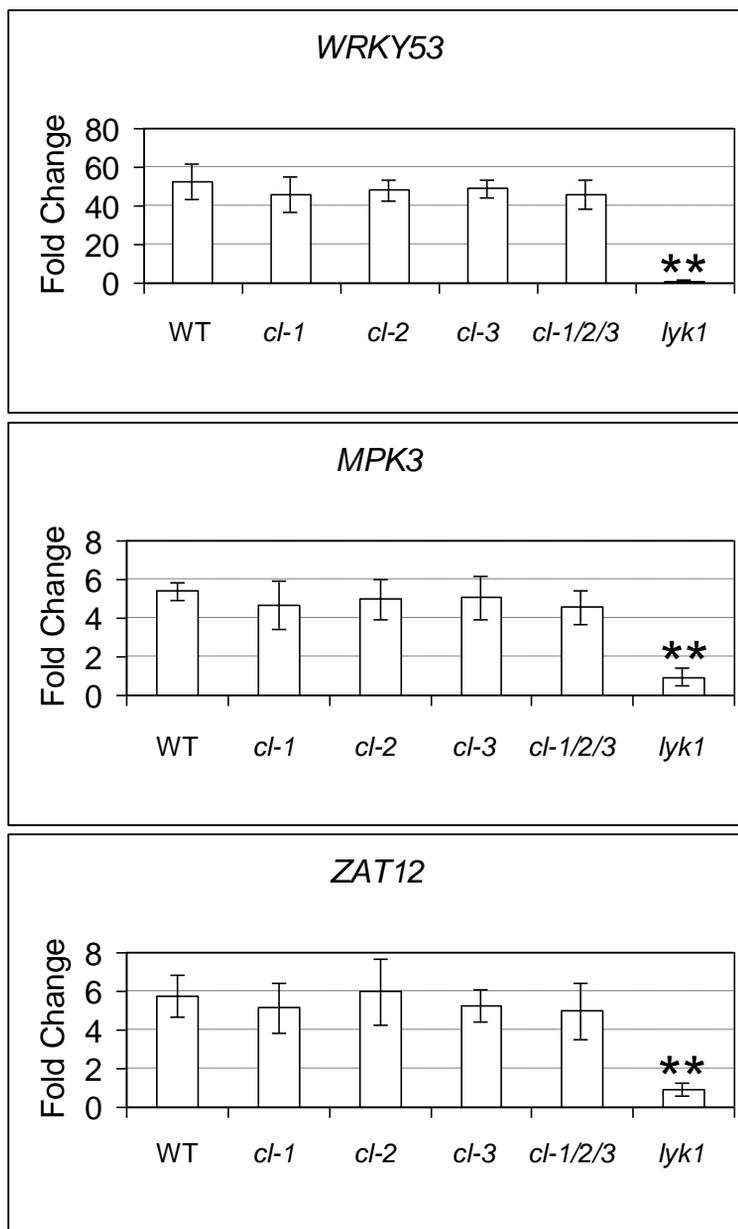
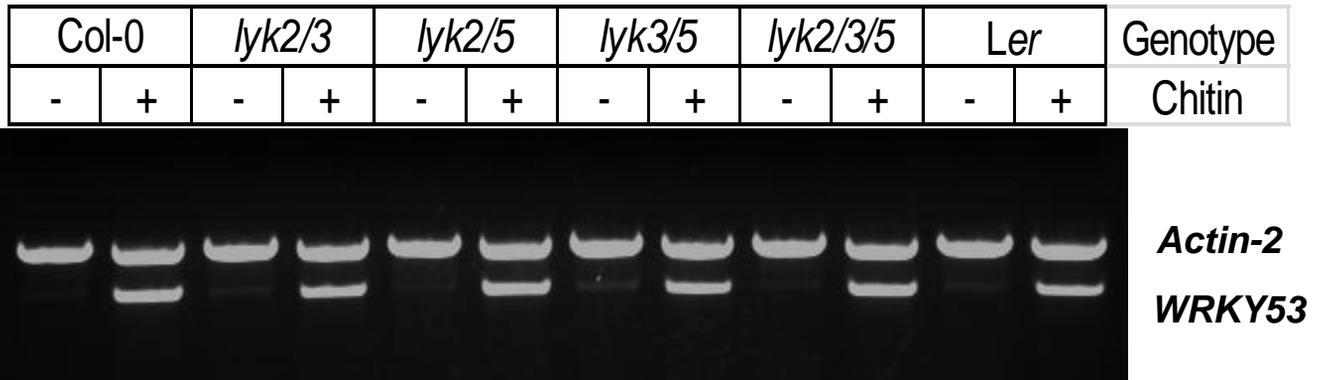


Supplemental Table S1. *Microsomal fraction proteins pulled down by chitin magnetic beads.*

AGI code	Protein name	MW (kDa)	No. of Peptides
At3g21630	LysM RLK1 (LYK1)	67	16
At2g33580	LysM RLK5 (LYK5)	73	13
At2g23770	LysM RLK4 (LYK4)	67	8
At2g17120	CEBiP-like protein 1	38	8
At1g07660	Histone superfamily protein	11	8
AtCg00120	ATPase alpha subunit (in the thylakoid membrane of the chloroplast)	55	7
At5g28540	Luminal binding protein BiP, an ER-localized member of the HSP70 family	74	6
At4g40030	Histone superfamily protein	15	4
At3g08580	Mitochondrial ADP/ATP carrier	41	3
At5g12250	Beta-tubulin	51	3
At5g08670	Mitochondrial ATP synthase beta-subunit	60	2
At2g37620	A member of the actin family	42	2

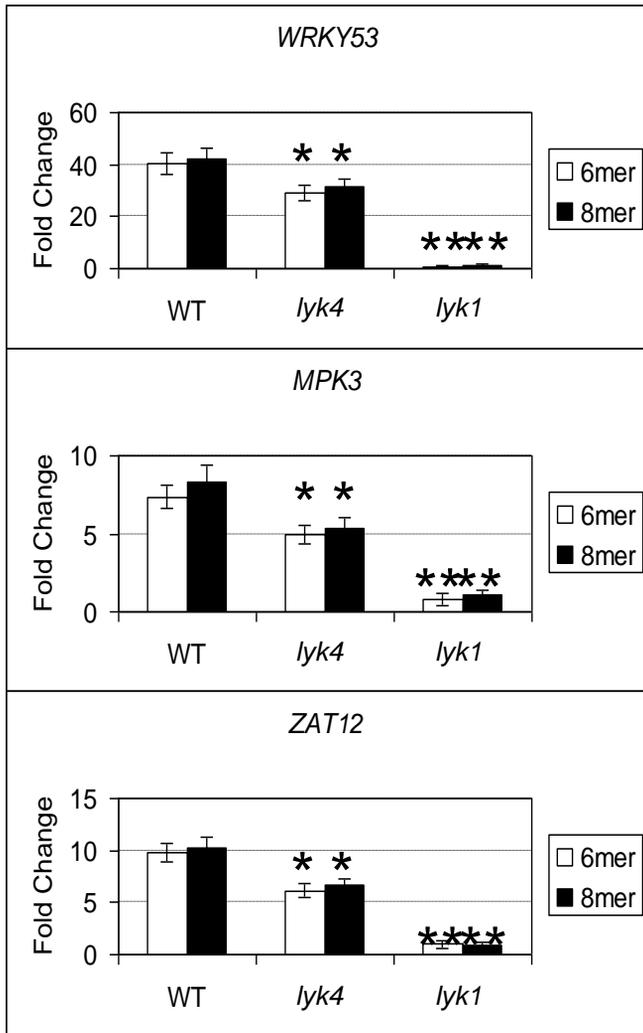


Supplemental Fig. S1. Analysis of chitin-responsive genes in the *CEBiP-like* mutants. Seedlings were treated with the purified chitin oligomer chitooctose at a final concentration of 1 μ M for 30 minutes, and then subjected to qRT-PCR analysis. The relative fold change (\pm SE) of the chitin-responsive genes (*WRKY53*, *MPK3*, and *ZAT12*) in a particular genotype was obtained from the comparison between the chitin-treated plants and the mock-treated plants after normalization with the reference gene, *SAND*. WT, wild-type plants; *cl-1*, *CEBiP-like-1* mutant; *cl-2*, *CEBiP-like-2* mutant; *cl-3*, *CEBiP-like-3* mutant; *cl-1/2/3*, a triple mutant of *CEBiP-like-1*, 2, and 3. Asterisks indicate statistically significant differences with respect to WT (** $P < 0.01$).

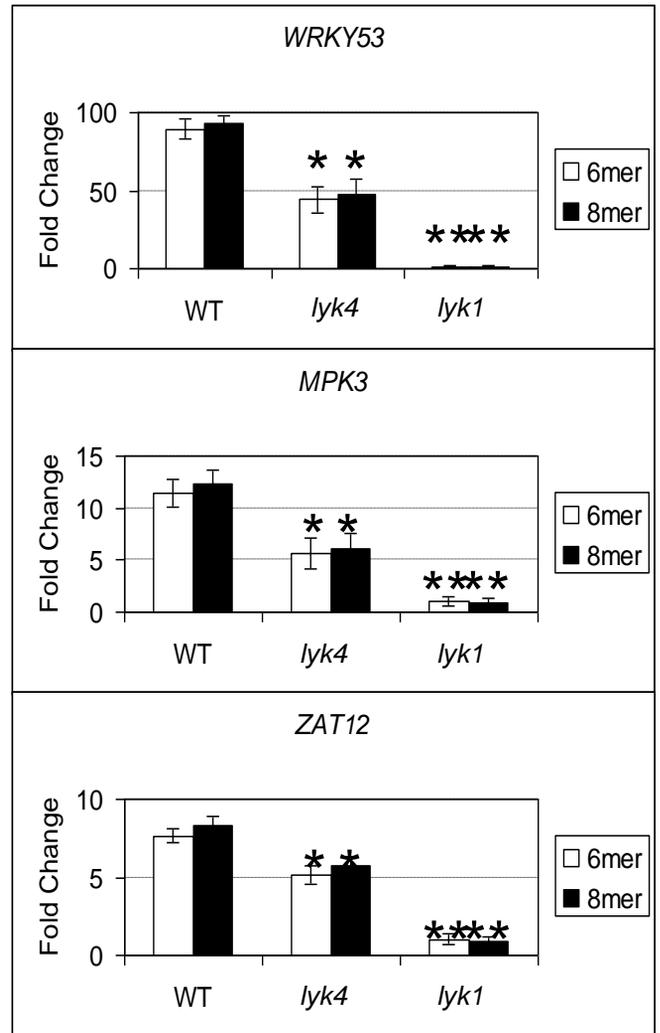


Supplemental Fig. S2. Induction of *WRKY53* by chitin is not blocked in the mutants of the *LYK2*, *LYK3*, and *LYK5* genes. Seedlings were treated with (+) or without (-) the purified chitin oligomer chitooctaose at a final concentration of 1 μ M for 30 minutes, then subjected to semi-quantitative RT-PCR analysis. *Actin-2* was included as an internal control. Col-0 and Ler, wild type plants; *lyk2/3*, double mutant of *lyk2* and *lyk3*; *lyk2/5*, double mutant of *lyk3* and *lyk5*; *lyk2/3/5*, triple mutant of *lyk2*, 3, and 5.

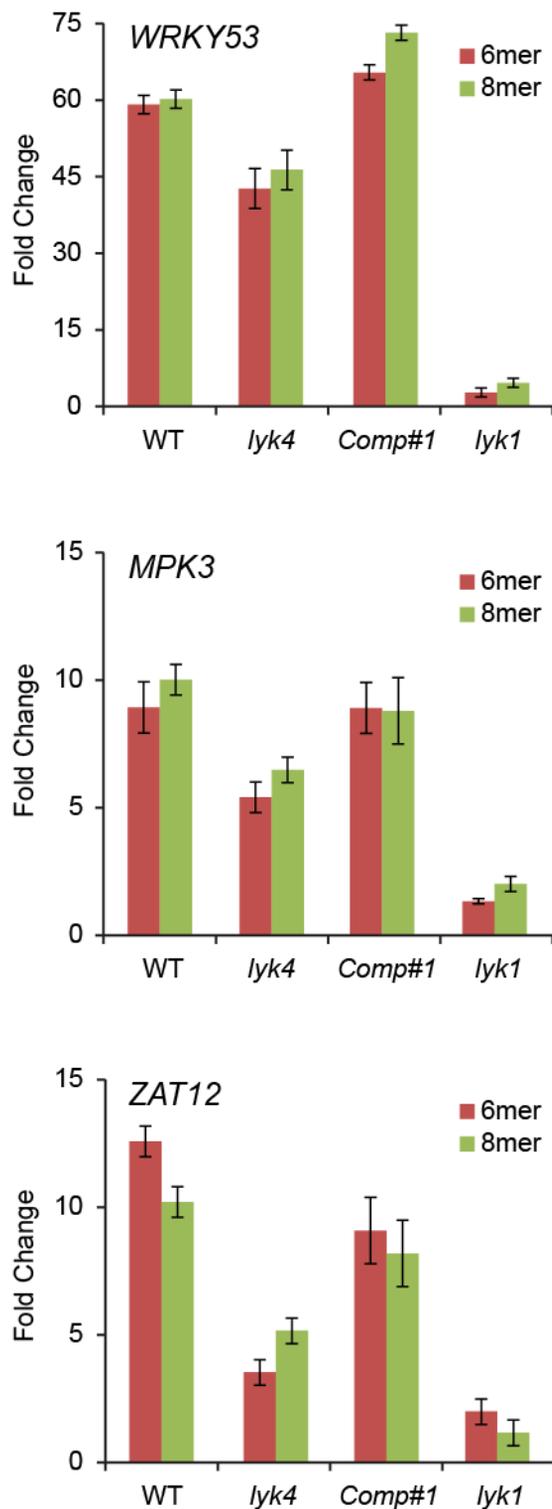
Aerial tissues



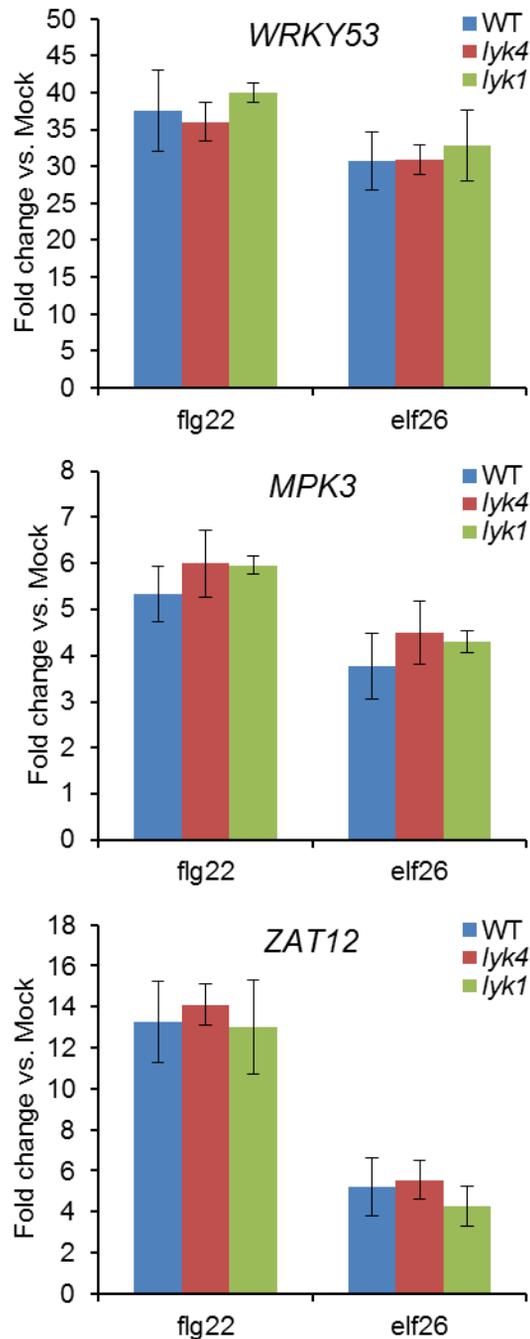
Root tissues



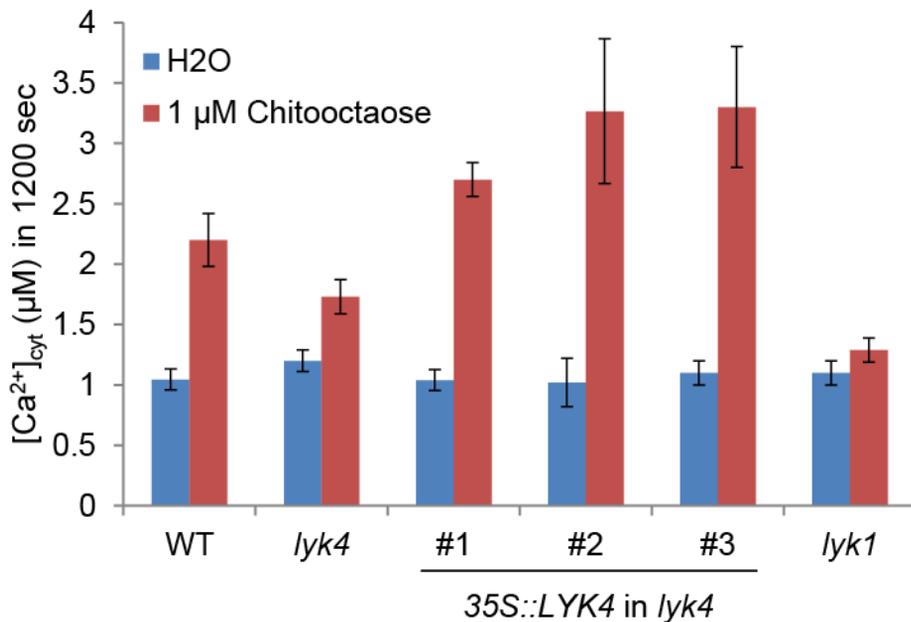
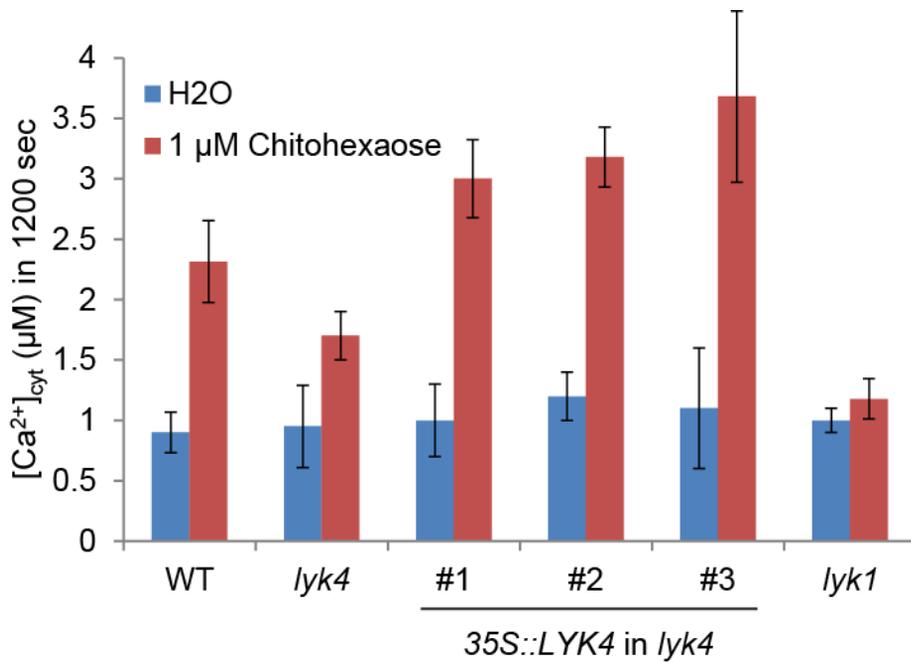
Supplemental Fig. S3. Expression levels of chitin-responsive genes in the aerial tissue or root tissues of *lyk4* mutant seedlings. Seedlings of *lyk4* and *lyk1* mutants were treated with the purified chitin oligomers, chitohexaose (6mer) and chitooctaose (8mer), at a final concentration of 1 μ M for 30 minutes, respectively, and then the aerial and root tissues were harvested separately for qRT-PCR analysis. The relative fold change (\pm SE) of chitin-responsive genes (*WRKY53*, *MPK3*, and *ZAT12*) in a particular genotype was obtained from the comparison between the chitin-treated plants and the mock-treated plants after normalization with the reference gene, *SAND*. Asterisks indicate statistically significant differences with respect to WT (*P < 0.05, **P < 0.01).



Supplemental Fig. S4. Analysis of chitin-responsive genes in complemented lines of the *lyk4* mutant. Seedlings (wild type, *lyk4*, *35S::LYK4* in *lyk4*, or *lyk1*) were treated with the purified chitin oligomer, chitohexaose (6mer) and chitooctaose (8mer), at a final concentration of 1 μ M for 30 minutes, and then subjected to qRT-PCR analysis. The relative fold change (\pm SE) of the chitin-responsive genes (*WRKY53*, *MPK3*, and *ZAT12*) in a particular genotype was obtained from the comparison between the chitin-treated plants and the mock-treated plants after normalization with the reference gene, *SAND*.



Supplemental Fig. S5. Analysis of flg22- or elf26-induced gene expressions in the *lyk4* and *lyk1* mutants. Seedlings were treated with flg22 or elf26 at a final concentration of 1 μ M for 30 minutes, and then subjected to qRT-PCR analysis. The relative fold change (\pm SE) of a target gene in a particular genotype was obtained using qRT-PCR with the comparison between the MAMP-treated plants and the mock-treated plants after normalization with the reference gene *SAND*.



Supplemental Fig. S6. Effects of chitin treatments on the $[Ca^{2+}]_{cyt}$ response in complemented lines of the *lyk4* mutant. Five-day-old seedlings were treated with 1 μM of the purified chitin chitohexaose or chitooctaose, or H₂O as a negative control. Histogram represents integrated $[Ca^{2+}]_{cyt}$ values over 1200 sec after chitin treatment. Each value shows a mean of 6 seedlings with SE.

```

PKA-Ca -----FERI 4
CaMK1 -----MLGAVEGPRWKQAEDIRDIYDFR 23
L4 -CLSKKTKTQTQEE TGNLDSFMGKKPPMSDQE----FDPLDGLSGMVVESLKVKYKFHE 54
L1 YAYRKNKSKGDSFSS SIPLSTKADHASSTS LQSGGLGGAGVSPGIAAISVDKSVEFSLEE 60

          |           |           |
PKA-Ca KTLGTGSFGRVMLVKHKETGNHYAMKILDKQKVVKLKQIEHTLN---EKRILQAVNFPFL 61
CaMK1  DVLGTGAFSEVILAE DKRTQKLVAI KCIAKEALE--GKEGSMEN---EIAVLHKIKHPNI 78
L4     LQSATSDFTSSSSSIG----GSGYIGKINGDGAMIKKIEGNAS---EEVNLLSKLNHLNI 106
L1     LAKATDNFNLSFKIGQGGFGAVYYAELRGEKAAIKKMDMEASKQFLAELKVLTRVHVNL 120
          * . *           : .           * : * : : . :

          IV          V          VIa
PKA-Ca VRLEFSFKDNSNLYMVMEYVPGGEMFSHLRRIGRFSEPHAR---FYAAQIVLTFEYLHSLD 119
CaMK1  VALDDIYESGGHLYL IMQLVSGGEL FDRIVEKGFYTERDAS--RLIFQVLDVAVKYLHDLG 136
L4     IRLSGFCFHEGDWYLVYEHASNGSLSEWIHTT-KSLLSLTQKLQIALDIATGLNYLHNFA 165
L1     VRLIGYCVVEG-SLFLVYEVVENGNLGQHLHGSGRE PLPWTKRVQIALDSARGLEYIHEHT 179
          : *           : : : . * : : :           :           : : : * :

          VIb          VII          VIII
PKA-Ca ---LIYRDLKPENLL---IDQQGYIQVTDFGFAKRVKG---RTWTLCGTPEYLAPEIILS 170
CaMK1  ---IVHRDLKPENLLYSLDEDSKIMISDFGLSKMEDPG-SVLSTACGT PGYVAPEVLAQ 192
L4     DPPYVHRDLNSMNVF---LDLEFRAKIGSLGSARS TTEDFVLTKHVEGTRGYLAPEYLEH 222
L1     VPVYVHRDIKSANIL---IDQKFRAKVADFGLTKLTEVGGSATRGAMGT FGYPAPETVYG 236
          : : * : : . * : :           : : * : :           * * * : * : :

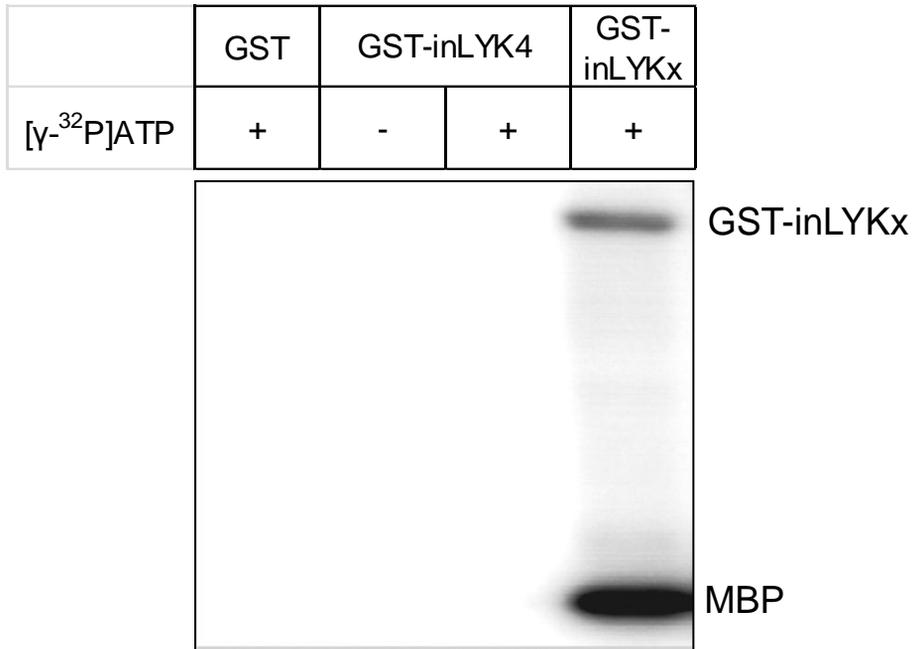
          IX          X
PKA-Ca KGYNKAVDWVWALGVLIYEMAAGYPPFFADQPIQIYEKIVSGKVRFPSPS----HFSSDLKDL 226
CaMK1  KPYSKAVDCWSIGVIAYILLCGYPPFYDENDAKLFEQILKAEYEFDSPYWDDISDSAKDF 252
L4     GLVSTKLDVYAFGVVLLEIVTGK-----EASELK-----KEIDEGKA 259
L1     -EVSAKVDVYAFGVVLYELISAKGAVVKMTEAVGEFRGLVGVFEESF-----KETDKEEA 290
          . * : : * : :           : .           . . .

          XI
PKA-Ca LRNLLQVDLTKRFGNLKNGVN-----DIKNHKWFATTDWIAIYORKVG 269
CaMK1  IRHLMEKDPEKRFTCEQALQHPWIAGDTALDKNIHQSVSEQIKKNFAKSKWQAFNATAV 312
L4     IDEILIH--GRLLPEGLTSSV-----ERLVVDCLKKDHLNRPSMDEN 299
L1     LRKIDPRLGDSYPFDSVYKM-----AELGKACTQENAQLRPSMRYI 332
          : : :           .           .

PKA-Ca LPSLSLLKIWQAKSQGGQPTR----- 292
CaMK1  VRHMRKLQLGTSQEGQGTASHGELLTPVAGGPAAGCCCRDCCVEPGTELSPTLPHQL 370
L4     VMSLSKILAATQNWESSY----- 318
L1     VVALSTLFSSTGNWDVGNFQNEDLVSLMSGR----- 363
          : : :           .

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Supplemental Fig. S7. Comparison of the LYK4 kinase domain with other kinases. ClustalW2 was used for the alignment. L1, LYK1; L4, LYK4; CaMK1, Ca²⁺/Calmodulin-dependent protein kinase I (NP_003647); PKA-Ca, PKA catalytic subunit alpha-form (XP_002761879).



Supplemental Fig. S8. Kinase assay of the recombinant LYK4 protein. The intracellular part of LYK4 was expressed as the GST fusion protein, GST-inLyk4. MBP (myelin basic protein) was used as the kinase substrate. LYKx: a LYK protein similar to LYK4.