

Supplementary information for manuscript

**Characterizing the role of rice NRAMP5 in Manganese, Iron and Cadmium Transport**

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**Supplementary Table 1. Gene expression patterns related to metal uptake transporters under Cd added condition**

The induction ratios for the WT plants were calculated as the relative increase or decrease in expression in response to the addition of 10  $\mu$ M CdCl<sub>2</sub> for two weeks, compared to control condition (+Cd/C). The values are the means of two biological replicates and color swaps.

Accession No.	Gene name	+Cd/C		+Cd/C		C Root	C Shoot
		Root		Shoot		signal	signal
<b>OsNRAMP transporters</b>							
AK103557	<i>OsNRAMP1</i>	15.0	$\pm 0.7$	95.5	$\pm 9.1$	274	29
AK065481	<i>OsNRAMP2</i>	0.9	$\pm 0.0$	1.3	$\pm 0.0$	603	553
AK070574	<i>OsNRAMP3</i>	1.2	$\pm 0.1$	1.1	$\pm 0.1$	5429	556
AK102180	<i>OsNRAMP4</i>	0.8	$\pm 0.0$	1.3	$\pm 0.6$	3119	3
AK070788	<i>OsNRAMP5</i>	0.2	$\pm 0.0$	0.4	$\pm 0.0$	14308	565
AK068118	<i>OsNRAMP6</i>	0.9	$\pm 0.0$	0.8	$\pm 0.1$	433	486
AK071485	<i>OsNRAMP7</i>	0.6	$\pm 0.0$	0.8	$\pm 0.0$	682	183
<b>OsZIP transporters</b>							
AK107681	<i>OsIRT1</i>	0.8	$\pm 0.0$	1.3	$\pm 0.0$	6938	188
CI162465	<i>OsIRT2</i>	5.0	$\pm 0.9$	10.2	$\pm 0.9$	49	6
AY302058	<i>OsZIP1</i>	0.7	$\pm 0.0$	1.1	$\pm 0.1$	10706	46
AK121551	<i>OsZIP2</i>	2.8	$\pm 0.0$	2.0	$\pm 0.4$	1126	382
AK069804	<i>OsZIP3</i>	80.4	$\pm 17.8$	4.9	$\pm 0.4$	3	24
AK105258	<i>OsZIP4</i>	1.5	$\pm 0.1$	1.0	$\pm 0.1$	293	61
AK070864	<i>OsZIP5</i>	1.1	$\pm 0.1$	1.0	$\pm 0.0$	1438	304
AK103730	<i>OsZIP6</i>	0.6	$\pm 0.0$	1.3	$\pm 0.0$	782	188
AK071272	<i>OsZIP7</i>	0.5	$\pm 0.1$	1.5	$\pm 0.2$	1168	125
AY327038	<i>OsZIP8</i>	3.4	$\pm 0.4$	4.6	$\pm 0.3$	187	324
Os05g0472400	<i>OsZIP9</i>	0.5	$\pm 0.1$	1.0	$\pm 0.4$	20	45
Os06g0566300	<i>OsZIP10</i>	1.0	$\pm 0.8$	1.0	$\pm 0.2$	10	4
AK099474	<i>OsZIP11</i>	0.7	$\pm 0.1$	1.0	$\pm 0.2$	1118	393

**OsYSL transporters**

AK121040	<i>OsYSL1</i>	1.1 ±0.7	1.0 ±0.4	3	3
CI446246	<i>OsYSL2</i>	2.3 ±1.9	1.1 ±0.8	3	4
AK068865	<i>OsYSL4</i>	1.0 ±0.2	0.9 ±0.2	3	3
AK108750	<i>OsYSL5</i>	1.0 ±0.1	1.0 ±0.1	405	145
AK100148	<i>OsYSL6</i>	0.9 ±0.1	1.0 ±0.1	2656	2180
AK070691	<i>OsYSL7</i>	1.2 ±0.0	0.6 ±0.1	41	26
AK072347	<i>OsYSL8</i>	0.8 ±0.1	1.7 ±0.2	14	78
AK120923	<i>OsYSL9</i>	0.7 ±0.0	0.9 ±0.0	1758	286
AK069645	<i>OsYSL10</i>	2.9 ±3.2	1.3 ±0.8	3	4
AK069437	<i>OsYSL12</i>	0.3 ±0.1	1.1 ±0.2	1541	127
AK067235	<i>OsYSL13</i>	2.0 ±0.1	1.9 ±0.0	98	31
AK063464	<i>OsYSL15</i>	1.8 ±0.1	4.4 ±0.8	7108	3
AK070304	<i>OsYSL16</i>	0.3 ±0.0	1.6 ±0.1	22152	3264
AK070618	<i>OsYSL18</i>	1.0 ±0.2	0.9 ±0.2	3	3

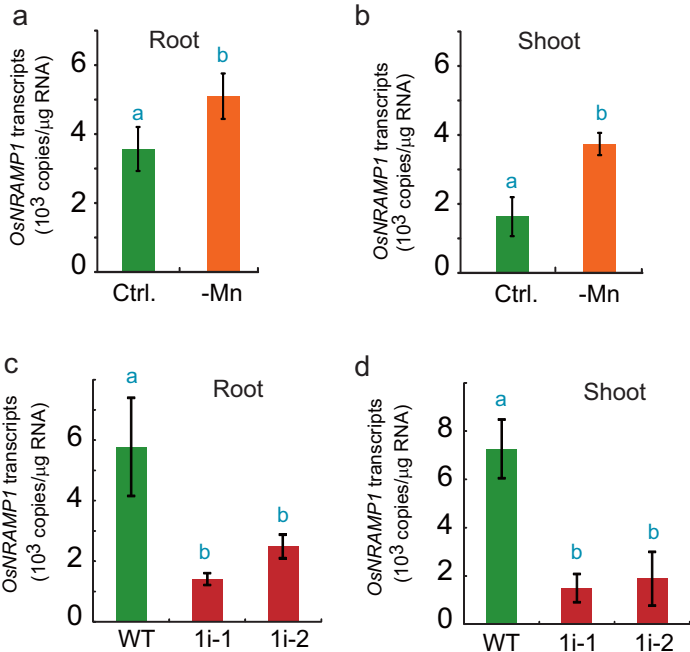
**OsCOPT transporters**

Os01g0770700	<i>COPT like</i>	0.4 ±0.1	2.1 ±0.1	17320	1793
AK109200	<i>COPT like</i>	1.0 ±0.2	0.9 ±0.2	4	4
AK107848	<i>COPT like</i>	2.9 ±0.2	1.3 ±0.3	808	14
AK069588	<i>COPT like</i>	2.8 ±0.3	1.5 ±0.2	3010	1352

**Supplementary Table 2. Fe-regulated gene expression patterns in OsNRAMP5i plants**

The induction ratios were calculated as the relative increase or decrease in expression of OsNRAMP5i plants as compared to WT (NRAMP5i/WT) in var. Tsukinohikari. The values are the means of three biological replicates and color swaps.

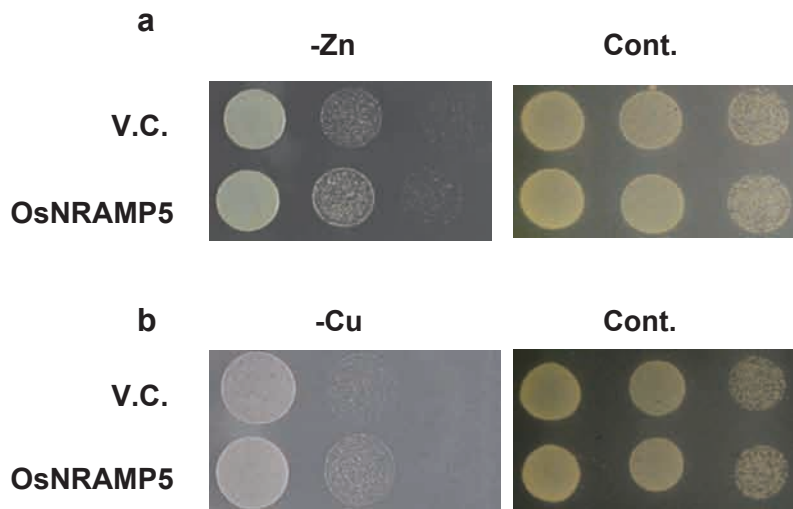
Accession No.	Gene name	NRAMP5i/WT		NRAMP5i/WT		WT Root	WT Shoot
		Root		Shoot		signal	signal
<b>Transporters</b>							
AK070788	<i>OsNRAMP5</i>	0.3	±0.0	0.3	±0.0	15827	200
AK103557	<i>OsNRAMP1</i>	7.0	±2.2	14.6	±11.2	765	21
AK107681	<i>OsIRT1</i>	2.7	±0.3	0.9	±0.1	13351	266
CI162465	<i>OsIRT2</i>	5.6	±1.4	1.5	±0.5	210	11
CI446246	<i>OsYSL2</i>	6.2	±2.6	3.5	±2.9	23	4
AK063464	<i>OsYSL15</i>	4.8	±0.8	0.9	±0.3	8946	3
<b>Iron deficiency related genes</b>							
AK103636	<i>MIR</i>	12.1	±7.1	4.4	±3.0	69	4
AK068159	<i>OsFRO2</i>	1.1	±0.4	8.2	±4.8	4	9
AK073385	<i>OsIRO2</i>	3.4	±0.4	15.0	±12.0	231	3
AK112069	<i>OsNAS1</i>	4.9	±2.0	1.2	±0.3	30366	29
AK112011	<i>OsNAS2</i>	5.6	±2.3	3.7	±3.0	25721	4
AB206814	<i>OsNAAT1</i>	5.2	±2.0	5.0	±3.0	4234	427
AK073738	<i>OsDMAS1</i>	4.2	±1.2	1.2	±0.2	1970	84



Supplementary Figure 1

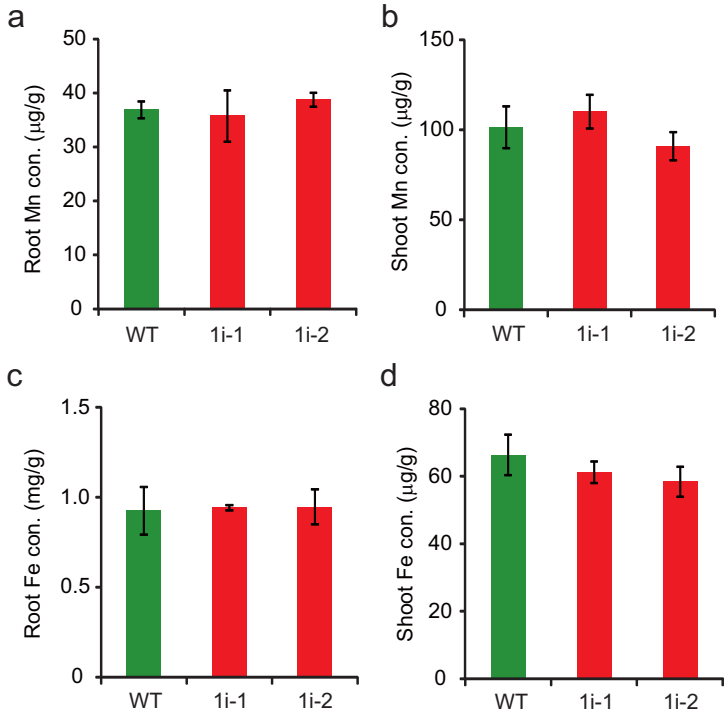
### *OsNRAMP1* expression in *OsNRAMP1i* plants

(a-b) Expression pattern of *OsNRAMP1* under Mn-deficient condition (-Mn) in roots (a) and shoot (b), compared to control condition (Ctrl.). (c-d) *OsNRAMP1* in the roots (c) and the shoot (d) of WT and *OsNRAMP1i* (1i-1, 1i-2) plants. Error bars represent S.D. Columns bars followed by different letters are statistically different according to analysis of variance followed by SNK test (a,  $p=0.0445$ ; b,  $p=0.0050$ ; c,  $p=0.0011$ ; d,  $p=0.0007$ );  $n=3$ .



Supplementary Figure 2

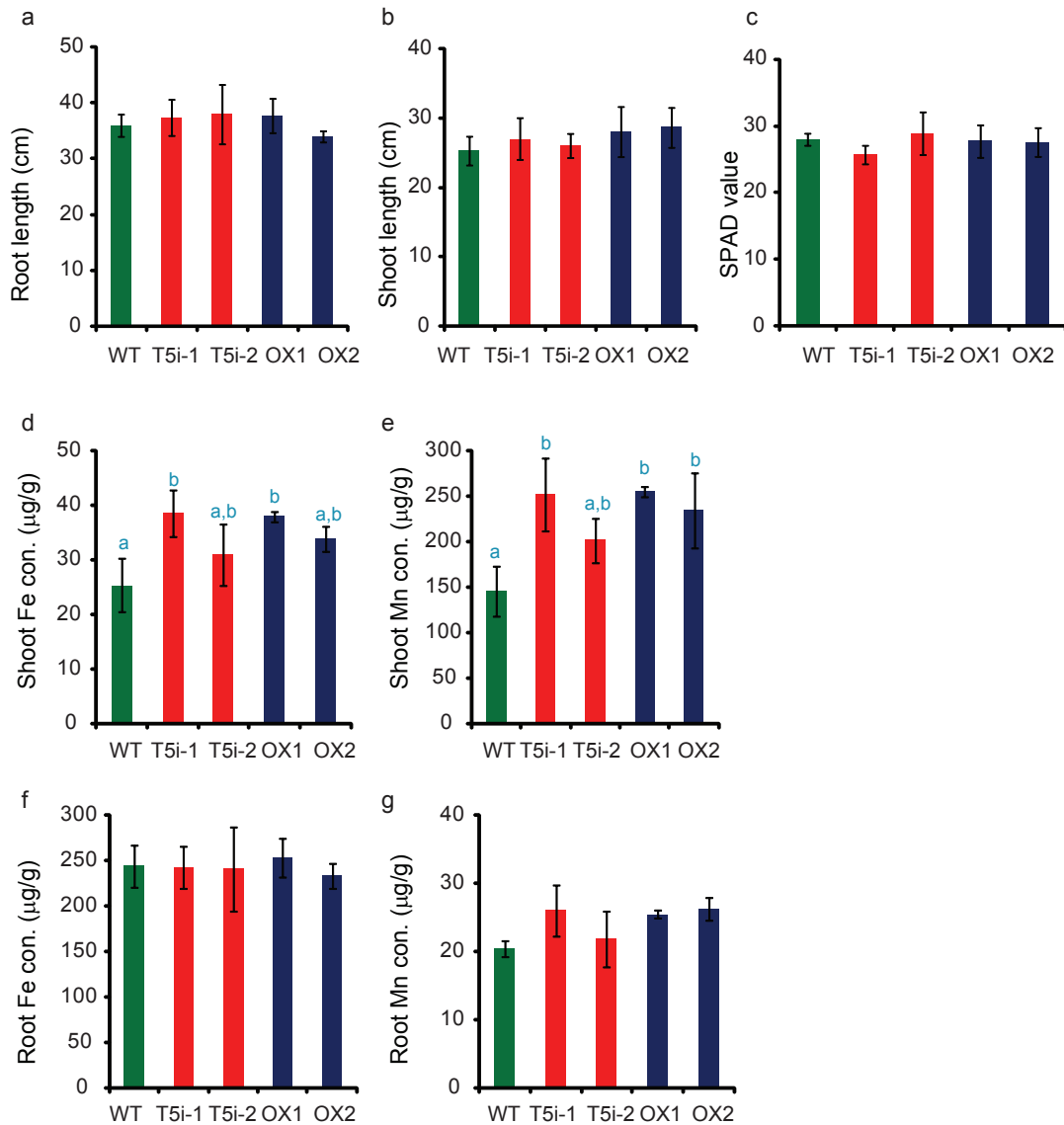
Serial dilutions of yeast cells for  $\Delta zrt3zrt4$  (Zinc uptake mutant) (a),  $\Delta ctr1$  (Cu uptake mutant) (b), transformed with empty vector (V.C.) or OsNRAMP5.



Supplementary Figure 3

Mn and Fe concentration of OsNRAMP1i plants

Root (a) and shoot (b) Mn and root (c) and shoot (d) Fe concentration of WT and OsNRAMP1i plants (1i-1, 1i-2). Error bars represent S.D. (n=3).



Supplementary figure 4

Phenotype and Metal concentration of WT, OsNRAMP5i and OsNRAMP5OX plants grown under Fe deficient conditions. Root length (a), shoot length (b), SPAD value (c), Shoot (d) and root (f) Fe concentration; shoot (e) and root (g) Mn concentration. Error bars represent the SD. Columns bars followed by different letters are statistically different according to analysis of variance followed by SNK test (d,  $p=0.0139$ ; e,  $p=0.0073$ );  $n=3$ .