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1 **Title page**

2

3 **Shade promotes phototropism through phytochrome B-controlled auxin**  
4 **production.**

5

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22

23 Running title: Shade promotes phototropism

24

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26 phototropin 1, phytochrome B, PHYTOCHROME INTERACTING FACTORS,  
27 YUCCAs, *Arabidopsis thaliana*.

28

29 **SUMMARY**

30

31 Phototropism is an asymmetric growth response enabling plants to optimally  
32 position their organs. In flowering plants, the phototropin (phot) blue light  
33 receptors are essential to detect light gradients. In etiolated seedlings the  
34 phototropic response is enhanced by the red/far-red (R/FR) sensing  
35 phytochromes (phy) with a predominant function of phyA. In this study, we  
36 analyzed the influence of the phytochromes on phototropism in green (de-  
37 etiolated) *Arabidopsis* seedlings. Our experiments in the laboratory and  
38 outdoors revealed that in open environments (high R/FR ratio) phyB inhibits  
39 phototropism. In contrast, under foliar shade where access to direct sunlight  
40 becomes important the phototropic response was strong. phyB modulates  
41 phototropism depending on the R/FR ratio by controlling the activity of three  
42 bHLH transcription factors of the PHYTOCHROME INTERACTING  
43 FACTORS (PIFs) family. Promotion of phototropism depends on PIF-  
44 mediated induction of several members of the *YUCCA* gene family leading to  
45 auxin production in the cotyledons. Our study identifies PIFs and YUCCAs as  
46 novel molecular players promoting phototropism in photoautotrophic but not  
47 etiolated seedlings. Moreover, our findings reveal fundamental differences in  
48 the phytochrome-phototropism crosstalk in etiolated versus green seedlings.  
49 We propose that in natural conditions where the light environment is not  
50 homogeneous the uncovered phytochrome-phototropin co-action is important  
51 for plants to optimize their growth strategy and hence photosynthetic light  
52 capture.

## 53 INTRODUCTION

54 Land plants respond to light cues with five photoreceptor families classified  
55 depending on their light absorption properties: UVR8 absorbing UV-B;  
56 phototropins, cryptochromes and Zeitlupes absorbing blue/UV-A and the  
57 phytochromes primarily absorbing red/far-red (R/FR) (reviewed in [1]). Some  
58 light responses are specifically mediated by a single photoreceptor while  
59 others depend on photoreceptor coordination to integrate various light cues to  
60 optimize plant growth and development [2, 3]. For example phytochromes and  
61 cryptochromes cooperatively promote de-etiolation, while phytochrome B  
62 (phyB) and cryptochrome 2 (cry2) antagonistically regulate the transition to  
63 flowering [2, 3]. Photoreceptor crosstalk also occurs during shade avoidance  
64 and phototropism, two growth responses enabling plants to maximize  
65 photosynthesis in low light conditions [4, 5]. Vegetative shade is detected by  
66 phytochromes and cryptochromes because light under a canopy is  
67 characterized by a low R/FR ratio and low blue light [5, 6]. Shade responses  
68 are inhibited in the presence of UV-B by the UVR8 photoreceptor [7, 8].  
69 Interestingly, these three photoreceptor families modulate the activity of  
70 PHYTOCHROME INTERACTING FACTORS (PIFs), identifying these bHLH  
71 transcription factors as potential point of integration [7, 9-12]. PIFs regulate  
72 the expression of a broad range of genes in shade conditions including genes  
73 involved in auxin biosynthesis, transport and signaling [12-15].

74

75 During phototropism plants shift the growth axis of organs such as stems to  
76 reorient themselves towards the light source [16]. This response is controlled  
77 by phototropins, phot1 and phot2 in Arabidopsis. phot1 functions across a

78 broad range of blue-light fluence rates while phot2 is important in high light  
79 intensities [17, 18]. Members of NRL (NPH3/RPT2-Like) and PKS  
80 (PHYTOCHROME KINASE SUBSTRATE) protein families play a major role in  
81 early steps of phototropin signal transduction [19-25]. Subsequently, a lateral  
82 auxin gradient is formed across the hypocotyl, by means of a complex  
83 process requiring auxin efflux carriers from the PINFORMED (PIN) family, the  
84 ABCB19 transporter and regulation of the apoplastic pH [26, 27]. Auxin re-  
85 distribution allows asymmetric growth in hypocotyls leading to phototropic  
86 bending.

87

88 In etiolated seedlings, phytochromes do not detect the light gradient per se,  
89 however, they manipulate the magnitude of the phototropic response [4].  
90 Phytochromes, with a predominant function of phytochrome A (phyA),  
91 enhance phototropism by modulating phototropin signaling at several steps [4,  
92 28, 29]. In particular, phyA promotes the expression of positive regulators of  
93 this signaling cascade including *PKS1*, *RPT2*, and *ABCB19* [30-32]. Given  
94 that for the past 150 years etiolated seedlings have been the model of choice  
95 to study phototropism [16], we do not know whether phytochromes modulate  
96 phototropin-mediated responses in green seedlings. Photoautotrophic  
97 *Cucumis sativus* and *Boehmeria nipoonivea* plantlets show a stronger  
98 reorientation of stem growth under canopy shade than in open places [33-35].  
99 However, this could be the result either of stronger blue light gradients in the  
100 presence of canopies or even of phytochrome perception of R/FR ratio  
101 gradients in de-etiolated tissues [33, 34, 36, 37]. Noteworthy, in sesame, blue  
102 light-induced phototropism is promoted by red light given as a pretreatment to

103 de-etiolate the seedling, however, red light given simultaneously with  
104 unilateral blue light inhibits bending compared to far-red red light [38]. These  
105 results suggest that in de-etiolated sesame seedlings reduced phytochrome  
106 activity simultaneously with the exposure to a blue-light gradient enhances  
107 phototropism which contrasts with what is typically observed in etiolated  
108 seedlings.

109

110 The aim of our study was to determine whether shade signals modulate  
111 phototropism in Arabidopsis and if so uncover the underlying molecular  
112 mechanisms. We found that the R/FR ratio has a strong impact on the  
113 phototropic potential of green Arabidopsis seedlings. phyB inhibits  
114 phototropism in open environments by limiting the activity of several members  
115 of the PIF family. In the shade PIFs promote phototropism by enhancing auxin  
116 production. Our work uncovered new actors regulating phototropism  
117 specifically in green seedlings and novel mechanisms underlying  
118 photoreceptor crosstalk.

119

120 **RESULTS**

121 **phyB modulates phototropism in green seedlings depending on the**  
122 **red/far-red ratio in the environment.**

123 Green (de-etiolated) Arabidopsis seedlings undergo phototropism [39] but  
124 whether phytochromes also regulate phototropin signaling at this  
125 developmental stage remains unknown. Unilateral blue (B) light but not R or  
126 FR provided a phototropic cue to both etiolated and de-etiolated seedlings  
127 (Figure S1A). When combined with B light, unidirectional R or FR did not  
128 modify phototropism of etiolated seedlings, however, modulated the  
129 phototropic response of green seedlings. In such photoautotrophic seedlings  
130 phototropism was inhibited in the presence of R light while FR light enhanced  
131 hypocotyl bending towards the blue light source (Figure S1A). In natural  
132 environments, plants sense the R/FR ratio as a cue about the presence of  
133 competitors. Thus, we tested the impact of the R/FR ratio on the blue light-  
134 induced phototropic response. The experiment was designed such that blue  
135 light was provided from the side and R/FR was provided from above in order  
136 to mimic growth towards a blue light maximum in open (e.g. sun in the  
137 morning) versus crowded habitats (e.g. canopy gap). We observed that de-  
138 etiolated seedlings were largely unresponsive toward the blue light gradient in  
139 high R/FR (Figure 1), similar to the simultaneous unilateral irradiation with R  
140 and blue light (Figure S1A). On the contrary, hypocotyl bending towards blue  
141 light in low R/FR was strong (Figure 1) in accordance with unilateral  
142 application of blue and FR light (Figure S1A). We conclude that in de-etiolated  
143 seedlings phototropism towards blue light is modulated by the R/FR ratio.

144



145 To identify the primary photoreceptors regulating this response in our  
146 experimental conditions, we analyzed several phytochrome and phototropin  
147 mutants. The hypocotyls of *phot1* and *phot1phot2* seedlings failed to grow  
148 towards the blue light direction in both high and low R/FR, while *phot2*  
149 behaved like the wild type (Figure 1A). These results indicate that *phot1* is the  
150 primary phototropin controlling hypocotyl growth re-orientation in green  
151 seedlings in our setup. The analysis of phytochrome mutants revealed that  
152 while *phyA* seedlings displayed a largely wild-type response, *phyB* and  
153 *phyAphyB* seedlings did not show differential phototropic bending in response  
154 to different R/FR ratios (Figure 1B). Moreover, *phyB* mutants in the high R/FR  
155 ratio and wild type in the low R/FR ratio showed a similarly strong  
156 reorientation towards blue light (Figure 1B). These findings suggest that *phyB*  
157 negatively regulates phototropism in the high R/FR ratio. We conclude that in  
158 de-etiolated seedlings *phot1* is essential for phototropic bending, while *phyB*  
159 does not perceive the light gradient. However, *phyB* is key to modulate the  
160 *phot1* response in different R/FR ratios.

161

162 **PIF4, PIF5 and PIF7, acting downstream of *phyB*, are necessary and**  
163 **sufficient to promote phototropism.**

164 Next we studied known signaling components acting downstream of *phot1*  
165 and *phyB* to understand the mechanisms underlying this photoreceptor  
166 crosstalk. First, we tested the importance of key phototropism signaling  
167 components in etiolated seedlings such as NPH3 and the PINs. The *nph3*  
168 mutant showed a marked reduction in phototropic bending (Figure 1A),  
169 indicating that its activity is important to respond to a blue light gradient in de-

170 etiolated seedlings. It has been shown that PIN3, PIN4 and PIN7 co-operate  
171 to enable hypocotyl phototropism in etiolated seedlings [40]. We observed  
172 that the *pin3pin4pin7* triple mutant was defective in phototropism in our green  
173 seedlings (Figure S1B), suggesting that *PIN* activity is also important in de-  
174 etiolated seedlings possibly to establish an auxin gradient. Thus, several  
175 phototropin signaling elements that are essential in etiolated seedlings are  
176 also important for phototropism in green seedlings irrespective of the R/FR  
177 condition.

178

179 PIF4, PIF5 and PIF7 play a major role downstream of phyB to promote shade-  
180 avoidance responses [5], prompting us to analyze their role during  
181 phototropism in green seedlings. Interestingly, in de-etiolated seedlings the  
182 phototropic response towards B light was reduced in *pif7*, *pif4pif5*, and  
183 *pif4pif5pif7* mutants (Figure S2A). In contrast, the etiolated *pif4pif5pif7* triple  
184 mutant showed a phototropic response that was undistinguishable from wild-  
185 type seedlings (Figure S2B). Therefore, PIF4/5/7 promote phototropism in  
186 green but not etiolated seedlings. The phototropic response of all three de-  
187 etiolated *pif* mutants was similar to that of the wild type under a high R/FR  
188 ratio (Figure 2A). However, under of a low R/FR ratio *pif4pif5* showed a  
189 normal response, *pif7* showed reduced phototropism while the *pif4pif5pif7*  
190 triple mutant had strongly reduced phototropism that no longer responded to  
191 the R/FR ratio (Figure 2A). To determine whether these transcription factors  
192 act downstream of phyB in modulating phototropism we generated a  
193 *phyBpif4pif5pif7* quadruple mutant. Similar to the *pif4pif5pif7* triple mutant,  
194 *phyBpif4pif5pif7* seedlings were largely insensitive to a blue light gradient both

195 in high and low R/FR (Figure 2B). This epistatic relationship suggests that in  
196 green seedlings these three PIFs act downstream of phyB to control  
197 phototropism. A prediction of this model is that PIF over-expression would  
198 result in a strong phototropic response irrespective of the R/FR ratio. Indeed,  
199 the phototropic response of *PIF4* and *PIF5* overexpressing lines was higher  
200 than that of the wild type and was no longer inhibited by a high R/FR ratio  
201 (Figure 2C). Together these results indicate that PIF4/5/7 are essential for  
202 phototropism in green but not etiolated seedlings (Figures 2, S2A, S2B).  
203 Moreover, they suggest that phyB-mediated control of PIF4/5/7 underlies  
204 shade modulation of phototropism (Figure 2).

205

206 **PIFs regulate phototropism by controlling the expression of *YUCCA***  
207 **genes.**

208 PIFs mediate shade-regulated auxin production by controlling the expression  
209 of *YUC2*, *YUC5*, *YUC8* and *YUC9* [12, 13] suggesting that in green seedlings  
210 PIF-regulated auxin production may control phototropism. To test this  
211 hypothesis, we first analyzed *YUC* expression in our experimental conditions.  
212 Our data revealed that *YUC2*, *YUC5*, *YUC8* and *YUC9* expression was  
213 induced by a low R/FR ratio in a PIF-dependent manner (Figure 3A).  
214 Moreover, a *yuc2yuc5yuc8yuc9* (*yucQ*) mutant was strongly impaired in  
215 shade-enhanced phototropism highlighting the importance of those four *YUC*  
216 genes for this process in green seedlings (Figure 3B). Similarly, when  
217 subjected to a blue light gradient in the absence of any additional R and/or FR  
218 light the green *yucQ* seedlings showed a weak phototropic response (Figure  
219 S2C).

220

221 We also tested the role of TRYPTOPHAN AMINOTRANSFERASE OF  
222 ARABIDOPSIS 1 (TAA1), an enzyme acting upstream of YUCCA in the auxin  
223 biosynthetic pathway because of its importance for several shade-induced  
224 responses [41]. The *sav3/taa1* and *yucQ* mutant showed a similar shade-  
225 induced hypocotyl elongation defect (Figure S3A). However, we observed  
226 robust shade-regulated phototropism in *taa1* but not in *yucQ* (Figure 3B).  
227 Moreover, the *taa1/sav3* mutant showed a marginal defect in responding to  
228 unidirectional blue light (Figure S2C). This indicates that modulation of  
229 phototropism under these experimental conditions did not depend on the  
230 activity of TAA1. Moreover, it suggests that shade-modulation of the  
231 phototropic response is not simply a consequence of the growth potential of  
232 the seedlings in different conditions. To test this further, we examined  
233 phototropism in mutants defective in hypocotyl growth. The analysis of a *bri1*  
234 mutant revealed that while it grew considerably slower than the wild type, its  
235 phototropic response was similar to that of the wild type in a low R/FR ratio  
236 (Figure S3B). On the contrary, the *hy5hfr1* mutant showed enhanced growth  
237 in a low R/FR ratio but had a reduced phototropic response (Figure S3B).  
238 Interestingly, the *hy5hfr1* mutant in a high R/FR ratio grew at a similar rate  
239 than wild type in a low R/FR ratio, but we observed a large difference in  
240 hypocotyl bending (Figure S3B). These results indicate that the differences in  
241 phototropic bending triggered by the R/FR ratio cannot simply be explained by  
242 the growth potential in different conditions and/or genotypes.

243

244 Our *YUC* gene expression analysis and the phenotype of the *yucQ* mutant  
245 suggest that PIF-mediated *YUC* expression, which primarily occurs in  
246 cotyledons [12, 41], is a key step in the modulation of phototropism by shade.  
247 To test this hypothesis we asked whether induction of *YUCCA* expression in  
248 green seedlings is sufficient to promote phototropism as we observed in *PIF*  
249 overexpressing lines. A cotyledon-specific estradiol-inducible  
250 *FRO6::XVE::YUC3* line (*YUC3i*) was analyzed to address this question [42].  
251 We found that while in control conditions the *YUC3i* line behaved like the wild  
252 type, upon induction of *YUC3* we did not observe inhibition of phototropism by  
253 a high R/FR ratio, suggesting that *YUC3* expression in cotyledons was  
254 sufficient to promote phototropism (Figure 3C). In order to determine whether  
255 the phenotype of the *pif4pif5pif7* triple mutant can be complemented by PIF-  
256 independent *YUC* transcription we crossed the *pif4pif5pif7* triple mutant with  
257 the *FRO6::XVE::YUC3* line and selected *YUC3i pif4pif5pif7* seedlings. We  
258 observed that induction of *YUC3* in the *pif4pif5pif7* triple mutant background  
259 rescued the inhibition of phototropism in both high and low R/FR (Figure 3C).  
260 This leads us to conclude that PIF-mediated *YUC* expression is a key step in  
261 PIF-mediated phototropism regulation.

262

263 We have previously shown that cotyledons, the major auxin production  
264 organs, are largely dispensable for phototropism in etiolated seedlings while  
265 in de-etiolated seedlings “decapitation” leads to a stronger phototropic defect  
266 [43]. This difference might be explained by the requirement of auxin  
267 production for phototropism in green seedlings (Figure 3), while in etiolated  
268 seedlings redistribution of auxin present in the hypocotyls might be sufficient

269 to promote phototropism. We therefore characterized etiolated *yucQ*  
270 seedlings and found that the *yucca* quadruple mutant displayed normal  
271 phototropism, if anything the mutant reoriented growth more efficiently than  
272 the wild type (Figure S2B). This suggests that auxin synthesis by YUC2,  
273 YUC5, YUC8 and YUC9 is important for phototropism specifically in  
274 photoautotrophic seedlings. Our characterization of the *pif4pif5pif7* and *yucQ*  
275 mutants and a previous analysis of phototropism in de-etiolated seedlings [39]  
276 reveal the existence of different signaling mechanisms controlling  
277 phototropism in etiolated versus green seedlings.

278

279 **PIFs are important to promote phototropism in natural conditions.**

280 In order to verify the relevance of our observations obtained in laboratory  
281 conditions using monochromatic light sources we decided to test the  
282 phototropic response of key genotypes in natural conditions where  
283 background light levels and temperature fluctuate. Because of their striking  
284 phenotype in the laboratory we focused on the *phyB* and *pif4pif5pif7* mutants  
285 (Figures 1, 2). De-etiolated seedlings grown on vertical plates were placed  
286 outdoors under unilateral vegetative shade from tall grasses (Figures S4A,  
287 S4C, S4D). Wild-type and *phyB* seedlings re-orientated hypocotyl growth  
288 away from the vegetative shade with a significantly stronger response in the  
289 *phyB* background (Figure 4A). In contrast, similar to our observation in the  
290 laboratory, the *pif4pif5pif7* triple mutant was severely defective in phototropic  
291 bending (Figure 4A). We further examined the impact of the R/FR ratio on  
292 phototropism by comparing phototropism in open field versus shade  
293 conditions. In order to create similar blue light gradients in both conditions, we

294 used a black filter placed on the north side (southern hemisphere) of the  
295 seedlings used for control condition (high R/FR) and a combination of tall  
296 grasses and an orange filter (cutting blue light) for low R/FR conditions  
297 (Figures S4B, S4E). This way the seedlings were subjected to a similar lateral  
298 blue light gradient but either in high R/FR (black filter) or low R/FR (vegetation  
299 + orange filter) conditions (Figure S4C). As observed in laboratory conditions  
300 wild-type seedlings showed enhanced phototropism in low R/FR (Figure 4B).  
301 Also consistent with observations made in the laboratory the *phyB* mutant was  
302 more phototropic than the wild type in high R/FR conditions. This trend was  
303 also observed in low R/FR, a difference that we did not observe in the  
304 laboratory (Figure 4B). However, as observed in the laboratory the response  
305 of the wild type in low R/FR was similar to the response of *phyB* in a high  
306 R/FR ratio. Finally, the *pif4pif5pif7* mutant had a reduced phototropic  
307 response when the R/FR ratio was low (Figure 4B). Globally these  
308 experiments confirmed the importance of the PIFs and phyB in the control of  
309 phototropism in realistic environmental conditions.

310

311 The *phyB* mutant showed a residual enhancement of phototropism by true  
312 canopy shade (Figure 4B), but not by low R/FR in the laboratory (Figure 2B).  
313 phyB primarily controls shade responses elicited by a reduction of the R/FR  
314 ratio that already occurs in the absence of direct shading (neighbor proximity)  
315 [44]. Foliar shade leads to lower blue light levels and a further reduction of the  
316 R/FR ratio, conditions that are sensed by phyB and the cryptochromes [5, 44].  
317 The difference between laboratory and outdoors experiments therefore  
318 suggested that the cryptochromes may also inhibit phototropism. When

319 analyzed in natural shade conditions we found that *cry1* and *cry1cry2* double  
320 mutants also displayed an exaggerated phototropic response (Figure S5A).  
321 We further investigated the role of the cryptochromes in a controlled  
322 environment where seedlings were grown in the presence of white light  
323 supplemented or not with additional FR light to mimic shade signals (Figure  
324 S5B). Under these conditions *cry1* displayed an enhanced phototropic  
325 response both in high and low R/FR while *phyB* displayed a constitutively  
326 strong bending response that was not enhanced by a reduction of the R/FR  
327 ratio (Figure S5C). Collectively these results confirm a role for the  
328 cryptochromes in the modulation of phototropism by shade. Moreover, since  
329 true shade leads to a stronger decline of the R/FR ratio than the presence of  
330 non-shading neighbors, these results suggest that shade-induced phototropic  
331 enhancement may be a gradual response with a stronger impact as the R/FR  
332 ratio declines. We tested this hypothesis by analyzing phototropism in  
333 seedlings exposed to white light with different R/FR ratios. Indeed, the  
334 phototropic response was inversely proportional to the R/FR ratio (Figure 5A).  
335 Moreover, in agreement with the importance of shade-induced *YUC*  
336 expression promoting phototropism (Figure 3), we observed that particularly  
337 *YUC2* and *YUC9* expression gradually increased with a declining R/FR ratio  
338 (Figure 5B). We conclude that shade-regulation of phototropism is a gradual  
339 response that is presumably tuned to the degree of shading.



## 340 **DISCUSSION**

341 Our results show that the R/FR ratio modulates phototropism under both  
342 controlled and field conditions (Figures 1, 4 and 5). Field observations and  
343 laboratory experiments have suggested that plants under vegetative shade  
344 show more pronounced phototropic responses compared to open field  
345 environments [33-35]. However, it was not possible to discriminate whether  
346 this pattern resulted from the lower R/FR ratios, differences in the B light  
347 gradient, reduced photosynthetic light found in the shade or even  
348 phytochrome perception of R/FR gradients within the canopy [33, 34, 36, 37].  
349 The experiments that we performed in the laboratory circumvent this problem  
350 and allowed us to test the effect of the R/FR ratio on phototropism by keeping  
351 the blue light stimulation and the amount of photosynthetic light equal (Figures  
352 1, S1A, 5 and S5C). We propose that phyB-mediated regulation of growth  
353 orientation in photoautotrophic plantlets contributes to their ability to fill  
354 canopy gaps, an important physiological response in dense plant communities  
355 (Figures 1, 2, 4 and S5C) [33-36]. In cucumber phyB controls this response in  
356 part by mediating phototropism away from FR-rich light [33, 34]. Our work in  
357 Arabidopsis indicates that phyB regulation of phototropism towards blue light  
358 promotes growth out of the shade (Figures 1, 2, 4 and S5C). This  
359 enhancement of the phototropic response gradually increases with a declining  
360 R/FR ratio (Figure 5). This suggests that this response is more pronounced in  
361 true shade compared to non-shading neighbors that moderately lower the  
362 R/FR ratio by reflecting FR light [44]. Moreover, the involvement of  
363 cryptochromes in the regulation of phototropism in light-grown seedlings also

364 supports the view that phototropic enhancement is particularly strong in true  
365 shade where blue light and the R/FR ratio are strongly reduced (Figure S5).

366

367 We identify PIF4, PIF5 and PIF7 as key factors promoting phototropism in low  
368 R/FR (Figures 2-4). Moreover, we show that four *YUC* genes whose  
369 expression is rapidly induced in a PIF-dependent manner are important PIF  
370 target genes regulating this process (Figures 3, 5 and S2C) [12, 13, 45].  
371 PIF4/PIF5 and PIF7 play a predominant role in shade-regulated hypocotyl  
372 elongation in response to low B and low R/FR respectively [6, 9, 46]. The low  
373 R/FR promotion of phototropism is also controlled by PIF7 with a clear  
374 contribution of PIF4 and PIF5 (Figure 2). Collectively YUC2, YUC5, YUC8 and  
375 YUC9 are essential for low R/FR-induced hypocotyl growth and phototropism  
376 (Figure 3) [45]. However, TAA1/SAV3, which is very important for low R/FR-  
377 induced hypocotyl elongation, plays a minor role in the regulation of  
378 phototropism (Figures 3, S3A) [41]. This reveals interesting difference  
379 between both low R/FR-induced responses and shows that promotion of  
380 phototropism in such conditions does not simply correlate with the growth  
381 potential (Figure S3).

382

383 Our study illustrates how development modifies the regulation of phototropism  
384 signaling and photoreceptor crosstalk. In etiolated seedlings, phytochromes  
385 promote phototropism with phyA playing a predominant role [4, 28-32]. In  
386 contrast, in photoautotrophic seedlings we observed no obvious role for phyA  
387 but phyB is a strong inhibitor of phototropism particularly in a high R/FR ratios  
388 (Figures 1, 4 and S5C). Such an antagonistic interaction between phyB and

389 the phototropins has also been observed in the control of leaf flattening [47].  
390 In this situation the phyB response partially depends on PIF4 and PIF5, but  
391 how those PIFs modulate leaf-flattening remains unknown [48]. PIF4 and  
392 PIF5 were also proposed to inhibit phototropism in etiolated seedlings [49].  
393 However, we did not observe a significant phototropic defect in etiolated  
394 *pif4pif5pif7* triple mutant while previously it was reported that *pif4pif5* has a  
395 very modest phenotype (Figure S2B) [49]. We conclude that in etiolated  
396 seedlings PIF4, PIF5 and PIF7 play a minor role. In contrast, in green  
397 seedlings these three PIFs are of great importance to enable phototropic  
398 reorientation in all tested conditions (Figures 2-4, S2). Based on the  
399 phenotypes of loss- and gain-of-function mutants we conclude that their role is  
400 rate limiting in this process (Figure 2). Our study shows that PIFs promote  
401 phototropism by YUC-mediated auxin production (Figure 3). Although it is  
402 likely that PIFs also regulate this process by controlling the expression of  
403 additional genes [13, 14, 49], our finding that *YUC3* induction in cotyledons  
404 can complement the phototropic defect of *pif4pif5pif7* shows that auxin  
405 production is an important aspect of PIF-mediated phototropic enhancement  
406 (Figure 3). Interestingly, phytochromes control the expression of regulators of  
407 the phototropic response in both etiolated and in green seedlings, but the  
408 nature of these signaling elements is developmental stage-dependent  
409 (Figures 3, 6) [30-32].

410

## 411 **Experimental procedures**

### 412 **Plant material and growth conditions**

413 Detailed descriptions of the plant material and growth conditions used in this  
414 study are provided in the supplemental experimental procedures.

415

### 416 **Physiological analysis of phototropism and measurements**

417 For phototropism experiments three-day-old de-etiolated seedlings grown in  
418 continuous white light were shifted at ZT0 to  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$  blue light from  
419 one side and  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$  red light from above at 22 °C for 24 hours.

420 Supplementary far-red light provided from above was adjusted such that R/FR  
421 ratio was 6.6 in high R/FR and 0.18 in low R/FR conditions. White light  
422 gradient experiments were performed by shifting three-day-old de-etiolated  
423 seedlings in  $180 \mu\text{mol m}^{-2} \text{s}^{-1}$  white light at 22 °C in a black box opened from  
424 only one side (Figure S5B). Varying amount of supplemental FR from LEDs  
425 was provided from above to obtain the required R/FR ratios. Phototropic  
426 bending angles and growth rates determined by a customized MATLAB script  
427 developed in Fankhauser lab (see supplemental experimental procedures for  
428 details).

429

### 430 **Outdoor phototropism analysis**

431 For outdoor phototropism experiments three-day-old de-etiolated seedlings  
432 were shifted at ZT4 to fields in Buenos Aires, Argentina. The seedlings were  
433 either placed to the south side of a grass canopy or a tilted screen was placed  
434 between the grasses and the seedlings (Figure S4). A black screen was used  
435 to prevent the projection of grass shade on control seedlings (R/FR ratio: 1.2).

436 An orange acetate filter was used to allow the projection of shade on low  
437 R/FR ratio (0.4)-treated seedlings.

438

#### 439 **Supplemental Information**

440 Supplemental Information includes Figures S1-S5, figure legends and  
441 supplemental experimental procedures.

442

#### 443 **Author Contributions**

444 Conceptualization, A.G., J.C. and C.F.; Investigation, A.G., and E.K.;  
445 Resources, V. C. G. and H. R.; Funding Acquisition, C.F.; Writing, A. G. and  
446 C. F.; Supervision, J.C. and C.F.

447

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633 response in Arabidopsis. *Plant Cell* **25**, 2102-2114.
- 634  
635

636 **Figure legends**

637

638 **Figure 1**

639 **The photoreceptors phot1 and phyB regulate phototropism in de-**  
640 **etiolated seedlings.**

641 A and B) Three-day-old de-etiolated seedlings of WT (Col-0), phototropin and  
642 *NPH3* mutants (A), and phytochrome mutants (B) were subjected to blue light  
643 from the side, while red and far-red lights were provided from above to  
644 simulate control and shade conditions as described in the experimental  
645 procedures. Bending angles were measured after 24 hours of light treatments.  
646 Bars represent mean bending angle  $\pm$  S.E. ( $n \geq 20$ ). Small alphabetic letters  
647 above each bar indicate statistically significant groups at  $p$  value  $< 0.01$   
648 obtained by ANOVA followed by the post-hoc Tukey's HSD. See also Figure  
649 S1

650

651 **Figure 2**

652 **PIF transcription factors act downstream of phyB to regulate**  
653 **phototropism.**

654 A-C) Three-day-old de-etiolated seedlings of the indicated genotypes were  
655 subjected to light conditions as described in figure 1. Bars represent mean  
656 bending angle  $\pm$  S.E. ( $n \geq 20$ ). Small alphabetic letters above each bar indicate  
657 statistically significant groups at  $p$  value  $< 0.01$  obtained by ANOVA followed  
658 by the post-hoc Tukey's HSD. See also Figure S2.

659

660 **Figure 3**

661 **PIFs modulate phototropism by transcriptional regulation of *YUCCA***  
662 **genes.**

663 A) Three-day-old de-etiolated seedlings of Col-0 and *pif4pif5pif7* were treated  
664 at ZT0 with light conditions as described in figure 1 for one hour. RNA was  
665 extracted from the untreated (W) and the light-treated seedlings and  
666 quantitative PCR was performed. Data are mean expression  $\pm$  S.E. of *YUCCA*  
667 genes normalized to two control genes (*UBC* and *YSL8*) and expressed  
668 relative to Col-0 in untreated condition. Mean values are obtained from three  
669 biological replicates each quantified with three technical replicates. Asterisks  
670 indicate the statistical significance compared to Col-0 untreated (p value <  
671 0.05, Student's t-test).

672 B and C) Three-day-old de-etiolated seedlings of the indicated genotypes  
673 were treated with light conditions as described in figure 1. Bars represent  
674 mean bending angle  $\pm$  S.E. (n $\geq$ 20). In part C, the seedlings were induced with  
675 10 $\mu$ M estradiol 16 hours prior to light treatments. Small alphabetic letters  
676 above each bar indicate statistically significant groups at p value < 0.01  
677 obtained by ANOVA followed by the post-hoc Tukey's HSD. See also Figures  
678 S2 and S3.

679

#### 680 **Figure 4**

681 **PIF4/5/7 are important for robust phototropism in natural environments.**

682 A) Three-day-old de-etiolated seedlings of Col-0, *phyB* and *pif4pif5pif7* grown  
683 on vertical plates were placed on the south side of vegetative shade from tall  
684 grasses for 5 hours before measurement of the phototropic bending angle.

685 Data were pooled from 3 independent experiments. Bars represent mean  
686 bending angle  $\pm$  S.E. ( $n \geq 130$ ).

687 B) Three-day-old de-etiolated seedlings of Col-0, *phyB* and *pif4pif5pif7* were  
688 subjected to light gradients with the help of black (control) or orange filters +  
689 vegetation (shade) placed on the north such that seedlings were exposed to  
690 more light coming from south. Bending angle towards the south was  
691 measured after 5 hours of the treatment. Data were pooled from six  
692 independent experiments. Bars represent mean bending angle  $\pm$  S.E.  
693 ( $n \geq 110$ ).

694 Small alphabetic letters above each bar indicate statistically significant groups  
695 at  $p$  value  $< 0.05$  obtained by ANOVA followed by the post-hoc Tukey's HSD.  
696 See also Figures S4 and S5.

697

## 698 **Figure 5**

699 **Gradual enhancement of phototropism and *YUC* expression with a**  
700 **declining R/FR ratio.**

701 A) Three-day-old de-etiolated Col-0 seedlings were subjected to similar white  
702 light gradients in the presence of the indicated R/FR ratios. Our white light  
703 source has a R/FR ratio of 1.4, which is close to the R/FR ratio of sunlight.  
704 Bending angles were measured after 6 hours of light treatments. Bars  
705 represent mean bending angle  $\pm$  S.E. ( $n \geq 95$ ). Small alphabetic letters above  
706 each bar indicate statistically significant groups at  $p$  value  $< 0.01$  obtained by  
707 ANOVA followed by the post-hoc Tukey's HSD. B) Three-day-old de-etiolated  
708 Col-0 seedlings were treated at ZT0 with the same light conditions as in the  
709 panel A for one hour. RNA was extracted from the samples and quantitative

710 PCR was performed. Data are mean expression  $\pm$  S.E. of *YUCCA* genes  
711 normalized to two control genes (*UBC* and *YSL8*) and expressed relative to  
712 R/FR ratio 1.4. Mean values are obtained from four biological replicates each  
713 quantified with three technical replicates. Asterisks indicate the statistical  
714 significance compared to R/FR ratio 1.4 (\* p value < 0.05, \*\* p value < 0.01,  
715 Student's t-test). See also Figure S5.

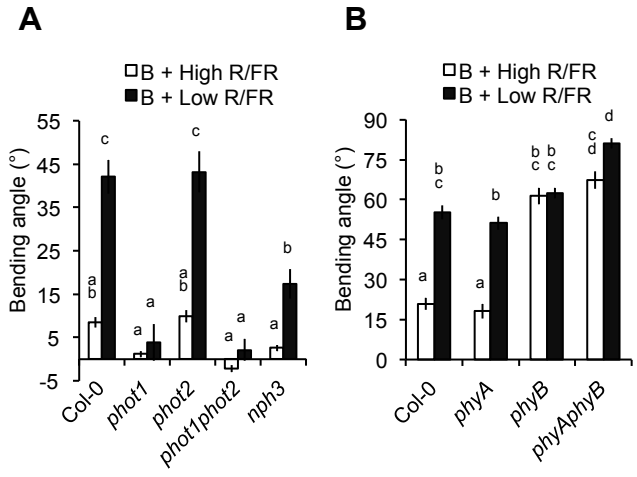
716

## 717 **Figure 6**

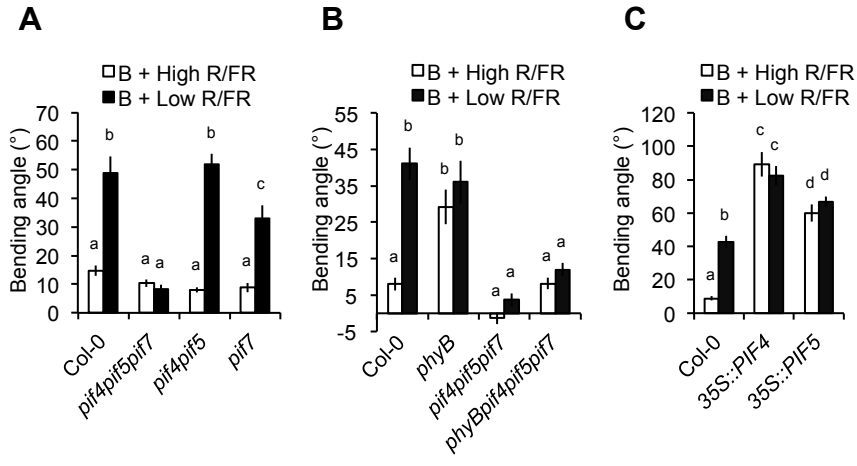
### 718 **Proposed model**

719 Schematic representation of a model of shade-regulated phototropism  
720 suggested by our studies. On the left the seedling in an open (high R/FR)  
721 environment where phyB is primarily in its active PfrB conformation. Active  
722 phyB inhibits the PIFs thereby leading to reduced *YUC* gene expression  
723 resulting in low auxin levels. On the right the seedling is in the shade (low  
724 R/FR) where phyB is primarily in its inactive PrB conformation. PIFs are  
725 released from the inhibitory activity of phyB leading to high expression of *YUC*  
726 genes resulting in increased auxin levels which promotes phototropism in de-  
727 etiolated seedlings.

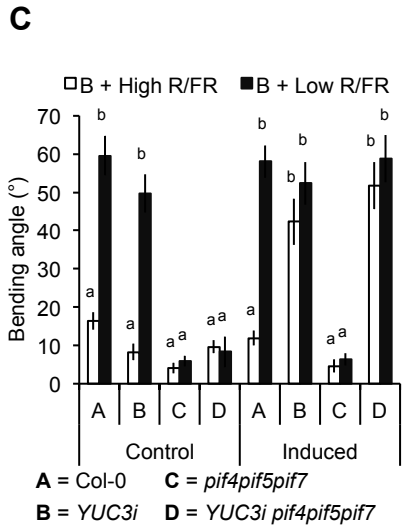
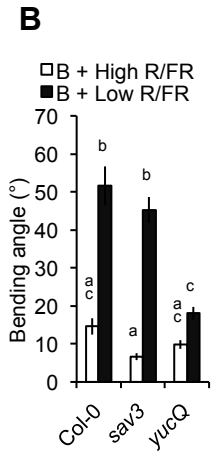
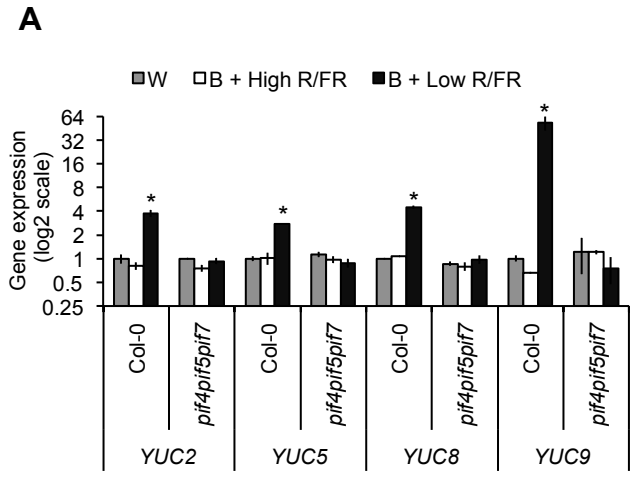
**Figure 1**



**Figure 2**

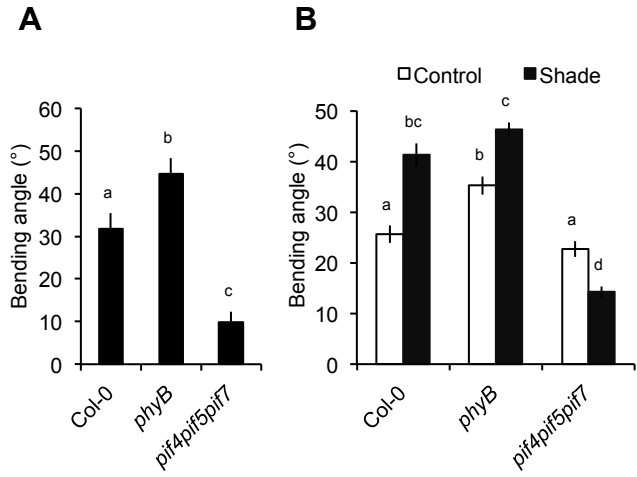


**Figure 3**





**Figure 4**



**Figure 5**

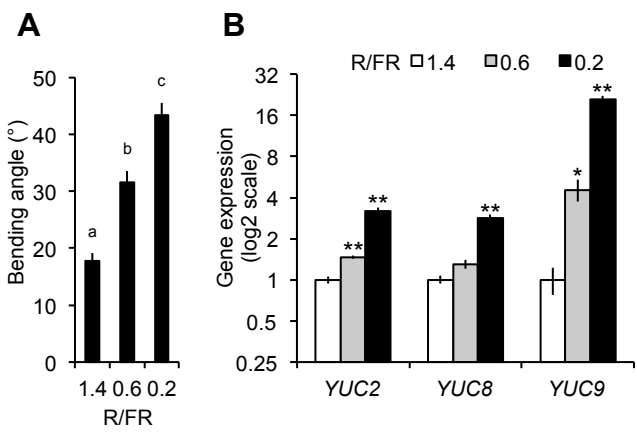
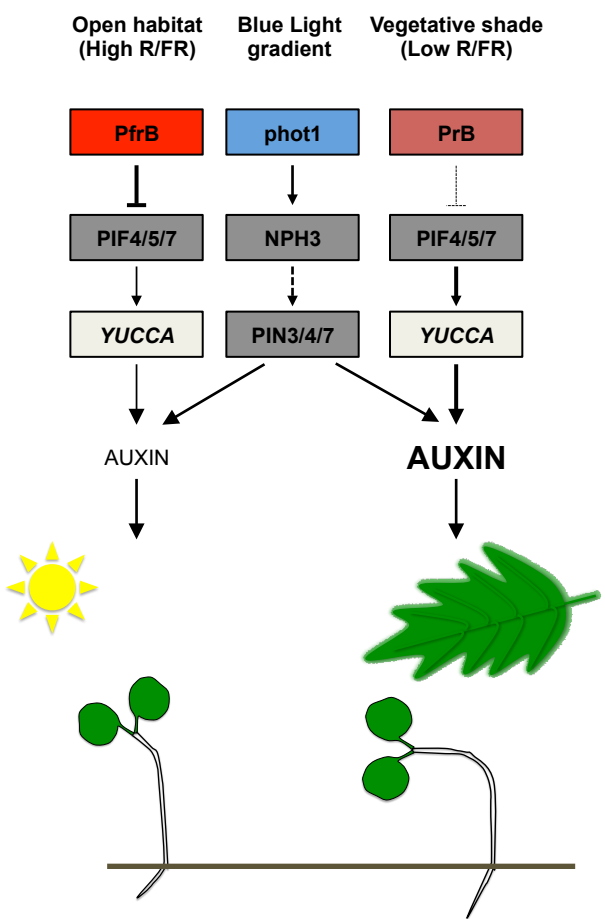
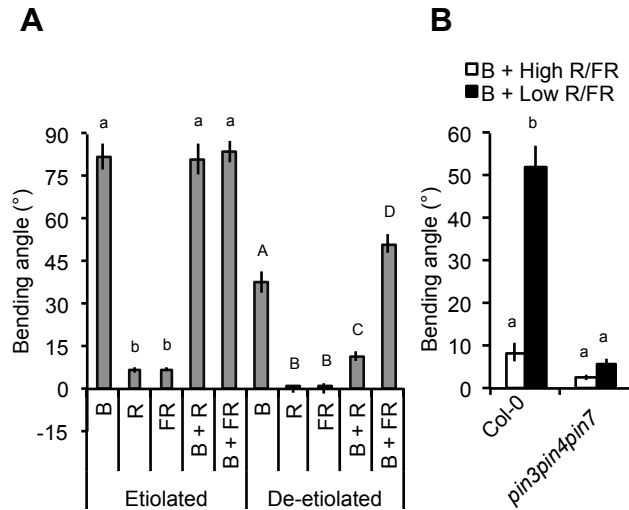


Figure 6

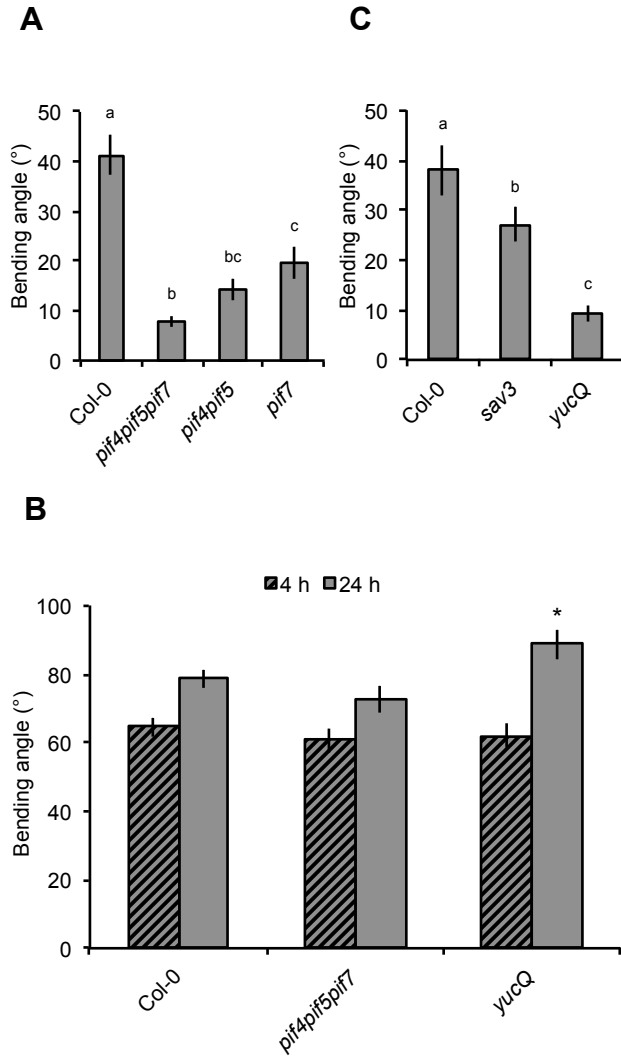




**Figure S1. Modulation of phototropism by red and far-red light in Arabidopsis de-etiolated seedlings.** Related to figure 1.

A) Three-day-old etiolated and de-etiolated Col-0 seedlings were subjected to the denoted combinations of unilateral lights (lateral illumination) for 24 hours. Bars represent mean bending angle  $\pm$  S.E. ( $n > 40$ ). Key for graph legend: B =  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$  blue light; R =  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$  red light; FR =  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$  far-red light. Small and capital alphabetic letters above each bar indicate statistically significant groups at  $p$  value  $< 0.01$  obtained by ANOVA followed by the post-hoc Tukey's HSD for etiolated and de-etiolated seedlings, respectively.

B) Three-day-old de-etiolated seedlings of Col-0 and *pin3pin4pin7* were treated with unilateral blue light from the side, while red and far-red lights were provided from above to simulate control and shade conditions. Bending angles were measured after 24 hours of light treatments. Bars represent mean bending angle  $\pm$  S.E. ( $n \geq 20$ ). Small alphabetic letters above each bar indicate statistically significant groups at  $p$  value  $< 0.01$  obtained by ANOVA followed by the post-hoc Tukey's HSD test.

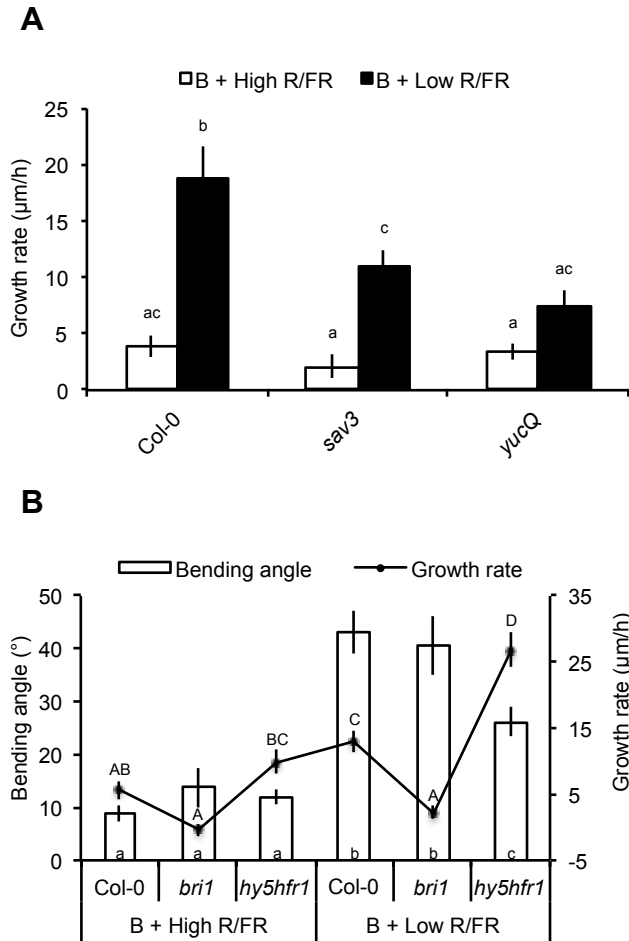


**Figure S2. Regulation of phototropism by PIFs and YUCCAs is dependent on the developmental stage of Arabidopsis seedlings.** Related to figures 2 and 3.

A) Three-day-old de-etiolated seedlings of the indicated genotypes were laterally illuminated with  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$  blue light for 24 hours. Bars represent mean bending angle  $\pm$  S.E. ( $n \geq 20$ ). Small alphabetic letters above each bar indicate statistically significant groups at  $p$  value  $< 0.01$  obtained by ANOVA followed by the post-hoc Tukey's HSD test.

B) Three-day-old etiolated seedlings of the indicated genotypes were irradiated with  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$  unilateral blue light. Phototropic bending angle was measured at 4 hours and 24 hours. Data indicates mean  $\pm$  S.E bending angle at each time point ( $n > 50$ ). Asterisk indicates statistical significance difference to Col-0 at the indicated time (\*  $p$  value  $< 0.01$ , Student's  $t$ -test).

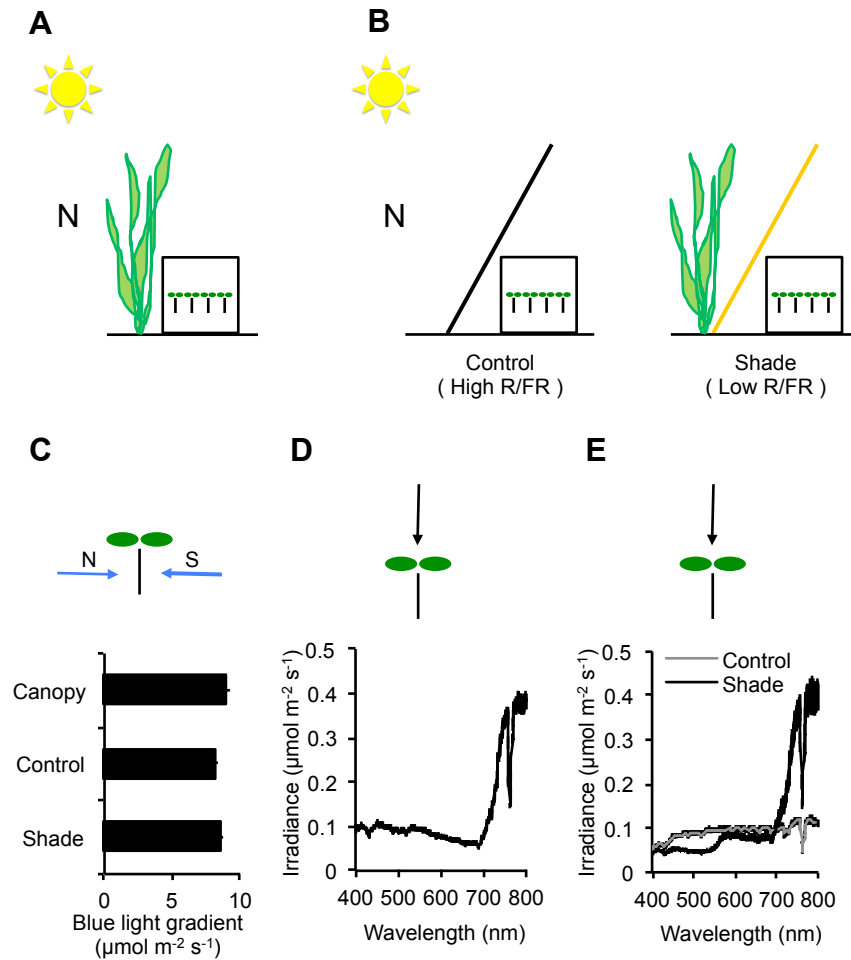
C) Same as part A.



**Figure S3. The growth potential of seedlings in high and low R/FR conditions does not strictly correlate with the magnitude of phototropic bending.** Related to figure 3.

A) The bar graph represents the hypocotyl growth rate  $\pm$  S.E of the seedlings of the indicated genotypes used for measurement of phototropic bending angle in figure 3B. Small alphabetic letters indicate statistically significant groups according to growth rate at p value  $< 0.01$  obtained by ANOVA followed by the post-hoc Tukey's HSD test.

B) Three-day-old de-etiolated seedlings of Col-0, *bri1* and *hy5hfr1* were subjected to light conditions as described in figure S1B. Bars represent mean bending angle  $\pm$  S.E. (n>20). Line graph indicates mean hypocotyl growth rate  $\pm$  S.E. during 24 hours of phototropism experiment for the same set of seedlings used to measure bending angles. Small and capital alphabetic letters indicate statistically significant groups according to bending angles and growth rate, respectively, at p value  $< 0.01$  obtained by ANOVA followed by the post-hoc Tukey's HSD test.



**Figure S4. Schematic representation of outdoor experimental set up.** Related to figure 4.

The figure schematically describes the experimental set up used for outdoor phototropism experiments. The experiments were performed in an open field in Buenos Aires, Argentina (southern hemisphere).

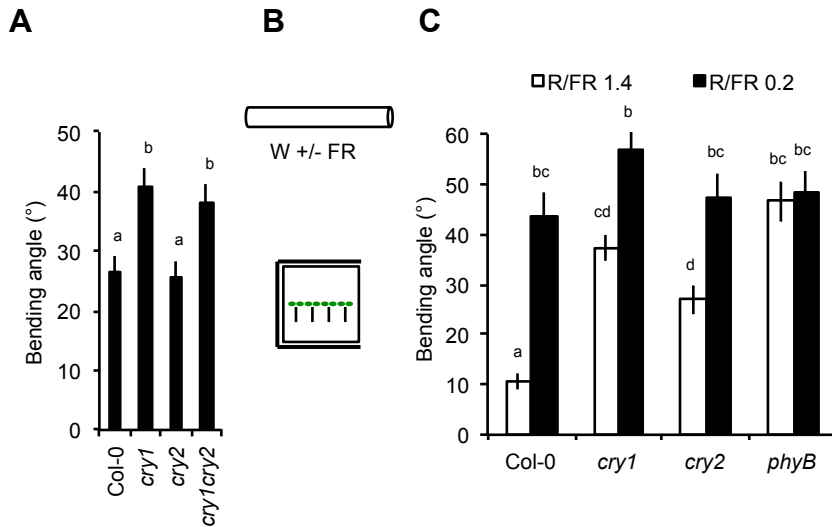
A) In this set up, three-day-old de-etiolated seedlings grown in lab conditions were shifted on the south side of a grass canopy (R/FR ratio 0.4) at ZT4.

B) In this set-up, de-etiolated seedlings were either subjected to control condition (High R/FR ratio = 1.2) by placing a black screen on the north side of seedlings or vegetative shade (Low R/FR ratio = 0.4) by placing an orange screen, at an angle of approximately 45°, between seedlings and tall grasses. While the black screen blocked the projection of grass shade on control seedlings, the orange screen enabled seedlings to be subjected to vegetative shade of a grass canopy. The blue light gradient was similar in both conditions (panel C), such that seedlings were subjected to more light from the south side.

C) Horizontal blue light gradient in the experimental setups. Canopy corresponds to the set-up shown in A. Control and shade correspond to the respective conditions presented in B. The horizontal blue light gradient was determined by subtracting the blue light irradiance coming from North from the irradiance coming from South. The light intensity was determined by placing the light sensor facing away from the filter (South) or towards the filter (North). Blue light irradiance is the integral of a Gauss curve with  $\lambda_{\text{max}}$  at 450 nm and band width of  $\pm 15$  nm at 0.5 the irradiance of  $\lambda_{\text{max}}$ . Six measurements were made in each direction; data are the average  $\pm$  S.E.

D) Scan of the light reaching the seedling from above (sensor facing upwards) in the set-up shown in A. Data are average  $\pm$  S.E. of six measurements.

E) Scan of the light reaching the seedling from above (sensor facing upwards) in the control (black filter) and shade (orange filter) of the set-up shown in B. Data are average  $\pm$  S.E. of six measurements.



**Figure S5. Cryptochromes negatively regulate phototropism in de-etiolated Arabidopsis seedlings.** Related to figures 4 and 5.

A) Three-day-old de-etiolated seedlings of Col-0, *cry1*, *cry2* and *cry1cry2* grown on vertical plates were placed on the south side of vegetative shade from tall grasses for 5 hours as described in figure S4A. Phototropic bending angle was then measured. Data were pooled from 3 independent experiments. Bars represent mean bending angle  $\pm$  S.E. ( $n \geq 230$ ).

B) The figure schematically describes the experimental set up used for white light gradient experiment. White light and varying amount of supplemental FR was provided from above to obtain the required R/FR ratios. Square plates with de-etiolated seedlings were put into a black box opened only from one side and kept under the light, thereby, subjecting seedlings to a gradient of light.

C) Three-day-old de-etiolated seedlings of Col-0, *cry1*, *cry2* and *phyB* were subjected to light conditions as described in the panel B. Bending angles were measured after 6 hours of light treatments. Bars represent mean bending angle  $\pm$  S.E. ( $n > 20$ ).

Small alphabetic letters above each bar indicate statistically significant groups at  $p$  value  $< 0.01$  obtained by ANOVA followed by the post-hoc Tukey's HSD test.



## Supplemental Experimental Procedures

### Plant material and growth conditions

The Columbia (Col-0) ecotype was used as the wild-type control. The mutants used in the study described previously are: *cry1-b104* (outdoor experiment) [S1], *cry1-301* (indoor experiment), *cry2-1* [S2], *FRO6::XVE::YUC3* [S3], *hy5-215hfr1* [S4], *nph3-6* [S5], *phot1-5*, *phot2-1*, *phot1-5phot2-1*, *phyA-211*, *phyB-9*, *phyA-211phyB-9* [S6], *pif4pif5pif7* [S7], *pin3-3pin4-101pin7-101* [S8], *sav3-2* [S9], *yuc2yuc5yuc8yuc9* [S10], *35S::PIF4* and *35S::PIF5* [S11]. The *bri1-235* mutant contains a point mutation at the nucleotide position 467 (C to T) in the *BRI1* protein coding sequence resulting in the substitution of S156F. The *phyBpif4pif5pif7* and *YUC3i pif4pif5pif7* mutants were obtained by crossing *pif4pif5pif7* with *phyB-9* and *FRO6::XVE::YUC3*, respectively.

Seeds were surface sterilized and plated on half-strength Murashige and Skoog medium with 0.8 % (w/v) agar and kept at 4 °C in the dark for 3 days. Square plates were then transferred to continuous white light ( $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) for 3 days at 22-22.5 °C in Percival AR-41L2 incubator to obtain de-etiolated seedlings. For outdoor experiments seedlings were grown in LD (16 h light / 8 h darkness) for 3 days in  $50 \mu\text{mol m}^{-2} \text{s}^{-1}$  white light (fluorescent lamps) at 22 °C on vertical plates. Etiolated seedlings were obtained by inducing germination in white light ( $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) for 6 hours after 3 days of cold and dark treatment and subsequently shifting plates to dark for 64 hours at 22 °C. For experiments involving induction of *YUC3* expression, de-etiolated seedlings were grown on nylon mesh (160 mm, Micropore) placed on the surface of plates as described above. The nylon mesh was transferred to a new plate containing 0.1% ethanol (control) or 10  $\mu\text{M}$  estradiol (Sigma) in 0.1% ethanol (induced) for performing the phototropism experiments.

Indoor unilateral light phototropism and white light gradient experiments were performed in Percival I-33NL and Percival SE-41L incubators, respectively. The LED light sources were from CLF Plant Climatics GmbH: blue ( $\lambda_{\text{max}}$ , 462 nm), red ( $\lambda_{\text{max}}$ , 664 nm) and far-red ( $\lambda_{\text{max}}$ , 730 nm). Light intensities were determined with an International Light IL1400A photometer (Newburyport, MA) equipped with an SEL033 probe with appropriate light filters or with an Ocean Optics (Dunedin, FL, USA) USB2000+ spectrometer. In the field, the vertically and horizontally incident radiation (R/FR ratio, R, FR and blue light) were measured for each experiment with the SKR 1850 four-channel sensor probe of a Skye Instruments SKL 904/I SpectroSense2 meter, respectively facing upwards or towards north and south. For a more detailed characterization we produced scans with an Ocean Optics USB4000-UV-VIS spectrometer configured with a DET4-200-850 detector and QP600-2-SR optical fiber in one of the experiments (Figures S4D, S4E).

### Phototropism experiment and measurements

De-etiolated seedlings for phototropism experiment were treated as described in the experimental procedures. Etiolated seedlings were subjected to unilateral light from the side. Plates were photographed at the indicated times in infra-red light.

The phototropic bending angles of de-etiolated seedlings were calculated by subtracting average angle of orientation of upper region (70% to 95% of total length) of each hypocotyl with respect to horizontal before and after blue light treatment determined by a customized MATLAB script developed in Fankhauser lab. Growth rates were determined by measuring hypocotyl length of the same seedlings used for phototropism measurements using the same MATLAB script.

Bending angles for etiolated seedlings were calculated by measuring hypocotyl angles relative to growth direction prior to light treatments by using National Institutes of Health ImageJ software version 1.45s.

## RNA extraction and RT-qPCR

50 de-etiolated seedlings were harvested and frozen in liquid nitrogen for each light condition. Total RNA was extracted using TRIzol reagent (Life Technologies) following the manufacturer's instructions. Samples were further treated with DNase I (New England Biolabs) and cleaned up using RNeasy Mini Kit (Qiagen). cDNA was prepared from 250 ng RNA per sample using Superscript II Reverse Transcriptase (Invitrogen, Life Technologies, Carlsbad, CA, USA) and random primers. RT-qPCR was performed in three technical replicates for each sample (ABI prism 7900HT sequence detection system, Applied Biosystems, Life Technologies, Carlsbad, CA, USA) using FastStart Universal SYBR green Master mix (Roche, Mannheim, Germany). Primer sequences used were described previously [S7]. The data was analyzed using the Biogazelle qbase software.

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