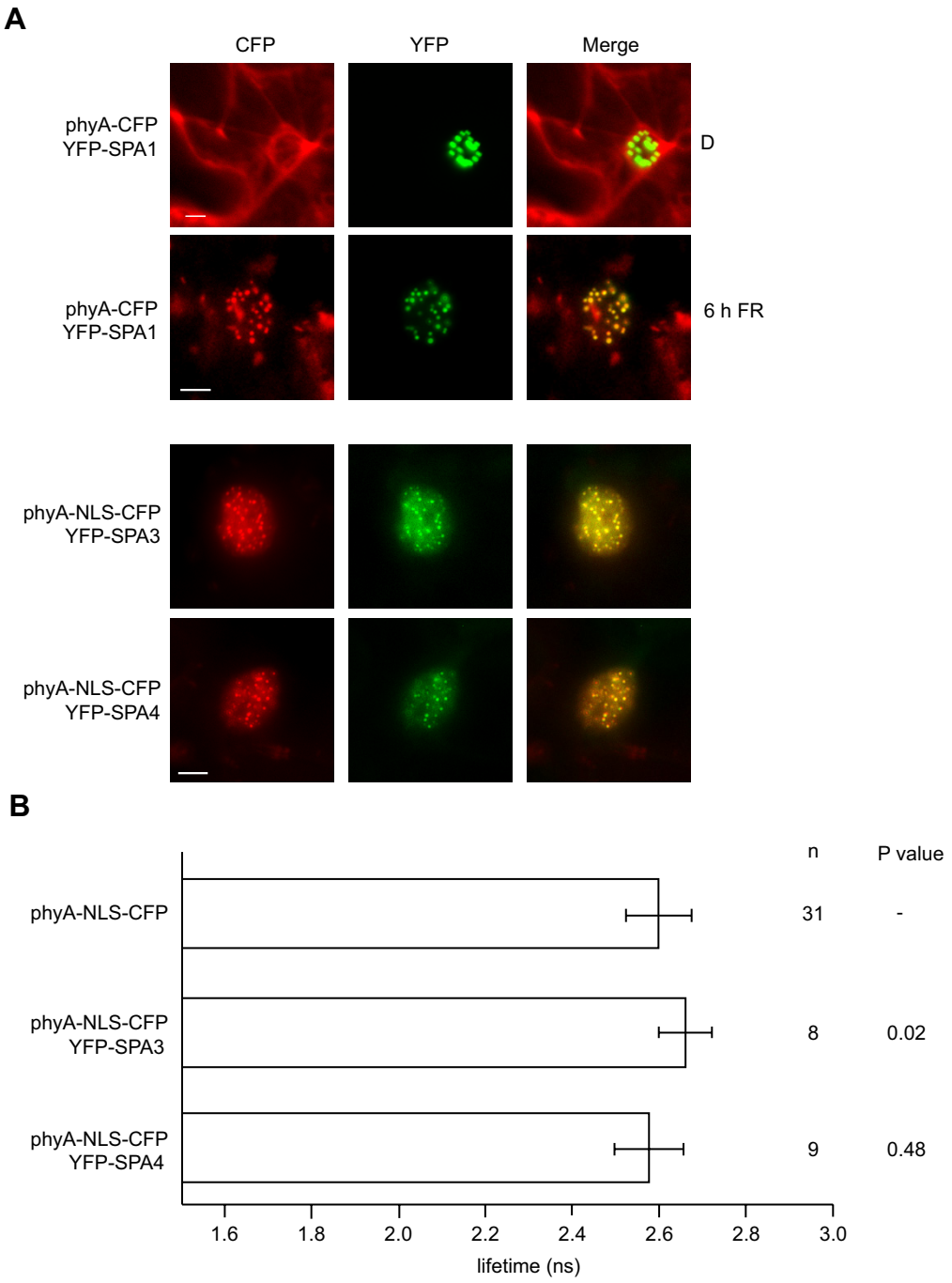
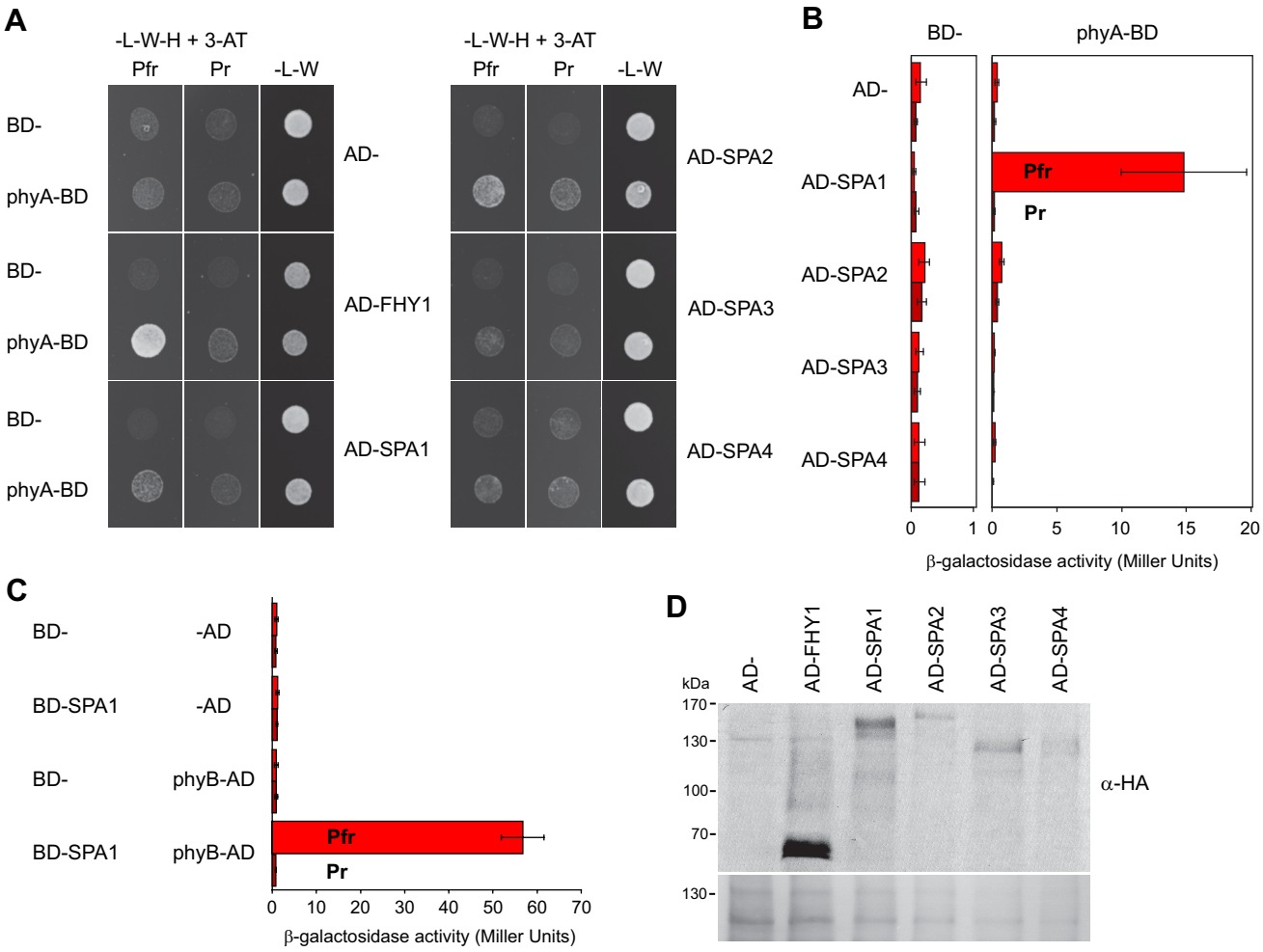
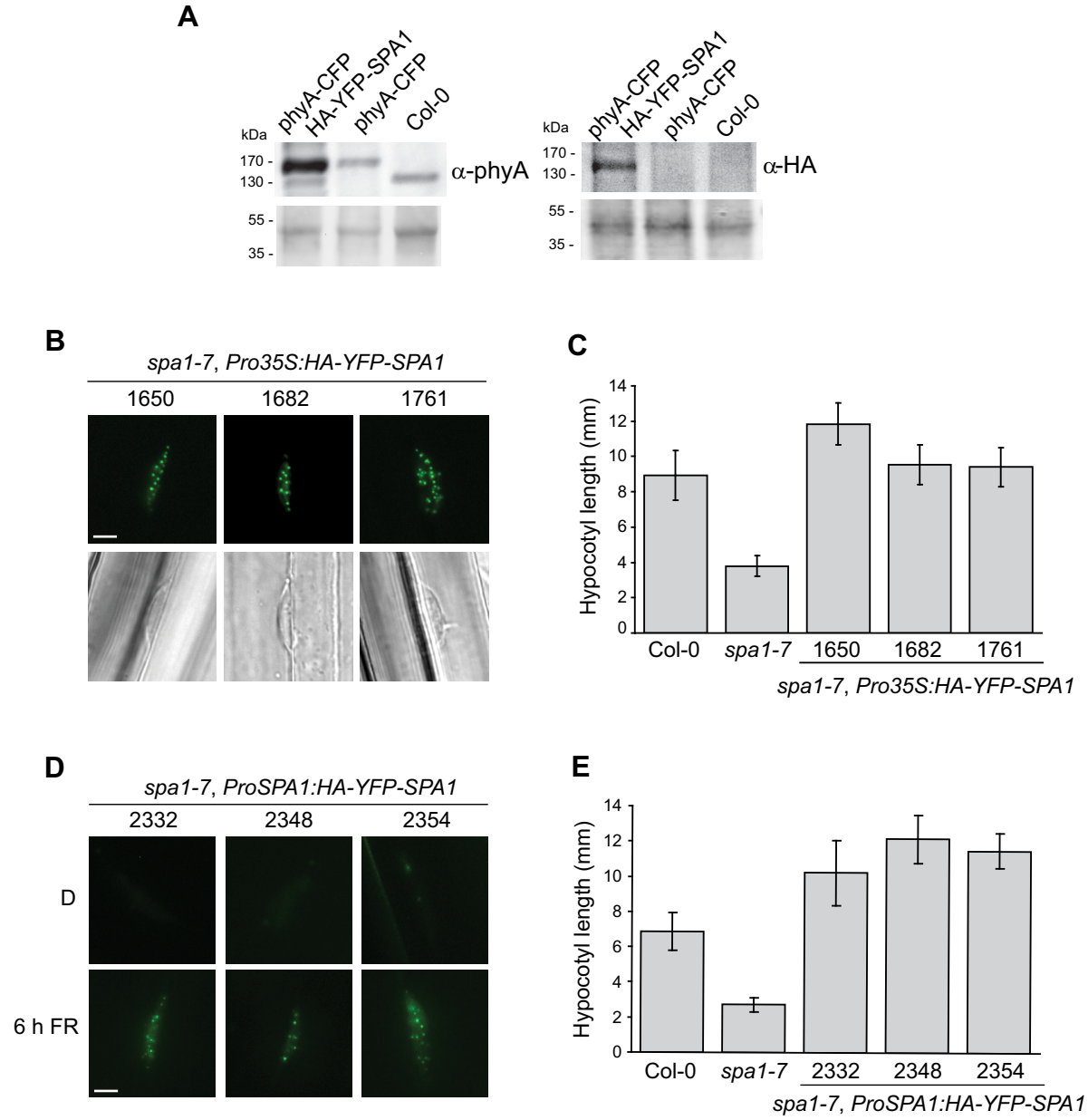


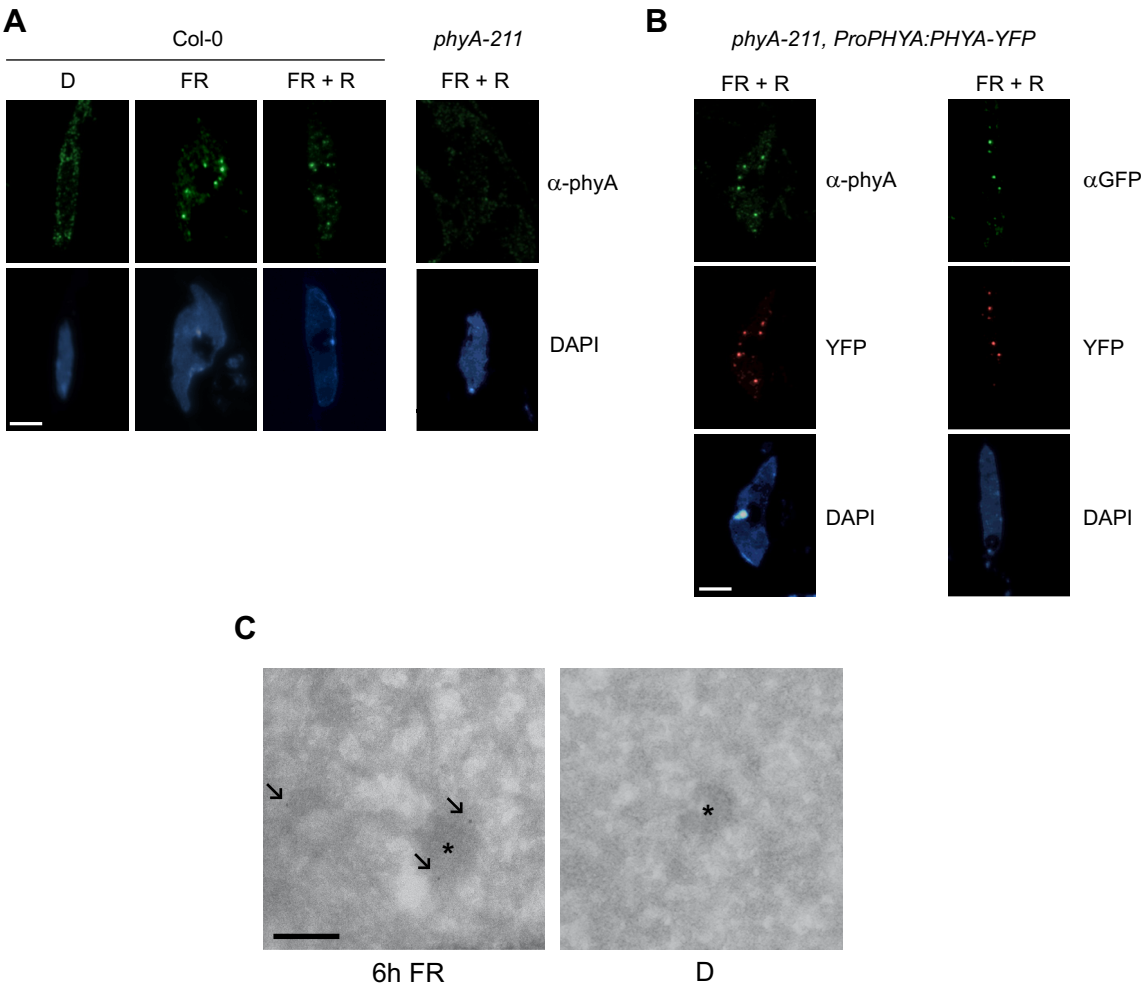
Supplemental Figure 1. PhyA co-localizes with SPA3 and SPA4 in *Nicotiana benthamiana* nuclear bodies. **(A)** Epi-fluorescence microscopy detection of phyA-CFP or phyA-NLS-CFP transiently co-expressed with YFP-SPA1, -SPA3, or -SPA4 in *Nicotiana benthamiana* leaf epidermal cells. Plants expressing phyA-CFP were exposed to FR light (18 $\mu molm^{-2}s^{-1}$) for 6 h prior to imaging, whereas those transformed with phyA-NLS-CFP were kept under constant darkness (D) prior to microscopy. The scale bars indicate 10 µm. **(B)** FRET-FLIM analysis of nuclear body localized phyA and SPA3/SPA4 CFP and YFP fusions transiently expressed in *Nicotiana benthamiana*. The fluorescence lifetime of the donor (CFP) is shown. n = number of measurements. Error bars show one standard deviation. P values indicate t-test analysis for statistically significant differences. Fusion proteins used in A and B were expressed under the control of the 35S promoter.



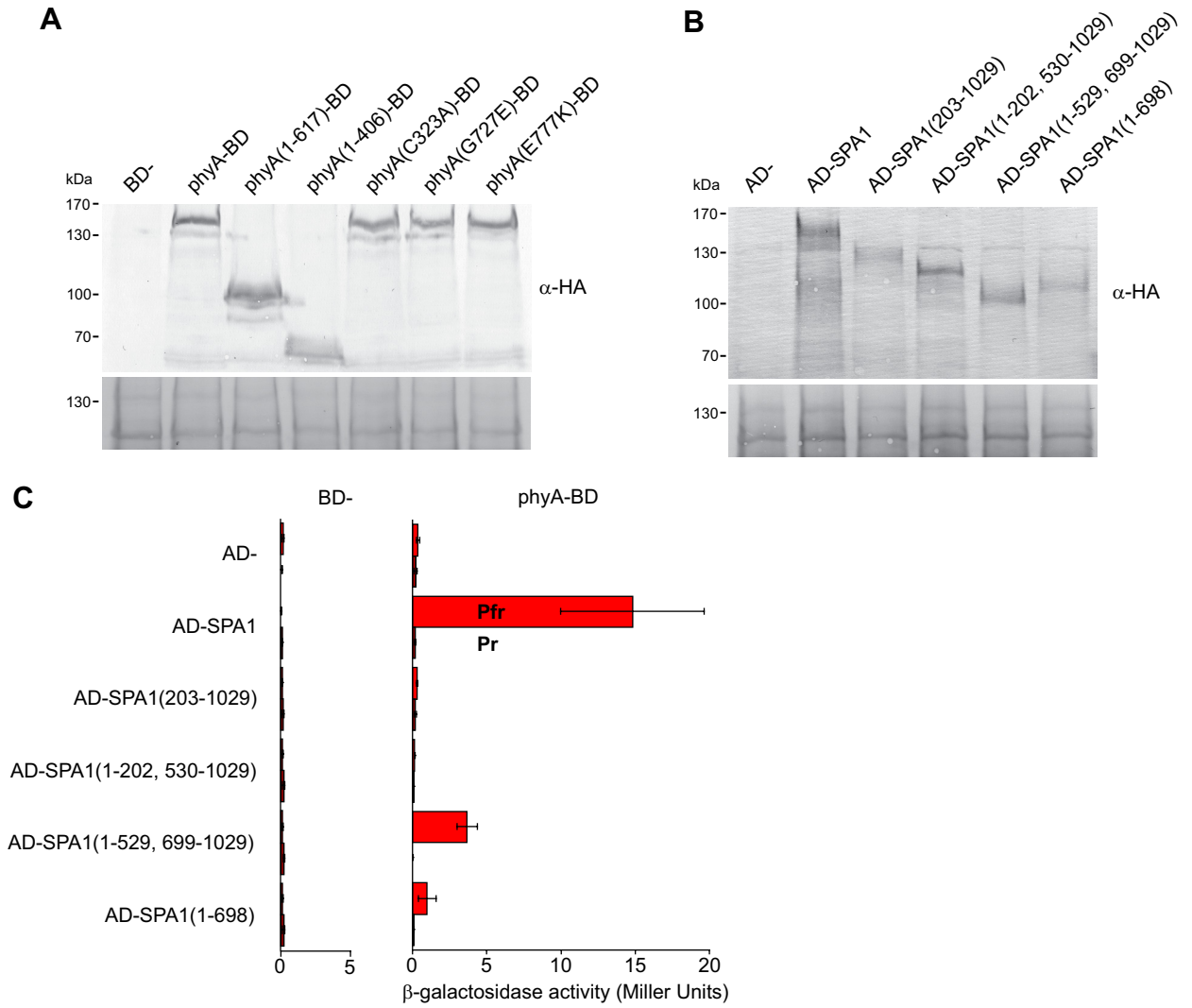
Supplemental Figure 2. Light-activated phyA interacts with SPA1 and SPA2 in yeast. **(A)** Yeast two-hybrid protein-protein interaction assay. The phyA-GAL4-DNA binding domain (phyA-BD) fusion was co-expressed with GAL4-activation domain (AD-) fusions of FHY1 and SPA1-4. Yeast were grown on selective media lacking histidine, supplemented with the histidine biosynthesis inhibitor 3-amino triazole (3-AT) and phycocyanobilin (PCB), under constant R (Pfr) or FR (Pr) light to assay activation of the HIS-reporter gene. **(B)** Yeast two-hybrid protein-protein interaction of phyA-BD and AD-SPA1-4. Yeast were grown on chromophore-supplemented plates for 48 h under either constant R (bright-red, Pfr) or FR (dark-red, Pr) light. Interaction was detected by a liquid o-nitrophenyl-β-galactoside (ONPG) assay. Values are the average of nine assays; error bars display one standard deviation. **(C)** as for (B) using BD-SPA1 and phyB-AD. **(D)** Immunoblot detection of yeast expressed FHY1 and SPA1-4. Yeast were harvested from chromophore supplemented plates that had been incubated for 48 h under constant R light. FHY1 and SPA1-4 AD-fusions contain a HA tag, and an α-HA antibody was used to detect these AD fusions in yeast protein extracts. The lower pane shows the membrane stained with amido-black as a loading control.



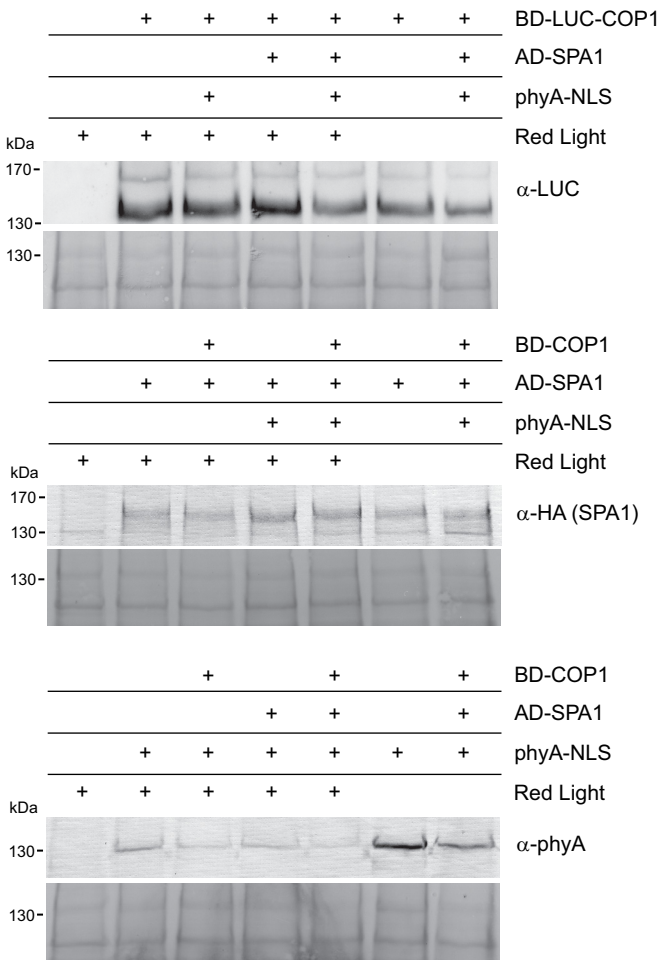
Supplemental Figure 3. *Pro35S:HA-YFP-SPA1* rescues *spa1-7*. **(A)** Immunoblot detection of phyA-CFP and HA-YFP-SPA1 proteins expressed in stable transformed Arabidopsis. The lower pane shows the membrane stained with amido-black as a loading control. **(B)** Localization of HA-YFP-SPA1 in dark-grown *spa1-7 Pro35S:HA-YFP-SPA1*. **(C)** Hypocotyl measurements of 4-day-old Arabidopsis *spa1-7 Pro35S:HA-YFP-SPA1* seedlings grown in weak FR (1 $\mu molm^{-2}s^{-1}$). **(D)** Localization of HA-YFP-SPA1 expressed from the native *SPA1* promoter in *spa1-7 ProSPA1:HA-YFP-SPA1* seedlings grown in darkness, or darkness followed by 6 h FR (10 $\mu molm^{-2}s^{-1}$). **(E)** Hypocotyl measurements of 4-day-old Arabidopsis *spa1-7 ProSPA1:HA-YFP-SPA1* seedlings grown in weak FR (1 $\mu molm^{-2}s^{-1}$). White scale bars indicate 4 μm. Error bars display one standard deviation for measurements from 25 or more seedlings. Data for three independent transgenic lines are shown in (B-E).



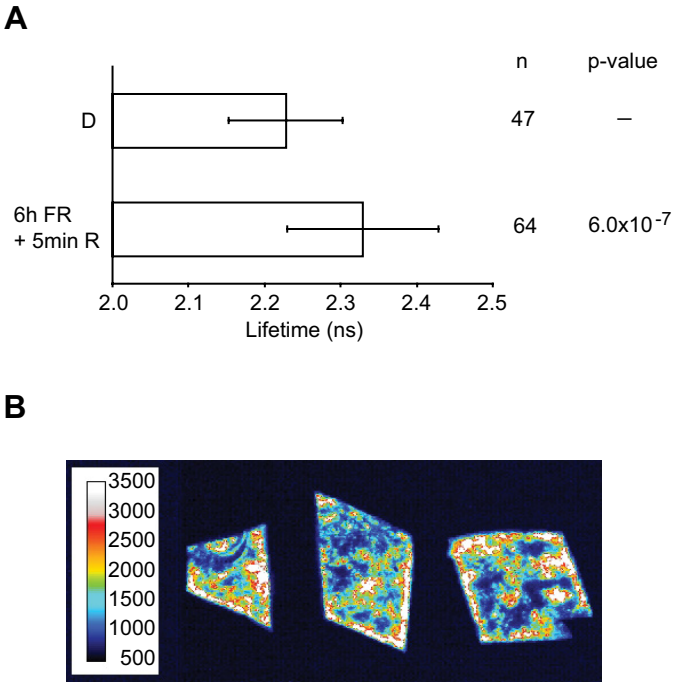
Supplemental Figure 4. Immuno-histochemistry of phyA and phyA-YFP nuclear bodies. **(A)** Detection of endogenous phyA in hypocotyl cell nuclei. Seedlings were grown in darkness for 4 d, followed by either no light (D), 6 h FR light (18 $\mu molm^{-2}s^{-1}$) (FR), or 6 h FR followed by 10 min R light (22 $\mu molm^{-2}s^{-1}$) (FR + R) and subsequently fixed with formaldehyde. phyA was detected using α-phyA and a Cy3-coupled secondary antibody. **(B)** Detection of phyA-YFP. As for (A), except YFP was additionally detected with α-GFP and the YFP fluorescence visualized directly. DAPI staining of DNA used to show nuclei. (A) and (B) The scale bar indicates 4 μm. **(C)** Immuno-electron microscopy-localization of phyA in wild-type Arabidopsis Col-0 hypocotyl nuclei. Seedlings were grown in darkness for 4 d and treated with either 6 h FR light followed by 5 min R light, or constant darkness prior to fixation. Endogenous phyA was probed with α-phyA antibodies, and detected with protein A-labelled 6-nm gold particles (indicated by arrows). Nuclear bodies are indicated with *. Black scale bar indicates 200 nm.



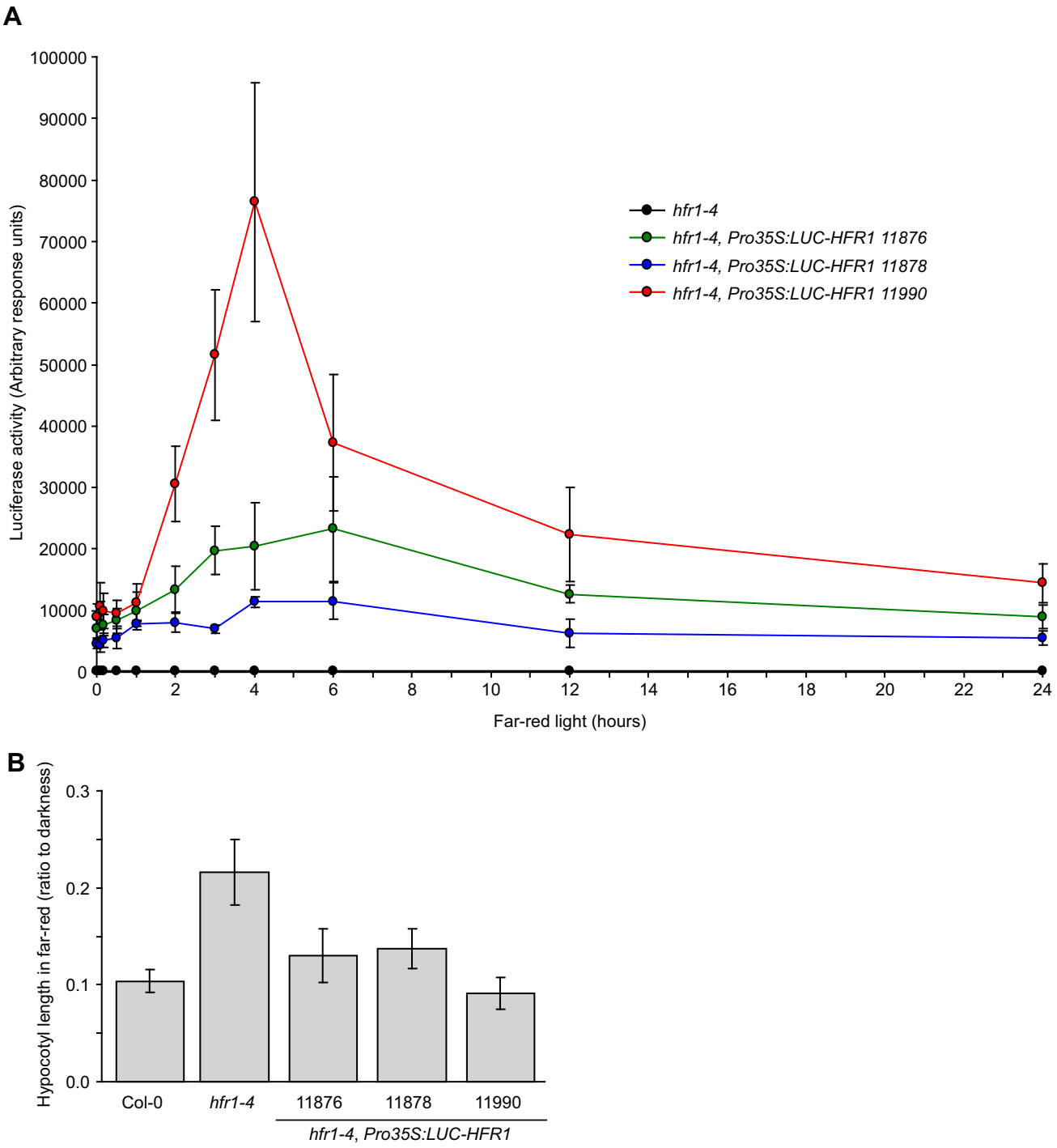
Supplemental Figure 5. Truncations and mutants of phyA and SPA1 are stable in yeast. **(A, B)** Yeast cells were harvested from chromophore-supplemented plates that had been incubated for 48 h under constant R light. All AD-SPA1 and phyA-BD fusions contain a HA tag, and an α-HA antibody was used to detect the SPA1 and phyA fusions in yeast protein extracts. The lower panels show the membrane stained with amido-black as a loading control. **(A)** Immunoblot detection of yeast-expressed phyA truncations and single amino acid substitution mutants. **(B)** Immunoblot detection of yeast-expressed SPA1-truncated proteins. **(C)** Yeast two-hybrid protein-protein interaction of phyA-BD and AD-SPA1 truncations. Yeast were grown under either constant R (bright-red, Pfr) or FR (dark-red, Pr) light. Interaction was detected by a liquid o-nitrophenyl-β-galactoside (ONPG) assay. Values are the average of nine assays; error bars display one standard deviation.



Supplemental Figure 6. Immunoblot detection of yeast three-hybrid proteins from Figure 4. Yeast cells were harvested from chromophore supplemented plates that had been incubated for 72 h under either constant R light or darkness. The AD-SPA1 fusion contains an HA; α-HA, α-LUC, and α-phyA antibodies were used to detect AD-SPA1, BD-LUC-COP1, and phyA-NLS in yeast protein extracts. Yeast co-expressed combinations of either BD-/BD-COP1/BD-LUC-COP1 with AD-/AD-SPA1 and -/phyA-NLS. Lower panels show amido-black stained membranes as a loading control. The upper band detected by α-LUC is the expected molecular weight for the full-length COP1-fusion protein (155 kDa).



Supplemental Figure 7 - Reorganization of the COP1/SPA1 complex by phyA upon irradiation with light. **(A)** FRET-FLIM analysis of the disruption of the interaction between COP1 and SPA1. CFP-SPA1, YFP-COP1 and phyA-LUC were co-transformed into *Nicotiana bethamiana* and plants were grown in darkness (D) or darkness followed by 6 h FR and 5 min R pulse to activate phyA nuclear transport and NB formation. Prior to microscopy, leaves were fixed to prevent effects of irradiance with fluorescent light during FRET measurement on the COP1/SPA1 complex. The fluorescence lifetime of the donor (CFP) is shown. Error bars show one standard deviation. n = number of measurements. P values indicate t-test analysis for statistically significant differences. **(B)** Detection of phyA-LUC in co-transformed *Nicotiana bethamiana*. To confirm expression of phyA-LUC, leaves were sprayed with 1 mM D-luciferin and detected by 5 min exposure using a CCD camera. Color scale indicates relative light emittance. Expression of constructs in A and B was driven by the 35S promoter.



Supplemental Figure 8. HFR1 accumulates in FR light. **(A)** Quantification of LUC-HFR1 abundance in three independent transgenic Arabidopsis *hfr1-4 Pro35S:LUC-HFR1* lines. Seedlings were grown in darkness after 6 h germination induction with white light, and transferred to FR light (10 $\mu molm^{-2}s^{-1}$) at various time points prior to harvesting at 4 d (96 h) post germination induction. Error bars show one standard deviation of nine measurements from three biological replicates. **(B)** Hypocotyl lengths of 4-day-old Arabidopsis *hfr1-4 Pro35S:LUC-HFR1* seedlings grown in continuous FR light (10 $\mu molm^{-2}s^{-1}$) following 16 h darkness, as a ratio to hypocotyl length in darkness. Error bars display one standard deviation of 30 or more seedlings.

SUPPLEMENTAL METHODS

Yeast interaction assays

For histidine-reporter assays, yeast harvested from overnight cultures were washed and suspended in sterile ddH_2O to an OD_{600} of 0.1. 3 µL spots were plated onto synthetic media lacking leucine, tryptophan and histidine, supplemented with 20 µM phycocyanobilin (PCB) and 20 mM 3-amino triazole, or control plates lacking leucine and tryptophan. Plates were incubated for four days at 26 °C in either constant R light (670 nm, 1.7 $\mu molm^{-2}s^{-1}$), or FR light (720 nm, 13 $\mu molm^{-2}s^{-1}$). Control plates were incubated for 48 h in normal light conditions. For o-nitrophenyl-β-galactoside (ONPG) assays yeast were cultured on plates lacking leucine and tryptophan, supplemented with 20 µM phycocyanobilin. Plates were incubated under constant R or FR light as above for 48 h at 26 °C. ONPG assays were otherwise performed as described in the Clontech yeast two-hybrid manual.

Immunoblotting

Total protein was extracted from yeast as previously described (Printen and Sprague, 1994), except yeast were harvested from plates supplemented with 20 µM phycocyanobilin, grown for 48 h at 26 °C in either darkness or constant R light (670 nm, 1.7 $\mu molm^{-2}s^{-1}$). Total protein was extracted from four day old Arabidopsis seedlings as previously described (Kircher et al., 2002). Protein transferred to membranes was detected by amido-black staining. Blocked membranes were incubated with primary antibodies for 16 h at 4 °C. Primary antibodies were diluted in 50 mM Tris:HCl pH 7.5, 150 mM NaCl, 0.005 % (v/v) Tween-20, 5 % (w/v) milk powder, with the following exceptions: 500 mM NaCl and 0.05 % Tween-20 were used for blots of Arabidopsis extracts, and 2 % (w/v) ECL advance blocking agent used in place of milk powder for detection of BD-LUC-COP1. Antibody dilutions were as follows: α-HA (Covance or Roche) 1:1000, α-phyA (Agrisera, for yeast) 1:2000, α-LUC (Sigma) 1:2000, and α-phyA ((Hiltbrunner et al., 2006), for plants) 1:3000. Secondary antibodies were used at either 1:50000 (alkaline-phosphatase conjugate, Sigma), 1:50000 (Horseradish-peroxidase conjugate, GE Healthcare) for yeast, or 1:1500 (Horseradish-peroxidase conjugate, Santa Cruz Biotechnology) for detection of Arabidopsis proteins. Immunoblots were developed using either Nitrotetrazolium Blue and Bromo-chloro-indolyl phosphate, ECL advance

reagents (detection of BD-LUC-COP1, GE Healthcare), or ECL reagents (Pierce).

Immuno-histochemistry

Sections were prepared as for immunogold-labeling, except 400-nm sections were cut at - 80 °C and transferred to coverslips. Sections were probed with either rabbit α-phyA (1:300, Agrisera) or rabbit α-GFP (1:500, Abcam) and washed 6 times. Bound antibodies were detected with goat α-rabbit IgG-Cy3 conjugate (1:400, Dianova). Following washes with phosphate buffered saline, sections were stained with DAPI (1 µg/mL, 4',6-Diamidino-2-phenylindole dihydrochloride, Sigma) for 10 min to detect DNA, embedded in Mowiol (Sigma), and visualized with an epi-fluorescent microscope (Zeiss). The primary antibodies were omitted in control experiments, resulting in negligible background.

Cloning of constructs

The plant expression vectors pCHF40-PHYA (encoding *Pro35S:PHYA-CFP-TerRbcS*) and pPHYA40-PHYA (encoding *ProPHYA:PHYA-CFP-TerRbcS*) were previously described (Genoud et al., 2008).

pCHF40-PHYA-NLS (encoding *Pro35:PHYA-NLS-CFP-TerRbcS*) was created by amplifying the C-terminal region of PHYA-NLS present in pPHYA-PHYA-NLS-GFP5 (Genoud et al., 2008) by PCR using 5'-TTA CAC CAT CCG GAG GTC AG-3' and 5'-GGA CTA GTT GCG GCC GCT CCT CCA ACC T-3', cut with XbaI/SpeI, and used to replace the XbaI/SpeI fragment of PHYA in the intermediary vector pBS II KS-PHYA (Hiltbrunner et al., 2005). PHYA-NLS was subsequently cut with BamHI/SpeI and cloned into BamHI/XbaI cut pCHF40 (Hiltbrunner et al., 2005).

To generate an eYFP tagged form of PHYA-NLS, the fragment coding for PHYA-NLS was cut from pBS II KS-PHYA-NLS using BamHI/SpeI and cloned into the BamHI/XbaI sites of pPPO30 (Rausenberger et al., 2011).

The plant expression vector pPPO70v1HA (encoding *Pro35S:HA-YFP-BamHI-AvrII-XbaI-TerRbcS*) was generated by first cutting pCHF5 (Hiltbrunner et al., 2005) with PmeI/NcoI, and ligating in a StuI/NcoI fragment from pYES2 (Invitrogen) to generate pCHF5v1. pWCO35 (Rausenberger et al., 2011) was then cut with PvuII/PstI and this fragment ligated into PmlI/SbfI cut pCHF5v1 to generate pPPO5v1. Finally, eYFP was amplified by PCR from pPPO30 using 5'-GAA GAT CTA AAA ATG GCC TAC CCA TAC GAC GTA CCA GAT TAC GCT GCT AGC ATG GTG AGC AAG GGC GAG-3'/5'-GGA CTA

GTT ATC TAG AGC CCT AGG ATC CGC CTT GTA CAG CTC GTC CAT G-3', cut with BglII/SpeI and cloned into BamHI/XbaI cut pPPO5v1 to generate pPPO70v1HA.

SPA1 was amplified by PCR with 5'-GCT CTA GAA AAA TGC CTG TTA TGG AAA GAG-3' and 5'-GCT CTA GAA ACA AGT TTT AGT AGC TTC-3' from cDNA clone pda17902 (Riken), cloned into pBS II KS (pBS II KS-SPA1), cut with XbaI and cloned into the AvrII/XbaI sites of pPPO70v1HA to generate pPPO70v1HA-SPA1 (*Pro35S:HA-YFP-SPA1-TerRbcS*), or into the SpeI site of pCHF40 to generate pCHF40-SPA1 (*Pro35S:CFP-SPA1-TerRbcS*).

SPA2/3/4 were each amplified by PCR with 5'-ACG CGG ATC CAA AAA TGA TGG ATG AGG GAT CAG T-3'/5'-ACG CAC TAG TGA CCA ACT GTA GAA CTT TGA TT-3' (SPA2), 5'-ACG CGG ATC CAA AAA TGG AAG GTT CTT CAA ATT CTA ACT-3'/5'-ACG CAC TAG TAG TCA TCA TCT CCA GAA TTT TTA TG-3' (SPA3), and 5'-ACG CGG ATC CAA AAA TGA AGG GTT CTT CAG AAT CTA-3'/5'-ACG CAC TAG TTA CCA TCT CCA AAA TCT TGA TAT TG-3' (SPA4) from cDNA clones obtained from Ute Hoecker (University of Cologne, Germany), cut with BamHI/SpeI and cloned into the BamHI/XbaI sites of pPPO70v1HA.

The FRET positive control pCHF30-CFP (*Pro35S:CFP-YFP-TerRbcS*), was generated by ligation of BamHI/SpeI cut CFP from pCHF40 (Hiltbrunner et al., 2005) into BamHI/XbaI cut pCHF30 (Hiltbrunner et al., 2006).

pSPA1-HA-YFP-SPA1 is a T-DNA vector containing a *ProSPA1:HA-YFP-SPA1-TerRbcS* cassette and was obtained as follows. A 1672-bp SPA1 promoter fragment including the first 12 bp of the SPA1 coding sequence was PCR amplified from genomic Col-0 DNA using the primers 5'-CAT GCC ATG GGA TAC AAT TAT TGG GAG CTA TTA GTC-3' and 5'-CGG GAT CCT CCA TAA CAG GCA TCA ACA CTC-3'. This fragment was cut with NcoI/BamHI and ligated into the NcoI/BamHI site of pCHF5 (Hiltbrunner et al., 2005) resulting in pSPA1-1672. In parallel, HA-YFP was amplified by PCR from pPPO70v1HA-SPA1 using primers including BglII (5'-CAT GCC ATG GCA TGG AAG ATC TTA TGG CCT ACC CAT ACG ACG-3') and BamHI/AvrII/SpeI (5'-GAC TAG TTA CCT AGG TGC CGG ATC CGC CTT GTA CAG CTC GTC CAT GC-3') sites, respectively. The PCR fragment was then cut with BglII/SpeI and ligated into the BamHI/XbaI site of pSPA1-1672 to obtain pSPA1-1672-HA-YFP. Next, SPA1 was cut from pBS II KS-SPA1 using XbaI and ligated in sense orientation into the AvrII site of pSPA1-1672-YFP, resulting in pSPA1-1672-HA-YFP-SPA1. Finally, a 2260 bp SPA1 promoter fragment was PCR amplified from

genomic Col-0 DNA using 5'-CAT GCC ATG GTT TAA ACC TAG GGA GCA GAG AAA ATA ATA CAA CAT GTT GCT G-3' and 5'-CGG GAT CCT CCA TAA CAG GCA TCA ACA CTC-3'. This fragment was cut with PmeI/AatII and ligated into the PmeI/AatII site of pSPA1-1672-HA-YFP-SPA1 to obtain pSPA1-HA-YFP-SPA1.

pPPO70v1HA-COP1 is a T-DNA vector containing a *Pro35S:HA-YFP-COP1-TerRbcS* cassette. COP1 was PCR amplified from total Col-0 cDNA with the primers 5'-GAA GAT CTA AAA ATG GAA GAG ATT TCG ACG-3' and 5'-GGA CTA GTC GCA GCG AGT ACC AGA ACT TTG-3'. The PCR fragment was then cut with BglII/SpeI and ligated into the BamHI/XbaI site of pPPO70v1HA.

pCHF91-HFR1 is a T-DNA vector containing a *Pro35S:LUC+-HFR1-TerRbcS* cassette. Firefly luciferase (LUC+) was PCR amplified from *ProPIF3:LUC+* (Viczian et al., 2005) using the primers 5'-AAG ATC TAA AAA TGG AAG ACG CCA AAA ACA-3' and 5'-GGA CTA GTT ATC TAG AGC TTA CCT AGG ATC CGC CAC GGC GAT CTT TCC GCC C-3'. The PCR fragment was cut with BglII/SpeI and ligated into the BamHI/XbaI site of pCHF5 (Hiltbrunner et al., 2005), resulting in pCHF91 (*Pro35S:LUC+-BamHI-AvrII-XbaI-TerRbcS*). The primers 5'-CGC GGA TCC AAA AAT GTC GAA TAA TCA AGC TTT-3' and 5'-GGA CTA GTT AGT CTT CTC ATC GCA TGG G-3' where then used to amplify the HFR1 coding sequence from total Arabidopsis cDNA. The PCR fragment was cut with BamH/SpeI and ligated into the BamHI/SpeI site of pBluescript II KS (Stratagene), from which it was cut with BamHI/SpeI and ligated into the BamHI/XbaI site of pCHF91.

pCHF90-PHYA is a T-DNA vector containing a *Pro35S:PHYA-LUC+-TerRbcS* cassette. LUC+ was amplified by PCR from *ProPIF3:LUC+* using the primers 5'-CGC GGA TCC CGG CTC TAG AAT GGA AGA CGC CAA AAA CA-3' and 5'-GGA CTA GTT ACA CGG CGA TCT TTC CGC CC-3'. The PCR fragment was cut with BamHI/SpeI and ligated into the BamHI/XbaI site of pCHF5, resulting in pCHF90 (*Pro35S:BamHI-XbaI-LUC+-TerRbcS*). PHYA was then cut from pBS II KS-PHYA (Hiltbrunner et al., 2005) using BamHI/SpeI and ligated into the BamHI/XbaI site of pCHF90, resulting in pCHF90-PHYA.

pCHF40-PHYB is a T-DNA vector containing a *Pro35S:PHYB-eCFP-TerRbcS* cassette. The PHYB coding sequence was amplified from *Pro35S:PHYB-GFP* (Hiltbrunner et al., 2005) using the primers 5'-CCC AAG CTT CTA GAA AAA TGG TTT CCG GAG TCG GG-3' and 5'-GGG GTA CCT TAT CTA GAA TAT GGC ATC ATC AGC ATC A-3'. The PCR fragment was then digested with XbaI and ligated in sense orientation into the XbaI site of pCHF40 (Hiltbrunner et al., 2005).

pPPO30A-PHYA is a T-DNA vector containing a *ProPHYA:PHYA-eYFP-TerRbcS* cassette and has been described previously (Rausenberger et al., 2011).

All pCHF T-DNA vectors confer resistance to Basta; pPPO T-DNA vectors contain a mutated version of *PPO* as selection marker that results in resistance to Butafenacil/Inspire. Selection of transgenic plants using Basta and Inspire has been described (Rausenberger et al., 2011).

The yeast three-hybrid vector, pBridge (Clontech), was modified to replace multiple cloning sites and to remove the N-terminal fusion from the second cloning site. Synthetic oligonucleotides 5'-AAT TGG ATC CAG AAT TCA CTA GTT AAT GCA-3' and 5'-TTA ACT AGT GAA TTC TGG ATC C-3' were annealed and ligated into EcoRI/PstI cut pBridge. Subsequently pBridge was cut with XbaI/BglII and the fragment replaced with a fragment generated by PCR using 5'-ACG TCT CTA GAG CAC ATT CTG CG-3', 5'-ACG TCG GAT CCT TAC CTA GGC TGC AGA GAT CTT GTA TGG ATG GGG GTA ATA G-3', and pBridge as a template, that was cut with XbaI and BamHI. COP1 was amplified by PCR from Arabidopsis total cDNA with 5'-CGC GGA TCC AAA AAT GGA AGA GAT TTC GAC GGA CCC GGT TG-3' and 5'-GGA CTA GTC GCA GCG AGT ACC AGA ACT TTG-3', creating a silent mutation in the internal BamHI site, cut with BamHI/SpeI, and cloned into the BamHI/SpeI sites of modified pBridge. PHYA-NLS as described above, was cut from pBS II KS using BamHI/SpeI, and cloned into the BglII/AvrII sites of modified pBridge. For generation of vectors for PHYB-NLS, the second multiple cloning site of pBridge was alternately modified by cutting XbaI/BglII, and ligating in a new XbaI/BamHI cut fragment generated from pBridge by PCR using 5'-ACG TCT CTA GAG CAC ATT CTG CG-3' and 5'-ACG TCG GAT CCT TAC CTA GGC TGC AGA GAT CTT GTA TGG ATG GGG GTA ATA G-3'. Subsequently full length PHYB, cut with XbaI from pCHF40-PHYB was cloned into the NheI site.

pCGADT7ah-PHYB is a yeast two hybrid vector coding for *PHYB-GAL4 AD*. To obtain it, PHYB coding sequence was amplified from *Pro35S:PHYB-GFP* (Hiltbrunner et al., 2005) using oligos 5'-CCC AAG CTT CTA GAA AAA TGG TTT CCG GAG TCG GG-3'/5'-GGG GTA CCT TAT CTA GAA TAT GGC ATC ATC AGC ATC A-3', cut with XbaI and cloned into the XbaI site of pCGADT7ah (Rausenberger et al., 2011).

The PHYA-, PHYA 1-406-, PHYA 1-617-, and PHYA C323A-binding domain vectors (pD153AH) have been previously described (Hiltbrunner et al., 2006; Rausenberger et al., 2011).

PHYA G727E and PHYA E777K were created by overlap extension PCR using primer pairs 5'-AGA CAC TCT TGT GCG ATA TG-3'/5'-ACA AAA CAC ACC **T**CA ACC ACG TTT T-3' + 5'-AAA ACG TGG TTG **A**GG TGT GTT TTG T-3'/5'-GGC AAG TTG CAG GAA ACA GA-3' (G727E), or 5'-AGA CAC TCT TGT GCG ATA TG-3'/ 5'-TGG ATT CCA CT**T** TGT GCA CCA TC-3' + 5'-GAT GGT GCA CA**A** AGT GGA ATC CA-3'/5'-GGC AAG TTG CAG GAA ACA GA-3' (E777K), cut with AvrII/SpeI and cloned into AvrII/SpeI cut pD153AH-PHYA.

The yeast two-hybrid activation domain vector, pGADT7 (Clontech), was modified using annealed oligonucleotides 5'-TAT GGA TCC CGG GAC TAG TTA AA-3' and 5'-GAT CTT TAA CTA GTC CCG GGA TCC A-3' ligated into NdeI/BamHI cut plasmid to replace the multiple cloning site. FHY1 was amplified by PCR with 5'-CGC GGA TCC AAA AAT GCC TGA AGT GGA AGT G-3' and 5'-ACG TCA CTA GTT TAC AGC ATT AGC GTT GAG AAG T-3', cut with BamHI/SpeI, and cloned into the BamHI/SpeI sites of modified pGADT7. SPA1 was cut from pBS II KS-SPA1 using XbaI and ligated into the SpeI site of modified pGADT7. SPA2/3/4 PCR products were each cut using BamHI/SpeI and cloned into the BamHI/SpeI sites of modified pGADT7.

Deletion constructs of SPA1 were generated by PCR using 5'-ACG TCG CTA GCG GCA TGT TAC TTA AAA GAG CTA TGA AAG G-3' and 5'-ACG TCG CTA GCA ACA AGT TTT AGT AGC TTC ATG TT-3' for SPA1 203-1029, 5'-ACG TCG CTA GCG GCA TGC CTG TTA TGG AAA GAG TAG-3' and 5'-ACG TCG CTA GCA TAC CGA GCA AAT TTG CAC AAC-3' for SPA1 1-698, both cut with NheI and cloned into the SpeI site of modified pGADT7. SPA1 203-529 was generated by PCR using 5'-ACG TCG GAT CCA GGA ATG GTT ACT TAA AAG AGC TAT GAA AG-3'/5'-ACG TCA CTA GTT ATC AAC TCT GAC TTT AGT ATA TC-3' and cloned into the BamHI/SpeI sites of modified pGADT7. SPA1 1-202, 530-1029 and SPA1 1-529, 699-1029 were generated by overlap extension PCR using primer pairs 5'-ACG TCG CTA GCG GCA TGC CTG TTA TGG AAA GAG TAG-3'/5'-CAT CCT CGC ACA ACT GAG AAA AAT TCG AAG-3' + 5'-TTC TCA GTT GTG CGA GGA TGA TTC AGT T-3'/5'-ACG TCG CTA GCA ACA AGT TTT AGT AGC TTC ATG TT-3' and 5'-ACG TCG CTA GCG GCA TGC CTG TTA TGG AAA GAG TAG-3'/5'-GAA CTT GCT TAT CAA CTC TGA CTT TAG T-3' + 5'-AGA GTT GAT AAG CAA GTT CGA AAC CTG TG-3'/5'-ACG TCG CTA GCA ACA AGT TTT AGT AGC TTC ATG TT-3'. Flanking primers as above were used for the second round. Products were cut with NheI and cloned into the SpeI site of modified pGADT7.

The yeast two-hybrid DNA binding domain vector pGBKT7 (Clontech) was modified as above for pGADT7, to replace the multiple cloning site. Full-length SPA1, cut with XbaI as above was cloned into the SpeI site of modified pGBKT7 to obtain pGBKT7-SPA1.

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