Auxin methylation is required for differential growth in *Arabidopsis*

Restriction of polar auxin transport by IAA methylation is necessary for correct differential growth in *Arabidopsis*

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Auxin gradients are instrumental for the differential growth that causes organ bending upon tropic stimuli and curvatures during plant development[1,2]. Local differences in auxin concentrations are mainly achieved by polarized cellular distribution of PIN auxin transporters[3,4] (Adamowski and Friml TPC), but it is not clear if other mechanisms involving auxin homeostasis are also relevant for the formation of auxin gradients. Here we show that auxin methylation is required for asymmetric auxin distribution across the hypocotyl, in particular during its response to gravity. We found that loss-of-function mutants in Arabidopsis *IAA CARBOXYL METHYLTRANSFERASE1* (*IAMT1*) prematurely open the apical hook and their hypocotyls are impaired in gravitropic reorientation. This defect is linked to an increased polar auxin transport and to the lack of asymmetric distribution of PIN3 in the *iamt1* mutant, which presumably causes the accumulation of auxin on either side of the gravistimulated hypocotyl. Partial inhibition of polar auxin transport in the *iamt1* mutant resulted in the restoration of normal gravitropic reorientation. We propose that IAA methylation is necessary to restrict polar auxin transport within the range of auxin levels that allow differential responses.

The plant hormone auxin has long been known to act not only as a key morphogenetic component of differentiation pathways, but also as a coordinator of plant growth in response to environmental stimuli[5,6]. Particularly interesting is the involvement of auxin in the generation of curvatures, such as the apical hook of etiolated seedlings[1,2], and in the reorientation of organ growth upon lateral illumination or in response to gravity[7-9]. An essential feature that explains

the relevant role of auxin in these processes is the robust mechanism that directs the movement of this hormone through the plant, known as polar auxin transport (PAT)[10-12]. Among other consequences, PAT allows the establishment of asymmetric distribution of auxin, which results in differential triggering of auxin responses in different parts of a given organ.

In the case of tropic responses, such as phototropism and gravitropism, it has been estimated that the gradient across the hypocotyl may range between 1.5- and a two-fold difference[13-15], similar to the two-fold difference in the root tip that triggers gravitropic reorientation[16]. The fact that this small difference is enough to cause differential growth responses implies that the levels of auxin must be well maintained within a very specific range, to ensure that this gradient is informative. Although the regulation of the expression, tissue distribution and cellular localization of the PINFORMED (PIN) auxin efflux is presumably the most important mechanism for the maintenance of auxin gradients[3,4], it is possible that other mechanisms such as the regulation of auxin homeostasis are also contributing to this effect[17-19].

In Arabidopsis ectopic overexpression of *IAMT1* disrupts gravitropic responses[20]. The specificity of IAMT1 on IAA has been demonstrated for the orthologs in rice and Arabidopsis[21,22]. It has been reported that silencing of *IAMT1* in Arabidopsis using an RNAi strategy causes a dramatic phenotype that might be explained by simultaneous repression of additional members of the SABATH family[20], which includes methyltransferases for jasmonic acid and other substrates (Fig. 1a). Currently, there is no indication about the physiological relevance of IAA methylation in the generation of auxin gradients. In this manuscript we show that conversion of indole-3-acetic acid (IAA) into

Methyl-IAA (Me-IAA) by an IAA CARBOXYL METHYLTRANSFERASE (IAMT) is relevant. Therefore, we selected two T-DNA insertion lines (*iamt1-1* and *iamt1-2* in Col and L*er* backgrounds, respectively) the first one of which would putatively render a truncated version of IAMT1 lacking part of the active site[20] (Supplementary Fig. 1a). Hormone quantification in etiolated seedlings (Fig. 1b) and in light-grown seedlings (Supplementary Fig. 1b) showed that there was at least a 50% decrease in the levels of Me-IAA in the *iamt1-1* mutant, confirming *in vivo* that *IAMT1* encodes an IAA methyltransferase and suggesting that other methyltransferases can also act on IAA, or alternatively that the truncated protein encoded by the *iamt1-1* allele might retain some activity. It is also important to remark that the decrease in Me-IAA was not accompanied by a significant increase in free-IAA levels (Fig. 1b), indicating that Me-IAA represents a small proportion in the total IAA pool, in accordance with the observation that *iamt1-1* mutant plants do not display any obvious morphological defects that resemble IAA overaccumulation (Supplementary Fig. 1c).

To investigate if a reduction in IAA methyltransferase activity has an impact in the formation of auxin gradients, we first examined the dynamics of apical hook development and the hypocotyl response to a gravitropic reorientation, two processes that involve auxin-dependent differential growth [2,7,8]. The *iamt1-1* mutant did not display any severe defect in the formation or maintenance of the apical hook showing only slightly faster opening of the hook (Fig. 1c). In contrast, the ability of the mutant hypocotyls to reorient after gravistimulation was largely impaired (Fig. 1d). Importantly, this different behavior of *iamt1-1* with respect to the two processes was correlated with the ability of the mutant to

generate auxin gradients across the hypocotyl in each situation. The asymmetry in the activity of the auxin signaling reporter DR5::GFP across the apical hook was similar in three-day-old etiolated wild-type and *iamt1-1* mutant seedlings, despite the reporter signal being overall higher in the mutant hook (Supplementary Figure 2), indicating that there is no apparent defect in the differential auxin distribution during hook formation in etiolated *iamt1-1* seedlings. Nonetheless, the seemingly high auxin levels in the mutant apical hook might be the cause of its premature opening (Fig. 1c). On the other hand, gravistimulation of *iamt1-1* mutant hypocotyls did not provoke the typical accumulation of the DR5 reporter observed on the lower side of wild-type hypocotyls [8], but the increased signal was observed on both sides (Fig. 2a,b). This defect in the asymmetric auxin response was very likely caused by the inability of the *iamt1-1* mutant to differentially accumulate auxin, as indicated by the loss of signal of more direct auxin reporter, DII-Venus on both sides of the hypocotyl (Fig. 2c,d).

The observation that the amount of free IAA in whole seedlings was not altered in the *iamt1-1* mutant (Fig. 1b) but there are defects in local auxin distribution upon gravistimulation (Fig. 2) suggests that IAA methylation may be relevant for the regulation of PAT. In fact, auxin transport along the hypocotyl, measured using ^{3}H-IAA, was two-fold higher in the *iamt1-1* mutant as compared to the wild type (Fig. 3a). In both cases, transport was inhibited by incubation with 1-naphthylphthalamic acid (NPA), confirming that enhanced IAA movement was due to increased PAT. To investigate if the observed increase in the IAA transport in *iamt1-1* correlates with the agravitropic phenotype of the mutant, we measured PAT in wild-type and mutant seedlings with or without

gravistimulation. Interestingly, PAT was enhanced in the wild type and in the *iamt1-1* mutant after reorientation (Fig. 3b). Next, we assayed the capacity of wild-type and mutant seedlings to reorient in the presence of NPA. As expected, the gravitropic reorientation of the hypocotyls of wild-type seedlings was gradually reduced with increasing doses of NPA (Fig. 3c). In contrast, low NPA doses promoted reorientation of *iamt1-1* seedlings and only a high concentration, 10 μM, of NPA abolished it (Fig. 3c). Remarkably, the same amount of NPA restored both the reorientation ability and auxin transport to wild-type levels in the mutant (Fig. 3a,c). Collectively, these results suggest that there is a causal connection between the increased PAT in the *iamt1-1* mutant and its agravitropic phenotype.

The confirmation that a reduction of PAT alleviates the agravitropic phenotype of the *iamt1-1* mutant suggests that PAT restriction by IAA methylation is an important element in the generation of lateral auxin gradients. Given that auxin has been proposed to indirectly regulate its own transport [23-25], we hypothesized that local alterations in IAA methylation would impact the expression of *PIN* genes. To test this hypothesis, we measured the transcript levels of *PIN1*, *PIN2*, *PIN3* and *PIN7* in 3-day-old etiolated seedlings and found that their expression level was at least two-fold higher in *iamt1-1* mutants (Fig. 3d and Supplementary Fig. 3). Moreover, transcriptional regulation of *PIN* genes by IAMT1 activity may be physiologically relevant for the formation of auxin gradients, since gravistimulation provoked not only an increase in PAT in wild-type seedlings (Fig. 3b), but also an increase in the expression of *PIN1* and *PIN3*, albeit with different kinetics (Fig. 3d), and to a lesser extent of *PIN2* and *PIN7* (Supplementary Fig. 3). More importantly, the expression of *PIN* genes in

the *iamt1-1* mutant followed the same transient induction upon reorientation as in the wild type, but with higher transcript levels (Fig. 3d and Supplementary Fig. 3).

The increase in *PIN3* gene expression in the *iamt1-1* mutant also resulted in higher levels of PIN3 protein, as indicated by the comparably stronger GFP signal in *PIN3::PIN3-GFP* lines throughout the mutant hypocotyls (Fig. 4). Gravistimulation of wild-type hypocotyls provokes the gradual asymmetrical re-distribution of PIN3 to the lower side of endodermal cells[8] (Rakusova CB 2016) being proposed as a major mechanism how the gravity vector is translated into the directional auxin fluxes both in roots and shoots (Rakusova COPB). However, we observed that the PIN3-GFP signal in the *iamt1-1* mutant remained in the upper side of endodermal cells even six hours after gravistimulation (Fig. 4). This explains the mutant defects in both the gravity-mediated asymmetric auxin distribution and hypocotyl bending (Fig) and is in agreement with the extended auxin accumulation in the upper half of reoriented hypocotyls in the mutant (Fig. 2).

These results suggest that IAA methylation participates in the establishment of adequate rates of PAT along the hypocotyl, this function being especially relevant during the gravitropic response. If Me-IAA is simply an inactive form of IAA, methylation could be a fine-tuning mechanism to correct local concentrations of auxin at or near the sources of auxin. In favor of this model, higher levels of *IAMT1* expression coincide with auxin accumulation tissues in the seedling, like cotyledons[20], and the upregulation of *IAMT1* expression by auxin could act as a negative feedforward loop to maintain auxin homeostasis. On the other hand, it cannot be ruled out that Me-IAA itself has a direct role as a

modulator of auxin signaling or transport. In order to evaluate this, we resorted to orthogonal systems, allowing the assessment of these processes in a context devoid of other endogenous components that may potentially affect the study. We first reconstructed the IAA perception complex in mammalian cells to observe the event of perception using a ratiometric sensor for auxin[26]. Results showed that Me-IAA neither mimicked the response to IAA in the activity of the auxin coreceptor complex, nor did it interfere with the response triggered by IAA (Supplementary Fig. 4). Similarly, the presence of intracellular Me-IAA in transport assays using Xenopus oocytes[27], reduced PIN1- and PIN3-mediated IAA efflux to the same extent as IAA itself (Supplementary Fig. 5), suggesting Me-IAA can be transported by PINs and compete with IAA and that it is rather unlikely that it acts as an allosteric inhibitor of these transporters.

In summary, we propose that IAA methylation is a biologically relevant mechanism for the maintenance of auxin homeostasis and the correct generation of the auxin gradient that directs differential growth responses under certain circumstances, such as during gravitropic reorientation.

Methods

Plant material and growth conditions. *Arabidopsis thaliana* ecotype Col-0 was used as wild-type. Published transgenic and mutant lines were used: *PIN3::PIN3-GFP, 35S::DII-VENUS*[28] and *DR5rev::GFP. PIN3::PIN3-GFP, DII-VENUS* and *DR5::GFP* were introgressed into the *iamt1-1* mutant background by crossing. The *iamt1-1* described in this work corresponds to the T-DNA insertion line SALK_072125 [29]. This line was genotyped with *IAMT1*-specific oligonucleotides and with an oligonucleotide specific for the T-DNA left border (Supplementary Table 1). The presence of transgenes in progenies of crosses was determined by the corresponding antibiotic resistance when possible, and by genotyping (Supplementary Table 1).

Seeds were sown on ½ MS plates with 1 % (w/v) sucrose, 8 g/L agar, pH 5.8. Seeds were stratified for 3 days at 4°C, exposed to light for 6-8 h at 20°C, and then cultivated in the dark. For experiments that include chemicals, wild-type and *iamt1-1* seedlings were grown for 3 days in darkness and then transfered to medium containing 0.125, 0.25, 0.5, 1 and 10 µM NPA (Sigma) for 2 h before rotating the plate 90º for 12 h.

***In vivo* plant imaging.** Apical hook development and gravitropic reorientation were monitored as previously described[30,31]. For the analysis of the gravity response, 3-day-old etiolated seedlings grown in vertical plates were imaged at 1 h intervals for 16 h after rotating the plate 90º. Hypocotyl angles were measured by ImageJ. Three replicates of at least 10 seedlings with a synchronized germination start were processed.

Confocal imaging and signal quantification. Seedlings were rinsed first for 2 min with 10 µg/ml propidium iodide (PI) and then 5 min with water. Fresh stained seedlings were mounted on slides only with water. Images were taken with a Zeiss 780 Axio Observer confocal microscope for *DR5::GFP* and with a Zeiss LSM 800 for *DII-VENUS* and *PIN3::PIN3-GFP*. For GFP and VENUS detection, channel 1 was configured between 500-540 nm; and for PI detection, channel 2 was configured between 590-660 nm.

Fluorescence intensity was measured in the apical hook and in the bent region of the hypocotyl. The *DII::VENUS* and *DR5rev::GFP* fluorescence intensity was compared between the inner and outer sides of the apical hook, and the lower and upper sides of the hypocotyl in the responsive part as described previously[8]. For quantification of the gravity-induced PIN3-GFP relocation, the PIN3-GFP fluorescence intensity was compared between the external sides of endodermal cells as described previously[8]. ImageJ software was used for all intensity measurements. Three replicates of at least 10 seedlings of similar size were processed. The presented value is the mean of the averages. T-test was used for statistic evaluation. Error bars in graphs represent SE.

Real-time quantitative RT-PCR. Total RNA from 3-day-old etiolated seedlings was extracted using the RNAeasy Plant Mini Kit (Qiagen). cDNA synthesis and

quantitative PCR, as well as primer sequences for amplification of *PIN1*, *PIN2*, *PIN3, PIN7*, *IAMT1* and *EF1α* genes, have been described previously [32,33].

Auxin transport assay. Three-day-old etiolated seedlings grown on vertical plates containing control medium were transplanted for 6 h to plates with mock or with NPA at the indicated concentrations. The upper half of seedlings was placed on top of a small strip of parafilm and a droplet containing 6.75 nM [^{3}H]-IAA (specific activity 25 Ci/mmol, 1 µCi/µl; Amersham) in 0.1 % tween-20 (Sigma) was applied to cotyledons for 3 h, after which seedlings were either allowed to grow straight for 6 more hours or plates were rotated 90° for the same time. The lowest 5 mm of the hypocotyl was collected and radioactivity was measured using previously described methods [34].

IAA and MeIAA quantification. Whole seedlings were immediately frozen in liquid N_2. Approximately 100 mg of tissue was pooled per sample and at least 3 biological replicates were harvested for each independent experiment. 1 ml of methanol and 50 pmol of [$^2H_2$]-IAA or 100 pmol [$^2H_5$]-MeIAA was added, heated for 2 minutes at 60 °C, followed by a further incubation without heating for at least 1 hour. The sample was taken to complete dryness.

For purification of IAA and MeIAA, the sediments were dissolved in 2 ml cold sodium phosphate buffer (50 mM, pH 7.0) containing 5% MeOH, followed by a 10 min ultrasonic treatment (Branson B5510DTH, Branson Ultrasonics, Dunbury, USA). Next, the pH was adjusted to 2.5 with 1 M hydrochloric acid, and the sample was purified by solid-phase extraction using 1 ml/30 mg Oasis™ HLB columns (Waters Corporation, Milford, USA) conditioned with 1 ml methanol and 1 ml water, and equilibrated with 0.5 ml sodium phosphate buffer (acidified with 1 M hydrochloric acid to pH 2.5). After sample application, the column was washed twice with 1 ml 5% methanol and then eluted with 2 ml 80% methanol. The elution fraction was taken to complete dryness by using a vacuum concentrator (Vacufuge® plus, Eppendorf, Hamburg, Germany). 20 µl of N,O-bis(trimethylsilyl) trifluoroacetamide containing 1% trimethylchlorosilane (BSTFA+TMCS, 99:1 (v/v), Supelco, Bellefonte, USA) were added to each extract. The extracts were transferred into 400 µl GC-MS vials and incubated for 70 min at 60º C. To analyze IAA and Me-IAA contents in the same samples, 1 µL of each sample was injected split less by a CombiPAL autoinjector (CTC

Analytics, Zwingen, Switzerland) into a Bruker Scion-455 gas chromatograph (BRUKER Daltonics, Bremen, Germany) equipped with a 30 m x 0.25 mm i.d. fused silica capillary column with a chemical bond 0.25-µm ZB35 stationary phase (Phenomenex, Torrance, USA). Helium at a flow rate of 1 mL/min served as the mobile phase. A pressure pulse of 25 psi over 1 min was used to force the transfer of compounds from the injector into the column. The injector temperature was 250ºC and the column temperature was held at 50ºC for 1.20 min. Thereafter, the column temperature was increased by 30º C/min to 120ºC. After reaching 120º C, the temperature was further increased by 10º C/min to 325º C and held at that temperature for another 5 min. The column effluent was introduced into the ion source of a Bruker Scion-TQ triple quadruple mass spectrometer. The mass spectrometer was used in EI-MRM mode. The transfer line temperature was set to 250 ºC and the ion source temperature to 200ºC. Ions were generated with -70 eV at a filament emission current of 80 µA. The dwell time was 100 ms, and the reactions m/z 247 to m/z 130 (endogenous IAA), m/z 249 to m/z 132 ([$^2H_2$]-IAA, internal standard), m/z 261 to m/z 202 (endogenous Me-IAA), and m/z 266 to m/z 207 ([$^2H_5$]-Me-IAA, internal standard) were recorded. Argon set at 1.5mTorr was used as the collision gas. The amount of the endogenous compound was calculated from the signal ratio of the unlabeled over the stable isotope-containing mass fragment observed in the parallel measurements.

Mammalian cell culture orthogonal platform for determination of Me-IAA and IAA signaling with an IAA sensor. Human embryonic kidney 293-T cells (HEK-293T) were cultivated and transfected as described previously (Wend et al 2013). For transfection of each well, 0.55 µg of a plasmid encoding rice TIR1 and 0.2 µg of a plasmid harbouring a ratiometric luminescent auxin sensor (with full length Arabidopsis AUX/IAA17 as the sensor module) was mixed and diluted in 50 µl of OptiMEM (Life Technologies, US) and subsequently mixed with 2.5 µl of PEI solution while vortexing. After 15 min at room temperature this mixture was added to the cells in a dropwise manner. To induce auxin-mediated protein degradation, appropriate IAA and MeIAA dilution series were prepared in DMEM and added to the cells 24 h post transfection and incubated for 3.5 h

prior to firefly and renilla luminescence analysis, completed as described previously[26].

References

1 Abbas, M., Alabadi, D. & Blazquez, M. A. Differential growth at the apical hook: all roads lead to auxin. *Front Plant Sci* **4**, 441 (2013).

2 Zadnikova, P. *et al.* Role of PIN-mediated auxin efflux in apical hook development of Arabidopsis thaliana. *Development* **137**, 607-617 (2010).

3 Grieneisen, V. A., Xu, J., Maree, A. F., Hogeweg, P. & Scheres, B. Auxin transport is sufficient to generate a maximum and gradient guiding root growth. *Nature* **449**, 1008-1013 (2007).

4 Kramer, E. M. PIN and AUX/LAX proteins: their role in auxin accumulation. *Trends Plant Sci* **9**, 578-582 (2004).

5 Vanneste, S. & Friml, J. Auxin: a trigger for change in plant development. *Cell* **136**, 1005-1016 (2009).

6 Woodward, A. W. & Bartel, B. Auxin: regulation, action, and interaction. *Ann Bot (Lond)* **95**, 707-735 (2005).

7 Friml, J., Wisniewska, J., Benkova, E., Mendgen, K. & Palme, K. Lateral relocation of auxin efflux regulator PIN3 mediates tropism in Arabidopsis. *Nature* **415**, 806-809 (2002).

8 Rakusova, H. *et al.* Polarization of PIN3-dependent auxin transport for hypocotyl gravitropic response in Arabidopsis thaliana. *Plant J* **67**, 817-826 (2011).

9 Spalding, E. P. Diverting the downhill flow of auxin to steer growth during tropisms. *Am J Bot* **100**, 203-214 (2013).

10 Muday, G. K. & DeLong, A. Polar auxin transport: controlling where and how much. *Trends Plant Sci* **6**, 535-542 (2001).

11 Swarup, R. & Bennett, M. Auxin transport: the fountain of life in plants? *Dev Cell* **5**, 824-826 (2003).

12 Blakeslee, J. J., Peer, W. A. & Murphy, A. S. Auxin transport. *Curr Opin Plant Biol* **8**, 494-500 (2005).

13 Esmon, C. A. *et al.* A gradient of auxin and auxin-dependent transcription precedes tropic growth responses. *Proc Natl Acad Sci U S A* **103**, 236-241 (2006).

14 Fuchs, I., Philippar, K., Ljung, K., Sandberg, G. & Hedrich, R. Blue light regulates an auxin-induced K+-channel gene in the maize coleoptile. *Proc Natl Acad Sci U S A* **100**, 11795-11800 (2003).

15 Hohm, T. *et al.* Plasma membrane H(+) -ATPase regulation is required for auxin gradient formation preceding phototropic growth. *Mol Syst Biol* **10**, 751 (2014).

16 Band, L. R. *et al.* Root gravitropism is regulated by a transient lateral auxin gradient controlled by a tipping-point mechanism. *Proc Natl Acad Sci U S A* **109**, 4668-4673 (2012).

17 Mellor, N. *et al.* Dynamic regulation of auxin oxidase and conjugating enzymes AtDAO1 and GH3 modulates auxin homeostasis. *Proc Natl Acad Sci U S A* **113**, 11022-11027 (2016).

18 Porco, S. *et al.* Dioxygenase-encoding *AtDAO1* gene controls IAA oxidation and homeostasis in Arabidopsis. *Proc Natl Acad Sci U S A* **113**, 11016-11021 (2016).
19 Zhang, J. *et al.* DAO1 catalyzes temporal and tissue-specific oxidative inactivation of auxin in Arabidopsis thaliana. *Proc Natl Acad Sci U S A* **113**, 11010-11015 (2016).
20 Qin, G. *et al.* An indole-3-acetic acid carboxyl methyltransferase regulates Arabidopsis leaf development. *Plant Cell* **17**, 2693-2704 (2005).
21 Zhao, N. *et al.* Structural, biochemical, and phylogenetic analyses suggest that indole-3-acetic acid methyltransferase is an evolutionarily ancient member of the SABATH family. *Plant Physiol* **146**, 455-467 (2008).
22 Zubieta, C. *et al.* Structural basis for substrate recognition in the salicylic acid carboxyl methyltransferase family. *Plant Cell* **15**, 1704-1716 (2003).
23 Peret, B. *et al.* Sequential induction of auxin efflux and influx carriers regulates lateral root emergence. *Mol Syst Biol* **9**, 699 (2013).
24 Sauer, M. *et al.* Canalization of auxin flow by Aux/IAA-ARF-dependent feedback regulation of PIN polarity. *Genes Dev* **20**, 2902-2911 (2006).
25 Chen, Q. *et al.* A coherent transcriptional feed-forward motif model for mediating auxin-sensitive PIN3 expression during lateral root development. *Nat Commun* **6**, 8821 (2015).
26 Wend, S. *et al.* A quantitative ratiometric sensor for time-resolved analysis of auxin dynamics. *Sci Rep* **3**, 2052 (2013).
27 Zourelidou, M. *et al.* Auxin efflux by PIN-FORMED proteins is activated by two different protein kinases, D6 PROTEIN KINASE and PINOID. *Elife* **3** (2014).
28 Brunoud, G. *et al.* A novel sensor to map auxin response and distribution at high spatio-temporal resolution. *Nature* **482**, 103-106 (2012).
29 Alonso, J. M. *et al.* Genome-wide insertional mutagenesis of Arabidopsis thaliana. *Science* **301**, 653-657 (2003).
30 Abbas, M. *et al.* Oxygen sensing coordinates photomorphogenesis to facilitate seedling survival. *Curr Biol* **25**, 1483-1488 (2015).
31 Gallego-Bartolomé, J., Kami, C., Fankhauser, C., Alabadí, D. & Blázquez, M. A. A hormonal regulatory module that provides flexibility to tropic responses. *Plant Physiol* **156**, 1819-1825 (2011).
32 Frigerio, M. *et al.* Transcriptional regulation of gibberellin metabolism genes by auxin signaling in Arabidopsis. *Plant Physiol* **142**, 553-563 (2006).
33 Javier Gallego-Bartolome´, David Alabadı´ & Bla´zquez, M. A. DELLA-Induced Early Transcriptional Changes during Etiolated Development in Arabidopsis thaliana. *Plos one* **6**, e23918 (2011).
34 Willige, B. C., Isono, E., Richter, R., Zourelidou, M. & Schwechheimer, C. Gibberellin regulates PIN-FORMED abundance and is required for auxin transport-dependent growth and development in Arabidopsis thaliana. *Plant Cell* **23**, 2184-2195 (2011).

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Author contributions

M.A., J.H.G., S.P., S.L.S. and M.K. performed experiments and analyzed the data; M.A., J.F., U.H., M.Z., M.A.B. and D.A. conceived the work and designed experiments; M.A.B. wrote the manuscript with the help of the rest of the authors.