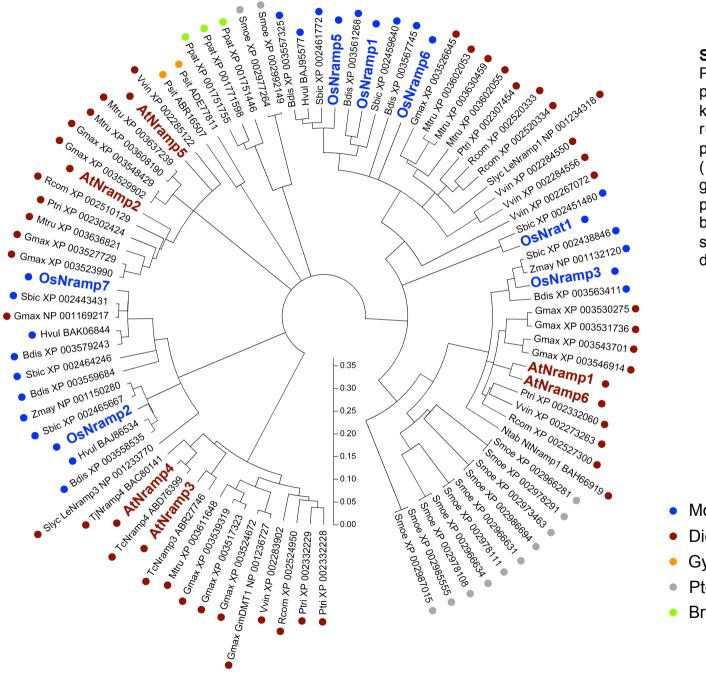


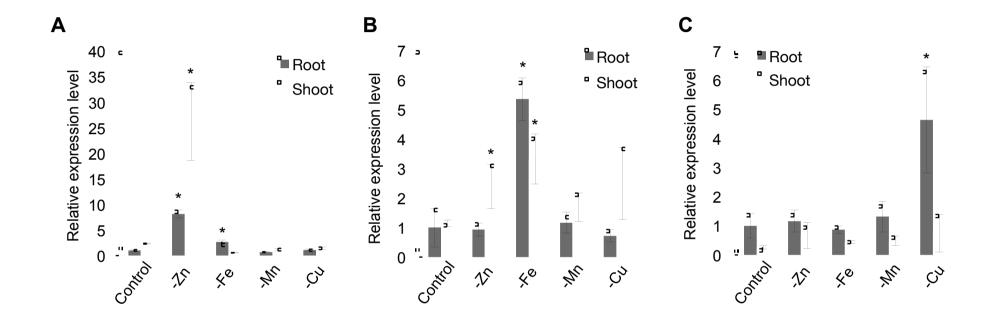
Supplemental Figure 1. Gene structure and knockout line of Os Nramp5.

(A) Gene structure of Os *Nramp5*. Black boxes, gray boxes and lines show exon of untranslated region, exon of coding region and intron, respectively. The location of the T-DNA insertions is indicated by a triangle. The arrows show the Os *Nramp5*-specific primers used for screening homozygous lines. (B) Transmembrane domains predicted with SOSUI program. (C) Expression of Os *Nramp5* in the roots of the wild-type rice (WT) and knockout line (*nramp5*). The expression level was examined by RT-PCR.



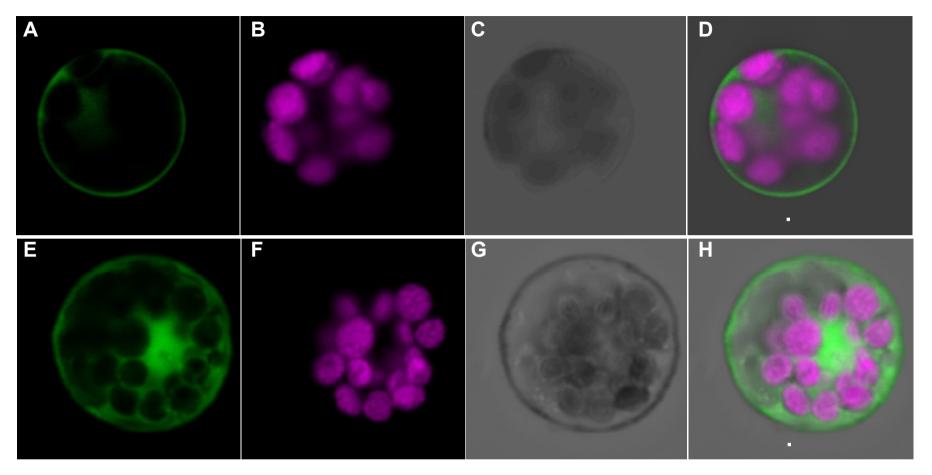
Supplemental Figure 2. Phylogenic tree of Nramp proteins in the plant kingdom. Phylogenetic relationship of Nramp proteins in monocot (blue), dicot (red), gymnosperm (orange), pteridophyta (gray) and bryophyta (green). The scale shows substitution distance.

- Monocot
- Dicot
- Gymnosperm
- Pteridophyta
- Bryophyta

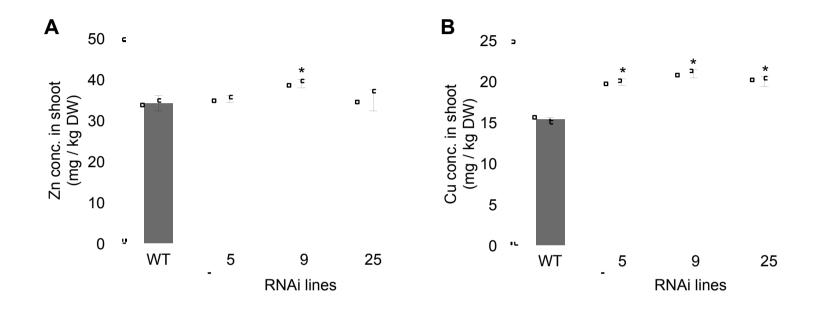


Supplemental Figure 3. Expression pattern of metal deficiency inducible genes.

(A-C) Response of Os *ZIP4* (A), Os *IRT1* (B) and Os *COPT5* (C) expression to metal deficiency. Rice was cultivated in a nutrient solution with (control) or without Zn, Fe, Mn or Cu for one week. The expression level was determined by quantitative real-time RT-PCR. *HistoneH3* and *Actin* were used as internal control. Data are means ±SD of three biological replicates. Asterisks indicate significant difference from the control condition at *P < 0.05 by Dunnett's test.

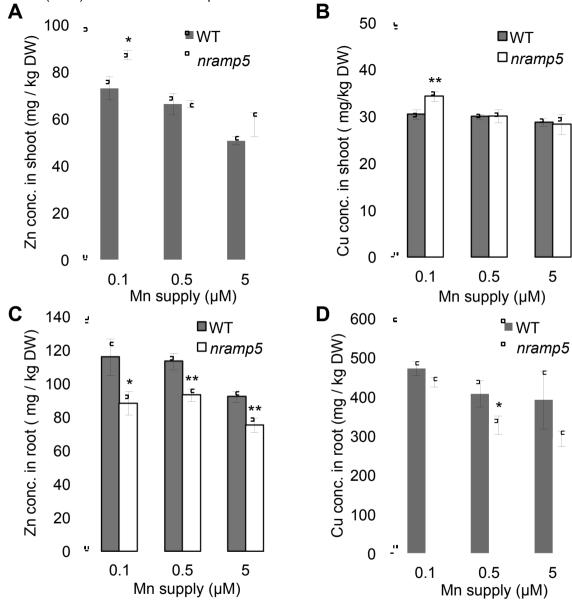


Supplemental Figure 4. Subcellular localization of Os Nramp5 in rice leaf protoplasts.
Os Nramp5-GFP (A-D) and GFP alone (E-H) were transiently expressed in rice leaf protoplasts. (A, E) GFP fluorescence, (B, F) chloroplast autofluorescence, (C, G) bright image and (D, H) merged image. Scale bar = 10 µm.

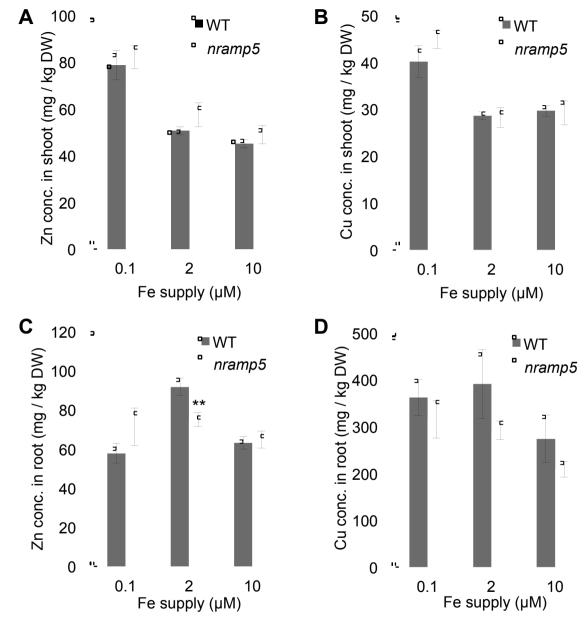


Supplemental Figure 5. Phenotypic analysis of the *Nramp5* knockdown line.

(A, B) Concentration of Zn (A) and Cu (B) in the shoots. Both wild-type rice and three independent RNAi lines were cultivated in a nutrient solution containing $0.5~\mu M$ MnCl₂ and $10~\mu M$ FeSO₄ for 4 weeks. The concentration of Zn and Cu in the shoots was determined by ICP-MS. Data are means $\pm SD$ of three biological replicates. Asterisks indicate significant difference from WT at *P < 0.05 by Dunnett's test.

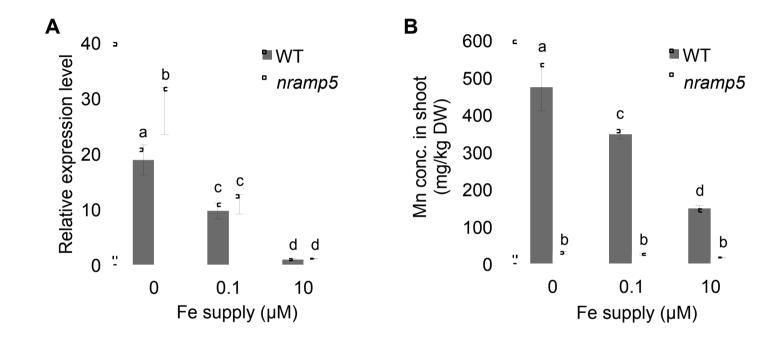


Supplemental Figure 6. Concentration of Zn and Cu in the shoots and roots at different Mn supply. (A, B) Concentration of Zn (A) and Cu (B) in the shoots. (C, D) Concentration of Zn (C) and Cu (D) in the roots. Both the wild-type rice and nramp5 were cultivated in a nutrient solution containing 0.1, 0.5 or 5 μ M MnCl₂ and 2 μ M FeSO₄ for 3 weeks. Data are means \pm SD of three biological replicates. Asterisks indicate significant difference from WT at *P < 0.05 and **P < 0.01 by Student's t-test.

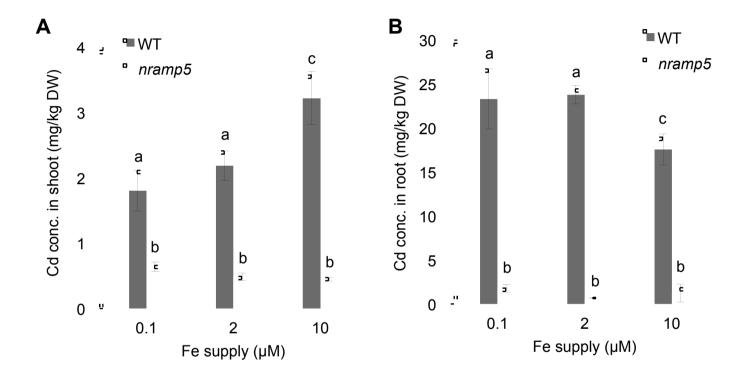


Supplemental Figure 7. Metal concentration of shoots and roots at different Fe supply.

(A, B) Concentration of Zn (A) and Cu (B) in the shoots. (C, D) Concentration of Zn (C) and Cu (D) in the roots. Both the wild-type rice and nramp5 mutant were grown in a nutrient solution containing 0.1, 2 or 10 µM FeSO₄ for 3 weeks. Data are means ±SD of three biological replicates. Asterisks indicate significant difference with WT at **P < 0.01 by Student's t-test.



Supplemental Figure 8. Expression of *IRT1* and Mn accumulation in the *nramp5* mutant at different Fe concentrations. Rice was grown in a nutrient solution with different concentrations of Fe (0, 0.1 or 10 μ M) as FeSO₄ for 7 days. The expression level of Os *IRT1* (A) and shoot Mn concentration (B) were determined by quantitative real-time RT-PCR and ICP-MS, respectively. Data are means \pm SD of three biological replicates. Different letters indicate significant difference at P < 0.05 by Tukey's test.



Supplemental Figure 9. Cd concentration at different Fe supply concentrations.

Concentration of Cd in the shoots (A) and roots (B). Both the wild-type rice and knockout line were grown in a nutrient solution containing 0.1, 2 or 10 μ M FeSO₄ for 3 weeks and further for 4 days in the presence of 0.1 μ M CdSO₄. Data are means ±SD of three biological replicates. Different letters indicate significant difference at P < 0.05 by Tukey's test.