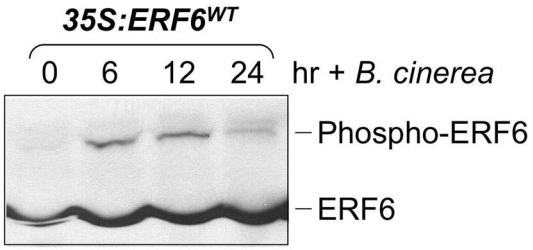
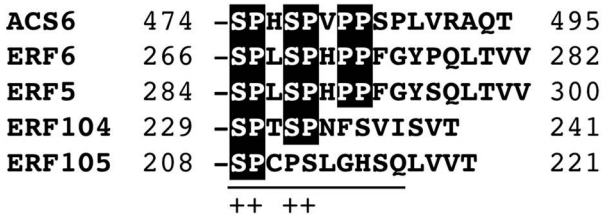
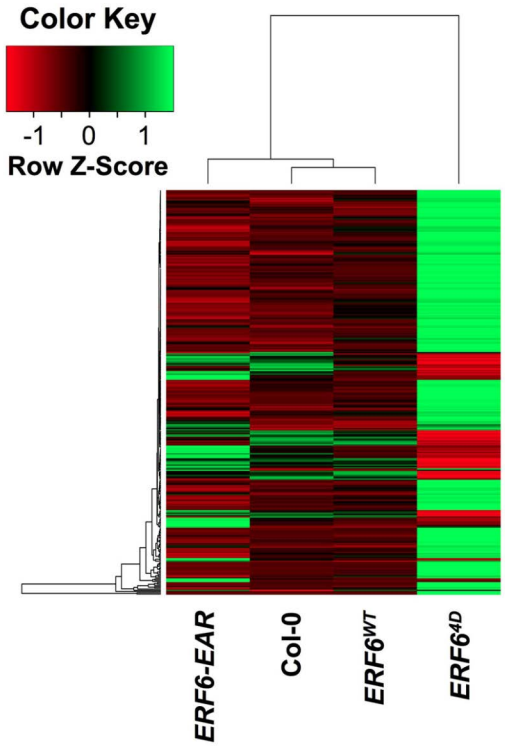


Supplemental Figure 1. Phos-Tag mobility shift detection of in vivo phosphorylated ERF6 in *35S:ERF6*^{WT} plants after *B. cinerea* inoculation.

Protein extracts from *35S:ERF6*^{WT} seedlings treated with *B. cinerea* for different times were separated in an SDS-PAGE gel with Phos-tag reagent. The samples are the same as the ones shown in Figure 2 (the first panel). After being transferred to a nitrocellulose membrane, myc-tagged ERF6^{WT} protein was detected by an anti-myc antibody.

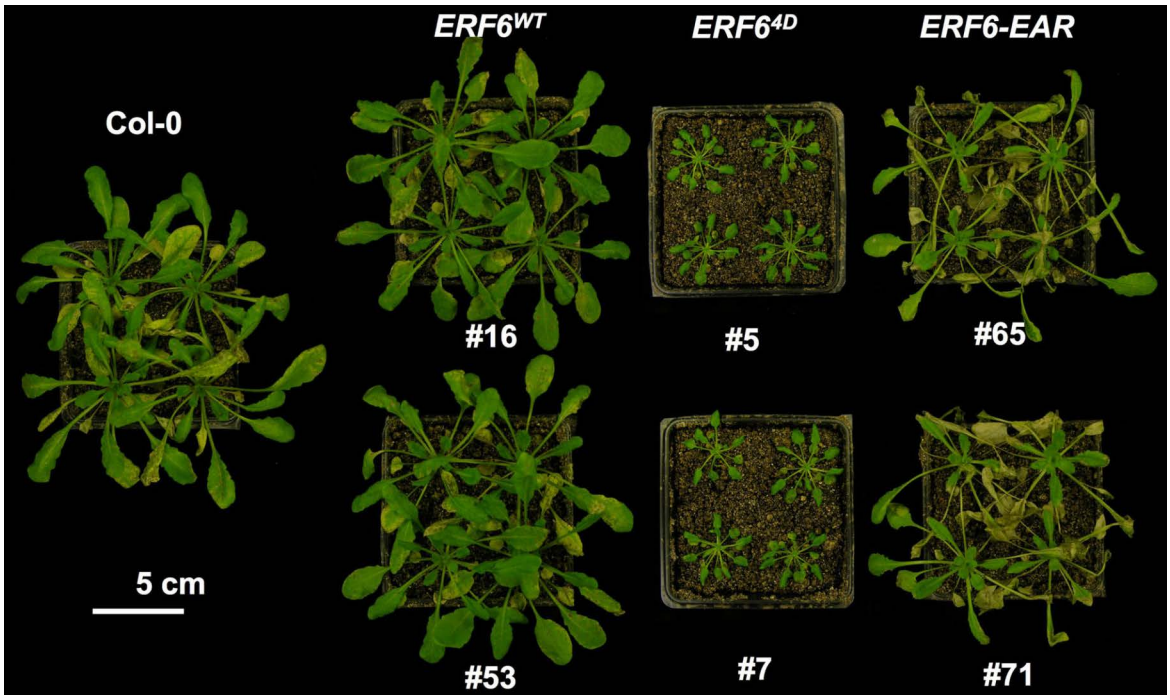


Supplemental Figure 2. Alignment of the C-termini of ERF6, ERF5, ERF104, and ERF105 with the C-terminus of ACS6 that contains the MPK3/MPK6-phosphorylation sites. Plus symbols indicate the conserved MAPK-phosphorylation sites.



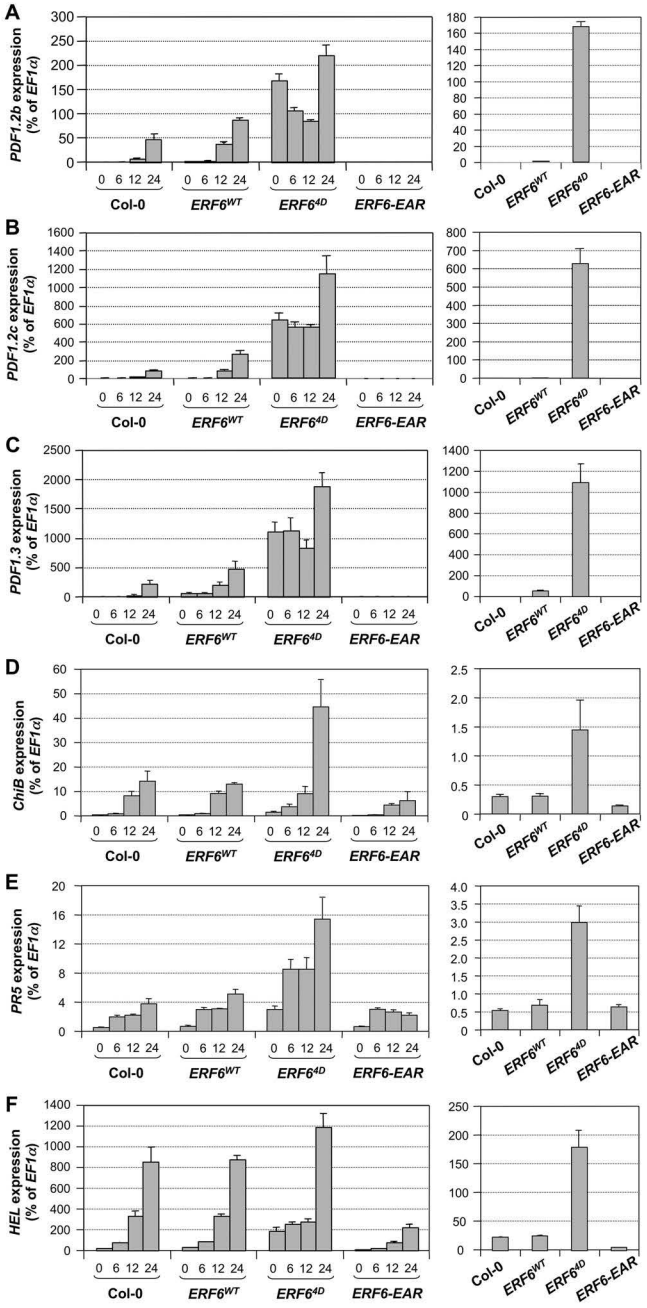
Supplemental Figure 3. Differential gene expression in twelve-day-old Col-0, *35S:ERF6*^{WT}, *35S:ERF6*^{4D}, and *35S:ERF6-EAR* seedlings.

Differential gene expression patterns of Col-0, *35S:ERF6*^{WT}, *35S:ERF6*^{4D}, and *35S:ERF6-EAR* seedlings based on Illumina RNA-Seq data. The heat map includes all genes that were differentially expressed in *ERF6*^{4D} seedlings (320 genes, p-value <= 0.001). The color key represents Z-Score transformed RPKM values of 320 specific genes. Green indicates an increase in expression, red indicates a decrease in expression; color intensity indicates the magnitude of the effect.



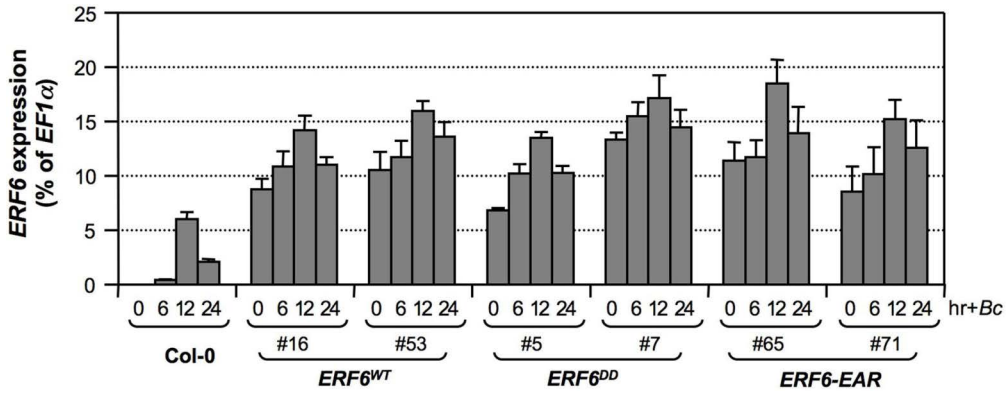
Supplemental Figure 4. Expression of phospho-mimicking *ERF6*^{4D} confers strong resistance to *B. cinerea*; whereas expression of a dominant negative *ERF6-EAR* results in an opposite phenotype.

Four-week-old soil grown Col-0, *35S:ERF6*^{WT}, *35S:ERF6*^{4D}, and *35S:ERF6-EAR* plants were spray-inoculated with *B. cinerea* spore suspension (1x10^5 spores/ml). Images were taken at 3 days after inoculation.



Supplemental Figure 5. Opposing effects of *ERF6*^{4D} and *ERF6-EAR* transgenes on defense gene expression.

Twelve-day-old Col-0, *35S:ERF6*^{WT}, *35S:ERF6*^{4D}, and *35S:ERF6-EAR* seedlings were inoculated with *B. cinerea* spores, and samples were collected at indicated times. Defense gene expression was quantified by real-time qPCR, and the transcript levels were calculated as a percentage of the *EF1α* transcript. Basal level expression is shown in panels on the right. Error bars indicate standard deviations (n = 3).



Supplemental Figure 6. The expression levels of endogenous *ERF6* and transgenes before and after *Botrytis* infection.

Twelve-day-old Col-0, *35S:ERF6*^{WT}, *35S:ERF6*^{4D}, and *35S:ERF6-EAR* seedlings (two lines each) were inoculated with *B. cinerea* spores, and samples were collected at indicated times. Defense gene expression was quantified by real-time qPCR, and the transcript levels were calculated as a percentage of the *EF1α* transcript. Primers used for qPCR detect both endogenous *ERF6* and transgene transcripts. Error bars indicate standard deviations (n = 3).

Supplemental Table 1. Primers used in this study.

Primers used for cloning of *ERF6* gene

ERF6-F1: 5'-catatggctacaccaaacgaagtatc-3'

ERF6-B1: 5'-attcaaacaacggtcaattgtgg-3'

ERF6-B2: 5'-attcaaacaacggtcaattgtgg-3'

Primer pairs used for mutagenesis

1A-F1/B1: 5'-ggcagccatatggctGcTccaaacgaagtatcag-3' and its complementary primer

2A-F1/B1: 5'- ctcctcgacgaattgGctccgttgcctactac-3' and its complementary primer

3A-F1/B1: 5'-gaaataatcgatctcgtcGctcccaaaccggag-3' and its complementary primer

4A-F1/B1: 5'-agcatgccgcttttaGcTccgttaGctccacacccaccg-3' and its complementary primer

4D-F1/B1: 5'-agcatgccgcttttaGATccgttaGAtccacacccaccg-3' and its complementary primer

Mutated nucleotides are marked with upper case letters.

Primer pairs used for qPCR

EF1α (At5g60390): 5'-tgagcacgctcttcttgctttca-3' and 5'-ggtggtggcatccatcttgttaca-3'

ERF6 (At4g17490): 5'-attgtctccgttgcctacta-3' and 5'-ggtttggtttcaaattcaga-3'

PDF1.1 (At1g75830): 5'-tggctaagtctgctaccatcgtt -3' and 5'-agatccatgtcgtgctttctca -3'

PDF1.2a (At5g44420): 5'-gcttccatcatcacccttatctt-3' and 5'-tagttgcatgatccatgtttgg -3'

PDF1.2b (At2g26020): 5'-tgcttccatcatcacctttatcta-3' and 5'-gcaagatccatgttttgctcct-3'

PDF1.2c (At5g44430): 5'-tgctaccatcatcaccttcctt-3' and 5'-taacatgggacgtaacagatacat-3'

PDF1.3 (At2g26010): 5'-ctaagtctgctgccatcatcact-3' and 5'-atgttttgccccctcaaggt-3'

ChiB (At3g12500): 5'-cagacttccatgaaactaca-3' and 5'-ctgttcttgcttgaaacagta-3'

HEL (At3g04720): 5'- cggcaagtgtttaagggtgaag-3' and 5'-ttgttgatagccaaaaccatcg-3'

PR5 (At1g75040): 5'-aggctgtgtctctgacctcaa-3' and 5'-acaagtttccggcttatcgtt-3'

Primer pairs used for ChIP-qPCR

PDF1.2a: 5'-ttgtcctaaccgcgagaatc-3' and 5'-cggctggttaatctgaatgg-3'

PDF1.2b: 5'-ttgcatcttgtccaagtcaga-3' and 5'-tgatggcttgtttatctaccaca-3'

Supplemental Methods

Phospho-protein mobility shift assay

Phos-tag™ reagent (NARD Institute, Ltd. http://www.phos-tag.com/english/index.html) was used for the phospho-protein mobility shift assay to detect *in vivo* phosphorylated ERF6 protein. Proteins (10 μg) were separated in a 10% SDS-PAGE gel containing 100 μM Phos-tag™ and 200 μM $MnCl_2$. After proteins were transferred to a nitrocellulose membrane, 4myc-tagged ERF6 was detected by using the anti-myc antibody as previously described (Mao et al., 2011).

References

Mao, G., Meng, X., Liu, Y., Zheng, Z., Chen, Z., and Zhang, S. (2011). Phosphorylation of a WRKY transcription factor by two pathogen-responsive MAPKs drives phytoalexin biosynthesis in *Arabidopsis*. Plant Cell **23,** 1639-1653.