Supplementary Information for:

PLETHORA gradient formation mechanism separates auxin responses

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1 SUPPLEMENTARY NOTES

1.1 PLT induction rapidly inhibits cell expansion

Cell expansion is generally considered as the first sign of differentiation. Therefore, we studied whether PLT2 regulates cell expansion. Cells progress quickly from the MZ to the DZ (Supplementary Notes, section 1.6; Extended Data Fig. 6a,b,g) so for local PLT levels to be instructive, cells near the MZ/EZ boundary must rapidly respond to PLT levels. Indeed, ubiquitous PLT2-YFP induction from a strong estradiol-inducible promoter[1] inhibited cell expansion rapidly, within 2 h (Extended Data Fig. 1d, e). These results indicate that the decline in PLT levels along the gradient allows for differentiation.

1.2 The 'initial' model: Requirement of prolonged, high auxin levels for PLT induction predicts a narrow transcription domain

Our 'initial' model predicts that the experimentally determined requirement of prolonged high level auxin exposure for PLT expression results in a short ranged, relatively non-gradual domain in which *PLT* transcription and PLT proteins occur (Fig. 2c and Supplementary Video S1). To further strengthen this finding, in Extended Data Fig. 4b we describe the PLT expression and protein domain as a function of the half-saturation constant of ARF for auxin (see Supplementary Computational Methods, Eq. 15, half-saturation constant of ARF determined by the combination of values used for ARF association and dissociation and AUX/IAA production and degradation rates). The half-saturation constant of ARF for auxin, which determines at which auxin level half maximal induction of *PLT* transcription occurs, is the main parameter impacting the size and shape of the PLT domain as a function of auxin levels. However, no experimental data are available to constrain the value of this parameter. Therefore, to determine a reasonable value for the half-saturation constant of ARF, we combine our experimental observation that PLT induction requires high auxin levels with our simulated auxin profile. This allows us to determine values corresponding to high auxin level by taking into account that auxin levels occurring near the MZ can be considered high, and decline first to intermediate and then low levels when moving shootward from this region.

From Extended Data Fig. 4b it follows that for half-saturation constants ranging from 50 till 400, neither the *PLT* transcription nor the PLT protein domains show the experimentally observed long range gradient shaped

distribution of the PLT protein. Instead, both transcription and protein patterns are limited in size and are relatively non-gradual. In Extended Data Fig. 4b it can be seen that auxin levels from 50 till 400 are typically found within or close to the meristematic region in our simulation model, and hence can be considered as intermediate to high auxin values. Only if we use a half-saturation constant of 25, which corresponds to an auxin level occurring close to the border of the MZ and EZ that hence can be considered low, a broad and graded *PLT* transcription and protein pattern arise. Thus, based on our experimental observation that PLT induction requires high auxin levels, a value of 100 for the auxin half-saturation constant of ARF, as used in all simulations including the one shown in Fig. 2c, is considered as a conservative value.

As a final remark, the sudden drop in transcription and protein levels that can be observed in the graphs of Extended Data Fig. 4b around a distance of 100 micrometer from the root tip is due to the simplified way in which we incorporated local variation in division rates. In reality division rates are likely to gradually change in the meristem from the low values observed in the stem cell niche to the high maximum values observed in transit amplifying cells shootward from the stem cell niche. In the model we approximated this as a discrete, sudden transition from a zero division rate in the stem cell niche (as a further approximation of the very low division rates found there) to a single high division rate for transit amplifying cells. As a consequence, there is a sudden transition from none to significant division driven growth dilution of transcript and protein, causing a drop in levels.

1.3 The *in vivo* role of PLT2 movement in zonation

Cell-to-cell movement of transcription factors in plants occurs through plasmodesmata (PD). Movement is controlled at least partly by the aperture of PD, and can be blocked when protein size is increased by adding several copies of fluorescent proteins[2]. When PLT2 was tagged with three copies of YFP (PLT2-3xYFP), and induced under the AHP6 promoter, the protein revealed more restricted expression within the AHP6 promoter activity domain (Fig. 3a). Interestingly, more pronounced spreading of PLT2-YFP signal occurred in the MZ, near the stem cells (Fig. 3a). Our data are consistent with movement through plasmodesmata which display decreased connectivity as stem cell daughters mature[3], and indicate that PLT2 moves from cell to cell in the root meristem thus widening its activity gradient.

We analysed the in vivo role of PLT2 cell-to-cell movement in the MZ by expressing PLT2-YFP and the movement-deficient version, PLT2-3xYFP, under the pPLT2 promoter. Both constructs displayed graded expression, however PLT2-3xYFP lines consistently showed reduced fluorescence in the vasculature compared to PLT2-YFP lines, suggesting that cell-to-cell movement of PLT2 is important for maintaining the normal PLT2 gradient (Extended Data Fig. 5f). Furthermore, although both constructs complemented the meristem defects of

plt1,2 mutant[4], thus demonstrating their functionality, meristem size of PLT2-3xYFP lines was shorter than that of PLT2-YFP lines, indicating that cell-to-cell movement of PLT2 contributes to meristem size (Extended Data Fig. 5g, h).

1.4 The 'PLT-spread' model: PLT protein stability allows intercellular movement and growth dilution to contribute to gradient formation

In Fig. 3c we studied the effect of PLT cell-to-cell movement on PLT gradient formation in our computational model. For an explanation on the determination of parameter values used for rates of cell-to-cell movement and PLT turnover rates see section 4.3.6 of the Supplementary Computational Methods. Simulations are performed in the absence of cell division, growth and expansion processes to ensure that all protein spread is due to cell-to-cell movement and no additional spread due to cellular growth processes occurs. We studied the effect of cell-to-cell movement both for a fast and a slow turnover rate of PLT proteins. We see that for fast PLT turnover, movement of PLT proteins through plasmodesmata hardly affects the protein profile. Due to the fast PLT turnover rate, hardly any PLT protein will last long enough to travel a substantial distance into the neighbouring cells given the slow rate of intercellular movement. In contrast, for slow PLT turnover, movement of the protein through plasmodesmata substantially changes the protein profile, changing it from a rather localised to a more gradient like distribution pattern. We thus find that the effect of PLT cell-to-cell movement crucially depends on the turnover dynamics (lifespan) of the PLT protein (Fig. 3c).

We studied the effect of growth (cell growth, division, expansion) on PLT gradient formation in the presence of slow PLT turnover alone or in combination with cell-to-cell movement (Fig. 3d). We saw that slow PLT turnover dynamics alone already resulted in a significant spreading out of PLT protein due to growth dilution. The long lifespan of PLT protein allows the protein to survive for a long time in daughter cells leaving the *PLT* transcription domain due to division and growth. Adding of cell-to-cell movement further extends and smoothens the PLT protein gradient. Assuming a PLT level of 15 or higher to designate the meristimatically active zone, we see that for slow PLT turnover growth dilution and movement of PLT through plasmodesmata both contribute to MZ size, consistent with our experimental results (Fig. 3e-h and Extended Data Fig. 5f-i). In Fig. 3d a rather abrupt initial drop in PLT levels appears to occur. As explained above, this drop results from the fact that the highest PLT levels maintain stem cells, for which no division was implemented in the model and hence no growth dilution occurs, whereas somewhat lower PLT levels stimulate division and hence lead to growth dilution.

Finally, in Fig. 4a and Supplementary Video S2 we show how the combination of growth dilution and cell-to-cell

movement occurring for a relatively stable PLT protein allows for a smooth PLT gradient capable of controlling root zonation dynamics in a coordinated manner. In the 'initial' model, where PLT protein occurred only in a small non-graded domain, dosage-dependent control of the transitions from slow dividing, to fast dividing to expanding and differentiating cells was impossible due to the short and relatively non-graded PLT pattern. Therefore, in order to produce proper zonation in the 'initial' model the PLT-dependent zonation rules needed to be replaced by implementing an independent position based zonation rule. In contrast, in the 'PLT-spread', 'auxin', 'gravitropism', and 'closed feedback' models PLT dosage dependent zonation can be implemented and functions in agreement with our experimental results (Supplementary Notes 1.7; Extended Data Fig. 6 and 7).

1.5 Cells within the PLT2 gradient, but outside the *PLT2* transcription domain are mitotically active

To investigate whether MZ cells outside the PLT2 transcription domain were still able to divide we transformed *PLT2* transcription domain-specific *pPLT2:CYCB1;1-YFP* into lines carrying the G2-M reporter *pCYCB1;1:CYCB1;1-GFP*. Both GFP and YFP co-expressed in a punctuate pattern in the proximal meristem, whereas only green fluorescence was present in a few cells near the MZ/EZ boundary (Extended Data Fig. 5i). These cells with only GFP signal also underwent mitosis (data not shown) indicating that cells which contain PLT2 protein, but are unable to transcribe *PLT2* themselves (Fig. 3f), are still capable of dividing.

1.6 PLT-independent effect of auxin on zonation

Because auxin only affected PLT expression over longer time scales, we could assess the in vivo roles of auxin in root zonation independent of PLT level changes by restricting our measurements to short time scales. High (5 µM) auxin levels rapidly inhibited cell expansion. Cell expansion rate in the early EZ was 28.5 µm/h ± 5.4 (n = 14 cells, s.d.) before, and 4.1 µm/h ± 1.8 (n = 13 cells, s.d.) 25 min after auxin application. As a consequence, root growth was rapidly inhibited (Extended Data Fig. 6a-c). The cell division rate also rapidly decreased as measured by G2/M phase cell cycle marker expression dynamics, (Extended Data Fig. 6c, Supplementary Video S3), as did the incorporation of the nucleotide analogue EdU in S-phase [5] (Extended Data Fig. 6d-e). In contrast, moderate levels of auxin (30 nM) inhibited only cell expansion but not cell division (Extended Data Fig. 6f). Cell expansion rate in the early EZ was 23.6 µm/h ± 2.7 (n = 14 cells, s.d.) before auxin application, and 17.2 µm/h ± 4.9 (n = 10 cells, s.d.) 210 min after auxin application. The average cell division rate for Root 1 was 7.9 mitosis/h before IAA application and 8.3 mitosis/h after 30 nM IAA application, whereas the corresponding values for Root 2 were 6.9 mitosis/h before and 4.7 mitosis/h after IAA application (Extended Data Fig. 6f). In support of these data, Rahman et al [6] similarly show that 30 nM IAA inhibit cell expansion, but not cell division. High auxin treatment did not prevent the differentiation zone from moving further towards the root tip, so that by 7 hours it reached the MZ/EZ boundary beyond which it did not proceed (Extended Data Fig. 6a,b; Extended

Data Fig. 7e, f; Supplementary Video S4). Consistently, we measured the same timespan (6-8 hours) for cells to leave the meristem (i.e. enter the EZ), expand and enter the DZ both in untreated and auxin treated roots (Extended Data Fig. 6a, g and h). Thus, high levels of auxin inhibit cell division and expansion, while moderate levels only inhibit expansion, whereas we detected no upper limit for auxin in cell differentiation.

Auxin has been reported to be required for cell division in cell suspensions, for cell expansion in stems and for root hair and xylem cell differentiation in roots [7-11]. To further study the in vivo effects of auxin levels on zonation, we expressed a stabilized inhibitor of Aux/IAA/SCF$^{TIR1/AFB}$ mediated auxin signalling, *axr3-1* [12], which inhibits xylem formation [8] and root growth [13] under an inducible promoter. 24 hours after induction of *axr3-1* cell division and expansion as well as root hair and protoxylem (px) differentiation were inhibited (Extended Data Fig. 7a,b,d-g). A shortened PLT2 domain however continued to mark the cells capable of division (Extended Data Fig. 7h). Similar effects were achieved by treatment with an auxin antagonist, auxinole [14] (Extended Data Fig. 7c,d,i,j). These results indicate that auxin signalling via the Aux/IAA/SCF$^{TIR1/AFB}$ pathway is required for cell division, expansion and differentiation, largely independent of the shape of the PLT gradient. As cell division and cell expansion depended on optimum auxin (signalling) levels, and cell differentiation required a minimum level of auxin (signalling), we concluded that auxin rapidly regulates all aspects of zonation.

1.7 The 'auxin' model: Auxin influences division, expansion and differentiation rates, which by growth dilution of PLT proteins provides a feedback between fast PLT-independent and slow PLT-dependent regulation

In Extended Data Fig. 8 we show the behaviour of the 'auxin' model, in which cell growth, division, expansion and differentiation rates are auxin-dependent. We show zonation dynamics for normal growth conditions (a), conditions of intermediate (b) and high level (c) auxin application, shoot cut (i.e. reduced auxin) (d), and auxin signalling inhibitor application (e). Shown are the auxin distribution, *PLT* transcription profile, PLT protein profile and zonation dynamics. We see that compared to Fig. 4a, under normal conditions comparable growth dynamics occur, the only difference being the non-homogeneity of growth, division, expansion and differentiation rates due to local differences in auxin levels.

Intermediate level auxin application shortens the elongation zone, while leaving the meristematic zone mostly intact (slight shortening) (Extended Data Fig. 8b). This shortening of the elongation zone is due to the decrease in expansion rates caused by elevated auxin levels, similar to the experimental data (Extended Data Fig. 6h; Supplementary Notes 1.6).

In contrast, 24 h of high level auxin application almost abolishes the EZ and substantially shortens the MZ

(Extended Data Fig. 8c), again in agreement with the experimental data (Extended Data Fig. 6a-c). Furthermore, we see that the PLT protein gradient becomes significantly shortened. This is due to the decrease in cell division rate that occurs under high auxin levels, thus reducing the spread of PLT through growth dilution, which consequently leads to shortening the MZ domain. Shortening of the PLT gradient with high levels of auxin is evident also in our experiments (Fig. 3g, h; Extended Data Fig.2c). The shortened MZ domain combined with the decreased cell division rate cause a substantially lower output of cells entering the EZ, leading to the near abolishment of the EZ. Thus, at this intermediate timescale of 24 h, a feedback occurs between the fast route that is not dependent on change of PLT levels (hours timescale) through which auxin influences rates of zonation processes and the slow route (timescale of days) through which auxin sets the transcription domain of *PLT*s and hence influences the PLT gradient determining size and location of zones. This feedback arises since sufficiently long lasting changes in division and expansion rates also influence the shape of the PLT gradient by affecting the extent of growth dilution.

Simulating the cutting of the root from the shoot results in a shortening of the MZ after 60hrs (Extended Data Fig. 8d). Shoot removal leads to decreased auxin concentrations in the meristem[15] which leads to reduced cell division rates and consequently reduced PLT protein spread (see above). Furthermore, on a longer timescale (this experiment lasted 60 rather than 24 hours) *PLT* transcription and protein levels become reduced due to the lower auxin levels. We see these two influences indeed reflected by a substantial shortening and lowering of the PLT gradient. Both effects thus contribute to the shortened MZ. Again these results agree with experimental observations [15] (Fig. 3g, h).

Finally, 24 h inhibition of auxin signalling (Extended Data Fig. 8e) results in a decrease in auxin dependent cell division, expansion and differentiation. The large decrease in cell division rates causes a decline of the PLT gradient. Furthermore, the decrease in expansion rates results in small cells interspersed with larger cells higher up in the root, as observed experimentally (Extended Data Fig. 7c), while the decrease in auxin dependent differentiation rate results in an apparent expansion of the elongation zone, resulting from the delayed beginning of the differentiation zone. Supporting the simulations, inhibition of auxin signalling in experiments leads to decrease in cell division (Extended Data Fig. 6d, e and 7g, j), expansion (Extended Data Fig. 7c, d) and differentiation (Extended Data Fig. 7a-c; e, f) as well as a decline of the PLT gradient (Extended Data Fig. 7h, i).

1.8 The 'gravitropism' model: Fast auxin changes combined with stable PLT patterns enable gravitropism while maintaining stable developmental zonation

We simulated zonation dynamics in root under gravitropism in the 'gravitropism' model (Fig. 4b, Supplementary

Video S5 and Extended Data Fig. 9a and b). Fig. 4b, right panel, depicts the leftward, apolar and rightward columella PIN orientations, the 12 hour cycle of PIN orientation changes, and resulting auxin, PLT and differentiation level left-right differences in distribution pattern. For the differentiation level, we measured, in the left and right epidermal cell file, the location of the first cell in which auxin or PLT drop below a threshold level or in which differentiation exceeds a specific level, and computed the distance between the cells found on the left and right sides. For auxin a threshold of 5 is used, above which elongation rate declines, while for PLT we use a threshold of 15 below which division stops and elongation and differentiation can occur, finally for terminal differentiation a level of 85 is used beyond which elongation stops and final differentiation occurs. Note that the stochasticity in measured differences is due to cellular growth and division events.
We see both from the zonation dynamics snapshots (Extended Data Fig. 9b) and the auxin, PLT and differentiation left-right difference dynamics graph (Fig. 4b) that switching from an apolar to a leftward or rightward PIN orientation in the columella leads to a fast occurrence of leftward or rightward oriented differences in the auxin distribution profile. In contrast, no significant changes in the PLT protein profile are observed, consistent both with the slow response of *PLT* transcription to changes in auxin levels and the additional smoothing out effect of PLT cell-to-cell movement and growth dilution on potential left-right differences. Our model findings agree with our experimental observations (Fig. 4c): while the DR5:GFP auxin response reporter shows a clear asymmetry in auxin distribution after gravistimulus, the PLT2-YFP gradient maintains a stable symmetric pattern. Furthermore, we observe that the gravity-induced asymmetric auxin accumulation causes an asymmetric inhibition of cell expansion in the lower part of the elongation zone that leads to a slow build-up of left-right differences in differentiation level (Fig. 4b), leading the differentiation zone to move in approximately 2 cells closer to the root tip. This is consistent with the experimental observation that root hairs, landmarks for epidermal differentiation, appear a few cells closer to the root tip on the lower than on the upper bending side of the root (Fig. 4c). The resulting left-right difference in differentiation has an opposite skew to the asymmetric auxin pattern causing it: while auxin levels extend higher upward at the lower side of the root (the side of the root towards gravity vector), differentiation is delayed at the upper side of the root. Finally, we see that upon switching from leftward to apolar PIN orientations, auxin distribution rapidly restores to a non-biased pattern, while the differentiation zone still shows a leftward bias since this is due to a slower timescale process of reduced expansion.

In Extended Data Fig. 9c and d we show the gravitropism dynamics in a “scaled-down” version of the 'gravitropism' model, in which cellular growth and division rates are constants not depending on auxin, and cellular expansion rate only decreases with increasing auxin level (not with decreasing auxin levels). We obtain similar gravitropism dynamics as before, illustrating that for gravitropism only the inhibitory effect of high auxin

levels on cell expansion is needed.

1.9 The 'closed-feedback' model: Closing the feedback loop from PLT back to auxin does not significantly affect model behaviour

In addition to the finding that auxin promotes *PLT* transcription, we have observed previously that PLTs promote PIN expression and auxin biosynthesis, therefore generating a feed-forward regulatory loop[16,17,18]. In order to study the role of PLT regulated auxin biosynthesis and transport, we developed a 'closed-feedback model'. In Extended Data Fig. 9e-g, we show zonation dynamics of the 'closed feedback' model under normal growth conditions (e), after 24 hours of high auxin application (f) and dynamic gravitropic stimulation (g). For comparison purposes arrows are used to indicate the location of transitions from MZ to EZ and from EZ to DZ in the auxin model. For gravitropism, both the minimum and maximum EZ lengths are indicated. As can be seen, the qualitative behaviour of the model remains similar upon adding the additional feedbacks from PLT level to PIN expression and expression of auxin synthesizing and degrading enzymes. Quantitatively, the sizes of zones also stay similar, with only the difference between minimum and maximum EZ length being somewhat smaller in the 'closed feedback' than the 'auxin' model. Furthermore, it can be seen that the dependence on PLT levels results in a graded expression of PINs, auxin synthesizing and auxin degrading enzymes. Since PLT suppresses the expression of auxin degrading enzyme the latter gradient runs opposite to the PLT gradient.

2 SUPPLEMENTARY DISCUSSION

2.1 The role of intercellular PLT movement

An important open question not addressed in the current study is how zonation transitions are coordinated across different cell files, and how auxin, PLTs and tissue mechanics (see section 4.3.7) play a role in this coordination. In this study we demonstrated how intercellular movement of PLT proteins contributes to the length of the axial PLT gradient (see section 1.3). An interesting potential additional function of PLT intercellular movement could be the smoothing of across cell file differences in PLT levels, thus contributing to radial coordination of zonation. It is however difficult to separate this role in our experimental system, given the large amount of redundancy between the different PLTs[16], and the potential involvement of other factors such as tissue mechanics in radial coordination.

In our experiments, we studied the role of PLT cell-to-cell movement by complementing the *plt1,2* mutant with *pPLT:PLT2-YFP* and the movement-deficient version *pPLT:PLT2-3xYFP* (Extended Data Fig. 5f-h). Even though the 3xYFP version complements meristem maintenance, it has a shorter meristem than when 1xYFP version is used, supporting the role of PLT movement in extension of the axial gradient. The 3xYFP version

shows reduced expression in the vasculature (Extended Data Fig. 5f), however we did not observe earlier cell expansion in vascular cells compared to ground tissue/epidermis cells in the same position (data not shown), seemingly suggesting that PLT movement is not involved in radial coordination of zonation. However, we can envision at least three alternative reasons for the absence of loss of coordination: first, the transition from meristematic cell to expanding cell is gradual and quite variable, making it difficult to identify a relatively small effect on differential cell expansion between 1xYFP and 3xYFP versions. Second, in the *plt1,2* mutant there are still redundantly acting PLT3 and PLT4 proteins left, both strongly expressing in the vasculature (Extended Data Fig. 2), and therefore potentially blocking premature cell expansion there in the *plt1,2 pPLT:PLT2-3xYFP* line. Third, remaining, higher PLT levels in one cell layer at the MZ/EZ boundary could physically restrain cell expansion on adjacent layers, similar to reported effects when gibberellin signaling was blocked in the endodermis[19]. Therefore, we take a conservative stand and assume that PLT movement mainly contributes to make the axial gradient longer. Although radial movement might contribute to the co-ordination of zonation at a local level, we currently cannot present clear evidence for this role. Future studies on *plt* mutants, and layer-specific expression of PLTs and its movement-deficient variants will be needed to understand the exact role of intercellular movement of PLTs in growth coordination across the cell layers.

2.2 Structure and function of the auxin-PLT regulatory network

In Extended Data Fig. 10a we summarize the structure of the regulatory architecture through which auxin and PLT control zonation and tropisms. We depicted the fast, direct route through which auxin influences the rates of zonation processes and the slow route in which auxin acts via PLTs to regulate the location of developmental zones. In Extended Data Fig. 10b we show how growth-dilution and intercellular movement contribute to PLT gradient formation, and make explicit where high auxin levels, where PLT transcription and where PLT proteins occur. Extended Data Fig. 10c summarizes how short-term manipulations in auxin levels or signalling influence PLT gradient formation and zonation processes and serves as a reference to both experiments and simulations. Finally, in Extended Data Fig. 10d and e we explore the functional importance of the slow PLT gradient formation mechanism and the partial separation in timescales this results in. In the regulatory design we uncovered in this study (Extended Data Fig. 10a) auxin influences zonation via a slow and a fast route, with the first influencing the size and location of zones and the second influencing the rates of the different processes occurring within the zones. We demonstrated (Fig. 4b, Extended Data Fig. 9) how during a transient gravitropic stimulus the PLT pattern stays stable, thus maintaining the boundaries between stem cell and transit amplifying cells within the MZ and the boundary between the MZ and EZ, while an asymmetric accumulation of auxin on the lower side of the root decreases expansion rates in the elongation zone. This decrease in expansion rates results in shorter EZ cells and therefore a shorter overall EZ length at the lower side of the root, thus allowing

for root bending and causing a downward movement of the boundary of the EZ and DZ. The number of cells residing inside the EZ and their progression through differentiation remains unaltered. In addition, the downward movement of the start of the DZ at the lower side of root may serve to partly compensate for the inward bending of the root at this side, thus maintaining an approximately horizontal boundary between EZ and DZ relative to the net direction of root growth.

To gain a better understanding of the functional significance of these findings we performed simulations where, instead of the regulatory design uncovered in this study, we assume that the PLT gradient is a relatively direct and proportionate readout of the auxin gradient (Extended Data Fig. 10d and e), as was earlier thought to be the case. For simplicity we simulate the limit case in which *PLT* transcription immediately follows auxin levels. Furthermore, there is no intercellular PLT protein movement, and PLT turnover is fast (half-life of ~1 h). As a consequence, neither intercellular movement nor growth dilution contribute to PLT gradient formation, and the PLT gradient is fully dependent on the auxin gradient. In this setting, gravitropism (Extended Data Fig. 10d) not only results in an asymmetric accumulation of auxin but also rapidly results in an asymmetric accumulation of PLT on the lower side (left in the snapshots) of the root. As a consequence, this ectopic PLT expression converts the cells recently progressed from division to expansion/differentiation back to meristematic cells (highlighted with brackets in Extended Data Fig. 10d, e). Indeed, both our current and earlier[16] experimental results indicate that when PLT is induced ubiquitously, the cells that have recently left the meristem will regain cell division. Thus, a decrease of expansion is achieved here through a PLT induced movement of the boundary between division and expansion zones at the lower side of the root shootward, thus reducing the number of elongating cells, rather than an auxin induced reduction of expansion rates that reduces cell sizes but not numbers. Indeed, the latter effect does not further contribute to a reduction of expansion, since the auxin elevation now occurs in the MZ, not EZ due to the upward shift of the MZ and EZ boundary.

However, this shift in MZ/EZ boundary also affects the progression of differentiation. For simplicity, we simulated here that as cells stop expanding and differentiating and revert back to the MZ, they rapidly and fully dedifferentiate (see section 4.3.5). Similar results are obtained with slower dedifferentiation rates (down to 0.001 a.u./s). We see in the differentiation snapshots and graphs that under a gravitropic stimulus, the cells that revert to the MZ dedifferentiate whereas more shootward cells continue to differentiate, causing a sudden jump from zero to substantial differentiation levels at the new MZ/EZ boundary (black line in the graph). At the end of the gravitropic stimulus (Extended Data Fig. 10e), as the auxin and PLT asymmetry has disappeared and the MZ/EZ boundary recovers, cells that reverted to the MZ now return to the EZ resuming their expansion and restarting their differentiation. Since this group of cells is collectively fully dedifferentiated and now collectively start

differentiating again, this results in a series of cells with the same differentiation level (indicated with brackets in the differentiation snapshots). Shootward are cells that during gravitropism remained in the EZ and hence continued their normal differentiation. Rootward of the group of simultaneously differentiating cells are cells that were always in the MZ (also in absence of gravitropic stimulus) and that leave the MZ zone in the normal, growth and division induced one by one manner, resulting in a gradual, sequential differentiation profile. Overall, if the PLT gradient were a direct readout of the auxin gradient, gravitropism results in a disruption of the normal, sequential differentiation profile. In contrast, under the regulatory architecture uncovered in this study, both during and after gravitropism a gradual differentiation profile is maintained (red lines in differentiation graphs) as no shifts in MZ/EZ boundaries and hence no temporary dedifferentiation phases occur.

From these results we infer that the function of the regulatory design uncovered in this study is that the slow route of auxin action operating through PLT level changes and determining zonation boundaries ensures a stable, coordinated progression of differentiation, while the fast auxin route allows for transient adjustment of expansion rates needed for adaptive tropisms. Furthermore, it illustrates the importance of PLT regulating the location of expansion and differentiation in a coordinated manner, while auxin regulates the rates of expansion and differentiation separately and in different manners.

3 SUPPLEMENTARY EXPERIMENTAL MATERIALS AND METHODS

3.1 Plant Materials and Constructs

All the DNA constructs were stably transformed in Col-0, Ler (DR5:nYFP) or Ws (plt1,2) plants. For each observation reported with newly made transgene constructs, multiple transgenic lines were analysed.To generate *pPLT1:gPLT1-YFP*, 7398 bp promoter sequence and the coding sequence of *PLT1* was isolated from P1 clone MOE17, ligated upstream of *Venus YFP*[20] and then assembled into pGreenII0226 binary vector[21]. *pPLT3:gPLT3-YFP-PLT3-3'* was previously described[22]. Three different lines of *pPLT2:PLT2-YFP* were used in this article. A line published in Galinha et al.[16] was used for the NPA+IAA and IAA time series experiment. We validated these results by using a new independent line in the *PLT2-YFP* 24h NPA+IAA experiment presented in the Extended Data Fig. 2c. Finally, to study dose-dependency of PLT in determining the high-division-rate domain (Fig.1c), we utilize a line that had strong PLT2-YFP expression and contained several copies of the transgene, therefore generating a long meristem. In our induction studies, we utilized the MultiSite Gateway (Invitrogen) compatible inducible system (XVE), which is based on the pER8 vector[1]. Three different promoters were used in the XVE inducible system: the ubiquitously expressing G1090 promoter[1,23], the epidermis-specific *WEREWOLF (WER)* promoter[23,24], and the protoxylem/protoxylem associated pericycle specific *AHP6* promoter[25]**.** For primer details,

see Supplementary Table 1. To create an inducible *PLT2* RNA interference construct (*pG1090:XVE>>PLT2 RNAi*), a 433bp fragment located within the last exon of PLT2 was amplified with primers PLT2iF-Xho1 and PLT2iR-BamH1 and ligated in both sense and antisense orientation in a modified, MultiSite Gateway compatible pHANNIBAL vector[26]. The following 433bp sequence was used in the *PLT2* RNAi construct: 5′-agcattcaacaacctttgagcatcttcatcatcatcagcctttacttactctacagaacaacaacgatatctctcagtatcatgattcctttagttacattcagacgcagctt catcttcaccaacaacaaaccaacaattacttgcagtcttctagtcacacttcacagctctacaatgcttatcttcagagtaaccctggtctgcttcatggatttgtctctga taataacaacacttcagggtttcttggaaacaatgggattggtattgggtcaagctctaccgttggatcatcggctgaggaagagtttccagccgtgaaagtcgattac gatatgcctccttccggtggagctacagggtatggaggatggaatagtggagagtctgctcaaggatcgaatccaggaggtgttttcacgatgtgga-3′. To generate the PLT2-RFP clonal activation line, *pUAS:PLT2-RFP* was combined with MultiSite Gateway technology, and then transformed into plants containing pCB1 vector and pHS:CRE[27]. Short heat shock (+37 ˚C) was used to induce a transient CRE recombinase expression that leads to random recombination at the lox sites and activation of GAL4 expression located in the pCB1 vector. GAL4 induce the expression of pUAS:erGFP, to mark the clone, and pUAS:PLT2-RFP. Tracking the residence time of a GFP clone in the EZ, the above mentioned clonal activation system without pUAS:PLT2-RFP was utilized. MultiSite Gateway technology (Invitrogen) was used to generate all other constructs. For primer details, see Supplementary Table 1.

3.2 Plant Growth and Microscopy

Seeds were germinated on ½ GM plates containing 0.5×MS salt mixture with vitamins (Duchefa), 1% sucrose, 0.5 g/l 2-(N-morpholino) ethanesulfonic acid (MES) pH 5.8 and 0.8% agar. Confocal imaging was carried out with Leica SP2 and SP5 microscopes. Adobe Photoshop CS6 was used to compile the figures and occasionally to process images by changing brightness or contrast. This processing was applied equally for entire images and it was applied equally to controls. The panels of vertically aligned roots were composed by rotating and subsequently cutting the images. This occasionally resulted in empty corners which were filled up for aesthetic reasons by extending the black background colour of the images to the corners. Confocal or light microscopy images presenting long stretches of root were compiled by merging two or more separate, adjacent images of the root after equalizing brightness. This procedure was applied for the following panels: Figs. 1a and 3e; Extended Data Figs. 1c; 5a,e; 6a,d and 7b,c,e,f. Panels of two or more individual roots (Fig. 2a, b; Extended Data Figs. 1e, f; 2a, b; 6a, b, d, g, h; 7e-j) did not grow side-by-side but were merged seamlessly to save space. For live imaging, 4-d-old seedlings were transferred to coverslip-bottomed imaging chambers (Thermo Scientific, item # 155361) containing water, and covered with a piece of ½ GM agar. An SP2 microscope with inverted objectives was programmed to take images every 2 min of horizontally growing roots. First, root growth was monitored for a period of 2-4 h to follow the initial growth and fluorescence dynamics, after which 17β-estradiol (for inducible PLT2-YFP) or IAA (S18 and CYCB1;1-GFP) was applied (t = 0 in the graphs) by pipetting the effector under

the piece of agar. Immediately after this, live imaging was resumed to follow the fast consequences of the applied effector. Root growth rate was determined with Image J software by measuring the distance the root had grown between consecutive frames (i.e. 2 min), or frames with longer time span (e.g. 20 min, after IAA, when roots grow slowly). Cell expansion rates were obtained from the root growth movies by following individual early EZ cell expansion. Average cell expansion rates were calculated by measuring the distance cells had grown within a 30 or 60 min time window. Cell lengths were measured with Image J software. For the inducible overexpression studies, 4-d-old seedlings were transferred either on a plate containing 5µM of the inducer 17β-estradiol (est, for the XVE lines), or on a plate containing an equal volume of DMSO (named 'ctrl' in the figures). Equal volumes of 70% ethanol and DMSO were used as control for IAA and auxinole experiments, respectively. To visualize DNA synthesis, 5 uM of 5-ethynyl-2'-deoxyuridine, EdU (Invitrogen)[5] was added to the seedlings for two last hours of the experiment (e.g. IAA for 4 hours means 2h IAA + 2h EdU+IAA), and samples were processed as described[28]. Whole-mount visualization of roots and GUS staining were performed as described[29].

3.3 Quantifying the effect of NPA+IAA on PLT2-YFP gradient shape

We selected a representative single locus insertion line (segregating 3:4) of *pPLT2:PLT2-YFP* (in Col background) for the quantification experiment. The seedlings homozygous for the construct were germinated for 4 days on ½ GM agar medium and then transferred for 24 hours on plates containing equal volume of DMSO or 20µM NPA + 5µM IAA. The seedlings were analysed with 20X immersion objective in confocal microscope Leica SP5 II HCS A. We used large pinhole (Airy 2) to receive signal from the majority of nuclei in a given focal plane. The same confocal settings were maintained for the NPA+IAA treated and control roots. The confocal images were analysed with ImageJ program by drawing a rectangle of 200 µm wide starting from the end of the last columella cell, and extending until the EZ. The average signal was quantified with the option "Plot Profile", and the results were averaged and plotted with the Microsoft Excel program.

3.4 Measuring mitotic events

Measuring the number of CYCB1;1-GFP[30] positive cells has been used as a standard for approximating cell division rate. This is because CYCB1;1 is present in dividing cells only from G2 to anaphase[31], and thus CYCB1;1-GFP is marking the cells destined for mitosis. However, in some conditions the cell cycle may be blocked at G2/M, or it may proceed more rapidly through G2, subsequently leading to wrong conclusions on cell division rates. To avoid this problem in the auxin application movies we monitored mitotic events directly by counting the disappearance of fluorescence signal of CYCB1;1-GFP in individual cells (Supplementary Video S3) in a given focal plane, after which these events were plotted below the x axis as a function of time (min)

(Extended Data Fig. 6c, f). As the fluorescence should disappear at the middle of mitosis (at anaphase), this method likely gives a good estimation of cell division rates. In order to confirm the accuracy of this method, CYCB1;1-GFP was crossed with H2B-RFP to monitor chromosome segregation during mitosis. 22/22 cells that lost GFP fluorescence, showed also chromosome segregation, demonstrating the accuracy by which CYCB1;1-GFP signal disappearance reports mitotic events.

3.5 Gravity-induction experiments

Seedlings were subjected for a 135 degree gravitystimulus for 4-5h. We used Imaris 7.6 (Bitplane Scientific Software) to visualize 3D confocal stacks of the PLT2-YFP pattern, and we failed to find a significant left-right asymmetry in PLT2-YFP gradient at the MZ/EZ boundary (Fig. 4c and data not shown).

3.6 General methodology and statistical analysis

For all the experiments, we first performed a preliminary analysis with a few samples, and this preliminary analysis was subsequently confirmed with a large enough number of samples to ensure statistical significance. We excluded samples that germinated poorly, or showed overall growth defects that were confirmed genetically not to be related to the genotype. These defects occur occasionally, and are caused by the seed sterilization method used.

Statistical analyses were carried out in R version 3.0.2 [http://www.r-project.org/]. In all analyses, a linear model including all fixed effects was constructed first and then a Shapiro-Wilk test for normality on the residuals was carried out. If the residuals were significantly different from normal, a non-parametric Mann-Whitney U test was applied, otherwise Welch's t-test was used. In ANOVA analysis, similarity of variances between groups was tested using Bartlett's test when constructing the linear model, and then groupwise comparisons were carried out using R package multcomp [http://cran.r-project.org/web/packages/multcomp/index.html]. In all cases, p-value adjustment for multiple comparisons was carried out with the Bonferroni method.

Table S1. Primers used in cloning and generation of the expression constructs

Primers to generate entry clones by using classical cloning methods; assembly with MultiSite Gateway LR reaction (Invitrogen)

Primer name	5' --> 3' sequence	To generate construct called:
PLT2iF-Xho1	ctatctcgagcattcaacaacctttgagcatcttc	pG1090:XVE>>**PLT2 RNAi**

PLT2iR-BamH1	taatggatccacatcgtgaaaacacctcctgg	

Primers to generate entry clones by using MultiSite Gateway BP reaction; assembly with MultiSite Gateway LR reaction (Invitrogen)

Primer name	5' --> 3' sequence	To generate construct called:
attB4-Kpn-pPLT2-f attB1-pPLT2-r	ggggacaactttgtatagaaaagttgcgggtacctggtttggtaagtttacttacgtat ggggactgctttttttgtacaaacttgcttccctttttcttggaatcaaagctt	**pPLT2**:gPLT2-YFP, **pPLT2**:gPLT2-3xYFP, **pPLT2**:H2B-YFP, **pPLT2**:CYCB1;1-YFP, **pPLT2**:CYCB1;1-RFP
attB1-gPLT2-f attB2-Kpn-gPLT2-r	ggggacaagtttgtacaaaaaagcaggcttcatgaattctaacaactggctcgcgt ggggaccactttgtacaagaaagctgggtcggtaccttcattccacatcgtgaaaacacct	pAHP6:XVE>>**PLT2**-YFP, pAHP6:XVE>>**PLT2**-3xYFP, pG1090:XVE>>**PLT2**-YFP, pPLT2:**gPLT2**-YFP, pPLT2:**gPLT2**-3xYFP , p6xUAS:**gPLT2**-mCherryRFP
attB4-pPLT4-f attB1-pPLT4-r	ggggacaactttgtatagaaaagttgcggaaagcttacgattacagagaccaa ggggactgctttttttgtacaaacttgcaataatattctaactactccttgtga	**pPLT4**:gPLT4-YFP
attB1-gPLT4-f attB2-BamHI-gPLT4-r	ggggacaagtttgtacaaaaaagcaggcttcatgaactcgatgaataactggttag ggggaccactttgtacaagaaagctgggtcgggatccccagtgtcgttccaaactgaaaacgtt	pPLT4:**gPLT4**-YFP
pUASF-attB4 pUASR-attB1	ggggacaactttgtatagaaaagttggaattcgatatgaagcttga ggggactgctttttttgtacaaacttgctgtcctctccaaatgaaatgaa	**p6xUAS**:gPLT2-mCherryRFP
H2BF-attB1 H2BR-attB2	ggggacaagtttgtacaaaaaagcaggctaaacaatggcgaaggcagataagaaaccagc ggggaccactttgtacaagaaagctgggtcagaactcgtaaacttcgtaaccgccttagtcc	pPLT2:**H2B**-YFP
dboxF-attB1 dboxR-attB2	ggggacaagtttgtacaaaaaagcaggctaaacaatgatgacttctcgttcgattgttcctcaac ggggaccactttgtacaagaaagctgggtccaattgcttctctcgagcagcaactaaaccaagttc	pPLT2:**CYCB1;1**-YFP, pPLT2:**CYCB1;1**-mCherryRFP
AXR3F-attB1 AXR3R-attB2	ggggacaagtttgtacaaaaaagcaggctctagaaaaatgatgggcagtgtcgagctgaatctg ggggaccactttgtacaagaaagctgggtcggtaccagctctgctcttgcacttctccatcg	G1090:XVE>>**axr3-1**-mCherryRFP
glyVenF-attB2 3ATR-attB3	ggggacagctttcttgtacaaagtgggtggtggtggcgcggtg ggggacaactttgtataataaagttgccctcgacacaaaaagcctatactgtac	pAHP6:XVE>>PLT2-**YFP**, pG1090:XVE>>PLT2-**YFP**, pPLT2:gPLT2-**YFP**, pPLT4:gPLT4-**YFP**, pPLT2:CYCB1;1-**YFP**, pPLT2:H2B-**YFP**
glyCheF-attB2 CheR-attB3	ggggacagctttcttgtacaaagtgggtgggggtggcgtgagcaagggcgaggagg ggggacaactttgtataataaagttgagatcttacgtaatcgatctggattttagtactggattttgg	p6xUAS:gPLT2-**mCherryRFP**, pPLT2:CYCB1;1-**mCherryRFP,** G1090:XVE>>axr3-1**-mCherryRFP**

4 SUPPLEMENTARY COMPUTATIONAL METHODS

4.0 Modeling aims and approach

The aim of this study is twofold, 1) to determine the roles of auxin and *PLT* transcription factors in the control of developmental zonation and 2) to unravel how auxin is capable of controlling both long term stable developmental zonation and short term transient tropisms necessary to adjust growth to environmental conditions. In order to achieve these two aims we developed a multiscale model of root zonation and growth.

In the root, four PLT transcription factors (PLT1, PLT2, PLT3 and PLT4) are involved in controlling root zonation[16]. While these four PLTs each have a gradient shaped distribution with the maximum near the root meristem, they each have a distinct expression pattern with more dominant expression in some than in other tissues[16]. These differences are likely to be caused by differences in the tissue-specific distribution of ARFs and cofactors involved in PLT expression and their differential action on distinct *PLT* promoter regions.

In this study we focus on the role of PLTs in controlling zonation transitions, for which the graded distribution of PLTs along the longitudinal axis of the root is relevant. The question of how these transitions are coordinated across different tissue types in which four different, but highly redundant[16] PLTs are expressed to different extents is left for future study. Therefore rather than simulating the precise tissue distributions of four PLT proteins, for which no quantitative data are available for the PLT patterns themselves, the factors other than auxin controlling these patterns, or how these distributions should be summed to determine their downstream effects, we here simply simulate a generalised PLT gradient by incorporating a single general ARF factor and a single general PLT transcription factor into our model. The expression of this single general PLT thus is solely dependent on ARF and hence auxin levels. In this approach, only a qualitative comparison is possible between our simulated general PLT gradient and our experimentally measured specific PLT2 gradient.

On a similar note, we incorporate the PIN pattern generating the auxin reflux pattern responsible for generating the longitudinal auxin gradient, while ignoring tissue specific auxin importer patterns that further enhance between cell file differences in auxin levels (see section 4.3.1).

Source code for the simulation models used is made publicly available at:
http://www-binf.bio.uu.nl/khwjtuss/DigitalRoot

4.1 Summary of the models

We constructed a series of zonation models. For brevity, and since the models are constructed in an incremental manner, the different models were named after the latest added mechanism and will be referred to as the 1) ***initial***; 2) ***PLT-spread***; 3) ***auxin*** 4) ***gravitropism*** and 5) ***closed feedback*** models, respectively. In all five root zonation models described in the manuscript, root tissue architecture was represented in a similar manner as in previous modelling studies[15,32]. All models were constructed based on the following experimental results and earlier model studies:

- Auxin metabolism and active and passive auxin transport dynamics were modelled in a similar manner as in earlier studies, incorporating the typical reflux PIN pattern generating the auxin maximum in the root tip[15,33]. However, to obtain a better agreement with experimentally observed PIN patterns[34,36], lateral PIN levels were substantially reduced relative to these earlier modelling studies (see section 4.3.1 and Extended Data Fig. 3c).

- Auxin promotes PIN expression[36]
- Auxin induces PLT expression at a slow timescale of ~24-72 h [this m/s]

- PLTs control in a dosage-dependent manner where the domains of stem cell maintenance and slow division, fast cell growth and division, and cell expansion and differentiation are located[4,16] (Extended Data Fig. 3e, second panel) [this m/s]. We implemented specific thresholds for this (Th_{PLT}):
    - High PLT levels define the stem cell and slow division domain ($PLT > Th_{PLT,A}$)
    - Intermediate PLT levels define the transit amplifying domain ($Th_{PLT,A} > PLT > Th_{PLT,B}$)
    - Low PLT levels define the transition to expansion and differentiation ($PLT < Th_{PLT,B}$)

- WT meristem size is ~30-35cells per file[37]

- Cell differentiation is completed in ~7 h. [this m/s]

- Root growth rate varies between 120-180 microm/h and average cortex cell length is ~170μm, implying a cell division rate of ~1-1.5 cells/cell file/h[this m/s]. Combined with the above this implies:
    - Elongation zone size is ~7-11 cells per file. This agrees with counted cell numbers in the EZ (this m/s, data not shown)
    - Average division rate is ~0.029-0.05cells/h, so average division time is around 20-35hrs (this m/s)

The above experimental observations were all incorporated to construct the so-called ***initial*** model. This model predicted the presence of a rather limited, non-graded PLT domain (Fig. 2c), inconsistent with experimental data. The failure of the ***initial*** model to correctly reproduce a smooth PLT gradient led us to investigate the role of PLT cell-to-cell movement, life span and growth dilution in the so-called ***PLT-spread*** model. In this model we first demonstrated theoretically the requirement of an extended PLT lifespan to facilitate significant PLT cell-to-cell movement (Fig. 3c), also showing that such an extended lifespan leads to additional PLT spread through growth dilution (Fig. 3d). Next, we experimentally confirmed the role of cell-to-cell movement, PLT stability and growth dilution. Subsequently, we adjusted the final parameter values used in the ***PLT-spread*** model for PLT cell-to-cell movement rate and PLT turnover rate to agree with our experimental observations (Fig. 4a). Thus, the following additions were made to extend our ***initial*** model into the ***PLT-spread*** model:

- PLT proteins display slow cell-to-cell movement. In our final model parameter settings we use a PLT cell-to-cell flux rate of 0.0008microm/s which is capable of reproducing the experimentally observed contribution of PLT movement to overall MZ size [this m/s].

- PLT proteins are stable such that due to growth dilution they occur a limited number of cells away from the PLT transcription domain. In our final model parameter settings we use a PLT half-life of ~16 h which is capable of reproducing the experimentally observed contribution of PLT growth dilution to the size of the PLT protein domain. This half-life value is further supported by the observed slow decay of induced PLT protein levels upon targeted RNAi [this m/s].

The thus obtained ***PLT-spread*** model correctly reproduced a smooth PLT gradient capable of controlling root zonation dynamics under normal conditions, as observed experimentally. However, in order to enable the model

to reproduce short term changes in zonation dynamics upon interference with auxin levels or signalling the model was extended with the following results to obtain the so-called ***auxin*** model:

- Apart from the slow, indirect PLT mediated effects of auxin on root growth, auxin also influences root growth directly and rapidly without affecting PLT levels, by influencing cell growth and division, expansion and differentiation rates[7,8,38,39] [this m/s]. Specifically:
    - Cell growth and division rates depend on optimum auxin levels, with rates decreasing both for high (level A in Extended Data Fig. 3e, third and fourth panel) and low (levels C and D in Extended Data Fig. 3e, third and fourth panel) auxin levels
    - Cell expansion rates depend on lower optimum auxin levels, decreasing for both intermediate (levels B in Extended Data Fig. 3e, third and fourth panel) and low auxin levels (level D in Extended Data Fig. 3e, third and fourth panel)
    - Cellular differentiation rate is constant for a wide range of auxin levels, and decreases only for low auxin levels (level D in Extended Data Fig. 3e, third and fourth panel).

To investigate the relevance of having both long term PLT-mediated auxin effects as well as more short term PLT-independent auxin effects on root growth dynamics we developed the ***gravitropism*** model. This model incorporates on top of all the above, the following experimental results:

- Columella PIN proteins polarize laterally according to the gravity vector within minutes, resulting in asymmetric shootward auxin transport[40].

- Due to compensatory growth, this left/right asymmetry is reversed every 6 h, producing a characteristic waving root pattern[41]

Finally, to investigate the importance of having a closed positive feedback between auxin and PLT levels, we developed the ***closed feedback*** model. This model incorporates the following additional experimental results:

- PLTs promote PIN expression[16,17], promote the expression of auxin synthesizing enzymes (e.g. YUC3)[18] (Ben Scheres lab, unpublished data) and repress the expression of auxin degrading enzymes (e.g. GH33) (Ben Scheres lab, unpublished data).

Extended Data Fig. 3a shows an overview of the variables and processes incorporated in the ***initial***, ***PLT-spread, auxin*** and ***closed feedback*** model, respectively. (Note that the ***gravitropism*** model is merely the ***auxin*** model extended with gravitropic conditions). We see how in the ***initial*** model only auxin induced gene expression governs PLT levels, while in the ***PLT-spread*** model PLT levels also depend on cell-to-cell movement and growth dilution. In both these models only PLT levels influence zonation dynamics. In contrast, in the ***auxin*** model, auxin and PLT levels together control zonation dynamics. Finally, in the ***closed feedback*** model, auxin influences PLT levels, while PLT also influences auxin levels. Extended Data Fig. 3b demonstrates how the spatiotemporal interplay between auxin and PLT gradients and growth processes leads to the unfolding of zonation processes in the ***auxin*** model.

The simulation code of the models was written in C++, and run on a Dell Precision T7400 workstation with two intel xeon X5482 quad core processors. Typical run times for simulations were on the order of 24hrs to reach steady state growth patterns (biological time of a few days), and on the order of 2-4 h to test specific model settings (biological time of 7-24hrs).

In the next sections (4.2 till 4.4), a detailed description of the how and why of the implementation of the different model parts described above will be provided.

4.2 Model robustness and parameter settings

It follows from the above section that the constructed models are fairly complex in terms of number of processes considered, entangledness of feedbacks, and range of relevant spatial and temporal scales. A major issue with models of this level of complexity is to find robust parameter settings generating the biologically relevant behaviour of interest.

Here we took a **three step** approach in finding parameter settings capable of generating realistic zonation dynamics in a robust manner. **First**, tissue topology, membrane PIN patterns, and parameters governing auxin transport dynamics -diffusion, influx, and efflux rates- were based on previous modelling studies[15,31] and experimental data.

Second, we set the parameters describing auxin metabolism, auxin dependent ARF activation, and ARF dependent PIN and PLT gene expression. Since no absolute measurements for auxin levels and protein concentrations are available, auxin, ARF, PIN and PLT levels are modelled in arbitrary units (a.u.). As a consequence, the ratios between auxin, PIN and PLT production and degradation, and between ARF activation

and inactivation that determine maximum auxin, PIN, PLT and free ARF levels need to be chosen without much constraining data. Auxin metabolism rates where chosen similar to earlier modelling studies[15,32], while PIN, PLT and ARF rates were chosen such that maximum PIN, PLT and free ARF levels are 100. Furthermore, assuming that the non-transcriptional activation and inactivation of ARF is significantly faster than transcriptional up and downregulation of PIN protein levels, ARF activation/inactivation rates are a factor of 10 faster than PIN production and degradation rates. PLT dynamics were set based on further experimental data (see step 3).

As a **third step**, we used the experimental data obtained in the current study to derive parameter values resulting in a realistic PLT induction time course (number and dynamics of intermediate transcription factors), PLT lifespan (PLT decay rate), cell-to-cell spread (PLT flux rate), realistic time courses for cell division, cell expansion and cell differentiation (division, expansion and differentiation rates), and realistic meristematic, expansion and differentiation domain sizes (dependencies of these processes on PLT and auxin levels) (for further details see later sections).

As explained above, auxin and protein concentrations are modelled in arbitrary units (a.u.). This prohibits us from obtaining a single unique parameter setting (with surrounding region) generating the biologically correct behaviour. For example, by using significantly different PLT production or decay rates the maximum PLT protein levels would change substantially (i.e. 1000 instead of 100). As a consequence the PLT protein profile would change significantly and hence we would need to rescale the PLT thresholds setting the locations of the stem cell, fast division, expansion and differentiation domains in step three to get the same zonation dynamics. On the other hand, PLT half-life and cell-to-cell movement rate can be maintained at the same values since these affect the percentage of PLT remaining after a certain time or the percentage of PLT moving to a neighbouring cell in a certain time window, and hence require no rescaling for the absolute amounts of PLT. On a similar note, changing auxin production or decay rates significantly would change the amplitude of the auxin maximum and gradient substantially and hence require a rescaling of the auxin dependency of division, expansion and differentiation rates to get the same zonation dynamics. Again, parameter values for auxin diffusion, influx and pumping rates can remain constant since these affect the fraction of auxin moving in a certain time window and hence require no rescaling with absolute auxin levels.

Summarizing, in step three, most parameters are constrained based on experimental observations (PLT lifespan, PLT cell-to-cell movement rate, PLT induction dynamics, mean division, expansion and differentiation rates). However, a small subset of the parameters determined in step 3 require scaling relative to the maximum PLT levels or relative to the maximum auxin levels as set in step 2 (PLT thresholds dictating locations of different

zones, and auxin levels modulating division, expansion and differentiation rates, respectively) in order to correctly reproduce experimentally observed sizes and locations of MZ, EZ and DZ zones and the observed impact of auxin levels on division, expansion and differentiation rates.

The final obtained model behaviour was robust for moderate parameter changes (that is parameter changes that do not change maximum auxin levels more than 3-fold and PLT levels more than 30% and hence do not require rescaling, see above). Similar model behaviour was observed for varying the auxin influx from the shoot two-fold, varying lateral PIN levels four-fold, varying auxin production or decay two-fold, or varying the shape of the rate dependency functions of cell expansion and division on auxin levels (simulation data not shown).

4.3 Detailed description of the models

The ***initial***, ***PLT-spread***, ***auxin***, ***gravitropism*** and ***closed feedback*** models all use the same root tissue architecture, auxin metabolism and transport dynamics, auxin dependent PLT expression and protein dynamics, and PLT dependent cellular growth, division, expansion and differentiation dynamics. These are described in detail below.

4.3.1 Root tissue architecture

We constructed a spatially-extended grid-based multiscale model of root zonation dynamics (Extended Data Fig. 3c). The root tissue was modelled as a rectangular grid (typically 80 by 925 grid points, with a spatial resolution of 2 micrometers), and grid points were either part of the cell wall or of the cytoplasm of a plant cell. For simplicity, cellular organelles such as the vacuole were not explicitly modelled. Earlier simulation studies have shown that exclusion of vacuoles does not significantly affect model results[15]. Cell walls were typically 2 grid points wide, whereas cells were typically 8 grid points wide and varied in height from cells in the meristematic growth zone being 4 grid points high to fully elongated and differentiated cells of 72 grid points height. Cytoplasmic grid points neighbouring cell wall grid points were used to store cell membrane properties such as auxin membrane permeability and levels of auxin efflux carriers, here represented as PIN levels, as well as cytoplasmic properties such as auxin concentrations. The reason for not using separate grid points to represent cellular membranes, as well as using a cell wall width that is larger than that observed in real plants, is that this would require a spatial resolution that would make model simulations prohibitively slow. Other model studies have taken a similar approach[15].

Extended Data Fig. 3c shows the tissue layout and reflux loop PIN polarity pattern applied in our model. For computational simplicity, we considered a total of 8 cell files in our tissue, and modelled all cell files to have

equal cell width, similar to the approach taken in Grieneisen et al.[15]. The leftmost and rightmost cell files are the epidermal cells (blue), the second leftmost and second rightmost cell files are the cortex files (green), the next left and right cell files represent the border files (pericycle/endodermis, yellow) and the two middle cell files represent the vasculature (red). In addition, in the lower part of the root we distinguish the quiescent center (grey) and columella cells (cyan).

The superimposed PIN topology (Extended Data Fig. 3c) is similar to those used in the models by Grieneisen et al.[15] and Laskowski et al.[31]. However, lateral PIN levels were decreased to obtain an improved agreement with experimentally observed PIN patterns[34,35] (see below). In the columella region cells have an apolar PIN pattern, with PINs residing on all membranes. In contrast, in the vascular and border cell files, PINs are located on the basal membranes, resulting in a rootward direction of net auxin transport. In addition, border cells have laterally inward oriented PINs resulting in auxin transport into the vasculature. In the epidermal cell files PINs have an apical and inward lateral polarisation resulting in shootward auxin flux and reflux of auxin into the vasculature. Finally, in the cortical cell files, cells in the MZ have a basal and cells in the rest of the root have an apical PIN pattern, thus contributing to rootward transport low in the root and to shootward transport higher up in the root (similar to the approach in Laskowski et al.[31]). In addition, the cortical cell files contain inward laterally oriented PINs resulting in auxin reflux into the vasculature.

Experimental data suggest the presence of lateral PINs in epidermal, cortical and border cells, with lateral PIN levels in border cells being somewhat lower than apical and basal PIN levels and lateral PIN levels in epidermal and cortex cells being much lower than apical and basal PIN levels[34,35]. Based on these observations we used as maximum levels for lateral PINs in border cells a factor 0.35 lower than maximal apical and basal PIN levels, whereas lateral PIN levels in epidermal and cortical cells were set to a factor 0.1 lower than apical and basal PIN levels.

Upon division both daughter cells inherit the PIN pattern of the mother cell. Furthermore, during cell growth, expansion and differentiation, the cellular PIN pattern is maintained. One exception holds for cortical cells, which upon transiting from the MZ to the EZ reorient their PINs from the basal (rootward-oriented) to the apical (shootward-oriented) surface while keeping the pattern of lateral PINs unchanged (flipping indicated with arrows in Extended Data Fig. 3c).

For simplicity, we refrained from incorporating known differential expression patterns for auxin importing proteins[32,42] and instead assumed a uniform level of auxin import across our simulated root tissue. Note that these

differential expression patterns mainly result in increasing auxin level differences across different cell files (i.e. the radial tissue direction), whereas in the current study we are mostly focusing on longitudinal auxin and PLT differences involved in controlling zonation transitions. However, if one would like to simulate root gravitropism in more extensive detail than done in the current study, incorporating these tissue specific auxin-importer patterns will become important[32,42].

4.3.2 Auxin metabolism and transport dynamics

Auxin dynamics were modelled on each grid point i, j with i indicating the horizontal location of the point on the simulated root tissue grid and running from 0 to n, and j indicating the vertical location on the grid and running from 0 to m. Auxin production and decay were incorporated inside cells (cytoplasm) but not in cell walls, and diffusion was implemented inside cells and inside walls. Across the plasma membrane, passive and active influx from walls to cytoplasm, and active PIN mediated efflux and marginal passive permeability from cytoplasm to walls were incorporated according to Mitchison[33] and Grieneisen et al.[15].

For a grid point i,j inside the wall bordered by only wall grid points we write:

$$\frac{\delta Aux_{i,j}}{\delta t} = \frac{D_{wall}}{\Delta x}\left(Aux_{i+1,j} + Aux_{i-1,j} + Aux_{i,j+1} + Aux_{i,j-1} - 4\,Aux_{i,j}\right) \quad \text{Eq. 1}$$

Here D_{wall} is the diffusion rate for auxin in the apoplast and Δx is the spatial resolution of the simulation.

For a grid point i,j inside the cytoplasm bordered by only other cytoplasmic grid points we write:

$$\frac{\delta Aux_{i,j}}{\delta t} = p_{Aux} - d_{Aux}\,Aux_{i,j} + \frac{D_{cell}}{\Delta x}\left(Aux_{i+1,j} + Aux_{i-1,j} + Aux_{i,j+1} + Aux_{i,j-1} - 4\,Aux_{i,j}\right) \quad \text{Eq. 2}$$

Here p_{Aux} is the rate at which auxin is produced per cell, d_{Aux} is the rate at which auxin is degraded per cell, and D_{cell} is the diffusion rate for auxin inside cells.

For a grid point i,j inside the wall, bordered by three other wall grid points and one cytoplasmic grid point (i,j-1) and hence membrane grid point we write:

$$\frac{\delta Aux_{i,j}}{\delta t} = -i_{pas+act}\,Aux_{i,j} + \left(e_{pas} + e_{PIN}\right)Aux_{i,j-1} + \frac{D_{wall}}{\Delta x}\left(Aux_{i+1,j} + Aux_{i-1,j} + Aux_{i,j+1} - 3\,Aux_{i,j}\right) \quad \text{Eq. 3}$$

Here, $i_{pas+act}$ is the combined passive and active auxin influx from walls to cytoplasm, e_{pas} is the small passive efflux of auxin from cytoplasm to walls, and e_{PIN} is the active pumping of auxin through PINs from cytoplasm to walls.

Finally, for a point i,j inside the cytoplasm, neighbouring three other cytoplasmic grid points and one cell wall grid point (i,j-1)we write:

$$\frac{\delta Aux_{i,j}}{\delta t} = i_{pas+act}\, Aux_{i,j-1} - \left(e_{pas} + e_{PIN}\right) Aux_{i,j} + \frac{D_{cell}}{\Delta x}\left(Aux_{i+1,j} + Aux_{i-1,j} + Aux_{i,j+1} - 3\mathrm{Aux}_{i,j}\right) \quad \text{Eq. 4}$$

For simplicity, auxin transport by influx and efflux carriers were modelled using linear mass action kinetics, similar to the approach taken in Grieneisen et al.[15].

Parameter settings are listed in Supplementary Table 2.

Note that e_{PIN} is not a parameter, but instead dependent on the product of the predefined PIN pattern ($PIN_{pattern}$,defining for each membrane segment how much PINs they can maximally contain as a relative level between 0 and 1) and the cellular PIN expression level (PIN , the concentration of PINs in the cell) (see Eqs. 19 and 20).

Due to the fast rates for auxin diffusion and flux, very small temporal integration steps (ht=0.0001s) would be required to solve the above equations stably using straightforward explicit integration. This would render our simulations extremely slow, considering that we are interested in modelling growth processes that occur on a timescale of multiple days. Moreover, additional time is needed for simulations to reach representative, steady state behaviour. To resolve this problem we implemented an alternating direction semi-implicit integration scheme[43], allowing us to use a temporal integration step of 0.2s instead of 0.0001s. To check the validity of this approach shorter test simulations were performed using both semi-implicit integration and long timesteps and explicit integration and short timesteps, producing very similar results (data not shown). Thus, we utilized this semi-implicit integration scheme in all of our further simulations.
To simulate the connection of our finite piece of root tissue with the shoot, we simulated efflux from the topmost non-vascular cells and influx to the topmost vascular cells[15].

4.3.3 Auxin induced PLT transcription

4.3.3.a TIR1-dependent auxin sensing

Transcriptional responses of genes to auxin levels occur through the TIR pathway via ARF response factors. ARF factors heterodimerize with their AUX/IAA repressors in the absence of auxin, thus making ARFs incapable to activate transcription of auxin-inducible genes. If present, auxin acts as a ‘molecular glue’ to

promote AUX/IAA interaction with the TIR1 F-box protein, which leads to AUX/IAA ubiquitination and degradation. As a consequence, the ARF factors are now free to induce gene expression[44].

In our model we considered a single, generalized ARF factor. Note that the amount of non-AUX/IAA bound ARF factor and resulting gene expression were modeled at the cell level rather than at the grid point level. We assumed that the concentration of ARF factor in cells is constant (i.e. no transcriptional effects). This model is a simplification as it is well-known that individual ARF and AUX/IAA genes display distinct, non-uniform expression patterns[45]. However, given that these expression patterns do not correlate clearly with zonation patterns, a simplified approach ignoring ARF pre-patterns is justified.

In our models, auxin signalling therefore only changes the fraction of a generalised, homogeneously expressed ubiquitous ARF that is free instead of bound by AUX/IAA repressors.

Thus we can write $ARF_{total} = constant$ and hence also

$$ARF_{total} = ARF_{free} + ARF_{complex} \quad \text{Eq. 5}$$

Given that total ARF is constant and the above conservation equation, we only need to write a single dynamic equation for ARF_{free} , $ARF_{complex}$ can then be derived as

$$ARF_{complex} = ARF_{total} - ARF_{free} \ . \quad \text{Eq. 6}$$

Thus we write for ARF_{free} the dynamic equation:

$$\frac{dARF_{free}}{dt} = dissARF_{complex} - assARF_{free}\, AUX/IAA \quad \text{Eq. 7}$$

which we can write as:

$$\frac{dARF_{free}}{dt} = diss\left(ARF_{total} - ARF_{free}\right) - assARF_{free}\, AUX/IAA \quad \text{Eq. 8}$$

here $diss$ is the rate with which $ARF_{complex}$ dissociates into ARF_{free} and AUX/IAA repressor, while ass is the rate at with which these two components associate into $ARF_{complex}$.

It follows from the above equation that to determine the amount of ARF_{free} available for the induction of gene expression, we need to know the amount of AUX/IAA , which will depend on the amount of auxin present.

For AUX/IAA we can write the following dynamic equation:

$$\frac{dAUX/IAA}{dt} = p_{AUX/IAA} - d_{AUX/IAA,basal}\, AUX/IAA - d_{AUX/IAA,TIR1}\, Aux\, AUX/IAA \quad \text{Eq. 9}$$

here $p_{AUX/IAA}$ is the rate at which AUX/IAA is produced, $d_{AUX/IAA,basal}$ is a low basal rate at which

AUX/IAA is being degraded, and $d_{AUX/IAA,TIR1}$ is the much faster TIR1 complex dependent degradation of AUX/IAA in the presence of auxin. Note that a single auxin molecule binds to a single AUX/IAA repressor to allow its TIR1 dependent degradation[41].

We assumed that AUX/IAA dynamics is fast, and use a quasi steady-state assumption (QSSA) to derive an expression for AUX/IAA :

$$AUX/IAA = \frac{p_{AUX/IAA}}{d_{AUX/IAA,basal} + d_{AUX/IAA,TIR1} Aux} \quad \text{Eq. 10}$$

This expression can now be substituted into our dynamic equation for ARF_{free} :

$$\frac{dARF_{free}}{dt} = diss\left(ARF_{total} - ARF_{free}\right) - assARF_{free} \frac{p_{AUX/IAA}}{d_{AUX/IAA,basal} + d_{AUX/IAA,TIR1} Aux} \quad \text{Eq. 11}$$

allowing us to model the level of ARF_{free} available for gene expression induction as a function of local auxin levels.

Although we used the above dynamic equation (Eq. 11) to simulate free ARF levels in our model, we next used a QSSA to derive an equation for free ARF in order to obtain a better insight in how free ARF levels depend on local auxin levels. For this we first rewrite the above dynamic equation as:

$$\frac{dARF_{free}}{dt} = dissARF_{total} - \left(diss + ass \frac{p_{AUX/IAA}}{d_{AUX/IAA,basal} + d_{AUX/IAA,TIR1} Aux}\right) ARF_{free} \quad \text{Eq. 12}$$

Using a QSSA we then obtain:

$$ARF_{free} = \frac{dissARF_{total}}{diss + ass \frac{p_{AUX/IAA}}{d_{AUX/IAA,basal} + d_{AUX/IAA,TIR1} Aux}} \quad \text{Eq. 13}$$

which can be rewritten as:

$$ARF_{free} = ARF_{total} \frac{d_{AUX/IAA,basal} + d_{AUX/IAA,TIR1} Aux}{d_{AUX/IAA,basal} + \frac{ass}{diss} p_{AUX/IAA} + d_{AUX/IAA,TIR1} Aux} \quad \text{Eq. 14}$$

or

$$ARF_{free} = ARF_{total} \frac{1 + \frac{d_{AUX/IAA,TIR1}}{d_{AUX/IAA,basal}} Aux}{1 + \frac{ass}{diss} \frac{p_{AUX/IAA}}{d_{AUX/IAA,basal}} + \frac{d_{AUX/IAA,TIR1}}{d_{AUX/IAA,basal}} Aux} \quad \text{Eq. 15}$$

From this equation we can see that the amount of free ARF depends in a saturating manner on local auxin levels. For very high auxin levels, free ARF levels approach the amount of total ARF, for very low auxin levels free

ARF levels approach $\frac{d_{AUX/IAA,basal} ARF_{total}}{d_{AUX/IAA,basal} + \frac{ass}{diss} p_{AUX/IAA}}$, free ARF levels are half maximal (half of total ARF) for

auxin levels satisfying $Aux = \frac{\frac{ass}{diss} p_{AUX/IAA} - d_{AUX/IAA,basal}}{d_{AUX/IAA,TIR1}}$

We use a constant, maximum protein level of 100 for ARF and AUX/IAA. Thus $ARF_{total} = 100$ and

$\frac{p_{AUX/IAA}}{d_{AUX/IAA,basal}} = 100$.

Furthermore, we assumed that the TIR1 mediated degradation of IAA/AUX is 10 times faster than its basal

degradation rate, giving us $\frac{d_{AUX/IAA,TIR1}}{d_{AUX/IAA,basal}} = 10$. Finally, assuming $\frac{ass}{diss} = 10$, that is that association of ARF

free into complex is 10 times as fast as dissociation of complex into free ARF, results in the following equation

for free ARF: $ARF_{free} = 100 \frac{1+10\text{Aux}}{1+1000+10\text{Aux}}$

which is half-maximal for auxin levels around 100. This way we ensure that PLT induction, which is dependent on free ARF levels, only occurs for the high auxin levels normally occurring close to the root tip (For more details see Extended Data Fig. 4 and section 4.5).

4.3.3.b PLT gene expression and protein dynamics

Gene expression dynamics were modelled on the cell level, and gene expression levels were modelled in terms of concentrations. Furthermore, rather than separately describing production of mRNA and protein, which would require the use of two variables and dynamic equations per gene, we choose to summarize gene expression dynamics as a single process, combining transcription and translation, using a single variable to describe the resulting protein levels.

During cell growth and expansion cell volume increases and we needed to determine how to incorporate this into the protein concentration dynamics. During cell expansion, cellular volume is assumed to increase predominantly through an increase of the vacuolar compartment, leaving the volume of the cytoplasmic compartment approximately constant. Therefore, during this growth phase we assumed that protein concentrations remain constant and hence no dilution of protein levels is applied. In contrast, during cell growth, cytoplasmic cell volume increases, implying a dilution of protein levels.

Given that in our model cellular growth was implemented as a stepwise process, producing stepwise increases in cellular volume, (see later sections on growth), this dilution was applied instantaneous upon the occurrence of such a volume increase. For this dilution we made a distinction between transcription factor and non-transcription factor proteins. Non-transcription factor proteins reside in the cytoplasm and on the cell-membrane. Their concentration was diluted proportional to the volume increase of the cell:

$$\left[non-TF_{new}\right]=\frac{volume_{old}}{volume_{new}}\left[non-TF_{old}\right] \qquad \text{Eq. 16}$$

For transcription factor proteins we assumed that they partly reside in the nucleus, which volume does not increase upon cellular growth, and partly in the cytoplasm, which volume does increase. Therefore, for these proteins dilution was only applied on the fraction residing in the cytoplasm:

$$\left[TF_{new}\right]=\left(fraccyto\frac{volume_{old}}{volume_{new}}+\left(1-fraccyto\right)\right)\left[TF_{old}\right] \qquad \text{Eq. 17}$$

We assumed that 50% of the TF protein resides in the cytoplasm and 50% in the nucleus.

These dilutions are applied directly after stepwise increases in cytoplasmic volume and thus result in an instantaneous lowering of cellular protein concentrations. Note however that for gene expression rates that are fast relative to cytoplasmic growth rates, ongoing gene expression leads to a fast subsequent recovery of cellular protein levels.

We experimentally observed that only prolonged exposure (24-72hrs) to high auxin levels induced PLT expression (Fig 2), implying a significant delay in the auxin-induced onset of PLT expression. Recent data on lateral root initiation suggest that downstream of auxin but upstream of the slowly responding PLTs 1, 2 and 4, two ARFs, one or two intermediate transcription factors, and the faster responding PLTs 3, 5 and 7 are involved[46] (Ben Scheres lab, unpublished data), suggesting the involvement of around 4 intermediate transcription factors in addition to the initially responding ARF in PLT 1, 2, 4 transcriptional activation. Together this led us to the following model for PLT expression induction by ARFs via a series of intermediate transcription factors:

$$\text{auxin} \longrightarrow \text{ARF} \longrightarrow \text{A} \longrightarrow \text{B} \longrightarrow \text{C} \longrightarrow \text{D} \longrightarrow \text{PLT}$$

The equation for PLT gene expression in our model can thus be written as:

$$\frac{dPLT}{dt} = max_{PLT} \frac{D^2}{D^2 + sat_{PLT}^2} - d_{PLT} PLT; \qquad \text{Eq. 18}$$

where max_{PLT} is the maximum PLT production rate, sat_{PLT} the concentration of transcription factor D for which PLT production occurs at half of its maximum rate and d_{PLT} is the rate of PLT degradation.

For the intermediate transcription factors A till D equations similar to the one described above for PLT dynamics were used.

Extended Data Fig. 3d, left and right panel show the time course of free ARF, A, B, C , D and PLT protein dynamics under application of constant high auxin levels (100, a.u.). In Extended Data Fig. 3d, left panel, PLT protein turnover dynamics was fast (half-life 1.4hr), as used in the ***initial*** model, while in Extended Data Fig. 3d, right panel, PLT protein turnover dynamics was slow (half-life 15.8hr), as used in the other models (see later sections). We see that the intermediate factors A, B, C, and D that need to become activated in between ARF activation and PLT activation in both cases ensure a delayed onset of PLT protein levels, with PLT starting to rise to significant levels only after 24hrs and reaching maximum levels after 70 to 80 h. A slow PLT turnover dynamics further contributes to this delay.

4.3.4 Auxin dependent expression of PIN proteins

Auxin is known to also regulate PIN expression in an ARF dependent manner[36], thus regulating the total amount of PIN protein available in a cell.

For the PIN expression dynamics we write:

$$\frac{dPIN}{dt} = max_{PIN} \left(a \frac{ARF^2}{ARF^2 + sat_{PIN}^2} + (1-a) \right) - d_{PIN} PIN \qquad \text{Eq. 19}$$

where max_{PIN} is the maximum PIN production rate, a is the fraction of ARF dependent PIN expression, sat_{PIN} is the ARF level at which (the ARF dependent) PIN production occurs at half of its maximum rate, and d_{PIN} is the rate of PIN degradation.

The a and $1-a$ terms reflect that while PIN protein levels depend on auxin levels[33], PIN expression does not

completely disappear in tissue regions with low auxin levels.

Furthermore, we assume that in addition to the dynamically regulated PINs, additional PINs are present, further ensuring the presence of PIN proteins for low auxin levels. Thus total cellular PIN levels were defined as:

$$PIN_{total} = a_{PIN} PIN_{regulated} + \left(1 - a_{PIN}\right) PIN_{basal} \quad \text{Eq. 20}$$

where $PIN_{regulated}$ is the auxin dependent PIN level following from Eq. 19, PIN_{basal} is a basal, auxin independent PIN level, and a_{PIN} determines their relative contribution to overall PIN levels.

From this we can now derive the local auxin efflux rate by PINs as:

$$e_{PIN} = max_{pump} PIN_{pattern} PIN_{total} \quad \text{Eq. 21}$$

where max_{pump} is the maximum pumping rate per unit of PINs, and $PIN_{pattern}$ defines the maximum relative level of PINs per membrane segment.

Parameter settings can be found in Supplementary Table 2.

4.3.5 PLT dependent regulation of cellular growth, division, expansion and differentiation dynamics

From the experiments described in the main text we derive how PLT levels impact cell growth, division, expansion and differentiation:

1. Increasing of PLT dosage shifts shootward both the upper and lower boundaries of the meristematic domain containing fast dividing cells (Fig. 1b, c; Extended Data Fig. 1a, b).
2. Clonal expression of PLTs outside their normal rootward expression domain locally inhibits cellular differentiation and expansion and leads to resumption of cell division (Extended Data Fig. 5a-c). Given the small numbers of cells in the clones locally expressing PLT, the inability of the auxin antagonist PEO-IAA to promote cell expansion in the clones (Extended Data Fig. 5e), and the absence of the auxin response reporter, DR5 (Extended Data Fig. 5d) in the clones, localised high auxin accumulation induced by the PLT expression is unlikely.
3. High auxin induced cellular differentiation fails to invade the PLT expression domain in the MZ (Extended Data Fig. 6a, b; 7e, f), while in the absence of PLT expression (*plt 1,3,4* triple mutant with RNAi against PLT2 experiment, Extended Data Fig 1f), the MZ cells expand and differentiate.

Together this indicates that PLT levels autonomously, largely independent of auxin, control the location of domains of slowly dividing stem cell, rapidly dividing transit amplifying cells, expanding and differentiating cells. Extended Data Fig. 3e, second panel, shows how the implementation of PLT threshold levels in our model

result in the PLT gradient subdividing the root into a succession of slow dividing stem cell, fast dividing transit amplifying cell and expansion and differentiation domains.

We implemented cell growth, division, expansion and differentiation dynamics as follows:

Cell growth

The actual growth of the cell was implemented as a stepwise increase of the cytoplasmic volume of the cell with one row of grid points (Extended Data Fig. 3f), shifting the cell membrane and shootward cells and cell walls upward with one row of grid points each time an interval $t_{growth}=\frac{1}{r_{growth}}$ has been exceeded. The rationale behind the temporally and spatially rather discrete approach taken here is it's computational simplicity.

In the ***initial*** and ***PLT-spread*** models, r_{growth} is a constant valued parameter. In contrast in the ***auxin***, ***gravitropism*** and ***closed feedback*** models r_{growth} is ARF and hence auxin level dependent, with r_{growth} having a maximum value of $r_{growth,max}$ and growth rate decreasing for both high and low auxin levels (for further details see section 4.3.7).

As explained above, to compensate for the increase in cytoplasmic volume that occurs during cell growth, a dilution of protein levels was applied.

Cell division

In our model, for simplicity cell division was assumed to take place for the same conditions as cytoplasmic growth (see above), once a critical cell size (actually cell height, as cells only grow in the axial direction and their width stays constant) of 2 times their initial size has been exceeded. Upon division, two rows of grid points in the middle of the cell were converted into new cell wall, and the neighbouring rows of cytoplasmic grid points were assigned to be new cell membranes. The two newly formed daughter cells were furthermore assumed to inherit the PIN polarity pattern of the original mother cell (Extended Data Fig. 3f). As all other variables (auxin, gene expression levels) were modelled in terms of concentrations, no further adjustments (i.e. dividing quantities over the two cells) are necessary.

Cell expansion

Similar to the approach for cell growth, cell expansion was implemented as a stepwise increase of cell length by

adding a row of cytoplasmic grid points each time an interval $t_{expansion} = \frac{1}{r_{expansion}}$ is exceeded (Extended Data Fig. 3f). As for cellular growth rates, $r_{expansion}$ has a constant value in the ***initial*** and ***PLT-spread*** models, while in the ***auxin***, ***gravitropism*** and ***closed-feedback*** models $r_{expansion}$ is dependent on the cellular ARF level, with a maximum value of $r_{expansion,max}$ and expansion rate decreasing for both high and low auxin levels (see section 4.3.7).

Once cell length has reached a certain maximum length or cell differentiation (see below) has reached a certain critical level, expansion ceases. Alternatively or in addition, an increase in auxin levels towards the DZ as observed in other modelling studies[29,44] could inhibit cell expansion independently from cell length and differentiation level. Although we have not modelled this increased auxin in the DZ explicitly, this mechanism would not change the zonation behaviour of our model at the MZ/EZ boundary, and would only potentially change the behaviour of our model at the EZ/DZ boundary under conditions of significant auxin (signalling) reduction.

As stated earlier in the section on gene expression, cell expansion was assumed to mainly be due to vacuolar growth, conserving cytoplasmic volume and hence (effective) protein levels.

Cell differentiation

To indicate the differentiation level of a cell we introduced an additional cell level variable DF in our model. Initially, this variable was set to zero for all cells, indicating their fully undifferentiated state. If PLT levels drop below 15, the value of DF was set to 0.001 to indicate the possible onset of differentiation. Once DF has reached this above zero value, the dynamics of DF were updated according to the following differential equation (Extended Data Fig. 3f):

$$\frac{dDF}{dt} = r_{DF} - d_{DF} DF \qquad \text{Eq. 22}$$

Here r_{DF} is the differentiation rate. Again, in the ***initial*** and ***PLT-spread*** models this rate is constant, while in the ***auxin***, ***gravitropism*** and ***closed-feedback*** models this rate is a function of ARF levels. In these models it has a maximum value of $r_{DF,max}$ for a wide range of auxin levels while decreasing for very low auxin levels (section 4.3.7). The values of r_{DF} / $r_{DF,max}$ and d_{DF} were chosen such that a fully differentiated cell has a

DF value of 100.

If cells that have recently left the MZ and progressed to differentiation and expansion (cells with a differentiation level less than 60) experience an increase in PLT levels of 15 or higher, cells stop expanding and dedifferentiate back to a DF level of 0. In contrast, cells that experience such an increase in PLT levels but that have left the MZ a while ago (cells with a differentiation level of 60 or higher) continue to expand and differentiate.

Cell growth, division, expansion and differentiation rates

First, differentiation rates (r_{DF} / $r_{DF,max}$) were chosen such that under normal conditions differentiation takes approximately 7-8 hours, the time we experimentally established for this process (Extended Data Fig. 6a, g, h). A threshold level of DF=85 was choosen above which cell expansion ceases.

Next, expansion rates ($r_{expansion}$ / $r_{expansion,max}$) were chosen such that under normal growth conditions, fully expanded cell lengths were reached before differentiation was completed and halted expansion (i.e. within ~7-8 hours).

Finally, cell growth and division rates (r_{growth} / $r_{growth,max}$) were chosen based on experimental observations and such that under normal growth conditions, and given a normal meristem size (~30-35 cells), the size of the generated elongation zone agrees with experimental observations (~7-11 cells long).

Extended Data Fig. 3f shows a schematized depiction of the root zonation dynamics algorithm implemented in our model, together with the relevant cell level processes incorporated.

4.3.6. PLT cell-to-cell movement and slow PLT turnover

In the ***initial*** model, the presence of the PLT protein is restricted to the cell in which it is being produced and PLT protein turnover is relatively fast (half-life of 1.4 h). In contrast, in the ***PLT-spread***, ***auxin***, ***gravitropism*** and ***closed feedback*** models, PLT proteins move between cells through plasmodesmata and PLT turnover is slow. The detailed implementation of the PLT movement and turnover dynamics and a derivation of used parameter settings is described below.

The movement of PLT protein through plasmodesmata was modeled as a flux process between the cytoplasms of neighbouring cells. As a simplification, we assumed equal plasmodesmatal connectivity between cell types and developmental stages. At each simulation time step, cell level PLT levels were first converted into grid level PLT levels, assuming homogeneous intracellular PLT levels. Next, flux was computed between all cytoplasmic grid

points at a cell's border (i,j) and the cytoplasmic grid points at the borders of its neighbouring cells (p,q) :

$$J_{PLT} = p_{flux}\left(PLT_{i,j} - PLT_{p,q}\right) \quad \text{Eq. 23}$$

with p_{flux} the rate of PLT flux between cells. As a consequence, PLT flux between cells is proportional to both their PLT concentration difference and to the length of cell wall and hence the amount of plasmodesmatal connections they share. Note that in these computations, we computed fluxes between points (i,j) and (p,q) which are not physical neighbours on our simulation grid, as there is a cell wall in between the cells. By thus skipping the cell walls (only for these computations), we implicitly modelled the cytoplasmic connection between the cells.

Finally, from the resulting grid level intracellular PLT distributions, a new cell level PLT concentration was computed, by averaging over the PLT concentrations of the individual cytoplasmic grid points belonging to a cell, that subsequently is used for other, cell-level based computations using cellular PLT levels (i.e. gene expression and root zonation dynamics).

PLT turnover dynamics are determined by the value of the parameter governing the PLT protein decay rate, with the half-life of PLT protein depending on this decay rate in the following manner: $t_{\frac{1}{2}} = \frac{\ln(2)}{d_{PLT}}$, allowing us to adjust PLT turnover in a simple manner.

How to determine the values for p_{flux} and d_{PLT} used in the ***PLT-spread***, ***auxin***, ***gravitropism*** and ***closed feedback*** models? Initially, we assumed a low value for p_{flux} as our clonal and tissue-specific PLT expression experiments reveal PLT proteins only 1 to 2 cells away from the transcription domain indicating a slow transport process (Fig. 3a, b). From this we found that effective cell-to-cell movement requires a low value for d_{PLT} and hence slow PLT turnover dynamics (Fig. 3c). PLT stability subsequently allows the protein to also spread through growth dilution (Fig. 3d).

After having confirmed experimentally the roles of both cell-to-cell movement and growth dilution in the formation of the PLT gradient (Fig. 3a, b, e-g; Extended Data Fig. 5f-h), we used these experiments in a more quantitative manner to further constrain the values of p_{flux} and d_{PLT} . First, our experimental data show that the *PLT2* transcription domain encompasses only the first approximately 1/3 of the PLT2 protein gradient (Fig.

3f), with the overall PLT protein gradient extending into the elongation zone, thus setting the size for the domain of PLT protein spread arising through a combination of cell-to-cell movement and growth dilution. Second, our experimental data also show that both cell-to-cell movement and growth dilution contribute to the PLT protein gradient formation (Fig. 3g; Extended Data Fig. 5f-h). Hence, values for p_{flux} and d_{PLT} need to be found that reproduce both these observations. Additional constraints arise from the fact that p_{flux} only affects the extent of PLT cell-to-cell movement, while d_{PLT} influences both PLT spread due to cell-to-cell movement and due to growth dilution.

Despite the lack of detailed measurements of the rate of PLT protein spread through plasmodesmata or of PLT protein half-life, the above data provide us with sufficient constraints to reasonably approximate the values of the p_{flux} and d_{PLT} parameters of our model. We found that for a PLT decay rate of 0.0000175s^{-1} resulting in a simulated half-life of ~16 h, and a plasmodesmatal flux speed of $0.0008\mu m$, our simulated PLT protein gradient approximately fulfilled these requirements (Fig. 3c and d: for a PLT cutoff level of 15 the PLT transcription domain has a size of 270 μm, the PLT protein domain in case of only growth dilution has a size of 352 μm and the PLT protein domain in case of both growth dilution and cell-to-cell movement has a size of 560 μm, so growth dilution contributes 88 μm, cell-to-cell movement 208 μm, which is within same order of magnitude, and the transcription domain of 270 μm encompasses a fraction of 0.48 of the total gradient size of 560 μm, which is close to 1/3). Finally, as an independent confirmation, the half-life value of ~16hr agrees well with our experimental observations of slow PLT2-YFP decay dynamics upon RNAi with fluorescence still detectable after 23 but gone after 49 h (Extended Data Fig. 1f).

4.3.7 Auxin dependent regulation of cellular growth, division, expansion and differentiation dynamics

For the ***initial*** and ***PLT-spread*** models, growth, division, expansion and differentiation processes are assumed to only depend on PLT levels and occur at constant rates. However, earlier experimental data[7,48] show that the rates at which these processes occur are auxin dependent, these data are confirmed and extended by our current experimental results :

1. High externally applied auxin levels significantly repress cell division after ~100 minutes and cell expansion rates after 25 minutes, while intermediate externally applied auxin levels repress only expansion rates (Extended Data Fig. 6c, f; Supplementary Notes 1.6). In both cases differentiation

proceeded normally.

2 Inhibition of auxin signalling (Extended Data Fig. 7) results in repression of cell division, expansion and differentiation rates on a timescale of 7-24 hours.

Together these data indicate that apart from slow, indirect, PLT mediated effects of auxin on root growth dynamics, auxin also has faster effects on root growth. Furthermore, they show that the dependence of cell division and expansion rates on auxin levels follows optimum functions, with rates decreasing for both high and low auxin levels. The cell division optimum range is located at higher auxin levels than the cell expansion optimum range (Extended Data Fig. 3e, fourth panel). For cellular differentiation, rates are constant for a broad range of auxin levels and decline only for very low auxin concentrations (Extended Data Fig. 3e, fourth panel). Therefore, for the ***auxin***, ***gravitropism*** and ***closed feedback*** models, auxin dependent cellular growth, division, expansion and differentiation rates are incorporated.

In our current models we incorporated only an auxin-ARF pathway for the auxin dependence of division, expansion and differentiation rates. In reality, other, potentially even faster acting, signalling pathways such as auxin mediated cell wall acidification affecting cell expansion rates[49], and the SKP2 cell cycle regulator affecting cell division rate[50] have been implicated. For simplicity, these additional regulatory pathways were not incorporated in our current model version. However, the currently included auxin-ARF pathway does allow for reasonably fast responses, with free ARF levels responding to changed auxin concentrations within 20 min. Note that we assumed here for simplicity that cell growth and division are coupled by a size threshold -once a cell reaches a certain size through growth, division occurs[51] - implying that cell growth and division depend in a similar manner on auxin levels. The equations used for the rate dependency functions schematically depicted in Extended Data Fig. 3e, fourth panel are:

For growth / division rate:

$$if\left(ARF<3\right)$$

$$r_{grow}=r_{grow,max}\frac{4^2}{4^2+\left(ARF-3\right)^2} \qquad \text{Eq. 24}$$

$$else \quad if\left(ARF<60\right)$$

$$r_{grow}=r_{grow,max}$$

$$else$$

$$r_{grow} = r_{grow,max} \frac{3.5^2}{3.5^2 + (ARF - 60)^2}$$

For expansion rate:

$$if\ (ARF < 0.5)$$

$$r_{expansion} = r_{expansion,max} \frac{2^2}{2^2 + (ARF - 0.5)^2} \quad \text{Eq. 25}$$

$$else\ \ if\ (ARF < 8)$$

$$r_{expansion} = r_{expansion,max}$$

$$else$$

$$r_{expansion} = r_{expansion,max} \frac{3^2}{3^2 + (ARF - 8)^2}$$

For differentiation rate:

$$if\ (ARF < 0.5)$$

$$r_{DF} = r_{DF,max} \frac{2^2}{2^2 + (ARF - 0.5)^2} \quad \text{Eq. 26}$$

$$else$$

$$r_{DF} = r_{DF,max}$$

Notably, these settings reproduced the experimentally observed effect that under auxin application expansion slows down and cell differentiation occurs before expansion is completed, blocking further expansion and resulting in shorter differentiated cells (Extended Data Fig. 6a-c).

The specific constant values used in the above equations were obtained by fitting overall model behaviour to experimentally observed root zonation dynamics under standard conditions, conditions of perturbed auxin (signalling) and gravitropism.

Note that for the cell growth and expansion rates, these rates are per unit of length (2 um, 1 grid row) rather than per cell length. As a result, longer cells will grow and expand faster than shorter cells. This is based on measurements by Van der Weele et al.[52] indicating a linear shallow root growth displacement velocity profile

across the division zone and a similar but steeper linear displacement velocity profile along the expansion zone. The linear shape of the displacement velocity profiles indicates that growth and expansion rates are constant per mm of root.

Radial coordination of growth dynamics:
- local, weighted averaging of growth and expansion rates as a proxy for mechanical constraints-
In plants, neighbouring cells share a cell wall, preventing them to slide past one another during growth. This property causes symplastic growth. In our current model, no mechanical constraints were implemented to ensure symplastic growth. As a consequence, due to the significant differences in auxin levels across different cell files, local auxin levels could cause different cellular growth and expansion rates and hence lead to unrealistic sliding of cells. Therefore, as a phenomenological proxy for the mechanical constraints arising from shared cell walls, we averaged growth rates locally between directly neighbouring cells. As a consequence, faster growing or expanding cells are being slowed down, while slower growing or expanding cells are being sped up by their neighbours, thus limiting, although not fully abolishing, unrealistic sliding of cells past one another. Note that a single rate variable is used to indicate either growth or expansion rate (dependent on which process is relevant for the particular cell), as in terms of mechanical constraints imposed by cells on one another these processes are similar. Thus, if slowly growing cells are neighbouring fast expanding cells, growth will be sped up while expansion will be slowed down.

Experimental data suggest that epidermal growth and expansion rates most strongly affect nearby root tissue growth. For example, during gravitropism, auxin differences are most pronounced in the epidermis and these are sufficient to result in differential expansion at the upper and lower side of the root causing root bending. Furthermore, our clonal PLT expression experiments show that expression of PLT in the epidermis of the elongation zone is sufficient to block overall root growth. Based on these observations, the local averaging of growth rates is done in a weighted manner, with outer cell files having higher weights than inner cell files, thus allowing them to more strongly influence local growth rates.

A schematic description of how the weighted, locally averaged growth and expansion rates are obtained from the ARF level based cellular rates is given in Extended Data Fig. 3g.

4.3.8 Gravitropism
In the ***gravitropism*** model, we used the ***auxin*** model settings and simulated gravitropism in the following manner:

It is well-established that upon gravistimulus root tips initiate bending towards the new gravity vector due to a fast redistribution of auxin into the direction of the vector[53].It has been shown that a change in the PIN3 polarity pattern in the columella cells (from apolar to the direction of the new gravity vector) drives this fast auxin redistribution[40]. Therefore, we simulated a gravitropic stimulus by a redistribution of columella PIN proteins.

For growth along the gravity vector, we assumed apolar columella PIN levels (maximum relative level of 1, cyan cells in Extended Data Fig. 3c). Under gravity-induced conditions, we assumed that the columella PIN levels of the apical and basal membranes and of the lateral membrane opposite to the gravity vector decline to a level that is a factor 0.35 of their normal, non-gravity stimulated levels, while the PIN levels on the lateral membrane in the direction of the gravity vector increase to a factor 2 of their non-gravity stimulated levels (Fig. 4b).
Next we take advantage of this in silico gravity-induced, asymmetric PIN distribution model to test the stability of the PLT protein gradient under physiologically relevant, dynamically changing auxin gradients. During normal root growth a typical waving pattern with leftward and rightward oriented bends produced at 6 hour intervals is observed due to left-right alternations in asymmetric cell expansion[41]. To approximate this physiological growth pattern, we set up a dynamic, gravity-stimulus switching procedure, containing 4 hours of leftward oriented columella PINs, 2 hours of apolar columella PINs, 4 hours of rightward oriented columella PINs, and then again 2 hours of apolar columella PINs, thus producing a repetitive 12 hours period in which leftward and rightward bends occur at 6 hour intervals (Fig. 4b). The rationale behind the rapid switching between different PIN orientation patterns and behind interspersing the leftward and rightward PIN orientations with periods of apolar PIN distribution is that upon sufficient gravistimulus a rather rapid response of auxin redistribution has been observed, while once gravitropic growth has reduced this bending below 40 degrees, also a rapid normalisation of auxin patterns has been observed, which has led to the postulation of a so-called tipping-point mechanism[54]. Therefore, we assumed that PIN patterns can respond rapidly to gravistimulus, justifying the fast switching between PIN patterns, and that there are interspersed periods of apolar PIN patterns corresponding to the periods of sub 40 degrees bending.

It should be noted that, although valuable for initial insights in auxin and PLT distribution patterns under gravitropism, a limitation of the current model is that it does not allow the differential expansion rates occuring for lower and upper sides of the root to result in actual root bending. As a consequence, shape change effects on auxin patterns such as those described in Laskowski et al.[32] or mechanical effects on auxin patterning[55,56] are currently not taken into account in this model. In addition, the cell shape changes accompanying bending may result in subtle changes in PLT distribution.

4.3.9 Closed feedback model

In the ***closed feedback*** model we incorporated the dependence of PIN expression, the expression of a generalised auxin synthesizing and auxin degrading enzyme on local PLT levels. These modifications are based on the findings that PIN expression is upregulated under high PLT expression[16,17], the auxin synthesizing enzyme YUC3 is upregulated under high PLT expression[18] (Ben Scheres lab, unpublished data), and the auxin degrading enzyme GH33 is downregulated under high PLT expression (Ben Scheres lab, unpublished data).

For PIN expression, which in the other model settings already was ARF dependent (Eq. 19) we now write the following equation:

$$\frac{dPIN}{dt} = max_{PIN}\left(0.5a\frac{ARF^2}{ARF^2+sat1_{PIN}^2}+0.5a\frac{PLT^2}{PLT^2+sat2_{PIN}^2}+(1-a)\right)-d_{PIN}\,PIN; \qquad \text{Eq. 27}$$

with max_{PIN} , and d_{PIN} the same as for Eq. 19, a the fraction of ARF and PLT dependent PIN expression, $sat1_{PIN}$ the ARF concentration at which ARF dependent PIN production reaches its half maximum rate, and $sat2_{PIN}$ the PLT concentration at which PLT dependent PIN production reaches its half maximum rate.

For the expression of the generalised auxin synthesizing enzyme we use the following equation:

$$\frac{dSE}{dt} = max_{SE}\frac{PLT^2}{PLT^2+sat_{SE}^2}-d_{SE}\,SE \qquad \text{Eq. 28}$$

where max_{SE} is the maximum production rate of the enzyme, d_{SE} the degradation rate of the enzyme, and sat_{SE} the level of PLT at which half the maximum production rate of the enzyme is reached. For simplicity, the values for the maximum production and degradation rates are set to the same values as for PIN production and degradation. For sat_{SE} a value of 40 was used.

Incorporating this PLT-dependent and hence spatially non-homogenous expression of an auxin synthesizing enzyme implies that the cellular production rate of auxin is no longer constant and identical for all cells but instead now depends on the local level of auxin synthesizing enzyme in that cell:

$$p_{Aux} = \left(a_{SE}\,0.01\,SE+\left(1-a_{SE}\right)\right)p_{max} \qquad \text{Eq. 29}$$

here a_{SE} reflects the fraction of auxin production that is assumed to be PLT dependent (value set to 0.7). Note that the 0.01 scaling factor serves to scale the expression level of SE which lies between 0 and 100 to a number between 0 and 1. As a consequence, for a maximum expression of SE of 100 p_{Aux} equals p_{max} , for lower levels of SE expression p_{Aux} is lower than p_{max} . The value of p_{max} is set to the constant value p_{Aux} has in

the earlier model versions.

For the expression of the generalised auxin degrading enzyme we use the following equation:

$$\frac{dDE}{dt} = max_{DE} \frac{sat_{DE}^{2}}{PLT^{2} + sat_{DE}^{22}} - d_{DE} DE \qquad \text{Eq. 30}$$

where max_{DE} is the maximum production rate of the enzyme, d_{DE} the degradation rate of the enzyme, and sat_{DE} the level of PLT at which half the maximum production rate of the enzyme is reached. For simplicity, the values for the maximum production and degradation rates are set to the same values as for PIN production and degradation. For sat_{DE} a value of 40 was used.

Similar to above, the incorporation of non-homogeneous expression of an auxin degrading enzyme implies that the cellular degradation of auxin is no longer constant but instead depends on local auxin degrading enzyme levels:

$$d_{Aux} = \left(a_{DE} 0.01\, DE + \left(1 - a_{DE}\right)\right) d_{max} \qquad \text{Eq. 31}$$

here a_{DE} reflects the fraction of auxin degradation that is assumed to be PLT dependent (value set to 0.7). Similar to above the 0.01 scaling factor serves to scale the expression level of DE to a number between 0 and 1, resulting in a maximum value for d_{Aux} of d_{max}, for lower levels of DE d_{Aux} is lower than d_{max}. The value of d_{max} is set to the constant value d_{Aux} has in the earlier model versions.

Supplementary Table 2. Parameter settings used for the initial, PLT-spread and auxin models. The gravitropism model has the same parameter settings as the auxin model, except for the described superimposed PIN3 dynamics. The closed feedback model also has the same parameter settings as the auxin model, except for the above described dependencies of p_{Aux} (Eq. 29) and d_{Aux} (Eq. 31) on PLT regulated enzyme levels."-" indicates that the same parameter value is used in a model as in the earlier model (to the left of it in the table).

Parameter	Initial model	PLT-spread model	Auxin Model	units
Timestep	0.2	-	-	s
spacestep	2	-	-	μm
cell width	16	-	-	μm
cell wall width	4	-	-	μm

initial cell height	8	-	-	μm
root tissue width	160	-	-	μm
root tissue height	1850	-	-	μm
D_{cell}	600	-	-	$\mu m^2 s^{-1}$
D_{wall}	40	-	-	$\mu m^2 s^{-1}$
$i_{pas+act}$	10	-	-	$\mu m \ s^{-1}$
e_{pas}	1	-	-	$\mu m \ s^{-1}$
p_{flux}	0	0.0008	-	$\mu m \ s^{-1}$
ARF_{total}	100	-	-	$[]$
ass	0.01	-	-	s^{-1}
$diss$	0.001	-	-	s^{-1}
$p_{AUX/IAA}$	0.01	-	-	s^{-1}
$d_{AUX/IAA,basal}$	0.0001	-	-	s^{-1}
$d_{AUX/IAA,TIR1}$	0.001	-	-	$[]^{-1} s^{-1}$
max_A	0.005	-	-	$[] s^{-1}$
sat_A	50	-	-	$[]$
d_A	0.00005	-	-	s^{-1}
max_B	0.005	-	-	$[] s^{-1}$
sat_B	50	-	-	$[]$
d_B	0.00005	-	-	s^{-1}
max_C	0.005	-	-	$[] s^{-1}$
sat_C	50	-	-	$[]$
d_C	0.00005	-	-	s^{-1}
max_D	0.005		-	$[] s^{-1}$
sat_D	60	-	-	$[]$
d_D	0.00005		-	s^{-1}
max_{PLT}	0.02	0.00175	-	$[] s^{-1}$
sat_{PLT}	40	-	-	$[]$
d_{PLT}	0.0002	0.0000175	-	s^{-1}

max_{PIN}	0.01	-	-	$[]s^{-1}$
a	0.5	-	-	dimensionless
sat_{PIN}	15	-	-	$[]$
d_{PIN}	0.0001	-	-	s^{-1}
a_{PIN}	0.6	-	-	dimensionless
PIN_{basal}	100	-	-	$[]$
max_{pump}	0.2	-	-	$\mu ms^{-1}[]^{-1}$
p_{Aux}	0.0005	-	-	$[]s^{-1}$
d_{Aux}	0.00005	-	-	s^{-1}
$i_{pas+act}$	10	-	-	μms^{-1}
e_{basal}	1	-	-	μms^{-1}
Max rel collumella PIN levels	1	-	-	dimensionless
Max rel apical PIN levels	1	-	-	dimensionless
Max rel basal PIN levels	1	-	-	dimensionless
Max rel lateral PIN levels, epidermal and cortex cells	0.35	-	-	dimensionless
Max rel lateral PIN levels, border cells	0.1	-	-	dimensionless
$H_{division}$	16	-	-	μm
$H_{maximum}$	144	-	-	μm
Th_{PLT1}	40	-	-	$[]$
Th_{PLT2}	15	-	-	$[]$
$r_{growth,max}$	0.0396	0.0491	-	$hrs^{-1}\mu m^{-1}$
$r_{expansion,max}$	0.000314	-	-	$min^{-1}\mu m^{-1}$
$r_{DF,max}$	0.0064	-	-	$[]s^{-1}$
d_{DF}	0.000064	-	-	s^{-1}

4.4 Model settings to simulate physiological experiments

The above described models were used to simulate root zonation dynamics under a series of different conditions that we shortly describe below.

4.4.1 Normal growth

Simulations of normal growth utilized all previously described model components. Simulations were run till steady state overall auxin levels were reached. Shown results are always for such steady state conditions.

4.4.2 Auxin application

External, global auxin application was simulated by adding an influx term i_{Aux} to Eq. 3 for those wall grid points that form the outer boundary of the tissue, thus emulating the influx that results from auxin present in the external medium. Auxin application experiments were performed after a steady state dynamics under normal growth conditions was reached. Experiments were performed both for high and intermediate external auxin levels, and were run for 24hrs of physiological time.

4.4.3 Auxinole application/AXR3 mutation

Application of the auxin signalling inhibitors auxinole/axr3 was simulated by reducing the rate of TIR mediated AUX/IAA repressor degradation tenfold. These application experiments were performed after a steady state dynamics under normal growth conditions was reached, and were run for 24 hours of physiological time.

4.4.4 Shoot cut

Cutting the shoot from the root was simulated by abolishing the influx from the (not-explicitly simulated) shoot vasculature to the root, while maintaining the efflux from the uppermost root tissues (which under normal growth conditions would go to the shoot), similar to the approach taken in Grieneisen et al.[15]. In our simulations the shoot cut was performed after a steady state dynamics under normal growth conditions had been reached. Shoot cut simulations were run for 72hrs of physiological time.

4.4.5 Non-growing

In addition to the above described simulated conditions, auxin, ARF and PLT expression patterns were also simulated in the absence of cell growth, division and expansion, to determine the effect of PLT lifespan and PLT cell-to-cell spread in the absence of cell displacement. We will refer to these simulations as 'non-growing', to distinguish them from the standard simulations incorporating growth processes.

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