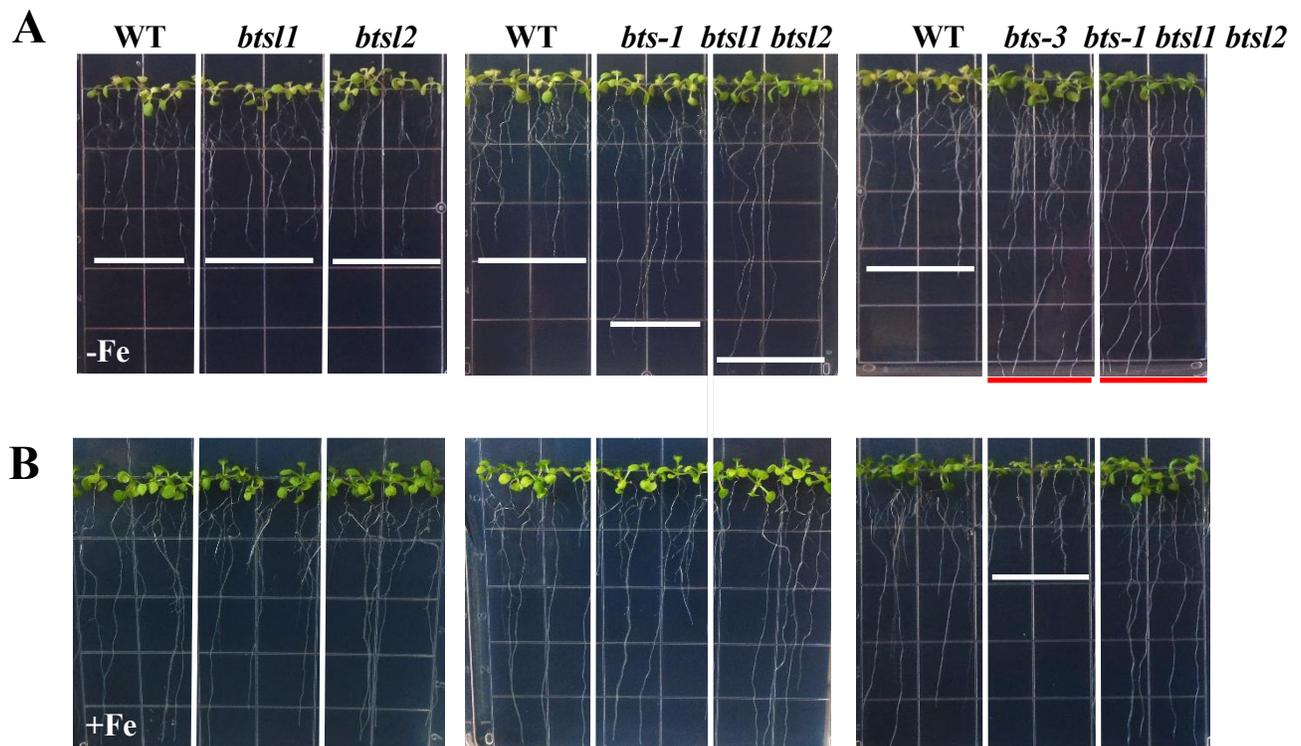
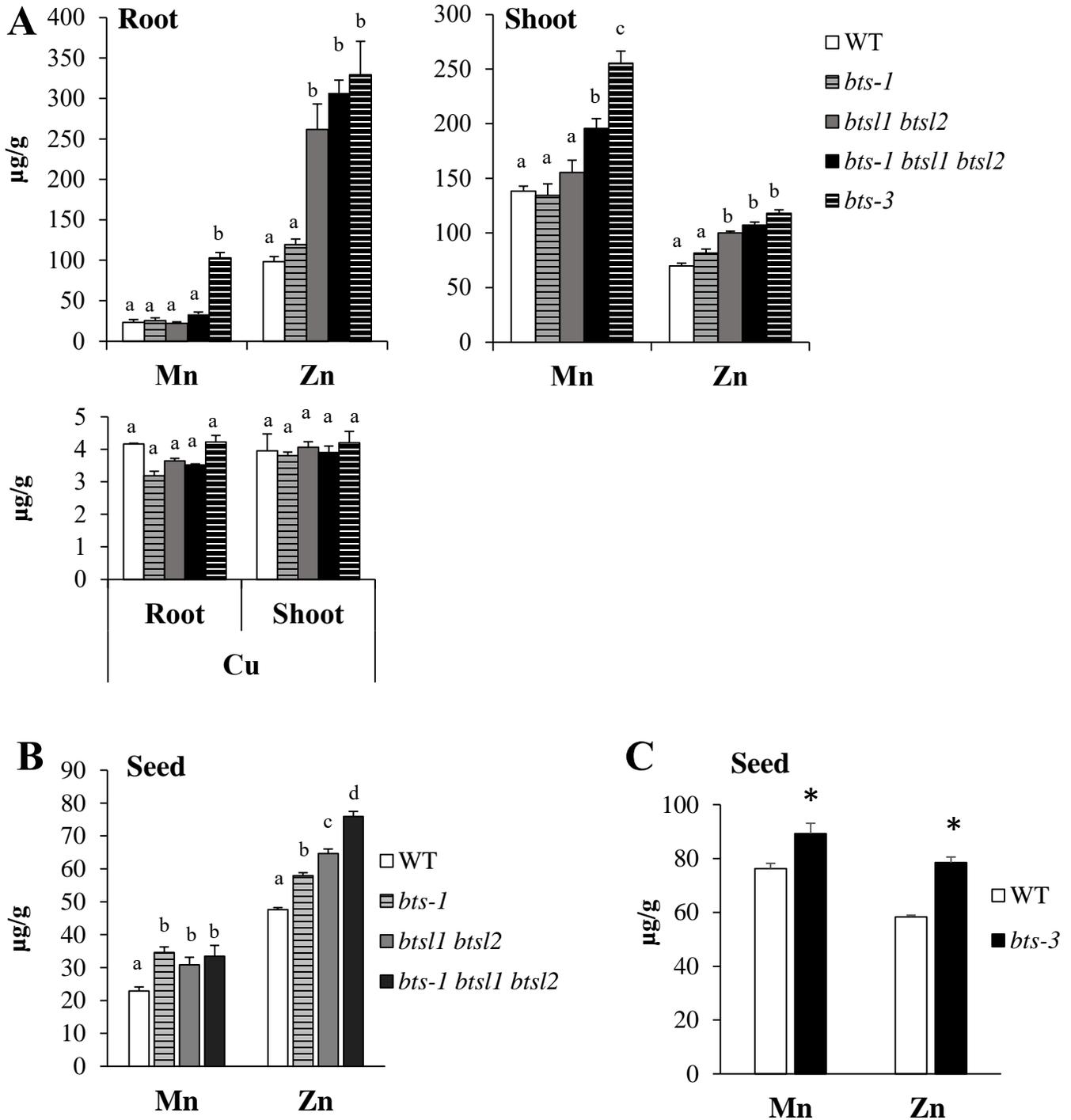


**Supplemental Figure 1. Location of T-DNA insertions of *bts-1*, *bts11* and *bts12* mutants.**

**A.** Arrows indicate position of T-DNA insertions within *BTS*, *BTSL1*, and *BTSL2* genes. Black boxes represent exons, lines indicate introns, and white boxes indicate UTRs. The *bts-1* insertion occurs in the 5'UTR, the insertion in *bts11* occurs in the 2<sup>nd</sup> exon, and the insertion in *bts12* occurs in the promoter just before the 5'UTR. **B.** Full length transcript amplified from cDNA of roots of plants grown for 2 weeks on B5 medium and then transferred to +Fe or -Fe medium for 3 days.

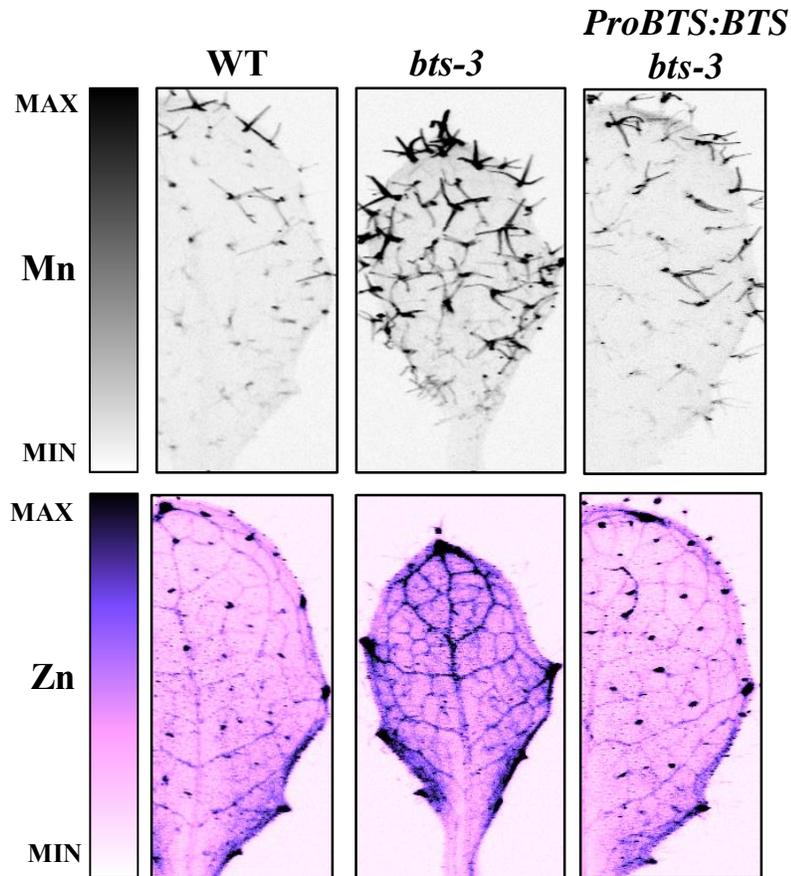


**Supplemental Figure 2. *bts-1*, *bts11 bts12*, *bts-1 bts11 bts12*, and *bts-3* mutants all exhibit tolerance to Fe-deficient growth conditions, but only *bts-3* is sensitive to Fe-sufficient conditions.** Plants were grown vertically for 5 days on B5 medium and then transferred to +Fe (A) or -Fe medium (B) for 7 days.



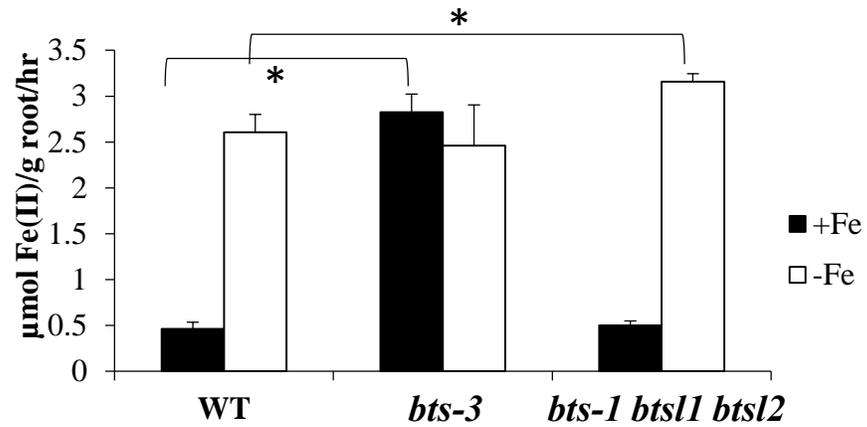
**Supplemental Figure 3. BTS family E3 ligase mutants have higher Mn and Zn concentrations in roots, shoots and seeds compared to wild type.**

**A.** Root and shoot ICP-MS measurements of plants grown for 2 weeks on B5 and then transferred to +Fe medium for 3 days. **B.** Seed ICP-MS measurements of harvest from soil grown plants (as in Figure 2). Lower case letters indicate significant differences at  $p < 0.05$  (ANOVA with Tukey's). **C.** Seed ICP-MS measurements of harvest from soil grown plants. \* indicates  $p < 0.05$  compared to WT (Student's T-test).



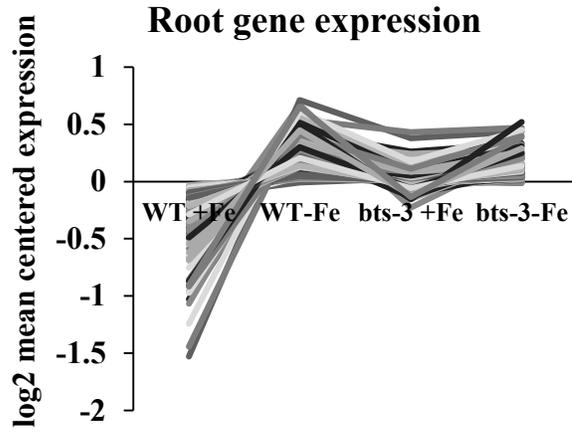
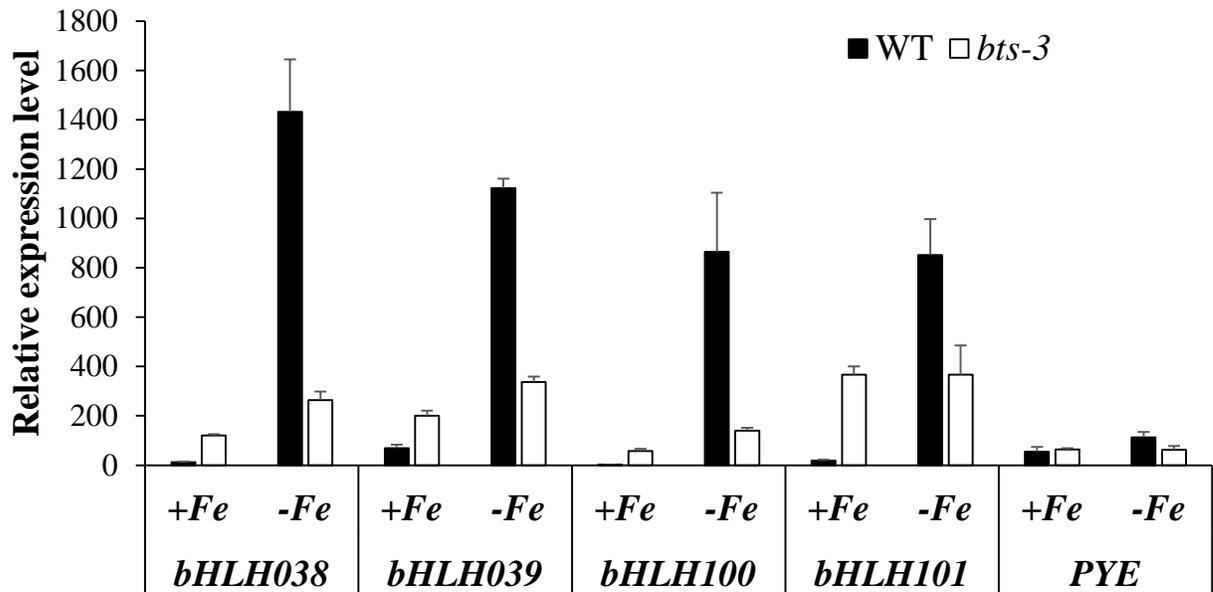
**Supplemental Figure 4. Expression of *BTS* controlled by its endogenous promoter in *bts-3* returns Mn and Zn levels to wild type levels.**

SXRF scans showing Fe localization in leaf #5 of plants grown for 2 weeks on B5 medium and then transferred to +Fe medium for 3 days. *ProBTS:BTS bts-3* is the complementation line where the *BTS* gene driven by its own promoter is expressed in the *bts-3* mutant.



**Supplemental Figure 5. Unlike *bts-3*, *bts-1 bts11 bts12* mutants do not exhibit induction of ferric chelate reductase activity when grown under Fe-sufficient conditions.**

Ferric chelate reductase activity of roots of plants grown for 2 weeks on B5 medium before transfer to +Fe or -Fe medium for 3 days. \* $p < 0.05$  (Student's t-test). Brackets indicate comparisons. Error bars indicate SE.

**A****B**

**Supplemental Figure 6. *bts-3* roots exhibit constitutive expression of Fe deficiency genes.**

A. Line graphs showing gene expression profiles of all genes from cluster 4 of root microarray analysis.

B. qPCR of bHLH transcription factor expression in roots of plants grown for 2 weeks on B5 medium and then transferred to +Fe or -Fe medium for 3 days. Expression is relative to EF1 $\alpha$ . Error bars represent SE (n=3).

### **Supplemental methods: SXRF analysis**

Plants for NSLS leaf SXRF experiments were grown for 2 weeks on B5 and 3 days on +Fe or -Fe medium. Leaf #5 from plants of each genotype was detached and mounted on the sample stage on metal free Kapton™ tape just prior imaging. X26A and X27A use Kirkpatrick-Baez (KB) mirror microprobes and a Ge detector. Incident energy for each leaf image was 11 keV, step size was 10 µm and dwell time was 100 milliseconds. Dry seeds from soil-grown plants imaged at NSLS were also mounted on Kapton™ tape and scanned with a 5 µm step size and 100 millisecond dwell time.

Roots, leaves, and siliques for SSRL experiments were detached and mounted on Kapton™ tape from plants just prior to analysis. Roots were from that were grown for 7 days on B5 medium and 3 days on -Fe medium. Leaf #8 for shoot images was from plants grown for 10 days on B5 medium. Green, developed siliques were from plants grown on soil. Beamline 2-3 also uses a KB mirror microprobe with a Ge detector. Root images had a step size of 1 µm and dwell time of 100 milliseconds. Leaves had a 7 µm step size and 30 millisecond dwell time. Siliques had a 7 µm step size and 50 millisecond dwell time.

Dry seeds imaged at XFM were from soil grown plants and mounted on metal free Ultralene thin window film®. A KB mirror microprobe was used with the Maia detector, a Si detector (Ryan et al., 2010). Incident energy was 11 keV, step size was 2 µm and dwell time was 2.6 milliseconds.

High resolution imaging of leaf sections were conducted at 2-ID-D of the Advanced Photon Source. Plants were grown on B5 medium for 10 days and leaf #1 was fixed for sectioning. Sample preparation was performed as described, using LR White embedding resin, 1µm thick sectioning, and mounting on silicon nitride windows (Punshon et al., 2012). The 2-ID-D beamline uses a zone plate insertion device to achieve a highly focused beam. Imaging was conducted using an incident energy of 10.1 keV, 0.15 µm step size, and 500 millisecond dwell time.

Data gathered at SSRL was analyzed with Sam's Microtool Analysis Kit (SMAK) ([microtoolkit.sams-xrays.com](http://microtoolkit.sams-xrays.com)), data from NSLS was processed with Xmap Plotter. ([bnl.gov/x26a/comp\\_download.shtml](http://bnl.gov/x26a/comp_download.shtml)) and data from the Australian Synchrotron was analyzed with Image J (<http://imagej.nih.gov/ij/>). Images are false colored to show fluorescence counts detected on a scale from minimum to maximum for specific elements. Images that are directly compared are scaled to the same minimum and maximum fluorescence counts.

### **Supplemental References**

- Punshon, T., Hirschi, K., Yang, J., Lanzirotti, A., Lai, B., and Guerinot, M.L.** (2012). The role of CAX1 and CAX3 in elemental distribution and abundance in Arabidopsis seed. *Plant Physiol.* **158**, 352-362.
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