Supporting Information

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SI Materials and Methods

Plant Material and Growth Conditions. All experiments were performed using *Arabidopsis thaliana* of the Columbia accession. The mutant line SALK_054242C (*jkd-4*) is designated *atidd10* in this paper. The homozygous *atidd10* transfer DNA (T-DNA) mutant line was identified by PCR (http://signal.salk.edu/tdnaprimers.2.html) (Table S1). Seedlings were established on Murashige and Skoog (MS) medium [half-strength MS salt mixture (pH 5.7; Wako), B5 vitamins, 1% sucrose, and 0.8% gellan gum (Wako)]. Plants, except when used for protoplast isolation, were grown under long-day conditions (16 h light/8 h dark) under white fluorescent light (40 $\mu mol \cdot m^{-2} \cdot s^{-1}$) on Rock Fiber (Nittobo) with 500-fold diluted Hyponex (Hyponex Japan) fertilizer saturated with water. More than three batches of plants were grown to provide independent biological replicates. Transformation of *Arabidopsis* was performed using the floral dip method (1). Plants used for protoplast isolation were grown in soil at 22 °C with a photoperiod of 12 h light/12 h dark for 4–5 wk.

Plasmid Construction. The sequences of primers for PCR amplification used in this study are listed in Table S1. All PCR fragments were sequenced to confirm that no mutations were introduced.

Plasmids for yeast hybrid analysis. We amplified the coding regions of the objective genes, which were fused to the pBridge and pGADT7 vectors (Clontech). The prey constructs containing the coding DNA sequence (CDS) of each of the *Arabidopsis* DELLA genes were a gift from Masatoshi Nakajima (The University of Tokyo, Tokyo). The promoter sequences were amplified and fused to the R4L1pDEST_HISi vector (2).

To generate the constructs expressing both REPRESSOR of *ga1-3* (RGA) and SCARECROW-LIKE 3 (SCL3) for yeast three-hybrid analysis, the coding region of RGA was cloned into the NotI site of the pBridge plasmid containing the SCL3 GAL4-binding domain (BD-SCL3).

Plasmids for bimolecular fluorescence complementation assay. The coding regions (lacking the stop codons) were produced by PCR using *Arabidopsis* cDNA as template and appropriate primers. The amplified fragments were subcloned into TOPO-D/pENTR (Invitrogen) to generate the entry clones. The *INDETERMINATE DOMAIN* (*IDD*) and *RGA* constructs were used to clone CDS into the vectors pGWcY and pGWnY by LR Gateway reaction to generate C- and N-terminal fusions to the two YFP fragments (3).

Plasmids for in vitro pull-down assay. For construction of SCL3 in the pGEX-TEV vector (pGEX-TEV-SCL3), SCL3 was PCR amplified from the plasmid used in yeast hybrid assay and cloned into the pGEX-TEV vector in which the PreScission protease site of pGEX-6P-1 (GE Healthcare) was replaced with the TEV protease site.

Plasmids for the transient reporter assay. To generate the pE2113_GW_SAS effector vector, we amplified the GATEWAY cassette using a reverse primer containing an original multiple cloning site (SpeI-ApaI-StuI) and replaced a coding region for β-glucuronidase (GUS) of pE2113-GUS, which contained a CaMV 35S core promoter, two enhancer sequences (El2 and Ω), and a terminator of a gene for nopaline synthase (4), with the amplified DNA fragment. The coding region of viral protein 16 (VP16) was amplified and inserted into the SpeI/StuI site of pE2113_GW_SAS to generate the effector vector for the protein fused with VP16, pE2113_GW_VP16. The amplified fragments in TOPO-D/pENTR were transferred into these vectors by Gateway LR reaction.

To generate the reporter vector, the coding region of the Renilla luciferase (rLUC) gene of 6xUAS-35S MP-rLUC/pUC19 (5) was replaced with that of the firefly LUC (fLUC) gene, which was amplified using pUGW35 as a DNA template (6). Then DNA containing each length of the promoter region of *SCL3* was amplified using the construct for yeast one-hybrid (Y1H) as template and replaced the promoter region of 6xUAS-35S MP-fLUC/pUC19.

Plasmid for EMSA. We amplified the coding region (amino acids 11–210) of the truncated *Arabidopsis* IDD3 (AtIDD3) and inserted it into pGEX-6P-1 (GE Healthcare).

Plasmids for transgenic plants. To generate transgenic plants overexpressing AtIDD3 (IDD3-ox plants), the AtIDD3 CDS were cloned by Gateway LR recombination into pGWB502 vectors (6). The construction used to generate IDD3-SRDX plants was made by the method described previously (7).

Bimolecular Fluorescence Complementation Assay. Plasmids were extracted as for the transient reporter assay. Transient gene expression using *Arabidopsis* mesophyll protoplasts was performed as previously described (8, 9) with the modification that the protoplasts were isolated using the Tape-sandwich method (10). YFP fluorescence was recorded by a LSM 700 confocal microscope (Zeiss).

In Vitro Pull-Down Assay. GST-RGA and GST-SCL3 were overexpressed using *Escherichia coli* Rosetta (pLysS) harboring pGEX-4T1-RGA and pGEX-TEV-SCL3 plasmids. The transformants were grown in LB medium containing 100 μg/mL ampicillin at 37 °C. Cells were grown to an A_{600} of 0.7–1.0 and then were induced with 0.1 mM isopropyl β-D-1-thiogalactopyranoside for 16–20 h at 16 °C. The recombinant protein was partially purified by affinity choromatography (Glutathione Sepharose 4B; GE Healthcare) and gel-filtration chromatography (Superdex 200; GE Healthcare). The protein in buffer [10 mM Tris·HCl (pH 7.5), 150 mM NaCl] was concentrated using an Amicon Ultra-15 concentrator unit with a molecular cutoff of 30 kDa (Millipore). The protein concentration was determined by Coomassie Brilliant Blue staining on the SDS/PAGE gel using BSA (Pierce) as a standard.

Flag-AtIDD3 and Flag-AtIDD3-C were produced by wheat germ cell-free translation using in vitro transcription/translation reagents (BioSieg) according to the manufacturer's instructions. The reaction mixture was centrifuged at 15,000 × *g* for 10 min and was used for the pull-down assay.

The pull-down assay was performed as follows. Synthesized Flag-AtIDD3 or Flag-AtIDD3-C (90 μL) and 10 μL of partially purified GST-RGA or GST-SCL3 were mixed (input). Then, 85 μL of the mixture was applied to 7.5 μL of Glutathione Sepharose 4B beads or anti-Flag M2 Affinity Gel (Sigma), and incubated for 16 h at 4 °C. After three washings with 600 μL of wash buffer [10 mM Tris·HCl (pH 7.5), 150 mM NaCl, and 1 mM DTT for Glutathione Sepharose 4B beads, and 10 mM Tris·HCl (pH 7.5) and 150 mM NaCl for anti-Flag M2 Affinity Gel], the bound protein was eluted with 20 mM Glutathione or 300 μg/mL Flag peptide (Sigma). The input and eluate were resolved by SDS/PAGE, and specific proteins were detected by immunoblot analysis using anti-Flag M2-HRP antibody (Sigma) or anti-GST HRP conjugate antibody (GE Healthcare).

EMSA. The recombinant truncated AtIDD3 protein (from amino acids 11–210) fused with GST was affinity purified from *E. coli* as described previously (11). The four eluted fractions were pooled and concentrated using Amicon ultra centrifugal filter devices (Millipore). The protein solution then was desalted using a

PD-10 column (GE Healthcare) equilibrated with KPi buffer containing 20 mM KPi (pH 9.0) and 5 mM DTT and was purified further by a MonoQ 5/50 GL (GE Healthcare) column equilibrated with KPi buffer and eluted with 0–1 M KCl gradient at a flow rate of 0.5 mL per min. The peak fractions for the truncated GST-AtIDD3 were loaded onto a Hi-Load 26/60 Superdex 200-pg column (GE Healthcare) equilibrated with the buffer containing 10 mM Tris·HCl (pH 7.5), 50 mM KCl, and 1 mM DTT. The peak fractions for the truncated GST-AtIDD3 were used.

DNA probes were generated by annealing a 5′ biotinylated oligonucleotide (Fasmac) to a complementary unmodified oligonucleotide (Fasmac), following the manufacturer's instructions (Sigma-Aldrich). The same procedure was followed to generate unmodified double-stranded DNA fragments for competition assays.

The Lightshift Chemiluminescent DNA EMSA kit (Pierce) was used according to the manufacturer's instructions to detect the biotinylated probes.

Analysis of Transgenic and Mutant *Arabidopsis* Plants. The rosette radius of each plant was determined by taking the mean of the lengths of the two largest leaves at 4 wk after germination, as previously described (12). Flowering time was defined as the number of days from sowing until flower buds became visible.

For paclobutrazol treatment, young seedlings (1 d after germination) were transferred to MS agar plates with or without 1 μM paclobutrazol (Wako).

Root length was measured as previously described ($n > 10$) (13).

To measure the number of leaf abaxial epidermal cells, the first true leaves from seedlings (8 d after germination) were used. Cell numbers in the first true leaves were counted as described previously (14). In this experiment, the first leaf pair was harvested from more than four plants in which the IDD function was suppressed by introduction of AtIDD3 fused with the plant-specific repression domain (IDD3-SRDX), from paclobutrazol-treated plants, and from untreated WT plants, and the data were analyzed using the Excel statistical package (Microsoft).

The formative cell divisions of ground tissue were observed as described previously (15). Roots of seedlings (4 d after germination) were used to observe additional periclinal divisions ($n > 30$). Laser-scanning confocal microscopy was performed using a Leica DMI6000 with propidium iodide (Sigma-Aldrich).

Expression Analysis by Quantitative RT-PCR. Total RNA was isolated from leaves of seedlings or whole seedlings sampled at 1 wk after germination using an RNeasy plant minikit (Qiagen). The first strand of cDNA was synthesized from 0.5 μg of total RNA using an Omniscript reverse transcription kit (Qiagen). Transcripts were quantified by quantitative RT-PCR (qRT-PCR) analyses using one-twentieth of the resulting cDNA as template. qRT-PCR was performed with a CFX96 real-time PCR detection system (Bio-Rad) with the SYBR Green PCR kit (Qiagen) and appropriate primers (Table S1). Relative transcript abundance was calculated by CFX96 Manager software (Bio-Rad).

Yeast Three-Hybrid Analysis. The yeast three-hybrid assays were performed following the manufacturer's instructions (Clontech). Plate assays were performed as previously described (16) with a modification of the selection medium.

1. Clough SJ, Bent AF (1998) Floral dip: A simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J* 16(6):735–743.
2. Mitsuda N, et al. (2010) Efficient yeast one-/two-hybrid screening using a library composed only of transcription factors in *Arabidopsis thaliana*. *Plant Cell Physiol* 51(12):2145–2151.
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5. Hirano K, et al. (2012) The suppressive function of the rice DELLA protein SLR1 is dependent on its transcriptional activation activity. *Plant J* 71(3):443–453.
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7. Oshima Y, et al. (2011) Novel vector systems to accelerate functional analysis of transcription factors using chimeric repressor gene-silencing technology (CRES-T). *Plant Biotechnol* 28(2):201–210.
8. Yoo SD, Cho YH, Sheen J (2007) *Arabidopsis* mesophyll protoplasts: A versatile cell system for transient gene expression analysis. *Nat Protoc* 2(7):1565–1572.
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13. Tsukagoshi H, Busch W, Benfey PN (2010) Transcriptional regulation of ROS controls transition from proliferation to differentiation in the root. *Cell* 143(4):606–616.
14. Achard P, et al. (2009) Gibberellin signaling controls cell proliferation rate in Arabidopsis. *Curr Biol* 19(14):1188–1193.
15. Heo JO, et al. (2011) Funneling of gibberellin signaling by the GRAS transcription regulator scarecrow-like 3 in the *Arabidopsis* root. *Proc Natl Acad Sci USA* 108(5): 2166–2171.
16. Ueguchi-Tanaka M, et al. (2005) *GIBBERELLIN INSENSITIVE DWARF1* encodes a soluble receptor for gibberellin. *Nature* 437(7059):693–698.

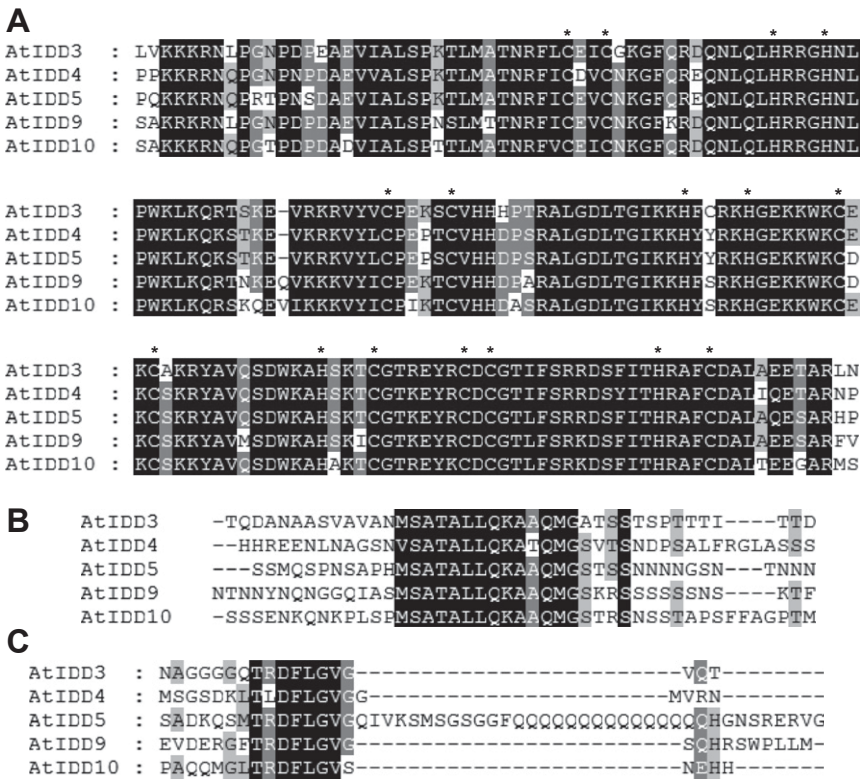

Fig. S1. Alignment of conserved domain amino acid sequence of IDDs. (*A*) Alignment of the ID domain. The asterisks indicate conserved cysteine (C) and histidine (H) residues. (*B*) The C-terminal MSATALLQKAA conserved domain. (*C*) The C-terminal TR/LDFLG conserved domain.

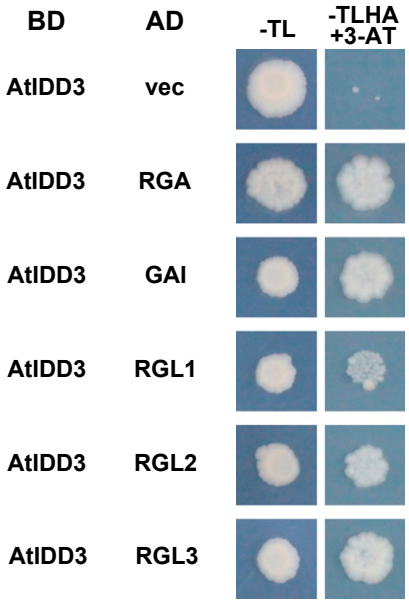

Fig. S2. AtIDD3 interacts with all DELLA proteins present in *Arabidopsis*. The selection medium contained 50 mM 3-AT. AD, GAL4-activating domain; BD, GAL4-binding domain; –TL, synthetic complete medium lacking Trp and Leu; –TLHA, synthetic complete medium lacking Trp, Leu, His, and adenine.

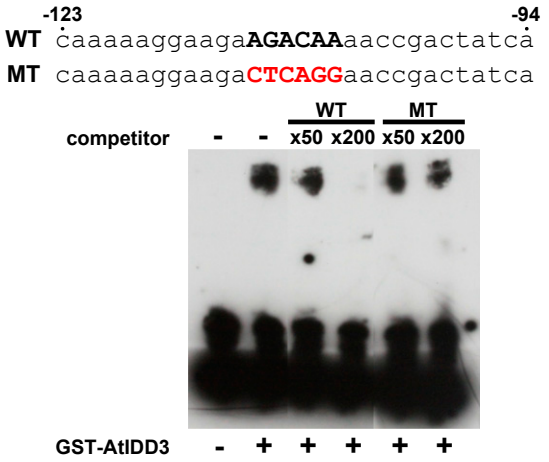

Fig. S3. Competitive gel mobility shift assays. (*Upper*) DNA fragments used in this EMSA. The oligonucleotide assigned as WT was used as the biotin-labeled probe. MT; mutant-type oligonucleotide. (*Lower*) Competition experiments were performed using increasing molar amounts (0.5 and 2 μM) of the indicated unlabeled fragments.

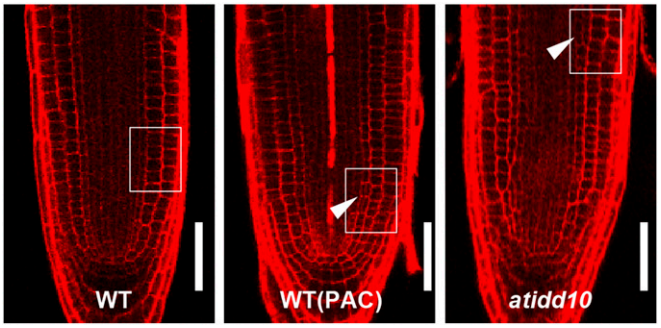

Fig. S4. Longitudinal confocal sections of the root meristem in seedlings at 4 d after germination. (Scale bar: 50 μm.) Arrowheads indicate periclinal cell division in the endodermis. Higher-magnification views of the boxed areas are shown in Fig. 3*A*.

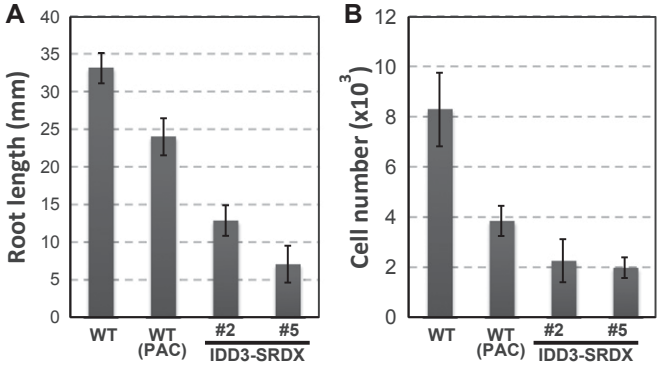

Fig. S5. GA-related phenotypes of IDD3-SRDX plants. (*A* and *B*) The root length (*A*) and total number of abaxial epidermal cells of the first true leaf pair from the seedlings (8 d after germination) (*B*) of IDD3-SRDX, WT (+1 μM paclobutrazol, PAC), and untreated WT plants. Error bars indicate SD of the mean.

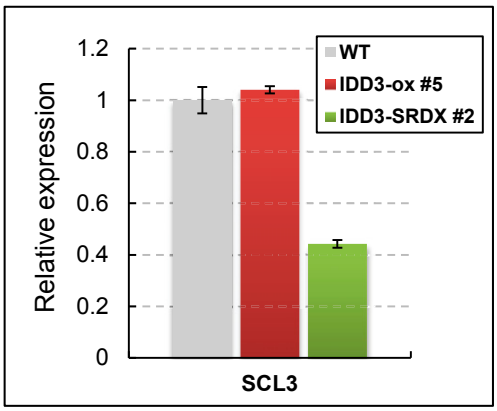

Fig. S6. Relative transcript levels of *SCL3* from 1-wk-old seedlings. Biological triplicate samples were averaged. Error bars indicate SD of the mean.

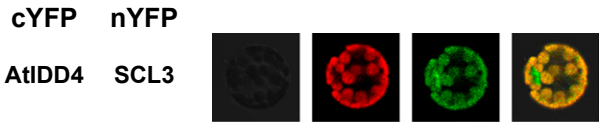

Fig. S7. IDD-SCL3 binding by bimolecular fluorescence complementation in *Arabidopsis* mesophyll protoplasts.

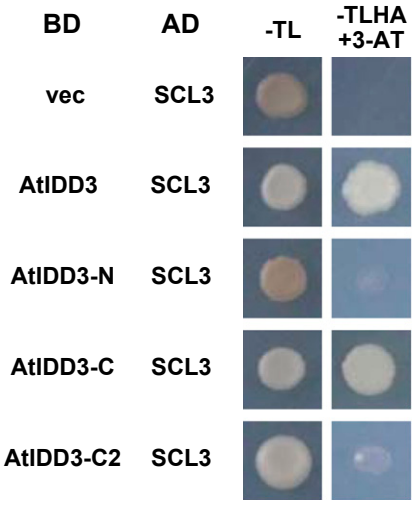

Fig. S8. Domain analyses of SCL3 in Y2H assays. The selection medium contained 50 mM 3-AT.

Table S1. Primers used in this study

Primer name	Sequence
T-DNA analysis	
SALK_054242.F1	GGTATGTGATGTACCTTGCGC
SALK_054242.R1	AGTCTGATTGGAAGGCACATG
SALK_LBb1.3	ATTTTGCCGATTTCGGAAC
Construction for yeast assays	
SmaI+RGA555.F	TCCCCCGGGTGGAGTCATAGGAACGACGGT
RGA.BamHI	CGGGATCCTCAGTACGCCGCCGTCGAGAGT
EcoRI+SCL3.prom.F	GGAATTCCCCACACCCAAGCCTCAGCCTCA
SCL3.prom.R+XbaI	GCTCTAGAGGTTCTCTCAATCTTTATCTCT
EcoRI+SCL3.F	GGAATTCATGGTGGCTATGTTTCAAGAAGA
SCL3.R+SmaI	TCCCCCGGGTCACTTCCTGCATCTCCAAGC
MGP687.R+BamHI	CGGGATCCTGTCCCCATGAGATAATGATAG
EcoRI+MGP688.F	GGAATTCCTTATCCCATCACCATCACTACC
EcoRI+MGP1201.F	GGAATTCACCGACCAATCAGCGTATCTTCA
NdeI+RGA.F	GGCATATGAAGAGAGATCATCACCAATTC
NdeI+RGAG186.F	GGCATATGGGAGTCATAGGAACGACGG
NdeI+RGAQ280.F	GGCATATGCAGAATCAGATCGATCATTG
NdeI+RGAL407.F	GGCATATGCTTAGACCGAGCGATACGG
RGA.R+BamHI	GCGGATCCTCAGTACGCCGCCGTCGAG
RGAe406.R+BamHI	GCGGATCCCTCAAGCATCGAAGCATCG
NotI+RGA.F	ataagaatGCGGCCGCaATGAAGAGAGATCATCACCA
RGA.R+NotI	atagtttaGCGGCCGCTCAGTACGCCGCCGTCGAGA
Construction of reporter plasmids	
HindIII+pAtSCL3.F	CCCAAGCTTCCCACACCCAAGCCTCAGCCT
AtSCL3.R+SmaI	TCCCCCGGGGGTTCTCTCAATCTTTATCTC
SmaI+fLUC.F	TCCcccgggATGGAAGACGCCAAAAACATA
fLUC.R+SacI	gagctcTTACACGGCGATCTTTCCGCCCTT
HindIII+pAtSCL3.397.F	CCCAAGCTTCTAAATCAAGAAAATTCTAGG
HindIII+pAtSCL3.748.F	CCCAAGCTTCAACCTTTTGTTTAATAATTC
HindIII+pAtSCL3.831.F	CCCAAGCTTACTATCACGCATTGTCTGGTA
HindIII+pAtSCL3.861.F	CCCAAGCTTAAAAGGAACAATGCCTCTGTT
Construction of the effector vector	
HindIII+GATEway.F	AAGCTTGCATGCCTGCAGGTCCCCAGATTA
GATEway.R+SpeI+ApaI+StuI+SacI	GAGCTCTAAGGCCTGGGCCCACTAGTAGCGCTGTTATCAACCACTTTGTA
SpeI+VP16.F	gactagtGATATCCCCCCGACCGATGTCAG
VP16.R+StuI	aaggcctTCAATCCCCACCGTACTCGTCAA
Construction of entry clones	
CACC+AtIDD3.F	CACCATGACAACTGAAGATCAGACAATTTC
AtIDD3.nonSTOP.R	AATCCATCCATTGATAGAGGAAGAATGGCA
CACC+AtIDD4.F	CACCATGTCGTCATCATCATATAACACAAG
AtIDD4nonSTOP.R	ACCTCTTCCAAATGGATAATTTTGCTCAGG
CACC+AtIDD5.F	CACCATGGCTGCTTCTTCATCCTCCGCTGC
AtIDD5nonSTOP.R	GAAACTCGCATGATGGATTCCATAAGGTGG
CACC+AtIDD9.F	CACCATGATGATGCCAGATGATCATCATCC
AtIDD9nonSTOP.R	CTGGTTCATGTCGGCGGTTGGTGTGCCGTC
CACC+AtIDD10.F	CACCATGCAGATGATTCCAGGAGATCCATT
AtIDD10nonSTOP.R	ACCCAATGGAGCAAACCTTGCGAGTTCTTG
CACC+RGA.F	CACCATGAAGAGAGATCATCACCAATTCCA
RGA.nonSTOP.R	GTACGCCGCCGTCGAGAGTTTCCAAGCGGA
CACC+AtSCL3.F	CACCATGGTGGCTATGTTTCAAGAAGATAA
AtSCL3.nonSTOP.R	CTTCCTGCATCTCCAAGCTGATACCGAGTA
Construction for plant transformation	
SmaI+MGP.F	TCCCCCGGGATGACAACTGAAGATCAGACA
MGP.R+SmaI	TCCCCCGGGAATCCATCCATTGATAGAGGA
Construction for protein expression in *E. coli*	
BamHI+IDD3_28-.F	CGGGATCCAGTGGAGGATATGTCCAAAGCT
IDD3_-630+EcoRI.R	GGAATTCTCAGAGATGCGAGGCTGCGTTTA
qRT-PCR	
GAPC F2	AGCTGCTACCTACGATG
GAPC R2	CACACGGGAACTGTAAC
EXP1 F	ACCTTCTTGTTTATTGCTACCCTTGG
EXP1 R	AATGTGGCGTGTGCGTTGAC
EXP8 QF	TCCTCCTCTTCAGCATTTCGACCT

Table S1. Cont.

Primer name	Sequence
EXP8 QR	CTTGCCACGACTGTGTTTTTGAGC
PRE1RTF	GTTCTGATAAGGCATCAGCCTCG
PRE1GWR	CATGAGTAGGCTTCTAATAACGG
PRE2 F	CCGTCGTTCCAACACGGTATCAG
PRE2 R	CTGCGGCTTGTGGGCTATTAGG
PRE5 F	AACGGCGTCGTTCTGATAAG
PRE5 R	CATGAGTAAGCTTCTAATCACGG
KIDARI F	TCCAACACCTCATCCCTGAACTTCGC
KIDARI R	CGGTCACTGAGGTCATCAACCTCTC
AtSCL3 RT.F2	CCTCAATGCTATTTGGGGTTTG
AtSCL3 RT.R2	ATTAGTGTGGAGCCGTTGTGG

F, forward; R, reverse.