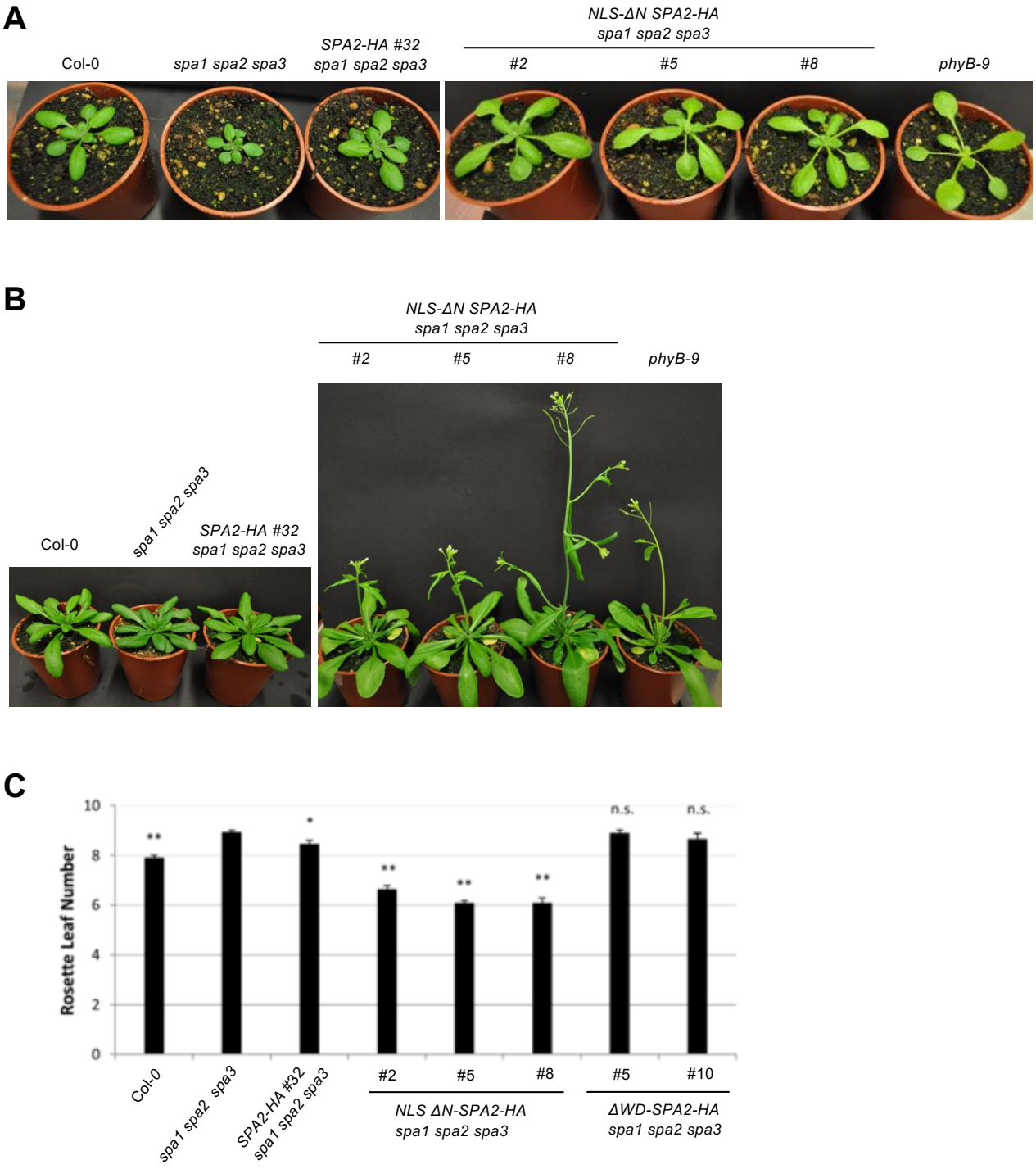

Figure S1. Transgenic lines expressing NLS-ΔN SPA2 exhibit longer petioles and early flowering.

A, B. Visual phenotype of the indicated genotypes grown in long day for three weeks (**A**) or four weeks (**B**).

C. Flowering time of the indicated genotypes, determined as number of rosette leaves produced at the time of bolting. Plants were grown in long day. Error bars represent SEM of 9-15 plants. All transgenes are in the *spa1 spa2 spa3* mutant background. Line numbers represent independent transgenic lines. Asterisks indicate statistically significant differences when compared to *spa1 spa2 spa3* (*P<0.05, **P<0.01, n.s. not significant at P<0.05).

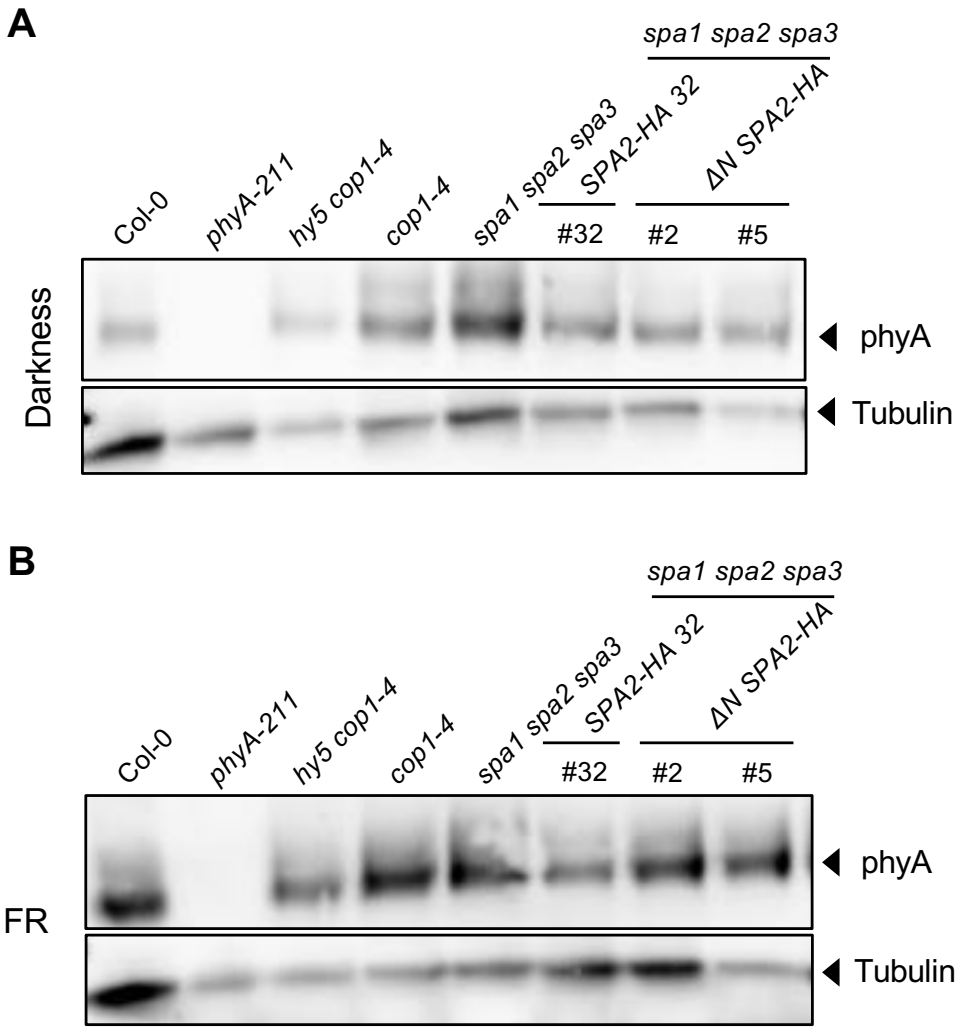

Figure S2. Immunodetection of phyA protein levels.

A, B. Seedlings were grown in darkness for 4 days **(A)** or shifted to FRc (1 μmol m^{-2} s^{-1}) for 6 h **(B)**. Proteins were detected using α-phyA antibody. Tubulin levels, detected by α-tubulin, are shown as a loading control.

Supplemental Materials and Methods

Plasmid constructions

The SPA2 N-terminal deletion fragment (NLS ΔN SPA2-HA) was amplified from full-length *SPA2* ORF using the primer pair SPA2 deltaN Apa1 NLS F and SPA2 deltaN Not1 R, introducing a ApaI restriction site and an artificial NLS at 5', and a 3' NotI restriction site lacking stop codon. The resulting fragments were introduced into the pJET1.2 vector using the manufacturer's instructions (Thermo Fisher Scientific, Schwerte, Germany). After sequencing to confirm the correct sequences, the deletion constructs were digested with ApaI and NotI and ligated into the ApaI and NotI sites of the pBS SK+ vector carrying the *SPA2* 5' and 3' regulatory sequences as described previously (Balcerowicz et al., 2011), resulting in the pBS NLS ΔN SPA2 construct. With the HA-specific primers 3xHA-NotI-F and 3xHA-NotITGA-R, a triplicate of HA was amplified with a stop codon at the end of its sequence using pJHA212-hpt SPA2::SPA1-HA (Balcerowicz et al., 2011) as a template. The HA PCR products were ligated into the pBS NLS ΔN SPA2 construct via NotI sites. The resulting constructs were digested with KpnI and ligated into the pJHA212-hpt binary vector (Yoo et al., 2005), as described in Balcerowicz et al. (2011), to generate the pJHA212-hpt NLS ΔN SPA2-HA which was used for transformation of the *spa1-7 spa2-1 spa3-1* mutant.

To generate the SPA2ΔWD-HA binary vector, the coding sequence of SPA2ΔWD was PCR-amplified from full-length *SPA2* ORF using the primer pair SPA2-Apa1-F and SPA2 delta WD NotI R. The resulting fragment was subsequently cloned as described above to generate pJHA212-hpt SPA2ΔWD-HA which was transformed into the *spa1-7 spa2-1 spa3-1* mutant.

To generate SPA2 deletion constructs for yeast two-hybrid assays, respective sequences corresponding to the deletion constructs described above were amplified by PCR using Gateway attachment sites and cloned into pDONR221 and subsequently into pAS 2.1 (Clontech) that had been modified to insert a Gateway cassette into the multiple cloning site. SPA2-NT expressed amino acids 1-579, retaining the complete kinase-like domain but excluding the coiled-coil domain.

To generate SPA3-HA- and SPA4-HA-expressing transgenic plants, the respective open-reading-frame of *SPA3* and *SPA4* was amplified using the primer pairs SPA3/SPA4-ApaI-F and SPA3/SPA4-NotI-R and fused to a triple-HA tag and *SPA2* 5´ and 3´ regulatory sequences as described above followed by subcloning into the pJHA212-hpt binary vector as described above.

References

Balcerowicz M, Fittinghoff K, Wirthmueller L, Maier A, Fackendahl P, Fiene G, Koncz C, Hoecker U (2011) Light exposure of Arabidopsis seedlings causes rapid de-stabilization as well as selective post-translational inactivation of the repressor of photomorphogenesis SPA2. Plant J **65:** 712-723

Yoo SY, Bomblies K, Yoo SK, Yang JW, Choi MS, Lee JS, Weigel D, Ahn JH (2005) The 35S promoter used in a selectable marker gene of a plant transformation vector affects the expression of the transgene. Planta **221:** 523-530

Supplemental Table 1: Oligonucleotides

Primer name	Sequence 5´ to 3´
3xHA-NotI-F	CCTAGCGGCCGCTTACCCATATGACGTTCCAGAC
3xHA-NotITGA-R	GGTAGCGGCCGCTCAAGCGTAGTCAGGTACGTCGTAAG
SPA2 deltaN ApaI NLS F	CCGAGGGCCCATGATGGATCTTCCTAAGAAGAAGAGAAAGGTTGGA GGATCTTTATCCATTGAACA AGAGGAC
SPA2 delta N NotI R	TTAAGCGGCCGCGACCAACTGTAGAACTTTGATTG
SPA2-Apa1-F	CCGAGGGCCCATGCCTGTTATGGAAAGAGTAGCTGAA
SPA2 delta WD NotI R	TTAAGCGGCCGCGGTTTCAAACTTACTATATCGAGC
TS_SPA2_attB1_for	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGATGGATGAGGGA TCAGTAGG
TS_SPA2_attB2_rev	GGGGACCACTTTGTACAAGAAAGCTGGGTCGACCAACTGTAGAACT TTGATTGAC
TS_SPA2_deltaWD_attB2_rev	GGGGACCACTTTGTACAAGAAAGCTGGGTCAGTTTCAAACTTACTAT ATCG
TS_SPA2_GA_rev	AGTGCGGCCGCGAATTGACCAACTGTAGAACTTTGATTGACC
TS_SPA2_WD_attB1_NLS_ATG _for_1	GAAAGGTTGGAGGAAAATATGCTCGATATAGTAAGTTTGAAACTCG
SPA3-ApaI-F	CCGAGGGCCCATGGAAGGTTCTTCAAATTC
SPA3-NotI-R	TTAAGCGGCCGCGAGTCATCATCTCCAGAA
SPA4-ApaI-F	CCGAGGGCCCATGAAGGGTTCTTCAGAATCT
SPA4-NotI-R	TTAAGCGGCCGCGATACCATCTCCAAAATCTTG