OsPIP2;4 aquaporin water channel primarily expressed in roots of rice mediates both water and nonselective Na^+ and K^+ conductance

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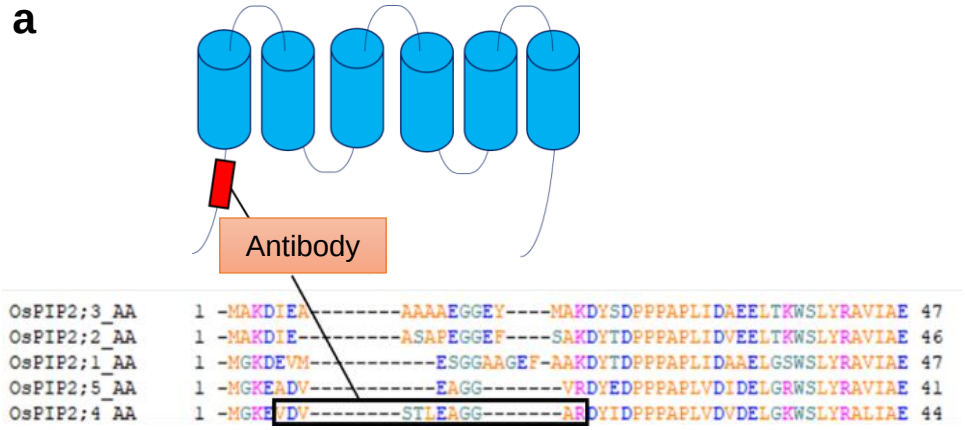
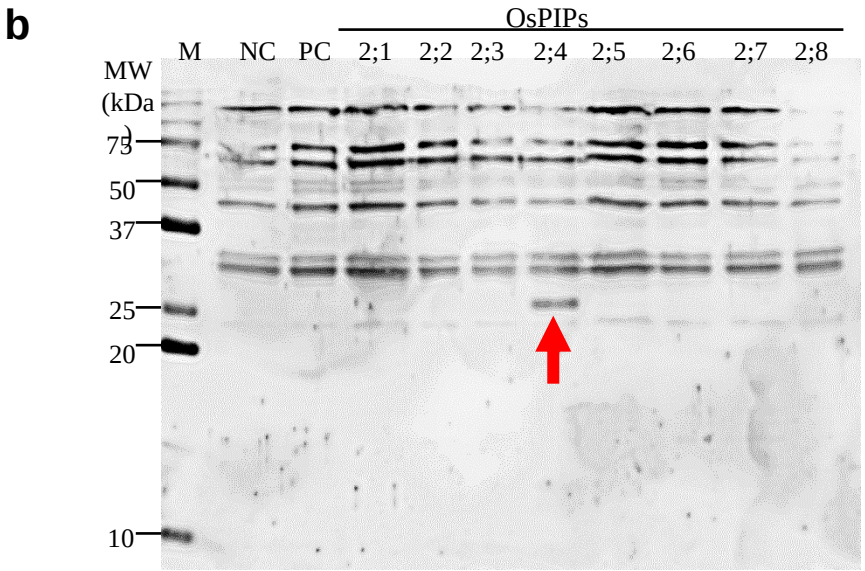
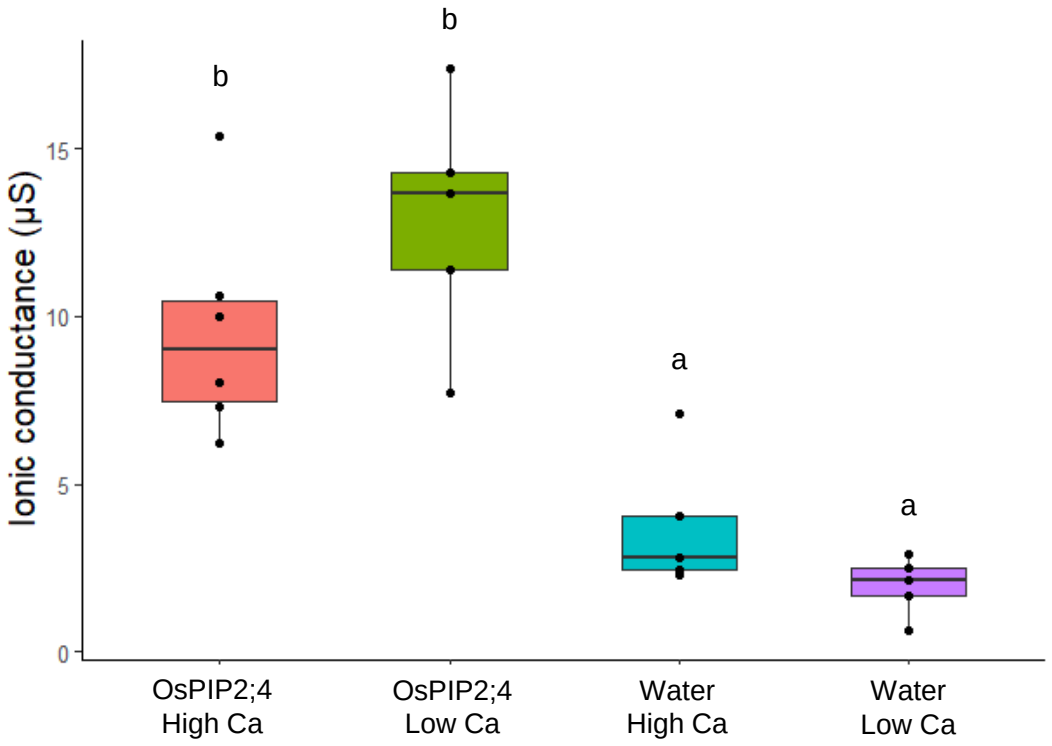
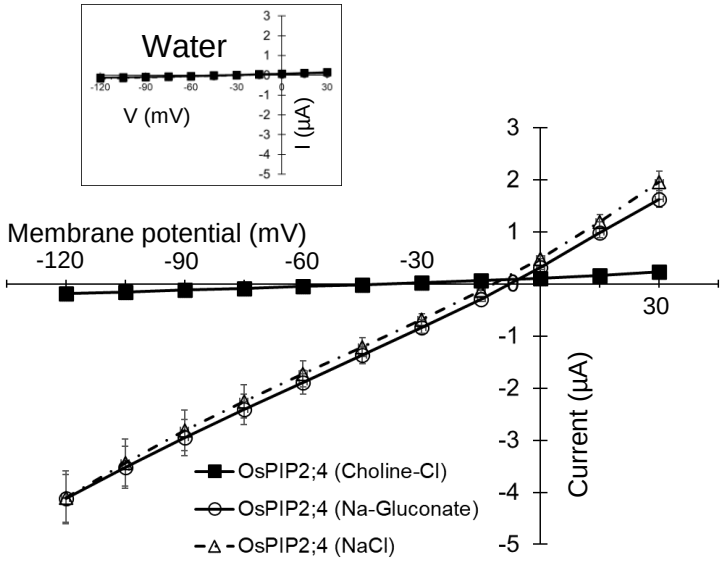




Supplementary Figure 1. Generation of the anti-OsPIP2;4 peptide antibody. (a) The anti-OsPIP2;4 antibody was designed to recognize the amino acid sequence "VDVSTLEAGGAR" that is in N-terminal of OsPIP2;4. (b) To check the specificity of the antibody, 50 ng of each *PIP2* cRNA or water (NC) was injected into *X. leavis* oocytes. The protein in the extracted membrane fraction was solubilized by incubation at 95℃ for 15 min within the sample buffer (4×XT sample buffer (BIO-RAD,USA), 1% Lithium Lauryl Sulfate (LDS, Nacalai, Japan) 240mM, DTT (Nacalai, Japan), 240mM mercaptoethylamine hydrochloride(Nacalai, Japan)). Proteins were separated by the SDS-PAGE and transcribed. The signal derived from the antibody was detected by Amersham ECL Anti-rabbit IgG, Horseradish Peroxidase-Linked Specific Whole Antibody from donkey (GE Healthcare, UK). Note that the red arrow indicates the signal derived from the OsPIP2;4 protein and that the upper extra bands, which can be observed in every sample, are considered non-specific signals.



Supplementary Figure 2. The ionic conductance of water- and *OsPIP2;4* cRNA-injected oocytes, obtained from TEVC recordings in the presence of 86.4 mM NaCl, 9.6 mM KCl, and 1.8 mM Ca^{2+} with or without 1.8 mM EGTA. 10 ng of *OsPIP2;4* cRNA was injected. Each value was calculated from V = -90 mV to -120 mV. Data are presented as means ± SD (n=5-6). Significant differences ($p < 0.05$) are analyzed using one-way ANOVA followed by Tukey HSD post-test and indicated by different alphabets.



Supplementary Figure 3. OsPIP2;4-mediated Na^+ transport is independent of Cl^-. Current (I)-voltage (V) relationships obtained from *X. laevis* oocytes expressing OsPIP2;4 or oocytes injected with water in the presence of 96 mM NaCl, 96 mM Choline-Cl, or 96 mM Na-gluconate. All solutions contained 30 µM Ca^{2+}. A step pulse protocol of -120 mV to +30 mV with a 15-mV increment was applied on every oocyte. Data are means ± SE (n = 7-8).