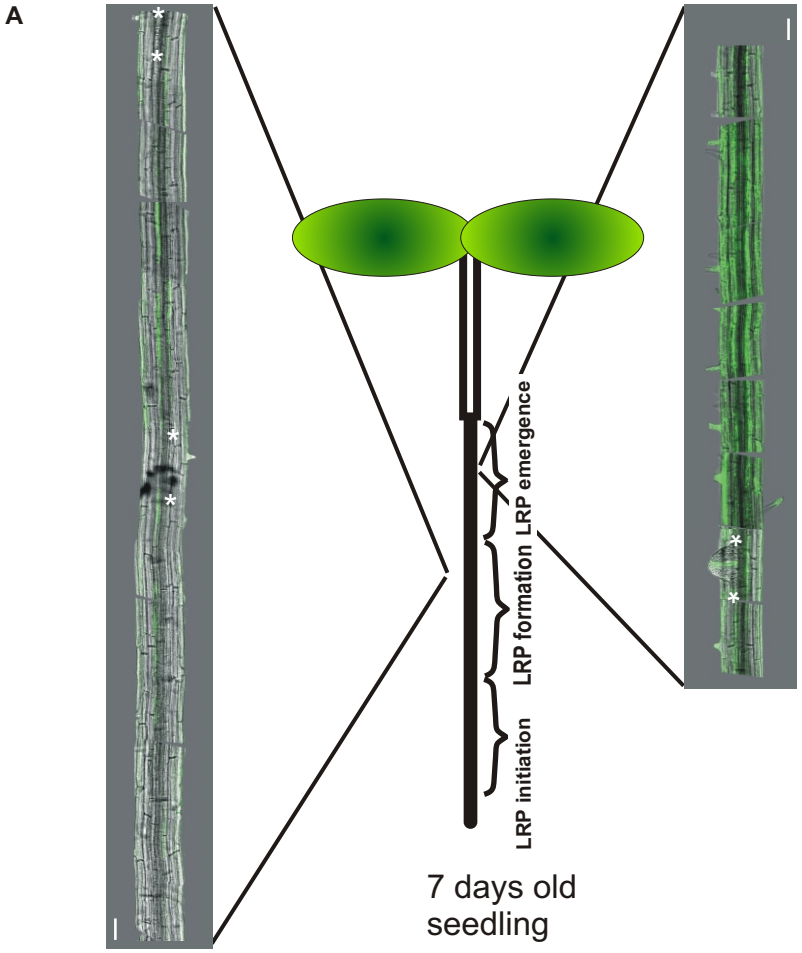
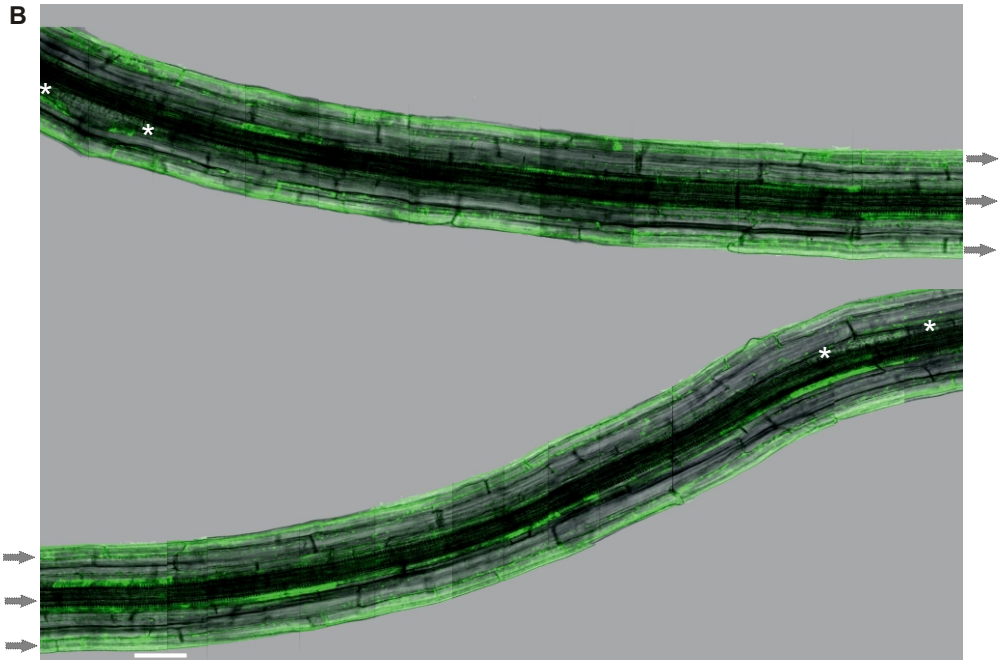
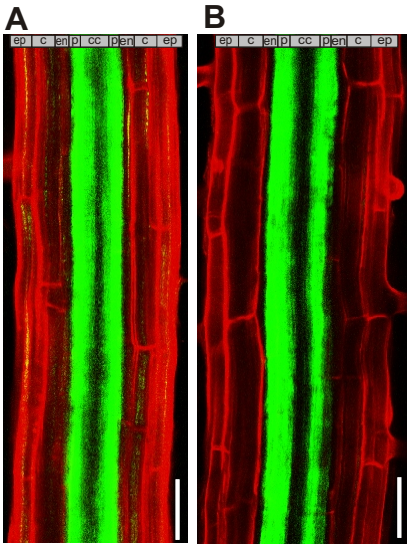




Supplemental Figure 1. Spatial distribution of cytokinin response along the root.

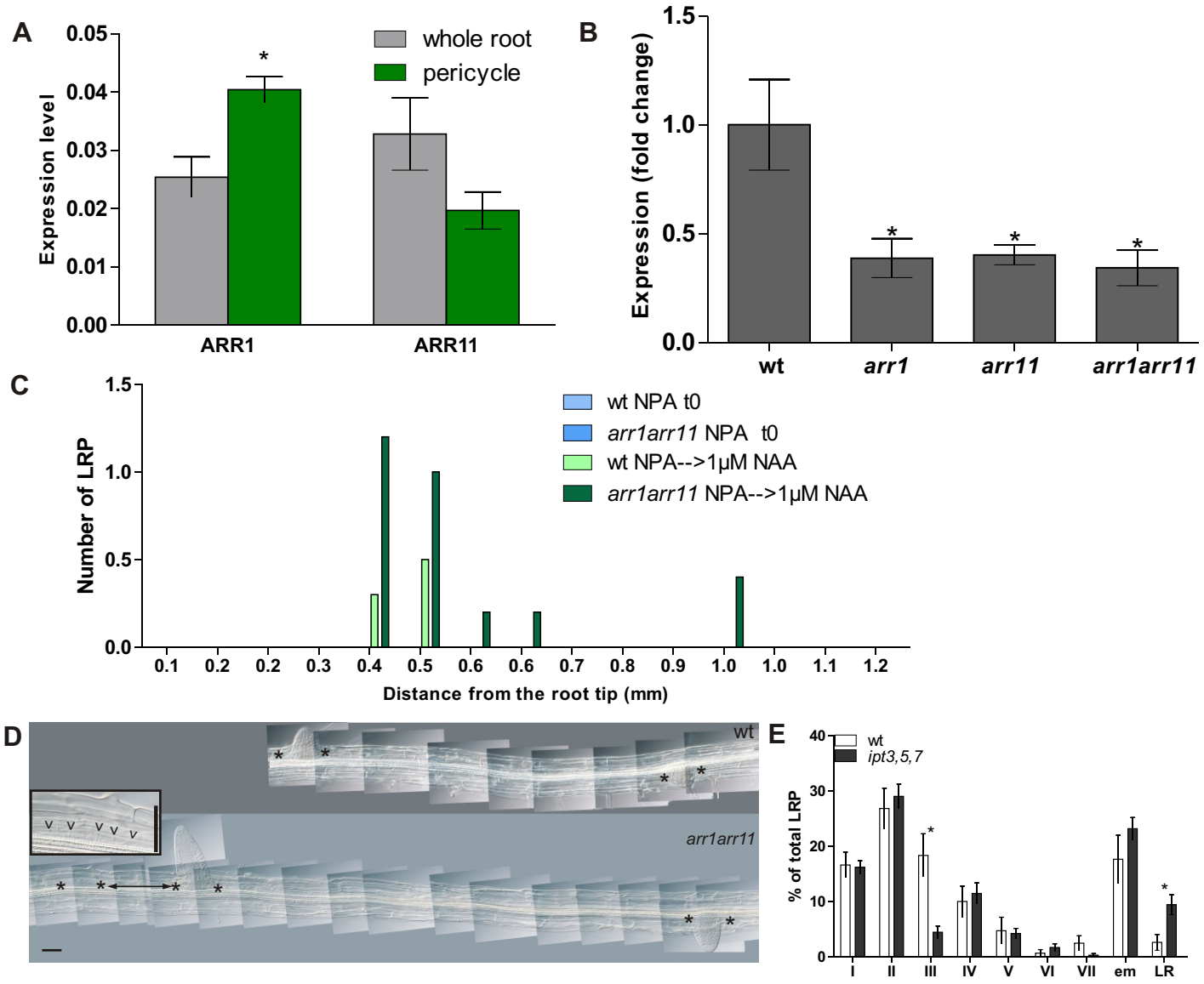
(A) and **(B)** *TCS:GFP* expression along the root. **(A)** *TCS:GFP* detected in pericycle xylem pole cells in the proximity of young or across LRP and in endodermal cells adjacent to early-stage LRP. *TCS:GFP* signal becomes stronger and continuous in the endodermis of the older root portion, where LR emerge. Bars = 50 µm

(B) Magnification of *TCS:GFP* expression between two LRP. White asterisks indicate borders of LRP. Bar = 50 µm.



Supplemental Figure 2. Xylem pole pericycle cell identity in control and NPA-treated roots. (**A)** and **(B**) Expression of *J0121* xylem pole pericycle cell identity marker in 7-day-old roots grown on control media **(A)** or supplemented with 10 μM NPA **(B)**.

c, cortex; cc, central cylinder; en, endodermis, ep, epidermis; p, pericycle. Bar = 50 μm.



Supplemental Figure 3. LRP initiation spacing defective in roots with compromised cytokinin responses.

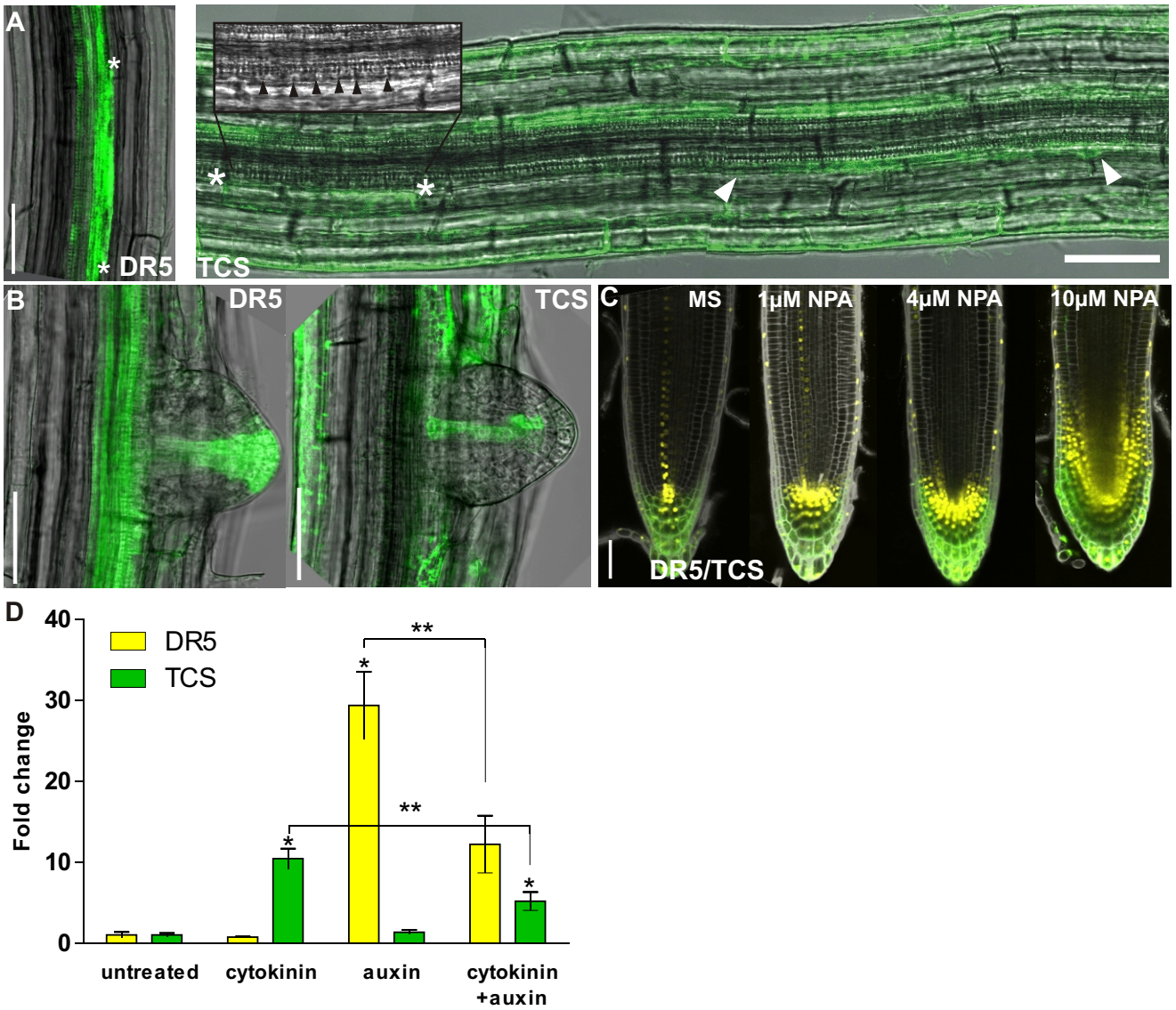
(A) B-type *ARR1* and *ARR11* transcription factors expressed in roots and the pericycle. Error bars denote standard errors (*p < 0.05 Student's *t* test; n =3).

(B) Reduced *TCS:LUC* expression in *arr1* and *arr11* mutants and their double combination Error bars denote standard errors (*p < 0.05 Student's *t* test; n =3).

(C) Auxin-induced LRP initiation in the arr1 arr11 roots pretreated with NPA inhibitor.

(D) Defective distance between neighboring LRP in the *arr1 arr11* mutant. White asterisks indicate borders of LRP. Bar = 50 µm**.** I to V, LRP stages; Em, emerged LRP

(E) Enhanced lateral root development in the *ipt3 ipt5 ipt7* mutant. Error bars denote standard errors (*p<0.05; Student's *t* test; n =10 roots).



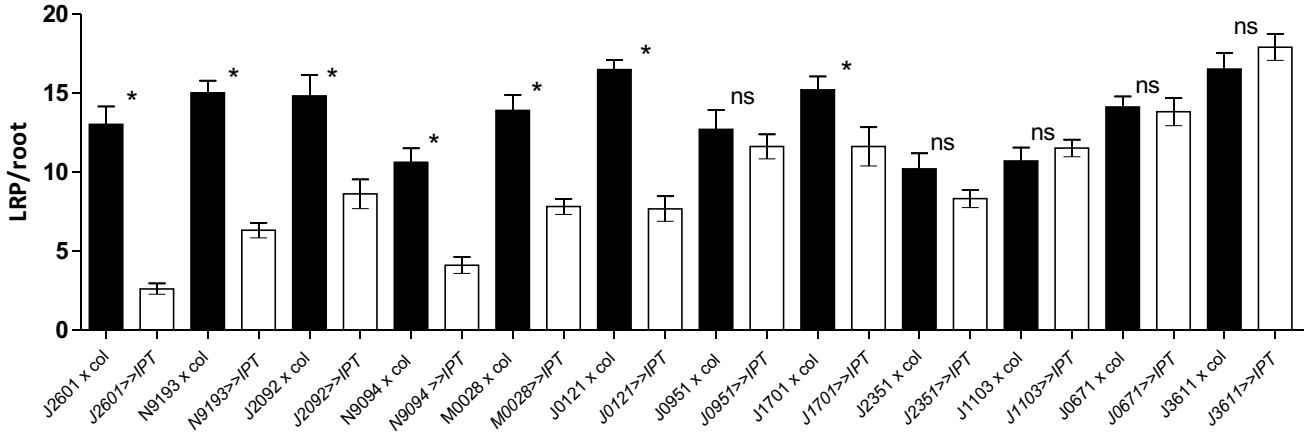
Supplemental 4. Cytokinin and auxin response distributions in roots.

(**A**) *DR5::GFP* expression in founder cells compared to the *TCS::GFP* signal in pericycle cells in the vicinity of LRP. White stars, position of the LRP; inset, LRP at stage I.

(**B**) *DR5::GFP* expression maxima at the tips and *TCS::GFP* in the provasculature of emerging LRP.

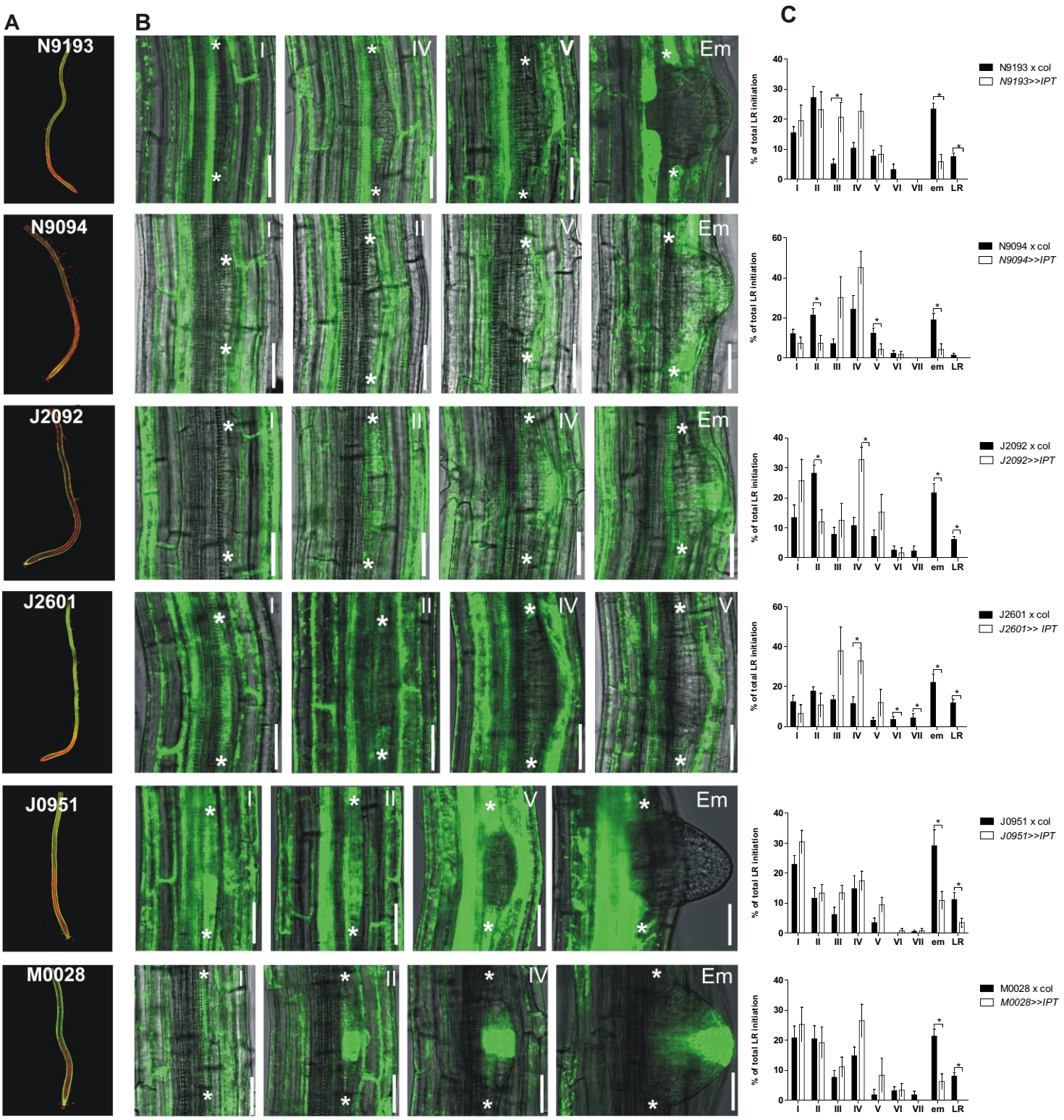
(C) *DR5::3XVENUS-N7 x TCS::GFP* expressions in root tips grown for 7 days on control or NPA-supplemented media. *DR::3XVENUS-N7* expressed in the quiescent center, columella initials, and central columella cells, and *TCS::GFP* in the outer columella cells and lateral root cap **(C)**. Enlarged *DR5:: 3XVENUS-N7* expression domain and *TCS::GFP* expression shifted toward the outer columella cells in NPA-treated roots **(C)**. Bar = 50 μm.

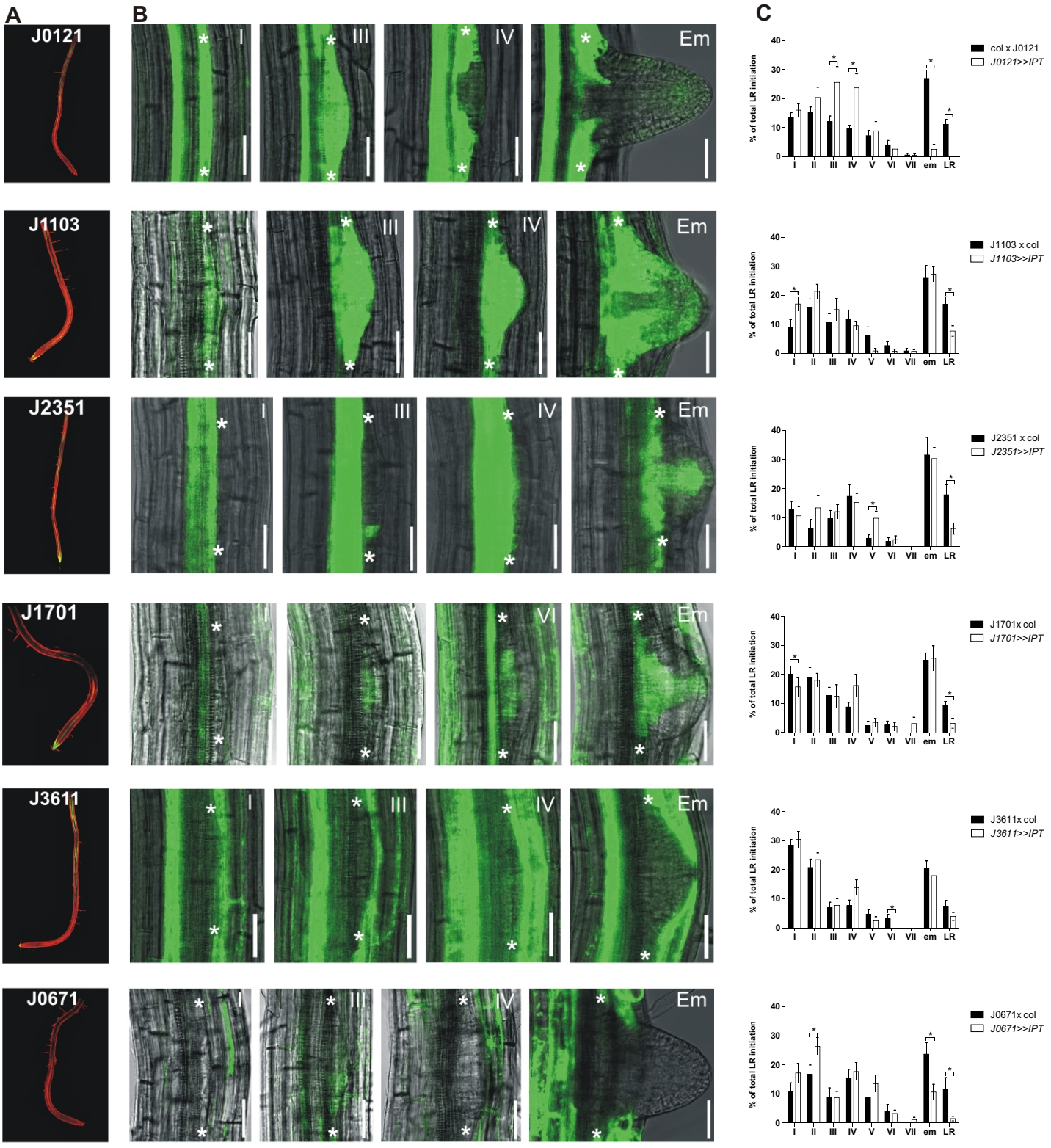
(D) Transient expression of cytokinin *TCS::LUC* and auxin *DR5::LUC* reporters in *Arabidopsis* protoplasts. *TCS::LUC* is upregulated by cytokinin, but not auxin. Cytokinin stimulatory effect is significantly compromised at simultaneous application of auxin. Vice versa, auxin, but not cytokinin stimulated expression of the *DR5::LUC* and simultaneous treatment with cytokinin reduces auxin induction. Error bars denote standard errors (*$p<0.05$ Student's *t* test; n =3).



Supplemental 5. Effect of *IPT* expression on LRI.

Reduced number of LRP in lines with *IPT* expression in the basal meristem (*J2601>>IPT*; *N9193>>IPT*; *J2092>>IPT*; *N9094>>IPT*; *M0028>>IPT*; *J0121>>IPT*; *J1701>>IPT*; and *J2351>>IPT*). Error bars denote standard errors (*$p<0.05$, Mann-Whitney test; n=10 roots).


A
B
C
N9193
N9094
J2092
J2601
J0951
M0028
I
II
III
IV
V
Em
% of total LR initiation
N9193 x col
N9193>>IPT
N9094 x col
N9094>>IPT
J2092 x col
J2092>>IPT
J2601 x col
J2601>> IPT
J0951 x col
J0951>>IPT
M0028 x col
M0028>>IPT



Supplemental 6. Spatiotemporal effect of *IPT* expression on LRP development.

(**A)** to **(C**) Monitoring of the expression of the *GAL4*-GFP enhancer trap lines in the root **(A),** during LRP development **(B),** and impact on the LRP development of tissue-specific *IPT* expression driven by different activator lines **(C)**. Most effective inhibition of LRP development detected when *IPT* was expressed prior LRI (*N9391>>IPT; N9094>>IPT*; and *J2092>>IPT*;) and/or at the very early stages of lateral root development (*J2601>>IPT*; *J0951>>IPT*; *M0028>>IPT*; *J0121>>IPT;* and *J1103>>IPT*). I to V, LRP stages; Em, emerged LRP. Error bars denote standard errors (*$p < 0.05$; Student's *t* test; n =10 roots). Bar = 50 µm.