



**Figure 4.** *sit1* mutant enhances salinity insensitivity by reducing Na<sup>+</sup> influx across the plasma membrane. Rice seeds of the *sit1* mutant and wild-type (WT) were grown for 1 week under half-strength KimuraB nutrient solution (NS) or deionized water (DW) conditions. Seedlings were then treated with NS or DW containing 0, 50, 100 mM NaCl for 1 week. (a) Representative images of the *sit1* mutant and WT at 7 days after salinity treatment. Boxes with broken lines indicate the third leaf of the *sit1* mutant and WT. Scale bar, 5 cm. Quantification of fresh weights of root, leaf sheath, and leaf blade tissues under (b) NS condition and (c) DW condition ( $n = 30$  with 3 replicates). Quantification of leaf chlorophyll content under (d) NS condition and (e) condition ( $n = 30$  with 3 replicates). (f) Representative DAB staining images of leaf blades of the *sit1* mutant and WT. Rice seeds of the *sit1* mutant WT were germinated and grown in half-strength NS for 1 week. Seedlings were then treated with NS containing 0, 50, 100 mM NaCl for 1 week and leaf blades were stained with DAB solution to assess the accumulation of H<sub>2</sub>O<sub>2</sub>. Scale bar, 1 mm. (g) Quantification of H<sub>2</sub>O<sub>2</sub> content in root tissues ( $n = 6$  with 3 replicates). (h) Quantification of H<sub>2</sub>O<sub>2</sub> content in leaf blade tissues ( $n = 6$  with 3 replicates). (i) Representative images of Na<sup>+</sup> accumulations in lateral roots of the *sit1* mutant and WT. One-week-old seedlings of the *sit1* mutant and WT were treated with NS containing 50 mM NaCl for 3 h. The lateral roots were detached and stained with CoroNa-green AM and FM4-64 to visualize the accumulations of Na<sup>+</sup> in the vacuole. (j) Representative images of Na<sup>+</sup> distribution in rice protoplasts of the *sit1* mutant and WT. Leaf protoplasts were isolated and treated with 0 and 50 mM NaCl solution for 1 h. The CoroNa-green AM was used to visualize Na<sup>+</sup> distribution in protoplasts. (k) Quantification of CoroNa green intensity ( $n = 3$  replicates with average intensity of 50 protoplasts per replicate). Value represent means  $\pm$  SD, ns = non-significant, \* $p < 0.05$  and \*\*\* $p < 0.001$ , two-way ANOVA with Sidak's multiple comparison test.