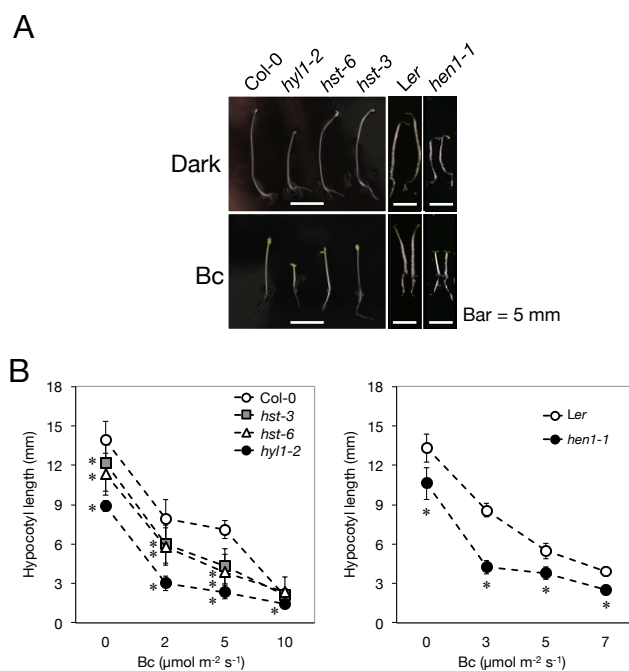
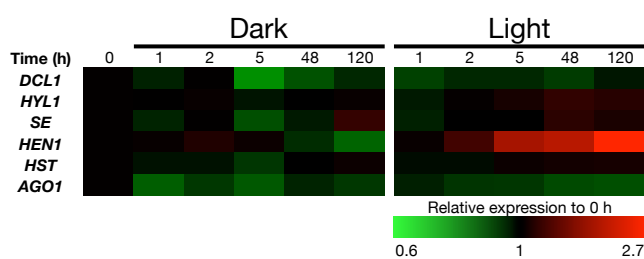


Supplemental Figure 1. Schematic overview of canonical miRNA biosynthesis (modified from Rogers and Chen, 2013).



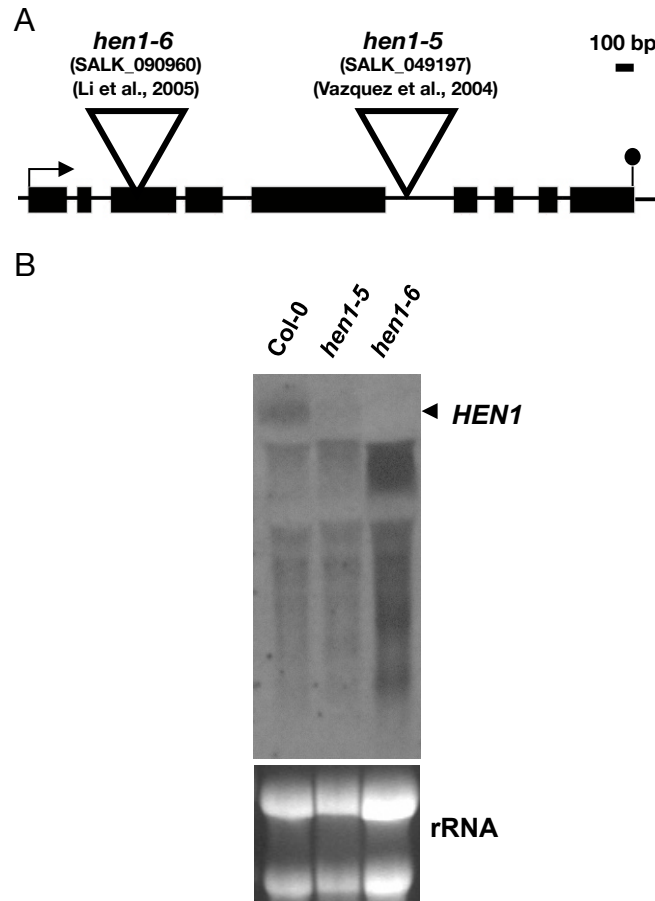
Supplemental Figure 2. Skotomorphogenesis and photomorphogenesis are defective in miRNA biogenesis mutants

The *hyl1-2*, *hen1-1*, *hst-3* and *hst-6* mutants show defects in hypocotyl elongation in the dark and light (A). However, the mutants are hypersensitive to continuous blue light (Bc)(B). Data are mean \pm SD. *, significantly different from the corresponding wild type (Col-0 and *Ler*)($p < 0.05$, Student's *t* test; $n = 16-32$).



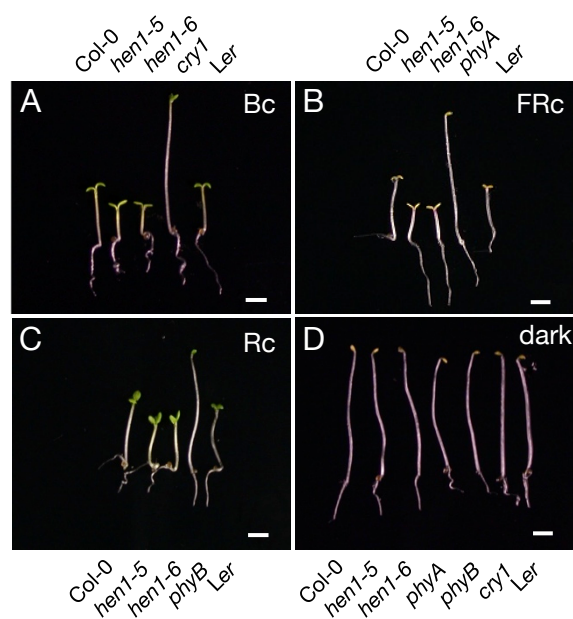
Supplemental Figure 3. The transcript level of *HEN1* is light-upregulated

Expression of miRNA biosynthetic genes *HEN1*, *DCL1*, *HYL1*, *SE*, *HST* and *AGO1* in etiolated seedlings under dark and light treatments at the indicated times from Genevestigator sample AT-00003 (repository name: PZ051203_02). Gene expression for the times indicated was normalized to time 0.



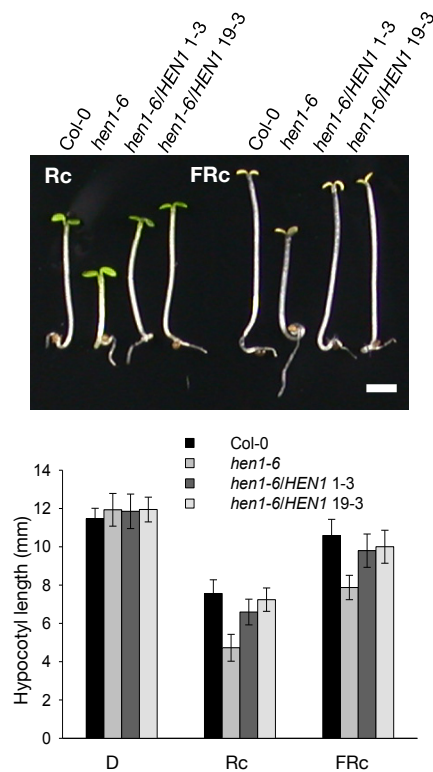
Supplemental Figure 4. **Molecular characterization of *hen1-5* and *hen1-6* mutants**

(A) SALK_049197 (*hen1-5*) and SALK_090960 (*hen1-6*) lines carried the T-DNA insertion in intron 5 and exon 3, respectively, of *HEN1*. The locations of the T-DNA and transcription start/end are marked on the *HEN1* gene model. (B) RNA gel blot analysis with a DNA probe spanning 1 to 500 bp of the *HEN1* coding region for profiling *HEN1* expression in the wild type (Col-0), *hen1-5* and *hen1-6*. Ethidium-bromide-stained rRNA is shown as a loading control.



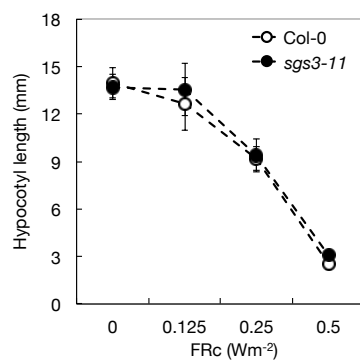
Supplemental Figure 5. The *hen1* mutants are hypersensitive to blue (B), far-red (FR), and red (R) light

Representative seedlings of 4-d-old seedlings of Col-0, *Ler*, *hen1-5*, *hen1-6*, *phyA*, *phyB* and *cry1* seedlings grown under continuous blue light (Bc) (A), FRc (B), Rc (C) or dark (D). Bar = 2 mm.



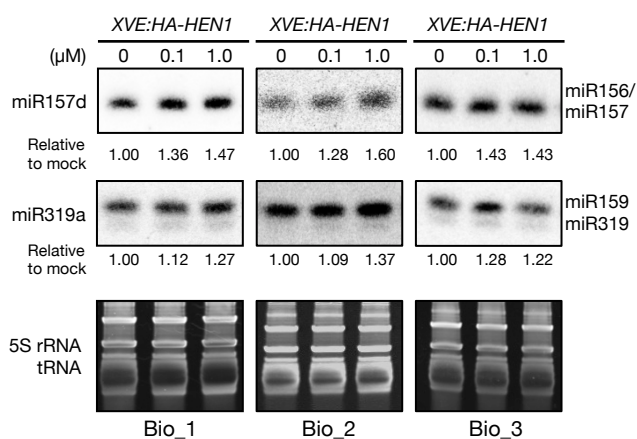
Supplemental Figure 6. Functional complementation of *hen1* light-hypersensitive phenotype

A genomic fragment harboring *HEN1* (-2,135 to +4,492 of *HEN1* locus) was generated by PCR with specific primers (Supplemental Table 1 online) and subcloned into the *SalI-PmlI* sites of the binary vector pCAMBIA1390 and introduced into *hen1-6*. Hypocotyl lengths of Col-0, *hen1-6*, and 2 representative complementation lines, *hen1-6/HEN1* 1-3 and *hen1-6/HEN1* 19-3, grown under dark (D), Rc ($5 \mu\text{mol m}^{-2} \text{s}^{-1}$) or FRc (0.125 Wm^{-2}). Data are mean \pm SD. Bar = 2 mm.



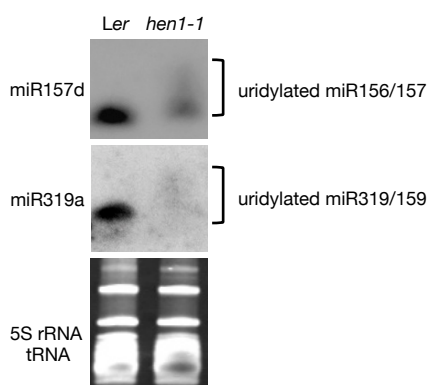
Supplemental Figure 7. Effect of FR fluence rate on hypocotyl extension in wild-type and *sgs3-11* mutant seedlings

Hypocotyl lengths of wild-type and *sgs3-11* seedlings grown under different FRC fluence rates for 4 days. Data are mean \pm SD.



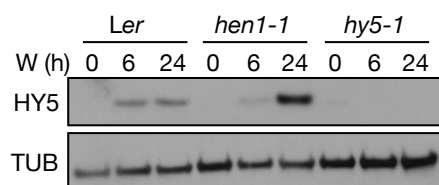
Supplemental Figure 8. The levels of miR157 and miR159/319 are increased accompanied with increased level of HA-HEN1 protein.

Three biological replicates of small RNA analyses of miR157 and miR159/319 expression levels. The replicate Bio_1 was shown in Figure 5C. The numbers below the blots showed 5S rRNA- and tRNA-normalized miRNA fold increase in HA-HEN1-induced seedlings.



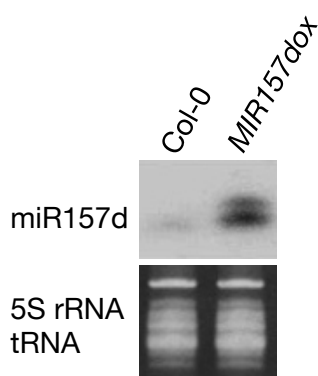
Supplemental Figure 9. The expressions of miR157d and miR319 are compromised in *hen1-1* mutant

Four-day-old etiolated wild-type (*Ler*) and *hen1-1* seedlings were treated with W ($50 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 24 h, and then RNA gel blot analysis was carried out with $1 \mu\text{g}$ total RNA. DNA oligos used for each blot are indicated to the left of each blot. Despite the clear detection of miR156/157 and miR319/159 in *Ler*, only traces of uridylated miR156/157 and miR319/159 were detected in *hen1-1*. Ethidium-bromide-stained rRNA/tRNA is shown as loading control.



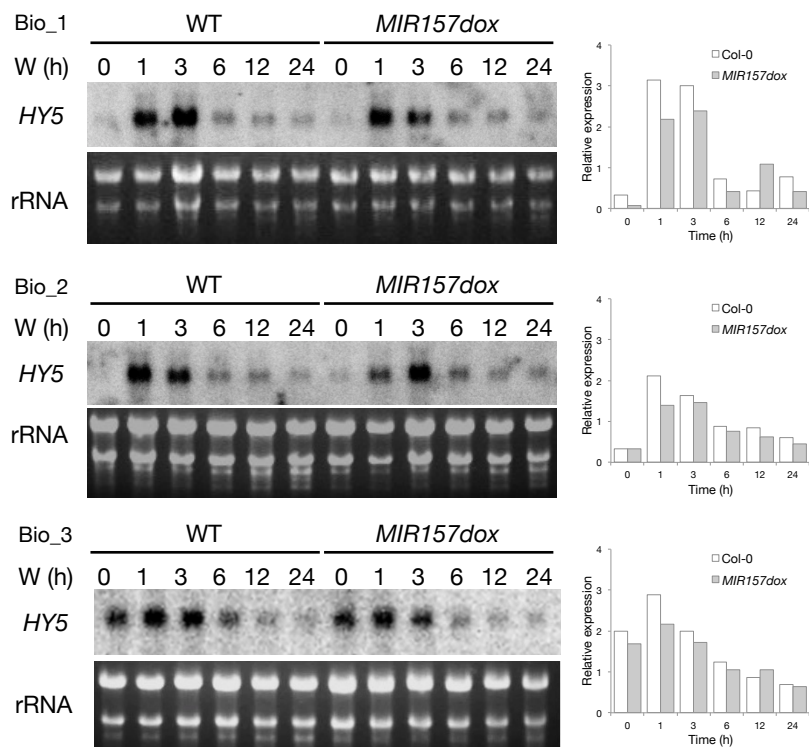
Supplemental Figure 10. HY5 is increased in the *hen1-1* mutant during photomorphogenesis

Protein levels of HY5 in 4-d-old de-etiolating seedlings of wild type (*Ler*) and *hen1-1* under W ($100 \mu\text{mol m}^{-2} \text{s}^{-1}$) at the indicated times. HY5 was not detected in the *hy5-1* mutant. TUB was used as a loading control.



Supplemental Figure 11. Over-accumulation of miR157d detected in the *MIR157dox* line

RNA gel blot analysis of the miR157d levels in 4-d-old Wc ($100 \mu\text{mol m}^{-2} \text{s}^{-1}$)-grown seedlings of the wild type (Col-0) and *MIR157d* overexpression line (*MIR157dox*). DNA oligos used for each blot are indicated to the left of each blot. Each sample contained 1 μg total RNA.



Supplemental Figure 12. The *HY5* transcript levels are decreased in the *MIR157dox* transgenic line

Three biological replicates of RNA gel blot analyses on *HY5* transcript levels in *MIR157dox* transgenic line during the de-etiolation. The replicate Bio_1 was shown in Figure 8A. The etiolated seedlings on the time 0 point were collected in the dark except those on Bio_3 time 0, which were collected under the light. The bar graphs show the *HY5* signals normalized to ribosomal RNA using Image J on each biological replicate.

Supplemental Table 1. **Primers and oligos used in this study**

Primer	Sequence (5' to 3')
<u>Quantitative RT-PCR analysis</u>	
<i>HEN1</i> -F	CAACGTCAAATCTGCTACACTTT
<i>HEN1</i> -R	AAGTGCCGATGTCAACGTCAT
<i>HY5</i> -F	ATGTTAAAATTATATGGGATGTGAGAGCTAA
<i>HY5</i> -R	TTCAATTAAGAGAAATCTAAGACTACAATAAGAGA
<i>TCP2</i> -F	GGTGGCTTCAGTGGTTTCAAC
<i>TCP2</i> -R	CCTCTGTAGATTAGCGAGGAATGAC
<i>TCP3</i> -F	CCGGAGGATTTGTGTTTGTCT
<i>TCP3</i> -R	CCCCTCTGTGATAAAAAGCTGACTT
<i>TCP4</i> -F	CAAAGCCAACAGCTTTATTCTCAGA
<i>TCP4</i> -R	GATGATGGTGAGGATCAAACCA
<i>TCP10</i> -F	TGTTTCTTGGTGGCCAACAA
<i>TCP10</i> -R	TGATCCCAAGAACGAAACGAAT
<i>TCP24</i> -F	ACCCATTCTCCTTTGTACCTGATT
<i>TCP24</i> -R	AAGAGAGATTGTGAATTGGACTGAAG
<i>UBQ10</i> -F (Col-0)	AGAAGTTCAATGTTTCGTTTCATGTAA
<i>UBQ10</i> -R (Col-0)	GAACGGAACATAGTAGAACACTTATTCA
<i>UBQ10</i> -F (Ler)	ACAGCTAGAGGATGGCCGTA
<i>UBQ10</i> -R (Ler)	GTTGGTCTGGCGGGATAC
<i>GFP</i> -F	GATGGCCCTGTCTTTTACCA
<i>GFP</i> -R	GGCAGATTGTGTGGACAGGTAAT
<i>UBI3</i> -F (N.b.)	GCCGACTACAACATCCAGAAGG
<i>UBI3</i> -R (N.b.)	TGCAACACAGCGAGCTTAACC
Northern blot probe	
<i>HEN1</i> -F	TCTCCATGGATTTTCAGGGGTATGCACATT
<i>HEN1</i> -R	CGCTCTAAGGTGAGCTCCCA
<i>HY5</i> -F	GTAGTCGACCAGAGATCTGACGGCGGTAG
<i>HY5</i> -R	GTAGGATCCCCTCCGCCGGTGTCTCCCT
miR157d-probe	GAGTGCTCTCTATCTTCTGTCATCAAAGAGAATCAATGA
miR156a-probe	GTGCTCACTCTCTTCTGTCA
miR319a-probe	AGGGAGCTCCCTTCAGTCCAA
<i>MIR</i> gene amplification	
<i>MIR156a</i> -F	GTATCTAGACTCTTATCTTCTTCTCATCAG
<i>MIR156a</i> -R	GTACCCGGGAACAAGAACAAGCCAGAGTT
<i>MIR157d</i> -F	GTATCTAGACCACTTACTCTTCACCGCCC
<i>MIR157d</i> -R	GTACCCGGGTATAACATGAACTGATGAACCT
<u><i>HEN1</i> gene amplification</u>	
<i>HEN1</i> genomic-F	CGAGGTACCATGGCCGGTGGTGGGAAGCA
<i>HEN1</i> genomic-R	TGTCTAGGTCAAAGATCAGTCTTTTTTC
<u><i>HEN1</i> complementation</u>	
<i>HEN1</i> -gDNA-SalI-S1	AGCGTCGACTGTTATGTGGTATGTATA
<i>HEN1</i> -gDNA-S1642	CATGAAAGTCCGCTGAACCTTTAGGTTTCGTTGTGCG
<i>HEN1</i> -gDNA-S3266	GATGTTTGGCAAAGCTTCTGTGGCTCTGAGTGTAG
<i>HEN1</i> -gDNA-S4862	CAAAGTTATGTAAGAACCCTCACGTAAAGTCTTCTGG
<i>HEN1</i> -gDNA-AS1678	TCGACAACGAACCTAAAGAGTTTCAGCGGACTTTTCAT
<i>HEN1</i> -gDNA-AS3302	TCTACACTCAGAGCCACAGGAAGCTTTGCCAAACAT
<i>HEN1</i> -gDNA-AS4897	CAGAAGACTTTACGTGAGGTTCTTACATAACTTTGC
<i>HEN1</i> -gDNA-PmlI-AS6627	AGCACGTGTTAAACTTCAACGGACCA

<i>HY5</i> 5'UTR amplification	
<i>HY5</i> -5'UTR-F	TCTAGAGTCCCCTCTTTTCCTCTTTATC
<i>HY5</i> -5'UTR-R	CTCGAGTTTTCTTACTCTTTGAAGATCGAT
<i>HY5</i> -5'UTR _m -F	TTCCTCCAATACCTTTTCACCAG
<i>HY5</i> -5'UTR _m -R	GCTGGTGAAAAGGTATTGGA
<i>mHY5</i> cDNA	
<i>HY5</i> -5'UTR _m - <i>Xba</i> I-F	GACTCTAGATCCCCTCTTTTCCTCCAATACCTTTTCACCA
<i>HY5</i> <i>stop</i> - <i>Eco</i> RI-R	TCGGAATTCTCAAAGGCTTGCATCAGCAT
5'-RACE	
<i>HY5</i> -R	TCCTCCCTCGCTTCCTTTGACTTTC
<i>TCP24</i> -R	TCTCCTTTCCTTTGCCTTGTCAT
