

Figure S1. Expression pattern of *WRKY* genes in response to Fe deficiency revealed by BAR Expression Browser. Numbers beside the heat map indicate the fold changes of gene expression, and the higher expression levels are indicated by more reddish colors. Genes in red boxes are indicated as Fe-deficiency inducible *WRKY*s.

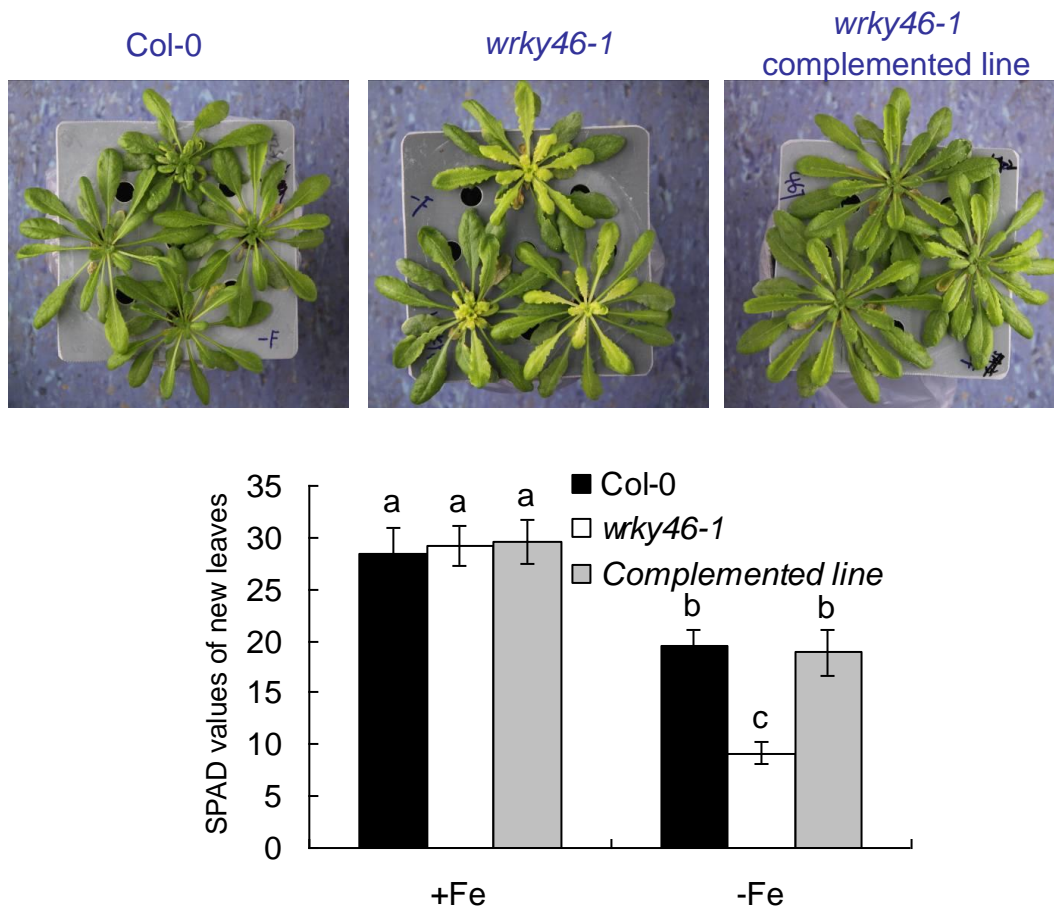


Figure S2. Lack of WRKY46 affects sensitivity to Fe deficiency. Growth of 6-week-old wild-type (WT), *wrky46-1* loss-of-function mutant and *wrky46-1* complemented plants under normal and Fe-deficiency treatments for 6 days. The chlorophyll content or leaf greenness in the new leaves was indicated as SPAD (Soil Plant Analysis Development) values. Three independent experiments were done, each containing at least 10 plants per genotype for one treatment. Data were analyzed by one-way ANOVA (Analysis of Variance) following Duncan's test. Error bars with different letters represent a statistical difference ($P < 0.05$, Duncan's test).

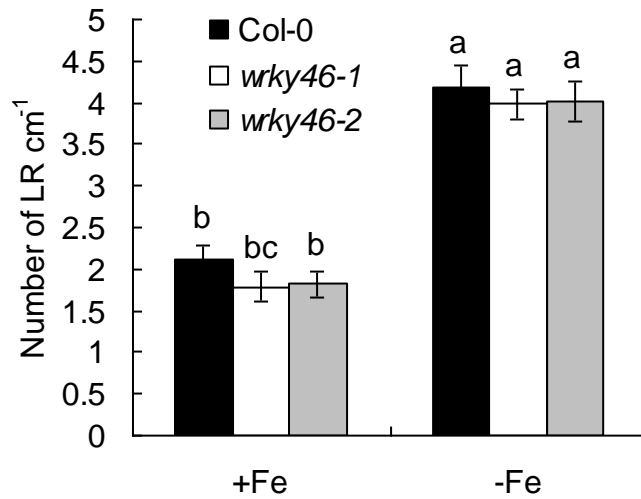


Figure S3. Lack of WRKY46 does not significantly affect lateral root development in Fe deficiency condition. 3-day-old WT and *wrky46* mutant seedlings were grown on half-strength MS medium with or without Fe supply for 10 days. The number of mature lateral roots (> 0.5mm long) emerged from primary root was counted. Three independent experiments were done, each containing at least 20 seedlings per treatment. Data were analyzed by one-way ANOVA (Analysis of Variance) following Duncan's test. Error bars with different letters represent a statistical difference ($P < 0.05$, Duncan's test).

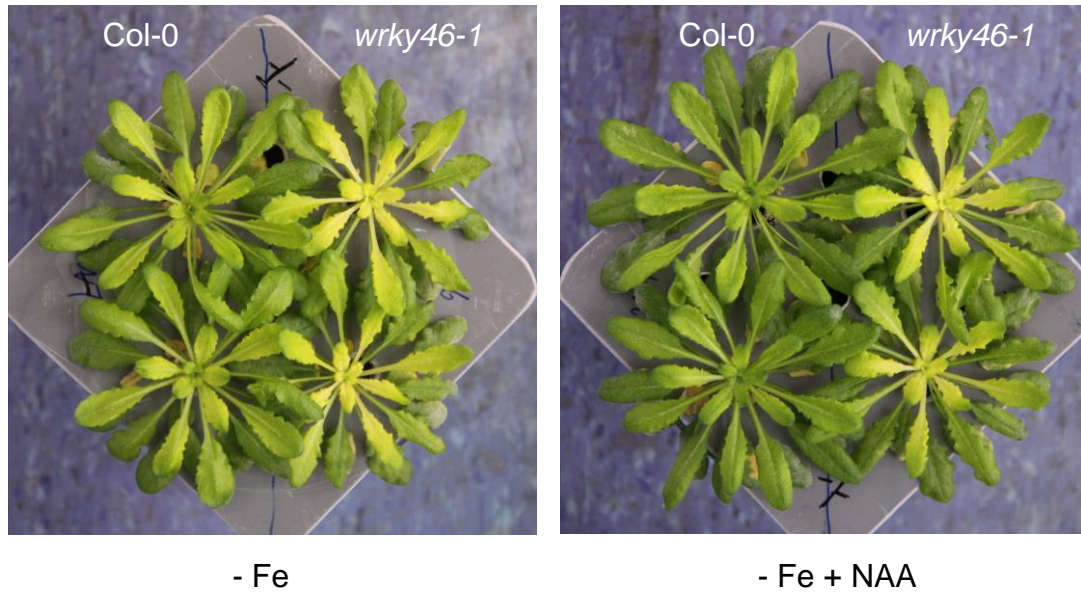
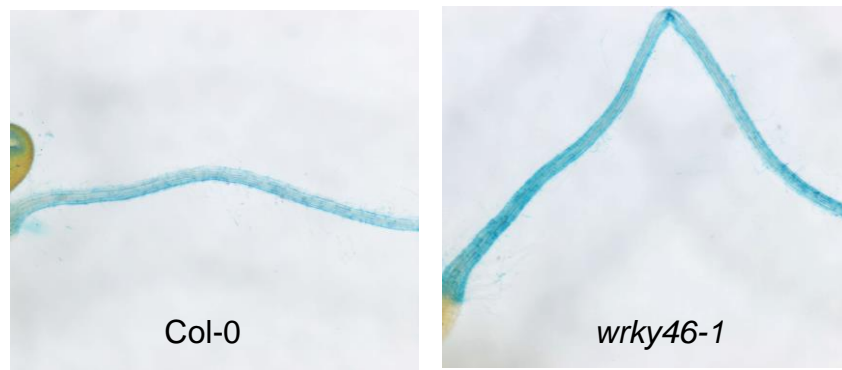


Figure S4. Auxin can not rescue response of *wrky46-1* to Fe deficiency. Growth of WT and *wrky46-1* mutant plants under Fe-deficient condition with or without 0.1 μ M 1-naphthylacetic acid (NAA) treatment for 6 days. Three independent experiments were done with similar results.

A



B

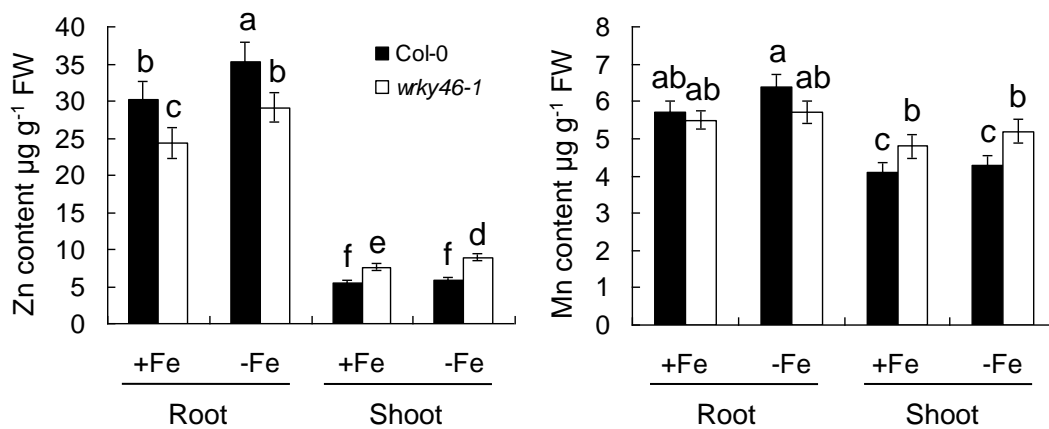


Figure S5. Micro-elements content analysis in WT and *wrky46-1* mutant plants. A, Perl-stained roots of 7-day-old WT and *wrky46-1* seedlings germinated and grown on Fe sufficient media. B, Zn and Mn content measured in roots and shoots of WT and *wrky46-1* mutant plants with or without Fe-deficiency treatment for 6 days. Three independent experiments were done. Data were analyzed by one-way ANOVA (Analysis of Variance) following Duncan's test. Error bars with different letters represent a statistical difference ($P < 0.05$, Duncan's test).

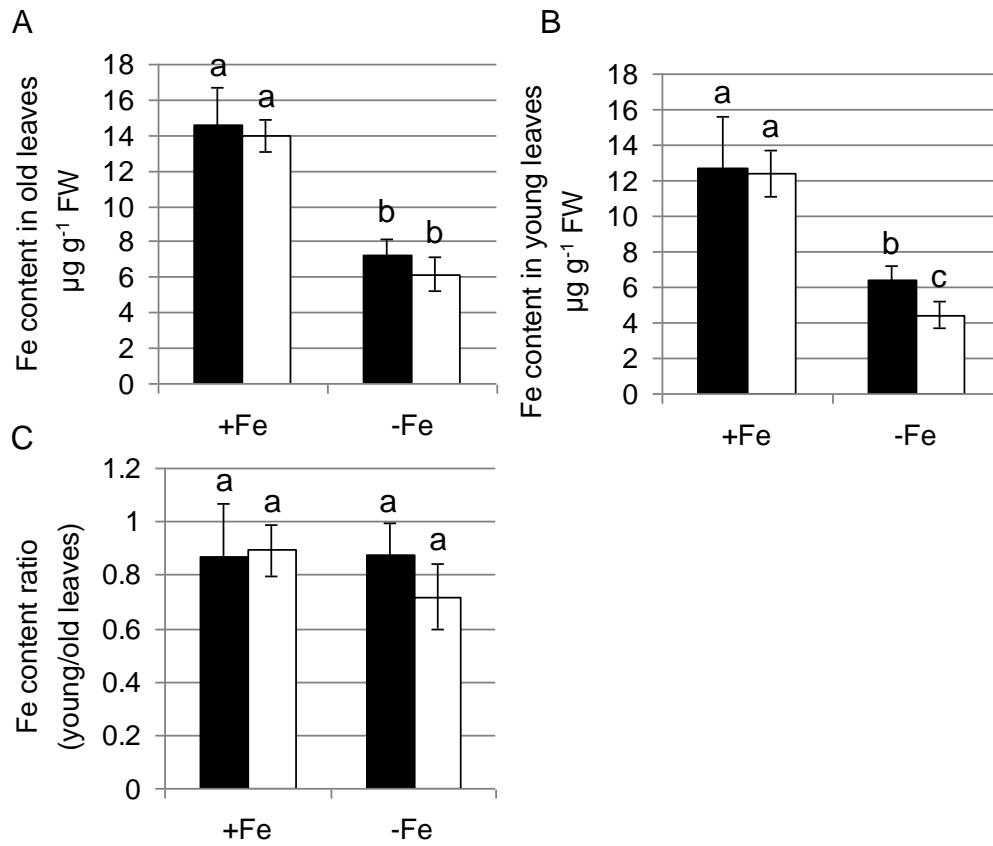


Figure S6. WRKY46 does not significantly affect old-to-young leaves Fe translocation. A and B, Fe content measured in old leaves (A) and young leaves (B) of WT and *wrky46-1* mutant plants with or without Fe-deficiency treatment for 6 days. C, The ratio of young leaves Fe content (in B) to old leaves Fe content (in A). Three independent experiments were done. Data were analyzed by one-way ANOVA (Analysis of Variance) following Duncan's test. Error bars with different letters represent a statistical difference ($P < 0.05$, Duncan's test).

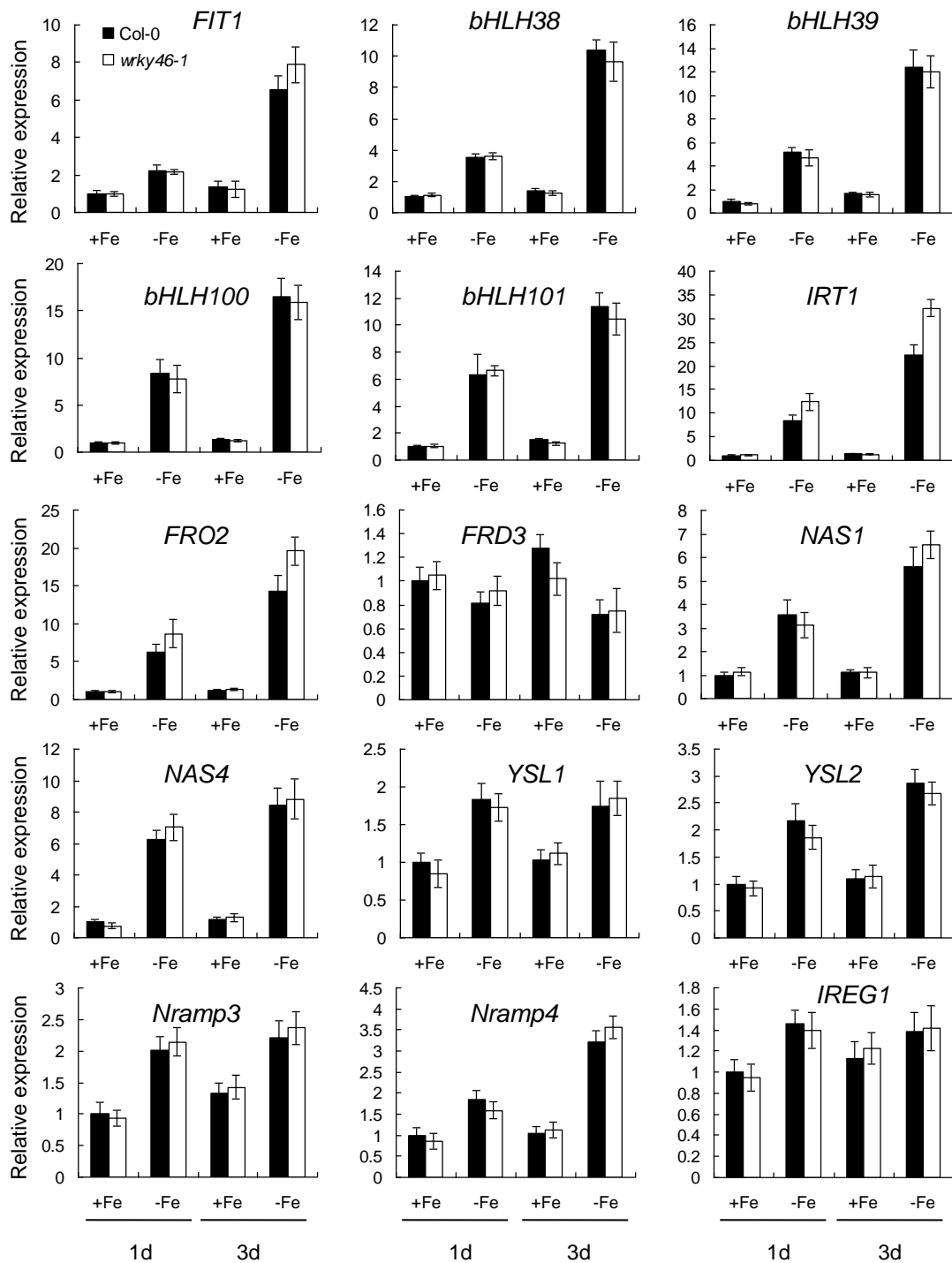


Figure S7. Expression analysis of Fe-deficiency responsive genes in WT and *wrky46-1* mutant plants. RNAs were extracted from roots of 6-week-old WT and *wrky46-1* mutant plants with or without Fe-deficiency treatment for indicated days. Gene expression was determined by RT-qPCR using *ACT* mRNA as internal reference. Three independent repeats were done with similar results. Error bars indicate SD (n=3).

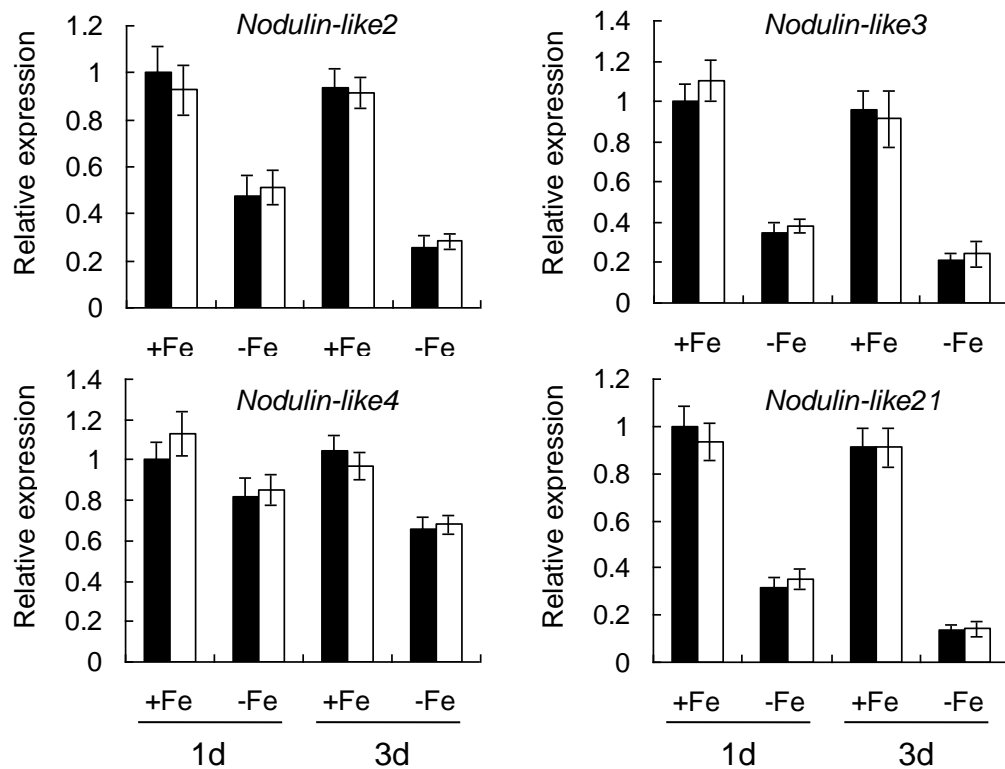


Figure S8. Expression of nodulin-like genes in WT and *wrky46-1* mutant plants. RNAs were extracted from roots of 6-week-old WT and *wrky46-1* mutant plants with or without Fe-deficiency treatment for indicated days. Gene expression was determined by RT-qPCR using *ACT* mRNA as internal reference. Three independent repeats were done with similar results. Error bars indicate SD (n=3).

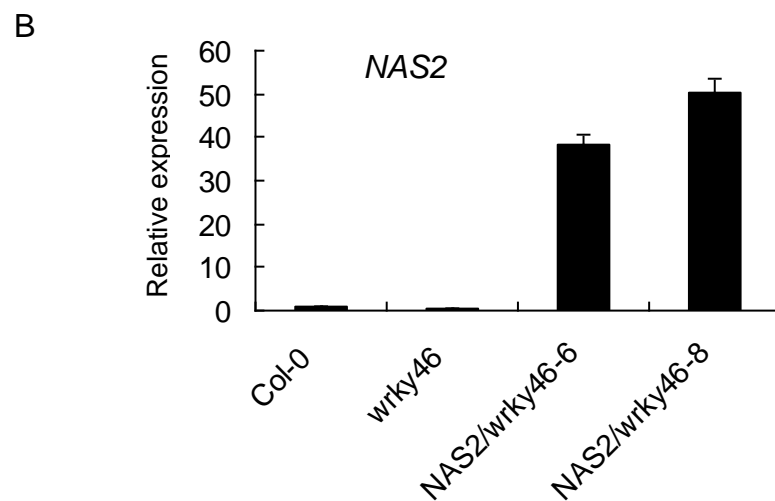
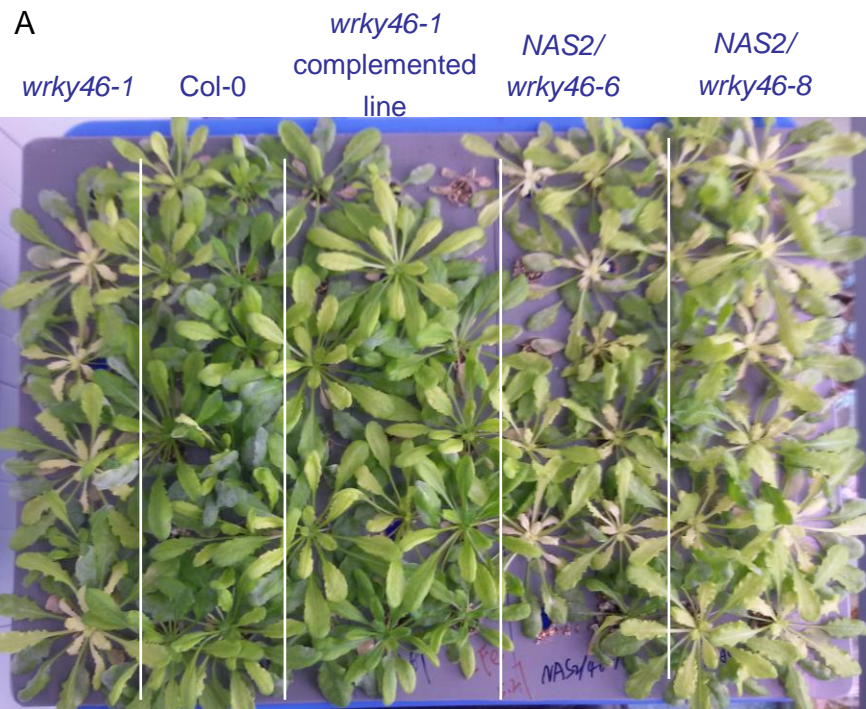


Figure S9. Increased expression of *NAS2* can't restore Fe-deficiency phenotype on *wrky46-1* mutant. A, Growth of 6-week-old indicated genotypes with or without Fe-deficiency treatment for 6 days. B, Expression of *NAS2* in roots of indicated genotypes. Three independent repeats were done with similar results. Error bars indicate SD (n=3).

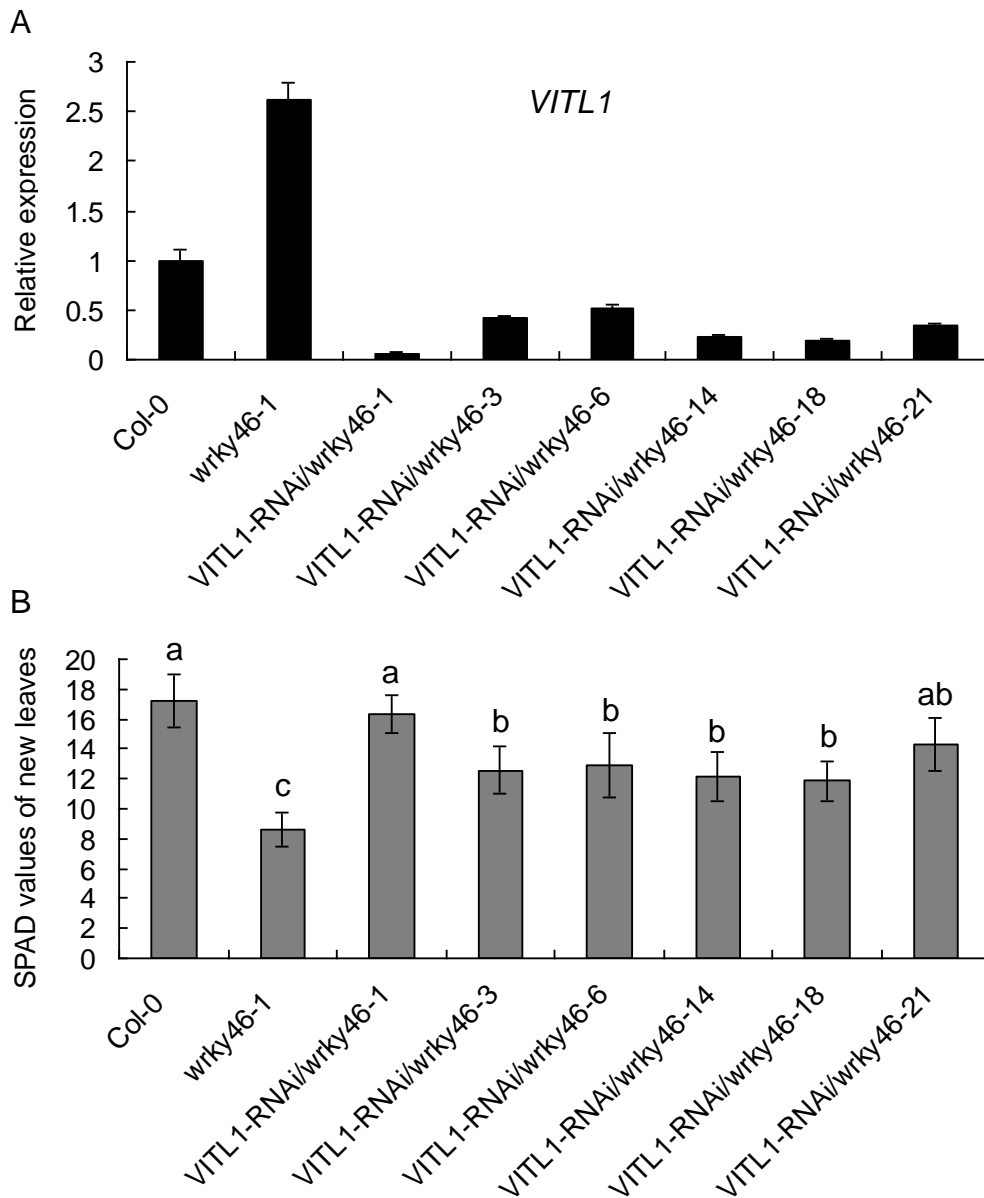


Figure S10. Lack of *VITL1* rescues *wrky46-1* sensitivity to Fe deficiency. A, Expression of *VITL1* in roots of indicated genotypes. B, SPAD values of new leaves from 6-week-old indicated genotypes with or without Fe-deficiency treatment for 6 days. Three independent experiments were done. Data were analyzed by one-way ANOVA (Analysis of Variance) following Duncan's test. Error bars with different letters represent a statistical difference ($P < 0.05$, Duncan's test).

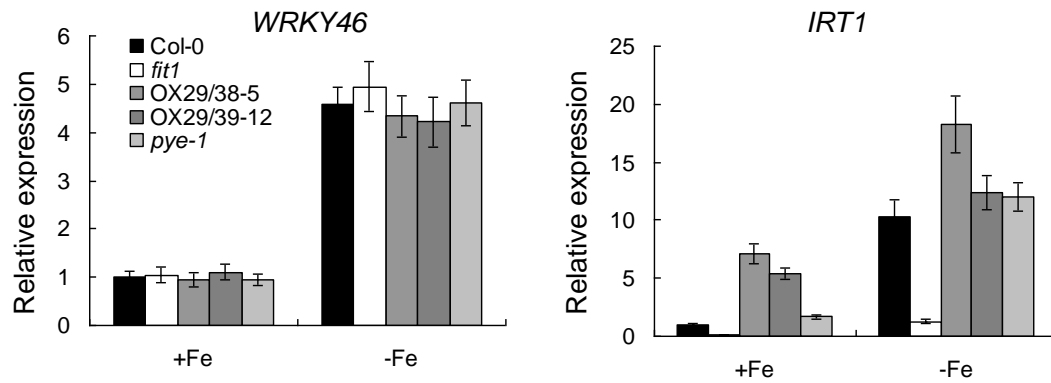


Figure S11. *WRKY46* expression is not regulated by FIT1 or PYE transcription factors. Expression of *WRKY46* and *IRT1* in 6-week-old WT, *fit1* loss-of-function mutant, the transgenic plants simultaneously overexpressing *FIT1* and *bHLH38* (OX29/38) or *FIT1* and *bHLH39* (OX29/39), and *pye-1* mutant plants with or without Fe-deficiency treatment for 3 days. Gene expression was determined by RT-qPCR using *ACT* mRNA as internal reference. Three independent repeats were done with similar results. Error bars indicate SD (n=3).

Table S1. Primers used in this study.

Name	Forward Primer	Reverse Primer	Process
<i>PYE</i>	LP- TTCAAGACCTCATTCACTGGC	RP- ATCGTCTGATGAAGCAAATGC	Mutant identification
<i>LB</i>	ATTTTGCCGATTCGGAAC		Mutant identification
<i>VITLRNAi1</i>	<u>ACTCGAGGGATCC-</u> GTTACTTTTGGTTTGACAAAAGTAT	<u>ACCGGTACC-</u> GACAGAATTTATATTAACACATTTGC	PCR cloning to pAGRIKOLA
<i>VITLRNAi2</i>	<u>ATCTAGAGGATCC-</u> GTTACTTTTGGTTTGACAAAAGTAT	<u>ACCATCGAT-</u> GACAGAATTTATATTAACACATTTGC	PCR cloning to pAGRIKOLA
<i>NAS2</i>	<u>AGGTACC</u> ATGGCTTGCGAAAACAACCT	<u>CGTCTAGA-</u> TTACTCGATGGCACTATACTCCTCG	PCR cloning to pCambia1301
<i>WRKY46</i>	<u>GCCATATG</u> ATGATGATGGAAGAGAAACTTGTG	<u>TTCTCGAG-</u> CTACGACCACAACCAATCCTG	PCR cloning for yeast one-hybrid
<i>WRKY46</i>	ACATCACATCCCCGAAGACG	ACTTCTTCGGAATTGGTCGG	Real-time qPCR
<i>VITL1</i>	ACAACGTGAGCAACAGCTTG	TGAAGCCGTCGAGACAAGAC	Real-time qPCR
<i>IRT1</i>	TCAAATGCACAGCTTTGCG	TCCAATGACCACCGAGTGAAC	Real-time qPCR
<i>FRO2</i>	TTGCTACCGCAATGGGATT	AGAGCTATCTCTCCGGCCAA	Real-time qPCR
<i>FIT1</i>	CCAACACCTGTCGATGACCT	TTCACCACCGGCTCTAACAC	Real-time qPCR
<i>bHLH38</i>	ACGGTGCCGGAGATAACCTA	GCTTCTTGACCACAACCGGA	Real-time qPCR
<i>bHLH39</i>	CCGTTCATGTCTTCTGCCT	GCCTTTGGTGGCTGCTTAAC	Real-time qPCR
<i>bHLH100</i>	CTCCCACCAATCAAACGAAGTT	ACGCATAGCTTGTAACCCCTT	Real-time qPCR
<i>bHLH101</i>	CGAGACCGCCGTAGAAAAGT	CGCTACCGTCATAGGAATGCT	Real-time qPCR
<i>FRD3</i>	TCATCTCAAGTTCACGTGACTACT	ATCTCTCGCCCAGTTGTGTC	Real-time qPCR
<i>NAS1</i>	TCCCACCAAGATTGCCTTC	TTGAAGCGAGTGTGTTTGGC	Real-time qPCR
<i>NAS2</i>	CGGTCCGATGCCACTTACTT	TTTGAAGCGAGTGTGTTGGC	Real-time qPCR
<i>NAS4</i>	CTTCCGTCGTTCTTGCCCTCT	TTGCTGATGGTTCGATGTC	Real-time qPCR
<i>YSL1</i>	TGGCTCCACACACAAGGAG	ACCACTGGAAGAAACCCAC	Real-time qPCR
<i>YSL2</i>	CTAACGATTTGGGGCCAGGT	GATCGCTCGAACCGTGATCT	Real-time qPCR
<i>NRAMP3</i>	GGGATTTTGCCTCTTTGGGC	GAACTGCAAACACAGCCTCG	Real-time qPCR
<i>NRAMP4</i>	GAGATAGCGGACACCATCGG	GGAAAAACCCACCCCGTAT	Real-time qPCR
<i>IREG1</i>	AAACCCACCACCAGCCTTAC	CCCATGTTCTGGCACTCCAT	Real-time qPCR
<i>Nodulin-L2</i>	TTCGGATGGTTAGGAGCAGC	AGCCAAACGTAACCGCCATA	Real-time qPCR
<i>Nodulin-L3</i>	CAGCATCTGCGTTAGCGTTTT	GTAACCGCTGCCACTATTGC	Real-time qPCR
<i>Nodulin-L4</i>	CGGTTTTGCCGTTTAGTGG	TTTCTTTGTCTCCCTCCGC	Real-time qPCR
<i>Nodulin-L21</i>	CCAACGATGGTCTGGTCACA	GGGTGCACACAGACACAAC	Real-time qPCR
<i>ACT</i>	GACCTTTAACTCTCCCGCTA	GGAAGAGAGAAACCTCGTA	Real-time qPCR
<i>VF1</i>	TGCAGTGTGTGAATCATAGGG	TGCATGCATACGTAGTCGCC	ChIP-qPCR
<i>VF2</i>	GTGTCCGTTTATCAACAAGGAGC	GTTTGACCAAAAAGTATCAACGG	ChIP-qPCR
<i>VF3</i>	ACCAACCACACTCCGATCAAA	CATTCTTTTGGCAGCTGTACT	ChIP-qPCR
<i>VF4</i>	TTTACCGATGGTACGCGGC	TGGGGAGCATCATCACCATA	ChIP-qPCR
<i>FIT1</i>	ATCCCAAAAATCCAATGTTGCTCA	GTCTTGGCCGGTTAGGACAAA	ChIP-qPCR