Expanded View Figures

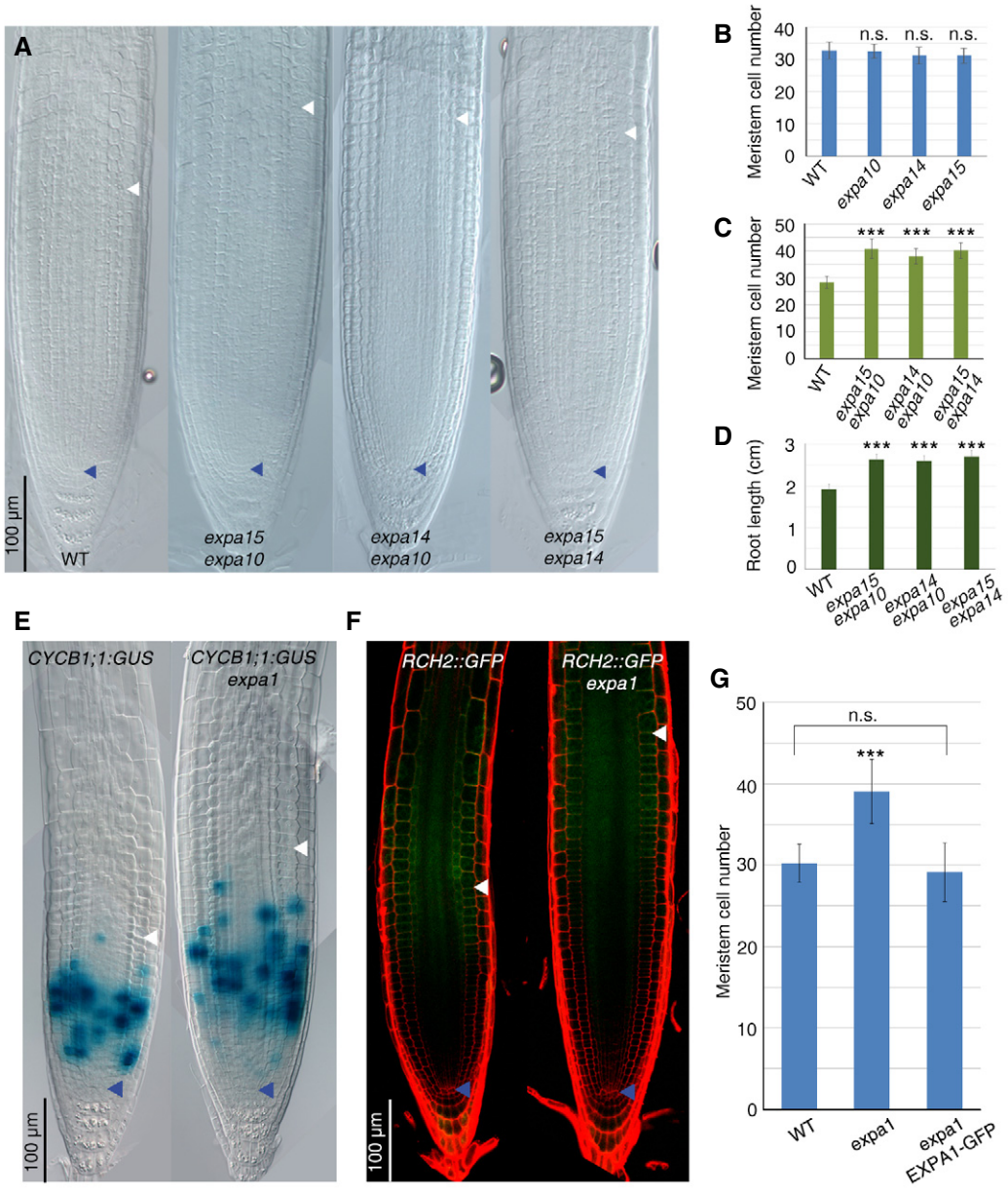

Figure EV1. *EXPA1*, *EXPA10*, *EXPA14*, and *EXPA15* control cell differentiation initiation at the TZ.

A DIC microscopy images of WT, *expa15;expa10*, *expa14;expa10*, and *expa15;expa14* root tips.

B Root meristem cell number of the WT, *expa10*, *expa14*, and *expa15* single mutants (n = 50. n.s. corresponds to not significant; Student's t-test).

C, D Measurement of root meristem cell number (n = 50) (C) and root length (n = 15) (D) of *expa15;expa10*, *expa14;expa10*, and *expa15;expa14* double mutants (***P < 0.001; Student's t-test).

E DIC microscopy images of *CYCB1;1:GUS* (n = 20) and *CYCB1;1:GUS;expa1* (n = 20) roots.

F Confocal microscopy images of *RCH2::GFP* (n = 10) and *RCH2::GFP;expa1* (n = 30) roots. Note changes in TZ position in the *expa1* mutant correspond to changes in *CYCB1;1:GUS* and *RCH2::GFP* domains.

G Measurement of root meristem cell number of WT, *expa1*, and *expa1;EXPA1-GFP* (n = 50. ***P < 0.001, n.s. corresponds to not significant; Student's t-test)

Data information: In (A–G), experiments were performed on seedlings at 5 dpg. Blue and white arrowheads indicate the QC and the cortex TB, respectively. Error bars indicate SD.

Figure EV2. Low pH-activated EXPA10, EXPA14, and EXPA15 proteins are expressed at the TZ and control cell differentiation.

A *EXPA1* fold enrichment deriving from ChIP-qPCR analysis performed on immunoprecipitated chromatin of *pARR1::ARR1:GFP* roots (***$P < 0.001$; Student's *t*-test; two technical replicates performed on two independent DNA batches).

B–D DIC microscopy images of WT roots expressing *pEXPA10-GUS* (B), *pEXPA14-GUS* (C), and *pEXPA15-GUS* (D) constructs, respectively.

E qRT–PCR analysis of *EXPA10*, *EXPA14*, and *EXPA15* mRNA levels in the root tip of WT plants upon cytokinin treatment (***$P < 0.001$; Student's *t*-test; three technical replicates performed on two independent RNA batches).

F *EXPA10*, *EXPA14*, and *EXPA15* fold enrichment deriving from ChIP-qPCR analysis performed on immunoprecipitated chromatin of *pARR1::ARR1:GFP* roots. (Two technical replicates performed on two independent DNA batches)

G, H Measurements of tracheids (G) and root hairs (H) distance from the QC in WT and *UBQ10-EXPA1* root tips grown on standard (5.8) or acidic (4.0) pH. ($n = 10$. **$P < 0.01$, ***$P < 0.001$; Student's *t*-test).

I Confocal microscopy images of WT ($n = 10$) root tips grown on standard (5.8) pH and of *UBQ10-EXPA10* ($n = 15$), *UBQ10-EXPA14* ($n = 20$), and *UBQ10-EXPA15* ($n = 15$) root tips grown on standard (5.8) or acidic (4.0) pH.

Data information: In (A–I), experiments were performed on seedlings at 5 dpg. Blue and white arrowheads indicate the QC and the cortex TB, respectively. Error bars indicate SD.

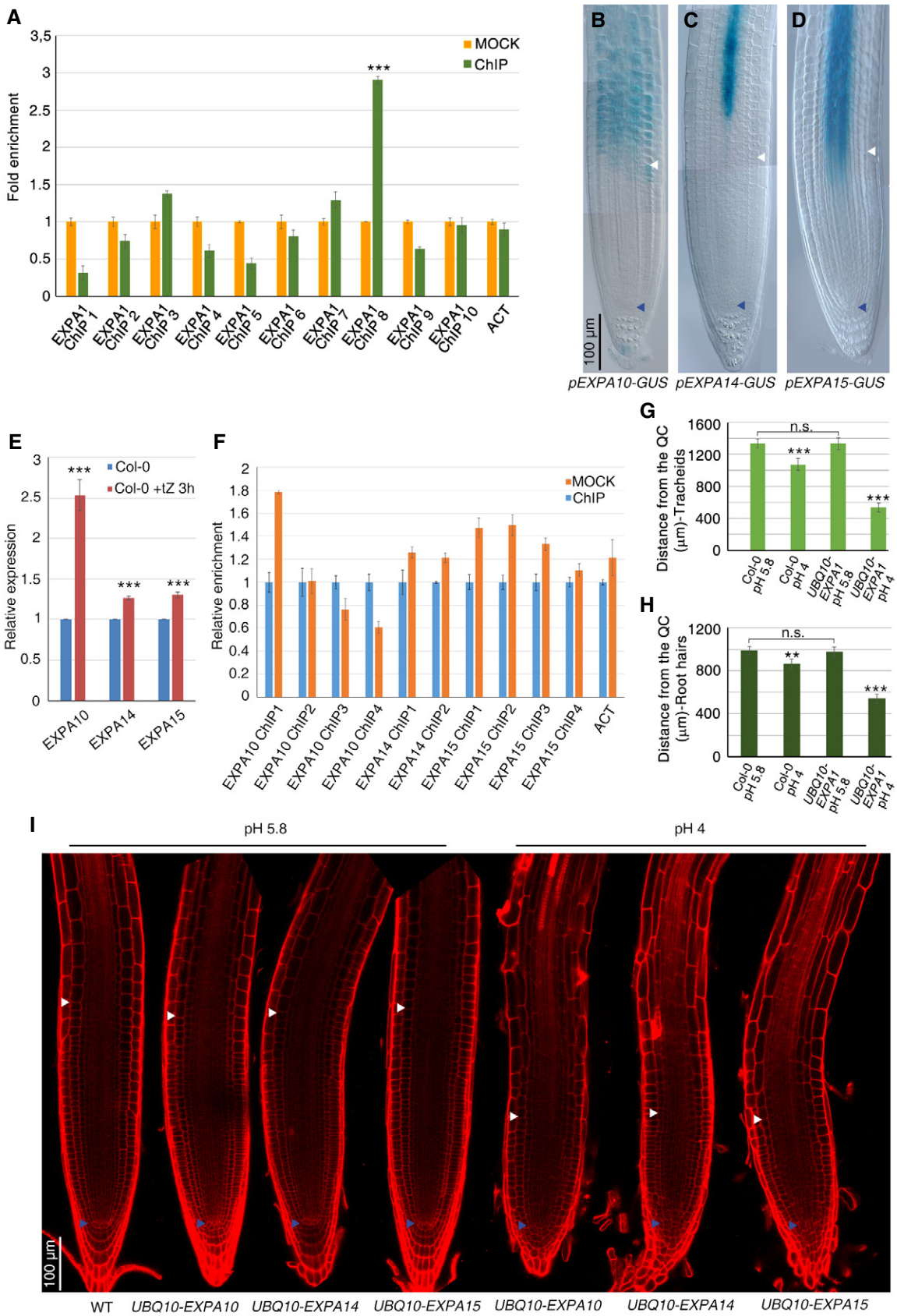

Figure EV2.

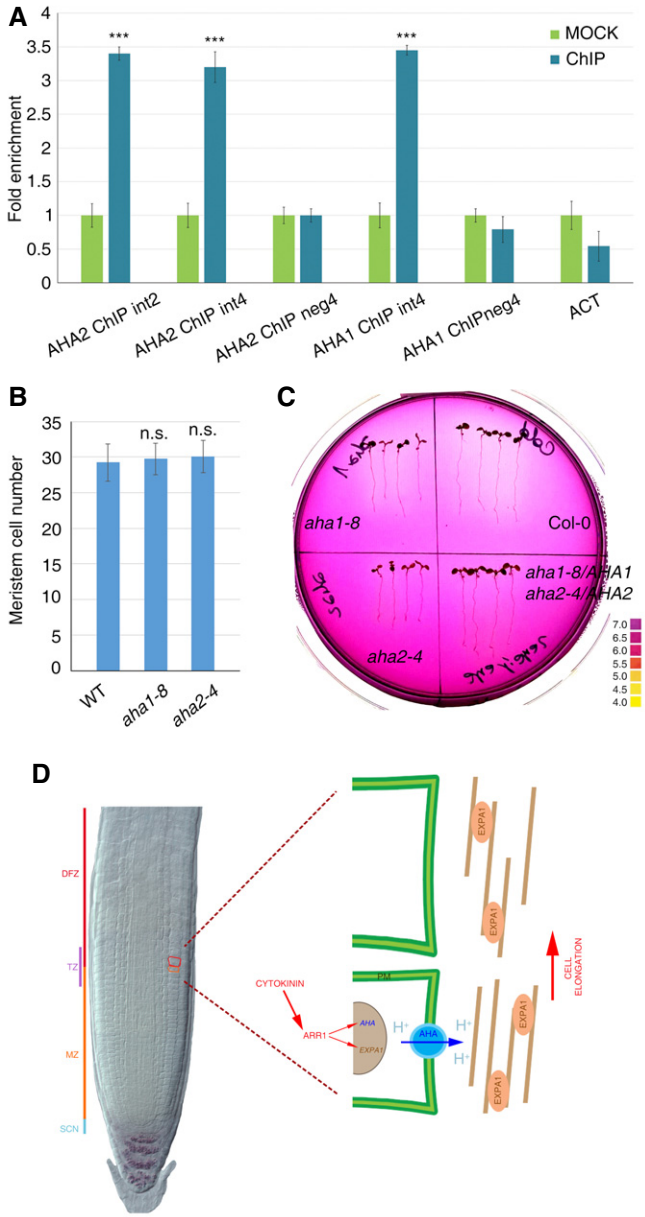

Figure EV3. AHA1- and AHA2-dependent apoplastic acidification at the TZ induces cell differentiation.

A *AHA1* and *AHA2* fold enrichment deriving from ChIP-qPCR analysis performed on immunoprecipitated chromatin of *pARR1::ARR1:GFP* roots. (***$P < 0.001$; Student's *t*-test; two technical replicates performed on two independent DNA batches). Error bars indicate SD.

B Measurements of meristem cell number of *aha1-8* and *aha2-4* mutants. ($n = 15$. n.s. corresponds to not significant; Student's *t*-test). Error bars indicate SD.

C Medium acidification through proton extrusion from WT, *aha1-8*, *aha2-4*, and *aha1-8/AHA1;aha2-4/AHA2* seedlings, 16 h after transfer onto fresh medium containing pH indicator. ($n = 4$; three replicates). Colored bar indicates pH values.

D Proposed model: cytokinin, via ARR1, controls both apoplastic acidification (via positive regulation of *AHA1* and *AHA2*) and cell wall (brown lines) loosening (via positive regulation of *EXPA1*). As a result, at the TZ, cells expand and initiate the differentiation program.

Data information: In (A–C), experiments were performed on seedlings at 5 dpg.