



Supplemental Figure 1. ERF family members function redundantly in the regulation of NRT1.8 expression

(A) Schematic map of the T-DNA insertion sites in *ora59-1* and *ora59-2* mutants. Closed box and open boxes represent coding region and untranslated regions (UTRs), respectively.

(B) Quantitative RT-PCR analysis of *ORA59* expression in *ora59* mutant plants. Values are mean \pm SD, n = 3.

(C) Transient transcriptional activity assay of $P_{NRTI.8}$:LUC expression. The $P_{NRTI.8}$:LUC-35S:REN reporter construct was transiently expressed in *Arabidopsis* protoplasts together with the control vector, 35S:ERF1A, 35S:ERF2, 35S:ERF6 or 35S:ERF13 effectors, respectively. The expression of REN was used as an internal control. LUC/REN ratio represents the relative activity of the *NRT1.8* promoter. Values are mean \pm SD, n = 3.

(D) and (E) Quantitative RT-PCR analysis of *NRT1.8* expression in roots of 4-week-old *ERF104*-overexpressing (D) or *ERF1B*-overexpressing (E) plants grown in hydroponics. Values are mean \pm SD, n = 3.

(F) Characterization of the triple mutant lines *tri-1* and *tri-2*, in which *ORA59* and *ERF104* showed undetectable mRNA, while *ERF1B* showed essentially reduced mRNA level. Values are mean \pm SD, n = 3.



Supplemental Figure 2. ERF expression upon stress/hormone treatment in the ET/JA mutants

Quantitative RT-PCR analysis of *ORA59* (A), *ERF1B* (B), *ERF1A* (C) and *ERF104* (D) expression in the roots of 4week-old WT, *ein2-50*, *coi1-1* and *ein2-50 coi1-1* plants treated with 20 μ M ACC, 50 μ M MeJA, 20 μ M ACC+50 μ M MeJA (A+J), 200 μ M CdCl₂ and 150 mM NaCl for 6 h. Data were normalized to those of *SAND*. Values are mean \pm SD, n = 3.



Supplemental Figure 3. NRT1.5 and NRT1.8 expression in response to SA or ABA treatment

Quantitative RT-PCR analysis of *NRT1.8* (A) and *NRT1.5* (B) expression in roots of 4-week-old Col-0 exposed to 50 μ M ABA or 1 mM SA for 6 h. Data were normalized to those of *SAND*. Values are mean \pm SD, n = 3.



Supplemental Figure 4. The expression of *ERFs* in the *ein3 eil1* mutant in response to different treatments

Quantitative RT-PCR analysis of *ORA59* (A), *ERF1B* (B), *ERF1A* (C) and *ERF104* (D) expression in the roots of 4-week-old WT, *ein3 eil1* plants treated with 20 μ M ACC, 50 μ M MeJA, 20 μ M ACC+50 μ M MeJA (A+J), 200 μ M CdCl_{2 or} 150 mM NaCl for 6 h. Data were normalized to those of *SAND*. Values are mean \pm SD, n = 3.



Supplemental Figure 5. Root growth of EIN3 overexpressing lines under non-stressed conditions

Seedlings were germinated and allowed to grow as described in Figure 8. Relative root growth was defined as the root elongation of *EIN3* overexpressing line *EIN3ox* plants against those of wild type control Col-0.Values are mean \pm SE, n = 5.



Supplemental Data. Zhang et al. (2014). Plant Cell 10.1105/tpc.114.129296

Supplemental Figure 6. Nitrate reallocation upon stresses in *nrt1.8* and *nrt1.5* mutants in the *nia1 nia2* background

(A) and (B) PCR-based genotyping of *nrt1.5-4 nia1 nia2* (A) and *nrt1.8-2 nia1 nia2* (B) mutant plants. A region containing the nucleotide mutation (G to A) of *nia1* was amplified and further digested with *HhaI* to identify *nia1* mutants. Specific primers located in the deleted region of *NIA2* were designed to identify *nia2* mutants. Both *nrt1.5-4* and *nrt1.8-2* contain T-DNA insertion and were identified as described (Krysan et al., 1999). Primer sequence information is available in Supplemental Table 1 online. (C) and (D) Nitrate reallocation to roots as determined by the root/shoot nitrate ratio. Plants in the *nia1nia2* background were grown hydroponically with NH₄HCO₃ as the only nitrogen source. At 4 weeks of age, plants were transferred to 2.25 mM NO₃⁻ solution and subjected to 200 μ M CdCl₂ or 150 mM NaCl for 12h (C), or 20 μ M CdCl₂ or 50 mM NaCl for 72h (D). Tissues were then sampled and nitrate was extracted and determined. Data were normalized to fresh weight and ratio between roots and shoots (R/S) were calculated. Values are mean \pm SD, n = 3.

Destination	Forward primer	Reverse primer	
RT-PCR			
SAND expression	GATGAGGATGATGCTTCTACG	CCTGAGCGTTGTATCTTGGT	
ORA59 expression	CAATCCTTCCTTTTATCTAGCCCTAC	CAGCACCTAAATCCTCAAGAACC	
ERF1B expression	GAATTCATGGATCCATTTTTAATTC	GTCGACTCACCAAGTCCCACTATTTTC	
ERF104 expression	ATGGCAACTAAACAAGAAGC	TGAAGTGACGGAGATAACGGAAAAGTTG	
NRT1.8 expression	тстттосттоотосттсст	ATCCGCTCTCTACTTTCTCAGC	
qRT-PCR			
SAND expression	ATATGACACCCTTGCTTGGAGGGA	TGAGAATAAGACACCAGACGCGCA	
ORA59 expression	AAGGGATAAGAGTGTGGCTTGGGA	CTTTCAAAGCGAAAGCCGCCTGAT	
ERF1B expression	GAGGAAACACTCGATGAGACG	GGAGCGGTGATCAAAGTCAC	
ERF104 expression	GAACCATCACCAACCAATCC	GTCCCAAGCCAGATCCTACA	
ERF1A expression	TCCGGTCAAGAAGGAGAAGA	GCCGTCTCAAACGTTCCTAA	
NRT1.8 expression	TCTTCATCTTCGCATACAGGCGGT	GCCATTATCGCAATCACAAGCCCA	
NRT1.5 expression	TGGAGCGTTTCTCAGCGATT	TCCATCATGGAATGTGAACCAC	
Genotyping			
Genotyping of	ATGCTGCAGACGGTAACAAAC	CTTCAAGCTCATGGAGCTCAC	
ora59-1			
Genotyping of	GCCACACTCTTATCCCTTTCC	TTACTTTGGGATTGGTTGCAG	
ora59-2			
Genotyping of erf104	CCTCTTGCTCAGTTGCTTGAC	GAAACAGGCCCTAGAACCATC	
Genotyping of	TGAAAATTGTGTATACGAAACGAGA	GGTGTCGATCATATATTTTCCTCC	
nrt1.5-4			
Genotyping of	AGTTAGACAGTTTGAGGTTTGCACTCAAG	GTAGAATCTCTCCAAGTGTCCTTTGTTGA	
nrt1.8-2			
Genotyping of ein3-1	TACCAAGTATCAAGCGGAG	AGGCCACCAATCCTCTTTC	
Genotyping of eil1-1	GGGAATGGTGGAAAGATAAG	CTTTCGCCGTCATCTTATCC	
Genotyping of nia1	TACGACGACTCCTCAAGCGAC	GGCTATAGATCCCGCATCGAC	
Genotyping of <i>nia2</i>	GTTCTTACAAACCTCCCGTTCC	CTCTGTTGTCCTTGAAATGGTAG	
Genotyping of ctr1-8	AGAGTCCACGACATATCTTG	AGGAATCAGGCTCCCATAAG	
Genotyping of eto1-1	GCAACACAACTTGACCCTCTT	GGGAGAATCCCTCAGAAAGG	
Protein expression and EMSA			
pET-30a (+) ORA59	GGATCC ATGGAATATCAAACTAACTTC	AAGCTT TCAAGAACATGATCTCATAAGC	
pET-30a (+) ERF1B	GAATTCATGGATCCATTTTTAATTC	GTCGAC TCACCAAGTCCCACTATTTTC	
pET-30a (+) ERF104	GTCGACTCATGGCAACTAAACAAGAAGC	GCGGCCGC TCAAGTGACGGAGATAAC	
		GGAAAAGTTG	
pGEX4T-1 EIN3(N)	GGATCCATGATGTTTAATGAGATGGGAATG	GAATTC ACCAGACAGAGAAAGAGGTGGAC	
<i>NRT1.8</i> promoter WT	GATTTTATCAGTCTCTTCCAGCAG	CAATTTAATTGTAAAGGTTTTGAAACG	
probe			

Supplemental Table 1. List of primer sequences.

Mutate NRT1.8	TTTATCAGTCTCTTCCATCATCCCTCC		
promoter WT	AGGTGTAACTGG		
probe GCC box B1			
Mutate NRT1.8	AATCCACCGAACATATATTTTATCATCCCGTT		
promoter WT	ТСААААССТТТАС		
probe GCC box B2			
NRT1.5 P5 fragment	TCTATAAGCATACGCATAAATGGA	CATATTGCCTCACAATCAATCTAG	
NRT1.5 P7 fragment	TATTGGTATTTAAACTTTGTTTTACTA	TAAATGATATAAACGTTTAAAGTACTT	
ChIP-qPCR			
TUB2 for ChIP	ATCCGTGAAGAGTACCCAGAT	AAGAACCATGCACTCATCAGC	
normalization			
NRT1.8 P1	CTATTTAAGAACAATTATTTGGGG	CATCCCTAAAATCTATGAAGAAGG	
fragment			
NRT1.8 P2	GCATATGATTATTATTGAGATAAC	GAGAGCTCAGAAATTGAGTCAG	
fragment			
NRT1.8 P3	CACTTCTTCACTTTCCTCGA	AGATTCGATTTGGTGTAGAG	
fragment			
ERFs binding sites in	TATACTTGTGTAACTATGGCTTGG	TGTTGATGGCTGGTTTCTCCAC	
PDF1.2 promoter			
EIN3 binding sites in	TGATGACAATTGGGTTCGGTG	GTTTGTGATATTGAGAAGAAC	
EBF2 promoter			
NRT1.5 P1 fragment	GGCATGCTTGCGTTTAGTTAGGT	ТСАСТСАААСТТААААТСАТАТТАС	
NRT1.5 P2 fragment	GTTGTTTTTAATCATGGGATTTATC	ТТАСАСАСТАСААТАТСТТТСАСТАА	
NRT1.5 P3 fragment	GTGTTTTGCATAGTAAATCGAATG	GCGGAGTAAAGTTTACATTATATC	
NRT1.5 P4 fragment	CAAGGGATTGACCTGTGGTAG	CGATTTCATGAGGTTGCAATATC	
NRT1.5 P5 fragment	TCTATAAGCATACGCATAAATGGA	CATATTGCCTCACAATCAATCTAG	
NRT1.5 P6 fragment	TAGATTGATTGTGAGGCAATATG	AATCGTTGAGATCTAATTTTAGCT	
NRT1.5 P7 fragment	TATTGGTATTTAAACTTTGTTTTACTA	TAAATGATATAAACGTTTAAAGTACTT	
NRT1.5 P8 fragment	ACAATGTTGTAGTTCACACAATGC	GTTGTTAGAAAAAAGAGGTACCAC	
	Transient expression & Plant tran	sformation	
<i>ERF1B</i> RNAi	CCCGGG GGCGCGCC CAATCCACTAAC	TCTAGA ATTTAAAT TTCTCTGACTTTC	
	GATCCCTAAC	TTGAGCTTAC	
pGreenII62-SK&	CTCGAG GGATCCATGGAATATCAA	GTCGACTGAAGAACATGATCTCATAAGCTC	
pCAMBIA 1300	ACTAACTTC		
ORA59			
pGreenII62-SK&	TCTAGA GAATTC ATGGATCCAT	GTCGAC TGACCAAGTCCCACTATTTTC	
pCAMBIA 1300	ТТТТААТТС		
ERF1B			
pGreenII62-SK	TCTAGA ATGGCAACTAAACAAGAAGC	ССССБСБ	
&pCAMBIA 1300		TGAAGTGACGGAGATAACGGAAAAGTTG	
ERF104			
pGreenII62-SK	GGATCCATGTCGATGACGGCGGATTC	CCCGGG TGATAAAACCAATAAACGAT	
ERF1A		CGCCACG	

pGreenII62-SKERF2	GGATCC ATGTACGGACAGTGCA	CCCGGG TGATGAAACCAATAACTCAT
	ΑΤΑΤΑGAATC	CAACACG
pGreenII62-SK <i>ERF6</i>	GGATCC ATGGCTACACCAAACGAAG	CCCGGG TGAAACAACGGTCAATTG
	TATCAGCTC	TGGATAACC
pGreenII62-SK	TCTAGA GAATTC ATGAGCTCATCTG	CCCGGG TGATATCCGATTATCAGAA
ERF13	ATTCCGTTAATAACG	TAAGAACATTC
pGreenII62-SK	GGATCC ATGGAATATTCCCAATC	CCCGGG TGAACATGAGCTCATAAG
ERF15	TTCCATGTATTC	AAGTTGTTC
NRT1.8 promoter in	AAGCTT CAATGAATCTAGACATTAATTCG	GGATCC AGATTCGATTTGGTGTAG
pGreenII0800-LUC		AGATATA