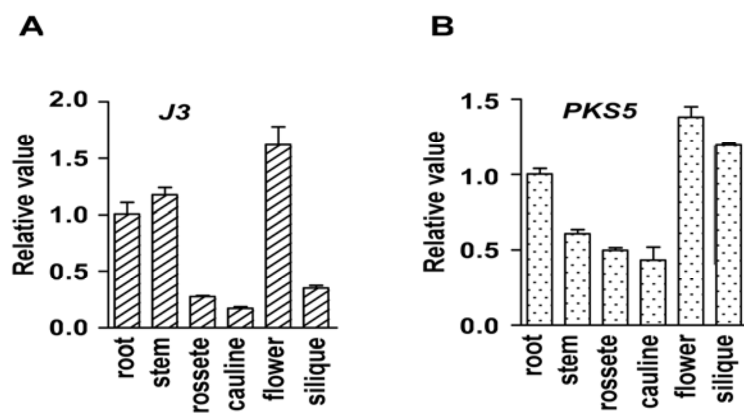
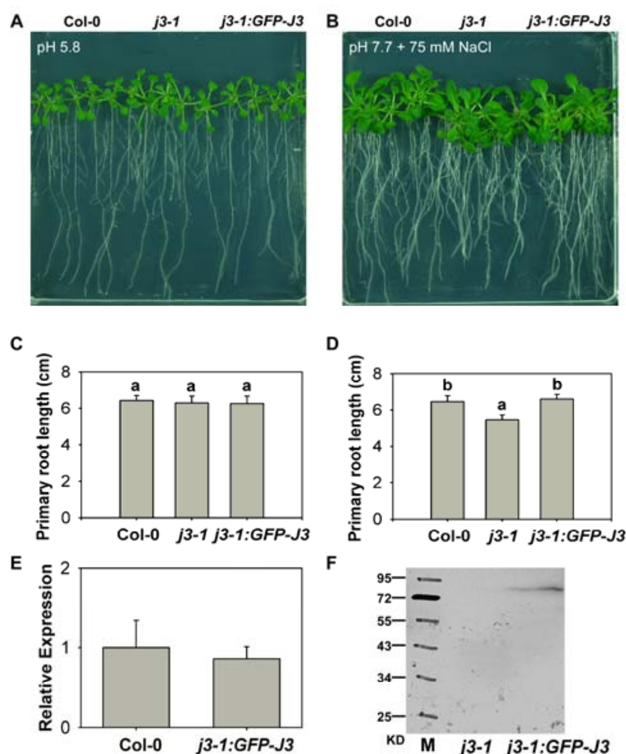


Supplemental Figure 1. Expression of *J3* (**A**) and *PKS5* (**B**) in roots, stems, rosette leaves, cauline leaves, flowers and siliques determined by quantitative real-time PCR using gene-specific primers.



Supplemental Figure 2. Phenotypic complementation of *j3-1* by expression of 35SP:GFP-J3.

Five-day-old Col-0, *j3-1*, and *j3-1* expressing 35SP:GFP-J3 seedlings grown on MS medium at pH 5.8 were transferred to MS medium at pH 5.8, at pH 7.7 with 75 mM NaCl.

Photograph in **A** was taken 7 days after transfer, in **B** was taken 14 days after transfer.

(C) Primary root elongation of seedlings transferred to MS medium at pH 5.8.

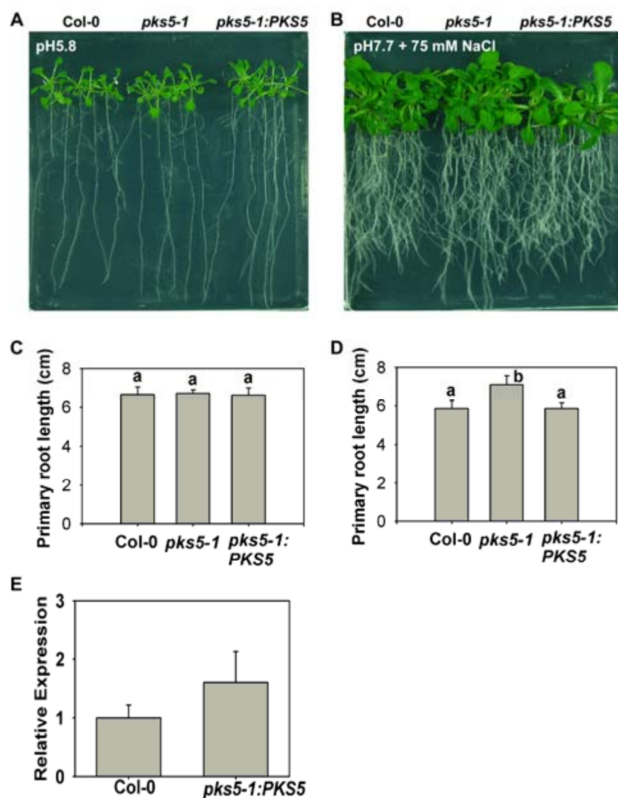
(D) Primary root elongation of seedlings transferred to MS medium at pH 7.7 with 75 mM NaCl.

(E) Relative expression of *j3-1* expressing 35SP:GFP-J3 with *J3* real-time quantitative RT-PCR.

(F) Immunoblot of *j3-1* expressing 35SP:GFP-J3 with anti-GFP antibody (Roche).

Primary root length was measured 7 days after transfer in **A**, 14 days after transfer in **B**.

Error bars represent SD (plant number >15). A Student's t-test was used for determining the statistical significance; significant differences ($P \leq 0.05$) are indicated by different lowercase letters.



Supplemental Figure 3. Phenotypic complementation of *pks5-1* by expression of DexP:3×flag-PKS5.

Five-day-old Col-0, *pks5-1*, and *pks5-1* plants expressing *DexP:3×flag-PKS5* seedlings grown on MS medium at pH 5.8 were transferred to MS medium at pH 5.8, at pH 7.7 with 75 mM NaCl.

Photograph in **A** was taken 7 days after transfer, in **B** was taken 14 days after transfer.

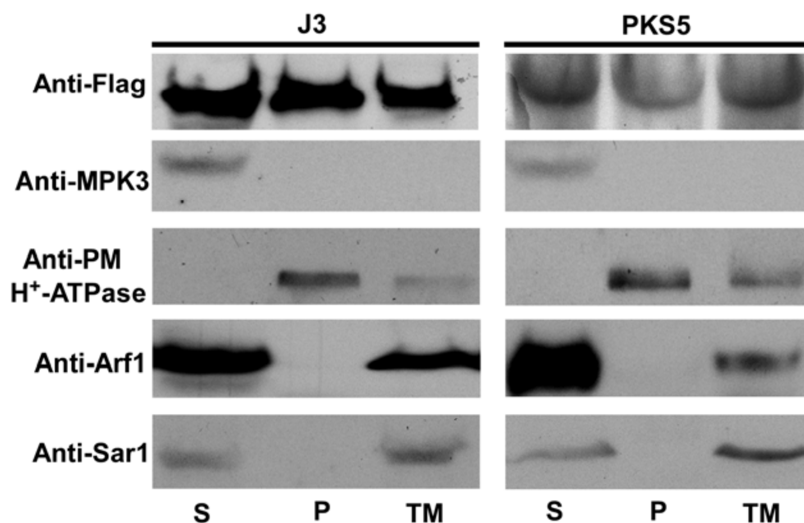
(C) Primary root elongation of seedlings transferred to MS medium at pH 5.8.

(D) Primary root elongation of seedlings transferred to MS medium at pH 7.7 with 75 mM NaCl.

(E) Relative expression of *pks5-1* plants expressing *DexP:3×flag-PKS5* with *PKS5* real-time quantitative RT-PCR.

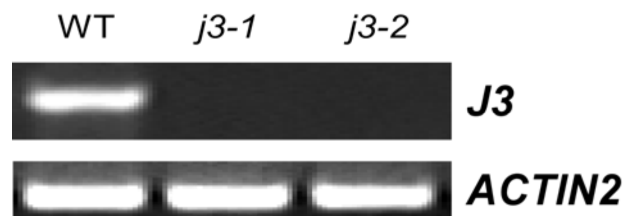
Primary root length was measured 7 days after transfer in **A**, 14 days after transfer in **B**.

Error bars represent SD (plant number >15). A Student's *t*-test was used for determining the statistical significance; significant differences ($P \leq 0.05$) are indicated by different lowercase letters.

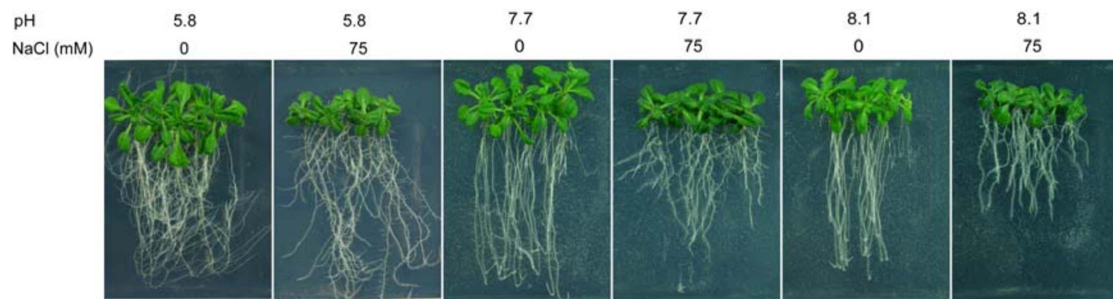


Supplemental Figure 4. PKS5 and J3 were detected in soluble and plasma membrane-enriched fractions.

Isolation of plasma membrane vesicles by two-phase partitioning from *j3-1* seedlings expressing *35SP:3×flag-J3* or *pks5-1* seedlings expressing *DexP:3×flag-PKS5*. Equal amounts of soluble (S), plasma membrane (P), and total membrane (TM) proteins were separated by SDS-PAGE followed by analysis with anti-flag, anti-MAPK3, anti-PM H^{+}-ATPase, anti-Arf1, or anti-Sar1 antibodies.

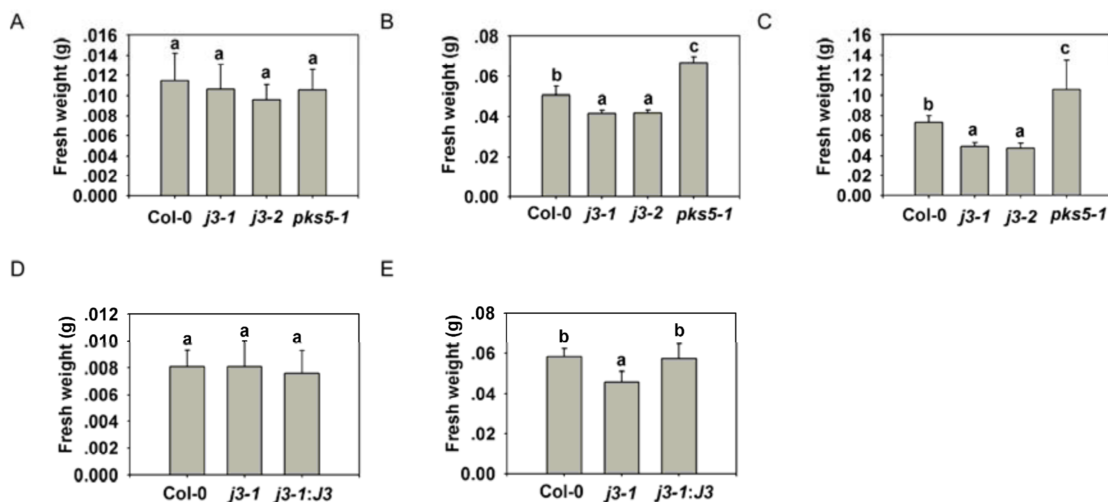


Supplemental Figure 5. RT-PCR analysis of *J3* in Col-0, *j3-1* and *j3-2* plants using *ACTIN2* as control.



Supplemental Figure 6. Alkaline conditions significantly enhance Arabidopsis sensitivity to salt.

Five-day-old wild-type seedlings grown on MS medium at pH 5.8 were transferred to MS medium at pH 5.8, 5.8 with 75 mM NaCl, pH 7.7 or pH 7.7 with 75 mM NaCl, pH 8.1 or pH 8.1 with 75 mM NaCl. Photographs were taken 12 days after transfer.

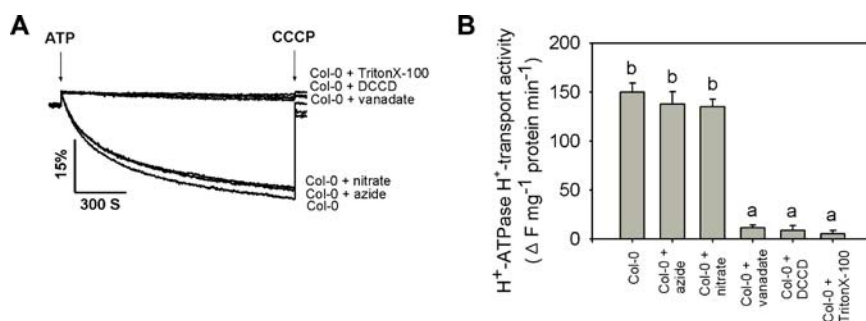


Supplemental Figure 7. Fresh weight of Col-0, *pks5-1*, *j3-1*, and *j3-2* under salt and alkalinity conditions.

Five-day-old Col-0, *pks5-1*, *j3-1*, *j3-2* and *j3-1* expressing 35SP:J3 seedlings grown on MS medium at pH 5.8 were transferred to MS medium at pH 5.8 (**A** and **D**), at pH 7.7 with 75 mM NaCl (**B** and **E**), or at pH 8.1 with 75 mM NaCl (**C**).

Fresh weight in **A** and **D** were measured 7 days after transfer; in **B** and **E**, 14 days after transfer; in **C** 21 days after transfer.

Error bars represent SD (plant number >15). A Student's *t*-test was used to determine statistical significance; significant differences ($P \leq 0.05$) are indicated by different lowercase letters.



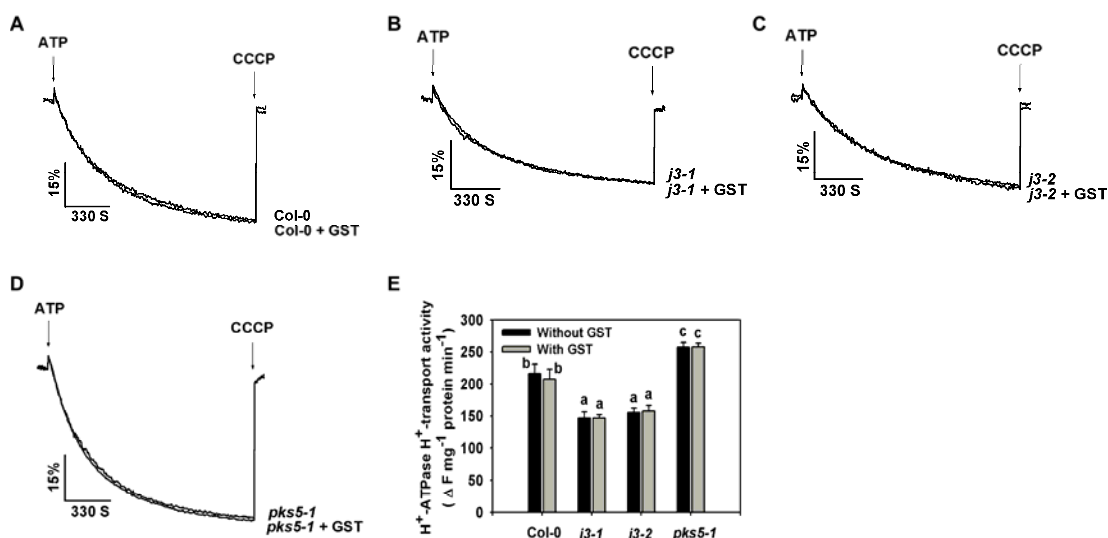
Supplemental Figure 8. Vesicles isolated from Arabidopsis leaves are transport-competent.

Plasma membrane vesicles were isolated from leaves of Col-0 treated with 250 mM NaCl for 3 days. PM H^+-ATPase activity was measured as a decrease (quench) in the fluorescence of the pH sensitive fluorescent probe quinacrine. PM H^+-ATPase activity was initiated by addition of 3 mM ATP, the ΔpH was collapsed by addition of 10 μM (final concentration) carbonyl cyanide m-chlorophenylhydrazone (CCCP). PM H^+-ATPase activity was measured in vesicles isolated from wild-type plants in the presence of different inhibitors as follows:

(A) PM H^+-ATPase activity was measured in the vesicles isolated from Col-0 plants with azide (1 mM, an F-type ATPase inhibitor), nitrate (50 mM, a V-type ATPase inhibitor), vanadate (1 mM, a P-type ATPase inhibitor), N,N'-dicyclohexylcarbodiimide (DCCD, 10 μM, a H^+ channel inhibitor) and Triton X-100 (0.1%, a detergent that causes the membranes to be leaky).

(B) Comparison of PM H^+-ATPase activity in vesicles isolated from Col-0 plants in the presence and absence of inhibitors (concentrations of inhibitors as indicated above for **A**).

The units of PM H^+-ATPase activity are ΔF/min per mg protein. All data represent means ± SE of at least three replicate experiments. Each replicate was performed using independent membrane preparations. One representative experiment of three replicates is shown in **A.** A Student's *t*-test was used to determine statistical significance; significant differences ($P \leq 0.05$) in **B** are indicated by different lowercase letters.

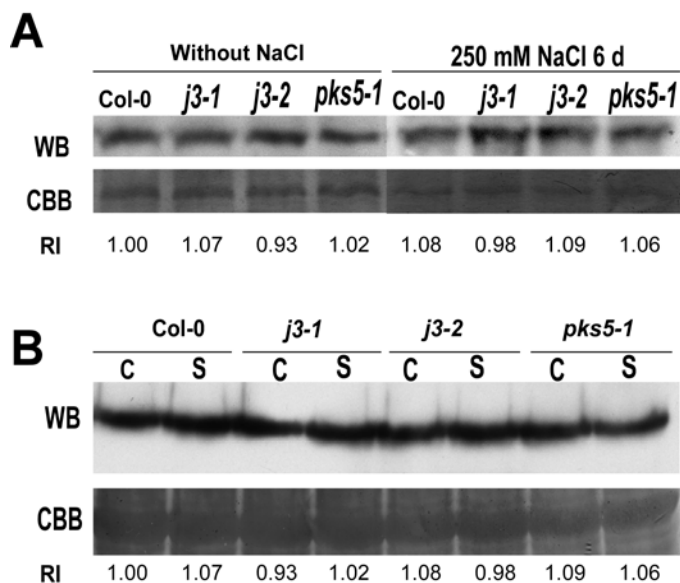


Supplemental Figure 9. GST protein has no effect on PM H^+-ATPase activity.

Plasma membrane vesicles were isolated from Col-0, *j3-1*, *j3-2* and *pks5-1* plants treated with 250 mM NaCl for 3 days. PM H^+-ATPase activity was initiated by addition of 3 mM ATP, the pH gradient was collapsed by addition of 10 µM CCCP. PM H^+-ATPase activity was measured in the vesicles of Col-0 (**A**), *j3-1* (**B**), *j3-2* (**C**) and *pks5-1* (**D**) in the presence or absence of 250 ng/mL GST protein.

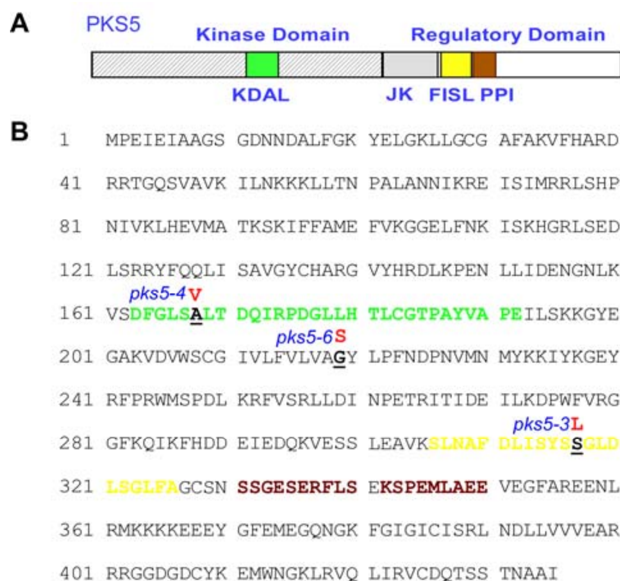
(**E**) Comparison of effect of GST protein on PM H^+-ATPase activity in vesicles isolated from Col-0, *j3-1*, *j3-2* and *pks5-1* plants treated with 250 mM NaCl.

The units of PM H^+-ATPase activity are ΔF/min per mg protein. All data represent means ± SE of at least three replicate experiments. Each replicate was performed using independent membrane preparations. One representative experiment of three replicates is shown in **A-D**. A Student's *t*-test was used to determine statistical significance; significant differences ($P \leq 0.05$) in E are indicated by different lowercase letters.



Supplemetal Figure 10. Immunoblot of PM H^+-ATPase protein from Col-0, *pks5-1*, *j3-1* and *j3-2* plants.

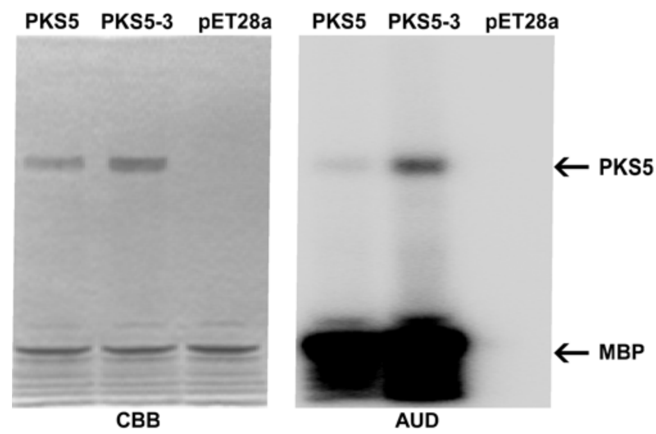
(A-B) Upper panel (WB), anti-PM H^+-ATPase immunoblot; lower panel (CBB), Coomassie Brilliant Blue staining. RI (Relative Intensity) values are normalized to the gray value of WB/CBB and calculated relative to the value for Col-0 without NaCl treatment in **(A)** and **(B)**, respectively. C, without NaCl treatment; S, 6-day 250 mM NaCl treatment. **A** and **B** are two independent biological replicates.



Supplemental Figure 11. Schematic diagram of domains and *pks5* point mutations' distribution in the PKS5 protein

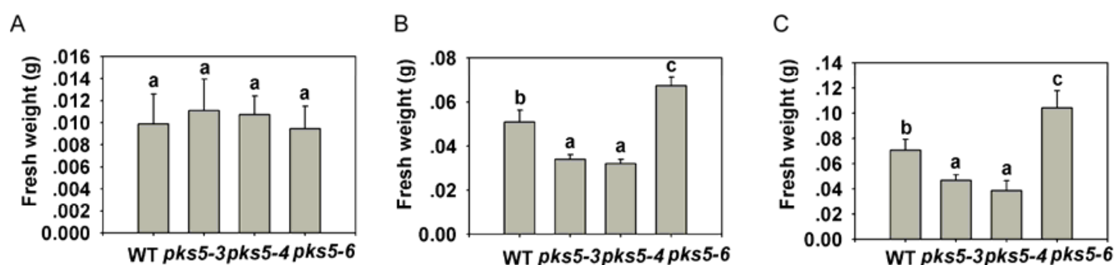
(A) Schematic diagram of domains in the PKS5 protein. The kinase activation loop (KDAL) is shown in green, the junction domain (JK) in blue, the FISL motif (FISL) in yellow and the protein phosphatase interaction domain (PPI) in brown.

(B) The amino acid sequence of the PKS5 protein. Positions of the *pks5-3*, *pks5-4* and *pks5-6* point mutations are indicated. The kinase activation loop sequence is shown in green, the junction domain sequence in blue, the FISL motif sequence in yellow and the PPI sequence in brown.



Supplemental Figure 12. Kinase assay for recommbinant proteins of PKS5, PKS5-3 and pET28a empty vector.

Left panel, Coomassie Brilliant Blue (CBB)-stained SDS-PAGE gel. Right panel, autoradiograph (AUD) of kinase activity assays shown in the left panel. Myelin Basic Protein (MBP).

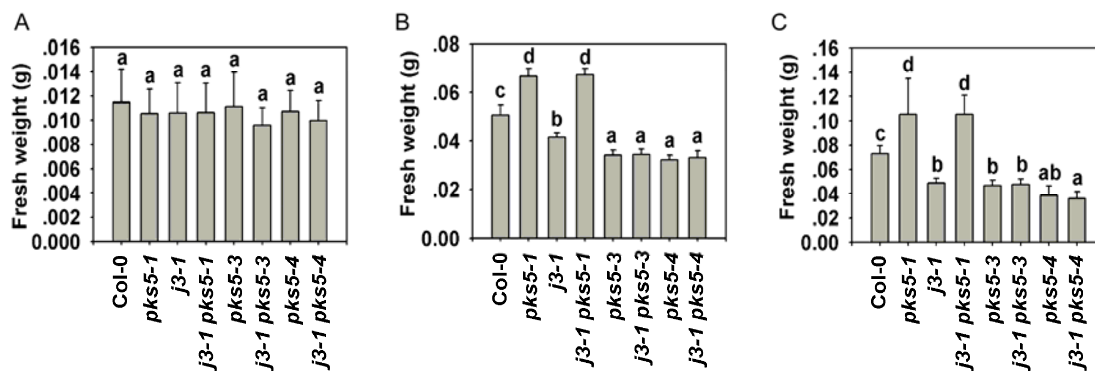


Supplemental Figure 13. Fresh weight of WT, *pks5-3*, *pks5-4* and *pks5-6* under salt and alkalinity conditions.

Five-day-old WT, *pks5-3*, *pks5-4* and *pks5-6* seedlings grown on MS medium at pH 5.8 were transferred to MS medium at pH 5.8 **(A)**, at pH 7.7 with 75 mM NaCl **(B)** or at pH 8.1 with 75 mM NaCl (**C**).

Fresh weight in **A** were measured 7 days after transfer; in **B**, 14 days after transfer; in **C**, 21 days after transfer.

Error bars represent SD (plant number >15). A Student's *t*-test was used to determine statistical significance; significant differences (P≤0.05) are indicated by different lowercase letters.

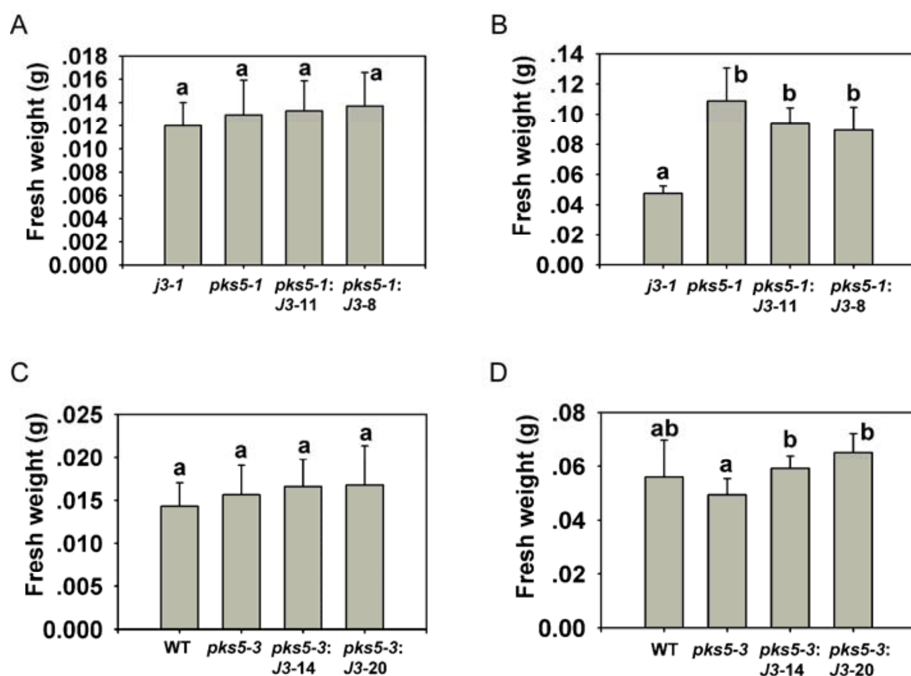


Supplemental Figure 14. Fresh weight of Col-0, *j3-1, pks5-1*, *j3-1 pks5-1*, *pks5-3*, *j3-1 pks5-3*, *pks5-4* and *j3-1 pks5-4* seedlings under salt and alkalinity conditions.

(A-C) Five-day-old Col-0, *j3-1, pks5-1*, *j3-1 pks5-1*, *pks5-3*, *j3-1 pks5-3*, *pks5-4* and *j3-1 pks5-4* seedlings grown on MS medium at pH 5.8 **(A)**, at pH 7.7 with 75 mM NaCl **(B)**, or at pH 8.1 with 75 mM NaCl **(C)**.

Fresh weight in **A** were measured 7 days after transfer; in **B**, 14 days after transfer; in **C**, 21 days after transfer.

Error bars represent SD (plant number >15). A Student's *t*-test was used to determine statistical significance; significant differences ($P \leq 0.05$) are indicated by different lowercase letters.



Supplemental Figure 15. Fresh weight of *j3-1, pks5-1*, *pks5-3*, and *pks5-1* (*pks5-1:J3*) or *pks5-3* over-expressing *J3* (*pks5-3:J3*) under salt and alkalinity conditions.

Five-day-old Col-0, WT, *j3-1, pks5-1*, *pks5-3*, *pks5-1:J3* and *pks5-3:J3* seedlings grown on MS medium at pH 5.8 (**A** and **C**), at pH 7.7 with 75 mM NaCl (**B** and **D**). Fresh weight in **A** and **C** were measured 7 days after transfer; in **B** and D, 14 days after transfer.

Error bars represent SD (plant number >15). A Student's *t*-test was used to determine statistical significance; significant differences ($P \leq 0.05$) are indicated by different lowercase letters.

Addition	H^+-ATPase activity (μM Pi mg^{-1} protein min^{-1}) and inhibit effect (%)
	Upper phase
Control (Without inhibitor)	4.610 ± 0.094 (0)
Na_3VO_4（1 mM)	0.506 ± 0.084 (89.03)
$NaNO_3$ (50 mM)	4.502 ± 0.116 (2.34)
NaN_3 (1 mM)	4.530 ± 0.091 (1.74)
$(NH_4)_6Mo_7O_{24}$ (0.1 mM)	4.543 ± 0.067 (1.46)

Supplemental Table 1. Vesicles isolated from Arabidopsis leaves are enriched in plasma membranes.

Plasma membrane vesicles were isolated from leaves of Col-0 treated with 250 mM NaCl for 3 days. Sodium ortho vanadate (Na_3VO_4), sodium nitrate ($NaNO_3$), sodium azide (NaN_3) or sodium molybdate ($(NH_4)_2MO_4$) were added to assays at the concentrations indicated to assess the percent of ATPase hydrolytic activity (ATP hydrolysis) originating from plasma membranes (vanadate), vacuolar membranes (nitrate), mitochondrial membranes (azide) or soluble phosphatases (molybdate). Azide-and nitrate-sensitive ATPase activities were measured at pH 7.5, vanadate-sensitive ATPase activity was measured at pH 6.5. Inhibitor-dependent changes in hydrolytic activity were calculated based on the activity of a boiled-membrane control at each assay pH. Values are means of five replicates ± SD.