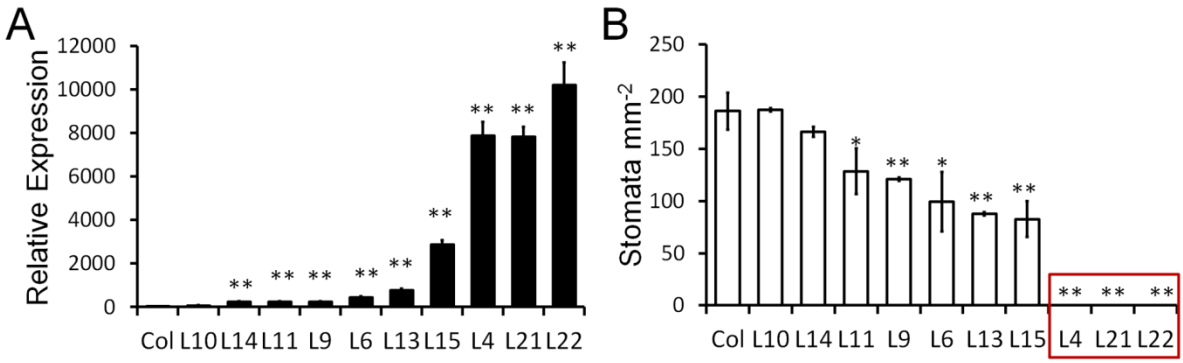
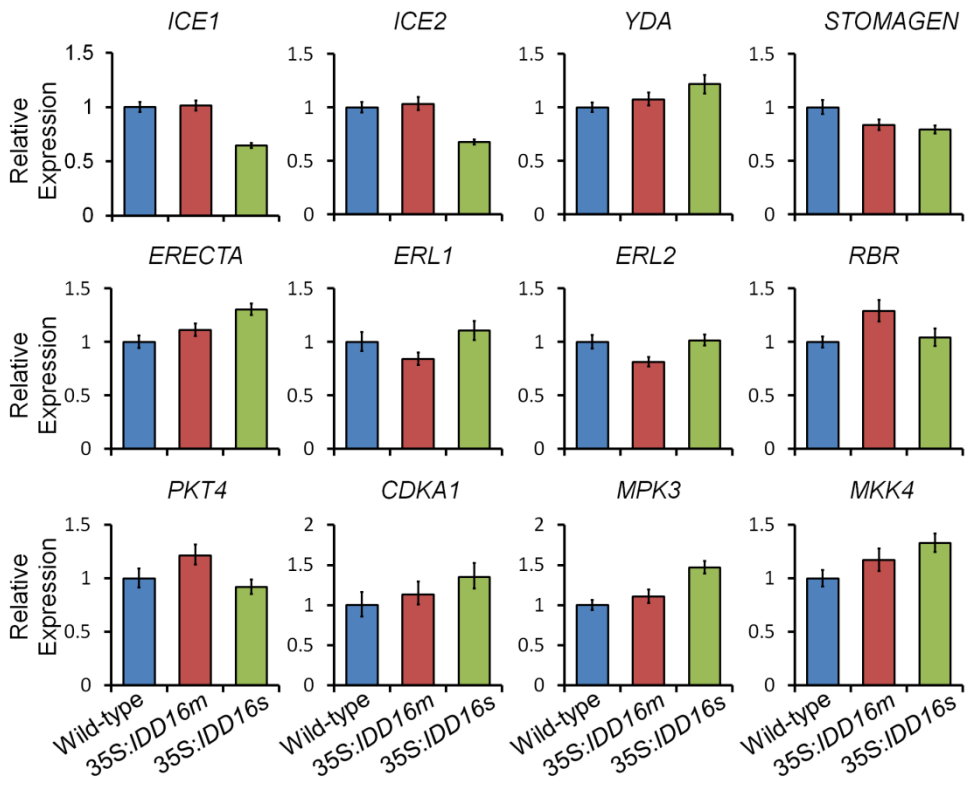
Supporting Information



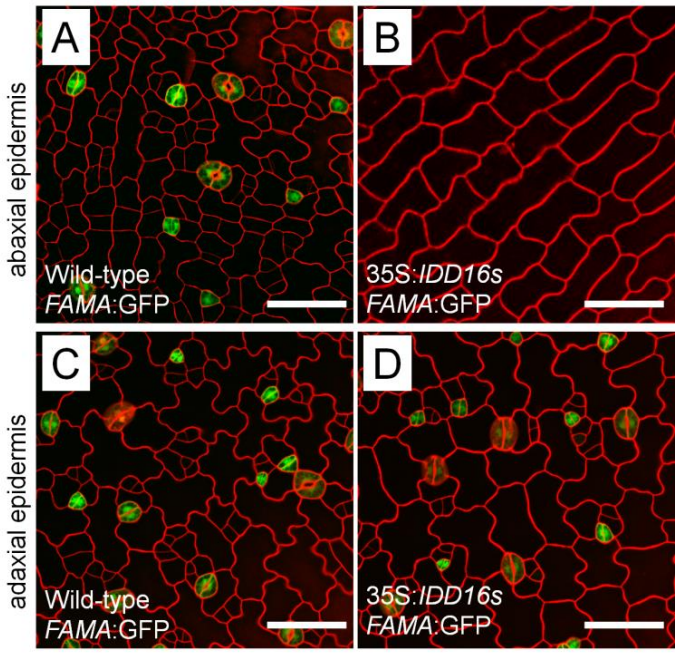
Supplemental Figure 1. Correlation between stomatal density in cotyledons and *IDD16* transcripts level.

(A) Expression level of *IDD16* in transgenic plants. qRT-PCR was performed on total RNA from 4-DAG wild-type and *IDD16*-OE plants. The expression level in wild-type was normalized as 1. **(B)** Stomatal density in cotyledons of 8-DAG *IDD16*-overexpression plants. Values are means ± SE from three biological replicates. Asterisks indicate statistical significance based on Student's t test; ** $P < 0.01$, * $P < 0.05$.



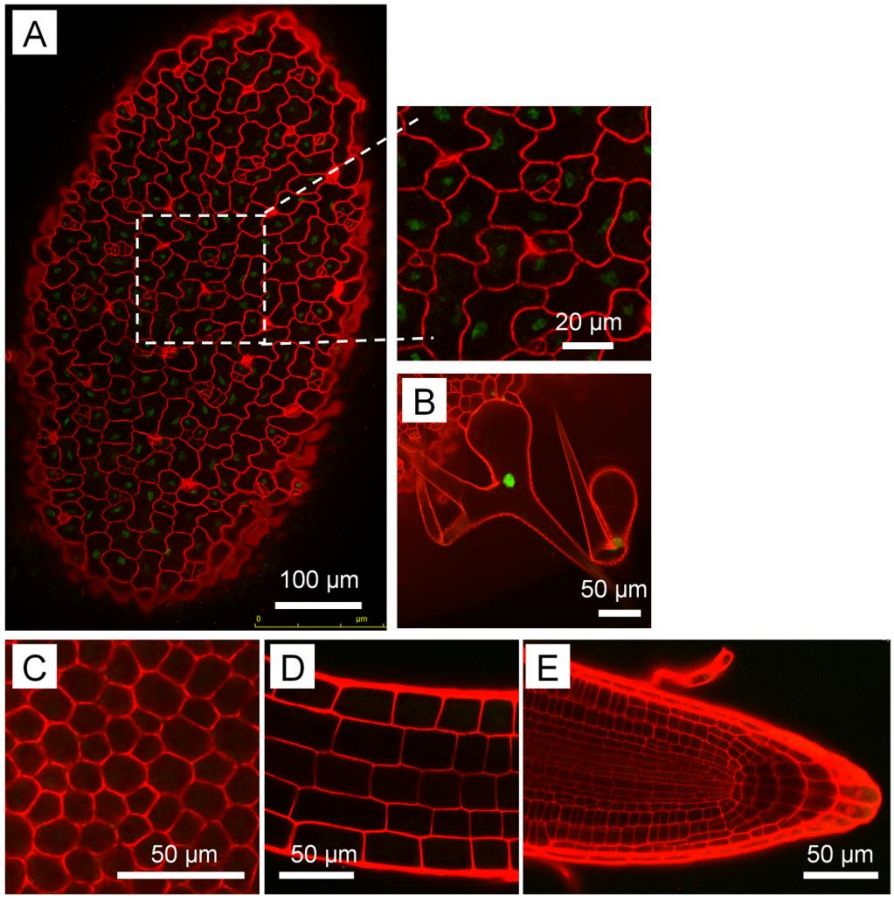
Supplemental Figure 2 Analysis of the expression of the genes related to stomatal development.

qRT-PCR was performed on total RNA from 4-DAG Wild-type and *IDD16*-OE plants. The expression level in wild-type was normalized as 1. Data represent means ± SE of three biological replicates.



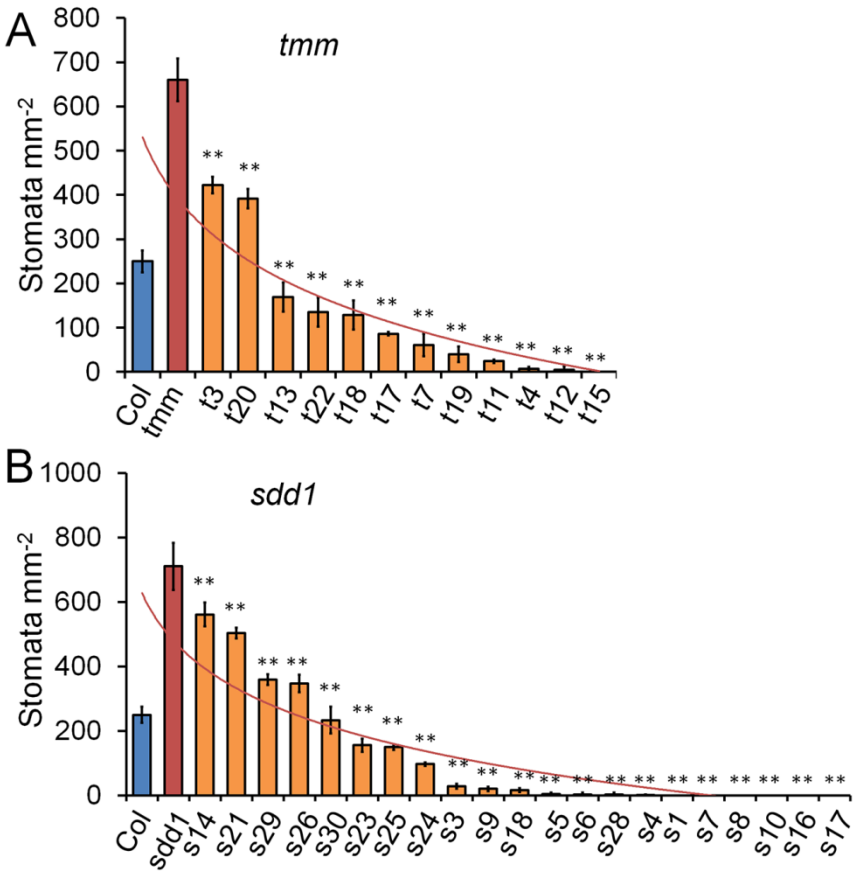
Supplemental Figure 3 The effect of *IDD16* overexpression on *FAMA* expression.

(A and B) Confocal images of *FAMA*:GFP abaxial cotyledons in 2-DAG wild-type and *35S:IDD16s* background, respectively. **(C and D)** show the images of *FAMA*:GFP on adaxial epidermis of cotyledons. Epidermal cell periphery is highlighted by propidium iodide (red) staining. Scale bars: 50 μm.



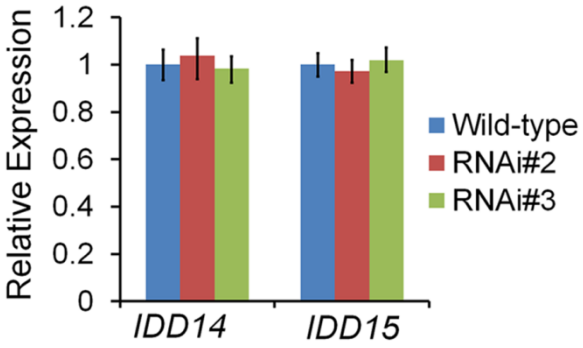
Supplemental Figure 4 The expression pattern of *IDD16* protein.

The expression of IDD16 protein was visualized in *IDD16pro:IDD16-GFP* transgenic lines under confocal microscopy. (A) The expression pattern of *IDD16* protein in adaxial epidermis of 2-DAG seedling cotyledons, GFP signal was absent in meristemoid cells and young guard cells. (B-E) The expression is strong in trichome of young leaves of 7 DAG seedlings (B), but almost undetectable in mesophyll cells of cotyledons (C), root elongation zone (D) and root tip (E) of 1-DAG seedlings. Cell periphery is highlighted by propidium iodide (red) staining.



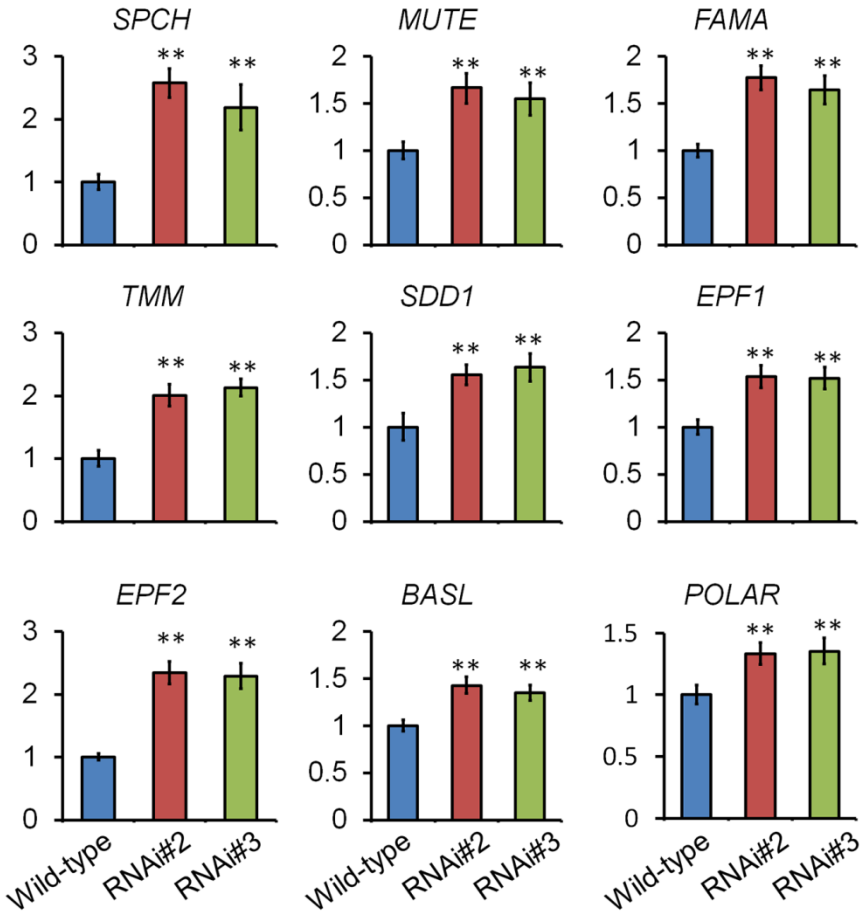
Supplemental Figure 5 Effect of *IDD16* overexpression on stomatal density in different genetic backgrounds.

Stomatal densities in abaxial epidermis of the cotyledons from 11-day-old *tmm* **(A)** and *sdd1* **(B)** seedlings containing *35Spro:IDD16* were analyzed. Values are means ± SE from three biological replicates. Asterisks indicate statistical significance from the background (*tmm* or *sdd1*) based on Student's t test; ** $P < 0.01$.



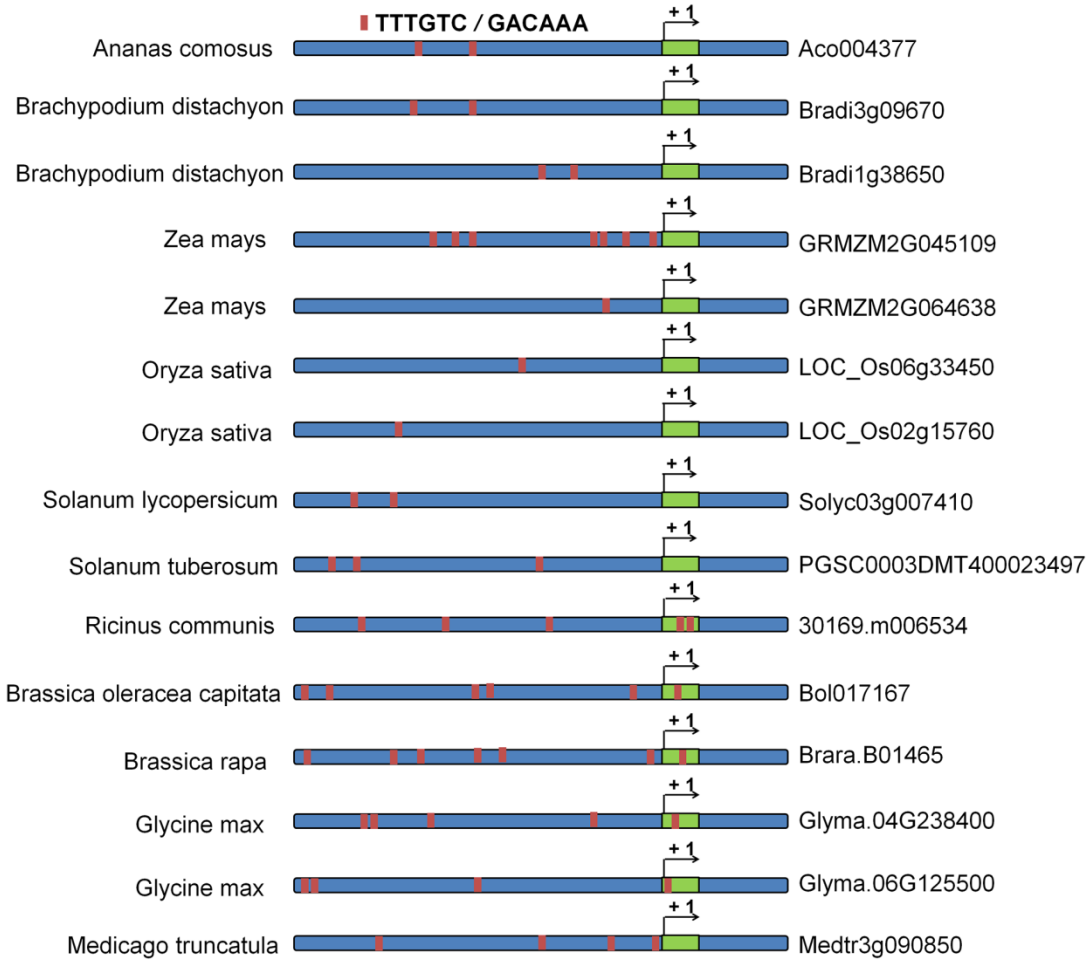
Supplemental Figure 6 The expression of *IDD14* and *IDD15* were not affected in *IDD16*-RNAi lines.

The expression levels of *IDD14* and *IDD15* in 4-DAG wild-type and *IDD16*-RNAi seedlings were determined by qRT-PCR. Values are means ± SE from three biological replicates. Primers are listed in Supplementary Table S1.



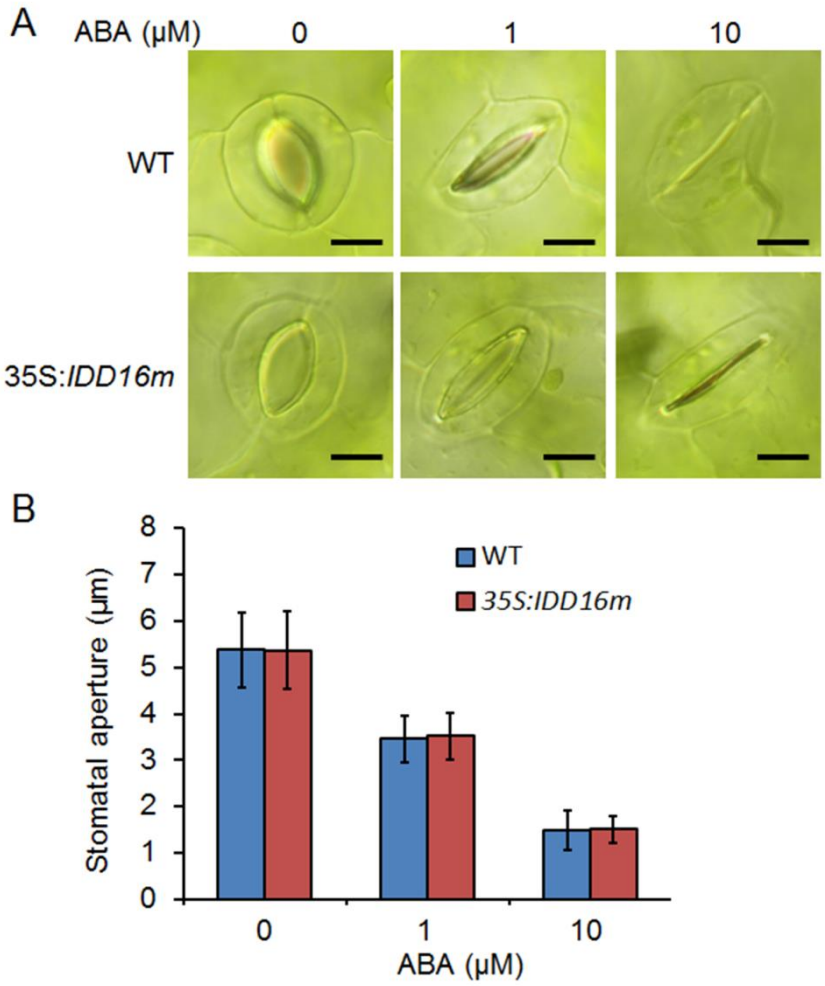
Supplemental Figure 7 The expression of genes related to stomatal development was enhanced in *IDD16*-RNAi seedlings.

qRT-PCR was performed on total RNA from 4-DAG wild-type and *IDD16*-RNAi seedlings. Values are means ± SE from three biological replicates. Asterisks indicate statistical significance based on Student's t test; ** $P < 0.01$.



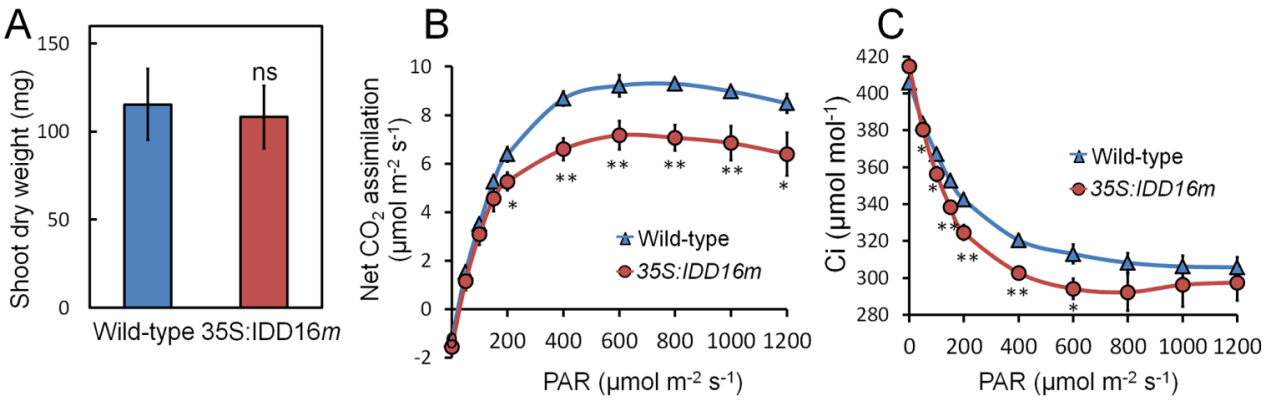
Supplemental Figure 8 The core binding sites of IDD proteins in promoter of *SPCH*-like genes from angiosperms.

The angiosperm genomes were searched through Phytozome v12 (https://phytozome.jgi.doe.gov/pz/portal.html#) by similarity search with the *SPCH* of *Arabidopsis*. 3-kb promoter region upstream of the initiation codon and the first exon were analyzed. Red bar represents the core binding sites (TTTGTC or GACAAA) of IDD proteins.



Supplemental Figure 9 35S:*IDD16m* plants exhibited similar stomatal aperture in response to ABA treatment compared with wild-type plants.

(A) Comparison of stomatal aperture in response to ABA treatment. (B) Stomatal aperture. Values are means ± SE from three biological replicates, and each replication with 30 stomata from leaves of three different plants.



Supplemental Figure 10 Growth and carbon assimilation of 35S:*IDD16m* plants were not affected under normal growth condition.

(A) Shoot dry weight was measured at the completion of the WUE experiment. Values are means ± SE from four biological replicates. The data are from three replicates, with each replicate include more than 20 plantlets. **(B)** Net CO_2 assimilation and **(C)** internal CO_2 concentration(Ci) were determined on individual leaves of 5-week-old wild-type (Col-0) and 35S:*IDD16m* plants using a Li-Cor 6400 gas exchange system, values are the Mean ± SE (n = 4). Asterisks indicate statistical significance from the WT based on Student's t test; ** $P < 0.01$, * $P < 0.05$.

Table S1. Primers used in this study

Primers		Sequence	Annotation
Primers for transgenetic plants			
	IDD16-F	CACCATGATACATTACGAACAAAACA	For the CDs of *IDD16*.
	IDD16-R	TCTCGCATTCTCCTTCAGT	
	*IDD16*i-F	GGGGACAAGTTTGTACAAAAAAGCAGGC TCACCATCCGGCAACCTCA	DNA fragments for *IDD16* silencing.
	*IDD16*i-R	GGGGACCACTTTGTACAAGAAAGCTGGG TATGGATAGTTGAAGTTCGAGGC	
	*IDD16*pro-F	AACTGCAGAAAGCAATAACCTAGATGAC	For the 2,621 bp promoter of *IDD16*.
	*IDD16*pro-R	GACTAGTTGATGCCAAGAAGAAGATCG	
Primers for qPCR			
AT3G13920	*eIF4a*-F	TGACCACACAGTCTCTGCAA	
	eIF4a-R	ACCAGGGAGACTTGTTGGAC	
AT1G25250	q*IDD16*-F	CAATGCTTCATCAGCTCCTTTC	
	q*IDD16*-R	CGTTCGCTCTCTCCTTTGTTA	
AT5G53210	q*SPCH*-F	ATCATAGGAGGAGTTGTGGAG	
	q*SPCH*-R	TAGAACAGGCGGTGAAGGAC	
AT3G06120	q*MUTE*-F	CGATCATCGGAGGAGTGATAGA	
	q*MUTE*-R	AAGGGAAAGATGGTCGGTTTAG	
AT3G24140	q*FAMA*-F	GAGCTCGAGCAACTCCTACAAT	
	q*FAMA*-R	GAAGTCGTTGTCGTTGTCATGT	
AT1G80080	q*TMM*-F	TCCTTCACCTAGAGGGCAATAA	
	q*TMM*-R	ACGGTACTGGTCCTGTCAGACT	
AT1G04110	q*SDD1*-F	AGCGGAATCACTGCTCTTATC	
	q*SDD1*-R	GCTGGTTTGTTACCATCCTTTATC	
AT2G20875	*qEPF1-F*	ATGCCGTCTTGTGATGGTTAG	
	qEPF1-R	TCAAGGGACAGGGTAGGACTT	
AT1G34245	*qEPF2-F*	TTTGGTCGTTAACTCCATTCG	
	qEPF2-R	ATCCGGTAAGCTTGATCCTGT	
At5g60880	q*BASL*-F	TCCTCTAGACGGAGATGAAGATGG	
	q*BASL*-R	TGGTGGGCTTAGGCTGAGTTTC	
At4g31805	q*POLAR-F*	TGAGTCACAAAGCAGAGAGTCACC	
	q*POLAR-R*	TCCATTTGCATTCGCAGGTTTGTC	
AT3G26744	q*ICE1*-F	TCTTTGCCTCCAACTTCATC	
	q*ICE1*-R	CACTGCTCTTCCTTCCCTTA	
AT1G12860	q*ICE2*-F	GTGTCCATCTTCCTCCTTGC	
	q*ICE2*-R	ACAGCTAATCACCGCTTGTT	
AT1G63700	q*YDA*-F	CACCATGAGATCACTGGACATT	
	q*YDA*-R	GCCATGTTTTAATCCTTTCTGC	
AT4G12970	q*STOMAGEN-F*	TGTAGTTCAAGCCTCAAGAC	
	q*STOMAGEN-R*	TTGATAGGGTCATTTCCTTC	

Table S1. Cont.

	Primers	Sequence	Annotation
AT2G26330	q*ERECTA*-F	GATAATGTCAAAGACGGGGAAC	
	q*ERECTA*-R	GGAAAACTTTCTTCACCACACC	
At5g62230	q*ERL1*-F	CGCATAACTTGCGGGAATTTG	
	q*ERL1*-R	AGTCCTTGTGCAGCTCCAACC	
AT5G07180	q*ERL2-F*	GCTGTGGATAACGAGGCCAAC	
	q*ERL2-R*	CATGGTGGGTCTCTCCAAAGG	
AT3G12280	q*RBR*-F	CAGATGGCTTGACCTACTTTGA	
	q*RBR*-R	CCTCTCATCAAGTTCGCCTTTA	
AT1G04710	q*PKT4*-F	ACGAGTTGCTTGCCTCTGTA	
	q*PKT4*-R	CACTGTCTGTTCACGGTTCTG	
AT3G48750	q*CDKA1*-F	GCCAAAAGCCCTTATTTCCT	
	q*CDKA1*-R	GTTACCCCACGCCATGTATC	
AT3G45640	q*MPK3*-F	TGACGTTTGACCCCAACAGA	
	q*MPK3*-R	CTGTTCCTCATCCAGAGGCTG	
AT1G51660	q*MKK4*-F	TCTTCCTCTCCCACCTACTTC	
	q*MKK4*-R	TGGAGCTGTTAGTGTTCGTTG	
AT1G68130	q*IDD14*-F	GGGACACCGGATCCGG	
	q*IDD14*-R	GTCTTGGTGCTCTATGAAACTTTCC	
AT2G01940	q*IDD15*-F	CCACAAACACATCCACTCAGA	
	q*IDD15*-R	TCTGGTCTCTTTGAAACCCTTG	
Primers for ChIP-qPCR			
	P1-F	ACTATGTCCGCTAAATCA	Used for detect the enrichment of regions 1, 2, 3, 4 of the *SPCH* gene.
	P1-R	ATGAAACATCAGCAATTAGA	
	P2-F	TAATTTGGGCTAGAAACAAAGC	
	P2-R	AGAAGATGGAGCCAAGAC	
	P3-F	ATGCAGGAGATAATACCG	
	P4-F	ATCATAGGAGGAGTTGTGGAG	
	P4-R	TAGAACAGGCGGTGAAGGAC	
At3g18780	ACTIN2-F	CGTTTCGCTTTCCTTAGTGTTAGCT	To detect the enrichment of *ACTIN2*, as internal control.
	ACTIN2-R	AGCGAACGGATCTAGAGACTCACC TTG	
AT3G30720	QQS-F	TCTGCAATTATGTAAACATA	Used for detect the enrichment of the fragment of *QQS* gene, as a positive control.
	QQS-R	ACAAATCAAAGGCCAATATC	