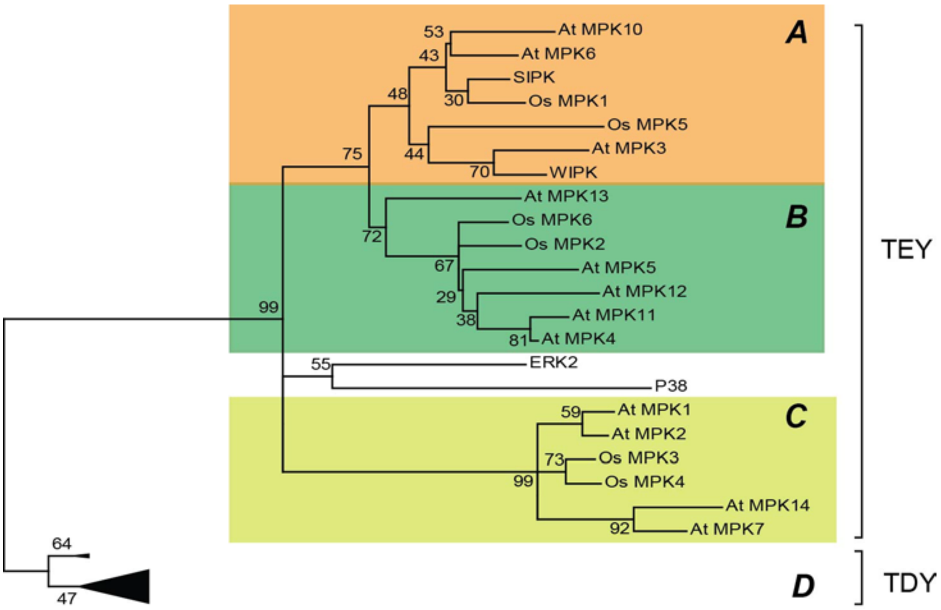
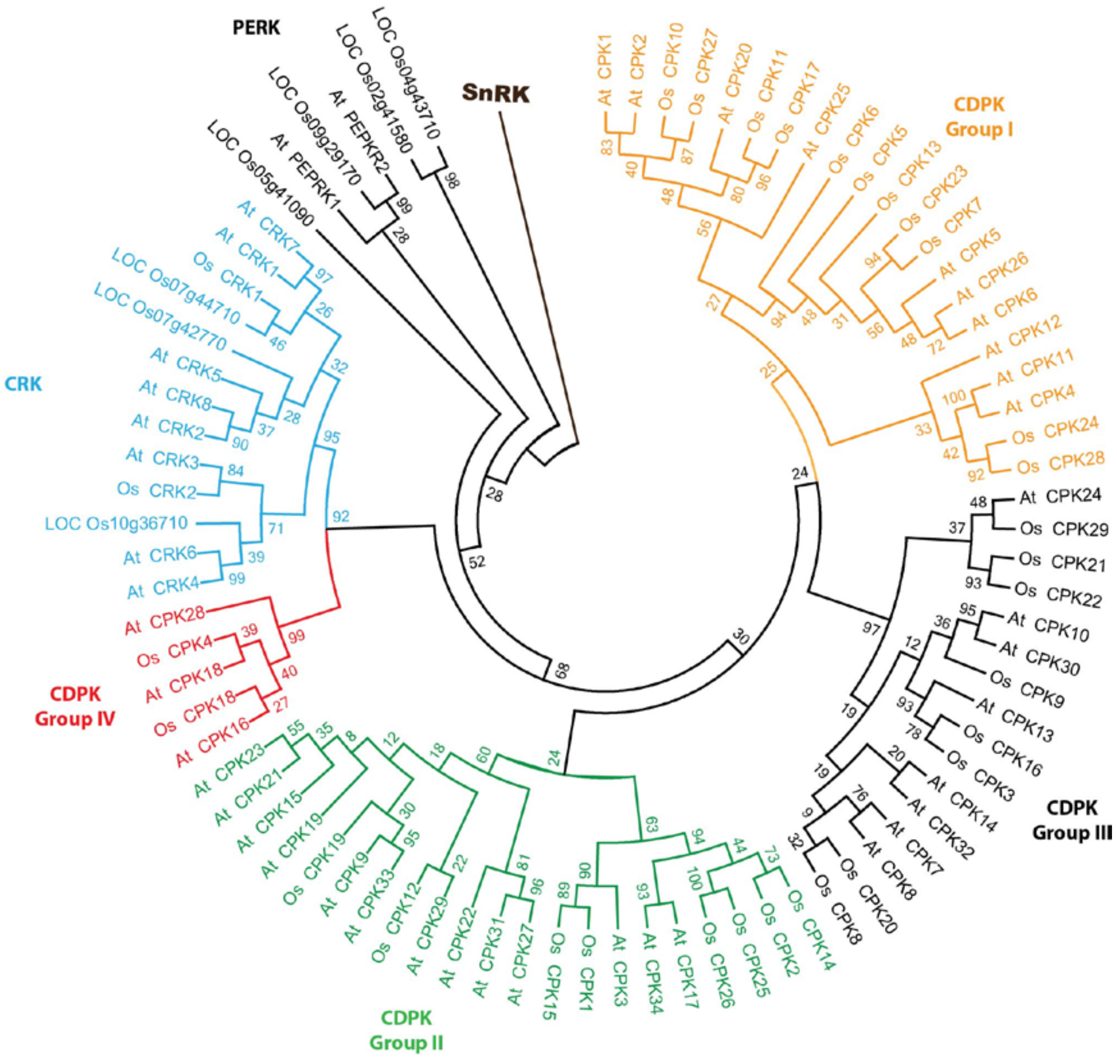
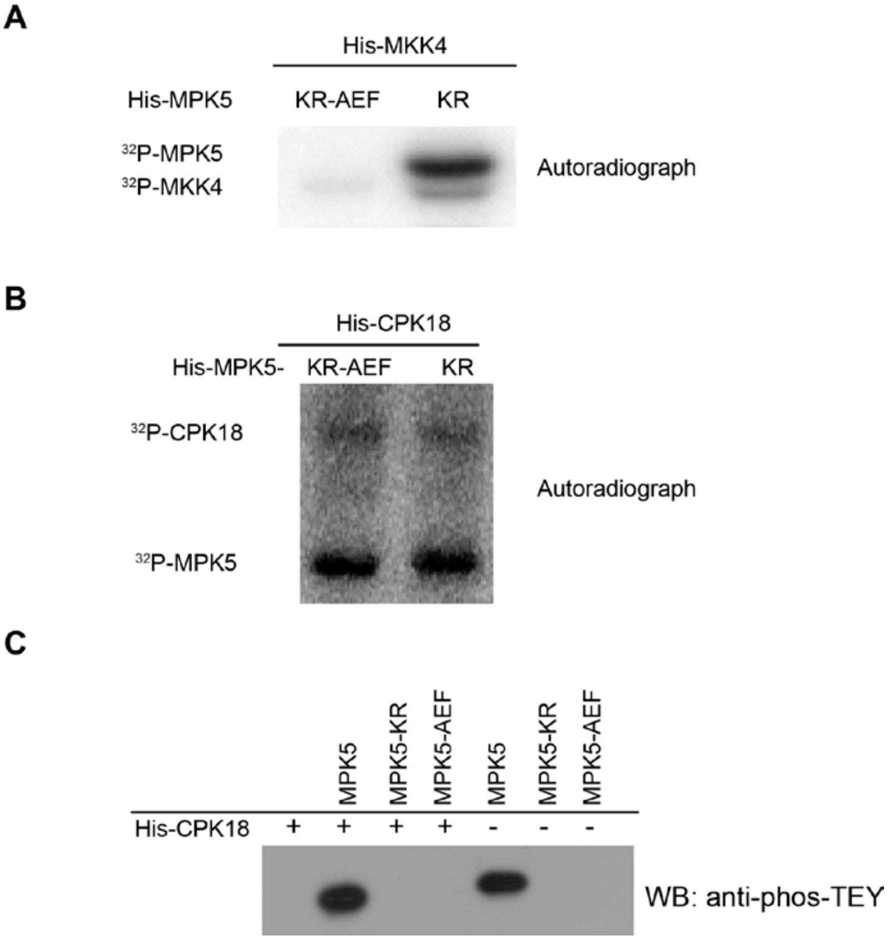


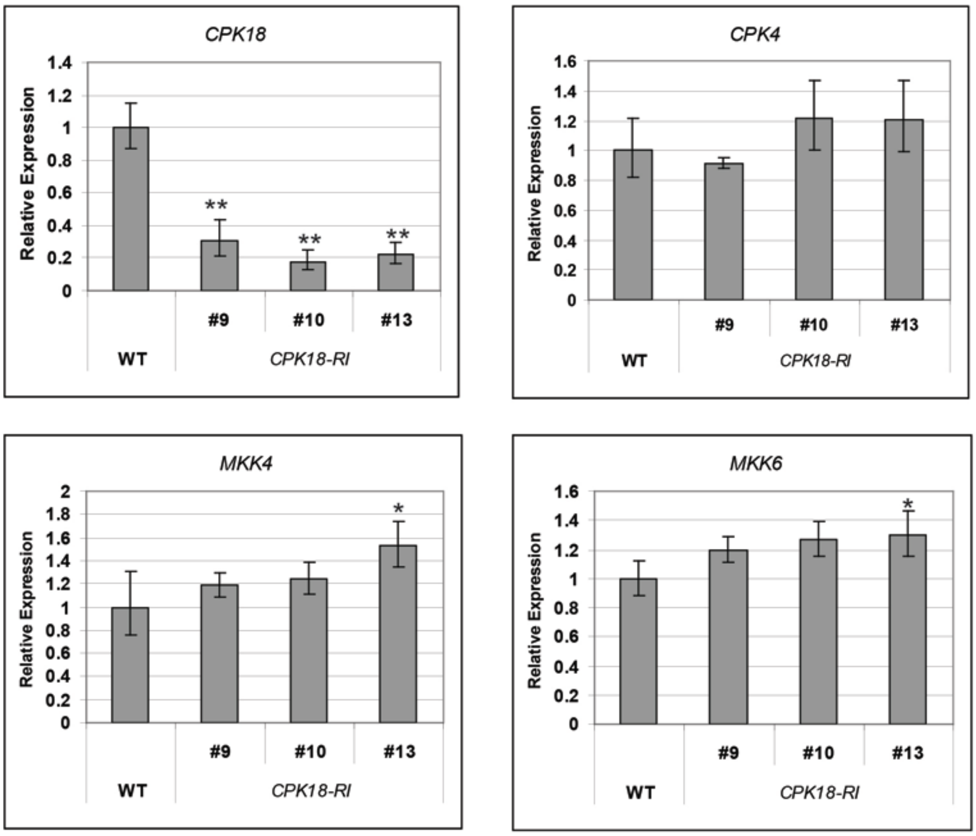
Supplemental Figure 1. The phylogenetic tree of rice and *Arabidopsis* Group A/B/C MAPKs that contain the TEY motif in their activation loop. Tobacco SIPK and WIPK, human ERK2 and P38 were also included in the tree. The numbers at the nodes indicate the bootstrap value. Rice MAPK nomenclature and accession numbers were the same as described in (Reyna and Yang, 2006). *Arabidopsis* MAPK accession numbers were the same as described in (MAPK-group, 2002). The Group D MAPKs, which contain the TDY motif in their activation loop, are schematically illustrated as triangles at the bottom. At, *Arabidopsis*; Os, rice. The alignment used to generate the phylogeny is shown in Supplemental Dataset 1.



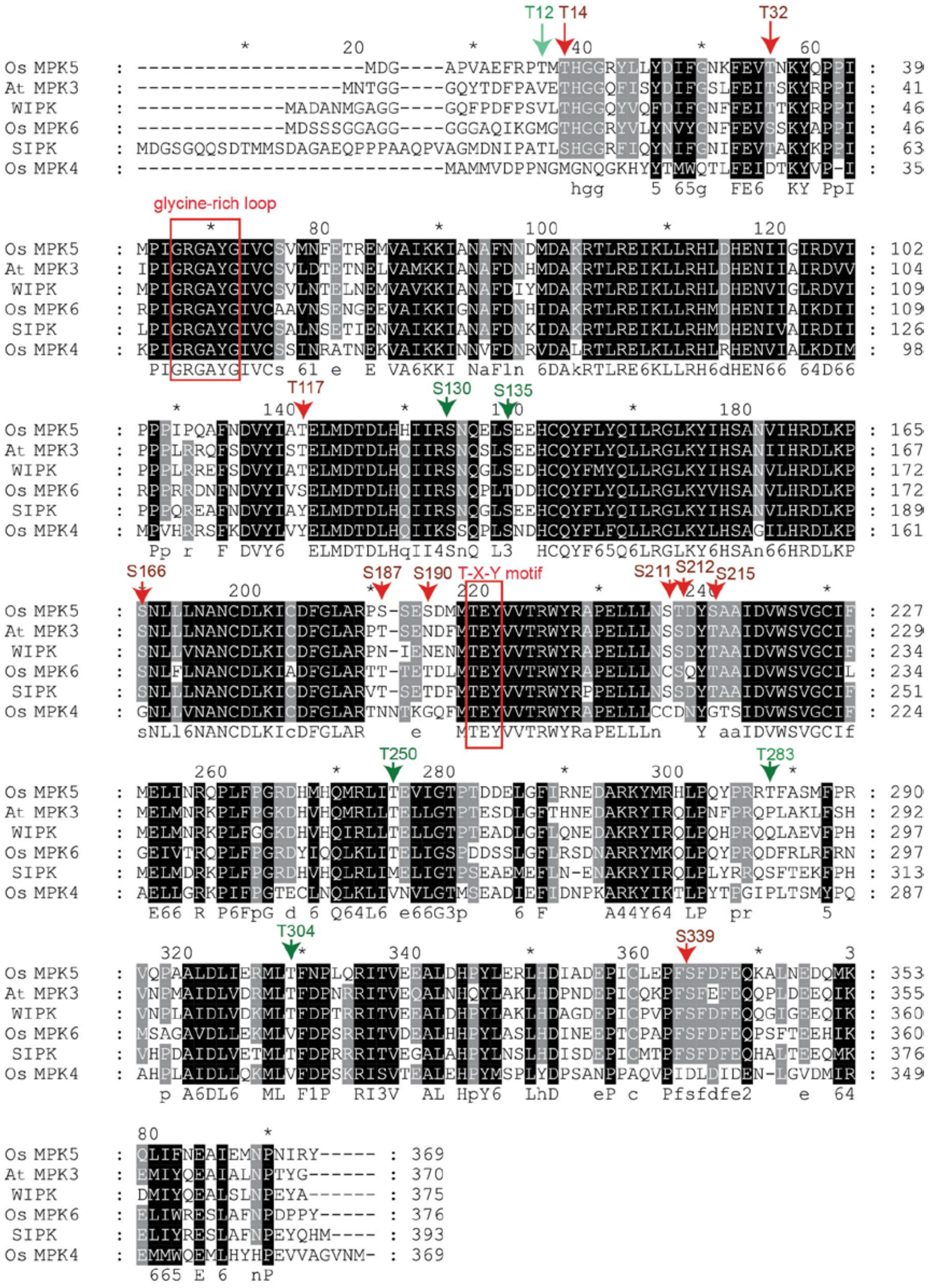
Supplemental Figure 2. Phylogenesis of rice (Os) and *Arabidopsis* (At) CDPKs. CDPKs were divided into four groups (Group I-IV). CRK, PEPRK and SnRK are the three plant protein kinase families closely related to CDPKs. CRK, CDPK-related kinase; PERPK, phosphoenolpyruvate carboxylase kinase-related kinase; SnRK, SNF1-related kinase. The numbers at the nodes indicate the bootstrap value. The LOC_Os indicates the locus ID of rice gene annotations (http://rice.plantbiology.msu.edu/). At, *Arabidopsis*; Os, rice. The alignment used to generate the phylogeny is shown in Supplemental Dataset 2.



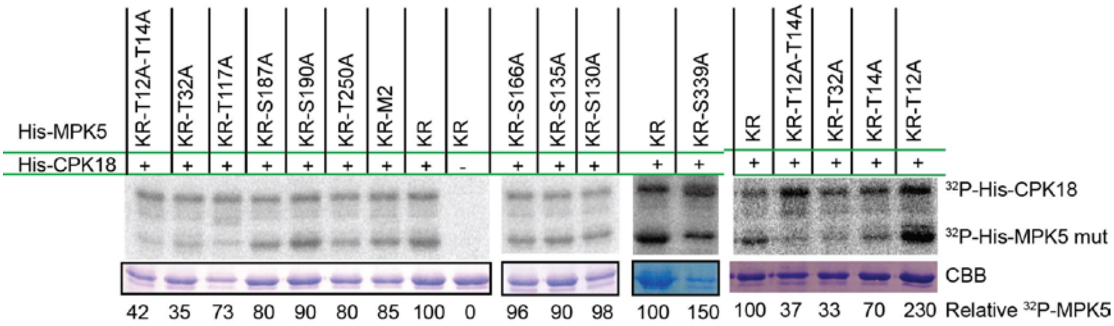
Supplemental Figure 3. The MPK5 TEY motif is phoshorylated by MKK4 and autophosphorylation, but not by CPK18. (**A**) MKK4 was capable of phosphorylating MPK5KR but not MPK5KR-AEF in which the TEY motif was substituted by AEF (MPK5KR-AEF). (**B**) CPK18 was capable of equally phosphorylating MPK5KR and MPK5KR-AEF. (**C**) Detection of the phos-TEY level of MPK5 after autophosphorylation (in the absence of CPK18) and phosphorylation by CPK18. MPK5KR and MPK5-AEF were used as negative controls in immunoblotting.



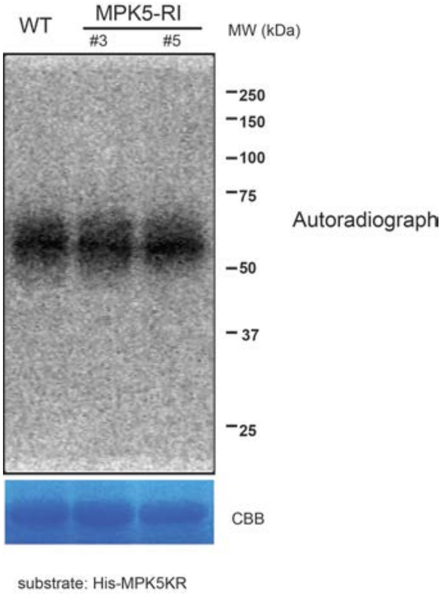
Supplemental Figure 4. Expression of *CPK18*, *CPK4*, *MKK4* and *MKK6* in three CPK18-RI lines (9, 10, and 13). The relative expression level of these genes was measured by RT-qPCR. Data presented as Mean ± SD (*n* = 3). Asterisks indicate statistically significant differences (* *p*<0.05; ***p*<0.01, Student’s *t*-test).



Supplemental Figure 5. Prediction of CPK18 phosphorylated sites on MPK5. Potential CPK18 phosphorylated residues on MPK5 were labelled with red arrows (predicted according to CPK18-MAPK phosphorylation specificity, see Figure 3) or blue arrows (predicted according to common CDPK phosphorylated motifs). The T-x-Y motif and ATP binding pocket (glycine-rich loop) were labelled with rectangles. The numbers and asterisks on the top indicate coordinates of aligned sequence. At, *Arabidopsis*; Os, rice.



Supplemental Figure 6. **Mapping CPK18 phosphorylated sites on MPK5.** MPK5-KR-M2 possessed five substitutions including S211A, T212A, S215A, T283A, and T304A. The amount of mutated His-MPK5 proteins was shown by Coomassie Brilliant Blue (CBB) staining. The loadings of MPK5KR-T117A and MPK5KR-S339A were lower than others because of their poor solubility. The relative phosphorylation level (% to MPK5KR) of MPK5 mutant proteins was shown at the bottom.



Supplemental Figure 7. Native CPK18 activities in WT and MPK5-RI lines. An in-gel kinase assay was performed to measure native CPK18 activities in WT and two MPK5-RI lines (#3 and #5). His-MPK5KR was used as substrate and embedded in SDS-PAGE.

Supplemental Table 1 List of genes and DNA oligos used in this study

Genes and primers used in cloning

Gene	Primer Name	Primer Sequence (5’->3’)	Comment
MPK5 (AF479883)	MPK5-ENTR-F	CACC GAATTCATGGACGGGGCGCCGGTG	TOPO Cloning
	MPK5-ENTR-R	CTAGTACCG GAT GTT TGG GTT CAT	With stop codon
	MPK5-CF-R	CCGGATGTTTGGGTTCA	Without stop codon
CPK18 (AK121471)	CPK18-ENTR-F157	CACC CAG CCG ACG ACG ATG GGA CT	TOPO cloning
	CPK18-ENTR-R1703	CGATCTGTGAACACTCCTCG	Full length
	CPK18-ENTR-R1121	TGC CTG TCC TCC TTC TCT CAC	OsCPK18AC constructs
CPK18	CPK18-ENTR-F1364	CACC GATTGTTGAGGCAATTGACAG	RNAi constructs
	CPK18-ENTR-R	GGATCC AATGACTGCCCTTTGTTCA	
CPK4 (AK060738)	CPK4-ENTR-F	CACC ATGGGCGCGTGCTTCTCATC	TOPO cloning
	CPK4-ENTR-R	TCA CAG GGGTTGTGGATTTGGAG	
CPK7 (AK066500)	CPK7-ENTR-F	CACC ATGGGGAATCAGTGCCAGAA	TOPO cloning
	CPK7-ENTR-R	TCA ATG TACTTGAGGTGCGTCTC	
MPK4 (AK071376)	MPK4-ENTR-F	CACC ATGGCGATGATGGTGGACCC	TOPO cloning
	MPK4-ENTR-R	TCA CATATTCACTCCTGCAACAA	
MPK6 (AK111579)	MPK6-ENTR-F	CACC ATGGATTCCTCCTCCGGCGG	TOPO cloning
	MPK6-ENTR-R	TTA AATGCCAAGGATTCCC	
MKK4 (AK120525)	MKK4-ENTR-F99	CACC GCCGTCGCG ATGCGACCGG	TOPO cloning
	MKK4-ENTR-R1292	TCCCACGTCTCAATGCCAAAC	
PR5 (AK241419) promoter	Pro-PR5-F	CACCATGGAAGTTATATTACGTCTAC	TOPO cloning
	Pro-PR5-R	TAACAATTTACTCTACTATA	
Hin1 (AK068115) promoter	Pro_Hin1-F	CACC GCATGCATGTGGACCC	TOPO cloning
	Pro_Hin1-R	GATCGTAGCTAGTTGTGACAATT	

Genes and primers used in qRT-PCR

Gene	Primer	Primer Sequence (5’->3’)
CPK4 (AK060738)	CPK4-qF1942	TGCTGTTCTAGTCTCCCATTCTCC
	CPK4-qR2032	CCCAGGCAACTTTATTGCGA
CPK18 (AK121471)	CPK18-qF1686	AGGAGTGTTCACAGATCGTAGC
	CPK18-qR1802	TTCTGGCAACAATTTTGATTACAC
UBQ10	UBQ10-qF1447	TGGTCAGTAATCAGCCAGTTTG

(AK101547)	UBQ10-qR1521	CAAATACTTGACGAACAGAGGC
MPK4 (AK071376)	MPK4-qF1605	GGCCACGTTTACACATATATTTGC
	MPK4-qR1666	CCGCCCTAAAACCAAGCA
MPK5 (AF479883)	MPK5-F	TCGAGCAGAAGGCTCTAAACG
	MPK5-R	CCGGATGTTTGGGTTCATCT
MPK6 (AK111579)	MPK6-qF1102	TTTGACCCAAGCAGACGGATA
	MPK6-qR1163	AGAGAAGCCAAGTATGGGTGATG
MKK4 (AK120525)	MKK4-qF1266	ACGGTGGTTTGGCATTGAG
	MKK4-qR1327	GAATACCCAAAATGGCTAGGAAGA
MKK6 (AK059461)	MKK6-qF1344	TCTTGGTAGCGGCACATGTTC
	MKK6-qR1407	CCTCCCGAATTTTCAACATCA
PR5 (AK241419)	PR5-qF	TACAACGTCGCCATGAGCTTCT
	PR5-qR	TGGGCAGAAGACGACTTGGTAGTT
PR10 (D38170)	PR10-qF	TGGCATGCTCAAGATGATCGAGGA
	PR10-qR	TTACTCTCACGGACTCAAACGCCA
Chitinase (AK104397)	CHI-qF830	GTTCATCTGGTCAGCGGATAGC
	CHI-qR896	CTGAGCCTTGGTCTCGTACTCA
Hin1 (AK068115)	Hin1-qF	ATTGACGTGTTCGTGTCACTGAT
	Hin1-qR	GTTCCCAGCCGAGGAGTTC

DNA oligos used for site-directed mutangenesis

Gene	Oligo Name (Gene-mutation)	Oligo for mutagenesis
MPK5	MPK5-T12A	AGTTCAGGCCG GCG ATGACGCACGG
	MPK5-T14A	GGCCGACGATG GCG CACGGCGGC
	MPK5-T32A	GAACAAGTTCGAGGTGGCG AACAAGTACCAGCC
	MPK5-T117A	ACGTCTACATCGCC GCG GAGCTCATGGAC
	MPK5-S187A	TGGCGCGGCCG GCG TCGGAGAGC
		ACCCGCGC CGG GCG TTCGCGAGC
	MPK5-S211A-T212A-S215A	TGCTGCTCAACGCCGCCGACTACGCCGCCGCCATC
	MPK5-S339A	GCCTGGAGCCCTTCGCCTTCGACTTCGAG
	MPK5-T250A	GATGCGCCTCATCGCCGAGGTGATCGG
	MPK5-S190A	GGCCGTCGTCGGAGGCCGACATGATGACGG
	MPK5-S188A	CGCGGCCGTCGGCGGAGAGCGAC
	MPK5-T304A	TCGAGAGGATGCTCGCCTTCAACCCGCTG
	MPK5-K65R	GAGATGGTGGCGATAAGGAAGATCGCCAACGC
	MPK5-T194A	GAGAGCGACATGATGGCGGAGTACGTGGTCA

	MPK5-T194A-E-Y196F	GAGCGACATGATGGCGGAGTTCGTGGTCACCCGG
	MPK5-T14D	TTCAGGCCGACGATGGATCACGGCGGCCGGTAC
	MPK5-T32D	GGGAACAAGTTCGAGGTGGATAACAAGTACCAGCCGCCC
CPK18	CPK18 (D178A)	GGTTTGGTTCATCGGGCCATGAAGCCTGAGAAC