

Autumn–winter progression triggers distinct transcriptional programs in *Arabidopsis thaliana*

Mélanie Ormancey^{1,5} · Benjamin J. M. Tremblay^{1,6} · Yajiao Cheng¹ · Myrthe Praat^{2,7} · Rea L. Antoniou-Kourounioti³ · Jenna Krumbach¹ · Martijn van Zanten^{2,4} · Julia I. Qüesta¹

Received: 14 November 2025 / Accepted: 17 September 2026
© The Author(s) 2026

Abstract

Background Temperate plants are adapted to survive winter chilling temperatures. However, the global regulatory landscape underlying acclimation to noisy and fluctuating natural cold conditions remains poorly investigated.

Results We explore the transcriptome dynamics of *Arabidopsis thaliana* plants grown over weeks of a gradually decreasing temperature regime, mimicking natural cold conditions of autumn and early winter, including daily temperature fluctuations. Our results define two distinct genetic programs responding to either moderate cold (*COOLING* genes) or to temperatures below 10 °C (*CHILLING* genes). Transient induction of *C-REPEAT/DEHYDRATION-RESPONSIVE ELEMENT BINDING FACTORS* (*CBFs*) lies in the boundary between *COOLING* and *CHILLING* activation, while the core circadian clock component *CIRCADIAN CLOCK-ASSOCIATED 1* (*CCA1*) is essential for transcriptional responses both at moderate cold and chilling conditions. By profiling genome-wide chromatin accessibility, we demonstrate that the induction of cold-responsive genes is associated with an increase in promoter accessibility. In contrast, down-regulated genes do not lose promoter accessibility, allowing plants to rapidly reactivate transcription after the return to warm conditions.

Conclusions Overall, our work provides a comprehensive view of the extent of transcriptome and promoter accessibility dynamics underlying plant adaptation to cooling and chilling conditions, revealing the function of key regulators at specific low temperature ranges.

Keywords *Arabidopsis thaliana* · Responses to cold temperature · Transcriptional regulation · Epigenetics · Chromatin accessibility

Mélanie Ormancey, Benjamin J.M. Tremblay and Yajiao Cheng contributed equally to this work.

This Article in Press is shared early to give you faster access to new research. It is citable and carries a permanent DOI. The final edited version will replace it automatically.



Background

Plants in temperate climates which undergo wintering must deal with progressive declines in daily temperatures over months and the fluctuations in the daily extremes of night and day temperatures. To survive such climates, plants have evolved fine-tuned mechanisms to sense both short and long-term temperature shifts and adapt their physiology accordingly. For example, upon exposure to low, but non-freezing temperatures (i.e., chilling temperatures; 0°C–15°C), many plants improve their freezing tolerance through a process known as cold acclimation [1]. In some species, long-term exposure to chilling temperatures is in turn used as a developmental cue to trigger the switch to reproductive competence, also known as vernalization [2–4]. While the molecular bases of both short and long-term cold responses in plants grown in standard laboratory settings has been relatively well characterized, our understanding of how plants sense and integrate both simultaneous short-term daily fluctuations and long-term gradual declines remains elusive.

In *Arabidopsis thaliana* (*Arabidopsis*), exposure to cold quickly results in the induction of the expression of *C-repeat/dehydration-responsive element binding factors* (*CBFs*) genes [5], which in turn activate a set of *COLD REGULATED* (*COR*) genes to promote chilling and freezing tolerance [6]. *CBF* genes are transiently induced under constant cold (i.e. 4 °C), reaching a peak of induction within 3 h, followed by a rapid decline after 8 h [5, 7, 8]. *CBF* expression also relies on the circadian clock, as central dawn-phased transcription factors, (i.e. *CIRCADIAN CLOCK-ASSOCIATED 1* (*CCA1*) and *LATE-ELONGATED HYPOCOTYL* (*LHY*)), play a key role in their induction during cold exposure [8, 9]. However, the pattern of *CBFs* expression during natural wintering conditions and how the clock contributes to cold acclimation in these conditions remain unclear.

Over the much longer timescale of months of winter cold, vernalization enables flowering in spring via the stable inactivation of the floral repressor *FLOWERING LOCUS C* (*FLC*). Silencing of *FLC* occurs by deposition of the histone mark H3K27me3 leading to increased chromatin compaction [2–4]. This involves the action of Polycomb Repressive Complex 2 (*PRC2*) in association with the *PLANT HOMEODOMAIN* (*PHD*) protein *VERNALIZATION INSENSITIVE 3* (*VIN3*), the latter of which is partially induced by *CCA1* and *LHY* during early winter upon exposure to chilling temperatures (12°C) [10]. *VIN3* mRNA increases quantitatively over weeks of cold under laboratory-controlled constant conditions and in natural fluctuating environments [11]. Thus, *VIN3* mRNA abundance provides a “memory” of time in the cold [11]. While the *FLC* locus and *VIN3* mRNA serve as well-described examples of chromatin dynamics in response to cold and the plants’ ability to record the duration of cold, it remains unclear whether other mechanisms unrelated to vernalization exist. Although cold is known to affect global chromatin accessibility in constant conditions, little is known about chromatin dynamics during naturally declining temperature conditions [12–16].

In the present study, we aimed to decipher the regulatory steps required for *Arabidopsis* adapting to winter cold using a temperature regime mimicking natural wintering conditions. We observed that progressive cold, whether fluctuating or not, induces two principal patterns of gene regulation that are sequentially activated as temperature decreases. We define these two distinct genetic programs as responding to either moderate cold (*COOLING* genes) or to temperatures below 10 °C (*CHILLING* genes). Furthermore, we assessed genome-wide chromatin accessibility dynamics in response to gradually declining temperature environments. Induction of cold-responsive genes was associated with an increase in promoter accessibility whereas down-regulated genes including those related to photosynthesis remained accessible, probably allowing for their rapid reactivation upon the return of favorable conditions. We also uncovered a complex network of transcription factors regulating cold-responsive genes. This analysis revealed that *CBF* genes are expressed and regulate genes at the interface between *COOLING* and *CHILLING* programs, while *CCA1* is required for both programs.

Results

Seasonal decreasing temperatures lead to major changes in the *Arabidopsis thaliana* transcriptome

To simulate a natural temperate autumn/early winter scenario in the lab, we grew *Arabidopsis thaliana* Col-0 plants in soil for two weeks at 22 °C in short-day conditions (SD; 8 h photoperiod) before exposing them to progressively declining temperatures from 22 °C to −2 °C for four additional weeks, with regular daily fluctuations (fluctuating, FLU) emulating the temperature pattern seen in a previous Swedish autumn (Fig. 1A and B; Additional file 1: Fig. S1A; Additional file 2: Table S1). Plants were sampled every week at midday (ZT4) for RNA-seq experiments, for a total of five sampling points. We also sampled from a set of control plants kept at constant

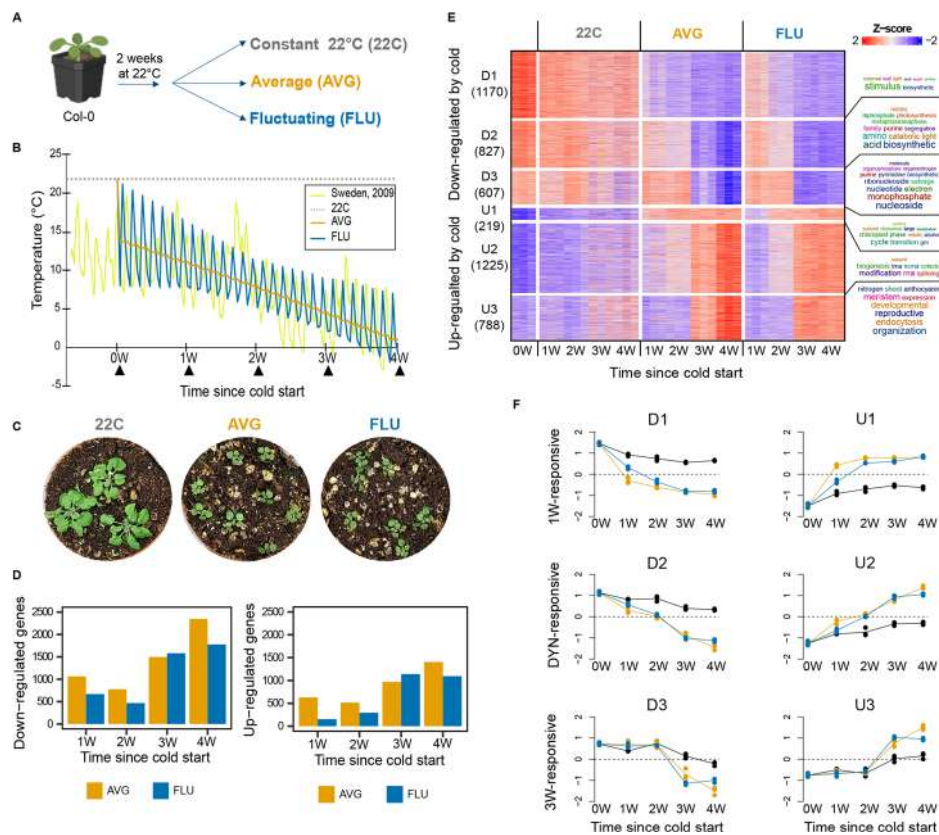


Fig. 1 Effects of a simulated autumn-to-winter scenario on the *Arabidopsis thaliana* transcriptome. **A** Experimental design. Plants were grown in a growth chamber in SD conditions at 22 °C for two weeks. After this period, the plants continued to grow for another four additional weeks either at 22 °C (22C; grey line) or in a decreasing temperature profile, i.e. fluctuating (FLU; blue line) or average (AVG; orange line) temperatures (adjusted daily). **B** Temperature profiles used during the experiment starting from two weeks after germination. FLU temperature regime (in blue) was designed according to natural days in autumn measured in Eden, Sweden (in green, sowing date in autumn 2009). The means of FLU temperatures were determined daily to generate the AVG temperature (in orange). Sampling time-points are indicated for both the RNA-seq and the ATAC-seq experiments (▲), with indication of the sampling week (W). **C** Representative pictures of wild-type *Arabidopsis thaliana* plants grown under FLU or AVG conditions for 4 weeks. **D** Number of differentially expressed genes (down-regulated: left panel; up-regulated: right panel) between control and cold-exposed plants by week (W) as obtained by RNA-seq experiments. All FLU and AVG comparisons were performed with the matching control samples (22C) from the same week. Genes with a fold-change of at least 2 and an FDR less than 0.01 were considered differentially expressed. **E** Heatmap of Z-scores of clustered gene expression data from the RNA-seq. Genes from each cluster were used for GO enrichment analysis, followed by semantic enrichment using enriched terms and plotted as a word cloud for each cluster. **F** Diagrammatic representation of cold-modulated genes belonging to the three distinct transcriptional programs, using the average Z-score expression of all genes per cluster

22 °C (22C). To further dissect the effect of daily fluctuations on longer term adaptation to cold temperature, we also exposed a set of plants to the average daily temperatures of cold-exposed plants without any fluctuations for additional RNA-seq experiments (average, AVG). Both FLU and AVG plants underwent similar severe reductions in growth rate in comparison to control plants (Fig. 1C; Additional file 1: Fig. S1B), underscoring the strong effect of declining temperature on plant development. Furthermore, we also detected a significant acceleration in flowering time for AVG and FLU samples (Additional file 1: Fig. S1C).

We generated 39 RNA-seq datasets with 3.37 billion reads in total from our sampled material (Additional file 2: Table S2), with high inter-replicate correlations (Additional file 1: Fig. S2A). Differential gene expression (DE) analyses revealed a gradual divergence in the transcriptomes of plants exposed to AVG or FLU declining temperature conditions compared to the plants exposed to 22 °C for the same period of time (Fig. 1D). A comparison between the initial week 0 samples with the AVG and FLU samples revealed a similar trend, with increased numbers of DE genes over time (Additional file 1: Fig. S2B; Additional file 3: Table S1). We also observed a sharp increase in the number of DE genes from week 2 to week 3 of the experiment, corresponding to 7.8 °C and 4.4 °C measured AVG temperatures, respectively (Fig. 1D). We found few significantly DE genes between the transcriptomes of AVG and FLU plants (Additional file 1: Fig. S2C), though AVG plants generally had an increased number of DE genes compared to FLU plants for most weeks (Fig. 1D). Possible explanations for this phenomenon may be related to the temperature fluctuations, with warmer (above average) temperatures attenuating transcriptional responses, or to the temperature of the plants at the time of sampling, which was consistently higher in FLU plants (Additional file 1: Fig. S1A; Additional file 2: Table S1). However, despite the differences in temperature at sampling, we detected equivalent numbers of DE genes under AVG and FLU conditions at week 3 of the experiment (Fig. 1D). Together, our results demonstrate that independent of the daily temperature patterns, the gradual drop in temperature across the autumn–winter causes a significant reprogramming of the plant transcriptome.

Distinct transcriptional programs underlie autumn–winter progression

We next tested for the possible existence of distinct transcriptional programs. To this aim, we clustered the RNA-seq data and detected three main transcriptional response patterns for both increasing and decreasing gene expression in AVG and FLU plants: 1W-responsive genes, which were down (D) or up-regulated (U) in week 1 upon decreasing temperature treatment (D1/U1); 3W-responsive genes, which changed in week 3 (D3/U3); and dynamically-responsive genes (DYN), which gradually changed over the course of the entire experiment (D2/U2) (Fig. 1E, F; Additional file 3: Table S2). Up-regulated genes (U1–U3) in these three patterns were enriched for distinct functional gene ontology (GO) pathways, with increased expression of cell cycle, external stimulus and chloroplast-related genes in the 1W-responsive cluster; developmental and cellular organization-related genes in the 3W-responsive cluster; and finally ribosome and transcription process-related genes in the DYN-responsive cluster (Fig. 1E; Additional file 3: Table S3). Down-regulated genes (D1–D3) were instead enriched for stress-related terms such as hypoxia among 1W-responsive genes; amino acid biosynthetic pathways among DYN-responsive genes; and photosynthesis-related terms among 3W-responsive genes (Additional file 3: Table S3). GO enrichment analysis of RNA-seq clusters revealed that cold stress response genes are enriched in U1 and U2 (Additional file 3: Table S3).

While the overall patterns of gene expression were highly similar between AVG and FLU conditions, we did observe a slight lag in the FLU gene expression per week between conditions (Additional file 1: Fig. S2B). Therefore, at the global scale, seasonal decreasing temperatures reshape the *Arabidopsis* transcriptome in three main patterns of sustained transcriptional responses over time. It is worth noting that the time-course experiment was carefully designed to capture cold-specific transcriptome responses. To this end, we fixed sampling time (ZT4) and day-length (SD) to minimize potential circadian and photoperiodic effects. While the possibility remains that our data do not accurately detect genes with maximum expression peaks outside ZT4, most of the thousands of DE

genes belonging to clusters U1-U3 and D1-D3 (Fig. 1E, F; Additional file 3: Table S2) are very likely uniquely responding to the continuously decreasing temperatures.

Progressive cold sequentially triggers the *COOLING* and *CHILLING* genetic programs

To explore the regulatory features of the three major transcriptional patterns observed, we focused our analysis on the clusters of up-regulated genes (U1-U3, Fig. 1E, Additional file 1: Fig. S3A). We selected three genes showing increased transcript accumulation in each transcriptional program from our RNA-seq data: 1W-responsive genes (*EMB2746*, *HCAR*, *SCPL49*), 3W-responsive genes (*RD29B*, *RD29A*, *TPR*) and DYN-responsive genes (*KIN1*, *EARL11*, *AZ11*) (Fig. 2A, Additional file 1: Fig. S4). For the 3W and DYN categories, we selected genes with previously reported function in Arabidopsis cold acclimation and freezing tolerance (*RD29B*, *RD29A*, *KIN1*, *EARL11* and *AZ11*) [18–20]. We also included the *TETRATRICOPEPTIDE REPEAT (TPR)-LIKE* gene (*AT1G80130*), which although its function remains undescribed, it appears within top hits in previous transcriptomic analyses [6, 21]. For the genes falling in the 1W category, we selected 3 genes out of the 219 genes falling in the U1 cluster (Fig. 1E, F; Additional file 3: Table S2), which had relatively constant expression over time in 22 °C conditions and significant activation from 1W onwards at both AVG and FLU. We estimate that nearly 50 percent of the genes within U1 show similar expression profiles as these selected candidate 1W genes (Additional file 1: Fig. S5). Despite some differences at individual time-points, all nine genes tested showed the expected and similar patterns both under AVG and FLU conditions at most weeks (Fig. 2A, Additional file 1: Fig. S4). These observations show the plants' ability to largely buffer against the noise of temperature fluctuations, instead integrating and responding to an average decreasing temperature signal. We validated this further using an additional cold time-course with plants exposed to stepwise decreasing temperatures, wherein the temperature was kept constant for a week (Fig. 2B; Additional file 1: Fig. S4). The weekly temperature setting corresponded to the mean temperature of each week under AVG conditions, lowering the temperature by 4 °C per week (14 °C in week 1, 12 °C in week 2, 6 °C in week 3 and 2 °C in week 4; Additional file 2: Table S1). Plants exposed to weekly decreasing temperatures (WEEK) as well as a control group maintained at 22 °C (CTL) were sampled every week and subjected to RT-qPCR analysis. The expression profiles of the 1W-, 3W- and DYN-responsive genes were highly similar between the three experimental conditions (WEEK, AVG and FLU), demonstrating that plants can effectively buffer transient temperature fluctuations, while responding to a cumulative signal of progressively decreasing temperature.

We also observed that 1W-responsive genes were significantly activated at 10 °C, whereas 3W- and DYN-responsive genes only responded when temperature drop to 6 °C (Fig. 2A and B; Additional file 1: Fig. S4). Therefore, the WEEK experiment helped us narrow down the temperature thresholds for significant transcript accumulation of 1W- (< 14 °C) and 3W-responsive genes (< 10 °C). Interestingly, the DYN genes behaved like 3W-responsive genes in the WEEK experiment, implying that the dynamic pattern may only be observed when temperature continuously changes over time. To further refine the temperature thresholds for activation of 1W-, 3W- and DYN-genes, we exposed two-week-old Arabidopsis plants to ten different temperatures varying from 5 °C to 21 °C for the same period of time (1 day; 1D) using a thermal gradient table [22]. We assessed transcript accumulation of the nine selected genes belonging to the distinct transcriptional programs at each specific temperature by RT-qPCR. The data demonstrated that expression of 1W-responsive genes become significantly activated and saturated at temperatures varying from 16.5 °C to 13 °C (Fig. 2C; Additional file 1: Fig. S6). In contrast, 3W-responsive genes required 10.9 °C or lower for significant activation. As in the WEEK experiment, dynamic genes fell within the 3W temperature range. These results demonstrate a clear distinction between the genetic programs primarily responding to either moderate cold temperatures (*COOLING* genes) or chilling temperatures (< 10 °C; *CHILLING* genes).

The distinction between the *COOLING* and *CHILLING* gene programs was further supported by comparing the expression profiles of genes in clusters U1–U3 (Fig. 1E; Additional file 3: Table S2) with a previously published

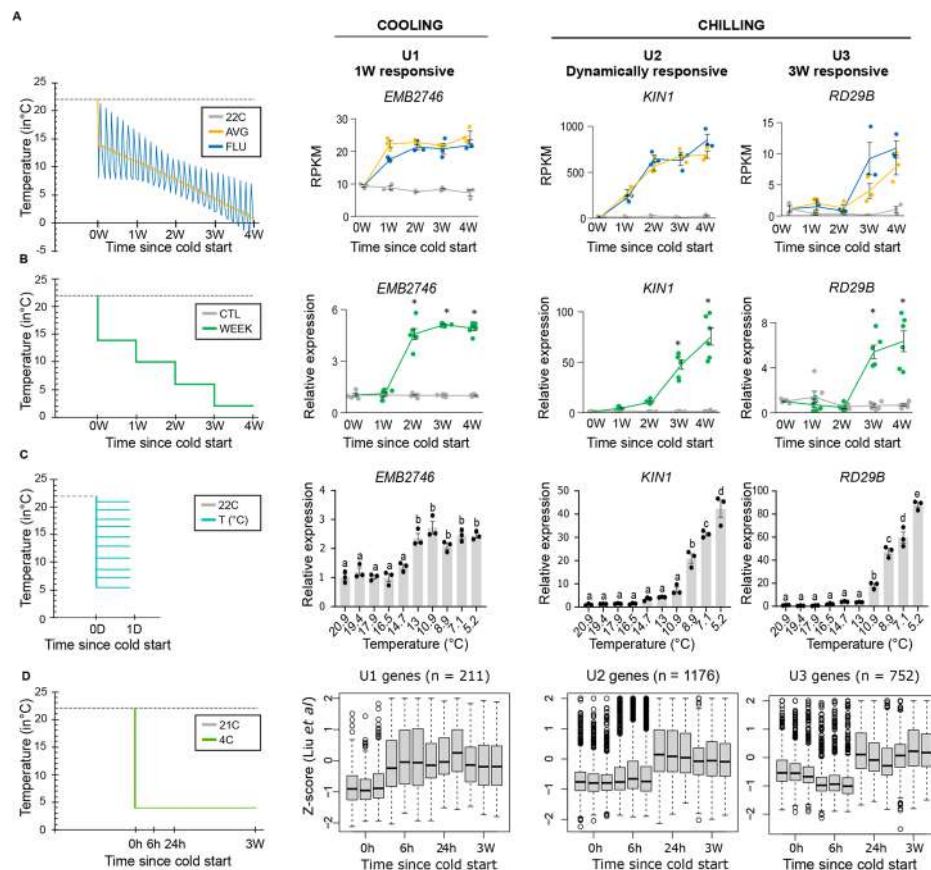


Fig. 2 Discrete temperature thresholds dictate distinct functional transcriptional programs. **A** Expression profiles of selected candidate genes, obtained from plants grown at 22 °C under SD conditions for 2 weeks before exposing them to FLU (blue), AVG (orange) or 22C (grey) conditions (visualized in the left-most panel). Error bars show the standard error of the mean (SEM) from three RNA-seq biological replicates. **B** RT-qPCR validation of transcriptomic data using selected candidate genes. Seedlings were grown at 22 °C (CTL; grey) under SD conditions for 2 weeks and then exposed to weekly temperature blocks (WEEK; green). Gene expression was normalized to the reference gene *PP2A*. Error bars indicate SEM from six biological replicates. Each sample contained more than 50 seedlings. Statistically significant differences were evaluated by a one-way ANOVA with a Tukey-HSD post hoc test. Asterisks indicate significant differences between two treatments ($p < 0.05$). **C** Relative expression of selected candidate genes upon exposure to specific temperatures along a gradient. Plants were grown at 22 °C in SD conditions on a thermal gradient table (grey) for 2 weeks and then exposed for 1D to selected temperatures across a gradient ranging from 5.2 °C to 20.9 °C (blue). Error bars indicate SEM. The experiment was performed three times and each sample contained at least 50 seedlings. Letters indicate statistically significant differences ($p < 0.05$) as determined by a one-way ANOVA followed by a Tukey-HSD post hoc test. **D** Boxplots of Z-score expression values from the Liu et al. (2022) RNA-seq [17] of genes from the up-regulated clusters (U1-U3) in this study. The most comparable subset of timepoints from Liu et al. [17] (up to 3 weeks of cold exposure) were used

RNA-seq dataset of *Arabidopsis* plants exposed to constant cold (4 °C) for three weeks [17] (Fig. 2D). This analysis revealed that most *COOLING* genes reached their peak expression within 6 h of exposure to constant cold, whereas *CHILLING* genes required prolonged low-temperature treatment for activation. These findings also highlight the graded nature of low-temperature perception in plants. After six hours at 4 °C, plants do not yet initiate the *CHILLING* program but instead engage a moderate cold (*COOLING*) response, suggesting an active mechanism distinguishing transient cooling from sustained cold exposure.

Temperature before time is the major driver of cold transcriptional reprogramming

Despite the presence of major (Figs. 1E, 2) temperature-related transcriptional patterns described above, we questioned whether some of their up-regulation could be explained in part by the progression of time, in addition to low temperature, as has been previously proposed for *VERNALIZATION INSENSITIVE 3* (*VIN3*) [4, 23]. *VIN3* is a key thermosensory component of the vernalization mechanism which slowly and progressively increases in response to weeks of winter cold [23]. In our dataset, *VIN3* appeared among the DYN-responsive genes in the *CHILLING* group (U2, Additional file 3: Table S2; Fig. 3A). Our analysis of the expression levels of *COOLING* and *CHILLING* genes in the Liu et al. RNA-seq dataset (Arabidopsis plants exposed to constant 4 °C for three weeks [17] suggested that they are primarily responsive to temperature before time (Fig. 2D). Among the *CHILLING* group, the 3W- and DYN-responsive genes (U2 and U3; Additional file 3: Table S2), which require at least two weeks for significant activation in the cold time-course, reach their maximum expression levels within 24 h of exposure to 4 °C. In contrast, *VIN3* required 3 weeks for significant activation under constant cold (Fig. 3B), further demonstrating the unique time-dependent regulation of *VIN3* expression [4, 11, 23]. Noteworthy, we could identify a small number of genes in each cluster with *VIN3*-like expression patterns in this experiment (Additional file 1: Fig. S3B).

To further test the impact of time and temperature on gene expression, we calculated the percent gene expression variance explained when modeled by these two variables. This revealed that temperature at sampling is the likely driver of gene expression changes for most genes, no matter the order of variables in the model or which cluster the genes are from (Fig. 3C; Additional file 1: Fig. S3C). Altogether, these data confirmed that the considerable changes to the plant transcriptome observed in a simulated autumn–winter scenario are caused primarily by temperature. Only a subset of cold-inducible genes also require time for activation (Additional file 1: Fig. S3B; Additional file 3: Table S4).

Transient *CBF* expression defines the boundary between *COOLING* and *CHILLING* program activation

To investigate whether any genes were regulated at the interface between *COOLING* and *CHILLING* transcriptional programs, we looked for genes which were significantly up-regulated in week 2, but not weeks 1 or 3, in either AVG or FLU compared to 22 °C and found 269 and 169 such examples, respectively (Additional file 3: Table S5). Curiously, among genes present at the temperature- and temporal-dependent separation in activation

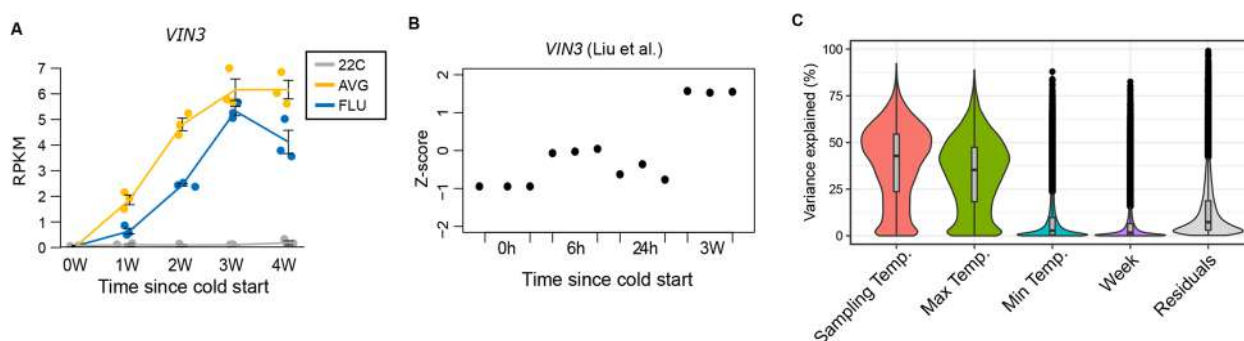


Fig. 3 Temperature before time is the major driver of cold transcriptional reprogramming. **A** Expression profile of *VIN3* derived from the RNA-seq dataset obtained from plants grown at 22 °C under SD conditions for 2 weeks before exposing them to FLU (blue), AVG (orange) or 22 °C (grey) conditions. Error bars show the standard error of the mean (SEM) from three RNA-seq biological replicates. **B** Z-score values of *VIN3* expression values in the Liu et al. (2022) RNA-seq dataset [17]. **C** Percent variance of gene expression of all genes in the RNA-seq experiment explained by temperature parameters at the moment of sampling, the maximum or minimum daily temperature of the sampling day, or time. Residuals represent the remaining unexplained percent of gene expression by the model for each gene

of these two programs, we identified the well described *CBF* cold-responsive transcription factors. In clear contrast to the thousands of cold-inducible genes defined by our clusters U1-U3 (Fig. 1E; Additional file 3: Table S2), whose expression is induced and sustained at different ranges of cold temperatures (Fig. 1F), *CBF* genes were only transiently activated during the cold time-course. We observed that *CBF2* and *CBF3* expression peaked under AVG conditions at week 2 of the experiment (Fig. 4A), with 7.8 °C measured at time of sampling (Additional file 2: Table S1), while *CBF1* was not induced in the cold time-course. Based on these observations, we hypothesized that activation of the *CHILLING* but not of the *COOLING* program was dependent on *CBF2/3* activity. To verify this, we tested expression of *COOLING* and *CHILLING* genes in WT and *cbf123* triple mutant plants [21]. Our data showed that functional *CBFs* are not required for the induction of the selected *COOLING* genes, while they appear essential for the full activation of some but not all of the *CHILLING* genes under our experimental conditions (Fig. 4B). These results suggest that transient activation of *CBF* transcription factors under natural conditions initiate the plant cold acclimation program by triggering the activation of a sub-group of *CHILLING* genes. Furthermore, we compared our data to the *CBF*-regulated genes defined by previous works. We observed that out of the 133 genes belonging to the “*CBF* regulon” [24], 45 (34%) were *CHILLING* genes

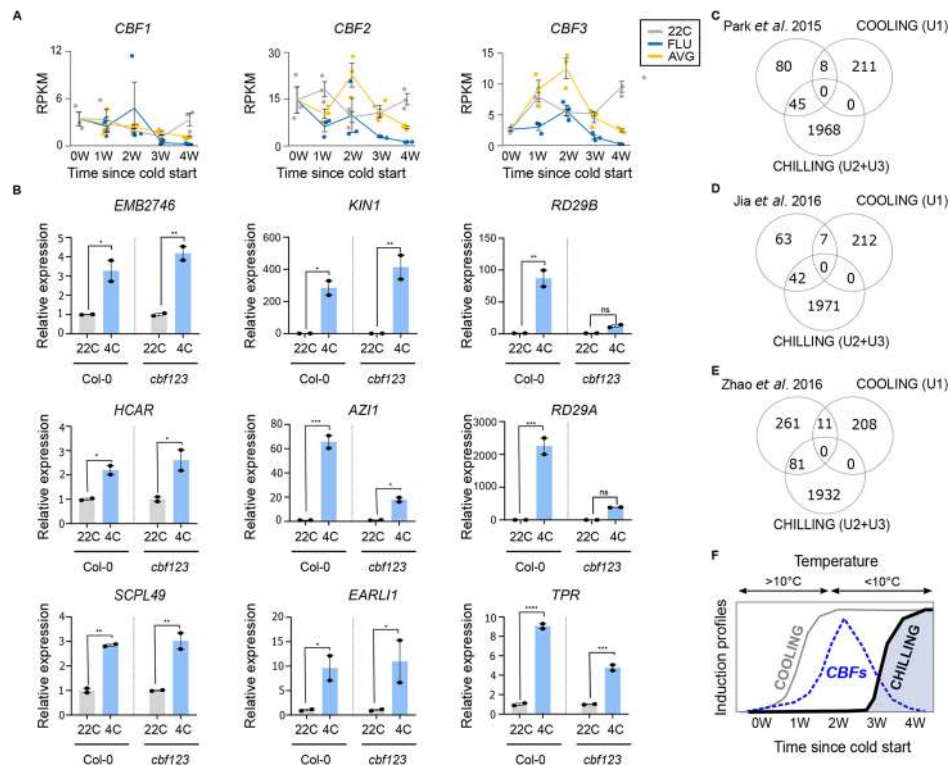


Fig. 4 Regulation of the *CHILLING* program by *CBF* genes. **A** Expression profiles of *CBF1*, *CBF2* and *CBF3* under AVG (orange), FLU (blue) and 22°C (grey) conditions from RNA-seq analysis. Error bars show the standard deviation of the mean from three RNA-seq biological replicates. **B** Relative expression of selected genes belonging to the *CHILLING* and *COOLING* transcriptional programs in Col-0 wild-type and *cbf123* triple mutant plants. Plants were grown at 22 °C under SD conditions for 2 weeks before being exposed either at 22 °C or at 4 °C for 1D. Gene expression was assessed by RT-qPCR in whole seedlings relative to the reference gene *PP2A*. Error bars indicate SEM. Three biological replicates were performed, and each sample contained more than 50 seedlings. Asterisks show significant differences between two samples (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$) as determined by a one-way ANOVA followed by a Tukey-HSD post hoc test. **C-E** Venn diagrams showing the gene overlaps of the *COOLING* and *CHILLING* programs (Supplementary Data Set 2) described in this work with three different publicly available datasets for *CBF* genes: **C** RNA-seq of *CBF1/2/3* overexpressing lines at 22 °C by Park et al. [24]; **D** RNA-seq analysis of *cbf* triple mutant at different times at 4 °C by Jia et al. [21]; **E** RNA-seq analysis of *cbf* triple mutant at different times at 4 °C by Zhao et al. [6]. **F** Schematic representation of transient expression of *CBFs* is at the interface of *COOLING* and *CHILLING* gene induction

while only 8 (6%) fell in the *COOLING* category (Fig. 4C). Likewise, 37.5% (42/112) and 23% (81/353) of the CBF-regulated genes reported by Jia et al. [21] and Zhao et al. [6] in their 4 °C transcriptome studies, respectively, belonged to the *CHILLING* group, while only 6% (7/112) and 3% (11/353) of the genes from each dataset fell into the *COOLING* category (Fig. 4D, E). This analysis further reinforces that CBF factors majorly regulate a subset of *CHILLING* genes, while only a reduced number of *COOLING* genes are affected by CBF function (Fig. 4F).

Functional circadian clock and light signaling is required for *COOLING* and *CHILLING* responses

Our results revealed that a subset of *CHILLING* genes require CBF for transcriptional activation in response to cold (Fig. 4F). However, if and which additional factors control the *CHILLING* program and what upstream regulatory factors lead to induction of the *COOLING* program remained in question. To answer this, we profiled changes in genome-wide chromatin accessible regions (ACRs) during the cold time-course, as promoter proximal ACRs could allow us to predict putative regulatory TFs. We thus performed ATAC-seq of a subset of plants grown in 22 °C and FLU conditions from the RNA-seq experiment (Fig. 1B). In total, 16 ATAC-seq libraries, with 2 replicates per week and condition were prepared and sequenced (Additional file 2: Table S3). Most detected promoter accessibility changes in plants exposed to increasing cold over the course of the entire experiment were associated with increasing accessibility (clusters A1 and A2; Fig. 5A; Additional file 3: Tables S6 and S7). Genes with increasing promoter accessibility were enriched for similar pathways as seen in the RNA-seq clusters of genes with increasing expression in cold conditions (Additional file 1: Fig. S7A; Additional file 3: Table S8).

We subsequently performed motif enrichment from the ACR sequences of promoters with increased accessibility in cold. Several known *cis* elements, including the G-box [25], TCP elements [26], ACE [27]/bZIP [28], CE3 [29], the Site II motif [30], and the E2F binding site [31] were found to be highly overrepresented (Fig. 5B; Additional file 3: Table S9). Next, we searched our RNA-seq data for cold-responsive TFs whose known binding sites were enriched at ACRs with increasing accessibility during cold (Fig. 5C). In order to validate these results, among the identified candidate TFs (Fig. 5C), we focused our attention on two key regulators, CCA1 and HY5, as these were previously reported to have a role in response to cold in Arabidopsis [32, 33]. Considering their expression profiles in our RNA-seq data, *CCA1* showed significant transcript accumulation at cooling temperatures, which was sustained at lower temperatures (Fig. 6A). In contrast, *HY5* was more responsive to chilling temperatures (Fig. 6A). Given their differential activation in the cold time-course, we hypothesized that CCA1 and HY5 could be involved in the regulation of *COOLING* and *CHILLING* genes, respectively. We therefore assessed expression of the 9 candidate genes described above in 2-week-old wild-type, and *cca1* and *hy5* mutant plants exposed to either 12 °C or 4 °C during 1D and 7D (Fig. 5D, E; Additional file 1: Figs. S8, S9 and S10). While HY5 seemed to regulate only a specific subset of genes, CCA1 showed a profound effect on transcript accumulation at both temperatures assayed. All genes, except for *AZII* at 12 °C, showed significantly less activation in *cca1* plants after 1D at both cooling and chilling temperatures. Likewise, both *COOLING* and *CHILLING* genes are significantly less induced in *cca1* after 7D at both 12 °C or 4 °C (Additional file 1: Fig. S10). These results further support the essential role of the circadian regulator CCA1 in gene activation at 12 °C and lower temperatures (Fig. 5F).

The association of discovered motifs from ATAC-seq clusters (Fig. 5B) with a broad range of cold-induced TFs with similar known binding sites revealed a complex network of regulators associated with distinct temperature activations (Fig. 5G; Additional file 3: Table S10). This analysis yielded additional candidate HY5 targets including *COR28*, which together are known co-regulators of photomorphogenesis [35], as well as the cold gene *COR47* [36]. We also found *CHE*, a key regulator of CCA1 [37] as a candidate repressor whose target ACRs gain accessibility as its expression decreases over time in the cold. Similar declining expression of highly connected TFs in the network such as *TCP7* and *HBII* may explain in part the reduction in leaf growth rate [38, 39]. Significant additional efforts should be devoted to determining which TFs specifically regulate each transcriptional program.

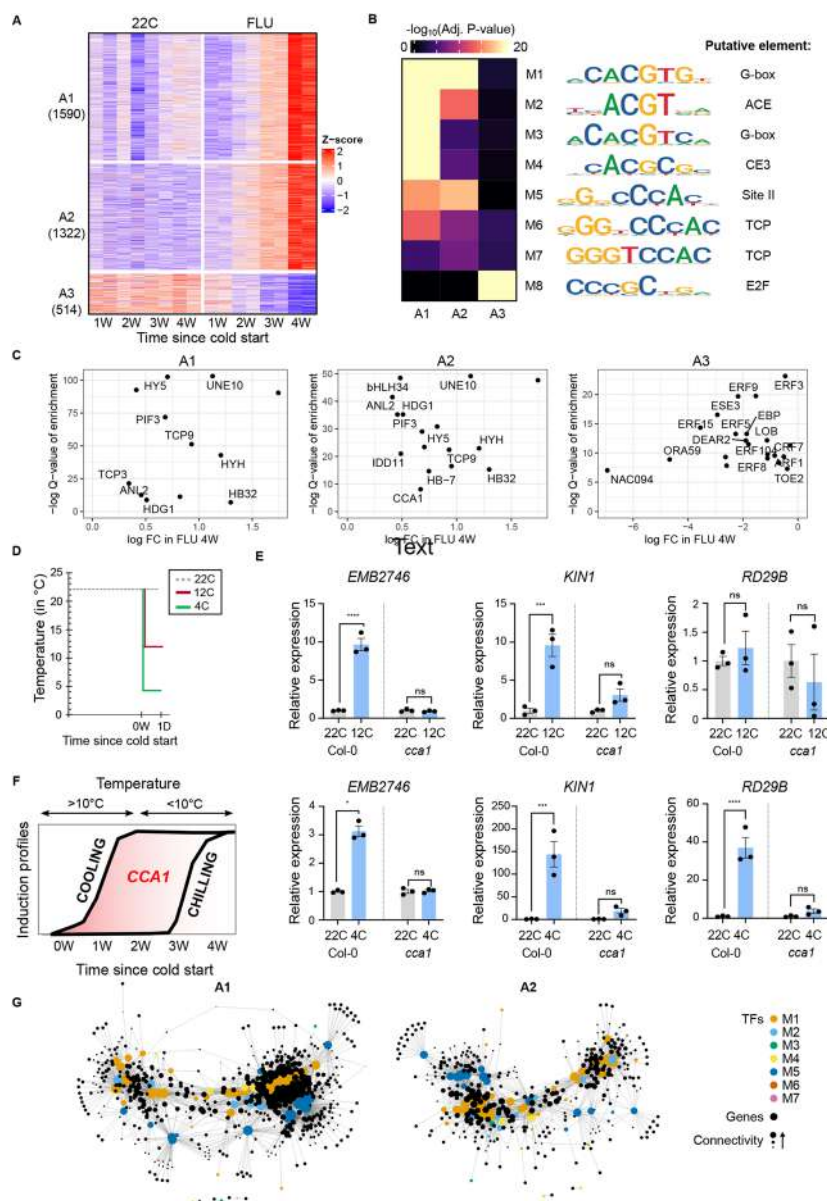
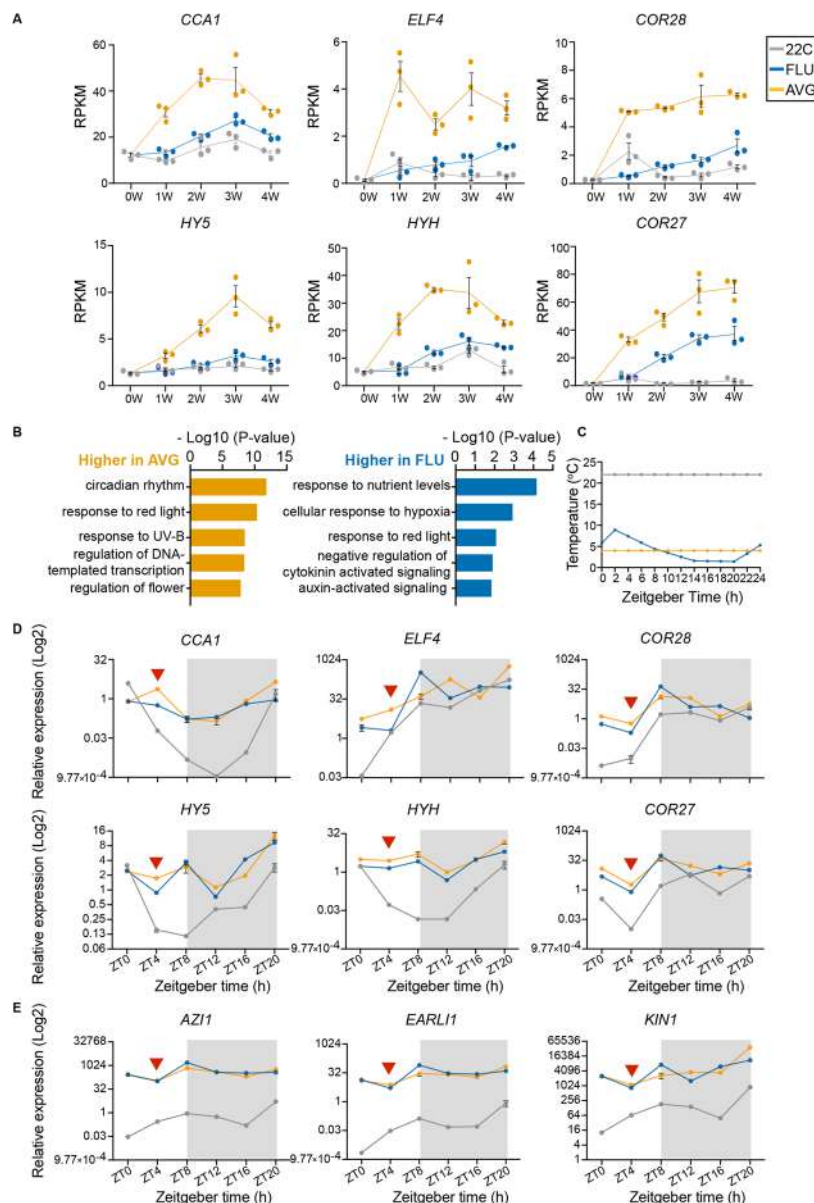


Fig. 5 A network of transcription factors drives increased promoter accessibility at cold-responsive genes. **A** Heatmap of Z-scores of clustered peak accessibility data from the ATAC-seq, showing clusters A1, A2 and A3. **B** Enrichment of de novo discovered motifs from ATAC-seq clusters using negative log₁₀-transformed adjusted overrepresentation *P*-values. The motifs (putative elements) are drawn as information content matrices to the right. **C** Known transcription factor binding sites that are significantly enriched in the ACRs of clusters A1, A2 and A3. Enrichment was determined using sea [34] and plotted as the negative log of the Q-value against the log fold-change of the transcription factor's expression in week 4 of the FLU RNA-seq data. **D** Temperature profile used for Fig. 6E. Plants were grown at 22 °C under SD conditions for 2 weeks and then exposed for 1D to either 4 °C (4C; green), 12 °C (12C; red) or constant 22 °C (22C; grey). **E** Relative expression of selected candidate genes from Col-0 wild-type and *cca1* mutant plants grown as described in Fig. 6D. Gene expression was assessed by RT-qPCR using whole seedlings relative to the reference gene *PP2A*. Error bars indicate SEM. At least 3 biological replicates were performed for each condition. Each sample contained more than 50 seedlings. A one-way ANOVA followed by a Tukey-HSD post hoc test was performed indicate the significant differences. Asterisks show significant differences between two samples (**p* < 0.05, ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001). 'ns' stands for non-significant. **F** Schematic representation of *CCA1* expression profile relative to genes belonging to the *CHILLING* and *COOLING* programs. **G** Connectivity network of transcription factors and target genes inferred from the integration of RNA-seq and ATAC-seq data, using the de novo discovered motifs (M1-M7). Transcription factor-gene pairs with a matching binding site in the promoter ACR and a Pearson correlation of expression greater or equal to 0.5 were used to create networks for ATAC-seq clusters A1 and A2

Fig. 6 Daily oscillations of circadian clock and light signaling components in long-term cold. **A** Expression profile from RNA-seq data of circadian clock and light signaling genes under 22 C (grey), AVG (orange), and FLU (blue) conditions. Error bars show the standard deviation of the mean from three RNA-seq biological replicates. **B** GO analysis of up-regulated genes in AVG (left; $n=95$) and FLU (right; $n=53$) conditions. Top 5 representative terms are listed and ranked by P -values. **C** Daily temperature profile at 3 W under 22 C (in grey), AVG (orange), and FLU (blue) conditions. **D** and **E** Col-0 seedlings (leaves and apices) were collected every 4 h over a 24-h period at 3 W under AVG, FLU, and 22 C at lights-on (Zeitgeber time 0) for RT-qPCR experiment. Transcript levels were normalized to *PP2A*. Error bars indicate mean $\pm$ SEM. 3 biological replicates were performed for each condition. Red arrowheads depict the equivalent time-point to 3 W in (A). Grey boxes indicate dark period (lights off)



Daily oscillations of circadian clock and light signaling components are dampened during autumn–winter progression

Our results showed that Arabidopsis plants largely buffer gradual seasonal temperature fluctuations at the transcriptomic scale (Fig. 1E, F), progressively activating *COOLING* and *CHILLING* genes (Fig. 2), partly dependent on the function of the regulatory TFs *CCA1* and *HY5*. However, we noticed that expression of *CCA1* and *HY5* appears particularly sensitive to FLU conditions (Fig. 6A). Furthermore, we identified 153 genes that were significantly DE (two-fold change) between AVG and FLU conditions (95 up-regulated in AVG and 58 in FLU; Additional file 3: Table S11). GO analysis revealed that genes with higher expression under AVG were enriched for circadian clock and light response functions (Fig. 6B; Additional file 3: Table S12), whereas genes with higher expression in FLU were related to nutrient metabolism and hormone signaling (Fig. 6B).

Like *CCA1* and *HY5*, several TF genes with functions linked to photoperiodic flowering *CONSTANS* (*CO*) and *FLAVIN-BINDING KELCH REPEAT F-BOX 1* (*FKF1*) or circadian regulation, including *EARLY FLOWERING*

4 (*ELF4*), *PSEUDO-RESPONSE REGULATOR 5* (*PRR5*), *PRR9*, *REVEILLE1* (*RVE1*), *RVE2*, *CYCLING DOF FACTOR 6* (*CDF6*) and *B-BOX DOMAIN* (*BBX*) genes (*BBX18/30/31*), were significantly more induced in AVG compared to FLU conditions (Fig. 6A; Additional file 1: Fig. S11A and S11B) [40, 41]. Similarly, expression of the light-signaling *HY5 HOMOLOG* (*HYH*), as well as of the temperature- and light-responsive genes *COR27* and *COR28*, was attenuated by daily temperature fluctuations [42, 43]. The reduced expression of these light- and circadian-responsive genes may, at least in part, reflect the higher temperature at sampling, which was consistently higher in FLU conditions. Conversely, the differential expression patterns could also be the result of diurnal oscillations.

To disentangle temperature effects from circadian regulation of this subgroup of light- and circadian-responsive genes, we carried out a second temperature time-course following the same settings as in Fig. 1. We collected samples every 4 h at the 3 W time-point (Fig. 6C) to test expression of *CCA1*, *COR27*, *COR28*, *HY5*, *HYH*, *ELF4*, *PRR5* and *PRR9* genes by RT-qPCR (Fig. 6D; Additional file 1: Fig. S11C). All genes presented diurnal oscillation in warm conditions (22C). As previously reported, cold temperature conditions (both AVG and FLU) attenuated the amplitude of diurnal oscillations (Fig. 6D; Additional file 1: Figure S11C; [44]). We repeatedly detected higher accumulation of all transcripts at ZT4 under AVG compared to FLU, showing the robustness of our experimental setting. However, these ZT4 differential expression levels were not maintained throughout the 24 h period. Instead, transcript accumulation at 22C intersected AVG and FLU levels in a time-of-the-day-dependent manner. In contrast, expression levels of the *CHILLING* genes *AZII*, *EARLI1* and *KIN1* at AVG/FLU were significantly different from 22C throughout the 24 h period (Fig. 6E). These results indicate that the seasonal, long-term exposure to low temperature, both under AVG or FLU regimes, dampens the diurnal oscillations of clock and light regulators. Thus, what looks as a cold-dependent induction of these genes over time at a specific time of the day (ZT4) is in fact the result of them reaching the maximum expression levels of each day in the warm (22C).

Long-term, progressive cold treatment confers robust cold acclimation

Although we did not detect major differences between AVG and FLU at the transcriptomic scale over weeks (Fig. 1E, F) or during a 24 h period in 3 W (Fig. 6D), we still wondered whether AVG or FLU would have a distinct effect on plant growth and freezing tolerance. To assess this, in the independent experimental repeat of the temperature time-course, we included mutants of the regulatory TFs *cbf123* (Fig. 4B), *cca1* (Fig. 5E; Additional file 1: Figs. S8 and S10) and *hy5* (Additional file 1: Fig. S9), as well as two alleles of the novel *COOLING* gene *HCAR*, and the previously characterized *CHILLING* gene *AZII* [45]. Due to the extremely poor germination of *cca1* under SD, we could not include it in our analysis. We quantified fresh weight (FW) and dry weight (DW) in AVG, FLU and 22C (Additional file 1: Fig. S12), observing a marked retardation of growth, and therefore decreased FW and DW, for all genotypes under AVG and FLU. Plants showed no significant differences in FW and DW under 22C. In AVG, *cbf123* and *hy5* showed significantly lower FW and DW compared to Col-0. In contrast, *azi1-1* appeared less sensitive to chilling, showing higher water retention (higher FW) and biomass (higher DW). Under FLU, *cbf123* and *azi1-1* were consistently more affected by cold, while *hy5* was indistinguishable from WT. These results reinforce the role of HY5 and CBF in Arabidopsis chilling tolerance, and revealed that *AZII* is required to induce growth retardation under chilling conditions. Conversely, *hcar* mutants behaved like Col-0 under all conditions tested, suggesting that the *COOLING* gene *HCAR* may not be related to chilling tolerance.

We next evaluated the effect of AVG and FLU on plant freezing tolerance by exposing plants to below zero temperatures at the end of the experiment. Surprisingly, all genotypes showed 100% survival to freezing following acclimation at FLU and AVG, while plants incubated at 22C were severely affected by the freezing treatment (Additional file 1: Fig. S13A). The only visible effect of the freezing treatment for both AVG and FLU plants was a delay in flowering time (Additional file 1: Fig. S13B and S13C). These results demonstrate that the reduced

tolerance to freezing of *cbf123*, *hy5* and *azi1-1* mutants after relatively short cold acclimation at constant cold [6, 19, 32] can be overcome by a longer decreasing temperature treatment that mimic autumn–winter progression.

Promoter accessibility of cold down-regulated genes is maintained during the cold season for fast reactivation in the spring

Progressive cold leads to significant transcriptional reprogramming (Fig. 1E). Under our experimental settings, we detected thousands of up- and down-regulated genes, with the number of down-regulated genes being almost double the count of up-regulated genes in week 4 under AVG and FLU conditions (Fig. 1D). However, this marked trend towards transcriptional repression was not reflected in the global levels of genome accessibility (i.e. cluster A3 is much smaller than A1 and A2; Fig. 5A). Likewise, when comparing the average accessibility changes of the most up-regulated genes in the final week of cold in FLU conditions, we observed a clear trend of increasing promoter accessibility (Fig. 7A). Conversely, there was no average loss of average promoter accessibility for the most down-regulated genes.

Down-regulated genes in our conditions were mainly associated with plant metabolism, biosynthesis and photosynthesis (Fig. 1E), processes known to be attenuated by the plant to survive winter conditions [46]. Thus, we wondered whether promoters remaining accessible was associated with plants needing to quickly reactivate their metabolic activity when the temperatures become favorable again. To answer this question, we monitored expression of five randomly selected photosynthesis-related genes belonging to clusters D1–3 (Fig. 1E). We repeated the WEEK time-course of Fig. 2B, but in this case, we moved the plants back to 22 °C for 1 d at the end of the cold treatment (Fig. 7B). RT-qPCR analysis revealed that all down-regulated genes tested are reactivated when plants return to warm conditions (Fig. 7C). These results suggest that cold adaptation is not associated with a genome-wide loss of accessibility despite the large number of down-regulated genes, which likely allows the plant to rapidly reactivate transcription after the return of favorable conditions.

Discussion

Plants have evolved complex mechanisms to tolerate and adapt to progressively declining average daily temperatures during the change of seasons from autumn to winter, while also contending with large daily fluctuations. However, how plants integrate and adapt to cold has so far mainly been investigated under constant temperatures (e.g. 4 °C). One pioneering transcriptomic study in *Arabidopsis halleri* has provided insight in how plants integrate environmental cues to adapt to seasonal changes [47]. Here, we show that the regulation of the transcriptome in *A. thaliana* during such changing conditions is highly complex, with a subset of genes responding to daily average temperatures irrespective of the temperature extremes from daily fluctuations (Fig. 1E, F), while the apparent sensitivity to fluctuating temperatures of a smaller number of genes related to light and circadian regulation (Additional file 3: Table S11; Additional file 3: Table S12) is actually the effect of cold temperature on dampening the diurnal oscillations of these gene subset (Fig. 6; Additional file 1: Fig. S11). We demonstrate how plants encode distinct regulatory pathways to control the transcription of genes in response to cooling and chilling (0–15 °C) conditions (Fig. 2A–C). Generally, the *Arabidopsis* transcriptome responds relatively quickly to temperature changes (within hours to a day) (Fig. 2D), allowing plants to adjust their metabolism to changes in the environment and consequently to survive winter. Progressively decreasing cold temperatures induces dynamic changes in gene expression that we classified into two distinct genetic programs that we defined as *CHILLING* and *COOLING* programs, i.e. genes responding at temperatures below 10 °C or at moderate cold (10–15 °C), respectively (Fig. 2). A key finding of this analysis is that plants exhibit a remarkable capacity to buffer temperature fluctuations over daily and weekly timescales.

One interesting question is what are the distinct functionalities of the *COOLING* vs *CHILLING* programs that are sequentially activated during autumn–winter progression. Multiple genes falling in the *CHILLING* category

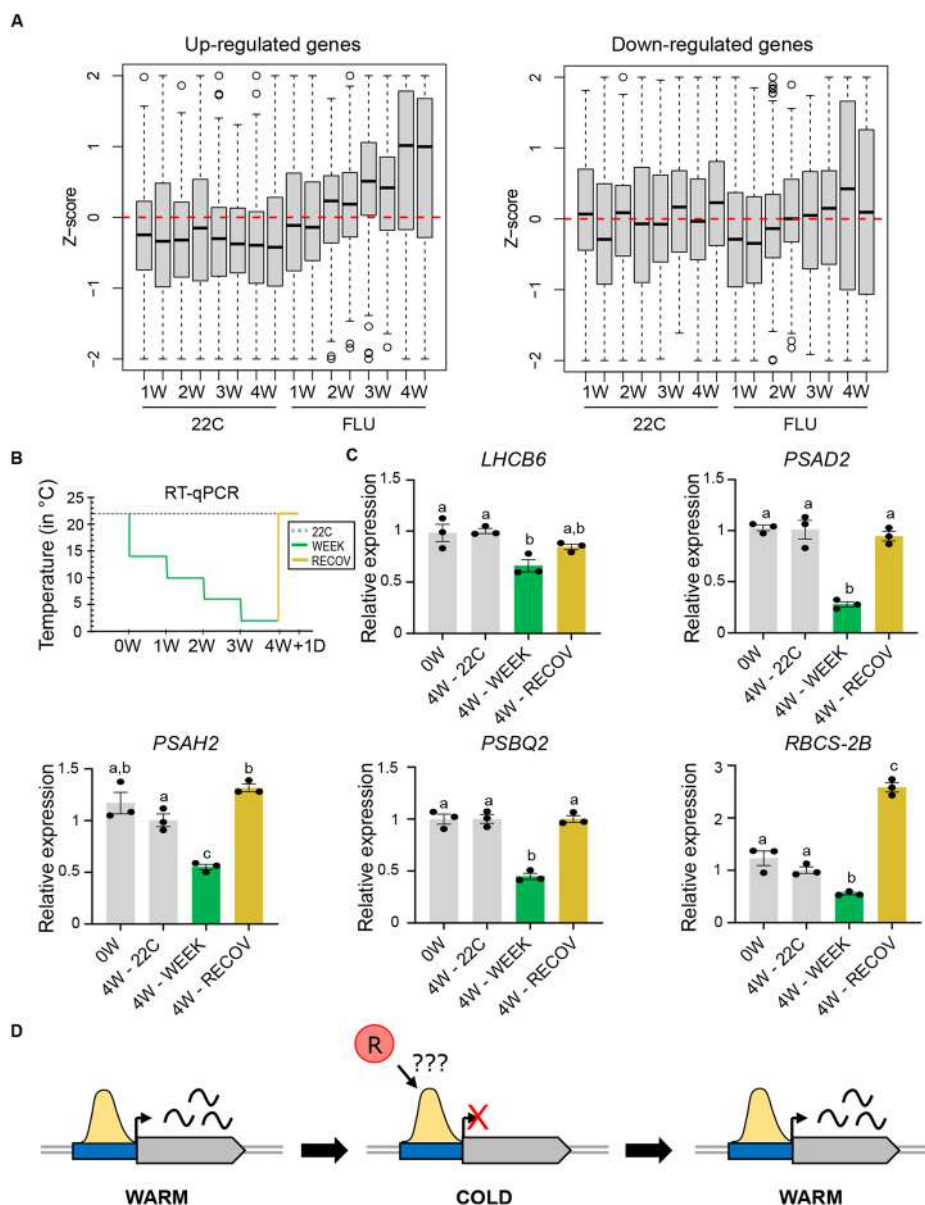


Fig. 7 Promoter accessibility of cold down-regulated genes is retained during the cold season, enabling fast reactivation in the spring **A** Boxplots of promoter accessibility Z-scores of genes with detected ATAC-seq peaks among the top 500 up- ($n=196$) and down-regulated ($n=209$) genes at in the FLU samples compared to 22°C at week 4 of the RNA-seq data. Z-score of 0 is indicated by a red line for reference. **B** Temperature profiles used for the experiment presented in Fig. 7C. Plants were grown at 22 °C under SD conditions for 2 weeks before being exposed to temperature blocks that changed per week (WEEK; green). Plants were finally moved back to 22 °C for 1D. **C** Relative expression of selected photosynthesis-related genes exposed to the temperature regime indicated in Fig. 7B. Gene expression was assessed by RT-qPCR using whole seedlings relative to the reference gene *PP2A*. Error bars indicate SEM. Three biological replicates were performed, and each sample contained more than 50 seedlings. Letters indicate statically significant differences ($p < 0.05$) as determined by a one-way ANOVA followed by a Tukey- HSD post hoc test. **D** Schematic model representing the observed promoter accessibility dynamics of photosynthesis-related genes over seasonal progression. In warm conditions, active gene expression is associated with fully accessible promoters. Despite the significant down-regulation of these genes during cold, their promoters retain full accessibility, likely to allow binding of specific transcriptional repressors (R), which maintain these genes transcriptionally inactive. On return to warm conditions in spring, gene expression is rapidly reactivated to resume active photosynthetic activity. Genes are represented by grey pentagons. Blue boxes depict promoters. Yellow shapes represent chromatin accessible regions. Arrows depict transcription initiation sites

have been characterized in Arabidopsis cold responses, conferring either plant cold and freezing tolerance (e.g. *RD29A/COR78/LTI78*, *RD29B/LTI65*, *KIN1*, *COR15A*, *AZ11*, *EARL11*, *COR27*, *RVE2*, *COR47*) or enabling the floral transition through vernalization (e.g. *VIN3*). In addition to these relatively well-characterized genes, GO analysis suggests that an important proportion of the chilling-induced transcriptome is enriched by terms related to cellular organization as well as RNA processing and modification (Additional file 3: Table S3). Thus, under temperatures below 10 °C plants devote their energy in increasing their capacity to overcome harsh winter conditions. In contrast, the *COOLING* category is enriched by terms related to cold temperature stimulus and response to sugar (Additional file 1: Table S3). Notably, several *COOLING* genes show chloroplast localization, suggesting plants adjust their photosynthetic activity at the initiation of the cold season. In line with this, the *COOLING* gene *RBD1* (AT1G70200, Additional file 3: Table S2), which encodes an RNA binding protein that localizes to the chloroplast, is required for chloroplast function under low temperature conditions [48]. *RBD1* loss-of-function mutants are defective in the generation of mature 23S RNA, presenting a deficiency in chloroplast protein synthesis especially under cold. It remains to be determined whether other chloroplast-localized genes such as *EMB2746* (Fig. 2A) and *HCAR* (Additional file 1: Fig. S4) also play a role in the preservation of chloroplast function in response to cold. Under our experimental setting, the mutant of the *COOLING* gene *HCAR* behaved like WT both regarding cold and freezing tolerance (Additional file 1: Figs. S12 and S13). Although more detailed analysis will be required to fully address the distinctive functions of *COOLING* and *CHILLING* genes, our data clarifies the stepwise changes in transcriptome dynamics as the cold season progresses.

We found that temperature is the primary driver of transcriptional responses during the autumn–winter transition (Fig. 3B, C). Only a small portion of genes also require time for activation (Supplementary Fig. S3B). Among them, the expression of the vernalization-induced *VIN3* progressively increases when the temperature is below 14 °C and according to the duration of cold exposure (over weeks) via the accumulation of transcription factor NTL8/14 to allow flowering under favorable conditions [23, 49]. Nevertheless, despite not being a major driver, time is required in the temperature sensing mechanism. It is probably important for spreading the temperature signal to the whole plant, since Arabidopsis plants exposed to constant 4 °C during 6 h only activate *COOLING* genes that respond to moderate cold, requiring a longer time to induce the *CHILLING* program (Fig. 2D).

By profiling genome-wide chromatin accessibility with ATAC-seq, we revealed a complex regulatory network of factors driving cold-responsive gene expression (Fig. 5G). A motif enrichment analysis of the sequences of promoters with increasing accessibility in cold led to the identification of known *cis* elements (Fig. 5B), for which we predicted TFs able to bind these motifs (Fig. 5C; Additional file 3: Table S9). Among the identified TFs, we focused our attention on CCA1 and HY5, which are both associated with cold acclimation and involved in the regulation of several clock-regulated genes [32, 33, 50, 51]. CCA1 and its paralogue LHY are also known to be involved in the activation of *VIN3* transcription under long-term cold response [10]. Cold-mediated *VIN3* induction and flowering acceleration were impaired in the *cca1 lhy* double mutant, mainly at 12 °C vernalization compared to vernalization at 4 °C [10]. *CCA1* is in fact quickly induced at 12 °C [52]. In our RNA-seq data, *CCA1* expression increased at cooling temperatures, and is sustained at chilling temperatures (Fig. 3B). Using *cca1*, we observed that CCA1 regulates all tested genes at both 12 °C and 4 °C, regardless of the transcriptional program they belong to (Fig. 6E; Additional file 1: Figs. S8 and S10), indicating that CCA1 represents a major regulator of *COOLING* and *CHILLING* programs allowing plants to readjust their transcriptome during the autumn–winter progression. This seasonal CCA1 function seems to be dependent solely on temperature decrease instead of on the progressive shortening of daylength as previously proposed [53].

Interestingly, the well described *CBF2* and *CBF3* genes were transiently activated after 2 weeks of cold exposure, at the interface between the *COOLING* and *CHILLING* genetic programs. Results from *cbf123* triple mutant confirmed that they are required for the activation of *CHILLING*, but not *COOLING* genes (Fig. 4). Accordingly, *cbf123* showed significant growth retardation in the cold time-course, further supporting their implication in plant cold tolerance. Notably, we did not detect significant activation of *CBF1* in the simulated autumn–winter progression. This distinct gene expression patterns of *CBF1* vs *CBF2/3* likely reflects specific

regulatory features within the *CBF* family. Previous studies highlighted both functional and regulatory divergence among *CBF* genes [54], including cross-regulation whereby *CBF2* can repress *CBF1* and *CBF3*, as well as partial overlap and specialization in their downstream target genes [55]. Consistent with this, *CBF2* and *CBF3* appear to be more important for environmental adaptation, whereas *CBF1* may play an essential role in plant survival as indicated by the fact that *CBF1* rarely harbors frameshift or premature stop codon mutations [56]. While all CBFs play a crucial role in cold acclimation and freezing tolerance through the redundant regulation of *COR* genes expression, the analysis of single *cbf* mutants revealed that each CBF protein controls overlapping as well as distinct sets of genes [6, 21, 24]. Besides, although all CBF genes are subject to circadian regulation, *CBF1* displays a distinct circadian expression profile compared to *CBF2* and *CBF3*, particularly in response to dark to light transitions, suggesting an interaction between temperature and diurnal signals [9]. Taken together, these regulatory and functional differences may contribute to the distinct induction dynamics observed for *CBF* genes under constantly decreasing temperatures.

Progressive cold induces changes in promoter accessibility, which are mainly associated with an increasing promoter accessibility of up-regulated genes (Figs. 6A and 7A), as previously reported at constant 4 °C in potato tubers [12]. Surprisingly, we also discovered that down-regulated genes generally do not lose chromatin accessibility (Fig. 7A), probably allowing the binding of repressive TFs. In clear contrast to the developmental regulator *FLC*, whose chromatin switches to a repressive state by the action of PRC2 during winter, the promoters of photosynthesis-related genes remain accessible during the cold season despite the strong downregulation of these transcripts, likely to ensure rapid reactivation of photosynthetic activity upon return to warmer temperatures (Fig. 7D). These observations demonstrate the use of different mechanisms for developmental vs metabolic adjustments across seasons. In line with this, genome-wide distribution of H3K27me3 is not dramatically affected by short- or long-term cold treatment (4 °C) [57]. The few genes gaining H3K27me3 upon cold exposure are mostly enriched for development related terms. Therefore, plants may anticipate a return to warmer temperatures by never fully engaging in long term transcriptional repression of metabolic genes during winter. It remains to be studied what specific mechanisms prevent the loss of promoter accessibility of this large group of genes during the cold season.

Beyond chromatin-level regulation, post-transcriptional mechanisms play central roles in plant responses to temperature. These include mRNA stability and RNA decay pathways [58], temperature-dependent alternative splicing [59], as well as RNA modifications such as *N*⁶-methyladenosine (m6A) methylation that is known to regulate gene translation efficiency under chilling condition [60]. In addition, small RNAs such as microRNAs are key regulators of stress responses by modulating transcript abundance and translation [61]. More recently, long non-coding RNAs (lncRNAs) have emerged as important regulators integrating transcriptional and post-transcriptional control under environmental stress conditions [62]. Collectively, these mechanisms may act in concert with chromatin accessibility to fine-tune transcriptional dynamics and contribute to the rapid recovery of gene regulatory networks upon warming.

Conclusions

Our characterization of the regulatory mechanisms underlying *A. thaliana* responses to gradually decreasing temperatures identified two sequentially activated genetic programs, one responding to moderate cold (*COOLING* genes) and the other to temperatures below 10 °C (*CHILLING* genes). While the *CHILLING* program is partially dependent on CBF function, a proficient circadian clock is essential for plant low temperature responses. Genome-wide chromatin accessibility analyses showed that induction of cold-responsive genes is accompanied by increased promoter accessibility, whereas down-regulated genes, including those related to photosynthesis, retain open chromatin. This pattern likely facilitates their rapid transcriptional reactivation upon warming. Overall, our findings uncover the intricate transcriptomic and chromatin accessibility changes associated with Arabidopsis adaptation to cooling and chilling conditions.

Methods

Plant materials

The Arabidopsis accession Columbia-0 (Col-0) was used as the wild-type control in all experiments. The null mutant *cca1-1* [63] and the *hy5-3* (SALK_096651C) [42] insertion mutant line were kindly provided by Dr. Paloma Mas and Dr. Elena Monte, respectively. The *cbf123* triple mutant was described in [21]. The *hcar* mutant alleles (*hcar-1*, SALK_018790C, N6558117; *hcar-2*, WiscDsLoxHs087_04A, N908281) were a gift from Ito Hisashi [64], and the *azi1-1* mutant was kindly provided by Nicolás Cecchini [45]. All gene identifiers are listed in Additional file 2: Table S4.

Growth conditions and sampling in fluctuating and average decreasing temperature profiles

Plants were grown over a 6-week regime of decreasing or steady temperatures. The temperature profile of the climate chambers (ARALAB 1200) was designed according to Hepworth and colleagues [11] and experiments were performed in short day conditions to minimize variations due to photoperiod. Briefly, Col-0 seeds were sown in soil, stratified for 72 h at 4 °C, incubated at 22 °C for 14 d to allow germination and seedling establishment (8 h/16 h, light/dark photoperiod; approximately 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$; RH 60%) and finally moved to three different plant growth cabinets: (1) at constant 22 °C (22C); (2) with decreasing, fluctuating temperatures (FLU); (3) with decreasing daily average temperature (AVG) (Supplementary Table S1). The FLU temperature regime was designed according to natural days in autumn measured in Eden, Sweden (sowing date in autumn 2009; <https://www.smhi.se/data/meteorologi/ladda-ner-meteorologiska-observationer/#param=airtemperatureInstant,stations=all,stationid=128390>). The means of FLU temperatures were determined daily to generate the AVG temperature. FLU, AVG and 22C plants were grown as indicated above, except a different light intensity ($\sim 100 \mu\text{mol m}^{-2} \text{s}^{-1}$). Aerial parts of seedlings/plants (leaves and shoot apical meristem) were collected at different timepoints (ZT4): firstly, 14 d at 22 °C (pre-growth at 22 °C, 0W); and subsequently, 21 d (1W), 28 d (2W), 35 d (3W) and 42 d (4W) after germination at AVG, FLU and 22 °C, respectively. These samples were used for both RNA sequencing (RNA-seq) and the Assay for Transposase Accessible Chromatin with high-throughput sequencing (ATAC-seq) to monitor the transcriptome and chromatin accessibility, respectively.

Growth conditions in a weekly decreasing temperature profile

Surface-sterilized seeds were sown on $\frac{1}{2}$ MS medium with 0.8% agar and stratified at 4 °C for 3 days. The plates were then transferred to 22 °C in a near-vertical position in a controlled environmental growth chamber under a 8 h/16 h, light/dark photoperiod, with $\sim 100 \mu\text{mol m}^{-2} \text{s}^{-1}$ for 2 weeks. Seedlings were then grown in climate chamber (ARALAB 1200) for 1 week at 14 °C, then 1 week at 10 °C, then 1 week at 6 °C and finally 1 week at 2 °C under controlled conditions (8 h/16 h, light/dark photoperiod; $\sim 100 \mu\text{mol m}^{-2} \text{s}^{-1}$; RH 60%). Seedlings were collected after 14 d (0W) at 22 °C and then 21 d (1W), 28 d (2W), 35 d (3W) and 42 d (4W) after germination at 22 °C and in weekly decreasing temperature conditions, respectively to perform RT-qPCR analyses. Six independent biological replicates were performed, and around 50 seeds were sown on each plate at the beginning of the experiment. For recovery assays (Fig. 7C), seedlings were finally moved back to the normal growth temperature condition of 22 °C for 24 h.

Growth conditions in the 12 °C/4 °C experiment

Surface-sterilized seeds were sown on $\frac{1}{2}$ MS medium with 0.8% agar and grown in the same conditions as above. 2-week-old seedlings were then moved for 1 day (Fig. 6E, Supplementary Figs. S8 and S9) or 1 week (Supplementary Fig. S10) at either to 12 °C or 4 °C under controlled conditions (8 h/16 h, light/dark

photoperiod; $\sim 100 \mu\text{mol m}^{-2} \text{s}^{-1}$). For experiments displayed in Fig. 5B, seedlings were only exposed to a 4 °C treatment for 1D.

Growth conditions for the thermal gradient table experiment

Surface-sterilized seeds were sown on ½ MS medium with 0.8% agar and stratified at 4 °C for 3 days. Stratified seeds were then moved to 22 °C under short day conditions (8 h/16 h light/dark photoperiod, $\sim 100 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 2 weeks then placed on a thermal gradient temperature table [22]. Subsequently the plants were exposed to the given temperatures for 1D: 20.9 °C, 19.4 °C, 17.9 °C, 16.5 °C, 14.7 °C, 13.0 °C, 10.9 °C, 8.9 °C, 7.1 °C or 5.2 °C, by adjusting the table settings. Three biological replicates were obtained for each specific temperature timepoint. Each plate contained more than 50 seeds.

RNA sequencing

Total RNA was extracted from 0.5 g of Arabidopsis leaves and shoots (above ground tissues without roots) with the RNeasy plant total RNA kit (Qiagen; Cat#: 74904) with the optional on-column DNase digestion step (Cat# 79254) according to manufacturer's instructions and resuspended in 30 µL of nuclease-free water. Isolated DNA-free RNAs were submitted to Novogene for stranded RNA-seq library preparation and paired-end sequencing (2 × 150 bp reads). RNA sequencing was performed in triplicates.

RT-qPCR

Total RNA was isolated using TRIZOL (Invitrogen) from around 100–150 mg Arabidopsis tissue powder according to the manufacturer's instructions and resuspended in 25 µL of nuclease-free water. 2 µg of total RNA in 7.8 µL nuclease-free water were then treated with a 2.2 µL DNase I mixture (1 µL of 10X DNase I buffer with MgCl_2 (ThermoFisher Scientific), 1 µL of 1U/µL DNase I (ThermoFisher Scientific) and 0.2 µL of RiboLock RNase Inhibitor (ThermoFisher Scientific; Cat#:EO0381)) for 30 min at 37 °C. To stop the enzymatic reaction, 1 µL of 50 mM EDTA was added and incubated at 65 °C for 10 min. Reverse transcription was performed with the addition of 9 µL Maxima Reverse transcriptase mastermix (1 µL of 100 µM oligo(dT)18, 1 µL of 10 mM dNTPs, 2.65 µL nuclease-free water, 4 µL of 5X RT buffer, 0.1 µL Ribolock, 0.25 µL maxima RT) and incubating for 30 min at 50 °C, before increasing the temperature at 85 °C for 5 min to stop the reaction. The cDNA was diluted by adding 180 µL of nuclease-free water to the RT reaction before use. RT-qPCR was performed in 10 µL reaction volume, by mixing 3 µL of cDNA with 5 µL of 2X SYBR Green mastermix (ThermoFisher Scientific), 0.5 µL of forward and reverse primers (10 µM), and 1.5 µL of nuclease-free water. RT-qPCR analysis was done on a LightCycler480 II (Roche) and data were normalized to *PP2A* (AT1G13320) [11]. Relative expression levels were determined using the $\Delta\Delta\text{Ct}$ method. All primers are listed in Additional file 2: Table S5.

Extraction of plant cell nuclei

Crude nuclei were extracted from Arabidopsis seedlings as previously described [65] with minor modifications. Briefly, approximately 0.4 g of frozen aerial parts of Arabidopsis plants were chopped with a double-sided blade in 4 ml of pre-chilled lysis buffer (15 mM Tris-HCl pH7.5, 20 mM NaCl, 80 mM KCl, 5 mM 2-mercaptoethanol, 0.5 mM spermine, 0.2% Triton X-100, 1X cOmplete Mini EDTA-free protease inhibitor cocktail (Roche; Cat#:11,836,170,001). After being passed twice through Miracloth into a 15 mL Falcon tube, the homogenate was then loaded on the surface of 4 mL sucrose buffer (20 mM Tris-HCl pH8, 2 mM MgCl_2 , 2 mM EDTA, 15 mM 2-mercaptoethanol, 1.8 M sucrose, 0.2% Triton X-100) into another 15 mL Falcon tube. The tube was centrifuged at 2200 g for 20 min at 4 °C. The supernatant was discarded and the pellet gently resuspended in 500 µl ice-cold lysis buffer.

Fluorescence-Activated Nuclei Sorting and ATAC-seq sequencing library preparation

The nuclei were stained with 1 µg/mL of 4,6-Diamidino-2-phenylindole (DAPI; Sigma-Aldrich; Cat#D9542) and the solution was filtered through a 35 µm cell strainer caps into a 5 mL round-bottom polystyrene tube (Falcon; Cat#352235), before being loaded into a flow cytometer (BD FACSARIA SORP, IDIBAPS Flow Cytometry Facility, <https://www.clinicbarcelona.org/en/idibaps/core-facilities/cytometry-and-cell-sorting-facility>). 50,000 nuclei were sorted, collected in a 2 mL Eppendorf tube with 500 µL of lysis buffer and finally centrifuged at 1,000 g at 4 °C for 10 min. The pellet was then washed with Tris-Mg buffer (10 mM Tris-HCl pH8, 5 mM MgCl₂). The supernatant was carefully removed and the nuclei pellet was resuspended in 50 µL tagmentation mastermix (5 µL 10X TMg Buffer (100 mM Tris-HCl pH 7.5, 50 mM MgCl₂), 5 µL DMF, 13.5 µL PBS pH 7.4, 0.5 µL 10% Tween-20, 0.5 µL 1% Digitonin, 3.5 µL 1 mM PitStop, 20 µL nuclease-free water, 2.5 µL recombinant Tn5 loaded tagmentase (purchased at the Dorothy Hodgkin Proteomics and Biochemistry Service, Universidad Pablo Olivade, Seville, Spain) and incubated at 37 °C for 35 min, on a thermomixer at 1000 rpm. After the tagmentation reaction, DNA was purified using Diagenode's MicroChIP DiaPure columns (Diagenode; Cat#C03040001) according to the manufacturer's instructions. ATAC-seq libraries were performed using the 24 UDI for tagmented libraries set I kit from Diagenode (Cat#01011034). Libraries were amplified using 2×NEBNext High fidelity PCR mix (New England Biolabs; Cat.#M0541L). The number of PCR cycles was determined as described previously [66]. ATAC-seq libraries were then purified with AMPure XP beads (Beckman Coulter; Cat#A63880) and their quality were assessed using a Bioanalyzer High Sensitivity DNA chip (Agilent). Libraries were finally sequenced on an Illumina NovaSeq with paired-end (2 × 150 bp) reads.

RNA-seq processing

Raws paired-end reads were trimmed with *cutadapt -a AGATCGGAAGAGCACACGTCTGAACTCCAGTCA -A AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT -m 15* (Martin 2011) and mapped onto the TAIR10 genome assembly [68] using *STAR --outSAMstrandField intronMotif --outMultimapperOrder Random --outSAMmultNmax 1 --outSAMtype BAM SortedByCoordinate --outFilterMultimapNmax 10,000* [69]. Gene counts were obtained using *stringtie* [70] and the Araport11 annotations [71]. The counts table was imported into R and normalized using the *RUVs* function from the *RUVSeq* package with $k = 2$ [72]. The normalized counts were then processed with the *edgeR* package [73], using the *calcNormFactors* and *estimateDisp* functions, and exported to RPKM quantification using the *rpkm* function. For differential expression analyses, the processed counts from *edgeR* were fitted using the *glmQLFit* function and using a design matrix considering the sample condition and week number, then comparisons between groups performed using the *glmQLFTest* and *topTags* functions.

Modelling the importance of time and temperature in gene expression

Percent variance explained analyses were performed using the *variancePartition* R package [74]. The temperature at sampling, minimum and maximum temperatures of the sampling day, and week number were used as variables to calculate percent variance using the *fitExtractVarPartModel* function with the following formula: $\sim \text{sampling_temperature} + \text{maximum_daily_temperature} + \text{minimum_daily_temperature} + \text{week}$. To ensure the resulting parameter estimates were not influenced by the order of variables in the model we tested different version with shuffled placement of each variable in the formula. As suggested in the *variancePartition* manual we also used the *colinearityScore* function to test whether the variables were too highly correlated and thus could result in degenerate parameter estimates. No variables had correlations greater than the suggested cutoff of 0.99 from the *variancePartition* manual. The final results were plotted using the *plotVarPart* function.

Gene expression clustering analyses

Normalized and $\log_2$ -transformed expression data was initially clustered with the *WGCNA* package [75], using the *blockwiseModules* function with parameters: *power*=3, *TOMType*="signed", *minModuleSize*=30, *networkType*="signed", *mergeCutHeight*=0.1, *deepSplit*=4. Clusters showed three broad patterns for both up- and down-regulated genes which eventually served as the classification system for the cold-responsive genes in our experiment: cooling-responsive or *COOLING* (with a shift in expression at weeks 1 and 2), chilling-responsive or *CHILLING* (with a shift in expression at weeks 3 and 4), and dynamic or *DYN* (gradually shifting over the whole experiments). Additionally, a small number of genes with transient expression patterns clustered together. To classify all genes into these patterns, we calculated the squared Pearson correlation coefficient (r) between all per condition gene expression profiles and the average expression patterns of the previously described clusters. Genes were classified with a matching pattern when the r^2 was greater than 0.8, or in cases of multiple matching patterns, the pattern with the highest r^2 . The sign of the correlation coefficient was used to annotate increasing or decreasing expression. Genes without a matching pattern were annotated as stable. To identify cold-responsive genes, we used genes annotated as stable in the constant 22 °C condition and one of the increasing or decreasing patterns in the average or fluctuating cold conditions. The Z-score transformed expression of these resulting cold-responsive genes were plotted as a heatmap using the *ComplexHeatmap* package [76]. Gene ontology (GO) enrichment was performed using the *gprofiler2* package [77] and plotted as semantic enrichment GO clouds with the *simplifyEnrichment* package [78].

ATAC-seq processing

Raw paired-end reads were trimmed using *fastp -a CTGTCTCTTATACACATCT* [79] and mapped onto the TAIR10 genome assembly with *bowtie2 --very-sensitive -X 2000 --dovetail* [80]. Read mate correction was applied with *samtools fixmate*, then reads coordinate sorted with *samtools sort*, duplicates removed using *samtools markdup -r*, and finally reads filtered and non-nuclear reads removed using *samtools view -F 3852 -q 10* [81]. Reads overlapping a blacklist [82] were removed using *bedtools intersect -v* [83]. Next, the reads were sorted by name using *samtools sort -n* and converted to BEDPE format using *bedtools bamtobed -bedpe*. Reads outside nucleosome-free regions (fragment size greater than 120 bp) were removed and the remaining reads converted to BED format using *awk*, then sorted using *bedtools sort*. BigWig files were generated by resizing the reads 100 bp from the 5' ends using *awk* and *bedtools slop -l 50 -r 49*, then first converting to bedGraph format with *bedtools genomcov -bg* before finally converting to BigWig with the UCSC tool *bedGraphToBigWig*. Peaks were called for all sample BED files using *macs3 callpeak --nomodel --shift -100 --extsize 200 -p 0.05 -f BED --keep-dup all -g 1.191e8* [84]. The peaks were filtered to only keep those with a q value less than 0.001 and present within at least 3 samples using *awk* and *bedtools merge -d 100 -c 4*. Read counts were obtained from the final merged peak set using *bedtools coverage -counts* and imported into R. Batch correction of the reads counts was performed by first obtaining the residuals with the *residuals* function (with *type*="deviance") from *edgeR* using counts fitted to a model considering the sample condition using the *glmFit* function from *edgeR*, then using them with the *RUVr* function from *RUVSeq* (with $k=2$). The corrected read counts were then processed using *edgeR* with the *calcNormFactors* and *estimateDisp* functions, then fitted to a model considering the sample groups using *glmQLFit* and differential abundance calculated with *glmQLFTest*. Normalized RPKM quantification tables were generated using the *rpkm* function from *edgeR* and exported from R.

ACR clustering, motif enrichment and transcription factor network analyses

The top 10% ACRs with the highest absolute difference in accessibility across the whole time-series were $\log_2$ and Z-score transformed then clustered and plotted using the *ComplexHeatmap* package with the *Heatmap* function and parameter *row_km*=3. The *streme* program [85] was used to perform de novo motif identification

in each ACR cluster after resizing the peaks to 500 bp and a random set of 3000 ACRs regions as background. These motifs were then enriched using the *sea* [34] and *centrimo* programs [86]. Motifs with a $-\log_{10}$ Q-value of at least 10 in one cluster from either *sea* or *centrimo* were kept and plotted using the *Heatmap* function from the *ComplexHeatmap* package and the *view_motifs* function from the *universalmotif* package [87]. To generate transcription factor (TF)-gene networks, TF-motif assignments were made using the *tomtom* program [88] and keeping matches with a Q-values less than 0.05. Target genes were then identified by scanning the clustered ACRs using the *scan_sequences* function with parameter *RC=TRUE* from the *universalmotif* package. The resulting TF-gene lists were then filtered, keeping those with a Pearson correlation coefficient greater than 0.5 between their normalized $\log_2$ -transformed RNA-seq expression values. The *igraph* package [89] was used to create and plot TF-gene networks, using $\log_{10}$ -transformed connectivity scores to control node size. ACRs which overlapped the promoters of genes (−400 and +100 upstream & downstream of the TSS) were considered promoter ACRs.

24-h time course

For the 24-h time-course, Col-0 seedlings (leaves and apices) were collected every 4 h over a 24-h period at the beginning of week 3 (3W) under AVG, FLU, and 22 °C. Sampling was initiated at lights-on (Zeitgeber time 0). RNA was isolated using TRIZOL (Invitrogen) reagent. Following cDNA preparation, transcript accumulation was measured by RT-qPCR. *PP2A* was used as a normalization control. Primers are listed in Supplementary Table S4.

Growth rate analysis and freezing tolerance

At the end of the 4 W treatment under AVG, FLU and 22 C, three plants were pooled as one biological replicate, and six biological replicates were collected for each plant genotype and condition to determine fresh weight. The samples were subsequently dried at 80 °C for 3 days before dry weight measurement. The remaining plants were used for freezing tolerance assays.

To assess freezing tolerance, the temperature was first decreased to 0 °C and maintained for 1 h, followed by a decrease to −4 °C and maintained for 2 h. The temperature was then further decreased to −8 °C and maintained for 3 h. After freezing treatment, plants were recovered overnight at 4 °C and subsequently transferred to 22 °C under long-day conditions for 1 week. Flowering time was assessed by recording the number of rosette leaves and the number of days to flowering at the time of bolting after the freezing experiment.

Statistical analyses

Statistical testing was performed using the base R *stats* package and *rstatix* package. Statistical significance was determined using one-way ANOVAs with Tukey HSD post-hoc tests and *P*-value cutoff of 0.05 (Figs. 2B, C, 5B, 6E, 7C; Additional file 1: Figs. S4, S6A-C, S8, S9, S10), repeated Mann–Whitney tests with Bonferroni correction for multiple testing (Additional file 1: Fig. S1C) or Student's T-test *P*-value cutoff of 0.05 (Additional file 1: Fig. S12).

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1186/s13059-026-04289-3>.

Acknowledgements We thank Paloma Mas and Elena Monte (CRAG, Spain), Ito Hisashi (Hokkaido University, Japan) and Nicolás Cecchini (University of Córdoba, Argentina) for providing Arabidopsis mutant seeds.

Peer review information Wenjing She was the primary editor of this article and managed its editorial process and peer review in collaboration with the rest of the editorial team. The peer-review history is available in the online version of this article.

Authors' contributions J.I.Q. conceptualized and supervised the study and acquired funding. J.I.Q., M.O., and B.J.M.T. designed the experiments and prepared the manuscript with input from all authors. R.L.A.K designed the temperature profile for the autumn-winter simulation. J.I.Q., J.K., and Y.C. grew the plants and sampled for the time-course progressive cold

experiment; Y.C. extracted RNA for RNA-seq and M.O. prepared ATAC-seq libraries. M.O. performed the weekly-decreasing and 12 °C / 4 °C cold experiments and performed RT-qPCR analyses. M.P. and M.v.Z. carried out the thermal gradient table experiment, and M.O. extracted RNA and performed RT-qPCR. B.J.M.T. performed the bioinformatic analyses. B.J.M.T. and R.L.A.K. performed the temperature-time modelling. Y.C. performed the second cold-time course and collected samples for phenotypic analyses. Y.C. collected samples for the 24-hour time course and performed the RT-qPCR experiments. All authors read and approved the final manuscript.

Funding This work was supported by the Severo Ochoa Excellence Program for Centers (CEX2019-000902-S) and grants from the Spanish Ministry of Science and Innovation (MCIN/AEI/10.13039/501100011033) awarded to J.I.Q. (EUR2021-122003 and CNS2023-145632). Work at CRAG was also supported by the Generalitat de Catalunya (AGAUR, GRE2021, Ref. SGR00873). J.I.Q. was a Ramón y Cajal Fellow (RYC2021-032539-I) and is currently member of the EMBO Young Investigator Program (2025–2028). M.O. is a recipient of the Postdoctoral Fellowship HORIZON-MSCA-2022-PF COOL-CASE—GA101108060 funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the EUROPEAN RESEARCH EXECUTIVE AGENCY (REA). Neither the European Union nor the granting authority can be held responsible for them. B.J.M.T. was supported by an FPI predoctoral fellowship (PRE2019-090156) from MICIN/AEI. M.P. and M.v.Z. were supported by a Graduate School Green Top Sectors grant GSGT.2018.007 of the Netherlands Organization for Scientific Research (NWO) and the Netherlands Plant Eco-phenotyping Centre (NPEC), funded by NWO grant 184.034.012 for large-scale scientific infrastructure.

Data availability All RNA-seq and ATAC-seq data generated in this study are available from the NCBI GEO repository under the accession GSE283018 [90]. The following published sequencing data was used in this study: Liu Y, Cai Y, Li Y, Zhang X, Shi N, Zhao J, and Yang H. Dynamic changes in the transcriptome landscape of *Arabidopsis thaliana* in response to cold stress. Datasets. SRA Database. <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA854395/> (2022; [17]). All bioinformatic analyses were performed using publicly available software packages, employing default function and program arguments unless otherwise specified. No other scripts and software were used other than those mentioned in the [Methods](#) section. This study reports no original code.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

1. Thomashow MF. PLANT COLD ACCLIMATION: Freezing Tolerance Genes and Regulatory Mechanisms. *Annu Rev Plant Physiol Plant Mol Biol.* 1999;50:571–99. <https://doi.org/10.1146/annurev.arplant.50.1.571>.
2. Costa S, Dean C. Storing memories: The distinct phases of Polycomb-mediated silencing of *Arabidopsis* FLC. *Biochem Soc Trans.* 2019;47(4):1187–96. <https://doi.org/10.1042/BST20190255>.
3. De Lucia F, Crevillen P, Jones AM, Greb T, Dean C. A PHD-polycomb repressive complex 2 triggers the epigenetic silencing of FLC during vernalization. *Proc Natl Acad Sci U S A.* 2008;105(44):16831–6. <https://doi.org/10.1073/pnas.0808687105>.
4. Sung S, Amasino RM. Vernalization in *Arabidopsis thaliana* is mediated by the PHD finger protein VIN3. *Nature.* 2004;427(6970):159–64. <https://doi.org/10.1038/nature02195>.

5. Gilmour SJ, Zarka DG, Stockinger EJ, Salazar MP, Houghton JM, Thomashow MF. Low temperature regulation of the *Arabidopsis* CBF family of AP2 transcriptional activators as an early step in cold-induced COR gene expression. *Plant J*. 1998;16(4):433–42. <https://doi.org/10.1046/j.1365-313X.1998.00310.x>.
6. Zhao C, Zhang Z, Xie S, Si T, Li Y, Zhu JK. Mutational evidence for the critical role of CBF transcription factors in cold acclimation in *Arabidopsis*. *Plant Physiol*. 2016;171(4):2744–59. <https://doi.org/10.1104/pp.16.00533>.
7. Medina J, Bargues M, Terol J, Pérez-Alonso M, Salinas J. The *Arabidopsis* CBF gene family is composed of three genes encoding AP2 domain-containing proteins whose expression is regulated by low temperature but not by abscisic acid or dehydration. *Plant Physiol*. 1999;119(2):463–70. <https://doi.org/10.1104/pp.119.2.463>.
8. Fowler SG, Cook D, Thomashow MF. Low temperature induction of *Arabidopsis* CBF1, 2, and 3 is gated by the circadian clock. *Plant Physiol*. 2005;137(3):961–8. <https://doi.org/10.1104/pp.104.058354>.
9. Dong MA, Farré EM, Thomashow MF. Circadian clock-associated 1 and late elongated hypocotyl regulate expression of the C-repeat binding factor (CBF) pathway in *Arabidopsis*. *Proc Natl Acad Sci U S A*. 2011;108(17):7241–6. <https://doi.org/10.1073/pnas.1103741108>.
10. Kyung J, Jeon M, Jeong G, Shin Y, Seo E, Yu J, et al. The two clock proteins CCA1 and LHY activate VIN3 transcription during vernalization through the vernalization-responsive cis-element. *Plant Cell*. 2022;34(3):1020–37. <https://doi.org/10.1093/plcell/koab304>.
11. Hepworth J, Antoniou-Kourounioti RL, Bloomer RH, Selga C, Berggren K, Cox D, et al. Absence of warmth permits epigenetic memory of winter in *Arabidopsis*. *Nat Commun*. 2018;9(1):639. <https://doi.org/10.1038/s41467-018-03065-7>.
12. Zeng Z, Zhang W, Marand AP, Zhu B, Buell CR, Jiang J. Cold stress induces enhanced chromatin accessibility and bivalent histone modifications H3K4me3 and H3K27me3 of active genes in potato. *Genome Biol*. 2019;20(1):123. <https://doi.org/10.1186/s13059-019-1731-2>.
13. Raxwal VK, Ghosh S, Singh S, Katiyar-Agarwal S, Goel S, Jagannath A, et al. Abiotic stress-mediated modulation of the chromatin landscape in *Arabidopsis thaliana*. *J Exp Bot*. 2020;71(17):5280–93. <https://doi.org/10.1093/jxb/eraa286>.
14. Ren C, Li H, Wang Z, Dai Z, Lecourieux F, Kuang Y, et al. Characterization of chromatin accessibility and gene expression upon cold stress reveals that the RAV1 transcription factor functions in cold response in *Vitis amurensis*. *Plant Cell Physiol*. 2021;62(10):1615–29. <https://doi.org/10.1093/pcp/pcab115>.
15. Li J, Liu H, Qian H, Lu S, Wu Y, Hua J, et al. Chromatin accessibility mediated by CHROMATIN REMODELING 11 promotes chilling tolerance in rice. *Plant Physiol*. 2024;197(1):kiaf018. <https://doi.org/10.1093/plphys/kiaf018>.
16. Han J, Dai Y, Zhou J, Tian J, Chen Q, Kou X, et al. Tissue-specific chromatin accessibility and transcriptional regulation in maize cold stress response. *Genomics*. 2025;117(1):110981. <https://doi.org/10.1016/j.ygeno.2024.110981>.
17. Liu Y, Cai Y, Li Y, Zhang X, Shi N, Zhao J, et al. Dynamic changes in the transcriptome landscape of *Arabidopsis thaliana* in response to cold stress. *Front Plant Sci*. 2022;13:983460. <https://doi.org/10.3389/fpls.2022.983460>.
18. Li F, Hu Q, Chen F, Jiang JF. Transcriptome analysis reveals vernalization is independent of cold acclimation in *Arabidopsis*. *BMC Genomics*. 2021;22(1):462. <https://doi.org/10.1186/s12864-021-07763-3>.
19. Xu ZY, Zhang X, Schläppi M, Xu ZQ. Cold-inducible expression of AZI1 and its function in improvement of freezing tolerance of *Arabidopsis thaliana* and *Saccharomyces cerevisiae*. *J Plant Physiol*. 2011;168(13):1576–87. <https://doi.org/10.1016/j.jplph.2011.01.023>.
20. Bubier J, Schläppi M. Cold induction of EARLI1 a putative *Arabidopsis* lipid transfer protein, is light and calcium dependent. *Plant Cell Environ*. 2004;27(7):929–36. <https://doi.org/10.1111/j.1365-3040.2004.01198.x>.
21. Jia Y, Ding Y, Shi Y, Zhang X, Gong Z, Yang S. The cbfs triple mutants reveal the essential functions of CBFs in cold acclimation and allow the definition of CBF regulons in *Arabidopsis*. *New Phytol*. 2016;212(2):345–53. <https://doi.org/10.1111/nph.14088>.
22. Praat M, Jiang Z, Earle J, Smeekeens S, van Zanten M. Using a thermal gradient table to study plant temperature signalling and response across a temperature spectrum. *Plant Methods*. 2024;20(1):114. <https://doi.org/10.1186/s13007-024-01230-2>.
23. Antoniou-Kourounioti RL, Hepworth J, Heckmann A, Duncan S, Qüesta J, Rosa S, et al. Temperature sensing is distributed throughout the regulatory network that controls FLC epigenetic silencing in vernalization. *Cell Syst*. 2018;7(6):643–655.e9. <https://doi.org/10.1016/j.cels.2018.10.011>.
24. Park S, Lee CM, Doherty CJ, Gilmour SJ, Kim Y, Thomashow MF. Regulation of the *Arabidopsis* CBF regulon by a complex low-temperature regulatory network. *Plant J*. 2015;82(2):193–207. <https://doi.org/10.1111/tpj.12796>.
25. Ezer D, Shepherd SJK, Brestovitsky A, Dickinson P, Cortijo S, Charoensawan V, et al. The G-box transcriptional regulatory code in *Arabidopsis*. *Plant Physiol*. 2017;175(2):628–40. <https://doi.org/10.1104/pp.17.01086>.
26. Giraud E, Ng S, Carrie C, Duncan O, Low J, Lee CP, et al. TCP transcription factors link the regulation of genes encoding mitochondrial proteins with the circadian clock in *Arabidopsis thaliana*. *Plant Cell*. 2010;22(12):3921–34. <https://doi.org/10.1105/tpc.110.074518>.
27. Armstrong GA, Weissshaar B, Hahlbrock K. Homodimeric and heterodimeric leucine zipper proteins and nuclear factors from parsley recognize diverse promoter elements with ACGT cores. *Plant Cell*. 1992;4(5):525–37. <https://doi.org/10.1105/tpc.4.5.525>.

28. Jakoby M, Weisshaar B, Dröge-Laser W, Vicente-Carbajosa J, Tiedemann J, Kroj T, et al. bZIP transcription factors in *Arabidopsis*. *Trends Plant Sci.* 2002;7(3):106–11. [https://doi.org/10.1016/S1360-1385\(01\)02223-3](https://doi.org/10.1016/S1360-1385(01)02223-3).
29. Shen Q, Zhang P, Ho TH. Modular nature of abscisic acid (ABA) response complexes: composite promoter units that are necessary and sufficient for ABA induction of gene expression in barley. *Plant Cell.* 1996;8(7):1107–19. <https://doi.org/10.1105/tpc.8.7.1107>.
30. Kosugi S, Ohashi Y. PCF1 and PCF2 specifically bind to cis elements in the rice proliferating cell nuclear antigen gene. *Plant Cell.* 1997;9(9):1607–19. <https://doi.org/10.1105/tpc.9.9.1607>.
31. Vandepoele K, Vlieghe K, Florquin K, Hennig L, Beemster GT, Grissem W, et al. Genome-wide identification of potential plant E2F target genes. *Plant Physiol.* 2005;139(1):316–28. <https://doi.org/10.1104/pp.105.066290>.
32. Catalá R, Medina J, Salinas J. Integration of low temperature and light signaling during cold acclimation response in *Arabidopsis*. *Proc Natl Acad Sci U S A.* 2011;108(39):16475–80. <https://doi.org/10.1073/pnas.1107161108>.
33. Nishio H, Cano-Ramirez DL, Muranaka T, de Barros Dantas LL, Honjo MN, Sugisaka J, et al. Circadian and environmental signal integration in a natural population of *Arabidopsis*. *Proc Natl Acad Sci U S A.* 2024;121(35):e2402697121. <https://doi.org/10.1073/pnas.2402697121>.
34. Bailey TL and Grant CE. SEA: Simple Enrichment Analysis of motifs. *bioRxiv.* 2021. Preprint: not peer reviewed. <https://doi.org/10.1101/2021.08.23.457422>
35. Li X, Liu C, Zhao Z, Ma D, Zhang J, Yang Y, et al. COR27 and COR28 are novel regulators of the COP1–HY5 regulatory Hub and photomorphogenesis in *Arabidopsis*. *Plant Cell.* 2020;32(10):3139–54. <https://doi.org/10.1105/tpc.20.00195>.
36. Guo W, Ward RW, Thomashow MF. Characterization of a Cold-Regulated Wheat Gene Related to *Arabidopsis* cor47. *Plant Physiol.* 1992;100(2):915–22. <https://doi.org/10.1104/pp.100.2.915>.
37. Pruneda-Paz JL, Breton G, Para A, Kay SA. A functional genomics approach reveals CHE as a component of the *Arabidopsis* circadian clock. *Science.* 2009;323(5920):1481–5. <https://doi.org/10.1126/science.1167206>.
38. Malinovsky FG, Batoux M, Schwessinger B, Youn JH, Stransfeld L, Win J, et al. Antagonistic regulation of growth and immunity by the *Arabidopsis* basic helix-loop-helix transcription factor homolog of brassinosteroid enhanced expression2 interacting with increased leaf inclination1 binding bHLH1. *Plant Physiol.* 2014;164(3):1443–55. <https://doi.org/10.1104/pp.113.234625>.
39. Zhang G, Zhao H, Zhang C, Li X, Lyu Y, Qi D, et al. TCP7 functions redundantly with several Class I TCPs and regulates endoreplication in *Arabidopsis*. *J Integr Plant Biol.* 2019;61(11):1151–70. <https://doi.org/10.1111/jipb.12749>.
40. Song YH, Smith RW, To BJ, Millar AJ, Imaizumi T. FKF1 conveys timing information for CONSTANS stabilization in photoperiodic flowering. *Science.* 2012;336(6084):1045–9. <https://doi.org/10.1126/science.1219644>.
41. Nagel DH, Kay SA. Complexity in the wiring and regulation of plant circadian networks. *Curr Biol.* 2012;22(16):R648–57. <https://doi.org/10.1016/j.cub.2012.07.025>.
42. Nawkar GM, Kang CH, Maibam P, Park JH, Jung YJ, Chae HB, et al. HY5, a positive regulator of light signaling, negatively controls the unfolded protein response in *Arabidopsis*. *Proc Natl Acad Sci U S A.* 2017;114(8):2084–9. <https://doi.org/10.1073/pnas.1609844114>.
43. Li X, Ma D, Lu SX, Hu X, Huang R, Liang T, et al. Blue Light- and Low Temperature-Regulated COR27 and COR28 Play Roles in the *Arabidopsis* Circadian Clock. *Plant Cell.* 2016;28(11):2755–69. <https://doi.org/10.1105/tpc.16.00354>.
44. Bieniawska Z, Espinoza C, Schlereth A, Sulpice R, Hinch DK, Hannah MA. Disruption of the *Arabidopsis* circadian clock is responsible for extensive variation in the cold-responsive transcriptome. *Plant Physiol.* 2008;147(1):263–79. <https://doi.org/10.1104/pp.108.118059>.
45. Cecchini NM, Steffes K, Schläppi MR, Gifford AN, Greenberg JT. *Arabidopsis* AZI1 family proteins mediate signal mobilization for systemic defence priming. *Nat Commun.* 2015;6:7658. <https://doi.org/10.1038/ncomms8658>.
46. Seydel C, Kitashova A, Fürtauer L, Nägele T. Temperature-induced dynamics of plant carbohydrate metabolism. *Physiol Plant.* 2022;174(1):e13602. <https://doi.org/10.1111/ppl.13602>.
47. Nagano AJ, Kawagoe T, Sugisaka J, Honjo MN, Iwayama K, Kudoh H. Annual transcriptome dynamics in natural environments reveals plant seasonal adaptation. *Nat Plants.* 2019;5(1):74–83. <https://doi.org/10.1038/s41477-018-0338-z>.
48. Wang S, Bai G, Wang S, Yang L, Yang F, Wang Y, et al. Chloroplast RNA-Binding Protein RBD1 Promotes Chilling Tolerance through 23S rRNA Processing in *Arabidopsis*. *PLoS Genet.* 2016;12(5):e1006027. <https://doi.org/10.1371/journal.pgen.1006027>.
49. Zhao Y, Antoniou-Kourounioti RL, Calder G, Dean C, Howard M. Temperature-dependent growth contributes to long-term cold sensing. *Nature.* 2020;583(7818):825–9. <https://doi.org/10.1038/s41586-020-2485-4>.
50. Green RM, Tobin EM. Loss of the circadian clock-associated protein 1 in *Arabidopsis* results in altered clock-regulated gene expression. *Proc Natl Acad Sci U S A.* 1999;96(7):4176–9. <https://doi.org/10.1073/pnas.96.7.4176>.
51. Hajdu A, Dobos O, Domijan M, Bálint B, Nagy I, Nagy F, et al. ELONGATED HYPOCOTYL 5 mediates blue light signalling to the *Arabidopsis* circadian clock. *Plant J.* 2018;96(6):1242–54. <https://doi.org/10.1111/tjp.14106>.
52. Yang X, Liu Y, Jia ZC, Li M, Hou XX, Shi XL, Gao B, Zhang DY, Zhu FY, Chen MX, et al. Moderate Cold Stress Enhance Drought Resistance through CCA1 via -an ABA independent Pathway. *bioRxiv.* 2024. Preprint: not peer reviewed. <https://doi.org/10.1101/2024.07.09.602734>

53. Kidokoro S, Yoneda K, Takasaki H, Takahashi F, Shinozaki K, Yamaguchi-Shinozaki K. Different cold-signaling pathways function in the responses to rapid and gradual decreases in temperature. *Plant Cell*. 2017;29(4):760–74. <https://doi.org/10.1105/tpc.16.00669>.
54. Novillo F, Medina J, Salinas J. Arabidopsis CBF1 and CBF3 have a different function than CBF2 in cold acclimation and define different gene classes in the CBF regulon. *Proc Natl Acad Sci U S A*. 2007;104(52):21002–7. <https://doi.org/10.1073/pnas.0705639105>.
55. Novillo F, Alonso JM, Ecker JR, Salinas J. CBF2/DREB1C is a negative regulator of CBF1/DREB1B and CBF3/DREB1A expression and plays a central role in stress tolerance in *Arabidopsis*. *Proc Natl Acad Sci U S A*. 2004;101(11):3985–90. <https://doi.org/10.1073/pnas.0303029101>.
56. Monroe JG, McGovern C, Lasky JR, Grogan K, Beck J, McKay JK. Adaptation to warmer climates by parallel functional evolution of CBF genes in *Arabidopsis thaliana*. *Mol Ecol*. 2016;25(15):3632–44. <https://doi.org/10.1111/mec.13711>.
57. Faivre L, Kinscher NF, Kuhlmann AB, Xu X, Kaufmann K, Schubert D. Cold stress induces rapid gene-specific changes in the levels of H3K4me3 and H3K27me3 in *Arabidopsis thaliana*. *Front Plant Sci*. 2024;15:1390144. <https://doi.org/10.3389/fpls.2024.1390144>.
58. Deragon JM, Merret R. Co-translational mRNA decay in plants: recent advances and future directions. *J Exp Bot*. 2025;9:eraf146. <https://doi.org/10.1093/jxb/eraf146>.
59. Alhabsi A, Ling Y, Crespi M, Reddy ASN, Mahfouz M. Alternative splicing dynamics in plant adaptive responses to stress. *Annu Rev Plant Biol*. 2025;76(1):687–717. <https://doi.org/10.1146/annurev-arplant-083123-090055>.
60. Wang S, Wang H, Xu Z, Jiang S, Shi Y, Xie H, et al. M6A mRNA modification promotes chilling tolerance and modulates gene translation efficiency in *Arabidopsis*. *Plant Physiol*. 2023;192(2):1466–82. <https://doi.org/10.1093/plphys/kiad112>.
61. Zhao X, Yang J, Wang H, Xu H, Zhou Y, Duan L. MicroRNAs in Plants Development and Stress Resistance. *Plant Cell Environ*. 2025;48(8):5909–29. <https://doi.org/10.1111/pce.15546>.
62. Chorostecki U, Bologna NG, Ariel F. The plant noncoding transcriptome: a versatile environmental sensor. *EMBO J*. 2023;42(20):e114400. <https://doi.org/10.15252/embj.2023114400>.
63. Nitschke S, Cortleven A, Iven T, Feussner I, Havaux M, Riefler M, et al. Circadian stress regimes affect the circadian clock and cause jasmonic acid-dependent cell death in cytokinin-deficient *Arabidopsis* plants. *Plant Cell*. 2016;28(7):1616–39. <https://doi.org/10.1105/tpc.16.00016>.
64. Meguro M, Ito H, Takabayashi A, Tanaka R, Tanaka A. Identification of the 7-hydroxymethyl chlorophyll a reductase of the chlorophyll cycle in *Arabidopsis*. *Plant Cell*. 2011;23(9):3442–53. <https://doi.org/10.1105/tpc.111.089714>.
65. Wang FX, Shang GD, Wu LY, Mai YX, Gao J, Xu ZG, et al. Protocol for assaying chromatin accessibility using ATAC-seq in plants. *STAR Protoc*. 2021;2(1):100289. <https://doi.org/10.1016/j.xpro.2020.100289>.
66. Buenrostro JD, Giresi PG, Zaba LC, Chang HY, Greenleaf WJ. Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. *Nat Methods*. 2013;10(12):1213–8. <https://doi.org/10.1038/nmeth.2688>.
67. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMnet J*. 2011;17:10–2. <https://doi.org/10.14806/ej.17.1.200>.
68. Lamesch P, Berardini TZ, Li D, Swarbreck D, Wilks C, Sasidharan R, et al. The Arabidopsis Information Resource (TAIR): Improved gene annotation and new tools. *Nucleic Acids Res*. 2012;40(Database issue):D1202–1210. <https://doi.org/10.1093/nar/gkr1090>.
69. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, et al. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics*. 2013;29(1):15–21. <https://doi.org/10.1093/bioinformatics/bts635>.
70. Pertea M, Pertea GM, Antonescu CM, Chang TC, Mendell JT, Salzberg SL. StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. *Nat Biotechnol*. 2015;33(3):290–5. <https://doi.org/10.1038/nbt.3122>.
71. Cheng CY, Krishnakumar V, Chan AP, Thibaud-Nissen F, Schobel S, Town CD. AraPort1.1: a complete reannotation of the *Arabidopsis thaliana* reference genome. *Plant J*. 2017;89(4):789–804. <https://doi.org/10.1111/tpj.13415>.
72. Risso D, Ngai J, Speed TP, Dudoit S. Normalization of RNA-seq data using factor analysis of control genes or samples. *Nat Biotechnol*. 2014;32(9):896–902. <https://doi.org/10.1038/nbt.2931>.
73. McCarthy DJ, Chen Y, Smyth GK. Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. *Nucleic Acids Res*. 2012;40(10):4288–97. <https://doi.org/10.1093/nar/gks042>.
74. Hoffman GE, Schadt EE. variancePartition: Interpreting drivers of variation in complex gene expression studies. *BMC Bioinformatics*. 2016;17(1):483. <https://doi.org/10.1186/s12859-016-1323-z>.
75. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics*. 2008;9:559. <https://doi.org/10.1186/1471-2105-9-559>.
76. Gu Z, Eils R, Schlesner M. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics*. 2016;32(18):2847–9. <https://doi.org/10.1093/bioinformatics/btw313>.
77. Kolberg L, Raudvere U, Kuzmin I, Vilo J, Peterson H. gprofiler2 – an R package for gene list functional enrichment analysis and namespace conversion toolset g:Profiler. *F1000Res*. 2020;9:709. <https://doi.org/10.12688/f1000research.24956.1>.

78. Gu Z, Hübschmann D. simplifyEnrichment: a Bioconductor package for clustering and visualizing functional enrichment results. *Genomics Proteomics Bioinformatics*. 2023;21(1):190–202. <https://doi.org/10.1016/j.gpb.2022.04.008>.
79. Chen S. Ultrafast one-pass FASTQ data preprocessing, quality control, and deduplication using fastp. *iMeta*. 2023;2(2):e107. <https://doi.org/10.1002/imt2.107>.
80. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods*. 2012;9(4):357–9. <https://doi.org/10.1038/nmeth.1923>.
81. Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, et al. Twelve years of SAMtools and BCFtools. *Gigascience*. 2021;10(2):giab008. <https://doi.org/10.1093/gigascience/giab008>.
82. Klasfeld S, Roulé T, Wagner D. Greenscreen: a simple method to remove artifactual signals and enrich for true peaks in genomic datasets including ChIP-seq data. *Plant Cell*. 2022;34(12):4795–815. <https://doi.org/10.1093/plcell/koac282>.
83. Quinlan AR, Hall IM. a flexible suite of utilities for comparing genomic features. *Bioinformatics*. 2010;26(6):841–2. <https://doi.org/10.1093/bioinformatics/btq033>.
84. Zhang Y, Liu T, Meyer CA, Eeckhoutte J, Johnson DS, Bernstein BE, et al. Model-based analysis of ChIP-Seq (MACS). *Genome Biol*. 2008;9(9):R137. <https://doi.org/10.1186/gb-2008-9-9-r137>.
85. Bailey TL. STREME: accurate and versatile sequence motif discovery. *Bioinformatics*. 2021;37(18):2834–40. <https://doi.org/10.1093/bioinformatics/btab203>.
86. Bailey TL, MacHanick P. Inferring direct DNA binding from ChIP-seq. *Nucleic Acids Res*. 2012;40(17):e128. <https://doi.org/10.1093/nar/gks433>.
87. Tremblay BJM. Universalmotif: an R package for biological motif analysis. *J Open Source Softw*. 2024;9(100):7012. <https://doi.org/10.21105/joss.07012>.
88. Gupta S, Stamatoyannopoulos JA, Bailey TL, Noble WS. Quantifying similarity between motifs. *Genome Biol*. 2007;8(2):R24. <https://doi.org/10.1186/gb-2007-8-2-r24>.
89. Csardi G and Nepusz T. The igraph software package for complex network research. *InterJournal, Complex Systems*. 2006;1695. <http://igraph.org>.
90. Ormancey M, Tremblay BJM, Cheng Y, Praat M, Antoniou-Kourounioti RL, Krumbach J, van Zanten M and Qüesta JI. Autumn-winter progression triggers distinct transcriptional programs in *Arabidopsis thaliana*. *Datasets. Gene Expression Omnibus*. 2026. <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi>.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Authors and Affiliations

Mélanie Ormancey^{1,5} · Benjamin J. M. Tremblay^{1,6} · Yajiao Cheng¹ · Myrthe Praat^{2,7} · Rea L. Antoniou-Kourounioti³ · Jenna Krumbach¹ · Martijn van Zanten^{2,4} · Julia I. Qüesta¹

✉ Julia I. Qüesta
julia.questa@cragenomica.es

¹ Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, Campus UAB, Bellaterra, Barcelona, Spain

² Plant Stress Resilience, Institute of Environmental Biology, Utrecht University, Padualaan 8, Utrecht 3584CH, The Netherlands

³ School of Molecular Biosciences, College of Medical, University of Glasgow, Veterinary & Life Sciences, Glasgow, UK

⁴ Netherlands Plant Eco-Phenotyping Centre, Institute of Environmental Biology, Utrecht University, Padualaan 6, Utrecht, The Netherlands

⁵ Present Address: Laboratoire Des Interactions Plantes- Microbes-Environnement (LIPME), Université de Toulouse, INRAE, CNRS, 31326 Castanet-Tolosan, France

⁶ Present Address: The Sainsbury Laboratory, University of East Anglia, Norwich Research Park, Norwich NR4 7UH, UK

⁷ Present Address: Green Biotechnology, Inholland University of Applied Sciences, Amsterdam, the Netherlands