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Supplementary Information for

The Evening Complex integrates photoperiod signals to control flowering in rice.

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Supplementary Information Text

Methods

Cloning of CRISPR/*Cas9* transformation cassettes

Binary vectors containing a hygromycin resistance gene driven by the cauliflower mosaic virus *35S* promoter were used to generate Cas9-mediated genomic insertion and/or deletion events at *LUX*, *ELF3-1*, and *ELF3-2* loci (Supplemental Fig. 1A and Supplemental table 1). The target region contained in *LUX* locus 5' – ATGCAGCTGATGAACGTGGA – 3' was synthesized and cloned into pMiao (1). To generate the *elf3-1* and *elf3-2* single mutants as well as the *elf3-1 elf3-2* double mutant, a two-guide RNA system was used (2). The targets 5' – GTTTCCTCTATGCCTCCTTG – 3' and 5' – GTGCGGACTGAGAGCAACAA – 3' were selected for the *ELF3-1* and *ELF3-2* loci, respectively. The final vectors were introduced into *Agrobacterium tumefaciens* EHA105 for rice transformation.

Cloning of LUX genomic tagged transformation cassette

For generating a genomic tagged line of *LUX*, a 2,935 bp sequence comprising 2,000 bp of the promoter sequence, 217 bp of 5'UTR, and 717 bp of *LUX* coding sequence was amplified by PCR (primers in Supplemental Table 2). The PCR products where cloned in tandem into linearized binary vector upstream and in the same open reading frame of a 5xMYC tag using NEBuilder® HiFi DNA Assembly Cloning Kit (New England Biolabs). The *LUX* coding sequence was modified by removing the last nucleotide in order to eliminate the formation of a stop codon. Then, a 500 bp fragment downstream of *LUX* stop codon (3'UTR) was amplified by PCR and cloned downstream of the 5xMYC tag using the same procedure. The sequence of the primers used can be found in Supplemental Table 2. The final vector was introduced into *Agrobacterium tumefaciens* EHA105 for rice transformation.

Rice *calli* culture and transformation

Stable transformations were generated using wild-type rice cv. Nipponbare following established methods (3).

Identification of tagged LUX, *lux, elf3-1, elf3-2,* and *elf3-1 elf3-2* mutants

Plant lines obtained from tissue culture harboring the hygromycin cassette were further tested. The *LUX* tagged lines were validated by testing the expression of *LUX::MYC* by RT-qPCR. *Cas9* transformed lines were then tested for the presence of insertions or deletions (InDels) in the target loci. The target region was amplified by PCR in WT and transformed plants. PCR products were Sanger sequenced and the resulting chromatograms were compared using the decomposition tool TIDE (4). Identified lines with InDels in the target loci are compiled in Supplemental table 2. Several lines for *lux* and *elf3-1 elf3-2* were generated, yet none flowered in our conditions and therefore lines were only maintained by vegetative propagation. Lines harboring a three nucleotide deletion in either *elf3-1* or *elf3-2* flowered and were not considered double mutants since this deletion does not cause a translation frame shift. The lines 246U2.7a

and 246U1.6a were used to generate *elf3-1* and *elf3-2* single mutants, respectively, by obtaining homozygous three nucleotide deletion in the loci we wanted to preserve. Hence we obtained 246U2.7a1 with two nucleotide deletion in *ELF3-1* locus and three nucleotide deletion in *ELF3-2* locus and 246U1.6a1 with three nucleotide deletion in *ELF3-1* locus and two nucleotide deletion in *ELF3-2* locus. These single mutants were used to access flowering time and for ELF3 antibody validation. The *elf3-1 elf3-2* double mutant was obtained from seed by germinating 246U1.6a and selecting plants harboring a one nucleotide deletion in *ELF3-1* locus and a two nucleotide deletion in *ELF3-2* locus (line named 246U1.6a2). Primers used in genotyping these lines are available in Supplemental table 2.

Rice growth conditions

For the measurement of flowering-time, wild-type, *elf3-1* (line 246U2.7a1), *elf3-2* (line 246U2.7a1), and *elf3-1 elf3-2* (line 246U2.7a2) rice seeds were germinated in water-soaked paper at 28 ºC for 3 days. Germinated seedlings were planted in containers with soil (soil:peat:vermiculite in 2:2:1 volume ratio) and placed in controlled conditions at 28 ºC under 12h/12h light/dark photoperiod (ND conditions) at 70 % humidity. Plants harboring a *lux* mutation were grown under similar conditions in order to compare flowering time, but the initial plant material used was prevenient from *calli* culture and not from seeds, therefore the time to flower was counted from the emergence of the second leaf. Additionally, WT and *lux* rice (from *calli* culture) were grown under LD conditions (14 h day, 10 h night) and SD conditions (10 h day, 14 h night). From 30 days of development, the plants were regularly monitored for panicle emergence. The flowering-time of each plant was considered when the first emerging seed was above the flag-leaf collar.

For assessing either gene expression by high-throughput RNA sequencing or protein accumulation, wild-type, *phyb-1* (generated elsewhere (5)), *elf3-1* (line 246U2.7a1), *elf3-2* (line 246U2.7a1) and *elf3-1 elf3-2* (line 246U2.7a2) rice seeds were surface sterilized and germinated under dark for 3 days at 28 ºC. Rice *lux* mutants (line 75Z3a) tillers were obtained by a donor plant and grown under similar conditions in order to compare results. Seedlings were transferred to tubes containing sterile ½ MS solid medium and placed in a controlled chamber at 28 ºC at either LD conditions or SD conditions with photoactive radiation of 300 µmol photons $m^{-2}s^{-1}$. Pools of 4-5 plants were collected 21 days after germination at specific time-points: wild-type plants were collected at ZT2, ZT6, ZT10, ZT14, ZT18, and ZT24; *phyb* and *elf3-1 elf3-2* mutant plants were collected at ZT6, ZT14, and ZT18; lux mutant plants were collected at ZT14 in SD conditions. Each sample was immediately frozen using liquid nitrogen and stored at -80 ºC for later use. These experiments were repeated to obtain a total of three biological replicates.

For the identification of EC direct targets, wild-type and *LUX* genomic 5 x MYC tagged line seeds were surface sterilized and germinated under dark for 3 days at 28 ºC. Germinated seedlings

were then transferred to tubes containing sterile ½ MS solid medium and placed in a growth chamber in SD conditions for 14 days. A pool of 20 plants was collected from each genotype at ZT14 using liquid nitrogen and stored at -80 ºC for later use.

For the night-break experiment, wild-type and *phyb-1* rice seeds were germinated as described above but placed in a growth chamber at SD conditions for 10 days. Part of the plants was exposed to a night-break (NB) of 300 µmol photons $m^{-2}s^{-1}$ of photoactive radiation for 15 minutes in the middle of the night (ZT17) while others remained under dark. Samples were collected in pools of 2 plants and the experiment was repeated to obtain a total of three biological replicates.

Extraction of total RNA

Total RNA was extracted from WT, *phyb-1*, *lux* (line 75Z3a) and *elf3.1 elf3.2* (line 246U2.7a2), sampled at multiple points in the day under SD or LD conditions, by homogenizing samples to powder using a freeze cold mortar and pestle followed by total RNA extraction using the RNeasy plant kit (Qiagen), followed by DNAse I treatment (Ambion), according to the manufacturer's instructions. Total RNA was quantified using Qubit4 (Thermo Fisher Scientific) according to the manufacturer's instructions and tested for integrity using an RNA screentape in an Agilent2200 tape station (Agilent), following the procedure recommended by the manufacturers.

Chromatin immunoprecipitation

3 g of plant material for each genotype was fixed in 1xPBS (10 mM PO_4^{3-}, 137 mM NaCl, and 2.7 mM KCl) containing 0.5 % Formaldehyde (SIGMA). The reaction was quenched by adding glycine to a final concentration of 62 mM. Chromatin immunoprecipitation (ChIP) was performed as described (6), with the exception that 100 µl of anti-cMyc agarose affinity gel (A7470-1ML) was used per sample or custom anti-ELF3 (Agrisera, AS184168, lot# 1808). 3 µl of anti-ELF3 was preincubated with Novex™ DYNAL™ Dynabeads™ Protein A and G (50 µl each, Thermo Scientific) for 1h on a rotating wheel. Sequencing libraries were prepared using TruSeq ChIP Sample Preparation Kit (Illumina) and prepared according to manufacturer instructions. DNA libraries were quantified using a Qubit fluorometer, and library profiles were analysed using a TapeStation 2200 with High Sensitivity D1000 ScreenTapes (Agilent). Libraries were sequenced on a NextSeq-500 (Illumina; single end, 75bp reads), according to Illumina guidelines.

Messenger RNA and DNA library preparation and sequencing

Ultrapure total RNA from wild-type, *phyb-1*, *lux* (line 75Z3a), and *elf3-1 elf3-2* (line 246U2.7a2) rice genotypes was used to prepare high-throughput mRNA sequencing libraries using NEBNext Ultra II Directional RNA Library Prep Kit (New England Biolabs). ChIP DNA libraries were performed using wild-type and *LUX* tagged line via TruSeq ChIP Sample Preparation Kit (Illumina). Nucleotide sequencing was performed in a NEXTSEQ550 (Illumina) using a high-throughput flow cell.

RNAseq and ChIPseq data processing

HISAT2 with parameters '--no-mixed, --rna-strandness RF --dta --fr' was used for aligning the raw RNA-seq reads to the rice genome assemblies (ftp://ftp.ensemblgenomes.org/pub/plants/release-47/fasta/oryza_sativa/dna/). TPM values for genes were calculated by StringTie with default settings directed by gene annotation file IRGSP-1.0 (ftp://ftp.ensemblgenomes.org/pub/release-47/plants/gtf/oryza_sativa/Oryza_sativa.IRGSP-1.0.47.gtf.gz). Mean TPM values were calculated from replicates.

For processing ChIP-seq fastq files, BWA was used to map raw reads to rice genome IRGSP-1.0. Unmapped reads, mate unmapped reads, non-primary alignment and duplicate reads were removed. Peaks were identified using MACS2 and filtered by *q*-value < 0.05. BigWig files for IGV tracks were generated using deepTools function *bamCoverage* and normalized using RPKM.

The accession number for the raw and processed data from RNA-seq and ChIP-seq in this paper is GEO: GSE181836.

RNAseq and ChIPseq data analysis

TPM values were transformed into log2(TPM+1). Genes with the maximum log2(TPM+1) > 2 were kept. Out of 37,849 reference genes, 21,175 were kept. To perform clustering of transcriptomic data, a time-course perturbation matrices was constructed between SD and LD in WT, for example, $\log2(\frac{TPM\_SD\_ZT6+1}{TPM\_LD\_ZT6+1})$. The selected perturbation matrices as follows:

[SD/LD, WT, ZT6]

[SD/LD, WT, ZT14]

[SD/LD, WT, ZT18]

Gaussian Mixture Models (GMM), a distribution-based clustering method, implemented by an R package *ClusterR* (https://github.com/mlampros/ClusterR), was used for performing the clustering. The Expectation-Maximization algorithm was used for fitting GMM to the given matrices. The Bayesian information criterion was used for estimating the number of clusters.

ELF3 and LUX bound genes were determined if ChIPseq peaks overlap with the genomic regions of gene body extended by 2 kb upstream and downstream, respectively.

Motifs were predicted using HOMER2 (*de novo* and known motifs), using the genomic regions of 100 bp upstream and downstream of peak summits as target sequences and permuted sequences (excluding target sequences) as background. R package *motifStack* was used for generating Figure S5 and motifs were filtered using the following criteria: *p-value* < 1e-5 for known motifs, *p-value* < 1e-10 for *de novo* motifs.

Software used for analysis

Graphpad Prism 8.0.2; Geneious Prime 2020.2.2; HISAT2 version 2.2.1; StringTie version 2.1.1; bwa version: 0.7.17-r1188; macs2 version 2.2.7.1; deeptools version 3.5.0; homer version 4.11; samtools version 1.11; bedtools version 2.30.0; R version 4.1.0;

Custom code for using R packages are deposited at https://github.com/yl-lu/Rice_EC.

Validation of sequencing data using real-time PCR

To validate the RNAseq results, the same samples were used for RNA extraction using RNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. To synthesize complementary DNA (cDNA), 1 µg of RNA was used in the Transcriptor First Strand cDNA synthesis kit (Roche Diagnostics). The obtained cDNA was diluted to 1:100 to obtain a working concentration. The PCR reaction mix was performed using 3 µL of diluted cDNA, gene-specific primers (available in Supplemental table 2), and LightCycler® 480 SYBR Green I Master (Roche Diagnostics) set up according to the manufacturer's instructions. The cDNA was then amplified using a LightCycler® 480 machine (Roche Diagnostics). Ubiquitin-conjugating enzyme E2 (UBC2) was used as an internal normalization for the cDNA abundance in each sample. The experiment was done in triplicate for each sample. The results are given as a normalization of the target of interest / UBC2 amplification using double delta Ct analysis (7).

To validate ChIPseq results, ChIP DNA and input DNA were de-crosslinked by submitting the samples to 65 ºC for 8 h, followed by a cleaning step using Ampure beads according to manual. The obtained DNA was diluted to 1:100 to obtain a working concentration. The PCR reaction mix was performed using 2 µL of diluted DNA, region-specific primers (available in Supplemental table 2), and LightCycler® 480 SYBR Green I Master (Roche Diagnostics) set up according to the manufacturer's instructions. The DNA was then amplified using a LightCycler® 480 machine (Roche Diagnostics). A region showing no particular enrichment was used as an internal control. The experiment was done in triplicate for each sample. Results are given as fold change enrichment relative to the internal control region using double delta Ct analysis (7).

Protein extraction and analyses

Protein extracts were prepared from wild-type, *elf3-1* (line 246U2.7a1), *elf3-2* (line 246U2.7a1), *elf3-1 elf3-2* (line 246U2.7a2), *phyb-1*, and *LUX::*MYC. Whole plants were homogenized to powder using a freeze cold mortar. For each 50 mg of plant homogenized material was added 150 µl of freshly made 2 x Laemmli buffer (Bio-rad) supplemented with 50 mM DTT. The samples were immediately vortexed for 1 min and incubated at 70 ºC with 1,500 rpm agitation for 10 min. Samples were centrifuged at 20,000 x g for 5 min at 4 ºC. The supernatant was recovered and centrifuged again for 10 min. 15 ul of the supernatant were immediately used to load a 7.5 % Mini-PROTEAN TGX Stain-free precast gel (Bio-rad) followed by gel electrophoresis. The semi-dry transfer was performed using a PVDF blot in the Trans-blot TURBO system (Bio-rad). After transfer, the blot was imaged using a GelDoc imaging system (Bio-rad) to record total protein, used as loading control. The blot was then blocked for 1 h at room-temperature using Tris Buffer Saline (TBS: 20 mM Tris, 137 mM sodium chloride pH 7.6) supplemented with 1 % Tween20 (v/v) and 5 % skimmed milk powder (TBS-Tm) with 30 rpm agitation. Then, the blot was incubated over-night at 4 ºC with 30 rpm agitation in TBS-Tm supplemented with 1:1000 ELF3 antibody

(Agrisera custom made antibody AS184168, lot# 1808) or 0.5 µg/ml c-MYC antibody (Sigma, C3956, Lot# 059M4801V). In the next day, the blot was washed twice with TBS-T and incubated for 1 h at room-temperature and 25 rpm in TBS-Tm supplemented with 1:25.000 anti-rabbit antibody conjugated with horse-radish peroxidase (Agrisera #AS09 602). The blot was washed twice with TBS-T followed by a single wash with TBS to remove Tween. The blot was developed for 5 min with chemiluminescent detection reagent Superbright ECL (Agrisera) following manufacturer's instructions. The images were captured with a GelDoc imaging system (Bio-rad) where the blot was exposed for 15 s.

Yeast 2-hybrid

To test *in-vitro* interaction, the coding sequence of *ELF3-1* and *ELF3-2* were cloned into pGADT7 vector (Clontech) whereas the coding sequence of *LUX* was cloned in pGBKT7 (Clontech). *LUX* in pGBKT7 and either *ELF3* in pGADT7 vectors were used to transform *Saccharomyces cerevisiae* Y2HGold (Clontech). Positively transformed yeast grew in a synthetic defined medium lacking the amino acids leucine and tryptophan (SD). Interaction screening was performed in SD without adenine (-Ade) and histidine (-His). The interactions were evaluated in three individual colonies transformed with both plasmids. Empty vectors were used as negative controls. The experiment was performed twice.

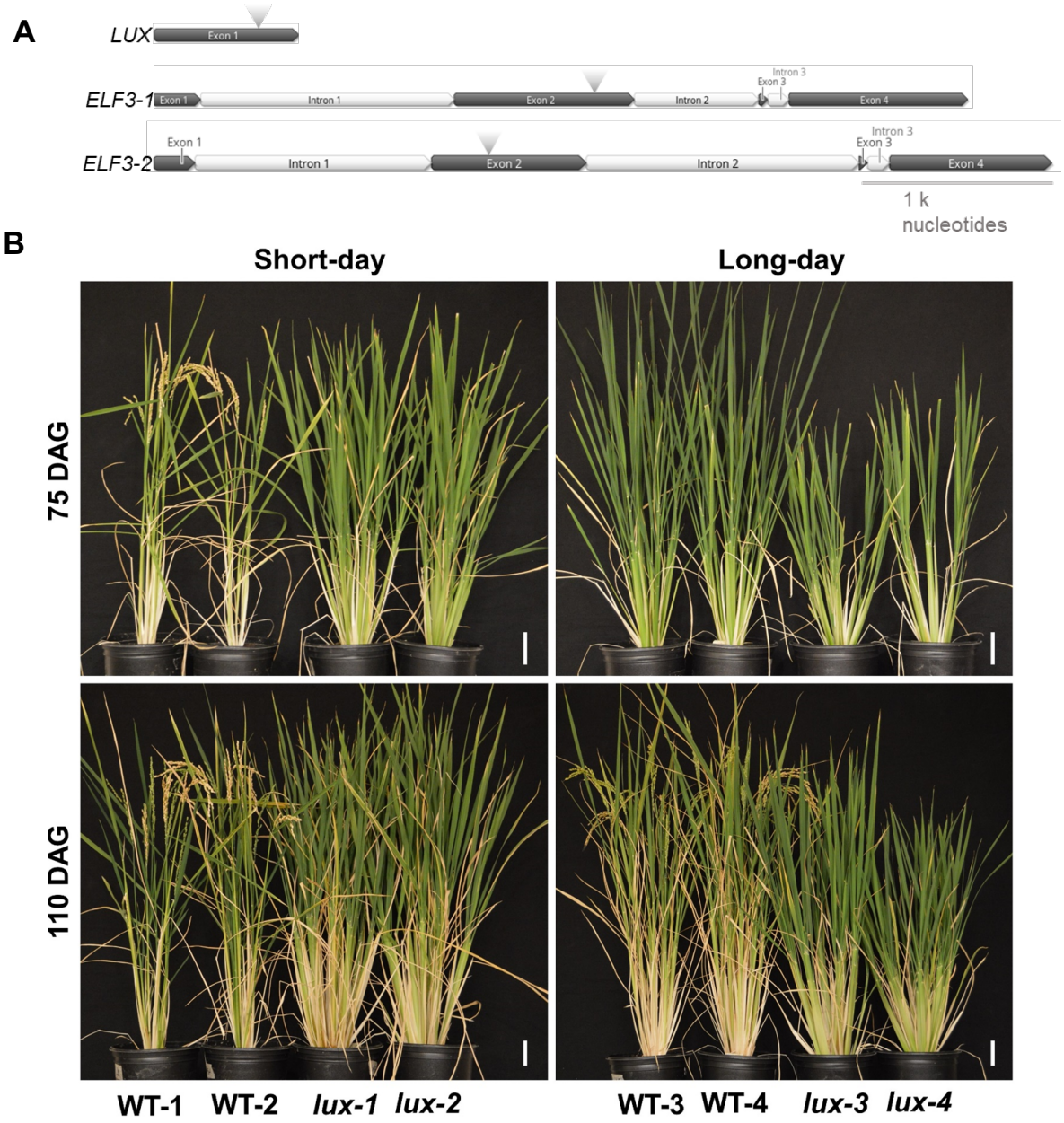

Fig. S1. Characterisation of CRISPR-Cas9 generated Evening Complex mutants in rice. A Schematic showing positions of CRISPR-Cas9 generated lesions in *LUX, ELF3-1* and *ELF3-2*. B Non-flowering phenotypes of *lux* plants. C Yeast 2-hybrid interactions between LUX and ELF3-1 and ELF3-2.

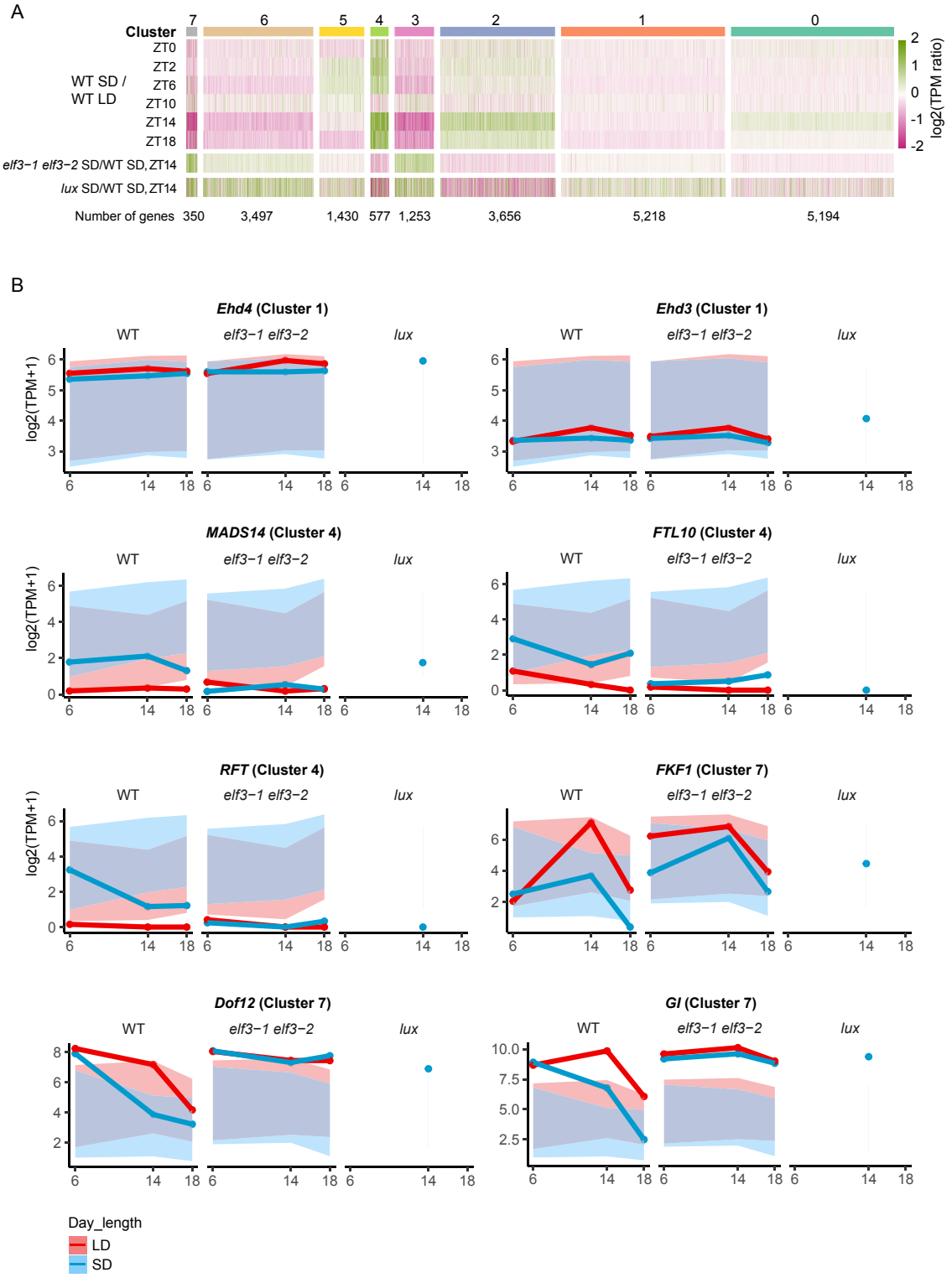

Fig. S2. Loss of Evening Complex activity renders rice insensitive to photoperiod at the transcriptome level. A Clustering of the rice photoperiod transcriptome in SD (10 h day, 14 h

night) compared to LD (14 h day, 10 h night). Values for log2(TPM ratio) > 2 or < -2 are transformed to the range of ± 2. B Key flowering genes that show a photoperiodic difference in their expression, especially in clusters 3, 4 and 7 lose this responseiveness in *elf3-1 elf3-2* (line 246U2.6a2: 1 nucleotide insertion in *ELF3-1* locus: 2 nucleotide deletion in *ELF3-2* locus).

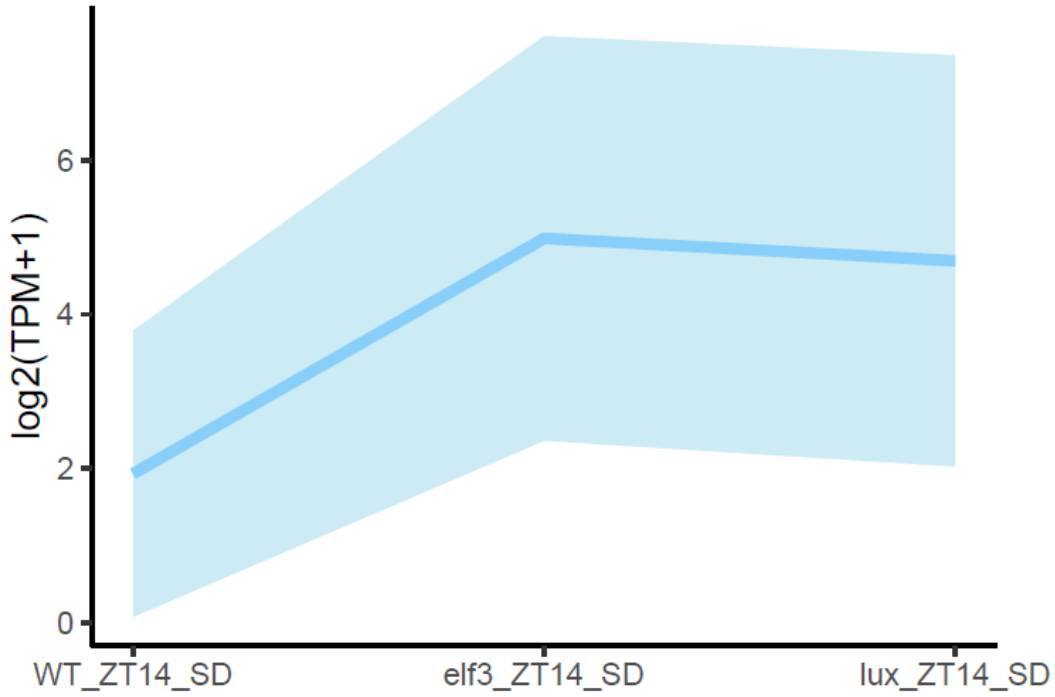

Fig. S3. **The *lux* mutant shows up-regulation of the same genes that are mis-regulated in *elf3-1 elf3-2* at ZT14 in SD**. Of the 54 genes up-regulated in *elf3-1 elf3-2*, we observe 40 of these are upregulated in *lux*, the blue line shows median expression and the shaded area is the range of expression for these 54 *elf3-1 elf3-2* dependent genes.

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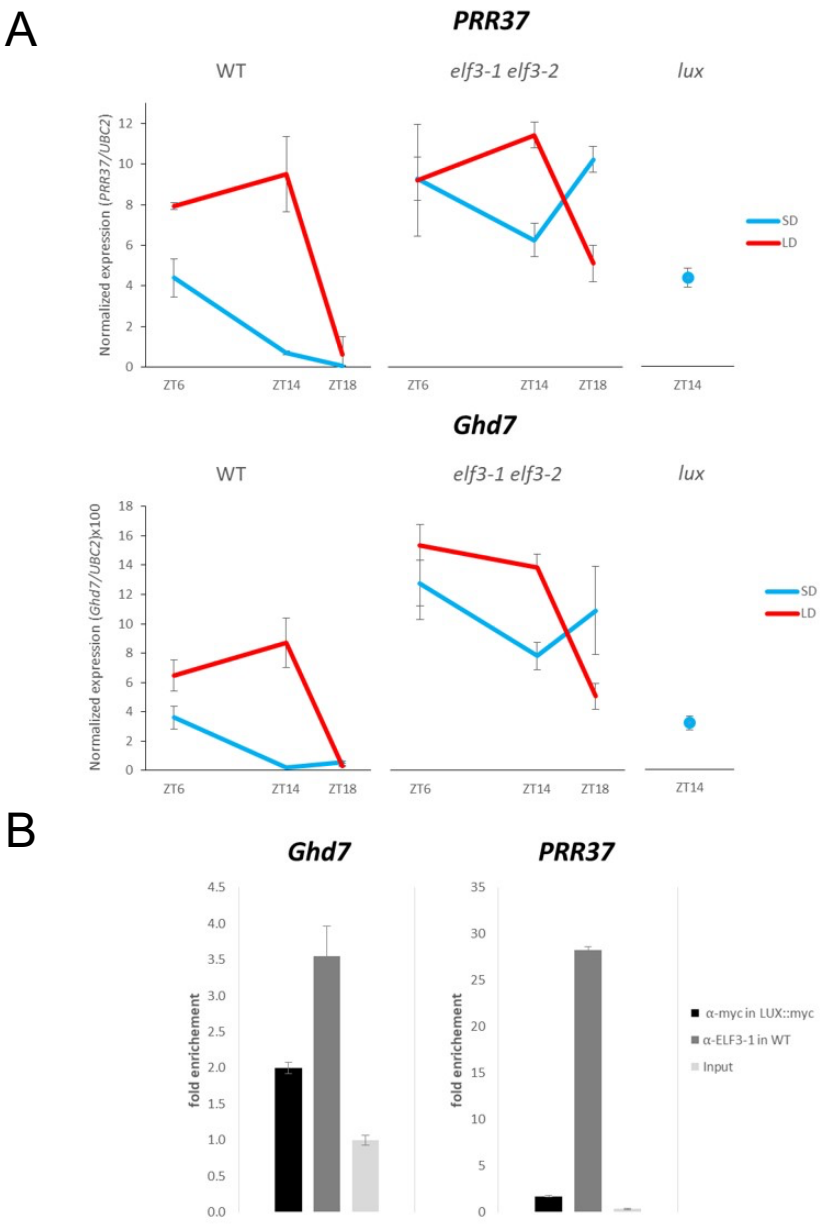

Fig. S4. **Confirmation of RNA-seq and ChIP-seq results**. **A** RT-qPCR validation of RNA-sequencing results showing *PRR37* and *Ghd7* up-regulation in *elf3-1 elf3-2* (line 246U2.6a2) and *lux* (line 120T7, 2 nucleotide insertion) mutants compared to wild-type. **B** RT-qPCR validation of chromatin immunoprecipitation-sequencing enrichment in *PRR37* and *Ghd7* promoter sequence in comparison to input (no antibody).

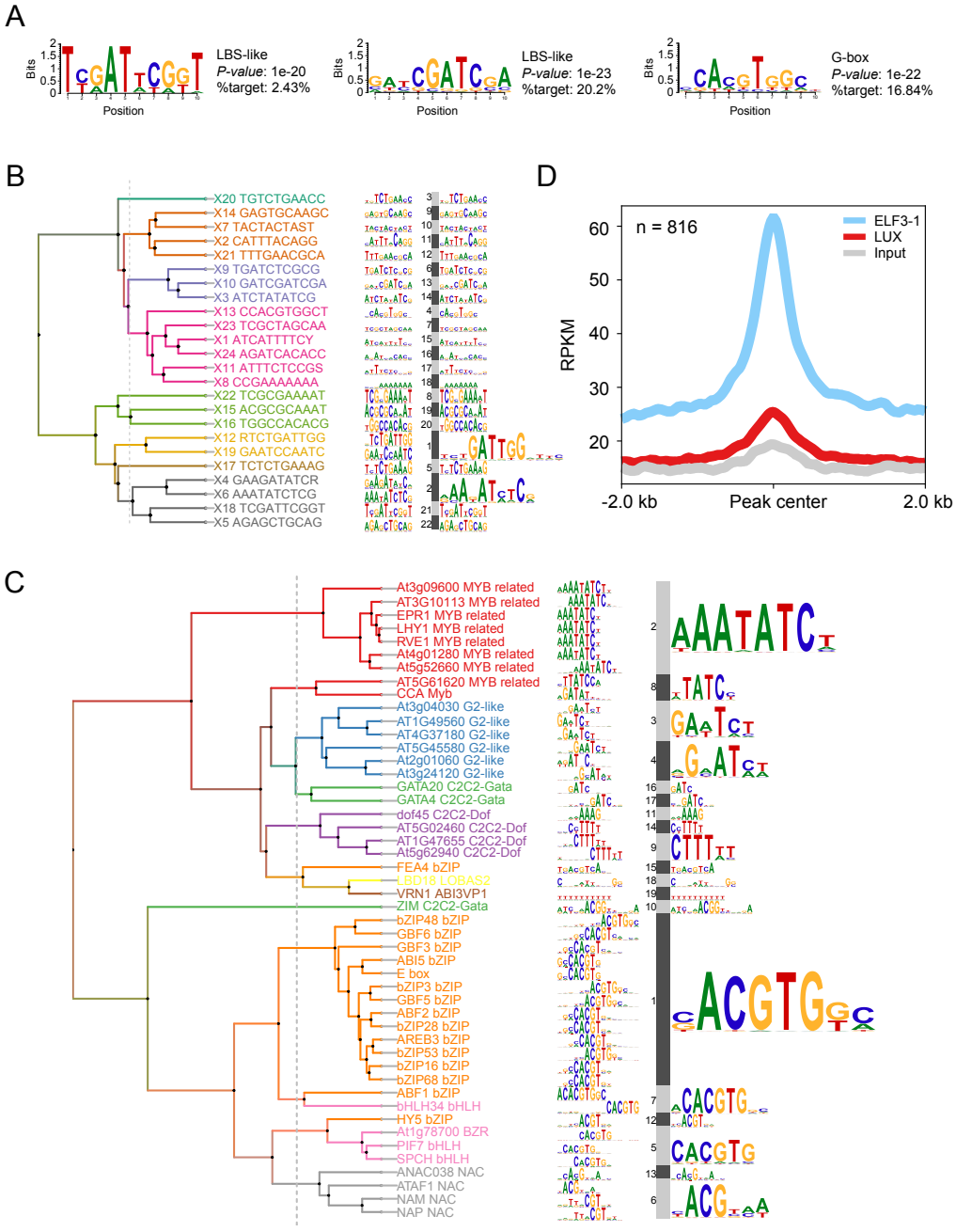

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Fig. S5. **ELF3-1 ChIP-seq peaks are enriched for LUX Binding Site (LBS) –like sequences. A** Representative LBS-like and G-box motifs identified *de novo* within ELF3-1 peaks using HOMER2. **B** Hierarchical clustering of *de novo* identified motifs within ELF3-1 peaks. **C** Hierarchical clustering of identified known motifs within ELF3-1 peaks. **D** Binding intensity profile for ELF3-1 peaks where LUX binding signal are also shown.

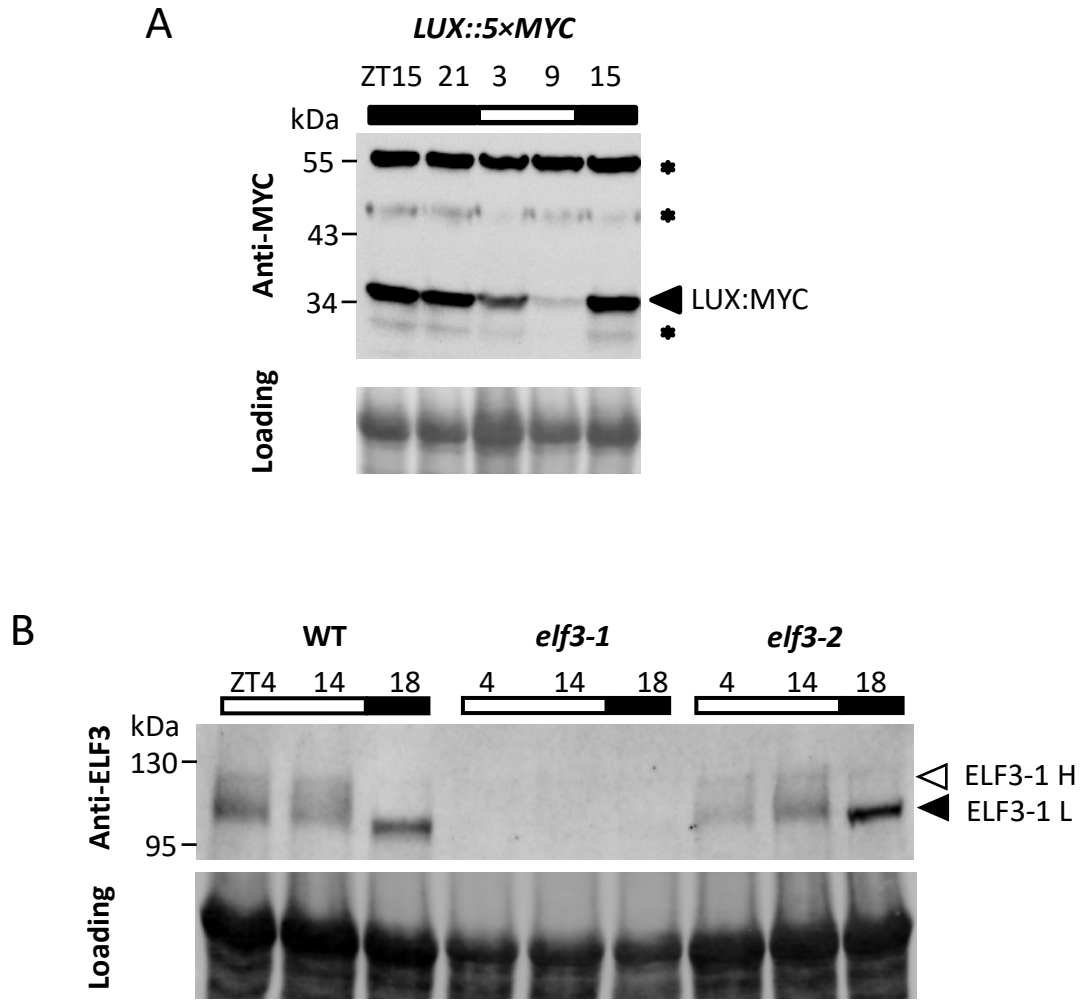

Fig. S6. Protein levels of Evening Complex proteins detected by immunoblotting. A Transgenic plants expressing LUX from its native promoter fused to a 5X Myc tag were immunoblotted with anti-Myc under ND (12 h day, 12 h night). Protein levels of LUX peak at the end of the day. B The ELF3 antibody is specific for ELF3-1, since no bands are detected in the *elf3-1* background (line 246U2.7a1: 2 nucleotide deletion in *ELF3-1* locus and 3 nucleotide deletion in *ELF3-2* locus). By contrast there is no apparent loss of signal in the *elf3-2* plants (obtained by segregating 246U1.6a1: 3 nucleotide deletion in *ELF3-1* locus and 2 nucleotide deletion in *ELF3-2* locus). Plants were grown under LD (14 h day, 10 h night) conditions and sampled at the times indicated. Note the considerable spreading and slower migration of the ELF3-1 signal in the day, consistent with a light mediated post-translational modification.

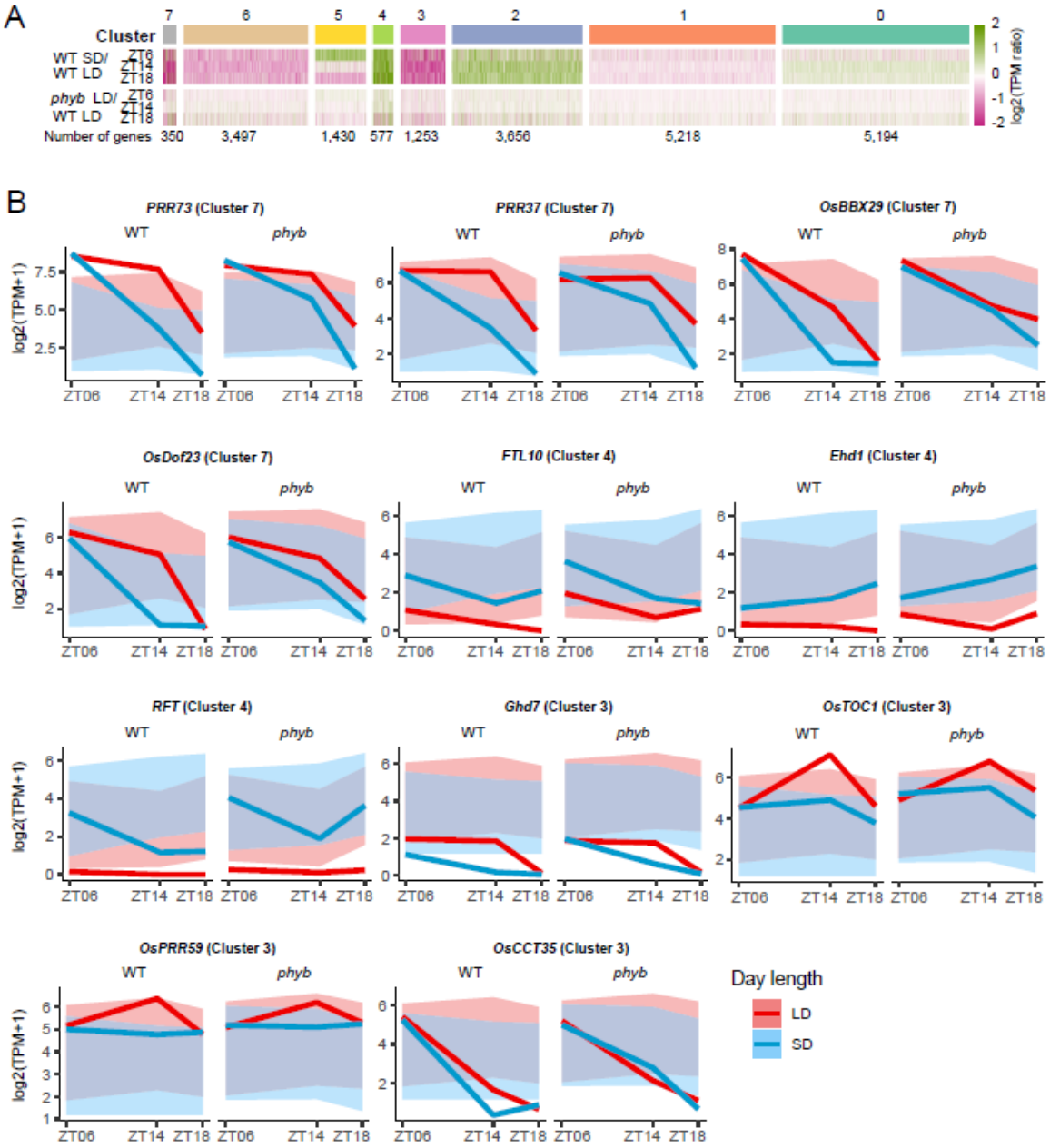

Fig. S7. ***phyb* causes a perturbation of the photoperiod transcriptome. A** Comparison of SD (10 h day, 14 h night) induced clusters expressed in WT with *phyb* shows those clusters that are repressed in WT in response to SD tend to be repressed in LD (14 h day, 10 h night) in *phyb* compared to WT, consistent with phyb having enhanced ELF3-1 activity and higher expression of the SD transcriptome. Values for log2(TPM ratio) > 2 or < -2 are transformed to the range of ± 2. **B** Specific genes that are bound by ELF3 directly show a reduced responsiveness to photoperiod in their expression in *phyb* compared to WT.

Fig. S8 GO terms enriched for cluster 0

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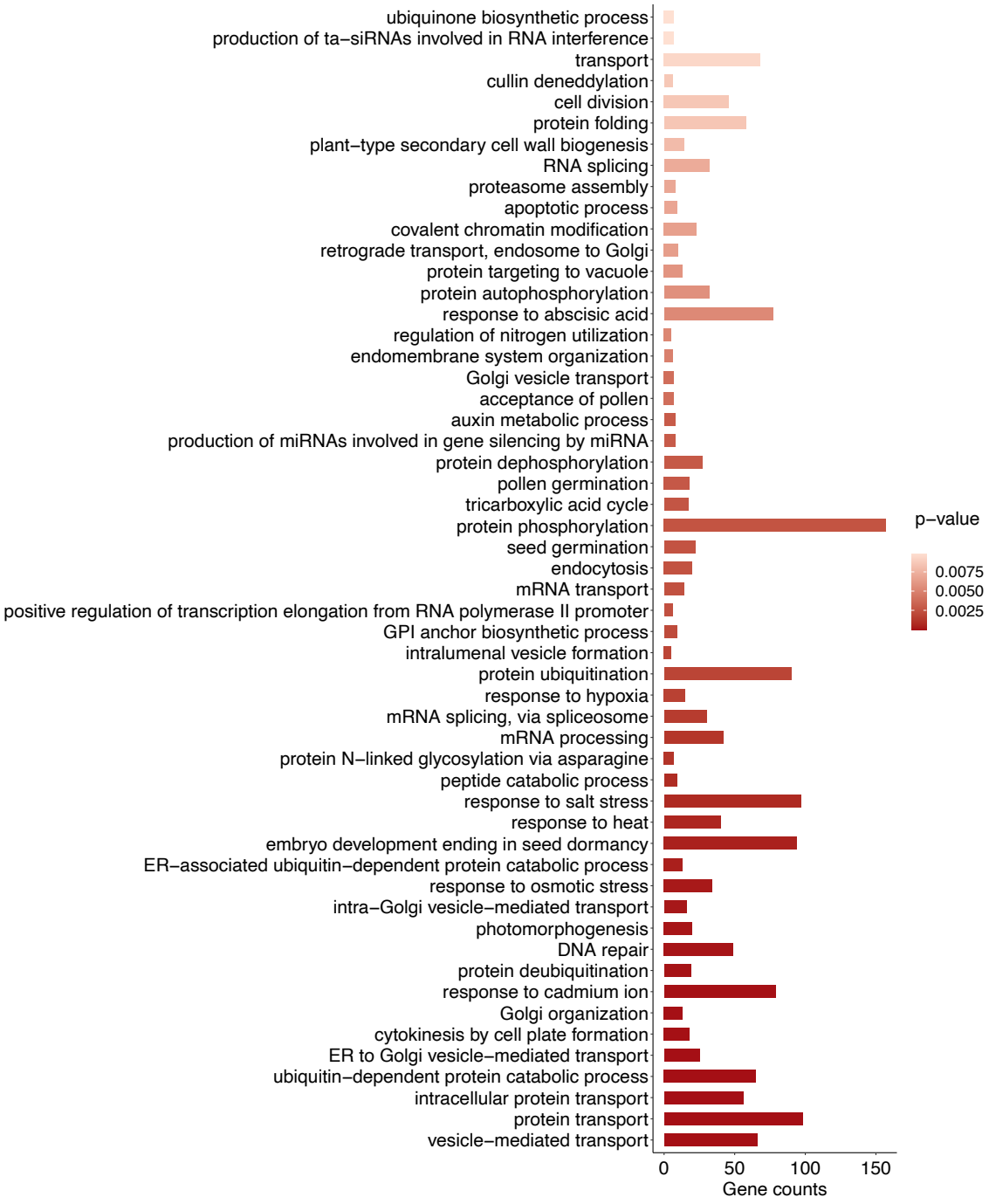

Fig. S9 GO terms enriched for cluster 1

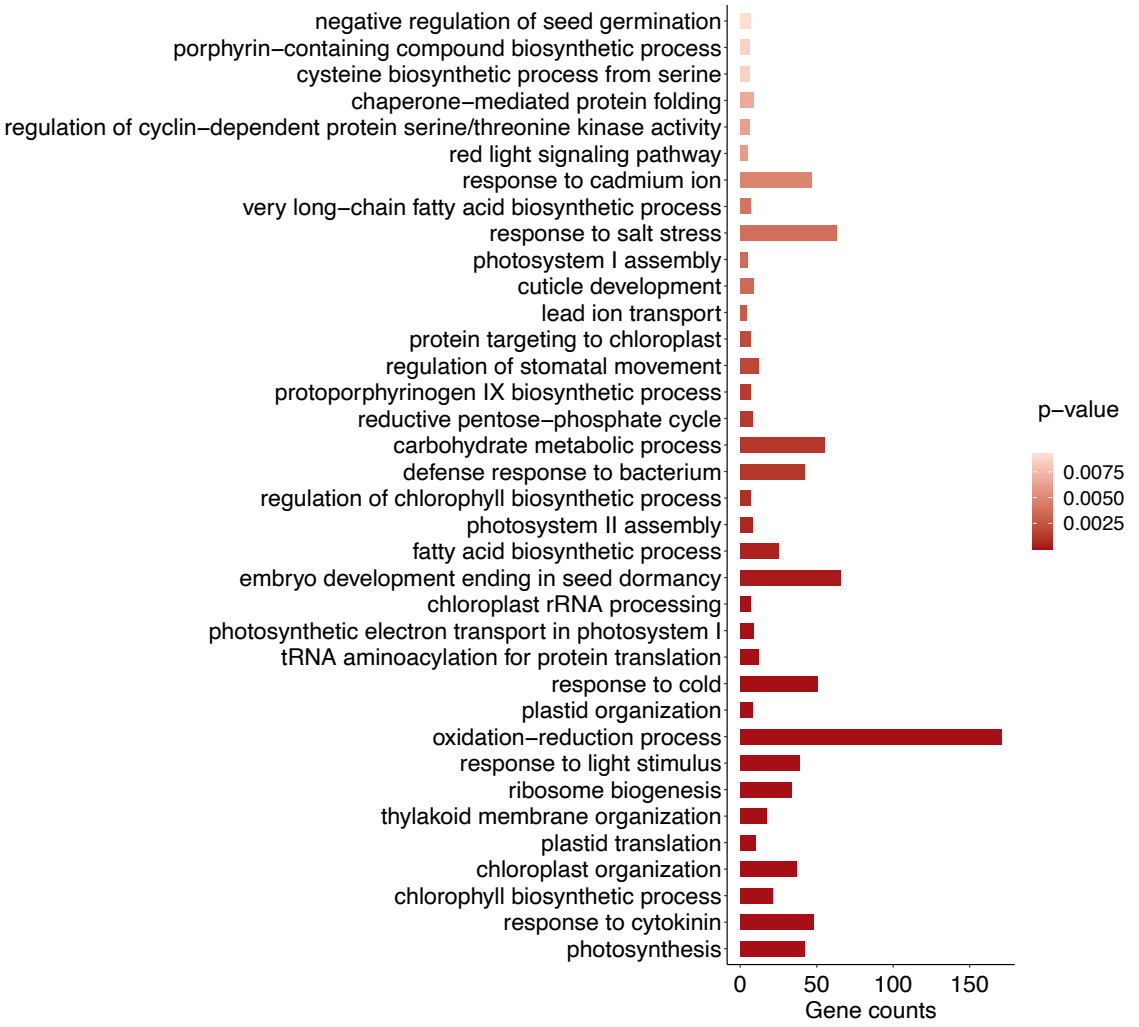

Fig. S10 GO terms enriched for cluster 2

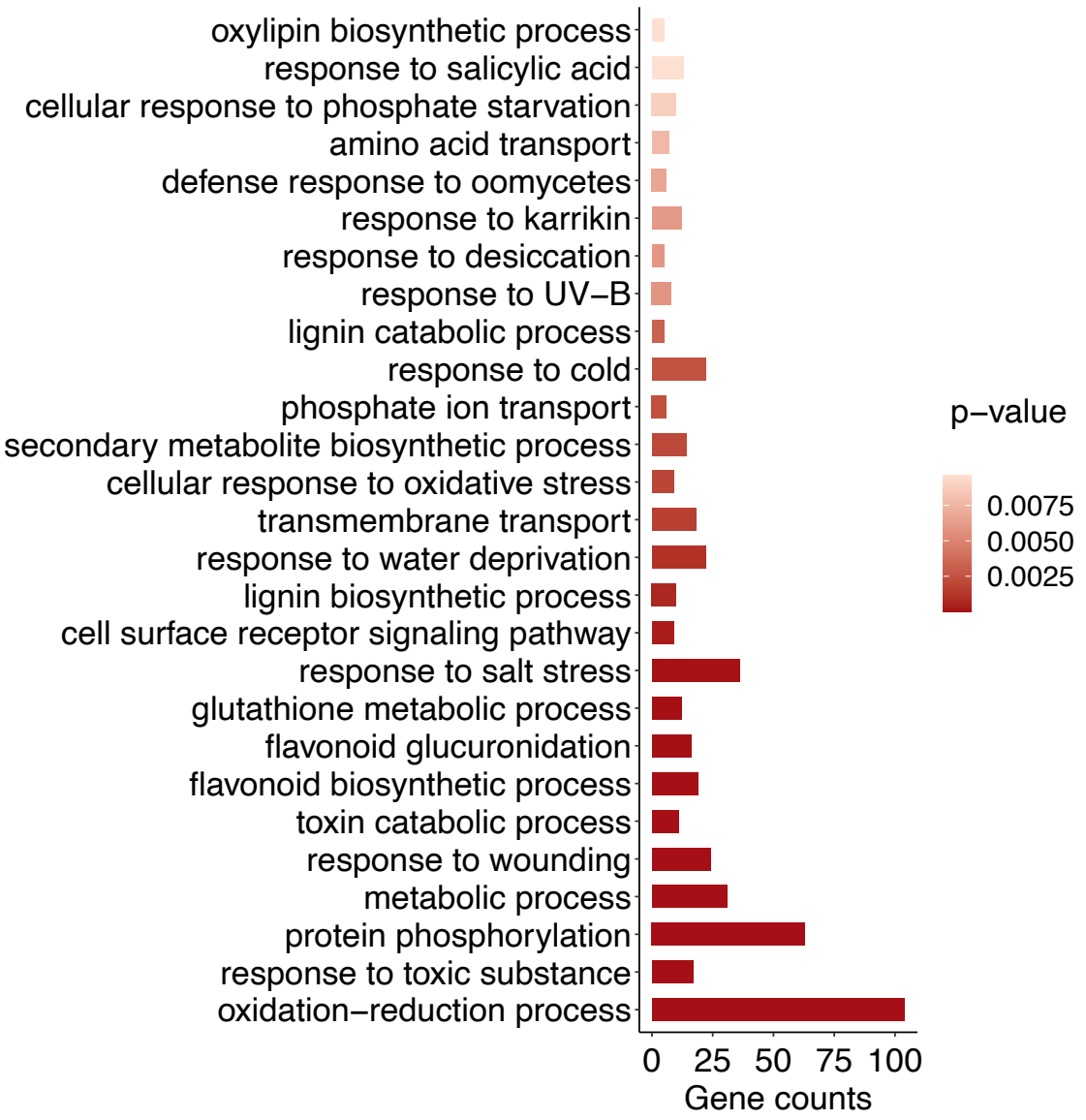

Fig. S11 GO terms enriched for cluster 3

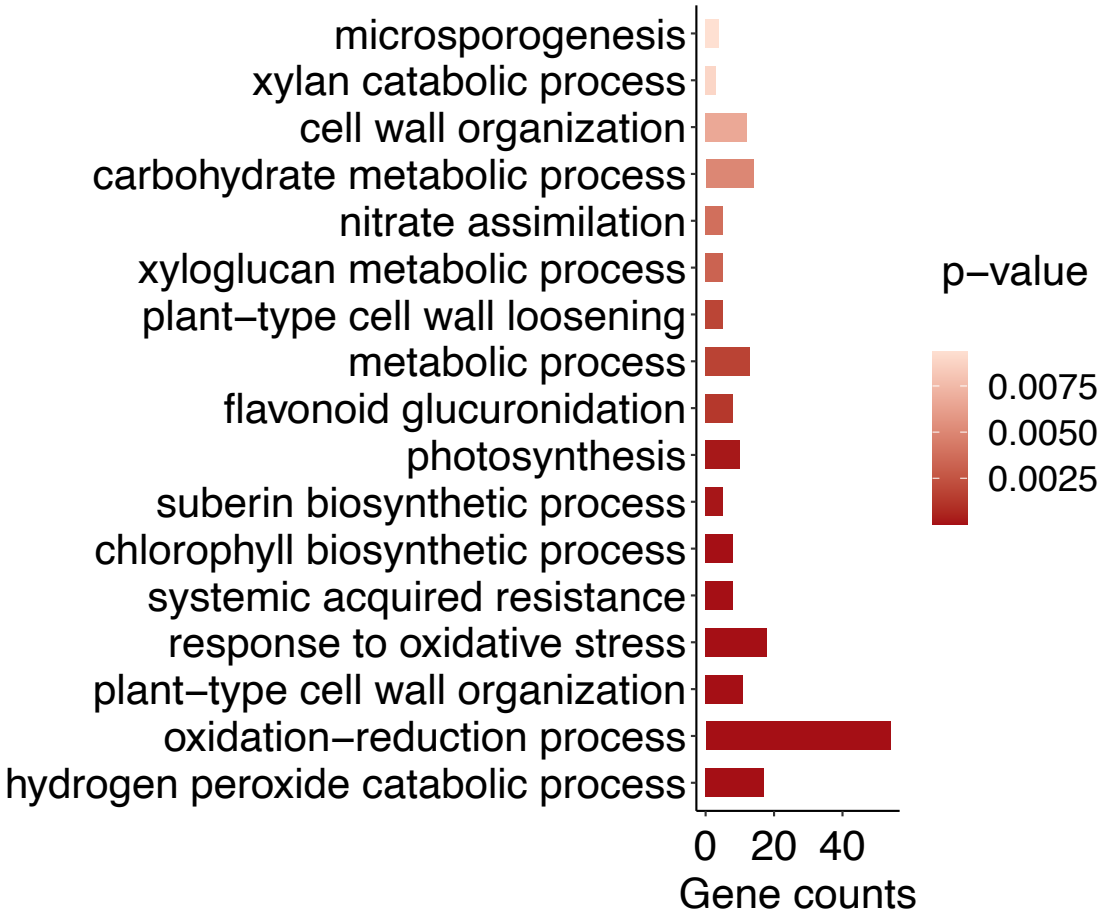

Fig. S12 GO terms enriched for cluster 4

Fig. S13 GO terms enriched for cluster 5

Fig. S14 GO terms enriched for cluster 6

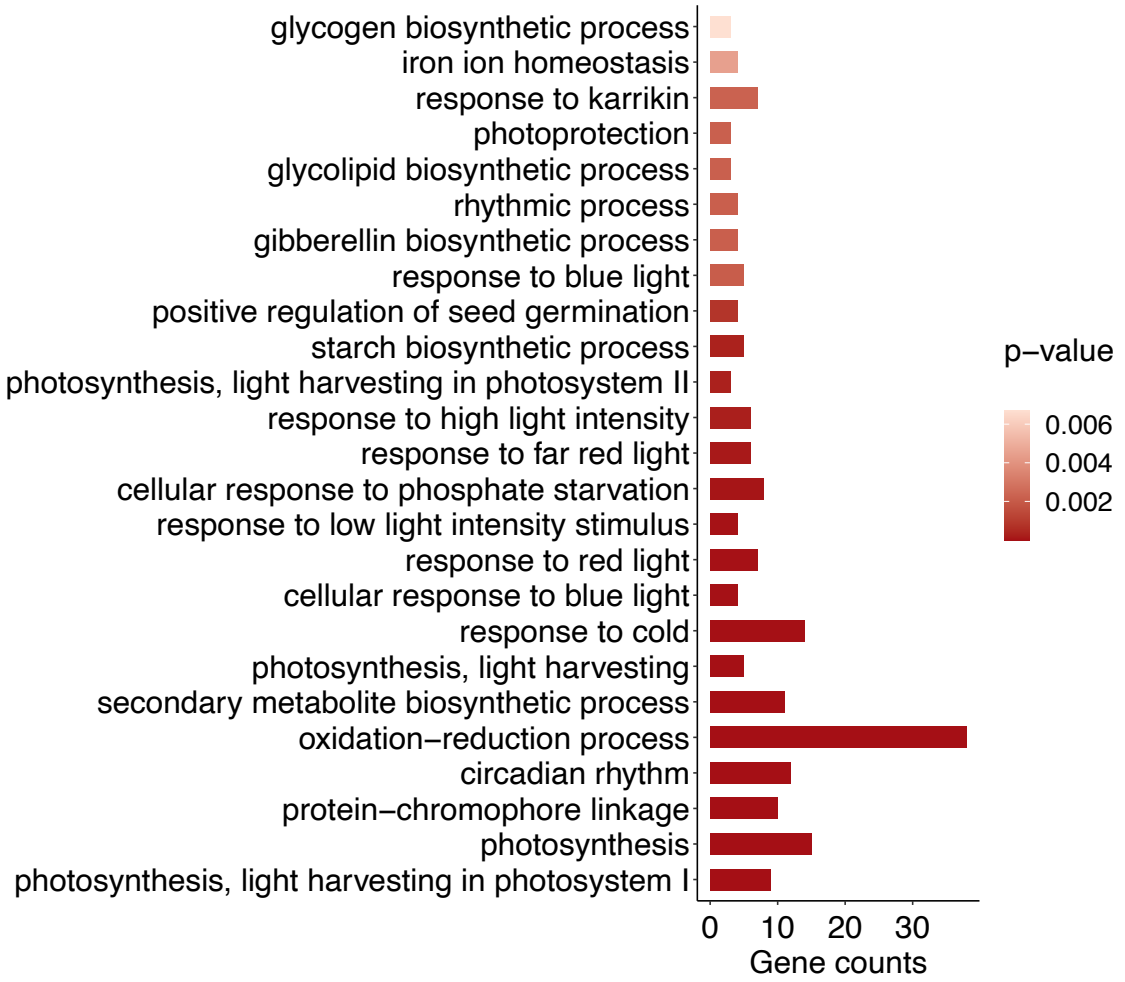

Fig. S15 GO terms enriched for cluster 7

Table S1. **Allelic mutations in *LUX*, *ELF3-1* and *ELF3-2* loci obtained by CRISPR/*Cas9*.** Cas9-induced mutations were verified in the T_0 generation of transformed rice plants by Sanger sequencing. The two sequences for each plant line represent the 2 copies for each gene, which may display the same mutation (homozygous) or, more commonly, be differentially mutated (heterozygous). Deleted nucleotides are represented by "-" while inserted nucleotides are represented by a letter in bold. In the InDel (insertions and deletions) column, the symbols “+” and “-” represent the addition or deletion of the represented number of nucleotides in the target region, respectively. In each sequence from the wild type. the Cas9 target site is represented in red and the PAM sequence is underlined in the wild-type sequence. Lines marked with "*" were selected for segregating *elf3-1* and *elf3-2* single mutants since one of the alleles did not cause a translation frame-shift.

Line	Mutations caused in *LUX* alleles by CRISPR/*Cas9*	InDel
wild-type	5′-GACGATCATGCAGCTGATGAACGTGGAGGGCCTCACCCGGGAGAACG-3′	0
69S4a-n	5′-GACGATCATGCAGCTGATGAACG----GGGCCTCACCCGGGAGAACG-3′ 5′-GACGATCATGCAGCTGATGAACGT**A**GGAGGGCCTCACCCGGGAGAACG-3′	-4 +1
70T4a	5′-GACGATCATGCAGCTGATGAACGT--------TCACCCGGGAGAACG-3′ 5′-GACGATCATGCAGCTGATGAACGT----GGCCTCACCCGGGAGAACG-3′	-8 -4
70T3a	5′-GACGATCATGCAGCTGATGAACGT----GGCCTCACCCGGGAGAACG-3′ 5′-GACGATCATGCAGCTGATGAACG--GAGGGCCTCACCCGGGAGAACG-3′	-4 -2
71U5c	5′-GACGATCATGCAGCTGATGAACG--GAGGGCCTCACCCGGGAGAACG-3′ 5′-GACGATCATGCAGCTGATGAACGT**T**GGAGGGCCTCACCCGGGAGAACG-3′	-2 +1
75Z3a	5′-GACGATCATGCAGCTGATGAACGT--AGGGCCTCACCCGGGAGAACG-3′ 5′-GACGATCATGCAGCTGATGAACGT--AGGGCCTCACCCGGGAGAACG-3′	-2 -2
120T7	5′-GACGATCATGCAGCTGATGAACGT**AG**GGAGGGCCTCACCCGGGAGAACG-3′ 5′-GACGATCATGCAGCTGATGAACGT**AT**GGAGGGCCTCACCCGGGAGAACG-3′	+2 +2
120T9	5′-GACGATCATGCAGCTGATGA--------------ACCCGGGAGAACG-3′ 5′-GACGATCATGCAGCTGATGAA--------------CCCGGGAGAACG-3′	-14 -14
	Mutations caused in *ELF3-1* alleles by CRISPR/*Cas9*	
wild-type	5′-CTGATAGGCATTATAATTTACCGCAAGGAGGCATAGAGGAAACAGGC-3′	0
246U1.2a	5′-CTGATAGGCATTATAATTTACCGCA-GGAGGCATAGAGGAAACAGGC-3′ 5′-CTGATAGGCATTATAATTTACCGCA--GAGGCATAGAGGAAACAGGC-3′	-1 -2
246U1.6a*	5′-CTGATAGGCATTATAATTTACCGC-AGGAGGCATAGAGGAAACAGGC-3′ 5′-CTGATAGGCATTATAATTTACCGCA---AGGCATAGAGGAAACAGGC-3′	-1 -3
246U2.4a	5′-CTGATAGGCA----------------GAGGCATAGAGGAAACAGGC-3′ 5′-CTGATAGGCA----------------GAGGCATAGAGGAAACAGGC-3′	-17 -17
246U2.6b	5′-CTGATAGGCATTATAATTTA------GGAGGCATAGAGGAAACAGGC-3′ 5′-CTGATAGGCATTATAATTTACCG----GAGGCATAGAGGAAACAGGC-3′	-6 -4
246U2.7a*-b	5′-CTGATAGGCATTATAATTTACCGC--GGAGGCATAGAGGAAACAGGC-3′ 5′-CTGATAGGCATTATAATTTACCG---GGAGGCATAGAGGAAACAGGC-3′	-2 -3
246U5.2a	5′-CTGATAGGCATTATAATTTACCGCA-GGAGGCATAGAGGAAACAGGC-3′ 5′-CTGATAGGCATTATAATTTACCGCA----GGCATAGAGGAAACAGGC-3′	-1 -4
246U6.2a	5′-CTGATAGGCATTATAATTTACCGCAA**T**GGAGGCATAGAGGAAACAGGC-3′ 5′-CTGATAGGCATTATAATTTACCGCAA**T**GGAGGCATAGAGGAAACAGGC-3′	+1 +1
	Mutations caused in *ELF3-2* alleles by CRISPR/*Cas9*	
wild-type	5′-GGTTCAAGAGGAATCAACACCCCTTGTTGCTCTCAGTCCGCACAAAAG-3′	0
246U1.2a	5′-GGTTCAAGAGGAATCAACACCCCTT------CTCAGTCCGCACAAAAG-3′ 5′-GGTTCAAGAGGAATCAACACCC-----TGCTCTCAGTCCGCACAAAAG-3′	-6 -5
246U1.6a*	5′-GGTTCAAGAGGAATCAACACCCCTT--TGCTCTCAGTCCGCACAAAAG-3′ 5′-GGTTCAAGAGGAATCAACACCCCTTG-TGCTCTCAGTCCGCACAAAAG-3′	-2 -1
246U2.4a	5′-GGTTCAAGAGGAATCAACACCCCTT--TGCTCTCAGTCCGCACAAAAG-3′ 5′-GGTTCAAGAGGAATCAACACCCCTTGT**T**TGCTCTCAGTCCGCACAAAAG-3′	-2 +1
246U2.6b	5′-GGTTCAAGAGGAATCAACACCCCTT--TGCTCTCAGTCCGCACAAAAG-3′ 5′-GGTTCAAGAGGAATCAACACCCCTTGT**T**TGCTCTCAGTCCGCACAAAAG-3′	-2 +1
246U2.7a*-b	5′-GGTTCAAGAGGAATCAACACCCCTTG---CTCTCAGTCCGCACAAAAG-3′ 5′-GGTTCAAGAGGAATCAACACCCCTTG---CTCTCAGTCCGCACAAAAG-3′	-3 -3
246U5.2a	5′-GGTTCAAGAGGAATCAACACCCC----TGCTCTCAGTCCGCACAAAAG-3′ 5′-GGTTCAAGAGGAATCAACACCCCTTGT**T**TGCTCTCAGTCCGCACAAAAG-3′	-4 +1
246U6.2a	5′-GGTTCAAGAGGAATCAACACCCCTT--TGCTCTCAGTCCGCACAAAAG-3′ 5′-GGTTCAAGAGGAATCAACACCCCTT--TGCTCTCAGTCCGCACAAAAG-3′	-2 -2

Table S2. Oligonucleotides used in this study. The locus names used are based on the rice annotation project database (RAP-DB). The upper and lower case letters in oligos intended for cloning purposes indicate the portion of the sequence used for PCR amplification and for fragment annealing when doing NEBuilder® HiFi DNA Assembly Cloning Kit (New Eand Biolabs), respectively.

Primer name	Locus	Sequence (5’ – 3’)	Objective
LUX_vFw1	Os01g0971800	cgcggtaccccgaATCCTGTGGAGCGTCAAG	Clone LUX promoter region
LUX_vRv1	Os01g0971800	tatactagcaattTCGAATCAAATGAAATATAGACTTC	
LUX_vFw2	Os01g0971800	tcatttgattcgaAATTGCTAGTATATTATTTATGGGATATGGGTG	Clone LUX genomic region; modify the last nucleotide of LUX gene to remove stop codon
LUX_vRv2	Os01g0971800	ttgctccatagccCAGTGGTTGGCGTGGTGG	
LUX_vFw3	Os01g0971800	ttgggcgacctcaccatggagtgacTCCATTCCTTCTCATCATC	Clone LUX 3' UTR region
LUX_vRv3	Os01g0971800	ttattgccaaatgtttgaacgatccATTAGTATTATCTATGATGAATGGATATG	
HPTII_Fw1	Non applicable	AATAGCTGCGCCGATGGTTTCTACA	Detect the transformation cassette in new lines
HPTII_Rv1	Non applicable	AACATCGCCTCGCTCCAGTCAATG	
ELF3-1_Fw1	Os06g0142600	TCAAGCGACAAGCAGAAATGG	Genotype ELF3-1 locus for Cas9-mediated mutation
ELF3-1_Rv1	Os06g0142600	AAACCTGGACAGCAAACACC	
ELF3-2_Fw1	Os01g0566100	TCGGGTGAACTAACAGGTGG	Genotype ELF3-2

ELF3-2_Rv1	Os01g0566100	ACTCCCGTTTCTACTTGTCGC	locus for Cas9-mediated mutation
LUX_Fw1	Os01g0971800	AGGAGTACGAGCTGGGCGGC	Genotype LUX locus for Cas9-mediated mutation
LUX_Rv1	Os01g0971800	TCAGTGGTTGGCGTGGTGGT	
LUX_Fw2	Os01g0971800	TCCACGACCAGGTTCCTTCT	Determine LUX::MYC expression
MYC_Rv1	Non applicable	GCTTTTGCTCCATAGCCCAG	
UBC2_qFw1	Os02g0634800	TTGCATTCTCTATTCCTGAGCA	RT-qPCR amplification of *UBC2* gene fragment for RNA-seq validation
UBC2_qRv1	Os02g0634800	CAGGCAAATCTCACCTGTCTT	
PRR37_qFw1	Os07g0695100	CCTCCTGTCGAAGGTCATGC	RT-qPCR amplification of *PRR37* gene fragment for RNA-seq validation
PRR37_qRv1	Os07g0695100	CTCCCATTCTGCCCATTGCT	
Ghd7_qFw1	OS07G0261203	GTGGTTGGCGCAATGGTG	RT-qPCR amplification of *Ghd7* gene fragment for RNA-seq validation
Ghd7_qRv1	OS07G0261203	TTCTCGTAGCACCTCTTCTTCC	
PRR37_qFw2	Os07g0695100	CCAGCATTGACGGGAGATG	RT-qPCR amplificati

PRR37_qRv2	Os07g0695100	CTTGCGCTCCCCGAGATCC	on of *PRR37* promoter fragment for ChIP-seq validation
Ghd7_qFw2	OS07G0261203	GCAGGATCTCTCTCTCGCTGG	RT-qPCR amplification of *Ghd7* promoter fragment for ChIP-seq validation
Ghd7_qRv2	OS07G0261203	CTTGTGGCTTCTCCCCGTC	
ChCtl_qFw1	Os07g0167100	AGACTAAATGCTGCGTAGTGC	RT-qPCR amplification of *desert region* for control ChIP-seq validation
ChCtl_qRv1	**Os07g0167100**	**ACAAAGAGACCTGCATGTCC**	

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Supplementary Datasets

Dataset S1: RNA-seq gene expression values used in this study

Dataset S2: Gene lists for every cluster described in Fig. 2A

Dataset S3: ELF3 bound genes identified by ChIP-seq