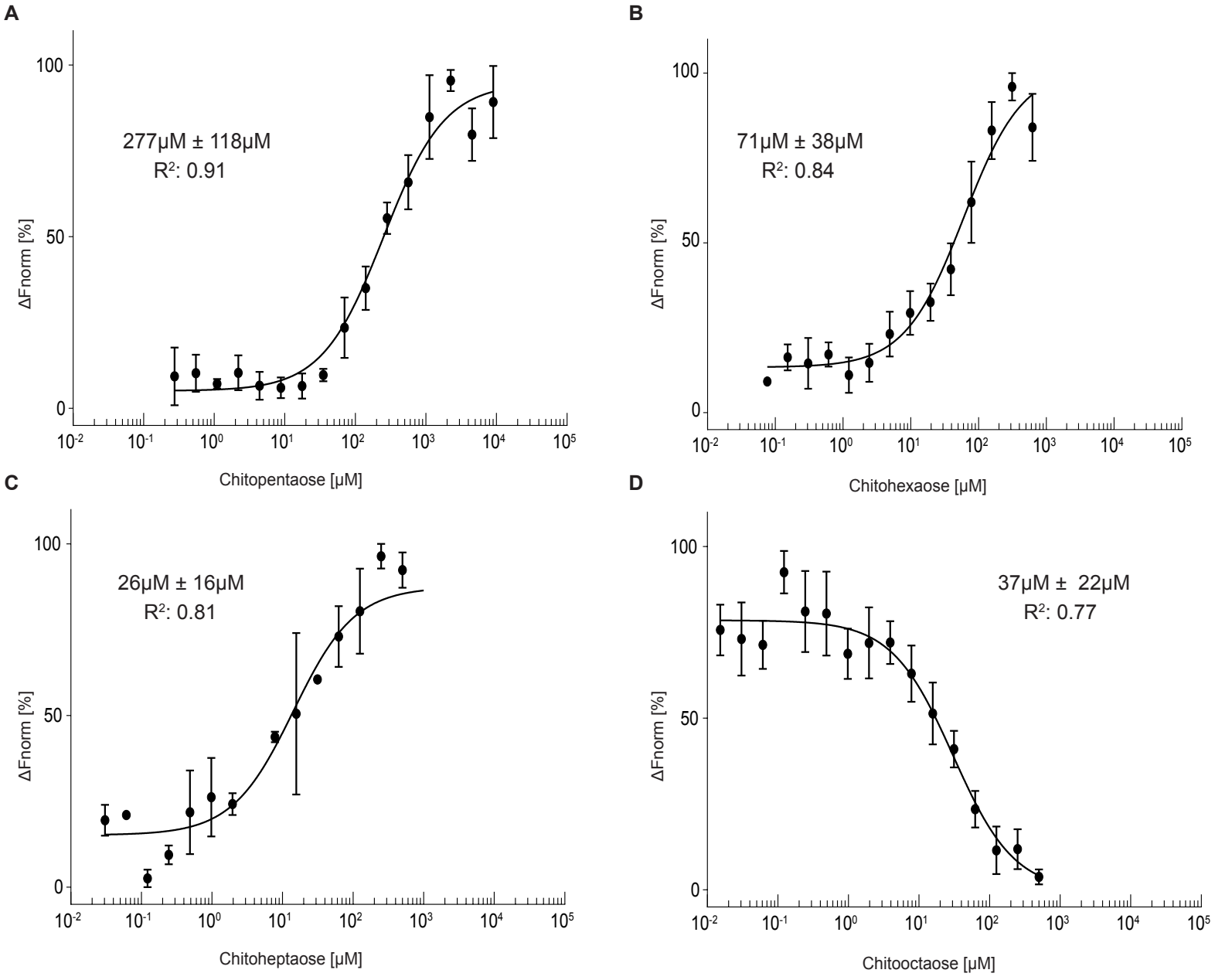

Fig. S1. Binding curves for the *Lj*LYS6 ectodomain obtained from Microscale Thermophoresis (MST) and used for calculating Kd values shown in Table 1.
Analysis of MST data of *Lj*LYS6 ectodomain binding to different lengths of chitin oligomers, (*A*) Chitopentaose, (*B*) Chitohexaose, (*C*) Chitoheptaose and (*D*) Chitooctaose. The change in fluorescence in a thermal gradient (ΔF) as a function of ligand concentration is plotted. The K_D values ± s.e.m. are indicated together with the R^2 fit. The binding curves represent measurements where each data point was derived from three (*A*, *C*) or four (*B*, *D*) biological replicates.

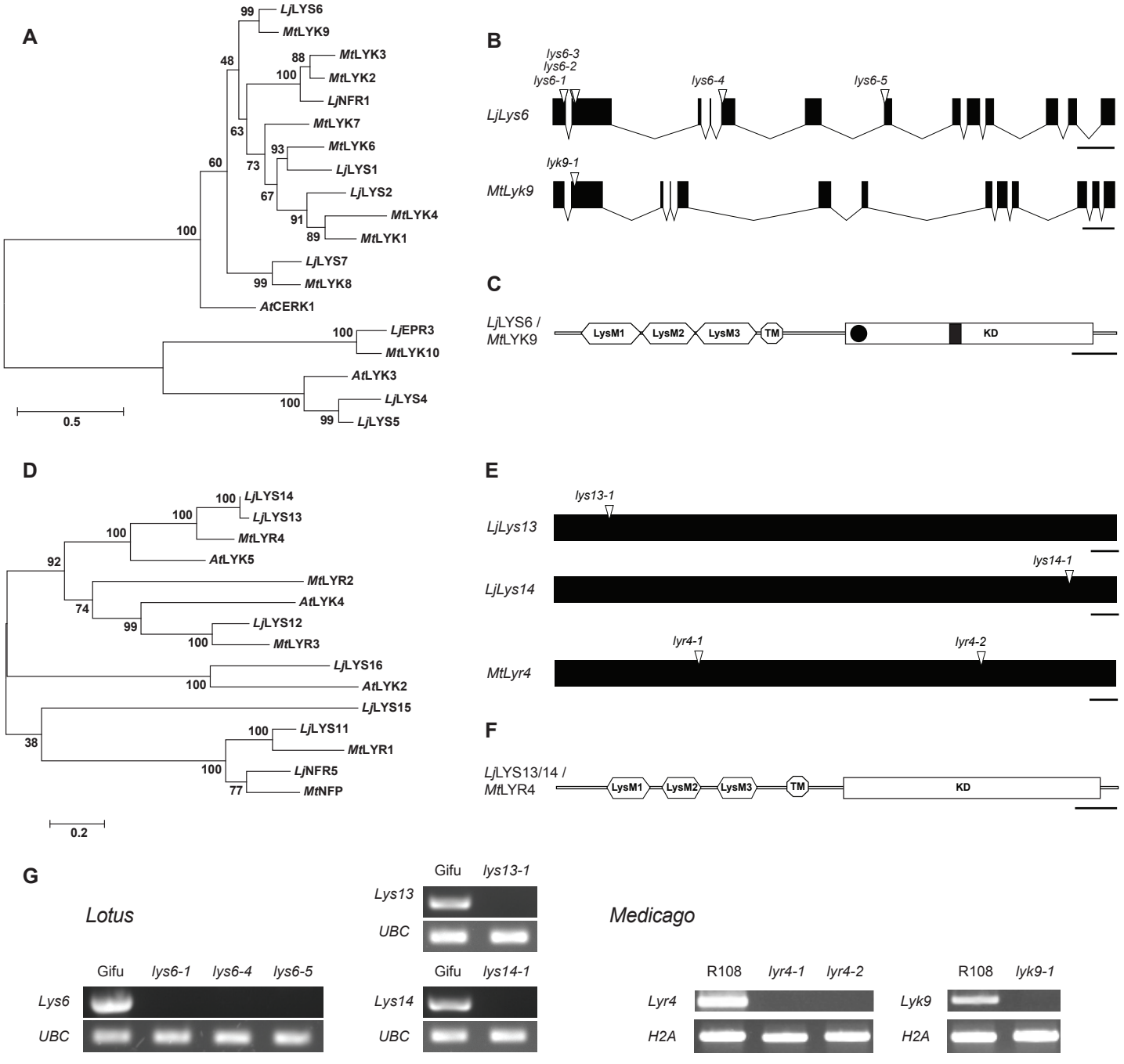

Fig. S2. Phylogeny, gene- and protein structures of *Lj*LYS6, *Mt*LYK9 and *Lj*LYS13, *Lj*LYS14 and *Mt*LYR4.
(A) Unrooted maximum likelihood (ML) phylogenetic tree of full length NFR1/LYK3 type LysM receptor kinase proteins from *Lotus* (*Lj*), *Medicago* (*Mt*) and the *Arabidopsis* (*At*) CERK1 and LYK3 proteins. For each node bootstrap values are shown as percentages based on 1000 repetitions. Branch lengths are proportional to the number of substitutions per site (bar). (*B*) Intron-exon structure of the *LjLys6* and *MtLyk9* genes. White triangles show the position of LORE1 and *Tnt1* retrotransposon insertions respectively. (*C*) Schematic domain structure of the LjLYS6 and MtLYK9 proteins. LysM1, 2, 3: LysM domains, TM: transmembrane domain, KD: kinase domain, black circle: kinase ATP binding site, black rectangle: Ser/Thr kinase active site. (*D*) Unrooted ML phylogenetic tree of full length NFR5/NFP type LysM receptor kinase proteins from *Lotus* (*Lj*), *Medicago* (*Mt*) and the *Arabidopsis* (*At*) LYK2, 4 and 5 proteins. For each node bootstrap values are shown as percentages based on 1000 repetitions. Branch lengths are proportional to the number of substitutions per site (bar). Note that *LjLys13* and *LjLys14* are tandem repeat genes located next to each other on *Lotus* chromosome 2 of while the corresponding *MtLyr4* is a single copy gene in *Medicago*. (*E*) Intron-exon structure of the *LjLys13*, *LjLys14* and *MtLyr4* genes. White triangles show the position of LORE1 and Tnt1 retrotransposon insertions respectively. (*F*) Schematic domain structure of the *Lj*LYS13, *Lj*LYS14 and *Mt*LYR4 proteins. LysM1, 2, 3: LysM domains, TM: transmembrane domain, KD: kinase domain. (*G*) Semiquantitative RT-PCR to detect transcripts of the receptor genes in wild type and mutant plants. Transcripts of the *L. japonicus* ubiquitin-conjugating enzyme (*UBC*) and *M. truncatula* histone *H2A* respectively were used as control.

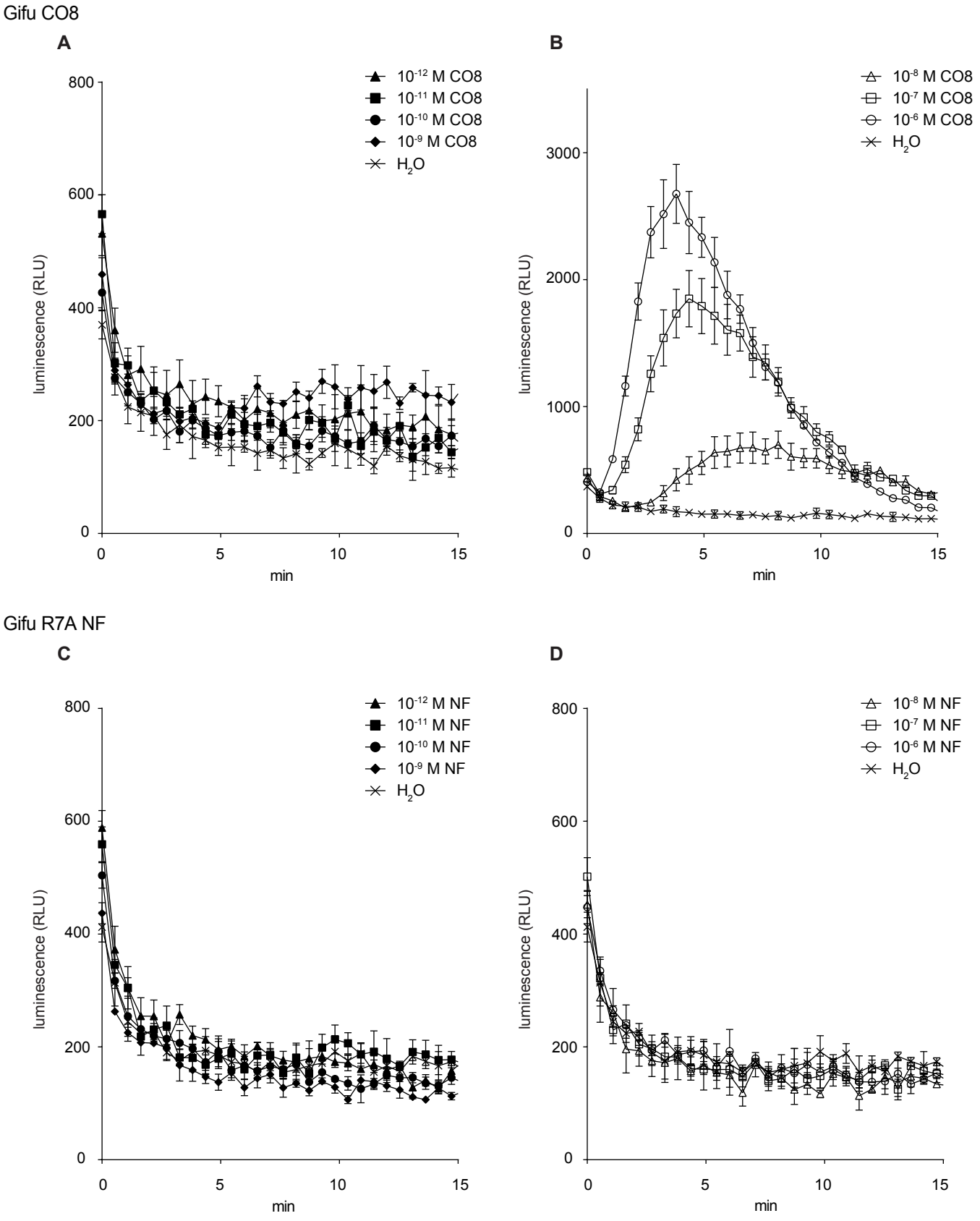

Fig. S3. Primary luminescence curves for ROS responses elicited by CO8 and Nod factor treatment of roots.
Gifu wild type plants were treated with different concentrations of CO8 or purified *M. loti* R7A Nod factor. (*A*) and (*B*) ROS response after 10^{-12}-10^{-6} M CO8 treatment. (*C*) and (*D*) ROS response after 10^{-12}-10^{-6} M Nod factor treatment. Values are plotted as relative light units (RLU) and error bars represent the s.e.m.

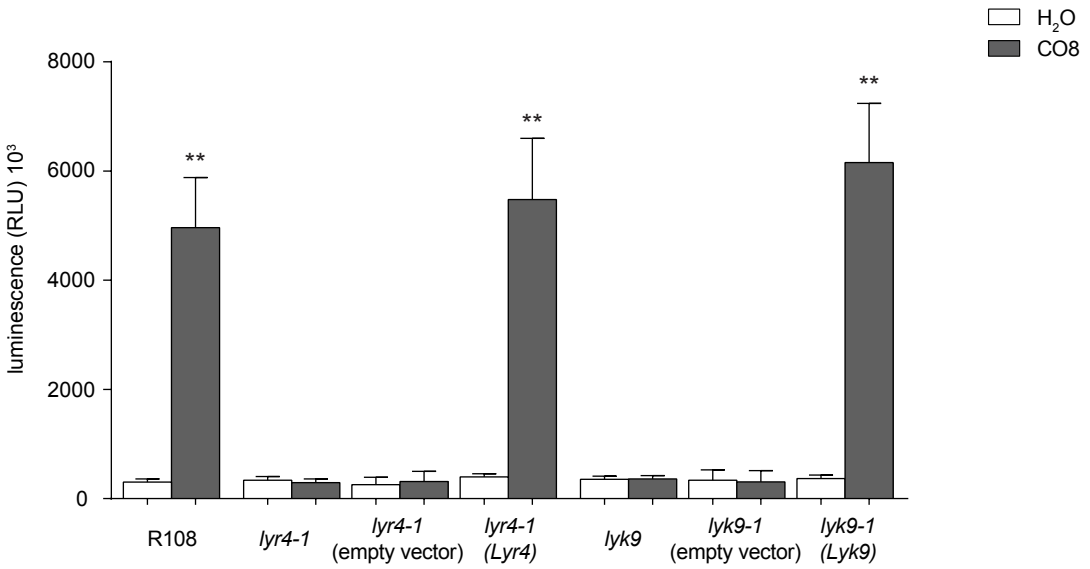

Fig. S4. ROS measurements on complemented *Mtlyr4* and *Mtlyk9* mutants.
The complementing genes are shown in brackets. Peak values are plotted as relative light units (RLU) and error bars represent the s.e.m. **P < 0.01 indicate significant difference (*t*-test) between the CO8 treated sample and the respective water treated control.

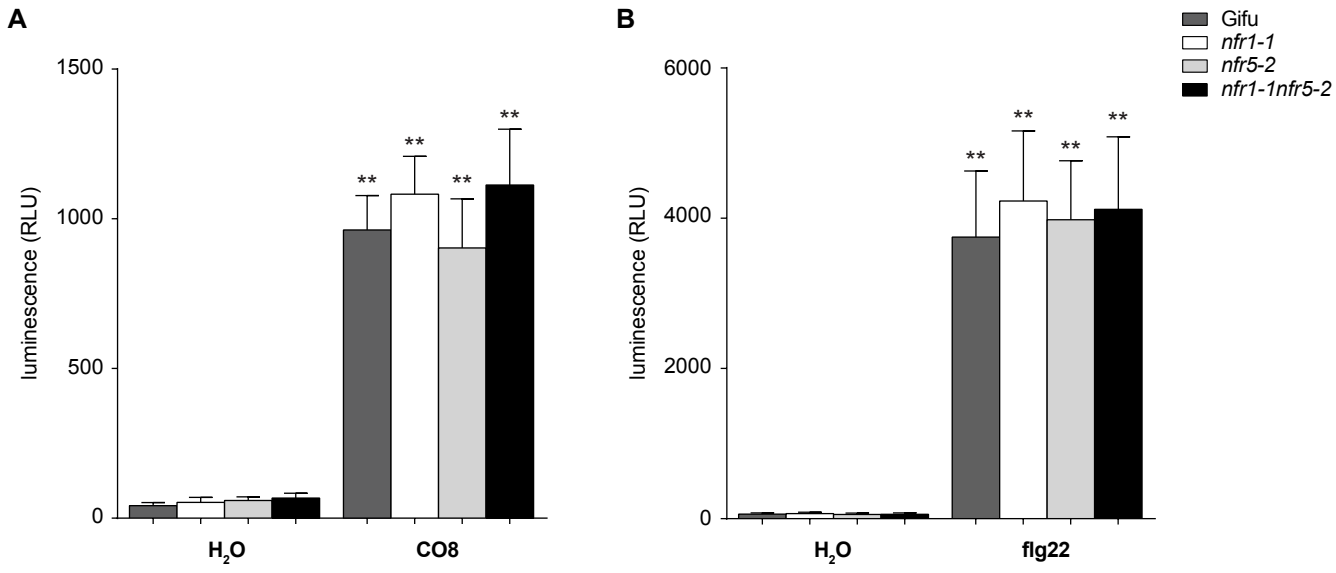

Fig. S5. The *Nfr1* and *Nfr5* nod factor receptors are not required for a chitin induced release of ROS.
(*A*) CO8 induced ROS in *nfr1*, *nfr5* and *nfr1 nfr5* double mutants. (*B*) flg22 induced ROS in *nfr1*, *nfr5* and *nfr1 nfr5* double mutants. Peak values are plotted as relative light units (RLU) and error bars represent the s.e.m. **P < 0.01 indicate significant difference (*t*-test) between the elicitor treated sample and the respective water treated control.

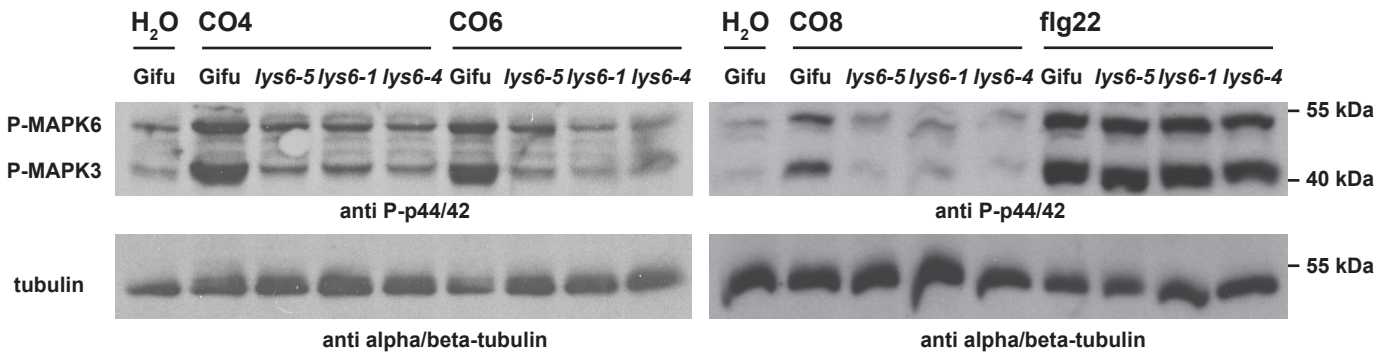

Fig. S6. Chitin induced MAPK3/6 phosphorylation in *Lotus* is dependent on *LjLys6*.
MAPK3/6 phosphorylation in roots of *Lotus* Gifu, *Ljlys6-1*, *Ljlys6-4* and *Ljlys6-5* mutants treated with 1 µM CO4, CO6 and CO8 or 0.5 µM flg22.

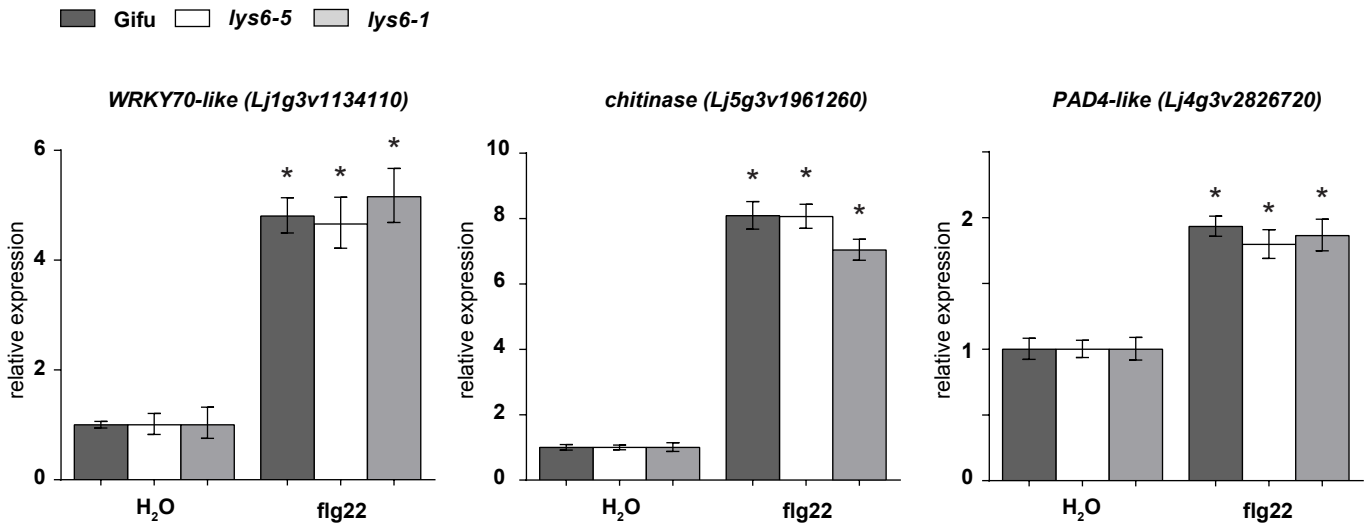

Fig. S7. flg22 treatment triggers defense-response gene upregulation in *Ljlys6* mutants similar to wild type.
QPCR determination of transcript levels in *Lotus* roots after 1 hour treatment with 0.5 µM flg22 or water. The values shown are geometric means of three biological and three technical replicates relative to the respective water treated control set as 1. Bars represent the 95% CI. Asterisk indicates significant difference compared to the respective water treated control.

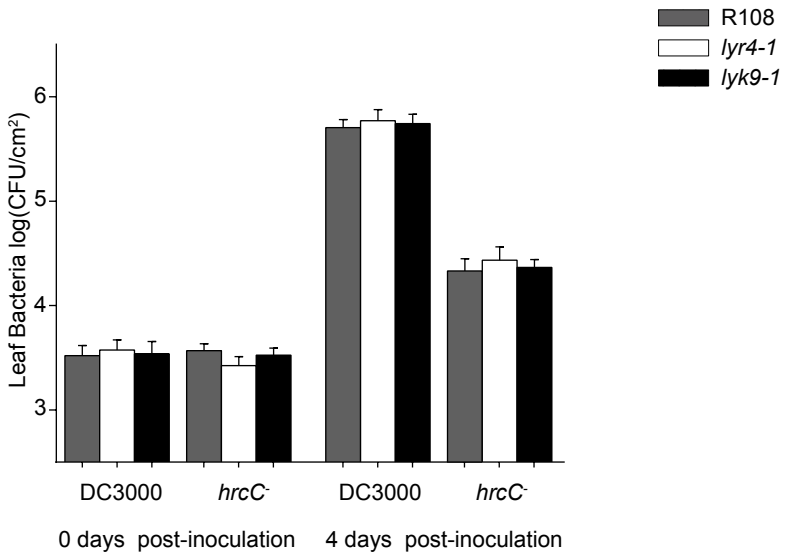

Fig. S8. *Mt*LYR4 and *Mt*LYK9 are not required for P. syringae triggered immunity.
Wild type R108 and *lyr4* or *lyk9* mutant *M. truncatula* leaves were infiltrated with *Pst* DC3000 or its type III secretion system mutant strain *Pst hrcC*$^{-}$. Bacterial population (colony-forming units, CFU) in the leaf was measured at the indicated times (error bars represent the s.e.m., n=6). The experiments were repeated three times with similar results.

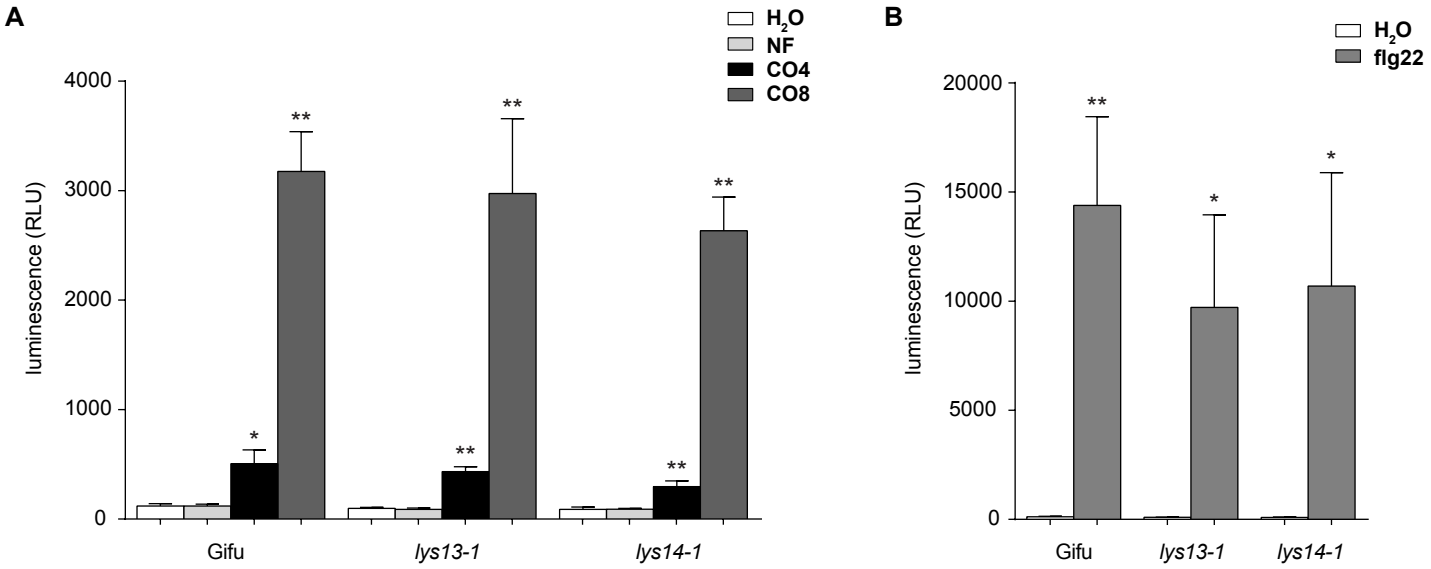

Fig. S9. Nod factor, CO4, CO8 and flg22 induced ROS response is unaffected in *Ljlys13* and *Ljlys14* single mutants. (*A*) Nod factor, CO4 and CO8 induced ROS in Gifu wild type, *Ljlys13* and *Ljlys14* single mutants. (*B*) flg22 induced ROS in Gifu, *Ljlys13* and *Ljlys14* single mutants. Peak values are plotted as relative light units (RLU) and error bars represent the s.e.m. *P < 0.05, **P < 0.01 indicate significant difference (*t*-test) between the elicitor treated sample and the respective water treated control.

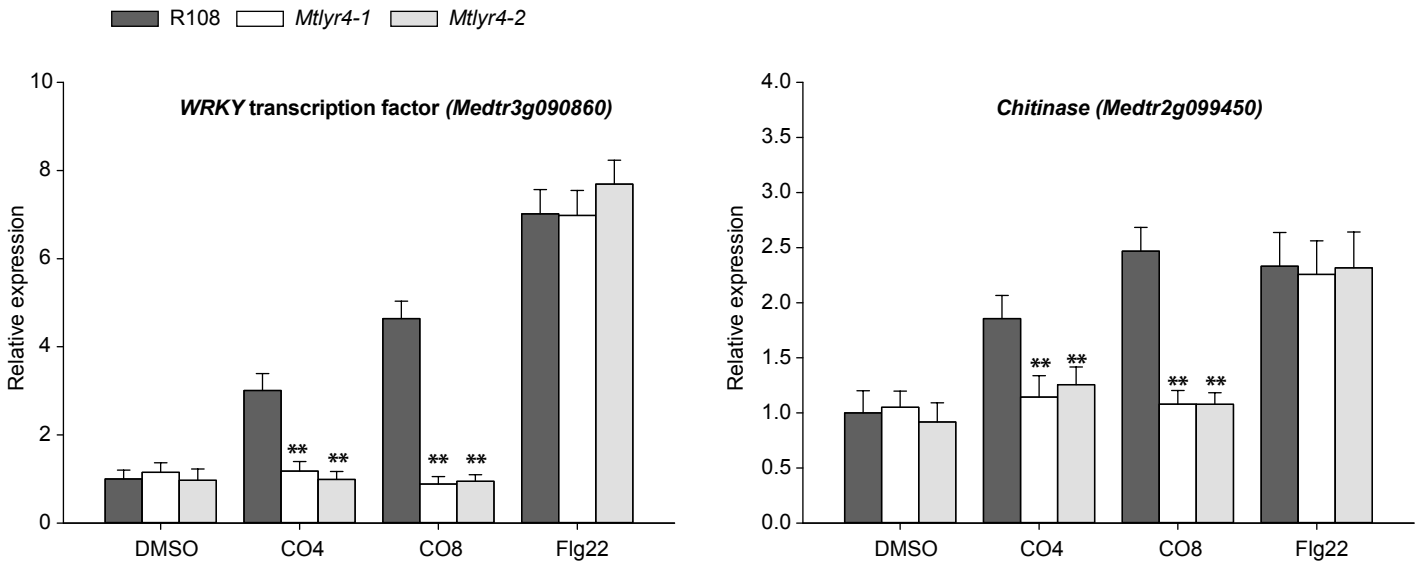

Fig. S10. *Mt*LYR4 is required for CO4 and CO8 induced defense-related gene expression.
Quantitative RT-PCR analysis of two defense-related genes *WRKY* and a *chitinase* in *M. truncatula* wild type R108 and *lyr4* mutants after treatment with 100 nM CO4, CO8, flg22 or DMSO as the negative control (mean+s.e.m., n=8; **indicates significant difference relative to wild type, $p<0.01$ measured using a Student's *t*-test).

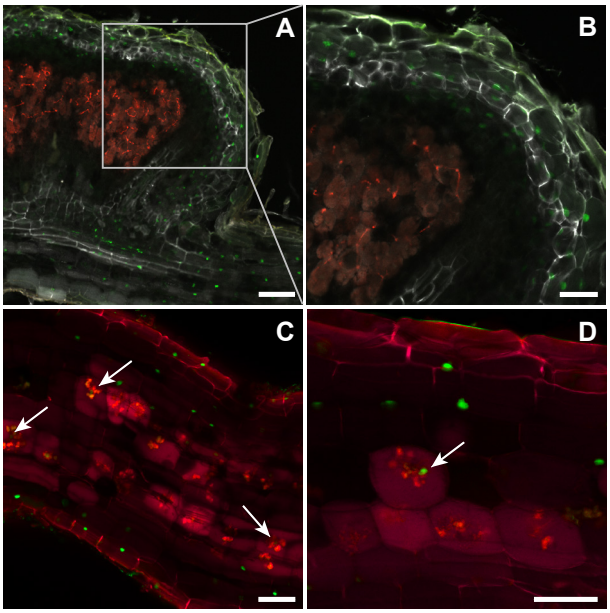

Fig. S11. *LjLys6* promoter expression pattern in root nodules and in arbusculated root cells.
(*A*) and (*B*) Lack of detectable activity of the *proLys6:NLS-tYFP* reporter in mature root nodules with *M. loti* bacteroids expressing DsRed. (*B*) Close up of the area marked in (*A*). (*C*) and (*D*) Activity of the *proLys6:NLS-tYFP* reporter in root cells with fungal arbuscules. Autofluorescence of arbuscules is shown in orange-red. Arrows label nuclei of arbuscule containing root cells showing *LjLys6* promoter activity. Scale bars: 100 µM (*A*) and 50 µM (*B-D*).

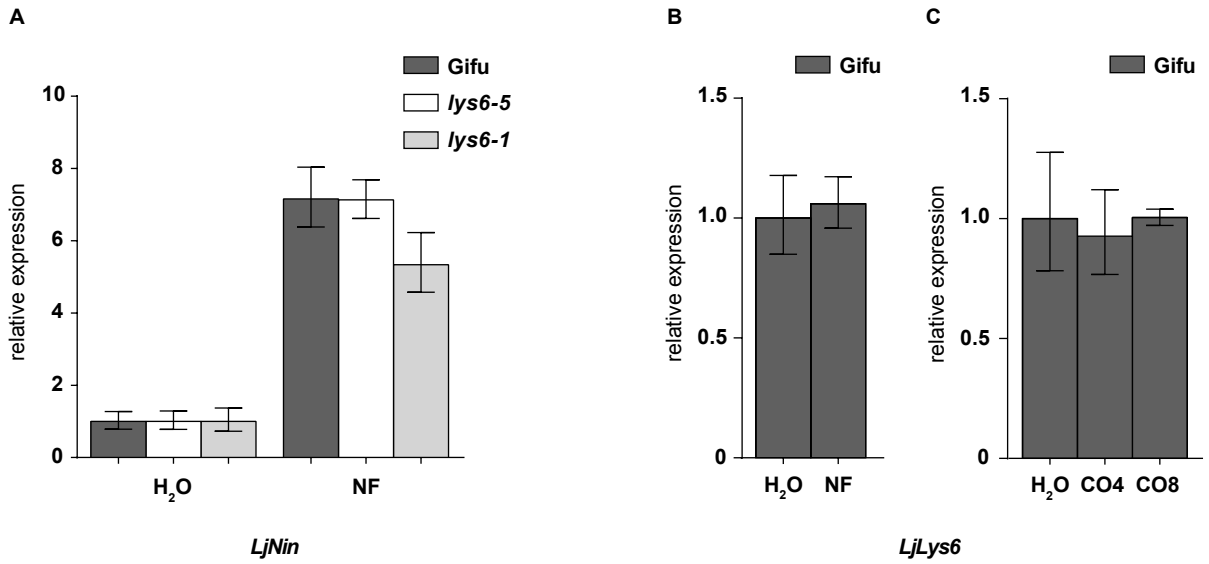

Fig. S12. QPCR determination of transcript levels after Nod factor, CO4 and CO8 treatment of *Lotus* roots.
(*A*) Nod factor induced LjNin expression in wild type Gifu and Ljlys6 mutants. *LjLys6* gene expression after (*B*) Nod factor and (*C*) CO4, CO8 treatment of Gifu roots. The values shown are geometric means of three biological and three technical replicates relative to the respective water treated control set as 1. Bars represent the 95% CI.

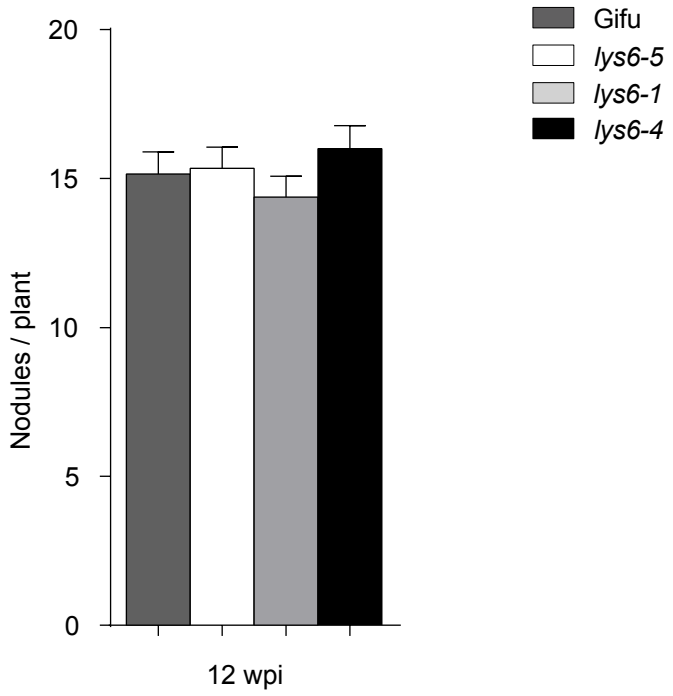

Fig. S13. Long term nodulation test on *Ljlys6* mutants.
Plants were inoculated with *M. loti* NZP2235 (OD*600*=0.02) and grown for 12 weeks in the greenhouse. Bars represent the s.e.m. (n=70). No significant difference was found between Gifu and the *Ljlys6* mutants (*t*-test).

DATA COLLECTION	
PDB accession	5LS2
Beamline	MAX-lab I911-3
Wavelength	0.98
Resolution range	28.23 - 2.3 (2.38 - 2.3)
Space group	P 1 21 1
Unit cell	45.29 130.69 53.63 90 107.702 90
Total reflections	111281 (11297)
Unique reflections	26292 (2625)
Completeness (%)	99.5 (99.8)
Mean I/sigma(I)	15.07 (2.20)
Wilson B-factor	44.64
R-meas	0.0795 (0.977)
REFINEMENT	
Reflections used in refinement	26273 (2624)
R-work	0.183 (0.267)
R-free	0.218 (0.303)
Number of non-hydrogen atoms	3310
macromolecules	3000
ligands	178
Protein residues	391
RMS(bonds)	0.002
RMS(angles)	0.46
Ramachandran favored (%)	98.2
Ramachandran allowed (%)	1.8
Ramachandran outliers (%)	0
Rotamer outliers (%)	0.89
Average B-factor	60.17
macromolecules	59.46
ligands	77.68
solvent	52.87

Table S1.
*Lj*LYS6 data collections and refinement statistics.
Values between parentheses are for the last resolution shell.

target gene	*L. japonicus* v3.0 gene ID	primer orientation	primer sequence (5'-3')	comment	reference
ATP	*Lj5g3v2169420*	fw	CAATGTCGCCAAGGCCCATGGTG	qPCR	
		rev	AACACCACTCTCGATCATTTCTCTG		
PP2A	*Lj2g3v0742070*	fw	GTAAATGCGTCTAAAGATAGGGTCC	qPCR	
		rev	ACTAGACTGTAGTGCTTGAGAGGC		
UBC	*Lj1g3v2063210*	fw	ATGTGCATTTTAAGACAGGG	qPCR	
		rev	GAACGTAGAAGATTGCCTGAA		
Lys6	*Lj6g3v1055580*	fw	CCTGTTGATTCAGTTCGCAAGATGGC	qPCR	
		rev	GCTTTACTGTCACCATCCTATCTTCC		
NIN	*Lj2g3v3373100*	fw	AGGAGCCCAAGTGAGTGCTA	qPCR	
		rev	GCCATCAAGGTATATGACGAG		
peroxidase	*Lj1g3v1183610*	fw	CTCTTTCGGGTTTTGTTCTGTGT	qPCR	(76)
		rev	CAGAAGCTAACTTGACTTGTTGATG		(76)
chitinase	*Lj5g3v1961260*	fw	GCAGTGTCTGATTAGTTAGTCTCA	qPCR	(76)
		rev	GAAGTGATCACATACAGTACTCAAC		(76)
RbohB-like	*Lj6g3v1549190*	fw	GAAATGGCGTACTGTCTTTAAACCA	qPCR	(76)
		rev	GGTGGTTTTCCTGGAAAAGTCAAGA		(76)
WRKY70-like	*Lj1g3v1134110*	fw	TCTCAATTCCCCAGGAGTTACTTC	qPCR	
		rev	TTGATCGGAGTGTCTTTGCATG		
PRp27-like	*Lj5g3v2112200*	fw	CATGGTTATGATGTTACTGCTCGT	qPCR	(76)
		rev	GATCACTATAACCAGTCCTCATCT		(76)
PAD4-like	*Lj4g3v2826720*	fw	CATAGCAAACCAGGAAACCACTATC	qPCR	
		rev	CATCACACCAAATTTTGTACCATTC		
MPK3	*Lj3g3v3087330*	fw	ATTGATCCCACCAAAAGAATCACAGTTGAA	qPCR	
		rev	CCAATGCTTCCCTGTAGATCATCTC		
WRKY53	*Lj3g3v2427550*	fw	GGAACTTCCCTCTCATGCTAGTTAAG	qPCR	
		rev	CTCTTGCTATTGCTTGAGCCAGAAG		
Lys6	*Lj6g3v1055580*	fw	GAACACCCCAGATTAGGGTTCCCC	semi-qPCR	
		rev	CAGGTTAAGATGATGAACACG		
Lys13	*Lj2g3v2899910*	fw	GCTATGCTCCTCTTCTACAAGC	semi-qPCR	
		rev	ACGGTGACCATGGTGTCGGTGATG		
Lys14	*Lj2g3v2899900*	fw	CAGGAAGAGAAGCCACAAGTAGTGGGG	semi-qPCR	
		rev	CACAGAATTTATTATGGCTTCAG		

target gene	*M. truncatula* gene ID	primer orientation	primer sequence (5'-3')	comment
Lyk9	*Medtr3g080050*	fw	ATGGAACATCAACCCAGATTCACCT	semi-qPCR
		rev	CTTTTCCCGGAACAAAAACAATGC	
Lyr4	*Medtr5g085790*	fw	ACATCTACCTATGCTACTAAC	semi-qPCR
		rev	GTTCATTAGCTTCATTAATGC	
		fw	CAGTGAAGAGAATAAGATTAAAG	semi-qPCR
		rev	CAACTTCTCTACCAGAAAGAAGC	
WRKY	*Medtr3g090860*	fw	ACCAGGAATGGCAATGGAAGGTC	qPCR
		rev	AATAGCCTCTTGGATGCATGGC	
chitinase	*Medtr2g099450*	fw	AACACCACCAAGTTTGCCTAGC	qPCR
		rev	GGGCACATGTGTACTCTGCATTG	
H2A	*Medtr4g097170*	fw	CTTTGCTTGGTGCTGTTTAGATGG	qPCR
		rev	ATTCCAAAGGCGGCTGCATA	

target gene	species	primer orientation	primer sequence (5'-3')	comment
CutA	*B. cinerea*	fw	ATTCCACAATATGGCATGAAATC	qPCR
		rev	ATGTTATCTCCAGCGTGACAAAT	

Table S2.
Primers used in the study.