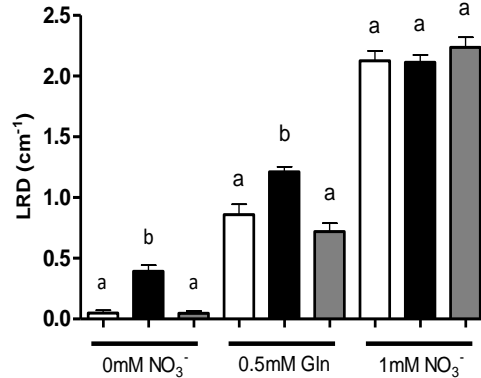
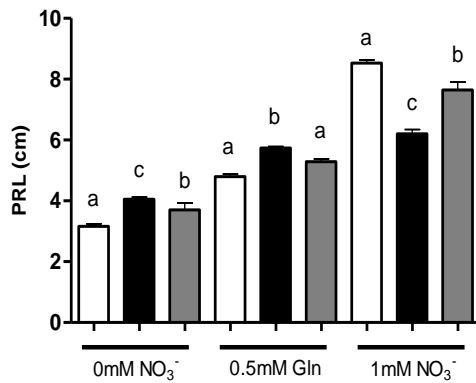


A

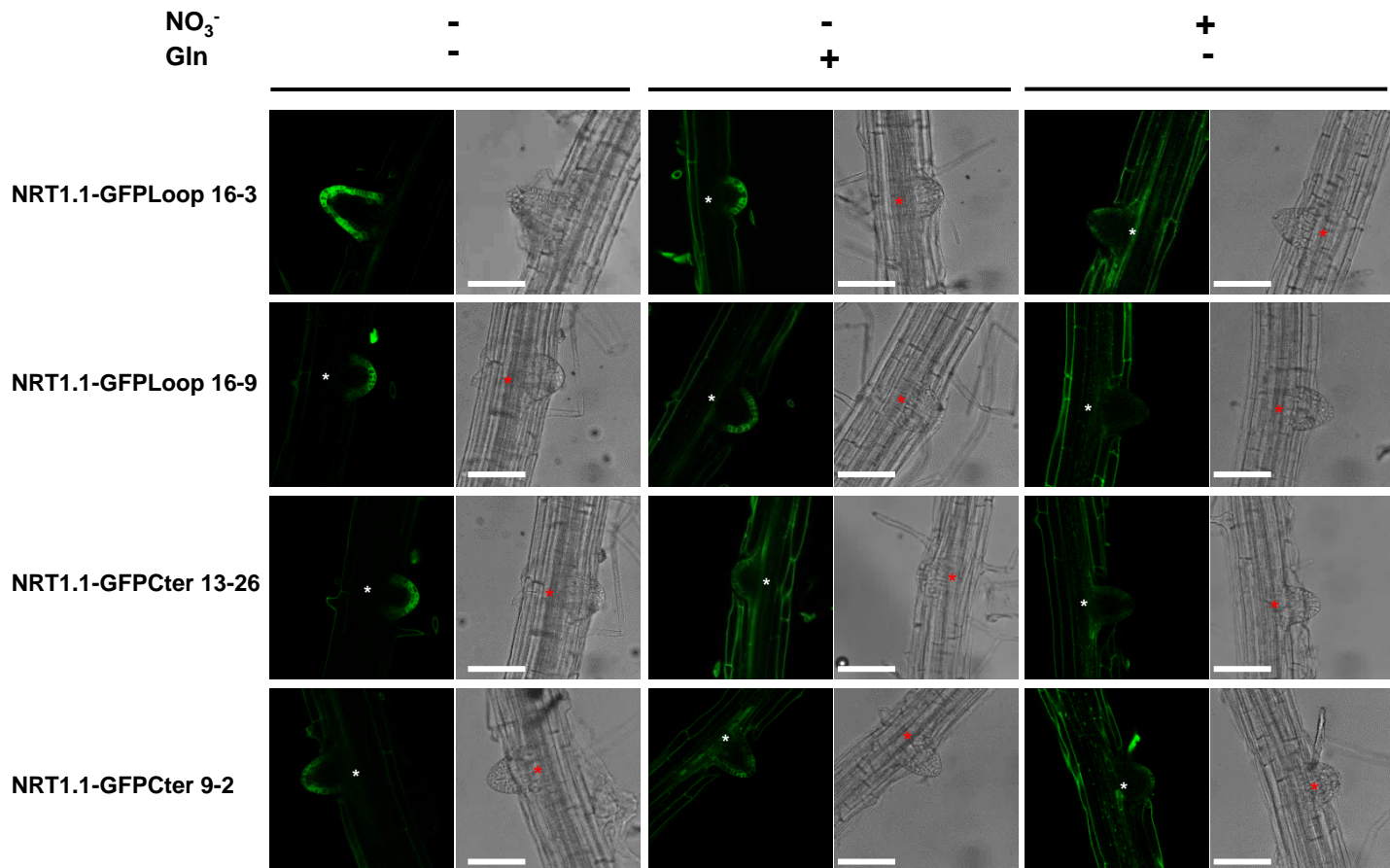


B



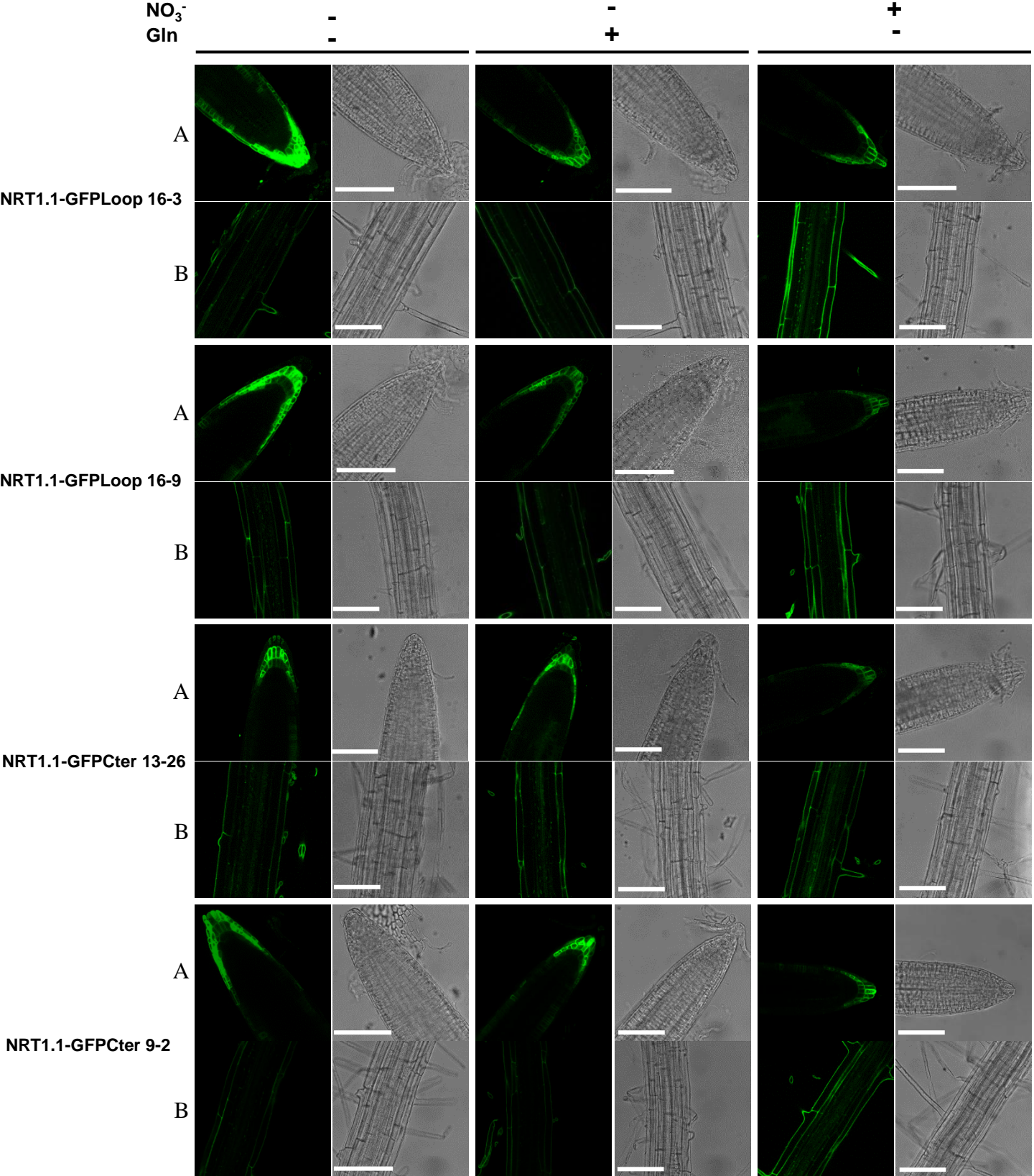
Supporting Figure 1 : *NRT1.1-GFPLoop* fusion protein fully complements the *chll-5* root development phenotype.

Density of visible (>0.5 mm) lateral roots (A) and primary root length (B) of plants grown for 8 days on basal medium without N or supplemented with 0.5mM glutamine or 1mM NO₃⁻. Open, black and shaded boxes represent respectively Col, *chll-5* mutant and transgenic line expressing *NRT1.1-GFPLoop* protein fusion in *chll-5* background (pNRT1.1::NRT1.1-GFPLoop16-3 line). Results (n = 30–52 plants) are representative of three independent experiments (error bars are s.e.). For each treatment, data were analyzed through one-way ANOVA, followed by a Tuckey test as a post hoc analysis are statistically significant at p < 0.05



Supporting Figure 2: NRT1.1-GFP accumulation in lateral root primordia is repressed by NO₃⁻.

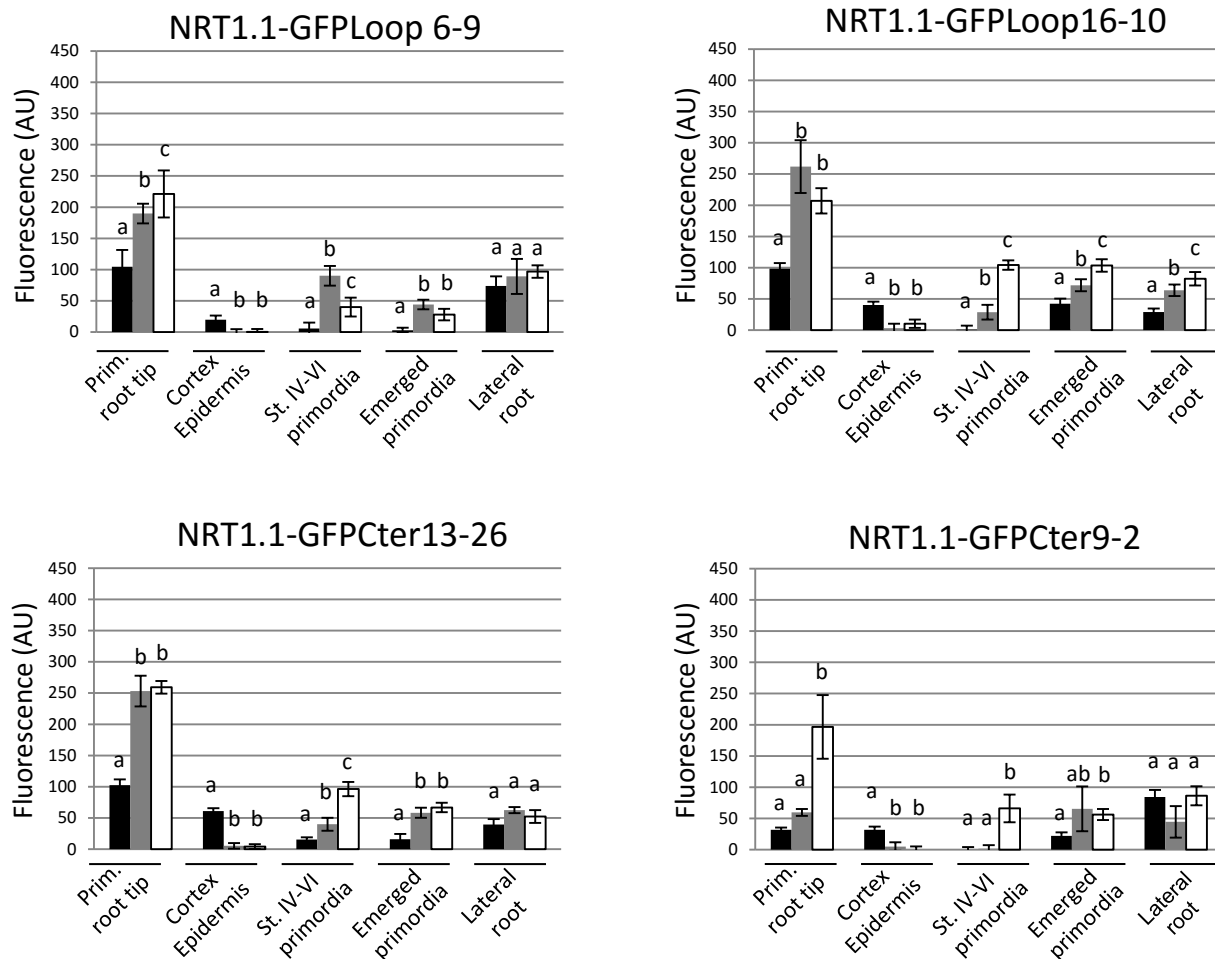
NRT1.1-GFP localization in fully emerged lateral root primordia of pNRT1.1::NRT1.1-GFPLoop16-3, pNRT1.1::NRT1.1-GFPLoop16-9, pNRT1.1::NRT1.1-GFPCter13-26 and pNRT1.1::NRT1.1-GFPCter9-2 transgenic lines. Plants were grown for 8 days on basal medium without N (left panel) or supplemented with 0.5mM glutamine (middle panel) or 1mM NO₃⁻ (right panel). The asterisk visualizes the location of the LRP. The pictures shown are representative of >20 primordia from >10 plants of 3 independent experiments. White bar represents 100µM.



Supporting Figure 3: NRT1.1-GFP accumulation in lateral root primordial is repressed by NO₃⁻.

NRT1.1-GFP localization in primary roots of pNRT1.1::NRT1.1-GFPLoop16-3, pNRT1.1::NRT1.1-GFPLoop16-9, pNRT1.1::NRT1.1-GFPCter 13-26 and pNRT1.1::NRT1.1-GFPCter 9-2 transgenic lines. Plants were grown for 8 days on basal medium without N (left panel) or supplemented with 0.5mM glutamine (middle panel) or 1mM NO₃⁻ (right panel). Each line corresponds respectively to primary root tip (A) and primary root above the first emerged primordial (B). The pictures shown are representative of >10 plants from 3 independent experiments. White bar represents 100µM.

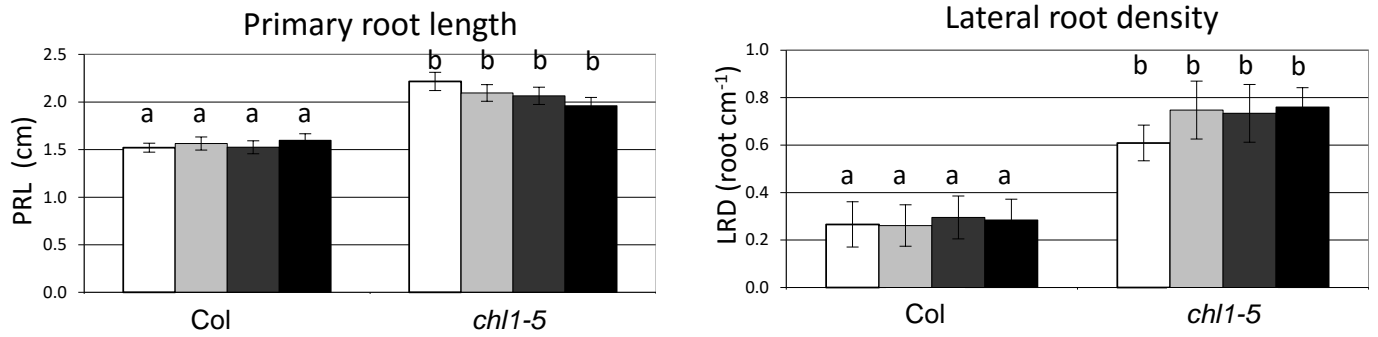
0 mM NO₃⁻
 0,5 mM gln
 1 mM NO₃⁻



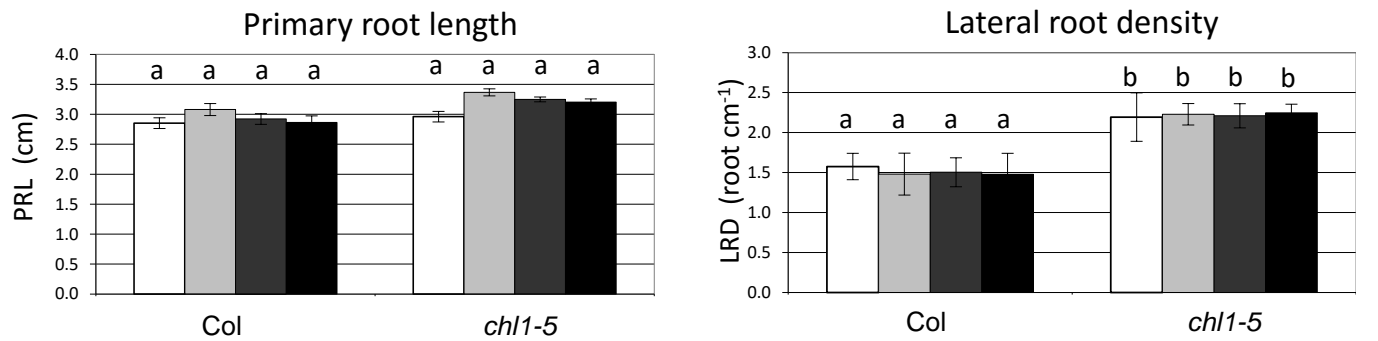
Supporting Figure 4: *NRT1.1-GFP* accumulation is differentially regulated by N in root tissues.

Fluorescence quantification in different root tissues of 8-day-old plants from pNRT1.1::NRT1.1-GFPLoop16-9, pNRT1.1::NRT1.1-GFPLoop16-10, pNRT1.1::NRT1.1-GFPCter13-26 and pNRT1.1::NRT1.1-GFPCter9-2 transgenic lines expressing NRT1.1-GFP protein fusion in the absence or presence of NO₃⁻ (1 mM) or 0,5 mM glutamine. Fluorescence was quantified respectively in primary root tip, cortex and epidermis of primary root (above the first emerged primordium), unemerged primordia stage IV to VI, emerged primordia and growing lateral roots +/-0,5mm (Values are the mean of 8–12 plants from two independent experiments and are normalized to Col (error bars are s.e.). For each tissue, data were analyzed through one-way ANOVA, followed by a Tuckey test as a post hoc analysis is statistically significant at p < 0.05.

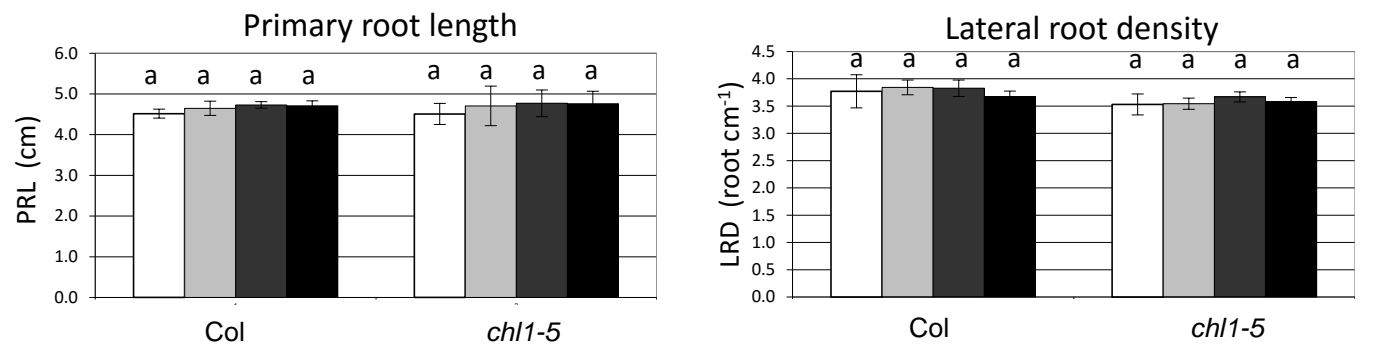
N free medium



0,5 mM Glutamine

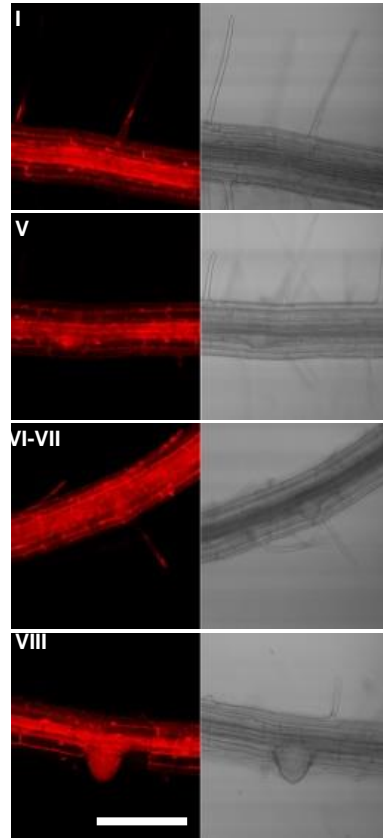


1 mM NO₃⁻



Supporting Figure 5 : Estradiol does not modify Root System Architecture.

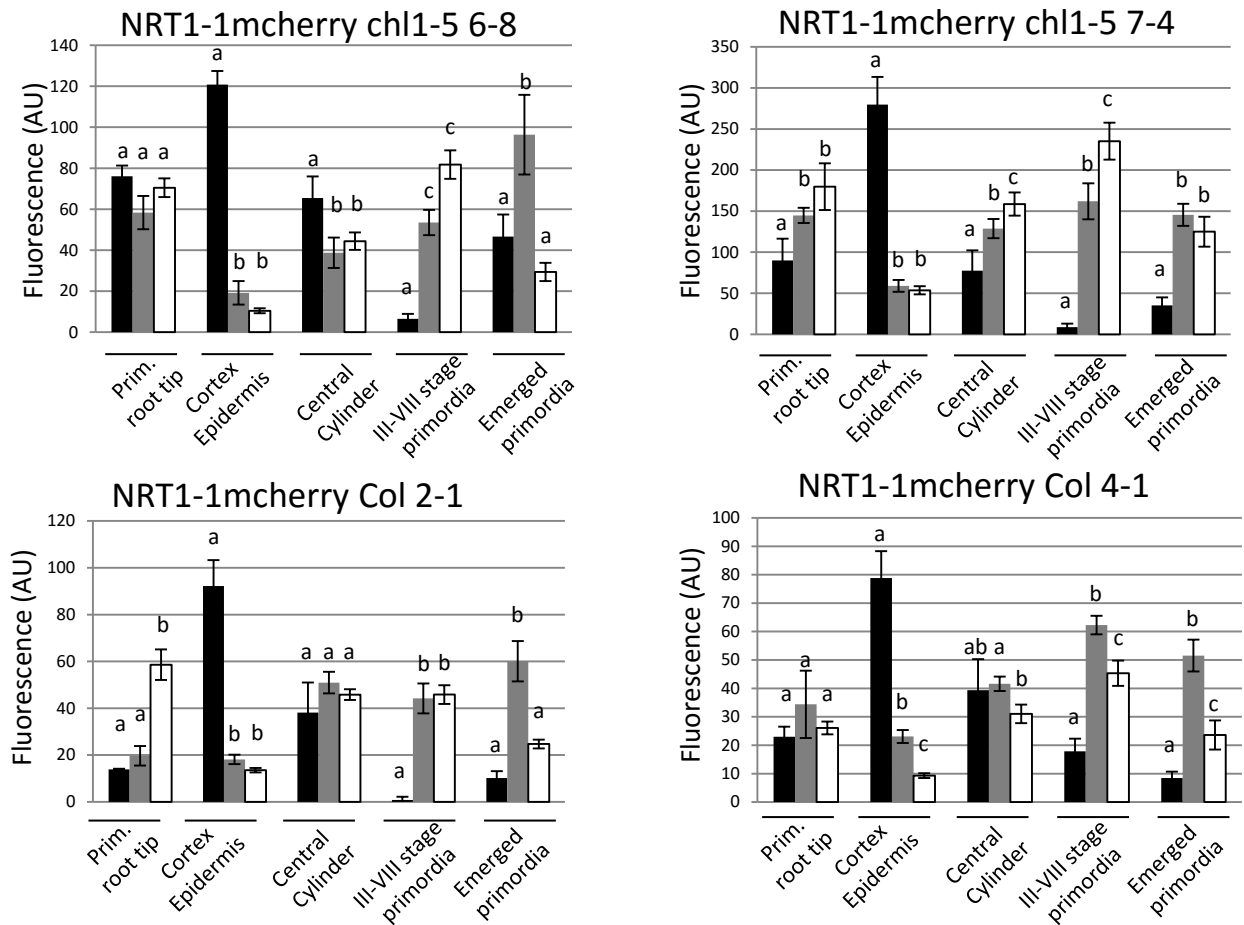
Primary root length and density of visible (>0.5 mm) lateral roots of *col* and *chl1-5* plants grown for 8 days on basal medium without N or supplemented with 0.5mM glutamine or 1mM NO₃⁻ supplemented with different concentrations of estradiol. Open, light grey, dark grey and black boxes represent respectively 0,mM, 1nM, 100nM and 5µM of Estradiol (n = 30–52 plants) are representative of two independent experiments. Data were analyzed through one-way ANOVA, followed by a Tuckey test as a post hoc analysis are statistically significant at p < 0.05.



Supporting Figure 6 : mCherry protein accumulation in roots of the *chl1-5* mutant.

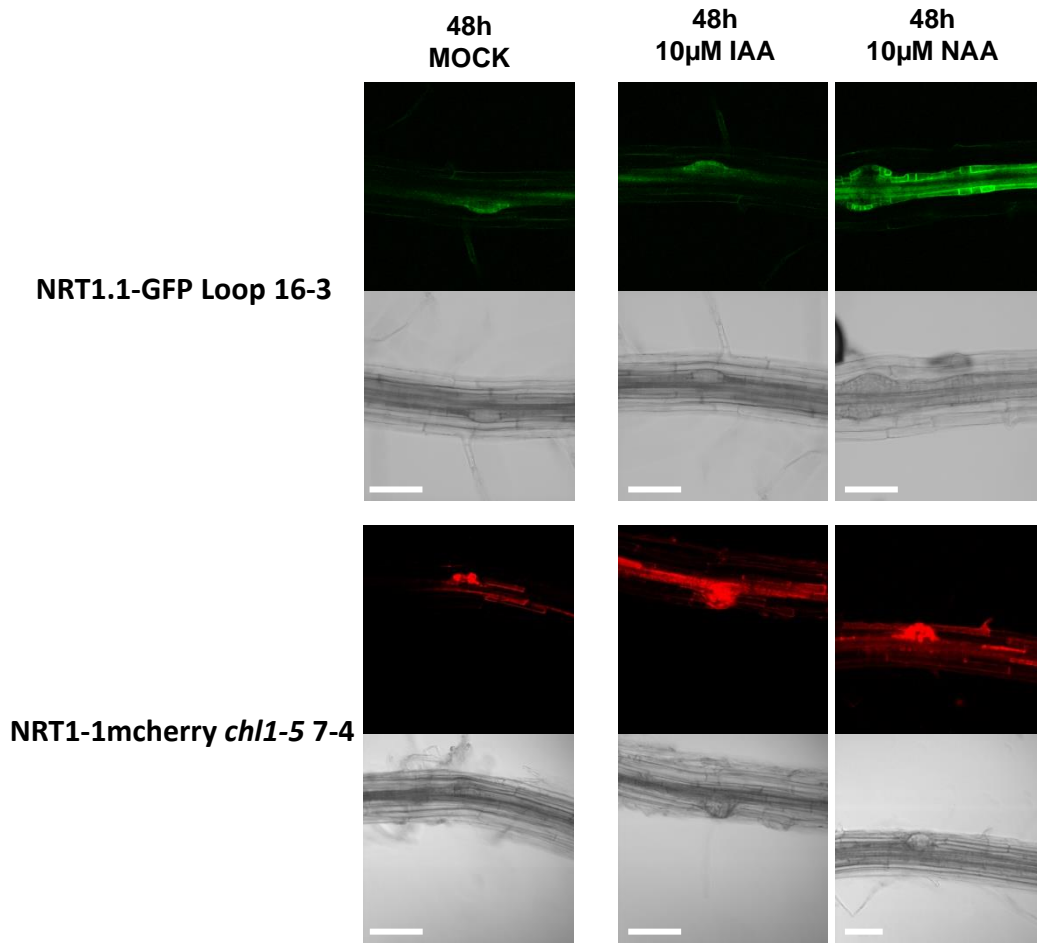
mCherry localization in root of transgenic line expressing mCherry under control of β -estradiol inducible promoter in *chl1-5* background. Seedlings were grown for 10 days on basal medium containing 1mM NO_3^- and transferred on the same medium supplemented with 5 μM of β -estradiol for 48 h. Each line of the panel corresponds to a LRP developmental stage from initiation (I) to emergence (VIII) as described by Malamy and Benfey (1997). The pictures shown are representative of >10 plants of 2 independent experiments. White bar represents 50 μm .

■ 1 mM NO₃⁻
 ■ 0,5 mM gln
 □ 0 mM NO₃⁻



Supporting Figure 7: NRT1.1-mCherry accumulation is differentially regulated by N in root tissues.

Fluorescence quantification in different root tissues of 8-day-old plants from pNRT1.1::NRT1.1-mcherry chl1-5 6-8, pNRT1.1::NRT1.1-mcherry chl1-5 7-4, pNRT1.1::NRT1.1-mcherry col2-1 and pNRT1.1::NRT1.1-mcherry col 4-1 transgenic lines expressing NRT1.1-mcherry protein fusion in the absence or presence of NO₃⁻ (1 mM) or 0,5 mM glutamine. Fluorescence was quantified after induction with 5µM of β-estradiol for 4 days. Fluorescence was measured respectively in primary root tip, cortex and epidermis or central cylinder of primary root tissues root above the first emerged primordium, unemerged primordia stage III to VIII, and emerged primordia (Values are the mean of 5–10 plants from two independent experiments and are normalized to Col (error bars are s.e.).



Supporting Figure 8: NRT1.1-GFP and NRT1.1-mcherry accumulation is enhanced by auxin in lateral root primordia.

NRT1.1-GFP and NRT1.1-Mcherry localization in unemerged lateral root primordia of pNRT1.1::NRT1.1-GFPLoop16-3 and pNRT1.1::NRT1.1-mcherry chl1-5 7-4 respectively.

pNRT1.1::NRT1.1-GFPLoop16-3 seedlings were grown for 10 days on basal medium without N and then transferred for 48H00 on the same medium supplemented with 10 μ M IAA or 10 μ M NAA. pNRT1.1::NRT1.1-mcherry chl1-5 7-4 seedlings were grown for 8 days on basal medium without N and then transferred for 48H00 on the same medium supplemented 5 μ M estradiol and then on the same medium supplemented with 10 μ M IAA or 10 μ M NAA.

The pictures shown are representative of >15 primordia from >12 plants of 2 independent experiments. White bar represents 100 μ M.