Supporting Information

Xin et al., (2016)

SI Materials and Methods

Whole genome sequencing

An F₂ mapping population was constructed by crossing *sfps-1* to the Ler accession, and 70 F₂ seedlings were selected based on the long hypocotyl observed in the mutant. We extracted genomic DNA from nuclei isolated from flowers (pooled from all plants used for mapping), and used 1 µg for library generation with the Illumina Truseq DNA PCR-free kit (San Diego, CA, USA) following manufacturer's protocol for 350-bp insert size, followed by a 12-cycle PCR amplification with the PCR primer cocktail provided in Illumina's TruSeq DNA sample preparation kit. Non-incorporated primers were removed with a Qiagen MinElute column. Library concentration was estimated by fluorescence measurement by Qubit fluorometer (Life Technologies, Grand Island, NY, USA), and quality assessed on a Bioanalyzer DNA chip (Agilent Technologies, Santa Clara, CA, USA). Sequencing was performed on an Illumina Hiseq2000 instrument with 2 x 101bp paired-ends reads. Reads were aligned to the Col-0 reference genome with the SHORE pipeline (1), and allele frequencies plotted along the genome with SHOREmap (2). The boost function of SHOREmap identified an interval on the top arm of chromosome 1 (between 10 - 12 Mbp) with highest Col-0 frequencies that coincides with a rough-mapping estimate obtained by conventional mapping means. A list of genes with mutations in their coding regions was then compiled, and candidates containing T-DNA insertion sites were obtained from ABRC for measuring hypocotyl lengths under dark and red light conditions as described above.

Protein extraction and immunoblot analyses

All plant materials were frozen and ground into a fine power in liquid nitrogen. Protein samples were extracted in 2X SDS-Loading buffer (1 M Tris-HCl pH 6.8, 1 ml 10% SDS, 4 ml Glycerol, 2 ml β -mercaptoethanol, 2.5 ml 1% Bromophenol blue, 500 μ l dH₂O, 1 mM PMSF, 1X Protease inhibitor cocktail, and 20mM MG132). Buffer was preheated at 95^oC and added in a 1:2 (w/v) ratio. Samples were then boiled for 10 mins, spun at 14000 rpm for 10 mins, and 40 μ l of total

protein supernatant was loaded onto 8% SDS-PAGE gels. Proteins were transferred onto PVDF membrane, and the membrane probed with anti-GFP antibody (Abcam; Cat ab290; Cambridge, MA).

Yeast two-hybrid analyses

The constructs used are as follows: for the bait vectors BD-PHYB, the full length and the C-terminal domain of PHYB were previously described (3, 4). For the prev vector AD-SFPS, full length SFPS ORF was amplified with forward and reverse primers containing XhoI restriction site and then cloned into pJG4-5 vector (Invitrogen Thermo Fisher Scientific Inc. Waltham, MA). For AD-SFPS full length and truncated versions, full length SFPS ORF, T1 (G-patch and RRM, 499bp-1098bp), T2 (RRM, 801bp-1098bp), T3 (N-TER w/o RRM 1bp-823bp, T4 (N-TER w/o G-patch and RRM, from 1bp-597bp) were amplified with forward and reverse primers containing BamHI and PstI restriction sites, respectively, and then cloned into pGAD424 (Clontech Laboratories Inc., Mountain View, CA). Yeast transformation was performed using the EZ-Yeast Transformation Kit (MP Biomedicals, LLC, Boston, MA). Yeast two-hybrid quantitative interaction assays were performed according to manufacturer's instructions (Matchmaker Two-Hybrid System, Clontech Laboratories Inc., Mountain View, CA).

Construction of vectors and generation of transgenic plants

To construct GFP fusion vector, *SFPS* (AT1G30480) genomic DNA fragment, including 1.8kb promoter region and 2.109kb gene body was cloned into pENTRY vector and recombined with pGWB4 (5). A stop codon was not included in the GFP fusion vector to allow C-terminal fusion protein expression. To construct *U2AF65B* (AT1G60900) MYC fusion vector, full-length *U2AF65B* open reading frame without the stop codon was cloned into pENTRY vector and recombined with pEARLEYGATE203 (6). These constructs were then transformed into wild type by Agrobacterium-mediated transformation as described (7). Single-locus transgenic plants were selected based on antibiotic resistance and several homozygous lines were produced for analysis for each construct.

In vivo/in vitro co-immunoprecipitation assays

For in vivo co-immunoprecipitation assay, we used *pSFPS:SFPS-GFP/sfps-1* and *35S:U2AF65B-MYC/*Col-0 single transgenic lines described above, and crossed them to create a double transgenic line. Double homozygous plants were selected for glufosinate and hygromycin

resistance, and used for the following SFPS and U2AF65B Co-IP assay. Total proteins were extracted from ~0.4g seedlings (either dark-grown or exposed to 6-hr pulse of R light (1.89 µmolm⁻²s⁻¹)) with 2 ml native extraction buffer (100 mM NaH₂PO₄, pH 7.8, 130 mM NaCl, 0.1% NP-40, 10% glycerol, 0.1mM EDTA, 1X Protease inhibitor cocktail [Sigma-Aldrich; catalog No. P9599], 1 mM phenylmethylsulfonyl fluoride, 20 μM MG132, 25 mM β-glycerophosphate, 10 mM NaF and 2 mM Na orthovanadate) and cleared by centrifugation at 14,000 rpm for 10 mins twice at 4°C. Anti-GFP antibody [Abcam; Cat ab290; Cambridge, MA] was incubated with Dynabead protein A [Invitrogen Inc., Carlsbad, CA; catalog No. 10002D] (2.5 µg antibody and 20 µl beads per sample) for 2 hrs at 4°C. The bound antibody-beads were added to a total of 2ml protein extracts and rotated for another 2 hrs at 4°C in the dark. The beads were collected using a magnet, then washed three times with washing buffer (100 mM NaH₂PO₄, pH 7.8, 130 mM NaCl, 0.1% NP-40, 10% glycerol, 0.1mM EDTA, 1X Protease inhibitor cocktail [Sigma-Aldrich; catalog No. P9599], 1 mM phenylmethylsulfonyl fluoride, 20 μM MG132, 25 mM βglycerophosphate, 10 mM NaF and 2 mM Na orthovanadate). We added 40 µl 2X SDS-Loading buffer to elute the immunoprecipitated samples and heated at 70°C for 10 mins. Immunoprecipitated samples were separated on an 6% SDS-PAGE gel, transferred onto PVDF membrane, and probed with α -myc antibody [Calbiochem/EMD; catalog No. OP10] to detect U2AF65B-MYC or with α -PHYB antibody [Santa Cruz; catalog No. sc-9996] to detect native PHYB protein. We re-probed the same membrane with a GFP antibody to detect the bait SFPS-GFP.

For the tobacco Co-IP assays, SFPS-GFP and Myc-U2AF65B constructs were generated as described above. These two constructs were transformed into Agrobacterium, and then coinjected in tobacco leaves using the infiltration method mentioned above (8). We used tobacco leaves injected with Myc-U2AF65B single construct only as control for the following Co-IP assay. After 48 hrs incubation, around 1g tobacco leaves was collected for coimmunoprecipitation assays as described above.

Co-localization analysis

For co-localization for SFPS-GFP and U2-mcherry fusions, full length U2A' and U2AF35A open reading frames (without stop codon) were PCR-amplified with forward and reverse primers containing BamHI and XbaI restriction sites, respectively, and cloned at the 5'

end of the mCherry vector, a plant-expression vector. The full length *U2AF65B* CDS (without stop codon) was PCR-amplified with forward and reverse primers containing BamHI restriction sites, and cloned at the 5' end of the mCherry vector. All fusion constructs were verified by restriction digestion and sequencing. The following three combination constructs, U2A'-mCherry and SFPS-GFP, U2AF35A-mCherry and SFPS-GFP, U2AF65B-mCherry and SFPS-GFP, were transformed into Agrobacterium and then co-injected in tobacco leaves using the infiltration method (8). After 48 hrs incubation, transformed epidermal cells were used for confocal microscopy.

For co-localization of SFPS-RFP and phyB-GFP, full-length SFPS open reading frame (without stop codon) was PCR-amplified with forward and reverse primers containing BamHI and XbaI restriction sites, respectively, and cloned at the 5' end of the RFP vector, a plant-expression vector. The full length *PHYB* CDS (without stop codon) was PCR-amplified with forward and reverse primers containing KpnI and SmaI restriction sites, and cloned into Pezs-NL vector (9). *35S:PHYB-GFP* cassette was then cloned into pART27 vector using the NotI site. All fusion constructs were verified by restriction digestion and sequencing. *35S:PHYB-GFP* was introduced into Col-0 background to obtain homozygous *35S:PHYB-GFP*/Col-0 transgenic plant. 35S:SFPS-RFP was then introduced into *35S:PHYB-GFP*/Col-0 plants. Primary transformants for *35S:SFPS:RFP* (in an otherwise homozygous *35S:PHYB:GFP* background) were selected based on resistance to glufosinate. Localization of GFP and RFP was observed on a confocal microscope using seedlings grown either under continuous white light or dark-adapted seedlings (light grown plants treated with saturated FRp for 5 mins and then grown under dark condition for 24 hrs) exposed to white light for 40 mins.

Bioluminescence assays

sfps-2 lines carrying the *ProPRR7:LUC* reporter construct was generated by crossing to Col-0 *ProPRR7:LUC* seedlings and selected for homozygous *sfps-2* and the *ProPRR7:LUC* construct. *ProPRR7:LUC* consisted of the *PRR7* promoter driving expression of firefly *luciferase* (10). After seed germination under white light at 22°C for 3 days, seedlings were transferred to entrainment for 5 days in cycles of 12 hrs light and 12 hrs dark at 22°C. Surface sterilization of seeds and all transfers (*i.e.*, germination, entrainment, and free run) occurred at ZT 0.

Each MS plate was sprayed with 1 ml of 5 mM firefly luciferin (Biosynth; Gold Biotechnology) prepared in 0.01% (vol/vol) Triton X-100 (Sigma Aldrich) applied 24 hrs before

imaging with a luciferase imaging system built by BioImaging Solutions, employing an ORCAII camera (Hamamatsu Photonics) housed in a Percival incubator (Percival Scientific Inc.) for temperature control. Each experiment had 9 plates imaged by the camera at once. Halogen bulbs provided 50 µmol m⁻² sec⁻¹ of white light. Seedlings were imaged every 2.5 hrs and bioluminescence of individual seedlings collected using MetaMorph software (Molecular Devices). Bioluminescence data from images was extracted with MetaMorph software and compiled in Microsoft Excel (Microsoft Corporation) with the Biological Rhythms Analysis Software System 3.0 (BRASS), an Excel workbook for the analysis of rhythmic data series (11, 12). Within BRASS, Fast Fourier Transform-Nonlinear Least Squares (FFT-NLLS) was used to estimate circadian period, RAE, and phase values from the rhythmic bioluminescence data (13).

References:

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Supplemental Data



Figure S1: Gene structure of *SFPS* and complementation of *sfps* with *pSFPS:SFPS-GFP*. **(A)** The gene structure of *SFPS* indicating the locations of the EMS mutation and the different T-DNA insertion sites. The arrowheads indicate the positions of the primers to determine the abundance of different exons in *SFPS*. **(B)** RT-qPCR shows the relative abundance of extron 1 and extron 2 of *SFPS* in different *sfps* mutant alleles. Total RNA was extracted from the Col-0, *sfps-1*, *sfps-2*, and *sfps-3* seedlings grown in white light for two weeks. *PP2A* was used as an internal control. Each bar is the mean \pm S.E.M. (n=3 independent biological repeats, and each biological repeat include three technical repeats). **(C)** Photographs of wild type Col-0, *sfps-2* mutant and a representative *pSFPS:SFPS-GFP/sfps-1* transgenic line grown for four days under red (1.2 µmol m⁻² s⁻¹), far-red (0.56 µmol m⁻² s⁻¹), or blue light (0.73 µ µmol m⁻² s⁻¹). White bar = 1 cm. **(D)** Hypocotyl elongation of Col-0, *sfps-2* mutant and *pSFPS:SFPS-GFP/sfps-I* transgenic line seedlings grown under different light conditions indicated above. Error bar = S.E.M. (n>30).



Figure S2: Different alleles of *sfps* display reduced cotyledon angle and cotyledon area in response to light.

(A) to (C) Different alleles of *sfps* show cotyledon opening defects under different light conditions. Cotyledon angle in four-day-old seedlings grown under 1.2 μ molm⁻²s¹ red light (A), 0.56 μ molm⁻²s¹ or 2.1 μ molm⁻²s¹ blue light (B), and 2.3 μ molm⁻²s¹ far-red light (C). Each bar is the mean ±S.E.M. (n>40). Chlorophyll (D) and anthocyanin (E) contents in Col-0 and *sfps-2* mutant. Seedlings were grown in the dark for four days with or without subsequent white light (3.5 hrs or 6.5 hrs) irradiation for chlorophyll measurement or grown under dark conditions for one day and then transferred to either dark or far red light conditions for additional three days for anthocyanin measurement. (F) Hypocotyl elongation of Col-0 and different *sfps* mutant alleles grown in the dark for 2 to 4 days.



Figure S3: Different *sfps* mutant alleles show early flowering compared to wild type under both short day and long day conditions. Rosette leaf number at 1-cm bolting time of wild type Col-0 and different *sfps* alleles grown under short days (8-h light/16-h dark) (**A**) and long days (16-h light/ 8-h dark) (**B**), Each bar represents the mean \pm S.E.M. (n>30 plants). Days to 1cm bolting time under short days (8-h light/16-h dark) (**C**) and long days (16-h light/ 8-h dark) (**D**). Each bar is the mean \pm S.E.M. (n>30 plants). The asterisk shows statistically significant differences to Col-0 wild type.



Figure S4: SFPS regulates the expression of flowering time genes under both long days and short days.

A) to H) *sfps* affects the expression of flowering time genes *CO*, *FLC*, *FT*, *SOC1* under short days **(A)** to **(D)** and long days **(E)** to **(H)**. RNA was extracted at different time points from two weeks seedlings grown in short days or long days , and reverse transcribed into cDNA. *PP2A* was used as an internal control. Each bar is the mean \pm S.E.M. (n=3 independent biological repeats, and each biological repeat include three technical repeats).





Seedlings were grown for five days in LD|22°C entrainment conditions of 12-hrs-light/12-hrs-dark and constant 22°C, then transferred at ZT 0 for five days of LL|22°C free-running conditions of constant light and 22°C. Light was provided as 50 µmol m⁻² s¹ of white light. Individual traces of *ProPRR7:LUC* activity in Col-0 (blue lines; n = 22) and *sfps-2* (red lines; n = 43) during entrainment (**A**) and in free-running conditions (**D**). Mean period length in hours, during entrainment (**B**), and in free-running conditions (**E**). Mean Relative Amplitude Error (RAE) of rhythms during entrainment (**C**), and in free-running conditions (**F**). RAE is a measure of the quality of the rhythm, with RAE = 0 corresponding to a perfect sine wave and RAE = 1 being expected for a straight line lacking any rhythmic component. Error bars are standard deviation and asterisk (*) indicates significance p-value <0.05 from two-tailed t-test between WT and *sfps-2*.

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Figure S6: Photographs showing the juvenile and mature plants of wild type and *sfps* mutant. Three *sfps* mutant alleles along with wild type Col-0 were grown under continuous white light at 22^oC for approximately three weeks (A), and 8 weeks (B), respectively.



Figure S7: SFPS-GFP and MYC-U2AF65B proteins co-immunoprecipitate in tobacco cells in a transient assay. Tobacco leaves were co-infiltrated with Agrobacterium carrying 35S:MYC-U2AF65B and 35S:SFPS-GFP and incubated for 16 hrs. Total proteins were extracted and incubated with anti-GFP (rabbit) or anti-IgG (rabbit) conjugated beads as indicated. The IP products were detected with anti-MYC and anti-GFP respectively. A separate sample only expressing MYC-U2AF65B was used as a control for co-IP.



Figure S8: Mapping of interaction domains in SFPS and phyB using yeast-two-hybrid assays and co-localization of SFPS and phyB in Arabidopsis. (**A**) The G-patch domain of SFPS is necessary for the interaction between phyB and SFPS in the yeast two-hybrid assays. Full length phyB fused with GAL4 DNA binding domain and various truncated SFPS proteins fused with GAL4 activation domain were co-transformed into yeast Y187 stain. T1 represents SFPS G-patch and RRM domain (499-1098 bp); T2 represents RRM domain (801-1098 bp), T3 represents N terminal domain of SFPS protein without RRM domain (1-823 bp), T4 represents N terminal domain of SFPS protein without RRM domain (1-823 bp), T4 represents N terminal domain of SFPS protein without the G-patch and RRM domains (1-597 bp). β -galactosidase activity was measured to quantify the interaction between phyB and SFPS. The error bar is the S.E.M. (n>3). (**B**) The C terminal domain of phyB is necessary for the interaction between phyB and SFPS and PIF1 (Phytochrome Interaction protein 1) full length proteins were fused to the B42 activation domain. β -galactosidase activity indicates that SFPS interact with the C terminal domain of phyB, but not PIF1 protein. The error bar is the S.E.M. (n>3). (**C**) Co-localization of phyB-GFP and SFPS-RFP in nucleoplasm as well as in nuclear photobodies in another independent transgenic Arabidopsis seedlings. 35S:phyB-GFP/35S:SFPS-RFP /Col-0 seedlings were grown under continuous white light for fours days before being imaged. Scale bar = 2 mm.



Figure S9: The transcription of *SFPS* and stability of SFPS are not regulated by light. (**A**) Transcription of *SFPS* is not regulated by light. Total RNA was extracted from four-day-old dark-grown Col-0 seedlings either kept in the dark or exposed to red light (7 μ mol m⁻² s¹) for 30 mins or 3 hrs. *PP2A* was used as an internal control. Each bar is the mean ±S.E.M. (n=3 independent biological repeats, and each biological repeat include three technique repeats). (**B**) SFPS protein abundance is not regulated by light. Total proteins were extracted from *pSFPS:SFPS-GFP/sfps-1* seedlings grown in darkness or under red (1.2 μ mol m⁻² s¹), far-red (0.56 μ mol m⁻² s¹) or blue light (0.73 μ mol m⁻² s¹) conditions for four days. The protein samples were separated in the 8% polyacrylamide gel and transferred onto PVDF membrane for the immunoblot. Anti-GFP and anti-RPT5 antibodies were used to detect the SFPS and internal control RPT5 protein level, respectively.



Figure S10: Differential gene expression analysis in Col-0 vs *sfps-2* seedlings grown in dark or red light conditions.

(A) Venn diagram of the number of genes that displayed altered expression in Col-0 vs *sfps-2* mutant in the dark (CM_D_TX), Col-0 vs Mut 3hrs Rc (CM_L_TX), Col-0 dark vs 3hrs Rc (C_DL_TX), and Mut dark vs 3hrs Rc (M_DL_TX). FDR<0.05 and log₂ (fold_change) is larger than 0.58 or smaller than -0.58. (B) Heatmaps of genome-wide gene expression changes between Col-0 and *sfps-2* mutant in the dark (left) and light (right) conditions. Blue color = higher log2 (RPKM), whereas red color = lower log₂ (RPKM). Red light intensity used was 7 μ mol m⁻² s⁻¹.



Figure S11: GO analysis of the differential expressed genes between Col-0 and *sfps-2* dark samples. The graph shows the proportion of the analyzed genes (FDR<0.05, and log₂(fold_change) larger than 0.58 or smaller than -0.58) in each category or that of all of the genes in each category to the annotated genes in the genome. The color represents the enrichment levels, and the arrows represent the relationship between each category. The analysis was done by agriGO (http://bioinfo.cau.edu.cn/agriGO/).



Figure S12: GO analysis of differential spliced genes between Col-0 and *sfps-2* in the dark (A) or light (B) samples. The graph shows the proportion of the analyzed genes (FDR<0.05, and PSI or PIR fold change is larger than 3%) in each category or that of all of the genes in each category to the annotated genes in the genome. The color represents the enrichment levels, and the arrows represent the relationship between each category. The analysis was done by agriGO (http://bioinfo.cau.edu.cn/agriGO/).



Figure S13: KEGG pathway enrichment analysis of genes that displayed AS pattern changes between Col-0 and *sfps-2* in the dark (A) or red light (B) samples (FDR<0.05, and Delta PSI or PIR is larger than 3%). The bar graph shows the $-\log (q \text{ value})$ for each category. The most significantly enriched pathways are listed in the graph. The analysis is done in David website (<u>https://david.ncifcrf.gov/</u>).



Figure S14: GO analysis to determine the molecular function of the phenotypic genes between Col-0 and *sfps-2* in the dark (A) or light (B) samples. The graph shows the proportion of the analyzed genes (FDR<0.05, and PSI or PIR fold change is larger than 3%) in each category or that of all of the genes in each category to the annotated genes in the genome. The color represents the enrichment levels, and the arrows represent the relationship between each category. The analysis was done by agriGO (http://bioinfo.cau.edu.cn/agriGO/).



Figure S15: Validation of SFPS-regulated alternative splicing.

Figure S15: Validation of SFPS-regulated alternative splicing. (A), (D), (G), (J) and (M) IGV viewer illustrating alternative splicing events detected by RNA-seq between Col-0 and *sfps-2* dark-grown or 3 hrs (7 μ mol m⁻² s¹) red light irradiated samples. The red bar indicates the position of AS events in the genes. (B), (E), (H), (K) and (N) RT-qPCR validation of RNA-seq data. Total RNA was extracted from Col-0 and *sfps-2* seedlings grown in the dark or following 3hrs red light (7 μ mol m⁻² s¹), respectively. AS patterns of the target genes are expressed as the proportion of one specific isoform in the total transcripts. *PP2A* was used as an internal control. Each bar is the mean ±S.E.M. (n=3 independent biological repeats, and each biological repeat include three technical repeats). (C), (F), (I), (L) and (O) The AS patterns indicated in the RNA-seq results. PSI and PIR are calculated according to formula described in the methods (n=3 independent biological repeats).



Figure S16: *sfps-2* shows defects in alternative splicing of *SR* genes. AS patterns of splicing factors, *RS31*, *SR34*, and *SR34b* have been changed in *sfps-2* mutant compared with Col-0 wild type. Total RNA was extracted from two-day-old dark-grown Col-0 and *sfps-2* seedlings transferred to darkness, white light, red (1.2 μ mol m⁻² s¹), or far-red (0.56 μ mol m⁻² s¹) for additional 48 hrs. *PP2A* was used as an internal control. The PCR products are separated in the 1.5% agarose gel.



Figure S17: RT-qPCR analyses of the mature and pre-spliced form of *ELF3* over time. Total RNA was extracted from Col-0 and *sfps-2* mutant seedlings grown under darkness or dark-grown seedling exposed to short term or long term continuous red light as indicated. The samples were collected at the time points indicated above. *PP2A* was used as an internal control. The error bar indicated S.E.M. (n = biological repeats, each repeat include three technical repeats).



Figure S18: The long hypocotyl phenotype of *sfps-2* mutant is not complemented by over expression of *ELF3*. **(A)** *ELF3* expression levels in two independent 35S::MYC-ELF3/*sfps-2* transgenic lines. Total RNA was extracted from seedlings of Col-0 and different transgenic lines grown under darkness or dark-grown seedlings exposed to red light (7 µmol m⁻² s¹) for 12 hrs. *PP2A* was used as an internal control. The bar is the mean \pm STD (n= 3 technical repeats). **(B)** Hypocotyl length of Col-0, *sfps-2*, *elf3-1*, *sfps-2elf3-1*, 35S::MYC-ELF3/*sfps-2* L27, and L31 seedlings. Seedlings were grown under red (1.2 µmol m⁻² s¹), far-red (0.56 µmol m⁻² s¹) or blue light (0.73 µmol m⁻² s¹) conditions for four days before being measured.