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A LysM Receptor-Like Kinase Plays a Critical Role in Chitin Signaling and Fungal Resistance in Arabidopsis

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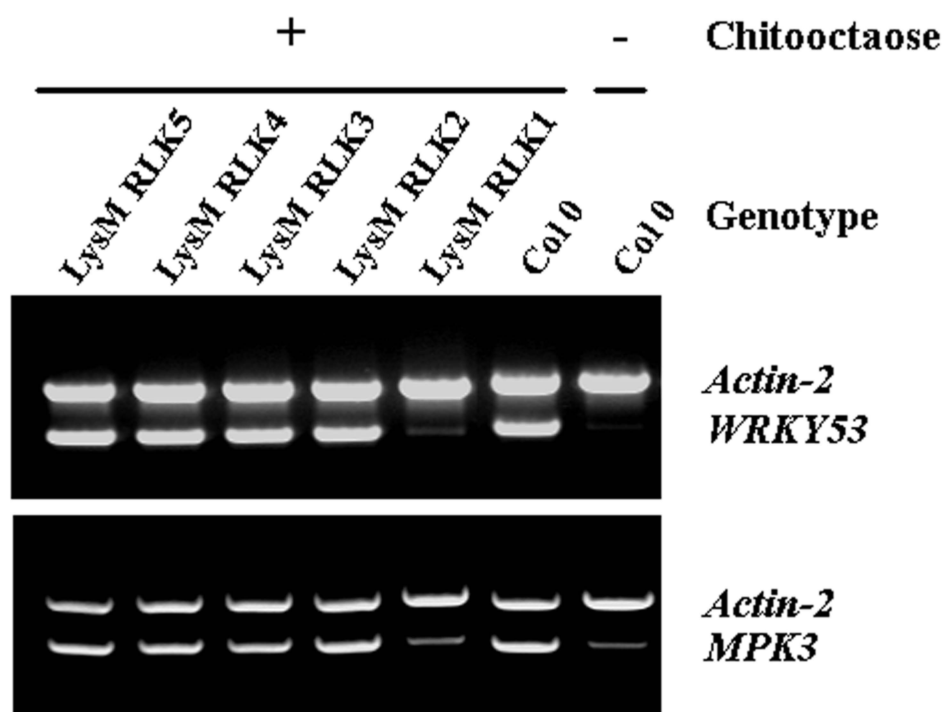
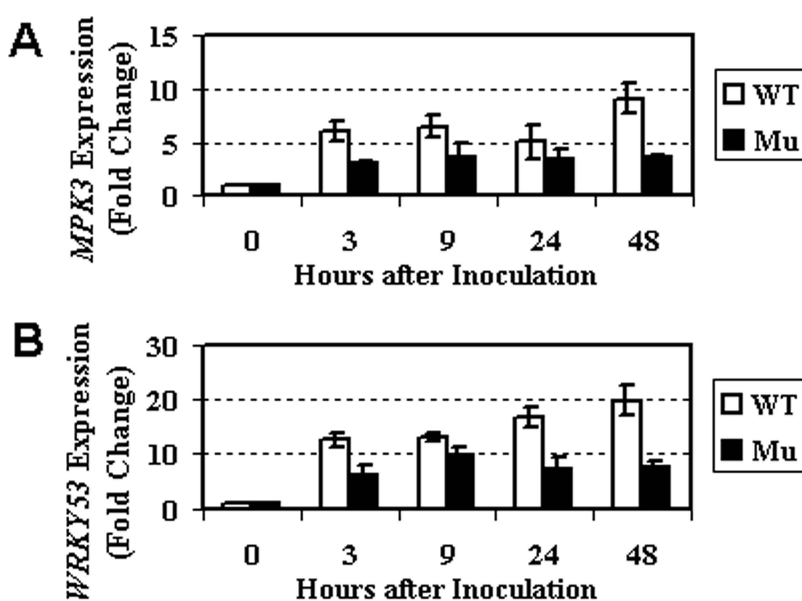
Supplemental Figure 1. Initial screen of the five LysM RLK mutants in response to chitin treatment. The Arabidopsis LysM RLK1 to 5 correspond to the genes At3g21630, At1g51940, At2g33580, At2g23770, and At3g01840, respectively. Both the mutant and wild-type Col 0 plants were treated with the purified chitin oligomer chitooctaose for 30 minutes. Meanwhile, wild-type Col 0 plants were also similarly treated with water (as a negative control). The expression of the selected chitin-responsive genes (CRGs) in both the mutant and wild-type plants was analyzed using RT-PCR. *Actin-2* was used as an internal control and the amplification of both *actin-2* and a CRG was conducted in the same PCR reaction tube.

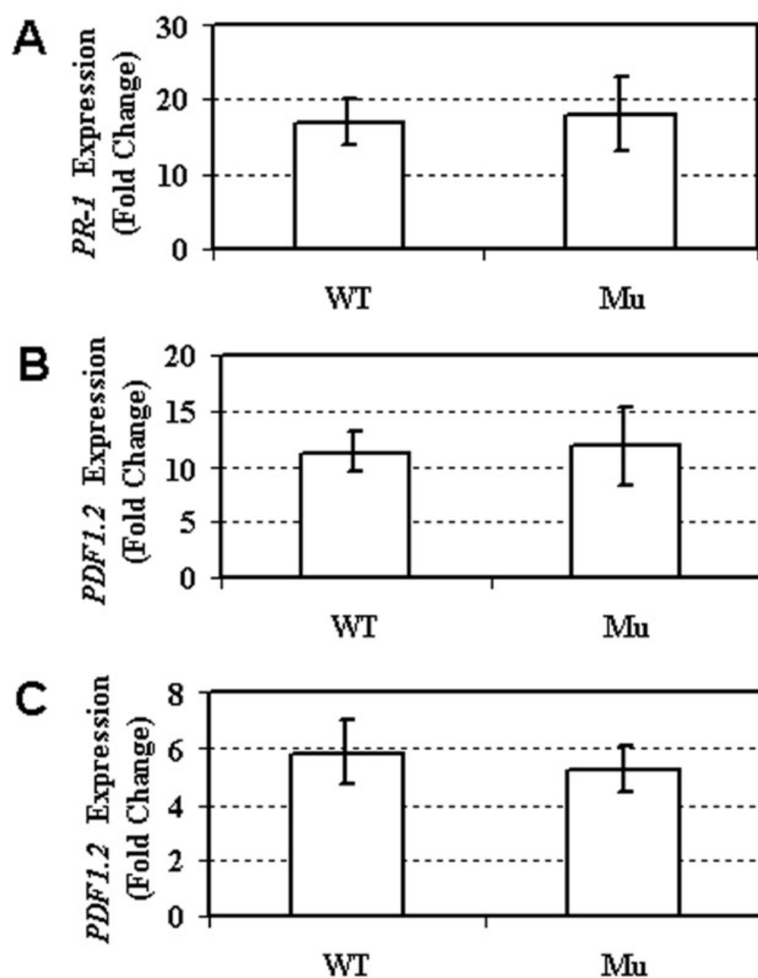


Supplemental Figure 2. Expression analysis of the *LysM RLK1* gene in different tissues or organs using RT-PCR. RT-PCR was conducted using equivalent amounts of cDNA derived from different tissues or organs. *Actin-2* served as an internal control and the amplification of both *actin-2* and the *LysM RLK1* gene was conducted in the same PCR reaction tube with 25 cycles.

Supplemental Figure 3. The selected CRGs were still induced in the *LysM RLK1* mutant by a fungal pathogen, but to a reduced level. (A) Analysis of the *MPK3* gene expression in both the mutant and wild-type plants in response to a fungal pathogen. (B) Analysis of the *WRKY53* gene expression in both the mutant and wild-type plants in response to a fungal pathogen. In both (A) and (B), the gene induction by the fungal pathogen *A. brassicicola* was monitored by quantitative RT-PCR at different time points after inoculation. Each data point was the average of the relative gene expression (fold change, normalized to *actin-2* and relative to the time 0 sample) from three biological replicates. Error bar = standard error. WT = wild-type Col-0; Mu = *LysM RLK1* mutant.



Supplemental Figure 4. The mutation in the *LysM RLK1* gene did not affect other defense-related pathways. (A) Analysis of *PR-1* expression in response to salicylic acid (SA) in both the mutant and wild-type plants by quantitative PCR. (B) Analysis of *PDF1.2* expression in response to methyl jasmonic acid (MeJA) in both the mutant and wild-type plants by quantitative PCR. (C) Analysis of *PDF1.2* expression in response to 1-aminocyclopropane-1-carboxylic acid (ACC) in both the mutant and wild-type plants by quantitative PCR. In the above experiments, each data point was the average of the relative gene expression (fold change, normalized to *actin-2* and relative to the control sample) from three replicates. Error bar = standard error. No statistically significant differences were found between the mutant and wild type in the induction of the above genes based on a Student's t-test.



Supplemental Figure 5. The mutations in the Nod factor receptor genes *NFR1* and *NFR5* in the legume *Lotus japonicus* did not affect the induction of the selected CRGs in the plant. Both the wild type (Gifu) and the Nod factor receptor mutants *nfr1-1* and *nfr5-1* were treated with chitooctaose for 30 minutes at a concentration of 1 μM or with water (as a control). The selected CRGS were detected using RT-PCR. Lj *Actin-2* was used as an internal control and the amplification of both Lj *actin-2* and a CRG was conducted in the same PCR reaction tube with 25 cycles. The experiment was repeated twice with similar results.