Supporting Information

Xia et al. 10.1073/pnas.1004949107

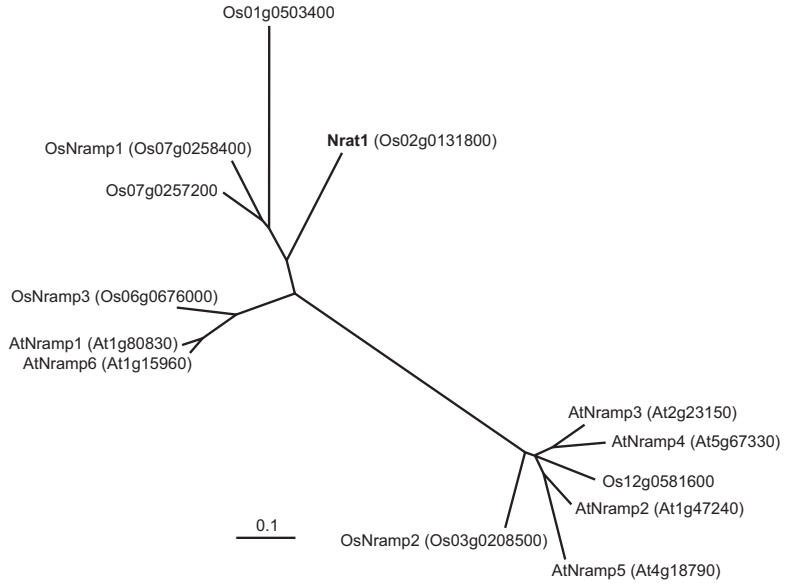

Fig. S1. Phylogenic tree of Nramp proteins in rice (Os-) and *Arabidopsis* (At-). The 0.1 scale shows substitution distance.

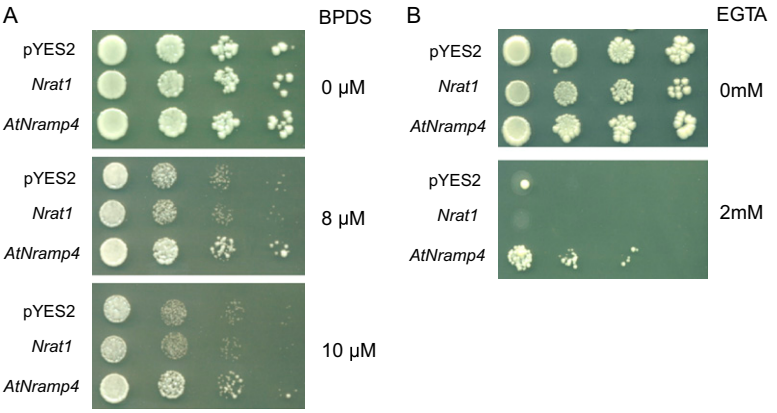

Fig. S2. Transport activity for iron and manganese in yeast. Yeast mutant (*fet3fet4* or *smf1*) transformed with the empty vector pYES2, Nrat1, or AtNramp4 were spotted on synthetic medium at serial dilutions (OD600, 0.2, 0.02, 0.002, and 0.0002). (*A*) Complementation of iron uptake. Transformed *fet3fet4* were grown on a medium (pH 5.5) buffered with 50 mM Mes in the presence or absence of BPDS. (*B*) Complementation of manganese uptake. Transformed *smf1* were grown on a medium (pH 6.0) buffered with 50 mM Mes in the presence or absence of 2 mM EGTA. The plates were incubated at 30 °C for 5 d.

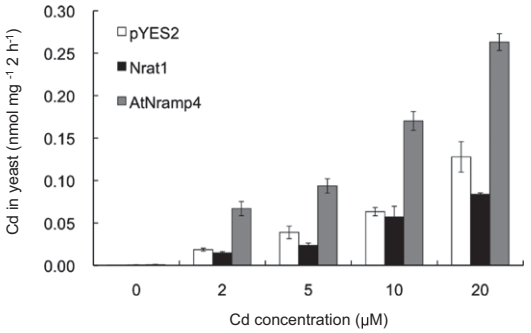

Fig. S3. Transport activity of Nrat1 for cadmium. Yeast strain (BY4741) transformed with empty vector pYES2, *Nrat1*, or *AtNramp4* were exposed to a solution (pH 4.6) containing 2, 5, 10, or 20 μM CdCl2 for 2 h. The Cd concentration was determined by atomic absorption spectrophotometer. Data are means ± SD of three biological replicates.

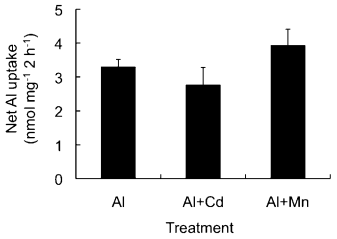

Fig. S4. Competition experiments in yeast. Yeast cells expressing Nrat1 were exposed to a solution with or without equal concentration of Cd or Mn in the presence of 50 μM aluminum (Al) for 2 h. The concentration of Al in the digest solution was determined by atomic absorption spectrophotometer. Data are means ± SD of three biological replicates.

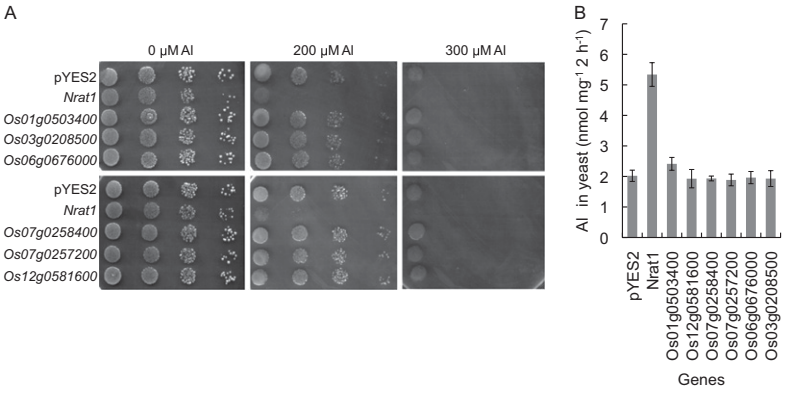

Fig. S5. Effect of expression of rice *Nramp* genes on Al sensitivity in yeast. (*A*) Yeast cells (BY4741) were transformed with the empty vector pYES2, *Nrat1*, *Os01g0503400*, *Os03g0208500*, *Os06g0676000*, *Os07g0258400*, *Os07g0257200*, and *Os12g0581600*. The transformed cells were spotted on the LPM-ura medium (pH 4.2) buffered with 5 mM succinic acid with or without AlCl3 at serial dilutions (OD600: 0.2, 0.02, 0.002, and 0.0002 from left to right). The plates were incubated at 30°C for 3 d. (*B*) Al uptake by yeast expressing different rice Nramp genes. Yeast cells expressing different rice Nramp genes were exposed to a solution containing 30 μM Al (pH 4.2) for 2 h. The concentration of Al in the digest solution was determined by atomic absorption spectrophotometer. Data are means ± SD of three biological replicates.

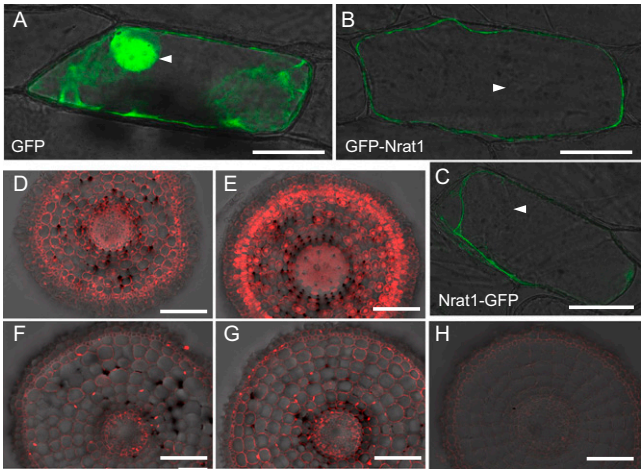

Fig. S6. Localization of Nrat1. (*A*–*C*) Subcellular localization of Nrat1. GFP alone (*A*), GFP fused with Nrat1 at the N terminal (*B*), or the C terminal (*C*) was expressed in the epidermal cells of onion. Plasmolysis was induced by 1 M mannitol. Arrowhead indicates the nucleus. (*D*–*H*) Immunostaining of GFP in the GFP transgenic rice under the control of Nrat1 promoter or wild-type. (*D* and *E*) Root tip without Al (*D*) or with Al treatment (*E*); (*F* and *G*) root segment at 15 mm without (*F*) or with Al (*G*) treatment. (*H*) wild-type root. (Scale bars in *A*–*H*, 100 μm.)

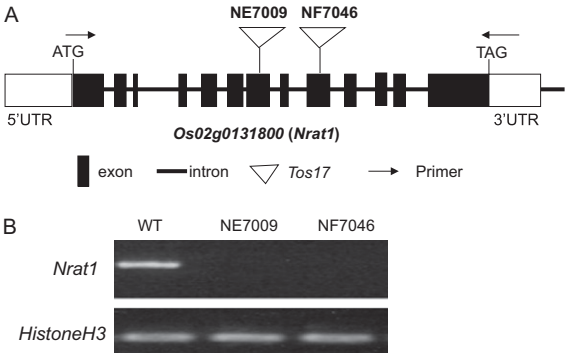

Fig. S7. Tos-17 insertion lines of *Nrat1*. (*A*) Insertion position of two Tos17 insertion lines (NE7009 and NF7046) indicated by triangles. (*B*) Expression of *Nrat1* mRNA in wild-type rice (WT), NE7009, and NF7046. The expression was examined by RT-PCR.

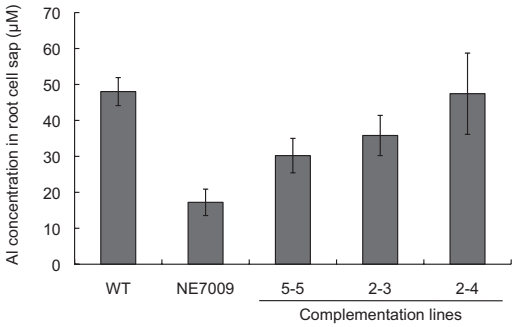

Fig. S8. Concentration of Al in the root-cell sap of complementation lines. Rice *Nrat1* gene was introduced into a Tos-17 insertion line (NE7009) by *Agrobacterium*-mediated transformation. Wild-type rice (WT), NE7009 and three independent transgenic lines were exposed to a solution containing 30 μM Al for 8 h. The root-cell saps were extracted by a frozen-thawed method and determined with atomic absorption spectrophotometer. Data are means ± SD of three biological replicates.

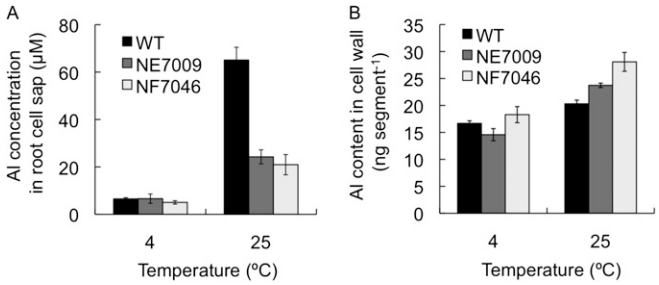

Fig. S9. Effect of temperature on Al uptake. (*A*) Al in the root-cell sap. (*B*) Al content in the cell wall. Wild-type rice and two knockout lines were exposed to 30 μM Al (pH 4.2) for 8 h. The root tips (0–1 cm) were excised for determination. Data are means ± SD of three biological replicates.

Table S1. Concentration of cations in the roots and shoots of wild-type rice (WT) and mutant (NE7009)

	Shoots		Roots	
	WT	NE7009	WT	NE7009
Macrocations (mg kg^{-1})				
K	28.5 ± 0.42	28.0 ± 0.89	18.2 ± 0.54	17.2 ± 0.76
Ca	2.18 ± 0.23	2.07 ± 0.08	0.58 ± 0.01	0.61 ± 0.03
Mg	2.55 ± 0.11	2.45 ± 0.02	1.43 ± 0.17	1.63 ± 0.19
Microcations (μg kg^{-1})				
Fe	83.1 ± 9.34	81.7 ± 2.08	191.0 ± 38.13	220.7 ± 4.66
Cu	14.6 ± 0.43	14.8 ± 0.20	204.4 ± 23.5	253.1 ± 26.5
Zn	33.3 ± 2.57	32.1 ± 0.85	54.8 ± 4.77	66.0 ± 7.2
Mn	99.2 ± 10.9	100.9 ± 4.0	—	—

The plants were cultured in half-strength Kimura B nutrient solution for 1 mo. The concentration of cations was determined by atomic absorption spectrophotometer after digestion with HNO_3. Data are means ± SD of three biological replicates. —, not detectable.