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The chromatin remodeling protein BAF60/SWP73A regulates the plant immune Receptor NLRs

--Manuscript Draft--

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Abstract:	In both plant and animal innate immune responses, surveillance of pathogen infection is mediated by membrane-associated receptors and intracellular nucleotide-binding domain & leucine-rich repeat receptors (NLRs). Homeostasis of NLRs is under tight multilayered regulation to avoid over-accumulation or over-activation, which often leads to autoimmune responses that cause detrimental effects on growth and development. How NLRs are regulated epigenetically at the chromatin level remains unclear. Here, we report that SWP73A, an orthologue of the mammalian switch/sucrose nonfermentable (SWI/SNF) chromatin remodeling protein BAF60, suppresses the expression of NLRs either directly by binding to the NLR promoters or indirectly by affecting the alternative splicing of some NLRs through the suppression of Cell Division Cycle 5 (CDC5), a key regulator of RNA splicing. Upon infection, bacteria-induced small RNAs silence SWP73A to activate a group of NLRs and trigger robust immune responses. SWP73A may function as a H3K9me2 reader to enhance transcription suppression.
Suggested Reviewers:	Gitta Coaker UC Davis glcoaker@ucdavis.edu World expert on NLR expression regulation, plant immunity against bacterial diseases.
	Xin Li UBC xinli@mail.ubc.ca She is a world expert on epigenetic regulation of NLRs and signal transduction of plant immune responses.
	Brad Day MSU bday@msu.edu He is a world expert on NLR signaling pathways and plant innate immunity.
Opposed Reviewers:	Jurriaan Ton

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Jan 04, 2020

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We have no additional edits on the Declaration of Interest form, Highlights and eTOC blurb.

Sincerely,

Hailing Jin
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December 15, 2020

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Supplemental material

We have reorganized all supplemental figures to 6 figures (Figure S1-S6). The Supplemental information file includes Figure S1-S6 with legends and Table S3 which is not over 3 pages.

- All references should be part of the main reference list, not included as part of the STAR Methods, Key Resources Table, or supplement. Remove References from Supplemental Document.

Corrected.

- Please include the STAR Methods text within the main article file. You may refer to our STAR Methods Guidelines for more information about the STAR Methods format. Star Methods in supplemental document, should be in main.

Corrected.

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Revised as required.

- The number of supplemental figures should not exceed the number of main items (figures and tables). For example, a paper with X main items can have up to X supplemental figures. There are 17 supplemental figures and 6 main items. It seems that the supplemental figures can be combined, especially if relating to the same main figure. If you have trouble reducing the number of supplemental figures, please let me know.

Now we combined the figures to have 6 final Supplemental figures.

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Fixed.

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Table S1 and S1 is upload as Excel files. Table S3 is add in Supplemental file.

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STAR Methods

- STAR Methods follows a standardized structure. Please include these specific headings in the following order: RESOURCE AVAILABILITY; EXPERIMENTAL MODEL AND SUBJECT DETAILS; METHOD DETAILS; QUANTIFICATION AND STATISTICAL ANALYSIS; ADDITIONAL RESOURCES. The list below details the information that should be reported in each section. Please see the STAR Methods guide for more information or contact me for help.
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- We require three subheadings in this section (Lead Contact, Materials Availability, and Data and Code Availability). Please be in touch if you have any questions about this and refer to the Information for Authors for specific details on Cell Press policy on reagents sharing.
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    - Growing conditions for plant models
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Sincerely,

Hailing Jin
Professor
University of California, Riverside.
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The chromatin remodeling protein BAF60/SWP73A regulates the plant immune receptor NLRs

Chien-Yu Huang[1,†], Diana Sanchez Rangel[1,2,†], Xiaobo Qin[1], Christine Bui[1], Ruidong Li[3], Zhenyu Jia[3], Xinping Cui[4], Hailing Jin[1,*]

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Summary

In both plant and animal innate immune responses, surveillance of pathogen infection is mediated by membrane-associated receptors and intracellular nucleotide-binding domain & leucine-rich repeat receptors (NLRs). Homeostasis of NLRs is under tight multilayered regulation to avoid over-accumulation or over-activation, which often leads to autoimmune responses that cause detrimental effects on growth and development. How NLRs are regulated epigenetically at the chromatin level remains unclear. Here, we report that SWP73A, an orthologue of the mammalian switch/sucrose nonfermentable (SWI/SNF) chromatin remodeling protein BAF60, suppresses the expression of NLRs either directly by binding to the NLR promoters or indirectly by affecting the alternative splicing of some NLRs through the suppression of *Cell Division Cycle 5 (CDC5)*, a key regulator of RNA splicing. Upon infection, bacteria-induced small RNAs silence *SWP73A* to activate a group of NLRs and trigger robust immune responses. *SWP73A* may function as a H3K9me2 reader to enhance transcription suppression.

Introduction

Intracellular nucleotide-binding domain and leucine-rich repeat receptors (NLRs) regulate innate immune responses against pathogen infection in both plants and animals (Jones et al., 2016; Zhou and Zhang, 2020). Typically, NLRs contain a central nucleotide-binding domain (NBD) and a C-terminal leucine-rich repeat (LRR) domain. Upon the recognition of pathogen effectors, NLR proteins oligomerize to form an oligomeric "resistosome" structure (Li et al., 2020; Wang et al., 2019), which induces rapid and robust effector-triggered immunity (ETI) (Lolle et al., 2020). Expression and activation of NLRs are precisely regulated at multiple levels, including transcriptional regulation, alternative mRNA splicing, small RNA (sRNA)-mediated post-transcriptional regulation, post-translational modifications, NLR dimerization or oligomerization, and proteasome-mediated degradation (Li et al., 2015; Lolle et al., 2020). This tight regulation is critical because over-accumulation or imbalanced activity of NLRs can lead to autoimmunity and serious fitness costs including reduced growth and development (Cui et al., 2015; Li et al., 2015; Lolle et al., 2020). Furthermore, heteromeric interaction between certain NLR alleles can also trigger autoimmunity, leading to cell death (Chae et al., 2014; Li et al., 2020; Tran et al., 2017).

To balance the trade-off between plant growth and defense, some sRNAs, including microRNA (miRNA) and small interfering RNA (siRNA), target NLRs and plant defense signaling genes, contributing to the precise regulation of plant immunity (Chen et al., 2010; Huang et al., 2019; Katiyar-Agarwal et al., 2007; Katiyar-Agarwal et al., 2006; Li et al., 2012; Shivaprasad et al., 2012). In this study, we identified two *Arabidopsis* sRNAs, including an miRNA and an siRNA, which were explicitly induced by a bacterium

Pseudomonas syringae pv. tomato (*Pst*) strain carrying the effector, AvrRpt2 (Chellappan et al., 2010; Zhang et al., 2011). Both sRNAs target *Arabidopsis SWP73A*, an ortholog of the mammalian BRG1-Associated Factor 60 (*BAF60*). SWP73/BAF60 is a subunit of the SWI/SNF (switch/sucrose non-fermentable) chromatin remodeling complex, which utilizes the energy of ATP hydrolysis for nucleosome positioning on the DNA (Hopfner et al., 2012; Jerzmanowski, 2007). SWI/SNF complexes are well characterized as transcriptional activators but can also physically repress gene expression, depending on their regulatory dynamics (Pulice and Kadoch, 2016; Raab et al., 2015). Chromatin remodeling mediated by SWI/SNF complexes plays a regulatory role in many cellular processes in mammals (Gatchalian et al., 2020). For example, mammalian BAF60 is a transcriptional coactivator with NF-κB and activates pro-inflammatory genes during infection (Tartey et al., 2014). Whether plant BAF60 homologs regulate host innate immunity against pathogen infection is still unknown.

There are two SWP73/BAF60 variants in *Arabidopsis*, SWP73A, and SW73B. SWP73B functions in leaf and flower development (Sacharowski et al., 2015; Vercruyssen et al., 2014), DNA repair (Campi et al., 2012), and flowering time (Jegu et al., 2014). In contrast, the functions of *SWP73A* are still unknown. Here, we discovered that, unlike mammalian BAF60 which acts as a positive regulator of gene transcription, plant *SWP73A* acts as a negative regulator of gene expression and inhibits plant innate immune response to avoid autoimmunity in the absence of pathogens. Upon bacterial infection, *SWP73A* is suppressed by two bacteria-induced sRNAs, and plant immune responses are activated through the accumulation of a set of NLRs.

Results

Two sRNAs are specifically induced by *Pst (AvrPpt2)* infection and target *SWP73A*

From the sRNA profiles of *Arabidopsis* Col-0 plants infected by bacterium (*Pst*) DC3000 strains, we identified a set of sRNAs that were specifically induced by the avirulent strain *Pst (AvrRpt2)* (Zhang et al., 2011). The bacterial effector AvrRpt2 is recognized by the NLR protein RPS2 in Col-0, triggering the ETI response (Bent et al., 1994; Chellappan et al., 2010; Zhang et al., 2011). Two sRNAs, including one miRNA, miR3440, and one siRNA, siRNA-*SWP73A,* were explicitly induced by *Pst (AvrRpt2)* but not by the type III secretion system mutant strain, *Pst* DC3000 *hrcC*, virulent strain *Pst* DC3000 which carries an empty vector (EV) or the mock solution (Fig. 1a). In addition, the two sRNAs were not induced by *Pst (AvrRpt2)* infection in the *RPS2* loss-of-function mutant, *rps2-101c* (Fig. S1a). Thus, the induction of the two sRNAs by *Pst (AvrRpt2)* is *RPS2*-dependent. miR3440 was predicted to target the stop codon region of the *SWP73A* gene, and siRNA-*SWP73A* targets the 5' UTR of *SWP73A* (Fig. S1b). Indeed, the transcript level of *SWP73A* decreased after *Pst (AvrRpt2)* infection when both sRNAs were induced (Fig. 1b and c). Transient co-expression of *SWP73A* mRNA with miR3440 in *Nicotiana benthamiana* (*Nb*) leaves resulted in reduced SWP73A protein in comparison to co-expression with the negative control, miR395, confirming that *SWP73A* cDNA is a real target of miR3440 (Fig. S1c). The other *SWP73/BAF60* variant in *Arabidopsis*, *SWP73B* (Fig. S2a), was not affected by *Pst (AvrRpt2)* infection at the transcript expression level (Fig. S2b).

SWP73A negatively regulates plant immune responses against *Pst (AvrRpt2)*

Because *SWP73A* is down-regulated upon *Pst (AvrRpt2)* infection, to characterize the function of *SWP73A* in plant immunity, we obtained the *swp73a* T-DNA insertion knockout mutant, which has no obvious vegetative developmental defect as reported before (Fig. 2a) (Sacharowski et al., 2015). The transcript levels of NLR gene *RPS2,* as well as the *Pathogenesis-Related gene 1 (PR1*), were elevated in the *swp73a* mutant (Fig. 2b). Furthermore, *swp73a* displayed accelerated hypersensitive response (HR) and reduced bacterial growth after *Pst (AvrRpt2)* infection compared with wild-type (WT) plants (Fig. 2c and d). These results suggest that SWP73A is a negative regulator of plant ETI.

In the *swp73a* mutant, *SWP73B* mRNA has a significantly higher expression level (Fig. S2d), suggesting that it has a partial compensatory effect on *SWP73A* when *SWP73A* is absent. Therefore, *SWP73A* and *SWP73B* double mutants *AmiRswp73a/b* were generated through silencing *SWP73B* using an artificial miRNA in the *swp73a* mutant (Fig. S2e). These plants displayed drastically reduced plant size (Fig. 2a), and elevated levels of *RPS2* and *PR1* compared to the *swp73a* single mutant plants (Fig. 2b). The *AmiRswp73a/b* double mutant plants also showed further accelerated HR and reduced bacterial growth after *Pst (AvrRpt2)* infection compared to the *swp73a* mutant (Fig. 2c and d). These characteristics indicate an autoimmune phenotype, which can be caused by the elevated expression of NLRs and PR genes leading to a strong fitness cost. The *swp73b* mutant has a defect in leaf and flower development and does not produce seeds (Sacharowski et al., 2015). Although the *swp73b* single mutant is also smaller than WT (Fig. S2f), the expression levels of *RPS2* and *PR1* exhibited no obvious changes compared to WT (Fig. S2g). Further, there was no difference between *Pst (AvrRpt2)* growth in WT and *swp73b* plants (Fig. S2h). Because *Arabidopsis* SWP73A and SWP73B are associated with the SWI/SNF complex in a

mutually exclusive manner (Vercruyssen et al., 2014), SWP73B mainly regulates plant development and is not involved in plant immunity unless *SWP73A* is knocked out.

To investigate the function of *SWP73A* and avoid the compensatory effect of *SWP73B,* we generated CaMV 35S promoter-driven SWP73A-FLAG overexpression (OE) plants (Fig. 2e and S3a). Compared to WT plants, the SWP73A OE plants had slightly curly leaves (Fig. 2e), and significantly lower expression levels of *RPS2* and *PR1* (Fig. 2f). The SWP73A OE plants also displayed delayed HR and increased bacterial growth after *Pst (AvrRpt2)* infection compared to WT (Fig. 2g and 2h). All these results demonstrate that plant *SWP73A* plays an important role in suppressing ETI to prevent autoimmunity.

SWP73A acts as a transcription suppressor of a set of NLRs and plant immunity related genes

SWP73A is one of the core subunits of the SWI/SNF chromatin remodeling complex (Sacharowski et al., 2015; Vercruyssen et al., 2014). To identify the genes regulated by SWP73A, we conducted expression profiling using the Arabidopsis Affymetrix microarray to identify differentially expressed mRNAs between *swp73a* or SWP73A OE plants and WT. We used a q-value of 0.03 as a cutoff and identified 447 differentially expressed genes in the SWP73A OE versus WT comparison and 9 differentially expressed genes in the *swp73a* versus WT comparison (Table S1). The small number of differentially expressed genes between *swp73a* and WT further indicates the compensatory effects of *SWP73B* in the *swp73a* mutant background. Among the 447 differentially expressed genes between SWP73A OE and WT plants, 427 genes were down-regulated, and 19 genes were up-regulated (Table S1), which suggested that

SWP73A may mostly act as a transcriptional suppressor. Gene ontology (GO) term enrichment analysis showed that 79 genes (18.5%) downregulated by SWP73A were related to biotic stress responses, including plant immune responses, defense responses to bacterium, systemic acquired resistance and the jasmonic acid biosynthetic process (Fig. S3b). Among them, seven NLRs represented 1.6% of the down-regulated genes (Table S1). In comparison, biotic stress-related genes and NLR genes only represent 3.7% and 0.5% of total genes in the *Arabidopsis* genome, respectively (Mondragon-Palomino and Gaut, 2005), making biotic stress-related genes five-fold enriched and NLRs 3.2 fold enriched in the SWP73A down-regulated genes list. In addition to *RPS2*, the expression levels of four more NLRs, *RPS4, RRS1*, *ZAR1,* and *RPP1-like,* were experimentally validated by RT-PCR in the SWP73A OE plants and the *swp73* single and double mutants (Fig. 3a). All four NLRs exhibit elevated transcript levels in *swp73a* and *amiRswp73a/b* plants, and reduced expression levels in SWP73A OE lines, confirming that SWP73A suppresses the expression of a set of NLRs. We also checked another NLR gene, *RPM1* (Grant et al., 1995; Lolle et al., 2020), whose response pathway shares some common upstream and downstream components with the signal transduction pathway of *RPS2*. The expression level of *RPM1* had no significant difference among *swp73a*, WT and the SWP73A overexpression lines (Fig. S3c), which is consistent with the microarray data. The *swp73a* single mutant, *swp73a/b* double mutant and SWP73A OE lines showed similar levels of bacterial growth as wild-type plants upon infection of *Pst (AvrRpm1)* (Fig. S3d). These results support that expression of *RPM1* is not regulated by *SWP73A*. Further, sRNA-mediated regulation of *SWP73A* was not altered after *Pst (AvrRpm1)* infection because miR3440 and siRNA-SWP73A were not induced by *Pst (AvrRpm1)* (Fig. S3e).

RPS4 and *RRS1* are a divergent pair of NLRs that share the same promoter region. The RPS4 and RRS1 proteins form a complex that recognizes the bacterial effector AvrRps4 and triggers ETI (Le Roux et al., 2015; Sarris et al., 2015). We examined the expression of miR3440 and siRNA-SWP73A in response to *Pst (AvrRps4)* infection. Both sRNAs were induced by *Pst (AvrRps4)* but not by *Pst* (*hrcC)* or *Pst* (EV) (Fig. 3b). The expression level of their target gene, *SWP73A*, descended after *Pst (AvrRps4)* infection in a manner that corresponded to the time course of the two gradually increasing sRNAs (Fig. 3c, d). Upon infection by *Pst (AvrRps4),* both the single and double mutants, *swp73a* and *amiRswp73a/b,* showed accelerated HR, whereas SWP73A OE plants displayed a delayed HR compared with WT (Fig. 3e and f). As expected, SWP73A OE plants were more susceptible to *Pst (AvrRps4),* whereas the *swp73a* and *amiRswp73a/b* mutants were more resistant (Fig. 3g). Taken together, these results demonstrate that SWP73A also negatively regulates RPS4 and RRS1-mediated ETI.

SWP73A directly binds to the promoters of a group of NLRs to suppress their expression

The SWI/SNF complex components regulate gene expression by modulating nucleosome positioning. Transient expression of YFP-tagged SWP73A in *Nb* leaves confirmed the nuclear localization of SWP73A (Fig. 4a). We hypothesize that SWP73A regulates the transcription of NLRs and defense-related genes through direct association with their promoters. To identify the target genes directly regulated by SWP73A, we generated a native antibody against the first 200 residues of SWP73A, a region with low similarity with SWP73B (Fig. S2a). This anti-SWP73A antibody is highly specific to

SWP73A protein as no signal was detected in the *swp73a* mutant (Fig. 4b). Using this anti-SWP73A antibody, we performed chromatin immunoprecipitation-sequencing (CHIP-Seq) in WT plants. We identified a total of 2061 peaks representing the potential SWP73A binding sites. When using a peak calling $q<0.005$ as a cutoff, we found 801 genes with SWP73A binding sites within 1 kb from their transcription start site (TSS) (Table S2). GO enrichment analysis indicates that these SWP73A-binding genes are enriched in biotic stress responses (Fig. S4a). The protein domain enrichment analysis showed that LRR, NB-ARC, and TIR domain are among the top 10 most enriched domains (Fig. S4b). We identified 21 NLRs and 95 biotic stress-related genes that had SWP73A binding peaks in their promoters (Table S2), including *RPS2, ZAR1, RPP1-like* (Fig. 4c and S4c). However, SWP73A was not found to be associated with the promoter region shared by *RPS4* and *RRS1*. We further validated these results using CHIP-qPCR and confirmed that SWP73A was indeed associated with the promoter and TSS regions of *RPS2, ZAR1* and *RPP-like* genes but not *RPS4* and *RRS1* (Fig. 4d, e and S4d). The binding of the *RPS2* promoter with SWP73A was reduced after infection by *Pst (AvrRpt2)* (Fig. 4f), which leads to increased expression of *RPS2* after *Pst(AvrRpt2)* infection (Fig. 2b and S4e). These results demonstrate that SWP73A directly binds to the promoters of *RPS2, ZAR1,* and *RPP1-like* genes to suppress their expression, whereas *RPS4* and *RRS1* might be regulated by SWP73A indirectly.

SWP73A regulates *RPS4* and *RRS1* expression indirectly via CDC5

In order to determine how SWP73A regulates the expression of *RPS4* and *RRS1*, we performed a Supernode network analysis to identify regulatory genes that connect

SWP73A with *RPS4* and *RRS1* via a systems biology platform, VirtualPlant (http://virtualplant.bio.nyu.edu/)(Katari et al., 2010). *CDC5,* an R2R3 MYB transcription regulator and an evolutionary conserved spliceosome-associated protein, was identified as a regulatory hub that links *SWP73A* with *RPS4* and *RRS1* and three additional biotic stress-related genes identified from our expression profiling dataset (Fig. 5a). *CDC5* was identified to be directly regulated by SWP73A in our CHIP-seq analysis (Table S2). Indeed, the *CDC5* transcript was down-regulated in the SWP73A OE plants and up-regulated in both the *swp73a* and *amiRswp73a/b* mutants (Fig. S5a). *CDC5* was reported to regulate the alternative splicing of *RPS4,* which is important for RPS4 activity (Palma et al., 2007; Zhang and Gassmann, 2007). The *cdc5* loss-of-function mutant was more susceptible to *Pst (AvrRps4)* (Fig. S5b). Direct binding of SWP73A to the promoter of *CDC5* was detected by both CHIP-seq and CHIP-qPCR analysis (Fig. 5b, c, and d), and the association was impaired by *Pst (AvrRps4)* infection (Fig. 5e).

Alternative splicing of *RPS4* is a critical regulatory step for producing a functional transcript (Zhang and Gassmann, 2007). The dominant functional transcript, TV3, is generated from transcript TV2A by excision of intron III (Fig. S5c). Since SWP73A suppresses the expression of *CDC5,* SWP73A likely regulates the alternative splicing of *RPS4* indirectly through *CDC5*. We examined the transcript levels of TV2A and TV3 in WT and SWP73A OE plants before and after *Pst (AvrRPS4)* infection. The transcription level of TV2A was reduced in WT but not in SWP73A OE plants after *Pst (AvrRPS4)* infection (Fig. S5d). *RRS1* also undergoes alternative splicing and generates two transcript variants of *RRS1*, a full-length functional *RRS1.1,* and a truncated *RRS1.2. RRS1.2* retains the 5th intron and translates a truncated protein without the WRKY domain (Fig. S5c)

(Noutoshi et al., 2005). After *Pst (AvrRPS4)* infection, *RRS1.2* expression was decreased in WT but remained in SWP73A OE plants. On the contrary, the functional *RPS4* TV3 and RRS1.1 transcripts were induced after *Pst (AvrRPS4)* infection in WT, but not in the SWP73A OE plants (Fig. S5e). Thus, these results demonstrate that SWP73A suppresses *RPS4* and *RRS1* expression by regulating their alternative splicing via *CDC5*.

SWP73A associates with histone marker H3K9me2

Previous CHIP-PCR analysis found that, in the *SWP73B* silencing mutants, the H3K27me3 histone markers on the *FLOWERING LOCUS C* promoter were decreased whereas the H3K9AC histone markers increased (Jegu et al., 2014). From the UCSC Genome Browser of histone marker CHIP-seq database (Karolchik et al., 2014; Stroud et al., 2012) of *Arabidopsis*, we found that *RPS2* and *CDC5* both have H3K9me2 markers associated with their promoter regions. H3K27me3 and H3K9me2 are transcriptional repression markers in plants (Pfluger and Wagner, 2007; Rosenfeld et al., 2009). H3K9me2 tends to span the entire gene and is correlated with low expression levels (West et al., 2014; Zhou et al., 2010). Co-immunoprecipitation (co-IP) analysis showed that SWP73A was associated with H3K9me2 but not with H3K9Ac (Fig. 6a). The promoter regions of *RPS2* and *CDC5,* which are associated with SWP73A (Fig. 6b), were also associated with H3K9me2 but not with H3K27me3 (Fig 6c, S6a and S6b). The promoter regions of *RPP1-like* and *ZAR1* were also associated with H3K9me2 (Fig. S6c). In *Arabidopsis*, H3K9me2 is mainly established by *Su(var)3–9* family histone methyltransferases, *SUVH4/KYP*, *SUVH5*, and *SUVH6,* which are likely to be functionally redundant (Ebbs et al., 2005; Ebbs and Bender, 2006). To genetically examine whether SWP73A-mediated suppression of

gene expression is associated with the histone repression marker H3K9me2, we performed CHIP-qPCR analysis on the triple mutant *suvh456,* which has impaired H3K9me2. The association between SWP73A and the *RPS2* or *CDC5* promoter was abolished or largely reduced in the *suvh456* mutant (Fig. 6b and c), suggesting that SWP73A binds to chromatin in an H3K9me2-dependent manner. The level of H3K9me2 on promoters of *RPS2* and *CDC5* was significantly reduced after infection by *Pst (AvrRpt2)* and *Pst (AvrRps4),* respectively (Fig. 6d), which reduced SWP73A association and activated the expression of *RPS2* and *CDC5*. In summary, the repression of *RPS2* and *CDC5* by SWP73A through H3K9me2 is abolished or largely reduced after pathogen infection in order to promote gene transcription and activate plant innate immunity.

Discussion

Precise control of NLR expression and homeostasis is essential for plant immune responses. Over-accumulation/activation of NLRs typically causes autoimmunity (McDowell and Simon, 2006; Todesco et al., 2010), whereas insufficient NLR expression can result in higher susceptibility to diseases. Here, we identified a chromatin-remodeling protein, SWP73A, which acts as a transcription suppressor to prevent NLR over-accumulation through both direct and indirect regulation. Upon infection by avirulent bacteria, *SWP73A* is silenced by two bacterial-induced sRNAs, which leads to the transcription of NLRs, triggering a robust immune response ETI. Furthermore, we discovered that the expression of the other *Arabidopsis* SWP73/BAF60 variant *SWP73B* was elevated in *swp73a* plants and showed a compensatory effect on the function of *SWP73A* in *swp73a* to avoid over-accumulation of NLRs. This suggests that the

compensatory effect of *SWP73B* in *swp73a* mutant could be a mechanism to protect the plant from autoimmunity when there is no pathogen challenge to ensure normal development.

In mammalian immune responses, BAF60 is a transcriptional co-activator and activates the promoters of pro-inflammatory genes in mouse macrophages during innate immune responses against viral or bacterial infection (Tartey et al., 2014). The mammalian SWI/SNF complex "reads" or "shapes" the chromatin landscape in order to epigenetically regulate target genes involved in the transition from myoblasts to myotubes and cardiac development (Gillette and Hill, 2015; Lange et al., 2008). On the contrary, plant BAF60/SWP73 acts mainly as a transcription repressor (Jegu et al., 2014). Here, we discovered that SWP73A associates with the repression marker H3K9me2 and may act as an H3K9me2 reader to potentiate its suppression function on NLRs and other defense signaling proteins. Our work has revealed a new layer of precise regulation of NLRs - epigenetic control at the chromatin level to ensure rapid induction of NLRs only upon bacterial infection and avoid autoimmunity when bacteria are absent.

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AUTHOR CONTRIBUTIONS

H.J. conceived the idea, designed and supervised the project. C.H. and D.S.R. planned and carried out most of the experiments. X.Q. generated SWP overexpression lines and performed the analysis. C.H. and R.L. performed the bioinformatics analysis with the support from Z.J. and X.C.. C.H. wrote the manuscript, H.J. and D.S.R. revised the manuscript, X.C. and Z.J. edited the paper.

Conflict of Interest: None of the co-authors have a conflict of interest to declare.

Figure Legend

Figure 1. SWP73A-targeting sRNAs, miR3440 and siRNA-SWP73A, are induced by *Pst(AvrRpt2)* infection.

a. Northern blot analysis of miR3440 and siRNA-SWP73A in *Pst (Empty-vector)*, *hrcC Pst*, and *Pst (AvrRpt2)* infected *Arabidopsis* at 14 hours post infection (hpi). Buffer inoculation was used as a mock control. U6 is the loading control. The relative abundance (RA) of the small RNAs detected is labeled under the U6 panel.

b. Expression level of *SWP73A* in *Arabidopsis* at various time points post *Pst(AvrRpt2)* infection analyzed by qRT-PCR and normalized to *Actin2.*

c. Northern blot analysis of miR3440 and siRNA-SWP73A in *Arabidopsis* infected by *Pst (AvrRpt2)* in a time course corresponding to panel b. U6 is the loading control. The RA of the small RNAs detected is labeled under the U6 panel.

Figure 2. SWP73A suppresses *Arabidopsis* defense response against *Pst(AvrPpt2).*

a. Morphological phenotype of SWP73A T-DNA insertion knockout mutant, *swp73a* and the *amiRswp73a/b* knockdown double mutant.

b. The expression levels of *RPS2* and *PR1* in *swp73* were analyzed by qRT-PCR and normalized to *Actin2* gene. Significant difference is indicated by * ($p < 0.01$; ANOVA Dunnett's multiple comparisons test to WT).

c. Accelerated HR response induced by *Pst (AvrPpt2)* in *swp73a* and *amiRswp73a/b*. Buffer inoculation was used as mock control.

d. The SWP73A mutants displayed enhanced resistance to *Pst (AvrPpt2).* Bacterial growth assay was performed at 0-, and 3-days post pathogen inoculation. Data are means ± SE.

Different letters indicate a significant difference between groups ($p < 0.01$; ANOVA Dunnett's multiple comparisons test to WT).

e. Morphological phenotype of SWP73A overexpressed plants (SWP73A OE).

f. The expression levels of *RPS2* and *PR1* in SWP73A OE plants compared to WT were analyzed by qRT-PCR and normalized to *Actin2*. Significant difference is indicated by * ($p < 0.05$; ANOVA Dunnett's multiple comparisons test to WT).

g. Delayed HR response upon *Pst (AvrPpt2)* infection was observed in the SWP73A OE plants. Buffer inoculation was used as a mock control.

h. The SWP73A OE plants are more susceptible to *Pst (AvrRpt2)*. Significant difference is indicated by * ($p < 0.01$; ANOVA Dunnett's multiple comparisons test to WT).

Figure 3. SWP73A suppresses the expression of a group of NLRs and negatively regulates *Arabidopsis* defense response against *Pst(AvrRps4).*

a. Verification of differentially expressed NLRs identified by microarray analysis by qRT-PCR. Expression level of *RPS4, RRS1 ZAR1,* and *RPP1-like* were analyzed by qRT-PCR and normalized to *Actin2*. Significant difference is indicated by * ($p < 0.01$; ANOVA Dunnett's multiple comparisons test to WT).

b. Northern blot analysis of miR3440 and siRNA-SWP73A in *Pst (Empty-vector, EV)*, *hrcC Pst*, and *Pst (AvrRpt2)* infected *Arabidopsis* at 20 hours post infection (hpi). Buffer inoculation was used as a mock control. U6 is the loading control. The relative abundance (RA) of the small RNAs detected is labeled under the U6 panel.

c. Expression level of *SWP73A* in *Arabidopsis* at various time points post infection by *Pst (AvrRpt2)* analyzed by qRT-PCR and normalized to *Actin2.*

d. Northern blot analysis of miR3440 and siRNA-SWP73A with *Arabidopsis* infected by *Pst (AvrRpt2)* in a time course corresponding to panel b. U6 is the loading control. The RA of the small RNA detected is labeled under the U6 panel.

e and f. HR response induced by *Pst (AvrRps4)* in *SWP73* mutants and SWP73 OE plants. Buffer inoculation was used as a mock control.

g. *Pst(AvrRps4)* growth assay on SWP73 mutants. Data are means ± SE. Significant difference is indicated by * ($p < 0.05$) or **($p < 0.01$; ANOVA Dunnett's multiple comparisons test to WT).

Figure 4. SWP73A suppresses the expression of *RPS2* by promoter and TSS occupation but does not regulate *RPS4* and *RRS1*.

a. YFP-SWP73A is co-localized with the DAPI stained nucleus in an *N. benthamiana* transient expression assay.

b. SWP73A was detected by anti-swp73A antibody in the nuclei isolated from *swp73a* and WT plants mutants. H3K4me3 was used as a loading control.

c. The promoter and TSS regions of three NLR genes, *RPS2, RPS4* and *RRS1*, were shown to associate with SWP73A by CHIP-seq analysis.

d. Diagrams show the promoter region of *RPS2* and *RRS1-RPS4*. The solid lines and gray boxes indicate the promoter and CDS regions, respectively. ATG indicates the start codon, and TSS represents the transcription start site. Arrows represent the primer sets for CHIP-PCR amplification. *RPS2* and *RRS1-RPS4* promoter regions, I and II, associated with SWP73A were analyzed with CHIP-qPCR in panel e.

e. CHIP-qPCR analyses were performed with anti-SWP73A antibody in WT or anti-FLAG

antibody in SWP73A OE plants with anti-IgG as a negative control. Significant difference is indicated by * ($P < 0.05$; T-test). The agarose gel analysis of CHIP-PCR amplified bands is shown in Fig. S6d.

f. CHIP-qPCR analysis of *RPS2* and *RPS4* promoter cross-linked with SWP73A in WT plants infected with *Pst (AvrRpt2)* (12hpi) or *Pst(AvrRps4)* (20hpi). Buffer inoculation was used as mock control. Significant difference is indicated by * ($P < 0.05$; T-test). The agarose gel analysis of CHIP-PCR amplified bands is shown in Fig. S6f.

Figure 5. SWP73A regulates *CDC5* by directly binding to its promoter region.

a. Supernode network analysis on SWP73A using the systems biology platform VirtualPlant (http://virtualplant.bio.nyu.edu/). *CDC5,* an R2R3 Myb transcription regulator, is regulated by SWP73A. The genes regulated by CDC5 and suppressed by SWP73A (Table S1) are represented by orange nodes. The biotic stress response genes are shown in red.

b. The promoter and TSS region of *CDC5* was found associated with SWP73A by CHIP-seq analysis.

c. Diagrams show the promoter region of *CDC5*. *CDC5* promoter regions, I and II, associated with SWP73A were analyzed by CHIP-qPCR in panel d.

d and e. The *CDC5* promoter was cross-linked with SWP73A in WT with or without *Pst (AvrRps4)* (20hpi) infection. Buffer inoculation was used as a mock control. CHIP was performed using the anti-SWP73A antibody with anti-IgG as a negative control. Significant differences are indicated by * (p-value < 0.05; T-tests). The agarose gel analysis of CHIP-PCR amplified bands is shown in Fig. S17.

Figure 6. SWP73A is associated with the histone marker H3K9me2.

a. SWP73A was found to be associated with H3k9me2 histone markers but not H3K9Ac histone markers by co-IP. Anti-AGO2 antibody was used as a negative control.

b and c. CHIP-qPCR analysis of *RPS2* and *CDC5* promoters cross-linked with SWP73A (**b**) and H3K9me2 (**c**) in WT vs. *suvh456.* Anti-IgG was used as a negative control. The agarose gel analysis of CHIP-PCR amplified bands is shown in Fig. S6e.

d. CHIP-qPCR analysis of *RPS2* and *CDC5* promoters cross-linked with H3K9me2 in WT infected by *Pst (AvrRpt2)* (12hpi) or *Pst (AvrRps4)* (20hpi) infection. Buffer inoculation was used as a mock control. Anti-IgG was used as a negative control for CHIP analysis. Significant difference was indicated by * in b, c and d (p-value < 0.05; T-tests). The agarose gel analysis of CHIP-PCR amplified bands is shown in Fig. S6f and S6g.

STAR METHODS

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KEY RESOURCES TABLE (uploaded separately)

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for resources and reagents should be addressed to the Lead Contact, Hailing Jin (hailingj@ucr.edu)

Materials Availability

All plasmids and plant lines generated in this study are available from the Lead Contact with a completed Materials Transfer Agreement.

Data and Code Availability

Microarray data of SWP73 OE plants compared to WT are available in ELIXIR Core Data Resources with ArrayExpress accession E-MTAB-9308 (https://www.ebi.ac.uk/fg/annotare/). CHIP-Seq data of SWP73A are available in the National Center for Biotechnology Information Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra) (SRA): PRJNA642248.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Plant Model

All Arabidopsis thaliana genotypes used in this study were in the Columbia wild-type (Col-0, N60000) background. Full information on all genotypes were included in Key Resources Table.

Arabidopsis mutants were used in this study including *swp73a* knockout mutant (CS117257 from NASC stock), *rps2-101c* mutant (Bent et al., 1994), *cdc5* knockout mutant (Palma et al., 2007), *suvh456* triple mutant (Ebbs et al., 2005; Ebbs and Bender, 2006). Wide type of *N. benthamiana* were used for transient expressed assay.

Plant Growth Conditions

Arabidopsis and *N. benthamiana* plants were grown in a growth room under short day conditions with a 12-h light/12-h dark photoperiod at 23±1 °C.

Bacterial Strains

Pseudomonas syringae pv. *tomato* DC3000 bacterial strains were used for analysis including those carrying empty vector pVSP61 (EV) (Innes et al., 1993); pVSP61 plasmid with avirulence gene *AvrRpt2* (Innes et al., 1993), or *AvrRps4* (Hinsch and Staskawicz, 1996); and *hrcC*$^{-}$ strain that has a mutation in its type III secretion system (Yuan and He, 1996).

METHOD DETAILS

Generation of Transgenic Plants

To generate the SWP73A OE plant, the *SWP73A* CDS was cloned into a pEarleyGate (pEG) 202 destination vector by gateway cloning system (Invitrogen). Artificial miRNA

to knockdown *SWP73B* in the *swp73a* mutant was designed according to WMD3-web miRNA Designer (Schwab et al., 2006). The amiRNA fragment was cloned into the pGWB402 destination vector using the gateway cloning system (Invitrogen). *Arabidopsis* plants were transformed using the floral dip method with *Agrobacterium tumefaciens* strain GV3101 carrying the cloned vectors.

Transient Expression Analysis in *N. benthamiana*

Transient co-expression assays were performed by infiltrating 3-week-old *N. benthamiana* plants with *Agrobacterium* GV3101 (OD600=1.0) carrying constructs containing the miR3440 or miR395 precursor (in PEG100) and *Agrobacterium* (OD600=1.0) containing binary vector with insertion of *SWP73A* cDNA (pEG202) or CDS (pEG104). The same amount of leaf tissue was collected 48 hours post infiltration and processed to perform western blotting.

RNA Extraction, Northern Blot, and qRT-PCR Analysis

Total RNA was extracted by TRIzol Reagent (Invitrogen) following the manufacturer's instructions. RNA is separated on a 14% denaturing 8 M urea-PAGE gel then transferred and chemically crosslinked onto a Hybond N+ membrane (GE Healthcare Life Sciences) with *N*-(3-Dimethylaminopropyl)-*N*′(3-Dimethylaminopr hydrochloride. Oligonucleotide probes used for miR3440 and siRNA-SWP73A detection were GAA+GTG+GAT+GGG+CCA+AGA+AAA (Chellappan et al., 2010) and AT+CTTC+TTCA+TCTT+CTTC+TTCT+AG, respectively. Oligonucleotide probes end-labeled with γ^{32}P were used to probe sRNAs and exposed to a phosphor imager screen.

Relative abundance levels between samples were measured by ImageQuant TL 7.0 software (GE Healthcare Life Sciences). For quantification of relative gene expression, cDNA was synthesized by reverse transcription (RT) with Superscript III (Invitrogen) according the manufacturer's instructions. Quantitative real-time RT–PCR was performed with SYBR green dye on a CFX detection system (Bio-Rad). The primers for all experiments are listed in Table S3.

Immunoblot

Plant tissue was ground in liquid nitrogen and total proteins were extracted by 1 × SDS sampling buffer. The protein samples were resolved with a 12% SDS–PAGE gel and transferred onto PVDF membranes in a Tris-Glycine transfer buffer. The membrane was blocked with TBS/0.5% (v/v) Tween 20/3% (w/v) fat-free milk power and immunoblotted with appropriate antibodies: monoclonal mouse anti-FLAG (Sigma-Aldrich, F3165, 1:3,000 dilution); monoclonal mouse anti-α tubulin (Sigma-Aldrich, T6074, 1:3,000 dilution); polyclonal rabbit anti-SWP73A (serum containing polyclonal antibodies was produced in rabbits immunized with peptide containing the first 200 amino acids of the SWP73A protein, AbMax Biotechnology Co., Ltd., 1:1,000 dilution); goat anti-mouse IgG-HRP (Santa Cruz Biotechnology, sc-2005, 1:3,000 dilution); and goat anti-rabbit IgG-HRP (Santa Cruz Biotechnology, sc-2030. 1:3,000 dilution). Enhanced chemiluminescence (ECL) reagents (Amersham) were used for detection. Relative abundance levels between samples were measured by ImageJ (Schneider et al., 2012).

***Pst* Growth Assay**

For bacterial growth assays, 4-week-old *Arabidopsis* plants were infiltrated with a 5×10^5 c.f.u. per ml bacterial suspension by syringe. From each treatment group, 12 leaf discs from 4 plants were collected 3 days post infection. Bacterial titer was determined by counting the colonies on Pseudomonas Agar F (BD Difco) plate with serial dilution and incubation. Three biological repeats were performed with similar results. For infection sample collected for northern and the half leaf HR assay, 1×10^7 c.f.u. per ml bacterial suspension was used. A total of 12 leaves were inoculated for the half leaf HR assay and monitored for the appearance of HR symptoms.

Nuclear Extraction and Immunoprecipitation

Ten grams of three week old leaf tissue was ground to a fine powder in liquid nitrogen and homogenized in lysis buffer (20 mm Tris-HCl, pH 7.4, 25% glycerol, 20 mm KCl, 2 mm EDTA, 2.5 mm MgCl2, 250 mm sucrose, Protease Inhibitor Cocktails[sigma, p9599]) at 4°C for 30 mins. The homogenate was sequentially filtered through a 70-μm nylon mesh. The nuclei were pelleted by centrifugation at 1500 ***g*** for 15 min and washed three times with nuclei resuspension buffer (20 mm Tris–HCl, pH 7.4, 25% glycerol, 2.5 mm MgCl2, 0.2% Triton X-100) at 4°C. The nuclei were then resuspended in 2 ml IP binding buffer (20 mm Tris-HCl pH 7.5, 150 mM NaCl,1% NP-40, 2 mM EDTA, 1 mm DTT and Protease Inhibitor Cocktails [sigma, p9599]) and sonicated with 5-sec on and 10-sec off intervals for 10 times to release nuclear proteins. The nuclear proteins solution was then used to perform western blot analysis or for protein co-immunoprecipitation. 1ml of nuclear proteins solution was pre-cleaned with 20 μl protein A agarose beads (Sigma) and mixed with 5 μl of anti-SWP73A and the other with 5 μl of anti-AGO2 antibody as a negative

control in 4^0C overnight than pulled-down with 20 µl protein A agarose beads by centrifuge in 300g for 5 minutes. The beads were then washed 3 times with washing buffer (20 mm Tris-HCl pH 7.5, 150 mM NaCl, 1 mm DTT, 0.3% Triton X-100, 0.2 mm EDTA, with protease inhibitors). Finally, proteins were eluted with 50 µl of 1XSDS sampling buffer and incubated at 95°C for 10 mins. The co-IP samples were subsequently analyzed with by western blot.

ChIP, ChIP-seq Library Preparation, Sequencing and Data Analysis

Chromatin was isolated from 2g of 3-week-old leaf tissue. Methods for CHIP analysis were adapted from Saleh et al. (2008)(Saleh et al., 2008). After cross-linking to DNA, proteins were subjected to immunoprecipitation using 25 µl of Anti-FLAG M2 Affinity gel (Sigma) or 5 mg anti-SWP73A, anit-H3K9me2 (Abcam, ab1220), or anti-H3k27me3 (Millipore 07-449) antibodies to pull-down with 25 µl protein A Agarose beads (Sigma), according to the manufacturer's protocol. The percentage of input was calculated using the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008). Shearing of chromatin used for preparing ChIP-seq libraries was conducted by Covaris S220 Focused-ultrasonicator (Covaris) and milliTUBE 1 ml AFA Fiber (Covaris) with standard settings that sheared the chromatin around 200bp. The resulting DNA was using for ChIP-seq libraries that were prepared by the NEBNext Ultra II DNA Library Prep Kit (New England Biolabs, cat. no. 7645) and sequenced (single-end read 150-bp) on a HiSeq 4000 machine (Illumina). Quality control of reads was performed with FASTQC. The reads were then mapped onto the TAIR10 assembly with 2-bp mismatch permission by Bowtie (Langmead and Salzberg, 2012). The Broad peak calling function of MACS2 was used to identify the significantly

enriched binding regions (Zhang et al., 2008). Visualization and analysis of genome-wide enrichment profiles were done with IGV (Robinson et al., 2011). Peak annotations including, proximity to genes and overlap on genomic features such as transposons and genes, were assigned using ChIPseeker (Yu et al., 2015).

Microarray Analysis

Microarray data were preprocessed using robust multiarray analysis (RMA) for background adjustment and normalization (Irizarry et al., 2003). Significant Analysis of Microarray (SAM) software was used for differential analysis with 0.03 as FDR cutoff (Tusher et al., 2001).

Subcellular Localization

Three-week-old *N. benthamiana* plants were infiltrated with *Agrobacterium* carrying pEG104 with SWP73A CDS after 48 hpi and staining with DAPI. Subcellular localization of fluorescent-tagged proteins was observed by using Leica SP5 confocal microscopy.

QUANTIFICATION AND STATISTICAL ANALYSISSUPPLEMENTAL INFORMATION

Details of data visualization, sample number and statistical analysis used for each dataset can be found in the corresponding figure legend. All plots display means ±SE. Statistic alanalyses were performed by Prism (https://www.graphpad.com/scientific-software/prism/) with ANOVA Dunnett's multiple comparisons test to WT or with

Student's T-test. P- values from analyses with multiple comparisons were adjusted using methods indicated in figure legends. Significance was indicated by asterisks.

Supplemental Tables:

Table S1: Genes are differentially expressed in SWP73A OE plants by microarray analysis. Related to Figure 3.

Table S2: Identification of SWP73A associated binding site by MACS2 with broad region calling. Related to Figure 4 and 5.

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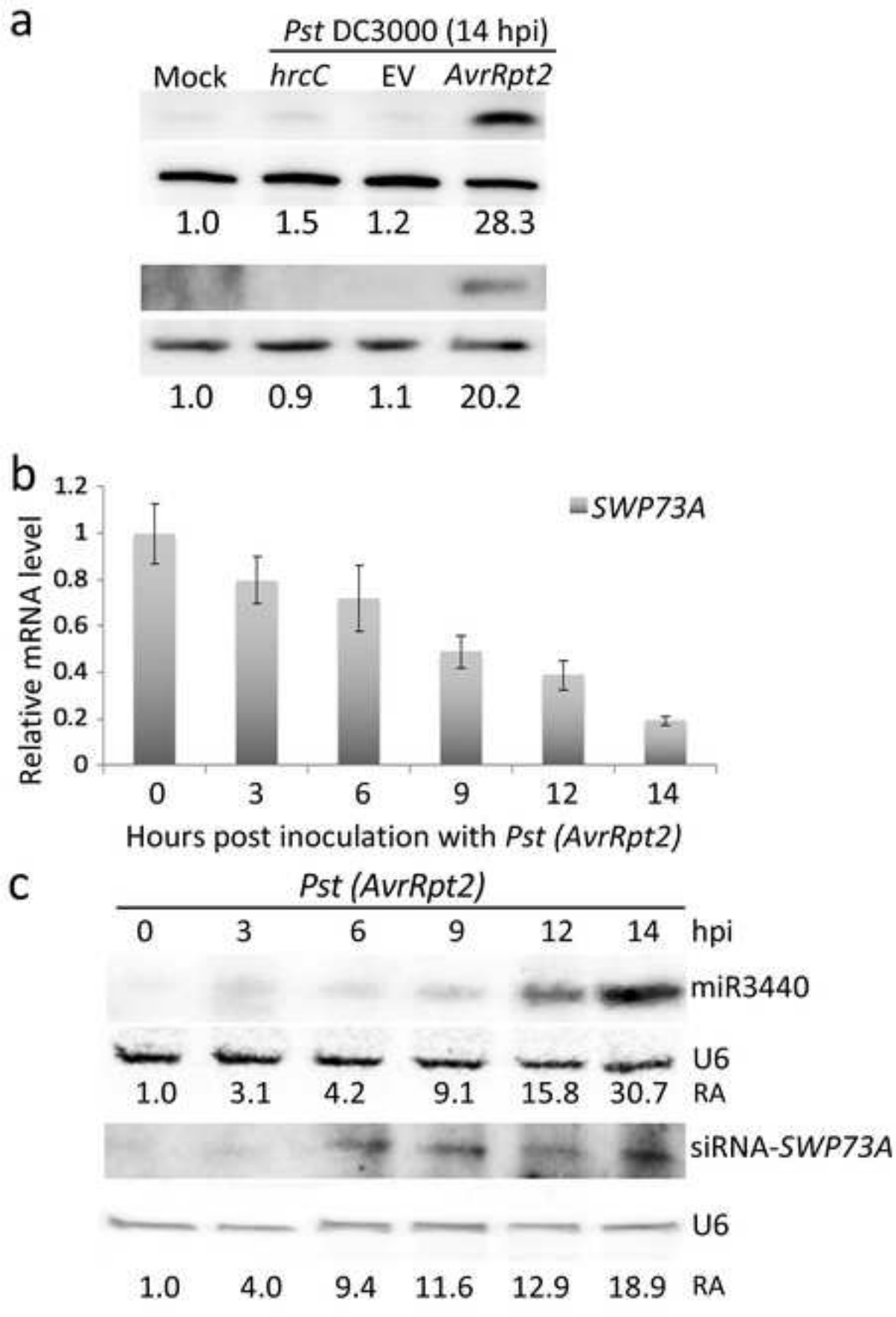
KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit polyclonal anti-AtSWP73A	This paper	N/A
Mouse monoclonal anti-Tubulin	Sigma-Aldrich	Cat# T6074, RRID:AB_477582
Rabbit polyclonal to Histone H3 (tri methyl K4)	Abcam	Cat# ab8580, RRID:AB_306649
Mouse monoclonal to Histone H3 (di methyl K9)	Abcam	Cat# ab1220, RRID:AB_449854
Rabbit Polyclonal to Histone H3 (tri methyl K27)	Millipore	Cat# 07-449, RRID:AB_310624
Rabbit polyclonal to Histone H3 (acetyl K9)	Abcam	Cat# ab10812, RRID:AB_297491
Mouse monoclonal anti-FLAG	Sigma-Aldrich	Cat# F3165, RRID:AB_25952
Goat anti-mouse IgG-HRP	Santa Cruz Biotechnology	Cat# sc-2005, RRID:AB_631736
Goat anti-rabbit IgG-HRP	Santa Cruz Biotechnology	Cat# sc-2030, RRID:AB_631747
Bacterial and Virus Strains		
Pst (EV)	Innes et al., 1993	N/A
Pst (*AvrRpt2)*	Innes et al., 1993	N/A
Pst (*AvrRps4)*	Hinsch and Staskawicz, 1996	N/A
Pst (*hrcC*$^{-}$*)*	Yuan and He, 1996	N/A
Chemicals, Peptides, and Recombinant Proteins		
Protease Inhibitor Cocktails	Sigma-Aldrich	Cat# p9599,
Anti-FLAG M2 Affinity gel	Sigma-Aldrich	Cat# 200-350-383, RRID:AB_10704031
Protein A Agarose	Roche	PROTAA-RO
Critical Commercial Assays		
NEBNext Ultra II DNA Library Prep Kit	New England Biolabs	Cat# 7645
Deposited Data		
Microarray data	This paper	E-MTAB-9308; https://www.ebi.ac.uk/fg/annotare/
CHIP-Seq data		SRA: PRJNA642248; https://www.ncbi.nlm.nih.gov/sra
Experimental Models: Organisms/Strains		
Arabidopsis swp73a knockout mutant	NASC	ID: CS117257
Arabidopsis rps2-101c mutant	Bent et al., 1994	N/A
Arabidopsis cdc5 knockout mutant	Palma et al., 2007	N/A
Arabidopsis suvh456 triple mutant	Ebbs et al., 2005; Ebbs and Bender, 2006).	N/A
Arabidopsis SWP73A overexpression line	This paper	N/A
Arabidopsis AmiRswp73a/b mutant	This paper	N/A
Oligonucleotides		

Primers for this study, see Table S3	This paper	N/A
LNA oligos for siRNA-SWP73A detection: AT+CTTC+TTCA+TCTT+CTTC+TTCT+AG	This paper	N/A
LNA oligos for miR3440 detection: GAA+GTG+GAT+GGG+CCA+AGA+AAA	Chellappan et al., 2010	N/A
Recombinant DNA		
Plasmid: pEG202-SWP73ACDS	This paper	N/A
Plasmid: pEG202-SWP73AcDNA	This paper	N/A
Plasmid: pEG104-SWP73ACDS	This paper	N/A
Plasmid: pEG100-miR3440	This paper	N/A
Plasmid: pGWB402- *AmiRswp73a/b*	This paper	N/A
Software and Algorithms		
Robust multiarray analysis	Irizarry et al., 2003	http://www.molmine.com/magma/loading/rma.htm
Significant Analysis of Microarray	Tusher et al., 2001	https://statweb.stanford.edu/~tibs/SAM/
Bowtie	Langmead and Salzberg, 2012	http://bowtie-bio.sourceforge.net/bowtie2/index.shtml
MACS2	Zhang et al., 2008	https://github.com/macs3-project/MACS
ChIPseeker	Yu et al., 2015	https://guangchuangyu.github.io/software/ChIPseeker/
IGV	Robinson et al., 2011	http://software.broadinstitute.org/software/igv/
ImageJ	Schneider et al., 2012	https://imagej.nih.gov/ij/

Figure 1



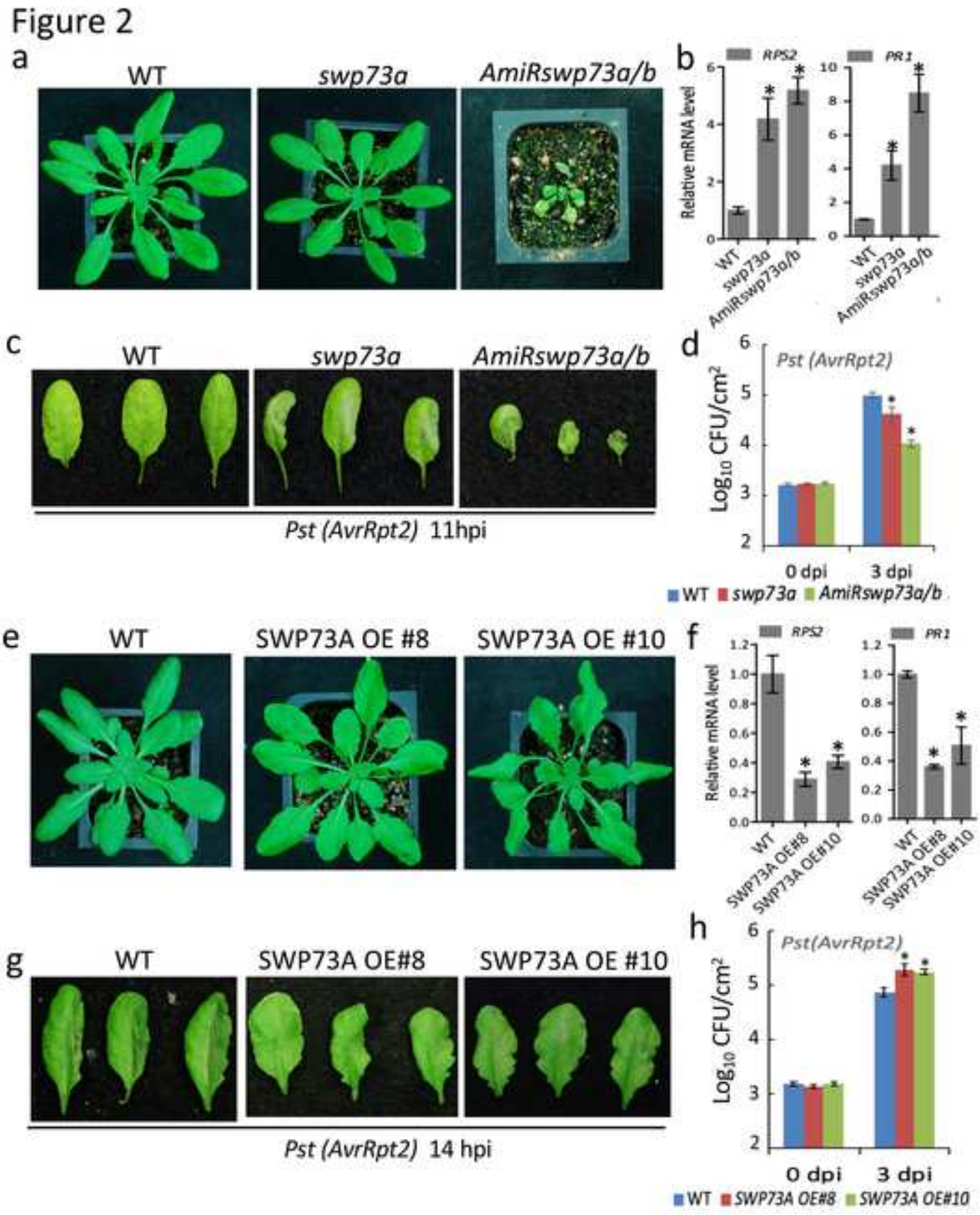
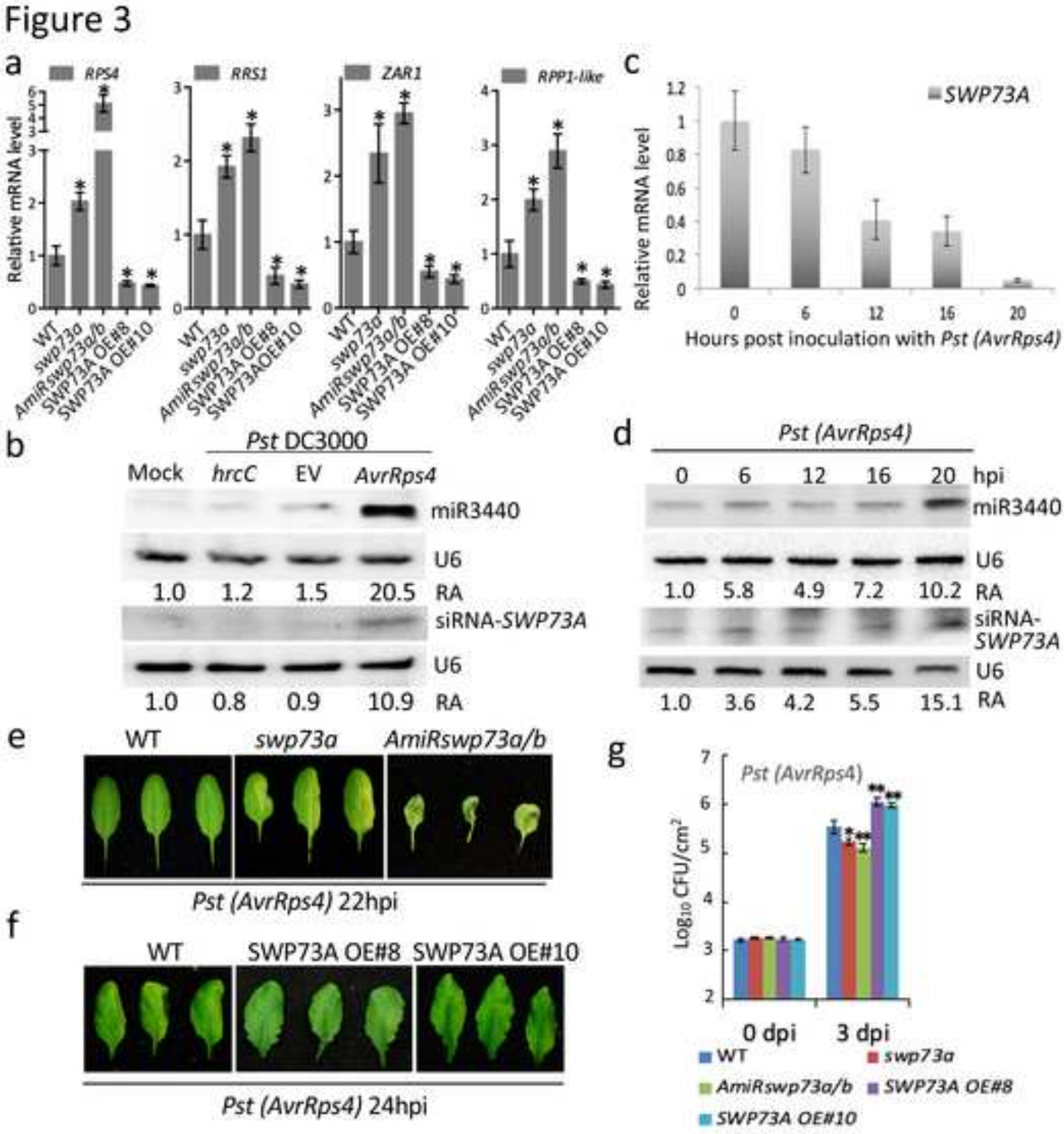


Figure 2
a
WT
swp73a
AmiRswp73a/b
b
RPS2
PR1
Relative mRNA level
WT
swp73a
AmiRswp73a/b
c
WT
swp73a
AmiRswp73a/b
Pst (AvrRpt2) 11hpi
d
Pst (AvrRpt2)
Log10 CFU/cm2
0 dpi
3 dpi
WT swp73a AmiRswp73a/b
e
WT
SWP73A OE #8
SWP73A OE #10
f
RPS2
PR1
Relative mRNA level
WT
SWP73A OE#8
SWP73A OE#10
g
WT
SWP73A OE#8
SWP73A OE #10
Pst (AvrRpt2) 14 hpi
h
Pst(AvrRpt2)
Log10 CFU/cm2
0 dpi
3 dpi
WT SWP73A OE#8 SWP73A OE#10



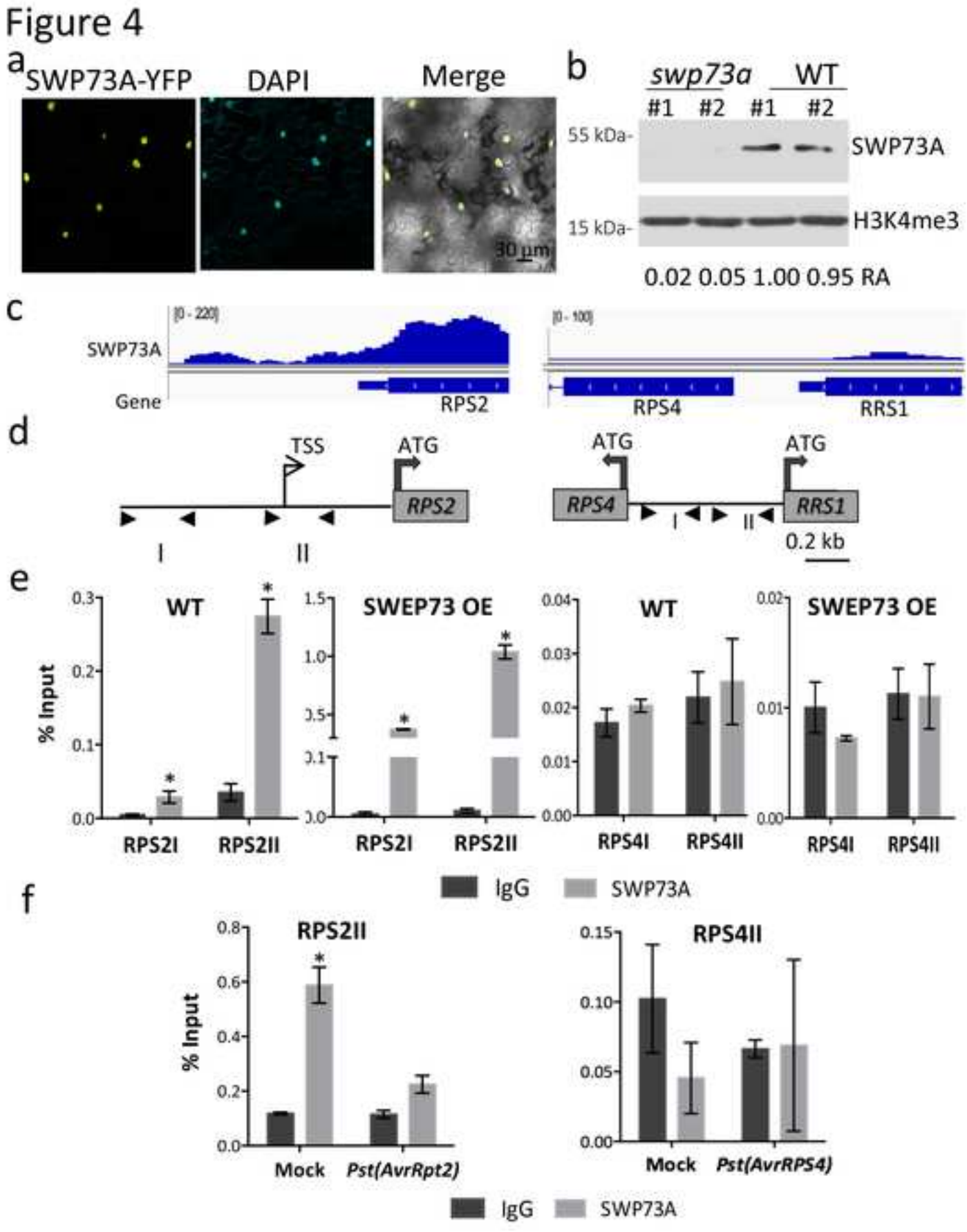


Figure 4
a
SWP73A-YFP
DAPI
Merge
30 μm
b
swp73a
WT
#1 #2 #1 #2
55 kDa-
SWP73A
15 kDa-
H3K4me3
0.02 0.05 1.00 0.95 RA
c
SWP73A
Gene
RPS2
RPS4
RRS1
d
TSS
ATG
RPS2
RPS4
RRS1
I
II
0.2 kb
e
WT
SWEP73 OE
% Input
RPS2I
RPS2II
RPS4I
RPS4II
IgG
SWP73A
f
RPS2II
RPS4II
% Input
Mock
Pst(AvrRpt2)
Pst(AvrRPS4)
IgG
SWP73A

Figure 5

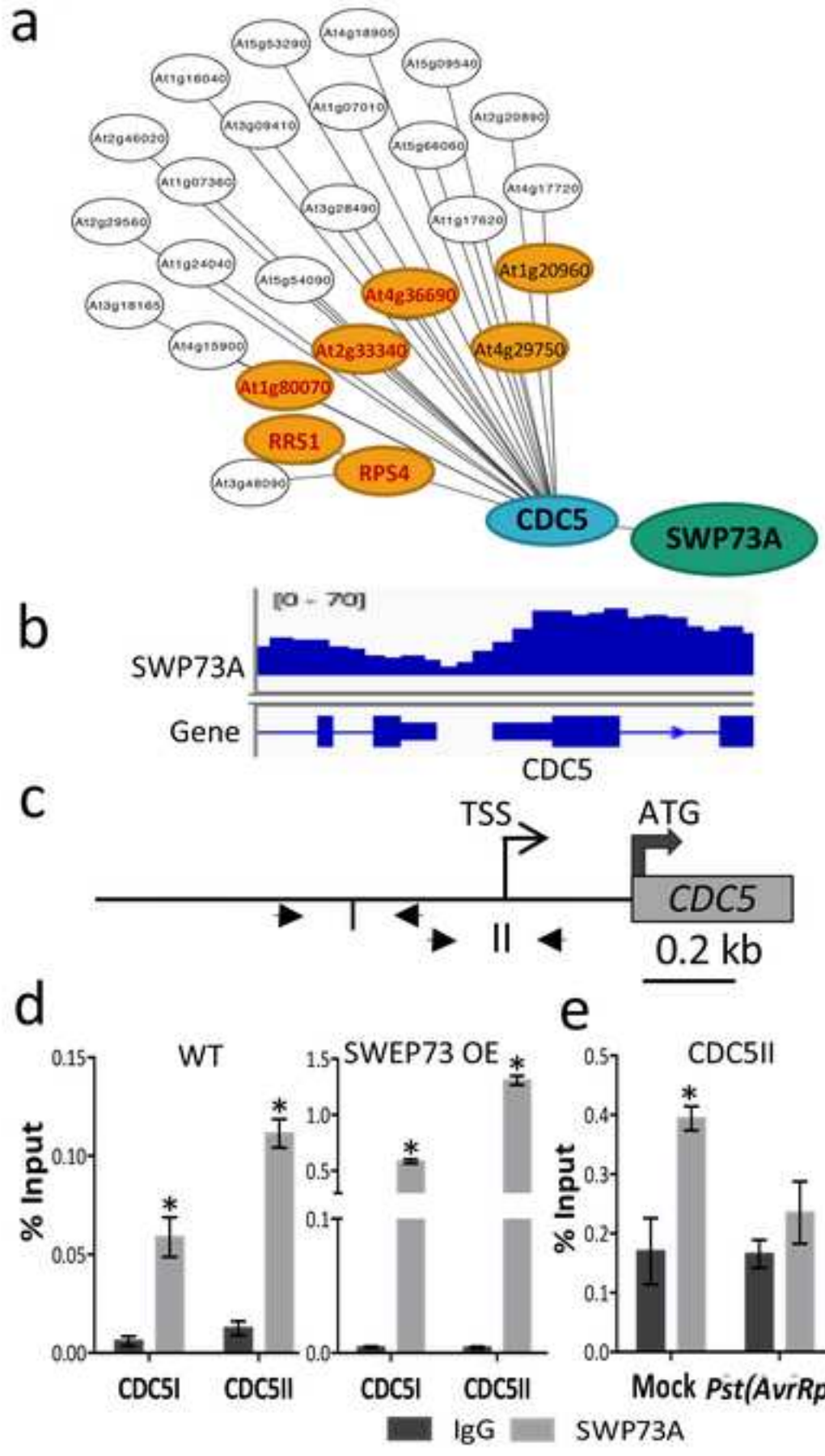


Figure 6

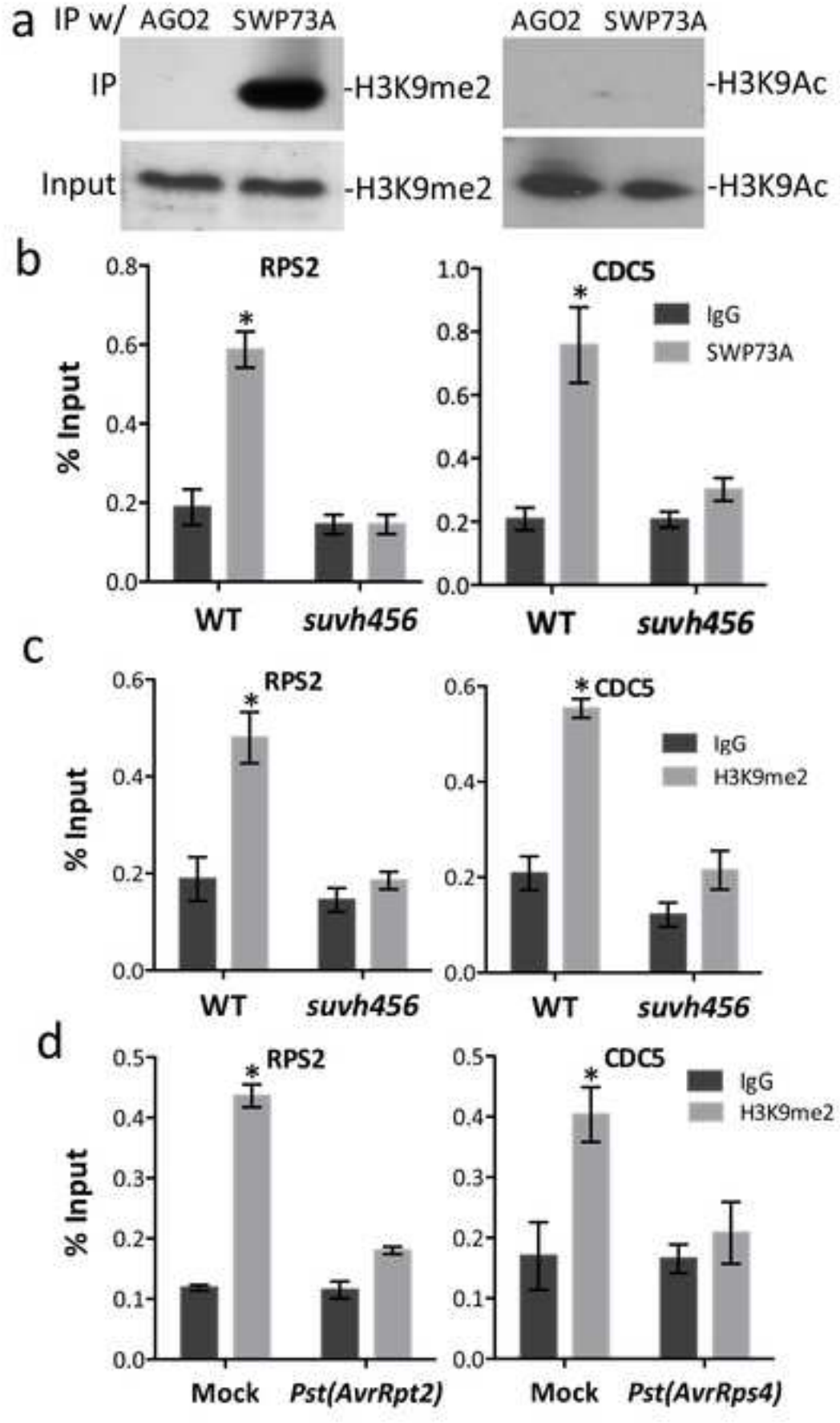


Table S2

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Supplemental Videos and Spreadsheets

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Table S1

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