Supporting Information

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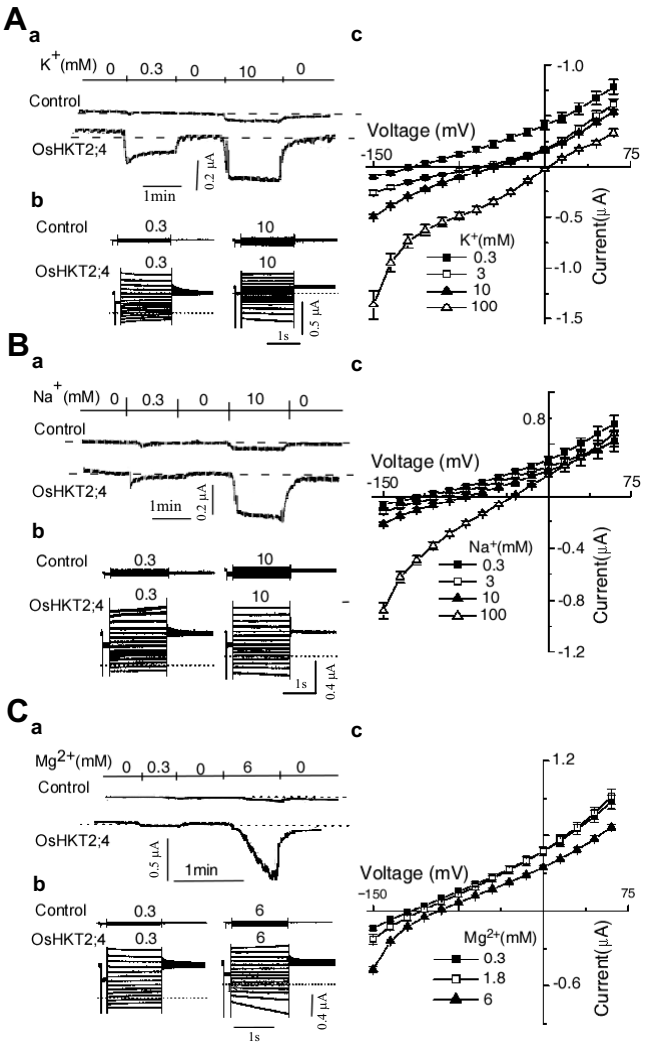

Fig. S1. OsHKT2;4 currents are sensitive to extracellular K^+ (*A*), Na^+ (*B*), and Mg^{2+} (*C*). (*a*) Typical traces recorded at −120 mV from a control oocyte and an oocyte expressing OsHKT2;4 perfused with the solution containing 185 mM mannitol, 10 mM Mes–Tris (pH 7.4) with 0, 0.3, or 10 mM K^+ (*A*), Na^+ (*B*), or 0, 0.3, or 6 mM Mg^{2+} (*C*) as indicated. (*b*) Typical traces recorded with voltages ranging from 60 to −150 mV (15-mV increments). Oocytes were perfused with a solution containing 185 mM mannitol, 10 mM Mes–Tris (pH 7.4) with 0.3 or 10 mM K–gluconate (or Na–gluconate or MgCl2). (*c*) The current–voltage relationship of control and OsHKT2;4 currents. Oocytes were perfused with a solution containing 185 mM mannitol, 10 mM Mes–Tris (pH 7.4) with the indicated concentrations of K–gluconate (or Na–gluconate or MgCl2). Summarized current data are from 10 cells/condition.

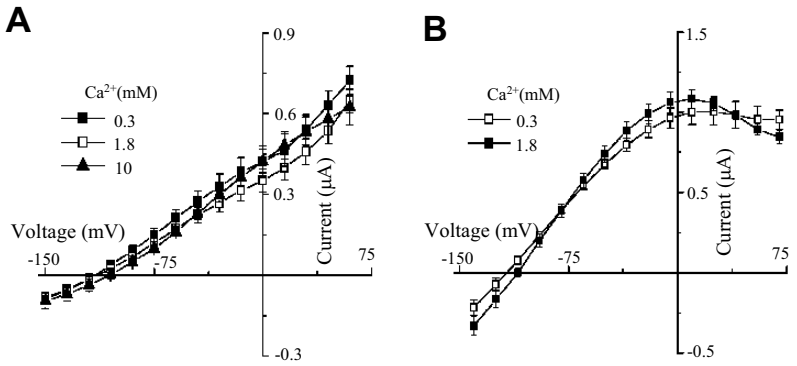

Fig. S2. AtHKT1;1 (*A*) and OsHKT2;2 (*B*) were not permeable to Ca^{2+}. The current–voltage relationship from the oocytes perfused with the solution containing 185 mM mannitol and 10 mM Mes–Tris (pH 7.4) with 0.3, 1.8, or 10 mM Ca^{2+}. Currents generated by the oocytes expressing AtHKT1;1 or OsHKT2;2 are outwardly rectifying, and the reversal potentials are not significantly different. Summarized current data are from seven cells per condition.

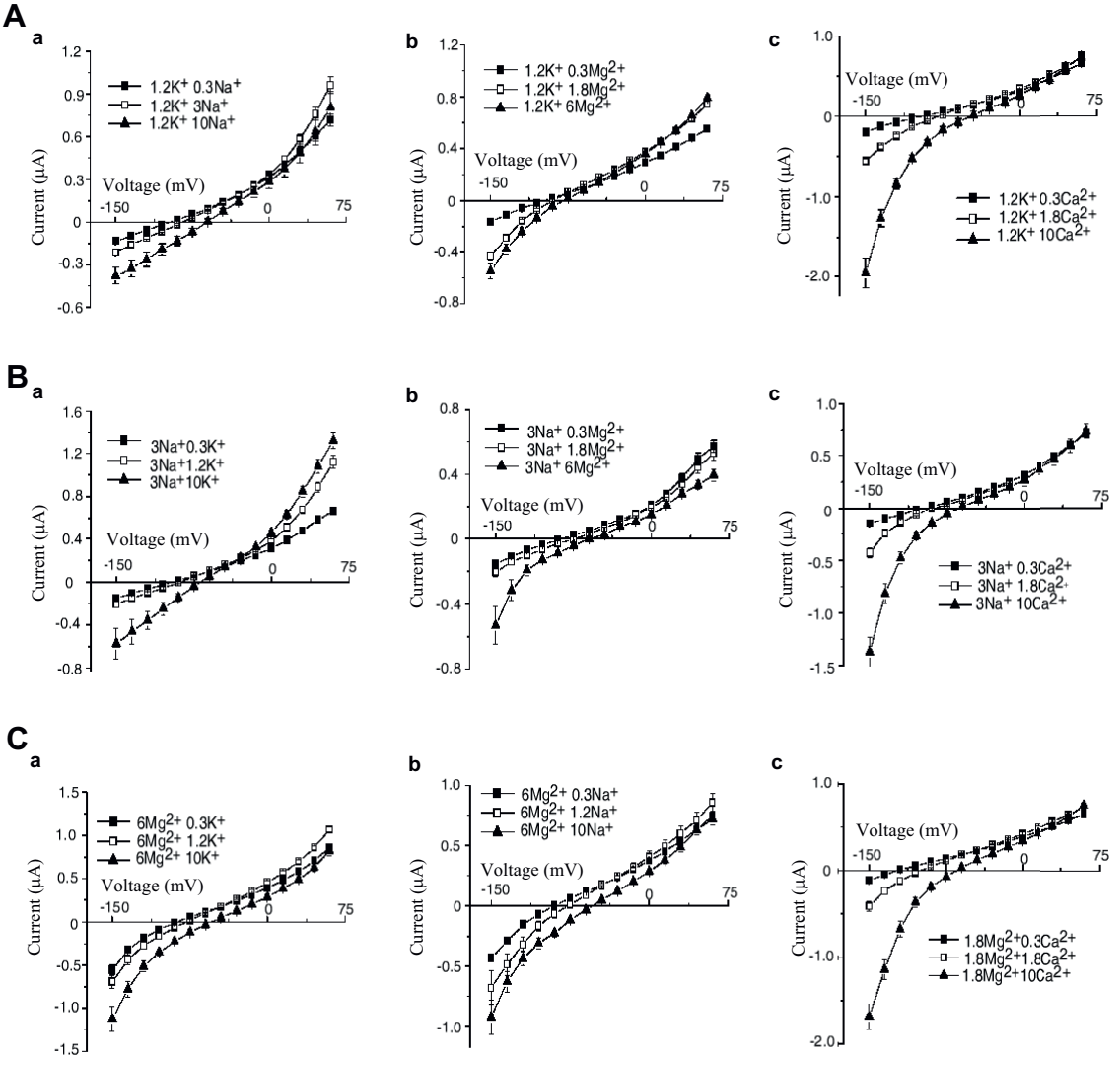

Fig. S3. OsHKT2;4 currents with various combination of cations. (*A*) Current–voltage relationship in the OsHKT2;4-injected oocytes bathed in 1.2 mM K^+ with indicated concentrations of Na^+ (*a*), Mg^{2+} (*b*), or Ca^{2+} (*c*). (*B*) Current–voltage relationship in OsHKT2;4-injected oocytes bathed in 3 mM Na^+ with the indicated concentrations of K^+ (*a*), Mg^{2+} (*b*), or Ca^{2+} (*c*). (*C*) Current–voltage relationship with OsHKT2;4-injected oocytes bathed in 6 mM Mg^{2+} with the indicated concentrations of K^+ (*a*), Na^+ (*b*), or 1.8 mM Mg^{2+} plus various concentrations of Ca^{2+} (*c*). Summarized current data are from seven cells per condition.

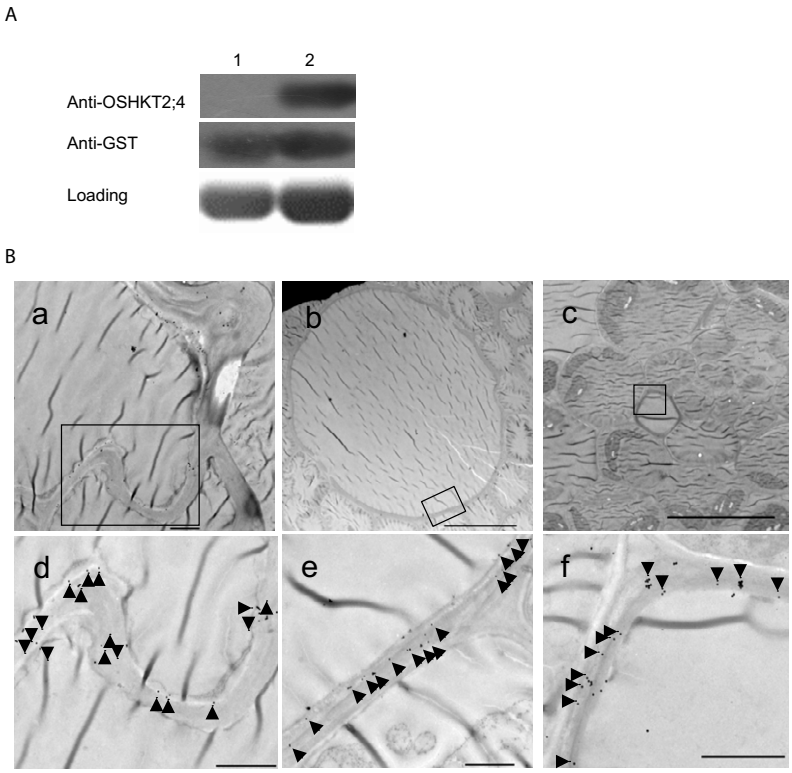

Fig. S4. (*A*) Antibody used in immunogold detection was specific for OsHKT2;4. *Escherichia coli*-expressed GST-OsHKT2;3 (lane 1) and GST-OsHKT2;4 (lane 2) were purified and assayed by Western blot using anti-OsHKT2;4 antibody. A GST antibody detected both fusion proteins. Coomassie brilliant blue-stained bands were used to show loading control. (*B*) Immunogold localization of the OsHKT2;4 protein to the plasma membrane of different cells. (*a*) Leaf epidermal cells. (*b*) Root vascular cells. (*c*) Leaf vascular cells. *d*, *e*, and *f* are enlarged sections of *a*, *b*, and *c*, respectively. Arrowheads indicate gold particles. (Scale bars: *a*, *d*, *e*, and *f*—1 μm; *b* and *c*—10 μm.)

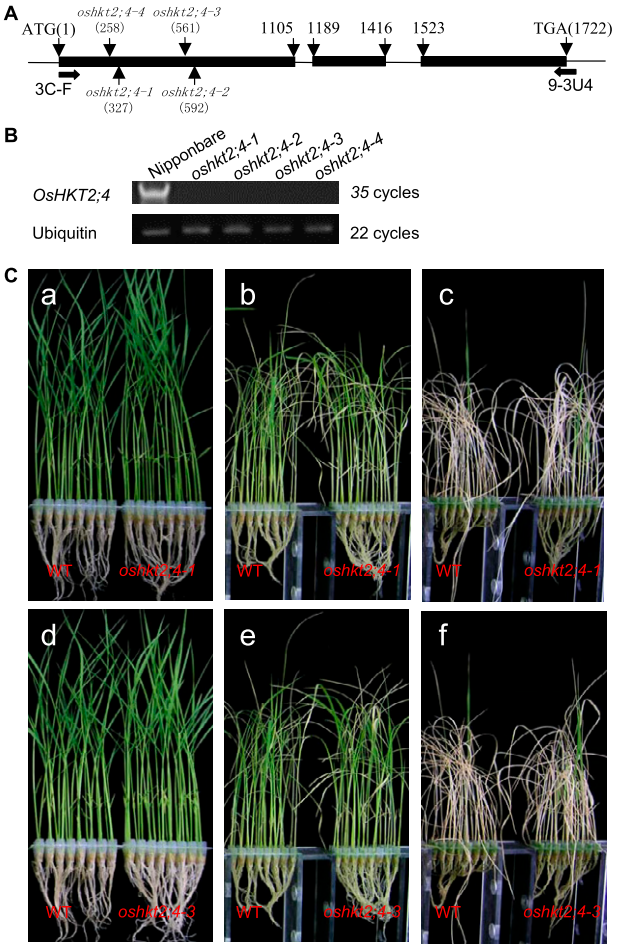

Fig. S5. The oshkt2;4 mutants and wild-type Nipponbare plants show the same phenotype under normal and salt stress conditions. (*A*) Four Tos17 insertion mutant lines—oshkt2;4–1, oshkt2;4–2, oshkt2;4–3, and oshkt2;4–4—were identified. Solid boxes represent exons and lines represent introns. The sites of tos17 insertions are indicated by arrowheads. 3C-F and 9–3U4 were primers used for RT–PCR to measure levels of OsHKT2;4 mRNA in *B*. Ubiquitin mRNA levels were used as loading control. (*C*) Two mutant lines, oshkt2;4–1 and oshkt2;4–3, were used to assess salt tolerance. Twenty-day-old seedlings grown under normal conditions (*a* and *d*) were treated with 100 mM NaCl for 8 days (*b* and *e*) and 18 days (*c* and *f*).