SUPPLEMENTAL INVENTORY

Supplementary Information contains: Supplementary Figures and Legends 1-5; Supplementary Methods and additional References.

Supplementary Figure 1 provides additional data on the genetic relationship between, and the tissue specificity of, SCR and the cytokinin signaling pathway. It is related to main Figure 1 because it provides further evidence that SCR suppresses AHK3-mediated differentiation input in the QC cells.

Supplementary Figure 2 provides additional data on the genetic relationship between SCR and the cytokinin-response transcription factor ARR1. It is related to main Figure 2 because it provides further evidence that SCR specifically suppresses ARR1-mediated differentiation input indicating that ectopic expression of *ARR1* in the *scr* QC induces cell differentiation in the SCN via a mechanism different from that in the TZ.

Supplementary Figure 3 provides additional data on the molecular relationship between SCR and ARR1. It is related to main Figure 3 because it provides further evidence that SCR directly suppresses ARR1 in the QC cells.

Supplementary Figure 4 shows how SCR maintains SCN activity. It is related to main Figure 4 since it further confirms that SCN and meristem defects of *scr* mutant are dependent on the ARR1-mediated control of *ASB1* expression.

Supplementary Figure 5 shows that both SCR and ARR1 are auxin inducible. It is related to main Figure 5 because it provides further evidence that SCR controls *ARR1* at the TZ from the QC, via auxin.

SUPPLEMENTAL INFORMATION

Figure S1

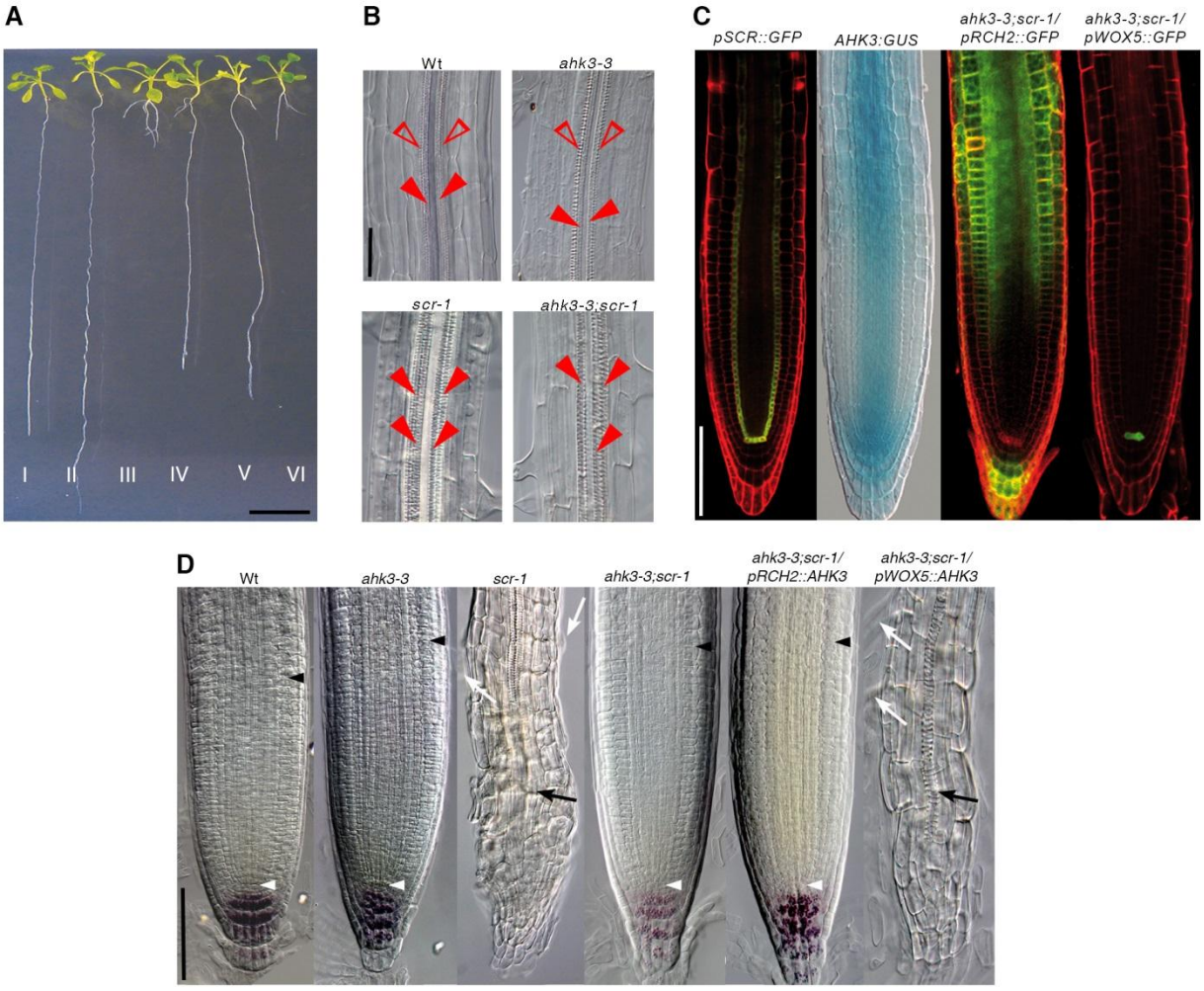


Figure S2

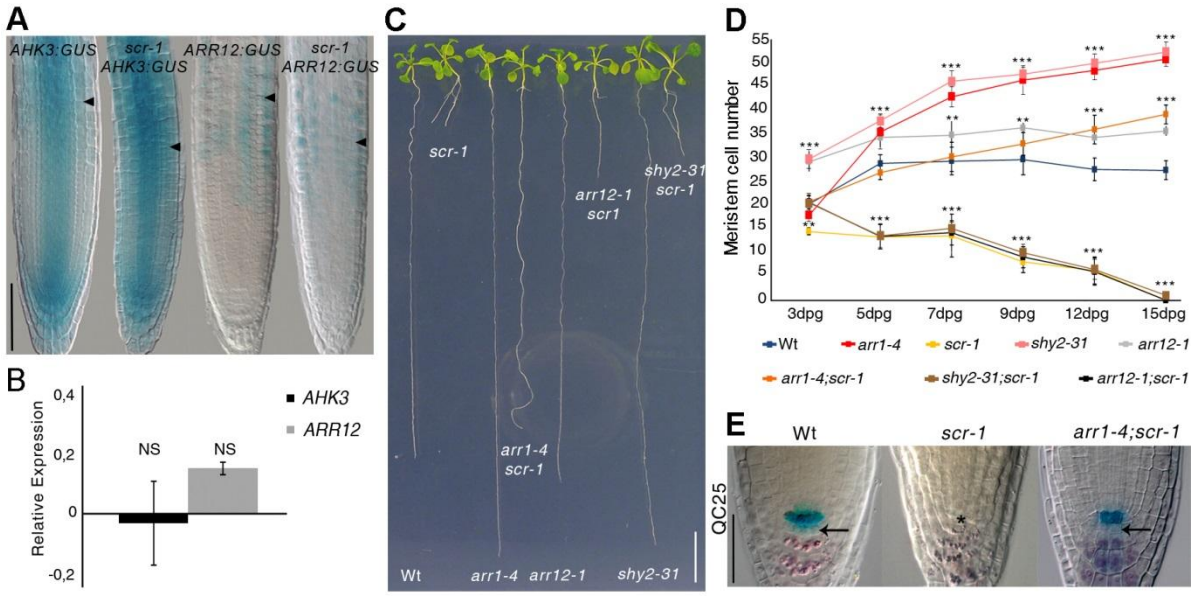

A
AHK3:GUS
scr-1 AHK3:GUS
ARR12:GUS
scr-1 ARR12:GUS
B
Relative Expression
0,4
0,2
0
-0,2
NS
NS
AHK3
ARR12
C
scr-1
arr12-1 scr1
shy2-31 scr-1
arr1-4 scr-1
Wt
arr1-4
arr12-1
shy2-31
D
Meristem cell number
55
50
45
40
35
30
25
20
15
10
5
0
3dpg
5dpg
7dpg
9dpg
12dpg
15dpg
Wt
arr1-4
scr-1
shy2-31
arr12-1
arr1-4;scr-1
shy2-31;scr-1
arr12-1;scr-1
E
Wt
scr-1
arr1-4;scr-1
QC25

Figure S3

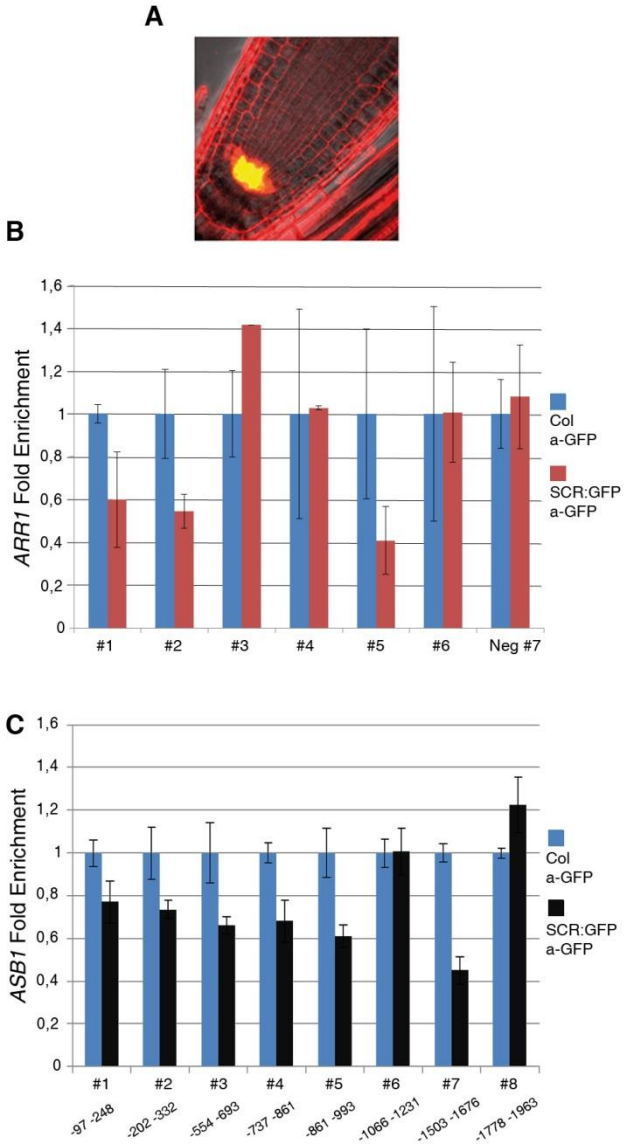

A
B
ARR1 Fold Enrichment
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
#1
#2
#3
#4
#5
#6
Neg #7
Col a-GFP
SCR:GFP a-GFP
C
ASB1 Fold Enrichment
#1
#2
#3
#4
#5
#6
#7
#8
-97 -248
-202 -332
-554 -693
-737 -861
-861 -993
-1066 -1231
-1503 -1676
-1778 -1963
Col a-GFP
SCR:GFP a-GFP

Figure S4

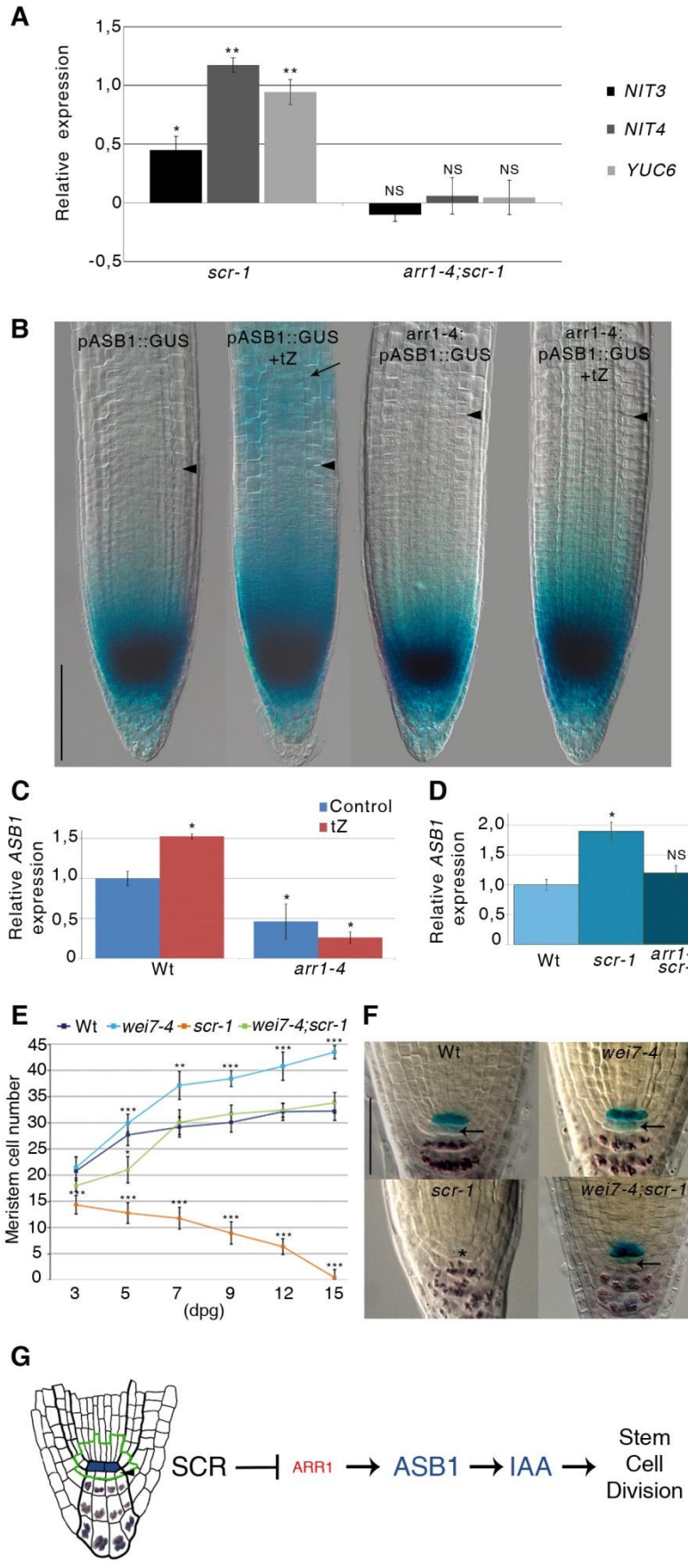
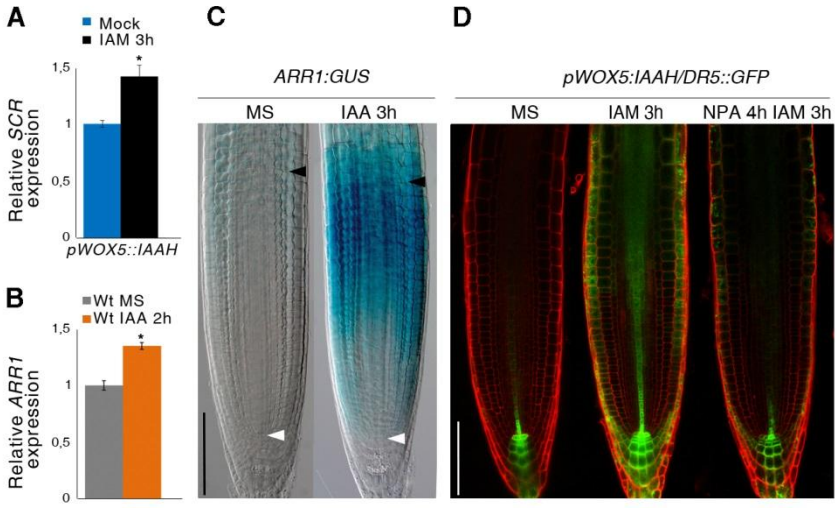

A
Relative expression
1,5
1,0
0,5
0
-0,5
NIT3
NIT4
YUC6
NS
scr-1
arr1-4;scr-1
B
pASB1::GUS
pASB1::GUS +tZ
arr1-4: pASB1::GUS
arr1-4: pASB1::GUS +tZ
C
Relative ASB1 expression
Control
tZ
Wt
arr1-4
D
Relative ASB1 expression
2,0
Wt
scr-1
arr1-4; scr-1
E
Wt
wei7-4
scr-1
wei7-4;scr-1
Meristem cell number
(dpg)
F
Wt
wei7-4
scr-1
wei7-4;scr-1
G
SCR
ARR1
ASB1
IAA
Stem Cell Division

Figure S5


A
Mock
IAM 3h
Relative SCR expression
1,5
1
0,5
0
*
pWOX5::IAAH
B
Wt MS
Wt IAA 2h
Relative ARR1 expression
1,5
1
0,5
0
*
C
ARR1:GUS
MS
IAA 3h
D
pWOX5:IAAH/DR5::GFP
MS
IAM 3h
NPA 4h IAM 3h

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. SCR sustains root growth and controls meristem size by suppressing cytokinin perception specifically in the QC. Related to Figure 1

(A) 15-day-old seedlings of, from I to VI, wild-type (Wt), *ahk3-3*, *scr-1*, *ahk3-3;scr-1*, *ahk3-3;scr-1/pRCH2::AHK3* and *ahk3-3;scr-1/pWOX5::AHK3*. Scale bar represents 1 cm. (B) Xylem patterning in Wt, *ahk3-3*, *scr-1* and *ahk3-3;scr-1*. Filled arrowheads indicate metaxylem, unfilled arrowheads indicate protoxylem. Note that in *ahk3-3;scr-1* double mutant metaxylem differentiates ectopically in the place of protoxylem, as in *scr-1* mutant (Carlsbecker et al., 2010). The same phenotype has been observed in *arr1-4;scr-1* and *wei7-4;scr-1* (data not shown). Roots were analyzed 5 days post germination. Scale bar represents 50 µm. (C) Expression of, from left to right, *pSCR::GFP* and *AHK3:GUS* in wild-type root meristem and *pWOX5::GFP* and *pRCH2::GFP* in *ahk3-3;scr-1* root meristem. Scale bar represents 100 µm. (D) 15-day old root meristems of Wt, *ahk3-3*, *scr-1*, *ahk3-3;scr-1*, *ahk3-3;scr-1/pRCH2::AHK3* and *ahk3-3;scr-1/pWOX5::AHK3*. Root meristem size is indicated as the number of cortex cells in a file extending from the QC (white arrowheads) to the first elongated cortex cell (black arrowheads). Black arrow indicates differentiated xylem strands, white arrows indicate root hairs of differentiated epidermal cells in *scr-1* and in *ahk3-3;scr-1/pWOX5::AHK3*. Note that lack of meristem activities in *scr-1* and *ahk3-3;scr-1/pWOX5::AHK3* leads to arrest of organ growth. Scale bar represents 100 µm.

Figure S2. *scr-1* root growth is not restored in the *arr12-1;scr-1* and *shy2-31;scr-1* double mutants. Related to Figure 2

(A and B) SCR doesn't affect neither *AHK3* nor *ARR12* expression. (A) Expression of, from left to right, *pAHK3::AHK3:GUS* construct in wild-type (Wt) roots and in *scr-1* mutant and *pARR12::ARR12:GUS* construct in Wt roots and in *scr-1* mutant. Black arrowheads indicate the position of transition zone. Scale bar represents 100 µm. (B) qRT-PCR confirming no changes of the *AHK3* and *ARR12* mRNA in the *scr-1* mutant background. Relative expression is normalized to

ACTIN2 and 0 corresponds to mRNA level in Wt roots. Error bars, SD; NS, not significant; Student's t-test. (C) 15-day-old seedlings of Wt, *scr-1*, *arr1-4*, *arr1-4;scr-1*, *arr12-1*, *arr12-1;scr-1*, *shy2-31* and *shy2-31;scr-1*. Scale bar represents 1 cm. (D) Root meristem cell number measured over time of genotype depicted in (C). Error bars, SD; $**p < 0.01$, $***p < 0.001$; Student's t-test. (E) *QC25* expression in, from left to right, 5-day old Wt, *scr-1* and *arr1-4;scr-1*. Double labeling of QC and differentiated columella cells visualized by QC25 and amyloplast staining, respectively, reveal columella stem cells activity restoration (black arrows) in the *arr1-4;scr-1* double mutant. Asterisk indicates position of QC cells in the *scr-1* mutant. Scale bar represents 50 µm.

Figure S3. ChIP analysis of SCR binding on the *ARR1* promoter using the entire root meristem. Related to Figure 3

(A) QC specific *GFP* expression of *pWOX5>>SCR:GR>>GFP* in *scr-4* root meristem. (B) ChIP-qRT-PCR analysis using the entire root meristem of SCR::GFP-SCR/*scr-4* plants. GFP-SCR DNA fragment were immuno-precipitated using GFP antibody and analyzed by qRT-PCR. PCR results are shown as enrichment compared to Col-0. Similar results were observed in five biological replicates. Error bars, SD. (C) ChIP-qRT-PCR of the *ASB1* promoter using 5 days old Col-0 (blue bars) and *pSCR::SCR:GFP/scr-4* (black bars) plants. ChIP samples were prepared from QC-enriched material. RT-qPCR results are shown as fold enrichment compared to Col-0. No fragments were bound by SCR by scanning the sequence upstream of ASB1. The data shown are representative of three independent biological experiments with similar results. Error bars show the standard deviations of the ChIP-qRT-PCR reactions performed in triplicate.

Figure S4. SCN and meristem defects in the *scr-1* mutant depend on the ARR1-mediated *ASB1* expression control. Related to Figure 4

(A) qRT-PCR shows mRNA up-regulation of NIT3, NIT4 and YUC6 genes in *scr-1* roots and reversion to normal mRNA levels in the *arr1-4;scr-1* double mutant. Relative expression is

normalized to *ACTIN2* and 0 corresponds to mRNA level in Wt roots. Error bars, SD; ∗p < 0.05, ∗∗p < 0.01, NS, not significant; Student's t-test. (B and C) *ASB1* expression is controlled by cytokinin. (B) Expression of the *pASB1::GUS* construct in 5-day old, from left to right, wild-type (Wt) roots, Wt roots treated for 4 hours with 5 µM transzeatin (tZ), *arr1-4* mutant and *arr1-4* mutant treated for 4 hours with 5µM tZ. Black arrow indicates ectopic *ASB1* expression at the transition zone. Scale bar represents 100 µm. (C) qRT-PCR confirming *ASB1* mRNA up-regulation after 3 hours of cytokinin treatment (5 µM tZ) in Wt roots and no up-regulation in the *arr1-4* mutant upon cytokinin treatment. Relative expression is normalized to *ACTIN2*. Error bars, SD; asterisk indicates statistical significance (P<0.05). (D) qRT-PCR confirming *ASB1* mRNA up-regulation in *scr-1* roots and reversion to normal mRNA levels in the *arr1-4;scr-1* double mutant. Relative expression is normalized to *ACTIN2*. Error bars, SD; ∗p < 0.05, NS, not significant; Student's t-test. (E and F) *scr-1* indeterminate root growth and SCN activity are restored by elimination of *WEI7* function. (E) Root meristem cell number measured over time in Wt, *wei7-4*, *scr-1* and *wei7-4;scr-1*. dpg: days post germination. Error bars, SD; ∗∗p < 0.01, ∗∗∗p < 0.001; Student's t-test. (F) *QC25* expression and lugol staining in, top from left to right, Wt and *wei7-4*, bottom from left to right, *scr-1* and *wei7-4;scr-1*. Black arrow indicates active stem cells in Wt, *wei7-4* and *wei7-4;scr-1*. Asterisk indicates the presumptive position of QC cells in *scr-1*. Roots were analyzed 5 days post germination. The same results have been obtained with the *wei7-1;scr-1* double mutant combination. Scale bar represents 50 µm. (F) Model showing how SCR maintains stem cell niche activity. In the QC (blue), SCR represses *ARR1*, which in turn controls auxin production via *ASB1*, thus enabling stem cell division.

Figure S5. *ARR1* is auxin inducible. Related to Figure 5

(A) qRT-PCR showing upregulation of *SCR* transcription in *pWOX5::IAAH* roots upon 3 hours of IAM treatment. Error bars, SD; ∗p < 0.05; Student's t-test. (B) qRT-PCR showing *ARR1* mRNA up-regulation after 2 hours of IAA treatment of 5-day old Wt roots. Relative expression is

normalized to *ACTIN2*. Error bars, SD; $*p < 0.05$; Student's t-test (C) Expression of the *ARR1:GUS* translational fusion in 5-day old wild-type (Wt) root meristem untreated (left) and treated for 3 hours with IAA (right). White and black arrowheads indicate, respectively, the QC and the first elongated cortex cell. Note that no changes in meristem size have been observed after auxin treatment. Scale bar represents 100 µm. (D) *DR5::GFP* expression in 5-day old mock-treated *pWOX5::IAAH* root meristems (left), treated for 3 hours with IAM (middle) or treated with IAM and NPA (right) (Blilou et al., 2003). Scale bar represents 100 µm.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Plasmids construction and plant transformation

AHK3 cDNA (Mähönen et al., 2006) was provided by Tatsuo Kakimoto (Osaka University, Japan). *pRCH2::AHK3* plants were obtained as follow. *AHK3* cDNA (3040 bp) was digested with *SpeI* and *SalI* from *pHM-1-AHK3* vector and cloned into *PGEM-T* vector (Promega). *RCH2* promoter (AT3g24240 - 2360 bp fragment) was digested with *Apa*I and *Xba*I from *pGreenII0229_RCH2::AtCKX1* vector (Dello Ioio et al., 2007) and cloned into the *Apa*I and *Spe*I sites upstream of the *AHK3* cDNA. The *pRCH2::AHK3* fragment was excised from pGEM-T vector with *Sal*I and *Apa*I and cloned into the *Kpn*I and *Apa*I sites of the binary vector *pGREEN0179*, incompatible ends made blunt by Klenow reaction. *pWOX5::AHK3* plants were obtained as follow. *WOX5* promoter (AT3g11260 – 4471 bp fragment) was excised with *Kpn*I and *BamH*I from *pGreenII229_WOX5::GFP* and cloned into pGREEN0179. Further, *AHK3* cDNA (3040 bp) was excised with *Sal*I and *Sma*I from *pHM-1-AHK3* vector and cloned downstream of the *WOX5* promoter into the *Sma*I-*Sal*I sites of the *pGREEN0179-pWOX5* vector. The p*WOX5>>SCR:GR>>GFP* transactivation system is constituted by a *pGreenII0229* vector harbouring a 4,4kb *WOX5* promoter driving *GAL4VP16*, a 6x*UAS* promoter driving *erGFP* and a

6x*UAS* promoter driving the *SCR* coding sequence fused at the 3' end to the ligand binding domain of the rat glucocorticoid receptor (GR).

Plants were transformed by floral-dip method (Clough et al., 1998) and transgenic plants were selected on the appropriate antibiotic. Transformants for p*WOX5>>SCR:GR>>GFP* into the *scr-4* mutant were phenotypically screened after dexamethasone induction.

IAA quantification

Root tips (3 mm) from 20, 4-day old seedlings were collected and pooled for IAA quantification. 150 pg of $^{13}C_6$-IAA was added as internal standard to each sample before purification and analysis by gas chromatography - selected-reaction-monitoring - mass spectrometry as described (Edlund et al., 1995; Ljung et al., 2005).

RNA isolation and qRT-PCR

Total RNA was extracted from 5-day old Wt and *scr-1* roots using the TRIsure reagent (Bioline), and the first strand cDNA was synthesized using the SuperScript® VILO™ cDNA Synthesis Kit (Invitrogen). Quantitative RT-PCR (qRT-PCR) analysis was conducted using the gene-specific primers listed below:

for *AHK3* (At1g27320): AHK3 FWD: GTCTGGGAAAGAAGATCGTGA and AHK3 REV: AATGTCAGGATCACCCCAAG

for *ARR12* (At2g25180): ARR12 FWD: ACCGGCTTCAGTAGATAATCACA and ARR12 REV: ATACCATGTAACAACGACGAACC

for *ARR1* (At3g16857): ARR1 FWD: TTTGGTTACCCAGAAGAGATTCA and ARR1 REV: AGCTCGAACCCAAGAAACAA

for *NIT3* (AT3G44320): NIT3 FWD: CGAAGTTGGTGTTGTTTCCC and NIT3 REV: GCCAACTCAGCCAATCTTTC

for *NIT4* (AT5G22300): NIT4 FWD: GCACTTGAGGGTGGATGTTT and NIT4 REV: GCTCTCTTCTGAACCGGAAA

for *YUC6* (AT5G25620): YUC6 FWD: AGGTCCACTCGAGCTCAAAA and YUC6 REV: CCTTCTTATCCCCGAACACA

for *ASB1* (At1g25220): ASB1 FWD: GGATTCGTAAGAGGGTTAACGA and ASB1 REV: GACAATGGCGGCTTCTACAT

for *ACTIN2* (At3g18780): ACT FWD: GACCAGCTCTTCCATCGAGAA and ACT REV: CAAACGAGGGCTGGAACAAG

PCR amplification was carried out in the presence of the double-stranded DNA specific dye SYBR Green (Quantace). Amplification was monitored in real time with a 7300 Real Time PCR System (Applied Biosystems). Amplification of *ACTIN2* served as control. Data are expressed either in Log_2 ratio or $2^{-\Delta\Delta ct}$ value. Three technical replicates of qRT-PCR were performed on two independent RNA batches. Results were comparable in all experiments. Student's t-test was performed to assess the significance of the difference between each sample and the control sample.

Chromatin immunoprecipitation followed *ASB1*-specific quantitative real-time PCR (ChIP-qRT-PCR)

ChIP was conducted dissecting Col-0, as our control, or *pSCR::SCR::GFP/scr-4* 5 days old roots which included longitudinal sections comprised of 3-5 cell lengths from the QC, as described in the main Materials and Methods section. Enrichment of the *ASB1* putative target promoter-region DNA was determined using RT-qPCR. A qPCR efficiency of 2-fold amplifications per cycle was assumed, and sequences from ubiquitin 10 (UBQ10-F: GGC CTT GTA TAA TCC CTG ATG AAT AAG; UBQ10-R: AAA GAG ATA ACA GGA ACG GAA ACA TAG T) were used to normalize the results between samples. Tiling along the At1g25220 ASB1 was done using the following sets of adjacent specific amplified regions along the putative *ASB1* promoter. In ascending order upstream from the ASB1 ATG:

97 - 248 bp: ASB1#1-F: TTGACTAGTCCTGCGAATGG; ASB1#1-R: GTGCGTTTTGTGGAACATGA

202 – 332 bp: ASB1#2-F: TCGGGCTAAAACAAGACTGG; ASB1#2-R: TGTCTAAAATGGCGTTTGGA

554 - 693 bp: ASB1#3-F: TGGCCATAGACCGCCTAATA; ASB1#3-R: TCTCAATTCCGGTTGATGTG

737 - 861 bp: ASB1#4-F: TGTACGAGCCATCTTCGTCA; ASB1#4-R: TTTTTGGTATGTGTTTGTTTGGTC

861 - 993 bp: ASB1#5-F: CACATGAAAATCAGTTAAAGCACA; ASB1#5-R: TGACGAAGATGGCTCGTACA

1066 - 1231 bp: ASB1#6-F: AATTTTGCAGCCCAATTCAG; ASB1#6-R: ATGCAAACTATTGCCGGAGA

1503 – 1676 bp: ASB1#7-F: AGATGTTTCGATTATTTCAAATGC; ASB1#7-R: CAACTCCCTTGCGTTTGTCT

1778 - 1963 bp: ASB1#8-F: CGCGAAAATCCTACCTTCAG; ASB1#8-R: ATGGTGACCCACCACAATTT

SUPPLEMENTAL REFERENCES

Clough, S.J. and Bent, A.F. (1998). Floral dip: A simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J. *16*, 735–743.

Edlund, A., Eklöf, S., Sundberg, B., Moritz, T. and Sandberg, G. (1995). A Microscale Technique for Gas Chromatography-Mass Spectrometry Measurements of Picogram Amounts of Indole-3-Acetic Acid in Plant Tissues. Plant Physiol. *108*, 1043–1047.

Mähönen, A.P., Higuchi, M., Törmäkangas, K., Miyawaki, K., Pischke, M.S., Sussman, M.R., Helariutta, Y., and Kakimoto, T. (2006). Cytokinins regulate a bidirectional phosphorelay network in Arabidopsis. Curr. Biol. *6*, 1116-1122.