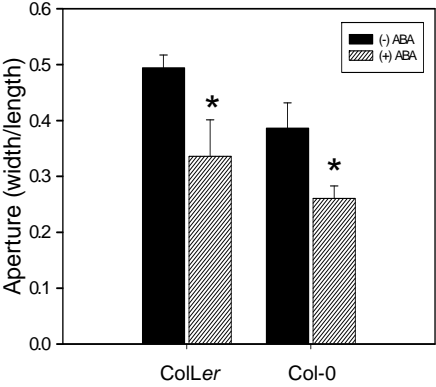


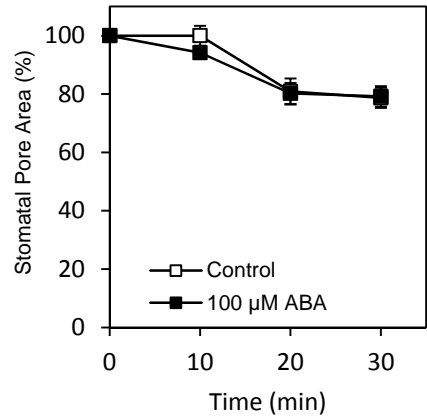
A Stomatal density, index and opening area in ABA and *sal1* mutants

Genotype	Stomatal characteristics			
	Stomatal index	Stomatal density (N per mm ²)	Epidermal cell density (N per mm ²)	Total stomatal opening area per leaf area
Col-0	20.4 ± 1.7	197 ± 37	759 ± 66	(1.09 ± 0.58) × 10 ⁻³
<i>sal1-8</i>	20.6 ± 1.8	307 ± 56	1176 ± 168	(1.80 ± 0.88) × 10 ⁻³
Ler	12.3 ± 1.7	127 ± 15	910 ± 93	(2.06 ± 0.68) × 10 ⁻³
<i>abi1-1</i>	17.2 ± 2.5	231 ± 35	1113 ± 75	(4.61 ± 1.01) × 10 ⁻³
<i>ost1-2</i>	18.0 ± 2.9	214 ± 33	985 ± 147	(5.96 ± 1.32) × 10 ⁻³
<i>abi1-1 sal1-8</i>	13.4 ± 0.9*	376 ± 45*	2428 ± 284*	(5.39 ± 1.17) × 10 ⁻³ (n.s.)
<i>ost1-2 sal1-8</i>	11.9 ± 0.7*	359 ± 33*	2666 ± 357*	(5.51 ± 1.07) × 10 ⁻³ (n.s.)

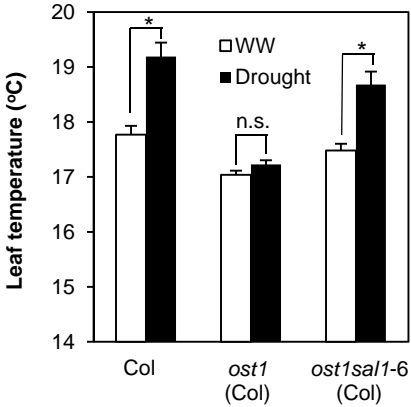
B ColLer and Col-0 responses to ABA



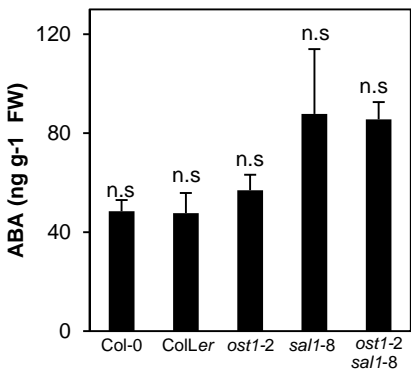
C ABA insensitivity in *ost1* (Col)



D Restoration of stomatal closure in drought-stressed *ost1sal1-6* (Col)



E ABA content in ABA and *sal1* mutants



F Stomatal closure in the chloroplastic calcium sensor mutant *cas1* in response to ABA and PAP

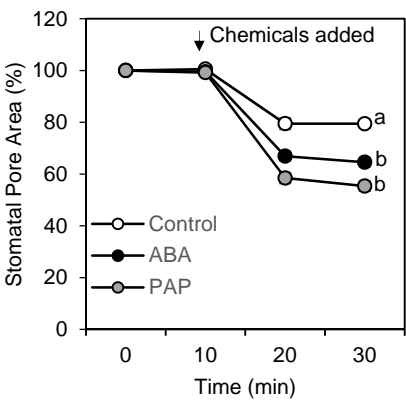


Figure 1 – figure supplement 3

Figure 1 – figure supplement 3. The biochemistry, physiology and stomatal features of single and double mutants, and testing of PAP action via pathogen signaling.

(A) Stomata density, epidermal cell density, stomatal index and total stomatal opening area per leaf area from CryoSEM images of abaxial surface of leaves of six week-old plants. Stomatal index is calculated according to the equation: $\text{Stomatal index} = [(\text{stomatal density}) / (\text{stomatal density} + \text{epidermal cell density})] * 100$. Calculations were performed from five different fields of view per leaf per genotype, $n > 30$. The experiments were performed twice with similar results. Significant differences at $p < 0.05$ (*) or no significant difference (n.s.) are shown for *abi1-1 sal1-8* to *abi1-1* and *ost1-2 sal1-8* to *ost1-2*, respectively. **(B)** Effect of ABA on the stomatal aperture of Col-0 vs ColLer hybrid of leaf peels from five- to six-week old plants after 2 h of 50 μM ABA. **(C)** Effect of ABA on the stomatal aperture of *ost1* (Col-0) treated with either mock control buffer or 100 μM ABA for 30 minutes. Results are mean \pm SE of 15 stomata per treatment. **(D)** Stomatal closure, as assayed by leaf temperature using infrared thermography, in well-watered and drought-stressed WT, *ost1* and *ost1sal1-6* (all in Col-0 background). Values are mean \pm SE of five individual plants per genotype. Significant differences are shown for $p < 0.05$ (*). **(E)** Effect of the *sal1-8* mutation on total leaf ABA content. The ABA content in *sal1-8* and *ost1-2 sal1-8* were marginally higher but not statistically different to ColLer or *ost1-2* (ANOVA, $p = 0.09$). Results are means and standard error of 3 x 100 mg pooled two-week old seedlings. **(F)** Stomatal closure in leaf peels of the chloroplastic calcium sensor mutant, *cas1*, after treatment with 100 μM ABA, 100 μM PAP or control measuring buffer (Means \pm SEM of >40 stomata per genotype). Significant differences at $t = 30$ minutes (a, b; $p < 0.05$) are shown.