Supplementary Material

Knock-down of a tonoplast localized low-affinity nitrate transporter *OsNPF7.2* affects rice growth under high nitrate supply

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Supplementary Figure legends

Figure S1

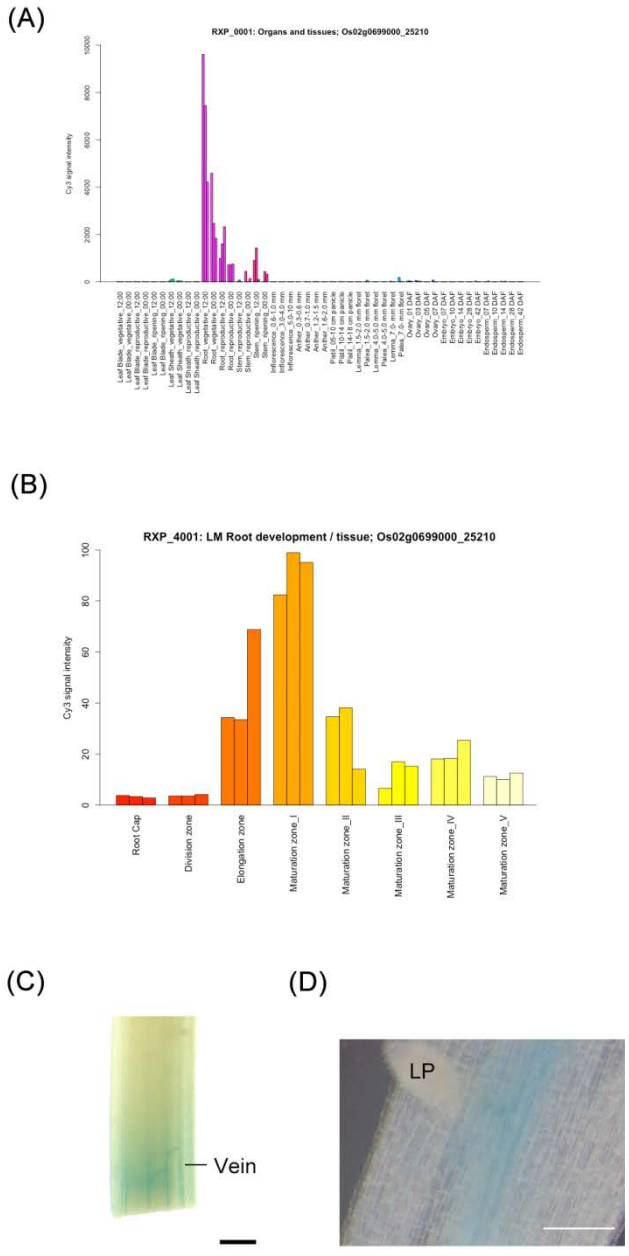

Figure S1. Tissue Expression pattern of *OsNPF7.2.* The gene is mainly expressed in the rice root elongation zone and mature zone. **(A)** Organ and tissue expression data of *OsNPF7.2* from RiceXPro. **(B)** Root development and tissue expression data of *OsNPF7.2* from RiceXPro. **(C)** and **(D)** Gus staining of $P_{OsNPF7.2}$*: GUS* in leaf (C) and lateral root primordium (D). Leaf and root were stained overnight. LP, lateral root primordium. Bar = 1 mm in (C) and 2 mm in (D).

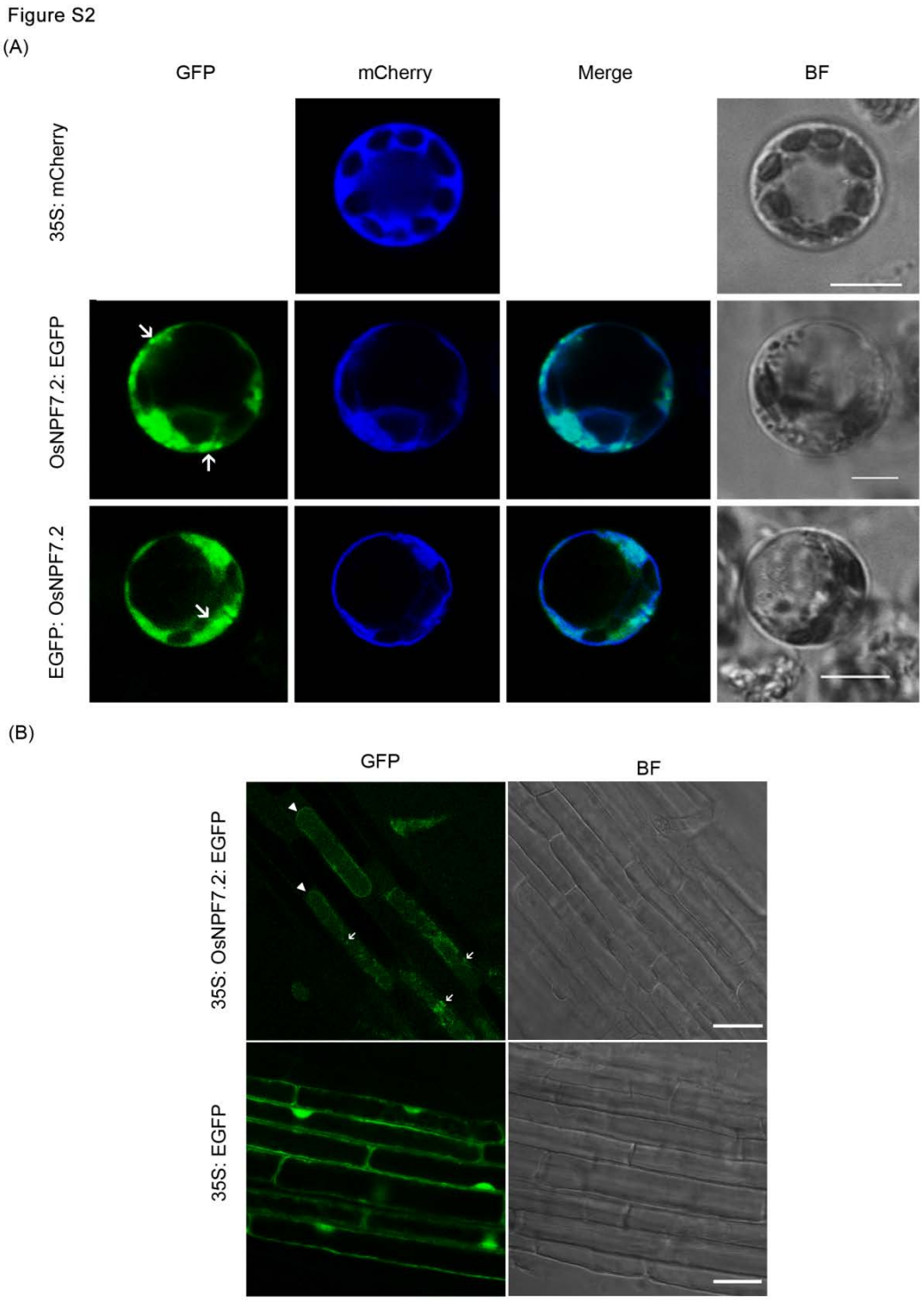

Figure S2. OsNPF7.2 is mainly localized on the tonoplast. (A) Co-localization of *35S: OsNPF7.2: EGFP* with a tonoplast localized marker *35S: OsTPKa: mCherry*. The images were coded green for GFP and blue for mCherry. Arrows indicate the small vacuoles. Scare bars are 7.5 µm, 5 µm, and 7.5 µm from upper to lower panels. TPKa is localized on lytic vacuole membrane of rice aleurone (Isayenkov *et al.*, 2011). For *35S: OsTPKa: mCherry* construction, the *mCherry* was cloned from the *vac-rk* (Nelson *et al.*, 2007) and substituted the *nEYFP* of *pSAT1A-nEYFP-N1* via *Xba*I and

*Kpn*I, then the CDS of *OsTPKa* was fused to *mCherry* via *Xho*I and *Eco*RI. Moreover, a linker $(GGGS)_2$ was inserted between the CDS of *OsTPKa* and *mCherry*. **(B)** Localization of OsNPF7.2 in rice root. Arrowheads indicate the lager vacuole. Arrows indicate the small vacuoles. Scare bars are both 20 μm. *OsNPF7.2: EGFP* fusion protein was drove by *35S* promoter and inserted into *pCAMBIA1301* to generate transgenic plants. Positive control was generated by *35S: EGFP* cloned into *pCAMBIA1301*. Roots of the transgenic plants were observed with confocal laser scanning microscope (Leica TCS SP5, Germany) with 488 nm exciting wavelength for GFP.

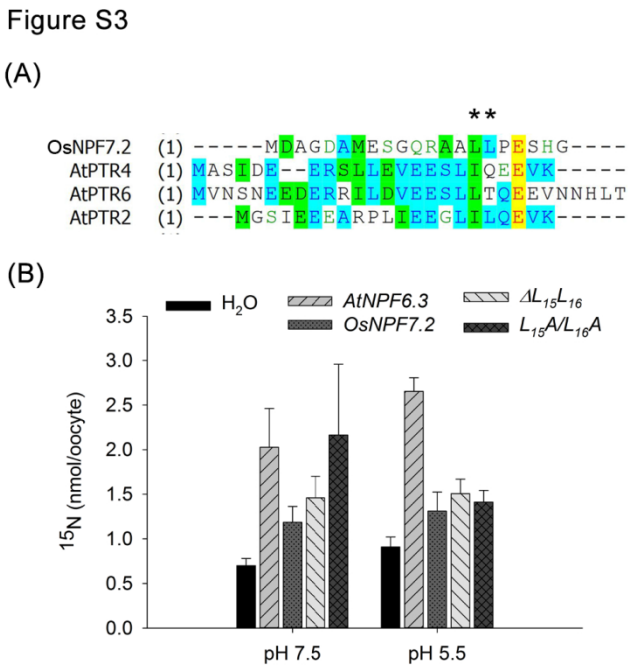

Figure S3. Mutation of the tonoplast-localization motif of OsNPF7.2 has no effect on nitrate uptake in *Xenopus* oocytes. (A) EX_6LL motif on N terminal of OsNPF7.2. Asterisks indicate the $L_{15}L_{16}$ of the motif. **(B)** Nitrate uptake of the $L_{15}L_{16}$ mutation of OsNPF7.2. $\Delta L_{15}L_{16}$ is the construct with a deletion of $L_{15}L_{16}$ of OsNPF7.2. $L_{15}A/L_{16}A$ is the construct mutated L_{15} to A and L_{16} to A of OsNPF7.2. For the mutation $L_{15}A/L_{16}A$ and deletion $\Delta L_{15}L_{16}$ of *OsNPF7.2*, overlapping PCR was used and the mutated sequences were cloned to the *pT7Ts-OsNPF7.2* via the *Hind*III and *Not*I. Data represent mean±SD (n=5).

Figure S4

Figure S4. Southern blot analysis of *OsNPF7.2-RNAi* plants and *osnpf7.2-2*. Genomic DNAs were digested with *Hind*III and *BamH*I, and hybridized to a probe of approximately 800-bp sequence from *Hpt* of T-DNA. R, RNAi lines; M, DNA Molecular-Weight Marker II DIG Labeled (Roche, Switzerland).

Figure S5

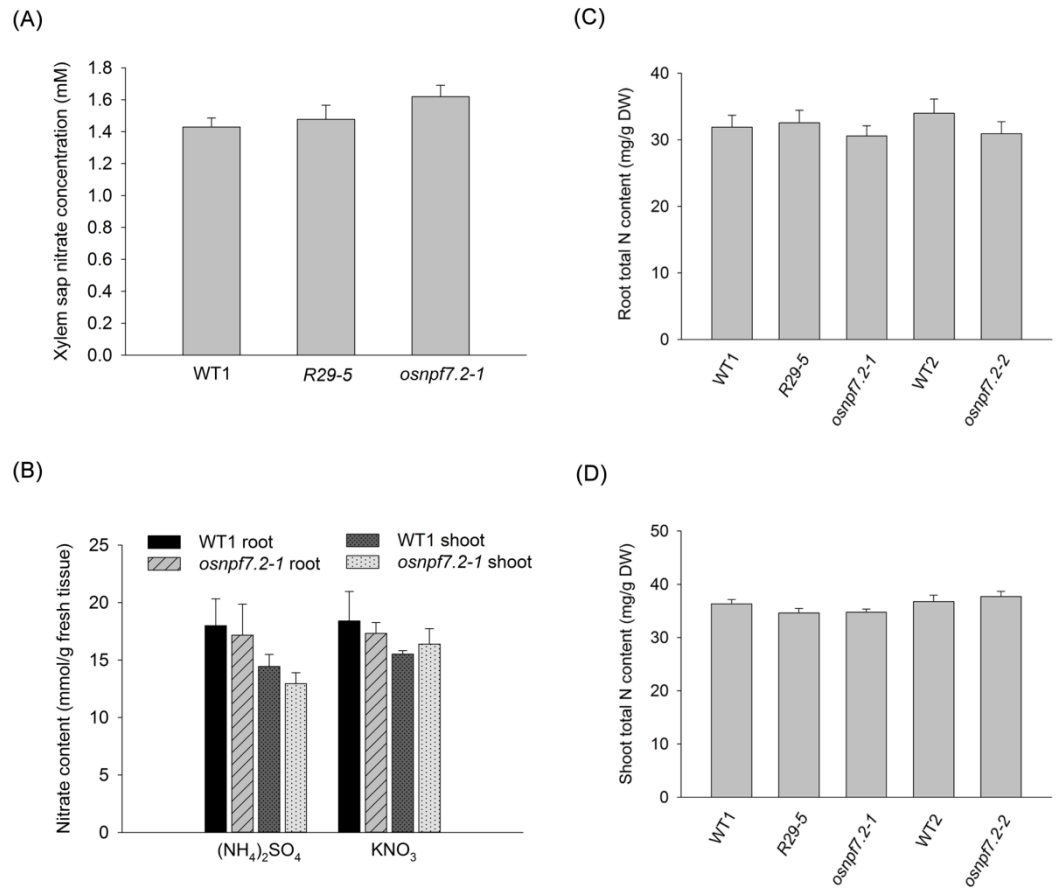

Figure S5. Knock-down plants of *OsNPF7.2* have no differences of xylem sap nitrate concentration, total N content, and tissue nitrate content compared with wild type. (A) Nitrate concentration in xylem sap. Six-week seedlings placed under N-starvation for 3 d, and then transferred to IRRI solution containing 5 mM $Ca(NO_3)_2$. Plants were cut 4 cm above roots immediately and xylem sap collected for 2 h. Data represent mean±SE (n=6). **(B)** Nitrate content in root and shoot. Two-week seedlings were N starved for 4 d, and transferred to IRRI solution containing 20 mM KNO_3 or 10 mM $(NH_4)_2SO_4$ for 24 h, then washed to measure nitrate content by HPLC. Data are mean±SE (n=12) from three replicates. **(C)** and **(D)** Total N content in roots and shoots. Six-week seedlings were transferred to IRRI solution containing 10 mM KNO_3 for 24 h, roots and shoots were separated to measure the total N content. Data represent mean±SE (n=3). Significant differences (P<0.05) between knock-down plants and their corresponding wild-type plants were calculated through LSD-test following one-way ANOVA.

Figure S6

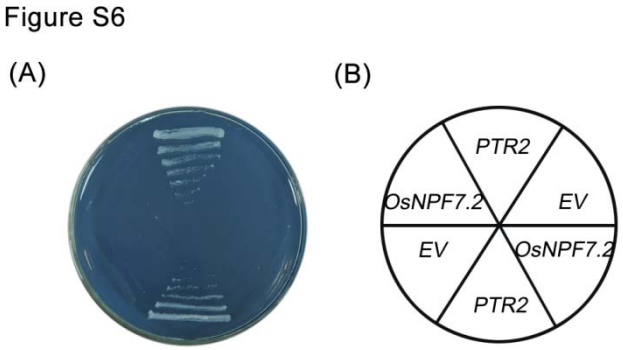

Figure S6. *OsNPF7.2* does not rescue growth of *Saccharomyces cerevisiae ptr2* mutant. Complementation of a *ptr2* mutant strain ABC738 deficient in the uptake of leucine. *EV* is empty vector *pYES260*. *ScPTR2* is positive control from *S. cerevisiae*. The *pYES260* (Melcher, 2000) with modified multi cloning site (MCS) was used as the backbone for all the yeast expression vectors. For *pYES260-OsNPF7.2*, the CDS of *OsNPF7.2* was cloned into *pYES260* via *Hind*III and *Xba*I. For *pYES260-PTR2*, the CDS of *PTR2* from yeast genomic DNA was cloned into *pYES260* via *Bam*HI and *Xba*I. *Saccharomyces cerevisiae* strain ABC738 (*MATa ura3-52 leu2-Δ1 lys2-801 his3-Δ200 trpl-Δ63 ade2-101 ptr2Δ::KanMX2*) was transformed according to Yeastmaker™ Yeast Transformation System 2 User Manual (Clontech, USA), and the transformants were selected on SC-Ura medium. To test peptide transport activity, the transformants were selected on SC-Ura/-Leu medium containing galactose as carbon source and 1mM Pro-Leu as sole source of the required amino acid leucine.

Reference

Isayenkov, S., Isner, J.-C., and Maathuis, F. J. M. (2011). Rice two-pore K^+ channels are expressed in different types of vacuoles. *Plant Cell*. 23, 756-768. DOI: 10.1105/tpc.110.081463

Melcher, K. (2000). A modular set of prokaryotic and eukaryotic expression vectors. *Analytical Biochemistry*. 277, 109-120. DOI: 10.1006/abio.1999.4383

Nelson, B. K., Cai, X., and Nebenfuehr, A. (2007). A multicolored set of in vivo organelle markers for co-localization studies in *Arabidopsis* and other plants. *Plant Journal*. 51, 1126-1136. DOI: 10.1111/j.1365-313X.2007.03212.x