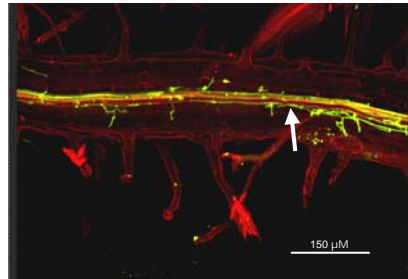
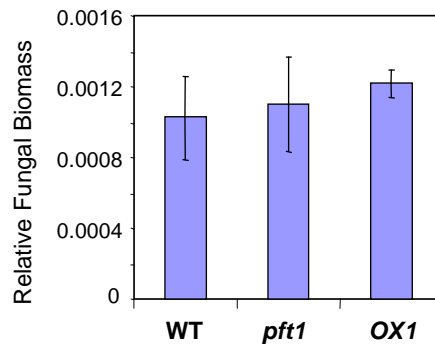


Supplemental Data. Kidd et al. (2009). The Mediator Complex Subunit, PFT1, is a Key Regulator of Jasmonate-dependent Defense in *Arabidopsis*

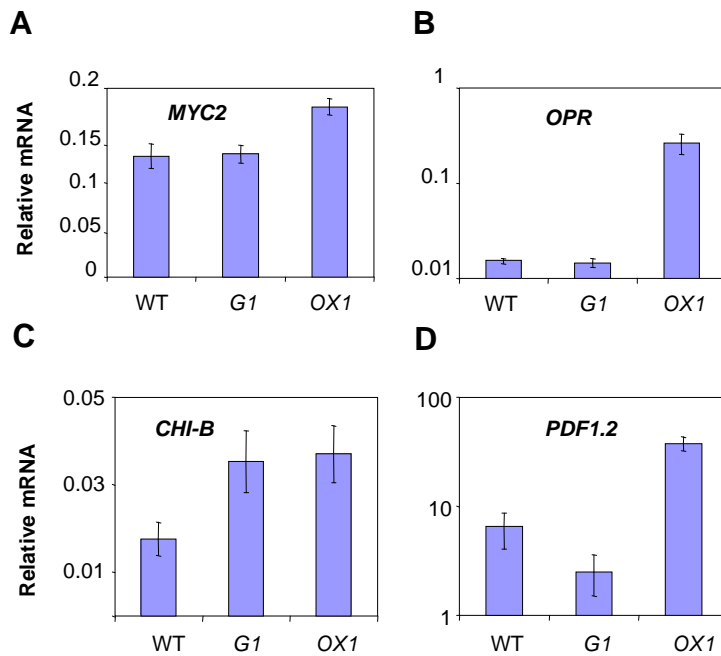
A



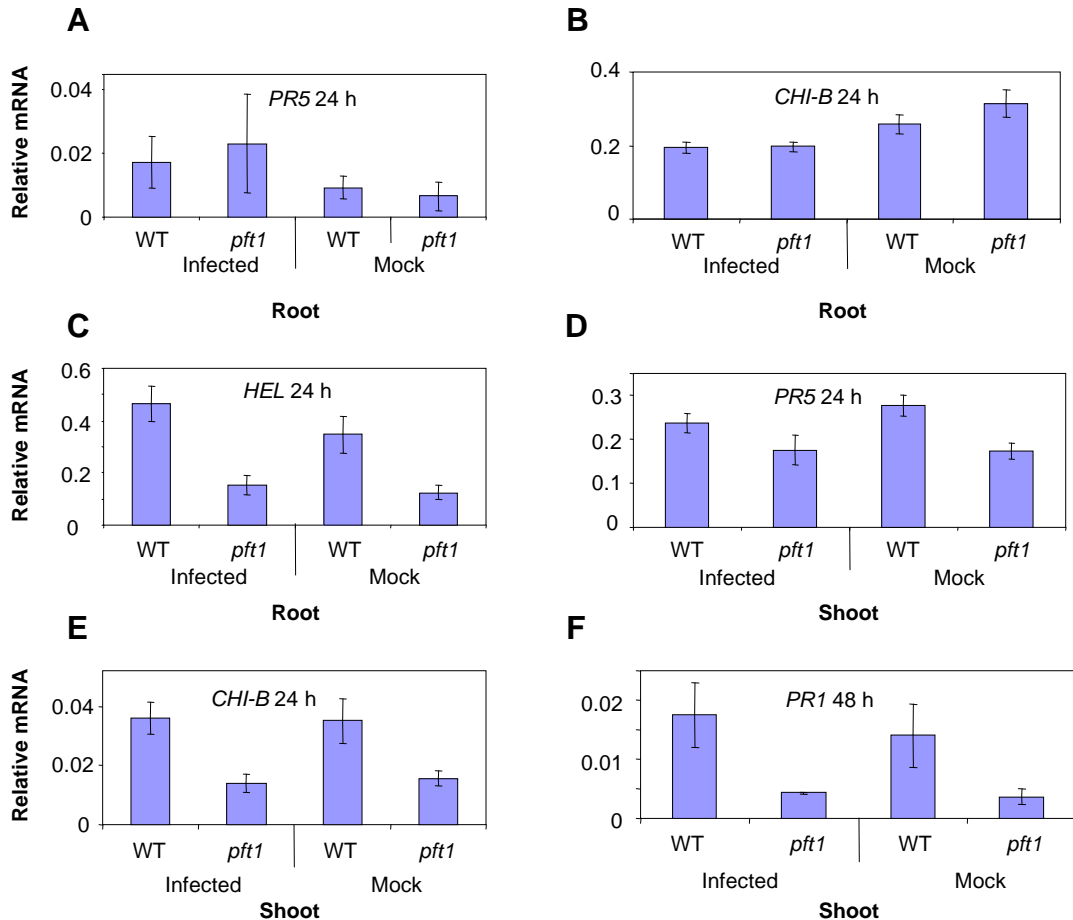
B



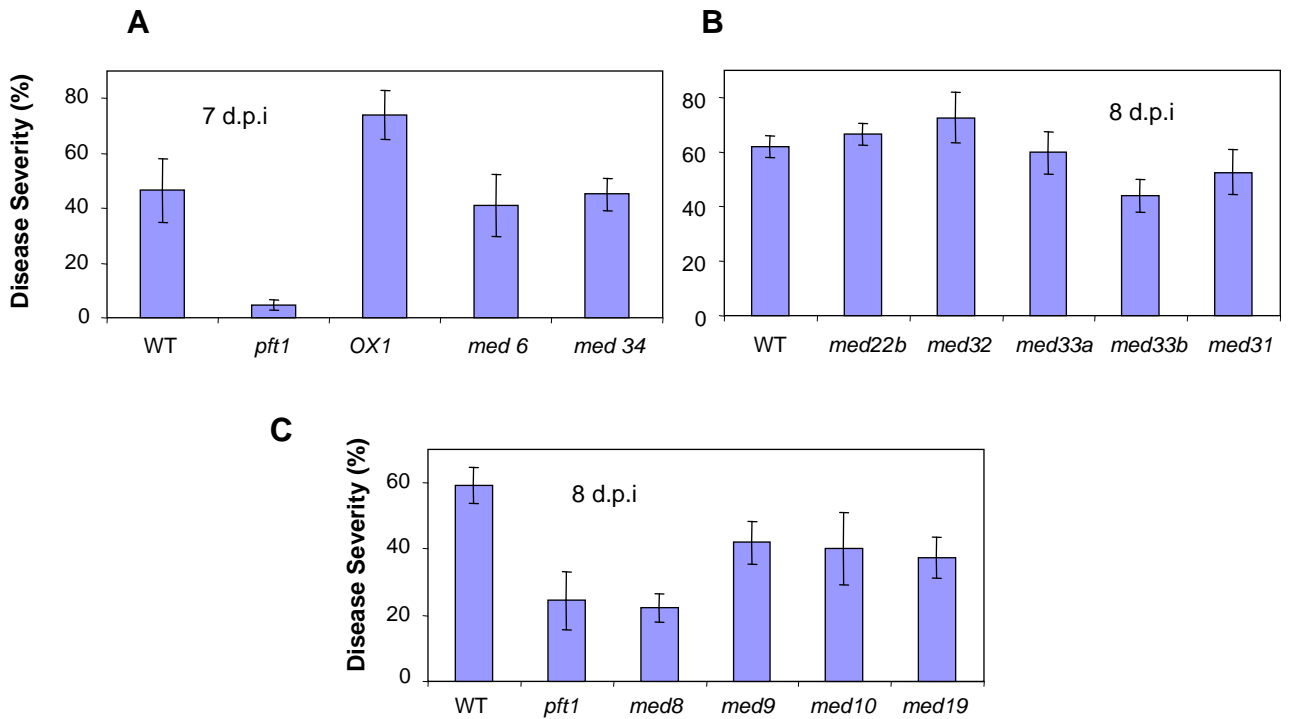
Supplemental Figure 1. Increased *F. oxysporum* resistance in *pft1* mutants is not associated with increased resistance of roots to pathogen infection or *in planta* inhibition of fungal growth. **(A)** A representative confocal microscope image of *pft1* roots inoculated with *F. oxysporum* at 6 days after inoculation (see Supplemental Methods). The arrow indicates the fungal tissue stained with wheat germ agglutinin (bright green fluorescence) in the root vasculature (yellow fluorescence). The diseased WT and *OX1* plants showed similar levels of fungal colonization (data not shown). **(B)** The relative fungal biomass in the leaves of *F. oxysporum* inoculated WT, *pft1* and *OX1* plants was measured by RT-Q-PCR using the *F. oxysporum* specific primers *FoGPD* and the *Arabidopsis* specific primers *iASK* (see Supplemental Methods). Error bars represent the standard error from three replicates of 40 plants each.



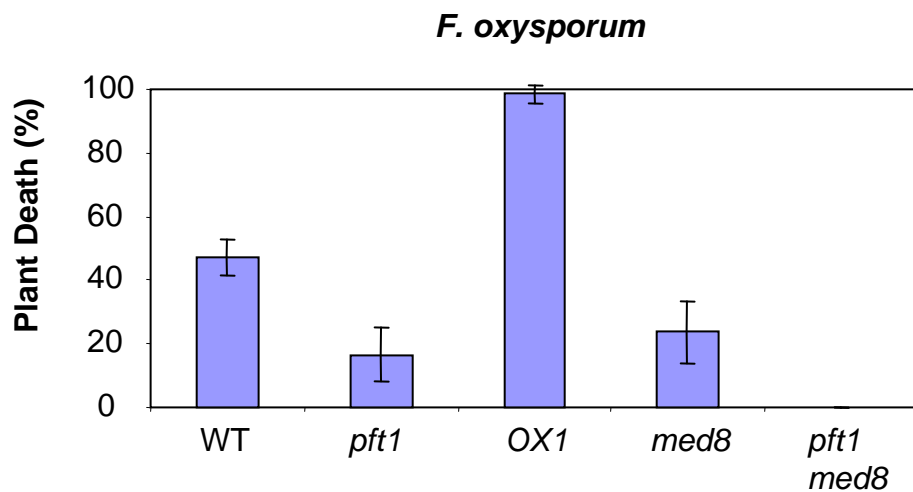
Supplemental Figure 2. The *OX1* genotype has increased expression of JA-associated genes. (A-D) RT-Q-PCR analysis reveals that *MYC2*, *PDF1.2*, *CHI-B* and *OPR* all have higher expression in *OX1* compared to the wild type, 6hr after MeJA treatment. *OPR* and *PDF1.2* are shown in logarithmic scale. Error bars represent the standard error of 3 replicated experiments with approximately 20 plants per replicate.



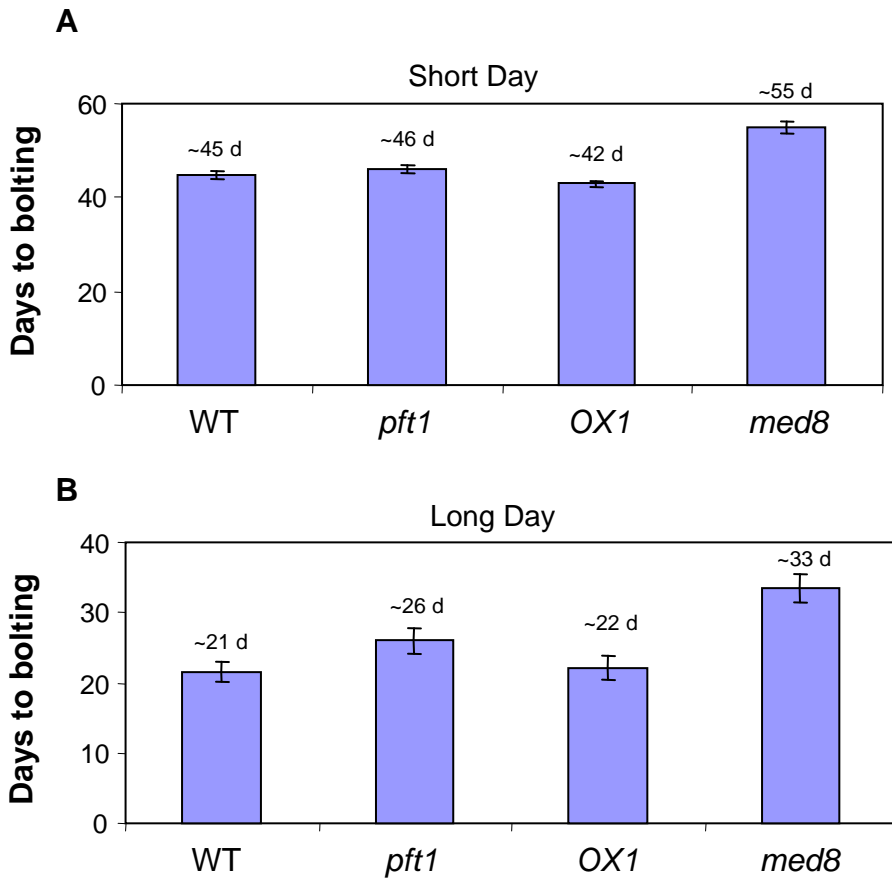
Supplemental Figure 3. The expression of defense genes in the roots and shoots of WT and *pft1* plants after infection with *F. oxysporum*. **(A-B)** There was no statistically significant difference in defense gene expression in the roots of *pft1*, compared to the WT, shown here for *PR5* and *CHI-B*. **(C)** The expression of *HEL*, however, showed induction by *F. oxysporum* yet decreased expression in *pft1* root tissue compared to the WT. **(D-F)** The expression of defense genes remained attenuated in the shoot tissue of *pft1* plants after infection with *F. oxysporum*. Data shown is from samples collected 24 hours after *F. oxysporum* inoculation with the exception of *PR1*, which is from 48 hours **(F)**. Error bars represent the standard error of 3 replicated experiments with approximately 20 plants per replicate.



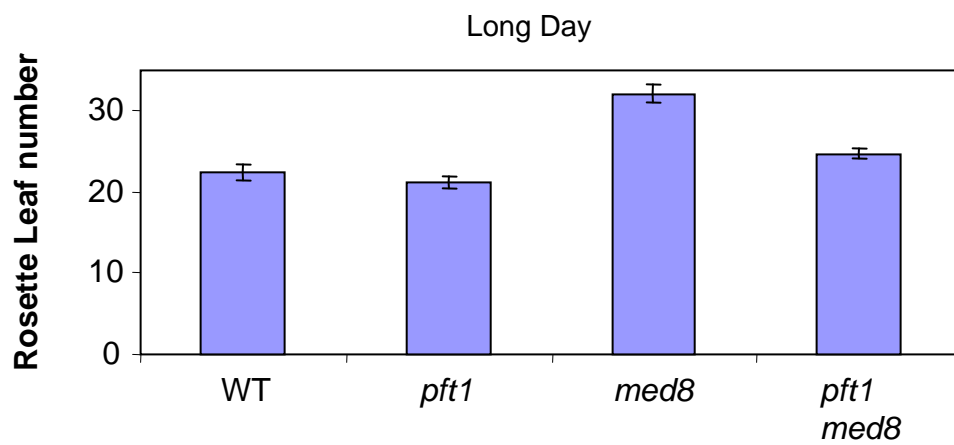
Supplemental Figure 4. Disease severity following the inoculation of 11 Mediator subunit mutant lines with *F. oxysporum*. (A-C) The average disease severity of *med6*, *med8*, *med9*, *med10*, *med19*, *med22b*, *med31*, *med32*, *med33a*, *med33b* and *med34* were compared to the WT (Col-0) with *pft1* and *OX1* also used as controls. Approximately 30 plants were used in three replicated experiments and the number of leaves showing chlorosis and necrosis were counted 7-8 days after inoculations (d.p.i) and expressed as a percentage of the total number of leaves. The lines showing either an increase or decrease in symptom development were repeated in separate inoculation experiments. With the exception of *pft1* and *OX1*, only the *med8* mutant showed a phenotype that was significantly different to the WT in replicated experiments.



Supplemental Figure 5. The survival of WT, *pft1*, *OX1*, *med8* and the *pft1 med8* double mutant after infection with *F. oxysporum*. Approximately 30 plants were used per genotype in three replicated experiments and the percentage of plant death was recorded after 14 d.p.i. The error bars represent the standard error from the three replicated experiments.



Supplemental Figure 6. The *pft1* and *med8* mutants have increased flowering time. (A-B) The *pft1* mutant show a slight delay in flowering time when using “days to bolting” as the determining factor whereas the *med8* mutant has a stronger delay in flowering. Flowering measurements were taken with 18 plants per line in each experiment and the error taken as the standard error of the 18 plants. The flowering date was recorded when the shoot bud had extended 5mm.



Supplemental Figure 7. The *pft1 med8* double mutant has a flowering phenotype similar to *pft1* and the WT when recorded as rosette leaf number. Flowering measurements were taken with 18 plants per line and the error taken as the standard error of the 18 plants. The rosette leaf number was recorded when the shoot bud had extended 5mm.

Supplemental Methods

Confocal Microscopy of *F. oxysporum* Infected *Arabidopsis* Roots

Intact roots and hypocotyl tissue of *F. oxysporum* infected plants were cleared in 10% KOH for 16 hrs and stained in wheat germ agglutinin Alexa Fluor 488 (Invitrogen) for 20 minutes at a concentration of 50 µg/ml. Stained tissue was then rinsed twice in distilled water and examined using a Leica TCS SP2 confocal laser scanning microscope (Leica Microsystems, Germany). The WGA stained *F. oxysporum* was visualized using an emission range between 505 to 540 nm while a false red channel (610-660 nm) was used for detecting plant autofluorescence. Excitation of both plant and WGA stained *F. oxysporum* was performed at 488 nm.

Fungal Biomass Measurements

For fungal biomass measurements, DNA was extracted from *F. oxysporum* infected plant material (three separate replicates containing 40 plants each) 6 and 7 days following inoculations using the QIAGEN DNeasy Plant Minikit (Qiagen, Germany). The following *F. oxysporum* specific primers were designed based on the glyceraldehyde 3-phosphate dehydrogenase (*GPD*) gene (FOXG_08006; <http://www.broad.mit.edu>) *FoGPD-F*, AAGGGTGCTTCTTACGACCA; *FoGPD-R*, ATCGGAGGAGACAACATCGT and analyzed with the Applied Biosystems 7900HT Fast Real-Time PCR System as per the gene expression experiments. Relative fungal biomass was calculated by normalization of the *F. oxysporum GPD* gene to the *Arabidopsis iASK* gene (At5g26751) as described by Gachon and Saindrenan (2004). As expected, no reliable amplification was detectable by *GPD* specific primers from the DNA isolated from un-inoculated plants.

Supplemental References

Gachon, C., and Saindrenan, P. (2004). Real-time PCR monitoring of fungal development in *Arabidopsis thaliana* infected by *Alternaria brassicicola* and *Botrytis cinerea*. *Plant Physiology and Biochemistry* **42**: 367-371.