Expanded View Figures

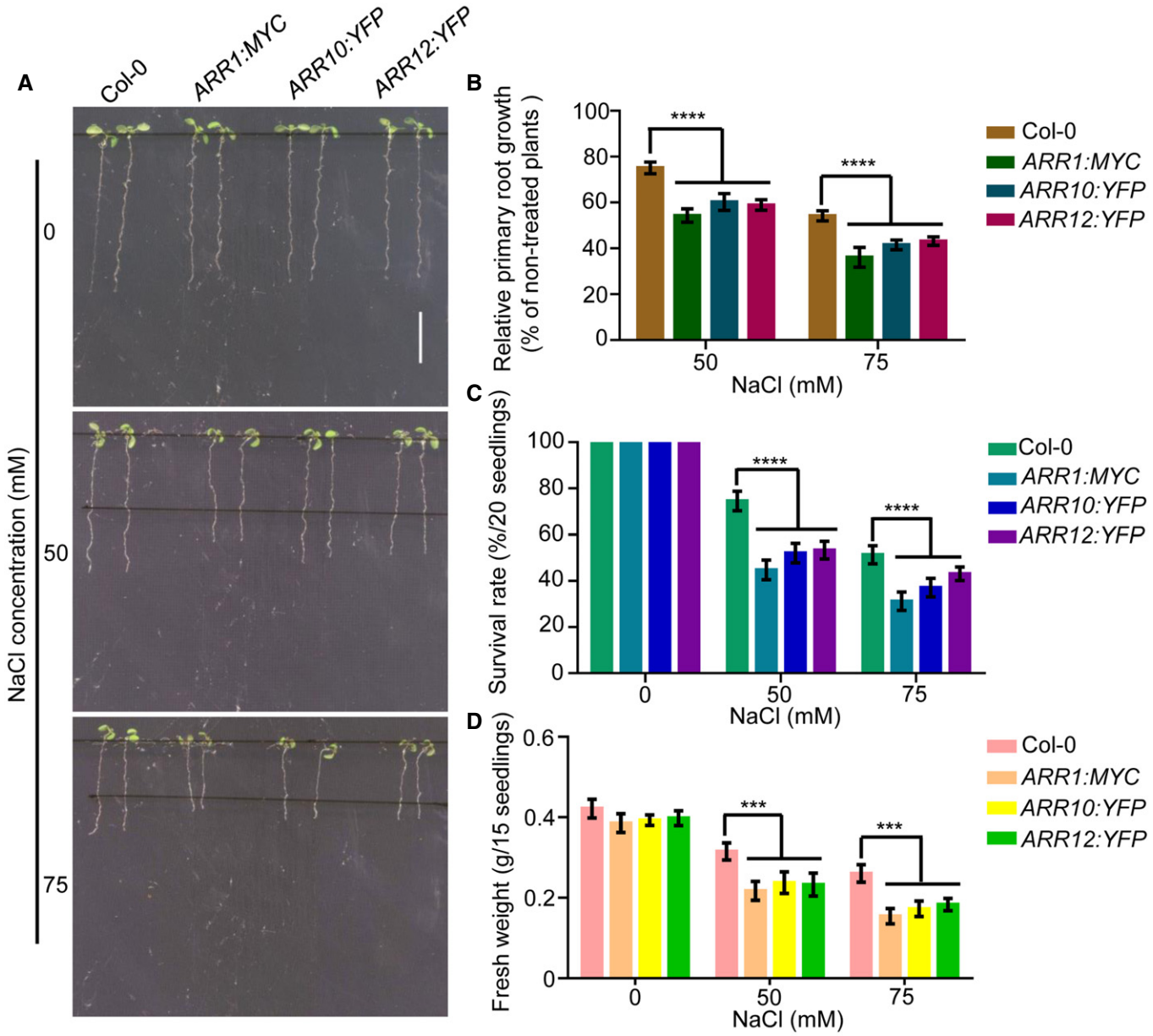

Figure EV1. Transgenic plants overexpressing ARR1/10/12 all show hypersensitivity to salt stress compared with that of wild type.

A–D Four-day-old seedlings of *35S:ARR1:MYC*, *35S:ARR10:YFP*, and *35S:ARR12:YFP* were transferred to 1/2 MS medium supplemented with 50 mM or 75 mM NaCl. Pictures were taken, and the elongated root length was determined 3 days later (A and B). Changes in survival rates (C) and fresh weights (D) were examined after 10 days of salt treatment. Scale bar, 1 cm.

Data information: In (B–D), data are means of three biological replicates ± SD (n = 60 for (B), and n = 12 for (C) and (D)). ***, and **** indicate significant difference to the corresponding controls with $P < 0.001$ and $P < 0.0001$, respectively (Student's t-test).

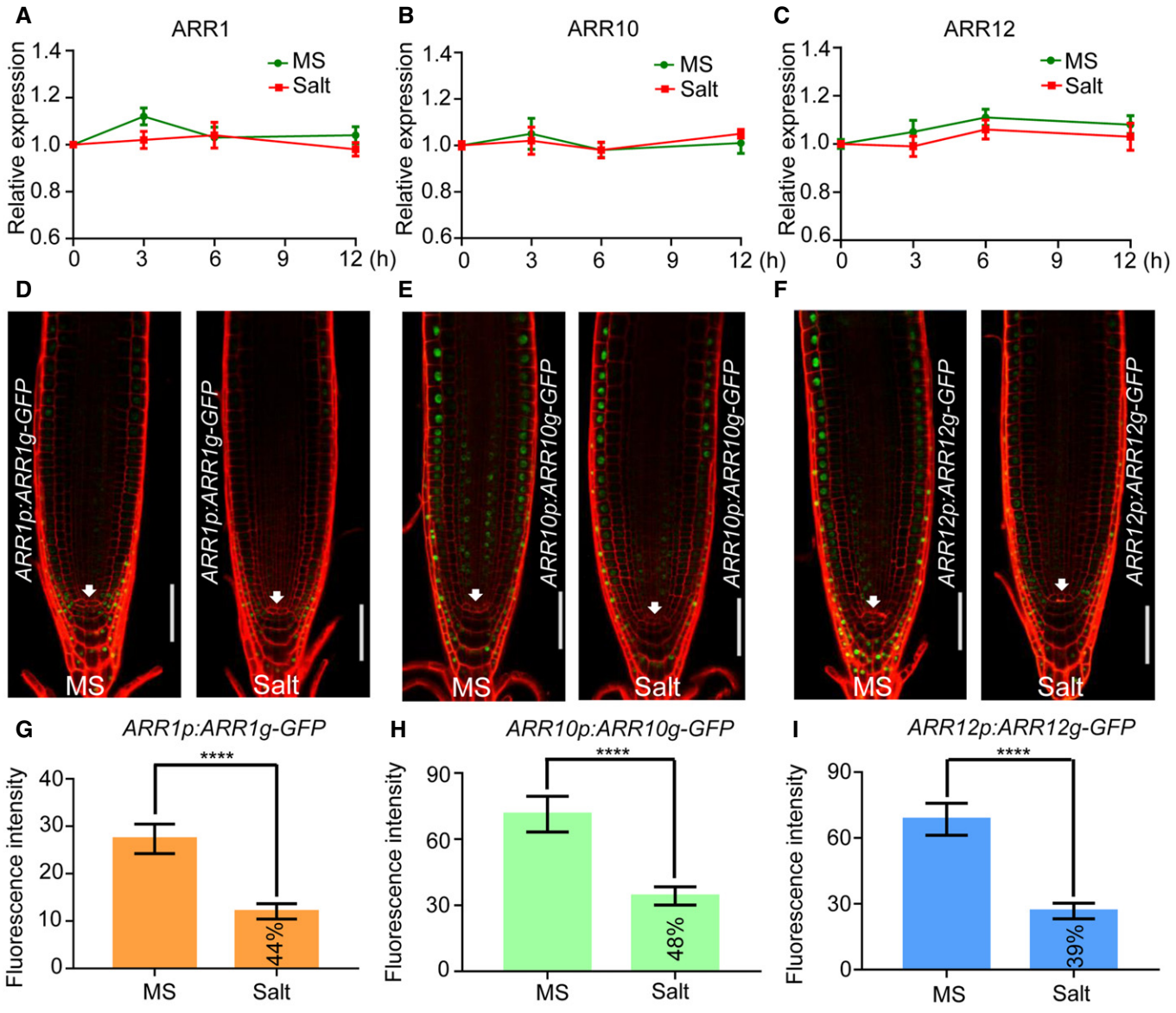

Figure EV2. Salt treatment decreases the protein levels of ARR1/10/12.

A–C Expression analysis of *ARR1*, *ARR10*, and *ARR12* under salt stress. Five-day-old wild-type Col-0 seedlings were treated with or without 200 mM NaCl for the indicated periods of time. Transcription levels were determined by qRT-PCR analysis of the indicated genes and normalized to the expression levels of *Actin2* and *UBQ1*.

D–F Five-day-old seedlings of *ARR1p:ARR1g-GFP*, *ARR10p:ARR10g-GFP*, and *ARR12p:ARR12g-GFP* transgenic plants were transferred to 1/2 MS plates with or without 50 mM NaCl for 12 h. Cell boundaries appear red following propidium iodide staining. The white arrowheads mark the position of the QC (Quiescent center) cells. Scale bar, 100 μm.

G–I Quantification of the fluorescence intensity in *ARR1p:ARR1g-GFP*, *ARR10p:ARR10g-GFP*, and *ARR12p:ARR12g-GFP* seedlings in (D–F). The fluorescence intensity of NaCl treated seedlings/fluorescence intensity of NaCl untreated seedlings ratios were labeled in the columns in (G–I).

Data information: In (A–C), data shown are means ± SD of three biological replicates. In (G–I), data are means of three biological replicates ± SD ($n = 45$). **** indicates significant difference to the corresponding controls with $P < 0.0001$ (Student's *t*-test).

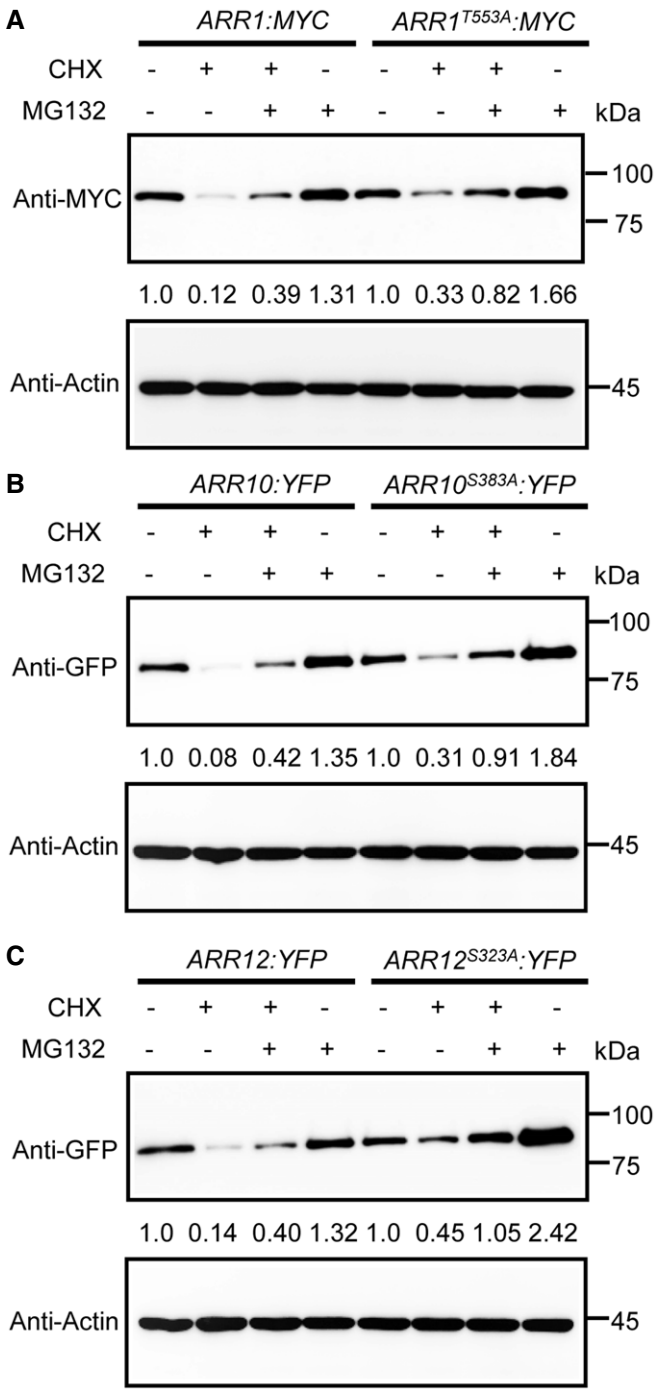

Figure EV3. Dephosphorylated form of ARR1/10/12 have a higher protein stability.

A–C Immunoblot analysis the stability of wild-type and dephosphorylated forms of ARR1, ARR10, and ARR12 proteins *in vivo*. Five-day-old *35S:ARR1:MYC*, *35S:ARR10:YFP*, *35S:ARR12:YFP*, *35S:ARR1^{T553A}:MYC*, *35S:ARR10^{S383A}:YFP*, and *35S:ARR12^{S323A}:YFP* transgenic plants were treated with 200 μM cycloheximide (CHX), 50 μM MG132, or 200 μM CHX, together with 50 μM MG132 for 3 h. ARR1, ARR10, and ARR12 were detected with anti-MYC or anti-GFP antibody. Actin was used as a control.

Data information: Similar results were obtained with three biological repeats.

Figure EV4. Cytokinin signal transduction is independent on mitogen-activated protein kinases MPK3/6 under normal growth conditions.

A, B Primary root phenotype (A) and primary root length (B) of Col-0, *MPK3SR*, and *arr1/12* seedlings grown on 0.25 μM NA-PP1-containing 1/2 MS medium supplemented with or without 25 or 50 nM 6-BA for 5 days. Scale bar, 1 cm.

C, D Transcript levels of *ARR5* and *ARR6* in Col-0, *MPK3SR*, and *arr1/12* were examined by qRT–PCR. Gene expression levels in wild-type control plants without 6-BA treatment were set to 1.0. *Actin2* gene and *UBQ1* gene were analyzed as internal controls.

Data information: In (B), data are means of three biological replicates ± SD ($n = 60$). In (C), and (D), values are means ± SD of three biological replicates. ns indicates no significant difference to the corresponding controls. *** and **** indicate significant difference to the corresponding controls with $P < 0.001$ and $P < 0.0001$, respectively (Student's *t*-test).

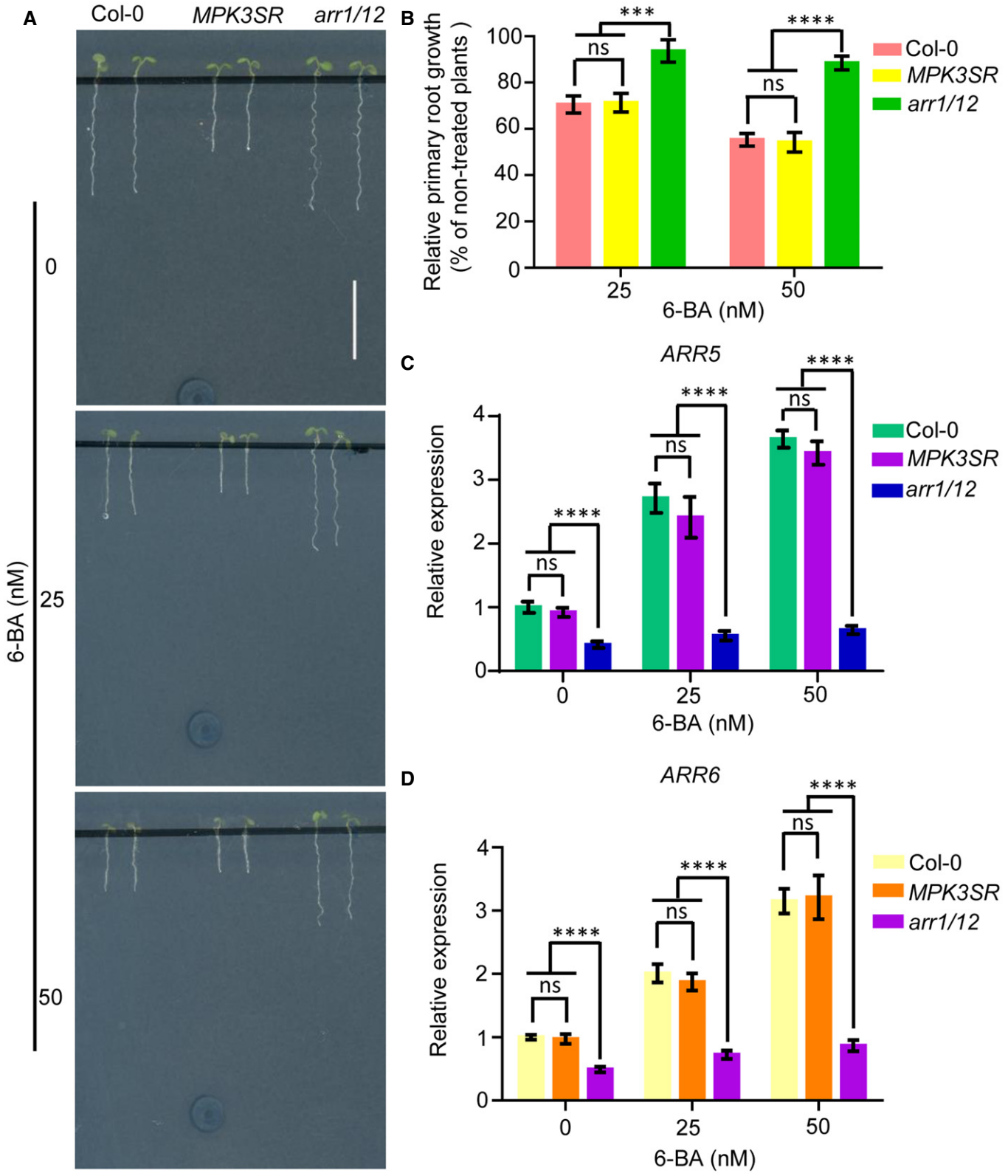
A
Col-0
MPK3SR
arr1/12
6-BA (nM)
0
25
50
B
Relative primary root growth (% of non-treated plants)
***
****
ns
ns
Col-0
MPK3SR
arr1/12
6-BA (nM)
C
ARR5
Relative expression
****
ns
****
ns
****
ns
Col-0
MPK3SR
arr1/12
6-BA (nM)
D
ARR6
Relative expression
****
ns
****
ns
****
ns
Col-0
MPK3SR
arr1/12
6-BA (nM)

Figure EV4.

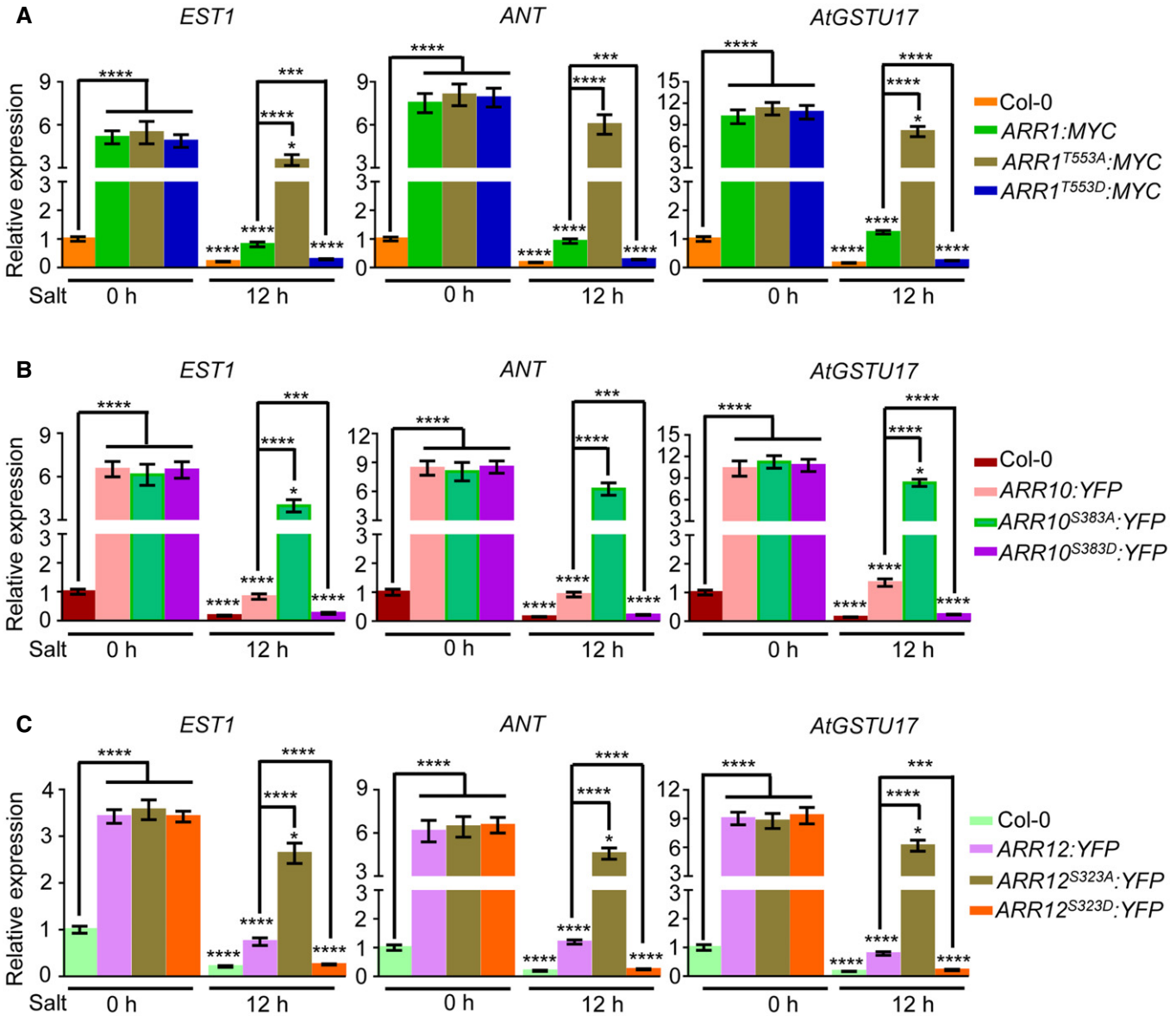

Figure EV5. Expression analysis of direct downstream targets of ARR1/10/12.

A–C The relative expression of direct downstream targets of ARR1/10/12 such as *EST1*, *ANT*, and *AtGSTU17* in 7-day-old seedlings treated with 200 mM NaCl for given time. *Actin2* gene and *UBQ1* gene were analyzed as internal controls. Gene expression levels in wild-type control plants treated with NaCl for 0 h were set to 1.0.

Data information: Data shown are means ± SD of three biological replicates. *$P < 0.05$, ***$P < 0.001$, ****$P < 0.0001$ (Student's *t*-test).