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The blue light receptor CRY1 interacts with GID1 and DELLA proteins to repress gibberellin signaling and plant growth

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ABSTRACT

Improvements in plant architecture, such as reduced plant height under high-density planting, are important for agricultural production. Light and gibberellin (GA) are essential external and internal cues that affect plant architecture. In this study, we characterize the direct interaction of distinct receptors that link light and GA signaling in *Arabidopsis* (*Arabidopsis thaliana*) and wheat (*Triticum aestivum* L.). We show that the light receptor CRY1 represses GA signaling through interaction with all five DELLA proteins and promotion of RGA protein accumulation in *Arabidopsis*. Genetic analysis shows that CRY1-mediated growth repression is achieved by means of the DELLA proteins. Interestingly, we find that CRY1 also directly interacts with the GA receptor GID1 to competitively inhibit the GID1-GAI interaction. We also show that overexpression of TaCRY1a reduces plant height and coleoptile growth in wheat and that TaCRY1a interacts with both TaGID1 and Rht1 to competitively attenuate the TaGID1-Rht1 interaction. Based on these findings, we propose that the photoreceptor CRY1 competitively inhibits the GID1-DELLA interaction, thereby stabilizing DELLA proteins and enhancing their repression of plant growth.

Key words: wheat, plant height, gibberellin, cryptochrome 1, GID1, DELLA

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INTRODUCTION

Cryptochromes (CRYs) are blue light receptors that mediate photoresponses in plants, and they are a group of evolutionarily conserved flavoproteins found in many organisms. In *Arabidopsis*, there are two homologous CRYs: CRY1 and CRY2. CRY1 is primarily responsible for the inhibition of hypocotyl elongation under blue light, whereas CRY2 mainly controls photoperiodic flowering under long-day conditions (Ahmad and Cashmore, 1993; Guo et al., 1998). CRYs are comprised of a conserved N-terminal photolyase-related (PHR) domain for chromophore FAD binding and a C-terminal domain (CCT) of variable length (Yang et al., 2000; Cashmore, 2003; Partch et al., 2005). Most currently known cryptochrome-interacting proteins, except CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1), physically interact with the PHR domain of cryptochromes (Wang and Lin, 2020). Both CRY1 and CRY2 interact with COP1/SUPPRESSOR OF PHYTOCHROME A-105 1 (SPA1), leading to a disruption of the COP1-SPA1 core complex and thus inhibiting the E3 ubiquitin ligase activities of COP1 in a blue light-dependent manner. They suppress COP1-dependent degradation of transcription factors, including Long Hypocotyl 5 (HY5), Long Hypocotyl in Far-Red 1, and CONSTANS, that regulate photomorphogenesis and photoperiodic flowering (Osterlund et al., 2000; Wang et al., 2001; Yang et al., 2001, 2005; Jang et al., 2008; Liu et al., 2008, 2011; Lian et al., 2011; Zuo et al., 2011). When illuminated by blue light, CRY1 interacts with BRASSINAZOLE-RESISTANT 1 (BZR1)/BRI1 EMS SUPPRESSOR 1 (BES1) and PHYTOCHROME INTERACTING FACTOR 4 (PIF4) to repress target gene

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expression and inhibit hypocotyl elongation (Ma et al., 2016; Wang et al., 2018; He et al., 2019). In addition, CRY1 also promotes photomorphogenesis through blue light-dependent interactions with AUX/IAA and AUXIN RESPONSE FACTOR (ARF) proteins (Xu et al., 2018; Mao et al., 2020).

The phytohormone gibberellin (GA) regulates a variety of plant growth and developmental processes, including seed germination, stem elongation, leaf expansion, and flower and seed development (Yamaguchi, 2008). The GA signaling pathway has been well established in the model plant species *Arabidopsis* and rice (*Oryza sativa*). In canonical GA signaling, the soluble receptor GA-INSENSITIVE DWARF 1 (GID1) senses and binds active GAs, thereby tethering DELLAs for poly-ubiquitination by the E3 ligase $SCF^{SLEEPY1/GID2}$ and subsequent degradation by the 26S proteasome, promoting plant growth (Sasaki et al., 2003; Ueguchi-Tanaka et al., 2005, 2007; Sun, 2011). DELLA proteins negatively regulate GA signaling and act as key repressors of GA-responsive growth (Peng et al., 1997; Silverstone et al., 1998). It is generally believed that the DELLA transcriptional regulators are nuclear-localized proteins that interact with multiple transcription factors, such as PIFs, and repress their activity (de Lucas et al., 2008; Feng et al., 2008). On the other hand, they are recruited by type-B ARABIDOPSIS RESPONSE REGULATORS to the promoters of cytokinin-regulated genes, where they act as transcriptional coactivators. DELLAs also function as transcriptional activators using the indeterminate domain family proteins as transcriptional scaffolds for DNA binding to regulate the expression of their downstream targets (Marin-de la Rosa et al., 2015; Yoshida et al., 2014).

The "green revolution" of the 1960s increased cereal crop yield through the cultivation of semi-dwarf plant varieties. The semi-dwarfism of green revolution wheat and rice varieties is conferred by the *Reduced height-1* (*Rht-1*) and *semi-dwarf1* (*sd1*) mutant alleles, respectively, and the genes responsible for these traits participate in the signaling and biosynthesis of the phytohormone GA (Peng et al., 1999; Sasaki et al., 2002). Gene cloning showed that the mutant wheat DELLA protein Rht-1 is insensitive to GA-triggered degradation (Peng et al., 1999), whereas the rice *sd1* allele reduces GA biosynthesis and consequently results in the accumulation of the rice DELLA protein SLR1 (Slender Rice 1) (Sasaki et al., 2002; Spielmeyer et al., 2002; Li et al., 2018). Therefore, these alleles repress plant growth by causing the accumulation of growth-repressing DELLA proteins.

Light and GA antagonistically regulate plant growth. Light regulation of GA biosynthesis is mediated through the COP1/HY5 pathway, and GA modulates light signaling to prevent de-etiolation of *Arabidopsis* seedlings in darkness (Alabadi et al., 2008; Weller et al., 2009). The direct interaction of DELLA proteins with the transcription factors PIF3 and PIF4 was shown to integrate light and GA signaling pathways (de Lucas et al., 2008; Feng et al., 2008). A recent report showed that DELLAs are destabilized not only by the canonical GA-dependent pathway but also by COP1 under shade and warm temperatures (Blanco-Tourinan et al., 2020). FLAVIN-BINDING KELCH REPEAT F-BOX 1 (FKF1) regulates photoperiodic flowering in part by reducing DELLA protein stability under long-day photoperiods in *Arabidopsis* (Yan et al., 2020).

In this study, we reveal a direct interaction of distinct receptors that links light and GA signaling in *Arabidopsis* and wheat. We find that CRY1 interacts directly with GID1 and DELLA proteins in a blue light-dependent manner to disrupt their interaction, thereby stabilizing DELLA proteins and enhancing DELLA repression of plant growth. Coincidentally, our findings in *Arabidopsis* and wheat are in line with two recent reports (Xu et al., 2021; Zhong et al., 2021) showing that blue light-dependent interactions of CRY1 with GID1 and DELLA proteins regulate gibberellin signaling and photomorphogenesis in *Arabidopsis*.

RESULTS

CRY1 negatively regulates GA response and genetically interacts with DELLA proteins in *Arabidopsis*

CRY1 plays a key role in the inhibition of hypocotyl elongation in response to blue light signaling (Ahmad and Cashmore, 1993), and the phytohormone GA promotes plant growth by stimulating the destruction of DELLAs (Harberd et al., 2009; Xu et al., 2014). Given the antagonistic roles of CRY1 and GA in the modulation of hypocotyl elongation, we were interested in exploring the relationship between CRY1 and GA signaling in this process. To this end, we investigated the sensitivity of hypocotyl elongation in *cry1* mutant and *CRY1*-overexpressing plants in response to exogenous GA3. To eliminate the influence of endogenous GA synthesis, we supplemented half-strength Murashige and Skoog ($^1/_2$ MS) medium with PAC (paclobutrazol, a GA biosynthesis inhibitor). The hypocotyl lengths of Col-0, *cry1* mutant, and *CRY1*-overexpressing plants were largely similar when grown on $^1/_2$ MS medium with 0.5 μM PAC (Figure 1A and 1B). The *cry1* mutant was more sensitive to exogenous GA3 than Col-0 when the long hypocotyl phenotype of *cry1* was abolished by the GA synthesis inhibitor PAC under blue light (Figure 1A–1C). By contrast, compared with Col-0, the *CRY1*-overexpressing plants *35S:GFP-CRY1* (He et al., 2019) and *35S:CRY1-Flag* generated in this study displayed decreased sensitivity to exogenous GA3 treatment under blue light (Figure 1A–1C). Taken together, these results indicate that CRY1 negatively regulates GA response in *Arabidopsis*.

Next, we investigated the genetic interaction relationship between CRY1 and the DELLAs. The GA-insensitive *gai-1* mutant lacks a short (17 amino acid) segment of the GAI protein, is relatively resistant to the effects of GA, and displays a reduced hypocotyl elongation phenotype (Fu et al., 2004; Peng et al., 1997). Here, we generated the *cry1 gai-1* double mutant by genetic crossing of the *cry1* and *gai-1* mutants. We observed that the *gai-1* mutant displayed markedly reduced hypocotyl elongation compared with wild-type (WT) plants under blue light (Figure 1D and 1E). Notably, the gain-of-function *gai-1* mutation could partially suppress the longer hypocotyl phenotype of the *cry1* mutant under blue light (Figure 1D and 1E). These results indicate that GAI acts downstream of CRY1 in the regulation of photomorphogenesis. We also generated *35S:AtCRY1-Flag* homozygous transgenic lines in the quadruple-*DELLA* (*della*) mutant background (Achard et al., 2007). As shown in Figure 1F–1H, overexpression of AtCRY1 significantly reduced hypocotyl elongation in the WT background under blue light, but it failed to repress hypocotyl elongation in the *della* mutant background, suggesting that CRY1 represses hypocotyl elongation by means of the DELLAs.

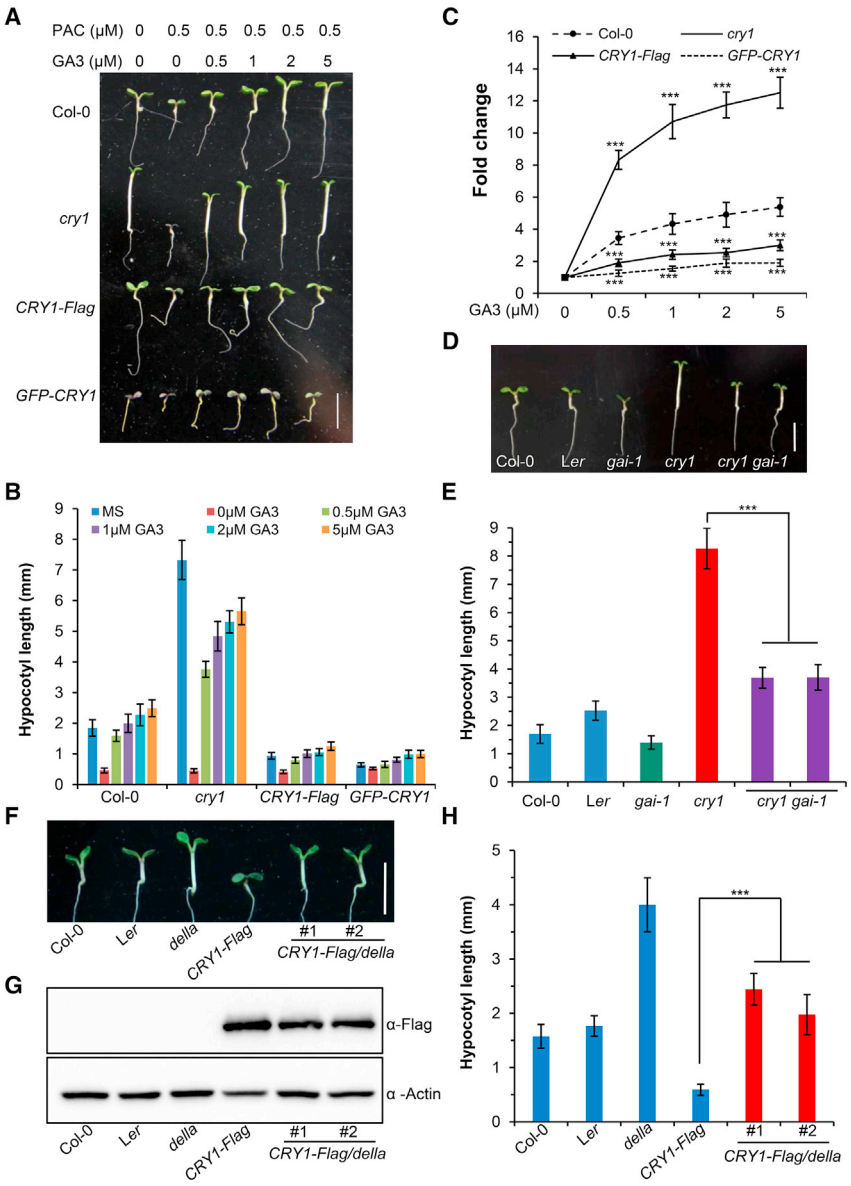

Figure 1. CRY1 negatively regulates GA response and genetically interacts with DELLA proteins in *Arabidopsis*.

(A) Hypocotyl elongation phenotype of the CRY1-overexpressing and *cry1* mutant plants in response to GA3 treatment. Representative images show Col-0, CRY1-overexpressing, and *cry1* mutant seedlings grown in $^1/_2$ MS medium with the indicated concentrations of exogenous GA3 and PAC under blue light for 5 days. Scale bars correspond to 5 mm.

(B) Quantification of the hypocotyl lengths of the indicated genotypes in **(A)**. Data are mean ± SD (n > 20).

(C) Fold change in hypocotyl length. The hypocotyl lengths of the indicated genotypes in response to different GA3 concentrations are shown relative to that of seedlings grown in $^1/_2$ MS medium with 0.5 μM PAC. Data are mean ± SD (n > 20). ***P < 0.001, Student's t-test.

(D and E) The hypocotyl lengths of the indicated seedlings grown under blue light. The image in **(D)** shows representative seedlings of the indicated genotypes. Scale bars correspond to 5 mm.

(E) Quantification of hypocotyl lengths of the indicated genotypes. Data are mean ± SD (n > 20). ***P < 0.001, Student's t-test.

(F–H) The hypocotyl lengths of the indicated seedlings grown under blue light. The image in **(F)** shows representative seedlings of the indicated genotypes. Scale bars correspond to 5 mm. **(G)** Immunoblotting showing CRY1-Flag protein levels in the indicated genotypes. Actin was used as the loading control. **(H)** Quantification of hypocotyl lengths of the indicated genotypes. Data are mean ± SD (n > 20). ***P < 0.001, Student's t-test.

Taken together, these genetic interaction assays demonstrate that CRY1 represses hypocotyl elongation at least in part through the function of DELLA proteins.

CRY1 physically interacts with the DELLA and GID1 proteins in *Arabidopsis*

Previous studies have shown that the DELLA family proteins are key repressors of GA responses (Lee et al., 2002; Richards et al., 2001). The degradation of DELLAs releases plants from DELLA-mediated growth restraint (Harberd, 2003). There are five DELLAs in *Arabidopsis*: GAI, RGA, RGL1, RGL2, and RGL3 (Dill and Sun, 2001; Hussain et al., 2005; Lee et al., 2002; Silverstone et al., 2001; Wen and Chang, 2002). Meanwhile, CRY1 is a master regulator of hypocotyl elongation in *Arabidopsis*. In this context, we speculated that CRY1 may interact directly with DELLAs to regulate hypocotyl elongation. To test this idea, we performed firefly luciferase complementation imaging (LCI) assays and co-immunoprecipitation (co-IP) assays to evaluate the physical interactions between CRY1 and DELLA proteins. For LCI assays, CRY1 was fused to nLUC, and five DELLA proteins were fused to cLUC. Strong interaction signals were clearly observed following co-expression of CRY1 and five DELLA proteins in *Nicotiana benthamiana* leaves under continuous blue light, whereas there was no signal in the negative controls (Figure 2A). By contrast, these interactions were not detectable under dark conditions, except for a very weak interaction between CRY1 and RGL3 (Figure 2A). These results suggest that CRY1 interacts with the five DELLA proteins in a blue light-dependent manner. To further confirm the interaction between CRY1 and DELLAs in *Arabidopsis*, we generated *pRGA:GFP-RGA/35S:CRY1-Flag* double transgenic plants by genetic crossing between the *pRGA:GFP-RGA* (Silverstone et al., 2001) and *35S:CRY1-Flag* lines. The *35S:CRY1-Flag*, *pRGA:GFP-RGA*, and *pRGA:GFP-RGA/35S:CRY1-Flag* transgenic plants were grown under continuous blue light for 7 days, then subjected to co-IP analysis. The co-IP assays revealed that the CRY1-Flag fusion protein was precipitated by GFP-RGA in the double transgenic *pRGA:GFP-RGA/35S:CRY1-Flag* seedlings, demonstrating the interaction between CRY1 and RGA in *Arabidopsis* (Figure 2B). Based on these results, we concluded that CRY1 interacts with DELLA proteins in a blue light-dependent manner.

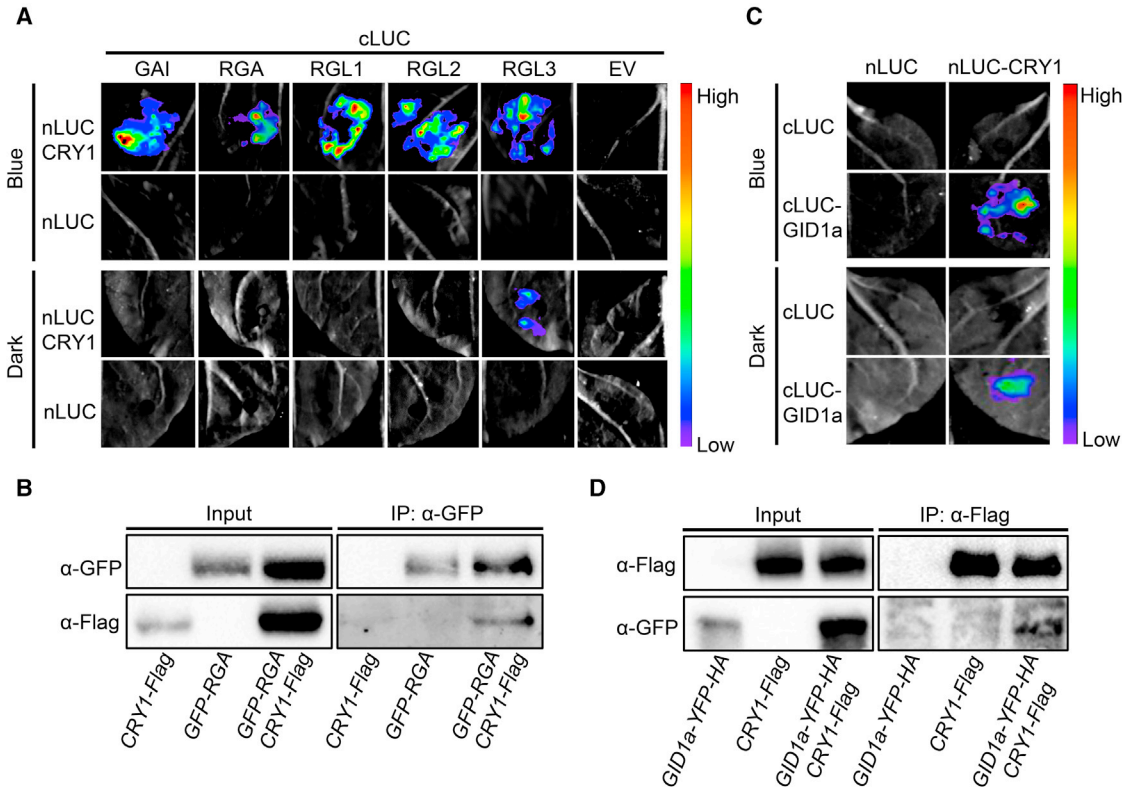

Figure 2. CRY1 physically interacts with the DELLAs and GID1a proteins in *Arabidopsis*.

(A) LCI assays showing the interactions between CRY1 and GAI/RGA/RGL1/RGL2/RGL3 in *N. benthamiana* leaves under blue light (40 μmol/m^2/s) and in darkness. Empty vectors were used as negative controls.

(B) Co-IP assays showing that CRY1 physically associates with RGA *in vivo*. The *35S*:*CRY1-Flag*, *pRGA*:*GFP-RGA*, and *pRGA*:*GFP-RGA*/*35S*:*CRY1-Flag* transgenic plants were grown under continuous blue light for 7 days.

(C) LCI assays showing the interactions between CRY1 and GID1a in *N. benthamiana* leaves under blue light and dark conditions. Empty vectors were used as negative controls.

(D) Co-IP assays showing that CRY1 physically associates with GID1a *in vivo*. The *35S*:*GID1a-YFP-HA*, *35S*:*CRY1-Flag*, and *35S*:*GID1a-YFP-HA*/*35S*:*CRY1-Flag* transgenic plants were grown under continuous blue light for 7 days.

Next, we sought to explore whether CRY1 also interacts with GID1. To investigate the physical interaction between CRY1 and GID1, we first performed LCI assays in *N. benthamiana* leaves. As shown in Figure 2C, strong LUC activity was detected in the samples that co-expressed nLUC-CRY1/cLUC-GID1a under blue light, but no or very weak LUC activity was detected in darkness (Figure 2C). These results suggest that CRY1 undergoes blue light-dependent physical interaction with GID1a in plant cells. We next conducted co-IP assays using the *35S*:*GID1a-YFP-HA*, *35S*:*CRY1-Flag*, and *35S*:*GID1a-YFP-HA*/*35S*:*CRY1-Flag* transgenic seedlings. Yellow fluorescent protein (YFP)-fused hemagglutinin (HA)-tagged GID1a was precipitated by CRY1-Flag (Figure 2D), demonstrating the physical interaction between CRY1 and GID1a in *Arabidopsis*. We also performed co-IP assays demonstrating the blue light-dependent interaction, at least in part, of CRY1 and GID1a in *Arabidopsis* (supplemental Figure 1). From these results, we concluded that the light and GA receptors CRY1 and GID1 interact directly in a blue light-dependent manner.

Based on the demonstration that CRY1 interacts with DELLA proteins, we sought to better understand the features of the interaction between CRY1 and DELLA proteins. CRYs are comprised of a conserved PHR domain and a CCT domain (Yang et al., 2000; Cashmore, 2003; Partch et al., 2005). The C-terminal domains of *Arabidopsis* CRY1 and CRY2, known as CCT1 (CRY1 C terminus) and CCT2 (CRY2 C terminus) (Yang et al., 2000), mediate CRY signaling via direct interactions with COP1 (Wang et al., 2001; Yang et al., 2001). However, it has been reported that most known cryptochrome-interacting proteins, except COP1, physically interact with the PHR domain of cryptochromes (Wang and Lin, 2020). To define the interaction domains of CRY1 with DELLA proteins, the full-length CRY1 protein was divided into N-terminal (CNT1, residues 1–489) and C-terminal (CCT1, residues 490–681) parts (He et al., 2019). The CNT1 and CCT1 parts of CRY1 were fused to nLUC to generate nLUC-CNT1 and nLUC-CCT1, respectively. The LCI assays showed strong interaction signals in the samples that co-expressed nLUC-CCT1/cLUC-RGA and weak interaction signals in the samples that co-expressed nLUC-CNT1/cLUC-RGA under continuous blue light. No signal was detected in the control samples (supplemental Figure 2A). These results suggest that the CCT1 of CRY1 is primarily responsible for mediating its interaction with RGA in *Arabidopsis*. We also investigated the interaction domains of RGA with CRY1: the full-length RGA was divided into the N-terminal domain (residues 1–213) and the C-terminal

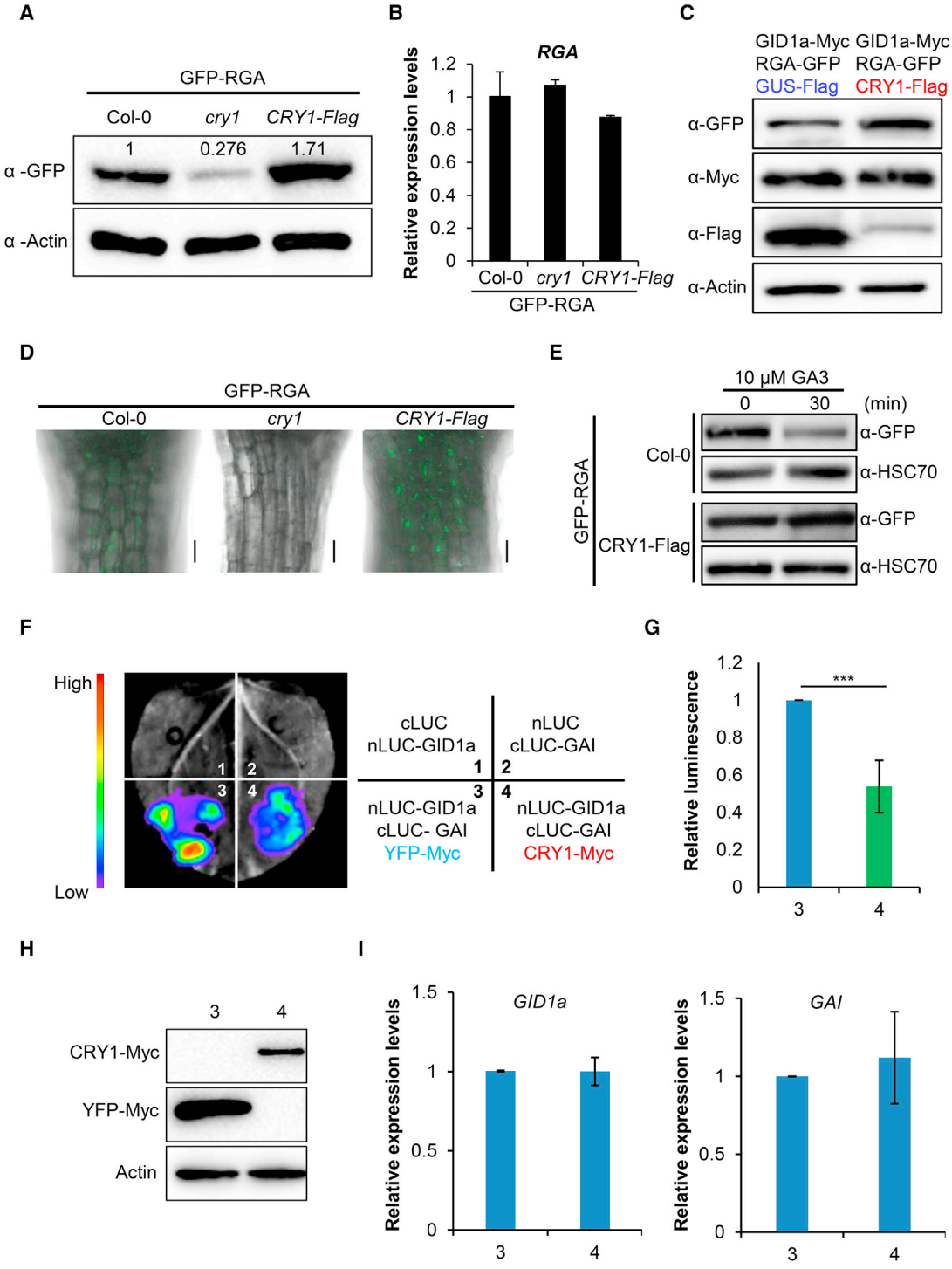

Figure 3. CRY1 promotes RGA protein accumulation and interferes with the interaction of GID1 and GAI.

(A and B) CRY1 promotes the protein accumulation of RGA. *pRGA*:*GFP-RGA*, *pRGA*:*GFP-RGA/35S*:*CRY1-Flag*, and *pRGA*:*GFP-RGA/cry1* seedlings were grown under continuous blue light for 6 days. **(A)** Immunoblotting showing the quantification of GFP-RGA in the indicated seedlings. Actin was used as the control. **(B)** Relative mRNA levels of RGA in the indicated seedlings. Data are mean ± SD (n = 3).

(C) Immunoblotting showing the regulation of RGA protein abundance by CRY1 in *N. benthamiana* leaves. Actin was used as the control.

(D) CRY1 promotes the nuclear accumulation of RGA. The subcellular localization of GFP-RGA in epidermal cells at the top region of the hypocotyls was observed in the indicated 6-day-old seedlings by laser confocal microscopy. *pRGA*:*GFP-RGA*, *pRGA*:*GFP-RGA/35S*:*CRY1-Flag*, and *pRGA*:*GFP-RGA/cry1* seedlings were grown under continuous blue light. Scale bars correspond to 50 μm.

(E) Immunoblotting showing that CRY1 represses the GA-induced degradation of RGA. *pRGA*:*GFP-RGA* and *pRGA*:*GFP-RGA/35S*:*CRY1-Flag* seedlings were grown on medium containing 0.01 μM PAC under continuous blue light for 5 days, then treated with 10 μM GA3 for the indicated times. Hsc70 was

(legend continued on next page)

domain (residues 214–587). The N-terminal and C-terminal domains of RGA were fused to cLUC to generate cLUC-RGA-NT and cLUC-RGA-CT, respectively. The LCI assays showed strong interaction signals in the samples that co-expressed nLUC-CRY1/cLUC-RGA-CT and weak interaction signals in the samples that co-expressed nLUC-CRY1/cLUC-RGA-NT under continuous blue light. No signal was detected in the control samples (supplemental Figure 2B). These results suggest that the C-terminal domain of RGA is primarily responsible for mediating its interaction with CRY1 in *Arabidopsis*.

CRY1 promotes RGA accumulation and interferes with the GID1-GAI interaction

GA-activated GID1 stimulates the destruction of DELLAs, thus promoting plant growth (Harberd et al., 2009; Xu et al., 2014). The above finding that CRY1 interacts with both GID1 and DELLAs in *Arabidopsis* prompted us to ask whether CRY1 modulates the stability of DELLA proteins. To address this question, we crossed an *Arabidopsis* transgenic line expressing GFP-RGA driven by an endogenous promoter fragment (Silverstone et al., 2001; Shani et al., 2013) into the *cry1* and *35S:CRY1-Flag* backgrounds. Western blot analysis showed that GFP-RGA protein levels were higher in the *CRY1*-overexpressing lines than in the control plants, whereas they were abrogated or lower in the absence of CRY1 (Figure 3A), suggesting that CRY1 is required for the accumulation of RGA protein. Notably, at the transcriptional level, no major perturbations in *RGA* expression were observed in the *cry1* mutant and *CRY1*-overexpressing lines compared with the control plants (Figure 3B). Furthermore, overexpression of CRY1 in a transient expression system increased RGA protein abundance (Figure 3C). Next, confocal microscopy observations of the GFP-RGA transgenic lines in the *cry1* mutant and *CRY1*-overexpressing backgrounds revealed remarkable differences in GFP-RGA accumulation in hypocotyls from the different backgrounds (Figure 3D). Immunoblotting analysis of the *pRGA:GFP-RGA and pRGA:GFP-RGA/35S:CRY1-Flag seedlings* demonstrated that CRY1 repressed the GA-induced protein degradation of RGA (Figure 3E). Based on these results, we concluded that CRY1 represses the GID1-mediated degradation of RGA proteins.

Because blue light-activated CRY1 interacts with both GID1 and DELLA, we asked whether CRY1 modulates the interaction between GID1 and DELLA. To test this possibility, we transiently co-expressed CRY1-Myc with nLUC-GID1a/cLUC-GAI in *N. benthamiana* leaves under blue light. The YFP-Myc fusion protein was expressed instead of CRY1-Myc to serve as a control. The intensity of luminescence generated by the interaction of nLUC-GID1a and cLUC-GAI was clearly reduced when CRY1-Myc was co-expressed (Figure 3F–3I). These observations indicate that CRY1 may reduce the physical interaction between GID1 and DELLA proteins (such as GAI) in plant cells.

TaCRY1 regulates wheat plant height

To explore whether the *Arabidopsis* CRY1 ortholog TaCRY1 regulates plant architecture in wheat, we identified nine putative CRY1 and CRY2 orthologous protein sequences from the wheat genome (Chinese spring [CS]) (supplemental Figure 3). We focused on *TaCRY1a* (TraesCS6A02G203300, supplemental Figure 5) for further study, as it is most closely related to *Arabidopsis CRY1* (supplemental Figures 3 and 4). We generated *pUbi:Flag-TaCRY1a* transgenic lines (OE 1 and OE 2) in the bread wheat cultivar 'Kelong199' (KN199) background. Overexpression of *TaCRY1a* resulted in a reduction of plant height in wheat (Figure 4A and 4D), and statistical analyses showed a dramatic decrease in plant height from ~70 cm in WT KN199 to ~55 cm in the transgenic lines (Figure 4D). We also compared plant stem length between KN199 and *pUbi:Flag-TaCRY1a* transgenic wheat lines, and we found that each internode was shorter in *pUbi:Flag-TaCRY1a* transgenic plants than in KN199 (Figure 4B and 4E). As shown in Figure 4C, immunoblotting analyses showed that TaCRY1a protein was highly expressed in the transgenic lines; notably, there were two TaCRY1 protein bands in the transgenic plants, one of which may represent phosphorylated TaCRY1 protein. Next, we investigated whether TaCRY1 affected coleoptile growth in wheat. The coleoptile lengths of TaCRY1-overexpressing lines were shorter than those of WT KN199 under blue light (Figure 4F and 4G). We also generated TaCRY1-overexpressing transgenic *Arabidopsis* plants, which displayed reduced hypocotyl elongation under blue light (Figure 4H and 4I). This genetic evidence clearly confirmed that TaCRY1 (TraesCS6A02G203300) is indeed one ortholog of *Arabidopsis* CRY1, both in terms of protein sequence and biological function. Taken together, these results suggest that TaCRY1 functions as a negative regulator of plant height in bread wheat.

Wheat CRY1 also interacts with wheat GID1 and the wheat DELLA protein Rht1

In light of our above results in *Arabidopsis*, we wondered whether TaCRY1 also interacts with the critical GA signaling regulators TaGID1 and Rht1 in wheat. To investigate this possibility, we performed LCI and yeast two-hybrid (Y2H) assays to assess the physical interaction between TaCRY1 and TaGID1/Rht1. For LCI assays in *N. benthamiana* leaves, TaCRY1a was fused to nLUC, and Rht-A1 (*Rht-A1*-CDS, supplemental Figure 6), Rht-B1 (*Rht-B1*-CDS, supplemental Figure 7), Rht-D1 (*Rht-D1*-CDS, supplemental Figure 8), and TaGID1 (*TaGID1-1B*-CDS, supplemental Figure 9) were fused to cLUC. The LCI assays showed that strong interaction signals were clearly observed between TaCRY1a and Rht-A1/Rht-B1/Rht-D1/TaGID1 under continuous blue light (Figure 5A and 5C). By contrast, only weak LUC activity was detected in darkness, and there were no signals in the negative controls under either continuous blue light or dark conditions (Figure 5A and 5C). These results

used as the loading control. **(F–I)** CRY1 competitively attenuates the GID1-DELLA interaction. **(F and G)** CRY1 reduces the interaction of GID1a and GAI in *N. benthamiana* leaves under blue light in LCI assays. YFP-Myc was used as a control. A representative image is shown in **(F)**, and the quantification of relative luminescence intensities is shown in **(G)**. Error bars denote ±SD (n = 10). ***P < 0.001, Student's t-test. **(H)** The protein levels of YFP-Myc and CRY1-Myc in the infiltrated *N. benthamiana* leaf areas (infiltrations 3 and 4) were determined by immunoblotting. Actin was used as the loading control. **(I)** The transcript levels of *GID1a* and *GAI* in the infiltrated *N. benthamiana* leaf areas (infiltrations 3 and 4) were quantified by qRT–PCR. Results were normalized to *NbACTIN1* (*NbACT1*). Data are mean ± SD (n = 3).

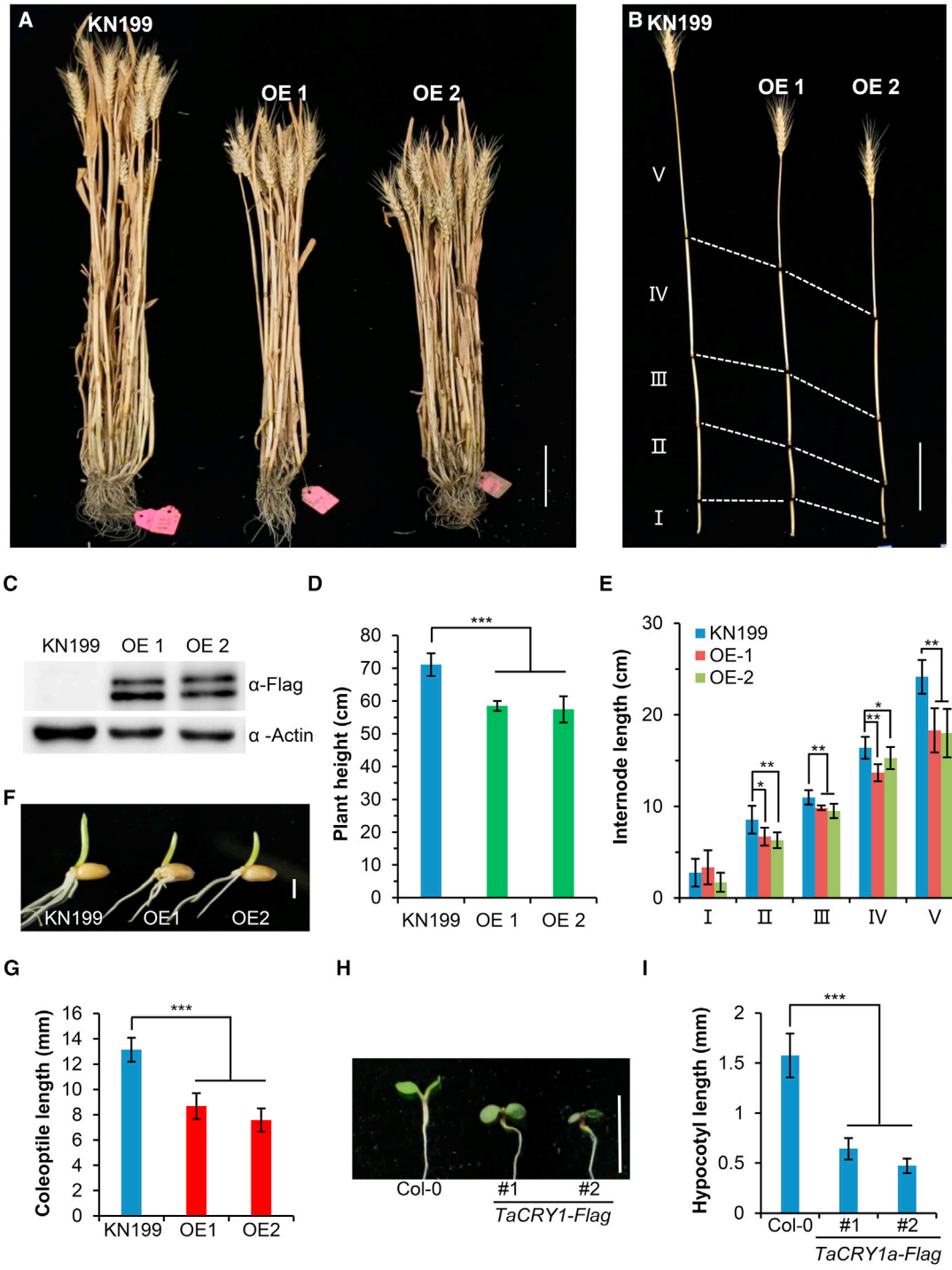

Figure 4. Overexpression of TaCRY1 reduces wheat plant height.

(A) Plant height of the *pUbi:Flag-TaCRY1a* transgenic wheat lines at the mature stage. Scale bars correspond to 10 cm.

(B) Comparison of each internode length between KN199 and *pUbi:Flag-TaCRY1a* transgenic wheat lines. Scale bars correspond to 10 cm.

(C) Immunoblotting showing the protein levels of Flag-TaCRY1a. Actin was used as the loading control.

(D) Quantification of plant height of the indicated genotypes in **(A)**. Error bars denote ±SD ($n > 10$). ***$P < 0.001$, Student's *t*-test.

(E) Quantification of each internode length of the indicated genotypes in **(B)**. Error bars denote ±SD ($n > 3$). *$P < 0.1$, **$P < 0.05$, Student's *t*-test.

(F and G) TaCRY1 represses coleoptile growth in wheat. The image in **(F)** shows the coleoptile phenotypes of KN199 and *pUbi:Flag-TaCRY1a* transgenic wheat lines. Scale bars correspond to 5 mm. The quantification of coleoptile lengths is shown in **(G)**. Error bars denote ±SD ($n > 20$). ***$P < 0.001$, Student's *t*-test. **(H and I)** TaCRY1 represses plant growth in *Arabidopsis*. The image in **(H)** shows the hypocotyl elongation phenotypes of the *35S:TaCRY1-Flag* transgenic lines. Scale bars correspond to 5 mm. **(I)** The quantification of hypocotyl lengths of the indicated genotypes in **(H)**. Error bars denote ±SD ($n > 20$). ***$P < 0.001$, Student's *t*-test.

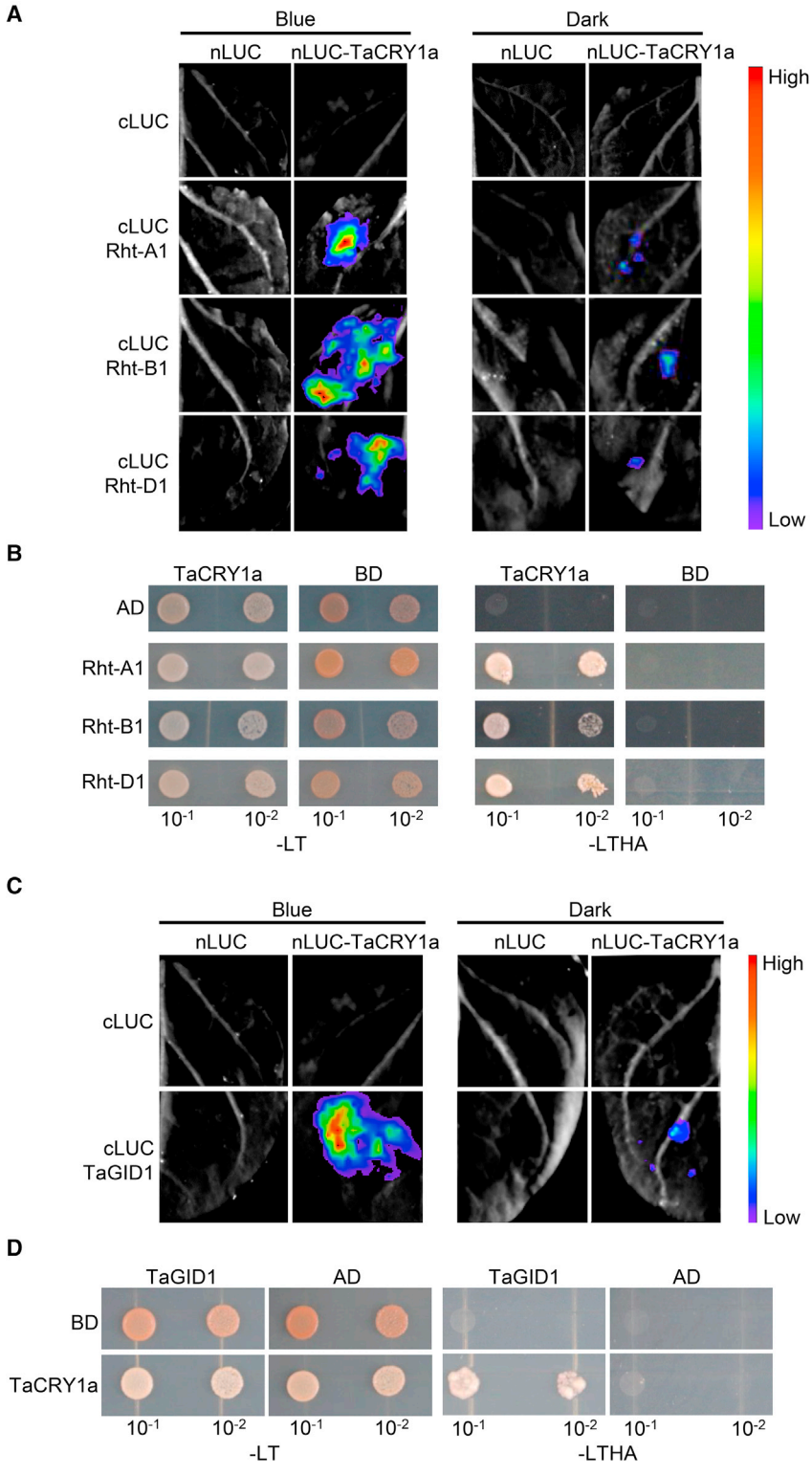

Figure 5. TaCRY1 physically interacts with TaGID1 and Rht1.

(A and B) TaCRY1 physically interacts with Rht1. **(A)** LCI assays showing the interactions between TaCRY1a and TaRht-A1/B1/D1 in *N. benthamiana* leaves under continuous blue light and dark conditions. Empty vectors were used as negative controls. **(B)** Y2H assays showing the interaction between TaCRY1a and TaRht-A1/B1/D1. SD-L/T, synthetic dextrose medium lacking Leu and Trp; SD-L/T/H/A, synthetic dextrose medium lacking Leu, Trp, His, and Ade.

(C and D) TaCRY1 physically interacts with TaGID1. **(C)** LCI assays showing the interactions between TaCRY1a and TaGID1 in *N. benthamiana* leaves under continuous blue light and dark conditions. Empty vectors were used as negative controls. **(D)** Y2H assays showing the interaction between TaCRY1a and TaGID1. SD-L/T, synthetic dextrose medium lacking Leu and Trp; SD-L/T/H/A, synthetic dextrose medium lacking Leu, Trp, His, and Ade.

suggest that TaCRY1 physically associates with TaGID1 and Rht1 in a blue light-dependent manner. For Y2H assays, TaCRY1a was fused to pGBDKT7 to generate pGBDKT7-TaCRY1a, and Rht-A1/Rht-B1/Rht-D1/TaGID1 were fused to pGADKT7 to generate pGADKT7-Rht-A1, pGADKT7-Rht-B1, pGADKT7-Rht-D1, and pGADKT7-TaGID1. The Y2HGold strain yeast cells transformed with BD-TaCRY1a and AD-Rht-A1/AD-Rht-B1/AD-Rht-D1/AD-TaGID1 grew better than the negative controls on limited medium (Figure 5B and 5D). Taken together, these results suggest that wheat CRY1 directly interacts with wheat GID1 and the wheat DELLA protein Rht1.

To define the interaction domains of TaCRY1 with Rht1, the full-length TaCRY1a protein was divided into N-terminal and C-terminal parts (supplemental Figure 10A) (He et al., 2019). The N-terminal (CNT1, residues 1–494) and C-terminal (CCT1, residues 495–698) parts of TaCRY1a were fused to nLUC to generate nLUC-CNT1 and nLUC-CCT1, respectively. The LCI assays showed strong interaction signals in the samples that co-expressed nLUC-CCT1/cLUC-Rht1 and weak interaction signals in the samples that co-expressed nLUC-CNT1/cLUC-Rht1 under continuous blue light. No signal was detected in the control samples (supplemental Figure 10B). These results suggest that the C-terminal region of TaCRY1 is primarily responsible for mediating its interaction with Rht1. To define the interaction domains of Rht1 with TaCRY1, the full-length Rht-B1 was divided into the N-terminal domain containing the DELLA motif and the C-terminal domain (supplemental Figure 10C). The N-terminal and C-terminal domains of Rht-B1 were fused to cLUC to generate cLUC-Rht-B1-NT (residues 1–223) and cLUC-Rht-B1-CT (residues 224–621), respectively. The LCI assays showed strong interaction signals in the samples that co-expressed nLUC-TaCRY1a/cLUC-Rht-B1-CT and weak interaction signals in the samples that co-expressed nLUC-TaCRY1a/

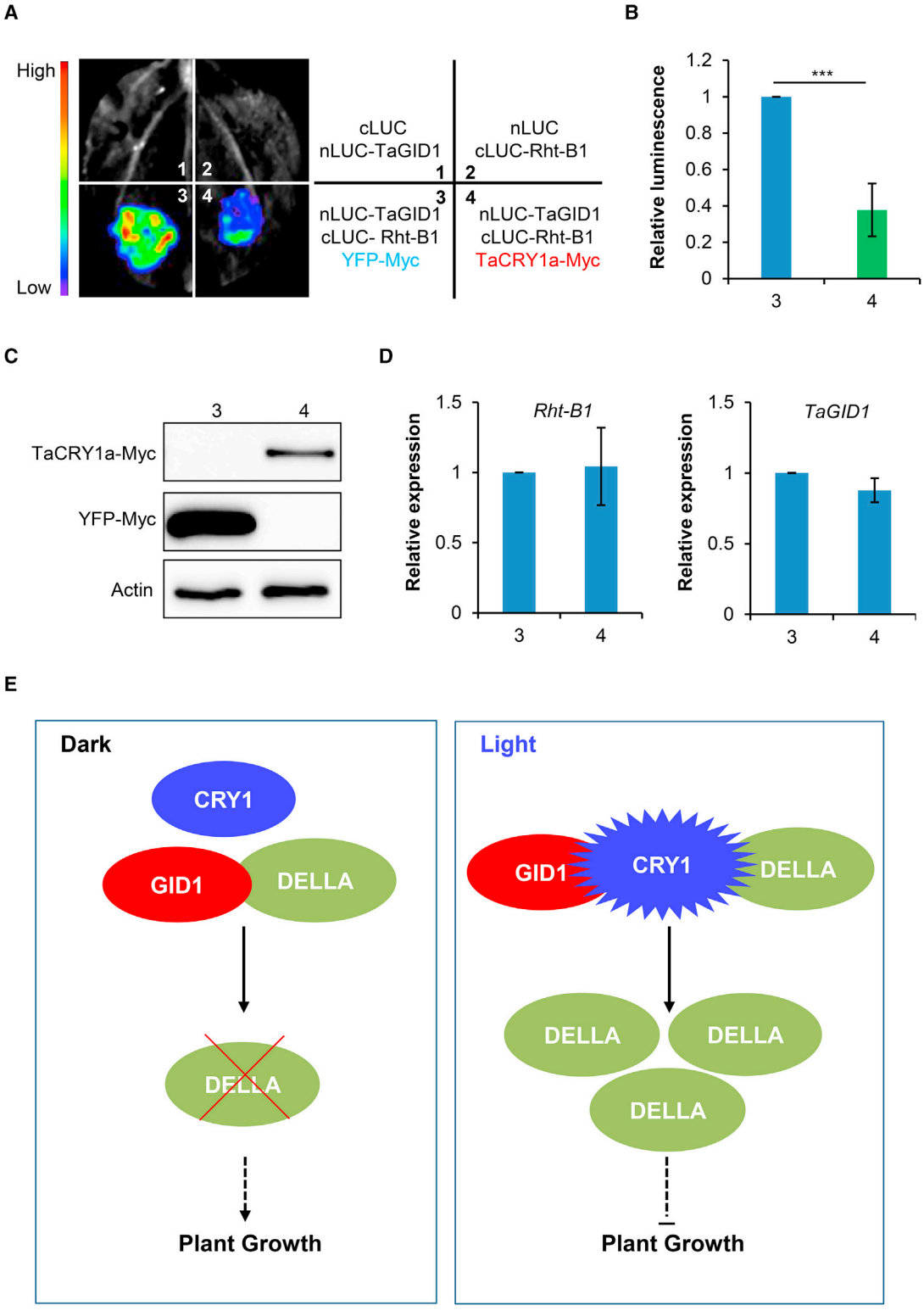

Figure 6. TaCRY1 competitively attenuates the TaGID1-Rht1 interaction.

(A and B) LCI assays showing that TaCRY1 reduces the interaction of TaGID1 and Rht1 in *N. benthamiana* leaves under blue light. YFP-Myc was used as a control. A representative image is shown in **(A)**, and the quantification of relative luminescence intensities is shown in **(B)**. Error bars denote ±SD ($n = 22$). ***$P < 0.001$, Student's *t*-test.

(C) The protein levels of YFP-Myc and TaCRY1a-Myc in the infiltrated *N. benthamiana* leaf areas (infiltrations 3 and 4) were determined by immunoblotting. Actin was used as the loading control.

(legend continued on next page)

cLUC-Rht-B1-NT under continuous blue light. No signal was detected in the control samples (supplemental Figure 10D). These results suggest that the C-terminal region of Rht1 is primarily responsible for mediating its interaction with TaCRY1.

TaCRY1 attenuates the TaGID1-Rht1 interaction

Because blue light-activated TaCRY1 interacts with both TaGID1 and Rht1, we wondered whether TaCRY1 affects the physical interaction between TaGID1 and Rht1. To test this idea, we transiently co-expressed TaCRY1a-Myc with nLUC-TaGID1/cLUC-Rht-B1 in *N. benthamiana* leaves under blue light. The YFP-Myc protein was expressed instead of TaCRY1a-Myc to serve as a control. The intensity of luminescence generated by the interaction of nLUC-TaGID1 and cLUC-Rht-B1 was clearly reduced when TaCRY1a-Myc was co-expressed (Figure 6A–6D). These observations indicate that TaCRY1 interferes with the physical interaction between TaGID1 and Rht1.

DISCUSSION

Light and GA are two essential external and internal cues that affect plant architecture and mediate many essential and partially overlapping plant developmental processes. Light induces GA biosynthesis in germinating seeds (Oh et al., 2006) but represses GA biosynthesis in de-etiolating seedlings (Achard et al., 2007). How light and GA signaling antagonistically regulate plant growth has been extensively studied. Two independent studies demonstrated that DELLA proteins sequester PIF3 and PIF4 and repress their DNA-binding ability, thereby explaining the antagonistic relationship between light and GA signaling (de Lucas et al., 2008; Feng et al., 2008). A recent study showed that DELLAs promote the degradation of PIF proteins by the ubiquitin-proteasome system to coordinate light and GA signaling during plant development (Li et al., 2016). In this study, we found that the blue light receptor CRY1 interacts with the GA receptor GID1 and the DELLA GA signaling repressors to coordinate plant growth. We propose a working model in which the antagonistic regulation of DELLA protein stability by the photoreceptor CRY1 and the GA receptor GID1 allows plants to optimize their growth (Figure 6E).

Wheat green revolution genes that encode mutant GA response repressor DELLA proteins give rise to semi-dwarf plant varieties (Peng et al., 1999). DELLA proteins function as suppressors of GA signaling because their degradation triggers various GA responses *in planta* (Harberd, 2003). In this study, we found that CRY1 negatively regulates GA response in *Arabidopsis* (Figure 1A–1C), and CRY1 represses hypocotyl elongation, at least in part, through the function of DELLA proteins (Figure 1D–1H). Moreover, overexpression of *TaCRY1* led to a reduction of plant height in wheat (Figure 4A and 4D). A previous study has shown that CRY1 mediates blue light inhibition of hypocotyl elongation by reducing GA levels in *Arabidopsis* (Zhao et al., 2007). Therefore, in addition to regulating GA homeostasis, CRY1 also directly represses GA signaling to modulate plant architecture.

In this study, we demonstrate that CRY1 interacts directly with the GA receptor GID1 and with DELLA proteins in *Arabidopsis* and wheat (Figures 2 and 5). We show that CRY1 attenuates the GID1-DELLA interaction in *Arabidopsis* and wheat (Figures 3F–3I and 6A–6D). As expected, CRY1 can enhance the stability of RGA proteins in *Arabidopsis* (Figure 3A and 3C–3E). These results led us to conclude that CRY1 directly interacts with and stabilizes DELLA proteins, at least in part by interfering with the GID1-DELLA interaction.

In past decades, there have been many intensive studies on the crosstalk between light and phytohormones (Lau and Deng, 2010). For example, brassinosteroid (BR) is a key phytohormone involved in the repression of photomorphogenesis. When illuminated by blue light, CRY1 interacts with and inhibits BZR1/BES1, master transcription factors of BR signaling, to repress hypocotyl elongation (Wang et al., 2018; He et al., 2019). The red light receptor phytochrome B (phyB) also interacts with and inhibits BZR1 to repress hypocotyl elongation (Dong et al., 2020). Recent research has shown that the UV-B photoreceptor UVR8 physically interacts with BIM1 (BES1-INTERACTING MYC-LIKE 1) and BES1, which are transcription factors that mediate BR signaling, to repress their DNA-binding activities (Liang et al., 2018). In this study, we found that the blue light receptor CRY1 interacts directly with the GA receptor GID1 to enhance the stability of DELLA proteins, thereby restricting plant growth. The two distinct receptors CRY1 and GID1 competitively prevent or promote the destruction of DELLA proteins, respectively, suggesting that CRY1 acts as a brake system to prevent the overactivation of GA signaling. The direct interaction of the light and GA receptors CRY1 and GID1 may mediate efficient crosstalk between light and GA signaling to regulate plant architecture.

During the revision of this manuscript, two papers reported similar findings in *Arabidopsis* (Xu et al., 2021; Zhong et al., 2021). These papers demonstrated that the blue light receptor CRY1 interacts with GID1 and DELLA proteins to repress GA signaling during photomorphogenesis in *Arabidopsis* (Xu et al., 2021; Zhong et al., 2021). In addition to similar findings in the dicot *Arabidopsis*, we also found that TaCRY1 plays a critical role in regulating plant height in the monocot wheat, potentially by interacting with TaGID1 and Rht-1. Here, we showed that the C-terminal regions of CRY1 and DELLA are primarily responsible for mediating their interaction in both *Arabidopsis* and wheat. However, the two previous papers reported that both the N-terminal and C-terminal regions of CRY1 and DELLA similarly mediate their interaction in *Arabidopsis*.

(D) The transcript levels of *TaGID1* and *Rht-B1* in the infiltrated *N. benthamiana* leaf areas (infiltrations 3 and 4) were quantified by qRT–PCR. Results were normalized to *TaGAPDH*. Data are mean ± SD (n = 3).

(E) A proposed working model describing a CRY1-GID1-DELLA signaling module. Under darkness (left panel), CRY1 is inactive and does not interact with the GID1 and DELLA proteins, thereby promoting the degradation of DELLA proteins by the 26S proteasome and plant growth. In the light (right panel), activated CRY1 competitively attenuates the GID1-DELLA interaction by directly interacting with the GID1 and DELLA proteins, leading to the accumulation of DELLA proteins and the repression of plant growth.

MATERIALS AND METHODS

Plant materials and growth conditions

The WT *Arabidopsis* ecotypes used in this study were Columbia-0 (Col-0) and Landsberg erecta (Ler). All transgenic and mutant lines used in this study were described previously: *35S*:*GFP-CRY1* (He et al., 2019), *cry1* (Gao et al., 2015), *pRGA*:*GFP-RGA* (Silverstone et al., 2001), *gai-1* (Koornneef and van der Veen, 1980), and *della* (quadruple-DELLA, *gai-t6 rga-t2 rgl1-1 rgl2-1*) (Achard et al., 2007). The full-length CRY1 and GID1a coding sequences were cloned into the p1300-35S-Flag and pEarleyGate101 (p35S-YFP-HA) vectors, respectively. Col-0 was transformed with *35S*:*CRY1-Flag*, *35S*:*TaCRY1-Flag*, and *35S*:*GID1a-YFP-HA* by floral dip transformation (Clough and Bent, 1998), and *della* was transformed with *35S*:*CRY1-Flag* by the same method. The *cry1 gai-1* double mutant and *pRGA*:*GFP-RGA/35S*:*CRY1-Flag* were generated by genetic crossing. *Arabidopsis thaliana* was grown under LD conditions (16 h light/8 h dark) at 22°C.

The bread wheat (*Triticum aestivum*) cultivar WT KN199 was used to amplify gene sequences, as the receptor plant for wheat gene transformation, and to analyze gene expression. The full-length *TaCRY1a* (TraesCS6A02G203300) gene sequence was ligated into the pUbi:cas vector and transformed into 1-month-old embryogenic calli of KN199 using a PDS1000/He particle bombardment system (Bio-Rad, Hercules, CA) with a target distance of 6.0 cm from the stopping plate at a helium pressure of 1100 psi, as described previously (Shan et al., 2013). The KN199 (WT) and *TaCRY1a*-OE transgenic lines (T2 generation) were planted in an experimental field (39°56′N, 116°20′E) at the Institute of Crop Sciences, CAAS, Beijing under natural growth conditions. Plant height was measured after harvest.

N. benthamiana was grown in a greenhouse at 22°C with a 16 h light/8 h dark cycle. Blue light conditions (40 μmol/$m^{2/s}$) were achieved using a light-emitting diode light incubator (He et al., 2019).

DNA constructs

All DNA constructs used in the study were generated by following classic molecular biology protocols or using Gateway technology (Invitrogen). For ligase-independent ligation, the constructs were based on ligation-free cloning MasterMix (Applied Biological Materials, E011-5-A) according to the manufacturer's instructions. For ligase-dependent cloning, the endonuclease digested vectors and PCR fragments were separately purified using a PCR cleanup kit (Axygen, AP-PCR-250) and ligated at 16°C with T4 DNA ligase (New England Biolabs, M2020) for at least 8 h. For Gateway cloning, all gene sequences were cloned into the pQBV3 vector (Gateway) and then recombined with the specific destination vectors using Gateway technology (Invitrogen). All the primers used for the generation of constructs are shown in supplemental Table 1.

Measurement of *Arabidopsis* hypocotyl length

The *Arabidopsis* seeds were sterilized with 75% (v/v) ethanol for 7 min and 100% (v/v) ethanol for 3 min (v/v), then grown on $^1/_2$ MS medium. After 3 days of incubation at 4°C, seedlings were irradiated with blue light (40 μmol/m^2/s) for 5 or 6 days. Seedlings were photocopied, and hypocotyl lengths were measured using ImageJ software (http://rsb.info.nih.gov/ij).

Measurement of wheat plant height and coleoptile length

Plant height of field-grown wheat was measured at the mature stage and was defined as the distance from the soil surface to the top of the main panicle. Each internode of field-grown transgenic wheat was measured at the mature stage. Seeds of transgenic wheat plants were incubated in humid conditions on sterilized filter paper at 4°C for 7 days. They were transferred to blue light growth conditions for 2 days, and then the coleoptile lengths were measured.

RNA extraction and qRT–PCR

Young, fresh leaves of *Arabidopsis*, *N. benthamiana*, and wheat seedlings were collected under the indicated conditions. Total RNA was extracted with TRIzol reagent (Invitrogen) according to the manufacturer's instructions. About 2 μg of total RNA was used for reverse transcription with the 5X All-In-One RT MasterMix system (Applied Biological Materials), and then 2 μl of 20% (v/v) complementary DNA (cDNA) diluent was used as a template. Gene-specific primers and the SYBR Premix Ex-Taq Kit (TaKaRa) were used for qPCR reactions with a total volume of 10 μl. qRT–PCR was performed using a LightCycler 96 instrument (Roche). Expression levels of target genes were normalized to those of *AtACTIN7*, *NbACTIN1*, or *TaGAPDH*. Each experiment was repeated independently three times. Primer sets for qRT–PCR are shown in supplemental Table 1.

LCI assay

The LCI assays for protein interaction detection were performed as described previously (Chen et al., 2008; Sun et al., 2013; Liu et al., 2017). For LCI assays in *N. benthamiana*, the full-length coding sequence or its truncated derivatives were amplified and cloned into the p1300-35S-nLUC and p1300-35S-cLUC vectors (Chen et al., 2008) for fusion with the N-terminal and C-terminal parts of the firefly luciferase reporter gene *LUC*. Primers are summarized in supplemental Table 1. *Agrobacterium tumefaciens* strain GV3101 bacteria carrying different constructs were co-infiltrated into *N. benthamiana* leaves. After infiltration, *N. benthamiana* plants were incubated at 22°C for 12 h in the dark and then transferred to dark or blue light growth conditions for an additional 36 h before analysis for LUC activity. A low-light-cooled CCD imaging apparatus (NightShade LB 985; Berthold Technologies) with Indigo software was used to capture the LUC image. For each analysis, at least eight independent *N. benthamiana* leaves were infiltrated and analyzed, and three biological replications were performed with similar results.

For LCI assays to determine the effects of CRY1 on the interaction of GID1 with DELLA proteins, GV3101 colonies harboring constructs expressing nLUC-GID1a or nLUC-TaGID1, cLUC-GAI or cLUC-Rht-B1, and CRY1-Myc or TaCRY1-Myc or YFP-Myc (negative control) were infiltrated into *N. benthamiana* leaves. After infiltration, *N. benthamiana* plants were incubated at 22°C for 12 h in the dark and then transferred to blue light growth conditions for an additional 36 h before analysis for LUC activity. A low-light-cooled CCD imaging apparatus (NightShade LB 985; Berthold Technologies) with Indigo software was used to capture the LUC image. For each analysis, at least eight independent *N. benthamiana* leaves were infiltrated and analyzed, and two biological replications were performed with similar results.

Y2H assay

For Y2H analysis, the full-length CDS of *TaCRY1a* was cloned into the pGBKT7 vector, and the full-length CDSs of *TaGID1*, *TaRht-A1*, *TaRht-B1*, and *TaRht-D1* were amplified and cloned into the pGADT7 vector. The plasmids were transformed into *Saccharomyces cerevisiae* strain Y2HGold. The presence of transgenes was confirmed by growth on SD-Trp/Leu (SD-T/L) medium. The yeast strains were then dropped onto synthetic dextrose selection medium SD-Trp/Leu/His/Ade (SD-T/L/H/A) at 30°C for 3 days to assess the protein interactions.

Protein extraction and immunoblotting

Total proteins were extracted using extraction buffer (125 mM Tris–HCl [pH 6.8], 4% SDS, 20% glycerol, 0.001% bromophenol blue) with freshly added 2% β-mercaptoethanol. Immunoblots were performed as described previously (Pedmale and Liscum, 2007). Different tagged proteins were detected by immunoblotting with the corresponding antibodies: anti-GFP (1:2000; Roche, 11814460001) and anti-mouse IgG (1:75 000; Sigma, A9044-2ML) or IP kine HRP anti-mouse IgG LCS (1:5000; Abbkine, A25012) antibodies were used to detect GFP-tagged

and YFP-tagged proteins; anti-Flag (1:5000; MBL, M185-3L) and anti-mouse IgG or IP kine HRP anti-mouse IgG LCS antibodies were used to detect Flag-tagged proteins; anti-MYC (1:5000; Roche, 11667149001) and anti-mouse IgG antibodies were used to detect MYC-tagged proteins; and actin (1:5000; CWBIO, CW0264M) or HSC70 (1:1000; Enzo Life Sciences, ADI-SPA-818-F) was used as the loading control.

Co-IP assays

To verify the interaction between CRY1 and GID1a/RGA *in vivo*, double transgenic *35S:GID1a-YFP-HA/35S:CRY1-Flag* and *pRGA:GFP-RGA/35S:CRY1-Flag* seedlings were grown under blue light for 7 days. Total proteins were extracted using lysis buffer (50 mM Tris–HCl [pH 7.5], 150 mM NaCl, 5 mM EDTA [pH 8.0], 0.1% Triton X-100, 0.2% NP-40) with freshly added phenylmethylsulfonyl fluoride (10 mM), protease inhibitor cocktail (Roche, 11873580001), and 20 μM MG132. Extracts were then clarified by centrifugation at 4°C for 20 min. For co-IP assays, extracts containing equal amounts of total proteins were mixed with protein G (18 μl/1 ml, Invitrogen, 00621751) by gentle rotation for 1 h at 4°C, then incubated with anti-GFP mAb-magnetic agarose (18 μl/1 ml, MBL, D153-10) or anti-DDDDK (Flag)-tag mAb-magnetic agarose (18 μl/1 ml, MBL, M185-10)-conjugated beads at 4°C overnight under blue light.

ACCESSION NUMBERS

Arabidopsis sequence data from this article can be found in the Arabidopsis Genome Initiative data library under the following accession numbers: *CRY1* (AT4G08920), *GID1a* (AT3G05120), *GAI* (AT1G14920), *RGA* (AT2G01570), *RGL1* (AT1G66350), *RGL2* (AT3G03450), and *RGL3* (AT5G17490). Wheat sequence data from this article can be found at EnsemblPlants (http://plants.ensembl.org/index.html) under the following accession numbers: *TaCRY1a* (TraesCS6A02G203300), *TaGID1* (TraesCS1B01G265900), *Rht-A1* (TraesCS4A02G271000), *Rht-B1* (TraesCS4B02G043100), and *Rht-D1* (TraesCS4D02G040400).

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xplc.2021.100245.

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AUTHOR CONTRIBUTIONS

J.S. designed the research project. B.Y., Z.Y., G.H., Y.J., H.D., L.J., and Yunwei Z. performed the experiments. Yingfang Z. and Yun Z. analyzed the data and revised the manuscript. B.Y. and J.S. analyzed the data and wrote the manuscript.

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