

APPENDIX Montesinos et al.

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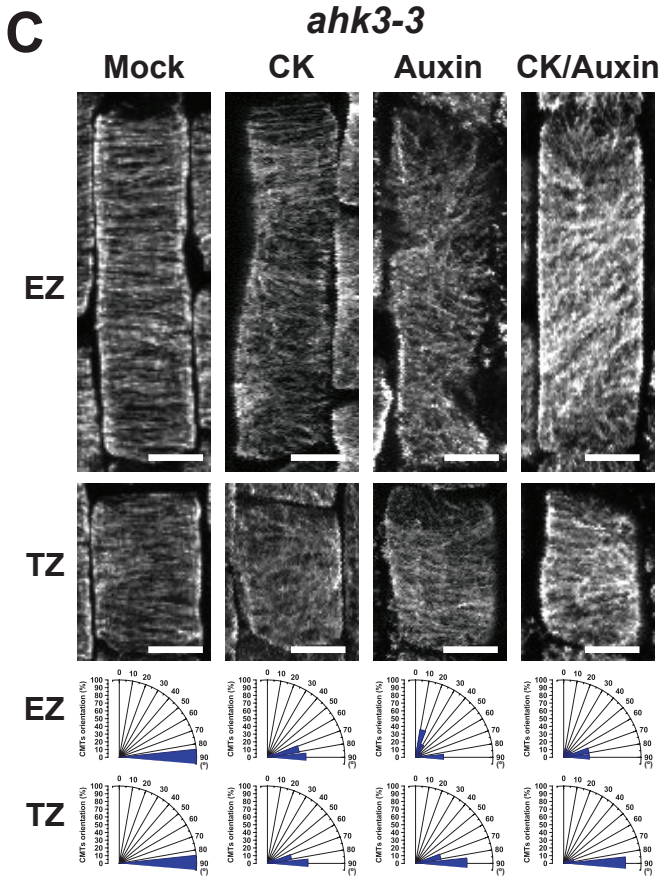
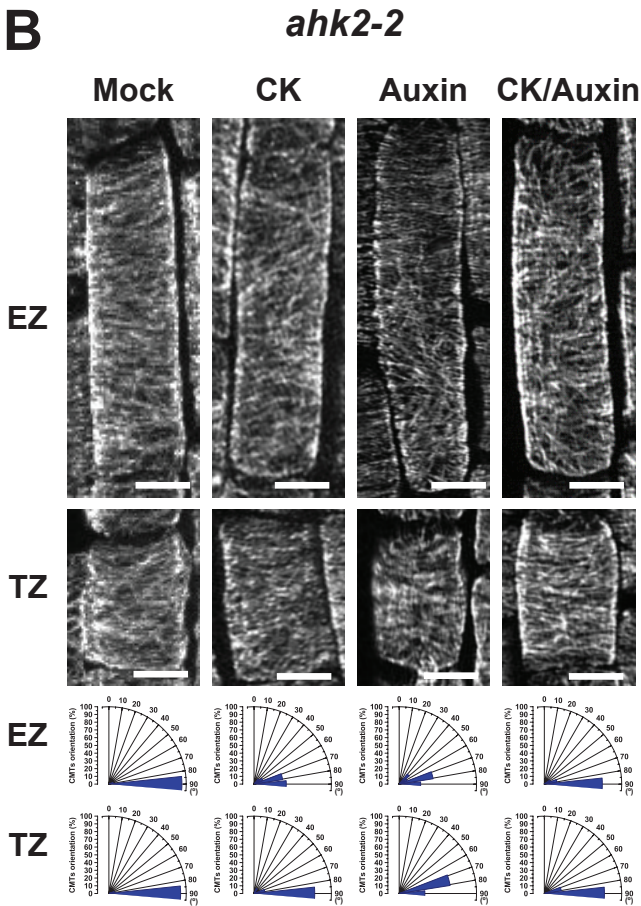
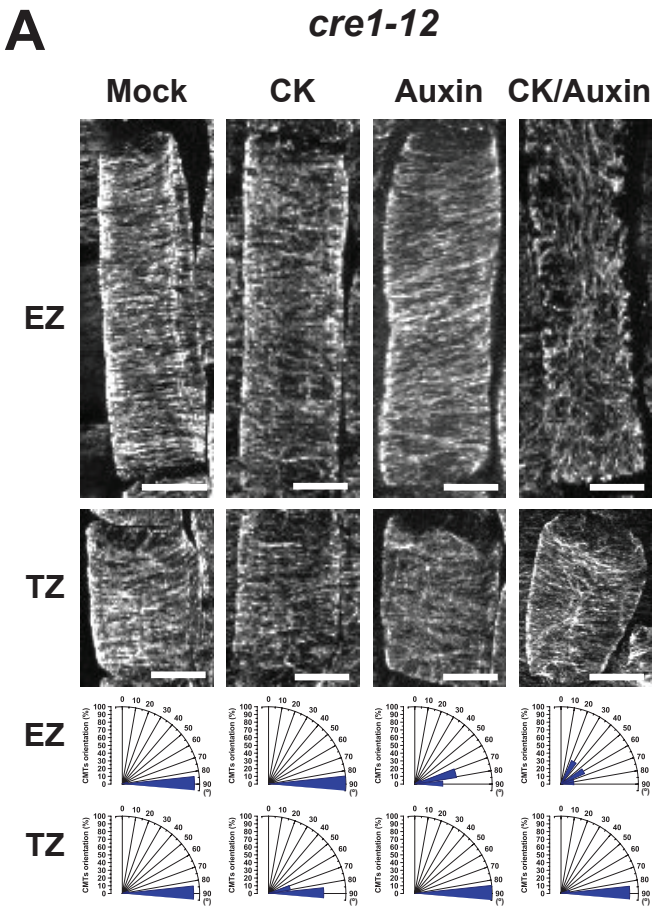
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Appendix Fig. S4 | Cytokinin prevents CMT depolymerization in *ahk2-2* and *ahk3-3* cytokinin receptor mutants.

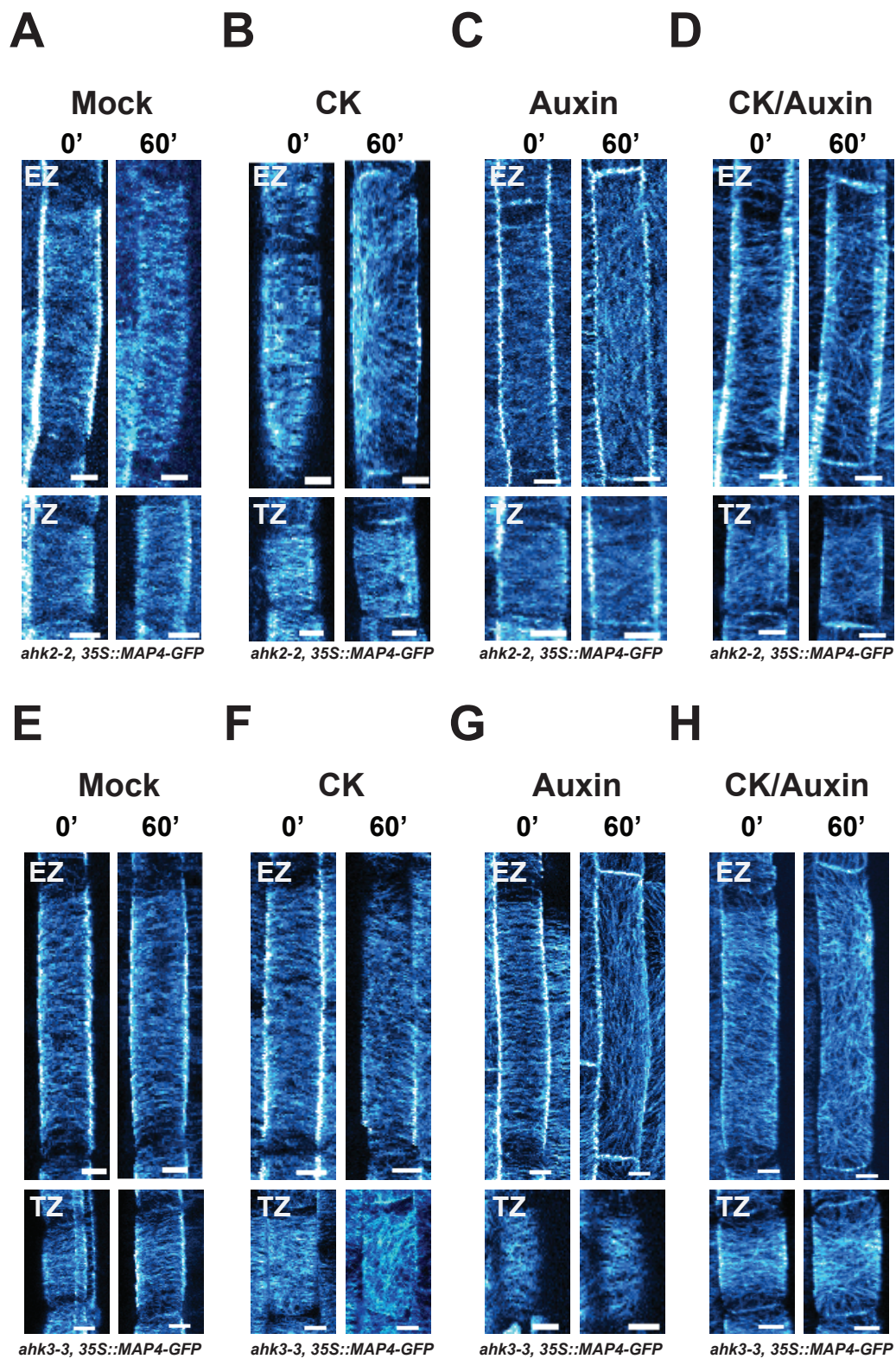
Appendix Fig. S5 | Cytokinin interferes with the auxin-mediated regulation of CMTs.

Appendix Figure S1



Appendix Fig. S1 | CMT patterns in the cytokinin receptor mutants. A-C. Monitoring of CMTs visualized by immunostaining of α -tubulin and histograms of CMT orientation distributions in epidermal cells of the transition (TZ) and the elongation (EZ) zone of *cre1-12* (**A**), *ahk2-2* (**B**) and *ahk3-3* (**C**) roots. CMTs were visualized in roots treated for 60 min with mock (DMSO), CK (10 μ M BAP), auxin (0.1 μ M NAA), or CK and auxin (10 μ M BAP and 0.1 μ M NAA provided after a 60-min pretreatment with 10 μ M BAP). n = 10-20 cells per root growth zone in 5-to 8 roots per condition. Scale bar 10 μ m.

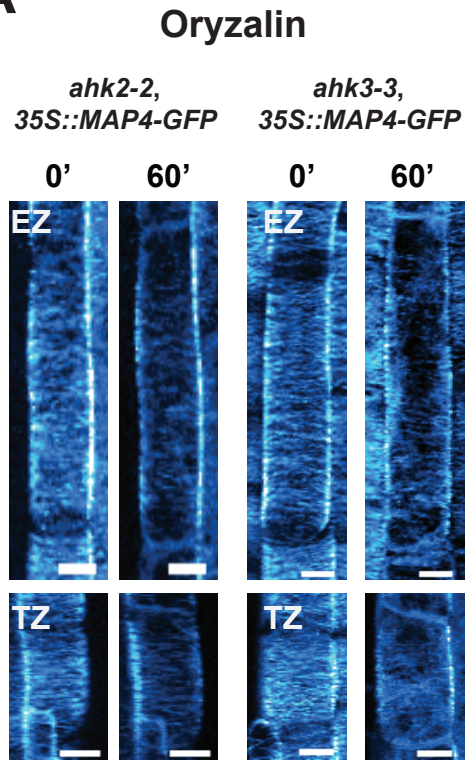
Appendix Figure S2



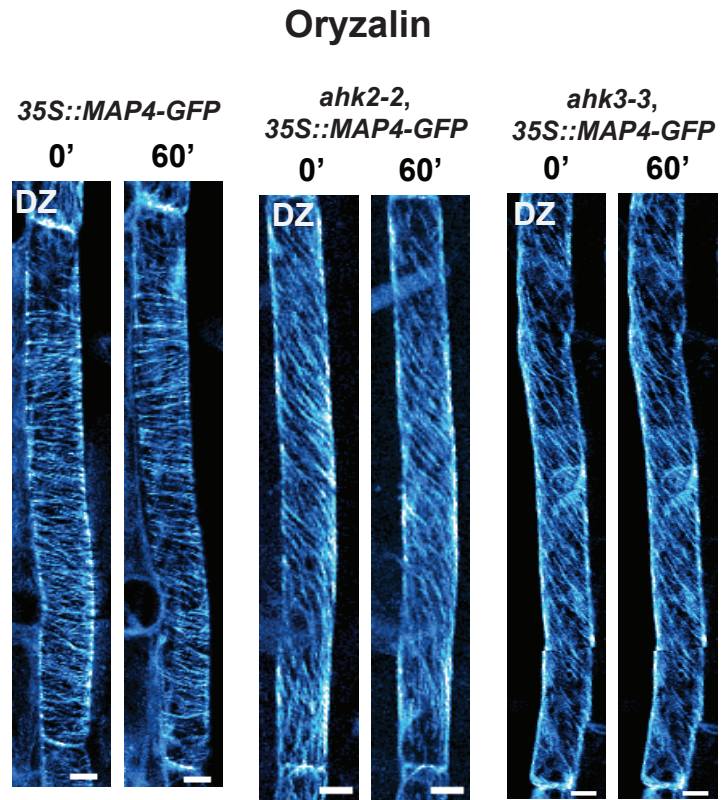
Appendix Fig. S2 | CMT arrangements in mutants lacking the cytokinin receptors AHK2 and AHK3. A-H. Monitoring of CMTs visualized by MAP4-GFP in epidermal cells of the transition (TZ) and elongation (EZ) zone of *ahk2-2* and *ahk3-3* roots. CMTs were visualized in roots treated for 60 min with mock (DMSO) (**A, E**), cytokinin (CK, 10 μ M BAP) (**B, F**), auxin (0.1 μ M NAA) (**C, G**) or CK and auxin (10 μ M BAP and 0.1 μ M NAA provided after a 60-min pretreatment with 10 μ M BAP) (**D, H**). Scale bar 10 μ m.

Appendix Figure S3

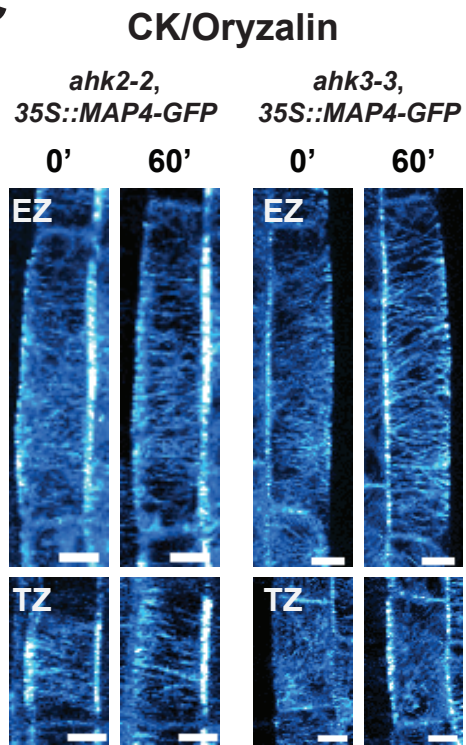
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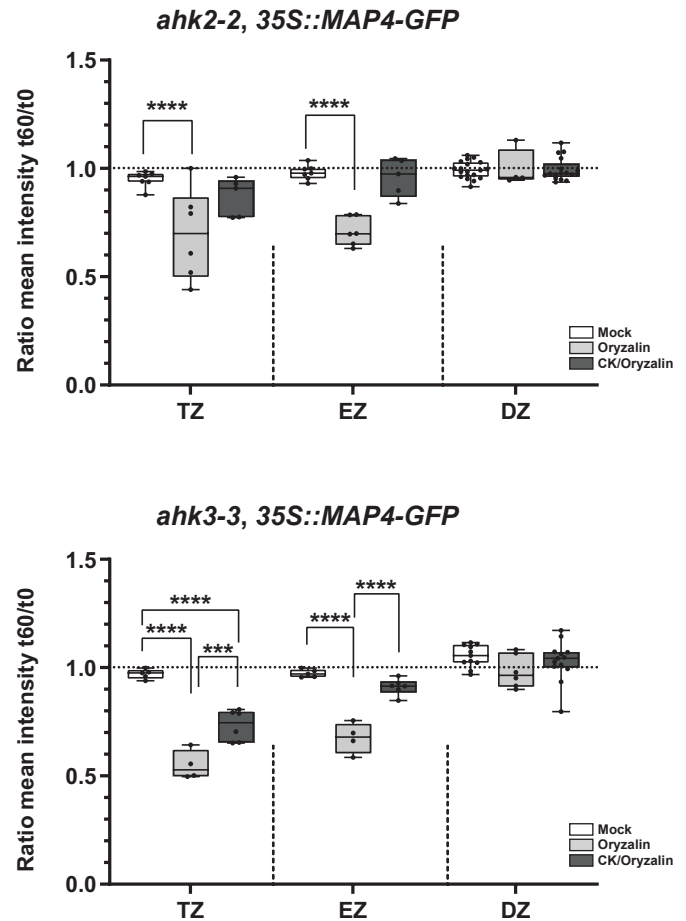
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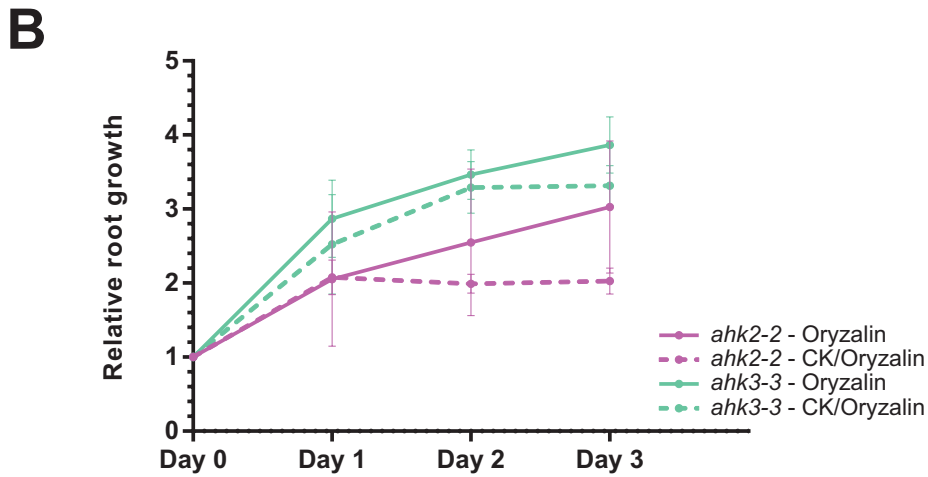
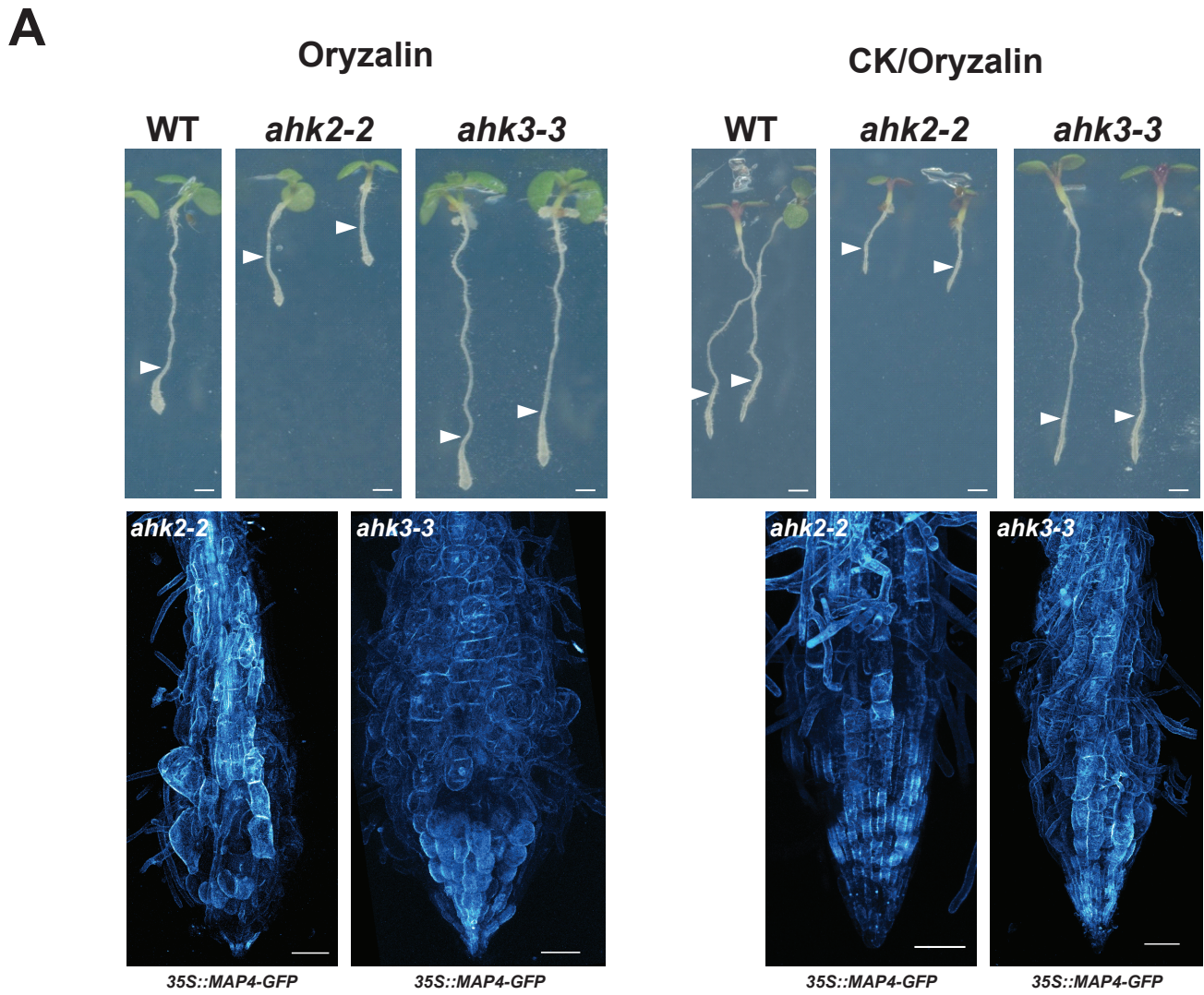


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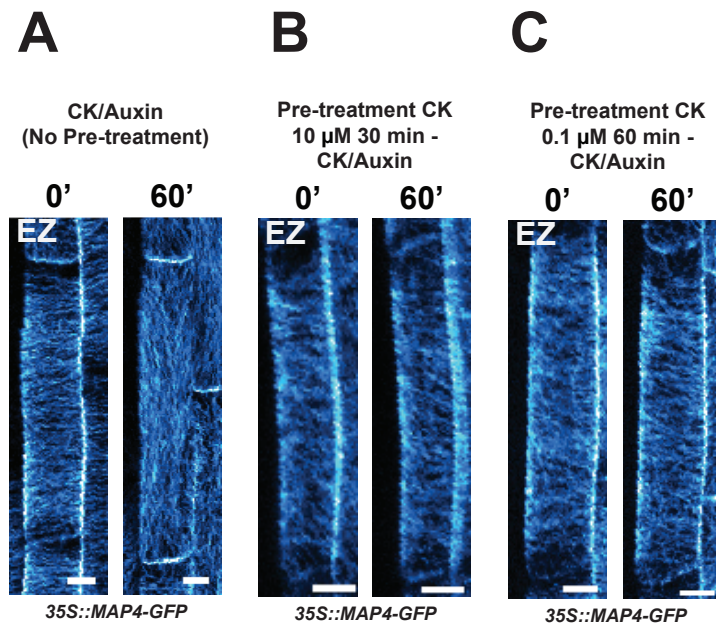
Appendix Fig. S3 | Cytokinin prevents CMT depolymerisation in the *ahk2-2* and *ahk3-3* cytokinin receptor mutants. **A-C.** CMTs visualized with MAP4-GFP in root epidermal cells of the transition (TZ) and the elongation (EZ) (**A, C**) and the differentiation (DZ) (**B**) zone treated with 1 μ M oryzalin (**A, B**), and cytokinin (CK, 10 μ M BAP) plus oryzalin (1 μ M) (**C**). For the double CK and oryzalin treatment, roots were pretreated for 60 min with CK and then transferred to medium supplemented with both compounds. Scale bar 10 μ m. **D.** Quantification of the MAP4-GFP reporter signal in *ahk2-2* and *ahk3-3* root epidermal cells of the TZ, the EZ and the DZ treated with mock (DMSO; white box), oryzalin (1 μ M; light grey box) and CK and oryzalin (10 μ M BAP and 1 μ M oryzalin; dark grey box). For the double treatments, roots were pretreated for 60 min with 10 μ M BAP prior to transfer to medium supplemented with both compounds. Boxplots represent ratio between mean fluorescence intensity (arbitrary units) measured in epidermal cells at 60 and 0 min. Ratio close to 1 (segmented line) corresponds to an unchanged MAP-GFP signal for 60 min. (***) $P < 0.001$ and (****) $P < 0.0001$ by Student's *t*-test, n= 3-10 cells per root growth zone per with 5-8 roots per condition in 3 independent replicates).

Appendix Figure S4



Appendix Fig. S4 | Cytokinin prevents CMT depolymerization in *ahk2-2* and *ahk3-3* cytokinin receptor mutants. **A.** Representative images of *ahk2-2,35S::MAP4-GFP* and *ahk3-3,35S::MAP4-GFP* seedlings (top) and root tips (bottom) grown for 5 days on mock (Murashige and Skoog) and then transferred to medium supplemented with 1 μ M oryzalin, or cytokinin (CK) and oryzalin (10 μ M BAP and 1 μ M oryzalin) for 3 days. For the double CK and oryzalin treatment, seedlings were pretreated with 10 μ M BAP for 60 min prior to transfer to medium supplemented with both compounds. White arrowheads indicate root length at the day of transfer. Confocal images of root tips (bottom) recorded 3 days after transfer. Root tips and CMTs, visualized by the MAP4-GFP reporter, were less affected by oryzalin when pretreated with cytokinin than those treated with oryzalin only in both *ahk2-2* and *ahk3-3*. Scale bar 50 μ m. **B.** Relative root growth of *ahk2-2,35S::MAP4-GFP* (pink lines) and *ahk3-3,35S::MAP4-GFP* (green lines) seedlings grown under the conditions described for (A) monitored for 3 days. Day 0, day of transfer. Mean $\pm$ s.d.; n = 10 roots.

Appendix Figure S5



Appendix Fig. S5 | Cytokinin interferes with the auxin-mediated regulation of CMTs. A-C. Monitoring of CMTs visualized by the MAP4-GFP reporter in epidermal cells of the elongation zone (EZ) of wild-type roots treated with cytokinin (CK, 10 μ M BAP) and auxin (0.1 μ M NAA) (**A**, **B**) without (**A**) or pretreated for 30 min with CK (**B**); CK (0.1 μ M BAP) and auxin (0.1 μ M NAA) applied after a 60-min pretreatment with CK (**C**).