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Supplemental Information

Spatial transcriptomics of a lycophyte root sheds light on root evolution

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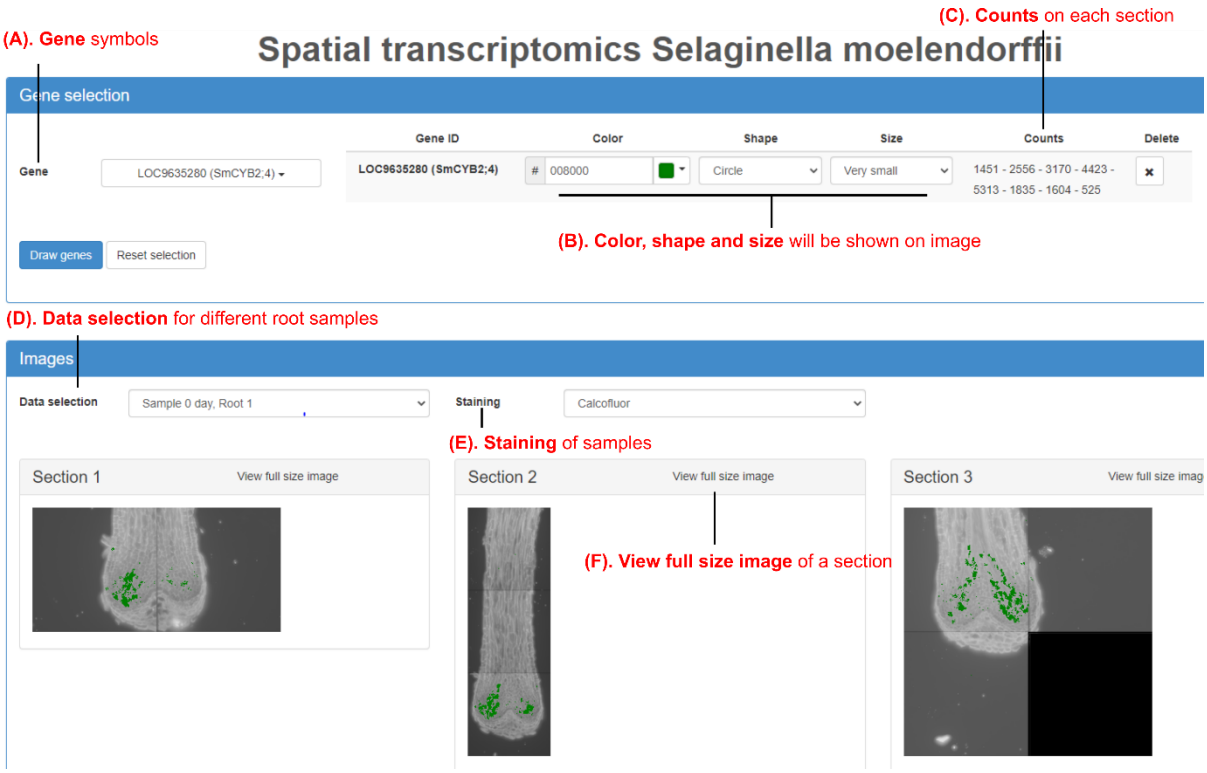


Figure S1. Spatial transcriptomics online tool, Related to Figure 1 to Figure 4.

Window illustrating the online tool to visualize gene expression patterns in *S. moellendorffii* roots (https://bioinformatics.psb.ugent.be/webtools/spatial-transcriptomics/selaginella). On the website, genes of interest can be selected in the **gene selection** panel, where **genes** are displayed by the LOCUS IDs from the National Center for Biotechnology Information (NCBI) and gene names in this study (A). Once a gene is selected, the "**draw genes**" button can be clicked. The **color, shape, size** of genes can be changed to facilitate the visualization of the transcripts (B) and the number of **counts** present how many dots or transcripts are presented in each displayed sections (C). In the **images** panel, all sections of the selected root (D) are shown and for display, DAPI, calcofluor or brightfield images can be chosen (E). "**View full size image"** can be selected to view a larger image of the selected section (F).

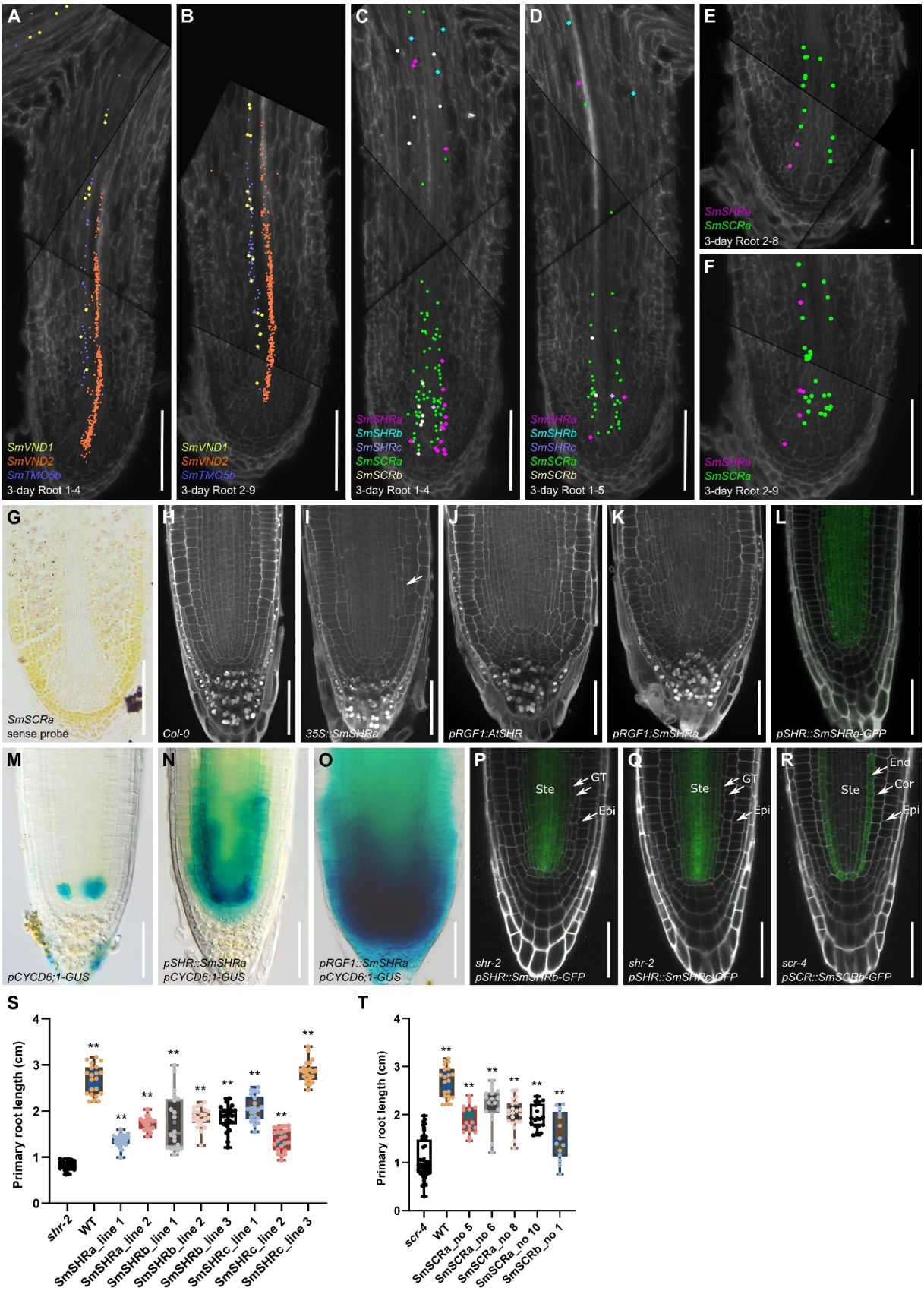


Figure S2. Examples of gene expression in *S. moellendorffii* roots as retrieved via spatial transcriptomics. Expression patterns of Selaginella SHRs are highly similar to the Arabidopsis SHR expression, Related to Figure 2 and Figure 3.

(A-B) The examples show that *SmVND2* and *SmTMO5b* were respectively expressed in the phloem and xylem cells.

(C-F) The examples show homologues of *SHR* and *SCR*.

(G) *In situ* hybridization of *SmSCRb* with sense probe.

(H-I) Overexpression of *SmSHRa* (I) show extra ground tissue divisions compared to wild type (H).

(J-K) Expression of *SmSHRa* under the QC and columella stem cell-specific *RGF1* promoter (K) shows, just as is the case for *AtSHR* (J), supernumerary cell layers.

(L) *pSHR::SmSHRa-GFP* in *Col-0* background shows nuclear localization in the endodermis.

(M-O) The *pCYCD6;1-GUS* expression as compared to the wild type (M), expanded into ground tissue layers in the *pSHR::SmSHRa* transgenic line (N), and into the whole root the *pRGF1::SmSHRa* transgenic line (O).

(P-R) *SmSHRs* and *SmSCRb* are able to complement the root cell files of Arabidopsis *shr-2* and *scr-4* mutant, respectively. GT, ground tissue; Ste, stele; Epi, epidermis; End, endodermis; Cor, cortex.

(S-T) The box plots show that *SmSHRs* and *SmSCRs* partially complement the root length of Arabidopsis *shr-2* and *scr-4* mutant. The T3 seedlings of pSHR::SmSHRs-GFP were measured (S). And the T2 seedlings of pSCR::SmSCRs-GFP were measured (T). The dots show repeats ($n > 14$) And significant difference ($p < 0.05$) was tested by a one-way ANOVA with Tukey's post hoc test.

Roots were treated with mPS-PI staining (H-K), GUS staining (M-O) and PI staining (L and P-R) with green indicating the localization of the protein. Arabidopsis roots are from 5 DAG (H-O) or 3 DAG (P-R) seedlings. Scale bars represent 100 μm (A-F) and 50 μm (G-R).

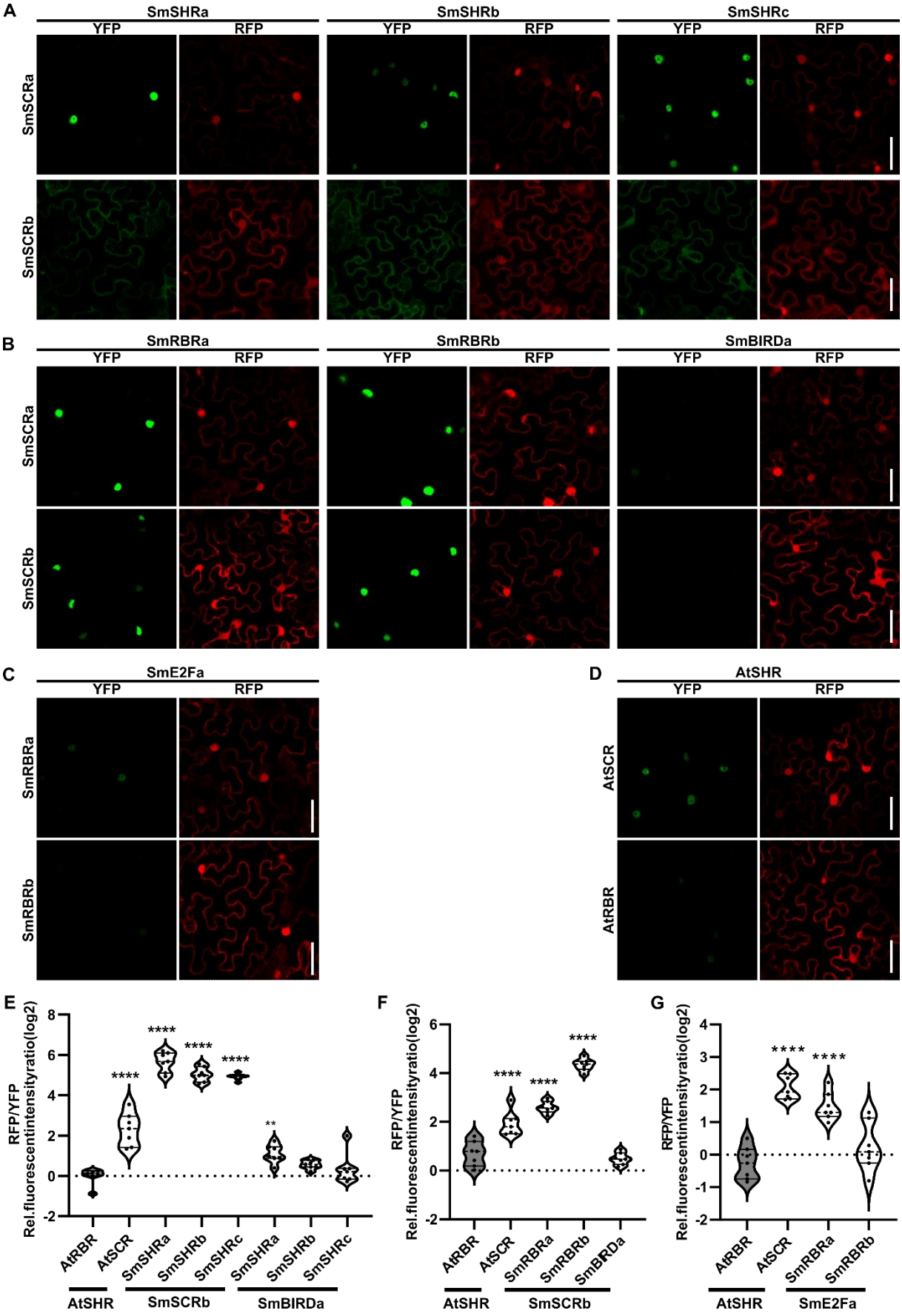
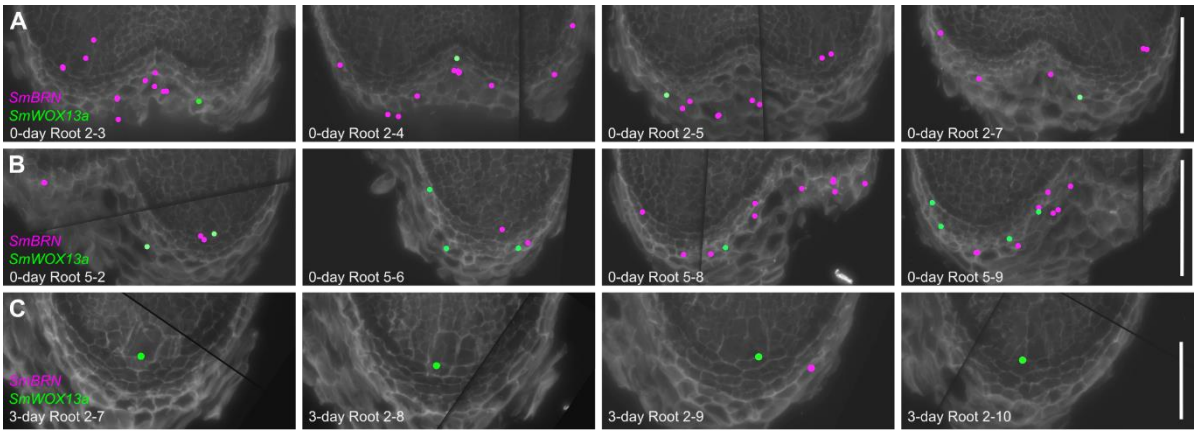
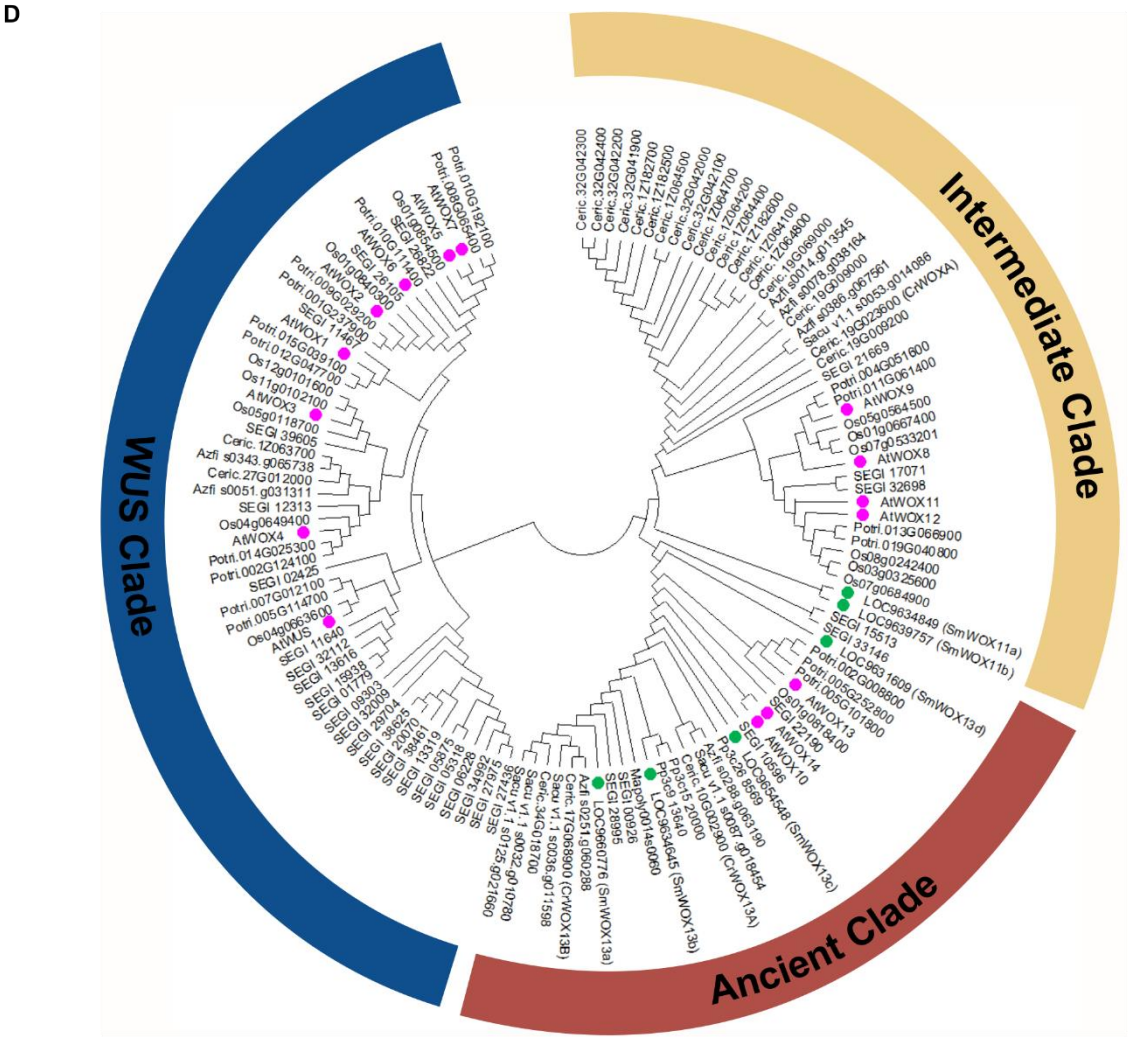


Figure S3. rBiFC experiments show strong interaction between SmSCRs and anticipated interactors, Related to Figure 3.

(A-D) Confocal images showing interactions between SmSCRa or SmSCRb and SmSHRs, SmRBRs or SmBIRDa in tobacco leaves as detected with rBiFC. The interactions between AtSHR and AtRBR and between AtSHR and AtSCR were used as negative and positive controls, respectively (D). The right channel is YFP, and left is RFP as control. Scale bars, 50 μm.

(E-G) Violin plots showing the quantification of the rBiFC. Dots show repeats (n = 7). Significance was tested via a one-way ANOVA with Tukey's post hoc test compared to the AtSHR-AtRBR control. All experiments were repeated independently three times.


A
SmBRN
SmWOX13a
0-day Root 2-3
0-day Root 2-4
0-day Root 2-5
0-day Root 2-7
B
SmBRN
SmWOX13a
0-day Root 5-2
0-day Root 5-6
0-day Root 5-8
0-day Root 5-9
C
SmBRN
SmWOX13a
3-day Root 2-7
3-day Root 2-8
3-day Root 2-9
3-day Root 2-10


D
WUS Clade
Intermediate Clade
Ancient Clade
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Potri.008G065400
AtWOX5
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SEGI 26822
Potri.010G114400
AtWOX16
SEGI 28105
Os01g0843000
AtWOX2
Potri.009G029200
Potri.001G237900
SEGI 11467
AtWOX1
Potri.015G039100
Potri.012G047700
Os12g0101600
Os11g0102100
AtWOX3
Os05g0118700
SEGI 39605
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Azfi s0343.g065738
Ceric.27G012000
Azfi s0051.g031311
SEGI 12313
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AtWOX4
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Potri.002G124100
SEGI 02425
Potri.007G012100
Potri.005G114700
Os04g0663600
AtWUS
SEGI 11640
SEGI 32112
SEGI 13616
SEGI 16918
SEGI 01779
SEGI 09303
SEGI 32009
SEGI 29704
SEGI 38625
SEGI 20075
SEGI 38461
SEGI 13319
SEGI 05875
SEGI 05318
SEGI 08628
SEGI 34992
SEGI 27875
SEGI 27436
Sacu v1.1 s0125.g021660
Sacu v1.1 s0032.g010780
Ceric.34G018700
Sacu v1.1 s0036.g011598
Ceric.17G068900 (CrWOX13B)
Azfi s0251.g060288
LOC9660776 (SmWOX13a)
SEGI 00926
SEGI 28995
MapoMv0014s0060
LOC9634645 (SmWOX13b)
Ppc15 13640
Ppc15 20000
Sacu v1.1 s0087.g018454
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Azfi s0228.g063190
Ppc28 8569
LOC9654548 (SmWOX13c)
SEGI 10596
AtWOX14
SEGI 22190
AtWOX10
Os01g0818400
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Potri.005G252800
AtWOX13
Potri.002G009800
LOC9631609 (SmWOX13d)
SEGI 33146
SEGI 15513
LOC9639757 (SmWOX11b)
LOC9634849 (SmWOX11a)
Os07g0684900
Os03g0325600
Os08g0242400
Potri.019G040800
Potri.013G066900
AtWOX12
AtWOX11
SEGI 32698
SEGI 17071
AtWOX8
Os07g0533201
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AtWOX9
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Potri.004G051600
SEGI 21669
Ceric.19G009200
Sacu v1.1 s0053.g014086
Azfi s0386.g067561
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Azfi s0014.g013545
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Ceric.1Z064100
Ceric.1Z182600
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Ceric.1Z064200
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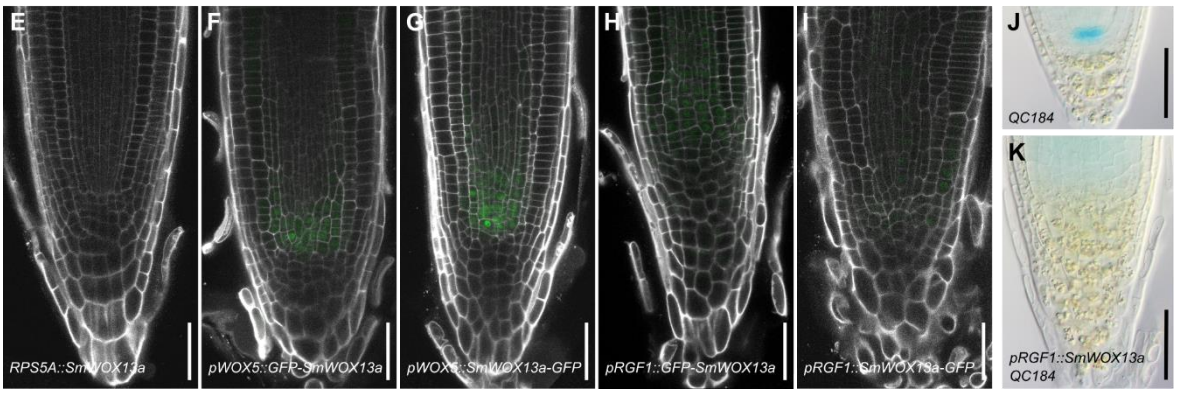

E
RPS5A::SmWOX13a
F
pWOX5::GFP-SmWOX13a
G
pWOX5::SmWOX13a-GFP
H
pRGF1::GFP-SmWOX13a
I
pRGF1::SmWOX13a-GFP
J
QC184
K
pRGF1::SmWOX13a
QC184

Figure S4. *SmWOX13a* is related to the root cap, Related to Figure 4.

(A-C) Additional examples of *SmWOX13a* and *SmBRN* expression as detected via spatial transcriptomics.

(D) Phylogenetic tree based on the HD domain of *WOX* genes from *Physcomitrium patens*, *Marchantia polymorpha*, *Selaginella moellendorffii* (Sm, green), *Ceratopteris richardii* (Cr), *Salvinia cucullata*, *Azolla filiculoides*, *Sequoiadendron giganteum*, *Oryza sativa ssp. japonica*, *Populus trichocarpa* and *Arabidopsis thaliana* (At, magenta).

(E-I) Different lines result in aberrant root meristems. The strongest effect is observed when *SmWOX13a* was expressed under control of the *RGF1* promoter (H, I)

(J-K) The *pRGF1::SmWOX13a* line crossed with QC184 (J) shows disappearance of the QC and extra columella cells compared with QC184 (K).

Roots were treated with PI staining (A-E) and GUS staining (F-G). Arabidopsis seedlings are 3 DAG. Scale bars represent 100 μm (A-B), 200 μm (C) and 50 μm (E-K).

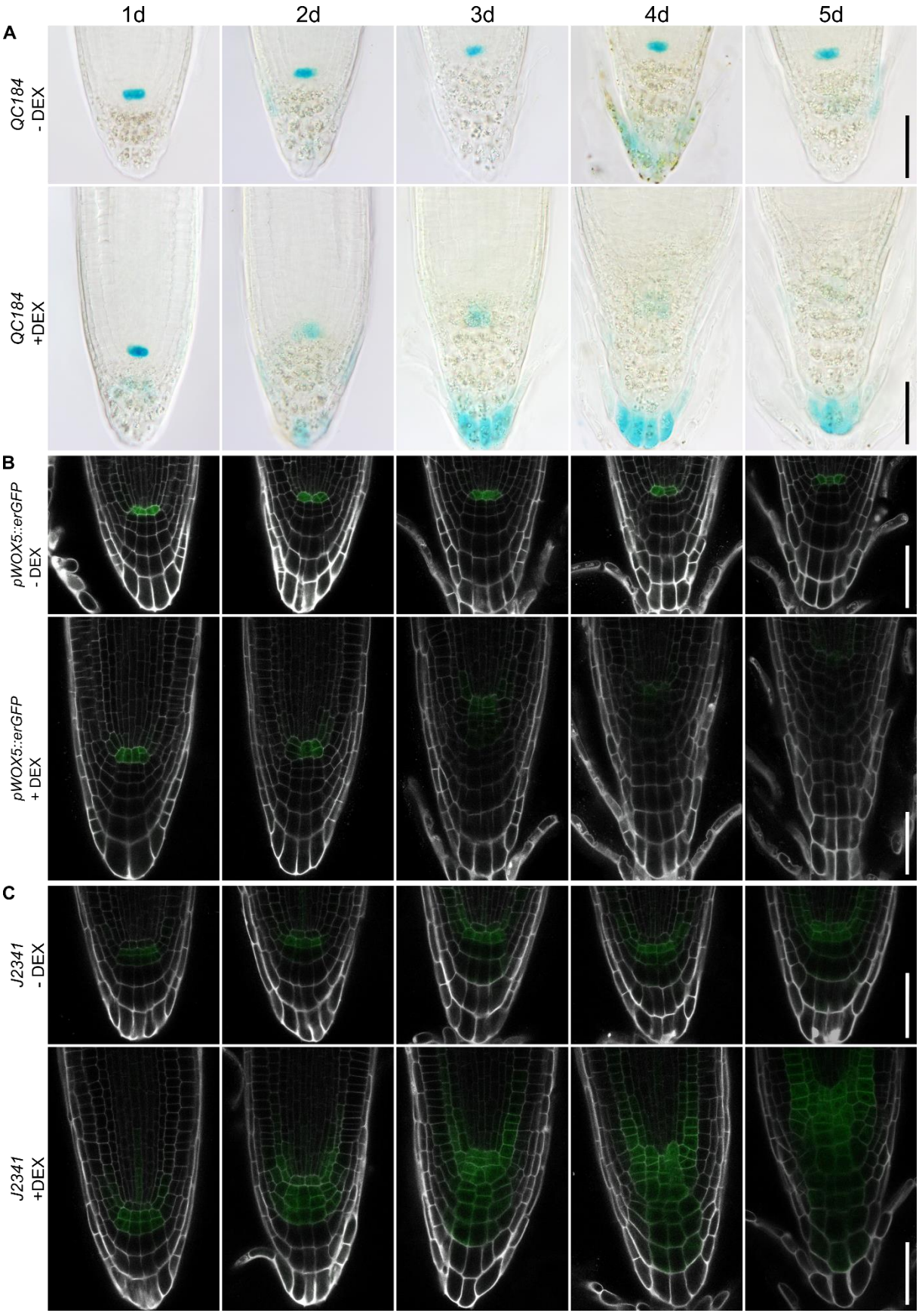


Figure S5. Root meristems of inducible *pRGF1::SmWOX13a-GR* lines combined with QC and columella stem cell marker lines. The different panels show a 5-day time-course of DEX treatment, Related to Figure 4.

(A) Effect of induced *SmWOX13a* expression (*pRGF1::SmWOX13a-GR*) on the *QC184* line indicating quiescent center cells (QC).

(B) Effect of induced *SmWOX13a* expression (*pRGF1::SmWOX13a-GR*) on the *pWOX5::erGFP* line indicating WOX5 expression in QC.

(C) Effect of induced *SmWOX13a* expression (*pRGF1::SmWOX13a-GR*) on the *J2341* line, indicating columella stem cells.

Roots were subjected to GUS staining (A) and PI staining (B-C). Arabidopsis seedlings were first geminated on ½ MS for 2 days, and then transferred to DEX medium. Scale bars, 50 μm.

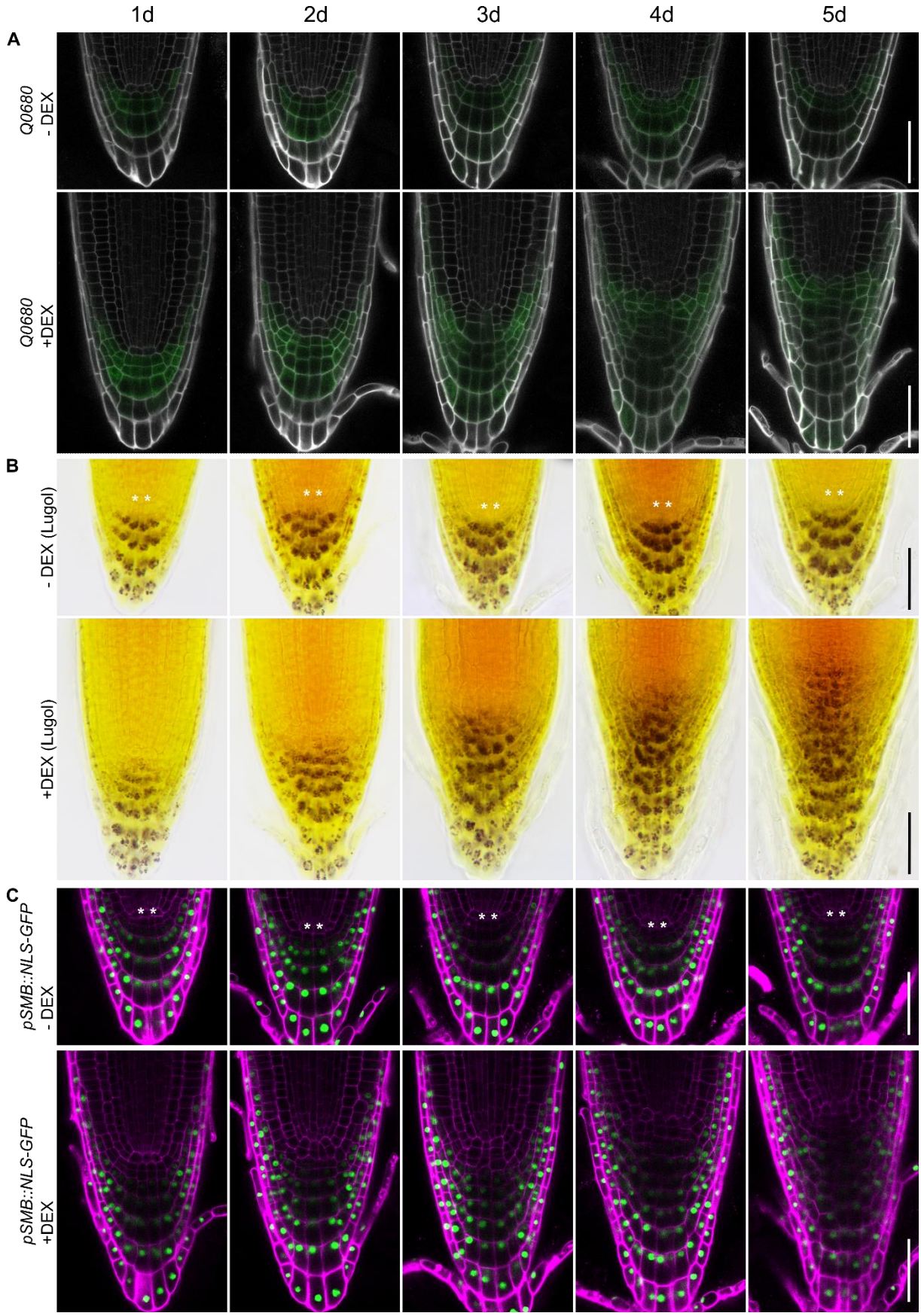

1d
2d
3d
4d
5d
A
Q0680
- DEX
Q0680
+DEX
B
- DEX (Lugol)
+DEX (Lugol)
C
pSMB::NLS-GFP
- DEX
pSMB::NLS-GFP
+DEX

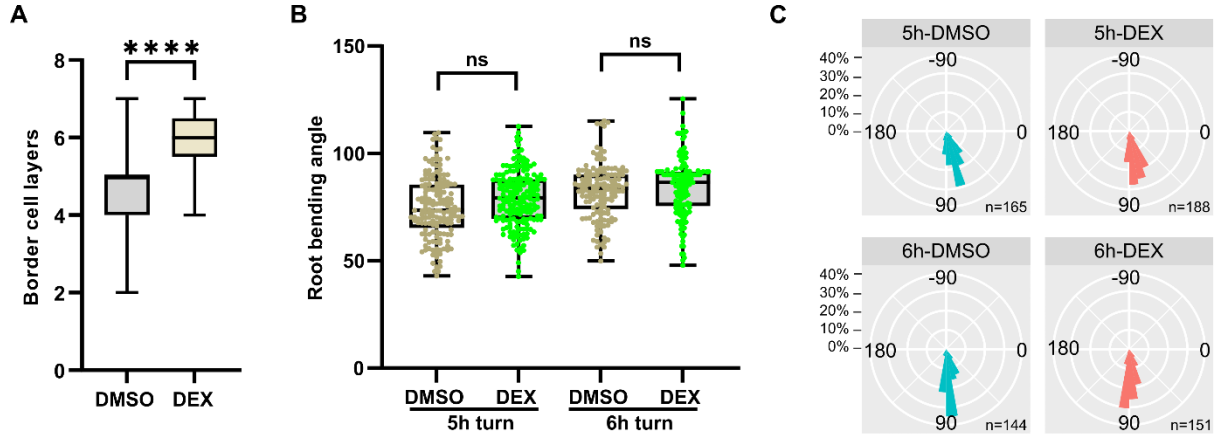
Figure S6. Root meristems of inducible *pRGF1::SmWOX13a-GR* lines and effects on columella cells and columella cell differentiation. The different panels show a 5-day time-course of DEX treatments, Related to Figure 4.

(A) Effect of induced *SmWOX13a* expression (*pRGF1::SmWOX13a-GR*) on the *Q0680* line indicating columella cells.

(B) Effect of induced *SmWOX13a* expression (*pRGF1::SmWOX13a-GR*) on starch accumulation, an indicator of differentiated columella cells, as shown via lugol staining. White asterisks indicate quiescent center cells (QC).

(C) Effect of induced *SmWOX13a* expression (*pRGF1::SmWOX13a-GR*) on the crossing F1 seedlings of *pSMB::nlsGFP/GUS*, indicating the formation of root cap.

Roots were subjected to PI staining (A-C) and lugol staining (B). Arabidopsis seedlings were first geminated on ½ MS for 2 days, and then transferred to DEX medium. Scale bars, 50 μm.



D

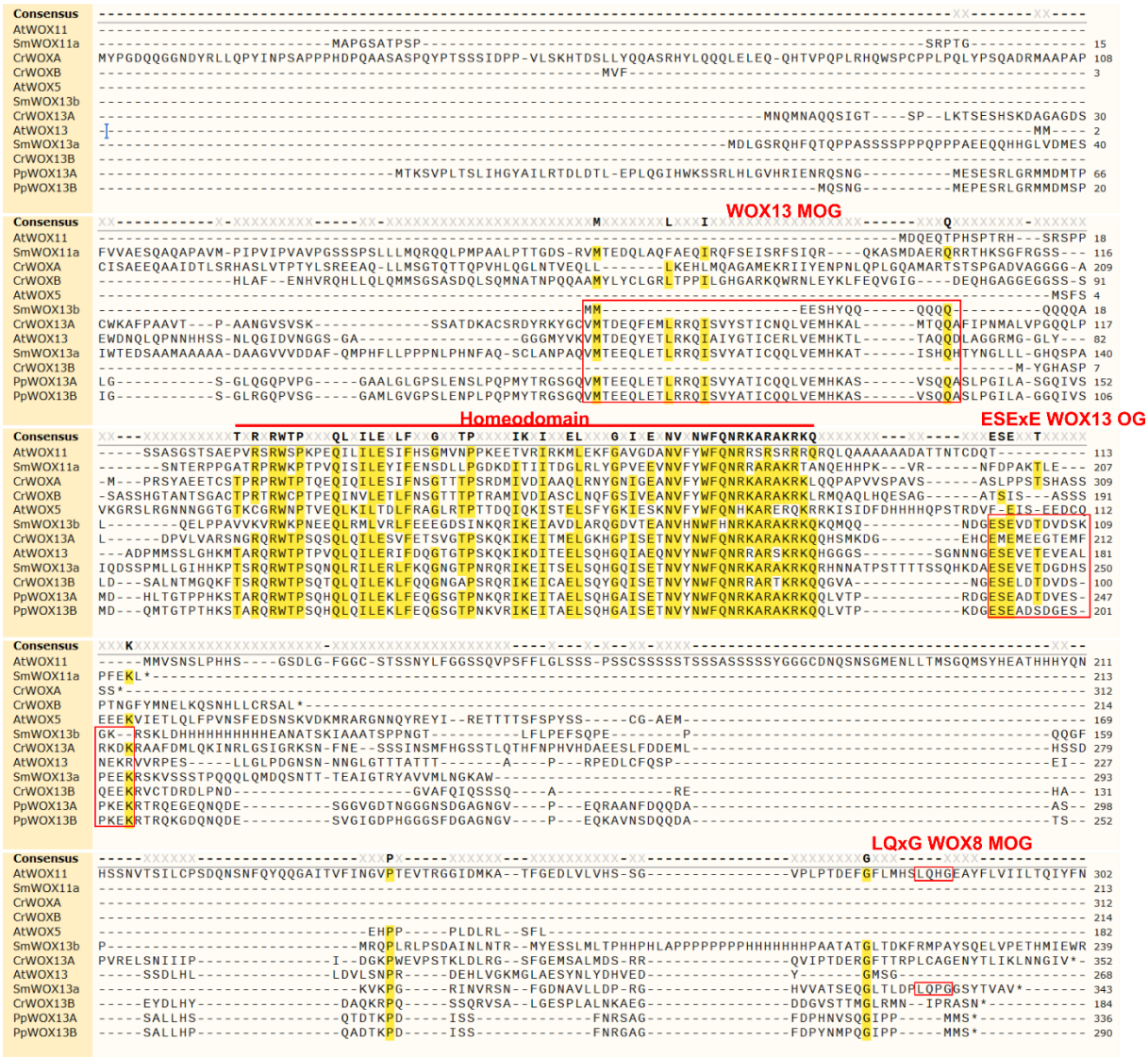


Figure S7. The effect of *SmWOX13a* on border cell layers and gravitropism, Related to Figure 4.

(A) The number of border cell layers of *pRGF1::SmWOX13a* after 7 days of DMSO or DEX treatment. Significance was tested via a Student's t-test.

(B) Distribution of the root bending angle after gravistimulation for the indicated hours and treatments. Significance was tested via a Student's t-test.

(C) Distribution of the root bending angle after gravistimulation for the indicated hours and treatments represented as nightingale plots. Angles are binned in 10 ° intervals, with each bar's length representing the percentage of the roots within that bin. As such, a bar reaching the second ring of each plot represents 10% roots within that bin. The total number of the roots within three repeats represented in each plot is indicated in the bottom-right corner.

(D) Sequence alignment of WOXs. Boxes indicate WOX motifs or domains, including the WOX homeodomain, the ESExE-WOX13 orthologous group (OG) motif, the WOX13 model plant OG (MOG) motif and the LQxG-WOX8 MOG motif. *WOX8* is a member of the *WOX11*-clade. No specific motifs associated to the root cap cell inducing action were found.