SUPPLEMENTARY INFORMATION

Title: HOS15 is a Transcriptional Corepressor of NPR1-mediated Gene Activation of Plant Immunity

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Materials and Methods

**Plant Materials and Growth Conditions.** *Arabidopsis thaliana* accession Columbia (Col-0) and C24 were used in this study. The hos15-2 (1) and cul4-1 (2) T-DNA insertion mutants were obtained from NASC (http://arabidopsis.info). The cul1-6 (3) and axr6-1 (4) mutant lines were obtained from ABRC and can be found at TAIR (http://www.arabidopsis.org). The npr1-1 (5), 35S::NPR1-GFP/npr1-1 (6), 35S::npr1S11/15D-GFP and 35S::npr1S11/15A-GFP (7) seeds were kind gifts from Xinnian Dong at Duke University, USA. 35S::Flag-CUL4 (8) was kind gifts from Xingwang Deng at Peking University, China. npr1-1 hos15-2, sid2-2 hos15-2, NahG hos15-2, 35S::NPR1-GFP/hos15-2, 35S::npr1S11/15D-GFP/hos15-2 and 35S::npr1S11/15A-GFP/hos15-2 were generated by genetic crossing. Genotypes were determined by genomic DNA PCR. The primers used in genotyping PCR are listed in supplemental table 4.

For plants growth, all seeds were sterilized with 5% sodium hypochlorite solution (Yakuri pure chemicals) or 20% clorox regular liquid bleach (Clorox) for 5 min and washed with sterilized water for 5 times. After stratification at 4 °C for 3 days, seeds were sown on 1/2 Murashige and Skoog salts (Sigma) plates or on soil pots. For adult plants, 2-week-old MS plates or soil pots grown seedlings plants were transferred to plants growth flats and continuous grown in growth room at 24°C/17°C under 8h light/16h dark cycle. Chemical treatments were performed as described in each experiment, see figure legend. For CHX or MG132 treatment, seedling plants were transferred to 1/2 MS liquid medium containing 100 μM CHX or 50 μM MG132 and incubated for the indicated times. *N. benthamiana* was grown in growth chamber at 23 °C under 16h light/8h dark cycle.

**Plasmid Construction.** The plasmid constructs used in this study are listed in supplemental table 3. The full-length indicated genes CDS were amplified by PCR using specific primers from Arabidopsis ecotype Col-0 cDNA. For luciferase complementation imaging (LCI) assays, full-length of HOS15, NPR1, ASK1, AKS2 and CUL1 CDS were cloned into pDONR zeo vector by in vitro recombination using Gateway BP reaction (invitrogen), then cloned in-frame to pCAMBIA13000-GWCLuc, pCAMBIA1300-GWNLuc, pCAMBIA1300-CLucGW or pCAMBIA1300-NLucGW vectors by in vitro recombination using Gateway LR reaction (invitrogen). For cloning TGA2-NLuc, SARD1-NLuc, CBP60g-NLuc, SNI1-NLuc, WRKY46-NLuc, CBNAC-NLuc, HDA6-NLuc and HDA19-NLuc, the coding sequences of indicated genes were cloned into T-blunt vector (SolGent) according to the manufacturer’s instructions, then cloned in-frame to pCAMBIA1300-NLuc. For Co-IP assays, the pDONRzeo-HOS15, NPR1, ASK1, ASK2 and
CUL1 entry clones were used to generate HOS15-Flag, HOS15-3xHA, NPR1-Flag, NPR1-GFP, ASK1-3xHA, ASK2-3xHA and CUL1-3xHA plasmid constructs by in vitro recombination using Gateway LR reaction (Invitrogen) with pGWB11, pGWB14 or pK7FWG destination vectors. The specific primers used for plasmid constructions are listed in supplemental table 4.

RNA Extraction and Quantitative PCR Analysis. Total mRNA was extracted using the RNeasy plant Mini kit (Qiagen) and reverse transcribed using SuperScriptⅡ reverse transcriptase (Invitrogen) or Reverse Transcription System (Promega). Quantitative PCR was performed using iQ SYBR Green Supermix (Bio-Rad) according to instructions with the CFX96 or CFX384 real-time PCR detection system (Bio-Rad). The relative expression levels were calculated using the comparative cycle threshold method. The sequences of primers used in qRT-PCR are listed in supplemental table 4.

Bacterial Growth Assay. The wild type Pseudomonas syringae pv. tomato DC3000 were used in this study. Short-day grown four- to five-week-old plant leaves were infiltrated with a suspensions of 0.5x10^5 colony forming units (CFU)/mL (OD_{600} = 0.0001) of Pst DC3000 using a 1 cc needleless syringe. After infiltration, the bacterial suspensions on the leaves were removed and allowed to dry for 3 hrs. Then the plants were returned to the growth room kept in 100% humidity by covering with a clear dome for 1day. After 4 days, a cork borer No.3 (0.282cm^2) were used to collect leaf discs, nine leaf discs were collected and separated into three tubes (each tube three leaf discs). Leaf discs were ground in 10 mM MgCl_2 using TissueLyser (Qiagen) and serially diluted in a 96-well plate to measure bacterial numbers. The serially diluted bacterial suspensions were plated on King’s B plates using a replicator for 96-well plate. The bacterial colonies grown on the plates were counted and performed statistical analysis. P values were calculated by One-way ANOVA with Tukey’s HSD. All growth assays were conducted at least three independent biological replicates.

Luciferase Complementation Imaging (LCI) Assay. The luciferase complementation imaging assay was performed as previously described (9) with minor modifications. The plasmid constructs used in this study are listed in supplemental table 3. The indicated constructs were transformed into Agrobacterium tumefaciens strain GV3101. Bacteria were grown in LB medium at 28 °C overnight and pelleted, washed one time with wash buffer (10 mM MgCl_2, 10 mM MES), and resuspended in infiltration buffer (10 mM MgCl_2, 10 mM MES, 100 μM Acetosyringone). After a 2 hr incubation, bacterial suspensions (OD_{600}=0.5) were infiltrated into four-week-old N. benthamiana leaves using a needleless 1cc syringe. The infiltrated plants were kept covered for 12 hr. After 2 or 3 days of incubation, bioluminescence
signals was detected by CCD imaging system (Andor iXon) or captured and quantified by a Bio-Rad ChemiDoc™ Imaging Systems.

**Yeast Two-Hybrid Assay.** The plasmid constructs used in yeast two-hybrid assay are listed in supplemental table 3. The full-length of HOS15 and NPR1 CDS was amplified by PCR from Arabidopsis ecotype Col-0 cDNA, and cloned in-frame to yeast two-hybrid GAL4 activation domain cloning vector pGAD424 and yeast two-hybrid GAL4 DNA-binding domain cloning vector pAS2-1 to generated prey and bait constructs of pGAD424-HOS15 and pAS2-1-NPR1, respectively. The plasmid constructs were transformed into the *Saccharomyces cerevisiae* strain PJ69-4A by heat shock according to Yeast protocols handbook (Clontech), and the interaction of HOS15 and NPR1 were observed on the synthetic complete medium lacking Trp, Leu, His plates. Photographs were taken after 5 days incubation at 30 °C. Empty vector was used as a negative control. For HOS15 with ASK interaction, The full-length of HOS15 and ASK CDS were cloned in-frame to pDEST22 vector and pDEST32 vector to generated prey and bait constructs of pDEST22-HOS15 and pDEST32-ASK, respectively. The specific primers for plasmid construction are listed in supplemental table 4.

**BiFC Assay.** The plasmid constructs used in BiFC assay are listed in supplemental table 3. HOS15 were fused in-frame to the N-terminal fragment of the eYFP fluorescent protein (Venus aa 1–173) in the pDEST-VYNE(W) vector (10) and the C-terminal fragment of eYFP (Venus aa 156–239) in the pDEST-VYCE(W) vector to generated VYNE-HOS15 and VYCE-HOS15, respectively. ASK1, NPR1, TGA2 and WRKY46 were fused in-frame to C-terminal fragment of eYFP in the pDEST-GWVE vector to generated ASK1-VYCE, NPR1-VYCE, TGA2-VYCE and WRKY46-VYCE, respectively. Once transferred to *Agrobacterium tumefaciens* (GV 3101) cells, the indicated constructs were transiently coexpressed in the four-week-old *N. benthamiana* leaves for 2 days. Fluorescence was detected by a confocal laser scanning microscopy (Olympus FV1000) with an excitation wavelength of 514 nm for YFP.

**Protein Extraction and Immunoblot Analysis.** The total proteins from 12-day or four- to five-week-old Arabidopsis plants or four-week-old *N. benthamiana* leaves were extracted in extraction buffer containing 100 mM Tris-Cl, pH 7.5, 150 mM NaCl, 0.5% NP-40, 1 mM EDTA, 3 mM DTT, 50 µM MG132 and Sigma protease inhibitor cocktail. After collection the total protein extracts by centrifuge at 18,000 g for 10 min at 4 °C, the protein extracts were mixed with 4 × SDS sample buffer and separated by SDS-PAG and then the proteins was transferred onto a polyvinylidene fluoride membrane (Immobilon-P, Millipore). The membrane were incubated in blocking buffer (1xTBS buffer including 5% dried nonfat milk) for 1hr at room temperature (RT), then incubated with indicated antibody (in blocking solution) for 2 hr at RT
and additionally incubated in secondary antibody for 1 hr at RT. The antigen proteins were visualized by chemiluminescence using ECL-detection reagent (Bio Rad) and imaged by a Bio-Rad ChemiDoc™ Imaging Systems. Immunoblotting for NPR1 protein was performed with anti-NPR1 antibody (Agrisera, AS12 1854) according to the manufacturer’s instructions.

Nuclear fractionation of Arabidopsis tissue was performed as previously described (6, 11) with minor modifications. Briefly, approximately 150 mg of Arabidopsis tissue was frozen in liquid nitrogen, ground into a fine powder using TissueLyser (Qiagen) and homogenized in two volumes (~600 μl) of Honda buffer (25 mM Tris-HCl, pH 7.4, 0.4 M sucrose, 10 mM MgCl₂, 2.5% Ficoll 400, 5% dextran 40, 10 mM β-mercaptoethanol, and a proteinase inhibitor cocktail). The homogenate was filtered through two layers of Miracloth (Millipore) and then filtered through 50 μm (pore-size) nylon mesh by centrifuge at 500 g for 1 min at 4°C. Triton X-100 was added to a final concentration of 0.5% and the mixture was incubated on ice for 15 min. The solution was centrifuged at 1,500 g for 5 min and the supernatant constitutes the “cytosolic” fraction. The pellet was resuspended gently in 1 mL of Honda buffer containing 0.1% Triton X-100 and centrifuged at 200 g for 1 min to pellet cell debris. The supernatant was centrifuged at 1,800 g for 5 min to pellet nuclei and gently washed twice with nuclei resuspension buffer (20 mM Tris-HCl, pH 7.4, 2.5 mM MgCl₂, 25% glycerol, 0.2% Triton X-100). This pellet was resuspended in 100 μl of protein extraction buffer (100 mM Tris-Cl, pH 7.5, 150 mM NaCl, 0.5% NP-40, 1 mM EDTA, 3 mM DTT and a protease inhibitor cocktail) to release nuclear proteins, centrifuged at 18,000 g for 10 min at 4 °C pellet remaining debris, and the supernatant constituted the “nuclear” fraction. For the “total” protein, approximately 50 mg of fine ground powder of Arabidopsis tissue was combined with 100 μl of protein extraction buffer and the supernatant collected after centrifugation at 18,000 g for 10 min at 4 °C. Immunoblotting with anti-Histone 3 antibody (Trans, HL102) was used as control for nuclear proteins.

Cell-Free Degradation Assay. Cell-free degradation assays for NPR1 protein, four- to five-week-old Arabidopsis plants were spray treated with or without 0.5 mM SA for 8 or 12 hours and the samples were ground in liquid nitrogen and extracted total protein in extraction buffer containing 100 mM Tris-Cl, pH 7.5, 150 mM NaCl, 0.5% NP-40, 1 mM EDTA, 3 mM DTT and 1 mM ATP. The protein extracts were centrifuged at 18,000 g for 10 min at 4 °C and incubated at room temperature for 0, 15 and 30 min with or without 100 μM MG132. Degradation reactions were stopped by mix with 4xSDS sample buffer and incubation at 65 °C for 15 min. Immunoblotting for NPR1 protein was performed with anti-NPR1 antibody (Agrisera, AS12 1854) according to the manufacturer’s instructions.

Colimmunoprecipitation Assay. For colimmunoprecipitation assays, the total proteins were
extracted from 12-day-old Arabidopsis seedling plants or four-week-old *N. benthamiana* leaves in extraction buffer (100 mM Tris-Cl, pH 7.5, 150 mM NaCl, 0.5% NP-40, 1 mM EDTA, 3 mM DTT, 50 µM MG132 and Sigma protease inhibitor cocktail). For immunoprecipitation, the protein extracts were centrifuged twice at 18,000 g for 10 min at 4 °C and incubated with anti-GFP (Invitrogen, A11120) or anti-FLAG (Sigma-Aldrich, F1804) or anti-HOS15 pre-cross-linked protein A agarose at 4 °C for 1.5 hours with gentle rotation. Followed washing twice with extraction buffer and once with 1xPBS, the protein samples were mixed with 4 × SDS sample buffer and heated to 90 °C for 2 min. Then proteins were separated by SDS-PAGE and transferred onto a PVDF membrane (Immobilon-P, Millipore). Immunoblotting was performed using anti-GFP (Abcam, ab6556), anti-HA (Roche, 11867423001), anti-FLAG (Sigma-Aldrich, F1804) or anti-HOS15 antibodies. The antigen proteins were detected using ECL-detection reagent (Bio Rad).

**Chromatin Immunoprecipitation (ChIP) Assay.** ChIP assays were carried out by following a protocol as previously described (12). In brief, four to five-week-old Arabidopsis plants were treated with 1% formaldehyde under vacuum for 15min at room temperature. The cross-linking reaction was terminated by adding with 2.5 M glycine to a final concentration of 0.125 M under vacuum for an additional 5min at room temperature. Plants were then washed with sterilized water and ground in liquid nitrogen. Arabidopsis nuclei were extracted in cold nuclei isolation buffer (0.25 M sucrose, 15 mM PIPES pH 6.8, 5 mM MgCl_2_, 60 mM KCl, 15 mM NaCl, 1 mM CaCl_2_, 0.9% Triton X-100, 1 mM PMSF and Sigma phosphatase inhibitor cocktail), lysed in nuclei lysis buffer (50 mM HEPES pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% SDS, 0.1% sodium deoxycholate, 1% Triton X-100 and Sigma phosphatase inhibitor cocktail), and sheared DNA to an average size of 500-1,000 bp by sonication with the Bioruptor. The sonicated chromatin samples were diluted tenfold with nuclei lysis buffer. Immunoprecipitations was performed with anti-HA antibody (Roche) pretreated salmon sperm DNA/protein A agarose beads (upstate) incubated with chromatin solution for 2 hours at 4 °C. Treatment with anti-rat IgG was used as a negative control to detect background levels in each ChIP experiment. After washing with low salt wash buffer (150 mM NaCl, 20 mM Tris—HCl pH 8.0, 0.2% SDS, 0.5% Triton X-100 and 2 mM EDTA), high salt wash buffer (500 mM NaCl, 20 mM Tris—HCl pH 8.0, 0.2% SDS, 0.5% Triton X-100 and 2 mM EDTA) and LiCl wash buffer (0.25 M LiCl, 1% sodium deoxycholate, 10 mM Tris—HCl pH 8.0, 1% NP-40 and 1 mM EDTA), eluted immuno-complexes from the agarose beads by adding freshly prepared elution (1% SDS and 0.1 M NaHCO_3_). Reverse crosslinking was achieved by incubation overnight at 65 °C, then treated with Proteinase K to remove all proteins. DNA was purified by phenol-chloroform-
isoamyl alcohol extraction and precipitated by ethanol. DNA were dissolved in 50ul TE buffer (1 mM EDTA and 10 mM Tris–HCl pH 8.0) and 1 μl aliquots were used for quantitative PCR. The specific primers used in ChIP qPCR are listed in supplemental table 4.

**Statistical Analyses.** Quantification experiment, the relative LUC activity was quantified with Image lab 5.2 software (Bio-Rad). Statistical analyses were performed using SPSS software and Microsoft Office Excel 2013. Error bars represent in the figures are standard deviation (SD) or the standard error of the mean (SEM= SD/√n). Numbers of sample and independent biological replicates are indicated in figure legends. Statistical comparison among different samples was carried out by one-way ANOVA or Student’s t-test. Samples with statistically significant differences indicated in the figure legends as *(p < 0.05) or ** (p < 0.01) or *** (p < 0.001). Different letters (a,b,c etc) indicate significant differences tested by One-way ANOVA with Tukey’s HSD. Details are shown in figure legends.
**Supplementary figures and tables**

**Figure S1.** HOS15 associated with ASKs and CUL1 form SCF<sub>HOS15</sub>.

(A) CLuc-HOS15 was coexpressed with ASK1-NLuc or ASK2-NLuc in *N. benthamiana* and bioluminescence signal in the leaves was quantified using a CCD imaging systems. 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 = 21).

(B and C) HOS15-Flag was coexpressed with ASK1-3xHA (B) or ASK2-3xHA (C) in *N. benthamiana*, and total protein was immunoprecipitated with anti-HOS15 antibody and immunoblotted with anti-HOS15 or anti-HA antibodies.

(D) HOS15 and ASK interaction was examined in a yeast two-hybrid assay. AD-HOS15 in the pDEST22 vector was used as prey and BD-ASK proteins in the pDEST32 vector were used as bait. Growth of yeast cells on media without tryptophan and leucine (-TL) indicated co-transformation and growth on media additionally lacking histidine (-TLH) indicated direct interaction between HOS15 and ASK. Shown are results representative of three independent transformants.

(E) NLuc-HOS15 was coexpressed with CUL1-CLuc in *N. benthamiana*. Error bars represent means ± SD. Different letters (a or b) indicate significant differences tested by One-way ANOVA with Tukey’s HSD (p < 0.001, n = 7).

(F) HOS15-Flag was coexpressed with ASK1-3xHA or ASK1-3xHA and CUL1-3xHA in *N. benthamiana* and total protein was immunoprecipitated with anti-HOS15 antibody and immunoblotted with anti-HOS15 or anti-HA antibodies.
Figure S2. HOS15 interacts with ASK1, NPR1 and TGA2 in nucleus.

Bimolecular fluorescence complementation (BiFC) analysis showing HOS15 interacts with ASK1, NPR1 and TGA2 in nucleus of *N. benthamiana* leaf epidermal cells. The coexpression of VYNE-HOS15 with WRKY46-VYCE was used as negative control. DAPI staining was used as a nuclear marker. Bars = 40 µm.
Figure S3. *hos15-1* plants display increased resistance to *Pst* DC3000.

(A) Morphology of four-week-old C24 and *hos15-1*.

(B) Autonomous cell death phenotype of *hos15-1* leaves compared with wild-type C24.

(C) Fresh weight of the rosette leaves of three- to four-week-old C24 and *hos15-1*. Error bars represent means ± SD. Asterisks indicate significant differences between C24 and *hos15-1* tested by Student’s *t* test (**p < 0.001; n = 9).

(D) Abundance of *PR1*, *PR2* and *PR5* in C24 and *hos15-1*, and *PR1* protein levels in C24 and *hos15-1*. RT-PCR was used for assess *PR* gene levels in three-week-old plants. The tubulin 2 was used as an internal control. Total protein was immunoblotted with anti-*PR1* antibody. CBB staining of membrane was used as a loading control.

(E) Bacterial growth on C24 and *hos15-1*. Four-week-old plants were inoculated with *Pst* DC3000 (OD*$_{600}$* =0.0001) and colony-forming units (cfu) were quantified at 0, 1 and 3 days after inoculation. Error bars represent means ± SD from three biological replicates, each with six 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).
Table S1. Microarray analysis of mRNA levels of defense-response genes in hos15-1 versus wild-type (C24) plants.

| Gene ID     | Description                                      | Fold difference* (hos15-1/C24) |
|-------------|---------------------------------------------------|---------------------------------|
| A2g40750    | WRKY family transcription factor, Group III (WRKR54) | 8.8                             |
| A3g57240    | Similar to glycosyl hydroxase family 17 (A13g57260.1)(BGG37) | 7.1                             |
| A2g17040    | No apical meristem (NAM) family protein           | 6.3                             |
| A3g57260    | Glycosyl hydroxase family 17 protein              | 6.1                             |
| A2g14610    | Pathogenesis-related protein 1 (PR-1)             | 5.6                             |
| A4g23140    | Receptor-like protein kinase 5 (RLK5)             | 5.4                             |
| A2g46400    | WRKY family transcription factor, Group III (WRKY46) | 4.4                             |
| A3g48090    | Defense response protein/lipase (EDS1)            | 4.4                             |
| A1g21250    | Wall-associated kinase 1 (WAK1)                  | 4.2                             |
| A3g56400    | Member of WRKY transcription factor, Group III (WRKY70) | 4.2                             |
| A1g08050    | Zinc finger (C3HC4-type Ring finger) family       | 4.1                             |
| A2g37710    | Induced in response to salicylic acid            | 3.9                             |
| A1g6510     | Disease resistance protein (TIR-NBS-LRR class) (WRR4) | 3.6                             |
| A1g74710    | Isochorismate synthase 1(IC51 ,SID2)             | 3.5                             |
| A3g52430    | Phytoalexin-deficient 4 protein (PAD4)            | 3.3                             |
| A2g43620    | Chitinase, putative                               | 3.1                             |
| A5g55450    | Lipid transfer protein (LTP) family protein       | 3.1                             |
| A1g03850    | Glutaredoxin family protein                       | 3.1                             |
| A2g20145    | Toll-interleukin-resistance domain-containing protein | 3.1                             |
| A4g44220    | Disease resistance protein family                 | 3.1                             |
| A4g39830    | Salicylic acid induction deficient 1(SID1,EDS5)   | 2.9                             |
| A4g33300    | Disease resistance protein (CC-NBS-LRR class,ADR1.1) | 2.8                             |
| A1g75040    | Pathogenesis-related protein 5 (PR-5)             | 2.6                             |
| A3g47570    | Leucine-rich repeat transmembrane protein kinase   | 2.5                             |
| A5g06320    | Harpin-induced family protein/HIN1 family protein | 2.4                             |
| A1g02890    | Glutathione S-transferase                         | 2                               |

*Relative levels of mRNA accumulated in hos15-1 versus wild-type C24 plants

Figure S4. RT-PCR analysis of defense-response genes.

Abundance of indicated genes in C24 and hos15-1. RT-PCR was used for detection of mRNA transcripts in three-week-old plants. The tubulin 2 was used as an internal control.
Table S2. The binding intensity between HOS15 and selected candidate proteins in LCI assay.

| NO. | Gene ID | Gene name                        | Description                                           | Relative LUC activity |
|-----|---------|----------------------------------|-------------------------------------------------------|-----------------------|
| 1   | A4g23570 | SGT1b                            | May function in SCF mediated protein degradation      | O                     |
| 2   | A5g06650 | TGA2                             | Transcription factor of the B-ZIP family               | O                     |
| 3   | A1t64280 | NFR1                             | SA receptor, Transcriptional coactivator              | O                     |
| 4   | A5g03740 | HD2C                             | HD2-type histone deacetylase HDAC                     | O                     |
| 5   | A4g38130 | HDA19                            | Encodes a histone deacetylase                        | O                     |
| 6   | A5g63110 | HDA6                             | Encodes a histone deacetylase                        | O                     |
| 7   | A5g01900 | WRKY02                           | Member of WRKY Transcription Factor, Group III        | O                     |
| 8   | A5g60410 | SIZ1                             | Small ubiquitin-like modifier (SUMO) E3 ligase         | O                     |
| 9   | A5g26920 | CBP50g                           | Encodes a calmodulin-binding protein CBP50g           | O                     |
| 10  | A0g45040 | MPK3                             | Encodes a mitogen-activated kinase                    | O                     |
| 11  | A4t01370 | MPK4                             | Encodes a mitogen-activated kinase                    | O                     |
| 12  | A5g51700 | RAR1                             | Encodes a resistance signalling protein with two zinc binding domains | O                     |
| 13  | A1g73805 | SARD1                            | Calmodulin binding protein-like                       | O                     |
| 14  | A4g16840 | SNI1                             | Encodes leucine-rich nuclear protein                  | O                     |
| 15  | A4g35580 | CBNAC                            | NAC transcription factor-like 9 (NTL9)                | O                     |
| 16  | A4g26840 | SUMO1                            | Encodes a small ubiquitin-like modifier (SUMO) polypeptide | O                     |
| 17  | A5g55170 | SUMO3                            | Encodes a small ubiquitin-like modifier (SUMO) polypeptide | O                     |
| 18  | A0g46400 | WRKY46                           | Member of WRKY Transcription Factor, Group III        | O                     |

Figure S5. LUC image of *N. benthamiana* leaves.

CLuc-HOS15 or HOS15-NLuc were coexpressed with the indicated constructs in the *N. benthamiana* leaves and bioluminescence signal in the leaves was visualized using a CCD imaging systems. Coexpression of SGT1-NLuc and CLuc-RAR1 was used as a positive control and coexpression of CLuc-HOS15 or HOS15-NLuc with empty vector were used a negative controls.
Figure S6. HOS15 limits post-transcriptional NPR1 protein accumulation.

(A and B) NPR1 protein levels in C24 and hos15-1. (A) Four-week-old plant leaves were collected 0 and 8 hours after spray treatment with 0.5 mM SA and total protein was immunoblotted with anti-NPR1 antibody. CBB staining of membrane was used as a loading control. (B) Quantification of three of independent biological replicates.

(C) Abundance of HOS15 in C24 and hos15-1. Plants were treated with SA as in (A) and total protein was immunoblotted with anti-HOS15 antibody. CBB staining of membrane was used as a loading control.

(D) NPR1 transcript abundance in C24 and hos15-1 plants. Total mRNA was extracted from plants treated as in (A) and NPR1 transcript was measured by quantitative RT-PCR analysis. Error bars represent means ± SD from four biological replicates.

(E and F) NPR1 protein levels in Col-0, hos15-2 and npr1-1. Four-week-old plant leaves were spray treated with 0.5 mM SA and NPR1 proteins was detected by anti-NPR1 immunoblotting. npr1-1 plants were used as negative control. CBB staining of membrane was used as a loading control. (F) Quantification of three of independent biological replicates.

(G) Abundance of HOS15 in Col-0, hos15-2 and npr1-1. Plants were treated with SA as in (E) and total protein was immunoblotted with anti-HOS15 antibody. CBB staining of membrane was used as a loading control.

(H) NPR1 transcript abundance in Col-0 and hos15-2 plants. Total mRNA was extracted from plants treated as in (E) and NPR1 transcript was measured by quantitative RT-PCR analysis. Error bars represent means ± SD from three biological replicates.

(I, J, K and L) Cell-free degradation of NPR1 proteins. C24 and hos15-1 plants (I and J) or Col-0 and hos15-2 plants (K and L) were treated as in (A) or (E), respectively. (I and K) Protein extracts were incubated with (M: MG132, 30 min) or without MG132 for the indicated time at room temperature (RT). Immunoblots were performed with anti-NPR1 antibody. CBB staining of membrane was used as a loading control. (J and L) Quantification of two of independent biological replicates.

In B, F, J and L, error bars represent means ± SD. Asterisks indicate significant differences between C24 and hos15-1 or Col-0 and hos15-2 tested by Student’s t test (* p < 0.05, ** p < 0.01, *** p < 0.001; ns, no significance).
Figure S7. HOS15 negatively regulates stability of the NPR1 protein.

(A) 12-day-old seedlings of 35S::NPR1-GFP/npr1-1 or 35S::NPR1-GFP/npr1-1/hos15-2 plants were treated with 0.5 mM SA. Total protein from leaves collected 0, 4 and 8 hours after treatment and NPR1-GFP protein levels were measured by anti-GFP immunoblotting. Immunoblotting with anti-α tubulin was used as a loading control.

(B) 12-day-old seedlings of 35S::NPR1-GFP/npr1-1 and 35S::NPR1-GFP/npr1-1/hos15-2 plants were treated with 100 µM CHX for the indicated number of hours prior to analysis of total protein for NPR1-GFP protein levels by anti-GFP immunoblotting. CBB staining of membrane was used as a loading control.

(C) 12-day-old seedlings of 35S::NPR1-GFP/npr1-1 or 35S::NPR1-GFP/npr1-1/hos15-2 plants were collected 0 and 8 hours after spray treatment with 0.5 mM SA. Total, cytosolic and nuclear proteins extracts were immunoblotted with anti-GFP antibody. Histone 3 was used as a control for nuclear fraction. CBB staining of membrane was used as a loading control.

(D) NPR1-3xHA and HOS15-Flag was coexpressed in N. benthamiana. Increase level of HOS15-Flag decrease level of NPR1-3xHA. Total protein was immunoblotted with anti-HA or anti-Flag antibodies. CBB staining of membrane was used as a loading control.
Figure S8. Expression of NPR1-target genes in wild-type and hos15 plants.

(A-E) Abundance of indicated NPR1-target genes in four-week-old C24 and hos15-1 plants before and 8 hours after spraying with 0.5 mM SA. Error bars represent means ± SD from three biological replicates. Asterisks indicate significant differences between C24 and hos15-1 tested by Student’s t test (** p < 0.01, *** p < 0.001; n = 10).

(F-J) Abundance of indicated NPR1-target genes in four- to five-week-old Col-0, hos15-2 and npr1-1 plants before and 12 hours 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, ** p < 0.01, *** p < 0.001; n = 3). The experiment was repeated three times with similar results.
Figure S9. NPR1 protein is targeted by SCF complex.

(A and B) NPR1-CLuc was coexpressed with ASK1-NLuc or ASK2-NLuc (A), and NPR1-NLuc was coexpressed with ASK1-CLuc or CUL1-CLuc (B) in N. benthamiana. Bioluminescence signal in the leaves was quantified using a CCD imaging systems. Error bars represent means ± SD 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).

(C) Morphology of four- to five-week-old Col-0, hos15-2, cul1-6 and heterozygous axr6-1^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^-^
Figure S10. NPR1 associated with DDB1A/B and CUL4 the major components of CUL4 E3 ligase complex.

(A and B) NPR1-GFP was coexpressed with DDB1A-3xHA or DDB1A-3xHA and HOS15-3xHA (A), and coexpressed with DDB1B-3xHA or DDB1B-3xHA and HOS15-3xHA (B) in *N. benthamiana*. Total protein was immunoprecipitated with anti-GFP antibody. Immunoblots were performed with anti-GFP or anti-HA antibodies.

(C and D) 35S::NPR1-GFP/npr1-1 was crossed with 35S::Flag-CUL4 and F1 plants were treated with 100 μM MG132 (C) or 0.5 mM SA (D). Total protein was immunoprecipitated with anti-GFP antibody and immunoblotted with anti-GFP or anti-Flag antibodies.

(E) Morphology of five-week-old Col-0, cul4-1 and 35S::Flag-CUL4.

(F) NPR1 protein levels in Col-0, hos15-2, cul4-1, 35S::Flag-CUL4 and npr1-1. Four- to five-week-old plant were spray treated with 0.5 mM SA for 24 hours. Total protein was immunoblotted with anti-NPR1 antibody. npr1-1 was used as a negative control. CBB staining of membrane was used as a loading control.

(G) HOS15 protein levels in Col-0, hos15-2, cul4-1, 35S::Flag-CUL4 and npr1-1. Plant were spray treated with 0.5 mM SA for 24 hours. Total protein was immunoblotted with anti-HOS15 antibody. hos15-2 was used as a negative control. CBB staining of membrane was used as a loading control.

(H) Bacterial growth on Col-0, hos15-2, cul4-1 and 35S::Flag-CUL4. Four- to five-week-old plants were inoculated with *Pst* DC3000 (OD<sub>600</sub> =0.0001) and colony-forming units (cfu) were quantified at 4days after inoculation. Error bars represent means ± SD from three biological replicates, each with six 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).
Figure S11. NPR1, HOS15 and PR1 protein levels in Col-0, hos15-2, sid2-2, sid2-2 hos15-2, NahG, NahG hos15-2, npr1-1 and npr1-1 hos15-2 plants.

(A and B) 10-day-old indicated seedling plants were treated with 0.5 mM SA for 12 hours. Immunoblots were performed with anti-NPR1 (A) or anti-HOS15 (B) antibodies. CBB staining of membrane was used as a loading control.

(C) Four- to five-week-old indicated plants were treated with 0.5 mM SA for 12 hours and total protein was immunoblotted with anti-PR1 antibody. CBB staining of membrane was used as a loading control.
Fig. S12. SA promotes formation of a HOS15-NPR1-TGA2 complex

(A) 12-day-old seedlings of 35S:NPR1-GFP/npr1-1 transgenic plants were treated with 50 μM MG132 or MG132 and 0.4 mM INA or 0.5 mM SA. Total protein prepared 8 hours later was immunoprecipitated with anti-GFP antibody and immunoblotted with anti-GFP or anti-HOS15 antibodies.

(B) NPR1-GFP was coexpressed with HOS15-3xHA or HOS15-3xHA and TGA2-Flag in N. benthamiana. At 3 days after agro-infiltration, N. benthamiana leaves were infiltrated with 50 μM MG132 and 0.5 mM SA and total protein prepared 8 hours later was immunoprecipitated with anti-GFP antibody and immunoblotted with anti-GFP, anti-HOS15 or anti-Flag antibodies.

(C) NLuc-HOS15 was coexpressed with NPR1-NLuc or NPR1-NLuc and TGA2-Flag in N. benthamiana. Bioluminescence signal in leaves that were untreated or sprayed 8 hours earlier with 0.5 mM SA was quantified using a CCD Imaging Systems. 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 < 0.05, n = 16).

(D) TGA2-Flag was coexpressed with NPR1-GFP or HOS15-3xHA and NPR1-GFP in N. benthamiana. At 3 days after agro-infiltration, N. benthamiana leaves were infiltrated with 50 μM MG132 and 0.5 mM SA and total protein prepared 8 hours later was immunoprecipitated with anti-Flag antibody and immunoblotted with anti-Flag, anti-HOS15 or anti-GFP antibodies.

(E and F) NPR1 and HOS15 accumulation in Col-0, hos15-2 and tga256 plants. Four-week-old plants were sprayed with 0.5 mM SA and total protein prepared 24 hours later was immunoblotted with anti-NPR1 (E) or anti-HOS15 (F) antibodies.
In unchallenged plant cells (left), NPR1 is predominantly localized in the cytoplasm as an inactive high molecular weight oligomeric complex, formed through intermolecular disulfide bonds. The small amount of monomeric NPR1 that translocates to the nucleus is rapidly targeted for proteasome dependent degradation by SCF<sup>HOS15</sup> E3 ubiquitin ligase to prevent inappropriate expression of defense genes in uninduced cells.

In SA-induced plant cells (right), monomeric NPR1 proteins, released from the oligomeric complexes, translocate to the nucleus. Phosphorylated, nuclear NPR1 forms a coactivating complex with TGA-family transcription factors to activate defense gene expression. Targeting of phosphorylated NPR1 by CUL3 E3 ubiquitin ligase promotes NPR1-target gene expression. Simultaneously, HOS15 targets NPR1-TGA activation complexes to the SCF<sup>HOS15</sup> E3 ubiquitin ligase for ubiquitination and subsequent degradation by the proteasome. However, unlike CUL3-mediated NPR1 degradation, NPR1 degradation by SCF<sup>HOS15</sup> E3 ubiquitin ligase limits expression of NPR1-target genes, and thus contributes to the dynamic balance between transcriptional gene activation and repression during SA-induced defense.
Table S3. Plasmid constructs used in this study.

| Construct name       | Description          | Plasmid backbone | Purpose | References   |
|----------------------|----------------------|------------------|---------|--------------|
| HOS15-Flag           | 35S HOS15-Flag       | pGW811           |         |              |
| HOS15-3xHA           | 35S HOS15-3xHA       | pGW814           |         |              |
| ASK1-3xHA            | 35S ASK1-3xHA        | pGW814           |         |              |
| ASK2-3xHA            | 35S ASK2-3xHA        | pGW814           |         |              |
| CUL1-3xHA            | 35S CUL1-3xHA        | pGW814           |         |              |
| NPR1-Flag            | 35S NPR1-Flag        | pGW811           |         |              |
| NPR1-GFP             | 35S NPR1-GFP         | pKCFWG           |         |              |
| DDB1A-3xHA           | 35S DDB1A-3xHA       | pGW814           |         |              |
| DDB1B-3xHA           | 35S DDB1B-3xHA       | pGW814           |         |              |
| TGA2-Flag            | 35S TGA2-Flag        | pGW811           |         |              |
| CLuc-HOS15           | 35S CLuc-HOS15       | pCAMBIA1300-CLuc |         |              |
| NLuc-HOS15           | 35S NLuc-HOS15       | pCAMBIA1300-NLuc |         |              |
| NPR1-CLuc            | 35S NPR1-CLuc        | pCAMBIA1300-CLuc |         |              |
| NPR1-NLuc            | 35S NPR1-NLuc        | pCAMBIA1300-NLuc |         |              |
| ASK1-NLuc            | 35S ASK1-NLuc        | pCAMBIA1300-NLuc |         |              |
| ASK2-NLuc            | 35S ASK2-NLuc        | pCAMBIA1300-NLuc |         |              |
| CUL1-CLuc            | 35S CUL1-CLuc        | pCAMBIA1300-NLuc |         |              |
| SGT1b-NLuc           | 35S SGT1b-NLuc       | pCAMBIA1300-NLuc |         |              |
| CLuc-RAR1            | 35S CLuc-RAR1        | pCAMBIA1300-CLuc |         |              |
| TGA2-NLuc            | 35S TGA2-NLuc        | pCAMBIA1300-NLuc |         |              |
| SARD1-NLuc           | 35S SARD1-NLuc       | pCAMBIA1300-NLuc |         |              |
| CBP60g-NLuc          | 35S CBP60g-NLuc      | pCAMBIA1300-NLuc |         |              |
| CLuc-SNI1            | 35S CLuc-SNI1        | pCAMBIA1300-CLuc |         |              |
| CLuc-WRKY46          | 35S CLuc-WRKY46      | pCAMBIA1300-CLuc |         |              |
| SIZ1-NLuc            | 35S SIZ1-NLuc        | pCAMBIA1300-NLuc |         |              |
| MPK3-NLuc            | 35S MPK3-NLuc        | pCAMBIA1300-NLuc |         |              |
| MPK4-NLuc            | 35S MPK4-NLuc        | pCAMBIA1300-NLuc |         |              |
| CLuc-SUMO1           | 35S CLuc-SUMO1       | pCAMBIA1300-CLuc |         |              |
| CLuc-SUMO3           | 35S CLuc-SUMO3       | pCAMBIA1300-CLuc |         |              |
| CLuc-CBNAC           | 35S CLuc-CBNAC       | pCAMBIA1300-CLuc |         |              |
| HD2C-NLuc            | 35S HD2C-NLuc        | pCAMBIA1300-NLuc |         |              |
| WRIKY62-NLuc         | 35S WRIKY62-NLuc     | pCAMBIA1300-NLuc |         |              |
| HD6-NLuc             | 35S HD6-NLuc         | pCAMBIA1300-NLuc |         |              |
| HD19-NLuc            | 35S HD19-NLuc        | pCAMBIA1300-NLuc |         |              |
| VYNE-HOS15           | 35S VYNE-HOS15       | pDEST-VYNE(R)wy  |         |              |
| ASK1-VYCE            | 35S ASK1-VYCE        | pDEST-3xVYCE     |         |              |
| NPR1-VYCE            | 35S NPR1-VYCE        | pDEST-3xVYCE     |         |              |
| TGA2-VYCE            | 35S TGA2-VYCE        | pDEST-3xVYCE     |         |              |
| WRIKY46-VYCE         | 35S WRIKY46-VYCE     | pDEST-3xVYCE     |         |              |
| AD-HOS15             | proADH1.GAL4AD-HOS15 | pGAD24           |         |              |
| BD-NPR1              | proADH1.GAL4BD-NPR1  | pAS2-1           |         |              |

References:
- Park et al., 2018
- Chan et al., 2008
Table S4. List of primers used in this study.

| Primer Name | Primer Sequence | Purpose |
|-------------|-----------------|---------|
| GABI-Kot    | CCAATTGGAGCTGAATGTAGACAC  | Genotyping |
| Nts15-2 F   | GATGGCCAACGACAGATCCTG  |         |
| Nts15-2 R   | TCCTGTAGGGCTCCATCTGA  |         |
| np1-1 F     | ATGTTCGGAATGTACATAAGGCG  |         |
| np1-1 R     | CATQAGTGCGGTCCATCTC  |         |
| sid2-2 F1   | QAAAGACGCACGCTGAGTTCTTCA  |         |
| sid2-2 R1   | CTTTCTCCTTAATCGAAAAGCCCTT  |         |
| sid2-2 F2   | AAAGCTGCaAGATGTCAACACTCA  |         |
| sid2-2 R2   | CGTAAAGCTCCTCAGGCAATTGAG  |         |
| NahG F      | TTTCAATTGCGACGTTGAG  |         |
| NahG R      | GTGCGGAAACTCTGATAACTCG  |         |
| PR1 RT-PCR F| CTCATCACCTCTGGTGAG  | RT-PCR |
| PR1 RT-PCR F| GAACTCATATTCACACAGGAG  |         |
| PR2 RT-PCR F| GTTCACTCTCTCAACACACAGC  |         |
| PR2 PT-PCR R| GTGTAATGTACGGAATGTGAC  |         |
| PR5 RT-PCR F| GTCAAGGACCCAAGCTCGG  |         |
| PR5 RT-PCR F| TCTTCAGGCGCAAGCAGCT  |         |
| WRR4 RT-PCR F| GTCTCACAAATTGAGGAGAG  |         |
| WRR4 RT-PCR R| GAACCGGAAATGTCTTCTCA  |         |
| ICS1 RT-PCR F| GTGCTCTGATGTCGTGTA  |         |
| ICS1 RT-PCR R| CAATTGACGTGTTGGAAGCTGAGA  |         |
| WRRKY48 RT-PCR F| CAAGCTGATGTCATCAACAA  |         |
| WRRKY46 RT-PCR R| TACAGCACAAGAATCCTG  |         |
| WRRKY54 RT-PCR F| AGATGCAAGCAAGCAGACC  |         |
| WRRKY54 RT-PCR R| CAGTGTCTTCTCAATCAATCGCA  |         |
| WRRKY70 RT-PCR F| GAGGAGCCTATTCTTGAGG  |         |
| WRRKY70 RT-PCR R| TGCTTTCACATTTGAGCTAAC  |         |
| EDS5 RT-PCR F| CCACTCTCTCAACGCGCTCA  |         |
| EDS5 RT-PCR R| CTCACCCTGCTATGGAACCTG  |         |
| PDF1.2 RT-PCR F| CACCTTATCTTCCGCTGCTCT  |         |
| PDF1.2 RT-PCR R| TACACTTGTCCTGCGGGAACAG  |         |
| EDS1 RT-PCR F| GTTCAACTCTGATACCAGGACA  |         |
| EDS1 RT-PCR R| GCAGAGGAGAATGGGATTGG  |         |
| PAD4 RT-PCR R| GGAGAAGATGGATTACGCATCT  |         |
| PAD4 RT-PCR R| CTGATGCTCATTGGGCTCATT  |         |
| NDR1 RT-PCR F| TTTACCCACATCAACACAGA  |         |
| NDR1 RT-PCR R| GCTCCACCTCACCACCATATA  |         |
| NPR1 RT-PCR F| TCCTGCTAGAGAAGAACA  |         |
| NPR1 RT-PCR R| TGAGAGGATTTACGGGTAGAC  |         |
| Tubulin2 RT-PCR F| AGCAAATGTGGGACCTCAGAAG  |         |
| Tubulin2 RT-PCR R| CACCTTCTCAGTCCGAGTTT  |         |
| PR1 qRT F    | GAAACTGTTGGTAGTACGGG  | qRT-PCR |
| PR1 qRT R    | GTTCACATAATTCCCACAGGGA  |         |
| NPR1 qRT F   | ATTAGGCACCTGATGCGGATGA  |         |
| NPR1 qRT R   | TCAGTTCTCTATGTTGGAACAC  |         |
| WRRKY18 qRT F| GCAAGGCTCTTGTCCACGTTAA  |         |
| WRRKY18 qRT R| TGGGTTTTTCCACACGCTGAC  |         |
| WRRKY38 qRT F| CGGGTCAACAGGACCAACTACT  |         |
| WRRKY38 qRT R| AACCAGTAAAGGAGAAGAAGACGTT  |         |
| WRRKY62 qRT F| TATGCAACTTTCTCCACATTGAC  |         |
| WRRKY62 qRT R| ACGGGGATGGGAGACCAATCCTC  |         |
| SAR1 qRT F   | AGCCACCTACCAAAGACACCTG  |         |
| SAR1 qRT R   | GAAAGATCGCGTGGAAGAACAATA  |         |
| ACTIN2 qRT F| TCTCGAGTTATGCGTGGACGTAT  |         |
| ACTIN2 qRT R| CTGGACCTGCTCTCATACATC  |         |
| Primer Name | Primer Sequence | Purpose |
|-------------|----------------|---------|
| HOS15 kpn I F | GGTACCATGCTTCCTACCATCCTGC | Cloning (LCI assay) |
| HOS15 Sal I R | GTCGACCCTAGATCTGAAATCAAGG | |
| HOS15 Sal I R2 | GTCGACCCATTCTGAAATCAAGGACGC | |
| TGA2 kpn I F | CAGGATCCATGGCGTATACCAAGTCCGGAGAACAC | |
| TGA2 Sal I R | CAGGATCCATGGCTTCAACGTGGCCGGAGAAGCCA | |
| SARD1 kpn I F | AGCTACATAGGCGGAAGGATTTTCAAGGA | |
| SARD1 Mlu I R | TACGCGTGAAGGAAGGTATATGATTTTGAAC | |
| CBP600 g kpn I F | AGGTACCATGAAAGATGCTGGGAACAGCCCTA | |
| CBP600 g Sal I R | AGTCGACAGACCTCTTCTTCCTGGATTCTCTGGAG | |
| SN11 kpn I F | GGTACCATGCTGCAGAAGACGAA | |
| SN11 Sal I R | GTCGACGACTTTCGCTGCACTTACCAT | |
| WRKY46 kpn I F | GGTACCATGATCAGAAGAAGAAACCTT | |
| WRKY46 Sal I R | GTATCGACGCGGTGATGAGTTT | |
| NTL9 BsuHI F | GGTACCATGCGCTCGTATGCTGAGG | |
| NTL9 Sal I R | GTCGACGACTTTCGCTGAAATCTTACCT | |
| HD6 kpn I F | GTTACATGCTGCAGAAGACGAA | |
| HD6 Mlu I R | AGCGCTAAGGCTAGGAGGCTAGTCAGTCC | |
| HD19 kpn I F | GTTACATGCTGCAGAAGACGAA | |
| HD19 Sal I R | GTCGACGACTTTCGCTGAAATCTTACCT | |
| attB1 adapter | GGGGAACTTGGTATACAAAAAAGCAGGCT | |
| attB2 adapter | GGGGAACTTGGTATACAAAAAAGCAGGCT | |
| HOS15 attB1 | AAAAAAGCAGGCTGATGCTGCTACCATTTACCT | Cloning (LCI and ColIP assays) |
| HOS15 attB2 | GGGGAACTTGGTATACAAAAAAGCAGGCT | |
| HOS15 w/o attB2 | GGGGAACTTGGTATACAAAAAAGCAGGCT | |
| NPR1 attB1 | AAAAAAGCAGGCTGATGCTGCTACCATTTACCT | |
| NPR1 w/o attB2 | AAAAAAGCAGGCTGATGCTGCTACCATTTACCT | |
| TG2 attB1 | AAAAAAGCAGGCTGATGCTGCTACCATTTACCT | |
| TG2 w/o attB2 | AAAAAAGCAGGCTGATGCTGCTACCATTTACCT | |
| ASK1 attB1 | AAAAAAGCAGGCTGATGCTGCTACCATTTACCT | |
| ASK1 w/o attB2 | AAAAAAGCAGGCTGATGCTGCTACCATTTACCT | |
| ASK2 attB1 | AAAAAAGCAGGCTGATGCTGCTACCATTTACCT | |
| ASK2 w/o attB2 | AAAAAAGCAGGCTGATGCTGCTACCATTTACCT | |
| AIC1 attB1 | AAAAAAGCAGGCTGATGCTGCTACCATTTACCT | |
| AIC1 w/o attB2 | AAAAAAGCAGGCTGATGCTGCTACCATTTACCT | |
| DDB1 attB1 | AAAAAAGCAGGCTGATGCTGCTACCATTTACCT | |
| DDB1 attB2 | AAAAAAGCAGGCTGATGCTGCTACCATTTACCT | |
| DDB1 w/o attB2 | AAAAAAGCAGGCTGATGCTGCTACCATTTACCT | |
| DDB1B attB1 | AAAAAAGCAGGCTGATGCTGCTACCATTTACCT | |
| DDB1B attB2 | AAAAAAGCAGGCTGATGCTGCTACCATTTACCT | |
| DDB1B w/o attB2 | AAAAAAGCAGGCTGATGCTGCTACCATTTACCT | |
| CUL4 attB1 | AAAAAAGCAGGCTGATGCTGCTACCATTTACCT | |
| CUL4 w/o attB2 | AAAAAAGCAGGCTGATGCTGCTACCATTTACCT | |
| PR1-P2 CHIP F | AGAGGCTGATGCTGCTACCATTTACCT | ChiP-qPCR |
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