Spatially selective hormonal control of RAP2.6L and ANAC071 transcription factors involved in tissue reunion in *Arabidopsis*.

Masashi ASAHINA[a,b,c,*], Katsuya AZUMA[b,*], Weerasak PITAKSARINGKARN[b], Takashi YAMAZAKI[b], Nobutaka MITSUDA[d], Masaru OHME-TAKAGI[d], Shinjiro YAMAGUCHI[c], Yuji KAMIYA[c], Kiyotaka OKADA[e], Takeshi NISHIMURA[f], Tomokazu KOSHIBA[f], Takao YOKOTA[a], Hiroshi KAMADA[b], Shinobu SATOH[b,†]

[a]Department of Biosciences, Teikyo University, Utsunomiya, Tochigi, 320-8551, Japan.
[b]Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki, 305-8572, Japan.
[c]RIKEN Plant Science Center, Yokohama, Kanagawa, 230-0045, Japan.
[d]Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki, 305-8562 Japan.
[e]National Institute for Basic Biology. Okazaki, Aichi, 444-8585, Japan.
[f]Department of Biological Sciences, Tokyo Metropolitan University, Hachioji, Tokyo, 192-0397, Japan.

Corresponding author: Shinobu Satoh. Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki, 305-8572, Japan.
Tel; +81-298-53-4871, Fax; +81-298-53-4579 <satoh.shinobu.ga@u.tsukuba.ac.jp>

Classification: BIOLOGICAL SCIENCES: Plant Biology

Number of text pages: 16
Number of figures: 5
Number of tables: 0

Abstract

When grafting or wounding disconnects stem tissues, new tissues are generated to restore the lost connection. In this study, the molecular mechanism of such healing was elucidated in injured stems of *Arabidopsis*. Soon after the inflorescence stems were incised, the pith cells started to divide. This process was strongly inhibited by the elimination of cauline leaves, shoot apices, or lateral buds that reduced the indole-3-acetic acid supply. Microarray and quantitative RT-PCR analyses revealed that genes related to cell division, phytohormones, and transcription factors were expressed due to incision. Among them, two plant-specific transcription factor genes, *ANAC071* and *RAP2.6L*, were abundantly expressed. *ANAC071* was expressed at 1-3 days after cutting exclusively in the upper region of the cut gap with concomitant accumulation of indole-3-acetic acid. In contrast, *RAP2.6L* was expressed at 1 day after cutting exclusively in the lower region with concomitant deprivation of indole-3-acetic acid. The expression of *ANAC071* and *RAP2.6L* were also promoted by ethylene and jasmonic acid, respectively. In transformants suppressing the function of *RAP2.6L* or *ANAC071*, the division of pith cells was inhibited. Furthermore, the ethylene signaling-defective *ein2* mutant showed incomplete healing. Hence, plant-specific transcription factors differentially expressed around the cut position were essential for tissue reunion of *Arabidopsis* wounded flowering stems, and were under opposite control by polar-transported auxin, with modification by the ethylene and jasmonic acid wound-inducible hormones.

Introduction

Functionally specialized leaves, flowers, and roots of vascular plants are integrated via stems to enable plant responses to changing environments (1). Plant stems provide essential mechanical support of the plant body and deliver nutrients as well as physiological chemical information through vascular bundles (1). Auxin is required to establish the vertical axis and is produced in shoot apices and transported to roots through stele parenchyma or procambial cells with auxin-specific transporters (2). Biotic or biophysical plant environmental stresses often result in wounding that may disintegrate and endanger plant tissues. Cellular responses to wounding involve reconstruction of damaged tissues and also activation of the synthesis of defense-related proteins, including basic pathogenesis-related (PR) proteins as well as wound hormones such as ethylene and jasmonic acid (JA) (3). During reconstruction of damaged tissues, vascular and/or other cells are transformed so as to physiologically connect the existing tissues (4, 5). Such activities have long been observed in grafting techniques, which are advantageous for agriculture and horticulture (6) and also for basic research on systemic physiological events such as flower induction (7) and hormone actions (8, 9). Low grafting efficiency and tissue incompatibility are often encountered, necessitating an understanding of the healing mechanism underlying grafting. In previous studies on grafting as well as repairing of wounded tissues, much effort has focused on understanding the regeneration of vascular elements, demonstrating the involvement of phytohormones such as auxin and cytokinin, and several NAC (NAM, ATAF1, 2 and CUC2) transcription factors (TFs) (4, 10).

Processes reuniting ground tissues such as the cortex and pith in injured regions comprise cell division and successive cell differentiation, although the underlying molecular mechanisms have been less studied than vascular regeneration. Active pectin biosynthesis occurs in cucumber and tomato hypocotyls, and gibberellin (GA), likely produced in and exported from cotyledons, is utilized for cell division during cortical tissue reunion (11-13). Such regeneration ability has been studied in terms of totipotency in tissue culture, suggesting that TFs are required for regulating gene expression related to regeneration (14, 15). However, the TFs and accompanying molecular processes involved in reuniting ground tissues remain to be determined.

This research aims to understand the molecular events during the tissue-reunion process in plant stems. To this end, a tissue-reunion inducible system was established by

incising *Arabidopsis* inflorescence stems. The incised tissues were investigated by microarray analysis followed by expression/functional analysis of the up-regulated genes. Two plant-specific TF genes, *ANAC071* and *RAP2.6L*, with localized expression in the upper and lower regions of the cut gap, respectively, were required for the division of pith cells in the reunion process. Differential controls of these genes by auxin and the wound-related ethylene and JA hormones are also described.

Results

Histological Time-Course of Cell Division during Wounding of the *Arabidopsis* Inflorescence Stems.

Arabidopsis inflorescence stems were used 7-10 days after bolting. The internodes between the first cauline leaves and rosette leaves, which were no longer capable of cell division and elongation, were incised to half-diameter depth with a microsurgical knife (Fig. S1*A* and *B*).

The pith cells around the cut site then began to randomly divide and elongate intrusively toward the cut surface (Fig. 1*A-D*). To obtain an estimate of cell division activity in the wounded stems, quantitative RT (QRT) -PCR analyses were performed using mitotic cyclin-encoding *cyclin B1;1* (At4g37490), a mitosis marker gene expressed only around the G2/M transition (16) (Fig. 1*E*). The cyclin gene was clearly up-regulated in the wounded region 3 days after cutting, where cell division was initiated in both pith and cortical cells neighboring the wounded regions (Fig. 1*B* and *E*), as was previously observed in wounded cucumber hypocotyls (11). Spatiotemporal histochemical changes in mitotic activity in response to tissue reunion were pursued using the *cyclin B1;1* promoter (designated as *pcyclin B*)::*GUS* transgenic plants (Fig. 1*F-I*). The GUS activity culminated 3 days after cutting (Fig. 1*G*), in agreement with both QRT-PCR analysis (Fig. 1*E*) and morphological observations (Fig. 1*A-D*). The GUS activity was localized in the pith, vascular tissues, and cortex in both the upper and lower regions of the cut gap, although the intensity was much higher in the upper region (Fig. 1*G*).

Roles of Auxin in Tissue Recovery in Incised *Arabidopsis* Stems.

The level of auxin was histochemically analyzed using artificial auxin-responsive promoter (*DR5*)::*GUS* transgenic plants (Fig. 1*J-M*). Strong GUS activity occurred at

1–5 days after cutting with higher intensity in the upper region (Fig. 1*J-L*), indicating that auxin accumulation may have occurred in the upper region immediately after cutting, lasting for 5 days and diminishing and then tissue reunion completed within almost 7 days (Fig. 1*L* and *M*). Auxin is derived from either cauline leaves, shoot apices, or lateral buds. Removal of both the shoot apex and lateral buds or only the cauline leaves resulted in minor inhibitory effects (Fig. S1*E* and *F*), but simultaneous removal of all three severely retarded cell division and *pcyclin B*::GUS activity in the pith tissue (Fig. S1*D* and *H*). Involvement of auxin in this phenomenon was substantiated by the fact that 10^{-3} M indole-3-acetic acid (IAA) application to the top of the decapitated stem nullified the inhibitory effect of decapitation (Fig. 2*E*). Furthermore, the healing processes in the wound tissue were also inhibited by the application of 10^{-3} M 2,3,5-triiodobenzoic acid (TIBA), an inhibitor for polar auxin transport, to the upper part of the cut gap (Fig. 2*B*). Similar effects were also observed in the polar auxin transport mutant *pin1-1* (17) (Fig. 2*C*). In contrast, cortical cell division was activated without regard to the removal of the auxin-delivering tissues (Fig. S1*C-J*).

Finally, the auxin level in the wound tissues was simultaneously measured by evaluating the expression of *IAA5*, an early auxin-inducible gene. Compared to the control, the IAA level was not altered in the upper region but was decreased by 50% in the lower region 1 day after cutting (Fig. 2*F*). Simultaneously, the *IAA5* gene was strongly up-regulated exclusively in the upper region, and then strongly reduced, possibly by negative feedback (Fig. S2*A*).

Microarray Analysis of the Tissue-Reunion Process and Gene Expression Profiles of *ANAC071* and *RAP2.6L*.

Explants shorter than 5 mm with or without nicks were collected from the same internodes 1, 3, and 5 days after cutting and subjected to an oligonucleotide-based microarray to determine what genes initiate and regulate the tissue-reunion process (Fig. S3 and Dataset S1). Table S1 shows selected genes with expression patterns that correlate with tissue reunion.

The microarray analysis led us to focus on the expression of TFs that were highly up-regulated at an early stage. Two plant-specific TF genes were selected, *ANAC071* (*Arabidopsis* NAC domain containing protein 71; At4g17980) encoding NAC domain-TF, and *RAP2.6L* (At5g13330) encoding an ERF/AP2 -TF (Table S1, Fig. 3*A*

and *B*). *ANAC071* belongs to the NAC domain-TF family known to include 105 predicted NAC proteins in *Arabidopsis* (18), which are involved in the formation of organ primordia and other biological functions including defense responses (19). *RAP2.6L* belongs to the *APETALA2* (AP2)/ERBP family, which is one of the large families of TFs in *Arabidopsis* involved in many different developmental processes and environmental response events (20, 21). The family is composed of 144 members in *Arabidopsis* and has been divided into five subfamilies: the AP-2 subfamily, RAV subfamily, DREB (A) subfamily, ERF (ethylene response factor; B) subfamily, and others (22).

The *ANAC071* transcript was highly expressed 1–3 days after cutting, and the transcript was much intense in the upper cut region (Fig. 3*A*). Intensive expression of *ANAC071* in the upper region was also observed in *pANAC071*::*GUS* transgenic plants, the promoter activity was predominantly observed in pith and vascular tissue (Fig. S2*B*). The transcript level was drastically reduced after decapitation, but was restored by IAA application (Fig. 3*C*).

The *RAP2.6L* transcript was transiently highly expressed 1 day after cutting, with exclusive expression in the lower region (Fig. 3*B*). The *RAP2.6L* transcript was scarce in the non-cut stem (Fig. 3*B*). Decapitation strongly enhanced the level of the *RAP2.6L* transcript in the cut and non-cut stems, and this effect was nullified by IAA application (Fig. 3*D*).

Histological Analysis of Transformants Defective in *ANAC071* and *RAP2.6L*.

In general, plant TFs constitute a large family that shares highly conserved DNA-binding domains, resulting in much redundancy. Although gene-suppressing transformants are difficult to obtain for such genes (23), this can be overcome by using *C*himeric *RE*pressor *S*ilencing *T*echnology (CRES-T) (24). To determine the roles of *ANAC071* and *RAP2.6L* in cell division, the gene-suppressing transformants of these genes, *ANAC071*-SRDX and *RAP2.6L*-SRDX, were prepared using the CRES-T method (Fig. S4*A* and *B*). In *ANAC071*-SRDX, cell division was strongly inhibited but cell elongation was enhanced around the lesion area, resulting in incomplete tissue reunion (Fig. 3*E* and *F* and S4*C* and *D*). In *RAP2.6L*-SRDX, moderate inhibition was observed (Fig. 3*E* and *G* and S4*E* and *F*). Transformants with the plant expression vector alone showed normal reunion.

Involvement of Ethylene in Tissue Reunion and Gene Expression of *RAP2.6L* and *ANAC071*.

QRT-PCR analyses revealed that stem cutting promoted the expression of the aminocyclopropane carboxylic acid (ACC) synthase gene, *ACS2* (At1g01480), shortly (1 day) after cutting in both the lower and upper regions (Fig. 4*A*). Likewise, nearly equal promoter activity was observed in the upper and lower regions in the *pACS2*::*GUS* transgenic plants 1 day after cutting (Fig. S5*A*). ACS2 is one of the ACC synthases encoded by a multi-gene family in many plant species and produced in response to various environmental stimuli (25). Decapitation suppressed *ACS2* transcript expression, although such suppression was not restored by exogenous IAA (Fig. S6*A*). It is likely that the *ACS2* gene is spatially regulated by either IAA or injury.

To determine if ethylene was involved in tissue reunion, the ethylene-insensitive mutant *ein2* was investigated. Five days after cutting the *ein2* stem, cell division was observed only in the cortex neighboring the cut and not in the pith, unlike in the wild-type stem (Fig. S5*B*-*D*). Complete reunion was not observed up to 7 days after cutting the *ein2* stem (Fig. 4*B*). *ANAC071* expression in the *ein2* cut was significantly lower than in the wild type (Fig. 4*C*), indicating that *ANAC071* was up-regulated by ethylene. In contrast, *RAP2.6L* expression level was ~2 times higher in the *ein2* than in wild type 1 day after cutting (Fig. 4*D*), indicating that *RAP2.6L* was down-regulated by ethylene.

Regulation of *RAP2.6L* by Jasmonic Acid.

Microarray and QRT-PCR analyses showed that some jasmonic acid (JA) biosynthesis genes were up-regulated during the tissue-reunion process. *LOX2* (encoding lipoxygenase 2 (At3g45140), a JA biosynthesis enzyme) was selected because this gene was exclusively expressed in wounded tissue (Fig. 5*A*) and is known to be wound-inducible (26). *LOX2* expression peaked 1 day after stem cutting, with higher expression in the lower cut region (Fig. 5*A*) and a superimposable expression profile with *RAP2.6L* (Fig. 3*B*). *RAP2.6L* was up-regulated by JA methyl ester upon administration to the intact flowering stem (Fig. 5*B*), in accord with the AtGenExpress microarray database (27).

Discussion

The Recovery of Wounded Inflorescence Stems in *Arabidopsis* is Different from that of Cucumber and Tomato Hypocotyls.

As found in our earlier work (11), injured cucumber or tomato hypocotyls never healed when cotyledons were removed. This was due to GA depletion caused by removal of the GA-supplying cotyledons (11, 12). Exogenous GA, but not IAA, can replace the cotyledons. Hypocotyls are embryonic organs consisting of the epidermis, cortex, endodermis, and vascular tissues, but lacking a pith. Its growth requires import of nutrients and hormones from cotyledons because of its juvenility. In cucumber and tomato hypocotyls, injury recovery is accompanied by cell division and elongation mainly in the cortical cells, which characteristically have pectin-rich thick cell walls.

Prior to the present study, no involvement of GA was found in wound healing because the wounded stems of the GA-deficient *Arabidopsis* mutant *ga3ox1*/*ga3ox2* recovered with no difficulty. The tissue-reunion mechanism likely changes as developmental stages proceed. *Arabidopsis* inflorescence stem internodes analyzed in this study had no dividing cells and consisted of the epidermis, cortex, endodermis, vascular tissues, and a pith occupying much of the stem center. In the stems, cell proliferation–based reunion of the wounded area proceeded mainly in the pith, in contrast to the events in hypocotyls (Fig. 1). Involvement of auxin in healing the injured stems was substantiated by the fact that auxin-deprived stems, due to TIBA treatment or *pin1-1* mutation, were not capable of wound healing. Interestingly, cortical cell division occurred in both the upper and lower cut stem regions and was not affected by decapitation, auxin transport inhibition (Fig. 2), or GA biosynthesis inhibition, or in GA-deficient mutants.

The Upper Cut Region of Wounded Inflorescence Stems was Healed by *ANAC071*.

Microarray analysis followed by QRT-PCR of wounded stems revealed two TF genes, *ANAC071*and *RAP2.6L*, that were strongly up-regulated immediately after artificial wounding. Furthermore, *ANAC071*and *RAP2.6L* were spatially selectively expressed in the upper and lower cut regions, respectively (Fig. 3*A* and 3*B*). The requirement of these TFs for pith cell division in tissue reunion was indicated by analyzing TF gene-suppressing transformants (Fig. 3*E*-3*G* and S4).

ANAC071 was most likely induced by auxin because auxin accumulated in the upper cut region and *ANAC071* was not present in the auxin-poor lower region. Furthermore,

this finding is supported by a previous report that *ANAC071*-TF and related NAM (no apical meristem)-like TFs are up-regulated in auxin-rich callus induction medium (28, 29). Furthermore, auxin and microRNAs may regulate the spatial patterns of the transcription of some NAC family genes including *CUC1* and *CUC 2* (30). A group of microRNAs was also predicted to target the *Populus* homolog of *ANAC071* (31). The biological functions of NAC-TFs remain to be determined, but several NAC-TFs may be involved in shoot meristem formation, organ boundary specification, and secondary wall thickening (10, 32, 33), together with abscisic acid-mediated stress responses against drought and high salinity (18-19, 34).

Ethylene, a wound-inducible hormone, was also an important factor activating *ANAC071* because *ANAC071*-TF expression was significantly reduced in the *ein2* mutant (Fig. 4*C*), which had unusual reunion morphology (Fig. 4*B*). ACC, the direct precursor of ethylene, is synthesized by ACSs in response to biotic or abiotic stimuli, and the ACSs are rate-limiting enzymes in ethylene production. Although numerous *ACS* genes are known, *ACS2* was specifically up-regulated in both the upper and lower cut regions in this study (Fig. 4*A*). Decapitation decreased *ACS2* expression in the *Arabidopsis* flowering stem, although this negative effect was not fully compensated after IAA application (Fig. S6*A*). Taken together, the results suggest that auxin activated *ANAC071* and ethylene enhanced *ANAC071* expression in the auxin-rich upper cut region, inducing pith cell division during tissue reunion (Fig. S7).

The Lower Cut Region of Wounded Inflorescence Stems was Healed by *RAP2.6L*-TF.

The *RAP2.6L* gene is thought to regulate genes involved in meristem maintenance during shoot regeneration, because T-DNA knockdown of *RAP2.6L* reduced the frequency of shoot development from root explants and hampered the expression of shoot meristem-specific genes (28, 29). In the present study, *RAP2.6L* gene expression appeared in the lower cut region, which had a lower IAA level due to blocked polar auxin transport. It is likely that *RAP2.6L* was up-regulated in response to wounding in a region below the cut where auxin concentrations were low. Furthermore, *RAP2.6L* was not expressed in the IAA-rich upper cut region, indicating that it was down-regulated by IAA. In support of this conclusion, Che et al. (2006, 2007) clearly showed that *RAP2.6L* is up-regulated in cytokinin-rich shoot-generating media for *Arabidopsis* root explants,

but not in auxin-rich root-inducing media (28, 29).

LOX2 was also thought to be involved in injury healing because it was up-regulated in the lower cut region immediately after cutting (Fig. 5*A*). *LOX2* is a lipoxygenase participating in JA synthesis that converts linolenic acid to its hydroperoxide. The plant hormone JA is a key regulator of plant responses to environmental stresses and biotic challenges (3). *RAP2.6L*-TF expression was enhanced upon application of the JA derivative methyl jasmonate to the flowering stems (Fig. 5*B*). However, decapitation with or without IAA application did not affect the expression of *LOX2* during the reunion process (Fig. S6*B*). It is most likely that this gene is activated by wounding independently of IAA. However, it is not known why *LOX2* was not expressed in the upper cut region. On the other hand, *RAP2.6L* seems to be down-regulated by ethylene because its expression was increased in the *ein2* mutant (Fig. 4*D*). In conclusion, in the lower cut region, *RAP2.6L* was activated due to auxin depletion and this activation process was positively regulated by JA but negatively regulated by ethylene, resulting in the net incremental increase in *RAP2.6L* expression (Fig. S7), facilitating pith cell division during tissue reunion.

Materials and Methods

Arabidopsis thaliana seeds were germinated and grown in artificial soil under continuous white fluorescent light. After 7-10 days of bolting, stem internodes between the first or second cauline leaves and rosette leaves were cut through half of their diameter with a micro-surgical knife (Fig. S1*A* and *B*) and the plant was then grown for an additional 14 days. Detailed experimental procedures are described in the supporting information Materials and Methods.

Footnotes

* M.A. and K. A. contributed equally to this work.
†To whom correspondence may be addressed: satoh.shinobu.ga@u.tsukuba.ac.jp;
tel: +81-298-53-4871; Fax: +81-298-53-4579.

Author contributions: M.A., K. A., and S.S. designed research and analyzed data; M.A., K. A., W.P., and T.Y performed research; N.M., M.O.-T., S.Y., Y.K., K.O., T.N., T.K., T.Y., and H.K. contributed new reagents/analytic tools; M.A., T.Y., and S.S. wrote the paper.

Acknowledgments

We thank Dr. Hiroshi Ezura (University of Tsukuba, Japan) and Dr. Eiji Nambara (University of Toronto, Canada) for the kind gifts of plant materials, Ms. Miho Shimizu (University of Tsukuba) for technical assistance, and Drs. Masao Tasaka and Miyo Terao-Morita (Nara Institute of Science and Technology, Japan) for valuable advice on auxin. This work was supported in part by the Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN) and Grant-in-Aid for Scientific Research on Priority Areas (21027004) to S. S., and by a Research Fellowship from Japan Society for the Promotion of Science for Young Scientists (04J11879 to M.A.) .

References

1. Satoh S (2006) Organic substances in xylem sap delivered to aboveground organs by the roots. *J Plant Res* 119:179-187.
2. Kramer EM, Bennett MJ (2006) Auxin transport: a field in flux. *Trends Plant Sci* 11(8):382-386.
3. Leon J, Rojo E, Sanchez-Serrano JJ (2001) Wound signaling in plants. *J Exp Bot* 52:1-9.
4. Sachs T (2000) Integrating cellular and organismic aspects of vascular differentiation. *Plant Cell Physiol* 41:649-656.
5. Flaishman MA, Loginovsky K, Lev-Yadun S (2003) Regenerative Xylem in Inflorescence Stems of *Arabidopsis thaliana*. *J Plant Growth Regul* 22:253-258.
6. Davis AR et al. (2008) Cucurbit Grafting. *Crit Rev Plant Sci* 27(1):50-74.
7. Kobayashi Y, Weigel D (2007) Move on up, it's time for change-mobile signals controlling photoperiod-dependent flowering. *Genes Dev* 21:2371-2384.
8. Katsumi M, Foard DE, Phinney BO (1983) Evidence for the translocation of gibberellin A3 and gibberellin like substances in graft between normal, *dwarf1* and *dwarf5* seedling of *Zea mays* L. *Plant Cell Physiol* 24: 379-388.
9. Notaguchi M, Daimon Y, Abe M, Araki T (2008) Graft-transmissible action of Arabidopsis FLOWERING LOCUS T protein to promote flowering. *Plant Signal Behav* 4 (2): 123-125.
10. Yamaguchi M, Kubo M, Fukuda H, Demura T (2008) Vascular-related NAC-DOMAIN7 is involved in the differentiation of all types of xylem vessels in *Arabidopsis* roots and shoots. *Plant J* 55, 652-664.
11. Asahina et al (2002) Gibberellin produced in the cotyledon is required for cell division during tissue-reunion in the cortex of cut cucumber and tomato hypocotyls. *Plant Physiol* 129: 201-210.
12. Asahina M et al (2007) Effects of the removal of cotyledons on endogenous gibberellin levels in hypocotyls of young cucumber and tomato seedlings. *Plant Biotech* 24: 99-106.
13. Asahina M, Gocho Y, Kamada H, Satoh S (2006) Involvement of inorganic elements in tissue reunion in the hypocotyl cortex of *Cucumis sativus*. *J Plant Res* 119: 337-342.
14. Zimmerman JL (1993) Somatic Embryogenesis: A Model for Early Development

in Higher Plants. *Plant Cell* 5:1411-1423.

15. Sena G, Wang X, Liu HY, Hofhuis, Birnbaum KD (2009) Organ regeneration does not require a functional stem cell niche in plants. *Nature* 457 (26) 1150-1153.
16. Colón-Carmona A, You R, Haimovitch-Gal T, Doerner P (1999) Spatio-temporal analysis of mitotic activity with a labile cyclin-GUS fusion protein. *Plant J* 20: 503-508.
17. Benkova E et al (2003) Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell* 115 (5): 591-602.
18. Ooka H et al (2003) Comprehensive analysis of NAC family genes in *Oryza sativa* and *Arabidopsis thaliana*. *DNA Res* 10 (6) 239-247.
19. Olsen AN, Ernst HA, Leggio LL, Skriver K (2005) NAC transcription factors: structurally distinct, functionally diverse. *Trends Plant Sci* 10:79-87.
20. Sakuma Y et al (2002) DNA-binding specificity of the ERF/AP2 domain of *Arabidopsis* DREBs, transcription factors involved in dehydration- and cold-inducible gene expression. *Biochem Biophys Res Commun* 25; 290 (3): 998-1009.
21. Iwase et al (2011) The AP2/ERF transcription factor WIND1 controls cell dedifferentiation in Arabidopsis. *Curr Biol*. 22;21(6):508-514.
22. Riechmann JL, Meyerowitz EM (1998) The AP2/EREBP family of plant transcription factors. *Biol Chem* 379 (6): 633-646.
23. Riechmann JL et al (2000) *Arabidopsis* transcription factors: genome-wide comparative analysis among eukaryotes. *Science* 290(5499): 2105-2110.
24. Hiratsu K, Matsui K, Koyama T, Ohme-Takagi M (2003) Dominant repression of target genes by chimeric repressors that include the EAR motif, a repression domain, in *Arabidopsis*. *Plant J* 34 (5): 733-739.
25. Tsuchisaka A, Theologis A (2004) Unique and overlapping expression patterns among the *Arabidopsis* 1-amino-cyclopropane-1-carboxylate synthase gene family members. *Plant Physiol* 136(2): 2982-3000.
26. Suza WP, Staswick PE (2008) The role of JAR1 in Jasmonoyl-L: -isoleucine production during *Arabidopsis* wound response. *Planta* 227(6): 1221-1232.
27. Goda H et al (2008) The AtGenExpress hormone- and chemical-treatment data set: Experimental design, data evaluation, model data analysis, and data access. *Plant J* 55: 526-542.

28. Che P, Lall S, Howell SH (2007) Developmental step in acquiring competence for shoot development in *Arabidopsis* tissue culture. *Planta* 226:1183-1194.
29. Che P, Lall S, Nettleton D, Howell SH (2006) Gene expression programs during shoot, root, and callus development in *Arabidopsis* tissue culture. *Plant Physiol* 141(2): 620-637.
30. Aida M, Tasaka M (2006). Genetic control of shoot organ boundaries. *Curr Opin Plant Biol* 9, 72-77.
31. Barakat A, Wall PK, DiLoreto S, dePamphilis CW, Carlson JE (2007) Conservation and divergence of microRNAs in *Populus*. *BMC Genomics* 8:481.
32. Mitsuda N et al (2005) The NAC transcription factors NST1 and NST2 of Arabidopsis regulate secondary wall thickenings and are required for anther dehiscence. *Plant Cell* 17, 2993-3006.
33. Mitsuda N et al (2007) NAC transcription factors, NST1 and NST3, are key regulators of the formation of secondary walls in woody tissues of Arabidopsis. *Plant Cell* 19, 270-280.
34. Jensen MK et al (2010) The *Arabidopsis thaliana* NAC transcription factor family: structure-function relationships and determinants of ANAC019 stress signaling. *Biochem J* 426(2): 183-196.

Figure Legends

Figure 1. Process of tissue reunion in the wounded flowering stem of *Arabidopsis*. (*A-D*) Light micrographs of the tissue-reunion process. Arrowheads: position of the cut. pi, pith; co, cortex; vb, vascular bundle. (*E*) Expression of *Cyclin B1;1*. Each value is the mean ± S.E. (N = 3). Bars show comparative expression between upper (U; red bar) or lower regions (L; blue bar) of cut stem. The non-cut (N; gray bar) control was arbitrarily set to 1, and the mean ± S.E. is shown (N = 4). (*F-I*) *pCyclin B*::*GUS* plants. (J-M) *DR5*::*GUS* plants. Photographs were taken at 1 day (*A, F, J*), 3 days (*B, G, K*), 5 days (*C, H, L*), and 7 days (*D, I, M*) after cutting. Scale bars: 100 µm (*A-D*), 1 mm (*F-M*).

Figure 2. Roles of auxin on tissue reunion in *Arabidopsis* wounded stems. (*A, B*) Control wild type without decapitation. (*C*) *pin1-1*. (*D, E*) Decapitated wild type. Lanolin paste containing distilled water (D.W.) (*A, D*), 10^{-3} M TIBA (*B*) or 10^{-3} M IAA (*E*) was applied. Arrowheads: position of cut. pi, pith; co, cortex; vb, vascular bundle. Images were taken 7 days after stem cutting. Scale bars: 100 µm. (*F*) Quantification of endogenous IAA in upper (U; red bar), lower (L; blue bar) or non-cut region (N; gray bar). Mean ± S.E. is shown (N = 3).

Figure 3. Gene expression of *ANAC071* and *RAP2.6L* TFs and phenotypes of gene-suppressing transformants. (*A, B*) Gene expression of *ANAC071* (*A*) and *RAP2.6L* (*B*). For time-course analysis, the mean ± S.E. is shown (N = 3). Bars show comparative expression between upper (U; red bar) or lower region (L; blue bar) of wounded stem. The non-cut (N; gray bar) control was arbitrarily set to 1, and mean ± S.E. is shown (N = 4). (*C, D*) Effects of decapitation and IAA application on the expression of *ANAC071* (*C*) or *RAP2.6L* (*D*). Mean ± S.E. is shown (N = 3). *, $P < 0.05$ (Fisher's test). (*E–G*) Representative phenotype of SRDX transgenic plants. (*E*) wild type. (*F*) *ANAC071*-SRDX. (*G*) *RAP2.6L*-SRDX. Images were taken 7 days after cutting. Arrowheads: position of cut. pi, pith; co, cortex; vb, vascular bundle. Scale bars: 100 µm (*E-G*).

Figure 4. Involvement of ethylene in tissue reunion and expression of *RAP2.6L* and *ANAC071*. (*A*) Expression of *ACS2*. For time-course analysis, the mean ± S.E. is shown

(N = 3). Bars show comparative expression between upper (U; red bar) and lower region (L; blue bar) of cut stem. The non-cut (N; gray bar) control was arbitrarily set to 1, and the mean ± S.E. is shown (N = 4). (*B*) Light micrograph of *ein2* cut stem. Images were taken 7 days after cutting. Arrowheads: position of cut. pi, pith; co, cortex; vb, vascular bundle. Scale bars: 100 µm. The image is a composite to form the complete figure. (*C*, *D*) Relative expression of *ANAC071* (*C*) and *RAP2.6L* (*D*) in cut stem (white bar) or non-cut stem (gray bar) of wild type, and cut stem (blue bar) or non-cut stem (black bar) of *ein2*. Mean ± S.D. (N = 2) is shown.

Figure 5. Regulation of *RAP2.6L* expression by jasmonic acid. (*A*) Gene expression of *LOX2*. For time-course analysis, the mean ± S.E. is shown (N = 3). Bars show comparative expression between upper (U; red bar) and lower region (L: blue bar) of cut stem. The non-cut stem control (N; gray bar) was arbitrarily set to 1, and the mean ± S.E. is shown (N = 4). (*B*) Effects of methyl jasmonate (MJ) application on the expression of *RAP2.6L* in the intact flowering stem. The distilled water (D.W.) control was arbitrarily set to 1, and the mean ± S.E. is shown (N = 3).

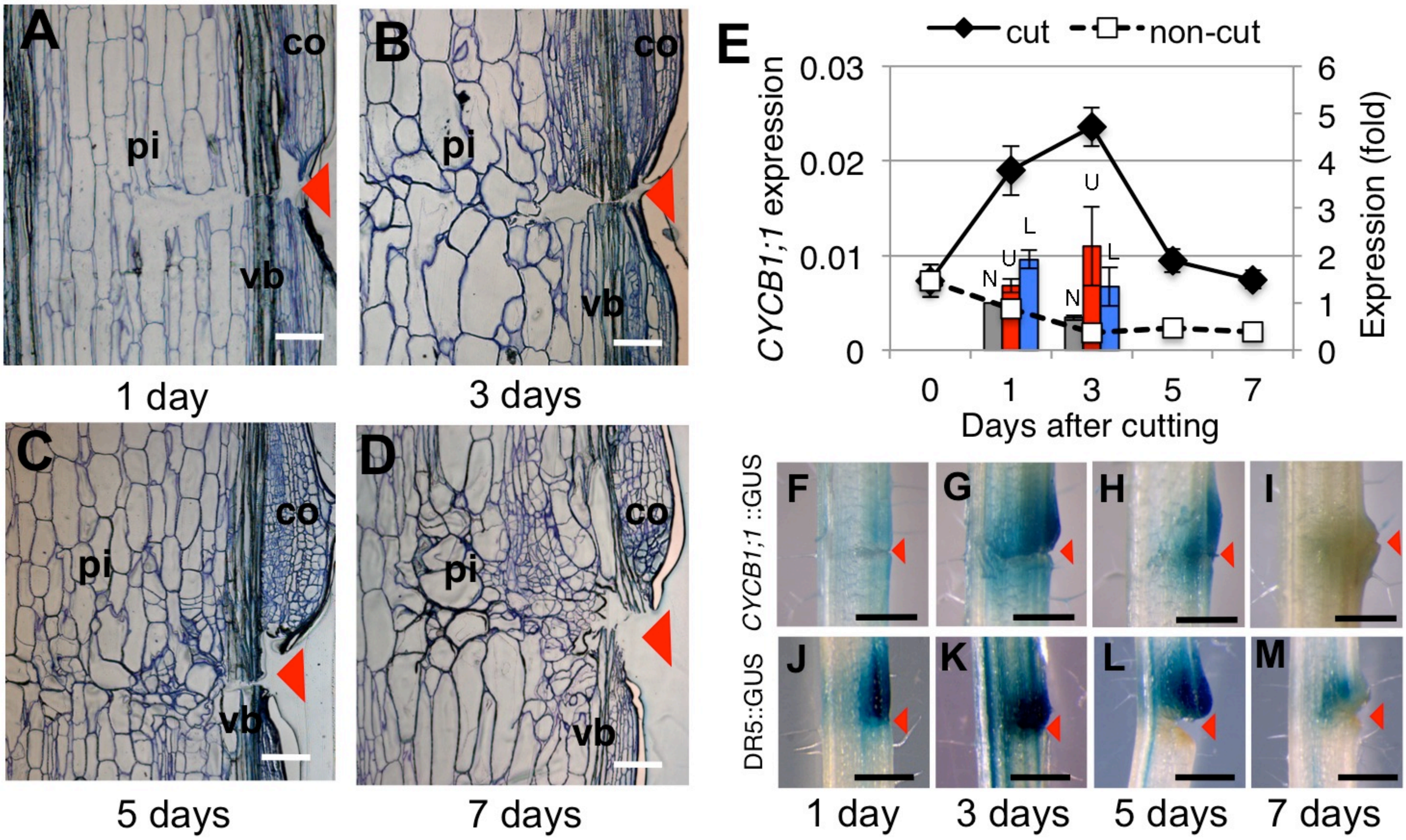
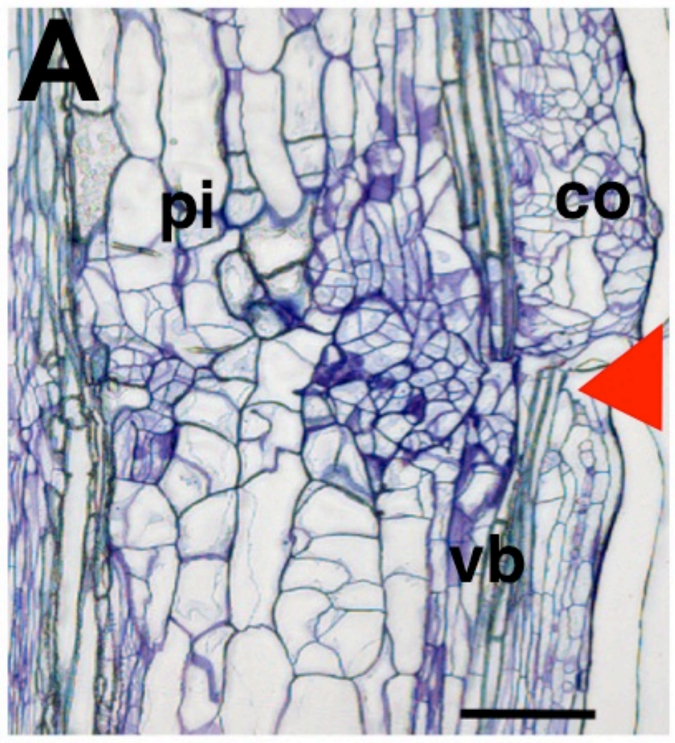
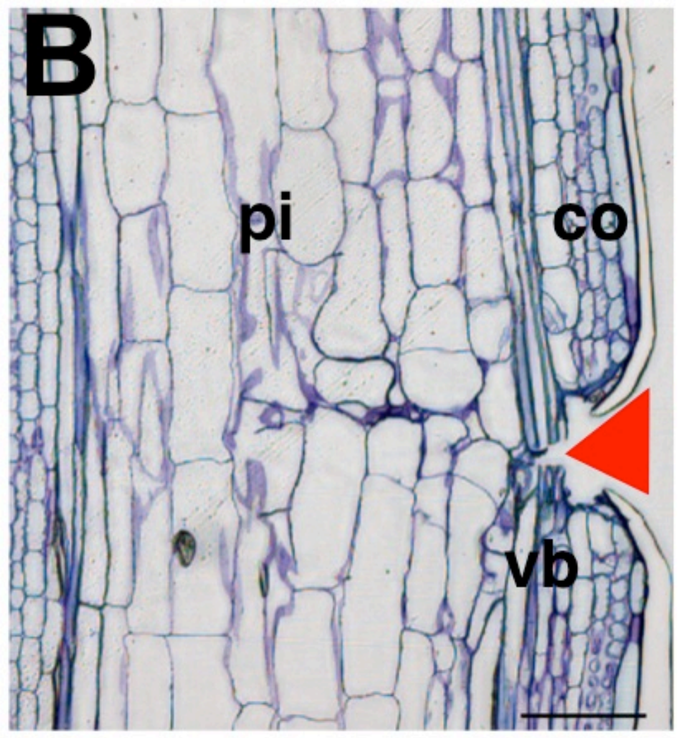

A
co
pi
vb
1 day
B
co
pi
vb
3 days
C
co
pi
vb
5 days
D
co
pi
vb
7 days
E
cut
non-cut
CYCB1;1 expression
0.03
0.02
0.01
0
Expression (fold)
6
5
4
3
2
1
0
0
1
3
5
7
Days after cutting
N
U
L
CYCB1;1 ::GUS
DR5::GUS
F
G
H
I
J
K
L
M
1 day
3 days
5 days
7 days

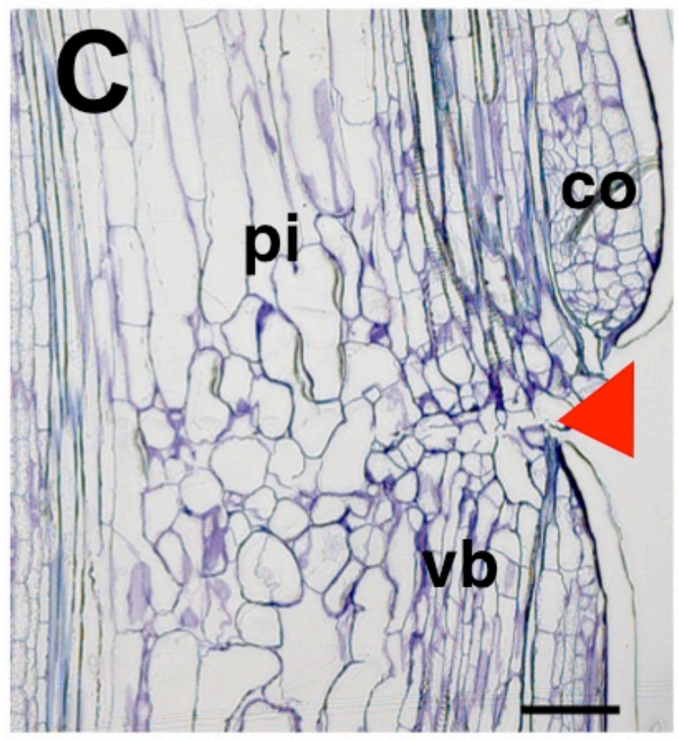
Figure 1



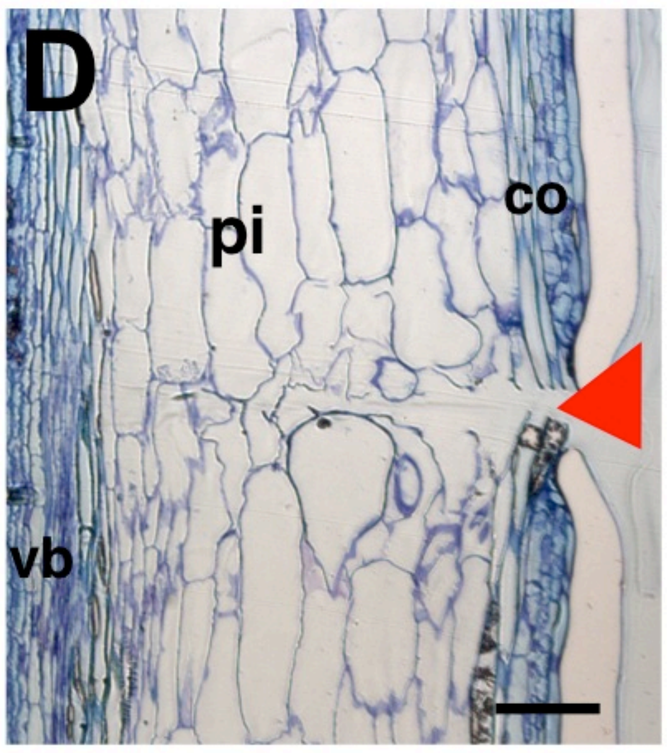
No decapitation



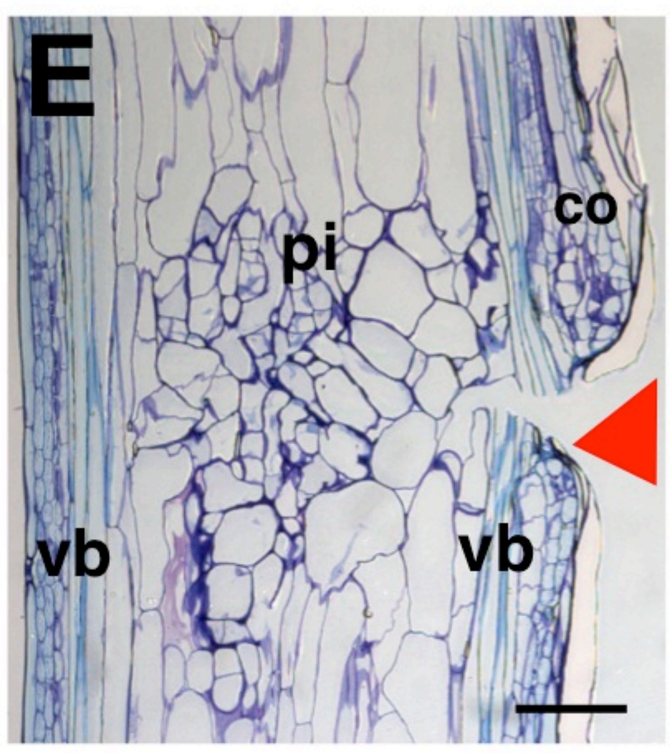
No decapitation
+ TIBA



No decapitation
pin1-1



Decapitation
+ D.W.



Decapitation
+ IAA

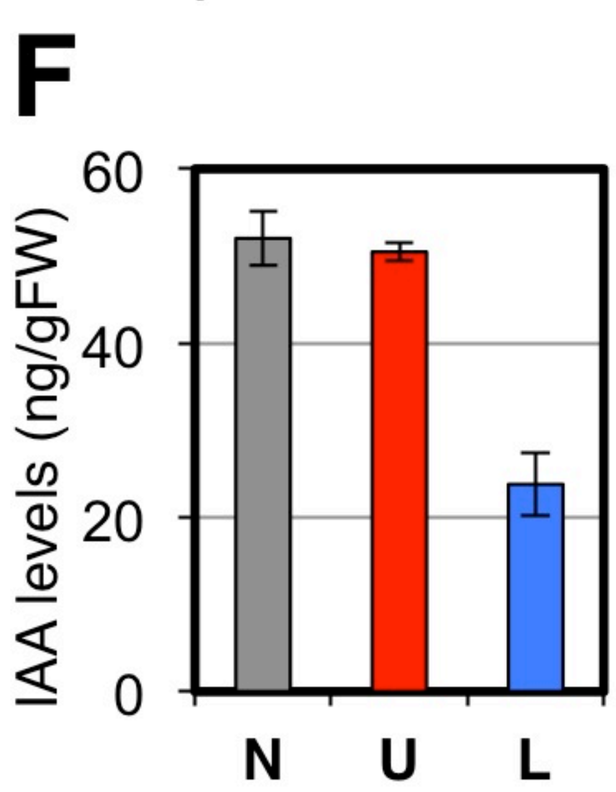
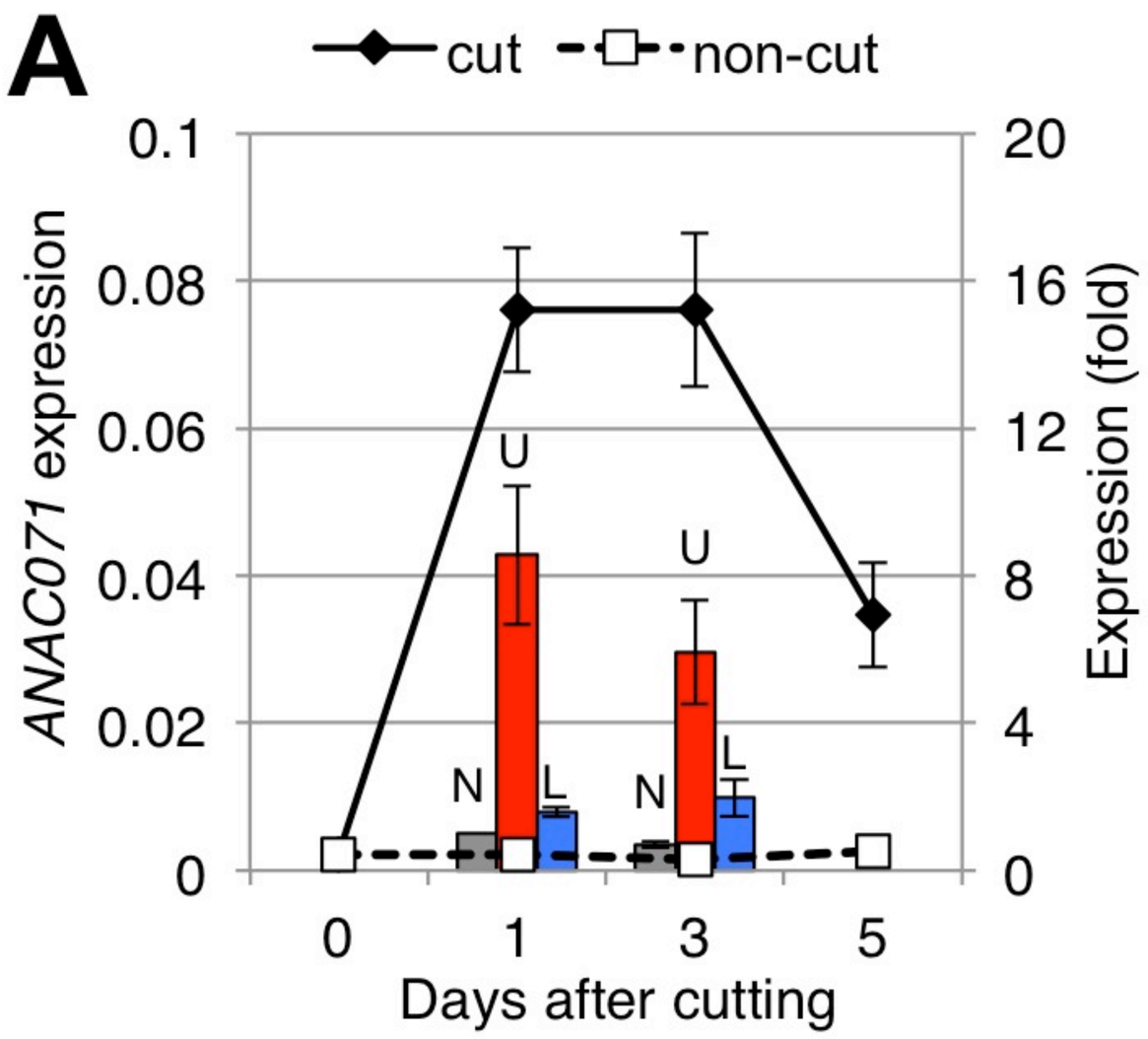
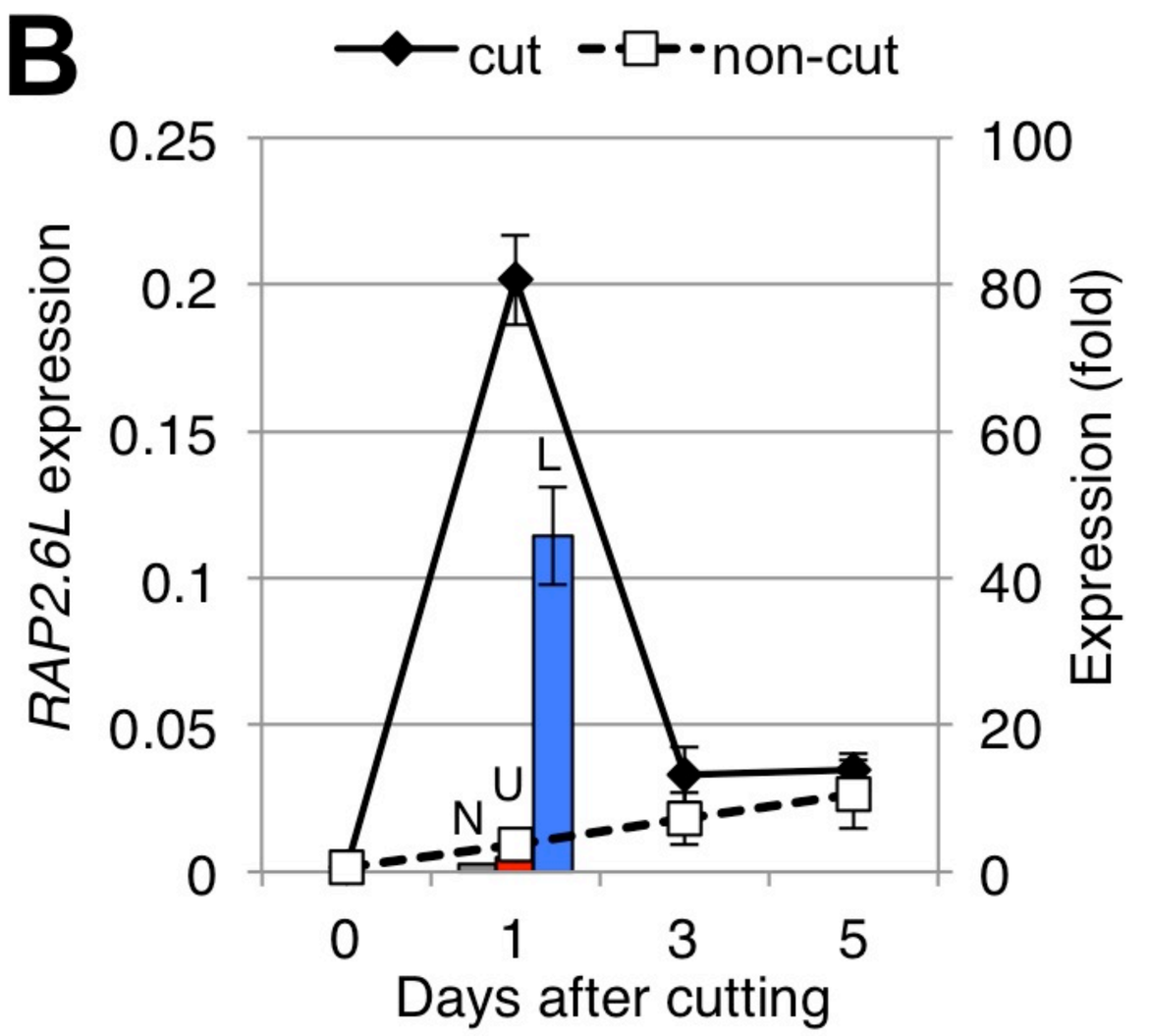
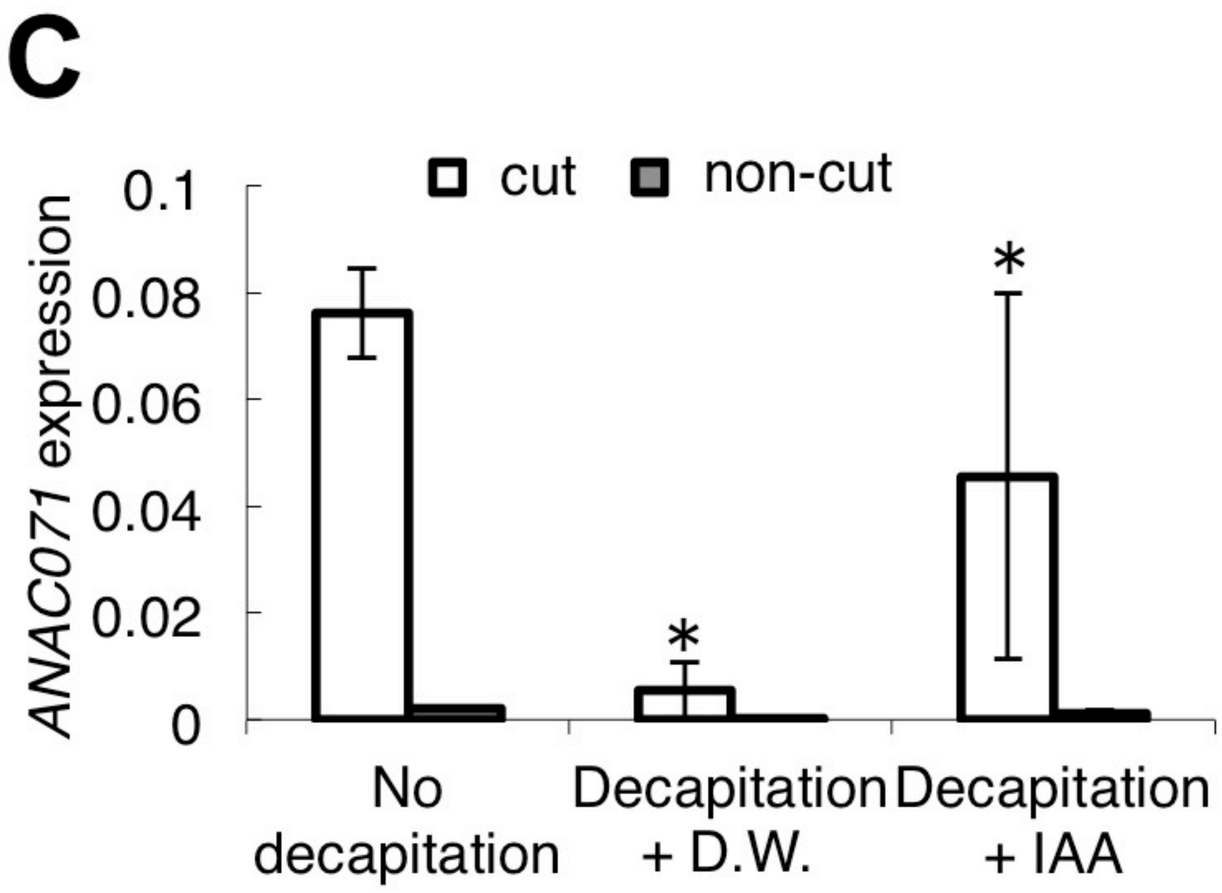
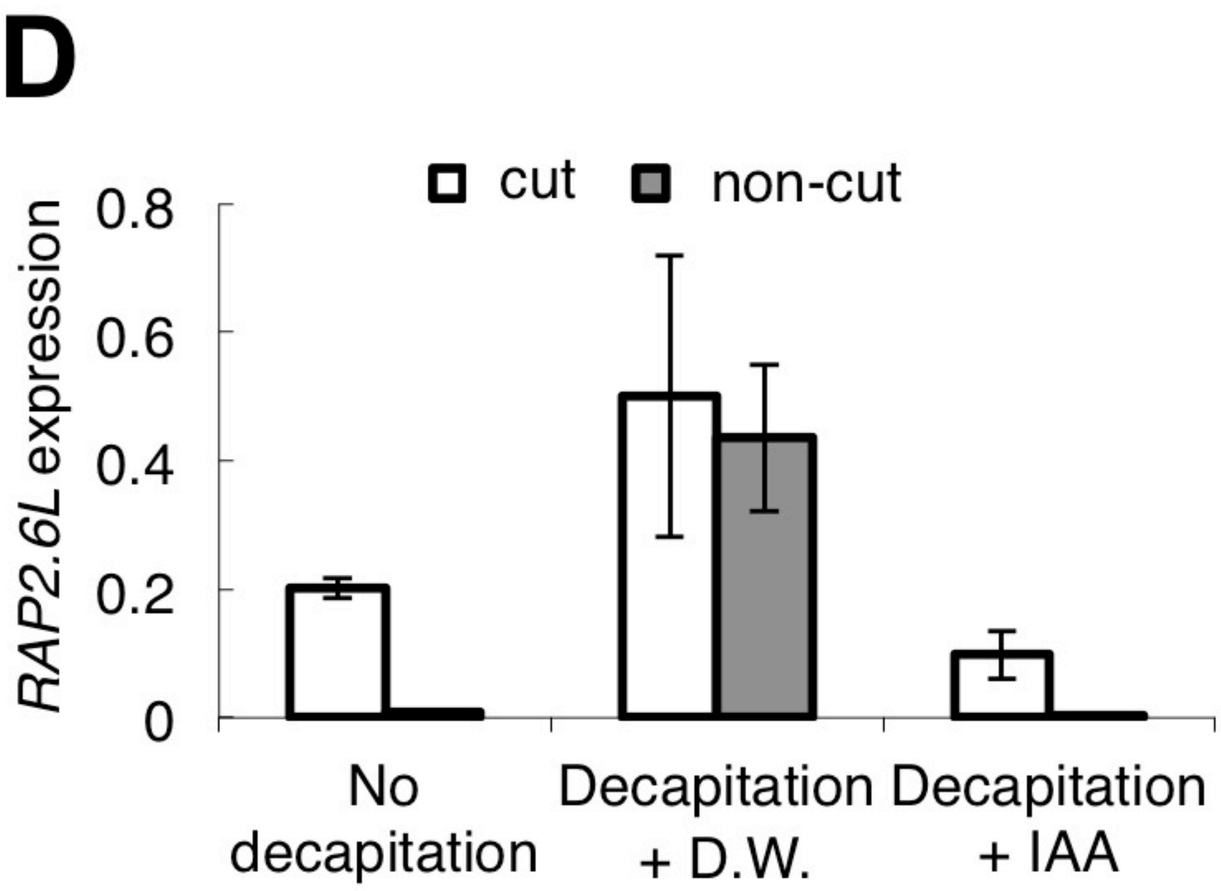


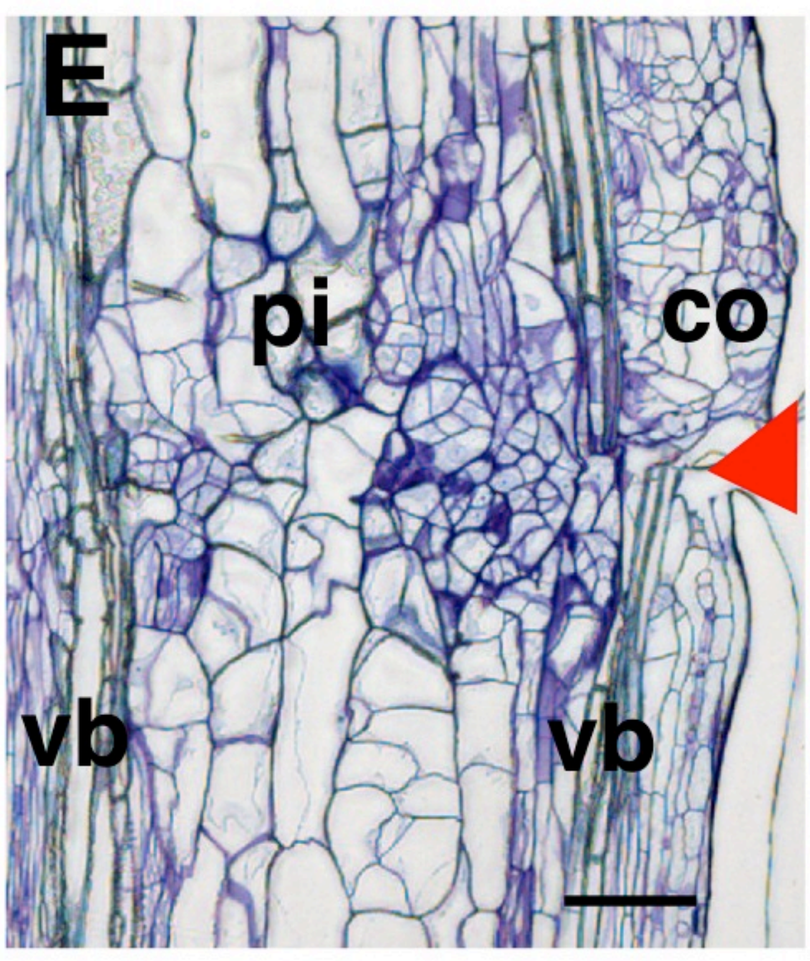
Figure 2

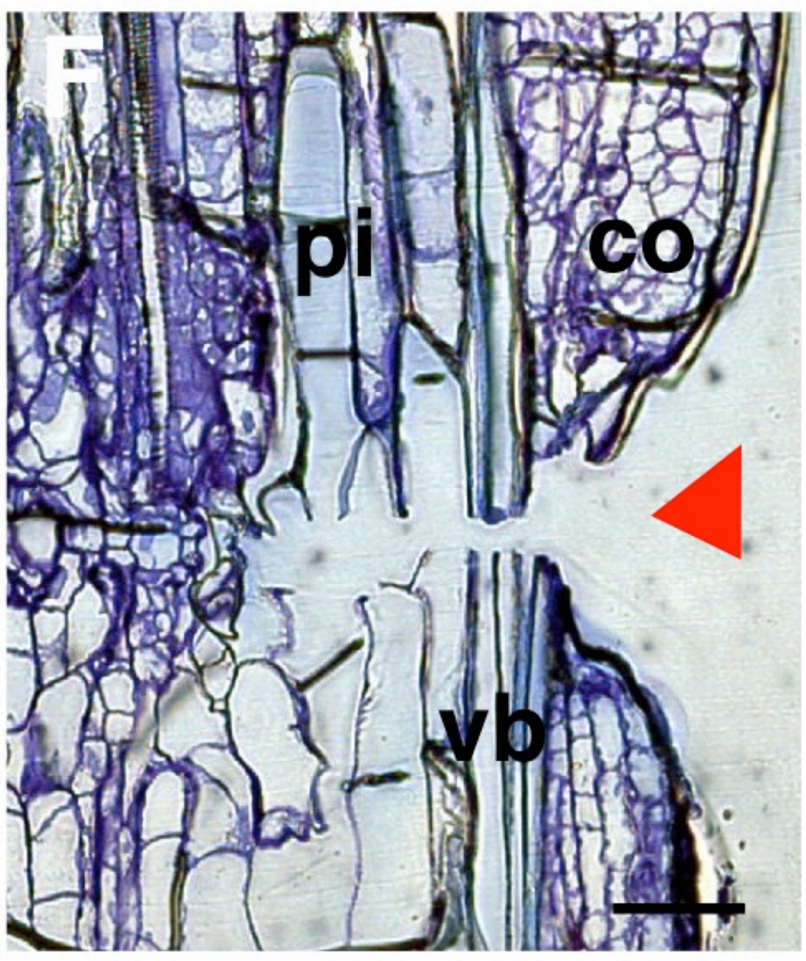


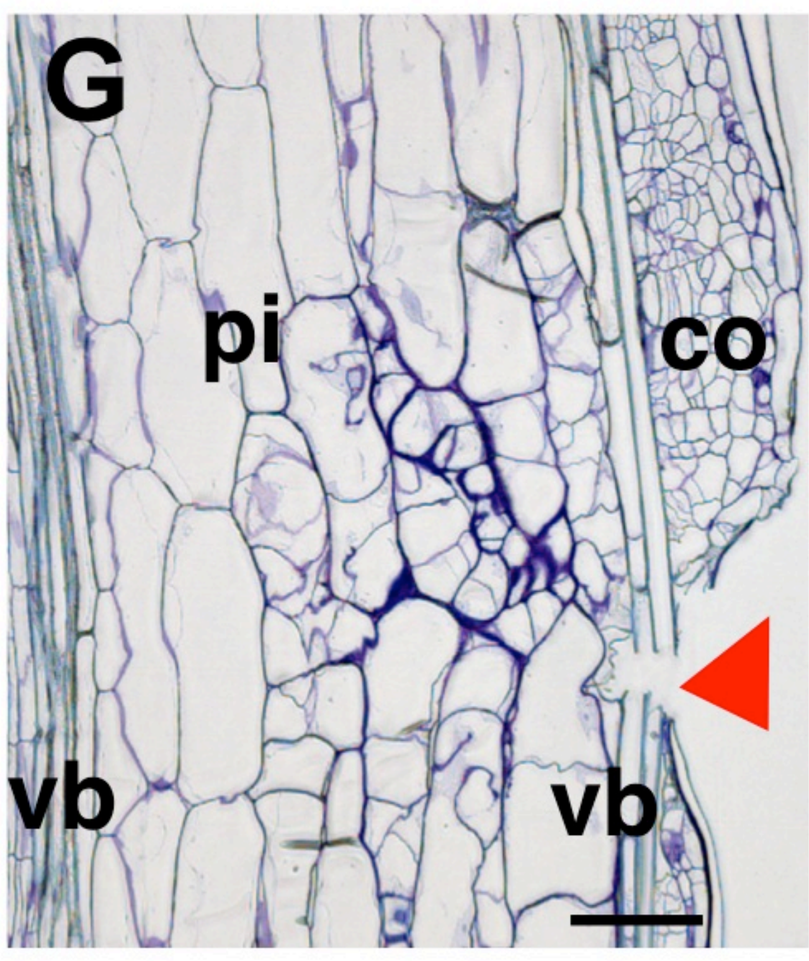






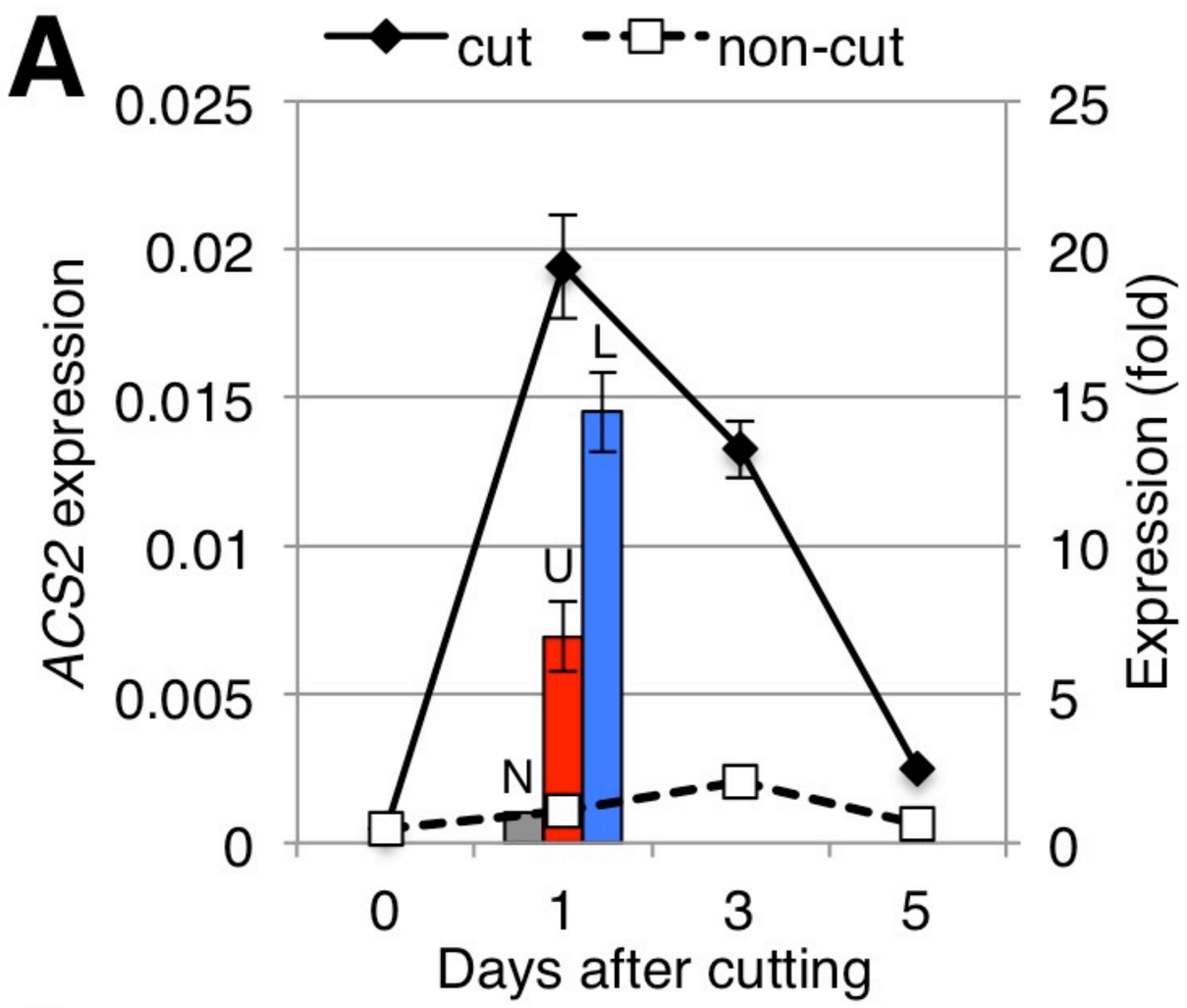


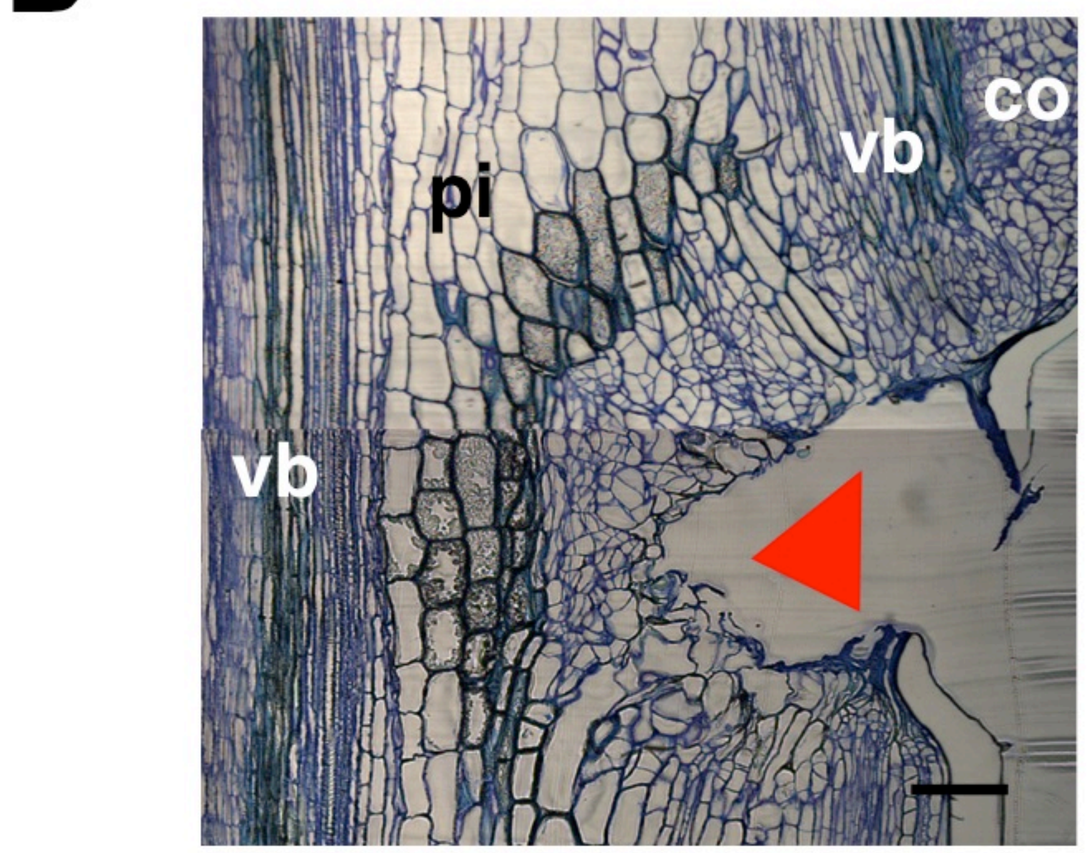


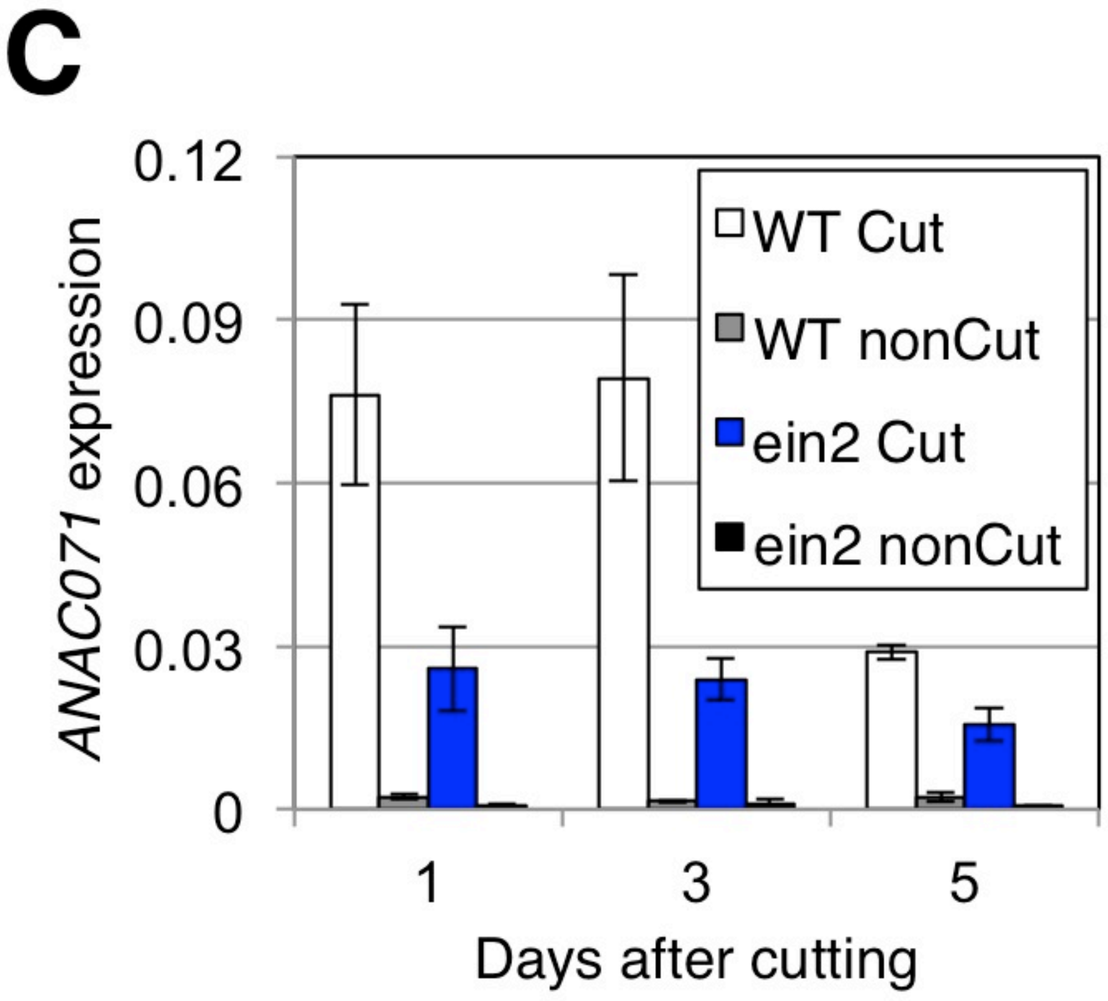


Wild-type ANAC071-SRDX RAP2.6L-SRDX

Figure 3


A
cut
non-cut
ACS2 expression
Expression (fold)
0.025
0.02
0.015
0.01
0.005
0
25
20
15
10
5
0
L
U
N
0
1
3
5
Days after cutting


B
pi
vb
co
vb


C
ANAC071 expression
0.12
0.09
0.06
0.03
0
WT Cut
WT nonCut
ein2 Cut
ein2 nonCut
1
3
5
Days after cutting

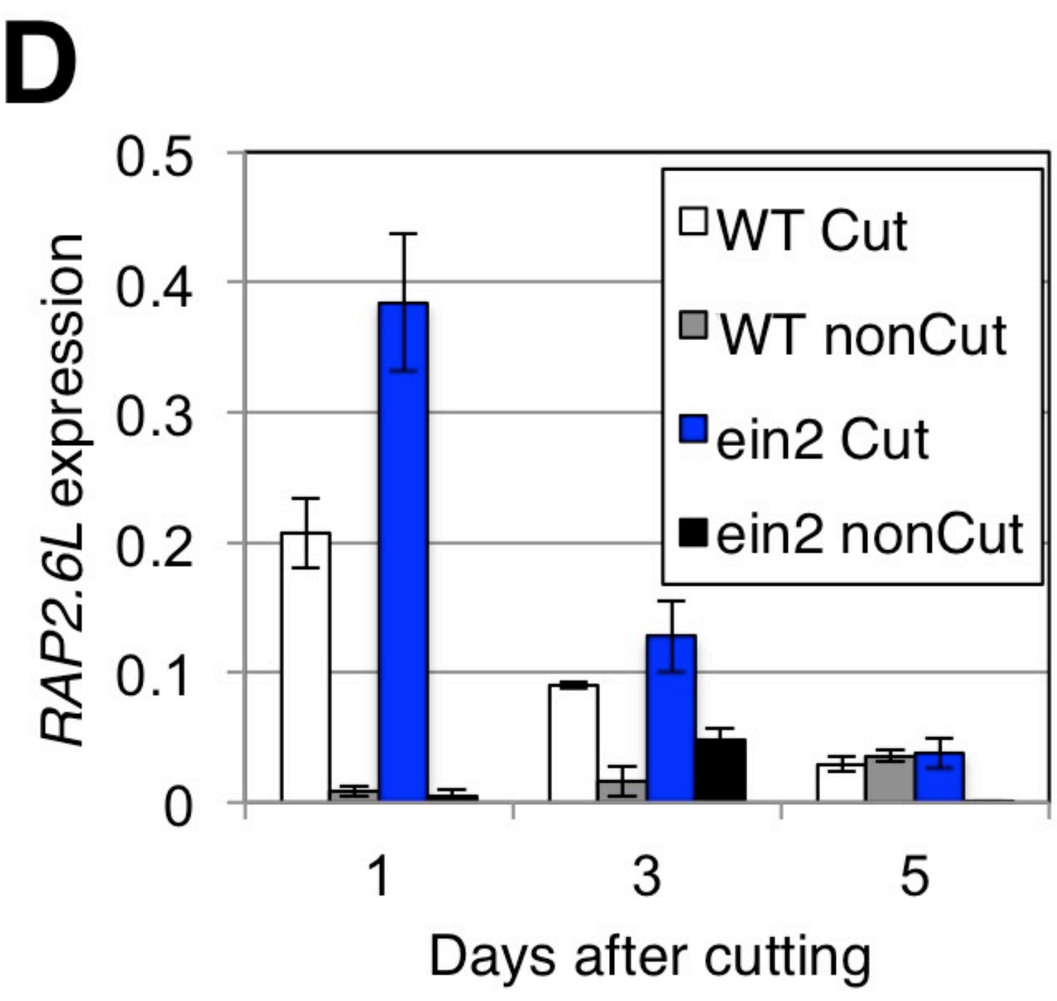

D
RAP2.6L expression
0.5
0.4
0.3
0.2
0.1
0
WT Cut
WT nonCut
ein2 Cut
ein2 nonCut
1
3
5
Days after cutting

Figure 4

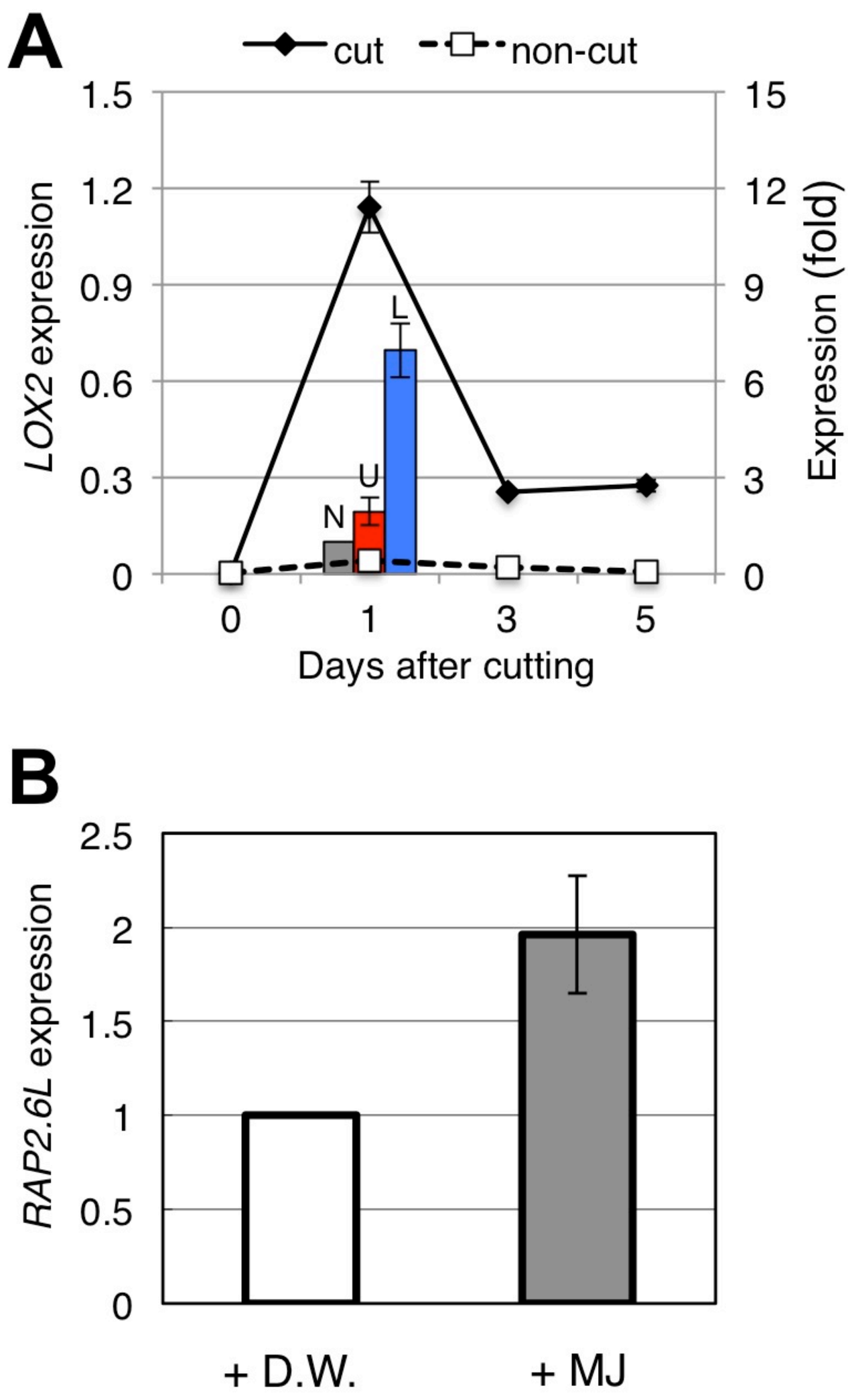

A
cut
non-cut
1.5
1.2
0.9
0.6
0.3
0
15
12
9
6
3
0
LOX2 expression
Expression (fold)
U
N
L
0
1
3
5
Days after cutting
B
2.5
2
1.5
1
0.5
0
RAP2.6L expression
+ D.W.
+ MJ

Figure 5

Supporting Information

Materials and Methods

Plant Materials and Growth Conditions.

The seeds of *Arabidopsis thaliana* (L.) Heynh ecotype Columbia (Col-0), promoter::GUS transgenic plants, *RAP2.6L*-SRDX and *ANAC071*-SRDX transformants, *ein2*, and *pin1-1* in the same background were germinated and grown in artificial soil (Kureha Co.) under continuous white fluorescent light (32 µmol $m^{-2}s^{-1}$) at 23°C, after stratification at 4°C for 2-4 days in the dark. After 7–10 days of bolting, the stems between the first or second cauline leaves and rosette leaves were cut through half of their diameter with a micro-surgical knife (Surgical Specialties) under a stereomicroscope (Fig. S1*A* and *B*). The plant was then grown as described above for an additional 14 days.

Quantitative Reverse Transcription (QRT)-PCR.

Cut flowering stem surrounding the cut surface and non-cut stem in the same internode were trimmed to ~5 mm segments at various stages, and total RNA was extracted using an RNAqueous RNA isolation kit with a plant RNA isolation aid (Ambion). For spatiotemporal gene expression analysis, stem segments surrounding the cut surface were further divided into upper or lower regions from the cut position. QRT-PCR was carried out as described previously (S1) with 7500 Fast Real-Time PCR System (Applied Biosystems). Experiments were replicated using independently grown plant materials.

The nucleotide sequences of gene-specific primers for QRT-PCR.

ACS2 (At1g01480)

Forward Primer; 5′-TCCGAAGAGGGTGGTTATGA-3′

Reverse Primer; 5′-CGCAAGGCAGAACATGATTG-3′

ANAC071 (At4g17980)

Forward Primer; 5′-CCTCTCCTTGTCGCGATGAA-3′

Reverse Primer; 5′-ATGCTTGAAGAGTCGTTTGTAGTAGAAG-3′

CYCB1;1 (At4g37490)

Forward Primer; 5′-GAACTGCAGCTTGTTGGTCTCA-3′

Reverse Primer; 5′-CACCTGTGGTGGCCAAATTT-3′

IAA5 (At1g15580)

Forward Primer; 5′-CCGGCGAAAAAGAGTCAAGT-3′

Reverse Primer; 5′-GACTGTTCTTTCTCCGGTACGAA-3′

LOX2 (At3g45140)

Forward Primer; 5′-CCCTGACAATGATCCTGAACTTG-3′

Reverse Primer; 5′-GCAACGCCTTGGATATGGA-3′

RAP2.6L (At5g13330)

Forward Primer; 5′-GAGGAAGCTGCTTTAGCCTATGA-3′

Reverse Primer; 5′-TGGACCCGTTCAGGGAAGT-3′

Light Microscopy of *Arabidopsis* Flowering Stems.

Sections were prepared as described previously (S2), and observations were made using a light microscope (DMRB, Leica). Experiments were replicated using biologically independent plant materials and representative results are shown.

Histochemical Analysis of Tissue Reunion in *Arabidopsis* Cut Flowering Stems.

For GUS staining, cut flowering stems of *pcyclin B*::*GUS* transgenic plants were immersed in X-Glu solution (1 mg/mL 5-bromo-4-chloro-3-indolyl-β-*D*-glucuronide in 50 mM sodium phosphate, pH 7.0) (Wako). Samples were then subjected to a vacuum for 5 min and incubated at 37°C for 8 h. Samples were longitudinally cut with a razor blade, and then visualized with a stereomicroscope (MZ-125, Leica) or a light microscope after the sectioning described above. Experiments were replicated using independently grown plant materials and representative results are shown.

Removal of Organs and Treatment with Phytohormones.

After 7-10 days of bolting, the shoot apex including a lateral bud and/or cauline leaf was removed using a razor blade. Decapitation involved excision of the shoot tip immediately below the oldest cauline leaf, with the rosette leaf and first internode remaining on the plant (Fig. S3*A*). Lanolin paste containing either indole-3-acetic acid (IAA) (Wako), or distilled water (D.W.) was applied to the apical tip of decapitated plants to cover the cut surface. The lanolin pastes were prepared by adding anhydrous lanolin to solutions of IAA or D.W. (3:1, v/v), and the final concentration of IAA was adjusted to 10^{-3} M. The flowering stem was then cut and the plants were grown under the same conditions.

Treatment with Phytohormone and Inhibitors.

Plants were treated with 2,3,5-triiodobenzoic acid (TIBA) (Wako), an inhibitor of polar auxin transport, by applying a ring of TIBA in lanolin around the stem immediately above the cut position in the same internode. The lanolin pastes were prepared in a similar way, and the final concentration was adjusted to 10^{-3} M. After 1 day of treatment, the flowering stem was then cut and the plants were grown under the same conditions. A solution of Triton-X 100 (0.1%, v/v) containing 2×10^{-3} M methyl jasmonate (Wako) was applied to the flowering stem twice a day. After 1 day of treatment, total RNA was extracted from the stem and QRT-PCR was carried out as described above.

Quantification of Endogenous IAA.

Stem segments of cut or non-cut flowering stems (~5 mm) were prepared as described above. Purification of IAA using HPLC and IAA quantification with gas chromatography-selected ion monitoring-mass spectrometry (GC-SIM-MS) were conducted according to Nishimura et al. (2006) (S3). Quantification of endogenous IAA in upper, lower, or non-cut regions of flowering stems was performed using independently grown plant materials. For each IAA measurement, 50-100 µg (fresh weight) of flowering stem was used.

Microarray Analysis.

Total RNA was extracted as described above. Double-stranded cDNA was synthesized from 8 µg of total RNA using a Super Script Choice cDNA synthesis kit (Invitrogen) with an oligo (dT)24-primer containing a T7 polymerase promoter site at the 3' end. Probe preparation, hybridization to the GeneChip *Arabidopsis* ATH1 Genome Arrays (Affymetrix), and subsequent processing steps were performed according to the manufacturer's procedure. Signals were scanned using a confocal microscope scanner (Gene Array Scanner; Hewlett-Packard) at 570 nm. The presence or absence of a reliable hybridization signal for each gene and signal values for individual genes were obtained using statistical algorithms on GCOS software (Affymetrix). The sum of signal values from all probe sets was used for normalization across different samples. Genes were classified as responsive genes if the signal values deviated either positively or negatively 2-fold or more between cut and control samples in duplicated experiments using independent plant materials for minimal statistical treatment. Genes for those transcripts determined to be undetectable (absent) in cut stems were eliminated from the list of

up-regulated genes. Furthermore, these genes were identified and categorized into functional groups based on the data from Salk Institution Genomic Analysis Laboratory (http://signal.salk.edu), National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov), Genevestigator (https://www.genevestigator.com), and The Arabidopsis Information Resource (http://www.arabidopsis.org). Advanced data analyses including hierarchical clustering and K-means clustering were performed using Microsoft Excel and Gene Spring software version 6.1 (Silicon Genetics). Genes were classified as responsive genes if the signal strength values deviated either positively or negatively 2-fold or more after cutting or decapitation with two independent replications (Fig S3*B*). To select genes more specific for the pith tissue (section labeled a in Fig S3*B*), the genes up-regulated in the lesion stem of decapitated plants (sections labeled b and c in Fig. S3*B*), in which tissue reunion never occurred, were eliminated (Fig. S3*B*). Using this procedure, up-regulated genes in the cortex were presumably removed because cell division also occurred in the cortex of decapitated plants (Fig. S1*C-J*). Among these genes, genes up-regulated due to cutting were examined, giving rise to 206, 246, and 289 genes at 1, 3, and 5 days after cutting, respectively (Student's *t*-test with $P < 0.05$) (Fig. S3*C*).

RT-PCR analysis for *ANAC071- or RAP2.6L-SRDX* expression.

Total RNA was extracted described as above. One μg of total RNA was used to synthesize the first stand cDNA using SuperScript First-Strand Synthesis System (Invitrogen) with an oligo$(dT)_{12–18}$ primer, according to the manufacturer's procedure. Experiments were replicated using independently grown plant materials and representative results are shown (transgenic line 1 and line 2). The *AtActin7* (*AtACT7*; AT5G09810) was used as internal control. The primer sets and cycles of amplification used for PCR were as follows:

ANAC071-SRDX (30 cycles)
Forward primer: 5′-CGCATGTTATAGCTGGAT-3′
Reverse primer: 5′-AGCGAAACCCAAACGGAGTTCTAG-3′
RAP2.6L-SRDX (30 cycles)
Forward Primer; 5′-ACTATAGAGGCGTAAGGCAGAGACC-3′
Reverse Primer; 5′-TTAAGCGAAACCCAAACGGAGTTCTAG-3′

AtACT7 (25 cycles for *ANAC071-SRDX* and 27 cycles for *RAP2.6L-SRDX*)
Forward Primer; 5′-CAATGTCCCTGCCATGTATG-3′
Reverse Primer; 5′-TGAACAATCGATGGACCTGA -3′

Figure legends.

Figure S1. Photographs showing how the flowering stems were treated. After 7-10 days of bolting, the flowering stem between the first cauline leaf and rosette leaves (arrow in *A*) was cut into half of its diameter (arrow in *B*) with a micro-surgical knife under the stereo-microscope. Scale bars: 10 mm (*A*) or 1 mm (*B*). (C-J) Effect of organ removal on the tissue-reunion process. (*C, G*) no organ removal control; (*D, H*) shoot apex (SA), lateral bud (LB), and cauline leaf (CL) removed; (*E, I*) shoot apex and lateral bud removed; (*F, J*) cauline leaf removed. (*C-F*) Longitudinal hand sections stained with toluidine blue of wild type 7 days after cutting the stem. (*G-J*) Longitudinal hand sections of *pcyclinB*::*GUS* transgenic plants 3 days after cutting the stem. pi, pith; co, cortex; vb, vascular bundle. Arrowheads: position of cut. Scale bars: 100 µm (*C-F*) or 1 mm (*G-J*).

Figure S2. Gene expression of *IAA5* and photograph of *pANAC071* during the tissue-reunion process. (*A*) For time-course analysis, each value is the mean ± S.E. (N = 3). Bars indicate relative expression levels of *IAA5* in upper (U; red bar) or lower region (L; blue bar) of cut stem, for comparative expression analysis. The non-cut control (N; gray bar) was arbitrarily set to 1 for normalization, and the mean ± S.E. is shown (N = 4). (*B*) Photograph of *pANAC071*::*GUS* transgenic plants 3 days after cutting the stem. Scale bar: 1 mm.

Figure S3. Comparison of up-regulated genes during tissue reunion between intact and decapitated plants. (*A*) Schematic illustrations showing how segments of cut flowering stems were collected from intact or decapitated plants. To select genes more specific for the pith tissue, the genes up-regulated in the lesioned stem of decapitated plants, in which tissue reunion never occurred, were eliminated (Fig. S2*C* and *D*). Of the genes that met these criteria, they were ranked by fold change in expression 1, 3, or 5 days after cutting versus 0 days (non-cut). (*B*) Schematic illustration of comparison of gene expression between intact and decapitated plants. (*C*) Number of genes in each category. Each number of genes (a, b, and c) was corresponding for sections labeled in Fig S4*B*. (*D*)

Functional classification of up-regulated genes determined to be responsible for the tissue-reunion process. After comparison analyses (*B* and *C*), the genes were classified into the following ten subgroups according to their deduced function at each time point: (1) cell division–related; (2) hormone-related and hormone synthesis; (3) signal-related; transcription factor; (4) signal transduction–related genes without transcription factors and phytohormone signal–related genes; (5) pathogenesis-related (PR) protein; (6) cell wall–related, cell wall synthesis and metabolism-related genes; (7) protease and protease-related genes; (8) other metabolism-related genes; (9) other functions; (10) unknown. Of the up-regulated genes determined to be responsible for tissue reunion, 11 (5%), 21 (9%), and 13 (5%) genes were cell division– or proliferation-related genes at 1, 3, or 5 days after cutting, respectively. Cell division–related genes were classified into subgroups: histone, cyclin, motor protein family, DNA polymerases, and others. At 1 day after cutting, a few genes encoding histone, cyclin, and DNA polymerase were up-regulated. At 3 and 5 days after cutting, genes belonging to all subgroup types were up-regulated, including the kinesin motor protein engaged in the transition from G1 to S and G2 to M phase, as well as transport of vesicles and organelles, spindle formation and elongation, chromosome segregation, and microtubule dynamics and morphogenesis during cytokinesis (S4 and S5). The cell wall–related genes included expansin (S6), lipid binding or transfer protein (S7), xyloglucan endotransglucosylase/hydrolase (S8), fucosyltransferase (S9), matrix metalloproteinase (S10), extensin (S11), and glycine-rich protein (S12) were also up-regulated at 3 and 5 days after cutting.

Figure S4. Representative phenotype of SRDX transgenic plants. (*A*, *B*) RT-PCR analysis for *ANAC071- (A)* or *RAP2.6L-SRDX* expression *(B)*. (*C, D*) *ANAC071*-SRDX. (*E, F*) *RAP2.6L*-SRDX. (*C, E*) 3 days after cutting. (*D, F*) 7 days after cutting. Arrowheads: position of cut. pi, pith; co, cortex; vb, vascular bundle. Scale bars: 100 µm.

Figure S5. Light micrographs of the tissue-reunion process in the cut flowering stem of *pACS2::GUS*-plant and the *ein2* ethylene signaling–deficient mutant. (*A*) *pACS2::GUS*-plant. (*B, C*) Wild type (WT); (*D, E*) *ein2*. (*B, D*) 3 days after cutting; (*C, E*) 5 days after cutting. Sections were stained with toluidine blue. Arrowheads: position of cut. pi, pith; co, cortex; vb, vascular bundle. Scale bars: 1 mm (*A*) or 500 µm (*B-E*).

Figure S6. Effects of decapitation and IAA application on *ACS2* and *LOX2*expression. (A) *ACS2*, (B) *LOX2* expression 1 day after cutting. White bar: cut stem; gray bar: non-cut stem. The mean ± S.E. is shown (N = 3). Experiments were repeated three times with different seed batches and normalized using 18S rRNA as the internal control.

Figure S7. Schematic model of phytohormone regulation of the expression of *ANAC071* and *RAP2.6L*-TFs during tissue reunion. The tissue-reunion process is operated differently between the upper and lower parts of the cut gap. In the upper part, *ANAC071*-TF was up-regulated by IAA and ethylene, a wound-inducible hormone. In the lower part, *RAP2.6L* was activated due to auxin depletion. JA, a key regulator of plant responses to environmental stresses and biotic challenges, also induced *RAP2.6L* expression.

Table S1. Selected genes determined to be responsive to tissue reunion. Table S1 shows selected genes with expression patterns that correlate with tissue reunion.

Dataset S1. List of genes responsive to tissue reunion. To select genes more specific for the pith tissue, the genes up-regulated in the lesion stem of decapitated plants, in which tissue reunion never occurred, were eliminated (Fig. S3*A* and *B*). Using this procedure, up-regulated genes in the cortex were considered to be removed because the same genes were presumably expressed in the cortex of both decapitated and intact plants (Fig. S1*C* and *D*). Among them, the genes up-regulated due to cutting were selected, giving rise to 206, 246, and 289 genes at 1, 3, and 5 days after cutting, respectively (Student's t-test with $P < 0.05$; Fig. S3*C*).

References

S1. Asahina M, et al. (2007) Effects of the removal of cotyledons on endogenous gibberellin levels in hypocotyls of young cucumber and tomato seedlings. Plant Biotechnol 24:99e106.

S2. Asahina M, et al. (2002) Gibberellin produced in the cotyledon is required for cell division during tissue reunion in the cortex of cut cucumber and tomato hypocotyls. Plant Physiol 129:201e210.

S3. Nishimura et al (2006). Red light causes a reduction in IAA levels at the apical tip by

inhibiting de novo biosynthesis from tryptophan in maize coleoptiles. *Planta* 224: 1427-1435.

S4. Nishihama R et al (2002) Expansion of the cell plate in plant cytokinesis requires a kinesin-like protein/MAPKKK complex. *Cell* 109: 87-99.

S5. Reddy AS, Day IS (2001) Kinesins in the *Arabidopsis* genome: a comparative analysis among eukaryotes. *BMC Genomics* 2: 1-13.

S6. Cho HT, Cosgrove DJ (2000). Altered expression of expansin modulates leaf growth and pedicel abscission in *Arabidopsis thaliana*. *PNAS* 15;97 (17):9783-9788.

S7. Carvalho AO and Gomes VM (2007) Role of plant lipid transfer proteins in plant cell physiology: a concise review. *Peptides* 28: 1144-1153

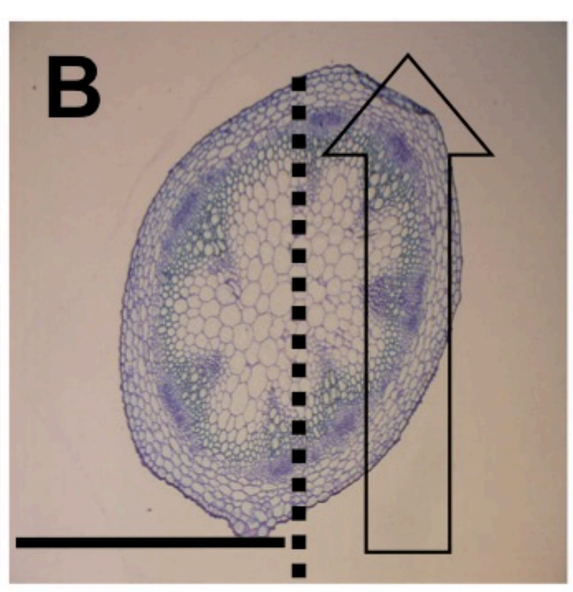
S8. Vissenberg K et al (2005) Differential expression of AtXTH17, AtXTH18, AtXTH19 and AtXTH20 genes in *Arabidopsis* roots. Physiological roles in specification in cell wall construction. *Plant Cell Physiol* 46(1):192-200.

S9. Sarria R et al (2001) Characterization of a family of *Arabidopsis* genes related to xyloglucan fucosyltransferase1. *Plant Physiol* 127(4):1595-1606.

S10. Golldack D, Popova OV, Dietz KJ (2002). Mutation of the matrix metalloproteinase At2-MMP inhibits growth and causes ate flowering and early senescence in *Arabidopsis. J Biol Chem* 15; 277(7): 5541-5547.

S11. Yoshiba Y et al (2001) Characterization of four extensin genes in Arabidopsis thaliana by differential gene expression under stress and non-stress conditions. *DNA Res* 30;8(3): 115-122.

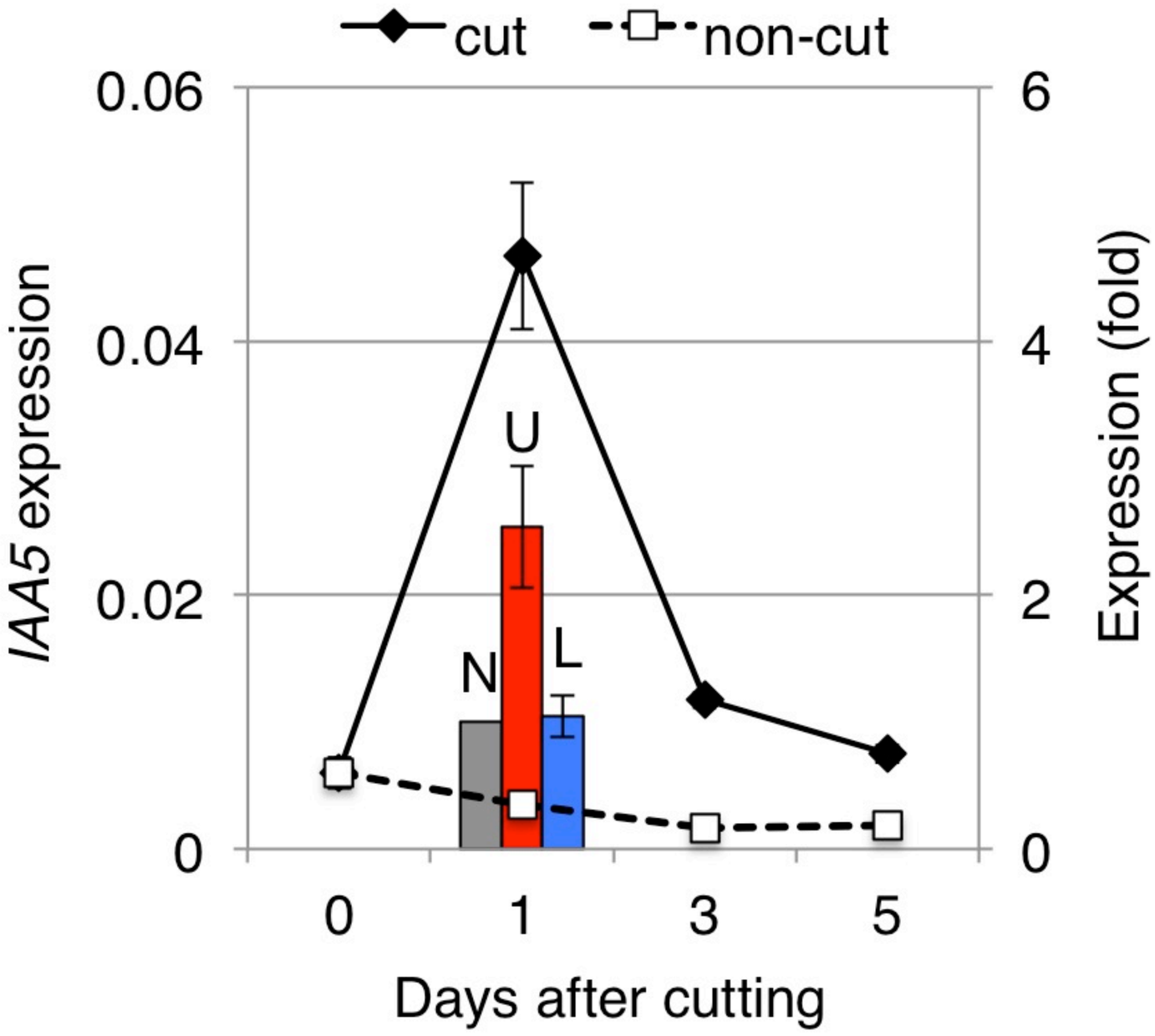
S12. Ringli C, Keller B, Ryser U (2001) Glycine-rich proteins as structural components of plant cell walls. *Cell Mol Life Sci* 58(10):1430-1441.



+ SA/ +LB/ +CL - SA/ -LB/ -CL - SA/ -LB/ +CL + SA/ +LB/ -CL

Figure S1

A


cut
non-cut
0.06
0.04
0.02
0
6
4
2
0
U
N
L
0
1
3
5
Days after cutting
IAA5 expression
Expression (fold)

B

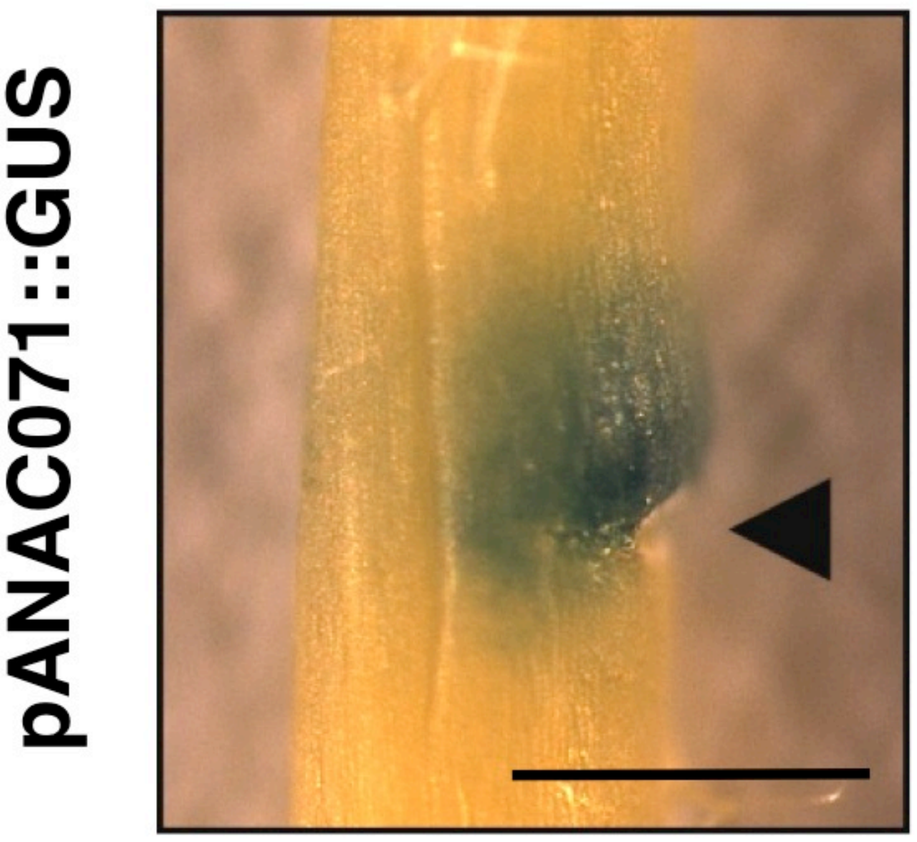
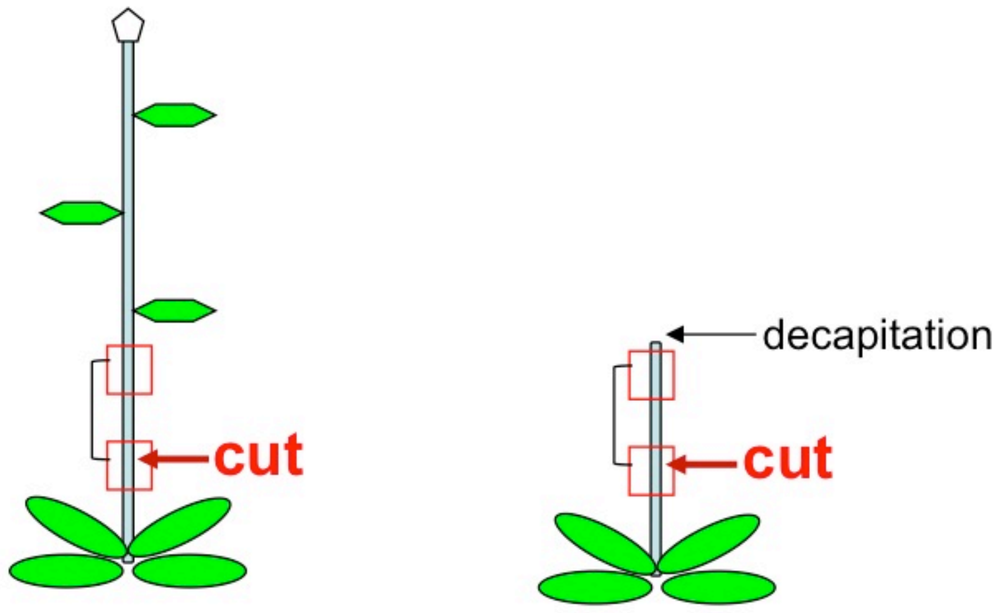
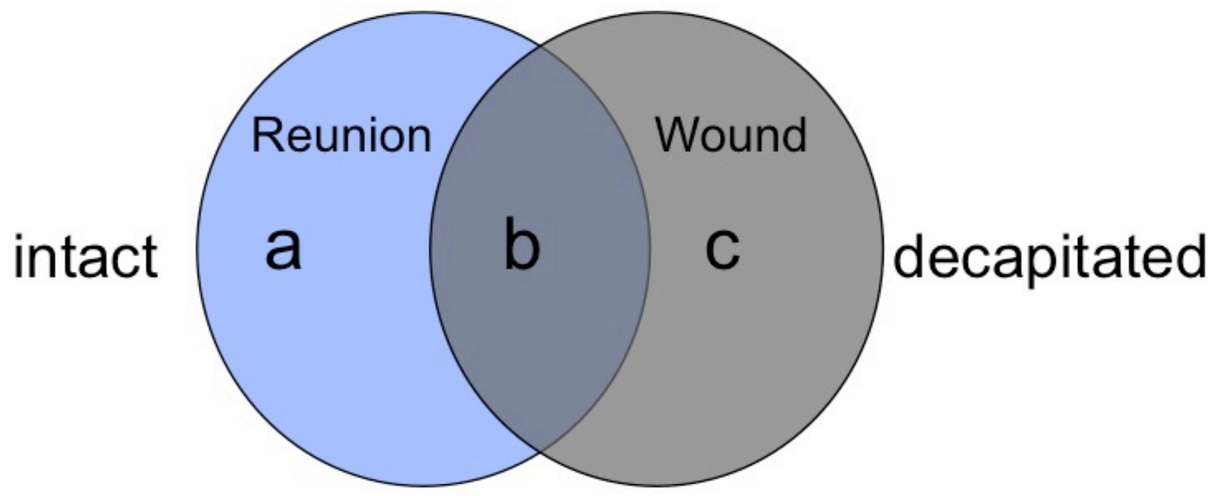

pANAC071::GUS
3 days

Figure S2

A



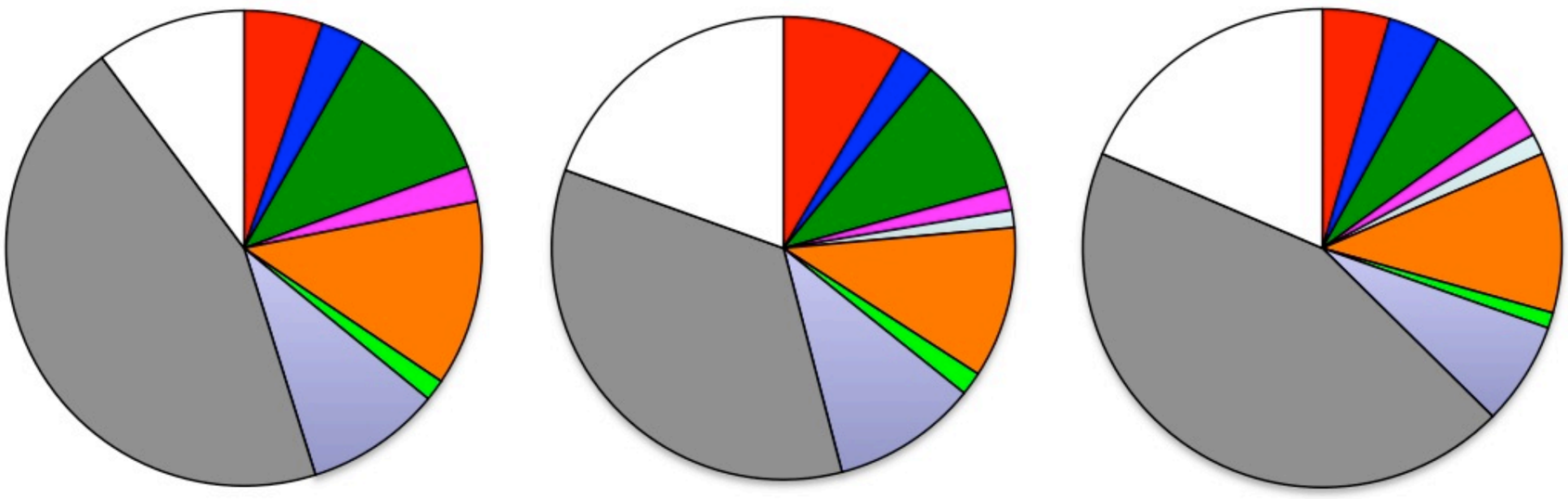
B



C

Days after cutting	Number of genes (a) Reunion	(b) Wound & Reunion	(c) Wound
1 day	206	30	154
3 days	246	89	95
5 days	289	107	81

D



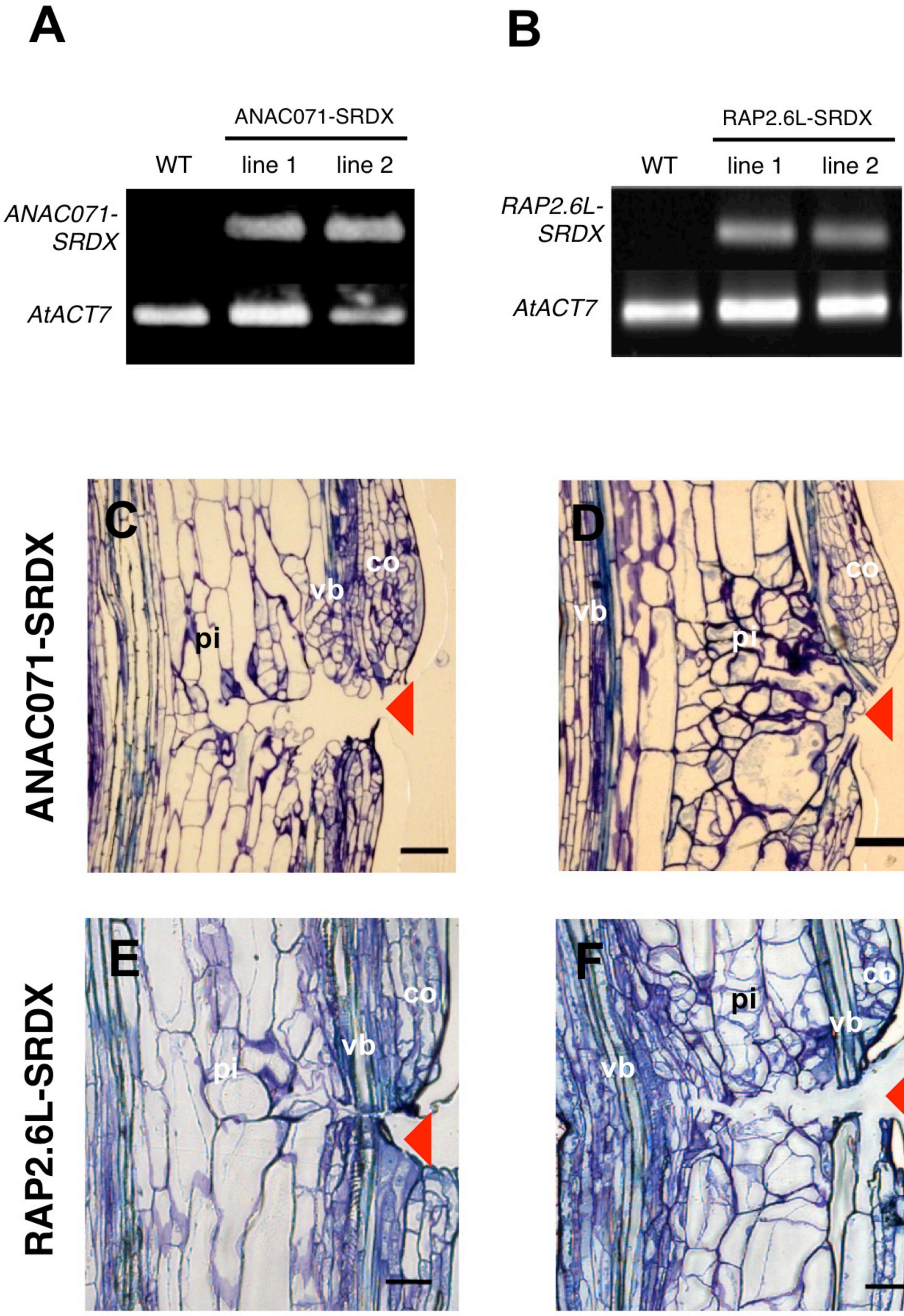

A
ANAC071-SRDX
WT
line 1
line 2
ANAC071-SRDX
AtACT7
B
RAP2.6L-SRDX
WT
line 1
line 2
RAP2.6L-SRDX
AtACT7
ANAC071-SRDX
C
co
vb
pi
D
vb
pi
co
RAP2.6L-SRDX
E
co
vb
pi
F
pi
co
vb
vb
3 days
7 days

Figure S4

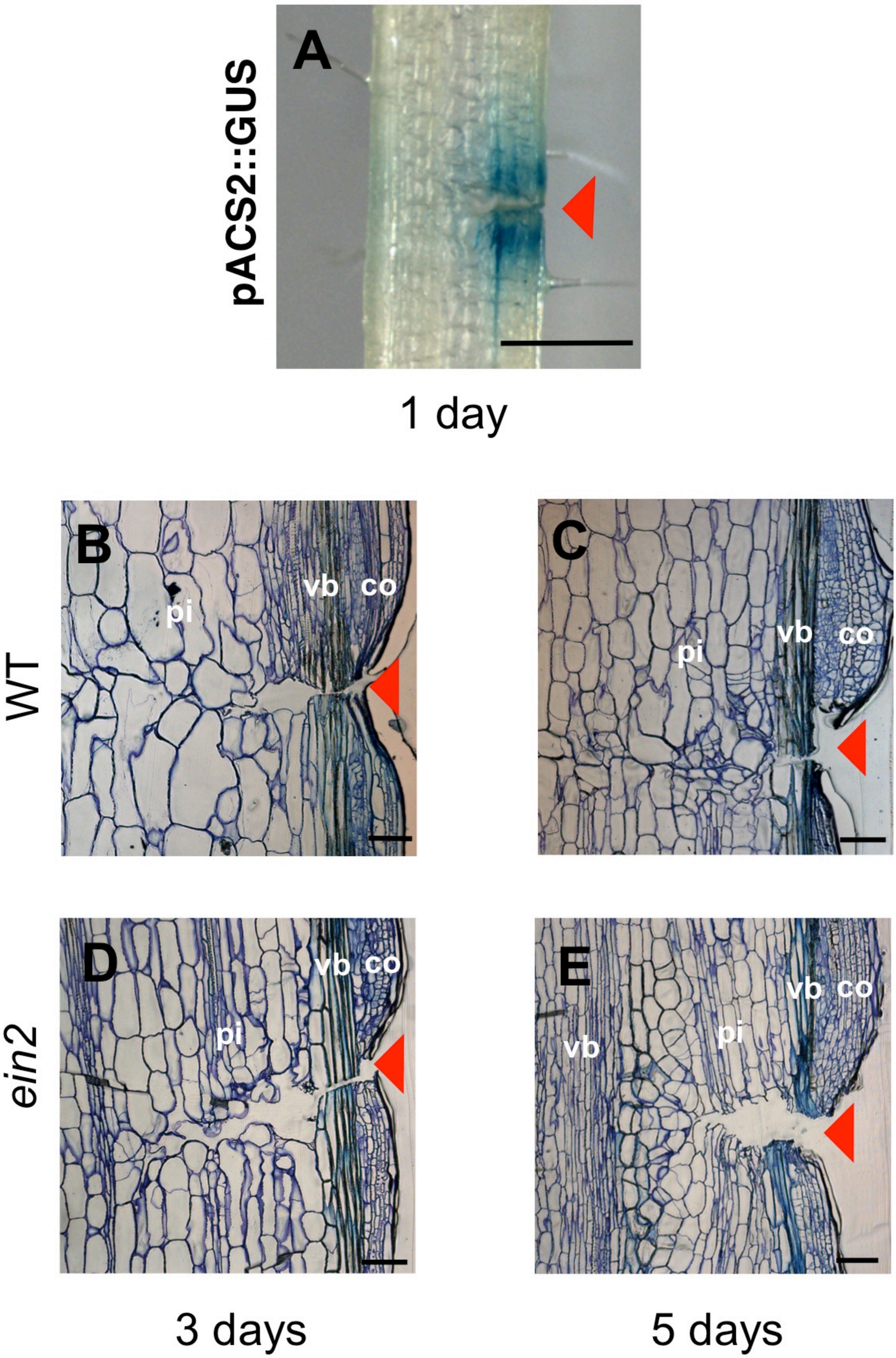
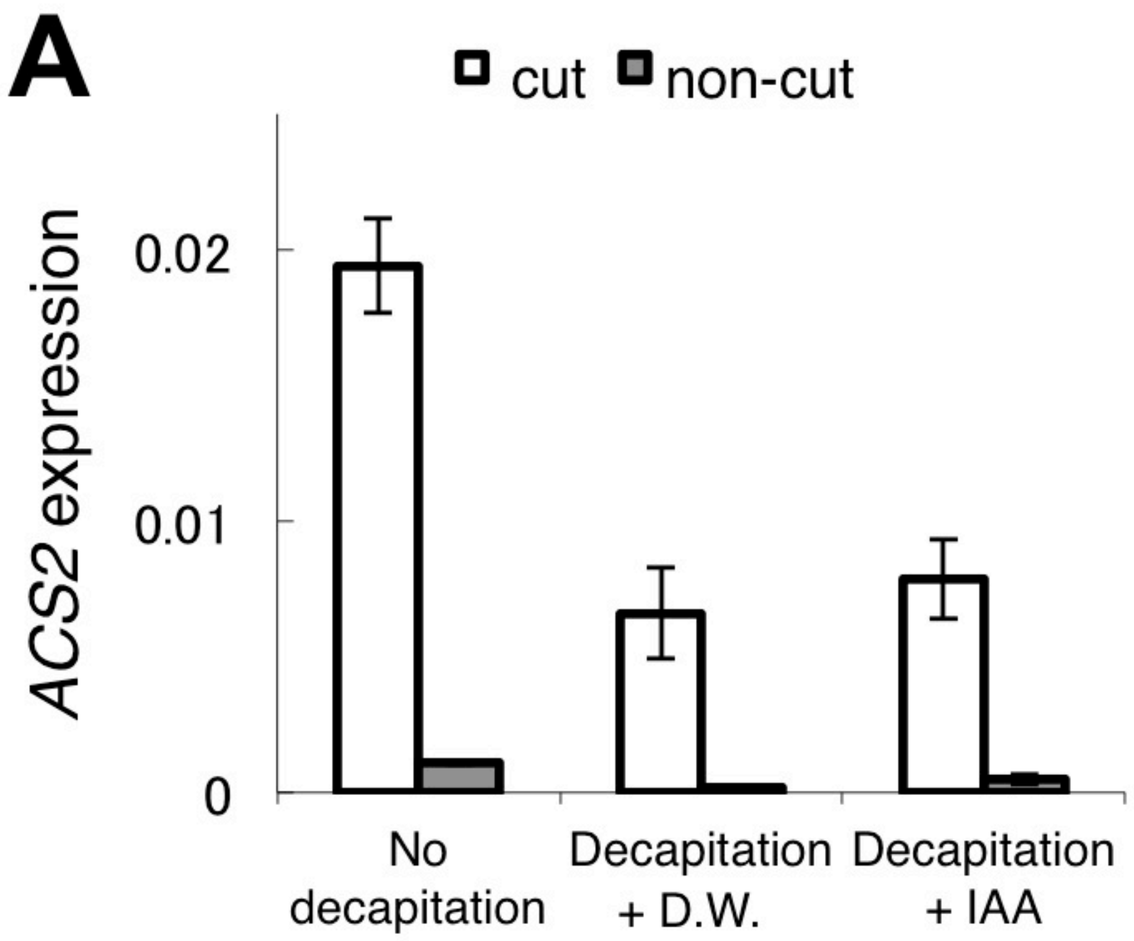

pACS2::GUS
A
1 day
WT
B
pi
vb
co
C
pi
vb
co
ein2
D
pi
vb
co
E
vb
pi
vb
co
3 days
5 days

Figure S5


A
cut
non-cut
ACS2 expression
0.02
0.01
0
No decapitation
Decapitation + D.W.
Decapitation + IAA

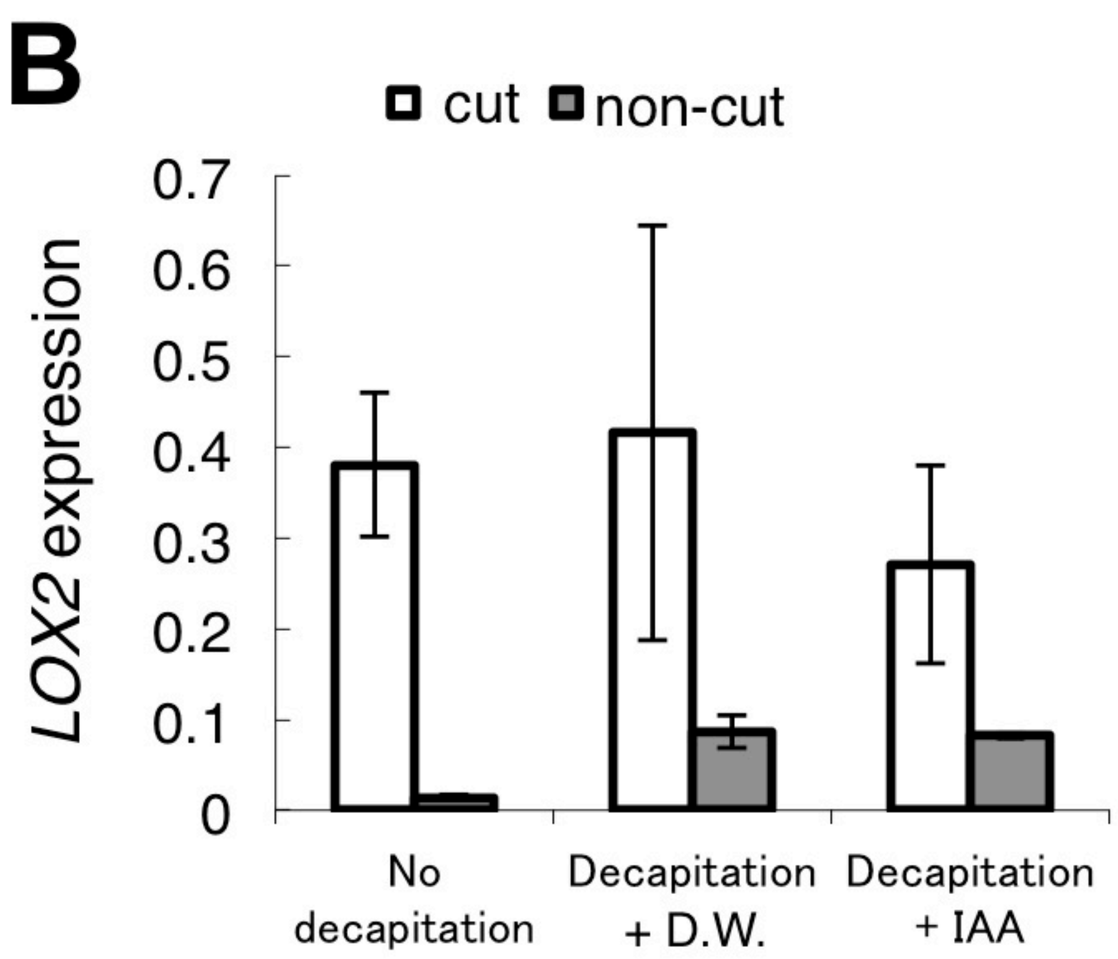

B
cut
non-cut
LOX2 expression
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
No decapitation
Decapitation + D.W.
Decapitation + IAA

Figure S6

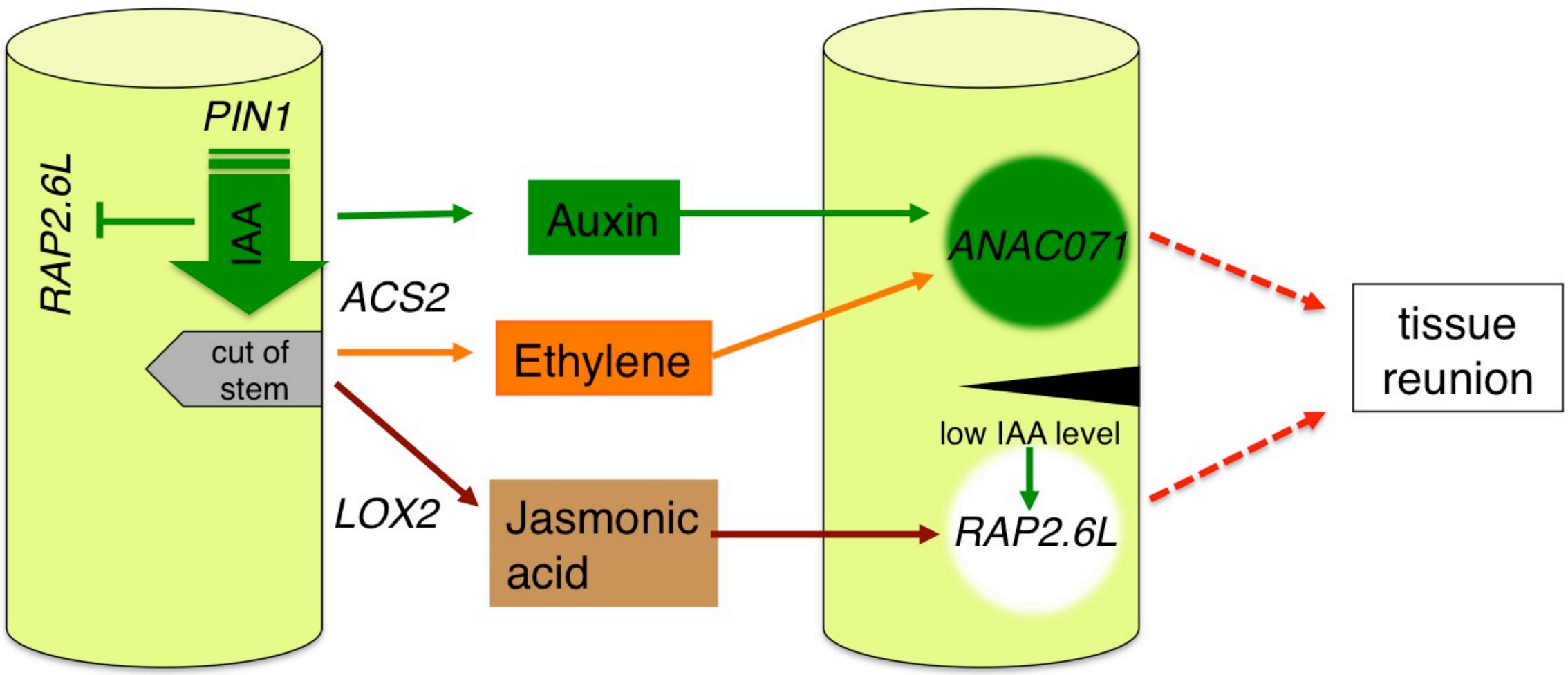

PIN1
RAP2.6L
IAA
cut of stem
ACS2
LOX2
Auxin
Ethylene
Jasmonic acid
ANAC071
low IAA level
RAP2.6L
tissue reunion

Figure S7

Table S1. Selected genes determined to be responsive to tissue reunion.

AGI No.	Gene name	Expression peak	Subgroups
At4g17980	NAC domain containing protein 71 (ANAC071)	1 day	transcription factor
At5g13330	AP2/ERF transcription factor (RAP2.6L)	1 day	transcription factor
At1g15580	AUX/IAA protein (IAA5)	1 day	hormone related
At1g01480	1-Aminocyclopropane-1-Carboxylate Synthase (ACS2)	1 day	hormone related
At3g45140	Lipoxygenase (LOX2)	1 day	hormone related
At1g26770	Expansin (EXP10)	1 day	cell wall related
At3g22600	Lipid transfer protein	1-3 days	cell wall related
At1g66830	Leucine-rich repeat protein kinase family protein	3 days	signal transduction
At4g37490	Cyclin-dependent protein kinase regulator (CYCB1;1)	3 days	cell division
At1g73690	Cyclin dependent kinase (CDKD1)	3 days	cell division
At1g59540	Kinesin (NACK1)	3 days	cell division
At2g39350	ABC-2 type transporter family protein (ABCG1)	3 days	other function (transporter)
At5g48070	Xyloglucan endotransglucosylase/hydrolase (XTH20)	3 days	cell wall related
At1g74420	Fucosyltransferase (FUT3)	3-5 days	cell wall related
At1g70170	Matrix metalloproteinase (MMP)	3-5 days	cell wall related
At1g76930	Extensin	3-5 days	cell wall related
At2g05510	Glycine rich protein	3-7 days	cell wall related
At2g04920	F-box protein (FBX9)	5 days	signal transduction
At4g37410	Cytochrome P450 Oxidase (CYP81F4)	5 days	other metabolism
At5g54230	myb-type transcription factor (myb 49)	5-7 days	transcription factor

Table S1 shows selected genes with expression patterns that correlate with tissue reunion.