

Supplemental Figure 1. Identification of a 7 bp deletion in At5g02600.

nakr1-1 and Col-0 WT genomic DNA was hybridized to Arabidopsis thaliana ATTILE 1.0R arrays. The vertical axis is the difference between the mean hybridization of *nakr1-1* and Col-0. Horizontal axis is the base pair position on chromosome 5. The data points represent hybridization to individual 25- mer features on the *A. thaliana* ATTILE 1.0R chip. Microarray data have been submitted to the GEO database with accession: GSE24385.



Supplemental Figure 2. AtNaKR1 and the C- terminal HMA domain failed to rescue the lysine auxotrophy phenotype of the yeast sod1 mutant. Lysine auxotrophy is an indication for reactive oxygen toxicity. Yeast sod1 cells transformed with p426GPD vector (vector) alone or Saccharomyces cerevisiae ATX1 (ScATX1), Arabidopsis ATX1 (AtATX1), AtNaKR1 and the C-terminal region (containing the HMA domain) were assayed for growth on SC-Ura and SC-Ura lacking lysine medium.

MKTEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTV **EHPDKLEEKFPQVAATGDGPDIIFWAHDRFGGYAQSGL** LAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEAL SLIYNKDLLPNPPKTWEEIPALDKELKAKGKSALMFNL QEPYFTWPLIAADGGYAFKYENGKYDIKDVGVDNAGAK AGLTFLVDLIKNKHMNADTDYSIAEAAFNKGETAMTIN **GPWAWSNIDTSKVNYGVTVLPTFK**GQPSKPFVGVLSAG INAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVA **LKSYEEELAKDPRIAATMENAQKGEIMPNIPQMSAFWY AVRTAVINAASGRQTVDEALKDAQTNSSSNNNNNNNN** NLGIEGRISEFLNQTSLYKKAEGPSRPEGDITMLCASQ ASTTTLCSTMDQTSQPSSSSSATIRLGGRAIDRHNPII RDGRRLTPPPSPNLNPSSSSSSSTYHTPLMTRLGLESSE QKRLAKRKSKKGDSDVGKSPVSCFSSDTPQGSSRYLLS NPVFFDGFVDSDPIPIPIDEPEITKADDLNNFHEDRLI INASKYLSTSASFLEKKQPDFFEGFLDYEPVLSPDNPF **SEPTKASPTASLSSLEDKDVSSPDFKFSPPPPPPPSPP QSSPPSPPEKNSSSDQVVVLRVSLHCKGCAGKVKKHLS KLKGVTSYNIDFAAKKVTVTGDVTPLTVLASISKVKNA** QFWPEIIQK

Supplemental Figure 3. The identity of the purified MBP-NaKR1 fusion protein was confirmed by mass spectrometry analysis.

Expression of *MBP-NaKR1* in *Escherichia coli* strain BL21-AI was induced by adding IPTG to the liquid culture. Cell lysate was incubated with amylose resin and MBP- tagged proteins eluted with 30 mM maltose and digested with trypsin. The underlined sequences were the unique peptides detected by LC-MS/MS analysis.



Supplemental Figure 4. *nakr1-1* developmental and Na⁺/K⁺/Rb⁺ accumulation defects were complemented with *NaKR1pro::NaKR1-GFP*.

(A) From left to right, *nakr1-1* expressing *NaKR1pro::NaKR1-GFP*, *nakr1-1* and Col-0. Plants were grown in the same tray under long day growth conditions. (B) Seedlings grown vertically on ATS media. From left to right: *nakr1-1* transformed with *NaKR1pro::NaKR1-GFP*, *nakr1-1* and Col-0. (C) Differences in leaf elemental content between individual NaKR1-GFP complementation lines and the average of Col-0 plants were plotted as z-values (standard deviations). Wild type and complemented lines were grown in the same tray.



Supplemental Figure 5. *nakr1-1* developmental and Na⁺/K⁺/Rb⁺ accumulation defects were complemented with *NaKR1pro::NaKR1-GUS*.

(A) From the left to the right, *nakr1-1* plants expressing *NaKR1pro::NaKR1-GUS*, *nakr1-1* and Col-0 plants grown in the same tray under long day growth conditions. (B) Seedlings grown vertically on ATS plate. From the left to the right: Col-0, *nakr1-1* mutants and *nakr1-1* plants complemented with *NaKR1pro::NaKR1-GUS*. (C-E) GUS staining of root tip (C), mature root (D) and the proximal region of root meristem (E). The scale bar is 100 μ m in (C) and 10 μ m in (D) and (E). (F) Differences in leaf elemental content between complementation lines and the mean of wild- type Col-0 plants were plotted as z-values (standard deviations).



Supplemental Figure 6. Cellular structures of SE/CC complexes were not affected by the *nakr1-1* mutation as shown by transmission electron microscopy.

(A) and (D) vascular region of Col-0 (A) and *nakr1-1* (D) roots. Xylem cells and phloem companion cells were labeled with asterisks and arrows, respectively. Scale bars are 10 μ m. (B) and (E) SE/CC complex of WT (B) and *nakr1-1* mutant (E), with the arrowheads pointing to the companion cells and the arrows pointing the protophloem (top) and metaphloem cells (bottom) respectively. Scale bars are 1 μ m. (C) and (F), the cellular structure of companion cells of WT (C) and *nakr1-1* mutant (F). Scale bars are 1 μ m.



Supplemental Figure 7. The differentiation of protophloem/ metaphloem cells and companion cells was not affected by the *nakr1-1* mutation.

The expression of *APLpro::GFP-APL* in Col-0 and *nakr1-1* was analyzed by confocal microscopy. (A) and (C) Expression of *APLpro::GFP-APL* in mature roots of Col-0 (A) and *nakr1-1* mutant (C). The GFP fluorescence specifically labeled the companion cells. (B) and (D) Expression of *APLpro::GFP-APL* in root meristem of Col-0 (B) and *nakr1-1* mutant (D), with the GFP fluorescence specifically localized in developing metaphloem and protophloem cells. Scale bars are 40 µm.



Supplemental Figure 8. Quantification of starch in Col-0 and *nakr1-1* rosette leaves.

(A) Leaf starch of *nakr1-1* and Col-0 at different time points during the night. The values at each time point are means from two independent experiments.
(B) Starch quantification during the day after 3 days of continuous darkness. A higher starch accumulation rate was detected in *nakr1-1* than in Col-0 leaves.



Supplemental Figure 9. Reciprocal grafting of Col-0 and *nakr1-1* was used to test the contribution of NaKR1 in root and shoot on leaf Na⁺ and K⁺ content of plants grown on soil.

(A) Leaf Na⁺ content analyses of different grafting types (indicated in the figure) and control plants grown in LG₃ soil. (B) Leaf K⁺ content of the same plants in (A). Na⁺ and K⁺ were assayed by ICP-MS. Data are presented as mean \pm SE, n= 9-12 plants for the reciprocal grafting types and n=5 for self-grafted plants and control plants.