A Novel Arabidopsis MYB-like Transcription Factor MYBH Regulates Hypocotyl Elongation by Enhancing Auxin Accumulation

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Supplementary figure S1

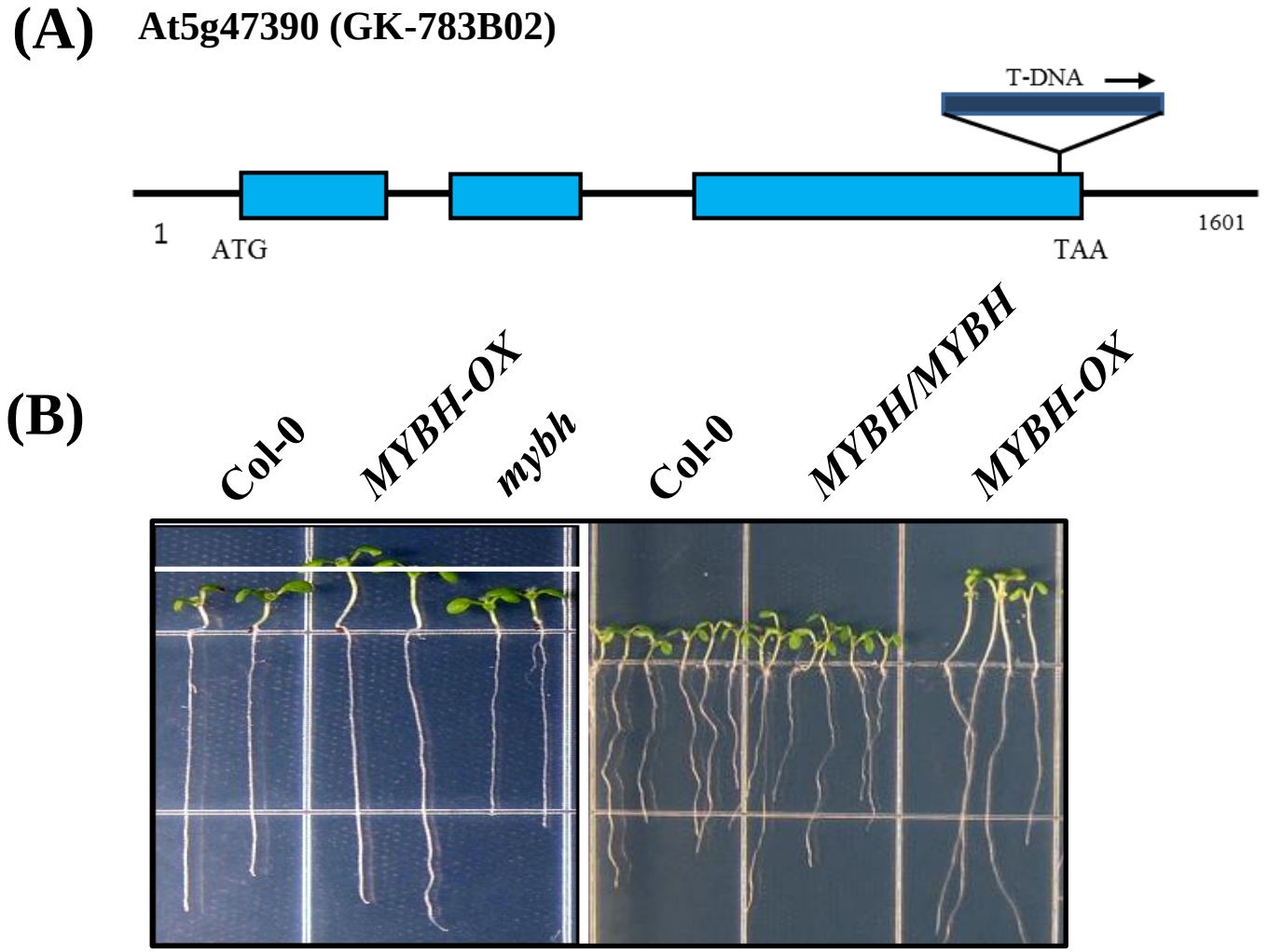


Figure S1. Complementation assay

Diagram of T-DNA localization in the *MYBH* gene (A) and a complementation assay in which wild-type *MYBH* was expressed under the control of the *MYBH* promoter (-1 kb) (B). The construct *MYBHpro::MYBH* was transferred to *mybh* plants, and the homozygous transgenic seeds were sown on Murashige and Skoog medium for 7 d.

Supplementary figure S2

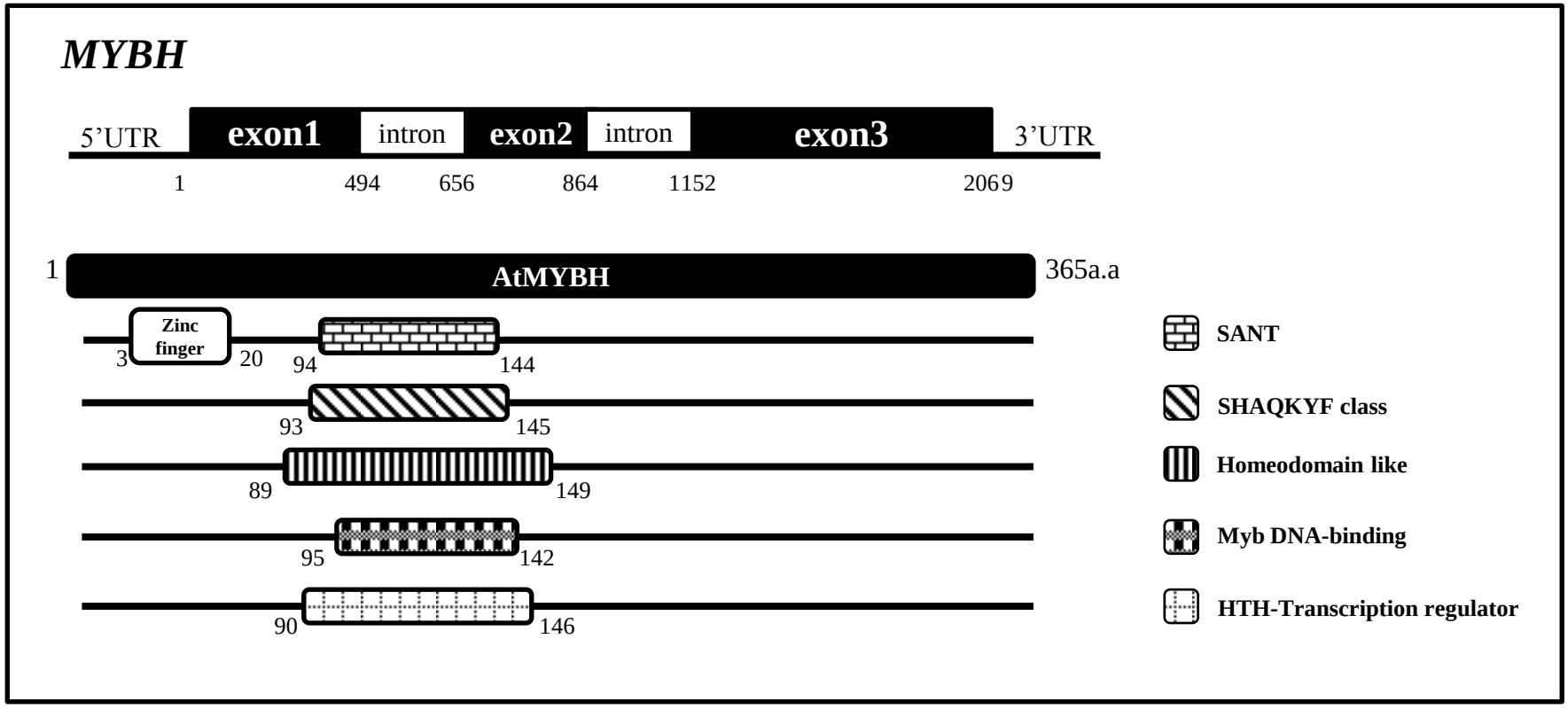


Figure S2. Schematic of the *MYBH* gene and protein.
MYBH (*AT5G47390*) belongs to the *MYB* family of genes that encode plant cell transcription factors consisting of three exons and two introns. *MYBH* is 2069 bp in length. The MYBH protein consists of a zinc finger, a SANT ('switching-defective protein 3 (Swi3), adaptor 2 (Ada2), nuclear receptor co-repressor (N-CoR), transcription factor (TF) IIIB'), and SHAQKYF class, homeodomain-like, MYB DNA-binding, and helix-turn-helix (HTH) transcription regulator domains.

Supplementary figure S3

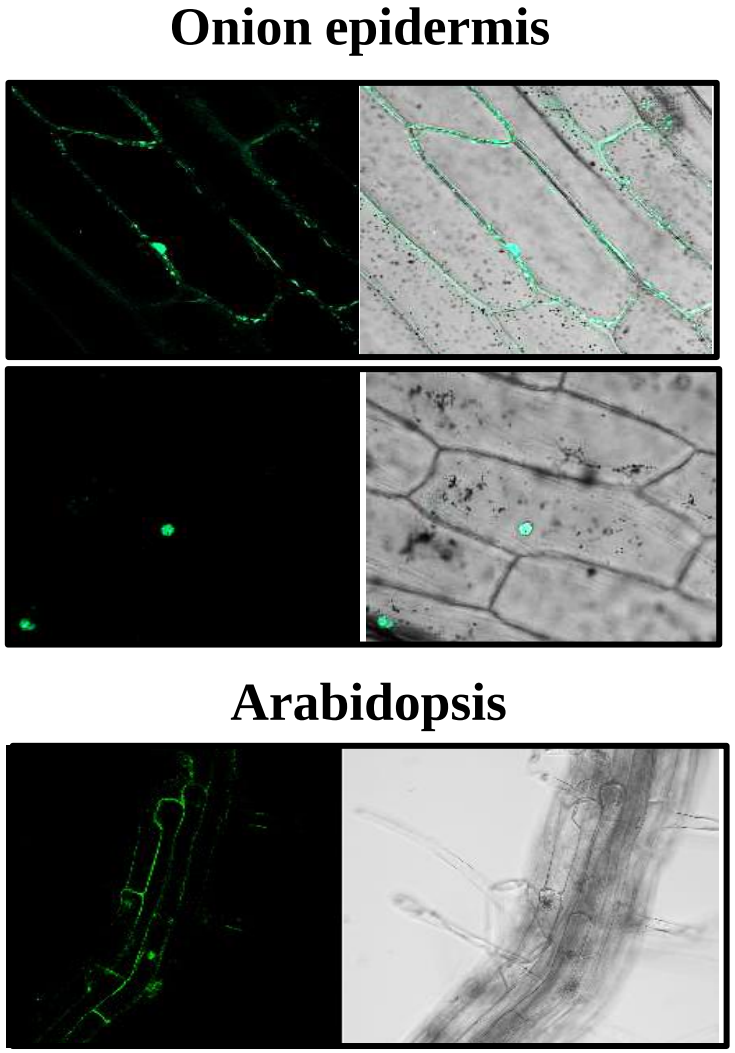


Figure S3. Subcellular localization of MYBH

The *CaMV35S::MYBH:GFP* fusion construct was introduced into onion epidermal cells by performing particle bombardment or stably expressed in Arabidopsis. After 18–48 h of incubation, the GFP signal of the MYBH:GFP fusion protein was detected in onion cell nuclei by confocal laser scanning microscopy.

Supplementary figure S4

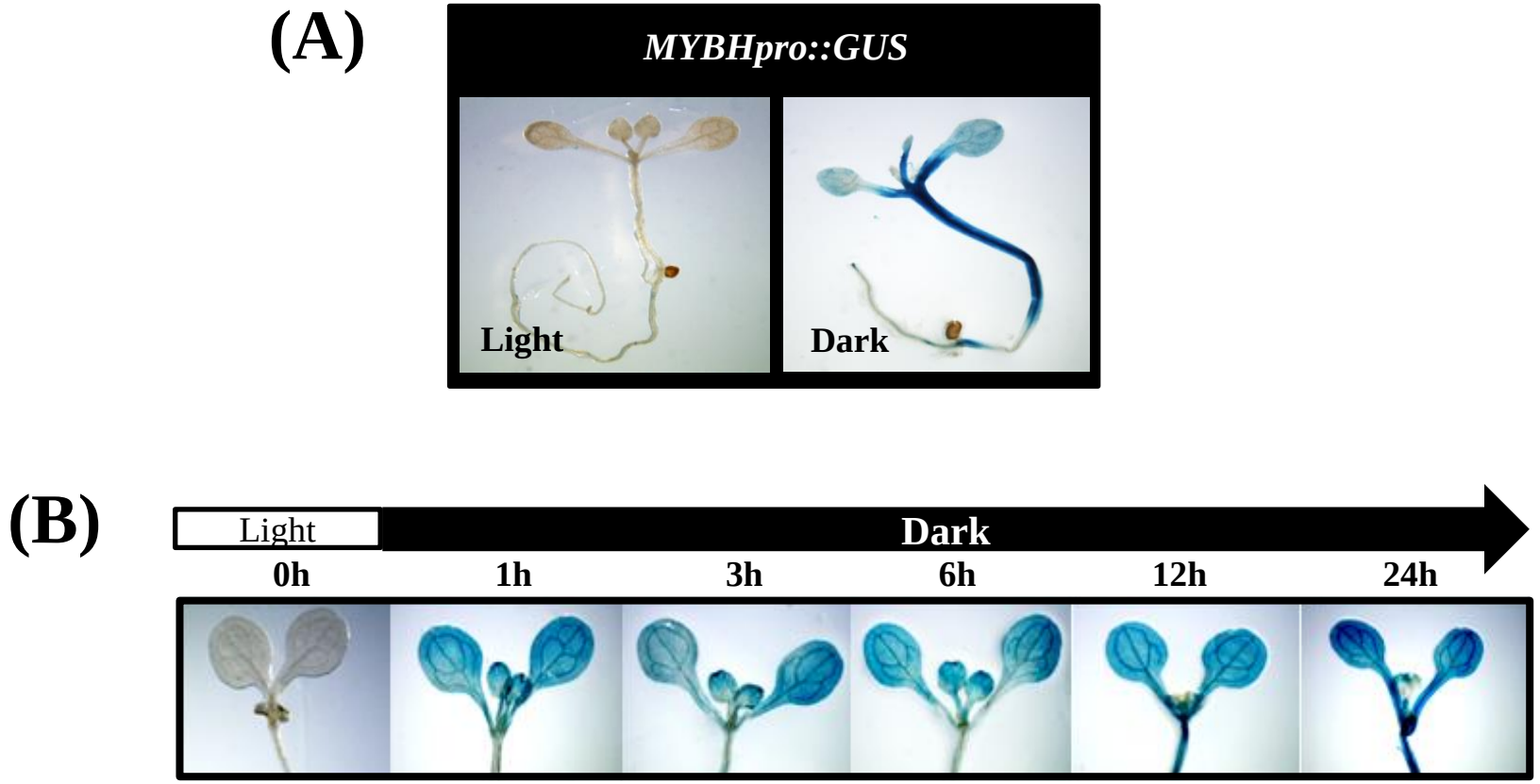
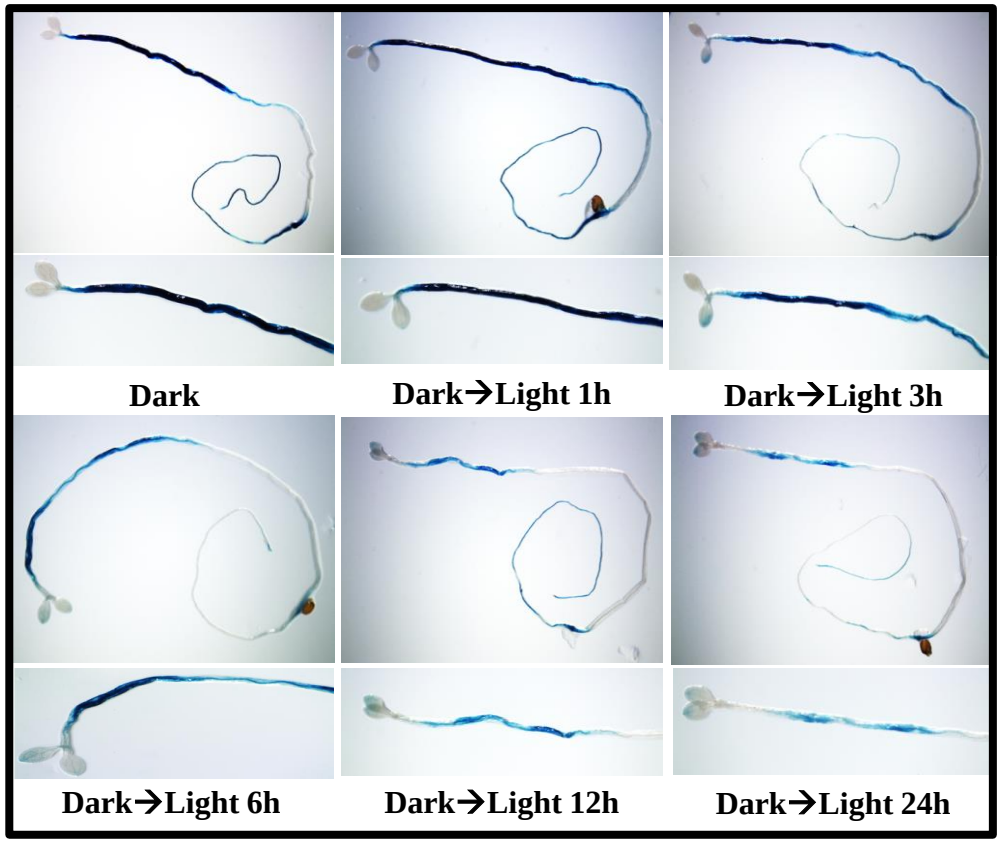


Figure S4. Light-dependent activity of the *MYBH* promoter.
(A) Transgenic plants that harbored *MYBHpro::GUS* were grown on Murashige and Skoog (MS) medium for 7 d after stratification in light or continuous darkness for histochemical GUS staining. The *MYBHpro::GUS* seeds were sown on MS medium under light (B) or continuous darkness (C). After 7 d, the light conditions were changed from light to dark (B) or from dark to light (C) for the indicated times. The *MYBHpro::GUS* transgenic seedlings were then collected for histochemical GUS staining at 0, 1, 3, 6, 12, and 24 h after exposure. Three independent experiments were conducted.

Supplementary figure S4

(C) *MYBHpro::GUS*



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Supplementary figure S5

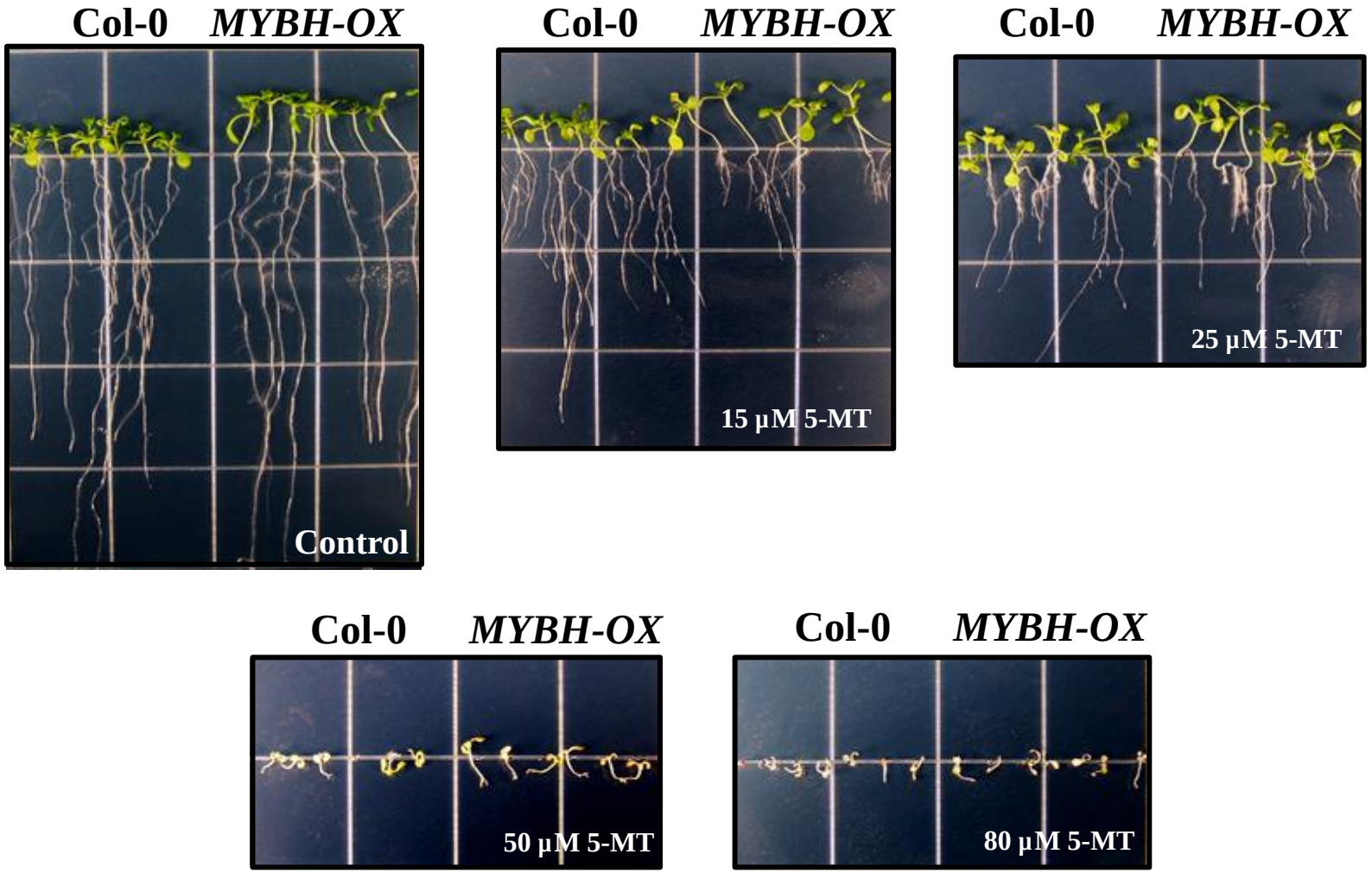

Figure S5. Effect of the auxin biosynthesis inhibitor 5-methyltryptophan on *MYBH-OX* seedling growth

Col-0 and *MYBH-OX* seedlings were grown on Murashige and Skoog medium that was supplemented with the indicated concentrations of 5-methyltryptophan (5-MT) for 7 d. Three independent experiments were conducted (n = 90).

Supplementary figure S6

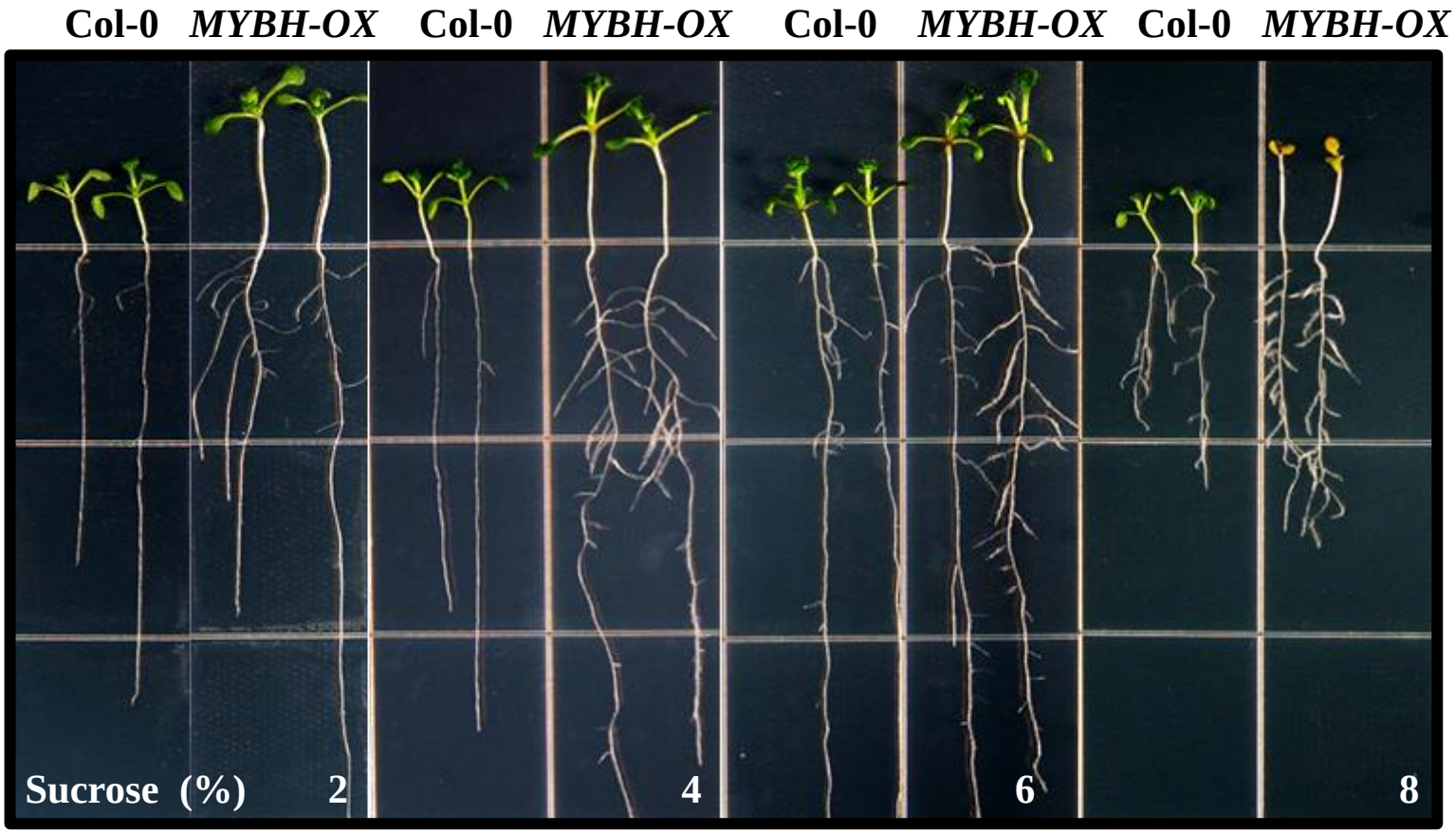

Figure S6. Increase in lateral root number of *MYBH-OX* in response to sucrose
Wild-type (Col-0) and *MYBH-OX* seedlings were grown on Murashige and Skoog medium that was supplemented with the indicated concentrations of sucrose for 7 d. Three independent experiments were conducted (n = 90).

Supplementary figure S7

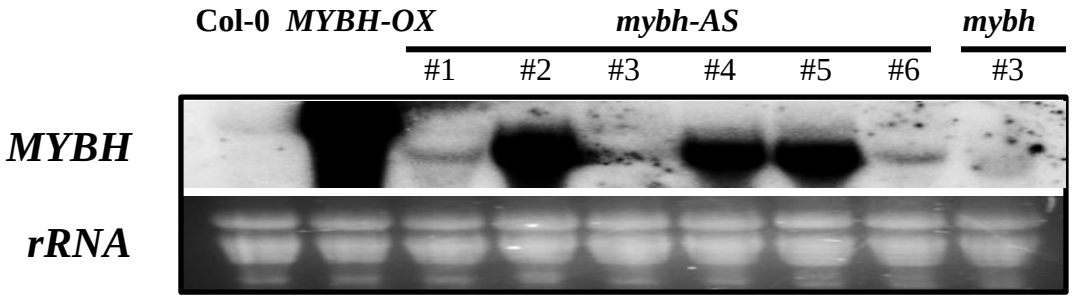

Figure S7. *MYBH* transcript levels in Col-0, *MYBH-OX*, *mybh-AS*, and *mybh* plants. Fourteen-day-old Col-0, *MYBH-OX*, *mybh*, and *mybh-AS* seedlings were harvested for RNA extraction and RNA blotting analysis. *MYBH* transcript levels are shown. *rRNA* was used as a loading control.

Supplementary figure S8

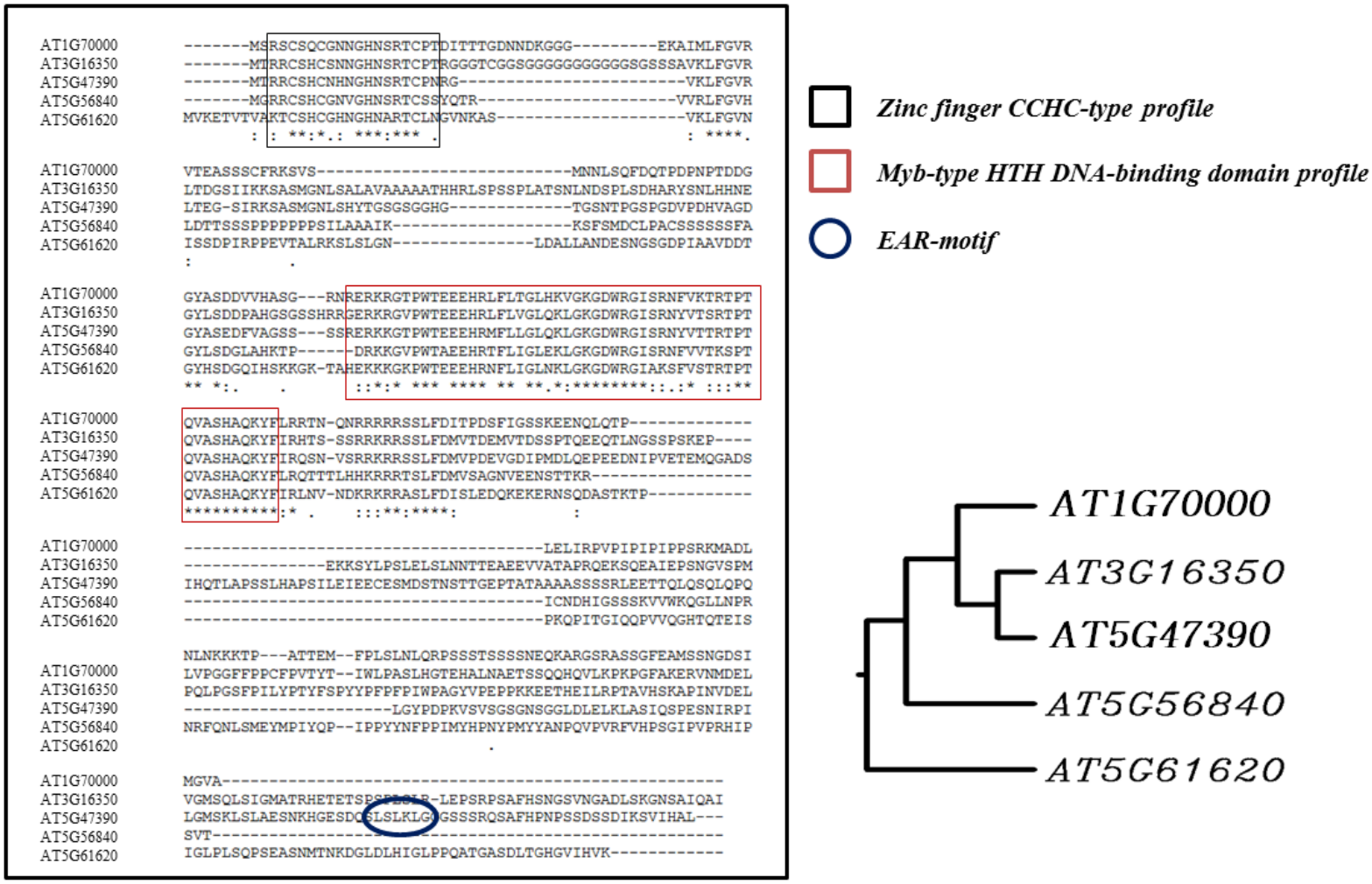

Figure S8. *MYBH* homologues in *Arabidopsis thaliana*

The homologues of *MYBH* in *Arabidopsis thaliana* are indicated by the protein sequence alignment.

Supplementary figure S9

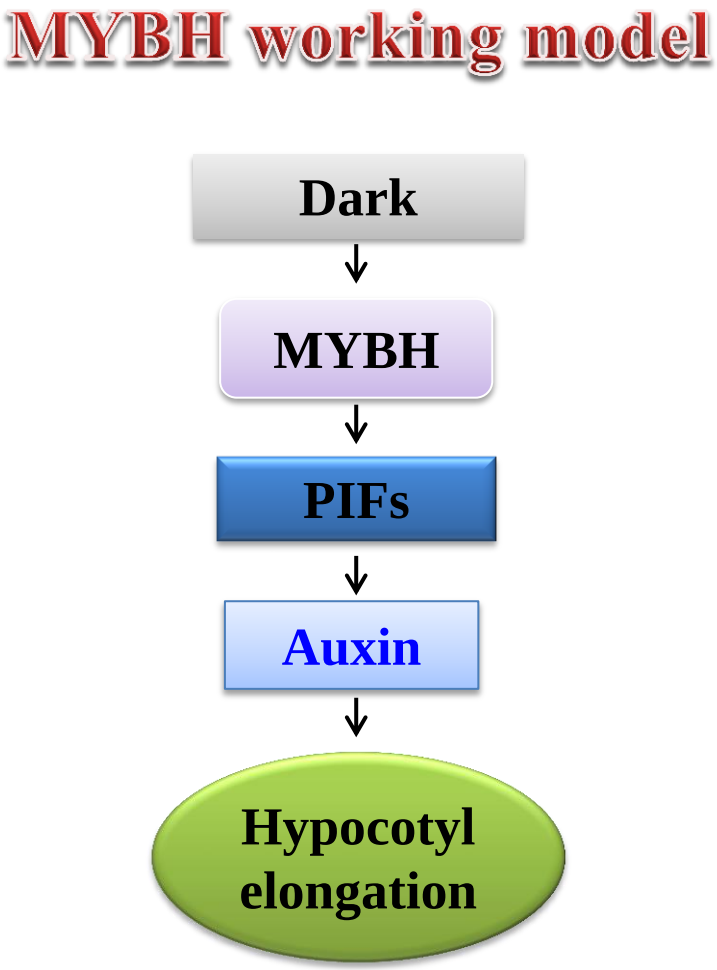

Figure S9. Working model of MYBH activity during dark-induced hypocotyl elongation

MYBH is likely to act as a transcriptional regulator during development of the hypocotyl. After induction in the dark, MYBH enhanced the expression of auxin-related genes such as *PIF4* and *PIF5*, and then induced auxin accumulation that could increase hypocotyl elongation.

Supplementary Table S1

Table S1. Primer sequences used in this current study

Gene	Forward (5'-3')	Reverse (5'-3')	
MYBH	ATGACTCGTCGATGTTCTCACTGC	TTATAAAGCGTGTATCACGC	**For cloning**
MYBHpro	ACAAACACTGGGATCCTCCA	ACCCGACCCGATTTGCTTCTCTTA	
PIF4	ATGGAACACCAAGGTTGG	CTAGTGGTCCAAACGAGA	**For *qRT-PCR***
PIF5	ATGGAACAAGTGTTTGCTG	CCATATGAAGACTGTCGG	
HY5	ATGCAGGAACAAGCGACTAGCT	TCAAAGGCTTGCATCAGC	
CAB	GACAAT GAGGAAGACTGTTGCC	TTTCCGGGAACAAAGTTGGTTGCG	
EXP3	GTTGGGCGTATTAGAGTC	ACAACCAGTTTCAGGAGG	
ACTIN7	ATGGCCGATGGTGAGGATAT	TCCTGTGAACAATCGATGGACC	
YUCCA8	ATCAACCCTAAGTTCAACGAGTG	CTCCCGTAGCCACCACAAG	