

Supplementary Figure S1. Genomic and transcriptional situation of *OsPCS1* locus (*Os6g0102300/LOC_Os6g01260*). Original images were captured from the RAP-DB database showing transcript abundance of *OsPCS1* in various rice tissues and callus. A, Transcript abundance in the region starting from TSS2 (predicted secondary transcript starting site specific to *OsPCS1b/1c*, Fig. 1A) and the flanking *OsPCS1s* common region separated by an orange line. In all tested tissues, the expression level is much lower in the

OsPCS1b/1c specific region than in the *OsPCS1* common region, suggesting higher expression of *OsPCS1full* and/or *OsPCS1a*. B, Transcript abundance in the 11 bp-region after the alternative splicing site (SS) specific to *OsPCS1a/1c* (Fig. 1A) and the flanking *OsPCS1s* common region separated by an orange line. The expression level is much lower in the *OsPCS1a/1c* specific region than in the *OsPCS1* common region. Taken together, these results suggest that *OsPCS1full* is a major variant with higher expression levels in various rice tissues.

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AtPCS1      1  -----MAMASLYRRSLPSPFAIDFSSAEGNLFNLSALQKQTEGFFNLSVFOQOSEPAFCGLASLSVVLNALSIDPGRKWKGPWRWFDE
AtPCS2      1  -----MSMASLYRRSLPSPFAIDFASPEGNLFNLSALQKQTEGFFNLSVFOQOSEPAFCGLASLSVVLNALSIDPGRKWKGPWRWFDE
OsPCS2      1  MASKPSSRAESNQAAAVPSLYGRALPSPFAVEFASAEGRRLFAALQGTMOGFFNLSVFOQOSEPAFCGLASLSVVLNALSIDPGRKWKGPWRWFDE
OsPCS1a     1  -----MAMASLYRRSLPSPFAVEFASAEGRRLFAALQGTMOGFFNLSVFOQOSEPAFCGLASLSVVLNALSIDPGRKWKGPWRWFDE
OsPCS1c     1  -----MAMASLYRRSLPSPFAVEFASAEGRRLFAALQGTMOGFFNLSVFOQOSEPAFCGLASLSVVLNALSIDPGRKWKGPWRWFDE
OsPCS1full  1  -----MAMASLYRRSLPSPFAVEFASAEGRRLFAALQGTMOGFFNLSVFOQOSEPAFCGLASLSVVLNALSIDPGRKWKGPWRWFDE
OsPCS1b     1  -----MAMASLYRRSLPSPFAVEFASAEGRRLFAALQGTMOGFFNLSVFOQOSEPAFCGLASLSVVLNALSIDPGRKWKGPWRWFDE

AtPCS1      86  SMLDCCEPLEVIVKGIIFGKVVCLAHSSGAKVEAFRTSSSTLIDDFRNFVVKCSSESNCHMISTYHRGVFKQTGTGHFSPIGGYNAERDMALILDVARFK
AtPCS2      85  SMLDCCEPLEVIVKGIIFGKVVCLAHSSGAKVEAFRTSSSTLIDDFRNFVVKCSSESNCHMISTYHRGVFKQTGTGHFSPIGGYNAERDMALILDVARFK
OsPCS2     101  SMLDCCEPLEVIVKGIIFGKVVCLAHSSGAKVEAFRTSSSTLIDDFRNFVVKCSSESNCHMISTYHRGVFKQTGTGHFSPIGGYNAERDMALILDVARFK
OsPCS1a     87  SMLDCCEPLEVIVKGIIFGKVVCLAHSSGAKVEAFRTSSSTLIDDFRNFVVKCSSESNCHMISTYHRGVFKQTGTGHFSPIGGYNAERDMALILDVARFK
OsPCS1c     1  -----MALILDVARFK
OsPCS1full  87  SMLDCCEPLEVIVKGIIFGKVVCLAHSSGAKVEAFRTSSSTLIDDFRNFVVKCSSESNCHMISTYHRGVFKQTGTGHFSPIGGYNAERDMALILDVARFK
OsPCS1b     1  -----MALILDVARFK

AtPCS1     186  YPPHWVPLKLLWEAMDSIDQSTCRRRGFMLISRPHREPGLLYTVSCRDESWIETAKLREKEDVPRLVSSQHVDSVEKISVVEKSLPSNFQPHRWVAEIR
AtPCS2     185  YPPHWVPLKLLWEAMDSIDQSTCRRRGFMLISRPHREPGLLYTVSCRDESWIETAKLREKEDVPRLVSSQHVDSVEKISVVEKSLPSNFQPHRWVAEIR
OsPCS2     201  YPPHWVPLKLLWEAMNTDDATGLLRGFMILSRPHREPGLLYTVSCRDESWIETAKLREKEDVPRLVSSQHVDSVEKISVVEKSLPSNFQPHRWVAEIR
OsPCS1a     187  YPPHWVPLKLLWEAMNTDDATGLLRGFMILSRPHREPGLLYTVSCRDESWIETAKLREKEDVPRLVSSQHVDSVEKISVVEKSLPSNFQPHRWVAEIR
OsPCS1c     12  YPPHWVPLKLLWEAMNTDDATGLLRGFMILSRPHREPGLLYTVSCRDESWIETAKLREKEDVPRLVSSQHVDSVEKISVVEKSLPSNFQPHRWVAEIR
OsPCS1full  187  YPPHWVPLKLLWEAMNTDDATGLLRGFMILSRPHREPGLLYTVSCRDESWIETAKLREKEDVPRLVSSQHVDSVEKISVVEKSLPSNFQPHRWVAEIR
OsPCS1b     12  YPPHWVPLKLLWEAMNTDDATGLLRGFMILSRPHREPGLLYTVSCRDESWIETAKLREKEDVPRLVSSQHVDSVEKISVVEKSLPSNFQPHRWVAEIR

AtPCS1     286  ITEDSNQNLSESEKSLKLLKQELKQVQEGKLLKRVDFK-----LSTVGYEDSLIYAKAKAGQGLLETISG--SPSKEKGCCHLGVVCKKGPDPDISE
AtPCS2     285  ITEDSNQNLSESEKSLKLLKQELKQVQEGKLLKRVDFK-----LSSV-YEDNLFYAAKVVQDGELETISG--YESDSECCNEKCVKCKKGLGEEK
OsPCS2      -----
OsPCS1a     -----
OsPCS1c     -----
OsPCS1full  287  HQSEGGSGLSSESEKSLKLLKQELKQVQEGKLLKRVDFKRLVRELQFTKQPCCSCSYSSDDDSFRRHAAASVCCQGAALLTGNLSSKRDGFCCHREKCFKCVQVDDGDP
OsPCS1b     112  HQSEGGSGLSSESEKSLKLLKQELKQVQEGKLLKRVDFKRLVRELQFTKQPCCSCSYSSDDDSFRRHAAASVCCQGAALLTGNLSSKRDGFCCHREKCFKCVQVDDGDP

AtPCS1     375  GVVVTCVVVRDGNKQVLLVPSQTQCECGPEAT-----YFAGNDVFNALLALPQGNWSGIKDQAMHBMKOLISMASPTLMOEVLHLRRQLQLL
AtPCS2     373  VVVVA-----YFAGNDVFNALLALPQGNWSGIKDQAMHBMKOLISMASPTLMOEVLHLRRQLQLL
OsPCS2      -----
OsPCS1a     -----
OsPCS1c     -----
OsPCS1full  387  KLVVTCVAVSGVNEQSDVMDLPISTLSTVVCNSNSSEVVKVPSRTDILVLLALHPSNHWGIKDERKAEFQSLSTDIHDDDKRREHLHLRRQLHYV
OsPCS1b     212  KLVVTCVAVSGVNEQSDVMDLPISTLSTVVCNSNSSEVVKVPSRTDILVLLALHPSNHWGIKDERKAEFQSLSTDIHDDDKRREHLHLRRQLHYV

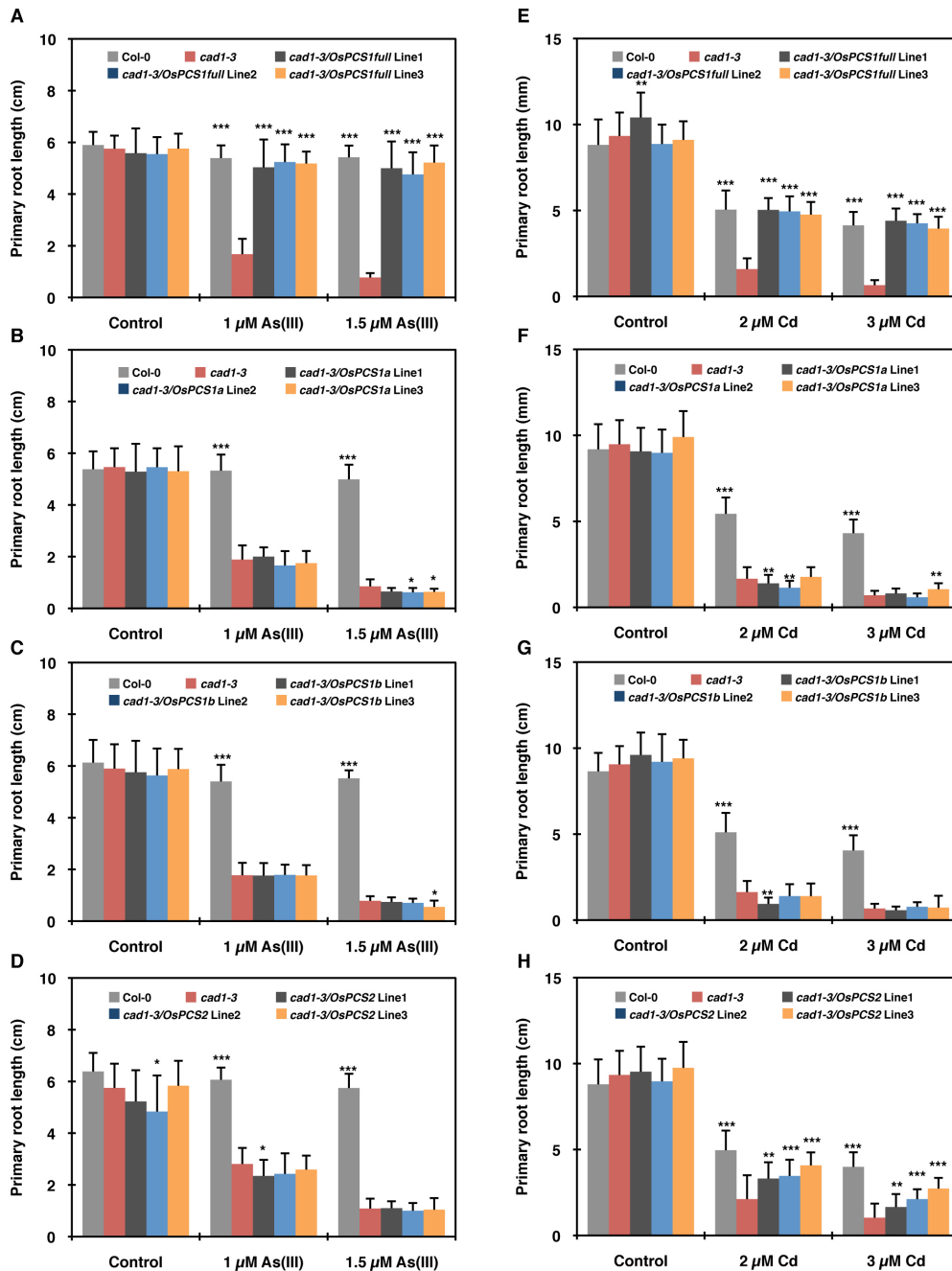
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AtPCS2     437  KRQENKEEDDLSAPA-
OsPCS2      -----
OsPCS1a     -----
OsPCS1c     -----
OsPCS1full  487  RSKREYGDVVPQSH-
OsPCS1b     312  RSKREYGDVVPQSH-

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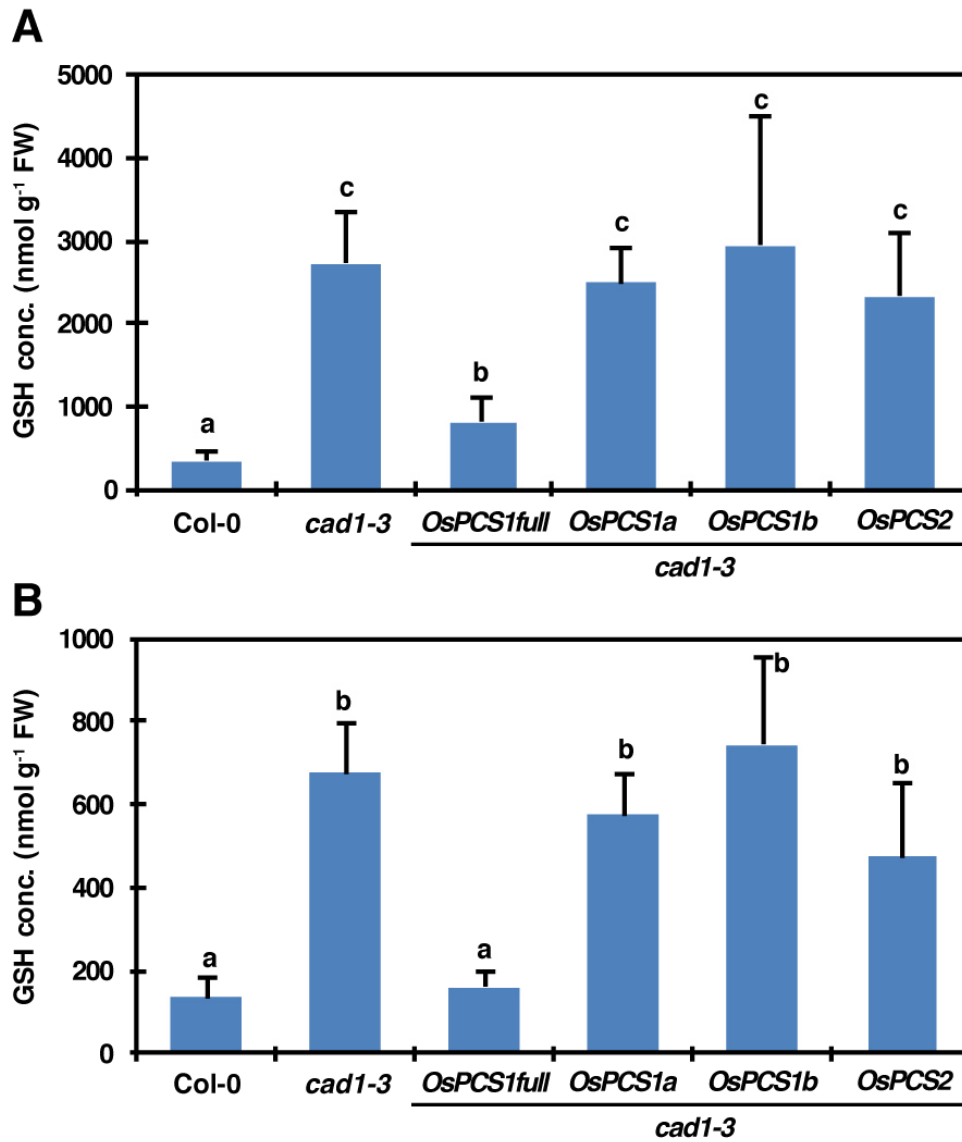
Supplementary Figure S2. Alignment of rice and Arabidopsis PCS amino acid sequences.



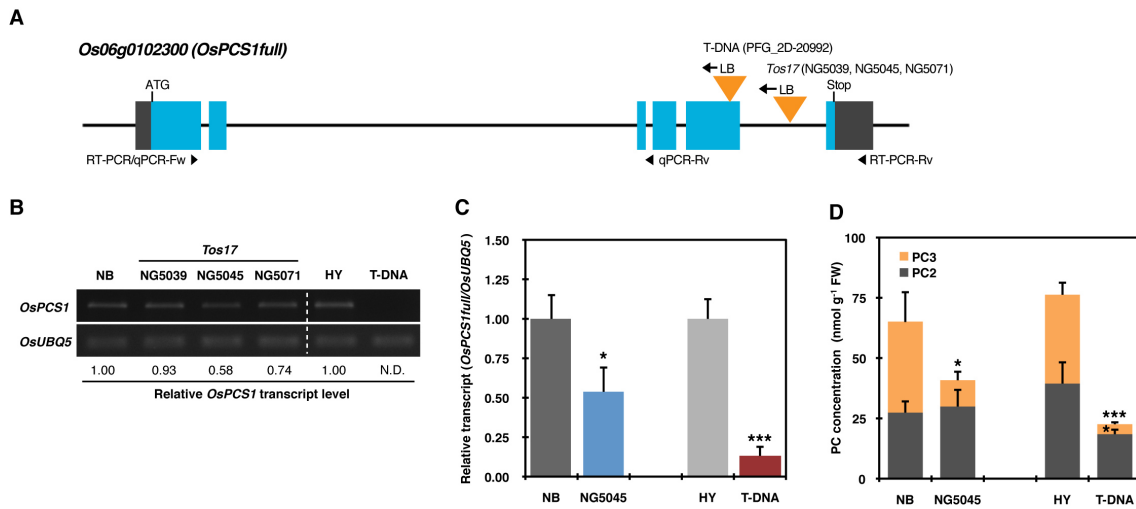
Supplementary Figure S3. Expression analyses of *OsPCS* transgenes in the *Arabidopsis* transgenic plants by RT-PCR.



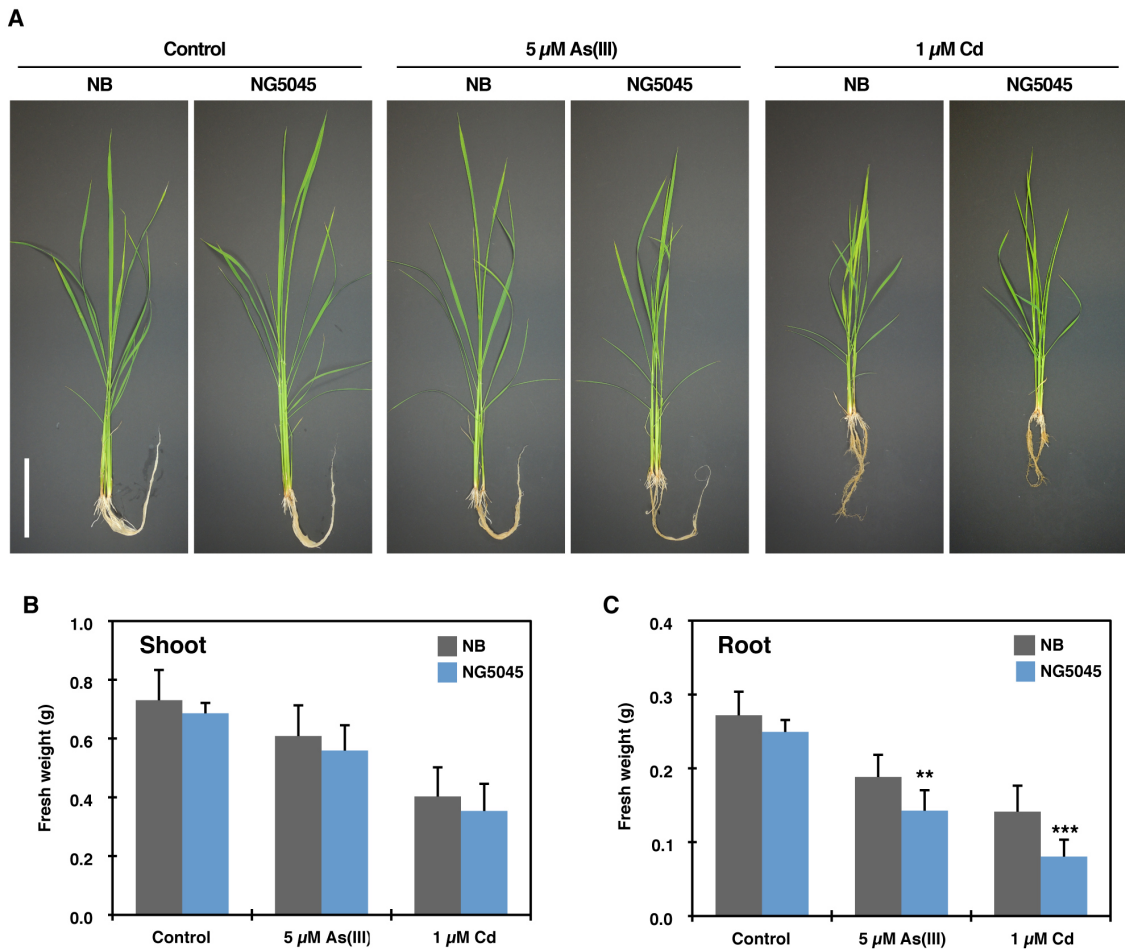
Supplementary Figure S4. As and Cd sensitivity assay of the Arabidopsis plants harboring *OsPCS* transgenes. Primary root lengths were measured after 12 d cultivation on the agar plates containing As(III) (A to D) or Cd (E to H). A and E, *cad1-3/OsPCS1full*. B and F, *cad1-3/OsPCS1a*. C and G, *cad1-3/OsPCS1b*. D and H, *cad1-3/OsPCS2*. Data represent means with SD of two independent experiments ($n = 13 - 25$). Asterisks indicate significant differences from *cad1-3* within each treatment (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; *t*-test).



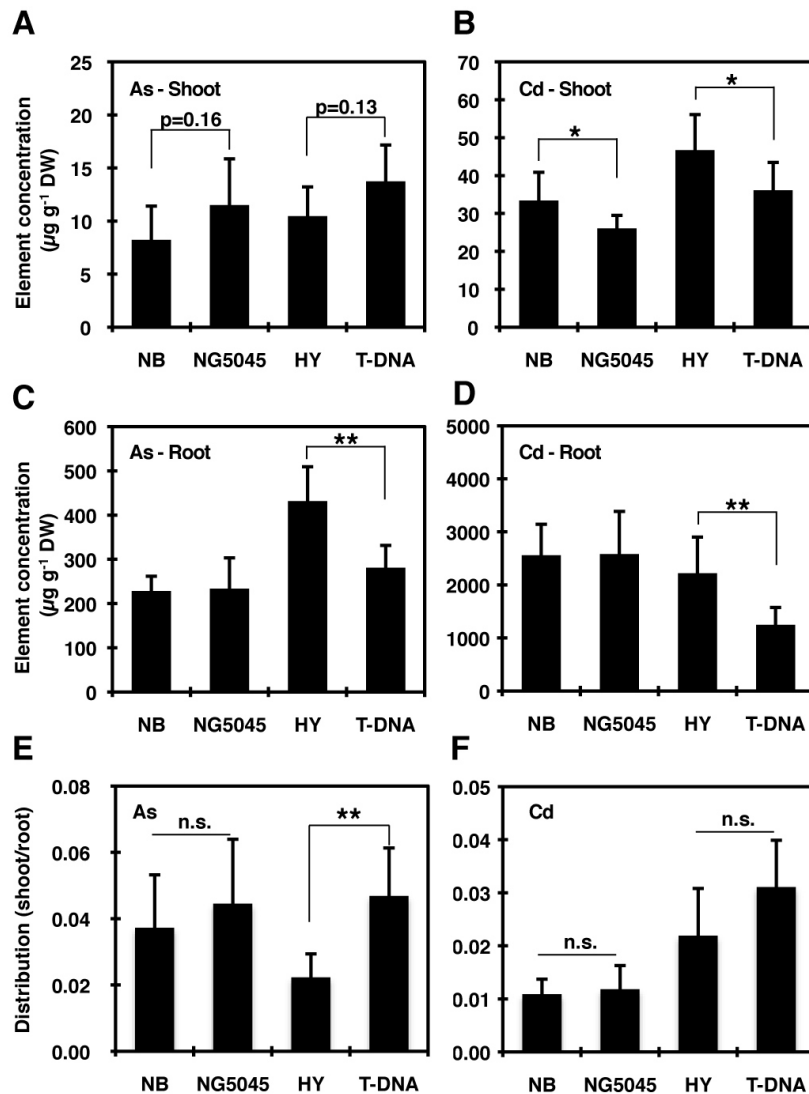
Supplementary Figure S5. GSH concentrations in roots of Col-0, *cad1-3* and *cad1-3* transgenic plants with *OsPCS* exposed to As(III) (A) or Cd (B). 7 d old seedlings were transferred from the control medium to the medium containing 5 μ M As(III) or 5 μ M Cd and grown for 7 d before harvest. Data represent means with SD of two independent experiments (n = 3 - 4). Means sharing the same lowercase (PC2) and uppercase (PC3) letter are not significantly different ($p < 0.05$, Tukey's HSD).



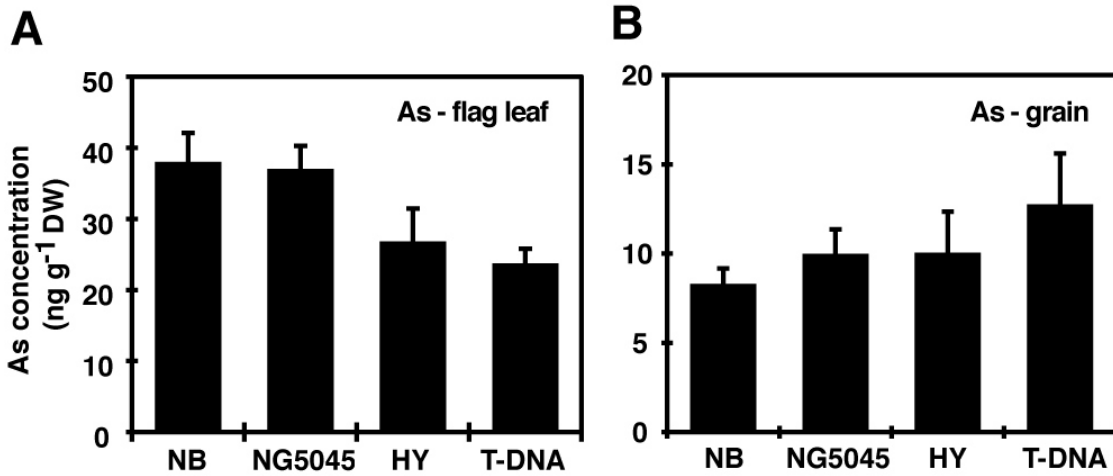
Supplementary Figure S6. Characterization of *OsPCS1* mutant rice. A, Insertion sites of T-DNA and *Tos17* in *Os06g0102300* (*OsPCS1full*). Black and blue boxes represent untranslated regions and coding regions, respectively. B and C, *OsPCS1* expression analyses in the wild-type and mutant plants by RT-PCR (B) and quantitative real-time PCR (C). D, PCs concentrations in the rice roots exposed to 10 μ M Cd for 3 d. C and D, Data represent means with SD of at least three biological replicates. Asterisks indicate significant differences from each wild-type line (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; *t*-test).



Supplementary Figure S7. As and Cd sensitivity assay of the *Tos17* insertion rice. A to C, the wild-type NB and *Tos17* mutant of *OsPCS1* (NG5045) were hydroponically grown for 2 weeks without As(III) or Cd addition and then grown further for 3 weeks with or without 5 μ M As(III) or 1 μ M Cd. A, Phenotypes of the NB and NG5045 after cultivation. A white bar = 10cm. B and C, Fresh weight of shoot (B) and root (C) after cultivation. Data represent means with SD of two independent experiments ($n = 6 - 10$). Asterisks indicate significant differences from the wild-type NB (** $p < 0.01$, *** $p < 0.001$; t -test).



Supplementary Figure S8. As and Cd accumulation in the hydroponically grown *OsPCS1* mutant rice and the respective wild-type plants. A to F, The wild-type and *OsPCS1* mutant rice were hydroponically grown for 2 weeks without As(III) or Cd addition and then grown further for 3 weeks with or without 5 μM As(III) or 1 μM Cd. A, As concentration in shoot. B, Cd concentration in shoot. C, As concentration in root. D, Cd concentration in root. E, As distribution ratio. E, Cd distribution ratio. Data represent means with SD of two independent experiments ($n = 6 - 8$). Asterisks indicate significant differences between each wild-type and mutant pair (* $p < 0.05$, ** $p < 0.01$; *t*-test).



Supplemental Figure S9. As concentrations in *OsPCS1* mutant rice and the respective wild-type plants grown under intermittent water conditions. The wild-type and *OsPCS1* mutant rice were grown in pots until grain ripening. After harvest, element concentrations in leaf blade and brown rice were measured by ICP-MS. A and B, As concentrations in flag leaf blade (A) and grain (B). Data represent means with SD of two independent experiments (n = 11 - 12).

Supplemental Table S1 List of primers used in the study

Primer set for	Primer name	Primer sequence (5' to 3')
Cloning of <i>OsPCS1</i> cDNA	OsPCS1full/a 5'UTR Fwd	TCGAGTGCAAGAAGAGGAAAGC
	OsPCS1b/c 5'UTR Fwd	CATTGCATTGCATTTTCAGAC
	OsPCS1 3'UTR Rev	CTTTTGAAGGACACCAGCC
Cloning of <i>OsPCS2</i> cDNA	OsPCS2 Fwd	ATGGCGTCTAAACCAAGCAG
	OsPCS2 3'UTR Rev	CCAGCATTGGGAGGAAGATG
Plasmid construction for Arabidopsis transformant	ProAtPCS1 HindIII Fwd	ccCAAGCTTCTCATGTTTATTGAAC
	ProAtPCS1 KpnI Rev	ggggtaccTTTTCACTGCTTGTITTTGGT
	OsPCS1full/a KpnI Fwd	ggggtaccATGGCAGCGATGGCATCCCT
	OsPCS1full/b PacI Rev	ccttaattaaTTAATGGGATTGTGGCACAG
	OsPCS1b/c KpnI Fwd	ggggtaccATGGCGCTTATCCTGGATGTC
	OsPCS1a/c PacI Rev	ccttaattaaCTACTCTCATCCTTAAGAAG
	sGFP KpnI Fwd sGFP PacI Rev	ggggtaccATGGTGAGCAAGGGCGAGG ccttaattaaTTACTTGTACAGCTCGTCCATGCC
Plasmid construction for yeast transformation	OsPCS1full/a XhoI Fwd	ccgctcgagATGGCAGCGATGGCATCCCT
	OsPCS1b/c XhoI Fwd	ccgctcgagATGGCGCTTATCCTGGATGT
	OsPCS2 XhoI Fwd	ccgctcgagATGGCGTCTAAACCAAGCAG
	OsPCS1full NotI Rev	ataagaatcgggcgccATGGGATTGTGGCACAG
	OsPCS1a NotI Rev	ataagaatcgggcgccCACTCTCATCCTTAAGAAGATCG
	OsPCS1b NotI Rev	ataagaatcgggcgccATGGGATTGTGGCACAGGAT
	OsPCS1c NotI Rev OsPCS2 NotI Rev	ataagaatcgggcgccCACTCTCATCCTTAAGAAGATC ataagaatcgggcgccATTTACCACTGCACGGATC
RT-PCR of Arabidopsis transgenic plants	OsPCS1 common Fwd	TTCAAATACCCTCCTCACTGG
	OsPCS1 common Rev	CACTGTGTAGAGCAATGAAGGAG
	OsPCS2 Fwd	TCGCTTCAAATACCCTCCTC
	OsPCS2 Rev	TGAGATAAGCATGAACCCCC
	AtEF1a Fwd	AGCAGCTCTTCTTGCTTTC
	AtEF1a Rev	AACGCCTGTCAATCTTGGTC
Genotyping of Tos17 mutant rice	Tos17 OsPCS1 Fwd	TGCTCCCTACTCCCTAGCAA
	Tos17 OsPCS1 Rev	TGTGGCACAGGATCTCCATA
	Tos17 LB	ATTGTTAGGTTGCAAGTTAGTTAAGA
Genotyping of T-DNA mutant rice	T-DNA OsPCS1 Fwd	GCAAGTCCGTGATACTGAGC
	T-DNA OsPCS1 Rev	GGATTAATACAACAAACCTCTCG
	POSTECH T-DNA RB	GCACCGTGGTAGTAAGAATGG
RT-PCR of <i>OsPCS1</i>	OsPCS1full/a Fwd OsPCS1 3'UTR Rev	GACTTCGGCCACCATCTC CTTTTGAAGGACACCAGCC
Real-time PCR of <i>OsPCSs</i>	OsPCS1full/a Fwd	GACTTCGGCCACCATCTC
	OsPCS1b/c Fwd	CATTGCATTGCATTTTCAGAC
	OsPCS1full/b Rev	CATCTCTGCAACTCACTGTG
	OsPCS1a/c Rev	GCAACTCTGCACAAAAGCAC
	OsPCS2 Fwd	ATGGCGTCTAAACCAAGCAG
	OsPCS2 Rev	CGGAGACGAGGCTGAAGAAC
Real-time PCR and RT-PCR of <i>OsUBQ5</i>	OsUBQ5 Fwd OsUBQ5 Rev	GAAGGAGGAGGAAATCGAAC CTTCACAGAGGTGATGCTAAGG

Lowercase sequences indicate additional sequences for restriction enzyme digestion.

Supplemental Table S2 Element concentrations and pH of the tested soil

Element concentration (mg kg ⁻¹ DW)	As	0.73 ± 0.03
	Cd	0.015 ± 0.001
	Fe	26.9 ± 2.2
	Mn	53.3 ± 2.4
	Cu	2.06 ± 0.10
	Zn	3.99 ± 0.08
pH (H ₂ O)		4.83 ± 0.03

Data represent mean with SD of 4 replicates.