HOS15 is a transcriptional corepressor of NPR1-mediated gene activation of plant immunity

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Edited by Pamela C. Ronald, University of California, Davis, CA, and approved October 15, 2020 (received for review August 12, 2020)

Transcriptional regulation is a complex and pivotal process in living cells. HOS15 is a transcriptional corepressor. Although transcriptional repressors generally have been associated with inactive genes, increasing evidence indicates that, through poorly understood mechanisms, transcriptional corepressors also associate with actively transcribed genes. Here, we show that HOS15 is the substrate receptor for an SCF/CUL1 E3 ubiquitin ligase complex (SCF^{HOS15}) that negatively regulates plant immunity by destabilizing transcriptional activation complexes containing NPR1 and associated transcriptional activators. In unchallenged conditions, HOS15 continuously eliminates NPR1 to prevent inappropriate defense gene expression. Upon defense activation, HOS15 preferentially associates with phosphorylated NPR1 to stimulate rapid degradation of transcriptionally active NPR1 and thus limit the extent of defense gene expression. Our findings indicate that HOS15-mediated ubiquitination and elimination of NPR1 produce effects contrary to those of CUL3-containing ubiquitin ligase that coactivate defense gene expression. Thus, HOS15 plays a key role in the dynamic regulation of pre- and postactivation host defense.

HOS15 | NPR1 | ubiquitin ligase | corepressor | plant immunity

Effective innate immune responses require that host cells quickly sense and respond to pathogen attack (1). Cellular immune responses are associated with rapid and intense induction of so-called defense genes (2, 3). However, inappropriate or excessive defense gene expression has negative consequences on plant growth and development (4). To balance these factors, host cells use fundamental yet complex mechanisms to tightly regulate defense gene expression. Control of transcription is not only dependent on transcription factors but also requires a coordinated regulation of multiprotein coregulator complexes, including coactivators and corepressors (2, 5, 6). Transcription factors bind to specific DNA sequences in regulatory regions of their target genes and participate in protein–protein interactions that regulate promoter activity and/or local chromatin remodeling. Collectively, these interactions control both the rate and timing of transcription.

NPR1 is a key regulator of plant immune responses. In response to redox changes mediated by the defense hormone salicylic acid (SA), NPR1 deoligomerizes and moves to the nucleus where it induces the expression of a suite of defense genes, including transcription factors and pathogenesis-related (*PR*) genes (7–11). NPR1, which functions as a transcriptional coactivator, has an N-terminal BTB/POZ domain, a central ankyrin repeat domain, and a C-terminal transactivation domain and nuclear localization sequence (12, 13). The interaction of nuclear-localized NPR1 with three functionally redundant basic leucine zipper TGA transcription factors, TGA2, TGA5, and TGA6, leads to SA-mediated *PR* gene activation in *Arabidopsis thaliana* (9, 14). Intriguingly, proteasome-mediated degradation of NPR1 both negatively and positively regulates its activity (15). In the cells of an uninduced leaf, basal levels of nuclear-localized NPR1 are degraded by the proteasome to limit inappropriate defense gene expression. Following defense induction, nuclear-localized NPR1 is phosphorylated at Ser11 and Ser15 and then is rapidly subjected to proteasome-mediated turnover in a CUL3-dependent manner. The counterintuitive observation that CUL3-mediated degradation of phosphorylated NPR1 is required for maximal expression of NPR1-targeted defense genes supports a model in which loading of "fresh" NPR1 at target promoters is required for new cycles of transcription initiation (15).

We have previously reported that HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENES 15 (HOS15) is a transcriptional corepressor that regulates plant acclimation and tolerance to cold stress through histone deacetylation at target genes in *Arabidopsis* (16). HOS15, which encodes an N-terminal LISH domain and C-terminal WD40 repeat protein, shares high sequence similarity with human transducin beta-like 1 (TBL1) that is a core component of Nuclear receptor Co-Repressor 1/Silencing Mediator of Retinoic acid and Thyroid hormone receptor (NCoR1/SMRT)–histone deacetylases 3

Significance

Immune responses protect organisms against biotic challenges but can also produce deleterious effects, such as inflammation and necrosis. This growth-defense trade-off necessitates fine control of immune responses, including the activation of defense gene expression. The transcriptional coactivator NPR1 is a key regulatory hub of immune activation in plant cells. Surprisingly, full activation of NPR1-activated defense genes requires proteasome-mediated degradation of NPR1 induced by a CUL3-based E3 ubiquitin ligase complex. Our work demonstrates that HOS15 is the specificity determinant of a CUL1-based E3 ubiquitin ligase complex that limits defense gene expression by targeting NPR1 for proteasome-mediated degradation. Thus, distinct ubiquitin-based degradation pathways coordinately modulate the timing and amplitude of transcriptional outputs during plant defense.

Author contributions: M.S., D.M., and D.-J.Y. designed research; M.S., C.J.L., J.P., and J.E.K. performed research; D.B., J.N., S.Y.L., J.M.P., and W.-Y.K. contributed new reagents/analytic tools; M.S., D.M., and D.-J.Y. analyzed data; and M.S., D.M., and D.-J.Y. wrote the paper.

The authors declare no competing interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2016049117/-/DCSupplemental.

First published November 16, 2020.

(HDAC3)–TBL1 corepressor complex in human (17–19). Similarly, HOS15 forms a core corepressor complex with histone deacetylase 9 (HDA9) and POWERDRESS (PWR) to regulate transcription and development in *Arabidopsis* (20, 21). A *hos15* mutant is dwarfed and displays histone hyperacetylation and methylation, similar to *hda9* and *pwr* mutants. The HOS15–HDA9–PWR corepressor complex controls gene expression via transcriptional repression, including genes that are responsive to biotic and abiotic stimuli (21). In addition, HOS15 also interacts with HDA9 to regulate photoperiodic flowering with the evening complex via transcriptional repression of the floral activator GIGANTEA (22). Taken together, these findings indicate that HOS15, together with HDA9 and PWR, plays an important role in transcriptional repression in *Arabidopsis*. Moreover, HOS15 contains a conserved DWD motif and acts as a substrate receptor of a CUL4–RING E3 ubiquitin ligase (CRL4) complex that targets HD2C and OST1 in responses to cold stress and ABA-signaling, respectively (23–25).

In addition to associating with transcriptionally inactive genes, accumulating evidence from studies in yeast and mammalian models indicates that transcriptional corepressors, such as the NCoR1/SMRT, Sin3-Rpd3, and NuRD corepressor complexes, also associate with actively transcribed genes (26–29). For example, NCoR1 and SMRT (NCoR2) were identified more than 20 y ago as interacting partners of the thyroid and retinoic acid nuclear receptors that mediate ligand-independent transcriptional repression (30, 31). NCoR1 and SMRT mediate transcriptional repression by recruiting a multiprotein complex including HDAC3, TBL1 and its homolog TBL-related 1 (TBLR1), and a G-protein pathway suppressor (GPS2) (17, 19). NCoR1 is required for cellular function in many biological processes, including metabolism, development, and glucocorticoid receptor signaling (32–35). Unexpectedly, NCoR1 is also a transcriptional activator of certain retinoic acid response elements (26). Similarly, SMRT was also observed to positively mediate agonist-dependent ERα transcriptional activation (28). HDACs associated with corepressor complexes have also been linked with active genes in yeast and mammals (27, 36, 37). However, most of these studies are based on genetic and genome-wide analysis, such as chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq), but the molecular mechanisms through which corepressor complexes function at active genes remain poorly understood.

In this study, we show that HOS15 is part of a transcriptional corepressor complex that limits NPR1-dependent defense gene expression by controlling NPR1 stability through proteasome-mediated degradation. Our findings revealed a mechanism by which the coordinated activity of a transcriptional coactivator and a transcriptional corepressor tunes the amplitude and duration gene expression during plant immunity.

Results

HOS15 Is a Component of an SCF E3 Ubiquitin Ligase Complex. HOS15 is a homolog of human transducin beta-like 1 proteins TBL1 and TBLR1 (16). In human, although the F-box motifs did not reach the threshold expected of a presumptive F-box (38), TBL1 and TBLR1 were reported to encode F-box–like motifs that link them to SKP1 and Cullin1 proteins to form SCF (SKP1–Cullin1–F-box) complexes. As substrate receptors for these SCF complexes, TBL1 or TBLR1 mediates ubiquitination and degradation of CtBP1/2 and NCoR1/SMRT, respectively (39). Similar to human TBL1/TBLR1, amino acid sequence alignment showed that HOS15 also contains an F-box–like motif at its N-terminal region (amino acids 42 to 88) (40). Therefore, to determine whether HOS15 also functions in an SCF complex through interaction with *Arabidopsis* SKP1-like proteins (ASKs) and Cullin1 proteins, we carried out luciferase complementation imaging (LCI) assays. Agrobacterium-mediated transient coexpression of CLuc-HOS15 with ASK1-NLuc or ASK2-NLuc in *Nicotiana benthamiana* leaves resulted in strong LUC activity compared to the negative controls of CLuc-HOS15 or ASK1/2-NLuc with empty NLuc or CLuc vectors, respectively (*SI Appendix*, Fig. S1*A*). To confirm the interactions between HOS15 and ASK1 or ASK2, we conducted in vivo coimmunoprecipitation (Co-IP) assays. For this assay, we coexpressed HOS15-Flag with ASK1-3xHA (*SI Appendix*, Fig. S1*B*) or ASK2-3xHA (*SI Appendix*, Fig. S1*C*) in *N. benthamiana* leaves. Total protein extracts were immunoprecipitated with anti-HOS15 antibody, and coprecipitation of ASK1-3xHA or ASK2-3xHA proteins was determined by anti-HA immunoblotting (*SI Appendix*, Fig. S1 *B* and *C*). In a bimolecular fluorescence complementation (BiFC) assay, we observed that HOS15 interacts with ASK1 in the nucleus of *N. benthamiana* leaf epidermal cells (*SI Appendix*, Fig. S2). To further assess HOS15 interaction with ASKs, we conducted a yeast two-hybrid assay. Five ASK proteins, ASK1, -2, -4, -11, and -18, were tested, and all except ASK1 directly interacted with HOS15 (*SI Appendix*, Fig. S1*D*). These results indicate that HOS15 associates with ASK proteins both in yeast and in planta.

Next, we used similar LCI and Co-IP assays to determine whether HOS15 associates with CUL1. Transient coexpression of NLuc-HOS15 with CUL1-CLuc in *N. benthami*ana leaves resulted in strong LUC activity compared to negative controls (*SI Appendix*, Fig. S1*E*). This interaction was confirmed in planta by a Co-IP assay following coexpression of HOS15-Flag with CUL1-3xHA or the positive control, ASK1-3xHA (*SI Appendix*, Fig. S1*F*). Taken together, these results indicate that HOS15 undergoes protein associations consistent with its function as an F-box–like protein that acts as a substrate receptor for plant SCF^{HOS15}, similar to TBL1 and TBLR1 in humans.

HOS15 Negatively Regulates Plant Immunity against Bacterial Pathogens. Emerging evidence indicates that the HOS15 play important roles in plant development and abiotic stress responses (16, 20–22, 24). However, it remains unknown whether HOS15 is also involved in plant immunity. The *hos15-1* plants display reduced growth and spontaneous cell death (*SI Appendix*, Fig. S3 *A*–*C*), similar to other lesion-mimic mutants that also display enhanced disease resistance to bacterial pathogen infection (41). In addition, previously reported microarray analysis shows increased expression of a set of defense-response genes in *hos15-1* mutant plants compared with wild-type C24 in the absence of any pathogen (*SI Appendix*, Table S1) (16). Increased expression of several defense-response genes from this set—including WRKY family transcription factors *WRKY46*, *WRKY54*, and *WRKY70* (10, 42–44); *ISOCHORISMATE SYNTHASE1* (*ICS1*, also known as *SID2*) (45, 46); *PHYTOALEXIN DEFICIENT4* (*PAD4*) (47, 48); *ENHANCED DISEASE SUSEPTIBILITY 5* (*EDS5*, also known as *SID1*) (49, 50); *WHITE RUST RESISTANCE4* (*WRR4*) (51); and *PATHOGENESIS-RELATED* genes (*PR1*, *PR2* and *PR5*) (52)—were confirmed by RT-PCR analysis (*SI Appendix*, Figs. S3*D* and S4). The expression of PR1 protein level was further confirmed by immunoblot analysis using anti-PR1 antibody (*SI Appendix*, Fig. S3*D*). We also examined other defense-response genes and found increased expression of *NON-RACE SEPCIFIC DISEASE RESISTANCE1* (*NDR1*) (53, 54) and *ENHANCED DISEASE SUSCEPTILILITY1* (*EDS1*) (55), decreased expression of *PLANT DEFENSIN1.2* (*PDF1.2*) (56), and no change in the expression of *NONEXPRESSOR OF PATHOGENESIS-RELATED GENES1* (*NPR1*) (7, 8) (*SI Appendix*, Fig. S4). This overall pattern of gene expression is consistent with enhanced SA-mediated disease resistance. Consistent with this prediction, increased resistance of *hos15-1* plants to *Pseudomonas syringae pv. tomato* DC3000 (*Pst* DC3000), compared to wild-type C24 (*SI Appendix*, Fig. S3*E*), indicates that HOS15 is a negative regulator of plant immunity.

To further support the conclusion that HOS15 acts as a negative regulator of plant immunity, a GABI-Kat transfer DNA (T-DNA) insertion line in the Col-0 ecotype, *hos15-2*, and two native-promoter *HOS15pro*::*HOS15-HA* complementation lines in the *hos15-2* mutant background were employed for further tests. Similar to *hos15-1*, reduced plant size and constitutive expression of *PR1* was detected in *hos15-2* plants compared with wild-type Col-0 and the two complementation lines (Fig. 1 *A*–*C*). To confirm elevated expression of the *PR1* gene, next we analyzed PR1 protein levels in wild-type Col-0 and *hos15-2* plants inoculated with *Pst* DC3000 and found that PR1 protein was more abundant in *hos15-2* than in Col-0 at 2 d after infection although expression was similar at 4 d after infection (Fig. 1*D*). Moreover, chromatin immunoprecipitation (ChIP) showed that HOS15 associates with the *PR1*-promoter region containing *as-1* elements, which are binding sites of TGA transcription factors, indicating that HOS15 directly regulates *PR1* gene expression (Fig. 1*E*). In addition, consistent with these findings, *hos15-2* displays greater resistance against *Pst* DC3000 compared to wild-type Col-0 or the two complementation lines (Fig. 1*F*). Thus, we conclude that HOS15 negatively regulates plant immunity and that, consistent with previous reports that HOS15 functions as a transcriptional corepressor in *Arabidopsis*, the enhanced disease resistance phenotype in *hos15* mutants appears to be based on derepression of *PR1* and misregulation of other defense genes (16, 22).

HOS15 Physically Interacts with NPR1. In order to identify the interacting partner or target proteins through which HOS15 1) acts as a transcriptional corepressor (16, 22), 2) functions as a substrate receptor in an E3 ubiquitin ligase complex (*SI Appendix*, Fig. S1), and 3) negatively regulates plant immunity (*SI Appendix*, Fig. S3 and Fig. 1), we selected a number of putative interacting proteins that also function in transcriptional gene regulation or posttranslational modifications during plant immunity. Eighteen candidate genes were tested by LCI for interaction with HOS15 (*SI Appendix*, Table S2 and Fig. S5). Among them, four proteins (SGT1b, TGA2, NPR1, and HD2C) were identified as the strongest interacting proteins according to LUC activity (*SI Appendix*, Fig. S5 and Fig. 2 *A* and *B*). Notably, Park et al. (24) previously used a yeast two-hybrid screen of an *Arabidopsis* complementary DNA library to identify HD2C as an interacting partner of HOS15. The interaction of HOS15 with SGT1b is perhaps unsurprising because SGT1 also associates with SKP1 and CUL1 (57–59). We were especially interested in the NPR1 protein, which is a widely studied transcriptional coactivator and positive regulator of plant immunity (7, 13, 60–62). Considering the biological functions of HOS15 in plants and the effect of mutation *HOS15* on *PR1* transcript abundance (*SI Appendix*, Fig. S1and Fig. 1), we hypothesized that HOS15 negatively regulates NPR1 by regulating its protein stability. Furthermore, the strong association of HOS15 with TGA2 (*SI Appendix*, Fig. S5), which interacts with NPR1 to mediate SA-induced *PR1* gene induction (9, 13, 61–64), led us to further speculate that TGA2 may participate in the HOS15–NPR1 interaction.

We first focused on investigating the interaction between HOS15 and NPR1. Co-IP following transient coexpression of HOS15-3xHA with NPR1-Flag in *N. benthamiana* leaves, performed using anti-Flag (Fig. 2*C*) or anti-HOS15 (Fig. 2*D*) antibodies, indicated that the two proteins interact in planta. The interaction of HOS15 with NPR1 and TGA2 was also confirmed by a BiFC assay. HOS15 was fused with the N-terminal fragment of eYFP (VYNE-HOS15) and NPR1 or TGA2 was fused to the C-terminal fragment of eYFP (NPR1-VYCE or TGA2-VYCE), and, following transient expression of the two pairs in *N. benthamiana* leaves, YFP fluorescent signals were detected in the nucleus (*SI Appendix*, Fig. S2). These results indicate that HOS15 interacts with NPR1 and TGA2 in the nucleus of plant cells. F-box proteins are substrate receptors that specifically recognize and recruit selected substrate proteins to be ubiquitinated by the SCF E3 ubiquitin ligase complex and subsequently degraded (65, 66). To determine if HOS15 directly interacts with NPR1, we conducted a yeast two-hybrid assay (Fig. 2*E*). The detected interaction between HOS15 and NPR1 indicates that HOS15 directly interacts with NPR1, possibly to recruit it to the SCF^{HOS15} E3 ubiquitin ligase.

HOS15 Regulates NPR1 Stability in *Arabidopsis*. Proteasome-dependent turnover regulates NPR1 protein levels during SA-mediated defense responses (15). Thus, our identification of HOS15 as an F-box–like protein capable of directly interacting with NPR1 led us to predict that HOS15 regulates NPR1 protein stability at steady state and/or during SA signaling. To begin investigating this hypothesis, NPR1 protein levels were measured by anti-NPR1 immunoblotting in wild-type C24 and *hos15-1* plants with or without exposure to SA. The NPR1 protein level was higher in *hos15-1* than in the C24 in both conditions (*SI Appendix*, Fig. S6 *A* and *B*). Consistent with previous reports (15, 67), NPR1 protein levels increase after treatment with SA. Even under these induced conditions, NPR1 protein still accumulated to higher levels in *hos15-1* compared to C24 (*SI Appendix*, Fig. S6 *A* and *B*), indicating that HOS15 not only continuously eliminates NPR1 protein in unchallenged cells but also does so in challenged cells. In contrast, HOS15 protein level is not affected by SA in the C24 (*SI Appendix*, Fig. S6*C*). Thus, constant levels of HOS15 protein regulate NPR1 protein levels during the SA-mediated cellular immune responses. Next, to check whether the higher level of NPR1 protein is due to increased *NPR1* gene transcription in *hos15-1* plants, we monitored the level of *NPR1* transcripts in the wild-type C24 and *hos15-1* plants (*SI Appendix*, Fig. S6*D*). Although SA treatment induced expression of *NPR1*, no significant differences in messenger RNA levels were found in the C24 and *hos15-1* plants either with or without SA treatments.

Additionally, NPR1 expression was analyzed in wild-type Col-0 and *hos15-2* plants before or after treatment with SA. Although we could not detect the NPR1 protein in the Col-0 and *hos15-2* in the absence of exogenously applied SA, results were otherwise similar to those in *hos15-1* and C24. NPR1 protein was more abundant in *hos15-2* plants compared with Col-0 following SA treatment (*SI Appendix*, Fig. S6 *E* and *F*), and the HOS15 protein level was not affected by SA in Col-0 and *npr1-1* plants (*SI Appendix*, Fig. S6*G*). Moreover, similar expression levels of *NPR1* transcripts were also detected in the Col-0 and *hos15-2* with or without SA treatment (*SI Appendix*, Fig. S6*H*). These results indicate that the hyperaccumulation of NPR1 in *hos15* is due to posttranscriptional regulation.

To further investigate the role of HOS15 in the regulation of NPR1 protein degradation, we employed a cell-free degradation assay. During a 30-min incubation at room temperature, the NPR1 protein degradation that was observed in C24 and in Col-0 was delayed in *hos15-1* and *hos15-2* samples, respectively (*SI Appendix*, Fig. S6 *I*–*L*). The Col-0/*hos15-2* samples (*SI Appendix*, Fig. S6 *K* and *L*) were from plants pretreated with SA, which is required to detect the NPR1 protein. In C24 and *hos15-1*, plants with and without SA pretreatment were compared. NPR1 was degraded more rapidly in samples from C24 plants that had been pretreated with SA, and the delayed degradation of NPR1 in *hos15-1* was observed in both cases. Consistent with previous reports of proteasome-mediated turnover of NPR1 and with the hypothesis that the HOS15-dependent degradation of NPR1 results in its being targeted by the SCF^{HOS15} E3 ubiquitin ligase, we observed that degradation of NPR1 in the cell-free degradation assay was delayed by the proteasome inhibitor MG132 (*SI Appendix*, Fig. S6 *I* and *K*) (15).

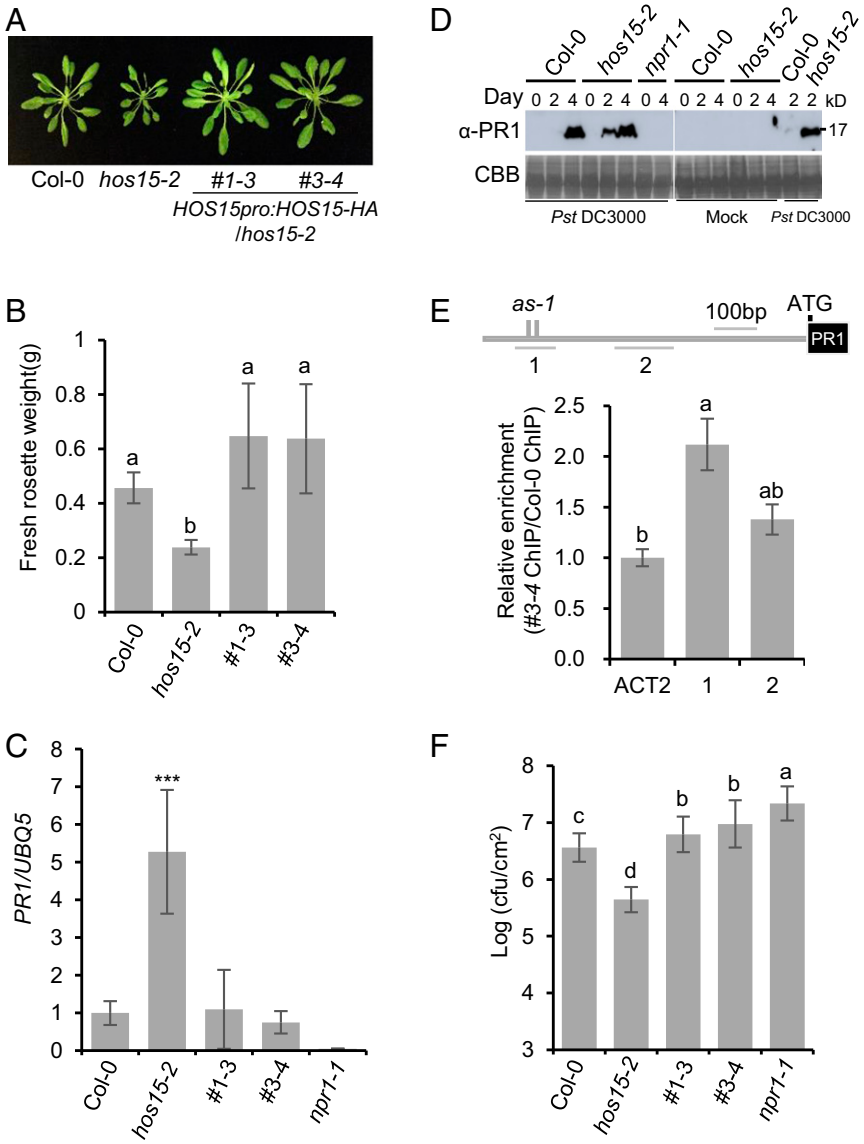

Fig. 1. The *hos15*-2 plants show increased resistance to the bacterial pathogen *Pst* DC3000. (*A*) Morphology of 5-wk-old Col-0, *hos15-2*, and two transgenic lines (#1–3 and #3–4) containing HOS15 transgene driven by native promoter in *hos15-2* (*HOS15pro:HOS15-HA/hos15-2*). (*B*) Fresh weight of the rosette leaves of 5-wk-old Col-0, *hos15-2*, and *HOS15pro:HOS15-HA/hos15-2* plants. Error bars represent means ± SD. Different letters (a or b) indicate significant differences tested by one-way ANOVA with Tukey's honestly significant difference (HSD) ($P < 0.01$, $n = 10$). (*C*) Abundance of *PR1* transcript in the 3-wk–old plants. Ubiquitin 5 was used as an internal control. Error bars represent means ± SD from four biological replicates. Asterisks indicate significant differences between Col-0 and *hos15-2* tested by Student's *t* test (***$P < 0.001$; $n = 12$). (*D*) Abundance of PR1 protein in Col-0, *hos15-2*, and *npr1-1*. Four-week-old plants were infiltrated with *Pst* DC3000 (optical density at 600 nm [OD_{600}] = 0.0001). Total protein was extracted from leaf tissues at the indicated times, and PR1 protein was detected by anti-PR1 immunoblotting. Samples from col-0 and *hos15* plants from 2 d after infection with *Pst* DC3000 were included as a positive control for the right-side blot. Coomassie brilliant blue (CBB) staining of the membrane was used as a loading control. (*E*) Chromatin immunoprecipitation (ChIP) assay showing HOS15 binding on the *as-1* element of the *PR1* promoter. ChIP assays were performed using anti-HA antibody and 4-wk-old Col-0 and *HOS15pro:HOS15-HA/hos15-2* (#3–4) plants. An Actin2 DNA fragment was used for normalization. Error bars represent means ± SEM from three biological replicates. Different letters (a or b) indicate significant differences tested by one-way ANOVA with Tukey's HSD ($P < 0.001$, $n = 15$). bp, base pairs. (*F*) Bacterial growth on Col-0, *hos15-2,* two transgenic *HOS15pro:HOS15-HA/hos15-2* lines (#1–3 and #3–4), and *npr1-1*. Rosette leaves of 4- to 5-wk-old plants were infiltrated with *Pst* DC3000 (OD_{600} = 0.0001), and colony-forming units (cfu) were quantified at 4 d after inoculation. Error bars represent means ± SD from five biological replicates, each with eight or nine technical replicates that consisted of three pooled tissue samples. Different letters (a or b) indicate significant differences tested by one-way ANOVA with Tukey's HSD ($P < 0.01$).

Because the differential protein stability of NPR1 could be confounded by inducible *NPR1* gene expression, the influence of HOS15 in NPR1 degradation was also tested using constitutively expressed NPR1-GFP protein. The *35S::NPR1-GFP/npr1-1/hos15-2* plants were generated by crossing *35S::NPR1-GFP/npr1-1* with *hos15-2*. As observed for native NPR1, *35S::NPR1-GFP/npr1-1/hos15-2* plants had elevated levels of NPR1-GFP compared to *35S::NPR1-GFP/npr1-1*, both prior to and following treatment with SA (*SI Appendix*, Fig. S7*A*). Furthermore, to determine if differences in NPR1-GFP accumulation were based on protein stability, *35S::NPR1-GFP/npr1-1* and *35S::NPR1-GFP/npr1-1/hos15-2* plants were treated with cycloheximide (CHX). As shown in *SI Appendix*, Fig. S7*B*, levels of NPR1-GFP decreased following CHX treatment, and the degradation was delayed in *35S::NPR1-GFP/npr1-1/hos15-2* relative to *35S::NPR1-GFP/npr1-1* plants. Since HOS15 is predominantly localized in the nucleus

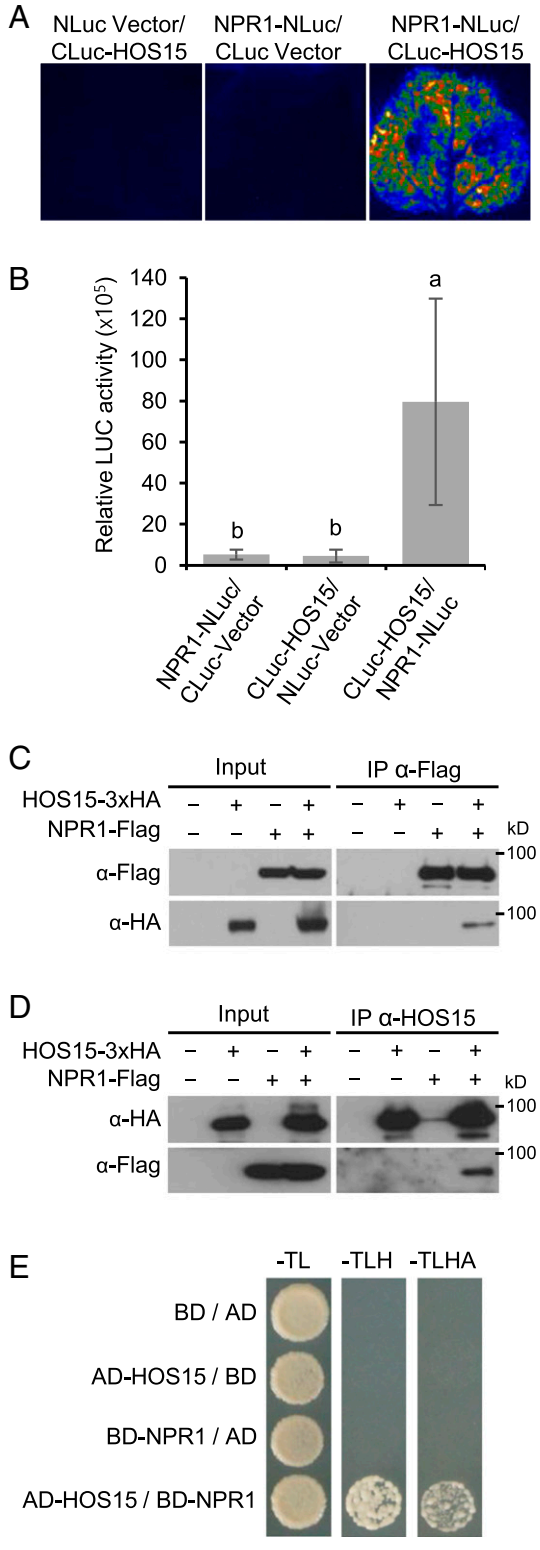

Fig. 2. HOS15 interacts with NPR1 in planta and in yeast. (*A* and *B*) CLuc-HOS15 was coexpressed with NPR1-NLuc in *N. benthamiana*, and the bioluminescence signal after 3 d was visualized (*A*) and quantified (*B*) using a charge-coupled device imaging system. Error bars represent means ± SD from four biological replicates. Different letters (a or b) indicate significant differences tested by one-way ANOVA with Tukey's HSD ($P < 0.001$, $n = 17$). (*C* and *D*) HOS15-3xHA was coexpressed with NPR1-Flag in *N. benthamiana*, and proteins immunoprecipitated with anti-Flag (*C*) or anti-HOS15 (*D*) antibodies were immunoblotted with anti-Flag or anti-HA antibodies. (*E*) HOS15 and NPR1 interaction was examined in a yeast two-hybrid assay. BD-NPR1 in the pAS2-1 vector was used as bait, and AD-HOS15 in the pGAD424 vector was used as prey. Growth of yeast cells on media without tryptophan and leucine (-TL) indicated cotransformation, and growth on media additionally lacking histidine (-TLH) or histidine and adenine (-TLHA) indicated direct interaction between HOS15 and NPR1. Empty vectors pAS2-1 (BD) and pGAD424 (AD) were used as negative controls. Shown are results representative of three independent transformants.

(16) and interacts with NPR1 in the nucleus (*SI Appendix*, Fig. S2), we measured cytosolic and nuclear levels of NPR1 in *35S::NPR1-GFP/npr1-1* and *35S::NPR1-GFP/npr1-1/hos15-2* plants, before and after treatment with SA. Levels of NPR1-GFP were higher in *35S::NPR1-GFP/npr1-1/hos15-2* relative to *35S::NPR1-GFP/npr1-1* in both conditions and in both subcellular fractions (*SI Appendix*, Fig. S7*C*). To further demonstrate the relationship between HOS15 and NPR1 protein levels, NPR1-3xHA was coexpressed with increasing levels of HOS15-Flag in *N. benthamiana* leaves. As the level of HOS15-Flag increased, the level of NPR1-3xHA decreased (*SI Appendix*, Fig. S7*D*), indicating again that HOS15 negatively regulates the stability of the NPR1 protein.

Based on the negative relationship between HOS15 and NPR1 protein stability, we predicted that NPR1-target genes *PR1*, *WRKY18*, *WRKY38*, *WRKY62*, and *SARD1* (10, 15, 68) would be more strongly induced in *hos15* mutant plants. The expression of these genes before and after SA treatment was examined by qRT-PCR in C24, *hos15-1*, Col-0, *hos15-2*, and *npr1-1* plants. As expected, SA induced NPR1-dependent expression of each of these genes. Also, consistent with elevated levels of NPR1 in *hos15*, transcripts of each NPR1-target gene accumulated to higher levels in *hos15* relative to C24 or Col-0 plants both before and after SA induction (*SI Appendix*, Fig. S8). Spoel et al. (15) demonstrated that proteasome-mediated degradation of NPR1 plays both inhibitory and stimulatory roles in activating expression of its target genes. Our results indicate that HOS15 functions as a corepressor by facilitating inhibitory, proteasome-mediated turnover of NPR1 that limits its activity as a transcriptional activator (16, 22). Taken together, we conclude that the corepressor HOS15 regulates the dynamic balance between transcriptional gene activation and repression in plant immunity by functioning as a substrate receptor in the E3 ubiquitin ligase complex that mediates degradation of the coactivator NPR1 to prevent target gene activation in unchallenged conditions and to limit activation during SA-mediated defense signaling.

NPR1 Degradation Is Mediated by the SCF E3 Ubiquitin Ligase Complex. In *Arabidopsis*, three cullin-based E3 ubiquitin ligase complexes have been best characterized, CUL1-based ubiquitin ligase complex (SCF), CUL3-based ubiquitin ligase complex (BCR/CLR3), and CUL4-based ubiquitin ligase complex (DCX/CRL4) (69–71). Here, we have shown that HOS15 displays protein interactions consistent with its function as a substrate receptor for an SCF E3 ubiquitin ligase complex (*SI Appendix*, Figs. S1 and S2). Furthermore, DDB1-binding WD40 (DWD) proteins are reported to act as substrate receptors for CRL4 E3 ubiquitin ligase complexes (25). HOS15 contains a conserved 16-amino acid DWD motif and also serves as a substrate receptor for CRL4 E3 ubiquitin ligase (24, 25). Therefore, we sought to determine the role of different E3 ubiquitin ligase complexes on NPR1 accumulation and plant innate immunity. To address this question, we first analyzed the relationship between NPR1 and the SCF E3 ubiquitin ligase complex. In an LCI assay following transient coexpression in *N. benthamiana* leaves, interactions between the NPR1-CLuc and ASK1-NLuc or ASK2-NLuc and between NPR1-NLuc and ASK1-CLuc or CUL1-CLuc indicated that NPR1 interacts with these SCF complex components (*SI Appendix*, Fig. S9 *A* and *B*). These

data further support the in planta interaction of NPR1 with an SCF^{HOS15} E3 ubiquitin ligase complex.

We next sought to test the role of CUL1-containing SCF E3 ubiquitin ligase complexes in the stability of NPR1 and plant immunity. The T-DNA insertion mutants of *cul1* (*cul1-1*, *cul1-2*, *cul1-3*, and *cul1-4*) are embryo lethal (72, 73). Thus, nonlethal *cul1* alleles were examined. Plants homozygous for the weak *cul1-6* allele display a dwarf phenotype due to disrupted interaction between CUL1 and CAND1 (*SI Appendix*, Fig. S9*C*) (74). Plants homozygous for the gain-of-function *axr6-1* allele are seedling lethal due to a lack of interaction between ASK1 and CUL1 while heterozygous plants exhibit shorter and more wrinkled phenotypes (*SI Appendix*, Fig. S9*C*) (73, 75). Similar to the observation in *hos15-2*, NPR1 protein levels in the *axr6-1*$^{-/+}$ and *cul1-6* plants were increased relative to Col-0 after treatment with SA (*SI Appendix*, Fig. S9*D*). Levels of *NPR1* transcript did not differ significantly between Col-0 and the *cul1* mutant plants either before or after SA treatment (*SI Appendix*, Fig. S9*E*), indicating that the increase in NPR1 protein levels was post-transcriptional. The change in NPR1 protein levels in the *cul1* mutant plants also did not result from a change in levels of HOS15 protein (*SI Appendix*, Fig. S9*F*). Additionally, similar to plants with a *cul1-7* knock-down allele, *cul1-6* and *axr6-1*$^{-/+}$ plants exhibited increased expression of *PR1* (*SI Appendix*, Fig. S9*G*) (76). As expected for a plant constitutively expressing *PR1*, *cul1-6* and *axr6-1*$^{-/+}$ displayed increased resistance against *Pst* DC3000 (*SI Appendix*, Fig. S9*H*). Collectively, these findings indicate that a CUL1-containing SCF E3 ubiquitin ligase complex negatively regulates NPR1 protein stability and plant immunity.

Next, we explored the relationship between CRL4 E3 ubiquitin ligase complexes and NPR1. DDB1A and DDB1B are substrate adaptors of CRL4 E3 ubiquitin ligase complexes (69). DDB1A-3xHA or DDB1B-3xHA was coexpressed in *N. benthamiana* leaves with NPR1-GFP or NPR1-GFP and HOS15-3xHA and subjected to anti-GFP Co-IP (*SI Appendix*, Fig. S10 *A* and *B*). DDB1A-3xHA was coimmunoprecipitated with NPR1-GFP in the presence or absence of HOS15-3xHA, but DDB1B was coimmunoprecipitated with NPR1-GFP only in the presence of HOS15-3xHA. This finding indicates that NPR1 associates with DDB1A and DDB1B in planta. To examine the interaction of NPR1 and CUL4 in *Arabidopsis*, we analyzed F1 progeny from a cross between *35S::NPR1-GFP/npr1-1* and *35S::Flag-CUL4* plants. Co-IP from leaves of these plants indicated that Flag-CUL4 could be coimmunoprecipitated with NPR1-GFP and that the association was increased after treatment with MG132, but not after treatment with SA (*SI Appendix*, Fig. S10 *C* and *D*). These results indicate that NPR1 associates with CRL4 E3 ubiquitin ligases in *Arabidopsis*. However, unlike *hos15-2*, the plants homozygous for *cul4-1* or *35S::Flag-CUL4* do not differ morphologically from Col-0 (*SI Appendix*, Fig. S10*E*). NPR1 protein levels following SA treatment were increased, relative to Col-0, in *cul4-1* and *35S::Flag-CUL4* plants to a similar level as observed in *hos15-2* (*SI Appendix*, Fig. S10*F*). These similar phenotypes for mutant and overexpression of *CUL4* are likely due to confounding pleiotropic effects. Neither mutation nor overexpression of *CUL4* altered the levels of HOS15 protein prior to or following SA treatment (*SI Appendix*, Fig. S10*G*). Considering the elevated levels of NPR1 observed in *cul4-1* plants, it was surprising that they did not display enhanced resistance to *Pst* DC3000 (*SI Appendix*, Fig. S10*H*). Though speculative, this result may indicate that, like CUL3-containing E3 ligase complexes, CRL4 E3 ligase complexes are involved in stimulatory turnover of NPR1 associated with defense gene activation in plant immunity (15). It is also possible that CRL4 E3 ubiquitin ligase may mediate NPR1 degradation under an abiotic stress because NPR1 also plays an essential role in response to abiotic stresses (77–81).

HOS15 Regulates Immune Function through NPR1- and SA-Dependent Pathways. The role of HOS15 in proteasome-mediated degradation of NPR1 is consistent with the prediction that enhanced disease resistance in *hos15* mutant plants is dependent on SA and NPR1. To test this hypothesis, we crossed plants to generate *sid2-2 hos15-2* and *npr1-1 hos15-2* double mutant plants, and a line of *hos15-2* expressing *NahG* (*SI Appendix*, Fig. S11 *A* and *B*). The *sid2-2* mutant fails to accumulate SA (45) whereas the bacterial NahG protein degrades SA (82). Mutations of *SID2* or *NPR1* or expression of *NahG* only partially suppressed the dwarf phenotype of *hos15-2* plants (Fig. 3*A*), indicating that this phenotype of *hos15-2* is not fully dependent on NPR1-dependent SA signaling. However, NPR1- and SID2-dependent accumulation of SA was essential for SA-induced expression of the NPR1-target genes, *PR1*, *WRKY62*, and *SARD1* (Fig. 3 *B*–*D*) and accumulation of the PR1 protein (*SI Appendix*, Fig. S11*C*). Since the increased expression of these defense genes observed in *hos15* mutant plants is entirely dependent on the SA-NPR1 signaling module, increased accumulation of NPR1 protein may account entirely for the phenotype. Similarly, infection of the single and double mutant/transgenic plants revealed that the enhanced susceptibility of *npr1-1*, *sid2-2*, and *NahG* plants to *Pst* DC3000 are epistatic to the enhanced resistance of *hos15-2* (Fig. 3*E*). Thus, both defense gene expression and resistance against a bacterial pathogen indicate that the enhanced resistance phenotypes of *hos15* are dependent on SA and NPR1.

HOS15 Targets Phosphorylated NPR1 for Degradation. Recruitment of phosphorylated substrates by F-box proteins for ubiquitination by an SCF E3 ubiquitin ligase complex and subsequent degradation is a common regulatory mechanism (65, 66, 83–85). Since phosphorylation of NPR1 facilitates its proteasome-dependent turnover (15), we sought to determine whether HOS15-dependent degradation was influenced by the phosphorylation status of NPR1. To this end, plants expressing phosphomimetic NPR1 (*35S::npr1S11/15D-GFP/hos15-2*) and nonphosphorylatable NPR1 (*35S::npr1S11/15A-GFP/hos15-2*) were generated by crossing *hos15-2* with the *35S::npr1S11/15D-GFP* and *35S::npr1S11/15A-GFP* transgenic plants. With these plants, we were then able to assess the abundance of phosphomimetic (S11/15D) and nonphosphorylatable (S11/15A) npr1-GFP proteins in *HOS15* and *hos15-2* plants. Consistent with previous findings that nonphosphorylatable mutants prevent NPR1 from proteasome-dependent degradation, the amount of npr1S11/15A-GFP proteins remained at similar, high levels in both *HOS15* and *hos15-2* plants prior to and following SA treatment (Fig. 4 *A* and *B*) (15). The npr1S11/15D-GFP protein displayed lower levels in the wild type and *hos15-2*. However, compared with wild-type plants, increased levels of npr1S11/15D-GFP proteins were detected in *hos15-2* with or without SA treatment (Fig. 4 *A* and *B*). Further, the interactions of HOS15 with wild-type NPR1-GFP and npr1-GFP derivatives were examined using transgenic plants *35S::NPR1-GFP, 35S::npr1 S11/15D-GFP*, and *35S::npr1 S11/15A-GFP*. The plants were treated with MG132 and the SA functional analog INA (2,6-dichloroisonicotinic acid), and the NPR1-HOS15 interaction was tested by Co-IP (Fig. 4*C*). Enhanced interaction between HOS15 and npr1S11/15D-GFP was revealed by the increased ratio of coimmunoprecipitated to input protein relative to the similar comparison for NPR1-GFP and npr1S11/15A-GFP (Fig. 4*C*). These results indicate that HOS15 likely contributes to phosphorylation-mediated degradation of NPR1.

Next, we tested the effect of the S11/15D and S11/15A derivatives of NPR1-GFP on SA-induced expression of NPR1-target genes. Interestingly, although npr1S11/15A-GFP proteins accumulated to higher levels in *35S::npr1S11/15A-GFP* and *35S::npr1 S11/15A-GFP/hos15-2* plants, NPR1-dependent, SA-induced expression of the NPR1-target genes *PR1*, *WRKY62*,

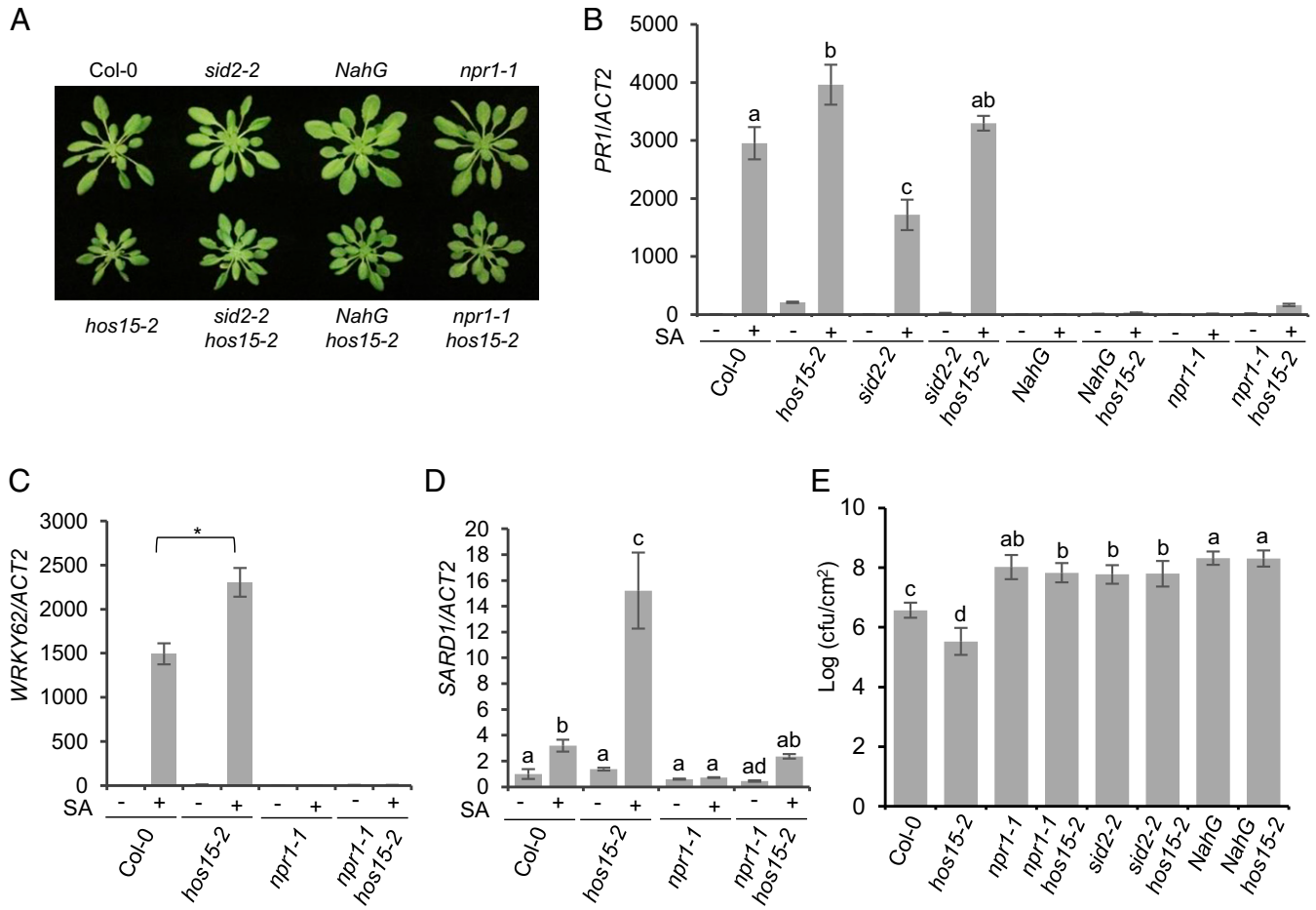

Fig. 3. The effect of HOS15 on plant immunity is dependent on NPR1. (*A*) Morphology of 4-wk-old Col-0, *hos15-2*, *sid2-2*, *sid2-2 hos15-2*, *NahG*, *NahG hos15-2*, *npr1-1*, and *npr1-1 hos15-2*. (*B*) Abundance of *PR1* transcript in 10-d-old seedlings of Col-0, *hos15-2*, *sid2-2*, *sid2-2 hos15-2*, *NahG*, *NahG hos15-2*, *npr1-1*, and *npr1-1 hos15-2* before and 12 h after spraying with 0.5 mM SA. Error bars represent means ± SD. Different letters (a, b, and c) indicate significant differences tested by one-way ANOVA with Tukey's HSD ($P < 0.001$, $n = 3$). The experiment was repeated three times with similar results. (*C* and *D*) Abundance of *WRKY62* and *SARD1* transcripts in 10-d-old seedlings of Col-0, *hos15-2*, *npr1-1*, and *npr1-1 hos15-2* seedlings before and 12 h after spraying with 0.5 mM SA. Error bars represent means ± SD. Asterisks indicate significant differences between Col-0 and *hos15-2* tested by Student's *t* test (*$P < 0.05$; $n = 3$) (*C*). Different letters (a, b, c, and d) indicate significant differences tested by one-way ANOVA with Tukey's HSD ($P < 0.01$, $n = 3$) (*D*). The experiment was repeated three times with similar results. (*E*) Bacterial growth on Col-0, *hos15-2*, *sid2-2*, *sid2-2 hos15-2*, *NahG*, *NahG hos15-2*, *npr1-1*, and *npr1-1 hos15-2* plants. Four- to five-week-old plants were inoculated with *Pst* DC3000 (OD_{600} = 0.0001), and bacterial growth was quantified 4 d after infiltration. Error bars represent means ± SD from three biological replicates, each with three or nine technical replicates that consisted of three pooled tissue samples. Different letters (a or b) indicate significant differences tested by one-way ANOVA with Tukey's HSD ($P < 0.001$). .

and *SARD1* was significantly reduced in these plants compared with the *35S::npr1 S11/15D-GFP* and *35S::npr1 S11/15D-GFP/hos15-2* (Fig. 4 *D–F*). Also, consistent with this observation, *35S::npr1S11/15A-GFP* and *35S::npr1S11/15A-GFP/hos15-2* plants exhibited increased susceptibility to *Pst* DC3000 compared to their S11/15D counterparts (Fig. 4*G*). These results indicate that increased expression of NPR1-target genes and resistance against a bacterial pathogen in *hos15* are largely dependent on NPR1 S11/15 phosphorylation.

SA Stimulates the Formation of the HOS15–NPR1–TGA2 Complex. The SA-NPR1 module is a central regulator in SA-dependent defense responses (68, 86). Given that HOS15 interacts with NPR1 (Fig. 2) and limits its accumulation during SA signaling (*SI Appendix*, Fig. S6), we further analyzed the interaction between HOS15 and NPR1 under the influence of exogenous SA and its synthetic, functional analog, INA. Coimmunoprecipitation with *Arabidopsis 35S::NPR1-GFP/npr1-1* plants revealed that, in the presence of the proteasome inhibitor MG132, exogenous application of INA or SA enhances the interactions between HOS15 and NPR1-GFP (*SI Appendix*, Fig. S12*A*). Since HOS15 and NPR1 share the ability to associate with TGA2 (*SI Appendix*, Figs. S2 and S5) (9, 63), we next tested the influence of TGA2 on the HOS15–NPR1 interaction. Following transient coexpression in *N. benthamiana* and treatment with SA, TGA2-Flag enhanced the interaction between NPR1-GFP and HOS15-3xHA (*SI Appendix*, Fig. S12*B*). Similarly, in the LCI assay, we observed that TGA2-Flag increased the interaction of NLuc-HOS15 and NPR1-CLuc and, notably, that this ability of TGA2 was only apparent in leaves treated with SA (*SI Appendix*, Fig. S12*C*). These results indicate that, dependent on SA, TGA2 positively influences HOS15–NPR1 interaction. Additional coimmunoprecipitation assays following transient coexpression in *N. benthamiana* were conducted to test the influence of HOS15 and SA on the interaction of TGA2 and NPR1 (*SI Appendix*, Fig. S12*D*). In the absence of HOS15, NPR1-GFP weakly coimmunoprecipitated with TGA2-Flag, but only if the leaves were treated with SA. Coexpression of HOS15-3xHA in the absence of SA had the counteracting effects of reducing the abundance of NPR1-GFP and TGA2-Flag in the input samples, while also promoting a

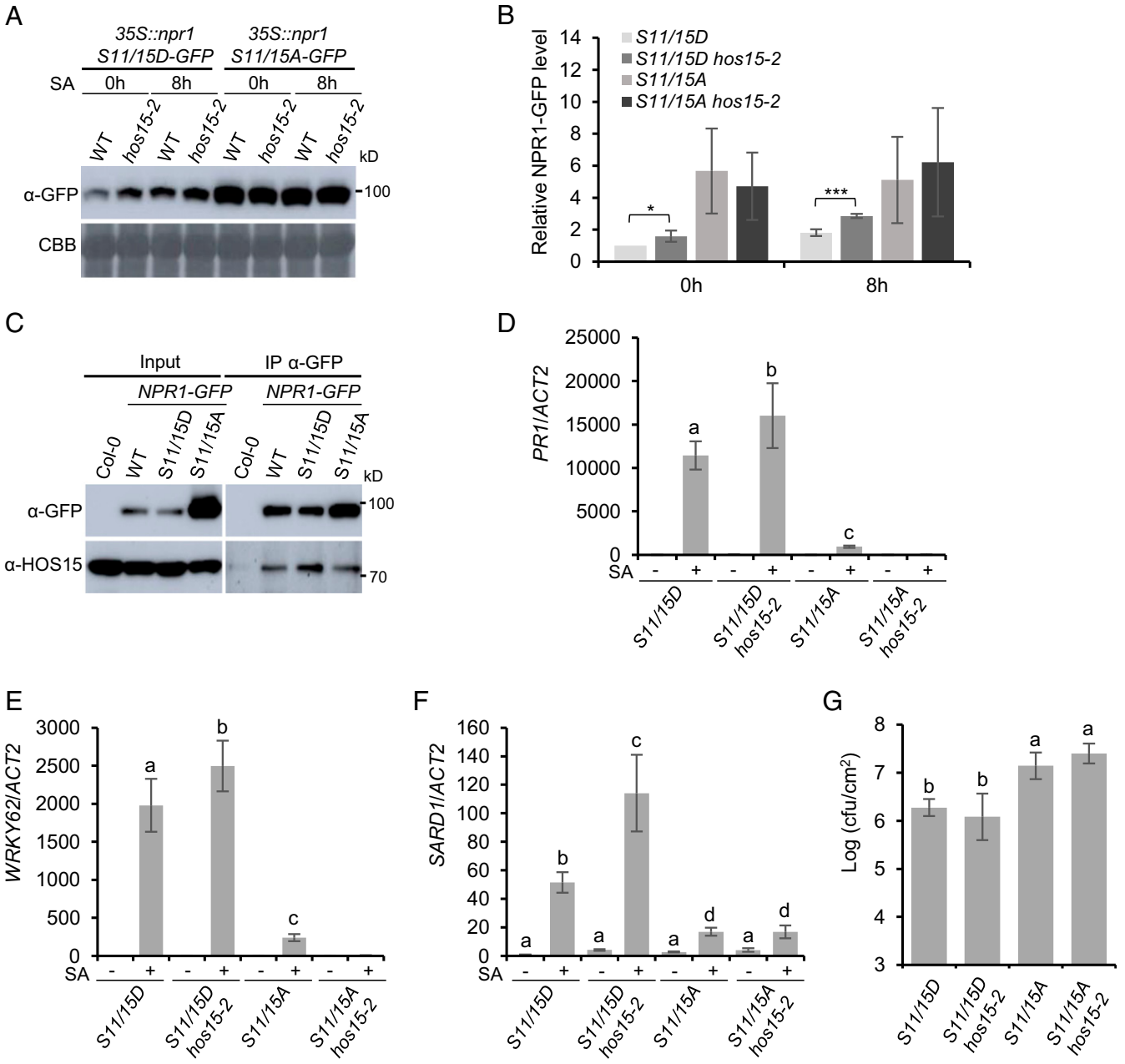

Fig. 4. NPR1 phosphorylation regulates its interaction with HOS15. (*A* and *B*) The 12-d-old seedlings of 35S::npr1 S11/15D-GFP(S11/15D), 35S::npr1 S11/15D-GFP/hos15-2 (S11/15D hos15-2), 35S::npr1 S11/15A-GFP(S11/15A), and 35S::npr1 S11/15A-GFP/hos15-2(S11/15A hos15-2) were treated with 0.5 mM SA. (*A*) Total protein was prepared from leaves collected at 0 and 8 h after treatment, and NPR1-GFP protein levels were measured by anti-GFP immunoblotting. CBB staining of membrane was used as a loading control. (*B*) Error bars represent means ± SD. Asterisks indicate significant differences between S11/15D and S11/15D/hos15-2 tested by Student's *t* test (**P* < 0.05, ****P* < 0.001). (*B*) Quantification of two of the independent biological replicates. (*C*) The 12-d-old seedlings of 35S::NPR1-GFP, 35S::NPR1S11/15D-GFP, and 35S::NPR1S11/15A-GFP were treated with 50 μM MG132 and 0.4 mM INA. Total proteins prepared 8 h later were immunoprecipitated with anti-GFP antibody and immunoblotted with anti-GFP or anti-HOS15 antibodies. (*D–F*) Abundance of PR1, WRKY62 and SARD1 transcripts in 4- to 5-wk-old 35S::npr1 S11/15D-GFP (S11/15D), 35S::npr1 S11/15D-GFP/hos15-2 (S11/15D hos15-2), 35S::npr1 S11/15A-GFP (S11/15A), and 35S::npr1 S11/15A-GFP/hos15-2 (S11/15A hos15-2) plants before or 12 h after spraying with 0.5 mM SA. Error bars represent means ± SD from two biological replicates. Different letters (a, b, c, or d) indicate significant differences tested by one-way ANOVA with Tukey's HSD (*P* < 0.001, *n* = 6). (*G*) Four- to five-week-old plants were infiltrated with Pst DC3000 (OD_{600} = 0.0001), and colony-forming units (cfu) were quantified at 4 d after inoculation. Error bars represent means ± SD from three biological replicates, each with six or eight technical replicates that consisted of three pooled tissue samples. Different letters (a or b) indicate significant differences tested by one-way ANOVA with Tukey's HSD (*P* < 0.001).

weak interaction of NPR1-GFP with TGA2-Flag. However, when the leaves coexpressing HOS15-3xHA were treated with SA, the interaction of NPR1-GFP and TGA2-Flag was enhanced more greatly than can be accounted for by their modest increase in abundance (*SI Appendix*, Fig. S12*D*). These data are consistent with the hypothesis that HOS15 is central to a nuclear complex mediating turnover of both NPR1 and TGA2 and that nuclear accumulation of NPR1 during SA-signaling increases the abundance of this complex. Since TGA2 enhances the formation of a complex between HOS15 and NPR1, we speculated that TGA

proteins might participate in HOS15-mediated NPR1 turnover. Accordingly, immunoblotting indicated that, following SA treatment, *tga2/5/6* triple mutant *Arabidopsis* plants overaccumulate NPR1 while displaying no change in the levels of HOS15 (*SI Appendix*, Fig. S12 *E* and *F*). Collectively, these findings indicate that TGA2, in complex with HOS15 and NPR1, promotes degradation of NPR1 by HOS15.

Discussion

Control of transcription is vital to cellular stress responses. Previously, we reported that HOS15 represses transcription through recruiting HDAC to target genes (16, 22) and regulates cold and ABA signaling as the substrate receptor of a CUL4-containing $CRL4^{HOS15}$ E3 ubiquitin ligase complex (23, 24). However, whether HOS15 corepresses transcriptionally active genes and the molecular mechanism by which it does so are largely unknown. Here, we show that HOS15, within SCF^{HOS15}, targets NPR1 for degradation. This activity accounts for the ability of HOS15 to limit the nuclear activity of NPR1 in uninduced cells and to corepress transcriptionally activated genes in defense-induced cells, which prevent the NPR1-mediated defense from pre- and hyperactivating, respectively (*SI Appendix*, Fig. S13).

Targeting of NPR1 by HOS15 Regulates Pre- and Postactivation Defense Gene Expression. Proteasome-mediated degradation of NPR1 plays dual roles in activation and repression of transcription. In unchallenged leaves, proteasome-mediated degradation of nuclear NPR1 prevents inappropriate expression of defense genes (15). Elevated expression of NPR1-target genes in unchallenged *hos15* mutant plants indicates a role for HOS15 in the basal repression of these genes in the absence of defense activation (*SI Appendix*, Fig. S13, Left). The NPR1 homologs NPR3 and NPR4 associate with TGA2/5/6 but, contrary to NPR1, act as corepressors of defense gene expression when SA levels are low (68). Thus, elevated levels of NPR1 in unchallenged *hos15* mutant plants may alter the competition between TGA/coactivator (TGA/NPR1) and TGA/corepressor (TGA/NPR3 or TGA/NPR4) complexes. In defense-activated cells, binding of SA to NPR3/NPR4 alleviates their corepressor activity. Simultaneously, SA enhances the coactivating role of NPR1 through increased nuclear protein accumulation and, surprisingly, its ubiquitin-dependent turnover (15, 60, 64, 68). Following SA treatment or pathogen exposure, HOS15 targets phosphorylated NPR1 for ubiquitin-dependent degradation that limits the extent of defense gene expression. Thus, the activity of HOS15 negatively regulates the expression of NPR1-target genes prior to and following defense activation.

HOS15 Serves as a Substrate Receptor in Distinct E3 Ubiquitin Ligase Complexes. Human F-box and WD-repeat domain containing 5 (FBXW5) protein contains a conserved N-terminally located F-box motif and two DWD motifs (87, 88). FBXW5 acts as a substrate receptor in both SCF and CRL4 E3 ubiquitin ligase complexes and plays an essential role in response to various pathophysiological and disease processes (89–94). HOS15, which also contains an F-box–like motif in its N terminus (40) and associates with ASK1/2 and CUL1, mediates NPR1 degradation as a substrate receptor for SCF^{HOS15}. HOS15 also acts as a substrate receptor for CRL4 E3 ubiquitin ligase complexes that regulate the stability of HD2C and OST1 during cold stress and ABA signaling, respectively (23, 24). Therefore, HOS15 functions as a substrate receptor in distinct E3 ubiquitin complexes to target various proteins involved in diverse biological processes. Accordingly, we and others recently found that HOS15 plays a crucial role in regulation of plant development, flowering, and response to environmental stresses (20–22, 24, 95). In this study, we found that the SCF^{HOS15} E3 ubiquitin ligase complex negatively regulates plant immunity against *Pst* DC3000 through destabilization of NPR1.

NPR1 Is Targeted by Multiple E3 Ubiquitin Ligases. A particular substrate can be targeted by multiple E3 ubiquitin ligases. For example, ABA-insensitive 5 (ABI5) is a basic leucine zipper transcription factor that plays an important role in the ABA-mediated inhibition of seed germination and is targeted by both RING E3 ligase, KEG, and $CRL4^{DWD1/2}$ E3 ubiquitin ligase in ABA signal transduction (96). Strikingly, ubiquitin-mediated turnover of NPR1 during plant immunity is mediated by at least three distinct E3 ubiquitin ligases. One is the bacterial effector AvrPtoB, which inhibits immunity by ubiquitinating and thus destabilizing cytosolic NPR1 monomers (97, 98). Plant E3 ubiquitin ligase complexes, based on CUL1 (SCF^{HOS15}) and CUL3, also target nuclear NPR1.

Posttranslational modifications of NPR1 contribute to its activity following defense induction (99). Notably, phosphorylation of S11/15 is critical for full induction of defense gene expression by NPR1 (15). S11/15 phosphorylation increases association of NPR1 with HOS15 and reduces its stability based on both SCF^{HOS15}-dependent and CUL3-dependent degradation (*SI Appendix*, Fig. S13, Right). SA-induced activation of gene expression by NPR1 is enhanced by a CUL3-based ubiquitin ligase through a proposed "promoter-refreshing" mechanism (15). Independently, HOS15-mediated degradation of NPR1 inhibits target gene expression and immunity. The opposite effects of HOS15- and CUL3-dependent elimination of NPR1 may reflect their different modes of action. CUL3-mediated degradation that supports promoter "refreshing" may be facilitated by high levels of nuclear NPR1 present early after pathogen challenge. On the contrary, HOS15-mediated inhibition may rely on limiting the overall abundance of TGA/NPR1 coactivating complexes. Additionally, the association of HOS15 with the *PR-1* promoter, presumably via its interaction with NPR1, raises the possibility that HOS15 also recruits HDA9 to further corepress NPR1-target genes through local chromatin modifications.

Surprisingly, we also found that NPR1 associated with not only SCF^{HOS15} E3 ubiquitin ligase, but also with CUL4-based $CRL4^{HOS15}$ E3 ubiquitin ligase (*SI Appendix*, Figs. S9 and S10). However, *cul4-1* plants did not display any enhanced resistance to *Pst* DC3000 (*SI Appendix*, Fig. S10). Since HOS15 and NPR1 are involved in regulation of various biological processes (20–22, 24, 77–81, 95), a CRL4 E3 ubiquitin ligase may mediate NPR1 stability in other biological contexts. Alternatively, an NPR1-dependent effect of *cul4-1* on resistance to *Pst* DC3000 may be obscured by the pleiotropic nature of the mutation.

Insight of HOS15 in the Regulation of Gene Expression. Previous studies have shown that HOS15 acts as a transcriptional corepressor by recruiting HDAC to regulate plant development, flowering, and response to environmental stresses (16, 21, 22, 95). Transcriptional repression can result from repressors maintaining transcriptional inactivity of a gene that is otherwise primed for derepression or from corepressors down-regulating an actively transcribed gene (100). Our study indicates that, by targeting NPR1, HOS15 functions as both a repressor in unchallenged conditions and a corepressor to limit the magnitude/duration of gene expression following defense activation (*SI Appendix*, Fig. S13). Transcriptional activation requires formation of a complex of general transcription machinery and coactivators at the promoter. The relationship between coactivators and corepressors at transcriptionally active genes is less well understood. Our findings indicate that the coactivator NPR1 and corepressor HOS15 coordinately regulate target genes active during plant immunity (*SI Appendix*, Fig. S13, Right). At these genes, HOS15 functions analogously to a "brake" that, perhaps through integration of other stress or developmental signals,

enforces the appropriate "speed limit" for defense gene expression. This biological rheostasis through graduated, quantitative regulation of signal output has recently been suggested for HOS15 in ABA signaling (23).

Regulation of gene expression in space and time is a fundamental biological process. Considering the diversity, versatility, and complexity of transcriptional gene regulation, we are now faced with the challenge of developing a coherent view of transcriptional gene regulation. Our finding that HOS15 is a multifunctional repressor of NPR1 gene activation provides a mechanistic platform for deeper understanding of transcriptional gene regulation.

Methods

Detailed information, including plant materials and growth conditions, plasmid construction, protein extraction and immunoblot analysis, RNA extraction and quantitative RT-PCR, and assays of bacterial growth, LCI, BiFC, yeast two-hybrid, cell-free degradation, Co-IP, and ChIP, is provided in *SI Appendix, Materials and Methods*. All of the data in this study are contained in the article and *SI Appendix*. All materials in the paper will be available from the corresponding authors upon request.

Data Availability. All study data are included in the article and *SI Appendix*.

ACKNOWLEDGMENTS. We thank Xinnian Dong (Duke University) for providing *npr1-1* and *NPR1* transgenic seeds; and Xing Wang Deng (Peking University) and Jae-Hoon Lee (Pusan National University) for providing 35S::*Flag-CUL4* seeds and *CUL4* plasmid construct. This work was supported by grants from the Agriculture and Food Research Initiative (2016-67013-24727) (to D.M.) and the Rural Development Administration, Republic of Korea, Systems & Synthetic Agrobiotech Center (PJ01326904 [to D.M.] and PJ01318205 [to J.M.P.]); the National Research Foundation of Korea funded by the Korean Government (2019R1A2C2084096) (to D.-J.Y.); and the Global Research Laboratory (2017K1A1A2013146) (to D.-J.Y.).

1. J. L. Dangl, J. D. G. Jones, Plant pathogens and integrated defence responses to infection. *Nature* **411**, 826–833 (2001).
2. T. Eulgem, Regulation of the Arabidopsis defense transcriptome. *Trends Plant Sci.* **10**, 71–78 (2005).
3. Z. Nimchuk, T. Eulgem, B. F. Holt III, J. L. Dangl, Recognition and response in the plant immune system. *Annu. Rev. Genet.* **37**, 579–609 (2003).
4. T. L. Karasov, E. Chae, J. J. Herman, J. Bergelson, Mechanisms to mitigate the trade-off between growth and defense. *Plant Cell* **29**, 666–680 (2017).
5. M. G. Rosenfeld, C. K. Glass, Coregulator codes of transcriptional regulation by nuclear receptors. *J. Biol. Chem.* **276**, 36865–36868 (2001).
6. J. N. Feige, J. Auwerx, Transcriptional coregulators in the control of energy homeostasis. *Trends Cell Biol.* **17**, 292–301 (2007).
7. H. Cao, S. A. Bowling, A. S. Gordon, X. Dong, Characterization of an Arabidopsis mutant that is nonresponsive to inducers of systemic acquired resistance. *Plant Cell* **6**, 1583–1592 (1994).
8. T. P. Delaney, L. Friedrich, J. A. Ryals, Arabidopsis signal transduction mutant defective in chemically and biologically induced disease resistance. *Proc. Natl. Acad. Sci. U.S.A.* **92**, 6602–6606 (1995).
9. C. Després, C. DeLong, S. Glaze, E. Liu, P. R. Fobert, The Arabidopsis NPR1/NIM1 protein enhances the DNA binding activity of a subgroup of the TGA family of bZIP transcription factors. *Plant Cell* **12**, 279–290 (2000).
10. D. Wang, N. Amornsiripanitch, X. Dong, A genomic approach to identify regulatory nodes in the transcriptional network of systemic acquired resistance in plants. *PLoS Pathog.* **2**, e123 (2006).
11. Y. Tada *et al.*, Plant immunity requires conformational changes of NPR1 via S-nitrosylation and thioredoxins. *Science* **321**, 952–956 (2008). Correction in: *Science* **325**, 1072 (2009).
12. H. Cao, J. Glazebrook, J. D. Clarke, S. Volko, X. Dong, The Arabidopsis NPR1 gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. *Cell* **88**, 57–63 (1997).
13. A. Rochon, P. Boyle, T. Wignes, P. R. Fobert, C. Després, The coactivator function of Arabidopsis NPR1 requires the core of its BTB/POZ domain and the oxidation of C-terminal cysteines. *Plant Cell* **18**, 3670–3685 (2006).
14. Y. Zhang, W. Fan, M. Kinkema, X. Li, X. Dong, Interaction of NPR1 with basic leucine zipper protein transcription factors that bind sequences required for salicylic acid induction of the PR-1 gene. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 6523–6528 (1999).
15. S. H. Spoel *et al.*, Proteasome-mediated turnover of the transcription coactivator NPR1 plays dual roles in regulating plant immunity. *Cell* **137**, 860–872 (2009).
16. J. Zhu *et al.*, Involvement of Arabidopsis HOS15 in histone deacetylation and cold tolerance. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 4945–4950 (2008).
17. M. G. Guenther *et al.*, A core SMRT corepressor complex containing HDAC3 and TBL1, a WD40-repeat protein linked to deafness. *Genes Dev.* **14**, 1048–1057 (2000).
18. J. Li *et al.*, Both corepressor proteins SMRT and N-CoR exist in large protein complexes containing HDAC3. *EMBO J.* **19**, 4342–4350 (2000).
19. J. Zhang, M. Kalkum, B. T. Chait, R. G. Roeder, The N-CoR-HDAC3 nuclear receptor corepressor complex inhibits the JNK pathway through the integral subunit GPS2. *Mol. Cell* **9**, 611–623 (2002).
20. M. Suzuki *et al.*, *OLIGOCELLULA1/HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENES15* promotes cell proliferation with *HISTONE DEACETYLASE9* and *POWERDRESS* during leaf development in *Arabidopsis thaliana*. *Front. Plant Sci.* **9**, 580 (2018).
21. K. S. Mayer *et al.*, HDA9-PWR-HOS15 is a core histone deacetylase complex regulating transcription and development. *Plant Physiol.* **180**, 342–355 (2019).
22. H. J. Park *et al.*, HOS15 interacts with the histone deacetylase HDA9 and the evening complex to epigenetically regulate the floral activator *GIGANTEA*. *Plant Cell* **31**, 37–51 (2019).
23. A. Ali *et al.*, Rheostatic control of ABA signaling through HOS15-mediated OST1 degradation. *Mol. Plant* **12**, 1447–1462 (2019).
24. J. Park *et al.*, Epigenetic switch from repressive to permissive chromatin in response to cold stress. *Proc. Natl. Acad. Sci. U.S.A.* **115**, E5400–E5409 (2018).
25. J. H. Lee *et al.*, Characterization of Arabidopsis and rice DWD proteins and their roles as substrate receptors for CUL4-RING E3 ubiquitin ligases. *Plant Cell* **20**, 152–167 (2008).
26. K. Jepsen *et al.*, Combinatorial roles of the nuclear receptor corepressor in transcription and development. *Cell* **102**, 753–763 (2000).
27. E. De Nadal *et al.*, The MAPK Hog1 recruits Rpd3 histone deacetylase to activate osmoresponsive genes. *Nature* **427**, 370–374 (2004).
28. T. J. Peterson, S. Karmakar, M. C. Pace, T. Gao, C. L. Smith, The silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) corepressor is required for full estrogen receptor alpha transcriptional activity. *Mol. Cell. Biol.* **27**, 5933–5948 (2007).
29. A. Miccio *et al.*, NuRD mediates activating and repressive functions of GATA-1 and FOG-1 during blood development. *EMBO J.* **29**, 442–456 (2010).
30. J. D. Chen, R. M. Evans, A transcriptional co-repressor that interacts with nuclear hormone receptors. *Nature* **377**, 454–457 (1995).
31. A. J. Hörlein *et al.*, Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. *Nature* **377**, 397–404 (1995).
32. L. Müller, D. Hainberger, V. Stolz, W. Ellmeier, NCOR1-a new player on the field of T cell development. *J. Leukoc. Biol.* **104**, 1061–1068 (2018).
33. T. I. Lima *et al.*, Role of NCoR1 in mitochondrial function and energy metabolism. *Cell Biol. Int.* **42**, 734–741 (2018).
34. S. Ramamoorthy, J. A. Cidlowski, Ligand-induced repression of the glucocorticoid receptor gene is mediated by an NCoR1 repression complex formed by long-range chromatin interactions with intragenic glucocorticoid response elements. *Mol. Cell. Biol.* **33**, 1711–1722 (2013).
35. S. M. Kyle, P. K. Saha, H. M. Brown, L. C. Chan, M. J. Justice, MeCP2 co-ordinates liver lipid metabolism with the NCoR1/HDAC3 corepressor complex. *Hum. Mol. Genet.* **25**, 3029–3041 (2016).
36. A. Wang, S. K. Kurdistani, M. Grunstein, Requirement of Hos2 histone deacetylase for gene activity in yeast. *Science* **298**, 1412–1414 (2002).
37. X. Chen *et al.*, Requirement for the histone deacetylase Hdac3 for the inflammatory gene expression program in macrophages. *Proc. Natl. Acad. Sci. U.S.A.* **109**, E2865–E2874 (2012).
38. J. Jin *et al.*, Systematic analysis and nomenclature of mammalian F-box proteins. *Genes Dev.* **18**, 2573–2580 (2004).
39. V. Perissi *et al.*, TBL1 and TBLR1 phosphorylation on regulated gene promoters overcomes dual CtBP and NCoR/SMRT transcriptional repression checkpoints. *Mol. Cell* **29**, 755–766 (2008).
40. J. Park *et al.*, Identification and molecular characterization of HOS15-interacting proteins in Arabidopsis thaliana. *J. Plant Biol.* **61**, 336–345 (2018).
41. Q. Bruggeman, C. Raynaud, M. Benhamed, M. Delarue, To die or not to die? Lessons from lesion mimic mutants. *Front. Plant Sci.* **6**, 24 (2015).
42. J. Li, G. Brader, E. T. Palva, The WRKY70 transcription factor: A node of convergence for jasmonate-mediated and salicylate-mediated signals in plant defense. *Plant Cell* **16**, 319–331 (2004).
43. Y. Hu, Q. Dong, D. Yu, Arabidopsis WRKY46 coordinates with WRKY70 and WRKY53 in basal resistance against pathogen Pseudomonas syringae. *Plant Sci.* **185-186**, 288–297 (2012).
44. J. Li, G. Brader, T. Kariola, E. T. Palva, WRKY70 modulates the selection of signaling pathways in plant defense. *Plant J.* **46**, 477–491 (2006).
45. M. C. Wildermuth, J. Dewdney, G. Wu, F. M. Ausubel, Isochorismate synthase is required to synthesize salicylic acid for plant defence. *Nature* **414**, 562–565 (2001).
46. C. Garcion *et al.*, Characterization and biological function of the ISOCHORISMATE SYNTHASE2 gene of Arabidopsis. *Plant Physiol.* **147**, 1279–1287 (2008).
47. J. Glazebrook *et al.*, Phytoalexin-deficient mutants of Arabidopsis reveal that PAD4 encodes a regulatory factor and that four PAD genes contribute to downy mildew resistance. *Genetics* **146**, 381–392 (1997).
48. N. Zhou, T. L. Tootle, F. Tsui, D. F. Klessig, J. Glazebrook, PAD4 functions upstream from salicylic acid to control defense responses in Arabidopsis. *Plant Cell* **10**, 1021–1030 (1998).
49. E. E. Rogers, F. M. Ausubel, Arabidopsis enhanced disease susceptibility mutants exhibit enhanced susceptibility to several bacterial pathogens and alterations in PR-1 gene expression. *Plant Cell* **9**, 305–316 (1997).
50. S. M. Volko, T. Boller, F. M. Ausubel, Isolation of new Arabidopsis mutants with enhanced disease susceptibility to Pseudomonas syringae by direct screening. *Genetics* **149**, 537–548 (1998).

51. M. H. Borhan *et al*., WRR4 encodes a TIR-NB-LRR protein that confers broad-spectrum white rust resistance in Arabidopsis thaliana to four physiological races of Albugo candida. *Mol. Plant Microbe Interact.* **21**, 757–768 (2008).
52. S. Uknes *et al*., Acquired resistance in Arabidopsis. *Plant Cell* **4**, 645–656 (1992).
53. K. S. Century, E. B. Holub, B. J. Staskawicz, NDR1, a locus of Arabidopsis thaliana that is required for disease resistance to both a bacterial and a fungal pathogen. *Proc. Natl. Acad. Sci. U.S.A.* **92**, 6597–6601 (1995).
54. K. S. Century *et al*., NDR1, a pathogen-induced component required for Arabidopsis disease resistance. *Science* **278**, 1963–1965 (1997).
55. J. E. Parker *et al*., Characterization of eds1, a mutation in Arabidopsis suppressing resistance to Peronospora parasitica specified by several different RPP genes. *Plant Cell* **8**, 2033–2046 (1996).
56. I. A. M. A. Penninckx *et al*., Pathogen-induced systemic activation of a plant defensin gene in Arabidopsis follows a salicylic acid-independent pathway. *Plant Cell* **8**, 2309–2323 (1996).
57. K. Shirasu, The HSP90-SGT1 chaperone complex for NLR immune sensors. *Annu. Rev. Plant Biol.* **60**, 139–164 (2009).
58. C. Azevedo *et al*., The RAR1 interactor SGT1, an essential component of R gene-triggered disease resistance. *Science* **295**, 2073–2076 (2002).
59. Y. Liu, M. Schiff, G. Serino, X. W. Deng, S. P. Dinesh-Kumar, Role of SCF ubiquitin-ligase and the COP9 signalosome in the N gene-mediated resistance response to Tobacco mosaic virus. *Plant Cell* **14**, 1483–1496 (2002).
60. M. Kinkema, W. Fan, X. Dong, Nuclear localization of NPR1 is required for activation of PR gene expression. *Plant Cell* **12**, 2339–2350 (2000).
61. X. Dong, NPR1, all things considered. *Curr. Opin. Plant Biol.* **7**, 547–552 (2004).
62. C. M. Pieterse, L. C. Van Loon, NPR1: The spider in the web of induced resistance signaling pathways. *Curr. Opin. Plant Biol.* **7**, 456–464 (2004).
63. J. M. Zhou *et al*., NPR1 differentially interacts with members of the TGA/OBF family of transcription factors that bind an element of the PR-1 gene required for induction by salicylic acid. *Mol. Plant Microbe Interact.* **13**, 191–202 (2000).
64. W. Fan, X. Dong, In vivo interaction between NPR1 and transcription factor TGA2 leads to salicylic acid-mediated gene activation in Arabidopsis. *Plant Cell* **14**, 1377–1389 (2002).
65. D. Skowyra, K. L. Craig, M. Tyers, S. J. Elledge, J. W. Harper, F-box proteins are receptors that recruit phosphorylated substrates to the SCF ubiquitin-ligase complex. *Cell* **91**, 209–219 (1997).
66. J. T. Winston, D. M. Koepp, C. Zhu, S. J. Elledge, J. W. Harper, A family of mammalian F-box proteins. *Curr. Biol.* **9**, 1180–1182 (1999).
67. Z. Q. Fu *et al*., NPR3 and NPR4 are receptors for the immune signal salicylic acid in plants. *Nature* **486**, 228–232 (2012).
68. Y. Ding *et al*., Opposite roles of salicylic acid receptors NPR1 and NPR3/NPR4 in transcriptional regulation of plant immunity. *Cell* **173**, 1454–1467.e15 (2018).
69. S. Biedermann, H. Hellmann, WD40 and CUL4-based E3 ligases: Lubricating all aspects of life. *Trends Plant Sci.* **16**, 38–46 (2011).
70. J. H. Lee, W. T. Kim, Regulation of abiotic stress signal transduction by E3 ubiquitin ligases in Arabidopsis. *Mol. Cells* **31**, 201–208 (2011).
71. S. Fonseca, V. Rubio, Arabidopsis CRL4 complexes: Surveying chromatin states and gene expression. *Front. Plant Sci.* **10**, 1095 (2019).
72. W. H. Shen *et al*., Null mutation of AtCUL1 causes arrest in early embryogenesis in Arabidopsis. *Mol. Biol. Cell* **13**, 1916–1928 (2002).
73. H. Hellmann *et al*., Arabidopsis AXR6 encodes CUL1 implicating SCF E3 ligases in auxin regulation of embryogenesis. *EMBO J.* **22**, 3314–3325 (2003).
74. J. Moon *et al*., A new CULLIN 1 mutant has altered responses to hormones and light in Arabidopsis. *Plant Physiol.* **143**, 684–696 (2007).
75. L. Hobbie *et al*., The axr6 mutants of Arabidopsis thaliana define a gene involved in auxin response and early development. *Development* **127**, 23–32 (2000).
76. Y. T. Cheng *et al*., Stability of plant immune-receptor resistance proteins is controlled by SKP1-CULLIN1-F-box (SCF)-mediated protein degradation. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 14694–14699 (2011).
77. E. Olate, J. M. Jiménez-Gómez, L. Holuigue, J. Salinas, NPR1 mediates a novel regulatory pathway in cold acclimation by interacting with HSFA1 factors. *Nat. Plants* **4**, 811–823 (2018).
78. M. Jayakannan *et al*., The NPR1-dependent salicylic acid signalling pathway is pivotal for enhanced salt and oxidative stress tolerance in Arabidopsis. *J. Exp. Bot.* **66**, 1865–1875 (2015).
79. Y. Ding, M. Dommel, Z. Mou, Abscisic acid promotes proteasome-mediated degradation of the transcription coactivator NPR1 in Arabidopsis thaliana. *Plant J.* **86**, 20–34 (2016).
80. M. Yasuda *et al*., Antagonistic interaction between systemic acquired resistance and the abscisic acid-mediated abiotic stress response in Arabidopsis. *Plant Cell* **20**, 1678–1692 (2008).
81. Y. S. Lai *et al*., Salicylic acid-independent role of NPR1 is required for protection from proteotoxic stress in the plant endoplasmic reticulum. *Proc. Natl. Acad. Sci. U.S.A.* **115**, E5203–E5212 (2018).
82. T. P. Delaney *et al*., A central role of salicylic Acid in plant disease resistance. *Science* **266**, 1247–1250 (1994).
83. E. E. Patton, A. R. Willems, M. Tyers, Combinatorial control in ubiquitin-dependent proteolysis: Don't Skp the F-box hypothesis. *Trends Genet.* **14**, 236–243 (1998).
84. H. Yu, J. Wu, N. Xu, M. Peng, Roles of F-box proteins in plant hormone responses. *Acta Biochim. Biophys. Sin. (Shanghai)* **39**, 915–922 (2007).
85. J. R. Skaar, J. K. Pagan, M. Pagano, Mechanisms and function of substrate recruitment by F-box proteins. *Nat. Rev. Mol. Cell Biol.* **14**, 369–381 (2013).
86. Y. Wu *et al*., The Arabidopsis NPR1 protein is a receptor for the plant defense hormone salicylic acid. *Cell Rep.* **1**, 639–647 (2012).
87. Y. J. He, C. M. McCall, J. Hu, Y. Zeng, Y. Xiong, DDB1 functions as a linker to recruit receptor WD40 proteins to CUL4-ROC1 ubiquitin ligases. *Genes Dev.* **20**, 2949–2954 (2006).
88. J. Hu *et al*., WD40 protein FBW5 promotes ubiquitination of tumor suppressor TSC2 by DDB1-CUL4-ROC1 ligase. *Genes Dev.* **22**, 866–871 (2008).
89. Y. Minoda, H. Sakurai, T. Kobayashi, A. Yoshimura, G. Takaesu, An F-box protein, FBXW5, negatively regulates TAK1 MAP3K in the IL-1beta signaling pathway. *Biochem. Biophys. Res. Commun.* **381**, 412–417 (2009).
90. T. Y. Kim *et al*., CRL4A-FBXW5-mediated degradation of DLC1 Rho GTPase-activating protein tumor suppressor promotes non-small cell lung cancer cell growth. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 16868–16873 (2013).
91. A. Werner *et al*., SCFFbxw5 mediates transient degradation of actin remodeller Eps8 to allow proper mitotic progression. *Nat. Cell Biol.* **15**, 179–188 (2013).
92. J. Y. Ha *et al*., Tnfaip8 l1/Oxi-β binds to FBXW5, increasing autophagy through activation of TSC2 in a Parkinson's disease model. *J. Neurochem.* **129**, 527–538 (2014).
93. A. Puklowski *et al*., The SCF-FBXW5 E3-ubiquitin ligase is regulated by PLK4 and targets HsSAS-6 to control centrosome duplication. *Nat. Cell Biol.* **13**, 1004–1009 (2011).
94. Y. T. Jeong *et al*., The ULK1-FBXW5-SEC23B nexus controls autophagy. *eLife* **7**, e42253 (2018).
95. R. K. Hammond, HOS15 coregulates photoperiodic flowering with the evening complex via transcriptional repression of *GIGANTEA*. *Plant Cell* **31**, 3–4 (2019).
96. A. Skubacz, A. Daszkowska-Golec, I. Szarejko, The role and regulation of ABI5 (ABA-Insensitive 5) in plant development, abiotic stress responses and phytohormone crosstalk. *Front. Plant Sci.* **7**, 1884 (2016).
97. H. Chen *et al*., A bacterial type III effector targets the master regulator of salicylic acid signaling, NPR1, to Subvert plant immunity. *Cell Host Microbe* **22**, 777–788.e7 (2017).
98. V. Göhre *et al*., Plant pattern-recognition receptor FLS2 is directed for degradation by the bacterial ubiquitin ligase AvrPtoB. *Curr. Biol.* **18**, 1824–1832 (2008).
99. J. Withers, X. Dong, Posttranslational modifications of NPR1: A single protein playing multiple roles in plant immunity and physiology. *PLoS Pathog.* **12**, e1005707 (2016).
100. V. V. Sridhar, A. Surendrarao, D. Gonzalez, R. S. Conlan, Z. Liu, Transcriptional repression of target genes by LEUNIG and SEUSS, two interacting regulatory proteins for Arabidopsis flower development. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 11494–11499 (2004).