SUPPORTING INFORMATION

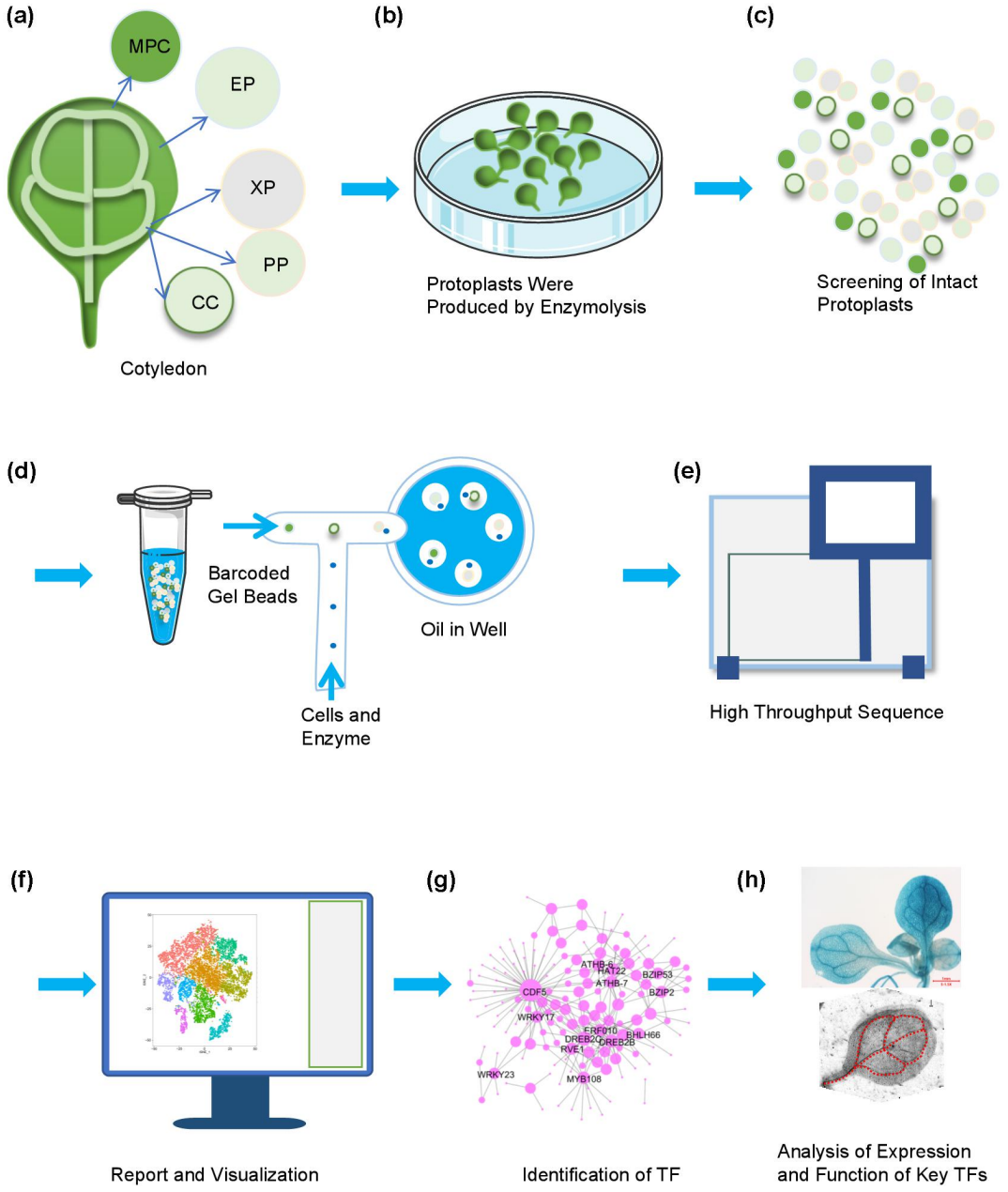

Figure S1 The experimental flow chart used in this study.

(**a**) Model of cotyledons and major representative cell types. MPC: mesophyll cell, EP:epidermis, PP: phloem parenchyma, XP: xylem parenchyma, CC: companion cell. (**b**) Protoplasts were prepared by enzymatic hydrolysis. (**c**) The protoplasts were screened by cell strainer. (**d**) Generation of single cell sequencing library by barcode technique. (**e**) Sequencing of the single cell library produced by second generation

sequencing system. (**f**) Analysis of the raw scRNA-seq data and visualization of the results. (**g**) Construction of transcriptional regulatory network of differentially expressed genes in specific cell types. (**h**) Analysis of the expression and physiological function of key transcription factors identified.

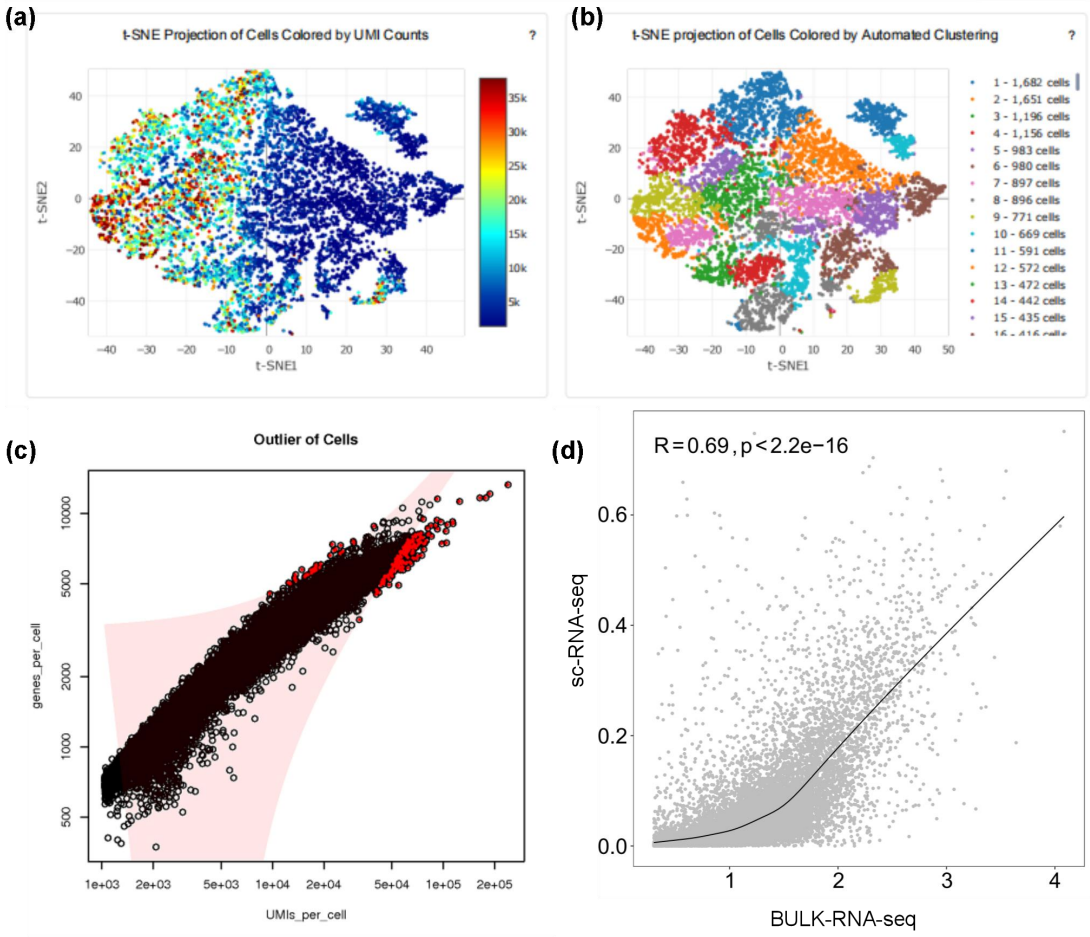

Figure S2 Quality control analysis of the raw data of scRNA-seq.

(**a**) Projection of cells colored by UMI (unique molecular identifier) counts onto a t-SNE plot. (**b**) Projection of cells colored by automated clustering onto a t-SNE plot. (**c**) Analysis of outlier cells by calculating the ratio of UMI/gene number. (**d**) Correlation analysis of gene expression in cotyledons, as inferred from scRNA-seq profiling of cotyledons (gene expression from all the cells was averaged) (r = 0.69, p< 2.2 e-16, Pearson; fit line by LOESS).

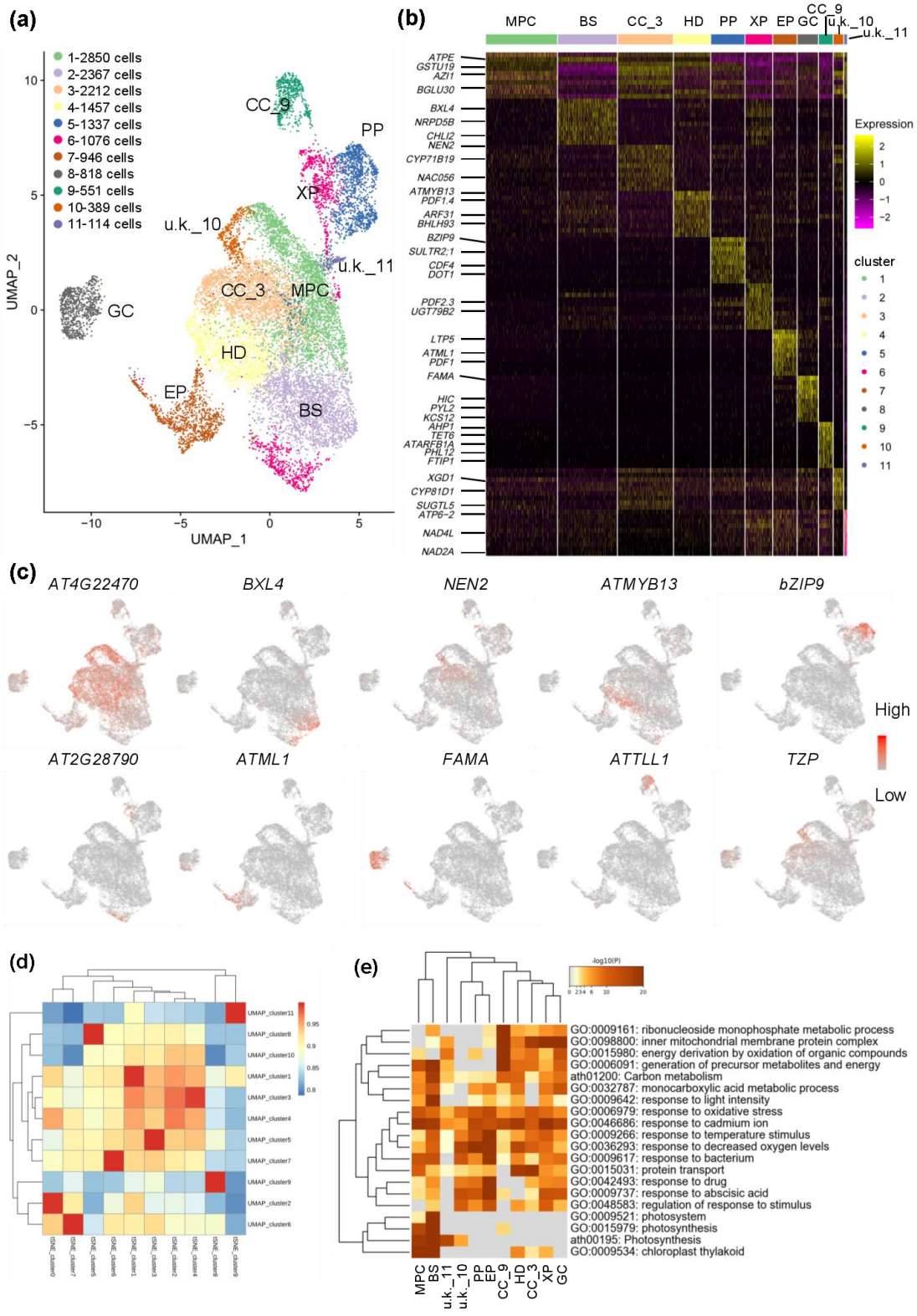

Figure S3 UMAP analysis of the cells.

(**a**) Visualization of the cell clusters by Uniform Manifold Approximation and Projection (UMAP) algorithm. Cell clusters identified by tSNE are marked on the UMAP plot. BS: bundle sheath; MPC: mesophyll cell; PP: phloem parenchyma; HD:

hydathode; CC: companion cell; GC: guard cell; EP: epidermis; XP: xylem parenchyma. (**b**) Expression of identified marker genes by UMAP is shown as a heatmap. (**c**) Feature plots show the expression of representative marker genes in each cell type. (**d**) Pearson correlation analysis between tSNE and UMAP was performed with the corresponding cell clusters. (**e**) GO enrichment of DEGs in all of cell clusters.

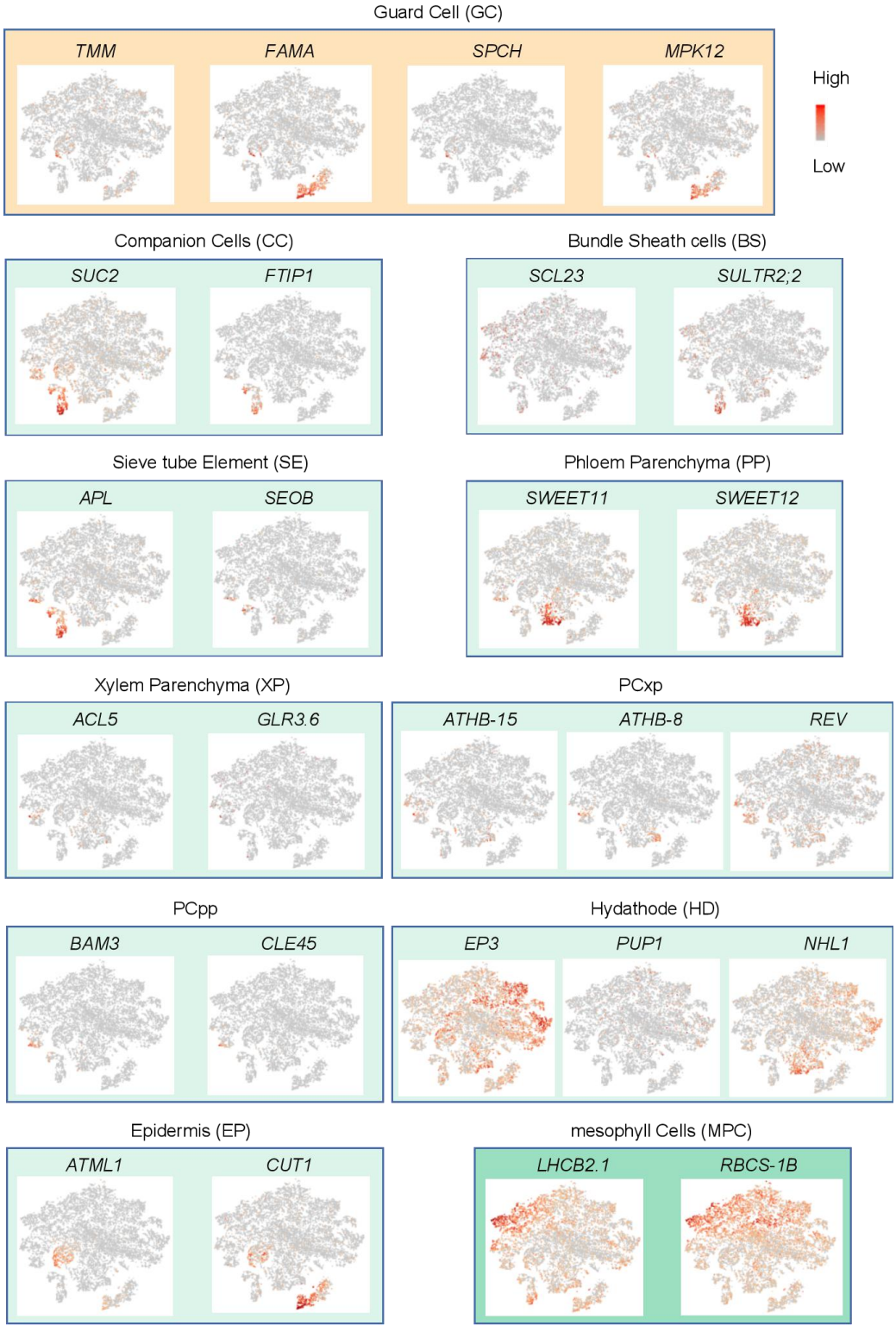

Figure S4 Analysis of the expression of known marker genes of different kinds of cell types on tSNE plot.

Feature plots of expression distribution for selected marker genes. Expression levels for each cell are color-coded and overlaid onto the tSNE plot.

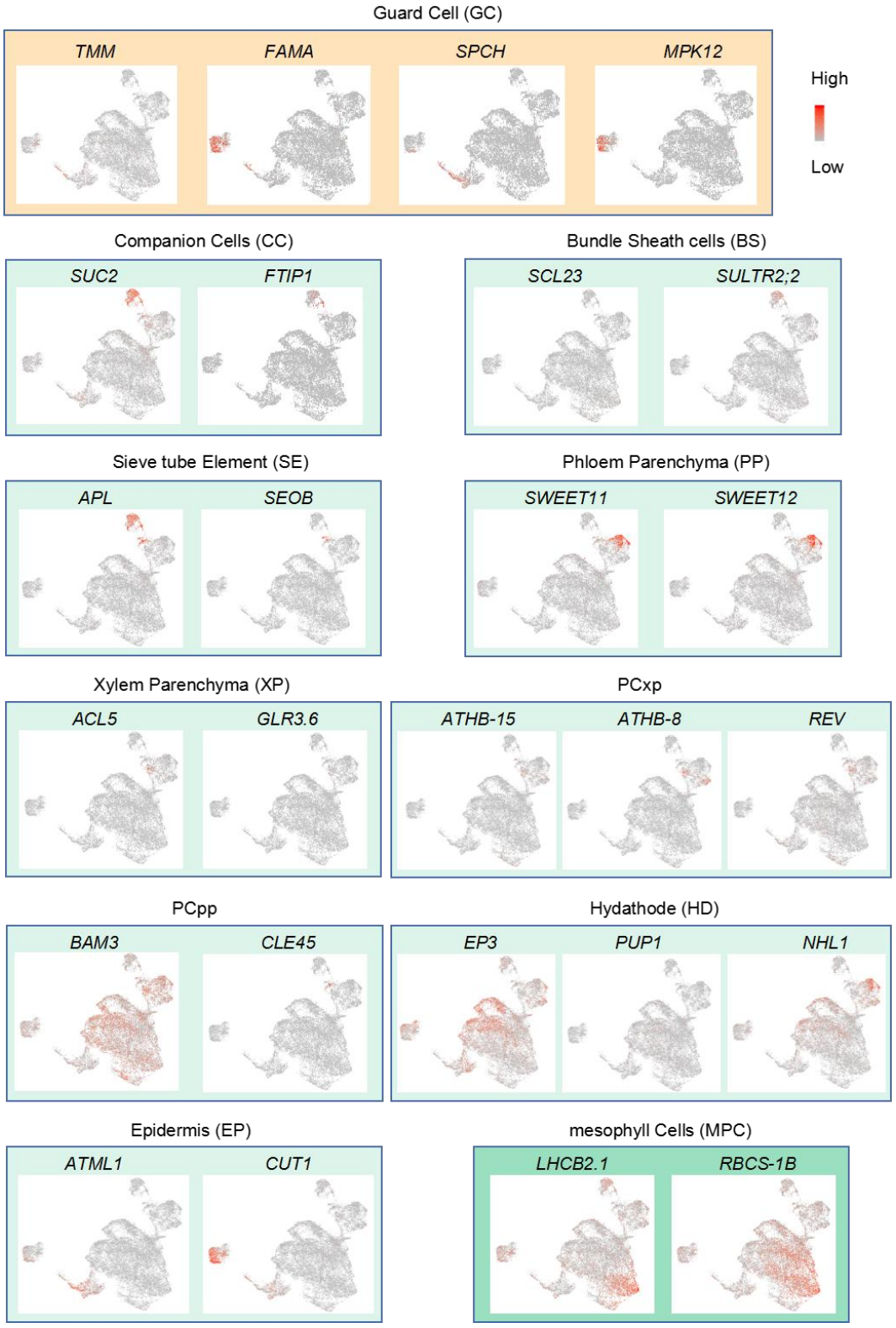

Figure S5 Analysis of the expression of known marker genes of different kinds of cell types on UMAP plot.

Feature plots of expression distribution for selected marker genes. Expression levels for each cell are color-coded and overlaid onto the UMAP plot.

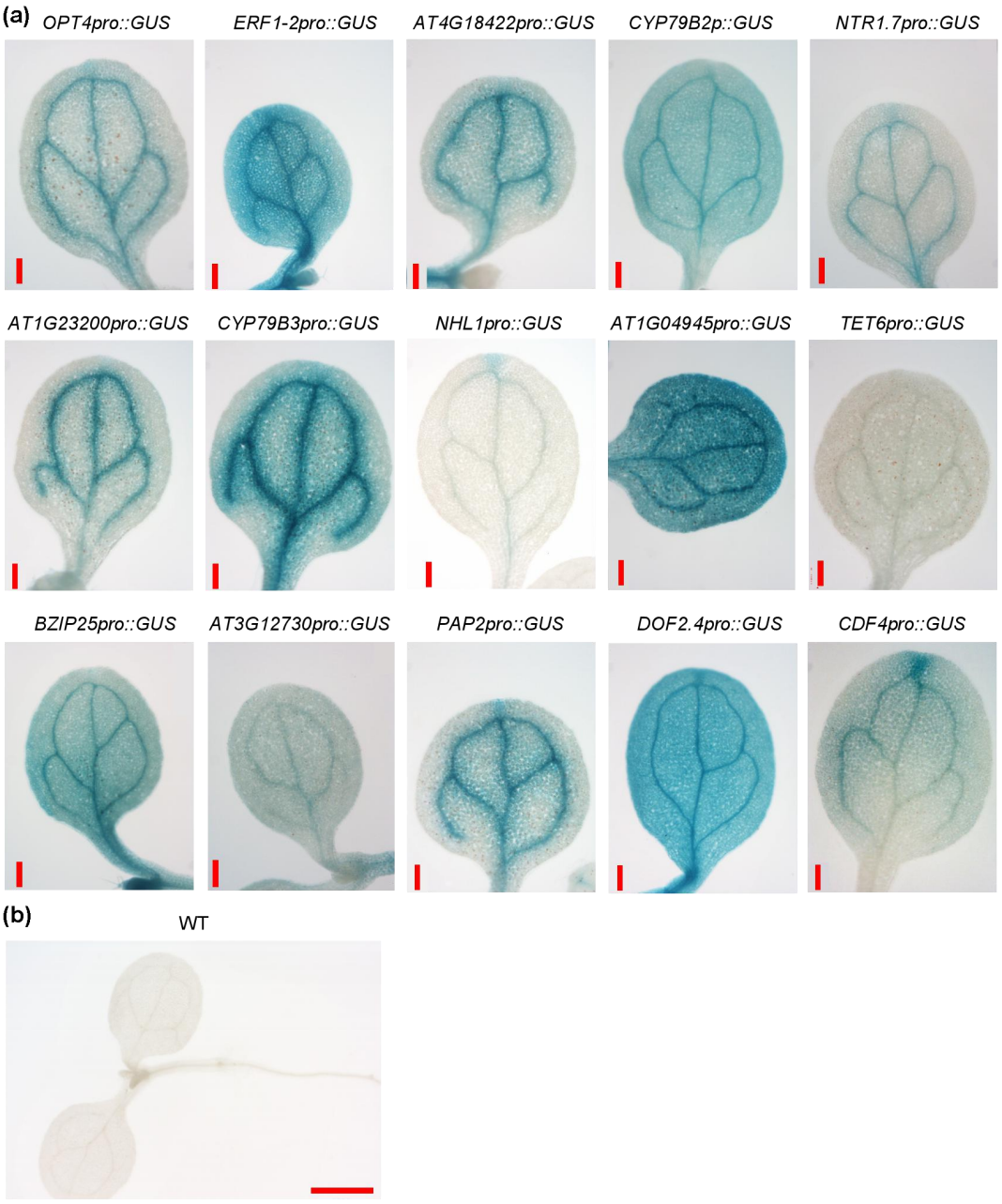

Figure S6 Analysis of the expression of representative vein marker genes.

To detect the expression patterns of representative vein marker genes, we generated transgenic plants expressing the GUS reporter driven by the promoter of these representative vein marker genes, respectively. (**a**) The GUS signals of representative vein marker genes were detected in the cotyledons. Scale bar: 200 μm. (**b**) Detection of the GUS activity in 3-day-old seedling of wild type (WT). Scale bar: 1000 μm.

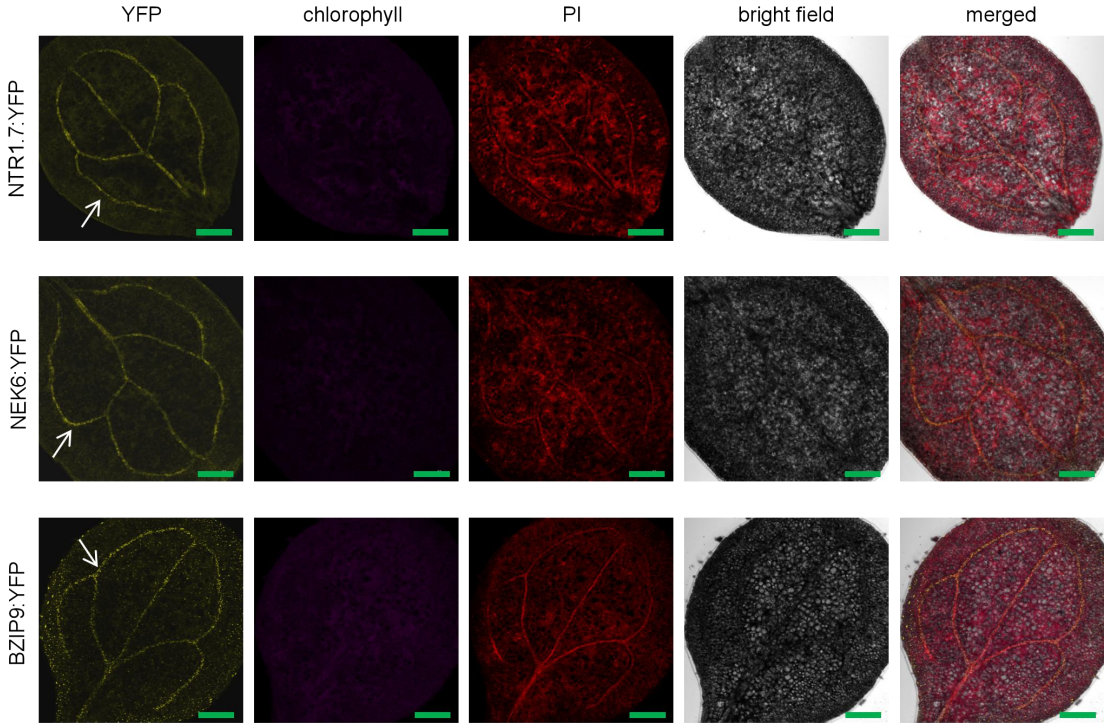

Figure S7 NTR1.7, NEK6, and BZIP9 were expressed in vein.

The expression of YFP of NTR1.7:YFP, NEK6:YFP and BZIP9:YFP can be detected in veins of 3-day-old cotyledons. Scale bar:200 μm. Write arrow showing the vein in cotyledons. PI: Propidium. YFP: Yellow Fluorescent Protein.

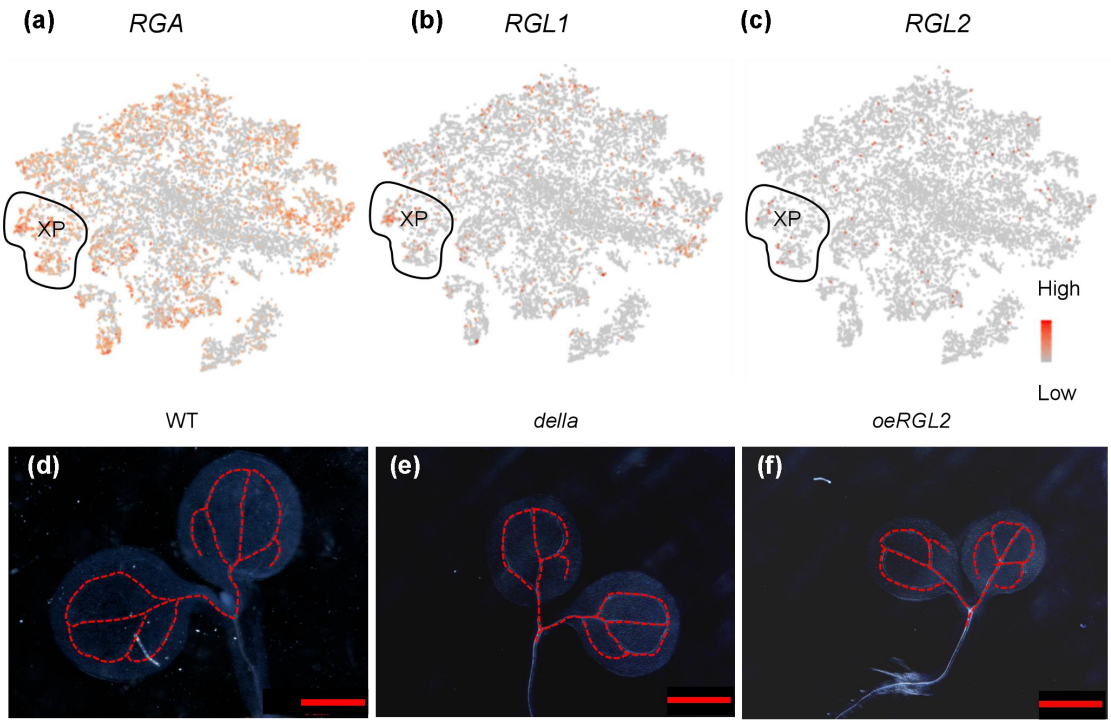

Figure S8 DELLA proteins are required for the regulation of the patterns of vascular systems in cotyledons.

(a) -(**c**) Feature plots of the expression of *RGA*, *RGL1* and *RGL2*. (**d**)-(**f**) Detection of the developmental patterns of vascular system in cotyledons of 3-day-old seedlings of wild-type (WT) seedlings, *della quintuple* mutant (*gai-t6*; *rga-t2*; *rgl1-1*; *rgl2-1*; *rgl3-1*), and *oeRGL2*， respectively. Scale bar: 500 μm.

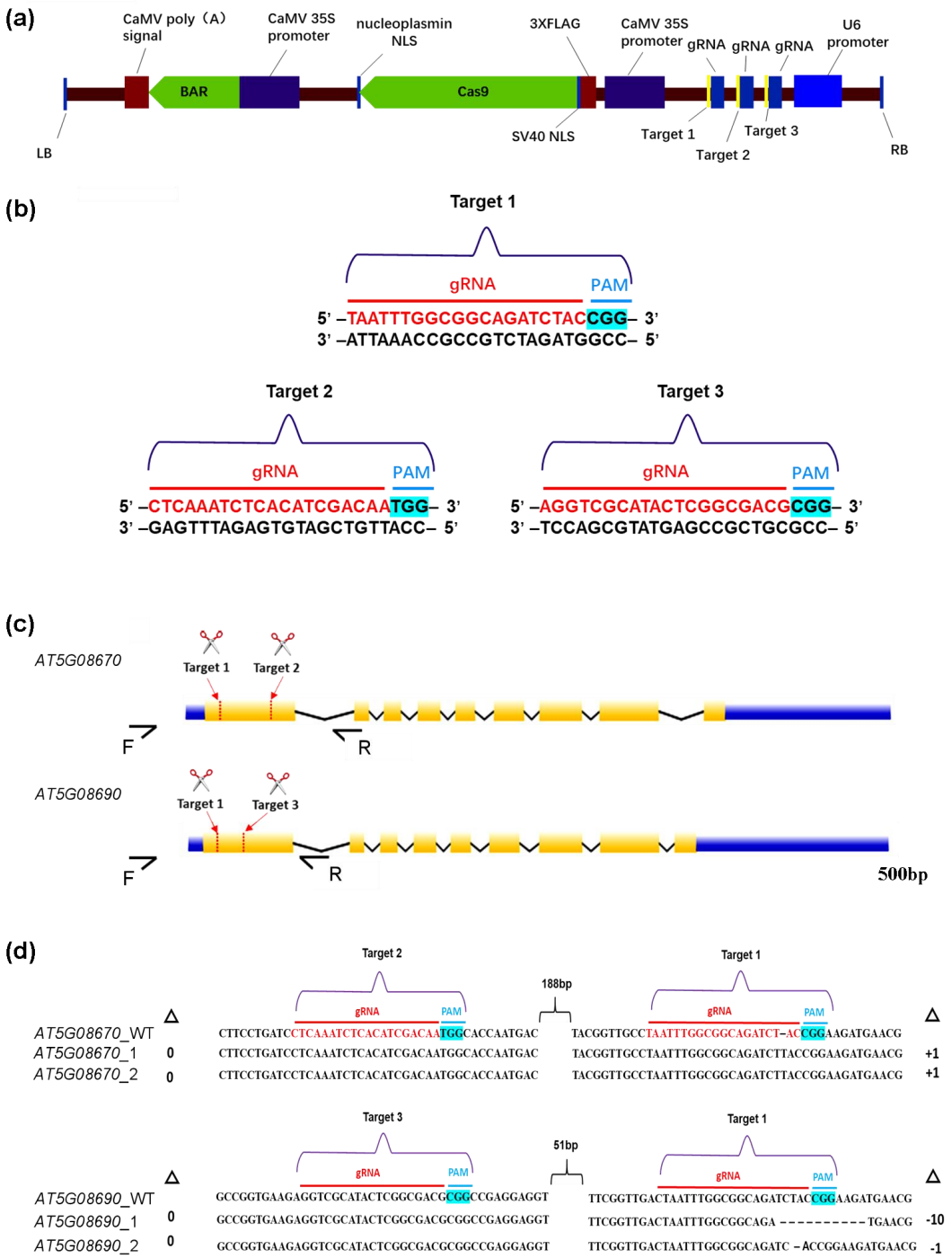

Figure S9 Vector information of gRNA CRISPR/Cas9 and double mutant verification.

(**a**) Crispr/cas9 vector for *Arabidopsis* transformation. The expression of *BAR* (anti herbicide gene that is a screening marker, and CAS9 protein was driven by 35S promoter, and the expression of three sgRNAs was driven by *Arabidopsis* U6 promoter (pU6). (**b**) sgRNA sequence, red letters are gRNA sequence, blue letters are protopackage adjacent motif (PAM) sequence. (**c**) The shear sites and identification

primers of *AT5G08670* and *AT5G08690* indicate that the lines represent intron region, blue represents UTR region, yellow represents exon region. (**d**) The results of target 1 and target 2 editing are identified by sequencing. The results of editing two T1 plants are shown. △ represents mutation results: -, deleted nucleotide; +, inserted nucleotide; 0, no deletion or insertion.

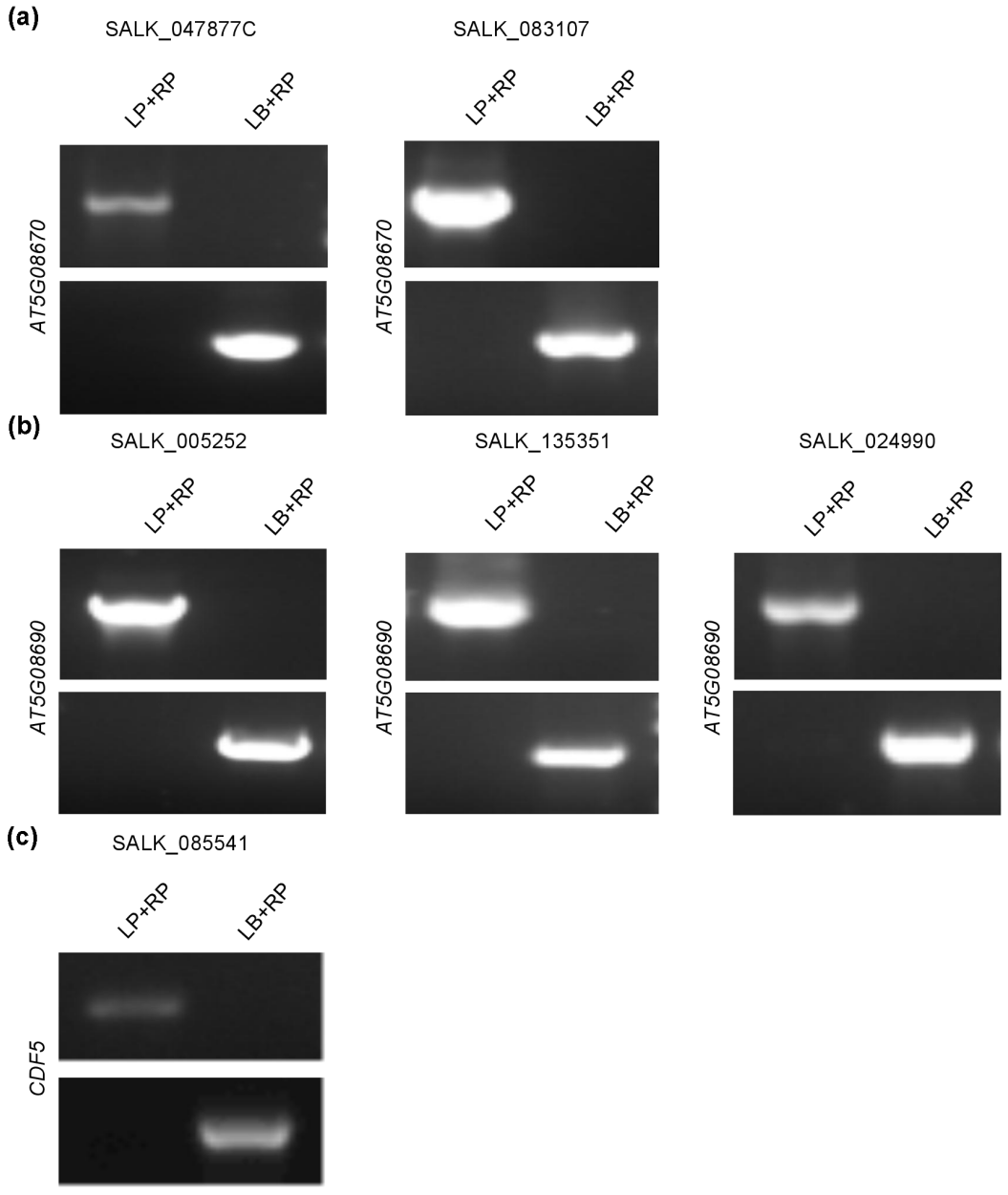

Figure S10 Verification of the T-DNA insertion of mutants.

DNA was extracted from the mutants and WT; the location of the T-DNA insertion in each mutant was verified by PCR with the corresponding primers. (**a**) Identification of

the homozygotes of *AT5G08670* mutants. (**b**) Identification of the homozygotes of *AT5G08690* mutants. (**c**) Identification of the homozygotes of *CDF5* mutant.

Table S6 List of mutant lines used in this study

Gene name	Accession number	Mutant line
AT5G08670		SALK_047877C
		SALK_083107
AT5G08690		SALK_005252
		SALK_135351
		SALK_024990
CDF5	AT2G34140	SALK_085541C

Table S7 List of oligonucleotides used in this study

Gene	**Name**	**Sequence (5' - 3')**
Plant transformant (cloned in PBGWFS7)		
ERF1-2	ERF1-2s	AAAAAGCAGGCTTCCAAGAAATACAATAGGTACT
	ERF1-2a	AGAAAGCTGGGTGGTTCACAATATTCAGAGATC
AT1G04945	AT1G04945s	AAAAAGCAGGCTTC TTGAATAGTATTACAATCTA
	AT1G04945a	AGAAAGCTGGGTG CCTTACCAACTAAAATGCCT
CDF4	CDF4s	AAAAAGCAGGCTTCAAAATTCATACTACAATTTA
	CDF4a	AGAAAGCTGGGTGGAAAACGTGCAGAGAATGGA
DOF2.4	DOF2.4s	AAAAAGCAGGCTTCCGTTCCTCGAGTTTTGCATT
	DOF2.4a	AGAAAGCTGGGTGGGTTATTCTCTTTTGATTTT
NHL1	NHL1s	AAAAAGCAGGCTTCTGCTTTTAAAAACTTGTTAA

	NHL1a	AGAAAGCTGGGTGGGTGGTTGTTTTTTTTCTTT
TET6	TET6s	AAAAAGCAGGCTTCGTCAAACGACTATTCGTTGC
	TET6a	AGAAAGCTGGGTGAGTAGTAATGTTATCAAGAA
BZIP25	BZIP25s	AAAAAGCAGGCTTCCATTCGCGGCGAGGATGATA
	BZIP25a	AGAAAGCTGGGTGCGCTTACCCAATCGCCTCAA
PAP2	PAP2s	AAAAAGCAGGCTTCAGTATAATTAAACCCTAGAG
	PAP2a	AGAAAGCTGGGTGTTTTTTCAAATATAATTGTG
CYP79B3	*CYP79B3s*	AAAAAGCAGGCTTCCCTCTGACGAAGAATTAAAA
	CYP79B3a	AGAAAGCTGGGTGTTGCAAGGAAGAAGGAGAAG
CYP79B2	*CYP79B2s*	AAAAAGCAGGCTTCGGAATATAAAGTTACTAATT
	CYP79B2a	AGAAAGCTGGGTGGTTTGTGTATAGAGAAGAAG
AT4G18422	*AT4G18422s*	AAAAAGCAGGCTTCCTAATTCGCTCAGCTTCGTT
	AT4G18422a	AGAAAGCTGGGTGAATTTTTCTAGGTTAAAAAA
AT1G23200	*AT1G23200s*	AAAAAGCAGGCTTCTATATAGTGACTCAAAACTG
	AT1G23200a	AGAAAGCTGGGTGTTGAGAAGTACCAATAGAGA
AT3G12730	AT3G12730s	AAAAAGCAGGCTTCACGATCTCAACAACCTGCGC
	AT3G12730a	AGAAAGCTGGGTGTTAACTAACAAAGTACTAAA
NRT1.7	NRT1.7s	AAAAAGCAGGCTTCATTAAAATGCATATGTTTGA
	NRT1.7a	AGAAAGCTGGGTGCTCTAAGATATTACTACACT
Plant transformant (cloned in pB7YWG2)		
BZIP9	BZIP9s	AAAAAGCAGGCTTCATGGATAATCACACAGCTAA
	BZIP9a	AGAAAGCTGGGTGTGGCCAGATGTCTGAGACGC
CDF4	CDF4s	AAAAAGCAGGCTTCATGGCGACTCAAGATTCTCA
	CDF4a	AGAAAGCTGGGTGGCACGATTGACCGTCGGAGT
AT3G12730	AT3G12730s	AAAAAGCAGGCTTCATGATGCAGTCGAGAGAGGA
	AT3G12730a	AGAAAGCTGGGTGACCGAGACAAACCGTACGGC

NEK6	NEK6s	AAAAAGCAGGCTTCATGGAGTCACGAATGGATCA
	NEK6a	AGAAAGCTGGGTGTGAACAATTCCTGGAGCTGC
ARFB1A	ARFB1As	AAAAAGCAGGCTTCATGGGAGCCAGATTTTCACG
	ARFB1Aa	AGAAAGCTGGGTGATACCTCGGACCTCGGACCA
ATNPF2.13	ATNPF2.13s	AAAAAGCAGGCTTCATGGTTTTGGAGGATAGAAA
	ATNPF2.13a	AGAAAGCTGGGTGTTTCATCGATTTCTTCGAAG
BZIP25	BZIP25s	AAAAAGCAGGCTTCATGCACATCGTCTTCTCTGT
	BZIP25a	AGAAAGCTGGGTGATGCTTGTGATTCCAATGGG
Mutant confirmed		
SALK_083107	LP	GACCACTTCTTCTGCGTAACG
	RP	AATGGTCCTGACGACATTCTG
SALK_005252	LP	CTGAGAGCAAATGTGCCCTAG
	RP	ACCTCATCGATACCTCCAACC
SALK_135351	LP	ACTGCAGCACTACATGCATTG
	RP	TCCATAGCAATGGTCCTAACG
SALK_024990	LP	GTATTTCCGTGATGCTGAAGG
	RP	CGAATGAATCTGTTTTCTCCG
SALK_047877	LP	TACAAAAGCTAAGAGCGACGC
	RP	GCAAGTACGATGATCTTTCCG
SALK_085541	LP	AAAATCATGCCGGAAACTACC
	RP	AATTTCGACAACCCTGAAATTC
	LBb1.3	ATTTTGCCGATTTCGGAAC
		Targets selection
AT5G08670	Target 1	TAATTTGGCGGCAGATCTACCGG
	Target 2	AGGTCGCATACTCGGCGACGCGG
AT5G08690	Target 1	TAATTTGGCGGCAGATCTACCGG
	Target 3	CTCAAATCTCACATCGACAATGG
		Primer for intermediate vectors

AT5G08670	Target 1	AT5G-Y1(+):cagtGGTCTCatgcataatttggcggcagatctac
		AT5G-Y1(-):cagtGGTCTCaaaacgtagatctgccgccaaatta
AT5G08670	Target 2	AT5G-B1(+):cagtGGTCTCatgcaaggtcgcatactcggcgacg
		AT5G-B1(-):cagtGGTCTCaaaaccgtcgccgagtatgcgacct
AT5G08690	Target 1	AT5G-Y1(+):cagtGGTCTCatgcataatttggcggcagatctac
		AT5G-Y1(-):cagtGGTCTCaaaacgtagatctgccgccaaatta
AT5G08690	Target 3	AT5G-A1(+):cagtGGTCTCatgcactcaaatctcacatcgacaa
		AT5G-A1(-):cagtGGTCTCaaaacttgtcgatgtgagatttgag