

Cell Systems, Volume 7

Supplemental Information

**Temperature Sensing Is Distributed
throughout the Regulatory Network that Controls
FLC Epigenetic Silencing in Vernalization**

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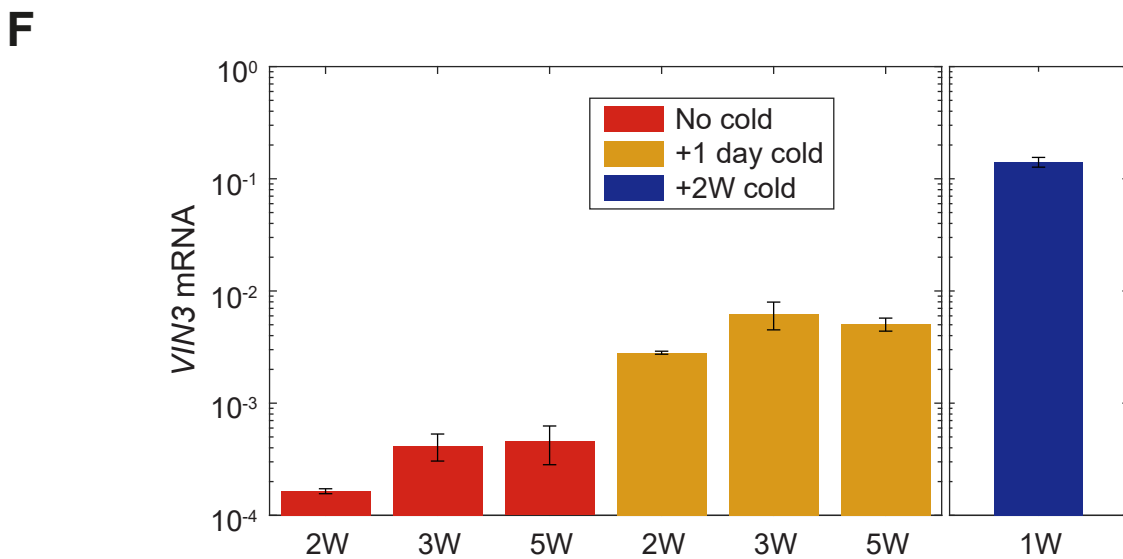
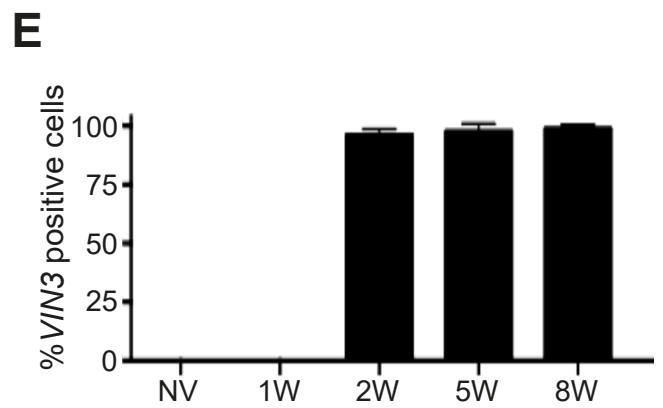
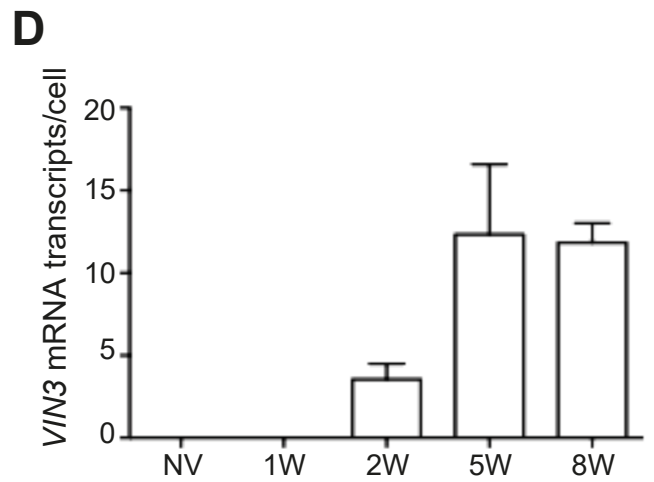
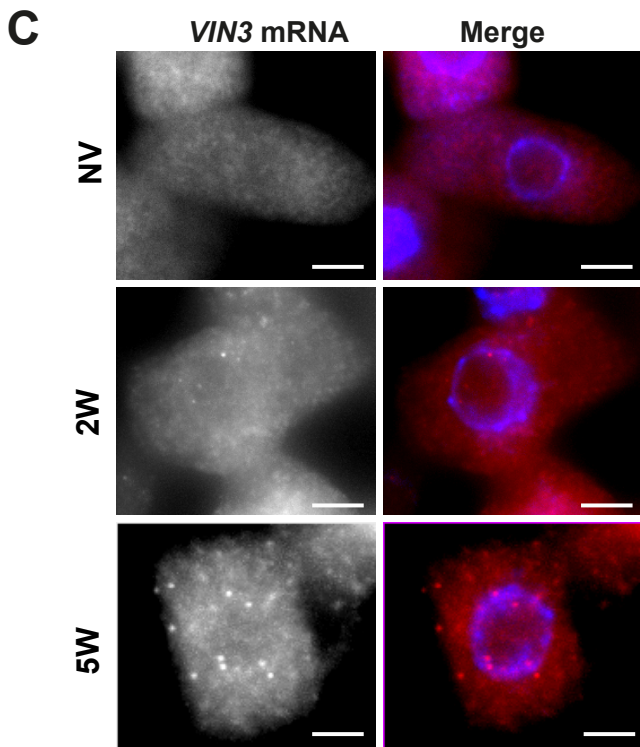
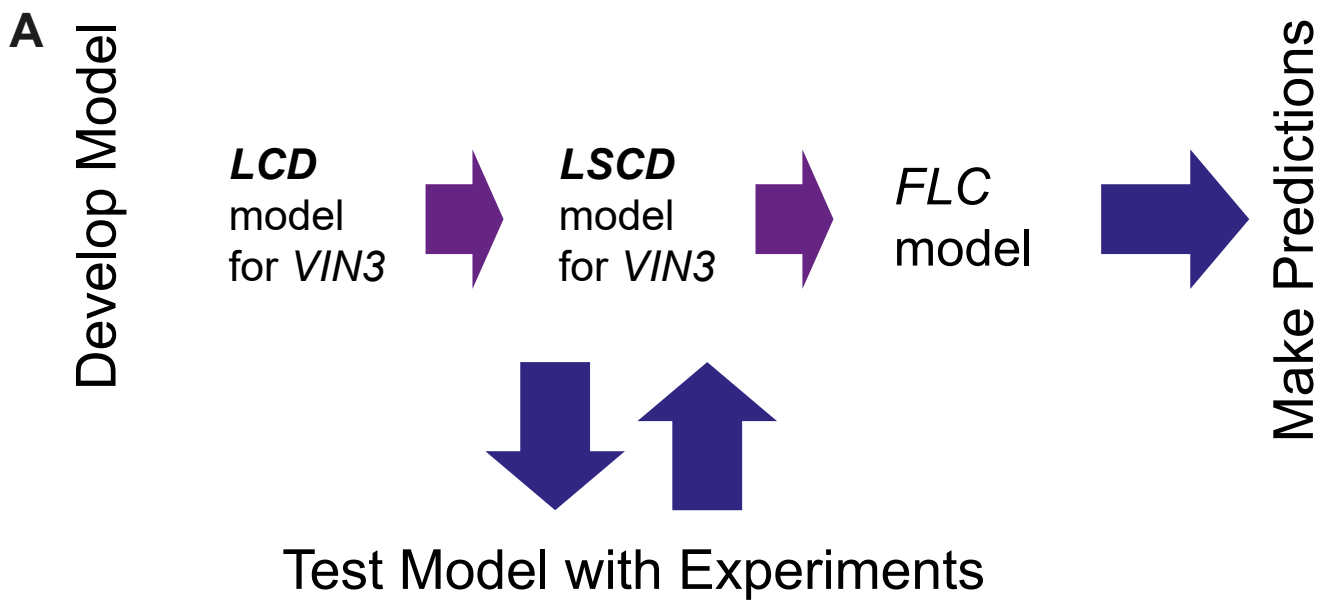


Figure S1. Long-term registers time in the cold using an analogue mechanism, related to Figure 3

A, Flow diagram of the work presented in this paper. **B**, Schematic showing smFISH probe design for *VIN3* mRNA detection. **C**, Representative images showing cellular distribution of *VIN3* mRNA (red) before cold (NV), and after 2 and 5 weeks of 5°C exposure. DNA labelled with DAPI (blue). Scale bars = 10 μ m. **D**, *VIN3* mRNA per cell data was determined by analysis of smFISH images. “NV” data was obtained from non-vernalized plants grown at 22°C, prior to any cold exposure. 1W, 2W, 5W and 8W refers to 1, 2, 5 and 8 weeks of cold treatment at 5°C. **E**, The percentage of *VIN3* positive cells was calculated by scoring each cell for the presence or absence of *VIN3* smFISH probe signal. For all time points, >70 cells were analysed. **F**, *VIN3* mRNA levels were measured using QPCR, from plants grown for different durations of time in warm conditions (20-22°C) and then in some cases transferred to cold (8°C). On the x-axis, 1W, 2W, 3W, 5W, indicates number of weeks in warm conditions. Colour of the bar corresponds to subsequent cold treatment. Red bar: “No cold” refers to plants without cold transfer; yellow bar: one day of cold; blue bar: shown as a reference for a short vernalizing treatment (2 weeks, data from Hepworth et al. (2018)). All samples collected at 3pm. RNA levels relative to *UBC*, *PP2A*. n=2-3, average >2.6. Error bars show standard error.

A**Unspliced *VIN3* concentration (v):**

$$\frac{dv}{dt} = p_v(L, C, D) - s_v v$$

Spliced *VIN3* concentration (V):

$$\frac{dV}{dt} = s_v v - d_V V$$

where $p_v(L, C, D) = LCD$ is the productive transcription, s_v is the splicing rate and d_V is the degradation rate of *VIN3*.

Long-term (L)

$$\frac{dL}{dt} = \begin{cases} 1 - d_L L, & T < 17^\circ C \\ -d_L L, & T \geq 17^\circ C \end{cases}$$

Diurnal regulation (D)

$$D(t) = \left[p_D + \sin \left(2\pi \left(t - \frac{t_m - 1}{24} \right) \right) \right]^2$$

where t_m is the time at dawn.

Current temperature (C)

$$C(T) = \begin{cases} p_{C1}, & T \leq 10^\circ C \\ c(T), & 10^\circ C < T < 17^\circ C, \\ p_{C1} - p_{C2}, & T \geq 17^\circ C \end{cases}$$

where

$$c(T) = p_{C1} - \frac{T - 10}{17 - 10} p_{C2}$$

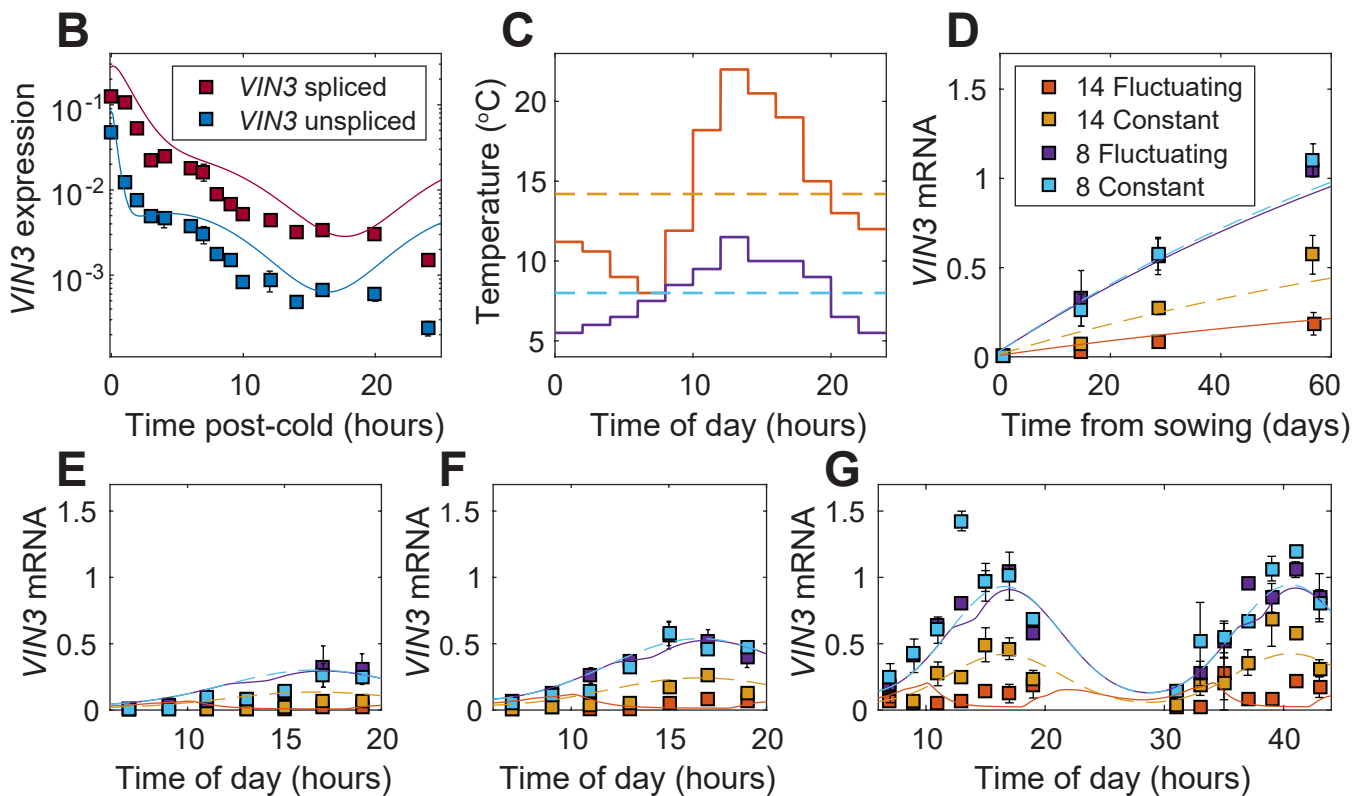


Figure S2. *LCD* model of *VIN3* expression fit to literature data, related to Figure 3

A, Equations for *LCD* model components. The parameter values used are $d_V = 18 \text{ day}^{-1}$, $s_v = 4.4d_V$, $d_L = 0.009 \text{ day}^{-1}$, $p_{C1} = 0.0416$, $p_{C2} = 0.0400$, $p_D = 2.05$. **B-G**, The *LCD* model of *VIN3* expression fit to data from the literature (Hepworth et al., 2018). Squares with error bars (standard error) represent experimental data, lines show the model fit. **B**, *VIN3* expression hours after plants are returned to the warm ($22^\circ C$) following 4 weeks at $5^\circ C$. **C**, Vernalization treatments that averaged $14.2^\circ C$ (orange and yellow) or $8^\circ C$ (purple and light blue), with either constant (dashed lines) or daily fluctuating (solid lines) patterns. **D-G**, *VIN3* mRNA expression from plants given the treatments of **C**. **D**, Maximum daily *VIN3* expression from plots **E-G** shown over the timecourse of weeks in the cold. For values at 8 weeks, measurements of two consecutive days (plot **G**) were first combined by averaging measurements from both days for each time of day, before selecting the maximum average. **E**, *VIN3* mRNA over a single day after 2 weeks vernalization. **F**, *VIN3* mRNA over a single day after 4 weeks vernalization. **G**, *VIN3* mRNA over two consecutive days after 8 weeks vernalization.

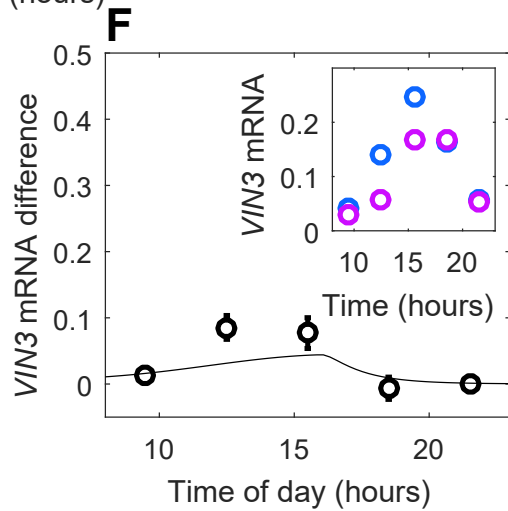
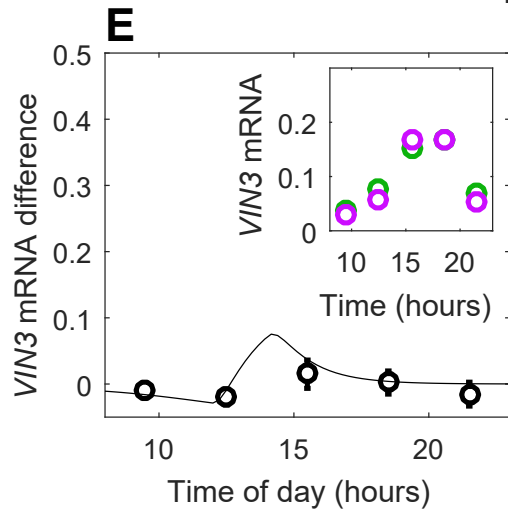
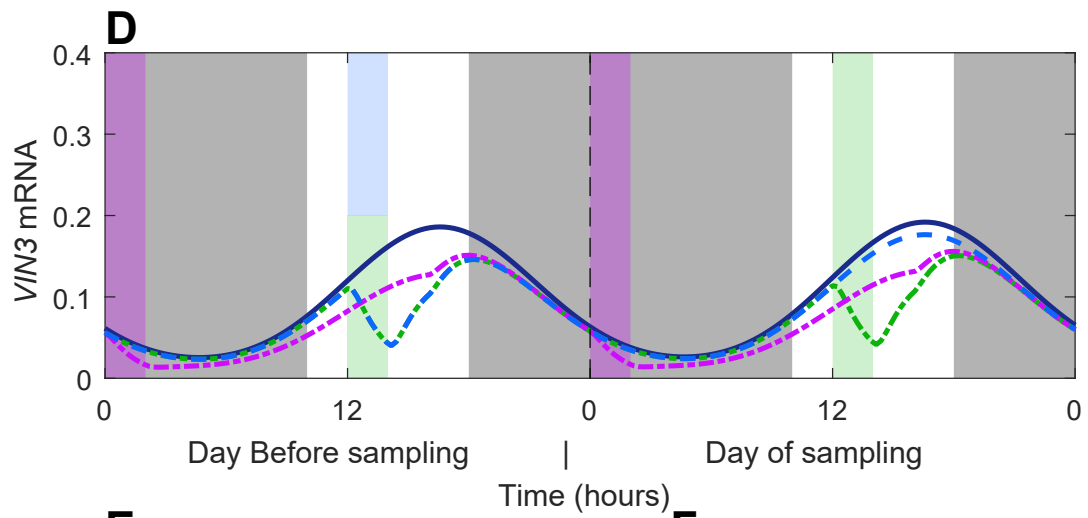
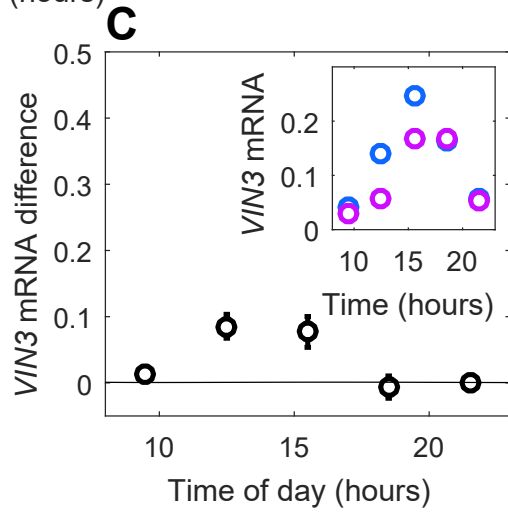
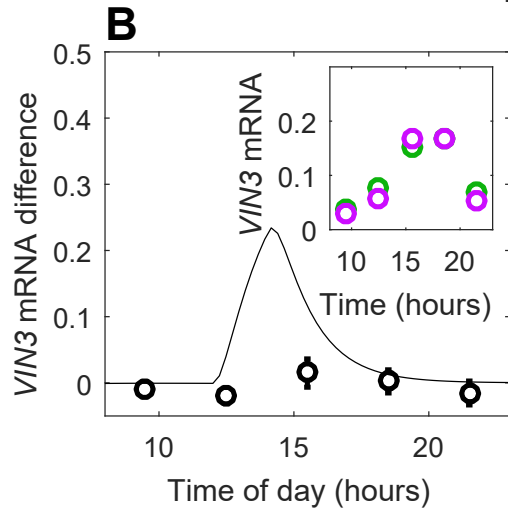
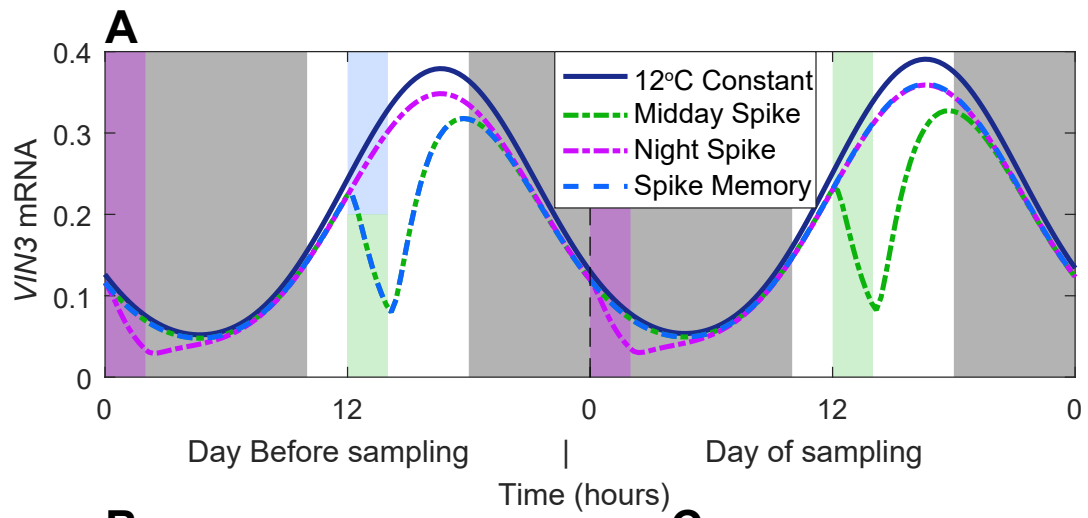


Figure S3. LCD model cannot fit the spike experiment data, related to Figures 2 and 3

A, The *LCD* model predictions for the spike conditions of **Fig. 2A** on the day before sampling and the day of sampling. **B**, Comparison of difference in *VIN3* mRNA level between “Night Spike” and “Midday Spike” (data from **Fig. 2B**). The model (solid line) predicts higher *VIN3* mRNA levels for the “Night Spike” compared to the “Midday Spike” treatment between approximately 12:00 and 16:00, whereas the experimental data shows no difference (empty circles, always close to 0). Inset of **B**: Experimental *VIN3* mRNA level for “Night Spike” (pink) and “Midday Spike” (green) treatments (data from **Fig. 2B**). **C**, Comparison of difference in *VIN3* mRNA level between “Spike Memory” and “Night Spike” (data from **Fig. 2B**). The model (solid line) predicts equal *VIN3* mRNA levels for the “Night Spike” and “Spike Memory” treatments on the day of sampling after 08:00, while the experimental data (empty circles) shows that the “Spike Memory” treatment gives mRNA levels higher than the “Night Spike”. Inset of **C**: Experimental *VIN3* mRNA level for “Night Spike” (pink) and “Spike Memory” (blue) treatments (data from **Fig. 2B**). **D**, The *LSCD* model predictions for the spike conditions of **Fig. 2A** on the day before sampling and the day of sampling. **E-F**, Comparison of difference in *VIN3* mRNA level between “Night Spike” and “Midday Spike” and between “Spike Memory” and “Night Spike”, respectively (data from **Fig. 2B**). In this case much better agreement is seen between the data and the model predictions. In all cases the green, blue and pink backgrounds indicate the times of the high temperature spike in the “Midday Spike”, “Spike Memory” and “Night Spike” conditions, respectively. Dark background indicates night-time. Circle and bars show mean and standard error, respectively, where the standard error of the difference is estimated by the sum of the standard errors. RNA levels normalised to *UBC*, *PP2A*.

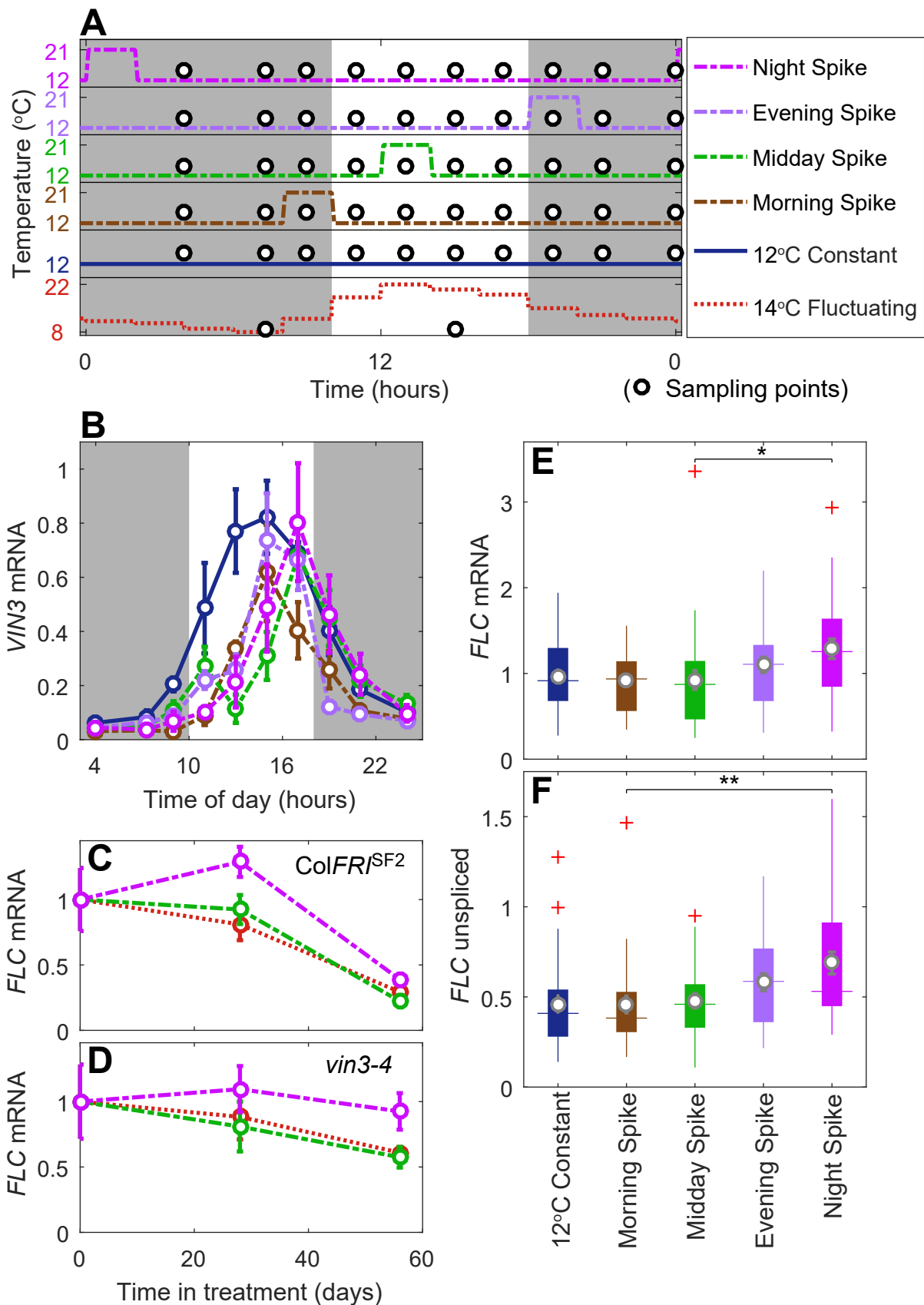


Figure S4. Spikes at particular times affect *VIN3* and *FLC* expression differently, related to Figure 2

A, Various temperature conditions given to plants following a pregrowth period in 20°C night, 22°C day 16hr photoperiod for one week. Dark background indicates night-time (8hr photoperiod). **B**, *VIN3* mRNA in *ColFRI^{SF2}*, after 4 weeks of 'cold' conditions as in **A**, sampled throughout the day as shown, n=3. **C-D**, Timeseries of *FLC* expression, normalised to non-vernalized (NV) levels, in *ColFRI^{SF2}* and *vin3-4*, respectively, under the Midday and Night Spike treatments, compared to the 14°C Fluctuating treatment, which is based on a natural autumn day in Norwich. Data points shown are averaged over all the sampling timepoints of one day. Full list of sampling timepoints and replicates are shown in **Supplementary Table 1**. n=6-29, average=11. **E-F**, *FLC* spliced and unspliced after 4 weeks cold, averaged over all the sampling

timepoints of one day in *ColFRI*^{SF2}. Box plot shows median and 25th and 75th percentiles of the samples. Ends of whiskers show maximum and minimum values excluding outliers, where an outlier (red +) is a value more than 1.5 times the interquartile range away from the top or bottom of the box. Kruskal-Wallis test with Dunn's post-hoc test between the Spike treatments (Morning, Midday, Evening and Night Spike, all with similar *VIN3* levels to allow testing for the *VIN3*-independent effect only) gives: $p < 0.05$ significant difference between Midday and Night Spike *FLC* mRNA (* in plot); $p < 0.01$ between Morning and Night Spike *FLC* unspliced (** in plot) (no other combinations were significant). In all cases, circle and bars show mean and standard error, respectively. $n=28-30$, average >29 . RNA levels normalised to *UBC*, *PP2A*.

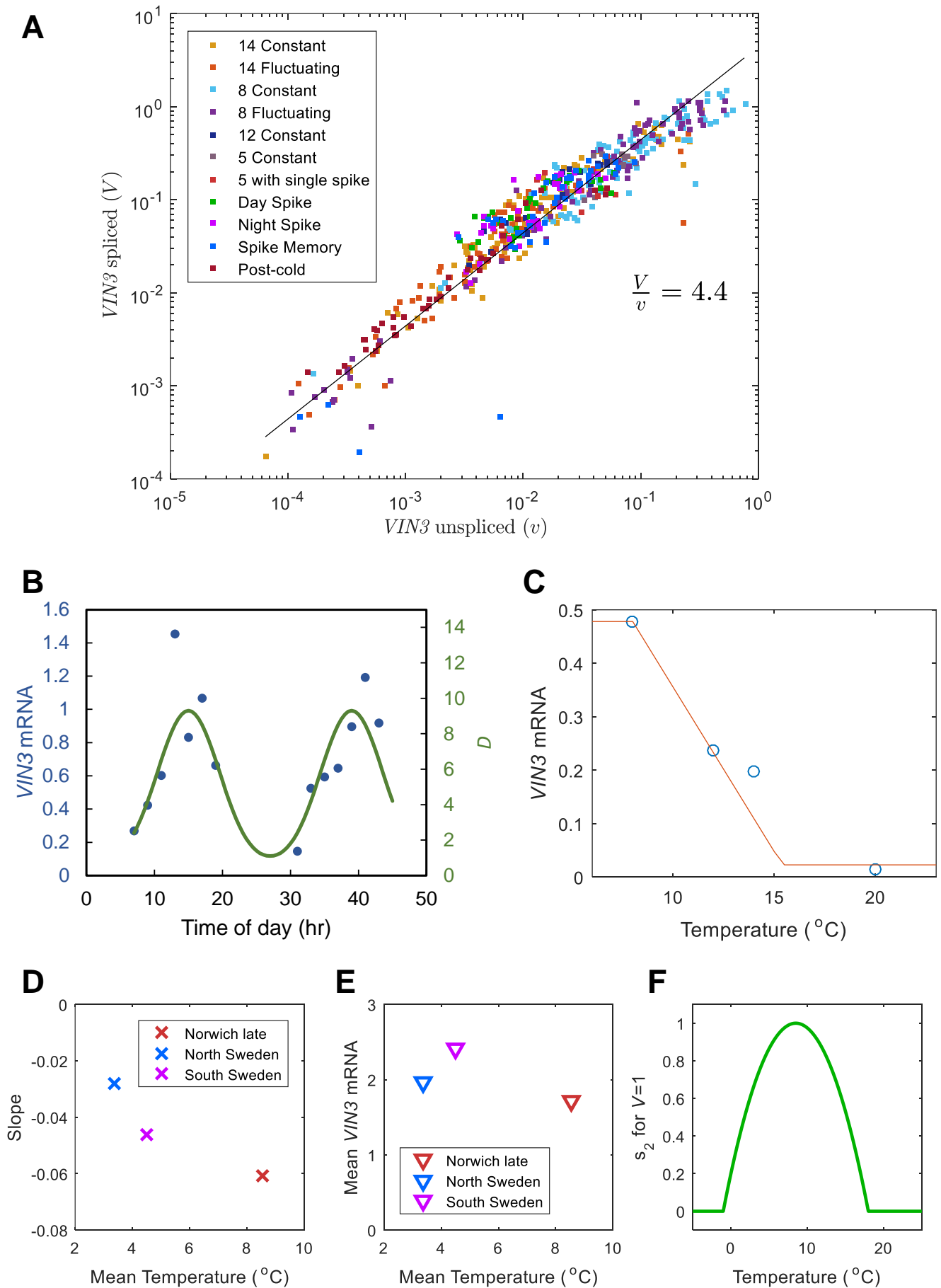


Figure S5. *VIN3/FLC* model description, related to Figures 3 and 4

A, Spliced *VIN3* levels plotted against unspliced *VIN3* levels from the same sample for the various experiments of **Fig. S7**. The black line shows $V=4.4v$, which captures the relationship between the two variables ($R^2=0.901$ on the log-log scale). **B**, Component D follows the diurnal pattern of the *VIN3* data. Data from Hepworth et al, (2018) is shown for two consecutive days (also in this paper in **Fig. S2G, S7D**). **C**, *VIN3* mRNA measurements (circles) after 4 weeks at cold temperatures (same input from L thermosensor), sam-

pled between 14:30 and 17:30 (similar input from component **D**) to determine contribution of thermosensor **C** to temperature sensing. The 20°C measurements are from samples transferred from 5°C for less than 24 hr, so input from **L** should also be approximately the same in those plants. In the **LSCD** model, the **S** thermosensor will take a different value for the 20°C measurement versus the others, but this is a comparatively minor effect. The line shows the result of the optimisation (not to these points alone but to all data) as explained in optimisation section of Methods. **D**, Slope of shutdown of *FLC* expression (estimated by linear regression) at the three field sites from the 2014-15 experiment (Hepworth et al., 2018) plotted against the mean temperature at the corresponding site during the time of the experiment. More negative values of the slope indicate a steeper slope and faster shutdown. Norwich late refers to measurements in Norwich after *VIN3* was induced (~55 days); the temperature data is also limited to that time period. **E**, As for **D** but mean *VIN3* mRNA is shown instead of slope. **F**, Behaviour of $s_2(V, T)$ in the model for constant *VIN3* expression.

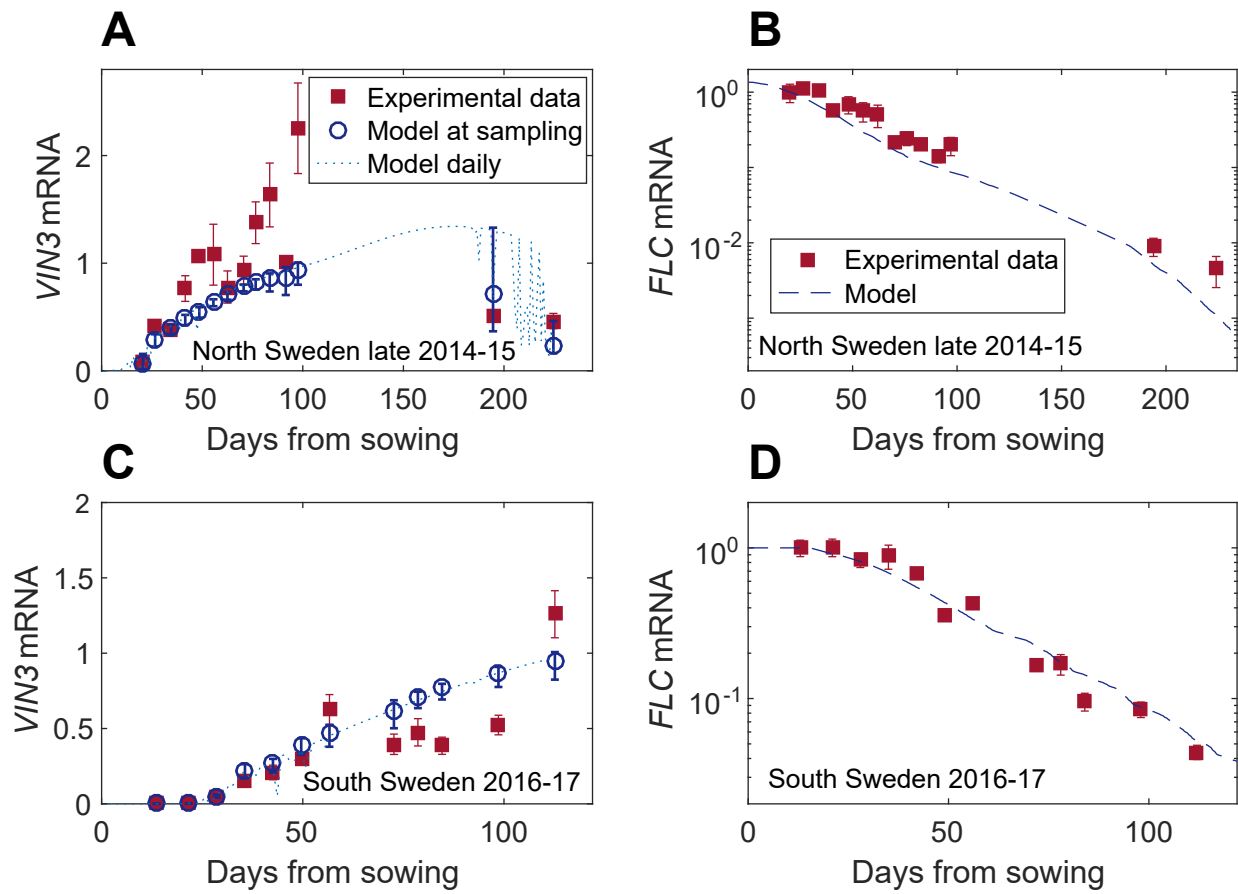


Figure S6. *VIN3/FLC* model fit and prediction for field sites, related to Figures 3, 4 and 5
A-B, Comparison of *VIN3/FLC* model and fitted experimental *VIN3* and *FLC* mRNA data, respectively, for the late planting in North Sweden, in 2014-15. Data from Hepworth et al (2018). **C-D**, Validation of *VIN3/FLC* model by prediction of *VIN3* and *FLC* behaviour under new field conditions, in South Sweden, in 2016-17. $n=5-6$, average >5.8 . In all cases, squares and error bars for experimental data show mean and standard error, respectively. In **A** and **C**, “Model at sampling” shows the mean of the model values of *VIN3* mRNA in the sampling time window, which is defined as the period from 2 hr before the recorded sampling time to 2 hr after due to the long duration of sampling. The error bars show the maximum and minimum model values of *VIN3* mRNA during that time window. “Model daily” shows the model value for *VIN3* mRNA at the same time every day (chosen as the time of the final sampling), to demonstrate the changes in amplitude of the *VIN3* daily peak. In **B** and **D**, the dashed blue line shows the model values of *FLC* mRNA.

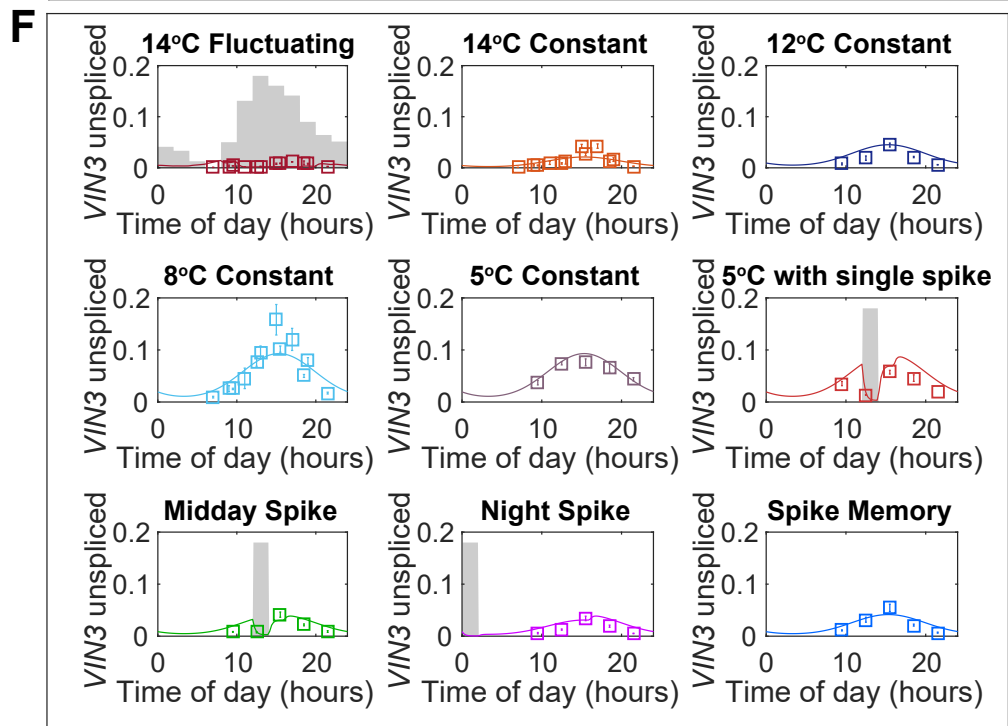
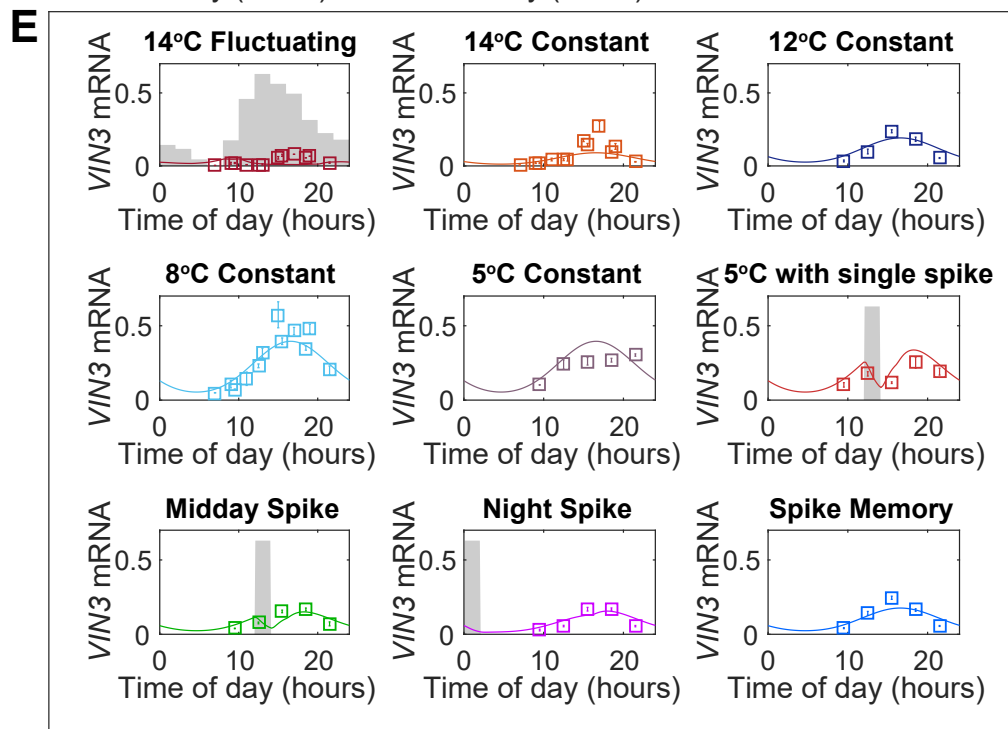
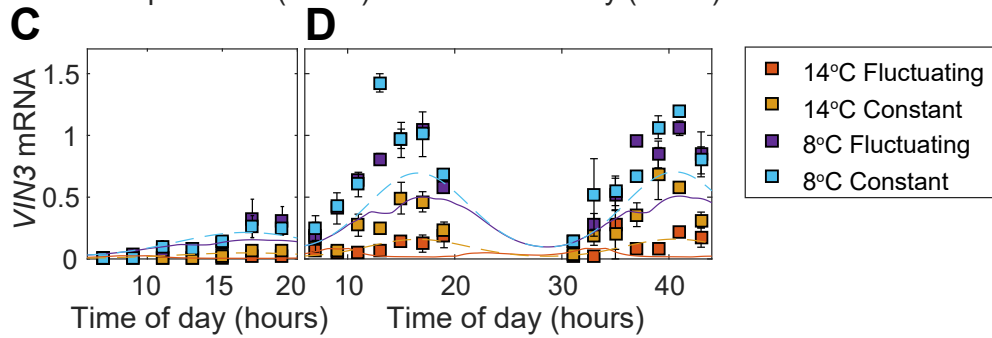
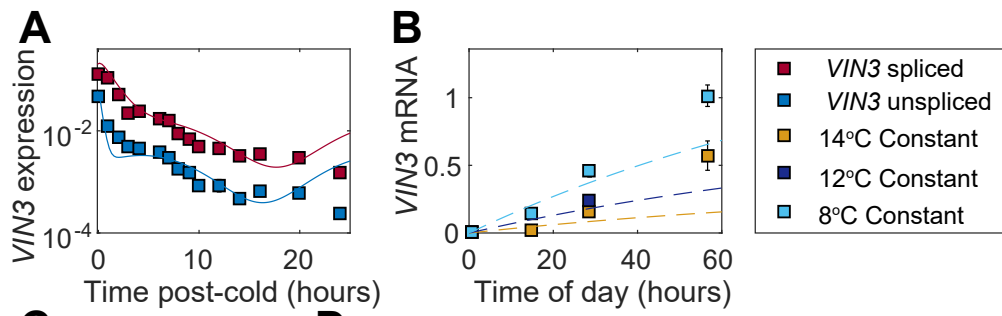


Figure S7. *LSCD* model fitted to *VIN3* data from wild-type (*ColFRI*^{SF2}) plants in lab conditions, related to Figure 3

A-F, The *LSCD* model of *VIN3* expression fit to data from the literature (Hepworth et al., 2018, same as presented in **Fig. S2**) and new data presented in this paper. Squares with error bars (standard error) represent experimental data, lines (dashed and full) show the model fit. **A**, *VIN3* expression hours after plants are returned to the warm at 22°C following 4 weeks at 5°C (data from Hepworth et al., 2018, same as presented in **Fig. S2B**). **B**, *VIN3* mRNA over weeks of cold treatment measured at the same time of day (3pm) for 3 different constant temperatures (data combined from Hepworth et al., 2018 and **Fig. 2**). **C**, *VIN3* mRNA over a single day after 2 weeks vernalization in the temperature conditions of **Fig. S2C** (data from Hepworth et al., 2018, same as presented here in **Fig. S2E**). **D**, *VIN3* mRNA over two consecutive days after 8 weeks vernalization in the temperature conditions of **Fig. S2C** (data from Hepworth et al., 2018, same as presented here in **Fig. S2G**). **E-F**, *VIN3* spliced and unspliced levels respectively, measured during the day after 4 weeks in the conditions shown (includes data from Hepworth et al., 2018 and **Fig. 2**, as well as new data: n=1-6, average >3). Grey background bars represent temperature profile in non-constant conditions.

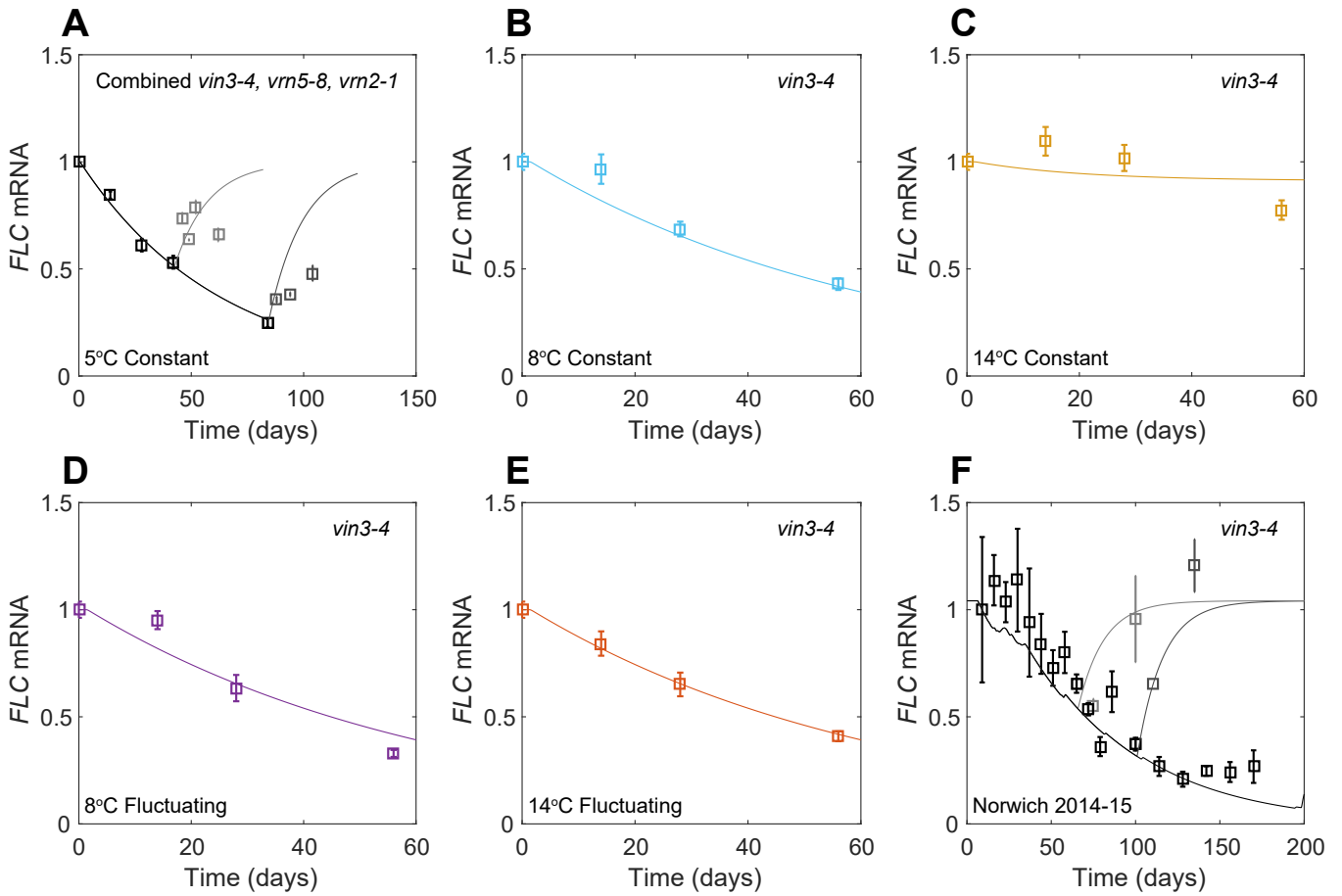


Figure S8. Comparison of VIN3-independent part of the *FLC* model and fitted experimental data for *vin3-4*, *vrn5-8* and *vrn2-1* mutants, related to Figure 4

A, Model simulated with $s_2 = s_3 = 0$ (for $I \rightarrow E$ and $H \rightarrow E$ transitions) (other parameters as in **Table S4**) at constant 5°C (black line) and post-cold at 22°C (grey lines), with transfer at times indicated by start of grey line. Experimental data shows combination of *vin3-4*, *vrn5-8* and *vrn2-1* mutants at constant 5°C (black squares) and after transfer to warm, 22°C (grey squares). Experimental data from Yang et al. (2017). **B-E**, Model simulated with $s_2 = s_3 = 0$ (other parameters as in **Table S4**) at 8°C constant, 14°C constant, 8°C fluctuating and 14°C fluctuating, respectively (lines). Experimental data shows *vin3-4* mutant with same conditions (squares). Experimental data and temperature profiles from Hepworth et al. (2018). **F**, Comparison of model and fitted experimental data for Norwich in 2014-15, for the *vin3-4* mutant (black squares; Hepworth et al., 2018). At times indicated by start of the grey lines, plants were transferred from ‘field’ conditions after 10 or 14 weeks to a heated, lit, long-day glasshouse, and continued to be sampled (grey squares). $n=6$ for timepoints taken in the ‘field’ glasshouse, $n=3$ for timepoints in the warm glasshouse. In all cases, squares and error bars show mean and standard error, respectively. RNA levels normalised to *UBC*, *PP2A* for **B-F**, normalised to *UBC* for **A**.

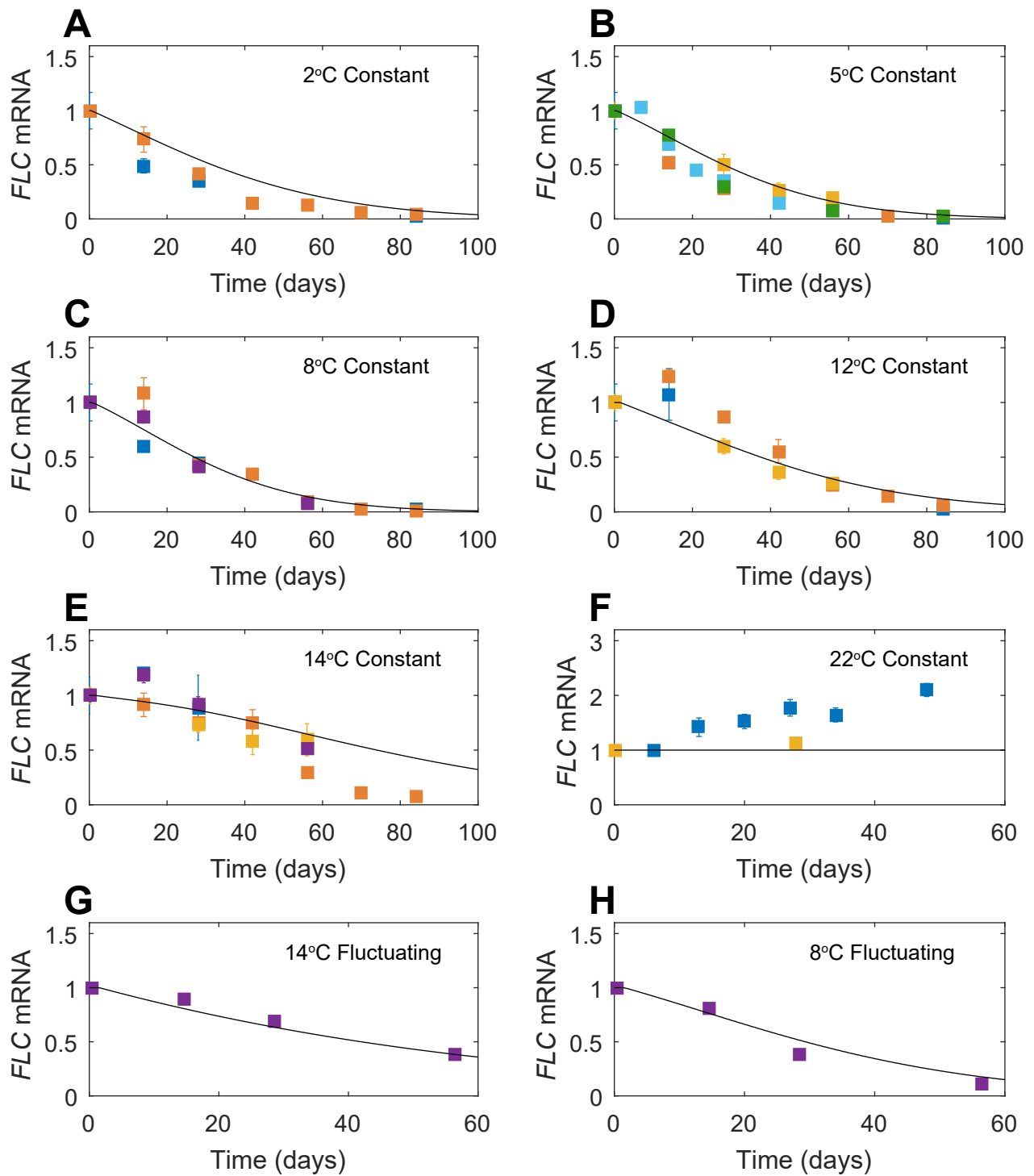


Figure S9. *VIN3/FLC* model fitted to experimental *FLC* data from wild-type (*ColFRI^{SF2}*) plants in lab conditions, related to Figure 4

A-F, Comparison of model (black lines) with experimental *FLC* data collected from plants treated at various constant temperatures, as indicated. Colour corresponds to individual who performed the experiment. Previously unpublished data: orange, blue, yellow, green (see Methods section for description) $n=1-27$, average >8.5 . Previously published data is also included: orange - Duncan et al., (2015) together with unpublished data from the same study; purple - Hepworth et al. (2018); light blue - Yang et al. (2017). **G-H**, Comparison of model (black lines) with experimental *FLC* data collected from plants treated at fluctuating temperatures, as indicated, (data from Hepworth et al., 2018). In all cases, squares and error bars show mean and standard error, respectively. RNA levels are normalised, as explained in the Methods.

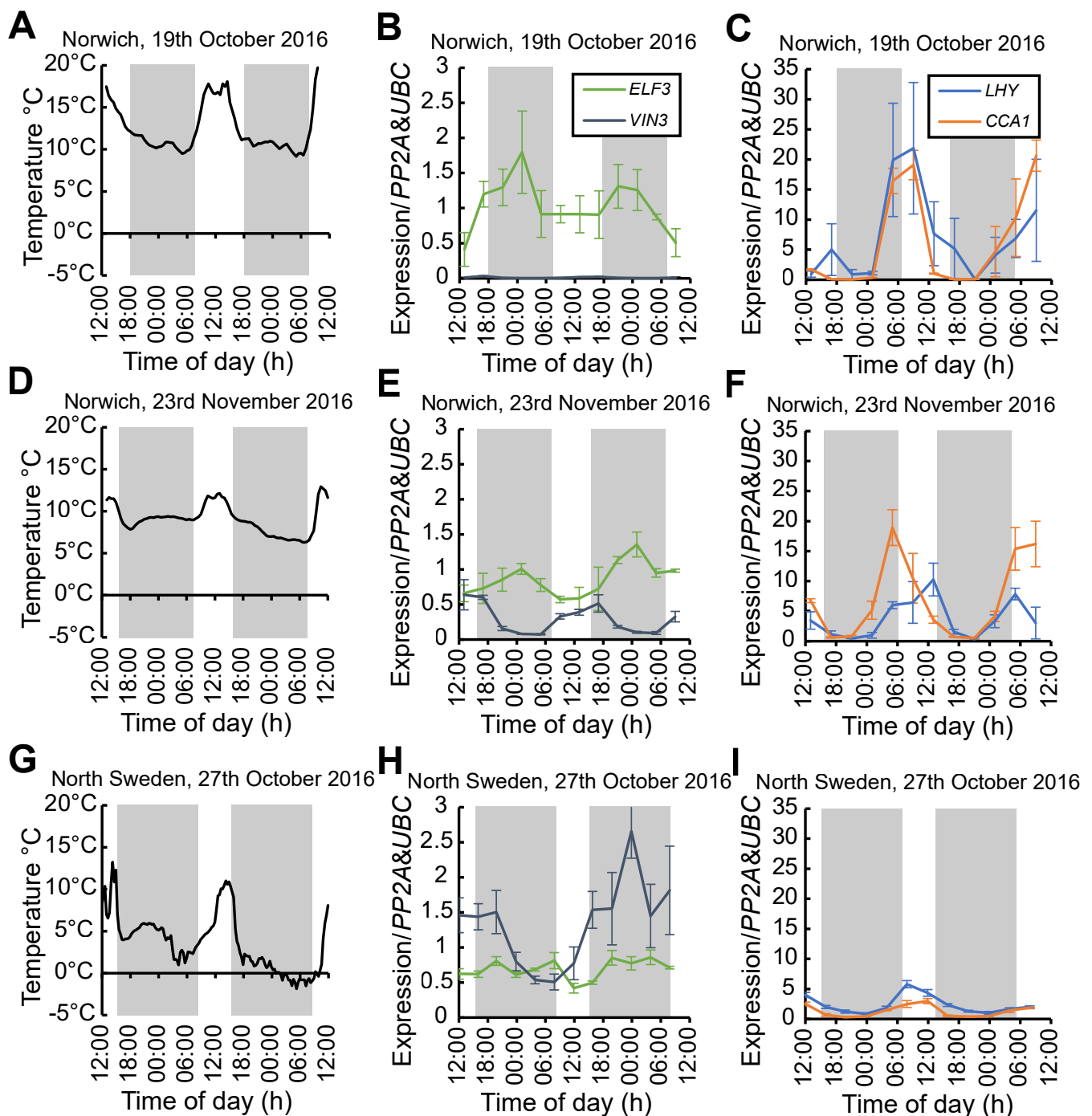


Figure S10. *VIN3* and some circadian clock genes show shifted expression in North Sweden in the 2016-17 winter, as compared to Norwich, related to Figure 5

Temperature and gene expression time-series including one complete day in Norwich in October 2016 (A, B, C), in Norwich in November 2016 (D, E, F), and in North Sweden in October 2016 (G, H, I). Ground temperatures (A, D, G), mean expression of *VIN3* and evening complex gene *ELF3* (B, E, H), and mean of morning-expressed circadian regulators *CCA1* and *LHY* (C, F, I). *VIN3* expression relative to control (see Methods) used for 2016-17, other expression relative to *PP2A* and *UBC*. Error bars show standard error. n=3 in Norwich, n=6 in Sweden. Dark shading indicates night-time.

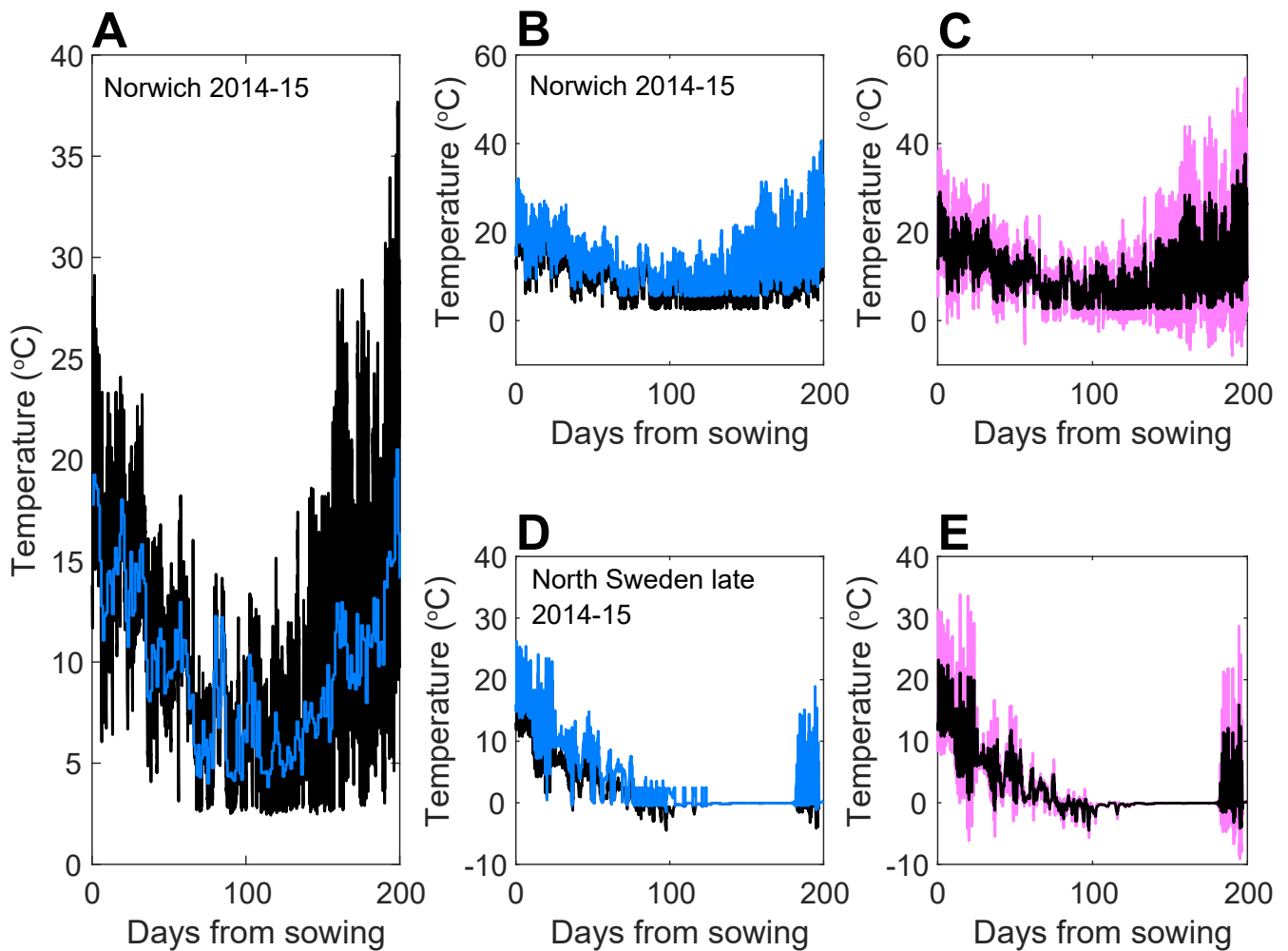


Figure S11. Modified temperature profiles, related to Figure 6

A, Temperature profile in Norwich 2014-15 (black) and day-mean profile (blue) of **Fig. 6A-F**. In the day-mean profile, the temperature measurements each day (initially every 30 min) are replaced by the mean value of that day. **B**, Temperature profile in Norwich 2014-15 (black) and “+3” profile (blue) of **Fig. 6G-I**. The original profile was modified by adding 3°C. **C**, Temperature profile in Norwich 2014-15 (black) and “x2” profile (pink) of **Fig. 6G-I**. The profile was modified by stretching the temperatures (T) above and below the daily mean temperature (T_m) for each day ($T \rightarrow 2(T - T_m) + T_m$). **D**, Temperature profile in North Sweden (late planting) 2014-15 (black) and “+3” profile (blue) of **Fig. 6J-L**. The original profile was modified by adding 3°C, with the exception of temperatures around 0°C, when the plants were mainly covered by snow. **E**, Temperature profile in North Sweden (late planting) 2014-15 (black) and “x2” profile (pink) of **Fig. 6J-L**. The profile was modified as in **C**.

Table S1. Primers used in this study, related to STAR methods

Primer name	Sequence 5'-3'	Used for RT reaction	From
UBC_qPCR_F	CTGCGACTCAGGGAATCTTCTAA		1
UBC_qPCR_R	TTGTGCCATTGAATTGAACCC	Y	1
FLC_4265_F (spliced sense)	AGCCAAGAAGACCGAACTCA		1
FLC_5683_R (spliced sense)	TTTGTCCAGCAGGTGACATC	Y	1
FLC_3966_F (unspliced sense)	CGCAATTTTCATAGCCCTTG		1
FLC_4135_R (unspliced sense)	CTTTGTAATCAAAGGTGGAGAGC		1
FLC unspliced RT (4029)	TGACATTTGATCCCACAAGC	Y	1
VIN3 qPCR 1 F	TGCTTGTGGATCGTCTTGTCA		1
VIN3 qPCR 1 R	TTCTCCAGCATCCGAGCAAG	Y	1
PP2A QPCR F2	ACTGCATCTAAAGACAGAGTTCC		1
PP2A QPCR R2	CCAAGCATGGCCGTATCATGT	Y	1
JF118-CCA1-F	CTGTGTCTGACGAGGGTTCGAA		2
JF119-CCA1-R	ATATGTAATAACTTTGCGGCAATACCT	Y	2
JF120-LHY-F	CAACAGCAACAACAATGCAACTAC		2
JF121-LHY-R	AGAGAGCCTGAAACGCTATACGA	Y	2
JF260-ELF3-F	GGAAAGCCATTGCCAATCAA		2
JF261-ELF3-R	ATCCGGTGATGCAGCAATAAGT	Y	2

1. Hepworth *et al.* (2018)

2. MacGregor *et al.* (2013)

Table S2. UPL Primers, related to STAR methods

UPL#65	CTGGAGGA
sFLC_UPL_F	GTGGGATCAAATGTCAAAAATG
sFLC_UPL_R	GGAGAGGGCAGTCTCAAGGT
UBC_UPL_F	TCCTCTTAACTGCGACTCAGG
UBC_UPL_R	GCGAGGCGTGTATACATTTG
UPL#9	TGGTGATG

Table S3. VIN3 smFISH probes, related to STAR methods

Probe Number	Sequence 5'-3'
1	TCTAAGGAGGAAACCCTCTG
2	TTTCTGTGATGGATGGTTCT
3	CTTCGTGTTCTTCGTTTTTT
4	CGAAGCAGCTTGCATTTTTT
5	TTACCATCGAAACGCCAGAT
6	AGAATCCATGTTCTCTGGAC
7	TCTCCTTTCACTTACATTCA
8	GGTTAGACAATGCGTGGATC
9	TCAAAGCTCCGAAGCTTCT
10	CCATCTCAGCACATATGATC
11	TAAGACCAGTGTACTTCCTT

12	GATTCTCTATGAGCTTTGGT
13	CGGTCAGAACAAGAGGTCTC
14	GTAACCGATCATCTTCTTCT
15	AGCAAGAACACCTTCTGCAA
16	AGCCATAAACTAGGATCCTT
17	AGACGATCCACAAGCATCAC
18	TGCTTCAAACCACATTCCAA
19	CCTACCATCAAGATCATCAC
20	TATCTTTACCGCAATACGCG
21	GTTCCAACAGATTCCGATAC
22	AAGTCTATTGACGATGCCTC
23	GAGAACACAGCTTCTGGACA
24	AGATTCTGATGGTGAGACCA
25	GCTTGAATCTCTTCTACTCT
26	TCTACTCTCACAGTGA
27	ACCTGTGATCTTGTTTTGTG
28	GTCCTTCGACTTTTCGACAAA
29	TGAGACAGAACTCGGTGTCG
30	AAGTCACCTTCCTCGTAAA
31	ATCATCCTTCAACGTTGTGA
32	CTTGAGTTTGTCAAAGGGCT
33	TTGCTGCAGCTTTTATTGAC
34	ACTACAGTGTTTCAGTGTGT
35	TCTCTTCTTCAAGCTCAGAT
36	GTTTGCTTTCTCTTTACAA
37	AAGCAAGTCTTCCATCTA
38	AATATCTCTTGCAGGGTG
39	TTGAATCTTTTATTCCCTCC
40	GGCATTATTGATCTCAGGTT
41	ATGACCCAAGTCTTTATCTC
42	CTTGTCTATATGTCCTTCTT
43	TGCAAGAACCTTTCCCTAA
44	CAACTCTTACTTCTCGGTGA
45	TCCTCCATAAACGTCTCAAC
46	CAAGCTGTTGTCCCAAAGAA
47	CACCATTTGTCGATGATCTC

Table S4. Model parameters, related to STAR methods

Name	Value	Units	Bounds	Fit based on data
d_V	18	day ⁻¹	$d_V \geq 0$	LSCD model VIN3 spliced and unspliced RNA data: Hepworth <i>et al.</i> (2018) and Fig. 2, 3, S6, S7.
S_1	0.75	dimensionless	$0 \leq S_1 \leq 1$	
T_L	17	°C	$14 \leq T_L \leq 20$	
d_L	0.009	day ⁻¹	$d_L \geq 0$	
T_{C1}	8	°C	$-10 \leq T_{C1} \leq T_{C2}$	
T_{C2}	15.4	°C	$T_{C1} \leq T_{C2} \leq 30$	
p_{C1}	0.0315	dimensionless	$p_{C1} \geq p_{C2}$	
p_{C2}	0.0300	dimensionless	$0 \leq p_{C2} \leq p_{C1}$	
p_D	2.05	dimensionless	$0.7 \leq p_D \leq 3$	
s_v	$4.4d_V$	day ⁻¹	Not applicable	Fig. S5A
T_S	15	°C	From the literature:	Hepworth <i>et al.</i> (2018).
s_1	0.016	day ⁻¹	$0 \leq s_1 \leq p_r$	VIN3-independent switch of FLC model FLC mRNA data for <i>vin3-4</i> , <i>vrn2-1</i> , <i>vrn5-8</i> mutants: Yang <i>et al.</i> (2017) and Hepworth <i>et al.</i> (2018), Fig. 2, S4, S8.
T_{r1}	11.5	°C	$-10 \leq T_{r1} \leq T_{r2}$	
T_{r2}	15	°C	$T_{r1} \leq T_{r2} \leq 30$	
p_r	0.05	day ⁻¹	$p_r \geq s_1$	
p_f	5.3	day ⁻¹	$p_f \geq 0$	VIN3-dependent switch of FLC model FLC mRNA data for <i>ColFR^{JSF2}</i> : Hepworth <i>et al.</i> (2018), Yang <i>et al.</i> (2017), Duncan <i>et al.</i> (2015), Fig. 2, 4, S6, S9.
T_1	-1	°C	$-10 \leq T_1 \leq T_2$	
T_2	18	°C	$T_1 \leq T_2 \leq 30$	
p_s	0.0111	day ⁻¹ (°C) ⁻²	$p_s \geq 0$	
p_{s3}	0.1	dimensionless	$0 \leq p_{s3} \leq 1$	

Table S5. Parameters for AIC comparison, related to STAR methods

Name	Value for LCD	Value for LSCD	Units
d_V	24.3	29.0	day ⁻¹
S_1	-	0.749	dimensionless
T_L	17.9	17.1	°C
d_L	1.46	0.105	day ⁻¹
T_{C1}	18.4	18.2	°C
T_{C2}	20.2	20.8	°C
p_{C1}	1.49	1.17	dimensionless
p_{C2}	1.76	0.230	dimensionless
p_D	0.712	0.710	dimensionless
s_v	$4.4d_V$	$4.4d_V$	day ⁻¹
T_S	-	15	°C