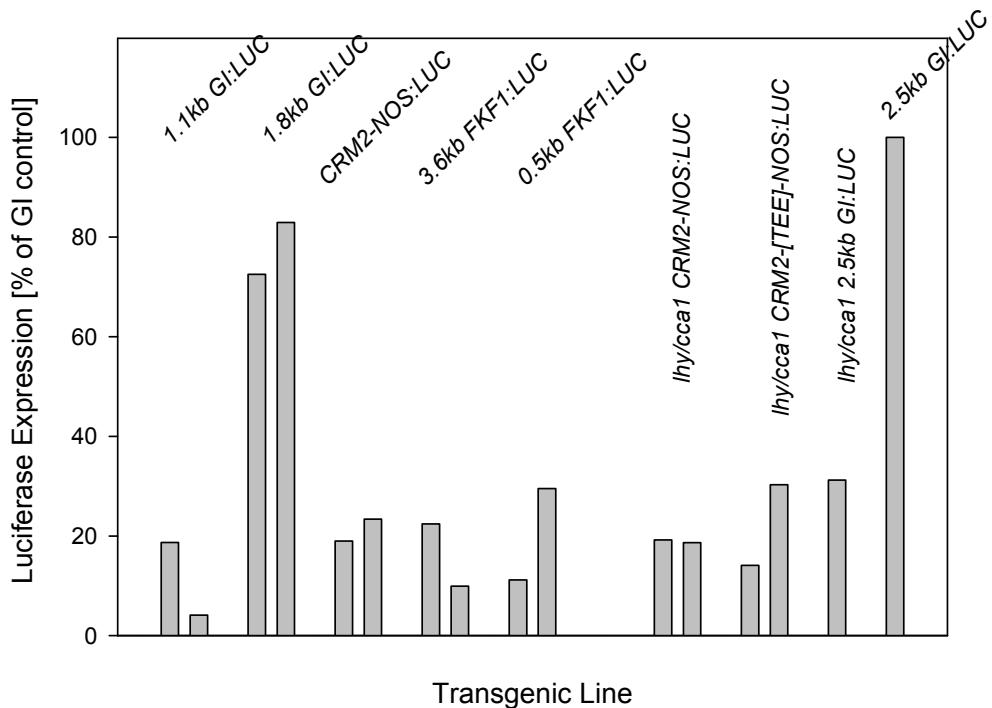
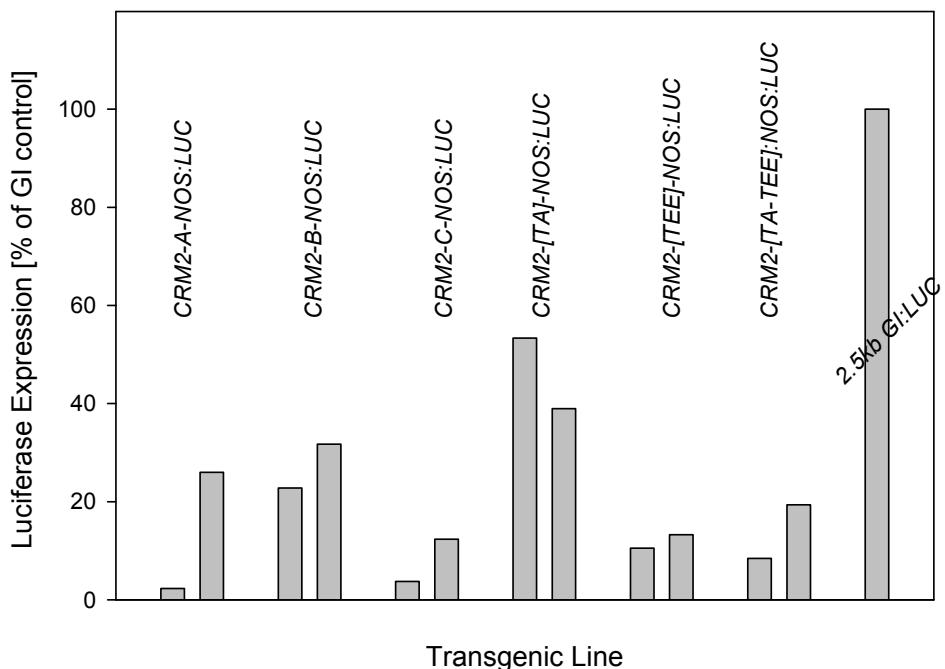


(A)



(B)

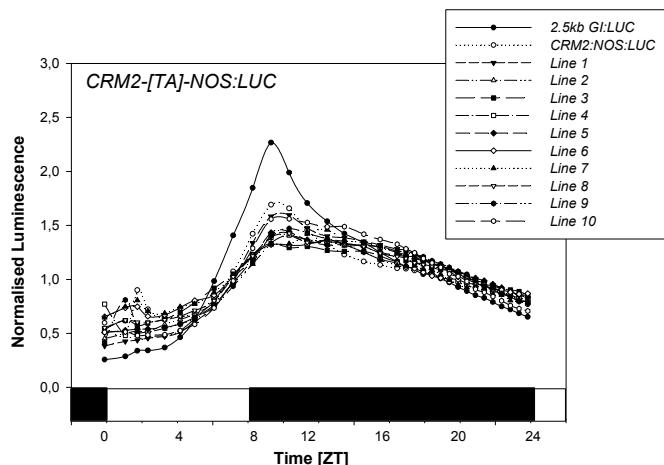


	Average Luc Expr.	SE	% of GI:LUC
1.1kb GI:LUC_1	725.09	98.36	18.71
1.1kb GI:LUC_2	159.37	19.91	4.11
1.8kb GI:LUC_1	2810.10	242.20	72.50
1.8kb GI:LUC_2	3212.50	435.19	82.88
CRM2-NOS:LUC_1	1074.68	124.12	18.96
CRM2-NOS:LUC_2	1170.38	97.67	23.37
2.5kb GI:LUC	5667.40	1334.14	100.00
CRM2-A-NOS:LUC_1	113.56	10.45	2.27
CRM2-A-NOS:LUC_2	1300.19	263.86	25.97
CRM2-B-NOS:LUC_1	1230.65	94.08	22.74
CRM2-B-NOS:LUC_2	1714.30	278.29	31.68
CRM2-C-NOS:LUC_1	186.83	22.70	3.73
CRM2-C-NOS:LUC_2	617.81	120.57	12.34
CRM2-[TA]-NOS:LUC_1	3021.41	326.33	53.31
CRM2-[TA]-NOS:LUC_2	2207.15	290.23	38.94
CRM2-[TEE]-NOS:LUC_1	595.81	56.05	10.51
CRM2-[TEE]-NOS:LUC_2	750.39	114.70	13.24
CRM2-[TA-TEE]-NOS:LUC_1	476.49	41.66	8.41
CRM2-[TA-TEE]-NOS:LUC_2	1095.89	151.94	19.34
3.6kb FKF1:LUC_1	1163.08	156.59	22.43
3.6kb FKF1:LUC_2	515.26	25.37	9.94
0.5kb FKF1:LUC_1	579.14	45.88	11.17
0.5kb FKF1:LUC_2	1528.56	155.04	29.48
Ihy/cca1 CRM2-NOS:LUC_1	743.72	137.46	19.19
Ihy/cca1 CRM2-NOS:LUC_2	723.27	59.11	18.66
Ihy/cca1 CRM2-[TEE]-NOS:LUC_1	545.78	41.85	14.08
Ihy/cca1 CRM2-[TEE]-NOS:LUC_2	1173.17	216.61	30.27
Ihy/cca1 2.5kb GI:LUC	1210.07	164.30	31.22

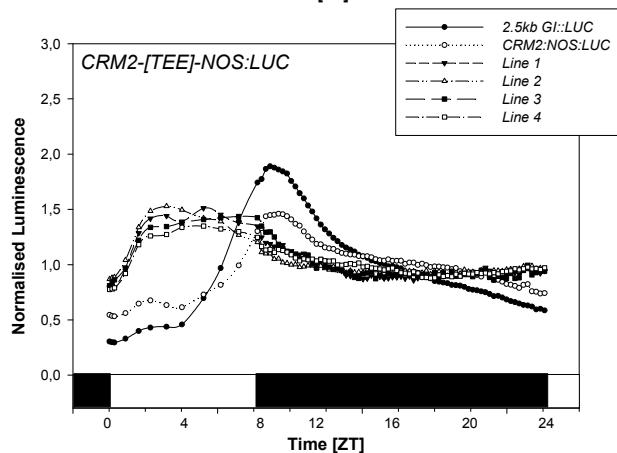
**Supplemental Figure 1.** Absolute gene expression patterns in all lines used in this study.

**(A)(B)(C)**Absolute luciferase activity was calculated from experiments shown in Figures 2, 3, 5, and 7. To make different independent experiments comparable. each absolute 24h expression pattern was normalised to the internal 2.5kb *Gl:LUC* control on the same respective plate. Details to the experimental settings are described in the respective figures and in the Methods section. **(C)** shows all absolute values as plotted in **(A)** and **(B)**.

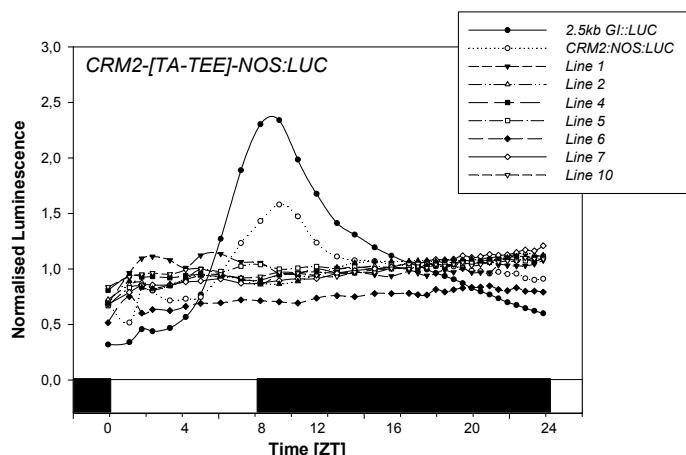
(A)



(B)

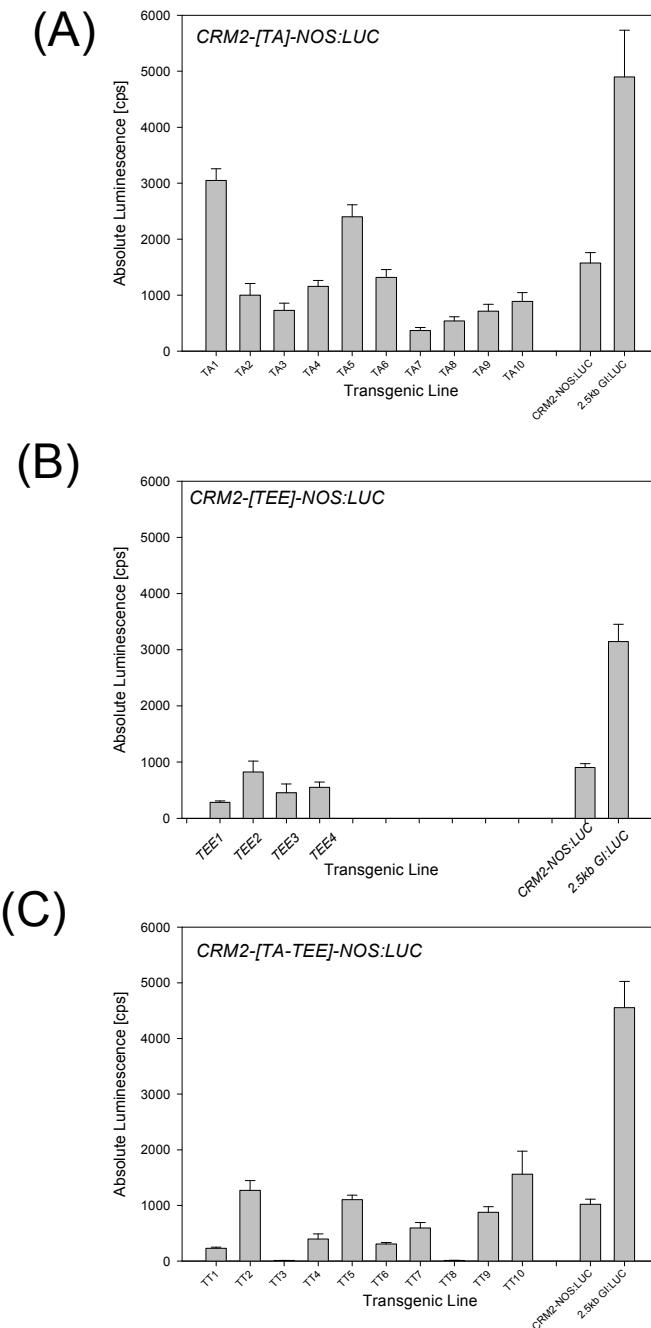


(C)



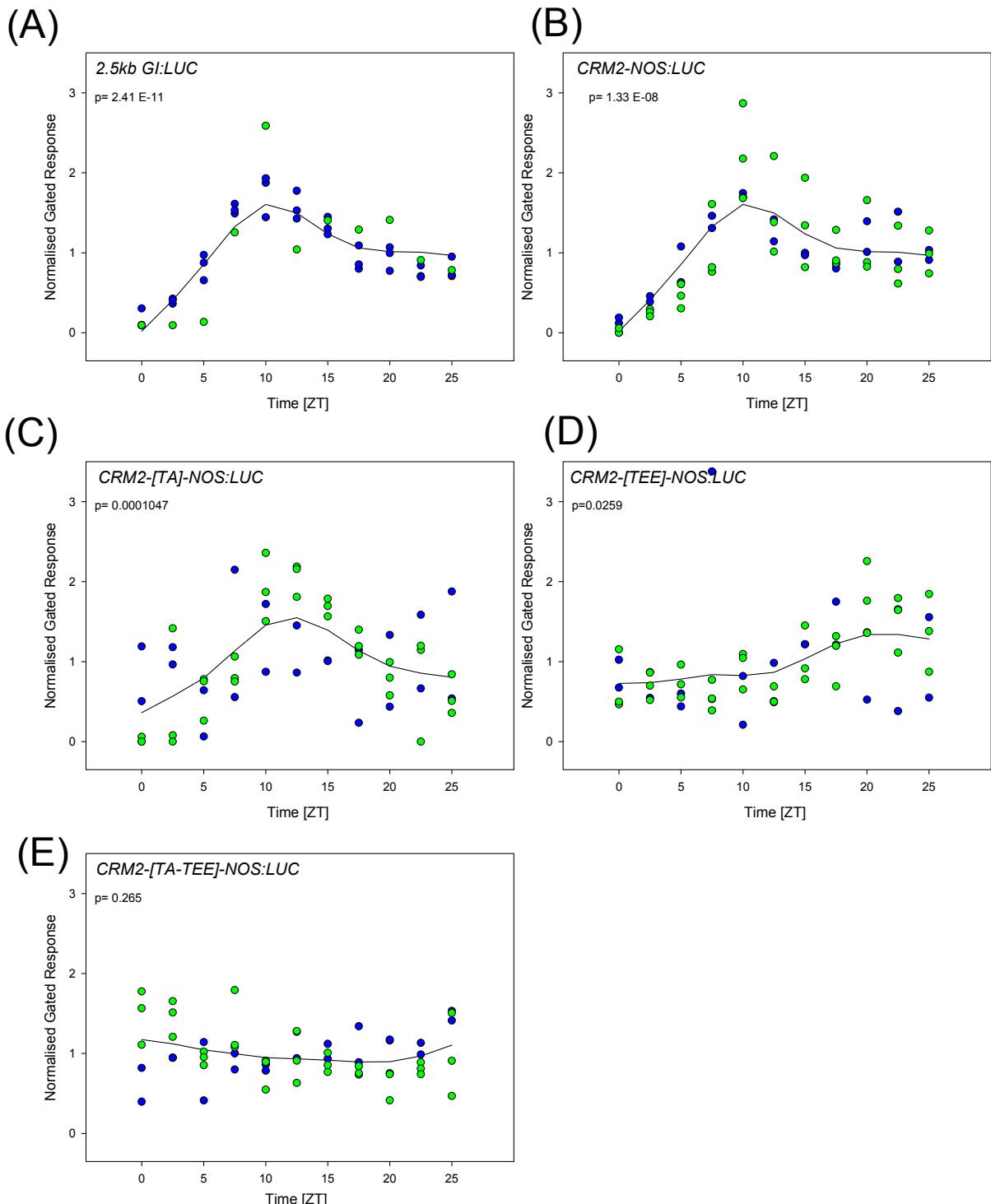
**Supplemental Figure 2.** Diurnal expression patterns of *GI* promoter constructs with point mutations in multiple independent lines

Diurnal expression patterns of different *GI* promoter fragments. Plants with the CRM2-[TA]-NOS:LUC (A), CRM2-[TEE]-NOS:LUC (B) and the CRM2-[TA-TEE]-NOS:LUC (C) were grown for 7d under SDs (8h L/16h D) and then measured under this light regime for 24h in a TopCount. 10 independent heterozygous T2 lines (4 for the CRM2-[TEE]-NOS:LUC construct) of the indicated construct were compared to CRM2-NOS:LUC and 2.5kb *GI*:LUC. All experiments represent the average of ~8 seedlings. Black and white boxes indicate dark/light cycles. ZT is Zeitgeber Time, and here represents time from lights on at dawn.



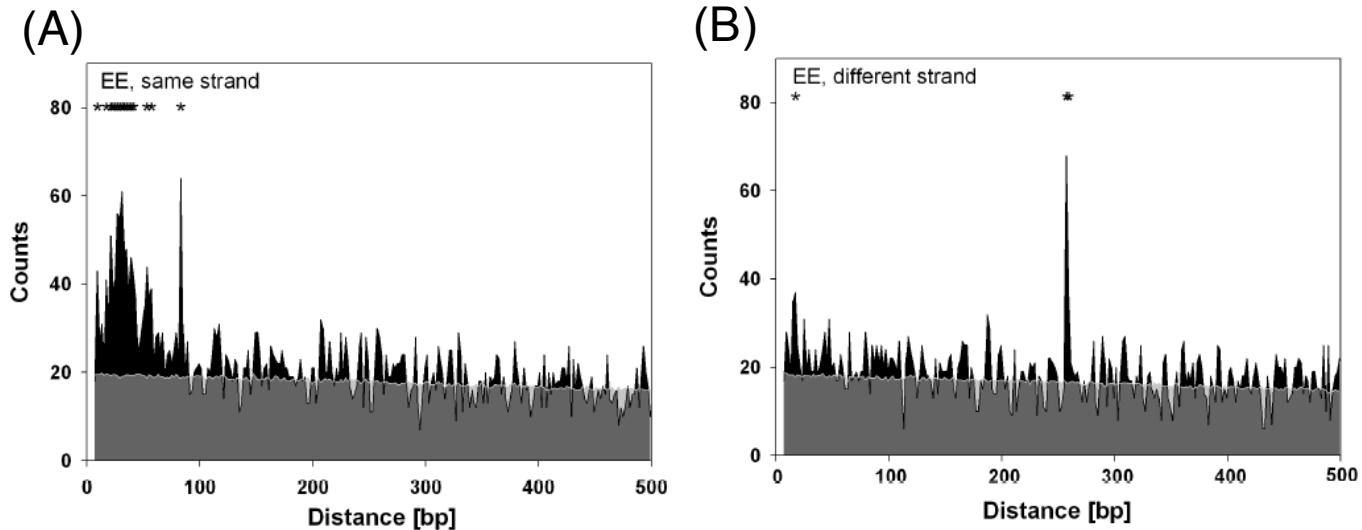
**Supplemental Figure 3.** Absolute expression level of *GI* promoter constructs with point mutations in multiple independent lines.

Absolute expression patterns of different *GI* promoter fragments. Plants with the CRM2-[TA]-NOS:LUC (**A**) . CRM2-[TEE]-NOS:LUC (**B**) and the CRM2-[TA-TEE]-NOS:LUC (**C**) were grown for 7d under SDs (8h L/16h D) and then measured under this light regime for 24h in a TopCount. 10 independent heterozygous T2 lines (4 for the CRM2-[TEE]-NOS:LUC construct) of the indicated construct were compared to CRM2-NOS:LUC and 2.5kb GI:LUC. All experiments represent the average absolute Luminescence of ~8 seedlings during 24h of measurement. Error Bars = SE



**Supplemental Figure 4.** Statistical analysis of light gating in different constructs with *cis*-element mutations

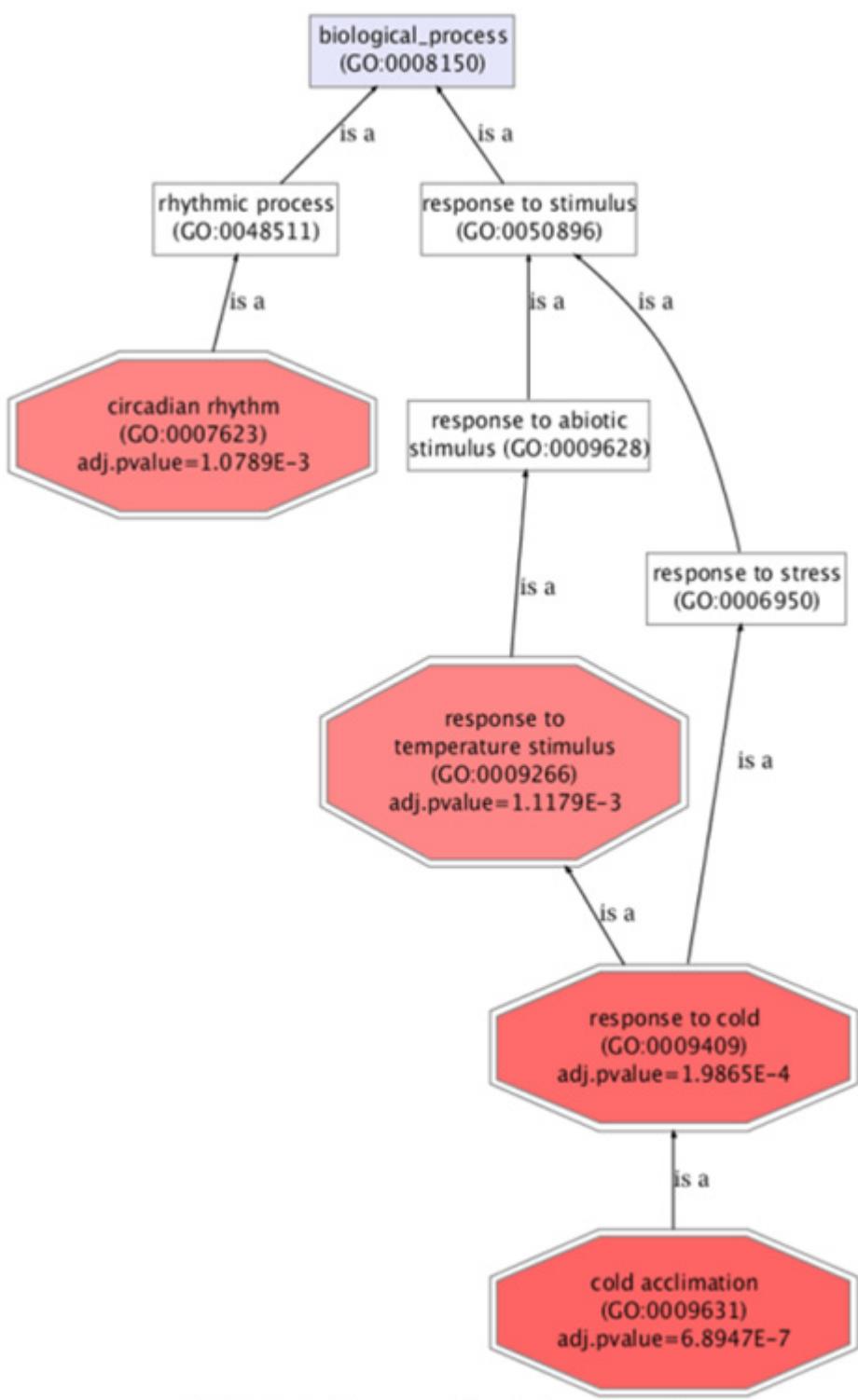
All values were normalised to their own 24h mean and the deviation from this mean was calculated for each timepoint. P values < 0.01 indicate a gated response. Each circle represents a normalised peak value from gating experiments as shown in Fig. 4. Blue circles represent same data as shown in Fig. 4, with an independent replicate of the same transformant line (n=12 seedlings). Green circles represent data from additional gating experiments with 3 independent transformant lines (n=3-6 seedlings). The black lines show regression curves as calculated by the generalised additive model. TA=Triple ABREL mutation. TEE=Triple EE mutation. **(A)** shows the same 2.5kb *GI:LUC* reference line from 4 independent experiments. ZT is Zeitgeber Time, and here represents time from lights on at dawn.



**Supplemental Figure 5.** Genome-wide analysis of EE positions

(A) and (B) EEs (containing the EE, the CBS and the SEE) were mapped within 3kb regions upstream of start codons of all *A. thaliana* genes. A random background was generated and compared to the actual EE distribution (details in the methods section). Analysis was done independently for EEs occurring on the same (A) or different (B) strand. X-axis distance of an EE from the closest neighbouring EE. Gray shade indicates the calculated background distribution. Asterisks indicate statistically significant over-representations ( $p < 0.05$ ).

(A)



(B)

**Genome:** *Arabidopsis thaliana*  
**Condition:** longday  
**Query Loci:** 71

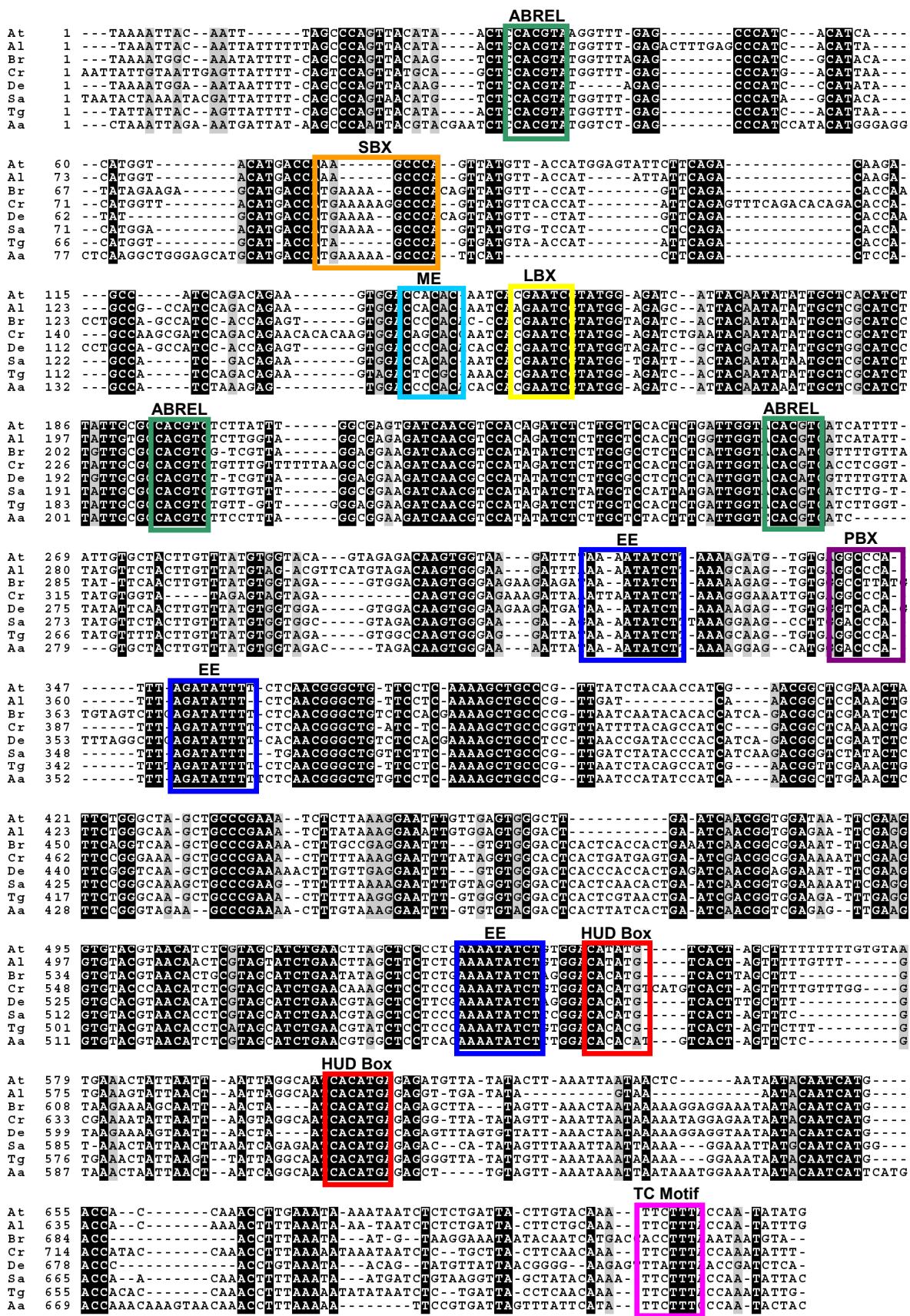
Phase	Count	Expected Count	P-value
no data	5		
10	10	2.64	2.85e-04
12	4	1.33	4.39e-02
8	5	2.12	6.11e-02
13	3	1.03	8.45e-02
11	3	1.42	1.71e-01
19	2	0.91	2.31e-01
16	3	1.74	2.52e-01
17	2	1.34	3.89e-01
3	2	1.46	4.29e-01
7	1	0.79	5.48e-01
21	2	2.11	6.28e-01
14	1	1.32	7.35e-01
5	1	1.51	7.82e-01
4	1	1.7	8.22e-01
15	1	1.81	8.41e-01
22	2	3.62	8.83e-01
non-rhythmic	23	31.11	9.84e-01

**Supplemental Figure 6.** Promoters with multiple EEs and ABRELs confer evening expression and cold responsiveness

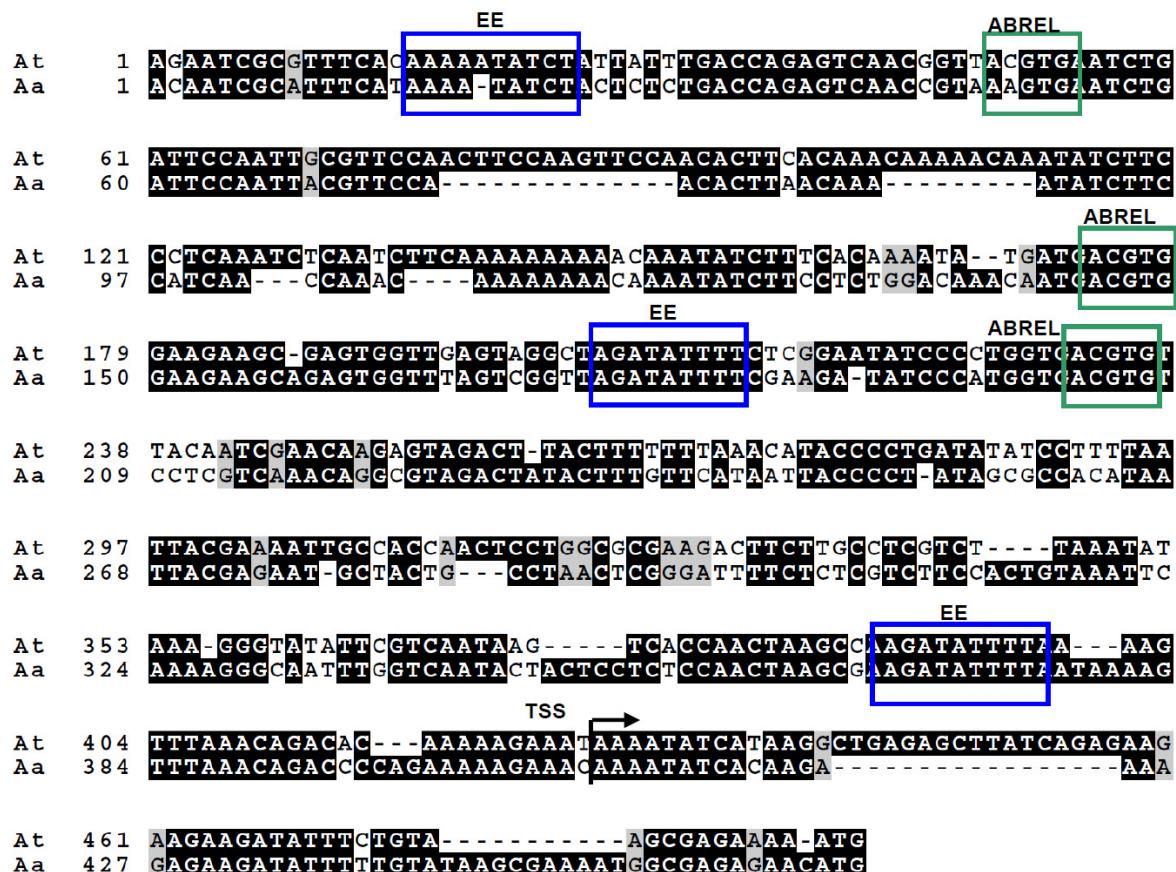
**(A)** Gene Ontology analysis of genes whose 3kb upstream region contains at least 3EEs. GO analysis was conducted with FatiGO from the Babelomics server using default parameters.

**(B)** Phase of expression of genes whose 3kb upstream region contains at least 3EEs. Rhythmicity was evaluated based on the long day microarray dataset published by Mockler et al. 2007. Statistical calculations were conducted with Phaser (Michael et al.. 2007 )

(A)



(B)



**Supplemental Figure 7.** Highly conserved modules within the *GI* and *FKF1* promoters contain multiple EEs and ABRELs

**(A)** Multiple sequence alignment of CRM2 within the *GI* promoter. CRM2s of *Arabidopsis thaliana* (At), *Arabidopsis lyrata* (Al), *Brassica rapa* (Br), *Capsella rubella* (Cr), *Diplotaxis erucoides* (De), *Sinapis alba* (Sa), *Turritis glabra* (Tr) and *Arabis alpina* (Aa) were aligned with DIALIGN and subsequently visualized with BOXSHADE. *Cis-regulatory* elements that are described in the paper are highlighted with colored boxes.

**(B)** Pairwise alignment of CRM\_FKF1. The conserved region of CRM\_FKF1 from *Arabidopsis thaliana* (At) and *Arabis alpina* (Aa) was aligned with DIALIGN and subsequently visualized with BOXSHADE. *Cis-regulatory* elements that are described in the paper are highlighted with colored boxes.

Module	Position (rel. to ATG)		Length (bp)
	from	to	
<b>CRM1</b>	-703	-524	179
<b>CRM2</b>	-1759	-1061	698
<b>CRM3</b>	-2303	-2123	180
<b>CRM2-A</b>	-1759	-1485	274
<b>CRM2-B</b>	-1484	-1227	257
<b>CRM2-C</b>	-1226	-1061	165

**Supplemental Table 1.** Positions and lengths of three CRMs and CRM2 sub-fragments in the *G1* promoter as described in the manuscript.

Conservation shown as WEBLOGO (all eight species included)	Name / Consensus	Position	Reference
<b>CACGT</b>	ABREL ACGTG	-1741 to -1737	Mikkelsen et al. Plant Journal (2009)
<b>AAGCCC</b>	Starch-Box AAGCCC	-1699 to -1694	Michael et al PLOS Genetics (2008)
<b>C<sub>c</sub> CAC</b>	Morning Element CCACAC	-1639 to -1694	Michael et al PLOS Genetics (2008)
<b>cGAATC</b>	LUX Binding Site CGAATC	-1628 to -1623	Helper et al.. Current Biology (2011)
<b>CACGT</b>	ABREL CACGT	-1580 to -1576	Mikkelsen et al. Plant Journal (2009)
<b>CAC<sub>G</sub>T</b>	ABREL CACGT	-1519 to -1515	Mikkelsen et al. Plant Journal (2009)
<b>AAATATCT</b>	Evening Element AAAATATCT	-1456 to -1448	Harmer et al. Nature (2000)
<b>G C<sub>c</sub>CAT</b>	Protein-Box GGCCCAT	-1433 to -1427	Michael et al PLOS Genetics (2008)
<b>AGATATT<sub>T</sub></b>	Evening Element AAAATATCT	-1424 to -1416	Harmer et al. Nature (2000)
<b>AAAATATCT</b>	Evening Element AAAATATCT	-1238 to -1230	Harmer et al. Nature (2000)
<b>CA<sub>c</sub>ATG</b>	HUD Box CACATG	-1224 to -1219	Michael et al. PLOS Biology (2008)
<b>CACATG</b>	HUD Box CACATG	-1170 to -1165	Michael et al. PLOS Biology (2008)
<b>TTC<sub>c</sub>TTT</b>	CT element	-1072 to -1067	Bernard et al. BMC Genomics (2010)

**Supplemental Table 2.** Conserved *cis*-regulatory elements in the *Gl* promoter of *A. thaliana*

Conservation of previously described *cis*-regulatory elements within CRM2 of *A. thaliana Gl* is shown as WEBLOGOS. CRM2s of *Arabidopsis thaliana*, *Arabidopsis lyrata*, *Brassica rapa*, *Capsella rubella*, *Diploptaxis erucoides*, *Sinapis alba*, *Turritis glabra* and *Arabis alpina* were aligned with DIALIGN and subsequently visualised with WEBLOGO. WEBLOGOS contain the sequence information of all 8 species. Positions relative to translational start site of *Gl*. (Red = CRM2-A. -1759 to -1485bp relative to the ATG; Cyan = CRM2-B. -1484 to -1227bp relative to *Gl*; Lilac: CRM2-C. -1226 to -1061bp relative to ATG)

Construct	p-Value	Gated
2.5kb <i>GI:LUC</i>	2.42E-11	yes
1.8kb <i>GI:LUC</i>	4.61E-05	yes
1.1kb <i>GI:LUC</i>	0.019	no
CRM2-NOS:LUC	1.33E-08	yes
3.6kb <i>FKF1:LUC</i>	0.000654	yes
CRM2-A-NOS:LUC	0.095	no
CRM2-B-NOS:LUC	0.0011	yes
CRM2-C-NOS:LUC	0.026	no
CRM2-[TA]-NOS:LUC	0.000104	yes
CRM2-[TEE]-NOS:LUC	0.025	no
CRM2-[TA-TEE]-NOS:LUC	0.265	no

**Supplemental Table 3.** ‘Gating Scores’ of all constructs used in this study.

Gating Assays were performed as described in Fig. 4, but with each 3 independent homozygous transgenic lines (number of individual seedlings: 3-6 for each line). All values were normalised to their own 24h mean and the deviation from this mean was calculated for each timepoint. P values are a cumulative calculation from 2 independent experiments with 1 homozygous line as shown in Figure 4 and 1 additional experiment with 3 independent homozygous lines. Statistical significance was determined in an ANOVA setting; P values < 0.01 indicate a gated response.

ID	# EE	# ABREL	Diurnal Rhythmicity	Description
AT1G10760	3	11	very strong	SEX1 (STARCH EXCESS 1); alpha-glucan, water dikinase
AT1G22770	3	4	very strong	GI (GIGANTEA)
AT1G48330	3	4	very strong	unknown protein
AT2G21660	4	4	very strong	CCR2 (COLD, CIRCADIAN RHYTHM, AND RNA BINDING 2)
AT3G20800	3	1	very strong	rcd1-like cell differentiation protein, putative
AT4G25480	3	10	very strong	DREB1A (DEHYDRATION RESPONSE ELEMENT B1A); CBF3
AT4G29190	3	2	very strong	zinc finger (CCCH-type) family protein
AT4G33980	4	7	very strong	unknown protein
AT5G24470	5	2	very strong	APRR5 (ARABIDOPSIS PSEUDO-RESPONSE REGULATOR 5)
AT5G48250	3	3	very strong	zinc finger (B-box type) family protein
AT1G68050	3	6	very strong	FKF1 (FLAVIN-BINDING, KELCH REPEAT, F BOX 1)
AT1G07040	5	3	robust	unknown protein
AT1G54410	3	1	robust	dehydrin family protein
AT2G15880	3	6	robust	leucine-rich repeat family protein / extensin family protein
AT2G33830	3	1	robust	dormancy/auxin associated family protein
AT3G05800	3	5	robust	transcription factor
AT3G07650	3	2	robust	COL9 (CONSTANS-LIKE 9); transcription factor
AT3G15450	3	3	robust	unknown protein
AT3G15830	3	2	robust	phosphatidic acid phosphatase-related / PAP2-related
AT3G51400	3	5	robust	unknown protein
AT3G61580	3	6	robust	delta-8 sphingolipid desaturase (SLD1)
AT4G16860	4	2	robust	RPP4 (recognition of peronospora parasitica 4)
AT4G17090	3	1	robust	CT-BMY (CHLOROPLAST BETA-AMYLASE); beta-amylase
AT4G25470	4	3	robust	CBF2 (C-REPEAT/DRE BINDING FACTOR 2)
AT4G25490	3	6	robust	CBF1 (C-REPEAT/DRE BINDING FACTOR 1)r
AT4G26530	3	2	robust	fructose-bisphosphate aldolase, putative
AT5G23570	3	0	robust	SGS3 (SUPPRESSOR OF GENE SILENCING 3)
AT1G28060	3	5	slightly rhythmic	small nuclear ribonucleoprotein family protein
AT1G42650	3	6	slightly rhythmic	transposable element gene
AT1G44100	3	0	slightly rhythmic	AAP5; amino acid transmembrane transporter
AT1G60270	3	4	slightly rhythmic	pseudogene, glycosyl hydrolase family 1
AT1G71710	3	2	slightly rhythmic	inositol polyphosphate 5-phosphatase, putative
AT2G25190	3	2	slightly rhythmic	unknown protein
AT2G34840	3	2	slightly rhythmic	coatomer protein epsilon subunit family protein
AT3G14270	3	3	slightly rhythmic	phosphatidylinositol-4-phosphate 5-kinase family protein
AT3G45190	3	1	slightly rhythmic	SIT4 phosphatase-associated family protein
AT3G61570	3	7	slightly rhythmic	GDAP1 (GRIP-RELATED ARF-BINDING DOMAIN-CONTAINING ARABIDOPSIS PROTEIN 1)
AT4G16890	3	1	slightly rhythmic	SNC1 (SUPPRESSOR OF NPR1-1, CONSTITUTIVE 1)
AT4G25500	3	10	slightly rhythmic	ATRSP35; RNA binding / nucleic acid binding
AT4G31360	3	2	slightly rhythmic	selenium binding
AT4G31875	3	2	slightly rhythmic	unknown protein
AT5G01050	3	2	slightly rhythmic	laccase family protein / diphenol oxidase family protein
AT5G09450	3	5	slightly rhythmic	pentatricopeptide (PPR) repeat-containing protein
AT5G12270	4	9	slightly rhythmic	oxidoreductase, 2OG-Fe(II) oxygenase family protein
AT5G15980	3	7	slightly rhythmic	pentatricopeptide (PPR) repeat-containing protein
AT5G23550	3	2	slightly rhythmic	FUNCTIONS IN: molecular function unknown
AT5G41830	3	2	slightly rhythmic	F-box family protein-related
AT5G47020	4	2	slightly rhythmic	glycine-rich protein
AT5G59820	3	5	slightly rhythmic	RHL41 (RESPONSIVE TO HIGH LIGHT 41)
AT1G21945	3	12	not rhythmic	transposable element gene
AT1G45332	3	8	not rhythmic	mitochondrial elongation factor, putative
AT1G45474	3	5	not rhythmic	LHCA5; pigment binding
AT1G65360	3	0	not rhythmic	AGL23 (AGAMOUS-LIKE 23); transcription factor
AT1G70650	3	12	not rhythmic	zinc finger (Ran-binding) family protein
AT1G75790	3	14	not rhythmic	sk18 (SKU518); copper ion binding / pectinesterase
AT2G21680	4	5	not rhythmic	FUNCTIONS IN: molecular function unknown
AT3G05790	3	4	not rhythmic	LON4 (LON PROTEASE 4); ATP binding
AT3G42050	3	2	not rhythmic	vacuolar ATP synthase subunit H family protein
AT4G03530	3	2	not rhythmic	transposable element gene
AT4G30190	3	1	not rhythmic	AHA2; ATPase/ hydrogen-exporting ATPase
AT4G31410	4	0	not rhythmic	unknown protein
AT5G03940	3	6	not rhythmic	CPSRP54 (CHLOROPLAST SIGNAL RECOGNITION PARTICLE 54 KDA SUBUNIT)
AT5G05400	3	6	not rhythmic	disease resistance protein (CC-NBS-LRR class), putative
AT5G39910	3	4	not rhythmic	glycoside hydrolase family 28 protein
AT5G50130	3	2	not rhythmic	short-chain dehydrogenase/reductase (SDR) family protein
AT5G51510	3	7	not rhythmic	unknown protein
AT5G56260	4	3	not rhythmic	dimethylmenaquinone methyltransferase family protein
AT5G56270	3	3	not rhythmic	WRKY2; transcription factor
AT5G56490	3	4	not rhythmic	FAD-binding domain-containing protein
AT5G59810	3	6	not rhythmic	SBT5.4; identical protein binding / serine-type endopeptidase
AT5G64400	3	1	not rhythmic	FUNCTIONS IN: molecular function unknown

**Supplemental Table 4.** Seventy-one Arabidopsis genes have at least 3 EEs in their promoters.

List of Arabidopsis genes that contain at least 3 EE within a 3kb upstream region of their translational start site. Rhythmicity was evaluated based on the long day microarray dataset published by Mockler et al.. 2007.

Cloning Primers		
ID	Sequence	Comment
CRM1-F	GGGGACAAGTTGTACAAAAAAGCAGGCTTGTGGGATGATGATGGTTATGG	to amplify CRM1. with GW site
CRM1-R	GGGGACCACTTGTACAAGAAAGCTGGTAAGAACATACACAGAAGAGATTG	to amplify CRM1. with GW site
CRM2-F	GGGGACAAGTTGTACAAAAAAGCAGGCTAGCCAGTACATAAC	to amplify CRM2. with GW site
CRM2-R	GGGGACCACTTGTACAAGAAAGCTGGGTATTGGTAAGGTTAGTCCAACG	to amplify CRM2. with GW site
CRM3-F	GGGGACAAGTTGTACAAAAAAGCAGGCTTGATGTCACGGCTTCTGT	to amplify CRM3. with GW site
CRM3-R	GGGGACCACTTGTACAAGAAAGCTGGGTAAAGTTAACAGTCAACG	to amplify CRM3. with GW site
CRM2-A-R	GGGGACCACTTGTACAAGAAAGCTGGGTACATAAACAGTAGCACAAATGATGAC	to amplify subfragment A of CRM2. with GW site
CRM2-B-F	GGGGACAAGTTGTACAAAAAAGCAGGCTGTACAGTAGAGACAAGTGGTAAGATT	to amplify subfragment B of CRM2. with GW site
CRM2-B-R	GGGGACCACTTGTACAAGAAAGCTGGGTACAGATTTGGGGAGCTAAG	to amplify subfragment B of CRM2. with GW site
CRM2-C-F	GGGGACAAGTTGTACAAAAAAGCAGGCTGACATATGTCAGCTTTTTGTG	to amplify subfragment C of CRM2. with GW site
NOS-min-F	AGACTCGTAAGCTTTGCGCGTCAAAAGTCG	to amplify nos minimal promoter -101 +4. +Hind 3. + 8bp random
NOS-min-R	ACGAGTCTAAGCTACTCTAATTGGATACCGAGGGAA	to amplify nos minimal promoter -101 +4. +Hind 3. + 8bp random
2.5kb GI-F	GGGGACAAGTTGTACAAAAAAGCAGGCTACCAGCATCTCTAATCAG	to amplify 2.5kb of GI Promoter. with GW site
1.8kb GI-F	GGGGACAAGTTGTACAAAAAAGCAGGCTAGCCAGTACATAAC	to amplify 1.8kb of GI Promoter. with GW site. same as CRM2F
1.1kb GI-F	GGGGACAAGTTGTACAAAAAAGCAGGCTATGCCATAGTGAATGTAATGG	to amplify 1.1kb of GI Promoter. with GW site
GI-R	GGGGACCACTTGTACAAGAAAGCTGGGTGAAACGAAACTAAACCCAAAC	to amplify truncation constructs of GI Promoter. with GW site
3.6kb FKF1-F	GGGGACAAGTTGTACAAAAAAGCAGGCTGACAAGTTGGCTGTGTT	to amplify FKF1 promoter in Arabidopsis. with GW site
0.5kb FKF1-F	GGGGACAAGTTGTACAAAAAAGCAGGCTAATCGCGTTCACAAAATATCT	to amplify FKF1 promoter in Arabidopsis. with GW site
FKF1-R	GGGGACCACTTGTACAAGAAAGCTGGGCTCGCTACAGAAATATCTCTC	to amplify FKF1 promoter in Arabidopsis. with GW site

Mutagenic Primers		
ID	Sequence	Comment
EE1-F	GTACAGTAGAGACAAGTGGTAAGATTTAGAGTGTCTAAAGATGTGAGGCC	to mutagenise EE1 in GI promoter
EE1-R	GGGCCTCACACATCTTTAACAGACACTCTAAATCTTACCACTGTCTACTGTAC	to mutagenise EE1 in GI promoter
EE2-F	TATCTAAAAGATGTGAGGCCATTAGACACTCTCAACGGGCTGTTC	to mutagenise EE2 in GI promoter
EE2-R	GAACAGCCCGTTGAGAGAGTGTCAAATGGGCTCACACATCTTAAAGATA	to mutagenise EE2 in GI promoter
EE3-F	GCATCTGAACCTAGCTCCCCTAGAGTGTCTGTGACATATGTC	to mutagenise EE3 in GI promoter
EE3-R	AGTGACATATGCCACAGACACTGTGAGGGAGCTAAGTTCAGATGC	to mutagenise EE3 in GI promoter
A1-F	AATTAGCCCAGTACATAACTCCTCGAAAGGTTGAGCCCACATCA	to mutagenise ABREL 1 in GI promoter
A2-F	ATATTGCTCACATCTATTGCGCCCTGACTCTATTGGCGAGTGTACAC	to mutagenise ABREL 2 in GI promoter
A3-F	CTCTTGCTCCACTCTGATGGTACTCGACATCTTATTGTGCTACTTG	to mutagenise ABREL 3 in GI promoter
A1-R	TGATGTGATGGGCTAAACCTTCGAGGAGTTATGTAACCTGGCTAAATT	to mutagenise ABREL 1 in GI promoter
A2-R	GTTGATCACTCGCCAAATAAGAGTCGAGGCGCAATAAGATGTGAGCAAT	to mutagenise ABREL 2 in GI promoter
A3-R	CAAGTAGCACAATAAAATGATGTCGAGTACCAATCAGAGTGGAGCAAGAG	to mutagenise ABREL 3 in GI promoter

Supplemental Table 5. Primer table