Supporting Information

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SI Materials and Methods

Plant Materials and Growth Conditions. The ecotype of all wild-type *Arabidopsis thaliana* used in this study was Columbia-0 (Col-0). The *phyB-9*, *cry1-304*, *pif3-3*, and *hid1* mutants (salk_017318) were reported previously (1–4). *phyA211* was ordered from the Arabidopsis Biological Resource Center. *Arabidopsis* seed sterilization, stratification, and standard seedling growth experiments were performed according to methods previously outlined (5). Different wavelength-specific conditions were as follows unless otherwise stated: 5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for far red light, 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for red light, and 7 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for blue light.

Northern Blot Analysis. Northern blot analysis was performed as previously reported (6). The sequences used for probing ncRNAs in this study are listed in Table S1.

Chlorophyll Content Measurement. Chlorophyll content was measured as previously described (7). The 50-mg seedlings were thoroughly homogenized in liquid nitrogen. Then 1 mL 80% (vol/vol) acetone was added to extract the chlorophyll. ODs at 645 nm and 663 nm were measured with a DU800 spectrophotometer (Beckman). Data are shown as the mean ± SD ($n = 4$).

Measurement of the Cotyledon Opening Angle. The cotyledon opening angle was defined as the angle between two cotyledon petioles (8). At least 30 seedlings grown on Murashiga and Skoog plates were examined for each biological replicate. Measurements were made using Leica microsystem software. Data are shown as the mean ± SD ($n \geq 30$).

Plasmid Construction and Generation of Transgenic Plants. To generate *35S:A* and *35S:B* constructs, DNA fragments containing full-length *nc3020–nc3017* coding sequences and full-length *nc3019–nc3017* coding sequences with attB sites were amplified from Col-0 genomic DNA and then were cloned into the gateway vector pDONR221 vector (Invitrogen) using BP Clonase. Each insert then was transferred into the Gateway pB2GW7 vector by LR reaction (Invitrogen).

To generate full-length (*pHID1: HID1*), mutated (*pHID1:M1* and *pHID1:M2*), and motif-deleted (*pHID1:dSLs*) *HID1* constructs, the fragment containing 1.5 kb upstream of *AtHID1* and the full-length *HID1* coding sequence plus the 101-bp downstream sequence was amplified from Col-0 genomic DNA and then was cloned into BamHI/PstI sites of pCambia1300. *pHID1:M1* (ATG to ATA in SL3), *pHID1:M2* (ATG to AG in SL3), *pHID1:dSL1* (deletion of SL1), *pHID1:dSL2* (deletion of SL2), *pHID1:dSL3* (deletion of SL3), and *pHID1:dSL4* (deletion of SL4) mutants were generated by PCR-based mutagenesis using a *pHID1:HID1* plasmid as the template and then were cloned into pCambia1300 (Cambia).

For the *pOsHID1:OsHID1* construct, the KpnI/HindIII sites containing 1.5 kb upstream of *OsHID1* and the full-length *OsHID1* coding sequence plus the 200-bp downstream sequence were amplified from *Oryza sativa* Japonica cv. Nipponbare genomic DNA and then were cloned into the binary vector pCambia1300 (Cambia).

For the *pHID1:S1-HID1* construct, the minimal S1 aptamer sequence (9) was inserted into the middle of the *HID1* SL3 motif as a possible RNA affinity tag. The modified fragment, which included 1.5 kb upstream of *HID1* and the *S1-HID1* plus the 100-bp downstream sequence, was cloned into the BamHI/PstI sites of pCambia1300.

Agrobacterium tumefaciens strain GV3101 was used to transform *A. thaliana* plants of the indicated backgrounds using the floral dip method. All transgenic plants were obtained through the use of antibiotic selection and were verified by Northern blot analysis.

To generate *pPIF3:LUC* (WT-p) and *pPIF3mut:LUC* (dI-p) reporter constructs, 1,574 bp upstream of the translation start site of *PIF3* (WT-p) and the same subfragment without the first intron sequence (dI-p) were cloned into the SalI /BamHI sites of reporter vector pGreen-0800-LUC (10). Both plasmids were transformed into *Agrobacterium tumefaciens* strain GV3101, which also contained the helper plasmid pSoup-P19. All the cloning primers are listed in Table S1.

Quantitative RT-PCR and RNA-Seq. Total RNA was isolated using RNeasy Plant Mini Kits (Qiagen). For quantitative RT-PCR (qRT-PCR), 3 μg of total RNA was used to synthesize cDNA with SSRII transcriptase (Invitrogen). Real-time PCR was performed using SYBR Premix Ex Taq Kits (Takara) on a 7500 Fast Real-Time PCR Amplifier (Applied Biosystems). The mRNA level of *ACTIN7* (*ACT7*) was used as our internal control.

Total RNA from 5-d-old WT and *hid1* seedlings grown in continuous red light (cR) was isolated using RNeasy Plant Mini Kits (Qiagen). Three biological replicates for each sample were subjected to RNA-seq. Poly (A) RNA from 1 μg total RNA was used to generate the cDNA library according to the TruSeq RNA Sample Prep Kit protocol. The library then was sequenced using a HiSeq 2000 system (Illumina). RNA-seq reads were mapped to TAIR10 using Tophat (version 2.0.9) (11). Differentially expressed genes were defined as those that differed by more than twofold with an adjusted P value ≤ 0.05 that was identified using Cuffdiff software (version 2.0.0) (12). We uploaded all the raw data to the Gene Expression Omnibus database under accession number GSE57806.

mRNA Decay Assay. For actinomycin D treatment, the time course started with untreated 5-d-old cR-grown seedlings. These seedlings then were incubated in half-strength Murashige and Skoog liquid medium (Sigma-Aldrich) with DMSO (mock) or 200 μM actinomycin D (Sigma-Aldrich) for the indicated period before being subjected to qRT-PCR.

Protein Extraction and Western Blot Analysis. Total protein extraction for detecting PIF3 was performed as previously described (13). Western blot analyses were performed as previously reported (5). Polyclonal anti-PIF3 antibodies were used at a 1:300 dilution. CSN6 was used as a loading control (14).

Biochemical Fractionation Assay. Nuclear cytoplasmic fractionation was carried out according to a previously reported protocol with some modifications (15). In brief, 0.5-g seedlings were ground into powder in liquid nitrogen and then were homogenized in 10 mL of Extraction Buffer 1 consisting of 0.4 M sucrose, 10 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 5 mM β-mercaptoethanol (β-ME), 1 mM phenylmethylsulfonyl fluoride (PMSF), and 1× complete protease inhibitor mixture (PI). The brei then was filtered through two layers of Miracloth (EMD Millipore) before being centrifuged at 3,000 × g for 20 min at 4 °C. Cytoplasmic RNA was isolated from 1 mL of supernatant using a TRIzol reagent. After being washed extensively with precooled Extraction Buffer 2 consisting of 0.25 M sucrose, 10 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 1% Triton X-100, 5 mM β-ME, 1 mM PMSF, and 1× PI, the pellet was resuspended in 250 μL of

precooled Extraction Buffer 3 consisting of 1.7 M sucrose, 10 mM Tris-HCl (pH 8.0), 2 mM $MgCl_2$, 0.15% Triton X-100, 5 mM β-ME, 1 mM PMSF, and 1× PI, which then was gently added on top of 300 μL Extraction Buffer 3. After centrifugation at 16,000 × *g* for 30 min at 4 °C, RNA was extracted from the pellet immediately using a TRIzol reagent. Then 1/10th of the isolated RNA was used as the nuclear RNA component for Northern blot analysis.

Isolation of chromatin-bound fractions was performed as previously described (16). RNA was extracted immediately from all fractions using a TRIzol reagent. Unless otherwise stated, 8 U/mL RNase inhibitor (Fermentas) were used in all buffers.

Gel Filtration Chromatography. Gel filtration analysis was performed as previously described, with some modifications (5). Five-day-old cR-grown seedlings were homogenized in a lysis buffer consisting of 50 mM Tris·HCl (pH 7.5), 450 mM NaCl, 1 mM EDTA, 10% (vol/vol) glycerol, 0.1% Tween 20, 1 mM PMSF, 1 × PI, and 100 U/mL RNase inhibitor. After centrifugation at 16,000 × *g* for 15 min at 4 °C, the supernatant was filtered through a 0.22-μm syringe filter and was fractionated using a Superose 6 column (Amersham Biosciences). After the void volume (8 mL) was achieved, consecutive fractions of 0.5 mL were collected. RNA in each fraction was extracted using a TRIzol reagent and subsequently was analyzed by Northern blot.

RNA Immunoprecipitation Assay. Five-day-old cR-grown *pHID1:S1-HID1/hid1* and *hid1* seedlings were homogenized in a lysis buffer consisting of 50 mM Tris·HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 10% (vol/vol) glycerol, 0.1% Tween 20, 1 mM PMSF, 1× PI, and 100 U/mL RNase inhibitor on ice for 10 min. After centrifugation at 16,000 × *g* for 15 min at 4 °C, the supernatant was collected, and its protein concentration was measured by the Bradford assay (Bio-Rad) at 595 nm on a DU800 spectrophotometer (Beckman). RNA extracted from 25 mg of total seedlings using a TRIzol reagent was used as the input sample. Prewashed streptavidin Sepharose beads (GE Healthcare) were mixed with 600 μg total protein extract in a total volume of 500 μL and then were rotated at 4 °C for 3 h. After extensive washing, the RNAs eluted from the beads using a TRIzol reagent were used as the immunoprecipitation (IP) sample. Input and IP samples were mixed with 2× RNA loading dye (Fermentas) and subjected to Northern blot analysis.

ChIP Assay. ChIP was performed based on previously reported protocols with some modifications (15, 17). A half gram of 5-d-old cR-grown *pHID1:S1-HID1/hid1* and *hid1* seedlings was cross-linked in 15 mL 1% fresh formaldehyde solution under vacuum twice for 10 min. To quench crosslinking, 2 M glycine was added to a final concentration of 0.125 M and was applied under vacuum for another 5 min. To protect RNA from degradation, RNase inhibitor was added. After a 1-h preclear step with prewashed protein G beads, streptavidin beads and protein G beads were used to precipitate the IP and control samples, respectively, and were rotated at 4 °C for 3 h. The subsequent steps were performed according to the methodology outlined in ref. 15.

Transient Transcription Dual-Luciferase Assay. A previously reported Dual-Luciferase (Dual-LUC) assay in *Arabidopsis* (18) was modified for our analysis. Agrobacteria containing WT-p and dI-p plasmids were collected and diluted to 0.5 OD_{600} in a resuspension buffer [10 mM $MgCl_2$, 10 mM Mes-KOH (pH 5.7), 200 μM acetosyringone]. After incubation at room temperature for 2 h, the Agrobacterial suspension was infiltrated gently into rosette leaves of ∼20-d-old long-day–grown (16 h light/8 h dark) WT and *hid1* mutant plants using a 5-mL syringe. Plants were maintained under light of the indicated wavelength for 3 d after infiltration. Infiltrated leaves were collected and used for the Dual-LUC assay, which was performed using the Dual-Luciferase Reporter Assay Kit (Promega) as previously reported (18). At least three biological repeats were measured for each sample.

Accession Numbers. Accession numbers obtained from the Arabidopsis Information Resource (TAIR) for the genes mentioned in this article are as follows: *PHYB*, AT2G18790; *CRY1*, AT4G08920; *PIF3*, AT1G09530; *PIF1*, AT2G20180; *PIF4*, AT2G43010; *PIF5*, AT3G59060; *PIF7*, AT5G61270; *ACT7*, AT5G09810; *CSN6*, AT5G56280; *SNRK2.5*, AT5G63650; *XTR7*, AT4G14130; *LHCB2.3*, AT3G27690; *PHYA*, AT1G09570; *RUP1*, AT5G52250; *SPT*, AT4G36930; *LHCB4.3*, AT2G40100; *ELIP2*, AT4G14690; *CP1*, AT4G36880.

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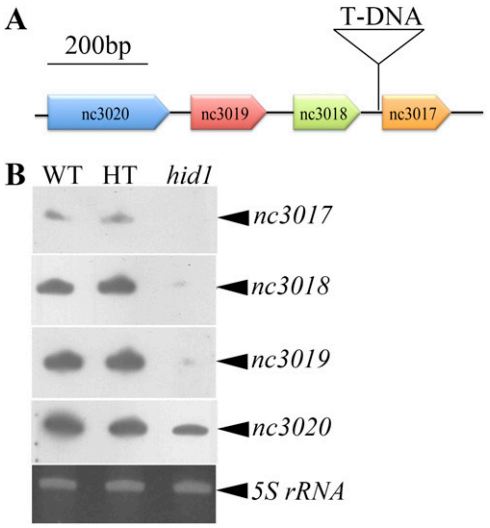

Fig. S1. Characterization of a T-DNA insertion mutant, *hid1*. (*A*) Schematic illustration of *Agrobacterium* transferred DNA (T-DNA) insertion in a noncoding RNA gene cluster. (*B*) Northern blot analysis of *nc3017*, *nc3018*, *nc3019*, and *nc3020* in 5-d-old seedlings grown in cR. *5S rRNA* is used as the loading control. HT, heterozygous seedlings.

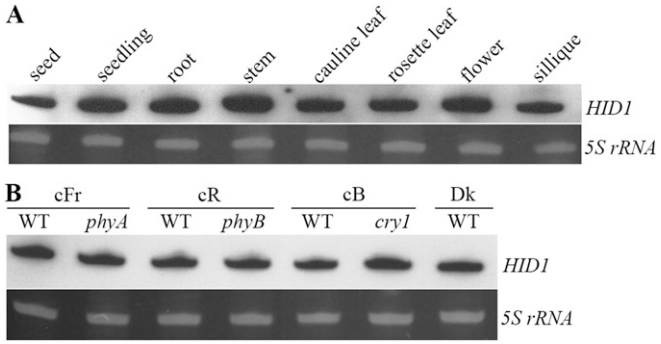

Fig. S2. *HID1* is expressed ubiquitously in different organs and various light conditions. Northern blot analyses show the expression of *HID1* in different organs (*A*) and various light conditions (*B*) as indicated. *5S rRNA* is used as the loading control.

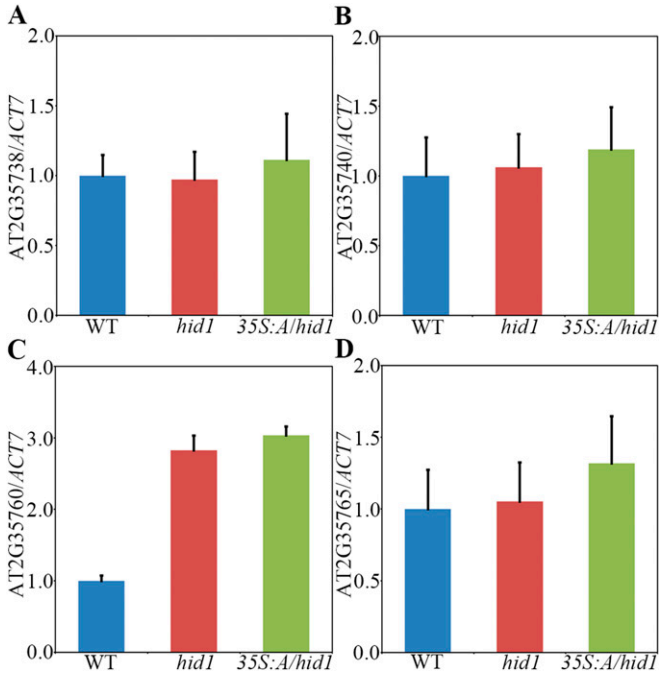

Fig. S3. The expression levels of neighboring genes are not affected by *HID1*. qRT-PCR analysis showing the expressions of AT2G35738 (*A*), AT2G35740 (*B*), AT2G35760 (*C*), and AT2G35765 (*D*) in 5-d-old WT, *hid1*, and *35S:A/hid1* seedlings grown in cR. cB, continuous blue light; cFr, continuous far-red light.

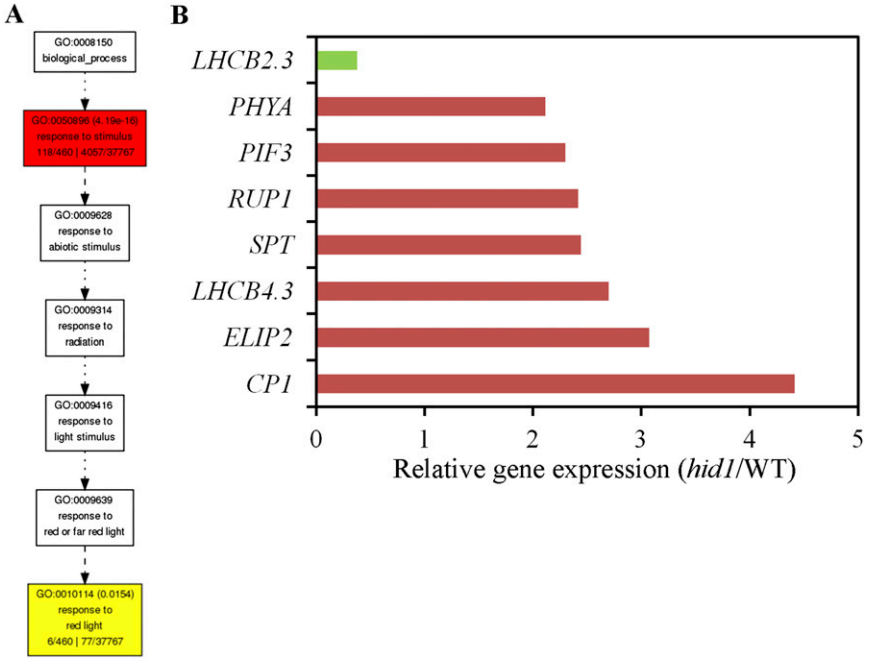

Fig. S4. A branch of red light-responsive genes exhibit significant expression changes in 5-d-old *hid1* seedlings grown in cR compared with WT control. (*A*) Gene Ontology classification using the agriGO web tool (http://bioinfo.cau.edu.cn/agriGO/) showing that a small set of genes that respond to red light are enriched in 5-d-old WT and *hid1* seedlings grown in cR. (*B*) Relative expression of genes responsive to red light in *hid1* compared with WT seedlings grown in cR light for 4 d (Q value < 0.05).

Table S1. Primers and probes used in this study

Name	Cloning primers
35S:A-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTAATCGACACTGAACACACACAC
35S:A-R	GGGGACCACTTTGTACAAGAAAGCTGGGTAGAAACGTCTCAAGCGGCT
35S:B-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTAAATGCCCATGGACGTTGAT
35S:B-R	GGGGACCACTTTGTACAAGAAAGCTGGGTAGAAACGTCTCAAGCGGCT
pHID1:HID1-F	ACTGGATCCATGGATTGGGGAGATACATAGATCAA
pHID1:HID1-R	ACTCTGCAGCAATGTTAATCAGTCCCGAAAGATG
pHID1:M1-F	GGGCATACCCATAGACGTTGATGGAGCTCTAGGG
pHID1:M1-R	GTCTATGGGTATGCCCAATTGCTTTGTGAC
pHID1:M2-F	CATGCCCAGGACGTTGATGGAGCTCTAGG
pHID1:M2-R	CAACGTCCTGGGCATGCCCAATTGC
pOsHID1:OsHID1-F	AATGGTACCCTGAACTGAGGCTATGATTTATCCTC
pOsHID1:OsHID1-R	AATAAGCTTCCTGGGATATGGGATATAATACAGAATG
HID1-dSL1-F	GTGACGAGCTATTACTAAAAAGATGGAGCAATCTGA
HID1-dSL1-R	AGTAATAGCTCGTCACGTAACTAATCCCTAACTTG
HID1-dSL2-F	GAATCTATAACGTCACAAAGCAATTGGG
HID1-dSL2-R	GTGACGTTATAGATTCCTTGGAGAGAAATCTCGT
HID1-dSL3-F	GTCACAAATATTGGCTCTCATATTCCATTGCC
HID1-dSL3-R	AGCCAATATTTGTGACGTTTACTAGTGGATTATGC
HID1-dSL4-F	TTGCTATTATATTCCATTGCCTTGCGGA
HID1-dSL4-R	GGGAATATAATAGCAAGATACCCAAAACGGAGA
S1-HID1-F	CGGGCCGGGGCTT
S1-HID1-R	CTGGTCGGAGCTCCATCAAC
S1-HID1-S	ATGGAGCTCCGACCAGAATCATGCAAGTGCGTAAGATAGTCGCGGGCCGGGGCTTCTC
S1-HID1-AS	GAGAAGCCCCGGCCCGCGACTATCTTACGCACTTGCATGATTCTGGTCGGAGCTCCAT
pPIF3-LUC-F	AATGTCGACTATAGTAGGATCACTTCCATTCCGAG
pPIF3-LUC-R	AATGGATCCGGTGTTGCGTTTTACAGAAACAAT
pPIF3mut-LUC-R	AATGGATCCCTCAAAGTATTAAAGTGGCATCAATCC
Name	**Genotyping primers**
hid1-LP	CCGTGATGTTAAAGGCGGTGT
hid1-RP	GGAAACGTCTCAAGCGGCTAG
LBb1.3	ATTTTGCCGATTTCGGAAC
pif3-3-F	AATTGTAATAATGCGAAGGACCTG
pif3-3-R	TAGTCCTGAGAAAGTAGGCGGAG
Name	**qRT-PCR primers**
ACT7-RT-F	GTATGCTCTTCCTCATGCTATCCTT
ACT7-RT-R	TTCCCGTTCTGCGGTAGTG
PIF1-RT-F	GTGGTGAATCTGGACCGTTGC
PIF1-RT-R	AACTGTCAGTACCATCCGTCCG
PIF3-RT-F	CAAAGACGACTATGGTGGACGAG
PIF3-RT-R	ACGAAGAAGCAGCAGGAGCTGAT
PIF4-RT-F	GGTAACGACCGTTGGACCTAGC
PIF4-RT-R	ACCAGAGGAGCCACCTGATGAG
PIF5-RT-F	AGATGGCTATGCAAAGTCAGATGC
PIF5-RT-R	TGTGCTTGGAGCTGCAACTGTAC
PIF7-RT-F	CGGAGCTTGAAGACAGCTAGAACC
PIF7-RT-R	GACTCGTTGTGAATCGCTGCTG
SNRK2.5-RT-F	CGCAGGAGTGCAGACACCTTC
SNRK2.5-RT-R	CCGCTTGAGCAGGCTCTGTAA
XTR7-RT-F	GGCACCGTCACTGCTTACTACG
XTR7-RT-R	CCCTTGAGCAAAGACATTGGTG
AT4G35720-RT-F	TCCGAGACTCCACAAAGCGTA
AT4G35720-RT-R	CCCAAAGCTCTAACCTTGGAGA
AT2G35738-RT-F	CATGGCTATCGAAACTGATTCTGC
AT2G35738-RT-R	GCACAGTAGAGCAGTGGAATCTTGA
AT2G35740-RT-F	TATCACAGAGGTGTGGACGACG
AT2G35740-RT-R	GACTCCCGTGTTGTATCCGAAG
AT2G35760-RT-F	TATTCTTTGGTTCAGGCGGTTC
AT2G35760-RT-R	CGCCACACACAAGTATGCTACTG
AT2G35765-RT-F	CCATCGGACAGAGGATAGACTCAG
AT2G35765-RT-R	ATGGAGATCAAACTTGATTGGCTG

Table S1. Cont.

Name	Northern Blot probes
HID1 (*nc3020*)	TCATGGCCGGTCCCAAACGGTAAAAGCAGCGACGCCGCTCCGCAAGGCAATGGAA
nc3019	GCCTCAGAATTGCAGAAGTATCATCAATGTTAATCAGTCCCGAAAGATGGTGTTCTTTT
nc3018	TGGGTTAGAGATGGTTATCAGCACTAATATCAGAGTGGTGCATGACATTCTCGTTCATC
nc3017	AACCATCAGCAACCAAAAGTTATCATCAAAGAAATACAGTCCGCATCAAGGTCAAGTCA
OsHID1	GGGAATATTCGCCTCATGGCCAATCCCAAATTTTCAAGGCAGCAACGCCGCTACA
tRNA0119	CGAACCGCTCACAGAAGGATTTACAGTCCTGCACTCTACCAGAGCTACTACCTGTTACC
dSL4	ATGCCCAATTGCTTTGTGACGTTTACTAGTGGATTATGCAATCACAATTTCAG
Name	**ChIP-qPCR primers**
pPIF3-ChIP-F1	TAATGATTCGGGCGACGCTAA
pPIF3-ChIP-R1	GGGGACCAAATCGTCTGTAACTG
pPIF3-ChIP-F2	TTGTGCGTCGATTTGTCTCCA
pPIF3-ChIP-R2	AGTGGCATCAATCCAAATGCAG
pPIF3-ChIP-F3	TGACTTCGATTGCTTCAGTACCC
pPIF3-ChIP-R3	AATGGCAACATGATTAGTCAAATCA
pPIF3-ChIP-F4	TTGCATATTGATGATTGAGACATTGA
pPIF3-ChIP-R4	CGAAGTCTTGATCCTAAAAAGGTGA
pPIF3-ChIP-F5	TGTAAAACGCAACACCATGCC
pPIF3-ChIP-R5	ATGGGACTCACGGAGAAGGG
ACT7-ChIP-F	GAGGCACCTCTTAACCCTAAAGC
ACT7-ChIP-R	GAGACACACCATCACCAGAATCG