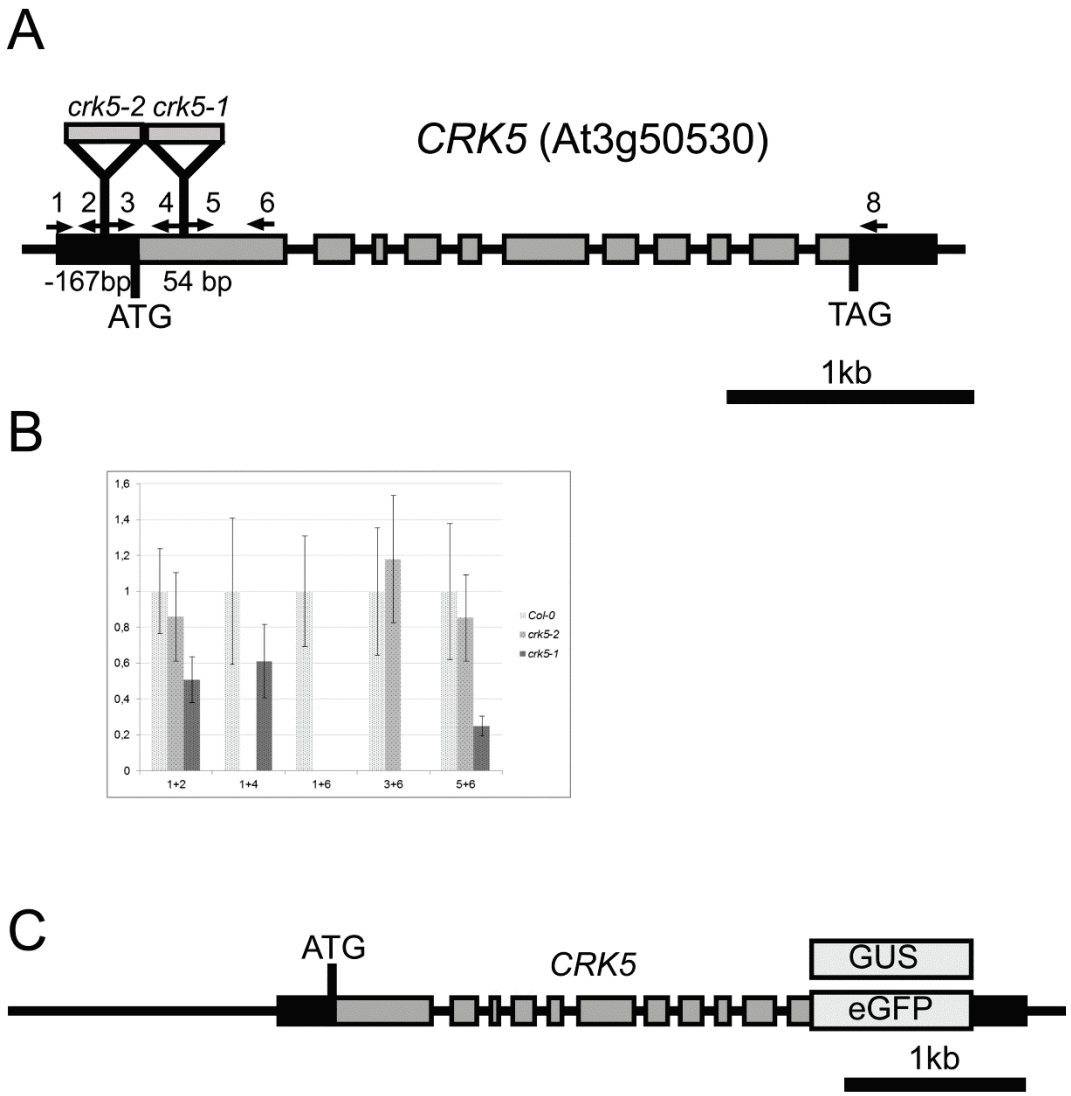
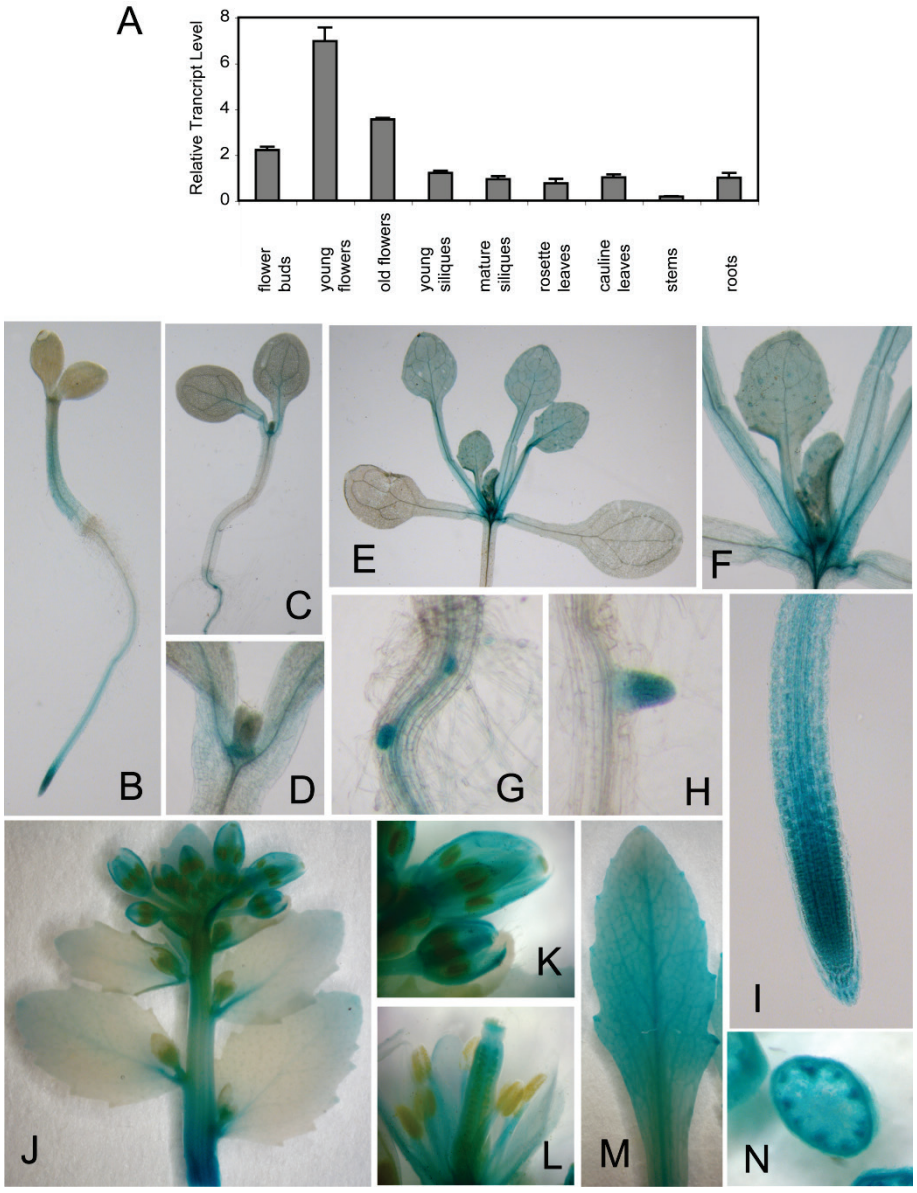


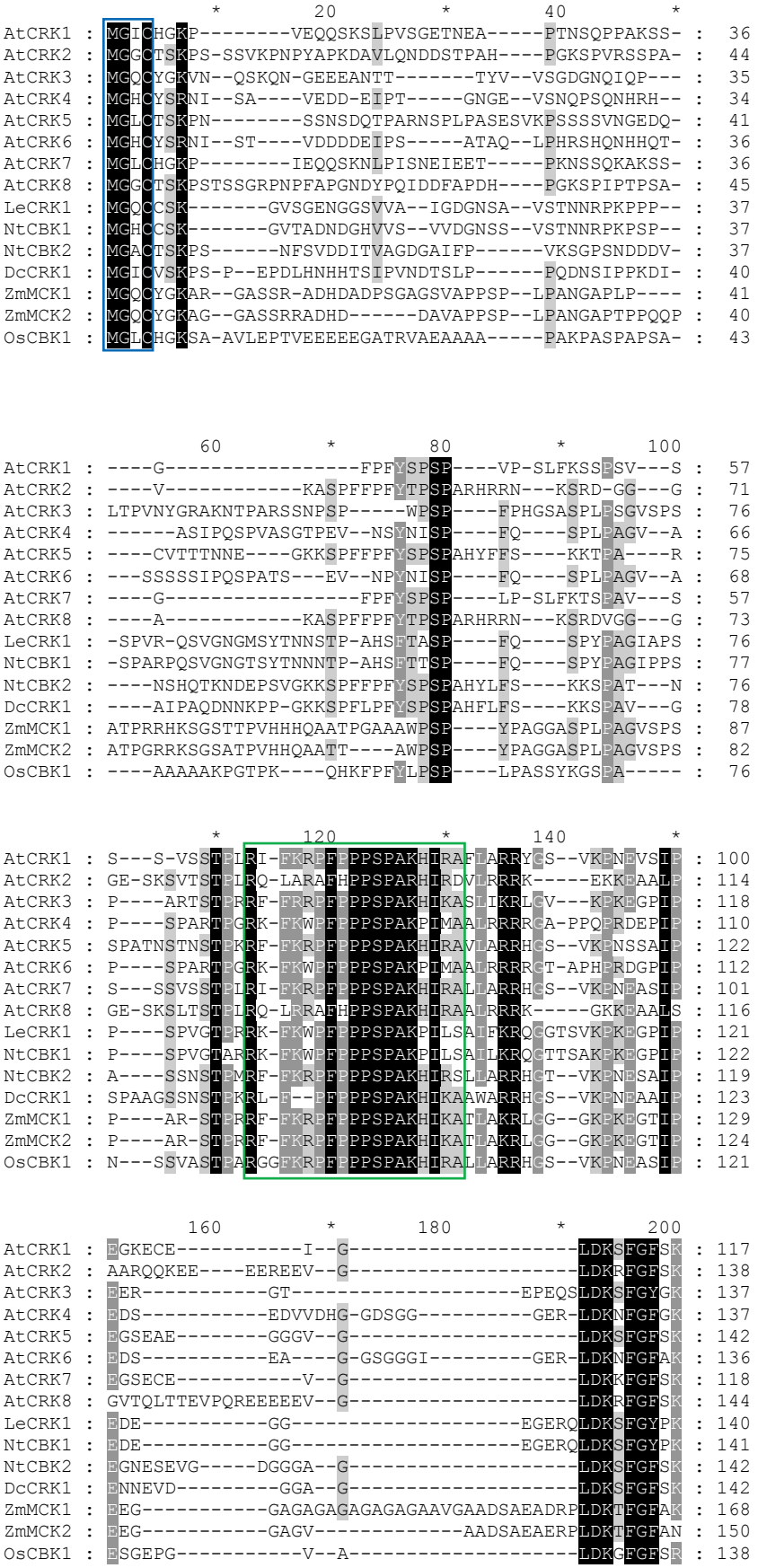
Supplemental Figure 1. Positions of T-DNA Insertion in *CRK5* and qRT-PCR Analysis of Transcription of *crk5* Mutant Alleles.

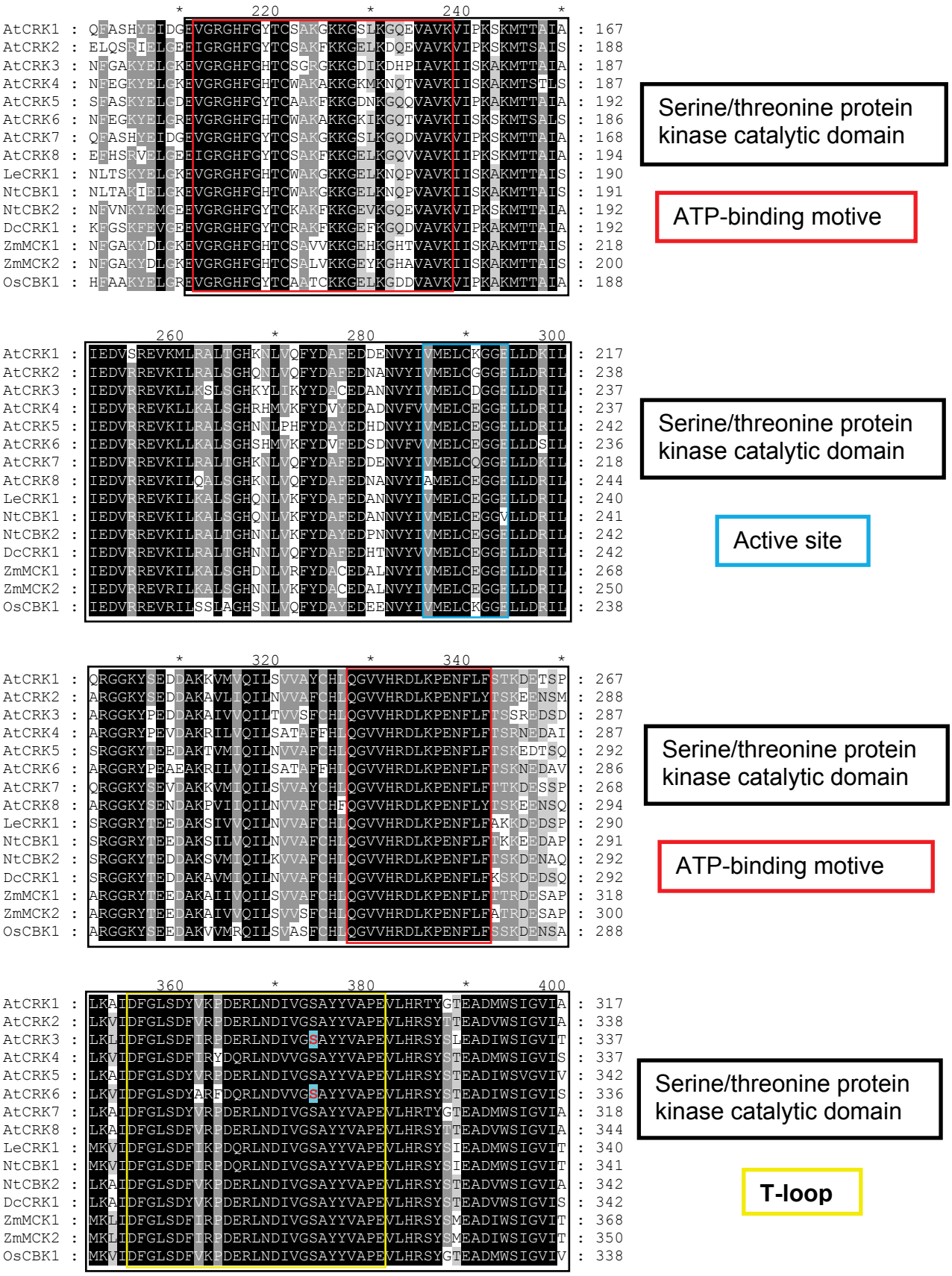
(A) Schematic map positions of T-DNA insertions in the *crk5-1* (MPIZ38225) and *crk5-2* (SALK_003774) alleles are indicated by triangles above the map. Dark grey boxes label 5' and 3' UTRs, light grey boxes are exons, and lines represent introns. Positions of oligonucleotide primers used in quantitative real-time PCR (qRT-PCR) analysis are indicated by arrows. **(B)** qRT-PCR measurement of *CRK5* transcript levels in wild type and the *crk5-1* and *crk5-2* insertion mutants. All qRT-PCR measurements were performed in triplicates. Bars label standard error (SE) of measurements performed with three biological replicates. **(C)** Schematic map of *CRK5-GFP* and *CRK5-GUS* gene fusions used for genetic complementation of *crk5-1* mutation and cellular localization studies.

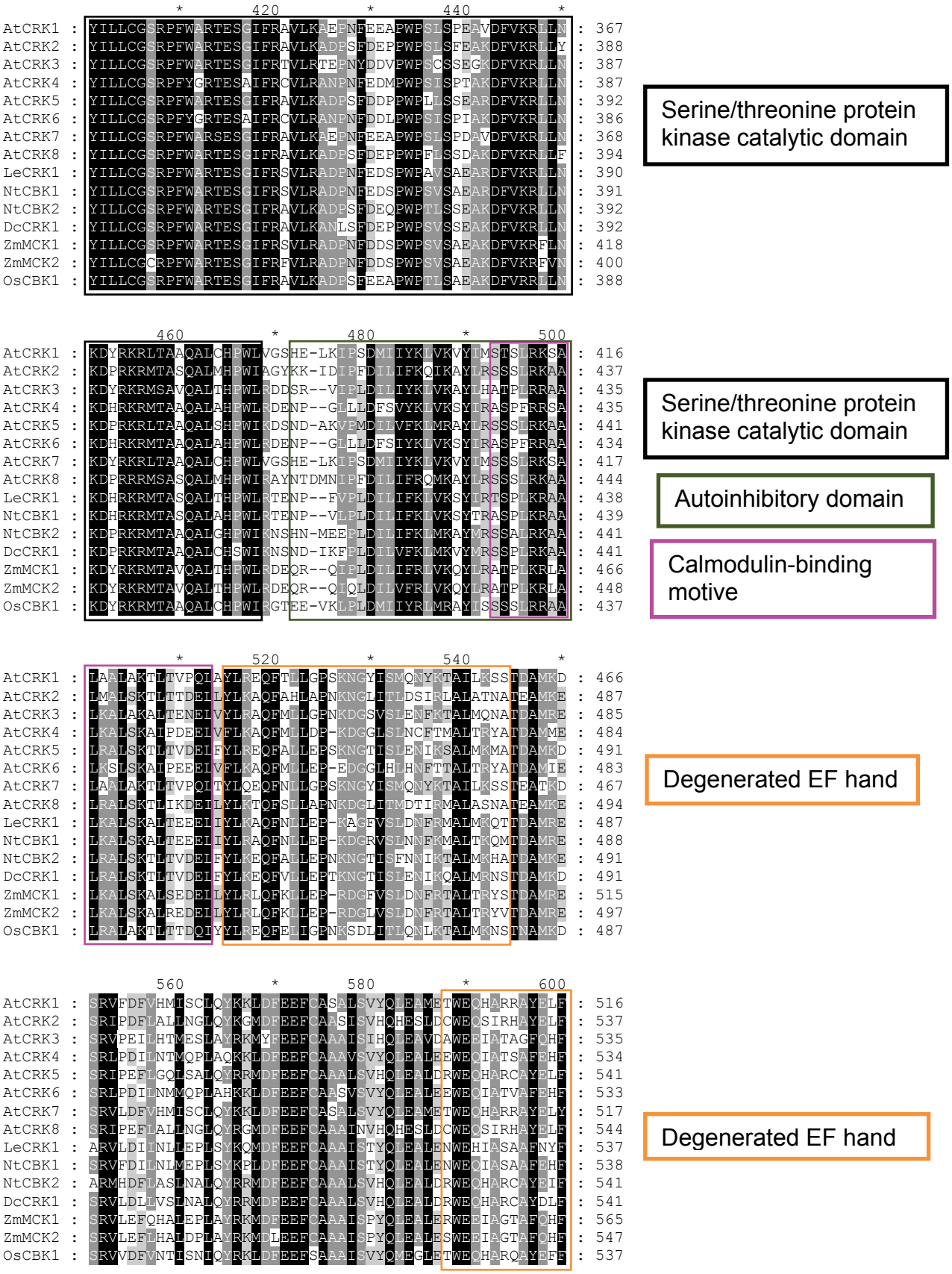


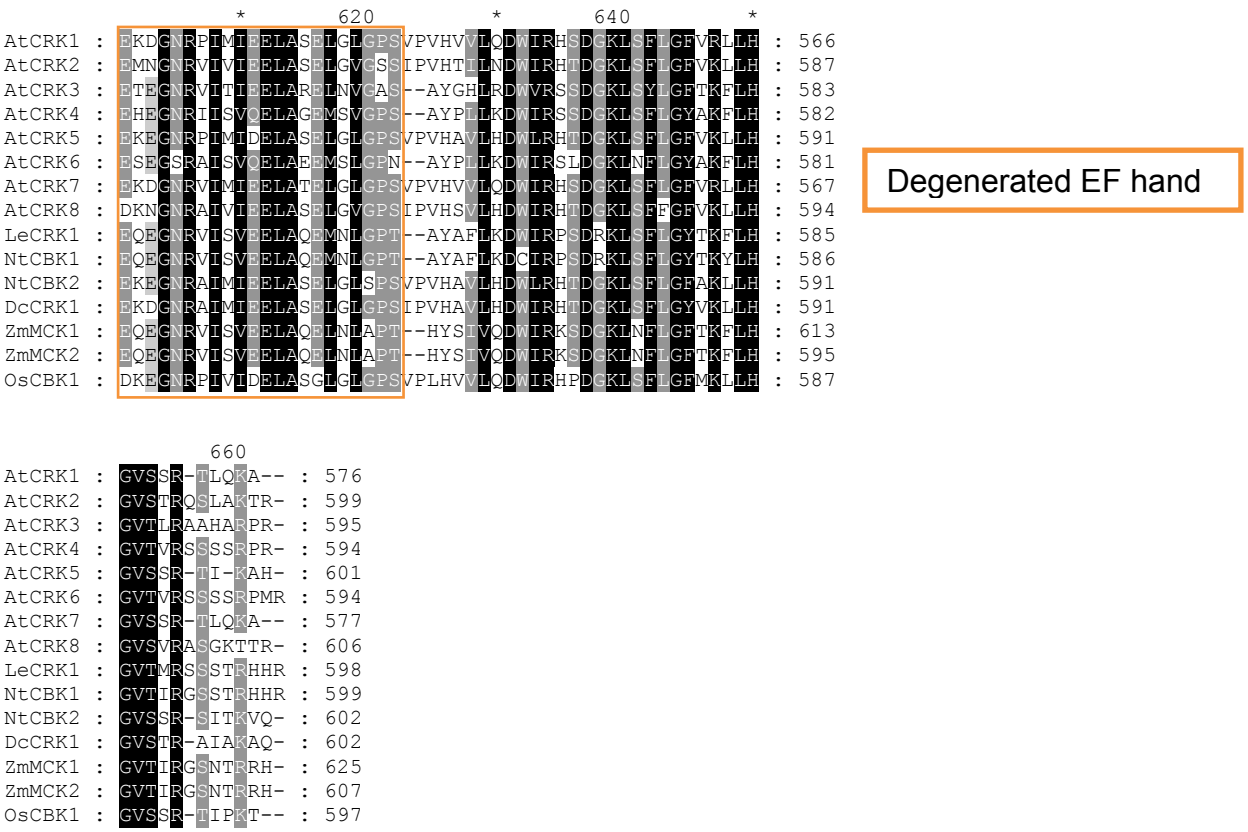
Supplemental Figure 2. qRT-PCR Measurement of *CRK5* Transcript Levels and Detection of CRK5-GUS Expression in Different Organs.

(A) qRT-PCR measurement of *CRK5* transcript levels in different organs of wild-type *Arabidopsis* plants. Relative transcript levels were standardized to *GAPDH-2* (At1g13440, Czechowski et al. 2005). The qRT-PCR measurements were performed in triplicates. Bars label standard error (SE) of measurements performed with three biological replicates. **(B-N)** Histochemical detection of CRK5-GUS expression in hypocotyls, roots, root apices, apical meristems and cotyledons of 7 (B-D) and 14 (E-F) days old light grown seedlings. CRK5-GUS shows high level of expression in the root stele and lateral root primordia (G-H), as well as in the root apex (I). In the inflorescence, CRK5-GUS is expressed in the vasculatures of cauline leaves, sepals, petals, anther filaments and pistils of flowers (J-M). In stem cross sections, CRK5-GUS activity is localized in the vascular bundles (N).


N-terminal myristoylation/ palmitoylation motive
Predicted NLS

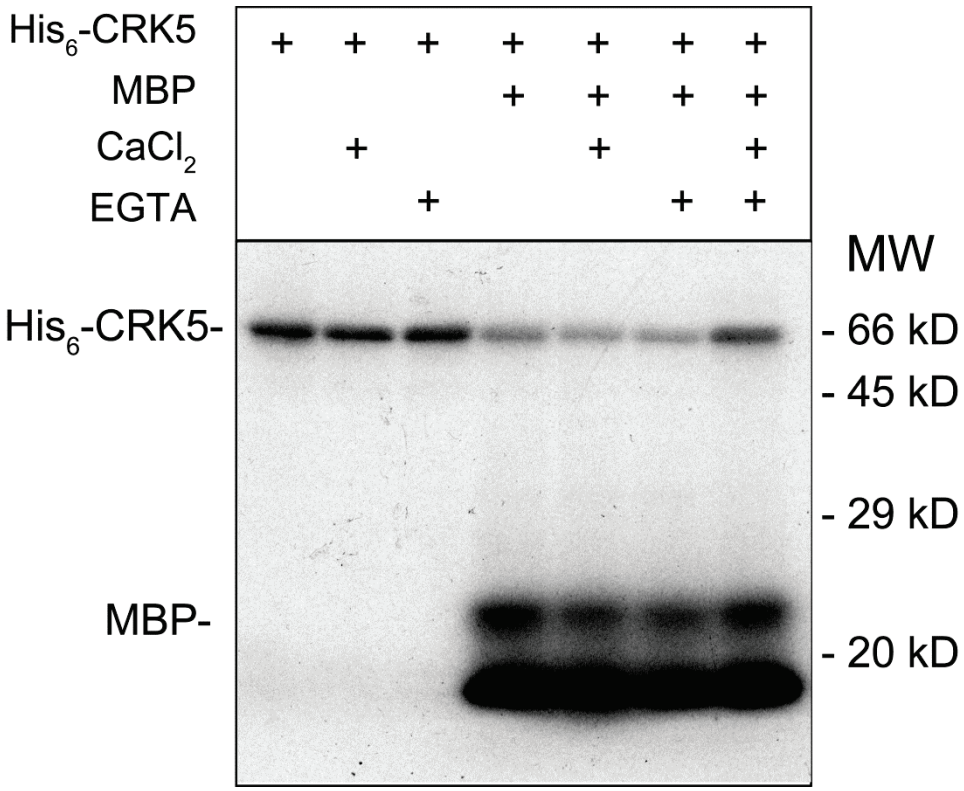

Serine/threonine protein kinase catalytic domain
ATP-binding motive
Serine/threonine protein kinase catalytic domain
Active site
Serine/threonine protein kinase catalytic domain
ATP-binding motive
Serine/threonine protein kinase catalytic domain
T-loop


Serine/threonine protein kinase catalytic domain
Serine/threonine protein kinase catalytic domain
Autoinhibitory domain
Calmodulin-binding motive
Degenerated EF hand
Degenerated EF hand

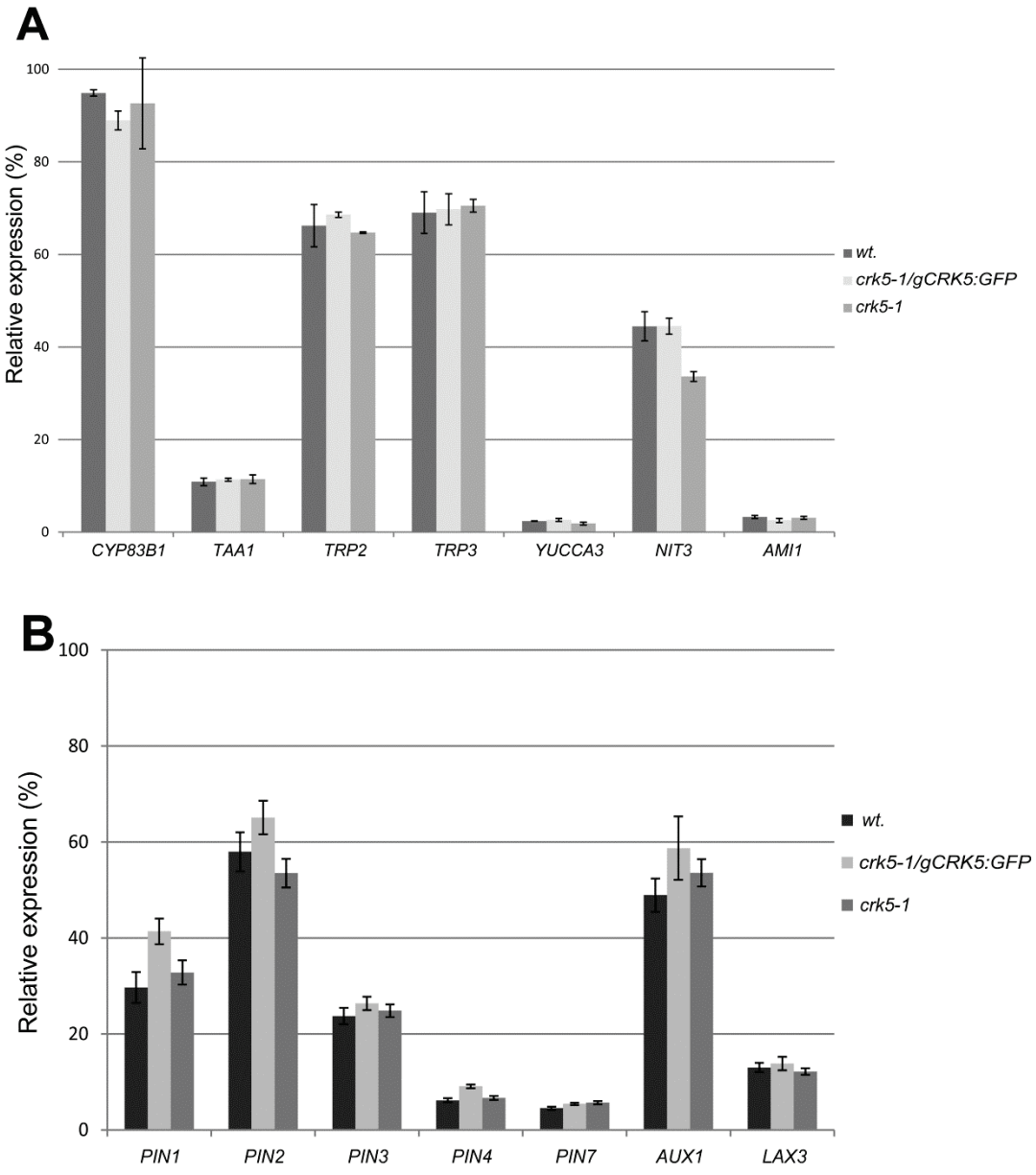


Supplemental Figure 3. Cobalt Sequence Alignment of Conserved Domains of CRK Family Members from Different Plant Species.

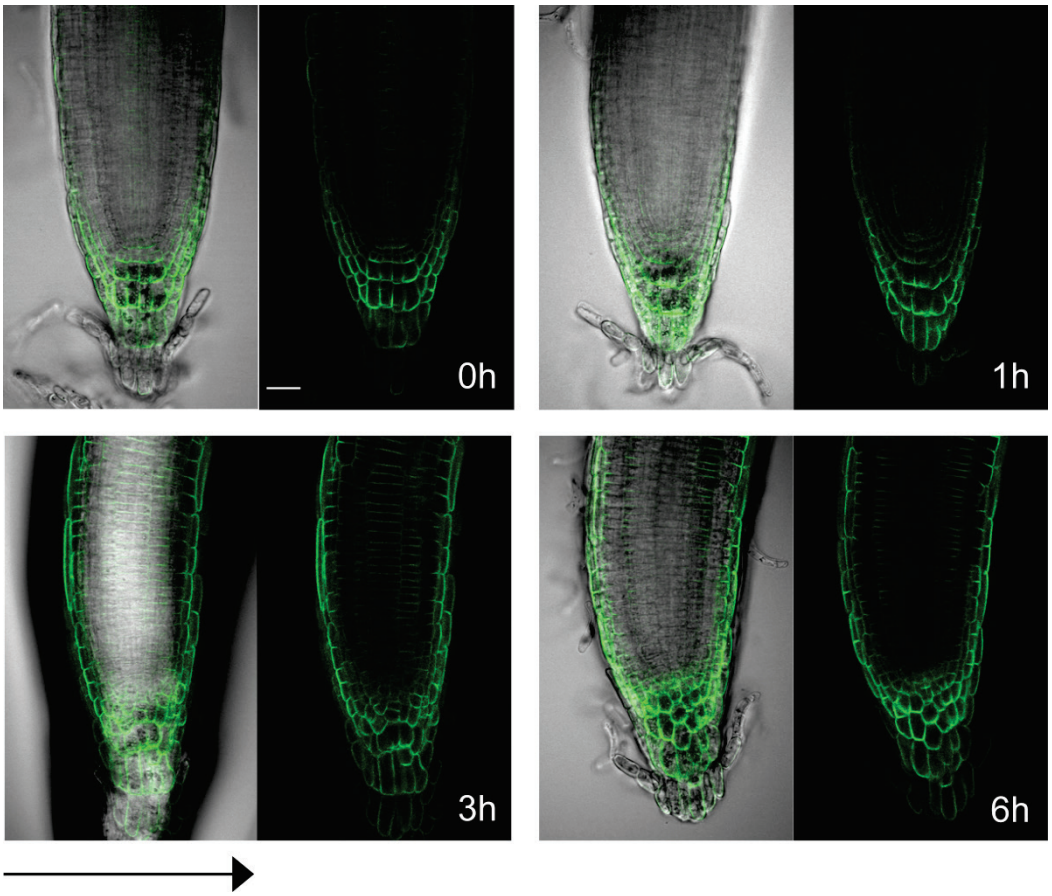
Members of the CRK family from different plant species carry highly conserved kinase catalytic domains but their N-terminal domains show a remarkable sequence divergence. Conserved myristolation, ATP-binding, T-loop, calmodulin-binding and degenerated EF hand motives of CRKs are indicated. GenBank (NCBI) accession numbers: CRK1 (At2g41140), CRK2 (At3g19100), CRK3 (At2g46700), CRK4 (At5g24430), CRK5 (At3g50530), CRK6 (At3g49370), CRK7 (At3g56760) CRK8 (At1g49580), LeCRK1 (AY079049), NtCBK1 (AF435450), NtCBK2 (AF435452), DcCRK1 (CAA58750), ZmMCK1 (AAB47181), ZmMCK2 (AF289237), OsCBK1 (AF368282). T-loop autophosphorylation sites identified in CRK3 and CRK6 (Hegeman et al., 2006) are indicated by blue shading.



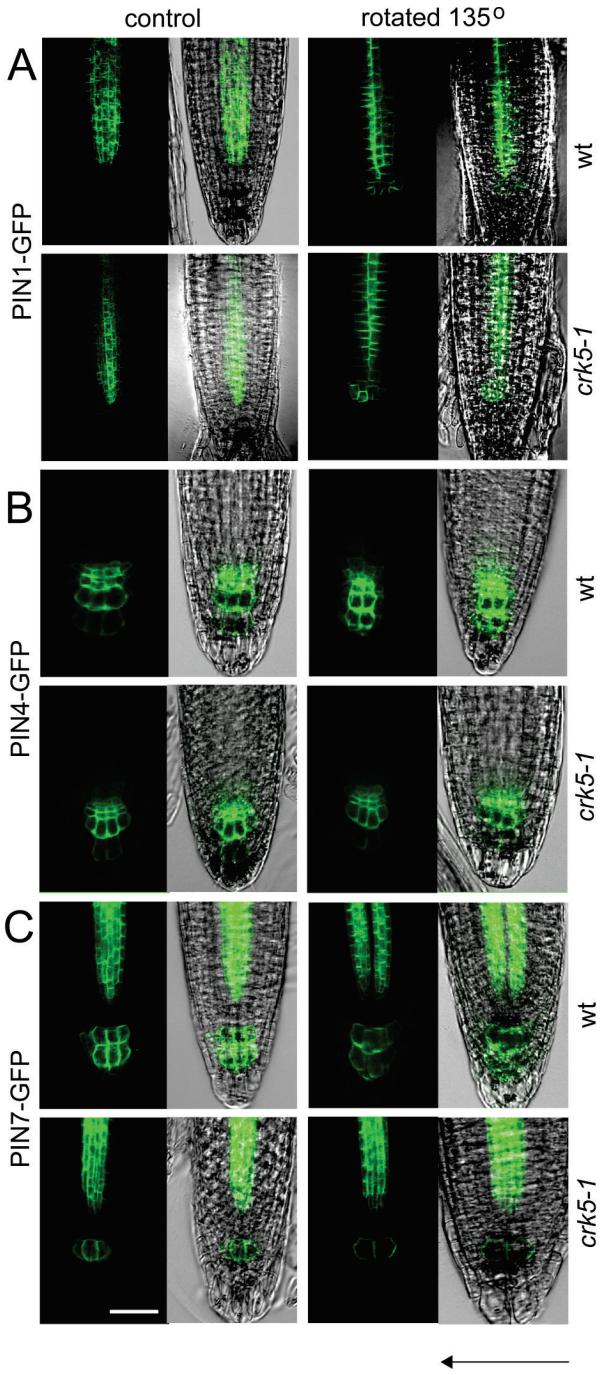
Supplemetal Figure 4. *In vitro* Kinase Assays with Purified His_6-CRK5.
The phosphorylation assays were performed with 1µg of His_6-CRK5 and 2µg myelin basic protein (MBP) as artificial phosphorylation substrate at room temperature for 0.5h with or without addition of 0.1 mM Ca^{2+} and 1 mM EGTA alone or in combination as described in the Methods. The reaction products were separated by 12% SDS-PAGE. CRK5 autophosphorylation and CRK5-mediated MBP phosphorylation were detected subsequently by autoradiography.



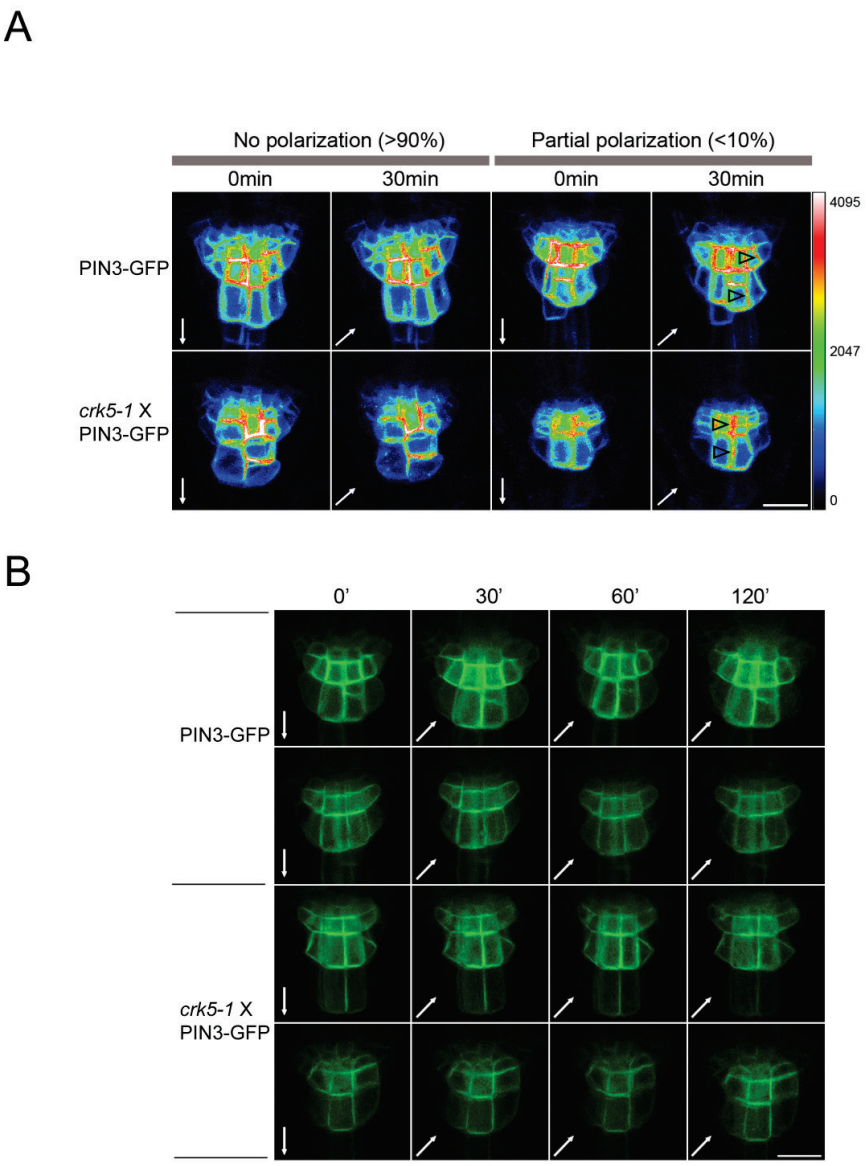
Supplemental Figure 5. qRT-PCR Comparison of mRNA Levels of Genes Involved in the Regulation of Auxin Biosynthesis and Encoding PIN Auxin Efflux and AUX/LAX Influx Carriers in Roots of Wild Type, *crk5-1* Mutant and Genetically Complemented *crk5-1/gCRK5-GFP* Seedlings. (A) Comparison of transcript levels of *CYP83B1* (At4g31500), *TAA1* (At1g70560), *TRP2* (At5g54810), *TRP3* (At3g54640), *YUCCA3* (At1g04610), *NIT3* (At3g44320), and *AMI* (At1g08980). **(B)** Relative transcript levels of *PIN1* (At1g73590), *PIN2* (At5g57090), *PIN3* (At3g70940), *PIN4* (At2g01420), *PIN7* (At1g23080), *AUX1* (At2g38120), and *LAX3* (At1g77690) normalized for *GAPDH2* (At1g13440). qRT-PCR measurements were performed in triplicates. Bars label standard error (SE) of measurements performed with three biological replicates.



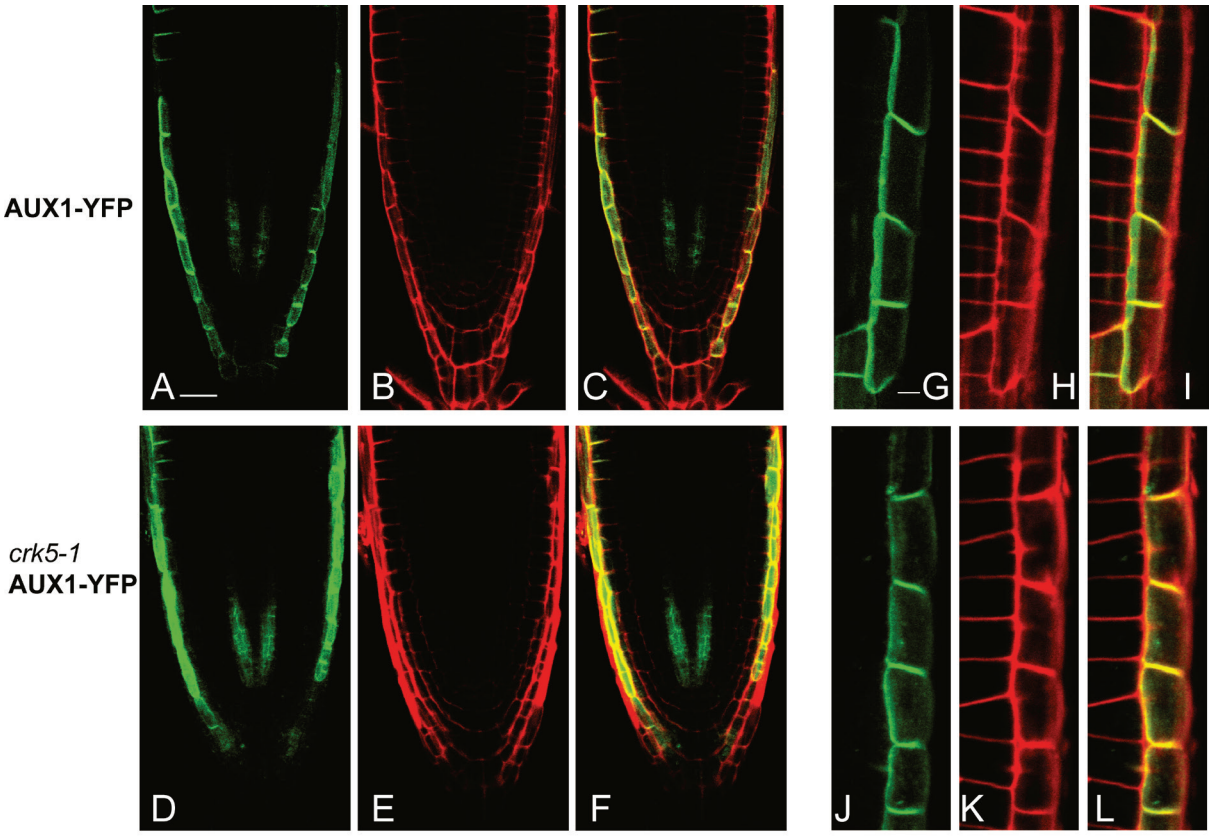
Supplemental Figure 6. Polar Localization of CRK5-GFP is not Changed in Root Cell Files in Response to Gravistimulation. Vertically grown 7-day-old seedlings were subjected to 135° rotation (0h) and CRK5-GFP localization was monitored at 1h, 3h and 6h after the start of gravistimulation. Bar 50 μm.



Supplemental Figure 7. Localization of PIN1-GFP, PIN4-GFP and PIN7-GFP in Vertically Grown and Gravistimulated Roots of Wild Type and *crk5-1* Mutant Plants.
(A to C) PIN1-GFP, (A) PIN4-GFP (B) and PIN7-GFP (C) expressing seedlings were grown for 7 days in vertical position, and then subjected to gravistimulation by 135° rotation for 20 h. Localization of PIN1-GFP is comparable between wild type and *crk5-1*. PIN4-GFP pattern is extended to the QC and adjacent layers of dividing cells during gravistimulation in wild type but not in the *crk5-1* mutant. Compared to wild type, the levels of PIN7-GFP is somewhat reduced in the vasculature of *crk5-1* roots.



Supplementary Figure 8. **Comparison of PIN3-GFP Localization in Vertically Grown and Gravistimulated Roots of Wild Type and *crk5-1* Mutant.**
(A) Localization of PIN3-GFP in columella and root cap cells of wild type (wt) and *crk5-1* mutant. Color-coded heat map of Z-stack projections of 6 slices are shown before (0 min) and after 135º gravistimulation (30 min). Seedlings were categorized to "No Polarization" (apolar; >90%) and "Partial Polarization" (<10%) classes. Regions of partial polarization of PIN3-GFP are indicated by black triangles. White arrows indicate the gravity vector. Scale bar is 20µm. **(B)** Time course analysis of PIN3-GFP localization in wild type (wt) and *crk5-1* mutant. Image at 0 min shows nonstimulated vertical grown root tips. Following gravistimulation of 4-day-old roots by 135º rotation, images were captured at 30, 60 and 120 minutes as mid optical sections of root columella cell layer. In both (A) and (B), at least 20 wild type and *crk5-1* seedlings were analyzed. Arrows indicate the gravity vector. Scale bar is 20 µm.



Supplementary Figure 9. Cellular Localization of AUX1-YFP in Wild Type and *crk5-1* Mutant Roots.

(A to F) Localization of AUX1-YFP in primary roots of 5-day-old wild type (A to C) and *crk5-1* (D to F) seedlings grown vertically in continuous light. (A, D) YFP signal, (B, E) counter-staining with propidium iodine, and (C, F) superimposed images of (A and B) and (D and E). **(G to L)** AUX1-YFP localization in the basal and internal lateral membranes of wild type (G to I) and *crk5-1* (J to K) lateral root cap cells. (G, J) YFP signal, (H, K) counter-staining with propidium iodine, and (I, L) superimposed images of (G and H) and (J and K).

Supplemental Table 1. List of PCR Oligonucleotide Primers.

Name	Sequence (5' to 3')	Gene number reference
CRK5BamHI-F	ctagggatccaaATGGGTCTATGTACTTCG	
CRK5XhoI-R	aggtactcgagCTAATGAGCTTTGATCG	
crk5-1 F	CCGAATTCTCCTATTTTCTAGCTTCGGC	
crk5-1 R	GGAGAATGAGACCTTAGAGCTCAGAC	
Fish1	CTGGGAATGGCGAAATCAAGGCATC	Ríos et al. (2002)
Fish2	CAGTCATAGCCGAATAGCCTCTCCA	Ríos et al. (2002)
crk5-2 F	CACCGAATTCTCCTATTTTCTAGC	
crk5-2 R	CCTCCTCTGTGTACTTCCCACC	
LBa1	TGGTTCACGTAGTGGGCCATCG	Alonso et. al. (2003)
1: CRK5-F1	AAGCGAACGTATCGTCGTTTC	
2: CRK5-R2	ATTGAGTACTTTGTCAATGGCGAAT	
3: CRK5-F3	GATTTCGTGTGATGTTGAGAGATT	
4: CRK5-R4	GAAGTACATAGACCCATTTAAGAATCTCTC	
5: CRK5-F5	CAACGAACAATGAAGGCAAAA	
6: CRK5-R6	GATCTCGCCGGAGTCTTCTT	
8: CRK5-R8	TCATAACAAAAGTCAAAAGCCACA	
ACT2/8-F	GGTAACATTGTGCTCAGTGGTGG	At3g18780, An et al. (1996)
ACT2/8-R	AACGACCTTAATCTTCATGCTGC	At3g18780, An et al. (1996)
CRK5 SauI	TAAACTTACCTCAGGAACTTGGT	
CRK5 stop to ApaI	TTCAAAGTTTCAAAACCGGGCCCATGAGCTTTG ATCGTGC	
T3 primer	ATTAACCCTCACTAAAGGGA	Stratagene
T7 primer	TAATACGACTCACTATAGGG	Stratagene
eGFP ApaI 5'	CATAAGGGCCCACCATGGTGAGCAAGGGCGAG GAGCTG	
eGFP ApaI 3'	AATATGGGCCCTTACTTGTACAGCTCGTCCATGC CGAG	
GUS ApaI 5’	CATAAGGGCCCACCATGTTACGTCCTGTAGAAA CCCCA	
GUS ApaI 3’	AATATGGGCCCTCATTGTTTGCCTCCCTGCTGCG GTTT	
CRK5-R1	GGGTCTATGTACTTCGAAACCGA	for real time PCR
CRK5-R2	TAACGGAAGAGCTCGAAGGC	for real time PCR
GAPDH-2-F	AATGGAAAATTGACCGGAATGT	At1g13440, Czechowski et al. (2005) for real time PCR
GAPDH-2-R	CGGTGAGATCAACAACTGAGACA	At1g13440, Czechowski et al. (2005) for real time PCR
PIN1-F	TGGAAGACAACCTTTGGAAACT	At1g73590
PIN1-R	TGAAGCATTAGAACGACGAACA	At1g73590
PIN2-F	CCTCGCCGCACTCTTTCTTT	At5g57090
PIN2-R	CGTACATCGCCCTAAGCAAT	At5g57090
PIN3-F	CAAGTGGAGATTTCGGAGGA	At3g70940
PIN3-R	GCGTCTTTTGGTCTCTCTGC	At3g70940
PIN4-F	GGCAACGGAACAATCTGAAC	At2g01420
PIN4-R	TCACCACCACCTCTAGCATTAC	At2g01420
PIN7-F	AAGGCGGTGCAAAAGAGATT	At1g23080
PIN7-R	CATCGGACCAGCTTTGTTTT	At1g23080
AUX1-F	CTTTCCTCCTCTGCACATTTCT	At2g38120

AUX1-R	AAGAGTGGTTTTTGTCCGTTTG	At2g38120
LAX3-F	TGCTTACCTTTGCTCCTGCT	At1g77690
LAX3-R	GTCCCCATCCATCCTCCTAC	At1g77690
CYP83B1-F	ACCCTAACCGCCCTAAACAAGA	At4g31500 Mei et al. 2011
CYP83B1-R	GTCAGTTCCCGGCACAACAATA	At4g31500 Mei et al. 2011
TAA1-F	TAAACACTATACAAACGACCAAACC	At1g70560 Mei et al. 2011
TAA1-R	TACACCTGTCACCCATCTTCCT	At1g70560 Mei et al. 2011
TRP2-F	TTGAATCCGCTTTCTATGCTCT	At5g54810 Mei et al. 2011
TRP2-R	CTGTAATGCTCCGTAAGCCTCT	At5g54810 Mei et al. 2011
TRP3-F	ATCATCTGTAAGCGGAAAGGTTC	At3g54640 Mei et al. 2011
TRP3-R	TTCAGTTGGCGACTTTGCATCAC	At3g54640 Mei et al. 2011
YUCCA3-F	CGTTCGTAGCGCTGTTCATG	At1g04610 Mei et al. 2011
YUCCA3-R	CTAACGGTCCAATTTTCGGC	At1g04610 Mei et al. 2011
NIT3-F	AGGTTATTGGCGTTGACCCAT	At3g44320 Mei et al. 2011
NIT3-R	ATCTTTCCACTTCAGGGCCAG	At3g44320 Mei et al. 2011
AMI-F	TCTACTTCCTCGTCGCCTCCT	At1g08980 Mei et al. 2011
AMI-R	GCGCATTTTCTCCGTTTATACTG	At1g08980 Mei et al. 2011

Supplemental Methods and References

Identification of *crk5* T-DNA Insertion Mutants

To screen for homozygous lines, segregation analysis of T2 families carrying T-DNA insertions in the *CRK5* gene was carried out as described (Ríos et al., 2002) using T-DNA and gene-specific primers (see Supplemental Table 1 online). By sequencing the PCR amplified left and right T-DNA border-plant DNA junctions, a single T-DNA insertion was localized 54 bp 3'-downstream of the ATG codon in exon 1. The T-DNA insertion generated a target site deletion of 10 bp. At the left border junction, facing the promoter, 21 bp was retained from the 25 bp border (capital letters) sequence (plant DNA-tcaaactccggcg/AGGATATATTCAA TTGTAAAT-T-DNA), while the right border junction contained 1 bp from the border repeat (T-DNA-T/cacttccggcg-plant DNA). In the *crk5-2* allele, a single T-DNA insertion 167 bp 5'-upstream of the ATG generated a target site deletion of 24 bp. At the right T-DNA junction facing the promoter, a single nucleotide from the right border repeat was linked through a filler DNA (bold) sequence of 29 bp to *CRK5* sequences (plant DNA-aagtactcaat/AACACATTGCGGACGTTATTGTGGTGTAAA-T-DNA). At the left T-DNA junction 9 bp was retained from the 25 bp border repeat (T-DNA-GTTTACACC/acaacaatttt-plant DNA).

Plasmid Constructs and *Agrobacterium*-mediated Plant Transformation

A BpmI-BstXI fragment of 7809bp, carrying the full length *CRK5* gene with a promoter region of 4414 bp, was isolated from the BAC clone T20E23 obtained from the Arabidopsis Biological Resource Center. After treatment with DNA polymerase Klenow fragment, the *CRK5* gene was cloned into the SmaI site of vector pBluescript SK (pBSK), from which the ApaI site was previously removed, to obtain pBSKΔApaIgCRK5. Translational stop codon of CRK5 was replaced by an ApaI site, and a SauI-SacII 3' segment of modified *CRK5* gene was amplified by a two-step PCR reaction using high fidelity Pfu DNA polymerase (Fermentas) and the pBSK T3, CRK5 SauI and CRK5 StoptoApaI primers (see Supplemental Table 1 online). Following digestion, the PCR amplified *CRK5* fragment was used for replacement of corresponding SauI-SacII segment of pBSKΔApaIgCRK5 to generate pBSKΔApaIgCRK5ApaI, carrying a single ApaI site replacing the *CRK5* stop codon. Coding regions of *GFP* and *uidA/GUS* were PCR amplified as ApaI fragments carrying 3' stop codons (see primers in Supplemental Table 1 online) and cloned into the single ApaI site of

pBSKΔApaIgCRK5ApaI to generate in frame 3'-translation gene fusions. The resulting gCRK5-GFP and gCRK5-GUS gene cassettes were isolated as PstI-NotI fragments and inserted into PstI-Ecl136II sites of the binary plant transformation vector pK7FWG2 (Karimi et al., 2002) by removing the Gateway cassette. Binary vectors were introduced into Agrobacterium GV3101 carrying either the pMP90 or pMP90RK Ti-helper plasmids as described (Koncz et al., 1994). Wild type and *crk5-1* mutant plants were transformed using the infiltration method (Bechtold et al., 1993). Primary (T1) transformants were selected on 0.5 MS medium containing either 30μg/ml kanamycin or 15.75 mg/l sulfadiazine in case of DR5-GFP (Ottenschläger et al. 2003). Following segregation analysis of T2 families, T3 lines carrying single T-DNA insertions were identified. AUX1-YFP (Swarup et al., 2004), PIN1-GFP (Benková et al., 2003), PIN2-GFP (Xu and Scheres, 2005), PIN3-GFP (Zádníková et al., 2010), PIN4 -GFP and PIN7-GFP (Blilou et al., 2005) were introgressed by crosses into the *crk5-1* mutant followed by PCR-based genotyping of *crk5-1* homozygous lines and screening for lines carrying the AUX1 and PIN reporters in homozygous forms.

To express N-terminal fusion of CRK5 with a His_6-tag, the coding sequence of *CRK5* cDNA was PCR amplified and inserted by BamHI-XhoI into pET28c (Novagen). The coding sequence of the PIN2 hydrophilic T-loop (Müller et al., 1998) was PCR amplified and cloned as an EcoRI-BamHI fragment into pET28a. The pET28c-CRK5 and pET28aPIN2loop were transformed into the *E. coli* strain BL21(DE3) Rosetta (Novagen).

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