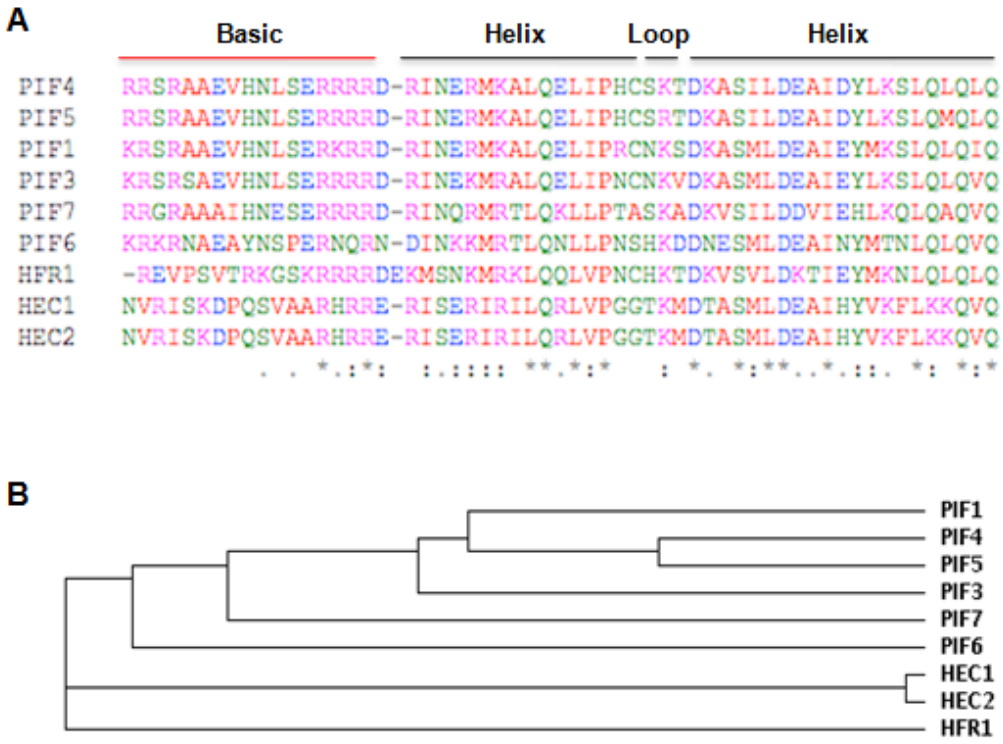
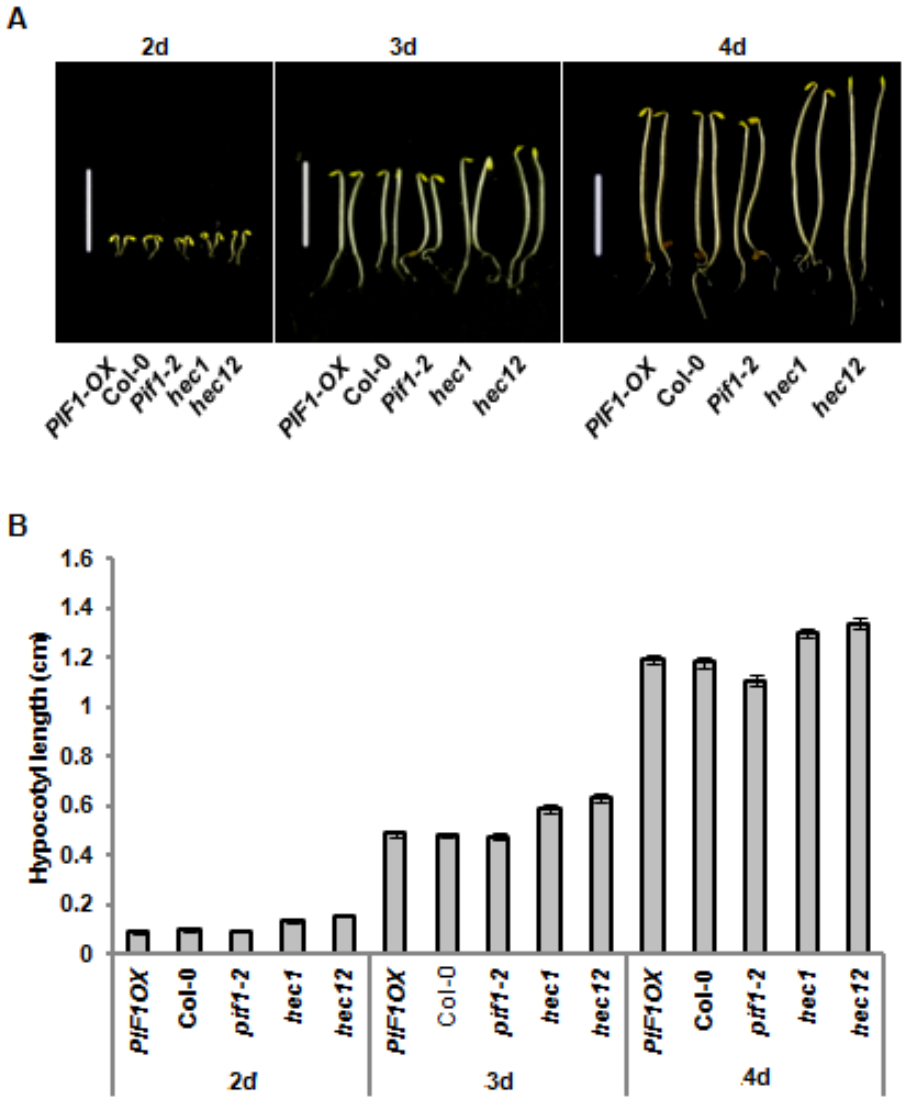


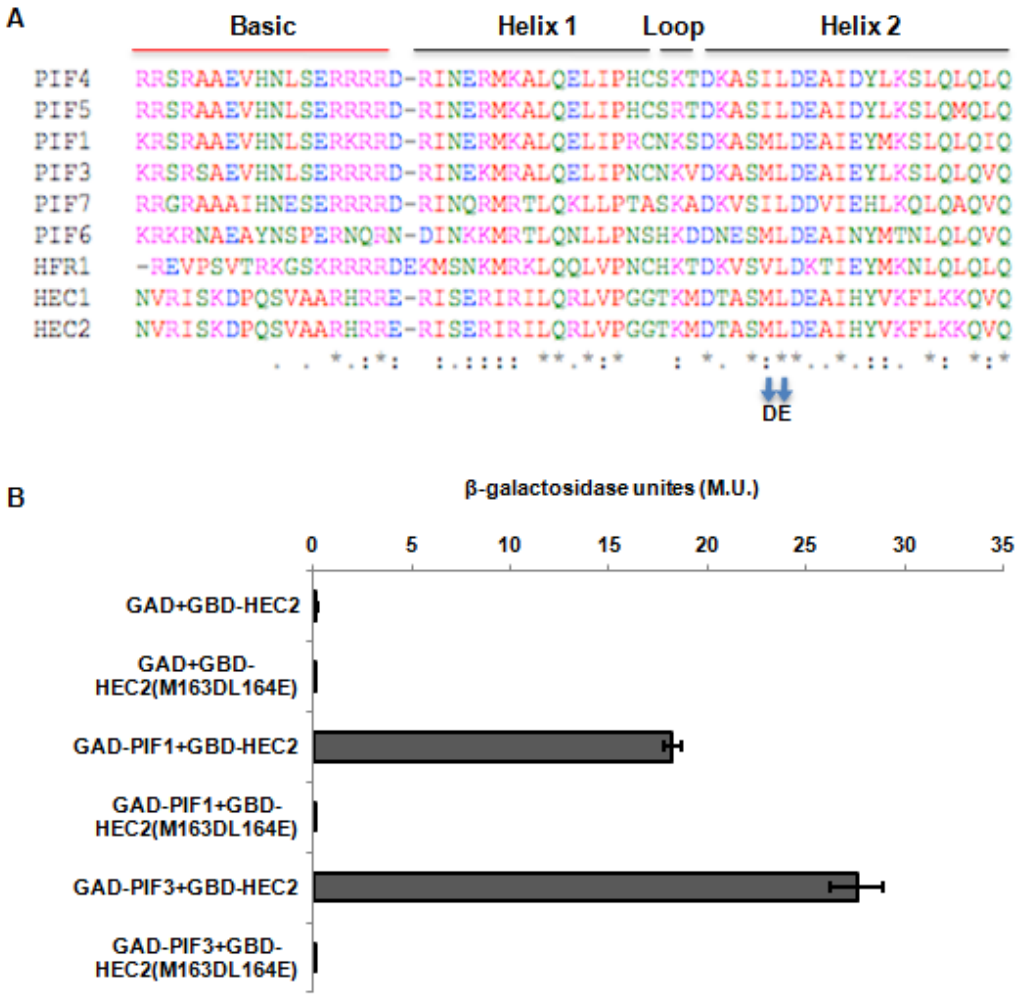

**Supplemental Figure 1**: **HECATE proteins belong to the HLH subclass of bHLH family.**

A) Sequence alignment of the bHLH domain of PIFs, HFR1, and HECATE proteins. *, indicates identical residues; :, indicates conserved residues and ., indicates similar residues. B) Phylogenetic analysis of HECATE proteins, PIFs and HFR1. Alignment provided as Supplemental Data Set 1.



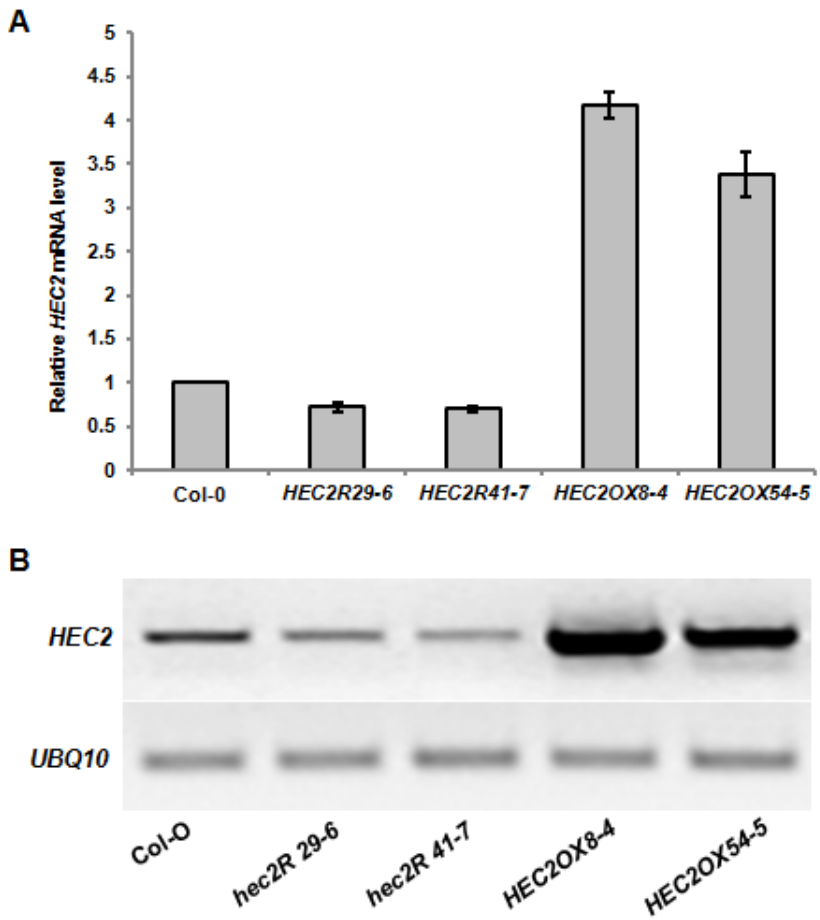

**Supplemental Figure 2**: **HEC1 and HEC2 promote hypocotyl elongation in the dark in Arabidopsis**

A) Photographs of wild-type Col-0, *pif1-2, hec1, hec12*, and *PIF1* overexpression line grown in the dark for two, three or four days. White bar = 5 mm. B) Hypocotyl length measurement of the wild- type Col-0, *pif1-2, hec1, hec12*, and *PIF1* overexpression line grown under the same conditions as shown in (A). For each genetic background under each condition, at least 40 seedlings were measured using ImageJ. Error bar = S.E.



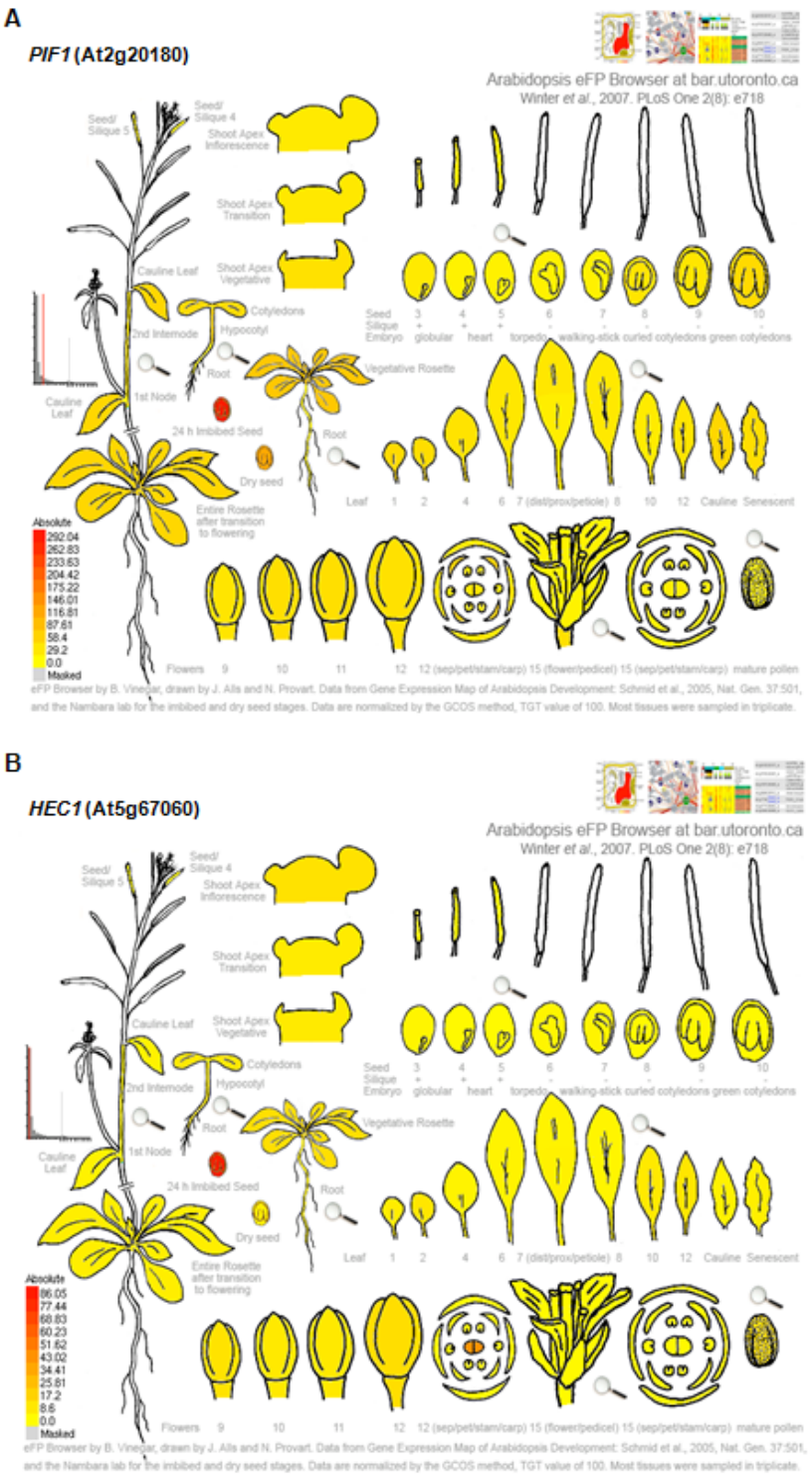

**Supplemental Figure 3: Yeast two-hybrid interaction assays between wild type and mutant HEC2 with PIF1 and PIF3.**

A) Sequence alignment of the bHLH domain of PIFs, HFR1, and HEC proteins. *, indicates identical residues; :, indicates conserved residues and ., indicates similar residues. Two arrows indicate two predicted key amino acids for dimerization of bHLH proteins. These amino acids were mutagenized to the amino acids indicated below the arrows in the mutant version of HEC2. B) The mutated HEC2 does not interact with both PIF1 and PIF3 in a quantitative yeast-two-hybrid assay. LacZ assays were performed in triplicate and the data represent mean ± s.e.m. b-Galactosidase units are Miller units (M.U.).



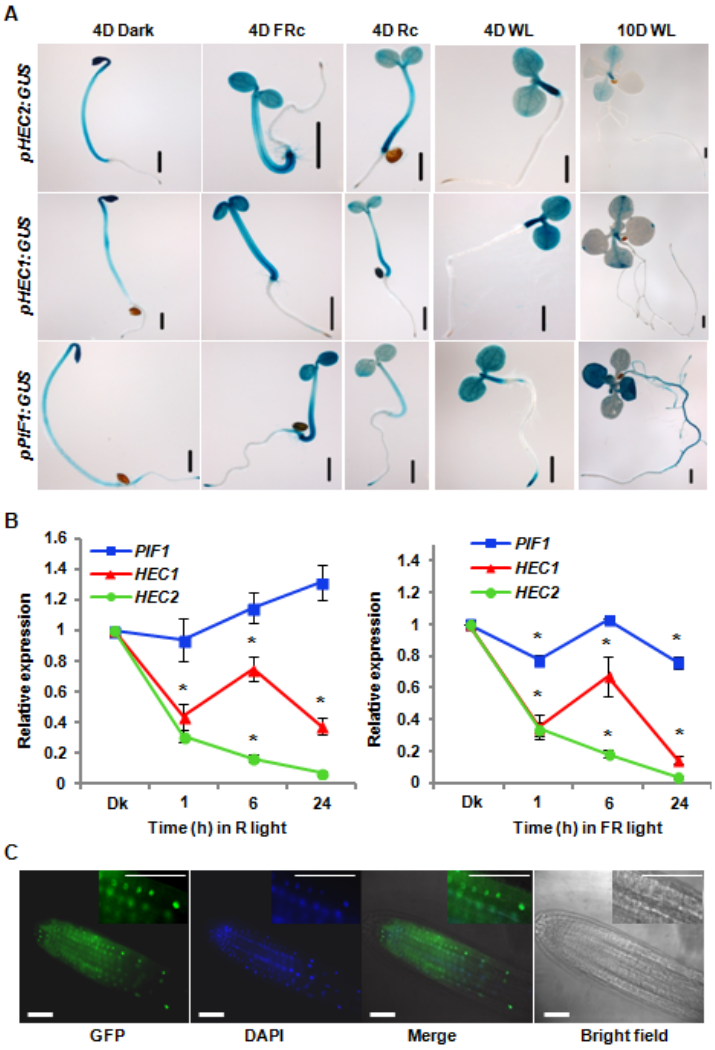

**Supplemental Figure 4: *HEC2* mRNA level in wild type, *HEC2* RNAi and overexpression lines.**

A) RT-qPCR data showing relative expression of *HEC2* gene in wt Col-0, two independent *HEC2* RNAi lines, and two independent *HEC2* overexpression lines at seed stage. RNA was extracted from 24-h imbibed seeds of individual genotypes and reverse transcribed into cDNA. RT-qPCR was performed using *HEC2* gene specific primers. *PP2A* was used as house keeping gene for normalization. *HEC2* expression level in Col-0 was normalized to 1. (N=3 biological repeats, each with 3 technical replicates, ± S.E.M). B) RT-PCR showing *HEC2* mRNA level in wt Col-0, two independent *HEC2* RNAi lines, and two independent *HEC2* overexpression lines at the seedling stage. Assays were performed using total RNA isolated from 4 day-old dark grown seedlings from different genotypes. RNA was reverse transcribed into cDNA and *HEC2* gene specific primers listed in the Supplemental Table 1 were used for *HEC2* detection in each genotype. *UBQ10* was used as a control.




**Supplemental Figure 5: Co-expression analyses of *PIF1* and *HEC1* in Arabidopsis.**

Digital expression patterns for *PIF1* (At2g20180) and *HEC1* (At5g67060) in various tissues were obtained from eFP Browser web site (http://www.bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi) (Schmid et al., 2005; Winter et al., 2007). Probe for *HEC2* is absent in the microarray chips.




**Supplemental Figure 6:** Co-expression analyses of *PIF1* and *HEC1/HEC2* and subcellular localization of HEC2-GFP.

A) Tissue-specific expression of *pPIF1:GUS*, *pHEC1:GUS* and *pHEC2:GUS*. Homozygous transgenic seedlings were grown either in dark or R/FR/WL for various times as indicated and GUS assays were performed as described (Shen et al., 2007). Black bar = 10 mm. B) The gene expression of *PIF1, HEC1* and *HEC2* in response to either red or far-red light. Four day-old dark-grown wild type Col-0 seedlings were either kept in darkness or exposed to red (7.8 $\mu molm^{-2}s^{-1}$) (left) or far-red light (1 $\mu molm^{-2}s^{-1}$) (right) for the indicated times. RNA was extracted and reverse transcribed to cDNA. RT-qPCR was performed using gene specific primers. (N=3 biological repeats, each with 3 technical replicates, ± S.E.M). C) HEC2 is localized to the nucleus. Photographs of HEC2-GFP fusion constructs expressed in stable transgenic wild type plants are shown. From the left, the first image shows GFP staining for HEC2-GFP driven by constitutively active 35S promoters, the second image shows DAPI staining for nuclei, the third image shows superimposition of the GFP and DAPI signals, and the fourth image shows the bright field photograph. White bar = 0.05 mm.

# Supplemental Table 1

**Supplemental Table 1: Primer sequences used in experiments described in the text.**

| Gene | Forward | Reverse |
|---|---|---|
| **Cloning** | | |
| *HEC1* for Y2H | agagaattcATGGATTCTGACATAATGAAC | cctgtcgacTCATCTAAGAATCTGTGCATTG |
| *HEC2* for Y2H | agagaattcATGGATAACTCCGACATTCTAATG | cctgtcgacTCATCTAAGAATCTGTGCATTTC |
| *HEC2RNAi* | caccGAACACTTCTCTAACTCAAACC | CTTTGGTGGCTTTACGGATTCC |
| *HEC2OX* | caccATGGATAACTCCGACATTCTAATG | cctgtcgacTCATCTAAGAATCTGTGCATTTC |
| *HEC2-GFP* | caccATGGATAACTCCGACATTCTAATG | TCTAAGAATCTGTGCATTTCC |
| *HEC2M163 DL164E* | GATACGGCGTCGgATgagGATGAGGCTATC | GATAGCCTCATCCTCATCCGACGCCGTATC |
| *pHEC1* | caccAGTCTTAAATGTGATTTTGTAC | AGAGAAAGATATGGAGAAGCTGA |
| *pHEC2* | caccGAGAGAGCAGCGAAACGTCATCG | CCTCCTTTTTTGTGGAATTTATAG |
| *pPIF1* | caccCACTTTGTTCACTAACTTGACTAC | GTCctgcagCTCTCTCTACAAAGATGATGATAATG |
| **RT-PCR** | | |
| *PIF1* | GTGATCGATATGTCAATGGGATGTGGAATGA | CTCGTCGACGCTTAACCTGTTGTGTGGTTTC |
| *HEC2* | CACCATGGATAACTCCGACATTCTAATG | CTTTGGTGGCTTTACGGATTCC |
| *UBQ10* | GATCTTTGCCGGAAAACAATTGGAGGATGGT | CGACTTGTCATTAGAAAGAAAGAGATAACAGG |
| **RT-qPCR** | | |
| *PP2A* | TATCGGATGACGATTCTTCGTGCAG | GCTTGGTCGACTATCGGAATGAGAG |
| *PIL1* | AAATTGCTCTCAGCCATTCGTGG | TTCTAAGTTTGAGGCGGACGCAG |
| *PIL2* | CACCACCATGGATGATACTCTTC | TTCTTGCAAAGGGCCAAAGATCC |
| *HFR1* | ATTGGCCATTACCACCGTTTAC | TGAGGAGAAGAAGCTGGTGATG |
| *FHL* | TCTGAGCATCAAGCCTCTCTTG | TCATCGCTGGTTTTTGTGTTCT |
| *SDR* | ATGAGCTCTCCCGTCAGCTTCAGG | CTCCCTTCACACTTGGATGCAGAGC |
| *XTR7* | CGGCTTGCACAGCCTCTT | TCGGTTGCCACTTGCAATT |
| *AtHB-2* | GTCGTTGCCGGTCAATGC | CCTAGGACGAAGAGCGTCAAAA |
| *PIF1* | TGAATCCCGTAGCGAGGAAACAA | TTCCACATCCCATTGACATCATCTG |
| *HEC1* | GAGGAAGGGTTTTGATCGGTGGAG | TGCATTGCCCACCATCTGATGAGT |
| *HEC2* | GAGGAATGACGGCGGTGGC | TGATCAGACCGCATAATGCCACAC |
| **ChIP-qPCR** | | |
| *PIL1* control | AATATGCAAGATCCGTTCTG | TCTTCAAGATGATGGCTGAC |
| *PIL1* G-box | ATAACACAAAGGGGTGGATG | GTGAGTGACATGCGAGAGAG |
| *XTR*control | TCGAGGTATGATGGGTGTAG | GCTGAGAACACTGAGTACTG |
| *XTR*G-box | CGCATGCCGGCTGGAATAGATAG | CGACGTGTCACTTCCCTCGTACC |
| *HFR1* control | ACGCAACAAACGAACCACAC | AGAGCGATCGGATCAGATAG |
| *HFR1* G-box | ACGTGATGCCCTCGTGATGGAC | GTCGCTCGCTAAGACACCAAC |
| *HEC1* control | TCACCCAACTACCAACTAAA | AACTTATATGAGCACATCGC |
| *HEC1* G-box | TGCCTTCTTTGCCTTTAGTG | CCGTCGATAATTGACCAATG |
| *HEC2* control | ATTTGTCGGAAAACGTGATT | TGTCCCAAGCATCATCTATG |
| *HEC2*G-box | CCTTAGTTCACAACTCGTG | TAGTCACTTGGTGTCACTAAA |