

Supporting Information

Materials and Methods

Plant Materials and Growth Conditions

All *Arabidopsis thaliana* plants used in this study were of the Columbia-0 ecotype. *35S:PIF3-Myc* (1), *35S:PIF4-Myc* (2), have been described previously. All the seeds were vernalized for 3 days at 4°C in the dark and grown on MS medium containing 1% sucrose. For phenotypic analysis under light, the seedlings were grown under continuous white light (20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). For phenotypic analysis in darkness, the plants were treated first with white light (70 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for 24h and then transferred into the dark. For statistical analysis, more than 20 seedlings were measured. ImageJ software was used to measure cotyledon areas and hypocotyl lengths.

Generation of Transgenic Plants

For overexpressing *SAURs* in the wild type background, the full length GFP was amplified from pUC18-sGFP (3) and cloned into the *StuI* and *SpeI* restriction sites of vector pJim19 (Bar) to construct pJim19-GFP (Bar). Then, the full length CDSs (coding sequences) of *SAUR14*, *SAUR50* and *SAUR65* without stop codons were amplified and inserted into the *XbaI* and *StuI* sites of vector pJim19-GFP to yield pJim19-SAUR-GFP. To generate transgenic plants overexpressing these genes in the *pifq* background, the full length *35S:SAUR14-GFP*, *35S:SAUR50-GFP* and *35S:SAUR65-GFP* sequences were amplified from pJim19-SAUR-GFP constructs and inserted into pCambia1300 using the *EcoRI* and *HindIII* sites. For generating *saur50saur16* mutants, the designed guide sequences targeting *SAUR50* and *SAUR16* were inserted into the *BbsI* sites of the pAtU6-26-SK vector (4), respectively. The product *SAUR50* sgRNA expression cassette was digested with *KpnI* and *SalI* and ligated into p35S-Cas9-SK. Then, the Cas9 cassette was cloned into pCambia1300 using *KpnI* and *EcoRI* to generate pCambia1300-SAUR50 Cas9. The *SAUR16* sgRNA expression cassette had *XbaI* and *KpnI* sites added to the ends by PCR with primers AtU6-sgRNA F and AtU6-sgRNA R, and was inserted into the vector pCambia1300-SAUR50 Cas9. All the constructs were transformed using *Agrobacterium* GV3101. Around 10 lines were obtained for each construct, and two representative independent lines were shown. We used T4 generation *saur50saur16* mutants which didn't contain the CRISPR/Cas9 construct for phenotypic characterization. The primers are listed in Table S1.

RNA-Seq Analysis

The seeds for RNA-seq were incubated under white light ($70 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for 3h to trigger germination and treated with FR ($2 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for 5 min before transfer into darkness. After growth in the dark for four days, the seedlings were harvested or transferred into white light ($70 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for 1h and 6h. At these time points, the cotyledons and hypocotyls were separately collected for RNA extraction. Total RNA was extracted using RNeasy Plant Mini Kits (Qiagen). mRNA deep sequencing was performed using Illumina HiSeq 2000 (Illumina, Inc., San Diego, CA, USA). Three biological repeats for each sample were prepared for RNA-seq analyses.

The paired-end reads generated by the Illumina HiSeq 2000 system were initially processed to remove the adapter sequences and low-quality (Q score < 20) reads. Next, the high-quality reads were mapped to the *Arabidopsis* TAIR10 genome using the spliced alignment identification tool TopHat (5). TopHat first mapped the reads using an unspliced aligner, and the remaining unmapped reads were split into shorter segments and aligned independently. Here, default TopHat parameters were used, and two mismatches per read were allowed. The sequence alignment files generated by TopHat were then used as inputs for Cufflinks (6), which assembled the mapping files into transcripts. After that, the significantly differentially expressed genes were identified by the program Cuffdiff with estimating FPKM (reads per kilobase of exon model per million mapped reads) values (6). The RNA-seq data generated in this study were submitted to NCBI's Gene Expression Omnibus (GSE79576).

qRT-PCR Analysis

Total RNA was extracted using RNeasy Plant Mini Kits (Qiagen). $1\mu\text{g}$ RNA were used as the templates and cDNA was synthesized using ReverTra Ace qPCR RT Master Mix (TOYOBO). qRT-PCR was performed using the 7500 real-time PCR system (Applied Biosystems). Gene-specific primers are listed in Table S1. *PP2A* was used as an internal control. At least three biological repeats for each experiment were performed and the representative result was shown.

Yeast Two-Hybrid Assays

Full length CDSs of *PP2C-D1* to *D9* were amplified and inserted into pLexA (Clontech) as baits.

Full length CDSs of *SAUR14*, *SAUR50* and *SAUR65* were cloned into vector pB42AD (Clontech) as preys. Specific bait and prey constructs were cotransformed into yeast EGY48 (containing p8op-lacZ). The transformants were grown on SD/-His-Trp-Ura medium for 3 days at 30°C. Then, six colonies were transferred onto SD/-His-Trp-Ura medium with the supplement of X-β-Gal, and two colonies were shown as representatives.

***In vitro* Phosphatase Assays**

PP2C-D1 was cloned into the BamHI and XhoI restriction sites of pGEX-4T-1 (Amersham). *SAUR14*, *SAUR50* and *SAUR65* were inserted into the BamHI and Sall sites of pGEX-4T-1. All the GST-tagged proteins were induced by IPTG for 16h at 16°C and purified using glutathione agarose beads (GE Healthcare). In phosphatase assays, 1μmol GST-PP2C-D1 was pre-incubated with 0.8μmol GST, GST-SAUR14, GST-SAUR50 or GST-SAUR65 for 10 min. The mixed proteins or control GST alone were then adjusted to a final volume of 500 μl assay buffer (7). The absorbance at 405 nm was measured every minute for 20 min on a Beckman DU800 spectrophotometer.

Confocal Microscopy

The fluorescence of VENUS was detected using a Zeiss LSM 710 confocal microscope. DII-VENUS transgenic lines were grown in darkness for four days and then either transferred to white light or kept in darkness for 0, 1, or 6h. The fluorescence at each time point was recorded using a 10× objective lens. The excitation wavelength was 514 nm and the emission wavelength was 520-560 nm.

Measurement of Free IAA Levels

The seeds were incubated under white light ($70 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for 3h and treated with FR ($2 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for 5 min. Next the seedlings were grown in the dark for 4 days, and then transferred into white light for 0h, 1h and 6h. At each time point, the cotyledons and hypocotyls were collected separately. About 60 mg cotyledons and 500 mg hypocotyls were used for the measurement of phytohormone IAA. The measurement was performed as previously described (8) in the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences.

IAA Treatment

Four-day-old dark-grown wild type and *DR5:GUS* seedlings were treated with 5 μ M IAA or mock (ethanol) in liquid MS medium (4.4g/L MS, 10g/L sucrose) for 1h. Then, each seedling was placed on a 3% agar plate, and cotyledons and hypocotyls were dissected and collected for RNA extraction.

Picloram Treatment

Four-day-old dark-grown wild type seedlings were transferred to solid MS plates with 10 μ M picloram or DMSO (mock), and then the plates were kept under white light (70 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for 1h, 6h, 12h, 24h and 48h. At each time point, cotyledons and hypocotyls were collected separately for RNA extraction. Seedlings were collected at 24h and 48h for hypocotyl length measurements. The gene expression and hypocotyl lengths of four-day-old dark-grown seedlings were used as the start points.

ChIP Assays

ChIP assays were carried out using about 2 g four-day-old dark grown PIF3-Myc and PIF4-Myc seedlings; wild type was used as a negative control. All samples were cross-linked in 1% formaldehyde solution for 30 min in a vacuum. Anti-Myc antibody (Sigma) and protein G beads (Invitrogen) were used for chromatin immunoprecipitation, and beads without antibodies (no antibody) were used as a negative control. The ChIP and input DNA precipitates were dissolved in 50 μ l autoclaved distilled water and 1 μ l was used for qPCR. Other procedures were performed as previously described (9). The detected promoter regions for SAUR14 , SAUR50 and SAUR65 were -488 to-321, -335 to -157, -365 to -266 upstream of ATG respectively, and the primers are listed in Table S1.

1. Kim J, Yi H, Choi G, Shin B, & Song PS (2003) Functional characterization of phytochrome interacting factor 3 in phytochrome-mediated light signal transduction. *Plant Cell* 15(10):2399-2407.

2. Dong J, *et al.* (2014) Arabidopsis DE-ETIOLATED1 represses photomorphogenesis by positively regulating phytochrome-interacting factors in the dark. *Plant Cell* 26(9):3630-3645.
3. Chiu W, *et al.* (1996) Engineered GFP as a vital reporter in plants. *Curr Biol* 6(3):325-330.
4. Feng Z, *et al.* (2013) Efficient genome editing in plants using a CRISPR/Cas system. *Cell Res* 23(10):1229-1232.
5. Trapnell C, Pachter L, & Salzberg SL (2009) TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics* 25(9):1105-1111.
6. Trapnell C, *et al.* (2012) Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nat Protoc* 7(3):562-578.
7. Spartz AK, *et al.* (2014) SAUR Inhibition of PP2C-D Phosphatases Activates Plasma Membrane H⁺-ATPases to Promote Cell Expansion in Arabidopsis. *Plant Cell* 26(5):2129-2142.
8. Sun J, *et al.* (2009) Arabidopsis ASA1 is important for jasmonate-mediated regulation of auxin biosynthesis and transport during lateral root formation. *Plant Cell* 21(5):1495-1511.
9. Wang Y, *et al.* (2014) Arabidopsis noncoding RNA mediates control of photomorphogenesis by red light. *Proc Natl Acad Sci U S A* 111(28):10359-10364.

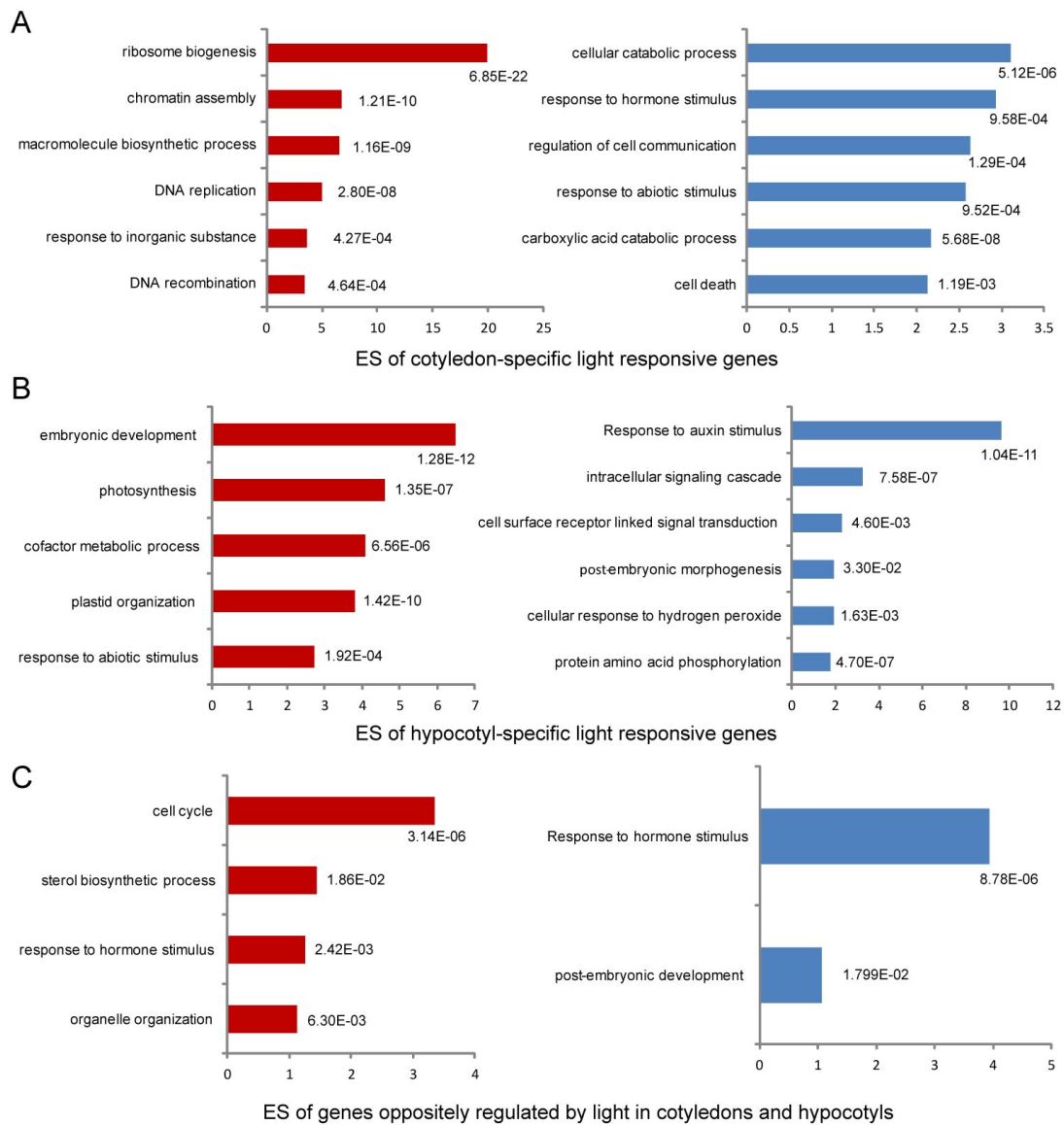


Fig.S1. GO Analysis of Organ-Specific Light-Regulated Genes.

(A-C) The enrichment score (ES) of highly enriched GO terms in cotyledon-specific light-responsive genes (Subgroup 1, 2, 3 in **Fig.1 C**), hypocotyl-specific light-responsive genes (Subgroup 4, 5, 6 in **Fig.1 C**), and genes oppositely regulated by light in cotyledons and hypocotyls (a portion of genes from Subgroup 7, 8, 15 in **Fig.1 C**). In (A and B), red indicates up-regulated genes and blue indicates down-regulated genes in cotyledons and hypocotyls respectively. In (C), red indicates genes up-regulated in cotyledons but down-regulated in hypocotyls, and blue indicates genes down-regulated in cotyledons but up-regulated in hypocotyls.

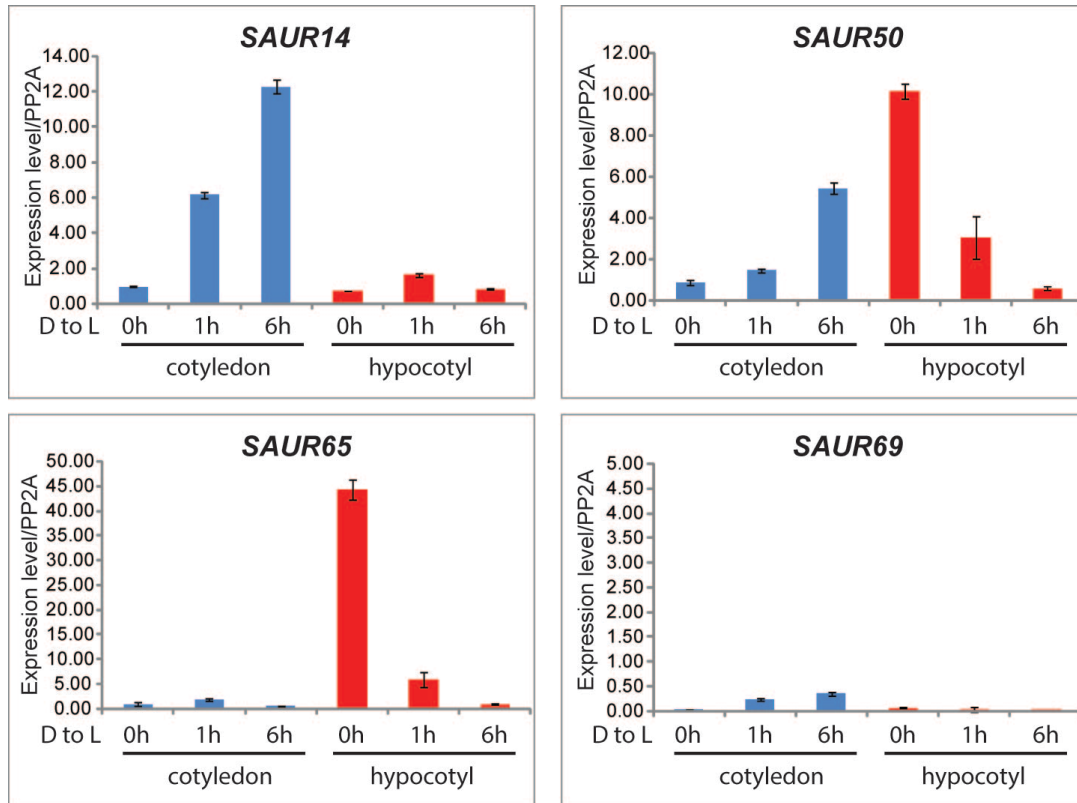


Fig.S2. qRT-PCR Verification of *SAUR* Genes Expression in Cotyledons and Hypocotyls During the Dark to Light Transition.

PP2A was used as an internal control. Data are shown as mean \pm SD. of three technical replicates.

More than three biological replicates were performed and one representative is shown.

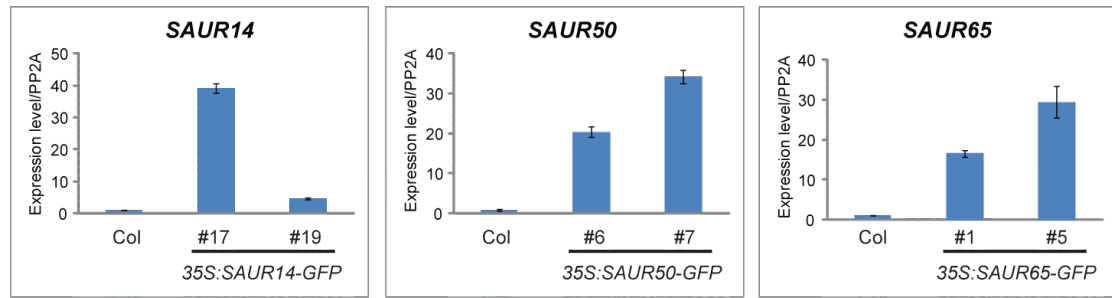


Fig.S3. Transcript Levels of *SAURs* in *35S:SAUR-GFP* Transgenic Lines.

Relative expression levels of *SAUR14*, *SAUR50* and *SAUR65* in seedlings of 4-day-old dark-grown wild type and *35S:SAUR-GFP* transgenic lines were measured by qRT-PCR. *PP2A* was used as an internal control. Data are shown as mean \pm SD. of three technical replicates. More than three biological replicates were performed and one representative is shown.

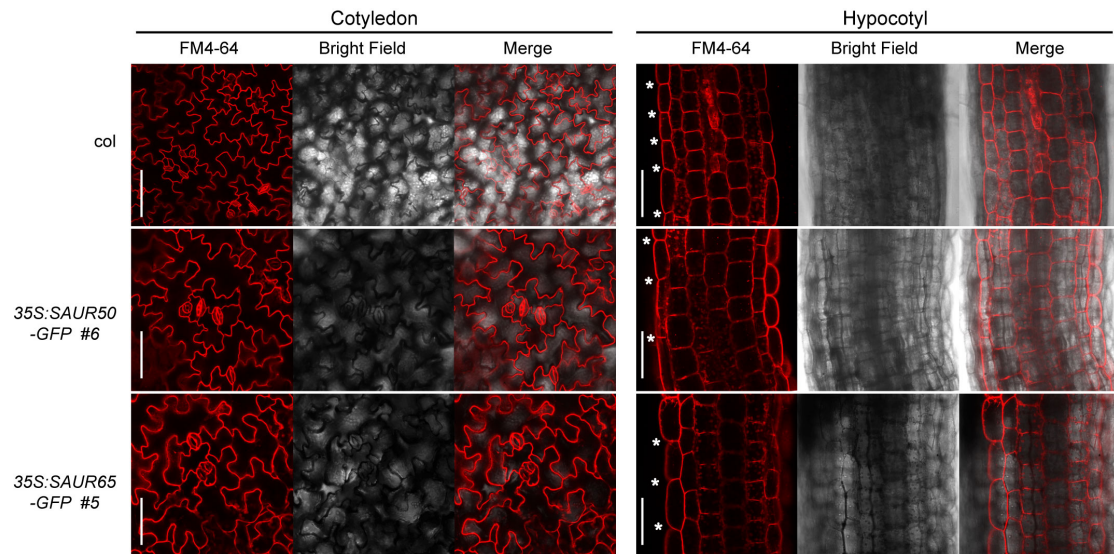


Fig.S4. Cell Size of Cotyledons and Hypocotyls in Wild Type and *lirSAUR* Overexpression Transgenic Lines.

The membranes were stained with FM4-64 to indicate cell boundaries. The adaxial epidermal cells of cotyledons are shown. Scale bar, 200 μ m. * indicates the boundaries of epidermal cells in hypocotyls.

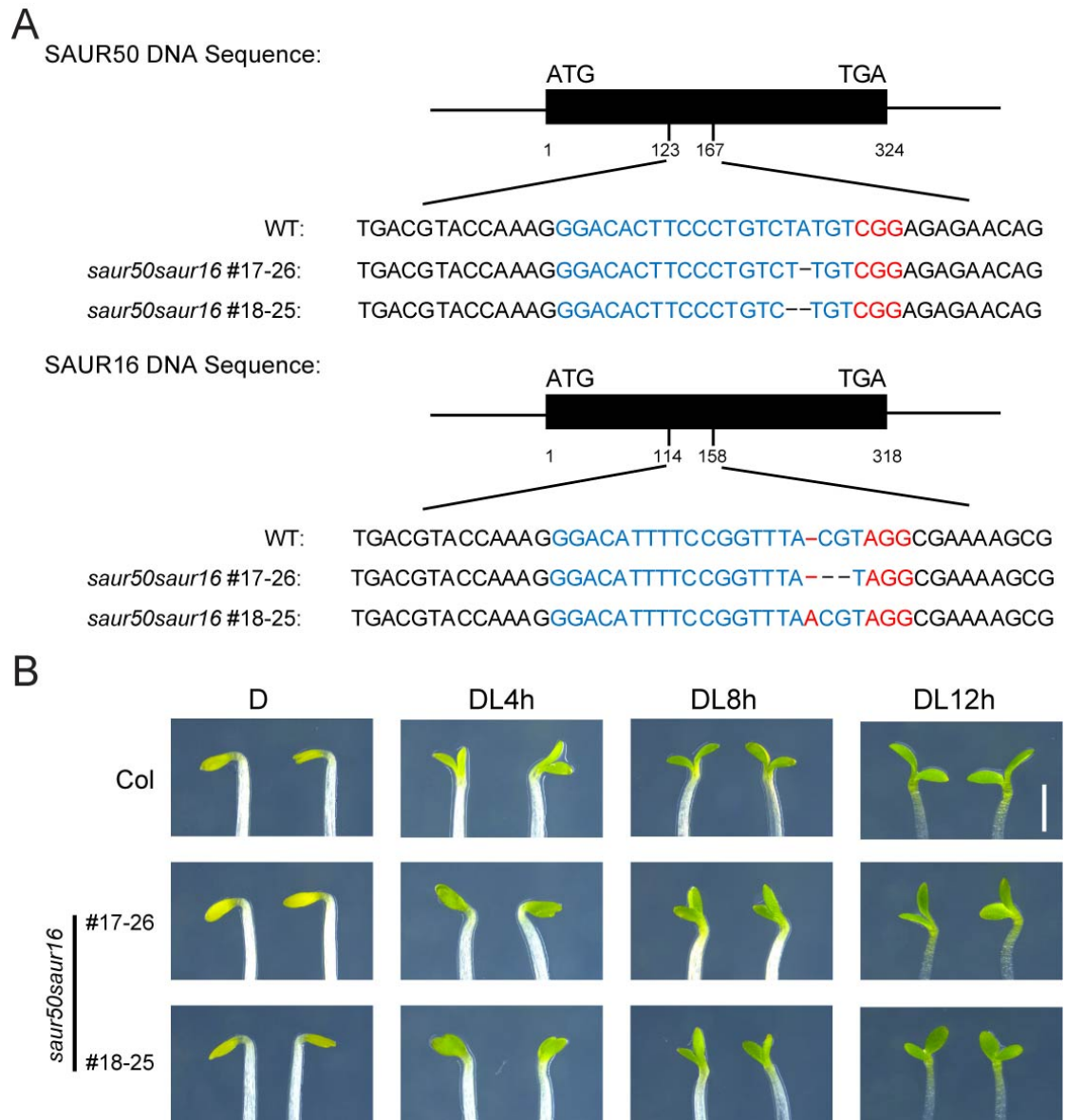


Fig.S5. Mutation Sites of *saur* Mutants and Their Phenotypes.

(A) The DNA sequences of *saur50saur16* mutants. Protospacer adjacent motif (PAM, NGG) is labeled in red. Guide sequences are labeled in blue.

(B) Images of cotyledon opening of Col and *saur* mutants during dark to light transition.

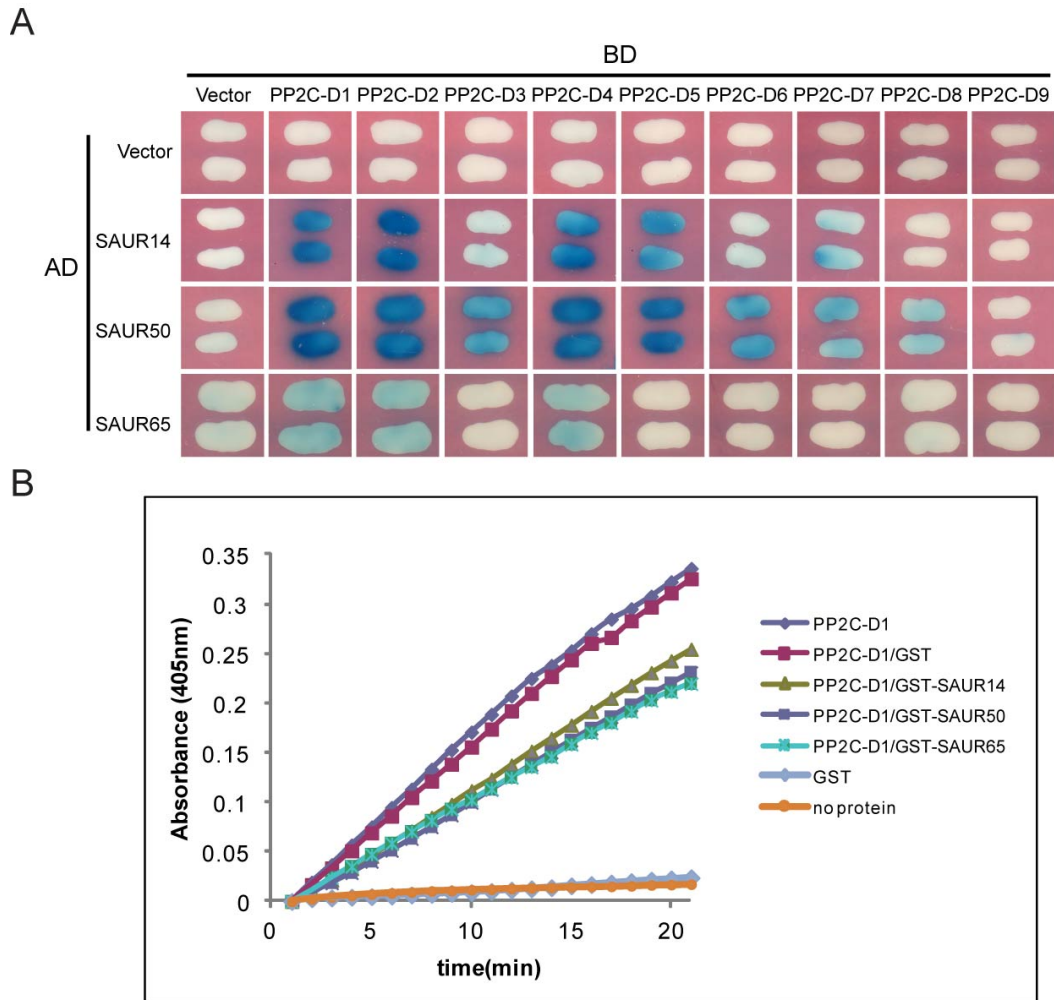


Fig.S6. lirSAUR Proteins Interact with PP2C-D Proteins and Inhibit Their Phosphatase Activities. (A) Yeast-two-hybrid assays showing the interactions between SAUR14, SAUR50, SAUR65 and D-clade PP2Cs. Bait (BD) and prey (AD) constructs were cotransformed into yeast as indicated. Cotransformants with empty vectors were used as negative controls. (B) SAUR14, SAUR50 and SAUR65 proteins inhibit the phosphatase activity of PP2C-D1 in pNPP phosphatase assays. 1 μ mol GST-PP2C-D1 was preincubated with 0.8 μ mol GST, GST-SAUR14, GST-SAUR50 and GST-SAUR65, and then the phosphatase activities were examined at indicated time points. PP2C-D1 alone was used as a positive control. GST alone or no protein didn't show phosphatase activity.

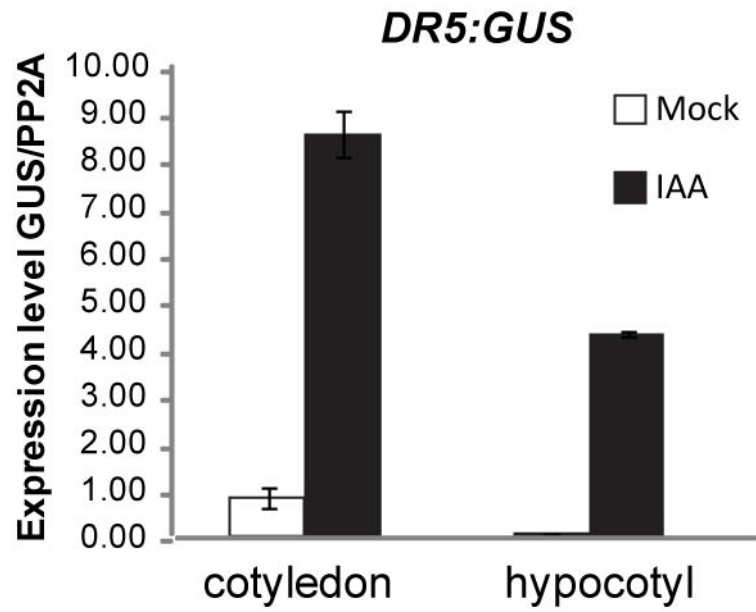


Fig.S7. IAA Treatment Induced the Expression of *GUS* in *DR5:GUS* Transgenic Lines.

PP2A was used as an internal control. Data are shown as mean \pm SD. of three technical replicates.

More than three biological replicates were performed and one representative is shown.

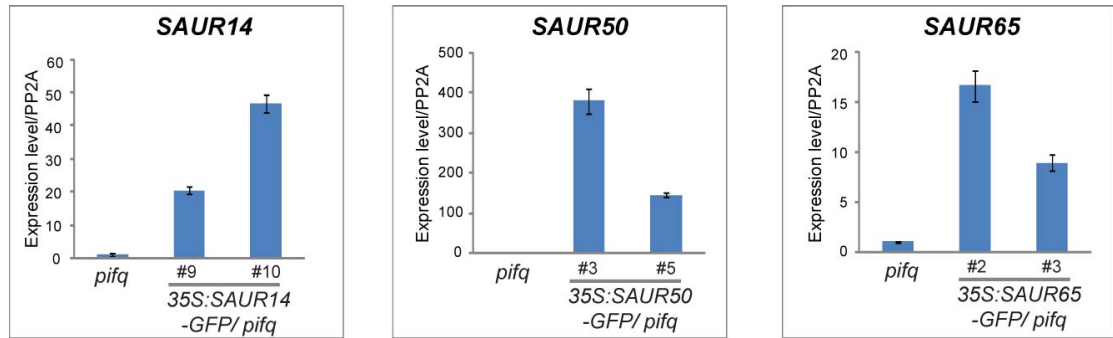


Fig.S8. Transcript Levels of *SAURs* in *35S: SAUR-GFP/pifq* Transgenic Lines. Relative expression levels of *SAUR14*, *SAUR50* and *SAUR65* were measured by RT-qPCR in *pifq* and *35S: SAUR-GFP/pifq* transgenic lines, which were grown in the dark for 4 days. *PP2A* was used as an internal control. Data are shown as mean \pm SD. of three technical replicates. More than three biological replicates were performed and one representative is shown.

Table S1. Primers used in this study

Name	Forward Primers (5' to 3')	Reverse Primers (5' to 3')
Primers for qPCR		
SAUR14	GATTCTCCGACAAGCCAAACT	TCTTGAAATGAAGGCTGGTCC
SAUR50	CACTTCCTGTCTATGTCGGA	TCTTCCTCGGCTCGTTGTAA
SAUR65	AACAAAGAGCTGCCCTCAAGA	AAACAGCCCTTCTCCACAGC
SAUR69	ATCCCGTGTGAAGAATCGGT	CACCGTGGCGATTCTCTTT
GUS	CCCTTACGCTGAAGAGATGC	GAGGTTAAAGCCGACAGCAG
PP2A	TATCGGATGACGATTCTTCGTGCAG	GCTTGGTCGACTATCGGAATGAGAG
Primers for transgenic materials		
GFP	GAAGGCCTATGGTGAGCAAGGGCGAG	GGACTAGT TTACTIONTACAGCTCGTC
SAUR14-CDS	GCTCTAGAATGGCGATCAGAATCCCT	GAAGGCCTGTTGAAGCGAGAAGCAAG
SAUR50-CDS	GCTCTAGAATGGCTATAATGAAGAAA	GAAGGCCTTCGGATCATGGATGTTAG
SAUR65-CDS	GCTCTAGAATGATCAACACTAAGAAA	GAAGGCCTAAATACAAGT AATTGTTG
SAUR50 guide sequence	GATTGGACACTTCCTGTCTATGT	AAACACATAGACAGGGAAGTGTC
SAUR16 guide sequence	GATTGGACATTTCCGGTTTACGT	AAACACGTAAACCGGAAAATGTCC
AtU6-sgRNA	GCTCTAGAAAGCTTCGTTGAACAACGGA	CGGGGTACCAAAAAAAGCACCGACTCGGTGC C
Primers for yeast two-hybrid		
PP2C-D1-BD	CACCATGGTTAAACCCTGTTGGAGAAT	TCATGATGTTGAATGCATCGGGTA
PP2C-D2-BD	CACCATGTCAGGTTTATTGATGAATCT	TCAGTGTTCAGAGCACTCCGTAT
PP2C-D3-BD	CACCATGGTATCATCGGCAACTATATTG	TCAAGTAGAAGGTCCAGCTAAATC
PP2C-D4-BD	CACCATGGTATCTACAACATTTAGGAGA	CTATAAACGGGATTATGGGCT
PP2C-D5-BD	CACCATGCTATCTGGGTTGATGAATTT	TCAGGAGGCCAGCAGCAGCA
PP2C-D6-BD	CACCATGTTATCAACGTTAATGAAACTC	TTAGATTTTCTTGGGGAATGTGAT
PP2C-D7-BD	CACCATGTTATCCCTTTTCTTCAACTT	TTAAAGTTTCTTAGGTAAGTGAT
PP2C-D8-BD	CACCATGTTGCGAGCTTAGCACGGC	TCAGTATAACACATTGAGTAACCG
PP2C-D9-BD	CACCATGTTCTCTGGTTAGCGAGAAT	CTAAGAGAGGAAGATACTGAACTGG
SAUR14-AD	CACCATGGCGATCAGAATCCCTCGT	TCAGTTGAAGCGAGAAGCAAG
SAUR50-AD	CACCATGGCTATAATGAAGAAAACT	TCATCGGATCATGGATGTTAG
SAUR65-AD	CACCATGATCAACACTAAGAAAACACTAC	CTAAAATACAAGTAATTGTTGG
Primers for in vitro phosphatase assay		
PP2C-D1-GST	CCGGAATTCATGGTTAAACCCTGTTGGAGA ATAG	CCGCTCGAGTCATGATGTTGAATGCATCGGG TAT
SAUR14-GST	CGCGGATCCATGGCGATCAGAATCCCT	ACGCGTCGACTCAGTTGAAGCGAGAAGC
SAUR50-GST	CGCGGATCCATGGCTATAATGAAGAAA	ACGCGTCGACTCATCGGATCATGGATGT
SAUR65-GST	CGCGGATCCATGATCAACACTAAGAAA	ACGCGTCGACCTAAAATACAAGTAATTG
Primers for ChIP assay		
SAUR14-ChIP	GACATCACATGCCAAATCTCTCT	TGAAGGCTTTTGTGACTTTGTC

SAUR50-ChIP	TAGTATTTATGGTGTAATCTGGTA	TGTAGGTAACGTATAGAGAGAGAGA
SAUR65-ChIP	TGAGAACATGAGAGGACCCG	AACATGGTGAGAAGAAACATGAC

Other Supporting Information Files

Dataset S1 (XLSX)

Dataset S2 (XLSX)

Dataset S3 (XLSX)

Dataset S4 (XLSX)

Dataset S5 (XLSX)

Dataset S6 (XLSX)