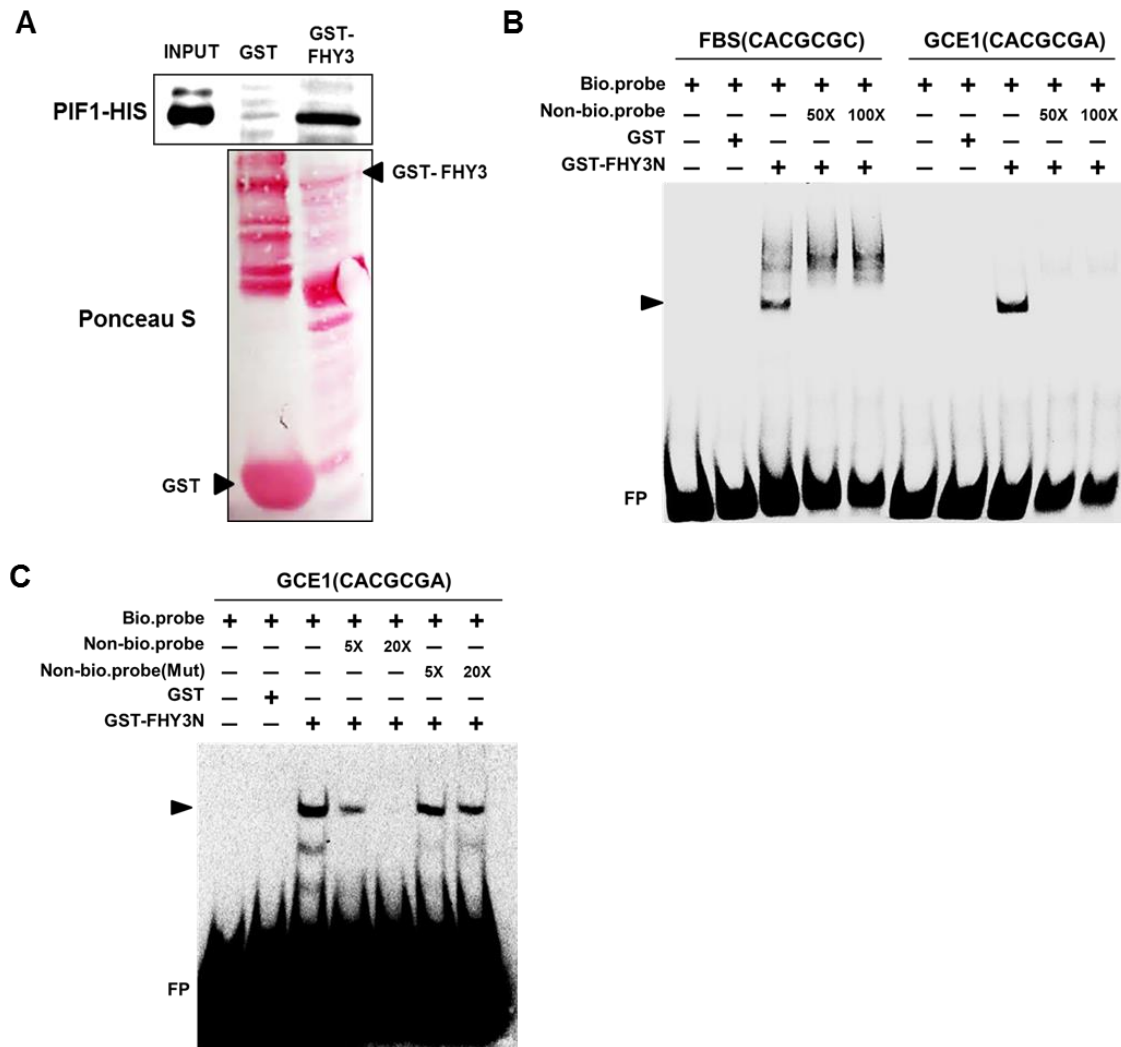


Supplemental Figure 1. T-DNA insertions do not affect PIF1 binding to independent loci.

(A) Similar enrichment of PBSs by PIF1 at both the *HFR1* and *BOI* loci in wild type (WT) and *pil2-sl158* seeds. This ChIP assay used an anti-PIF1 antibody on imbibed phyB_{off} seeds. Error bars: SD (n=2 biological replicates)

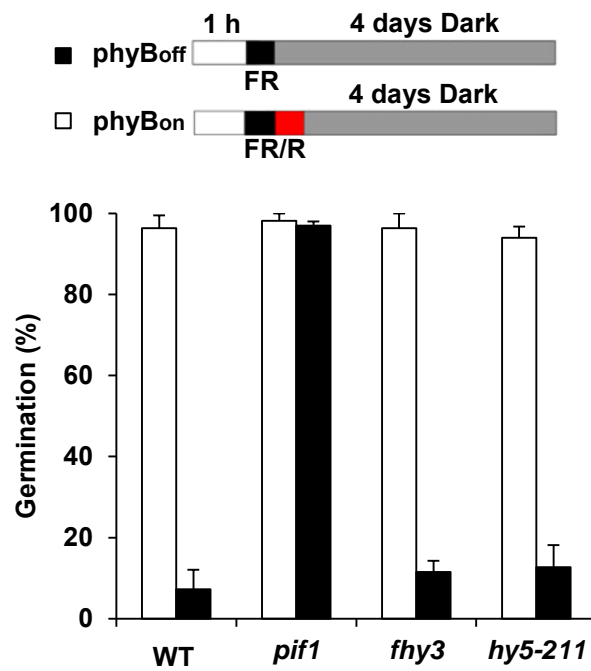
(B) Similar enrichment of PBSs by PIF1 at both the *HFR1* and *PIL2* loci in WT and *boi-gb012* seeds. This ChIP assay used an anti-PIF1 antibody on imbibed phyB_{off} seeds. Error bars: SD (n=2 biological replicates)



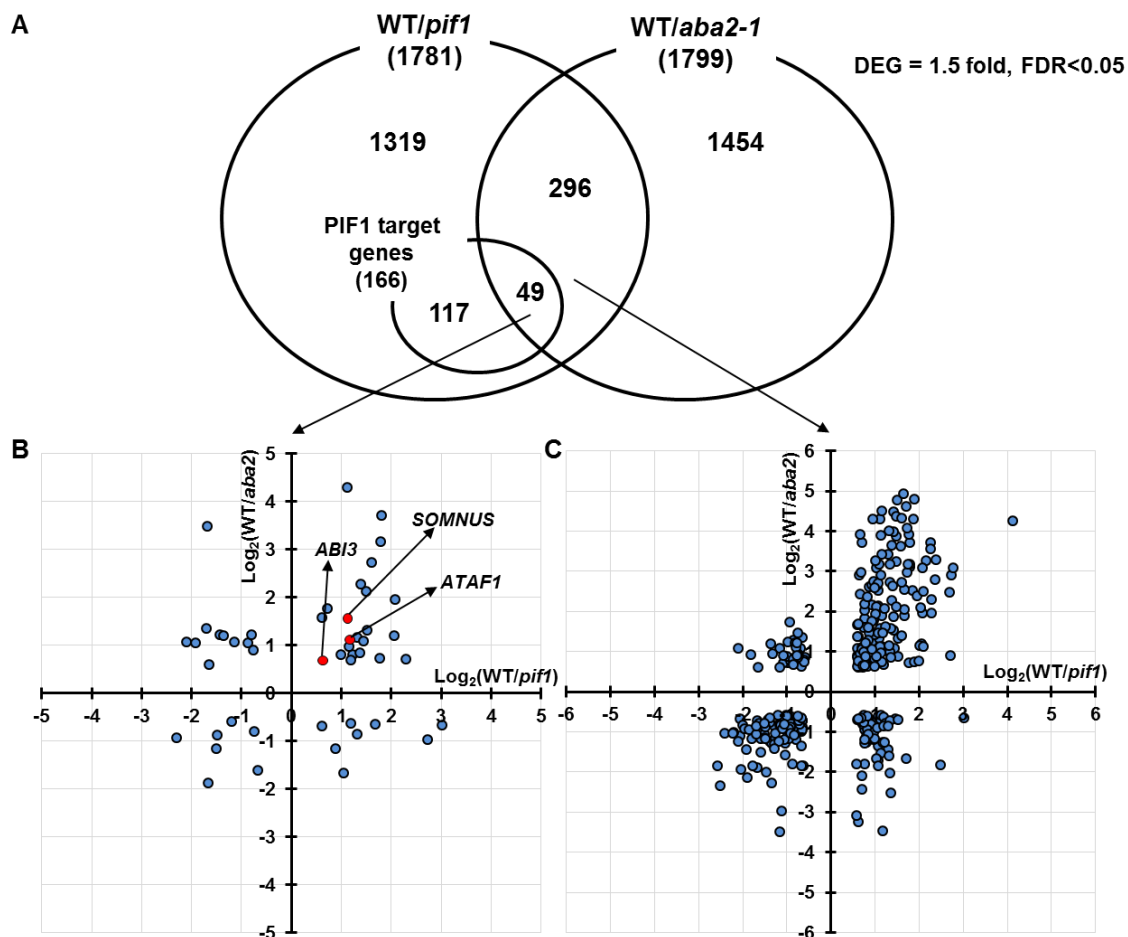
Supplemental Figure 2. FHY3 protein interacts with PIF1 and binds GCE1s.

(A) An *in vitro* binding assay demonstrates the interaction between FHY3 and PIF1. 6XHis-tagged PIF1 (PIF-HIS) is pulled-down by resin-bound GST or GST-FHY3 and detected with an anti-HIS antibody. Triangles indicate the GST or GST-FHY3 proteins.

(B and C) An EMSA showing the binding of FHY3 to the FHY3/FAR1 binding site (FBS) of the *FHY1* promoter and the GCE1 of the *At3g18080* promoter. Biotinylated and non-biotinylated double-stranded oligomers (Bio.probe and Non-bio.probe) were used for this assay. The Non-bio.probes were added at much higher concentrations (5x, 20x, 50x or 100x) than the Bio.probes. Non-bio.probe(Mut) indicates the addition of mutated non-biotinylated double-stranded oligomers at 5x or 20x the concentration of the Bio.probes. Triangles indicate the bands shifted by FHY3. FP indicates free, unbound DNA probes. This experiment used the N-terminal 200 amino acids of FHY3.



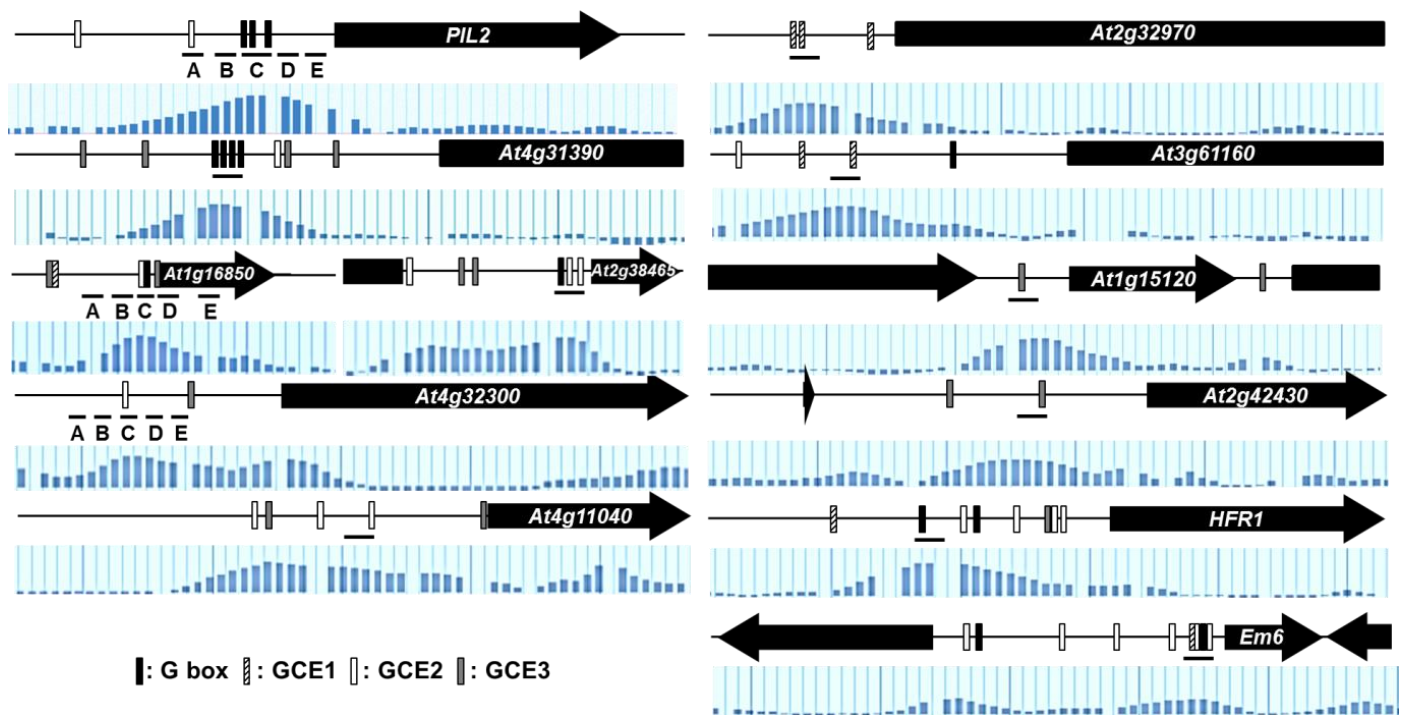
Supplemental Figure 3. *pif1* mutant but not *fhy3* and *hy5* mutant seeds germinate in the phyB_{off} condition. The upper diagram indicates the light treatment schemes for the phyB_{on} and phyB_{off} conditions. The lower graph indicates germination frequency of each mutant in different light conditions.



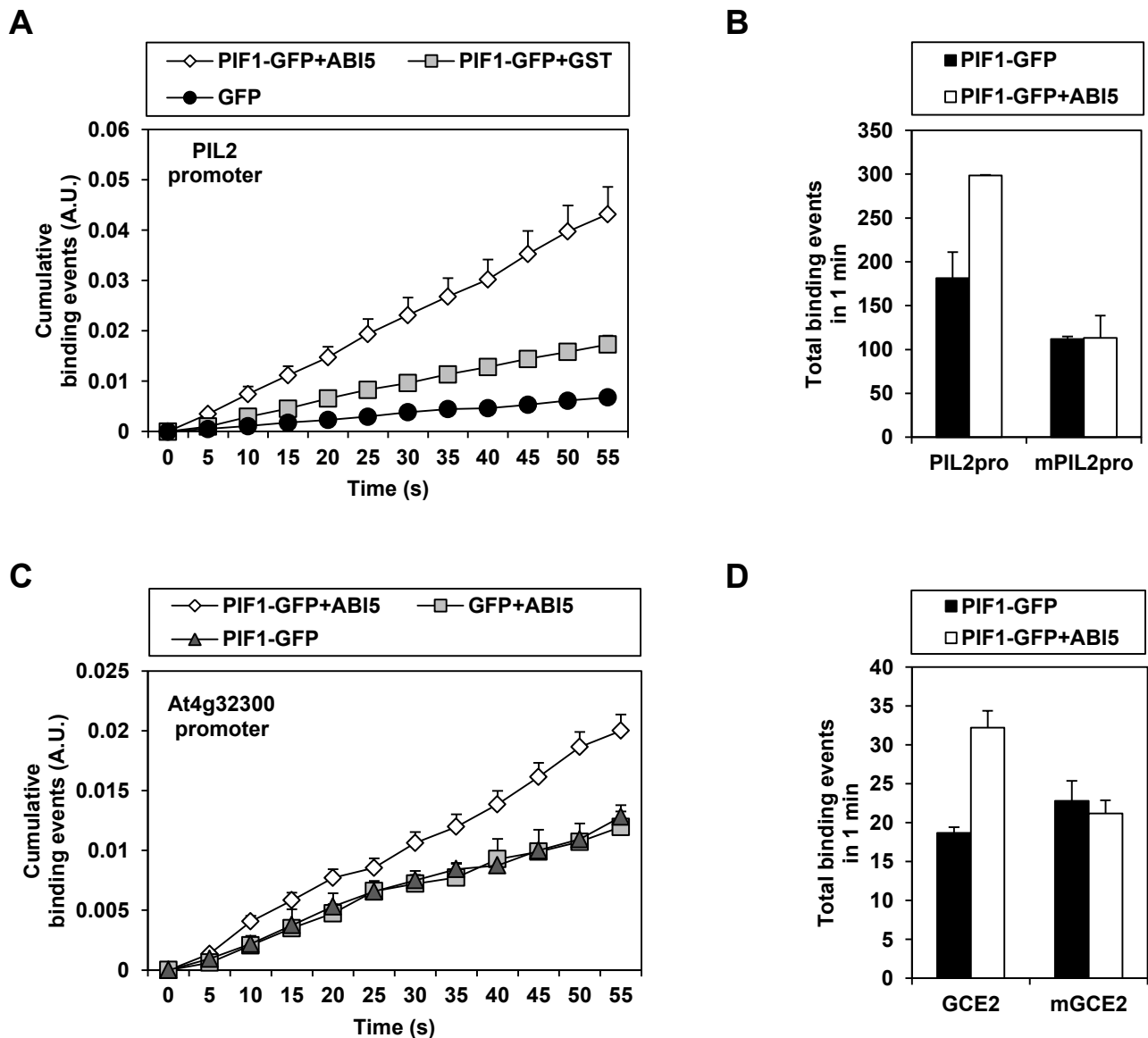
Supplemental Figure 4. PIF1 and ABA co-regulate a subset of genes in imbibed seeds.

(A) A Venn diagram indicating 345 overlapping differentially expressed genes (DEGs) between *WT/pif1* and *WT/aba2-1*. *WT/pif1* indicates *pif1* transcriptome data (fully dry, 12 hours, dark, GSE14374) and *WT/aba2* indicates *aba2* transcriptome data (freshly harvested, 24 hours, white light, GSE15700). The number of overlapping DEGs in the *pif1* and the *aba2* transcriptomes is statistically significant (hypergeometry test, $P = 1.226 \times 10^{-29}$). These overlapping DEGs also overlap significantly with PIF1 target genes (hypergeometry test, $P = 1.59 \times 10^{-24}$).

(B and C) Dot plots comparing the log₂-transformed expression levels of the indicated genes, which fall preferentially in quadrants 1 and 3 (proportion test, $P < 0.005$). Red dots indicate genes already known to inhibit seed germination.



Supplemental Figure 5. Diagrams showing various loci (upper panels), PIF1 binding signals from ChIP-chip assays (lower panels), PBS sequence elements, and ChIP-PCR amplicons. Vertical bars in the lower panels indicate PIF1 binding intensities for given sites in previously reported ChIP-chip assays. A, B, C, D, and E indicate ChIP amplicons. 'C' amplicons were used for *PIL2*, *At1g16850*, *At4g32300* enrichments in Figure 4B.



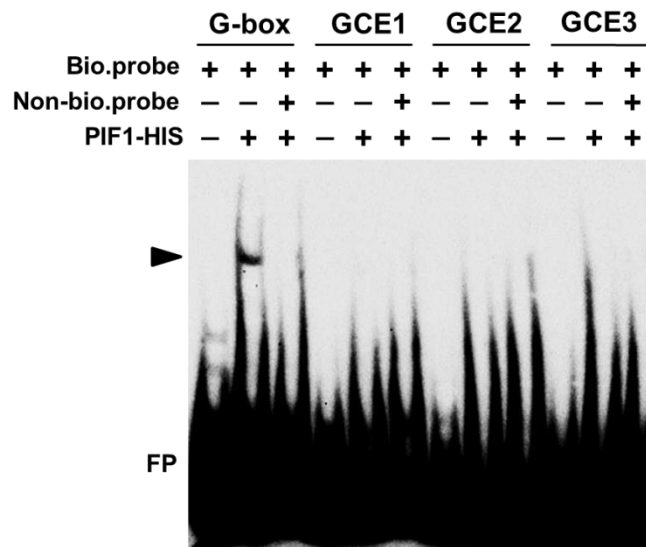
Supplemental Figure 6. ABI5 enhances PIF1 binding to PBSs – an experimental dataset distinct from that shown in Figure 7

(A) Real-time single molecule fluorescence imaging analysis shows that ABI5 enhances PIF1 binding to a *PIL2* promoter fragment containing three G-boxes. Promoter fragments are immobilized on a slide and binding events are detected by TIR fluorescence microscopy (TIRF) as GFP molecules approach the surface. Error bars: SEM (n=3 locations).

(B) The enhancement of PIF1 binding to the *PIL2* promoter fragment by ABI5 requires intact G-boxes. PIL2pro indicates immobilized *PIL2* promoter fragments containing three intact G-boxes, while mPIL2pro indicates the same fragment with three mutated G-boxes. Error bars: SEM (n=3 locations).

(C) ABI5 enhances PIF1 binding to an *At4g32300* promoter fragment containing a GCE2. Error bars: SEM (n=3 locations).

(D) The enhancement of PIF1 binding to the *At4g32300* promoter fragment by ABI5 requires an intact GCE2. GCE2 indicates immobilized promoter fragments containing an intact GCE2, while mGCE2 indicates the same fragments with a mutated GCE2. Error bars: SEM (n=20 locations).



Supplemental Figure 7. PIF1 binds G-boxes but not GCEs *in vitro*. EMSA assay demonstrating PIF1 binding to G-boxes but not GCEs. PIF1-HIS and biotinylated double-stranded oligomers (Bio.probe) were used for this assay. Non-biotinylated double-stranded oligomers (Non-bio.probe) were used at 100x the concentration of the Bio.probes. The triangle indicates a shifted band. FP indicates free, unbound DNA probes. The probe sequence sources are as follows: G-box, the *PIL2* promoter; GCE1, the *At3g18080* promoter; GCE2, the *At1g16850* promoter; GCE3, the *At1g17830* promoter.

Supplemental Table 1. Classification of PIF1 target genes based on GCEs and expressional direction.

Expression	Total PIF1 target genes	G-box-containing	GCE1-containing	GCE2-containing	GCE3-containing
Activated	105	71	41	69	44
Repressed	61	34	22	31	27
<i>*P-value</i>		0.1726	0.8291	0.08435	0.894

* The proportion of Activated and Repressed genes of total PIF1 target genes is compared with those of each GCE-containing target (proportion test).

Supplemental Table 2. List of primers

Locus	Primer name	Primer sequences	Purpose
<i>PP2A</i> (At1g13320)	PP2A	Forward : CTGGCGTGTGCGTTATATG Reverse : CAAACATGGACTTCCAAGTACC	ChIP
<i>PIL2</i> (At3g62090)	P0	Forward : GGTTATGTATAGTATCGATTGCCC Reverse : AAGATTGCCCTCCTAGTCCTAG	
	A	Forward : GAGGTTTCGTTTCGTGCCTTTTAGA Reverse : ATCGGCACAAGGGCAAATCA AATG	
	B(P1)	Forward : CTGAATCATTGTTGGTGTAAACCGGCT Reverse : GTACCCGTATGCAGTGTATTAGAC	
	C	Forward : ACATTGAATCACGTGGCTTTCACG Reverse : GTGTTGGGGAGGAGAGAGAGAGAGAG	
	D(P2)	Forward : GTCGTAATCGGTTTGTGAAACA Reverse : TTAAATGCAAGGGATGTCGTTTCC	
	E	Forward : TCTCAAAGATGCTAAGATTCGACT Reverse : TCTTCAGTGGAAAGTAAGTGAAAGCT	
<i>BOI</i> (At4g19700)	B0	Forward : GATCGATATTACAAAGCACCCG Reverse : TTACGGGTCTAGTCACTCTAGC	
	B3	Forward : TGTCTCAACGACATTTTCAGTTC Reverse : TAACTCGTGAATTCACACATCGT	
	B5	Forward : GACCAGACGACAATTCAAGA AGCA Reverse : GAGGCCACGTCTAAGTGGGACCAA	
At4g31390	At4g31390	Forward : ATAAAATGATGGGCGCACGTGACA Reverse : AGGAAGTGACGAAGTGGGGGTAAT	
At1g16850	A	Forward : ATAAGCACAA ACGTGGTTACCTGT Reverse : CATCTCAAGAGACGTATATACTGTG	
	B	Forward : CCTAATGGTTACGTTGCAGTACCG Reverse : GTGGCAGGTGGCTCATGAAACACC	
	C	Forward : TCATTCACGTCCGGTCATTTTGTCTG Reverse : GAAGCGAGTCCGGCAGCGAAA	
	D	Forward : TGCCGACTCGCTTCGCTTTGCAAA Reverse : CACCGCTCTCTCAGAATCGACATC	
	E	Forward : CCCACTTCTTTCCTCCGAACACAT Reverse : CGTCGCCGCCTCTTTCATATTTTC	
At2g38465	At2g38465	Forward : AGCAACTCTTGCCACGTCTGTTTT Reverse : TGGTATGGTTCTTACGTGGAACC	
At4g32300	A	Forward : AGCATACCCCTCCAATAAGAACC Reverse : TCTTTAGCTTTCAGCTCTTCCCTC	
	B	Forward : AAGAGCTGAATCTTATAAGAAATTAGCA Reverse : CAACGGCCAACGTTATTAACACAA	
	C	Forward : AAAAGTCAAACCAAACCCACACGT Reverse : GTGGGGAAGTGTGAAAGAAAGAGG	
	D	Forward : CTCCTTAATCTCTATTTTCAAGTGCC Reverse : GATTC AATACAGA ACTGCTTCTCCTC	
	E	Forward : CCGTCAAAGTAGACACAATACCAA Reverse : AGAGGAATACCAATTGAAGCATCC	
At4g11040	At4g11040	Forward : GTTGATCACTATCCCTGCTCATAT Reverse : CGTCTGTTACATAACTCTTG CATG	
At2g32970	At2g32970	Forward : GCCAGGGTCTCGTCCGTTTTTAAAT Reverse : GTTTGCGGCTTTTCAGAAGACATC	
At3g61160	At3g61160	Forward : GTCATATGAATCTTGTCGCGTGTG Reverse : CACAAGCCAG AAAAAGCTCA AGGG	

Supplemental Table 2. (Continued)

At1g15120	At1g15120	Forward : CTGCCATTA ACTCCATTACCACCA Reverse : GATACCATGTGACATGTTGTTGGG	ChIP
At2g42430	At2g42430	Forward : GAAGTCTCATGTTGCAGTCTCCAT Reverse : ATCTACTGAGATTCTGGTTCTTAC	
<i>EM6</i> (At2g40170)	EM6	Forward : ACACGCGGCGAAGAAGTACGGCCA Reverse : ACGAAGAAGACTATAGTAGCTCGC	
<i>HFR1</i> (At1g02340)	HFR1	Forward : TACCCAAGTGTCAGACATAGCACC Reverse : ACTTTGTAGCGTTGAATCAAATGG	
<i>PP2A</i>	PP2A	Forward : TATCGGATGACGATTCTTCG GCAG Reverse : GCTTGGTCGACTATCGGAATGAGAG	qRT-PCR
<i>PIL2</i>	PIL2	Forward : CCGTGGTTGCTGATAGGTCGTTCC Reverse : CGCTTCTGCATTTTCGTTTTCTTTTG	
<i>BOI</i>	BOI	Forward : GTAAACGGTGCGGTGAGAGAGAAG Reverse : TGACCATATCGCAAACCGGACAAG	
<i>ABI5</i> (At2g36270)	ABI5	Forward : GGTTAGAGAACATCCACTAATCC Reverse : TTAGCCCTCCCATATCTACTCCAT	
<i>PIL2</i>	G box	TCTTCCCACAACCACGTGGGCTTTTTGGCC	EMSA
At3g18080	GCE1	CGCTTTACGATTTCGCGTGATGACTTTTTCT	
At3g18080 (Mut)	Non-bio.probe (Mut)	CGCTTTACGATTCAAAGATGACTTTTTCT	
At1g16850	GCE2	GCCACCTCATTACGTCGGTCATTTTGTCG	
At1g17830	GCE3	CAGCTTCGACGACATGTGGAGACCAGATTA	
<i>FHY1</i> (At2g37678)	FBS	GTAGACTCTTTTCACGCGCCAAATCAAACA C	
<i>PIL2</i>	PIL2	CCGGTCTAGATGGTCGGACACCAAATATTA TAG	Imaging
At4g32300	At4g32300	GAAGATTTACACATAAAAGTCAAACCAAACCCAC ACGTCGGTCGTGAAAA AGC	
	Mut	GAAGATTTACACATAAAAGTCAAACCAAACCCAC TTTTCGGTCGTGAAAA AGC	