Development 140, 185-194 (2013) doi:10.1242/dev.084582

Auxin controls petal initiation in *Arabidopsis*

Edwin R. Lampugnani*, Aydin Kilinc and David R. Smyth‡

SUMMARY

Floral organs are usually arranged in concentric whorls of sepals, petals, stamens and carpels. How founder cells of these organs are specified is unknown. In *Arabidopsis*, the PETAL LOSS (PTL) transcription factor functions in the sepal whorl, where it restricts the size of the inter-sepal zone. Genetic evidence suggests that PTL acts to support a petal initiation signal active in the adjacent whorl. Here we aimed to characterise the signal by identifying enhancers that disrupt initiation of the remaining petals in *ptl* mutants. One such enhancer encodes the auxin influx protein AUX1. We have established that auxin is a direct and mobile petal initiation signal by promoting its biosynthesis in the inter-sepal zone in *ptl* mutant plants and restoring nearby petal initiation. Consistent with this, loss of PTL function disrupts DR5 expression, an auxin-inducible indicator of petal-initiation sites. The signalling network was extended by demonstrating that: (1) loss of RABBIT EARS (RBE) function apparently disrupts the same auxin influx process as PTL; (2) the action of AUX1 is supported by AXR4, its upstream partner in auxin influx; (3) polar auxin transport, which is controlled by PINOID (PID) and PIN-FORMED1 (PIN1), functions downstream of PTL; and (4) the action of *pmd-1d*, a dominant modifier of the *ptl* mutant phenotype, is dependent on auxin transport. Thus, loss of PTL function disrupts auxin dynamics, allowing the role of auxin in promoting petal initiation to be revealed.

KEY WORDS: AUX1, Auxin, Petal development, PETAL LOSS, Polar auxin transport, *Arabidopsis*

INTRODUCTION

The floral blueprint is established early in flower development (Endress, 2011; Smyth, 2005). The number and location of floral organs depends on the specification of founder cells in the flower meristem (Chandler, 2011). These cells are first destined to grow into organ primordia, which later differentiate appropriately as sepals, petals, stamens or carpels. The identity of each is defined by combinations of well-known transcription factor genes (Krizek and Fletcher, 2005). However, the mechanism by which founder cells are specified is not known.

By contrast, signals involved in defining the sites of origin of leaves that arise from the shoot apical meristem, phyllotaxy, and of flowers from the inflorescence meristem, are beginning to be characterised. Auxin plays a key role in this process. It is sufficient to define the sites of leaf and flower primordia, as its exogenous application sets their development in train (Reinhardt et al., 2000). Disruption of its biosynthesis results in loss of most floral organs (Cheng et al., 2006; Stepanova et al., 2008; Tobeña-Santamaria et al., 2002). In *Arabidopsis*, auxin is apparently accumulated in the epidermal (L1) layer by the auxin influx protein AUX1 and transported in this layer towards the sites of organ initiation by the polar auxin transporter PIN-FORMED1 (PIN1) (Reinhardt et al., 2003). This process is positively regulated by auxin concentration, so that foci of increased auxin are reinforced (Heisler et al., 2005). Thus new primordia act as auxin sinks, and reduce the surrounding auxin concentration so that the next primordium arises at the furthest distance from pre-existing primordia where the auxin concentration remains relatively high. As a primordium continues to grow, auxin flow reverses and it moves away from the primordium (Heisler et al., 2005), and into the underlying provasculature (Reinhardt et al., 2003). Later it acts at the apex of the primordium itself (Benková et al., 2003). The cycle of auxin concentration and depletion radially around the meristem is sufficient to define the phyllotactic pattern of leaf and flower initiation (Jönsson et al., 2006; Smith et al., 2006).

Other factors are also involved. Loosening of cell walls by the application of expansin or pectin methyl esterase can promote primordium initiation from shoot and inflorescence meristems (Fleming et al., 1997; Peaucelle et al., 2008). Disruption of intrinsic physical forces within meristems by regional laser ablation also influences phyllotaxy (Hamant et al., 2008), a process linked with auxin dynamics (Heisler et al., 2010). Cytokinin signalling is also involved in maintaining phyllotaxy, again in association with polar auxin transport (Lee et al., 2009). In addition, mobile peptides, already known to be involved in meristem maintenance (Butenko et al., 2009), may also trigger organ initiation. Whether any of these factors are directly involved in specifying organ founder cells is not established.

One approach to identifying signals is to specifically disturb the signalling process by mutation. Disruption of petal initiation is frequent among floral mutants of *Arabidopsis* (Irish, 2008), suggesting that the threshold of response to signalling may be finely balanced. This could be a consequence of the relatively few founder cells involved (Bossinger and Smyth, 1996).

Mutations of *PETAL LOSS* (*PTL*) specifically affect the initiation of petals (Griffith et al., 1999). There are normally four petals in *Arabidopsis* flowers, arising at stage 5 internally and alternately with the four sepals (Smyth et al., 1990). In *ptl* mutants, petals arise sporadically, and the number falls to zero in later arising flowers. The few petals that do occur are initiated over a wider area and an extended time interval during flower development. It is likely that the petal initiation process itself is disrupted, as there is no indication of primordial cell divisions where petals fail to appear, and Griffith et al. (Griffith et al., 1999) have proposed that the signalling of petal initiation is disrupted. A dominant modifier of petal initiation, *petal loss modifier-1d* (*pmd-1d*), was also

School of Biological Sciences, Monash University, Melbourne, Victoria 3800, Australia.

*Present address: School of Botany, University of Melbourne, Victoria 3010, Australia
‡Author for correspondence (david.smyth@monash.edu)

Accepted 1 October 2012

uncovered specifically in the Landsberg *erecta* (L*er*) background, resulting in a boost to petal numbers in *ptl* mutants, although they were still variably initiated. This allele may amplify the petal initiation signal, or the response to it. Finally, petal orientation is also disrupted in *ptl* mutant flowers although independently of petal initiation, and a second, independent signal may be involved (Griffith et al., 1999).

The *PTL* gene encodes a trihelix transcription factor (Kaplan-Levy et al., 2012) that is expressed in newly arising flower primordia, especially between sepals as they develop from stage 3 (Brewer et al., 2004). Expression occurs in the epidermis and underlying cell layers. Overexpression of PTL results in growth suppression wherever it is expressed (Brewer et al., 2004; Li et al., 2008). Conversely, loss of PTL function leads to overgrowth of the inter-sepal zone (Lampugnani et al., 2012). These findings indicate that the role of PTL is to help define the inter-sepal zone by keeping cell division there in check. PTL is not expressed in the second whorl, and so disruption of petal initiation in *ptl* mutants is apparently non-cell autonomous, perhaps by influencing the strength of a mobile founder cell signal (the movement of PTL protein itself has not been examined).

The aim of this study was to uncover such a petal initiation signal. To this end, we conducted a genetic screen to identify second site mutants that enhanced the loss of petals in *ptl* mutant flowers, where such a signal may already be partially disrupted. We revealed that the auxin influx gene *AUX1* is directly associated with petal initiation, although detectable only when PTL function is lost. The signalling role of auxin was confirmed because petal initiation could be restored by the synthesis of auxin in the *PTL* expression zone. Also, the sites of petal initiation, indicated by expression of the DR5 auxin activity reporter, were specifically disrupted in *ptl* mutants. Genetic interaction studies including other genes involved in petal initiation and auxin transport have allowed a petal initiation pathway to be proposed.

MATERIALS AND METHODS

Source and growth of plants

The mutants *ptl-1* (in Columbia, Col) (Griffith et al., 1999) and *ptl-3* (in Landsberg *erecta*, L*er*) (Brewer et al., 2004), and *pin1-3*, *pin1-5*, *pid-1* (all in L*er*) and *pid-3* (Col) (Bennett et al., 1995), were from our laboratory stocks. The dominant modifier *pmd-1d* is naturally present in all L*er* lines (Griffith et al., 1999). The mutant lines *aux1-7*, *aux1-21*, *axr4-1*, *axr4-2* and *rbe-2* (all in Col) were obtained from the *Arabidopsis* Biological Resources Center, Columbus, Ohio, USA (CS3074, CS9584, CS8018, CS8019 and CS6396). Seeds of all combinations of *aux1-21*, *lax1-1*, *lax2-1* and *lax3-1* mutants were generously provided by Morag Whitworth and Malcolm Bennett (University of Nottingham, UK). Plants were grown under continuous Cool White light supplemented with daylight at 20-26°C. For comparisons of floral organ numbers, all plants were grown together. Counts involved a specified number of the first flowers to develop in the primary inflorescence. Variant organs, such as mosaics and filaments, were included in the counts for each whorl.

Genetic procedures

Seeds of *ptl-3* mutants (L*er*) were mutagenised with ethyl methanesulfonate (EMS), planted, and pools of 3-6 M1 plants were collected after seed set. These pools were generously provided by Stuart Baum and John Bowman (University of California Davis, USA). M2 progeny of 32 M1 pools were screened. Suppressors were backcrossed either two or three times to the *ptl-3* parent line before further characterisation.

Multiple mutants were derived from F2 families, usually by selecting known homozygotes of one mutant and progeny testing for segregation of the other(s) in the F3. PCR genotyping tests were also developed for all mutants except *pin1* and *pid* alleles (supplementary material Table S1). In the case of *ptl-1 rbe-2* double mutants, where single mutant phenotypes are similar and the loci only three map units apart, F2 plants homozygous for *ptl-1* and heterozygous for *rbe-2*, or vice versa (i.e. carrying a single cross-over) were identified by DNA testing (7 of 78), and double mutants identified among the F3 progeny by DNA testing.

Origin and generation of transgenes

Seeds carrying pAUX1:AUX1-YFP116 (Reinhardt et al., 2003) were obtained from Malcolm Bennett. This carries YFP inserted in AUX1 at codon 116, driven by 2.2 kb of upstream promoter. We generated pAUX:GUS with a longer, 3.62 kb promoter, using the same approach and vectors as Brewer et al. (Brewer et al., 2004). A PCR-generated promoter sequence from BAC F16M14, obtained from ABRC (2575396), was inserted immediately upstream of the GUS sequence, transferred into the vector pMLBART, and transformed directly into Col and *ptl-1* plants. Transgenes containing *AUX1* or *iaa*H under the control of the pPTL(FI313) promoter (Lampugnani et al., 2012) were generated using the same strategy and vectors. The *AUX1* genomic DNA was generated from BAC F16M14, and the *iaa*H sequence (in TOPO-iaaH) was provided by Lars Østergaard (John Innes Centre, Norwich, UK). The presence of appropriate transgenes in T1 plants was confirmed in all cases by PCR analysis (supplementary material Table S1). The origins of pPTL(FI313):VENUS-N7 and pPTL(1.3i):GUS have been described (Lampugnani et al., 2012; Brewer et al., 2004). Seeds of DR5rev:GFP-ER in Columbia, and of pPIN1:PIN1-GFP in Columbia or *ptl-1* (Benková et al., 2003), were kindly provided by Philip Brewer and Jiří Friml (University of Tübingen, Germany).

Microscopy

For confocal fluorescence microscopy, live inflorescences were prepared as described previously (Lampugnani et al., 2012). pAUX1:AUX1-YFP116 plants were imaged using an Olympus FV500 microscope with a Uplan Fl 40X objective. Fluorescence was excited by a 488 nm laser line, and detected using a 515/530 band pass channel. pPIN1:PIN1-GFP plants were viewed using a Nikon C1 microscope, and plants carrying both pPTL(FI313):VENUS-N7 and DR5rev:GFP-ER (and homozygous for *erecta-109*) were imaged with a Leica SP5 microscope, as described earlier in each case (Lampugnani et al., 2012). Images were collected at 1 or 2 μm intervals and *z*-stacks generated and analysed as before (Lampugnani et al., 2012), applying linear unmixing where appropriate. Methods for observing expression of GUS reporters followed Brewer et al. (Brewer et al., 2004).

RESULTS

no petals mutants enhance the *petal loss* phenotype

To identify enhancers of the *petal loss* mutant phenotype, an EMS mutagenesis of *ptl-3 pmd-1d* seeds was screened. Two independent recessive mutations resulted in the loss of almost all petals (Fig. 1A-D,G; supplementary material Table S2). When these two mutant lines were inter-crossed, loss of petals still occurred, indicating their allelism. They were given the working names *no petals-1* (*nop-1*) and *nop-2*. Plants homozygous for *nop-1* or *nop-2* but wild type for *PTL* almost always had the wild-type number of petals, although one was occasionally absent (Fig. 1E-G). Thus NOP is a modifier of petal initiation, and its function is only uncovered when PTL function is compromised.

NO PETALS encodes the auxin influx carrier AUX1

To clone the *NOP* gene from its map position, a cross was made between *ptl-3 pmd-1d nop-1 er* in L*er* background and *ptl-1 PMD NOP ER* in Col background, and F2 plants were examined. Scoring of 866 unselected plants revealed that *NOP* is linked to the *ERECTA* (*ER*) locus on chromosome 2, separated by about 35-40 map units. Focusing on 990 homozygous *nop-1* mutant plants from the same F2 family and designing appropriate DNA markers, the location of *NOP* was ultimately constrained to a 41 kbp region between 15,960 kbp and 16,001 kbp (supplementary material Fig.

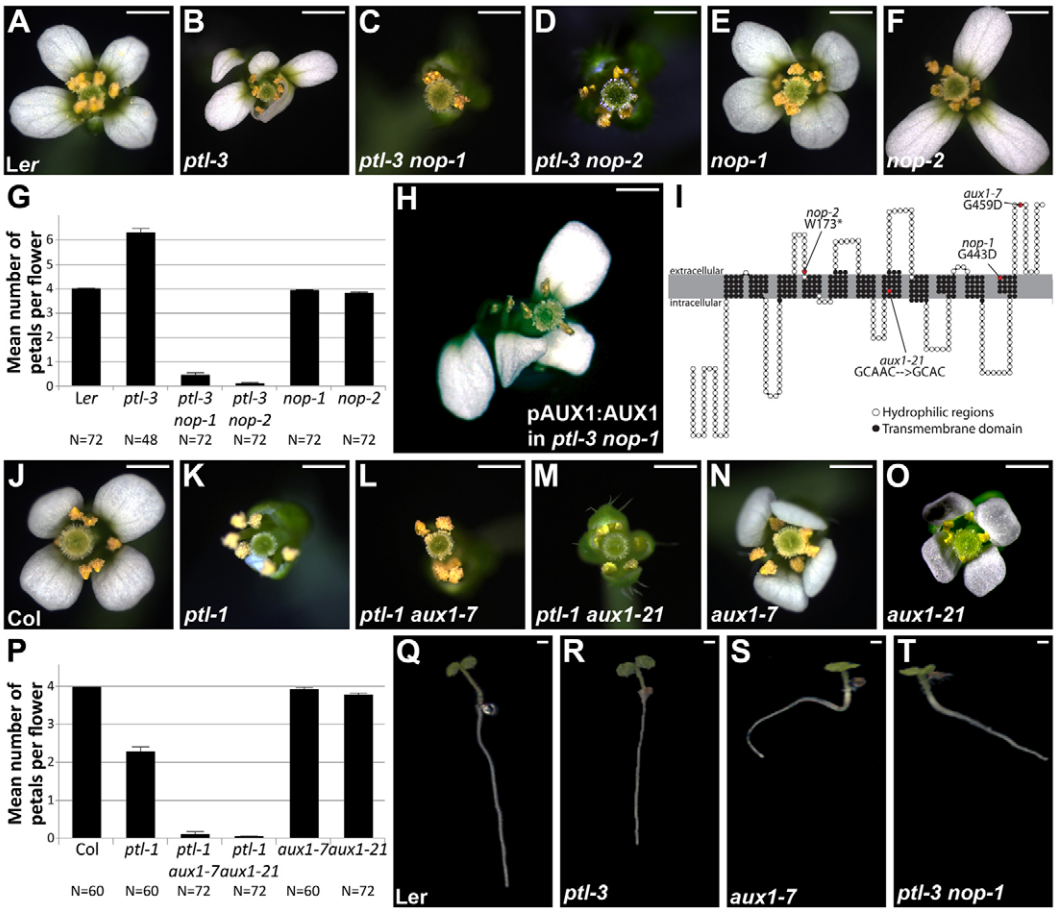

Fig. 1. Phenotype of *no petals* (*nop*) enhancers of the *petal loss* mutant phenotype, and demonstration of their allelism with *aux1*. (**A-F**) Floral phenotypes of wild-type (L*er*) (A) and *ptl-3* (B), *ptl-3 nop-1* (C), *ptl-3 nop-2* (D), *nop-1* (E) and *nop-2* (F) mutant plants. All plants carry the modifier *pmd-1d*. (**G**) Mean number of petals per flower in single and double mutant combinations of *ptl-3* and *nop-1* or *nop-2*. N flowers were scored, the first six on each plant. All plants carry the modifier *pmd-1d*. Bars represent s.e.m. (**H**) Floral phenotype of *ptl nop* double mutant flower complemented with pAUX1:AUX1-YFP116. (**I**) Topological representation of the AUX1 protein, showing predicted amino acid substitutions of *aux1-7*, *aux1-21*, *nop-1* (*aux1-n1*) and *nop-2* (*aux1-n2*). White circles, hydrophilic residues; black circles, predicted transmembrane domains. After Swarup et al. (Swarup et al., 2004). (**J-O**) Floral phenotypes of wild-type (Col) (J) and *ptl-1* (K), *ptl-1 aux1-7* (L), *ptl-1 aux1-21* (M), *aux1-7* (N) and *aux1-21* (O) mutant plants. (**P**) Mean number of petals per flower in single and double mutant combinations of *ptl-1* and *aux1-7* or *aux1-21*. N flowers were scored, the first six on each plant. Bars represent s.e.m. (**Q-T**) Root phenotypes of wild-type (L*er*) (Q), *ptl-3* (R), *aux1-7* (S) and *ptl-3 nop-1* (*aux1-n1*) (T) seedlings. All flowers shown are the first one formed on the primary inflorescence. Scale bars: 0.5 mm.

S1, Tables S10, S11). This includes seven annotated genes, of which *AUX1* (At2g38120) was a candidate.

NOP was confirmed to be the same gene as *AUX1* by five criteria. First, AUX1 expression driven by its own promoter was able to complement the severe loss of petal phenotype in *ptl nop* double mutant plants (Fig. 1H). Second, *nop-1* was shown to be allelic with *aux1-7* in that petals were almost always lacking in progeny of crosses between *ptl-3 nop-1* and *ptl-1 aux1-7* plants. Third, *nop-1* and *nop-2* mutants each showed single amino acid changes in the deduced AUX1 sequence that are likely to disrupt function (G443D and W173*, respectively) (Fig. 1I). Fourth, the characteristic loss of petals in *ptl-3 nop-1* plants was reproduced in *ptl-1 aux1-7* and *ptl-1 aux1-21* double mutant plants (Fig. 1J-P; supplementary material Table S3). These are unmodified *PMD* lines in Columbia background, so the effect of loss of AUX1 function is not confined to the modified *pmd-1d* genotype. Finally, *aux1* mutants show agravitropic root growth (Bennett et al., 1996), and *ptl-3 nop-1* seedlings were also agravitropic, although *ptl-3* plants were not (Fig. 1Q-T). Thus the two mutants define alleles of *aux1*, and they were named *aux1-n1* and *aux1-n2*.

AUX1 has three close relatives in *Arabidopsis*, *LIKE AUX1* (*LAX*) genes, that are functionally redundant with AUX1 in maintaining leaf and floral bud phyllotaxis (Bainbridge et al., 2008). However, redundancy in the control of petal initiation was relatively weak, as the mean number of petals in *aux1 lax1 lax2 lax3* quadruple mutant flowers was 3.65±0.09, close to the normal 4 (supplementary material Table S4).

aux1 mutations are semi-dominant in *ptl* mutant homozygotes, and vice versa

To examine the effects of *PTL* and *AUX1* gene dosage on phenotype, plants from an F2 family derived from a cross between *ptl-1* and *aux1-7* were individually genotyped, and petal numbers were scored (Table 1, left). When PTL function was lost (in *ptl-1* homozygotes), the additional loss of one dose of *AUX1* (*aux1-7*/+ heterozygotes) resulted in a significant further drop in the mean

Table 1. Effect of *PTL* and *AUX1* gene dose on petal numbers

Genotype (all *PMD*)	Number of plants (flowers)*	Mean number of petals±s.e.m.	Genotype (all *pmd-1d*)	Number of plants (flowers)*	Mean number of petals±s.e.m.
Homozygotes			Homozygotes		
Wild type (Col)	18 (180)	4.00±0.01	Wild type (L*er*)	18 (180)	4.02±0.01
ptl-1	24 (237)	1.35±0.08	*ptl-3*	21 (210)	5.44±0.09
ptl-1 aux1-7	24 (240)	0.02±0.01	*ptl-3 aux1-n1*	30 (300)	0.18±0.02
aux1-7	10 (100)	3.92±0.03	*aux1-n1*	10 (100)	3.95±0.02
Heterozygotes			Heterozygotes		
ptl-1 aux1-7/+	9 (90)	0.86±0.11	*ptl-3 aux1-n1/+*	26 (260)	3.99±0.06
ptl-1/+ aux1-7	10 (100)	2.72±0.09	*ptl-3/+ aux1-n1*	10 (99)	3.82±0.04
ptl-1/+ aux1-7/+	10 (94)	3.71±0.06	*ptl-3/+ aux1-n1/+*	10 (100)	3.99±0.01
ptl-1/+	16 (160)	3.90±0.05	*ptl-3/+*	18 (180)	4.01±0.01
aux1-7/+	18 (180)	4.00±0.00	*aux1-n1/+*	22 (220)	4.00±0.01

*The first 10 flowers on each plant were scored (with an occasional flower missed). All plants were F2 siblings within each series.

number of petals per flower, from 1.35±0.08 to 0.86±0.11 (t_{135}=3.50, P<0.001). Similarly, *aux1-7* homozygotes had close to wild-type petal numbers (3.92±0.03) unless *ptl-1* was heterozygous, in which case the mean number fell significantly to 2.72±0.09 (t_{198}=12.77, P<0.001). Similar results were obtained from parallel studies of *ptl-3* and *aux1-n1* in *pmd-1d* background (Table 1, right), although differences were not as marked. Thus the two functions, PTL and AUX1, are sensitive to a reduction in the function of the other, as expected if they act additively to promote petal initiation.

AUX1 is expressed in the epidermis of newly arising flower and floral organ primordia

Expression of the fluorescent reporter pAUX1:AUX1-YFP116 (Swarup et al., 2004) was investigated by live imaging the developing inflorescences of Columbia wild-type plants (Fig. 2A-E). Expression was localised to the epidermis (L1) and was strong throughout flower primordia from their inception (stage 1) until at least stage 4 (Fig. 2A-B). At stage 4, expression was relatively strong in inter-sepal zones (Fig. 2C-E, arrows), although not noticeably so in locations where petal primordia will arise.

To further define expression at stage 4, we also examined a new reporter construct, pAUX1:GUS with a longer upstream promoter region (3.67 kbp compared with 2.2 kbp). Serial transverse sections of stage 4 buds (supplementary material Fig. S2) confirmed that strong expression occurred in the inter-sepal zones (Fig. 2F), where it matched *PTL* expression (Fig. 2G), although the latter was also strong in the underlying cell layers (Lampugnani et al., 2012). The same construct was transformed into *ptl-1* mutant plants, and the localisation of expression in early flowers of 12 independent transformants was not detectably different from that in nine wild-type transformants.

Generation of auxin in the inter-sepal zone restores petal initiation in *ptl* mutant flowers

To test if auxin deficiency is the primary cause of loss of petals in *ptl* mutant plants, we generated ectopic auxin in the inter-sepal zones using the bacterial auxin biosynthesis gene *iaa*H under the control of the PTL promoter. *iaa*H expression can restore petal initiation in *Arabidopsis* flowers doubly mutant for the auxin biosynthesis genes *YUCCA1* (*YUC1*) and *YUC4* (Cheng et al., 2006). The PTL promoter used, pPTL(FI313), is sufficient to complement sepal and petal defects in *ptl* mutant plants when driving PTL expression (Lampugnani et al., 2012). When pPTL(FI313):iaaH was transformed into wild-type plants, there was no effect on petal development. However, in seven transformed *ptl-1* mutant plants petal numbers were boosted almost to wild-type (Col) levels (Fig. 3A; supplementary material Table S5). In many flowers four petals now occurred, one per position. By contrast, the mis-orientation defect in petals was not significantly ameliorated (69% were mis-oriented in the seven transformed *ptl-1* plants compared with 71% in ten untransformed controls).

We next tested whether driving AUX1 expression using the same *PTL* regulatory sequence could restore petal initiation in *ptl aux1* double mutants. There was no effect on petal numbers when the pPTL(FI313):AUX1 construct was inserted into wild-type plants. However, in five out of nine *ptl-1 aux1-7* transformants, the mean petal number per flower was raised from only 0.07±0.02 in untransformed controls to now match the *ptl-1* single mutant control (1.45±0.11) (Fig. 3B; supplementary material Table S6). In 12 *ptl-3 aux1-n1* transformants (carrying the *pmd-1d* modifier), the mean number was mostly increased although not matching the *ptl-3* single mutant control (5.63±0.15) (Fig. 3C; supplementary material Table S7). Even so, four of the T1 lines had a mean of between three and four petals per flower, and some early flowers had five or six. Thus even the localised restoration of auxin influx to the inter-sepal zone in *ptl aux1* mutants is able to promote significant petal initiation.

Loss of PTL function disrupts DR5 expression in petal initiation zones

The sites of organ initiation can be indicated by expression of the auxin-inducible reporter DR5rev:GFP-ER (Friml et al., 2003). In wild-type buds at stage 4, DR5 expression occurs at four sites where petal primordia will arise (Chandler et al., 2011; van Mourik et al., 2102). These lie immediately internal to the inter-sepal zones where *PTL* is expressed (Lampugnani et al., 2012), and each often involved two adjacent cells (Fig. 4A-I), as predicted by sector boundary analysis (Bossinger and Smyth, 1996). In *ptl-1* mutants, these DR5-expressing sites were disrupted in four ways (Fig. 4J-R). First, their location was more variable, including occasionally occurring within the inter-sepal zone (Fig. 4P-R). Second, the number of cells in each was sometimes increased or decreased, and their orientation distorted. Third, their relative intensity was reduced. Finally, they could not be detected in some petal initiation zones. These changes are consistent with loss of PTL function causing disruption to the auxin signalling of petal initiation. Further, the petal phenotype of mature *ptl* mutant flowers (Griffith et al., 1999) reflects the

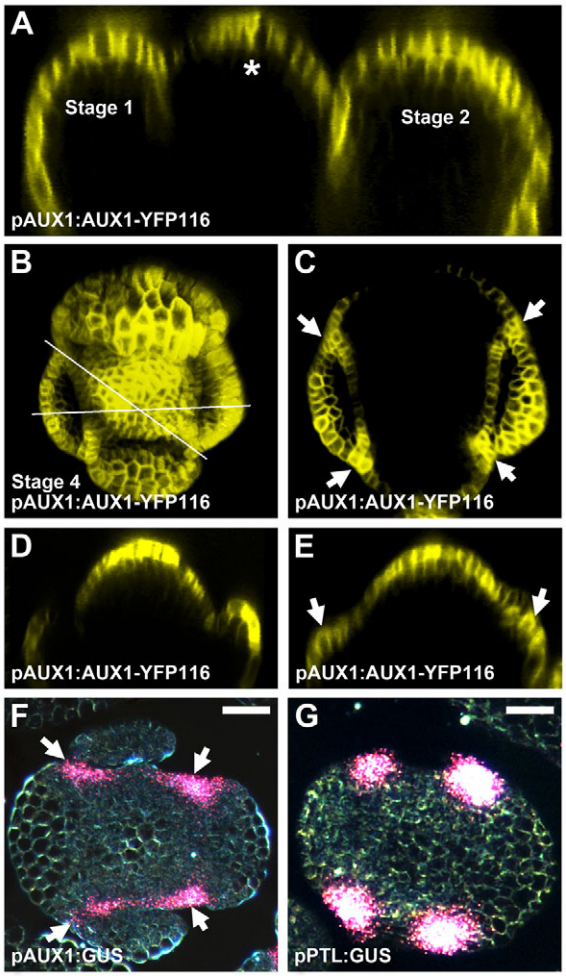

Fig. 2. Expression of *AUX1* in wild-type floral meristems. (**A**) Confocal image showing pAUX1:AUX1-YFP116 fluorescence in a virtual *z*-section through an inflorescence meristem (asterisk) and stage 1 and 2 buds. (**B**) A 3D reconstruction (vertical view) of a bud at stage 4 expressing the same construct. (**C-E**) Virtual *z*-sections through this bud taken transversely (C) or longitudinally in the medial (D) or diagonal planes (E) (indicated by lines in B). Arrows indicate stronger expression in inter-sepal zones. (**F**) GUS expression in a transverse section of a stage 4 bud carrying pAUX1(3.67kbp):GUS showing strong expression in the inter-sepal zones (arrows). See supplementary material Fig. S2 for further serial sections. (**G**) pPTL(1.3i):GUS expression in a transverse section of a stage 4 bud, showing localised expression in the inter-sepal zones. Scale bars: 20 μm.

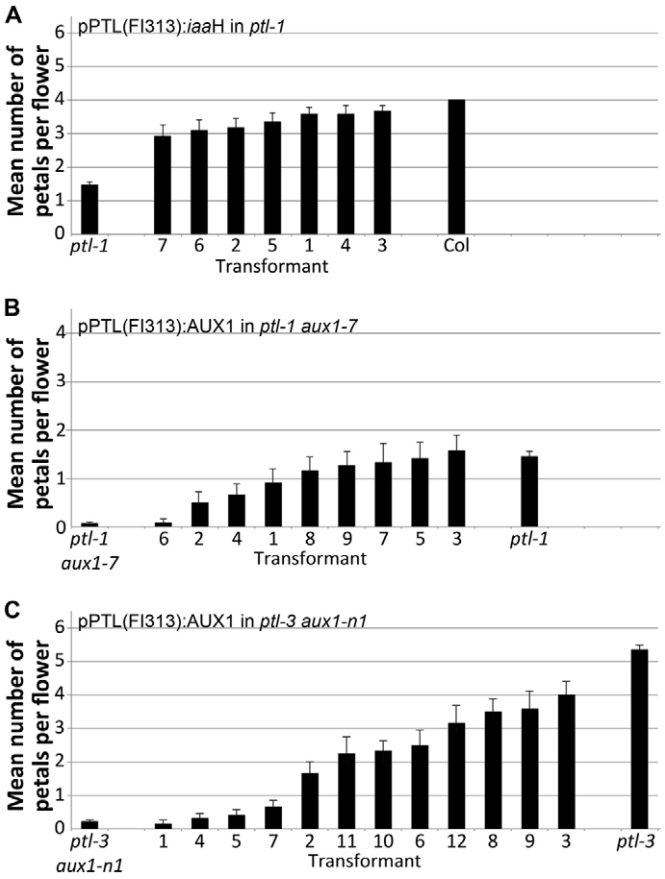

Fig. 3. Restoration of petals in *ptl* mutants by a transgene controlling auxin biosynthesis, and in *ptl aux1* mutants by a transgene controlling auxin influx. The first 12 flowers of each plant were scored. Results for untransformed control plants are shown in the left and right columns. Bars represent s.e.m. (**A**) Mean number of petals per flower in seven *ptl-1* T1 plants transformed with pPTL(FI313):iaaH. (**B**,**C**) Mean number of petals per flower in nine *ptl-1 aux1-7* T1 plants (A), or in 12 *ptl-3 aux1-n1* T1 plants (in *pmd-1d* background) (B), transformed with pPTL(FI313):AUX1 in each case.

variability in size, orientation and location of these DR5-expressing cells at stage 4.

Petal initiation is also sensitive to the loss of AXR4 function in the auxin influx pathway

To test if other disruptions to auxin influx also abolish petal initiation in *ptl* mutants, the consequences of loss of AUXIN RESISTANT4 (AXR4) function were examined. This acts upstream of AUX1 in the auxin influx pathway (Dharmasiri et al., 2006). Two mutant alleles, *axr4-1* and *axr4-2*, in Col background were examined alone and in combination with *ptl-1* (Fig. 5A-D). In each case, like *aux1*, they had a very minor effect on petal numbers when singly homozygous. However, double mutants of either with *ptl-1* resulted in an almost complete loss of petals, much fewer than the 2.31±0.13 per flower in *ptl-1* single mutants (Fig. 5E; supplementary material Table S7). Thus AXR4, just like AUX1, is required for petal initiation when PTL function is compromised.

RABBIT EARS and PTL share petal initiation function

RABBIT EARS (RBE) is a zinc finger regulatory protein that promotes petal initiation in many ways like PTL. Loss of RBE function also results in an acropetal decrease in petal initiation (Krizek et al., 2006; Takeda et al., 2004), although petal orientation is unaffected. It is reported to act downstream of PTL, as *RBE* expression was not observed in stage 5 *ptl* mutant flowers. Thus we were interested to examine whether loss of RBE function also revealed a role for auxin influx in petal initiation. Before doing this, however, we showed that *PTL* is expressed normally in *rbe-2* mutant flowers (Fig. 5F,G), and also that there is no further loss of petals in *ptl-1 rbe-2* double mutants (Fig. 5H-L; supplementary material Table S8) (t_{112}=0.68, P>0.05 comparing *ptl-1 rbe* with *ptl-*

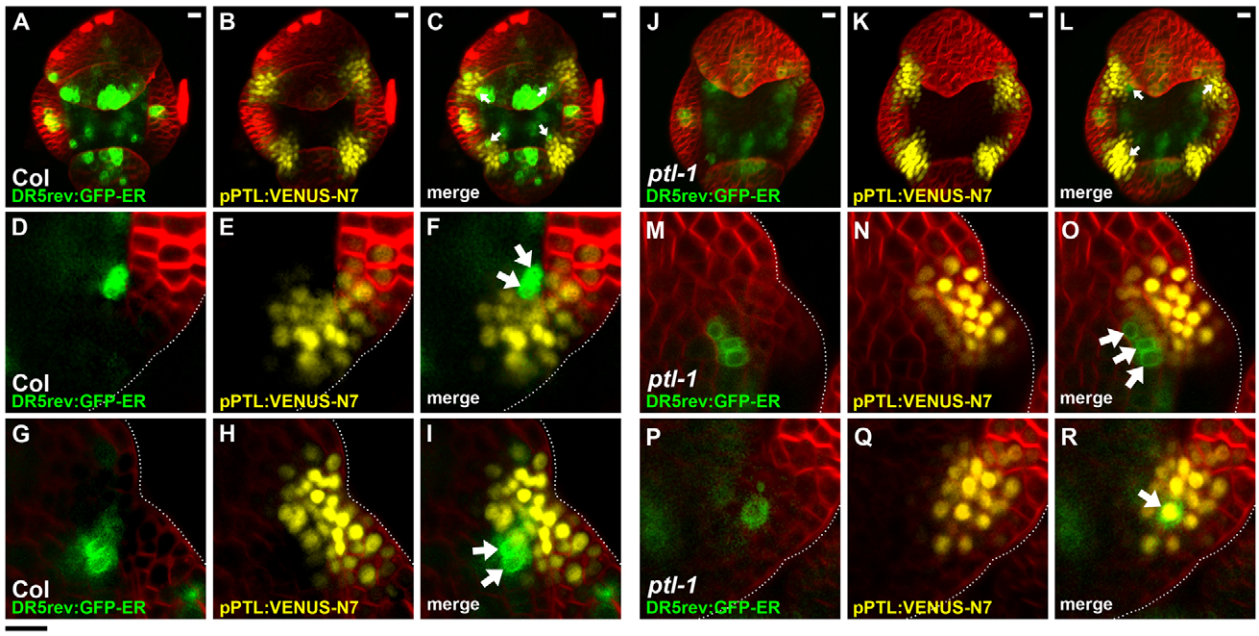

Fig. 4. Location of sites of DR5 expression in petal-initiation zones of wild-type and *ptl1* mutant buds at stage 4. Col and *ptl-1* plants were grown together and their buds imaged using the same settings to show DR5rev:GFP-ER cytoplasmic fluorescence (green; left panels), and pPTL:VENUS-N7 nuclear expression (yellow; centre panels), and the images merged (right panels). Cell membranes were stained with FM 4-64 (red). Images are 3D confocal reconstructions. (**A-C**) A wild-type bud showing DR5-expressing cells at presumed petal initiation sites (arrows) lying immediately internal to the *PTL*-expressing inter-sepal zone. DR5 expression also occurs at the apex of sepal margins. (**D-I**) Two further examples of wild-type inter-sepal zones at higher magnification showing the locations of DR5-expressing cells (arrows). (**J-L**) A *ptl-1* mutant bud showing weaker disrupted DR5 expression at presumed petal intiation sites (arrows). (**M-R**) Two further examples of *ptl-1* inter-sepal zones at higher magnification showing that DR5-expressing cells (arrows) are more variable in number and location, and have reduced intensity. Scale bar: 10 μm for D-I, M-R.

1), as expected if loss of PTL or RBE functions affect the same petal initiation pathway.

When the *rbe-2* mutant was combined with *aux1-7*, a striking loss of petals was observed (Fig. 5L), closely similar to that in *ptl-1 aux1-7* double mutants (Fig. 1P). A mean of only 0.22±0.04 petals per flower arose compared with the 2.02±0.10 observed in *rbe-2* single mutants. Thus petal initiation in *rbe* mutants is now dependent on AUX1-controlled auxin influx function, just as it is in *ptl* mutants.

Loss of PTL function also disrupts petal initiation in *pin1* and *pid* polar auxin transport mutants

Finally we assessed the contribution of polar auxin transport by generating multiple mutant combinations of *ptl* and *aux1* with *pin1* and *pinoid* (*pid*) (Figs 6, 7; supplementary material Table S9).

Flowers arise sporadically from inflorescence meristems of all *pin1* mutants, and the mean number of petals is significantly increased (Bennett et al., 1995; Okada et al., 1991). This may occur through a defect in the later depletion of auxin from the second floral whorl, stimulating additional petal initiation. Strikingly, when PTL function was removed from strong *pin1-3* mutant plants (in *ptl-3 pin1-3* doubles) (Fig. 6A-D), the mean number of petals per flower was greatly reduced from over seven to around one (Fig. 7A, left) (t_{43}=9.78, P<0.001). This also occurred when the weaker *pin1-5* mutant was used, although to a lesser extent (Fig. 7A, right) (t_{70}=10.8, P<0.001).

The *PID* gene acts upstream of PIN1 as the PID kinase activates it by phosphorylation (Christensen et al., 2000; Friml et al., 2004; Michniewicz et al., 2007). As in *pin1* mutants, the number of petals in *pid* single mutant flowers is significantly increased (Bennett et al., 1995). Also as in *pin1*, we found that the additional loss of PTL function resulted in loss of many of these petals (Fig. 6E-J; Fig. 7B).

The *pinoid* mutant results also provide evidence that the *pmd-1d* modifier requires PID function. For the *ptl-3 pid-1* mutant combination involving the modifier, the mean number of petals (1.28±0.15) was much reduced compared with the *ptl-3* single mutant control (5.22±0.14) (Fig. 7B, left) (t_{116}=18.2, P<0.001). However, for the *ptl-1 pid-3* observations in unmodified background, the mean number per flower (2.20±0.31) was not reduced further than that seen in *ptl-1* single mutants (2.60±0.20) (Fig. 7B, right) (t_{92}=1.53, P>0.05).

Thus disruption of polar auxin transport generally boosts petal numbers, but this boost is dependant on PTL function, which presumably acts upstream of it. In light of this, we tested whether PTL function influences PIN1 expression. pPIN1:PIN1-GFP is expressed throughout the epidermis of wild-type buds up to at least stage 5, but there were no detectable differences in its relative intensity or tissue localisation in *ptl-1* mutants, including in the inter-sepal and petal initiation zones at stage 4 (supplementary material Fig. S3; Movies 1-4).

Lastly we examined the consequences on petal initiation of the joint disruption of auxin influx and polar auxin efflux. For the strong *pin1-3* allele, petal numbers in the *aux1-n1 pin1-3* double mutant (Fig. 6K) were not significantly different from *pin1-3* single mutants (Fig. 7C, left) (t_{35}=0.076, P>0.05). However, when PTL function was also lost, in *ptl aux1 pin1* triple mutants (Fig. 6L), petal numbers now fell to the low level seen in *ptl aux1* double mutants (Fig. 7C, compared with Fig. 1G). Thus auxin influx

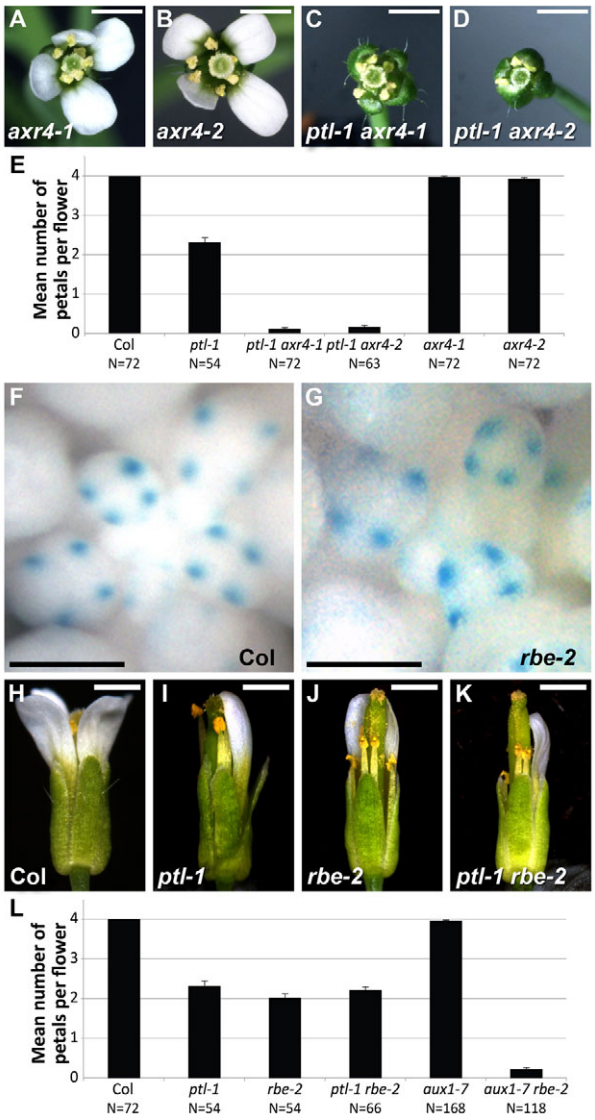

Fig. 5. Interactions of AXR4 with PTL, and of RBE with PTL and AUX1. (**A-D**) Floral phenotypes of *axr4-1* (A), *axr4-2* (B), *ptl-1 axr4-1* (C) and *ptl-1 axr4-2* (D) mutant plants. (**E**). Mean number of petals per flower in single and double mutant combinations of *ptl-1* and *axr4-1* or *axr4-2*. N flowers were scored, the first six on each plant. Bars represent s.e.m. (**F**,**G**) *PTL* expression in wild-type (B) and *rbe-2* mutant (C) plants carrying the pPTL(1.3i):GUS transgene. Vertical views of the inflorescence and young buds to stage 4 show four spots in the inter-sepal zones of both wild-type and *rbe-2* buds. (**H-K**) Floral phenotypes of wild-type (D) and *ptl-1* (E), *rbe-2* (F) and *ptl-1 rbe-2* (G) mutant plants. (**L**) Mean number of petals per flower in single and double mutant combinations of *rbe-2* and *ptl-1*, and of *rbe-2* and *aux-1-7*. N flowers were scored, the first six on each plant. Bars represent s.e.m. Flowers shown are the first one formed on the primary inflorescence. Scale bars: 100 μm.

regulated by AUX1 does not seem to be required for petal initiation in *pin1* mutants unless PTL function is also compromised. This does not apply for the weaker allele *pin1-5*, because loss of AUX1 function in this case does result in a modest but significant reduction in the number of petals (from 6.43±0.26 in *pin1-5* to 5.09±0.21 in *pin1-5 aux1-n1* double mutants) (Fig. 7C, right) (t_{83}=3.50, P<0.001). Thus examination of this mutant allele with partially reduced PIN1 function (Bennett et al., 1995) has confirmed the role AUX1 plays in petal initiation, a role that is masked if PIN1 function is fully lost.

DISCUSSION

Proposed genetic pathway controlling petal initiation

Integrating our observations with predicted patterns of auxin transport (Heisler et al., 2005; Reinhardt et al., 2003), we propose the following pathway (Fig. 8A). Petal initiation (red) may require localised auxin accumulation (green). Auxin may be made available from two sources: one dependent on regional growth suppression jointly promoted by PTL and RBE (long green arrow), and another by a weaker AXR4-AUX1 influx pathway (dashed green arrow). If PTL or RBE function is lost, regional overgrowth may inhibit the first pathway, but some auxin may still be supplied by the AUX1 pathway for limited and variable petal initiation. If AXR4 or AUX1 function is lost, then sufficient auxin may be supplied by the alternative pathway for full petal initiation. However, if both pathway functions are lost, insufficient auxin may be provided and almost no petals would arise.

Polar transport of this available auxin is likely to be directed by the PID-PIN1 pathway. This may first promote auxin accumulation in the petal initiation zone from available sources, and its later depletion. When PID or PIN1 function is lost, additional petals arise, perhaps because auxin still accumulates independently but that its subsequent depletion is now compromised. When PTL function is also lost in *pin1* or *pid* mutants, petal numbers are now low, indicating that only small amounts of auxin accumulate. However, when AUX1 and PIN1 functions are both lost, sufficient auxin may accumulate through the PTL-RBE promoted source to maintain high petal numbers. Finally, in *ptl aux1 pin1* triple mutants, available auxin may be almost abolished so that polar auxin transport would now be irrelevant and, as in *ptl aux1* double mutants, few if any petals would arise.

The role of the dominant allele of *PETAL LOSS MODIFIER*, *pmd-1d*, uniquely present in L*er* background, apparently occurs after all these steps. The boost in petal numbers it provides is only apparent when PTL function is lost, suggesting that its function is normally repressed by PTL. However, its effect in *ptl* mutants is not significant when AUX1 or PID function is also absent (PIN1 was not tested), indicating that its action is dependent upon auxin accumulation. One possibility is that *pmd-1d* is a gain-of-function allele of an auxin response pathway gene that amplifies the response to the petal initiation signal.

Loss of PTL function disrupts auxin availability

We have shown that loss of PTL function uncovers a role for auxin influx in signalling petal initiation. Further, generation of auxin between developing sepals can restore nearby petal initiation in *ptl* mutant flowers. Finally, auxin-inducible DR5 expression in the petal initiation zones is disrupted when PTL function is lost. Thus loss of PTL function interferes with the normal action of auxin in promoting petal initiation.

How does PTL influence auxin dynamics? An established role of PTL is to repress cell division in the developing inter-sepal zone (Lampugnani et al., 2012). This region overgrows somewhat in *ptl* mutants buds, and this morphological distortion alone may be sufficient to incidentally disrupt auxin flow and thus nearby petal initiation (Fig. 8B).

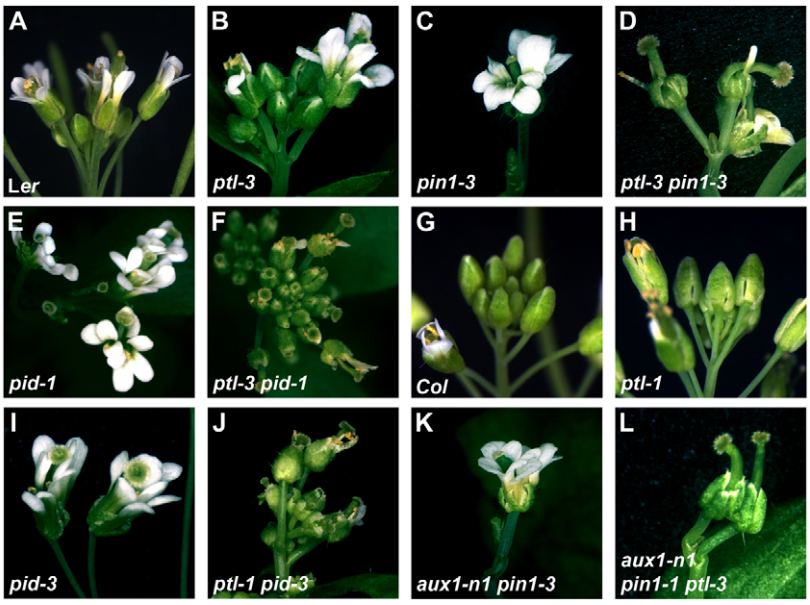

Fig. 6. Flower phenotypes in mutants of the polar auxin transport genes *pin1* and *pid* showing the effects of loss of PTL and AUX1 function. (**A-D**) Flowers of L*er* wild type (A), *ptl-3* (B), *pin1-3* (C) and *ptl-3 pin1-3* (D), showing that the increase in petal numbers in *pin1-3* (C) is strongly disrupted in *ptl-3 pin1-3* double mutants (D). All plants carry the modifier *pmd-1d*. (**E**,**F**) Flowers showing that the increase in petal numbers in *pid-1* (E) is almost fully disrupted in *ptl-3 pid-1* double mutants (F). Both plants carry *pmd-1d*. (**G-J**) Flowers of Col wild type (G), *ptl-1* (H), *pid-3* (I) and *ptl-1 pid-3* (J), showing that the increase in petal numbers in *pid-3* (I) is almost fully disrupted in *ptl-1 pid-3* double mutants (J). All plants have the unmodified *PMD* background. (**K**,**L**) Flowers showing that the increase in petal numbers in *pin1-3* (C) is unaffected in *aux-n1 pin1-3* double mutants (K), but almost fully disrupted in *aux-n1 pin1-3 ptl-3* triple mutants (L). Both plants carry *pmd-1d*.

Alternatively, PTL may play a more direct role. One possibility is that PTL normally supports auxin transport by another system, non-polar auxin efflux through the ATP-binding cassette B (ABCB) channels (Noh et al., 2001). In this case, auxin may be unable to escape from inter-sepal zone cells in *ptl* mutants, generating a sink that traps surrounding auxin, depleting it from the petal initiation zone. Such accumulation may also stimulate the additional cell divisions between sepals seen in *ptl* mutants (Lampugnani et al., 2012). However, we found no evidence for widespread DR5 expression in the inter-sepal zone in *ptl* mutant buds that might indicate such auxin activity.

Like PTL, loss of RBE function also sensitises petal initiation to reduced auxin influx. The defects associated with their loss of function are apparently shared as petal numbers are not further compromised in *ptl rbe* double mutants. As with PTL, RBE also dampens growth between developing sepals (Krizek et al., 2006). However, the mechanism of its growth suppression differs from that of PTL (Lampugnani et al., 2012) because RBE promotes the action of *CUP-SHAPED COTYLEDON* boundary genes through direct repression of their negative regulator *EXTRA EARLY PETALS1* (Huang et al., 2012). Also, RBE normally represses *AGAMOUS* expression in the second whorl (Krizek et al., 2006), but PTL does not (Griffith et al., 1999). Even so, it may be that either form of mutant overgrowth in the inter-sepal zone incidentally disrupts auxin-induced petal initiation.

The location of sepals defines the sites of petal initiation

Generally, the sites of petal initiation are dependent on the location of earlier arising sepals (Endress, 2011; Smyth, 2005). If petal initiation sites are controlled by the same processes as leaves and flowers, they would arise where auxin levels are relatively high. Newly growing sepal primordia may be auxin sinks, depleting it in their vicinity but less so in the inter-sepal zone (Reinhardt et al., 2003). In addition, reversal of polar auxin transport may occur around newly arising sepal primordia (Heisler et al., 2005), driving auxin outward and generating an interference peak internal to the inter-sepal zone (Fig. 8B).

A role for sepal-controlled auxin dynamics in defining sites of petal initiation is suggested by their uncoupling in polar auxin transport mutants. In *pin1* and *pid* flowers, extra sepals and extra petals both occur in variable numbers and sizes, but the sites of petals are now not strictly internal to inter-sepal zones (Bennett et al., 1995). PTL is also apparently involved in this auxin signalling process, either indirectly or directly, because loss of PTL function in *pin1* or *pid* mutants greatly reduces petal initiation. However, there is no effect on the number of sepals (Brewer et al., 2004) (supplementary material Table S9).

Petal orientation is controlled by a different signal

Loss of PTL function also disrupts petal orientation (Griffith et al., 1999), a process that precedes the imposition of dorsoventral polarity on developing organs (Siegfried et al., 1999). This was not affected by loss of other gene functions examined here, including RBE. Also, the restoration of petals in *ptl* mutants resulting from ectopic auxin was not associated with the amelioration of petal orientation. The only other mutants with a similar reported disruption to petal orientation are those of the *ASYMMETRIC LEAVES1* (*AS1*) and *AS2* organ growth genes (Xu et al., 2008). Double mutants of *as1* and *ptl* result in a strengthening of petal mis-orientation, pointing to overlap in their orientation function (Xu et al., 2008). A similar strengthening was seen in double mutants of *ptl* and B function organ identity genes (Griffith et al., 1999). The nature of this possible signal remains unknown.

Is auxin the primary signal for founder cell specification?

Auxin may be universally involved in regulating organ initiation in plants (Petrášek and Friml, 2009). In addition to the primordia of petals (present study), leaves and flowers (Reinhardt et al., 2000), it is required for development of the hypophysis in the developing embryo (Friml et al., 2003), and for lateral root initiation (Dubrovsky et al., 2008).

Organ initiation requires the prior specification of founder cells that are competent to respond to a specific induction signal

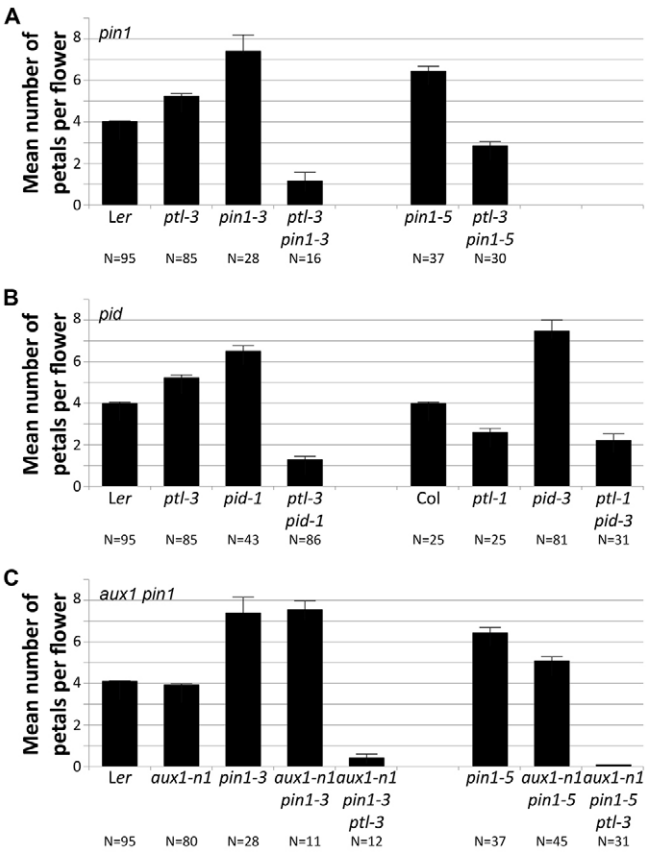

Fig. 7. Petal numbers in mutants of the polar auxin transport genes *pin1* and *pid* showing the effects of loss of PTL and AUX1 function. The number of flowers scored (N) is shown. Bars represent s.e.m. (**A**) Single and double mutant combinations of *ptl-3* with the strong *pin1-1* mutant (left), or the weaker *pin1-5* mutant (right), all in modified *pmd-1d* background. (**B**) Single and double mutant combinations of *ptl-3* and *pid-1* (in modified *pmd-1d* background) (left), and of *ptl-1* and *pid-3* (in unmodified *PMD* background) (right). (**C**) Single and multiple mutant combinations of *aux1-n1* and *ptl-3* with the strong *pin1-3* mutant (left), or the weaker *pin1-5* mutant (right), all in modified *pmd-1d* background.

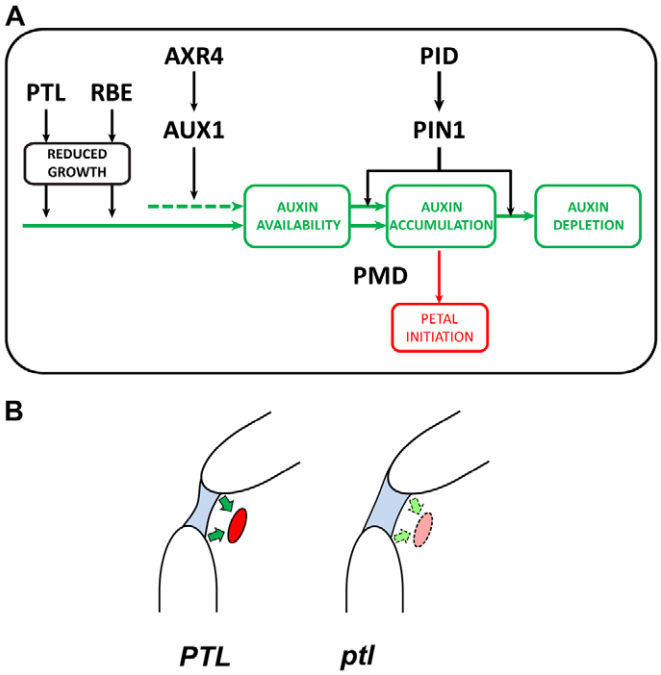

Fig. 8. Proposed pathways leading to the signalling of petal initiation by auxin. (**A**) Auxin availability may depend partly on regional growth suppression mediated by PTL and RBE, and more weakly by the AXR4-AUX1 pathway. Auxin may be accumulated in the petal initiation zone, in part by PID-PIN1 mediated polar auxin transport. This accumulated auxin may promote petal initiation, and be subsequently depleted by the same polar auxin transport function. The PMD modifier function is not normally detected, but if PTL function is absent and the *pmd-1d* allele is present, *pmd-1d* may boost response to the auxin petal initiation signal. (**B**) An inter-sepal zone where *PTL* is expressed (blue), and the nearby petal initiation zone (red). In wild type, auxin (green) may be available from the vicinity to support nearby petal initiation. In *ptl* mutants, the inter-sepal zone is enlarged and auxin signalling of nearby petal initiation may be compromised. This may occur indirectly as a consequence of the increase in bulk of the inter-sepal zone disrupting auxin availability, or perhaps directly if PTL normally supports auxin efflux from this zone.

(Chandler, 2011). Thus induction occurs before any morphological evidence of organ initiation. Despite strong evidence in favour, it is not proved that auxin itself is the induction signal. In developing flowers, one complication is that relevant auxin events occur in the epidermis (Reinhardt et al., 2003), but the first signs of organogenesis are seen in the underlying layers. For *Arabidopsis* petals, this is a periclinal division in the L2 (Hill and Lord, 1989). Expression of the DR5 auxin response reporter consistently indicates the sites of future organogenesis, and its expression occurs in the L1 (Chandler et al., 2011; Heisler et al., 2005; Lampugnani et al., 2012; van Mourik et al., 2012). Whether the DR5-expressing epidermal cells receive the induction signal, or whether it occurs earlier, either in the epidermis or in underlying cells, remains to be established. Study of genes that are expressed earlier than DR5 where organ primordia will arise, such as *DORNRÖSCHEN-LIKE* (Chandler et al., 2011), may help uncover mechanisms of specification.

In conclusion, we have revealed that auxin is required for petal initiation and the possible pathways involved. Future studies are needed to determine whether auxin is the ultimate inducer of floral organ founder cells, how the cells have become competent to respond, and the molecular and cellular mechanisms of their specification.

Acknowledgements
We thank Stuart Baum and John Bowman for providing M2 families of mutagenised *ptl-3* seeds. We especially thank Morag Whitworth and Malcom Bennett for providing *aux1* and *lax* mutant lines, and an insertion line carrying pAUX1:AUX1-YFP116. Phil Brewer and Jiří Friml kindly provided DR5rev:GFP-ER and pPIN1:PIN1-GFP seeds. Lars Østergaard donated the plasmid TOPO-*iaa*H. We obtained seeds of *aux1-7*, *aux1-21*, *axr4-1*, *axr4-2* and *rbe-2* from ABRC. We thank Monash Micro Imaging for providing excellent confocal microscopy facilities and advice; Sandy Floyd, John Golz and Marcus Heisler for experimental advice; Ed Newbigin for making time available to ERL for manuscript preparation; and Annette Becker, John Bowman, Phil Brewer, Ruth N. Kaplan-Levy, Tezz Quon and Marnie Soso for comments and interest.

Funding
This work was funded by the Australian Research Council [DP0451208, DP0879251 and DP0880214].

Competing interests statement
The authors declare no competing financial interests.

Supplementary material
Supplementary material available online at
http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.084582/-/DC1

References

Bainbridge, K., Guyomarc'h, S., Bayer, E., Swarup, R., Bennett, M., Mandel, T. and Kuhlemeier, C. (2008). Auxin influx carriers stabilize phyllotactic patterning. *Genes Dev.* **22**, 810-823.

Benková, E., Michniewicz, M., Sauer, M., Teichmann, T., Seifertová, D., Jürgens, G. and Friml, J. (2003). Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell* **115**, 591-602.

Bennett, S. R. M., Alvarez, J., Bossinger, G. and Smyth, D. R. (1995). Morphogenesis of pinoid mutants of Arabidopsis thaliana. *Plant J.* **8**, 505-520.

Bennett, M. J., Marchant, A., Green, H. G., May, S. T., Ward, S. P., Millner, P. A., Walker, A. R., Schulz, B. and Feldmann, K. A. (1996). Arabidopsis AUX1 gene: a permease-like regulator of root gravitropism. *Science* **273**, 948-950.

Bossinger, G. and Smyth, D. R. (1996). Initiation patterns of flower and floral organ development in Arabidopsis thaliana. *Development* **122**, 1093-1102.

Brewer, P. B., Howles, P. A., Dorian, K., Griffith, M. E., Ishida, T., Kaplan-Levy, R. N., Kilinc, A. and Smyth, D. R. (2004). PETAL LOSS, a trihelix transcription factor gene, regulates perianth architecture in the Arabidopsis flower. *Development* **131**, 4035-4045.

Butenko, M. A., Vie, A. K., Brembu, T., Aalen, R. B. and Bones, A. M. (2009). Plant peptides in signalling: looking for new partners. *Trends Plant Sci.* **14**, 255-263.

Chandler, J. W. (2011). Founder cell specification. *Trends Plant Sci.* **16**, 607-613.

Chandler, J. W., Jacobs, B., Cole, M., Comelli, P. and Werr, W. (2011). DORNRÖSCHEN-LIKE expression marks Arabidopsis floral organ founder cells and precedes auxin response maxima. *Plant Mol. Biol.* **76**, 171-185.

Cheng, Y., Dai, X. and Zhao, Y. (2006). Auxin biosynthesis by the YUCCA flavin monooxygenases controls the formation of floral organs and vascular tissues in Arabidopsis. *Genes Dev.* **20**, 1790-1799.

Christensen, S. K., Dagenais, N., Chory, J. and Weigel, D. (2000). Regulation of auxin response by the protein kinase PINOID. *Cell* **100**, 469-478.

Dharmasiri, S., Swarup, R., Mockaitis, K., Dharmasiri, N., Singh, S. K., Kowalchyk, M., Marchant, A., Mills, S., Sandberg, G., Bennett, M. J. et al. (2006). AXR4 is required for localization of the auxin influx facilitator AUX1. *Science* **312**, 1218-1220.

Dubrovsky, J. G., Sauer, M., Napsucialy-Mendivil, S., Ivanchenko, M. G., Friml, J., Shishkova, S., Celenza, J. and Benková, E. (2008). Auxin acts as a local morphogenetic trigger to specify lateral root founder cells. *Proc. Natl. Acad. Sci. USA* **105**, 8790-8794.

Endress, P. K. (2011). Evolutionary diversification of the flowers in angiosperms. *Am. J. Bot.* **98**, 370-396.

Fleming, A. J., McQueen-Mason, S., Mandel, T. and Kuhlemeier, C. (1997). Induction of leaf primordia by the cell wall protein expansin. *Science* **276**, 1415-1418.

Friml, J., Vieten, A., Sauer, M., Weijers, D., Schwarz, H., Hamann, T., Offringa, R. and Jürgens, G. (2003). Efflux-dependent auxin gradients establish the apical-basal axis of Arabidopsis. *Nature* **426**, 147-153.

Friml, J., Yang, X., Michniewicz, M., Weijers, D., Quint, A., Tietz, O., Benjamins, R., Ouwerkerk, P. B. F., Ljung, K., Sandberg, G. et al. (2004). A PINOID-dependent binary switch in apical-basal PIN polar targeting directs auxin efflux. *Science* **306**, 862-865.

Griffith, M. E., da Silva Conceição, A. and Smyth, D. R. (1999). PETAL LOSS gene regulates initiation and orientation of second whorl organs in the Arabidopsis flower. *Development* **126**, 5635-5644.

Hamant, O., Heisler, M. G., Jönsson, H., Krupinski, P., Uyttewaal, M., Bokov, P., Corson, F., Sahlin, P., Boudaoud, A., Meyerowitz, E. M. et al. (2008). Developmental patterning by mechanical signals in Arabidopsis. *Science* **322**, 1650-1655.

Heisler, M. G., Ohno, C., Das, P., Sieber, P., Reddy, G. V., Long, J. A. and Meyerowitz, E. M. (2005). Patterns of auxin transport and gene expression during primordium development revealed by live imaging of the Arabidopsis inflorescence meristem. *Curr. Biol.* **15**, 1899-1911.

Heisler, M. G., Hamant, O., Krupinski, P., Uyttewaal, M., Ohno, C., Jönsson, H., Traas, J. and Meyerowitz, E. M. (2010). Alignment between PIN1 polarity and microtubule orientation in the shoot apical meristem reveals a tight coupling between morphogenesis and auxin transport. *PLoS Biol.* **8**, e1000516.

Hill, J. and Lord, E. (1989). Floral development in Arabidopsis thaliana: a comparison of the wild type and the homeotic pistillata mutant. *Can. J. Bot.* **67**, 2922-2936.

Huang, T., López-Giráldez, F., Townsend, J. P. and Irish, V. F. (2012). RBE controls microRNA164 expression to effect floral organogenesis. *Development* **139**, 2161-2169.

Irish, V. F. (2008). The Arabidopsis petal: a model for plant organogenesis. *Trends Plant Sci.* **13**, 430-436.

Jönsson, H., Heisler, M. G., Shapiro, B. E., Meyerowitz, E. M. and Mjolsness, E. (2006). An auxin-driven polarized transport model for phyllotaxis. *Proc. Natl. Acad. Sci. USA* **103**, 1633-1638.

Kaplan-Levy, R. N., Brewer, P. B., Quon, T. and Smyth, D. R. (2012). The trihelix family of transcription factors – light, stress and development. *Trends Plant Sci.* **17**, 163-171.

Krizek, B. A. and Fletcher, J. C. (2005). Molecular mechanisms of flower development: an armchair guide. *Nat. Rev. Genet.* **6**, 688-698.

Krizek, B. A., Lewis, M. W. and Fletcher, J. C. (2006). RABBIT EARS is a second-whorl repressor of AGAMOUS that maintains spatial boundaries in Arabidopsis flowers. *Plant J.* **45**, 369-383.

Lampugnani, E. R., Kilinc, A. and Smyth, D. R. (2012). PETAL LOSS is a boundary gene that inhibits growth between developing sepals in Arabidopsis thaliana. *Plant J.* **71**, 724-735.

Lee, B. H., Johnston, R., Yang, Y., Gallavotti, A., Kojima, M., Travençolo, B. A. N., Costa, L. F., Sakakibara, H. and Jackson, D. (2009). Studies of aberrant phyllotaxy1 mutants of maize indicate complex interactions between auxin and cytokinin signaling in the shoot apical meristem. *Plant Physiol.* **150**, 205-216.

Li, X., Qin, G., Chen, Z., Gu, H. and Qu, L.-J. (2008). A gain-of-function mutation of transcriptional factor PTL results in curly leaves, dwarfism and male sterility by affecting auxin homeostasis. *Plant Mol. Biol.* **66**, 315-327.

Michniewicz, M., Zago, M. K., Abas, L., Weijers, D., Schweighofer, A., Meskiene, I., Heisler, M. G., Ohno, C., Zhang, J., Huang, F. et al. (2007). Antagonistic regulation of PIN phosphorylation by PP2A and PINOID directs auxin flux. *Cell* **130**, 1044-1056.

Noh, B., Murphy, A. S. and Spalding, E. P. (2001). Multidrug resistance-like genes of Arabidopsis required for auxin transport and auxin-mediated development. *Plant Cell* **13**, 2441-2454.

Okada, K., Ueda, J., Komaki, M. K., Bell, C. J. and Shimura, Y. (1991). Requirement of the auxin polar transport system in early stages of Arabidopsis floral bud formation. *Plant Cell* **3**, 677-684.

Peaucelle, A., Louvet, R., Johansen, J. N., Höfte, H., Laufs, P., Pelloux, J. and Mouille, G. (2008). Arabidopsis phyllotaxis is controlled by the methyl-esterification status of cell-wall pectins. *Curr. Biol.* **18**, 1943-1948.

Petrášek, J. and Friml, J. (2009). Auxin transport routes in plant development. *Development* **136**, 2675-2688.

Reinhardt, D., Mandel, T. and Kuhlemeier, C. (2000). Auxin regulates the initiation and radial position of plant lateral organs. *Plant Cell* **12**, 507-518.

Reinhardt, D., Pesce, E. R., Stieger, P., Mandel, T., Baltensperger, K., Bennett, M., Traas, J., Friml, J. and Kuhlemeier, C. (2003). Regulation of phyllotaxis by polar auxin transport. *Nature* **426**, 255-260.

Siegfried, K. R., Eshed, Y., Baum, S. F., Otsuga, D., Drews, G. N. and Bowman, J. L. (1999). Members of the YABBY gene family specify abaxial cell fate in Arabidopsis. *Development* **126**, 4117-4128.

Smith, R. S., Guyomarc'h, S., Mandel, T., Reinhardt, D., Kuhlemeier, C. and Prusinkiewicz, P. (2006). A plausible model of phyllotaxis. *Proc. Natl. Acad. Sci. USA* **103**, 1301-1306.

Smyth, D. R. (2005). Morphogenesis of flowers – our evolving view. *Plant Cell* **17**, 330-341.

Smyth, D. R., Bowman, J. L. and Meyerowitz, E. M. (1990). Early flower development in Arabidopsis. *Plant Cell* **2**, 755-767.

Stepanova, A. N., Robertson-Hoyt, J., Yun, J., Benavente, L. M., Xie, D.-Y., Dolezal, K., Schlereth, A., Jürgens, G. and Alonso, J. M. (2008). TAA1-mediated auxin biosynthesis is essential for hormone crosstalk and plant development. *Cell* **133**, 177-191.

Swarup, R., Kargul, J., Marchant, A., Zadik, D., Rahman, A., Mills, R., Yemm, A., May, S., Williams, L., Millner, P. et al. (2004). Structure-function analysis of the presumptive Arabidopsis auxin permease AUX1. *Plant Cell* **16**, 3069-3083.

Takeda, S., Matsumoto, N. and Okada, K. (2004). RABBIT EARS, encoding a SUPERMAN-like zinc finger protein, regulates petal development in Arabidopsis thaliana. *Development* **131**, 425-434.

Tobeña-Santamaria, R., Bliek, M., Ljung, K., Sandberg, G., Mol, J. N. M., Souer, E. and Koes, R. (2002). FLOOZY of petunia is a flavin mono-oxygenase-like protein required for the specification of leaf and flower architecture. *Genes Dev.* **16**, 753-763.

van Mourik, S., Kaufmann, K., van Dijk, A. D. J., Angenent, G. C., Merks, R. M. H. and Molenaar, J. (2012). Simulation of organ patterning on the floral meristem using a polar auxin transport model. *PLoS ONE* **7**, e28762.

Xu, B., Li, Z., Zhu, Y., Wang, H., Ma, H., Dong, A. and Huang, H. (2008). Arabidopsis genes AS1, AS2, and JAG negatively regulate boundary-specifying genes to promote sepal and petal development. *Plant Physiol.* **146**, 566-575.

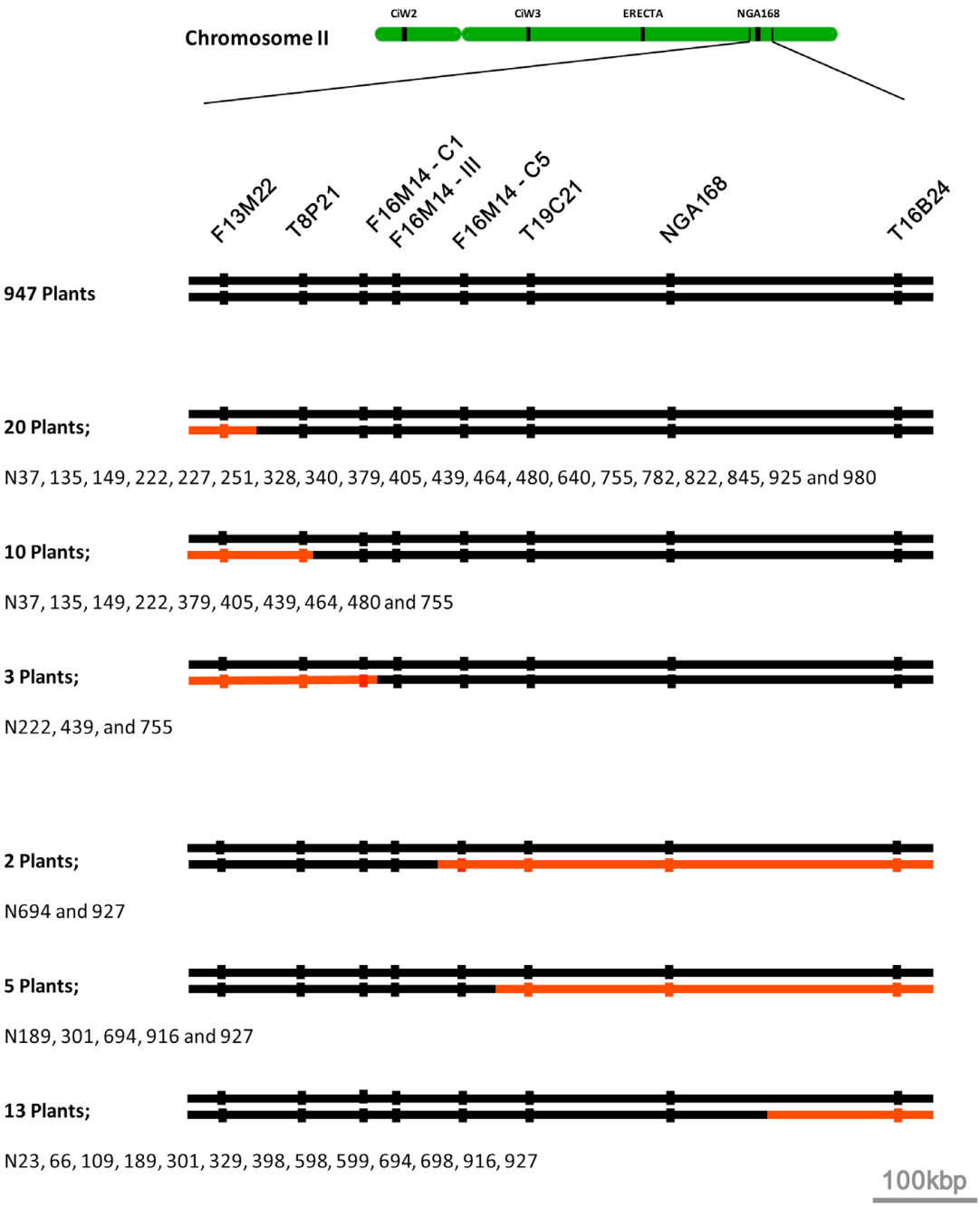

Fig. S1. Fine scale mapping of the *NOP* locus. 990 *ptl nop-1* mutant F2 plants from a cross between *ptl-3 pmd-1d nop-1 er* (in L*er* background) and *ptl-1 PMD NOP ER* (in Col background) were scored for DNA markers on chromosome 2 lying between F13M22 and T16B24. The strand diagrams show the deduced two chromosomes of 43 plants with recombination detected in this interval (black represents the Ler derived strand; red represents the Col-derived strand). The *NOP* locus must lie between DNA markers F16M14-C1 and F16M14-C5.

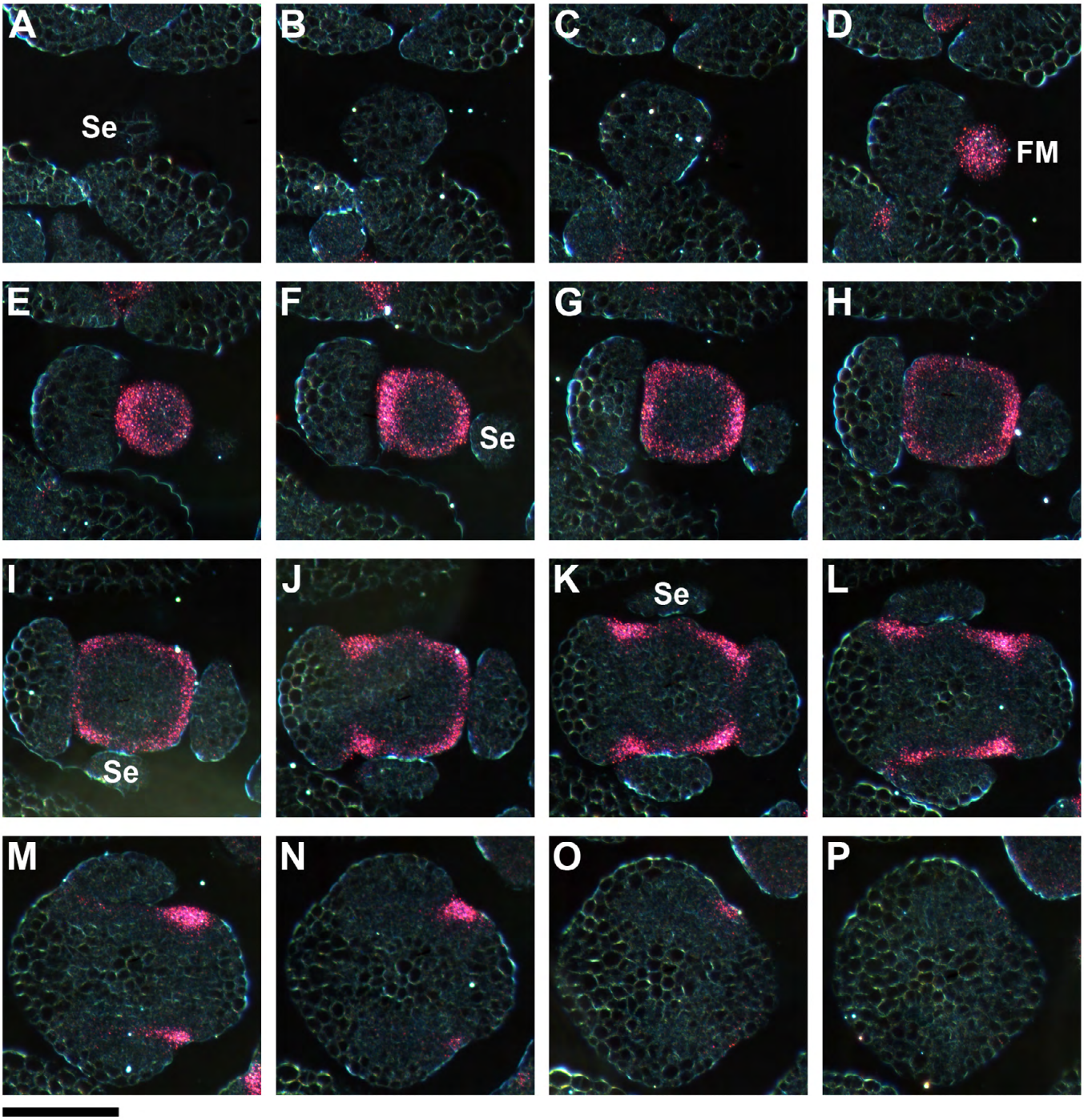

Fig. S2. pAUX1:GUS expression in serial transverse sections of a wild-type stage 4 bud. (**A-P**) Strong *AUX1* expression occurs in the epidermis of the floral meristematic dome (FM) and the inter-sepal zones (Se, sepals). (L) This panel is shown in Fig. 2. Scale bar: 50 μm.

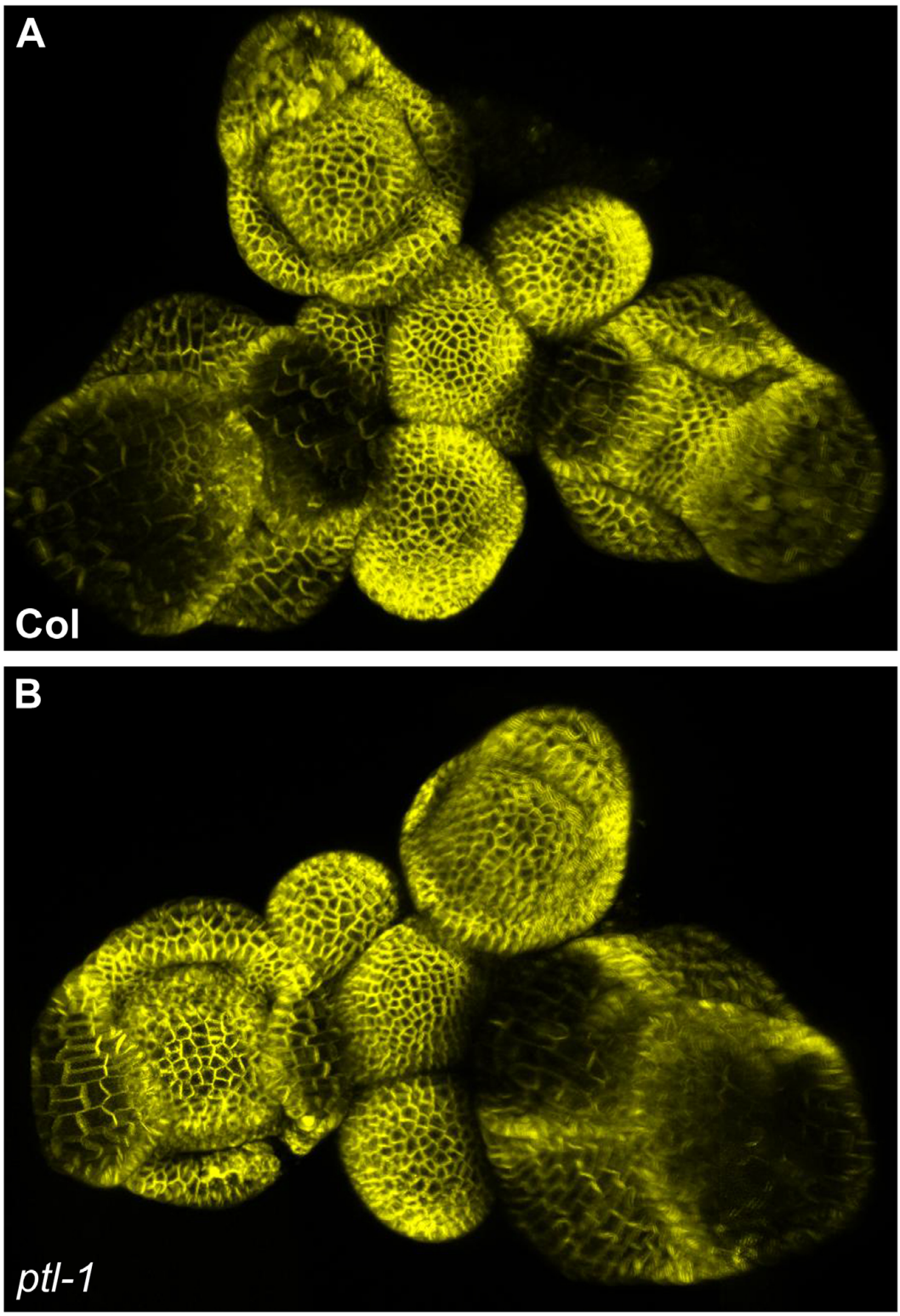

Fig. S3. pPIN1:PIN1-GFP expression in wild type and *ptl-1* mutant inflorescences. (**A**) Columbia wild type inflorescence. (**B**) *ptl-1* mutant inflorescence.

Table S1. DNA primers used

(A) For allele identification

Gene	Primer	Sequence	Restriction enzyme	Product size (bp)	
AUX1	A25F	5'-TCTTTCTTGTAGAATGCGGCG-3'	*Sau*3AI	*AUX1*	*aux1-7*
	A26R	5'-TCAAAGACGGTGGTGTAAAGC-3'		246	84, 162
	A48F+600	5'-CATTTAATTGCACTTTCCTCTTGTT-3'	*Apa*LI	*AUX1*	*aux1-21*
	A49R+1043	5'-CATATACTGTCACCTCAATGCAAAG-3'		443	220, 223
	A18F	5'-AGCTTTGGATTTTGTCCATTAC-3'	*Taq*I	*AUX1*	*aux1-n1*
	A50+3206R	5'-ACTTGACGAACAAAGTTGGTTACA-3'		250	39, 211
AXR4	axr4-144F	5'-AAACACGCTCCTTGAATGATG-3'	-	*AXR4*	*axr4-1,2*
	axr4-2147R	5'-TCCCAAAAGAAAAATCTCAAAA-3'		2,291	-
LAX1	lx1-1	5'-ATATGGTTGCAGGTGGCACA-3'	-	*LAX1*	*lax1-1*
	lx1-2	5'-GTAACCGGCAAAAGCTGCA-3'		1,193	-
LAX2	lx2-3	5'-ACAGCTGGGGATGCTCTCA-3'	-	*LAX2*	*lax2-1*
	lx2-4	5'-TCAGCACGTAGAGTGTTGCA-3'		1,193	-
LAX3	lx3-1	5'-TACTTCACCGGAGCCACCA-3'	-	*LAX3*	*lax3-1*
	lx3-2R	5'-ACTTAGAAATGGAGAGGGAAGAAGA-3'		1,043	-
PTL	ptl-30F P1	5'-TCTCTTTCGGCTGAATATAAATCTG-3'	*Hae*III	*PTL*	*ptl-1*
	ptl+498R P1	5'-ATTAGGGTAAAATCGAGAGAGGAGA-3'		138, 390	528
	ptl+1624 P3	5'-GAAGAAATGCAGAGAGAAGTTTGAG-3'	-	*PTL*	*ptl-3*
	ptl+2315R P3	5'-ATTGTTTCGGATCTCATTATTACCA-3'		691	599
RBE	rbe+74F	5'-CTTTATTTTGGCCGTTTAGGG-3'	-	*RBE*	*rbe-2*
	rbe+1105R	5'-GGAGTTCACATGGAATACCAGAC-3'		1,031	959
	Lbb1.3	5'-ATTTTGCCGATTTCGGAAC-3'			

(B) For insert generation

Construct	Primer	Sequence*	Substrate	Size (bp)
pPTL(FI313):iaaH	iaah-bamhi-F	5'-ttaggatccATGGTGGCCATTACCTCGTTA-3'	TOPO-iaaH	1,404
	iaah-xbai-R	5'-atgtctagaTTAATTGGGTAAACCGGC AAA-3'		
pPTL(FI313):AUX1	aux1-bamhi-F	5'-agcggatccATGTCGGAAGGAGTAGAAGCG-3'	F16M14	4,188
	aux1-ncoi-R	5'-tgcccatggTCAAAGACGGTGGTGTAAAGC-3'		
pAUX1(3.67bp):GUS	A33F-3770	5'-TGTTCATAGTGCGCATGTTTC-3'	F16M14	3,770
	Am32R+7	5'-CCGggATccTTTTAGCTTCTAGATCTGAG-3'		

*Lower case represents added restriction sites

(C) For confirmation of inserts in T1 plants

Construct	Primer	Sequence
pPTL(FI313):iaaH	ptl-30F P1	5’-TCTCTTTCGGCTGAATATAAATCTG-3’
	3'OCSR	5'-CATGCGATCATAGGCGTCTC-3'
pPTL(FI313):AUX1	ptl-30F P1	5’-TCTCTTTCGGCTGAATATAAATCTG-3’
	A49R+1043	5’-CATATACTGTCACCTCAATGCAAAG-3’
pAUX1(3.67bp):GUS	A01F-525	5’-AAAGGAGGAGTAGGGGGTGA-3’
	GUS84R	5’-ACAGTTTTCGCGATCCAGAC-3’

Table S2. Mean number of floral organs per flower in single and double mutant combinations of *ptl-3* and *nop-1* (*aux1-n1*) or *nop-2* (*aux1-n2*)

	Mean number of organs per flower±s.e.m.			
	Sepals	**Petals**	**Stamens**	**Carpels**
Ler *n*=72	4.00±0.00	4.00±0.00	6.00±0.00	2.00±0.00
***nop-1 n*=72**	4.00±0.00	3.93±0.03	6.00±0.00	2.00±0.00
***nop-2 n*=72**	4.00±0.00	3.82±0.05	5.86±0.06	2.00±0.06
***ptl-3 n*=48**	4.01±0.01	6.29±0.18	6.01±0.01	2.00±0.01
***ptl-3 nop-1 n*=72**	4.00±0.00	0.44±0.09	5.97±0.03	2.00±0.03
***ptl-3 nop-2 n*=72**	3.89±0.05	0.11±0.04	5.83±0.07	2.00±0.07

The first six flowers of the indicated number of plants (*n*) were scored.

Table S3. Mean number of floral organs per flower in single and double mutant combinations of *ptl-1* and *aux1-7* or *aux1-21*

	Mean number organs per flower±s.e.m.			
	Sepals	**Petals**	**Stamens**	**Carpels**
Col *n*=60	4.00±0.00	4.00±0.00	6.00±0.00	2.00±0.00
***aux1-7 n*=60**	4.00±0.00	3.93±0.03	5.88±0.06	2.00±0.00
***aux1-21 n*=72**	3.92±0.04	3.79±0.05	5.81±0.07	2.00±0.00
***ptl-1 n*=60**	4.00±0.00	2.30±0.11	6.00±0.00	2.00±0.00
***ptl-1 aux1-7 n*=72**	4.00±0.00	0.11±0.04	5.82±0.06	2.00±0.00
***ptl-1 aux1-21 n*=72**	3.89±0.04	0.00±0.00	5.85±0.06	2.00±0.00

The first six flowers of the indicated number of plants (*n*) were scored.

Table S4. Mean number of floral organs per flower in single and multiple mutant combinations of *aux1-21*, *lax1-1*, *lax2-1* and *lax3-1*

	Mean number organs per flower±s.e.m.			
	Sepals	**Petals**	**Stamens**	**Carpels**
Col *n*=72	4.00±0.00	3.99±0.01	6.00±0.00	2.00±0.00
***aux1-21 n*=119**	4.00±0.00	3.98±0.01	5.79±0.05	2.00±0.00
***lax1 n*=84**	4.00±0.00	4.00±0.00	5.86±0.05	2.00±0.00
***lax2 n*=72**	4.00±0.00	4.00±0.00	6.00±0.00	2.00±0.00
***lax3 n*=72**	4.00±0.00	4.00±0.00	6.00±0.00	2.00±0.00
***aux1-21 lax1 n*=95**	4.00±0.00	3.72±0.06	4.84±0.12	2.00±0.00
***aux1-21 lax2 n*=71**	4.00±0.00	3.94±0.03	5.63±0.08	2.00±0.00
***aux1-21 lax3 n*=72**	4.00±0.00	3.97±0.02	5.51±0.09	2.00±0.00
***aux1-21 lax2 lax3 n*=72**	4.00±0.00	3.96±0.03	5.63±0.09	2.00±0.00
***aux1-21 lax1 lax2 n*=59**	4.00±0.00	3.63±0.10	4.17±0.18	2.00±0.00
***aux1-21 lax1 lax3 n*=54**	4.00±0.00	3.70±0.07	4.53±0.16	2.00±0.00
***aux1-21 lax ;lax2 lax3 n*=60**	3.90±0.06	3.65±0.09	4.34±0.17	2.00±0.00

The first six flowers of the indicated number of plants (*n*) were scored.

Table S5. Restoration of petals in *ptl* mutant plants by a pPTL:iaaH transgene

Genotype	Construct	Number of independent lines	Mean number of organs per flower±s.e.m.			
			Sepals	Petals	Stamens	Carpels
Col	**Nil**	10	4.00±0.00	4.00±0.00	6.00±0.00	2.00±0.00
Col	**pPTL(FI313):iaaH**	10	4.00±0.00	4.01±0.01	5.96±0.02	2.00±0.00
ptl-1	**Nil**	10	4.00±0.00	1.45±0.11	5.99±0.01	2.00±0.00
ptl-1	**pPTL(FI313):iaaH**	7	4.00±0.00	3.33±0.10	5.93±0.04	2.00±0.00

The mean number of floral organs per flower in flowers 1-12 of T1 plants carrying the pPTL(FI313):iaaH transgene in either Col or *ptl-1* are shown. Controls without the transgene are also shown.

Table S6. Restoration of petals in *ptl aux1* mutant plants by a pPTL:AUX1 transgene

Genotype	Construct	Number of independent lines	Mean number organs per flower±s.e.m			
			Sepals	Petals	Stamens	Carpels
Col	**Nil**	10	4.00±0.00	4.00±0.00	6.00±0.00	2.00±0.00
Col	**pPTL(FI313):AUX1**	7	4.00±0.00	4.00±0.00	6.00±0.00	2.00±0.00
ptl-1	**Nil**	10	4.00±0.00	1.45±0.11	5.99±0.01	2.00±0.00
ptl-1 aux1-7	**Nil**	10	4.00±0.00	0.07±0.02	5.99±0.01	2.00±0.00
ptl-1 aux1-7	**pPTL(FI313):AUX1**	9	4.02±0.01	0.99±0.10	5.99±0.01	2.00±0.00
Ler	**Nil**	10	4.02±0.01	4.01±0.01	6.00±0.00	2.00±0.00
Ler	**pPTL(FI313):AUX1**	7	4.01±0.01	4.01±0.01	6.00±0.00	2.00±0.00
ptl-3	**Nil**	10	4.02±0.01	5.36±0.15	5.95±0.03	2.00±0.00
ptl-3 aux1-n1	**Nil**	10	4.01±0.01	0.23±0.04	5.93±0.03	2.00±0.00
ptl-3 aux1-n1	**pPTL(FI313):AUX1**	12	4.01±0.01	2.05±0.15	5.97±0.02	2.00±0.00

The mean number of floral organs per flower in flowers 1-12 of T1 plants carrying the pPTL(FI313):AUX1 transgene in either Col or *ptl-1 aux1-7*, and in either Ler or *ptl-3 aux1-n1*, are shown. Controls without the transgene are also shown.

Table S7. Mean number of floral organs per flower in single and double mutant combinations of *ptl-1* and *axr4-1* or *axr4-2*

	Mean number organs per flower±s.e.m.			
	Sepals	**Petals**	**Stamens**	**Carpels**
Col *n*=72	4.00±0.00	4.00±0.00	6.00±0.00	2.00±0.00
***ptl-1* *n*=54**	4.00±0.00	2.31±0.13	6.00±0.00	2.00±0.00
***axr4-1* *n*=72**	4.00±0.00	3.90±0.04	5.26±0.10	2.00±0.00
***axr4-2* *n*=72**	4.00±0.00	3.93±0.03	5.76±0.06	2.00±0.00
***ptl-1 axr4-1* *n*=72**	4.00±0.00	0.11±0.04	5.57±0.09	2.00±0.00
***ptl-1 axr4-2* *n*=63**	4.00±0.00	0.16±0.05	5.51±0.11	2.00±0.00

The first six flowers of the indicated number of plants (*n*) were scored

Table S8. Mean number of floral organs per flower in single and double mutant combinations of *ptl-1* and *rbe-2*, or *aux1-7* and *rbe-2*

	Mean number organs per flower±s.e.m.			
	Sepals	**Petals**	**Stamens**	**Carpels**
Col *n*=72	4.00±0.00	4.00±0.00	6.00±0.00	2.00±0.00
***ptl-1* *n*=54**	4.00±0.00	2.31±0.13	6.00±0.00	2.00±0.00
***rbe-2* *n*=54**	4.00±0.00	2.02±0.10	5.96±0.03	2.00±0.00
***ptl-1 rbe-2* *n*=66**	4.00±0.00	2.22±0.08	5.98±0.02	2.00±0.00
aux1-7* *n=168	4.00±0.00	3.96±0.02	5.83±0.04	2.00±0.00
aux1-7 rbe-2* *n=118	4.00±0.00	0.22±0.04	5.88±0.04	2.00±0.00

The first six flowers of the indicated number of plants (*n*) were scored.

Table S9. Mean number of floral organs per flower in mutant combinations of *ptl* with *pin1*, *pid* or *pin1 aux1*

Genotype All *pmd-1d* except Col strains*	Number of flowers	Mean number organs per flower±s.e.m.		
		Sepals	Petals	Stamens
PIN-FORMED1				
Ler	95	4.00±0.00	4.00±0.00	5.91±0.00
ptl-3	85	4.01±0.01	5.22±0.14	5.99±0.01
pin1-3	28	8.11±0.78	7.39±0.77	0.18±0.16
ptl-3 pin1-3	16	8.19±1.32	1.13±0.41	1.31±0.53
pin1-5	37	5.45±0.13	6.43±0.26	0.89±0.15
ptl-3 pin1-5	30	5.33±0.14	2.83±0.18	2.43±0.27
PINOID				
pid-1	43	4.51±0.15	6.51±0.27	2.65±0.28
ptl-3 pid-1	86	4.29±0.10	1.28±0.15	4.18±0.13
Col*	25	4.00±0.00	4.03±0.03	5.93±0.05
ptl-1*	25	4.00±0.00	2.60±0.20	6.00±0.00
pid-3*	81	5.22±0.21	7.47±0.52	3.01±0.35
ptl-1 pid-3*	69	4.53±0.18	2.20±0.31	5.10±0.31
AUXIN1 PIN-FORMED1				
aux-n1	80	4.00±0.00	3.95±0.02	5.51±0.08
aux1-n1 pin1-3	11	6.00±0.50	7.55±0.41	0.27±0.19
aux1-n1 ptl-3	60	4.02±0.02	0.78±0.10	5.67±0.07
aux1-n1 pin1-3 ptl-3	12	6.25±0.30	0.42±0.19	0.67±0.19
aux1-n1 pin1-5	45	4.18±0.11	5.09±0.21	0.71±0.13
aux1-n1 pin1-5 ptl-3	31	4.48±0.20	0.06±0.04	1.00±0.18

PMD

Fourth whorl organs were severely disrupted in many genotypes; numbers were not scored.

Table S10. Deduced genes lying between DNA markers F16M14-C1 and F16M14-C5.

Location	Locus	Gene Model	Description
15972993-15977180	AT2G38120	*AUX1*	Auxin influx transporter.
15978512-15980749	AT2G38130	*ATMAK3*	*Arabidopsis* homolog of the yeast protein MAK3, a component of the N-terminal acetyltransferase complex C.
15980883-15981612	AT2G38140	*PSRP4*	Plastid-specific ribosomal protein 4 (PSRP4) mRNA.
15981700-15982917	AT2G38150	AT2G38150	Putative transferase involved in transferring glycosyl groups.
15986643-15988522	AT2G38160	AT2G38160	Unknown protein (best Arabidopsis protein match is AT2G40070 (also an unknown protein)).
15989083-15993276	AT2G38170	*CAX1*	High affinity vacuolar calcium antiporter.
15997114-15998906	AT2G38180	AT2G38180	GDSL-motif lipase/hydrolase family protein.

Table S11. DNA primers used for positional cloning of *NOP*

Primer Name	Coordinates (kbp)	Sequence	Type	Notes
CIW2 F	1,194,606	5'-CCCAAAAGTTAATTATACTGT-3'	SSLP	15 bp deletion in Ler
CIW2 R	1,194,710	5'-CCGGGTTAATAATAAATGT-3'		
CIW3 F	6,402,846	5'-GAAACTCAATGAAATCCACTT-3'	SSLP	30 bp deletion in Ler
CIW3 R	6,403,081	5'-TGAACTTGTTGTGAGCTTTGA-3'		
F13M22 F	15,769,483	5'-TTCTTCTTGCTTAGCATGTTCTTG-3'	SSLP	12 bp deletion in Ler
F13M22 R	15,769,678	5'-CCCGCTCCCATTAGGATG-3'		
T8P21 F	15,829,952	5'-AACTTGGATTTATTGACATGATGTTT-3'	SSLP	9 bp deletion in Ler
T8P21 R	15,830,155	5'-CAGTAACATTGAAAAACAAAAGAC-3'		
F16M14 - C1 F	15,960,540	5'-ATGTTGGAAGCTGAGCCTGT-3'	CAPS	*Hin*cII cleavage site lost in Ler
F16M14 - C1 R	15,960,789	5'-GGCAACTGTAGCCATTTGAA-3'		
F16M14 - III F	15,989,704	5'-CAATGAACGTGAAGACCAAATG-3'	SSLP	10 bp deletion in Ler
F16M14 - III R	15,989,908	5'-CGCCAAAACCGAAAAATAAC-3'		
F16M14 - C5 F	16,000,894	5'-TGTTGTCTTTGTATACTTCACATTGAT-3'	CAPS	*Tsp*RI cleavage site lost in Ler
F16M14 - C5 R	16,001,135	5'-CTTCATCGACCACAAGCTGA-3'		
T19C21 F	16,102,876	5'-TGAATGCCTTTGCTCACAGT-3'	SSLP	19 bp deletion in Ler
T19C21 R	16,103,085	5'-GCCAGTCTTTCCAATGCCTA-3'		
NGA168 F	16,291,841	5'-TCGTCTACTGCACTGCCG-3'	SSLP	16 bp deletion in Ler
NGA168 R	16,291,991	5'-GAGGACATGTATAGGAGCCTCG-3'		
T16B24 F	16,422,953	5'-TTGACACTTTCGCGTAGAACA-3'	SSLP	14 bp deletion in Ler
T16B24 R	16,423,101	5'-AACAAGCTATTGGTGATGAACG-3'		