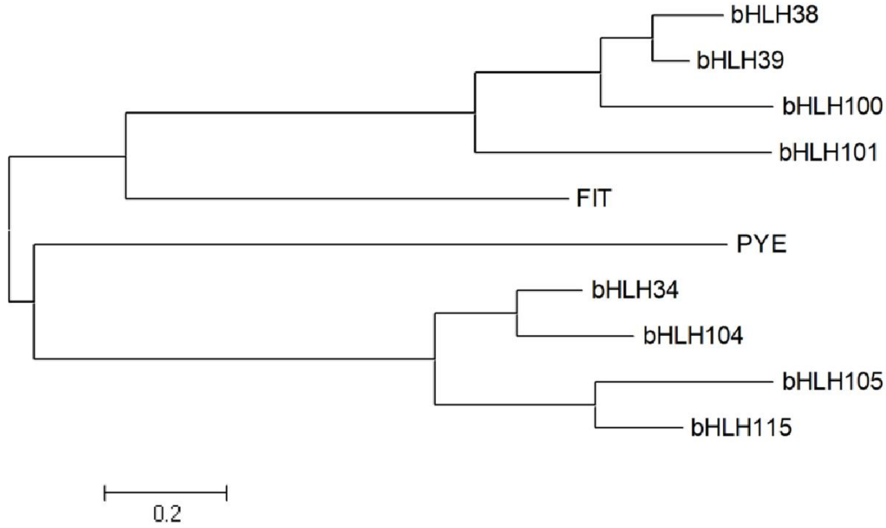
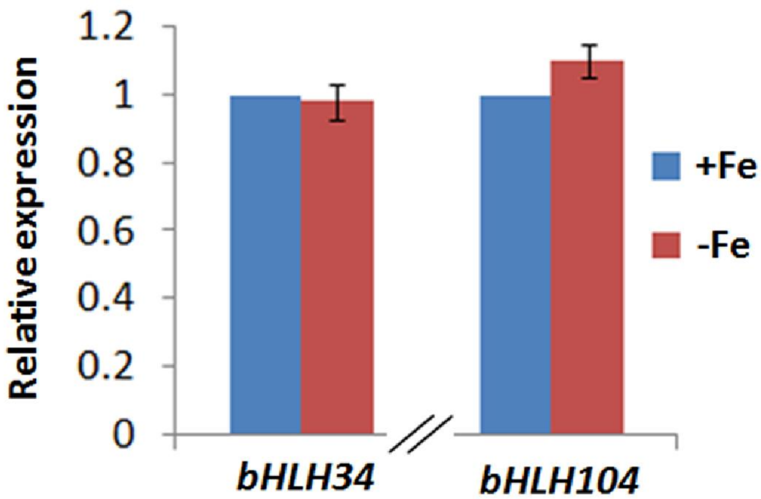
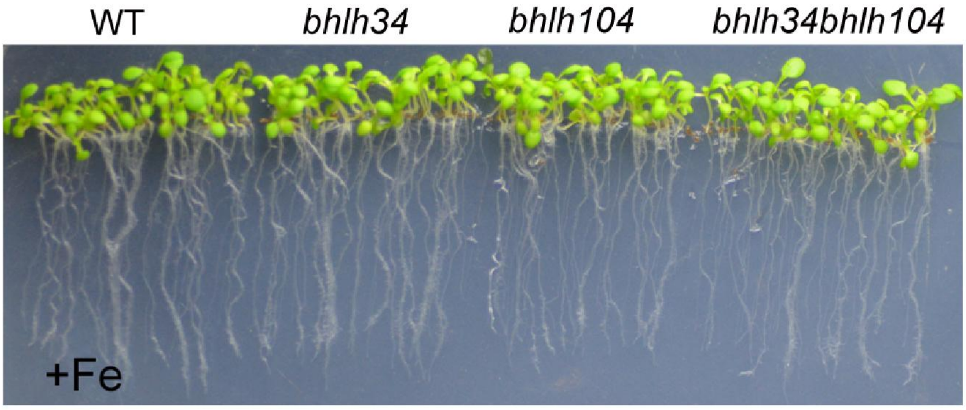


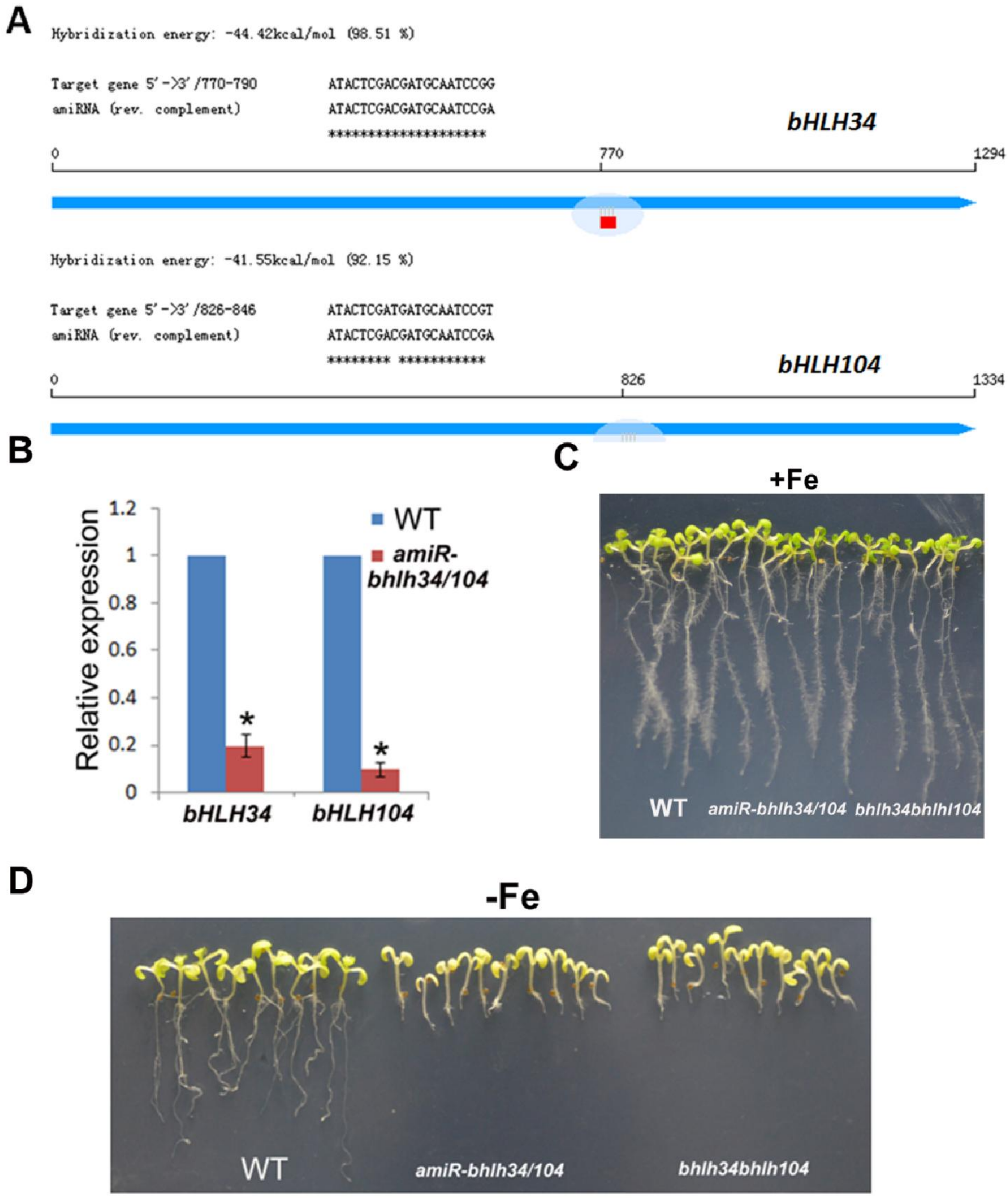
Supplemental Figure 1. Phylogenetic tree of bHLH proteins involved in Fe homeostasis. The protein sequences were retrieved from TAIR (The Arabidopsis Information Resource). Sequences were aligned by Clustal X1.5. The phylogenetic tree was constructed by MEGA 3.1.



Supplemental Figure 2. Expression of *bHLH34* and *bHLH104* in response to Fe deficiency. 10-day-old wild type seedlings grown on +Fe media were transferred to +Fe media or –Fe media for 3 days. Roots were harvested for the extraction of RNA and qRT-PCR. Values are means (±SD) of three technical repeats from one of three biological repeats.



Supplemental Figure 3. Growth status of wild-type and mutant plants. 10-day-old seedlings grown on +Fe media.



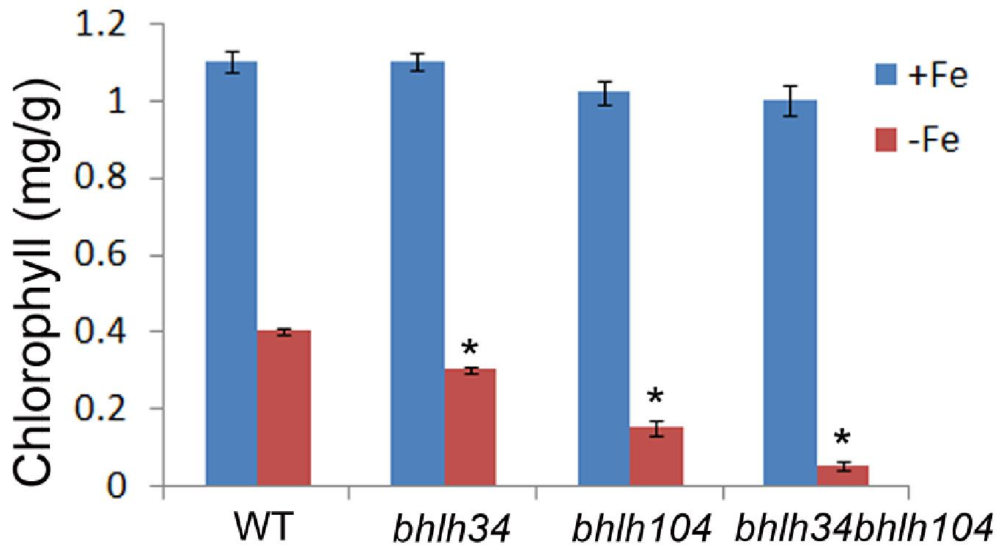
Supplemental Figure 4. Analysis of *amiR-bhlh34/104* and double mutant plants.

(A) The target site of *amiR-bhlh34/104* in *bHLH34* and *bHLH104.*

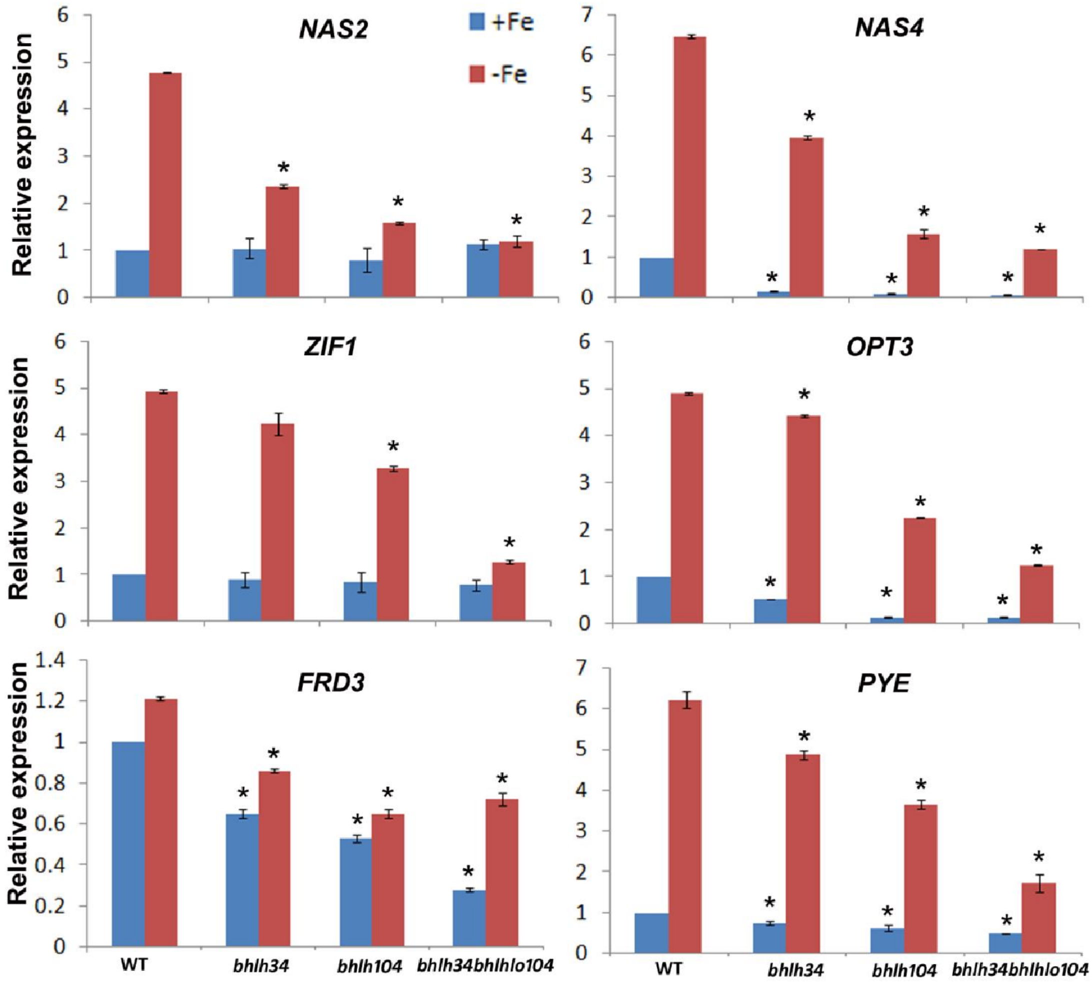
(B) Relative expression levels of *bHLH34* and *bHLH104* in *amiR-bhlh34/104* plants. Significant differences from the wild type are indicated by * ($P < 0.05$).

(C) 10-day-old seedlings grown on +Fe media.

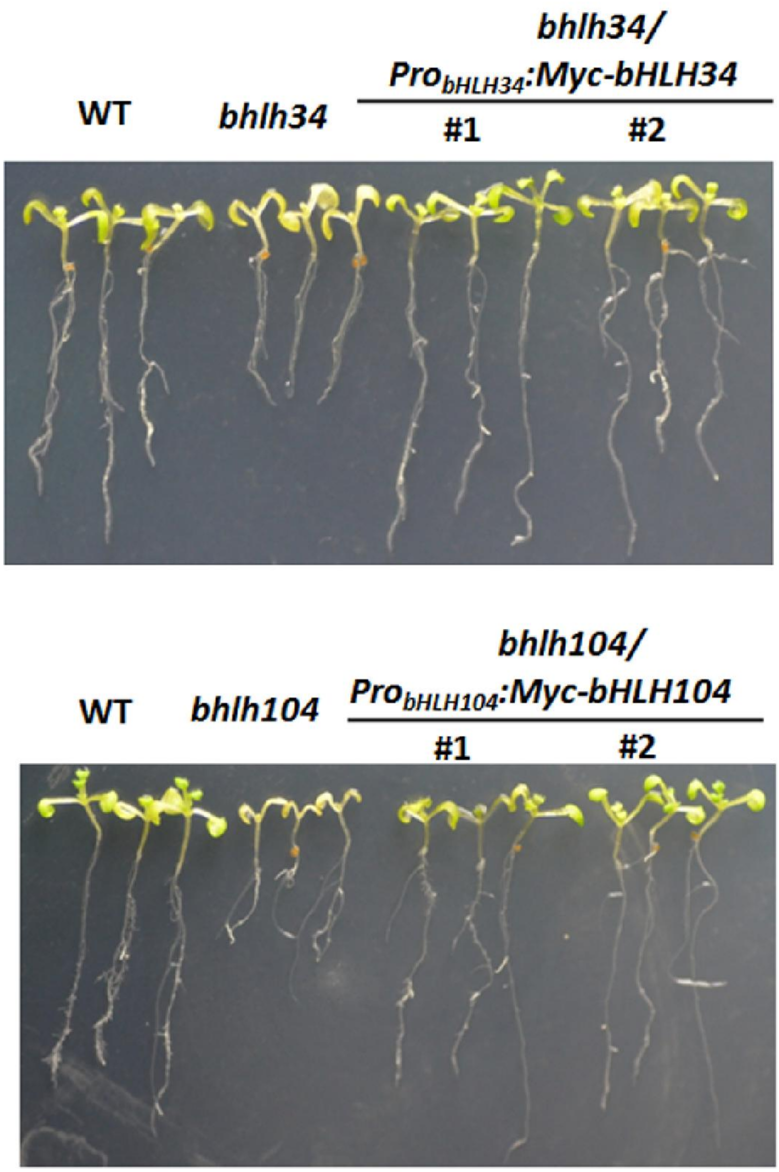
(D) 10-day-old seedlings grown on -Fe media.



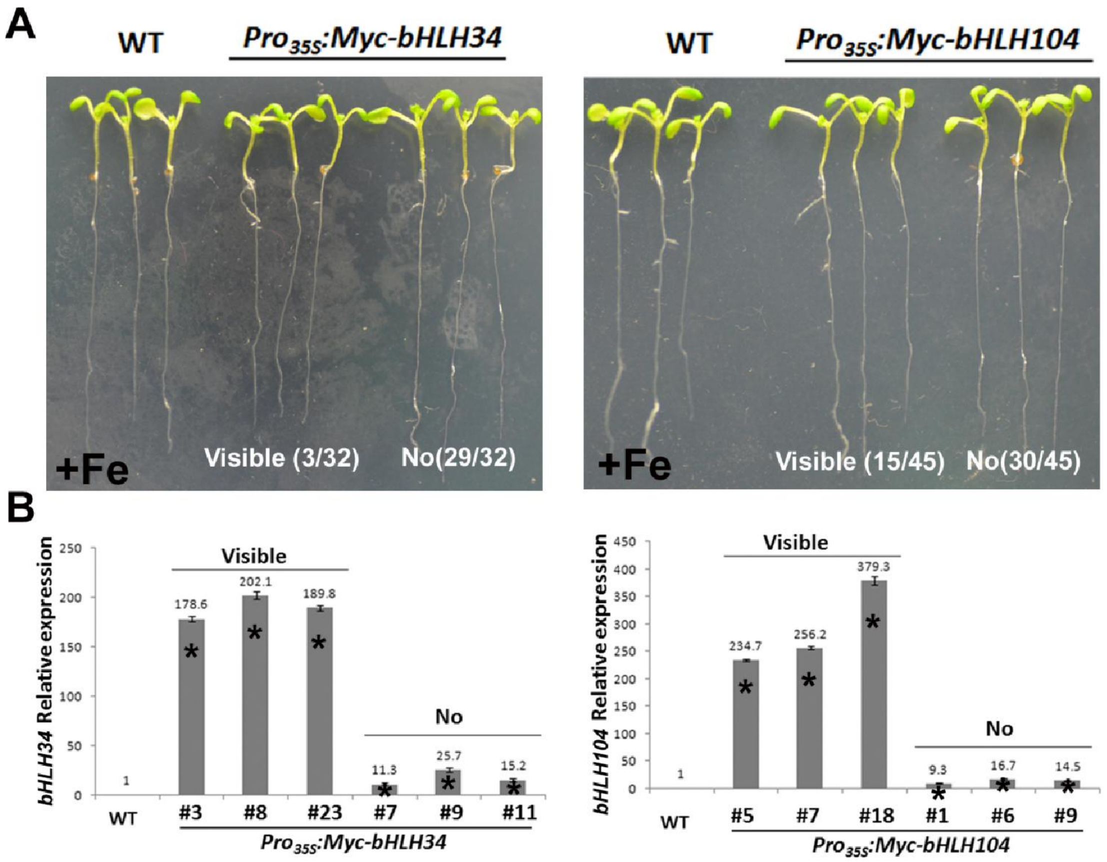
Supplemental Figure 5. Chlorophyll content of mutant seedlings on +Fe or –Fe media. Values are means (±SD) of three technical repeats from one of three biological repeats. Significant differences from the wild type are indicated by * ($P < 0.05$).



Supplemental Figure 6. *NAS2*, *NAS4*, *ZIF1*, *FRD3*, *OPT3* and *PYE* transcript levels in various mutant plants. Wild-type and mutant plants were grown on +Fe media for 10 days, then transferred to +Fe or –Fe media for 3 days. RNA was prepared from root tissues. The data represent means (±SD) of three technical repeats from one of three biological repeats. Significant differences from the wild type are indicated by * ($P < 0.05$).



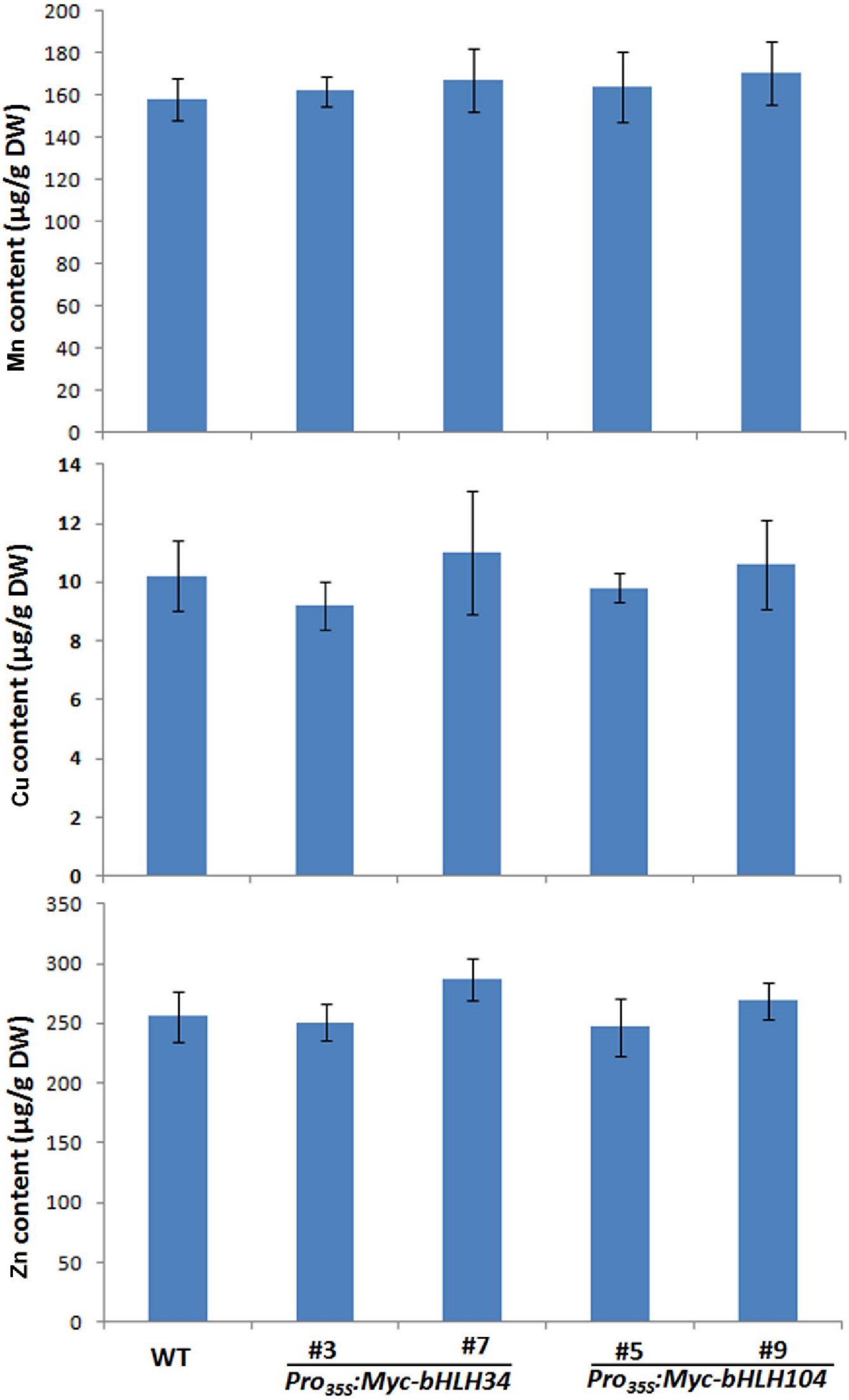
Supplemental Figure 7. Complementation of *bhlh34* and *bhlh104* mutants. 10-day-old seedlings grown on –Fe media were shown.



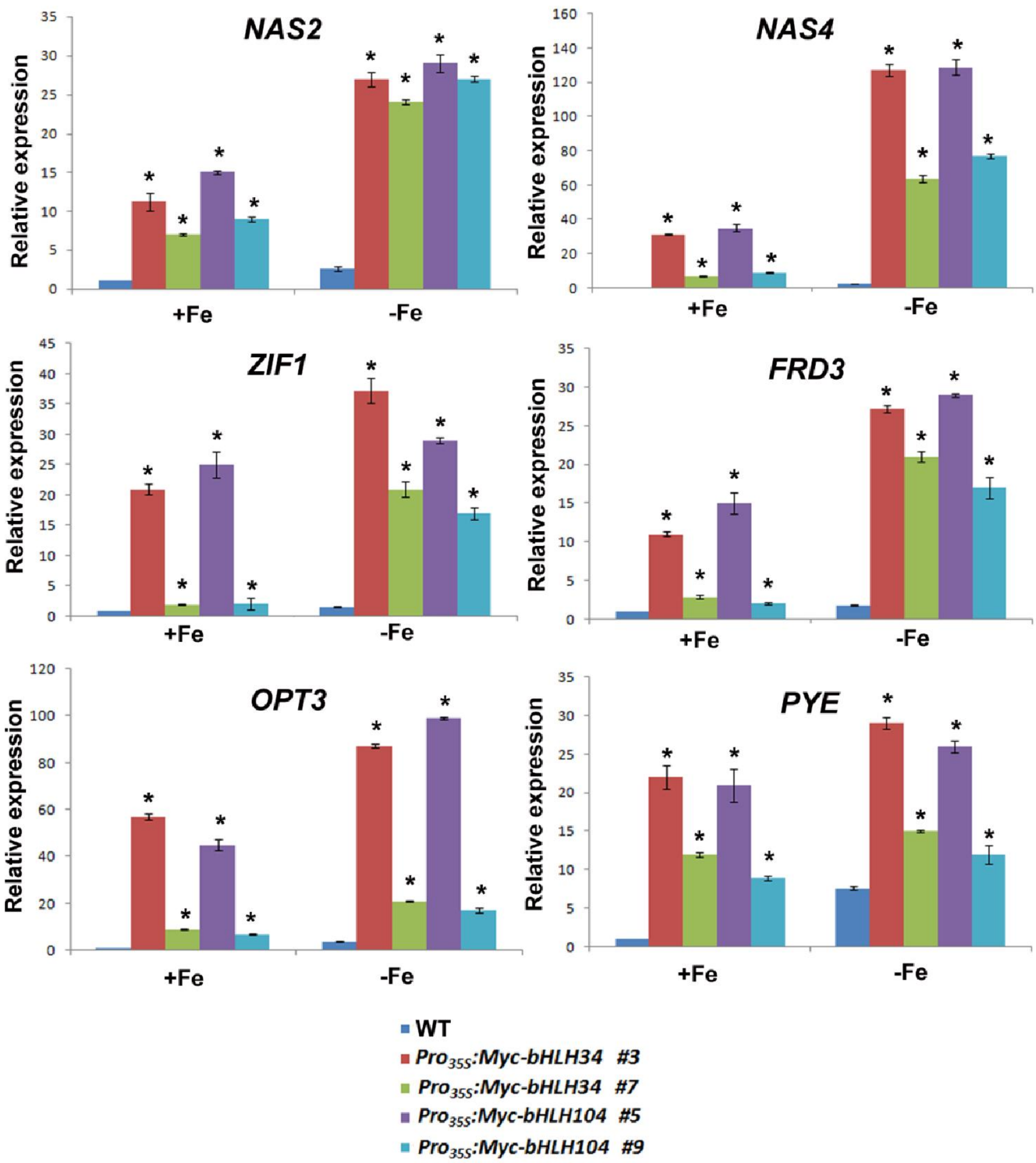
Supplemental Figure 8. Analysis of *bHLH34* and *bHLH104* overexpression plants.

(**A**) 10-day-old seedlings germinated directly on +Fe media. “Visible’ indicates the plants with visible phenotypes (short roots and leaf chlorosis) in normal soils or on –Fe media. “No” indicates the plants with phenotypes similar to wild type on in normal soils or –Fe media.

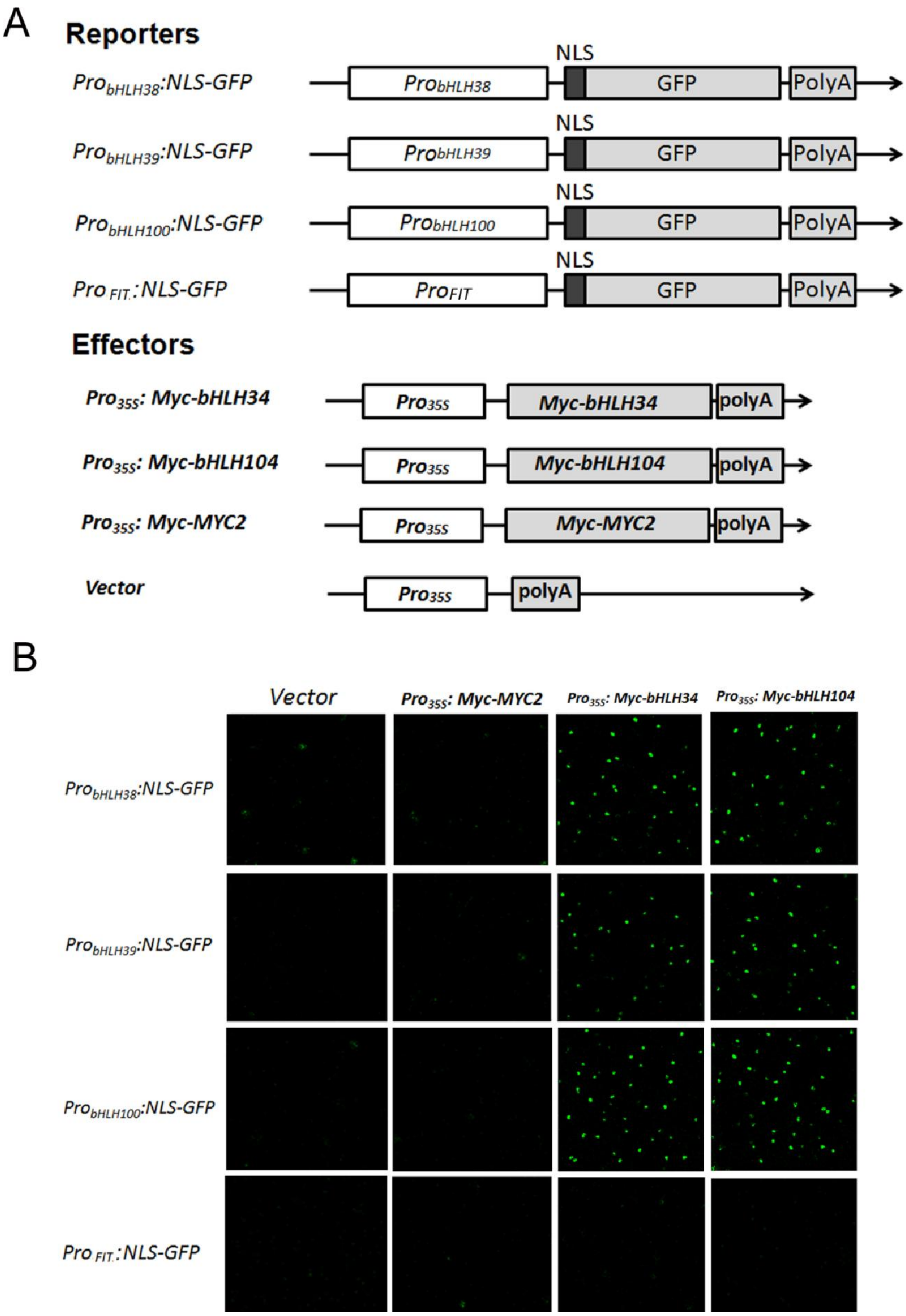
(**B**) Relative expression of *bHLH34* and *bHLH104* in overexpression plants. RNA was prepared from shoot tissues of 10-day-old seedlings grown on +Fe media. The data represent means (±SD) of three technical repeats from one of three biological repeats. Significant differences from the wild type are indicated by * ($P < 0.05$).



Supplemental Figure 9. Concentration of other metals in overexpression plants. Leaves of 4-week-old plants grown in normal soils were used for metal content measurement.



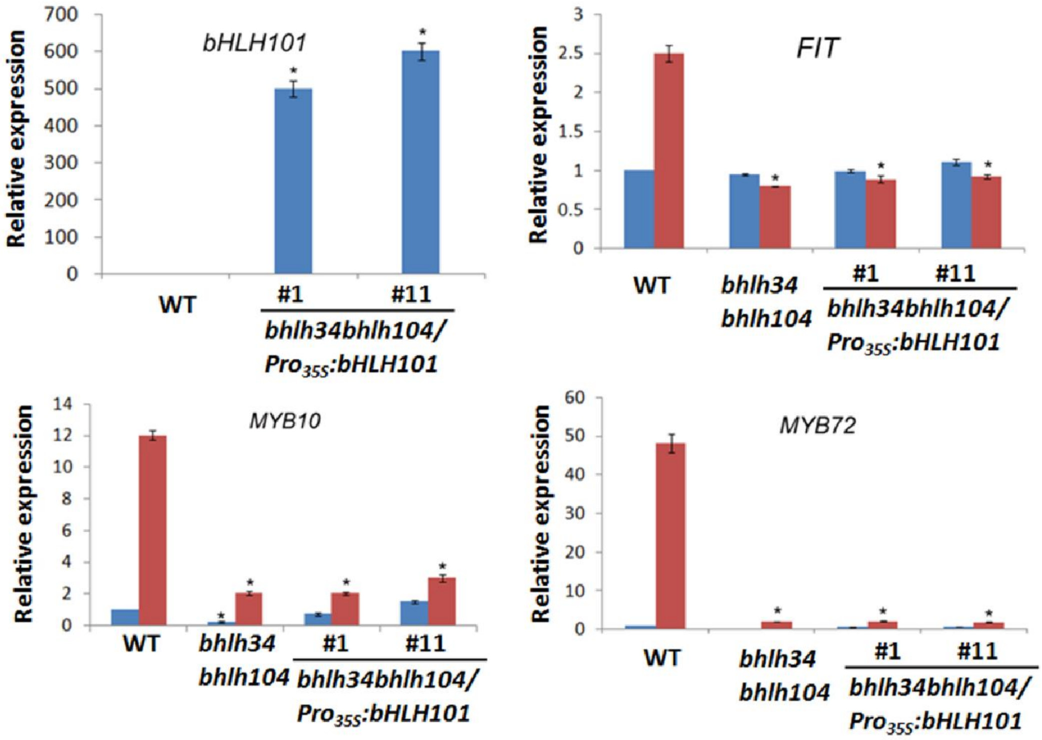
Supplemental Figure 10. *NAS2*, *NAS4*, *ZIF1*, *FRD3*, *OPT3* and *PYE* transcript levels in overexpression plants. Wild-type and overexpression plants were grown on +Fe media for 10 days, then transferred to +Fe or –Fe media for 3 days. RNA was prepared from root tissues. The data represent means (± SD) of three technical repeats from one of three biological repeats. Significant differences from the wild type are indicated by * ($P < 0.05$).



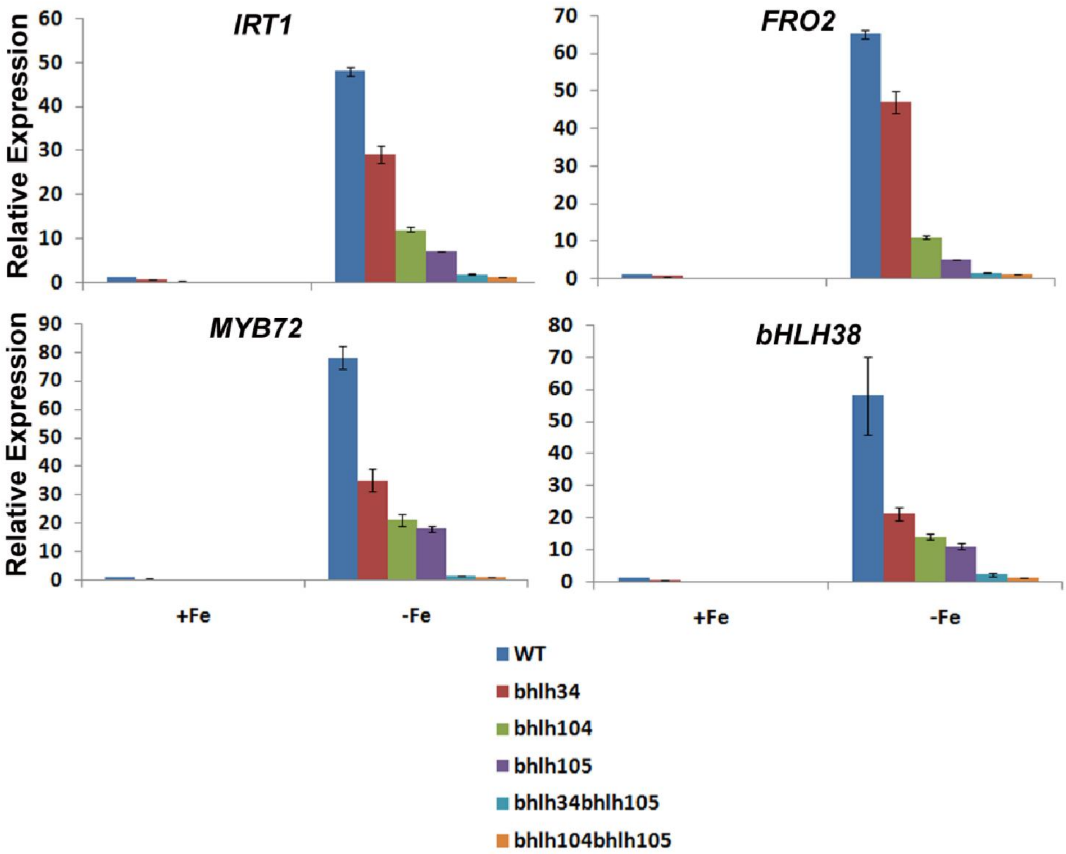
Supplemental Figure 11. bHLH34 and bHLH104 activate the promoter of *bHLH38/39/100*.

(A) Schematic representation of the constructs used for transient expression assays. The reporter construct consists of *bHLH38/39/100/FIT* promoter, NLS sequence fused with *GFP* coding sequence, and ployA terminator. Effector constructs express *Myc-bHLH34, Myc-bHLH104*, or *Myc-MYC2* under the control of the CaMV 35S promoter.

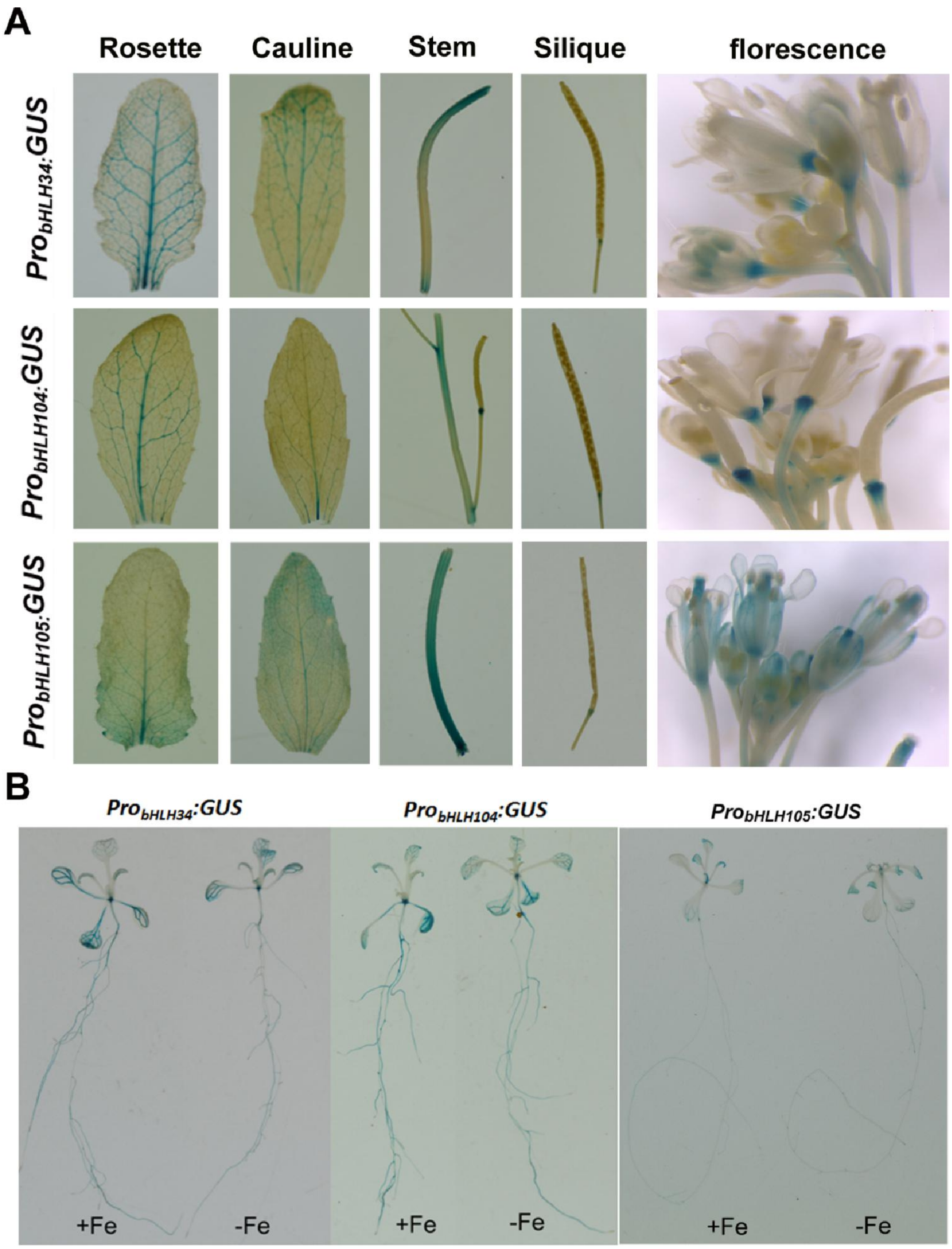
(B) bHLH34 and bHLH104 activate the promoter of *bHLH38/39/100* in transient expression assays. The results are one representative of three biological repeats



Supplemental Figure 12. Analysis of *bhlh34bhlh104/Pro*$_{35S}$*:bHLH101* Plants.
(**A**) Transcript levels of *bHLH101* in *bhlh34bhlh104/Pro*$_{35S}$*:bHLH101* Plants.
(**B**) (**C**) and (**D**) Transcript levels of *FIT*, *MYB10* and *MYB72* in *bhlh34bhlh104/Pro*$_{35S}$*:bHLH101* Plants. The data represent means (±SD) of three technical repeats from one of three biological repeats. Significant differences from the wild type are indicated by * ($P < 0.05$).



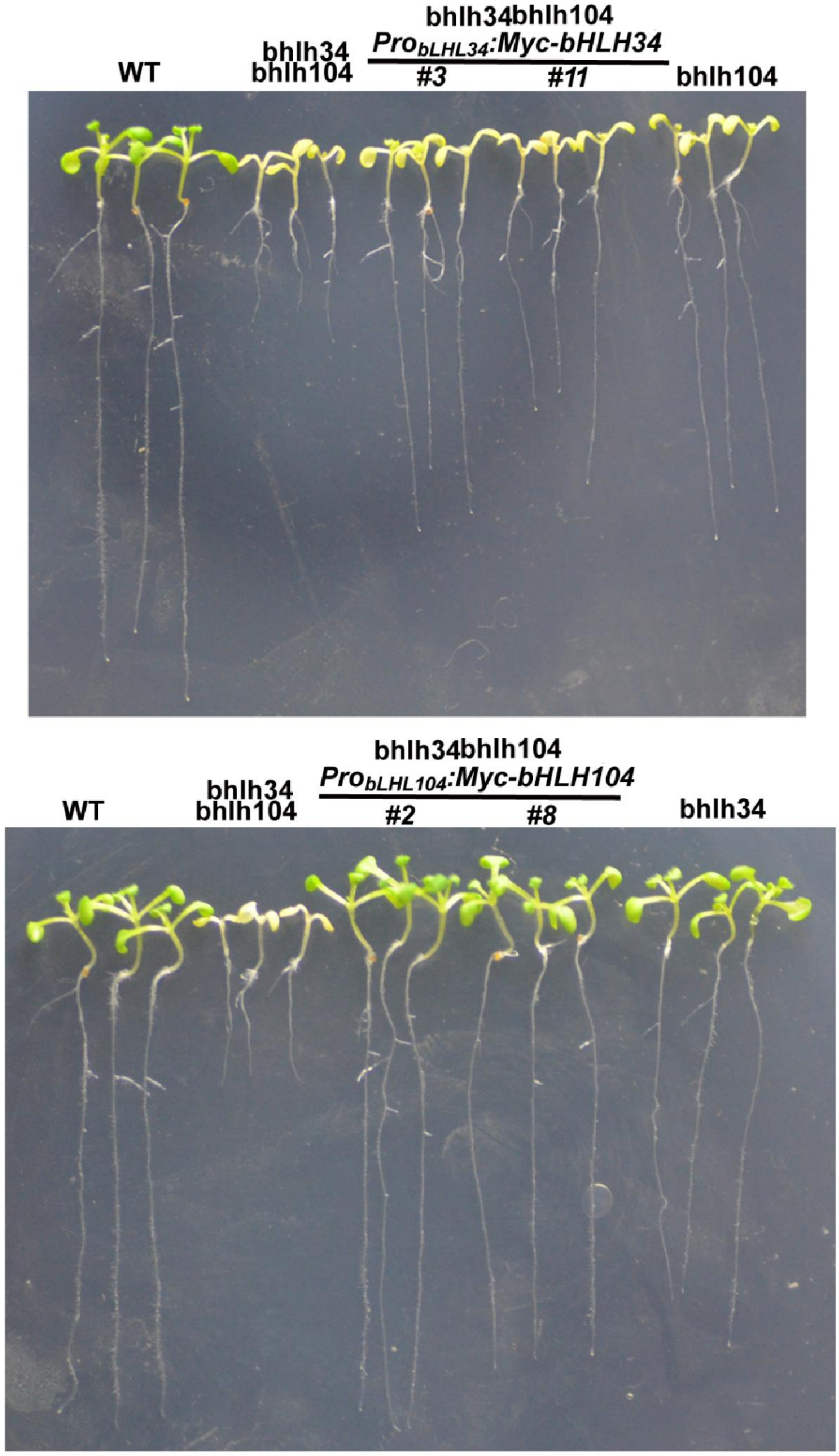
Supplemental Figure 13. Expression of Fe-deficient responsive genes in various single and double mutants. Wild-type and mutant plants were grown on +Fe media for 10 days, then transferred to +Fe or –Fe media for 3 days. RNA was prepared from root tissues.



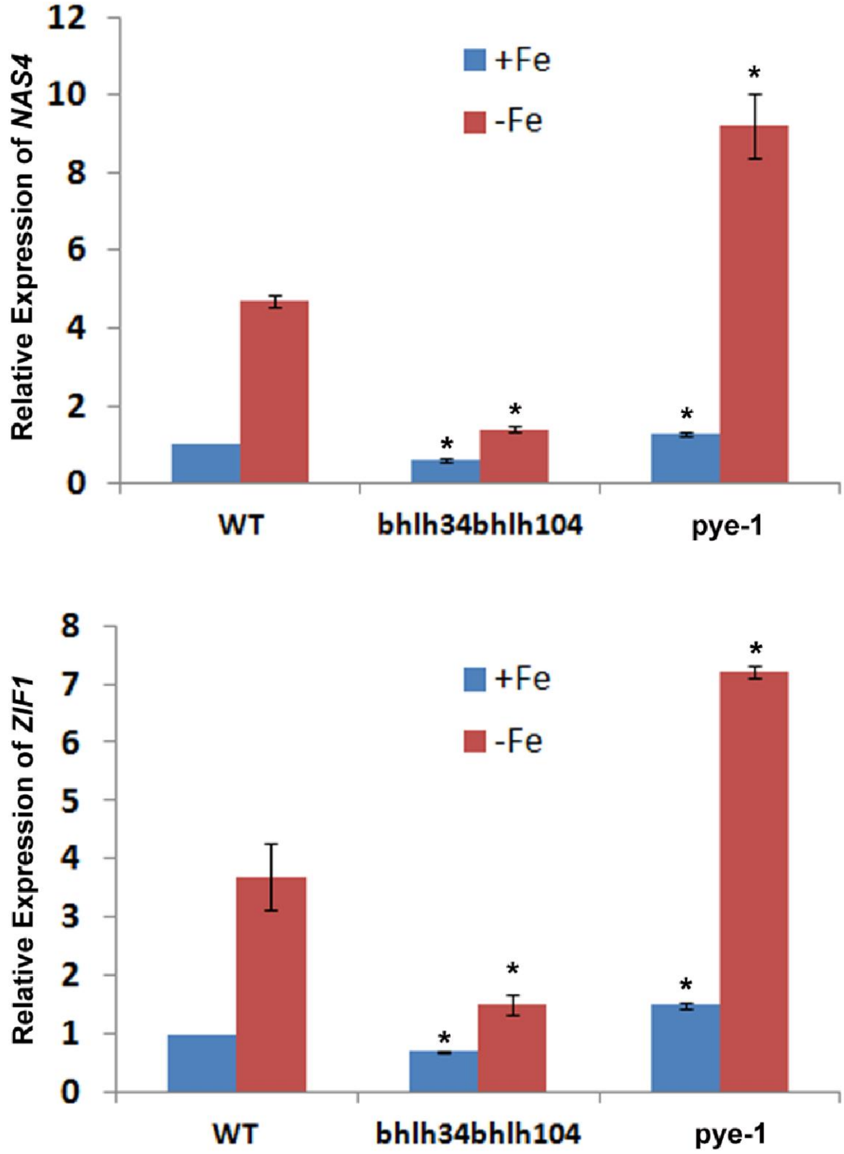
Supplemental Figure 14. GUS staining of *Pro$_{bHLH34}$:GUS*, *Pro$_{bHLH104}$:GUS* and *Pro$_{bHLH105}$:GUS* plants.

(**A**) GUS staining of rosette leaves, cauline leaves, stems, siliques and inflorescences was shown.

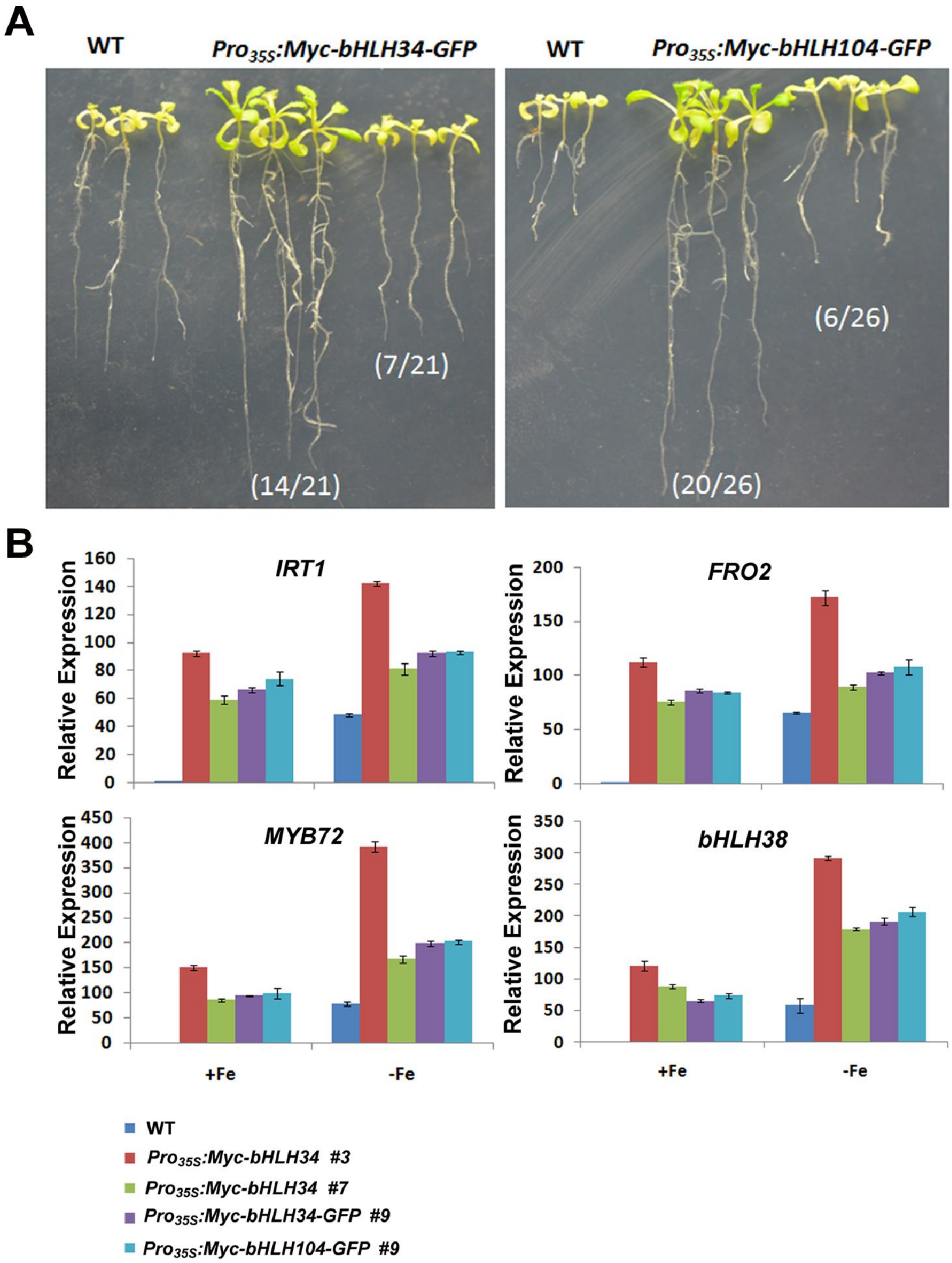
(**B**) GUS staining of seedlings treated with +Fe or –Fe for three days. Two-week-old seedlings grown on +Fe media were transferred to +Fe media or –Fe media for 3 days. The whole seedlings were used for GUS staining.



Supplemental Figure 15. Partially complementation of *bhlh34blhlh104* double mutants by *Prob*$_{HLH34}$*:Myc-bHLH34* and *Pro*$_{bHLH104}$*:Myc-bHLH104*. 10-day-old seedlings grown on –Fe media were shown.



Supplemental Figure 16. Expression of *NAS4* and *ZIF1* in *bhlh34blhlh104* and *pye-1* mutants. The data represent means (±SD) of three technical repeats from one of three biological repeats. Significant differences from the wild type are indicated by * ($P < 0.05$).



Supplemental Figure 17. Phenotypes of *Pro$_{35S}$:Myc-bHLH34-GFP* and *Pro$_{35S}$:Myc-bHLH104-GFP* plants.

(A)10-day-old seedlings grown on –Fe media were shown. The number indicates the frequency of transgenic plants with the corresponding phenotypes.

(B) Expression of Fe-deficient responsive genes in different transgenic plants. Plants were grown on +Fe media for 10 days, then transferred to +Fe or –Fe media for 3 days. RNA was prepared from root tissues.

Supplemental Table 1. Primers used in this paper.

Mutant screening	
bhlh34-A	TGGAAATCTTGAGCAAGTTTGT
bhlh34-B	ATCACATCAAACAACGAAATGG
bhlh104-A	TGAATCTGTGATGGTTGTTGATAGG
bhlh104-B	GGAAGAATCCTAAACCAAGTGATTC
bhlh105-A	CATGAATACTTGCCACTATACCGA
bhlh105-B	CAGGCACACACAGTTGACTTTAGAT
Promoter	
ProbHLH34-F	TATGTCGACACCAAAAGATACTCTCAGCCTCTCAC
ProbHLH34-R	TATGGATCCCAGAAGAAGAACAGAGAATGCAGAAC
ProbHLH104-F	TATGTCGACGTTTTCTTTAATAATCAGTAACAGCTACGT
ProbHLH104-R	TATGAGCTCTTGAGAGTCTCCAAACAAATTTGAG
ProbHLH105-F	TTTGGATCCATGGCAAAATTCATGCCTTCA
ProbHLH105-R	TTTGAGCTCTCCGGAAACTTCCACCGGT
ProbHLH38-F	ATAGTCGACTTAATTTTCGCCTAGACTTTGAATG
ProbHLH38-R	ATAGGATCCTTTTTGCTTAATCAAGGACAAGGT
ProbHLH39-F	ATAGGATCCATGAAGTGAAAGATGAGCGGC
ProbHLH39-R	ATAGAGCTCTTTTGCTTACTAAGGACAAGTATTGTAGA
ProbHLH100-F	ATAGTCGACGGAAATTTAGAGTTGAACCTAGGCA
ProbHLH100-R	ATAGGATCCTTTGAGTTTTAGATAGTTACTCTGTTCTTT
ProbHLH101-F	ATAGTCGACAAGTGGCCGATGAATGATGTG
ProbHLH101-R	ATAGGATCCTGTTGAAGCAGAGTACCATTCTTG
ProFIT-F	ATAGTCGACCGGTTTTCAAAATTGTAACCGATA
ProFIT-R	ATAGGATCCTTGTGTGTTTTTGTCAATGAAGAAA
Overexpression	
bHLH34-Myc-F	ATACCCGGGATGGAGGAGCAGAAGCTGATCTCAGAGGAGGACCTGtatccatcaatcgaagacgatg
bHLH34-Flag-F	ATACCCGGGATGGACTACAAAGACGATGACGACAAGtatccatcaatcgaagacgatg
bHLH34-R	ATAGGATCCAGCAACAGGAGGAAGATTTTTG
bHLH104-Myc-F	ATACCCGGGATGGAGGAGCAGAAGCTGATCTCAGAGGAGGACCTGTATCCTTCTCTCGACGATGATT
bHLH104-R	AAAGTCGACAAACAATTGAGAGTTTAAGCAGCA
MYC2-Myc-F	AACCCGGGATGGAGGAGCAGAAGCTGATCTCAGAGGAGGACCTGATGACTGATTACCGGCTACAACC
MYC2-R	TTACTAGTTAACCGATTTTTGAAATCAAACTTG
bHLH101-F	ATAGGATCCATGGAGTATCCATGGCTGCAG
bHLH101-R	ATAGTCGACTTATGATTGGCGTAATCCCAAG
BiFC	
YN-bHLH34-F	ATAATTTAAATATGTATCCATCAATCGAAGACGATG
YN-bHLH34-R	ATAGGATCCAGCAACAGGAGGAAGATTTTTG
YC-bHLH104-F	ATATCTAGAATGTATCCTTCTCTCGACGATGATT
YC-bHLH104-R	ATAGGATCCAGCAGCAGGAGGCCTGA
Yeast	
BD-bHLH34-F	ATAGAATTCGGTGTAGTGGAAATCAATTCTTCGT
BD-bHLH34-R	ATAGGATCCTTAAGCAACAGGAGGAAGATTTTTG
AD-bHLH34-F	ATAGAATTCatgtatccatcaatcgaagacgatg

AD-bHLH34-R	ATAGGATCCtTaagcaacaggaggaagatttTtg
BD-bHLH104-F	ATAGAATTCGACGATTGCTCCAGAAAGCG
BD-bHLH104-R	ATAGGATCCtTaagcaGcaggaggCCtGA
AD-bHLH104-F	ATAGAATTCATGTATCCTTCTCTCGACGATGA
AD-bHLH104-R	ATAGGATCCTTAAGCAGCAGGAGGCCTGA
BD-bHLH105-F	TTTGAATTCATGGTGTCACCCGAAAACG
BD-bHLH105-R	TTTGTCGACTTAAGCAACAGGAGGACGAAG
AD-bHLH105-F	TTTGAATTCATGGTGTCACCCGAAAACG
AD-bHLH105-R	TTTGTCGACTTAAGCAACAGGAGGACGAAG
qRT-PCR	
qbHLH34-F	TCGTCATCTGTTGGAGCTGT
qbHLH34-R	GTTTCTCGCGACAGGCTTTG
qbHLH104-F	CAGAGGAGGAGGAACTAAAGCG
qbHLH104-R	CGGTTTATCAGTCTTAGGAGTCCTC
qIRT1-F	GCCCCGCAAATGATGTTACC
qIRT1-R	TCCAATGACCACCGAGTGAA
qFRO2-F	ATCGAAAGTCGCCACACCAT
qFRO2-R	GAGCCACAAACATCGCCAAG
qMYB10-F	GGGGAAATCTTGGTGGAGCA
qMYB10-R	AGGAGGAACCTGGCTATCGT
qMYB72-F	TCGAGAGGTAACCAAATCGCA
qMYB72-R	CAGCTGTCTCCTCAAGTCGG
qbHLH38-F	ACGGTGCCGGAGATAACCTA
qbHLH38-R	GTCGGTCACGTTCACTAGCA
qbHLH39-F	CCGTTCATGTCTTCCTGCCT
qbHLH39-R	GCCTTTGGTGGCTGCTTAAC
qbHLH100-F	CTCCCACCAATCAAACGAAGAAG
qbHLH100-R	TGTTTTGGTCGGTGTAAACGAG
qbHLH101-F	AAGAAGATCGAGGAGCGGTG
qbHLH101-R	AGAGGCAAGAGAGCACGAAG
qFIT-F	CCAACACCTGTCGATGACCT
qFIT-R	TTCACCACCGGCTCTAACAC
qNAS2-F	CGACGTGGTTAATTCGGTGG
qNAS2-R	CATAACCACACACCGTCCGA
qNAS4-F	CTTCCGTCGTTCTTGCCTCT
qNAS4-R	TTCGCTGATGGGTCGATGTC
qZIF1-F	CGATATGCTGGGGCACTGAT
qZIF1-R	CCGGTTATGGCAGACACACT
qFRD3-F	TTGAGCATACGAGCTTTGGG
qFRD3-R	GAAGATGGGGTCGAGAACTATG
qOPT3-F	AAGCTTACTATAAACAGAGCCTTAGCTT
qOPT3-R	ACAGGATCAACAAGGTACCTCCTC
qPYE-F	CAGGACTTCCCATTTTCCAAG
qPYE-R	CTTGTGTCTGGGGATCAGGTT

qACT2-F	TGTGCCAATCTACGAGGGTTT
qACT2-R	TTTCCCGCTCTGCTGTTGT
qGFP-F	CACTACCAGCAGAACACCCC
qGFP-R	GAACTCCAGCAGGACCATGT
qGUS-F	TACGGCGTGGATACGTTAGC
qGUS-R	GGTCGCAAAATCGGCGAAAT