

Supplemental information

Phototropin-mediated perception of light direction in Arabidopsis leaves regulates blade flattening

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Supplemental figure S1 – Light treatments and growth conditions

Supplemental figure S2 – Expression levels and distribution pattern of *pAS2* and *pFIL* lines

Supplemental figure S3 – PHOT1 levels decrease with BL in the leaf blade

Supplemental figure S4 – Phototropins control flattening reversibly and late in development

Supplemental figure S5 - DR5:VENUS signal is detected in the epidermis.

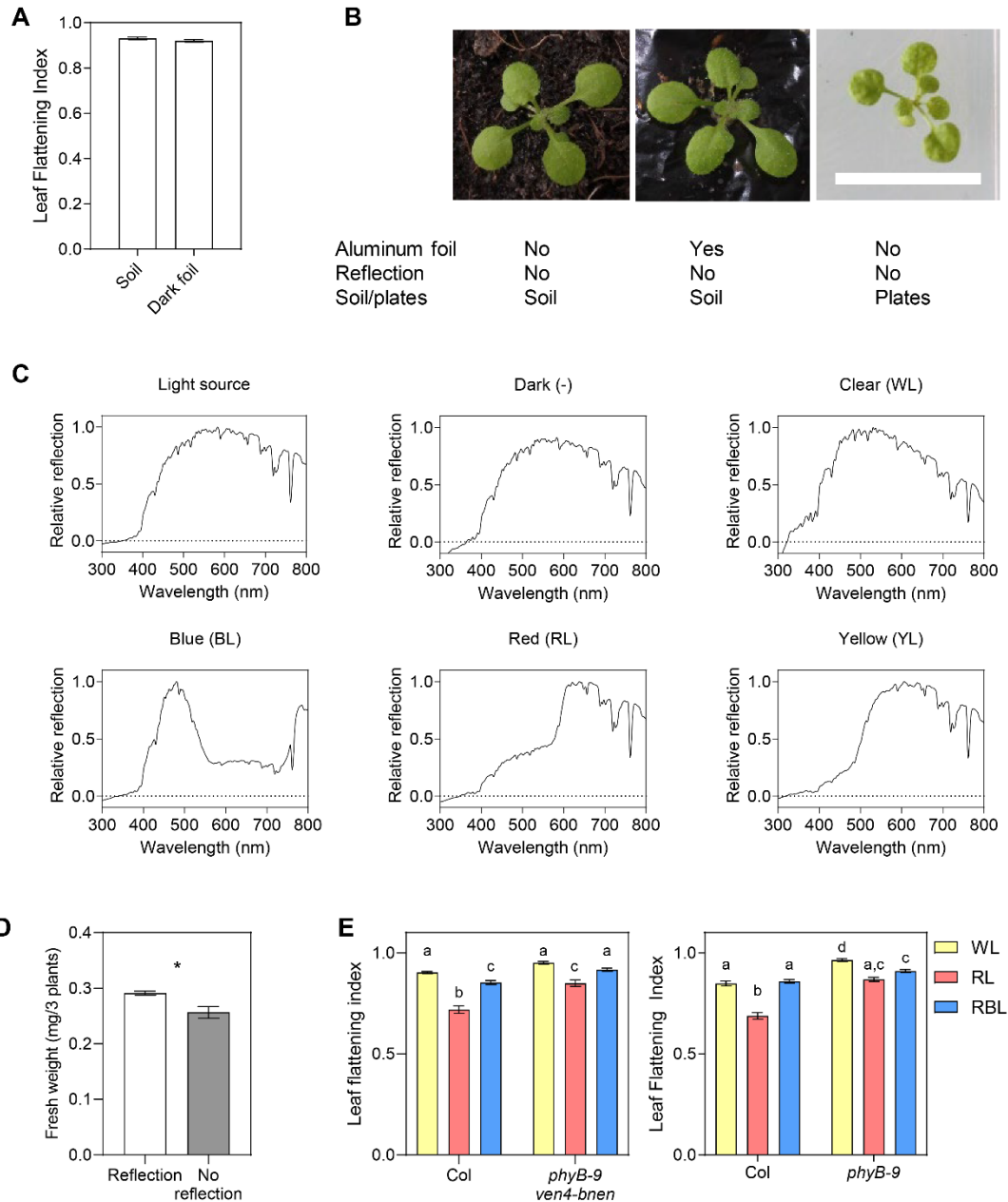
Supplemental table S1 - Genotyping conditions to select mutations in crosses

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Supplemental table S3 – Full ANOVA results for figure 1E

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Supplemental figure S1 – Light treatments and growth conditions

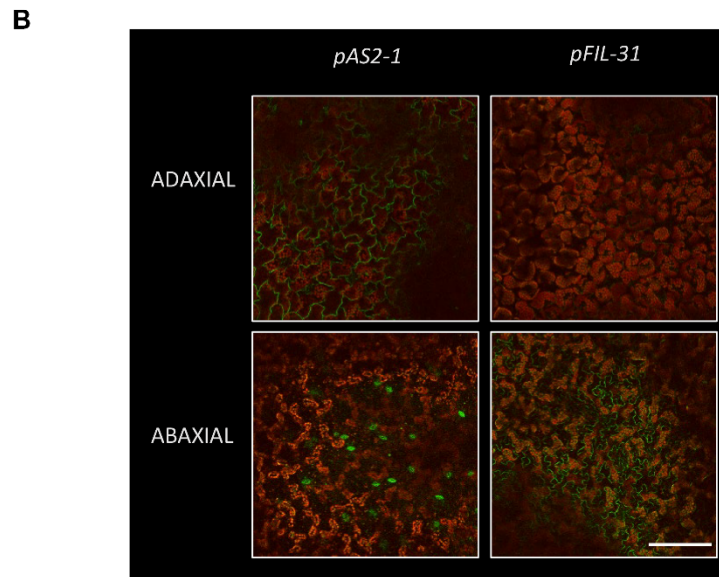
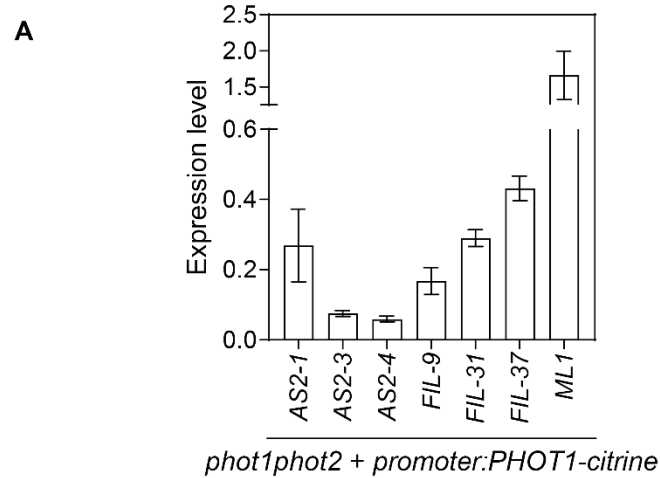
A- Dark aluminum foil does not affect leaf shape compared to soil. Col plants grown for three weeks on soil or on soil covered with dark aluminum foil. Leaf flattening index (mean \pm SE) of 22-32 leaves.

B- Images of Col plants grown in various conditions. Note that the presence of dark aluminum foil does not affect plant morphology, while plants grown in plates have smaller round leaf blades, with irregular surfaces, and thinner petioles than those grown on soil. Scale bar: 2cm.

C- Spectra of reflected light by colored aluminum foil. Colored aluminum foil was placed in front of a light source and the reflected light was measured. Top left, spectrum of the light source. To facilitate comparison of the shape of the spectra among colors in each case the spectrum plotted is relative to the maximum value.

D- Fresh weight of Col plants grown for 19 days on soil covered by dark (No reflection) or clear (Reflection) aluminum foil. Data are mean \pm SE of 10 replicates, each consisting of 3 plants. * $p < 0.05$ in a T-test.

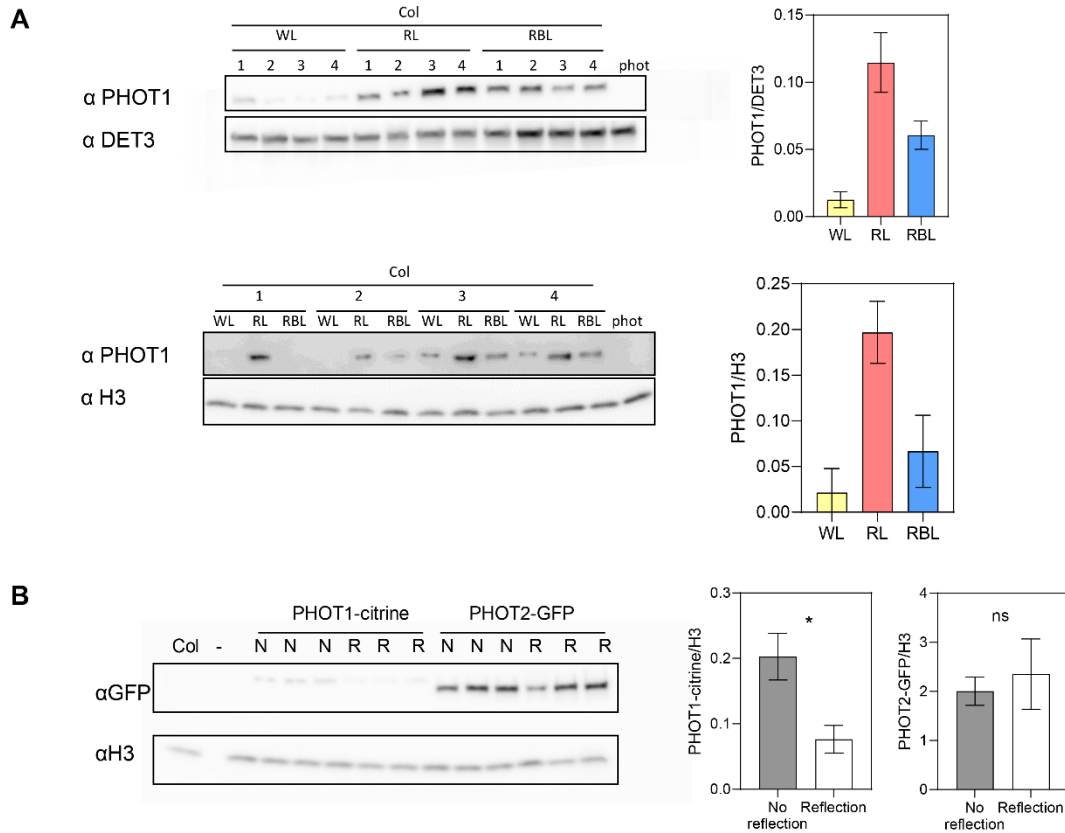
E- The *ven4-bnen* mutation does not affect phyB leaf flattening. Plants grown for two weeks and transferred to WL, RL or RBL for one week. Left panel: *phyB-9 ven4-bnen*. Right panel: *phyB-9* without the *ven4* mutation. Bars represent mean \pm SE of 22-28 leaves (left) and 37-48 leaves (right). Different letters represent significant differences among means ($p < 0.05$) in an ANOVA followed by Tukey's test.



Supplemental figure S2 – Expression levels and distribution pattern of *pAS2* and *pFIL* lines

A- Expression levels of *pAS2* and *pFIL* lines determined by RT-qPCR. Each bar represents mean \pm SE of three biological replicates.

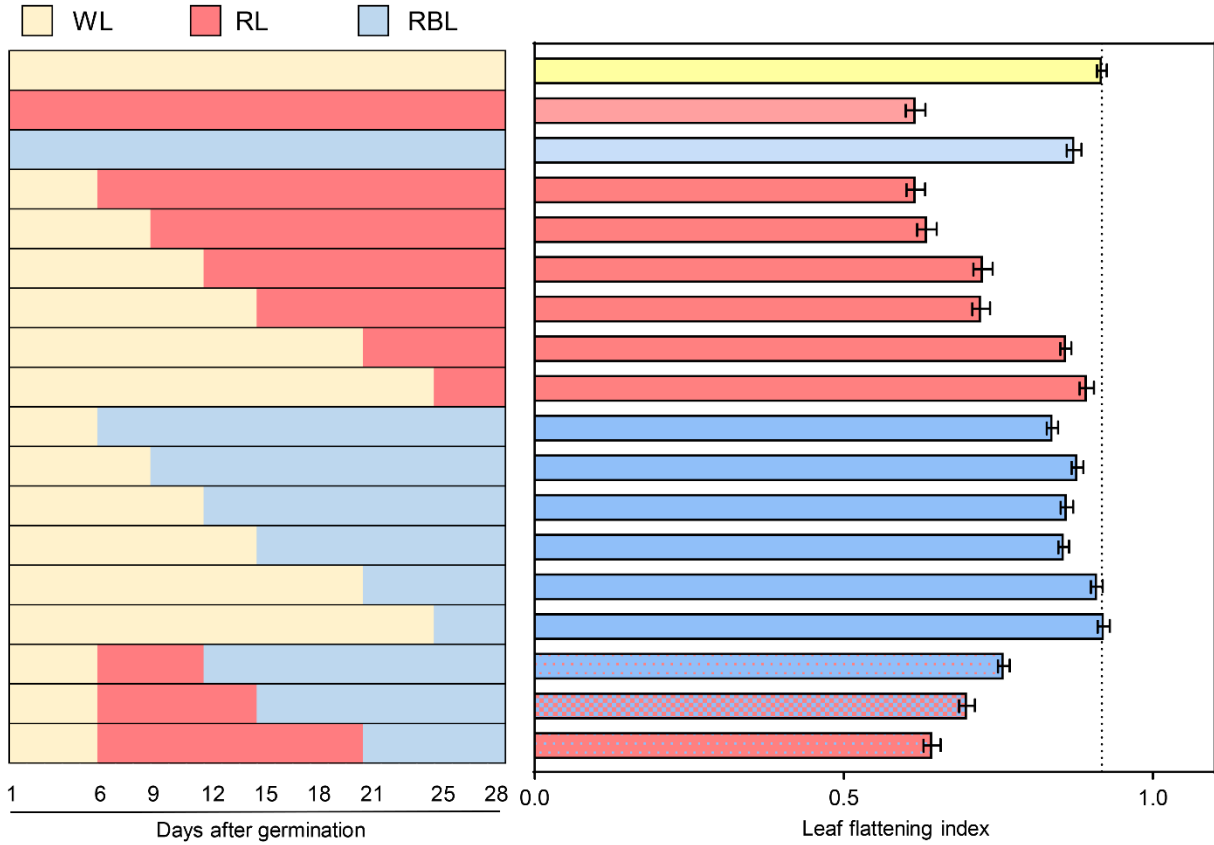
B- Confocal microscopy images of leaf blades of plants expressing *PHOT1-citrine* under various promoters. The same leaf was imaged on the adaxial and the abaxial side. Scale bar: 200 μ m.



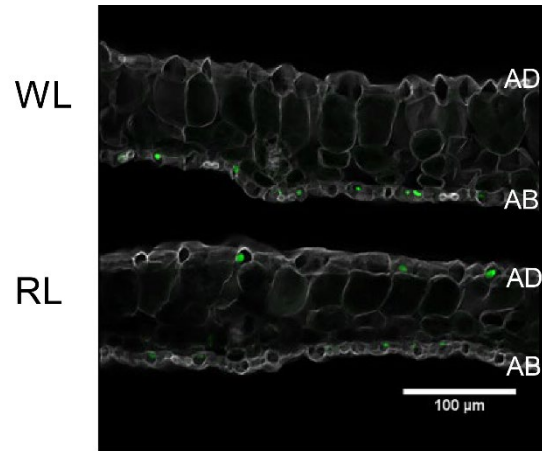
Supplemental figure S3 – PHOT1 levels decrease with BL in the leaf blade

A- PHOT1 levels decrease with BL in Col Plants grown for two weeks and transferred to WL, RL or RBL for one day. Membranes were probed with anti-PHOT1 and anti-DET3 or anti-Histone3 (H3) antibodies. Right: Quantification of the western blot. Each bar represents mean \pm SE of four replicates.

B- Plants expressing *pML1:PHOT1-citrine* or *pCER6:PHOT2-GFP* grown for two weeks in soil covered with dark (No reflection) or clear (Reflection) aluminum foil. Membranes were probed with anti-GFP and anti-H3 antibodies. N: No reflection, R: Reflection. Right: Quantification of the western blot. Each bar represents mean \pm SE of three replicates. * $p < 0.05$ in a T-test. The experiment was repeated three times with similar results.



Supplemental figure S4 – Phototropins control flattening reversibly and late in development
 Leaf flattening index of Col plants grown on soil and transferred to WL, RL or RBL at different time points. Measurements were done 28 days after germination. Bars represent mean \pm SE of 24 – 48 leaves. Left: scheme of the treatments.



Supplemental figure S5 - DR5:VENUS signal is detected in the epidermis.

Plants expressing DR5:VENUS (green) were grown for two weeks and transferred to WL or RL for one week. Leaves 1 and 2 were fixed and stained with calcofluor white (grays). Adaxial and abaxial epidermal layers are labeled as AD and AB respectively.

Allele	PCR conditions	Digestion enzyme	Result
pks1-1	CF82 + CF93 55°C JMRB1 + CF123 55°C		WT = 400bp mutant = 600pb
pks2-1	CF136 + CF330, 55°C CF136 + LB1, 55°C		WT= 306bp mutant=250bp
pks3-9	CF403 + CF522, 55°C	Hinf1	WT : 69 + 133 pks3-9 : 202
pks4-1	CF329 + AH021, 55°C CF329 + LB1, 55°C		WT= 560bp mutant= 450bp
phot1-5	CF342 + CF343 + CF344 + CF345, 55°C		WT = 250 bp + 450 bp <i>phot1-5</i> = 250 bp
phot2-1	CF346 + CF347, 55°C	Mbo1	WT = 398 +230+120 <i>phot2-1</i> = 530+230

Supplemental table S1 - Genotyping conditions to select mutations in crosses

Gene/Allele	Primer	Sequence
Citrine	LAP038	TACGGCCTGATGTGCTTCG
	LAP045	GTCTTGTAGTTGCCGTCGTC
GAPDH	FW	GCAAAATGGCTGACAAGAAGATC
	RV	AGCAACCAAACGACCGATTTC
UBC	FW	CAGTCTGTGTGTAGAGCTATCATAGCAT
	RV	AGAAGATTCCCTGAGTCGCAGTT
YLS8	FW	TCATTTCGTTTCGGCCATGA
	RV	CTCAGCAACAGACGCAAGCA
pks1-1	CF82	CTGGGTTTGTTCAGAGACAGA
	CF93	CCCTAATTCCACATATCTACACACAAGCAA
	JMRB1	GCTCATGATCAGATTGTCGTTTCCCGCCTT
	CF123	TCCTTTCTTTTGTGGTCACGGGGGTAACA
pks2-1	CF136	GCAAGGCAAGGAAGTAGTGA
	CF330	AGCTCGGTGTTCTGTTTCATG
	LB1	AATCAGCTGTTGCCCGTCTC
pks3-9	CF403	CTGACAATGACGCCGAGATTG
	CF522	GATCTTGACGACGTCGTTTTTCG
pks4-1	CF329	CTTGGGACTCGTAGGATTCA
	AH021	ACGAGGAATCTAAGTGGTCC
	LB1	AATCAGCTGTTGCCCGTCTC
phot1-5	CF342	TGTTGGCATCAGGAAGTT
	CF343	TGTGGCAGGAAAGAAGTT
	CF344	TGCCTGCAAACCAATAAC
	CF345	CCGGAGCAGGACATACG
phot2-1	CF346	CTGCCTCACAATAAGGAGAG
	CF347	GAACCTTGCAGAGTCTTCTG

Supplemental table S2 - Primers used in this study.

Source of Variation	P value
Interaction	<0,0001
Genotype	<0,0001
Light treatment	<0,0001

Tukey's multiple comparisons test	Adjusted P Value
<i>Col</i>	
WL vs. RL	<0,0001
WL vs. R+BL	0.8155
RL vs. R+BL	<0,0001
<i>phyB</i>	
WL vs. RL	<0,0001
WL vs. R+BL	0.0068
RL vs. R+BL	0.0499
<i>phot1</i>	
WL vs. RL	<0,0001
WL vs. R+BL	<0,0001
RL vs. R+BL	<0,0001
<i>phot2</i>	
WL vs. RL	<0,0001
WL vs. R+BL	0.1923
RL vs. R+BL	<0,0001
<i>phot1phot2</i>	
WL vs. RL	0.3582
WL vs. R+BL	0.9948
RL vs. R+BL	0.3941
<i>cry1</i>	
WL vs. RL	<0,0001
WL vs. R+BL	0.6667
RL vs. R+BL	<0,0001
<i>cry2</i>	
WL vs. RL	<0,0001
WL vs. R+BL	0.9001
RL vs. R+BL	<0,0001
<i>cry1cry2</i>	
WL vs. RL	<0,0001
WL vs. R+BL	0.9466
RL vs. R+BL	<0,0001

Supplemental table S3 – Full ANOVA results for figure 1E

Source of Variation	P value
Interaction	<0,0001
Genotype	<0,0001
Light treatment	<0,0001

Tukey's multiple comparisons test	Adjusted P Value	
<i>Col</i>	WL vs. RL	<0,0001
	WL vs. RBL	0.9927
	RL vs. RBL	<0,0001
<i>phot2</i>	WL vs. RL	<0,0001
	WL vs. RBL	0.2957
	RL vs. RBL	<0,0001
<i>phot1phot2</i>	WL vs. RL	0.9941
	WL vs. RBL	0.8402
	RL vs. RBL	0.7454
<i>AS2-1</i>	WL vs. RL	<0,0001
	WL vs. RBL	0.0545
	RL vs. RBL	<0,0001
<i>AS2-3</i>	WL vs. RL	<0,0001
	WL vs. RBL	0.1749
	RL vs. RBL	<0,0001
<i>AS2-4</i>	WL vs. RL	<0,0001
	WL vs. RBL	0.106
	RL vs. RBL	<0,0001
<i>FIL-9</i>	WL vs. RL	<0,0001
	WL vs. RBL	<0,0001
	RL vs. RBL	0.0023
<i>FIL-31</i>	WL vs. RL	<0,0001
	WL vs. RBL	0.6321
	RL vs. RBL	<0,0001
<i>FIL-37</i>	WL vs. RL	<0,0001
	WL vs. RBL	0.0274
	RL vs. RBL	<0,0001
<i>ML1</i>	WL vs. RL	<0,0001
	WL vs. RBL	0.133
	RL vs. RBL	<0,0001
<i>PHOT1</i>	WL vs. RL	<0,0001
	WL vs. RBL	0.8321
	RL vs. RBL	<0,0001

Supplemental table S4 – Full ANOVA results for figure 2A

Source of Variation	P value
Interaction	<0,0001
Genotype	<0,0001
Light treatment	<0,0001
Tukey's multiple comparisons test	
Adjusted P Value	
Col	
WL vs. RL	<0,0001
WL vs. RBL	0.0033
RL vs. RBL	<0,0001
<i>phot1phot2</i>	
WL vs. RL	0.1217
WL vs. RBL	0.049
RL vs. RBL	0.9848
<i>pks3-9</i>	
WL vs. RL	0.0002
WL vs. RBL	0.4926
RL vs. RBL	0.0221
<i>pks3-10</i>	
WL vs. RL	0.0002
WL vs. RBL	0.6879
RL vs. RBL	0.0028
<i>pks2</i>	
WL vs. RL	<0,0001
WL vs. RBL	0.2157
RL vs. RBL	<0,0001
<i>pks2 pks3</i>	
WL vs. RL	<0,0001
WL vs. RBL	0.0375
RL vs. RBL	0.014
<i>pks1,2,3,4</i>	
WL vs. RL	0.9988
WL vs. RBL	0.7449
RL vs. RBL	0.7089
<i>nph3-6</i>	
WL vs. RL	<0,0001
WL vs. RBL	<0,0001
RL vs. RBL	0.0658

Supplemental table S5 – Full ANOVA results for figure 3D

