

COP1 mediates the coordination of root and shoot growth by light through modulation of PIN1- and PIN2-dependent auxin transport in *Arabidopsis*

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SUMMARY

When a plant germinates in the soil, elongation of stem-like organs is enhanced whereas leaf and root growth is inhibited. How these differential growth responses are orchestrated by light and integrated at the organismal level to shape the plant remains to be elucidated. Here, we show that light signals through the master photomorphogenesis repressor COP1 to coordinate root and shoot growth in *Arabidopsis*. In the shoot, COP1 regulates shoot-to-root auxin transport by controlling the transcription of the auxin efflux carrier gene *PIN-FORMED1* (*PIN1*), thus appropriately tuning shoot-derived auxin levels in the root. This in turn directly influences root elongation and adapts auxin transport and cell proliferation in the root apical meristem by modulating PIN1 and PIN2 intracellular distribution in the root in a COP1-dependent fashion, thus permitting a rapid and precise tuning of root growth to the light environment. Our data identify auxin as a long-distance signal in developmental adaptation to light and illustrate how spatially separated control mechanisms can converge on the same signaling system to coordinate development at the whole plant level.

KEY WORDS: *Arabidopsis*, Root growth, Light, Auxin transport, COP1, PIN1, PIN2

INTRODUCTION

A striking example of the plasticity of plant development (Casal et al., 2004; Matesanz et al., 2010; Nicotra et al., 2010) is revealed when a seedling emerges through the soil. In the absence of light, the hypocotyl elongates whereas cotyledons remain closed. When the seedling emerges from the soil, hypocotyl elongation stops and the cotyledons open, exposing the young leaves to light (Nemhauser and Chory, 2002; Quail, 2002). Two distinct developmental programs, known as photomorphogenesis and skotomorphogenesis (or etiolation) in light and in darkness, respectively, control these radically different growth patterns (Nemhauser and Chory, 2002; Quail, 2002).

Genetic analysis of the *CONSTITUTIVE PHOTOMORPHOGENIC/DE-ETIOLATED/FUSCA* (*COP/DET/FUS*) loci of *Arabidopsis thaliana* (hereafter *Arabidopsis*) led to substantial insights into the molecular mechanisms regulating light-mediated morphogenesis (Yi and Deng, 2005; Li et al., 2011). The *cop/det/fus* mutants display a de-etiolated phenotype when grown in darkness and most of them are defective in the components of a macromolecular complex named the COP9 signalosome (CSN).

The CSN regulates the 26S proteasome-mediated degradation of photomorphogenesis-promoting transcription factors, a central step in the regulation of developmental responses to light (Yi and Deng, 2005; Li et al., 2011). The RING E3 ubiquitin ligase *CONSTITUTIVE PHOTOMORPHOGENIC1* (*COP1*) ubiquitinates these transcription factors, allowing their recognition by the CSN and their subsequent degradation by the 26S proteasome (Yi and Deng, 2005; Henriques et al., 2009; Lau and Deng, 2010). In this process, COP1 acts as the central integrator of light responses downstream of photoreceptors (Yi and Deng, 2005; Lau and Deng, 2010).

The plant hormone auxin (indole-3-acetic acid; IAA) plays a major role in morphogenesis and mediates some of the growth responses to light, including hypocotyl elongation, apical hook maintenance, phototropic responses and the shade avoidance syndrome (Boerjan et al., 1995; Zhao et al., 2001; Stepanova et al., 2008; Esmon et al., 2006; Tao et al., 2008). Auxin action depends on a tightly regulated distribution across the plant. Auxin is transported from cell to cell in a polar fashion through the concerted action of several carrier proteins (Friml, 2010). Among them, the *PIN-FORMED* (*PIN*) efflux carriers control the direction of auxin flux through their polar localization onto the plasma membrane (PM), thereby promoting the establishment and subsequent maintenance of instructive auxin gradients (Wisniewska et al., 2006; Benková et al., 2003; Friml et al., 2003). In light-grown seedlings, auxin synthesized in young leaf primordia and transported to the roots has been shown to promote the emergence of lateral root primordia (Bhalerao et al., 2002; Salisbury et al., 2007). This phenomenon is inhibited in etiolated seedlings, leading to the hypothesis that light influences lateral root formation through an effect on shoot-to-root polar auxin transport (PAT) (Bhalerao et al., 2002). Moreover, recent data show that darkness rapidly triggers a change in the intracellular distribution of several PIN

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proteins in the root apical meristem (RAM), which was proposed to lead to a local alteration of auxin transport and to a decrease in root growth (Laxmi et al., 2008). In the case of PIN2, strong evidence indicates that this occurs through a decrease in PIN2 protein levels at the PM that is triggered by degradation through vacuolar targeting of the protein, similarly to gravitropic responses (Kleine-Vehn et al., 2008; Leitner et al., 2012).

Although these findings suggest that modulation of root growth by light might be achieved by influencing local auxin fluxes in the root tip, how this regulation is achieved is entirely unknown. In natural environments, roots are not directly exposed to sunlight, being buried deep down in the soil and how light can directly signal to the root tip remains to be established. Here, we report a novel long-distance mechanism by which light coordinates root growth with shoot development through COP1-mediated regulation of *PIN1* transcription in the shoot and of PIN1 and PIN2 intracellular distribution in the root, and identify auxin as a key long-distance signal in light-regulated development.

MATERIALS AND METHODS

Plant materials and growth conditions

Arabidopsis Col-0 ecotype was used as wild type (WT) unless otherwise noted. Previously published lines are described in supplementary material Table S1. CLV3>>iaaL lines were generated by transforming the CLV3:AlcR line (Deveaux et al., 2003) with the AlcA:iaaL binary plasmid using the floral dipping method (Clough and Bent, 1998). Seedlings were germinated on soil (covered with a thin layer of sand to shield roots from light) or on vertical half-strength MS agar plates at 22°C, under long-day conditions (16 hours light/8 hours darkness) or continuous darkness.

Chemical treatments

N-1-naphthylphthalamic acid (NPA) applications on hypocotyls were performed as described (Geldner et al., 2004), during 3 days in light or 4 days in darkness. For lactacystin treatments, 5-day-old seedlings were incubated for 4 hours, in light or in darkness, with 50 µM lactacystin or DMSO as mock. For ethanol induction, CLV3:AlcR and CLV3>>iaaL seeds were germinated and grown on half-strength MS agar plates containing 0.1% ethanol.

Phenotypic and expression analyses

Seedlings were imaged with a Nikon Coolpix 990 camera and root length was measured using ImageJ (<http://rsbweb.nih.gov/ij/>). Quantitative analyses of RAMs were performed as previously described (Dello Ioio et al., 2007).

β-Glucuronidase (GUS) staining was performed as previously described (Scarpella et al., 2004). Images were taken with a Jenoptik ProgRes C10 camera mounted either on a Zeiss Axioskop 2+ microscope equipped with Nomarski optics or on a Leica MZ12 stereomicroscope.

RNA extraction and quantitative reverse transcription PCR (qRT-PCR) were performed as previously described using the *ACTIN 2* gene as a reference (Ciarbelli et al., 2008) and analyzed according to Pfaffl (Pfaffl, 2001). Primer sequences and Universal Probe Library (UPL) probes are given in the supplementary material Table S2.

Epifluorescence images were taken with a Leica M205FA stereomicroscope or an Axioskop 2+ microscope and Leica DFC310 FX or ProgRes C10 camera, respectively. Confocal imaging was performed using Zeiss LSM510 or Leica TCS SP5 microscopes. Propidium iodide (PI; 10 µg/ml in water) was used to counterstain root cells. FM4-64 staining was performed as previously described (Xu and Scheres, 2005). For fluorescence quantification, the same confocal settings were used to compare different genotypes/treatments. All the samples from a single experiment were analyzed serially on the same day. ImageJ was used to quantify fluorescence intensities.

Statistical analyses (pairwise two-tailed Student's *t*-test) were performed using Microsoft Excel. Correction for multiple testing was carried out using the Bonferroni correction.

RESULTS

Darkness reduces root growth in a reversible manner

To gain insight into how root growth is controlled by light, we compared the root development of light-grown and dark-grown (etiolated) *Arabidopsis* seedlings. Etiolated seedlings had shorter roots than light-grown controls from 6 days after plating on culture dishes (Fig. 1A,B), indicating reduced root growth in darkness. To establish whether this is due to alterations in cell expansion and/or proliferation, we measured the length of differentiated epidermal cells and analyzed the size of the RAM. We found no significant difference in epidermal cell length between light-grown and etiolated seedlings at 4 days after germination and only a slight difference at 12 days (Fig. 1D). However, we observed a strong reduction of the RAM size in darkness (Fig. 1C,E) that was not due to changes in meristem patterning (supplementary material Fig. S1), suggesting that only cell proliferation is inhibited in darkness. We confirmed this by using the *CYCBI*;1:*GUS* cell proliferation marker (Colón-Carmona et al., 1999) (Fig. 1F). This inhibition is fully reversible as RAM cell proliferation and root growth was recovered upon light exposure of etiolated seedlings (supplementary material Fig. S2).

Signals from the shoot are involved in adapting root growth to light conditions

The effect of dark treatments on PIN protein intracellular distribution in the root tip has led to the hypothesis that root growth might be regulated by light through changes in PIN intracellular localization (Laxmi et al., 2008). Unexpectedly, dark-induced changes in PIN-GFP subcellular localization have also been observed in isolated roots (Laxmi et al., 2008), indicating that removal of the shoot apex does not block root responses to changes in light environments. To establish whether roots underground autonomously perceive light or whether signals from the shoot are involved in this regulation, we used the subcellular localization of PIN1-GFP (Xu et al., 2006) and PIN2-GFP (Xu et al., 2006) driven from their endogenous promoter in soil-grown roots (that are in constant darkness) as a proxy for light-induced changes in root development. When the shoot was exposed to light, we observed association of PIN1-GFP with vacuoles [as confirmed by colocalization with the vacuolar marker mRFP-VAM3 (Ebine et al., 2008)] in addition to its PM localization (Fig. 2A), whereas PIN2-GFP was strictly associated with the PM (Fig. 2C). This indicates that, despite the stabilizing effect of darkness on GFP in the lytic vacuoles (Tamura et al., 2003), having the root in darkness is not sufficient to trigger high vacuolar PIN1-GFP or PIN2-GFP fluorescence. We observed a strong vacuolar targeting of both PIN-GFP markers only when shoots were also placed in darkness (Fig. 2B,D). In these conditions, PIN1-GFP almost completely disappeared from membranes (Fig. 2B), whereas PIN2-GFP membrane association was reduced (Fig. 2D). These data indicate that under physiological conditions the regulation of the intracellular localization of PIN proteins by light is not root-autonomous but rather involves signals from the shoot.

Darkness regulates root growth by impairing shoot-to-root PAT

The root phenotype of etiolated seedlings closely resembles that of light-grown seedlings in which the auxin supply from the shoot apex was removed by shoot decapitation or inhibited by local application of the PAT inhibitor NPA on the hypocotyl (Grieneisen et al., 2007; Reed et al., 1998). We thus hypothesized an

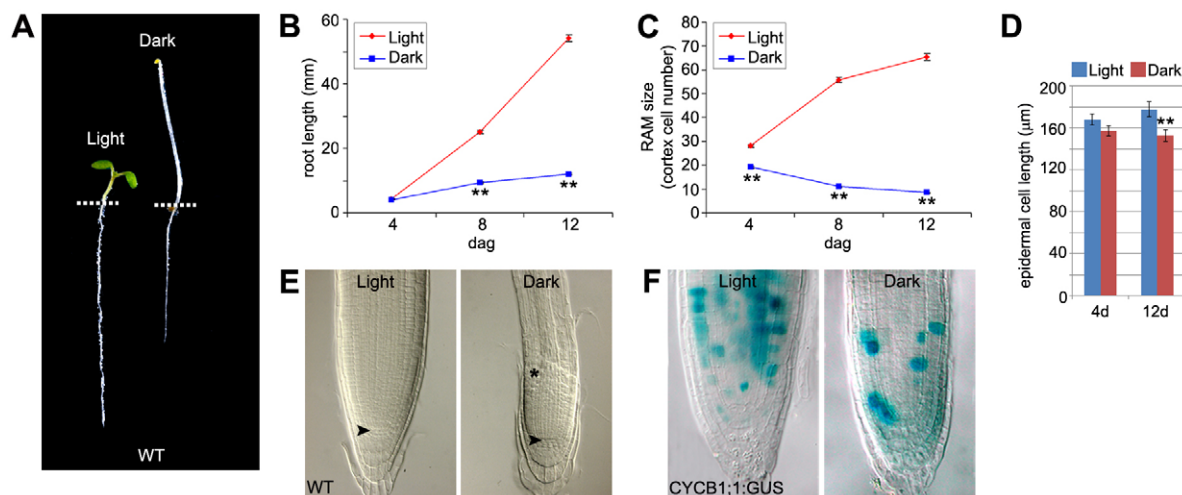


Fig. 1. Light controls root growth in *Arabidopsis*. (A) Phenotypes of 6-day-old light- and dark-grown WT seedlings. Dotted lines indicate the shoot-root junction. (B,C) Root length (B) and RAM size (C) of WT seedlings grown as indicated. All the differences between light and dark are statistically significant (*t*-test, $**P < 0.01$). (D) Epidermal cell length in roots of seedlings grown as indicated. $**P < 0.01$, *t*-test. (E) RAM phenotypes of 6-day-old light- and dark-grown seedlings. Asterisk, RAM boundary; arrowheads, quiescent centers. (F) CYCB1;1:GUS expression patterns in RAMs of 4-day-old light- and dark-grown seedlings. Error bars indicate s.e.m. dag, days after germination.

involvement of shoot-derived auxin and shoot-to-root PAT in the control of root growth by light. To verify this hypothesis, we first lowered auxin levels in the shoot apical meristem through ethanol-inducible expression of the bacterial gene *iaaL*, which reduces free auxin by conjugating IAA to Lys (Jensen et al., 1998), in the *CLAVATA3* (*CLV3*) (Fletcher et al., 1999) domain (*CLV3>>iaaL*). Upon induction, *CLV3>>iaaL* seedlings displayed shorter roots and less expanded RAMs compared with *CLV3:AlcR* controls (Deveaux et al., 2003) when grown in light (Fig. 3A; supplementary material Fig. S3), indicating that shoot-derived auxin is crucial for root growth. By contrast, etiolated *CLV3>>iaaL* and *CLV3:AlcR* seedlings showed no obvious differences after induction (Fig. 3A; supplementary material Fig. S3). Coherently, similar results were also obtained when shoot-to-root PAT was inhibited by local application of NPA on hypocotyls of WT seedlings (Fig. 3B; Fig. 4B,C; supplementary material Fig. S9). Taken together, these data support the idea that darkness inhibits shoot-to-root PAT.

To provide further evidence that shoot-to-root PAT is impaired in darkness, we used the DII-VENUS auxin sensor (Brunoud et al., 2012). DII-VENUS allows detection of dynamically relative changes in auxin distribution with cellular resolution (Brunoud et al., 2012). Whereas DII-VENUS could not be detected in light-grown hypocotyls (Fig. 3C,D), we observed a gradient of DII-VENUS fluorescence in etiolated hypocotyls, with the highest fluorescence in the basal region of the hypocotyl (Fig. 3E-J). Similarly, DII-VENUS fluorescence was greatly increased in dark-grown RAMs compared with light controls (Fig. 3K,L). These changes were not observed with the mDII-VENUS auxin-insensitive version of the sensor (supplementary material Fig. S4A). In addition, the tissue-specific expression along the hypocotyl axis or in the RAMs of GUS translational fusions to the TIR1/AFB1-3 auxin co-receptors (Parry et al., 2009) was not affected by the light conditions (supplementary material Fig. S4B), indicating that the differences in DII-VENUS fluorescence are not due to altered auxin sensitivity in response to light. These results demonstrate a decrease in auxin concentration along the hypocotyl

axis and in the RAM. Further strengthening our observations, expression of the synthetic auxin-inducible marker *DR5:GUS* (Ulmasov et al., 1997) was strongly enhanced in the aerial tissues

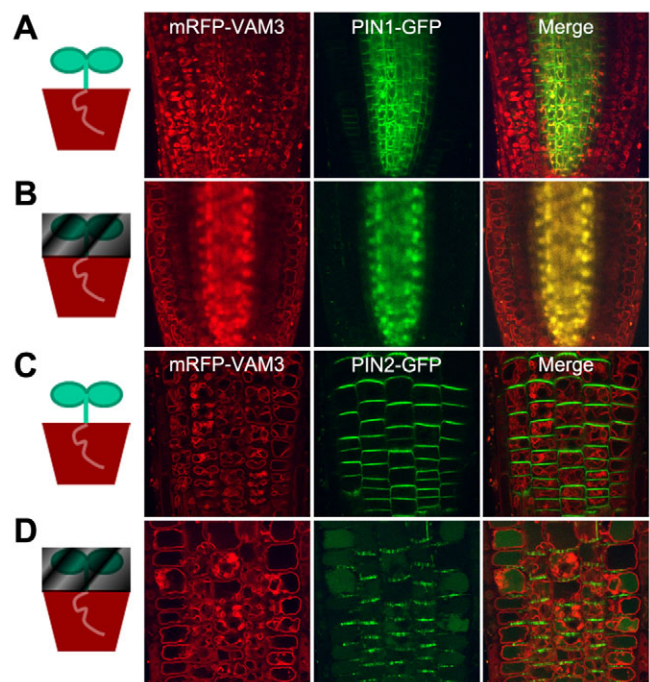


Fig. 2. Light regulates PIN1-GFP and PIN2-GFP intracellular localization in roots of *Arabidopsis* plants grown on soil. (A,B) PIN1-GFP and mRFP-VAM3 expression in the root vascular tissue of 7-day-old plants grown on soil with the shoot in light (A) or in darkness (B). (C,D) PIN2-GFP and mRFP-VAM3 expression in the root epidermis of 7-day-old plants grown on soil with the shoot in light (C) or in darkness (D). Note that the morphology of the vacuoles visualized with mRFP-VAM3 is different between tissues and that it changes with the light conditions.

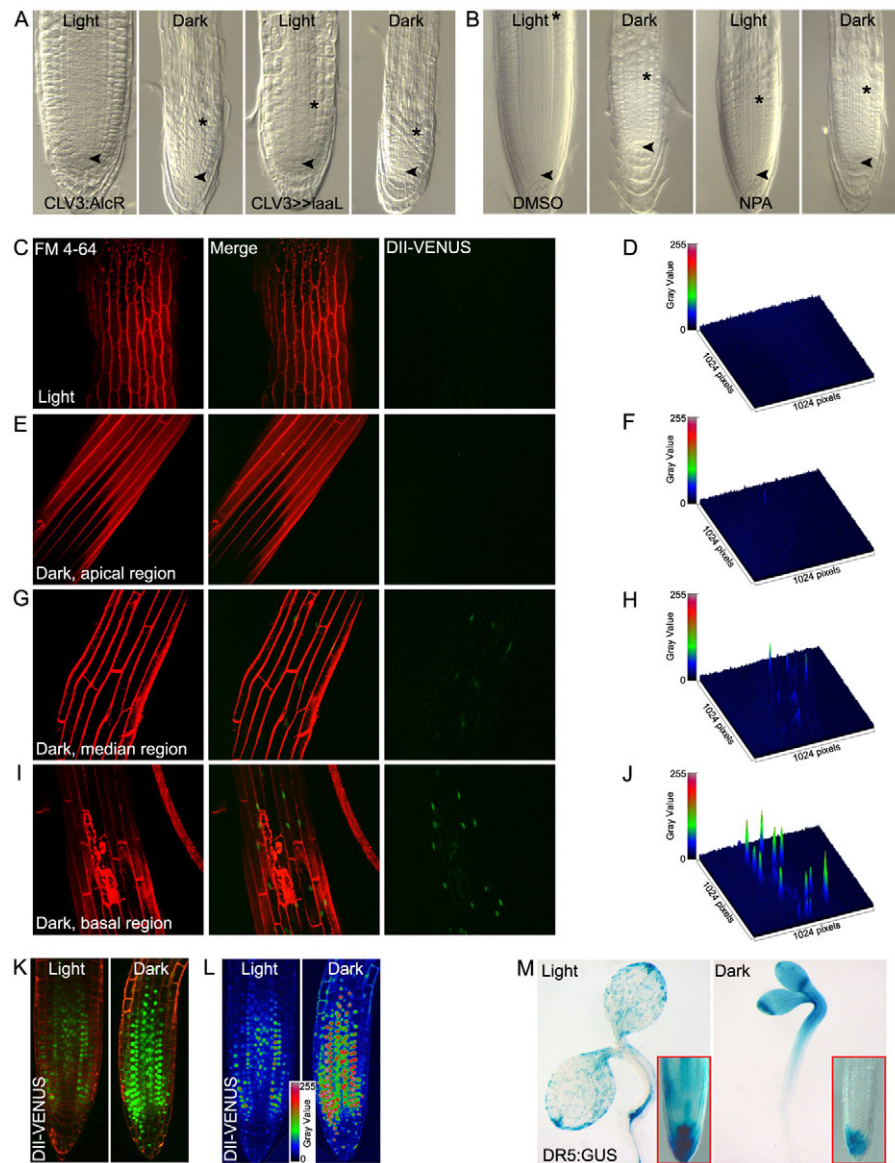


Fig. 3. Light regulates shoot-to-root PAT in *Arabidopsis*. (A,B) RAM phenotypes of 4-day-old light- and dark-grown CLV3:AlcR and CLV3>>iaaL seedlings upon ethanol induction (A) and of 7-day-old light-grown and 9-day-old dark-grown WT seedlings with and without (DMSO) NPA application on the hypocotyls (B). Asterisks, RAM boundaries; arrowheads, quiescent centers. (C,D) DII-VENUS expression (C) and fluorescence intensity (D) in 4-day-old light-grown hypocotyls. Red channel, FM4-64 staining. (E-J) DII-VENUS expression (E,G,I) and fluorescence intensity (F,H,J) in the apical (E,F), median (G,H) and basal (I,J) regions of 4-day-old etiolated hypocotyls. Red channel, FM4-64 staining. (K,L) DII-VENUS expression (K) and fluorescence intensity (L) in RAMs of 4-day-old light- and dark-grown seedlings. Red channel, FM4-64 staining; inset, false color scale. (M) DR5:GUS expression in shoots and RAMs (insets) of 4-day-old light- and dark-grown seedlings.

of etiolated seedlings, just above the apical hook, and markedly reduced in the RAM compared with light-grown controls (Fig. 3M). Our data thus suggest a decrease in shoot-to-root PAT in etiolated hypocotyls that leads to auxin depletion in the RAM and concurrent accumulation in the shoot apex.

Darkness regulates *PIN1* transcription in the hypocotyl

To identify the mechanisms underlying the changes in shoot-to-root PAT, we next investigated whether light could modulate the activity of the PIN family of auxin efflux carriers in the hypocotyl. Among the PIN genes, only *PIN1*, *PIN3* and *PIN7* have been reported to be expressed in hypocotyls (Blakeslee et al., 2007; Vieten et al., 2005). We therefore analyzed the expression patterns of *PIN1:GUS*, *PIN3:GUS* and *PIN7:PIN7-GUS* (Vieten et al., 2005) in light-grown and etiolated seedlings. *PIN1:GUS* expression was dramatically reduced in the hypocotyls of etiolated seedlings relative to the strong staining observed in light-grown seedlings (Fig. 4A). By contrast, GUS expression in both *PIN3:GUS* and *PIN7:PIN7-GUS* hypocotyls, albeit lower compared with light-grown seedlings, was

largely retained in darkness (Fig. 4A). Similar results were obtained when *PIN1*, *PIN3* and *PIN7* expression was analyzed using qRT-PCR, but a *PIN2* control showed no regulation by light (supplementary material Fig. S5A,B). In addition, we observed that the PIN1-GFP fluorescence also decreased in dark-grown *PIN1:PIN1-GFP* hypocotyls, indicating reduced PIN1 protein levels (supplementary material Fig. S5C). Thus, our results suggest that light might control shoot-to-root PAT mainly through modulating *PIN1* transcription in the hypocotyl.

PIN1-dependent shoot-to-root PAT is crucial for root growth

To establish a role for *PIN1*-dependent shoot-to-root PAT in root growth, we applied NPA locally to the hypocotyls of light-grown WT and *pin1-6* (Vernoux et al., 2000) seedlings, and analyzed their root phenotypes. NPA strongly reduced root length and RAM size in WT plants (Fig. 4B,C), whereas in *pin1* mutants RAM size was unaffected and root length was only mildly reduced (Fig. 4B,C). RAM size of untreated *pin1* mutants was nearly identical to that of NPA-treated WT plants (Fig. 4C),

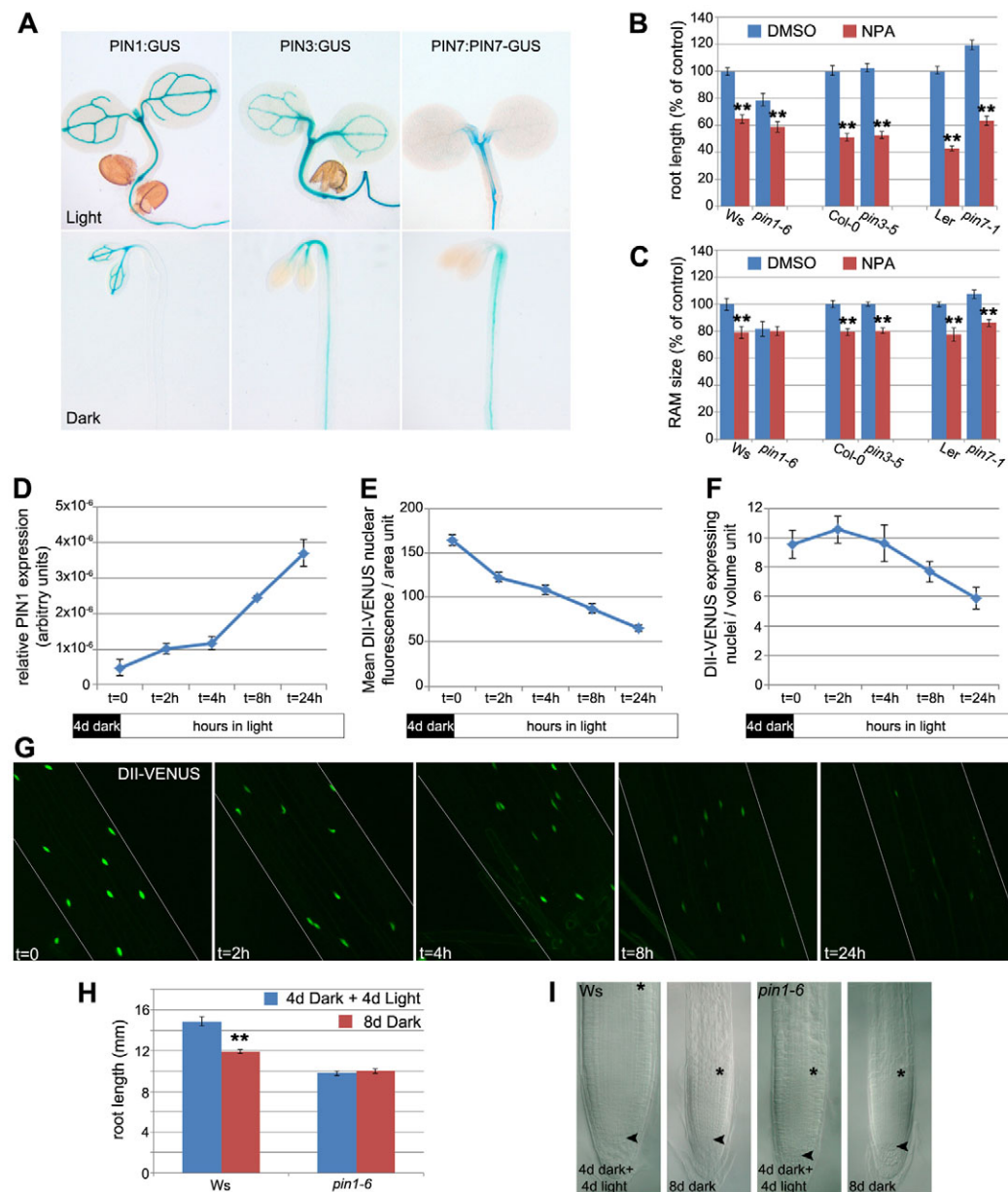


Fig. 4. PIN1 expression, auxin levels and root growth phenotypes during de-etiolation are closely correlated. (A) PIN1:GUS, PIN3:GUS and PIN7:PIN7-GUS expression in shoots of 4-day-old light- and dark-grown *Arabidopsis* seedlings.

(B, C) Root length (B) and RAM size (C) of light-grown *pin1-6*, *pin3-5* and *pin7-1* mutants and their WT controls (Ws, Col-0 and Ler, respectively) with or without (DMSO) NPA application on the hypocotyl. Error bars indicate s.e.m. ** $P < 0.01$, t-test. (D) PIN1 expression, determined by qRT-PCR, in shoot tissues of seedlings grown as indicated. Error bars indicate s.d. (E-G) DII-VENUS expression (G), mean nuclear fluorescence (E) and number of DII-expressing nuclei (F) in basal hypocotyls of seedlings grown as indicated. White lines indicate hypocotyl boundaries. Error bars indicate s.e.m. (H, I) Root length (H) and RAM size (I) of WT and *pin1-6* grown for 4 days in darkness and then either exposed to light, or kept in darkness for the following 4 days. In H, ** $P < 0.01$, t-test. In I, asterisks, RAM boundaries; arrowheads, quiescent centers.

supporting a key role for *PIN1*-dependent shoot-to-root PAT in the regulation of RAM size. By contrast, *pin3-5* and *pin7-1* (Benková et al., 2003) mutants had a WT-like response to NPA (Fig. 4B,C), suggesting that *PIN3* and *PIN7* play a negligible role in the light-regulation of root growth.

To provide further evidence for the role of *PIN1* transcriptional regulation in the hypocotyl, we analyzed by qRT-PCR its expression in hypocotyls after shifting etiolated seedlings back into light and observed a rapid and continuous induction of *PIN1* over a 24 hour period (Fig. 4D). This induction correlated with a progressive decrease of DII-VENUS fluorescence (suggesting an increase in auxin concentration) in the basal region of the hypocotyl (Fig. 4E-G). In addition, we observed that the *pin1* mutants could not recover root growth and RAM size during de-etiolation, demonstrating that *PIN1* is essential for this process (Fig. 4H,I). Taken together, our data indicate that the regulation of *PIN1* transcription by light is crucial for controlling shoot-to-root PAT and root growth in response to light.

Genetic evidence indicates that *PIN2* is also involved in the control of root growth by light

The effect of darkness on the intracellular distribution of PINs suggests that these proteins might also act directly in the RAM to regulate root growth in different light conditions. Previous observations have shown that at least *PIN1*, *PIN2*, *PIN3*, *PIN4* and *PIN7* are expressed in the RAM where they act partially redundantly to modulate root growth in light (Blilou et al., 2005). We thus analyzed root phenotypes of single and multiple *pin* mutants grown in light and in darkness. As previously observed (Blilou et al., 2005), light-grown *pin2* (*eirl-1*) (Luschnig et al., 1998) mutants showed the most severe reduction of RAM size (Fig. 5B; supplementary material Fig. S6), supporting a key-role for *PIN2* in regulating RAM cell proliferation (Blilou et al., 2005). However, all the etiolated *pin* single mutants exhibited reduced root length and RAM size, suggesting possible redundancy between *PIN* genes in this process (Fig. 5A,B). Among higher order mutants, *pin1 pin2* double mutant (Blilou et al., 2005) displayed

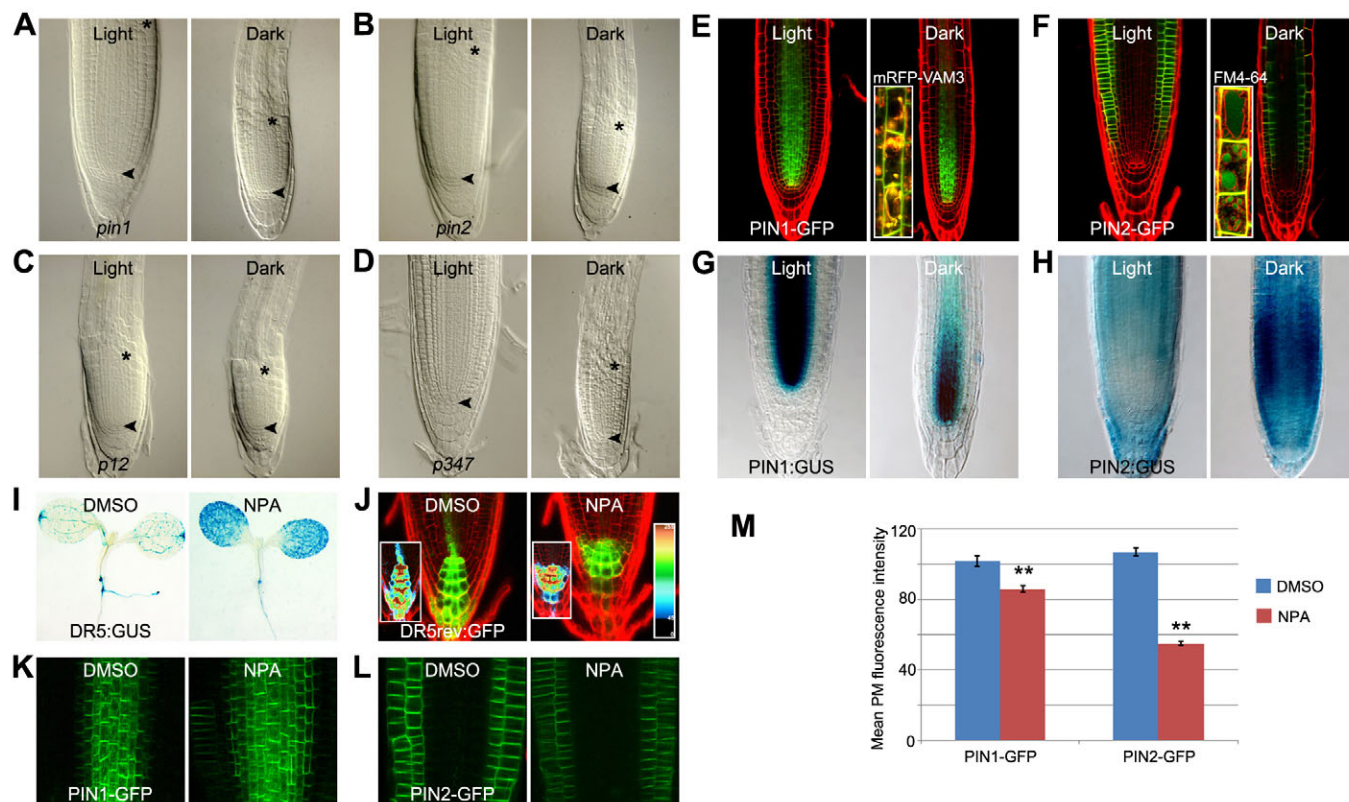


Fig. 5. Light controls root growth through PIN1 and PIN2. (A–D) RAM phenotypes of 6-day-old light- and dark-grown *pin1* (A), *pin2* (B), *pin1 pin2* (*p12*) (C) and *pin3 pin4 pin7* (*p347*) (D) *Arabidopsis* seedlings. Asterisks, RAM boundaries; arrowheads, quiescent centers. (E–H) PIN1:PIN1-GFP (E), PIN2:PIN2-GFP (F), PIN1:GUS (G) and PIN2:GUS (H) expression in 6-day-old RAMs. Inset in E: colocalization of PIN1-GFP (green) and mRFP-VAM3 (red) in etiolated roots; inset in F: colocalization of PIN2-GFP (green) and FM4-64 staining (red). (I–L) DR5:GUS (I), DR5rev:GFP (J), PIN1:PIN1-GFP (K) and PIN2:PIN2-GFP (L) expression in light-grown seedlings with and without (DMSO) NPA application on the hypocotyls. Insets in J depict pseudocolorimetric quantifications of GFP intensities. (M) Quantification of PM PIN1-GFP and PIN2-GFP levels in RAMs grown as shown in K and L, respectively. Error bars indicate s.e.m. ** $P < 0.01$, *t*-test.

severely reduced root length and RAM size in both light and dark conditions (Blilou et al., 2005; Vieten et al., 2005) (Fig. 5C; supplementary material Fig. S6). By contrast, *pin3 pin4 pin7* triple mutants (Blilou et al., 2005) were indistinguishable from the WT regardless of the light condition (Fig. 5D; supplementary material Fig. S6). Together, our results reveal that *PIN1* and *PIN2* (but not *PIN3*, *PIN4* and *PIN7*) are crucial regulators of root growth in response to light conditions. Our data further suggests that root-autonomous modulation of PAT through changes in both *PIN1* and *PIN2* activity might also control root growth in response to light.

PIN1-dependent shoot-to-root PAT modulates PIN1 and PIN2 intracellular distribution in the root tip

As demonstrated previously, darkness induces drastic changes in *PIN1* and *PIN2* intracellular localization in agar-grown roots (Laxmi et al., 2008; Kleine-Vehn et al., 2008) (Fig. 5E,F). This leads to a reduction in the abundance of PM-localized *PIN1* and *PIN2* and of the total pool of protein in the case of *PIN2* (Fig. 5E,F; Fig. 6G,H). Transcription was unchanged for *PIN1* and increased for *PIN2*, suggesting that these changes do not result directly from modifications in the transcription of the two genes (Fig. 5G,H). Because auxin can modulate *PIN* intracellular localization and stability (Paciorek et al., 2005; Sauer et al., 2006; Abas et al., 2006; Sieberer et al., 2000; Vieten et al., 2005), we wondered whether reduced shoot-to-root PAT in darkness could cause these changes. To

test this, we inhibited shoot-to-root PAT in light-grown *DR5:GUS*, *DR5rev:GFP* (Friml et al., 2003), *PIN1:PIN1-GFP* and *PIN2:PIN2-GFP* seedlings using local NPA application to the hypocotyl (Fig. 5I–M). This treatment caused a dramatic reduction of the PM abundance of *PIN2-GFP*, and to a lesser extent of *PIN1-GFP*, in the RAM compared with the untreated controls (Fig. 5K–M). The same treatment also led to enhanced *DR5:GUS* expression in the cotyledons (Fig. 5I) and lateral expansion of the *DR5rev:GFP* domain in the RAM (Fig. 5J), resembling that observed during etiolation (Fig. 3M) or in light-grown *pin2* mutant (Ottenschläger et al., 2003). Our results support a scenario in which dark-induced reduction in *PIN1* activity in the hypocotyl leads to lower auxin levels in the root, which in turn promotes a reduction in *PIN1* and *PIN2* levels at the PM, thus impairing root growth. Consistently, NPA application at the hypocotyls of *pin2* seedlings triggered a reduction in root length but did not cause any further inhibition of RAM size (supplementary material Fig. S7), providing additional evidence that shoot-derived auxin requires *PIN2* function in the root tip to control the RAM size and root growth in response to light.

Mutations in *COP1* improve root growth in darkness

Having established that light conditions act by modulating auxin transport both in the hypocotyl and in the root tip, we next investigated the molecular pathways involved in this regulation. To

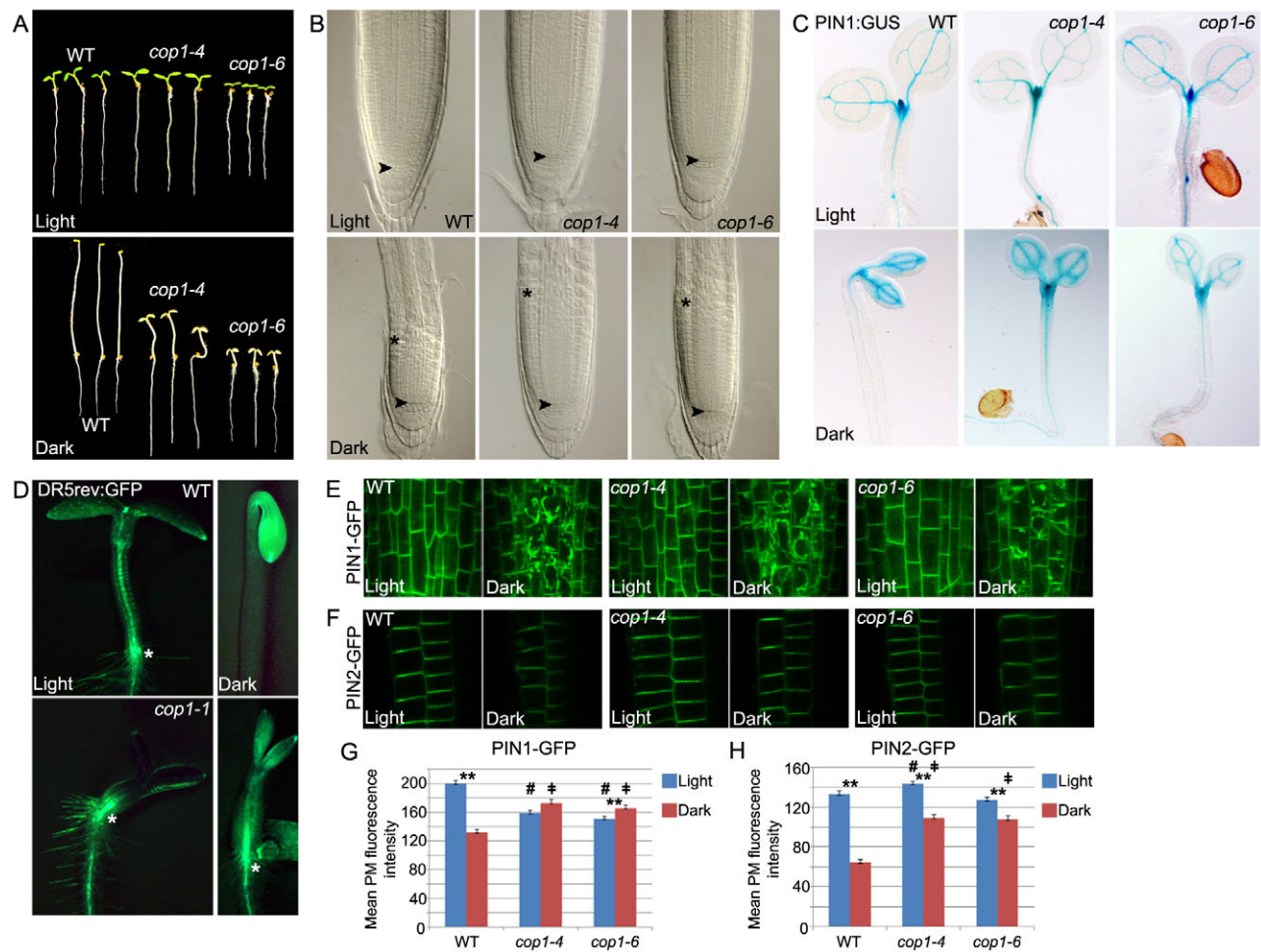


Fig. 6. COP1 regulates root growth, PIN1 expression and PIN1-GFP and PIN2-GFP in response to change in light conditions. (A) Seedling phenotypes of 6-day-old light- and dark-grown WT, *cop1-4* and *cop1-6* *Arabidopsis* plants. (B) RAM phenotypes of 6-day-old light- and dark-grown WT, *cop1-4* and *cop1-6* plants. Asterisks, RAM boundaries; arrowheads, quiescent centers. (C) PIN1:GUS expression in shoots of 6-day-old light- and dark-grown WT, *cop1-4* and *cop1-6* plants. (D) DR5rev:GFP expression in 6-day-old WT and *cop1-1* seedlings. Asterisks, shoot-root junctions. (E,F) PIN1-GFP (E) and PIN2-GFP (F) localization in RAMs of 6-day-old light- and dark-grown WT, *cop1-4* and *cop1-6* plants. (G,H) Quantification of PM PIN1-GFP (G) and PIN2-GFP (H) levels in WT, *cop1-4* and *cop1-6* plants grown as shown in E,F. Significant differences are indicated ($P < 0.05$ with Bonferroni correction): **, dark versus light; # or †, genotype versus WT in the light or the dark, respectively. Error bars indicate s.e.m.

this end, we analyzed the root phenotypes of the *cop1-4* and *cop1-6* (McNellis et al., 1994) mutant alleles, which have hypomorphic mutations in the *COP1* gene, which encodes a master regulator of light-controlled shoot development (Yi and Deng, 2005). Compared with WT, *cop1* mutants displayed reduced root length in light but longer roots in darkness (Fig. 6A; supplementary material Fig. S8A,C), suggesting a role for COP1 in both light and dark conditions. Strikingly, all the *cop1* mutants displayed more expanded RAMs compared with WT controls (Fig. 6B; supplementary material Fig. S8B,D) regardless of the light conditions. These phenotypes suggest that COP1 plays a key role in mediating the regulation of root growth in response to light.

COP1 acts together with both PIN1 and PIN2 to regulate root growth

To test whether COP1 controls root growth in response to light by regulating PIN1 and PIN2, we next generated *cop1-4 pin1-1*, *cop1-6 pin1-1*, *cop1-4 pin2* and *cop1-6 pin2* double mutant combinations. The *pin1-1* mutation significantly affected the

cop1 root phenotype. Both *cop1-4 pin1-1* and *cop1-6 pin1-1* displayed reduced root length and smaller RAM size compared with the parental *cop1* alleles both in light and in darkness (supplementary material Fig. S8A,B). However, *cop1 pin1* double mutants always showed longer roots and more expanded RAMs compared with *pin1-1* in darkness (supplementary material Fig. S8A,B), indicating that combining the *cop1* and *pin1* mutations partially rescue the capacity of the plant to adapt root growth to light conditions. Similarly to *cop1 pin1* double mutants, both *cop1-4 pin2* and *cop1-6 pin2* mutants displayed shorter roots and smaller RAMs compared with the parental *cop1* alleles regardless of the light conditions (supplementary material Fig. S8C,D), whereas dark-grown *cop1 pin2* root phenotypes were not as severe as those of the *pin2* mutant (supplementary material Fig. S8C,D). The combination of the *pin2* and *cop1* mutations thus also rescues, in part, the capacity of the plant to adapt root growth to light conditions, indicating that COP1, PIN1 and PIN2 participate together in the regulation of root growth by light.

COP1 regulates *PIN1* transcription in the hypocotyl to control *PIN1*-dependent shoot-to-root PAT

We then investigated whether COP1 could control the regulation of *PIN1* transcription in the hypocotyl and analyzed *PIN1:GUS* expression in light- and dark-grown *cop1-4* and *cop1-6* hypocotyls. Whereas *PIN1:GUS* expression was dramatically reduced in the hypocotyls of etiolated WT seedlings compared with light-grown controls (Fig. 6C), expression of the transgene was retained in the hypocotyls of dark-grown *cop1* mutants, resembling what we observed in light-grown WT and *cop1* seedlings (Fig. 6C). COP1 might thus be involved in downregulating *PIN1* transcription in the hypocotyls of dark-grown seedlings and in the inhibition of shoot-to-root PAT and root growth. To confirm this hypothesis, we introgressed *DR5rev:GFP* in a *cop1* background. We observed that, whereas *DR5rev:GFP* expression was restricted to the apical organs and markedly reduced in the hypocotyl below the apical hook region in WT (Fig. 6D), its expression was retained in the hypocotyls of dark-grown *cop1-1* seedlings, similarly to light-grown WT seedlings (Fig. 6D). This further substantiates a role for COP1 in the regulation of *PIN1*-dependent shoot-to-root PAT.

COP1 modulates *PIN1* and *PIN2* intracellular distribution in the root

As COP1 interacts genetically with both *PIN1* and *PIN2*, it is unlikely to act only through the regulation of *PIN1* transcription in the hypocotyl in response to light and might also play additional roles in the RAM. To confirm this, we applied NPA to the hypocotyls of dark-grown plants with WT and *cop1* alleles, to test whether the inhibition of shoot-to-root PAT is sufficient to impair root growth and RAM size in the mutants. In line with the downregulation of *PIN1* expression in darkness, NPA-treated WT seedlings did not display any difference in root length and in RAM size compared with untreated controls. By contrast, NPA strongly inhibited root length in dark-grown *cop1* mutants, but their RAM size was unchanged compared with untreated controls (supplementary material Fig. S9A,B). It should be noted that local application of NPA inhibited hypocotyl elongation in both WT and *cop1* mutants (supplementary material Fig. S9C), indicating that NPA effectively inhibits shoot-to-root PAT in *cop1* hypocotyls. Together, our results indicate that COP1 might also play a role locally in the root to regulate RAM size.

We next tested whether COP1 also regulates the intracellular localization of *PIN1* and *PIN2* in the RAM in response to light. To do so, we compared *PIN1*-GFP and *PIN2*-GFP PM abundance and intracellular distribution in RAMs of WT and *cop1* plants in both light and dark conditions. The *cop1* mutation did not affect the changes in intracellular localization of *PIN1*-GFP induced by darkness. However, quantification of PM fluorescence showed that, unlike WT roots (Fig. 6E,G), the PM abundance of *PIN1*-GFP in *cop1-4* and *cop1-6* roots (Fig. 6E,G) was not reduced by darkness. Dark-induced accumulation of *PIN2*-GFP in the vacuolar lumen (Laxmi et al., 2008) was, by contrast, blocked by mutations in *COP1* (Fig. 6F). Similarly to *PIN1*-GFP, in darkness both *cop1-4* and *cop1-6* had a higher intensity of *PIN2*-GFP at the PM than did WT controls (Fig. 6F,H). We thus conclude that, in the RAM, COP1 regulates the decrease in PM-associated *PIN1* and *PIN2* and also the changes in intracellular localization of *PIN2* triggered by darkness. In addition, in light-grown seedlings, NPA application at the hypocotyls of the *cop1-4* mutant did not cause any reduction in the amounts of PM-localized *PIN1*-GFP and *PIN2*-GFP, as

observed for WT plants (supplementary material Fig. S10), further supporting a root-autonomous role of COP1 in the regulation of *PIN1* and *PIN2* intracellular localization and, thus, of RAM size.

COP1 regulates *PIN2* stability

The changes in *PIN2* intracellular distribution in darkness have been proposed to result from a higher ubiquitination rate of *PIN2*, leading to degradation in vacuoles (Kleine-Vehn et al., 2008; Leitner et al., 2012). As previously reported using MG132 (Abas et al., 2006; Laxmi et al., 2008), specific inhibition of proteasome activity using lactacystin blocked *PIN2*-GFP targeting to the vacuole in darkness (supplementary material Fig. S11). This confirms an involvement of the ubiquitin pathway in dark-mediated *PIN2* degradation. However, this treatment had no visible effect on the intracellular distribution of *PIN1*-GFP, suggesting that *PIN1* might be regulated differently (supplementary material Fig. S11).

Given that *PIN2* degradation is also essential for root gravitropism (Abas et al., 2006), we reasoned that a role for COP1 in the regulation of this developmental response would provide independent evidence for its involvement in the regulation of *PIN2* stability. *cop1* mutants indeed demonstrated a reduced capacity to reorientate root growth in response to a change in the gravity vector (Fig. 7A). In addition, *cop1* mutations also interfered with the degradation of *PIN2*-GFP on the upper side of gravistimulated roots (Abas et al., 2006) (Fig. 7B-D). Our data thus demonstrate that COP1 is involved in regulating *PIN2* stability during gravitropism, further supporting a similar role in the RAM in response to changes in light conditions.

DISCUSSION

Although they are not directly exposed to light in most plant species, light influences the growth characteristics of roots (Feldman, 1984; Halliday et al., 2009). How root growth is controlled by light and integrated with developmental responses in the above-ground organs to shape the overall plant architecture is still largely unknown. Here, we provide strong evidence that light regulates root growth by modulating shoot-to-root PAT and identify auxin as a crucial long-distance signal in the regulation of root growth by light. We show that chemical inhibition of PAT in the hypocotyl, or decreasing shoot-derived auxin by genetic approaches, mimic the effect of darkness on root growth. Our results using the *DII-VENUS* and *DR5* auxin signaling sensors (Brunoud et al., 2012; Ulmasov et al., 1997; Friml et al., 2003), further suggest that dark-induced inhibition of shoot-to-root PAT in the hypocotyl results in auxin accumulation in the shoot apex and in a reduction in auxin levels at the base of the hypocotyl and in the root apex. This reversibly impairs RAM cell proliferation and root elongation in a manner that allows recovery of root growth upon re-exposure to light. The possible accumulation of auxin in aerial tissues resulting from the inhibition of shoot-to-root PAT might also participate in the regulation of light-regulated auxin responses in the shoot, such as the maintenance of the apical hook (Stepanova et al., 2008; Zádňíková et al., 2010), and hence to the coordination of growth responses at the level of the entire organism.

At the molecular level, we demonstrate that darkness leads to a strong reduction in *PIN1* expression in the hypocotyl that might lead to a decrease in shoot-to-root PAT (although direct measurements of auxin transport would be necessary to definitively prove this). Conversely, *PIN1* is induced during de-etiolation with kinetics that correlate with a progressive increase in auxin levels at the base of the hypocotyl as suggested by *DII-VENUS*

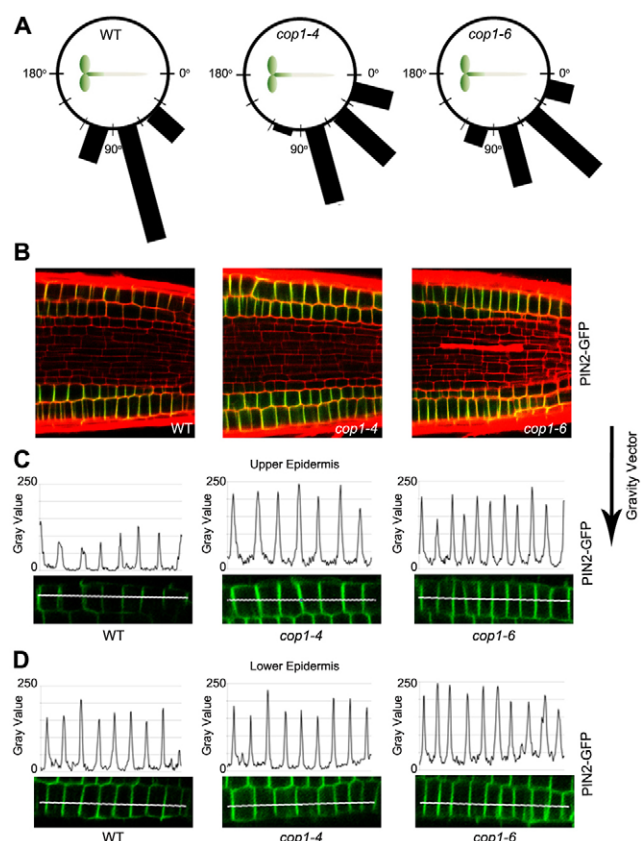


Fig. 7. Impaired gravitropic responses in *cop1* mutants. (A,B) Root reorientation (A) and PIN2-GFP expression (B) in WT, *cop1-4* and *cop1-6* *Arabidopsis* plants after a 90° shift of the gravity vector for 8 hours (A) or 3 hours (B). The length of black bars in A represents the relative frequency of the corresponding classes of angles. Red channel in B, propidium iodide. (C,D) Quantification of PM PIN2-GFP levels in upper (C) and lower (D) epidermis of roots grown as described for B. Graphs represent PM PIN2-GFP fluorescence intensity calculated along the white lines shown below.

fluorescence. Changes in *PIN1* transcription have also been reported using genome-wide expression analysis (López-Juez et al., 2008) and light might then control shoot-to-root PAT in the hypocotyl primarily by regulating *PIN1* transcription. This conclusion is further supported by the observation that *pin1* mutants have reduced root length and RAM defects identical to those of plants with inhibited shoot-to-root PAT and that the *pin1* mutation severely impairs the reversibility of root growth inhibition induced by darkness. Our data thus suggest that PIN1 function in the shoot is not restricted to the inflorescence (Gälweiler et al., 1998; Vernoux et al., 2000; Reinhardt et al., 2003), but is also responsible for the long-distance delivery of shoot-derived auxin to the RAM.

RAM cell proliferation is regulated by PIN-mediated recirculation of shoot-derived auxin across the root apex (Blilou et al., 2005; Grieneisen et al., 2007). Of all the PIN proteins, PIN2 plays a pivotal role in the RAM as it regulates non-redundantly the root basipetal transport of auxin (Blilou et al., 2005). Mutations in *PIN2* result in the most severe defects in root growth and RAM proliferation among single *pin* mutants (Blilou et al., 2005). In roots of etiolated seedlings both in vitro and in soil, the intracellular localization of PIN2-GFP is severely altered, being greatly reduced

at the PM and accumulating in the lytic vacuole (Kleine-Vehn et al., 2008; Laxmi et al., 2008) (our data). Furthermore, we have demonstrated that inhibition of shoot-to-root PAT using NPA application on hypocotyls of light-grown seedlings decreases PIN2 levels at the PM in the RAM, and causes root phenotypes similar to those of etiolated seedlings. This suggests that depletion of shoot-derived auxin in darkness is sufficient to decrease PIN2 levels at the PM, a step that might be essential for the inhibition of cell proliferation in the RAM. This role for shoot-derived auxin is further supported by the fact that auxin promotes PIN2 localization to the PM (Paciorek et al., 2005) and by a recent work demonstrating that dark-induced PIN2 targeting to the lytic vacuole can be prevented by exogenous IAA (Rosado et al., 2010).

Besides PIN2, changes in intracellular localization of PIN1, PIN3 and PIN7 in the RAM of dark-grown plants have also been demonstrated (Laxmi et al., 2008) (our data). However, our genetic analysis indicates that, among these, only PIN1 plays a relevant role locally in the RAM. PIN1 contributes to the regulation of cell proliferation together with PIN2, as demonstrated by the increased severity of the root phenotype of *pin1 pin2* double mutants compared with the single mutants and independently of the light conditions (Blilou et al., 2005; Vieten et al., 2005) (our data). The PM abundance of PIN1 is also reduced in darkness, although to a lesser extent than PIN2. Similarly to PIN2, PIN1 endocytosis is inhibited by auxin (Paciorek et al., 2005), indicating that this reduction in darkness might result from the decrease in shoot-to-root PAT. Dark-mediated changes in PIN1 activity might then function in reducing acropetal transport in etiolated roots. Together with the reduced basipetal flux resulting from higher rates of PIN2 degradation, this could lead to a global decrease in the recirculation of auxin in the RAM, explaining the reduction in cell proliferation and the reversibility of the growth arrest in darkness.

The E3 ubiquitin ligase COP1 has been shown to function as the master regulator of photomorphogenesis (Yi and Deng, 2005). Our data reveal a dual role for COP1 in the regulation of light-mediated root growth, as it regulates both long-distance transport and local RAM fluxes of auxin through different mechanisms. Upon changes in light conditions, COP1 regulates *PIN1* expression in the hypocotyl. How this is achieved is still unknown but an attractive possibility is that one of the targets of COP1-mediated degradation might directly activate *PIN1* transcription. The bZIP transcription factor LONG HYPOCOTYL 5 (HY5), which is the best-characterized target of COP1-mediated proteolysis (Osterlund et al., 2000), would be a good candidate as several functional links between HY5 and auxin have been established (Cluis et al., 2004; Sibout et al., 2006). However, although HY5 binding sites were found in the *PIN1* locus (Lee et al., 2007), genome-wide expression analyses did not detect significant changes in *PIN1* expression in the *hy5* mutant (Cluis et al., 2004; Zhang et al., 2011). This could still be explained by a functional redundancy between HY5 and HYH in the regulation of *PIN1* (Sibout et al., 2006) but further investigations will be needed to clarify this.

Our data also demonstrate a role for COP1 in the control of PIN1 and PIN2 intracellular distribution and PM abundance in the RAM. We provide genetic evidence that COP1 regulates the decrease in both PIN1-GFP and PIN2-GFP localized at the PM, indicating a role for the ubiquitin pathway in this regulation. However, although treatment with a proteasome inhibitor in darkness clearly stabilized PIN2 association to the PM, it did not affect PIN1 localization. This suggests that the two proteins might be regulated differently, and it also points at a role of the ubiquitin pathway in the regulation of PIN2 activity by light. It has indeed

been proposed that darkness reduces association of PIN2 to the PM by increasing degradation of the protein (Kleine-Vehn et al., 2008), similarly to what is observed during gravitropism (Abas et al., 2006; Kleine-Vehn et al., 2008). In *cop1* mutants, we did not observe any PIN2-GFP fluorescence associated with vacuolar compartments, suggesting a possible role for COP1 in targeting PIN2 to the vacuole. In addition, we provide evidence that COP1 activity is also necessary for PIN2 degradation during the gravitropic response, a function that might be independent from the role of COP1 in photomorphogenesis. This strongly supports the idea that plants use a common module involving both COP1 and PIN2 for the regulation of root growth in response to different environmental stimuli (Kleine-Vehn et al., 2008) (our data). The existence of a permanent cytoplasmic pool of COP1 (Jang et al., 2010) raises the possibility that, in the RAM, COP1 might directly target PIN2 for degradation through ubiquitination. Such a mechanism remains to be proven but is plausible given the recent demonstration of direct ubiquitination of PIN2 during gravitropism (Leitner et al., 2012). A role for COP1 in the regulation of root growth by light, through affecting the organization of the actin cytoskeleton (Dyachok et al., 2011), has also been demonstrated. Actin is known to be essential for the intracellular trafficking of PIN proteins (Geldner et al., 2001; Boutté et al., 2006) and COP1 could affect PIN1 and PIN2 intracellular localization by modulating the activity of the actin cytoskeleton. Elucidating the mode of action of COP1 will need further experiments but our findings suggest an elegant model that explains how light regulates shoot-to-root PAT and auxin distribution over the entire plant to coordinate root and shoot development in response to light in a COP1-dependent manner (supplementary material Fig. S12).

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Competing interests statement

The authors declare no competing financial interests.

Supplementary material

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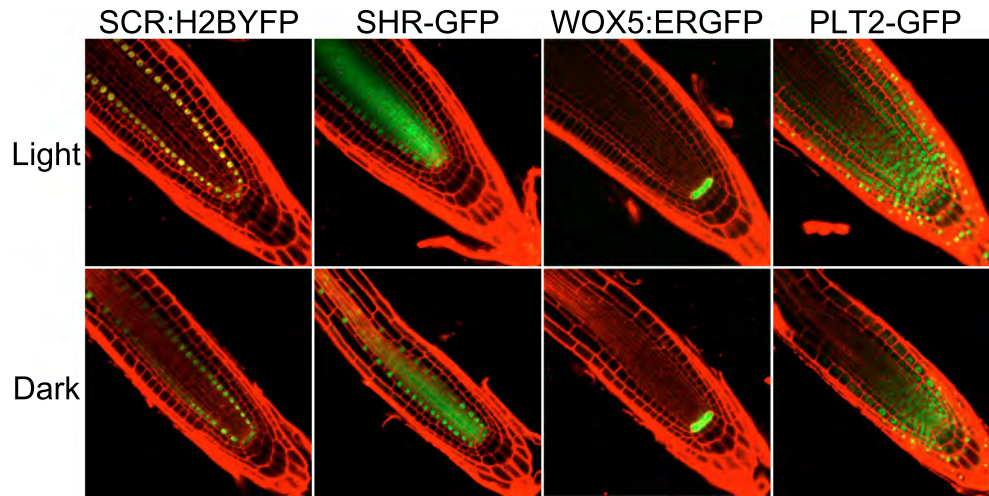


Fig. S1. The expression patterns of root-specific developmental markers are not altered upon etiolated growth. Confocal laser scanning images of 7-day-old light- and dark-grown seedling roots expressing SCR:H2BYFP, SHR-GFP, WOX5:ERGFP and PLT2-GFP. Roots were counterstained with propidium iodide (red channel).

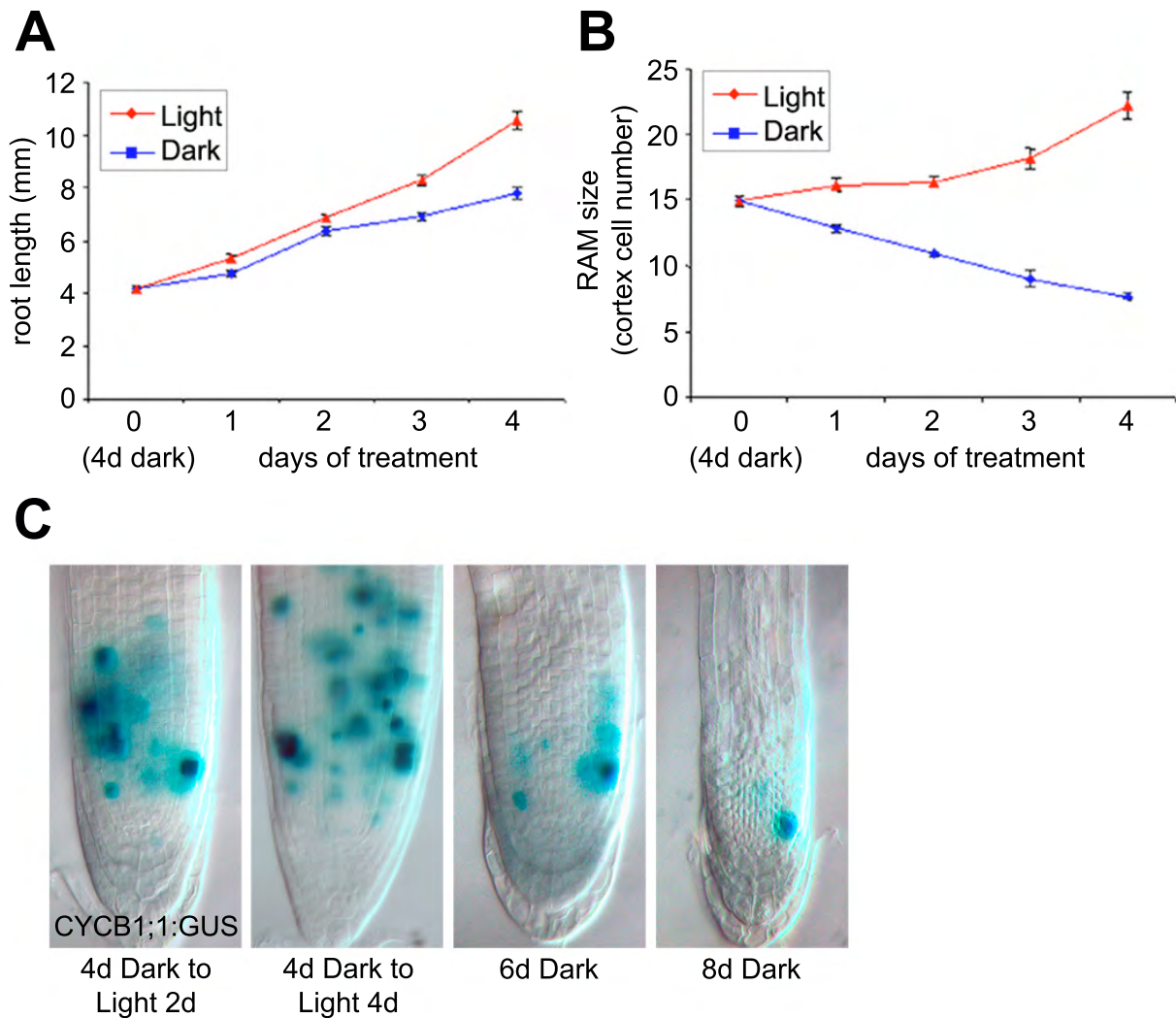


Fig. S2. Darkness reduces root growth in a reversible manner. (A,B) Primary root length (A) and RAM size (B) of WT seedlings grown for 4 days in the dark and either exposed to light or kept in the dark for additional 4 days. Measurements were taken at the indicated times. Error bars represent s.e.m. dag, days after germination. (C) CYCB1;1:GUS staining in seedling roots grown as indicated. The exposure of etiolated seedlings to light induces CYCB1;1:GUS expression and leads to a replenishment of the RAM, thereby allowing the recovery of root growth.

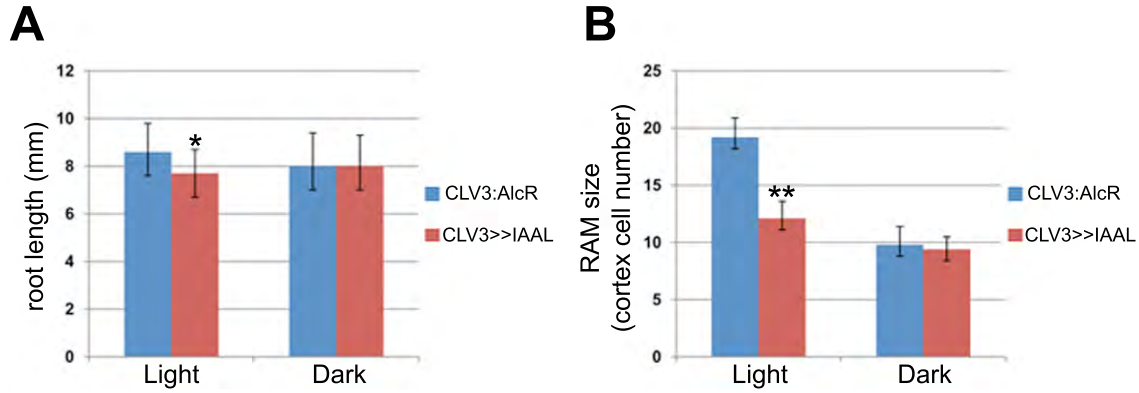


Fig. S3. Inhibition of STRAT results in reduced RAM size. (A,B) Primary root length (A) and RAM size (B) of 4-day-old light- and dark-grown CLV3:AlcR and CLV3>>IAAL seedlings upon induction by ethanol. Error bars represent s.d. * $P < 0.05$, ** $P < 0.01$, t -test.

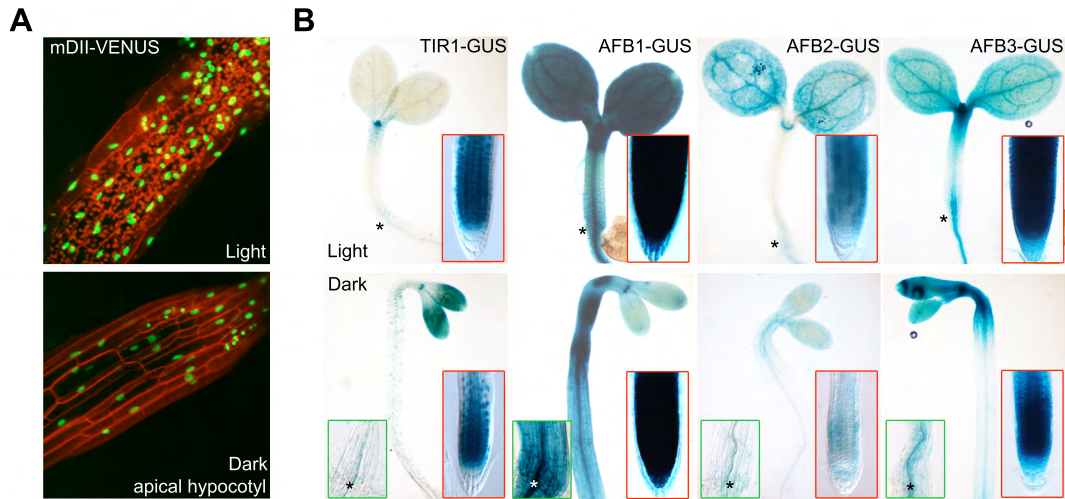


Fig. S4. Controls for DII-VENUS. (A) mDII-VENUS (green channel) expression in 4-day-old seedlings grown in light and in the dark. The pictures show that the mDII-VENUS auxin-insensitive version of the sensor is expressed in tissues where DII-VENUS is not detected, such as light-grown hypocotyls and the apical region of etiolated hypocotyls. Red channel, FM4-64 staining. (B) Expression patterns of TIR1:TIR1-GUS, AFB1:AFB1-GUS, AFB2:AFB2-GUS and AFB3:AFB3-GUS in 4-day-old seedlings grown in light or in the dark. Red-bordered insets: marker expression in the root apices of light- and dark-grown seedlings. Green-bordered insets: marker expression in the basal region of etiolated hypocotyl. Asterisks indicate the shoot-root junction. Samples were stained for 24 hours.

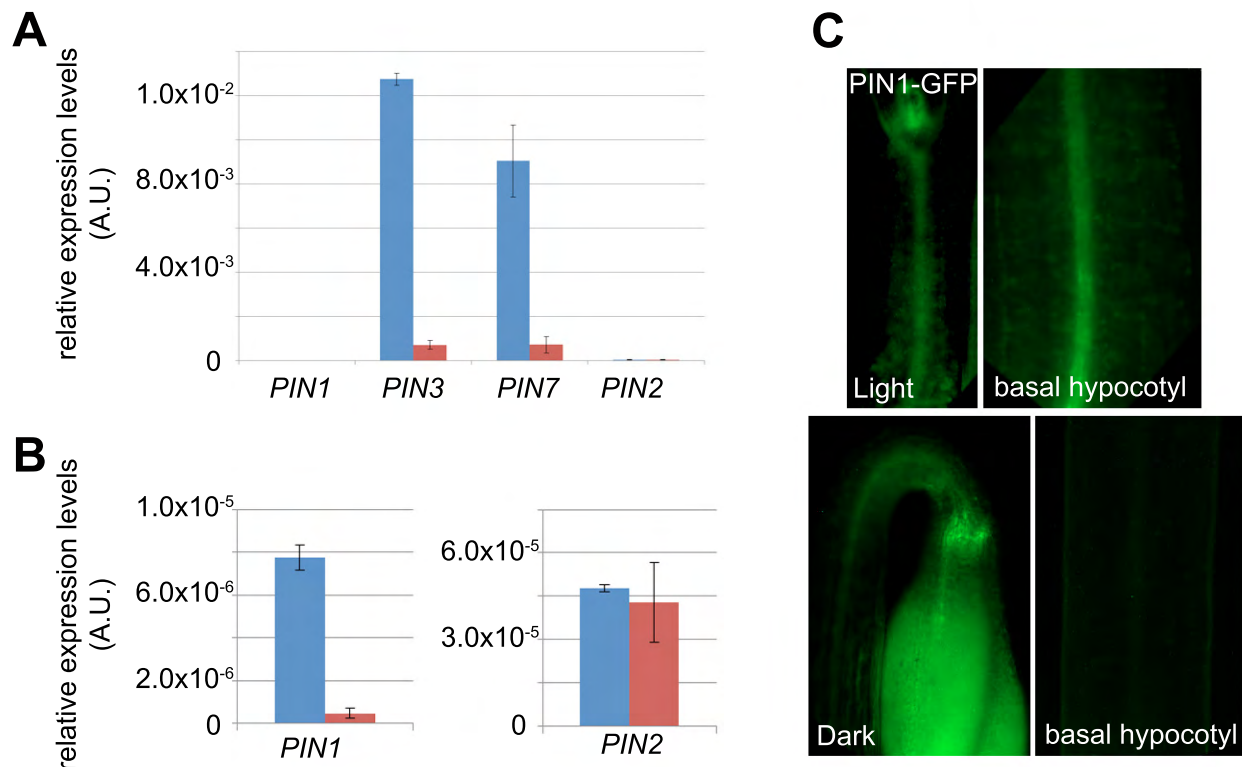


Fig. S5. Light regulation of *PIN* expression. (A) Expression levels of *PIN1*, *PIN2*, *PIN3* and *PIN7* determined by qRT-PCR on RNAs extracted from shoot tissues of 4-day-old seedlings grown in the light and in the dark. Note the low level of *PIN1* and *PIN2* relative expression compared with *PIN3* and *PIN7*. A.U., arbitrary units. (B) Expression levels of *PIN1* and *PIN2* determined as described for A, represented with the correct scales. Note that *PIN1*, but not *PIN2*, is strongly induced in the shoot tissues of light-grown seedlings compared with dark-grown ones. A.U., arbitrary units. (C) Epifluorescence pictures showing the expression of pPIN1:PIN1-GFP in whole-mount light and dark-grown hypocotyls. Pictures on the right show details of basal region of the hypocotyl. Note the lack of PIN1-GFP expression in the basal region of etiolated hypocotyls.

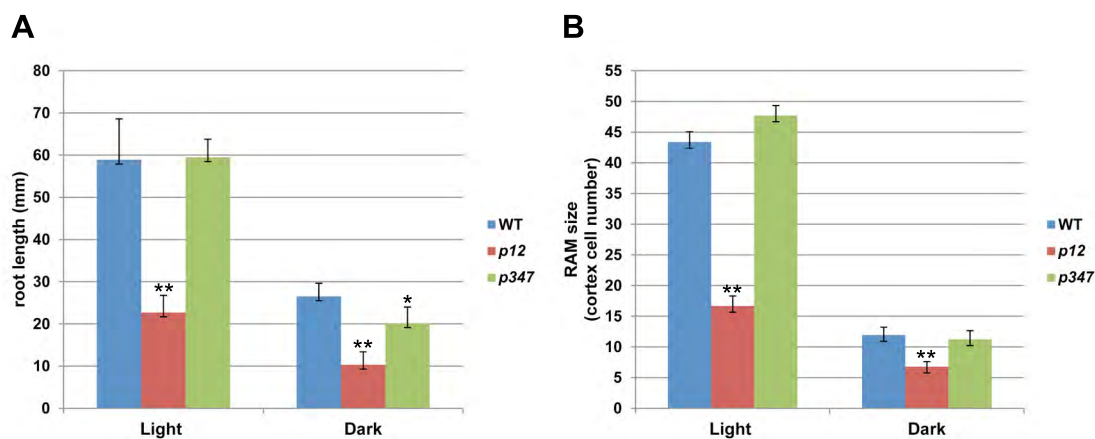


Fig. S6. A key role for *PIN1* and *PIN2*, but not *PIN3*, *PIN4* and *PIN7*, in the control of root growth. (A,B) Primary root length (A) and RAM size (B) of 6-day-old WT, *p12* and *p347* seedlings. Error bars represent s.d. * $P < 0.05$, ** $P < 0.01$, *t*-test.

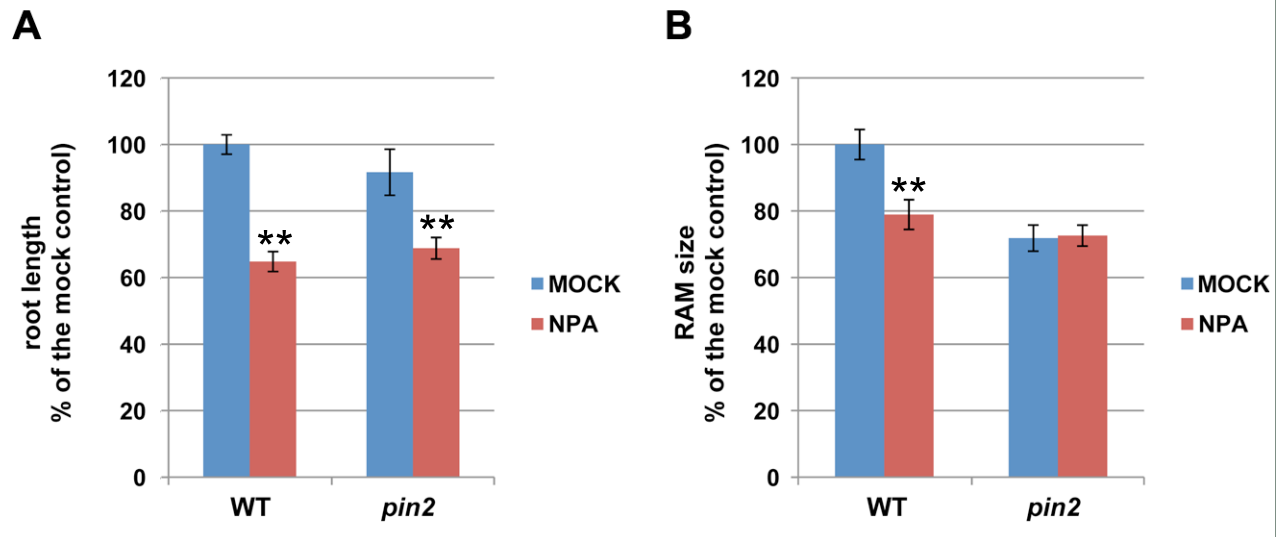


Fig. S7. The RAM of *pin2* mutants is less sensitive to NPA. (A,B) Primary root length (A) and RAM size (B) in 7-day-old light-grown WT and *pin2* (*eir1*) seedlings with and without (MOCK) NPA application on the hypocotyls. Data are expressed as percentage of the mock control, arbitrarily set to 100. Error bars represent s.e.m. ** $P < 0.01$, *t*-test.

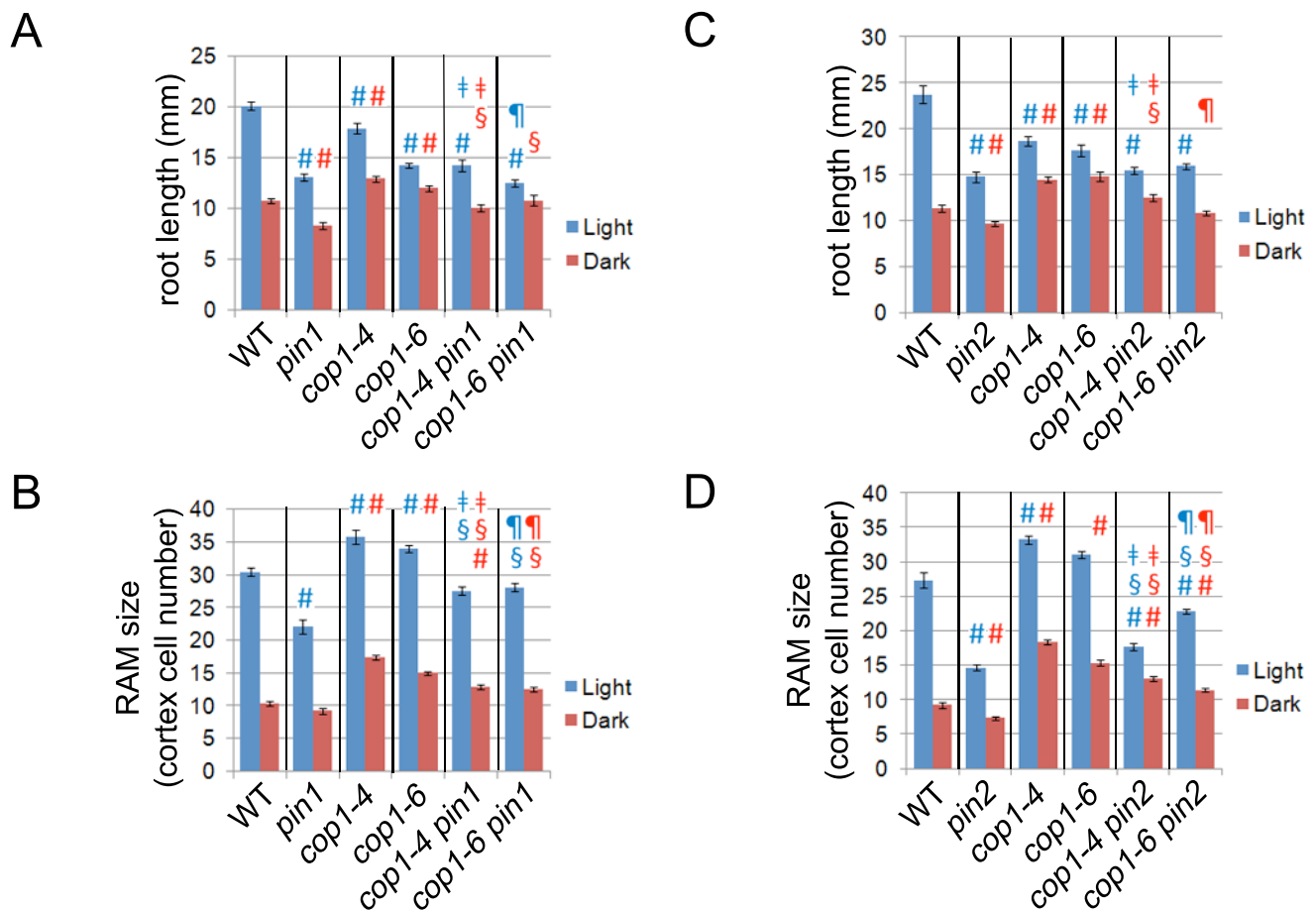


Fig. S8. *COPI1* acts together with both *PIN1* and *PIN2* to regulate root growth. (A-D) Primary root length (A,C) and RAM size (B,D) of 8-day-old WT, *pin1*, *cop1-4*, *cop1-6*, *cop1-4 pin1*, *cop1-6 pin1*, *pin2*, *cop1-4 pin2* and *cop1-6 pin2* plants. Error bars represent s.e.m. The significance of the differences were assessed using *t*-tests and *P*-values were corrected with Bonferroni correction ($\alpha = 26$). Differences between light and dark for a given genotype were all significant ($P < 0.05$), except for root length of *cop1-6 pin1* (panel A). Symbols highlight significant differences between genotypes ($P < 0.05$) in the light (blue) or in the dark (red): #, genotype versus WT; §, genotype versus *pin1* (A,B) or *pin2* (C,D); ‡, genotype versus *cop1-4*; ¶, genotype versus *cop1-6*.

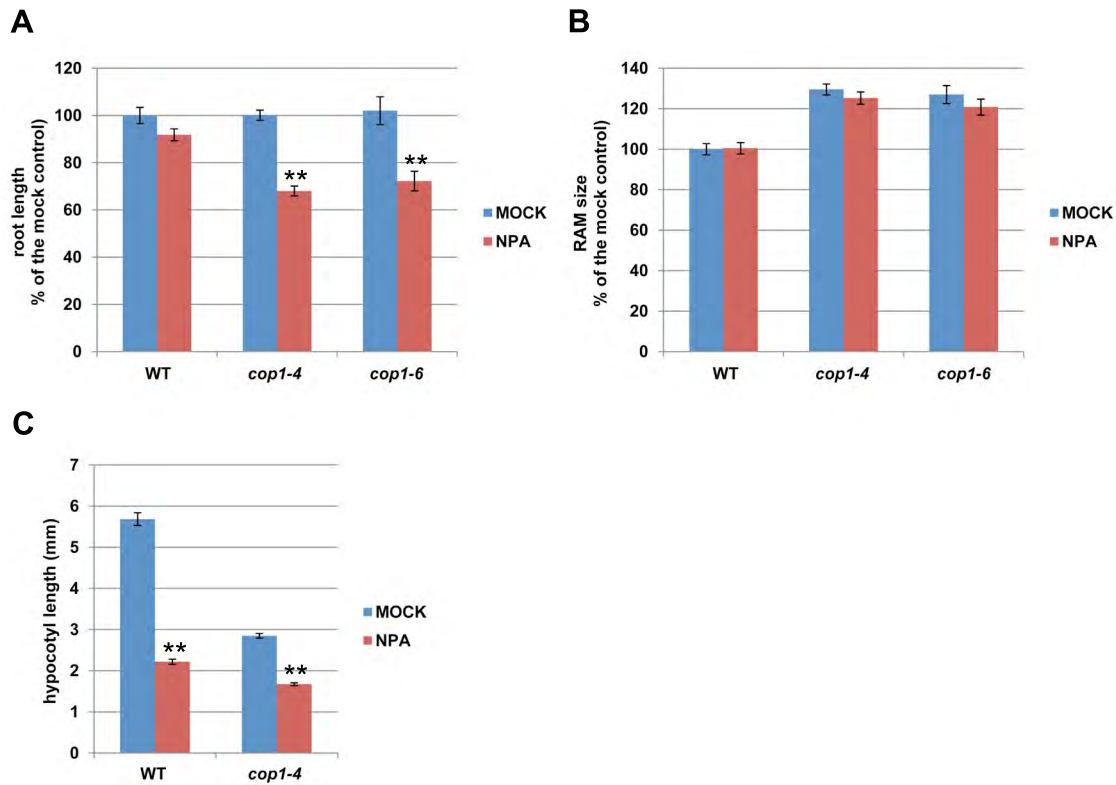


Fig. S9. COP1 regulates shoot-to-root PAT and root growth. (A,B) Primary root length (A) and RAM size (B) in 9-day-old dark-grown WT, *cop1-4* and *cop1-6* seedlings with and without (MOCK) NPA application on the hypocotyls. Data are expressed as percentage of the mock control, arbitrarily set to 100. Error bars represent s.e.m. (C) Hypocotyl length in 9-day-old dark-grown WT and *cop1-4* seedlings with and without (MOCK) NPA application on the hypocotyls. Note that NPA effectively inhibited hypocotyl elongation in both WT and *cop1* mutants. Error bars represent s.e.m. ** $P < 0.01$, *t*-test.

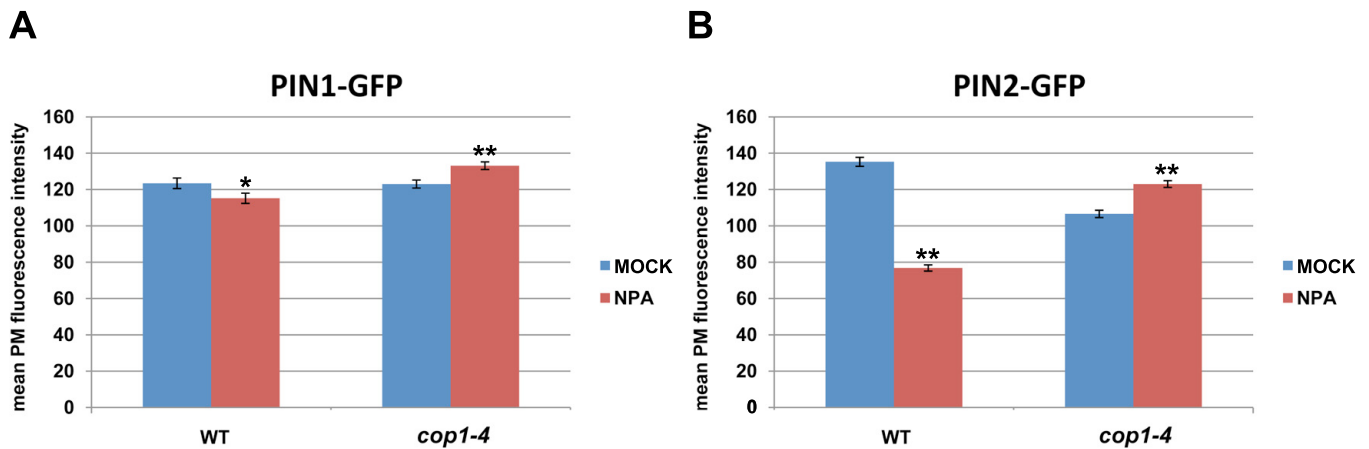


Fig. S10. COP1 function in the root regulates PIN1 and PIN2 stability. (A,B) Quantification of PM intensity of PIN1-GFP (A) and PIN2-GFP (B) in 7-day-old WT and *cop1-4* seedling roots with and without (MOCK) NPA application on the hypocotyls. Note that NPA application at the hypocotyls of *cop1-4* seedlings did not cause any reduction in the amounts of PM-localized PIN1-GFP and PIN2-GFP as observed for WT plants. Error bars represent s.e.m. * $P < 0.05$, ** $P < 0.01$, *t*-test.

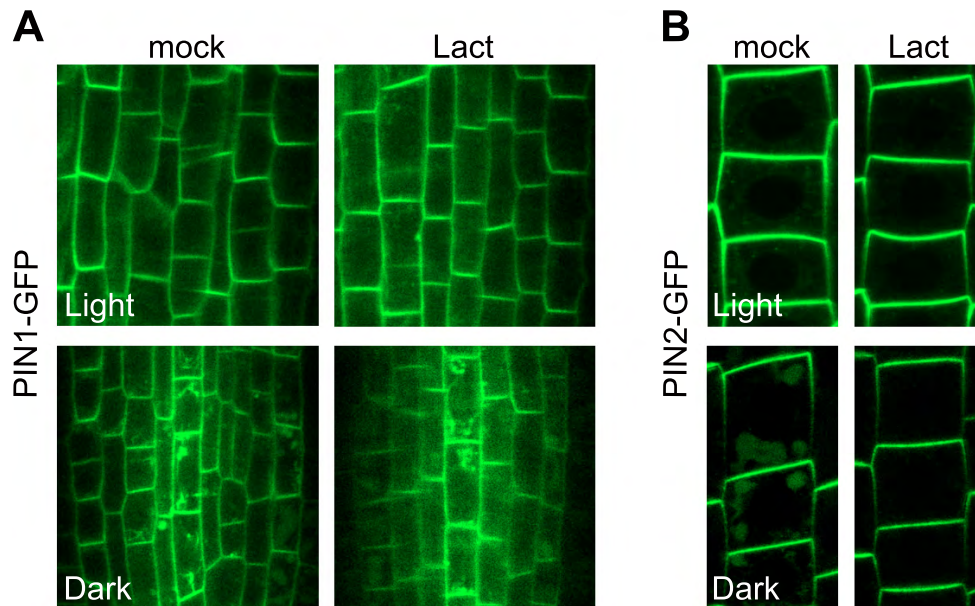


Fig. S11. The vacuolar targeting of PIN2-GFP, but not that of PIN1-GFP, is blocked by proteasome inhibition. (A) PIN1-GFP localization in RAMs of 5-day-old light- and dark-grown seedlings untreated (mock) or treated with 50 μ M lactacystin for 4 hours (Lact). (B) PIN2-GFP localization in RAMs of 5-day-old light- and dark-grown seedlings untreated (mock) or treated with 50 μ M lactacystin for 4 hours (Lact).

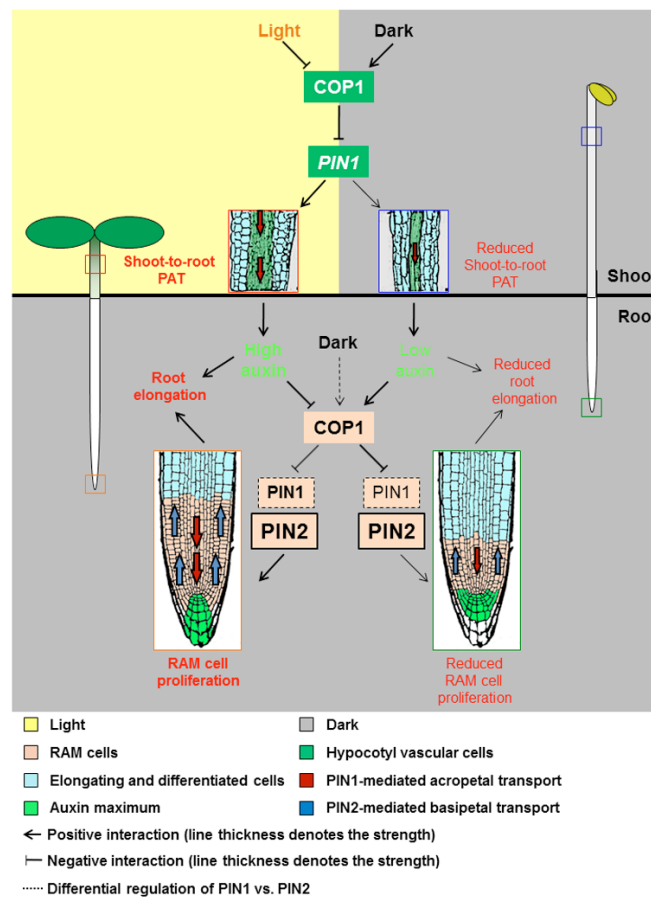


Fig. S12. Model for COP1-mediated coordination of root and shoot growth to changing light environments. The master photomorphogenesis repressor COP1 coordinates *Arabidopsis* root and shoot growth in response to changes of light condition. COP1 function in the shoot regulates shoot-to-root PAT by controlling the transcription of the auxin efflux carrier gene *PIN1*, resulting in an appropriately tuned level of shoot-derived auxin in the root, which directly influences primary root elongation and also adapts auxin transport and cell proliferation in the RAM by modulating root COP1-dependent PIN1 and PIN2 stability, thus permitting rapid and precise modulation of root growth in changing light environments.

Table S1. List of mutants and transgenic lines used in this study

Line	Ecotype	Reference
<i>pin1-1</i>	Originally derived from the En-2, backcrossed many times into Col-0	Okada et al., 1991
<i>pin1-6</i>	Ws-2	Vernoux et al., 2000
<i>pin2 (eir1-1)</i>	Col-0	Luschnig et al., 1998
<i>pin3-5</i>	Col-0	Benková et al., 2003
<i>pin7-1</i>	Ler	Benková et al., 2003
<i>pin1 pin2</i>	Col-0	Blilou et al., 2005
<i>pin3 pin4 pin7</i>	Col-0	Blilou et al., 2005
<i>cop1-1</i>	Col-0	McNellis et al., 1994
<i>cop1-4</i>	Col-0	McNellis et al., 1994
<i>cop1-6</i>	Col-0	McNellis et al., 1994
CYCB1;1:GUS	Col-0	Colon-Carmona et al., 1999
CLV3:AlcR	Ws-2	Deveaux et al., 2003
PIN1:GUS	Col-0	Vieten et al., 2005
PIN2:GUS	Col-0	Vieten et al., 2005
PIN3:GUS	Col-0	Vieten et al., 2005
PIN7:PIN7-GUS	Col-0	Vieten et al., 2005
DR5:GUS	Col-0	Ulmasov et al., 1997
DR5rev:GFP	Col-0	Friml et al., 2003
35S:DII-VENUS	Col-0	Brunoud et al., 2012
35S:mDII-VENUS	Col-0	Brunoud et al., 2012
PIN1:PIN1-GFP	Col-0	Xu et al., 2006
PIN2:PIN2-GFP	Col-0	Xu and Scheres, 2005
VAM3:mRFP-VAM3 PIN1:PIN1-GFP	Col-0	Ebine et al., 2008; Shirakawa et al., 2009
SCR:H2BYFP	Col-0	Heidstra et al., 2004
SHR-GFP	Col-0	Nakajima et al., 2001
WOX5:ERGFP	Col-0	Blilou et al., 2005
PLT2-GFP	Col-0	Aida et al., 2004
TIR1:TIR1-GUS	Col-0	Parry et al., 2009
AFB1:AFB1-GUS	Col-0	Parry et al., 2009
AFB2:AFB2-GUS	Col-0	Parry et al., 2009
AFB2:AFB2-GUS	Col-0	Parry et al., 2009

Table S2. qPCR primers used in this study

Gene	Agi Code	Left primer sequence	Right primer sequence	Amplicon length (nt)	UPL	Primer efficiency
<i>PIN1</i>	At1g73590	cctcaggggaatagtaacgaca	tcacgtctttgttaccgaaact	71	103	1.891
<i>PIN2</i>	At5g57090	ggcgaagaaagcaggaaga	ggtgggtacgacggaaca	78	29	1.792
<i>PIN3</i>	At1g70940	cccagatcaatctcacaacg	ccggcgaaactaaattgtg	90	42	1.904
<i>PIN7</i>	At1g23080	tgggctcttgttctttca	tcacccaaactgaacattgc	110	159	1.843

The table show primers couples, amplicon length, UPL probes as obtained by the software ProbeFinder by Roche Diagnostic (v 2.41; <http://qpcr.probefinder.com/organism.jsp>) with the intron-spanning assay option checked on. Primer efficiencies were calculated with the LightCycler480 software (Roche) on the basis of standard curves obtained by serially diluting a cDNA obtained from 4 day-old light-grown seedlings. Details on the ACTIN2 (Eff = 1.80) primer sequences and UPL can be found in Ciarbelli et al. (Ciarbelli et al., 2008).