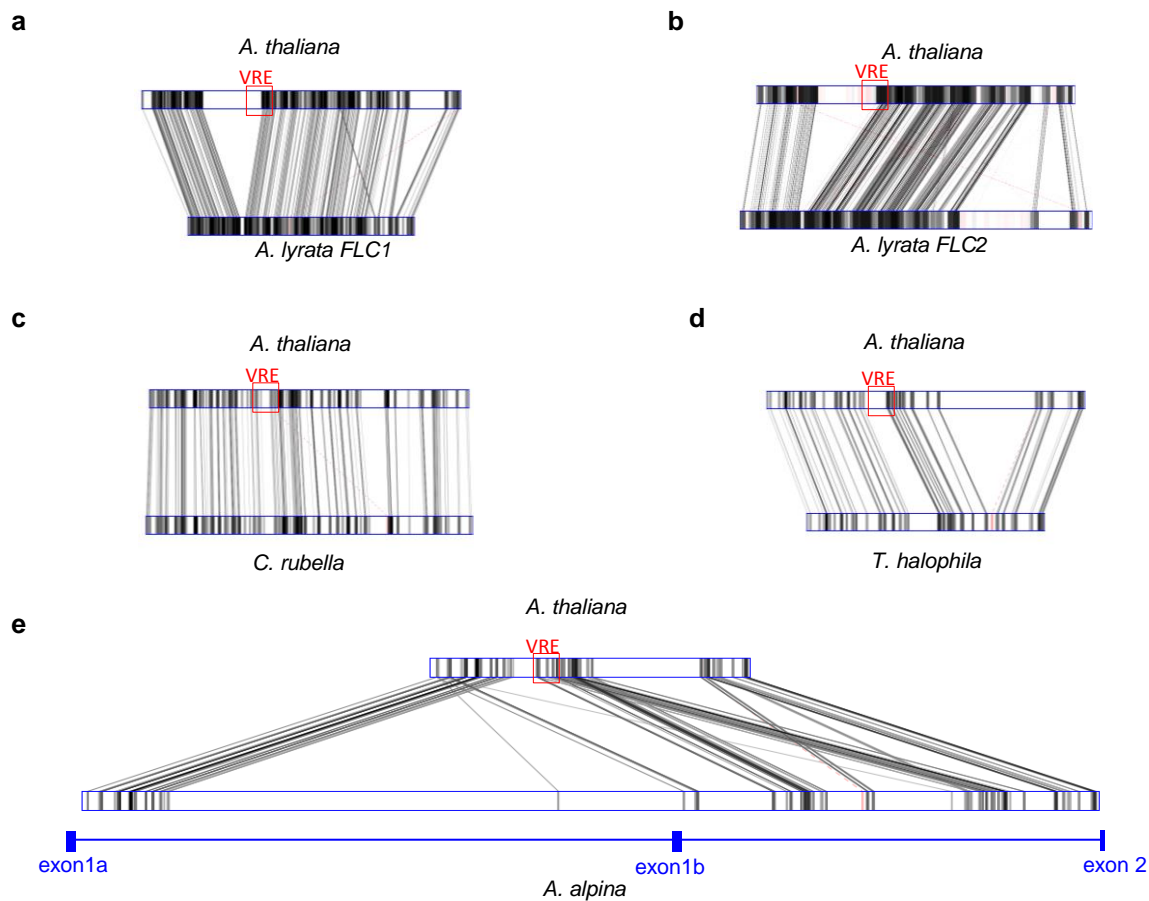


Supplementary Figure 1: 5' promoter conservation among *FLC* orthologues

Schematic representation of phylogenetic relationship of Brassicaceae species used in this study (a). GATA alignment of *A. thaliana* to *A. lyrata FLC1* 5' intergenic region (b), to *A. lyrata FLC2* 5' intergenic region (c), to *C. rubella FLC* 5' intergenic region (d) and to *T. halophila FLC* 5' intergenic region (e). GATA alignment of *A. lyrata FLC1* to *C. rubella FLC* 5' intergenic regions (f) showing that the low level of homology observed in b, c, d, e and g is not due to *A. thaliana* containing a unique *FLC* 5' intergenic region. GATA alignments of *A. thaliana FLC* 5' intergenic region to the full *PEP1a* and *b* 5' intergenic regions (g). The position of *PEP1* exon 1a and 1b on the *A. alpina* alignment is indicated by blue boxes on a blue line under the alignment.

Grey boxes are homologous regions whereas the red boxes indicate homologous inverted regions (the darker the box, the higher the similarity). Scale bars, 1 kb.



f

```

AIVRE-Like_FLC1 -----
AIVRE-Like_FLC2 -----
AtVRE --ATAGATTGCTCATATTTATGTGATTGATATCAATTATCGCCCTTAATCTTATCATCGTGTGTTTCATT-TATGACTTTGTTCCATTCGTTAAATGACAATCCACAACCTCAA
ThVRE-Like -----
CrVRE-Like ACATAGATATGCTACATATCTATGTGTTTATCAATTATCACTCTTCGCTCTTATAAGGCATATGTGCATTATAAGACTTTGTCCTTATT-----ATTTACTATGTATTAATTGA
AaVRE-Like1 -----TAAATATTTAAAGTGATTGGATATCAATTATAACCTTAAATCT-ATATCTGTTGTCGAATGAAATTT-----AACTGGTAGCTTTGG
AaVRE-Like2 -----GGTA--AATTTTCAACCTTAAATCTTATATCT--GTGTCGAATGCATAAAT-----AGCTTAGCTTTGG

AIVRE-Like_FLC1 -----TCACACAACCTTTGTATCTTGTG-TCFTTTGTCATAGAAATGTCAA
AIVRE-Like_FLC2 -----TCACACAACCTTTGTATCTTGTG-TCFTTTGTCATAGAAATGTCAA
AtVRE TCTTTTGTGTGAA-----AATCGACAATCACACAACCTTTGTATCTTGT--GTCFTTTGTCACACAACCTTTGTATCTTGTG-TCFTTTGTCATGAAATGTGCAT
ThVRE-Like -----ATCACACAAC-TTGTATCTTGTG-TCFTTTGTCATGAAATGTGCAT
CrVRE-Like CATTGTAGTGTGATCATTTGGAATCAACATGAATCCACAACCTTTGTAACCTTTGTTGTGTAA--ATCAACAATCATAAAGCTTTGTATCTTGGGGCTTTTGTGATGATTATTGTCAA
AaVRE-Like1 TCGTTGAAATGACA-----ATCACACAACCTTTGTGCTATTCCCCATCAACCAATCGATAATTACACTAC-CTTGTATCTTGGG--TTTTTTCATGAAATGGACAA
AaVRE-Like2 TCACATAAACCGACC-----ACCACAACCTTTGTATCTATTGCA-GTGAA--AATAGACAATTACGCAAC-CTTTTGTCTTGGG-TCFTTTGTCATGAAATGGACAA
* * * * * * * * * * * * * * * * * * * * * * * *
AIVRE-Like_FLC1 TAACACA--GCC-TTGTTCCTTTGGTGCCTCTAG-GAAATTGAAATCCCTCAAAA-----CTTGTCTTCATATAAGAAATA--
AIVRE-Like_FLC2 TAACACA--GCC-TTGTTCCTTTGGTGCCTCTAG-GAAATTGAAATCCCTCAAAA-----CTTGTCTTCATATAAGAAA--
AtVRE TCACACA--GCC-TTGTTCCTTTGGTGCCTCTAG-GAAATTGAAATCCCAACA-----CTTGTCTTCATGTAAGAAATA--
ThVRE-Like TAACATG--CCCTTGTATCTTTGGGCTCTAG-GAAATCGAGAACCCTACAGAAAAACTTTTGTCTTCATGGAAGAAATACC
CrVRE-Like TAACACTA--ACC-TTGTTCCTTGTGCTCTAATGAAATGAAAACCTTCAAAAA-----CTTGTCTTCATGTAAGAAATAC-
AaVRE-Like1 TAACACT--AAC---TTTCTTGTGCTCTTGG--GAAATCGACAATCCCAC-----CAAAAA
AaVRE-Like2 TAACACTGTACC---TTTCTTGTGCTCTTGG--GAAATCAATAATCCCAC-----CA-----
* * * * * * * * * * * * * * * * * *

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Supplementary Figure 2: First intron conservation among *FLC* orthologues and alignment of VRE-Like sequences

GATA alignment of *A. thaliana FLC* first intron to first intron of *A. lyrata FLC1* orthologue (**a**), *A. lyrata FLC2* orthologue (**b**), of *C. rubella FLC* orthologue (**c**) and of *T. halophila FLC* orthologue (**d**). GATA alignment of *A. thaliana* first intron to the region spanning exon 1a to exon 2 of *A. alpina PEP1* (**e**). Multiple alignment of the AtVRE, AIVRE-Like from *AIFLC1*, AIVRE-Like from *AIFLC2*, CrVRE-Like, ThVRE-Like, AaVRE-Like 1 and AaVRE-Like 2 nucleotide sequences (**f**).

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AlFLC1      GACAAAAAGGTTGATGA-----AACTTTGTACCTTATTCGTGAGAGAA-TTGCA 48
AlFLC2      GACAAAAAGGTTGATGA-----AACTTTGTACCTTATTCGTGAGAGAA-TTGCA 48
AtFLC       GACAAAAAGG-TTGATG-----AACTTTGTACCTTATTCGTGAGAGAA-TTGCA 46
PEP1        GACATAACTATTTATGG-----ATCTTCGTACCCTTGTTCGAGAGAAGTTGCA 49
CrFLC       GACAAAACATAACTACTGTACGATGAAACGTATTGTACCTATCCGAGGGAGAG-TTTTT 59
ThFLC       GACAAGAGTATGTAAC-----TTGTACCTTTTCGAGAGAGAA-TTCTT 44
          **** *                * ***** *      **** **

AlFLC1      TCGAGATCTTGCAGTATGTGTTCT-TCTCTTCTCT--CTAAAACCTGTGTTTGCTTCAC 105
AlFLC2      TCGAGATCTTGCAGTACGTGTTCT-TCTCTTCTCT--CTAAAACCTGTGTTTGCTTCAC 105
AtFLC       TCGAGATCTGAGTGTATGTGTTCT-TCACTTCTGT--CAAAAACCTGTGTTTGCTTCAC 103
PEP1        GCGAG--CTT--GTTTCATGTGTTCT-TGTGTTCTAT--CAAAAACCTGAGTTTGCTTCAC 102
CrFLC       GCA-----TCGACATGTTGTGTGTTCTTCTTCTCTCTTTGTGTTTCCCTTCAC 109
ThFLC       -CAGT-----TCTTTGTGTTCT-TCCGTTCCGC--CAAAAACCTGTGTTTGCTTCAG 92
          *                * **** * * ** *      *** **** *****

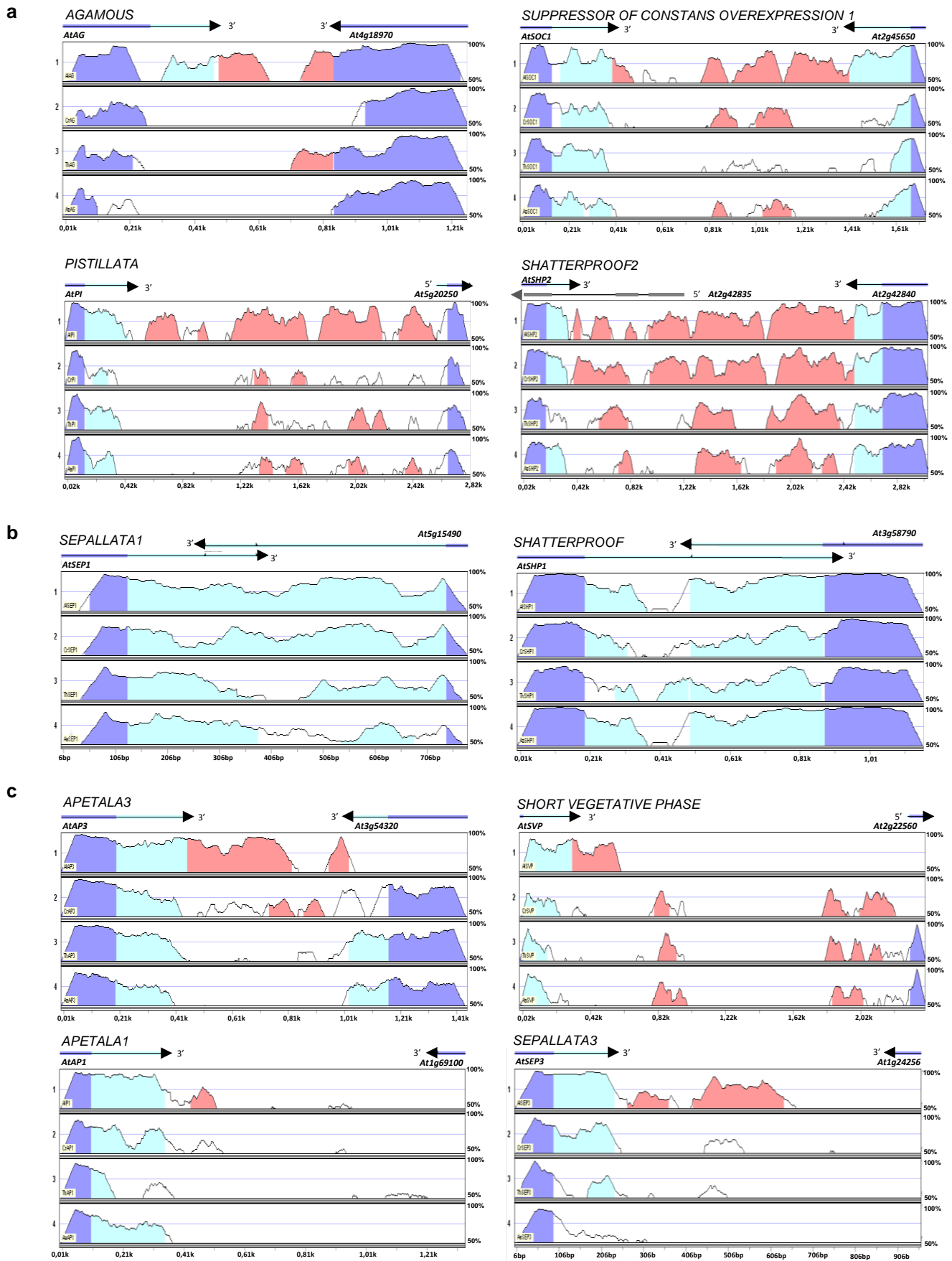
AlFLC1      TGTGAAGAAG--CCTACGGCCTATTTTGCAAAGAAAACGTGGC-----GCTCTCT-CAG 156
AlFLC2      TGTGAAGAAG--CCTACGGCCTATTTTGCAAAGAAAACGTG-----GCTCTCT-CAG 154
AtFLC       AGTGAAGAAG--CCTACGGCTTATTTTGCAACAGGGACGTGGCTCTCTCTCTCTCT-CTG 160
PEP1        GGTGAAGAAGCCCTACGGCTTATTTTGCAAACCGACGTG-----GCACACTTCTC 154
CrFLC       AGTGAAGAAG-CCTTCTGCTTATTTTGCAATGGAGGCGTG-----GCACAATCTG 160
ThFLC       GGTGAAGAAG-CCT-ACGGCTTATTTTGCAATCGAAAACGTG-----GC-CGTCTCTG 141
          ***** * * ** ***** ****          * * *

AlFLC1      TCGCTTTTTTTTCTCGTCGTAATTAAT-----AAACGCG-AATGGTTGGAAT 202
AlFLC2      TCGCTTTTTTTTCTCGTCGTAATTAATTTGTTTTTATCCTAAAAGCG-TATGGTTGGAAT 213
AtFLC       CGCGTTTTTTTCTCGTCGTAATTAATTTGTTTTTATCCTAACGCG-TATGGTTGGCAT 219
PEP1        TGCTGGCTTCTCTCGTAATTAATAATTGGGTTTTGTTTTTATTTAG-AACG--CGTTTT 211
CrFLC       CTATTT-ATGTCCTCGTG-AGTTAGTTTGTTTTATCTTAAAACGCGTGTGGTCTGCAT 218
ThFLC       CTTTTTCTGATATTTTG-TCTCAG-----TTTTAATTTAAAACGCG-TATGGCTGTGCAT 194
          * * *                * * * * *

AlFLC1      GG--GTTTATTTGGGCCATGTCGGTCACCT-- 231
AlFLC2      GG--GTTTCTTGGGCC----- 228
AtFLC       GG--GTTTTTGGGCCTATGTCGGTCACATT-- 248
PEP1        GGTGTGTGCATGGGTTTCATGTCGGTCAGCT-- 242
CrFLC       GGGTTTCTCTTGGGCCCATGTCGGTCATTTTT 251
ThFLC       GGGTTTCTAT-GGGCCCTCGTCGGTCACCTG-- 224
          ** * * ***

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Supplementary Figure 3: Conserved block at the 3' end of *FLC* homologues
Multiple alignments of the nucleotide sequences present in the conserved regions at the 3' ends of *FLC* orthologues and shown in Fig. 1c. Stars indicate 100% conserved bases.

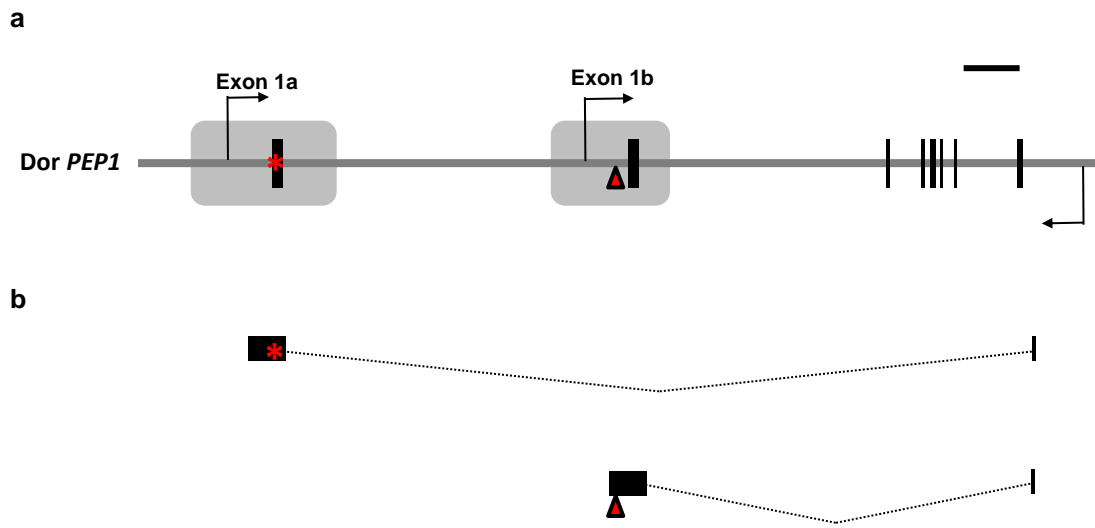


Supplementary Figure 4

Supplementary Figure 4: Alignment of 3' intergenic region of 10 randomly chosen MADS box genes

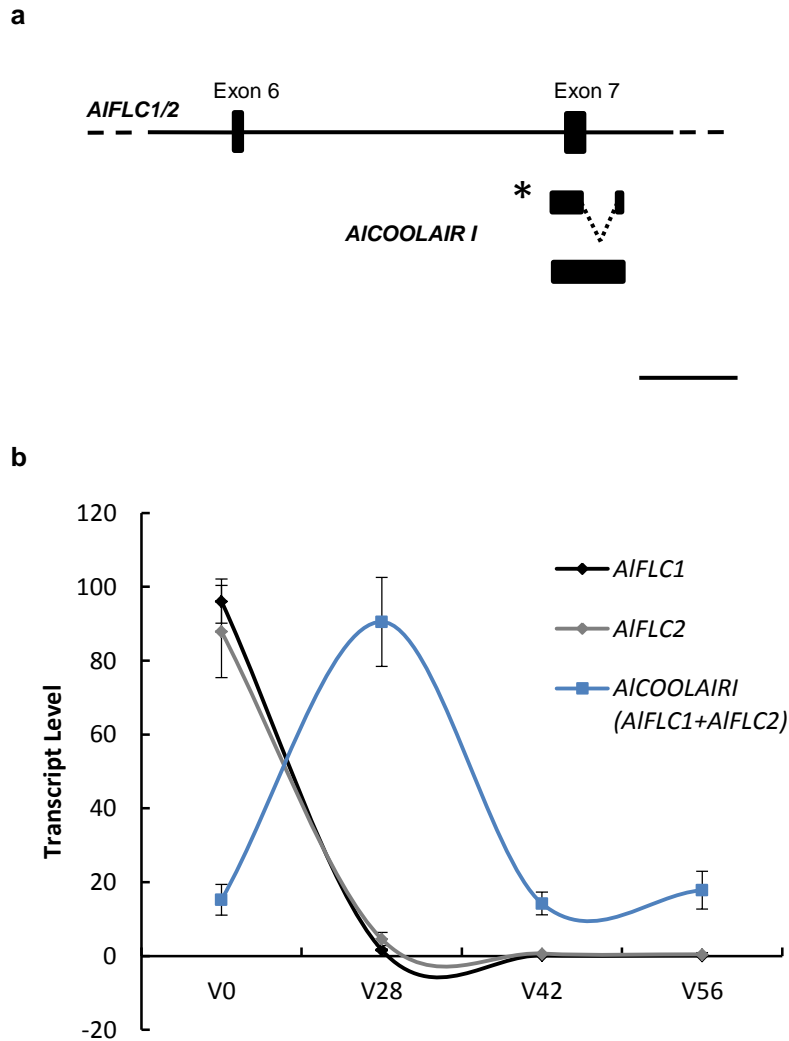
3' Intergenic region (spanning the last exon of the MADS box gene on the left to the first exon of the downstream gene on the right) from from *A. lyrata* (1), *C. rubella* (2), *T. halophila* (3) and *A. alpina* (4) MADS box genes have been aligned to their *A. thaliana* homologue (based line) using mVista pairwise alignment tool. **(a)** Genes with conserved synteny with *A. thaliana*. For *SHP2*, the antisense non coding gene exons are represented by grey boxes, **(b)** Genes with conserved synteny with *A. thaliana* and overlapping untranslated regions, **(c)** Genes for which the synteny with *A. thaliana* is not conserved in one, two or all species aligned.

Gene names and transcript models are presented on top of the alignments. The arrow points towards the 3' end of the gene. Colored areas illustrate stretches of homology greater than 75% identity at the nucleotide level. Pink, regions of homology; dark blue, exonic sequences; light blue, untranslated region.



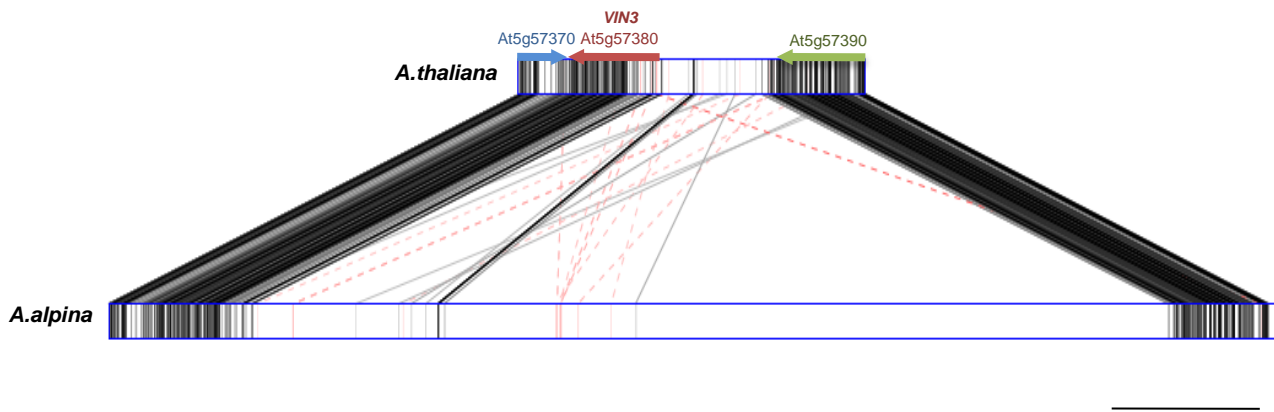
Supplementary Figure 5: Class II antisense transcripts at the Dor*PEP1*

(a) Schematic representation of *PEP1* locus in the Dor accession. Black boxes are exons, lines introns and non-coding regions. Grey boxes indicate the region tandemly duplicated. The red star marks a G to A substitution and the red triangle a 248 bp insertion as compared to the Pajares sequence in exon 1a and the 5'UTR of exon 1b respectively. Arrows indicate transcriptional start sites at *PEP1*. **(b)** Class II antisense transcripts obtained by cloning and sequencing of qRT-PCR products in non-vernalized 21 days old Dor leaf samples. Scale bar, 1kb.



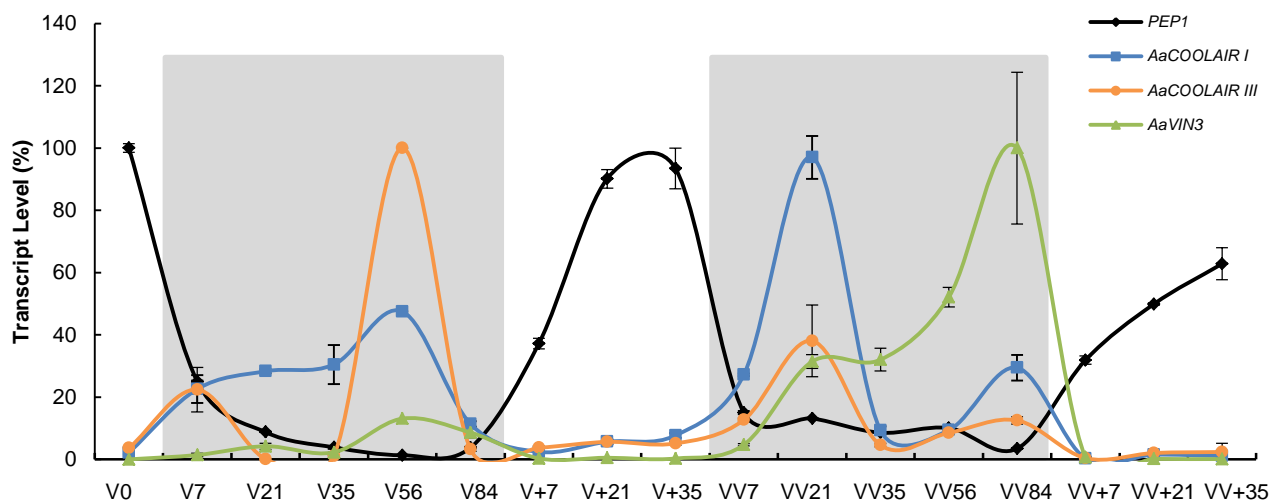
Supplementary Figure 6: Class I antisense transcript at *A. lyrata* *FLC* genes

(a) Black boxes are exons; lines are introns and other non-coding regions. A star indicates the most abundant form among splicing variants obtained by qRT-PCR product sequencing. Scale bar, 0.5 kb. **(b)** *AICOOLAIR I* (*AIFLC1*+*AIFLC2*), *AIFLC1* and *AIFLC2* qRT-PCR (see Supplementary Table 1 for primers) on leaf RNA of *A. lyrata* plants exposed to 0, 28, 42 and 56 days of vernalization (V0, V28, V42, and V56). Transcript levels were expressed relative to those of the reference gene *AIRAN3* \pm SD (n=3 to 5 individuals) and plotted as a % of their maximal expression.

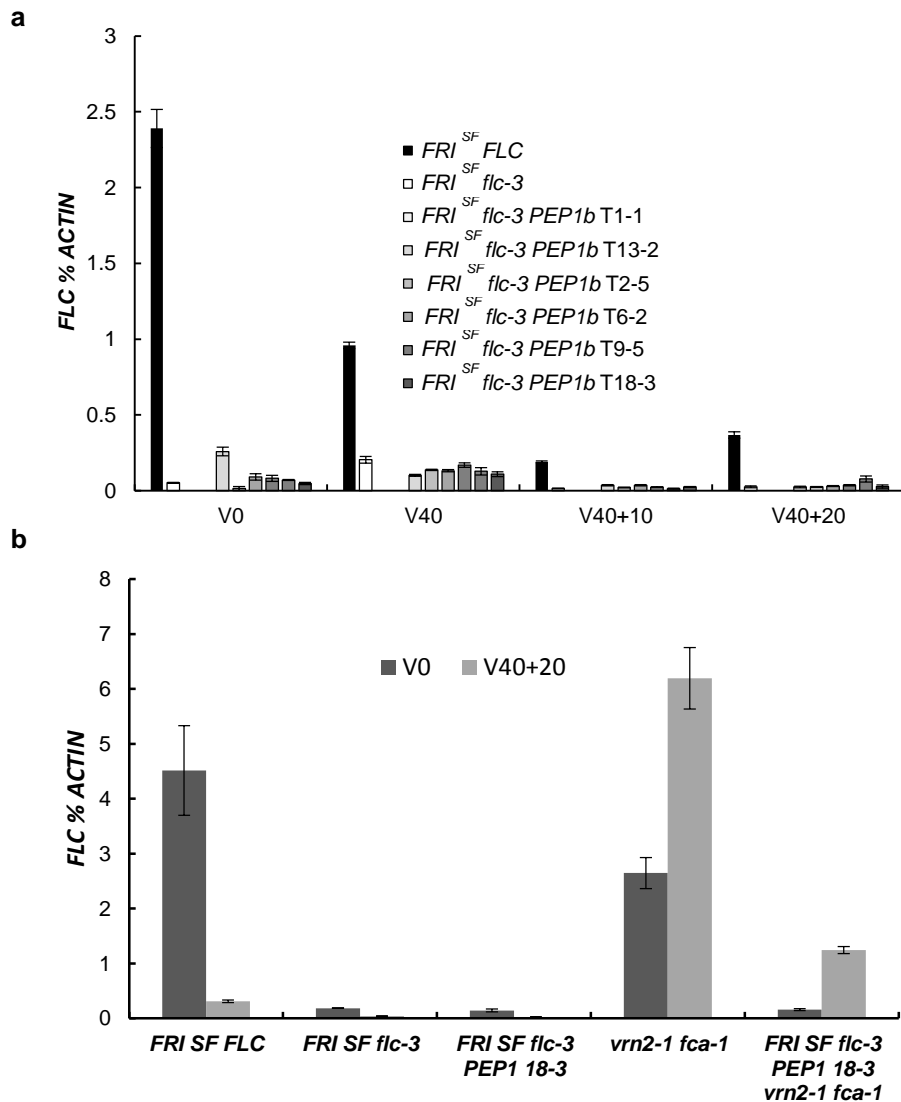


Supplementary Figure 7: Conservation of synteny between *A. thaliana* and *A. alpina* genomic regions carrying *VIN3* orthologues

GATA plot showing the conservation of synteny between the *A. thaliana* chromosome V region containing *VIN3* (At5g57380) and its two neighboring genes (At5g57370 and At5g57390) and the corresponding region in *A. alpina*. Scale bar, 5kb.



Supplementary Figure 8: Seasonal expression patterns of *PEP1*, *AaCOOLAIR I*, *AaCOOLAIR III* and *AaVIN3* expression over two successive vernalization treatments
A. alpina plants were vernalized for 0, 7, 21, 35, 56 and 84 days (V0, V7, V21, V35, V56, V84) followed by 7, 21 and 35 days of growth in normal temperature (V+7, V+21, V+35) then vernalized again for 7, 21, 35, 56 and 84 days (VV7, VV21, VV35, VV56, VV84) and grown for 7, 21 and 35 more days in normal growth temperatures (VV+7, VV+21, VV+35). Grey areas indicate the cold treatments. Transcript levels were measured by qRT-PCR relative to those of the reference gene *RAN3* \pm SD (n=3 technical replicates). The maximum level of expression of each gene is set at 100%.



Supplementary Figure 9: FLC expression in $FRI^{SF} flc3 PEP1$ transformants and $FRI^{SF} flc3 PEP1 18-3 vrn2-1 fca-1$ line

(a) FLC mRNA levels in *A. thaliana* $FRI^{SF} flc-3 PEP1b$ independent transformants as compared to those of the parental line $FRI^{SF} flc-3$ and the control line $FRI^{SF} FLC$ before vernalization (V0), after 40 days of vernalization (V40), and after 40 days of vernalization followed by 10 or 20 days of growth at normal growth temperatures (V40+10, V40+20).

(b) FLC mRNA levels in $vrn2-1 fca-1$ and $FRI^{SF} flc-3 PEP1b vrn2-1 fca-1$ as compared to those in $FRI^{SF} flc-3 PEP1b 18-3$ parental line, $FRI^{SF} flc-3$ and $FRI^{SF} FLC$ the control lines before vernalization (V0) and after 40 days of vernalization followed by 20 days of growth at normal growth temperatures (V40+20).

Transcript levels were measured by qRT-PCR relative to those of the reference gene $ACTIN \pm SD$ (n=3 technical replicates).

PEP1 cloning		
	forward	reverse
<i>EcoRI-Flank1-Sall</i>	GGGGTGTGGGAATTCTGGATTCTTGTAAATAATTTGTTTTTCAC	GGAAGAGGGTCGACCGTAGTATAAGAACACCAAGAAATCAAG
<i>Sall-Flank2-BamHI</i>	GGGGTGTGGTCGACGCGGCCGCAATAATTGAGTTCAAGTGATTTCCAAC	GGGAAGAGGGGATCCAAGCATTTTAACTTAACTACGATACG
PEP1 genotyping		
	forward	reverse
<i>EcoRI-Flank1-Sall</i>	GGGGTGTGGGAATTCTGGATTCTTGTAAATAATTTGTTTTTCAC	GGAAGAGGGTCGACCGTAGTATAAGAACACCAAGAAATCAAG
<i>Set1</i>	CTGTCTTGCCGGAGTATATTG	GAAGAGATGATGAAACAATAGC
<i>Set2</i>	GGGCCTTACAGTCAAAACAAG	AAGGACATTCTCCCAAGG
<i>Set3</i>	TGAGTACATGCATGGTTTG	CTTTGACTGAAGATCCTGTCC
<i>Sall-Flank2-BamHI</i>	GGGGTGTGGTCGACGCGGCCGCAATAATTGAGTTCAAGTGATTTCCAAC	GGGAAGAGGGGATCCAAGCATTTTAACTTAACTACGATACG
3'RACE PCR		
	forward	reverse
<i>AaCOOLAIR</i>	ACCGTGAAGCAAACACTCAAGT	UAP
<i>AaCOOLAIR nested</i>	GATAGAACACAAGAACACAT	AUAP
<i>AaCOOLAIR II 3''tiling'' PCR</i>		
	forward	reverse
<i>Set1</i>	ACCGTGAAGCAAACACTCAAGT	TCCTCCGGTGATAAGTATGA
<i>Set2</i>	ACCGTGAAGCAAACACTCAAGT	AACGCTTAGTATCTCAGGCGAC
<i>Set3</i>	ACCGTGAAGCAAACACTCAAGT	AAGAGGGTAATTATGGAATCTT
Q-PCR		
	forward	reverse
<i>VIN3</i>	AGAAGCTGTGTTCTCAGGCAATGG	TCTTCGCTTCGACTTTCGACAAA
<i>FLC</i>	TGATAAGGGCGAGCGTTT	CACGAATAAGGTACAAGTTC
<i>ACTIN</i>	GGTAACATTGTGCTCAGTGGTGG	AACGACCTTAATCTTCATGCTGC
<i>AtCOOLAIR I</i>	CTCGATGCAATTCTCACACG	TCCTTGGATAGAAGACAAAAAGAGA
<i>AtCOOLAIR II</i>	CTCGATGCAATTCTCACACG	TTCTCCTCCGGCGATAAGTA
<i>AaVIN3</i>	AGGACTTGAACCCGAGACC	GGGCAAAGATGGATTACTGC
<i>PEP1</i>	CTTGTCTCTCTCTCTCTGG	ACTACGGCGAGAGCAGTTTC
<i>AaRAN3</i>	CACAGGAAAAACCACATTCTGT	CCATCCCTAAGACCACCAAAT
<i>AaCOOLAIR I</i>	ACCGTGAAGCAAACACTCAAGT	CAGAAGCCAAAACAAGAAAGTG
<i>AaCOOLAIR II</i>	ACCGTGAAGCAAACACTCAAGT	TCCTCCGGTGATAAGTATGA
<i>AaCOOLAIR III</i>	ACCGTGAAGCAAACACTCAAGT	GAAGAATGTCTTTCAAGCTG
<i>AIFLC1</i>	GAGTGCCGAAACTCTTCTTCAACTA	GCCAAAACCTGGTCTCTCTCT
<i>AIFLC2</i>	GGGAAACAACATGCTGATGA	CAATTGAACAAGAGTATCGACACTC
<i>AIRAN3</i>	CACAGGGAAAAACACATTTGT	CCATCCCTCAGACCACAAA
<i>AICOOLAIR I</i>	CTCGATGCAATTCTCTCACG	TGATGGTATGAAAAAGATATTCC

Supplementary Table 1: Primer list

VRE-Like1 5'RACE PCRs		
	forward	reverse
PCR1	AAP	TGTCATTTCCAACGACCAAA
PCR2	AAP	GATTTCCAAGAGGCACCAAA
PCR3	AAP	GACCAGCATGGCCAAACTAC
PCR4	AAP	TCCAAGGTTGGACCTAGCAT
PCR5	AAP	GAGTATATAGAAGGAAACGACA
PCR6	AAP	TGGAAACGCAACTGAAAACA
VRE-Like1 5'RACE nested PCRs		
	forward	reverse
nested PCR1	AUAP	TACAATTCTCCCGGATTTGC
nested PCR2	AUAP	TGTCATTTCCAACGACCAAA
nested PCR3	AUAP	GATTTCCAAGAGGCACCAAA
VRE-Like1 5'RACE PCRs combinations		
	PCR	nested PCRs
PCR followed by nested PCR	1	1, 2
PCR followed by nested PCR	2	2,3
PCR followed by nested PCR	3	1,2,3
PCR followed by nested PCR	4	2
PCR followed by nested PCR	5	2
PCR followed by nested PCR	6	2
VRE-Like2 5'RACE PCRs		
	forward	reverse
PCR1	AAP	TTTTAGTGACCAAAGC
PCR2	AAP	GATTTCCAAGAGGCACCAAA
PCR3	AAP	ACTACTGGTTAATGTT
PCR4	AAP	TTGCACCAAATCAAGGATG
VRE-Like2 5'RACE nested PCRs		
	forward	reverse
nested PCR1	AUAP	AAGGACATTCTCCCAAGG
nested PCR2	AUAP	GATTTCCAAGAGGCACCAAA
VRE-Like2 5'RACE PCRs combinations		
	PCR	nested PCRs
PCR followed by nested PCR	1	1
PCR followed by nested PCR	2	1, 2
PCR followed by nested PCR	3	1, 2
PCR followed by nested PCR	4	1, 2

Supplementary Table 2: *Arabis alpina* 5'RACE PCR primers and PCR combinations

Genotype	<i>AtFRI^{SF} flc3</i>	<i>AtFRI^{SF} FLC</i>	<i>AtFRI^{SF} flc3</i> <i>PEP1b 1-1</i>	<i>AtFRI^{SF} flc3</i> <i>PEP1b 2-5</i>	<i>AtFRI^{SF} flc3</i> <i>PEP1b 9-5</i>	<i>AtFRI^{SF} flc3</i> <i>PEP1b 6-2</i>	<i>AtFRI^{SF} flc3</i> <i>PEP1b 13-2</i>	<i>AtFRI^{SF} flc3</i> <i>PEP1b 18-3</i>
Rosette Leaf Number of individuals	8	51	9	9	8	8	9	9
	8	50	9	9	9	7	9	10
	9	55	9	9	9	9	9	9
	8	57	9	9	9	9	9	9
	9	43	9	10	9	9	9	9
	8	56	8	9	10	9	9	9
	8	50	9	9	9	8	9	8
	9	50	9	9	9	9	8	9
	8	45	8	9	8	9	10	9
	7	40	9	9	9	9		9
	7	44	8	9	9	9		9
	8	40		10	9	9		9
	8	55			9	9		9
	7	50			9	9		
	7	40			9	9		
	8	43			9	9		
	7	47			8	9		
	8	40				9		
	8							
	9							
8								
8								
8								
Mean	7.96	47.56	8.73	9.17	8.88	8.78	9.00	9.00
SD	0.64	5.92	0.47	0.39	0.49	0.55	0.50	0.41
p value (Student T-test vs <i>AtFRI^{SF} flc3</i>)		1.418E-29	1.172E-03	9.951E-07	1.316E-05	9.627E-05	1.289E-04	7.058E-06

Supplementary Table 3: Rosette leaves number of *AtFRI^{SF} flc3 PEP1* transformants at bolting and statistical analysis

Mean value and standard deviation per genotype are represented in Fig. 3b. A star indicate genotypes with a p value <0,005.

Supplementary Methods

***Arabidopsis lyrata* growth conditions, RNA extraction and qRT-PCR**

A. lyrata plants from the North American Mayodan population ¹ were grown (after three days of stratification at 4°C) for 35 days at 20°C in daylength of 14 hours (14h light/10h dark) and vernalized at 4°C up to 56 days in short days (8h light/16h dark). Total RNA from leaf samples was isolated with a phenol-based 96-well extraction method ² from three to five individuals and subsequently digested with DNase I Amplification grade (Invitrogen) according to the manufacturer's protocol. Reverse transcription was performed with oligo (dT) primers on 1µg of total RNA using SuperScript II Reverse Transcriptase (Invitrogen) according to the manufacturer's protocols. qPCRs for *AIFLC1* and *AICOOAIR I* were performed on the Roche Light Cycler 480II instrument using the primer sets listed in Supplementary Table 1 and the IQ SYBR Green Supermix (BIO-RAD). Transcripts levels were measured relatively to the references genes *AIRAN3*.

***VIN3* synteny analysis**

A. thaliana chromosome 5 region spanning At5g57370 and At5g57390 was aligned to part of *A. alpina* genome containing *AaVIN3* (GenBank KC162887) using GATAligner and plotter in order to check synteny conservation.

Analysis of sequences of *FLC* orthologues

Multiple sequence alignments were performed with T-coffee using the default settings ³.

5'RACE PCRs and 5' RACE "tiling"PCRs

5'RACE PCRs were carried out with the 5' RACE System for Rapid Amplification of cDNA Ends Version 2 (Invitrogen, catalog # 18374-058) on *A. alpina* and *A. thaliana FRI^{SF} FLC* samples collected during the vernalization time courses described in Fig. 2b and Fig. 4b

respectively. cDNA were obtained by reverse transcription of 5µg of total RNAs with random primers and were subsequently tailed according to the manufacturer protocols. Real time PCRs were performed with the IQ SYBR Green Supermix (BIO-RAD) in the Roche Light Cycler 480II instrument. To amplify COLDAIR from Arabidopsis *FRI^{SF} FLC* 5'RACE samples, the Abridge Anchor Primer (AAP) provided with the 5'RACE kit was used together with AtCOLDAIR specific reverse primer as described by Heo *et al.*⁴. *PEP1* “tiling” PCRs were performed on 5'RACE samples with a series of successive reverse primers located downstream of the VRE-like 1 and VRE-like 2 sequences and coupled to the AAP (see Supplementary Table 2). Each PCR was used as a template for nested PCRs to improve detection (see Supplementary Table 2). PCR amplification products, when detected, were cloned and sequenced to test their validity.

Supplementary References

- 1 Leinonen, P H, Remington, D L, Leppälä J & Savolainen O. Genetic basis of local adaptation and flowering time variation in Arabidopsis lyrata. *Mol Ecol.* **22**, 709-723, doi: DOI 10.1111/j.1365-294X.2012.05678.x (2013).
- 2 Box M S, Coustham V, Dean C & Mylne J S. Protocol: A simple phenol-based method for 96-well extraction of high quality RNA from Arabidopsis. *Plant Methods.* **13**; 7:7. doi: DOI 10.1186/1746-4811-7-7 (2011).
- 3 Notredame, C., Higgins, D. G. & Heringa, J. T-Coffee: A novel method for fast and accurate multiple sequence alignment. *J Mol Biol* **302**, 205-217, doi:DOI 10.1006/jmbi.2000.4042 (2000).
- 4 Heo, J. B. & Sung, S. Vernalization-Mediated Epigenetic Silencing by a Long Intronic Noncoding RNA. *Science* **331**, 76-79, doi:DOI 10.1126/science.1197349 (2011).