Supplemental Materials

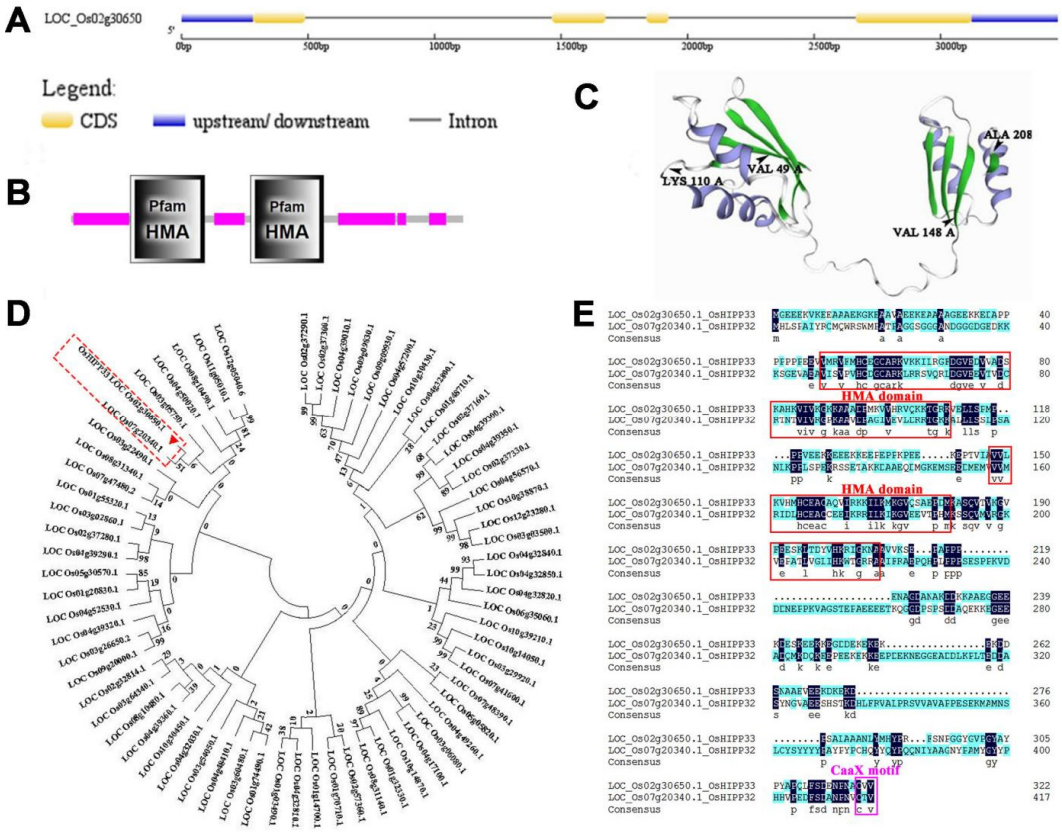


Figure S1. Basic information of *OsHIPP33* gene and protein structures. (**A**) Diagram of the OsHIPP33 gene structure. (**B**) Predicted HMA domains of *OsHIPP33* by Simple Modular Architecture Research Tool (SMART, http://smart.embl-heidelberg.de). (**C**) Three-dimensional structure of *OsHIPP33*. (**D**) Phylogenetic analysis of *OsHIPP33* homologous genes in rice. The numbers at branching sites indicate the posterior probability values for nodal support. The sequences of rice genes are from rice genome database (http://rice.plantbiology.msu.edu). The red triangles indicate *OsHIPP33*. (**E**) Alignment of amino acid sequences for *OsHIPP33* (LOC_Os02g30650.1) and *OsHIPP32* (LOC_Os07g20340.1). The red rectangle represents two HMA domains and CaaX notif. The neighbor-joining phylogenetic tree was constructed using MEGA 6.0. Sequence alignment was performed by DNAMAN.

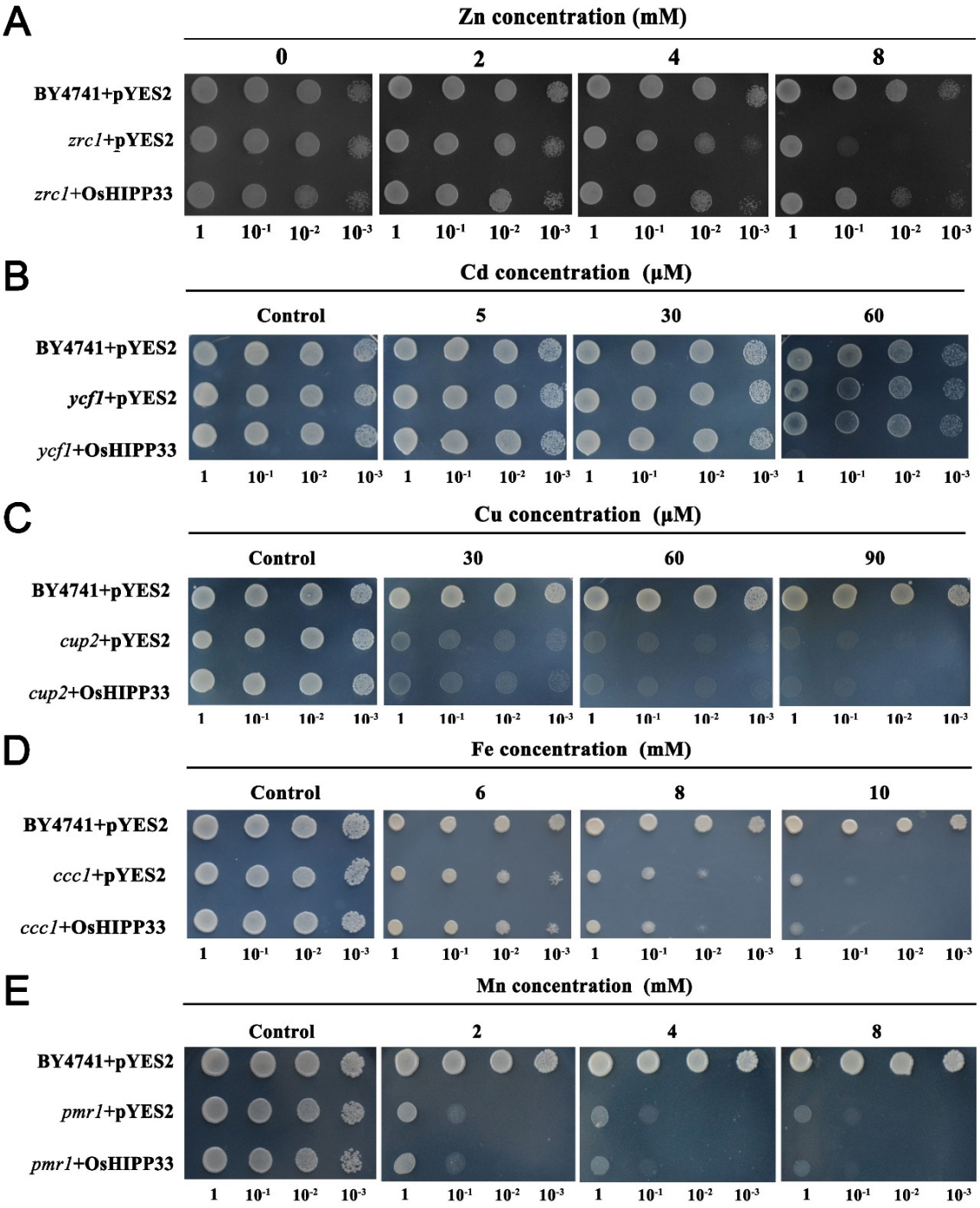


Figure S2. Transport activity of *OsHIPP33* in yeast. Yeast strains BY4741, *Δycf1*, *Δcup2*, *Δccc2* and *Δpmr1* transformed with empty vector pYES2 (Vector) or *OsHIPP33* were cultivated on varied concentrations of Cd, Cu, Fe and Mn as indicated. Eight microliters of cell suspension with an OD 600 nm of 0.5 and four serial 1:10 dilutions were spotted and incubated at 30 °C for 3 d.

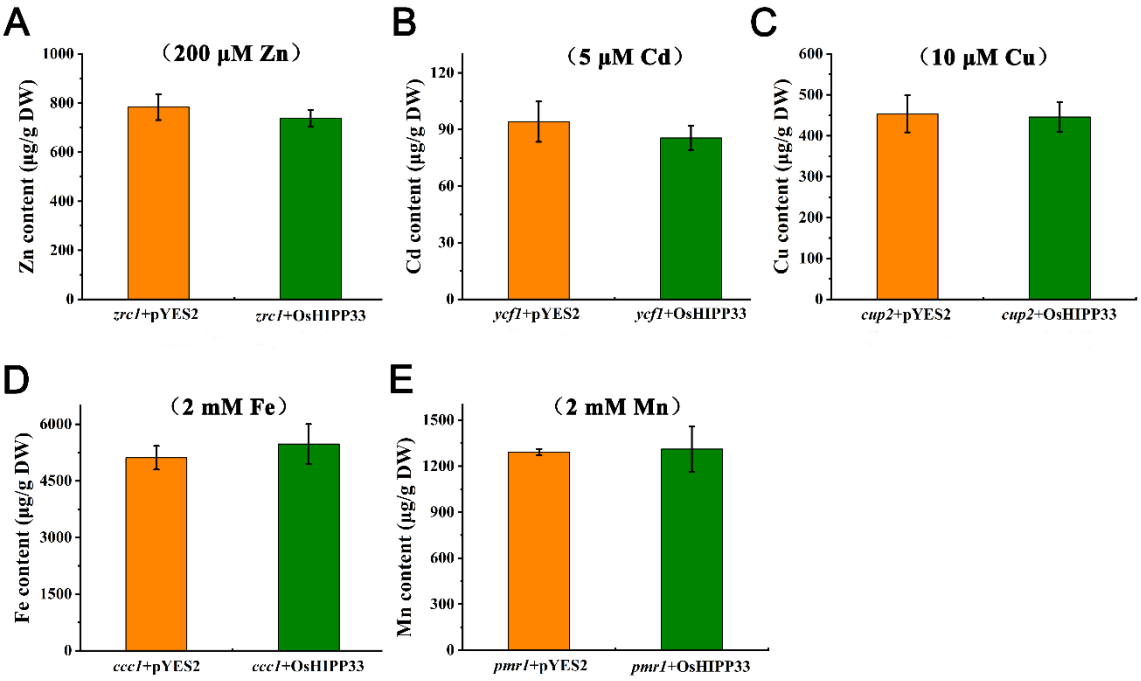


Figure S3. The metal concentrations in the yeast cells after *OsHIPP33* transformation. Zn, Cd, Cu, Fe, and Mn were absorbed by cells in the liquid medium. The yeast BY4741 and *zrc1*, *ycf1*, *cup2*, *ccc1* and *pmr1* carrying pYES2 or with pYES2 vectors containing the full *OsHIPP33* cDNA were cultured in a liquid medium containing 2% Gal in the presence of 200 μM Zn, 5 μM Cd, 10 μM Cu, 2 mM Fe, and 2 Mm Mn for 48 h, respectively. The metal concentration in the yeast was determined with ICP-MS after digestion. Data are means of three biological replicates.

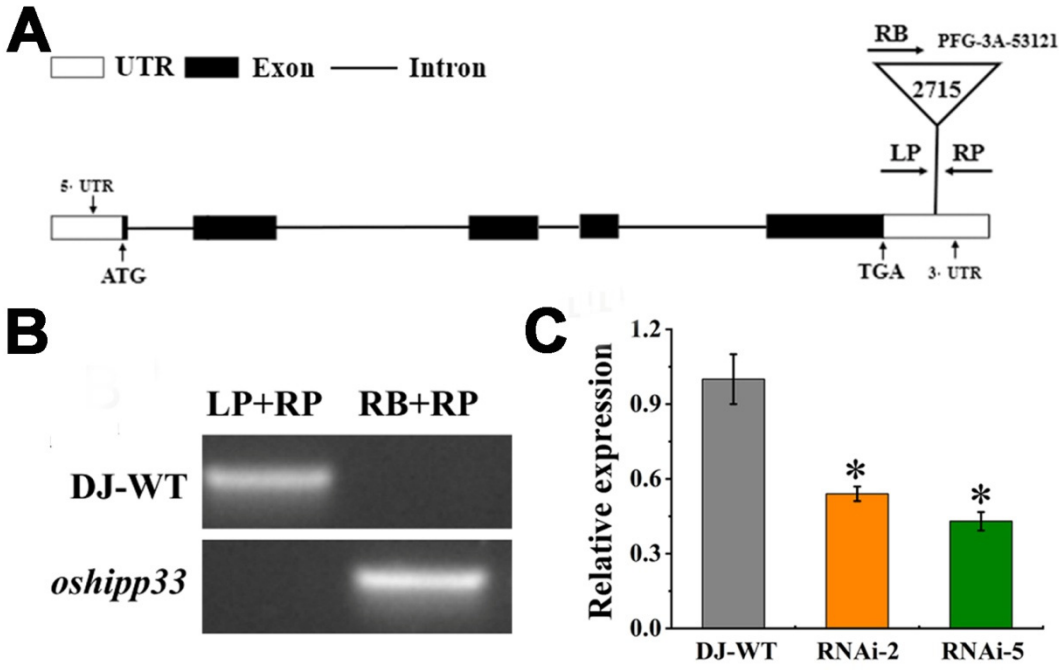


Figure S4. Analysis of *OsHIPP33* transcriptional expression in the mutant lines by qRT-PCR. (**A**) Schematic diagram of *OsHIPP33* mutant insertion sites. The triangle indicates the insertion site of the T-DNA. LP and RP are the primers on the genome near the insertion site. RB is the universal identification primer on the T-DNA. The white square boxes are the UTR region. The black square boxes are exons, and the black lines are introns. (**B**) Homogeneous identification of *OsHIPP33* mutants. (**C**) qRT-PCR analyses of *OsHIPP33* transcripts in the two RNAi lines. The vertical bars indicate the means of biological triplicates. Asterisks show a significant difference between the mutants and WT ($p < 0.05$).

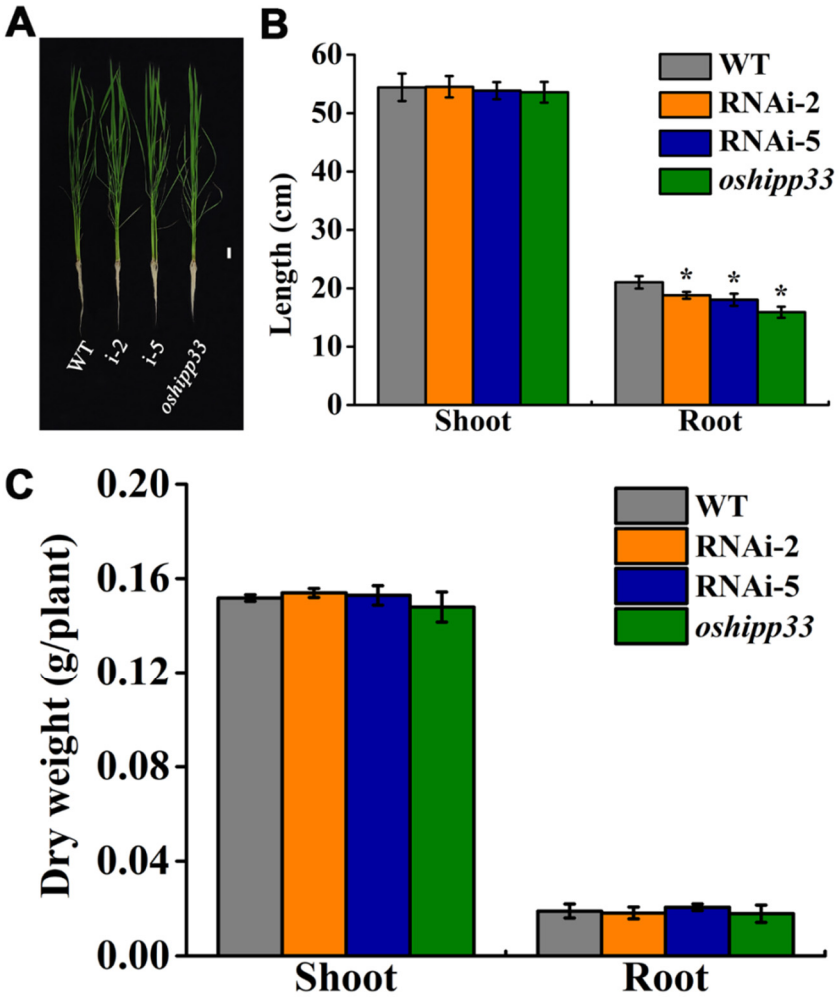


Figure S5. Growth phenotypes of *OsHIPP33* mutant, RNAi lines and wild-type under the normal Zn, Fe, Cu and Mn supply conditions. The germination rice seedlings were grown in the nutrient solution with the normal metals as mineral nutrients (0.4 μM Zn, 20 μM Fe, 0.2 μM Cu and 0.5 μM Mn) for four weeks. The rice plants were photographed. **A**: Growth phenotypes, **B**: the shoot and root length and **C**: dry weight of *OsHIPP33* mutant and RNAi lines (scale bars: 3 cm). The vertical bars indicate the means of biological triplicates. Asterisks show a significant difference between the mutants and WT ($p < 0.05$).

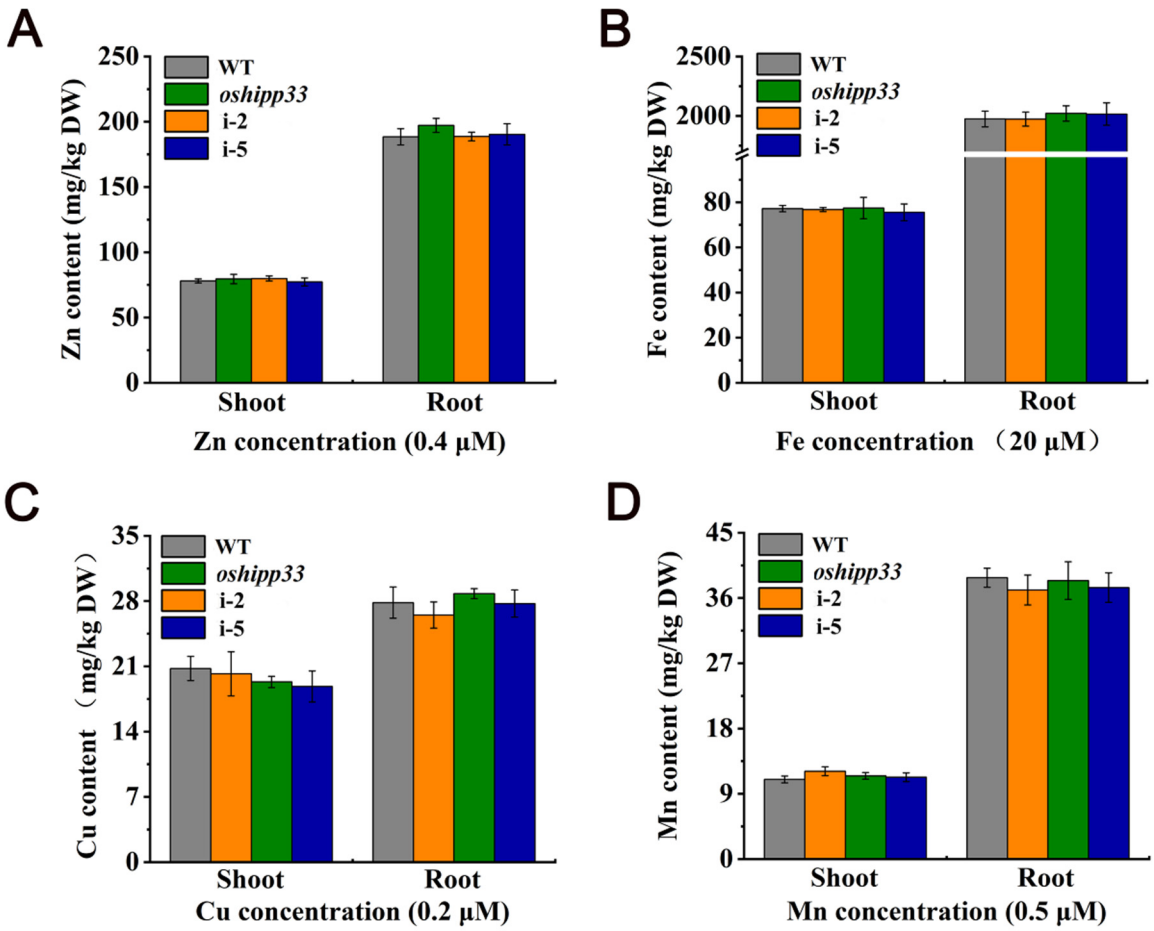


Figure S6. The concentrations of Zn, Fe, Cu and Mn in shoots and roots of *OsHIPP33* mutant, RNAi (i2 and i5) lines and wild-type (WT) under the normal conditions of Zn, Fe, Cu and Mn supply. The germination rice seedlings were grown in the nutrient solution with the normal metals as mineral nutrients (0.4 μM Zn, 20 μM Fe, 0.2 μM Cu and 0.5 μM Mn) for four weeks. (**A–D**) Zn, Fe, Cu and Mn concentrations analyzed in WT and *OsHIPP33* mutants and RNAi lines. The vertical bars indicate the means of biological triplicates.

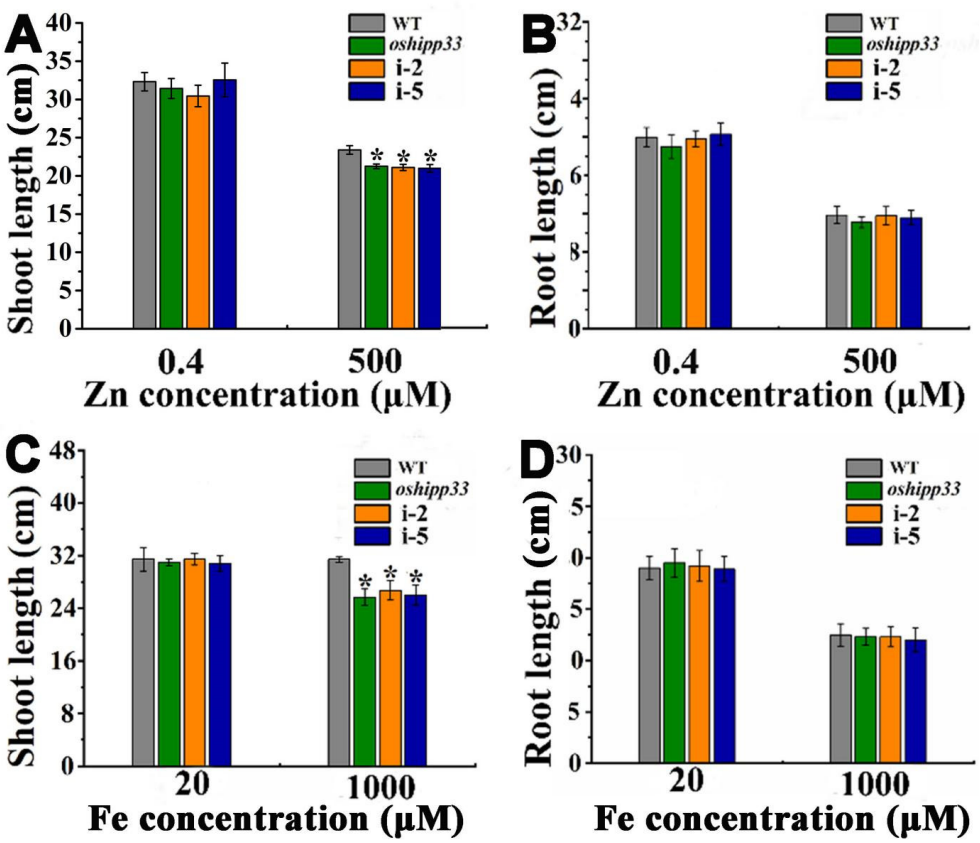


Figure S7. Growth phenotypes of *OsHIPP33* mutant, RNAi lines and wild-type (WT) under excessive Zn and Fe stress. Two-week-old seedlings were exposed to the nutrient solution containing 0.4 μM Zn (normal) and 500 μM Zn (excess)(**A, B**) or 20 μM Fe (normal) and 1000 μM Fe (excess)(**C, D**) for two weeks. When harvested, the rice plants were photographed and phenotypically analyzed with shoot and root length. The vertical bars indicate the means of biological triplicates. Asterisks show a significant difference between the mutants and WT ($p < 0.05$).

Table S1. Primer sequences used for this study.

Primer name	Used for	Primer sequence (5′ to 3′)
OsHIPP33-F	qRT-PCR	GCTGCTGCTAATCTGTATATGC
OsHIPP33-R	qRT-PCR	TTTGGATTCTCGTCACTGAAGA
OsHIPP33-F	eGFP vector	GGGGTACCATGGGCGAGGAGGAGAAGAAG
OsHIPP33-R	eGFP vector	CGGGATCCTCACATCACAACACAGGCG
OsHIPP33-F	*pan*580-GFP	GACTAGTATGGGCGAGGAGGAGAA
OsHIPP33-R	*pan*580-GFP	CGGGATCCCATCACAACACAGGCG
OsHIPP33-F	ORF	ATGGGCGAGGAGGAGAAGAAG
OsHIPP33-R	ORF	TCACATCACAACACAGGCGTTTG
33-RNAi-Forward-F	RNAi vector	GGGGTACCAGCTCACCGATTACGTG
33-RNAi-Forward-R	RNAi vector	CGAGCTCCATCACAACACAGG
33-RNAi-Reverse-F	RNAi vector	CGGGATCCAGCTCACCGATTACGTG
33-RNAi-Reverse-R	RNAi vector	AACTGCAGCATCACAACACAGG
OsHIPP33-LP	mutant-identification	TACCCAGCGTTTACCGTAGG
OsHIPP33-RP	mutant-identification	TGCCATACTCCCAGGAAAAG
OsActin		GCCATTCGCCTCCTTCTTG
OsActin		CCTACTTCAGCAACTCAGTTC
OsUbi10		TCACCAGGCTCAGGAAGGA
OsUbi10		AGCAGCAGCAAGCATCAAC