Supporting Information

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SI 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 d in the dark. After 7–10 d of bolting, the stems between the first or second cauline leaves and rosette leaves were cut through half of their diameter with a microsurgical knife (Surgical Specialties) under a stereomicroscope (Fig. S1 *A* and *B*). The plant was then grown as described above for an additional 14 d.

Quantitative RT-PCR (qRT-PCR). Cut flowering stem surrounding the cut surface and noncut 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 (1) with 7500 Fast Real-Time PCR System (Applied Biosystems). Experiments were replicated using independently grown plant materials.

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 (2), 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 d 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, vol/vol), 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 d 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%, vol/vol) containing 2×10^{-3} M methyl jasmonate (Wako) was applied to the flowering stem twice per day. After 1 d 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 noncut 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) was conducted according to Nishimura et al. (3). Quantification of endogenous IAA in upper, lower, or noncut 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 twofold 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 according to data from the 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 twofold 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 d after cutting, respectively (Student *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 microgram of total RNA was used to synthesize the first-strand 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′.

1. 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* 24:99–106.
2. 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* 129:201–210.
3. Nishimura T, Mori Y, Furukawa T, Kadota A, Koshiba T (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.

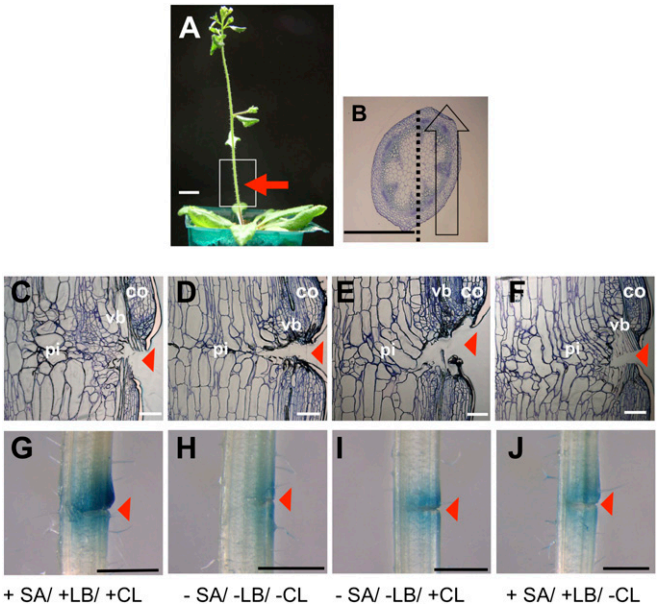


Fig. S1. Photographs showing how the flowering stems were treated. After 7–10 d of bolting, the flowering stem between the first cauline leaf and rosette leaves (arrow in *A*) was cut into half of its diameter (arrow in *B*) with a microsurgical knife under the stereomicroscope. (*C–J*) Effect of organ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

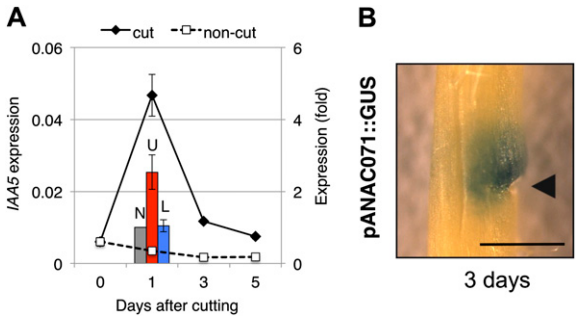
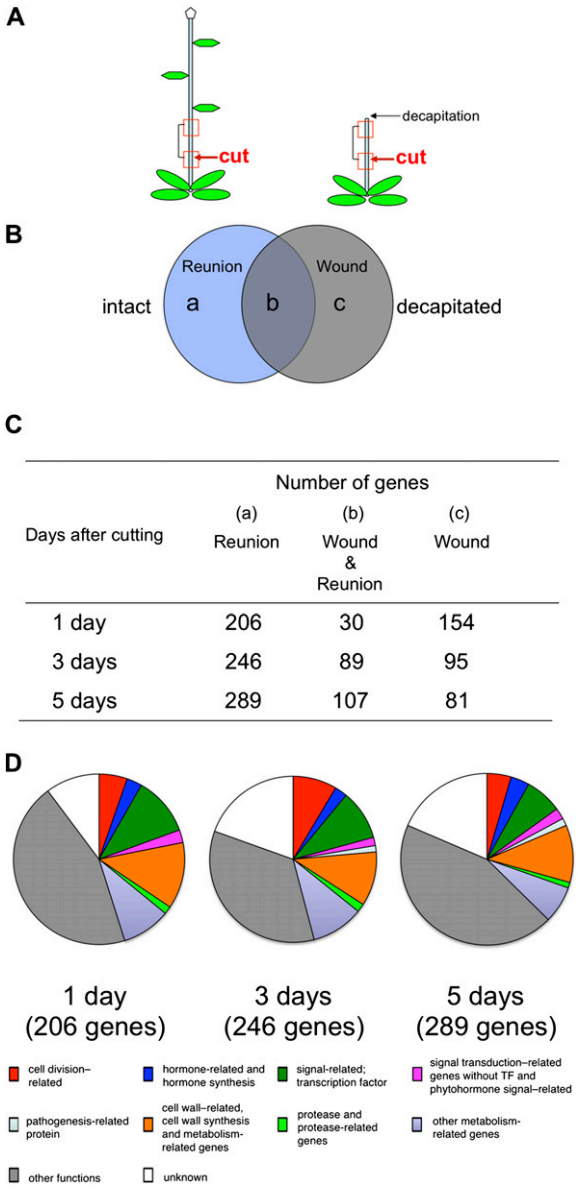


Fig. S2. Gene expression of *IAA5* and photograph of *pANAC071* during the tissue-reunion process. (*A*) For time-course analysis, each value is the mean ± SE (n = 3). Bars indicate relative expression levels of *IAA5* in upper (U; red bar) or lower region (L; blue bar) of cut stem, for comparative expression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



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

Fig. 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*). The genes that met these criteria were ranked by fold change in expression 1, 3, or 5 d after cutting vs. 0 d (noncut). (*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) corresponds to sections labeled in *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 10 subgroups according to their deduced function at each time point: (*i*) cell division–related; (*ii*) hormone-related and hormone synthesis; (*iii*) signal-related; transcription factor; (*iv*) signal transduction-related genes without transcription factors and phytohormone signal-related genes; (*v*) pathogenesis-related protein; (*vi*) cell wall-related, cell wall synthesis and metabolism-related genes; (*vii*) protease and protease-related genes; (*viii*) other metabolism-related genes; (*ix*) other functions; (*x*) 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 d after cutting, respectively. Cell division-related genes were classified into subgroups: histone, cyclin, motor protein family, DNA polymerases, and others. At 1 d after cutting, a few genes encoding histone, cyclin, and DNA polymerase were up-regulated. At 3 and 5 d 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 (1, 2). The cell wall-related genes, including expansin (3), lipid binding or transfer protein (4), xyloglucan endotransglucosylase/hydrolase (5), fucosyltransferase (6), matrix metalloproteinase (7), extensin (8), and glycine-rich protein (9) were also up-regulated at 3 and 5 d after cutting.

1. Nishihama R, et al. (2002) Expansion of the cell plate in plant cytokinesis requires a kinesin-like protein/MAPKKK complex. *Cell* 109:87–99.
2. Reddy AS, Day IS (2001) Kinesins in the *Arabidopsis* genome: A comparative analysis among eukaryotes. *BMC Genomics* 2:2.
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5. 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* 46:192–200.
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7. 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* 277:5541−5547.
8. 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* 8:115−122.
9. Ringli C, Keller B, Ryser U (2001) Glycine-rich proteins as structural components of plant cell walls. *Cell Mol Life Sci* 58:1430−1441.

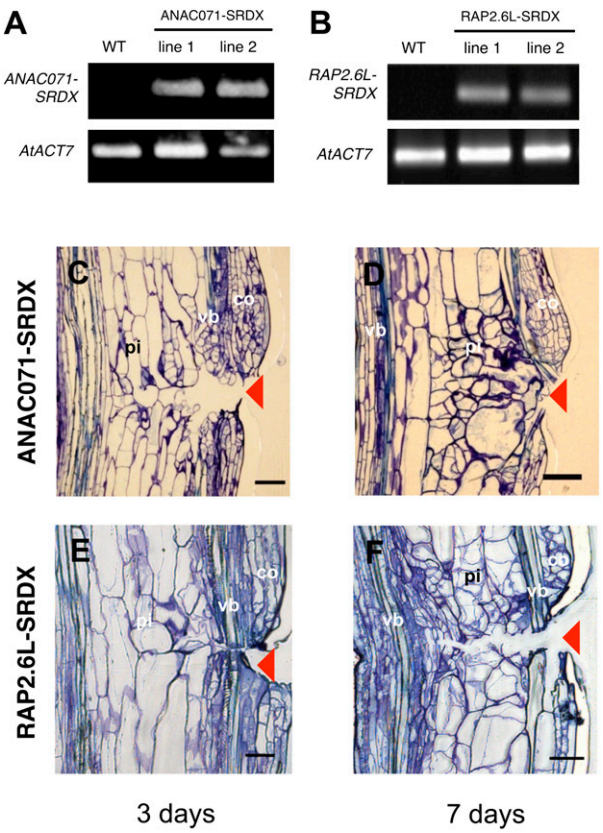

Fig. S4. Representative phenotype of SRDX transgenic plants. (*A* and *B*) RT-PCR analysis for *ANAC071- (A)* or *RAP2.6L-SRDX* expression *(B)*. (*C* and *D*) *ANAC071*-SRDX. (*E* and *F*) *RAP2.6L*-SRDX. (*C* and *E*) Three days after cutting. (*D* and *F*) Seven days after cutting. Arrowheads indicate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

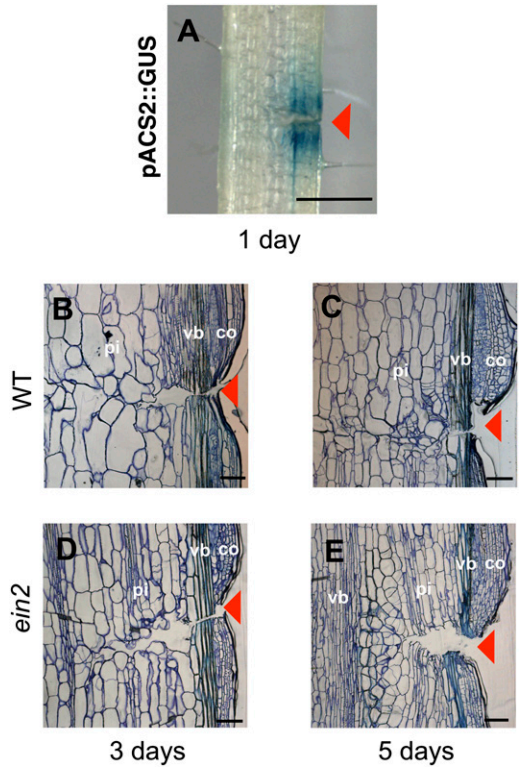

Fig. S5. Light micrographs of the tissue-reunion process in the cut flowering stem of *pACS2::GUS*-plant and the *ein2* ethylene signaling-deficient mutant. (*A*) *pACS2::GUS*-plant. (*B* and *C*) Wild type; (*D* and *E*) *ein2*. (*B* and *D*) Three days after cutting. (*C* and *E*) Five days after cutting.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

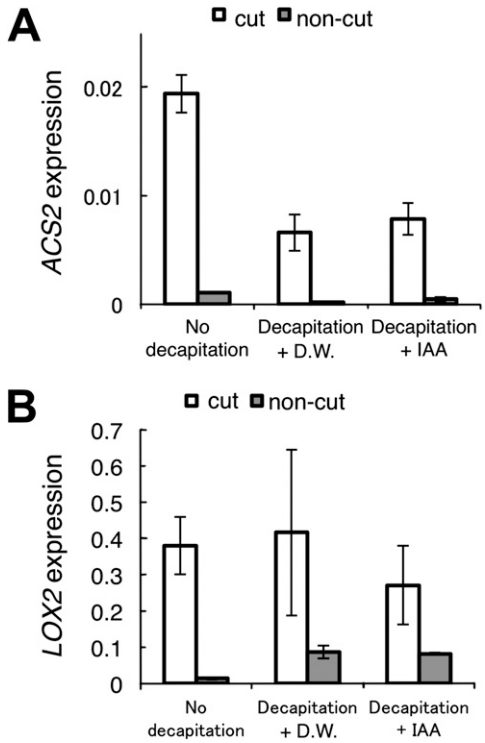

Fig. S6. Effects of decapitation and IAA application on *ACS2* and *LOX2*expression. (*A*) *ACS2*, (*B*) *LOX2* expression 1 d after cutting. White bar: cut stem; gray bar: noncut stem. The mean ± SE is shown (n = 3). Experiments were repeated three times with different seed batches and normalized using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

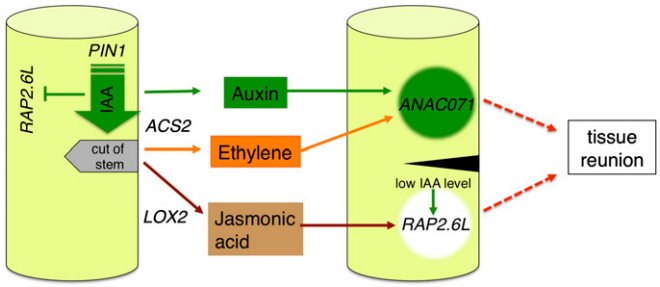

Fig. S7. Schematic model of phytohormone regulation of the expression of *ANAC071* and *RAP2.6L*-TFs during tissue reunion. The tissue-reunion process is operated differently between the upper and lower parts of the cut gap. *Upper: ANAC071*-TF was up-regulated by IAA and ethylene, a wound-inducible hormone. *Lower: RAP2.6L* was activated because of auxin depletion. Jasmonic acid, 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

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

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

Other Supporting Information Files

Dataset S1 (XLSX)