***New Phytologist* Supporting Information**

Article title: **Arabidopsis C-terminal Binding Protein ANGUSTIFOLIA modulates transcriptional co-regulation of *MYB46* and *WRKY33***

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The following Supporting Information is available for this article:

Fig. S1 Overexpression of Myc tagged AN (AN-Myc) in *an-t1* (*an-t1 35S:AN*) reverses its narrow-leaf phenotype to wild type morphology.
(**a**) qPCR analysis of *AN* transcript in Col-0, *an-t1*, and *an-t1 35S:AN*. Values represent means ± SEM, n = 3. Statistical significance was determined by two tailed students t-tests against Col-0 (** $P<0.01$).
(**b**) Phenotypes of three-week-old seedlings of Col-0, *an-t1*, and *an-t1 35S:AN*.

Fig. S2 AN antagonistically regulates gene expression in SA and JA/ET pathways.
(**a**) qPCR analysis showing expression changes of *PR-1*, *PAL1*, *PAL2*, *PAL3*, *PAL4*, *ICS1*, *PBS3*, and *EDS5* in Col-0, *an-t1*, and *an-t1 35S:AN* at 0 and 6 hpi of *P. syringae* treatment.
(**b**) qPCR analysis showing expression changes of *PDF1.2*, *ACS2*, *ACS6*, *ERF1A*, and *ORA59* in Col-0, *an-t1*, and *an-t1 35S:AN* at 0 and 24 hpi of *B. cinerea* treatment. Gene expressions were normalized against the expression of *EF1α*. For each gene, its expression in Col-0 at 0 hpi was set as 1. Values represent means ± SEM, n = 3. Statistical significance was determined by two tailed students t-tests against Col-0 (** $P<0.01$, * $P<0.05$, *ns* $P>0.05$).

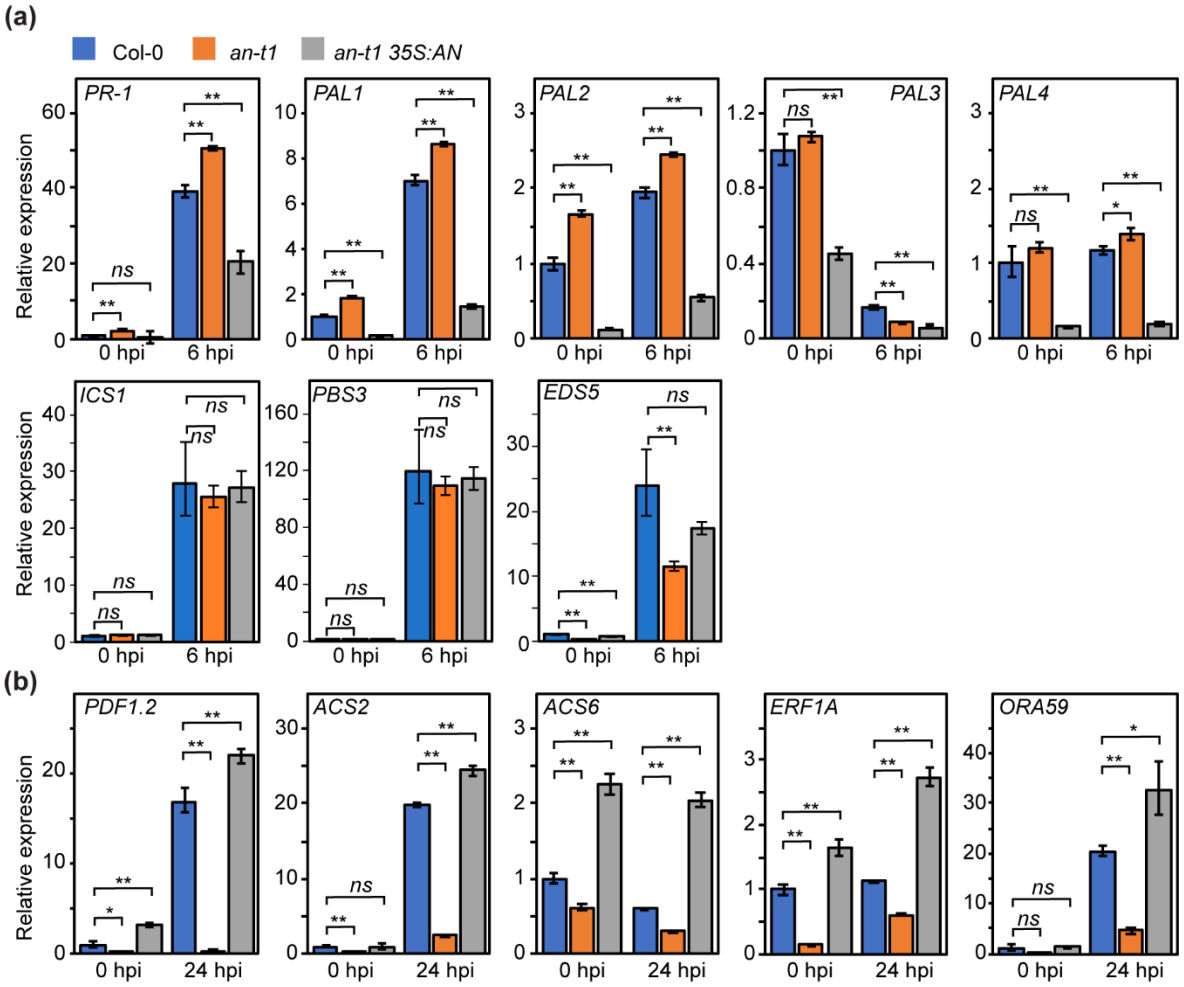

Fig. S3 Yeast two-hybrid analysis of AN and TDP1 interaction.
AN was fused with Gal4 activation domain (AD). Full-length and truncated TDP1 were fused with Gal4 DNA binding domain (BD). SD-T-L, indicates SD plate without Trp and Leu. SD-T-L-H-A, indicates SD plate without Trp, Leu, His and Ade.

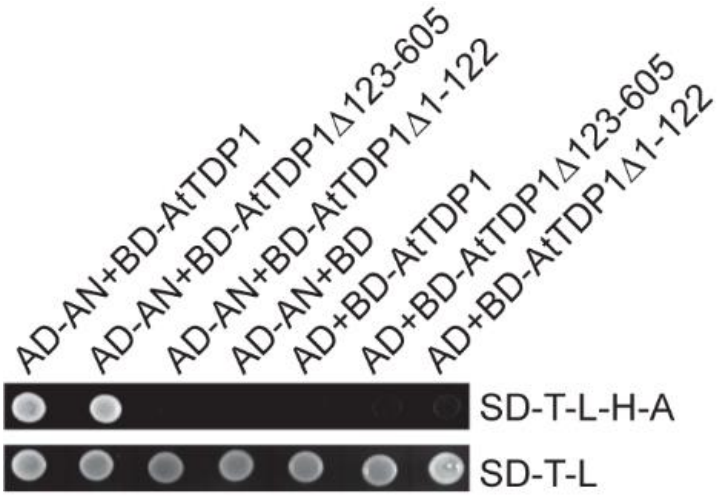

Fig. S4 µChIP-qPCR analysis using protoplasts co-expressing AN-Myc and HA-TDP1.
(**a**) Western blot results showing AN and TDP1 co-expression did not change protein expression levels of AN-Myc or HA-TDP1. ACTIN was blotted as a loading control.
(**b**) µChIP-qPCR analysis demonstrating the enhancement of association of TDP1 with *MYB46* promoter by AN. Assays without antibody (no ab) were performed as negative controls. Values represent means ± SEM, n = 3. Statistical significance was determined by two tailed students t-tests (** P<0.01).

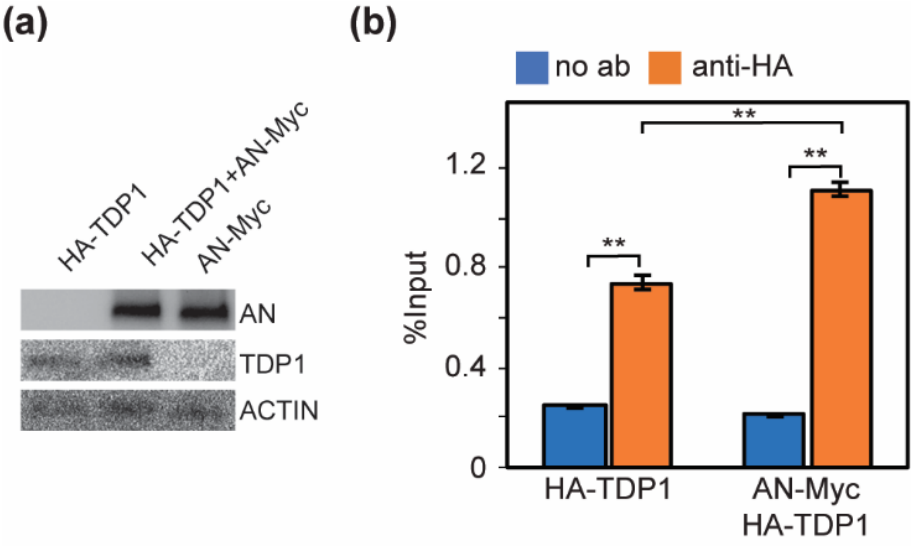

Fig. S5 AN has no transcriptional activator activity.
Left scheme displays the two vectors used in transactivation analysis: reporter construct contains one Gal4 binding site upstream of *GUS* reporter gene; effector construct expressing GD fused AN. GD fused VP16 (GD-VP16) was used as a positive control. Values represent means ± SEM, n = 3. Statistical significance was determined by two tailed students t-tests (** P<0.01).

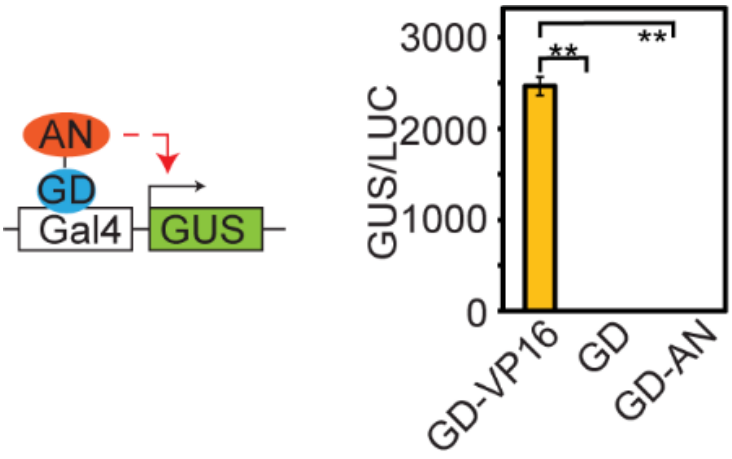

Fig. S6 Transcription factors up- and down-regulated in *an-t1* mutant.
Published RNA-seq data of *an-t1* mutant (Bryan AC, Zhang J, Guo J, Ranjan P, Singan V, Barry K, Schmutz J, Weighill D, Jacobson D, Jawdy S, et al. 2018. A Variable Polyglutamine Repeat Affects Subcellular Localization and Regulatory Activity of a *Populus* ANGUSTIFOLIA Protein. *G3 (Bethesda)* **8**(8): 2631-2641) is used to plot the bar graph.

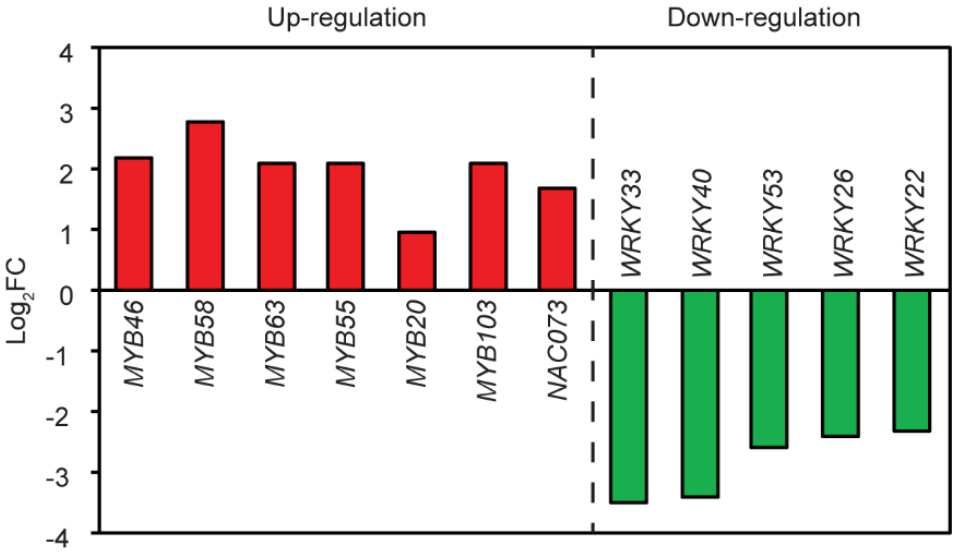

Fig. S7 Amino acid sequence alignment of Arabidopsis TDP1 (AT5G15170) and Medicago TDP1 (XM_003622639).
*, identical amino acid. :, similar amino acid. Solid black line indicates the FHA domain and dashed black line indicates the TDP domain.

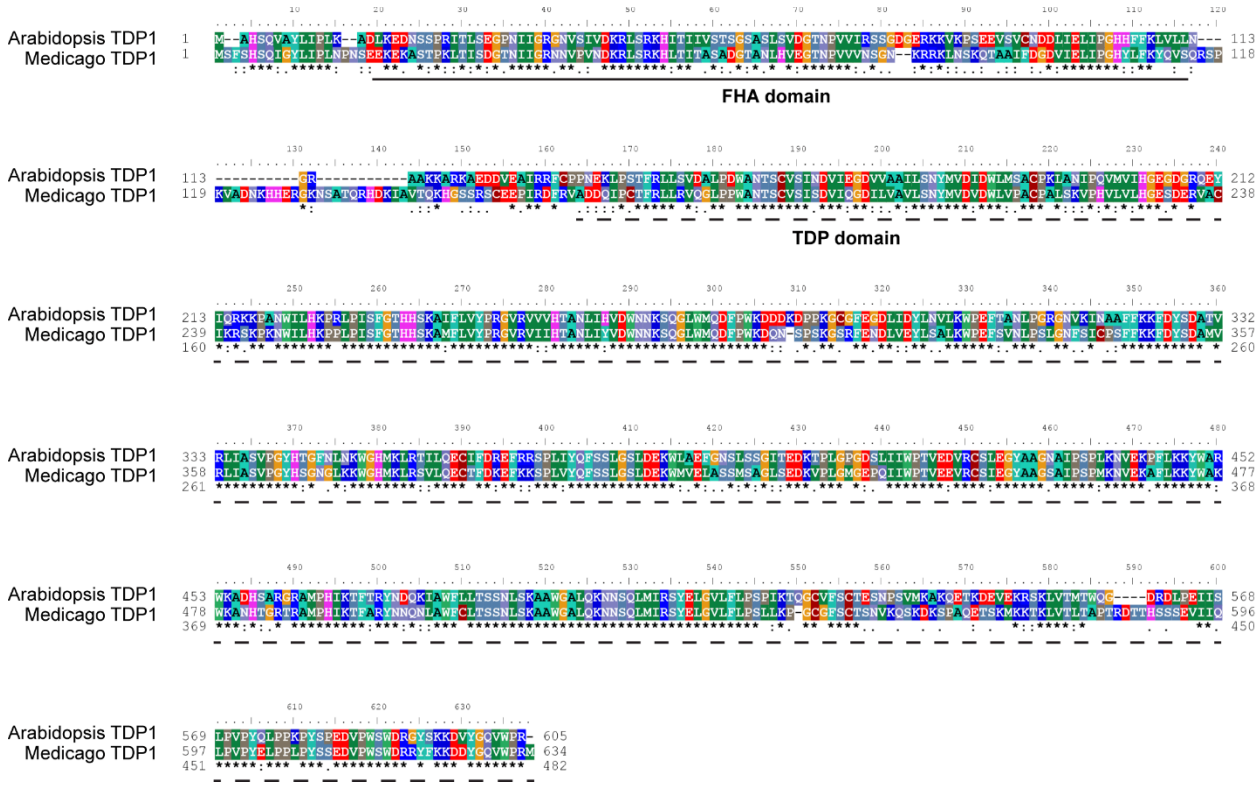

Fig. S8 A model illustrating AN-mediated transcriptional regulation of plant resistance to *P. syringea* and *B. cinerea*.
The AN-TDP1 interaction translocates AN into the nucleus to repress the expression of *MYB46*. On the other hand, the AN-TDP1 interaction releases the transcriptional repression of *WRKY33* by sequestering TDP1 away from the *WRKY33* promoter. The AN-mediated antagonistic regulation of *MYB46* and *WRKY33* leads to the antagonistic regulation of expression of genes in the SA pathway (*PR-1*, *PAL1*, *PAL2*, etc.) and JA/ET pathway (*PDF1.2*, *ACS2/6*, *ERF1A*, *ORA59*, etc.), which results in elevated resistance to *B. cinerea* and increased susceptibility to *P. syringae*.

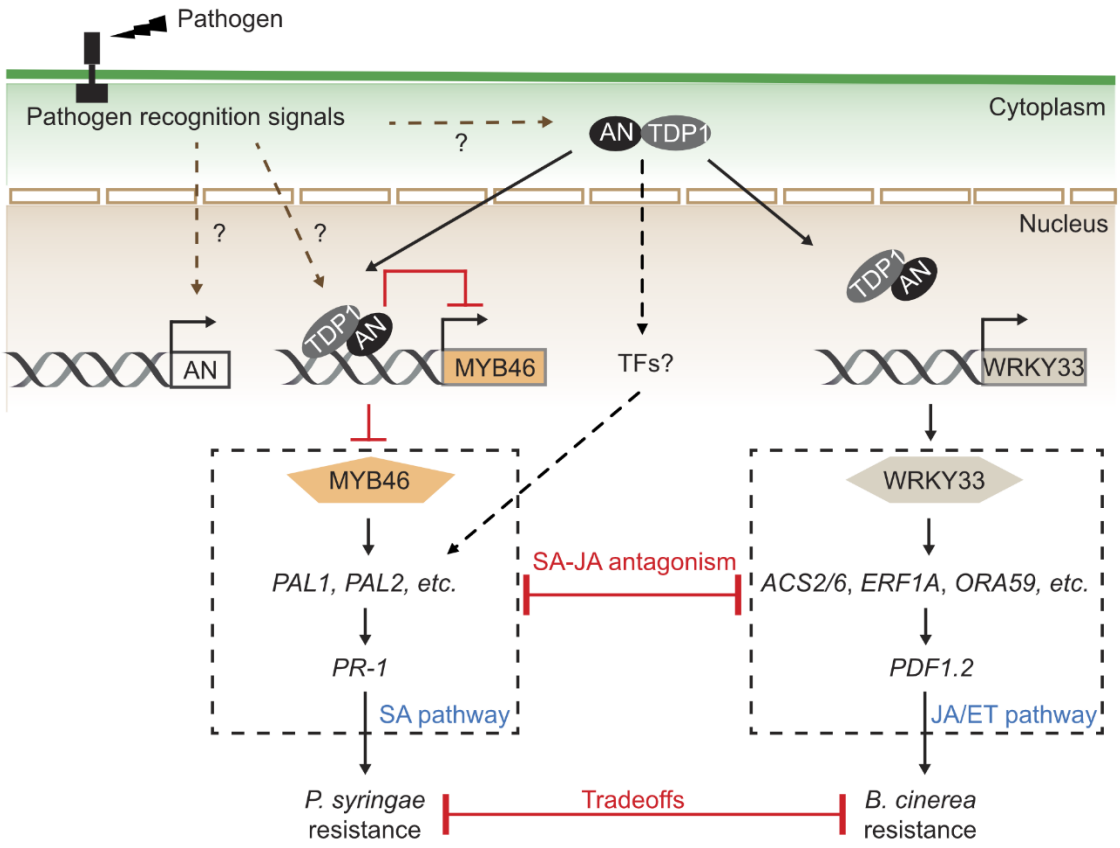

Fig. S9 Total amounts of salicylic acid (SA) and salicylic acid 2-O-glucoside (SAG) in Col-0, *an-t1*, and *an-t1 35S:AN* in 0 hpi and 24 hpi of *P. syringae* inoculation.
The unit of total amount is μg/g FW (sorbitol equivalents). Values represent means ± SEM, n ≥ 2. Statistical significance was determined by two tailed students t-tests (** $P<0.01$, * $P<0.05$, *ns* $P>0.05$).

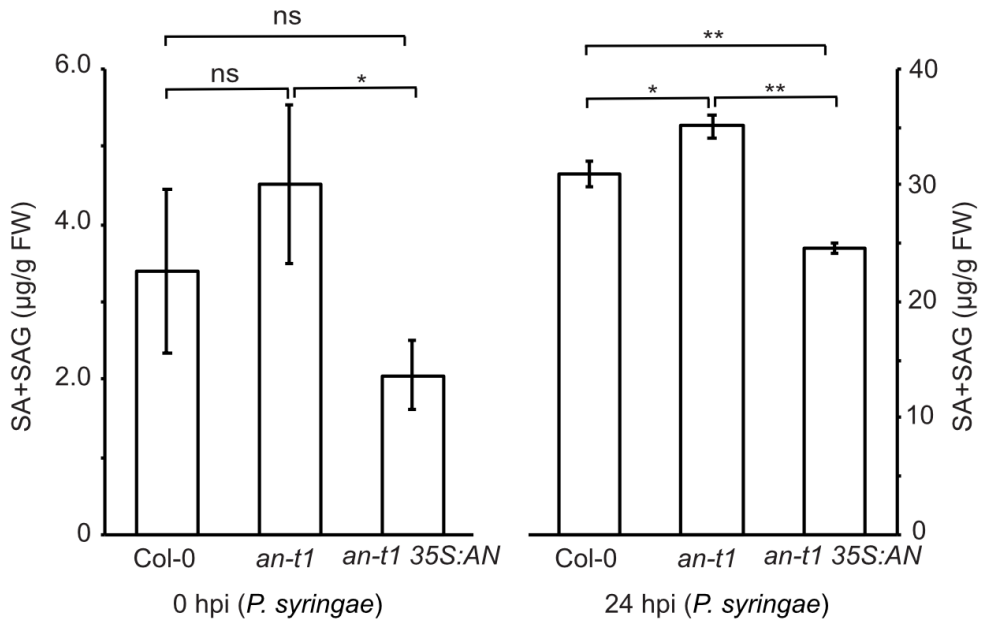

Fig. S10 Pathogen infection changes the transcription and nuclear accumulation of AN.
(**a**) qPCR analysis showing expression changes of *AN* in Col-0 at 0 and 6 hpi of *P. syringae* treatment.
(**b**) qPCR analysis showing expression changes of *AN* in Col-0 at 0, 24, 48, and 72 hpi of *B. cinerea* treatment. Gene expressions were normalized against the expression of *EF1α*. Gene expressions in 0 hpi of pathogen treatment were set as 1. Values represent means ± SEM, n = 3.
(**c**) western blotting indicating *P. syringae* treatment reduces the nuclear accumulation of AN. AN-Myc, HA-TDP1, UGPase (cytosolic marker), histone H3 (nuclear marker) are examined in cytosolic (C) and nuclear (N) fractions. AN-Myc signals were quantified using Image Lab (Bio-rad) and then normalized against corresponding nuclear or cytosolic marker to calculate the relative ratio of AN signals in cytosolic and nuclear fractions.

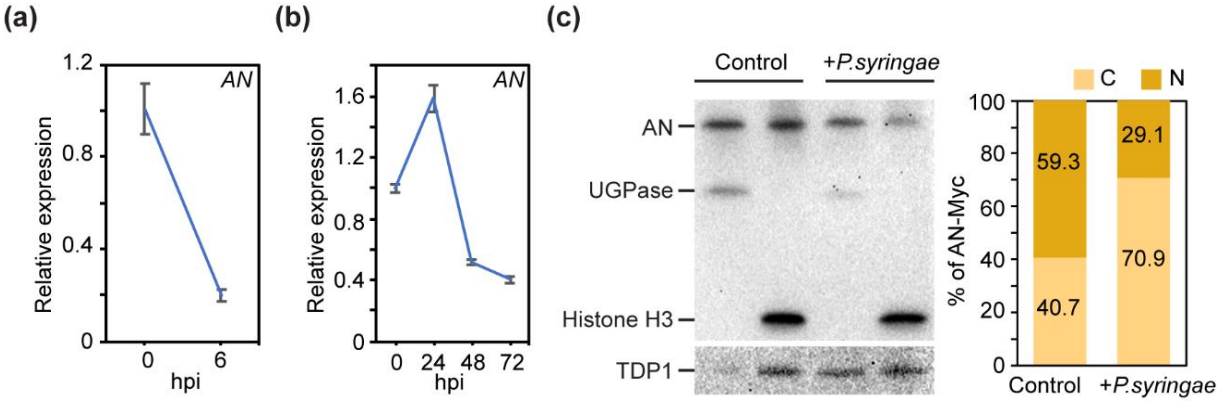

Fig. S11 *tdp1-2* mutant has increased resistance to *P. syringae* and *B. cinerea*.
(**a**) *P. syringae* inoculation of Col-0 and *tdp1-2*. Bacterial titers in 0 hpi and 72 hpi are shown in the bar graph. Values represent means ± SEM, n = 15.
(**b**) *B. cinerea* inoculation of Col-0 and *tdp1-2*. Scale bar: 1 mm. Lesion areas (% of total leaf area) in 0 hpi and 72 hpi are shown in the bar graph. Values represent means ± SEM, n = 40. Statistical significance was determined by two tailed students t-tests against Col-0 (** $P<0.01$, * $P<0.05$, *ns* $P>0.05$).

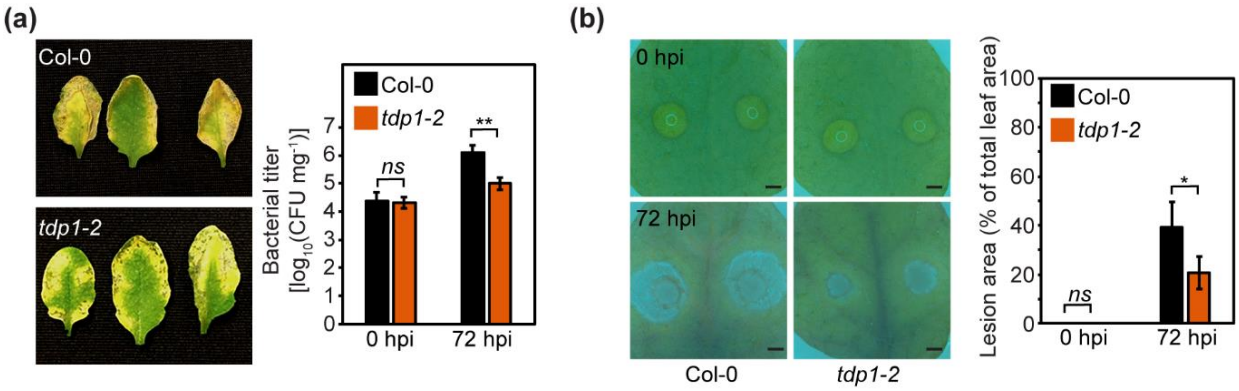

Table S1 Primers used in this study.

Name	Sequence (5'-3')	Applications
AN CDS-F	CACCATGAGCAAGATCCGTTCGTCTG	AN cloning
AN-CDS-R	TTAATCGATCCAACGTGTGATA	
TDP1-pGBKT7-F	CACCATGGCTCACTCTCAGGTTGCTT	TDP1 cloning
TDP1-pGBKT7-R	ATGTCGACCTATCTTGGCCAGACTTGTCCA	
P-MYB46-F	CACCAAAAGGAGTATAACATTTTCTT	Promoter cloning
P-MYB46-R	CTATCTTGGCCAGACTTGTCCA	
P-WRKY33-F	CACCCAAACTCACTTCTAAATACTAA	Promoter cloning
P-WRKY33-R	CTGTATATTTGTTGGTTATGTC	
AN qRT-F	GTCTTCCCCAAATCAGCTTG	qPCR
AN qRT-R	CCGAGAGGTCTTTCGCATAC	
TDP1 qRT-F	TTACCTGGCCGTGGTAATGT	qPCR
TDP1 qRT-R	GGTATCCAGGGACTGAAGCA	
EF1α qRT-F	TGAGCACGCTCTTCTTGCTTTCA	qPCR
EF1α qRT-R	GGTGGTGGCATCCATCTTGTTACA	
PR-1 qRT-F	TTCTTCCCTCGAAAGCTCAA	qPCR
PR-1 qRT-R	AAGGCCCACCAGAGTGTATG	
PR-3 qRT-F	GGCAAACGCTACTACGGAAG	qPCR
PR-3 qRT-R	GCAACAAGGTCAGGGTTGTT	
AtPAL1 qRT-F	GTGTCGCACTTCAGAAGGAA	qPCR
AtPAL1 qRT-R	GGCTTGTTTCTTTCGTGCTT	
AtPAL2 qRT-F	GTGCTACTTCTCACCGGAGA	qPCR
AtPAL2 qRT-R	TATTCCGGCGTTCAAAAATC	
AtPAL3 qRT-F	CAACCAAACGCAACAGCA	qPCR
AtPAL3 qRT-R	CTCCAGGTGGCTCCCTTTTA	
AtPAL4 qRT-F	GGTGCACTTCAAAATGAGCT	qPCR
AtPAL4 qRT-R	CAACGTGTGTGACGTGTCC	
AtCESA3 qRT-F	ACAGCCAACACAGTGCTCTC	qPCR
AtCESA3 qRT-R	TGGTACCCATTTACGAGCAA	
AtCESA4 qRT-F	CTGTGGTTATGAAGAGAAGACTGAA	qPCR
AtCESA4 qRT-R	TGCATTCTAAATCCAGTGAGGA	
AtCESA7 qRT-F	TTGTTGCAGGCATCTCAGATG	qPCR
AtCESA7 qRT-R	GCAGTTGATGCCACACTTGGA	

AtCESA8 qRT-F	TGAGCTTTACATTGTCAAATG	qPCR
AtCESA8 qRT-R	GCAATCGATCAAAAGACAGTT	
MYB46 ChIP-P1-F	CCATGACCGATCAACTAACG	ChIP-qPCR,
MYB46 ChIP-P1-	GAACCCTGGCTCTTTTTCAA	
MYB46 ChIP-P2-F	CCCAGAATTGTAAGCAAACCA	ChIP-qPCR
MYB46 ChIP-P2-	GGATCCATTGATGTGAACGA	
WRKY33 ChIP-F	TCATACGTGTCAGAACGAGACA	ChIP-qPCR
WRKY33 ChIP-R	CAGACCTTGTGGCCTTGACT	
ACS6 ChIP-F	ATGAAAAGAATTCCGGTCCA	ChIP-qPCR
ACS6 ChIP-R	TTGGAAAAGAAATGAGACATCAA	
ACS2 ChIP-F	AAATTCCCTTCCCAAATGGT	ChIP-qPCR
ACS2 ChIP-R	ACAAGCGAACCAAGGAAAAA	
ERF1A ChIP-F	CCAATCACAACATTGCTTCG	ChIP-qPCR
ERF1A ChIP-R	AAACACGTGCGTTTTATCCA	
ACTIN ChIP-F	CGTTTCGCTTTCCTTAGTGTTAGCT	ChIP-qPCR
ACTIN ChIP-R	AGCGAACGGATCTAGAGACTCACCTTG	
MYB58 ChIP-F	CGTCGAGAAATGTTGTGTGTG	ChIP-PCR
MYB58 ChIP-R	TGGGTCCTATAACCCTGTAACAT	
MYB103 ChIP-F	TTTATAAAATAATAGGTCAACCTCGAA	ChIP-PCR
MYB103 ChIP-R	CATGTATTATCCACTGTTTTCCTCT	
MYB63 ChIP-F	TGCATCGGTGTTAGAAGGAA	ChIP-PCR
MYB63 ChIP-R	TTGTTGAGTGGGAAAAGGTTG	
MYB55 ChIP-F	TCTACAATACTACCAAACAGAACCAAA	ChIP-PCR
MYB55 ChIP-R	GAGAGGAGGATTTGGGGAAT	
MYB20 ChIP-F	GATTGAGCTCATAGTCCCGTTT	ChIP-PCR
MYB20 ChIP-R	TTTTCTTATTTCGTGTCACTTTGG	
NAC073 ChIP-F	TTTGTTTGATCAGTCTTTGTCCA	ChIP-PCR
NAC073 ChIP-R	TTGCTTGGGTTTTAAGTTTGG	
WRKY53 ChIP-F	CACTCTGGCCCTATACTTCCTT	ChIP-PCR
WRKY53 ChIP-R	TTGACCAAATGACCAAACCA	
WRKY26 ChIP-F	ATTCAGCCGCCTTACACAAA	ChIP-PCR
WRKY26 ChIP-R	TCCAAGGAAAAGCAAGCAAT	
WRKY22 ChIP-F	ACAAACCGAACCGCTTTTTA	ChIP-PCR
WRKY22 ChIP-R	AGAACAAACCGCTGCAAACT	

WRKY40 ChIP-F	GCCGGCTATGCTATAACGAA	ChIP-PCR
WRKY40 ChIP-R	TATGACGCTCTCCACGTTTG	