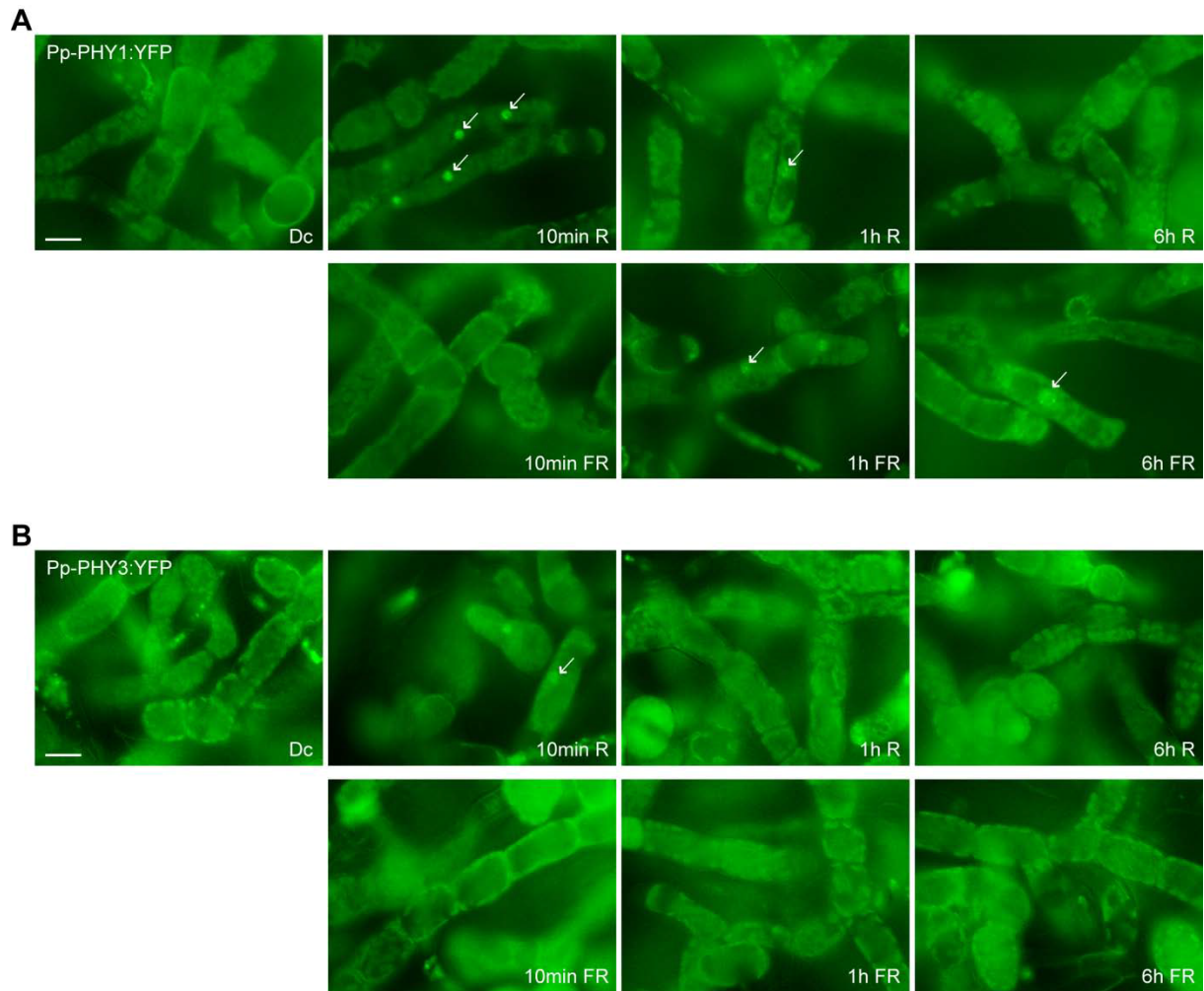


**Supplemental Figure 1.** Nuclear localization of *Physcomitrella* phytochromes.

**(A)-(D)** Nuclear localization of Pp-PHY2, Pp-PHY3, Pp-PHY4 and Pp-PHY5a in protonema filaments. Dark-adapted protonema filaments of *Physcomitrella* plants expressing YFP-tagged Pp-PHY2 **(A)**, Pp-PHY3 **(B)**, Pp-PHY4 **(C)** or Pp-PHY5a **(D)**, of which Pp-PHY2:YFP and Pp-PHY5a:YFP lines had been bleached with Norflurazone, were used for fluorescence microscopy. Images were acquired after

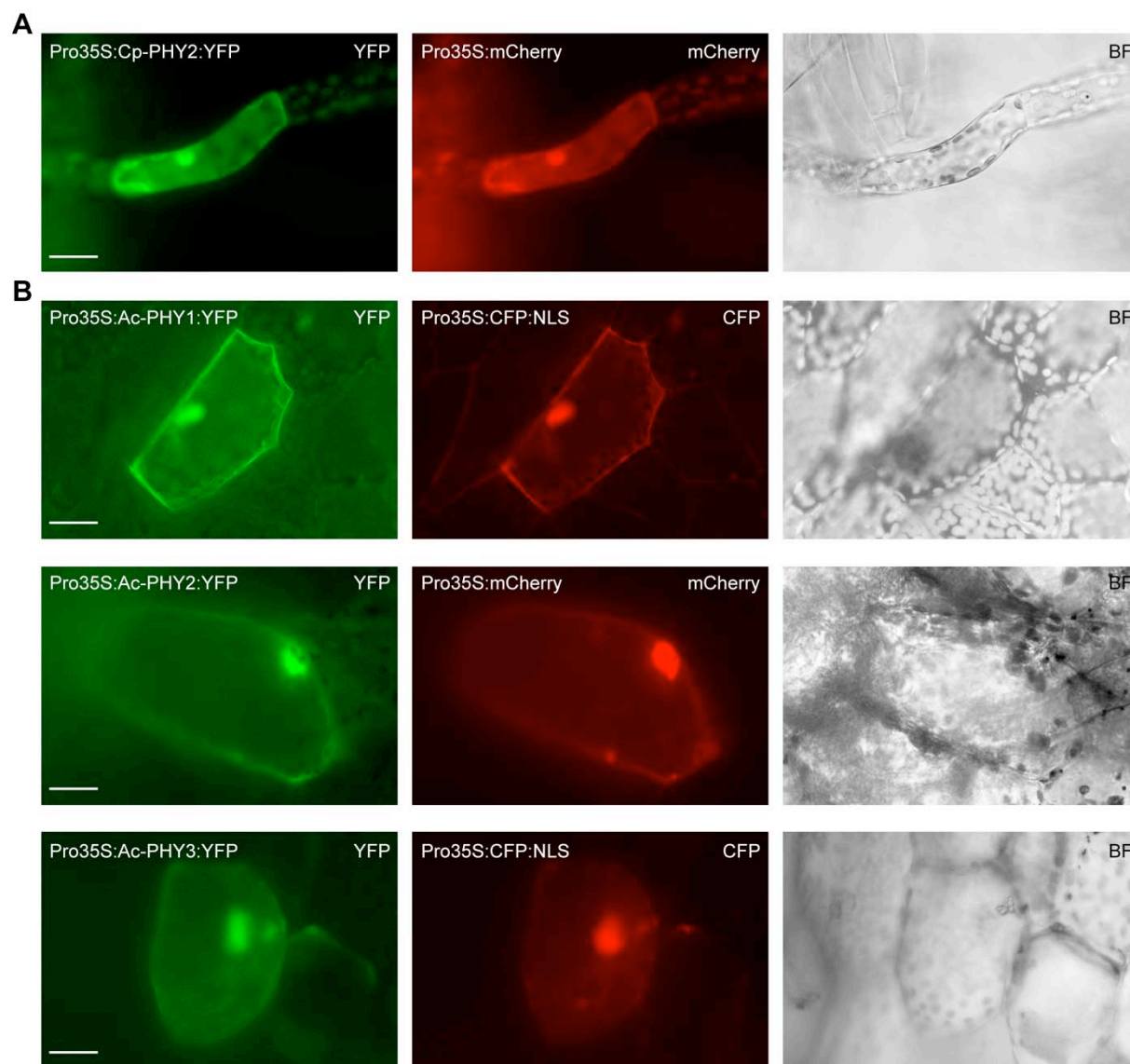
10-30 min irradiation with W light. The scale bars represent 20  $\mu\text{m}$ . Arrows indicate nuclei. BF, bright field.

**(E)** (Duplicates images in Figure 5E) Immunoblot analyses for Pp-PHY:YFP expressing lines. Dark-adapted protonema cultures of *Physcomitrella* lines expressing YFP-tagged Pp-PHY1, Pp-PHY2, Pp-PHY3, Pp-PHY4 or Pp-PHY5a were used for protein extraction. Total protein was analyzed by SDS-PAGE and immunoblotting with anti-YFP antibody. Protein extracts from dark-adapted wild-type *Physcomitrella* cultures were used as negative controls. An unspecific signal was used as loading control.



**Supplemental Figure 2.** (Duplicates images in Figure 1D) Pfr-dependent degradation of PHY1 and PHY3 in *Physcomitrella*.

Dark-adapted protonema filaments of Pp-PHY1:YFP (**A**) or Pp-PHY3:YFP (**B**) expressing *Physcomitrella* lines were used for fluorescence microscopy. Images were acquired before (dark control, D) and after irradiation with either R light ( $22 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; Pfr) or FR light ( $18 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; Pr). The duration of irradiation is indicated in the figure. Before microscopic analysis, the samples were fixed with formaldehyde. The scale bar represents  $20 \mu\text{m}$ . Arrows indicate nuclei.

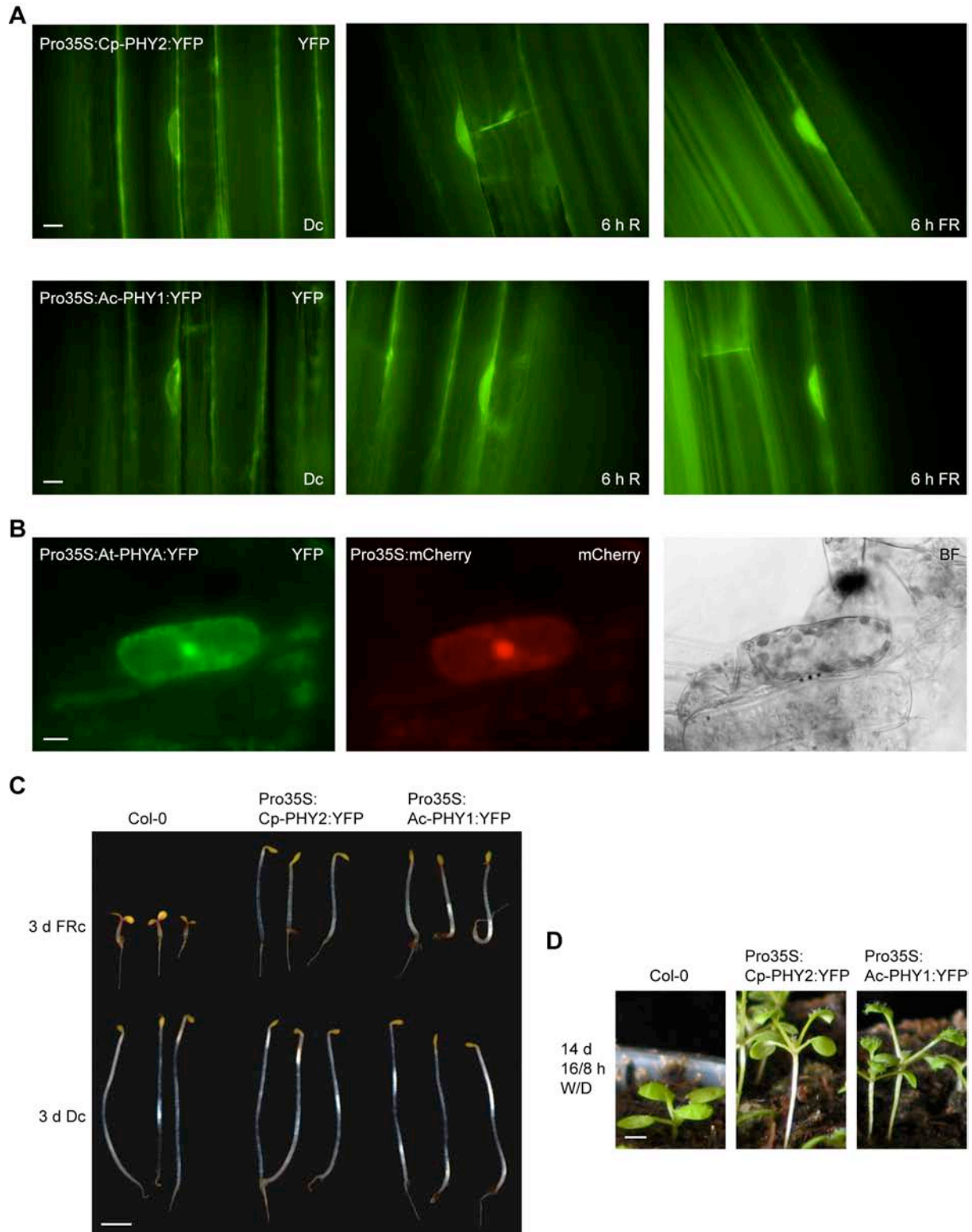


**Supplemental Figure 3.** Nuclear localization of *Ceratodon* and *Adiantum* phytochromes.

**(A)** Nuclear localization of *Ceratodon purpureus* PHY2 in protonema filaments. Protonema filaments of *Physcomitrella* were transiently transformed with Pro35S:Cp-PHY2:YFP and Pro35S:mCherry using particle bombardment. After transformation, the protonema filaments were grown in the dark (D) for 1 day and used for epifluorescence microscopy with filter sets specific for YFP and mCherry. The images were acquired after 15-30 min irradiation with microscope light. The scale bar represents 20  $\mu\text{m}$ . BF, bright field.

**(B)** Nuclear localization of phytochromes in fern gametophytes. *Adiantum capillus-veneris* gametophytes were transiently transformed by particle bombardment with

Pro35S:Ac-PHY1:YFP, Pro35S:Ac-PHY2:YFP or Pro35S:Ac-PHY3:YFP. Pro35S:CFP:NLS or Pro35S:mCherry were co-transformed as a control. The gametophytes were grown for 2-5 days in D after transformation and used for microscopy with YFP, CFP and mCherry-specific filters. The images were acquired after 15-30 min irradiation with microscope light. The scale bar represents 20  $\mu\text{m}$ . BF, bright field.



**Supplemental Figure 4.** Conserved nuclear transport mechanisms for cryptogam and seed plant phytochromes.

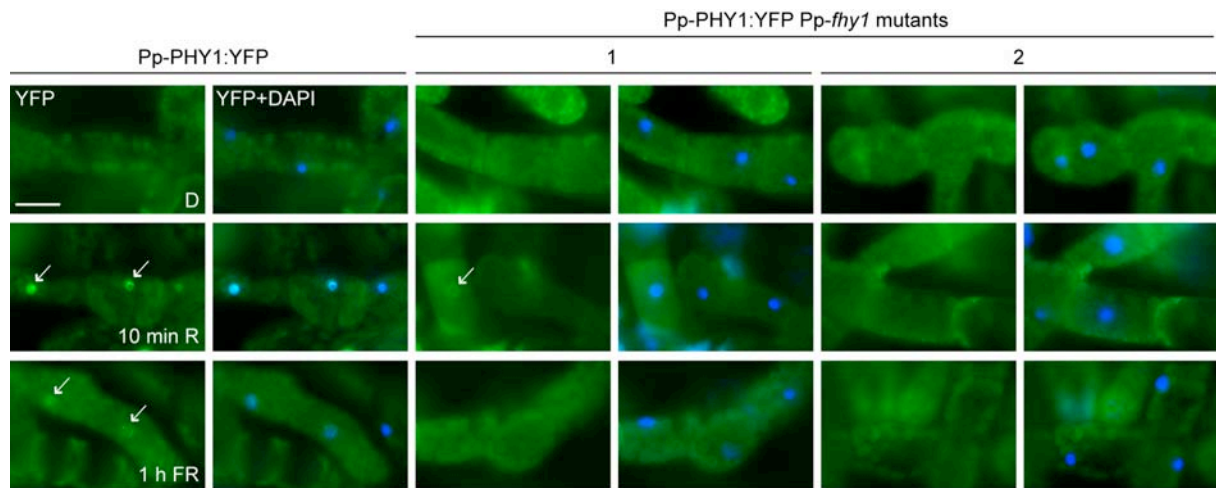
**(A)** Light enhanced nuclear accumulation of moss and fern phytochromes in *Arabidopsis*. Four-day-old etiolated *Arabidopsis* seedlings expressing 35S promoter

driven Cp-PHY2:YFP or Ac-PHY1:YFP were used for fluorescence microscopy. Images were acquired before (dark control, D) or after a six-hour irradiation with R light ( $15 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) or FR light ( $15 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). The scale bar represents 10  $\mu\text{m}$ .

**(B)** Nuclear localization of At-PHYA in *Physcomitrella*. *Physcomitrella* protonema filaments were transiently transformed with a Pro35S:At-PHYA:YFP construct using particle bombardment. After transformation, the protonema filaments were incubated in D for 1 day and used for microscopy. The images were acquired after 15-30 min irradiation with microscope light. The scale bar represents 10  $\mu\text{m}$ . BF, bright field.

**(C)** Wild-type (Col-0) seedlings as well as transgenic lines expressing 35S promoter driven Cp-PHY2:YFP or Ac-PHY1:YFP in the Col-0 background were grown for 3 days in D or FR ( $15 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). The scale bar represents 2 mm.

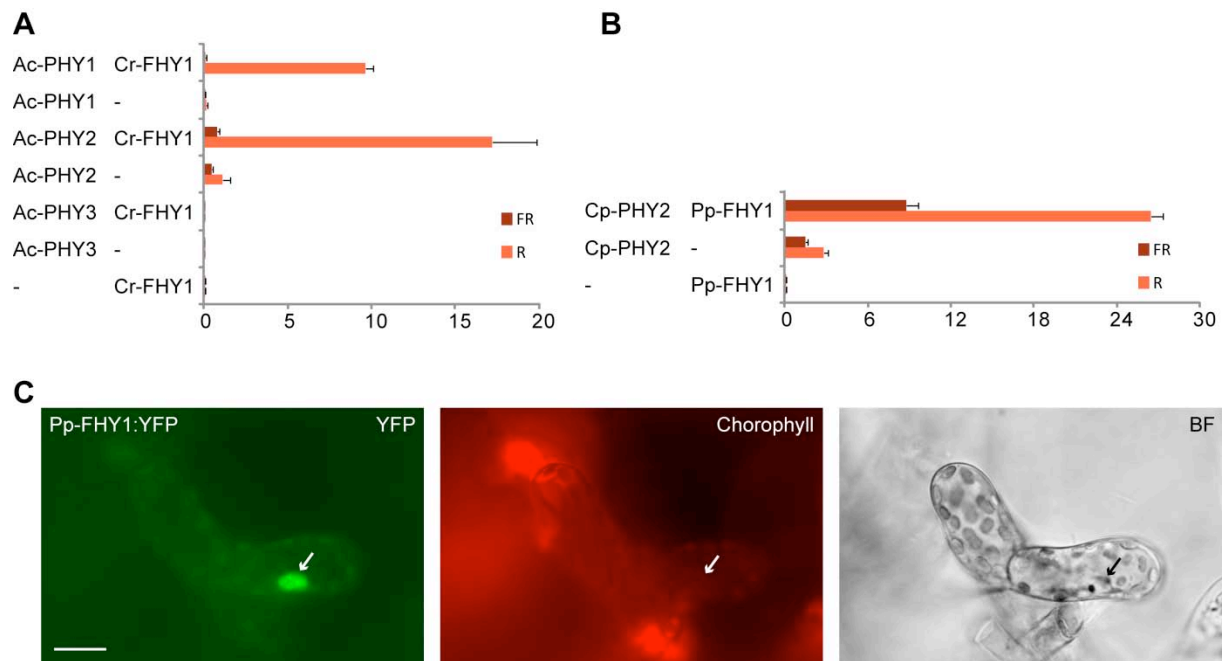
**(D)** Wild-type (Col-0) as well as Col-0 plants expressing Pro35S:Cp-PHY2:YFP or Pro35S:Ac-PHY1:YFP were grown for 14 days in the green house (16 h/8 h L/D cycles). The scale bar represents 2 mm.



**Supplemental Figure 5.** Light-induced nuclear transport of Pp-PHY1 depends on Pp-FHY1.

Dark-adapted protonema filaments of *Physcomitrella* wild type or *Pp-fhy1* mutants expressing Pp-PHY1:YFP were used for microscopy. Images were acquired before (dark control, D) and after irradiation with either R light (10 min,  $22 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) or FR light (1 h,  $18 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). The samples were fixed and stained with DAPI before microscopic analysis. Arrows indicate nuclei. The scale bar represents 20  $\mu\text{m}$ .





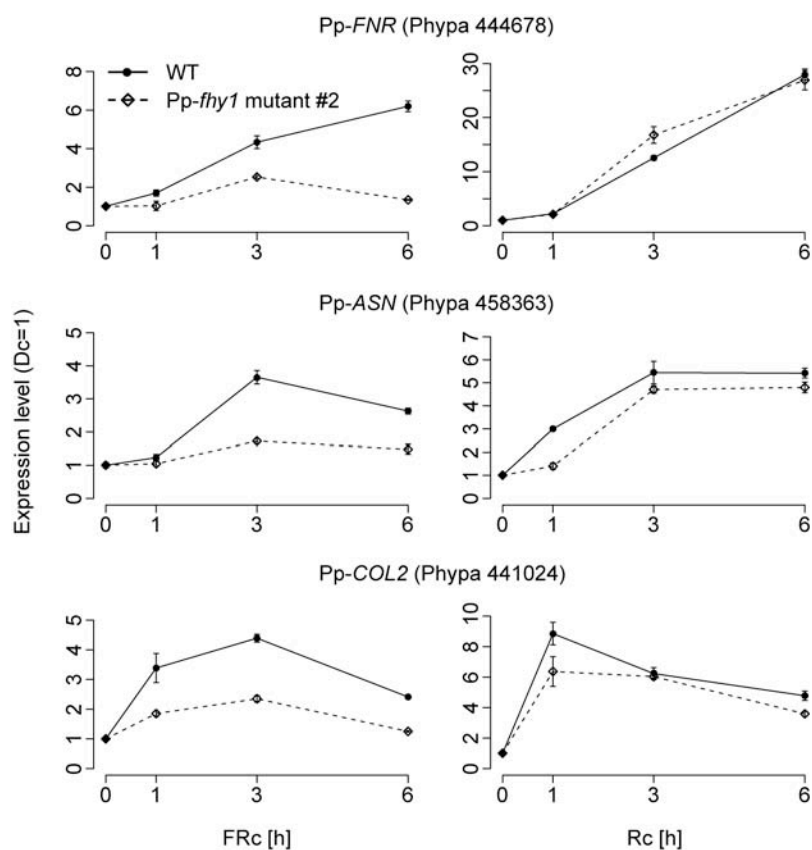
**Supplemental Figure 6.** Light regulated interaction of FHY1 and phytochromes from mosses and ferns.

**(A)** *Adiantum* phytochromes interact with Cr-FHY1. FHY1:AD and BD:PHY constructs were transformed into yeast strain Y187 and used for yeast two-hybrid assays. To convert PHYs to the Pfr or Pr form, yeast cultures were irradiated for 5 min with R light ( $12 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), either followed by a 5 min FR light pulse ( $12 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) or not. After 4 hours incubation in the dark, the  $\beta$ -galactosidase activity was measured using the ONPG assay. Error bars represent SE;  $n=3$ . AD, GAL4 activation domain; BD, GAL4 DNA binding domain; Ac, *Adiantum capillus veneris* (fern); Cr, *Ceratopteris richardii* (fern).

**(B)** Pfr-dependent interaction of Pp-FHY1 and PHY2 from *Ceratodon purpureus*. Constructs coding for AD:FHY1 and PHY:BD were used for yeast two-hybrid analysis as described in (C). SE;  $n=3$ . AD, GAL4 activation domain; BD, GAL4 DNA binding domain; Cp, *Ceratodon purpureus* (moss); Pp, *Physcomitrella patens* (moss).

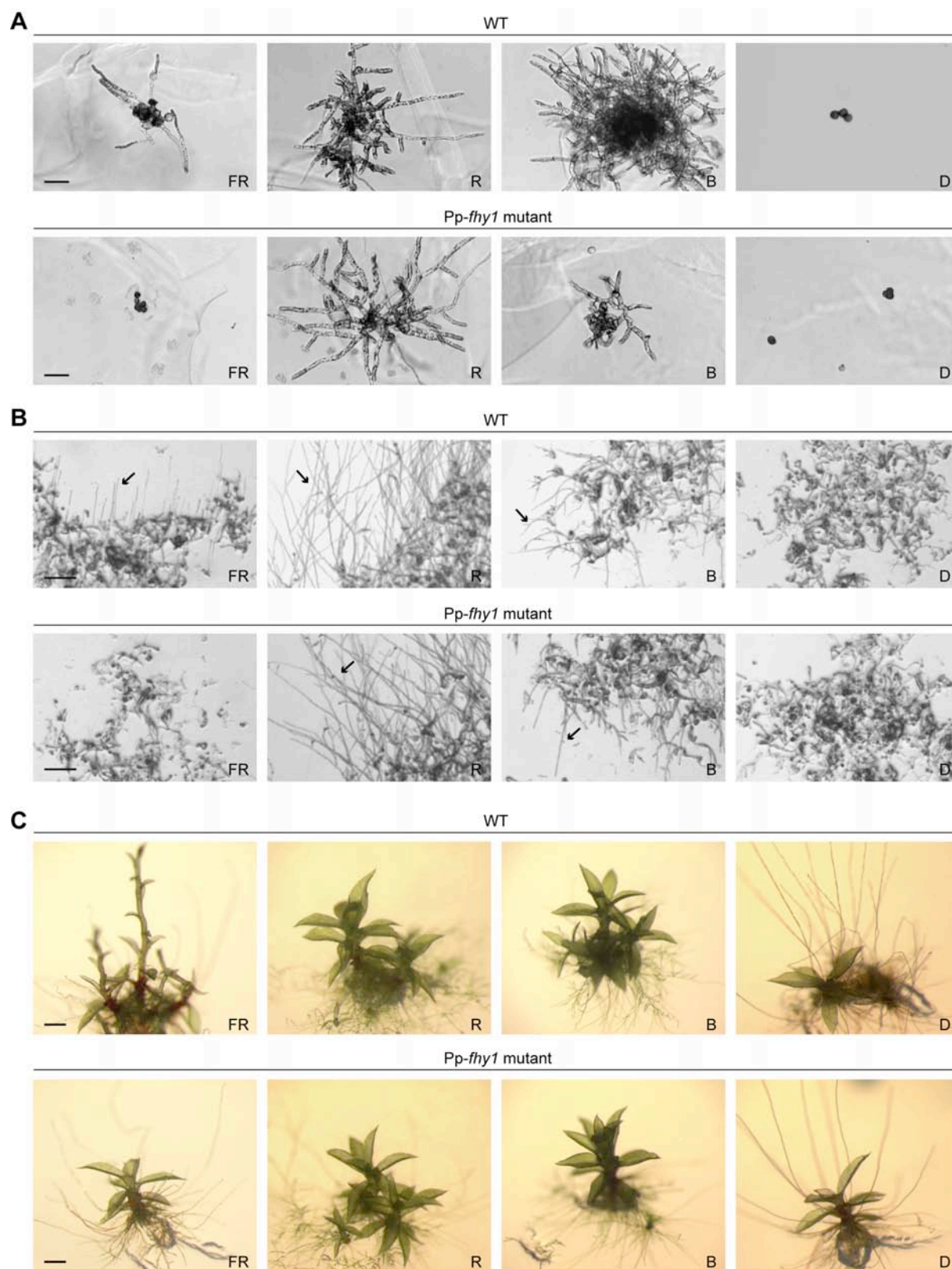
**(C)** Pp-FHY1 localizes to the nucleus in *Physcomitrella* and forms nuclear bodies. *Physcomitrella* protonema filaments were transiently transformed with Pro35S:YFP:Pp-FHY1 using particle bombardment. Transformed protonema filaments were grown for 3 days in D and used for microscopy. The images were acquired after 15 min irradiation with microscope light. The scale bar represents

20  $\mu\text{m}$ . Arrows indicate nuclei.



**Supplemental Figure 7.** Pp-FHY1 is essential for FR light-induced gene expression.

Protonemata cultures of *Physcomitrella* wild type and Pp-*fhy1* mutant lines were dark adapted and exposed to either R light ( $28 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) or FR light ( $16 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Samples for qRT-PCR analyses were harvested after 1, 3 and 6 hours of light treatment or darkness. The expression levels of *FNR*, *ASN* and *COL2* were normalized to the levels of *26S rRNA*. Expression levels in D were set to 1. Error bars represent SE of technical replicates,  $n=3$ . An independent biological replicate is shown in Figure 4A.



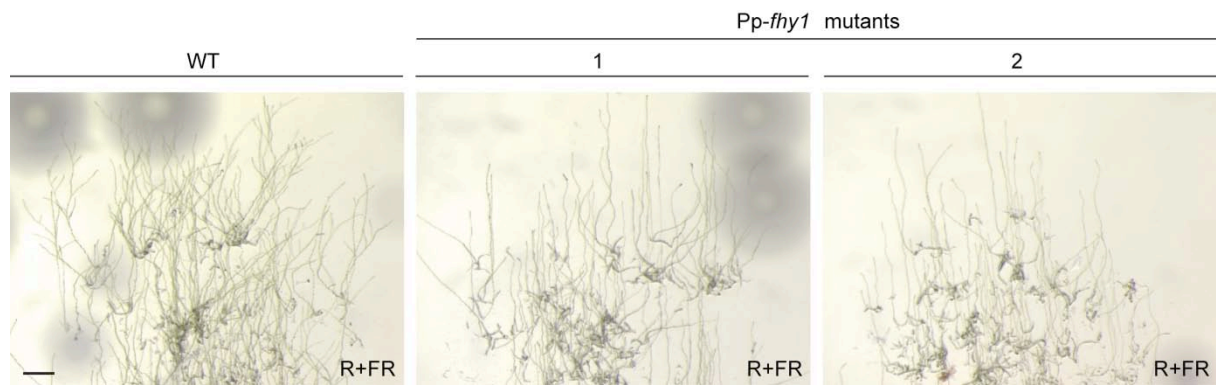
**Supplemental Figure 8.** Phenotype of the Pp-*fhy1* mutant under different light conditions.

**(A)** Spore germination depends on Pp-FHY1 in FR but not in R and B light. Spores

from the wild type and Pp-*fhy1* mutant were kept in darkness, D, or irradiated for 6 days with continuous FR light ( $18 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), R light ( $22 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) or B light ( $7 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). The scale bar represents 100  $\mu\text{m}$ .

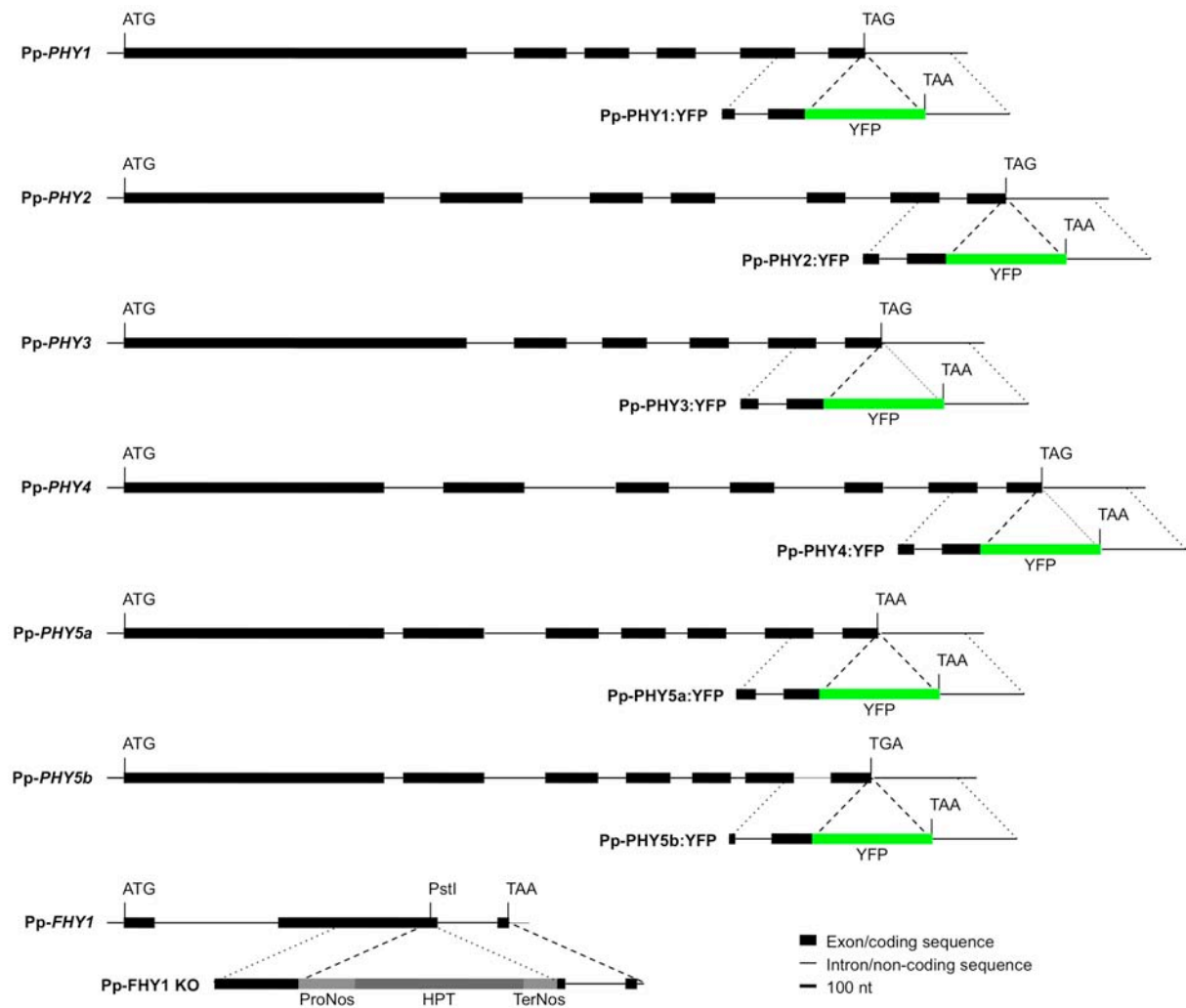
**(B)** Protonemata growth depends on Pp-FHY1 in FR but not in R and B light. Wild-type and Pp-*fhy1* mutant *Physcomitrella* cultures were grown on vertical plates for 10 days in continuous FR light ( $18 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), R light ( $28 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) or B light ( $7 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) or in D. The scale bar represents 200  $\mu\text{m}$ .

**(C)** Pp-FHY1 is essential for gametophore growth in FR but not in R or B light. Wild-type and Pp-*fhy1* mutant *Physcomitrella* gametophores were grown for 11 days in FR light ( $18 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), R light ( $22 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), B light ( $7 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) or D. The scale bar repres

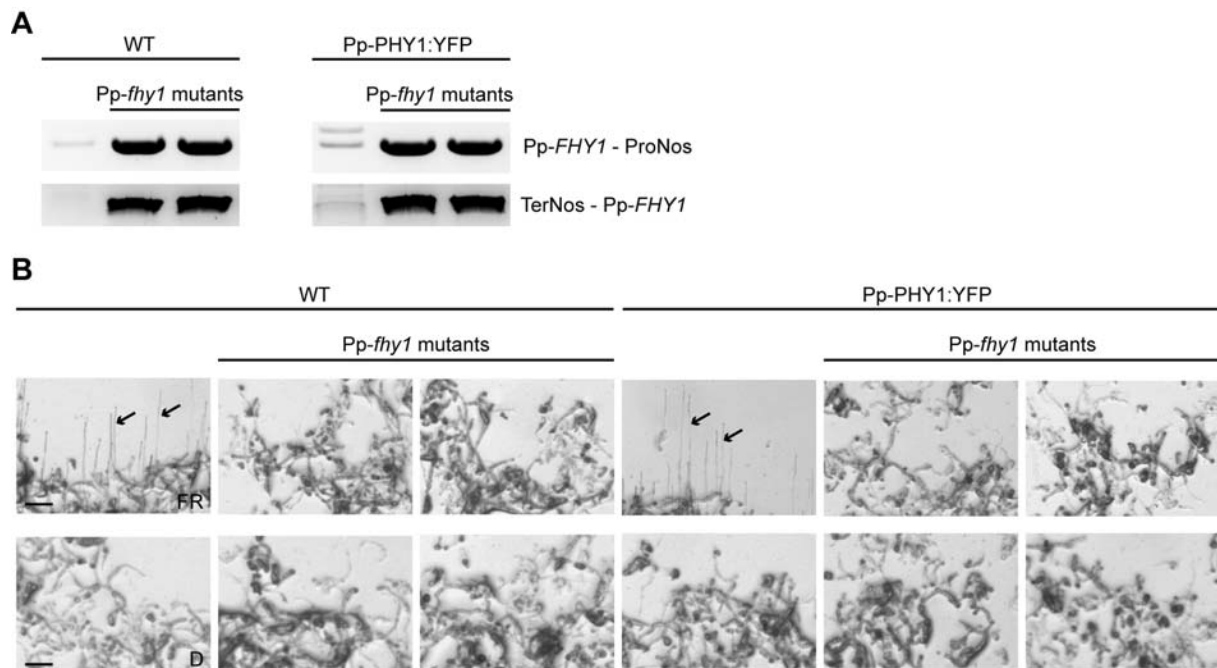


**Supplemental Figure 9.** Pp-FHY1 is required for branching of protonema filaments in a R-FR light mixture.

Wild-type and *Pp-fhy1* mutant protonema filaments were grown for 10 days in continuous R light ( $22 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) supplemented with FR light ( $16 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). The scale bar represents  $500 \mu\text{m}$ .



**Supplemental Figure 10.** Schematic representation of genomic loci (*Pp-PHY1-5b*, *Pp-FHY1*) and targeting cassettes (*Pp-PHY1-5b:YFP*, *Pp-FHY1 KO*). The targeting cassettes were cut from pBS II KS vectors using NotI.



**Supplemental Figure 11.** Molecular and phenotypal characterization of independent *Pp-fhy1* mutant lines.

**(A)** *Pp-fhy1* mutants and *Pp-fhy1* mutants expressing Pp-PHY1:YFP were analysed in PCR experiments using primers specific for the targeting cassette and genomic *Pp-FHY1*. The correct insertion of the targeting construct was verified by sequencing the PCR products.

**(B)** (Duplicates images in Supplemental Figure 8B) *Pp-fhy1* mutants and *Pp-fhy1* mutants expressing Pp-PHY1:YFP were tested for the protonemata growth phenotype in FR light. *Physcomitrella* protonemata were grown on vertical plates for 10 days in continuous FR light ( $18 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) or in D. The scale bar represents 200  $\mu\text{m}$ . Arrows indicate new-grown protonema filaments.



## Supplemental Table 1

Primer list; restriction sites are underlined.

Name	Sequence (5' → 3')	Restriction Sites
ah042	CGC <u>GGA TCC</u> CGC <u>TCT AGA</u> ATG GTG AGC AAG GGC GAG G	BamHI, XbaI
ah043	CGG <u>GGT ACC</u> <u>GCT AGC</u> TTA <u>ACT AGT</u> CTT GTA CAG CTC GTC CAT G	KpnI, NheI, SpeI
ah070	AGA AGT CGT GCT GCT TCA TG	-
ah094	<u>GGA CTA GTT</u> <u>ATC TAG AGC</u> <u>CCT AGG ATC CGC</u> CTT GTA CAG CTC GTC CAT G	SpeI, XbaI, AvrII, BamHI
ah309	CGC <u>GGA TCC</u> AAA AAT GGA TCA CAG CGG CAG CG	BamHI
ah310	<u>GCT CTA GAA</u> AGC ATG AGC CTT GTA AA	XbaI
ah312	<u>GGA CTA GTC</u> <u>CTA GGC</u> ATT TGG CTT GCA GCG TCA	SpeI, AvrII
ah349	CGC <u>GGA TCC</u> AAA AAT GGG GTT GCG TCG TCT G	BamHI
ah350	<u>GGA CTA GTT</u> TGA TTC AAC AAC AGT GA	SpeI
ah351	CGC <u>GGA TCC</u> AAA AAT GTT ACA GTT ATA TTC A	BamHI
ah352	<u>GGA CTA GTT</u> AGC ATA AGC GTT GAA AA	SpeI
ah357	<u>GGA CTA GTG</u> AGC ATA AGC TTG GCG AAG	SpeI
ah383	<u>GCT CTA GAA</u> AAA TGT TTG TCC TGT CCT CTG GCC G	XbaI
ah384	<u>GGA CTA GTC</u> AAG AGC AGC TGC GCA AAG	SpeI
ah404	<u>GAA GAT CTA</u> AAA ATG ACG ACG GGG AAG GTG	BglII
ah406	<u>GGA CTA GTC</u> ACC TGA AGG TTG GGT CA	SpeI
ah407	<u>GCT CTA GAC</u> ATT TGA CTT GAA GCA TCA	XbaI
ah408	<u>GCT CTA GAG</u> AGT TTA TAC TCC TAT TTG	XbaI
ah409	CGG <u>GGT ACC</u> <u>GCG GCC GCT</u> AAG TAT ATA ACC TAA AG	KpnI, NotI
ah410	<u>GGA CTA GTA</u> ATG CTG TGC GAT TCA CCC CA	SpeI
ah411	<u>GCT CTA GAG</u> ACA ACA GTA GAT CTC ACA	XbaI
ah412	<u>GCT CTA GAT</u> TTA TTA GAG TAA CAA TCT A	XbaI
ah413	CGG <u>GGT ACC</u> <u>GCG GCC GCT</u> GAA TCT CAT GTA AAA ACC A	KpnI, NotI
ah414	<u>GGA CTA GTT</u> TCT TAT TAA ACG CAG TG	SpeI
ah415	<u>GCT CTA GAC</u> ATT TGA CTC GCA GCA TCA	XbaI
ah416	<u>GCT CTA GAG</u> AGC TCA TAT ACC CTT TT	XbaI
ah417	CGG <u>GGT ACC</u> <u>GCG GCC GCA</u> ATG AGT TAT CAG TAT ATA	KpnI, NotI
ah418	<u>GGA CTA GTA</u> TTC ACC CCA TCC TCT GG	SpeI
ah419	<u>GCT CTA GAT</u> CTC ACA CTG CCT GCA TCA	XbaI
ah420	<u>GCT CTA GAA</u> GCT TTA TGA TAG TGG GCA	XbaI
ah421	CGG <u>GGT ACC</u> <u>GCG GCC GCG</u> ATC GTC ACA AAG ATC TA	KpnI, NotI
ah426	<u>GGA CTA GTG</u> GAA ACG CCT TGG TGG TG	SpeI
ah427	<u>GCT CTA GAG</u> CAA TTC AAA GGG CCC GA	XbaI
ah428	<u>GCT CTA GAT</u> GAT CCT GTT TTT GAA GA	XbaI
ah429	CGG <u>GGT ACC</u> <u>GCG GCC GCA</u> CAA GAC CTG CAG AAC ACG	KpnI, NotI
ah430	<u>GGA CTA GTT</u> TTA CTC CCT CTT CCG AG	SpeI
ah431	<u>GCT CTA GAC</u> CGC ATA GTA CCG GTA TCA TCT CGT ACA GCC A	XbaI
ah432	<u>GCT CTA GAA</u> TGG CCA CTA GAA TGC CAA TGA ACC TTG TTT G	XbaI
ah433	CGG <u>GGT ACC</u> <u>GCG GCC GCA</u> GCT GTT CAT CTA CAG CTT A	KpnI, NotI
ah434	<u>GGA CTA GTA</u> CCG GAG GCA GCA ACC TA	SpeI
ah435	CGG <u>GGT ACC</u> GCG GCC GCC CAG GAG CGC TGG GGA GG	KpnI
ah483	CGC <u>GGA TCC</u> TGC <u>AGG GCC</u> CCG AAG GAT CTG ATG GC	BamHI, SbfI
ah484	<u>GGA CTA GTC</u> CTG <u>CAG GCG</u> ATC TAG TAA CAT AGA TGA	SpeI, SbfI
ah441	CGC <u>GGA TCC</u> AAA AAT GTC GAC TCC CAA GAA G	BamHI
ah442	<u>GGA CTA GTA</u> GCG GCT AAC TCG ACC TCC A	SpeI
ah443	<u>GAA GAT CTA</u> AAA ATG TCG ACC CCC AAG TTG	BglII
ah444	<u>GGA CTA GTA</u> GCT GCA AGC TCA ACC TCC A	SpeI
ah445	CGC <u>GGT ACC</u> AAA AAT GTC GGC TCC GAA GAA G	BamHI
ah447	<u>GAA GAT CTA</u> AAA ATG TCG ACC ACC AAG TTG	BglII
ah448	<u>GGA CTA GTA</u> GCT GCA AGT TCA ACT TCC A	SpeI
ah455	CGC <u>GGA TCC</u> ACG GAG AAT GAG CTG AAG GG	BamHI
ah456	<u>GAA GAT CTT</u> TTC AGG ATA TTG ATG ACA G	BglII
ah457	CGC <u>GGA TCC</u> AAA AAT GTC GGC TCC CAA GAA G	BamHI
ah471	CGC <u>GGA TCC</u> AAA AAT GTC GAG TAC AAG ACA CAG	BamHI
ah472	<u>GGA CTA GTT</u> CTT ACG CTA GTA GCA TCA TCT CTG	SpeI
ah474	<u>GGA CTA GTC</u> AAA AAA TGT CCT CCA AAA CCA TG	SpeI
ah475	<u>GGA CTA GTG</u> TCA TCT TCT TGA ACA AG	SpeI
ah477	<u>GGA CTA GTC</u> AAA AAA TGG CGA CTC CAG GGG GG	SpeI
ah478	<u>GGA CTA GTG</u> AAT GTA TCT TGA AAG GA	SpeI
ah527	<u>GCT CTA GAA</u> AAA TGG GGC GAA CAA GAG GA	XbaI
ah528	<u>GGA CTA GTG</u> AGC ATT AAC ATG GAA AA	SpeI
ah791	AGC TGA TCT CAG AGG AGG ACC TGG CTA GCA TGG TGA GCA AGG GCG AG	-
ah792	<u>GAA GAT CTA</u> AAA ATG GCC GAG GAG CAG AAG CTG ATC TCA GAG GAG G	BglII

Supplemental Table 1 (continued)

Name	Sequence (5' → 3')	Restriction Sites
ah-nls	GGA CTA GTT TAT CCT CCA ACC TTT CTC TTC TTC TTA GGC TGA AGC CTA GTC TTG TAC AGC TCG TCC ATG CCG	SpeI
p023	AAG GTC TGC AAT TGA TAC GGG A	-
p026	CAA GTT CGT TTA CAA CAG GTC CT	-
p028	CAA TTA ATC CGG GAG ACT CCC A	-
p030	GTG AAA CGC CTC GGG AA	-
p046	GCG GTT CTG TCA GTT CCA A	-
p047	GGT TGT AGT GAG CAA AAC CTC CA	-
p050	GTG ATG CTC AAC GGA CTC GCT T	-
p051	GAG GTG AAG TTC GAG GGC GA	-
p062	CGC CGT TAT CAG TCA AGG TAT GA	-
p065	AGC GTG GTA TCA CAA TTG AC	-
p066	GAT CGC TCG ATC ATG TTA TC	-
p069	CCG AAG AGC GAC TTT ATT CAC T	-
p070	GGC GAA GTA TTC ATC GAA GTC T	-
p158	GTG CTT CGC ACC TCG AAT TG	-
p159	TTG TTC GCT ATC GGT CTC TTG	-
p118	ACA GGA ATT CAA CCC GAC AG	-
p119	GAG CAC CTT GAG AAT CCA GTG	-
p186	GGC AAT TAT CGA TCC CAC GTC	-
p187	ATC GCG AGC AGC AAT GAA TG	-
p188	GCG CGC ACA TCT ACT TCT G	-
p189	TTG ACA GCC TCA CAC ACC TG	-
p223	GAT AAA TTA TCG CGC GCG GTG	-
p225	GAT TCC ACC AGG TTC GGA C	-

## Supplemental Methods

### *Cloning of constructs*

A list including all primers used in this work can be found in Supplemental Table 1. Schematic representations of targeting constructs and genomic loci can be found in Supplemental Figure 10.

The gene targeting cassettes used to generate transgenic *Physcomitrella* lines containing a YFP tag fused to the endogenous phytochromes were obtained as follows. First, pBS II KS:YFP was generated by PCR amplifying EYFP from pEYFP (Clontech, Mountain View, CA, USA) using the primers ah042 and ah043 and ligating the resulting fragment into the BamHI/KpnI site of pBS II KS (Stratagene, La Jolla, CA, USA). In the next step, the region of the *Physcomitrella* phytochrome genes between the stop codon and approximately 500 bp upstream of it were PCR amplified from genomic *Physcomitrella* DNA using the primers ah406/ah407 for Pp-*PHY1*, ah410/ah411 for Pp-*PHY2*, ah414/ah415 for Pp-*PHY3*, ah418/ah419 for Pp-*PHY4*, ah430/ah431 for Pp-*PHY5a* and ah426/ah427 for Pp-*PHY5b*. These fragments were cut with SpeI/XbaI and ligated in sense orientation into the XbaI site of pBS II KS:YFP. Finally, the region of the *Physcomitrella* phytochrome genes between the stop codon and approximately 500 bp downstream of it were amplified by PCR from genomic *Physcomitrella* DNA using the primers ah408/ah409 for Pp-*PHY1*, ah412/ah413 for Pp-*PHY2*, ah416/ah417 for Pp-*PHY3*, ah420/ah421 for Pp-*PHY4*, ah432/ah433 for Pp-*PHY5a* and ah428/ah429 for Pp-*PHY5b*. These fragments were digested with XbaI/KpnI and ligated into the NheI/KpnI site of the pBS II KS:YFP vectors already containing the first fragment of the respective phytochrome gene, resulting in gene-targeting cassettes for each of the seven *Physcomitrella* phytochromes. These gene-targeting cassettes were flanked by NotI sites, which were used to cut the cassettes for transformation of *Physcomitrella*.

pRT101neo (provided by R. Reski, University of Freiburg, Germany and W. Frank, University of Munich, Germany), which confers resistance to G418 in *Physcomitrella*, was used for co-transformation with the YFP constructs described above.

The gene-targeting cassette used to generate the transgenic Pp-*phy1* knock out *Physcomitrella* lines was obtained as follows. First, part of Pp-*FHY1* gene sequence

of about 1000 bp (500 bp upstream and 500 bp downstream of the PstI restriction site) was PCR-amplified from genomic *Physcomitrella* DNA using the primers ah434/ah435. This fragment was cut with SpeI/KpnI and ligated into the XbaI/KpnI site of pBS II KS:YFP to replace YFP. A hygromycin resistance cassette, ProNOS:HPT:TerNOS (hygromycin phosphotransferase), was PCR amplified from pGAP:Hyg (GenBank Acc. EU933993; provided by R. Reski, University of Freiburg, Germany and W. Frank, University of Munich, Germany) using the primers ah483/ah484. The fragment was cut with BamHI/SpeI and ligated in pBS II KS cut with BamHI/SpeI. The HPT-cassette was cut from the resulting plasmid with SbfI and ligated into the PstI site of pBS II KS containing the 1000 bp fragment of Pp-FHY1. The gene-targeting cassette was flanked by NotI sites, which were used to cut the cassettes for transformation of *Physcomitrella*.

D153ah:At-PHYA and D153ah:At-PHYA 1-406, which have been described previously (Hiltbrunner et al., 2006), are yeast two-hybrid vectors coding for At-PHYA:GAL4 BD and At-PHYA 1-406:GAL4 BD, respectively. D153ah containing fragments of the *Physcomitrella* phytochromes corresponding to At-PHYA 1-406 were obtained as follows. These fragments were PCR amplified from genomic *Physcomitrella* DNA using the primers ah441/ah442 for Pp-PHY1 399, ah443/ah444 for Pp-PHY2 402, ah442/ah445 for Pp-PHY3 398 and ah447/ah448 for Pp-PHY4 402. The fragments for Pp-PHY1 399 and Pp-PHY3 398 were cut with BamHI/SpeI, and those for Pp-PHY2 402 and Pp-PHY4 402 with BglII/SpeI. Finally, the fragments were ligated into D153ah:At-PHYA digested with BamHI/SpeI to replace At-PHYA.

D153ah:At-FHY1 is a yeast two hybrid vector, which contains At-FHY1:GAL BD. To generate it we cut At-FHY1 from pGADT7:At-FHY1 (Hiltbrunner et al., 2005) using BamHI/SpeI and ligated it into D153ah:At-PHYA digested with BamHI/SpeI to replace At-PHYA. D153ah:Ac-PHY1, D153ah:Ac-PHY2 and D153ah:Ac-PHY3 are yeast two-hybrid vectors containing Ac-PHY1:GAL4 BD, Ac-PHY2:GAL4 BD and Ac-PHY3:GAL4 BD (= *Adiantum capillus-veneris* PHY). Ac-PHY1, Ac-PHY2 and Ac-PHY3 were PCR-amplified using the primers ah471/ah472 for Ac-PHY1, ah474/ah475 for Ac-PHY2 and ah477/ah478 for Ac-PHY3. As templates for the PCR, we used vectors containing the cDNA fragments for Ac-PHY1, Ac-PHY2 and Ac-PHY3, respectively, which were provided by M. Wada (Kyushu University, Fukuoka,

Japan). The Ac-PHY1 fragment was digested with BamHI/SpeI and the Ac-PHY2 and Ac-PHY3 fragments with SpeI and ligated into D153ah:At-FHY1 cut with either BamHI/SpeI or XbaI/SpeI to replace At-FHY1.

pPPO30:Ac-PHY1 is a T-DNA vector containing a Pro35S:Ac-PHY1:YFP:TerRbcS cassette and a mutated version of *PPO* as a marker for selection using Butafenacil (Rausenberger et al., 2011). To generate it, Ac-PHY1 was cut from D153ah:Ac-PHY1 using BamHI/SpeI and ligated into the BamHI/XbaI site of pPPO30 (Rausenberger et al, 2011).

pUC1930:Ac-PHY2 and pUC1930:Ac-PHY3 are vectors containing Pro35S:Ac-PHY2:YFP:TerRbcS and Pro35S:Ac-PHY3:YFP:TerRbcS, respectively. Ac-PHY2 and Ac-PHY3 were cut from D153ah:Ac-PHY2 and D153ah:Ac-PHY3 with XbaI/SpeI and ligated in sense orientation into pUC1930 cut with XbaI.

D153ah:Cp-PHY2 is a yeast two-hybrid vector containing Cp-PHY2:GAL4 BD (= *Ceratodon pupureus* PHY2), which we generated as follows. First, a C-terminal fragment of Cp-PHY2 was PCR amplified from plasmid p781\_a2 (provided by T. Lamparter, Karlsruhe Institute of Technology, Karlsruhe, Germany) using the primers ah312/ah456. This PCR fragment was cut with BglII/SpeI and ligated into the BglII/SpeI site of pBS II KS:At-PHYA (Hiltbrunner et al., 2005) to replace part of At-PHYA. To obtain the full-length cDNA of Cp-PHY2, the N-terminal fragment was amplified by PCR from plasmid p781\_a2 using the primers ah455/ah457. This PCR fragment was cut with BamHI and ligated in sense orientation into the BamHI/BglII site of the pBS II KS vector already containing the C-terminal part of Cp-PHY2. Finally, full-length Cp-PHY2 was cut from pBS II KS:Cp-PHY2 using BamHI/SpeI and this fragment was ligated into the BamHI/SpeI site of D153ah:At-PHYA to replace At-PHYA.

pPPO30:Cp-PHY2 is a T-DNA vector containing a Pro35S:Cp-PHY2:YFP:TerRbcS cassette and was obtained as follows. Cp-PHY2 was cut from pBS II KS:Cp-PHY2 using BamHI/SpeI and ligated into the BamHI/XbaI site of pPPO30 (Rausenberger et al, 2011).

pGADT7ah:Pp-FHY1 is a yeast two hybrid vector coding for GAL4 AD:Pp-FHY1 (= *Physcomitrella patens* FHY1) and pCHF70:Pp-FHY1 is a T-DNA vector containing a Pro35S:YFP:Pp-FHY1:TerRbcS cassette and a Basta resistance gene as selection

marker. Pp-FHY1 was PCR amplified from *Physcomitrella* total cDNA using the primers ah357/ah404. This PCR fragment was cut with BglII/SpeI and ligated into the BamHI/SpeI site of pGADT7:At-FHY1 to replace At-FHY1 as well as into the BamHI/XbaI site of pCHF70 (Hiltbrunner et al., 2005).

pPPO70 contains a mutant version of *PPO*, which confers resistance to Butafenacil (Hanin et al., 2001; Li et al., 2003). A PvuII-PstI fragment containing *PPO* was cut from pWCO35 (Hanin et al., 2001) (provided by J. Paszkowski, University of Geneva, Switzerland) and ligated into pCHF70 cut with PmlI/SbfI to replace the Basta resistance gene as selectable marker.

pGADT7ah:Cr-FHY1 is a yeast two-hybrid vector, which codes for GAL4 AD:Cr-FHY1 (= *Ceratopteris richardii* FHY1) and pPPO70:Cr-FHY1 is a T-DNA vector containing a Pro35S:YFP:Cr-FHY1:TerRbcS cassette. Cr-FHY1 was PCR amplified from cDNA clone Cri2\_1\_J13\_SP6 (GenBank Acc. BE640872; provided by S. J. Roux, University of Texas, Austin, TX, USA) using the primers ah349/ah350, cut with BamHI/SpeI and ligated into the BamHI/SpeI site of pGADT7:At-FHY1 to replace At-FHY1 as well as into pPPO70 cut with BamHI/XbaI.

pGADT7ah:Os-FHY1 is a yeast two-hybrid vector, which contains GAL4 AD:Os-FHY1 (= *Oryza sativa* FHY1) and was obtained as follows. Os-FHY1 was PCR amplified from cDNA clone J023050K07 (GenBank Acc. AK070454; provided by the Rice Genome Resource Center, Tsukuba, Japan) using the primers ah309/ah310, cut with BamHI/XbaI and ligated into the BamHI/SpeI site of pGADT7:At-FHY1 to replace At-FHY1.

pGADT7ah:To-FHY1 is a yeast two-hybrid vector containing GAL4 AD-To-FHY1 (= *Taraxacum officinale* FHY1) and was generated as follows. Total RNA was extracted from a dandelion (*Taraxacum officinale*) leaf collected in front of the Institute of Biology II, University of Freiburg, Germany, using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). This RNA was reverse transcribed using the ProtoScript First Strand cDNA Synthesis Kit (New England Biolabs, Beverly, MA, USA). To-FHY1 was PCR amplified from the resulting total cDNA using the primers ah351/ah352, cut with BamHI/SpeI and ligated in sense orientation into the BamHI/SpeI site of pGADT7:At-FHY1 to replace At-FHY1.

pGADT7ah:Cl-FHY1 CT is a yeast two-hybrid vector coding for GAL4 AD:Cl-FHY1

CT (= *Closterium* sp. FHY1 CT), which was obtained as follows. CI-FHY1 CT was PCR amplified from cDNA clone CL20\_G09 (GenBank Acc. BW647715; provided by H. Sekimoto, Japan Women's University, Tokyo, Japan) using the primers ah383/ah384, cut with XbaI/SpeI and ligated in sense orientation into the XbaI/SpeI site of pGADT7:At-FHY1 to replace At-FHY1.

pGADT7ah:Pg-FHY1 is a yeast two-hybrid vector coding for GAL4 AD:Pg-FHY1 (= *Picea glauca* FHY1), which was obtained as follows. Pg-FHY1 was PCR amplified from cDNA clone GQ03235\_G10 (GenBank Acc. BT111284; provided by the Center for Forest Research, Université Laval, Canada) using the primers ah527/ah528, cut with XbaI/SpeI and ligated in sense orientation into the XbaI/SpeI site of pGADT7:At-FHY1 to replace At-FHY1.

pGADT7:At-FHY1 is a yeast two-hybrid vector containing GAL4 AD-At-FHY1 and has been described previously (Hiltbrunner et al., 2006).

pUC1940:At-PHYA, which was used for transient transformation of mustard seedlings, contains a Pro35S:At-PHYA:CFP:TerRbcS cassette and has been described (Hiltbrunner et al., 2005).

pUC1930 contains a Pro35S:BamHI:XbaI:YFP:TerRbcS cassette and has been obtained as follows. The DNA fragment containing the Pro35S:BamHI:XbaI:YFP:TerRbcS cassette was cut from pCHF30 (Hiltbrunner et al., 2006) with EcoRI/HindIII and ligated into the EcoRI/HindIII site of pUC19 (Fermentas, St. Leon-Rot, Germany).

pUC1930:At-PHYA contains a Pro35S:At-PHYA:YFP:TerRbcS cassette and was used for transient transformation assays. At-PHYA was cut from pBS II KS:At-PHYA with BamHI/SpeI and ligated into the BamHI/XbaI site of pUC1930.

pCHF70:At-FHY1 is a T-DNA vector, which contains a Pro35:YFP:At-FHY1:TerRbcS cassette (Hiltbrunner et al., 2005) and was used for transient expression in mustard seedlings.

pCHF150myc is a T-DNA vector containing a Pro35S:myc:mCherry:BamHI:AvrII:XbaI:TerRbcS cassette and *bar* as selection marker and was obtained as follows. First, mCherry was PCR amplified from pBinAR-DCP2:mCherry (provided by A. Wachter, University of Tübingen, Germany) using the primers ah094/ah791. To add the myc tag to mCherry, this fragment was

purified and used as template for a second PCR with the primer pair ah094/ah792. This PCR fragment was digested with BglII/SpeI and ligated into the BamHI/XbaI site of pCHF5 (Hiltbrunner et al., 2005).

pUC1942 is a vector containing a Pro35S:CFP:NLS:TerRbcS cassette and was obtained as follows. CFP:NLS was PCR amplified from pUC1940:At-PHYA (Hiltbrunner et al., 2005) using the primers ah042 and ah-nls, which included the NLS described by Matsushita et al. (2003). The PCR fragment was digested with BamHI/SpeI and ligated into pCHF5 (Hiltbrunner et al., 2005) cut with BamHI/XbaI to obtain pCHF42. The EcoRI/HindIII fragment of pCHF42 containing the Pro35S:CFP:NLS:TerRbcS cassette was then ligated into the EcoRI/HindIII site of pUC19 (Fermentas, St. Leon-Rot, Germany), resulting in pUC1942.



### Supplemental References

- Hanin, M., Volrath, S., Bogucki, A., Briker, M., Ward, E., and Paszkowski, J.** (2001). Gene targeting in Arabidopsis. *Plant J.* **28**, 671-677.
- Hiltbrunner, A., Tscheuschler, A., Viczian, A., Kunkel, T., Kircher, S., and Schäfer, E.** (2006). FHY1 and FHL act together to mediate nuclear accumulation of the phytochrome A photoreceptor. *Plant Cell Physiol.* **47**, 1023-1034.
- Hiltbrunner, A., Viczian, A., Bury, E., Tscheuschler, A., Kircher, S., Toth, R., Honsberger, A., Nagy, F., Fankhauser, C., and Schäfer, E.** (2005). Nuclear accumulation of the phytochrome A photoreceptor requires FHY1. *Curr. Biol.* **15**, 2125-2130.
- Li, X., Volrath, S.L., Nicholl, D.B., Chilcott, C.E., Johnson, M.A., Ward, E.R., and Law, M.D.** (2003). Development of protoporphyrinogen oxidase as an efficient selection marker for *Agrobacterium tumefaciens*-mediated transformation of maize. *Plant Physiol.* **133**, 736-747.
- Matsushita, T., Mochizuki, N., and Nagatani, A.** (2003). Dimers of the N-terminal domain of phytochrome B are functional in the nucleus. *Nature* **424**, 571-574.
- Rausenberger, J., Tscheuschler, A., Nordmeier, W., Wüst, F., Timmer, J., Schäfer, E., Fleck, C., and Hiltbrunner, A.** (2011). Photoconversion and nuclear trafficking cycles determine phytochrome A's response profile to far-red light. *Cell* **146**, 813-825.