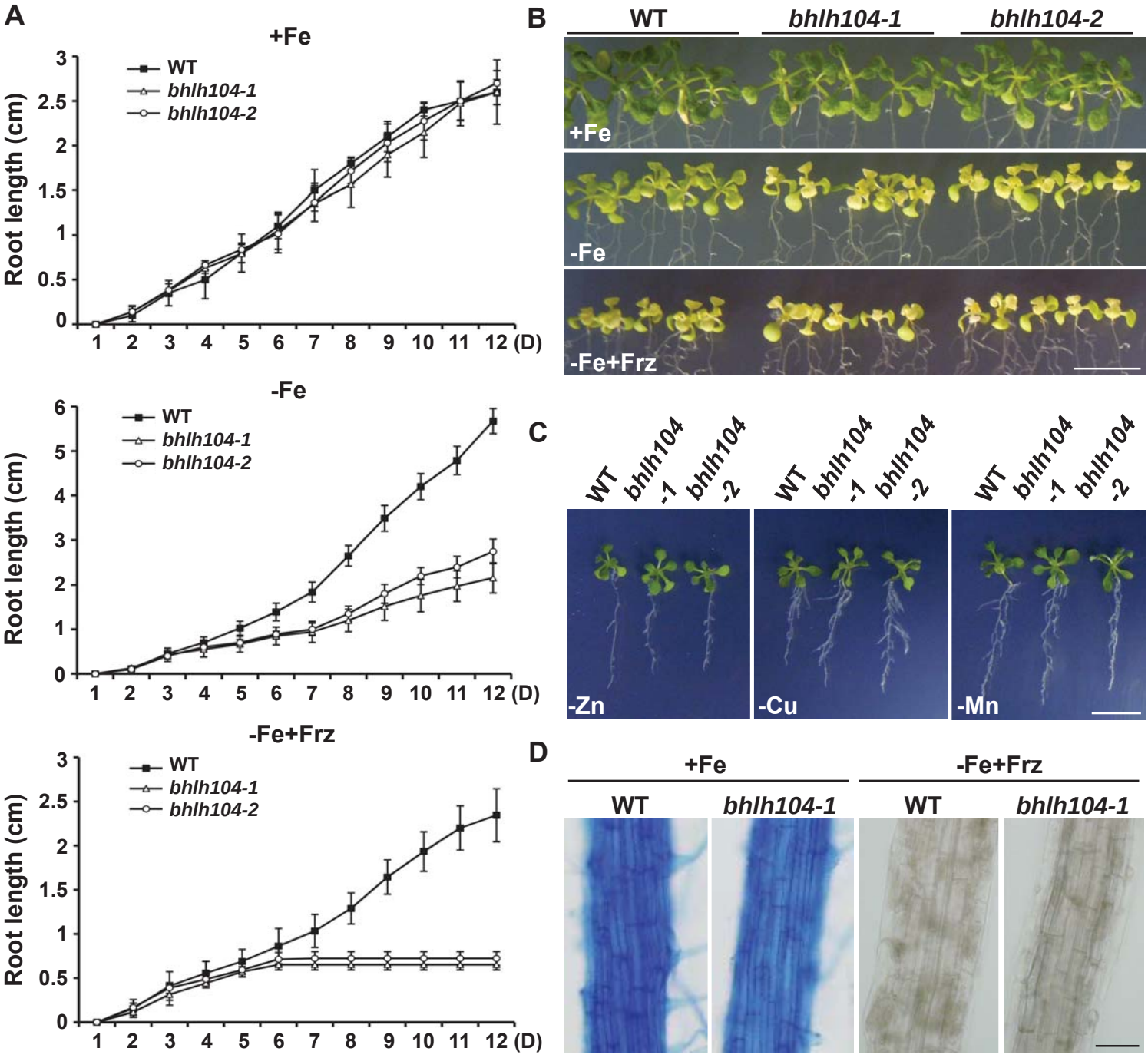
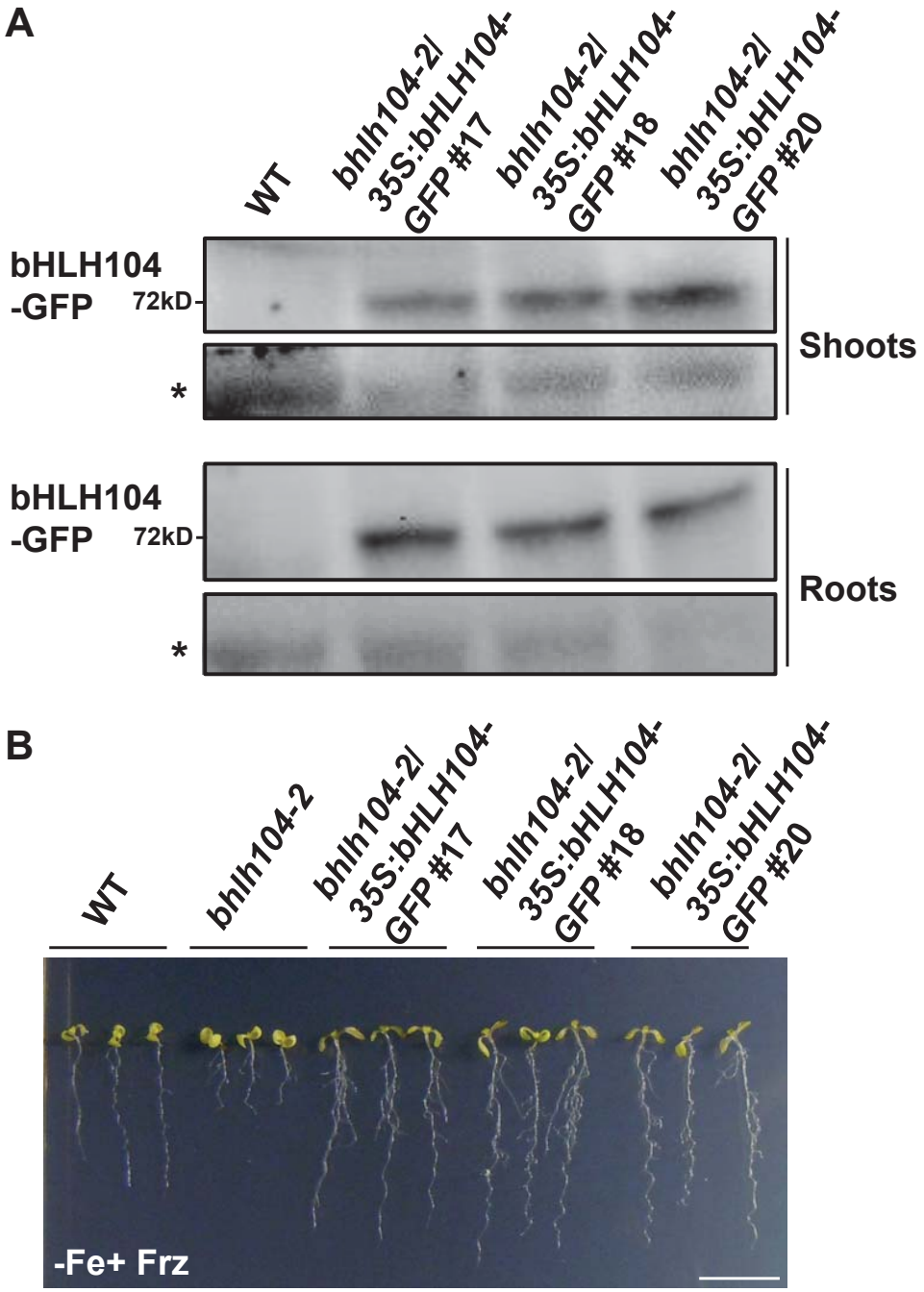


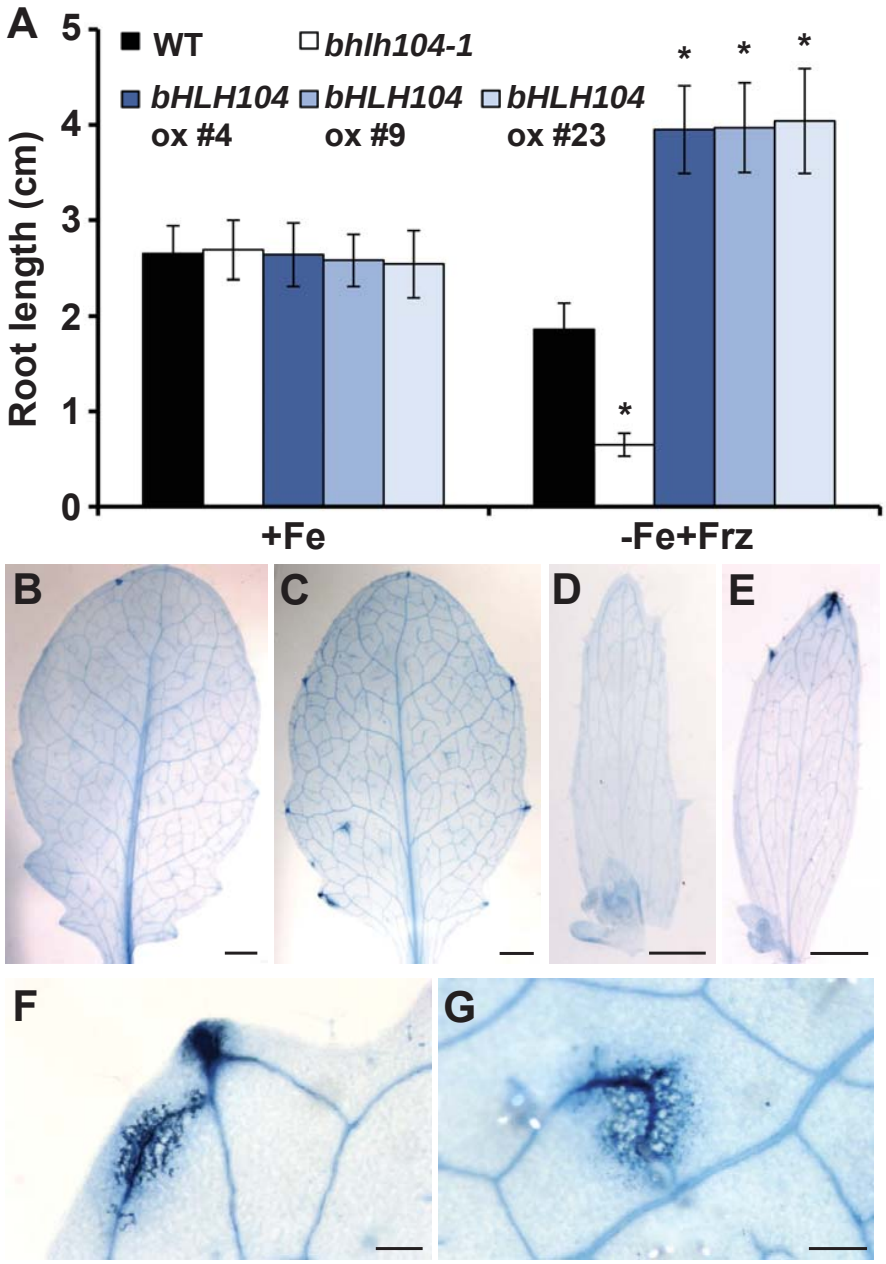
Supplemental Figure 1. Phenotypic Analyses of WT, *bhlh104-1* and *bhlh104-2* in Response to Fe Deficiency.

(A) Time course quantification of root length of WT and *bhlh104* mutants on Fe-sufficient (+Fe) or Fe-deficient (-Fe and -Fe+Frz) media. Values are means ± SD of ten plants for every line. **(B)** Phenotypes of WT, *bhlh104-1* and *bhlh104-2* plants germinated and grown on Fe-sufficient media for 4 days and then shifted to Fe-sufficient (+Fe) or Fe-deficient (-Fe and -Fe+Frz) media for 10 days. Bar = 1 cm. **(C)** Phenotypes of WT, *bhlh104-1* and *bhlh104-2* plants germinated and grown on Zn-, Cu-, Mn-deficient media (-Zn, 1/2 MS media without $ZnSO_4$; -Cu, 1/2 MS media without $CuSO_4$; -Mn, 1/2 MS media without $MnSO_4$) for two weeks. Bar = 1 cm. **(D)** Perls Fe stain in roots of 7-day-old WT and *bhlh104-1* plants germinated and grown on Fe-sufficient (+Fe) or Fe-deficient (-Fe+Frz) media. Bar = 100 μm.



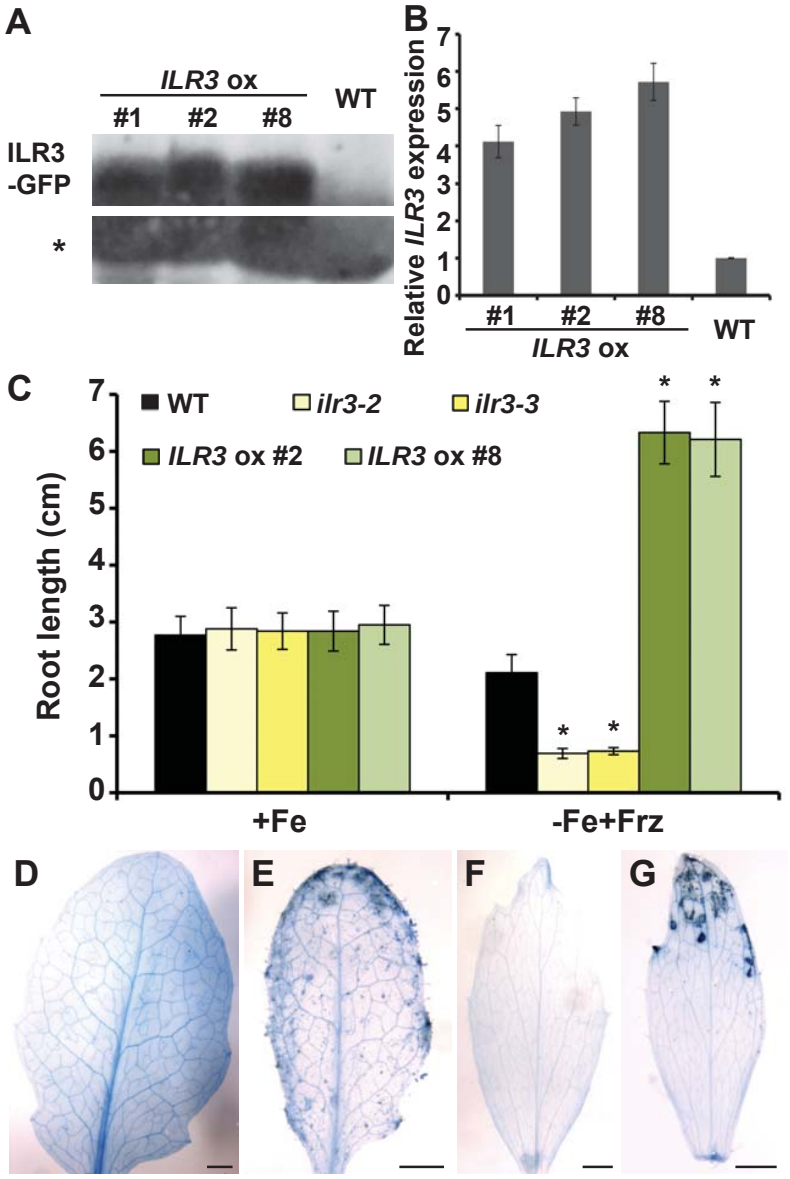
Supplemental Figure 2. Phenotypic Analyses of WT and *bhlh104-2* Complemented Plants in Response to Fe Deficiency.

(A) Levels of bHLH104-GFP fusion protein in shoots and roots of 7-day-old *bhlh104-2* complemented plants grown on 1/2 MS media, as determined by immunoblot analysis using an anti-GFP antibody. The asterisks label the non-specific signal used as loading control. **(B)** Phenotypes of WT and *bhlh104-2* complemented plants grown for two weeks on Fe-deficient (-Fe+Frz) media. Bar = 1 cm.



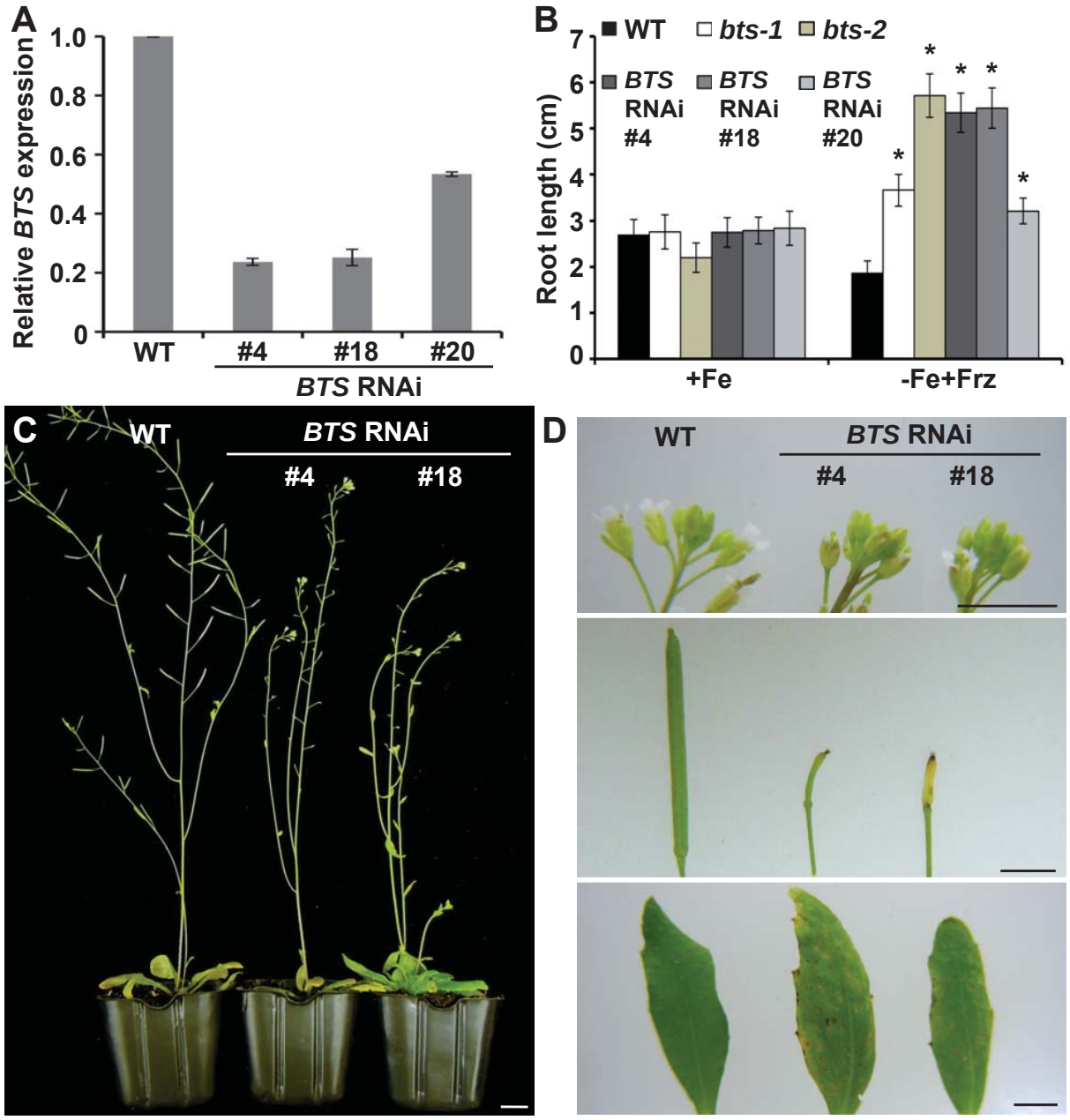
Supplemental Figure 3. Quantification of Root Length of *bHLH104* ox Plants and Trypan Blue Staining of Necrosis in *bHLH104* ox Leaves.

(A) Quantification of root length of WT, *bhlh104* mutants and *bHLH104* ox plants grown on Fe-sufficient (+Fe) or Fe-deficient (-Fe+Frz) media for two weeks. Values are means ± SD of ten plants for every line. Significant differences from the WT are indicated by * ($P < 0.05$), as determined by Student's *t* test. **(B-G)** Trypan blue staining of WT (B and D) and *bHLH104* ox (C, E, F and G) leaves. Bars = 1 mm in B, C, D and E; 200 μm in F and G.



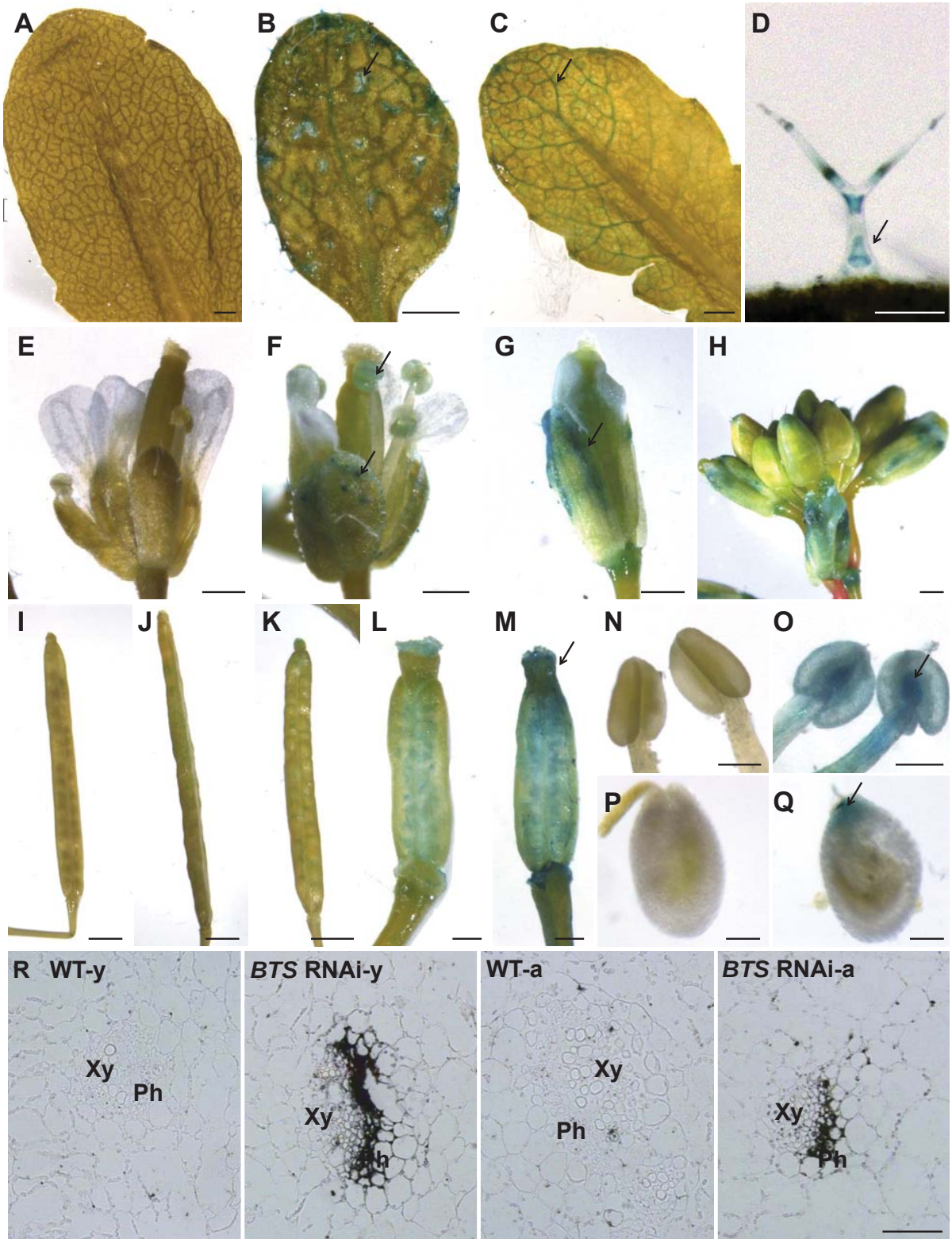
Supplemental Figure 4. Identification of *ILR3* ox Plants and Trypan Blue Staining of Necrosis in *ILR3* ox Leaves.

(A) Level of the ILR3-GFP fusion protein in 4-week-old soil-grown *ILR3* ox plants, as determined by immunoblot analysis using an anti-GFP antibody. The asterisk labels the non-specific signal used as loading control. **(B)** Determination of *ILR3* expression in *ILR3* ox plants grown for four weeks in normal soil, assessed by qPCR. Values are means ± SD of three independent experiments. **(C)** Quantification of root length of WT, *ilr3* mutants and *ILR3* ox plants grown on Fe-sufficient (+Fe) or Fe-deficient (-Fe+Frz) media for two weeks. Significant differences from the WT are indicated by * ($P < 0.05$), as determined by Student's *t* test. **(D-G)** Trypan blue staining of WT (D and F) and *ILR3* ox (E and G) leaves. Bars = 1 mm.

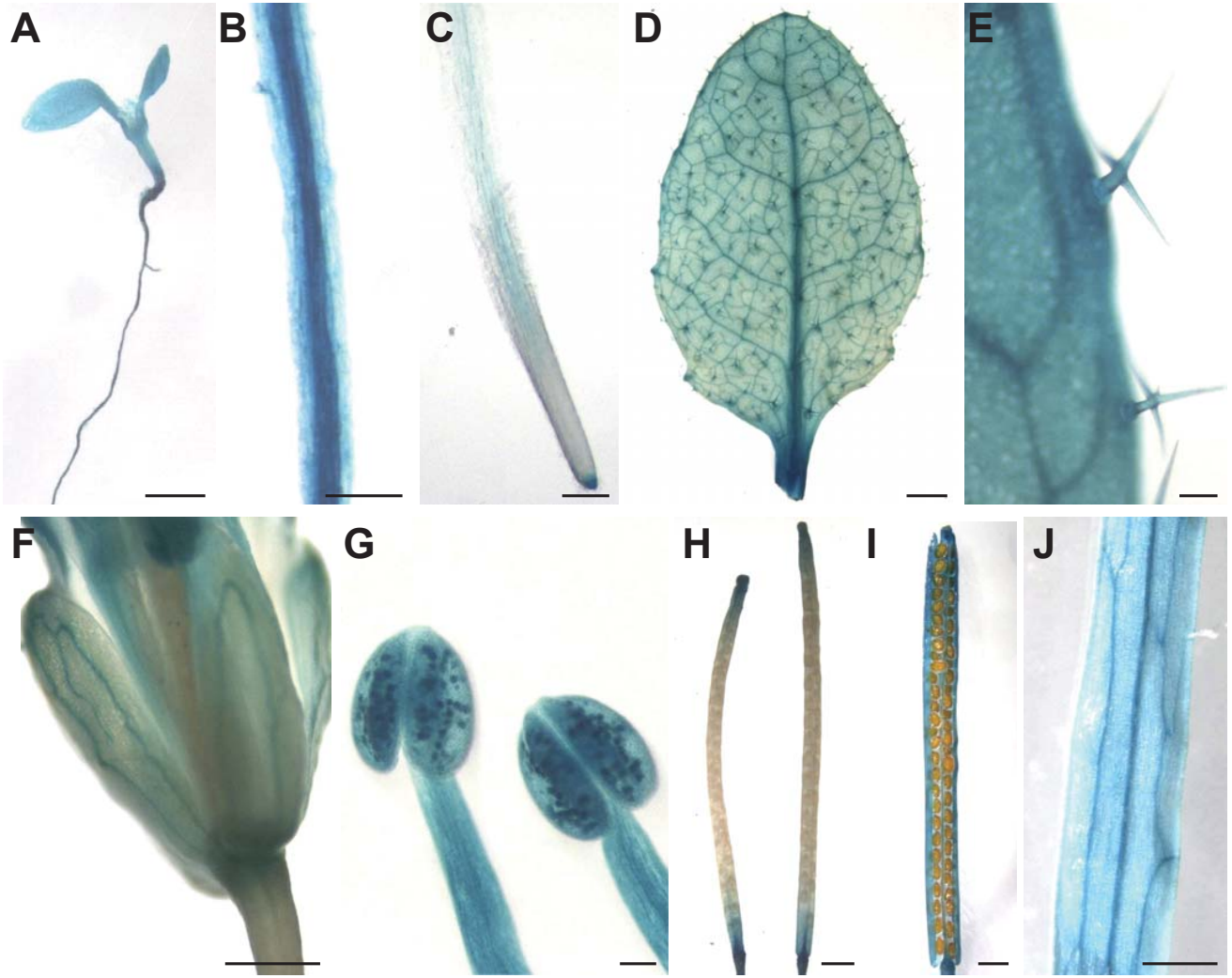


Supplemental Figure 5. Plants Silenced with *BTS* Exhibit Reduced Fertility.

(A) Determination of *BTS* expression in WT and *BTS* RNAi plants grown for four weeks in normal soil, assessed by qPCR. Values are means ± SD of three independent experiments. **(B)** Quantification of root length of WT, *BTS* RNAi and *bts-2* plants grown on Fe-sufficient (+Fe) or Fe-deficient (-Fe+Frz) media for two weeks. Significant differences from the WT are indicated by * ($P < 0.05$), as determined by Student's *t* test. **(C)** Phenotypes of WT and *BTS* RNAi plants at the reproductive stage. The *BTS* RNAi plants showed obviously decreased fertility compared with the WT. Bar = 1 cm. **(D)** The magnification picture of flowers, siliques and cauline leaves in WT and *BTS* RNAi plants. Bars = 500 μm.



Supplemental Figure 6. Perls Staining for Fe^{3+} of *BTS* RNAi Plants.
(A-Q) Perls Fe stain signals in rosette leaves (A-C), trichomes (D), flowers (E-H), siliques (I-M), stamens (N-O) and embryos (P-Q) of WT (A, E, I, J, N and P) and *BTS* RNAi plants (B-D, F-H, K-M, O and Q). Bars = 1 mm in A, B, C, I, J and K; 100 μm in D, P and Q; 500 μm in E, F, G and H; 200 μm in L, M, N and O. **(R)** Perls/DAB stain for Fe^{3+} signals in WT and *BTS* RNAi young- (y) and aged- (a) leaves, showing Fe deposits in phloem of the vessels in *BTS* RNAi plants. Ph, phloem; Xy, xylem. Bar = 50 μm.



Supplemental Figure 7. Expression Pattern Analysis of *bHLH104*.
(A-J) GUS expression in 7-day-old Pro*bHLH104:GUS* transgenic seedlings (A-B) and 5- to 7-week-old adult plants grown in soil (C-J), showing GUS accumulation in tissues of leaves (A, D), roots (B, C), trichomes (E), sepals (F), stamens (G), siliques (H, I) and seed pots (J). Bars = 1 mm in A, D, H and I; 100 μm in B, C, E and G; 500 μm in F and J.

Supplemental Table 1 Primers Used to Identify of T-DNA Insertion Mutants, Gene Cloning and qPCR

Name	Sequence
T-DNA identification	
bhlh104-1-F	GGGGAAAGGTTGTGTCTTTTG
bhlh104-1-R	GCCTGAGTTCTTGATCACGAG
bhlh104-2-F	TCATCGGTTTTATTCGGTCTG
bhlh104-2-R	GAGGAACTAAAGCGTGTCGTG
ilr3-2-F	GAATTCACTAGGTTAATGCCCTG
ilr3-2-R	TGCTAAGGTCAAACCATCCAC
ilr3-3-F	TCAATCAATTCCCGAATCAAG
ilr3-3-R	CTTGCCACTATACCGATTTTG
Transcript identification	
bHLH104-F	ATGTATCCTTCTCTCGACGATGATT
bHLH104-R	TTAAGCAGCAGGAGGCCTGAGT
Gene cloning	
bHLH104-F	ACTAAGCTTATGTATCCTTCTCTCGACG
bHLH104-R	TACCCATGGAGCAGCAGGAGGCCTGA
ILR3-F	ACTAAGCTTATGGTGTCACCCGAAAACGC
ILR3-R	TACCCATGGAGCAACAGGAGGACGAAGG
GFP-F	GGACCATGGGTAGATCTGACTAGTAAAGGA
GFP-R	CGTGGATCCTCATTTGTATAGTTCATCCATGC
BTS-RNAi-F-F	TGAAGCTTCAGAGCTTACTAACTTTGGC
BTS-RNAi-F-R	ACCTGCAGTTTAGCGAAGAAGATCTCAT
BTS-RNAi-R-F	ATGTCGACTTTAGCGAAGAAGATCTCAT
BTS-RNAi-R-R	ATACGCGTCAGAGCTTACTAACTTTGG
Promoter cloning	
Pro*bHLH104*-F	GCGAATTCAATCTCTCGAATTTATGAAC
Pro*bHLH104*-R	ATGGATCCTTGAGAGTCTCCAAACAAAT
QPCR	
FER1-F	ACGTTGCTATGAAGGGACTAGC
FER1-R	TAGGTGAGACGATAGGGTGGAG
bHLH38-F	CGTTCATGTCTTCCAGCTTCTG
bHLH38-R	GACCCGATACTCGTACCAAAAT
bHLH39-F	TCCGTTCATGTCTTCCTGCCTCT
bHLH39-R	CACTTGCTCTTGCAGCTCTGGT
bHLH100-F	CTTGTCTTCCTCCCACCAATCA
bHLH100-R	TGCTCTTGCAGCTCTGGTATGT
bHLH101-F	ACGCCTTGTACTCTTCACTTCG
bHLH101-R	CTTGCTTCTGCTCTGGTATGTATTTC
bHLH34-F	TCAATTCTTCGTCATCTGTTGGA
bHLH34-R	TCAAGTCCATGAACTTGTCATTTAG
bHLH104-F	CCAGCTGCATTTAACCACAACA
bHLH104-R	TTAAGCAGCAGGAGGCCTGAG
ILR3-F	CACAATCCAAGGTCCTGGTTTC
ILR3-R	GGATCCAGGTTCTTTGCTAGCTT
bHLH115-F	TACGAACTGGCTTAGTGATTACCC
bHLH115-R	AACCAGTGCAAGATTCAGTTTTGA
PYE-F	CAGGACTTCCCATTTTCCAA
PYE-R	CTTGTGTCTGGGGATCAGGT
FIT-F	TCGGTCTAGGACTTTGATCTCTG
FIT-R	TCTTGAACATACAACACTGCATCT
BTS-F	ACCATGTCGATCTCCGGCTG
BTS-R	CAAGAGAATATGTTTGCGCTACATT
FRO2-F	TTCACCGTTCATGGTCTTTGTT
FRO2-R	GAGCTATCTCTCCGGCCAAATT
IRT1-F	CTCTTTGCTTCCATCAAATGTTC
IRT1-R	CCTAACGCTATTCCGAATGG
IRT2-F	TCATAGTCACGGTCATGGTGTAG
IRT2-R	GAATGAAATAAAATGCCAACCTC
YSL1-F	CGAAACCATTCACAAGACAAGAG
YSL1-R	TTACCTTCCAAGTTCACACCAGAC
NAS4-F	TGTTCTTGGCTGCTCTTGTAGG
NAS4-R	CAAGGCTCAACGATTGGATAGA
FRD3-F	CGTCTAGGGATCATCGGTGCA
FRD3-R	TCCCCGAAGTTTGGTGGAATC
ZIF1-F	GCTGTAAGGTGGAGCAGATGAA
ZIF1-R	TAGTAGAGGAAGGGATAGAGTGAGG
MYB10-F	CACCATGCTGTGACAAAAGCCA
MYB10-R	AGACGACAACTCTTTCCGCATCT
MYB72-F	GGATAAACTATCTGAGACCGGACG
MYB72-R	GAGATGCGTGTTCCACACGTTT
TUB2-F	GGCCTTGTACGATATTTGCTTC
TUB2-R	TCGGAGGTCAGAGTTGAGTTGA

Supplemental Table 2 Primers Used in ChIP-qPCR

Name	Sequence
bHLH38	
Pro*bHLH38*-a-F	AGGATAAATGATAAATGAGAAGCCA
Pro*bHLH38*-a-R	CACTCTGATTAAAAAAACTGCCTCT
Pro*bHLH38*-b-F	CGAATGTTGGAAACTTCATTGATTC
Pro*bHLH38*-b-R	TTTTAATTCCACAATGACGATGGTC
Pro*bHLH38*-c-F	ACAACATAAAAAATGTATGGGACGA
Pro*bHLH38*-c-R	TTGTGTAAGAATCAAACATGTATAT
Pro*bHLH38*-d-F	CATGTTTGATTCTTACACAATATGC
Pro*bHLH38*-d-R	TCGTTGAGATTATATGATTGTGTTAT
bHLH39	
Pro*bHLH39*-a-F	GGAGGTCAACAAATAAATAAAATGC
Pro*bHLH39*-a-R	ACATATTGAAAGATGACTCAGCCTG
Pro*bHLH39*-b-F	CCAGTCTACTTGTGACTAGACCTTG
Pro*bHLH39*-b-R	AACCAAAACTTTAAAAATTCGCAAA
bHLH100	
Pro*bHLH100*-a-F	AACCGAAGTGTTGTACTGTTTTCGA
Pro*bHLH100*-a-R	AGATTTATCCATTGATATGTTGGCA
Pro*bHLH100*-b-F	ATGGGCTACAAACATGTAAACTCAT
Pro*bHLH100*-b-R	TCATTCTATTTACGCCTCTTTAAAT
Pro*bHLH100*-c-F	AAAATAAACGATAGACACTACACCA
Pro*bHLH100*-c-R	GGTGGACGTGGACTATTGAGA
Pro*bHLH100*-d-F	ATTTGCTCGTGCCTCTCAATAGT
Pro*bHLH100*-d-R	GAATATGCAATCACATTTTTCTACC
Pro*bHLH100*-e-F	AAGAACATTAGGATATTAATGCCTG
Pro*bHLH100*-e-R	TAAATTAAAATACATTGGGTCACGA
bHLH101	
Pro*bHLH101*-a-F	ATTTTGGAAGATGAAGGTAAAAGAG
Pro*bHLH101*-a-R	TCATCGATCTATATATCCATTTGGA
Pro*bHLH101*-b-F	TCTTTGGTACGACCCTACGGCT
Pro*bHLH101*-b-R	AAGGTTCTTTCTTTCACATGTTTTG
Pro*bHLH101*-c-F	TGAAGGTGATAAACACAAACAGACT
Pro*bHLH101*-c-R	TGGTATTTCGTACCAGAGTCTAGTT
Pro*bHLH101*-d-F	TTGATAGTGGATCATTAATTACTTGTA
Pro*bHLH101*-d-R	ATTTACCATTCAGAGATCCATCC
Pro*bHLH101*-e-F	ACAGCAAACATAAAACTTCATGTGG
Pro*bHLH101*-e-R	GTTATATTTTGAACATGTGAACGCA
FIT	
Pro*FIT*-a-F	AGATGTGATAGGTACAGCAAAATTG
Pro*FIT*-a-R	ATTTGATACATGTGAAGCTAGCATA
Pro*FIT*-b-F	GCTTGTGACAACTAAACCAGTTGAC
Pro*FIT*-b-R	ATCGATCAGACCGTATTAAAAAGGT
Pro*FIT*-c-F	CTCTTGTTTCTAATCTGCATTCCCT
Pro*FIT*-c-R	TTACTCGAATGATTAATTTGTCGTG
PYE	
Pro*PYE*-a-F	AGTTTCTAACAGATGTTTTTCCGG
Pro*PYE*-a-R	TTTTCATCAATTGTTTGCATTATAA
Pro*PYE*-b-F	GTTTGTGGGAAACACACGACAG
Pro*PYE*-b-R	AGATGAAAAGTAACATTTTCACAAAA
Pro*PYE*-c-F	GAGATGAGCTTTAGTGGCACGC
Pro*PYE*-c-R	GAAGGTCCGAAGTTGAGGAGGG
TUB2	
Pro*TUB2*-F	TTTCGCTTTCTTGTTGGTCAATTAT
Pro*TUB2*-R	CTTAACGATCCAAGTTTATGGATTG

Supplemental Methods

Trypan Blue Staining

To detect leaf necrosis, lactophenol-trypan blue staining was performed, as previously described (van Wees, 2008). *Arabidopsis* leaves were soaked in trypan blue working solution (10 mL phenol, 10 mL glycerol, 10 mL lactic acid, 10 mL distilled water, 80 mL ethanol and 0.02 g trypan blue, Sigma) in a boiling water bath for 5 min and cleared with saturated chloral hydrate solution (800 g of chloral hydrate dissolved in 200 mL distilled water and 100 mL glycerol).

van Wees, S. (2008). Phenotypic analysis of Arabidopsis mutants: trypan blue stain for fungi, oomycetes, and dead plant cells. CSH Protoc **2008,** pdb prot4982.