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RESEARCH ARTICLE

COR27 and COR28 are Novel Regulators of the COP1–HY5 Regulatory Hub and Photomorphogenesis in Arabidopsis

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Short title: COR27 and COR28 Regulate Photomorphogenesis

One-sentence summary: COR27 and COR28 act as key regulators in the COP1–HY5 regulatory hub by regulating HY5 activity to ensure proper skotomorphogenic growth in the dark and photomorphogenic development in the light.

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ABSTRACT

Plants have evolved sensitive signaling systems to fine-tune photomorphogenesis in response to changing light environments. Light and low temperatures are known to regulate the expression of the *COLD REGULATED* (*COR*) genes *COR27* and *COR28*, which influence the circadian clock, freezing tolerance, and flowering time. Blue light stabilizes the COR27 and COR28 proteins, but the underlying mechanism is unknown. We therefore performed a yeast two-hybrid screen using COR27- and COR28 as bait, and identified the E3 ubiquitin ligase CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) as an interactor. COR27 and COR28 physically interact with COP1, which is in turn responsible for their degradation in the dark. Furthermore, COR27 and COR28 promote hypocotyl elongation and act as negative regulators of photomorphogenesis in Arabidopsis. Genome-wide gene expression analysis showed that HY5, COR27, and COR28 co-regulate many common genes. COR27 interacts directly with HY5 and associate with the promoters of the HY5 target genes *HY5* and *PIF4*, and regulates their transcription together with HY5. Our results demonstrate that COR27 and COR28 act as key regulators in the COP1–HY5 regulatory hub, by regulating the transcription of HY5 target genes together with HY5 to ensure proper skotomorphogenic growth in the dark and photomorphogenic development in the light.

INTRODUCTION

Light is critical for plants, not only as an energy source for photosynthesis, but also because it regulates the plant development program known as photomorphogenesis. In Arabidopsis (*Arabidopsis thaliana*), at least five types of photoreceptors are involved in the regulation of overlapping physiological functions essential to plant development, such as de-etiolation and photoperiodic flowering. The main photoreceptors include blue light photoreceptors, known as cryptochromes (CRYs) (Lin, 2002); the red/far-red light photoreceptors, called phytochromes (Phys) (Quail, 2002); the blue light/UV-A photoreceptors phototropin (PHOTs) (Briggs and Christie, 2002); the LOV-domain/F-box proteins ZEITLUPE (ZTL), FLAVIN BINDING, KELCH REPEAT, F-BOX PROTEIN 1 (FKF), and LOV KELCH PROTEIN2 (LKP2) (Demarsy and Fankhauser, 2009); and the UV-B photoreceptor UV RESISTANCE LOCUS 8 (UVR8) (Rizzini et al., 2011).

Photomorphogenesis is critical for seedling development. Buried seeds will germinated into etiolated seedlings that develop long hypocotyls, keep their cotyledons closed, and maintain curved apical hooks to emerge from the soil unscathed. However, once they reach the soil surface, hypocotyl elongation is inhibited while cotyledons quickly expand in response to light (Sullivan and Deng, 2003). Mnay key factors have been reported to be involved in de-etiolation. CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) is a RING finger E3 ubiquitin ligase that acts downstream of the Phys, CRYs, and UVR8 (Ang and Deng, 1994; Christie et al., 2012). COP1 is responsible for the degradation of various photomorphogenesis-promoting transcription factors in the dark, including the bHLH transcription factor LONG HYPOCOTYL IN FAR RED 1 (HFR1) and the basic leucine-zipper (bZIP) factor ELONGATED HYPOCOTYL 5 (HY5) (Yi and Deng, 2005; Jiao et al., 2007; Foreman et al., 2011; Liu et al., 2011), thus promoting skotomorphogenesis (seedling development in the dark) (Lau and Deng, 2012). Arabidopsis *cop1* mutant seedlings exhibit a constitutive photomorphogenic phenotype, with expanded cotyledons and short hypocotyls even when grown in constant darkness (Deng et al., 1991). The COP1-related protein SUPPRESSOR OF PHYTOCHROME A (SPA1) interacts with COP1 to positively regulate COP1 activity, whereas CRYs and Phys suppress the E3 ubiquitin ligase activity of COP1 by forming a complex with SPA1 and COP1 in a light–dependent manner (Deng et al., 1991; Lian et al., 2011; Liu et al., 2011; Zuo et al., 2011).

The perception of light by the photoreceptors results in the regulation of the activity of many transcription factors. For example, CRYs interact with the transcription factors CRYPTOCHROME-INTERACTING BASIC-HELIX-LOOP-HELIX 1 (CIB1) and PHYTOCHROME-INTERACTING FACTOR (PIF) PIF4/ and PIF5 to regulate transcription (Liu et al., 2008; Liu et al., 2013a; Liu et al., 2013b; Ma et al., 2016; Pedmale et al., 2016). Similarly, Phys also interact with PIFs to regulate transcription (Ni et al., 1998; Leivar and Quail, 2011). HY5 promotes photomorphogenesis downstream of CRYs, Phys, and UVR8, and plays a critical role during de-etiolation (Jiao et al., 2007). In the dark, HY5 is a target of COP1 and is degraded via the proteasome, but remains highly abundant in the light, as COP1 is repressed by the photoreceptors (Osterlund et al., 2000; Hoecker, 2017; Podolec and Ulm, 2018). HY5 positively or negatively regulates the expression of over 3,000 genes, a large fraction of which are involved in photomorphogenesis (Zhang et al., 2011). HY5 is also involved in a positive feedback loop promoting *COP1* transcription by binding to its promoter (Huang et al., 2012). HY5 and the related HY5-HOMOLOGY (HYH) proteins interact directly with a T/G-box cis-acting element within the *HY5* promoter, activating its transcription in response to visible light and UV-B (Binkert et al., 2014). It was reported very recently that the primary activity of HY5 is to promote transcription and that this function relies on other, likely light-regulated, factors (Bischof, 2020; Burko et al., 2020).

COLD-REGULATED GENE 27 (*COR27*) and *COR28* were identified as cold-responsive genes in Arabidopsis transcriptome profiling studies (Fowler and Thomashow, 2002; Mikkelsen and Thomashow, 2009). The expression of *COR27* and *COR28* is regulated by both low temperatures and light, and represents a trade-off between flowering and freezing tolerance, as the *cor27* and *cor28* mutants show delayed flowering and increased resistance to cold stress (Li et al., 2016). Furthermore, COR27 and COR28 are involved in regulating the period length of the circadian clock and associate with the chromatin regions surrounding the clock genes *PSEUDO-RESPONSE REGULATOR 5* (*PRR5*) and *TIMING OF CAB2 EXPRESSION 1* (*TOC1*) to regulate their transcription (Li et al., 2016). Here, we show that COR27 and COR28 physically interact with COP1 and undergo COP1-mediated degradation in the dark. COR27 and COR28 negatively regulate photomorphogenesis by repressing the transcriptional activity of HY5, thereby fine-tuning the COP1–HY5 regulatory hub to ensure proper

photomorphogenic development in the light. COR27, COR28, and HY5 are all degraded in the dark, and COR27 inhibits the transcriptional activity of HY5 even in the dark to promote skotomorphogenic growth.

RESULTS

COR27 and COR28 Physically Interact with COP1

We previously showed that COR27 and COR28 protein levels were low in plants kept in the dark (Li et al., 2016). Here, we used transgenic lines overexpressing COR27 fused to the Yellow Fluorescent Protein (YFP) from the construct *35S:YFP-COR27* and determined that COR27 protein levels increased significantly within 1 h of a white-light treatment or 2 h of a blue-light treatment (Supplemental Figure 1A-D). Furthermore, *COR27* was highly expressed in seedlings pretreated with blue light, but COR27 protein levels decreased markedly within 3 h after the seedlings were transferred from white or blue light to darkness (Supplemental Figure 1A-D).We next examined the effects of blue light on the levels of the COR27 protein in the presence or absence of the 26S proteasome inhibitor MG132. The abundance of COR27 decreased markedly in darkness in the absence of MG132; by contrast, COR27 levels remained constant in darkness in the presence of MG132 (Supplemental Figure 1E-H), suggesting that the decrease of COR27 protein in the absence of blue light is due to its proteolysis by the 26S proteasome. The increase in COR27 in response to blue light and its decrease in the absence of blue light in the *cry1 cry2* double mutant were not as pronounced as in the wild type (Supplemental Figure 1I-L). Therefore, the CRY1 and CRY2 photoreceptors at least partially mediate the blue light suppression of COR27 degradation.

To elucidate the mechanism by which blue light stabilizes the COR27 and COR28 proteins, we performed a yeast two-hybrid screen on an Arabidopsis cDNA library to identify COR27- and COR28-interacting proteins. We identified COP1 and SPA1 (a COP1 interacting protein) in screens using COR27 or COR28, respectively, as the bait. The full list of identified COR27 and COR28 interactors is shown in Supplemental Table 1. In yeast cells, both COR27 and COR28 interacted with COP1 (Figure 1A). Bimolecular luminescence complementation (BiLC) assays also indicated that COP1 interacts with COR27 and COR28 in plant cells. Indeed, we detected strong luciferase activity in *Nicotiana benthamiana* leaves co-infiltrated with cLUC-

COP1 and COR27-nLUC or COR28-nLUC plasmids (Figure 1B). We also used an in vitro pulldown assay to test the interaction between COP1 and COR27 or COR28 produced and purified from *E. coli*. Both COR27 and COR28 pulled down COP1 in this assay (Figure 1C), further indicating that they physically interact in vitro. We performed co-immunoprecipitation (co-IP) analyses in transgenic Arabidopsis seedlings expressing YFP or Green Fluorescent Protein (GFP)-tagged COR27 or COR28. COP1 co-precipitated with COR27 or COR28 (Figure 1D). These results indicate that COR27 and COR28 interact with COP1 in planta.

COP1 is Responsible for the Degradation of COR27 Protein in the Dark

COP1 physically interacts with COR27 and COR28. COR27 is degraded in a 26S proteasome–dependent pathway in the absence of blue light; therefore, we hypothesized that COR27 might also be a substrate for COP1, and that COP1 may be responsible for the ubiquitination and degradation of COR27 in darkness. To explore this hypothesis, we first analyzed the protein stability of COR27 in the *cop1-6* mutant. COR27 protein levels (as reported by YFP) increased in response to blue light and decreased in the absence of blue light in the Col-0 but not in the *cop1-6* background (Figure 1E-H). Therefore, COP1 is responsible for the degradation of COR27 in the absence of blue light. Furthermore, COR27 showed high levels of ubiquitination in Col-0, but much reduced ubiquitination levels in *cop1-6* (Figure 1I). These results indicate that COP1 is responsible for the degradation of COR27 in the absence of light. CRY1 and CRY2 might regulate the protein stability of COR27 by repressing COP1, consistent with the finding that we observed no significant differences in COR27 protein levels between the *cry1 cry2* double mutant and Col-0 (Supplemental Figure 1I-L).

COR27 and COR28 Promote Hypocotyl Elongation

To investigate whether COR27 and COR28 might play a role in regulating photomorphogenesis, we grew Col-0, *cor27*, *cor28*, *cor27 cor28*, and transgenic seedlings overexpressing *COR27* or *COR28* in constant white light (LL), long-day conditions (LD), short-day conditions (SD), or in darkness. *cor27 cor28* double mutant seedlings exhibited a dramatic short hypocotyl phenotype in both LD and SD conditions compared to Col-0, but not in darkness or in constant light (Figure 2A and 2B; Supplemental Figure 2A and 2B). The

hypocotyl lengths of *cor27* and *cor28* single mutant seedlings were slightly shorter than those of Col-0 in SD conditions (Supplemental Figure 2C and 2D), consistent with the partial redundancy of COR27 and COR28 in the regulation of photomorphogenesis. The *COR27*- and *COR28*-overexpression lines showed longer hypocotyls than Col-0 under both LD and SD conditions, but not in constant darkness or in constant light (Figure 2A and 2B; Supplemental Figure 1A and 2B, 2E). All genotypes tested (Col-0, *cor27 cor28*, and transgenic lines overexpressing *COR27* or *COR28*) showed similar hypocotyl lengths when grown in constant darkness; therefore, we next grew them in SD conditions with white, blue, red, and far-red light for 7 d to analyze which aspect of light regulated hypocotyl growth. *cor27 cor28* double mutant seedlings developed shorter hypocotyls than Col-0 when grown under blue, red, or far-red light in SD conditions, whereas the overexpression lines produced significantly longer hypocotyls, similar to their respective patterns under white light (Figure 2C and 2D). These results indicate that COR27 and COR28 promote hypocotyl elongation and act as negative regulators of the blue-, red-, and far-red light–mediated repression of hypocotyl elongation.

COR27 and COR28 Regulate the Expression of COP1-Regulated Genes

To elucidate the mechanism by which COR27 and COR28 regulate photomorphogenesis, we performed RT-qPCR to analyze the expression of *HY5* and *PACLOBUTRAZOL RESISTANCE1* (*PRE1*) in LD-grown Col-0 and *cor27 cor28* seedlings. HY5 loss of function leads to dramatically elongated hypocotyls under all light conditions (Ang et al., 1998), while PRE1 was reported to regulate cell elongation (Lee et al., 2006). *HY5* transcript levels were higher in the *cor27 cor28* double mutant relative to Col-0, especially at night, while *PRE1* mRNA levels were lower in *cor27 cor28* compared to Col-0 during both the morning and night (Supplemental Figure 2F and 2G).

We then performed deep sequencing of the transcriptome (RNA-seq) to identify downstream genes affected in the *cor27 cor28* double mutant at night when grown in SD. We collected samples at Zeitgeber Time (ZT) 20, that is 12 h after lights off, late into the dark period. We identified 1,440 differentially expressed genes between *cor27 cor28* and Col-0 (Figure 3A, Supplemental Data Set 1). As shown above, COP1 is responsible for the degradation of COR27 in darkness, and COR27 is involved in photomorphogenesis. COR27 should therefore influence

the expression of genes downstream of COP1. Indeed, our RNA-seq datasets of the genes differentially expressed in *cor27 cor28* significantly overlapped with RNA-seq datasets of genes differentially expressed in the *cop1-6* mutant (Figure 3A–D, Supplemental Data Set 2). For 87% of these genes, the effects of the *cor27 cor28* double mutant on gene expression were the same as in *cop1-6* (compare *cor27 cor28* vs. Col-0 and *cop1-6* vs. Col-0 in Figure 3B and 3C). These results indicate that COP1, COR27 and COR28 exert similar effects on many commonly regulated genes. A Gene Ontology (GO) analysis revealed a marked enrichment in light-responsive, growth-related functions in the *cop1-6* and *cor27 cor28* co-regulated genes, including *PIF4* and *HYH* (Figure 3D). These genomic data thus provide direct evidence for the important roles of COR27 and COR28 in photomorphogenesis, particularly in their regulation of genes involved in cell elongation.

We verified the transcriptomic data using RT-qPCR. We selected *PIF4, HYH, PRE1, INDOLE-3-ACETIC ACID INDUCIBLE* 19 (*IAA29)*, and *ARABIDOPSIS COLUMBIA SAUR GENE23 (SAUR23)* from the list of genes commonly regulated by COR27, COR28 and COP1 (Figure 3E–J). *HY5* and its homologue *HYH* were both up-regulated in *cor27 cor28* and *cop1-6* mutants, but down-regulated in the transgenic lines overexpressing *COR27* or *COR28*. Conversely, *PIF4, PRE1, IAA29*, and *SAUR23* were all up-regulated in seedlings overexpressing *COR27*, *COR28,* or *COP1* compared to Col-0, but down-regulated in the *cor27 cor28* and *cop1-6* mutants.

COR27 and COR28 Promote Hypocotyl Elongation in a COP1-Dependent Manner

To further determine the relationship between COP1 and COR27, we investigated the genetic interactions between the *COR27* and *COP1* loci. We crossed the *35S:YFP-COR27* transgenic line with the *cop1-6* mutant, resulting in the YFP-COR27 *cop1-6* line. The long-hypocotyl phenotype of seedlings overexpressing *YFP-COR27* was mostly suppressed in the *cop1-6* background (Figure 4A and 4B), which suggested that COR27-promoted hypocotyl elongation is dependent on COP1. The hypocotyl phenotype of GFP-COR28 *cop1-6* was also similar to that of *cop1-6* (Supplemental Figure 3A and 3B). Consistent with these observations, *HYH* and *HY5* transcript levels were markedly higher in YFP-COR27 *cop1-6* seedlings than in YFP-COR27 in the Col-0 background, while the transcript levels of *PIF4, IAA29, PRE1*, and

SAUR23 were significantly lower in *YFP-COR27 cop1-6* seedlings than in *YFP-COR27* in the Col-0 background (Figure 4C–H). These results indicate that COR27 regulates the transcription of these elongation-related genes in a COP1-dependent manner.

COR27 Physically Interacts with HY5

COR27 and COR28 indirectly bind to the chromatin surrounding *PRR5* and *TOC1* to repress their expression and regulate the circadian clock (Li et al., 2016). We previously proposed that COR27 and COR28 might form a protein complex with other transcription factors to associate with the chromatin at these regions (Li et al., 2016). To determine the mechanism by which COR27 and COR28 regulate photomorphogenesis and the expression of downstream elongation-related genes, we tested the interaction between COR27, COR28, and various proteins known to be involved in regulating hypocotyl elongation using a yeast two-hybrid assay. Among the 32 proteins we tested (gene names and accession numbers are given in Supplemental Table 2), HY5 interacted with COR27 and COR28 in yeast cells (Figure 5A). We also pulled down HY5 with both COR27 and COR28 in an in vitro pull-down assay (Figure 5B), indicating that the proteins physically interact in vitro. We further confirmed the interaction between HY5 and COR27 and COR28 in plant cells by BiLC assay. Indeed, we detected strong luciferase activity in *N. benthamiana* leaves co-infiltrated with cLUC-HY5 and COR27-nLUC or COR28-nLUC plasmids (Figure 5C). Finally, we performed co-IP analyses in transgenic Arabidopsis seedlings expressing YFP-tagged COR27 and GFP-tagged COR28 in the Col-0 background, which revealed that HY5 co-precipitated with COR27 and COR28 (Figure 5D).

COR27 and COR28 Influence the Expression of HY5-Regulated Genes

COR27 physically interacts with HY5, suggesting they may act together to regulate transcription and photomorphogenesis. To explore how COR27 and COR28 might influence the expression of HY5-target genes, we performed RNA-seq to identify downstream genes affected in the *hy5-215* mutant at night (SD, ZT20). Our RNA-seq datasets of differentially expressed genes in *cor27 cor28* relative to Col-0 significantly overlapped with RNA-seq datasets of differentially expressed genes in *hy5-215* (Figure 5E-G, Supplemental Data Set 3). There were 235 genes co-regulated by both COR27 and COR28 and HY5. For 53.2% of these genes, the *cor27 cor28* double mutant had the opposite effect from that in *hy5*, with 17.9%

promoted by HY5 but repressed by COR27 and COR28, including *HYH* and *ELF4*; the remaining 35.3% were repressed by HY5 but promoted by COR27 and COR28, including *PIF4*, *IAA29* and *SAUR23*. These results indicated that COR27, COR28 and HY5 act in an opposite manner on the regulation of a large number of common genes. At the same time, we compared the list of COR27- and COR28-regulated genes with the genes whose promoters are bound by HY5, previously identified by Lee et al. (2007) (Figure 5D): 20% of the genes affected by the loss of COR27 and COR28 were direct targets of HY5 (Supplemental Figure 4E and 4F). Collectively, these results indicate that HY5 and COR27 and COR28 regulate a large number of common genes. Gene Ontology analysis showed that the molecular function categories stimulus and absence of light response genes were markedly changed in the list of genes commonly regulated by COR27, COR28 and HY5, including *HYH* and *PIF4* (Figure 5H; Supplemental Figure 4F). These genomic data thus provide direct evidence for the important role of COR27 in HY5-mediated regulation of gene expression.

Similarly, HY5 and PIFs co-regulated photomorphogenesis in both LD and SD conditiosn. When we grew Col-0, *hy5-215*, and *pifq* (a quadruple *pif* mutant) seedlings in LD and SD conditions, *pifq* exhibited a dramatically short hypocotyl phenotype relative to the wild type, and in sharp contrast to the long hypocotyl phenotype displayed by *hy5-215* (Supplemental Figure 4A–C). Consistent with these phenotypes, *PRE1* and *IAA29* transcript levels were higher in *hy5-215* compared to Col-0, but lower in *pifq* seedlings. HY5 also affects *PIF4* expression, *hy5-215* caused the up-regulation of *PIF4* (Supplemental Figure 4D).

COR27 Associates with the Promoters of HY5 Targets to Regulate their Expression

COR27 physically interacts with HY5 and regulates the expression of HY5-target genes. To achieve this role, we hypothesized that COR27 might physically associate with the same genomic regions to which HY5 binds. To test this hypothesis, we performed chromatin immunoprecipitation (ChIP)-qPCR assays, which revealed that COR27 did associate with the same chromatin region of the *HY5* and *PIF4* promoters as HY5 does in vivo (Figure 6A–F). We used the same PCR primer pairs to detect chromatin binding by HY5 and COR27. A yeast one-hybrid assay revealed that HY5, but not COR27, bound to the *PIF4* promoter (Supplemental Figure 5A and 5B), indicating that COR27 physically interacts with HY5 to then associate with

the genomic regions to which HY5 binds.

We then analyzed whether COR27 affected the transcriptional activity of HY5 on its target promoters using a transient transcription assay in Arabidopsis protoplasts and *N. benthamiana*. We used a dual-LUC reporter plasmid encoding the firefly luciferase (*LUC*) gene driven by the *HY5* or *PIF4* promoter and a Renilla luciferase (*REN*) gene driven by the constitutive cauliflower Mosaic Virus (CaMV) *35S* promoter (Figure 6G; Supplemental Figure 5C). We transiently expressed the *HY5pro:LUC* reporter in *hy5-215* protoplasts together with either *35S:HY5-GFP* or *35S:COR27-GFP* or both. The LUC activity level from the *HY5pro-LUC* reporter was about 2.5-fold higher when we co-expressed *HY5* in the protoplasts relative to co-expressed *GFP*, while the combination of HY5 and COR27 induced the reporter very modestly (Figure 6H). We also infiltrated *N. benthamiana* leaves with an Agrobacterium strain containing the reporter construct (pGreen-PIF4pro:LUC) alone or mixed with an Agrobacterium strain containing the indicated effector plasmid (pCambia1300-HY5-YFP*,* pEGAD-MYC-COR27, or pCambia1306-VP16-HY5-Flag). We fused HY5 to the VP16 activation domain (from Herpes simplex virus protein vmw65) to make it a strong constitutive activator of *PIF4* transcription rather than its typical function as a repressor (Supplemental Figure 4D), allowing us to more easily analyze the effect of COR27 on HY5 transcriptional activity. COR27 repressed the transcriptional activity of VP16-HY5 on the *PIF4pro:LUC* reporter gene (Supplemental Figure 5D). COR27 itself did not significantly affect the transcription of *PIF4pro:LUC* or *HY5pro:LUC* (Figure 6H; Supplemental Figure 5D); however, it did inhibit the transcriptional activity of HY5.

To further study the relationship between COR27, COR28, and HY5, we investigated the genetic interactions between the corresponding genes. We crossed the *cor27 cor28* double mutant to *hy5-215*, to generate the *hy5 cor27 cor28* triple mutant (Supplemental Fig 6A). Bringing the *hy5-215* mutation into the *cor27 cor28* double mutant background dramatically suppressed its short hypocotyl phenotype, resulting in hypocotyls that were almost as long in the triple mutant as in the *hy5-215* single mutant (Figure 7A and 7B). Transcript levels of *HYH* were also markedly lower in *hy5 cor27 cor28* compared to *cor27 cor28*, and were similar to those seen in *hy5-215* (Figure 7C), indicating that COR27 and COR28 regulate hypocotyl elongation at least partially via HY5, and that COR27 and COR28 also regulate hypocotyl

growth through HY5-independent pathways. Consistent with this, YFP-COR27 *hy5-215* showed longer hypocotyls than YFP-COR27 in the Col-0 background, and slightly longer hypocotyls than in the *hy5-215* mutant. *HYH* transcript levels were also lower in YFP-COR27 *hy5-215* seedlings than in YFP-COR27 seedlings, and comparable to those seen in *hy5-215*, indicating that COR27 and COR28 regulate the transcription of *HYH* through HY5 (Figure 7D-F).

DISCUSSION

Dark growth (skotomorphogenesis) and light-mediated growth (photomorphogenesis) are critical for germination and seedling development. Buried seeds use elongated hypocotyls, closed cotyledons, and curved apical hooks (skotomorphogenic growth) to limit mechanical damage after germination as they grow through soil particles, until they reach the soil surface. Once top-side, seedlings are exposed to light, which then inhibits hypocotyl elongation and promotes cotyledon expansion (photomorphogenesis). COP1 is a central repressor of light signaling, acting downstream of multiple photoreceptors (Ang and Deng, 1994; Christie et al., 2012). COP1 negatively regulates photomorphogenesis by mediating the degradation of various photomorphogenesis-promoting transcription factors in the dark, including HY5 (Yi and Deng, 2005; Jiao et al., 2007; Foreman et al., 2011; Liu et al., 2011). Light represses COP1 by activating multiple photoreceptors, stabilizing the HY5 protein (Ang and Deng, 1994; Christie et al., 2012). The COP1–HY5 module plays a critical role in regulating photomorphogenesis. *COR27* and *COR28* were identified as cold-responsive genes during Arabidopsis transcriptome analysis, and the expression of *COR27* was shown to be clock- and light-regulated (Fowler and Thomashow, 2002; Mikkelsen and Thomashow, 2009; Li et al., 2016). Light- and low temperature–regulated COR27 and COR28 are involved in the regulation of the circadian clock, freezing tolerance, and flowering time, representing a trade-off between flowering and freezing tolerance (Li et al., 2016).

Here, we showed that COR27 and COR28 are novel regulators of photomorphogenesis, and are involved in the COP1–HY5-mediated elongation of Arabidopsis seedlings in response to light. Like the HY5 protein, COR27 and COR28 are degraded in the dark via the 26S proteasome pathway. We also showed that COP1 physically interacts with COR27 and COR28,

that it is responsible for their degradation, and that their protein levels are relatively low in etiolated seedlings. Light promotes the accumulation of HY5, COR27, and COR28, most likely due to the light-mediated inactivation of COP1 (Ang and Deng, 1994; Christie et al., 2012). COP1 regulates the protein stability of HY5, COR27, and COR28, while COR27 or COR28 form a protein complex with HY5 on the chromatin of HY5 target genes to modulate their transcription and ultimately photomorphogenesis. COR27 inhibits the transcriptional activity of HY5 even in the dark to promote skotomorphogenic growth, and COR27 and COR28 fine-tune photomorphogenesis by modulating the COP1–HY5 module in response to changing light environments (Figure 6F).

Multiple HY5-interacting proteins have been identified and characterized, such as HYH, G-BOX BINDING FACTOR1 (GBF1), CALMODULIN7 (CAM7), B-BOX DOMAIN PROTEIN21 (BBX21), BBX22, BBX24, BBX25, BBX28, and BBX32. These factors interact with HY5 to positively or negatively regulate its transcriptional activity (Holm et al., 2002; Datta et al., 2006; Datta et al., 2008; Holtan et al., 2011; Singh et al., 2012; Gangappa et al., 2013; Abbas et al., 2014; Zhang et al., 2017; Lin et al., 2018). HY5 is a key transcription factor in photomorphogenesis, functioning alongside multiple factors to fine-tune HY5 biochemical activity and gene expression in response to the changing light environment. COP1 also regulates the protein stability of BBX28, which represses photomorphogenesis by inhibiting the DNA-binding activity of HY5 (Lin et al., 2018). Different proteins thus regulate HY5 via different mechanisms, with some regulating its DNA-binding activity while others form complexes with HY5 at its target chromatin sites to regulate its transcriptional activity. The expression of *COR27* and *COR28* is also regulated by cold temperatures and the circadian clock; they may integrate multiple signals to fine-tune skotomorphogenesis and photomorphogenesis. The transcriptome data indicates that cold-responsive genes are enriched in the COP1, HY5, COR27 and COR28 co-regulated genes, suggesting that these proteins may also be involved in the joint regulation of cold tolerance.

COR27 and COR28 are both small proteins with unknown biochemical functions. They do not have any known DNA-binding domains, and cannot bind DNA themselves in vitro, indicating that they are unlikely to be transcription factors (Supplemental Figure 5A and 5B). We previously showed that they physically associate with the genomic regions of the clock

genes *TOC1* and *PRR5* to directly regulate their transcription, and hypothesized that they may form a protein complex with other DNA-binding transcription factors to associate with chromatin and regulate the transcription of these and other clock genes (Li et al., 2016). Here, we showed that COR27 interacts with HY5 and physically associates with the genomic regions to which HY5 binds, regulating the transcriptional activity of HY5 and its gene expression. Our results confirm that COR27 and COR28 work as transcriptional regulators and interact with other transcription factors to regulate the expression of at least 1,840 genes. When compared with Col-0, about evenly split between up-regulation (53%) and down-regulation (47%) in the *cor27 cor28* mutant. Only a fraction of these genes (12.8 %) were also regulated by HY5, indicating that COR27 and COR28 may interact with multiple transcription factors to regulate transcription.

HY5 protein also accumulated to higher levels in *cor27 cor28* than in Col-0, consistent with the fact that COR27 and COR28 inhibited the transcription of *HY5* (Supplemental Fig 6B and 6C). The long-hypocotyl phenotype of seedlings overexpressing *YFP-COR27* was mostly suppressed in the *cop1-6* background. It is interesting that COP1 is responsible for the degradation of COR27 in the dark but is also required for its function. One possible explanation for the suppression of the long hypocotyl phenotype of YFP-COR27 seedlings by *cop1-6* would invoke an accumulation of HY5 protein. To test this possibility, we checked the protein levels of HY5 in Col-0, *cop1-6*, YFP-COR27 and YFP-COR27 *cop1-6* (Supplemental Fig 6D and 6E). Our results indicated that indeed, there was more HY5 protein in *cop1-6* and also in YFP-COR27 *cop1-6* relative to Col-0 and YFP-COR27.

In summary, we showed that COR27 and COR28 are negative regulators of photomorphogenesis. COR27 and COR28 undergo COP1-mediated degradation in the dark. Light promotes the accumulation of HY5 and COR27, while COR27 physically interacts with HY5 to inhibit its transcriptional activity and fine-tune skotomorphogenesis development in the dark and photomorphogenic development in the light (Supplemental Fig 6F).

MATERIALS AND METHODS

Plant Materials and Growth Conditions

We used the Arabidopsis Columbia (Col-0) accession as wild type. The *cor27* (CS834545), *cor28* (SALK_137155), *cor27 cor28*, *35S:YFP-COR27*, *35S:GFP-COR28, cop1-6, pro35S:MYC-COP1, hy5-215, hy5* (SALK_096651), and *pifq (pif1 pif3 pif4 pif5)* lines have been described previously (Ang and Deng, 1994; McNellis et al., 1994; Leivar et al., 2008; Li et al., 2016; Lin et al., 2016). Additional lines (YFP-COR27 *cop1-6*, GFP-COR28 *cop1-6*, and YFP-COR27 *hy5-215*) were generated by genetic crosses and combined phenotyping and genotyping of F_2 progeny.

All seeds were surface-sterilized in 75% ethanol for 10 min, washed four times with sterile water, then sown on half-strength Murashige and Skoog (MS) medium supplemented with 0.8% agar and 1% sucrose. Plates were then stratified for 4 d in the dark at 4°C before transfer to a Percival growth chamber (AR-22L for white light,E-30LEDL3 for blue, red or far-red light, Percival Scientific, Perry, IA, USA) under either constant light, LD (long days, 16 h light/8 h dark), SD (short days, 8 h light/16 h dark), or constant darkness conditions at 22°C. The lights used were white light (Fluorescent light, Philips F17T8/TL841, 90 μmol/m_2/s), blue light (LED light 40 μmol/m_2/s), red light (LED light 40 μmol/m_2/s), or far-red light (LED light 8 μmol/m_2/s).

Immunoblot Analysis

Seeds were grown for 7 d in SD conditions (white light) before being moved to blue or white light or darkness for 24 h, after we exposed seedlings to a defined light treatment (light to dark or dark to light for the indicated times). After treatment, we collected seedlings and prepared whole protein extracts with extraction buffer (25.2g Glycerol, 0.02g Bromophenol Blue, 4g SDS, 20 mL 1M Tris-HCl, pH6.8, 3.1g DTT, to 50mL ddH_2O). Equal protein amounts were loaded and separated on a 10% or 12% SDS-PAGE gel and transferred to Nitrocellulose Blotting Membrane (P/N66485, PALL, USA)VDF membrane. We then probed the membrane with an anti-GFP antibody (AE012, Abclonal, China, 1:3,000 dilution), stripped the blot, and re-probed with the anti-ACTIN antibody (AC009, Abclonal, China, 1:3,000 dilution) as loading control.

Transcriptome Analysis

We extracted total RNA from 7-d-old Col-0, *cor27 cor28*, *cop1-6* and *hy5-215* seedlings

grown in SD conditions and collected at ZT20 (12 h after lights off) under dim green light, using the mirVana miRNA Isolation Kit (Ambion-1561). We then generated mRNA sequencing libraries (with three independent biological replicates), which were sequenced by OE Biotech Co., Ltd. (Shanghai, China). We evaluated RNA integrity on an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The samples with RNA Integrity Number (RIN) $\geqslant$ 7 were subjected to further analysis. We generated libraries using the TruSeq Stranded mRNA LT Sample Prep Kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. Then these libraries were sequenced on an Illumina sequencing platform (Illumina HiSeq X Ten) as 125bp/150bp paired-end reads. Sequenced reads were processed using Trimmomatic (Version 0.36). Reads containing multiple Ns and low-quality reads were removed to obtain clean reads. We then mapped the clean reads to the Arabidopsis reference genome (TAIR10.1_NCBI) using hisat2 (Version 2.2.1.0). We then quantified read counts per gene and Fragments Per Kilobase exon per Million reads mapped (FPKM) values using Cufflinks (version:2.2.1), while read count values per transcript (protein_coding) were calculated using bowtie2 and eXpress. We identified differentially expressed genes (DEGs) using DESeq (Version1.18.0) (Anders et al., 2013). The R package functions 'Estimate Size Factors' and 'nbinom Test', Padjvalues<0.05 and Fold Changes >1.5 were set as the threshold for significantly different expression. We downloaded the ChIP-chip (Chromatin immunoprecipitation (ChIP) followed by microarray hybridization) data for HY5 from the Gene Expression Omnibus database (accession number GSE6510). We performed a GO enrichment analysis (Biological processes) using the Gene Ontology website (http://geneontology.org) with the released version 2019-07-01.

RT-qPCR Analysis

We performed all RT-qPCR expression analyses as described previously (Ma et al., 2016). Briefly, we isolated total RNAs using a RNAiso Plus kit (Takara Bio, Shiga, Japan). We synthesized first-strand cDNAs from 500 ng of starting total RNA using a Prime Script RT Reagent Kit with a genomic DNA Eraser (Takara Bio). We then used SYBR Premix Ex Tag (Takara Bio) and the MX3000 Real Time PCR System (Stratagene, San Diego, CA, USA) for the qPCR reactions. We used the *ISOPENTENYL DIPHOSPHATE ISOMERASE 2* (*IPP2*,

At3g02780) and *ACTIN 7* (At5g09810) genes as internal controls. Following initial denaturation of the first-strand cDNAs, we executed the following PCR program: denaturation at 95°C for 30 s, 2 step PCR for 40 cycles (95°C for 5 s, 60°C for 20 s per cycle) with fluorescence read at the end of each cycle, a dissociation program was performed after the reaction. The biological replicates represent three independent experiments involving about 20 seedlings per experiment. Three technical replicates were performed for each PCR reaction. The primers used are listed in Supplemental Data Set 4.

Yeast Two-Hybrid Analysis

We cloned the coding sequences of *COR27* and *COR28* in-frame with the *GAL4* DNA-binding domain (BD) sequence in the bait vector pBridge (Takara Bio). We obtained the Arabidopsis cDNA library clones in the prey vector pACT from Dr. Joe Ecker (Salk Institute, CA, USA). We co-transformed the bait plasmid pBridge-COR27/COR28 and the prey plasmid library DNA into yeast strain Y190. Yeast cells were then grown on synthetic dropout medium (SD –Trp –Leu) plates for 4 d. We tested the interactions using β-galactosidase assays (168 μg/mL substrate), checking the activity every h for eight h, and verified positive yeast clones by PCR and sequencing. The full list of identified COR27 and COR28 interactors is given in Supplemental Table 1. We fused the coding sequence of *COP1* in-frame with the sequence of the *GAL4* activation domain (AD) in the prey vector pGADT7 (Takara Bio).We cloned the coding sequence of *HY5* into the pGADT7 or pDEST22 (Takara Bio) vectors to verify the interaction in yeast strain AH109 against pBridge-COR27, pBridge-COR28 or pDEST32-COR28.

Bi-Luminescence Complementation (BiLC) Assays

We fused COR27/COR28 or COP1/HY5 to the N- or C-terminus of firefly luciferase, then transformed the constructs into Agrobacterium (*Agrobacterium tumefaciens*) strain GV3101. We collected overnight Agrobacterium cultures by centrifugation at 3200g for 20 min and resuspended the cells in MES buffer (10 mM MES,10 mM $MgCl_2$, and 100 mM acetosyringone) to a final OD_{600} = 0.8~1 before infiltration of *Nicotiana benthamiana* leaves. We then returned infiltrated plants to LD conditions for 3 d. Before luciferase activity observation, we infiltrated

leaves with luciferin solution (1 mM luciferin and 0.01%Triton X-100) and captured images immediately using a CCD camera (Tanon-5200, BioTanon, China).

in vitro Pull-downs

We performed in vitro pulldown protein–protein interaction assays as previously described (Liu et al., 2008; Liu et al., 2013b; Ma et al., 2016; Liang et al., 2018; Yang et al., 2018), with the following modifications. We cloned the full-length coding sequences of *COR27* or *COR28* into the pGEX4T-1 vector, and the full-length coding sequences of *COP1* or *HY5* into the pCold-TF vector. We then produced and purified the proteins in *Escherichia coli* BL21 strain by using Glutathione Sepharose 4B (17-0756-01, GE Healthcare, USA) or Ni-NTA Agarose (R901-15, invitrogen, USA). We then incubated equal amounts of eluted TF-His-COP1 or TF-His-HY5 with GST-COR27 or GST-COR28 in XB buffer (50 mM Tris (pH7.8), 500 mM NaCl, 0.5% Triton X-100, 1 mM PMSF, 4.7mM β-ME), used to pull down the protein complexes with glutathione beads. We removed unbound proteins by washing with WB buffer (50 mM Tris (pH7.8), 500 mM NaCl,0.1% Triton X-100, 1 mM PMSF), after which we eluted bound proteins and analyzed the resulting eluates by immunoblot analysis an anti-TF antibody (M201; Takara Bio, Japan, 1:3,000 dilution) or anti-GST antibody (G018, Abcam, China, 1:3,000 dilution).

Co-Immunoprecipitations (Co-IPs)

The co-IP procedure has been described previously (Liu et al., 2008; Liu et al., 2013b; Ma et al., 2016; Liang et al., 2018; Yang et al., 2018). Briefly, we grew seedlings for 10 d from the genotypes Col-0, YFP-COR27 (in Col-0), GFP-COR28 (in Col-0) in SD conditions, at which point we treated them with 50 mM MG132 at ZT 7.5 (30 min before lights off). We then moved seedlings into darkness at ZT8 for 4 h, collected and ground tissue in liquid nitrogen, which we then homogenized in binding buffer (20 mM HEPES (pH 7.5), 40 mM KCl, 1 mM EDTA, 1% Triton X-100, 1 mM PMSF). We incubated protein extracts at 4°C for 10 min, before centrifugation at 14,000*g* for 10 min. We mixed the supernatant with 35 µL of anti-GFP-IgG-coupled protein A Sepharose (KTSM1301, Alpalife, Shenzhen, China), incubated the mixtures at 4°C for 30 min, and washed the beads three times with washing buffer (20 mM HEPES (pH 7.5), 40 mM KCl, 1 mM EDTA, 0.1% Triton X-100). We eluted bound proteins from the affinity

beads with 4× SDS/PAGE sample buffer and analyzed the eluates by immunoblotting with anti-COP1 (YKCP938; Youke Biotech, China, 1:2,000 dilution) and anti-HY5 (PHY1908, QWBIO, China, 1:2,000 dilution) antibodies to detect the target proteins.

Yeast One-Hybrid Analysis

We cloned *PIF4* promoter fragments (–1,703bp to –1,231bp, –1,286bp to –785bp, –870bp to –435bp, –545bp to –1bp) into the pLacZi destination vector and transformed the resulting constructs into yeast strain YM4271. We also cloned the *COR27* and *HY5* coding sequences into the pDEST22 vector. We then transformed the resulting constructs into YM4271 cells containing the various promoter reporter plasmids. We grew cells in synthetic dropout medium (SD –Trp –Ura), and analyzed protein-promoter interactions using a β-galactosidase assay (with 168 μg/mL substrate added).

Chromatin Immunoprecipitation (ChIP) Assays

We performed ChIP experiments as described previously (Liu et al., 2008; Liu et al., 2013b; Ma et al., 2016; Liang et al., 2018; Yang et al., 2018), using 10-d-old Col-0, YFP-COR27 (in Col-0), and HY5-HA (in the *hy5*-215 background) seedlings grown in SD conditions. We harvested 2 g plant material, which we then cross-linked with 1% formaldehyde (Sigma-Aldrich, St. Louis, MO, USA) for 15 min under a vacuum. We stopped cross-linking by the addition of glycine to the solution, to a final concentration of 0.125 M. We rinsed seedlings with water, froze them in liquid nitrogen, and ground them into a fine powder. We sonicated chromatin fragments (~500 bp) with a bioruptor (Bioruptor Plus,Diagenode SA, Belgium, at program 30 sec on and 30 sec off for 15 min) before immunoprecipitation with anti-GFP (AE012, Abclonal, China) or anti-HA (clone3F10, Roche, USA) antibodies, and analyzed the precipitated DNA by qPCR with the indicated primer pairs (Supplemental Data Set 4). The level of binding was calculated as the ratio between the IP and Input proteins.

Transient Transcription Dual-LUC Assays

We performed transient transcription dual-LUC assays using *N. benthamiana* plants as previously described (Liu et al., 2008). We used Agrobacterium cultures containing both the

reporter construct (pGreen-PIF4pro:LUC) and the helper construct pSoup-P19, alone or mixed with Agrobacterium cultures containing the effector plasmids (pCambia1300-HY5-YFP, pEGAD-MYC-COR27, or pCambia1306-VP16HY5-Flag). We cloned the full length coding sequences of *COR27* and *HY5* into pEGAD (ABRC) at the EcoRI and XhoI restriction sites, resulting in pEGAD-MYC-COR27, into pCambia1300 and pCambia1306 (CAMBIA, Canberra, Australia) at the BamHI and SalI sites, resulting in pCambia1300-HY5-YFP and pCambia1306-VP16HY5-Flag.. We collected overnight Agrobacterium cultures by centrifugation at 3200g for 20 min and resuspended pellets in infiltration buffer to a final OD_{600} = 0.8~1, and infiltrated cell suspensions into healthy *N.benthamiana* leaves.

We carried out protoplast isolation and PEG-mediated transformation as previously described (Liang et al., 2018; Yang et al., 2018). We isolated protoplasts from three-week-old *hy5-215* plants grown in SD conditions. We transfected protoplasts with a total of 20 μg DNA (effector constructs 35S:COR27-GFP and 35S:HY5-GFP, and pGreen-HY5pro:LUC reporter) and incubated overnight. We measured luciferase activity using a luminometer (GloMax 20/20; Promega, Madison, WI, USA) with Dual-Luciferase Reporter Assay System (E1910, Promega, USA), according to the manufacturer's instructions.

ACCESSION NUMBERS

Sequence data for genes described in this article can be found in The Arabidopsis Information Resource (TAIR) under the following accession numbers：

COR27 (At5g42900), *COR28* (At4g33980), *COP1* (At2g32950), *HY5* (At5g11260), *CRY1* (At4g08920), *CRY2* (At1g04400), *HYH* (At3g17609), *PIF4* (At2g43010), *PRE1* (At5g39860), *IAA29* (At4g32280), *SAUR23* (At5g18060).

Accession numbers for 32 photomorphogenesis-related genes tested for interaction with COR27 are listed in Supplemental Table 2.

RNA-seq data have been deposited into the Gene Expression Omnibus with the following accession numbers: *cor27 cor28* and *cop1*_RNA-seq GSE154409, *hy5*_RNA-seq GSE154416.

AUTHOR CONTRIBUTIONS

X.L. and H.L. conceived the project. X.L. performed most of the experiments, C. L. performed the genomic expression analysis. Z.Z and Y.L performed the RNA-seq analysis. D.M. J.Z and Y.Y made some of the constructs. X.L and H.L analyzed the data and wrote the manuscript.

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SUPPLEMENTAL DATA

Supplemental Figure 1. The COR27 protein is degraded in the dark by the 26S proteasome and in CRY1 and COP1-dependent manner.

Supplemental Figure 2. COR27 and COR28 inhibit photomorphogenesis in photoperiod condition.

Supplemental Figure 3. COR28 inhibits photomorphogenesis in a COP1 dependent manner.

Supplemental Figure 4. HY5 and PIFs regulate hypocotyl elongation in both LD and SD.

Supplemental Figure 5. HY5, but not COR27, binds the *PIF4* promoter.

Supplemental Table 1. Full list of identified COR27 or COR28 interactors.

Supplemental Table 2. Accession numbers for 32 photomorphogenesis-related genes tested for interaction with COR27.

Supplemental Data Set 1. List of 1,440 COR27 and COR28 regulated genes.

Supplemental Data Set 2. List of 3,854 COP1 regulated genes.

Supplemental Data Set 3. List of 2,157 HY5 regulated genes.

Supplemental Data Set 4. Primers list used in this study.

Supplemental Data Set 5. Statistical analysis of *t*-test and ANOVA results for the data shown in figures.

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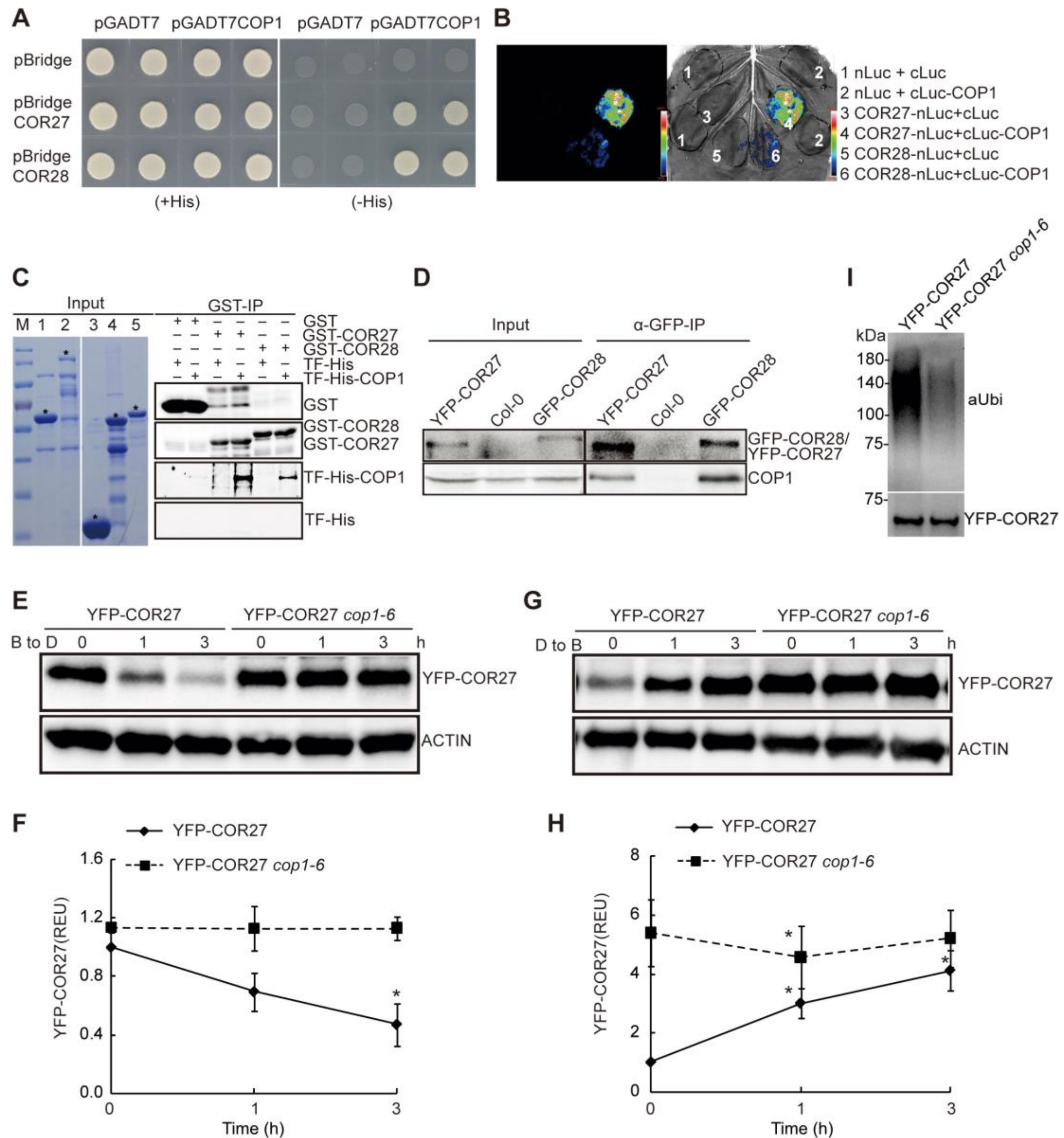

Figure 1. COR27 and COR28 Interact with COP1.

(A) Histidine auxotrophy assays showing the interaction between COR27 or COR28 and COP1. Yeast cells (strain AH109) containing plasmids encoding the indicated proteins were grown on medium in the presence (+) or absence (–) of histidine (His) in the dark for 3 d.

(B) Bimolecular luminescence complementation assays showing COR27 and COR28 interacting with COP1. Leaf epidermal cells of *Nicotiana benthamiana* were co-infiltrated with nLuc, COR27-nLuc, or COR28-nLuc and cLuc or cLuc-COP1 as indicated.

(C) In vitro pulldown assays showing the interaction of COR27 or COR28 with COP1. GST or GST-tagged COR27 or COR28 bound to glutathione-agarose beads was mixed with His-TF or His-TF-COP1 purified from *Escherichia coli*, as shown in the Coomassie-stained SDS-PAGE gel (left): (M) size marker, (1) His-TF tag, (2) His-TF-tagged COP1, (3) GST tag, (4) GST-tagged COR27, (5) GST-tagged COR28. The asterisk indicates the respective target protein. The pulldown products were analyzed using immunoblots probed with anti-GST or anti-TF antibodies (right).

(D) Co-immunoprecipitation assays showing in vivo protein interactions. Proteins were extracted from 10-d-old SD-grown YFP-COR27, GFP-COR28, or Col-0 seedlings. Input: immunoblots showing the level of YFP-COR27, GFP-COR28, COP1 in total protein extracts. α-GFP-IP: immunoprecipitation products precipitated with anti-GFP antibody. Total proteins (input) or immunoprecipitation products were probed in immunoblots using antibodies against GFP or COP1.

(E) Immunoblots showing that the *cop1-6* mutation blocks the degradation of COR27 in the dark. “B to D”

stand for blue light to darkness.

(F) Quantified protein levels in (**E**) with three biological repeats are shown. The relative level of YFP-COR27 accumulation as presented in relative expression units (REU) is calculated based on the formula [YFP-$COR27_t$/$ACTIN_t$]/[YFP-$COR27_0$/$ACTIN_0$ in YFP-COR27 (in Col-0)], in which “YFP-COR27” and “ACTIN” denote the digitized band intensities of YFP-COR27 or ACTIN in the respective samples collected at time 0 or at the indicated time (t) after dark treatment. Error bars represent standard deviation (n=3). Asterisks indicate a significant difference compared with the protein level at time 0 (*P<0.05, paired samples *t*-test).

(G) Immunoblots showing that a lack of light-induced COR27 accumulation in *cop1-6* mutant seedlings. “D to B” stand for darkness to blue light.

(H) Quantified protein levels in (**G**) are shown. The relative level of YFP-COR27 accumulation as presented in relative expression units (REU) is calculated by the formula [YFP-$COR27_t$/$ACTIN_t$]/[YFP-$COR27_0$/$ACTIN_0$in YFP-COR27 (in Col-0)], in which “YFP-COR27” and “ACTIN” denote the digitized band intensities of YFP-COR27 or ACTIN in the respective samples collected at time 0 or at the indicated time(t) after blue light exposure. Error bars represent standard deviation (n=3). Asterisks indicate a significant difference compared with the protein level at time 0 (*P<0.05, paired samples *t*-test).

(I) SD-grown 10-d-old YFP-COR27 (in Col-0) and YFP-COR27 *cop1-6* transgenic lines treated with 50 μM MG132 (26S proteasome inhibitor) at Zeitgeber Time (ZT) 7.5, then moved into darkness at ZT8 for 4 h.YFP-COR27 protein was extracted by immunoprecipitation with anti-GFP-conjugated sepharose, then separated on a pre-poured gradient gel. The ubiquitinated COR27 molecules between 75 kDa and 180 kDa in size were detected using an anti-ubiquitin antibody(α-Ubi). Anti-GFP antibody was used to detect non-ubiquitinated YFP-COR27 to ensure equal loading.

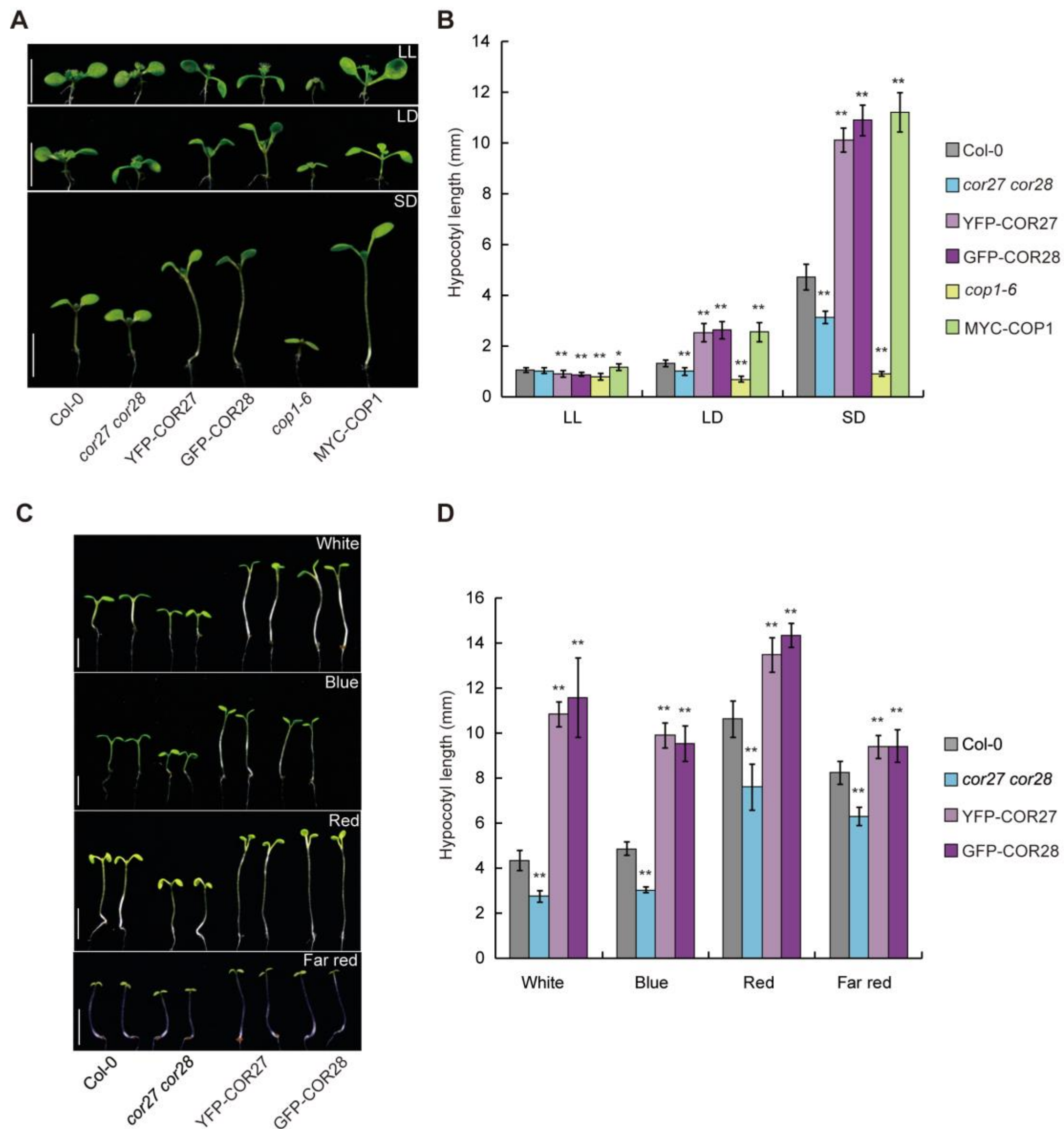

Figure 2. COR27 and COR28 Promote Hypocotyl Elongation.

(A) Phenotypes of 7-d-old Col-0, *cor27 cor28*, *35S:YFP-COR27* (YFP-COR27), *35S:GFP-COR28* (GFP-COR28), *35S:MYC-COP1* (MYC-COP1), and *cop1-6* seedlings grown in constant light (LL), long-day (16h/8h; LD), and short-day (8h/16h; SD) conditions. Bars = 5 mm.

(B) Quantification of hypocotyl lengths of the genotypes indicated in **(A)**. Error bars represent standard deviation (n=15). Asterisks indicate a significant difference compared with the wild type under the same treatment conditions (*P<0.05; **P<0.01, paired samples t-test).

(C) Phenotypes of 7-d-old Col-0, *cor27 cor28*, YFP-COR27, GFP-COR28, *cop1-6*, and MYC-COP1 seedlings grown under SD conditions with white (90 μmol/m$_2$/s), blue (40μmol/m$_2$/s), or red light (40 μmol/m$_2$/s), or far-red light (8 μmol/m$_2$/s). Bars = 5 mm.

(D) Quantification of hypocotyl lengths indicated in **(C)**. Error bars represent standard deviation (n=15). Asterisks indicate a significant difference compared with the wild type under the same treatment conditions (**P<0.01paired samples t-test).

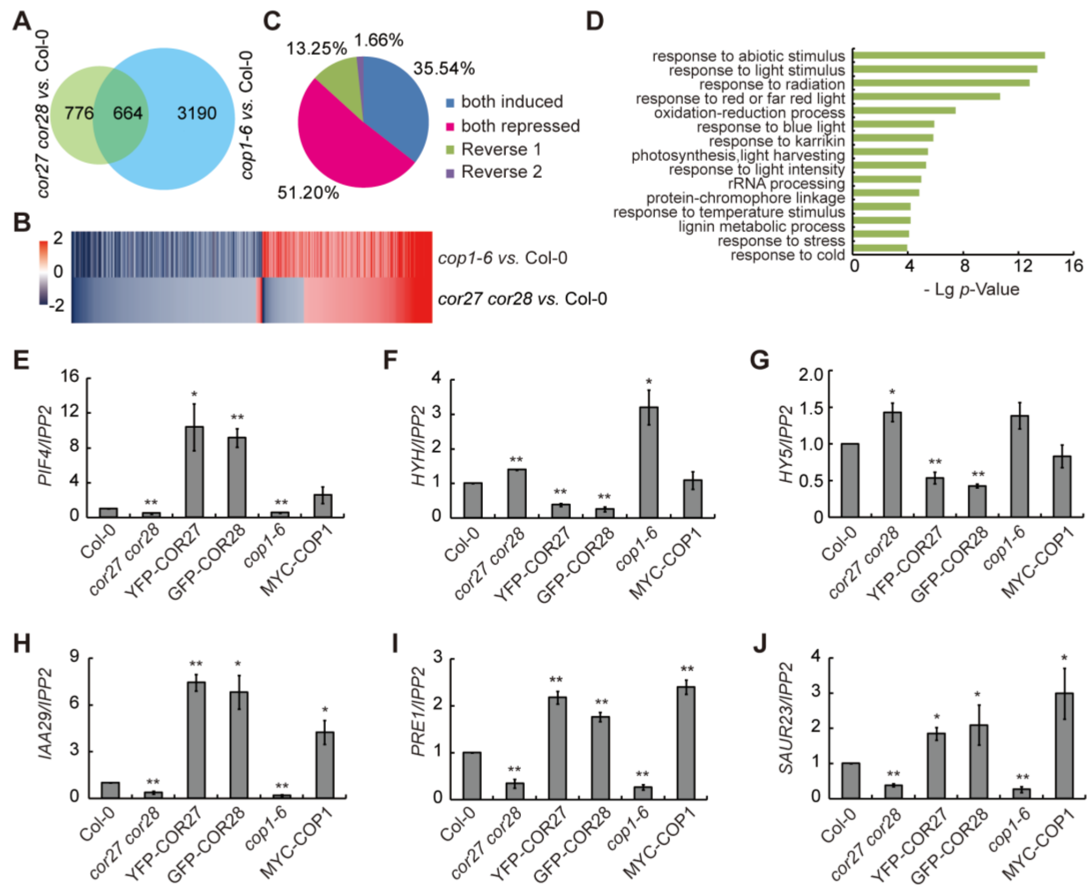

Figure 3. Genome-Wide Transcriptomic Analysis of Genes Coregulated by COR27, COR28, and COP1.

(A) Venn diagram showing the overlap between the sets of differentially expressed genes in Col-0 vs. *cor27 cor28* and Col-0 vs. *cop1-6*.

(B) Heatmap of COR27 and COR28 and COP1-regulated genes. The scale bar shows fold changes (log2 value).

(C) Distribution of genes co-regulated by COR27, COR28 and COP1. Reverse 1: COP1-repressed and COR27 and COR28-induced gene. Reverse 2: COP1-induced and COR27 and COR28-repressed gene.

(D) Gene Ontology analysis of the coregulated genes in **(B)**.

(E) to **(J)** COR27, COR28, and COP1 co-regulate the expression of the cell elongation genes *PIF4*, *HYH*, *HY5*, *PRE1*, *IAA29*, and *SAUR23*. RT-qPCR analysis of gene expression of 7-d-old Col-0, *cor27 cor28*, *35S:YFP-COR27* (YFP-COR27), *35S:GFP-COR28* (GFP-COR28), *cop1-6,* and *35S:MYC-COP1* (MYC-COP1) seedlings grown in SD conditions. Samples were collected at ZT 20. The *IPP2* gene was used as internal control. Gene expression in different genotypes were normalized to Col-0 (*gene*/*IPP2* in Col-0 was set to 1). Error bars represent standard deviation of three biological replicates. Asterisks indicate a significant difference compared with Col-0 (*P<0.05, **P<0.01, paired samples t-test).

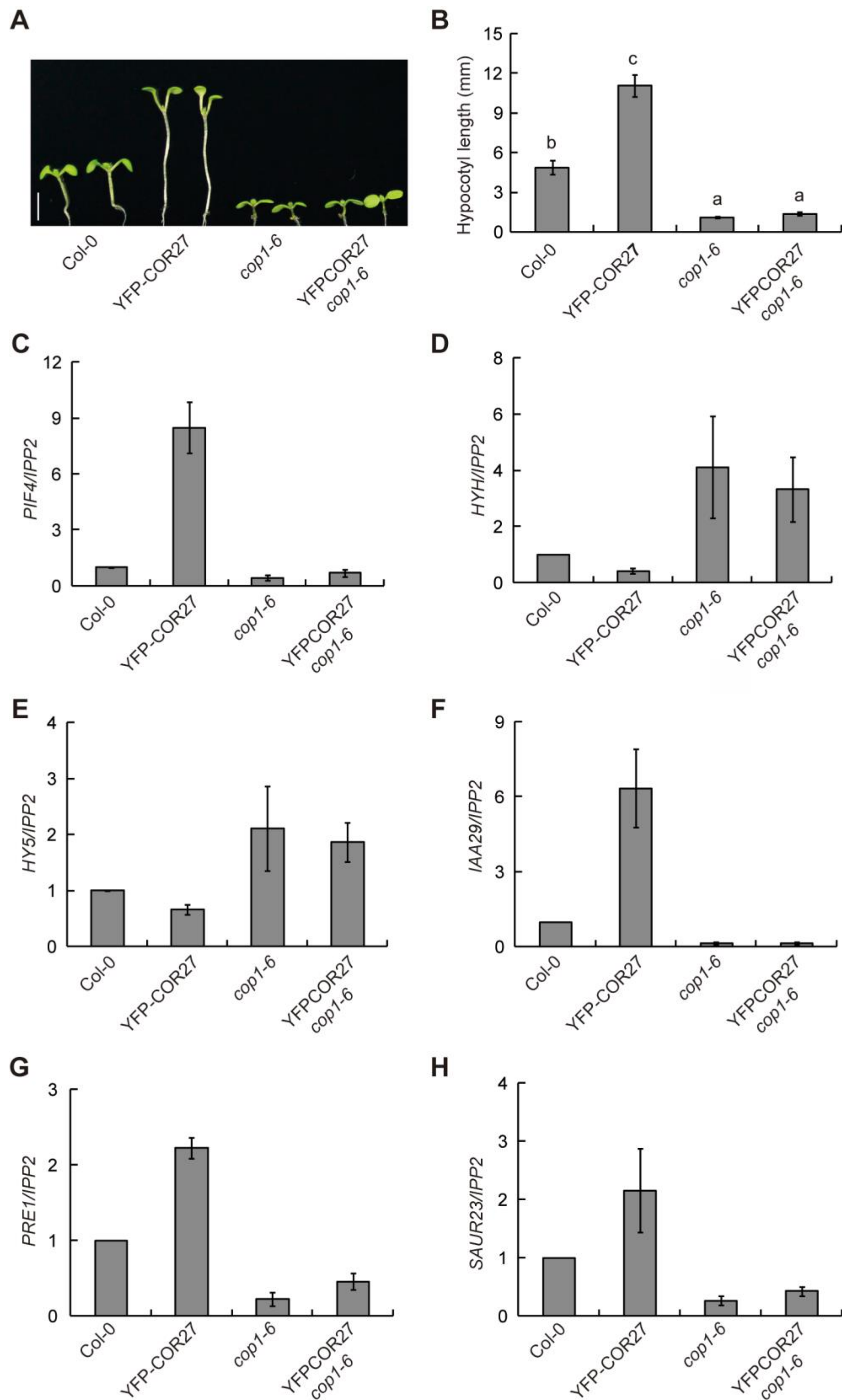

Figure 4. COR27 Inhibits Photomorphogenesis in a COP1-Dependent Manner.

(A) Phenotypes of 7-d-old Col-0, *35S:YFP-COR27* (YFP-COR27), *cop1-6*, YFP-COR27 *cop1-6* seedlings grown in SD conditions. Bars = 5 mm.

(B) Quantification of hypocotyl lengths of the genotypes indicated in **(A)**. Error bars represent standard

deviation (n=15). The letters “a” to “d” indicate statistically significant differences between the indicated genotypes, as determined by Turkey’s HSD test (p<0.05).

(C) to **(F)** The function of COR27 in regulating elongation-related gene expression depends on COP1. RT-qPCR analysis of gene expression of 7-d-old Col-0, YFP-COR27, *cop1-6*, and YFP-COR27 *cop1-6* seedlings grown in SD conditions at ZT 20. The *IPP2* gene was used as internal control. Gene expression in different genotypes was normalized to Col-0 (*gene*/*IPP2* in Col-0 was set to 1). Error bars represent the standard deviation of three biological replicates.

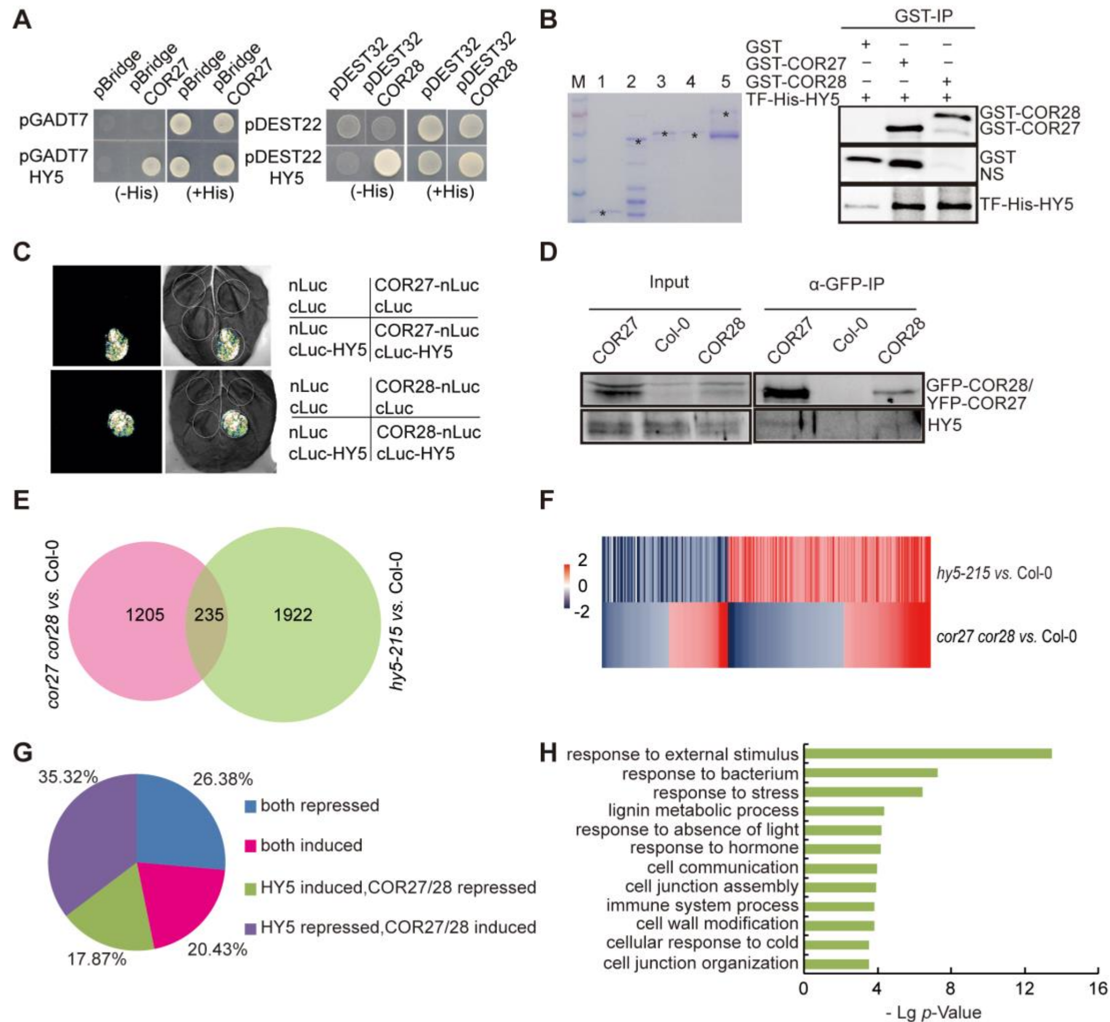

Figure 5. COR27 and COR28 Interact with HY5 to Regulate the Expression of Various Genes.

(A) Histidine auxotrophy assays showing the interaction between COR27 or COR28 and HY5. Yeast cells (strain AH109) containing plasmids encoding the indicated proteins were grown on medium in the presence (+) or absence (–) of histidine (His) in the dark for 3 d.

(B) In vitro pulldown assays showing that COR27 and COR28 interact with HY5. GST or GST-tagged COR27 or COR28 bound to glutathione-agarose beads were mixed with His-TF or His-TF-HY5 purified from *Escherichia coli*, as shown on the Coomassie-stained SDS-PAGE gel (left): (M) marker, (1) GST tag, (2) GST-tagged COR27, (3) GST-tagged COR28, (4) His-TF tag, and (5) His-TF-tagged HY5. The pulldown products were analyzed by immunoblot with anti-GST or anti-HY5 antibodies (right). NS: Non-specific band.

(C) Bimolecular luminescence complementation assays showing that COR27 and COR28 interact with HY5. Leaf epidermal cells of *Nicotiana benthamiana* were co-infiltrated with nLuc, COR27-nLuc, or COR28-nLuc and cLuc or cLuc-HY5, as indicated.

(D) Co-immunoprecipitation assays showing that COR27 and COR28 interact with HY5 in vivo. Ten-day-old SD-grown YFP-COR27, GFP-COR28, or Col-0 seedlings were collected and treated with MG132 (50 μM) at ZT 8, then moved to the dark for 4 h, after which the proteins were extracted for co-immunoprecipitation. Input: immunoblots showing the level of YFP-COR27, GFP-COR28, or HY5 in total protein extracts. α-GFP-IP: immunoprecipitation products precipitated using anti-GFP antibody. Total proteins (input) or immunoprecipitation products were probed in immunoblots with antibodies against GFP or HY5.

(E) Venn diagram showing the overlap between the sets of differentially expressed genes in Col-0 vs. *cor27 cor28* and Col-0 vs. *hy5-215*.

(F) Heatmap of COR27 and COR28 and HY5-regulated genes. The scale bar shows fold changes (log2 value).

(G) Distribution of genes co-regulated by COR27, COR28 and HY5.
(H) Gene Ontology analysis of the co-regulated genes indicated in **(F)**.

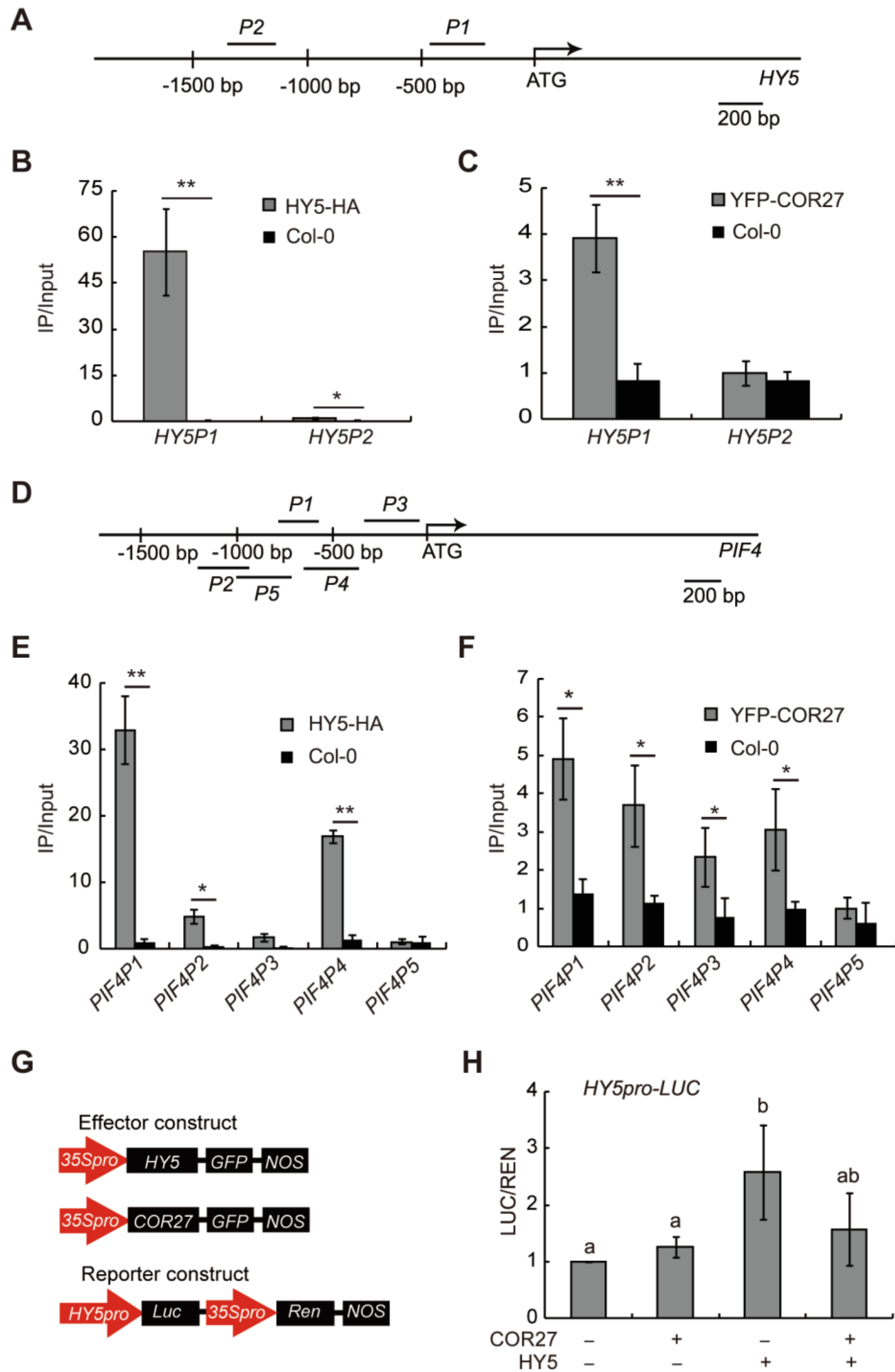

Figure 6. COR27 Associates with HY5 to Regulate Gene Expression During Photomorphogenesis.
(A) Diagram depicting the *HY5* promoter, including the two promoter sites studied (P1 and P2).
(B) and **(C)** ChIP-qPCR results showing that (**B)** HY5-HA and **(C)** YFP-COR27 bind to the *HY5* promoter. The P1 region contains various binding sites of the *HY5* promoter, including the ACG box, T/G box, and E box. The error bars represent the standard deviation of three biological replicates. Asterisks indicate a significant difference compared in the comparisons shown (**P*<0.05, ***P*<0.01, paired samples *t*-test).
(D) Diagram depicting the *PIF4* promoter, including the five promoter sites studied (P1–5).
(E) and **(F)** ChIP-qPCR results showing that **(E)** HY5-HA and **(F)** YFP-COR27 bind to the *PIF4* promoter. The error bars represent the standard deviation of three biological replicates. Asterisks indicate a significant difference compared in the comparisons shown (**P*<0.05, ***P*<0.01, paired samples *t*-test).
(G) Structure of the *HY5* promoter-driven dual-luciferase (LUC) reporter gene and two effector genes (*35S:COR27-GFP, 35S:HY5-GFP*). For the reporter constructs: *35S* promoter, *HY5* promoter (–700 bp to –1 bp), REN luciferase (REN), firefly luciferase (LUC) are indicated.

(H) Arabidopsis protoplasts isolated from the *hy5-215* mutant were transfected with reporter DNA together with empty effector DNA (GFP), COR27, HY5 or COR27+HY5.After transfection, the protoplasts were kept in low light for 12 h. LUC activity was normalized to REN activity. Error bars represent the standard deviation of three biological replicates. Letters “a” to “c” indicate statistically significant differences for the indicated values, as determined by a one-way analysis of variance (ANOVA), followed by a Tukey’s HSD test ($P < 0.05$).

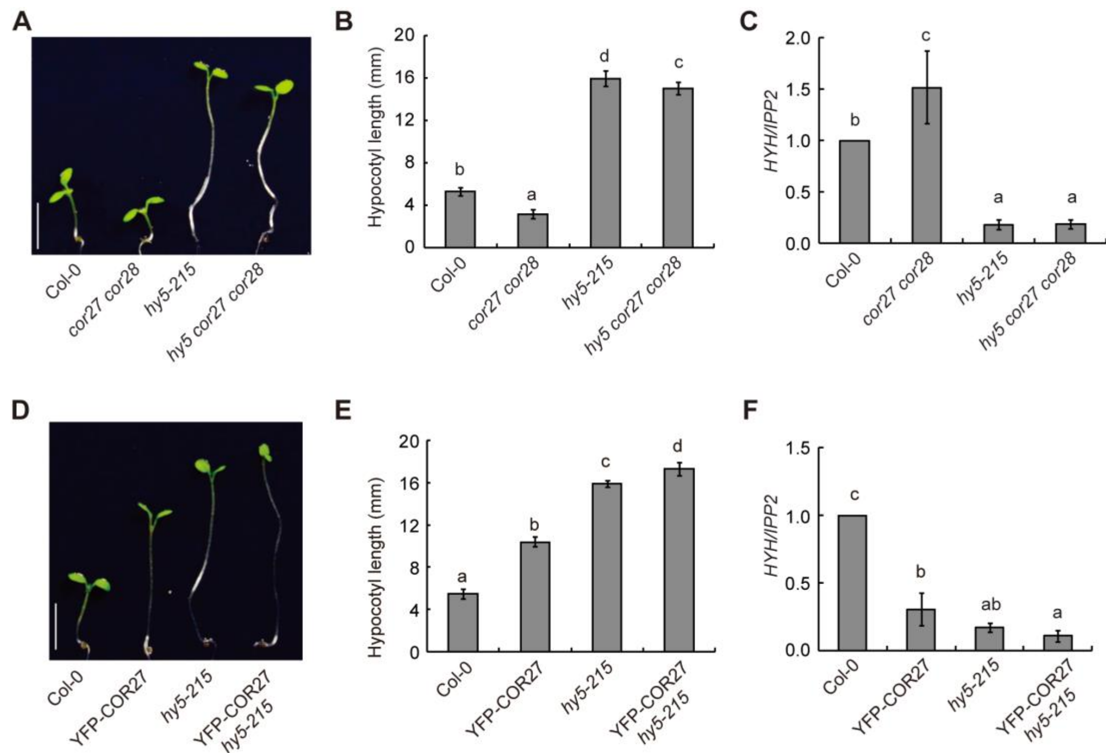

Figure 7. COR27 and COR28 Interact with HY5 to Regulate Gene Expression and Photomorphogenesis in Arabidopsis.

(A) Phenotypes of 7-d-old Col-0, *cor27 cor28*, *hy5-215*, and *hy5-215 cor27 cor28* seedlings grown in SD conditions. Bars = 5 mm.

(B) Quantification of hypocotyl lengths of Col-0, *cor27 cor28*, *hy5-215* and *hy5-215 cor27 cor28*. 7-d-old seedlings of the indicated genotypes were grown in SD conditions. Error bars represent the standard deviation (*n*=25). Different letters indicate statistically significant differences between the indicated genotypes, as determined using Tukey's HSD test (p<0.05).

(C) RT-qPCR analysis of *HYH* expression of 7-d-old Col-0, *cor27 cor28*, *hy5-215*, and *hy5-215 cor27 cor28* seedlings grown in SD conditions and collected at Zeitgeber time (ZT) 20. The *IPP2* gene was used as internal control. *HYH* expression in different genotypes was normalized to Col-0 (*gene*/*IPP2* in Col-0 was set to 1). Error bars represent the standard deviation of three biological replicates. Different letters indicate statistically significant differences between the indicated genotypes, as determined using Tukey's HSD test (p<0.05).

(D) Phenotypes of 7-d-old Col-0, YFP-COR27, *hy5-215*, and YFP-COR27 *hy5-215* seedlings grown in SD conditions. Bars = 5 mm.

(E) Quantification of hypocotyl lengths of Col-0, YFP-COR27, *hy5-215*, and YFP-COR27 *hy5-215*. 7-old seedlings of the indicated genotypes were grown in SD conditions. Error bars represent the standard deviation (*n*=25). Different letters indicate statistically significant differences between the indicated genotypes, as determined using Tukey's HSD test (p<0.05).

(F) RT-qPCR analysis of *HYH* expression of 7-d-old Col-0, YFP-COR27, *hy5-215*, and YFP-COR27 *hy5-215* plants grown in SD conditions and collected at ZT20. The *IPP2* gene was used as internal control. Error bars represent the standard deviation of three biological replicates. Different letters indicate statistically significant differences between the indicated genotypes, as determined using Tukey's HSD test (p<0.05).

COR27 and COR28 are Novel Regulators of the COP1-HY5 Regulatory Hub and Photomorphogenesis in Arabidopsis
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