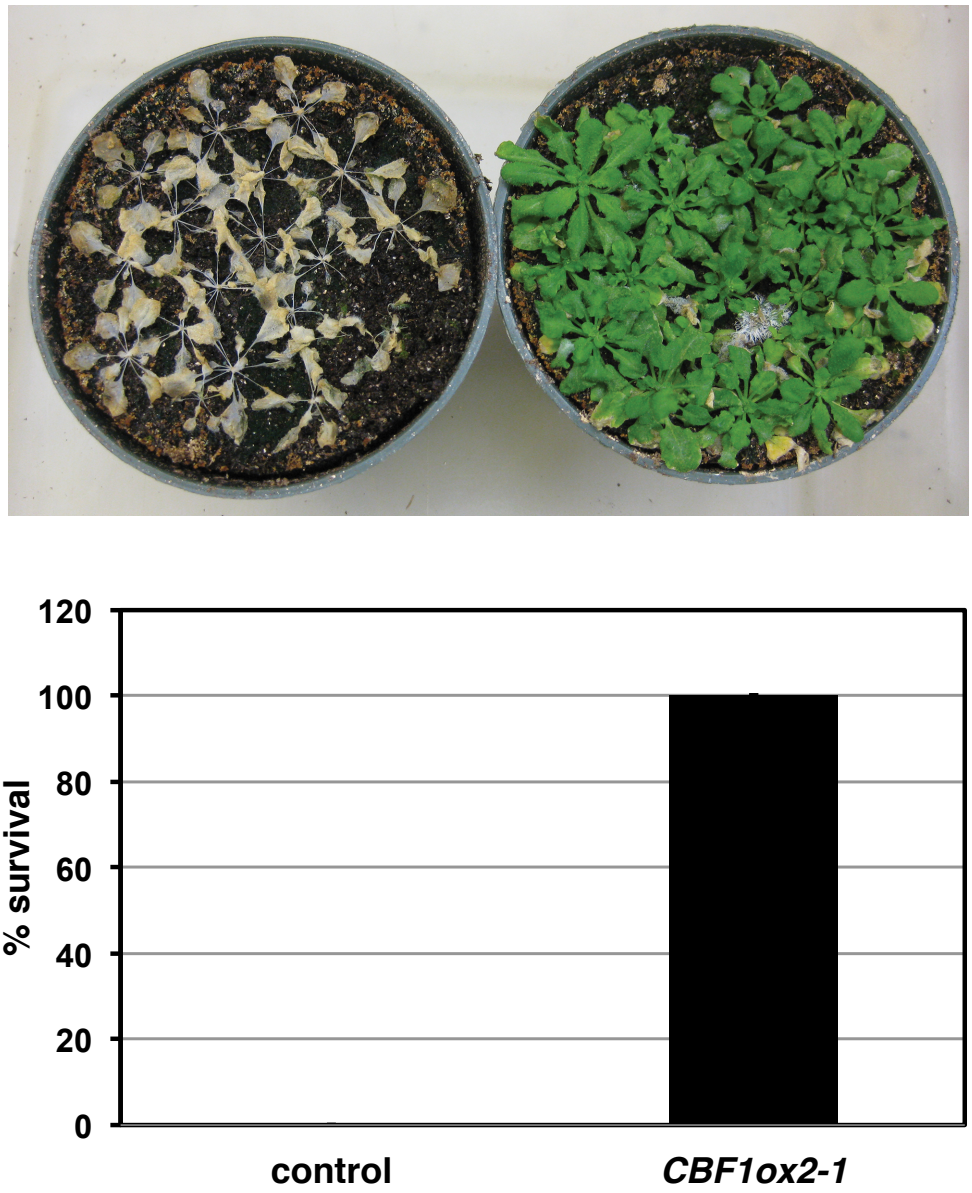

Figure S1 (Related to Figure 1). *CBF1* overexpressor line 2-1 has enhanced freezing tolerance

Freezing tolerance is expressed as percent (%) survival. Control (*LUX::LUC* line 139) and *CBF1* overexpressor line 2-1 (*CBF1ox2-1)* seedlings were grown under 12:12 at 22°C for approximately 6 weeks. Plants were then transferred at 16 hours after lights on to 4°C for 3 days cold acclimation before freezing at -5°C for 24 hours. After 24 hours recovery at 4°C, plants were returned to 12:12 at 22°C for 7 days. Data is the average of three biological replicates with error bars representing standard error.

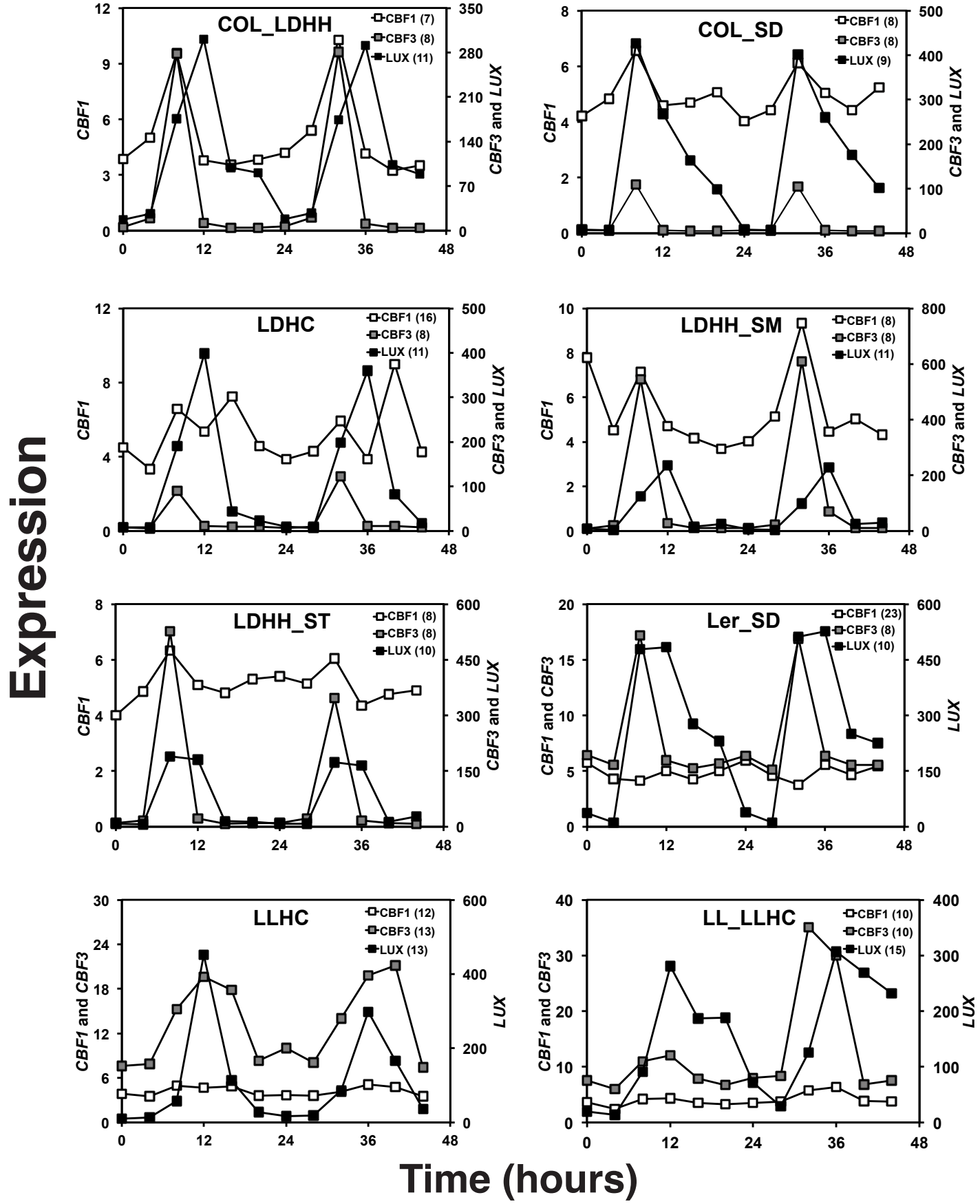

Figure S2. *CBF1* and *LUX* expression overlap under driven and circadian conditions
Expression data from Diurnal time-course arrays where *CBF1*, *CBF3*, and *LUX* were all called cycling (correlation cut-off >0.7). (A-G) Driven, (H) circadian conditions. Bracketed numbers in legends are phase peaks called by Diurnal. Information about arrays is available at http://diurnal.mocklerlab.org.

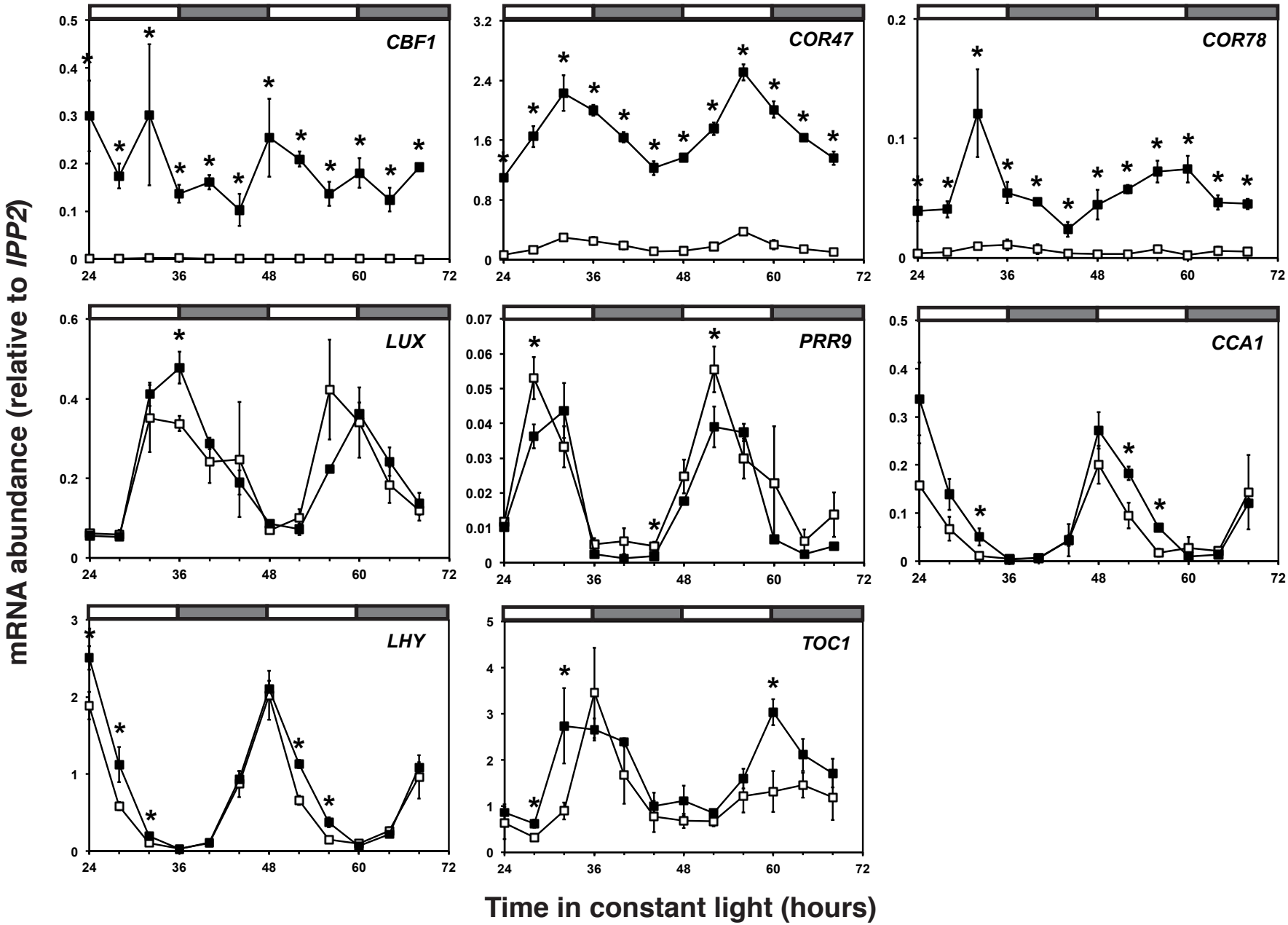

Figure S3 (Related to Figure 2). *CBF1* overexpression affects *LUX* and other clock gene transcripts
mRNA abundance was measured in control (*LUX::LUC* -660/+156 reporter line 139; open squares) and the *CBF1* overexpressor line 2-1 (*CBF1ox2-1*; filled squares). Seedlings were entrained under 12:12 at 22°C for 10 days before transfer to constant light. mRNA levels were quantified by real-time reverse transcription PCR. Data is the average of three biological replicates with error bars representing standard error. Student's t-test was used to determine the significance of transcript levels relative to control (* p-value ≤0.05).

Experimental Procedures

Yeast one-hybrid analysis

All reporter strains were in the YM4271 yeast strain and generated according to the manufacturer's protocols (Clontech). Truncated fragments of the *LUX* promoter were cloned into pENTR5'TOPO or pENTR-DTOPO (Invitrogen) before transfer to the Gateway-compatible pMW3 LacZi and pGLacZi [1,2]. Regions analyzed are as follows with primers listed 5' to 3' (forward primers listed first): *LUX -660/-412* (CACCGGGGAA ATCTCAGAGAATCAGAAATTT; ACGCAATGTGATCTTTCCACCGTTGGATTG); *LUX-441/-222* (CACCCAATCCAACGGTGGAAAGATCACATTGC; ACGTAAGATGGAG CCACGTGGAGTGAAAAGAGC); *LUX-253/-51* (CACCCTCTTTTCACTCCACGTGGCT CCATCTTACGTGC; GAAAAAGCGCAATATTTTAATTCCAACGTGGACG); *LUX-86/+156* (CACCCTCGTCCACGTTGGAATTAAAATATTGCGCTTTTTCC; CAAACT CTCTAATTTCTCGAAATTCAGTTAGAG); and *LUX-441/-222mutCRT* (CACCCAATCC AACGGTGGAAAGATCACATTGC; ACGTAAGATGGAGCCACGTGGAGTGAAAAGAG CCAAGTCAGCTATTTGTGAGGGTTTTTCAGTAAATTACTCAATCATTAGCAAG). β-galactosidase activity was measured in 96-well plates and quantified by spectrophotometry using o-nitrophenyl-D-galactoside (ONPG) as substrate [3].

Chromatin immunoprecipitation (ChIP)

Tissues were processed as previously reported [1] except for the following minor changes. Seedlings were grown under photocycles of 12-hours light/12-hours dark (12:12) and collected in the dark at 16 hours after lights on. Chromatin was sheared using the Bioruptor Plus sonicator (Diagenode) set for 10 cycles of 30 seconds on / 1 min off, which was repeated twice. Following immunoprecipitation, quantitative PCR was performed using SsoFast EvaGreen Supermix (Bio-Rad) to detect the following loci with primers listed 5' to 3' (forward primers listed first in each pair): *ACTIN* (CTTGCACC AAGCAGCATGAA; CCGATCCAGACACTGTACTTCCTT); *UBQUITIN* (GGCCTTGTAT AATCCCTGATGAATAAG; AAAGAGATAACAGGAACGGAAACATAGT); *COR78* -225/-144 (ATACCGACATCAGTTTGAAAGAAAAGG; TGCTTTTTGGAACTCATGTCG GTAG); and *LUX* -237/-129 (TGGCTCCATCTTACGTGCCTAG; ACCTAACCAATCTC TTTTCAACACG).

Plant materials and growth conditions

The Columbia accession was used in all experiments except those employing the previously published *CBF1* overexpressor in *Landsberg erecta* (kind gift from Dr. Pascal Genschik) [4]. The *CAB2::LUC* reporter line, *lux-1* and *lux-4* mutants were also previously reported [5,6]. To generate the *CBF1* overexpressor in Columbia, the coding sequence of *CBF1* was amplified from genomic DNA and cloned into pENTR/D-TOPO (Invitrogen). After sequence confirmation, the fragment was recombined into pEarlyGate101 [7] using Gateway LR Clonase II (Invitrogen). The construct was introduced into Arabidopsis *LUX::LUC* -660/+156 line 139 background by Agrobacterium-mediated transformation. *CBF1ox2-1* was used as a representative single insertion overexpression line.

For all experiments unless otherwise noted, seeds were gas sterilized and stratified in darkness at 4°C for 2-3 days on 1x Murashige and Skoog basal salt medium with 1.5% agar (no sucrose) plates. Seedlings were then transferred to 22°C under 12:12 for 10 days entrainment and either maintained or transferred to test conditions (22°C constant light or 4°C under 12:12). All light intensities were 80-100 μmol/m^2/sec.

Transcript analysis

For expression analysis of the previously published *CBF1* overexpressor [4], total RNA was extracted from 10-days old seedlings grown on Whatman filter paper using Qiagen RNeasy plant mini kit with on-column DNAse treatment (Roche). *CBF1ox2-1* and control seedlings were grown on medium additionally supplemented with 1% sucrose and 10μM GA_3 to enhance germination and total RNA was extracted using TRIzol (Life Technologies). For cDNA synthesis, 1μg RNA was reverse transcribed using the iScript cDNA synthesis kit (Bio-Rad). cDNA was quantified using Bio-Rad CFX Real-Time System and analyzed with the Bio-Rad CFX Manager software. *IPP2*/At3g02780 was used for normalization in all experiments. The following transcripts were measured with primers listed 5' to 3' (forward primers listed first): *IPP2*/At3g02780 (GTATGAGTTGCTT CTCCAGCAAAG; GAGGATGGCTGCAACAAGTGT); *CBF1* (CCGCCGTCTGTTCAATG GAATCAT; TCCAAAGCGACACGTCACCATCTC); *COR47* (ACAAGCCTAGTGTCATC GAAAAGC; TCTTCATCGCTCGAAGAGGAAG); *COR78* (GCACCAGGCGTAACAGGTA AAC; AAACACCTTTGTCCCTGGTGG); *LUX* (TGTTGGAGGTGGAAGCGCAAA; TCCA CGAGCAATAACAAGCTCACC); *PRR9* (GCCAGAGAGAAGCTGCATTGA; CCTGCTCT GGTACCGAACCTT); *CCA1* (CCGCAACTTTCGCCTCAT; GCCAGATTCGGAGGTGAG TTC); and *TOC1* (TCTTCGCAGAATCCCTGTGAT; GCTGCACCTAGCTTCAAGCA).

Histochemical detection of GUS activity

CBF1::GUS and *LUX::GUS* constructs were generated by cloning -1845/+163 and -660/+156 bp respectively (relative to the transcriptional starts of each gene) into pENTRD-TOPO before transfer to pMDC163 [8]. Primers were as follows (5' to 3' with forward primer listed first for each pair): *LUX* (CACCGGGGAAATCTCAGAGAATCAGA AATTT; CAAACTCTCTAATTTCTCGAAATTCAGTTAGAG) and *CBF1* (CACCAAAGAA GGCAAGAGAGATCATTTGGAT; TGATCAGAGTACTCTGTTTCAAGAAACTGG). T3 seedlings were grown for 10-days under 12:12 at 22°C and stained for GUS activity [9]. Five independent transgenic lines were analyzed for each construct and produced comparable results. *CBF1::GUS* line 27 and *LUX::GUS* line 21 were photographed as representative lines.

Luciferase expression

For *LUX* promoter analysis in Arabidopsis, fragments were generated using the same primers as used in yeast one-hybrid analysis but in the appropriate combination. Fragments were cloned into pENTR/D-TOPO before recombination into the Gateway compatible pFLASH binary vector [10]. The total number of plants imaged per construct was as follows: $n_{-660/+156}$ = 26; $n_{-441/+156}$ = 21; $n_{-253/+156}$ =36; and $n_{-86/+156}$ = 41.

Multimeric sequences containing tandem repeats of wildtype or mutant motifs (separated by 6-7 flanking nucleotides per motif) were repeated three times. Fragments were created by annealing complementary forward and reverse primers (Valuegene Inc). Primers used are as follows (5' to 3' with forward primers listed first in each pair): CRT+EE (CACCTGCTAAGTCGGTAGTAATAGATCTAAAATATCTAGCTAATGCTAAG TCGGTAGTAATAGATCTAAAATATCTAGCTAATGCTAAGTCGGTAGTAATAGATCTA AAATATCTAGCTAA; TTAGCTAGATATTTTAGATCTATTACTACCGACTTAGCATTAG CTAGATATTTTAGATCTATTACTACCGACTTAGCATTAGCTAGATATTTTAGATCTAT TACTACCGACTTAGCAGGTG) and mCRT+EE (CACCTGCTAATGATTGAGTAATAGA TCTAAAATATCTAGCTAATGCTAATGATTGAGTAATAGATCTAAAATATCTAGCTAAT GCTAATGATTGAGTAATAGATCTAAAATATCTAGCTAA; TTAGCTAGATATTTTAGAT CTATTACTCAATCATTAGCATTAGCTAGATATTTTAGATCTATTACTCAATCATTAGC ATTAGCTAGATATTTTAGATCTATTACTCAATCATTAGCAGGTG). After annealing, multimers were cloned into pENTR/D-TOPO (Invitrogen) before recombination upstream of the nopaline synthase (NOS) gene and the *LUC* reporter in the Gateway-compatible pGATM-Nos binary vector. pGATM-Nos was generated by blunt-ligation of an EcoRV-digested attRI/attRII cassette into the pATM-Nos vector [11] that was treated with XhoI and Klenow (NEB). Transgenic seedlings were grown for 7 days on selective medium in 22^{o}C under 12:12 before transfer to non-selective medium for imaging. The total number of plants imaged per construct was as follows: $n_{CRT+EE22^{\circ}C} = 46$; $n_{mutCRT+EE22^{\circ}C} = 44$; $n_{CRT+EE4^{\circ}C} = 102$; and $n_{mutCRT+EE4^{\circ}C} = 95$.

Seedlings were sprayed with 2.5mM luciferin (Biosynth AG) and imaged every 2.5 hours using a Hamamatsu VIM CCD camera [5]. Bioluminescence was analyzed as previously reported [5].

In addition to T1 lines, the following single insertion lines were analyzed as representative lines: *LUX::LUC* -660/+156 line 123, *LUX::LUC* -441/+15 line 98, *LUX::LUC* -253/+156 line-41, and *LUX::LUC* -86/+156 line 27.

Freezing tolerance assays

Plants were grown in pots at 22^{o}C under 12:12 for approximately 4-6 weeks and tested before bolting. For cold acclimation, plants were transferred at 4 hours after lights on to 4^{o}C constant darkness for 3 days (covered by foil) before transfer to -5^{o}C constant darkness for 5 hours or 24 hours (uncovered). After freezing, plants were re-covered and transferred to 4^{o}C for 24 hours recovery. Subsequently plants were uncovered and returned to 22^{o}C under 12:12 for 7 days and photographed. Survival was measured as the presence of green tissue. Experiments were performed in triplicate with 20 plants per pot.

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