

Supplemental Fig 1. Western blot of the tagged EC components and phyB in the lines used in this study under the different conditions used for ChIP-Seq. (A) a, LUX-GFP elf3-1; b, LUX-GFP. (B) a, ELF3-MYC; b, ELF3-MYC lux-4. (C) ELF4-HA. (D) PHYBpro::PHYB-myc phyB-9 (PHYB-MYC). (E) ELF3-MYC.



Supplemental Fig 2. *The genomic ELF3-MYC construct rescues the long hypocotyl phenotype of elf3-1.* The *ELF3-MYC* transgenic plant, which was used in ChIP-seq experiments, was constructed by expressing a 7.8 kb genomic fragment of *ELF3* including its promoter in the *elf3-1* mutant. (A) Seedlings were grown at the indicated temperatures for 7 days under short photoperiods. Scale bars, 5mm. (B) Hypocotyl length boxplots for the indicated genotypes grown at different temperatures as in A. Each box is bounded by the lower and upper quartiles, the central bar represents the median, and the whiskers indicate minimum and maximum values. (C) Expression of *ELF3* at 22°C. Ten-day-old whole seedlings grown under short photoperiods were harvested at both ZT0 and ZT8 time points for total RNA extraction. All RT-qPCRs were performed in biological triplicates, as described in Matt et al, 2015. Please note that the *ELF3* gene is expressed in the *elf3-1* and the *ELF3-MYC* transgenic plant.



Supplemental Fig 3. *Follow-up ChIP-seq experiments*. (A) A temperature shift experiment was conducted in which plants were shifted from 17°C to 27°C at ZT8 and were sampled at ZT9 and ZT10. Note that the plants that were grown at constant 17°C and were harvested at ZT10 are the samples analyzed in **Fig 1B and 2A,B**. (B) There was a reduction in peak intensity (as calculated by MACS2) for the plants that were shifted to 27°C (each line represents a peak identified in the LUX ChIP-seq at 22°C) (C) Additional LUX ChIP-seq experiments were conducted at constant 22°C at ZT8 and ZT12—here they are compared to the ZT10 data. (D) Additional ELF3 ChIP-seq experiments were conducted at 22°C over a 24-hour time course.

Here we show peaks near PRR7 (i), PRR9 (ii), GI (iii) and PIF4 (iv), which are representative of the pattern we see at most EC target genes.



Supplemental Fig 4. *LUX is capable of binding to target promoters in the absence of ELF3*. (A) The predicted target genes of a LUX ChIP-seq in *elf3-1* are compared with those from a control LUX ChIP-seq at ZT8. (B) A comparison is also made between these predicted target genes at ZT8 and the predicted target genes of LUX, ELF3, and ELF4 at ZT10, all at 22°C— these three samples are the ones shown in **Fig2A**. It is important to remember that two of these samples were collected at ZT8 ("LUX in *elf3-1*" and "LUX (control)") and the rest were collected at ZT10. (C) These plots using the Integrated Genome Viewer (IGV) show that the LUX peaks appear similar in the control and *elf3-1* backgrounds near key circadian targets.

	Rank Motif		P-	log P-	% of	% of	STD(Bg	Best Match/Details
LUX 22°C:			value	pvalue	Targets	Background	STD)	
	1	<u><u><u></u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u><u></u></u>	Ie- 17	-3.993c+01	76.19%	4.50%	36.5bp (83.4bp)	E-box/Arabidopsis-Promoters/Homer(0.954) More Information Similar Motifs Found
	2*	CGAGATAGGCTA	1e- 10	-2.340e+01	19.05%	0.03%	18.5bp (67.0bp)	ARR18/MA0948.1/Jaspar(0.626) More Information Similar Motifs Found
	3*	ASAATCSAAIGC	1e-7	-1.789c+01	14 .29%	0.02%	77.1bp (38.4bp)	MF0011.1_HMG_class/Jaspar(0.593) More Information Similar Motifs Found
	4*	T <u>STEASAATS</u>	10-7	-1.719e+01	38.10%	2.63%	65.0bp (69.9bp)	MF0011.1_HMG_class/Jaspar(0.627) More Information Similar Motifs Found
	5*	CGACTAAATAAA	1e-7	-1.710e+01	14. <mark>29%</mark>	0.03%	69.8bp (62.2bp)	TBP(- other)/several species/AthaMap(0.595) More Information Similar Motifs Found
ELF4 22°C:	Rank Motif		P- value	log P-	% of Targets	% of Backgroup	STD(Bg	Best Match/Details
	1	ISCACCTSI	1e- 26	-6.200e+0	68.57%	3.36%	47.2bp (85.6bp)	ABI5/MA0931.1/Jaspar(0.956) More Information Similar Motifs Found
	2 *	AAAAAGATAT	le-1	1 -2.643e+0	1 34.29%	2.07%	39.0bp (79.2bp)	SD0003.1_at_AC_acceptor/Jaspar(0.744) More Information Similar Motifs Found
	3 •	AAAGEGGGCEII	le-1	1 -2.637e+0	1 14.29%	0.04%	45.7bp (61.5bp)	Dof3/MA0021.1/Jaspar(0.706) More Information Similar Motifs Found
	4•	CASGTCASCT	le-1	I -2.607c+0	1 20.00%	0.26%	50.4bp (93.3bp)	TGA2/MA1068.1/Jaspar(0.853) More Information Similar Motifs Found
	5*	ALLETAAGCCAC	le-1	1 -2.566e+0	17,14%	0.13%	47.3bp (47.0bp)	POL004.1_CCAAT-box/Jaspar(0.560) More Information Similar Motifs Found
ELF3 22°C:	Rank Motif		P- val	log P- ue pvalue	% of Target	% of Backgrou	nd STD(Bg	Best Match/Details
	1	FFCACGTCACE	1e- 40	-9.360e+0	01 25.079	\$ 4.36%	52.5bp (70.4bp)	bZIP68/MA0968.1/Jaspar(0.914) More Information Similar Motifs Found
	2	<u><u><u>CTTETAAATCT</u></u></u>	le- 19	-4.522c+0	91 8.45%	0.86%	55.9bp (78.2bp)	AGP1(GATA)/Nicotiana tabacum/AthaMap(0.663) <u>More Information</u> 1 <u>Similar Motifs Found</u>
	3	<u>GTGGACCAATGA</u>	1e- 19	-4.431e+()1 4.79%	0.16%	57.3bp (59.2bp)	CBF(- other)/several species/AthaMap(0.658) More Information Similar Motifs Found
	4	<u>GTGGGGGCCCA</u>	1e- 17	-3.993e+6	01 7.61%	0.80%	51.4bp (76.0bp)	TCP19/MA1063.1/Jaspar(0.878) More Information Similar Motifs Found
	5	TTTAAAAgeeit	1e- 16	-3.744e+	01 7.04%	0.73%	52.7bp (88.7bp)	POL012.1_TATA-Box/Jaspar(0.583) More Information Similar Motifs Found
	6	AICCCTTGTA	1e- 15	-3.556e+4	01 6.48%	0.64%	58.6bp (89.4bp)	HMG-1/MA0044.1/Jaspar(0.579) More Information I Similar Motifs Found
	7	TTGTGGCTCA	1e- 12	-2.971c+(01 6.20%	0.76%	63.3bp (61.2bp)	RAV1(1)(AP2/EREBP)/Arabidopsis thaliana/AthaMap(0.609) More Information Similar Motifs Found

Supplemental Fig 5. *Homer2 top motifs for 22°C, ZT10 ChIP-seqs in Fig. 1B.*



Supplemental Fig 6. *A closer look at G-boxes under EC peaks.* (A) This figure illustrates the positioning of G-boxes and LBS motifs (see Fig 1E), relative to the centre of the predicted peak. The score against the PWM is determined by its transparency: > indicates that the motif is only identified on the forward strand, < indicates that the motif is only identified in the reverse strand and >< indicates bi-directionality. (B) This is the same as **Fig S5A**, except centred on the strongest G-box. (C) This is a subset of the DAP-seq data showing the occurrence of binding *in vitro* of transcription factors (rows) with ELF4 peaks (columns)—red indicates a binding event. Colour bars in the top indicate whether the peak contains a G-box, LBS, both or none. Color bars in the side indicate the transcription factor family. Note that bZIPs bind to G-boxes.



Supplemental Fig 7. The EC targets genes overlap extensively between *independent experiments*. A number of genes related to photosynthesis (green), the circadian cycle (blue), temperature (red) and growth (orange) are specifically mentioned in the text as possible EC targets. This Venn diagram indicates which ChIP-seq experiments predicted each of these target genes. This is not an exhaustive list of the target genes (See **Table S6**), but it highlights all of the key genes mentioned in the main text.



log-fold change in expression in *lux-4/Col-0*

Supplemental Fig. 8: Gene expression pattern of putative targets

discussed in main text. (A) Putative targets discussed in the main text are highlighted, color-coded by their functional category. (B) All of the color-coded genes are labelled by name for ZT16. Recall that the natural log is used throughout this manuscript.



Supplemental Fig 9. *Expression pattern in lux-4 and elf3-1 across entire time course.* This figure contains the complete set of scatterplots comparing the log-fold change in expression across all time points. Recall that the natural log is used throughout this manuscript.



Supplemental Fig 10. *Expression pattern of LUX 17*°C *predicted gene targets*. The same scatterplots are drawn as in Fig. S9, except that LUX 17°C target genes are highlighted. Recall that the natural log is used throughout this manuscript.



Supplemental Fig 11. *Genome-wide expression pattern in elf3-1 and lux-***4.** All genes that were within the top 5% most differentially expressed in *elf3-1* or *lux-4* in any time point were clustered. In particular, the genes were clustered by their z-score (their TPM's were normalised so that the mean is zero and the standard deviation in 1). Note that the z-scores were calculated across *all* of the samples (i.e. the entire row including samples from Col-0, *elf3-1*, and *lux-4*). The hierarchical clustering was done using default parameters in heatmap.2 in R. There are a total of 9186 genes that were in the top 5% most differentially expressed in at least one time point—of these 877 are within 3000bp of at least one EC component (ELF3, ELF4 and LUX at 22°C or LUX at 17°C)







(so you can use the slope to fit these models)

Supplemental Fig 13. Predicting the rate of repression by EC for circadian targets. Given that we see similar degree of change of expression in elf3-1 vs. Col-0 and lux-4 vs. Col-0 among circadian targets of the EC (note that the line plot is taken from Fig. 3A) we wanted to know what determines the rate of repression by the EC. To do so, we fit a linear model of the slope shown in the figure, given the expression of each EC component (interpolated to the midpoint). The equation listed near "recall" explains how this linear regression is roughly equivalent to fitting the models listed under "alternative models"-the form of these "alternative models" is reminiscent of the type of models that are used to analyse simple gene expression networks with a system of Ordinary Differential Equations (ODEs). Please note that +EC models were used to form the "ELF3+EC", "ELF4+EC", and "LUX+EC" models in Fig. 3Ci and -EC models were used to form the "ELF3", "ELF4", and "LUX" models. The +EC models assume that the presence of all components in the EC is a necessary condition for the function of the EC-i.e. if one component of the EC is missing, the expression of the other components is effectively zero. On the other hand, the -EC models assume that the gene expression of only a single member of the EC is sufficient for explaining the rate of repression. There are a few other models that were not tested. For instance, we do not test whether ELF3, ELF4 and LUX have an additive impact of the rate of repression.



Supplemental Fig 14. Additional comparisons of temperature and *phytochrome-dependent transcriptional changes in EC targets.* These additional scatterplots compare EC target gene expression changes in *phyABCDE* and *elf3-1*, which should be compared to Fig. 5Bi-ii. Also, we compare log-fold change in expression at 27°C vs. 22°C in wild types to log-fold change in expression in *phyABCDE*, *elf3-1*, and *lux-4* in the day time (ZT4)—please compare to the results in Fig. 5Biii-iv. Again, note that all seedlings were grown in SD conditions.



Supplemental Fig 15. *elf3-1 and lux-4 transcriptomes correlate with elevated temperature transcriptome at the end of the night.* For each time point, for all genes in the differentially expressed set (all) or genes that were near ELF3, ELF4, and LUX peaks the log-fold change in expression *elf3-1* vs. Col-0 at 22°C (A) or *lux-4* vs. Col-0 at 22°C (B) were compared with the logfold change in expression of Col-0 at 27°C vs. 22°C, and Pearson's R was calculated. The dark bars indicate the night and the white bars indicate the day. In both cases, there is the greatest genome-wide transcriptome correlation at the end of the night, but the greatest correlation among EC targets is in the middle of the night.



Supplemental Fig 16. Overlap between PhyB and LUX binding sites.

These IGV snapshots further demonstrate that phytochromes can co-localise on the DNA near key EC targets, in a temperature dependent fashion—GI (A), *PRR9* (B), and CDF5 from the circadian cluster in **Fig. 2D** (C) are shown. The blue bars represent the peaks for ELF4, ELF3, and LUX at 22°C ZT10 as determined by MACS2. The phytochrome data comes from Jung et al 2016.





Supplemental Fig 17. Temperature affects night time expression of ELF4 targets in lux-4 and phyABCDE mutant, providing further evidence for additive model of temperature integration at EC loci. These heatmaps depict log-fold changes in expression in *phyABCDE* at 27°C vs. 22°C (i) and *lux-4* at 27°C vs. 22°C. Although Figure 5B demonstrates that *phyABCDE* and *lux-4* both have similar effects on the expression of EC targets as changing the temperature of WT Arabidopsis to 27°C, this figure demonstrates that EC target genes have change their expression in response to temperature at night in either a *lux-4* or *phyABCDE* background. This is consistent with the

phenotypic data in Figure 6A-B, which suggest that phytochromes and the EC provide additive temperature responsiveness.



Supplemental Fig S18. *Strategy for generating Venn diagram for Fig. 1B.* All contiguous genomic regions in which there is at least one ChIP-seq peak are determined using bedtools 'merge'. Then, this merged peak file is compared to the ChIP-seq peaks for each individual transcription factor, which is used to generate the Venn diagram. Note that this means that the total number of peaks in the Venn diagram is smaller than the total number of peaks identified—for instance, see segment A.