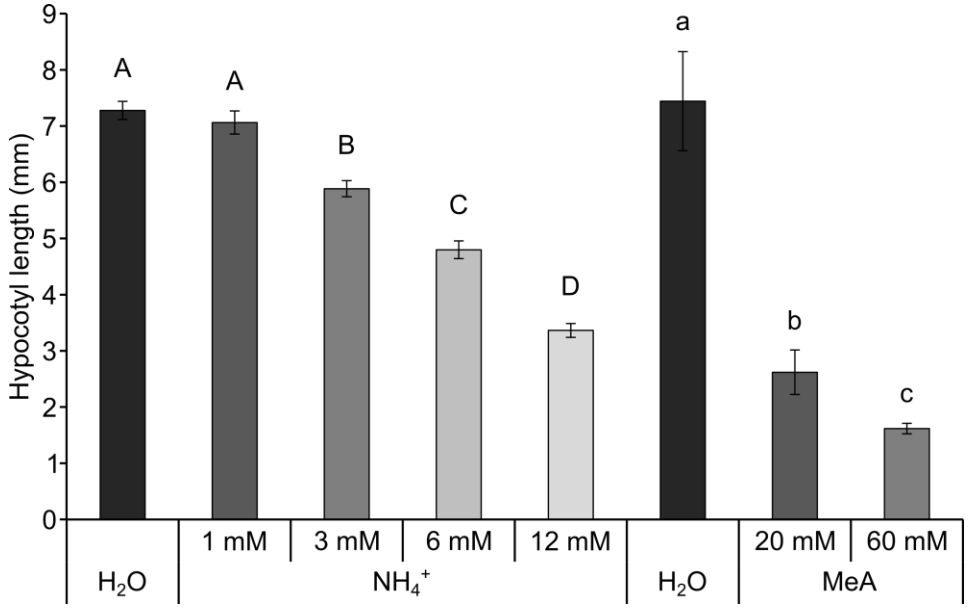
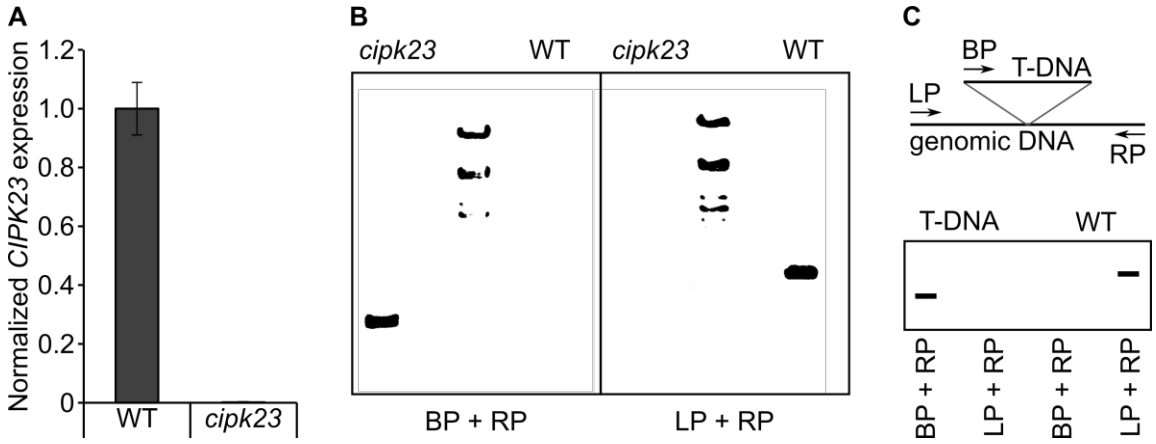
Supplemental Data. Straub et al. Plant Cell (2017) 10.1105/tpc.16.00806.



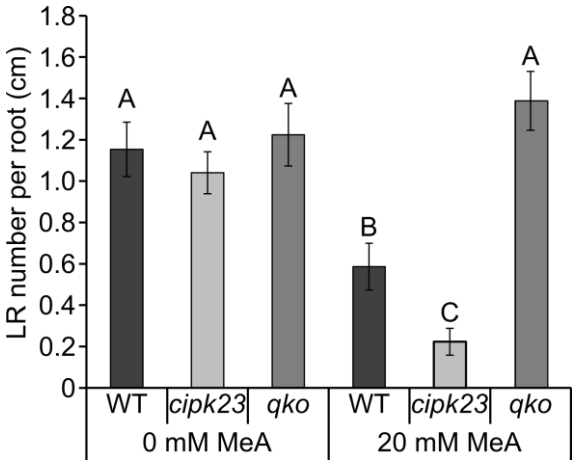
Supplemental Figure 1. Susceptibility of Col-0 seedlings grown in the dark to toxic concentrations of ammonium and methylammonium (MeA).

Seeds were sown on filter papers soaked with water containing ammonium or methylammonium in the indicated concentration. After breaking dormancy in 4°C for two days, seedlings were exposed to light for 6 hours and subsequently incubated in the dark for 6 days. Growth was assayed by the computer program *ImageJ*. Values are means ± SE. n ≥ 60. Upper and lower letters were used for comparison of different experiments, different letters indicate significant differences at the level of p-value ≤ 0.05.



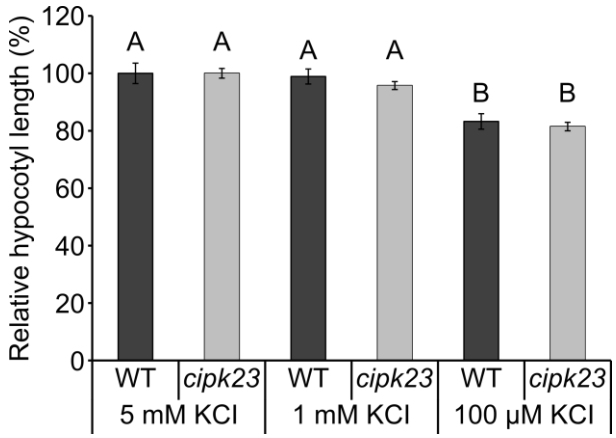
Supplemental Figure 2. Test for homozygousity of cipk23 and CIPK23 expression in cipk23.

(A) *CIPK23* expression in *cipk23* plants was tested by qPCR relative to wild type (WT). The experiment was reproducible (4 times repeated) and representative data is shown ± SD; 1g of 14 day old seedlings was pooled to gain one sample; n=4.
(B) Test for zygousity of *cipk23* T-DNA insertion plant line. Left: T-DNA insertion band with RP (right primer) and BP (left T-DNA border primer). Right wild type band with RP (right primer) and LP (left primer).
(C) Cartoon showing the binding sites for the T-DNA and gene specific primers and the expected genotyping result for a homozygous insertion line.



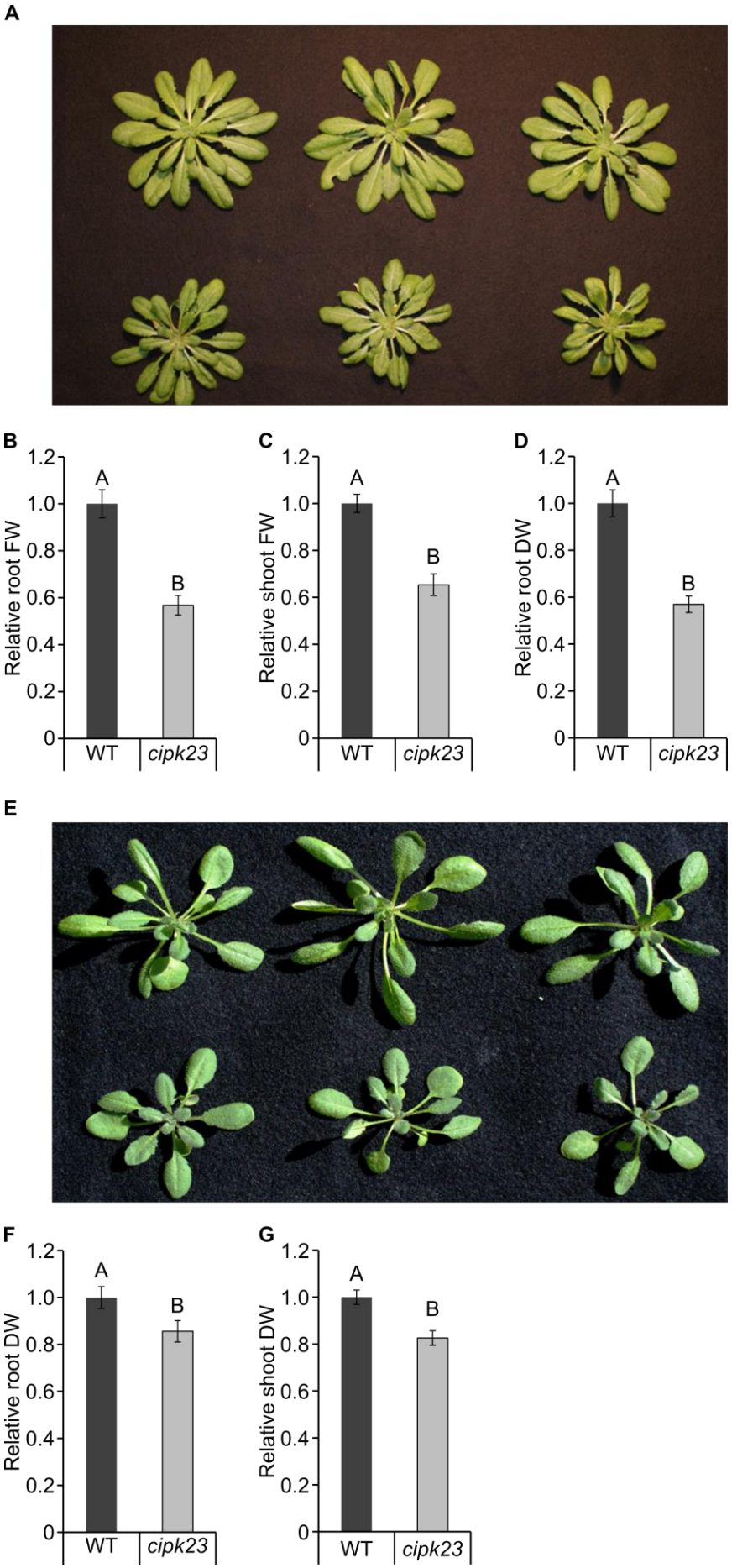
Supplemental Figure 3. Root phenotypes of the *cipk23* and *qko* mutants.

Number of the first order lateral roots relative to the primary root length in wild type, *cipk23* and *qko* plants grown on 20 mM MeA. Plants were precultured on HL medium containing 2 mM potassium nitrate as sole nitrogen source for 4 days and then transferred on agar plates containing 0, 20, or 30 mM MeA in the presence of 2 mM KNO_3 for 14 days. Values are means ± SD; n≥27. A/B/C ANOVA test p-value $\leq$ 0.001.



Supplemental Figure 4. Influence of potassium on hypocotyl elongation.

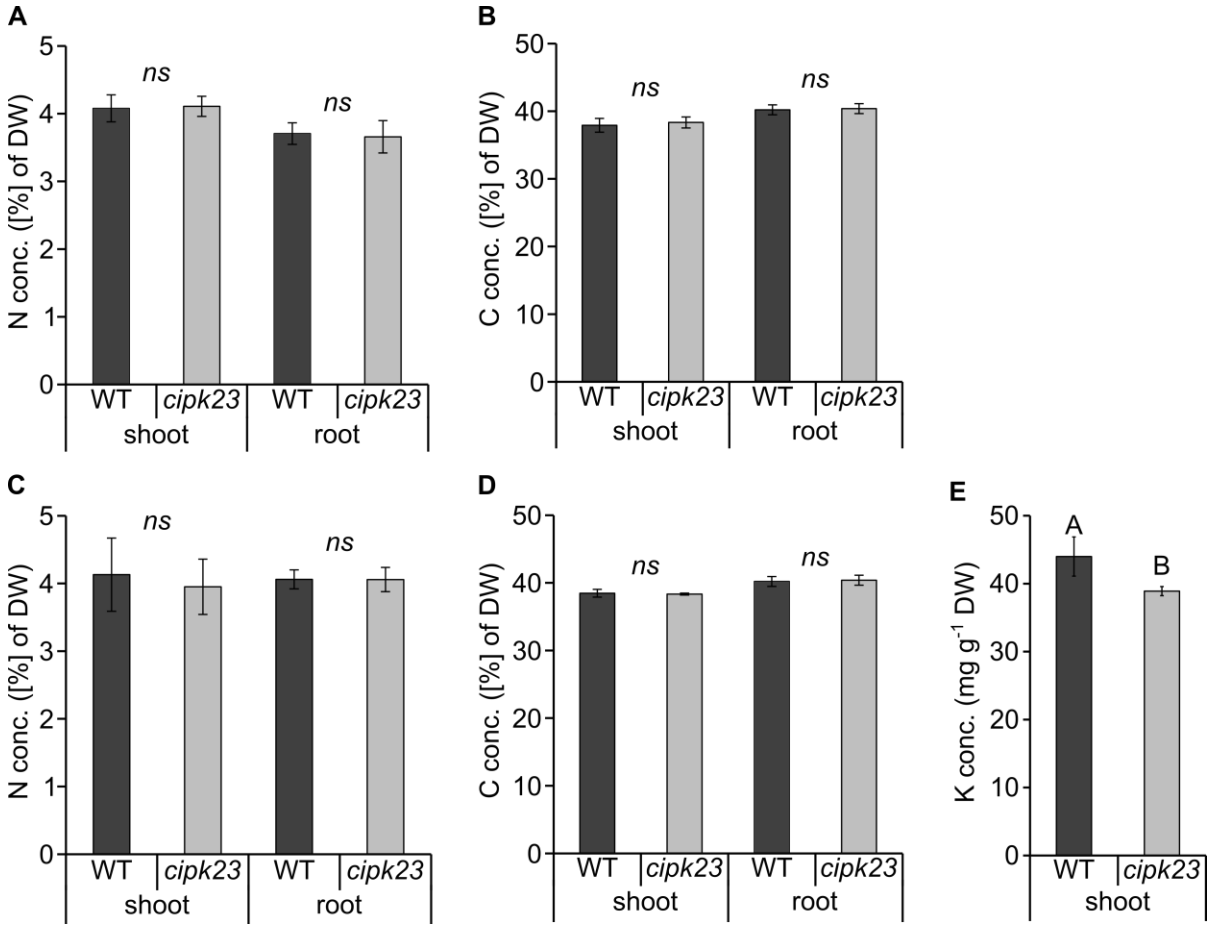
Seeds were sown on filter papers soaked with water containing potassium in the indicated concentration. After breaking dormancy in 4°C for two days, seedlings were exposed to light for 6 hours and subsequently incubated in the dark for 6 days. Growth was assayed by the computer program *ImageJ* and normalized on the 5 mM mean. Values are means ± SE. n ≥ 60. A/B t-test p-value ≤ 0.05.


A
B
Relative root FW
1.2
1.0
0.8
0.6
0.4
0.2
0
A
B
WT
cipk23
C
Relative shoot FW
D
Relative root DW
E
F
Relative root DW
G
Relative shoot DW

Supplemental Data. Straub et al. Plant Cell (2017) 10.1105/tpc.16.00806.

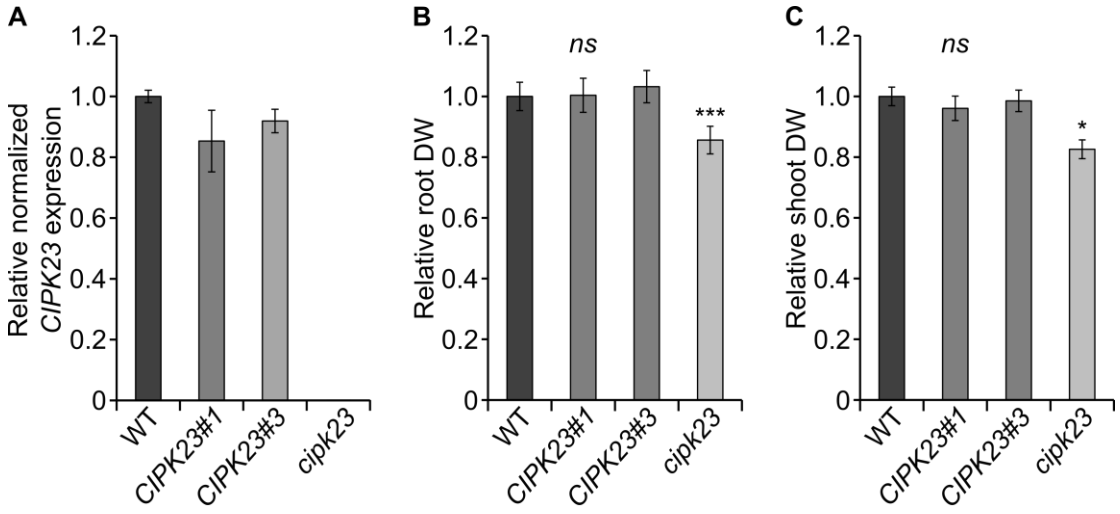
Supplemental Figure 5. Comparative growth under normal (1 mM) and elevated potassium conditions (5 mM potassium).

(A) Rosette size of wild type (top) and *cipk23* (bottom) plants cultivated for 6 weeks in HL with 1 mM KNO_3 and 1 mM NH_4NO_3 hydroponically. Relative fresh weight (FW) shoot **(B)**, fresh weight root **(C)** and dry weight (DW) root **(D)**. Data shown are means ±SD; n=24. A/B students T-test p-value $\leq$ 0.001.
(E) Plant (wild type and *cipk23*) growth after 4 weeks under elevated potassium nutrition (5 mM potassium) wild type (top) and *cipk23* (bottom). **(F)** Root and **(G)** shoot dry weight of 6 week old plants, data is shown as means ±SD; n≥60. Significance tested with T-test and indicated by A/B, p $\leq$ 0.001. WT, wild type plants.



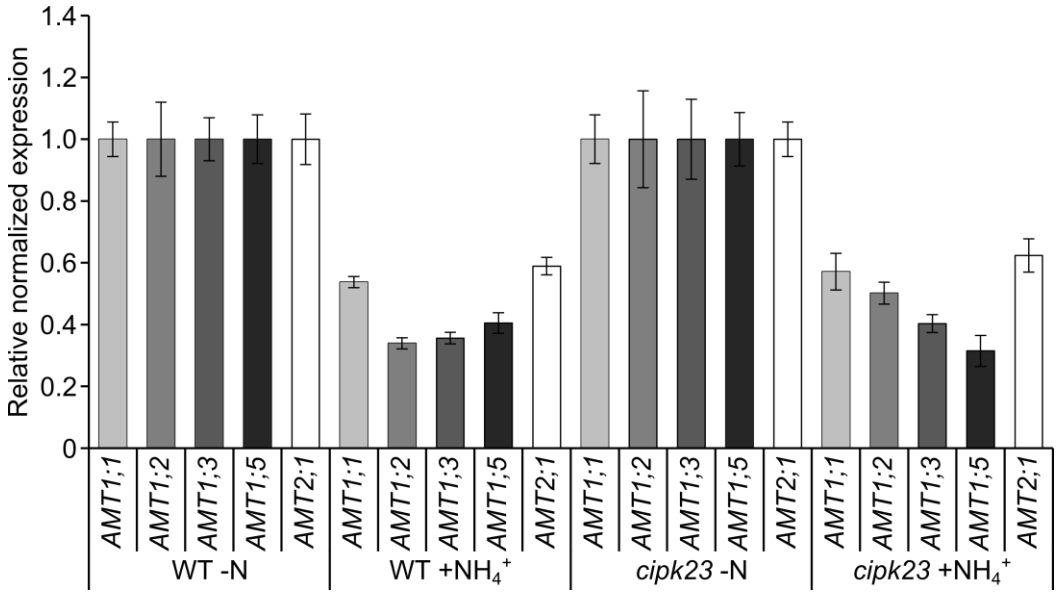
Supplemental Figure 6. Nutrient concentrations in 6-week-old wild type and *cipk23* plants grown under normal (1 mM) and elevated potassium conditions (5 mM potassium).

(A) Nitrogen (n=24) and **(B)** Carbon (n=24) concentration in the shoot and root of wild type (WT) and *cipk23* plants grown under normal potassium nutrition and elevated potassium nutrition **(C)** and **(D)** respectively (n≥60).
(E) Potassium concentration in the shoots of wild type and *cipk23* plants (n=15) under elevated potassium nutrition. Plants were grown hydroponically in HL solution for 6 weeks. Three independent experiments were performed, and values are means ± SD. Concentrations are given in % of dry weight (DW). A/B students T-test p-value $\leq$ 0.05; ns, not significant.



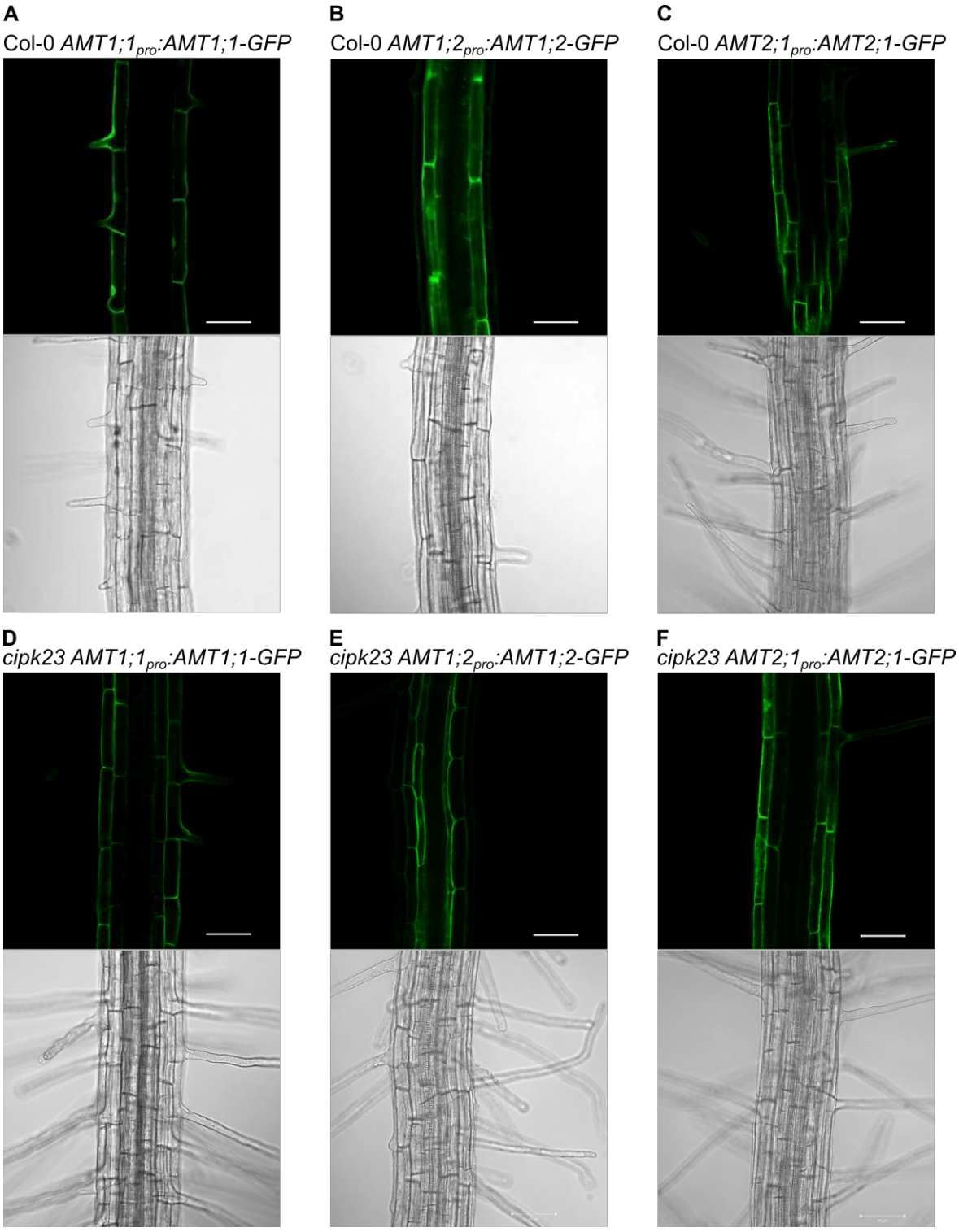
Supplemental Figure 7. Complementation of the *cipk23* line.

(A) *CIPK23* expression in wild type, *cipk23*, and two complementation lines $CIPK23_{pro}$*:CIPK23-1*, $CIPK23_{pro}$*:CIPK23-3*. Using quantitative PCR, *CIPK23* gene expression in complementation plant lines was tested relative to the expression in wild type. Data is shown as means ±SD; 1g of 14 day old seedlings was pooled in one sample; n=4; experiment was repeated 3 times.
(B) Root and **(C)** shoot dry weight of wild type, *cipk23*, and two complementation lines $CIPK23_{pro}$*:CIPK23-1*and $CIPK23_{pro}$*:CIPK23-3*. Plants were grown hydroponically (5 mM potassium in HL) and harvested after 6 weeks. The experiment was repeated twice, values are means ± SD; n≥60. Significance was tested using student T-test and indicated by stars; *, $p \leq 0.05$; *** $p \leq 0.001$; ns, not significant. WT, wild type plants. $CIPK23_{pro}$*:CIPK23-1*, *CIPK23#1*; $CIPK23_{pro}$*:CIPK23-3*, *CIPK23#3.*



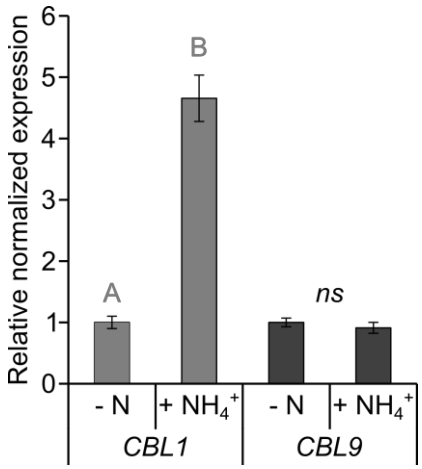
Supplemental Figure 8. Relative normalized expression of ammonium transporter genes in wild type and *cipk23* plants after nitrogen starvation and after subsequent ammonium shock.

Plants were grown hydroponically in HL solution for 6 weeks and then transferred in HL solution without nitrogen. After 4 days of nitrogen starvation (WT-N; *cipk23*-N) some of the plants were subjected to HL with 1mM $(NH_4)_2SO_4$ for 30 min – ammonium shock (WT+NH_4^+; *cipk23*+ NH_4^+;). The experiment was repeated 3 times values are means ±SD; n=3; always 10 plants were pooled in one sample. WT, wild type plants; *cipk23*, *cipk23* plants.



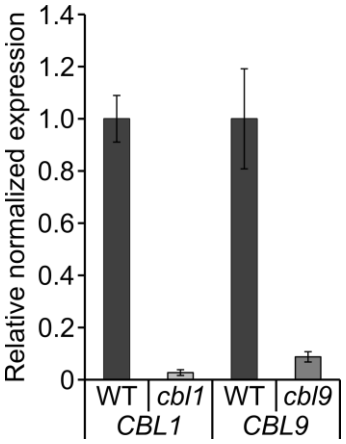
Supplemental Figure 9. AMT localization in the *Arabidopsis thaliana* Col-0 (upper panel) and *cipk23* (lower panel) backgrounds.

AMTs are expressed under their endogenous promoter and fused to the green fluorescent protein. AMT1;1 **(left; A/D)** and AMT2;1 **(right; C/F)** are expressed in root cortex and epidermis, while AMT1;2 **(middle; B/E)** is found in the endodermal and cortical cells regardless of the background. Scale bar size = 50µM.



Supplemental Figure 10. Relative normalized expression of *CBL1* and *CBL9* in 6-week-old *cipk23* plants after 4 days of nitrogen starvation (-N) and after 30 min of 2 mM NH_4^+ (as 1 mM $(NH_4)_2SO_4$) shock.

Significance was tested using students T-test, p≤0.001. Data is shown as means ±SD. Always 5 plants were pooled in sample, three biological replicates (each from a separate experiment) were used and for each replicate two technical replications were performed.



Supplemental Figure 11. *CBL1* and *CBL9* expression in *cbl1* and *cbl9* T-DNA insertion lines.

Using quantitative RT-PCR, two different T-DNA insertion lines (*cbl1* and *cbl9*) were tested for their expression of the respective gene relative to wild type (WT). The experiment was reproduced 3 times and data is shown as means ± SD. 1g of 14 day old seedlings was pooled in one sample, two biological replicates were used and for each replicate two technical replications were performed.

Supplemental Data. Straub et al. Plant Cell (2017) 10.1105/tpc.16.00806.

Supplemental Table 1. T-DNA insertion lines used in the hypocotyl screen.

NASC: Line number given by the *European Arabidopsis Stock Centre*, T-DNA Line: Line number given by the producing institute, AGI-Code and Gene Name of the kinases used are given.

NASC	T-DNA Line	AGI-Code	Gene Name
N669412	Salk_014699C	At1g01140	AtSnRK3.12/AtPKS6/AtCIPK9
N663934	Salk_127158C	At1g08720	AtEDR1
N901418	WiscDsLoxHs015_10B	At1g11280	
N659985	Salk_076543C		
N655245	Salk_007756C	At1g18040	
N662100	Salk_036154C	At1g30270	AtSnRK3.23/AtPKS17/AtCIPK23
N667044	Salk_112091C		
N661978	Salk_029403C	At1g48260	AtSnRK3.21/AtPKS20/AtCIPK17
N665133	Salk_003412C		
N677316	Salk_146186C	At1g50030	AtTOR
N669165	Salk_142042C	At1g51660	AtMKK4/AtMAPKK4
N671393	Salk_018804C		
N872509	SAIL_251_B02	At1g54960	AtANP2
N661330	Salk_004748C	At1g67520	
N858701	WiscDsLox497_10B		
N657698	Salk_081193C	At1g72180	
N661575	Salk_014533C		
N661655	Salk_017378C	At1g73500	AtMKK9/AtMAPKK9
N657637	Salk_059205C	At1g73660	
N669306	Salk_001982C		
N668518	Salk_077975C	At1g79670	AtWAKL22
N829571	SAIL_675_F09		
N654919	Salk_137779C	At2g26980	AtSnRK3.17/AtPKS12/AtCIPK3
N672732	Salk_074944C		
N669693	Salk_051317C	At2g28990	
N667130	Salk_118231C	At2g30360	AtSnRK3.22/AtPKS5/AtCIPK11
N656985	Salk_131251C	At2g41890	
N659911	Salk_058928C		
N657979	Salk_081990C	At3g06030	AtANP3
N668209	Salk_047797C	At3g21220	AtMKK5/AtMAPKK5
N911333	WiscDsLoxHs119_01E	At3g23000	AtSnRK3.10/AtPKS7/AtCIPK7/AtSR2
N660127	Salk_116983C	At4g00340	
N664674	Salk_069473C	At4g08500	AtMEKK1
N658593	Salk_029496C	At4g14780	
N658925	Salk_135277C		
N852223	WiscDsLox345-348N24	At4g18700	AtSnRK3.9/AtPKS8/AtCIPK12
N664463	Salk_018985C	At4g24400	AtSnRK3.13/AtPKS11/AtCIPK8
N656005	Salk_140054C	At4g26070	AtMKK1/AtMEK1/AtMAPKK1
N852195	WiscDsLox345-348H12	At4g30960	AtSnRK3.14/AtPKS4/AtCIPK6
N663562	Salk_105027C	At4g32300	
N661249	Salk_000367C	At5g01820	AtSnRK3.15/AtPKS24/AtCIPK14/AtSR1
N672594	Salk_066741C	At5g03730	AtCTR1
N913020	WiscDsLoxHs136_08D	At5g07070	AtSnRK3.2/AtPKS16/AtCIPK2
N664585	Salk_046150C	At5g11850	
N673571	Salk_000085C	At5g21326	putative SnRK3-type protein kinase
N655063	Salk_047425C	At5g50000	
N664697	Salk_076181C		
N660005	Salk_084332C	At5g56580	AtMKK6/AtMAPKK6
N667034	Salk_111320C	At5g58380	AtSnRK3.8/AtPKS2/AtCIPK10
N318666	GK-149A04.02		
N655352	Salk_022711C	At5g59650	
N848612	SAIL_1297_H07		

Supplemental Table 2. Combined ^{15}N ammonium uptake rates from Figure 2.

Uptake values are given after starvation and after shock for the respective ^{15}N concentration, uptake duration and genotype. Uptake is given as the complete quantity of ^{15}N taken up during the short term uptake experiment by the plants per g DW. Values are means ± SD. Reduction gives the reduction of the uptake due to the ammonium shock in % of the uptake before the shock.

^{15}N concentration	Uptake duration	Genotype	NH_4^+ uptake µM g^{-1} DW		Reduction (%)
			4d starvation	30 min shock	
0.5 mM ^{15}N	6 min	WT	21.8 ± 0.67	13.2 ± 1.52	31
		CIPK23#1	21.3 ± 3.02	11.7 ± 1.93	44
		CIPK23#3	18.5 ± 3.13	10.0 ± 2.89	41
		cipk23	24.1 ± 2.77	15.0 ± 2.86	35
	30 min	WT	54.2 ± 7.63	33.1 ± 0.80	27
		CIPK23#1	48.0 ± 5.13	36.9 ± 16.70	27
		CIPK23#3	56.8 ± 5.96	32.3 ± 11.57	27
		cipk23	65.5 ± 5.55	53.9 ± 4.73	13
5 mM ^{15}N	6 min	WT	32.0 ± 0.73	22.5 ± 3.9	17
		CIPK23#1	32.0 ± 6.3	18.6 ± 4.70	40
		CIPK23#3	31.3 ± 4.46	17.3 ± 5.69	39
		cipk23	31.8 ± 2.07	28.7 ± 3.36	11
	30 min	WT	68.2 ± 4.76	53.4 ± 10.84	20
		CIPK23#1	75.6 ± 5.23	57.9 ± 2.78	24
		CIPK23#3	74.8 ± 5.05	45.7 ±10.10	39
		cipk23	73.1 ± 16.81	72.9 ± 12.33	0.07

Supplemental Table 3. Modified Hoagland medium used for plant growth. pH adjusted to 6.0.

Salt	Concentration, µM
KH_2PO_4	1000
$MgSO_4$	500
$CaCl_2$	1000
$MnCl_2$	9
$ZnSO_4$	0.765
$CuSO_4$	0.32
H_3BO_3	46
Na_2MoO_4	0.016
FeNaEDTA	50

Supplemental Table 4. Primers used in qRT PCR.

Gene	Locus	Forward Primer (5'→3')	Reverse Primer (5'→3')
AMT 1;1	AT4G13510	CGCGGCGCTGACAACCCTAT	GAGGACTAGGGCCGCCACGA
AMT 1;2	AT1G64780	GGCCGGTCCGTGGCTTTACG	GACCGCGGTGCGACCTACAG
AMT 1;3	AT3G24300	CGGCCACTCTGCCTCGCTAG	CCGCACACAATCGCTGCCCA
AMT 1;5	AT3G24290	TTCAACCCTGGTTCCTTCAC	ACGTTTTCCGAAGAGTGTGG
AMT 2;1	AT2G38290	GGTGCTCCTTACGCGGCCAA	CGGGAGTGACGCCGGCTAAG
CIPK23	AT1G30270	CGTTTTGGAATTCGTCACTG	TGTTGGAAATACTTCCTCGC
CBL1	AT4G17615	CATTGAACGACAAGAGGTCA	CTTGATTCACGTCTGCATCT
CBL9	AT5G47100	ATTGAGCGCCAAGAGGTGAA	CCATCCCGATCCACATCTGC
PDF	AT1G13320	TAACGTGGCCAAAATGATGC	GTTCTCCACAACCGCTTGGT
SAND	AT2G28390	CAGACAAGGCGATGGCGATA	GCTTTCTCTCAAGGGTTTCTGGGT

Supplemental Table 5. Newly generated plant lines in this study. The combination of plasmids used for the transformation and the corresponding resistance are shown.

Plant line	Plasmids used for plant transformation	Resistance
cipk23-AMT1;1:GFP	*pTbar pAtAMT1;1:AMT1;1-GFP*	MSX
cipk23-AMT1;2:GFP	*pTbar pAtAMT1;2:AMT1;2-GFP*	MSX
cipk23-AMT2;1:GFP	*pTbar pAtAMT2;1:AMT2;1-GFP*	MSX
AMT1;1:NY/**AMT1;1:CY**	*pTkan+pAtAMT1;1:AMT1;1-NY/* ***pTbar pAtAMT1;1:AMT1;1-CY***	Kanamycin MSX
AMT1;2:NY/**AMT1;2:CY**	*pTkan+pAtAMT1;2:AMT1;2-NY/* ***pTbar pAtAMT1;2:AMT1;2-CY***	Kanamycin MSX
AMT1;1:NY/**CIPK23:CY**	*pTkan+pAtAMT1;1:AMT1;1-NY/* ***pTbar pAtCipk23:Cipk23-CY***	Kanamycin MSX
CIPK23:NY/**AMT1;1:CY**	*pTkan+pAtCIPK23:CIPK23-NY/* ***pTbar pAtAMT1;1:AMT1;1-CY***	Kanamycin MSX
AMT1;2:NY/**CIPK23:CY**	*pTkan+pAtAMT1;2:AMT1;2-NY/* ***pTbar pAtCIPK23:CIPK23-CY***	Kanamycin MSX
Cipk23:NY/**AMT1;2:CY**	*pTkan+pAtCipk23:Cipk23-NY/* ***pTbar pAtAMT1;2:AMT1;2-CY***	Kanamycin MSX
AMT2;1:NY/**CIPK23:CY**	*pTkan+pAtAMT2;1:AMT2;1-NY/* ***pTbar pAtCIPK23:CIPK23-CY***	Kanamycin MSX
AMT1;1:NY/**PromCIPK23:CY**	*pTkan+pAtAMT1;1:AMT1;1-NY/* ***pTbar pAtCipk23-CY***	Kanamycin MSX
AMT1;2:NY/**PromCIPK23:CY**	*pTkan+pAtAMT1;2:AMT1;2-NY/* ***pTbar pAtCipk23-CY***	Kanamycin MSX
AMT2;1:NY/**PromCIPK23:CY**	*pTkan+pAtAMT2;1:AMT2;1-NY/* ***pTbar pAtCipk23-CY***	Kanamycin MSX
cipk2- amiRNA 8-2-1	*pUTbar amiRNA*	MSX
k1-K1(1)	*pTbar pAtCIPK23:CIPK23*	MSX
k1-K1(3)	*pTbar pAtCIPK23:CIPK23*	MSX