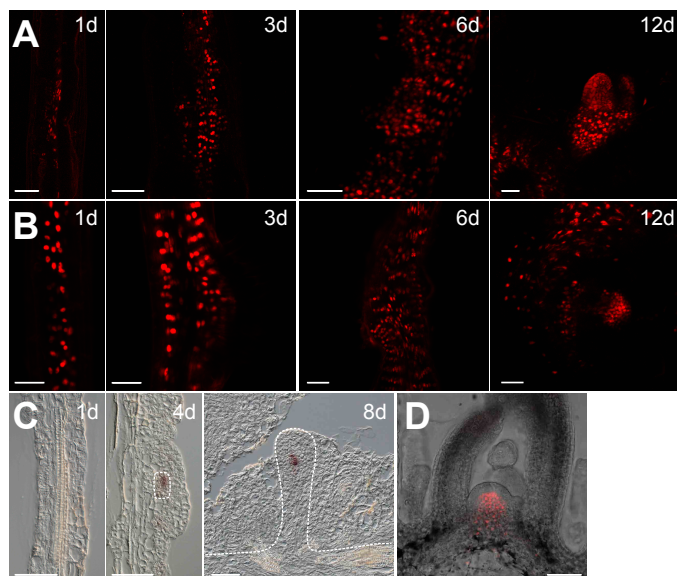


Supplemental Figure 1. Shoot Regeneration in *clv3*, *stm* and *cuc2 cuc3*. (Supports Figure 1).

The regenerative capacity in (A) was calculated as the number of shoots/total number of explants. Data are expressed as mean ± s.d.. $n=24$; p-value: Student's *t* test; ** $p < 0.01$. Bar = 0.5 cm.

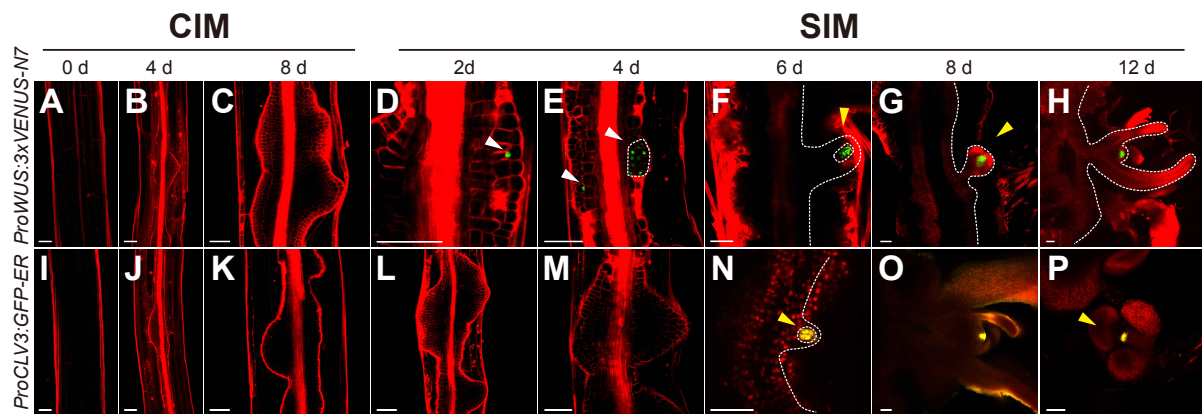


Supplemental Figure 2. Comparison of the Expression Patterns of *WUS* Reporters. (Supports Figure 1).

(A) and (D) The expression pattern of *ProWUS:dsRED-N7* (Gordon et al., 2007) reporter during shoot regeneration **(A)** and in the SAM **(D)**. The hypocotyls of *ProWUS:dsRED-N7* were used as explants. During shoot regeneration **(A)**, WUS^+ cells (red) were visible after 1 day transfer to SIM and did not mark shoot progenitor cells. The difference between the mRNA accumulation pattern revealed by our *in situ* hybridization (Figures 1D to 1I) and protein localization revealed by live imaging analyses of Gordon's *ProWUS:dsRED-N7* reporter is not caused by the movement of DsRED because DsRED was fused to the N7 nuclear localization sequence. We speculate that the difference between our and Gordon's *WUS* reporter was due to the 35S promoter in the binary construct pPZP222 used by Gordon et al (Gordon et al., 2007). However, this reporter does mimic endogenous *WUS* expression pattern in the SAM **(D)**. Bar = 50 μm.

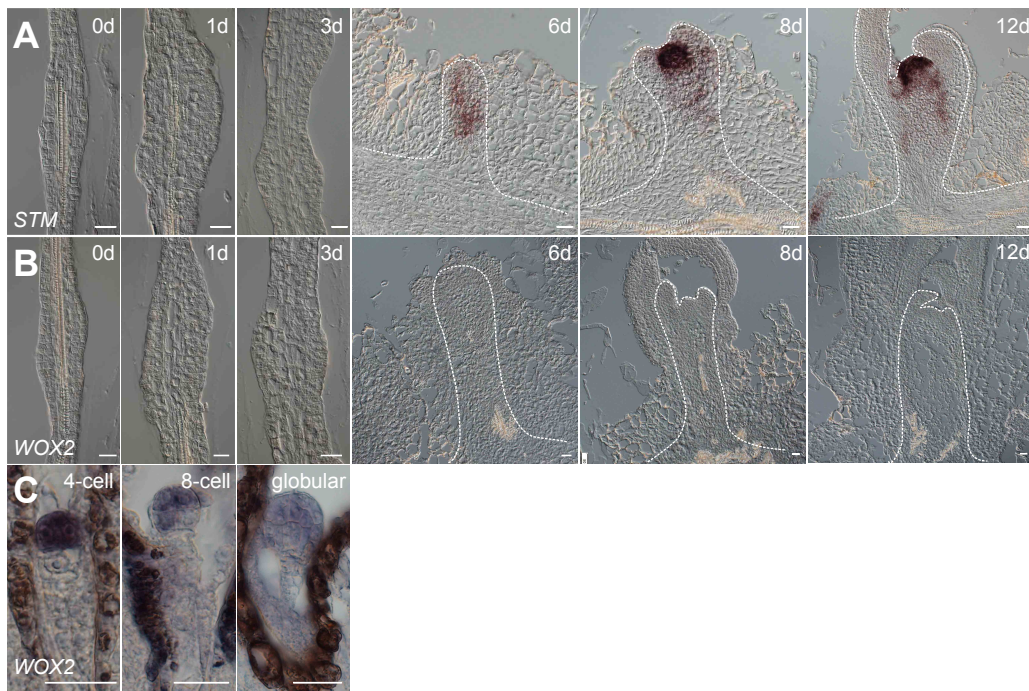
(B) The expression pattern of *ProWUS:dsRED-N7* (Gordon et al., 2007) reporter on SIM. The roots were used as explants. Bar = 50 μm.

(C) RNA *in situ* hybridization analyses. The roots of *ProWUS:dsRED-N7* were used as explants. Note when comparing **(B)** and **(C)**, it appears that *ProWUS:dsRED-N7* reporter does not mimic the *WUS* mRNA expression pattern. Bar = 50 μm.



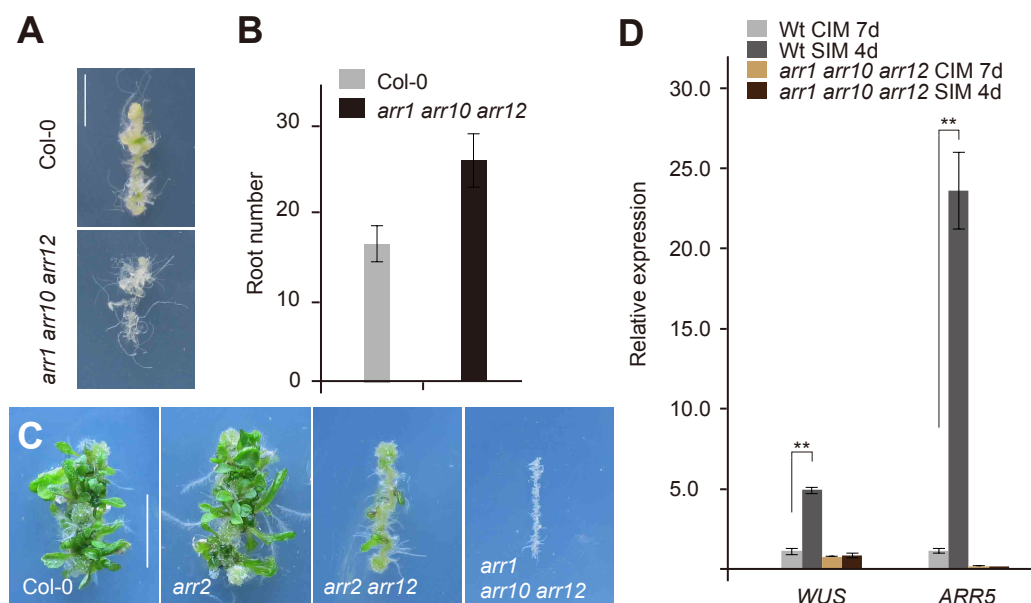
Supplemental Figure 3. Dynamic Expression Patterns of *WUS* and *CLV3* during Shoot Regeneration. (Supports Figure 1).

Hypocotyls from *ProWUS:3xVENUS-N7* (A) to (H) and *ProCLV3:GFP-ER* (I) to (P) were used for shoot regeneration. White and yellow arrows indicate *WUS*⁺ cell and the developing SAM respectively. Cell outlines were stained by propidium iodide (PI, red). Bar = 50 μ m.



Supplemental Figure 4. Expression Patterns of *STM* and *WOX2*. (Supports Figure 1).

(A) Expression of *STM* is detectable beginning at stage III during shoot regeneration. Bar = 20 μm.
(B) and **(C)** The transcripts of *WOX2* (blue) could be detected in the proembryo during embryogenesis **(C)** but not in calli during shoot *de novo* regeneration **(B)**. Bar = 20 μm.

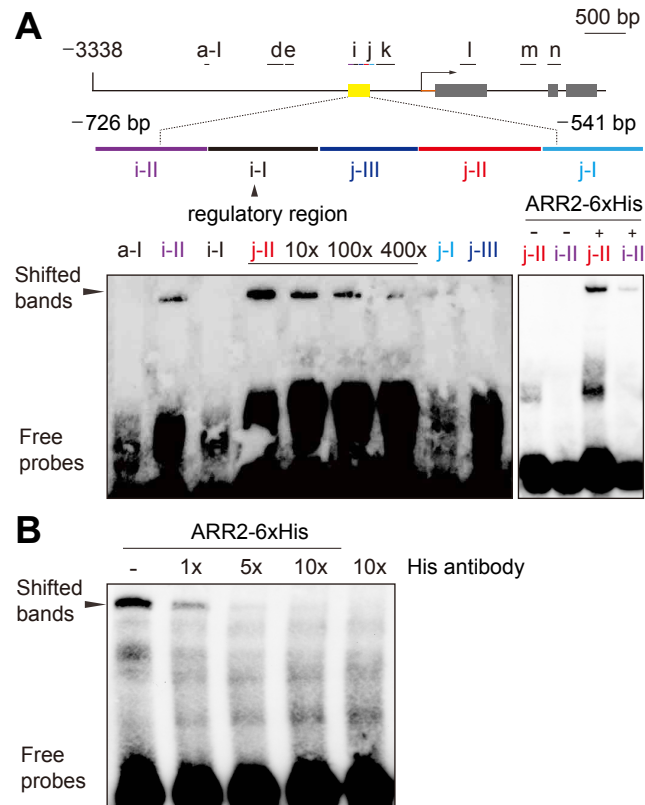


Supplemental Figure 5. Regeneration Assay in the *arr* Mutant. (Supports Figure 2).

(A) and (B) The hypocotyls of wild type and *arr1 arr10 arr12* mutant were used for the root regeneration assay. Note that the loss of function of *B-ARRs* did not affect root regenerative capacity. The regenerative capacity in (B) was calculated as the number of rooted explants/total number of explants. Data are expressed as mean \pm s.d.. $n=32$; Bar = 0.5 cm.

(C) Shoot regeneration of wild type (Col-0), *arr2*, *arr2 arr12* and *arr1 arr10 arr12*. While *arr2* and *arr12* single mutant regenerated shoots normally (Mason et al., 2005), *arr2 arr12* double mutant showed reduced shoot regenerative capacity. Bar = 0.5 cm.

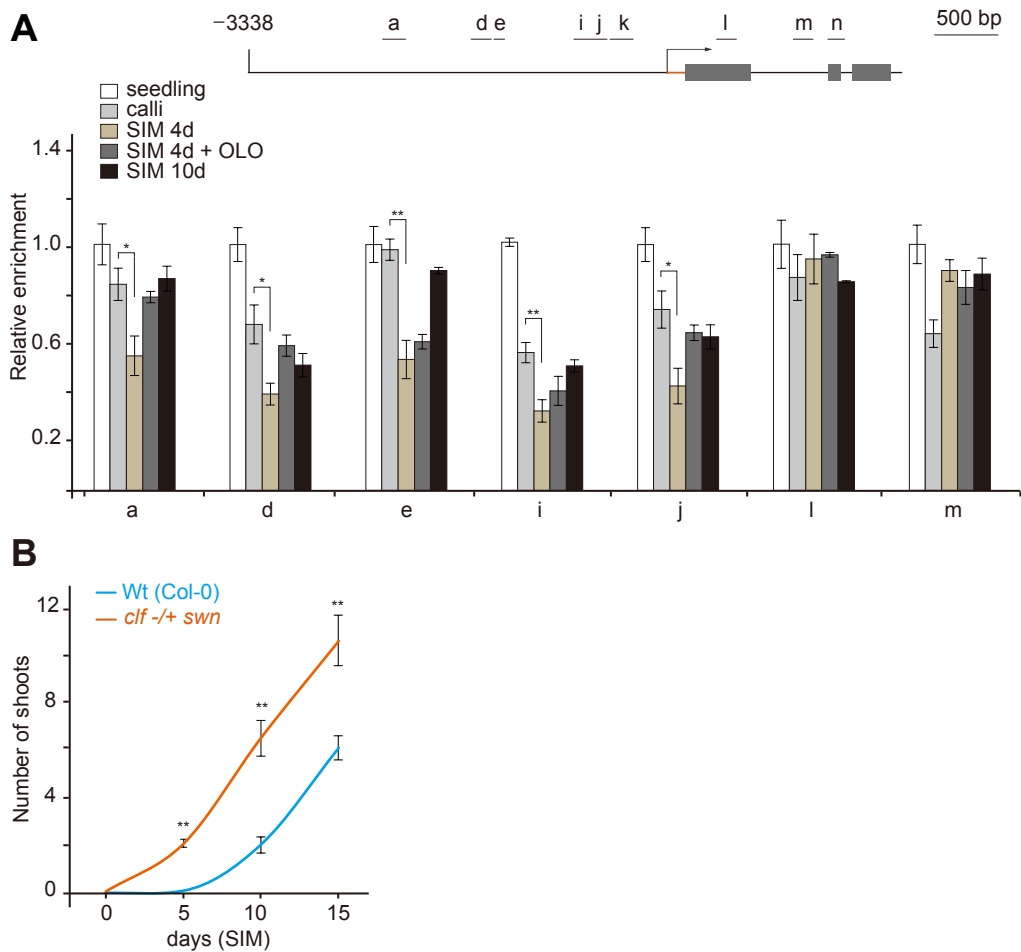
(D) The expression level of *WUS* and *ARR5* in the wild-type and *arr1 arr10 arr12* explants. The expression of *WUS* and *ARR5* in wild type was normalized to that of *TUB*. Data are means \pm s.d.. $n=3$. Student's *t* test; ** $p<0.01$.



Supplemental Figure 6. EMSA Assays. (Supports Figure 2).

(A) EMSA assays. Competitive EMSA showing binding of ARR2 to two DNA fragments [-550 to -620 bp (j-II) and -700 to -760 bp (i-II)] of the *WUS* promoter (left panel). Relative amounts (labeled oligonucleotide was set to 1.0) of the un-labeled competitive oligonucleotide used in the reactions are indicated on the top. The positions of probes (a-l, i-l, i-II, j-l, j-II, j-III) are labeled with different colors. Shifted bands are indicated. The D5 region (-726 to -541 bp) and a 57-bp regulatory region (-712 to -655 bp, i-l) identified by Baurle and Laux are shown (Baurle and Laux, 2005). As a control, we performed the EMSA assays without (-) or with (+) ARR2 protein (right panel). Note that there is no shifted band for probe j-II and i-II in the absence of ARR2 protein.

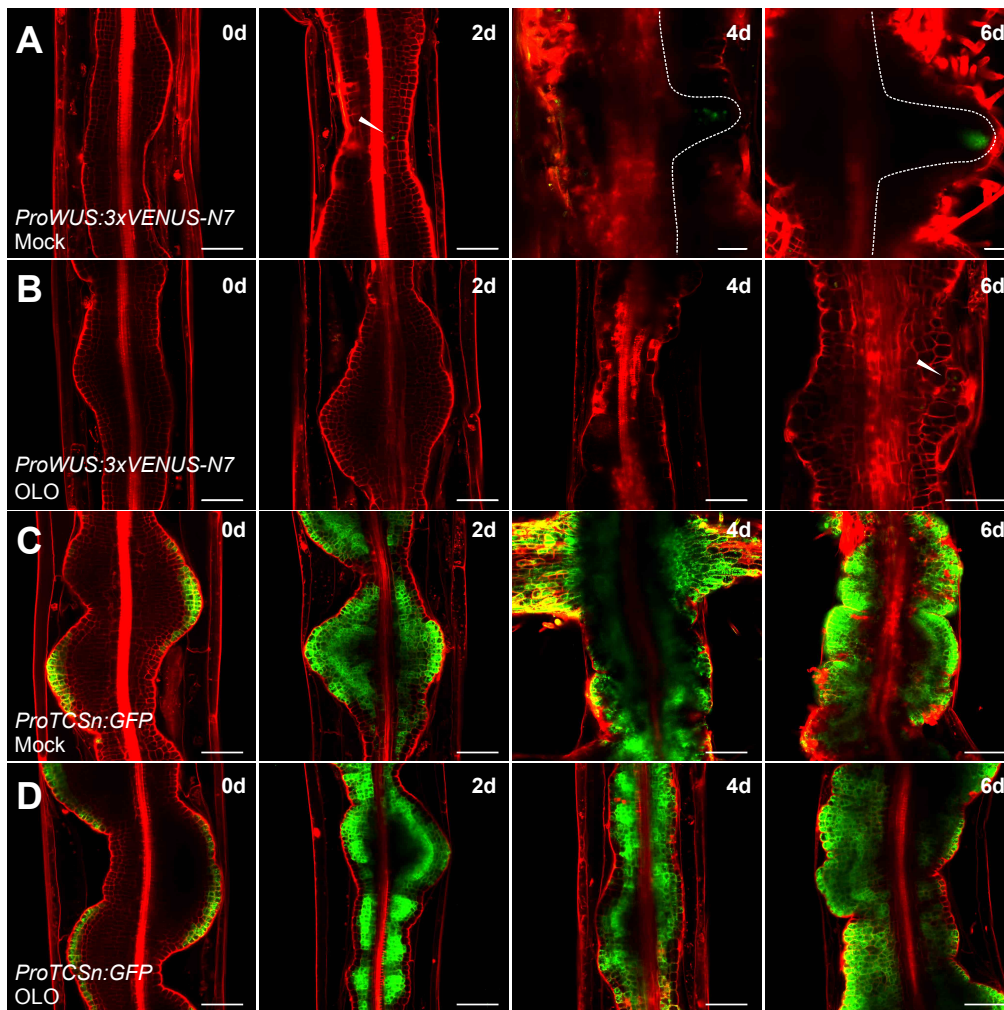
(B) Supershift assays of j-II segment. The purified ARR2 proteins were mixed with different amounts of His antibody. The binding of His antibody with ARR2-6xHis proteins caused the decreased amounts of j-II shifted band.



Supplemental Figure 7. The Progressive Decrease in H3K27me3 Marks at the *WUS* Locus is Delayed by OLO Treatment. (Supports Figure 3).

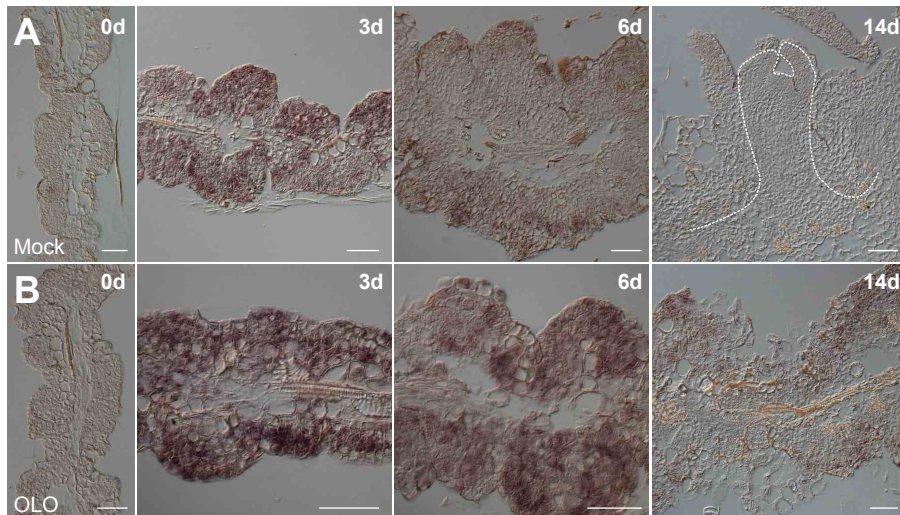
(A) ChIP analyses. Wild-type seedlings and explants cultured on SIM with or without OLO were used. Eight fragments (a to n) were analyzed. Error bars represent s.e.m. ($n=3$ biological replicates); p-value: Student's t test; * $p<0.05$, ** $p<0.01$.

(B) Shoot regeneration of the wild-type (Col-0) and *swn clf +/-* explants. Student's t test; ** $p<0.01$, $n=18$.



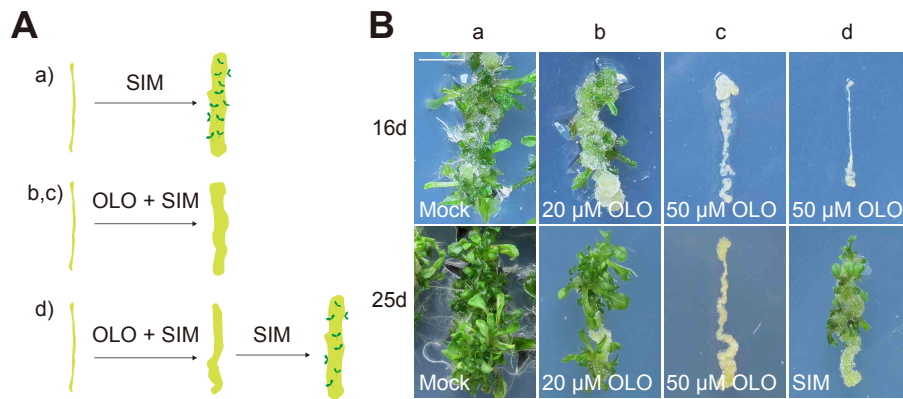
Supplemental Figure 8. The Induction of *WUS* by Cytokinin is Delayed by OLO Treatment. (Supports Figure 3).

(A) to (D) The *ProWUS:3xVENUS-N7* and *ProTCSn:GFP* explants were regenerated on SIM with (B) and (D) or without OLO (A) and (C). White arrows indicate *WUS*⁺ cells. Cell outlines were stained by PI (red). Bar = 50 μm.



Supplemental Figure 9. The Induction of *ARR5* by Cytokinin is not Delayed by OLO Treatment. (Supports Figure 3).

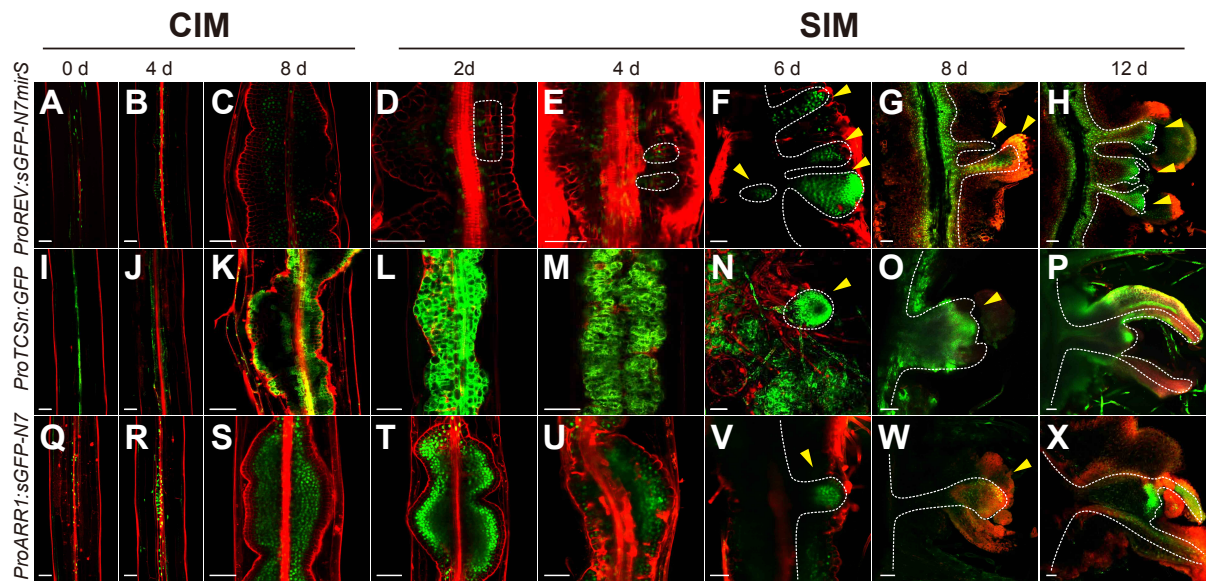
(A) and (B) The expression of *ARR5*. The wild-type explants were regenerated on SIM with (B) or without OLO (A). The expression of *ARR5* was analyzed at day 0, 3, 6 and 14. Bar = 50 μ m.



Supplemental Figure 10. The Induction of Shoot Regeneration by Cytokinin is Delayed by OLO Treatment. (Supports Figure 3).

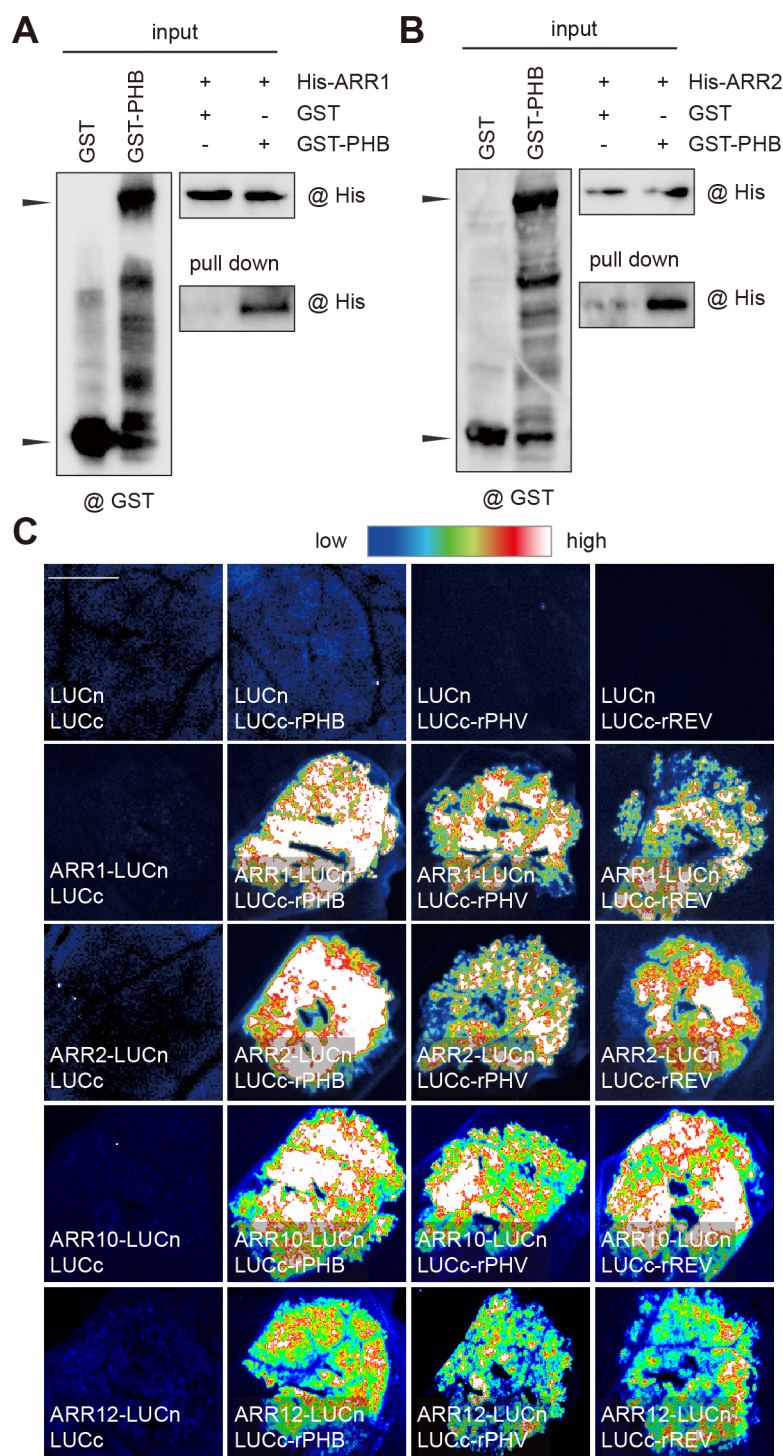
(A) Set-up for the experiment to test the role of cell cycle in shoot regeneration. Four experiments (a-d) were designed.

(B) Shoot regeneration assays according to the experimental designs **(A)**. Bar = 0.5 cm.



Supplemental Figure 11. Dynamic Expression Patterns of *REV*, *TCS* and *ARR1* during Shoot Regeneration. (Supports Figures 2, 4 and 7).

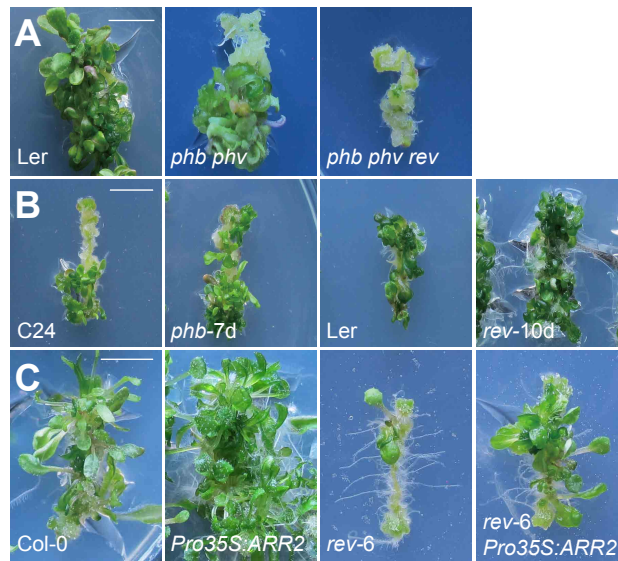
The hypocotyls from *ProREV:sGFP-N7mirS* (**A**) to (**H**), *ProTCSn:GFP* (**I**) to (**P**) and *ProARR1:sGFP-N7* (**Q**) to (**X**) were used for shoot regeneration. Yellow arrows indicate the developing SAM. Cell outlines were stained by propidium iodide (PI, red). Bar = 50 μ m.



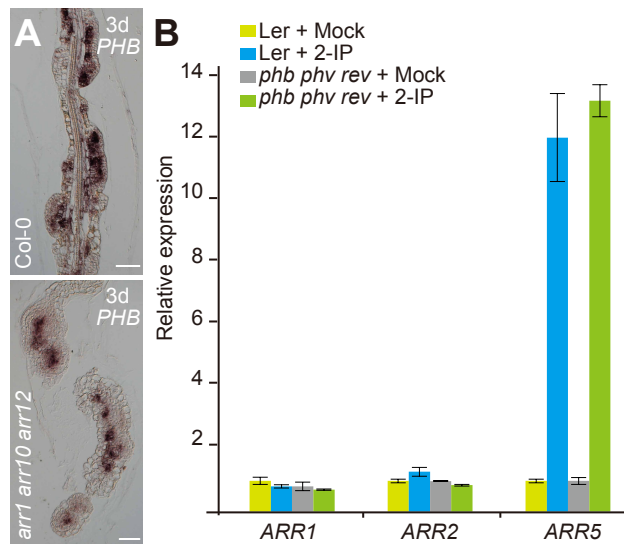
Supplemental Figure 12. ARR1, ARR2, ARR10 and ARR12 Bind to HD-ZIP III Proteins. (Supports Figure 4).

(A) and (B) *In vitro* pull-down assay. 6xHis-ARR1, 6xHis-ARR2 and GST-PHB were expressed in *E. coli*. Purified proteins were mixed and immunoprecipitated with glutathione sepharose 4B resins and blotted against anti-His or anti-GST antibody.

(C) BiLC assay in *N. benthamiana* leaves. ARR1, ARR2, ARR10 and ARR12 were fused to the amino-terminal domain of LUC (LUCn) and PHB/PHV/REV fused to the carboxyl-terminal domain of LUC (LUCc). Bar = 1.0 cm.



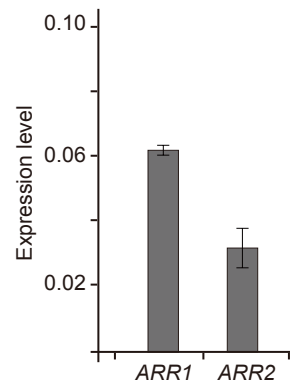
Supplemental Figure 13. Shoot Regeneration Assay of *HD-ZIP III* Mutants. (Supports Figure 4).
(A) and **(B)** Shoot regeneration assay of *HD-ZIP III* mutants. Bar = 0.5 cm.
(C) Shoot regeneration assay of Col-0, *Pro35S:ARR2*, *rev-6* and *Pro35S:ARR2 rev-6*. Bar = 0.5 cm.



Supplemental Figure 14. Expression of HD-ZIP III Transcription Factors and B-type ARRs. (Supports Figure 4).

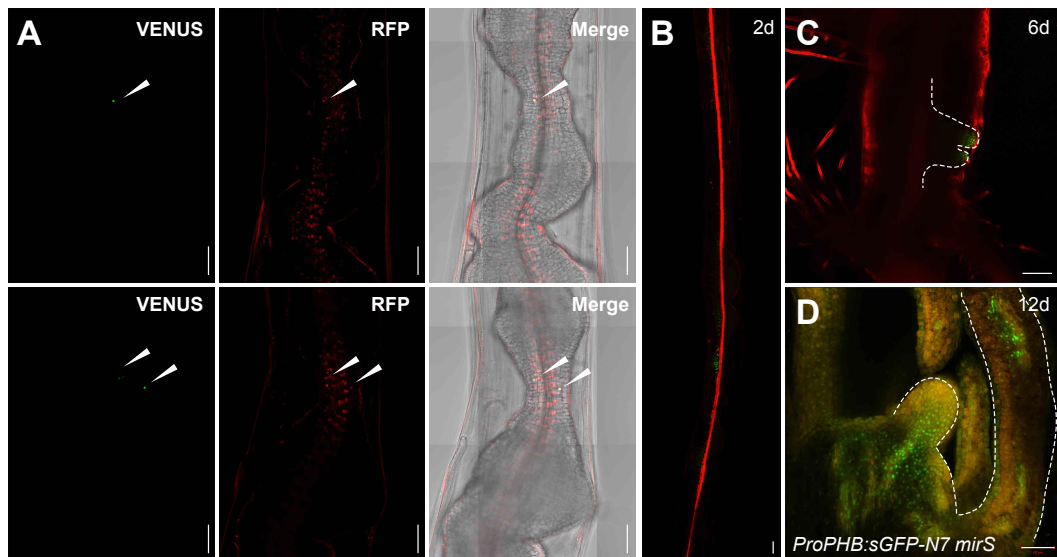
(A) Expression of *PHB* in the wild-type and *arr1 arr10 arr12* explants. The explants were cultured on SIM for 3 days. Bar = 50 μ m.

(B) Expression of A-type and B-type ARRs in wild type and *phb phv rev* explants. Expression was examined by qRT-PCR and normalized to that of *TUB*. Data are means \pm s.d.. $n=3$.



Supplemental Figure 15. Expression of *ARR1* and *ARR2* in Wild-Type Explants. (Supports Figure 6).

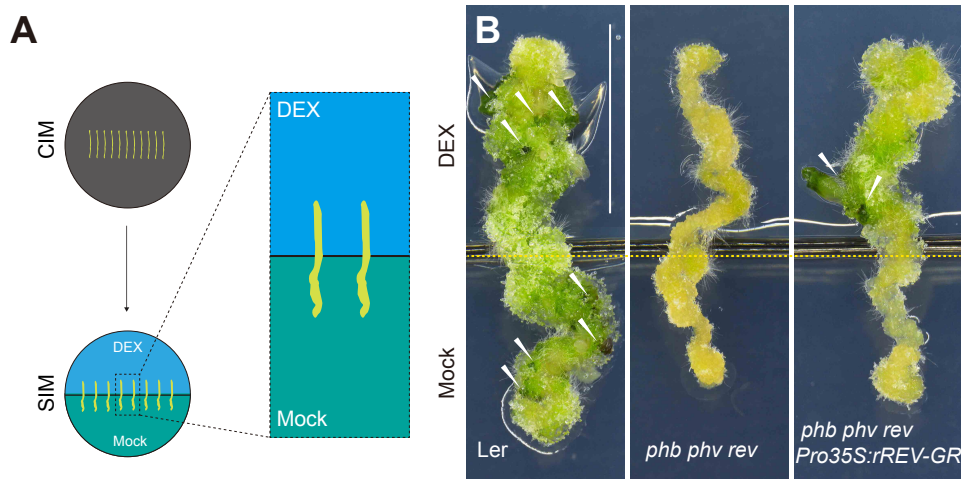
Expression was examined by qRT-PCR and normalized to that of *TUB*. Data are means \pm s.d.. $n=3$.



Supplemental Figure 16. Spatial Activation of *WUS* by *REV* and *PHB*. (Supports Figure 7).

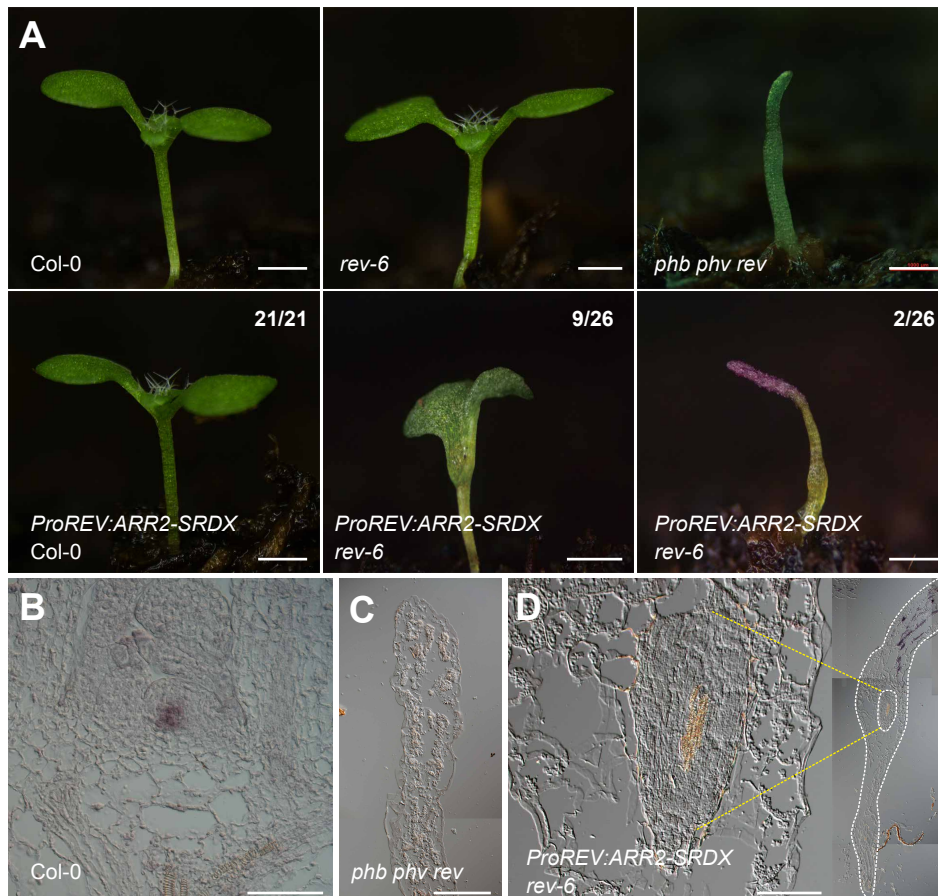
(A) Expression of *ProREV:DsRED-N7 mirS ProWUS:3xVENUS-N7*. The explant was cultured on SIM for 2 days. The *WUS*⁺ cell (green) is indicated by white arrows. Bar = 50 μm.

(B) to (D) Expression of *ProPHB:sGFP-N7 mirS* (green). The explants were culture on SIM for 2, 6 and 12 days. The expression domain of *PHB* is marked with dashed lines. Bar = 50 μm.



Supplemental Figure 17. Local Induction of *HD-ZIP III* Promotes Shoot Regeneration on SIM. (Supports Figures 4 and 7).

(A) and **(B)** Shoot regeneration assay of wild type (Ler), *phb phv rev* and *phb phv rev Pro35S:rREV-GR*. **(A)** Schematic diagram shows the experimental procedure for local induction of *HD-ZIP III*. A biplate (Thermo fisher, PB5220E) which allows two separate media formulations was used. **(B)** Explants of different genotypes were cultured on SIM with or without 10 μ M DEX for 10 days. White arrows indicate regenerated shoots. Bar = 0.5 cm.



Supplemental Figure 18. Genetic Interaction between *ARR2* and *REV* during Shoot Development. (Supports Figures 4 and 7).

(A) Phenotypes of wild type and mutants. Bar = 1 cm. The proportion of transgenic plants showing phenotype is shown.

(B) to (D) Expression of *WUS* in wild type and mutants. The *WUS* transcripts were detected by *in situ* hybridization assay. Bar = 50 μ m.

Supplemental Table 1. Yeast Two-Hybrid Assay using ARR2 as Bait.

| Gene | Interaction? | Biological Function |
|-------------------------|---------------------|---|
| <i>PHB</i> (At2g34710) | Yes | HD-ZIP III transcription factor, defines adaxial leaf fates (Prigge et al., 2005) |
| <i>REV</i> (At5g60690) | Yes | HD-ZIP III transcription factor, defines adaxial leaf fates (Prigge et al., 2005) |
| <i>PHV</i> (At1g30490) | Yes | HD-ZIP III transcription factor, defines adaxial leaf fates (Prigge et al., 2005) |
| <i>CUC2</i> (At5g53950) | No | Transcription factor of the <i>NAC</i> gene family, required for the embryonic SAM (Daimon et al., 2003) |
| <i>CUC3</i> (At1g76420) | No | Transcription factor of the <i>NAC</i> gene family, required for embryonic apical meristem formation (Daimon et al., 2003; Vroemen et al., 2003; Hibara et al., 2006) |
| <i>TCP4</i> (At3g15030) | No | Target gene of miR319, regulate leaf differentiation (Efroni et al., 2013) |
| <i>ESR1</i> (At1g12980) | No | ERF/AP2 transcription factor, regulates shoot regeneration and meristem activity (Banno et al., 2001) |
| <i>MP</i> (At1g19850) | No | Auxin response factor, play roles in the development of shoot primordia (Zhao et al., 2010) |
| <i>BRC1</i> (At3g18550) | No | TCP transcription factor, functions in axillary bud development (Aguilar-Martinez et al., 2007) |
| <i>TFL2</i> (At5g17690) | No | TFL2 recognizes specifically H3K27me3 <i>in vivo</i> (Turck et al., 2007) |
| <i>LCR</i> (At1g27340) | No | F-box protein, involved in the regulation of leaf morphology and meristem activity (Knauer et al., 2013) |

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