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Supplemental information

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Transcriptional landscapes of *de novo* root regeneration from detached *Arabidopsis* leaves revealed by time-lapse and single-cell RNA sequencing analyses

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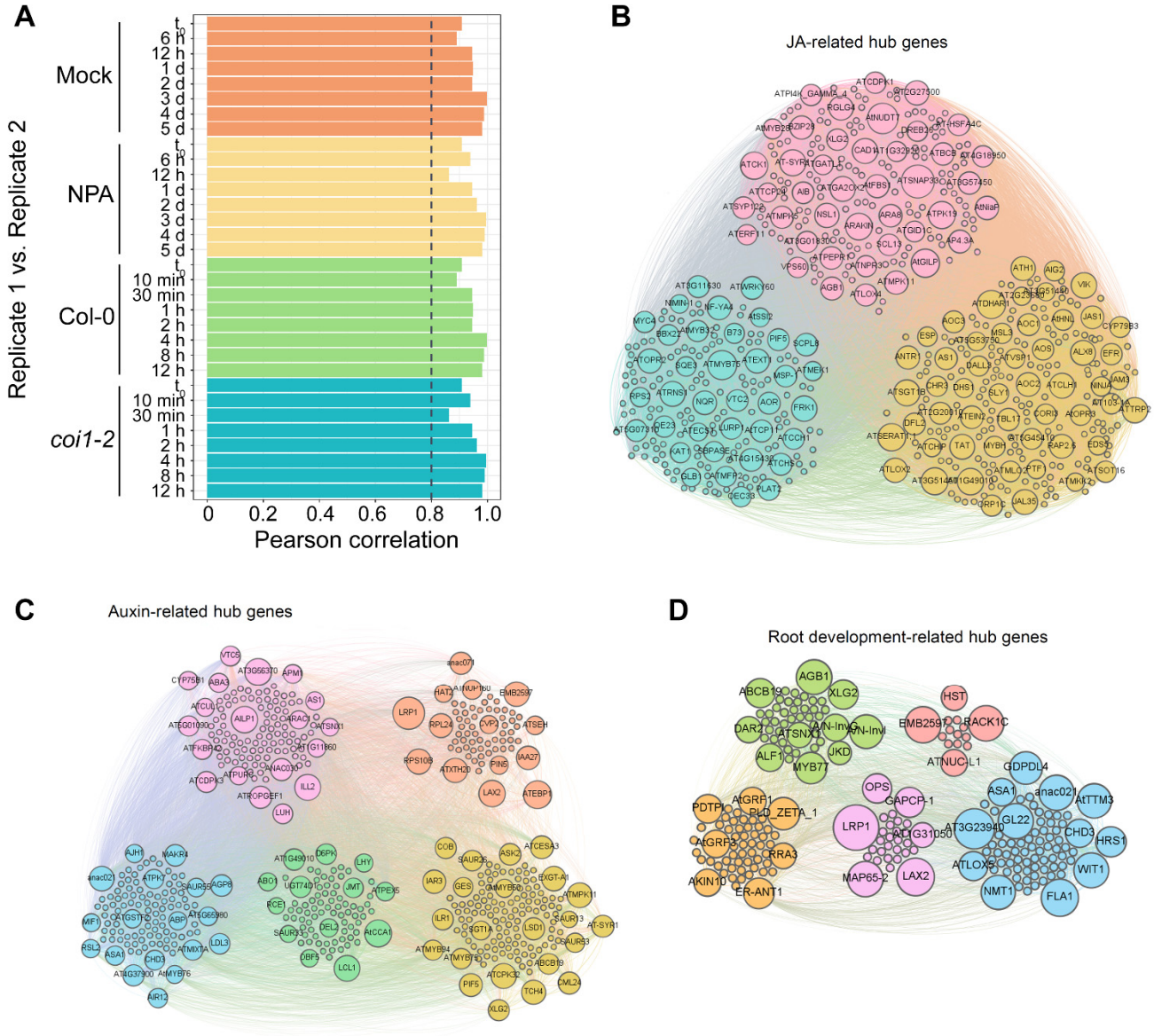
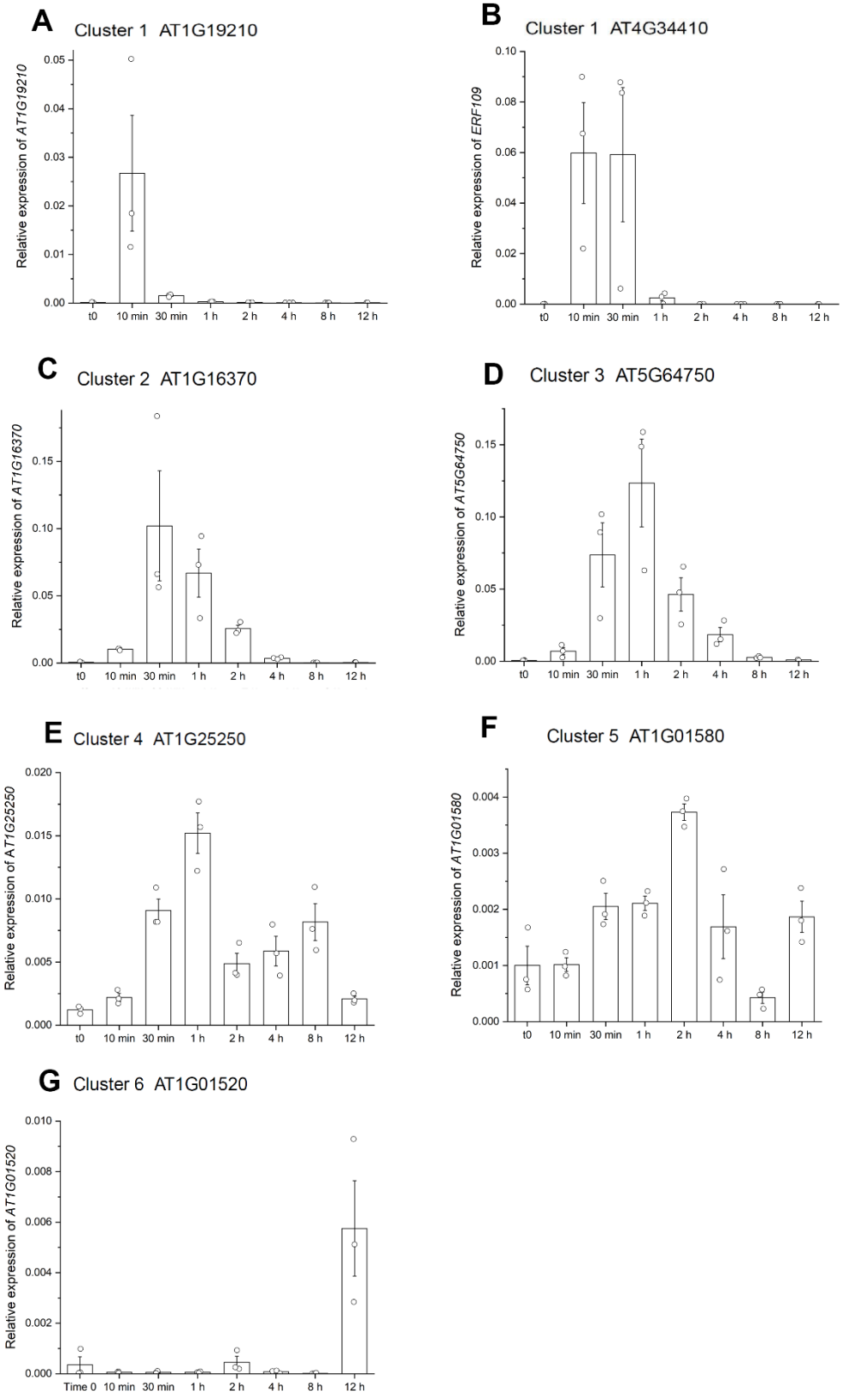


Supplemental Figure 1. Quality control of RNA-seq data and examples of co-expression sub-networks.

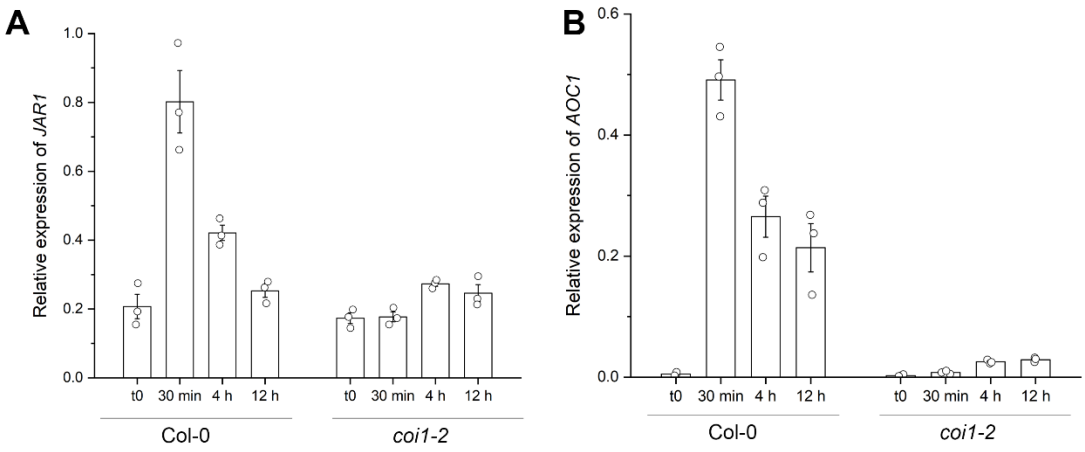
(A) Correlation between biological replicates in time-lapse RNA-seq analysis indicated by Pearson correlation coefficients between gene expression values (TPM) in two replicates.

(B–D) Examples of JA-related (B), auxin-related (C) and root development-related (D) hub genes in the co-expression sub-network. Size of dots represents the membership of genes. Color of dots represents the class of genes. The JA-related co-expression sub-network was built using the time-lapse RNA-seq data of Col-0 and *coi1-2*, and the auxin- and root development-related co-expression sub-networks were built using the time-lapse RNA-seq data of mock and NPA treatment. See Table S1 for the full list of hub genes.



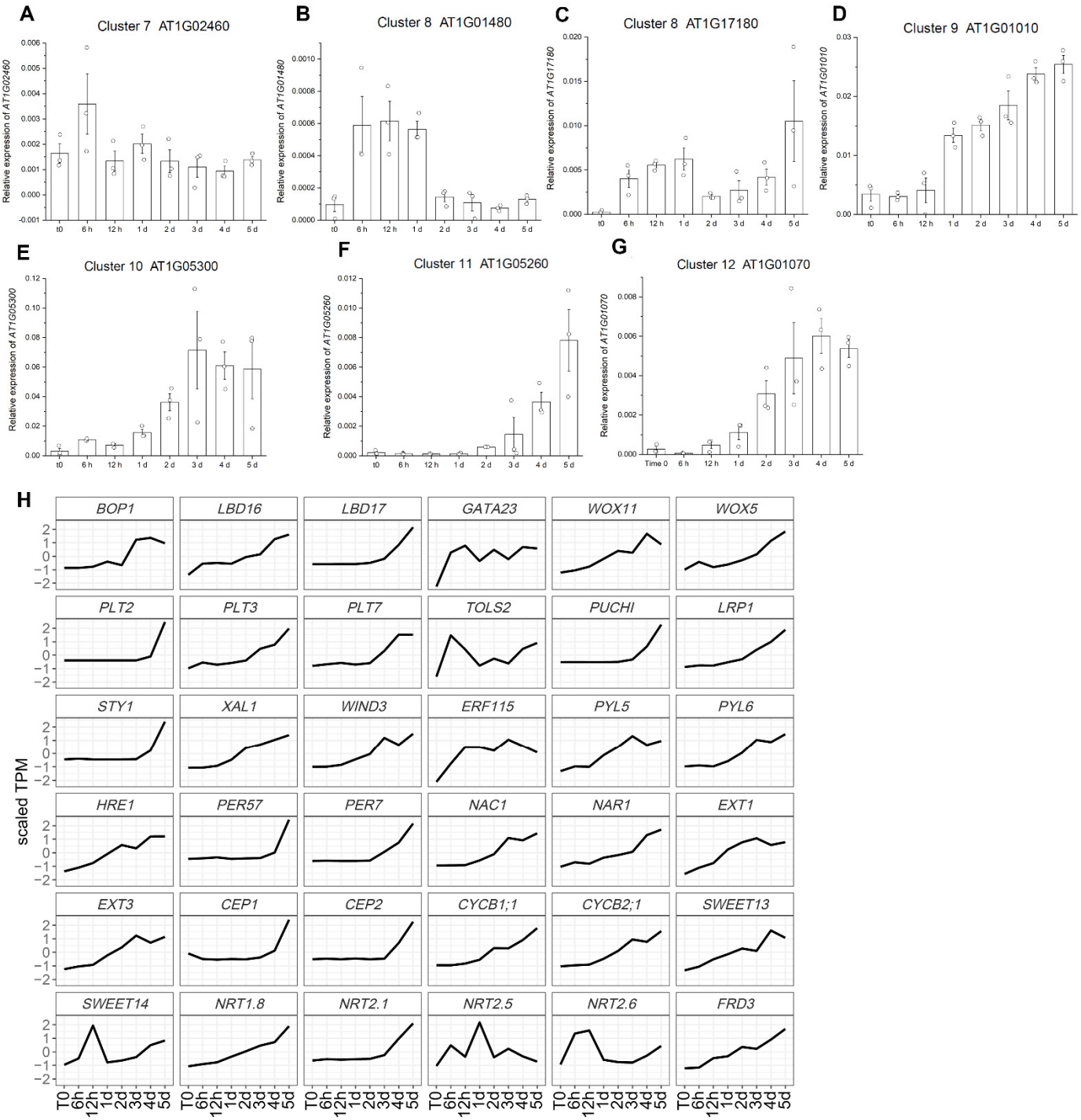
Supplemental Figure 2. Analysis of gene expression levels in gene-clusters 1 to 6.

(A–G) Selected genes from the gene-clusters 1 to 6 and qRT-PCR analysis of their expression levels. The whole leaf explants of Col-0 were used for qRT-PCR. Error bars show SEM with three biological replicates.



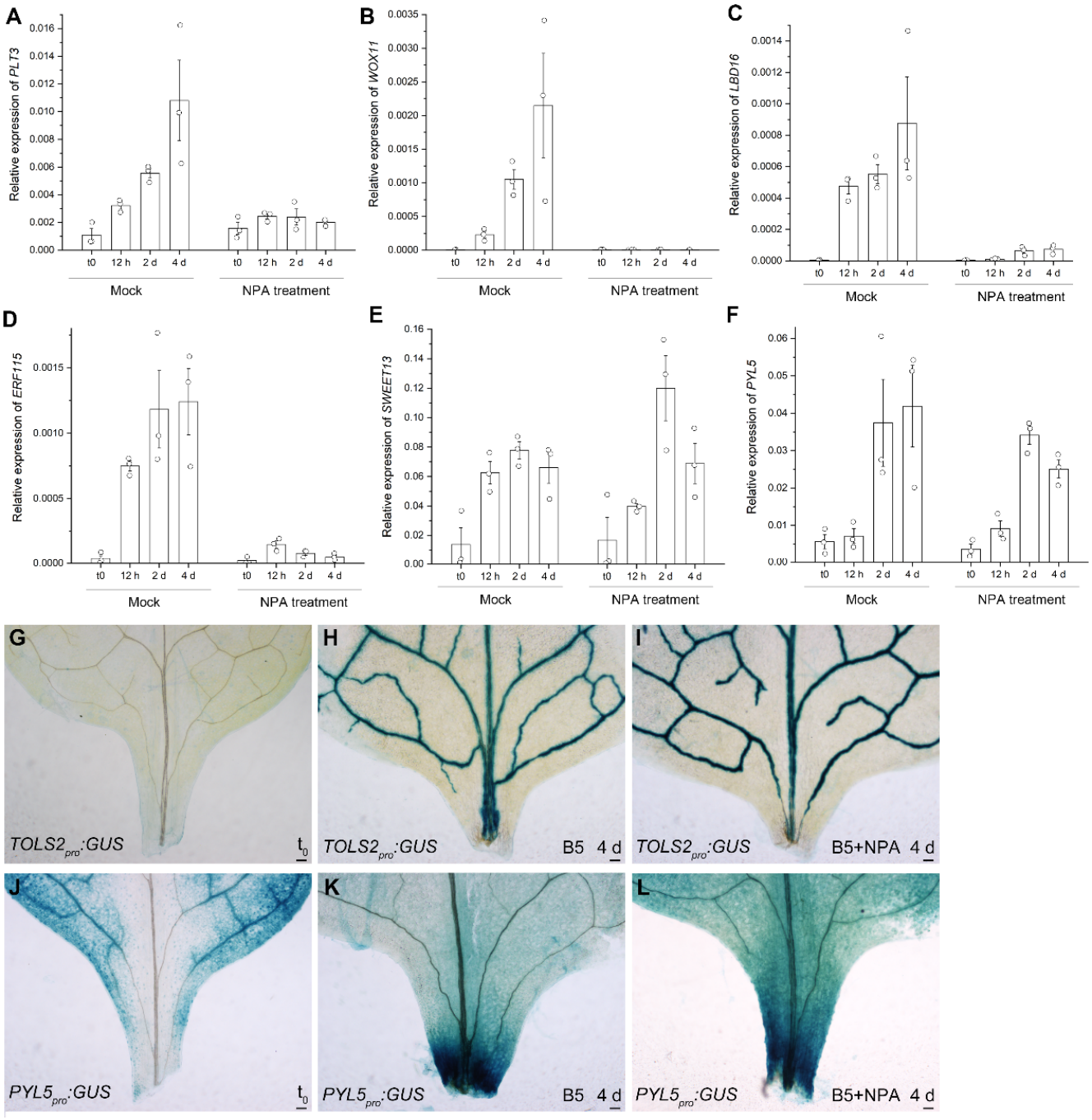
Supplemental Figure 3. Analysis of gene expression levels in Col-0 and *coi1-2*.

(A, B) qRT-PCR analysis of the expression levels of *JAR1* (A) and *AOC1* (B) in Col-0 and *coi1-2*. The whole leaf explants were used for qRT-PCR. Error bars show SEM with three biological replicates.



Supplemental Figure 4. Analysis of gene expression levels in gene-clusters 7 to 12.

(A–G) Selected candidate genes from the gene-clusters 7 to 12 and qRT-PCR analysis of their expression levels. The wounded region of Col-0 leaf explants was used for qRT-PCR. Error bars show SEM with three biological replicates.
(H) Expression patterns of selected candidate genes in time-lapse RNA-seq data.

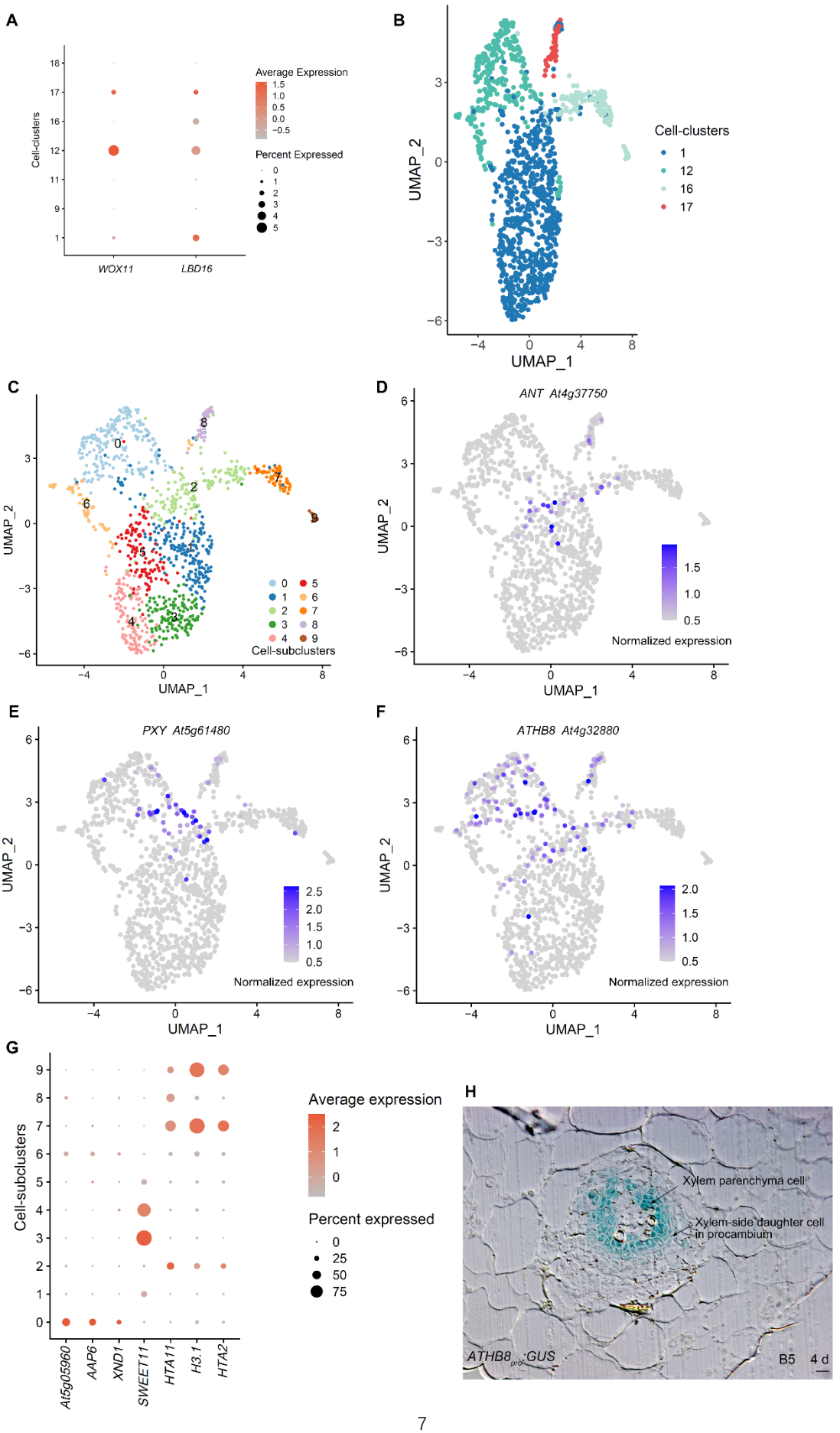


Supplemental Figure 5. Analysis of gene expression levels by NPA treatment.

(A–F) qRT-PCR analysis of the expression levels of *PLT3* (A), *WOX11* (B), *LBD16* (C), *ERF115* (D), *SWEET13* (E) and *PYL5* (F) by NPA treatment or in mock control. The wounded region of Col-0 leaf explants was used for qRT-PCR. Error bars show SEM with three biological replicates.

(G–L) GUS staining of *TOLS2*$_{pro}$*:GUS* (G–I) and *PYL5*$_{pro}$*:GUS* (J–L) leaf explants at t_0 (G,J) and 4 d (H,I,K,L) cultured on B5 medium (H,K) or B5 medium with 5 μM NPA treatment (I,L).

Scale bars, 100 μm (G–L).


A
Cell-clusters
WOX11
LBD16
Average Expression
Percent Expressed
B
UMAP_2
UMAP_1
Cell-clusters
C
UMAP_2
UMAP_1
Cell-subclusters
D
ANT At4g37750
Normalized expression
E
PXY At5g61480
Normalized expression
F
ATHB8 At4g32880
Normalized expression
G
Cell-subclusters
At5g05960
AAP6
XND1
SWEET11
HTA11
H3.1
HTA2
Average expression
Percent expressed
H
Xylem parenchyma cell
Xylem-side daughter cell in procambium
ATHB8pro::GUS
B5 4 d

Supplemental Figure 6. Gene expression patterns in single-cell RNA-seq.

(A) Dot plot of *WOX11* and *LBD16* showing their expression patterns in the vascular (cell-clusters 1, 9, 11, 12, and 18) and dividing cells (cell-clusters 16 and 17). Note that the two marker genes for adventitious root primordium initiation are highly enriched in cell-clusters 1, 12, 16, 17.
(B) Original cell-clusters in the new UMAP of cell-subclusters. Cell-clusters 1, 12, 16, and 17 were selected for reconstruction of UMAP analysis. Note that cell-subcluster 8 was mainly from cell-cluster 17, cell-subclusters 0 and 6 were mainly from cell-cluster 12, cell-subclusters 1, 3, 4, and 5 were mainly from cell-cluster 1, cell-subcluster 7 was mainly from cell-cluster 16, and cell-subcluster 2 was mainly from cell-clusters 1, 12, 16.
(C–F) UMAP plots showing cell-subclusters 0 to 9 (C), and *ANT* (D), *PXY* (E), and *ATHB8* (F) expression patterns.
(G) Dot plot of marker genes showing the cell identities of cell-subclusters.
(H) GUS staining of $ATHB8_{pro}$*:GUS* leaf explants at 4 d cultured on B5 medium.
Scale bar, 100 μm (H).

Supplemental Table 1. Overview of time-lapse RNA-seq data.

Overview of time-lapse RNA-seq data in Col-0 and *coi1-2*. Genes with significantly increased or decreased transcript levels at each time point after leaf detachment compared with that at t_0 in Col-0 were listed (log_2[fold change] > 1 or < -1 and FDR < 0.05). The gene normalized counts in *coi1-2* were listed, showing their regulations by the JA pathway.
GSEA of GO terms in up- or down-regulated genes in Col-0. Full list of GO terms significantly enriched in up- and down-regulated genes at each time point after leaf detachment compared with that at t_0 in Col-0.
List of hub genes in time-lapse RNA-seq data of Col-0 and *coi1-2*. Genes with high membership in each module were defined as hub genes of the co-expression network. Transcription factors were marked.
Overview of time-lapse RNA-seq data in mock and NPA treatment. Genes with significantly increased or decreased transcript levels at each time point after leaf detachment compared with that at t_0 in mock were listed (log_2[fold change] > 1 or < -1 and FDR < 0.05). The gene normalized counts in NPA treatment were listed, showing their regulations by the auxin pathway.
GSEA of GO terms in up- or down-regulated of genes in mock. Full list of GO terms significantly enriched in up- and down-regulated genes at each time point after leaf detachment compared with that at t_0 in mock.
List of hub genes in time-lapse RNA-seq data of mock and NPA treatment. Genes with high membership in each module were defined as hub genes of the co-expression network. Transcription factors were marked.

Supplemental Table 2. Analysis of gene-clusters 1 to 6.

List of gene-clusters 1 to 6. In Col-0 leaf explants, genes with undetectable or very low transcript levels (TPM < 2) at t_0 but significantly increased transcript levels (log_2[fold change] > 2 and FDR < 0.05) at any time point after leaf detachment compared with that at t_0 were grouped into gene-clusters 1 to 6 based on their expression patterns.
Full list of genes and their GO terms in gene-clusters 1 to 6.

Supplemental Table 3. Analysis of differentially expressed genes between Col-0 and *coi1-2* at 1 h.

Full list of differentially expressed genes between Col-0 and *coi1-2* at 1 h and their GO terms.
Full list of cis element analysis of differentially expressed genes between Col-0 and *coi1-2* at 1 h.

Supplemental Table 4. Analysis of gene-clusters 7 to 12.

List of gene-clusters 7 to 12. In the wounded region of the mock control, genes with undetectable or very low transcript levels (TPM < 2) at t_0 but significantly increased

transcript levels (log_2[fold change] > 2 and FDR < 0.05) at any time point after leaf detachment compared with that at t_0 were grouped into gene-clusters 7 to 12 based on their expression patterns.
Full list of genes and their GO terms in gene-clusters 7 to 12.

Supplemental Table 5. Analysis of differentially expressed genes between mock and NPA treatment at 4 and 5 d.

Full list of differentially expressed genes between mock and NPA treatment at 4 and 5 d and their GO terms.
Full list of cis element analysis of differentially expressed genes between mock and NPA treatment at 4 and 5 d.

Supplemental Table 6. List of cell-clusters 0 to 18 and cell-subclusters 0 to 9.

Analysis of specific marker genes in cell-clusters 0 to 18 in single-cell RNA-seq data.
Analysis of specific marker genes in cell-subclusters 0 to 9 in single-cell RNA-seq data.

Supplemental Table 7. Information of this study.

Primers used in this study. Note that lower case letters represent additional nucleotides to introduce restriction sites.
Mapping efficiency of RNA-seq.
List of auxin-, JA- and root development-related genes.
List of gene accession numbers.

Supplemental Data 1. Scripts used in this study.