Supporting Information

Sang et al. 10.1073/pnas.1411859111

SI Materials and Methods

Plant Materials and Growth Conditions. Rice (*Oryza sativa* L.) plants were grown in paddy fields in Beijing in summer or in Hainan province in winter. The *la1*, *sol1*, and *sols la1* mutants are in the *ZH11* background, *d14* is in the *Shiokari* background, and *d27* is in the *Nipponbare* background, all of which are *japonica* subspecies. Seedlings were grown on 0.4% agar plates at 28 °C under a 16-h light/8-h dark cycle.

Arabidopsis seedlings were grown in soil or on 1/2 Murashiga and Skoog (MS) medium plates containing 1% sucrose and 0.8% agar at 22 °C under a 16-h light/8-h dark cycle. All *Arabidopsis* mutants used in this study are in the Columbia (Col-0) background. The *Atla1* (GABI_591A12) mutant was obtained from the *Arabidopsis* Biological Resource Center. The transfer DNA (T-DNA) insertion sites were verified by PCR and sequencing (Fig. S7), indicating that the genotype is identical to a previously reported allele (1). The *max2* and *max4* mutants were provided by Ottoline Leyser (University of Cambridge, Cambridge, UK) (2). *Atla1 max2* and *Atla1 max4* double mutants were generated from the genetic crosses of *Atla1* × *max2* and *Atla1* × *max4*, and homozygous lines were confirmed by PCR genotyping and/or sequencing. Primers used for identifying T-DNA insertion sites and genotypes of double mutants are given in Table S1.

Map-Based Cloning of Suppressors of *LA1*. To isolate suppressors of *LA1* (SOLs), we carried out map-based cloning. The *sol* mutants were crossed to *la1* in the background of *ZF802*, an *indica* variety. The InDel and cleaved amplified polymorphic sequences markers were generated based on nucleotide polymorphisms between the genome sequences of Nipponbare and 93-11, an *indica* variety. The *SOL1* locus was placed within an 80-kb region between M8 and M10 on chromosome 6 by using 220 F_2 plants showing a compact plant type. The molecular lesions of *sol1-1* and *sol1-2* were identified by PCR amplification of the LOC_Os06g06050 (*D3*) genomic region from *la1* and *sol1-1* and *sol1-2* mutant plants and sequence comparison using DNASTAR. The primer sequences used for mapping are listed in Table S1.

Assay of Shoot Response to Gravity. The gravitropic assay was carried out as described previously (3). For rice shoot gravitropism, 3- or 4-d-old light-grown seedlings were grown on 0.4% agar containing different concentrations of GR24 as indicated and then were transferred to darkness and reoriented by 90° for a series of time periods at 28 °C. To examine gene expression upon gravity stimulation by qRT-PCR analyses, 7-d-old light-grown seedlings were reoriented by 90° for 6 h, and then 1.5 cm of the basal shoot was dissected into lower and upper sides. For *Arabidopsis* shoot gravitropism, 4-d-old etiolated seedlings were grown on 1/2 MS medium with or without 2.5 μM GR24 and then were reoriented by 90° for up to 24 h, or 1-mo-old inflorescence stems were transferred to darkness with 24 h gravistimulation.

Generating *D3*RNAi Transgenic Lines. To construct the *D3*RNAi plasmid, two 350-bp DNA fragments were amplified from the *D3* cDNA using two pairs of primers, D3RNAi-F1 and D3RNAi-R1 and D3RNAi-F2 and D3RNAi-R2 (Table S1) and were cloned into the binary vector 1460. This recombined plasmid then was introduced into *Agrobacterium tumefaciens* EHA105, and the *la1* mutant was transformed as previously reported (4). The phenotypes were scored in the homozygous T_3 progeny.

Quantitative RT-PCR Analysis. Total RNA was extracted using a TRIzol RNA extraction kit (Invitrogen). One microgram of total RNA was treated with DNase I and used to synthesize cDNA with an Avian Myeloblastosis Virus Reverse Transcriptase (Promega). Quantitative RT-PCR (qRT-PCR) experiments were performed using the SsoFast EvaGreen Supermix kit (Bio-Rad) on the CFX96 real-time system (Bio-Rad) following the manufacturer's instructions. The expression levels were normalized to the expression of a rice ubiquitin gene. The gene-specific primers are listed in Table S1.

Measurement of Free Indoleacetic Acid Content. Indoleacetic acid (IAA) extraction and measurement were performed as previously described (5) with minor modifications. Briefly, 7-d-old seedlings were reoriented by 90° for 12 h. After gravistimulation, 150-mg shoot tissues from the lower and upper sides of the 1.5-cm shoot base were collected for analysis. After extraction and purification, the samples were subjected to LC/MS-MS analysis using a system consisting of an Acquity Ultra Performance Liquid Chromatograph (Acquity UPLC; Waters) and a triple quadruple tandem mass spectrometer (Quattro Premier XE; Waters).

Lateral Auxin Transport Assay. Lateral auxin transport was assayed as previously described with minor modifications (3). Briefly, 5-d-old dark-grown coleoptiles (1 cm) were harvested and deprived of endogenous IAA. The apical ends of coleoptiles were inserted horizontally into agar blocks that contain 100 nM ^{3}H-IAA. After transport in darkness at 28 °C for 2.5 h, sections of the 0.5-cm segments from the apex were split evenly into upper and lower halves. After incubation in 2 mL scintillation liquid overnight, the radioactivity of each half was counted by a liquid scintillation counter (1450 MicroBeta TriLux; Perkin-Elmer).

1. Yoshihara T, Spalding EP, Iino M (2013) AtLAZY1 is a signaling component required for gravitropism of the *Arabidopsis thaliana* inflorescence. *Plant J* 74(2):267–279.
2. Stirnberg P, Furner IJ, Ottoline Leyser HM (2007) MAX2 participates in an SCF complex which acts locally at the node to suppress shoot branching. *Plant J* 50(1):80–94.
3. Li P, et al. (2007) LAZY1 controls rice shoot gravitropism through regulating polar auxin transport. *Cell Res* 17(5):402–410.
4. Hiei Y, Ohta S, Komari T, Kumashiro T (1994) Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA. *Plant J* 6(2):271–282.
5. Fu J, Chu J, Sun X, Wang J, Yan C (2012) Simple, rapid, and simultaneous assay of multiple carboxyl containing phytohormones in wounded tomatoes by UPLC-MS/MS using single SPE purification and isotope dilution. *Anal Sci* 28(11):1081–1087.

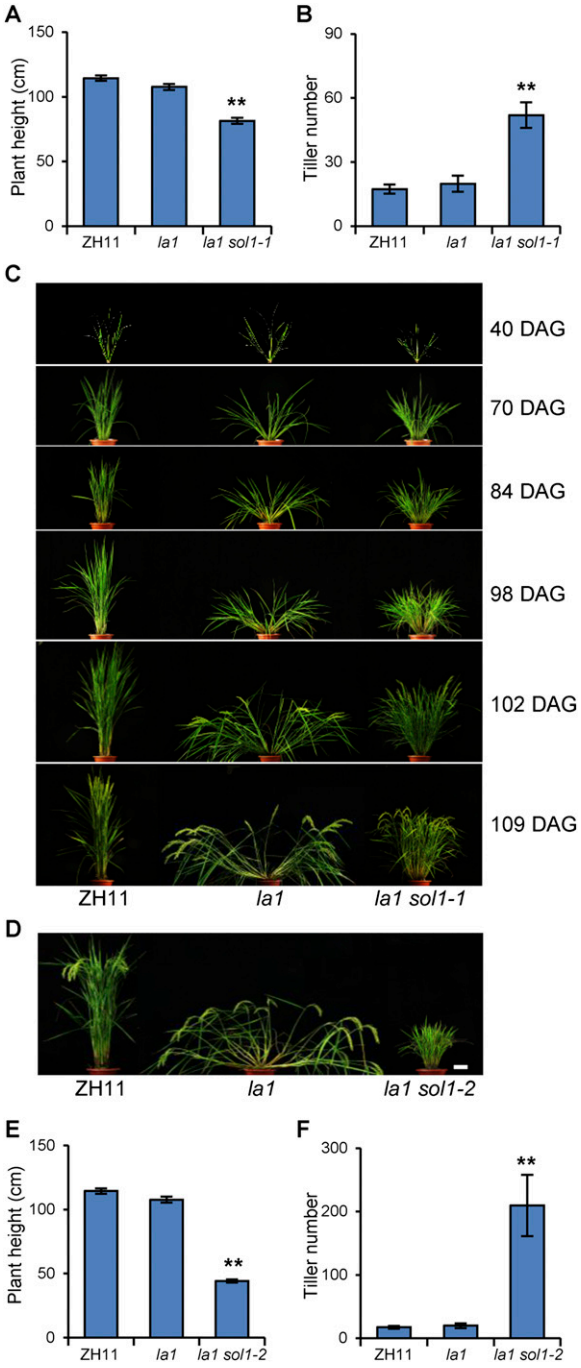

Fig. S1. Phenotypic comparison of wild-type (ZH11), *la1*, *la1 sol1-1*, and *la1 sol1-2* plants. (*A* and *B*) Statistical analysis of plant height (*A*) and tiller number (*B*) of wild-type, *la1*, and *la1 sol1-1* plants. (*C*) Phenotypic comparison of wild-type (ZH11), *la1*, and *la1 sol1-1* plants after the seedling stage. DAG, days after germination. (*D*) Phenotypic comparison of wild-type (ZH11), *la1*, and *la1 sol1-2* plants at the adult stage. (*E* and *F*) Plant height (*E*) and tiller number (*F*) of wild-type, *la1*, and *la1 sol1-2* plants. Error bars indicate SEM; $n = 10$. $^{**}P < 0.01$, Student *t* test.

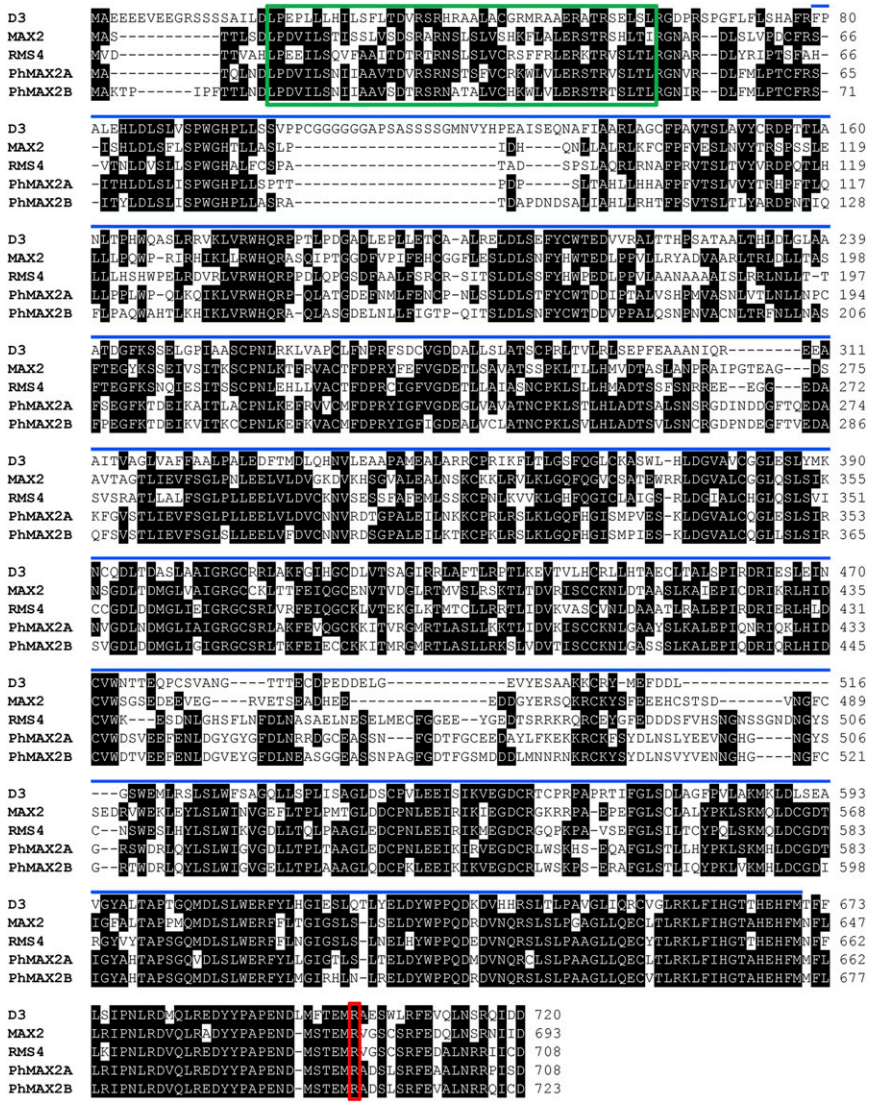

Fig. S2. Sequence alignment of rice *D3*, *Arabidopsis MAX2*, pea *RMS4*, *petunia PhMAX2A*, and *PhMAX2B*. The conserved R702 is marked by the red box. The green box indicates the F-box, and the blue lines above the sequence indicate leucine-rich repeats.

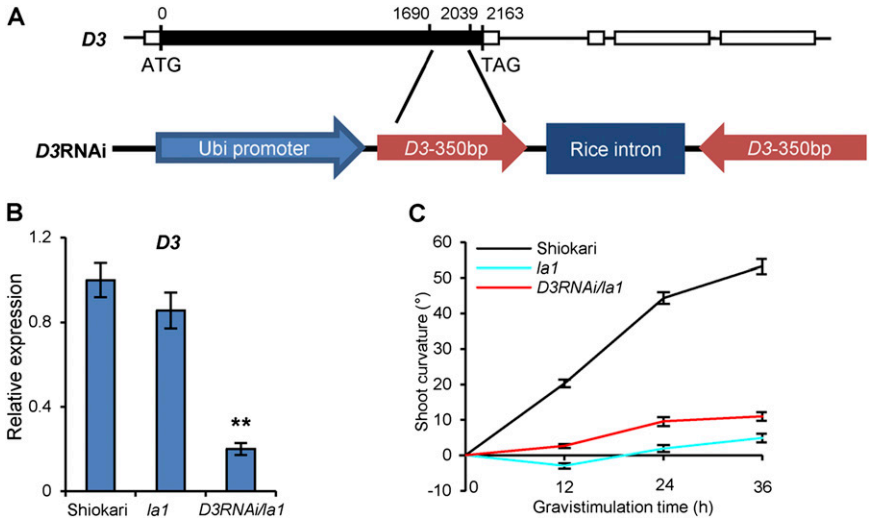

Fig. S3. Confirmation of the involvement of D3 in shoot gravitropism. (*A*) Schematic representation of *D3* and *D3*RNAi constructs. The closed box indicates an ORF, and open boxes indicate UTRs. The red arrows indicate the fragment selected from the corresponding sequence in *D3*. (*B*) qRT-PCR analysis of *D3* expression levels in *D3RNAi/la1* transgenic seedlings. Error bars indicate SEM; $n = 4$. $^{**}P < 0.01$; Student *t* test. (*C*) Kinetic analysis of wild-type (Shiokari), *la1*, and *D3RNAi/la1* shoot curvature upon gravistimulation. Three-day-old light-grown seedlings were transferred to darkness and reoriented by 90°. Error bars indicate SEM; $n = 10$.

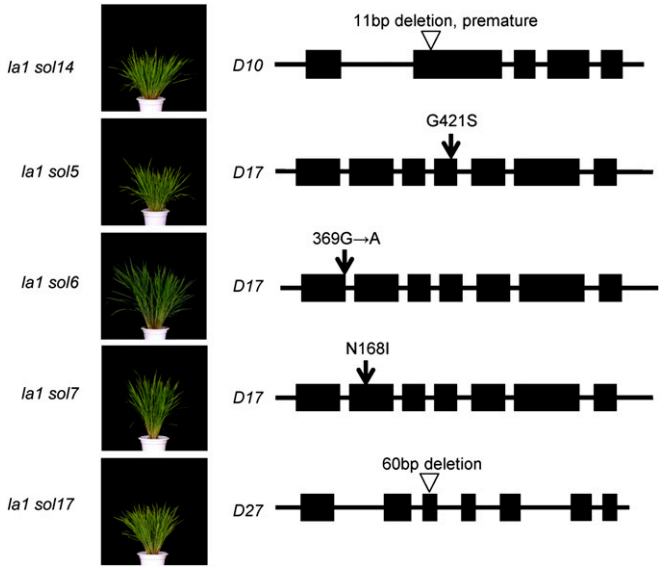

Fig. S4. Identification of other *la1 sols*. The phenotypes of *la1 sols* and their mutation sites at the corresponding *D* loci. The black boxes represent exons. An 11-bp deletion occurred at the second exon in *D10* in *la1 sol14*, resulting in premature translation. The 421st amino acid G of D17 is replaced by S in *la1 sol5*. The 369th base G, which occurs at the splicing site after the first exon, is replaced by A in *D17* in *la1 sol6*. The 168th amino acid N of *D17* is replaced by I in *la1 sol7*. A 60-bp deletion occurs in *D27* in *la1 sol17*, resulting in a truncated protein.

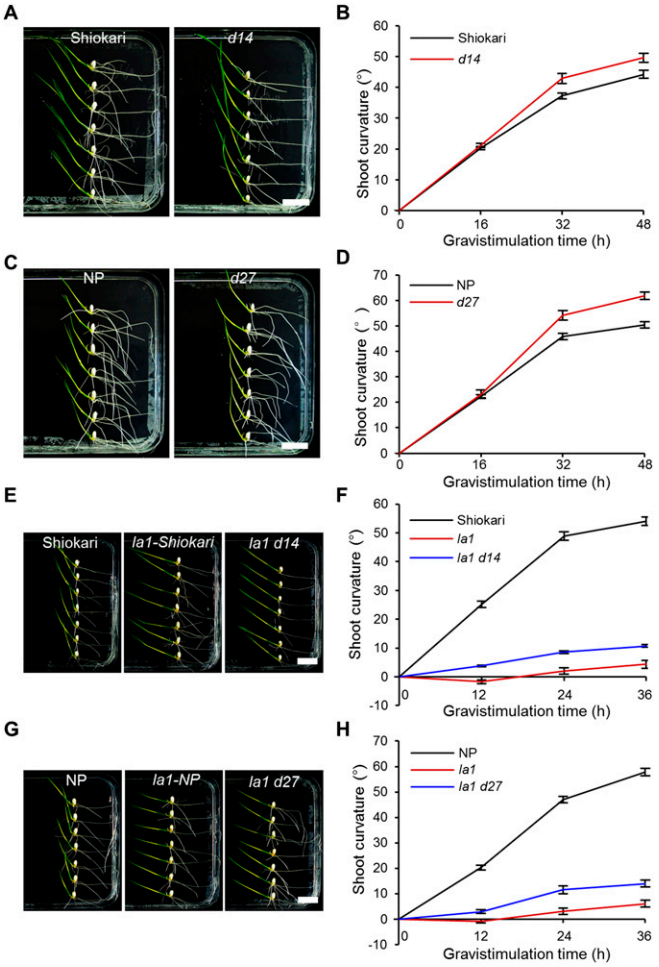

Fig. S5. Analysis of gravitropic responses. (*A* and *B*) Photographs (*A*) and kinetic analysis (*B*) of shoot gravitropism of the wild type (Shiokari) and *d14* mutants upon 48-h gravistimulation. (*C* and *D*) Photographs (*C*) and kinetic analysis (*D*) of shoot gravitropism of the wild type (NP) and *d27* mutant upon 48-h gravistimulation. (*E* and *F*) Photographs (*E*) and kinetic analysis (*F*) of shoot gravitropism of the wild type (Shiokari), *la1*, and *la1 d14* mutants upon 36-h gravistimulation. (*G* and *H*) Photographs (*G*) and kinetic analysis (*H*) of shoot gravitropism of the wild type (NP), *la1*, and *la1 d27* mutants upon 36 h gravistimulation. The 3-d-old light-grown seedlings were transferred to darkness and reoriented by 90° for gravistimulation. Error bars indicate SEM. $n = 10$. (Scale bar: 2 cm.)

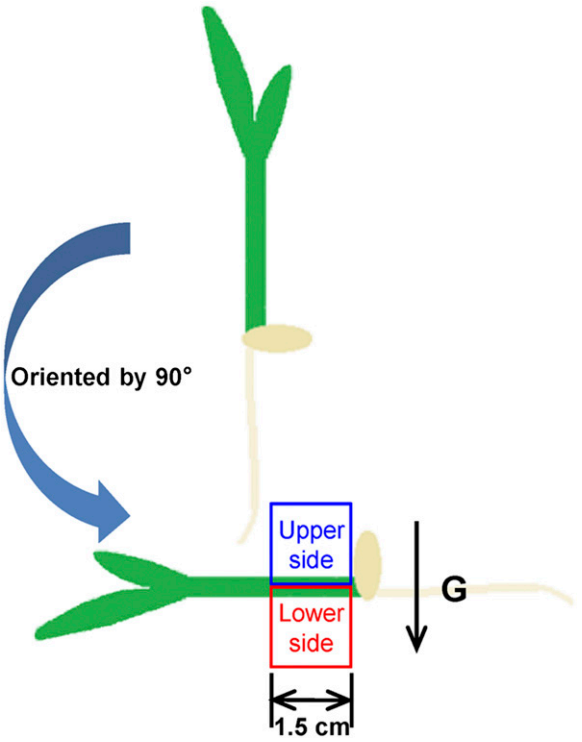

Fig. S6. Graphical representation of rice seedling upon gravistimulation. The blue and red boxes indicate the upper and lower sides of a shoot base, respectively, for gene-expression analysis and measurement of IAA content.

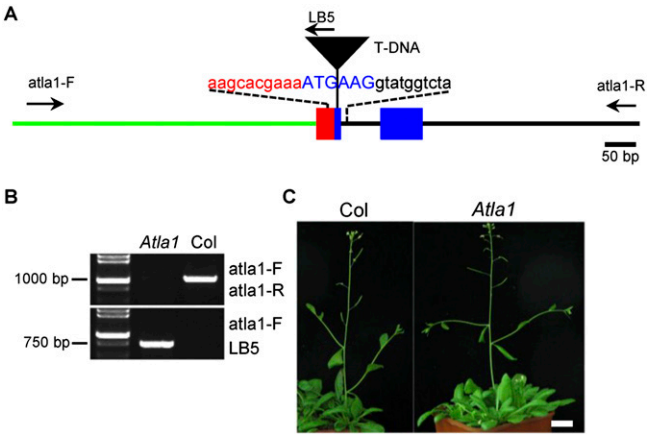

Fig. S7. Characterization of the *Atla1* mutant. (*A*) Diagram of *AtLA1* and the mutation site in the T-DNA insertion line. The sequence above the genomic structure shows the wild-type sequences flanking the T-DNA insertion site. Blue boxes indicate coding exons, the red box indicates the 5′ UTR, the black lines between the boxes indicate introns, and the green line indicates the promoter. The triangle represents T-DNA insertion, and the arrows indicate the positions of primers used for PCR genotyping. (*B*) T-DNA insertion was confirmed by the genomic PCR and sequencing. (*C*) The phenotype of the *Atla1* mutant at the adult stage, showing the larger branch angle. (Scale bar: 2 cm.)

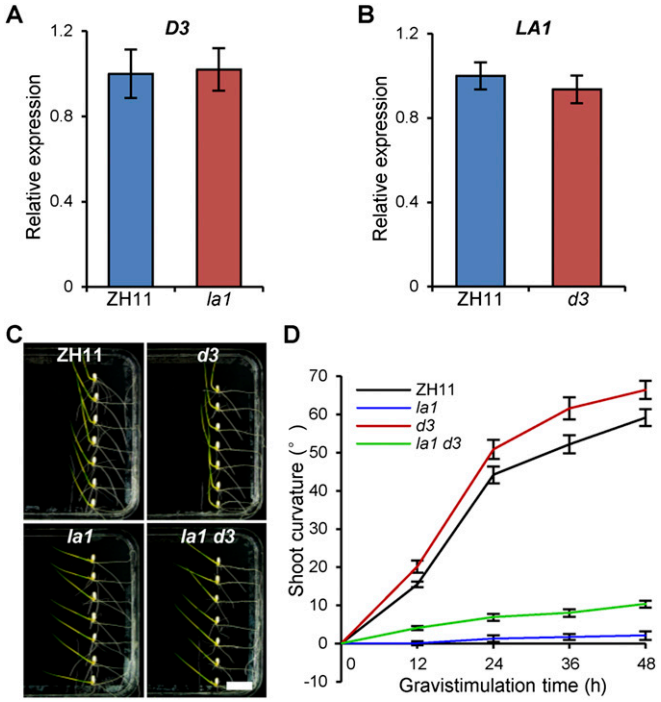

Fig. S8. Gene-expression analysis of *LA1* and *D3* by qRT-PCR and gravitropic responses of the wild type, *d3*, *la1*, and *la1 d3*. (*A*) Comparison of *D3* levels expressed in the wild-type and *la1* seedlings. (*B*) Comparison of *LA1* levels expressed in wild-type and *d3* seedlings. (*C* and *D*) Photographs (*C*) and kinetic analysis (*D*) of shoot gravitropism of wild-type, *d3*, *la1*, and *la1 d3* seedlings upon 48-h gravistimulation. (Scale bar: 2 cm.) Error bars indicate SEM. $n = 3$ in *A* and *B*; $n = 10$ in *D*.

Table S1. Primers used in this study

Primer name	Sequence (5′-3′)
Genotyping	
LA1-F	CAGATATTTAGAAACGGAGGGAG
LA1-R	TACCGGCAAACAATTGAACTC
D14-F	ATGCTGCGATCGACGCATCC
D14-R	TTAGTACCGGGCGAGAGCG
D27-F	TGCCTGATACCTGATTAG
D27-R	GTTGTGTTTACCCACTGA
atla1-F	CACTCTAAGTGAGCAAGGAG
atla1-R	GGCAAATTGAGTAGGTGAGC
LB5	ATATTGACCATCATACTCATTGC
Map-based cloning	
M1F	AGGCTTGCTCCGTTTGAT
M1R	CATACAGACAGGTGGTACAGTAAAT
M2F	TCGCCGAGGGAATACAAAT
M2R	GCACGGAGAACCAAGCGGGAAAC
M3F	CAGGTGGGAGAAAGAAAGCC
M3R	GAGGAAGAAGCCAAGGAGG
M4F	TCTTTCTCCTCCTCAATACCTG
M4R	GGCAAGAAACACGCTAAGAT
M5F	AAACGGAATACCAAACAGGTG
M5R	TCTGCTGCTCGCATCACG
M6F	AAATCTTACGATATGGCACGG
M6R	CAGTTTTCCCTTCGCCAC
M7F	AACACGGCTGCCTGCCTGAG
M7R	GGCGGTGGTGGTCGAGGTAA
M8F	CACGGTGTGGATGGATCG
M8R	CTTACCGTCAGGATGAAGAACAT
M9F	AAATCCCTCAACGGCAGC
M9R	CCGGCAGGTCCAGTATCG
M10F	GGATTGGTTTTATGCCGTAA
M10R	ATGAGGACGACGAGCAGATT
M11F	TGCTCGCCATATCTTCCC
M11R	TTCTTCTCCTGAGGCTCTACT
Transgenic constructs	
D3RNAi-F1	AAGGATCCCCACGACCTGCTCCAAGAAC
D3RNAi-R1	AAGGTACCCGCAAGTTTGGAATTGAAAGGA
D3RNAi-F2	AAGAGCTCCCACGACCTGCTCCAAGAAC
D3RNAi-R2	AAACTAGTCGCAAGTTTGGAATTGAAAGGA
qRT-PCR	
OsUBQ1-QF	AACCAGCTGAGGCCCAAGA
OsUBQ1-QR	ACGATTGATTTAACCAGTCCATGA
OsIAA20-QF	TGGCGGATATGTGAAGGTGAA
OsIAA20-QR	TATGAGCCGAGGATGGACAAG
LA1-QF	GCAACGCCGAGATGAACG
LA1-QR	ATAATTCCAGCACCAAGTAGTCG
D3-QF	CCACCATTTGCTGATTCGTTCT
D3-QR	ATGTTCTGATGCTGATCTTGTTCC