SUPPORTING INFORMATION

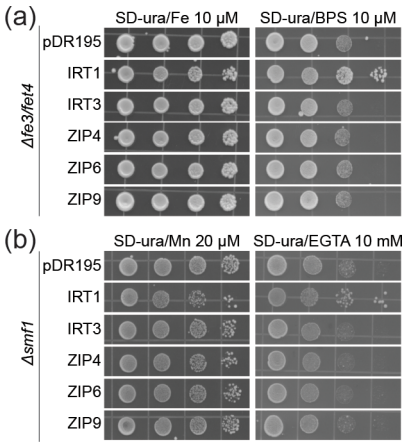

Figure S1. Complementation of yeast metal uptake mutants. Serial dilutions (OD_{600} = 0.1, 0.01, 0.001, and 0.0001) were grown for 3 days at 30 C. (a) Cells of the iron uptake mutant *fet3fet4* transformed with empty vector (pDR195) or *ZIP* cDNA constructs were spotted on to medium supplemented with 10 µM Fe or 10 µM Bathophenanthroline disulfonate (BPS, Fe chelator). IRT1 served as the positive control. (b) Cells of the Mn uptake mutant *smf1* transformed with the empty vector (pDR195) or *ZIP* cDNA constructs were spotted on to medium supplemented with 20 µM $MnSO_4$ or 10 mM EGTA.

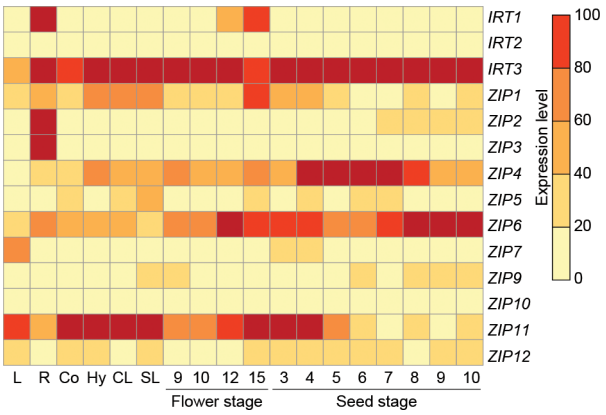

Figure S2. *In Silico* expression analysis of *ZIP* genes during development. Publicly available microarray data were obtained from BAR (The Bio-Analytic Resource for Plant Biology, https://bar.utoronto.ca/efp). Data sets were mined to generate an expression heat map using the heatmap.plus. The heat map represents the expression of *ZIP* genes during the indicated developmental stages and the color bar represents the expression levels. L, leaf; R, root; Co, cotyledon; HY, hypocotyl; CL, cauline leaf; SL, senescing leaf.

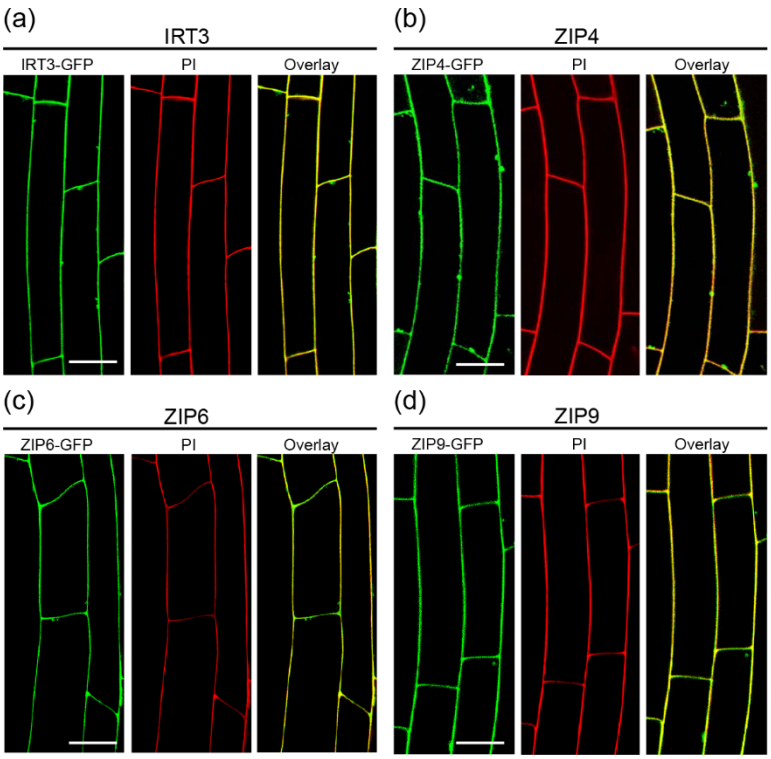

Figure S3. ZIP-GFP translational fusion proteins localize to the plasma membrane. Confocal fluorescence image of IRT3-GFP fusion protein (A), ZIP4-GFP (B), ZIP6-GFP (C), and ZIP9-GFP (D) in root cells. The image triplets consist of GFP signal (left panel), propidium iodide (PI) red fluorescence (center) and overlapping images of GFP and PI signal. Bars = 10 μm.

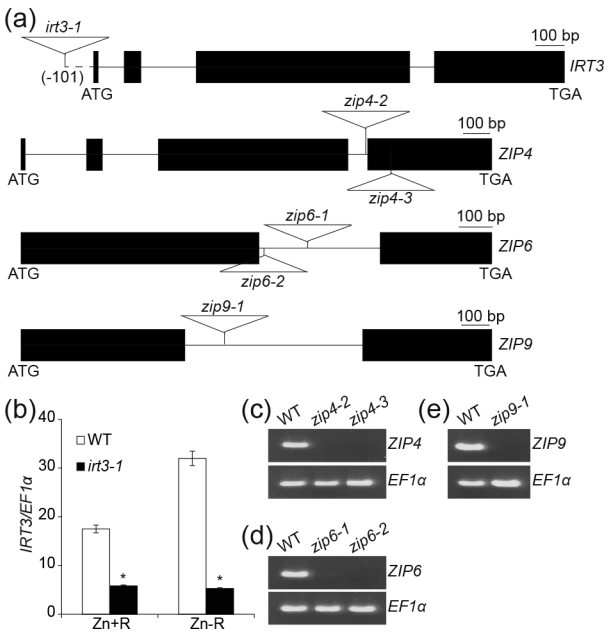

Figure S4. T-DNA mutants. (a) Schematic diagrams of genomic structures of four *ZIP* genes along with the location of T-DNA insertions. Block boxes represent an exon, black lines represent introns and dotted line indicate 5'-untranslated regions. (b) Real-time PCR analyses of *IRT3* expression in roots grown under Zn-sufficient (Zn+R) or Zn-deficient (Zn-R) conditions. Expression is shown relative to *EF1α*. Error bars represent mean +/- SE of three independent experiments. *, $P < 0.05$ for significant difference from WT. (c) RT-PCR analyses of WT, *zip4*-2 and *zip4-3* using RNA samples from Zn-deficient roots. (d) Detection of *ZIP6* mRNA. RT-PCR was performed on total RNA extracted from Zn-deficient roots of WT, *zip6-1 and zip6-2*. (e) RT-PCR analysis of WT and *zip9-1* in Zn-deficient roots. *EF1α*-specific primers were included as a control.

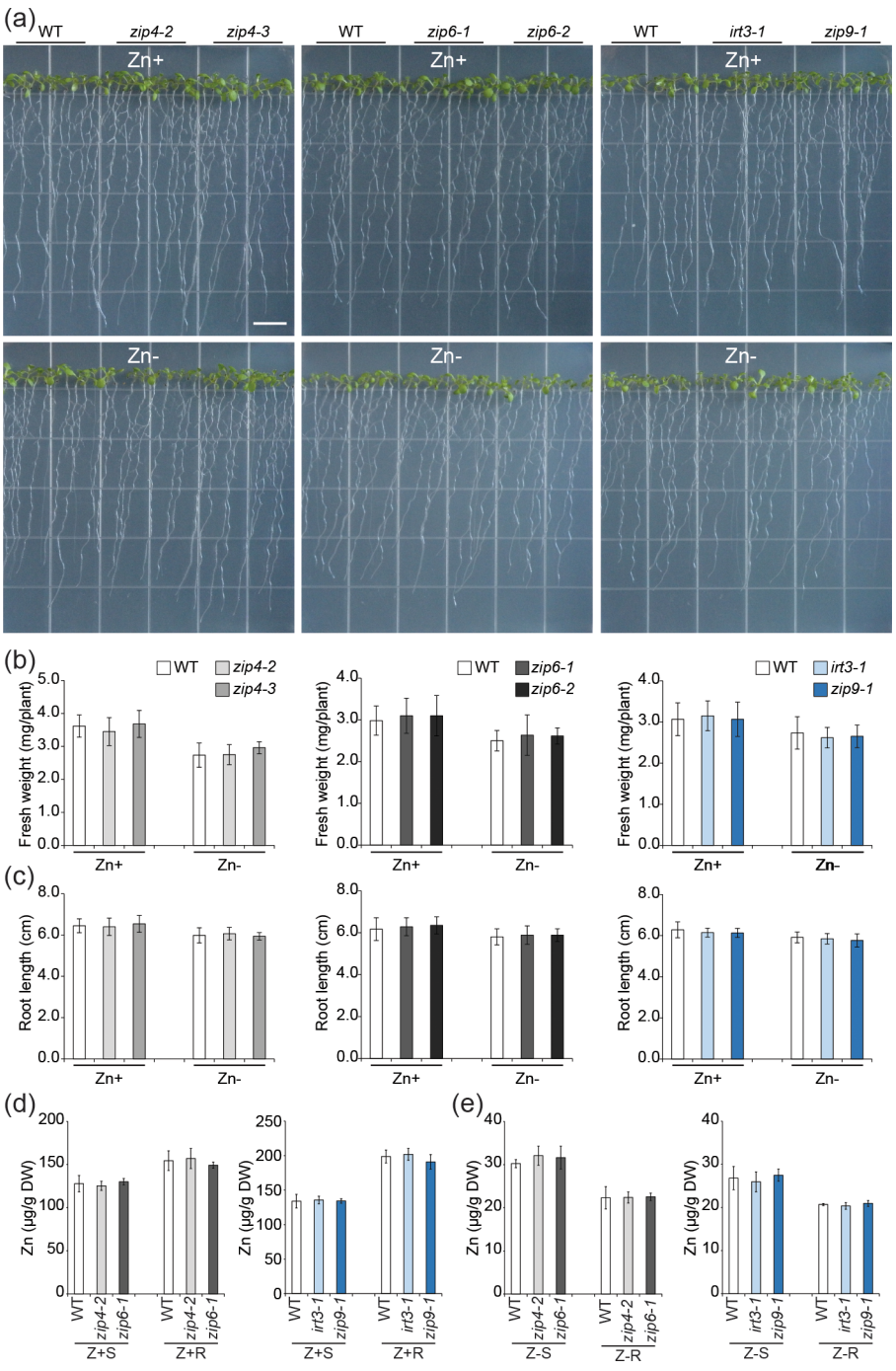

Figure S5. Seedling phenotypes of single T-DNA mutants grown under Zn-sufficient or Zn-deficient growth conditions. (a) The growth performance of WT, *zip4-2*, *zip4-3*, *zip6-1*, *zip6-2*, *irt3-1* and *zip9-1* seedlings grown under Zn-sufficient (Zn+) or Zn-deficient (Zn-) medium. Photographs were taken 8 d after germination. Bar = 1 cm. Fresh weight (b) and root length (c) of seedlings from each line grown under Zn+ or Zn- medium. Mean values and standard errors were obtained from 10 seedling plants of each genotype. (d) Zn concentration in shoots and roots

from WT, *zip4-2*, *zip6-1*, *irt3-1* and *zip9-1* when grown under Zn sufficient growth medium. (e) Zn distribution in shoots and roots of WT and single mutants under Zn deficiency. Z+S denotes shoots from Zn sufficient medium; Z+R, roots from Zn sufficiency; Z-S, shoots from Zn deficiency; Z-R, roost from Zn deficiency. Shoots and roots from 2~3 plants were pooled and then 4~5 replicates were used for ICP-MS analyses.

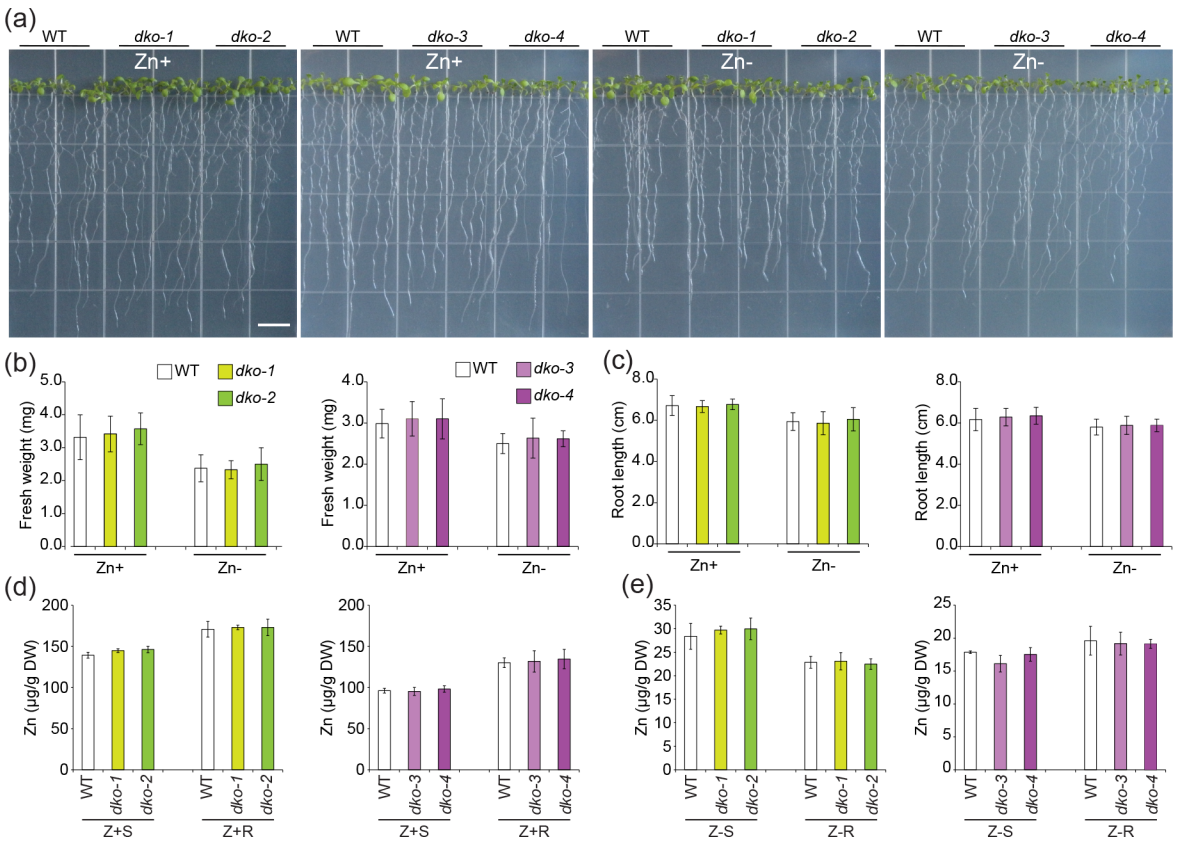

Figure S6. Seedling phenotypes of double mutants grown under Zn-sufficient or Zn-deficient growth conditions. (a) Growth comparison of WT and double mutants when grown under Zn-sufficient (Zn+) or Zn-deficient (Zn-) medium for 8 days. *dko-1* indicated the *irt3-1/zip4-2* double knockout mutant. *dko-2, zip4-2/zip6-1*; *dko-3, irt3-1/zip9-1*; *dko-4, zip4-1/zip9-1.* Bar = 1 cm. Fresh weight (b) and root length (c) of seedlings from each line grown under Zn+ or Zn- medium. Mean values and standard errors were obtained from 10 seedling plants of each genotype. Zn concentration in shoots and roots from WT and double mutants (*dko-1, dko-2, dko-3* and *dko-4)* when grown under Zn-sufficient (d) or Zn-deficient growth medium (e). Data represent mean ± SE (n = 4 or 5). Z+S denotes shoots from Zn sufficient medium; Z+R, roots from Zn sufficiency; Z-S, shoots from Zn deficiency; Z-R, roots from Zn deficiency. Data represent mean ± SE (n = 4 or 5).

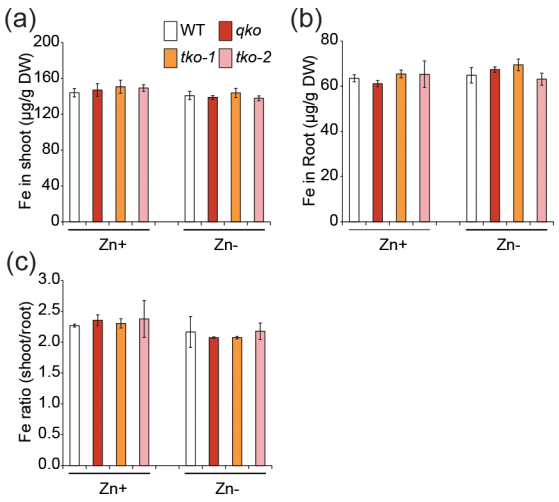

Figure S7. Measurement of Fe levels of WT, triple and quadruple mutants. Fe concentrations in shoots (a) and roots (b) of WT, and quadruple and triple mutants were grown in 1/2 MS medium containing (Zn+) or without Zn (Zn-) for 8 days. Tissues were harvested, digested and analyzed by ICP-MS. For shoot/root ratios of Fe concentrations (c) were calculated from the data shown in a and b. Error bars represent the mean +/- SE of four independent experiments.

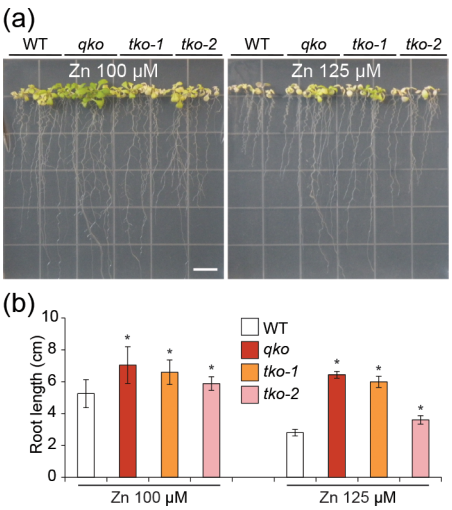

Figure S8. Increased Zn resistance of triple and quadruple mutants. (a) Plants were grown in half-strength MS medium containing excess Zn (100 µM or 125 µM) for 10 days. Bar = 1 cm. (b) Root length of WT and mutant lines were grown under excess Zn. Data are mean +/- SE and significant differences from WT are indicated (*$P < 0.05$).

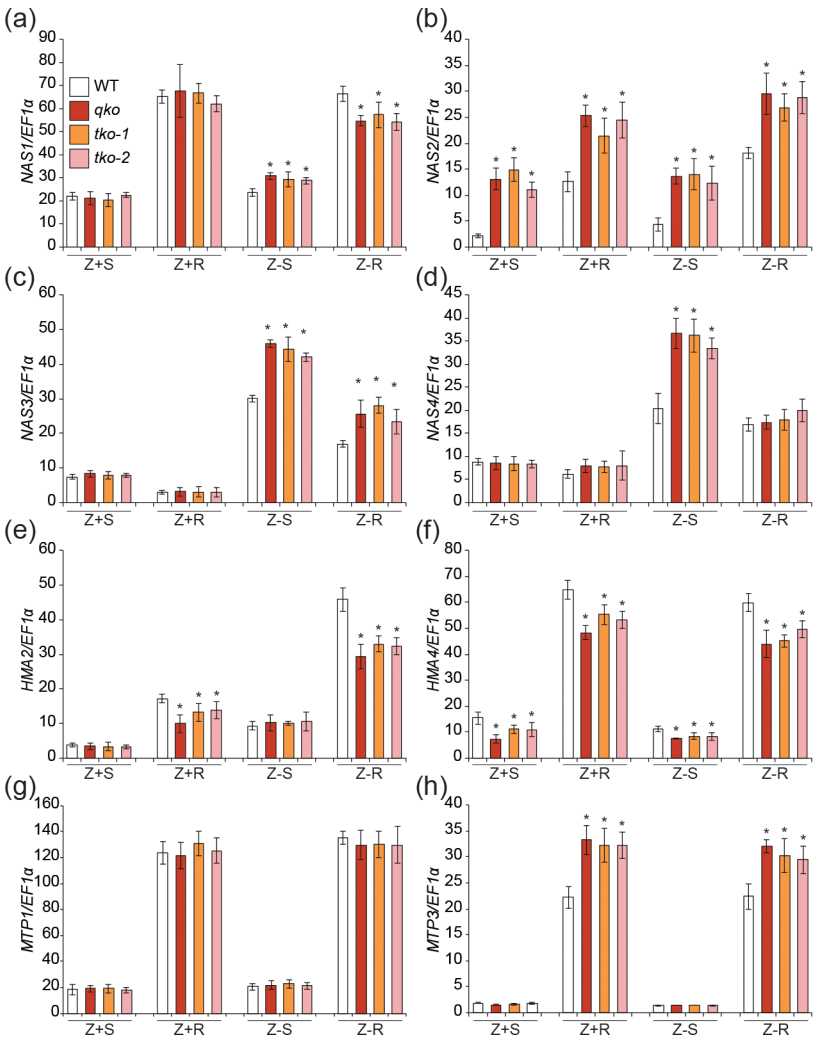

Figure S9. Quantitative RT-PCR analysis of Zn homeostasis related genes at the seedling stage. RNA samples were prepared from roots and shoots from WT, quadruple and triple mutants grown on Zn-sufficient (Zn+) or Zn-deficient (Zn-) medium for 8 days. (a-d) Expression of four *NAS* genes. Expression analysis of *HMA2* (e), HMA4 (f), *MTP1* (g) and *MTP3* (h) in seedling shoots and roots. Z+S denotes shoots from Zn sufficient medium; Z+R, roots from Zn sufficiency; Z-S, shoots from Zn deficiency; Z-R, roots from Zn deficiency. Error bars represent the standard error of 4 replicates. Asterisks above the bars indicate significant differences between WT and mutants ($P < 0.05$).

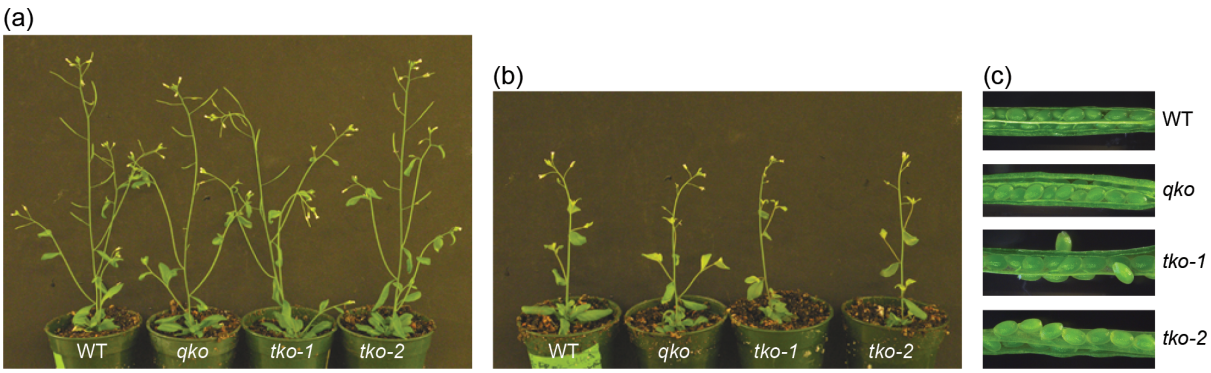

Figure S10. Growth morphology of WT, quadruple and triple mutants. Plants were grown under long-day conditions in normal soil (a), and watered 1mM $ZnSO_4$ every week (b) up to 7 weeks. (c) Morphology of siliques from WT, quadruple and triple mutants in b.

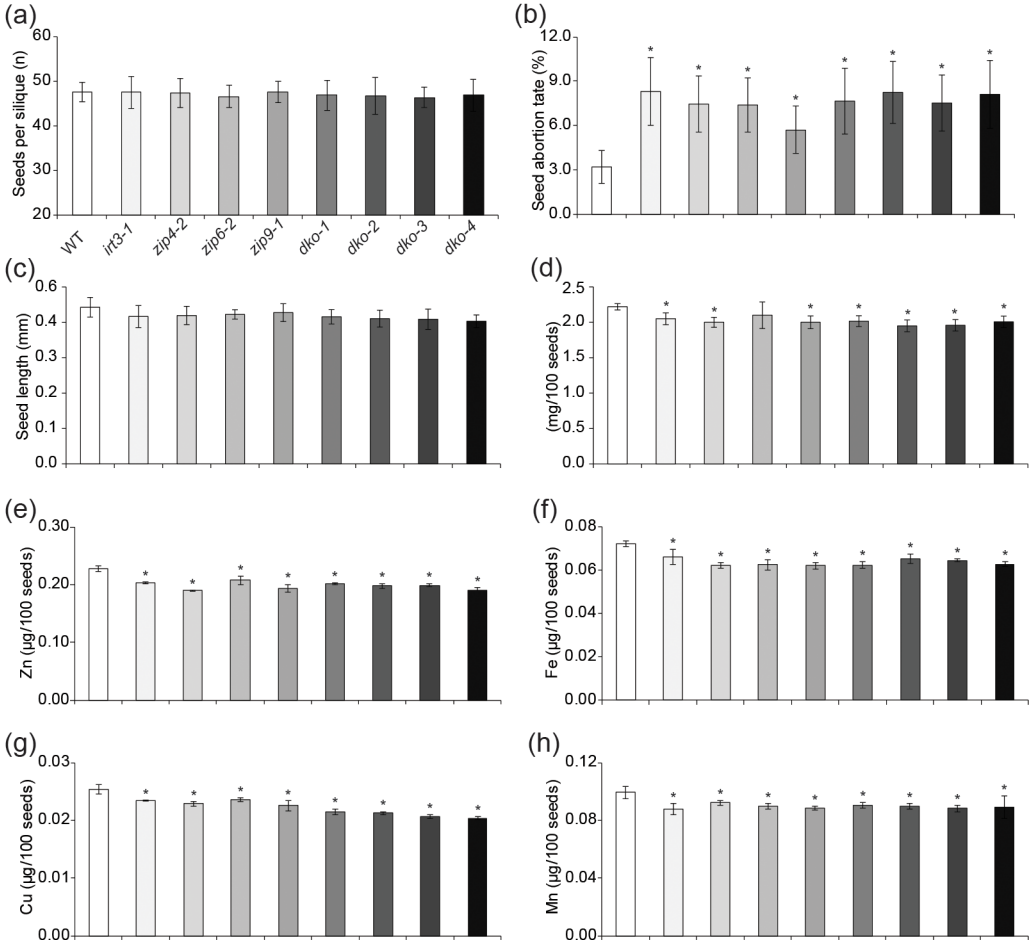

Figure S11. Characterization of seed phenotypes and metal contents in single and double mutants. The number of seeds per silique (a) and quantification of seed abortion in WT and mutants (b). Values are mean ± SE of 15 to 20 siliques from four independent plants. (c) Average seed length was measured after scanning the seeds via Image*J* software (https://imagej.nih.gov/ij/). Data represent mean ± SE of a minimum of 30 seeds. (d) Seed weight obtained by measuring 100 seeds (n = 5, mean ± SE). Measurement of Zn (e), Fe (f), Mn (g) and Cu (h) from WT, single and double mutants. Values are mean ± SE of 100 seeds from four independent plants. Asterisks above the bars indicate significant differences between WR and knockout mutants ($P < 0.05$).

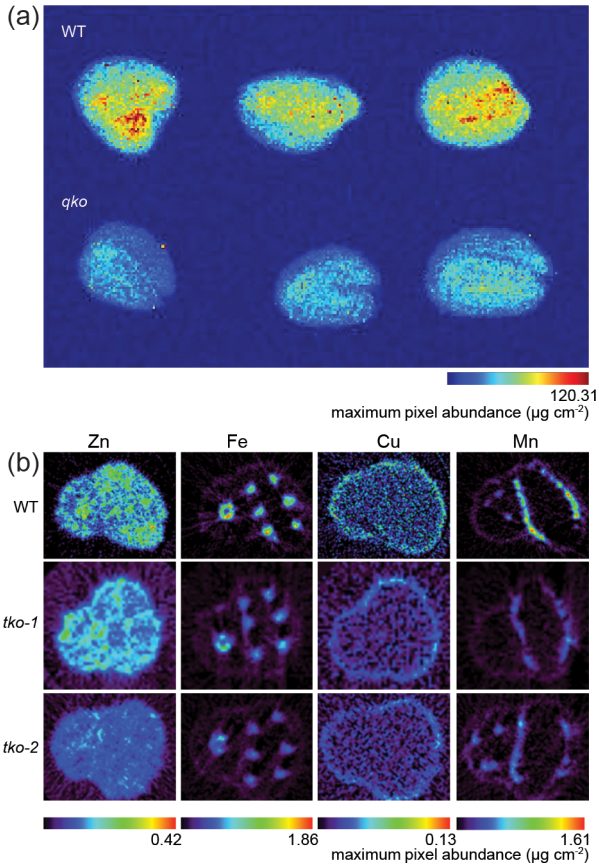

Figure S12. Synchrotron X-ray fluorescence (SXRF) elemental imaging of triple and quadruple mutants. (a) 2D map of whole quadruple mutant seeds and (b) quantified 3D tomographic slices of seeds from the triple mutants.

Table S1. Primers used in this study.

Name	Primer sequence (5′→3′)
Primers used for yeast expression	
IRT3-YF	CTCGAGCGAGGACATCTTAGACCCCAA
IRT3-YR	GGATCCAAACTATGGAATCTTATACTC
ZIP4-YF	CTCGAGGAAGAATAAACTCTTGTTCCC
ZIP4-YR	GGATCCAGTAGATAATATGTAATCTAA
ZIP6-YF	CTCGAGCCTCTCTTTCTTCTTCTTCCACAA
ZIP6-YR	GGATCCTTTTTGTCTAAGCCCAAAGAGC
ZIP9-YF	CTCGAGCTTACCCTCAAGTTCGCAGCT
ZIP9-YR	GCGGCCGCGGCATGCCTAAATTTATTACT
Primers used for qRT-PCR	
IRT3-QF	GGTTTATCCCTGGGGGTATCACA
IRT3-QR	GCAGCTGACAGCGAGTCCAGTA
ZIP4-QF	CAGAGCAGCGGCATGTAGAGA
ZIP4-QR	TACTCCATCCCTCCGTCACCA
ZIP6-QF	TTCTTACTCTGCTCGCGGATTTC
ZIP6-QR	GAAACTTGCGTACCAAGCGACTC
ZIP9-QF	TGCTTACCGGATTTCCCTTGG
ZIP9-QR	GTGTACGTGTCCATGTCCGTGTC
NAS1-QF	ATCTTCCACACAACGGACG
NAS1-QR	ATCTTCCACACAACGGACG
NAS2-QF	AGATCGGACGGTGTGTGG
NAS2-QR	CCTCGATCAAATTCTTCTCCAT
NAS3-QF	CAATTGGGAATGTTGGTGG
NAS3-QR	TGTTCCTCCCTAGCTCCG
NAS4-QF	TGTAATCTCAAGGAAGCTAGGTG
NAS4-QR	CAGTTACACGCGAGATCCG
HMA2-QF	TAAGCCTGAAGCGGTCGAGGAC
HMA2-QR	AGCGGCATGATTATCTCCGGTA

HMA4-QF	CTTAACGGGCGAAGCATTTCC
HMA4-QR	TGCCGCTTTAGTAAGTGCACAGAA
MTP1-QF	GGCTGTTCTGCTAGGGCATGA
MTP1-QR	CCATTCCGGATTGTACCAAATGA
MTP3-QF	GCAGCCTTTGCAATATCTTTG
MTP3-QR	AGATCCCTCCACTTCACCACT
EF1α-QF	GATTGCCACACCTCTCACATTGCAG
EF1α-QR	GCTCCTTCTCAATCTCCTTACCAG
Primers used for promoter-GUS	
IRT3-PF	GAATTCCTTGAGGGACTCTTTGATGCAG
IRT3-PR	ACTAGTCATTTGGGGTCTAAGATGTCCTCGG
ZIP4-PF	GAATTCGTCGAAAACGCTTAAGAAGCTCAA
ZIP4-PR	ACTAGTCATGGGAACAAGAGTTTATTCTTCTTCTTC
ZIP6-PF	AAGCTTGTTGCAGGACGGTGATATTTTC
ZIP6-PR	CATACTAGTCATTTGTGGAAGAAGAAGAAAGAGAGGGAG
ZIP9-PF	GGTACCCACCTGCAAAAACAAAAGCA
ZIP9-PR	AGATCTGCCATAGCTGCGAACTTGAG
Primers used for GFP-fusion	
IRT3-GF	CCATGGTCTTCGTCGATGTTCTTTGGAA
IRT3-GR	ACTAGTAGCCCAAATGGCAAGAGAAGAC
ZIP4-GF	CCATGGTCTTCGTCGATGTTCTTTGGAA
ZIP4-GR	ACTAGTAGCCCAAATGGCGAGAGCAGAC
ZIP6-GF	AGATCTTTCTTGCGTCACCGGAACAGAG
ZIP6-GR	ACTAGTAGCCCAAAGAGCAAGTAGTGAC
ZIP9-GF	AGATCTGTCGATCCTTATCTCCGGAGCT
ZIP9-GR	ACTAGTAGCCCAAATTGCAAGAGCAGAC
Primers used for genotyping	
irt3-1f	TGTCGACATTTAGTTGGCGTGTGG
irt3-1r	TGCAGCGGAATCATCACGACAAAG
Wisc-p745	AACGTCCGCAATGTGTTATTAAGTTGTC
zip4-2f	AAATGGAGCTAGGCATGTTTTGT

zip4-2r	GTAATCTAAGAGCTAAGCCCAAATGGCG
zip6-1f	TCTAAAGATAGTCGCCGTCTTCG
zip6-1r	GTTACCGCGAACATTAAGCACATA
zip9-1f	TGTGAGGCACGTTGTTGTTTC
zip9-1r	ATCGGAGTAGTGATGGCGAAG
LBb1	GCGTGGACCGCTTGCAACT