

Supplemental Data Figure 1

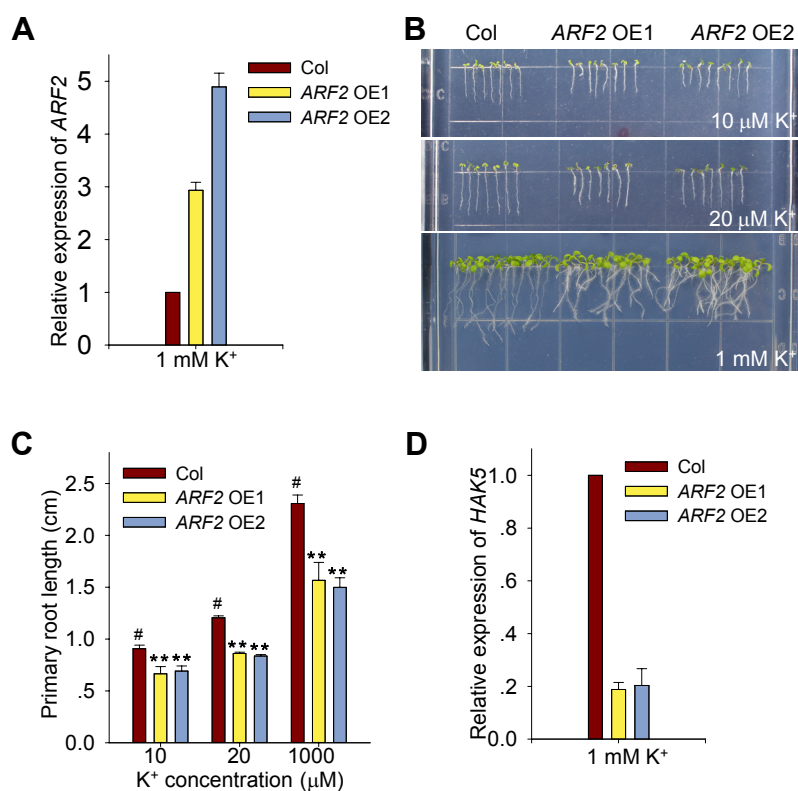


Figure S1. Phenotype Analysis of *ARF2*-overexpressing Lines

(A) RT-qPCR analysis of *ARF2* expression in *ARF2*-overexpressing lines (OE1 and OE2) driven by the 35S promoter. Seedlings were germinated and grown on K⁺ sufficient medium for 7 days. Data are means \pm SE (n=3).

(B) Phenotype comparison between wild-type and *ARF2*-overexpressing lines. Seedlings were germinated and grown on low-K⁺ medium or K⁺ sufficient medium for 7 days.

(C) Primary root length of various plants tested in **(B)**. Data are means \pm SE (n=15). Student's *t*-test (**P<0.01) was used to analyze statistical significance, and “#” represents control.

(D) The qPCR analyses of *HAK5* expression in *ARF2*-overexpressing lines. Seedlings were germinated and grown on K⁺ sufficient medium for 7 days. Data are means \pm SE (n=3).

Supplemental Data Figure 2

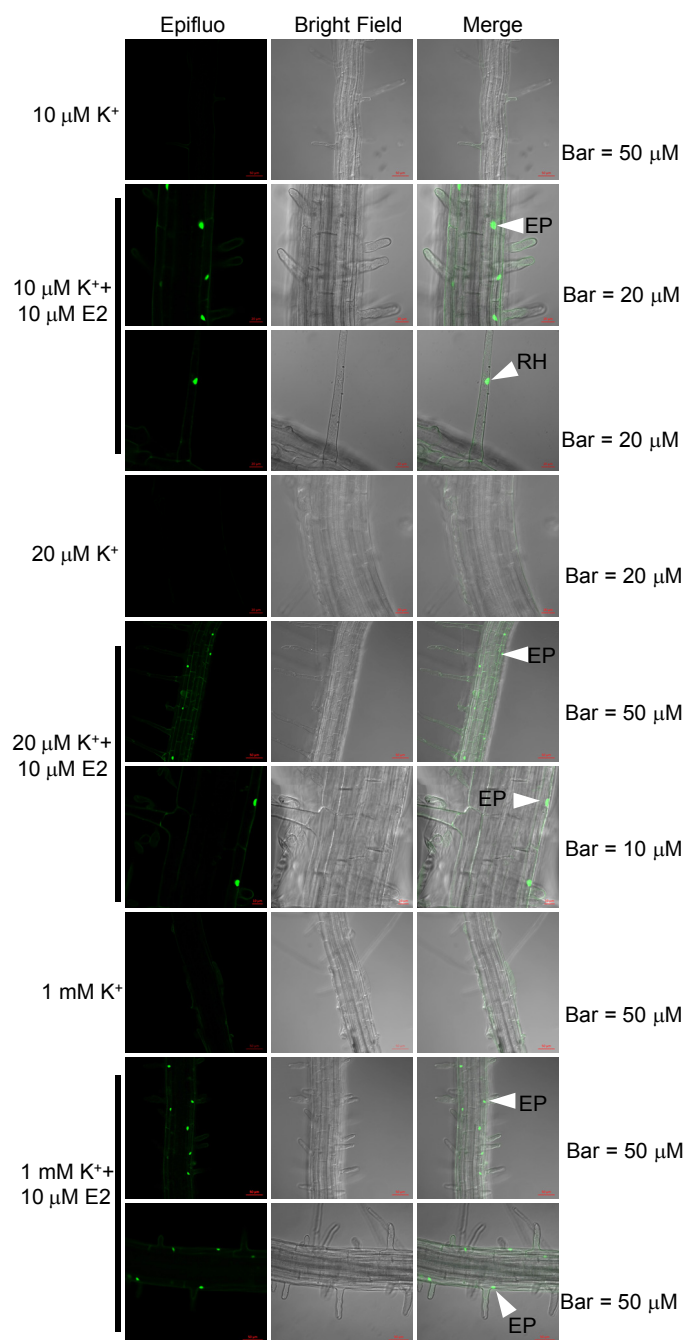


Figure S2. ARF2 Proteins Are Targeted to the Nucleus in the Root Epidermis and Root Hairs

The inducible *ARF2*-overexpressing lines (OE3 and OE4) were germinated and grown on low- K^+ medium or K^+ sufficient medium with or without 10 μ M β -estradiol (E2) for 7 days. ARF2-GFP proteins were induced and expressed after application of β -estradiol (E2). ARF2-GFP proteins are mainly targeted to the nucleus in the root epidermis (EP) and root hairs (RH).

Supplemental Data Figure 3

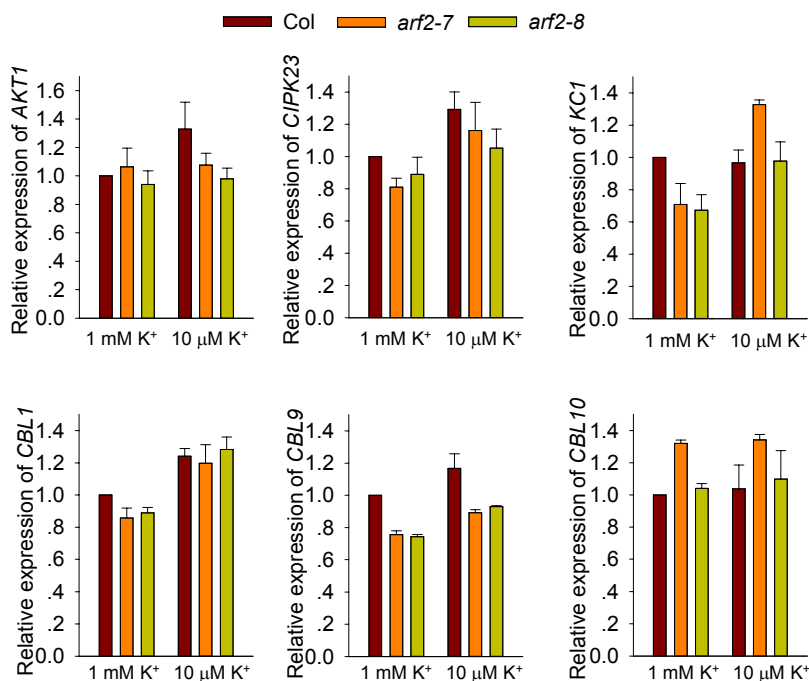


Figure S3. Expression Analyses of Potassium Relevant Genes in *arf2* Mutants

Seedlings were germinated and grown on K⁺ sufficient medium for 5 days, and then transferred to low-K⁺ medium or K⁺ sufficient medium for 12 h. Total RNA of plants was extracted and used for RT-qPCR analyses. The expression levels of *AKT1*, *CIPK23*, *KC1*, *CBL1*, *CBL9* and *CBL10* were determined in wild-type and *arf2* mutants. Data are means \pm SE (n=3). Student's *t*-test (**P<0.01) was used to analyze statistical significance, and “#” represents control.

Supplemental Data Figure 4

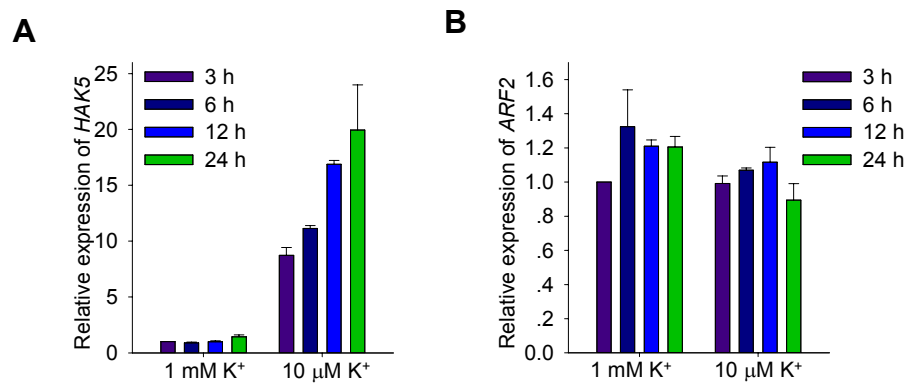


Figure S4. Expression Analyses of *HAK5* and *ARF2* in Response to Low-K⁺ Stress

Wild-type plants were germinated and grown on K⁺ sufficient medium for 5 days, and then transferred to low-K⁺ medium or K⁺ sufficient medium for the indicated times. Total RNA of plants was extracted and used for RT-qPCR analyses. The expression levels of *HAK5* and *ARF2* were determined. Data are means \pm SE (n=3).

Supplemental Data Figure 5

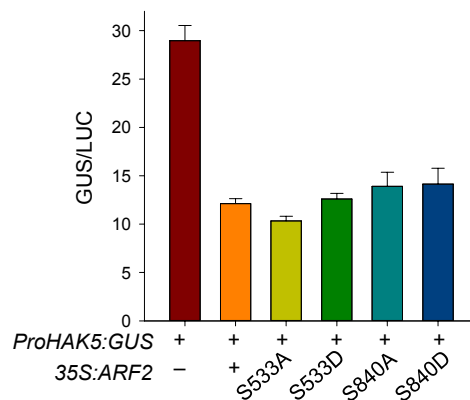


Figure S5. GUS/LUC Assay in Tobacco Leaves to Test the Activity of Mutant ARF2 Proteins

S533 and S840 on ARF2 were mutated to Ala (A) or Asp (D). The mutated 35S:ARF2 were co-expressed with *ProHAK5:GUS* in tobacco leaves. Then the GUS activity was measured. *LUC* gene was taken as an internal control. Data are means \pm SE (n=6).

Supplemental Data Figure 6

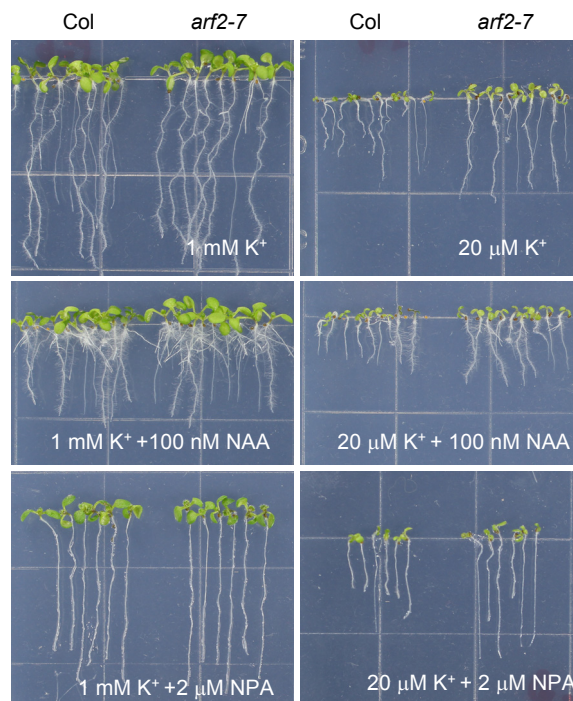


Figure S6. Phenotype Test of *arf2* Mutant in Response to NAA or NPA

Wild-type and *arf2-7* mutant were germinated and grown on low-K⁺ medium or K⁺ sufficient medium for 8 days. NAA (100 nM) and NPA (2 μM) were added in the medium respectively.

Supplemental Table 1

Primer sequences and enzyme sites used in this study.

Primer name	Vector	Primer sequence (5'–3')
Primers used in transgenic construction		
ARF2-1305-BamHI-F	pCAMBIA1305	CGGGATCCAAAGAAAGAGAGATGAA GGTGAGATGAATGAAAGAGTCG
ARF2-1305-Sall-R		ACGCGTCGACAGAGTTCCCAGCGCT GGACAATG
ARF2-SpeI-F	SUPERR:sXVE:GFP _c :Bar	GGACTAGTATGGCGAGTTCGGAGGT TTC
ARF2-XhoI-R		CCGCTCGAGAGAGTTCCCAGCGCTG GAC
ARF2-F	pCXSN	ATGGCGAGTTCGGAGGTTTCAATGA
ARF2-R		TTAAGAGTTCCCAGCGCTGGACAA
ProHAK5-1391-SmaI-F	pCAMBIA1391	ATCGATCCCGGGTTTTTGTGTGTTT TTTTTTTTTTTTTTTTGTGTTGTG
ProHAK5-1391-PstI-R		ATCGATCTGCAGTAGGCTAATGTAAT GGCTTCGTGTGAAAAATCATATGCT
HAK5-1391-SmaI-F	pCAMBIA1391	ATCGATCCCGGGATGGATGGTGAGG AACATCAAATAG
HAK5-1391-SpeI-R		ATCGATACTAGTTAACTCATAGGTCA TGCCAACCTTG
Primers for RT-PCR		
ARF2-F		ATGGCGAGTTCGGAGGTTTCAATGA
ARF2-R		TTAAGAGTTCCCAGCGCTGGACAA
HAK5-RT-F		CGGGATCCCGATGGATGGTGAGGAA CATCAAATAG
HAK5-RT-R		CGAGCTTTATAACTCATAGGTCATG CCAACCTT
EF1 α -F		ATGCCCCAGGACATCGTGATTTTCAT
EF1 α -R		TTGGCGGCACCCTTACGTGGATCA
Primers for qPCR		
HAK5-qRT-F		AAGAGGAACCAAATGCTGAGACA
HAK5-qRT-R		GCCCCGATGAAGGGACAT
ARF2-qRT-F		CCTCATCCGAAGGATGCTCAAACG
ARF2-qRT-R		GGAGCCATCAACTCTCCATTGAACTC
Actin2/8-qRT-F		ACGGTAACATTGTGCTCAGTGGTG
Actin2/8-qRT-R		CTTGGAGATCCACATCTGCTGGA

Primer name	Vector	Primer sequence (5'–3')
Primers for qPCR		
CBL1-qRT-F		AATGTTGATCGCGCTTCTCT
CBL1-qRT-R		CATGTGGCAATCTCATCGAC
CBL9-qRT-F		AGCACCTATGGGGTTTGATG
CBL9-qRT-R		CTCTTCGTCGATGGTTTTCC
CBL10-qRT-F		TCTGCGCCGTCTTTATACCT
CBL10-qRT-R		AACAGCGCCAGTCTAAGCTC
KC1-qRT-F		ATTGCTTCCAAGGGTGTGAG
KC1-qRT-R		GCGTCGACGTCATTATCAGA
AKT1-qRT-F		TGACGAATGTTCTGCTGGAG
AKT1-qRT-R		TGCCATTGTTATCCGATTCA
CIPK23-qRT-F		CAAGTTCGAGAGGATGGGTTAC
CIPK23-qRT-R		TCTTAGCACTTGCAGAGAACCA
Primers for GUS/LUC assay		
ProHAK5-1381-HindIII-F	pCAMBIA1381	ATCGATAAGCTTTTTTTGCTGTGTTTTTT TTTTTTTTTTTTTTGTGTTGTG
ProHAK5-1381-Sall-R		ATCGATGTCGACTAGGCTAATGTAATGG CTTCGTGTGAAAAATCATATGCT
ARF2-F	pCXSN	ATGGCGAGTTCGGAGGTTTCAATGA
ARF2-R		TTAAGAGTTCCCAGCGCTGGACAA
840-SA-F	pCXSN	AGTTGTTGGGAAGGAGCAGATGCAAA GGACGC
840-SA-R		GCGTCCTTTGCATCTGCTCCTTCCCCAA CAACT
840-SD-F	pCXSN	GACTTGGCGTCCTTTGCATCGTCTCCTT CCCCAACAACTGCTT
840-SD-R		AAGCAGTTGTTGGGAAGGAGACGATG CAAAGGACGCCAAGTC
689-SA-F	pCXSN	GTCCTGAACCTTTGGTGCTGCTATCTGT GTAAGCC
689-SA-R		GGCTTACACAGATAGCAGCACCAAAGG TTCAGGAC
689-SD-F	pCXSN	AGGTCCTGAACCTTTGGGTCTGCTATCT GTGTAAGCCCCGC
689-SD-R		GCGGGGCTTACACAGATAGCAGACCCA AAGGTTTCAGGACCT

Primer name	Vector	Primer sequence (5'–3')
Primers for GUS/LUC assay		
533-SA-F	pCXS	AGGCATAGAAGGTGATGATGCATGGTC ATAAAAAGGTATCC
533-SA-R		GGATACCTTTTTATGACCATGCATCATC ACCTTCTATGCCT
533-SD-F	pCXS	AGGCATAGAAGGTGATGAGTCATGGTC ATAAAAAGGTATCCGCTGACCA
533-SD-R		TGGTCAGCGGATACCTTTTTATGACCAT GACTCATCACCTTCTATGCCT
Primers for ChIP		
HAK5-ChIP-P1-F		TAACCCTCAATAATACGTATTTG
HAK5-ChIP-P1-R		ATTTTGTACCAATTAGCTAATCTT
HAK5-ChIP-P2-F		ACTCACTAATGCACACAATGACA
HAK5-ChIP-P2-R		GGTAACATGACGAGTTGGTGG
HAK5-ChIP-P3-F		TCCGTTATCCACCCTTACCA
HAK5-ChIP-P3-R		TGGGCAAAGTAACTAATGATGG
HAK5-qRT-F		AAGAGGAACCAAATGCTGAGACA
HAK5-qRT-R		GCCCCGATGAAGGGACAT
Primers for EMSA		
CKS-ARF2-N-BamHI-F	pTrc-CKS	CGGGATCCATGGCGAGTTCGGAGGTTT C
CKS-ARF2-N-EcoRI-R		GGAATTCCTCAGGAGCATCACACTCTAC ACTCTC
CKS-ARF2-BamHI-F	pTrc-CKS	CGGGATCCATGGCGAGTTCGGAGGTTT C
CKS-ARF2-NheI-R		CTAGCTAGCAGAGTTCAGCGCTGGA CA
HAK5-P1-F		TAACCCTCAATAATACGTATTTG
HAK5-P1-R		ATTTTGTACCAATTAGCTAATCTT
HAK5-mutant P1-F		TGACTATTTGCAAGAGACAATAGAATAG ACGATGCAAAAAC
HAK5-mutant P1-R		TGACTATTTGCAATTTTTTATAGAATAGA CGATGCAAAAAC
HAK5-P3-R		CGATAGGATTGTCTATTTAAGATATCTG
HAK5-P3-R		CGATAGGATTGTCTATTTAAGATATCTG
HAK5-mutant P3-1-F		AATTATCCGTTATCCACCCTTACCAATAT TGTTTTTTCAGATATCTTAAATAGACAA TC

Primer name	Vector	Primer sequence (5'–3')
Primers for EMSA		
HAK5-mutant P3-1-R		GATTGTCTATTTAAGATATCTGAAAAAA CAATATTGGTAAGGGTGGATAACGGATA ATT
HAK5-mutant P3-2-F		TATTGTTTTTTTCAGATATCTTAAATAAA AAATCCTATCGAATCGAATCCCGCGG
HAK5-mutant P3-2-R		CCGCGGGAATTCGATTCGATAGGATTTT TTATTTAAGATATCTGAAAAAACAATA
689-SA-F	pTrc-CKS	GTCCTGAACCTTTGGTGCTGCTATCTGT GTAAGCC
689-SA-R		GGCTTACACAGATAGCAGCACCAAAGG TTCAGGAC
689-SD-F	pTrc-CKS	AGGTCCTGAACCTTTGGGTCTGCTATCT GTGTAAGCCCCGC
689-SD-R		GCGGGGCTTACACAGATAGCAGACCCA AAGGTCAGGACCT
Primers for cell free phosphorylation		
501-700 BamHI-F	pTrc-CKS	CGGGATCCTCAGCCAGGCATGAACCTA CTTAC
501-700 EcoRI-R		CGGAATTCTTTTGACTGATCTGAAAGGT CCTGAA