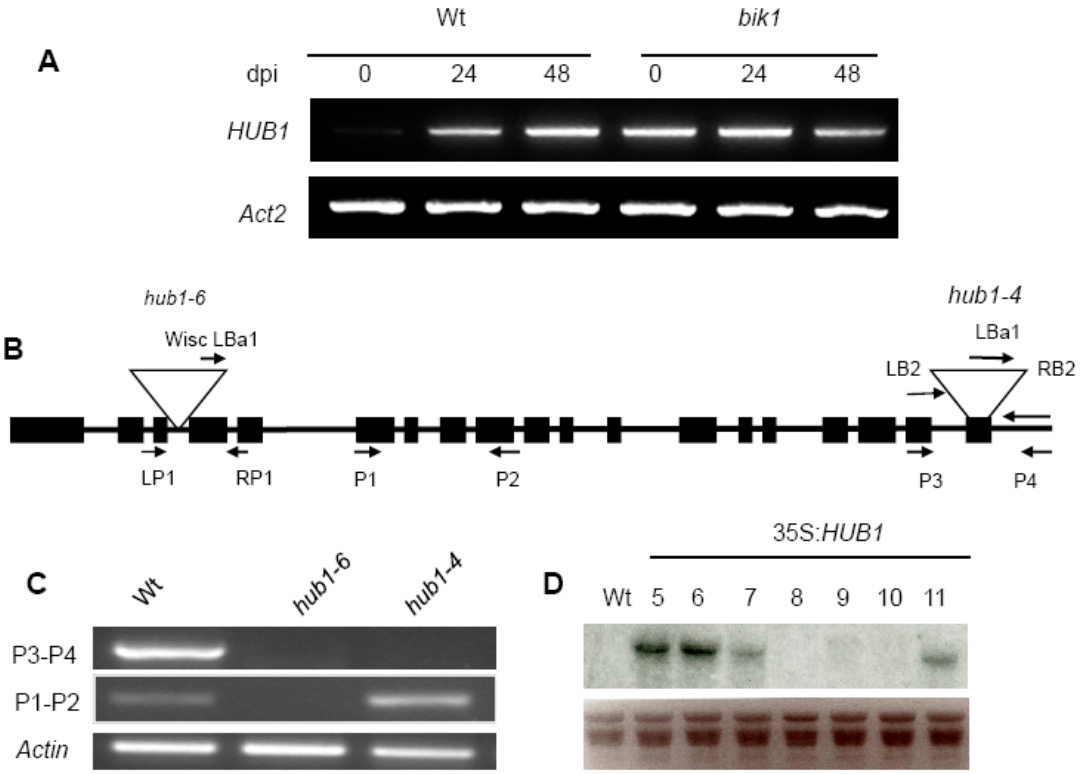
Supplemental data. Dhawan et al (2009). HISTONE MONOUBIQUITINATION 1 interacts with a subunit of the Mediator complex and regulates defense against necrotrophic fungal pathogens.



Supplemental Figure 1. Expression of *HUB1*, characterization of the *hub1* mutations and the 35S:*HUB1* plants.

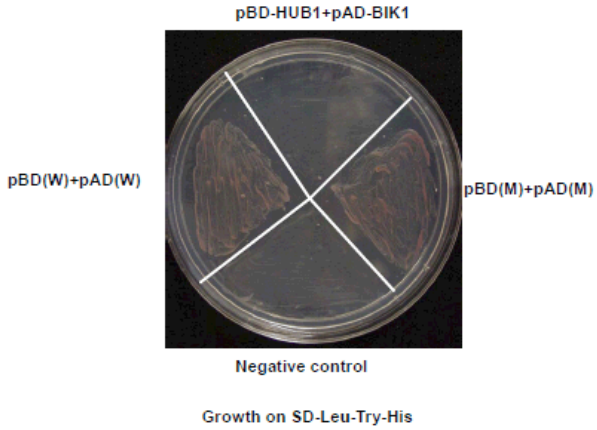
(a) RT-PCR data showing increased basal expression of *HUB1* in the *bik1* mutant.

(b) Genomic organization of the *hub1-4* and *hub1-6* T-DNA insertion alleles.

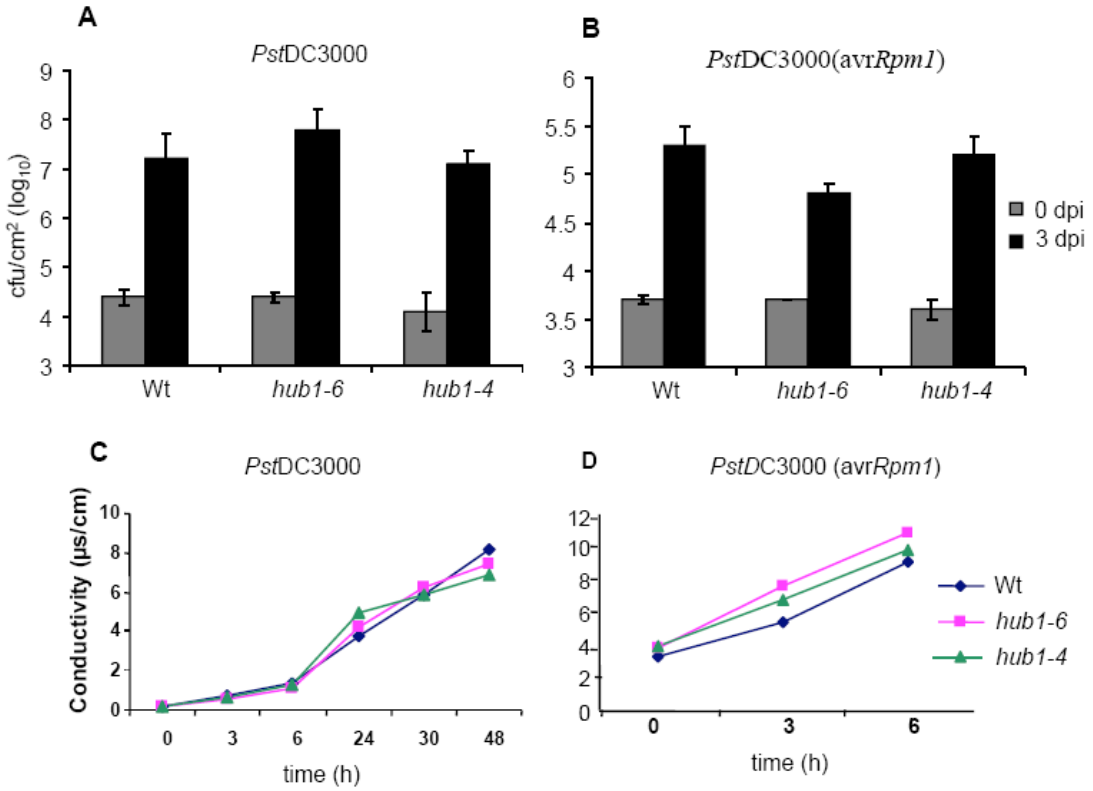
(c) RT-PCR from wild type, *hub1-4* and *hub1-6* showing altered transcripts of the *HUB1* gene in the *hub1* mutant alleles.

(d) RNA-blot of 35S:*HUB1* transgenic plants showing selected lines with higher constitutive *HUB1* expression. In (d) 10 µg of total RNA was loaded. Numbers above the lanes show independent transgenic lines.

Arabidopsis ATH1 oligonucleotide microarray was used to identify a list of *Botrytis* induced genes. The expression profiling experiment was designed to compare expression at 0 and 24 hours after inoculation with *B. cinerea*. The expression data was analyzed to determine changes in expression profiles after *B. cinerea* infection as described (Abuqamar et al., 2006). Initially, genes with >2.5 fold expression in wild type plants at 24 h after *Botrytis* infection relative to expression in healthy plants were selected based on data from two biological replicates. Among these, *HUB1* was identified due to its increased *Botrytis* induced expression at 24 h after inoculation in wild type plants. The expression of these induced genes was further examined in the Arabidopsis *bik1* mutant in a microarray experiment that compared expression profiles in wild type and *bik1* plants at 0 and 24 hours after inoculation with *B. cinerea*. This microarray array experiment was performed using RNA pooled from three independent disease assays hybridized to single ATH1 arrays for each time point. The HUB1 expression data was further confirmed by RT-PCR experiments. *HUB1* was constitutively expressed in the *bik1* mutant.

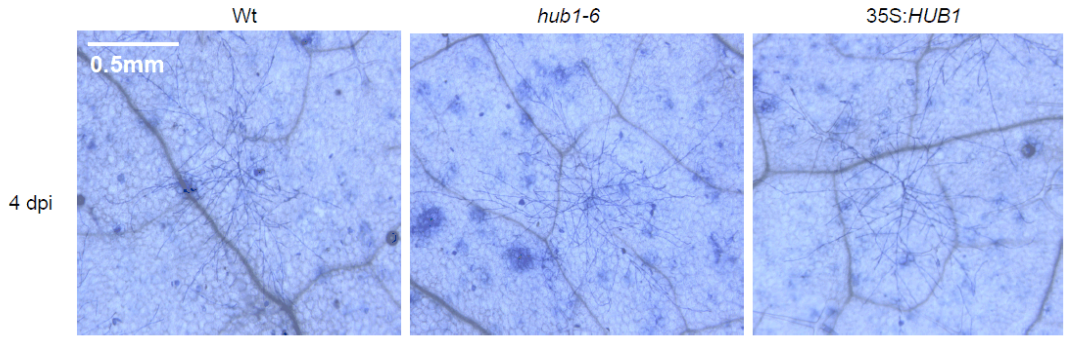


Supplemental Figure 2. HUB1 does not interact with BIK1 in yeast two hybrid assays. The picture shows growth of yeast cells on selective media (-Leucine, Tryptophan, -Histidine) showing lack of growth of yeast strains containing HUB1 and BIK1. The positive and negative controls from the Stratagene yeast two hybrid kit are shown for comparison.

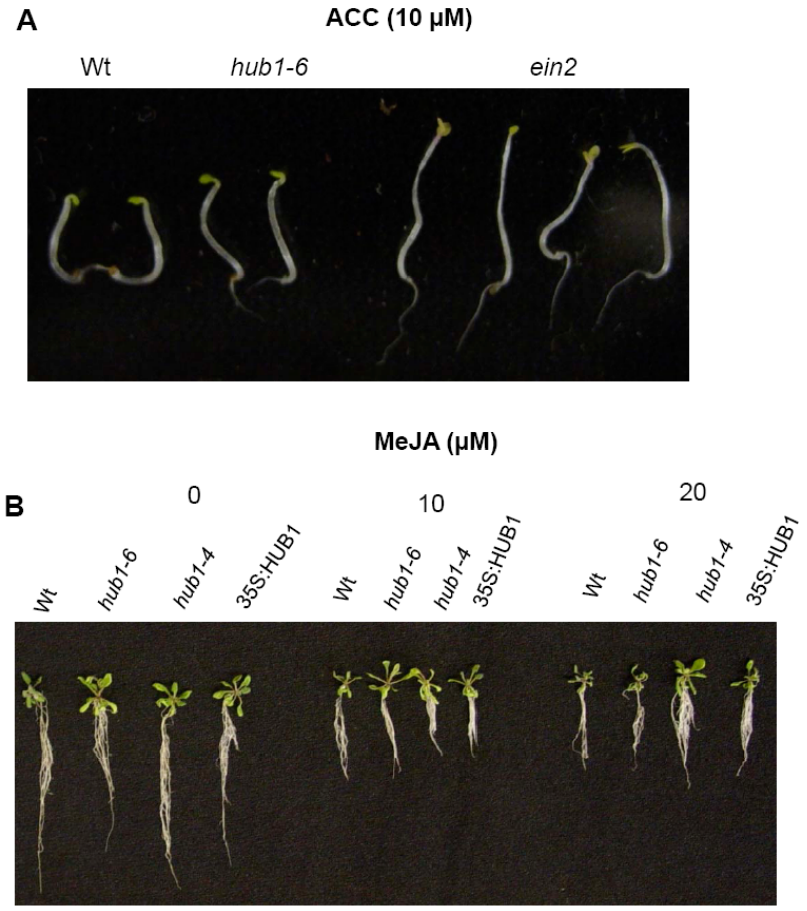


Supplemental Figure 3. HUB1 has no role in resistance to *P. syringae*
Growth of (a) virulent, (b) avirulent strains, and
Electrolyte leakage after inoculation with (c) virulent, and (d) avirulent strains of *P. syringae* pv tomato in *hub1* and wild type plants.

The diseases assays were repeated at least three times. *cfu*, colony forming units.



Supplemental Figure 4. The *hub1* plants show no altered responses to *Erysiphe cichoracearum* infection. Pictures show representative leaves from inoculated plants stained with trypan blue at 4 dpi to reveal fungal growth.

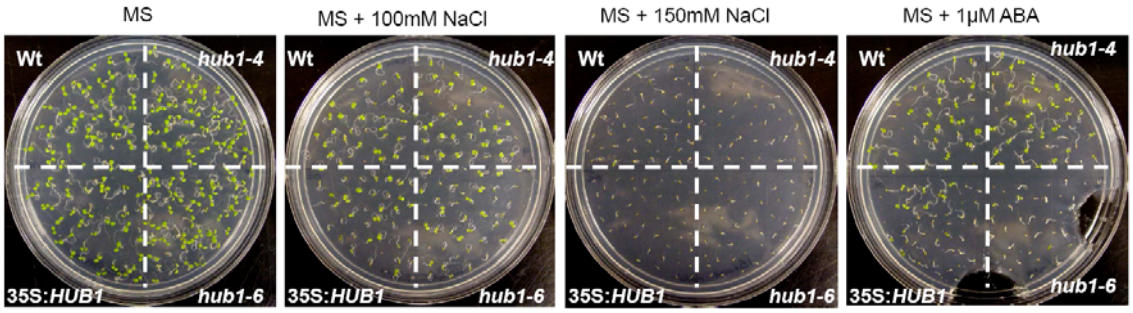


Supplemental Figure 5. *hub1* shows wild type responses to plant hormones.

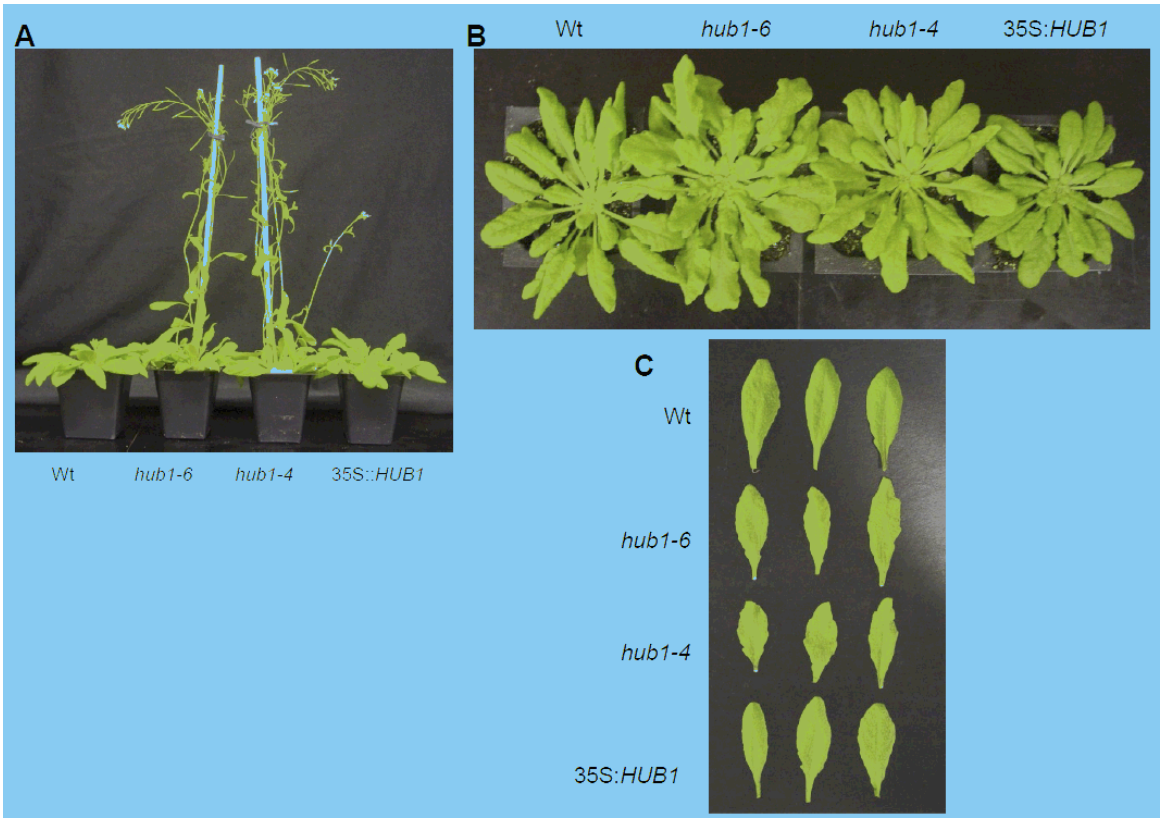
(a) Lack of altered triple responses in *hub1* seedlings in response to ACC.

(b) Lack of altered responses to MeJA.

Pictures are from 4 days after ACC or 10 days after transfer to MeJA.

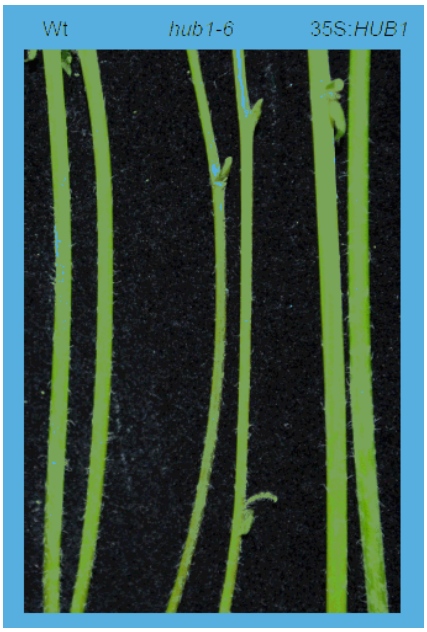


Supplemental Figure 6. *hub1* shows wild type responses to NaCl and ABA. Pictures were taken at 6 days after the start of the experiment.

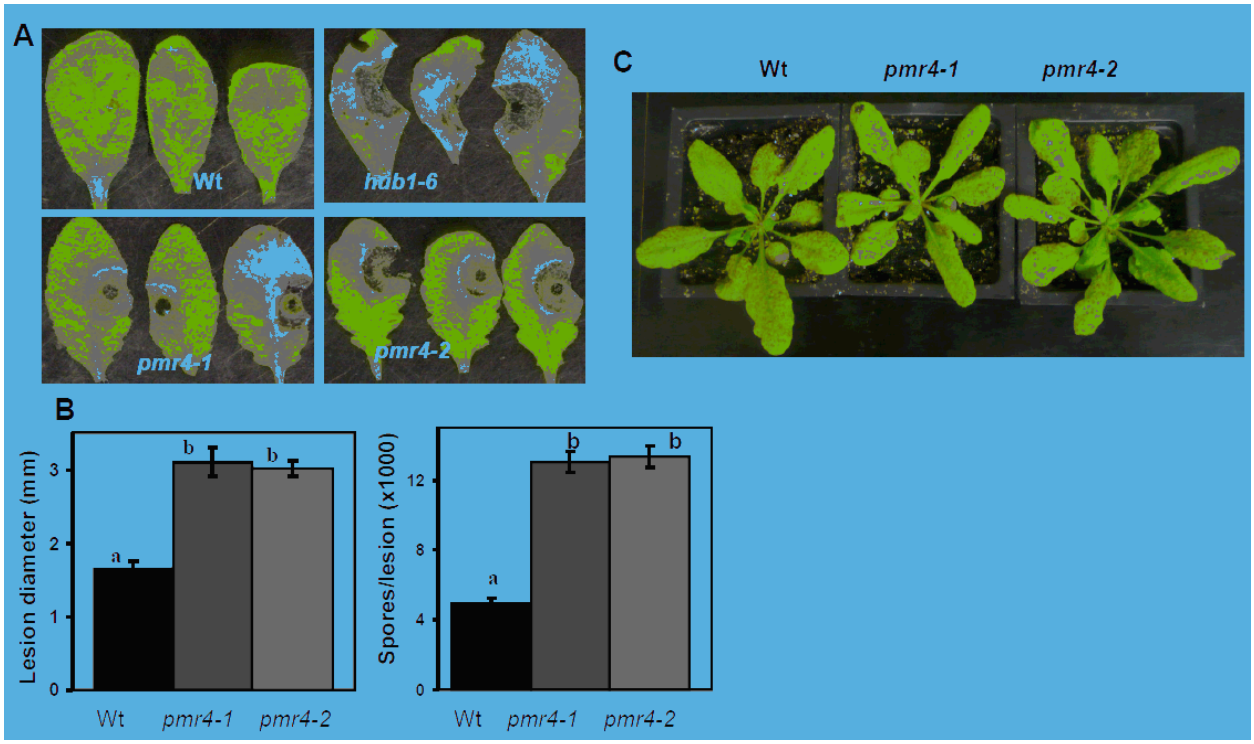


Supplemental Figure 7. Flowering, growth and senescence of *hub1*.

(a) *hub1* plants showing early flowering under 12 h light/dark photoperiod,
(b) Six week old *hub1* plants grown under short day (SD) conditions showing vigorous vegetative growth,
(c) *hub1* leaves show no altered senescence in detached leaves. The pictures in (c) were taken 7 days after leaves were detached and the leaves remained green.

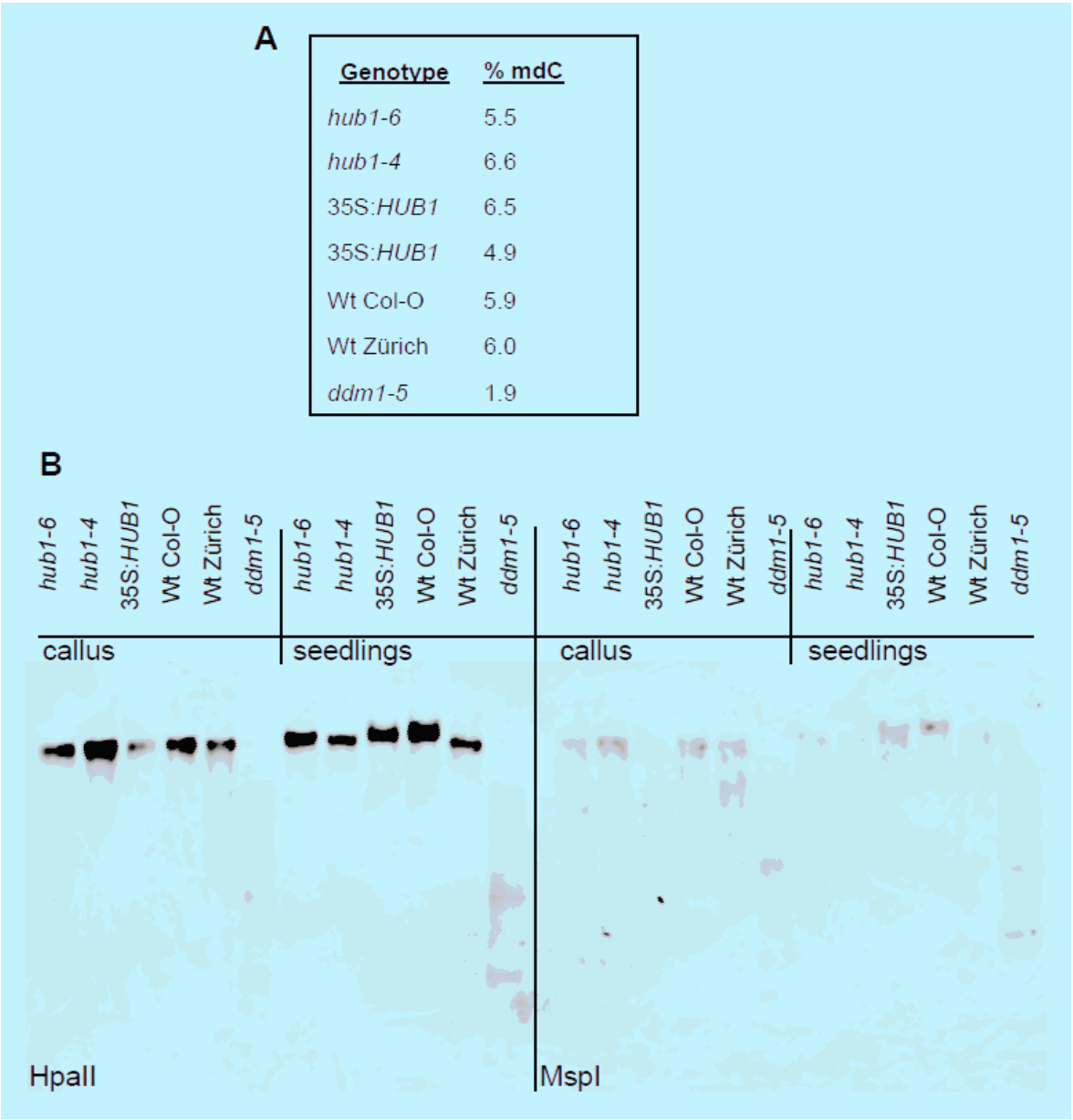


Supplemental Figure 8. Reduced stem thickness in *hub1* plants. Pictures were taken close to maturity when the stem has fully grown.



Supplemental Figure 9. The *PMR4* gene is required for resistance to *A. brassicicola*

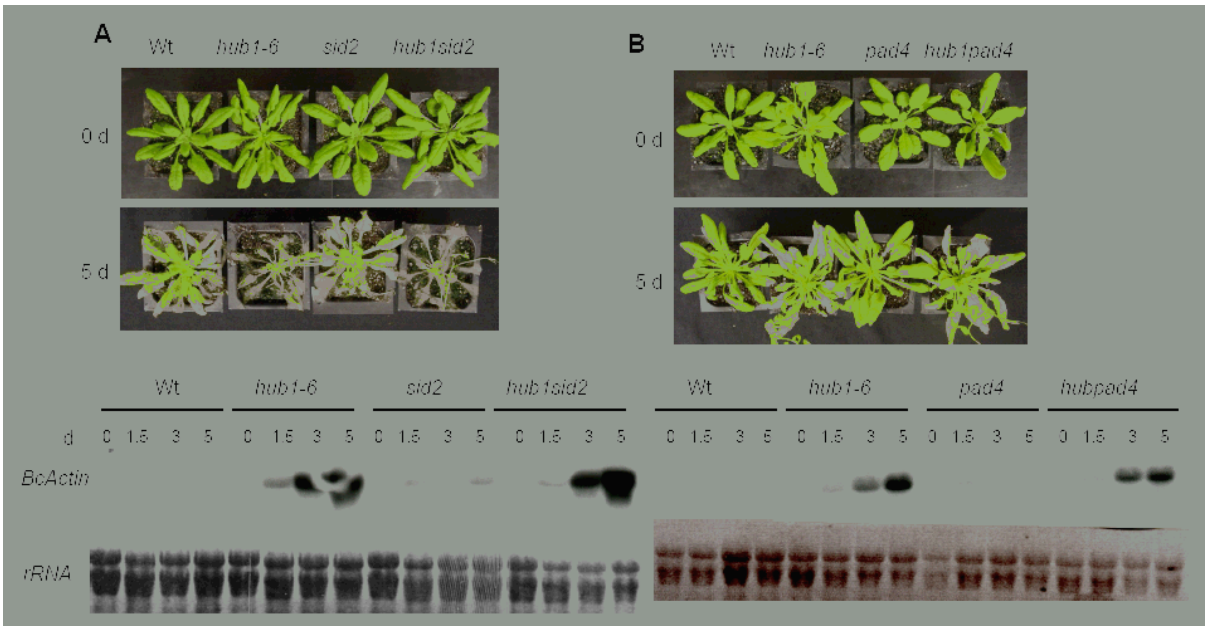
(a) Disease symptoms, (b) Disease lesion size (left), sporulation (right) in *pmr4* alleles at 4 dpi with *A. brassiciola*, (c) *B.cinerea* disease symptom in *pmr4* mutant alleles at 3 dpi after spray inoculations.



Supplemental Figure 10. Global and locus specific DNA methylation

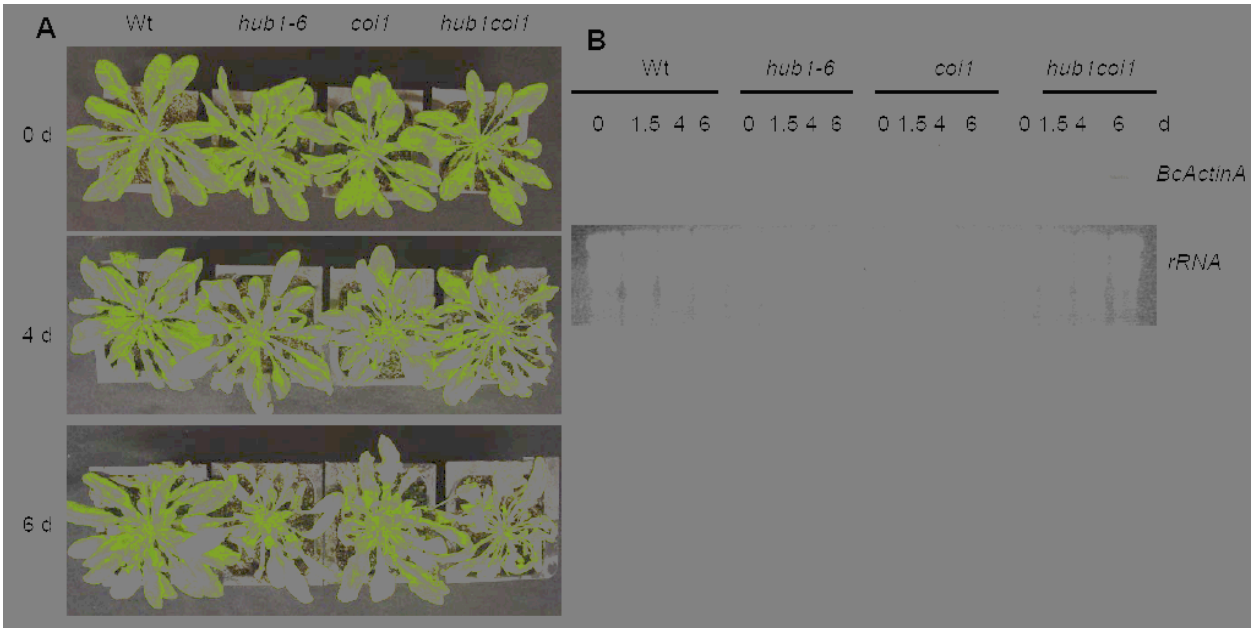
(a) Total cytosine methylation was determined from genomic DNA using HPLC as described by (Rozhon et al., 2008). The global DNA methylation in wild type plants is about 6%. A severe reduction in cytosine methylation could be detected in the control plant (*ddm1-5*). No effect in *hub1* plants was determined on the genome wide methylation level.

(b) DNA gel blot analysis using the methylation-sensitive restriction enzymes (HpaII, blocked by mCG and MspI blocked by mCHG) and a probe specific for TSI. TSI is significantly methylated in wildtype background whereas the *ddm1-5* control shows severe reduction in DNA methylation. HUB1 does not affect cytosine methylation at the TSI loci; Wt Zürich: *A. thaliana* accession Zürich; *ddm1-5*: mutant with reduced DNA methylation at pericentromeric repeats (Kakutani et al., 1995).

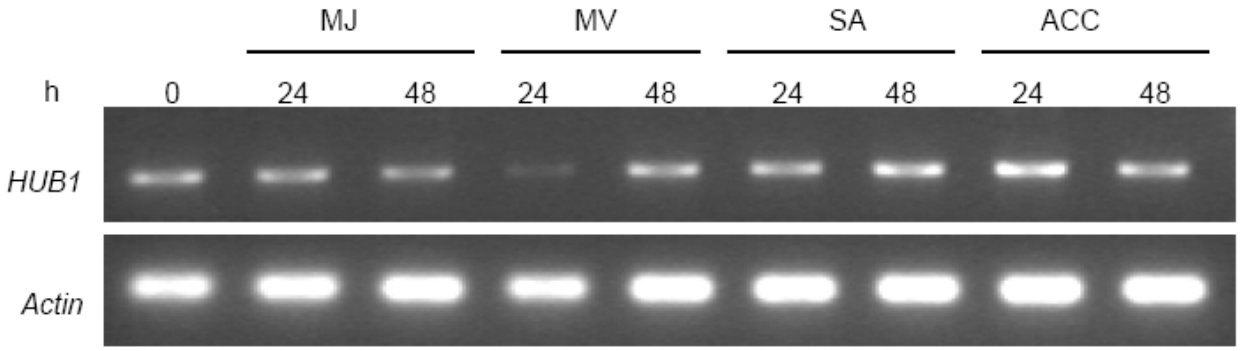


Supplemental Figure 11. SA has only a marginal effect on the functions of HUB1 in limiting *Botrytis* growth and disease symptoms.

(a, b, top panels) Single and double mutants showing *Botrytis* disease symptom
(a, b, lower panels) RNA-blots from *Botrytis*-inoculated single and double mutants showing the accumulation of *Botrytis ActinA* RNA as a measure of fungal growth. d, days after inoculation.



Supplemental Figure 12. COI1 function in *Botrytis* resistance is additive to HUB1.
(a) Single and double mutants showing *B. cinerea* disease symptom, (b) RNA-blot from *B. cinerea* inoculated single and double mutants showing the accumulation of *B. cinerea ActinA* RNA.
B. cinerea inoculation was done at 10^5 spores/ml. d, days after inoculation, *BcActinA, Botrytis cinerea ActinA* gene.



Supplemental Figure 13. RT-PCR showing the *HUB1* gene expression in response to plant hormones and Methyl viologen (paraquat)

A

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MmMED21 MADRLTQLQDAVNSLADQFCNAIGVLQQCGPPASFS------------------------
HSMED21 MADRLTQLQDAVNSLADQFCNAIGVLQQCGPPASFN------------------------
DrMED21 MADRLTQLQDAVNSLADQFCNAIGVLQQCAPPASFS------------------------
AgMED21 MADRLTQLQDTVNQQAEHFCNSIGILQQCSVPSKFAGFERTGSQT---------------
CeMED21 MADRMTQLQDMINEMAGLMTNAIGVLQATAPPCEFG------------------------
AtMED21 -MDIISQLQEQVNTIAAITFNAFGTLQRDAPPVQLSPNYPEPPAT---------------
PpMED21 -MDIIAQLQEHASAIAYIAFNAAGTIQRDAPPSQLAQDYPKP-----------------
SbMED21 -MDIITQLQDQLDEMAVLAVNTFGTLQRDAPPDRLSNSYPDPLNP------NPKPED-VS
OsMED21 -MDIISQLQEQLNEMAMVAVNTFGTLQRDAPPVRLSNNYPDPLNPAAAAAANPNPDDPAQ
ScMED21 MTDRLTQLQICLDQMTEQFCATLNYIDKNHGFERLTVNEP--------------------
SPMED21 MACRCTQLQDTIDEVATQFYSSIHYLSSHHDFVPLPGQEK--------------------

MmMED21 ---------------NIQTAINKDQPANP--TEEYAQLFAALIARTAKDIDVLIDSLPSE 79
HSMED21 ---------------NIQTAINKDQPANP--TEEYAQLFAALIARTAKDIDVLIDSLPSE 79
ZdMED21 ---------------NIQTAINKDQPSNP--TEEYAQLFAALIARTAKDVDVLIDSLPSE 79
AmMED21 --------PQQQVHQQQQLPQQQQQQQQPQQQEDFPQLFSTLISRCAKDIDTLIESLPSE 97
CeMED21 --------------------AISQELEDEP-----NCAIFAAGIAKAAKNIEILIDSFPIE 72
AtMED21 ----------------------TTVTDDATPFPEQPKQLSAGLVKAAKQFDALVAALPLS 82
PpMED21 ----------------------TGITTEAL---EQPKEMATAFVQAVQRFDALVSALPDI 76
SbMED21 TKP--QVQGQPGAP------PPAQAQPPAPDLSEQPKAMSHALVLAAKKFDALVAALPLS 104
OsMED21 PQPGAAAAAAAGAPAAQAQAPPAQAQPPALDLAEHPKAMSHALVLAAKKFDALVSALPLS 119
ScMED21 -----------------QMSDKHATVVPPEEFSNTIDELSTDIILKTRQINKLIDSLPGV 83
SPMED21 -----------------VSDSKVNPISAEELQFAQRDLAKDLVTKFMQIDTLINQLPGI 82

MmMED21  ESTAALQAAS-LYKLEEENHEAATCLEDVVYRGDMLLEKIQSALADIAQSQLKTRSVTHS 138
HSMED21  ESTAALQAAS-LYKLEEENHEAATCLEDVVYRGDMLLEKIQSALADIAQSQLKTRSGTHS 138
ZdMED21  ESTAALQAAS-LRQLEEENQEAAARLEEVVYRGDALLEKIQTALADIAQSQLRTRSGAPS 138
AmMED21  ESSIELQVQS-LQRLEAENKESAEKLEEIVRKGELLLEKIQAALSDIAQSQLDMQYSS-- 154
CeMED21  AGNQEAEVEEKMIKNDEKQREKVNELVGLVGDSNRLVGVVQKKLAEISKTQMSSRPSE-- 130
AtMED21  EGGEGAQLKR-IAELQVENDLVGQELQKQLEAAEKELKQVQELFGQAADNCLNMKKPE-- 139
PpMED21  QGGEEAQLKR-IAELEAENEAFGQELQRELEAADSELNQIQELFDMATDNWLQMKPP--- 132
SbMED21  --SEEDQVKR-IQELQAENEVVGLELQKQLEAAERELKQVEVLFNEATDNCINFKRLD-- 159
OsMED21  --SEEDQLKR-IKELQAENEVVGTELQKQLEAAELELKQVEALFNEATDHCINLKKPE-- 174
ScMED21  DVSAEEQLRK-IDMLQKKLVEVEDEKIEAIKKKEKLLRHVDSLIEDFVDGIANSKKST-- 140
SPMED21  STAPKHQLEK-IKKLQNSIEEKQLERKSLESENEDLKLQLAKRIETFGRLSCVLFQP--- 138
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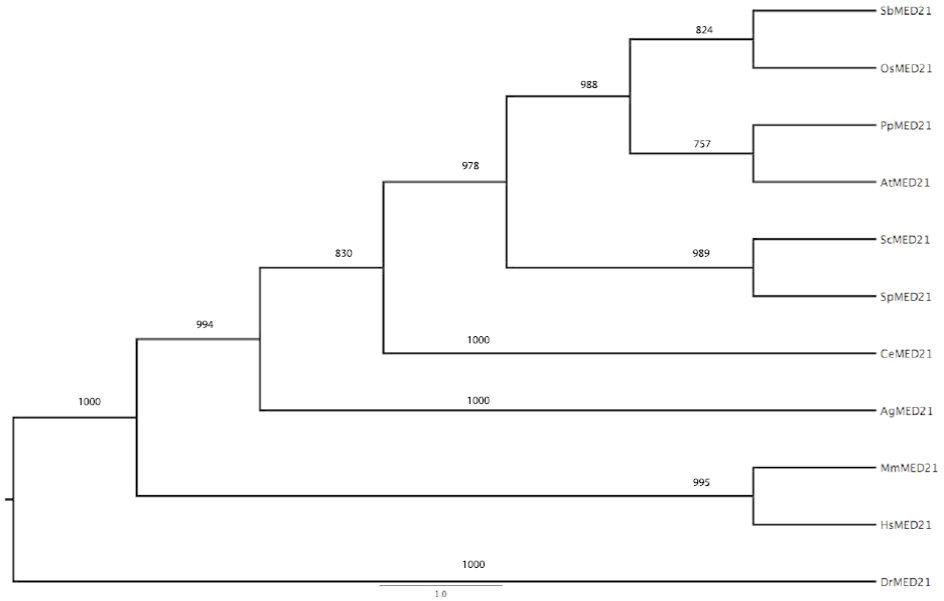
B



Supplemental Figure 14. MED21 is an evolutionarily conserved eukaryotic protein.
(a) Comparisons of MED21 proteins from various organisms.
(b) Neighbor-joining tree of MED21 from Arabidopsis and other eukaryotic organisms.

The MED21 amino acid sequences from various organisms were used for building the phylogenetic tree. Sequences were aligned using ClustalW (Thompson et al., 1994) with default gap penalties, and the alignment was manually adjusted where necessary. Mean character distances were used to construct a midpoint-rooted neighbor-joining phylogeny (Saitou and Nei, 1987) from the PHYLP version 3.67 package (Felsenstein, 1993). Statistical support of the branches was tested with 1000 bootstrap resamples.

The MED21 sequences are from *Sorgum bicolor* (Sb MED21, AAL73528), *Oryza Sative* (OSMED21, NP_001060943), Arabidopsis (At MED21, AAD03443, At4g04780), *Mus Musculus* (MSMED21, AAH12286), *H. sapiens* (HpMED21, NP_004255), *Caenorhabditis elegans* (Ce MED21, NP_001021184), *Danio rerio* (Zebra fish, Dr MED21, NP_998588), *S. cervisae* (Sc MED21, P47822), and *Schizosaccharomyces pombe* (Sp MED21, CAA22343), *Physcomitrella patens* (Pp MED21, XP_001763794), *Anopheles gambiae* (Mosquito, AgMED21, XP_307937). Sequence alignment was performed using the Clustal W (Thompson et al., 1994). The green shading indicates amino acid residues conserved in at least 10 out of the 11 proteins compared. Yellow shading indicates residues shared between Arabidopsis MED21 and other sequences.

Supplemental Figure 15. *MED21* is required for resistance to *B. cinerea*.
Pictures show representative *B.cinerea* disease symptoms at 3 dpi after inoculation with $2.5x10^5$ spores/ml. MI, *MED21* RNAi lines; MO, *MED21* overexpression lines

Table 1. List of primers

Description	Primer sequence
B. cinerea ActinA (Bc ActA)	FP (5'-ACTCATATGTTGGAGATGAAGCGCA-3') RP (5'-AATGTTACCATACAAATCCTTACGGA-3')
A. brassicicola CutA	FP (5'-CACTGCGCCCAATGATGAAC-3') RP (5'-GTAGCCGAACAACACGACACC-3')
Arabidopsis *Actin2*	FP (5'-GTCGTACAACCGGTATTGTGCTG-3') RP (5'-CTCTCTCTGTAAGGATCTTCATGAGGT-3')
HUB1 promoter	FP (5'-TATCCGTCGACGCTTTATCACTATACTGGC-3') RP (5'-CTAGGGATCCTGAAAAACCCTAGAATCGCC-3')
T-DNA primer	LBA1: 5'-TGGTTCACGTAG TGGGCCATCG-3')
hub1-4 allele	FP (5'-TCACAGGAACT CGACAAAAGAAG-3') RP (5'-AAGAGAAAAAGAACATGTGCGG-3')
Homozygous *hub1-6* allele	FP (5'-GCGATGACTCTGCAATTCTCTAG-3') RP (5'-TCTTTCTTCTTCATCTCCCCC-3')
M*ED21* T-DNA allele	*MED21-FP* (5'-TATTT ATTGGGCCTATTCGGG-3') RP (5'-TTACCTGCAGCCTCTAGTTGC-3')
MED21 RNAi fragment	FP (5'-AGACCATGGAT GGATATAATCTCACAGTTGCAAG-3') RP (5'-AGAGGATCCTTAACTAAGCCAGCACTGAGC-3')
MED21 QRT-PCR	FP (5'-ACTGATGCTACGCCTTTTCCT-3') RP (5'-ACAGATCATTTTCCACCTGGAGTTCAG 3')
HUB1 RT-PCR	FP (5'-TGTGCGGTCAGCTAGCTCTGA-3') RP (5'-TGCCTTCATATCTGCA AAGAT-3')
SOC-QRT-PCR	FP (5'-AGAATGCAACAAGCAGACAAGTGAC-3') RP (5'-TATCTTGCATATTGGAGCTGGCGAAT-3')
FT-QRT-PCR	FP (5'-TTGTTGGAGACGTTCTTGATCCGTTTAA-3') RP (5'-CATCTGGATCCACCATAACCAAAGTATAGAAG-3')
MAF4 QRT-PCR	FP (5'-AAGCTCTCGACAAGTTACTTTCTGTAAACG-3') RP (5'-GATCTTGGCCATGCTATCACCTGA-3')
MAF1 QRT-PCR	RP (5'-GTTCTTCAAGCTTGCTTTGGACTGTTTC-3') FP (5'-GCCTCCGGAAAACTCTATGACTCTT-3')
WRKY29 RT-PCR	FP (5'-CAACCCAAACCAACCTTAAC-3') RP (5'-CATACACCTAATGCTCTCTAG-3')
WRKY33 RT-PCR	FP (5'-TCTCTCCTTCTCTTGTCTCTCCTT-3') RP (5'-CGTCACCAACAGAATCAGAAGTAGT-3)
WRKY25 RT-PCR	FP (5'-TTGGTTCTTCCGGCGTTGACTGTTA-3') RP (5'-TTCTGTTCCAAAACTTTG ATTGT TG-3')
MED21 RT-PCR	FP (5'-TCGCGAGCTCATGGATATAATCTCACAGTTGCAAGAA-3') RP (5'-AAAAGGATCCTTCAGGTTTCTTCATGTTCAGGCAAT-3')
HUB1 RT-PCR	FP (5'-TGTGCG GTCAGCTAGCTCTGA-3') RP (5'-TGCCTTCATATCTGCAAAGAT-3')
Cloning in BiFC vectors	MED21 FP (5'-ATGAGCTCATGGATATAATCTCACAG-3') RP (5'-ATTCTAGATCATTCAGGTTTCTTCAT-3') HUB1 FP (5'-ATACTAGTATGGCGAGCACAGGCGA-3') RP (5'-ATGAATTCTCATATGTAGATAGGTT-3')
ChIP	*MAF1* FP (5'-GCACTACTTTCCGATCAAAACCAATGAGA-3') RP (5'-AGCGAAACGATTGCGTTTTGAAGCCACT-3')
Double mutant isolation	*sid2-5* isolated using (5'- TTCATAACCAATCTCGATACAC-3) and a T-DNA primer (5'-TTCATAACCAATCTCGATACAC-3'). PAD4/LP (5'-GCGATGCATCAGAAGAG-3') RP (5'-TTAGCCCAAAAGCAAGTATC-3') COI1/LP (5'-GGTTCTCTTTAGTCTTTAC-3') RP 5'-CAGACAACTATTTCGTTACC-3')

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Saitou, N., and Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4,** 406-425.

Thompson, J.D., Higgins, D.G., and Gibson, T.J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22,** 4673-4680.