild-type plants. The *Pst* DC3000 *hrcC*$^-$ strain is defective in Type III secretion but retains PAMP molecules through which it is able to activate PAMP-triggered immunity. The *hls1* mutant supported a level of bacterial growth comparable to that in wild-type plants after inoculation with the virulent bacterial strain *Pst* DC3000 (Figure 1F). By contrast, inoculation with the avirulent bacterial strain *Pst* DC3000 (*avrRpm1*) resulted in significantly more bacterial growth in the *hls1* mutants than the wild-type plants. Thus, HLS1 suppresses disease symptom expression regardless of the bacterial strain but also is required to limit growth of *Pst* DC3000 (*avrRpm1*), suggesting that HLS1 modulates some specific effector-triggered immunity responses.

HLS1 Regulates Expression of Defense-Related Genes in Response to *B. cinerea*

To gain insight into the molecular function of HLS1 in plant defense responses, the expressions of immune response marker genes were studied. *PLANT DEFENSIN1.2* (*PDF1.2*) is a well-known defense-related gene induced by fungal infection in JA- and ET-dependent pathways (Penninckx et al., 1998). The expression of *PDF1.2* significantly increased in the *hls1* mutant after *B. cinerea* infection (Figure 2A), while that of *PATHOGENESIS-RELATED PROTEIN1* (*PR1*) increased after *Pst* DC3000 (*avrRpm1*) infiltration. The expression of *PR1* correlates with activation of the SA pathway that mediates responses to *Pst* (Delaney et al., 1994), implying HLS1-mediated *Pst* resistance is unlikely to be due to loss of the SA-mediated defense pathway but rather due to the increase in SA levels in response to increased bacterial growth in the *hls1* mutant (Figure 2A). The *R* genes *P. syringae* pv *maculicola 1* (*RPM1*) and *RPM1-INTERACTING PROTEIN4* (*RIN4*) are positive and negative regulators of resistance to *Pst* DC3000 (*avrRpm1*), respectively. However, the expression of *RPM1* was reduced and *RIN4* expression increased after *Pst* DC3000 (*avrRpm1*) inoculation in the *hls1* mutant. Together, a distinct regulation of *RPM1* and *RIN4* expression by HLS1 correlated with resistance to *Pst* DC3000 (*avrRpm1*).

Based on genetic evidence, HLS1 is predicted to function at the boundary between ethylene and auxin pathways (Li et al., 2004). Therefore, we tested the expression of genes in auxin perception, signaling, and transport, which also affect plant immunity (Kazan and Manners, 2009). The expression of *CYP79B3*, encoding an enzyme that catalyzes the conversion from tryptophan to indole-3-acetaldoxime, a precursor of camalexin and

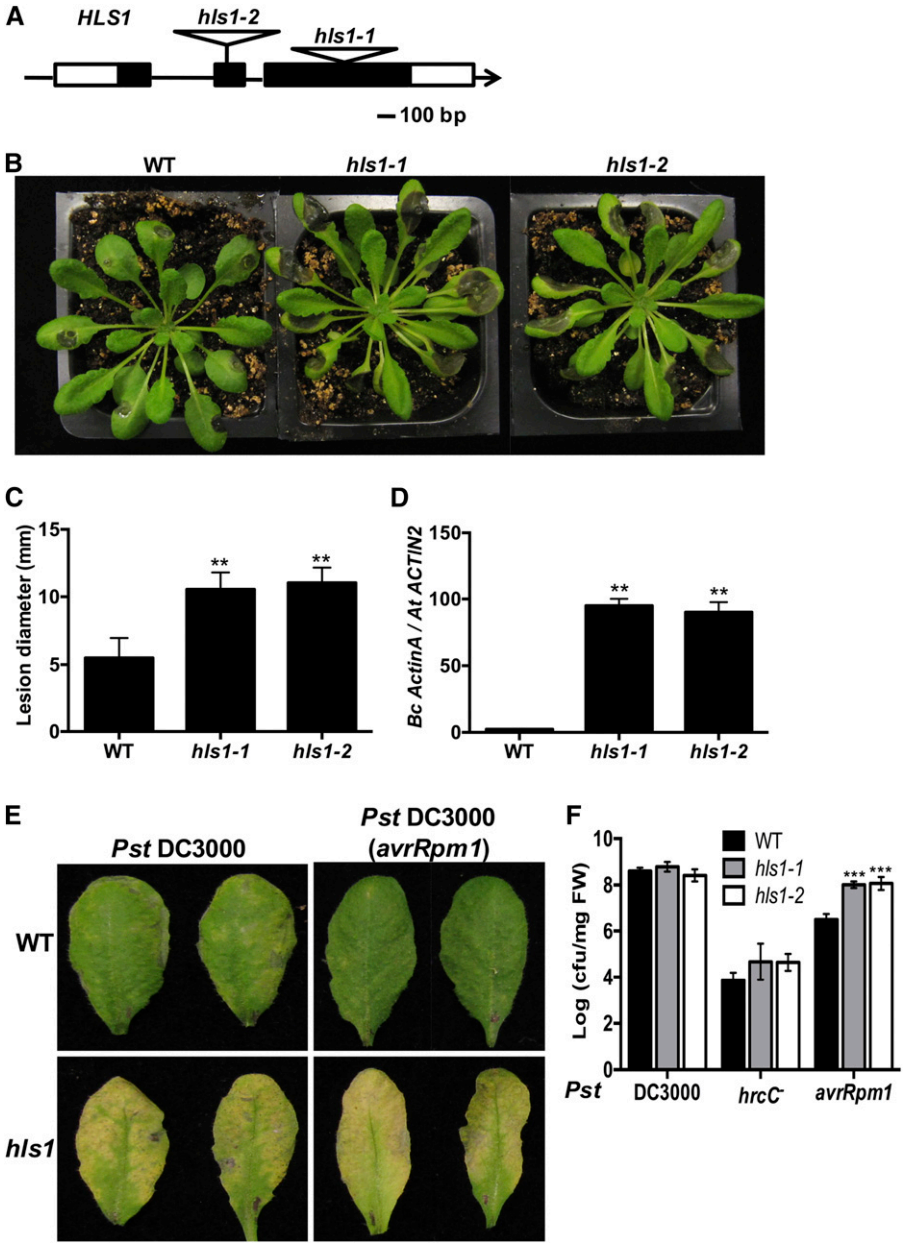

Figure 1. Arabidopsis HLS1 Mediates Responses to Fungal and Bacterial Pathogens.

(A) Schematic diagram showing T-DNA insertion alleles of the *HLS1* gene. *HLS1* gene and T-DNA insertions are shown in the *hls1* mutant alleles. Black and white squares are exons and untranslated regions, respectively.

(B) *B. cinerea* disease symptoms showing susceptibility of *hls1* mutant and

(C) Disease lesion size in the wild-type and *hls1* mutants. The data represent mean values ± SD from (n = 36).

(D) Enhanced *B. cinerea* growth in *hls1* mutants as measured by qPCR. Fungal growth was determined by qPCR amplification of the *B. cinerea ActinA* gene relative to Arabidopsis *ACTIN2* gene. The data represent mean values ± SE (n = 3).

(E) Enhanced disease symptoms in *hls1* mutant after inoculation with *Pst* strain. Inoculated leaves were detached for photographing at 3 days after inoculation (dai). Plants were infiltrated with a bacterial suspension (OD_{600} = 0.0005, ~2.5 × 10^5 CFU/mL).

(F) Bacterial growth in the wild-type and *hls1* mutants showing altered responses to bacterial infection. Bacterial growth is expressed in colony-forming units per mg fresh weight (cfu/mg FW). The data represent mean values ± SD (n = 24).

In **(C)**, **(D)**, and **(F)**, the mean values with statistically significant differences are indicated by asterisks (ANOVA test: **P < 0.01 and ***P < 0.001). The experiment was repeated three times with similar results.

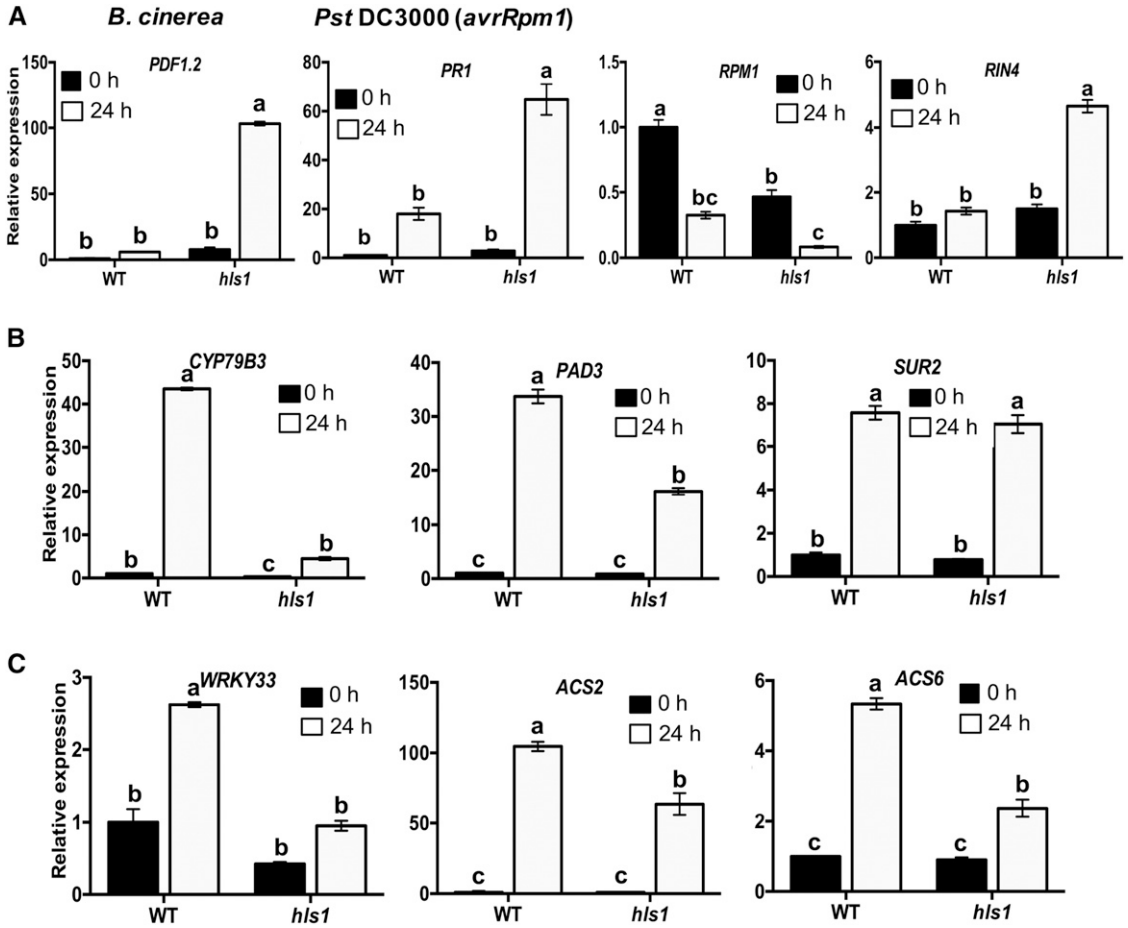

Figure 2. HLS1 Regulates Expression of Genes in Different Defense Pathways.

(A) The expression of defense genes: *PDF1.2* after inoculation with *B. cinerea* and *PR1*, and *RPM1* and *RIN4* after inoculation with *Pst* DC3000 (*avrRpm1*).
(B) Phytoalexin and glucosinolate biosynthesis genes *CYP79B3*, *PAD3*, and *SUR2* in *B. cinerea*-inoculated plants.
(C) Transcription factor *WRKY33* and ethylene biosynthesis genes *ACS2* and *ACS6* in response to *B. cinerea*.
In **(A)** to **(C)**, relative gene expression is normalized to Arabidopsis *ACTIN2* (*ACT2*) gene. The data represent mean values ± SE ($n = 3$) from at least two independent experiments. The gene expression in the wild type at 0 h after inoculation is set to 1. Statistically significant differences are marked by different letters (least squares means post hoc test: $P < 0.05$).

indole-glucosinolates (IGs), was reduced in the *hls1* mutant (Figure 2B). IG also contributes to fungal resistance and SUR2 is required for conversion of indole-3-acetaldoxime to IG. The expression of *SUR2* displayed no difference between the wild type and the *hls1* mutant in response to *B. cinerea* (Figure 2B). PHYTOALEXIN DEFICIENT3 (PAD3) is an enzyme required for camalexin biosynthesis and widely known to contribute to fungal resistance (Zhou et al., 1999). *PAD3* expression was significantly reduced in the *hls1* mutant consistent with the susceptibility of the *hls1* mutant to fungal infection (Figure 2B) (Petersen et al., 2008). The transcription factor WRKY33 regulates expression of camalexin biosynthesis genes, including *PAD3* and *CYP79B3* (Zheng et al., 2006; Mao et al., 2011). Pathogen-induced *WRKY33* expression was attenuated in the *hls1* mutant (Figure 2C). The *1-AMINOCYCLOPROPANE-1-CARBOXYLATE SYNTHASE* (*ACS2* and *ACS6*) genes, which function in the WRKY33-regulated ethylene biosynthesis pathway, also showed reduced expression in the *hls1* mutant after *B. cinerea* inoculation (Figure 2C). The results further confirm the effects of HLS1 on WRKY33-regulated pathway. These gene expression data indicate that HLS1 regulates *WRKY33* to modulate the expression of downstream genes.

HLS1 Is a Nuclear Protein That Associates with *WRKY33*

HLS1 is a putative GCN5 acetyltransferase based on amino acid sequence alignment to other known acetyltransferases but its molecular function has not been determined (Lehman et al., 1996). Epitope-tagged HLS1 predominantly localized to the nucleus in Arabidopsis protoplasts consistent with its potential function as a histone acetyltransferase (Figure 3A). To determine whether HLS1-dependent expression of target genes is through direct association with target genes, the recruitment of HLS1 to specific genes was tested by chromatin immunoprecipitation-qPCR (ChIP-qPCR) experiments with transgenic plants that express

hemagglutinin (HA)-tagged HLS1 under the control of the CaMV 35S promoter (HLS1-HA). *WRKY33* is a potential target of HLS1 due to the loss of basal and induced *WRKY33* expression in the *hls1* mutant. HLS1 associated with the transcription start site (TSS) and 3′-coding sequence (CDS) regions of *WRKY33* as revealed by the precipitation of HLS1 and WRKY33 protein-DNA complex (Figure 3B). Interestingly, the association between HLS1 and *WRKY33* was enriched after *B. cinerea* inoculation consistent with their functions in regulating responses to pathogens (Figure 3C). Thus, HLS1 directly regulates *WRKY33* expression through association with its regulatory regions. However, HLS1-HA failed to associate with the TSS or CDS region of the *RPM1* gene despite the reduced expression of *RPM1* in the *hls1* mutant (Supplemental Figure 2). Hence, the effects of HLS1 on *RPM1* expression and its role in *Pst* DC3000 (*avrRpm1*) resistance appear to be indirect.

HLS1 Mediates Histone Acetylation on *WRKY33* Chromatin

Previously, Arabidopsis HLS1 was studied in connection with plant growth, but its biochemical function was not determined. In light of the predicted HAT functions of HLS1, we tested the acetyltransferase activity of recombinant HLS1 protein tagged with glutathione *S*-transferase (GST-HLS1) purified from *Escherichia coli.* Autoradiographs labeled with tritium of the acetyl group revealed no detectable histone acetyltransferase activity from recombinant HLS1 protein when core histones were supplied as substrates (Figure 4A). The control reaction with the P300/CBP-associated factor (PCAF), a protein with known HAT activity, clearly acetylated histones. Global histone H3 and H4 acetylation was analyzed in *hls1* mutants to clarify the impact of HLS1 on this process in vivo. There were no significant differences in the levels of global histone acetylation in the *hls1* mutant before or after inoculation with *B. cinerea* (Figure 4B).

The histone acetylation at chromatin of the *WRKY33* locus was tested in the *hls1* mutant with antibodies that recognize acetylated histones (AcH3 and AcH4). Interestingly, the histone H3 acetylation at *WRKY33* chromatin was reduced significantly in the *hls1* mutant relative to the wild type (Figure 4C). However, histone H4 acetylation at the *WRKY33* promoter region was increased significantly relative to other regions that show no difference between the wild type and the *hls1* mutant. The results suggest that *WRKY33* chromatin is mainly acetylated by HLS1 on histone H3 (Figure 4D). To eliminate the possibility that the reduction of histone H3 acetylation in the *hls1* mutant results from the loss of histone H3 occupancy, we normalized the histone H3 acetylation to total histone H3 on the *WRKY33* TSS region. The significantly increased histone H3 acetylation in wild-type plants correlated with *WRKY33* induction triggered by *B. cinerea* (Figures 2C and 4E). Interestingly, the increased histone H3 acetylation level in wild-type plants was suppressed in the *hls1* mutant. By contrast, histone H3 acetylation in the HLS1-overexpressing plants was enriched significantly more than in wild-type plants (Figure 4E; Supplemental Figure 3). These results suggest that the regulatory impact of HLS1 on *WRKY33* gene expression is mediated through histone H3 acetylation. To determine the biological function of this regulation, transgenic plants overexpressing WRKY33 in *hls1* mutant background were tested for their responses to *B. cinerea*. WRKY33 rescued the *B. cinerea* susceptibility of *hls1* mutant to wild-type levels (Figure 4F), suggesting that *WRKY33* is a direct target of HLS1 during plant responses to pathogens.

HLS1 Mediates Responses to ABA through Direct Association and Histone Acetylation of *ABI5*

The *med18* and *hls1* mutants share similar phenotypes such as disease susceptibility and hookless seedlings in response to ethylene. To determine whether the functional overlap between MED18 and HLS1 extends to other biological functions, the role of HLS1 in response to ABA was tested in seedling germination and growth assays. The role of MED18 in plant response to ABA has been studied previously (Lai et al., 2014). In the presence of 0.5 or 1 μM ABA, the germination of wild-type seedlings was reduced, but *med18* and *hls1* mutants were less affected, exhibiting insensitivity to ABA similar to the *abi5* mutant used as a positive control (Figures 5A and 5B). By contrast, transgenic seedlings expressing HLS1-HA were hypersensitive to ABA relative to the wild-type plants. In addition, *HLS1* expression was induced by ABA treatment (Figure 5C), confirming that HLS1 is positively regulated by ABA and contributes to ABA response.

To assess the impact of HLS1 on the ABA response pathway at the molecular level, the expression of genes involved in the ABA response pathway was examined. Expression of *WRKY40*, *RD29a*, *HY5*, *ABI3*, and *ABI5* genes that are involved in the ABA signaling pathway were attenuated in the *hls1* mutant in response to ABA (Figure 5D). Interestingly, HLS1 directly associated with the *ABI5* gene, particularly with the TSS and CDS regions, but the region for a transcription factor ABI4 recruitment (ABI4 binding site [ABS]) remained at background level (Figure 5E). Thus, *ABI5* expression is directly regulated by HLS1 similar to our previous observation showing MED18 directly regulates *ABI5* expression (Supplemental Figure 4A) (Lai et al., 2014). However, the recruitment of HLS1 to the *ABI5* promoter was not enhanced by *B. cinerea* (Supplemental Figure 4B), which suggests that HLS1 association with *ABI5* is not directly related to plant defense against *B. cinerea*. ChIP-qPCR experiments with AcH3 antibody revealed that the *hls1* mutant has significantly reduced AcH3 levels at the *ABI5* TSS and CDS regions, whereas the H3 acetylation level at Arabidopsis *ACTIN2*, used as a control, was comparable between *hls1* and wild-type plants (Figure 5F), suggesting that HLS1 is required for histone H3 acetylation. Therefore, we concluded that HLS1-mediated *ABI5* expression correlates with the HLS1-dependent histone H3 acetylation.

Functional and Molecular Convergence of HLS1 and MED18 on Target Genes *ABI5* and *WRKY33*

Due to the overlapping biological functions of HLS1 and MED18, we tested their physical interactions by coimmunoprecipitation (co-IP) assays. MED18-MYC precipitated with HLS1-HA in *Nicotiana benthamiana* after transient coexpression, suggesting their presence in the same complex (Figure 6A). MED18-MYC immunoprecipitated with HLS1-HA but not with the empty vector expressing MYC alone. The interaction was confirmed in transgenic Arabidopsis plants coexpressing MED18-MYC and HLS1-HA (Figure 6B). Our current and previous data demonstrated that HLS1 and MED18 are required for responses to ABA and that both proteins associate with the *ABI5* gene (Figures 6A and 6B; Lai et al., 2014). To confirm their functional interdependence, the *HLS1-HA* and *MED18-MYC* plasmids were transfected together with a reporter construct,

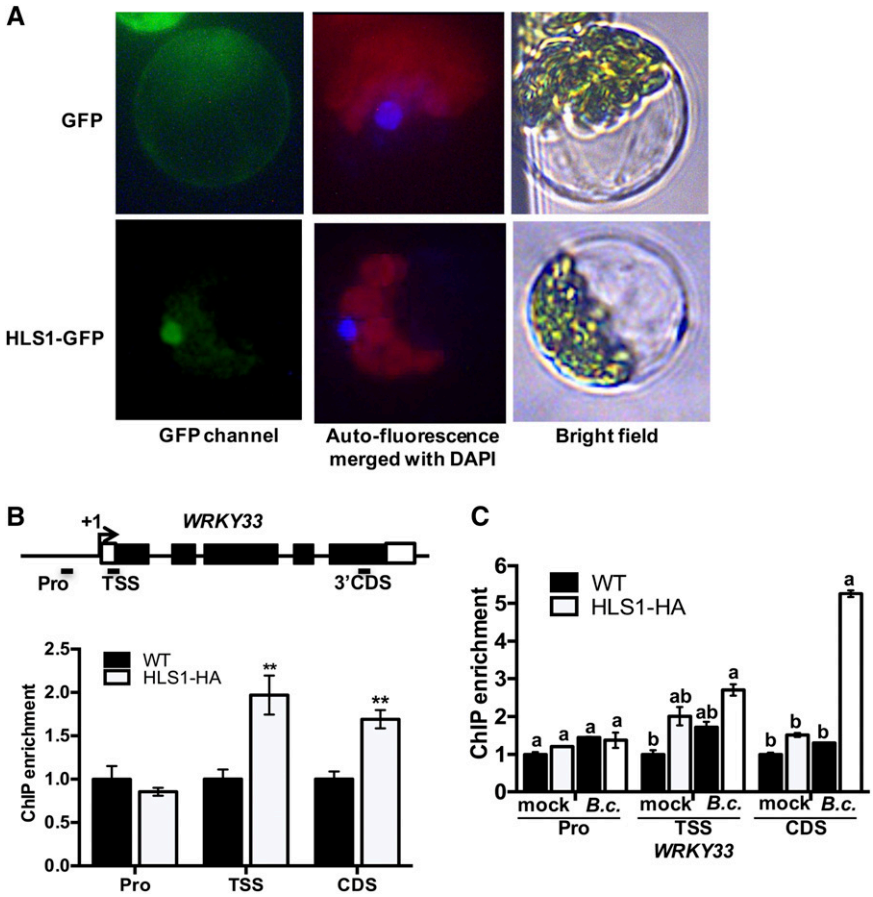

Figure 3. Subcellular Localization of HLS1 and Its Association with Target Genes.

(A) HLS1 is predominantly localized to the nucleus. A plasmid expressing HLS1-GFP fusion under the control of the CaMV 35S promoter was transfected into Arabidopsis protoplasts. The fluorescence signals were observed by epifluorescence microscopy. The red, green, and blue signals are autofluorescence from chloroplasts, GFP, and DAPI, respectively. The merged image is an overlap of DAPI staining and autofluorescence channels.

(B) HLS1 associates with the *WRKY33* gene. The upper panel shows a schematic diagram of the genomic structure of the *WRKY33* gene. The association of HLS1 in the wild type with *WRKY33* is set to 1 as a background control. Statistically significant differences are indicated by asterisks (Student's *t* test: **P < 0.01). Black squares and +1 represent exons and the transcription start site for WRKY33 gene, respectively. The short black bars represent qPCR amplicons in different regions of the *WRKY33* gene.

(C) HLS1 association with the *WRKY33* is enhanced in *B. cinerea*-inoculated plants. The association of the wild type with the *WRKY33* gene under mock treatment is set to 1 as a background control. Statistically significant differences are marked by different letters (least squares means post hoc test: P < 0.05). In the ChIP-qPCR assay (**[B]** and **[C]**), the chromatin and protein complexes were prepared from Arabidopsis wild-type and HLS1-HA-overexpressing plants. The anti-HA antibody was used for immunoprecipitation, and IgG antibody was used as a negative control for immunoprecipitation. The *ACT2* gene represented an internal control for qPCR. The data represent mean values ± SE ($n = 3$). The experiment was repeated three times with similar results. HLS1-HA, HLS1-tagged HA epitope driven by CaMV 35S promoter; Pro, promoter region; Mock, treated with 1% Sabouraud Maltose Broth; *B.c.*, *B. cinerea* inoculation.

ABI5 promoter fused with GUS (pABI5:GUS) into protoplasts. HLS1-HA or MED18-MYC induced *ABI5-GUS* expression in protoplast transactivation assay (Figure 6C). Furthermore, ABI5-GUS accumulation significantly increased when MED18-HA and HLS1-MYC were coexpressed, suggesting their synergistic action on *ABI5* expression. Similarly, the synergistic action of HLS1 and MED18 on *WRKY33* expression was determined in parallel protoplast transactivation experiments (Figure 6C). Transient HLS1 expression enhanced WRKY33-GUS accumulation, confirming the regulatory role of HLS1 on *WRKY33* expression (Figure 2C). However, MED18 failed to activate *WRKY33* gene expression when expressed alone. Interestingly, MED18 enhanced expression of *WRKY33* when coexpressed with HLS1 in protoplasts, suggesting it enhances the function of HLS1 on transcriptional regulation of *WRKY33* (Figure 6C). RT-qPCR data indicated that the *B. cinerea*-induced *WRKY33* expression is reduced in the *hls1* mutant and in MED18-MYC; *hls1* transgenic plants comparable to the *hls1* mutant, implying that MED18-mediated expression of *WRKY33* induction requires HLS1 (Figure 6D). In addition, ChIP-qPCR assay further confirmed that MED18-MYC was associated with the *WRKY33* TSS and 3′CDS regions after *B. cinerea* inoculation, but this association was lost in the absence of HLS1 (Figure 6E). However, disease assays on MED18-MYC; *hls1* transgenic plants revealed that MED18-MYC rescued the *B. cinerea* susceptibility of the *hls1* mutant to wild-type levels,

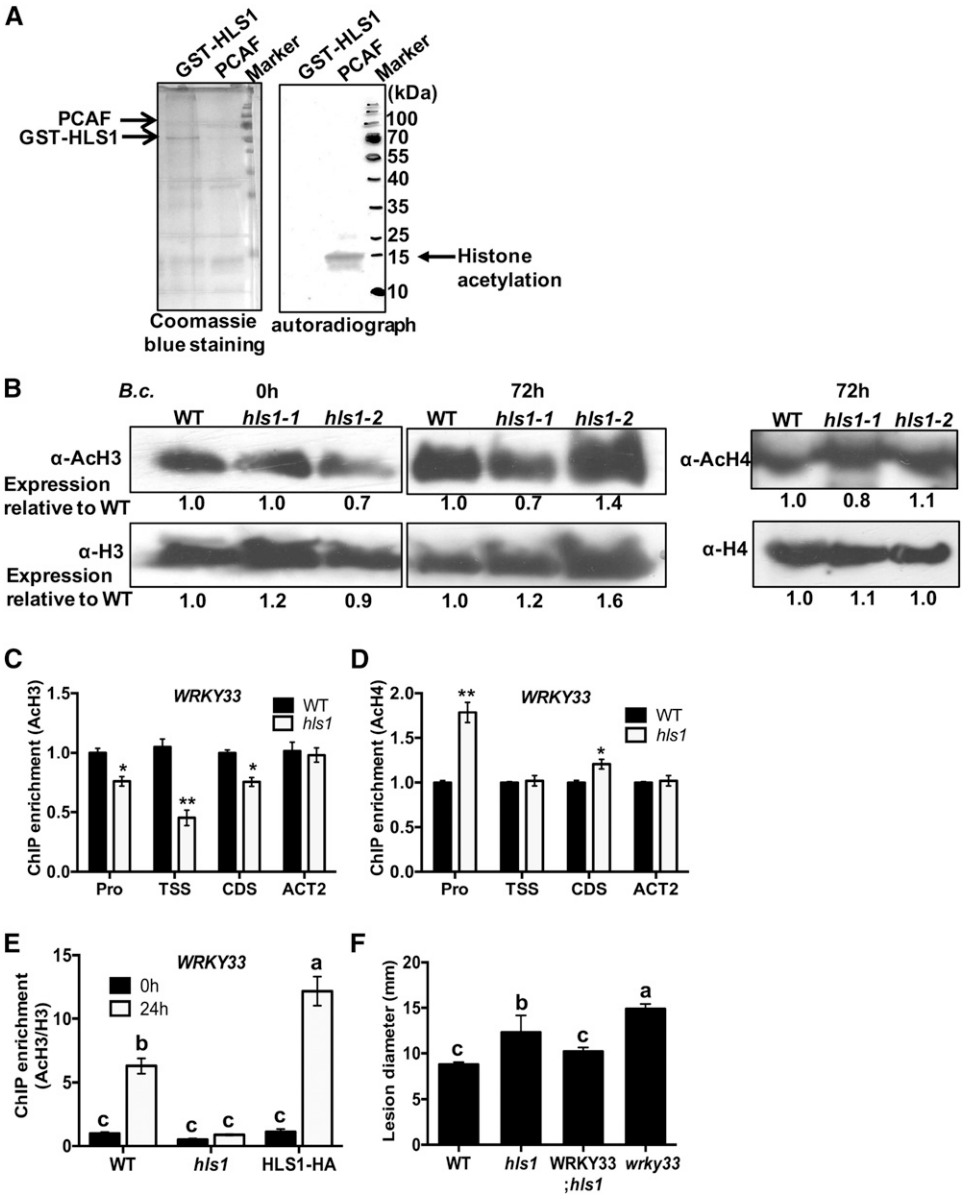

Figure 4. HLS1 Modulates Histone H3 Acetylation on *WRKY33* Chromatin.

(A) Histone acetyltransferase activity assay with recombinant GST-HLS1. The recombinant protein expressing full-length HLS1 tagged with GST (GST-HLS1) was produced in *E. coli* and affinity purified. The recombinant acetyltransferase PCAF protein was used as a positive control in the HAT assay. The chicken core histone used as substrate was obtained from Millipore. The recombinant GST-HLS1 protein run on 15% SDS-PAGE gel and stained with Coomassie blue is shown as a loading control (left panel). The H^3-labeled acetyl-CoA was detected on acetylated histones by autoradiography. The autoradiograph shows that PCAF, but not HLS1, transferred acetyl groups labeled with H^3 from acetyl-CoA to core histones.

(B) Global H3 and H4 acetylation is not altered in *hls1* mutant plants. Histone proteins were extracted from plants inoculated with *B. cinerea.* The global histone H3, acetyl-histone H3, histone H4, and acetyl-histone H4 accumulations were detected using specific antibodies as indicated. The signals on immunoblots were quantified by ImageJ software and numbers are shown corresponding to each lane on the blot.

(C) and **(D)** ChIP-qPCR showing histone H3 **(C)** and H4 **(D)** acetylation at *WRKY33* chromatin. ChIP-qPCR was performed using antibodies that recognize acetylated histone H3 or H4. The different regions of *WRKY33* were amplified by qPCR. The enrichment of *WRKY33* in wild-type plants was set to 1 as a background control in ChIP-qPCR assay. The data represent mean values ± SE ($n = 3$). Statistically significant differences are indicated by asterisks (Student's *t* test: *$P < 0.05$ and **$P < 0.01$).

(E) Histone H3 acetylation at *WRKY33* chromatin is enhanced in *B. cinerea*-inoculated plants. The chromatin complexes were collected from wild-type, *hls1* mutant, and HLS1-HA plants at 0 or 24 h after *B. cinerea* inoculation. The H3 acetylation status is normalized with histone H3 from each sample. The enrichment of *WRKY33* in the wild type at 0 h is set to 1 as a background control in the assay. The data represent mean values ± SE ($n = 3$). Statistically significant differences are marked by different letters (least squares means post hoc test: $P < 0.05$).

suggesting that MED18-mediated plant immunity may be partially independent of HLS1 (Figure 6F). The expression of *LOCUS OF INSENSITIVITY TO VICTORIN* (*LIV1*, *TRX-h5*), a defense-associated thioredoxin and direct target for the victorin toxin (Lorang et al., 2012), was elevated in the *med18* mutant, which contributes to its susceptibility (Lai et al., 2014). *TRX-h5* expression is reduced in MED18 overexpression plants with a similar pattern of expression in MED18-MYC; *hls1* and MED18-MYC; HLS1 plants, demonstrating that MED18-modulating plant defense may be HLS1 independent (Supplemental Figure 5). In sum, our data suggest that HLS1 and MED18 coregulate *WRKY33* and *ABI5* genes. Interestingly, MED18-mediated plant resistance appears to be partially dependent on HLS1 function.

ABA-Induced HLS1 Accumulation Enhances Resistance to *B. cinerea*

To test whether overexpression of HLS1 is sufficient for resistance to *B. cinerea*, we generated transgenic Arabidopsis plants. Two independent HLS1-HA transgenic lines with increased HLS1 expression were selected (Figure 7A). The transgenic plants showed enhanced resistance to *B. cinerea* as well as an exaggerated hook in seedlings germinated in the dark consistent with previous reports (Lehman et al., 1996) (Figure 7B; Supplemental Figure 6A). The transgenic plants also displayed delayed flowering (Supplemental Figure 6B). Interestingly, significantly more HLS1 protein accumulated at 3 d after inoculation (dai) with *B. cinerea* relative to mock-inoculated plants (Figure 7C). The results suggest that either HLS1 is induced by a posttranslational mechanism involving the removal of a repressor or the rate of HLS1 turnover is decreased in response to infection.

In addition, the transgenic HLS1-HA plants treated with ABA accumulated significantly more HLS1 protein than the mock-treated plants (Figure 7D), suggesting that ABA modulates HLS1 at the protein level. To establish a functional link between ABA-induced accumulation of HLS1 and *B. cinerea* resistance, we infiltrated ABA into Arabidopsis leaves 1 d prior to *B. cinerea* inoculation. In wild-type plants, ABA pretreatment enhanced resistance to *B. cinerea*, resulting in significantly reduced disease lesion size (Figures 7E and 7F). The expansion of disease lesions in the *hls1* mutant was comparable in treated and nontreated plants, whereas the HLS1-HA plants exhibited further increase in resistance to *B. cinerea* after ABA treatment. The *B. cinerea* responses of the *hls1* mutant after ABA treatment confirm the *hls1* insensitivity to ABA on seedling germination. The *wrky33* mutant also displayed a loss of ABA-induced resistance to *B. cinerea* similar to *hls1*. The ABA biosynthetic mutants *aba2* and *aba3* and the ABA insensitive mutant *abi5*, which is impaired in ABA response, were selected to determine the role of ABA in induced resistance to *B. cinerea*. The ABA biosynthesis mutants displayed enhanced resistance to *B. cinerea* consistent with previous studies (Adie et al., 2007), but *abi5* was comparable to the wild type (Figure 7F). ABA increased susceptibility to *B. cinerea* in *aba2* and *aba3* relative to mock-treated plants. It has been suggested that ABA pretreatment suppresses callose deposition triggered by flagellin 22 through downregulation of gene expression in ET signaling and indole glucosinolate biosynthesis pathways (Clay et al., 2009). Consistent with this observation, ABA suppressed plant defense gene expression and increased susceptibility to *Pst* (Supplemental Figure 7). In our case, ABA-induced resistance to *B. cinerea* did not correlate with these defense pathways. Overall, plant resistance to fungal infection is enhanced through ABA-induced HLS1 protein accumulation, suggesting a priming effect of ABA though the stabilization of HLS1 protein.

HLS1-Regulated Plant Immunity Is Associated with Senescence

The *hls1* mutant plants displayed enhanced disease symptoms independent of increased pathogen growth and flowered earlier than the wild type regardless of the duration of light. To address a potential link between senescence, early flowering, and plant immunity, we tested dark-induced leaf senescence in *hls1* mutant and wild-type plants. The *hls1* mutants displayed early leaf senescence marked by extensive chlorosis when detached leaves were placed in the dark for 7 d (Figure 8A). The loss of chlorophyll increased significantly in the *hls1* mutant consistent with the dark-induced senescence phenotype (Figure 8B). Interestingly, senescence-like responses occurred in systemic (noninoculated) leaves of *hls1* plants when lower leaves were drop-inoculated with *B. cinerea* (Figure 8C). Symptoms of leaf senescence were increased in *hls1* mutants inoculated with *B. cinerea* as measured by total chlorophyll content (Figure 8D). Whether the early flowering of *hls1* mutants is due to the increased senescence and disease susceptibility was also analyzed. Compared with *hls1* mutants grown in long day (12 h:12 h, light:dark cycle), plants grown in short day (8 h:16 h, light:dark cycle) exhibited increased leaf number indicative of delayed flowering. However, plant susceptibility to *B. cinerea* was unchanged under short day, implying that the *hls1* susceptibility is likely not caused by its early flowering (Supplemental Figure 8). ABA pretreatment increased leaf senescence in *hls1* and *aba2* mutants, which may explain the ABA-induced susceptibility to *B. cinerea* (Figures 7F and 8E). Staining with 3,3′-diaminobenzidine revealed that *hls1* and *aba2* mutants accumulated more H_2O_2, while wild-type plants accumulated lower levels after ABA treatment, suggesting H_2O_2 accumulation is associated with enhanced leaf senescence (Figure 8F). Overall, HLS1-mediated plant resistance may partially be explained by its function in maintaining normal levels of H_2O_2 and senescence.

Figure 4. (continued).

(F) Overexpression of *WRKY33* restored the disease susceptibility of the *hls1* mutant to wild-type levels. The data represent mean disease lesion size ± SE ($n = 12$). The statistical significance of the differences in the mean values is indicated by different letters (least squares means post hoc test: $P < 0.05$). The experiment was repeated at least two times with similar results.

Pro, promoter region; *WRKY33*; *hls1*, overexpressing *WRKY33* in the *hls1* mutant background.

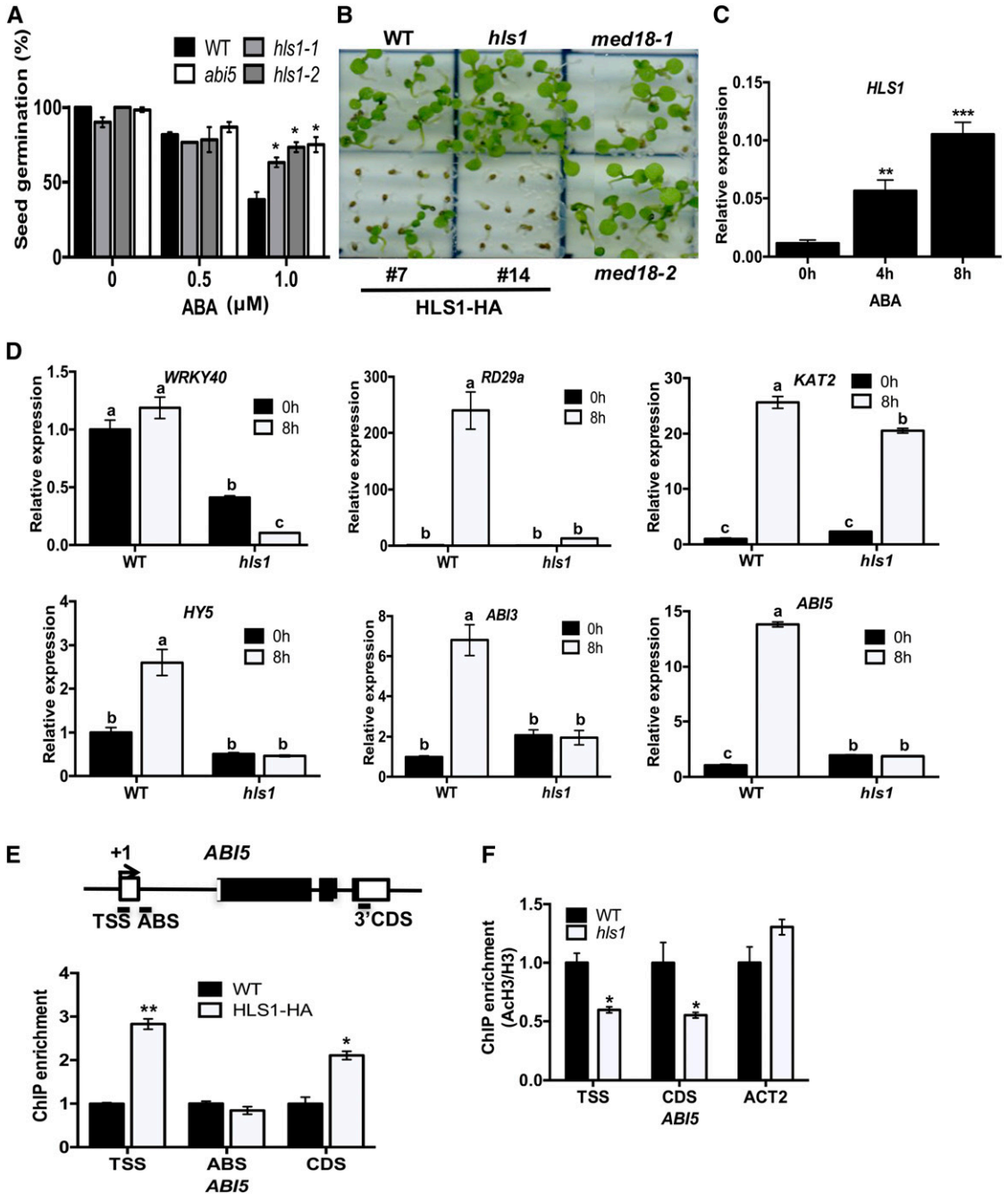

Figure 5. HLS1 Associates with *ABI5* through Histone Acetylation Regulating Responses to ABA.

(A) HLS1 is required for ABA responses as measured by seed germination assays on ABA-containing media. The data represent mean values ± SD ($n = 30$), and the statistically significant difference is indicated by an asterisk (ANOVA test: *P < 0.05).

(B) Seed germination and seedling growth on media supplemented with ABA.

(C) Expression of *HLS1* is induced by ABA. The data represent mean values ± SE ($n = 3$) from three independent biological replicates, and the statistically significant differences are indicated by asterisks (ANOVA test: **P < 0.01 and ***P < 0.001).

(D) HLS1 is required for ABA-induced expression of ABA response and regulatory genes. The expression of ABA response genes in the wild type after mock inoculation is set to 1. The data represents mean values ± SE ($n = 3$) from three independent experiments, and statistically significant differences are indicated by different letters (least squares means post hoc test: P < 0.05).

(E) Schematic diagram showing the genomic structure of *ABI5*. HLS1 associates with TSS and CDS regions on the *ABI5* gene. The association of the wild type with *ABI5* gene is set to 1 as a background control.

ABA-Induced *WRKY33* Expression Is Dependent on HLS1 Association and Chromatin Acetylation

We investigated genetic and molecular interactions between HLS1 and WRKY33 in ABA responses. Overexpressing WRKY33 in the *hls1* mutant background restored the ABA-insensitive phenotype in the *hls1* mutant to wild-type levels, suggesting that WRKY33 is a downstream target of HLS1 during ABA responses (Figures 9A and 9B). In response to ABA, the expression of *WRKY33* was significantly reduced in the *hls1* mutant, suggesting that ABA-induced expression of *WRKY33* is also dependent on HLS1 (Figure 9C). Consistent with this, the association of HLS1 protein with *WRKY33* TSS and CDS regions is enriched by ABA treatment, indicating that HLS1-dependent *WRKY33* induction is also modulated by ABA (Figure 9D). Interestingly, ABA significantly enhanced the H3 acetylation at the *WRKY33* locus in wild-type plants, whereas the *hls1* mutant remained at the nontreated level even after ABA treatment (Figure 9E). This result suggested that HLS1-mediated *WRKY33* expression responds to ABA through histone acetylation. Together, ABA-induced plant resistance to *B. cinerea* may go through HLS1-mediated *WRKY33* activation. In sum, HLS1 association with *WRKY33* is enhanced after *B. cinerea* inoculation, and both HLS1 and WRKY33 contribute to ABA-mediated priming of plant defense responses.

DISCUSSION

We describe the functions of Arabidopsis HLS1 and its role in plant immunity and responses to ABA primarily through its function in histone acetylation and interaction with the transcription coregulatory protein complex Mediator. We show that (1) HLS1 and the MED18 subunit of the Mediator complex share significant biological function and interact physically. (2) HLS1 is required for histone acetylation at the *ABI5* and *WRKY33* loci, critical regulators of ABA and pathogen responses, respectively. (3) Ectopic expression of HLS1 is sufficient for increased resistance to *B. cinerea.* Enhanced HLS1 protein accumulation in response to ABA and *B. cinerea* results in increased fungal resistance. ABA primes resistance to *B. cinerea*, likely through pathogen- and ABA-induced accumulation of HLS1 protein and upregulation of *WRKY33* expression. (4) HLS1 and MED18 coregulate *ABI5* and *WRKY33* target genes through direct association in response to ABA and *B. cinerea.* This observation is further supported by synergistic actions of MED18 and HLS1 on *ABI5* and *WRKY33* gene expression. HLS1 is required for MED18 association with *WRKY33* but not with *ABI5* upstream regulatory regions, suggesting a complex relationship with these partners. (5) The *hls1* mutant plants show enhanced senescence with particularly striking symptoms of senescence in systemic leaves in response to *B. cinerea*. (6) HLS1 is required for H3 acetylation at *WRKY33* and *ABI5* chromatin. The histone H3 acetylation at *WRKY33* chromatin increased in response to pathogen infection and ABA treatment consistent with positive regulation of the *WRKY33* and *ABI5* by HLS1. In sum, HLS1 is a major regulator in priming plant immune responses through transcriptional and posttranslational mechanisms.

HLS1 Is a Critical Transcriptional and Posttranscriptional Regulator of ABA-Mediated Priming of Plant Defense

The function of HLS1 in ABA responses is supported by genetic and molecular data. HLS1 is induced by ABA, and expression of ABA-responsive genes, including *ABI5*, a direct target of HLS1 and a known ABA response regulator. Germination and seedling growth of the *hls1* mutant are insensitivity to ABA, whereas the overexpression line is hypersensitive to ABA. Interestingly, ABA primes resistance to fungal infection in wild-type plants, whereas in *hls1*, ABA failed to prime resistance, consistent with the loss of ABA sensitivity. In addition, the HLS1-interacting protein MED18 and the common target gene *WRKY33* were implicated in ABA responses. The overexpression of *WRKY33* confers ABA hypersensitivity in Arabidopsis, similar to the overexpression of *HLS1*, whereas *med18* and *hls1* mutants are insensitive to ABA (Jiang and Deyholos, 2009). All three partners, HLS1, MED18, and WRKY33, are involved in pathogen and ABA response pathways. In particular, the critical role of HLS1 as a transcriptional activator of defense is underlined by its relationship with WRKY33, a major immune response regulator that operates through multiple mechanisms, including the regulation of phytoalexin biosynthesis, autophagy, and interaction with the MAPK pathway (Lai et al., 2011; Mao et al., 2011). WRKY33 also modulates ABA responses and biosynthesis (Liu et al., 2015) and its expression is mediated through ABA-induced HLS1 recruitment and histone acetylation. *WRKY33*, similar to *ABI5*, is regulated by direct association with HLS1 and potentially functions through ABA-dependent and ABA-independent pathways, consistent with its multifunctionality (Liu et al., 2015; Mao et al., 2011).

Multiple lines of genetic evidence suggest a link between ABA and pathogen responses. However, the role of ABA in plant defense responses is complex and varies depending on the nature of pathogens, the types of tissues, and the infection stages (Ton et al., 2009). Previous observations suggest that ABA promotes plant susceptibility to disease. For example, genes in the ABA biosynthesis pathway promote plant susceptibility to pathogens. The ABA-deficient tomato mutant *sitins* is resistant to *B. cinerea* and displayed increased basal and induced JA/ET-dependent defense gene expression and enhanced cuticle permeability (Asselbergh et al., 2007; Curvers et al., 2010). ABA-deficient mutants, such as *aba1* and *aba2*, modulate JA/ET-responsive genes to enhance plant resistance to necrotrophs (Anderson et al.,

Figure 5. (continued).

(F) Histone H3 acetylation at *ABI5* chromatin is reduced in the *hls1* mutant. The histone H3 acetylation levels in the wild type are set to 1 as a background control.

In **(E)** and **(F)**, the data represent mean values ± SE ($n = 3$) from three independent biological replicates. The statistically significant difference is indicated by asterisks (Student's *t* test: *P < 0.05 and **P < 0.01). HLS1-HA, HLS1-tagged HA epitope driven by CaMV 35S promoter; ABS, ABI4 binding region.

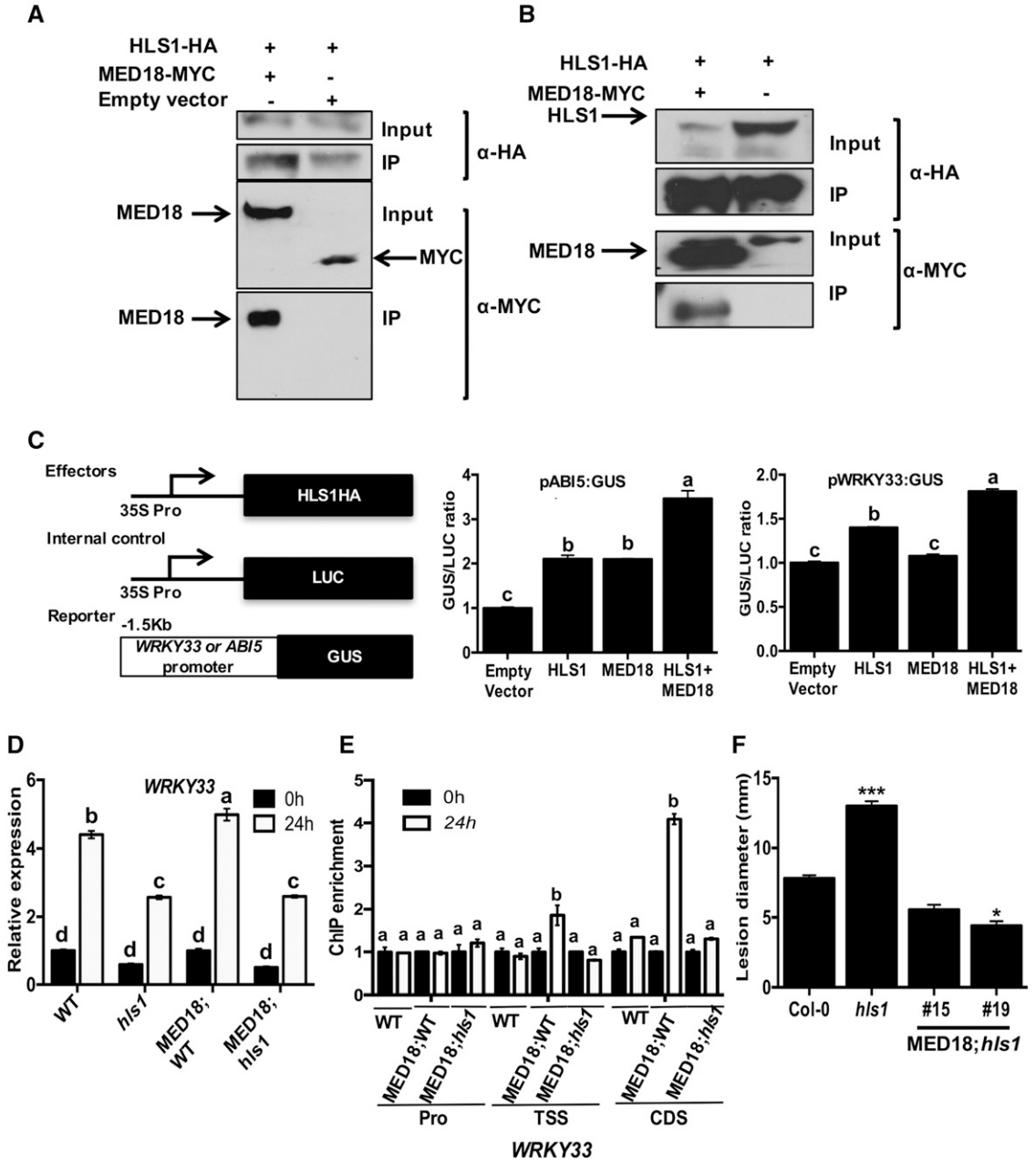

Figure 6. Molecular Interaction between MED18 and HLS1 and Their Synergistic Action on Target Gene Expression.

(A) and **(B)** Interaction between MED18 and HLS1 in co-IP assay in *N. benthamiana* **(A)** and transgenic Arabidopsis **(B)** plants. In **(A)**, HLS1-HA was transiently coexpressed with MED18-MYC by agroinfiltration in *N. benthamiana* leaves. The empty vector expressing MYC was used as a negative control. In **(B)**, transgenic Arabidopsis plants stably expressing *HLS1-HA* and *MED18-MYC* were used in the co-IP assays. Anti-HA beads were used to precipitate HLS1-HA protein. Anti-HA (α-HA) and anti-MYC (α-MYC) antibodies were used to detect protein accumulation in input or immunoprecipitated (IP) samples.
(C) Synergistic action of HLS1 and MED18 in the regulation of *ABI5* or *WRKY33* expression. The schematic diagram shows plasmid constructs used in transcriptional activation assay. The CaMV 35S promoter driving the luciferase reporter gene (35S:LUC) and the *WRKY33* or *ABI5* promoter fused with *GUS* reporter gene (pWRKY33/pABI5:GUS) are used as an internal control and a reporter, respectively. 35S promoter driving expression of HLS1 tagged with HA (HLS1-HA) is used as an effector. The bar graphs show the mean relative GUS activity from expression of the various plasmids depicted in the schematic. The mean values from protoplasts transfected with empty vector, pWRKY33/pABI5:GUS, and 35S:LUC were set to 1 as an internal control. The GUS signal is normalized with the LUC signal. The data represent mean values ± SE (n = 3) from two independent biological replicates, and statistically significant differences are indicated by different letters (least squares means post hoc test: $P < 0.05$).
(D) MED18-mediated *WRKY33* expression is dependent on HLS1. Relative gene expression is normalized to *ACT2*. The relative expression in wild-type plants at 0 h is set to 1.

2004). WRKY33 suppresses downstream target genes *NCED3* and *NCED5*, thus abrogating ABA biosynthesis and increasing disease resistance (Liu et al., 2015).

In contrast to the above data, pathogens hijack ABA, either by manipulating its biosynthesis or antagonizing the SA-mediated resistance pathway to attenuate plant immunity (Xu et al., 2013; de Torres-Zabala et al., 2007; Jiang et al., 2010). ABA signaling mutants *abi1-1* and *abi2-1* increase susceptibility to *Ralstonia solanacearum* (Hernández-Blanco et al., 2007). Arabidopsis mutants, such as *med25* and *med18*, with enhanced disease susceptibility phenotypes also displayed altered responses to ABA (Chen et al., 2012; Lai et al., 2014), but how the ABA function relates to the pathogen response functions of the genes is unclear. The non-protein amino acid β-amino-butyric acid (BABA) primes resistance to necrotrophic pathogens (Ton et al., 2005) based on primed callose accumulation, controlled by an ABA-dependent defense pathway. BABA-induced resistance was blocked in the ABA-deficient mutant *aba1-5* and the ABA-insensitive mutant *abi4-1* (Ton and Mauch-Mani, 2004). Application of ABA mimicked the effects of BABA on callose accumulation and resistance. Thus, ABA is required for BABA-induced resistance to pathogens by enhancing callose deposition. The phenotypes of ABA pretreatment and plant susceptibility in ABA biosynthetic mutants are similar to those in *hls1* mutants. This suggests that the plant resistance to pathogens is a consequence of ABA pretreatment. ABA suppressed expression of defense genes in wild-type plants, consistent with previous reports (Supplemental Figure 7; Clay et al., 2009). However, ABA-induced plant resistance to *B. cinerea* showed no correlation with ABA induced expression of defense genes. Instead, plant susceptibility in *hls1* was associated with ABA-induced senescence and accumulation of H_2O_2.

ABA is a well-known regulator in abiotic and biotic stress responses. Many studies implicate exogenous ABA treatment in increased plant tolerance to abiotic stresses such as chilling and osmotic stress (Jiang and Zhang, 2002; Guo et al., 2012; Ozfidan et al., 2012), but ABA is often implicated as a suppressor of plant resistance (Curvers et al., 2010). The mechanism underlying this disparity between plant responses to biotic and abiotic stress responses is unclear. ABA increases reactive oxygen species (ROS) production, which then activates an antioxidative defense response in maize (*Zea mays*) seedlings (Jiang and Zhang, 2001). *hls1* and ABA biosynthesis mutants accumulate increased ROS and display enhanced leaf senescence, likely without the concomitant increase in the appropriate antioxidant systems. This contention is consistent with the role of WRKY33 on ROS detoxification and scavenging, suggesting that WRKY33 may modulate ROS turnover in response to ABA (Jiang and Deyholos, 2009; Golldack et al., 2014). HLS1 may share the function of MED18 in the control of ROS homeostasis.

Application of ABA at the time of pathogen inoculation enhanced susceptibility (Liu et al., 2015); thus, the timing of ABA treatment may be important to determine defense functions in plants. Many genes in the ABA and defense pathways displayed altered expression in the *hls1* mutant. *PR1* expression is highly activated in the *hls1* mutant, consistent with previous reports that ABA signaling antagonizes SA-dependent responses (Yasuda et al., 2008; Pieterse et al., 2012; Liu et al., 2015). The induction of *PR1* in the *hls1* mutant does not correlate with resistance but implies that some pathways leading to *PR1* expression are affected. Alternatively, due to the susceptibility of the *hls1* mutant and increased fungal growth, some genes displayed increased gene expression. ABA antagonizes the ETHYLENE RESPONSE FACTOR (ERF) branch of the JA pathway and regulates defense marker gene *PDF1.2* (Anderson et al., 2004). However, the *hls1* mutant displayed increased expression of *PDF1.2*, which is linked to fungal resistance (Penninckx et al., 1996), but the mutant remained susceptible to *B. cinerea*. HLS1-mediated gene expression is not a function of the antagonism between JA- and SA-regulated pathways, since markers of both pathways are also upregulated in the mutant. Together, the HLS1-mediated plant immunity works through the ABA signaling pathway but is independent of the ET/JA- and SA-regulated pathways as well as independent of their antagonistic interactions.

The presented data and discussions in the preceding sections imply loss of HLS1-regulated senescence, which may account for the enhanced susceptibility of the mutant. Senescence-like responses are triggered by dark or ABA treatment in the *hls1* mutant. In particular, the senescence phenotype observed in secondary (noninoculated) leaves in mutant plants implies that HLS1 is important for restricting senescence-like symptoms that include extensive chlorosis and death of tissue away from the infection site. The increased susceptibility to *B. cinerea* may stem from impaired cell death control, including senescence.

HLS1-Mediated Histone Acetylation of Target Genes *ABI5* and *WRKY33*

HLS1 is required for H3 acetylation at *ABI5* and *WRK33* chromatin based on changes in acetylation status in *hls1* and HLS1-HA plants. However, acetyltransferase activity, measured through a standard HAT assay using recombinant protein, revealed no

Figure 6. (continued).

(E) MED18 recruitment to transcription start site and 3′-coding regions of *WRKY33* is enhanced by inoculation with *B. cinerea* in an HLS1-dependent manner. The enrichment of the *WRKY33* gene in the wild type at 0 h is set to 1 as a background control in the ChIP-qPCR assay.

(F) Ectopic expression of MED18 rescues disease phenotype of *hls1* mutant. The disease lesion size was determined after drop inoculation with *B. cinerea*. The data represent mean values ± SE ($n = 20$). Statistically significant differences are indicated by asterisks compared with wild-type plants (Student's *t* test: *P < 0.05 and ***P < 0.001).

In **(D)** and **(E)**, the data represent mean values ± SE ($n = 3$), and the statistically significant differences are marked by different letters (least squares means post hoc test: P < 0.05). *pABI5:GUS* and *pWRKY33:GUS*, reporter *GUS* fused with *ABI5* or *WRKY33* promoter region, respectively; HLS1, HLS1 tagged with HA; MED18, MED18 tagged with MYC. MED18; WT, overexpressing *MED18* in wild-type background. MED18; *hls1*, overexpressing *MED18* in the *hls1* mutant background.

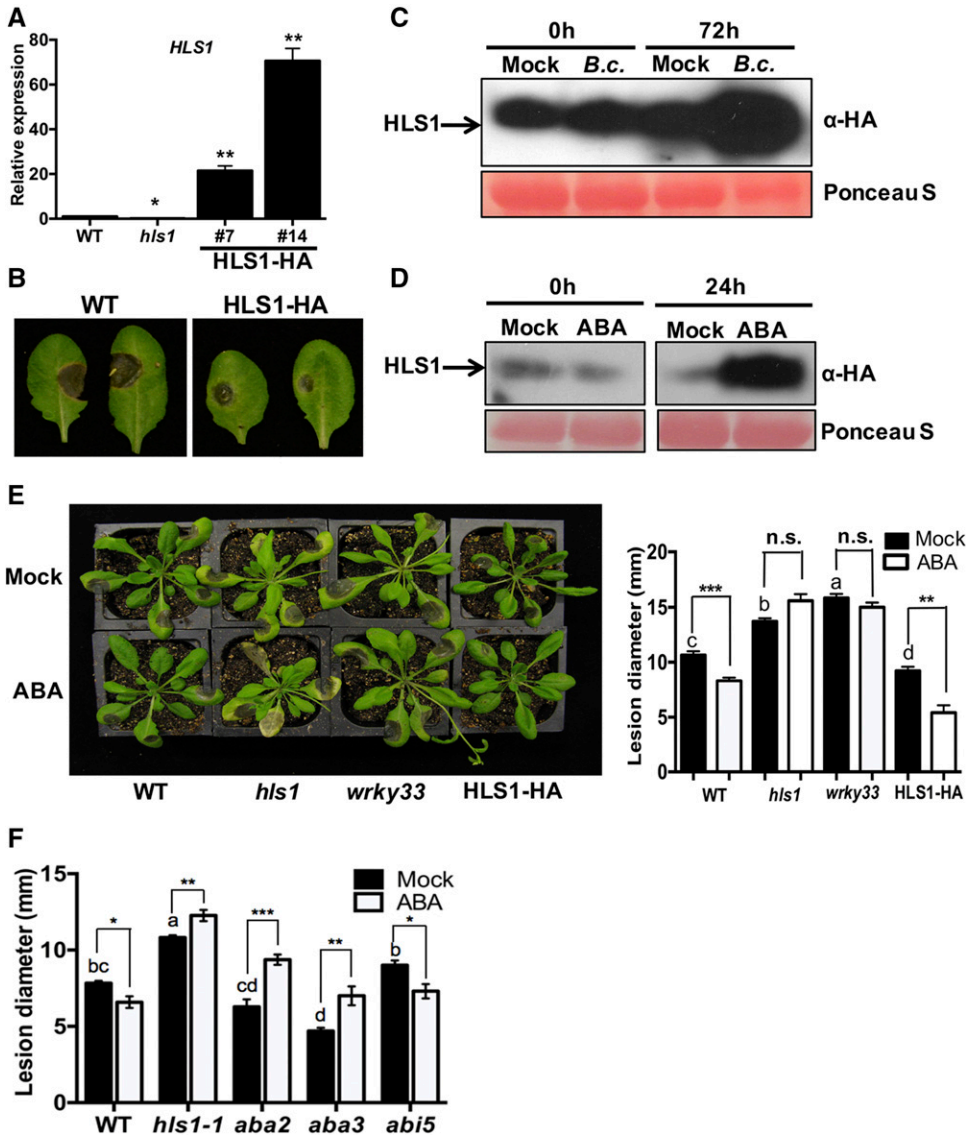

Figure 7. Resistance to *B. cinerea* Is Enhanced through ABA-Induced HLS1 Protein Accumulation.

(A) *HLS1* expression in wild-type, *hls1*, and *35S:HLS1-HA* plants.

(B) Increased disease resistance evaluated by disease symptoms in *B. cinerea* drop-inoculated plants.

(C) and **(D)** *B. cinerea*- **(C)** and ABA- **(D)** induced accumulation of HLS1 protein. *HLS1-HA* plants were inoculated with *B. cinerea* (top panel) or infiltrated with 100 μM ABA (bottom panel). Mock-treated plants were infiltrated with 0.5% methanol. Total protein was extracted from HLS1-HA plants at 0 or 72 h after *B. cinerea* inoculation or 24 h after treatment with ABA. HLS1 protein level was detected on immunoblot with anti-HA antibody. Equal loading is shown by Ponceau S staining of total protein.

(E) Disease symptoms (left panel) and disease lesion size (right panel) in ABA-treated plants. Plants were pretreated by infiltration with ABA 1 d prior to *B. cinerea* inoculation. Disease symptoms and lesion size were recorded at 3 d after *B. cinerea* inoculation. The data represent mean values ± SE ($n = 20$). Statistically significant differences are marked by asterisks (Student's *t* test: **P < 0.01 and ***P < 0.001; n.s., not significant) and by different letters (least squares means post hoc test: P < 0.05).

(F) Enhanced *B. cinerea* disease lesions in *hls1*, *aba2*, and *aba3* mutants in response to ABA pretreatment. The disease lesions were measured at 2 dai. The *aba2* and *aba3* mutant plants displayed enhanced resistance to *B. cinerea*, while *abi5* was comparable to wild-type plants. The data represent mean values ± SE ($n = 24$). Statistically significant differences are marked by asterisks (Student's *t* test: *P < 0.05, **P < 0.01, and ***P < 0.001) and by different letters (least squares means post hoc test: P < 0.05).

HAT activity, possibly due to either the GST tag in the GST-HLS1 fusion affecting the structure of the protein and its acetyltransferase activity, or the requirement for HLS1 to recruit other cofactors for activity. Histone acetylation alters the structure of defense and non-defense genes that underlie plant responses to the environment. Histone H4 deacetylase, HDT701, reduces

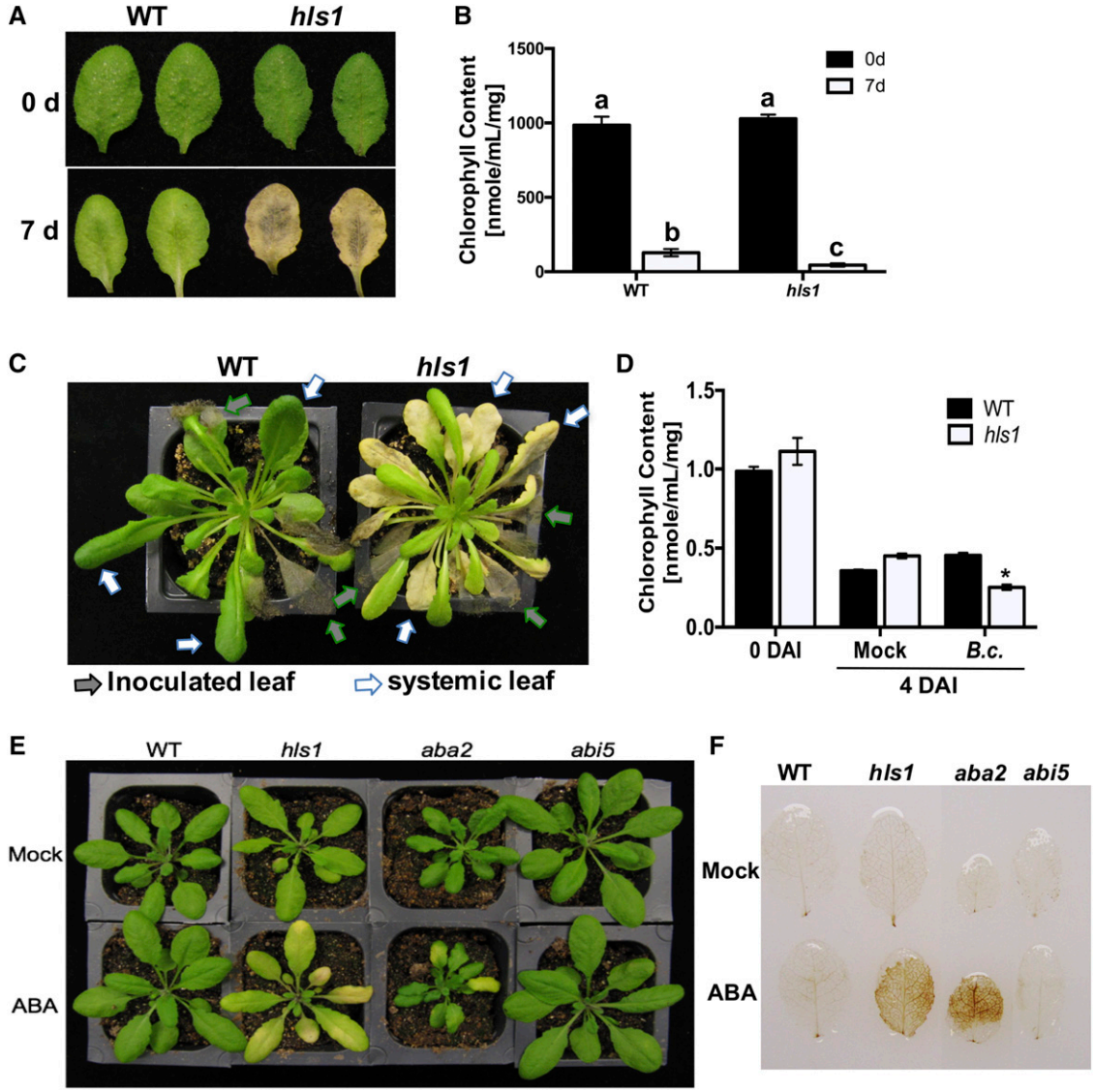

Figure 8. Accelerated Dark- or Pathogen-Induced Leaf Senescence in the *hls1* Mutant.

(A) and **(B)** Enhanced dark-induced leaf senescence **(A)** and reduced chlorophyll content **(B)** in *hls1* mutant. The photo was taken at 7 d after plants were incubated in the dark. Leaves from similar positions were detached from plants, and senescence was compared through analysis of chlorophyll content. The data represent mean values ± SD from (n = 5). The experiment was repeated two times with similar results.

(C) Enhanced systemic leaf senescence and extensive chlorosis are induced by *B. cinerea* in the *hls1* mutant. Leaf senescence is observed on noninoculated systemic leaves of the *hls1* mutant after inoculation of lower leaves with *B. cinerea*. The photo was taken 10 d after inoculation.

(D) Chlorophyll contents in plants showing senescence-like symptoms at 4 d after inoculation with *B. cinerea*. The data represent mean values ± SD from (n = 5). The experiment was repeated two times with similar results. Statistically significant differences are marked with an asterisk (ANOVA test: *$P < 0.05$).

(E) ABA-induced leaf senescence in *aba2* and *hls1* mutants. Leaf senescence in the *abi5* mutant was comparable to wild-type plants. The photo was taken 24 h after ABA infiltration.

(F) Increased accumulation of H_2O_2 in *aba2* and *hls1* mutants in response to ABA. Plants were stained 24 h after ABA treatment. The leaves from similar positions were infiltrated with ABA and H_2O_2 was detected by 3,3′-diaminobenzidine staining.

global histone H4 acetylation and modulates defense-related genes in rice resistance to *Magnaporthe oryzae* and *Xanthomonas oryzae* pv *oryzae* (Ding et al., 2012). The elongator complex subunit 2 (ELP2) and ELP3 regulate resistance to *P. syringae* pv *maculicola* (*Psm*) ES4326 through their HAT activity on defense-related genes (Defraia et al., 2013; Wang et al., 2013). As shown in this study, HLS1 modulates H3 acetylation on specific loci required for plant immunity and ABA responses.

Plant defense genes are poised to counteract attempted pathogen infection through priming, which has been linked to posttranslational modification of histone tails. The promoter regions of defense-related transcription factor genes *WRKY6*,

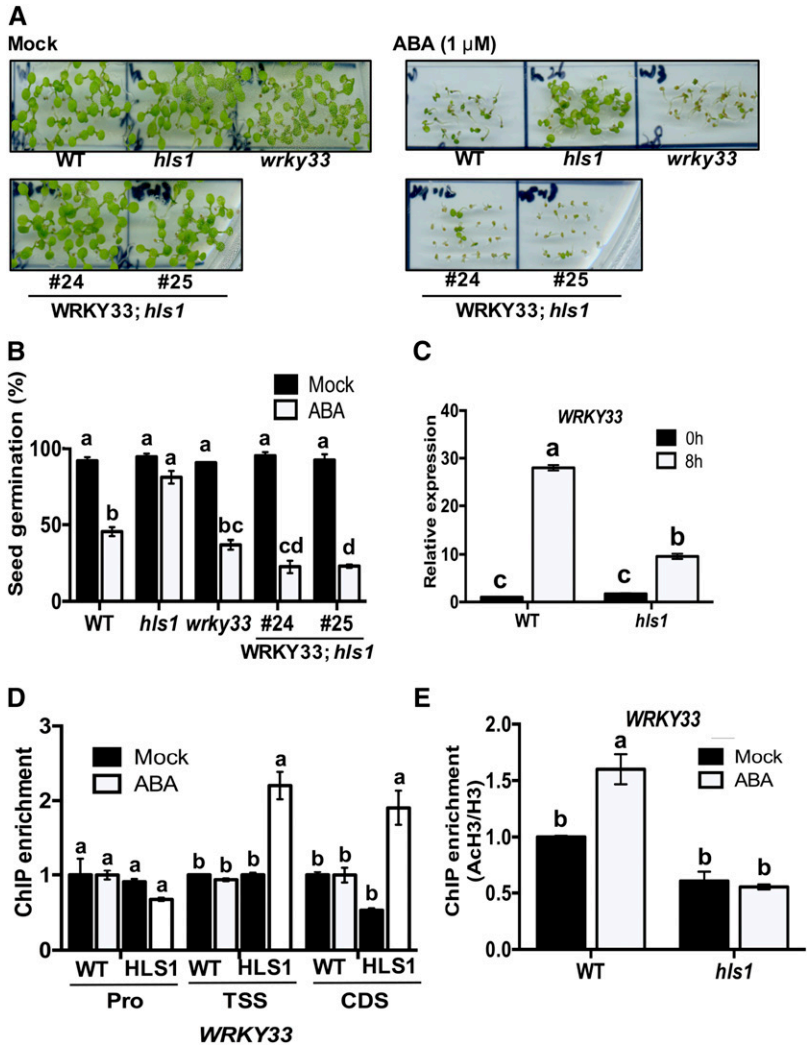

Figure 9. ABA-Mediated *WRKY33* Expression Is Modulated by HLS1.

(A) Ectopic expression of WRKY33 restores the ABA insensitivity of the *hls1* mutant to the wild-type level. Seeds germinated on 1/2 MS medium supplemented with 0.005% methanol (mock) or 1 μM of ABA. The photos were taken at 5 d.

(B) Seed germination and seedling growth on media supplemented with ABA. The data represent mean values ± SD ($n = 30$) and the statistical significance of the difference in mock or ABA treatment is indicated by different letters (least squares means post hoc test: $P < 0.05$).

(C) ABA-induced expression of *WRKY33* is attenuated in *hls1* mutant. The expression of *WRKY33* in wild-type plants at 0 h is set to 1.

(D) ABA mediates the recruitment of HLS1 to the *WRKY33* transcription start and coding regions. The association of HLS1 with *WRKY33* under mock treatment is set to 1 as a background control.

(E) ABA-enhanced H3 acetylation at the *WRKY33* locus is HLS1 dependent.

In **(C)** to **(E)**, the data represents mean values ± SE ($n = 3$), and statistically significant differences are indicated by different letters (least squares means post hoc test: $P < 0.05$). HLS1, HLS1 tagged with HA; *WRKY33*; *hls1*, overexpressing *WRKY33* in the *hls1* mutant background; Pro, promoter region.

WRKY26, or *WRKY53* were either acetylated or methylated in primed plants treated with the SA analog, benzothiadiazole (Jaskiewicz et al., 2011). Arabidopsis HAT1 mediates activation of PTI-related genes *WRKY53*, *FRK1*, or *NHL10* primed by environmental stresses (Singh et al., 2014). BABA-triggered chromatin modification activates defense-related gene transcription (Po-Wen et al., 2013). HLS1 mediates *WRKY33* expression through histone acetylation in response to ABA and pathogens, supporting the dynamic chromatin modification in response to stimuli. The results are consistent with previous studies demonstrating that histone modification enzymes stand by on specific target loci and modulate gene expression following attempted infection (Jaskiewicz et al., 2011).

Histone deacetylases reverse acetylation status on histones to remove acetyl group from substrates, resulting in repression of gene expression. Arabidopsis *HDA6* encodes histone deacetylase and the *hda6* mutation results in hypersensitivity to ABA, delayed senescence, and flowering (Wu et al., 2008). HDA6

recruits a JA-Zim domain (JAZ) protein to repress EIN3/EIL1-dependent transcription (Zhu et al., 2011b). Arabidopsis histone deacetylase HDA19 is another histone-modifying enzyme and the *hda19* mutant results in early senescence, hypersensitivity to ABA, and susceptibility to *Alternaria brassicicola* (Wu et al., 2000; Tian et al., 2005; Zhou et al., 2005). HDA6 and HDA19 share contrasting biological functions with HLS1, consistent with their distinct roles in histone modifications. Whether HLS1, HDA6, and HDA19 target the same set of genes for reversible modification of histone acetylation is unclear.

Functional Interaction between Histone Acetylation and Mediator

Current and previous data show that HLS1 and MED18 are positive regulators of ABA signaling and resistance to *B. cinerea* (Lai et al., 2014). HLS1 and MED18 interact and are associated with *WRKY33* and *ABI5* regulatory regions. This association increases in response to ABA and *B. cinerea* as determined through ChIP-qPCR and transcription activation assays. Many studies in yeast and mammalian cells have shown that mediator complexes modulate histone modification. The Spt-Ada Gcn5-acetyltransferase (SAGA) complexes require a mediator complex to be recruited to the GCN4-regulated promoters of *ARG1*, *ARG4*, or *SNZ1* (Yoon et al., 2003; Qiu et al., 2005). In mammalian cells, MED25 affects methylation or acetylation at H3K27 at *CYP2C9* promoter region by dissociation from Polycomb repressive complex 2 and activates *CYP2C9* expression (Englert et al., 2015). The Arabidopsis E3 ligase HUB1 is required for resistance to fungal pathogens and regulates ABA responses and biosynthesis (Peeters et al., 2002; Liu et al., 2007; Dhawan et al., 2009). HUB1 interacts with MED21 and activates gene transcription through H2B ubiquitination, implying it functions as a component of transcriptional activation complexes. In another report, mediator localization is determined by the interaction between mediator and histone tails. The interaction is relieved by the acetylation of H4K16 (Zhu et al., 2011a). MED18 is also associated with *WRKY33* TSS and CDS regions, similar to the genomic localization of HLS1. Interestingly, MED18 is unable to associate with *WRKY33* in the absence of HLS1, suggesting that HLS1 is required for MED18 recruitment to specific loci.

Proposed Model of HLS1 Function

Collectively, we demonstrate that HLS1 associates with MED18 at the *ABI5* and *WRKY33* loci and modulates their expression through acetylation of chromatin at these loci. Although many genes are regulated by HLS1 in response to ABA or pathogens, some of these are affected only indirectly. HLS1 associates with the *WRKY33* gene and activates its expression through histone acetylation after *B. cinerea* inoculation or ABA treatment. HLS1 recruits MED18 to the *WRKY33* locus where MED18 enhances the role of HLS1 in transcriptional activation of *WRKY33* (Figure 9). MED18 also enhances the HLS1-regulated expression of *ABI5*, but its recruitment to the *ABI5* locus is independent of HLS1. Interestingly, HLS1 is required and sufficient for the histone acetylation at *WRKY33* chromatin, consistent with the high sequence similarity of HLS1 to the GCN5 histone acetyl transferase. Other components that potentially associate with the two proteins are not known, but transcription factors, co-activators, or other chromatin remodeling components may be involved to form a preinitiation complex. Other non-histone proteins may be recruited with HLS1 and MED18 for initiation of gene expression. Identifying additional proteins that interact with HLS1 will help us understand the acetylation mechanism that modulates responses to biotic and abiotic stresses. In response to ABA or *B. cinerea*, the complex enhances histone acetylation to remodel chromatin structure, favoring increased gene expression. The consequences of these will be enhanced transcriptional activation of genes that requires the recruitment of HLS1, which then recruits MED18 to target sites. Biologically, HLS1 participates in different response pathways (light, sugar, and pathogen) and is regulated by hormone crosstalk (JA, gibberellin, ET, and ABA). Therefore, it will be important to investigate the global targets of HLS1 through ChIP-seq analysis to identify additional targets bound by HLS1 to decipher its regulatory impact and to determine histone acetylation mechanisms dynamically responding to environmental challenges.

METHODS

Plant Materials and Growth Conditions

Arabidopsis thaliana plants used in this study are in the Col-0 ecotype background. Plants were grown in a growth chamber at 24°C, 70% relative humidity, 110 to 130 μE m^{-2} s^{-1} light intensity by fluorescence tubes (model F32T8/TL741) with a 12-h-light/12-h-dark cycle unless stated otherwise. T-DNA insertion Arabidopsis mutants *hls1-1* (SALK_136528C) and *hls1-2* (SALK_009473) in the Col-0 background were obtained from the ABRC and confirmed by PCR to verify their T-DNA insertion. *HLS1* expression in *hls1* mutant plants was confirmed in 3-d-old seedlings compared with wild-type or transgenic plants by qPCR. Transgenic plants overexpressing *HLS1* tagged with HA under the CaMV 35S promoter were generated by *Agrobacterium tumefaciens*-mediated transformation. Plants were screened on half-strength Murashige and Skoog (1/2 MS) medium supplemented with hygromycin or the herbicide basta. Protein or mRNA levels of HLS1 were verified by immunoblotting analysis with anti-HA-specific antibody or qPCR assays, respectively. Transgenic plants coexpressing *HLS1* and *MED18* were generated by Agrobacterium-mediated transformation and HLS1 and MED18 expression was detected by immunoblotting. Transgenic plants overexpressing *MED18-MYC* or *WRKY33-MYC* in the *hls1* mutant background were generated by Agrobacterium-mediated transformation and *MED18* or *WRKY33* transgenic plants were screened on 1/2 MS medium supplemented with basta and protein expression was detected by immunoblotting.

Seed Germination, Dark-Induced Senescence, ABA Treatment, and Disease Assay

For seed germination assays, Arabidopsis mutant or transgenic seedlings were germinated on 1/2 MS medium supplemented with different concentrations of ABA and grown in a room at 22°C, 110 to 130 μE m^{-2} s^{-1} light intensity with a 16-h-light/8-h-dark cycle. For dark-induced senescence, comparable leaves from 4-week-old plants were detached and placed in water-saturated plates and incubated in the dark. The total chlorophyll content was measured by absorbance at 647 and 665 nm on a NanoDrop 2000c spectrophotometer (Thermo Scientific).

Fungal and bacterial disease assays were conducted as previously described (Laluk et al., 2011). In brief, for the *Botrytis cinerea* disease assay, 4-week-old plants were spray or drop inoculated with a conidial suspension (2.5×10^5 spores/mL) of *B. cinerea* strain B05.10 in 1% Sabouraud Maltose Broth and maintained under a transparent cover at high humidity. For the *Pseudomonas syringae* disease assay, plants were infiltrated with the bacterial strains and bacteria were extracted from inoculated leaves. The colony growth was determined and expressed in colony forming units on King's B medium supplemented with the antibiotics rifampicin and kanamycin. For ABA treatment, 4-week-old plants were infiltrated with 100 μM ABA. The accumulation of H_2O_2 in leaves was detected by 3,3′-diaminobenzidine staining (Daudi and O'Brien, 2012).

RNA Extraction and RT-qPCR Assay

Total RNA was extracted from leaves or seedlings with Trizol reagent according to the manufacturer's instructions (Sigma-Aldrich). The procedures for RNase-free DNase I treatment (Promega) and cDNA synthesis (New England Biolabs) from total RNA were conducted following the manufacturer's instructions. qPCR was performed with SYBR green supermix reagents (Bio-Rad) using gene-specific primers (Supplemental Table 1) and the Arabidopsis *ACTIN2* gene as an internal reference for normalization.

co-IP Assay

The co-IP was conducted following the previously described procedure (Lai et al., 2014; Zhu et al., 2014). Briefly, the plasmids containing full-length *HLS1-HA* and *MED18-MYC* driven by the CaMV 35S promoter were generated and transformed into Agrobacterium. The Agrobacterium strains were then infiltrated into *Nicotiana benthamiana*. After 36 h, total protein was extracted from infiltrated leaves with extraction buffer (50 mM HEPES, pH 7.5, 100 mM NaCl, 5 mM EDTA, 50 mM EGTA, 25 mM NaF, 1 mM $NaVO_3$, 50 mM β-glycerophosphate, 20% [v/v] glycerol, 1 mM PMSF, 0.1% [v/v] Triton X-100, 1 mM DTT, and 1× protease inhibitor cocktail [Sigma-Aldrich]). After removing debris by centrifugation at 12,000*g* for 10 min, 1 mL of supernatant mixed with anti-HA antibody-conjugated agarose beads (Sigma-Aldrich) and rotated overnight at 4°C. Then, beads with immunoprecipitates were washed four times with extraction buffer. Immunoprecipitates were detected by immunoblotting with anti-HA-specific (Covance) or anti-MYC-specific (Abcam) antibodies. A similar co-IP procedure was employed in Arabidopsis plants expressing *HLS1-HA* and *MED18-MYC*.

ChIP-qPCR Assay

ChIP assay was conducted as described previously with minor modifications (Saleh et al., 2008). Briefly, chromatin complexes with proteins were cross-linked and isolated from 4-week-old Arabidopsis plants. After sonication, protein complexes were precipitated with anti-HA (Abcam), anti-H3, anti-acetyl-H3, or anti-acetyl-H4 (Millipore) antibody at 4°C overnight and then captured with salmon sperm DNA/Protein A agarose (Millipore). Beads were washed and reverse cross-linked, and proteins were digested prior to DNA purification. The immunoprecipitated DNA was amplified with specific primers listed in Supplemental Table 1. ChIP enrichment was normalized with input from a non-precipitated sample and promoter region of *ACTIN2* as an internal control. Wild-type plants treated with the same procedure were used as a background control and IgG was used for the immunoprecipitation control. Primers at transcription start site and C-terminal sequences of Arabidopsis *ACTIN7* gene were used as a background control in ChIP-qPCR assay for histone H3 acetylation.

In Vitro HAT Assay

The HAT assay was conducted as described (Qian et al., 2012). The full-length HLS1 fused with GST was generated and purified in *Escherichia coli*. The GST-HLS1 recombinant proteins (5 μg), purified by glutathione Sepharose 4B beads (GE Healthcare Life Science), were mixed with 10 μg of chicken core histones (Millipore) and 1 μCi of H^3-acetyl-CoA (Perkin-Elmer Life Science) in HAT buffer containing 50 mM Tris-Cl, pH 8.0, 100 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, 10% glycerol, 10 mM butyric acid, and 1 mM PMSF, and incubated at 30°C for 2 h. After incubation, samples were run on 15% SDS-PAGE gel and the gel was fixed with 40% methanol-10% acetic acid. The gel was treated with an autoradiographic enhancer (Perkin-Elmer Life Science) and vacuum dried. The signals were detected after 2 weeks of exposure at −80°C. The same amount of PCAF (Abcam) with a known acetyltransferase activity was used as a positive control in parallel with GST-HLS1 in the reaction.

Transcriptional Activation Assays

The *ABI5* or *WRKY33* promoter region was fused with the *GUS* reporter gene to generate a transcriptional fusion. Two effector plasmids, *HLS1-HA* and *MED18-MYC*, were generated and each cotransfected with the reporter construct into $\sim 2 \times 10^4$ protoplasts isolated from 4-week-old Arabidopsis plants as described (Yoo et al., 2007). Protoplasts were lysed in lysis buffer containing 50 mM phosphate buffer, pH 7.0, 1 mM DTT, 2 mM trans-1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid monohydrate, 10% glycerol, and 1% Triton X-100. The lysate was mixed with 4-methylumbelliferyl-β-D-glucuronide (MUG) substrate buffer (10 mM Tris-Cl, pH 8.0, 1 mM MUG, and 2 mM $MgCl_2$) and incubated at 37°C. The reaction was stopped by adding 0.2 M Na_2CO_3, and the LUC substrates (Promega) were mixed with lysates. GUS and LUC activities were detected with a VICTOR 3V Multilabel plate reader (Perkin-Elmer). LUC reading was used as an internal control in each sample normalized to GUS reading.

Accession Numbers

Sequence data from this article can be found in the GenBank/EMBL data libraries under the following accession numbers: *HLS1* (AT4G37580), *WRKY33* (AT2G38470), *ABI5* (AT2G36270), *MED18* (AT2G22370), *ACT2* (AT3G18780), *ACT7* (AT5G09810), *PR1* (AT2G14610), *ERF1* (AT3G23240), *PDF1.2* (AT5G44420), *RPM1* (AT3G07040), *RIN4* (AT3G25070), *CYP79B3* (AT2G22330), *CYP81F2* (AT5G57220), *PAD3* (AT3G26830), *SUR2* (AT4G31500), *ACS2* (AT1G01480), *ACS6* (AT4G11280), *ABI3* (AT3G24650), *RD29a* (AT5G52310), *KAT2* (AT2G33150), *WRKY40* (AT1G80840), and *HY5* (AT5G11260).

Supplemental Data

Supplemental Figure 1. The *hls1-1* and *hls1-2* mutant alleles showing lack of *HLS1* transcript.

Supplemental Figure 2. HLS1 protein is not associated with resistance gene *RPM1*.

Supplemental Figure 3. The histone H3 acetylation at *ACTIN7* chromatin shows no difference between wild-type and *hls1* plants.

Supplemental Figure 4. The recruitment of HLS1 protein to the *ABI5* locus is not enhanced by *B. cinerea* inoculation.

Supplemental Figure 5. MED18-mediated plant resistance through *TRX-h5* regulation is HLS1 independent.

Supplemental Figure 6. Overexpressing *HLS1-HA* in Arabidopsis shows developmental phenotypes opposite to the *hls1* mutant.

Supplemental Figure 7. Expression of defense related genes is induced in *hls1* mutant in response to ABA.

Supplemental Figure 8. Leaf number, but not disease phenotype, is affected by HLS1 under short-day conditions.

Supplemental Table 1. Primer sequences used in this study.

ACKNOWLEDGMENTS

This research was funded by grants from the National Science Foundation (IOS-1456594) and the Next-Generation BioGreen 21 Program (SSAC Project No. PJ01137902), Rural Development Administration. We thank Zhixiang Chen (Purdue University) for the WRKY33-MYC construct used in our studies.

AUTHOR CONTRIBUTIONS

C.-J.L. conducted most of the experiments. C.-J.L. and T.M. designed most of the experiments and directed the project. Z.L. and S.L. performed the initial mutant screen that identified the *hls1* and other mutants. T.M., C.J.L., and D.-J.Y. wrote the article.

Received February 8, 2016; revised May 13, 2016; accepted June 16, 2016; published June 17, 2016.

REFERENCES

Adie, B.A., Pérez-Pérez, J., Pérez-Pérez, M.M., Godoy, M., Sánchez-Serrano, J.J., Schmelz, E.A., and Solano, R. (2007). ABA is an essential signal for plant resistance to pathogens affecting JA biosynthesis and the activation of defenses in Arabidopsis. Plant Cell **19:** 1665–1681.

An, C., and Mou, Z. (2013). The function of the Mediator complex in plant immunity. Plant Signal. Behav. **8:** e23182.

Anderson, J.P., Badruzsaufari, E., Schenk, P.M., Manners, J.M., Desmond, O.J., Ehlert, C., Maclean, D.J., Ebert, P.R., and Kazan, K. (2004). Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in Arabidopsis. Plant Cell **16:** 3460–3479.

Asselbergh, B., Curvers, K., Franca, S.C., Audenaert, K., Vuylsteke, M., Van Breusegem, F., and Höfte, M. (2007). Resistance to *Botrytis cinerea* in sitiens, an abscisic acid-deficient tomato mutant, involves timely production of hydrogen peroxide and cell wall modifications in the epidermis. Plant Physiol. **144:** 1863–1877.

Benhamed, M., Bertrand, C., Servet, C., and Zhou, D.X. (2006). Arabidopsis GCN5, HD1, and TAF1/HAF2 interact to regulate histone acetylation required for light-responsive gene expression. Plant Cell **18:** 2893–2903.

Bertrand, C., Bergounioux, C., Domenichini, S., Delarue, M., and Zhou, D.X. (2003). Arabidopsis histone acetyltransferase AtGCN5 regulates the floral meristem activity through the WUSCHEL/AGAMOUS pathway. J. Biol. Chem. **278:** 28246–28251.

Chen, R., Jiang, H., Li, L., Zhai, Q., Qi, L., Zhou, W., Liu, X., Li, H., Zheng, W., Sun, J., and Li, C. (2012). The Arabidopsis mediator subunit MED25 differentially regulates jasmonate and abscisic acid signaling through interacting with the MYC2 and ABI5 transcription factors. Plant Cell **24:** 2898–2916.

Chen, Z.J., and Tian, L. (2007). Roles of dynamic and reversible histone acetylation in plant development and polyploidy. Biochim. Biophys. Acta **1769:** 295–307.

Clay, N.K., Adio, A.M., Denoux, C., Jander, G., and Ausubel, F.M. (2009). Glucosinolate metabolites required for an Arabidopsis innate immune response. Science **323:** 95–101.

Curvers, K., Seifi, H., Mouille, G., de Rycke, R., Asselbergh, B., Van Hecke, A., Vanderschaeghe, D., Höfte, H., Callewaert, N., Van Breusegem, F., and Höfte, M. (2010). Abscisic acid deficiency causes changes in cuticle permeability and pectin composition that influence tomato resistance to *Botrytis cinerea*. Plant Physiol. **154:** 847–860.

Daudi, A., and O'Brien, J.A. (2012). Detection of hydrogen peroxide by DAB staining in Arabidopsis leaves. Bio Protoc. **2:** e263.

Defraia, C.T., Wang, Y., Yao, J., and Mou, Z. (2013). Elongator subunit 3 positively regulates plant immunity through its histone acetyltransferase and radical S-adenosylmethionine domains. BMC Plant Biol. **13:** 102.

Delaney, T.P., Uknes, S., Vernooij, B., Friedrich, L., Weymann, K., Negrotto, D., Gaffney, T., Gut-Rella, M., Kessmann, H., Ward, E., and Ryals, J. (1994). A central role of salicylic acid in plant disease resistance. Science **266:** 1247–1250.

de Torres-Zabala, M., Truman, W., Bennett, M.H., Lafforgue, G., Mansfield, J.W., Rodriguez Egea, P., Bögre, L., and Grant, M. (2007). *Pseudomonas syringae* pv. tomato hijacks the Arabidopsis abscisic acid signalling pathway to cause disease. EMBO J. **26:** 1434–1443.

Dhawan, R., Luo, H., Foerster, A.M., Abuqamar, S., Du, H.N., Briggs, S.D., Mittelsten Scheid, O., and Mengiste, T. (2009). HISTONE MONOUBIQUITINATION1 interacts with a subunit of the mediator complex and regulates defense against necrotrophic fungal pathogens in Arabidopsis. Plant Cell **21:** 1000–1019.

Ding, B., Bellizzi, Mdel.R., Ning, Y., Meyers, B.C., and Wang, G.L. (2012). HDT701, a histone H4 deacetylase, negatively regulates plant innate immunity by modulating histone H4 acetylation of defense-related genes in rice. Plant Cell **24:** 3783–3794.

Englert, N.A., Luo, G., Goldstein, J.A., and Surapureddi, S. (2015). Epigenetic modification of histone 3 lysine 27: mediator subunit MED25 is required for the dissociation of polycomb repressive complex 2 from the promoter of cytochrome P450 2C9. J. Biol. Chem. **290:** 2264–2278.

Golldack, D., Li, C., Mohan, H., and Probst, N. (2014). Tolerance to drought and salt stress in plants: Unraveling the signaling networks. Front. Plant Sci. **5:** 151.

Guo, W.L., Chen, R.G., Gong, Z.H., Yin, Y.X., Ahmed, S.S., and He, Y.M. (2012). Exogenous abscisic acid increases antioxidant enzymes and related gene expression in pepper (*Capsicum annuum*) leaves subjected to chilling stress. Genet. Mol. Res. **11:** 4063–4080.

Hernández-Blanco, C., et al. (2007). Impairment of cellulose synthases required for Arabidopsis secondary cell wall formation enhances disease resistance. Plant Cell **19:** 890–903.

Jaskiewicz, M., Conrath, U., and Peterhänsel, C. (2011). Chromatin modification acts as a memory for systemic acquired resistance in the plant stress response. EMBO Rep. **12:** 50–55.

Jiang, C.J., Shimono, M., Sugano, S., Kojima, M., Yazawa, K., Yoshida, R., Inoue, H., Hayashi, N., Sakakibara, H., and Takatsuji, H. (2010). Abscisic acid interacts antagonistically with salicylic acid signaling pathway in rice-*Magnaporthe grisea* interaction. Mol. Plant Microbe Interact. **23:** 791–798.

Jiang, M., and Zhang, J. (2001). Effect of abscisic acid on active oxygen species, antioxidative defence system and oxidative damage in leaves of maize seedlings. Plant Cell Physiol. **42:** 1265–1273.

Jiang, M., and Zhang, J. (2002). Involvement of plasma-membrane NADPH oxidase in abscisic acid- and water stress-induced antioxidant defense in leaves of maize seedlings. Planta **215:** 1022–1030.

Jiang, Y., and Deyholos, M.K. (2009). Functional characterization of Arabidopsis NaCl-inducible WRKY25 and WRKY33 transcription factors in abiotic stresses. Plant Mol. Biol. **69:** 91–105.

Jones, J.D., and Dangl, J.L. (2006). The plant immune system. Nature **444:** 323–329.

Kazan, K., and Manners, J.M. (2009). Linking development to defense: auxin in plant-pathogen interactions. Trends Plant Sci. **14:** 373–382.

Kornet, N., and Scheres, B. (2009). Members of the GCN5 histone acetyltransferase complex regulate PLETHORA-mediated root stem cell niche maintenance and transit amplifying cell proliferation in Arabidopsis. Plant Cell **21:** 1070–1079.

Lai, Z., Wang, F., Zheng, Z., Fan, B., and Chen, Z. (2011). A critical role of autophagy in plant resistance to necrotrophic fungal pathogens. Plant J. **66:** 953–968.

Lai, Z., Schluttenhofer, C.M., Bhide, K., Shreve, J., Thimmapuram, J., Lee, S.Y., Yun, D.J., and Mengiste, T. (2014). MED18 interaction with distinct transcription factors regulates multiple plant functions. Nat. Commun. **5:** 3064.

Laluk, K., Luo, H., Chai, M., Dhawan, R., Lai, Z., and Mengiste, T. (2011). Biochemical and genetic requirements for function of the immune response regulator BOTRYTIS-INDUCED KINASE1 in plant growth, ethylene signaling, and PAMP-triggered immunity in Arabidopsis. Plant Cell **23:** 2831–2849.

Lehman, A., Black, R., and Ecker, J.R. (1996). HOOKLESS1, an ethylene response gene, is required for differential cell elongation in the Arabidopsis hypocotyl. Cell **85:** 183–194.

Li, H., Johnson, P., Stepanova, A., Alonso, J.M., and Ecker, J.R. (2004). Convergence of signaling pathways in the control of differential cell growth in Arabidopsis. Dev. Cell **7:** 193–204.

Liu, S., Kracher, B., Ziegler, J., Birkenbihl, R.P., and Somssich, I.E. (2015). Negative regulation of ABA signaling by WRKY33 is critical for Arabidopsis immunity towards *Botrytis cinerea* 2100. eLife **4:** e07295.

Liu, X., Vorontchikhina, M., Wang, Y.L., Faiola, F., and Martinez, E. (2008). STAGA recruits Mediator to the MYC oncoprotein to stimulate transcription and cell proliferation. Mol. Cell. Biol. **28:** 108–121.

Liu, Y., Koornneef, M., and Soppe, W.J. (2007). The absence of histone H2B monoubiquitination in the Arabidopsis hub1 (rdo4) mutant reveals a role for chromatin remodeling in seed dormancy. Plant Cell **19:** 433–444.

Lorang, J., Kidarsa, T., Bradford, C.S., Gilbert, B., Curtis, M., Tzeng, S.C., Maier, C.S., and Wolpert, T.J. (2012). Tricking the guard: exploiting plant defense for disease susceptibility. Science **338:** 659–662.

Mao, G., Meng, X., Liu, Y., Zheng, Z., Chen, Z., and Zhang, S. (2011). Phosphorylation of a WRKY transcription factor by two pathogen-responsive MAPKs drives phytoalexin biosynthesis in Arabidopsis. Plant Cell **23:** 1639–1653.

Ozfidan, C., Turkan, I., Sekmen, A.H., and Seckin, B. (2012). Abscisic acid-regulated responses of aba2-1 under osmotic stress: the abscisic acid-inducible antioxidant defence system and reactive oxygen species production. Plant Biol. (Stuttg.) **14:** 337–346.

Pandey, R., Müller, A., Napoli, C.A., Selinger, D.A., Pikaard, C.S., Richards, E.J., Bender, J., Mount, D.W., and Jorgensen, R.A. (2002). Analysis of histone acetyltransferase and histone deacetylase families of *Arabidopsis thaliana* suggests functional diversification of chromatin modification among multicellular eukaryotes. Nucleic Acids Res. **30:** 5036–5055.

Peeters, A.J., Blankestijn-De Vries, H., Hanhart, C.J., Léon-Kloosterziel, K.M., Zeevaart, J.A., and Koornneef, M. (2002). Characterization of mutants with reduced seed dormancy at two novel rdo loci and a further characterization of rdo1 and rdo2 in Arabidopsis. Physiol. Plant. **115:** 604–612.

Penninckx, I.A., Thomma, B.P., Buchala, A., Métraux, J.P., and Broekaert, W.F. (1998). Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defensin gene in Arabidopsis. Plant Cell **10:** 2103–2113.

Penninckx, I.A., Eggermont, K., Terras, F.R., Thomma, B.P., De Samblanx, G.W., Buchala, A., Métraux, J.P., Manners, J.M., and Broekaert, W.F. (1996). Pathogen-induced systemic activation of a plant defensin gene in Arabidopsis follows a salicylic acid-independent pathway. Plant Cell **8:** 2309–2323.

Petersen, K., Fiil, B.K., Mundy, J., and Petersen, M. (2008). Downstream targets of WRKY33. Plant Signal. Behav. **3:** 1033–1034.

Pieterse, C.M., Van der Does, D., Zamioudis, C., Leon-Reyes, A., and Van Wees, S.C. (2012). Hormonal modulation of plant immunity. Annu. Rev. Cell Dev. Biol. **28:** 489–521.

Po-Wen, C., Singh, P., and Zimmerli, L. (2013). Priming of the Arabidopsis pattern-triggered immunity response upon infection by necrotrophic Pectobacterium carotovorum bacteria. Mol. Plant Pathol. **14:** 58–70.

Qian, W., et al. (2012). A histone acetyltransferase regulates active DNA demethylation in Arabidopsis. Science **336:** 1445–1448.

Qiu, H., Hu, C., Zhang, F., Hwang, G.J., Swanson, M.J., Boonchird, C., and Hinnebusch, A.G. (2005). Interdependent recruitment of SAGA and Srb mediator by transcriptional activator Gcn4p. Mol. Cell. Biol. **25:** 3461–3474.

Reeves, W.M., and Hahn, S. (2003). Activator-independent functions of the yeast mediator sin4 complex in preinitiation complex formation and transcription reinitiation. Mol. Cell. Biol. **23:** 349–358.

Saleh, A., Alvarez-Venegas, R., and Avramova, Z. (2008). An efficient chromatin immunoprecipitation (ChIP) protocol for studying histone modifications in Arabidopsis plants. Nat. Protoc. **3:** 1018–1025.

Singh, P., Yekondi, S., Chen, P.W., Tsai, C.H., Yu, C.W., Wu, K., and Zimmerli, L. (2014). Environmental history modulates Arabidopsis pattern-triggered immunity in a HISTONE ACETYLTRANSFERASE1-dependent manner. Plant Cell **26:** 2676–2688.

Tian, L., Fong, M.P., Wang, J.J., Wei, N.E., Jiang, H., Doerge, R.W., and Chen, Z.J. (2005). Reversible histone acetylation and deacetylation mediate genome-wide, promoter-dependent and locus-specific changes in gene expression during plant development. Genetics **169:** 337–345.

Ton, J., and Mauch-Mani, B. (2004). Beta-amino-butyric acid-induced resistance against necrotrophic pathogens is based on ABA-dependent priming for callose. Plant J. **38:** 119–130.

Ton, J., Flors, V., and Mauch-Mani, B. (2009). The multifaceted role of ABA in disease resistance. Trends Plant Sci. **14:** 310–317.

Ton, J., Jakab, G., Toquin, V., Flors, V., Iavicoli, A., Maeder, M.N., Métraux, J.P., and Mauch-Mani, B. (2005). Dissecting the beta-aminobutyric acid-induced priming phenomenon in Arabidopsis. Plant Cell **17:** 987–999.

Vlachonasios, K.E., Thomashow, M.F., and Triezenberg, S.J. (2003). Disruption mutations of ADA2b and GCN5 transcriptional adaptor genes dramatically affect Arabidopsis growth, development, and gene expression. Plant Cell **15:** 626–638.

Wang, Y., An, C., Zhang, X., Yao, J., Zhang, Y., Sun, Y., Yu, F., Amador, D.M., and Mou, Z. (2013). The Arabidopsis elongator complex subunit2 epigenetically regulates plant immune responses. Plant Cell **25:** 762–776.

Wu, K., Malik, K., Tian, L., Brown, D., and Miki, B. (2000). Functional analysis of a RPD3 histone deacetylase homologue in *Arabidopsis thaliana*. Plant Mol. Biol. **44:** 167–176.

Wu, K., Zhang, L., Zhou, C., Yu, C.W., and Chaikam, V. (2008). HDA6 is required for jasmonate response, senescence and flowering in Arabidopsis. J. Exp. Bot. **59:** 225–234.

Xing, J., Wang, T., Liu, Z., Xu, J., Yao, Y., Hu, Z., Peng, H., Xin, M., Yu, F., Zhou, D.X., and Ni, Z. (2015). GCN5-mediated histone

acetylation of FRD3 contributes to iron homeostasis in *Arabidopsis thaliana*. Plant Physiol. **168:** 1309–1320.

Xu, J., Audenaert K., Hofte, M., and De Vleesschauwer, D. (2013) Abscisic acid promotes susceptibility to the rice leaf blight pathogen pv by suppressing salicylic acid-mediated defenses. PLoS One **8:** e67413.

Yang, Y., Ou, B., Zhang, J., Si, W., Gu, H., Qin, G., and Qu, L.J. (2014). The Arabidopsis Mediator subunit MED16 regulates iron homeostasis by associating with EIN3/EIL1 through subunit MED25. Plant J. **77:** 838–851.

Yasuda, M., Ishikawa, A., Jikumaru, Y., Seki, M., Umezawa, T., Asami, T., Maruyama-Nakashita, A., Kudo, T., Shinozaki, K., Yoshida, S., and Nakashita, H. (2008). Antagonistic interaction between systemic acquired resistance and the abscisic acid-mediated abiotic stress response in Arabidopsis. Plant Cell **20:** 1678–1692.

Yoo, S.D., Cho, Y.H., and Sheen, J. (2007). Arabidopsis mesophyll protoplasts: a versatile cell system for transient gene expression analysis. Nat. Protoc. **2:** 1565–1572.

Yoon, S., Qiu, H., Swanson, M.J., and Hinnebusch, A.G. (2003). Recruitment of SWI/SNF by Gcn4p does not require Snf2p or Gcn5p but depends strongly on SWI/SNF integrity, SRB mediator, and SAGA. Mol. Cell. Biol. **23:** 8829–8845.

Zhang, X., Wang, C., Zhang, Y., Sun, Y., and Mou, Z. (2012). The Arabidopsis mediator complex subunit16 positively regulates salicylate-mediated systemic acquired resistance and jasmonate/ethylene-induced defense pathways. Plant Cell **24:** 4294–4309.

Zheng, Z., Qamar, S.A., Chen, Z., and Mengiste, T. (2006). Arabidopsis WRKY33 transcription factor is required for resistance to necrotrophic fungal pathogens. Plant J. **48:** 592–605.

Zhou, C., Zhang, L., Duan, J., Miki, B., and Wu, K. (2005). HISTONE DEACETYLASE19 is involved in jasmonic acid and ethylene signaling of pathogen response in Arabidopsis. Plant Cell **17:** 1196–1204.

Zhou, N., Tootle, T.L., and Glazebrook, J. (1999). Arabidopsis PAD3, a gene required for camalexin biosynthesis, encodes a putative cytochrome P450 monooxygenase. Plant Cell **11:** 2419–2428.

Zhu, X., Zhang, Y., Bjornsdottir, G., Liu, Z., Quan, A., Costanzo, M., Dávila López, M., Westholm, J.O., Ronne, H., Boone, C., Gustafsson, C.M., and Myers, L.C. (2011a). Histone modifications influence mediator interactions with chromatin. Nucleic Acids Res. **39:** 8342–8354.

Zhu, Y., Schluttenhoffer, C.M., Wang, P., Fu, F., Thimmapuram, J., Zhu, J.K., Lee, S.Y., Yun, D.J., and Mengiste, T. (2014). CYCLIN-DEPENDENT KINASE8 differentially regulates plant immunity to fungal pathogens through kinase-dependent and -independent functions in Arabidopsis. Plant Cell **26:** 4149–4170.

Zhu, Z., et al. (2011b). Derepression of ethylene-stabilized transcription factors (EIN3/EIL1) mediates jasmonate and ethylene signaling synergy in Arabidopsis. Proc. Natl. Acad. Sci. USA **108:** 12539–12544.