Supporting Information

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SI Materials and Methods

Plant Materials and Growth Conditions. Seeds of wild-type rice (cv. Dongjin) and two T-DNA insertion mutant lines (*osabcc1-1* and *osabcc1-2*) were soaked in water overnight at 30 °C in the dark and then transferred to a net floating on a 0.5 mM $CaCl_2$ solution. On day 7, seedlings were transferred to a 3.5-L plastic pot containing half-strength Kimura B solution (1). The pH of this solution was adjusted to 5.6, and the nutrient solution was renewed every 2 d. Plants were grown in a closed greenhouse at 25–30 °C under natural light. At least three replicates were performed for each experiment.

Isolation of *OsABCC1* Knockout Lines. Two *OsABCC1* T-DNA insertion lines, *osabcc1-1* (PFG_4A-02789) and *osabcc1-2* (PFG_3A-13532), were obtained from the Rice T-DNA Insertion Sequence Database center. Each homozygous line was selected from heterogeneous *osabcc1-1* and *osabcc1-2* individual plants using T-DNA border primers [left border (LB), AACGTCGGCAATGTGTTATTAAG, and right border (RB), CCACAGTTTTCGCGATCCAGACTG] and gene-specific primer sets (LP1, GAGCAGATTTGCCCTGAGAG, and RP1, ATGTAACCGCTCCAAGATGG for *osabcc1-1*; LP2, CAATCAGCTGCATTTTTCTACCA, and RP2, CCAAAAGAAAGAAGGCCGTA, for *osabcc1-2*). To confirm that the T-DNA mutant lines were *OsABCC1* knockouts, RT-PCR was performed using gene-specific primer sets (RT-F, GGTTGATGATTGTGACACTTC, and RT-R, AGCGAGTGTATCATCAATTTCGG), and cDNA was synthesized from total RNA of each knockout line. Actin gene was used for RT-PCR control.

Expression Patterns. To investigate the expression pattern of *OsABCC1*, different organs were sampled from plants grown in a paddy field at different growth stages, and cDNA was prepared as described before (2). Quantitative real-time PCR was performed with the following primer sets for *OsABCC1*: 5′-AACAGTGGCTTATGTTCCTCAAG-3′ and 5′-AACTCCTCTTTCTCCAATCTCTG-3′ on CFX384 (Bio-Rad). *HistoneH3* and *Actin* were used as internal standards with primer pairs 5′-AGTTTGGTCGCTCTCGATTTCG-3′ and 5′-TCAACAAGTTGACCACGTCACG-3′ for *HistoneH3*, and 5′-GACTCTGGTGATGGTGTCAGC-3′ and 5′-GGCTGGAAGAGGACCTCAGG-3′ for *Actin*. The relative expression was normalized based on these two genes by ΔΔCt method using the CFX Manager software (Bio-Rad).

The effect of arsenic (As) or cadmium (Cd) on *OsABCC1* expression was investigated by exposing 17-d-old plants to half-strength Kimura B solution containing 0, 0.5, or 5 μM As (III), or 5 μM Cd. The seedlings were cultured in a growth chamber (12 h light/28 °C, 12 h dark/28 °C). After 24 h, the roots, shoot basal region (8 mm), and shoots were sampled and subjected to RNA extraction. Total RNA was extracted using an RNeasy Plant Mini Kit (Qiagen), subjected to DNase I treatment, and converted to cDNA using ReverTra Ace (TOYOBO). For quantitative real-time RT-PCR analysis, the primers designed to amplify *OsABCC1* (described above) were used. *HistoneH3* (amplified with primers 5′-GGTCAACTTGTTGATTCCCCTCT-3′ and 5′-AACCGCAAAATCCAAAGAACG-3′) was used as an internal control. Experiments were performed using the Mastercycler ep Realplex Real-Time PCR System (Eppendorf).

Furthermore, node I was separated by laser microdissection (Applied Biosystems Arcturus Laser Capture Microdissection System, Life Technologies) into enlarged vascular bundles (phloem and xylem regions) and diffuse vascular bundles, after the samples were fixed in a solution containing ethanol/acetic acid at a ratio of 3:1 (3). Total RNA was extracted using a PicoPure RNA Isolation Kit (Applied Biosystems) followed by DNase I (Qiagen) treatment and was then converted into cDNA using SuperScript III (Invitrogen). The expression level of *OsABCC1* and two marker genes (*OsLsi6* and *OsMHA2*) in different tissues was investigated as described above. The primers used for *OsLsi6* and *OsMHA2* were described previously (4, 5).

As Resistance Test in Yeast Strains Expressing *OsABCC1*. To express *OsABCC1* in yeast, the coding sequence (CDS) of *OsABCC1* was synthesized commercially (Cosmogentech.com). Because cloning of *OsABCC1* cDNA was unsuccessful, the DNA structure of the endogenous *OsABCC1* CDS (*LOC_Os04g52900*) was analyzed using the DNASIS (V7.06) program, to determine whether the gene had many hairpin loops and palindrome structures. Interestingly, the *OsABCC1* CDS had 21 hairpin loops (>6 bp of stem length) and 22 palindrome structures (>10 bp in length), which could give rise to strong secondary structures. To remove the secondary structures, 25% of the gene sequence was changed. The synthesized *OsABCC1* was cloned into the BamHI and XhoI sites of the pPGK vector, a modified pYES2/NTC (Invitrogen) vector in which the galactokinase promoter was substituted with the phosphoglycerate kinase (PGK) promoter. SM4 (*ycf1::His3, yhl035c::HIS3-MX6,yll015w::Kan-MX6, yll048c::TRP1-MX6*) and SM7 (*ycf1:: His3, yhl035c::HIS3-MX6, yll015w::Kan-MX6, yll048c::TRP1-MX6, TaPCS1::cup1-1*) strains (6) transformed with pPGK–OsABCC1 or pPGK empty vector were cultured in synthetic glucose liquid medium without uracil amino acid (SD^{ura-}), spotted on SD^{ura-} agar plates supplemented with or without $NaAsO_2$ or $CdCl_2$, and cultured at 30 °C for 3 d.

Preparation of Transgenic Plants. To analyze *OsABCC1* expression in rice tissue, the fragment 2,062 bp upstream of the *OsABCC1* start codon was PCR amplified using a primer set (OsABCC1gF, TGGATTACCTCTCTGTGGATCA; OsABCC1gR, GTCAAACCGTCCCCTTAGC) and genomic DNA from Dongjin cultivar rice, and cloned into the pCR8/GW/TOPO vector (Invitrogen). The promoter region was transferred into pMDC163 (6) using LR Clonase (Invitrogen), and the p*OsABCC1*::*GUS* construct was introduced into the Dongjin rice cultivar using Agrobacterium-mediated transformation (7). To express *OsABCC1* in *Arabidopsis*, the *OsABCC1* CDS from *pPGK–OsABCC1* was cloned into the BamHI and XbaI sites of the pMPC3300 vector (8). The MPC3300–OsABCC1 vector was introduced into the *atabcc1 atabcc1* double knockout plant by the flower dipping method (8).

Immunostaining with GUS and Anti-OsABCC1 Antibody. For immunostaining of the GUS reporter protein, an anti-GUS antibody (β-Glucuronidase, rabbit IgG antibody fraction; Molecular Probes) was used as the primary antibody for GUS immunostaining (at a dilution of 1:1,000). Immunostaining was performed in different organs of transgenic rice harboring pOsABCC1–GUS and nontransgenic rice as a negative control at different growth stages. For immunostaining of the endogenous OsABCC1 protein, the synthetic peptide C-NGGVWSDVENAFGAYTP (positions 14–30 of OsABCC1) was used to immunize rabbits and obtain antibodies against OsABCC1. The obtained antiserum was purified through a peptide affinity column before use (2.74 mg protein per 1 mL, diluted at 1:300). The root, basal node, leaf blade, and leaf sheath at the vegetative stage (1-mo-old seedlings) and node I at

the flowering stage of wild-type, *abcc1-1*, and *abcc1-2* plants were sampled. For double staining with DAPI and OsABCC1 antibody, DAPI (7.2 μM) was added. Four sequential optical sections with a 2.5-μm interval were observed using *z* axis scan. Other procedures were the same as described previously (9). Secondary antibody fluorescence (Alexa Fluor 555 goat anti-rabbit IgG; Molecular Probes) was observed with a confocal laser-scanning microscopy (LSM700; Carl Zeiss).

Immunoblot Analysis. To test the specificity of the OsABCC1 antibody, soluble and microsomal proteins were extracted from the roots of wild-type rice and abcc1-1 mutant (6 wk old) at 4 °C according to a previously described method (10). For density gradient fractionation, microsomal fractions extracted from the roots of wild-type seedlings (2 mo old) were used. The microsomes were fractionated using discontinuous sucrose gradients (20–60%). The protein concentration was measured using the Bradford assay (Biorad). Samples of each fraction were mixed with the same volume of sample buffer that contained 250 mM Tris·HCl (pH6.8), 8% (wt/vol) SDS, 40% (vol/vol) glycerol, 0.01% (wt/vol) bromophenol blue, and 200 mM β-mercaptoethanol. The mixtures were denatured for 10 min at 37 °C for OsABCC1 samples and 10 min at 65 °C for all membrane marker protein samples. SDS/PAGE was then performed using 5–20% gradient polyacrylamide gels (ATTO). The proteins were transferred to a PVDF membrane using a semidry blotting system, and the membrane was treated with purified primary rabbit anti-OsABCC1 (1:500) as described above, V-type ATPase (TP marker; 1:5,000; Agrisera), H^+-ATPase (PM marker; 1:5,000; Agrisera), and the ER marker Bip (1:2,000; Cosmo Bio). Anti-rabbit IgG (H+L) conjugated to horseradish peroxidase (1:10,000; Promega) was used as a secondary antibody, and an ECL Plus Western Blotting Detection System (GE Healthcare) was used for chemiluminescence detection.

Phenotypic Analysis of Knockout Lines. Seedlings (5 d old) of both wild-type rice and two *OsABCC1* mutants were exposed to a 0.5 mM calcium (Ca) solution containing 0, 1, 2, 5, or 10 μM arsenite for 48 h. The root length was measured before and after treatment. Ten replicates were performed for each treatment. To compare As tolerance, wild-type, *abcc1-1*, and *abcc1-2* plants were grown in half-strength Kimura B solution containing 1, 2, 5, or 10 μM of As (III). After 2 wk, the roots were washed three times with deionized water and separated from the shoots. Both roots and shoots were dried at 70 °C for 2 d and subjected to digestion as described below.

For soil culture, 17-d-old seedlings grown hydroponically were transplanted to a 3.5-L plastic pot filled with paddy soil containing 1.3 mg As per 1 kg soil. Tap water was supplied daily. At the ripening stage, the plant was separated into brown rice, husk, rachis, peduncle, node I and II, flag leaf blade, flag leaf sheath, and straw (containing the internodes and leaf II to leaf VI). The concentration of As was determined in each organ as described below.

For the stem-feeding experiment, plants grown hydroponically were cut below node II 1 wk after flowering. The cut ends were placed in a solution containing 10 μM As (III) and 10 μM Rb as a control. After 1 d of treatment, the upper parts were separated into spikelet, rachis, peduncle (inter node I), node I, flag leaf blade, and flag leaf sheath, which were subjected to As determination as described below.

Monobromobimane Staining. Seedlings (3 d old) of the wild-type, *abcc1-1*, and *abcc1-2* mutants were exposed to 0.5 mM $CaCl_2$ containing 0.5 μM As (III). After 3 h, the roots were washed with a 0.5 mM $CaCl_2$ solution and then stained with a 0.5 mM $CaCl_2$ solution containing 3.5 μM monobromobimane (mbbr), a thiol-reactive fluorescent dye, and 5 μM propidium iodide (PI), which stains the cell wall, for 10 min. Plasmolysis was induced by placing the roots in 8% (wt/vol) mannitol for 1 min. Fluorescence was observed with a confocal laser-scanning microscope (LSM700; Carl Zeiss).

As Determination. Dried samples were digested with concentrated HNO_3 (60%) at a temperature of up to 140 °C. The metal concentration in the digested solution was determined by inductively coupled plasma mass spectrometry (7700X; Agilent Technologies) after dilution.

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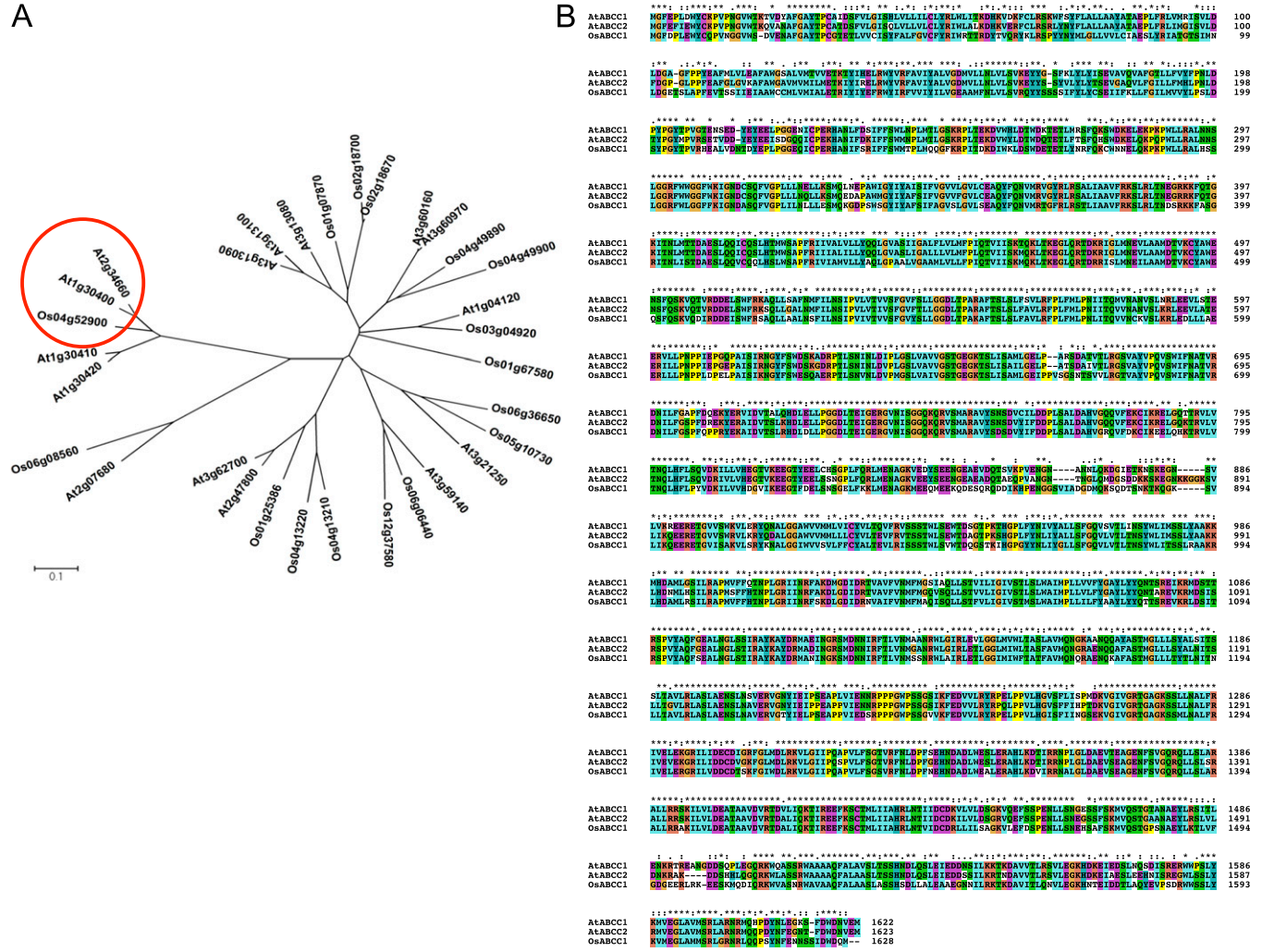

Fig. S1. Phylogenetic comparison and alignment of ABCC subfamily members in *Arabidopsis thaliana* and *Oryza sativa*. (*A*) Phylogenetic tree. Red circle indicates a cluster of *Arabidopsis* phytochelatin (PC)–As transporters (AtABCC1; At1g30400 and AtABCC2; At2g34660) and OsABCC1 (LOC_Os04g52900). The scale represents amino acid substitution distance. (*B*) Alignment of AtABCC1, AtABCC2, and OsABCC1.

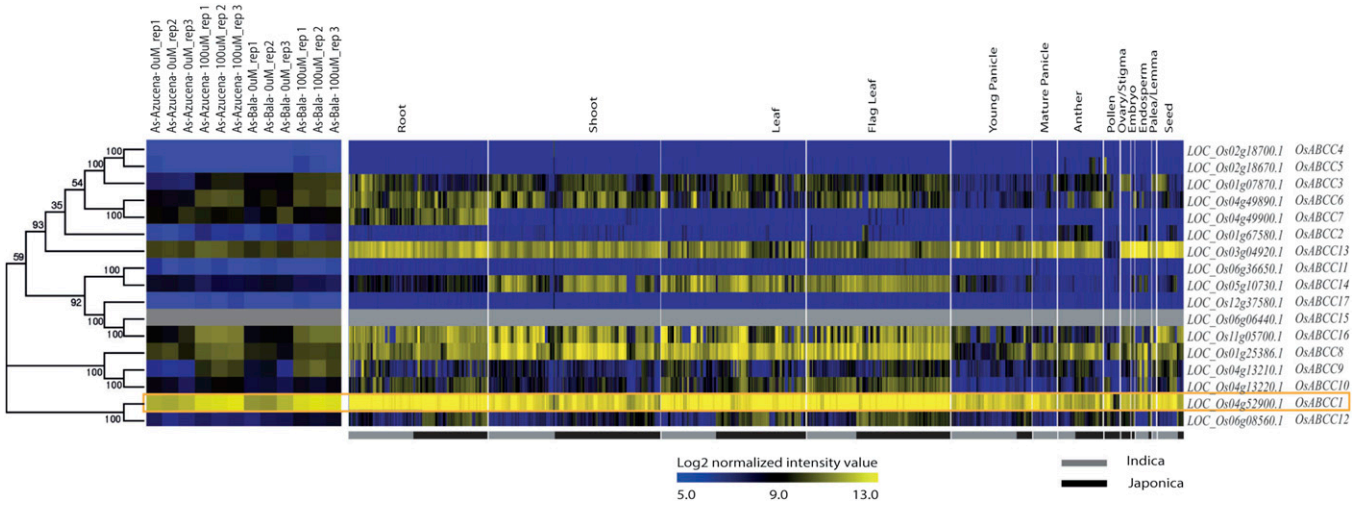

Fig. S2. Publicly available microarray data of transcriptional changes in rice cultivars subjected to As. Metaexpression analysis of ABCC subfamily genes in rice plants subjected to As treatment (National Center for Biotechnology Information Gene Expression Omnibus data series GSE4471) (1) and in various anatomical tissues/organs based on a large collection of Affymetrix microarray data, including 983 microarray datasets developed previously (2). Orange box indicates the expression patterns of *OsABCC1*. Rice varieties Bala and Azucena were grown for 7 d in hydroponics either with or without 1 ppm As. The numbers below the heat map indicate the $\log_2$ normalized intensity of the microarray data. Gray boxes, Indica samples used in the microarray experiment; black boxes, Japonica samples.

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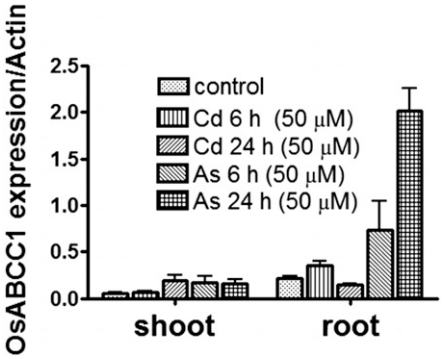

Fig. S3. Validation of the *OsABCC1* expression response to high As concentrations in wild-type rice. Seedlings were exposed to a solution containing 50 μM As or Cd. After 6 and 24 h, the roots and shoots were sampled and subjected to RNA extraction and real-time RT-PCR analysis. The *y* axis indicates the transcript level relative to *OsActin1*, the internal control.

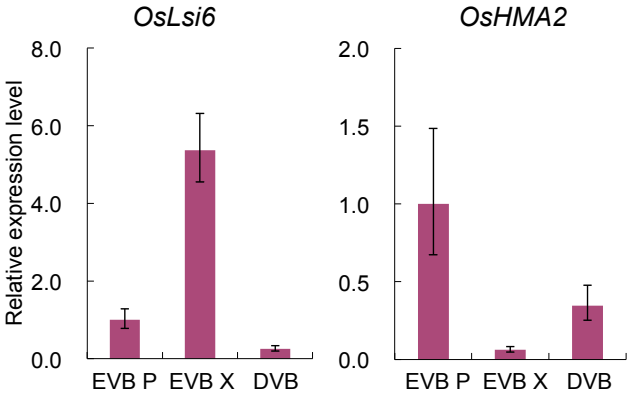

Fig. S4. Expression pattern of marker transporter genes in different vascular bundles of node I. Different vascular tissues of node I were separated by laser microdissection (LMD) at the flowering stage. The expression level of two marker genes (*OsLsi6 and OsHMA2*) was determined by quantitative real-time RT-PCR. *Actin* was used as an internal standard. Data are means ± SD of three biological replicates. DVB, diffuse vascular bundle; EVB P, enlarged vascular bundle phloem; EVB X, enlarged vascular bundle xylem.

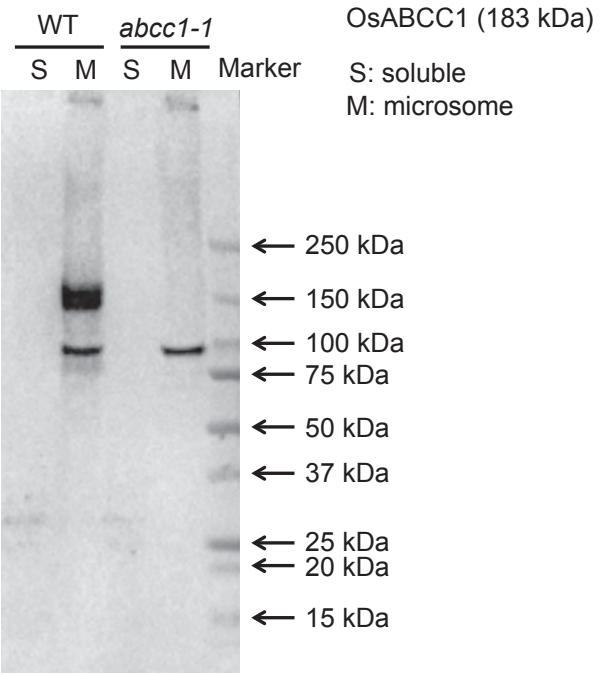

Fig. S5. Western blot analysis of OsABCC1 protein in wild-type rice and *abcc1-1* mutant. Soluble (S) and microsomal (M) fractions were extracted from the roots of wild-type rice and *osabcc1-1* mutant, respectively. An antibody against OsABCC1 was used for Western blot.

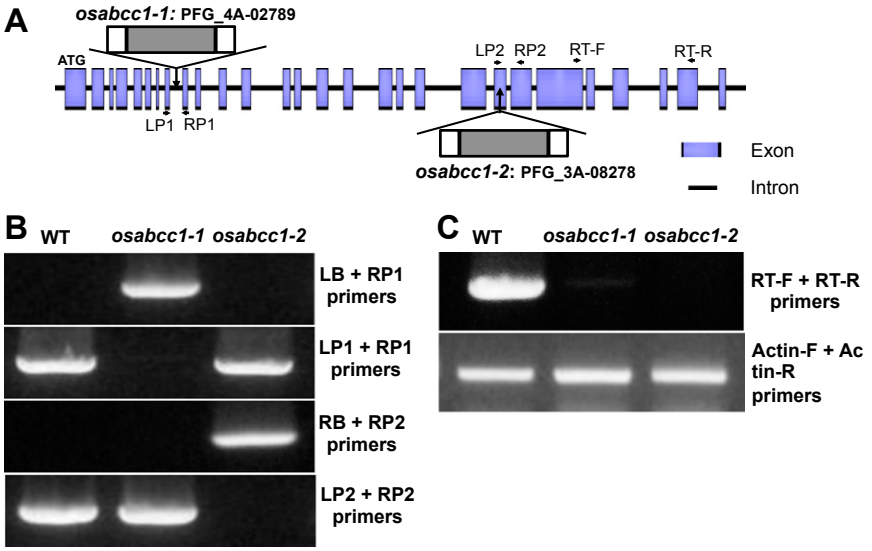

Fig. S6. Isolation of *osabcc1* knockout rice mutants. (*A*) A map of the *OsABCC1* genomic sequence with two T-DNA insertion sites marked. The positions of primers used for genotyping the mutants and for RT-PCR are shown. (*B*) *OsABCC1* genotyping using genomic PCR to identify homozygous T-DNA insertion lines. Different primers were used to genotype homogenous lines. (*C*) RT-PCR analysis using gene-specific primers (RT-F and RT-R) confirmed the absence of *OsABCC1* transcripts in the two T-DNA homozygous mutant plants.

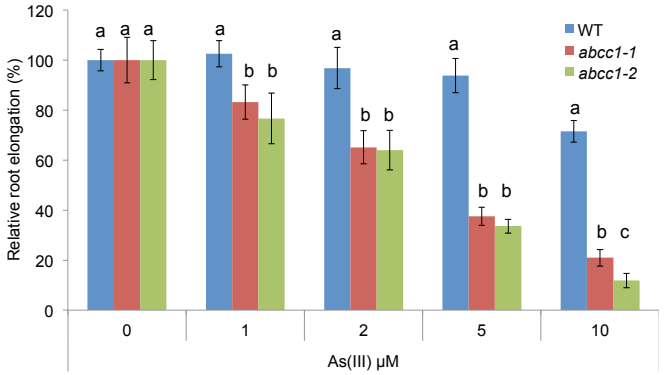

Fig. S7. Effect of As on root elongation in wild-type rice and *OsABCC1* knockout lines. Seedlings (5 d old) of both wild-type (WT) rice and two *OsABCC1* mutants (*abcc1-1* and *abcc1-2*) were exposed to a 0.5 mM Ca solution containing 0, 1, 2, 5, or 10 μM arsenite for 48 h. The root length was measured before and after treatment. Data are means ± SD of 10 biological replicates. Means with different letters are significantly different ($P < 0.05$, Tukey's test).

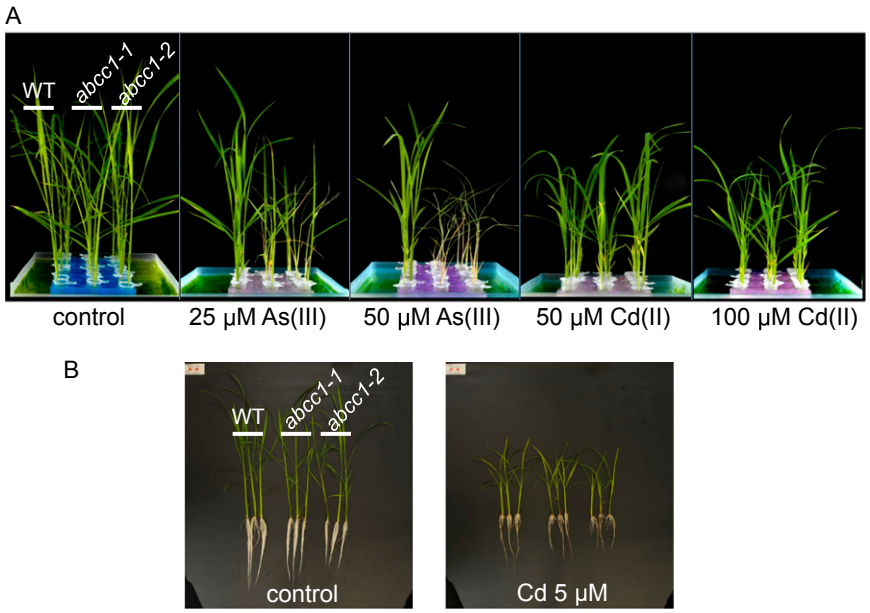

Fig. S8. The As and Cd tolerance test. (*A*) Tolerance to high As and Cd concentrations. Four-week-old wild-type rice plants and two *OsABCC1* knockout lines were exposed to a solution containing 0, 25, or 50 μM As or 50 or 100 μM Cd for 1 wk. (*B*) Tolerance to a low concentration of Cd. Seventeen-day-old seedlings of wild-type (WT) rice and two *OsABCC1* knockout lines (*abcc1-1* and *abcc1-2*) were exposed to a nutrient solution containing 5 μM Cd for 2 wk.

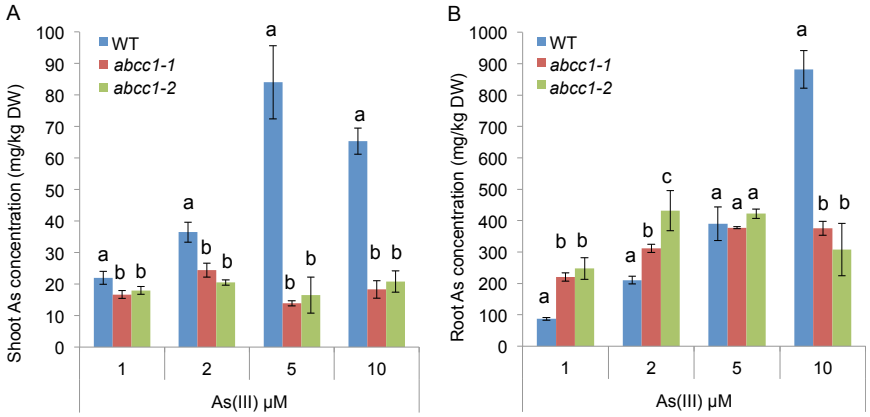

Fig. S9. The As concentration in shoots and roots. Seventeen-day-old seedlings of wild-type (WT) rice and two OsABCC1 knockout lines (*abcc1-1* and *abcc1-2*) were exposed to a nutrient solution containing different As concentrations. After 2 wk, the shoots (*A*) and roots (*B*) were separately harvested and subjected to As determination. Data are means ± SD of three biological replicates. Means with different letters are significantly different ($P < 0.05$, Tukey's test).

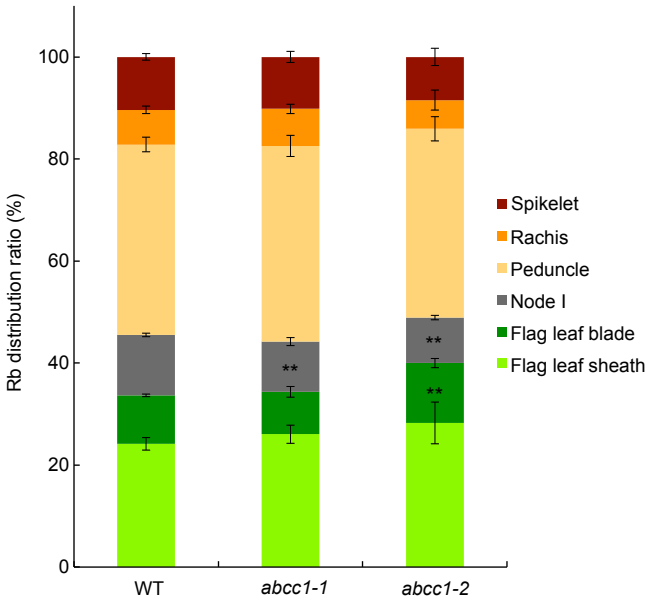

Fig. S10. Distribution of stem-fed Rb in different organs. Plants in the flowering stage were cut at the second internode below node II and were then fed with 10 μM As and 10 μM Rb solution from the cut end. After 24 h, each organ was separately harvested for Rb determination. Data are means ± SD of four to five biological replicates. Significant differences from WT at $*P < 0.05$ and $**P < 0.01$ by Tukey's test.