SUPPLEMENTAL FIGURES

by

Zdarska, Cuyacot et al., 2019

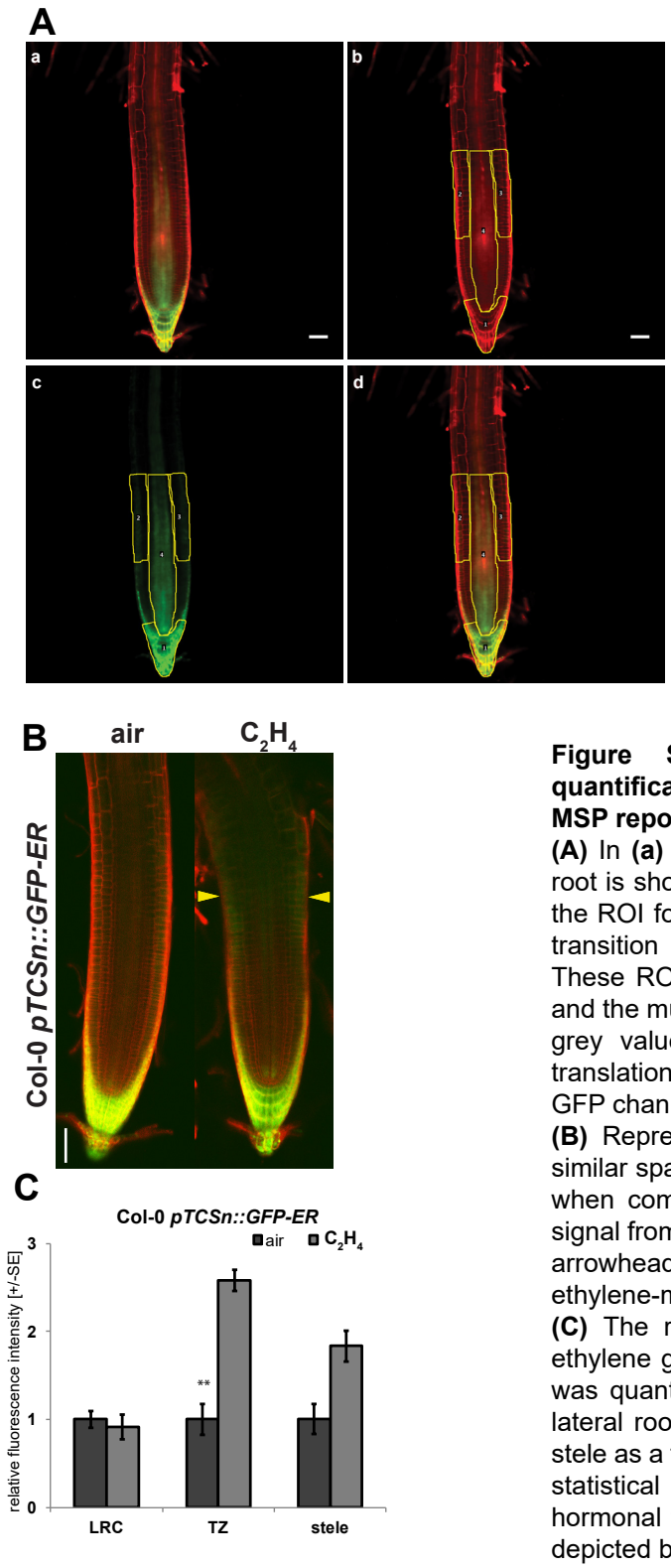

Figure S1. Representative ROI selection for the quantification of the *pTCSn::GFP-ER* and ethylene induces MSP reporter in the TZ .

(A) In **(a)** a processed image of BAP treated *pTCSn::GFP-ER* root is shown. **(b)** The PI channel was used for the selection of the ROI for lateral root cap and columella (1), the left and right transition zone (2 and 3, respectively), and the stele (4). **(c)** These ROI were translated onto the *pTCSn::GFP-ER* channel and the multi-measure tool was used for the quantification of the grey values. **(d)** Representative merge image showing the translation of the ROIs onto the composite image with the PI and GFP channels.

(B) Representative images of ethylene gas treatment shows similar spatially-specific effects on MSP activation in the root TZ when compared with ACC treatment. The plasma membrane signal from PI staining is shown in red; GFP in green. The yellow arrowheads point to the specific localization of the ethylene-mediated responses. Scale bar represents 50 µm.

(C) The relative fluorescence intensity quantification data of ethylene gas treatment is presented.The fluorescence intensity was quantified separately in three regions of interest (ROIs) - lateral root cap and columella (LRC), transition zone (TZ) and stele as a fold change relative to DMSO control +/- SE n=5). The statistical significance of differences between control and hormonal treatments (t test) at alpha < 0.05, 0.01 and 0.001 is depicted by asterisks (*, ** and ***, respectively).

Figure S2

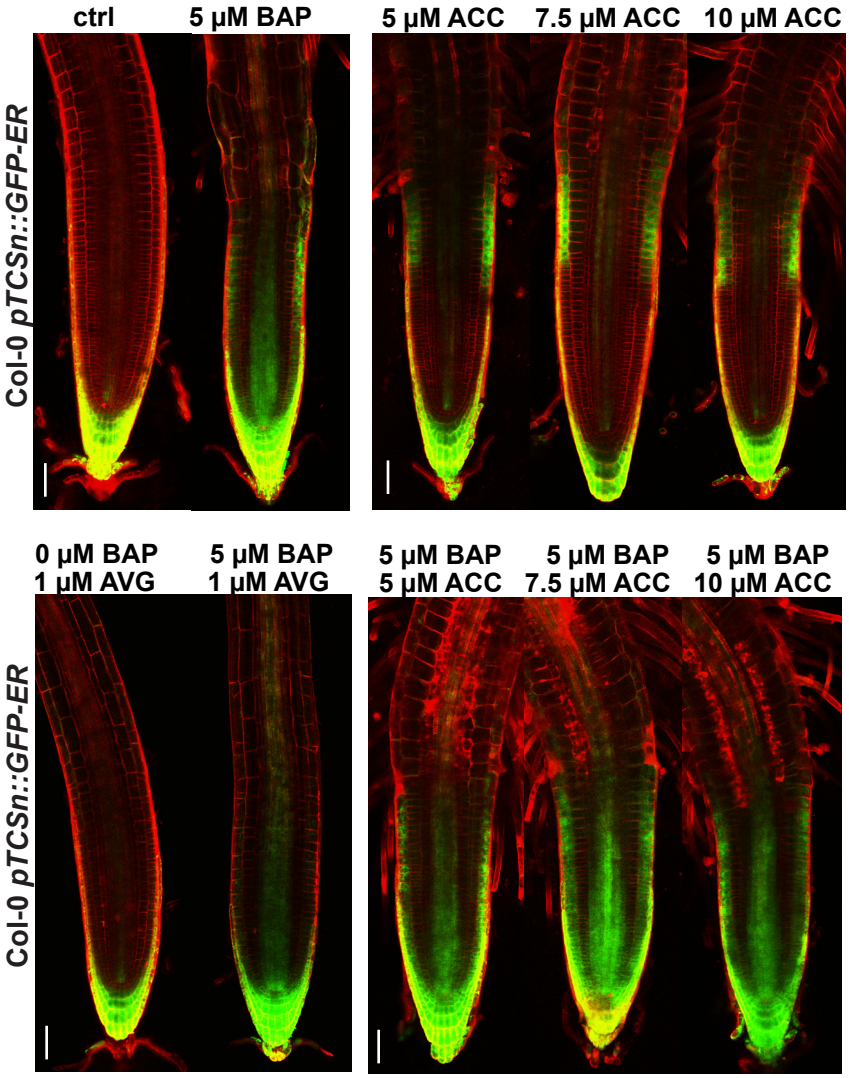

Figure S2. Hormonal dosage treatment of *pTCSn::GFP* reporter.
Six-day-old seedlings following 24h of hormonal treatment were used for imaging the expression pattern of *pTCSn::GFP* reporter line in the root apical meristem zone. Representative figures of ER-localized *pTCSn-driven GFP* signal in Col-0 are shown. The membrane signal from PI staining is shown in red; GFP in green. ctrl, mock-treated control (0.1% DMSO); BAP, benzyladenine; ACC, 1-aminocyclopropane-1-carboxylic acid; AVG, aminoethoxyvinylglycine. Scale bars represent 50 µm.

Figure S3

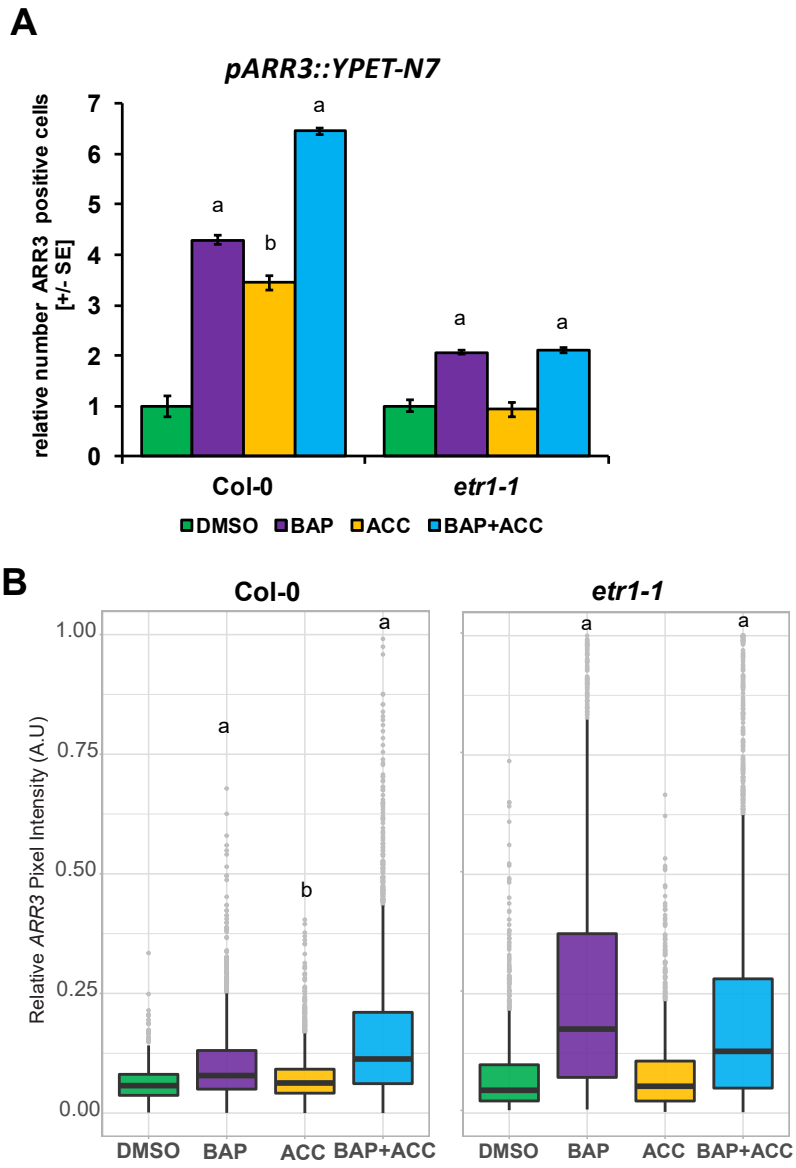

Figure S3. Detailed quantification of *ARR3* expression in the root tip.

Six-day-old seedlings following 24h of hormonal treatment (5 µM BAP, 5 µM ACC, 5 µM BAP + 5 µM ACC and 0.1% DMSO as a control) were used for Z-stack imaging of the *pARR3::YPET-N7* expression pattern in Col-0 and *etr1-1* background.

(A) Relative average number of *pARR3::YPET-N7* positive cells per root sample (mean +/- SE, n=5). Data presented are normalized to mock-treated samples. Statistical analysis of the number of *ARR3* positive cells was performed using the student's t-test.

(B) The pixel intensity for each *pARR3::YPET-N7* expressing cell in treated roots was calculated by the following transformation [raw pixel intensity/((2^16)-1)]. Plotting of the individual nuclei data distribution was done in R using ggplot2. Statistical analysis of nuclei pixel intensity distribution was conducted using the Kolmogorov-Smirnov test (n=5).

In **(A)** and **(B)** error bars show standard error, (a) indicates p value < 0.001 and (b) indicated p value ≤ 0.01.

(C) Representative images of *pARR3::YPET-N7*, nuclear-localized YPET (green signal) expressed from the *ARR3* promoter in the *etr1-1* background is shown. The white arrowheads point to the specific localization of the BAP-induced signal. Scale bars represent 50 µm.

Figure S4

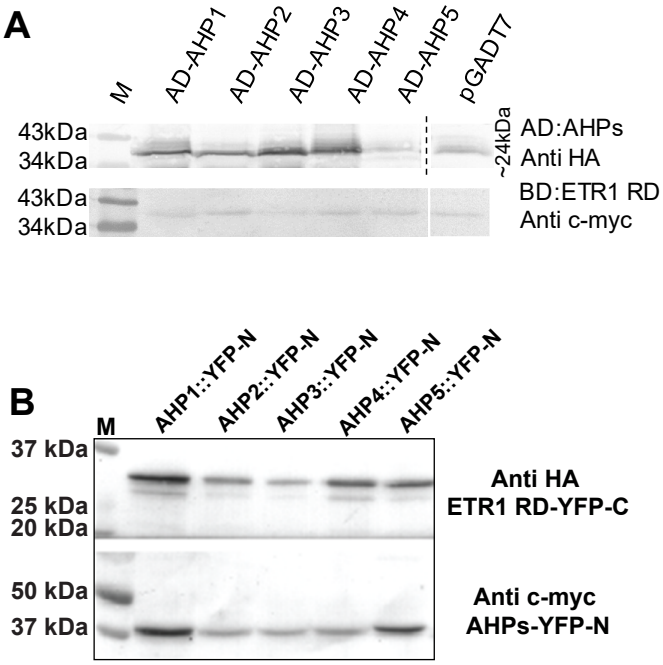

Figure S4. ETR1 RD interacts with a specific subset of AHP proteins in yeast two-hybrid assays and in planta.
(A) For yeast two-hybrid assay (see Figure 5A) recombinant protein expression was confirmed on immunoblots of protein extracts from corresponding yeast clones. M, protein marker. The empty (HA only expressing) pGADT7 was used as a positive control. **(B)** Immunoblot confirming production of all interacting partners in abaxial tobacco leaf cells used for the in planta interaction assay of AHP::YFP-N fusion proteins with ETR1 RD::YFP-C (see Figure 5B).

Figure S5

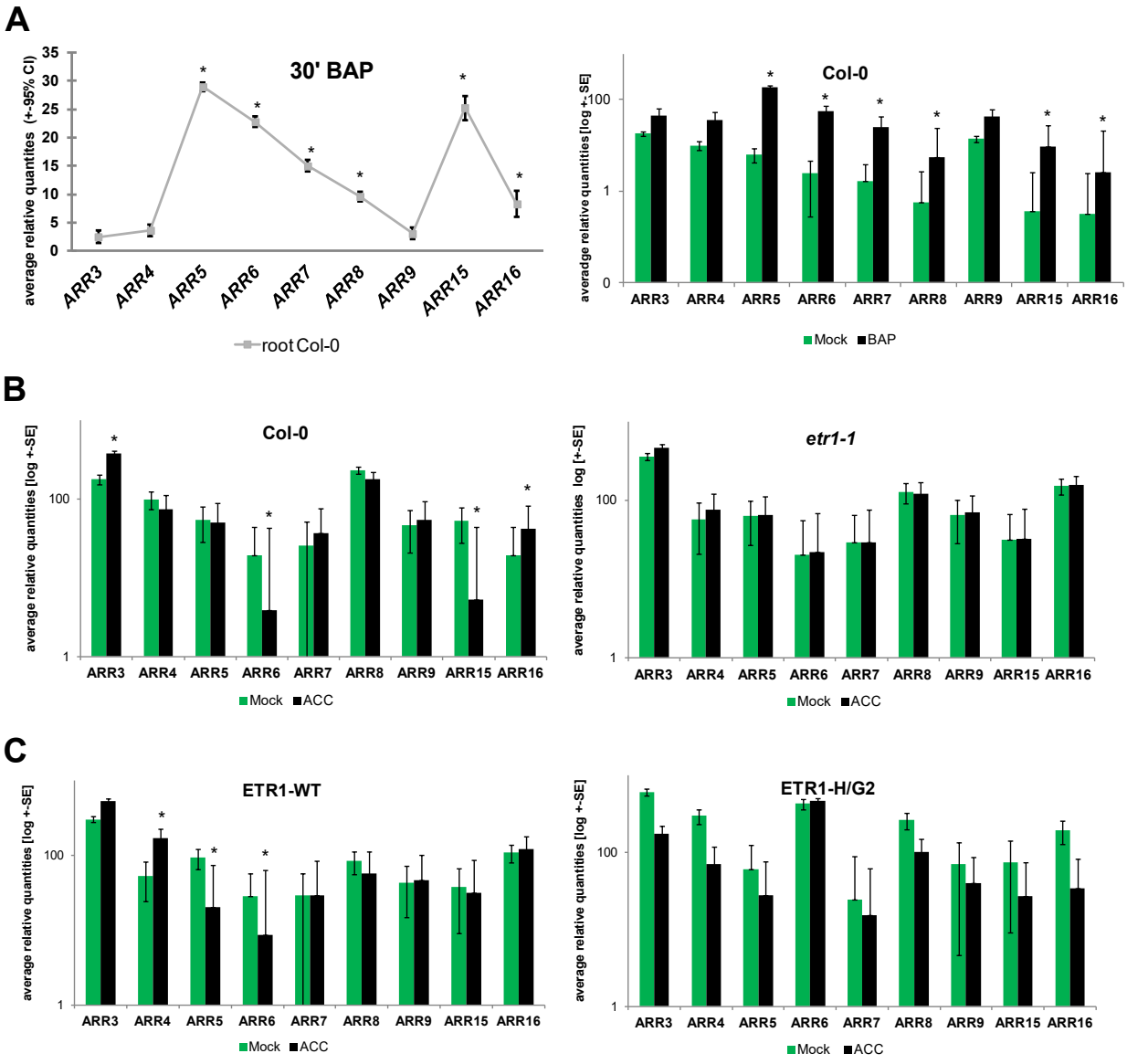

Figure S5. Ethylene induces *ARRs-A* gene expression in the root.
Relative gene expression of *ARRs-A* in the presence of BAP in Col-0 analyzed via qRT-PCR **(A):** double normalized to the internal control (*UBQ10)* and the *ARRs-A* transcription level in the mock-treated plants showing the BAP-induced fold change in the expression of individual *ARRs-A* (left chart) and single normalized to the internal control (*UBQ10*) and depicted in log scale (right chart, Biogazelle). The line connecting individual ARRs highlights the pattern of *ARRs-A* expression in the given genotype (left chart only).
(B) Relative expression of *ARRs-A* in the absence (left) and presence (right) of ACC normalized to the internal control (UBQ10) and depicted in log scale (Biogazelle) in Col-0 and *etr1-1* and **(C)** ETR1-WT and ETR1-H/G2.
Statistical significance of differences observed between mock- and hormone- treated roots (Mann-Whitney) at alpha < 0.05 is depicted by asterisks (*, qbase+, Biogazelle).

Figure S6

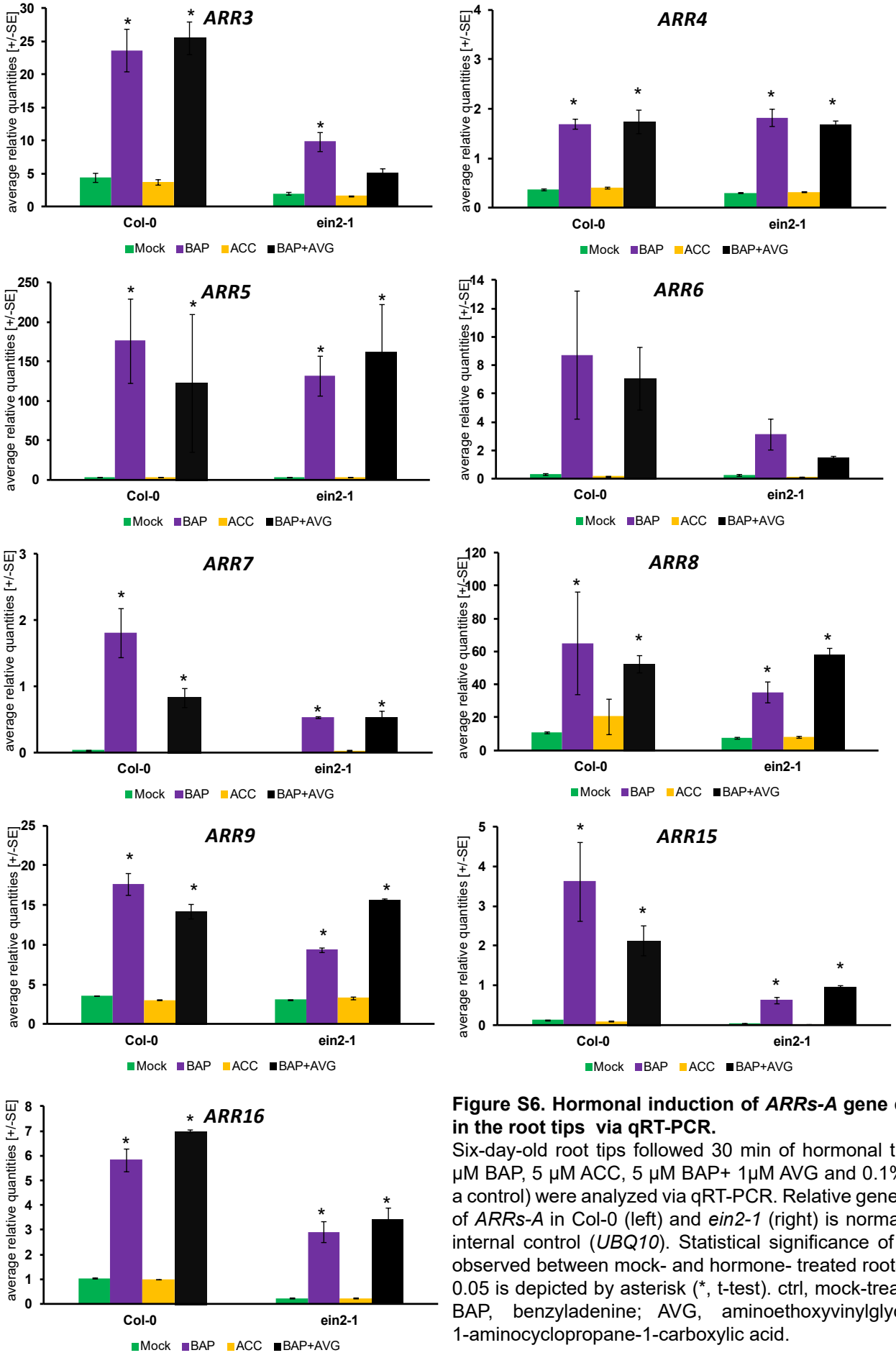

Figure S6. Hormonal induction of *ARRs-A* gene expression in the root tips via qRT-PCR.

Six-day-old root tips followed 30 min of hormonal treatment (5 µM BAP, 5 µM ACC, 5 µM BAP+ 1µM AVG and 0.1% DMSO as a control) were analyzed via qRT-PCR. Relative gene expression of *ARRs-A* in Col-0 (left) and *ein2-1* (right) is normalized to the internal control (*UBQ10*). Statistical significance of differences observed between mock- and hormone- treated roots at alpha < 0.05 is depicted by asterisk (*, t-test). ctrl, mock-treated control; BAP, benzyladenine; AVG, aminoethoxyvinylglycine; ACC, 1-aminocyclopropane-1-carboxylic acid.

Figure S7

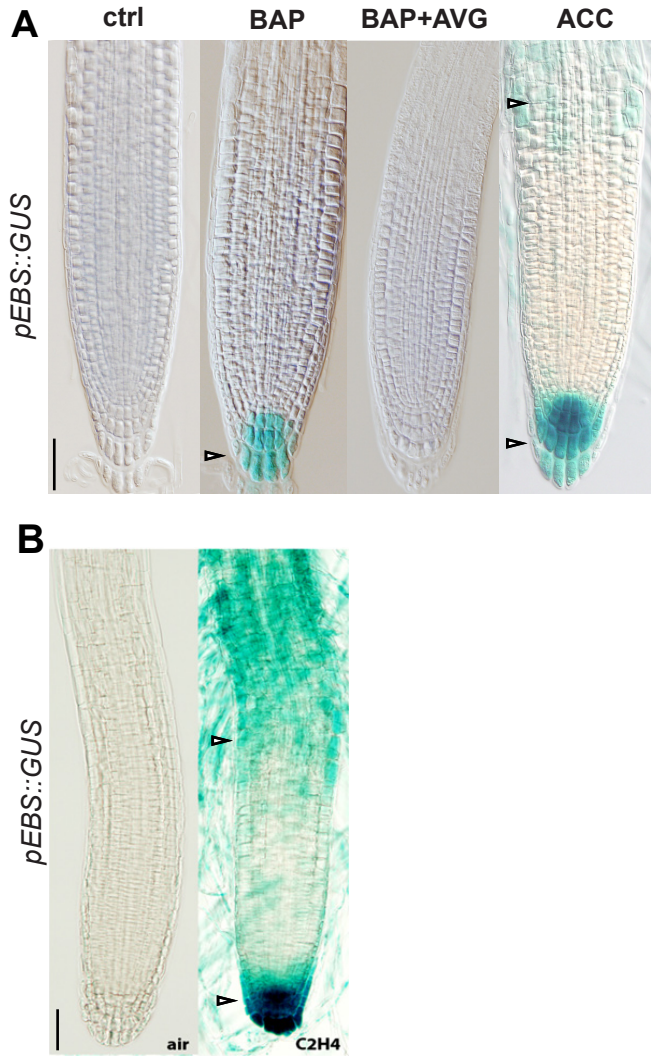

Figure S7. Cytokinin and ethylene effects on *pEBS::GUS* expression in the root tip.
Six-day-old seedlings followed 24 h of hormonal treatment of 5 μM BAP, 5 μM BAP + 5 μM AVG, 5 μM ACC, and 0.1% DMSO as a control in **(A)** and in the presence of 10 ppm ethylene gas and air as a control **(B)**. ctrl, mock-treated control; BAP, benzyladenine; AVG, aminoethoxyvinylglycine; ACC, 1-aminocyclopropane-1-carboxylic acid. The arrowheads indicate the specific localization of the GUS signal in columella cells, arrows show weak activation in the root transition zone. Scale bar represents 50 μm.