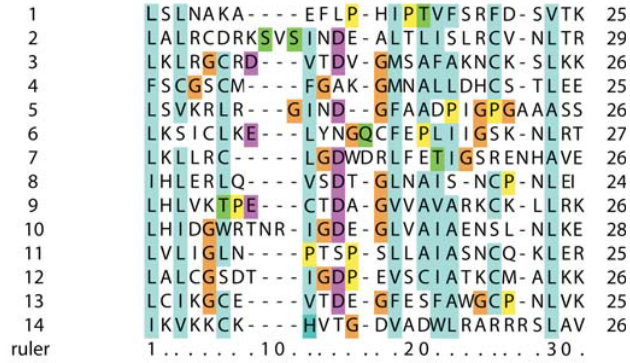
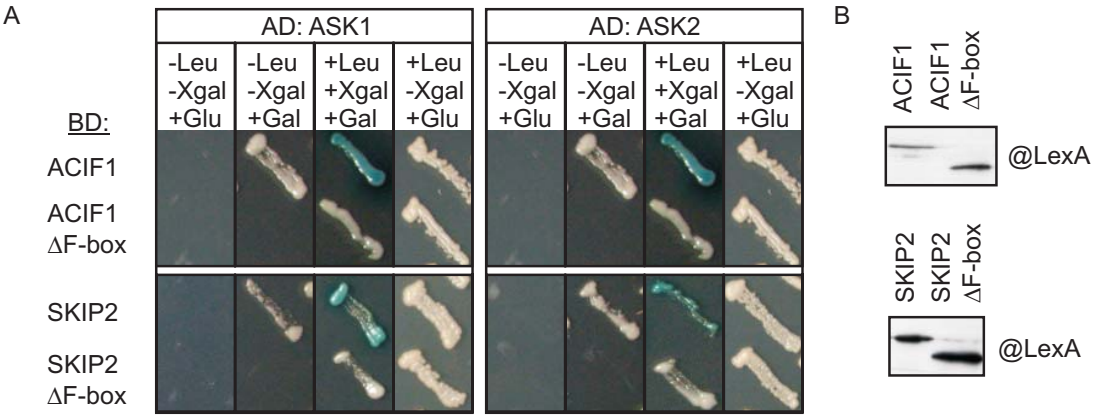
Supplemental Data. van den Burg et al. (2008). The F-box protein ACRE189/ACIF1 regulates cell death and defense responses activated during pathogen recognition in tobacco and tomato.



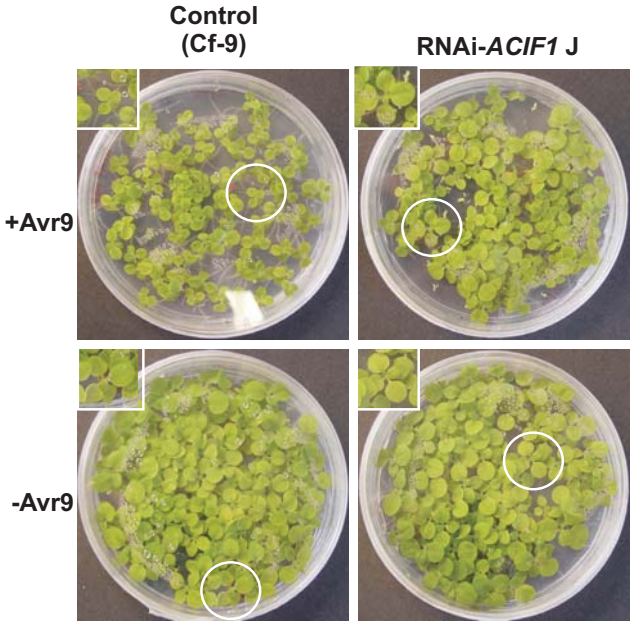
Supplemental figure 1. Alignment of the 14 putative leucine-rich repeats in tobacco ACIF1



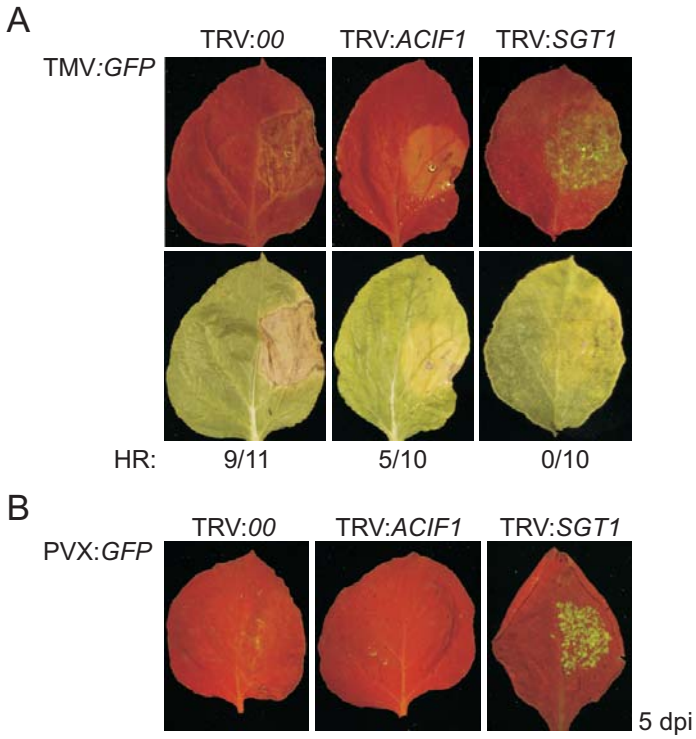
Supplemental Figure 2. ACIF1 and SKIP2 interact with subunits of the SCF complex in a F-box dependent manner in the yeast LexA two-hybrid assay.

(A) Yeast two-hybrid assay of interactions between ASK proteins and the F-box proteins ACIF1 and SKIP2. Deletion of the F-box (deltaF-box) results in loss of the interaction. Yeast is shown after growth for five days on non-inducing media (+Glu) and inducing media (+Gal) while selecting for two protein-protein interaction markers (+X-Gal /+Leu and -Leu).

(B) Western blot demonstrating expression of the pGilda LexA-F-box fusion proteins in yeast. Protein fusions were detected using anti-LexA mouse monoclonal antibody.



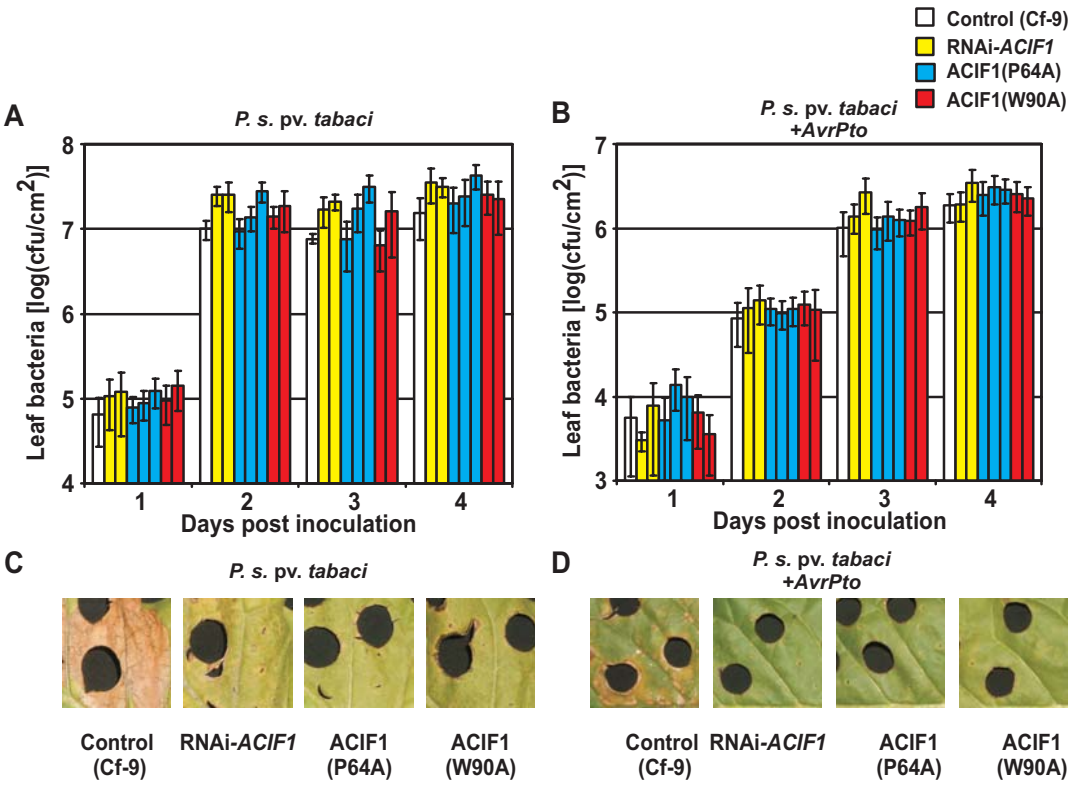
Supplemental Figure 3. RNAi-*ACIF1* seedlings (line J, parental line heterozygous for the transgene construct) show less growth inhibition in response to growth on IF+Avr9 compared to the control (*Cf-9* seedlings). The seedlings in the circle are enlarged in the top left insert. IF concentration: 2.5uL IF per mL medium; 24 day old seedlings.



Supplemental Figure 4. TRV-based VIGS of *ACIF1* compromises *N*-mediated HR in *N. benthamiana*, but not resistance to infection by TMV:GFP or PVX:GFP.

(A) *N. benthamiana* leaves were silenced with TRV. Seedlings were inoculated with TRV:ACIF1, TRV:SGT1, or control TRV:00. Three weeks after TRV delivery, the right leaf half received *A. tumefaciens* expressing TMV:*GFP*. HR was not observed in 5/10 plants in TRV:ACF1, while 9/11 plants showed HR for TRV:*00* (empty vector). *top* panel: Accumulation of TMV:GFP monitored by GFP fluorescence (TRV:SGT1 was included as positive control for loss-of-resistance; Peart et al., 2002); *bottom* panel: HR triggered by expression of TMV. Photographs were taken 6 days after Agroinfiltration. The plants used carry both the *N* and *Rx* resistance genes.

(B) Experimental conditions similar as in (A), except that PVX:GFP was delivered to test viral accumulation. Photographs were taken 6 days after Agroinfiltration. Only TRV:SGT1 plants showed viral accumulation of PVX:GFP.



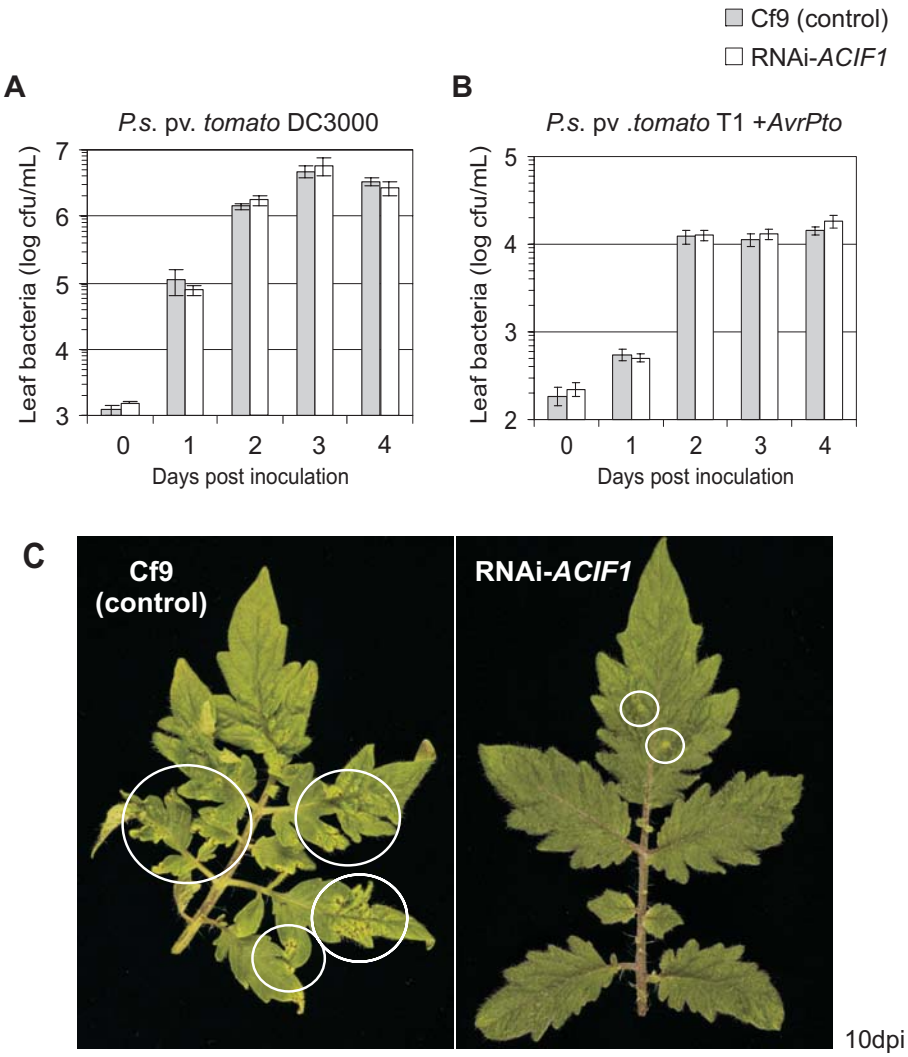
Supplemental Figure 5. Bacterial growth of *Pseudomonas syringae* pv. *tabaci* (+/- AvrPto) is not affected by silencing *ACIF1* or expression of mutant ACIF1 in tobacco.

(A) Bacterial growth in leaves of 4-week-old *Cf-9* tobacco (control, RNAi-*ACIF1* lines D/J, ACIF1(P64A) lines A/D/E, and ACIF1(W90A) lines A/E) following infiltration with *Pst* (empty vector; 10^4 CFU/mL). Bacterial counts are the mean of at least 6 samples, while the error bars represent SD. The shown experiment was performed three times with similar result. Analyses of variance (ANOVA) in bacterial growth amongst the different plant lines was tested using a two-way randomized block design for biological replicates at t=3 dpi. Prior to ANOVA, data were transformed using the 10logarithm function. No significant trial interaction was observed (*p=0.115*). The Tukey HSD (Honestly Significantly Different) multiple comparison test did not support any clustering of datasets.

(B) Similar as in (A), except that *Pst+AvrPto* was infiltrated. No significant trial interaction was observed (*p=0.853*) using ANOVA (statistical analysis similar to A).

(C) Disease symptoms 10 days after infection with *Pst*. Shown leaves were also used in (A). From this stage onwards, disease symptoms did not develop any further.

(D) HR symptoms 10 days after infection with *Pst+AvrPto*. The leaves shown were also used in (B). From this stage onwards, HR did not develop any further.

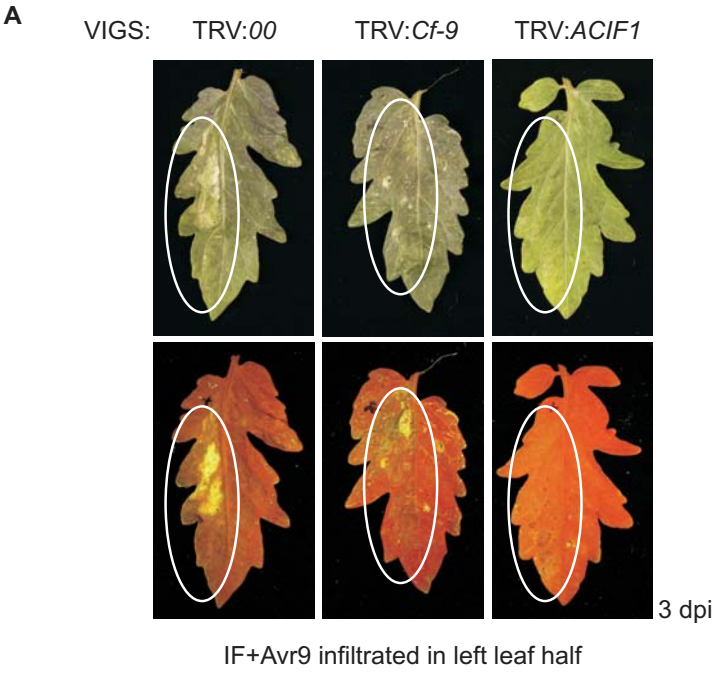


Supplemental Figure 6. Tomato *ACIF1* function is required for *AvrPto*-induced HR and disease-associated cell death following infection by *Pseudomonas syringae* pv. *tomato* DC3000 and T1.

(A) Bacterial growth of *P.s.* pv. *tomato* strain DC3000 in leaves of four-week-old Cf9 tomato cv. Moneymaker (RNAi and control). Bacterial values are the mean of at least 6 samples, while the error bars represent SE. The experiment was performed three times with similar result.

(B) Similar as (A), except that tomato was infected with the incompatible *P.s.* pv. *tomato* strain T1 +*AvrPto*.

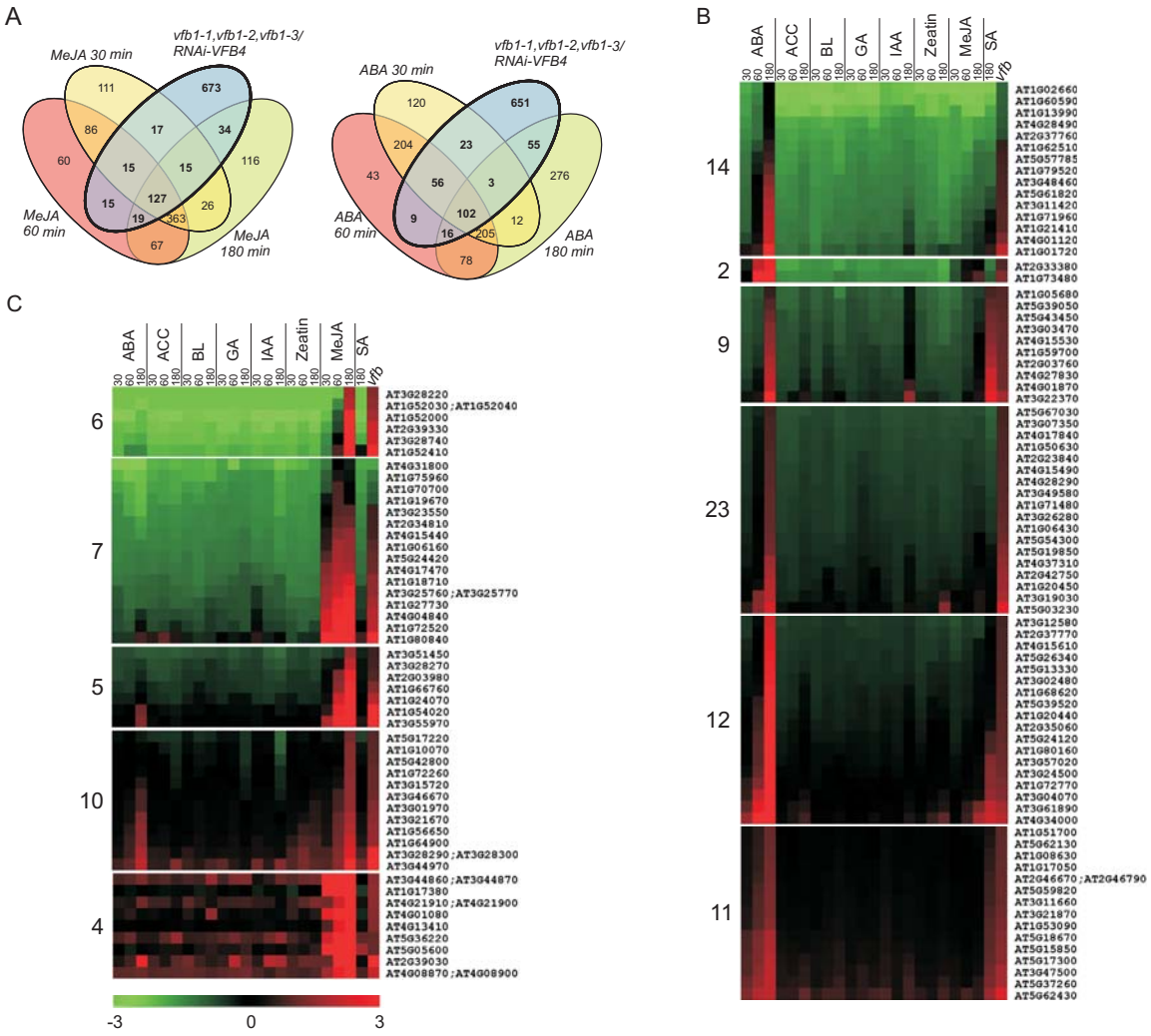
(C) Development of speck disease symptoms 10 days after infecting four-week-old Cf9 tomato with a low inoculum of *P.s.* pv. *tomato* DC3000 (10^4 CFU/mL) using vacuum-infiltration.



Supplemental Figure 7. TRV-based VIGS of *ACIF1* compromises Avr9-dependent HR in *Cf-9* tomato.

(A) TRV-based VIGS was administered by infiltrating the transgenic *Cf-9* cotyledons with Agrobacterium carrying the indicated TRV constructs. Three weeks later, the left leaf half was infiltrated with IF+Avr9 and HR was scored after three days. *Cf-9*- and *ACIF1*-silenced tomatoes showed compromised HR in response to Avr9. TRV:*00* (empty vector control), TRV:*Cf-9* (positive control for loss-of-resistance due to silencing). *Bottom* panel, UV light; *top* panel, bright light. Shown leaves were photographed 3 days after IF infiltration.

(B) Transgenic *Cf-9* or parental Cf0 tomato seedlings were silenced with the indicated TRV construct as described in (A). Three weeks later, plants were infected with *C. fulvum* race 4 GUS. Leaves were stained with X-gluc 3 weeks after *C. fulvum* inoculation, when pictures were taken. Only the TRV:*Cf-9* shows a few instances of *C. fulvum* infection (besides the susceptible control Cf0).

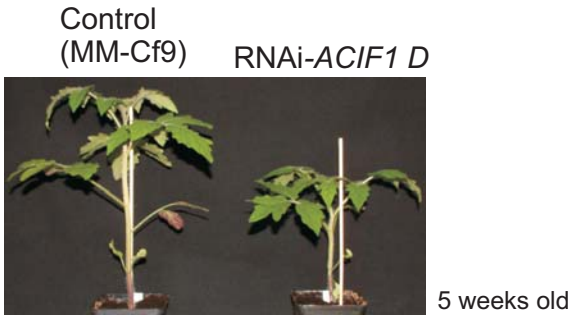


Supplemental Figure 8. Knockdown of the Arabidopsis *ACIF1* homologues (*VFBs*) positively regulates a set of MeJA- and ABA-responsive genes.

(A) Venn diagrams representing the overlap in genes differentially expressed in the *vfb* knock down mutant and genes differentially expressed during ABA and MeJA responses.

(B) Heat map representation of gene clusters containing genes constitutively up-regulated in the *vfb* knock down and genes induced after ABA application (predominantly 180 min). Gene clustering was performed for genes differentially regulated in the v*fb* knock down and compared with gene expression levels obtained after individual plant hormone treatments of wild-type Arabidopsis (30, 60, and 180 min)(Nemhauser et al., 2006). Complete clustering data are available in the supplementary data.

(C) Similar as (B), except that heat map representations are shown for gene clusters that are both constitutively up-regulated in the *vfb* knock down mutant and are progressively induced after MeJA application (30, 60, and 180 min).



Supplemental Figure 9. Tomato RNAi-*ACIF1* line D plant is slightly stunted compared to control (cv. MM-Cf9).