Matters arising

Cold induction of nuclear FRIGIDA condensation in *Arabidopsis*

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In over-wintering annuals of *Arabidopsis thaliana* grown in temperate regions, prolonged cold exposure in winter, through the physiological process of vernalization, represses the expression of the potent floral repressor *FLOWERING LOCUS C* (*FLC*) to enable the transition to flowering in spring[1,2]. Recently, Zhu et al.[3] reported that cold induced nuclear condensation of FRIGIDA (FRI) for *FLC* repression and that cold-induced antisense RNA *COOLAIR* promoted FRI condensation during prolonged cold exposure. Here we report that the cold-induced formation of nuclear FRI condensates is independent of *COOLAIR*.

Before exposure to cold, *FRI* activates *FLC* expression, and long-term continuous winter cold exposure (typically lasting more than a month) results in *FLC* repression in *FRI*-bearing winter annuals grown at high latitudes[1,4]. Prolonged cold exposure induces the expression of *COOLAIR*, a group of non-coding antisense RNAs initiating from a region downstream of the 3′ end of *FLC* that is composed of proximally polyadenylated class I and distally polyadenylated class II transcripts[5]. *COOLAIR* expression reaches a high level after around 3 weeks of cold exposure and subsequently declines under constant cold temperature[6]. Using a CRISPR–Cas9 system, we previously constructed several lines in which a large part of the core *COOLAIR* promoter region was removed[7], resulting in the elimination of both class I and class II *COOLAIR* transcripts before cold exposure (Fig. 1a,b and Extended Data Fig. 1a,b). Furthermore, consistent with a recent study[8], cold induction of *COOLAIR* expression was eliminated in these core promoter deletion lines (Fig. 1b), partly because the *cis*-acting cold-responsive elements located in the promoter region have been removed. To examine the role of *COOLAIR* in FRI condensation, we introduced a functional *FRI-GFP* into two lines in which the *COOLAIR* promoter was deleted—*ΔCOOLAIR-1* and *ΔCOOLAIR-2*—in the rapid-cycling accession Col-0 (bearing a loss-of-function *fri* allele[9]) by genetic transformation. Subsequently, independent *FRI-GFPΔCOOLAIR* lines (numbers 2, 5 and 7) were backcrossed to Col-0 and *ΔCOOLAIR-1* or *ΔCOOLAIR-2*, respectively, resulting in F_1 progeny of *FRI-GFPΔCOOLAIR*$^{-/-}$ and *FRI-GFPΔCOOLAIR*$^{+/-}$. In these lines, FRI–GFP is fully functional and acts to activate *FLC* expression before cold exposure (Extended Data Fig. 1c–f).

We next determined whether the loss of *COOLAIR* expression might reduce nuclear FRI condensation. We measured the fluorescence intensity of FRI–GFP in the root tips and the size and number of FRI–GFP condensates in root tip nuclei of cold-treated *FRI-GFPΔCOOLAIR*$^{+/-}$ and *FRI-GFPΔCOOLAIR*$^{-/-}$ seedlings and found that there was no difference between these two genotypes (Fig. 1e–i). Furthermore, we crossed a *FRI-GFP* line[3] to both *ΔCOOLAIR-1* and *ΔCOOLAIR-2* and obtained homozygous *FRI-GFPΔCOOLAIR* lines. Subsequently, we measured the size and number of FRI–GFP condensates in root tip nuclei in these lines after cold exposure and found that there was no statistically significant difference (Fig. 2a–f). Together, these results show that the cold-induced formation of nuclear FRI condensates is independent of *COOLAIR* expression, given that, before and during cold exposure, *COOLAIR* expression (including class I and class II transcripts) was eliminated in both the *ΔCOOLAIR-1* and *ΔCOOLAIR-2* lines.

Zhu et al.[3] reported that, in the cold, the FRI protein enriched class II.ii *COOLAIR* transcripts and that the FRI–class II.ii interaction was closely connected with cold-induced FRI condensation. Using a transgenic *FLC* terminator exchange (*TEX*) line in which the *COOLAIR* promoter was replaced with an *RBCS3B* (encoding Rubisco small subunit 3B) terminator, Zhu et al.[3] found that the size and number per nucleus of FRI–GFP condensates were reduced in the *TEX* line compared with those in the non-transgenic background (Col-0). In the *ΔCOOLAIR* lines that we used in this study, the class II transcripts are eliminated before cold exposure and in the cold. Thus, we conclude that the FRI-class II.ii interaction and *COOLAIR* expression are not involved in cold-induced FRI condensation. The cause for the discrepancy between the two studies is unclear.

In addition to the *COOLAIR* transcripts initiated downstream of the 3′ end of *FLC*, there are other antisense transcripts (ASTs) initiated within the *FLC* locus (see, for example, ref. 10). We examined these ASTs in *FRI*-Col (a reference winter-annual line[11]) and *FRIΔCOOLAIR* seedlings, and found that they were at low levels before cold exposure (Fig. 2g). After cold exposure for 3 days, the expression of ASTs in all three examined regions declined in *FRI*-Col, and was apparently reduced in two examined regions in the *FRIΔCOOLAIR* seedlings (Fig. 2g); cold exposure for 14 days strongly suppressed the expression of ASTs in both the *FRI*-Col and *FRIΔCOOLAIR* lines (Fig. 2h). Thus, in contrast to *COOLAIR*, the expression of ASTs is repressed along the early phase of long-term cold exposure or vernalization. The function of ASTs in the vernalization-mediated *FLC* repression remains to be seen.

Cold induction of *COOLAIR* expression in the early phase of vernalization was reported to mediate *FLC* repression[5,6]. We measured the levels of *FLC* transcripts (both spliced and unspliced) in the cold-treated *FRIΔCOOLAIR* seedlings, and found that loss of *COOLAIR* expression in either *FRIΔCOOLAIR-1* or *FRIΔCOOLAIR-2* had no effect on the progression of transcriptional shutdown of *FLC* during cold exposure or on post-cold stable silencing of *FLC* (Fig. 1c,d and Extended Data Fig. 1g), consistent with observations in a recent study[8]. Notably, in our vernalization study, like several other studies reporting a role of

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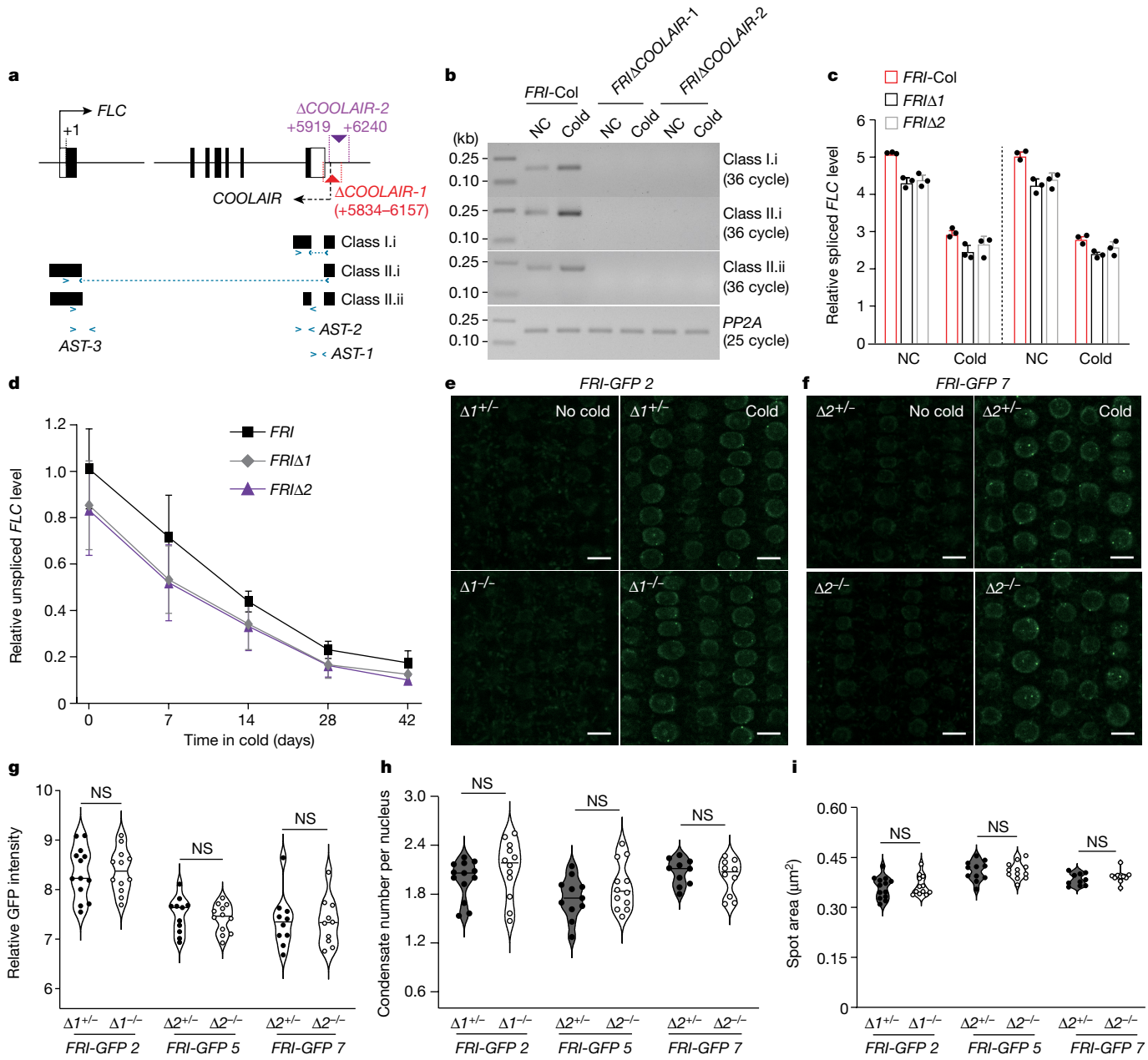

Fig. 1 | Functional analysis of *COOLAIR* in cold-mediated nuclear FRI condensation and *FLC* repression. a, Schematic of the *FLC* locus. The A of ATG is indicated (+1), the blue arrows show the primer positions and the dashed lines show the interexon primers. **b**, *COOLAIR* expression is eliminated in *FRIΔCOOLAIR* lines (*FRIΔCOOLAIR-1* (*FRIΔ1*) and *FRIΔCOOLAIR-2* (*FRIΔ2*)). Seedlings were exposed to cold for 14 days or no cold (NC). *FRI*-Col is a winter-annual reference line. The constitutively expressed *PP2A* (*At1G13320*; *PROTEIN PHOSPHATASE 2A SUBUNIT A3*) was used as an internal control. **c**,**d**, Quantification of spliced (**c**) and unspliced *FLC* (**d**) transcripts in cold-treated seedlings. Seedlings were cold-treated for 14 days and two biological replicates were conducted in **c**. The levels of *FLC* transcripts were normalized to *PP2A*. Data are mean ± s.d. of three technical replicates (**c**) or biological replicates (**d**). The relative expression to *FRI*-Col (before cold exposure) is presented in **d**. **e**,**f**, Confocal microscopy images of FRI–GFP in the root tip nuclei of *FRI-GFP 2* (**e**) and *FRI-GFP 7* (**f**) seedlings. Seedlings were treated with cold for 14 days. Scale bars, 10 μm. **g**, The fluorescence intensity of FRI–GFP in the root tips treated with cold for 14 days. Data points are plotted on bar graphs. **h**,**i**, Quantification of FRI–GFP condensates (condensate number per nucleus (**h**) and spot area (**i**)) in the nuclei of cold-treated root tips. For **g**–**i**, 10–13 seedlings per F_1 population were scored and data were analysed using two-tailed *t*-tests with Welch's correction; NS, not significant ($P > 0.05$).

COOLAIR for *FLC* repression[5,6], seedlings were exposed to a constant cold temperature, the mechanisms uncovered through which may not fully represent *FLC* regulation by winter cold in the fields with fluctuating cold temperatures.

In summary, our study shows that the cold-induced formation of nuclear FRI condensates is independent of *COOLAIR*. Moreover, our vernalization study with constant cold temperature shows that *COOLAIR* is not involved in *FLC* repression by prolonged cold exposure. Thus, more in-depth experiments would be required to resolve the role of *COOLAIR* in vernalization.

Methods

Arabidopsis thaliana FRI-Col, Col-0, *ΔCOOLAIR-1* and *FRIΔCOOLAIR-1* were described previously[7]. Treatment of the seedlings with constant cold and quantification of the expression of genes of

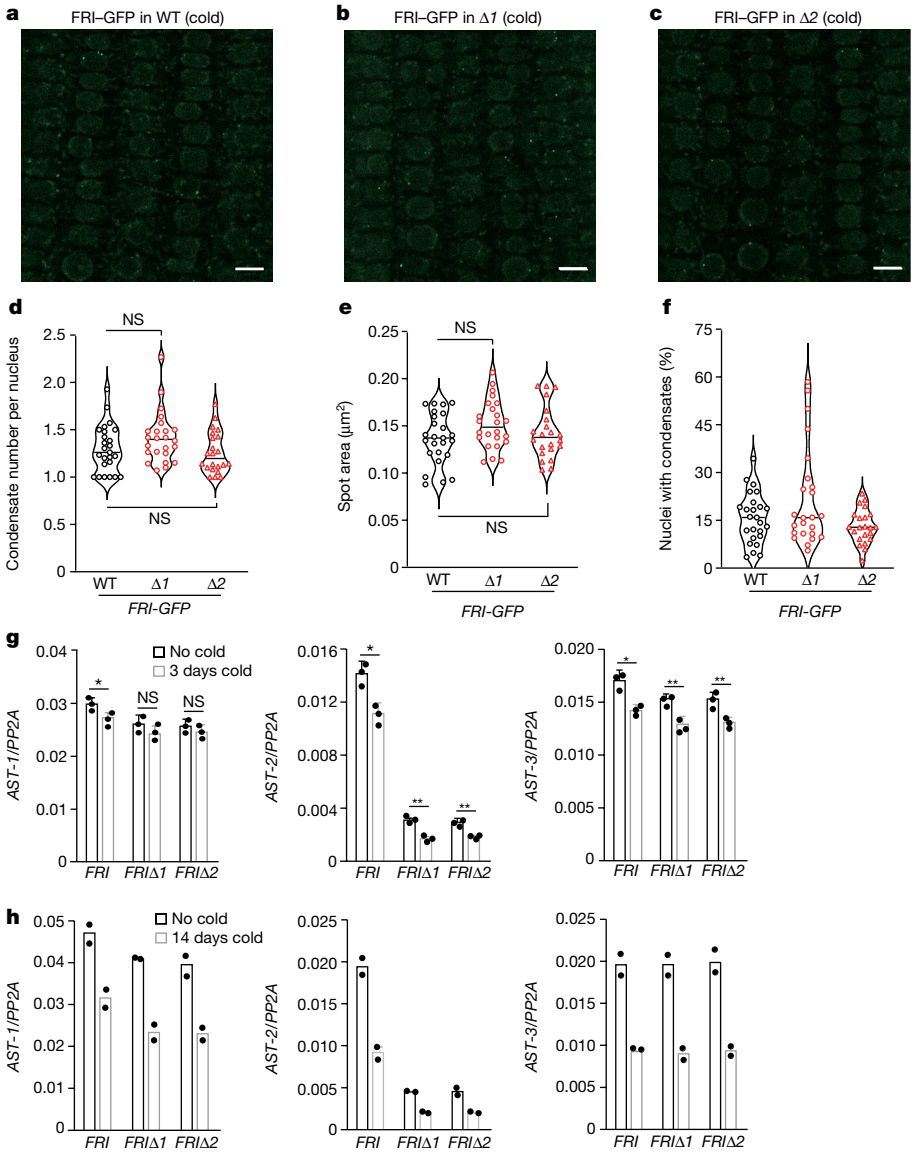

Fig. 2 | Characterization of nuclear FRI–GFP condensation and antisense transcription at *FLC* in the absence of *COOLAIR* expression. a–c, Confocal microscopy images of FRI–GFP in root-tip nuclei from the indicated seedlings (WT (**a**), Δ*COOLAIR-1* (**b**) and Δ*COOLAIR-2* (**c**)) exposed to cold for 14 days. Scale bars, 10 μm. **d–f**, Quantification of FRI–GFP condensates (number (**d**), spot area (**e**) and percentage of nuclei with condensates (**f**)) in the nuclei of cold-treated root tips. 22–25 seedlings per line were scored. For **d** and **e**, statistical analysis was performed using two-tailed *t*-tests with Welch's correction. **g**,**h**, Quantification of ASTs at *FLC* in the indicated seedlings exposed to cold for 3 days (**g**) and 14 days (**h**). The examined regions with ASTs are indicated in Fig. 1a. AST-3 is known as CAS (convergent antisense transcript). For **g**, data are mean ± s.d. of three biological replicates. For **g**, statistical analysis was performed using two-tailed *t*-tests; $^{*}P < 0.05$, $^{**}P < 0.01$. Data points in **h** denote two biological replicates.

interest using quantitative PCR were performed as previously described[12]. *COOLAIR* expression was examined by semiquantitative PCR, after reverse transcription using transcript-specific primers (5′-TGGTTGTTATTTGGTGGTGTGAA-3′ for class I; and 5′-GCCCGACGAAGAAAAAGTAG-3′ for class II[10]). A list of the PCR primers is provided in Extended Data Table 1.

FRI$_{pro}$*:FRI-GFP* was constructed by cloning a 4.8 kb genomic *FRI* fragment (2.5 kb promoter plus the 2.3 kb entire coding region without the stop codon) upstream of the *GFP*-coding region in the binary vector pMDC110[13]. Microscopy analysis and image quantification of nuclear FRI–GFP condensates were performed as follows. Root tips of the seedling samples were imaged using the Zeiss LSM900 confocal microscope with a ×40/1 NA water objective and an Airyscan detector of GaAsP-PMT. GFP fluorescence was excited at a wavelength of 488 nm (argon ion laser and laser power 8.0%), and detected at 490–620 nm in lambda mode. All of the images were obtained with a pixel size of 0.119 μm, and exported using the ZEN3.1 software (Zeiss) for quantitative analysis. The number of spots (with an area of larger than 0.1 μm^2) per nucleus and the spot area in the F_1 seedlings were obtained by outlining the spots using Graphics from ZEN3.1. Similarly, the fluorescent spots with an area of larger than 0.05 μm^2 were scored in the root tips of the seedlings bearing the homozygous *FRI-GFP*[3].

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-023-06189-z.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this Article.

Data availability

Source data are provided with this paper.

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Author contributions Y.H. conceived the study. Z.Z., X.L. and Y.Y. conducted the experiments. Y.H. wrote the paper with help from Z.Z. and X.L.

Competing interests The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41586-023-06189-z.

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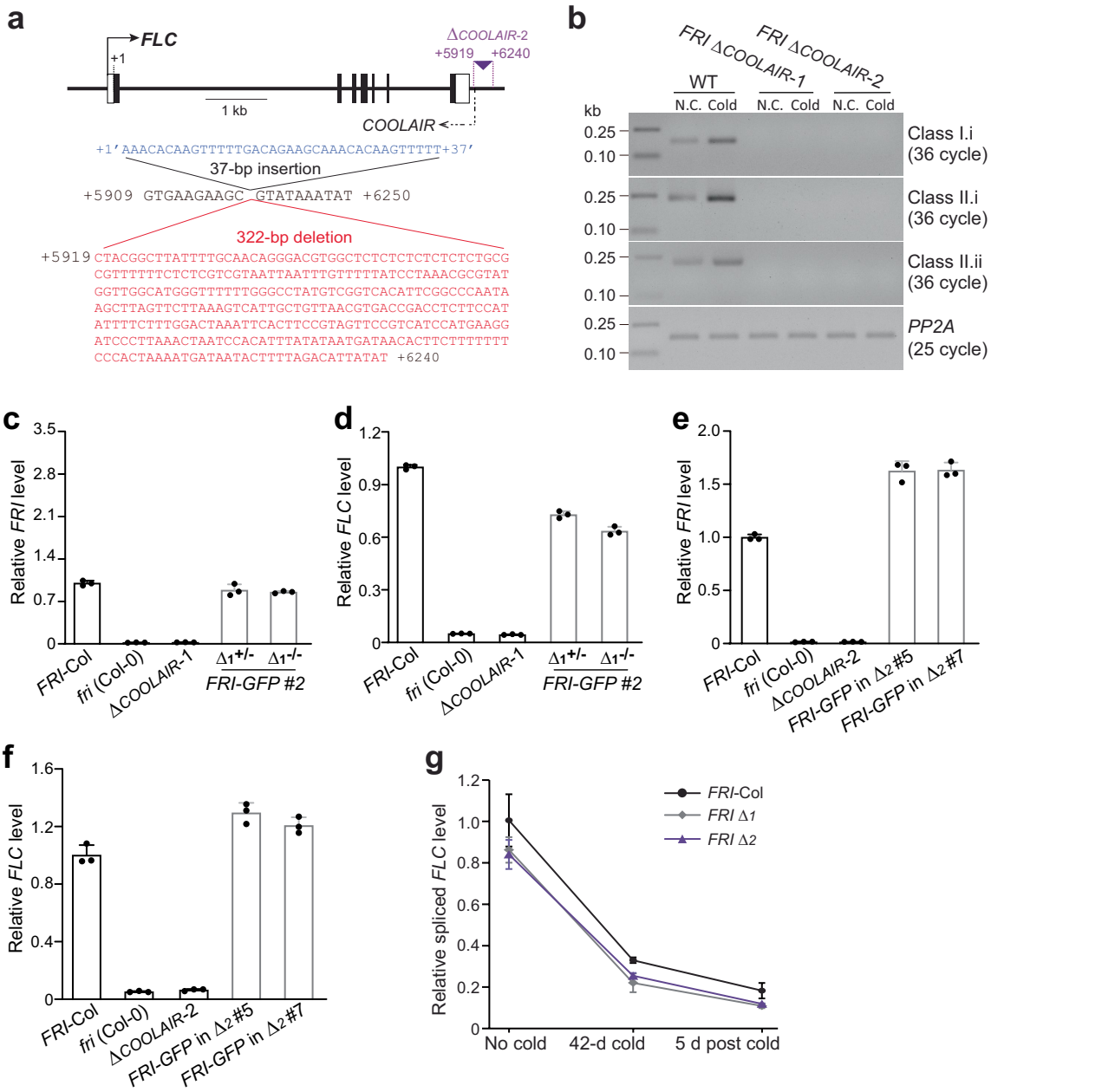

Extended Data Fig. 1 | Characterization of *COOLAIR* promoter deletion lines and functional analysis of *FRI-GFP*. **a**, Genomic sequences around the 322-bp promoter deletion in the *ΔCOOLAIR*-2 line. Notably, there is a 37-bp insertion at +5919. **b**, *COOLAIR* expression is eliminated in the *COOLAIR* promoter deletion lines. Shown is a biologically-independent repeat experiment of Fig. 1b. **c**–**f**, Relative *FRI* (**c**,**e**) and *FLC* (**d**,**f**) expression in the indicated seedlings. Transcript levels of *FRI* and *FLC* (spliced) were normalized to *PP2A*. Relative expression to *FRI*-Col is presented. Individual data points are shown. Error bars for s.d. of three biological replicates. **g**, Relative levels of spliced *FLC* transcripts upon 42-d cold exposure. 5-d post cold, return to warmth for 5 d after 42-d constant cold exposure. Error bars for s.d. of three biological replicates.

Extended Data Table 1 | Primers used in the study

Amplified Region	Sequence (5'-3')
Class I.i	F: TCACACGAATAAGGTGGCTAATTAAG
	R: TCCTTGGATAGAAGACAAAAAGAGA
Class II.i	F: TGCAATTCTCACACGAATAAGAAAAGT
	R: TAGCCGACAAGTCACCTTCTCCAA
Class II.ii	F: TAGTGGGAGAGTCACCGGAAG
	R: TTCTCCTCCGGCGATAAGTAC
AST-1	F: AAATAAGATATGTAATTATTCCGCTGA
	R: ACAAAGTTCATCAACCTTTTGTCTT
AST-2	F: TCCTTGGATAGAAGACAAAAAGAGA
	R: ATTGTCGGAGATTTGTCCAGCA
AST-3 (CAS)	F: ATCTCATGTATCTATCATGGTCGCAGA
	R: TTCTCCTCCGGCGATAAGTAC
FRI	F: TTCTTGTCCCTATGGTCTCAGG
	R: AGCCGCTTCTTTAAATGCCAG
FLC (spliced)	F: GCAACGGTCTCATCGAGAAAGCT
	R: GATCATCAGCATGCTGTTTCCCAT
FLC (unspliced)	F: CGCAATTTTCATAGCCCTTG
	R: CTTTGTAATCAAAGGTGGAGAGC
PP2A	F: TATCGGATGACGATTCTTCGTGCAG
	R: GCTTGGTCGACTATCGGAATGAGAG

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☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Confocal microscopic images were collected by Zeiss LSM900.
Data analysis	ZEN3.1 (Zeiss), GraphPad Prism (v9.3.1), and Microsoft Excel.

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All source data (for Fig. 1, Fig. 2 and Extended Data Fig. 1), are provided with this paper.

Human research participants

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Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	FRI:GFP condensates in 154-297 root tip nuclei of 10-13 F1 seedlings or over 300 nuclei of 22-25 F3 seedlings per sample were examined. The sample size was chosen based on prior studies (e.g. DOI: 10.1038/s41586-021-04062-5 and DOI: 10.1038/s41586-020-2644-7). Statistical methods were not used to pre-determine sample size.
Data exclusions	No data from the experiments were excluded.
Replication	All attempts at replication in the experiments reported in this study were successful.
Randomization	In the experiments involving several lines, petri plates with plants were placed alongside and randomly in the experimentation.
Blinding	Investigators were not blinded to group allocation during experiments or data analysis. Blinding is unnecessary as bias could not be introduced in the experiments with samples growing alongside and treated identically.

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Materials & experimental systems

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Methods

n/a	Involved in the study
☒	☐ ChIP-seq
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Reply to: Cold induction of nuclear FRIGIDA condensation in *Arabidopsis*

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Pan Zhu[1] & Caroline Dean[1]✉

REPLYING TO: Z. Zhang et al. *Nature* https://doi.org/10.1038/s41586-023-06189-z (2023)

We write in response to Zhang et al.[1]. Using their mutant we confirm our original findings. Zhang et al.[1] analyse both F_1 populations and *FRI-GFP* homozygous lines[2] (but using different criteria for each) and report that "the cold-induced formation of nuclear FRI condensates is independent of *COOLAIR*". Zhang et al.[1] also find that "*COOLAIR* is not involved in *FLC* repression by prolonged cold exposure" because "loss of *COOLAIR* expression in either *FRIΔCOOLAIR-1* or *FRIΔCOOLAIR-2* had no effect on the progression of transcriptional shutdown of *FLC* during cold exposure or on post-cold stable silencing of *FLC*".

We have combined the He laboratory Δ*COOLAIR-1* deletion[3] with our *FRI-GFP* transgene[2] (*FRI-GFP* Δ*COOLAIR-1*) and repeated the analysis. In contrast to their data, we show that deletion of the *COOLAIR* promoter significantly attenuates cold-induced formation of FRI–GFP nuclear condensates, changing the overall size distribution[4] (Fig. 1a–c and Extended Data Fig. 1a–c). This fully confirms our original findings that antisense transcription is one component of the multiple cold responsive factors regulating FRI condensates[2]. FRI–GFP condensates show concentration dependency and plasticity to environmental conditions, both well-known properties of condensate dynamics[5].

We disrupted *COOLAIR* expression in our original report using a terminator exchange construct (*TEX1.0*)[6] and, indeed, the level of FRI–GFP is reduced in this line as it is in the *frl1-1* mutant (discussed in our original paper), a component of the FRI complex[2]. These effects are not due to transgene-induced RNA silencing of *FRI-GFP* (Extended Data Fig. 1d). We also confirm that the repression of spliced *FLC* RNA levels, which is particularly sensitive to widely fluctuating cold conditions[6], is attenuated in the *FRI ΔCOOLAIR-1* line (Fig. 1d,e). Over the years of study, we find that *FLC* downregulation in the cold, even in wild-type plants, is very dependent on growth conditions and seedling density, with growth being essential for RNA reduction. Cold-induced downregulation of *FLC* expression is mediated through several different mechanisms[7–13] with *COOLAIR* affecting the dynamics of many of these, and not only through FRI condensation.

The *ΔCOOLAIR-1* line still produces abundantly expressed antisense transcripts[6] (Extended Data Fig. 1e–k). The marked robustness of antisense/non-coding expression at loci such as *FLC* shows the intrinsic connection of sense/antisense transcription, as has been found at many yeast loci[14,15].

These antisense transcripts are inducible by short cold exposure (Fig. 1f–i) but are indeed downregulated after longer exposure (Extended Data Fig. 1f,h,i). The *TEX1.0* line has different alternative antisense transcript levels compared with the *ΔCOOLAIR-1* line (Extended Data Fig. 1e–k). We chose to use the *TEX1.0* line in our original study because it has the lowest levels of alternative antisense transcripts[6] (Extended Data Fig. 1e–k). The sequence of events during cold-induced *FLC* silencing is very dynamic and condition dependent due to the nonlinearity of *FLC* transcriptional shutdown and epigenetic silencing dynamics. This nonlinearity emerges from the complex feedback mechanisms interconnecting non-coding transcription, chromatin modifications and RNA stability.

Methods

The reference genotype Col *FRI*[2], the *FRI-GFP*[2] transgenic plants, *TEX1.0*[2,6] and *ΔCOOLAIR-1*[3] have been described previously. *ΔCOOLAIR-1* was crossed with Col *FRI* and *FRI-GFP* to generate the *FRI ΔCOOLAIR-1* and *FRI-GFP ΔCOOLAIR-1* lines. Imaging and quantification of FRI–GFP condensates were performed as described previously[2]. The experiments under fluctuating conditions and all of the qPCR with reverse transcription analyses were performed as previously described[6]. A list of all of the primers used in the qPCR assay are provided in Extended Data Table 1.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-023-06190-6.

Reporting summary

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Data availability

Source data are provided with this paper.

1. Zhang, Z., Luo, X., Yang Y. & He, Y. H. Cold induction of nuclear FRIGIDA condensation in *Arabidopsis*. *Nature* https://doi.org/10.1038/s41586-023-06189-z (2023).
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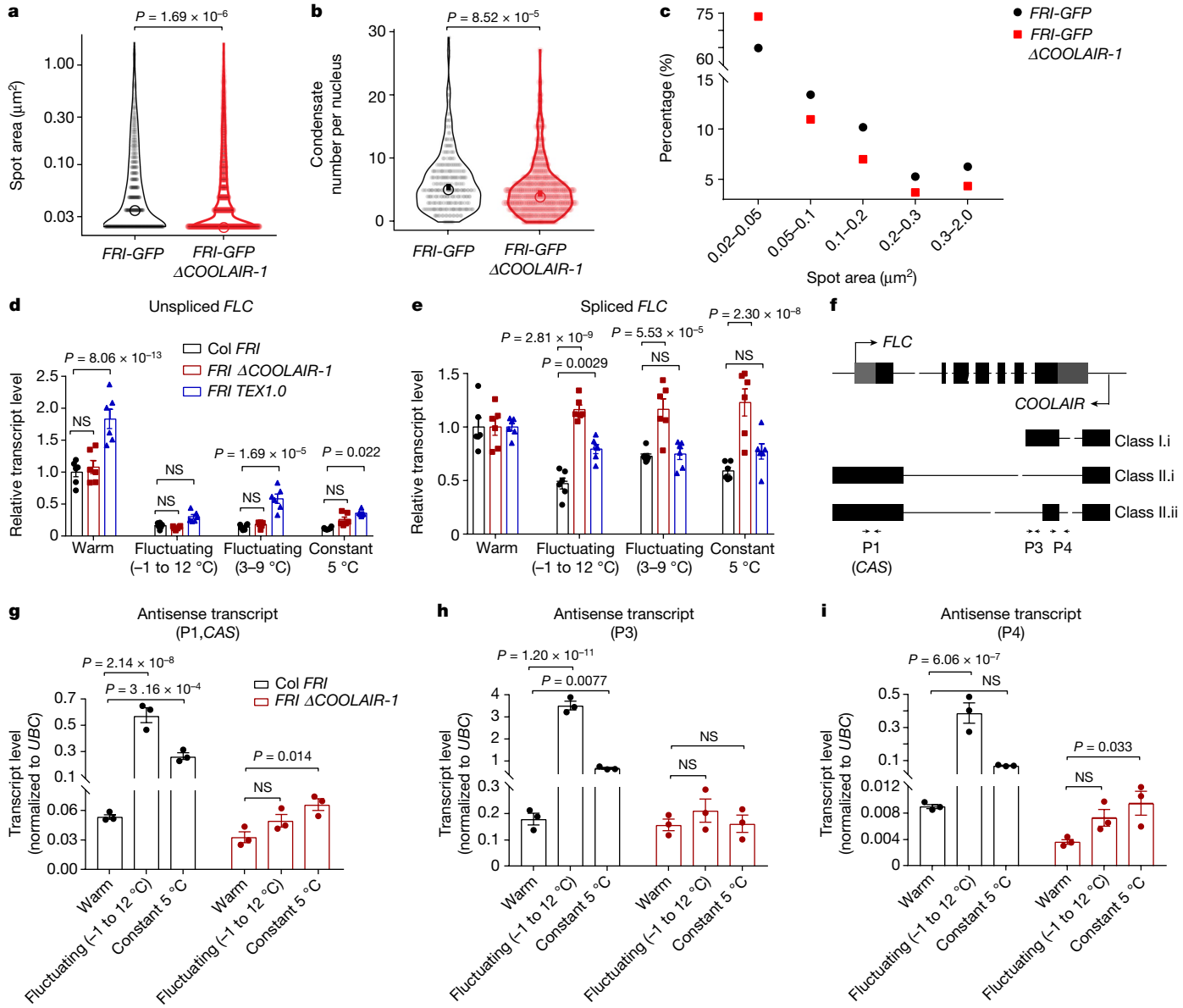

Fig. 1 | Cold-induced FRI–GFP condensate formation and *FLC* repression are attenuated in *ΔCOOLAIR-1*. **a**–**c**, Quantification of the FRI–GFP nuclear condensate area (spot area (**a**) and percentage (**c**)) and number per nucleus (**b**) in the roots of *FRI-GFP* and *FRI-GFPΔCOOLAIR-1* homozygous F_3 lines. Plants were exposed to cold treatment for 4 days at a constant temperature of 5 °C. For **a** and **b**, the open circles indicate the median of the data and the vertical bars indicate the 95% confidence interval determined by bootstrapping. $n = 1{,}729$ and 2,548 condensates (**a**) and $n = 271$ and 529 (**b**) nuclei in $n = 15$ and 26 roots, respectively. Individual data points are shown as black or red dots. Comparison of mean values was performed using two-tailed *t*-tests with Welch's correction. **d**,**e**, The relative expression level of unspliced *FLC* (**d**) and spliced *FLC* (**e**) in Col *FRI*, *FRI ΔCOOLAIR-1* and *FRI TEX1.0* plants with 2 weeks of growth under the indicated temperature conditions[6]. Data are mean ± s.e.m. of $n = 6$ biologically independent experiments. **f**, Schematic of *FLC* and *COOLAIR* transcripts at the *FLC* locus. Untranslated regions are indicated by grey boxes and exons by black boxes. Head-to-head arrows indicate primers used for antisense transcript level analysis by quantitative PCR (qPCR). **g**–**i**, The relative expression level of antisense transcripts at *FLC*, including but not limited to *CAS*[6], by the indicated primers (P1 (*CAS*) (**g**), P3 (**h**) and P4 (**i**)) in Col-*FRI* and *FRIΔCOOLAIR-1* plants with 24 h of growth under the indicated temperature conditions. The primers used are indicated in **f**. Data are mean ± s.e.m. of $n = 3$ biologically independent experiments. NS, not significant; the exact *P* values are shown at the top of each comparison.

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Competing interests The authors declare no competing interests.

Additional information
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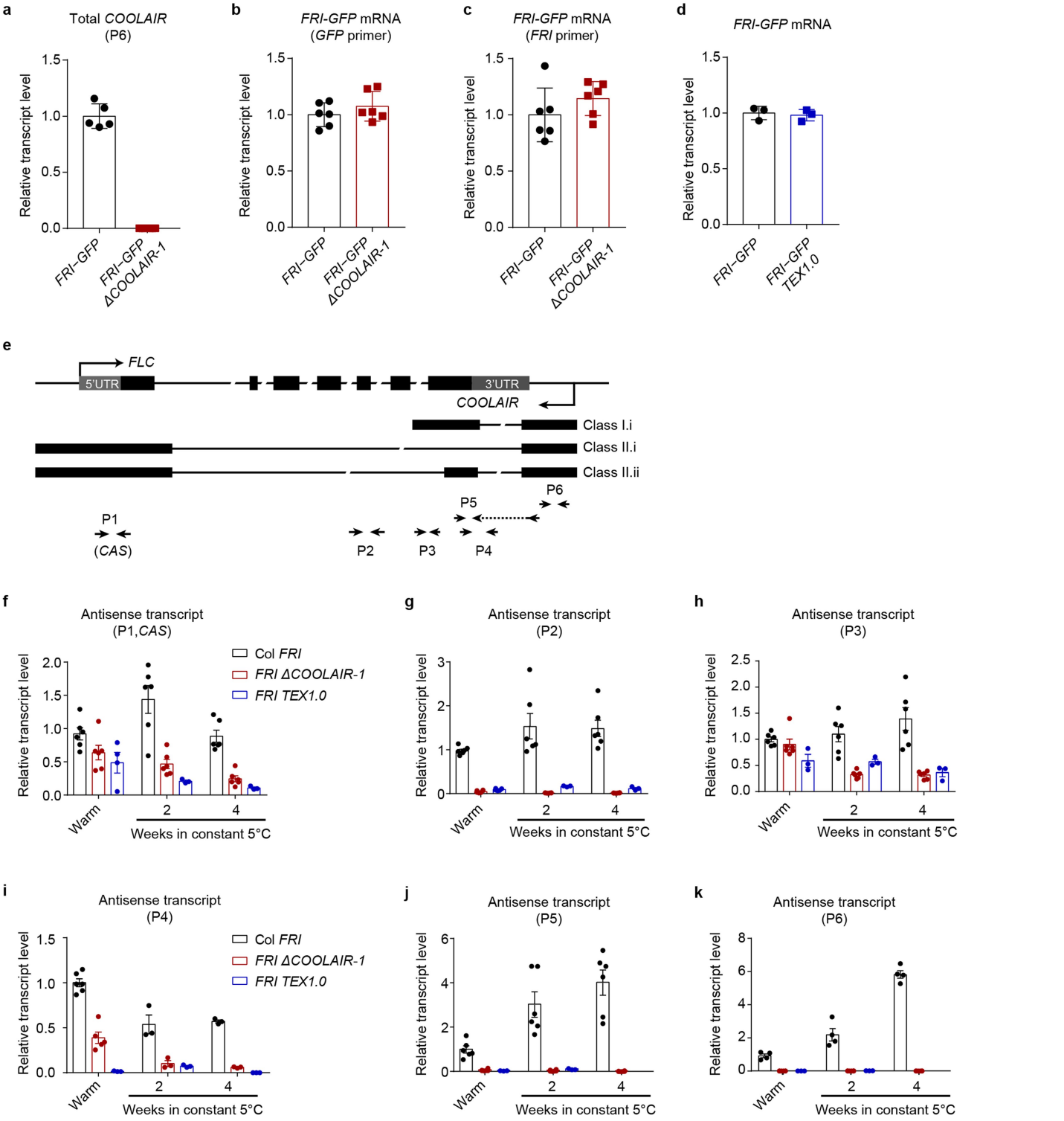
Correspondence and requests for materials should be addressed to Caroline Dean.



Extended Data Fig. 1 | *FRI-GFP* and antisense transcript levels in *ΔCOOLAIR-1* and *TEX1.0*. **a**–**c**, Relative expression level of total *COOLAIR* and *FRI-GFP* mRNA assayed using the indicated primers in *FRI-GFP* and *FRI-GFP ΔCOOLAIR-1* F3 lines. Plants were given 4 days of cold treatment at constant 5 °C as in Fig. 1a–c. **d**, Relative expression level of *FRI-GFP* mRNA assayed in *FRI-GFP TEX1.0*. (**a**–**d**) Mean ± s.e.m.; n = 3 biologically independent experiments. **e**, Schematic of *FLC* and *COOLAIR* transcripts at the *FLC* locus. Untranslated regions are indicated by grey boxes and exons by black boxes. Head-to-head arrows indicate primers used for antisense transcript level analysis by qPCR. **f**–**k**, Relative expression level of antisense transcripts at *FLC* by the indicated primers in Col *FRI*, *FRI ΔCOOLAIR-1*, and *FRI TEX1.0* plants with the indicated lengths of cold treatment at constant 5 °C. The previously reported *CAS*[6] was detected by P1 primer in (**f**). The primers used are indicated in (**e**). Mean ± s.e.m.; n = 3 or 6 biologically independent experiments as shown by the individual data points.

Extended Data Table 1 | List of primers used in this study

Name	Sequence (5'-3')
Unspliced FLC-F	CGCAATTTTCATAGCCCTTG
Unspliced FLC-R	CTTTGTAATCAAAGGTGGAGAGC
Spliced FLC-F	AGCCAAGAAGACCGAACTCA
Spliced FLC-R	TTTGTCCAGCAGGTGACATC
GFP-F	CGTGCAACTCGCTGATCATT
GFP-R	CATGTGTAATCCCAGCAGCTG
FRI-F	CTGCTGTTGCTTGGAGGAAAAG
FRI-R	ACCTGAGACCATAGGGACAAGAA
Antisense P1-F	GTATCTCCGGCGACTTGAAC
Antisense P1-R	GGATGCGTCACAGAGAACAG
Antisense P2-F	CCGGTTGTTGGACATAACTAGG
Antisense P2-R	CCAAACCCAGACTTAACCAGAC
Antisense P3-F	TGGTTGTTATTTGGTGGTGTG
Antisense P3-R	ATCTCCATCTCAGCTTCTGCTC
Antisense P4-F	CCTGCTGGACAAATCTCCGA
Antisense P4-R	TACAAACGCTCGCCCTTATC
Antisense P5-F	CCTGCTGGACAAATCTCCGA
Antisense P5-R	TCACACGAATAAGGTGGCTAATTAAG
Antisense P6-F	TGCATCGAGATCTTGAGTGTATGT
Antisense P6-R	ACGTCCCTGTTGCAAAATAAGC

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Software and code

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Data collection	All QPCR reactions were run with LightCycler® 480; all confocal images were collected on Zeiss LSM880 confocal microscope.
Data analysis	Microsoft Excel (version 2102, 64-bit), GraphPad Prism 7, Fiji-ImageJ 1.52i (Java 1.8.0_172, 64-bit)). Web service: PlotsOfData (https://huygens.science.uva.nl/PlotsOfData/).

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Sample size	The sample size and the results of statistical analyses are described in the relevant figure legends. No statistical approach was used to predetermine sample size. Sample sizes were determined based on previous publications on similar experiments. The determined sample size was adequate as the differences between experimental groups was significant and reproducible. RNA expression analysis (https://doi.org/10.1038/s41586-020-2485-4) Microscopy and image quantification (https://doi.org/10.1038/s41586-019-1165-8; https://doi.org/10.1038/s41586-020-2485-4)
Data exclusions	No data was excluded from analysis.
Replication	All data contained at least three biologically independent replicates.
Randomization	Plants of different genotypes were grown side by side to minimize unexpected environmental variations during growth and experimentation. Different treatments were carried out in parallel, with minimum covarying factors. Seedlings at the same developmental stage were collected and assessed randomly for each genotype/treatment.
Blinding	Blinding was not applicable for this study because plants grown at different temperature conditions need to be collected at different growing time points and require specific handling temperatures.

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