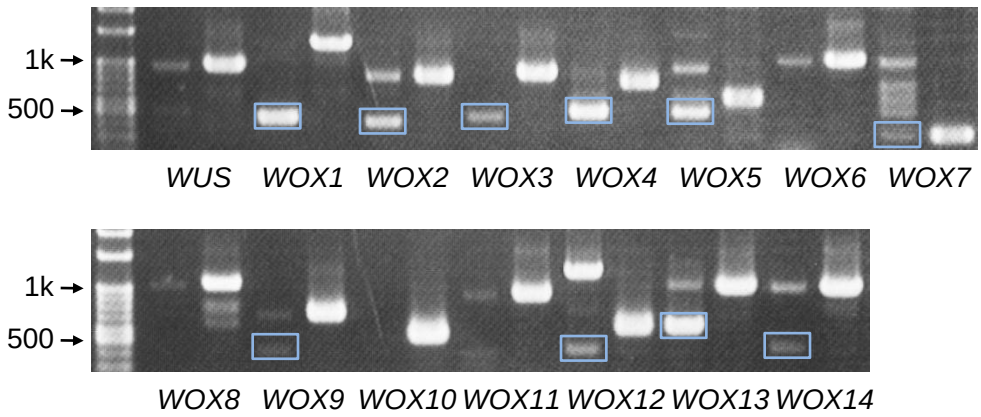
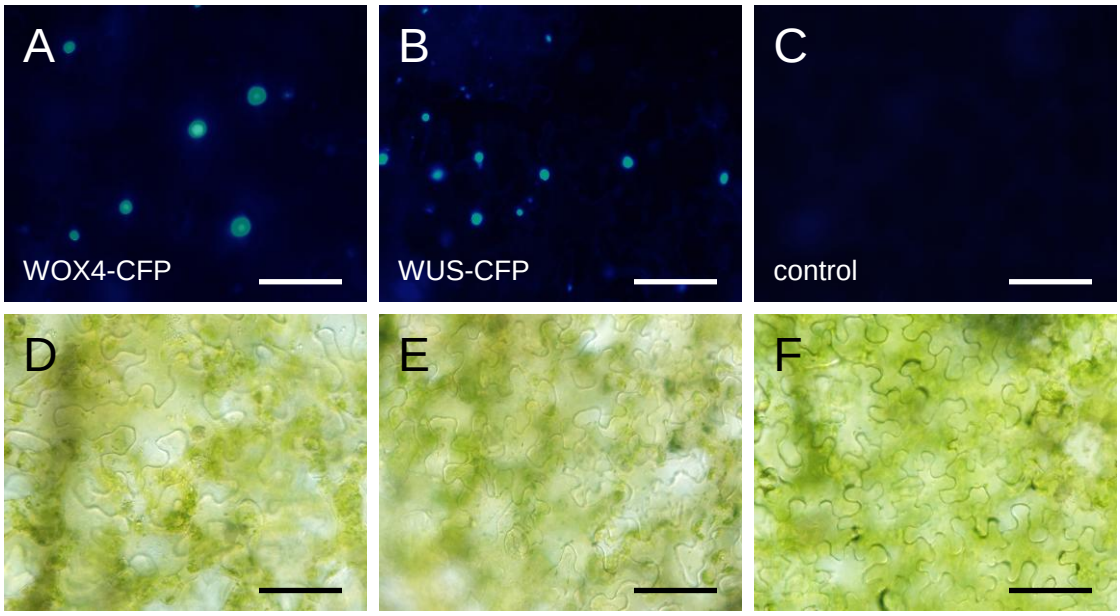
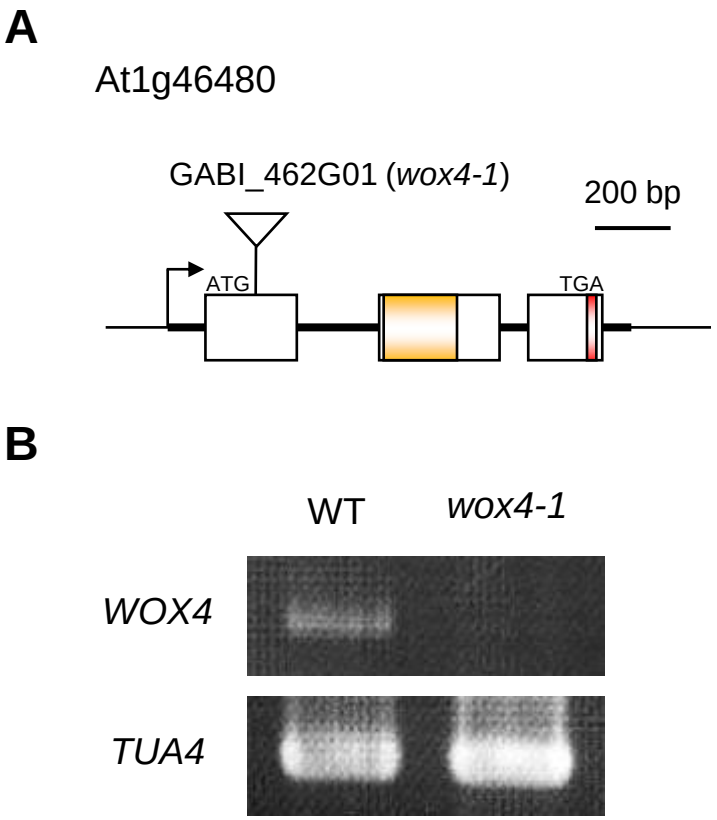


Supplemental Figure 1. Expression of WOX Family Genes in *Arabidopsis* Seedlings.

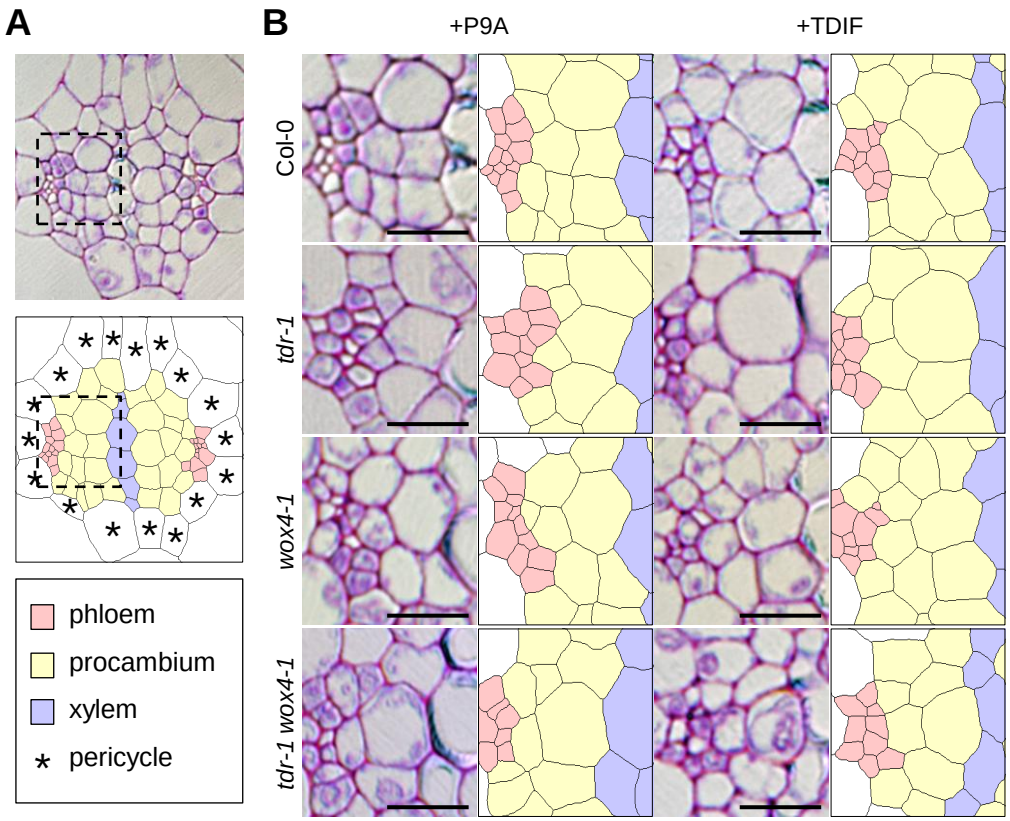
Expression levels of WOX family genes in the upper part of 7-day-old seedlings were examined by RT-PCR. The two lanes for each gene are cDNA-derived (left) and genomic DNA-derived (right) PCR products. Blue boxes indicate the band corresponding to the length of correct cDNA-derived product. The primers used are given in Supplemental Methods.



Supplemental Figure 2. Subcellular Localization of WOX4.
Subcellular localization of WOX4 and WUS was analyzed by using CFP-fusion proteins in leaf disks of *Nicotiana Benthamiana*. Leaf disks were visualized under fluorescence **(A)** to **(C)**, and optical microscopy **(D)** to **(F)**. **(A)** and **(D)** WOX4-CFP. **(B)** and **(E)** WUS-CFP. **(C)** and **(F)** no infection. Scale bars: 100 μm.



Supplemental Figure 3. Gene Structure of *WOX4*.
(A) Schematic illustration of the exon–intron structure of *WOX4* (At1g46480) and the position of the T-DNA insertion in the *wox4-1* allele (GABI_462G01). Sequences encoding the homeodomain (orange) and WUS-box (red) are highlighted. **(B)** The *WOX4* transcript was not detected in *wox4-1* by RT–PCR. *TUA4* was used as a control. WT: wild type.

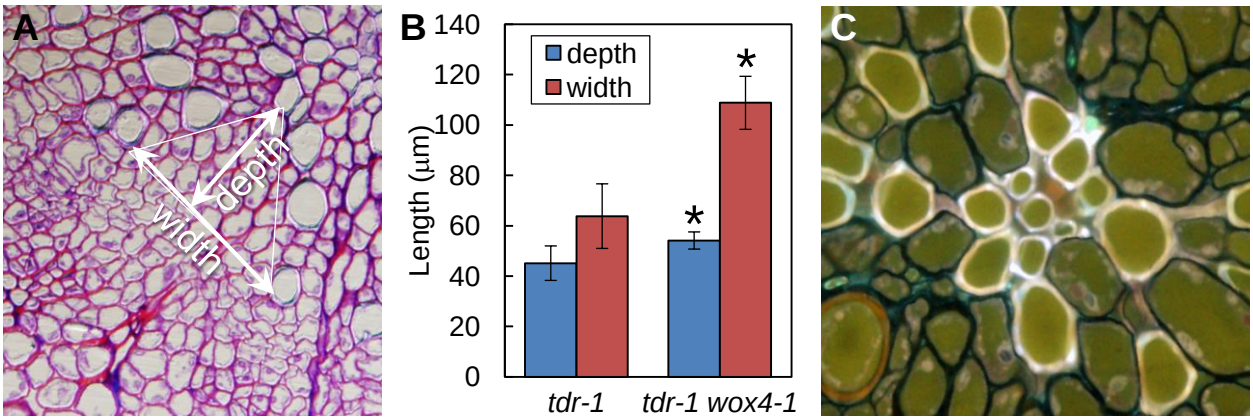


Supplemental Figure 4. TDIF Signaling Does not Affect the Radial Structure of Primary Vascular Tissues.

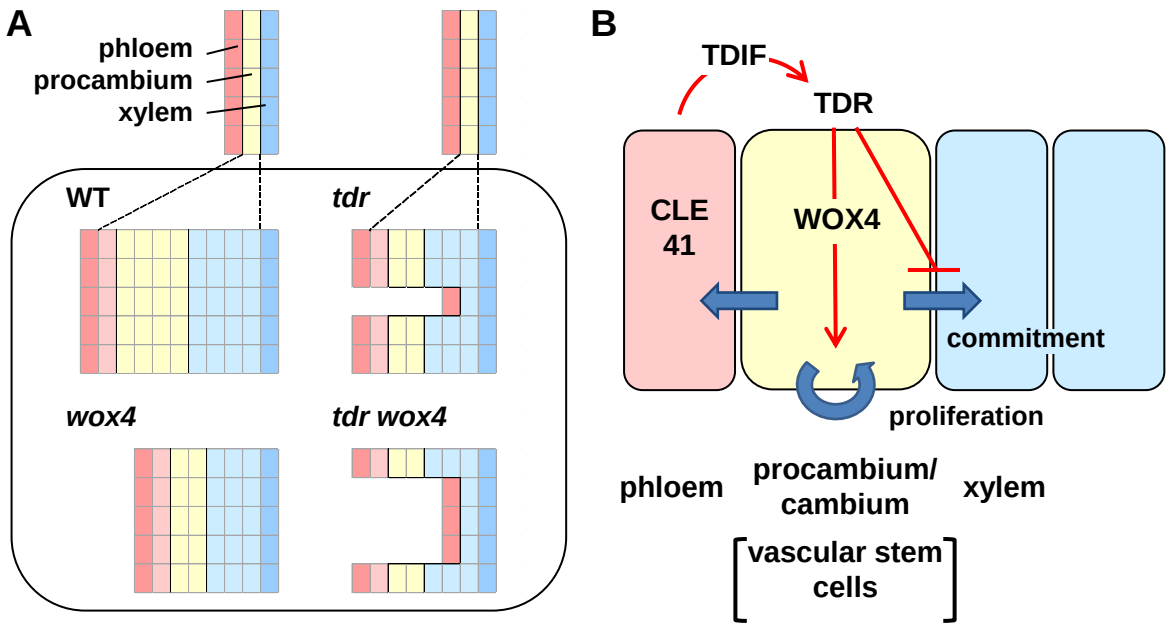
(A) The radial structure of the stele in the 5-day-old *Arabidopsis* hypocotyl and its schematic illustration. The primary vascular tissues, including three types of tissues/cells, phloem (red), xylem (blue) and procambium (yellow), are surrounded by a pericycle cell layer. Small cells clustered in the two phloem poles are defined as phloem cells. Large cells that have or start producing thick secondary cell walls are defined as xylem cells. The others in the vascular cylinder are defined as procambial cells. Procambial cells divide periclinally and some of them differentiate into phloem or xylem cells.

(B) Magnification of intervening procambium located between the phloem and xylem tissues in different genotypes grown with 1 μ M of P9A (left) or TDIF (right) for five days.

Scale bars: 10 μm.



Supplemental Figure 5. Cavity Formation in *tdr* Mutants.
(A) A method for quantifying the size of cavities.
(B) Width and depth of the cavities in the *tdr-1 wox4-1* double mutants compared to those in the *tdr-1* mutants.
(C) Central region of the vascular cylinder in *tdr-1 wox4-1*. Xylem vessel cells and sieve elements show orange and green fluorescence respectively.
Error bars: SEM, n = 7–8, *Student's *t*-test at $p < 0.05$ for different means, .



Supplemental Figure 6. Schematic Diagrams of Mutant Phenotypes and a Model for Stem Cell Maintenance in the Vascular Meristem.

(A) A schematic diagram of mutant phenotypes on cambial development. The radial organization of cells in each vascular tissue type—phloem (red), procambium/cambium (yellow) and xylem (blue)—is illustrated. The young seedlings have the same set of cell layers in all genotypes (upper). The procambial/cambial cells continue to divide and add new layers for both phloem and xylem (lower, light red and blue). There are fewer new cell layers in *tdr* and *wox4* mutants than in the wild type. This could be caused by the suppression of cell proliferation in the procambium/cambium. Additionally, only *tdr* mutants have cavities in the vascular tissues, where only a few xylem cells were produced newly. This could have been caused by the differentiation of procambial/cambial cells into xylem adjacent to phloem cells. The *tdr/wox4* double mutant had broader cavities than did the *tdr* single mutant. Thus, *WOX4* seems to promote stem cell maintenance in a *tdr* mutant background. **(B)** A model for vascular stem cell maintenance by TDIF signaling. TDIF, which is secreted from *CLE41*-expressing phloem cells, is perceived by TDR on procambial/cambial cells. This signal contributes to maintain these cells as vascular stem cells by promoting their proliferation and suppressing their xylem commitment through two distinct intracellular signaling pathways. *WOX4* mediates one signaling pathway toward promoting the proliferation.

Supplemental Methods

RT-PCR Analyses on *WOX* Genes

Sample RNAs were isolated from the upper part of 7-day-old seedlings as described in the main text. Primers used for RT–PCR analyses were as follows:

TUA4 (5′-TCCTCCTCGACAATGAAG-3′, 5′-TCATCGTCACCACCTTCA-3′);

WUS (5′-ACAACAATGCAATCCGGTCACC-3′,

5′-CACATTCAGTACCTGAGCTTGC-3′);

WOX1 (5′-GGTACGGGAAGATAGAAGGC-3′,

5′-GCTGTTGGACATGTTGGAGG-3′);

WOX2 (5′-TCCAGAACCATAAGGCTAGGC-3′,

5′-GAGTGTTTTCCGTCCACTGG-3′);

WOX3 (5′-GGAGTGGTATACGGACTCC-3′,

5′-GCATCAATATCTCTTCAGCTCCAC-3′);

WOX4 (5′-GTCACTTCAGCCACTTTTGACC-3′,

5′-TCCAAGTTCTCAAATCCCCAGC-3′);

WOX5 (5′-AAGGTCGAAGCTTACGTGGC-3′,

5′-GTGGATGTTCCATTTCAGCTCC-3′);

WOX6 (5′-GGAGAACACGATATTGATGAACGG-3′,

5′-CTGATCAGATTCTTCAGTCGTCC-3′);

WOX7 (5′-AATAACAACAACGGAGGAGGAGG-3′,

5′-ACCTTTCGCTGGTAGTTGATGAC-3′);

WOX8 (5′-GGTTATTCCTAGTACTGACGCTG-3′,

5′-GATCAAAACGGCATCGTTTCCG-3′);

WOX9 (5′-CCAATTAGGGTTTCTCTCCGG-3′,
5′-TCCCTCACATTGAACGGTCC-3′);
WOX10 (5′-CAAGAGAGCCTAAACGGTAGG-3′,
5′-ATCATCCGCCTGACTCGAGG-3′);
WOX11 (5′-CAGCTTACCACATCATAGTGGG-3′,
5′-CCTCTCGTCACTTCTGTCG-3′);
WOX12 (5′-CGGCCTCTCTTCTTCAAGTGG-3′,
5′-GGGAAGAGGAAGACCAGAGG-3′);
WOX13 (5′-GAGGAGGAATGTACGTGAAGG-3′,
5′-CTAGCAAGTGAAGGTCTGAGC-3′);
WOX14 (5′-AGAGAGATCCAAAACGGTGCG-3′,
5′-ATCGTCAGCCTGACCATTAGC-3′).

For detecting gene expression in the *wox4-1* allele, we used the same primer set for *TUA4* and a different primer set for *WOX4* (5′-CACCATGAAGGTTCATGAGTTTTCGAATGG-3′ and 5′-TCATCTCCCTTCAGGATGGAG-3′).

Primers and TaqMan probes (Universal ProbeLibrary Probe; Roche) for the qRT–PCR experiments were as follows:
TUA4 (5′-TCTTGAACATGGCATTCAGC-3′,
5′-CGGTTTCACTGAAGAAGGTGTT-3′, probe #22);
WOX1 (5′-AGGTCAAGAACTGGATATGTTCG-3′,
5′-CAGATGATAATCACGGGAAGG-3′, probe # 126);
*WOX*2 (5′-AGCGCATGGCTTACTTCAAT-3′,

5′-GCTGTAAATAGTACGGACTGACACA-3′, probe #25);

*WOX*3 (5′-CCTTCTCCCATGTGTCTTCC-3′,

5′-TCACTTTGCTTGGAGCTTCTC-3′, probe #154);

WOX4 (5′-CAAGAACATCATCGTCACTAGACA-3′,

5′-GTACTCATTCTCTTCCACTAACTCCTC-3′, probe #22);

WOX5 (5′-CTATTGGTTTCAGAATCATAAGGCTA-3′,

5′-TGACAATCTTCTTCGCTTATTTCA-3′, probe #34);

WOX7 (5′-TCGTCGAGAGGATTCAACATT-3′,

5′-GATTCCACCGTCCACACTTC-3′, probe #63);

WOX9 (5′-CCGATCTTCTCCTTTCTCTTCA-3′,

5′-CTGGCTTTGGATTCCATCTT-3′, probe #21);

WOX12 (5′-GGTGTTACTTCATTCCTCTGGTC-3′,

5′-CCAGTTCATGTCTGTCTCGGTA-3′, probe #103);

WOX13 (5′-CAACAGCAACAACTACTGCTCCT-3′,

5′-CAGTCCCATCTTTCCAACAAG-3′, probe #124);

*WOX*14 (5′-CCGTTATTTGTGACCAACTCG-3′,

5′-AAAGTATCCGCCAACCATTG-3′, probe #25);

ATHB8 (5′-CTCAAGAGATTTCACAACCTAACG-3′,

5′-TCACTGCTTCGTTGAATCCTT-3′, probe #60);

ATHB15 (5′-CCGTCAACATACTCCAAATCC-3′,

5′-CTCGTCACCACCGATTCAC-3′, probe #33);

TDR (5′-TGGTGGAAGTTACTTTGAAGGAG-3′,

5′-TTCAATCTCTGTAAACCACCGTAA-3′, probe #151);

CLE41 (5′-TCTCGTACTCTTCTCCTTCTCTTCA-3′,

5′-TGGGGATTGTAAGGCTACTGA-3′, probe #134);

CLE44 (5′-TCATCACCTTCATGAATCCTCA-3′,

5′-GTGGAAGGTTGTAGAAGGAACC-3′, probe #12).

Transient Expression of CFP Fusion Proteins in *Nicotiana Benthamiana* Leaf Disks

The coding sequences (CDSs) for *WUS* and *WOX4* were amplified by RT-PCR using primer sets as follows: *WUS* (5′-CACCATGGAGCCGCCACAGCATC-3′, 5′-GTTCAGACGTAGCTCAAGAGAAG-3′); *WOX4* (5′-CACCATGAAGGTTCATGAGTTTTCGAATGG-3′, 5′-TCTCCCTTCAGGATGGAGAGG-3′).

Estrogen-inducible translational CFP fusion protein constructs were produced by cloning the CDSs into the pER8-CFP vector using the Gateway Cloning System (Invitrogen). Leaves of four-week-old *N. Benthamiana* plants were infected with agrobacteria harboring these constructs with p19K suppressor for three days. Leaf disks from the infected leaves were incubated with β-estradiol overnight to induce the expression of the fusion proteins. The leaf disks were observed using a light microscope (BX51, Olympus) equipped with the U-MCFPHQ mirror unit (Olympus).

Confocal Imaging of Roots

For imaging roots, 7-day-old roots were briefly stained with 20 μg/ml solution of

propidium iodide (Invitrogen) and washed with water. Samples were observed under an inverted fluorescence microscope (IX70; Olympus) equipped with a confocal unit (CSU10; Yokogawa, Tokyo, Japan).