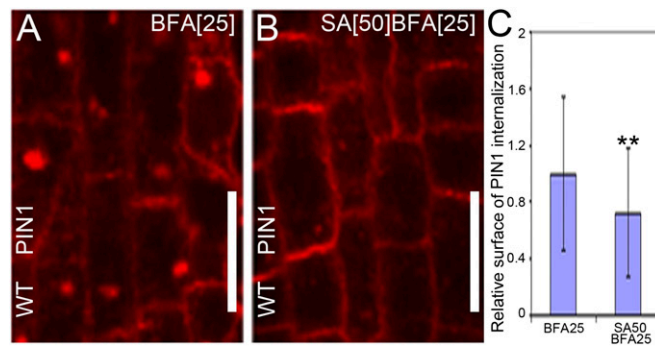
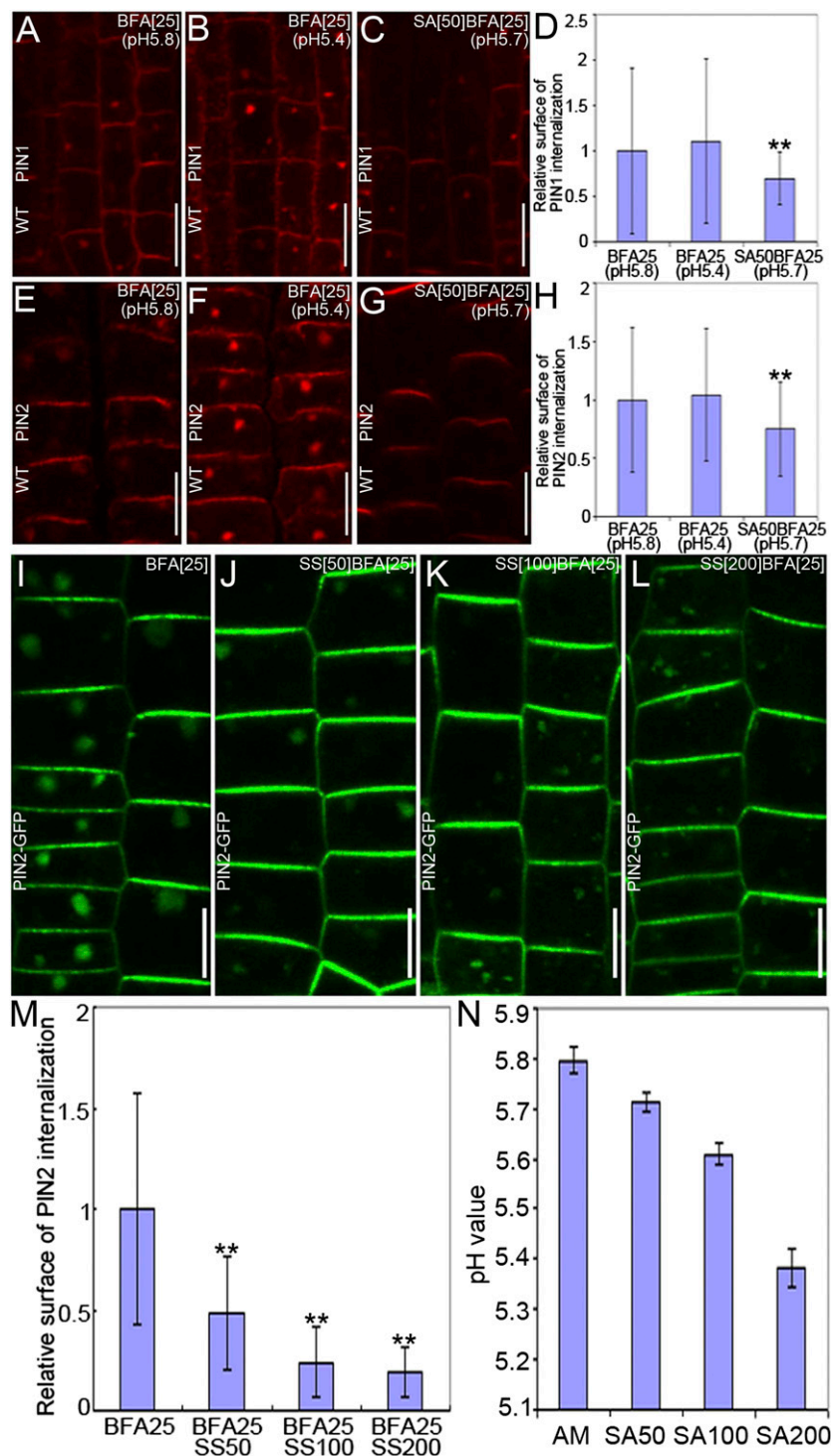


# Supporting Information

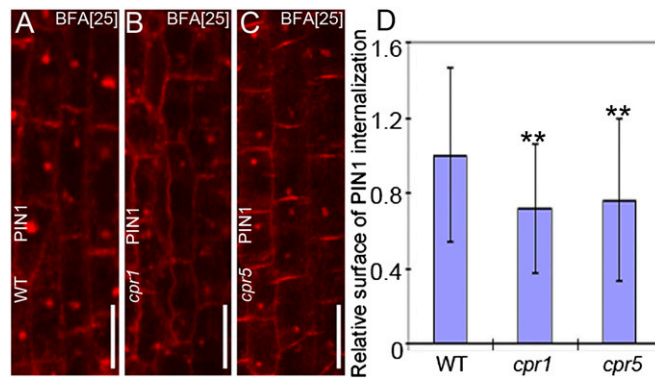
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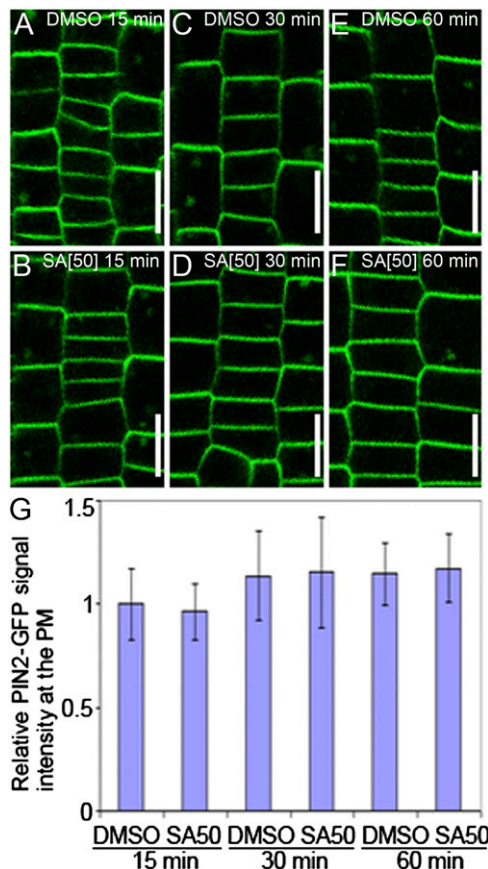
**Fig. S1.** Interference of salicylic acid (SA) with the brefeldin A (BFA)-visualized internalization of PIN1 proteins. (A and B) Immunolocalization of PIN-FORMED (PIN) 1 in root stele cells after treatment with 25  $\mu$ M BFA ( $n = 352$  BFA bodies; 19 roots) (A) or cotreated with 50  $\mu$ M SA for 90 min after a 30-min 50  $\mu$ M SA pretreatment ( $n = 284$  BFA bodies; 23 roots) (B). (C) Quantification of the relative surface of the internalized PIN1 under conditions described in A and B. Values represent the relative mean surface area normalized to the respective control treatments. Data are means  $\pm$  SD; \*\* $P < 0.01$  (Student's  $t$  test). (Scale bars: 10  $\mu$ m).



**Fig. S2.** Low pH does not interfere with BFA-visualized internalization of PIN1 and PIN2 proteins. (A–C) Subcellular localization of PIN1 in young stele root cells treated with 25  $\mu$ M BFA for 90 min in control media (pH 5.8) ( $n = 155$  BFA bodies; 11 roots) (A), in media adjusted to pH 5.4 ( $n = 130$  BFA bodies; eight roots) (B), or cotreated with 50  $\mu$ M SA (SA addition shifted pH to 5.7) ( $n = 99$  BFA bodies; 10 roots) (C) for 90 min after pretreatment with 50  $\mu$ M SA for 30 min. (D) Quantification of the relative surface of the internalized PIN1 in A–C, showing no significant differences between treatment at pH 5.8 and pH 5.4 (Student's  $t$  test,  $P > 0.05$ ). (E–G) Subcellular localization of PIN2 in young epidermal root cells treated with 25  $\mu$ M BFA for 90 min in control media (pH 5.8) ( $n = 251$  BFA bodies; 17 roots) (E), acidified media adjusted to pH 5.4 ( $n = 386$  BFA bodies; 21 roots) (F), or cotreated with 50  $\mu$ M SA (SA addition shifted pH to 5.7) ( $n = 168$  BFA bodies; 18 roots) (G) for 90 min after a 30-min pretreatment with 50  $\mu$ M SA. (H) Quantification of the relative surface of the internalized PIN2 in E–G, without significant differences between treatment at pH 5.8 and pH 5.4 (Student's  $t$  test,  $P > 0.05$ ). (I–L) Subcellular localization of PIN2-GFP in young epidermal root cells treated with 25  $\mu$ M BFA for 90 min ( $n = 612$  BFA bodies; 40 roots) (I) or cotreated with either 50  $\mu$ M sodium salicylate (SS) ( $n = 480$  BFA bodies; 40 roots) (J), 100  $\mu$ M SS ( $n = 590$  BFA bodies; 38 roots) (K), and 200  $\mu$ M SS ( $n = 547$  BFA bodies; 37 roots) (L) for 90 min after pretreatment with 50, 100, and 200  $\mu$ M SS for 30 min. (M) Quantification of the relative surface of the internalized PIN2-GFP under conditions described in I–L. (N) pH values of *Arabidopsis* medium (AM) supplemented with different concentrations of SA (50, 100, and 200  $\mu$ M). Values in D, H, and M represent the relative mean surface area in comparison with the control for each individual experiment. Data are means  $\pm$  SD; \*\* $P < 0.01$  (Student's  $t$  test). (Scale bars: 10  $\mu$ m).

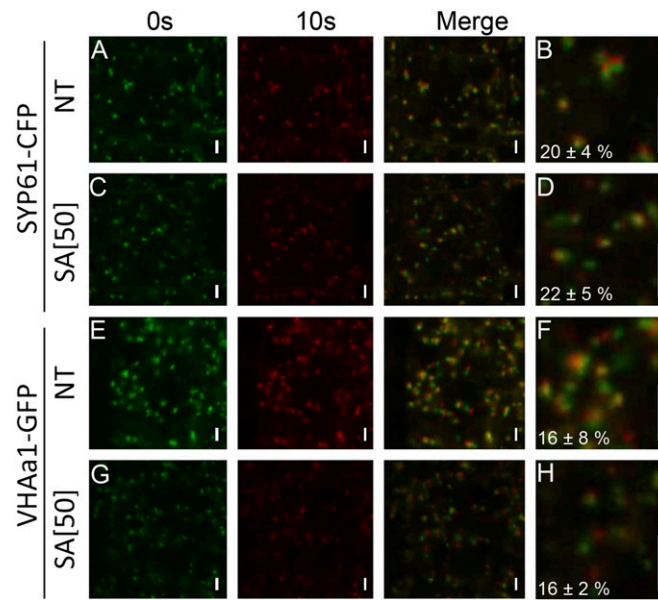


**Fig. 53.** SA-accumulating mutants interfere with the BFA-visualized internalization of PIN1 protein. Immunolocalization of PIN1 in young stele root cells of WT (Col-0) ( $n = 336$  BFA bodies; 16 roots) (A), an allele of *constitutive expressor of PR gene 1* (*cpr1*) ( $n = 543$  BFA bodies; 25 roots) (B), and *cpr5* ( $n = 385$  BFA bodies; 18 roots) (C) mutants after treatment with 25 μM BFA for 90 min. (D) Quantification of the relative surface of the internalized PIN1 under conditions described in A–C. Values in D represent the relative mean surface area in comparison with the control for each individual experiment. Data are means ± SD; \*\* $P < 0.01$  (Student's  $t$  test). (Scale bars: 10 μm).



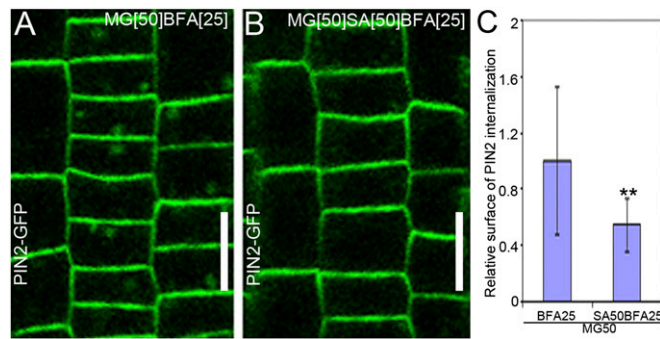
**Fig. 54.** SA does not affect PIN2-GFP recycling to the plasma membrane (PM). (A–F) Recovery of PIN2-GFP at the PM after treatment with 25 μM BFA for 60 min followed by a transfer to medium without BFA supplemented with either DMSO (A, C, and E) or 50 μM SA (B, D, and F) for 15 min (A and B), 30 min (C and D), or 60 min (E and F). (G) Quantification of the PIN2-GFP signal intensity at the PM during the BFA washout in the absence of SA ( $n = 150, 145,$  and 130 cells from 12, 14, and 13 roots for the DMSO treatment for 15, 30, and 60 min, respectively) and the presence of 50 μM SA ( $n = 149, 133,$  and 145 cells from 16, 13, and 17 roots for the SA treatment for 15, 30, and 60 min, respectively). No significant differences were observed. Data are means ± SD and were normalized to the DMSO control at 15 min (Scale bars: 10 μm).



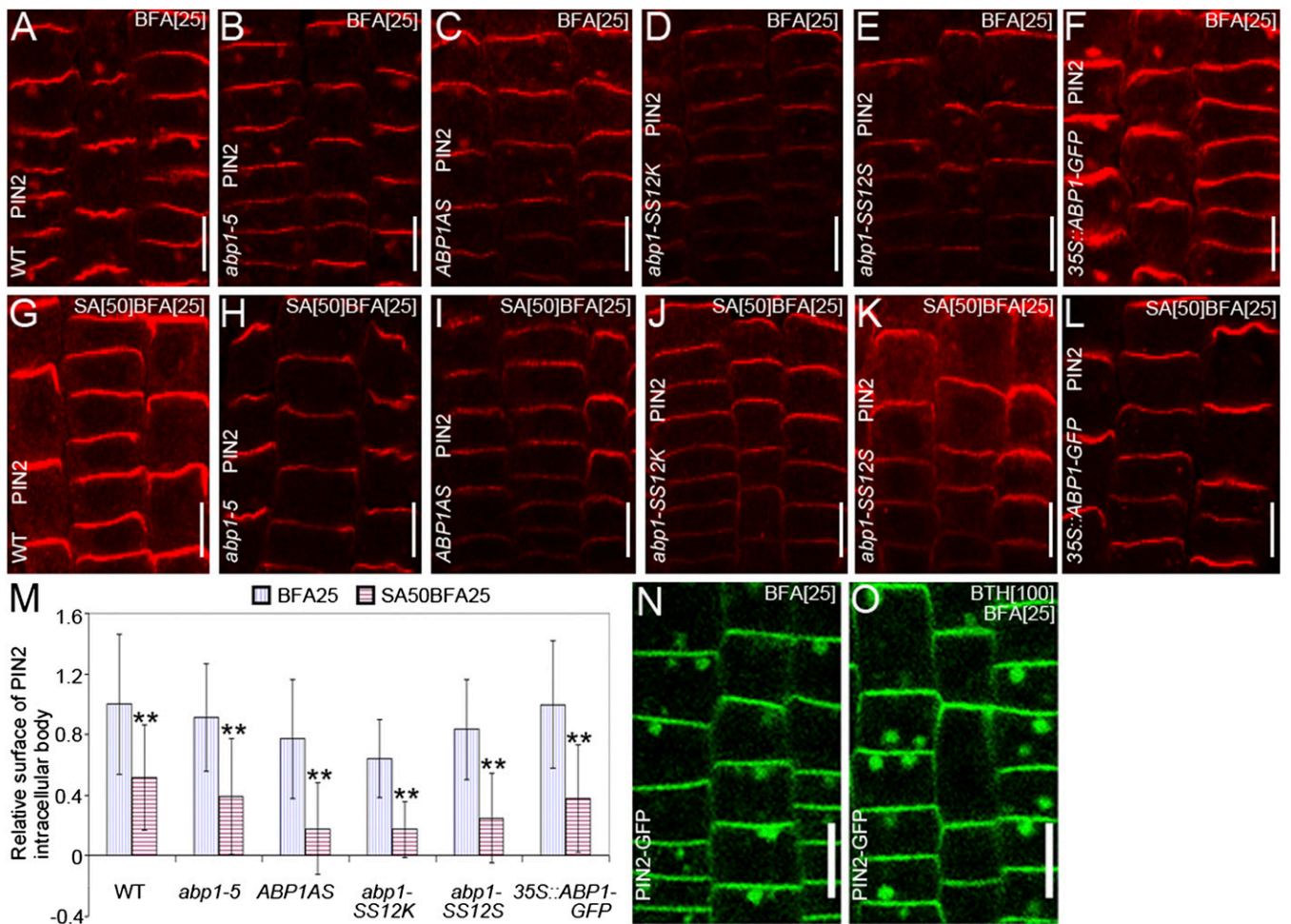


**Fig. S6.** SA has no impact on the endosomal motility. Qualitative assessment of the movement of endosomes labeled with syntaxin of plants 61 (SYP61)-CFP (A–D) or VHAa1-GFP (E–H) in a 10-s time frame in untreated controls (A, B, E, and F) and in seedlings treated with 50  $\mu$ M SA (C, D, G, and H) for 1 h. Images represent sequential imaging at 0 or 10 s after the indicated treatment. Green and red panes represent false-colored 0-s and 10-s situations, respectively. Merged green (0s) and red (10s) images were exposed to yellow immobile endosomes. Distinct red and green signals indicate endosomal movement. (B, D, F, and H) Fivefold digital enlargement of the merged images not treated and treated with 50  $\mu$ M SA. Quantifications of the mean percentage of immobile endosomes revealed that the mobility did not differ after treatment with or without SA. The data are means  $\pm$  SD of the percentage of colocalized immobile vesicles after 10 s of observation from five cells. (Scale bars: 2  $\mu$ m).

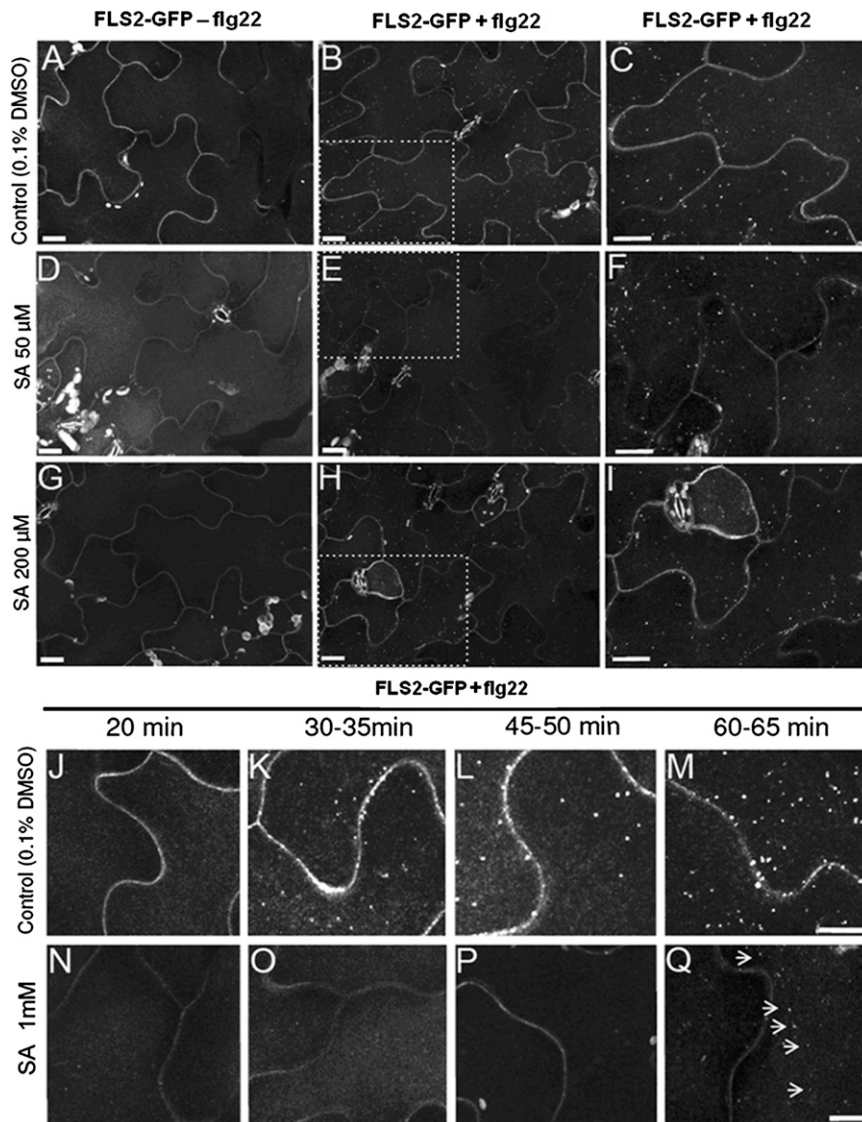




**Fig. 58.** SA does not require proteasome activity for endocytosis inhibition. (A and B) Internalization of PIN2-GFP in root cells cotreated with 50  $\mu$ M MG132 (MG) and 25  $\mu$ M BFA in combination with either DMSO for 90 min ( $n = 393$  BFA bodies; 17 roots) (A) or 50  $\mu$ M SA for 90 min after a 30-min 50  $\mu$ M SA pretreatment ( $n = 174$  BFA bodies; 30 roots) (B). (C) Quantification of the effect of 50  $\mu$ M MG132 on the inhibitory effect of SA on the BFA-visualized PIN2-GFP internalization. Values represent the relative mean surface area in comparison with the control. Data are means  $\pm$  SD; \*\* $P < 0.01$  (Student's  $t$  test). (Scale bars: 10  $\mu$ m).



**Fig. 59.** Effect of SA on endocytosis is independent of auxin binding protein 1 (ABP1) and benzothia-diazole *S*-methyl ester (BTH) cannot inhibit PIN2 internalization. (A–L) Immunolocalization of PIN2 in WT (A and G), *abp1-5* (B and H), induced *A1c::ABP1AS* (C and I), induced *A1c::abp1-SS12K* (D and J), induced *A1c::abp1-SS12S* (E and K), and *35S::ABP1-GFP* (F and L) seedlings after cotreatments with 25  $\mu$ M BFA and either DMSO for 90 min (A–F) or 50  $\mu$ M SA for 90 min after pretreatment with 50  $\mu$ M SA for 30 min (G–L). (M) Quantification of the effect of SA on the BFA-visualized PIN2 internalization in mutants and transgenic lines with altered ABP1 levels or activity as estimated by the relative surface of the BFA bodies in A–L for the DMSO treatment ( $n = 227, 251, 103, 74, 102,$  and  $276$  BFA bodies from 18, 16, 17, 20, 31, and 19 roots for WT, *abp1-5*, *ABP1AS*, *abp1-SS12K*, *abp1-SS12S*, and *35S::ABP1-GFP*, respectively) and for the SA treatment ( $n = 43, 21, 8, 17, 24,$  and  $20$  BFA bodies from 29, 25, 22, 26, 45, and 26 roots for WT, *abp1-5*, *ABP1AS*, *abp1-SS12K*, *abp1-SS12S*, and *35S::ABP1-GFP*, respectively). Values represent the relative mean surface area in comparison with the control for each individual experiment. Data are means  $\pm$  SD; \*\* $P < 0.01$  (Student's  $t$  test). (N and O) Internalization of PIN2-GFP in root cells cotreated with 25  $\mu$ M BFA and either DMSO for 90 min (N) or 100  $\mu$ M of the active SA analog BTH for 90 min after pretreatment with 50  $\mu$ M BTH for 30 min (O). (Scale bars: 10  $\mu$ m).



**Fig. S10.** SA does not inhibit flg22-induced FLS2-GFP endocytosis. (A–I) Cotyledon cells of 14-d-old seedlings pretreated with either DMSO or the indicated concentration of SA. Plants in B, E, and H were subsequently treated with 10  $\mu$ M flg22 for 40–50 min. Images C, F, and I are enlargements of the FLS2-GFP foci from the boxed regions in B, E, and H, respectively. (J–Q) FLS2-GFP internalization in cotyledon cells pretreated with either DMSO or 1 mM SA for 30 min, followed by incubation with 10  $\mu$ M flg22 for the indicated time intervals. Arrows show representative FLS2-GFP endosomes. (Scale bars: 10  $\mu$ m).