Supporting Information

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SI Experimental Procedures

Plant Material, Growth Conditions, and Tissue Sampling. WT hybrid aspen (*Populus tremula* × *tremuloides*, clone T89) and transgenic plants were grown on half-strength Murashige–Skoog medium (Duchefa) under sterile conditions for 4 wk and then transferred to soil and cultivated for 4 wk in a greenhouse (16-h light, 22 °C long days). Plants were fertilized weekly when growing in the greenhouse. To induce growth cessation, the plants were moved to a climate-controlled growth chamber and subjected to SDs (8-h day/16-h night, 20 °C during day/15 °C at night). SD responses were investigated by monitoring bud set and counting numbers of leaves formed between initiation of SDs and growth cessation. Tissue samples for gene expression analyses were collected from shoot apices after 0, 3, 6, and 10 wk of SDs, frozen in liquid nitrogen, and stored at −80 °C. Tissue samples for RNA isolation were collected at the same time of the day (i.e., 1400 hours), frozen, and stored at −80 °C until further use. Pictures of apices were taken using a Canon EOS digital camera to monitor bud formation.

Construction of Transgenic FDL Plants and Plasmid Constructs. FDL1 and the genomic sequence of FDL2 transcription factor were cloned into the pENTR/D-TOPO donor vector (Invitrogen), and subsequently into the pK2GW7 plant transformation vector (1), to the generate plasmids pK2GW7-FDL1 and pK2GW7-FDL2. To construct FDL1RNAi plants, a 351-bp fragment was amplified using primers 5′-CACCCCTAGCTTCTCTTCATGAACA-TCC-3′ and 5′-AGTTCCAACTCGGTCGTGTAA-3′ from the full-length FDL1 cDNA as a template, and to construct FDL2RNAi plants, a 156-bp fragment was amplified using primers 5′-CACC-GGTCATCGCCAGGAGCA-3′ and 5′-TTGAGGCTCCGTTCA-TTGAT-3′ from the full-length FDL2 genomic fragment as a template. The templates were cloned into the donor vector pENTR/D-TOPO and then cloned into pK7GWIWG2 (I) (1) to generate pK7GWIWG2 (I)-FDL1 and pK7GWIWG2 (I)-FDL2 constructs. The plant transformation constructs were introduced into *Agrobacterium* strain GV3101pmp90RK and then used to transform hybrid aspen clone T89 as previously described (2). Construction and characterization of ABI3oe plants have been described previously (3).

To generate recombinant constructs for coimmunoprecipitation assays, cDNA fragments corresponding to FDL1 and ABI3 coding regions were amplified from cDNAs prepared from mRNA extracted from hybrid aspen apex as templates. Amplified cDNA fragments for triple c-Myc epitope-tagged ABI3 (3X–c-Myc–ABI3) and HA-tagged FDL1 (3x-HA-FDL1) were cloned into BamHI/EcoRI and BamHI/SalI sites of plasmid pRT104 (4) to generate pRT104–3c-Myc–FDL1 and pRT104-3HA-ABI3 plasmids.

Transgenic *Arabidopsis* plants expressing poplar FDL1 cDNA under 35S promoter in *fd-2* background were generated by *Agrobacterium*-mediated transformation using the floral dip method (5). *Arabidopsis* plants were grown in growth chambers under long day conditions (16-h day/8-h night), and flowering time was assessed by counting total leaf number (rosette and cauline leaves produced on the main inflorescence stem) at the time of flowering.

Transient Expression Assays and Cloning of *FDL*s and *FT*s. Coding sequences of poplar FT1 and FT2 and FDLs were amplified by PCR, ligated into pENTR/D-TOPO cloning vector, and sequenced. Poplar FTs and FDLs were then transferred from pENTR into pUbq-GW destination vector (described in ref. 6) by Gateway (Invitrogen) recombination reaction with LR Clonase II (Invitrogen), and inserts were sequenced. Transient expression vector for OsGF14b, p35S:OsGF14b-GFP, was described previously (6).

Transient expression of rice protoplasts was performed as described previously (3). Briefly, 8 μg of plasmids for expressing hybrid aspen or rice FT and 16 μg of poplar or rice FDL cDNA were used for transfecting 500-μL portions of protoplast suspension (2 × 107 protoplasts per milliliter) by PEG-mediated transfection (7). After 24 h of incubation at 30 °C, the protoplast suspension was centrifuged and the cell pellet was frozen at −80 °C for RNA extraction. Total RNA was extracted using an RNeasy Plant Mini Kit (Qiagen), following the manufacturer's recommended protocol. cDNA was synthesized from 1 μg of RNA using oligo dT primer (21-mer) and SuperScript II Reverse Transcriptase (Invitrogen). cDNA (1 μL) was utilized for quantitative analysis of gene expression using SYBR Green PCR Master mix (Applied Biosystems) with gene-specific primers. Data were collected using the StepOnePlus sequence detection system (Life Technologies) in accordance with the instruction manual.

Transient Expression and Coimmunoprecipitation Assay in *Arabidopsis* Protoplasts. An *Arabidopsis* cell suspension culture derived from Col-0 roots was used in all experiments. Protoplast isolation and transient transfection were carried out as described by Dóczi et al. (8) with some modifications. Briefly, *ca.* 5 × 105 protoplasts were used for each transfection with 3–5 μg of plasmid DNA. Transfection was followed by 16 h of incubation in the darkness. Protoplasts were harvested by centrifugation at 300 × *g* for 8 min. Obtained pellets were resuspended and lysed (by vortex mixing and two freeze/thaw cycles in liquid nitrogen and ice) in buffer containing 25 mM Tris·HCl (pH 7.8), 5 mM EGTA, 10 mM $MgCl_2$, 75 mM NaCl, 10% (vol/vol) glycerol, 0.2% Tween-20, 2 mM DTT, 60 mM β-glycerophosphate, 0.1 mM activated Na_3VO_4, 1 mM benzamidine, and 1% (vol/vol) plant protease inhibitor mixture (P9599; Sigma).

In coimmunoprecipitation assays, 100 μg of proteins from transfected protoplasts was incubated in 100 μL of extraction buffer (final volume) containing 150 mM NaCl and 1.5 μg of anti–c-Myc antibody (clone 9E10 for c-Myc; Covance). After 2 h, 10 μL of Protein G/Sepharose matrix (GE Healthcare) was added, and the mixture was further incubated for another 2 h on a rotating wheel at 4 °C. The matrix was then washed in 3 × 500 μL of washing buffer [1× TBS, 5% (vol/vol) glycerol, and 0.1% Igepal CA-630 (Sigma Aldrich)], and bound proteins were eluted by boiling in 25 μL of 1.5× Laemmli sample buffer, separated by SDS/PAGE, and then blotted onto a PVDF transfer membrane (Millipore). Proteins of interest were immunodetected using rat anti–HA-peroxidase (3F10; Roche) or chicken anti–c-Myc primary antibody (A2128; Invitrogen) and rabbit anti-chicken IgY HRP conjugate (Thermo Scientific), respectively, in conjunction with ECL Reagents (GE Healthcare).

RNA Isolation and qRT-PCR Analysis. Total RNA was extracted using an Aurum Total RNA Kit (Bio-Rad). RNA (5 μg) was treated with RNase-free TURBO DNase (Life Technologies, Ambion), and 1 μg was then utilized for cDNA synthesis using an iScript cDNA Synthesis Kit (BioRad). In all experiments, TIP41-like was selected by GeNorm (Biogazelle) software (9) as a reference gene. Quantitative RT-PCR experiments were conducted using LightCycler 480 SYBR Green I Master mix and a Light Cycler 480 II instrument (both supplied by Roche). The Δ-cq method was used

to calculate relative expression values of genes of interest. Primer sequences used are as follows: TIP4-like (GCTGCACTTGCATCAAAAGA and GCAACTTGGCATGACTCTCA), LAP1 (TGGGAAGAGGTAGGGTTCAGT and CAAAGCAACCTCAGCGTCAC), FDL1 (GTTAGGAGAGGAGAATGCCAAG and TGAGGTTCGATAGAGGGTGTG), FDL2 (TCCCGGGCTAGAAAGCAG and TAAGCCTGCAAAATGAGTAAAAGA), OSM-like (ACCCATGCACTGTGTTCAAA and ATGTGCTTGAAGGGTCATCC), LEA-like (ATACGGTGGGTGAAGCGTTA and ACCTGCCTTTTCTGAAACCA), CHS-like (TACATGCACTTGACGGAGGA and CGCCTCTTTGCCTAACTTTG), and C4H-like (AGCTGCCCTGGGATTATTCT and AAAACCATCCCGCTTTTCTT).

BiFC Assay. For BiFC assay, full-length cDNA of FDL1, FDL2, FT1, FT2, and ABI3 was cloned in pUC-SPYNE or pUC-SPYNE vector (10). *Arabidopsis* protoplasts were transfected as described by Dóczi et al. (8), and fluorescence was visualized 24 h after transfection on a Carl Zeiss LSM780 confocal laser scanning microscope. YFP fluorescence was detected with the excitation/emission combination of 514/525–535 nm. Empty vectors expressing C-terminal YFP and N-terminal YFP were used as controls.

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2. Nilsson O (1992) Spatial pattern of cauliflower mosaic virus 35S promoter-luciferase expression in transgenic hybrid aspen trees monitored by enzymatic assay and non destructive imaging. *Transgenic Res* 1(5):209–220.
3. Petterle A (2011) ABA and chromatin remodelling regulate the activity-dormancy cycle in hybrid aspen. *Acta Universitatis Agriculturae Sueciae* 17:1652–6880.
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6. Taoka K, et al. (2011) 14-3-3 proteins act as intracellular receptors for rice Hd3a florigen. *Nature* 476(7360):332–335.
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10. Walter M, et al. (2004) Visualization of protein interactions in living plant cells using bimolecular fluorescence complementation. *Plant J* 40(3):428–438.
11. Larkin MA, et al. (2007) Clustal W and clustal X version 2.0. *Bioinformatics* 23(21): 2947–2948.

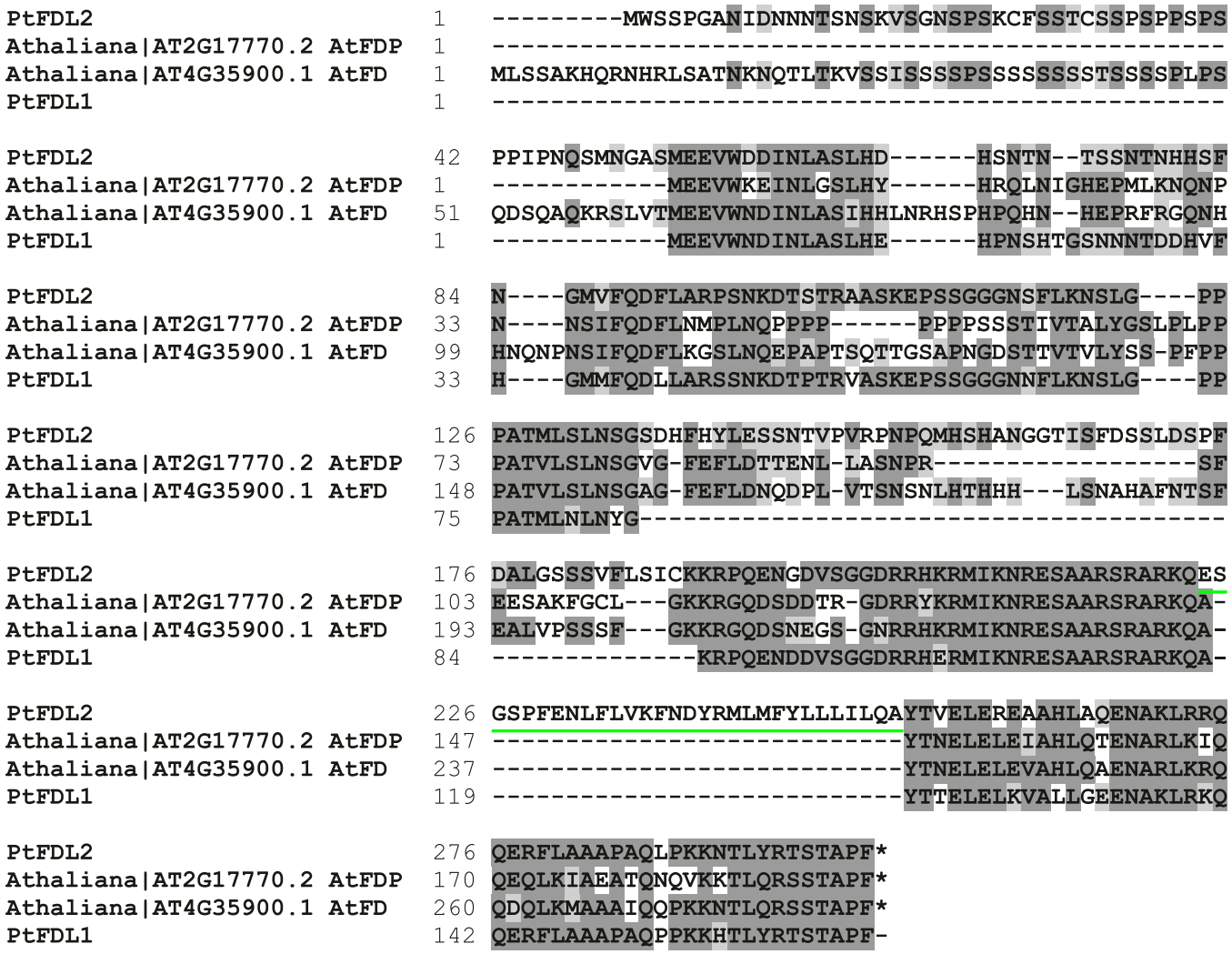

Fig. S1. Alignment of amino acid sequences of FD family members from *A. thaliana* and *Populus trichocarpa* generated using ClustalW (11), with a gap opening penalty of 10 and a gap extension penalty of 0.2, and then visualized using BioEdit software.

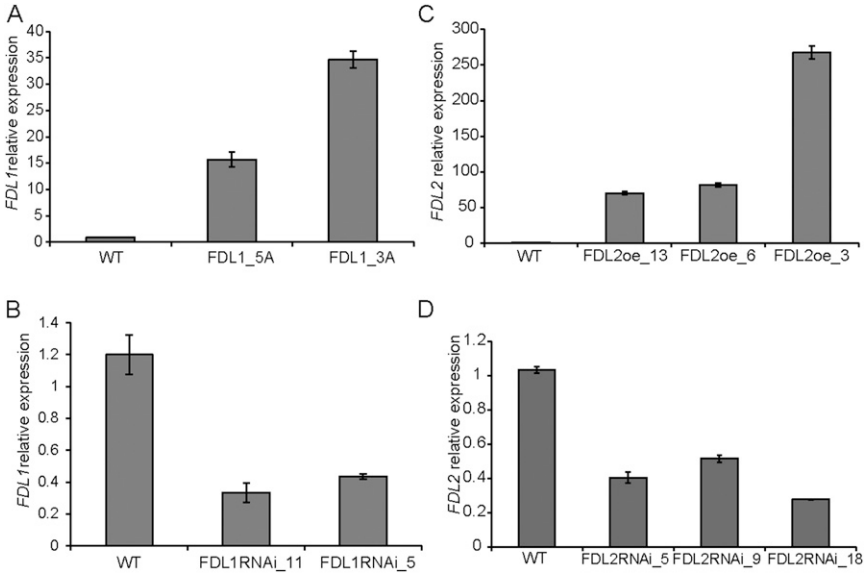

Fig. S2. *FDL1* expression in FDL1oe plants (*A*; lines 5A and 3A) and *FDL1* down-regulated (FDL1RNAi) plants (*B*; lines 5 and 11) is shown. *FDL2* expression in FDL2oe plants (*C*; lines 3, 6, and 13) and *FDL2* down-regulated (FDL2RNAi) plants (*D*; lines 5, 9, and 18) is shown. RT-PCR analysis was performed to analyze *FDL1* and *FDL2* expression in WT, FDL1oe, and FDL1RNAi lines and in FDL2oe and FDL2RNAi plants, respectively. Expression of *FDL1* or *FDL2* is plotted relative to the reference gene TIP41-like and normalized to transcript levels in WT controls (average from three biological replicates ± SE).

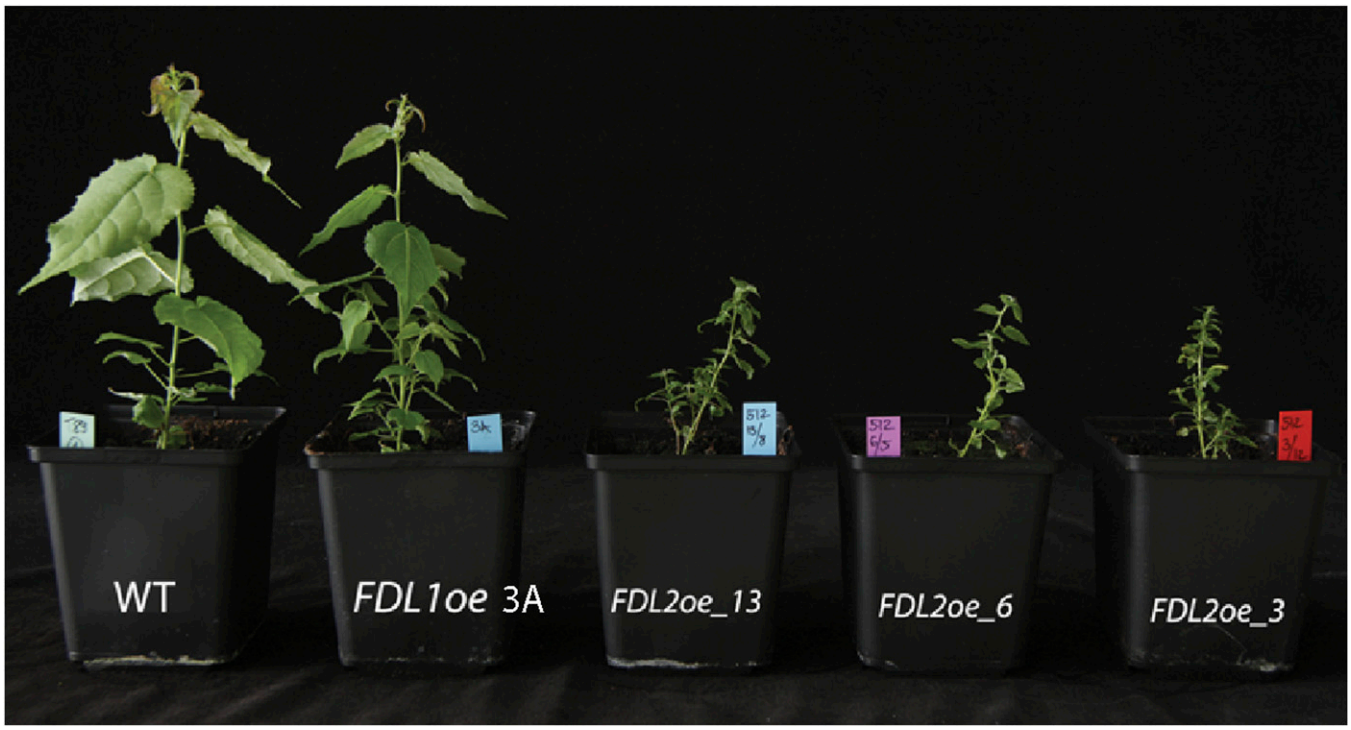

Fig. S3. Phenotypes of long day-grown hybrid aspen plants FDL1oe and FDL2oe cDNA compared with WT controls.

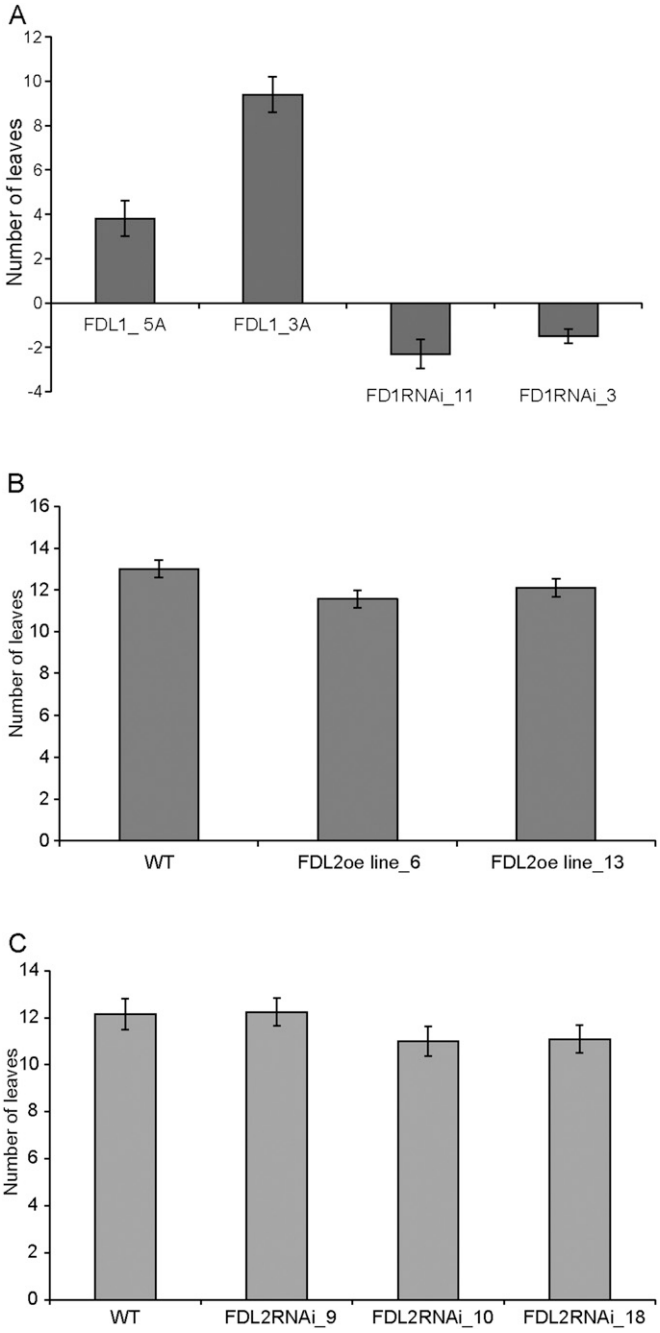

Fig. S4. (*A*) Analysis of SD response in FDL1oe and FDL1RNAi plants. Numbers of leaves formed between initiation of the SD treatment and growth cessation were counted in the WT, FDL1oe (lines 3A and 5A), and FDL1RNAi (lines 3 and 11) plants, and differences in the number of leaves produced by FDL1oe and FDL1RNAi plants relative to WT controls are plotted on the *x* axis. The values are average ± SE ($n = 10$). The SD response in FDL2oe (*B*; lines 6 and 13) and FDL2RNAi (*C*; lines 9, 10, and 18) plants is shown. Numbers of leaves formed between initiation of the SD treatment and growth cessation were counted in WT, FDL2oe, and FDL2RNAi plants. The values are average ± SE ($n = 10$).

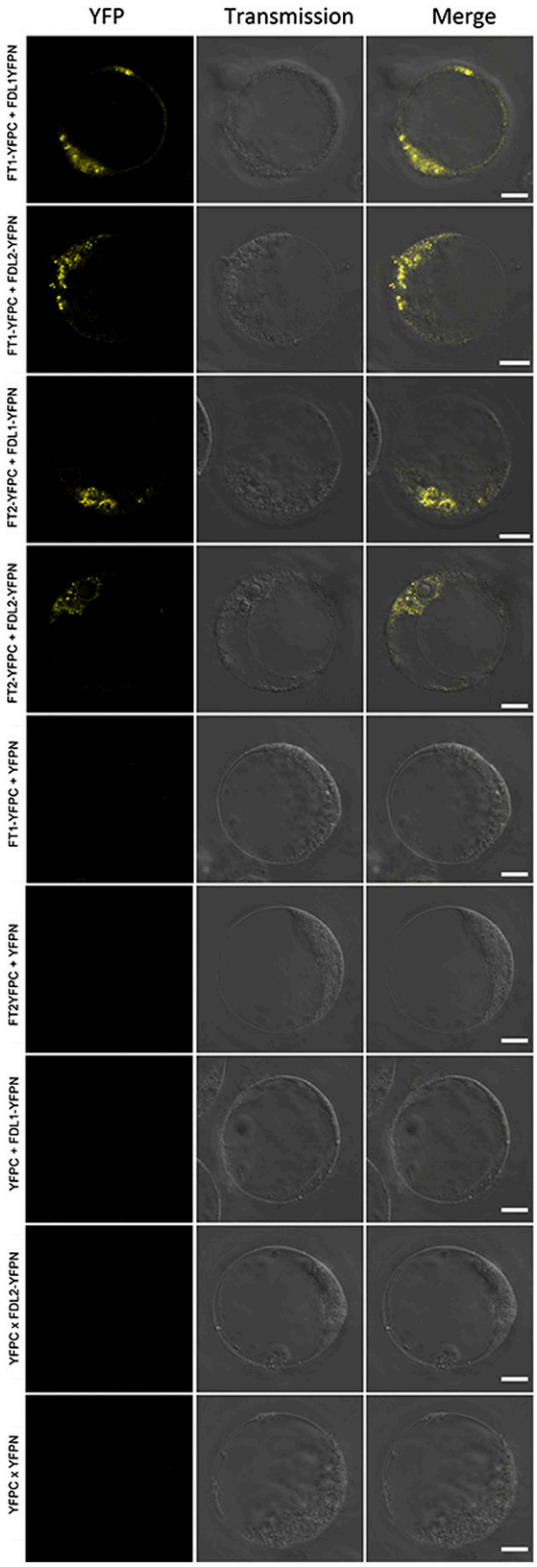

Fig. S5. FDL1 and FDL2 interact with FT1 and FT2. Interaction between FDL1 or FDL2 with FT1 and FT2 was analyzed using a BiFC assay (top four rows). The YFP channel (*Left*), transmission channel (*Middle*), and merging of the two channels (*Right*) are indicated. Empty vectors (bottom row) and a combination of empty vector with one of the constructs (rows 5 to 8) were used as controls and are indicated. (Scale bar: 10 μm.)

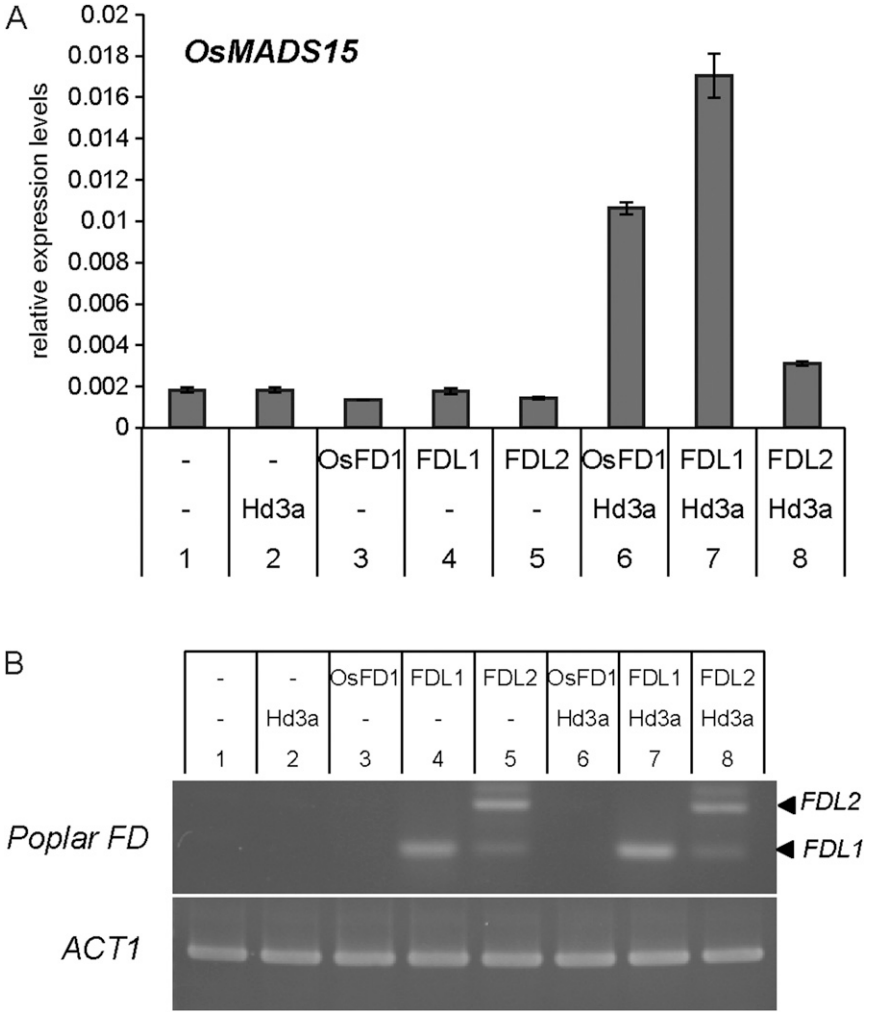

Fig. S6. (*A*) Hybrid aspen FDL1 interacts with rice FT homolog Hd3a in rice protoplasts. Rice FT homolog Hd3a and hybrid aspen FD homologs (FDL1 and FDL2) were coexpressed in rice protoplasts, and induction of OsMADS15 expression was analyzed by quantitative RT-PCR. The *x* axis shows the cDNAs expressed, and the *y* axis shows the OsMADS15/ubiquitin expression ratio (measured by quantitative RT-PCR analysis) 24 h after transformation. Error bars indicate standard deviations of triplicate measurements. (*B*) Plasmids for expressing FT and FD homologs from rice or hybrid aspen were transformed into rice protoplasts. Expression of FDL1 and FDL2 was confirmed by RT-PCR analysis, using the primer sequences listed in *SI Experimental Procedures*.

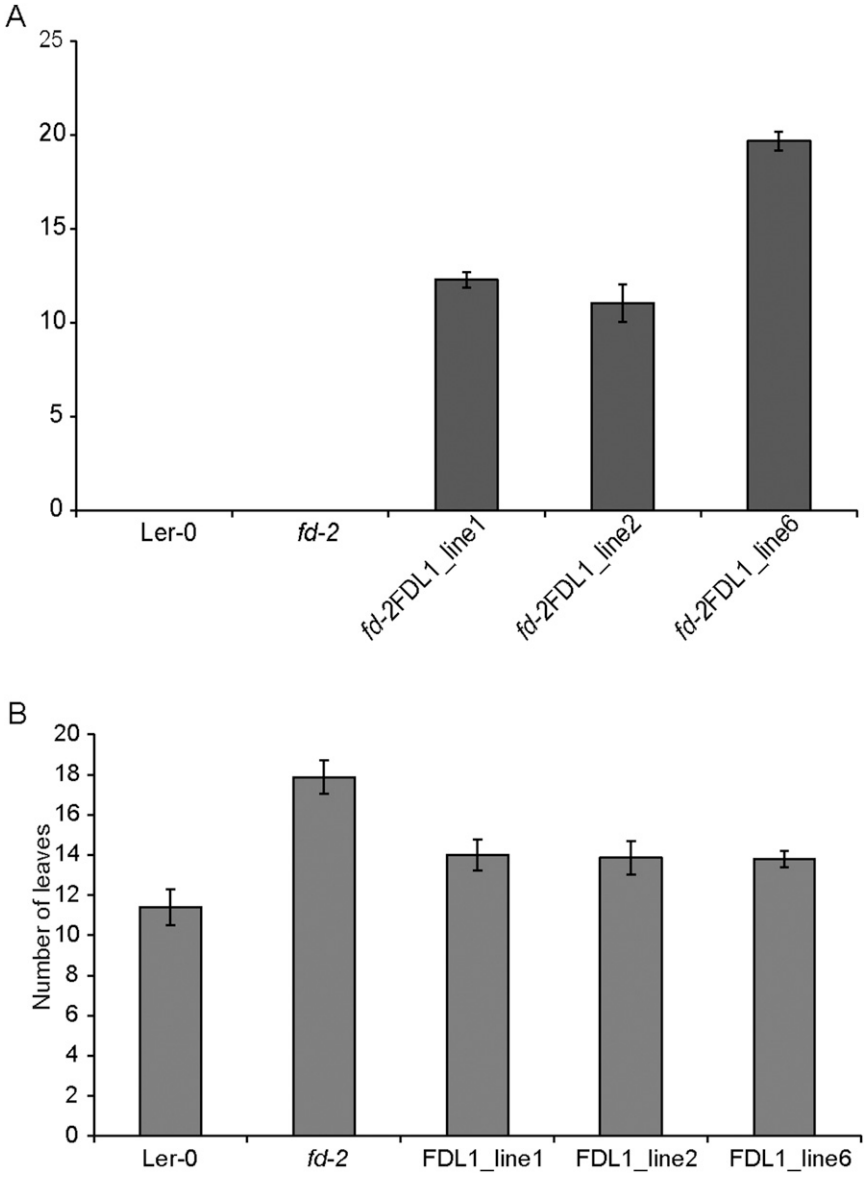

Fig. S7. (*A*) Relative expression of *FDL1* in the *Arabidopsis fd-2* mutant background overexpressing *FDL1* (lines 1, 2, and 6), *fd-2* mutants, and WT (Ler-0). The values are the average ± SE ($n = 3$). (*B*) Flowering time of the WT (Ler-0), *fd-2* mutant, and *fd-2* mutant expressing *FDL1 cDNA* in an *fd-2* mutant background (lines 1, 2 and 6) grown under long day conditions. The number of leaves to flowering is plotted on the *y* axis (average of at least 15 randomly selected plants ± SD). Ler-0, Landsberg erecta -0.

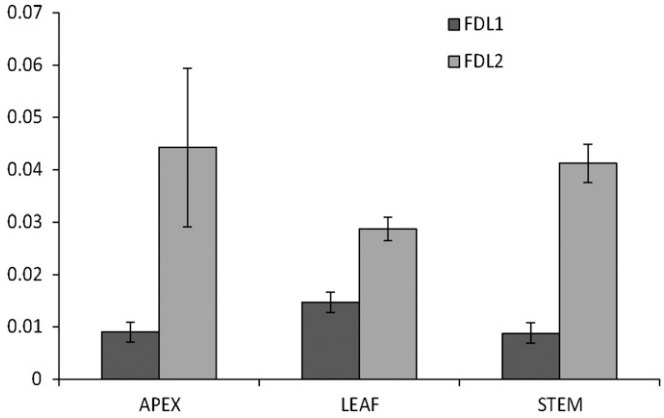

Fig. S8. Relative expression of *FDL1* and *FDL2* in different tissues of hybrid aspen (apex, leaf, and stem). The values represent the average of three biological replicates ± SE.

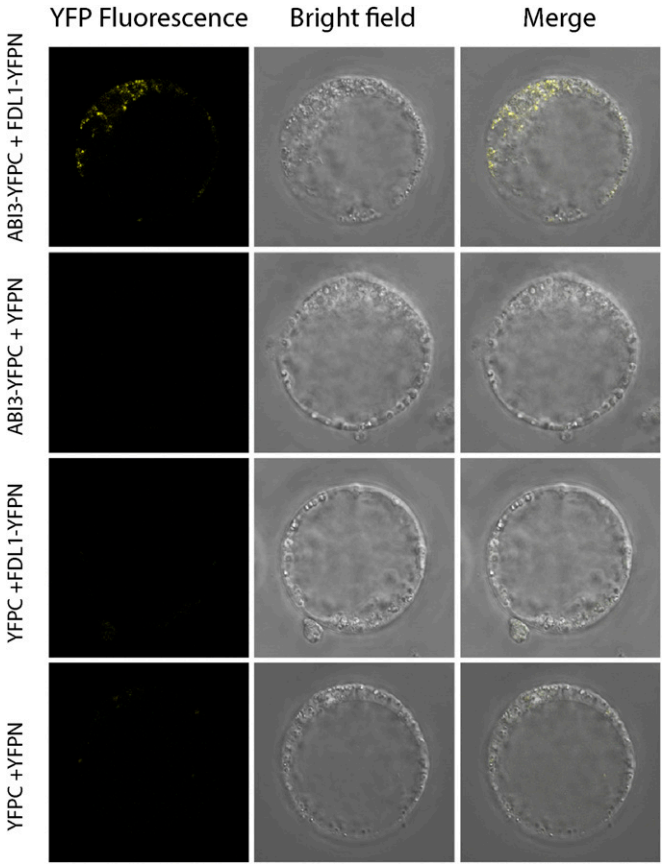

Fig. S9. FDL1 interacts with ABI3. Interaction between FDL1 and ABI3 was assayed using BiFC assay (top row). The YFP channel (*Left*), transmission channel (*Middle*), and merging of the two channels (*Right*) are shown. Empty vectors (bottom row) and a combination of empty vector with one of the constructs (rows 2 and 3) were used as controls and are indicated (bottom three rows). (Scale bar: 10 μm.)

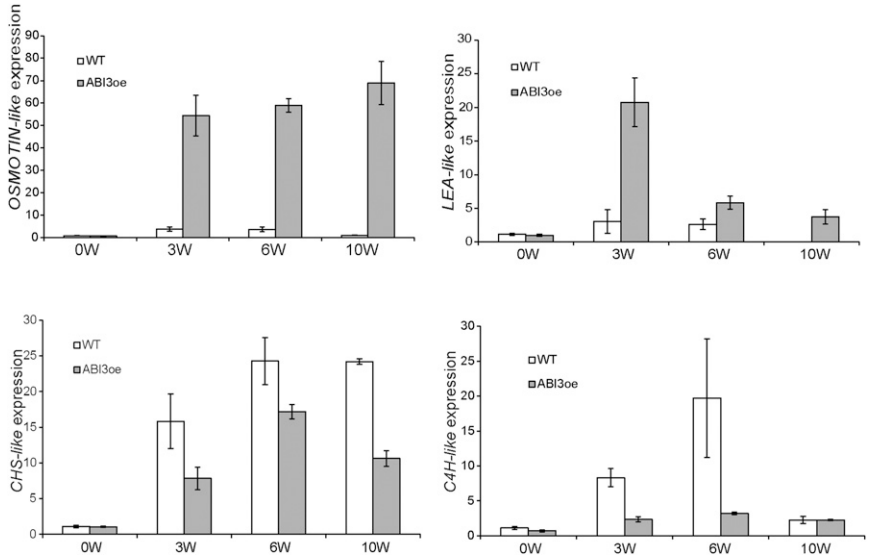

Fig. S10. ABI3 mediates in SD-controlled adaptive response and bud maturation pathways. Expression patterns of adaptive response (*LEA*, *LTP*, and *OSM*) and bud maturation (*CHS* and *C4H*) pathway markers in ABI3oe apices compared with WT plants after SD treatment. Expression values relative to the reference gene *TIP41-like* (average from three biological replicates ± SE) are shown on the *y* axis, and duration of the SD treatment (in weeks) is shown on the *x* axis. W, weeks.