pin2 mutant agravitropic root phenotype is conditional and nutrient-sensitive

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Abstract

Plants have the capacity to sense and adapt to environmental factors using the phytohormone auxin as a major regulator of tropism and development. Among these responses, gravitropism is essential for plant roots to grow downward in the search for nutrients and water. We discovered a new mutant allele of the auxin efflux transporter *PIN2* that revealed that *pin2* agravitropic root mutants are conditional and nutrient-sensitive. We describe that nutrient composition of the medium, rather than osmolarity, can revert the agravitropic root phenotype of *pin2*. Indeed, on phosphorus- and nitrogen-deprived media, the agravitropic root defect was restored independently of primary root growth levels. Slow and fast auxin responses were evaluated using DR5 and R2D2 probes, respectively, and revealed a strong modulation by nutrient composition of the culture medium. We evaluated the role of PIN and AUX auxin transporters and demonstrated that neither PIN3 nor AUX1 are involved in this process. However, we observed the ectopic expression of *PIN1* in the epidermis in the *pin2* mutant background associated with permissive, but not restrictive, conditions. This ectopic expression was associated with a restoration of the asymmetric accumulation of auxin necessary for the reorientation of the root according to gravity. These observations suggest a strong regulation of auxin distribution by nutrients availability, directly impacting root's ability to drive their gravitropic response.

Key words: Polar auxin transport, PIN1, PIN2, gravitropism, nutrients, conditional phenotype

1. Introduction

Gravitropism is essential to monitor gravity and allow roots to anchor themselves in the soil, where they navigate heterogeneous environments to uptake water and nutrients. The key phytohormone auxin controls many stages of plant development and tropism, including gravitropism (Friml et al., 2002). The gravitropic response mechanism can be divided into three sequential phases, i) perception of a change in gravity vector, ii) establishment of an asymmetric auxin distribution and iii) asymmetric growth response (Sato et al., 2015, Singh et al., 2017). Roots have been suggested to use a tipping point mechanism to reverse the asymmetric auxin flow at midpoint of bending, allowing to fine tune the root apex position (Band et al., 2012). During the asymmetry acquisition phase, auxin fluxes are altered through the differential relocalization of auxin efflux transporter PIN-FORMED proteins (PINs), in particular PIN3 and PIN2, observed 30 min to 2 h after root reorientation (Friml et al., 2002; Rahman et al., 2010). Gravistimulation induces changes in PIN3 polar localization in root columella where it relocates towards the lower part of the columella cells (Friml et al., 2002, Grones et al., 2018). Differential regulation of PIN2 trafficking between the upper and lower surfaces of a gravistimulated root is crucial to maintain modifications of the concentration of auxin on each side of the root, firstly initiated by PIN3 in the columella cells. On the lower root side, endocytosis of PIN2 is inhibited, resulting in plasma membrane maintenance of PIN2 in expanding epidermal cells, while on the upper root side, PIN2 is rapidly internalized and degraded (Abas et al., 2006). Although PIN1 does not play a direct role in the differential accumulation of auxin, it is responsible for providing an auxin pathway to feed the root tip. The asymmetric redistribution of auxin between the lower and upper part of a gravistimulated root can be revealed by markers like the auxin response reporter DR5 (Ulmasov et al., 1997), the auxin sensor DII (Brunoud et al., 2012) and its ratiometric version R2D2 (Liao et al., 2015). A decrease in the upper to lower auxin ratio is responsible for the differential root growth and thus the reorientation of the apex in the downward direction.

The *pin2* mutant was firstly described as an agravitropic mutant with a curling of the primary root, amongst other phenotypes, identified from various genetic screens (Chen et al., 1998; Luschnig et al., 1998; Müller et al., 1998, Utsuno et al., 1998). This multiple origin is reflected in its various given names prior to its molecular characterization: *wavy 6* (*wav6*), *ethylene-insensitive root 1* (*eir1*), *agravitropic 1* (*agr1*) and finally *pin-formed 2* (*pin2*). An extensive analysis of the published descriptions of *pin2* root phenotype gives a wide range of gravitropic responses, from almost entirely gravitropic to a strong loss of gravitropic response (Fig. 1A). This raises the question of the expressivity of the gravitropic phenotype in *pin2* and other *pin* mutants. In this study, we identified a new allelic mutant in *PIN2* that we named *pin2-2* and revealed that *pin2* phenotype is conditional and nutrient-sensitive, providing an explanation for its phenotypic discrepancy in the scientific literature (Fig. 1B,C).

Previous studies have shown that auxin and nutrient transports can compete, resulting in differential growth or development in various environmental conditions. The nitrate transporter NRT1.1 has been shown to transport auxin in heterologous systems (Krouk et al., 2010) providing an elegant explanation of its lateral root growth phenotype. Its role as a transceptor also suggests pathways for the regulation of PIN proteins. The root coiling on horizontal growth in nitrate deficiency has been shown to be caused by asymmetric auxin response (Chai et al., 2020). Presence of nitrate suppresses asymmetric root growth mediated by the transporter NRT1.1, indeed PIN2-mediated auxin transport is epistatic to NRT1.1 during nitrate deficiency (Chai et al., 2020). Our study does not suggest similar competing properties for PIN proteins but demonstrates a differential expression pattern in various conditions. Indeed, the conditional phenotype of *pin2* is revealed in the presence and absence of phosphate or nitrate and does not result from osmotic variations. Using slow and fast auxin reporters, respectively DR5 and R2D2, we showed that auxin asymmetric accumulation in the root tip is altered by the nutrient's composition of the medium. Further investigation revealed that ectopic expression of *PIN1* rather than changes in *PIN3* or *AUX1* is associated with a reversion of the gravitropic phenotype. Taken together, our study provides insight into the conditional agravitropic phenotype of *pin2* and exposes redundancy in the PIN family, with an important role for PIN1 in restoration of *pin2* phenotype as an adaptation to nutrients availability.

2. Materials and Methods

2.1. Plant materials, growth conditions, quantification of PR length and growth rate

Arabidopsis thaliana Columbia-0 (Col-0) ecotype was used as the wild-type. Mutants and transgenic lines including *pin2*/*eir1-1*/*eir1-4* (Chen et al., 1998; Luschnig et al., 1998), *pin3-5* (Blilou et al., 2005), *pin1-3* (Bennet et al., 1995), *SALK_122916 (*Alonso et al., 2003*)* were described previously. The following transgenic lines were used for expression studies: DR5:GFP (Benkova et al., 2003), R2D2 (Liao et al., 2015), pPIN3::PIN3-GFP (PIN3:GFP) (Dello Ioio et al., 2008), pAUX1::AUX1-YFP (AUX1:YFP) (Swarup et al., 2005), pPIN1::PIN1-GFP (PIN1:GFP) (Omelyanchuk et al., 2016) and were introgressed into the *pin2-2* genetic background by crossing to generate homozygous lines. For seedlings growing on plates, Arabidopsis seeds were surface-sterilized with a solution containing 12.5: 37.5 : 50 (v/v/v) of bleach/water/ethanol for 5 min with agitation. Seeds were rinsed three times with 96% ethanol before drying. Seeds were then germinated on 1/2 Murashige and Skoog basal medium (MS) supplemented with Gamborg's vitamins (Murashige and Skoog Basal Medium M0404 - Sigma Aldrich), 0.8% agar, 1% sucrose, 0.05% MES, pH adjusted to 5.7. Plants were grown under long-day photoperiods (16 h light/8 h dark and a temperature of 21°C with

light intensity of 120 µmol cm^{-2} s^{-1} provided by Osram, Berlin, Germany; 18-W 840 Lumilux neon tubes). For the experiments carried out on the Root Phenotyping platform using the The HIgh Resolution ROot Scanner (HIRROS) setup (Fernandez et al., 2022) the seedlings were grown with 1% agar, under long-day photoperiods with LED lighting (between 40 and 350 µmol m^{-2} s^{-1}) and a temperature of 23°C.

Plants were treated with a supplementation in the medium of 150 mM sorbitol or 75 mM NaCl or a range (0 g/L, 100g/L, 250 g/L) of PEG-8000 (P2139 - Sigma Aldrich) using a protocol described previously (Verslues et al., 2006).
The different medium compositions are in Materials and Methods Supplemental S1.
The primary root (PR) length was quantified using ImageJ software and presented in graphs with n=30 seedlings. The growth rate was calculated using seedling growth for 24h.

2.2. Sequencing

Bulk sequencing of *lasso* mutant was done from an F2 after a backcross with Col-0. Sequencing was produced by BGI with the sequencing platform BGISEQ-500, the read length used was paired-end 100 bp and the data output was 4G clean data per sample. Low quality bases were removed using cutadapt, and reads were mapped on Arabidopsis genome using bwa mem. Then GATK was used to call variants, along with SNPeff to predict their effect, allowing to quickly find differences between *lasso* and Col-0. All the variations (SNP or small deletion or insertion) with a potentially large impact on the structure of a gene was looked.

2.3. Gravistimulation

Seeds were sowed in sterile condition on MS/2 medium then vernalized for 2 days at 4°C in the dark. They were placed in culture chamber in vertical position. After 6 days, the seedlings were transferred to a new plate containing various media or control and rotated 90° with respect to the gravitational vector. A quantification of the position of the apex is carried out at different times and categorized into 8 different orientations on a circular graph (Swarup et al., 2004 ; Petrášek et al., 2006), detail of the category is in Materials and Methods Supplemental S2. Adobe Photoshop CS6 was used to overlay gravistimulation images.

2.4. Confocal imaging, and fluorescence signal quantification

For fluorescence visualization, Leica SP8 (Leica microsystems, Wetzlar, Germany) coupled with the LASX software and equipped either with HC PL APO CS2 40x/1.10 water or HC PL

APO CS2 63x/1.40 oil was used. Image captures were performed with the same confocal settings (gain, laser strength, pinhole) to generate comparable images among different treatments or genetic backgrounds. 6 DAG seedlings were mounted on a slice of medium. Fluorescence signals for GFP (excitation 488 nm, emission 500 to 540 nm), YFP (excitation 514 nm, 520 to 540 nm) and propidium iodide (excitation 561 nm, emission 580 to 630 nm) were detected. For image quantification (R2D2, PIN3:GFP, AUX1:YFP, PIN1:GFP, fluorescence intensity measurements), maximum intensity projections of confocal pictures were used. Roots were observed respectively 30 min, 1h or 1h30 after gravistimulation for PIN3:GFP, PIN1:GFP, AUX1:YFP and R2D2. The image analyzes and quantification were performed using Fiji-ImageJ. The quantification of GFP intensity for DR5:GFP was performed with the Plot Profile. The intensity of the signal was normalized with area when it was different and represented as mean signal intensity in arbitrary units (a.u). For R2D2 , nuclear signal was quantified in the first 9 cells of epidermis layer as previously described (Liao et al., 2015). Minimum 10 to 16 independent biological replicates were performed. For PIN3:GFP, the quantification was done as previously described (Grones et al., 2018). For AUX1:YFP, the quantification was done along a line passing through the lower and upper face of the root in response to 1h30 of gravitropism. For PIN1:GFP at first the quantification was done in a square in the stele after 1h of gravitropism and then in the total epidermis or directly at the plasma membrane.

2.5. Statistical analysis

The number of independent repetitions of experiments, as well as exact sample sizes, is described in the figure legends. Statistical analysis (Student's t-test) were performed using the software R. Statistical significance was tested as described in the figure legends.

3. Results

3.1. Identification of the *lasso (pin2-2)* mutant

A mutant was identified by screening an activation tagging population (Weigel et al., 2000) looking for plants with a skewed root in response to salt stress. This mutant displayed a curled primary root in half MS medium (MS/2) and in presence of NaCl (Fig. S1A). It was named *lasso* to describe the coiling of its root like a rope (Fig. 2A). Further gravistimulation experiments were performed on regular half MS medium supplemented with NaCl or sorbitol to understand how root coiling was impacted. Wild-type Col-0 plants displayed a fully functional response to gravity in all conditions whereas the *lasso* mutant root was agravitropic on MS/2 medium, supplemented or not with NaCl. Intriguingly, gravity response of the mutant was partially restored when grown on MS/2 supplemented with sorbitol (Fig. S1B). No activation tagging T-DNA was found to co-segregate with the *lasso* phenotype.

Instead, bulk sequencing of F2 *lasso* mutants from a wild-type backcross identified a deletion/insertion event in *PIN2* (AT5G57090). A 30bp deletion from C_{638} to A_{668} is replaced by a 19bp insertion (TAACTCCTCCATGATAACG) creating a stop codon in the third exon (Fig. S1C). Introgression of the *proPIN2:PIN2-GFP* construct into the *pin2-2* genetic background fully reverted the agravitropic root phenotype, further confirming the identification of the causal mutation (Fig. S1D). The *lasso* mutant was subsequently renamed *pin2-2* and was shown to also display a 30% reduction in primary root length (Fig. S2A) like previously described *pin2* alleles. Similarly to known *pin2* alleles (Ottenschlager et al., 2003), an accumulation of the auxin reporter DR5-GFP was observed in the root tip (columella and lateral root cap) of the *pin2-2* mutant (Fig. 2B). Upon gravistimulation, a lack of asymmetry in auxin distribution was observed in the *pin2-2* background, compared to wild-type where auxin signal asymmetry is measurable in the lateral root cap (Fig. 2C).

3.2. *pin2* agravitropic root phenotype is conditional and nutrient-sensitive

Since *pin2-2* displays sensitivity to the composition of the medium, we tested whether osmolarity impacts root gravity response in *pin2-2*. Polyethylene glycol (PEG), a non-metabolized polymer, was used to alter osmolarity in the medium. It was previously demonstrated that root growth is increased at 100 g/L of PEG and inhibited at 250 g/L of PEG (Rosales et al., 2019). We therefore used both concentrations to dissociate root growth effects from osmolarity. Position of the root apex was monitored 48h after a 90° reorientation of the vertical plates and grouped into 8 angular sections (Swarup et al., 2004). Wild-type seedlings displayed a normal response to gravity in all conditions tested and the *pin2-2* mutant displayed a strong agravitropic phenotype in all conditions tested (Fig. S1E). Increasing osmotic pressure did not revert the agravitropic phenotype of *pin2-2* mutant suggesting that osmolarity alone does not represent a permissive condition.

In order to determine which nutrient alters *pin2-2* phenotype, we tested the impact of deficiency in three major nutrients, nitrogen (MS/2-N), phosphorus (MS/2-P) and iron (MS/2-Fe). A global dilution of the culture medium was also tested by comparing half MS (MS/2) to one-tenth MS (MS/10). In all conditions tested, wild-type (Col-0) plants responded to gravity (Fig. 2D). The *pin2-2* mutant showed an agravitropic root growth on MS/2 and MS/2-Fe medium, thus defining restrictive conditions. However, when grown on MS/2-P, MS/2-N and MS/10 media the *pin2-2* mutant partially reverted to a wild-type gravitropic response, defining permissive conditions. It is worth noting that although the phenotype difference is unambiguous between the 2 conditions, the response to gravity in permissive conditions is not exactly as total as wild-type. Indeed, the root apex is orientated mostly in sector 6 for *pin2-2* compared to sectors 5 and 6 for wild-type (Fig. 2D). Also, the primary root of the *pin2-*

2 mutant shows a slight curvature whereas that of the wild-type plants is perfectly straight (Fig. 2D). This conditional phenotype was tested on the available allelic series of *pin2* mutants: *eir1-1*, *eir1-4* and a T-DNA mutant from SALK (*SALK_122916*). All *pin2* alleles tested showed a conditional agravitropic root phenotype with identical permissive and restrictive conditions (Fig. S2B,C). These results confirm that the *pin2* mutant agravitropic root phenotype is conditional and nutrient-sensitive.

3.3. Conditional *pin2* phenotype does not result from defects in growth or early gravitropism and is not observed in *pin1* and *pin3*

We tested whether mutants in the main PIN transporters expressed in the root tip could share a similar conditional root gravitropic phenotype as *pin2* (Table 1). PIN1 controls the main auxin flow to the root tip and PIN3 has been shown to relocalize auxin upon gravistimulation in the columella cells (Omelyanchuk et al., 2016; Friml et al., 2002). In both permissive and restrictive conditions, we observed a wild-type gravitropic response of *pin1* and *pin3,* 48 hours after induction (Fig. S3). We next tested the very early gravitropic response (up to 9 hours) and whether growth defects could be linked with the conditional phenotype of *pin2*. The kinetics of root curvature were monitored 0, 3, 6 and 9 h after gravistimulation (Fig. S4). All mutants tested (*pin1*, *pin2* and *pin3*) showed a root bending response similar to wild-type (Table 1) demonstrating that the early gravitropic response was not altered in any of the 3 *pin* mutants. Growth rate was evaluated over 24 hours post-gravitropic induction and showed a global reduction of root growth for all *pin* mutants tested (Fig. S5). However, no correlation with permissive and restrictive conditions were observed, suggesting that root growth alteration is not responsible for the phenotypic expression and reversion of *pin2* (Table 1). These results suggest that *pin2*, but not *pin1* or *pin3*, display a conditional agravitropic root phenotype that does not involve early gravitropic response or root growth defects.

3.4. Auxin accumulation is modulated by nutrient availability

The late auxin response marker DR5 fused to GFP (Ulmasov et al., 1997) was used to monitor auxin accumulation in the root tip 4 hours after plant gravistimulation. Permissive conditions (MS/10, MS/2-P and MS/2-N) had no effect on the wild-type auxin profile but resulted in a global reduction of the intensity of DR5-GFP both in the central zone and in the lateral root cap of *pin2* compared to restrictive conditions (MS/2 and MS/2-Fe) (Fig. S6). However, no asymmetric accumulation could be revealed using this marker in the mutant plant whereas it was observed in the wild-type plants in both conditions, suggesting either

that auxin asymmetry is not restored in the *pin2-2* mutant in permissive conditions or that DR5 is not a dynamic enough marker to monitor such changes.

We therefore used the ratiometric version of the DII marker (R2D2) to observe dynamic changes in auxin response as the degron (DII) motif of Aux/IAA is degraded upon auxin application within minutes (Liao et al., 2015). We were able to monitor auxin changes 90 minutes after plant gravistimulation (Fig. 3A,S7A). Data are presented as the mDII/DII (modified non-degraded DII to DII) ratio therefore showing an increase in ratio when more auxin response is occurring and DII is degraded (Fig. 3B,C). Wild-type (Col-0) plants displayed an increased auxin response on the lower epidermal layer (first nine epidermal cells) compared to the upper side (Fig. 3B,C). Some medium compositions slightly altered the auxin response levels in the wild-type plants. Indeed, conditions MS/2-Fe globally reduced both upper and lower auxin response whereas conditions MS/2-N and MS/10 reduced only auxin response in the lower side. These reductions were not associated with any root gravitropic defects. In the *pin2-2* mutant background, auxin signals were also reduced depending on the conditions. Only permissive conditions were associated with a reduction on the auxin response both in the upper and lower epidermal cell files (first nine epidermal cells). In this case, reduction of the mDII/DII ratio was associated with the phenotypic reversion of the root gravitropic response in *pin2-2*. However, this change in auxin response was not associated with a strong reduction of the up to down ratio demonstrating that auxin levels in the *pin2-2* mutant remain high compared to wild-type plants (Fig. S7B). Altogether, these results suggest that a reduction of the auxin signal in the *pin2-2* elevated auxin background would be responsible for the phenotypic reversion.

3.5. PIN3 and AUX1 protein accumulation is not altered in *pin2*

In order to identify a molecular mechanism responsible for the changes in auxin response in various nutrient conditions, we monitored the expression of the PIN3 and AUX1 proteins, two key players of the root gravitropic response in plants (Friml et al., 2002; Grones et al., 2018). The PIN3:GFP reporter line was used to monitor PIN3, which is localized to the plasma membrane in the columella and known for its rapid relocation following gravistimulation. In both the wild-type (Col-0) and *pin2-2* backgrounds, observation of PIN3-GFP after 30 minutes of gravistimulation (Fig. 4A,S8) showed a greater accumulation on the lower side independently of the medium composition used (Fig. 4B). We next used the AUX1:YFP reporter line to monitor AUX1 accumulation, an auxin influx transporter localized in the lateral root cap and epidermis whose expression mediates auxin transport from the root cap towards the epidermal cells during the gravitropic response (Bennet et al., 1996 ; Swarup et al., 2001). Observation of AUX1-YFP in both the wild-type (Col-0) and *pin2-2* backgrounds after 1h30 of gravistimulation (Fig. 4C,S9A) showed no changes in localization pattern. The

293 measure up to down ratio was close to 1, suggesting the absence of asymmetric distribution
294 of AUX1 (Fig. 4D). Medium composition still had an impact on AUX1:YFP intensity without
295 affecting this ratio (Fig. S9B). These results show that the symmetry of AUX1 and PIN3
296 protein accumulation profiles is not affected by either the *pin2-2* mutation or changes in
297 medium composition, and likely do not play a role in the conditional reversion of the *pin2*
298 phenotype.
299
300 **3.6. Ectopic expression of PIN1 in the epidermis is associated with the phenotypic**
301 **reversion of *pin2***
302 We next monitored the localisation of PIN1 using the PIN1-GFP reporter line (Huang et al.,
303 2010). The auxin efflux transporter is present in the basal plasma membrane in the stele
304 cells where it directs auxin transport to the apex (Omelyanchuk et al., 2016). Expression of
305 PIN1-GFP was observed 1 hour after gravistimulation. A decrease in the presence of PIN1
306 in the stele was observed on MS/2-Fe, MS/2-P, MS/2-N, MS/10 in wild-type plants (Fig.
307 S10A,B). There was a reduction in PIN1 accumulation in the stele in *pin2-2* compared to
308 Col-0 but because auxin levels remain high in the *pin2-2* columella (Fig. 2B), this suggests
309 that this reduction has no impact on auxin accumulation in the tip.
310 Previous reports have shown that ectopic expression of PIN1 can be seen in the epidermal
311 cells in the *pin2* mutant background (Vieten et al., 2005). We therefore monitored PIN1
312 protein localization in the epidermis and found that there is no accumulation in wild-type
313 plants in this tissue that could be detected by laser scanning confocal microscopy (Fig.
314 5A,S10C). Quantification therefore provided a background noise level to which other
315 conditions were compared (Fig. 5B,C). In the *pin2-2* mutant background, in all condition
316 tested, the signal of epidermal PIN1-GFP was systematically detected (Fig. 5B) and the
317 number of cells with a positive GFP signal was between 30 and 40 compared to less than 10
318 in wild-type (equivalent to noise level – Fig. 5C). Using higher magnification, we were able to
319 confirm PIN1-GFP signal at the membrane of epidermal cells in the *pin2-2* mutant only (Fig.
320 5D). Quantification of this signal revealed that PIN1-GFP is expressed almost symmetrically
321 in restrictive conditions (MS/2 and MS/2-Fe) with a ratio of 1 to 1.5 and a strong asymmetry
322 is established in permissive conditions (MS/2-P, MS/2-N and MS/10) with a ratio value
323 ranging from 3 to 5 (Fig. 5E). These observations demonstrate that nutrient composition can
324 alter PIN1 protein accumulation in the *pin2* mutant background, associated with a restoration
325 of the root gravitropic response.
326
327 4. Discussion
328 **4.1. PIN proteins play a complex and redundant role in physiology and development**

Despite being a well-characterized family of proteins, PINs still keep secrets regarding their mode of action. Recently, the structures and mechanisms of PIN1, PIN3 and PIN8 were reported (Yang et al., 2022; Su et al., 2022, Ung et al., 2022) but how their biochemical activity is further integrated in a complex multicellular organism, largely controlled by direct environmental interactions remains poorly understood. The early identification of *pin* mutants led to the description of major developmental events controlled by this redundant family of genes and to a better understanding of the underlying role of the major phytohormone auxin (reviewed by Krecek et al, 2009; Sauer and Kleine-Vehn, 2019). Surprisingly, the *pin2* mutant phenotype has suffered a lack of detailed description at the root level and has certainly come across as a weak gravitropic mutant due to the heterogeneity of observations, as reflected in the literature and summarized in Figure 1.

4.2. The *pin2* mutant lacks the ability to establish asymmetric auxin response…

Here, we report that *pin2* root agravitropic phenotype is conditional and nutrient-sensitive, providing an explanation for previous observations. Our study pinpoints that the wide heterogeneity of growth conditions used in the many laboratories involved (nutrients but also light intensity, temperature, photoperiod etc…) strongly affects conclusions about the role of given protein/gene families. In the case of *pin2*, we demonstrate that early response to gravity is intact (Table 1). However, *pin2* roots coil like a lasso in restrictive conditions after this brief period of gravitropic primary root growth (Fig. 1B,2A). The intensive coiling of this mutant suggests a loss of the ability to establish asymmetric auxin flows and probably also to return to a symmetric repartition. Indeed, it is well known that *pin2* accumulates auxin at the root tip (Ottenschläger et al., 2003) due to the lack of transport back towards the epidermis, where it is normally expressed (Blilou et al., 2005). This overaccumulation of auxin triggers events that are unique amongst other agravitropic mutants such as *aux1*. Indeed, the *aux1* mutant root is agravitropic from germination onward. This is due to the lack of auxin transport from the lateral root cap towards the epidermis (Swarup et al., 2005), however no overaccumulation of auxin is present in the root tip suggesting that the rapidity of auxin redistribution rather than its actual long-term transport is responsible for the agravitropic phenotype. In the present study, we found that AUX1 protein accumulation profile was not altered in the *pin2* mutant background suggesting that this protein does not contribute to the conditionality of the phenotype. The high levels of auxin in the *pin2* mutant apex most likely prevent the action of other PIN transporters (such as PIN3) to promote asymmetric redistribution of auxin. In the first few days of *pin2* mutant growth, plants still manage to grow along the gravity vector, probably due to the time it takes to build up the auxin accumulation in the apex. Then the primary root starts to coil continuously.

4.3. …but nutrients can revert *pin2* phenotype by modulating auxin fluxes

The reversibility of *pin2* root phenotype suggests that environmental conditions can modify auxin fluxes within the plant. It has been reported previously that nutrients can alter the gravitropic response of plants. Indeed, studies on the effect of nitrate on primary root coiling have shown that *pin2* mutants are less sensitive to nitrate-induced coiling (Chai et al., 2020). Since these experiments were performed on horizontal plates, they encompass both gravitropic and thigmotropic responses, and their effect in the longer term. This study nevertheless suggests a link between gravitropism and nutrient availability.

This result is reminiscent of the ability of the nitrate transporter NRT1.1 to transport auxin competitively, providing an explanation for the lateral root growth phenotype of the *chl1/nrt1.1* mutant (Krouk et al., 2010). Here, we observed the ectopic expression of PIN1 in the epidermal cells in the *pin2* mutant background as reported previously (Vieten et al., 2005) and seen in the wild-type background upon application of aluminium or flavonols (Li et al., 2021; Santelia et al. al., 2008). Interestingly, the asymmetric distribution of PIN1 in this tissue remains at a ratio value close to 1-1.5 in restrictive conditions. However, in permissive conditions, this up-to-down ratio is higher (3 to 5) suggesting that the subsequent restoration of asymmetric auxin fluxes would be responsible for the phenotypic change. Although PIN1 accumulation in the apical membrane of the epidermal cells is always at least slightly asymmetric, we suggest the existence of a threshold value below which no phenotypic restoration occurs (in restrictive conditions) and above which agravitropic restoration happens (in permissive conditions). This ratio threshold is situated between 2 and 3. Subsequent studies should further define how changes in nutrient composition can modulate PIN1 expression in a direct or indirect manner. In this study, we limited the analysis to major nutrients such as nitrate, phosphate and iron because they are major elements regulating root growth and development (Xuan et al., 2017; Liu 2021; Liang 2022). We defined permissive conditions based on a widely used medium, which is the half-diluted Murashige and Skoog medium but virtually infinite modulations of medium composition could be tested.

Since both nitrate and phosphate starvations, as well as one tenth diluted MS, define permissive conditions, we did not investigate the downstream pathways of particular elements. Numerous regulators have been identified as regulators of starvation response to nitrate (Kiba et al., 2018) and phosphate (Rouached et al., 2010) and even common elements are known (Ristova and Kopriva, 2022). However, no molecular link has been shown between these pathways and PIN1 expression in the root epidermis. Further studies should provide more information towards this goal. These would help understand how gravitropism is modulated by nutrients in the *pin2* mutant.

Indeed, our observations show that the phenotypic change only occurs in the mutant background, pinpointing the need for the ectopic expression of PIN1 in this context for a

plant root facing an heterogeneous soil. This observation fits with known characteristics of the PIN gene family. Indeed, PIN genes are known to show strong redundancy and changes in expression based on auxin induction (Vieten et al., 2005). Moreover, our results show that in the wild-type plants, nutrients have a measurable impact on auxin response (using R2D2 reporter) that could be the basis for a fine modulation of root gravitropism in soil where numerous factors can be altered simultaneously (nutrients, physical contact, water…). These observations suggest that the relation between root tropisms and nutrients, but also water and physical stress should be further investigated.

5. Conclusion

In this study, we provide evidence for a conditional phenotype of the *pin2* mutant and a link between nutrients and auxin transport/response (Fig. 6). Modulation of auxin transport through differential PIN expression in various environmental conditions alters root gravitropic response and suggests that this tropic response has been under strong selective pressure to promote a fine-tuning of soil exploration. Interestingly, the rice mutant in *PIN2*, *Ospin2,* as well as that of *AUX1*, *Osaux1* are not totally agravitropic, but instead display a root growth angle defect (Inahashia et al., 2018, Giri et al., 2018). This suggests that defining root growth angle by modulating the gravitropic response can be controlled both genetically and environmentally. These observations open the way to improving root exploration in crops for a better use of resources.

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Credit authorship contribution statement

BP, FD, and MaT contributed to conception and design of the study. MaT, FD, CO performed the acquisition, analysis and interpretation of data. AC and PN were in charge of creating and maintaining the phenotyping platform for dynamic root growth analysis. AS and FD analyzed the sequencing of the *lasso* mutant. MeT and EH contributed to the initial forward genetics screen to discover the new *lasso* mutant. BP and MaT wrote the article.

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Declaration of Competing Interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Figure captions

Figure 1. Literature description of the agravitropic root phenotype of *pin2* is heterogeneous

(A) Representative drawing of *pin2* mutant’s phenotype drawn from previous reports **1.** Allele *Atpin2::En701* grown on Murashige and Skoog (MS) medium **(**Müller *et al*., 1998) **2.** Allele *eir1-3* grown on unsupplemented plant nutrient agar (PNA) without sucrose (Luschnig *et al*., 1998) **3.** Allele not specified grown on MS medium (Blakeslee, *et al*., 2007) **4.** Allele *eir1-4* grown on MS/2 medium or PNS **(**Retzer *et al*., 2017) **5.** Allele N591142 (*pin2-T*) grown on MS medium (Liu *et al*., 2018) **6-**. Allele *eir1-4* grown on PNS (Retzer *et al*., 2019) **7.** Allele *eir1-4* grown on Hoagland medium (Ashraf *et al*., 2020) **8.** Allele not specified grown on MS/2 medium (Wu *et al*., 2021) **(B)** Representative drawing of *pin2-2* mutant phenotype used in the present study **1.** Plants grown in repressive conditions (MS/10). **2.** Plants grown in permissive conditions (MS/2) **(C)** Representative picture of *pin2-2* in repressive (MS/10) or repressive (MS/2) conditions, stars indicate plants selected for drawing.

Figure 2. The agravitropic root growth phenotype of *pin2-2* is conditional and nutrient-sensitive

(A) Agravitropic root phenotype of the *pin2-2* mutant allele **(**11-day-old seedlings grown on MS/2 medium). Inlay: zoom in on the “*lasso*” root coil. Black scale bar = 1 cm, white scale bars = 1mm. **(B)** Representative confocal images of the root apex stained with propidium iodide (cyan) of DR5-GFP expressing plants (fuchsia). White arrows indicate auxin accumulation in the lateral root cap. Dotted white lines indicate the position where GFP intensity was measured. Seedlings were grown vertically for 6 days and then transferred horizontally for 4 h on an MS/2 medium. g: gravity vector, scale bar = 50 microns. **(C)** DR5-GFP intensity was quantified with the Plot ImageJ profile and displayed along the position of the root, $16<n<20$ **(D)** Gravitropic response of wild-type (Col-0) and *pin2-2* mutants. 6-day-old seedlings were grown on vertical plates then rotated 90° and imaged after 48 hours. Circular diagrams display the primary root apex orientation as colored bars representing the percentage of plants. n=30, scale bar = 1cm.

Table 1. Gravitropic response and growth rate of *pin1*, *pin2* and *pin3* in permissive and restrictive conditions

Summarized table of *pin1*, *pin2-2* and *pin3* early and late gravitropic response and root growth. Early gravitropism: 6-day-old seedlings were subjected to a 90° gravitropic stimulus and imaged at 3, 6 and 9 hours. Representative image of data presented in Figure S4. Gravitropism: 6-day-old seedlings were subjected to a 90° gravitropic stimulus and imaged after 48h. Representative image of data presented in Figure S3. Growth: primary root growth of 6-day-old seedlings was monitored for 24 hours (n=20) and expressed as the average +/- standard error in mm per day. Representative graph of data presented in Figure S5. Green ticks indicate gravity response while a red cross indicates an agravitropic response. Scale bar = 1cm.

Figure 3. Auxin accumulation is modulated by nutrient availability

(A) Representative confocal image of the ratiometric sensor R2D2 showing DII-n3xVenus (green) and mDII-ndtTomato (purple) signals. White areas represent the first 9 epidermal cells chosen for quantification. 6-day-old seedlings were imaged on a microscopy slide with a block of MS/2 medium 1h30 after gravistimulation. g = gravity vector, scale bar = 50 microns **(B,C)** Quantification of the mDII/DII ratio in the first 9 epidermal cells in the up or down part of the root tip 1h30 after gravistimulus in wild-type (Col-0) and *pin2-2* in various media (MS/2, MS/10, MS/2-P, MS/2-N, MS/2-Fe). The p-values are based on Student's t-tests for a pairwise comparison relative to reference medium (MS/2) $p<0.0001$ (***), $p<0.001$ (**), and $p<0.05$ (*), n=15, error bars represent standard deviation.

Figure 4. Localization of PIN3 and AUX1 proteins is not altered in the *pin2* mutant background in various growth medium

(A) Representative confocal image of PIN3:GFP. 6-day-old seedlings were transferred on a horizontal microscopy slide with a block of MS/2 medium and imaged 30 min after gravistimulation. White lines represent the position where GFP intensity was measured. g = gravity vector, scale bar = 50 microns **(B)** Graph representing PIN3:GFP average lower outer/upper outer signal ratio after 30min of gravitropism of Col-0 and *pin2-2* in response to different media (MS/2, MS/10, MS/2-P, MS/2-N, MS/2-Fe). The p-values are based on Student's t-tests and the comparison is made for each genotype in MS/2 compared to the different media tested $P<0.0001$ (***), $P<0.001$ (**), and $P<0.05$ (*), n=15, error bars represent standard deviation **(C)** Representative confocal image of AUX1:YFP. 6-day-old seedlings were transferred on a horizontal microscopy slide with a block of MS/2 medium and imaged 90 min after gravistimulation. g = gravity vector, scale bar = 50 micron **(D)** Graph representing the intensity of AUX1:YFP on the lower and upper face of the root in response to 1h30 of gravitropism of Col-0 and *pin2-2* in response to different media (MS/2, MS/10, MS/2-P, MS/2-N, MS/2-Fe). The p-values are based on Student's t-tests and the comparison is made for each genotype in MS/2 compared to the different media tested $p<0.0001$ (***), $p<0.001$ (**), and $p<0.05$ (*), n=15, error bars represent standard deviation.

Figure 5. PIN1 is present in the epidermis in the *pin2* mutant background and is modulated by nutrient availability

(A) Representative confocal image of PIN1:GFP in Col-0 and *pin2-2*. 6-day-old seedlings were transferred on a horizontal microscopy slide with a block of MS/2 medium and imaged 1h after gravistimulation. White squares represent the position where GFP intensity was measured. g = gravity vector, scale bar = 50 micron. **(B)** Graph representing the mean of intensity of PIN1:GFP up + down in response to 1h of gravitropism in Col-0 and *pin2-2* in response to different media (MS/2, MS/10, MS/2-P, MS/2-N, MS/2-Fe). The p-values are based on Student's t-tests and the comparison is made for each genotype in MS/2 compared to the different media tested P <0.001 (b), and P <0.05 (a), n=15, error bars represent standard deviation. **(C)** Graph representing the epidermal cell count with PIN1:GFP signal (up+down) in response to 1h of gravitropism in Col-0 and *pin2-2* in response to different media. The p-values are based on Student's t-tests and the comparison is made for each genotype in MS/2 compared to the different media tested P <0.0001 (c), P <0.001 (b), and P <0.05 (a), n=15, error bars represent standard deviation. **(D)** Representative confocal image of PIN1:GFP in Col-0 and *pin2-2*. 6-day-old seedlings were transferred on a horizontal microscopy slide with a block of MS/2 medium and imaged 1h after gravistimulation. White squares represent a zoom of up and down epidermis. g = gravity vector, scale bar = 20 micron and scale bar in the zoom = 5 micron. **(E)** Graph representing the ratio down/up of the intensity of PIN1:GFP at the plasma membrane in response to 1h of gravitropism in Col-0 and *pin2-2* in response to different media. The p-values are based on Student's t-tests and the comparison is made for each genotype in MS/2 compared to the different media tested P <0.0001 (***), P <0.001 (**), and P <0.05 (*), 10<n<16

Figure 6. A model for *pin2* conditional agravitropic root phenotype

Proposed model explaining how epidermal ectopic expression of *PIN1* in the *pin2* mutant in permissive conditions re-establishes asymmetrical auxin fluxes. In the wild-type (Col-0) background, PIN2 protein localization in the epidermis drives auxin fluxes and promotes asymmetrical distribution after a gravistimulus in all conditions tested in this study (restrictive and permissive conditions). In the *pin2* mutants, PIN1 ectopic expression in the epidermis is observed in all conditions tested. In permissive conditions, PIN1 up-to-down ratio reaches a threshold value and restores an asymmetric auxin flux that is responsible for the gravitropic response. This mechanism is absent in restrictive conditions where *pin2* root still grows agravitropically and forms a lasso shape.

Figure S1. A new allele of *pin2* mutant discovered in a forward genetics screen

(A) Representative picture of the new mutant *lasso* on a forward genetics screening on MS/2 and MS/2 supplemented with 75 mM of NaCl. **(B)** Representative picture of Col-0 and *lasso* on MS/2 and MS/2 supplemented with 75 mM of NaCl or 150 mM of Sorbitol. 6-day-old seedlings were transferred on medium and subjected to a 90° gravitropic stimulus and imaged after 48h in the phenotyping robot HIRROS. **(C)** Representation of the PIN2 gene with alignment of reads for Col-0 and *lasso*. The inlay represents a zoom of the *pin2-2* mutation area noted with the red star on the gene. **(D)** Represensative picture of 7-day-old seedlings of Col-0 and *pin2-2* and the introgression of *proPIN2:PIN2-GFP* construct into

pin2-2 genetic background. scale bar = 1cm **(E)** Osmotic stress mimicked with the presence of a range of PEG for Col-0 and *pin2-2*. 6-day-old seedlings were transferred on medium and subjected to a 90° gravitropic stimulus and imaged after 48h. Circular diagrams display the primary root apex orientation as colored bars representing the percentage of plants. n=15, scale bar = 1cm

Figure S2. Several *pin2* allelic mutants have a conditional agravitropic phenotype

(A) Graph representing length of Col-0 and *pin2-2* primary root in MS/2. The p-values are based on Student's t-tests and the comparison is made between Col-0 and *pin2-2* P<0.0001 (***), P <0.001 (**), and P <0.05 (*), n=15, error bars represent standard deviation. **(B)** Representative picture of 10-day-old seedlings of Col-0, *pin2-2*, *eir1-1*, *eir1-4* and *SALK_122916* mutants on MS/2. Inlay: zoom in on the root coil. **(C)** Gravitropic response *eir1-1*, *eir1-4* and *SALK_122916* mutants. 6-day-old seedlings were grown on vertical plates then rotated 90° and imaged after 48 hours. Circular diagrams display the primary root apex orientation as colored bars representing the percentage of plants. n=15, scale bar = 1cm.

Figure S3. Gravitropism response of *pin1*, *pin2* and *pin3* in permissive and restrictive conditions

Representative image of 6-day-old seedlings of Col-0, *pin1*, *pin2-2* and *pin3* mutants subjected to a 90° gravitropic stimulus and imaged after 48h in response to different nutrient deficient media (MS/2, MS/2-Fe, MS/2-P, MS/2-N, MS/10). Circular diagrams display the primary root apex orientation as colored bars representing the percentage of plants.15<n<30, scale bar = 1cm.

Figure S4. Early gravitropism of *pin1*, *pin2* and *pin3* in permissive and restrictive conditions

Representative image of 6 day-old seedlings of Col-0, *pin1*, *pin2-2* and *pin3* mutants subjected to a 90° gravitropic stimulus and imaged at 3, 6 and 9 hours in response to different nutrient deficient media (MS/2, MS/2-Fe, MS/2-P, MS/2-N, MS/10).

Figure S5. Growth rate of *pin1*, *pin2* and *pin3* in permissive and restrictive conditions

Graph representing the primary root growth of 6 day-old seedlings of Col-0 , *pin1*, *pin2-2* and *pin3* mutants monitored for 24 hours (15<n<30) *2* in response to different media (MS/2, MS/10, MS/2-P, MS/2-N, MS/2-Fe) . The p-values are based on Student's t-tests and the comparison is made for each genotype in MS/2 compared to the different media tested P <0.0001 (***), P <0.001 (**), and P <0.05 (*), error bars represent standard deviation

Figure S6. Auxin accumulation is modulated by nutrient availability

Seedlings of Col-0 and *pin2-2* were grown for 6 days vertically and then transferred horizontally for 4 h on the different medium. DR5-GFP intensity was quantified with the Plot ImageJ profile and displayed along the position of the root, 16<n<20

Figure S7. Auxin accumulation is modulated by nutrient availability

(A) Representative confocal image of the ratiometric sensor R2D2 showing DII-n3xVenus (green) and mDII-ndtTomato (purple) signals in Col-0 and *pin2-2* in response to various media (MS/2, MS/10, MS/2-P, MS/2-N, MS/2-Fe). 6-day-old seedlings were imaged on a microscopy slide with a block of MS/2 medium 1h30 after gravistimulation. g = gravity vector, scale bar = 50 microns **(B)** Quantification of the lower/upper ratio of mDII/DII ratio in the first 9 epidermal cells in the up or down part of the root tip 1h30 after gravistimulus in wild-type (Col-0) and *pin2-2* in various media (MS/2, MS/10, MS/2-P, MS/2-N, MS/2-Fe). The p-values are based on Student's t-tests for a pairwise comparison relative to reference medium (MS/2) $p<0.0001$ (***), $p<0.001$ (**), and $p<0.05$ (*), n=15, error bars represent standard deviation.

Figure S8. Localization of proteins is not altered in the *pin2* mutant background in various growth medium

Representative confocal image of PIN3:GFP in response to various media (MS/2, MS/10, MS/2-P, MS/2-N, MS/2-Fe). 6-day-old seedlings were transferred on a horizontal microscopy slide with a block of medium and imaged 30 min after gravistimulation. g = gravity vector, scale bar = 50 micron

Figure S9. Localization of AUX1 proteins is not altered in the *pin2* mutant background in various growth medium

(A) Representative confocal image of AUX1:YFP. 6-day-old seedlings were transferred on a horizontal microscopy slide with a block of various medium and imaged 90 min after gravistimulation. g = gravity vector, scale bar = 50 micron. **(B)** Graph representing the intensity of AUX1:YFP on the lower and upper face of the root in response to 1h30 of gravitropism of Col-0 and *pin2-2* in response to different media (MS/2, MS/10, MS/2-P, MS/2-N, MS/2-Fe). The p-values are based on Student's t-tests and the comparison is made for each genotype in MS/2 compared to the different media tested $p<0.0001$ (***), $p<0.001$ (**), and $p<0.05$ (*), n=15, error bars represent standard deviation.

Figure S10. PIN1 is present in the epidermis in the *pin2* mutant background and is modulated by nutrient availability

(A) Representative confocal image of PIN1:GFP in Col-0 and *pin2-2*. 6-day-old seedlings were transferred on a horizontal microscopy slide with a block of MS/2 medium and imaged 1h after gravistimulation. White squares represent the position where GFP intensity was measured. g = gravity vector, scale bar = 50 micron. **(B)** Graph representing the mean of intensity of PIN1:GFP in the stele in response to 1h of gravitropism in Col-0 and *pin2-2* in response to different media (MS/2, MS/10, MS/2-P, MS/2-N, MS/2-Fe). The p-values are based on Student's t-tests and the comparison is made

for each genotype in MS/2 compared to the different media tested P <0.001 (b), and P <0.05 (a), n=15, error bars represent standard deviation. **(C)** Representative confocal image of PIN1:GFP in Col-0 and *pin2-2* in response to different media (MS/2, MS/10, MS/2-P, MS/2-N, MS/2-Fe). 6-day-old seedlings were transferred on a horizontal microscopy slide with a block of medium and imaged 1h after gravistimulation. g = gravity vector, scale bar = 50 micron

Materials and Methods Supplemental S1. Media composition

Materials and Methods Supplemental S2. Circular diagram used for gravitropic phenotyping of the root apex upon gravistimulation.

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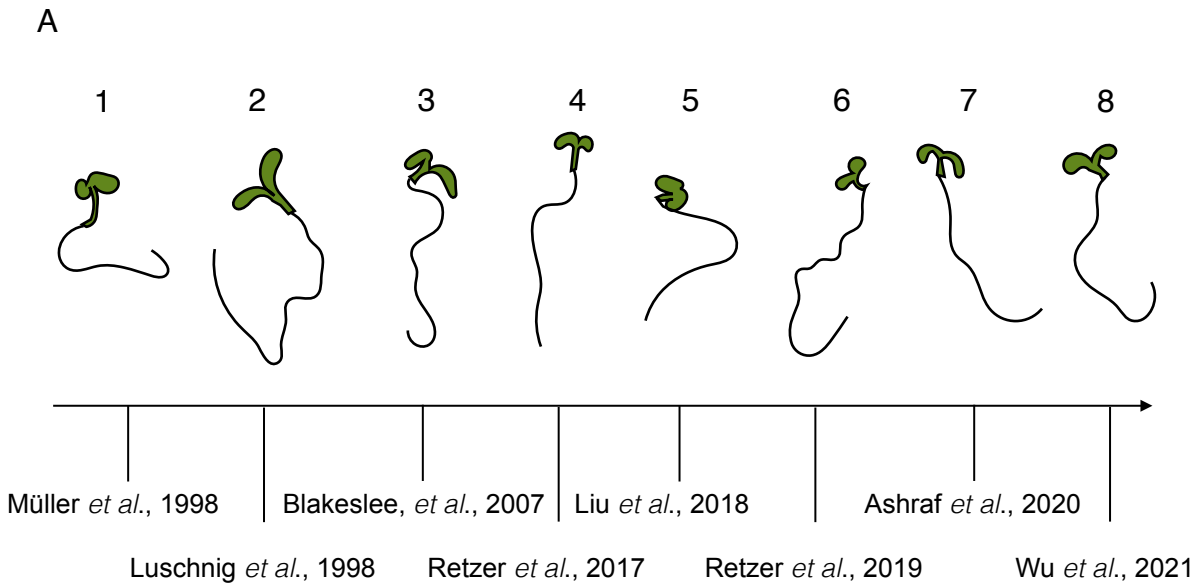
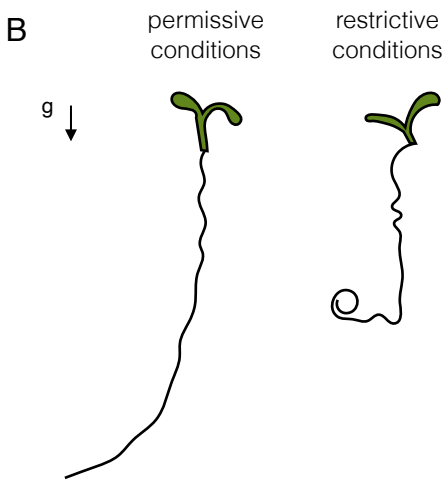
Yang Z, Xia J, Hong J, Zhang C, Wei H, Ying W, Sun C, Sun L, Mao Y, Gao Y, et al (2022) Structural insights into auxin recognition and efflux by Arabidopsis PIN1. Nature. doi: 10.1038/s41586-022-05143-9

Figure 1.





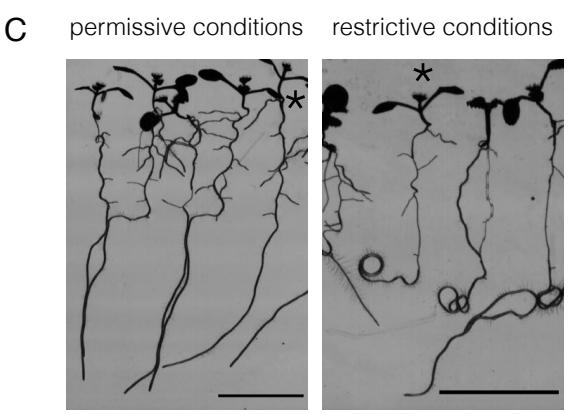


Figure 2.

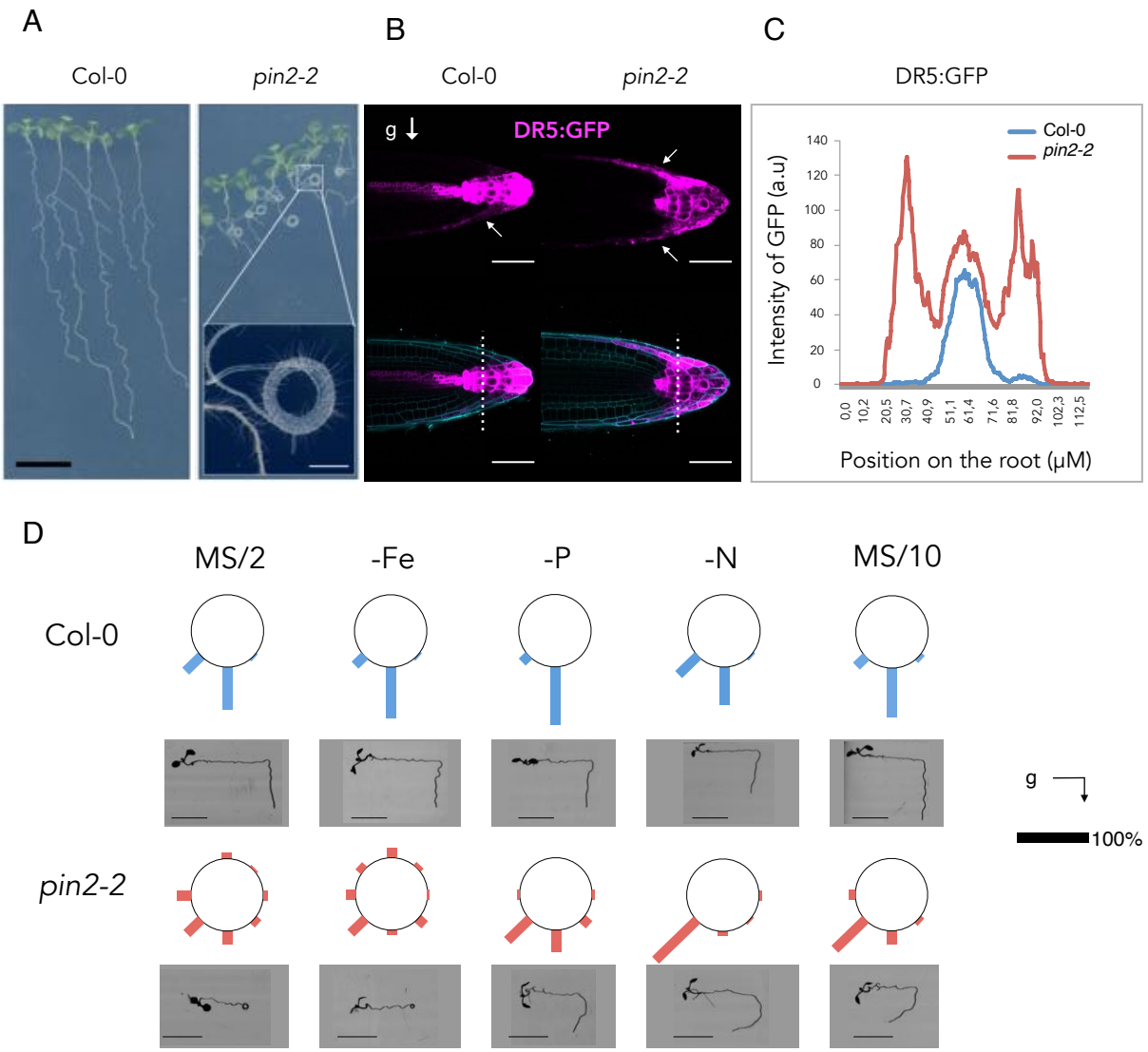

A
Col-0
pin2-2
B
Col-0
pin2-2
g
DR5:GFP
C
DR5:GFP
Col-0
pin2-2
Intensity of GFP (a.u)
140
120
100
80
60
40
20
0
0,0
10,2
20,5
30,7
40,9
51,1
61,4
71,6
81,8
92,0
102,3
112,5
Position on the root (µM)
D
MS/2
-Fe
-P
-N
MS/10
Col-0
pin2-2
g
100%

Table 1.

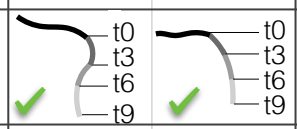
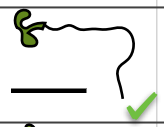
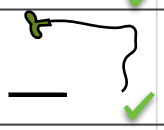
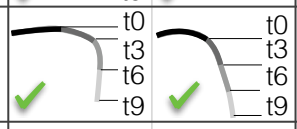
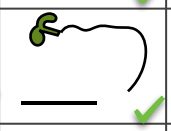
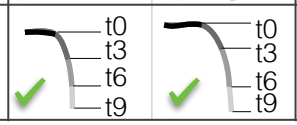
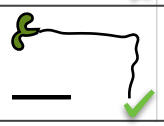
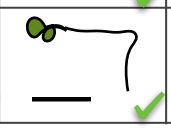
	Early gravitropism (9h)		Gravitropism (48h)		Growth (mm/24h)				
	restrictive conditions	permissive conditions	restrictive conditions	permissive conditions	restrictive conditions		permissive conditions		
	MS/2 , -Fe	-P , -N , MS/10	MS/2 , -Fe	-P , -N , MS/10	MS/2	- Fe	-P	-N	MS/10
Col-0					7,6 +/- 0,03	4,5 +/- 0,05	1,5 +/- 0,02	2,4 +/- 0,03	8,2 +/- 0,03
pin1					6,5 +/- 0,02	4,3 +/- 0,03	0,4 +/- 0,02	1,8 +/- 0,02	6,5 +/- 0,02
pin2					4,5 +/- 0,02	2,9 +/- 0,04	0,6 +/- 0,03	1,2 +/- 0,02	4,2 +/- 0,02
pin3					5,4 +/- 0,02	3,3 +/- 0,04	0,8 +/- 0,02	0,8 +/- 0,02	5,8 +/- 0,03

Figure 3.

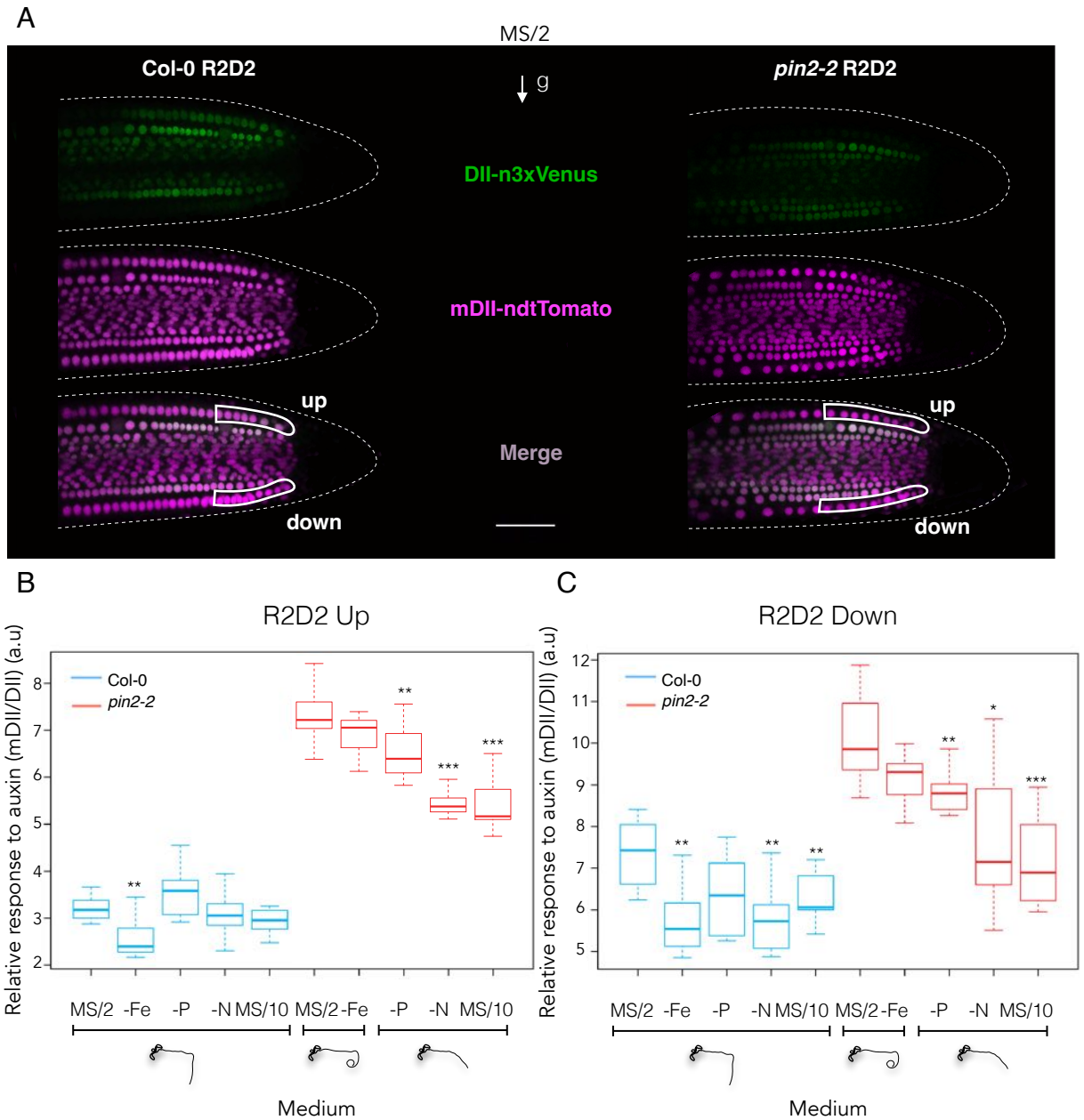

A
MS/2
Col-0 R2D2
g
pin2-2 R2D2
DII-n3xVenus
mDII-ndtTomato
Merge
up
down
up
down
B
R2D2 Up
Relative response to auxin (mDII/DII) (a.u)
Col-0
pin2-2
MS/2 -Fe -P -N MS/10 MS/2 -Fe -P -N MS/10
Medium
C
R2D2 Down
Relative response to auxin (mDII/DII) (a.u)
Col-0
pin2-2
MS/2 -Fe -P -N MS/10 MS/2 -Fe -P -N MS/10
Medium

Figure 4.

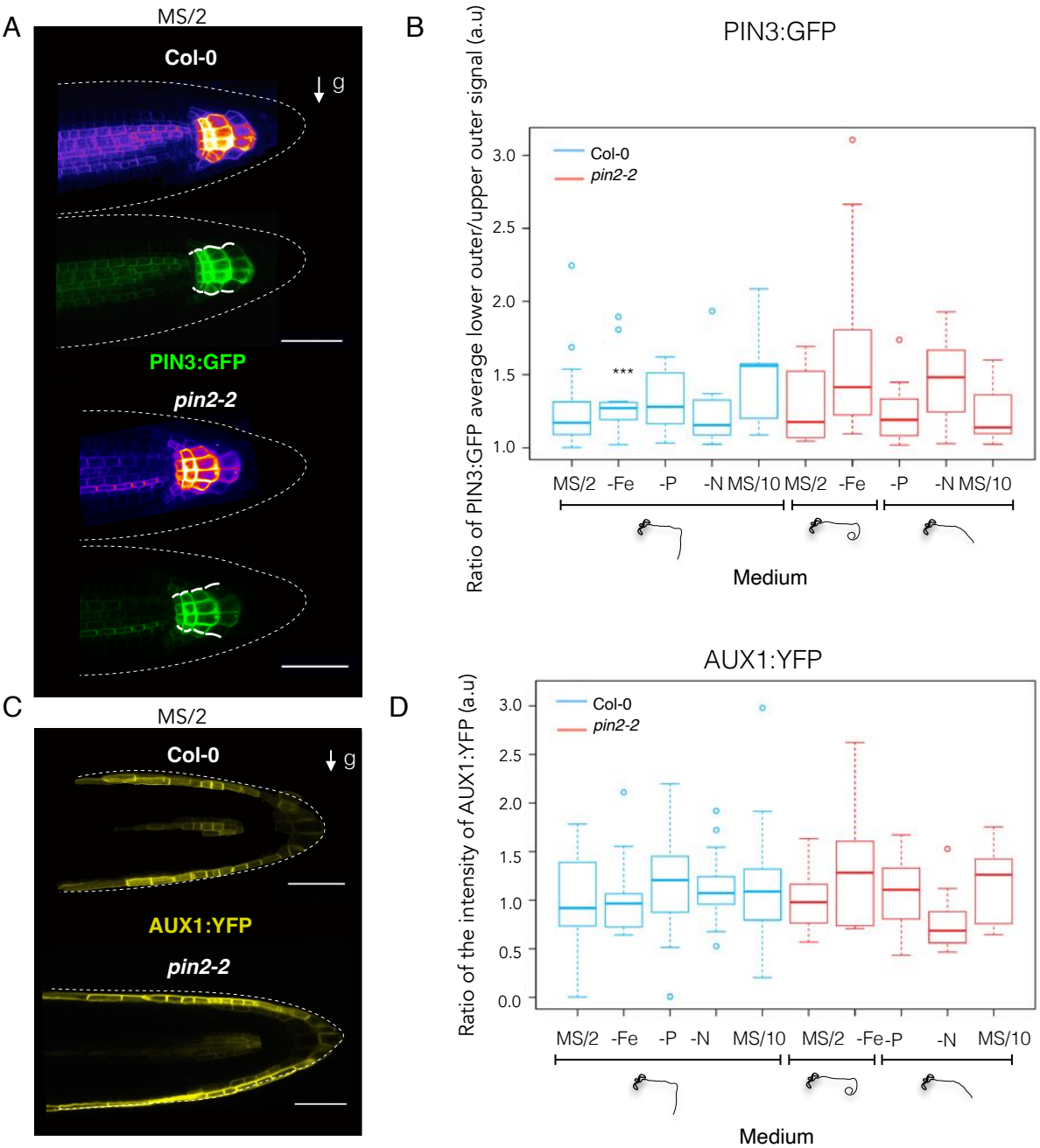

A
MS/2
Col-0
g
PIN3:GFP
pin2-2
B
PIN3:GFP
Ratio of PIN3:GFP average lower outer/upper outer signal (a.u)
Col-0
pin2-2
***
MS/2 -Fe -P -N MS/10 MS/2 -Fe -P -N MS/10
Medium
C
MS/2
Col-0
g
AUX1:YFP
pin2-2
D
AUX1:YFP
Ratio of the intensity of AUX1:YFP (a.u)
Col-0
pin2-2
MS/2 -Fe -P -N MS/10 MS/2 -Fe-P -N MS/10
Medium

Figure 5.

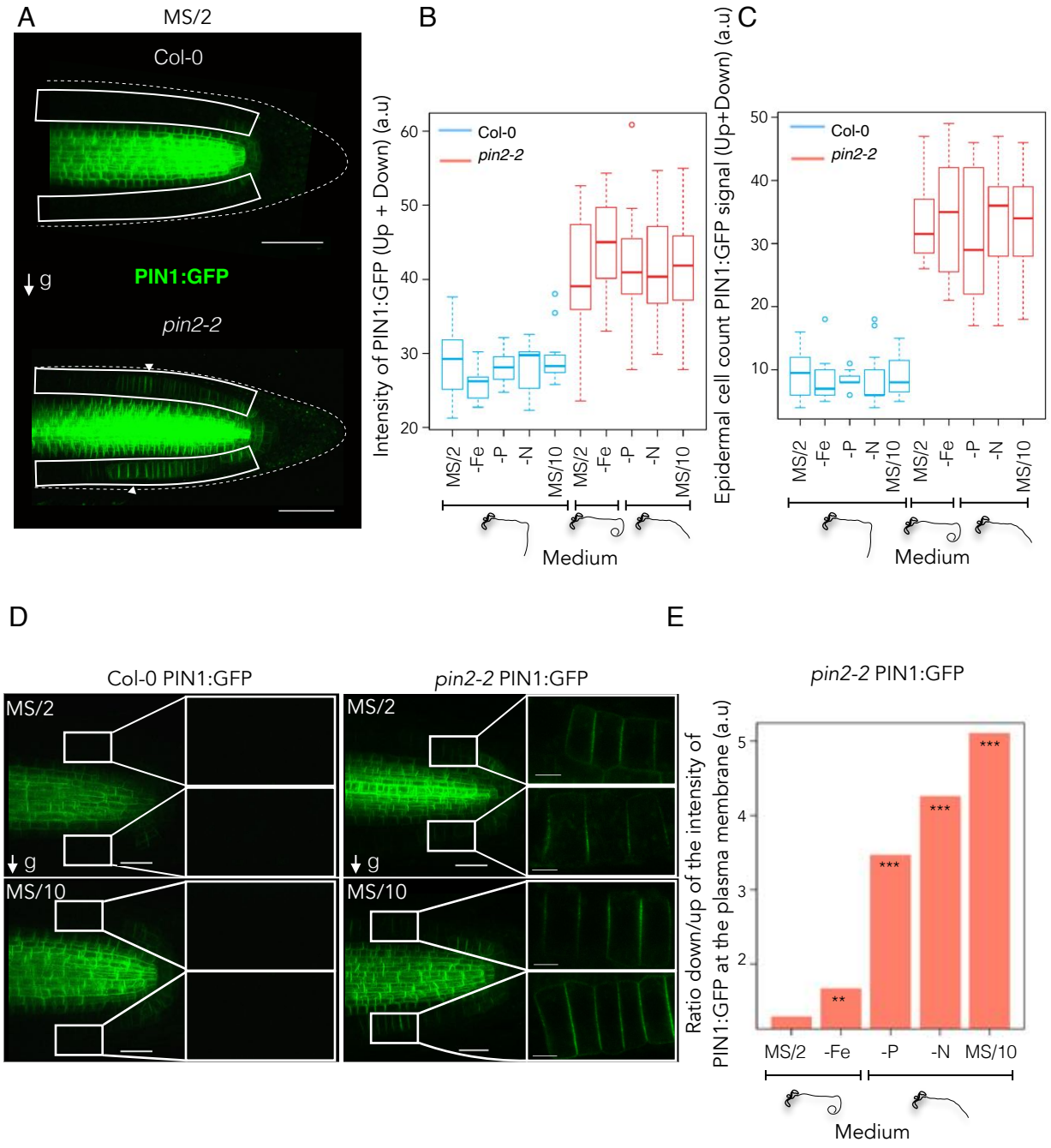

A
MS/2
Col-0
g
PIN1:GFP
pin2-2
B
Intensity of PIN1:GFP (Up + Down) (a.u)
Col-0
pin2-2
MS/2
-Fe
-P
-N
MS/10
Medium
C
Epidermal cell count PIN1:GFP signal (Up+Down) (a.u)
D
Col-0 PIN1:GFP
pin2-2 PIN1:GFP
E
pin2-2 PIN1:GFP
Ratio down/up of the intensity of PIN1:GFP at the plasma membrane (a.u)

Figure 6.

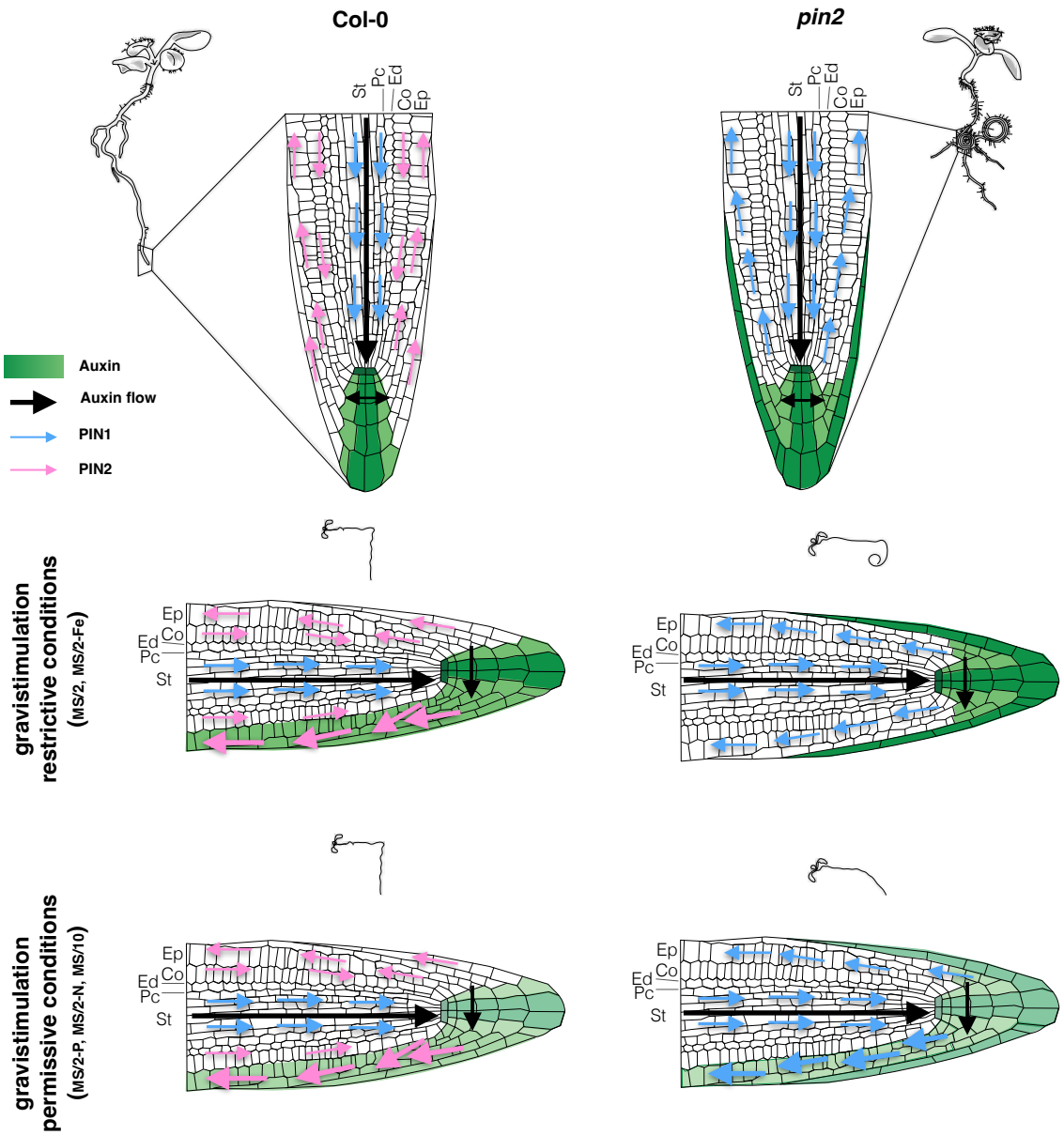

Col-0
pin2
St
Pc
Ed
Co
Ep
Auxin
Auxin flow
PIN1
PIN2
gravistimulation
restrictive conditions
(MS/2, MS/2-Fe)
gravistimulation
permissive conditions
(MS/2-P, MS/2-N, MS/10)

Figure S1.

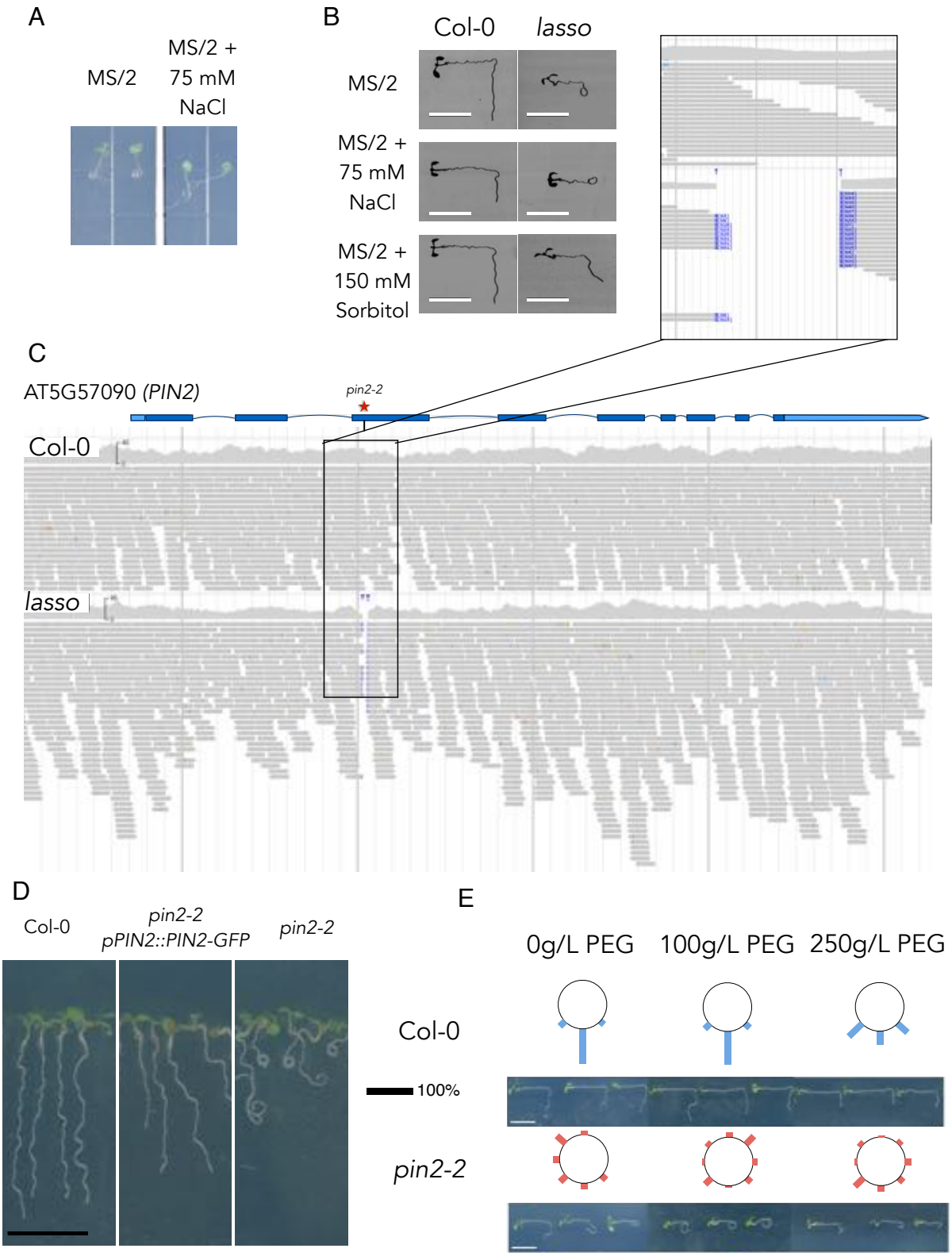
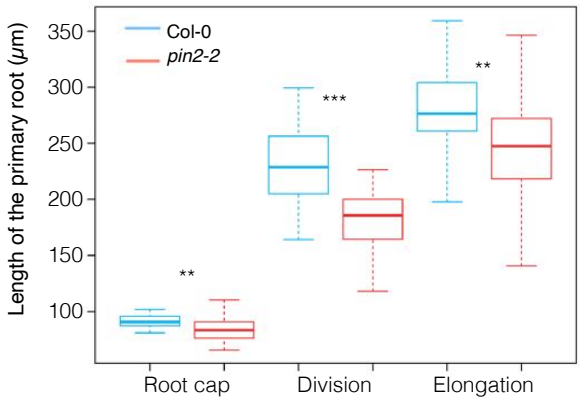
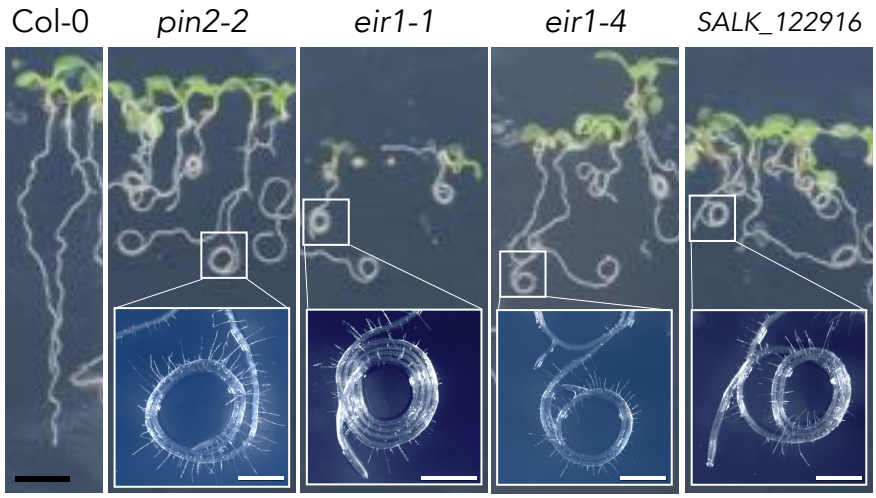


Figure S2.

A



B



C

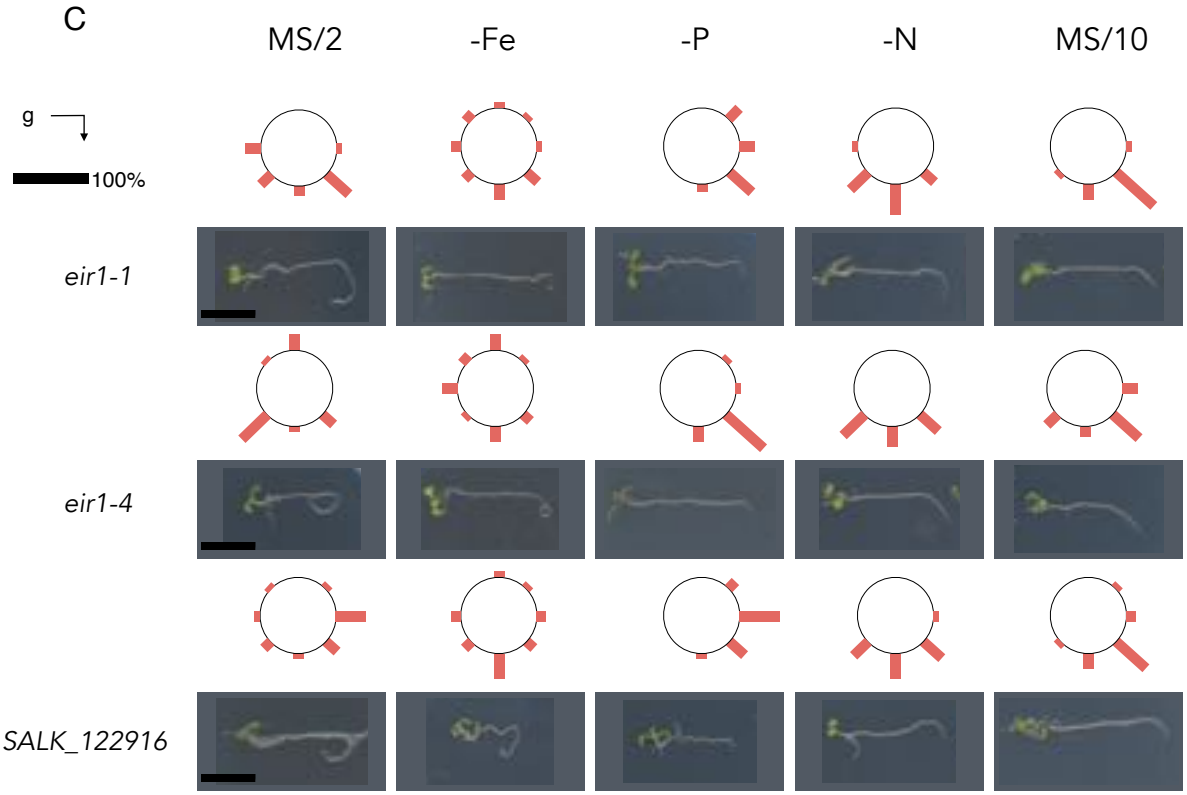


Figure S3.

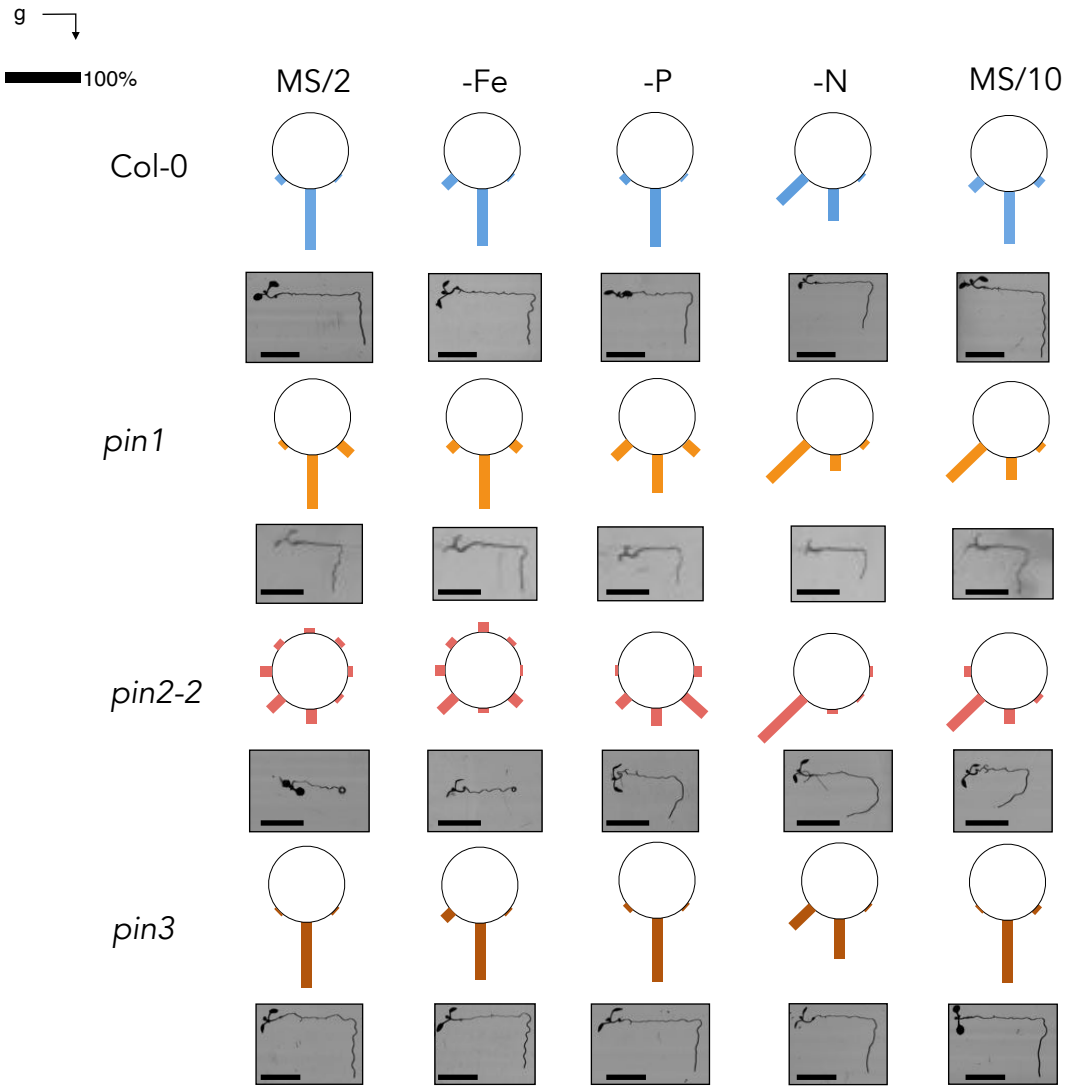


Figure S4.

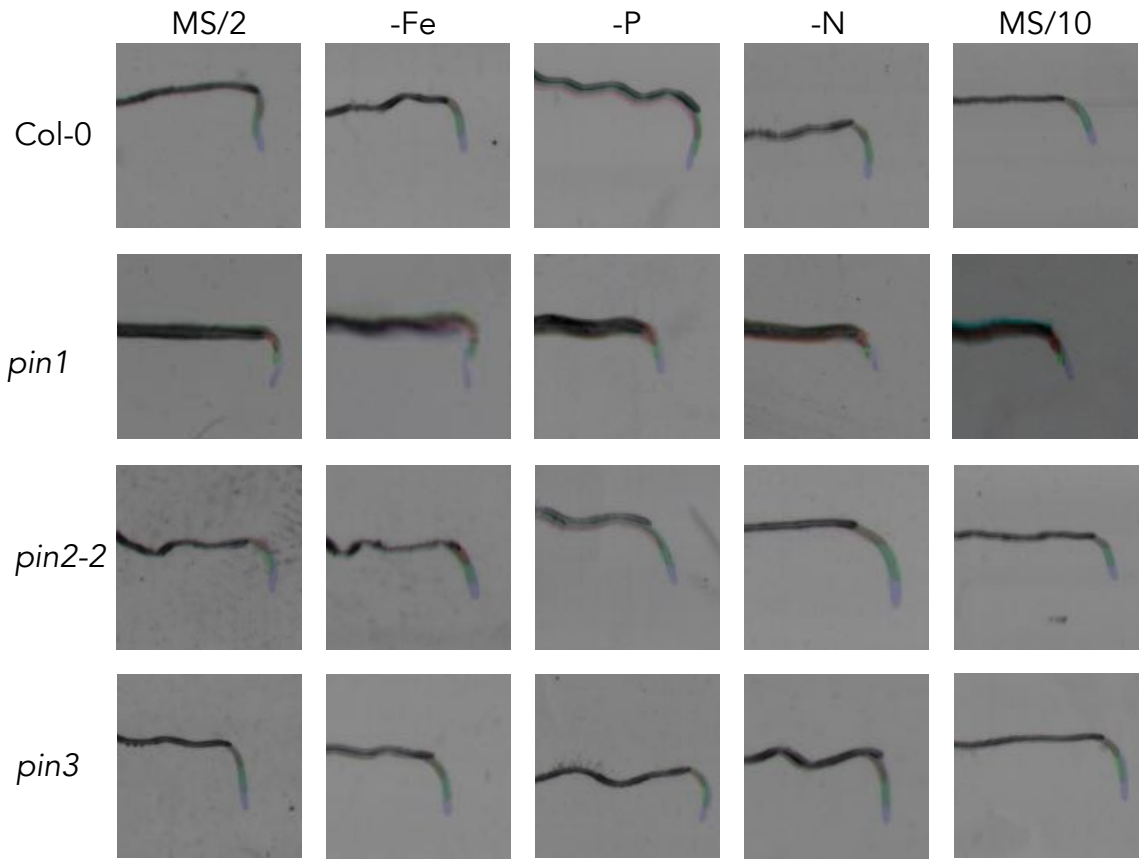


Figure S5.

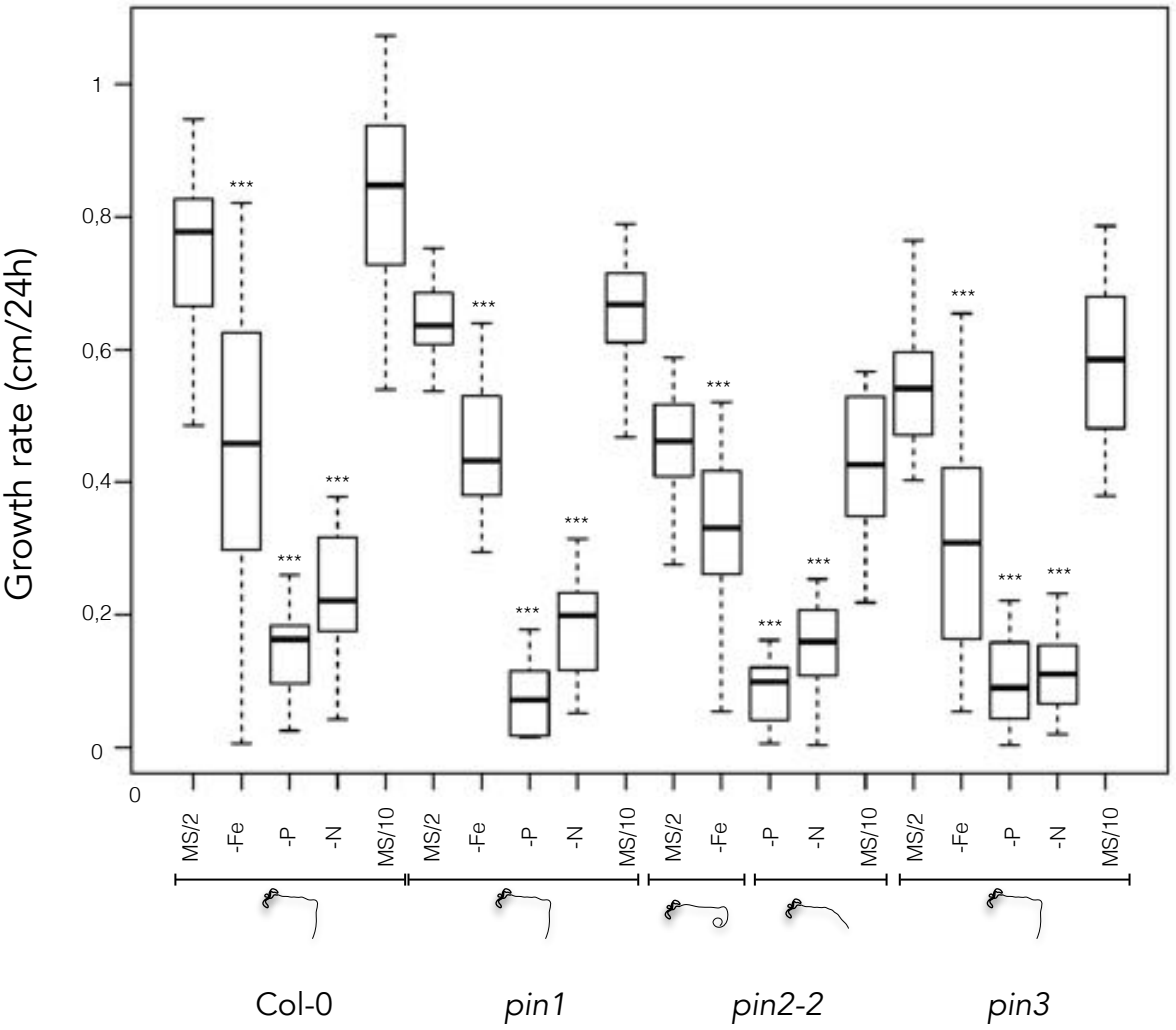

Growth rate (cm/24h)
0
0,2
0,4
0,6
0,8
1
***
MS/2
-Fe
-P
-N
MS/10
Col-0
pin1
pin2-2
pin3

Figure S6.

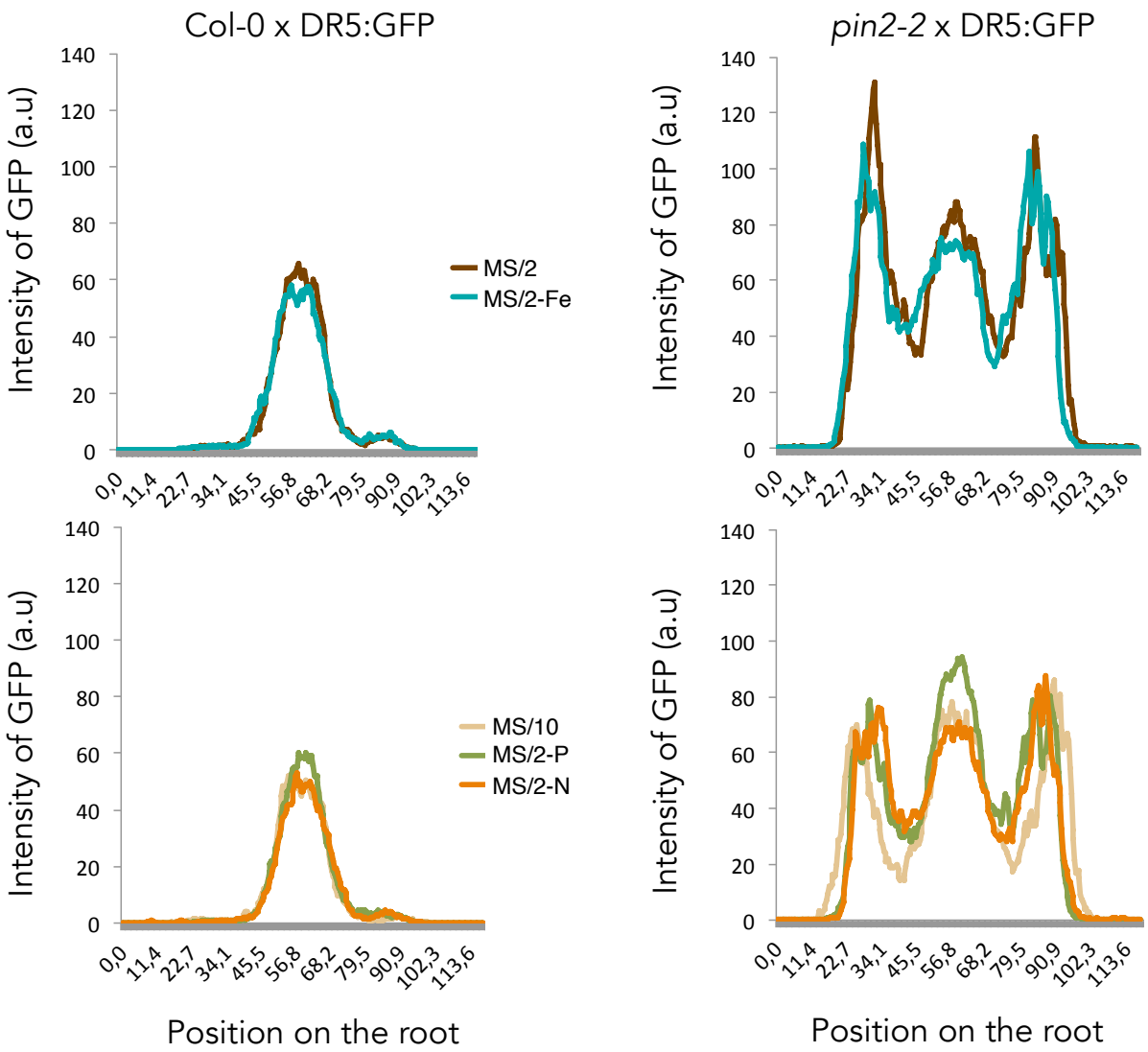
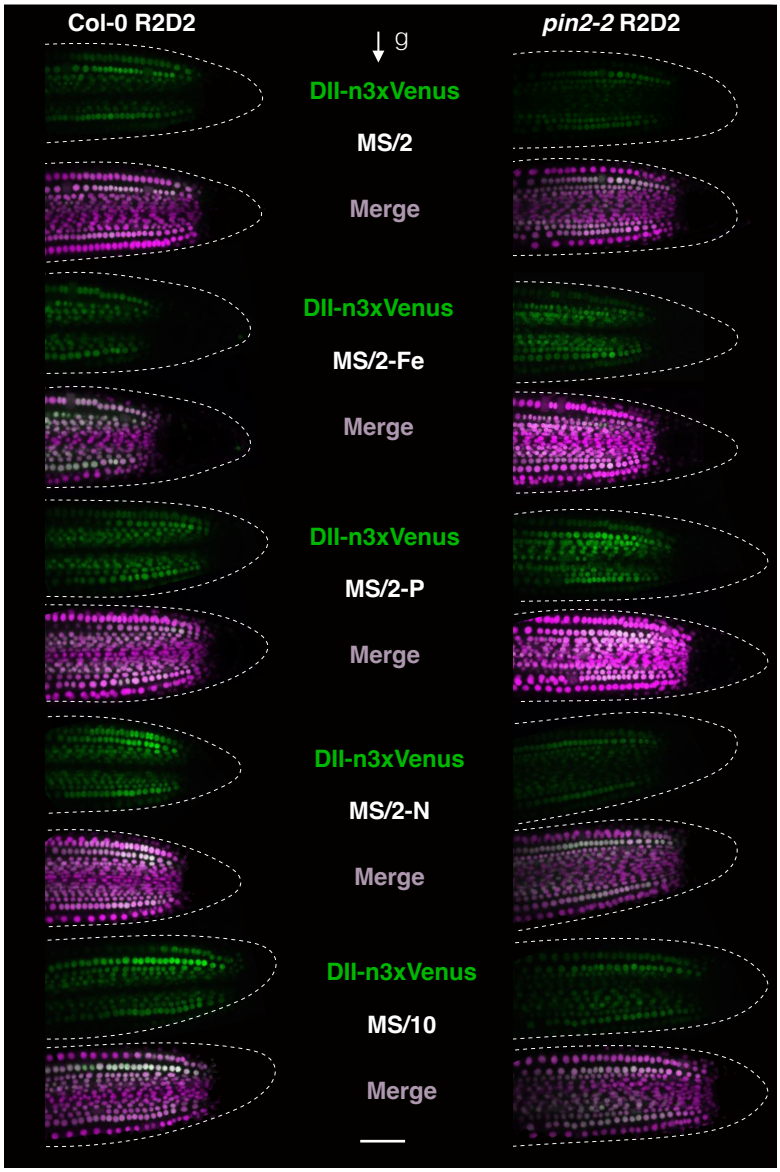

Col-0 x DR5:GFP
pin2-2 x DR5:GFP
Intensity of GFP (a.u)
140
120
100
80
60
40
20
0
MS/2
MS/2-Fe
MS/10
MS/2-P
MS/2-N
0,0
11,4
22,7
34,1
45,5
56,8
68,2
79,5
90,9
102,3
113,6
Position on the root

Figure S7.

A


Col-0 R2D2
g
pin2-2 R2D2
DII-n3xVenus
MS/2
Merge
DII-n3xVenus
MS/2-Fe
Merge
DII-n3xVenus
MS/2-P
Merge
DII-n3xVenus
MS/2-N
Merge
DII-n3xVenus
MS/10
Merge

B

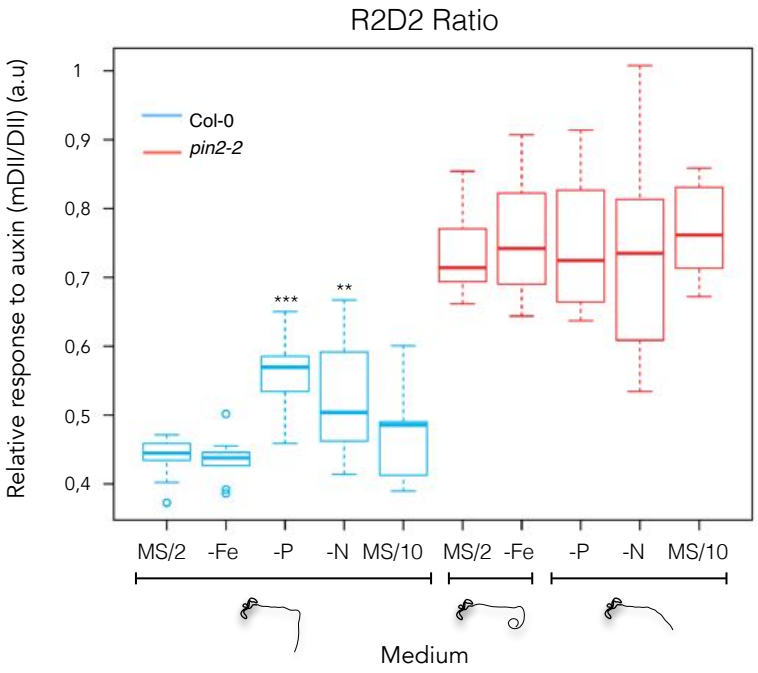

R2D2 Ratio
Relative response to auxin (mDII/DII) (a.u)
1
0,9
0,8
0,7
0,6
0,5
0,4
Col-0
pin2-2
***
**
MS/2
-Fe
-P
-N
MS/10
MS/2
-Fe
-P
-N
MS/10
Medium

Figure S8.

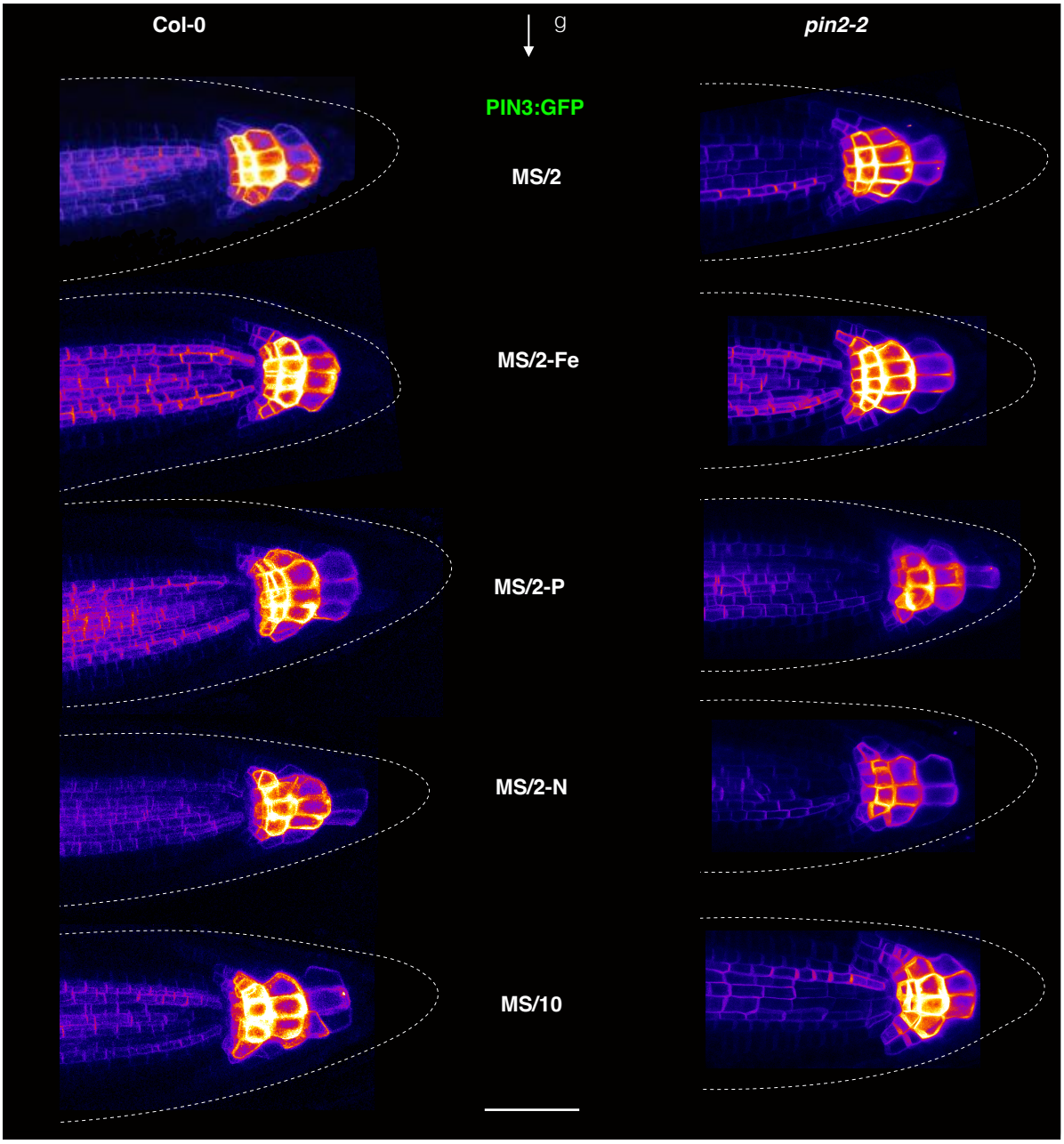
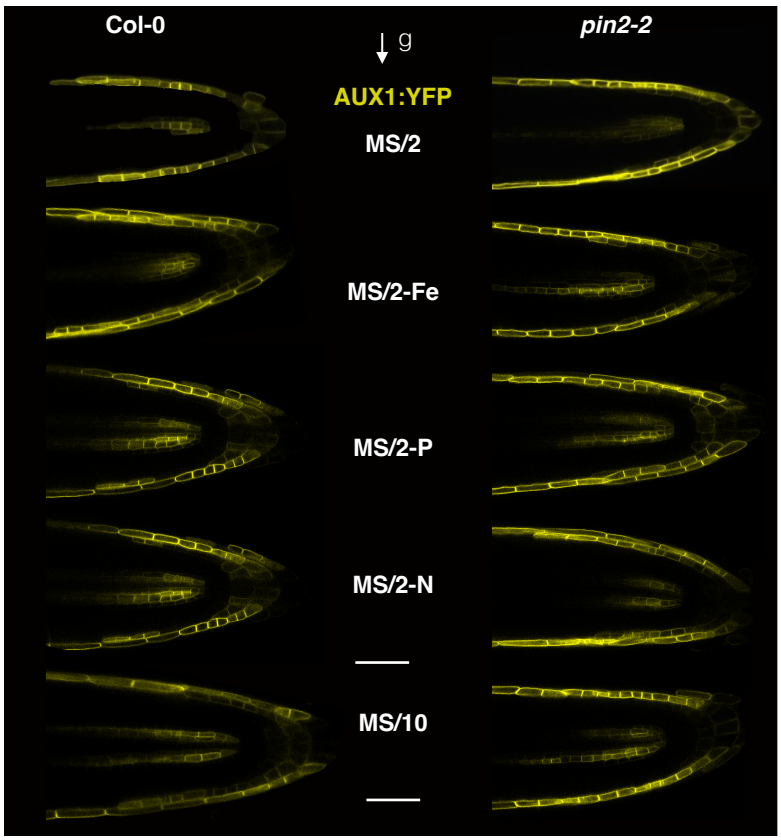


Figure S9.

A


Col-0
g
pin2-2
AUX1:YFP
MS/2
MS/2-Fe
MS/2-P
MS/2-N
MS/10

B

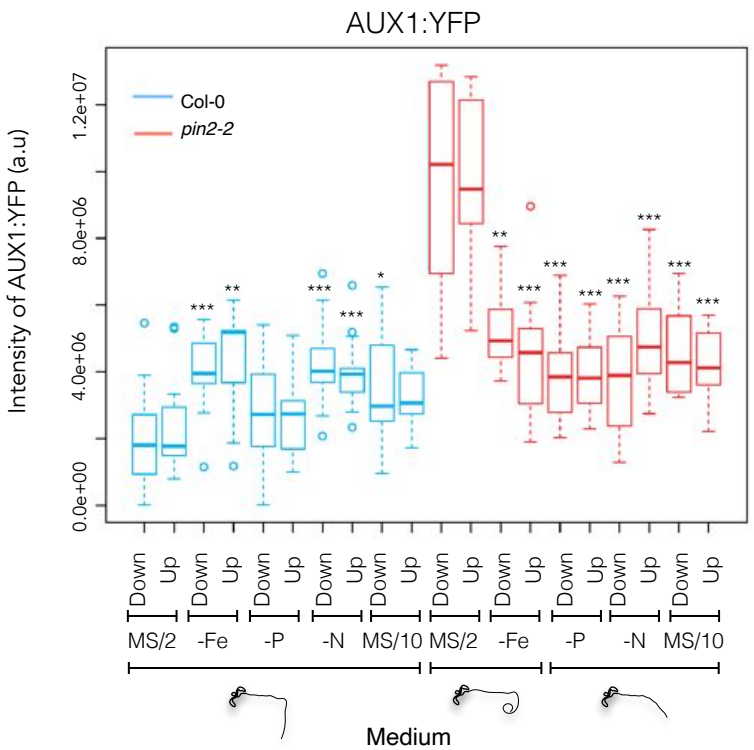
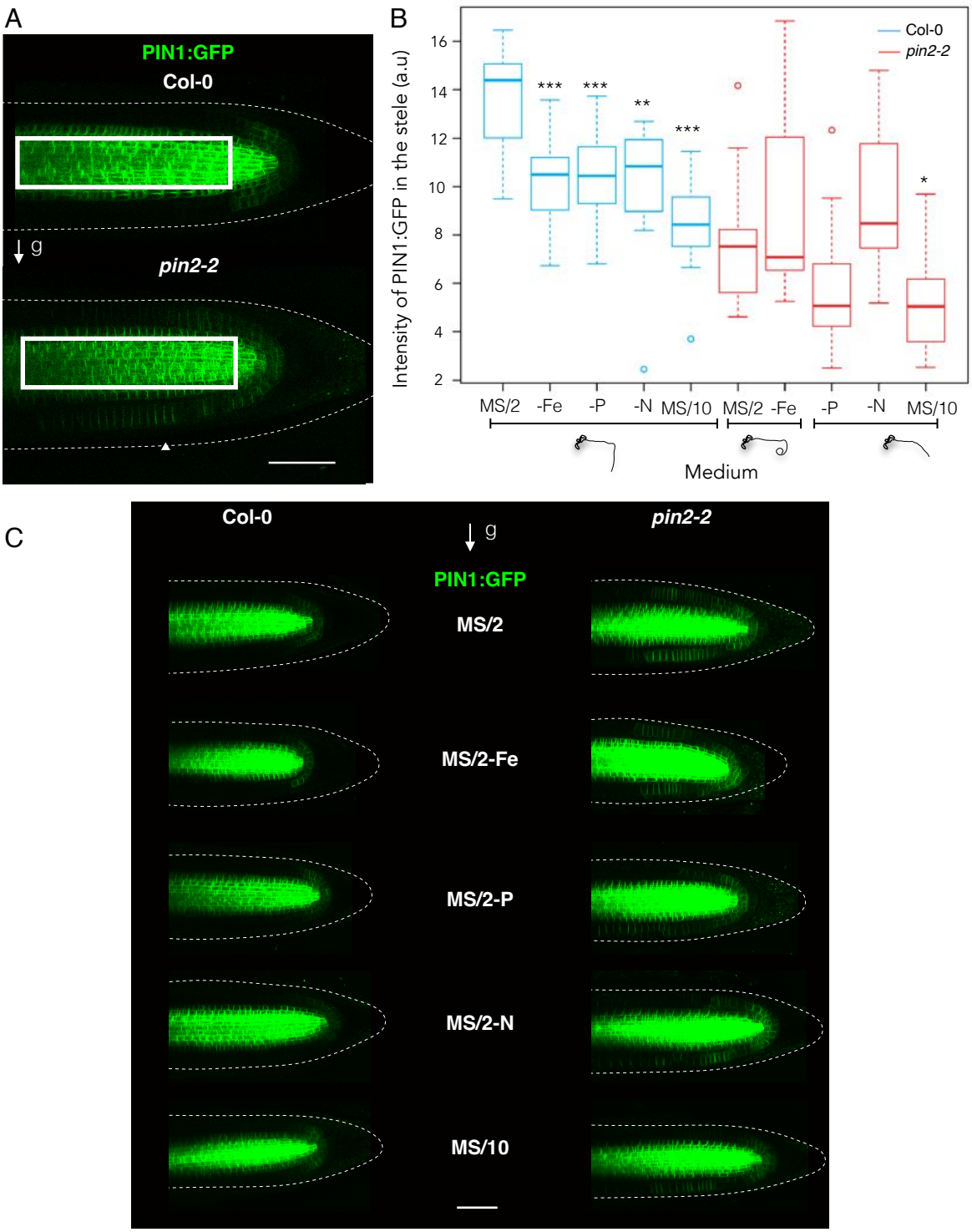

AUX1:YFP
Col-0
pin2-2
Intensity of AUX1:YFP (a.u)
0.0e+00
4.0e+06
8.0e+06
1.2e+07
Down
Up
MS/2
-Fe
-P
-N
MS/10
Medium

Figure S10.


A
PIN1:GFP
Col-0
g
pin2-2
B
Intensity of PIN1:GFP in the stele (a.u)
16
14
12
10
8
6
4
2
***
***
**
***
*
Col-0
pin2-2
MS/2
-Fe
-P
-N
MS/10
MS/2
-Fe
-P
-N
MS/10
Medium
C
Col-0
g
pin2-2
PIN1:GFP
MS/2
MS/2-Fe
MS/2-P
MS/2-N
MS/10