Supplemental Figure 1. Genetic analysis of *sls1* mutant.

(A) and **(B)** Phenotype assay of F_1 **(A)** and F_2 **(B)** progenies of *sls1* and *lks1-2* on LK medium. The photographs were taken 10 days after transferring from MS to LK medium. The table showed the phenotype segregation of F_2 progenies.

Genetic Analysis of *sls1* Mutants.

| Cross ($♀ \times ♂$)	Generation	Total seedlings tested	Low-K$^+$ sensitive	Low-K$^+$ insensitive	χ^2
lks1-2 $\times$ *sls1*	F_2	782	589	193	0.00012

Supplemental Figure 2
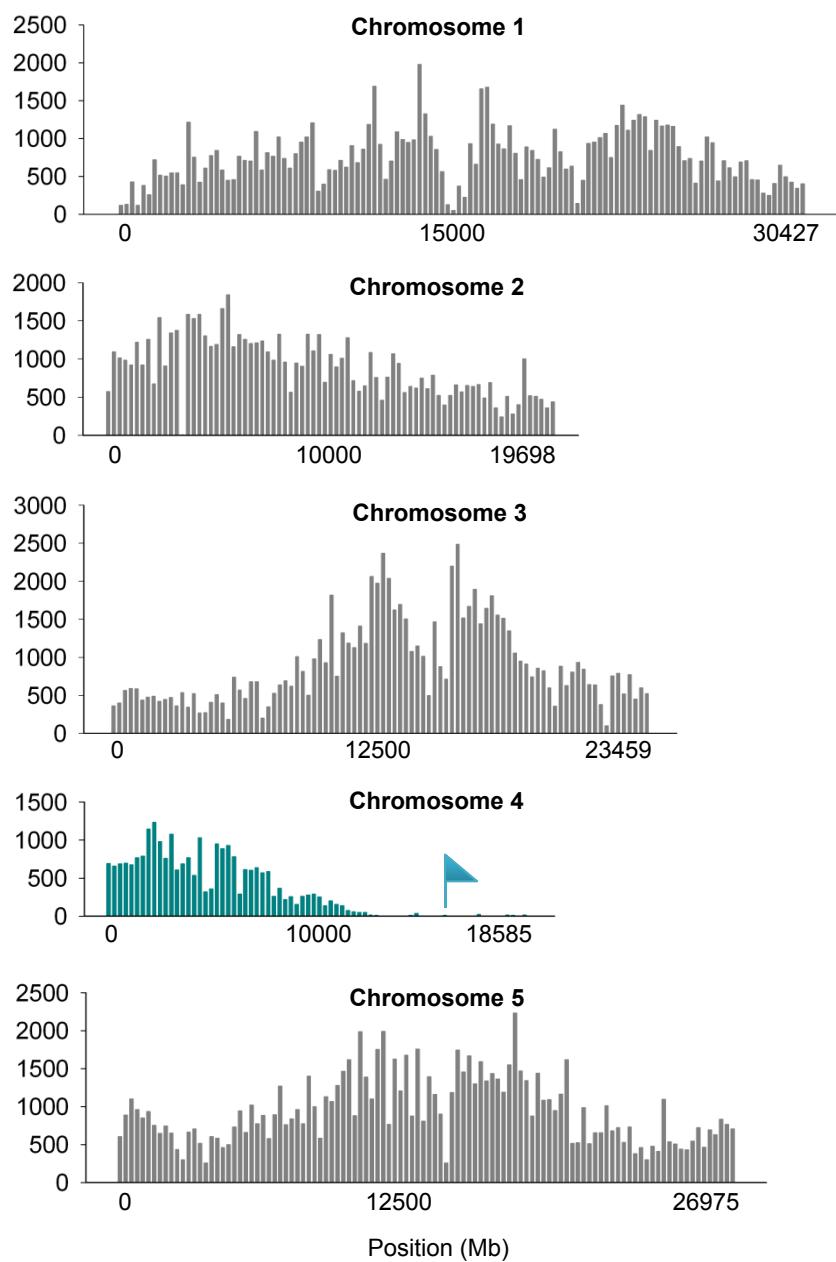
Supplemental Figure 2. Genome-wide SNP analysis of *sls1* mapping population using next generation mapping technique.

Genome-wide SNPs are shown by their chromosome positions. Filtered SNPs were collected over 250 kb intervals. There was non-recombinant block near the end of chromosome 4 (marked with a flag), which was consistent with the results from traditional map-based cloning.

Supplemental Figure 3. The *atkc1* could not suppress the LK-sensitive phenotype of *lks1-2*. Phenotype comparison of the various mutant plants after grown on MS or LK medium for 10 days.
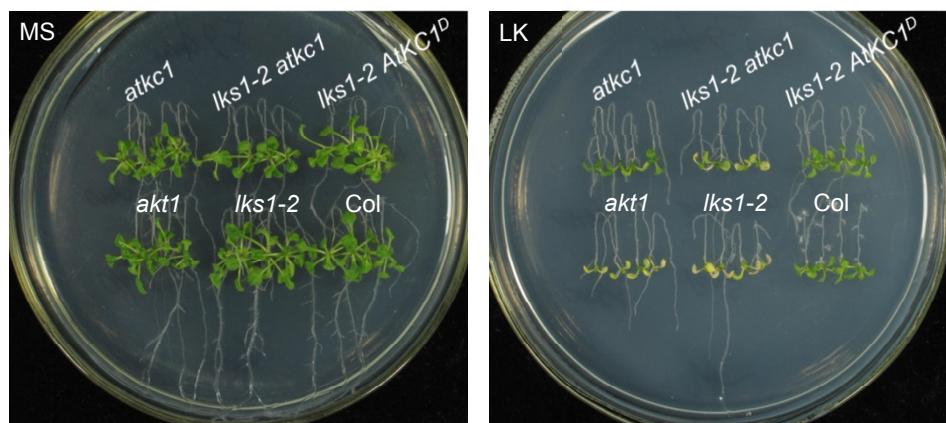
Supplemental Figure 4. The *AtKC1^D* and *sls1* showed a LK-tolerant phenotype on LK medium.
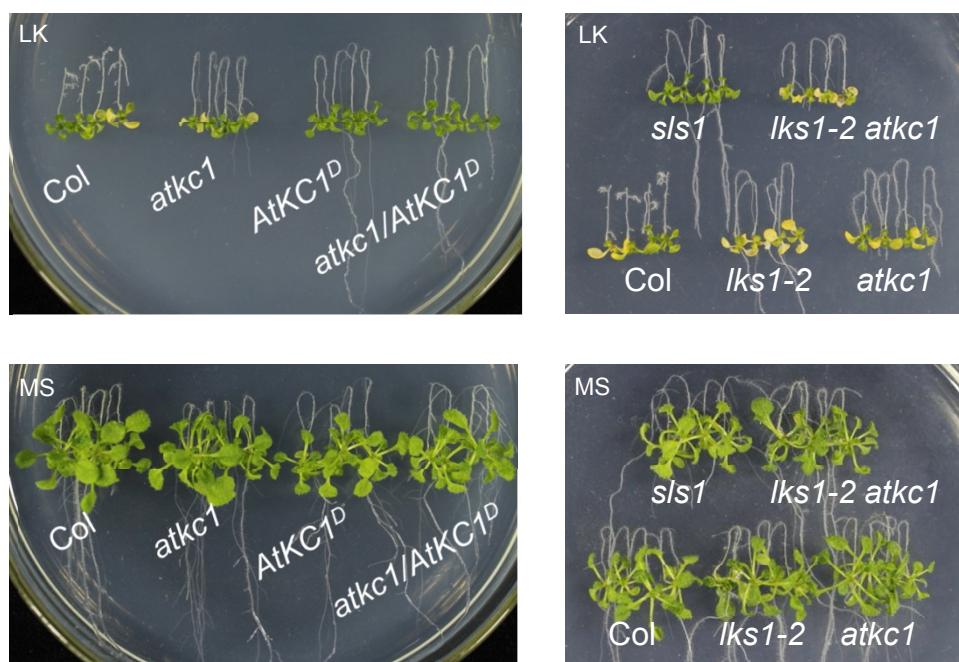
Phenotype comparison of various mutant plants after growth on LK or MS medium for 12 days.

Supplemental Figure 5. Total K^+ content and biomass analyses of *lks1 AtKC1^D* and *AtKC1^D*. The total K^+ content and biomass were measured after the plants were transferred to MS or LK medium for 10 days. Data are shown as means $\pm$ SE (n=4). The Student's *t* test (*P<0.05, **P<0.01) was used to determine the statistical significance.
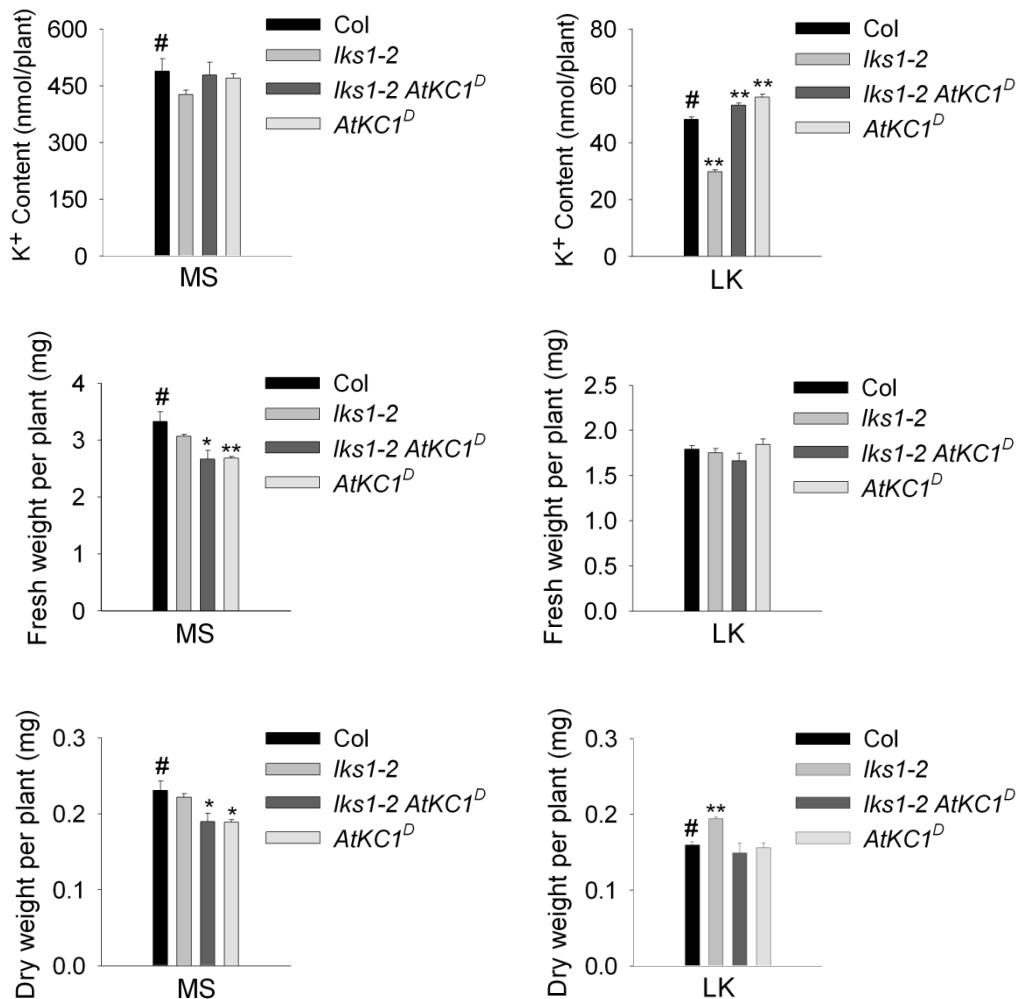
GFP

mCherry

mCherry-HDEL

AKT1-mCherry

Supplemental Figure 6. Subcellular localization of mCherry-HDEL and AKT1-mCherry in tobacco leaves.
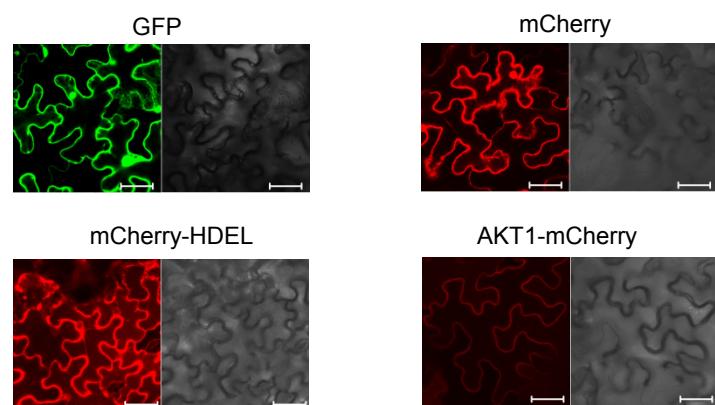
The mCherry-HDEL and AKT1-mCherry were located at the ER and PM respectively. GFP and mCherry showed nonspecific subcellular localization. Bars=50 μm.

Supplemental Figure 7. The protein interaction between AKT1 and AtKC1 or AtKC1^D in *Xenopus* oocytes using the BiFC method. Co-expression of AtKC1-YN or AtKC1^D-YN (YN, N-terminus of YFP) with AKT1-YC (YC, C-terminus of YFP) complemented YFP fluorescence at the plasma membrane. The point mutations in AtKC1^D did not affect its interaction with AKT1. Scale bars=100 μm.
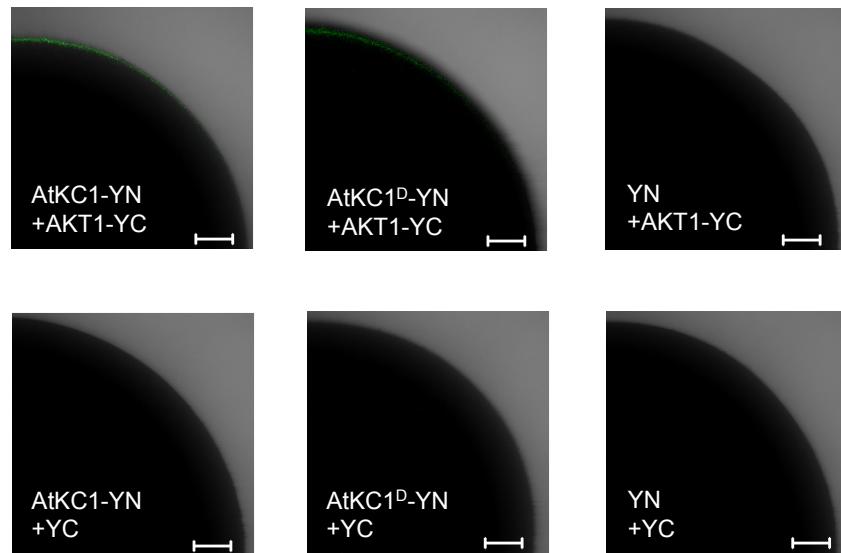
Supplemental Figure 8. Neither *AtKC1^D* nor *atkc1* could suppress the LK-sensitive phenotype of *akt1*.
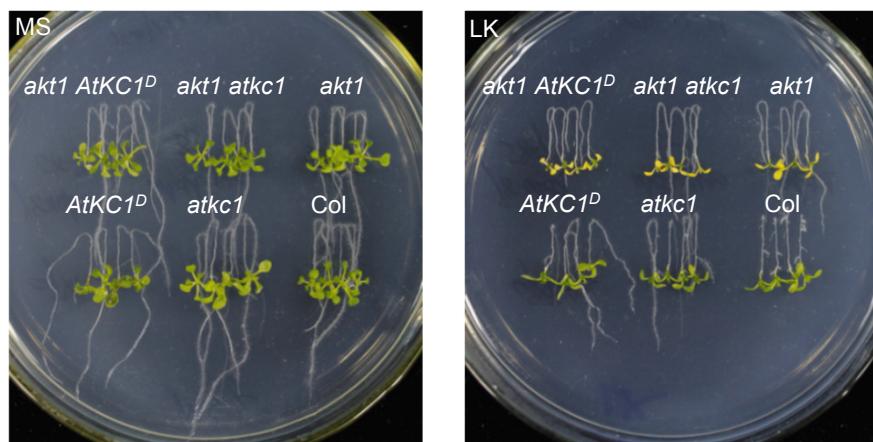
Phenotype comparison of various mutant plants after growth on MS or LK medium for 7 days.

A

B

Supplemental Figure 9. The open probability and cord conductance analyses of AKT1 in *Xenopus* oocytes.
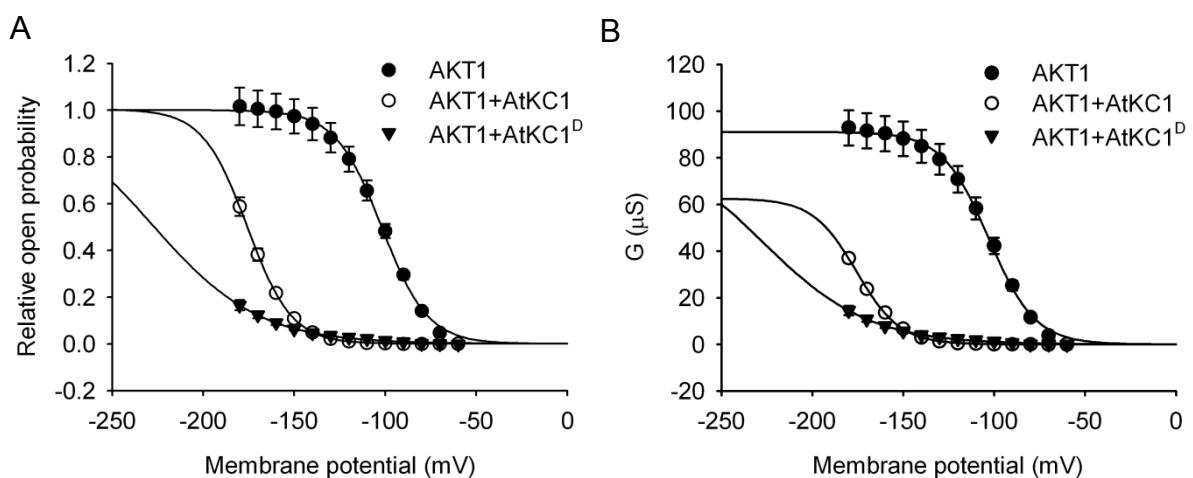
(A) The voltage dependence of inward K^+ currents. The solid lines fits to the Boltzmann functions: relative open probability $(G/G_{max}) = 1/(1 + exp((V_m - V_{1/2})/S))$.

Data are shown as means $\pm$ SE (n=4).

(B) The G-V relationship of AKT1 steady-state currents in different combination. G (chord conductance) were calculated as $G=I/(V_m-E_K)$.

Supplemental Figure 10. The dosage effect of AtKC1 or AtKC1^D on AKT1 activity.
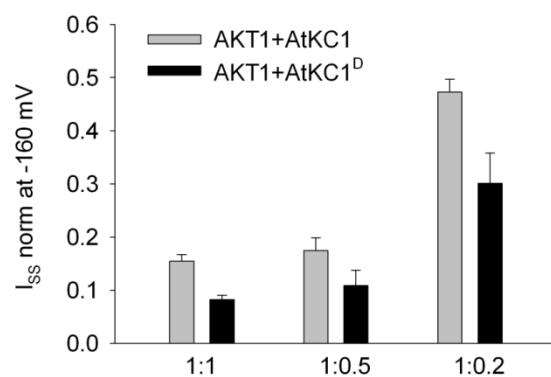
Normalized AKT1 steady-state currents (I_{ss}) in oocytes injected with different ratios of AKT1:AtKC1 or AtKC1^D at -160 mV. The currents under different ratio were normalized to the currents value of AKT1, respectively. CIPK23 and CBL1 are included in all combinations. Data are shown as means $\pm$ SE (n≥4).

Supplemental Figure 11. Phylogenetic analysis of AKT1 and AtKC1 homologs in different crops.
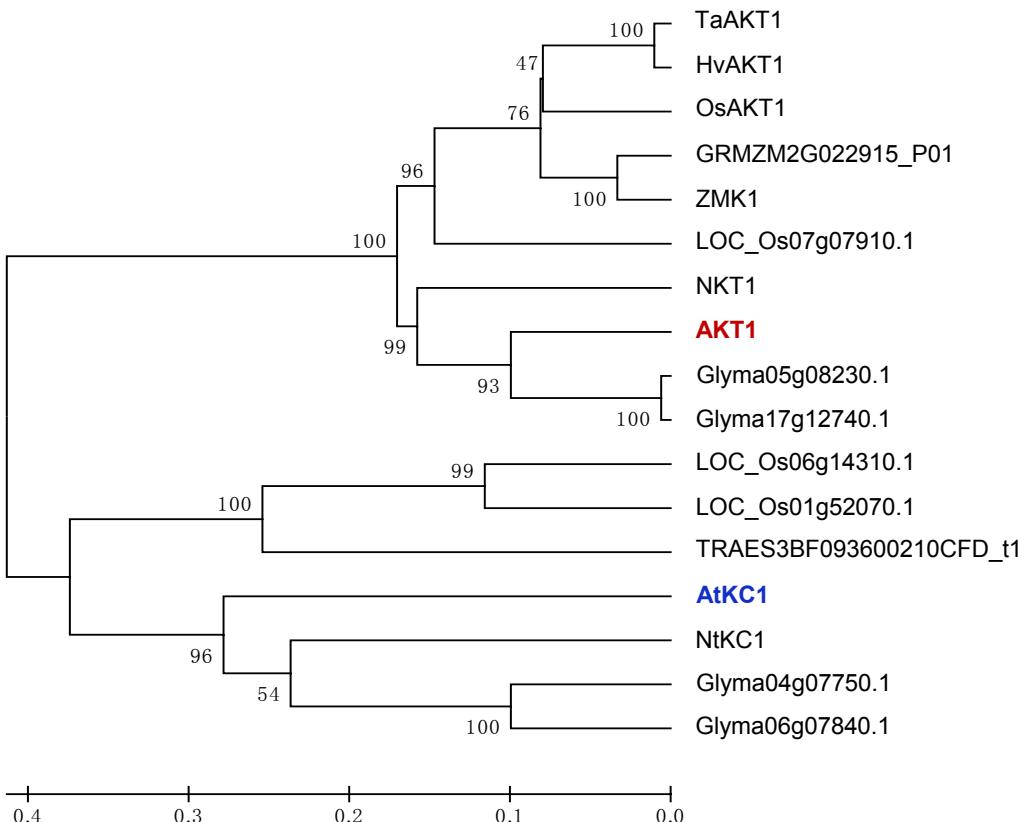
The sequences were aligned in ClustalX2 program, and the phylogenetic tree was constructed using MEGA Software. The phylogenetic analysis included 17 sequences from seven plant species, including

Arabidopsis thaliana, *Nicotiana tabacum*, *Glycine max*, *Oryza sativa*, *Zea mays*, *Triticum aestivum*, and

Hordeum vulgare. The sequence information was collected form the following websites:

Nicotiana tabacum (http://www.ebi.ac.uk/) AB196790 (NKT1), AB196791 (NtKC1);

Glycine max (http://www.plantgdb.org/GmGDB/) Glyma05g08230.1, Glyma17g12740.1, Glyma04g07750.1,

Glyma06g07840.1;

Oryza sativa (http://rice.plantbiology.msu.edu/) LOC_Os01g45990.1 (OsAKT1), LOC_Os07g07910.1,

LOC_Os06g14310.1, LOC_Os01g52070.1;

Zea mays (http://plants.ensembl.org/index.html) GRMZM2G171279_P01 (ZMK1), GRMZM2G022915_P01;

Triticum aestivum (http://plants.ensembl.org/index.html)

TRAES3BF087600020CFD_t1/Traes_3B_1795B9E0B.1 (TaAKT1), TRAES3BF093600210CFD_t1;

Hordeum vulgare (http://plants.ensembl.org/index.html) MLOC_74879.2 (HvAKT1).

Supplemental Figure 12. Molecular verification of various plant materials.
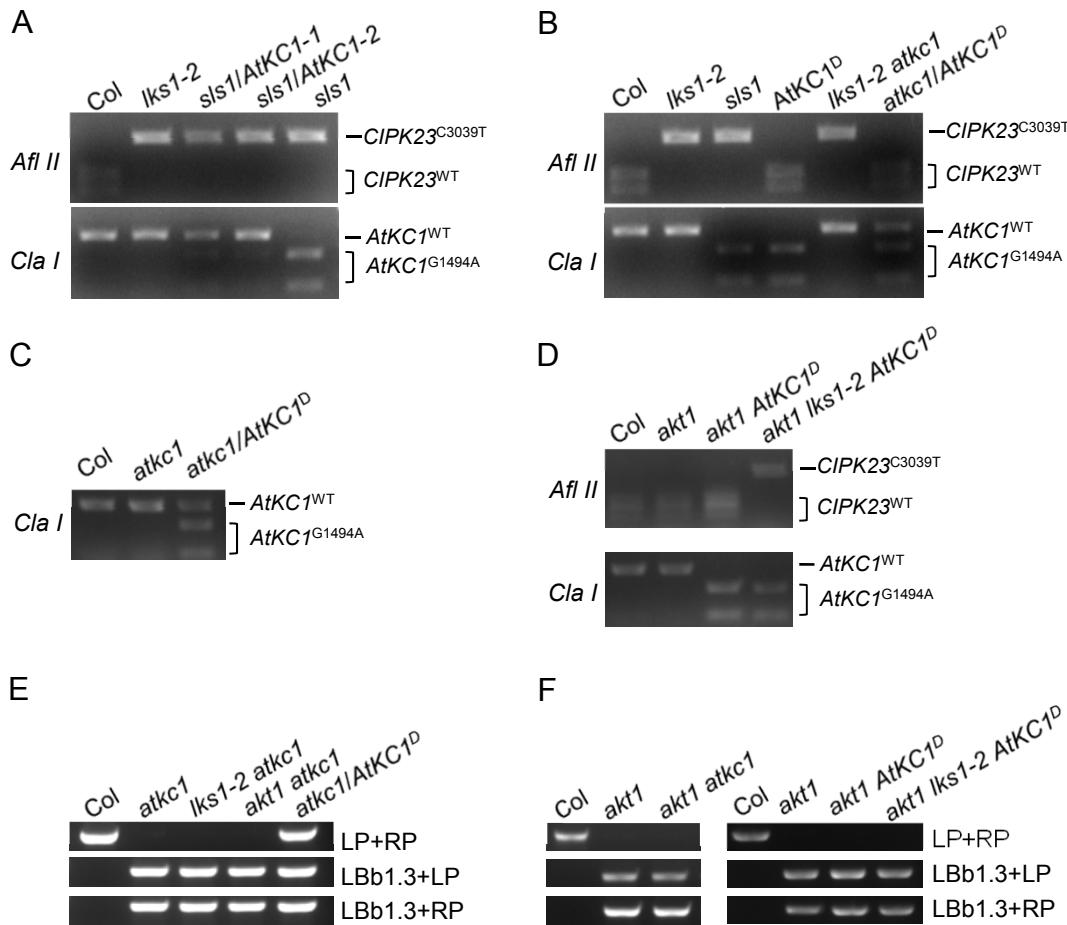
(A) to **(D)** The genotypes of *CIPK23* and *AtKC1* in different plant materials were confirmed using cleaved amplified polymorphic sequences (CAPS) markers. The restriction enzymes *Afl II* and *Cla I* were used to identify wild-type and mutant forms of *CIPK23* and *AtKC1*, respectively.

(E) T-DNA insertion verification of *atkc1* (SALK_140579).

(F) T-DNA insertion verification of *akt1* (SALK_071803).

Supplemental Table 1. The primer sequences used in this study.

Gene	Primer Name	Primer Sequence (5'-3')	Assay or Vector Design
For Genotyping			
CIPK23	C3039T-F	TGGAGTTGTGTATTGGTTTGTTG	CAPS
	C3039T-R	TCTGCTCCACTTCTTGACGC	
AtKC1	G1494A-F	GCACATTTCAACTATTTCG	CAPS
	G1494A-R	TTCTTGCTTGTGTATCGC	
AtKC1	AtKC1-LP	AACCAACATCTATTCCGCTCC	T-DNA
	AtKC1-RP	TCCTCATCGTTACTTTGTGGTG	
AKT1	AKT1-LP	TCCATGTCAAGCTAAGAAGACG	T-DNA
	AKT1-RP	TCGGTGATAGATAGTGGTGGC	
For Real-time PCR			
AtKC1	KC1-F	ATTGCTTCCAAGGGTGTGAG	Q-PCR
	KC1-R	GCGTCGACGTCATTATCAGA	
ACTIN2/8	ACTIN2/8-F	ACGGTAACATTGTGCTCAGTGGTG	Q-PCR
	ACTIN2/8-R	CTTGGAGATCCACATCTGCTGGA	
For Cloning			
AtKC1	AtKC1pg-F	GG<u>GGTACC</u>TGCCAAACAAAACAAAGAG	pCAMBIA1300
	AtKC1pg-R	CG<u>GGATCC</u>TAGGAGTCCTTACAGAAGTTTC	
AtKC1	AtKC1c-F	C<u>GGATCC</u>ATGTCTACGACGACTAC	SUPERR:sXVE:GFP_C:Bar
	AtKC1c-R	G<u>GGTACC</u>GAAAATATATAAATGATCG	
AKT1	AKT1c-F	GC<u>TCTAGA</u>ATGAGAGGAGGGGCTTTG	SUPERR:sXVE:mCherry_C:Bar
	AKT1c-R	TCC<u>CCCGGG</u>AGAATCAGTTGCAAAGATG	
AtKC1	AtKC1c2-F	TTTT<u>ACTAGT</u>ATGTCTACGACGACTACTGAGG	pGEMKN-YN-MCS
	AtKC1c2-R	TTTT<u>GCGGCCGC</u>TTAGAAAATATATAAATGATCG	
AKT1	AKT1c2-F	CACC<u>CCCGGG</u>ATGAGAGGAGGGGCTTTG	pGEMKN-MCS-YC
	AKT1c2-R	CG<u>GAATTC</u>AGAATCAGTTGCAAAGATG	
For Site-Directed Mutagenesis			
AtKC1	KC1 G1494A-F	GCCTCACTTCTTATATCATCGATATCATGACCAATCTAGTTGT	pCAMBIA1300
	KC1 G1494A-R	ACAACTAGATTGGTCATGATATCGATGATATAAGAAGTGAGGC	