

# Supporting Information

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## SI Materials and Methods

**Plasmid Construction.** The cDNA fragment containing the *OsLCT1* ORF was amplified from cDNA synthesized from rice seedlings (*Oryza sativa* L., cv. Nipponbare) by PCR using the primers 5'-CACCATGACCGGCGAGGCAAGCAAAG-3' and 5'-CTATGACTTCATAACTTCGACGCC-3'. The forward primer contained the sequence CACC (underlined) for subsequent TOPO cloning. The resulting product was cloned into a Gateway pENTR/D-TOPO cloning vector (Invitrogen) to produce pSU01. To construct the yeast *OsLCT1* expression vector, *OsLCT1* cDNA was subcloned into pESC-URA (Agilent Technologies) to produce pSU05; however, yeast transformed with pSU05 did not show *OsLCT1* accumulation by Western blotting using anti-Myc antibodies (MBL). To facilitate *OsLCT1* expression in yeast, a construct was designed containing *OsLCT1* cDNA with optimized codon use for yeast in the N-terminal region of the cDNA. The fragment containing *OsLCT1* cDNA was first amplified from pSU01 with the primers 5'-GAAGGTA-GAGCTTCTTCTTCTAGACAAAGAAGTATGTTGCTTC-TGAATTGGAATTGCAAGAAGCATCAAGCTCCGC-3' and 5'-CTAATACGACTCACTATAGG-3'. The obtained product was used as a template for PCR to amplify the full-length *OsLCT1* cDNA by using modified codons with the primers 5'-ccgctcgag-ATGACTGGTGAAGCTTCTAAAGGTAGATCTGGTGGTGGTAGAGAAGGTAGAGCTTC-3' and 5'-gactagtCTATGACTTCATAACTTCGACGCC-3'. The underlined sequences were designed to optimize the codon use for yeast without amino acid substitutions. The sequences shown in lowercase contained XhoI and SpeI recognition sites, respectively. The obtained product was digested with XhoI and SpeI and then inserted into the XhoI-NheI site in pESC-URA (Agilent Technologies). The resultant plasmid (pSU28) was used for yeast transformation.

To construct *35S-LCT1-sGFP*, *OsLCT1* cDNA without a stop codon was amplified using the primers 5'-CACCATGACCGGCGAGGCAAGCAAAG-3' and 5'-TGACTTCATAACTTCGACGCC-3'. The forward primer contained the sequence CACC (underlined) for subsequent TOPO cloning. The resulting product was cloned into a Gateway pENTR/D-TOPO cloning vector (Invitrogen); the obtained plasmid, pSU39, was sequenced and then subcloned into pGWB505 (1) using Gateway technology. The resultant plasmid was named pSU40. To construct *35S-sGFP*, the *sGFP* ORF was amplified from pTF521 (2) using the primers 5'-CACCATGGTGAGCAAGGGCGAGG-3' and 5'-TTACTTGTACAGCTCGTCCATGCC-3'. The resulting product was cloned into a Gateway pENTR/D-TOPO cloning vector (Invitrogen); the obtained plasmid, pTS99, was then sequenced. Next, *sGFP* cDNA was subcloned into pMDC32 (3) using Gateway technology to produce pTS100. The obtained plasmids were used to transform tobacco BY-2 cells.

To construct the RNAi vector for *OsLCT1*, a 527-bp fragment of *OsLCT1* cDNA was amplified with the primers 5'-CACC-ATGACCGGCGAGGCAAGCAAAG-3' and 5'-TAGAAGAG-GCACTTGGAGATGG-3'. The forward primer contained the sequence CACC (underlined) for subsequent TOPO cloning. The resulting PCR product was cloned into a Gateway pENTR/D-TOPO cloning vector (Invitrogen), and the obtained plasmid (pSU15) was sequenced then subcloned into pG121-RNAi-DEST (4) using Gateway technology. The resulting plasmid, pSU19, was used for rice transformation.

**Transformation of BY-2 Cells.** Tobacco BY-2 Cells (7-10 d after transfer) were transformed with *Agrobacterium tumefaciens* strain

EHA101 (Rif<sup>r</sup>) pEHA101 (Km<sup>r</sup>) containing pGWB505 with the *OsLCT1* ORF without a stop codon (*35S-OsLCT1-sGFP*) or pMDC32 with the *sGFP* ORF (*35S-sGFP*). After 3 d of co-incubation, the cells were washed with 3% sucrose then cultured in 30 mL of liquid medium with 500  $\mu\text{g mL}^{-1}$  cefotaxime for 5 d. The cells were then transferred to liquid medium containing 500  $\mu\text{g mL}^{-1}$  cefotaxime and 50  $\mu\text{g mL}^{-1}$  hygromycin for 5 d.

**Yeast Transformation and Functional Analyses.** pAG32 (5) was amplified with 5'-CATGTGGGTTGGCGTGATTATACTAGTT-ATTATGATGCCAAGCTGAAGCTTCGTACGCTG-3' and 5'-CCAGATAAGGAGATCCCTTTCTCGCCAACTAATGTCTT-ATCTCCATAGGCCACTAGTGGATCTG-3'. The amplified fragment was transformed into *Saccharomyces cerevisiae* strain W303-1A (*MATa, ura3, leu2, ade2, his3, trp1, can1-100*) to disrupt *Ycf1*. The transformants (named *W $\Delta$ ycf1*) were selected using yeast/peptone/dextrose (YPD) medium containing 300  $\mu\text{g mL}^{-1}$  hygromycin. The obtained yeast cells (*W $\Delta$ ycf1*) were then transformed with pESC-URA (Agilent Technologies) or pSU28 and the transformants were selected on SD-URA medium.

For the plate assay, the yeasts were grown with the arginine-phosphate medium containing 2% galactose for over-night. Five microliters of the cell suspension (OD = 1) and 10-fold dilution series were spotted on arginine-phosphate plates containing 2% galactose and 1% agar with 5 or 20  $\mu\text{M CdCl}_2$ . The plate without Cd was prepared as a control. The yeast was grown on the plates at 30 °C for 3 d.

For transport activity assay, the yeasts were grown in arginine-phosphate medium containing 2% galactose until the mid-log phase. The harvested and resuspended cells were incubated with arginine-phosphate medium containing 10, 20, or 100  $\mu\text{M CdCl}_2$  for 75 min. For excess Ca or Mg treatments, the cells were incubated with the arginine-phosphate medium supplemented with 1 mM  $\text{CaCl}_2$  or 10 mM  $\text{MgSO}_4$  for 1 h before Cd treatment. The cells were then incubated with excess Ca or Mg medium supplemented with 10  $\mu\text{M CdCl}_2$  for 75 min and harvested. After three washes with ice-cold 2 mM  $\text{CaCl}_2$ , the metal concentrations in the acid-digested cells were determined by inductively coupled plasma mass spectrometry (ICP-MS) (Model SPQ9700; SII NanoTechnology, Seiko).

**Preparation of the Microsomal Fraction and Western Blotting.** Yeast strain *W $\Delta$ ycf1* was transformed with pSU28 or pESC-URA, and microsomal fractions were prepared as described (6). Five micrograms of the prepared microsomes were immunoblotted with anti-Myc antibodies (MBL).

**Microarray Analysis.** The dataset from an *OsLCT1* microarray analysis was obtained from RiceXPro (7). Briefly, total RNAs were extracted from various rice organs at different stages of growth and used for analysis. A rice 44K oligo microarray (Agilent Technologies) with 42,000 oligonucleotides based on the Rice Annotation Project was used. The 3'-UTR of *OsLCT1* on the 44K oligo microarray was used to detect the *OsLCT1* signal.

**Histochemical Cd Staining.** Histochemical staining of Cd was performed according to a previous method (8) with a slight modification. Grains of vector control (108-1) and RNAi (118-9) plants were randomly selected from the main panicle and used for histochemical analysis after husking. The grains were immersed in 70% then 99.5% ethanol for 1 h, respectively, and washed with 50 mM borate buffer (pH 10). The samples were then incubated for 1.5 h in 0.33 mM Tris(2-aminoethyl)amine and 0.66 mM triethylenetetramine to prevent the staining of other heavy metals, in-

cluding Zn, Cu, Cr, and Pb. After masking, the samples were washed with 50 mM borate buffer (pH 10) and immersed in a 2-(8-quinolyazo)-4,5-diphenylimidazole (QAI) solution for 3 d.

**Collection and Analysis of Xylem Sap and Phloem Sap.** For xylem sap collection, vector control and RNAi plants (1-wk-old;  $T_1$ ) were grown for 3 wk hydroponically and exposed to 0.2  $\mu\text{M}$   $\text{CdCl}_2$  for 6 h and then xylem sap was collected for 2 h (9). Concentration of Cd in the sap was analyzed by ICP-MS.

For phloem sap collection, vector control and RNAi plants (1-wk-old;  $T_1$ ) were grown for 2 mo with a hydroponic solution containing 0.2  $\mu\text{M}$   $\text{CdCl}_2$ . The seventh and eighth leaf blades were removed from the plants, and the cut end was recut under 20 mM  $\text{Na}_2\text{EDTA}$  (pH 7.5) solution and kept for 1 min. Then, the leaves were left for 3 h in a dark growth chamber at 25 °C and >85% relative humidity, with the cut end of the leaf immersed in 100  $\mu\text{L}$  of the EDTA solution. The amount of Cd and K in the EDTA solution was determined by ICP-MS.

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OsLCT1      1  -----MTGEASKGRSGGREGRASSS-----RQRTDV
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TaLCT1     61  AGDGLMKAPPPPPPPPPPTARWSVAGGSLMRAPPIPLSRERLALPYQDGEPPATTTDDL

OsLCT1     59  AIPPAPAPLHDLAQGAEAAS-----AAGRSNEKE---EQV
TaLCT1    121  SMRPTSSPPPTSAEETQGARRSSVSPAPVTTGMATSRGPSTLIEAEEGRATERKEIVVKL

OsLCT1     91  ELRKVQKDIELGALVAGFSFSVAMTGFFLSQATGRQAIYIDISMFLAFSSFVCGCTFML
TaLCT1    181  LKARAKDNLELGGIAAIFGFAVLFGWSCFPEEMKRPGNLKFIFSLLLAIATFFSGTALTL

OsLCT1    151  LRMQRLSAREEHISGFHHAISKCLFYLCCVPVLTILCLLLVMPRKPYIYVGLGVLAAAV
TaLCT1    241  LSMNIVGLPESLVSAGQLVASKCLFLICTALSAMTLVSLLALLPSMLYLCLGLVVMTVV

OsLCT1    211  VEFVALMHWYVSRKTQLETNDTAPEDVEQNAMSRKTQETNGTAPEDDDEQKAMESSYKITS
TaLCT1    301  LPAIVVHCYMRRETEGGD-----EAAALEEHKEELEAASKITS

OsLCT1    271  AIVPMSLAGLVGVLFGVYKGSSSGGAGDISGSVHVVIMCMFITSMLSMLLMLMLWMKVL
TaLCT1    339  CVTNSAFGGLVGVLFSASKSKVSG-----APTAVYTAMFFMFTAIFGMVVMTMSKKVS

OsLCT1    331  ESKKPKLREFFVRATIPRANAALLALLAIAAFAASFGILRWYMVAAFLTLALAATVQFVI
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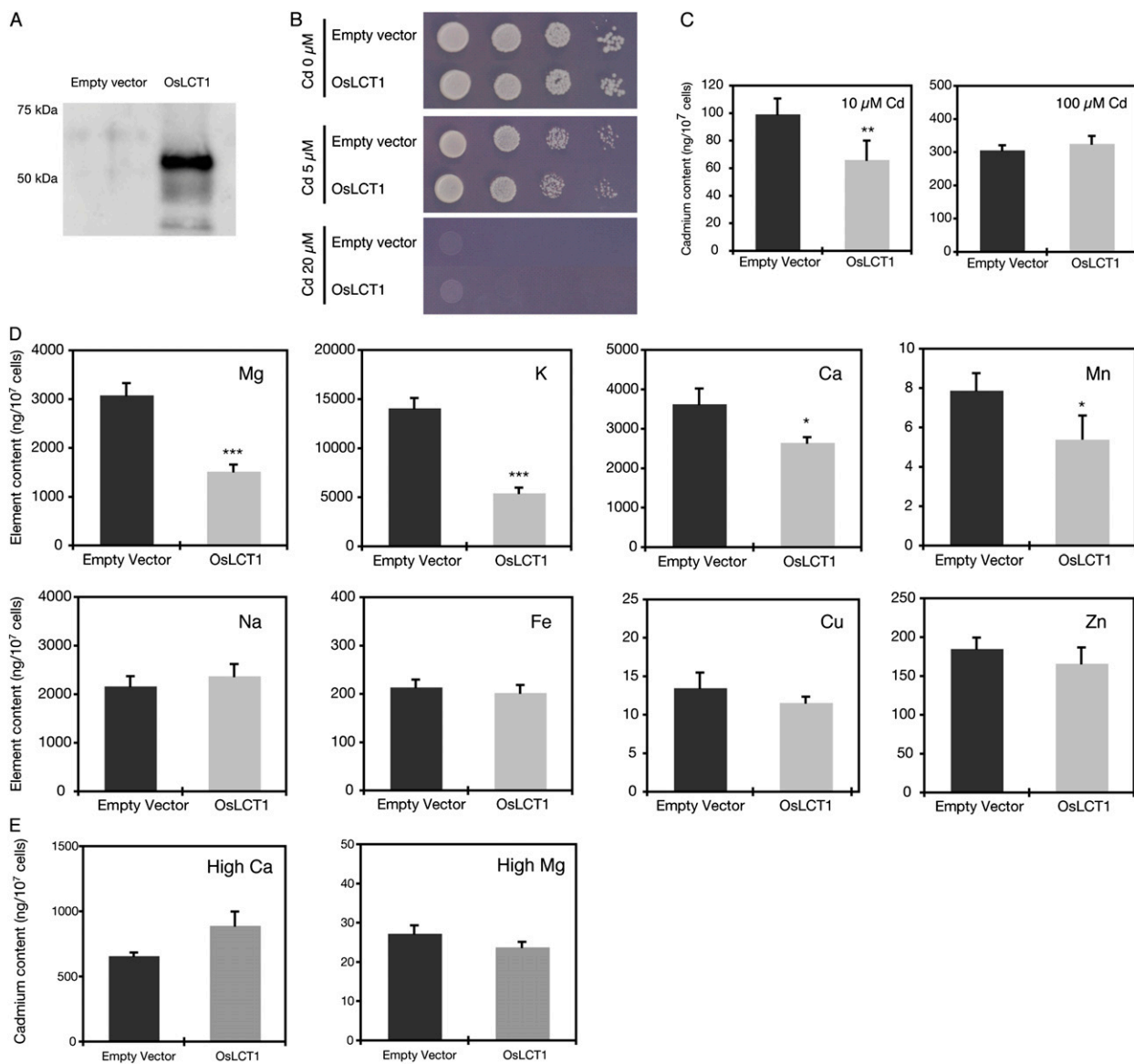
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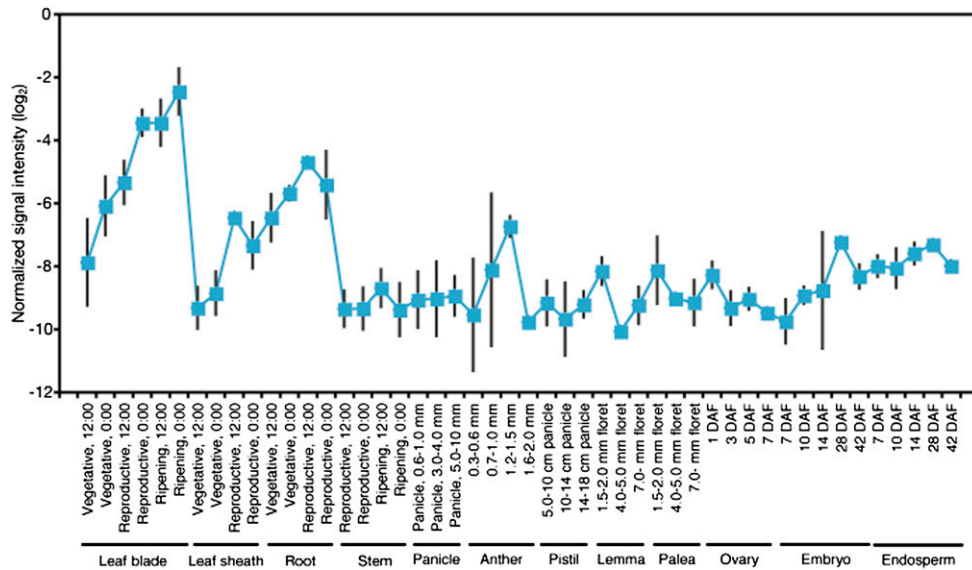
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TaLCT1     568  ISRAVSO

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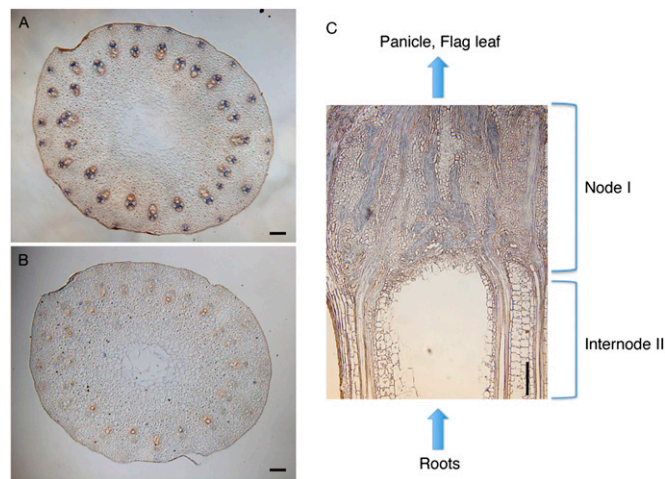
Fig. S1. Alignment of OsLCT1 and TaLCT1 amino acid sequences. Transmembrane domains in OsLCT1 and TaLCT1 (underlined) were predicted using ConPred II (8).



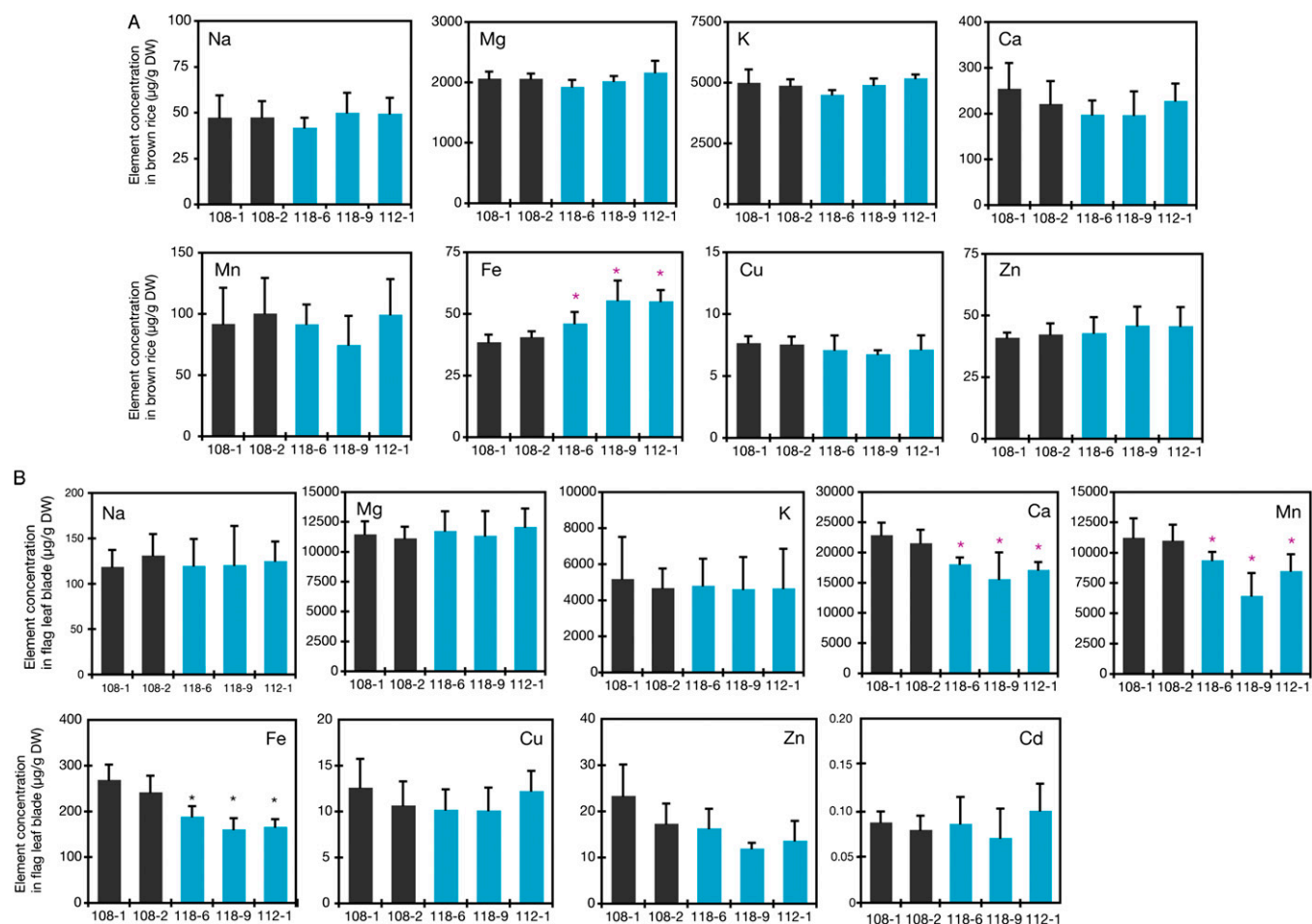
**Fig. S2.** Analysis of OsLCT1 in yeast. (A) Western blot analysis of microsomes prepared from *W $\Delta$ ycf1* yeast carrying Myc-tagged *OsLCT1* cDNA or empty vector. Anti-Myc antibodies were used for immunoblotting. (B) Growth of the yeast cells with empty vector or with OsLCT1 on the arginine-phosphate medium containing 2% galactose and 5 or 20  $\mu\text{M}$   $\text{CdCl}_2$ . The medium without Cd was prepared as a control. Yeasts were incubated for 3 d at 30  $^\circ\text{C}$ . (C) Cd content in yeasts with or without OsLCT1 after a 75-min exposure to 10 or 100  $\mu\text{M}$   $\text{CdCl}_2$ . Asterisks represent a significant difference from the empty vector cells ( $P < 0.01$ ). The data are presented as means  $\pm$  SD ( $n = 3$ ). (D) Mineral contents in yeasts with or without OsLCT1. Asterisks represent a significant difference from the empty vector cells ( $*P < 0.05$ ;  $***P < 0.001$ ). The data are presented as means  $\pm$  SD ( $n = 3$ ). (E) Cd content in yeasts with or without OsLCT1 exposed to the arginine-phosphate medium containing 10  $\mu\text{M}$   $\text{CdCl}_2$  and excess Ca (1 mM  $\text{CaCl}_2$ ) or Mg (10 mM  $\text{MgSO}_4$ ) supplementation. The data are presented as means  $\pm$  SD ( $n = 4$ ).



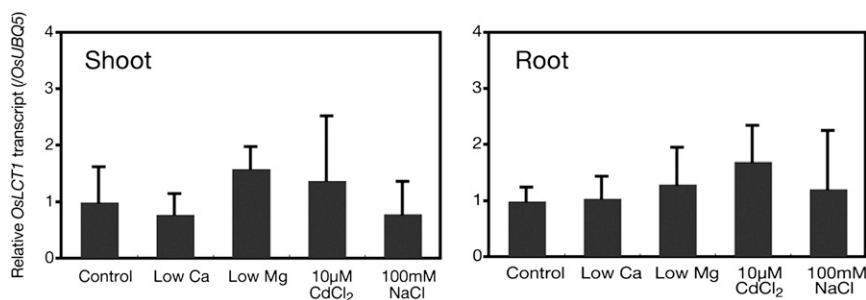
**Fig. S3.** *OsLCT1* expression analysis using a rice 44K oligo microarray. The 3'-UTR of *OsLCT1* on the 44K oligo microarray was used to detect the *OsLCT1* signal. Total RNAs extracted from various tissues of cv. Nipponbare were used for hybridization. The normalized (75 percentile) data were plotted with a SE bar of three biological replicates.



**Fig. 54.** *OsLCT1* expression in node I by in situ hybridization. (A and B) Cross-section of the bottom of node I treated with the antisense probe (A) or the sense probe (B). (C) Longitudinal section of node I and internode II treated with the antisense probe. (Scale bars = 100  $\mu$ m.)



**Fig. S5.** Mineral nutrient concentrations in grains (A) and flag leaf blades (B) of vector control and RNAi-mediated knockdown plants. Plants were grown in a pot until grain ripening, and harvested plant samples were used for elemental determination by ICP-MS. The data are presented as means  $\pm$  SD ( $n = 4-6$ ). Asterisks represent a significant difference from both control lines ( $P < 0.05$ ).



**Fig. S6.** *OsLCT1* expression in shoots and roots of rice under various mineral stress conditions. Plants were grown with a complete hydroponic solution, and 2-wk-old plants were subjected to each treatment for 24 h. Expression of *OsLCT1* was quantified by real-time PCR. The data are presented as means  $\pm$  SD ( $n = 4$ ). There was no significant difference between control and each treatment ( $P > 0.05$ ).

**Table S1.** Metal concentrations in the soil used for the pot experiment

	Metal concentration (mg kg <sup>-1</sup> dry wt.)				
	Cd	Cu	Fe	Mn	Zn
Mean	0.198	0.803	66.0	95.3	2.81
SD	0.003	0.011	0.6	0.6	0.02