Supplementary Information For

Vernalization-triggered intragenic chromatin-loop formation by long noncoding RNAs

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This includes:

Supplementary Table 1
Supplementary Figs. 1 to 6

Supplementary Table 1. Related to Figures 1 ~ 6 and Supplementary Figures S1 ~ S6.
Sequence information of primers used in this study.

Name	Forward	Reverse	Use
FLC	gccaagaagaccgaactcatgttga	caaccgccgatttaaggtggcta	qRT-PCR
PP2A	tatcggatgacgattcttcgtgcag	gcttggtcgactatcggaatgagag	qRT-PCR
FT	ggaacaacctttggcaatgagat	ctgccaagctgtcgaaacaa	qRT-PCR
COOLAIR	tgtatgtgttcttcacttctgtcaa	gccgtaggcttcttcactgt	qRT-PCR
COLDAIR	ggccacgcgtcgactagtac	agtagacactacaccagattcaattttgac	qRT-PCR
COLDWRAP	aggaaagatagttttcatttagcaacgaaagtg	gacgagcgtctttgctacttttgcattg	qRT-PCR
COLDWRAP-KD	caccaggaaagatagttttcatttagcaacgaaagtg	caagattgccacgtgtaccgcatgac	RNAi construct
COLDWRAP-Full	aggaaagatagttttcatttagcaacgaaagtg	ctttgtgccctaatttgatcctcaggtttg	RNA binding assay
COLDWRAP-5'RACE	gaaagtgaaaactaaggcaatgcaaaagtagc	gttgtgttttgaagacaagattgccacg	5'RACE
COLDWRAP-pBLUE	aaggtaccgggggtaaacgagagtgatgcaaaaaaa	aagagctcaggaaagatagttttcatttagcaacg	RNA blot probe
P5	gaaagtgaaaactaaggcaatgcaaaagtagc	gttgtgttttgaagacaagattgccacg	RT-PCR
P8	atgggaagaaaaaaactagaaatcaagcgaattg	tgcgtcacagagaacagaaagctgacg	RT-PCR
FLC-P1	cgtgagtccgcccctgatagc	ggaccaaaccaaacctacaaagactttc	ChIP
FLC-P2	cttagtatctccggcgacttgaacc	gcgtcacagagaacagaaagctga	ChIP
FLC-P3	acacaacctttgtatcttgtgtctttg	agtagacactacaccagattcaattttgac	ChIP
FLC-P4	gtgaatagtgattttgacctatgattatcgtacag	ggtggctaattaagtagtgggagagtcac	ChIP
AG	gtgaaacaaatttttcctgcagaatgtcact	agtttttgaggcactaaaatctttgggtaaatc	ChIP & RIP
UBQ10	aggtggaaagctccgacaccatcg	agctgcttgccggcgaaaataagcc	ChIP & RIP
PP2A	agcctttatacccgattgctgtgcttatcg	cctctcctctccaagagcacgagc	ChIP & RIP
A	aggaaagatagttttcatttagcaacgaaagtg	gttgtgttttgaagacaagattgccacgtg	RIP
B	cttagtatctccggcgacttgaacc	gcgtcacagagaacagaaagctga	RIP
C	tacaaccctccaatataataaccaaatggttg	cacgttctaaaaggcttcttctttattaaatc	RIP
D	gtgaatagtgattttgacctatgattatcgtacag	ggtggctaattaagtagtgggagagtcac	RIP
3C-F1	catgaaaatcatttggtcagtactcaatttttgtgg		3C
3C-F3	ggtctagatacgatggtagccaaagatttggg		3C
3C-F4	cgcttagtatctccggcgacttgaacc		3C
3C-F5	ccttttatcttctgttttgtgctcttttacttttctgag		3C
3C-F7	gaagagttaattatatatcacaagactaatgattaatgtct		3C
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3C-F10	cacgtccgggagatttataaataaaattagtatg		3C
3C-F11	gcctctaggaaattgaaaatcccacaac		3C
3C-F13	gcaaagagcttaacttcacaataggactgatatc		3C
3C-F15	cacgctttgtaaaggatataggtgtttttgagc		3C
3C-F17	ctgtcttttaccgcttcttctgtcc		3C
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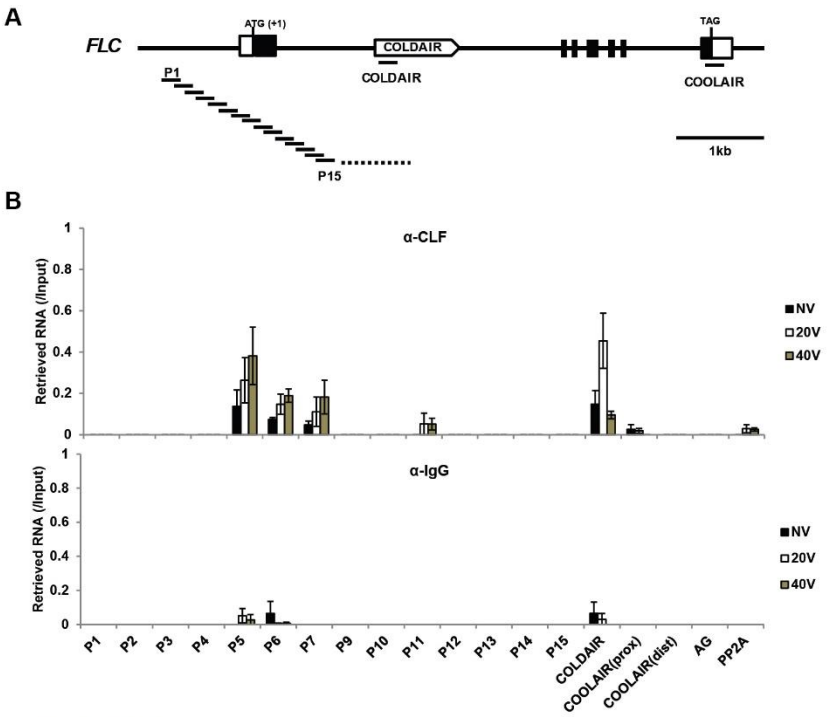
3C-R9		gtatatatacattgttcagcattaaccccatagcaa	3C
3C-R11		gacactacaccagattcaattttgacaacttatatat	3C
3C-R13		cttccatagaaggaagcgactaagacag	3C
3C-R15		ctaattgcaacctctatcagagattcagagcttcc	3C
3C-R17		gtagctcatagtgtgaaccatagttcagagc	3C
3C-R19		cctggttctcttctttcagcattttctcctg	3C
3C-R20		gcactacttctagacacttggagttggaac	3C
3C-R21		gggaaaaaaagaagtgttatcattatataaatgtg	3C
3C-R22		ggtcaggtgtaagtgtatcgcatcttgtg	3C
PP2A-LC	Ctctctagattgggacggcacaaagtttc	ctcaattccgcctctgagataattcaaagg	3C
P1	TAACTTTGAGCTATTGCCATATGTG	ATAGTAAAAACAAAGAACCAC	Tiling PCR
P2	TGTGTTAATCTCCCGAACATTATTA	GTAGACATATCGAAACCTAAA	Tiling PCR
P3	TGTAGGCACGACTTTGGTAACAC	TTTTCACTTTCGTTGCTAAATG	Tiling PCR

P4	CATAGTTCAAAGATGATGTAGAGT	GCAAAAAAACCAAATATGTGA	Tiling PCR
P5	ACTAAGGCAATGCAAAAGTAGCA	TTCCTTTTCTCGCTTTATTTCTT	Tiling PCR
P6	CATCACTCTCGTTTACCCCCA	TGGCTTCTCTCCGAGAGG	Tiling PCR
P7	AAAAAAAAAATAGAAAGAGAAAA	TGACGAGCTTTCTCGATGAGA	Tiling PCR
P8	ATGGGAAGAAAAAAACTAGAAAT	AAAAGGCGTACTTATCGCCGG	Tiling PCR
P9	AGCTTTCTGTTCTCTGTGACG	AACCAAACGGTATATTAATTGT	Tiling PCR
P10	TCCTTACCTGGGTTTTCATTTGTTC	GTTCTTCCTTAAATTTGGTTAT	Tiling PCR
P11	TTTTTCCGGCGGATCTCTTG	TTCCAAGGAAATAATGAAATC	Tiling PCR
P12	CAATGTCGTGAAGAAGCTTTTTAG	CTAAAGGTAAGAAACAATCAA	Tiling PCR
P13	AAAAAATTGCATGTCATTCACGAT	ACAAATCCGAGAGATCCAATG	Tiling PCR
P14	GAGATTCCTTAAGTTTTTGAAGAG	ATAGTCTTATATAGTCAATAGCT	Tiling PCR
P15	TATGCAATGCACTTACGGGAG	CTACTGTGTATGAAAAATATGA	Tiling PCR
P16	TTGTTAATCTTCTATGAATTCCTAT	AAAGAATCGCAATCGATAACC	Tiling PCR
P17	GTTTTGAATTTTGGTAGCTTCAAA	ACAAATCCAAGAGAACAATGA	Tiling PCR
P18	TGAATCACAATCGTCGTGTGCTAT	TTCCCAATTAATGTGGCTTACA	Tiling PCR
P19	tatatgcacgtccgggagatttataaataaa	ATAAACCTTTGGACAATCTTTA	Tiling PCR
P20	AACTATGACTAAAAGATGAATTGG	TAAATGAACACAACGATGATA	Tiling PCR
P21	TAGTTTCCCACTCTTGCAGTTACA	TGACAAAAGACACAAGATACA	Tiling PCR
P22	ATGACTTTGTTCCTATTCGTTAAAA	GACAAGTGTTGTGGGATTTTC	Tiling PCR
P23	ACACAACCTTTGTATCTTGTGTCTT	AGTAGACACTACACCAGATTC	Tiling PCR
P24	CTTCATGTAAGAAATACCAACCTC	CAAATCCTAACAAAGTATGCAT	Tiling PCR
P25	TACAACCCTCCAATATAATAACCA	CACGTTCTAAAAGGCTTCTTCT	Tiling PCR
P26	CAAGTGGGGATCAATCCAGG	ACTTAATTTGTTTTGTTAATAG	Tiling PCR
P27	GAACCCTTAGTTACTCAGTTACTC	CATATCTATAAATATATCTTCCA	Tiling PCR
P28	ACTTCAAAACAGTTTTAACAACCT	CAATTTCCTATATTTAAACCCC	Tiling PCR
P29	GTGTGATAAGTTTCCTACTAATATT	TTTTTTTTGCAAATATCAAAAG	Tiling PCR
P30	GGAACCTCACAGTTTCTATAAACG	GATGTGGCGGTAACCAGATAA	Tiling PCR
P31	CATATACAATAGAAATATGAGTTTT	AACATATACGAGAAAACTTTT	Tiling PCR
P32	CATCATTATCATCTTATGGGTCATC	TAAAAAGAATTTTTTAAAAAC	Tiling PCR
P33	TGATATGGTATTACTTACAAACAAA	CCTCTATTTTCTATCATGTTTAC	Tiling PCR
P34	AAAACTGGGATACAAAAAGAAAA	ATTTTGGTTTTGATCCATACCA	Tiling PCR
P35	GGTAAATAGGTTTTGTTCTTATAAT	TCCCTAACAATAGCAAATAGTG	Tiling PCR
P36	TGGAAGCTCTGAATCTCTGATAGA	ATGTGATGACATGGAAGTAAA	Tiling PCR
P37	AAGTCTTTCAGTTAATTTCAGAAA	TAAACGCAGCCCCAATCTTAA	Tiling PCR
P38	TTGTGGCTCATCAATATATGTGTGT	GAAAATGACATTTTCCCTCAA	Tiling PCR
P39	ACATTTTATATTGCATCAATTATTTC	GAAAATGACATTTTCCCTCAA	Tiling PCR
P40	CCTTAACTAGTTTGACTTTAAGTTA	GAAAAGAAAAGAACCAAAAC	Tiling PCR
P41	CAATCTGCCGAAATATATAATAAAT	GTAGCACATCTGAATTTCCACT	Tiling PCR
P42	CTTGAGAGAGAAAAAAAATATCA	ATGAAACATTGAGAGAACACC	Tiling PCR
P43	CTGCTTAAACATGAATATTAAGATT	ATCGATCAAGGATCTTGACCA	Tiling PCR



C

COLDWRAP

aggaaagatagttttcatttagcaacgaaagtgaaaactaaggcaatgcaaaagtagcaaagacgctcgtcatg

C2

cggtacacgtggcaatcttgtcttcaaaacacaacgttttttattcacatatttggttttttttgcatcactctcg

C2-2 (P5R) 9R

Tttacccccaaaaaaaaaaaaaatatctggcccgacgaagaaaaagtagataggcacaaaaaaatagaaagaaata

FLCmRNA

15R

aagcgagaaaaggaaaaaaaaaaaatagaaagagaaaacgcttagtatctccggcgacttgaacccaaacctgag

20R

gatcaaattagggcacaaagccctctcggagagaagccATGGGAAGAAAAAAACTAGAAATCAAGCGAATTGAG

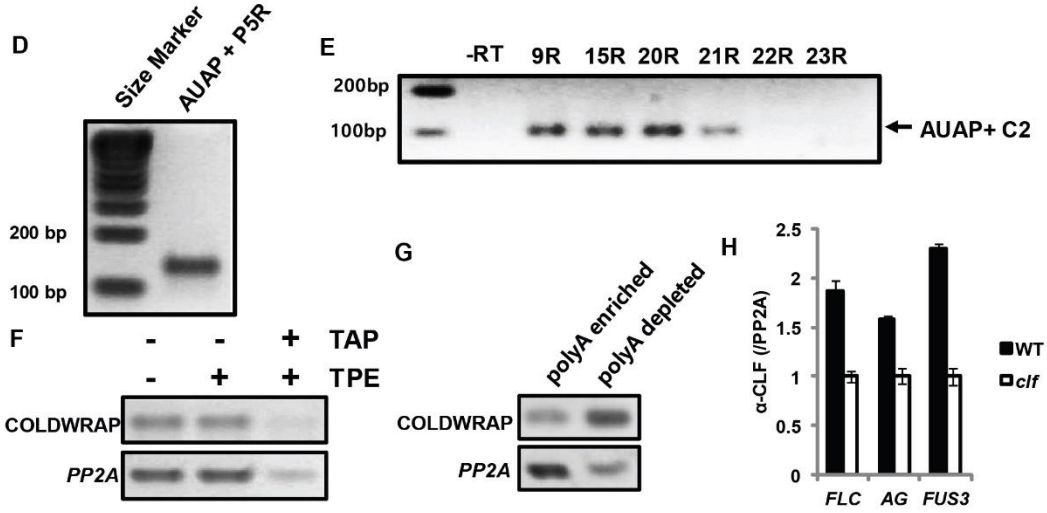
21R 22R

AACAAAAGTAGCCGACAAGTCACCTTCTCCAAACGTCGCAACGGTCTCATCGAGAAAGCTCGTCAGCTTTCTGT

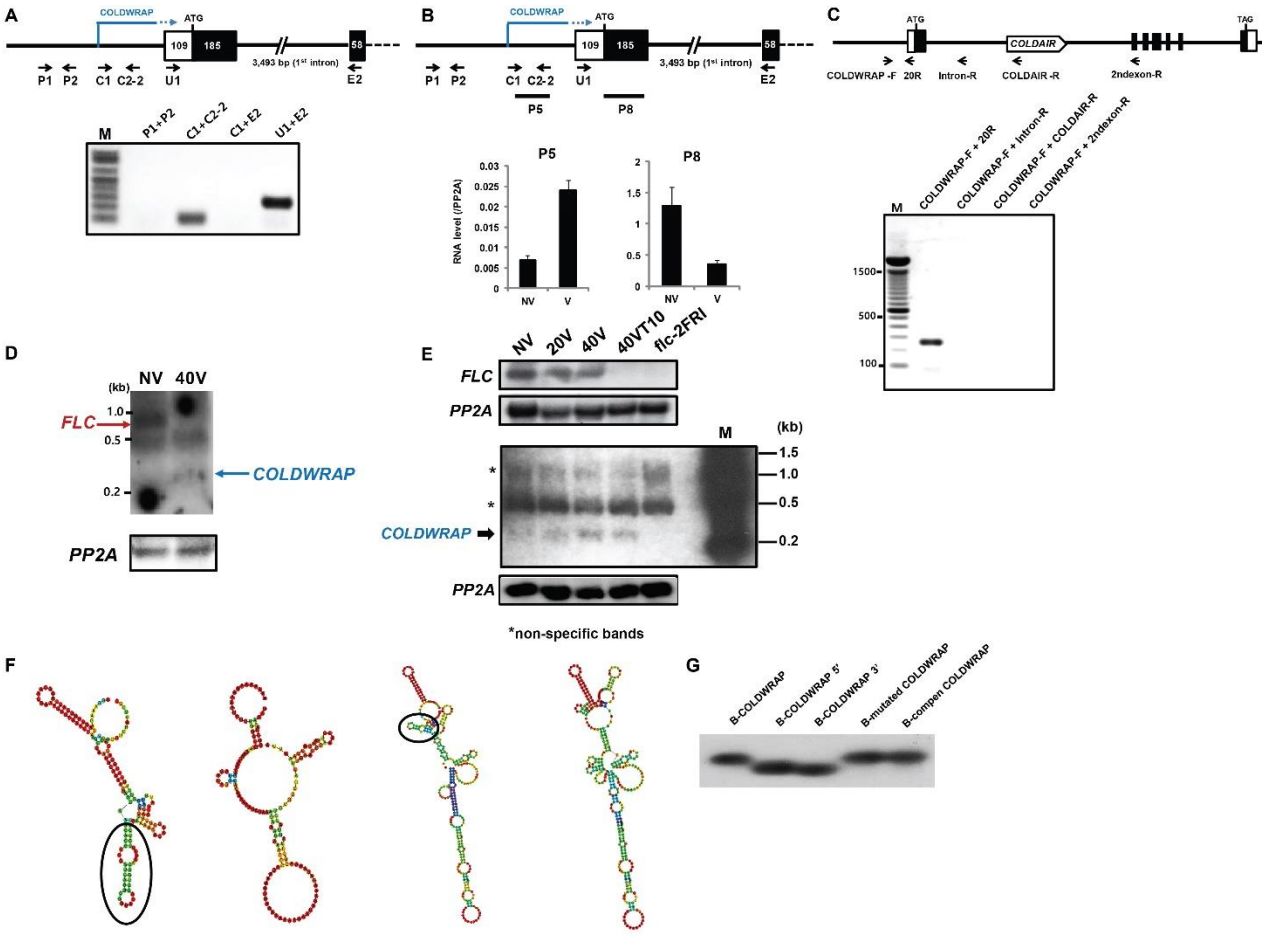
23R

TCTCTGTGACGCATCCGTCGCTCTTCTCGTCGTCTCCGCCTCCGGCAAGCTCTACAGCTTCTCCTCCGGCGATA

Italic : 5'UTR
Capital : Translated region
Underlined arrows : primers used

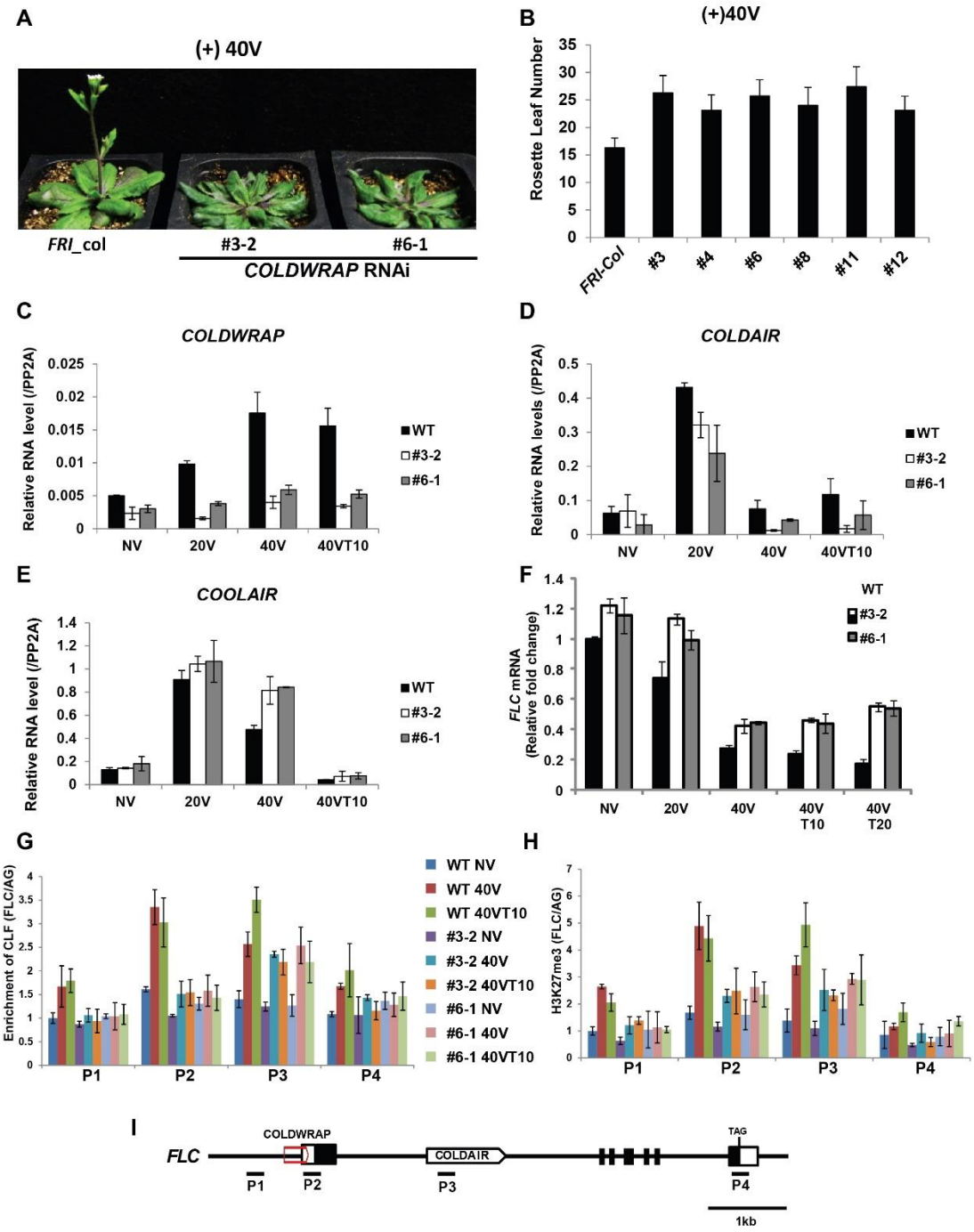


Supplementary Figure S1. Related to Figure 1. Identification of COLDWRAP. **(A)** Schematic representation of RNA immunoprecipitation (RIP) followed by tiled quantitative RT-PCR across *FLC* region. **(B)** Relative levels of RNA retrieved by RIP using anti-CLF (upper panel) and IgG (lower panel) followed by quantitative RT-PCR. AG: AGAMOUS, NV, non-vernalized. 20V, 20 days of vernalization. 40V, 40 days of vernalization. Data (mean ± SD of quantitative PCR; *n*=2). **(C)** The sequence of COLDWRAP-containing region. The transcription start site of COLDWRAP is indicated as a green arrow. The transcription start site of *FLC* mRNA is indicated as a red arrow. Locations of primers used are indicated as underlined black arrows. **(D)** Strand-specific amplification of COLDWRAP using 5'RACE to determine the direction and the 5' end of COLDWRAP. Abridged Upstream Anchor primer (AUAP) with a COLDWRAP-specific P5 reverse primer was used to detect sense strand of COLDWRAP. P5 forward primer did not produce any detectable amplification (data not shown). Location of P5 reverse primer is indicated in (A&B). (**C~D**) COLDWRAP is transcribed in a sense direction relative to *FLC* mRNA. COLDWRAP is ~ 316 bases long and overlaps with 5' end of *FLC* mRNA. **(E)** 5' RACE followed by 3' end tiled RT-PCR to determine the 3' end of COLDWRAP. Total RNA purified from 40 days-vernalized samples was used for this analysis. Locations of primers used are indicated in (A). –RT; without reverse transcription. **(F)** Treatment of Terminator 5'-Phospho dependent exonuclease (TPE) alone does not degrade COLDWRAP, but the treatment with both TPE and Tobacco Acid Pyrophosphatase (TAP) does degrade the COLDWRAP. PP2A was used as a 5' capped mRNA control. **(G)** COLDWRAP is detected from ploy (A)–depleted fraction. Polyadenylated *PP2A* (At1g13320) was used as a control. **(H)** Chromatin immunoprecipitation using anti-CLF antibody. Relative levels of enrichments were shown between wild type and *clf* mutant (*clf-29*) at known CLF target loci, *FLC*, *AGAMOUS* (*AG*) and *FUSCA3* (*FUS3*). Data (mean ± SD of quantitative PCR; *n* = 3).



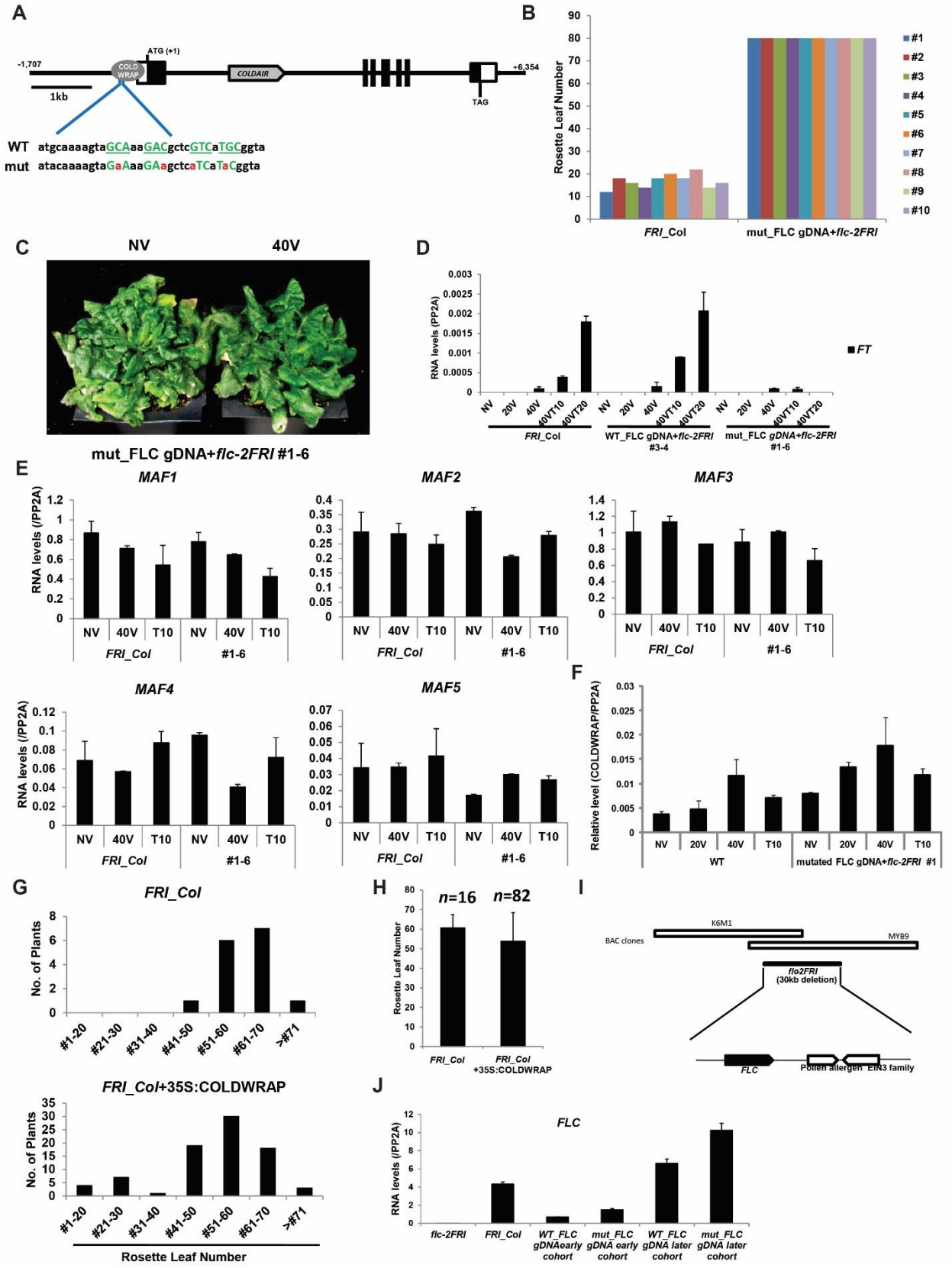
Supplementary Figure S2. Related to Figure 1. Characterization of COLDWRAP. **(A)** COLDWRAP is not an alternative *FLC* mRNA transcript. Relative positions of primer sets used in RT-PCR are indicated around the 5' part of *FLC* region (top). RT-PCR analyses using different combinatorial primer sets are shown (bottom). The *FLC* mRNA transcript was detected by primer set of forward primer U1 (5'UTR) and reverse primer E2 (the 2nd exon of *FLC*). Total RNA purified from 40 days-vernalized samples was used for this analysis. M, size marker. **(B)** Expression patterns of two different transcribed regions (P5 and P8) at 5' end of *FLC*. Schematic representation of relative positions of primer sets used in RT-PCR are indicated around 5' part of *FLC* region (top). Relative levels of RNA expressions were calculated compared to the control, *PP2A* (bottom). Mean ± SD of quantitative RT-PCR data are shown (biological replicates $n = 3$). NV, non-vernalized. V, 40 days of vernalization treatment. **(C)** COLDWRAP is not a part of COLDAIR. Schematic representation of relative positions of primer sets used in RT-PCR are indicated at *FLC* region (top). RT-PCR analyses using different combinatorial primer sets are shown (bottom). **(C, D)** COLDWRAP is not an unspliced or alternately spliced version of *FLC* mRNA. COLDWRAP is not co-amplified with either *FLC* mRNA or unspliced *FLC* **(D)** RNA blot analysis using full length COLDWRAP anti-sense RNA probe. 316 bases of full-length antisense COLDWRAP overlap with 5'UTR of *FLC*, and, therefore, detect both COLDWRAP and *FLC* mRNA transcript. NV, non-vernalized. 40V, 40 days of vernalization treatment. **(E)** RNA blot analysis using *FLC* anti-sense probe (top) and non-overlapping COLDWRAP (157 bp of 5' COLDWRAP) anti-sense RNA probe (middle), and PP2A anti-sense probe (bottom). RNA samples were prepared from the wild type and *flc-2FRI* (in which the COLDWRAP region is deleted). Asterisks indicate non-specific bands. NV, non-vernalized. 10V, 10 days of vernalization. 20V, 20 days of vernalization. 40V, 40 days of vernalization. 40VT10, 40 days of vernalization followed by 10 days of normal growth temperature. Total RNA from vernalized *flc-2FRI* was used as a negative control. **(D, E)** *PP2A* was used as a loading control. M; RNA size marker. **(F)** Predicted secondary structure of COLDWRAP. Stem-and-loop structures subject to mutations shown in black circle. **(G)** RNA stability was not

affected by introduced mutations used for *in vitro* RNA binding assays (Fig. 1D, E). Equal amount of biotin-labeled RNAs (three microgram) were incubated in incubation buffer as described in M&M. Biotin-labeled RNAs were separated on RNA gel and visualized using BrightStar Biotin detection Kit (Ambion, TX, USA).



Supplementary Figure S3. Related to Figure 2. COLDWRAP knockdown compromises vernalization response. **(A)** Representative COLDWRAP knockdown lines (#3-2, #6-1) flower later compared to the wild type (WT) after vernalization. **(B)** Flowering times of multiple COLDWRAP knockdown lines at the second generation (T2) compared to the wild type (WT; *FRI*-Col) after 40 days of vernalization. More than 9 plants for each line were used to count the number of rosette leaves at the timing of flowering. **(C)** Expressions of COLDWRAP in two representative COLDWRAP knockdown lines (#3-2, #6-1) and in the wild type (WT) during the course of vernalization. **(D)** Expressions of COLDAIR transcripts in two representative COLDWRAP knockdown lines (#3-2, #6-1) and in the

wild type (WT) during the course of vernalization. **(E)** Expressions of COOLAIR transcripts in two representative COLDWRAP knockdown lines (#3-2, #6-1) and in the wild type (WT) during the course of vernalization. **(F)** Expressions of *FLC* in two representative COLDWRAP knockdown lines (#3-2, #6-1) and in the wild type (WT) during the course of vernalization. **(C-F)** Relative levels were calculated compared to the control, *PP2A*. Mean ± SD of quantitative RT-PCR data are shown (biological replicates n =3). NV, non-vernalized. 20V, 20 days of vernalization treatment. 40V, 40 days of vernalization treatment. 40VT10, 40 days of vernalization treatment followed by 10 days of normal growth temperature. **(G)** Changes in occupancy of CLF at *FLC* chromatin during the course of vernalization. **(H)** Changes in enrichment of H3K27me3 at *FLC* chromatin during the course of vernalization. **(G, H)** Data (mean ± SD of quantitative PCR; biological replicates n = 3). **(I)** Schematic representation of the *FLC* genomic region. Relative positions of primer sets used for ChIP assays are shown (P1 ~ P4).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ATG (+1)
-1,707
COLD WRAP
COLDAIR
+6,354
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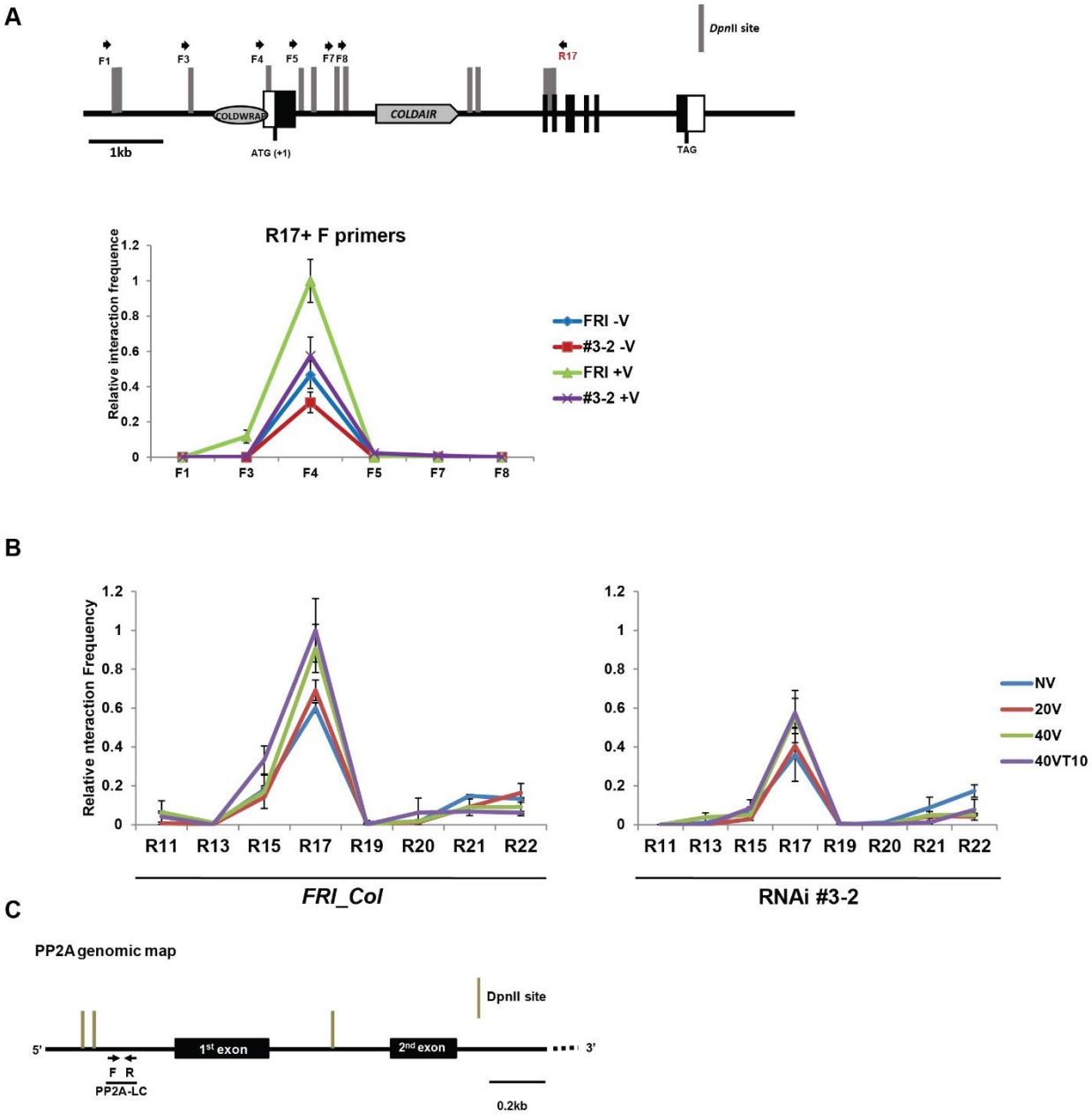
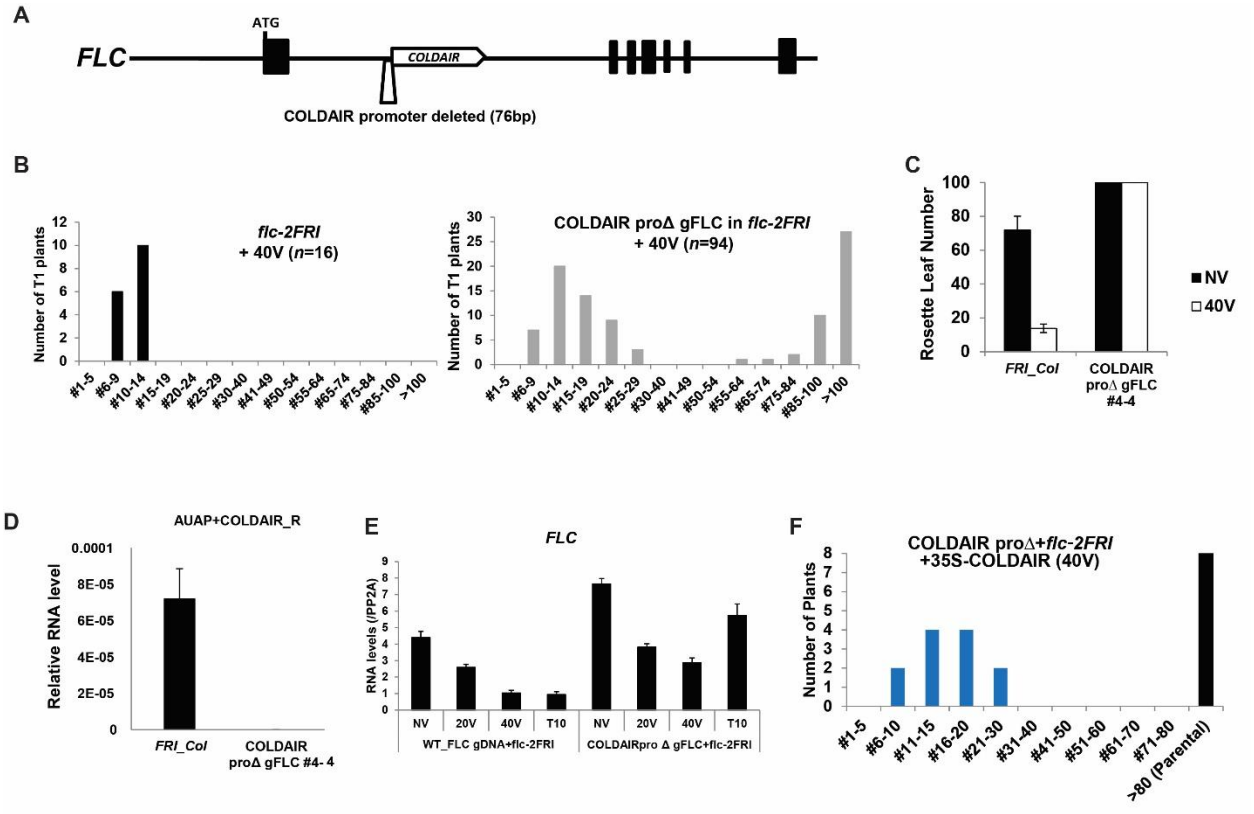
mut atacaaaagtaGaAaaGAagctcaTCaTaCggta
B
Rosette Leaf Number
#1
#2
#3
#4
#5
#6
#7
#8
#9
#10
FRI_Col
mut_FLC gDNA+flc-2FRI
C
NV
40V
mut_FLC gDNA+flc-2FRI #1-6
D
RNA levels (PP2A)
FT
NV
20V
40V
40VT10
40VT20
FRI_Col
WT_FLC gDNA+flc-2FRI #3-4
mut_FLC gDNA+flc-2FRI #1-6
E
MAF1
MAF2
MAF3
MAF4
MAF5
RNA levels (/PP2A)
NV
40V
T10
FRI_Col
#1-6
F
Relative level (COLDWRAP/PP2A)
NV
20V
40V
T10
WT
mutated FLC gDNA+flc-2FRI #1
G
FRI_Col
No. of Plants
#1-20
#21-30
#31-40
#41-50
#51-60
#61-70
>#71
FRI_Col+35S:COLDWRAP
Rosette Leaf Number
H
n=16
n=82
Rosette Leaf Number
FRI_Col
FRI_Col +35S:COLDWRAP
I
BAC clones
K6M1
MYB9
flo2FRI (30kb deletion)
FLC
Polen allergen EIN3 family
J
FLC
RNA levels (/PP2A)
flc-2FRI
FRI_Col
WT_FLC gDNAearly cohort
mut_FLC gDNA early cohort
WT_FLC gDNA later cohort
mut_FLC gDNA later cohort

Supplementary Figure S4. Related to Figures 2 ~ 4. **(A)** Diagrams to depict the extent of transgenes carrying the wild-type *FLC* and the mutant COLDWRAP. Nucleotides forming stem part of stem-loop structure are in green capital letters. Mutated nucleotides disrupting stem structure are in red lower case letters. **(B)** Flowering times of 10 randomly selected transgenic lines carrying the "mutant COLDWRAP in *flc-2* mutant background at the second generation (T2) compared to the wild type (WT; *FRI*-Col) after 40 days of vernalization. More than 9 plants for each line were used to count the number of rosette leaves at the timing of flowering. **(C)** Flowering behavior of the representative transgenic lines carrying the mutant COLDWRAP in *flc-2* mutant background. **(D)** *FT* mRNA expression in wild type (*FRI*-Col), a representative transgenic line carrying the wild-type *FLC* in *flc-2* mutant background (WT_FLC gDNA +*flc-2FRI* #3-4), and a representative transgenic lines carrying the mutant COLDWRAP in *flc-2* mutant background (mut_FLCgDNA + *flc-2FRI* #1-6) during the course of vernalization. Expression of *FLOWERING LOCUS T* (*FT*), a downstream target of *FLC*, remains at low levels even after vernalization, consistent with late flowering phenotype of the mutant COLDWRAP lines compared to wild type. **(E)** Expressions of five *FLC*-related genes in wild type (*FRI_Col*) and a representative transgenic line carrying the mutant COLDWRAP in *flc-2* mutant background (#1-6) during the course of vernalization. The transgene carrying the mutant COLDWRAP expresses COLDWRAP at comparable levels during the course of vernalization compared to the wild type. Data (fold change; mean ± SD of quantitative RT-PCR; biological replicates $n = 3$) is relative to the level of *PP2A* mRNA. Expressions of five *FLC*-related genes are not affected in the transgenic lines carrying the mutant COLDWRAP compared to the wild-type plants, and there is no COLDWRAP-like sequences found in five *FLC*-related gene loci. Therefore, COLDWRAP specifically affects the regulation of *FLC*. **(F)** The transgene carrying the mutant COLDWRAP expresses COLDWRAP at comparable levels during the course of vernalization compared to the wild type. Data (fold change; mean ± SD of quantitative RT-PCR; biological replicates $n = 3$) is relative to the level of PP2A mRNA. **(D-F)** Data (fold change; mean ± SD of quantitative RT-PCR; biological replicates $n = 3$) is relative to the level of *PP2A* mRNA. NV, non-vernalized. 20V, 20 days of vernalization treatment. 40V, 40 days of vernalization treatment. 40VT10, 40 days of vernalization treatment followed by 10 days of normal growth temperature. 40VT20, 40 days of vernalization treatment followed by 20 days of normal growth temperature. (**G**) Flowering times of the wild type (*FRI_Col*) and overexpression of COLDWRAP (*FRI_Col* + 35S::COLDWRAP) without vernalization. Distribution of flowering times in the wild type (upper panel) and the primary transgenic lines (lower panel). (**H**) average flowering times of the wild type and the primary transgenic lines. **(I)** Genomic structure showing the deleted region in *flc-2FRI* (Michaels and Amasino, 1999). **(J)** The level of *FLC* expression of 12 randomly selected T2-pools of transgenic lines carrying the mutant COLDWRAP (mut_FLC) in *flc-2* mutant background and the wild-type COLDWRAP (WT_FLC) at the second generation (T2) compared to the wild type (WT; *FRI*-Col). Due to the *FLC* transgene variability, transgenic lines were grouped together based on their flowering time before vernalization.



Supplementary Figure S5. Related to Figures 5 and 6. Chromatin conformation capture (3C) assay at *FLC* by vernalization. **(A)** Relative fold changes of interaction frequency in 3C assay between different regions of *FLC* by vernalization were indicated. Relative interaction between F4 and R17 are set as 1. Data (mean ± SD of quantitative 3C; biological replicates $n = 2$). Schematic representations of the *FLC* genomic region were given at upper panel. **(B)** Relative fold changes of interaction frequency in 3C assay between WT and COLDWRAP RNAi line (#3-2) of *FLC* during the course of vernalization. NV, non-vernalized, 10V, 10 days vernalized, 20V, 20 days vernalized, 30V, 30 days vernalized, 40V, 40 days vernalized, 40VT10, 40 days vernalized and followed by 10 days of normal growth temperature. Relative interaction between F4 and R17 of WT sample is set as 1. Data (mean ± SD of quantitative 3C; biological replicates $n = 2$). **(C)** Schematic representation of the *PP2A* regions used as a control (see materials and methods for the detail).



Supplementary Figure S6. Related to Figure 6. COLDAIR promoter deletion lines. **(A)** Schematic representation of the transgene in which 76 bp of the putative COLDAIR intrinsic promoter is deleted. **(B)** Flowering times of *flc-2FRI* (left) and the primary transgenic lines carrying the COLDAIR promoter deletion in *flc-2* mutant background after vernalization (right; *n* = 94). *X*-axis; range of rosette leaf numbers. **(C)** Flowering times of a representative transgenic lines carrying the COLDAIR promoter deletion in *flc-2* mutant background without (NV) and with (40V) vernalization. **(D)** COLDAIR is not expressed in a representative transgenic line (#4-4) carrying the COLDAIR promoter deletion in *flc-2* mutant background. Data (fold change; mean ± SD of quantitative RT-PCR; biological replicates *n* = 3) is relative to the level of *PP2A* mRNA **(E)** FLC mRNA expression patterns in a representative transgenic line (#4-4) carrying the COLDAIR promoter deletion in *flc-2* mutant background. *FLC* is de-repressed by vernalization in a representative transgenic line (#4-4) carrying the COLDAIR promoter deletion in *flc-2* mutant background. Data (fold change; mean ± SD of quantitative RT-PCR; biological replicates *n* = 3) is relative to the level of PP2A mRNA. **(F)** Restoration of the vernalization response by 35S::COLDAIR in a transgenic line (#4-4) carrying the COLDAIR promoter deletion. Flowering times of the primary 35S::COLDAIR transgenic lines (*n* = 12; blue bars) compared to the prenatal (#4-4; *n* = 8; black bar) after vernalization. *X*-axis; range of rosette leaf numbers