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# *Arabidopsis* NF-YCs Mediate the Light-Controlled Hypocotyl Elongation via Modulating Histone Acetylation

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## ABSTRACT

**Light is a crucial environmental signal that promotes photomorphogenesis, the developmental process with a series of light-dependent alterations for plants to adapt various external challenges. Chromatin modification has been proposed to be involved in such light-mediated growth, but the underlying mechanism is still elusive. In this study, we identified four *Arabidopsis thaliana* Nuclear Factor-YC homologs, NF-YC1, NF-YC3, NF-YC4, and NF-YC9 (NF-YCs), which function redundantly as repressors of light-controlled hypocotyl elongation via histone deacetylation. Obvious etiolation phenotypes are observed in *NF-YCs* loss-of-function mutant seedlings grown under light conditions, including significant elongated hypocotyls and fewer opened cotyledons. We found that NF-YCs interact with histone deacetylase HDA15 in the light, co-target the promoters of a set of hypocotyl elongation-related genes, and modulate the levels of histone H4 acetylation on the associated chromatins, thus repressing gene expression. In contrast, NF-YC-HDA15 complex is dismissed from the target genes in the dark, resulting in increased level of H4 acetylation and consequent etiolated growth. Further analyses revealed that transcriptional repression activity of NF-YCs on the light-controlled hypocotyl elongation partially depends on the deacetylation activity of HDA15, and loss of *HDA15* function could rescue the short-hypocotyl phenotype of *NF-YCs* overexpression plants. Taken together, our results indicate that NF-YC1, NF-YC3, NF-YC4, and NF-YC9 function as transcriptional co-repressors by interacting with HDA15 to inhibit hypocotyl elongation in photomorphogenesis during the early seedling stage. Our findings highlight that NF-YCs can modulate plant development in response to environmental cues via epigenetic regulation.**

**Key words:** photomorphogenesis, hypocotyl elongation, *NF-YC*, histone deactylation

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## INTRODUCTION

Light, one of the most important environmental factors for autotrophic plants, triggers a series of photomorphogenic processes during various developmental processes. At the post-germination stage, compared with dark-grown seedlings with elongated hypocotyls, apical hooks, unexpanded cotyledons, and undifferentiated chloroplasts, light-grown seedlings undergoing photomorphogenesis are characterized by short hypocotyls, open and expanded cotyledons, and green chloroplasts (de Wit et al., 2016). In addition, under shade conditions, neighbor plants usually adjust their architectures for light perception by accelerating elongation of internodes and petioles, elevating leaf angles from the horizontal, and reducing shoot branching, which are collectively referred to as the shade avoidance syndrome (Casal, 2012). These photomorphogenic phenotypes/processes confer plants more flexibility in survival when facing various light conditions.

Light signals need to be perceived and transduced to trigger photomorphogenic growth of plants. It has been revealed that plants

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precisely sense light intensity, duration, wavelength, and direction by several kinds of photoreceptors (Kami et al., 2010). Phytochromes are well known as the red/far-red photoreceptors. Phytochrome A (phyA), a light labile phytochrome, mediates the far-red light-dependent response, whereas the light-stable phytochrome phyB is involved in the red light-dependent response (Chen and Chory, 2011). Red light triggers the conversion of inactive Pr form of phyB to an active Pfr form, which then translocates to the nucleus and interacts with PHYTOCHROME INTERACTING FACTORs (PIFs), a group of negative regulators in photomorphogenesis. The interaction between phyB and PIFs results in phosphorylation of PIFs and their subsequent degradation by the 26S proteasome, thereby promoting photomorphogenesis (Leivar and Quail, 2011). The active Pfr form of phyB induced by red light can be reversed by far-red light. Far-red light also triggers the translocation of phyA into the nucleus by interacting with FAR-RED ELONGATED HYPOCOTYL 1 (FHY1) and FHY1-LIKE (FHL), and promotes photomorphogenesis as active Pfr-phyB does (Wang and Wang, 2015). On the other hand, accumulation of CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) proteins in the nucleus, which is inhibited by light, mediates the degradation of ELONGATED HYPOCOTYL 5 (HY5), a pivotal positive regulator of photomorphogenesis (Sheerin et al., 2015). The light-dependent PIF degradation and HY5 accumulation contribute to the repression of cell elongation-related genes, such as auxin-responsive genes *INDOLE-3-ACETIC ACID INDUCIBLE PROTEIN*s (*IAA*s), gibberellin-responsive gene *PACLOBUTRAZOL RESISTANCE 1* (*PRE1*), and cell wall loosening enzymes *XYLOGLUCAN ENDOTRANSGLUCOSYLSE/HYDROLASE*s (*XTH*s), thus inhibiting hypocotyl growth (Vandenbussche et al., 2005; Lee et al., 2006; Bai et al., 2012; Okello et al., 2016). Similar to phytochromes, the blue light receptors CRYPTOCHROME 1 (CRY1) and CRY2 also regulate photomorphogenic development by suppressing COP1-mediated degradation of HY5 and activity of PIFs (Liu et al., 2011; Pedmale et al., 2016).

Eukaryotic DNA is packaged with nuclear histone proteins to form chromatin, in which histone modifications, such as acetylation and methylation, are crucial for eukaryotic transcriptional regulation (Luo et al., 2012a). Histone acetylation is essential for light-dependent gene activation through the relaxation of chromatin by histone acetyltransferases (HATs) (Fisher and Franklin, 2011). For example, the loss-of-function mutant of *GENERAL CONTROL NON-REPRESSIBLE 5* (*GCN5*), a HAT in *Arabidopsis*, produced a long hypocotyl phenotype under light conditions due to reduced H3 and H4 acetylation (H3ac and H4ac) on target loci and the activation of early light-responsive genes (Benhamed et al., 2006, 2008). On the other hand, histone acetylation can be removed by histone deacetylases (HDACs), resulting in chromatin condensation and repression of gene expression. Based on sequence similarity and co-factor dependency, HDACs are grouped into three families: REDUCED POTASSIUM DEPENDENCE 3/HISTONE DEACETYLASE 1 (RPD3/HDA1), SILENT INFORMATION REGULATOR 2 (SIR2), and HISTONE DEACETYLASE 2 (HD2)-related protein families (Ma et al., 2013). Among 18 HDACs identified in *Arabidopsis*, 12 of them belong to the RPD3/HDA1 superfamily. RPD3/HDA1 members have been revealed to play vital roles in different biological processes, including seed development and dormancy (HDA6, HDA7, and HDA19) (Tanaka et al., 2008; Cigliano et al., 2013; Zhou et al., 2013), leaf development (HDA6) (Luo et al., 2012c), flowering-time control (HDA5, HDA6, HDA9, and HDA19) (Yu et al., 2011; Krogan et al., 2012; Kang et al., 2015; Luo et al., 2015), abiotic stress response (HDA6 and HDA19) (Chen and Wu, 2010; Luo et al., 2012b), and light signaling response (HDA6, HDA15, and HDA19) (Benhamed et al., 2006; Tessadori et al., 2009; Liu et al., 2013). Substantial evidence showed that HDACs function as transcriptional co-repressors with other regulators (Wang et al., 2014). A previous study showed that HDA15 is recruited by PIF3 to co-repress the expression of light-responsive genes by reducing H4ac levels and, thus, inhibiting chlorophyll biosynthesis and photosynthesis in the dark (Liu et al., 2013). Since PIF3 does not contribute to HDA15-mediated photomorphogenic hypocotyl growth under light conditions (Liu et al., 2013), it remains to be investigated how HDA15 functions in photomorphogenesis with other unidentified co-factors.

NUCLEAR FACTOR-Y C proteins (NF-YCs), of which core regions are closely related to histone H2A, functionally act as one subunit of the NF-Y heterotrimeric transcriptional factor (TF) that specifically recognizes and helps to establish permissive chromatin modifications at the CCAAT box containing promoters in eukaryotes (Petroni et al., 2012; Nardini et al., 2013). In plants, NF-YCs function as important regulators in various developmental and stress-related processes, including flowering-time control (NF-YC3, NF-YC4, and NF-YC9) (Kumimoto et al., 2010; Hou et al., 2014), seed germination (NF-YC3, NF-YC4, and NF-YC9) (Liu et al., 2016), abiotic stress response (NF-YAs and NF-YC2) (Hackenberg et al., 2012; Leyva-Gonzalez et al., 2012), and endoplasmic reticulum stress (NF-YC2) (Liu and Howell, 2010). The diverse roles of NF-YCs, together with those of the other two NF-Y subunits NF-YA and NF-YB, imply the widely flexible formation of NF-Y complexes spatially and temporally regulated by diverse developmental and growth conditions. It was reported that the NF-YC closet homologs *NF-YC1*, *NF-YC3*, *NF-YC4*, and *NF-YC9* were expressed in light- and dark-grown young *Arabidopsis* seedlings (Siefers et al., 2009). A most recent study demonstrated that NF-YC3, NF-YC4, and NF-YC9 function as positive regulators of photomorphogenesis in *Arabidopsis* probably via an HY5-independent light signaling pathway (Myers et al., 2016).

In this study, we show that the NF-YC homologs *NF-YC1*, *NF-YC3*, *NF-YC4*, and *NF-YC9* regulate photomorphogenic growth via histone modifications. NF-YCs physically interact with the histone deacetylase HDA15 to repress hypocotyl elongation-related gene expression in a light-dependent manner through modulating histone H4ac levels. In the dark, the DNA affinity of NF-YC-HDA15 complex to the target gene loci is reduced or abolished, resulting in increased H4ac level and consequent etiolated growth. Thus, our results establish that through interaction with HDA15 NF-YCs could function as transcriptional co-repressors of light control of hypocotyl elongation via histone deacetylation during the early seedling stage.

## RESULTS

### NF-YC Homologs Play Positive Roles in Photomorphogenic Growth

In *Arabidopsis*, *NF-YC* belongs to a multi-gene family containing 10 members. *NF-YC1*, *NF-YC3*, *NF-YC4*, and *NF-YC9* are the

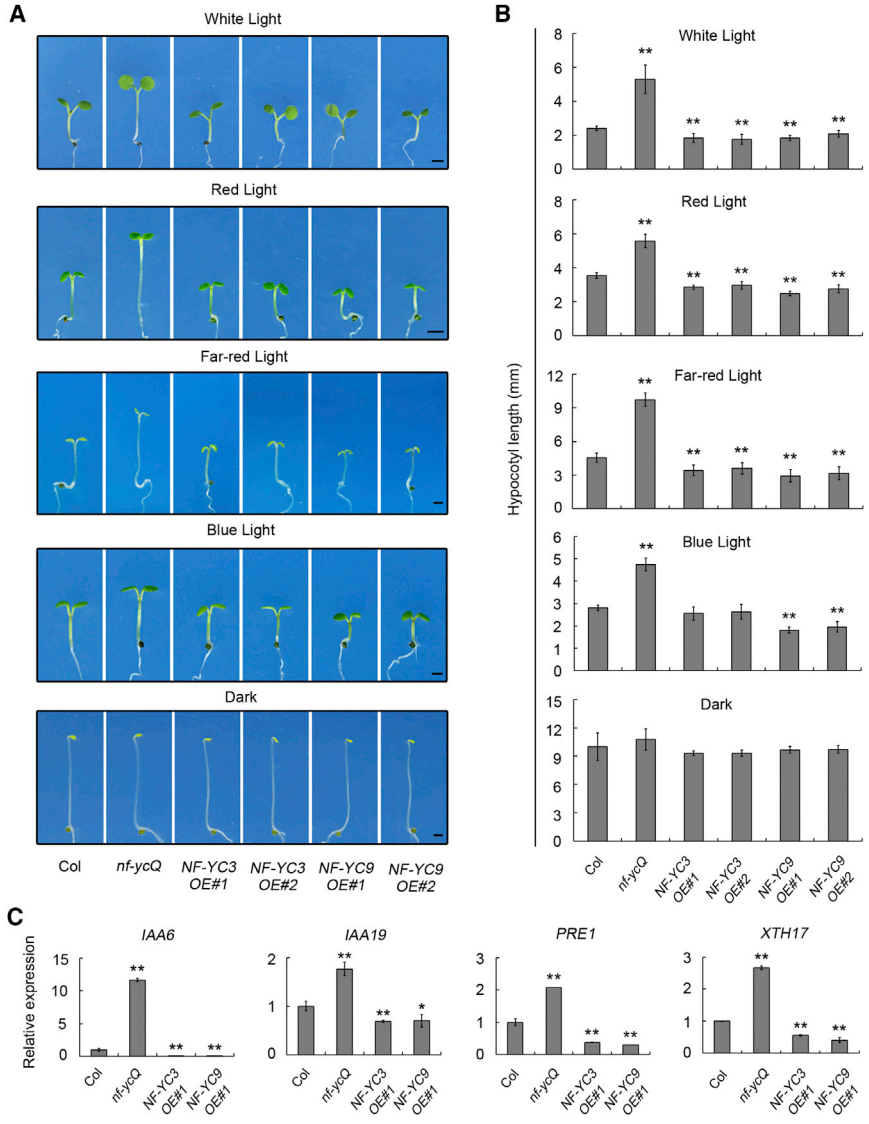


**Figure 1. NF-YCs Repress Hypocotyl Elongation under Various Light Conditions.**

**(A)** Hypocotyl phenotypes of Col, *nf-ycQ*, *NF-YC3OE*, and *NF-YC9OE* seedlings grown under red light, far-red light, blue light, or dark conditions for 4 days and white light for 7 days. *nf-ycQ* indicates *nf-yc1-1 nf-yc3-2 nf-yc4-1 nf-yc9-1* quadruple mutant; *OE#1* and *OE#2* indicate different individual overexpression lines of *NF-YC3* and *NF-YC9*. Scale bar, 1 mm.

**(B)** Hypocotyl length statistics of the seedlings shown in **(A)**. Data represent means ± SD of at least 30 seedlings. Asterisks indicate significant differences in *NF-YC* mutants and overexpression seedlings compared with Col wild-type (***P* < 0.01, Student's *t*-test).

**(C)** Relative expression analysis of hypocotyl elongation-related genes in Col, *nf-ycQ*, *NF-YC3OE*, and *NF-YC9OE* transgenic seedlings grown under red light for 2 days. Asterisks indicate significant differences in *NF-YC* mutants and overexpression seedlings compared with Col wild-type (**P* < 0.05 and ***P* < 0.01, Student's *t*-test). Relative gene expression was calculated by comparing the values with that of Col. *TUB2* was amplified as an internal control.

closest *NF-YC* homologs in the *NF-YC* family (Petroni et al., 2012). To investigate whether NF-YC function in photomorphogenesis, we analyzed the light-mediated growth phenotype of *nf-yc1-1*, *nf-yc3-2*, *nf-yc4-1*, and *nf-yc9-1* single and combinatorial T-DNA insertion mutants. There was little difference in hypocotyl elongation between all single mutants, various double mutants of *nf-yc3/4/9*, and Columbia (Col) wild-type seedlings under diverse light or dark conditions (Supplemental Figure 1A and 1B; data not shown). Strikingly, *nf-yc3-2 nf-yc4-1 nf-yc9-1* triple mutant (*nf-ycT*) and *nf-yc1-1 nf-yc3-2 nf-yc4-1 nf-yc9-1* quadruple mutant (*nf-ycQ*) seedlings exhibited longer hypocotyls than Col under white, red, far-red, or blue light conditions, while they showed no differences in the dark (Figure 1A and 1B; Supplemental Figure 1A and S1B). Considering that *nf-ycQ* had a longer hypocotyl than *nf-ycT*, which indicates a redundant function of *NF-YC1* with other *NF-YCs* in light control of growth (Supplemental Figure S1A and 1B), we hence employed *nf-ycQ* mutant to investigate NF-YC function in our subsequent experiments. To further verify the possible role of NF-YC in photomorphogenesis, we generated several individual *NF-YC3*- and *NF-YC9*-overexpressing lines to examine the hypocotyl elongation phenotype. As expected, *35S:NF-YC3-6HA* (*NF-YC3OE*) and *35S:NF-YC9-6HA* (*NF-YC9OE*) exhibited shorter hypocotyls than the wild-type in the light, whereas they showed similar phenotype in the dark (Figure 1A and 1B). Unexpectedly, *NF-YC3OE* showed no statistical difference with the wild-type in blue light. We then examined the NF-YC3/9 protein levels in *NF-YC3*- and *NF-YC9*-overexpression lines under red and blue light and found that NF-YC3 and NF-YC9 similarly expressed in different transgenic lines of *NF-YC3OE* and *NF-YC9OE*, respectively, suggesting that NF-YC3 might exert a weaker role in blue light-dependent suppression of hypocotyl than that in red and far-red light (Supplemental Figure 2). Interestingly, *nf-ycQ* seedlings grown in low-frequency red light also exhibited decreased cotyledons angles, together with elongated hypocotyls, mimicking an etiolated phenotype in the dark (Supplemental Figure 3A). Moreover, adult *nf-ycQ* plants grown under standard white light conditions showed the classic shade avoidance features including significantly elongated petioles and elevated leaf angles from horizontal compared with the normal phenotype of wild-type (Supplemental Figure 3B–3D). These observations indicate the overlapping function of *NF-YC1*, *NF-YC3*, *NF-YC4*, and *NF-YC9* in photomorphogenic growth.

To further confirm the role of NF-YCs in photomorphogenesis, we examined the expression of several hypocotyl elongation-related genes by real-time quantitative PCR (qPCR). Consistent with the seedlings phenotype, auxin-responsive genes *IAA6* and *IAA19*, GA-responsive gene *PRE1*, and the cell wall loosening enzyme

gene *XTH17* were significantly upregulated in *nf-ycQ* and downregulated in *NF-YC3OE* and *NF-YC9OE* in red light, respectively (Figure 1C). Although *nf-ycQ* seedlings only exhibited different hypocotyl length with the wild-type in the light, all of *NF-YC1*, *NF-YC3*, *NF-YC4*, and *NF-YC9* expressed in both red light- and dark-grown *Arabidopsis* seedlings (Supplemental Figure 4A and 4B). These results support that *NF-YC* homologs play positive roles in photomorphogenesis through functioning as repressors in light control of hypocotyl elongation.

## NF-YCs Interact with HDA15

It has been reported that *Arabidopsis* NF-YCs regulate flowering via direct protein-protein interaction with co-factors (Kumimoto et al., 2010; Hou et al., 2014). To test whether NF-YCs function with other partners in light control of hypocotyl growth during photomorphogenic development, we performed yeast two-hybrid screening and found that NF-YC3, NF-YC4, and NF-YC9 strongly interacted, and NF-YC1 weakly interacted with HDA15, an *Arabidopsis* histone deacetylase, in yeast (Figure 2A).

Next, we performed pull-down assays to test the *in vitro* interaction between NF-YCs and HDA15. Purified His-NF-YC recombinant proteins were incubated with glutathione *S*-transferase (GST)-HDA15 fusion protein and GST protein, respectively. The results showed that His-NF-YC1, His-NF-YC3, His-NF-YC4, and His-NF-YC9 were co-precipitated by GST-HDA15 but not by GST, demonstrating the physical interaction between NF-YCs and HDA15 (Figure 2B). When crossing *pNF-YC9*:*NF-YC9-FLAG* (*NF-YC9-FLAG*) into *nf-ycT* mutant, we found that *NF-YC9-FLAG* was sufficient to rescue the elongated hypocotyl phenotype of *nf-ycT* in the light (Supplemental Figure 5), suggesting the significant biological function of NF-YC9 in photomorphogenic growth. Hence, we hereafter used NF-YC9 as the representative of the four NF-YC homologs for further investigation.

A bimolecular fluorescence complementation (BiFC) assay was next performed in tobacco leaf epidermal cells to examine the interaction between NF-YC9 and HDA15 *in vivo*. The results showed that co-expression of $EYFP^N$-HDA15 and NF-YC9-$EYFP^C$ reconstituted a functional YFP in the nucleus, but the control did not (Figure 2C). Notably, co-immunoprecipitation of protein extracts from *nf-yc9-1 NF-YC9-FLAG 35S*:*HDA15-GFP* seedlings further confirmed that the interaction between NF-YC9 and HDA15 in plants occurred under light but not dark conditions (Figure 2D). Taken together, these results support that NF-YCs interact with HDA15 both *in vitro* and *in vivo*.

Previous study reported that the loss-of-function mutant *hda15* has a longer hypocotyl than the wild-type (Liu et al., 2013). We here confirmed the role of HDA15 in light control of hypocotyl elongation. *hda15* showed an elongated hypocotyl phenotype, while *35S*:*HDA15-GFP* had shorter hypocotyls than the wild-type, under white, red, far-red, and blue light conditions (Figure 3A and 3B). By contrast, both *hda15* and *35S*:*HDA15-GFP* exhibited no difference from the wild-type seedlings in the dark (Figure 3A and 3B). These observations imply the possibility that NF-YCs might exert function in repression of hypocotyl elongation in the light together with HDA15, thus prompting us to further investigate the biological relationship of NF-YCs and HDA15.

## NF-YCs and HDA15 Synergistically Regulate Light-Controlled Hypocotyl Elongation by Directly Binding the Promoters of Downstream Genes

To determine whether the interaction between NF-YCs and HDA15 regulates photomorphogenic growth, we first examined the mRNA levels of hypocotyl elongation-related genes under different *HDA15* genetic backgrounds. Similar to that in *nf-ycQ* and overexpression lines of *NF-YCs*, the expression of *IAA6*, *IAA19*, *PRE1*, and *XTH17* was significantly upregulated in *hda15* and downregulated in *35S*:*HDA15-GFP* under red light conditions (Figures 1C and 3C). By contrast, these gene expressions showed no difference in *hda15* and *nf-ycQ* seedlings grown in the dark (Supplemental Figure 6), consistent with the observations on hypocotyl phenotype, indicating that NF-YCs and HDA15 co-suppress hypocotyl elongation-related gene expression in a light-dependent manner.

Given that NF-Y and HDA15 have been respectively shown to mediate gene expression via association with DNA in plants (Liu et al., 2013; Cao et al., 2014), to learn how NF-YC and HDA15 co-regulate the expression of genes involved in light-responsive growth we performed a chromatin immunoprecipitation assay (ChIP) to investigate the binding enrichment of these proteins in *IAA19* and *XTH17* genes, the common downstream genes of NF-YC9 and HDA15. The results showed that NF-YC9 strongly bound to the regions at P1 and P2 in *IAA19* promoter and P2 and P3 in *XTH17* promoter in the light-grown seedlings. Similarly, enrichment of HDA15 to the promoter regions of *IAA19* and *XTH17* was also detected in *35S*:*HDA15-GFP* seedlings grown in the light (Figure 4A–4C). The chromatin affinity of NF-YC and HDA15 to the target gene loci was dramatically reduced or abolished in the dark-grown seedlings when compared with those in the light (Figure 4B and 4C). These results raise the possibility that NF-YC9 and HDA15 may co-repress hypocotyl elongation-related gene expression in the light via direct binding to promoters of these genes.

To further examine the repressive activity of NF-YCs and HDA15 on target gene expression, we performed transient expression assays using an ~2-kb fragment of *IAA19* promoter fused to the *β-glucuronidase* (*GUS*) gene as the reporter. The effector constructs of *35S*:*$EYFP^N$-HDA15* and *35S*:*NF-YC9-$EYFP^C$* were transformed individually or together with the reporter into *Arabidopsis* mesophyll protoplasts (Figure 4D). After 2 h of culture in darkness to activate the reporter gene expression, the protoplasts were then transferred to culture under red light overnight. The results showed that either $EYFP^N$-HDA15 or NF-YC9-$EYFP^C$ alone presented a significant repressive effect on the *IAA19* gene expression. Remarkably, a more dramatic decrease of GUS activity was observed when co-expressing HDA15 and NF-YC9 (Figure 4E), demonstrating that NF-YC9 and HDA15 synergistically repress target genes expression.

Because both NF-YC and HDA15 contribute to the hypocotyl elongation repression in light-grown seedlings (Figures 1 and 3), to investigate the genetic relationship between *HDA15*

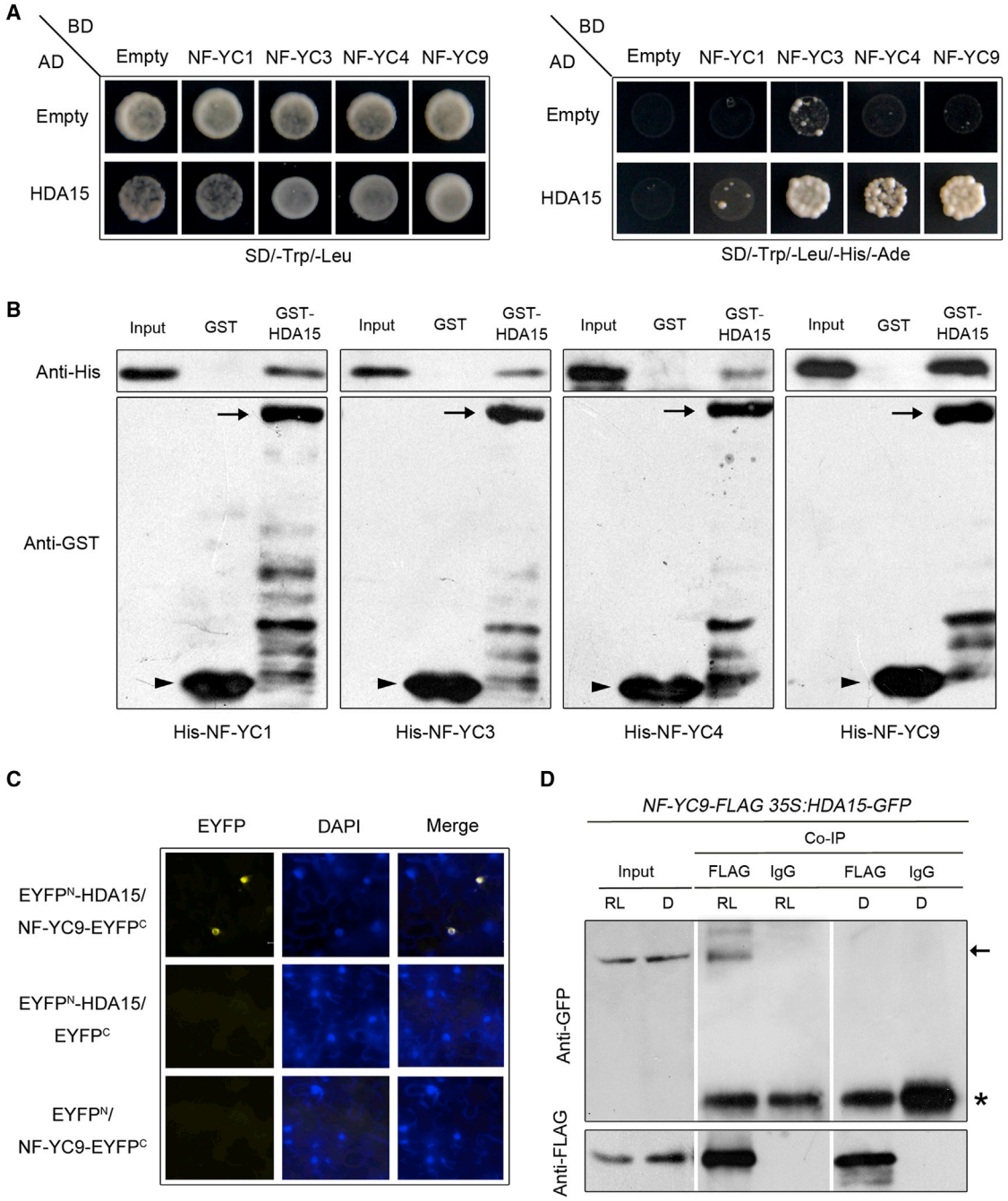


**Figure 2. NF-YCs Interact with HDA15 *In Vitro* and *In Vivo*.**

**(A)** Yeast two-hybrid assays showing the interactions between NF-YC homologs and HDA15. Transformed yeast cells were grown on SD/-Trp/-Leu/-His/-Ade and SD/-Trp/-Leu medium.

**(B)** *In vitro* pull-down assay showing the direct interaction between His-NF-YC1, His-NF-YC3, His-NF-YC4, or His-NF-YC9 and GST-HDA15 recombinant proteins. His-tagged proteins were incubated with GST or GST-HDA15 proteins and the immunoprecipitated fractions were detected by anti-His antibody. Arrows and arrowheads indicate GST-HDA15 and GST, respectively.

**(C)** BiFC analysis of the interaction between NF-YC9 and HDA15 in tobacco epidermal cells. EYFP, fluorescence of enhanced yellow fluorescent protein; DAPI, fluorescence of 4′,6-diamino-2-phenylindol, used as the nucleus indicator; Merge, merge of EYFP and DAPI.

**(D)** Co-immunoprecipitation assay of the interaction between NF-YC9 and HDA15 in plants. Plant nuclear extractions from 2-day-old *nf-yc9-1 pNF-YC9:NF-YC9-FLAG 35S:HDA15-GFP* seedlings grown under continuous red light or darkness were immunoprecipitated by anti-FLAG antibody or preiummune serum (IgG). The co-immunoprecipitated proteins were detected by either anti-GFP or anti-FLAG antibody. Arrow indicates HDA15-GFP bands and asterisk indicates non-specific bands.

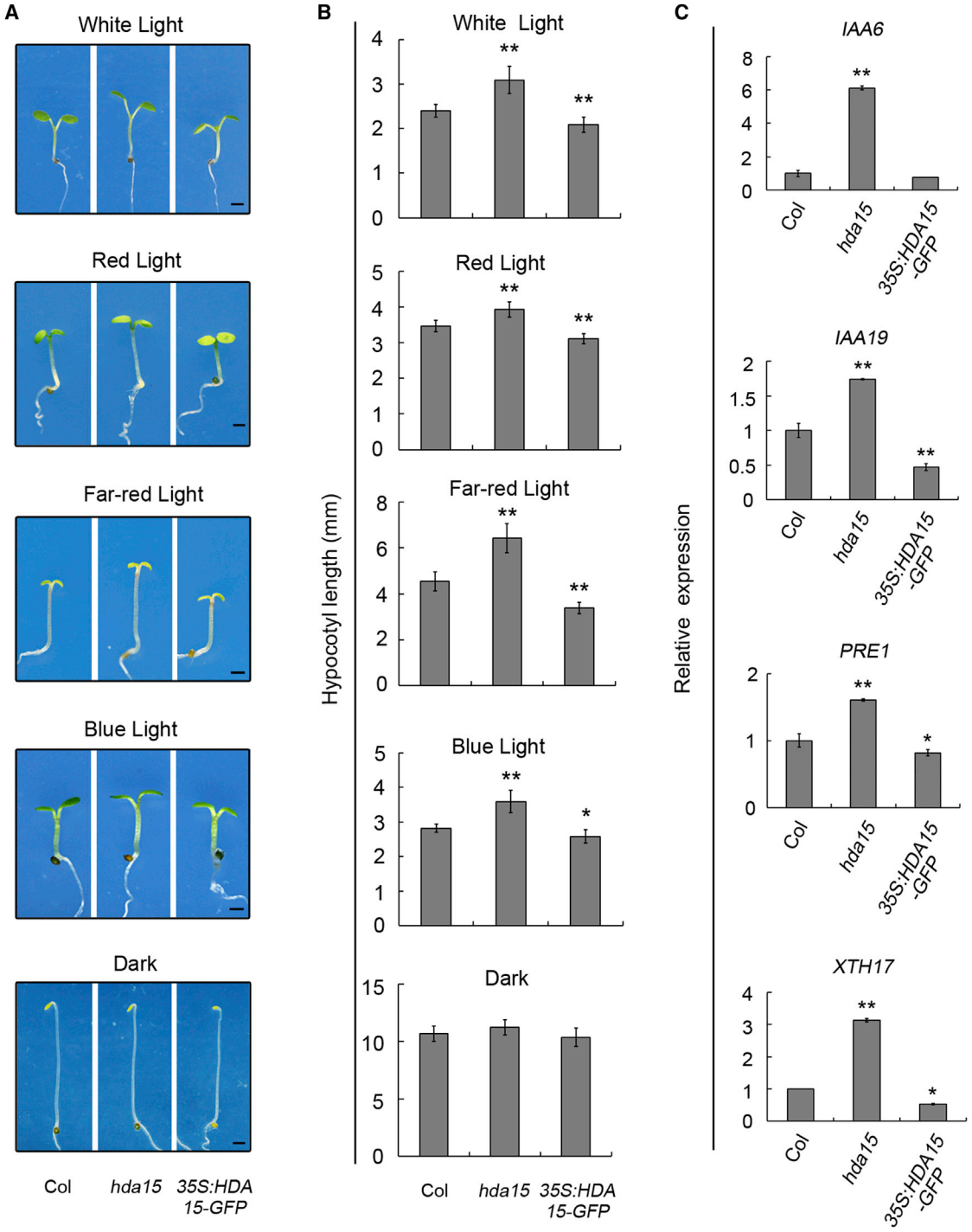


**Figure 3. HDA15 Represses Hypocotyl Elongation under Various Light Conditions.**

**(A)** Hypocotyl phenotypes of Col, *hda15*, and *35S:HDA15-GFP* seedlings grown under red light, far-red light, blue light, or dark conditions for 4 days and white light for 7 days. Scale bar, 1 mm.

**(B)** Hypocotyl length statistics of the seedlings shown in **(A)**. Data represent means ± SD of at least 30 seedlings. Asterisks indicate significant differences in *HDA15* mutants and overexpression seedlings compared with Col wild-type (*$P < 0.05$ and **$P < 0.01$, Student's *t*-test).

**(C)** Relative expression analysis of hypocotyl elongation-related genes in Col, *hda15*, and *35S:HDA15-GFP* transgenic seedlings grown under red light for 2 days. Asterisks indicate significant differences in *HDA15* mutants and overexpression seedlings compared with Col wild-type (*$P < 0.05$ and **$P < 0.01$, Student's *t*-test). Relative gene expression was calculated by comparing the values with that of Col. *TUB2* was amplified as an internal control.

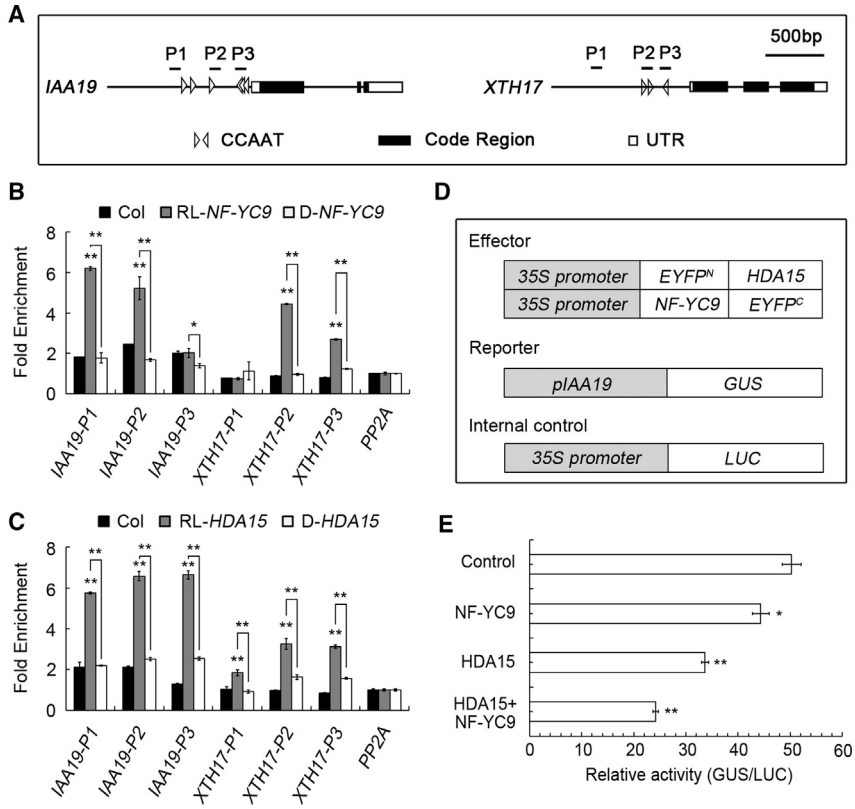


**Figure 4. NF-YCs and HDA15 Co-target to the Promoters of Hypocotyl Elongation Genes in the Light.**

**(A)** Schematic of *IAA19* and *XTH17* for ChIP–qPCR analysis. Black boxes indicate the exons and white boxes indicate the untranslated regions (UTR). P1 to P3 indicate fragments for ChIP–qPCR amplification.

**(B and C)** ChIP analysis of binding of NF-YC9 **(B)** and HDA15 **(C)** to the *IAA19* and *XTH17* promoter regions. The *nf-yc9-1 pNF-YC9:NF-YC9-FLAG* (*NF-YC9*), *35S:HDA15-GFP* (*HDA15*), and Col wild-type seedlings were grown under red light (RL) or darkness (D) for 2 days and then harvested for ChIP–qPCR analysis. Asterisks indicate significant differences in fold enrichment between RL-*NF-YC9-FLAG* or RL-*HDA15* and Col wild-type, or between the red light-grown seedlings and dark-grown seedlings, respectively (*$P < 0.05$ and **$P < 0.01$, Student's *t*-test). The values are means ± SD of triplicates.

**(D)** Schematics of the effector and reporter constructs used in transient expression assay in **(E)**.

**(E)** Transient expression assay indicating that the expression of *IAA19* is synergistically repressed by NF-YC9 and HDA15. *pIAA19:GUS* and *LUC* internal control were co-transformed with effectors or empty vector (Control) into *Arabidopsis* Col mesophyll protoplasts. Asterisks indicate significant differences compared with Control (*$P < 0.05$ and **$P < 0.01$, Student's *t*-test). After culturing in the dark for 2 h, the transformed protoplasts were transferred to red light overnight. The *IAA19* activity was calculated by relative GUS activity (GUS/luciferase [LUC]). The values are means ± SD of three biological replicates.

and *NF-YC* we generated several combinatorial lines with different *HDA15* and *NF-YC* genetic backgrounds. The double mutant *hda15 nf-ycQ* displayed an extremely elongated hypocotyl phenotype under light conditions compared with *hda15*, *nf-ycQ*, and the wild-type seedlings (Figure 5A and 5B; Supplemental Figure 7). Consistent with this, expression of the hypocotyl elongation-related genes was substantially increased (Figure 5C), confirming the synergistic regulation of HDA15 and NF-YC in inhibition of hypocotyl elongation. Furthermore, the shortened hypocotyl phenotype of *NF-YC3OE* and *NF-YC9OE* was significantly rescued by loss of function of *HDA15* under red, far-red, and blue light conditions (Figure 5A and 5B; Supplemental Figure 7). Expression of the hypocotyl elongation-related genes in *hda15 NF-YC3OE* or *hda15 NF-YC9OE* was also obviously higher than that in *NF-YC*-overexpression lines (Figure 5C), indicating that the repressive role of NF-YC on hypocotyl elongation in the light partially depends on HDA15 function.

## NF-YCs and HDA15 Co-regulate a Set of Downstream Genes Involved in Hypocotyl Elongation

To clarify the regulatory expression profile by NF-YC in the light signaling pathway, we performed transcriptomic analyses of Col wild-type and *nf-ycQ* seedlings grown under red light conditions. Based on the criteria of 1.5-fold cutoff with statistically significant *P* value of <0.05, 1000 genes were upregulated and 1067 genes were downregulated in *nf-ycQ* mutants, respectively, compared with the wild-type (Supplemental Tables 2 and 3). The genes upregulated and downregulated in *nf-ycQ* mutants are involved in a variety of biological processes, including the following gene ontology (GO) categories: response to biotic, abiotic, and endogenous stimuli, reproduction, embryonic and post-embryonic development, cell death, and some metabolic processes, such as photosynthesis, carbohydrate, lipid, and secondary metabolisms (Supplemental Figure 8).

In combination with the RNA-sequencing (RNA-seq) data of *hda15* mutant, we found that NF-YCs and HDA15 co-repressed 116 genes and co-activated 130 genes in de-etiolated seedlings, respectively (Figure 6A; Supplemental Tables 4 and 5). Bioinformatics analysis using DAVID (Huang et al., 2009) with high stringency revealed that the genes co-repressed by NF-YC and HDA15 are involved in the glycosinolate biosynthetic process, seed storage protein, leucine biosynthetic process, heme, cell wall, thioredoxin fold, water transporter activity, flavonoid biosynthetic process, and oxygen binding (Figure 6B). Notably, the hypocotyl elongation-related genes, such as *IAA6*, *IAA19*, *XTH17*, and *PRE1*, which have been identified as common downstream genes of NF-YCs and HDA15 in preceding expression analyses, were also confirmed to be upregulated in both *hda15* and *nf-ycQ* mutants in these transcriptome data. Collectively, these findings suggest that NF-YCs and HDA15 mediate light control of hypocotyl elongation by co-regulating a set of downstream genes.

## NF-YCs Modulate Histone Acetylation via HDA15

HDA15 is identified as a histone deacetylase in *Arabidopsis*, which functions together with other TFs to repress

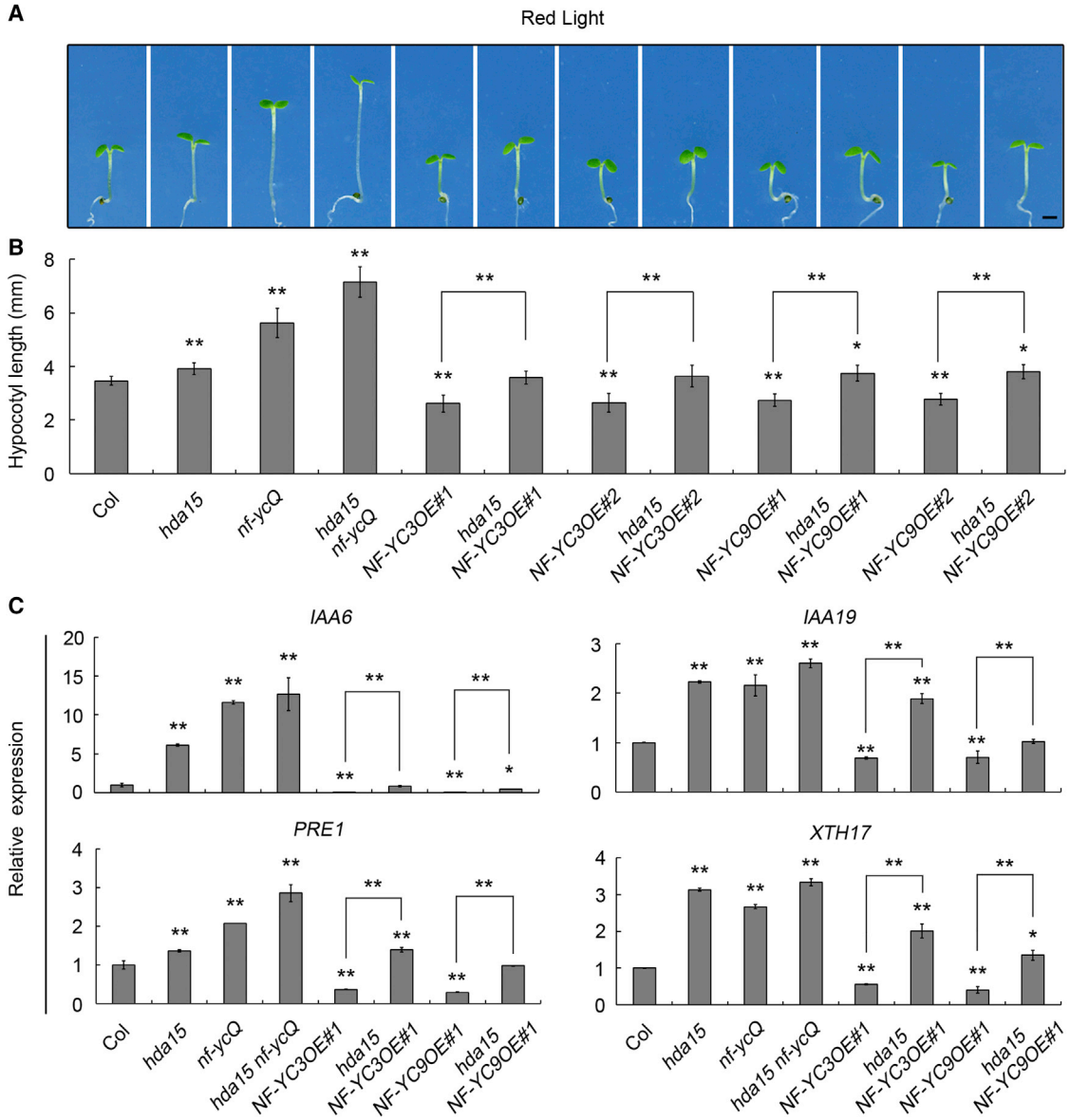


**Figure 5. Repressive Role of NF-YCs in Hypocotyl Elongation Depends on HDA15 Function.**

**(A)** Hypocotyl phenotypes of Col, *hda15*, *nf-ycQ*, *hda15 nf-ycQ*, *NF-YC3OE*, *hda15 NF-YC3OE*, *NF-YC9OE*, and *hda15 NF-YC9OE* seedlings grown under red light for 4 days. Scale bar, 1 mm.

**(B)** Hypocotyl length statistics of the seedlings shown in **(A)**. Data represent means ± SD of at least 30 seedlings. Asterisks indicate significant differences between mutants and Col wild-type, or as indicated (*$P < 0.05$ and **$P < 0.01$, Student's *t*-test).

**(C)** Relative expression analysis of hypocotyl elongation-related genes in Col, *hda15*, *nf-ycQ*, *hda15 nf-ycQ*, *NF-YC3OE*, *hda15 NF-YC3OE*, *NF-YC9OE*, and *hda15 NF-YC9OE* seedlings grown under red light for 2 days. Relative gene expression was calculated by comparing the values with that of Col. Asterisks indicate significant differences between mutants and Col wild-type, or as indicated (*$P < 0.05$ and **$P < 0.01$, Student's *t*-test). *TUB2* was amplified as an internal control.

transcription through removing acetyl groups from lysine residues of the histone tail (Liu et al., 2014). We thus examined whether interaction between NF-YCs and HDA15 are involved in histone acetylation modification in their direct targets. ChIP assays were performed to evaluate the distribution of H4ac, a favored substrate of HDA15 in *Arabidopsis* (Liu et al., 2013). The H4ac levels in *IAA19* and *XTH17* chromatins were increased in red light-grown *hda15* seedlings compared with those in the wild-type (Figure 7A). Strikingly, high deposition of H4ac in these target loci was similarly observed in *nf-ycQ* and *hda15 nf-ycQ* under red light conditions. By contrast, deposition of H4ac in dark-grown *hda15* and *nf-ycQ* seedlings was significantly reduced or even abolished compared with those grown in red light (Figure 7A and 7B). Consistent with the increasing expression of NF-YC and HDA15 target genes and the elongated hypocotyl phenotype in these mutants under light conditions (Figure 5), the results suggest the possibility that NF-YC and HDA15 play repressive roles on gene transcription probably by mediating H4ac modification.

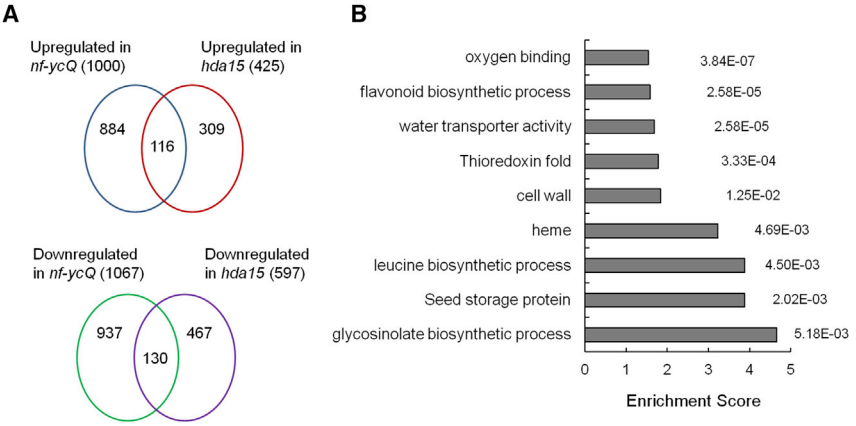


**Figure 6. Genome-wide Transcriptomic Analysis of NF-YCs and HDA15 Co-regulated Genes.**

**(A)** Venn diagrams show the overlaps of the co-repressed and co-activated genes by NF-YC and HDA15. The overlaps are significantly enriched ($\chi^2$ test, $P < 0.01$).

**(B)** DAVID functional clustering of NF-YCs and HDA15 co-regulated genes ($P < 0.01$).

To verify this hypothesis, we measured the H4ac levels in *NF-YC* overexpression transgenic seedlings. In contrast to the intensive deposition of H4ac in *nf-ycQ*, overexpression of *NF-YC9* dramatically attenuates H4ac levels of *IAA19* and *XTH17* chromatin in the light (Figure 7A and 7C). Interestingly, loss of function of *HDA15* completely restored the reduced H4ac levels in *NF-YC9OE* seedlings (Figure 7C), indicating that the effect of NF-YCs on H4 deacetylation depends on HDA15 function. Together with the expression and genetic analyses (Figure 5 and Supplemental Figure 7), these results strongly support the conclusion that NF-YCs and HDA15 co-repress light control of hypocotyl elongation by mediating histone acetylation on target loci in the light.

## DISCUSSION

NF-YC serves as one of subunits of the NF-Y TF complex, which is composed of three subunits, NF-YA, NF-YB, and NF-YC, and plays diverse roles together with transcriptional factors in various developmental processes including flowering, root growth, embryogenesis, and abiotic stress response (Laloum et al., 2013). Several pieces of evidence showed that NF-YC genes are involved in light-mediated gene regulation (Warpeha et al., 2007; Stephenson et al., 2010). In particular, a most recent study reported that *Arabidopsis* NF-YC3, NF-YC4, and NF-YC9 play redundant positive roles in photomorphogenesis (Myers et al., 2016). However, the detailed regulatory mechanism of how NF-YCs function in photomorphogenesis so far remains unidentified. Here, we showed that the four *NF-YC* homologs *NF-YC1*, *NF-YC3*, *NF-YC4*, and *NF-YC9* mediate light control of hypocotyl elongation during early seedling stage by interacting with HDA15, an RPD3/HDA1-type HDAC. HDA15 acts as a co-repressor to regulate gene expression in multiple development processes by modulating chromatin structure status (Ma et al., 2013). Similar to *hda15*, loss of function of *NF-YCs* exhibits elongated hypocotyl, while *NF-YC* overexpression inhibits hypocotyl growth, under various light conditions. Based on the findings in this study, we propose a model describing that in the light, the NF-YC subunits of NF-Y complex interact with HDA15, and recognize the promoter of hypocotyl elongation-related genes, such as *IAA19* and *XTH17*, repressing their expression via H4 deacetylation and thus promoting photomorphogenic growth. In the dark, HDA15 is dismissed from the target genes, resulting in elevated H4ac levels in these loci. The "opened" chromatin subsequently recruits the dark-related TFs to promote the expression of hypocotyl elongation-related genes and etiolated growth (Figure 7D). Thus, our findings elucidate that NF-YCs promote photomorphogenic growth via a chromatin modification.

It is well documented that epigenetic regulation plays fundamental roles in most biological processes including photomorphogenesis (Wu, 2014). Among them, histone acetylation is a reversible chromatin modification mediated by HATs and HDACs. HAT is considered one of the major factors that facilitate the chromatin relaxation and subsequent gene transcription activation, while HDAC has a reverse function (Liu et al., 2014). Studies of HAT and HDAC in *Arabidopsis* reveal the role of histone acetylation in light-activated gene expression. Two HATs, GCN5 and HAF2, both function in the HY5-related pathway to regulate light-responsive gene expression in plant development (Bertrand et al., 2005; Benhamed et al., 2008). The *HDAC* gene *HDA19* is involved in the negative regulation of light-responsive genes (Benhamed et al., 2006), while HDA6 acts as positive regulator of light-controlled chromatin compaction (Tessadori et al., 2009). HDA15, belonging to the RPD3/HDA1 superfamily together with HDA6 and HDA19, was reported to regulate chlorophyll biosynthesis and photosynthesis in the dark via PIF3 (Liu et al., 2013). Dark-accumulated PIF3 recruits HDA15 via protein interaction and then represses gene expression by decreasing the histone acetylation levels, while the interaction between HDA15 and PIF3 is dissociated under light conditions due to the light-triggered degradation of PIF3 proteins (Liu et al., 2013). However, loss-of-function mutant and overexpression lines of *HDA15* exhibit PIF3-independent changes on hypocotyl growth in the light (Liu et al., 2013; Figure 3), suggesting a novel role of HDA15 in light-mediated hypocotyl elongation. Our findings shed light on such a mechanism whereby NF-YCs associate with HDA15 to co-repress hypocotyl elongation in a light-dependent manner. Interestingly, the protein levels of HDA15 were reported to be constant during dark–light transition (Liu et al., 2013), but another study described that light drives the nucleocytoplasmic shuttling of HDA15 in hypocotyls (Alinsug et al., 2012). The possibility that the light-induced translocation of HDA15 facilitates NF-YC function in photomorphogenesis should be considered.

The NF-Y complex recognizes the CCAAT box in promoter through the NF-YA subunit to regulate gene expression in eukaryotes (Laloum et al., 2013). Here our ChIP analysis showed that NF-YC9 and HDA15 were associated with CCAAT box-containing regions in promoters of *IAA19* and *XTH17*, the targets of

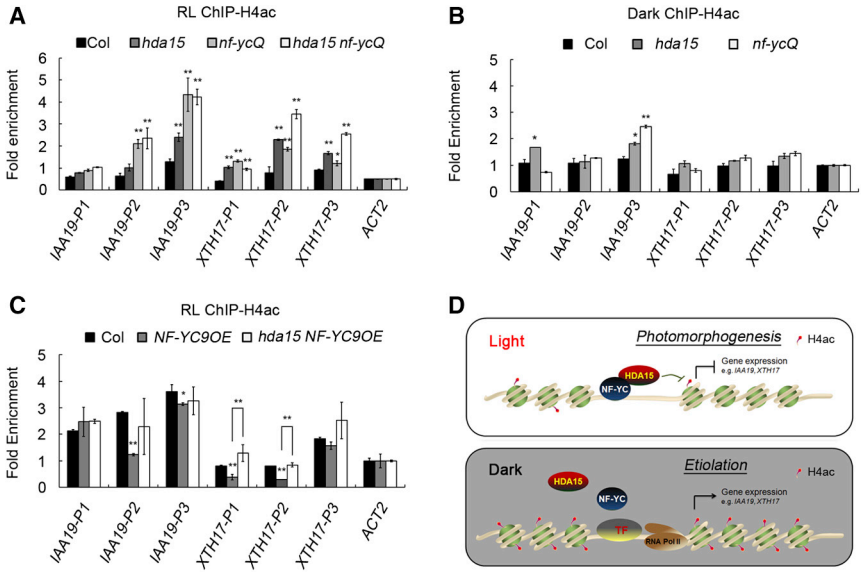


**Figure 7. NF-YCs and HDA15 Modulate H4 Acetylation Levels in the Light.**

**(A and B)** ChIP analysis of H4ac levels in *IAA19* and *XTH17* genes in Col, *hda15*, *nf-ycQ*, and *hda15 nf-ycQ* mutants under red light **(A)** and darkness **(B)**. Asterisks indicate significant differences in fold enrichment between mutants and Col wild-type (*$P < 0.05$ and **$P < 0.01$, Student's *t*-test).

**(C)** ChIP analysis of H4ac levels at the promoters of *IAA19* and *XTH17* in Col, *NF-YC9OE#1* (*NF-YC9OE*), and *hda15 NF-YC9OE#1* (*hda15 NF-YC9OE*) seedlings under red light. ChIP assay was performed using seedlings grown under red light or darkness for 2 days. Asterisks indicate significant differences in fold enrichment between *NF-YC9OE* and Col wild-type, or between *NF-YC9OE* and *hda15 NF-YC9OE* (*$P < 0.05$ and **$P < 0.01$, Student's *t*-test). Values are means ±SD of triplicates.

**(D)** Hypothetical model of NF-YCs and HDA15 cooperatively regulation in light control of cell elongation. In the light, NF-YC subunits of NF-Y complex interact with HDA15, and recognize the promoters of hypocotyl elongation-related genes, such as *IAA19* and *XTH17*, thus repressing their expression via H4 deacetylation and promoting photomorphogenic growth. In the dark, HDA15 is dissociated from the target genes, resulting in elevated H4ac levels in these loci. The "open" chromatin subsequently recruits the dark-related transcriptional factors (TF) to promote the expression of hypocotyl elongation-related genes and etiolated growth.

NF-YC and HDA15 (Figure 4A–4C), suggesting that NF-YC appears to work together with NF-YA and NF-YB as NF-Y trimer, and recruits HDA15 to regulate light-responsive gene expression. According to previous knowledge on the regulatory mode of NF-Y (Laloum et al., 2013), it is possible that other light-responsive TFs might be also involved in such regulation. The fact that NF-YC and HDA15 play similar roles under different light conditions to mediate hypocotyl elongation indicates their function as general negative regulators in light control of hypocotyl elongation. HY5 is the master positive regulator in photomorphogenic growth. Light triggers red/far-red/blue photoreceptors shuttling into the nucleus and suppressing COP1, which promotes HY5 accumulation through preventing COP1-mediated degradation by proteasome (Jiao et al., 2007). Remarkably, HY5 shares a significant portion of targets, including *IAA19* and *XTH17*, with NF-YCs and *HDA15* (Jing et al., 2013). Although a recent study reported a physical interaction between NF-YC9 and HY5 by FRET-FLIM analysis, those authors proposed that NF-YCs likely represent a parallel light signaling pathway independent of HY5 since *nf-yc3 nf-yc4 nf-yc9 hy5* mutants had significant synergistic defects in light control of hypocotyl growth, including both cell elongation and cell division (Myers et al., 2016). Instead, COP1-mediated degradation might be involved in the regulation of NF-Y (Myers et al., 2016). Therefore, it is still doubtful whether the regulation of NF-YC and HDA15 in hypocotyl elongation-related genes needs HY5 or not. In addition, since HY5 is degraded in the dark, and PIFs act as the main positive regulators of skotomorphogenesis through competing with HY5 to recognize the same targets (Toledo-Ortiz et al., 2014), PIFs might play the potential role of TFs in the dark described in the model (Figure 7D). These hypotheses need further investigation.

NF-Y, with nucleosome-like protein properties, has been shown to be important for the establishment of histone post-translational modifications such as methylation and acetylation on active promoters through recruitment of relevant enzymes (Dolfini et al., 2012; Nardini et al., 2013). Previous studies mainly focused on the roles of NF-Y in flowering control and abiotic stress response in plants, but NF-Y function and the relevant epigenetic regulation in photomorphogenesis remain obscure. We demonstrated that NF-YCs regulate light-dependent hypocotyl growth inhibition via HDA15-mediated histone H4 deacetylation. Consistent with this, the genome-wide analysis revealed that NF-YCs and HDA15 co-regulate a subset of downstream genes in the light. However, the NF-YC-regulated transcriptome profile also contains a significant number of genes unregulated by HDA15, which are either upregulated or downregulated by NF-YCs. Previous study has shown that NF-Y directly activates the expression of *SOC1*, an important flowering activator, partly through the H3K27 demethylase REF6 (Hou et al., 2014). We therefore suppose that NF-YC may function together with different epigenetic factors to play a dual role in transcriptional repression or activation during various developmental stages. It is intriguing to examine whether NF-YC also mediates photomorphogenic growth via histone methylation modification. A previous study reported that PICKLE (PKL) directly regulates the expression of hypocotyl elongation-related genes in photomorphogenesis, including *IAA19* and *XTH17* (Jing et al., 2013). *PKL* encodes an ATP-dependent chromatin remodeling factor. In the light, PKL interacts with HY5 and represses gene expression by depositing more histone H3 lysine 27 (H3K27) trimethylation marks in targeted chromatin (Jing et al., 2013). Further investigation of the possible relationship between NF-Y and PKL will provide insights into understanding how TFs control plant development response to light via chromatin modification.

We previously reported that PIF3-HDA15 co-represses the expression of chlorophyll biosynthesis- and photosynthesis-related genes in the dark (Liu et al., 2013). Notably, light-grown *nf-ycQ* seedlings exhibited etiolated phenotypes except a normal chlorophyll biosynthesis. As light triggers degradation of PIF3

(Leivar and Monte, 2014), it is plausible that HDA15 and NF-YCs are probably not involved in chlorophyll biosynthesis in the light. Apart from the etiolated phenotypes, including elongated hypocotyls and reduced cotyledon angles, in early light-grown seedlings of *nf-ycQ* remarkably longer petioles and elevated leaf angles from the horizontal were also observed in *nf-ycQ* adult plants compared with the wild-type, indicating that NF-YCs might be involved in the regulation of shade avoidance (Supplemental Figure 3A–3D). However, while *hda15* exhibited similar elongated hypocotyls with *nf-ycQ* during the early seedling stage, we did not observe the shading syndrome in adult *hda15* plants. This indicates that HDA15 is not involved in the NF-YC-mediated shade avoidance response. When plants are grown under shaded conditions, the photoreceptor phyB perceives less ratio of red/far-red and converts to the inactive form, thus maintaining the stabilization of PIFs (Pierik and de Wit, 2014; Pacin et al., 2016). In addition, shading signals are also perceived by the blue light photoreceptor CRY1, which triggers the degradation of HY5 (Pedmale et al., 2016). These changes in TF stability induce a series of gene expressions, conferring in plants accelerated growth of the hypocotyls, stems, and leave petioles, increased leaf hyponasty, and reduced shoot branching (Liu et al., 2011). Whether and how NF-YCs function with these TFs in the shade avoidance response remains to be investigated in the future.

## METHODS

### Plant Materials and Growth Conditions

All *Arabidopsis thaliana* plants used in this study are in Col background. *nf-yc1-1* (SALK_086334), *nf-yc4-1* (SALK_032163), *nf-yc9-1* (SALK_058903), and *hda15* (SALK_004027) were obtained from the Arabidopsis Biological Resource Center (http://www.arabidopsis.org/), while nf-yc3-2 (GK_051E10) was obtained from the European Arabidopsis Stock Center (http://arabidopsis.info/). Transgenic lines of *35S:NF-YC3-6HA*, *35S:NF-YC9-6HA*, *nf-yc9-1 pNF-YC9:NF-YC9-FLAG*, and *35S:HDA15-GFP* were described previously (Liu et al., 2013; Hou et al., 2014).

Surface-sterilized *Arabidopsis* seeds were plated on half-strength Murashige–Skoog (1/2 MS) medium containing 0.3% sucrose and 0.8% agar. After incubation for 3 days at 4°C in the dark, the seeds were irradiated with 6 h white light (100 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for germination, then were grown under continuous red light (660 nm, 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$), far-red light (740 nm, 5 $\mu mol \cdot m^{-2} \cdot s^{-1}$), blue light (470 nm, 5 $\mu mol \cdot m^{-2} \cdot s^{-1}$), or darkness at 22°C. The adult plants were grown in soil under full-spectrum white fluorescent light (16 h light/8 h dark) with a frequency of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 22°C.

### Phenotypic Analysis

The seedlings grown under various light conditions as indicated were photographed with a digital camera. The hypocotyl length, cotyledon angle, petiole length, and leaf angle from the horizontal soil surface were measured using ImageJ software (http://imagej.nih.gov/ij/). For petiole length measurement, the largest fully expanded rosette leaf (leaf 5 and leaf 6) were excised from each plant at day 21. Data were collected from at least 30 seedlings per genotype per treatment.

### GUS Staining Assay

For GUS staining experiments, seedlings were grown under continuous red light for 4 days and harvested to incubate in GUS staining solution (50 mM $NaPO_4$ (pH 7.0), 0.5 mM $K_3Fe(CN)_6$, 0.5 mM $K_4Fe(CN)_6$, and 2 mM X-Gluc 5-bromo-4-chloro-3-indolyl-b-D-glucuronic acid) at 37°C overnight. The stained tissues were then maintained in destaining solution (70% ethanol and 30% ethanoic acid) for observation. Pictures were taken with a Leica M165C stereoscope.

### RNA Extraction and Real-Time qPCR Analysis

Total RNA from various seedlings grown under indicated growth conditions was isolated using the E.Z.N.A. Total RNA Kit I (Omega) and reverse transcribed to cDNA using MMLV-RTase (Promega). The transcript level was determined by real-time qPCR with KAPA SYBR Fast qPCR Kit Master Mix (KAPA BIO) and a LightCycler 480 thermal cycler (Roche). The relative quantification method ($2^{-\Delta\Delta Ct}$) was used to evaluate quantitative variation between the replicates. Each sample was quantified at least in triplicate and normalized using *TUB2* as an internal control. All primers used are listed in Supplemental Table 1.

### Yeast Two-Hybrid Assay

The coding regions of *NF-YC1*, *NF-YC3*, *NF-YC4*, *NF-YC9*, and *HDA15* were amplified and subcloned into pGBKT7 and pGADT7 vectors. Yeast two-hybrid assay was performed using the Yeastmaker Yeast Transformation System 2 (Clontech). For library screening, the *BD-NF-YC9* was chosen as bait to screen an *Arabidopsis* cDNA library (CD4-30, from ABRC) (Fan et al., 1997). Yeast AH109 cells were co-transformed with specific bait and prey constructs and grown on SD/-Trp/-Leu/-His/-Ade medium for screening or interaction test.

### Pull-Down Assay

The coding regions of *HDA15*, *NF-YC1*, *NF-YC3*, *NF-YC4*, and *NF-YC9* were cloned into pGEX-4T-1 (Pharmacia) and pQE30 (Qiagen) vectors to produce GST-HDA15, His-NF-YC1, His-NF-YC3, His-NF-YC4, and His-NF-YC9 proteins, respectively. Primers used for the construction are listed in Supplemental Table 1. These recombinant proteins were then expressed in *Escherichia coli* Rosetta cells and purified using Glutathione Sepharose beads (Amersham Biosciences) or Ni–nitrilotriacetic acid agarose beads (Qiagen). The purified His-NF-YC1, His-NF-YC3, His-NF-YC4, and His-NF-YC9 proteins were incubated with immobilized GST or GST-HDA15 in binding buffer (50 mM Tris-HCl [pH 8.0], 150 mM NaCl, 1 mM EDTA. and 1% Triton X-100) at 4°C overnight. After being washed three times with binding buffer, proteins retained on beads were resolved in 5× SDS loading buffer by boiling for 5 min and separated by 12% SDS–PAGE for immunoblotting with anti-GST (Abcam) and anti-His (Abcam) antibodies.

### BiFC Analysis

The coding regions of *HDA15* and *NF-YC9* were amplified and subcloned into YN vector (pEarleyGate201-YN) and a pGreen binary vector HY105 containing the C-terminal of EYFP as described previously, respectively (Liu et al., 2013; Hou et al., 2014). The constructs were co-transformed into tobacco (*Nicotiana tabacum*) leaf epidermal cell by infiltrated with *Agrobacterium tumefaciens* strains (GV3101-psoup). After infiltration, tobacco was grown for 60 h before detection of the YFP signals using an inverted fluorescence microscope (Leica). DAPI (4′,6-diamidino-2-phenylindole) staining was used as the nucleus indicator.

### Co-immunoprecipitation Assay

The *nf-yc9-1 pNF-YC9:NF-YC9-FLAG 35S:HDA15-GFP* seedlings were grown in continuous red light or darkness for 2 days. These seedlings were ground and washed several times by wash buffer (50 mM potassium phosphate [pH 7.0], 100 mM NaCl, 10 mM β-mercaptoethanol, 1 M hexylene glycol, 10 mM $MgCl_2$, and 0.5% Triton X-100). After centrifugation, nuclear proteins were extracted by further washing with a resuspension buffer (10 mM potassium phosphate [pH 7.0], 100 mM NaCl, 10 mM EDTA [pH 8.0], and 0.5% sarkosyl). Nuclear proteins were then incubated with anti-FLAG antibody (Sigma) or preimmune serum (immunoglobulin G [IgG]) in the co-immunoprecipitation buffer (50 mM HEPES [pH 7.5], 150 mM KCl, 10 μM $ZnSO_4$, 5 mM $MgCl_2$, and 1% Triton X-100) at 4°C

overnight. After incubation, the beads were washed three times before eluted with 5× SDS loading buffer. The immunoprecipitated proteins were separated by SDS–PAGE gel and detected by anti-GFP (Abcam) and anti-FLAG (Sigma) antibodies.

### Transient Expression Assay

The reporter construct *pIAA19-GUS* was described previously (Huang et al., 2015). *35S:EYFP$^N$-HDA15* and *35S:NF-YC9-EYFP$^C$* were used as the effector constructs, and a construct containing the firefly luciferase (LUC) driven by 35S promoter in pGreen-35S was used as an internal control to evaluate the protoplast transfection efficiency. The reporter, effector, and internal control plasmids were co-transformed into *Arabidopsis* mesophyll protoplast. After culturing in darkness for 2 h to express reporter gene and then transfer to the red-light conditions overnight, the GUS activity was determined by measuring the content of 4-methylumbelliferone, which is catalyzed by GUS enzyme using 4-methylumbellif-cryl-β-D-glucuronide as a substrate. The LUC activity was detected with a Multiscan Spectrum using Luciferase Assay System (Promega). The GUS/LUC ratio was presented as the relative reporter gene expression.

### ChIP–qPCR Assay

ChIP assays were performed as previously described (Hou et al., 2014). Seedlings (0.5 g) grown in red light or darkness for 2 days were collected for fixation by 1% formaldehyde, and chromatin was extracted and sheared to an average DNA fragment size to an average size of ~500 bp by sonication. The sonication chromatins were immunoprecipitated by Protein G PLUS agarose (16-201, Millipore) with anti-FLAG (Sigma) and anti-H4ac (Abcam) antibodies or GFP-Trap (ChromoTek), and the precipitated DNA fragments were recovered and analyzed by qPCR with SYBR Premix ExTaq Mix (Takara Bio) using specific primers (Supplemental Table 1). Relative fold enrichment was calculated by normalizing the amount of a target DNA fragment against that of a *PP2A* or *ACT2* genomic fragment and then against the respective input DNA samples.

### Transcriptomic Analysis

Seedlings of Col wild-type and *nf-ycQ* were grown on 1/2 MS plates under continuous red light for 2 days before harvesting. Total RNA was isolated as described above. The RNA-seq library was constructed according to the manuals as provided by Illumina and sequenced on HiSeq2000. DEGs were defined as genes with a false discovery rate of <0.05 and with at least a 1.5-fold difference in expression level. Three valid biological replicates were used for the transcriptomic analysis. GO analysis was performed using the BiNGO software suite (www.psb.ugent.be/cbd/papers) with default parameters (Maere et al., 2005). The enrichment significance of HDA15 and NF-YCs co-regulated genes was determined by $\chi^2$ test analysis as previously described (Zhang et al., 2015).

## ACCESSION NUMBERS

Sequence data from this article can be found in the Arabidopsis Genome initiative or GenBank/EMBL databases under the following accession numbers: *NF-YC1* (AT3G48590), *NF-YC3* (AT1G54830), *NF-YC4* (AT5G63470), *NF-YC9* (AT1G08970), *HDA15* (AT3G18520), *IAA6* (AT1G52830), *IAA19* (AT3G15540), *PRE1* (AT5G39860), *XTH17* (AT1G65310), *TUB2* (AT5G62690), *PP2A* (AT1G69960), and *ACT2* (AT3G18780). The original expression profiling data have been deposited in the Gene Expression Omnibus database under accession number GEO: GSE89850 and in NCBI's Biosample (accession numbers: SAMN05920288, SAMN05920290, SAMN05920292, SAMN05920293, SAMN05920295, and SAMN05920296; http://www.ncbi.nlm.nih.gov/biosample/5920296).

## SUPPLEMENTAL INFORMATION

Supplemental Information is available at *Molecular Plant Online*.

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## AUTHOR CONTRIBUTIONS

Y.T., Xuncheng Liu, and X.H. designed the research. Y.T., Xu Liu, and Y.L. performed research. Y.T., Xuncheng Liu, K.W., and X.H. analyzed data. Y.T. and X.H. wrote the paper.

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