RESEARCH ARTICLE

HY5 Regulates GLK and GNC Transcription Factors to Orchestrate Photomorphogenesis in *Arabidopsis thaliana*

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Short title: GLK and GNC regulate photomorphogenesis

One-sentence summary: GLK and GNC act downstream of HY5, and cooperate with HY5 and DET1, to regulate both chloroplast development and hypocotyl elongation during the transition from skotomorphogenesis to photomorphogenesis.

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ABSTRACT

Light induced de-etiolation is an important aspect of seedling photomorphogenesis. GOLDEN2 LIKE (GLK) and GATA NITRATE-INDUCIBLE

CARBON-METABOLISM-INVOLVED (GNC) are master transcriptional regulators involved in chloroplast development, but whether they participate in photomorphogenesis is largely unknown. Here we show that ELONGATED HYPOCOTYL5 (HY5) binds to *GLK* and *GNC* promoters and activates their expression. Chlorophyll content in the de-etiolating seedlings of the *hy5 glk2* and *hy5 gnc* double mutants was lower than that in the single mutants. It was further found that GLK inhibited hypocotyl elongation, whereas GNC promoted hypocotyl elongation, and that both could superimpose on the *hy5* phenotype. Correspondingly, GLK and GNC differentially regulate the expression of cell elongation genes. DE-ETIOLATED 1 (DET1) destabilises GLK proteins. The light-grown traits exhibited by etioplast of *det1* mutant were attenuated in *det1 glk2* double mutant, while similar to *det1* mutant, photosystem genes were up-regulated to different extent in etiolated seedlings overexpressing *GLK2* or *GNC*. Our study reveals that GLK and GNC act downstream of HY5 and likely cooperate with HY5 and DET1 to orchestrate multiple developmental traits during the light-induced skotomorphogenesis to photomorphogenesis transition.

INTRODUCTION

Light serves as an indispensable resource and the essential regulator for plant growth. In addition to photosynthesis, light also play roles in regulating seed germination, seedling de-etiolation, organ development, flowering and other important physiological processes (Jiao et al., 2007). After germination in darkness, seeds first grow into etiolated seedlings, displaying long hypocotyls with hooked tops and closed cotyledons. These characteristics are called skotomorphogenesis. Once the etiolated seedlings break through the soil surface and receive light, hypocotyl elongation is inhibited, cotyledons open, chloroplasts develop, and photosynthesis begins. This series of processes is referred to as

64 photomorphogenesis. Skotomorphogenesis and photomorphogenesis together
65 ensure that etiolated seedlings emerge from the soil and develop into normal
66 healthy seedlings, and are both a prerequisite for plant growth and development
67 (Sullivan and Deng, 2003; Jiao et al., 2007).

68 As a positive regulator of photomorphogenesis, HY5 acts downstream of
69 photoreceptors, such as cryptochromes, phytochromes and UV RESISTANCE
70 LOCUS8 (UVR8), and plays important roles in seedling de-etiolation (Jiao et al.,
71 2007; Stracke et al., 2010). Studies have shown that over 3000 genes can be
72 directly regulated by the basic leucine zipper (bZIP) transcription factor HY5
73 (Jakoby et al., 2002; Lee et al., 2007; Zhang et al., 2011). It regulates many basic
74 plant growth and development processes, including cell elongation, cell
75 proliferation, chloroplast development, pigment accumulation, and nutrient uptake
76 (Oyama et al., 1997; Shin et al., 2007; Jing et al., 2013). HY5 binds to promoters
77 through G-box elements and inhibits the expression of cell elongation genes, such
78 that *hy5* mutants show a typical long hypocotyl phenotype (Jing et al., 2013; Xu et
79 al., 2016b). HY5 is important for chlorophyll and carotenoid accumulation under
80 light as it regulates the expression of key enzymes such as PHYTOENE
81 SYNTHASE (PSY) and PROTOCHLOROPHYLLIDE OXIDOREDUCTASE C
82 (PORC). It also regulates the expression of photosynthetic pigment proteins, such
83 as light-harvesting chlorophyll-protein complex I subunit A4 (LHCA4)
84 (Toledo-Ortiz et al., 2014). Moreover, HY5 can bind to promoters of flavonoid
85 synthesis genes, activating their expression and promoting anthocyanin synthesis
86 (Shin et al., 2007; Song et al., 2008). HY5 functions through many different
87 regulatory interactions. For example, it interacts with HY5-HOMOLOGY (HYH) or
88 CALMODULIN7 (CAM7) protein to binds to the HY5 promoter and activate its own
89 expression (Kushwaha et al., 2008; Abbas et al., 2014; Binkert et al., 2014). The
90 interaction between HY5 and B-box-containing (BBX) protein BBX21-BBX22

enhances the promoting effect of HY5 on photomorphogenesis, whereas the interaction between HY5 and BBX24-BBX25 does the opposite, suggesting that BBX protein acts as coregulator of HY5, for fine-tuning of photomorphogenesis (Datta et al., 2007; Datta et al., 2008; Xu et al., 2016a). Furthermore, HY5 interacts with PIF1 and PIF3 proteins to coordinate the production of reactive oxygen species and the temperature control of photosynthetic gene expression (Chen et al., 2013; Toledo-ortiz et al., 2014). In the case of excess HY5 protein, it can activate the expression of COP1, thus inducing the degradation of HY5 protein and maintaining it at a relatively stable level (Huang et al., 2012).

GOLDEN2 (G2)-LIKE (GLK) and GATA NITRATE-INDUCIBLE CARBON METABOLISM-INVOLVED (GNC) are two types of transcription factors directly involved in the regulation of chloroplast development. GLK transcription factors belong to the GARP superfamily (Fitter et al., 2002), which consists of GLK (initially from maize), Arabidopsis RESPONSE REGULATOR-B (ARR-B) and PHOSPHATE STARVATION RESPONSE 1 (PSR1) from Chlamydomonas (Hall et al., 1998; Imamura et al., 1999; Wykoff et al., 1999). GLKs were first found to regulate chloroplast development in maize, with a pair of GLK homologs ZmG2 and ZmGLK1 expressed in bundle sheath and mesophyll cells respectively (Hall et al., 1998; Rossini et al., 2001). When maize G2 and GLK1 were overexpressed in rice, chloroplasts in the bundle sheath of transgenic leaves were larger than those in the wild type (Wang et al., 2017). In Arabidopsis, GLK1 and GLK2 are regulated by light, and function redundantly in regulating chlorophyll biosynthesis and chloroplast development. The leaves and siliques of the double mutant are pale green, and thylakoid development in the chloroplasts is retarded (Fitter et al., 2002). Overexpression of GLK1 or GLK2 in *glk1 glk2* double mutants can restore the defect in chloroplast development (Waters et al., 2008). Ectopic expression of GLKs was also able to induce increased chloroplast numbers in non-green

tissues such as roots and callus of Arabidopsis and rice (Nakamura et al., 2009; Kobayashi et al., 2012a), and in tomato fruits (Nguyen et al., 2014). GLK target genes are mainly related to chlorophyll biosynthesis, light-harvesting, and electron transport (Waters et al., 2009; Kobayashi et al., 2012b). It also regulates the expression of thylakoid membrane galactolipids monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) synthesis related genes (Kobayashi et al., 2014). In the process of seedling de-etiolation, GLKs interact with and are phosphorylated by BRASSINOSTEROID INSENSITIVE2 (BIN2) protein, which promotes the stability and transcriptional activity of GLK1 (Zhang et al., 2021). GLK also interacts with ORESARA 1 (ORE1) protein during senescence, with ORE1 antagonizing GLK induction of target genes (Rauf et al., 2013). The degradation of GLK protein itself is related to ubiquitination, with GLK2 shown to interact with DET1 protein in tomato prior to processing by CUL4-DDB1 ubiquitin E3 ligase for degradation (Tang et al., 2016).

The *GATA NITRATE-INDUCIBLE CARBON-METABOLISM-INVOLVED* (*GNC*) gene was identified by screening mutants on nitrogen-deficient medium, with *gnc* mutants accumulating lower chlorophyll than wild type (Bi et al., 2005). The *CYTOKININ-RESPONSIVE GATA FACTOR1* (*CGA1*) gene is strongly induced by cytokinin, and its expression in Arabidopsis histidine kinase mutants *ahk2* or *ahk3* is significantly reduced, suggesting that CGA1 is involved in the cytokinin signaling (Naito et al., 2007). The *gnc cga1* double mutant exhibits decreased chlorophyll content and decreased chloroplast number. Overexpression of *GNC* or *CGA1* in Arabidopsis resulted in obvious chloroplast development in seedling roots and hypocotyls, and increased chloroplast number in cotyledon and leaf epidermal cells (Chiang et al., 2012). Overexpression of *CGA1* in rice increases chloroplast number as well as starch biosynthesis in the leaves (Hudson et al., 2013; Lee et al., 2021). In spite of the well-known

145 downstream targets of GLK and GNC, their upstream regulation, particularly in
146 the context of light signaling is not clear. As HY5 is a regulatory hub in the light
147 signaling pathway, and it has been reported that GLK and GNC are dependent on
148 HY5 in regulating root greening (Kobayashi et al., 2012a, 2017), the molecular
149 relationships between HY5, GLK, and GNC are in need of systematic analysis. In
150 addition, as the stability of GLK and GNC proteins is crucial for their activities, it is
151 also important to consider the role of the COP/DET/FUS complex in regulating
152 GLK and GNC function. DE-ETIOLATED 1 (DET1) is involved in the ubiquitination
153 and degradation of positive regulators of photomorphogenesis in darkness. Apart
154 from shorter hypocotyls and opened cotyledons, the dark-germinated *det1* mutant
155 seedlings show part of light-grown traits, such as expression of photosynthetic
156 genes and development of thylakoid membranes (Chory et al., 1989). The
157 molecular mechanism behind such phenotypes in the dark is not fully understood
158 and the potential involvement of GLK or GNC is worth considering.

159 Therefore, this study focused on the molecular and functional linkages
160 among HY5, GLK, and GNC/CGA1 transcription factors, aiming to analyze and
161 clarify the local regulatory network connecting them, so as to better understand
162 the relationships between light signaling, seedling formation, and chloroplast
163 development during photomorphogenesis. At the same time, relationships
164 between GLK, GNC, and DET1, and their effects on the expression of
165 photosynthetic genes in etiolated seedlings were examined. The results shed light
166 on the concerted involvement of HY5, GLK, and GNC/CGA1 transcription factors
167 in both skotomorphogenesis and photomorphogenesis.

168

169 **RESULTS**

170 **HY5, GLK1/2, and GNC/CGA1 Regulate Chlorophyll Content to Different**
171 **Extents in De-etiolating Arabidopsis Seedlings**

HY5 and GLK both regulate the expression of chlorophyll biosynthesis and photosynthesis related genes (Fitter et al., 2002; Lee et al., 2007; Waters et al., 2009; Toledo-ortiz et al., 2014). To explore the significance of these regulations during photomorphogenesis, we generated combined mutations in Arabidopsis, and systematically investigated the effects of HY5 and GLK on chlorophyll biosynthesis in de-etiolating seedlings. All plants were grown in darkness for 4 days, and then sampled at different time points after white light irradiation to determine the content of chlorophyll. It was found that the chlorophyll content in *glk1* mutant had no or little difference from the wild type, while the chlorophyll content in *glk2* mutant was significantly lower than that in the wild type, and the chlorophyll content in *glk1 glk2* double mutant decreased more significantly than that in *glk2* mutant **(Figures 1A and 1C; Supplemental Figures 1A and 1C)**. This is consistent with the decrease of chlorophyll content in mature leaves of *glk1 glk2* double mutant reported in previous literature (Fitter et al., 2002), indicating that both GLKs affect chlorophyll biosynthesis in seedling de-etiolation process and GLK2 may play a dominant role. Overexpression of GLK1 or GLK2 in double mutants can compensate for the phenotype of decreased chlorophyll **(Supplemental Figures 1A and 1C)**. Compared with the above, we measured the changes in the chlorophyll of *gnc* and *cga1* mutants during seedling de-etiolation, and found that chlorophyll content of *cga1* mutants was not notably different from the wild type, chlorophyll content of *gnc* and *gnc cga1* mutants was lower than that of the wild type, while the difference was much smaller than that between *glk2* related mutants and the wild type **(Figures 1B and 1D; Supplemental Figure 1B and 1D)**. The chlorophyll content of *35S:GNC* overexpressed material was significantly higher than that of the wild type in the greening process, while the chlorophyll content of *35S:CGA1* overexpressed material showed no difference with that of the wild type at this stage

199 **(Supplemental Figures 1B and 1D)**. The chlorophyll content of *hy5* mutant was
200 lower than that of the wild type, and overexpression of HY5 in the mutant could
201 effectively compensate for the phenotype of reduced chlorophyll **(Figure 1;**
202 **Supplemental Figure 1)**, which supports the function of *hy5* regulating
203 chlorophyll biosynthesis (Tolede-Ortiz et al., 2014). In fact, we checked the
204 expression of *GLK1*, *GLK2*, *GNC*, and *CGA1* genes in the de-etiolation process of
205 wild type seedlings, and found that the expression levels of *GLK2* and *GNC* were
206 more prominent in response to light **(Figure 1E)**, which again demonstrates the
207 relative greater importance of them to seedling greening. Therefore, we conclude
208 here that HY5, GLKs and GNC/CGA1 all positively regulate chlorophyll
209 biosynthesis, but to different extents, during the greening process of seedling
210 photomorphogenesis.
211

212 **Loss of *GLK1/2* or *GNC/CGA1* Function Combined with *HY5* Mutation Has**
213 **Limited Further Effects on Seedling Chlorophyll Content**

214 In order to elucidate the genetic upstream or downstream relationships between
215 HY5, GLKs and GNC/CGA1, we constructed double and triple mutants, and
216 investigated chlorophyll content in seedlings during de-etiolation. It was found that
217 the chlorophyll content of *hy5 glk1* double mutant was not different from that of the
218 wild type and *glk1* during de-etiolation. Similarly, there was no notable difference
219 between *glk1* and wild type during de-etiolation from previous measurements
220 **(Figures 1A and 1C)**. We noticed that the *glk1* single mutant used was caused by
221 an insertion in the promoter region, and thus the phenotypes in *glk1* single mutant
222 and *hy5 glk1* double mutant may not be representative of null alleles. On the other
223 hand, the chlorophyll content of *hy5 glk2* double mutant was lower than that of
224 *glk2* or *hy5* mutant at 12 h in the process of greening. However, there was no
225 difference in chlorophyll content between *hy5 glk1 glk2* triple mutant and *glk1 glk2*

double mutant during de-etiolation **(Figures 1A and 1C)**. Compared with *hy5* mutant, *hy5 gnc* and *hy5 cga1* mutants showed little or no difference in chlorophyll content during de-etiolation, and only *hy5 gnc cga1* triple mutant exhibited notably lower chlorophyll content than *gnc cga1* and *hy5* mutants at 12 h during greening. Moreover, chlorophyll content of these mutants was generally higher than that of *glk* related double or triple mutants **(Figures 1B and 1D)**.

In order to clarify the chlorophyll phenotype of *hy5* and *glk2* mutants, we verified the expression levels of some chlorophyll biosynthesis and photosystem genes in de-etiolating seedlings. At 12 h of light exposure, the expression levels of chlorophyll biosynthesis genes (*HEMA1*, *PORB*, *PORC* and *CAO*) in *hy5*, *glk2* and *hy5 glk2* mutants were all remarkably lower than those in the wild type. The decrease in *glk2* and *hy5 glk2* mutants was not lower than that in the *hy5* mutant, except for *PORB* **(Figure 2A)**. The expression levels of photosystem light harvesting genes (*LHCB1.2*, *LHCB2.2*, *LHCA1* and *LHCA4*) was also significantly lower in *hy5*, *glk2*, and *hy5 glk2* mutants than in the wild type, with their expression in *hy5* mutant considerably higher than those in *glk2* and *hy5 glk2* mutants **(Figure 2B)**. These results indicate that during de-etiolation of seedlings GLK2 has stronger regulation on photosystem light harvesting genes than HY5, whereas their regulation on chlorophyll biosynthesis may be comparable.

To further complement the observed phenotype, we moved on to analyse the changes of chlorophyll in the mutant seedlings grown under continuous white light for 4 days, and found that the trend of chlorophyll content among single mutant and double mutant was similar to that in the de-etiolation process of seedlings. However, chlorophyll content in *hy5 glk1 glk2* triple mutant was lower than that in *glk1 glk2* double mutant **(Figures 2C and 2D)**. We further investigated chloroplast development in wild type, *glk1 glk2*, *hy5*, and *hy5 glk1 glk2* mutants under sustained light exposure. Transmission electron microscopy showed that there

253 was no significant difference in mesophyll cell chloroplast ultrastructure between
254 *hy5* and wild type at this stage. The chloroplast size of *glk1 glk2* and *hy5 glk1 glk2*
255 mutants was smaller than that of the wild type and *hy5* mutant, the accumulation
256 of starch grains was decreased, and the thylakoid stacking of *glk1 glk2* mutants
257 was sparser. Even worse, *hy5 glk1 glk2* mutant showed obvious fractured stromal
258 thylakoids **(Figure 2E)**. These results suggest that under certain conditions, *HY5*
259 mutation combined with *GLK1* and *GLK2* double mutations can lead to more
260 severe defects in chloroplast development. Because of the functional redundancy
261 of *GLK1* and *GLK2*, the double mutant shows severe perturbations in chlorophyll
262 biosynthesis and chloroplast development, with the superimposed mutation of
263 *HY5* causing limited further defects. As such, we hypothesized that HY5 is either
264 located upstream of the GLK1 and GLK2 co-regulatory module, or that HY5 has
265 only a minor role in the regulation of chlorophyll biosynthesis.
266

267 **HY5 Directly Regulates *GLK1*, *GLK2*, *GNC* and *CGA1* Gene Expression**

268 According to ChIP-Seq analysis for HY5, its target genes include *GLK2*, *GNC*,
269 and *CGA1* (Lee et al., 2007). Through RT-qPCR verification, we found that the
270 expression levels of *GLK1*, *GLK2*, *GNC* and *CGA1* in *hy5* mutants were
271 significantly lower than those in the wild type **(Figures 1F)**, indicating that HY5
272 positively regulates their expression. Typical *cis*-elements to which HY5 binds to
273 regulate target gene expression include G-box (CACGTG), T/G-box (CACGTT),
274 E-box (CAATTG), ACE-Box (ACGT), and Z-Box (ATACGGT) (Shin et al., 2007;
275 Chang et al., 2008; Catala et al., 2011; Abbas et al., 2014). We performed
276 promoter analysis for *GLK1* and *GLK2*, and found that their promoter regions
277 contained HY5-binding *cis*-elements. These regions were synthesized to conduct
278 electrophoretic mobility shift assays (EMSA). It was shown that HY5 could bind to
279 *GLK1* and *GLK2* promoter sequences, competitive probes weakened such

280 binding, while the binding ability of mutant probes was much lower than that of
281 normal probes **(Figure 3A)**. In parallel, by transforming protoplasts dual-luciferase
282 assay displayed that HY5 could activate *GLK1* and *GLK2* expression **(Figures 3B**
283 **and 3C)**. While obtaining the above *in vitro* results, we also validated the binding
284 *in vivo* via Chromatin immunoprecipitation (ChIP) coupled with quantitative PCR
285 (qPCR) assay. Three or two regions containing HY5 binding elements were
286 selected from the promoters of *GLK1* and *GLK2* respectively, and the results
287 demonstrated that the P2 region from *GLK1* promoter and the P1 region from
288 *GLK2* promoter were most strongly bound by HY5 **(Figure 3D)**. *In vitro*
289 experiment EMSA and dual-luciferase assays confirmed that HY5 could also bind
290 to *GNC* and *CGA1* promoters to activate their transcription, again followed by *in*
291 *vivo* experiment ChIP-qPCR showing that the highest enrichment of P1 on *GNC*
292 and *CGA1* promoters by HY5 **(Figure 3)**. In conclusion, HY5 directly binds to the
293 promoters of *GLK1*, *GLK2*, *GNC*, and *CGA1* to activate their gene expression.
294

295 **GLK1/2 and GNC/CGA1 oppositely Regulate Hypocotyl Elongation of**
296 **Light-grown Seedlings**

297 HY5 is known to inhibit hypocotyl elongation during photomorphogenesis, as *hy5*
298 seedlings exhibit longer hypocotyls (Sullivan and Deng, 2003; Jiao et al., 2007).
299 Since we found that HY5 directly regulates the expression of *GLK*, *GNC*, and
300 *CGA1* genes, whether GLK, GNC and CGA1 also play roles in seedling
301 morphological development upon light regulation became the next question.
302 Therefore, we systematically investigated the hypocotyl phenotypes of a series of
303 *glk*, *gnc*, and *cga1* mutants. The hypocotyl length of *glk* mutants was measured
304 under different light quality, and those in *glk1*, *glk2* and *glk1 glk2* mutants were
305 longer than in wild type, while shorter than in the *hy5* mutant, after germinating
306 and growing under white, blue or red light conditions for 4 days. When *GLK1* or

307 *GLK2* were overexpressed in *glk1 glk2* mutants, the hypocotyl length was
308 restored to the level of wild type. There was no significant difference in hypocotyl
309 length between the mutants and wild type after germinating and growing for 4
310 days in darkness **(Supplemental Figure 3)**. Together the above results indicate
311 that GLK inhibits hypocotyl elongation during light-induced seedling development.
312 We further investigated the hypocotyl phenotype in *hy5* and *glk* double and
313 triple mutants. Under white or blue light, compared with *hy5*, hypocotyls of *hy5*
314 *glk1* were slightly longer, while hypocotyls of *hy5 glk2* and *hy5 glk1 glk2* were
315 significantly longer than that of *hy5*. The hypocotyls of all materials were generally
316 longer under red light, and those of *hy5 glk1*, *hy5 glk2* and *hy5 glk1 glk2* were still
317 longer than those of *hy5*, but with smaller differences. No notable differences in
318 hypocotyl length were observed in dark-grown seedlings between wild type and
319 these mutants **(Figures 4A and 4B)**. We measured the cell length of wild type,
320 *glk1 glk2*, *hy5*, and *hy5 glk1 glk2* materials in the middle segment of hypocotyls
321 growing under white light for 4 days, and found that the hypocotyl cell length of
322 *glk1 glk2* mutant was longer than that of the wild type, and that of *hy5 glk1 glk2*
323 mutant was longer than that of *hy5* mutant **(Figures 4C and 4D)**. In general, there
324 is an obvious trend of *hy5* < *hy5 glk1* < *hy5 glk2* < *hy5 glk1 glk2* in hypocotyl
325 length, indicating that GLK2 may work independent (at least partly) of HY5 to
326 inhibit hypocotyl elongation, but again GLK2 plays a more important role than
327 GLK1.
328 Different from the hypocotyl phenotype of *glk* series mutants, after
329 germinating and growing under white light for 4 days, the hypocotyl length of *gnc*,
330 *cga1* and *gnc cga1* mutants was shorter than that of the wild type, while the
331 hypocotyl length of *35S:GNC* and *35S:CGA1* overexpressed materials was longer
332 than that of the wild type. There was no difference in hypocotyl length between
333 these mutants and wild type after germinating and growing in darkness

(Supplemental Figure 4), indicating that GNC and CGA1 promoted hypocotyl elongation under white light. In addition, the hypocotyl length of *hy5 gnc*, *hy5 cga1* and *hy5 gnc cga1* mutants was shorter than that of *hy5* under white light, but significantly longer than that of *gnc*, *cga1* and *gnc cga1* mutants **(Figures 4E and 4F)**, indicating that GNC and CGA1 were able to reduce the inhibition of HY5 on hypocotyl elongation. Therefore, during seedling development GLK transcription factors can inhibit hypocotyl elongation, while GNC and CGA1 are able to promote hypocotyl elongation, such that their functions as photomorphogenesis regulators are expanded.

Transcriptome and RT-qPCR Analysis Reveals Differential Regulation of Cell Elongation Genes by HY5, GLK2, and GNC

The above results showed that the phenotype of the *hy5 glk2* mutant was more severe than that of the *hy5 glk1* mutant, both in regulating chlorophyll content during seedling de-etiolation and inhibiting hypocotyl elongation during light-induced seedling development. Hence, we selected wild type, *glk2*, *hy5* and *hy5 glk2* seedlings that germinated and grew for 4 days under white light for transcriptome sequencing, and analysed the genes jointly or differentially regulated by HY5 and GLK2. As shown in a Venn diagram, *glk2*, *hy5* and *hy5 glk2* mutants jointly affected the expression of 142 genes, including 138 down-regulated genes and only 2 up-regulated genes **(Figure 5A)**. Deletion of *HY5* or *GLK2* in most cases resulted in down-regulation of genes, and K-means clustering showed the same trend **(Figure 5B)**, in line with the expectation of them as positive regulators of photomorphogenesis. Most up-regulated genes were expressed in *hy5* and *hy5 glk2* mutants. Among the down-regulated genes, 385 were down-regulated together in *hy5* and *hy5 glk2* mutants, while 201 were down-regulated together in *glk2* and *hy5 glk2* mutants. This is consistent with

361 HY5's upstream position in activating *GLK* gene expression mentioned above.
362 The remaining 91 down-regulated genes only in *hy5 glk2* mutants may represent
363 targets of combined HY5 and GLK activity **(Figure 5A)**. According to the trend of
364 *glk2* < *hy5* < *hy5 glk2* in hypocotyl length, K4 and K7 groups from K-means
365 clustering could be considered for investigating GLK2 and HY5 regulated
366 hypocotyl elongation **(Figure 5B)**. In addition, a heat map was generated from the
367 differential expression of a list of known cell elongation related genes, and a
368 series of up-regulated genes were considered for subsequent validation **(Figure**
369 **5C)**.

370 Elongation genes from **Figure 5C** were selected for RT-qPCR verification.
371 Many of them expressed higher in *glk2* than in the wild type, and higher in *hy5*
372 than in *glk2*, but the expression in *hy5 glk2* double mutant was more variable.
373 Among them, the expression levels of *EXPA5*, *IAA19*, *SAUR20*, and *PME16* were
374 detected higher in *hy5 glk2* than in *hy5* **(Figure 5D)**. We hypothesized that the
375 interaction between GLK2 and HY5 proteins could enhance the inhibitory effect on
376 these elongation genes, but GLK2 may independently inhibit other elongation
377 genes. We also analysed the expression levels of selected elongation genes from
378 **Figure 5C** in the *gnc* and *hy5 gnc* materials. The expression level of *IAA7* in *gnc*
379 mutant was lower than that in wild type, and the expression levels of several other
380 genes in *gnc* mutant tended to be lower than in wild type, although not significant
381 **(Figure 5E)**. In addition, the expression levels of these elongation genes,
382 including *IAA7*, *IAA19*, *XTH4*, and *PME16*, appeared lower in the *hy5 gnc* double
383 mutant than in *hy5* mutant **(Figure 5E)**. These results indicate that GNC could
384 promote the expression of these elongation genes to less and varying degrees,
385 both independently or on top of HY5. It also suggests that the targeted hypocotyl
386 elongation genes by GNC/CGA1 may be largely different from those by GLKs.
387

388 **Potential Protein Interaction of HY5 and GLK, and the Effect on**
389 **Photosystem and Cell Elongation Genes**

390 Apart from directly targeting many genes to promote their expression, HY5
391 also interacts with other proteins, HYH or HFR1 for instance, to mutually enhance
392 protein stability, or to bind to *HY5* or downstream gene promoters (Lee et al., 2007;
393 Ciolfi et al., 2013; Jang et al., 2013). The interaction between HY5 and G-box
394 binding factor 1 (GBF1) attenuates the activation of *RBCS-1A* by HY5. They share
395 a common G-box *cis*-element, which enables them to bind to the same target
396 promoter. This may enable more precisely regulated expression of downstream
397 genes and better adapts to the environment. Since HY5 interacts with GBF1, and
398 GBF1 interacts with GLK (Tamai et al., 2002; Singh et al., 2012; Ram et al., 2014),
399 we then examined the possibility of HY5 interacting with GLK proteins. In
400 pull-down experiments, the experimental group GST-GLK1 and GST-GLK2 could
401 pull down HY5 protein, while the control group could not **(Supplemental Figure**
402 **7A)**, indicating that HY5 binds to GLK1 and GLK2. Through bimolecular
403 luminescence complementation assays, it was found that luciferase signal could
404 be detected when HY5 was co-transfected with GLK1 and GLK2 proteins, while
405 the signal was not present in the negative controls **(Supplemental Figure 7B)**,
406 indicating that HY5 interacts with GLK1 and GLK2. The above experiments
407 provide *in vitro* evidence of HY5 and GLK interaction. HY5 might first activate the
408 expression of *GLK*, and then interacts with GLK protein to regulate common
409 targets. Dual-luciferase assay displayed that both HY5 and GLK2 could activate
410 *LHCB1.2* expression, and GLK2 could activate to higher extent, but HY5 and
411 GLK2 together did not show an additive effect **(Supplemental Figure 7C)**, which
412 is consistent with the result shown in **Figure 2B**. **Supplemental Figure 7D**
413 showed that GLK2 could slightly activate *PME16* while HY5 inhibited its
414 expression, as indicated in **Figure 5C**. When HY5 and GLK2 were applied

together, it was more similar to the effect of GLK2 on *PME16* expression. This appears discordant with the gene expression result in *hy5 glk2* mutant **(Figures 5C and 5D)**, which might indicate that extra factors exist to coordinate HY5 and GLK2 on their effects on *PME16*.

GLK2 Content Affects the Plastid Ultrastructure and Photosystem Gene Expression in Dark-grown *det1* Mutant of Arabidopsis

Compared to the traits of photomorphogenesis, the morphology and gene expression changes of skotomorphogenesis are relatively less addressed by previous researches, although seedling development before they were exposed to light represents a preparative and important stage. One of the few but well known observations is that, the stromal thylakoid membrane was found sufficiently extended in the plastids of etiolated *det1* mutant seedlings rather than the default form of prolamella body, and some photosynthetic genes were already expressed in the dark (Chory et al., 1989). On account of the emphasis on GLK2 from above, we constructed *det1 glk2* mutant and comparative observations were made on the plastid ultrastructure in the cotyledons of dark-grown *det1*, *glk2*, and *det1 glk2* mutants. As expected, the cotyledons were open in the etiolated *det1 glk2* mutant, and paler in the light-grown *det1 glk2* mutant **(Figure 6A)**. We found that the thylakoid lamellae in the plastids of *det1 glk2* mutant were less developed than in *det1* mutant in the dark, although in both cases there was little evidence of a prolamellar body. By contrast, there was no significant structural difference between the prolamellar body of the *glk2* mutant, the *GLK2*-overexpressing line, and the wild type, but the size of the prolamellar body was smaller and larger than that of the wild type respectively **(Figure 6B)**. The changes of plastid ultrastructure in *det1 glk2* mutant suggested that developed chloroplasts in etiolated *det1* mutant were related to GLK2 function, while in the dark-grown wild

442 type, DET1 may affect GLK2 protein stability, thus preventing chloroplast
443 development.

444 It is known that in the dark, HY5 ubiquitination and degradation are mediated
445 by the COP/DET/FUS complex (Saijo et al., 2003). In order to study the stability of
446 GLK proteins in Arabidopsis, we examined the dark-grown wild type and *det1*
447 mutant using Arabidopsis GLK1 and GLK2 antibodies for Western-blots. The
448 results showed that both GLK1 and GLK2 protein contents in *det1* mutant were
449 higher than those in the wild type **(Figure 7A)**. The accumulation of GLK1 and
450 GLK2 proteins after DET1 deletion suggests that DET1 may promote the
451 degradation of GLK proteins. It should be noted that Rubisco large subunit (RbcL)
452 content was higher in *det1* than in the wild type, which is consistent with the
453 increased accumulation of *RbcL* transcript in dark-grown *det1* mutant (Chory et al.,
454 1989). We also grew the wild type and *det1* mutant in darkness for 4 days and
455 treated them with MG132, a proteasome inhibitor, for 12 h in darkness with DMSO
456 as the control. After MG132 treatment, the accumulation of GLK proteins in the
457 wild type was higher than that in the control group, but still much lower than in the
458 MG132 treated *det1* mutant **(Figure 7A)**. These results suggest that MG132 is
459 beneficial to the accumulation of GLK1 and GLK2 proteins in the wild type by
460 inhibiting proteasome activity, but the deficiency of DET1 may have blocked the
461 upstream steps of protein degradation such as ubiquitination, so that the
462 significant accumulation of GLK proteins in *det1* mutant maybe less affected. We
463 therefore assessed whether GLK1 and GLK2 interact with DET1 protein in
464 Arabidopsis. *In vitro* protein binding experiments support the expectation that
465 DET1 interacts with both GLK1 and GLK2 proteins **(Supplemental Figure 8A)**.
466 We also checked whether DET1 interacted with GNC and CGA1 proteins, and
467 again positive results were obtained from *in vitro* protein binding experiments
468 **(Supplemental Figure 8B)**.

469 The expression of some photosynthetic genes in dark-grown *det1* mutant
470 seedlings is an interesting phenomenon (Chory et al., 1989), but the mechanism
471 of the dark activation of these genes, or the significance of being turned off in
472 skotomorphogenesis, has not been elucidated. We measured the expression of
473 photosystem genes in a series of dark-grown *det1* and *glk2* related mutants, and
474 found that the expression levels of *LHCB1.2* and *LHCA1* were much higher in
475 *det1* mutant than those in wild type, but were lower in *glk2* mutant than in the wild
476 type. The down-regulation of these genes by GLK2 was also found in *det1 glk2*
477 double mutant comparing with the *det1* mutant **(Figure 7B)**. This indicates that
478 GLK2 contributes significantly to the expression of photosystem genes in
479 dark-grown seedlings, and the effects could be additive to the loss of DET1
480 function. At the same time, the notably elevated expression of photosynthetic
481 genes in *det1* mutant should be contributed by the increased amount of GLK1,
482 GNC, or CGA1 as well. As supportive results, in the etiolated materials
483 overexpressing *GLK1*, *GLK2*, *GNC* or *CGA1*, the expression of *LHCB1.2* and
484 *LHCA1* genes were significantly promoted in *GLK1* or *GLK2* overexpression lines,
485 less promoted in the *GNC* overexpression line, and least changed in the *CGA1*
486 overexpression line **(Figure 7C)**. When grown this same set of materials in the
487 light, increased expression of *LHCB1.2* and *LHCA1* genes were still observed to
488 larger extent in *GLK1* or *GLK2* overexpression lines than in *GNC* and *CGA1*
489 overexpression lines relative to the wild type **(Figure 7D)**. Notably, expression
490 levels of *LHCB1.2* and *LHCA1* genes also increased in both dark and light-grown
491 *HY5* overexpression lines, but were not higher than those in *GLK1* or *GLK2*
492 overexpression lines, indicating again that GLKs function at least partly
493 independent of HY5 during seedling photomorphogenesis.
494
495 **DISCUSSION**

496 In this work, we have constructed a series of mutant materials to systematically
497 study the regulatory relationships between HY5, GLK, and GNC/CGA1
498 transcription factors, as well as their stabilities and functions within the scenario of
499 skotomorphogenesis and photomorphogenesis. We find that: (1) HY5 directly
500 activates *GLK*, *GNC*, and *CGA1* expression, and together they positively regulate
501 chlorophyll biosynthesis and photosystem formation; (2) GLK proteins inhibit
502 hypocotyl elongation, whereas GNC and CGA1 promote hypocotyl elongation, by
503 differentially regulating the expression of cell elongation genes; (3) In the dark,
504 DET1 destabilises GLK proteins, and the light-grown traits exhibited in the
505 etioplast of *det1* mutant seedlings can be attributed to GLK2 activity; (4) GLK2
506 maybe the predominant regulator of photosynthetic genes, during both
507 skotomorphogenesis and photomorphogenesis. At present, the molecular
508 mechanism of GLK and GNC in regulating hypocotyl elongation is not entirely
509 clear. These need to be further investigated in combination with specific genetic
510 material construction. In addition, GLK2 function has been standing out in multiple
511 ways, partially recruited by HY5 in the light, and effectively compensates for HY5
512 function, so that it deserves targeted studies in order to guide the potential
513 application of GLK2 and HY5 in crop enhancement.
514

515 **GLK and GNC Function Downstream but Partially Independent of HY5 in**
516 **Seedling Photomorphogenesis**

517 HY5 and GLK both functions in regulating chlorophyll biosynthesis and chloroplast
518 development, but targeted studies and clear conclusions are missing on whether
519 or how the two are related, especially during photomorphogenesis. Studies have
520 shown that PHYTOCHROME-INTERACTING FACTOR 4 (PIF4) in Arabidopsis
521 binds to the promoters of *GLK1* and *GLK2* to inhibit their expression (Song et al.,
522 2014), while HY5 antagonises PIFs to regulate the biosynthesis of photosynthetic

523 pigments (Tolede-Ortiz et al., 2014). Besides, microarray studies showed that
524 both phytochrome A and phytochrome B regulate GLK transcription (Tepperman
525 et al., 2006). Considering that HY5 acts downstream of the photoreceptors, and is
526 a high-level regulator (many HY5 targets are other transcription factors) of
527 photomorphogenesis (Lee et al., 2007), there may be a direct positive regulation
528 of HY5 to *GLKs*. We proved in this work that HY5 indeed binds to the promoters of
529 *GLK* genes and activates their expression, through EMSA *in vitro* assay, dual
530 luciferase assay, and ChIP-qPCR *in vivo* assay. Similarly, *in vitro* and *in vivo*
531 experiments also confirmed that HY5 binds to the promoters of *GNC* and *CGA1*,
532 and regulates their expression **(Figure 3)**.

533 We have constructed double and triple mutants of HY5, GLK1/2, and
534 GNC/CGA1 to study their functional relationships. Only limited decrease of
535 chlorophyll content and related gene expression were observed when *hy5 glk2*
536 mutant was compared to *glk2* mutant, or *hy5 glk1 glk2* mutant was compared to
537 the pale green *glk1 glk2* mutant **(Figures 1 and 2)**. Our transcriptome data
538 indicate that, compared with HY5, GLK2 tends to positively affect more PSII and
539 PSI related genes **(Supplemental Data Set 1-3)**. Furthermore, gene expression
540 analysis reveals that GLK2 exhibits stronger regulation on light harvesting genes
541 than HY5, while their regulatory strength on chlorophyll biosynthesis may be
542 similar during seedling greening **(Figure 2A and 2B)**. When overexpressing
543 *GLK2* in the *hy5* mutant, the chlorophyll level increased significantly, although it
544 was still lower than that in the *GLK2* overexpression line with a wild type
545 background (Liu et al., 2021). HY5 was suggested to play an important role for
546 GLK2 function, while our study clarifies the functional downstream location of
547 GLKs to HY5, and implies that the complementary function of GLK1 and GLK2
548 contributes more intensively than HY5, to chlorophyll biosynthesis and chloroplast
549 development during Arabidopsis seedling greening.

550 It has been reported that HY5 and GLK2 are involved in the greening of
551 detached root in Arabidopsis, and it was suggested that the GLK2 regulation of
552 root greening depends to some extent on the presence of HY5 (Kobayashi et al.,
553 2012a). Using *hy5 glk* series mutants, we observed that the detached roots could
554 still turn green in the absence of a single GLK or both GLKs, but not in the
555 absence of HY5 **(Supplemental Figure 5)**. Endogenous cytokinin promotes the
556 greening process of detached roots (Kobayashi et al., 2012a). Our results confirm
557 that HY5 is essential in the process of root greening induced by cytokinin
558 accumulation. In order to investigate the effect of HY5 on cytokinin regulated leaf
559 greening, we added 6-Benzylaminopurine (6-BA) during seedling greening of *hy5*
560 *glk* series mutants. Results showed that 6-BA treatment promoted the
561 accumulation of chlorophyll during this process, with or without GLKs or HY5
562 **(Supplemental Figure 6)**. These results suggest that GLKs and HY5 play similar
563 roles in response to cytokinin to promote chlorophyll biosynthesis in leaves,
564 whereas HY5 plays a more significant role in detached roots.

565 GLKs seem to also exert stronger regulation than GNC/CGA1 in chlorophyll
566 biosynthesis, as *glk1 glk2* mutants are much paler than *gnc cga1* mutants relative
567 to wild type **(Figure 1; Supplemental Figure 1)**. No difference in chlorophyll
568 content between *glk1 glk2 gnc cga1* quadruple mutant and *glk1 glk2* mutant was
569 found, and *CGA1* overexpression in *glk1 glk2* mutant could not restore the
570 chlorophyll content, while overexpression of *GLK1* partially restores the
571 chlorophyll content in *gnc cga1* mutants (Bastakis et al., 2018). According to our
572 work, the reduction of chlorophyll contents in *hy5 gnc* and *hy5 gnc cga1* mutants
573 compared to *hy5* mutant, was less obvious than that of *hy5 glk2* and *hy5 glk1 glk2*
574 mutants compared with *hy5* mutant **(Figures 1B and 1D)**. Zubo et al. suggested
575 that GLK1/GLK2 and GNC/CGA1 play both overlapping and independent roles in
576 regulating chlorophyll biosynthesis and chloroplast development (Zubo et al.,

577 2018). We further propose that GLKs play more prominent roles than GNC/CGA1,
578 downstream but partly independent of HY5, to regulate various targets during the
579 de-etiolation process of photomorphogenesis.
580
581 **Differential Participation of GLK and GNC in HY5 Regulated Hypocotyl**
582 **Elongation**
583 The inhibition of hypocotyl elongation, opening of cotyledon, chlorophyll
584 biosynthesis and chloroplast development are continuous and inseparable
585 processes during seedling photomorphogenesis. Since HY5 inhibits hypocotyl
586 elongation, we were also curious about the potential GLK and GNC regulation of
587 hypocotyls. It has been mentioned that *glk1* seedlings displayed longer hypocotyls
588 and less separated cotyledons (Martín et al., 2016, Alem et al., 2022). By
589 systematically analysing the hypocotyl phenotype of mutants and overexpression
590 lines, we certified that both GLK1 and GLK2 inhibit hypocotyl elongation, with
591 additive effects on the function of HY5 **(Figures 4A and 4B; Supplemental**
592 **Figure 3)**. Transcriptomic sequencing and RT-qPCR verification showed that
593 GLK2 itself could inhibit the expression of elongation genes, and different
594 elongation genes exhibited different regulatory responses to GLK2 and HY5, such
595 that the inhibition of hypocotyl elongation in photomorphogenesis was the result of
596 comprehensive regulations.
597 Contrary to GLK's inhibition of hypocotyl elongation, we found that GNC and
598 CGA1 promote hypocotyl elongation **(Figures 4E and 4F; Supplemental Figure**
599 **4)**. We noticed that the expression levels of some elongation genes were reported
600 lower in *gnc cga1* mutants than those in the wild type (Xu et al., 2017). Other
601 clues were found in the *quintuple* mutant of *GNC*, *GNL*, and *B-GATA* genes, that
602 the seedlings had shorter hypocotyl than wild type seedlings (Ranftl et al., 2016).
603 We further found in this work that the mutation of GNC or CGA1 displayed

negative effects on the inhibition of hypocotyl lengths by HY5 **(Figures 4E and 4F)**. The expression of some tested elongation genes in *hy5 gnc* mutant was decreased relative to that of *hy5* mutant **(Figure 5E)**. Our results indicate that GNC could promote the expression of elongation genes to varying degrees, independently or on top of HY5. It should be pointed out that the tested elongation genes for GNC were initially from differentially expressed genes in *glk2* mutants, so that they may not be the best regulated genes by GNC, and larger scale gene expression analysis is needed to uncover the GNC/CGA1 specific elongation genes. In addition, the changes of elongation genes in *glk1 glk2*, *gnc cga1*, *hy5 glk1 glk2*, and *hy5 gnc cga1* mutants are worth exploring in the future, to clarify the potential redundant or additive effect of these transcription factors on hypocotyl length.

Significance of GLK2 and GNC through the Context from Skotomorphogenesis to Photomorphogenesis

Traits of photomorphogenesis are negatively regulated by the COP/DET/FUS complex in the dark, which mediates the ubiquitination and proteasome degradation of positive regulators such as HY5, HYH, LAF1, and HFR1 (Osterlund et al., 2000; Holm et al., 2002; Seo et al., 2003; Yang et al., 2005). Interestingly, *det1* and *cop1* mutants exhibit some of the characteristics of photomorphogenesis during skotomorphogenesis, with shorter hypocotyls, open cotyledons, and expression of photosynthetic genes (Chory et al., 1989; Deng et al., 1992). These phenotypes should be related to the accumulation of positive regulators of photomorphogenesis in darkness, but the specific regulators and their influence on the phenotypes are not clear. It has been reported that long-term ABA treatment activates the activity of COP1, which then interacts with GLK1 protein to mediate its ubiquitination and degradation (Tokumaru et al., 2017;

631 Lee et al., 2021). Further, brassinolide or darkness induces GLK1 degradation by
632 inhibiting the BIN2 phosphorylation of GLK1, and it was speculated that
633 BIN2-mediated phosphorylation prevents the interaction of GLK1 and E3 ligases
634 (Zhang et al., 2021). In this study we further showed that DET1 is responsible for
635 the protein degradation of GLK1 and GLK2 in Arabidopsis seedlings **(Figures 8A**
636 **and 8B)**. As for GLK2, we have constructed *det1 glk2* mutant in Arabidopsis to
637 compare its characters with *det1* mutant during skotomorphogenesis. Extended
638 thylakoid lamellae were found in the cotyledon protoplast of both mutants, but in
639 *det1 glk2* mutant the overall number of lamellae was significantly reduced **(Figure**
640 **6B)**. This is likely related with the GLK2 regulation on photosynthetic genes in
641 etiolated seedlings **(Figures 8C)**. In fact, both GLK2 and GLK1 contribute
642 significantly to the expression of photosystem genes, not only in the light but also
643 in dark-grown seedlings, while GNC and CGA1 seem to contribute less than
644 GLKs **(Figures 8D and 8E)**. Thus, the appropriate function of GLKs and
645 GNC/CGA1 is important to the whole process from skotomorphogenesis to
646 photomorphogenesis. They could be regulated at protein level by DET1 or COP1
647 related pathway in etiolated seedlings, while upon light HY5 takes the
648 transcriptional control and at least partly coordinates their functions, in a
649 regulation cascade of light, HY5, and GLKs or GNC/CGA1.
650
651 We constructed a working model as shown in **Figure 8**. In dark-grown
652 seedlings, GLK, GNC, CGA1 and HY5 proteins are subjected to the
653 COP/DET/FUS complex mediated degradation. Photosynthetic genes are inactive
654 and plastid inner membranes stay at prolamellar body status. After exposure to
655 light, COP/DET/FUS complex activity is inhibited and GLK, GNC, CGA1 and HY5
656 proteins accumulate. HY5 activates *GLK*, *GNC* and *CGA1* genes in a
657 light-dependent manner, and may interact with their proteins to cooperatively

658 regulate downstream gene expression to promote photomorphogenesis. GLKs
659 are master regulators of chloroplast development and able to inhibit hypocotyl
660 elongation independent of HY5. GNC and CGA1 are less dominant in chloroplast
661 development and able to promote hypocotyl elongation. These could be the
662 compensation for the relatively weak regulation of HY5 on chloroplast
663 development, and an efficient way to orchestrate light induced seedling
664 development, by recruiting multitasking yet complementary regulators. Our study
665 systematically reveals the new function of GLK and GNC regulating hypocotyl
666 length under light, refines the local network of HY5, GLK, GNC and CGA1, and
667 provides new perspective for better understanding the transition between dark
668 and light-grown seedlings.
669

670 **MATERIALS AND METHODS**

671 **Plant Materials and Growth Conditions**

672 All mutants used in this study were in the Columbia background. The *glk1*, *glk2*,
673 *glk1 glk2*, *gnc*, *cga1*, *gnc cga1*, *hy5*, and *det1* mutants have been previously
674 described (Chory et al., 1989; Oyama et al., 1997; Fitter et al., 2002; Chiang et al.,
675 2012). *35S:GLK1 glk1 glk2*, *35S:GLK2 glk1 glk2*, *35S:GNC*, and *35S:CGA1*
676 overexpression plants were also described previously (Waters et al., 2008;
677 Chiang et al., 2012). Other mutants described in this study were created by
678 crossing from the above materials. Surface-sterilized seeds were sown onto
679 half-strength Murashige and Skoog (MS) medium, which contained 1% sucrose
680 and 0.8% agar, and were cold-treated at 4°C in darkness for 3 days to ensure
681 synchronized germination, followed by growing in dark or light chambers
682 maintained at 22°C.
683

684 **Chlorophyll Measurements**

685 To measure the chlorophyll content, 4 d-old seedlings grown in the darkness or
686 continuous light were used. Total chlorophylls were extracted by homogenizing
687 the seedlings in 80% acetone, and leaving the solutions at 4°C for overnight
688 incubation. The absorbance was detected at wavelength OD_{652} with a
689 spectrophotometer. Chlorophyll concentration Ca+b was calculated by OD_{652}/34.5
690 (mg/mL), and then converted according to the fresh weight of the material (Arnon,
691 1949; Zhao et al., 2016).
692
693 **Hypocotyl Length Measurement**
694 To measure the hypocotyl length of seedlings, seeds were sown on plates and
695 stratified at 4°C in darkness for 3 d, and then kept in white light for 4 h in order to
696 induce uniform germination. The seeds were then transferred to white, blue, red
697 light, or dark conditions and incubated at 22°C for 4 d. The hypocotyl length were
698 measured using ImageJ software after photographing the seedlings.
699
700 **Quantitative RT-PCR**
701 Total RNA was extracted from wild type and mutant seedlings using an RNAiso
702 Plus kit (Takara). Complementary DNAs (cDNA) were synthesized from 2 μg of
703 total RNA using a Prime Script RT Reagent Kit, with a genomic DNA Eraser
704 (Yeasen) according to the manufacturer's instructions. Then, cDNA was subjected
705 to RT-qPCR assays, using the Step One Plus RT-PCR detection system (Applied
706 Biosystems) and SYBR Green PCR Master Mix (Yeasen). PCR was performed in
707 triplicate for each sample, and the expression levels were normalized to that of
708 *ACT7* gene.
709
710 **Immunoblot Analysis**
711 For immunoblot analysis, wild type and mutant seedlings were homogenized in a

712 protein extraction buffer containing 100 mM Tris (pH 6.8), 10% Glycerol, 0.5%
713 SDS, 0.1% Triton, 5 mM EDTA, 0.01 M DTT, and 1 × complete protease inhibitor
714 cocktail (Roche). Equal amount of proteins were loaded and separated on a 10%
715 SDS-PAGE gel and transferred to polyvinylidene fluoride membrane. The
716 membrane was blocked with 5% milk and incubated with primary antibody
717 overnight at 4°C. Primary antibodies anti-AtGLK1/2 were used with 1:2000 dilution.
718 The membrane was then washed four times with 1 × Phosphate Buffered Saline
719 Tween-20 (PBST) for 5 minutes, and incubated with secondary antibody (1:10000
720 dilution) for 1 h at room temperature. After four washes with PBST for 5 minutes,
721 signal was detected with ECL kit (Millipore) according to manufacturer's protocol.
722

723 **EMSA Assay**

724 Synthetic complementary oligonucleotides of *GLK1*, *GLK2*, *GNC*, and *CGA1* (or
725 mutant probe with HY5 binding motif mutation) were obtained, and probes were
726 PCR amplified using Cy5-labeled primers. For proteins, the sequence encoding
727 HY5 was cloned into the pCold-TF vector, expressed, and purified with
728 Capturem™ His-Tagged Purification Maxiprep Kit (Takara). The binding reaction
729 was performed in 20 μL binding buffer (10 mM Tris pH 8.0, 1 mM KCl, 4 mM
730 $MgCl_2$, 0.5mM DTT, 5% glycerol, 0.2 mM EDTA, and 0.01% BSA) using 15 nM
731 probes and 200 ng proteins, and incubated at room temperature for 30 min. The
732 reactions were resolved by electrophoresis in a 6% (v/v) native polyacrylamide gel
733 at 4°C. Cy5-labeled DNA in the gel was scanned by an Amersham Typhoon 5
734 Biomolecular Imager.
735

736 **Dual-luciferase Reporter System**

737 Promoter sub-fragments of *GLK1*, *GLK2*, *GNC*, and *CGA1* were cloned into the
738 pGreenII 0800-LUC vector to drive the firefly luciferase gene. p2GWY7-HY5-YFP

739 was used as the effector construct. Arabidopsis mesophyll cell protoplasts were
740 isolated and transfected as described previously (Yoo et al., 2007). We used a
741 Dual Luciferase kit (Yeasen) for transient expression analysis to detect reporter
742 activity. The Renilla luciferase gene, driven by the cauliflower mosaic virus 35S
743 promoter, was used as an internal control. The ratio of LUC/REN was calculated
744 as an indicator of the final transcriptional activity.
745
746 **Chromatin Immunoprecipitation (ChIP)**
747 The ChIP assay was performed as described by Xu et al. (2016). Chromatin
748 isolation was performed using Col and *HA-HY5 hy5-215* transgenic seedlings
749 grown under constant white light for 4 d. The resuspended chromatin was
750 sonicated at 4°C to 250- to 500-bp fragments. The sheared chromatin was
751 immunoprecipitated, washed, reverse cross linked, and finally amplified. About 10%
752 of sonicated but non-immunoprecipitated chromatin was reverse cross linked and
753 used as an input DNA control. Both immunoprecipitated DNA and input DNA were
754 analyzed by RT-PCR (Applied Biorad). Monoclonal anti-HA antibody (ab9110,
755 Abcam, 1:100 dilution) was used for the immunoprecipitation. The level of binding
756 was calculated as the ratio between the IP and Input groups.
757
758 ***In vitro* Pull-downs**
759 The full-length coding sequences of *GLK1*, *GLK2*, *GNC*, and *CGA1* were cloned
760 into pGEX-6P-1 vector, and the full-length coding sequences of *HY5* and *nDET1*
761 (26-87 aa) were cloned into pCold-TF vector. Two mL of transformed bacteria
762 cultures were grown over night, diluted 1:100 in the next morning, and grown for
763 another 3 hours at 37°C. When the cells reached logarithmic phase, 0.1 mM IPTG
764 was added to induce the expression of proteins at 37°C for 4 hours. Then 0.5 mL
765 of each selected *E. coli* cultures was added in the same tube, resuspended in

766 lysis buffer (1 × PBS, pH 7.4, 1 × complete protease inhibitor cocktail), and the
767 cells were broken with a sonicator for 3 times (30 s on and 60 s off) on ice. After
768 the cell debris were removed by centrifugation at 18000 g for 30 min, 20 μL
769 Glutathione Sepharose 4B was added to the mixture sample and rotated at 4°C
770 for 6 hours. After 4 washes with PBST buffer (1 × PBS, pH 7.4, 0.1% Triton X-100),
771 the pellet fraction was boiled in 5 × SDS protein loading buffer, and the input and
772 pull-down prey proteins were detected by immunoblot analysis using anti-His
773 (M201, Takara, 1:3000 dilution) and anti-GST (G018, Abcam, 1:3000 dilution)
774 antibodies respectively.
775
776 **Bi-luminescence Complementation (BiLC) Assay**
777 GLK or HY5 was fused to the N- or C-terminus of firefly luciferase, and the
778 constructs were transformed into agrobacterium (*Agrobacterium tumefaciens*)
779 strain GV3101. Overnight cultures of agrobacteria were collected by
780 centrifugation at 4000 g for 10 min, resuspended in MES buffer (10 mM MES,10
781 mM $MgCl_2$, and 100 mM acetosyringone), mixed with GV3101 colonies
782 expressing pSoup-P19 to a final OD_{600} = 0.5, and incubated at room temperature
783 for 3 h in the dark before infiltration. The agrobacterium suspension in a 1 mL
784 syringe (without the metal needle) was carefully press-infiltrated onto healthy
785 leaves of 3-week-old *N. benthamiana*. The infiltrated plants were returned to
786 long-day conditions for 3 d. Leaves were infiltrated with luciferin solution and left
787 for 10 min, before observing luciferase activity by imaging with a CCD camera
788 (Tanon-5200, BioTanon, China).
789
790 **RNA-seq Analysis**
791 Total RNA was extracted from the 4-d-old Col-0, *glk2*, *hy5*, and *hy5 glk2* seedlings
792 grown in constant white light (100 μmol photons m^{-2} s^{-1}) conditions. Sequencing

793 libraries were generated with three independent biological replicates for each
794 material, and the sequencing was performed using Illumina platform by Biomarker
795 company (Beijing). Clean reads were mapped to the Arabidopsis reference
796 genome (TAIR11.0_NCBI) using Hisat2 (Version 2.2.1.0). Read counts per gene
797 and Fragments Per Kilobase exon per Million reads mapped (FPKM) values were
798 quantified using Cufflinks (Version 2.2.1), while read count values per transcript
799 (protein coding) were calculated using Bowtie2 and eXpress. Differentially
800 expressed genes (DEGs) were identified using DESeq (Version1.18.0) (Anders et
801 al., 2013). The R package functions 'Estimate Size Factors' and 'nbinom Test',
802 Padj values<0.05 and Fold Changes >1 were set as the threshold for significantly
803 different expression.
804
805 **Accession Numbers**
806 Sequence information from this article can be found in The Arabidopsis
807 Information Resource (TAIR) under the following accession numbers: *GLK1*
808 (AT2G20570), *GLK2* (AT5G44190), *GNC* (AT5G56860), *CGA1* (AT4G26150),
809 *DET1* (AT4G10180), *HY5* (At5G11260), *ACT7* (At5G09810), *HEMA1*
810 (AT1G58290), *PORB* (AT4G27440), *PORC* (AT1G03630), *CAO* (AT1G44446),
811 *LHCB1.2* (AT1G29910), *LHCB2.2* (AT2G05070), *LHCA1* (AT3G54890), *LHCA4*
812 (AT3G47470), *EXPA5* (AT3G29030), *IAA19* (AT3G15540), *SAUR20*
813 (AT5G18020), *PME16* (AT2G43050), *IAA7* (AT3G23050), and *XTH4*
814 (AT2G06850).
815
816 **Supplemental Data**
817 Supplemental Figure 1. HY5, GLK, GNC and CGA1 regulate the chlorophyll
818 biosynthesis.
819 Supplemental Figure 2. Phenotypes of the mutants and overexpression lines
820 under continuous white light conditions.

821 Supplemental Figure 3. GLK inhibits hypocotyl elongation.

822 Supplemental Figure 4. GNC and CGA1 promote hypocotyl elongation.

823 Supplemental Figure 5. HY5 but not GLKs is crucial for the greening of detached
824 roots.

825 Supplemental Figure 6. 6-BA promotes GLK and HY5 dependent chlorophyll
826 biosynthesis.

827 Supplemental Figure 7. *In vitro* Evidence of HY5 Interacting with GLK and
828 Potential Co-regulation of Target Genes.

829 Supplemental Figure 8. *In vitro* evidence of DET1 interacting with GLK and
830 GNC/CGA1.

831 Supplemental Dataset 1. List of 516 GLK2 regulated genes.

832 Supplemental Dataset 2. List of 656 HY5 regulated genes.

833 Supplemental Dataset 3. List of 716 HY5 and GLK2 regulated genes.

834 Supplemental Data 4. Primers used in this study.

835 Supplemental Data 5. Statistical analysis for the data shown in figures.

836

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845

846 **Author Contributions**

847 TZ and PW designed the study. TZ and RZ performed most of the experiments.

848 XZ worked on the transcriptional activation and protein interaction. LY worked on
849 the ChIP-qPCR experiments. ST analyzed the RNA-seq data. PW, YZ, WZ, and
850 XZ wrote and revised the manuscript.
851
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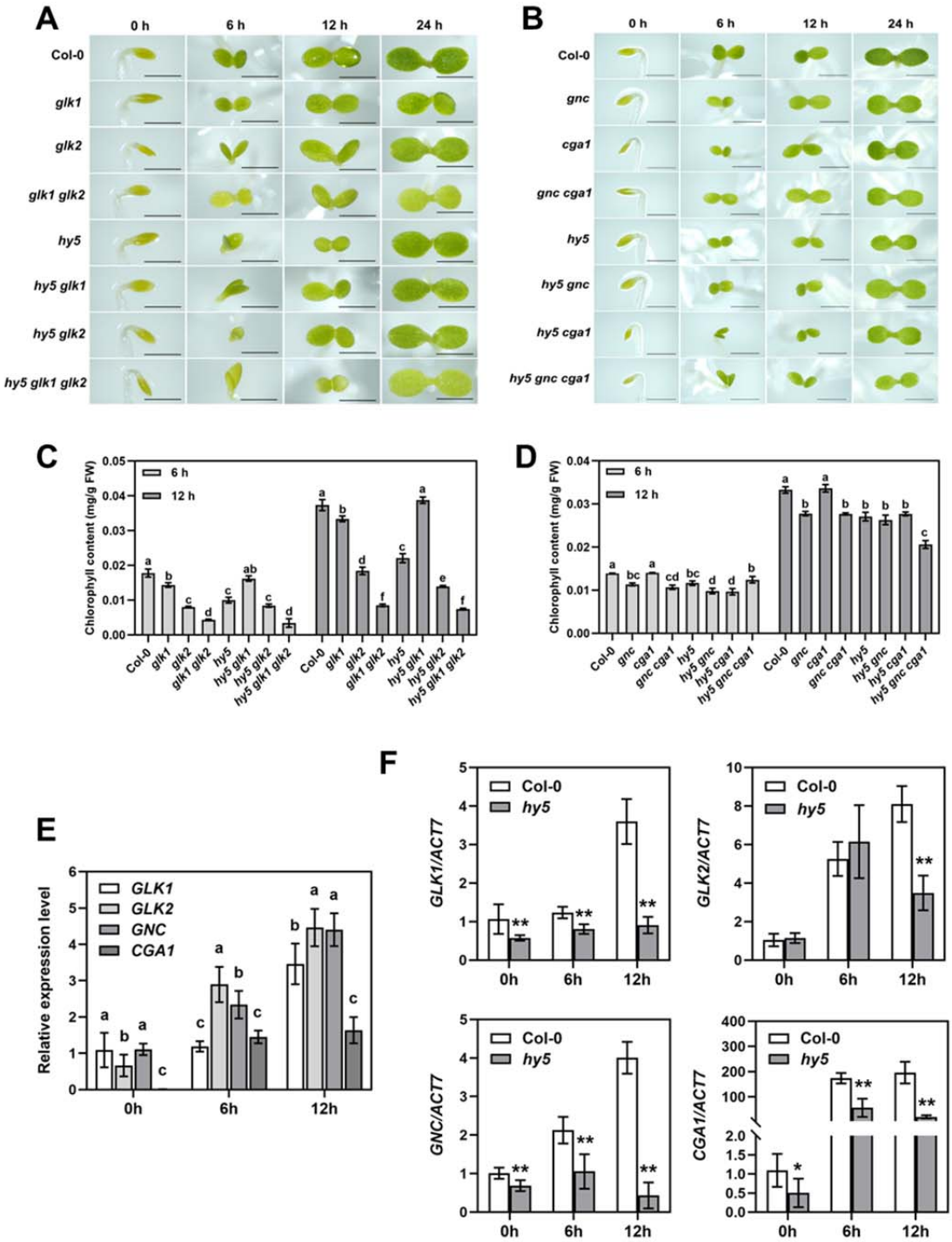


Figure 1. GLK1/2 and GNC/CGA1 Positively Regulate Chlorophyll Content Downstream of HY5 during De-etiolation.

(A) Phenotypes of 4-day-old etiolated seedlings of Col-0, *glk1*, *glk2*, *glk1 glk2*, *hy5*,

hy5 glk1, *hy5 glk2*, and *hy5 glk1 glk2* during transition from dark to light conditions for 6 h, 12 h, 24 h. Scale bars = 1 mm.

(B) Phenotypes of 4-day-old etiolated seedlings of Col-0, *gnc*, *cga1*, *gnc cga1*, *hy5*, *hy5 gnc*, *hy5 cga1*, and *hy5 gnc cga1* during transition from dark to light conditions for 6 h, 12 h, 24 h. Scale bars = 1 mm.

(C, D) Chlorophyll contents of de-etiolating seedlings during transition from (A) and (B) dark to light for 6 h and 12 h. The data represent means ± SD (n = 3) and letters above the bars indicate significant differences ($P < 0.05$), as determined by one-way ANOVA with Turkey's HSD test.

(E) The expression levels of *GLK1*, *GLK2*, *GNC* and *CGA1* in 4-d-old dark-grown Col-0 upon being transferred to white light at indicated time points. The *ACT7* gene was used as internal control. The data represent means ± SD (n = 3) and letters above the bars indicate significant differences ($P < 0.05$), as determined by one-way ANOVA with Turkey's HSD test.

(F) The expression levels of *GLK1*, *GLK2*, *GNC* and *CGA1* in 4-d-old dark-grown Col-0 and *hy5* seedlings upon being transferred to white light at indicated time points. The data represent means ± SD (n = 3), and asterisks indicate a significant difference compared with Col-0 (*P<0.05, **P<0.01, paired samples *t*-test).

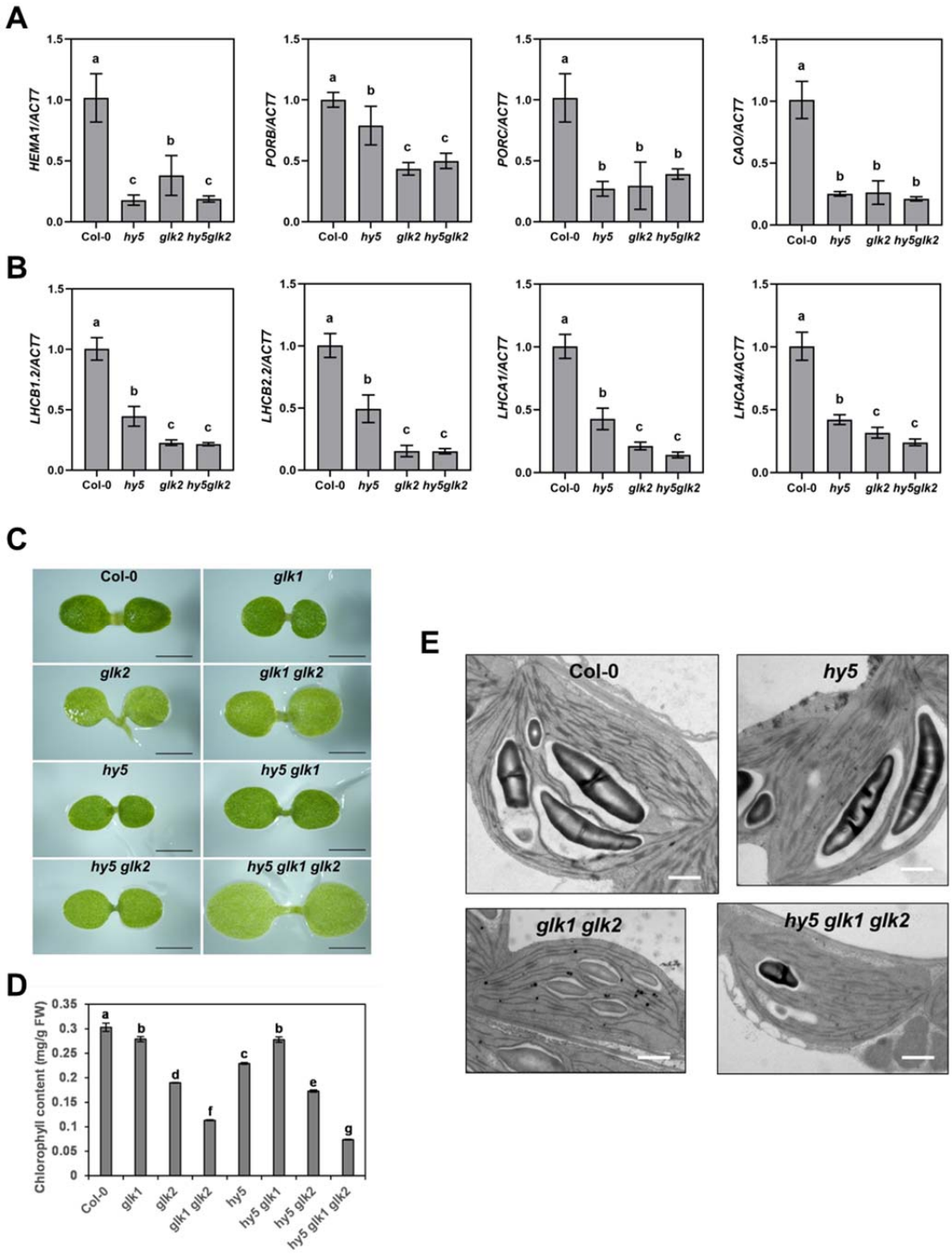


Figure 2. Chloroplast Development is Further Impaired in *hy5* and *glk* Multiple Mutants.

(A, B) RT-qPCR analysis of chlorophyll biosynthesis and photosystem genes

expressed in 4-d-old etiolated seedlings of Col-0, *hy5*, *glk2* and *hy5 glk2* upon being transferred to white light for 12 h. The *ACT7* gene was used as internal control. The data represent means ± SD (n = 3) and letters above the bars indicate significant differences ($P < 0.05$), as determined by one-way ANOVA with Turkey's HSD test.

(C) Phenotypes of 4-day-old Col-0, *glk1*, *glk2*, *glk1 glk2*, *hy5*, *hy5 glk1*, *hy5 glk2*, and *hy5 glk1 glk2* seedlings grown on half-strength MS plates under continuous white light conditions. Scale bars = 1 mm.

(D) Chlorophyll contents of the genotypes shown in **(A)**. The data represent means ± SD (n = 3) and letters above the bars indicate significant differences ($P < 0.05$), as determined by one-way ANOVA with Turkey's HSD test.

(E) The ultrastructure of mesophyll cell chloroplasts of Col-0, *hy5*, *glk1 glk2*, and *hy5 glk1 glk2* seedlings grown under continuous white light for 7 days. Scale bar = 1 μm.

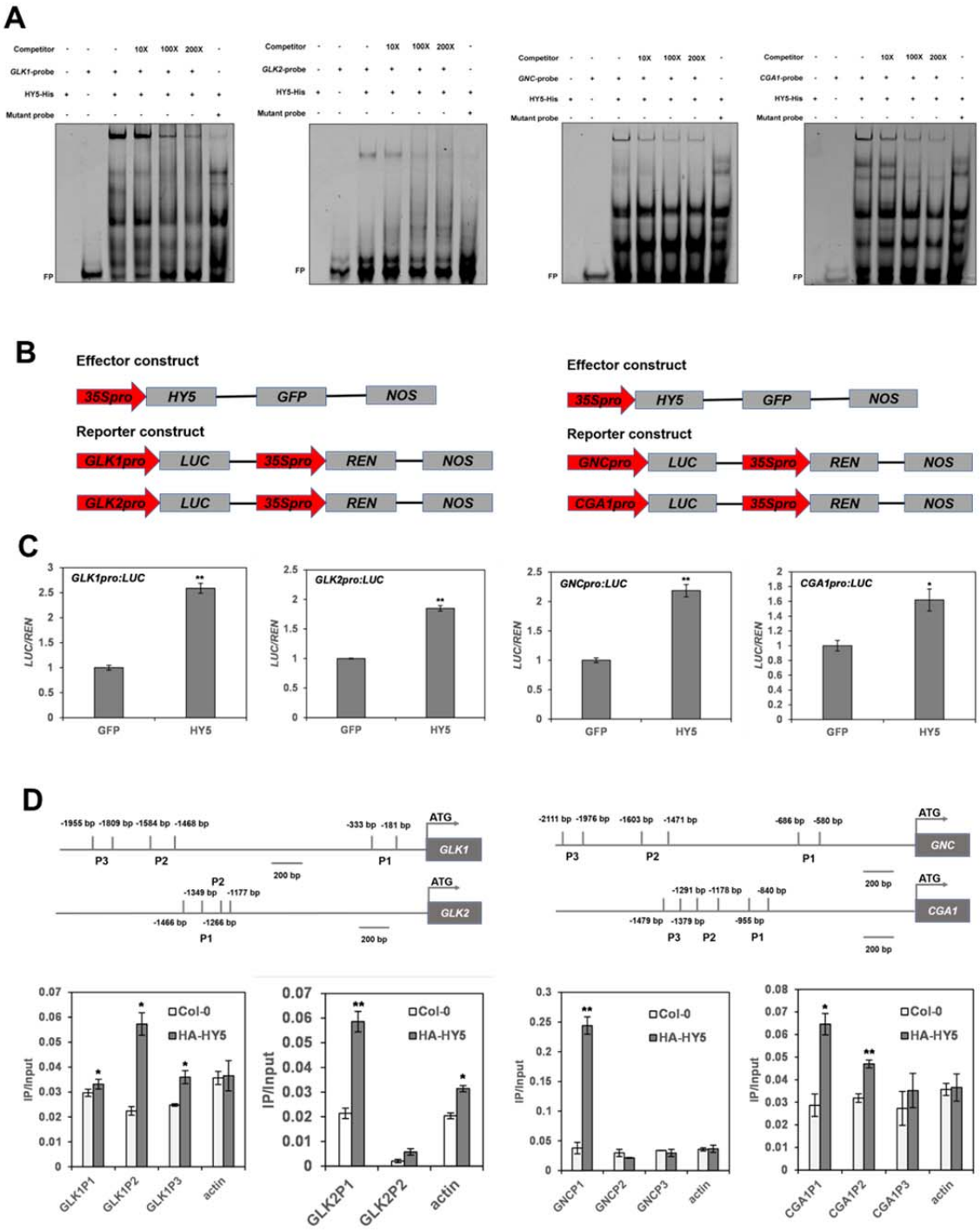


Figure 3. Binding of HY5 to the Promoters and Transcriptional Activation of *GLK1, GLK2, GNC* and *CGA1*.

(A) EMSA showing HY5 binds to the sub-fragments of promoters of *GLK1*, *GLK2*, *GNC* and *CGA1 in vitro*. "-" and "+" indicate the absence and presence of

corresponding probes or proteins. FP means free probe.

(B) Schematic structures of effector and reporter constructs used in dual-luciferase (LUC) reporter system. *REN*, renilla luciferase gene.

(C) Bar graphs showing HY5 induces the activation of *GLK1pro:LUC*, *GLK2pro:LUC*, *GNCpro:LUC* and *CGA1pro:LUC*. The data represent means ± SD (n = 4), and asterisks indicate a significant difference compared with control (*P<0.05, **P<0.01, paired samples *t*-test).

(D) Illustration of *GLK1*, *GLK2*, *GNC* and *CGA1* promoter regions with the indicated positions of primers used in ChIP-qPCR. Bar graphs showing ChIP-qPCR assays that HY5 associates with the promoters in vivo. Col-0 material and *ACTIN* gene were used as negative controls. The data represent means ± SD (n = 3), and asterisks indicate a significant difference compared with Col-0 (*P<0.05, **P<0.01, paired samples *t*-test).

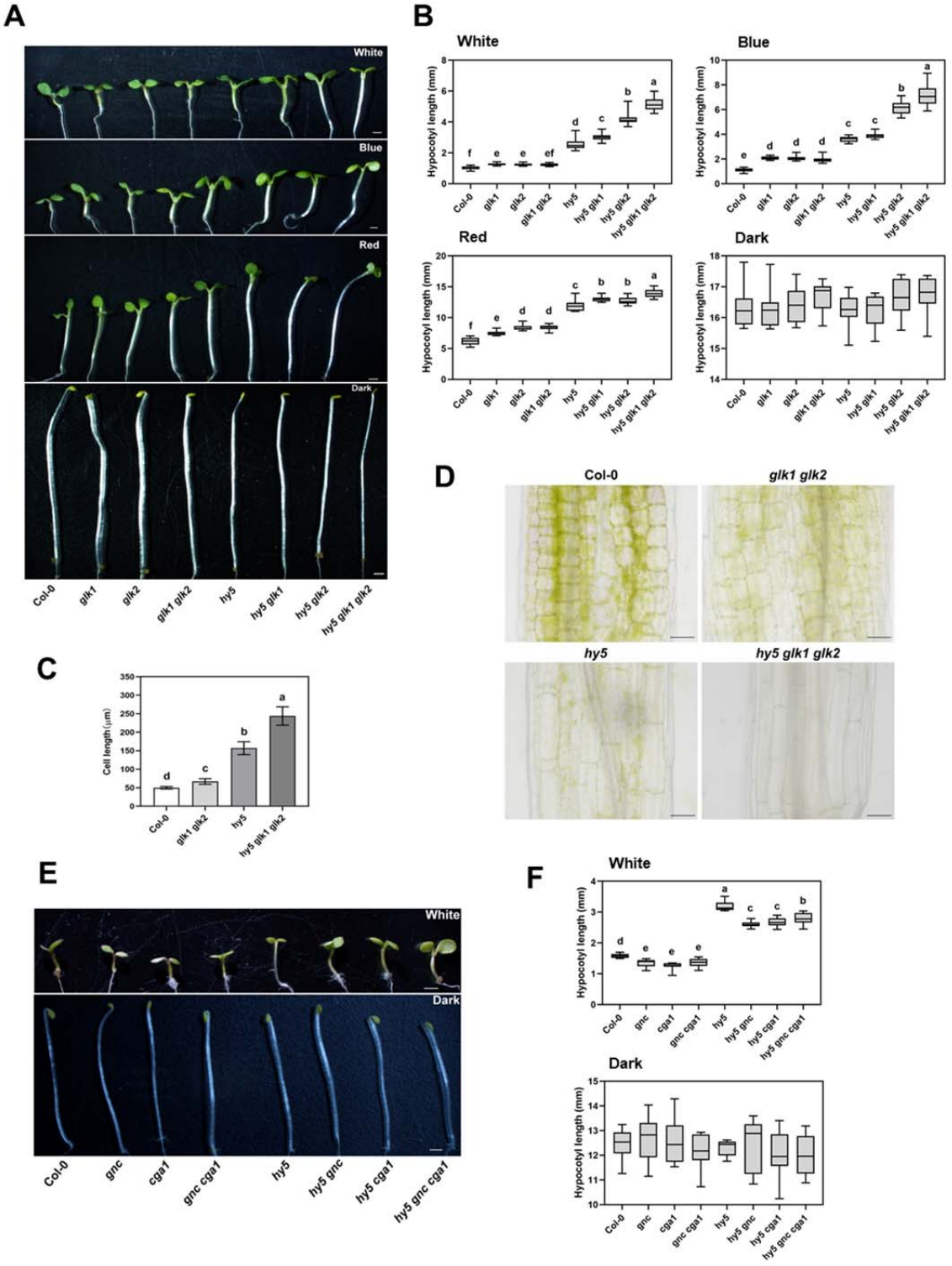


Figure 4. GLK1/2 Negatively and GNC/CGA1 Positively Regulate Hypocotyl Elongation.

(A) Phenotypes of 4-d-old Col-0, *glk1*, *glk2*, *glk1 glk2*, *hy5*, *hy5 glk1*, *hy5 glk2*, and *hy5 glk1 glk2* seedlings grown in white (100 μmol/m^2/s), blue (60 μmol/m^2/s), red (90 μmol/m^2/s) light and dark conditions. Scale bar = 1 mm.
(B) Quantification of hypocotyl lengths indicated in (A). The data represent means ± SD (n ≥17) and letters above the bars indicate significant differences ($P < 0.05$), as determined by one-way ANOVA with Turkey's HSD test.
(C) Quantification of hypocotyl cell length from 4-d-old Col-0, *glk1 glk2*, *hy5*, and *hy5 glk1 glk2* seedlings, grown in white light (100 μmol/m^2/s) conditions. The data represent means ± SD (n = 15) and letters above the bars indicate significant differences ($P < 0.05$), as determined by one-way ANOVA with Turkey's HSD test.
(D) Hypocotyl cell length phenotypes from 4-d-old Col-0, *glk1 glk2*, *hy5*, and *hy5 glk1 glk2* seedlings. Scale bar = 20 μm.
(E) Phenotypes of 4-d-old Col-0, *gnc*, *cga1*, *gnc cga1*, *hy5*, *hy5 gnc*, *hy5 cga1*, and *hy5 gnc cga1* seedlings grown in white light (100 μmol/m^2/s) and dark conditions. Scale bar = 1 mm.
(F) Quantification of hypocotyl lengths indicated in (E). The data represent means ± SD (n ≥15) and letters above the bars indicate significant differences ($P < 0.05$), as determined by one-way ANOVA with Turkey's HSD test.

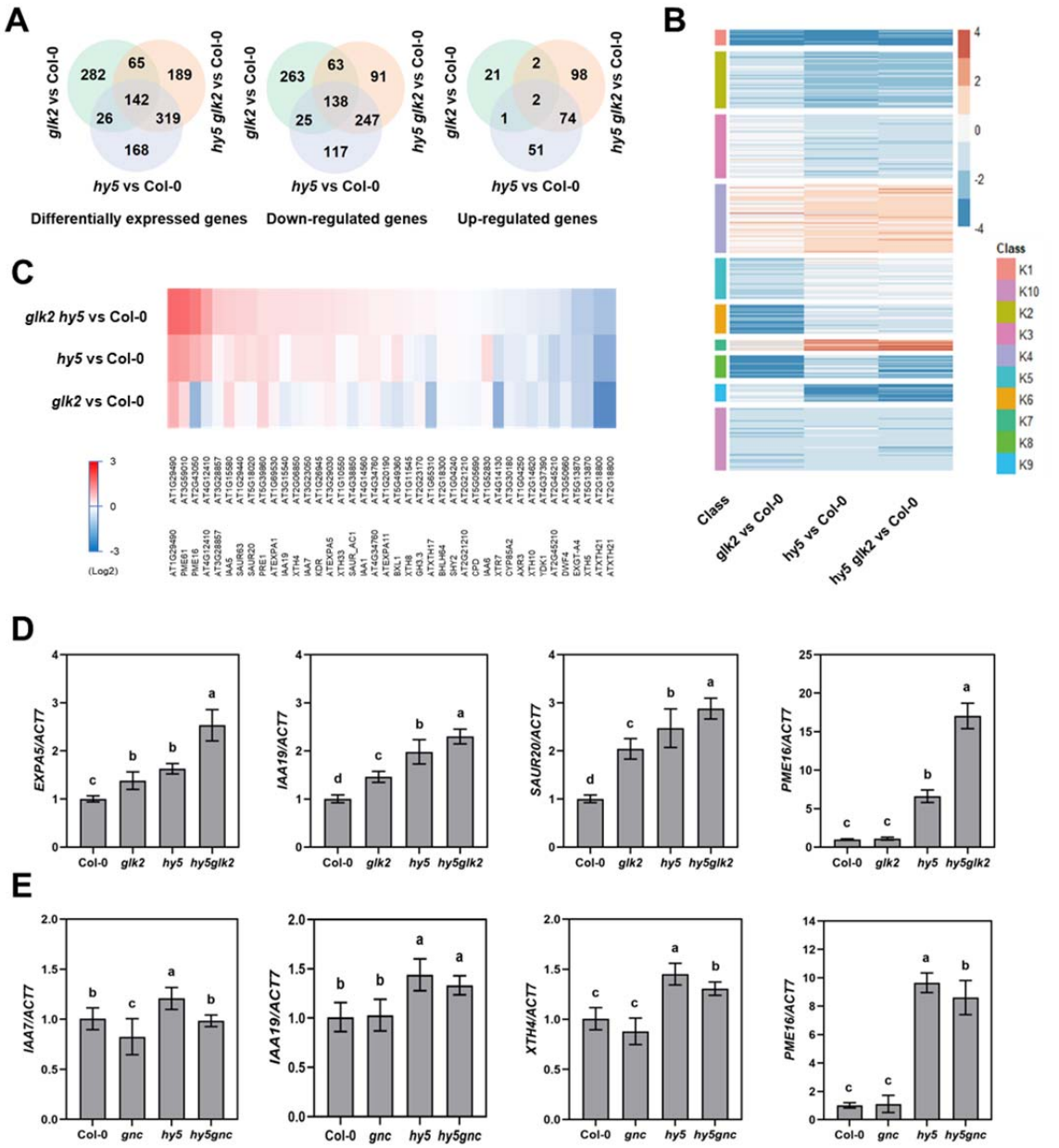


Figure 5. Different Expression Patterns of HY5, GLK2, and GNC Regulated Elongation Genes.

(A) Venn diagram showing overlaps between sets of differentially expressed genes in *glk2*, *hy5* and *hy5 glk2* mutants, relative to Col-0.
(B) K-means clustering of differentially expressed genes regulated by GLK2, HY5 and both. The scale bar shows fold changes (log2 value).
(C) Expression patterns of cell elongation genes in *glk2*, *hy5* and *hy5 glk2* mutants, relative to Col-0. The scale bar shows fold changes (log2 value). Heat

map was aligned according to fold changes in *hy5 glk2* mutant.

(D, E) RT-qPCR analysis of cell elongation genes expressed in 4-d-old Col-0, *hy5*, *glk2*, *hy5 glk2*, *gnc*, and *hy5 gnc* seedlings grown under continuous white light. The *ACT7* gene was used as internal control. The data represent means ± SD (n = 3) and letters above the bars indicate significant differences ($P < 0.05$), as determined by one-way ANOVA with Turkey's HSD test.

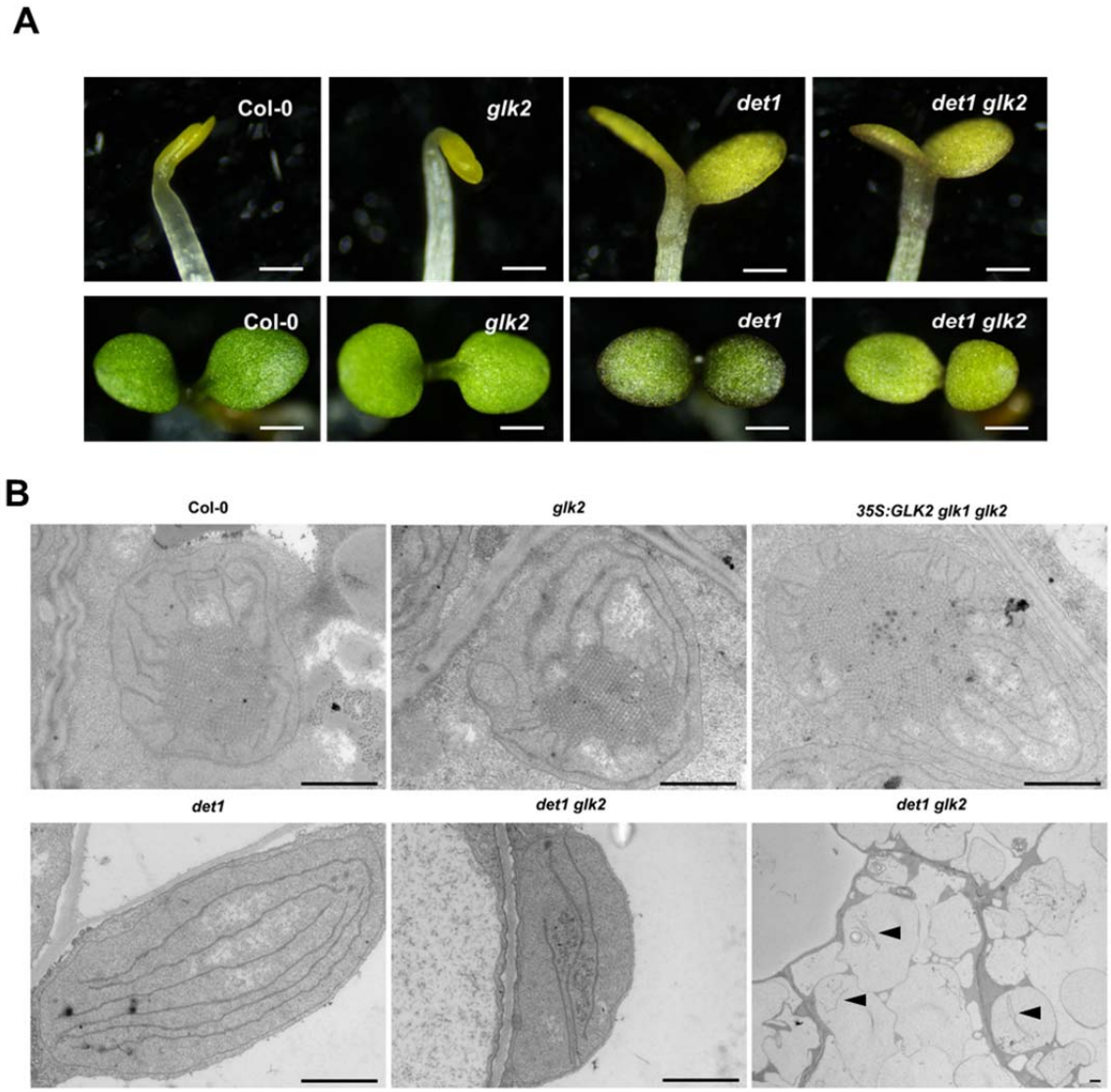


Figure 6. GLK2 Is Responsible for the Developed Thylakoid Structures in the Etiolated *det1* Mutant.

(A) Phenotype of 4 d dark (upper panel) or light (lower panel) grown Col-0, *glk2*, *det1*, and *det1 glk2* seedlings. Scale bars represent 500 μm (upper panel) or 1 mm (lower panel).
(B) The ultrastructure of mesophyll chloroplasts of Col-0, *glk2*, *35S:GLK2 glk1 glk2*, *det1*, and *det1 glk2* seedlings grown under dark condition for 4 days. Scale bar = 1 μm. Black arrow heads in the bottom right image point to the thylakoid lamellae in very few amout.

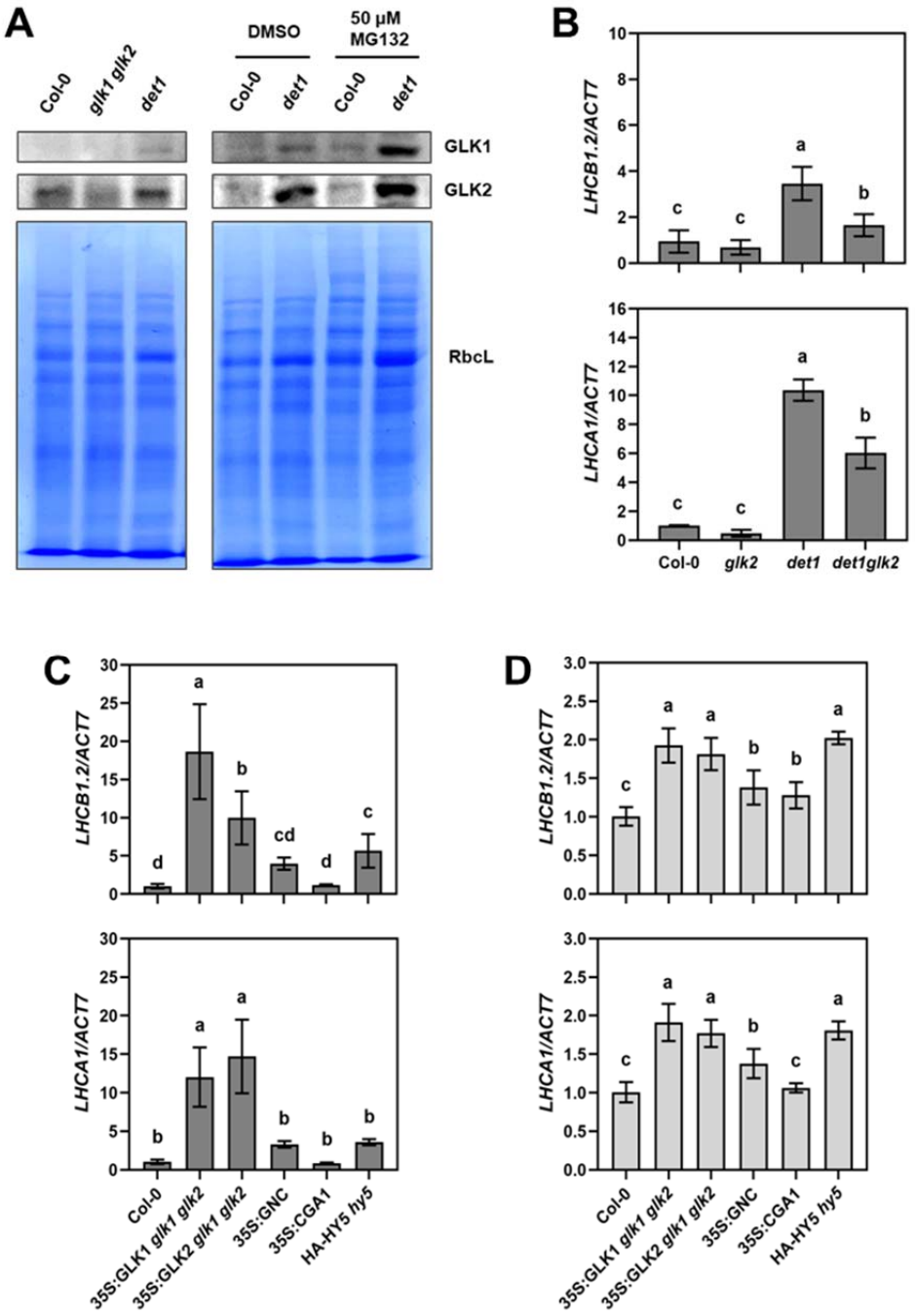


Figure 7. DET1 Promotes the Degradation of GLK Protein, and GLK Is Responsible for Photosystem Gene Expression in the Etiolated *det1* Mutant.

(A) Immunoblot detection of GLK1 and GLK2 in Col-0, *glk1 glk2*, and *det1* seedlings grown in the dark for 4 d (left). GLK protein levels in 4-d-old dark-grown Col-0 and *det1* seedlings treated with 50 μM MG132 or DMSO (right). Coomassie

stained PAGE gel was shown as loading control, and the band of Rubisco large subunit was indicated.

(**B**) RT-qPCR analysis of photosystem genes expressed in 4-d-old etiolated Col-0, *glk2*, *35S:GLK2 glk1 glk2*, *det1*, and *det1 glk2* seedlings.

(**C, D**) RT-qPCR analysis of photosystem genes expressed in 4-d-old etiolated (D) or light-grown (E) Col-0, *35S:GLK1 glk1 glk2*, *35S:GNC*, *35S:CGA1*, and *HA-HY5 hy5* seedlings. The *ACT7* gene was used as internal control. The data represent means ± SD (n = 3) and letters above the bars indicate significant differences ($P < 0.05$), as determined by one-way ANOVA with Turkey's HSD test.

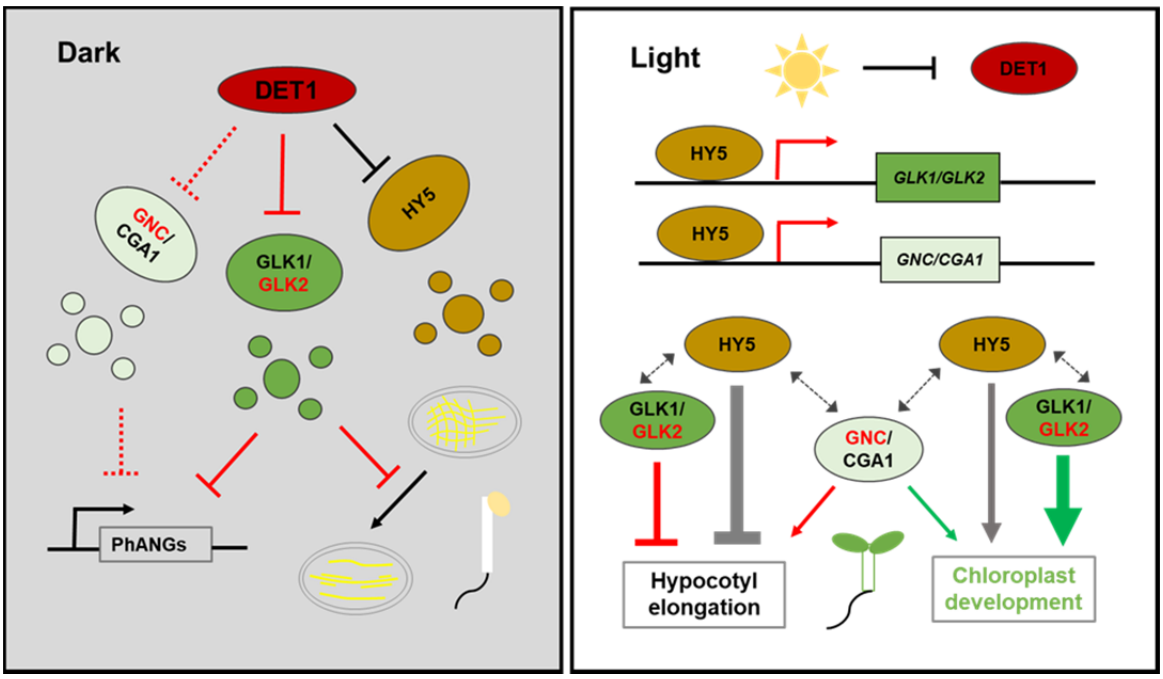
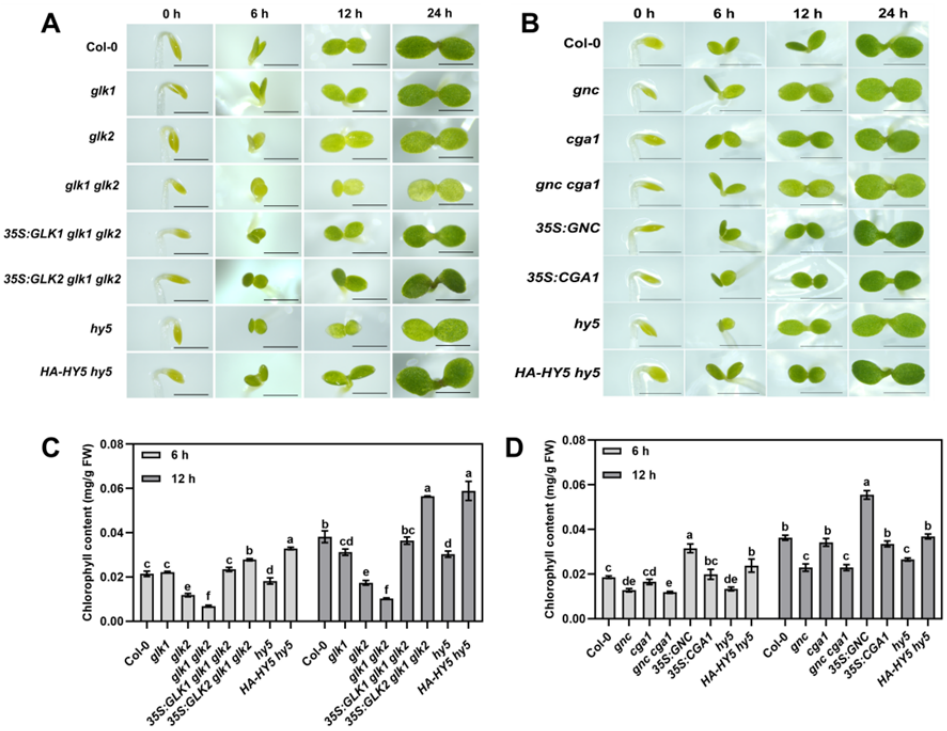


Figure 8. A Working Model Depicting GLK, GNC/CGA1, and HY5 in the Regulation of Skotomorphogenesis and Photomorphogenesis.

GLK, GNC, CGA1 and HY5 undergo DET1-mediated degradation in the dark, so that the expression of photosynthesis associated nuclear genes (PhANGs), and the transformation of prolamellar body towards extended lamellae are inhibited. Light inhibits DET1 activity, and HY5 directs the promoter of *GLK*, *GNC* and *CGA1*, inducing their activities to promote chloroplast development. GLK inhibits hypocotyl elongation while GNC and CGA1 promote hypocotyl elongation. Thick green arrow indicates strong promotion of chloroplast development by GLK. Thick gray line indicates strong inhibition of hypocotyl elongation by HY5. Thinner arrows indicate less strong regulation. Dashed lines in the dark indicate GNC/CGA1 related regulations that pending to be further validated. Dashed double sided arrows in the light indicate potential protein interactions of HY5 with GLKs and GNC/CGA1.



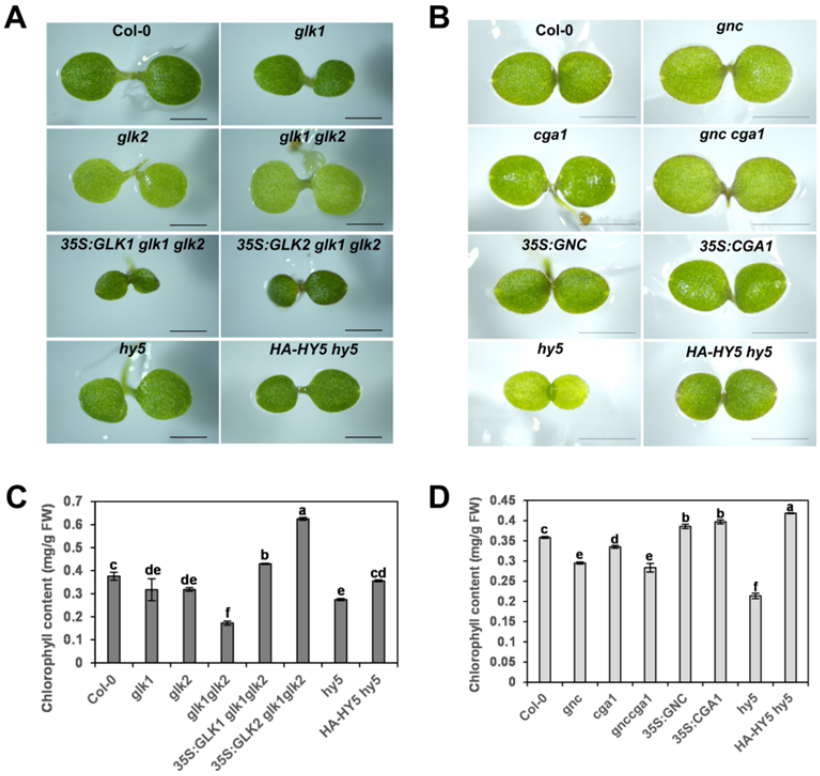
Supplemental Figure 1. HY5, GLK, GNC and CGA1 Regulate the Chlorophyll Biosynthesis. (Supports Figure 1)

(A) Representative images of 4-day-old etiolated seedlings of Col-0, *glk1*, *glk2*, *glk1 glk2*, *35S:GLK1 glk1 glk2*, *35S:GLK2 glk1 glk2*, *hy5*, and *HA-HY5 hy5* during the transition from dark to light conditions for 6 h, 12 h, and 24 h. Scale bars = 1 mm.

(B) Representative images of 4-day-old etiolated seedlings of Col-0, *gnc*, *cga1*, *gnc cga1*, *35S:GNC*, *35S:CGA1*, *hy5*, and *HA-HY5 hy5* during the transition from dark to light conditions for 6 h, 12 h, and 24 h. Scale bars = 1 mm.

(C) Chlorophyll contents of 4-day-old etiolated seedlings from **(A)** during the transition from dark to light conditions for 6 h and 12 h. The data represent means ± SD (n=3) and letters above the bars indicate significant differences ($P < 0.05$), as determined by one-way ANOVA with Turkey's HSD test. The experiments were performed three times with similar results.

(D) Chlorophyll contents of 4-day-old etiolated seedlings from **(B)** during the transition from dark to light conditions for 6 h and 12 h. The data represent means ± SD (n=3) and letters above the bars indicate significant differences ($P < 0.05$), as determined by one-way ANOVA with Turkey's HSD test. The experiments were performed three times with similar results.



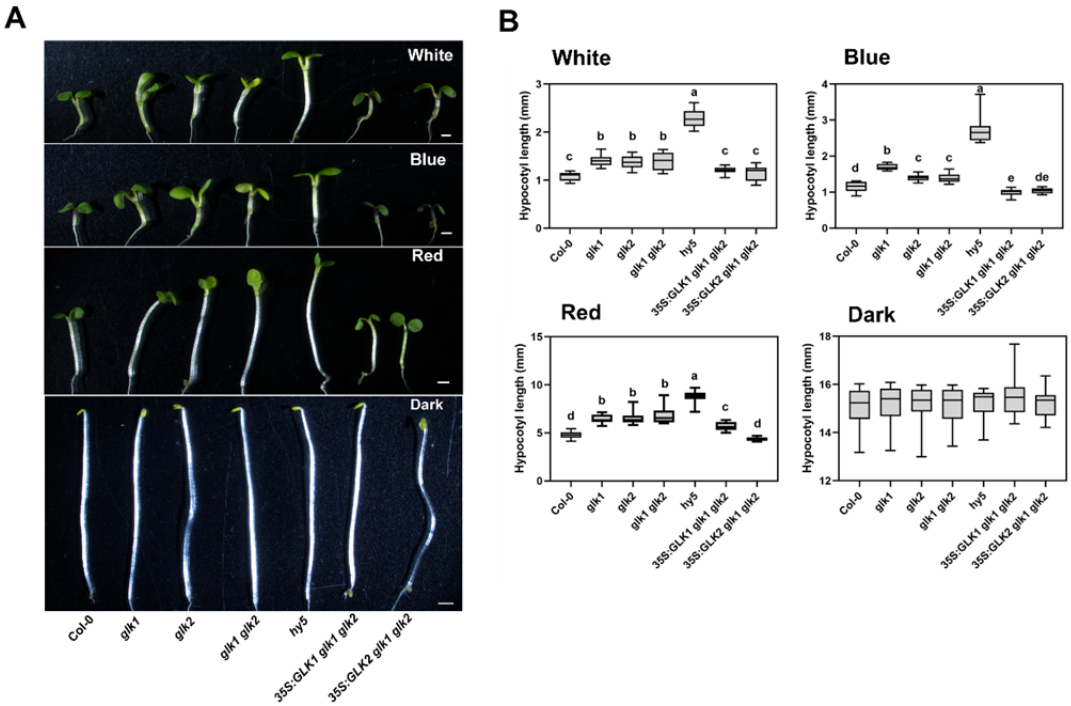
Supplemental Figure 2. Phenotypes of the Mutants and Overexpression Lines under Continuous Light Conditions. (Supports Figure 2)

(A) Representative images of 4-day-old Col-0, *glk1*, *glk2*, *glk1 glk2*, *35S:GLK1 glk1 glk2*, *35S:GLK2 glk1 glk2*, *hy5*, and *HA-HY5 hy5* seedlings grown on half-strength MS plates for 4 days under continuous white light conditions. Scale bars = 1 mm.

(B) Representative images of 4-day-old Col-0, *gnc*, *cga1*, *gnc cga1*, *35S:GNC*, *35S:CGA1*, *hy5*, and *HA-HY5 hy5* seedlings grown on half-strength MS plates for 4 days under continuous white light conditions. Scale bars = 1 mm.

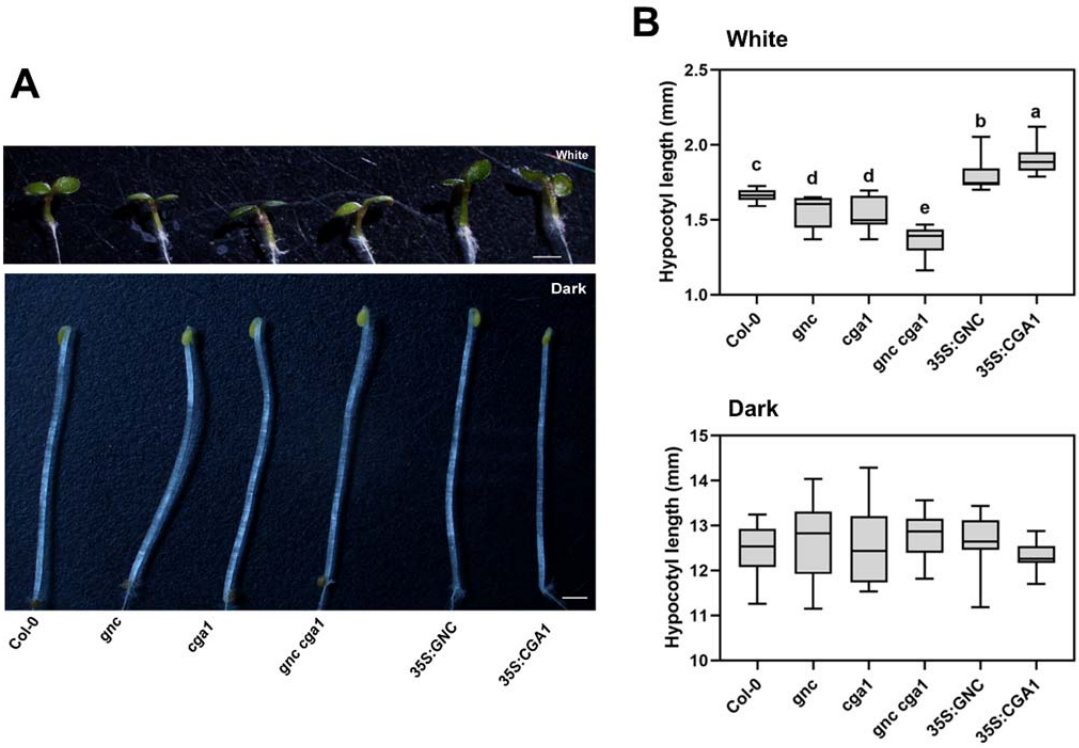
(C) Chlorophyll contents of the genotypes shown in **(A)**. The data represent means ± SD (n=3) and letters above the bars indicate significant differences ($P < 0.05$), as determined by one-way ANOVA with Turkey's HSD test. The experiments were performed three times with similar results.

(D) Chlorophyll contents of the genotypes shown in **(B)**. The data represent means ± SD (n=3) and letters above the bars indicate significant differences ($P < 0.05$), as determined by one-way ANOVA with Turkey's HSD test. The experiments were performed three times with similar results.



Supplemental Figure 3. GLK Inhibits Hypocotyl Elongation. (Supports Figure 4)

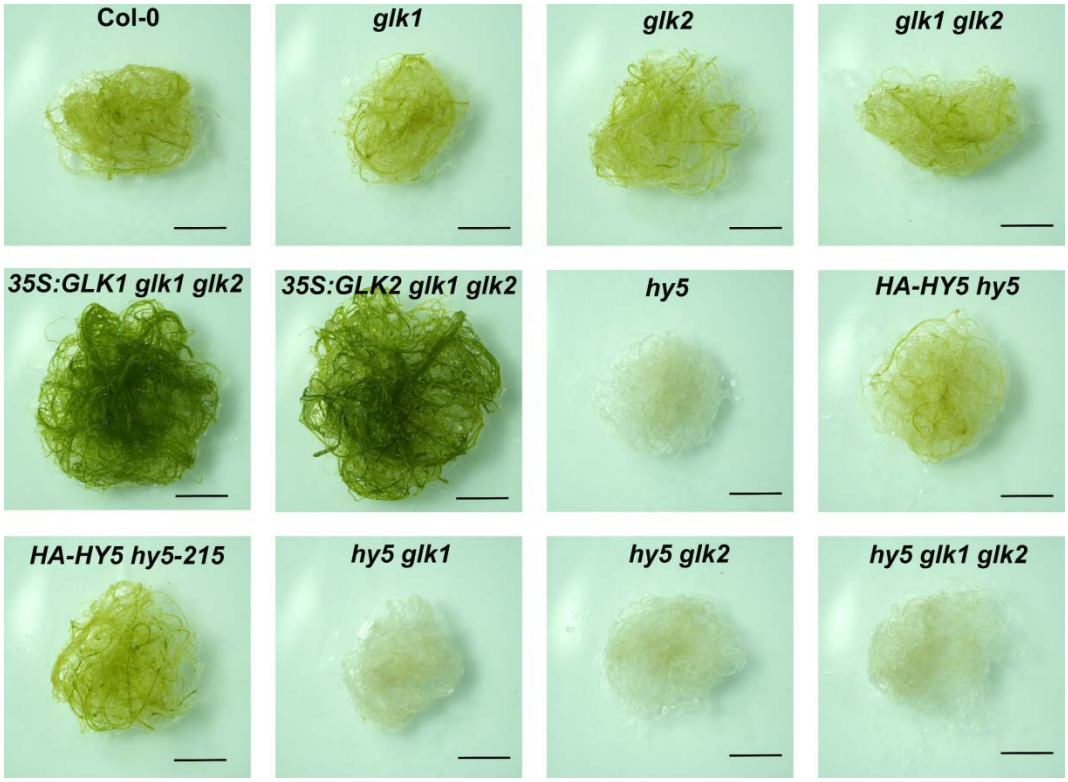
(A) Hypocotyl phenotypes of 4-d-old Col-0, *glk1*, *glk2*, *glk1 glk2*, *hy5*, *35S:GLK1 glk1 glk2*, and *35S:GLK2 glk1 glk2* seedlings grown in white (100 μmol/m^2/s), blue (60 μmol/m^2/s), red (90 μmol/m^2/s) light and dark conditions. Scale bar = 1 mm. **(B)** Quantification of hypocotyl lengths indicated in **(A)**. The data represent means ± SD (n ≥18) and letters above the bars indicate significant differences ($P < 0.05$), as determined by one-way ANOVA with Turkey's HSD test. The experiments were performed three times with similar results.



Supplemental Figure 4. GNC and CGA1 Promote Hypocotyl Elongation. (Supports Figure 4)

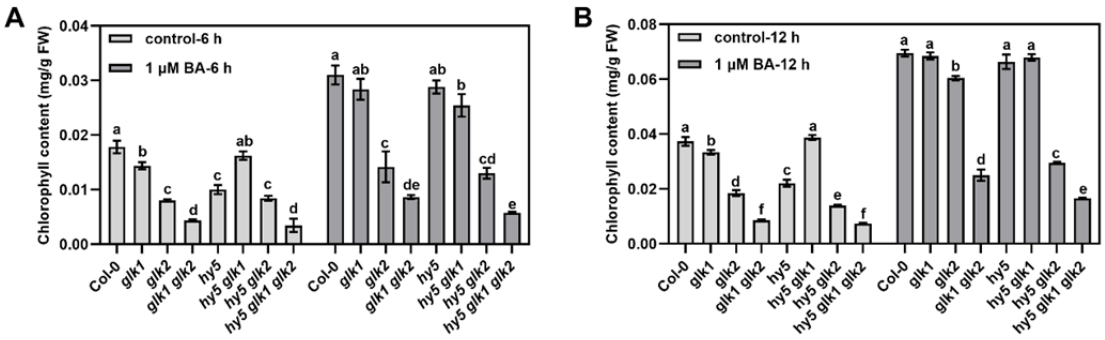
(A) Hypocotyl phenotypes of 4-d-old Col-0, *gnc*, *cga1*, *gnc cga1*, *35S:GNC*, and *35S:CGA1* seedlings grown in white light (100 μmol/m^2/s) and dark conditions. Scale bar = 1 mm.

(B) Quantification of hypocotyl lengths indicated in **(A)**. The data represent means ± SD (n ≥15) and letters above the bars indicate significant differences ($P < 0.05$), as determined by one-way ANOVA with Turkey's HSD test. The experiments were performed three times with similar results.



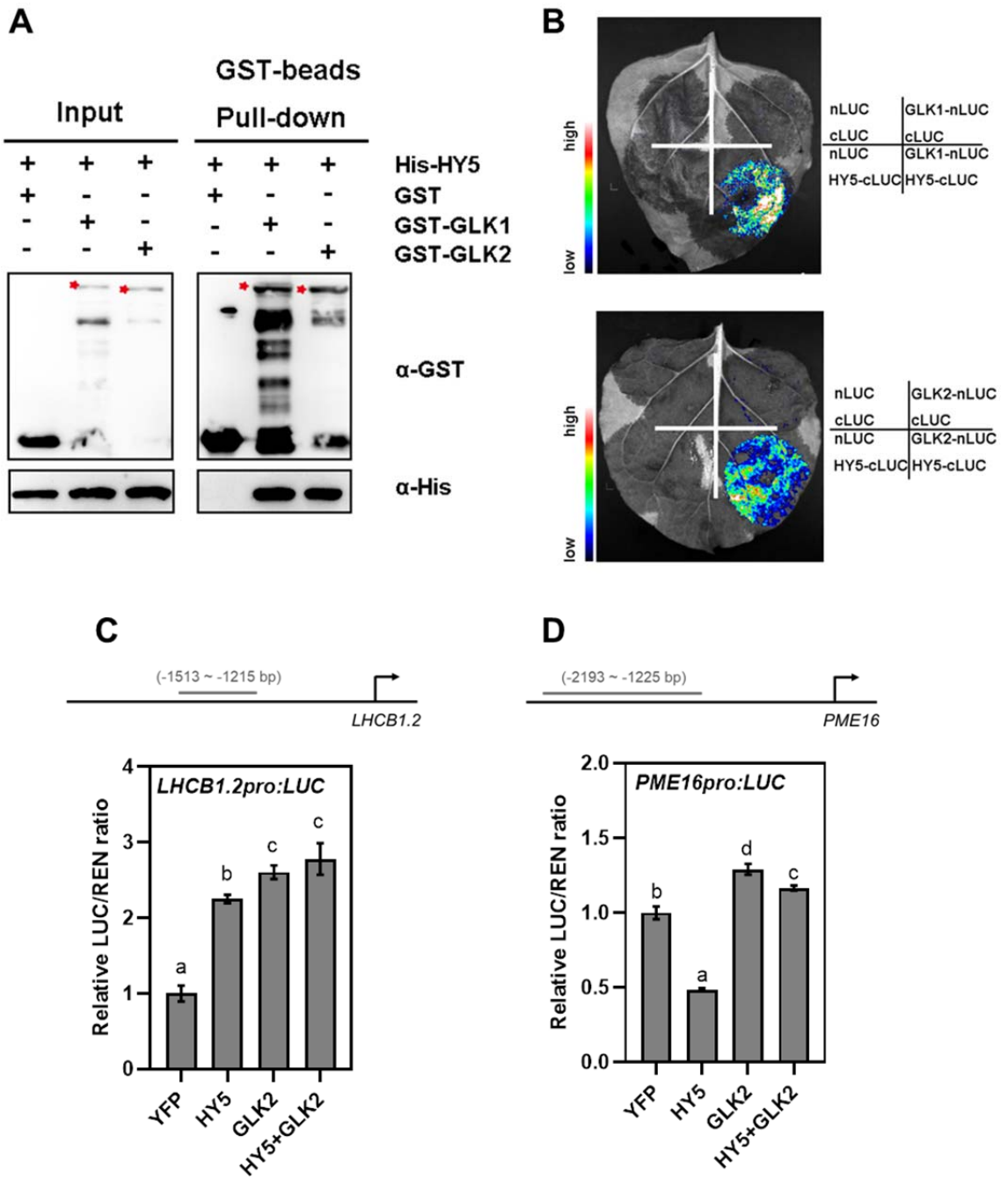
Supplemental Figure 5. HY5 but Not GLKs Is Crucial for the Greening of Detached Roots.

Root samples were detached from 7-d-old seedlings, and then cultured on hormone-free MS medium for 14 d. Scale bar = 5 mm.



Supplemental Figure 6. 6-BA Promotes GLK and HY5 Dependent Chlorophyll Biosynthesis.

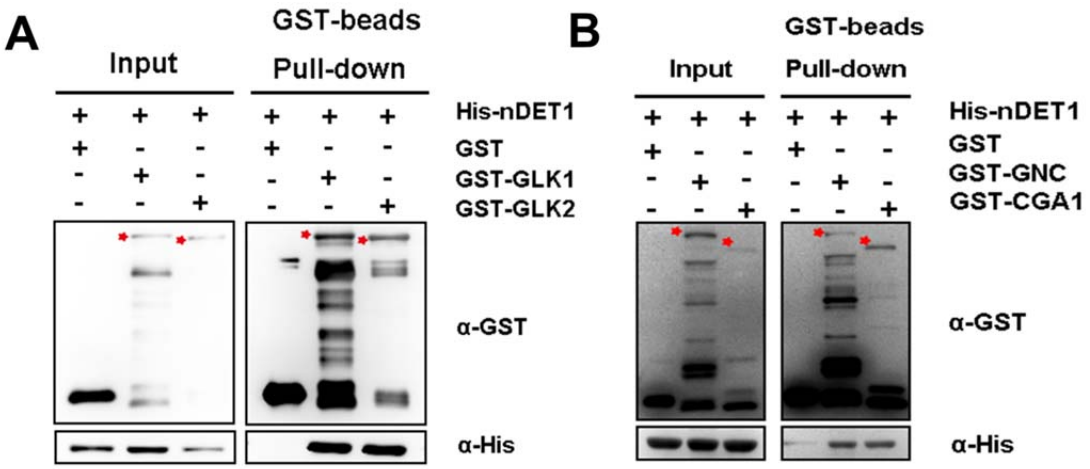
Chlorophyll content of 4-day-old etiolated seedlings of Col-0, *glk1*, *glk2*, *glk1 glk2*, *hy5*, *hy5 glk1*, *hy5 glk2*, and *hy5 glk1 glk2* grown on medium with or without 6-BA during transition from dark to light conditions for 6 h **(A)** and 12 h **(B)**. The data represent means ± SD (n=3) and letters above the bars indicate significant differences ($P < 0.05$), as determined by one-way ANOVA with Turkey's HSD test. The experiments were performed three times with similar results.



Supplemental Figure 7. *In vitro* Evidence of HY5 Interacting with GLK and Potential Co-regulation of Target Genes.

(A) *In vitro* pulldown assays showing the interaction of GLK1 and GLK2 with HY5. GST-GLK protein or GST protein were used to pull down His-HY5 protein using GST beads. Anti-GST and anti-His antibodies were used for immunoblot analysis.

"-" and "+" indicate the absence and presence of corresponding proteins.
(B) Bimolecular luminescence complementation assays showing GLK1 and GLK2 can interact with HY5. nLUC and cLUC served as negative controls.
(C, D) Illustration of *LHCB1.2* and *PME16* promoter regions used in dual-luciferase assay. Bar graphs showing HY5 and GLK2 induce the activation or inhibition of *LHCB1.2pro:LUC* and *PME16pro:LUC*. The data represent means ± SD (n = 4), and asterisks indicate a significant difference compared with control (*P<0.05, **P<0.01, paired samples t-test).



Supplemental Figure 8. *In vitro* Evidence of DET1 Interacting with GLK and GNC/CGA1.

In vitro pulldown assays showing the interactions of GLK1, GLK2 (A), GNC and CGA1 (B) with DET1. GST-GLK1, GST-GLK2, GST-GNC, GST-CGA1 or GST proteins were used to pull down His-nDET1 protein using GST beads. Anti-GST and anti-His antibodies were used for immunoblot analysis. “-” and “+” indicate the absence and presence of corresponding proteins.

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