

## SUPPLEMENTARY MATERIAL

Zarza et al., 2019

### **Supplementary Figure S1 | Spm induced-<sup>32</sup>P-PA response in Arabidopsis mature leaves.**

Leaf disks from 3-weeks-old Arabidopsis plants were labelled O/N and the treated for 30 min at indicated concentrations of Spm. Lipids were subsequently extracted and the levels of <sup>32</sup>P-PA quantified. Data are the mean ± SD of three independent experiments (n = 9).

### **Supplementary Figure S2 | PA response in LAT knock-out mutants.**

Spm induced-PA responses in L-type amino acid transporter (LAT) quadruple knock-out lines *lat1/2/3/5* and *lat1/2/4/5*. Seedlings were <sup>32</sup>P-labelled O/N and the next day treated for 30 min with or without 60 μM Spm and the PA response calculated.

### **Supplementary Figure S3 | Effect of Spm on root growth of Arabidopsis seedlings.**

(A) Inhibitory effect of root growth in Arabidopsis seedlings grown for 9 days on plates with increasing concentrations of Spm. (B) Comparison between wild-type and *pldδ* seedlings grown for 5 days on plates containing standard medium and then transferred to plates with or without 150 μM Spm. The image was taken 4 days after transfer (DAT). White dashes indicate the position of the root tip when the seedlings were transferred. (C) Quantification of the growth ratio of the main root (MR) at 4 DAT in response to 0, 60 and 150 μM Spm. Five independent plates per treatment were used, containing eight seedlings each. All experiments were repeated twice with similar results.

### **Supplementary Figure S4 | Gadolinium blocks Spm induced-K<sup>+</sup> flux.**

Quantification of the average K<sup>+</sup> flux measured over 30 min in 5 days-old seedlings pre-treated for 60 min with or without 100 μM GdCl<sub>3</sub> prior to 60 μM Spm application (t = 0). Mean ± SD (n = 6 - 7). Negative values represent net efflux of ions to the apoplast.

### **Supplementary Figure S5 | Expression of *SPMS*, *ACL5* and *PLDδ* in roots.**

Comparison of cell- and tissue-specific expression between *SPMS* (A), *ACL5* (B) and *PLDδ* (C) as indicated by the Arabidopsis eFP Browser (Brady et al., 2007; Winter et al., 2007).

### **Supplementary Figure S6 | *SPMS*-, *ACL5*- and *PLDδ* expression in response to abiotic stress.**

Comparison tissue-specific expression between *SPMS* (A), *ACL5* (B) and *PLDδ* (C) in response to several abiotic stresses as predicted by the Arabidopsis eFP Browser (Kilian et al., 2007; Winter et al., 2007; Dinneny et al., 2008).

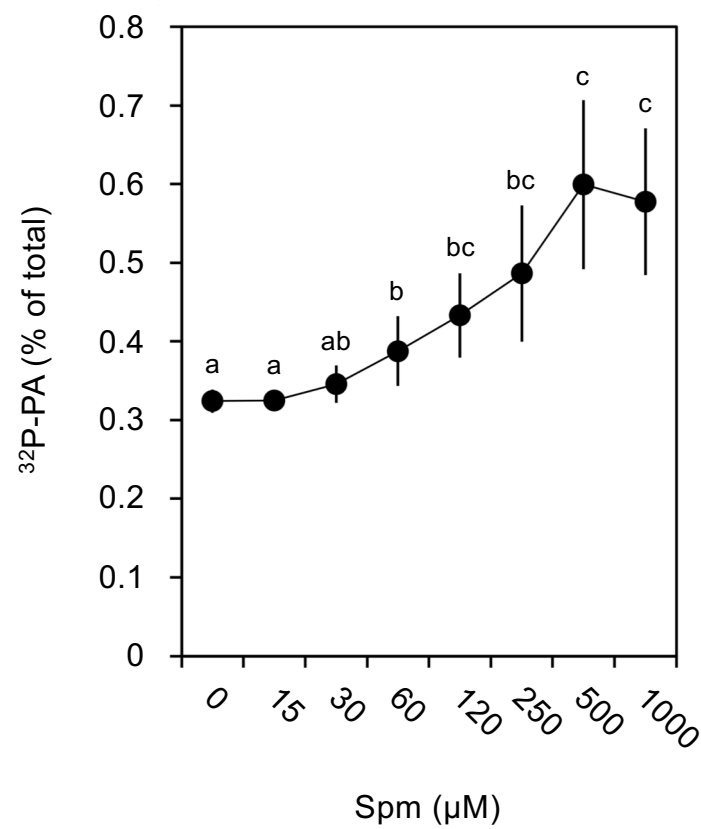
### **Supplementary Figure S7 | Salt stress-induced PA responses are stronger in an *SPMS*-overexpressor line and smaller in an *spms* KO- mutant.**

NaCl induced-PA responses in (A) *Pro35S::SPMS-9* and (B) *spms-2* KO lines. Seedlings were <sup>32</sup>P-labelled O/N and the next day treated with or without 500 mM NaCl for 30 min.

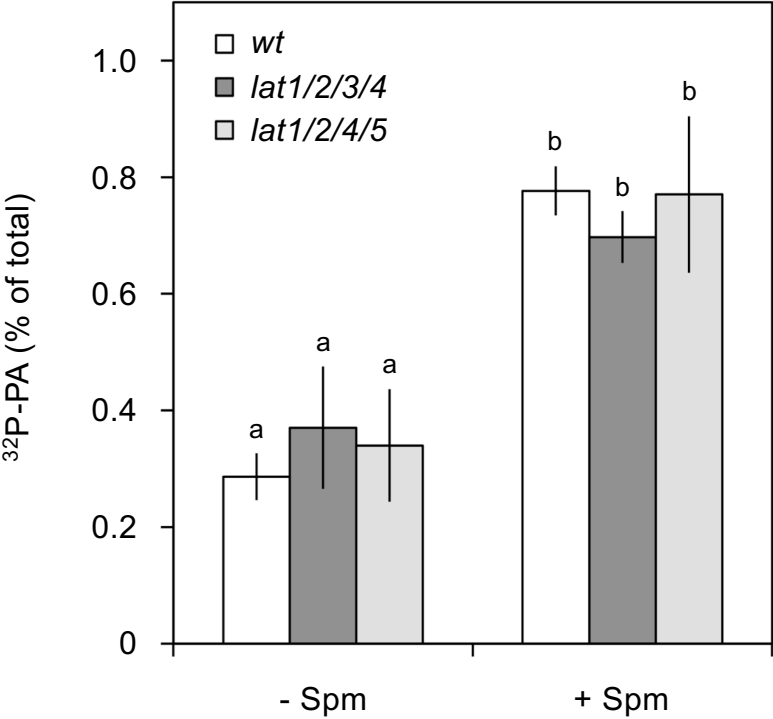
### **Supplementary Figure S8 | H<sup>+</sup> flux kinetics in seedlings treated with Spm**

(A) Dose-response analyses of H<sup>+</sup> flux kinetics measured by MIFE at the elongation zone of the root tip of 5 days-old Arabidopsis wt seedlings. Spm was added at indicated concentrations at t=0. Six to seven seedlings were analysed per treatment. (B) Correlation of the dose-response experiments between time-to-peak for influx H<sup>+</sup> and time-to-peak for efflux K<sup>+</sup>. For all MIFE data, negative values represent a net efflux of ions to the apoplast.

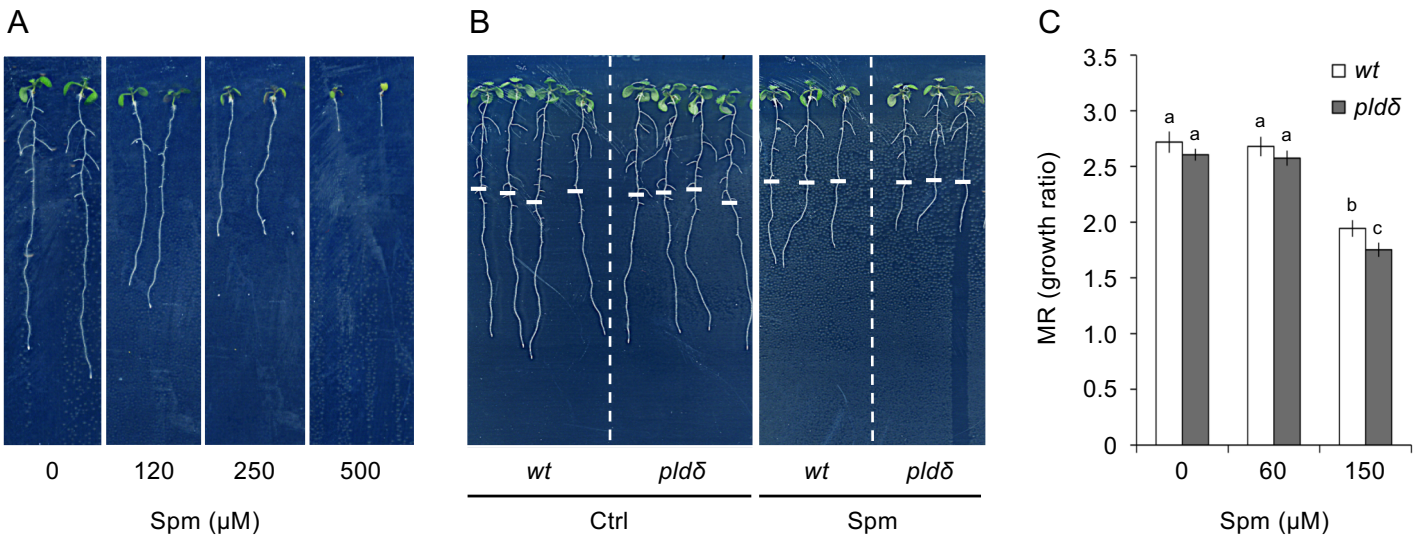
Supplementary Figure S1 – Zarza *et al.*



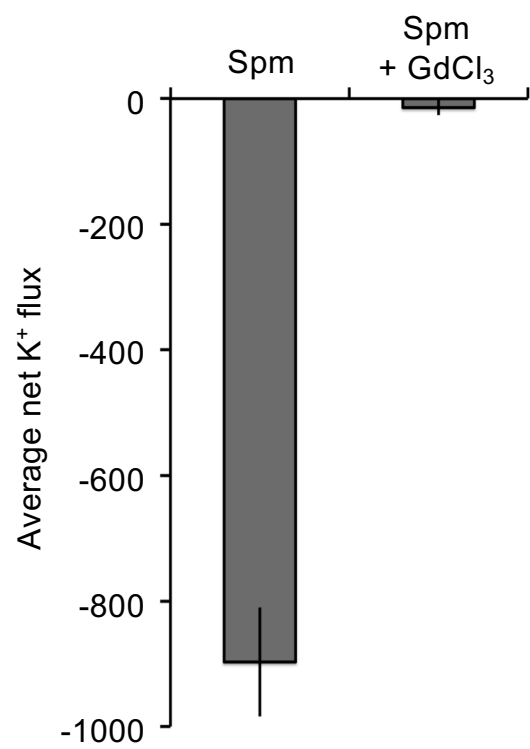
Supplementary Figure S2 – Zarza *et al.*



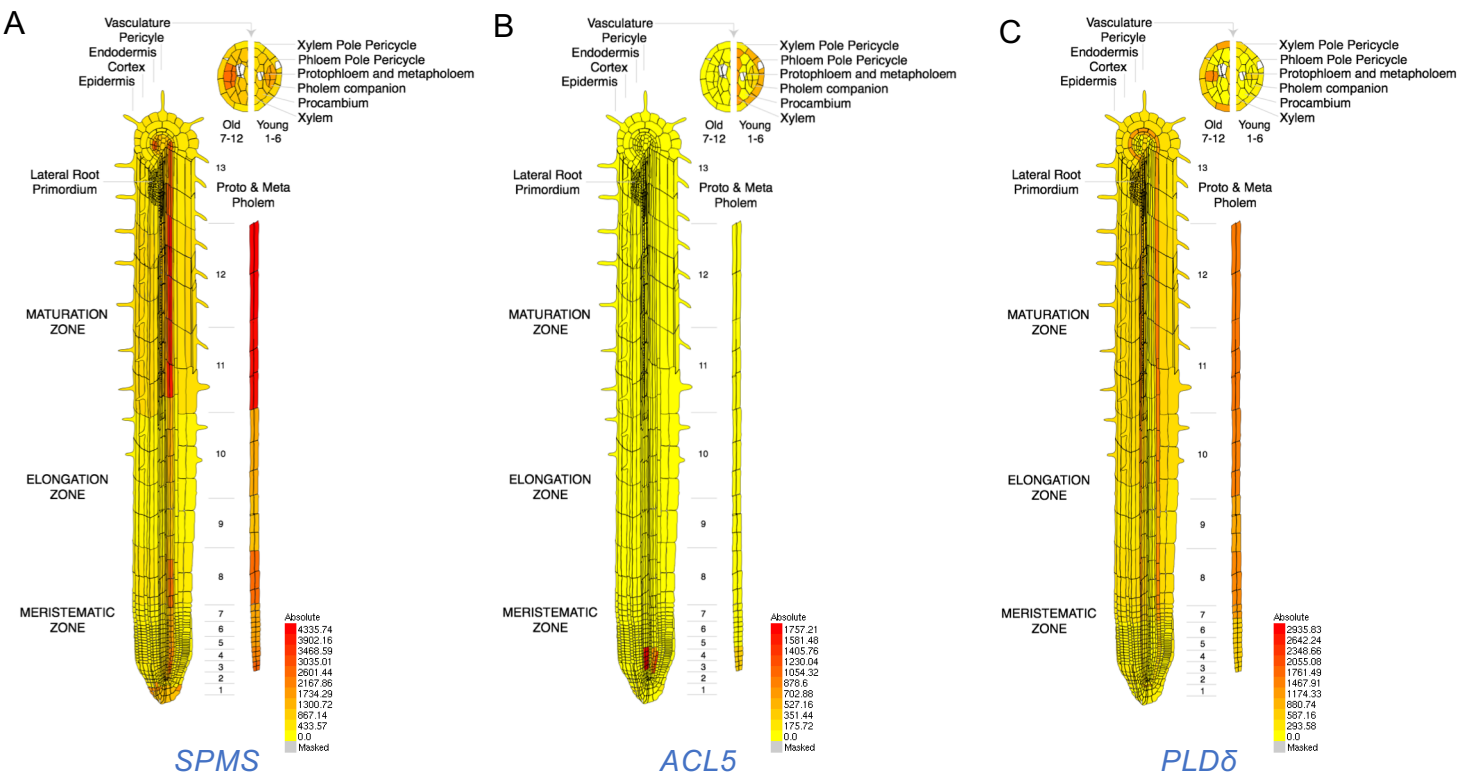
Supplementary Figure S3 – Zarza *et al.*



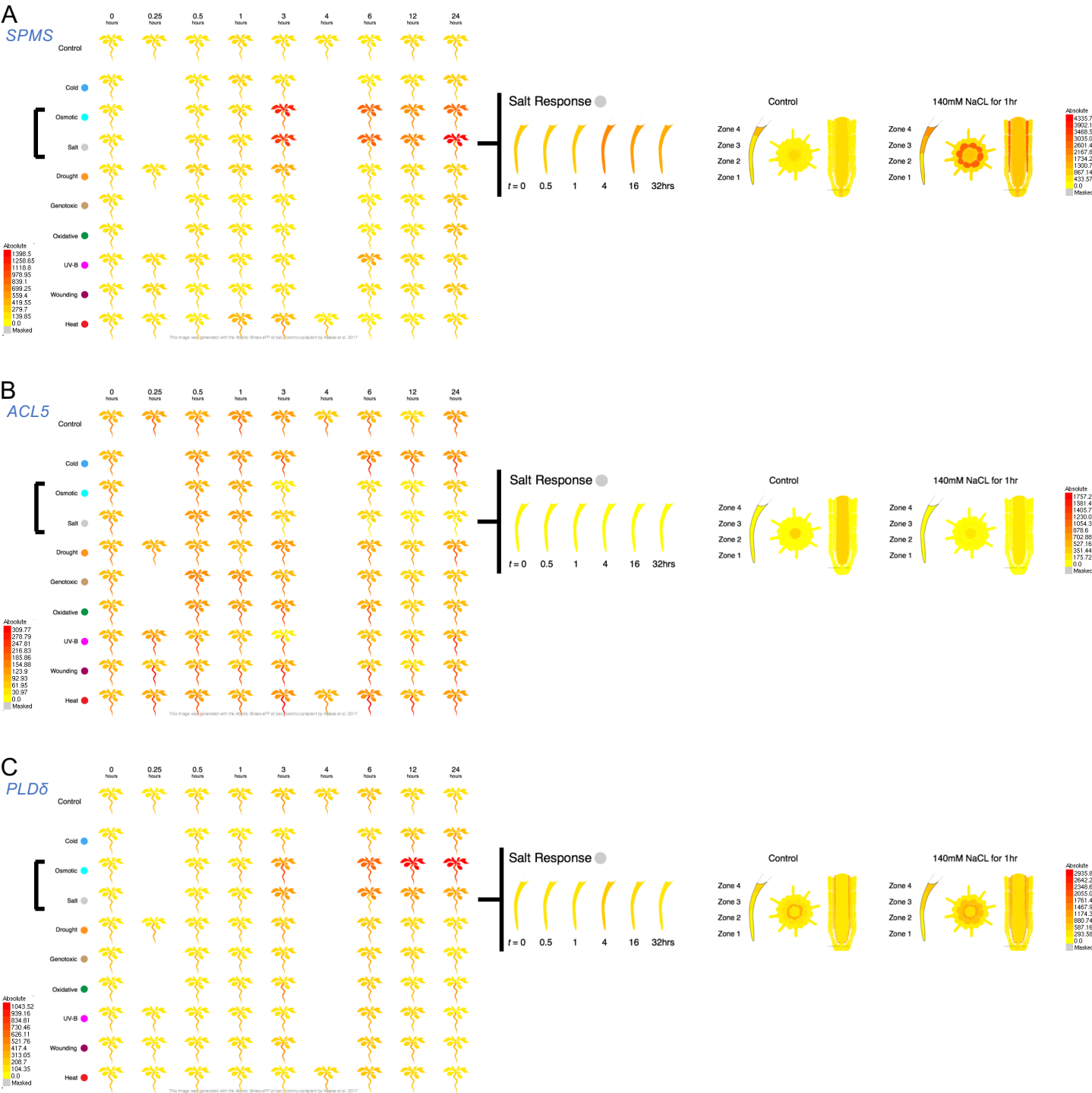
Supplementary Figure S4 – Zarza *et al.*



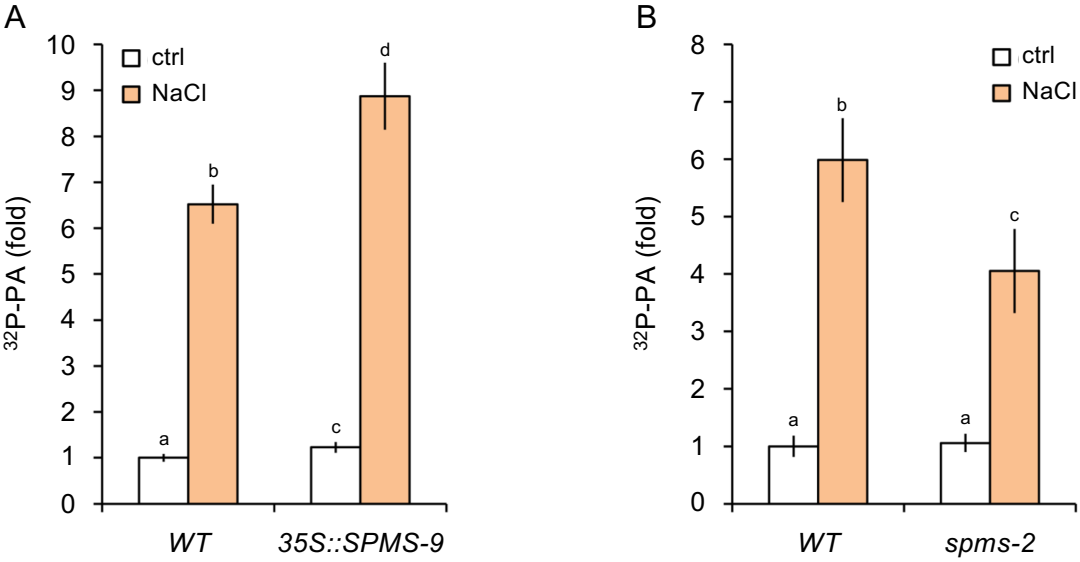
Supplementary Figure S5 – Zarza *et al.*



Supplementary Figure S6 – Zarza *et al.*



Supplementary Figure S7 – Zarza *et al.*



Supplementary Figure S8 – Zarza *et al.*

