LETTERS

# Conserved factors regulate signalling in *Arabidopsis thaliana* shoot and root stem cell organizers

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**Throughout the lifespan of a plant, which in some cases can last more than one thousand years, the stem cell niches in the root and shoot apical meristems provide cells for the formation of complete root and shoot systems, respectively. Both niches are superficially different and it has remained unclear whether common regulatory mechanisms exist. Here we address whether root and shoot meristems use related factors for stem cell maintenance. In the root niche the quiescent centre cells, surrounded by the stem cells, express the homeobox gene *WOX5* (*WUSCHEL-RELATED HOMEOBOX 5*)[1], a homologue of the *WUSCHEL* (*WUS*) gene that non-cell-autonomously maintains stem cells in the shoot meristem[2]. Loss of *WOX5* function in the root meristem stem cell niche causes terminal differentiation in distal stem cells and, redundantly with other regulators, also provokes differentiation of the proximal meristem. Conversely, gain of *WOX5* function blocks differentiation of distal stem cell descendents that normally differentiate. Importantly, both *WOX5* and *WUS* maintain stem cells in either a root or shoot context. Together, our data indicate that stem cell maintenance signalling in both meristems employs related regulators.**

Higher organisms evolved the ability to keep founder cells undifferentiated and pluripotent by signals provided in specialized stem cell niches[3]. In the model plant *Arabidopsis thaliana*, root and shoot meristem stem cell niches are organized differently compared with each other[4]. In the root meristem, the stem cells surround a small group of rarely dividing cells, termed the quiescent centre (Fig. 1a), and give rise to distal (columella), lateral (lateral root cap and epidermis) and proximal (cortex, endodermis and stele) cell types. Ablation studies show that short range signals from the quiescent centre keep only the directly abutting stem cells undifferentiated[5], similar to most animal stem cell niches studied so far[3]. In contrast, in the shoot meristem, a zone of three stem cell layers is maintained by an underlying organizing centre[2] (Supplementary Fig. 1a), which regulates the stem cell pool as a whole. In addition, the regulatory genes described so far for root and shoot stem cell niches are different[6].

In the shoot meristem, a feedback mechanism between the organizing centre and stem cells dynamically regulates maintenance of the stem cell pool: *WUS* activity in the organizing centre keeps stem cells undifferentiated and induces expression of the signal peptide CLAVATA3 (CLV3), which in turn restricts the size of the *WUS* expression domain (Supplementary Fig. 1a)[7,8]. We asked whether a related stem cell maintenance mechanism might operate in the root by analysing the *WOX* family for functions in the root meristem[1]. Expression of *WOX5* messenger RNA[1] and a *WOX5-GUS* reporter gene initiates in the embryonic cell lineage that gives rise to the quiescent centre (Supplementary Fig. 1b), and persists in the quiescent centre (Fig. 1b; and Supplementary Fig. 1c) during post-embryonic root growth. This expression pattern is strikingly similar to that of *WUS* in the shoot meristem[2] (Supplementary Fig. 2), raising the question of whether both genes might have similar functions in the respective stem cell niches.

In seedlings of the putative null allele *wox5-1* (Supplementary Fig. 1d, e), the cells at the quiescent centre position have abnormal shape and are enlarged in comparison to wild type (Fig. 1c, d, arrows, and Supplementary Table 1). Notably, adjacent columella stem cells are even larger, suggesting that they have undergone differentiation (Fig. 1d, bottom arrow). No other abnormality was observed in *wox5-1* mutants. Complementation of the mutant defects by a *WOX5* complementary DNA construct confirmed that the *wox5-1* mutation causes the observed phenotype (Supplementary Fig. 3a).

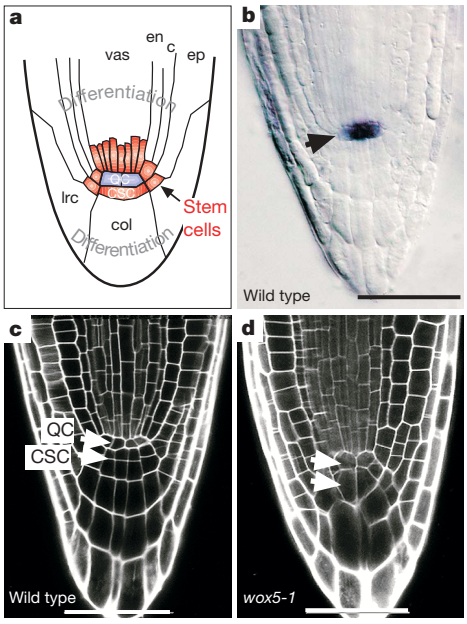


**Figure 1 | *WOX5* expression in the quiescent centre is required for root meristem development. a**, Schematic of *A. thaliana* root meristem with columella (col), columella stem cells (CSC), quiescent centre (QC), lateral root cap (lrc), epidermis (ep), cortex (c), endodermis (en) and vascular bundle (vas), redrawn with permission from ref. 4. **b**, *WOX5* mRNA expression in the quiescent centre. **c–d**, *wox5-1* roots display enlarged cells at the quiescent centre and columella stem cell (CSC) positions. Scale bars, 50 μm.

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Of several quiescent-centre-specific reporter genes[9], only *QC184* is not expressed in *wox5-1* and expression is lacking as early as the heart embryo stage (Fig. 2a, b, d, e; and Supplementary Table 2), indicating that *WOX5* functions in root development from early embryogenesis. In contrast, *QC25*, *QC46* and *WOX5-GUS* are expressed in slightly expanded domains compared to wild type (Fig. 2c, f; Supplementary Fig. 3c–h; and Supplementary Table 2). These data indicate that *WOX5* is required for some aspects of quiescent-centre-specific gene expression, but seems not to be a major factor in quiescent centre specification.

We asked whether in *wox5-1* roots the distal stem cells might have undergone premature differentiation, which in the columella can be visualized by the accumulation of starch-granule-containing organelles involved in gravitropic sensing. Indeed, in *wox5-1* roots, the cells at the stem cell position, but not the quiescent centre, accumulate starch granules (Fig. 2e, f; and Supplementary Table 2). In contrast, the proximal meristem appears normal. These findings indicate that *WOX5* gene expression in the quiescent centre is required to non-cell-autonomously maintain the distal stem cells as undifferentiated, similar to the role of *WUS* in the shoot[2].

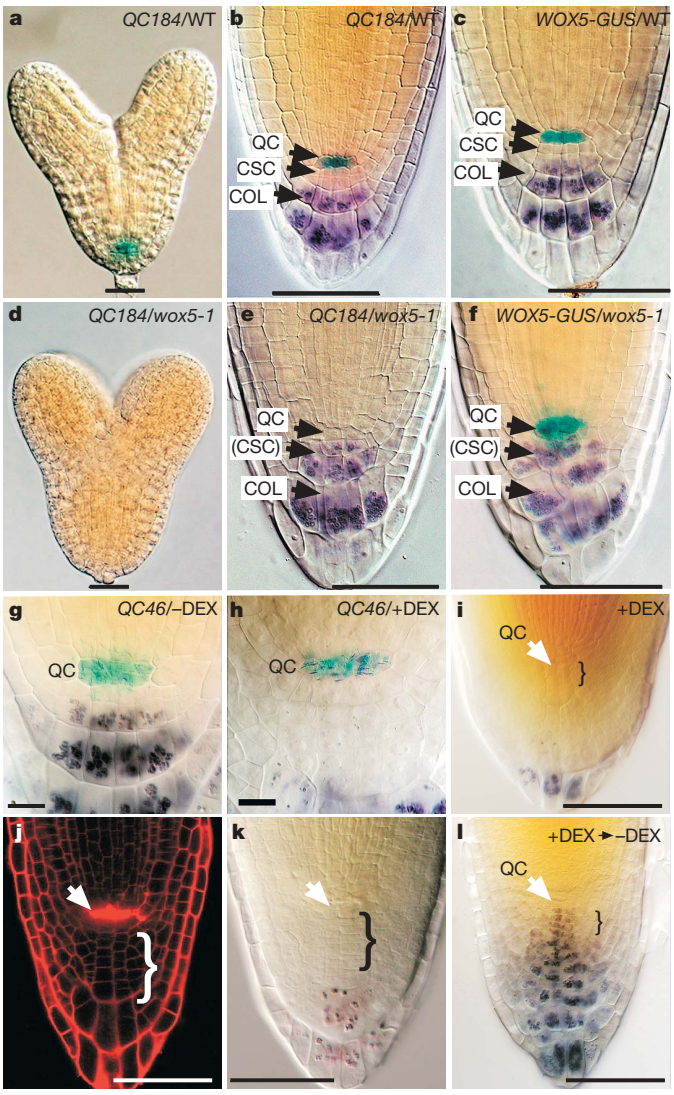


**Figure 2 | *WOX5* represses differentiation in the columella.** **a–f**, *wox5-1* heart-torpedo stage embryos (**d**) and root tips (**e**) lack *QC184* expression, express *WOX5-GUS* (**f**) in an enlarged domain and accumulate starch at the CSC position (**e**, **f**). **g**, **h**, *35S-WOX5* expression gives small cells in the columella, which lack *QC46* expression and starch grains (**h**). **j–l**, Quiescent centre ablation (arrow) does not induce differentiation (**j**, **k**), whereas transfer to non-inducing medium does (**l**; control, **i**). Nomarski (**a–i**, **k**, **l**) and confocal (**j**) median longitudinal images are shown. GUS staining, blue; starch, violet; waved brackets, extra small cells; COL, columella; CSC, columella stem cells; QC, quiescent centre; DEX, dexamethasone. Scale bars, 20 μm (**a**, **d**), 50 μm (**b**, **c**, **e**, **f**, **i–l**) and 10 μm (**g**, **h**).

We then investigated whether *WOX5* activity is also sufficient to repress columella cell differentiation, by ubiquitously expressing a dexamethasone (DEX)-inducible *WOX5* gene. DEX induction results in small cells that lack starch granules in the place of differentiated columella and lateral root cap cells (Fig. 2h, i; Supplementary Fig. 4a; and Supplementary Table 1), indicating that they have not undergone normal differentiation. Unlike quiescent centre cells, the small cells divide, as shown by the continuously increasing cell number and expression of the cell cycle marker *CYCB1-GUS* (Supplementary Fig. 4b) and quiescent centre markers are not induced (Fig. 2h). Consistent with a loss of differentiated columella cells, *35S[GVG]-WOX5* (*35S-WOX5*; Supplementary Methods) roots display reduced gravitropism (Supplementary Fig. 4c). After withdrawal of DEX, however, the small cells readily accumulate starch grains typical for differentiated columella cells (Fig. 2l). Normally, laser ablation of the quiescent centre causes differentiation of stem cells[9,10], but this did not have any effect in *35S-WOX5* (Fig. 2j, k). Thus, ectopic *WOX5* expression is sufficient to block differentiation of columella stem cell daughters without the requirement of any further quiescent centre signalling.

Previous studies highlighted auxin-dependent transcription of the *PLETHORA* (*PLT*) genes and *SHORTROOT/SCARECROW* (*SHR/SCR*) functions as essential components in root meristem maintenance and we asked how *WOX5* interacts with these pathways[9,11–13]. In mutants for the auxin response regulators *BODENLOS* (*BDL*) or *MONOPTEROS* (*MP;* Fig. 3d–f; and Supplementary Table 3), *WOX5* expression is rarely detected, consistent with the notion that *BDL*/*MP*-mediated auxin signalling is required for the embryonic initiation of the quiescent centre[13,14]. Auxin accumulation in the quiescent centre region provides patterning information to the root meristem mediated through *PLT* genes[9,12,15]. In *wox5-1* mutants, auxin accumulation seems normal based on the expression of the auxin response reporter gene *DR5-GUS* (Supplementary Fig. 5a, b). In *plt1 plt2* double mutants, *WOX5* expression is occasionally slightly expanded (Fig. 3g–i; and Supplementary Table 3), whereas *PLT1* expression is normal in *wox5-1* (Supplementary Fig. 5c, d and Supplementary Table 2).

The SHR protein moves from provascular cells into the quiescent centre and activates transcription of the *SCR* gene[16], which is required to specify quiescent centre identity and to maintain stem cells[11]. *WOX5* expression is reduced or undetectable in *shr* and *scr* mutants (Fig. 3j–l; Supplementary Fig. 5g–i; and Supplementary Table 3), whereas an *SCR* reporter gene is correctly expressed in *wox5-1* roots (Supplementary Fig. 5e, f; and Supplementary Table 2). Collectively, our data indicate that *WOX5* expression depends on the induction in the root pole by *MP*-mediated auxin signalling and on *SHR/SCR* activity, whereas *PLT1* and *PLT2* have only a minor role in confining *WOX5* expression to the quiescent centre.

The requirement of *WOX5* for only columella stem cell maintenance suggests unidirectionality of this signalling pathway in stem cell control, similar to *WUS*-mediated stem cell control in the shoot stem cell niche. We investigated whether effects of the *wox5-1* mutation on the proximal meristem might be masked by redundant functions. Indeed, in *scr-4*, *shr-1* single mutants and *plt1 plt2* double mutants, the *wox5-1* mutation promotes differentiation in the proximal region of the root meristem in addition to its effects on distal stem cells (Fig. 3m; and Supplementary Fig. 5j, k). This suggests that *WOX5* gene expression in the quiescent centre redundantly contributes to proximal stem cell activity or alternatively that *WOX5* has a stem-cell-independent function in the proximal meristem, as has been reported for its upstream regulator SCR[11] (Supplementary Fig. 7).

The striking similarities of *WOX5* and *WUS* expression patterns and functions in root and shoot stem cell niche organizers raised the



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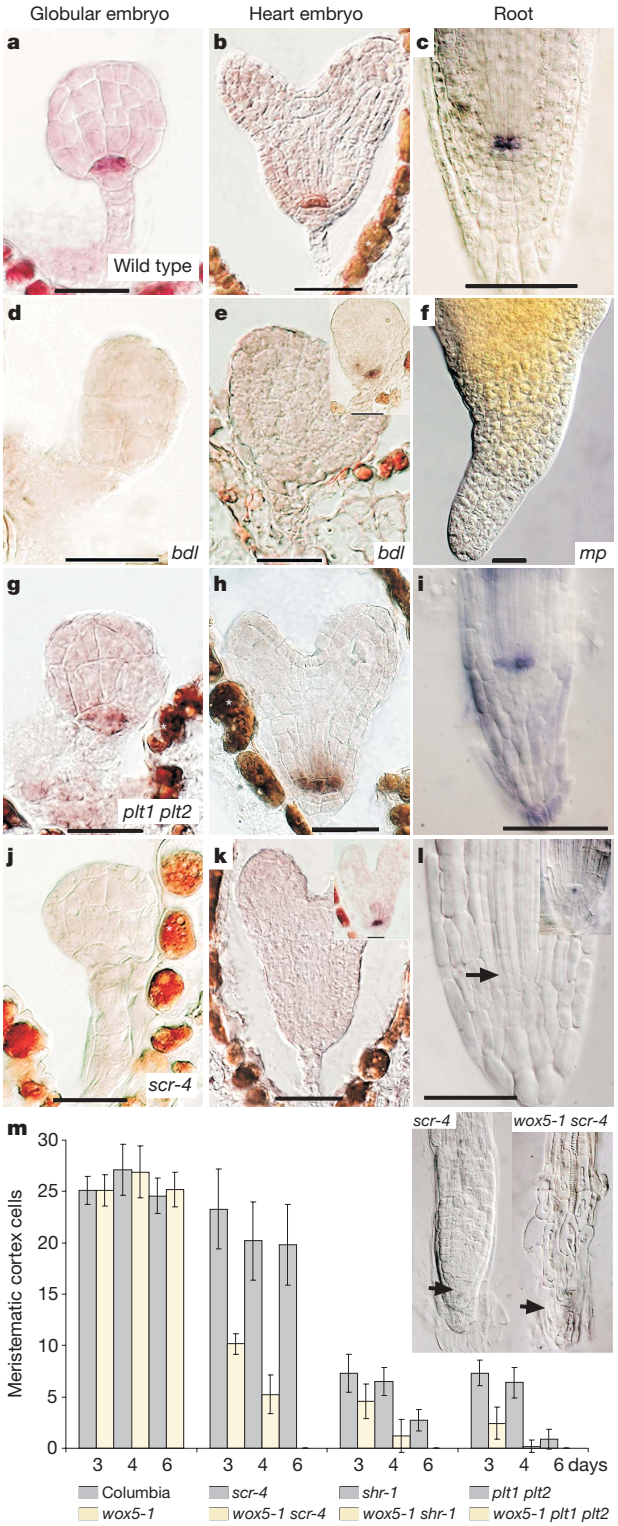


**Figure 3 | *WOX5* interaction with *SHR/SCR* and auxin pathways.** **a**–**l**, Hybridization with *WOX5* antisense probe. *WOX5* expression was detected in wild type (**a**–**c**), absent in *bdl* and *mp* (**d**–**f**, inset shows sporadic exceptions), deregulated in *plt1 plt2* (**g**–**i**), and undetectable in *scr-4* (**j**–**l**, insets show sporadic exceptions). **m**, *wox5-1* enhances differentiation in the proximal meristem in *scr-4* (inset), *shr-1* and *plt1 plt2* mutants. Error bars, s.d. Longitudinal median histological (**a**, **b**, **d**, **e**, **g**, **h**, **j**, **k**) and optical (**c**, **f**, **i**, **l**, **m**) sections are shown. Four-day-old roots (**c**, **i**, **l**) or middle stage embryos (**f**) were used. Hybridization signal is red–blue; asterisk, reddish colour is staining independent of hybridization reaction. Arrows, quiescent centre position. Scale bars, 25 μm (**a**, **b**, **d**–**f**, **g**, **h**, **j**, **k**) and 50 μm (**c**, **i**, **l**).

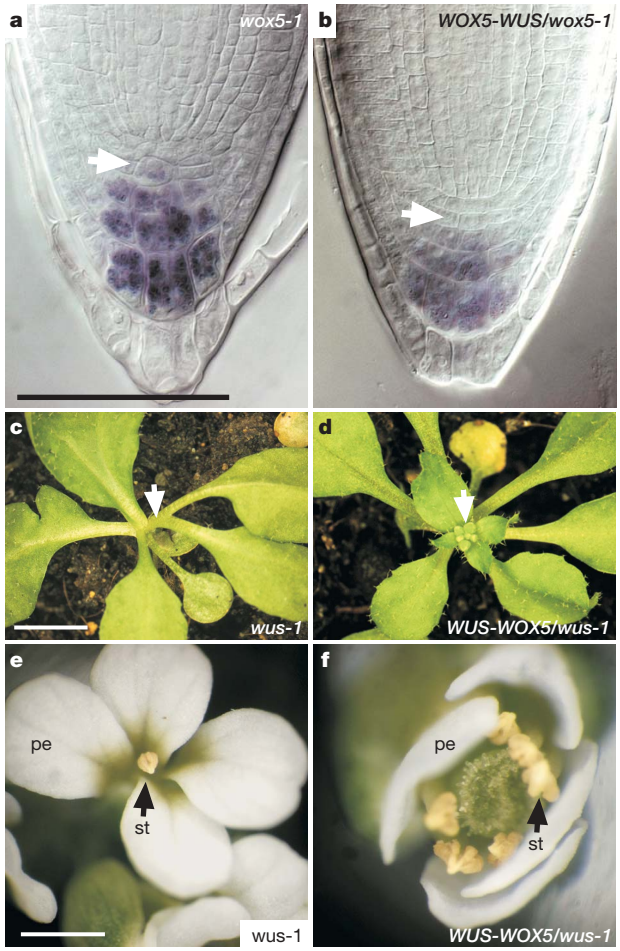


**Figure 4 | *WOX5* and *WUS* are interchangeable in stem cell control.** **a**, **b**, *WOX5-WUS* expression restores quiescent centre and columella stem cells (white arrows) in *wox5-1*. Violet, starch grains. **c**–**f**, Rescue of the shoot meristem stem cell niche. *wus-1* shoot (**c**) and floral meristem (**e**) fail to maintain stem cells and terminate prematurely. *WUS-WOX5* expression restores an indeterminate inflorescence meristem (**d**) and complete flowers (**f**). Twenty-six-day-old plants (**c**, **d**) and flowers (**e**, **f**) are shown. White arrows indicate shoot apex (**c**, **d**). pe, petal; st, stamen. Scale bars, 1 cm (**c**, **d**), 1 mm (**e**, **f**) and 50 μm (**a**, **b**).

question of whether both genes are functionally equivalent. Indeed, expression of the *WUS* cDNA from the *WOX5* promoter completely restores quiescent centre and stem cells in the *wox5-1* root meristem (Fig. 4a, b; and Supplementary Fig. 6a, b). Interestingly, ectopic shoot tissue was never observed when *WUS* was expressed in the quiescent centre. This contrasts to previous studies expressing *WUS* in a broader range of cell types[17] and favours the interpretation that organizer signalling only maintains stem cells as undifferentiated, whereas the fate of differentiating daughters is determined by the tissue context. Conversely, expression of a *WUS-WOX5* transgene rescues premature termination of inflorescence meristems and occasionally of floral meristems in the putative null allele *wus-1* (Fig. 4c–f; and Supplementary Fig. 6d–g). The rescued *wus-1* mutants resemble the weak *wus-6/jam* allele[18], suggesting that the *WUS-WOX5* transgene complements *WUS* function but provides a reduced level of organizing centre signalling. Defective ovule development in *wus* mutants is not rescued by *WUS-WOX5* (Supplementary Fig. 6c), indicating that both regulators are interchangeable in only stem cell maintenance. Significantly, the early embryo genes *WOX8* and *WOX9* are not able to rescue *wus-1* defects (H. Breuninger and T.L., unpublished observations), showing that the ability to regulate stem cell maintenance is not a general property of WOX proteins.



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The extent of mechanistic similarity and evolutionary relationship between root and shoot stem cell niches has remained unclear thus far. Here we show that the organizers of both niches employ related regulators to provide stem cell maintaining signals. However, there also seem to be differences between both niches. For example, *CLV3* homologues promote meristem differentiation but do not influence stem cell maintenance in the root on overexpression and it is thus unknown whether roots, similar to shoots, employ a *WUS–CLV3* related feedback mechanism[19,20].

Our results suggest that *WOX5* in the root stem cell niche has a more direct function in stem cell signalling, rather than in specifying quiescent centre identity (Supplementary Fig. 7). The nature of the signals that are induced by *WUS*/*WOX5* expression in the stem cell organizers is presently unresolved. WOX5 protein itself or a downstream factor might move to stem cells as the long-postulated short-range factor[5]. To date, WOX5 protein has not been robustly detected, presumably owing to very low expression levels. However, on the basis of localization of a functional WUS–GUS fusion protein, movement of WUS protein out of the organizing centre seems not to be necessary for its function in shoot meristem stem cell maintenance, indicating that factors downstream of *WUS* act as signals (Supplementary Fig. 6h, i).

Palaeobotanical evidence for early root structures is controversial, but the current view is that roots have evolved independently at least twice in Lycophytina and Euphyllophytina[21,22]. It remains to be shown whether *WUS* and *WOX5* shared an ancestral function in stem cell control since root and shoot separation, or whether they have been recruited for this function after the diversification of stem cell niches.

## METHODS

**Plant work.** *wox5-1* (SALK038262) and *wox5-3* (SALK147644) are transfer (T)-DNA insertion alleles in the Columbia (Col) accession and were obtained from the *Arabidopsis* Biological Resource Center (ABRC, USA) and the Nottingham *Arabidopsis* Stock Center (NASC, UK). *7×DR5-GUS*[23] was kindly provided by J. Marfett, and $mp^{U51}$ (ref. 14) by G. Jürgens (Tübingen). Root length and meristem size were measured as described[10]. The number of meristematic cells was obtained by counting cortex cells showing no signs of rapid elongation. Quiescent centre laser ablations were performed in 4-day-old roots using a Leica SP2 inverted confocal laser scanning microscope[5]. Roots were stained for amyloplasts using Lugol (Sigma) 24-h after ablation[24]. For confocal microscopy, root tips were mounted in 10 μg ml$^{-1}$ propidium iodide solution. Microscope settings were as described[5].

**Expression analysis.** Starch granules[24] and β-glucuronidase activity[7] were visualized as described. *35S-WOX5* seedlings were cultured on standard 0.5× MS medium supplemented with or without 1 μM dexamethasone. *In situ* hybridization of sections, whole-mount embryos, and 4-day-old seedlings were performed as described[1,24]. The *PLT1* riboprobe has been described previously[12]. The *WOX5* riboprobe was prepared from a cDNA without the homeobox to avoid cross-hybridization with related mRNAs.

Further experimental details are provided as Supplementary Information.

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**Supplementary Information** is linked to the online version of the paper at www.nature.com/nature.

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**Author Information** Sequence of *WOX5* mRNA is available in GenBank under accession numbers NM_111961 and AY251398. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to T.L. (laux@biologie.uni-freiburg.de).



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