New Phytologist Supporting Information

Title: HOS15 and HDA9 negatively regulate immunity through histone deacetylation of intracellular immune receptor NLR genes in Arabidopsis

Leiyun Yang, Xiangsong Chen, Zhixue Wang, Qi Sun, Anna Hong, Aiqin Zhang, Xuehua Zhong, Jian Hua

For correspondence:
Jian Hua Tel (+1) 607-255-5554, email jh299@cornell.edu
Xuehua Zhong Tel (+1) 608-316-4421, email xuehua.zhong@wisc.edu

Article acceptance date: 8 December 2019

The following Supporting Information is available for this article

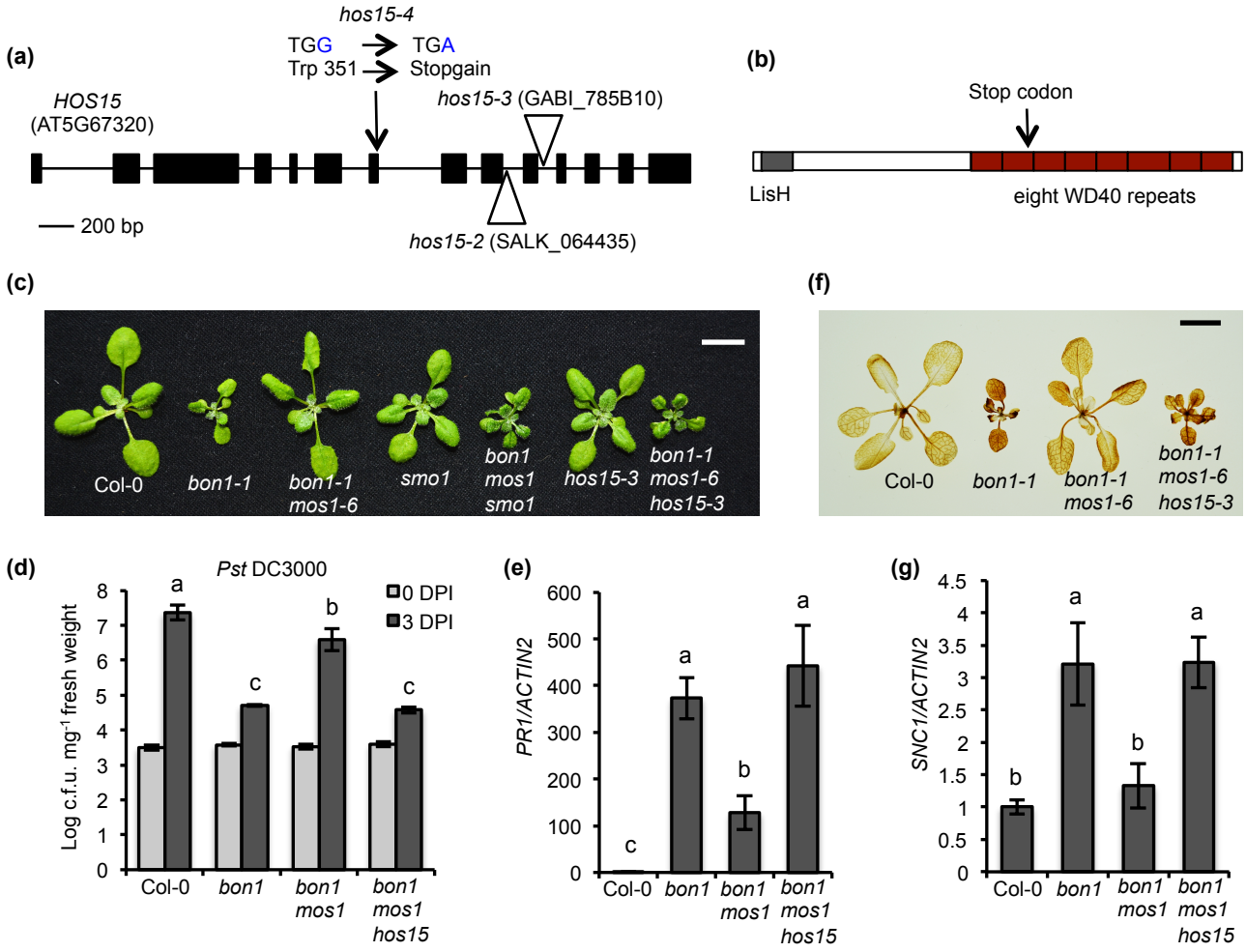

Fig. S1 The *hos15-3* mutant suppressed *bon1 mos1*. (a) Intron-exon structure of *HOS15* gene. The point mutation in *hos15-4* and T-DNA insertion in *hos15-2* and *hos15-3* are indicated. (b) The LisH domain and eight WD40 repeats of HOS15 protein. (c) Morphology of Col-0, *bon1-1*, *bon1-1 mos1-6*, *smo1*, *bon1 mos1 smo1*, *hos15-3*, *bon1-1 mos1-6 hos15-3*. (d) Growth of bacterial pathogen *Pst* DC3000 in Col-0, *bon1-1* , *bon1-1 mos1-6* and *bon1-1 mos1-6 hos15-3*. Statistical analysis was performed with infected plants. (e, g) Analysis of *PR1* (e) and *SNC1* (g) gene expression in Col-0, *bon1-1* , *bon1-1 mos1-6* and *bon1-1 mos1-6 hos15-3 by* qRT-PCR. (f) DAB staining of Col-0, *bon1-1* , *bon1-1 mos1-6* and *bon1-1 mos1-6 hos15-3*. Error bars represent S.D. from three biological replicates for (d), (e) and (g). Different letters indicate significant difference tested by One-way ANOVA/ Duncan ($P < 0.05$). Scale bar for (c) and (f), 1 cm.

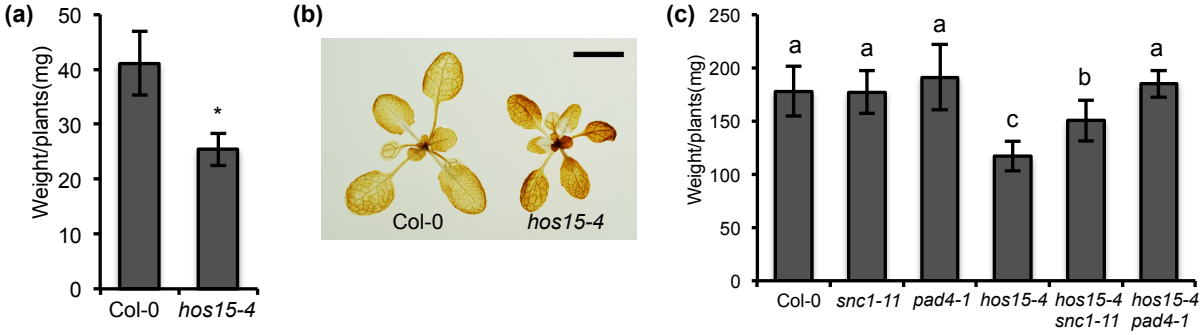

Fig. S2 The *hos15-4* is dwarf and has H_2O_2 accumulation. (a) Weight of Col-0 and *hos15-4*. * indicates significant difference tested by Student's t-test ($P < 0.05$), n >= 15. (b) DAB staining of Col-0 and *hos15-4*. Scale bar, 1 cm. (c) Weight of Col-0, *snc1-11*, *pad4-1*, *hos15-4*, *hos15-4 snc1-11*,and *hos15-4 pad4-1*. Different letters indicate significant difference tested by One-way ANOVA/ Duncan ($P < 0.05$), n >= 14. Error bars in (a) and (c) represent S.D..

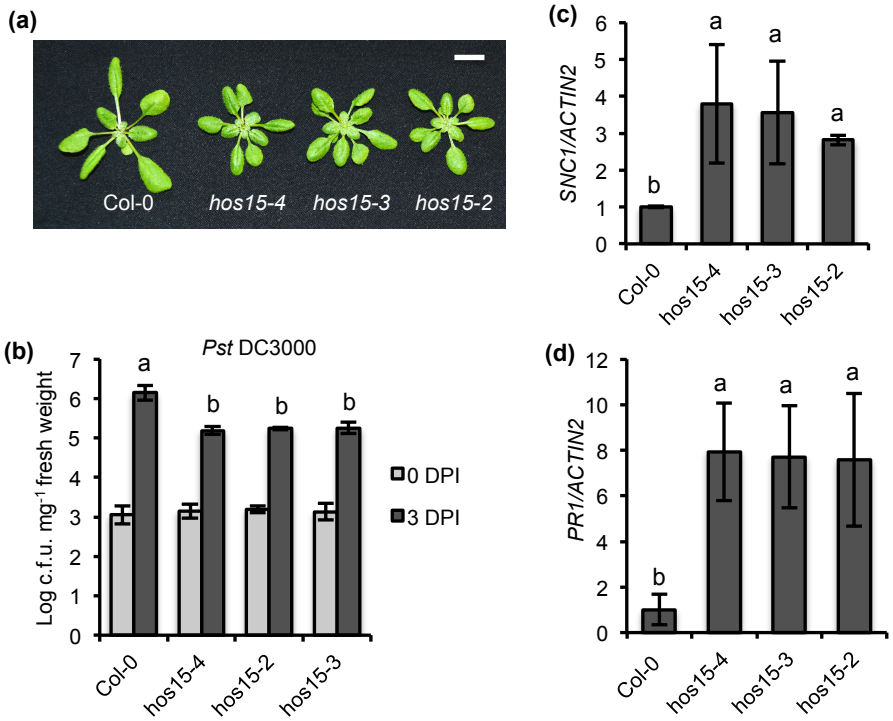

Fig. S3 The *hos15-2*, *hos15-3* and *hos15-4* showed similar growth and immune phenotypes. (a) Morphology of Col-0, *hos15-4*, *hos15-3* and *hos15-2*. Scale bar, 1 cm. (b) Growth of bacterial pathogen *Pst* DC3000 in Col-0, *hos15-4*, *hos15-3* and *hos15-2*. Statistical analysis was performed with infected plants. (c, d) Analysis of *SNC1* (c) and *PR1* (d) gene expression in Col-0, *hos15-4*, *hos15-3* and *hos15-2* by qRT-PCR. Error bars represent S.D. from three biological replicates. Different letters indicate significant difference tested by One-way ANOVA/ Duncan ($P < 0.05$).

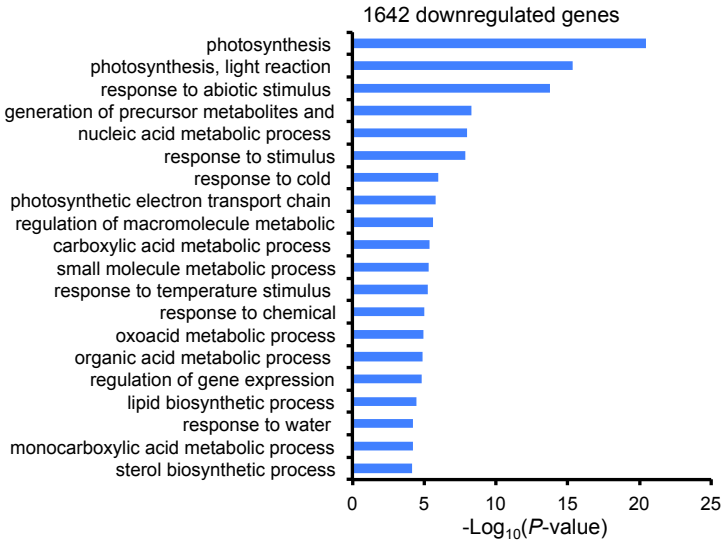

Fig. S4 GO term enrichment analysis of downregulated genes in *hos15-4*. Gene Ontology analysis of significantly downregulated genes (Fold change > 2, FDR < 0.05) using Panther (http://www.pantherdb.org). Shown is the Top 20 most significant groups.

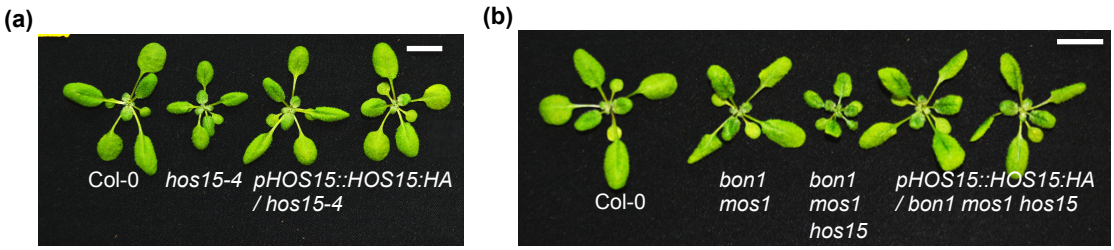

Fig. S5 *pHOS15::HOS15:HA* complements *hos15-4* and *bon1 mos1 hos15*. (a) Morphology of Col-0, *hos15-4*, two independent transgenic lines of *pHOS15::HOS15:HA* in *hos15-4*. (b) Morphology of Col-0, *bon1-1 mos1-6*, *bon1-1 mos1-6 hos15-4* and two independent transgenic lines of *pHOS15::HOS15:HA* in *bon1-1 mos1-6 hos15-4*. Scale bar, 1 cm.

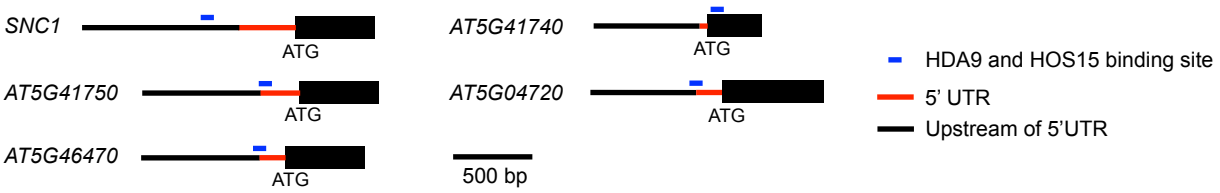

Fig. S6 Predicted WRKY53 binding sites on NLR genes. Approximately 700-1000bp upstream of 5'UTR, 5'UTR and the "ATG" site of NLR genes were shown. The sequence of each NLR genes was extracted from TAIR (https://www.arabidopsis.org).

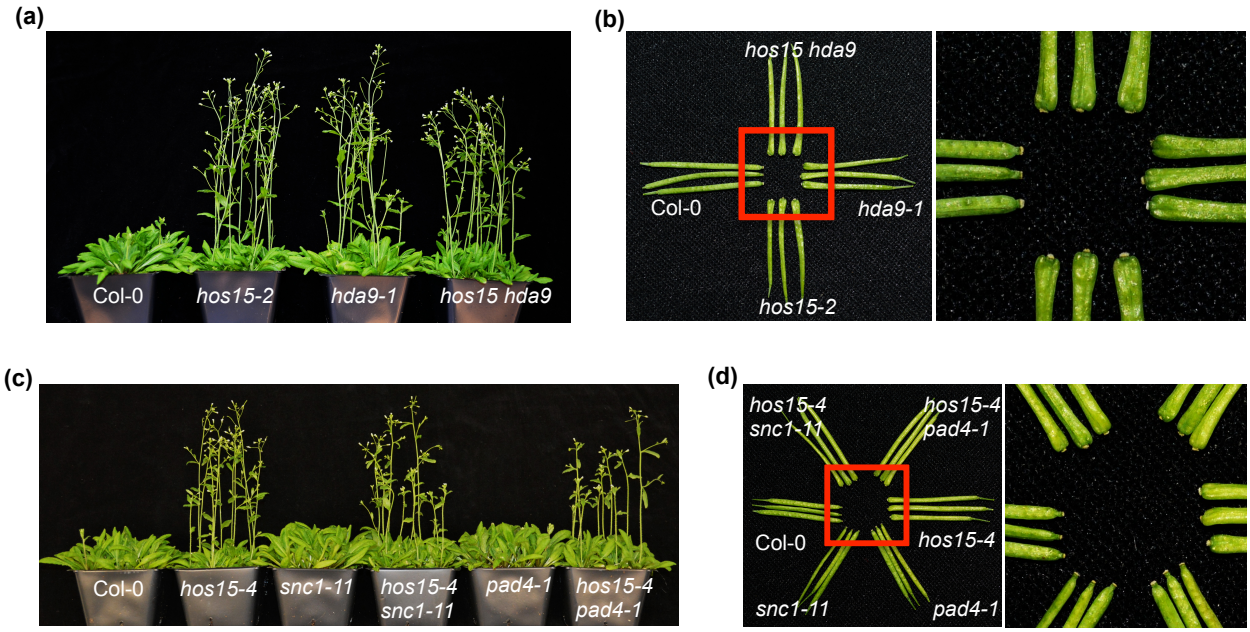

Fig. S7 HOS15 regulates flowering time and silique development independent of its regulation on immunity. (a, b) Early flowering (a) and enlarged silique tips (b) in Col-0, *hos15-2*, *hda9-1* and *hos15-2 hda9-1*. (c, d) Flowering phenotype (c) and enlarged silique tips (d) of Col-0, *hos15-4*, *snc1-11*, *hos15-4 snc1-11*, *pad4-1* and *hos15-4 pad4-1*. The right panel in (b) and (d) is the zoom in of the red square in the left panel.

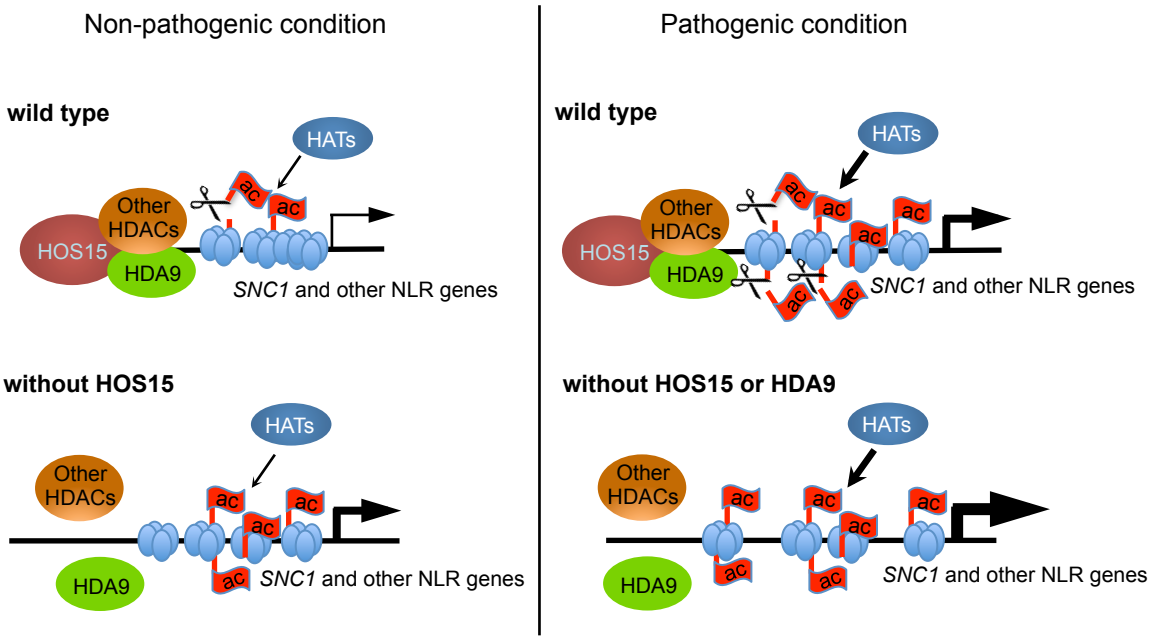

Fig. S8 Working model for HOS15 and HDA9 in plant immunity. In non-pathogenic condition, the expression of *SNC1* and other NLR genes are low due to activities of HOS15 and its associated HDACs. In the absence of HOS15, the NLR genes are expressed at a high level causing autoimmunity. HAD9 may have overlapping function with other HDACs or play no significant role under this normal condition. Under pathogen infection, the expression of *SNC1* and other NLR genes is induced likely due to the increased activity of HATs. HOS15 and HDA9 prevent the over-induction of these genes through deacetylation activities. Without HOS15 or HDA9, the expression of NLR genes is hyper-induced, and the over-activation of immune response might have deleterious consequences on the growth and survival of plants.

Table S1 Primers used in this study.

Primer name	Sequence (5' to 3')	Purpose
HOS15-gDNA F	GTTCCACGTTGATCTTCCGAATC	Complementation analysis
HOS15-gDNA R	GCCACGTCAGAATAAGAAGTG	
HOS15-dCAPs F	CGAGAAGAGCAAGGATGTGAC	Isolating *hos15-4* mutant from F2
HOS15-dCAPs R	GAGTACTTATCAGTTCACCATTTAAAAT	
HOS15pro-F	GGGGACAAGTTTGTACAAAAAAGCAGGCT TCGTTCCACGTTGATCTTCCGAATC	For HOS15-HA tag, BP cloning
HOS15beforeSTP	GGGGACCACTTTGTACAAGAAAGCTGGGT TCATTCTGAAATCAAGAACGCAAACTG	
GABI_785B10 LP	TTCGAATATCCCTCCATTTCC	Genotyping *hos15-3*
GABI_785B10 RP	GCTGTTGTTTGGGACGTAAAG	
SALKseq_064435 LP	AACCGGTTCCCTTCATATCAC	Genotyping *hos15-2*
SALKseq_064435 RP	TTAAGTGGAACAAGAAGGGGG	
SALK_007123 LP	TTCTTGTTGATGATTGGAGCC	Genotyping *hda9-1*
SALK_007123 RP	TTGAAACCGTCCTCACAAATC	
AT5G04720 qRT-F	TGGGTCTCCGTAGACTTTACCA	ChIP-qPCR for HDA9/HOS15 binging on NLR genes that upregulated in *hos15-4*
AT5G04720 qRT-R	GTAAACAAGTAGGTGACGTTGTGG	
AT5G46470 qRT-F	GGCTTGTGTGGTGGTGATTAAG	
AT5G46470 qRT-R	TGGTCCCTAGCAATTGCATCATT	
AT5G41740 qRT-F	CACCATGTCTTTTCGCGCTT	
AT5G41740 qRT-R	AGTCGTGATACCTTTGCTTGC	
AT5G41750 qRT-F	TGGTCCTCGACGACTATAACAA	
AT5G41750 qRT-R	TCGAAGAAATTGGAATGTAACTGGA	
SNC1 qRT-F	TTGAACCACTGACAGCCACA	
SNC1 qRT-R	AGACAGAAACTTCCTCGAGAGC	
PR1 qPCR F	CGAGAAGGCTAACTACAACTACG	qRT-PCR of gene expression
PR1 qPCR R	ACACCTCACTTTGGCACATC	
SNC1 qPCR F	GAATCGAATGTCTCTATCTGC	
SNC1 qPCR R	CTGTAAAGTCGGCGAGCTCA	
AT5G41740 qRT-F2	GCTGAGGGTGACAGTAGCAG	
AT5G41740 qRT-R2	TCCATCTCCATCAAGCTTCCG	
AT5G46470 qRT-F2	GCAGTTGCTCGAGTTTGGTG	
AT5G46470 qRT-R2	AACCGTGAGCATCCACTGAG	
ACT2 qPCR F	CACCACCTGAAAGGAAGTACAG	qRT-PCR internal control
ACT2 qPCR R	TGGACCTGCCTCATCATACT	
SNC1CHIP1-F	GTTTCGTGGCCATCTTGTCA	qRT-PCR for ChIP-H3K9ac at *SNC1*
SNC1CHIP1-R	CTGAGGAATGAGTCACGGACATC	
Spike-in ChIP F	TCCATGACAACTTTGGTATCGTG	Spike-in amplification used in ChIP
Spike-in ChIP R	GGAGCCAGTCTTGGATGAGAAAG	