RESEARCH ARTICLE

Figures and figure supplements

Natural variation in autumn expression is the major adaptive determinant distinguishing Arabidopsis FLC haplotypes

Jo Hepworth *et al*

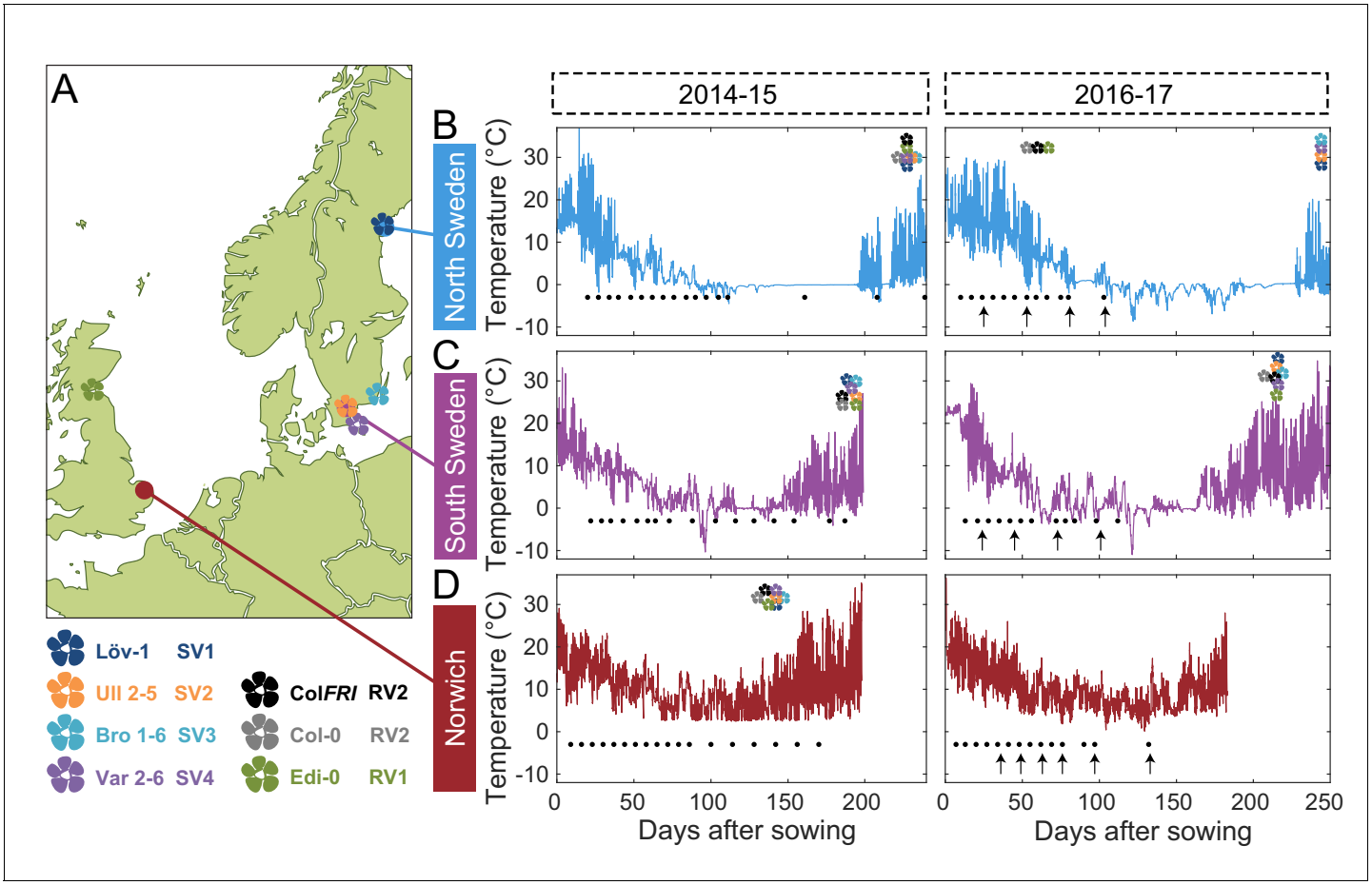

Figure 1. Field experimental setup. (**A**) Map showing locations of field sites (dots) and the origins of five of the accessions (flowers) used in this study. These accessions, with the addition of Col-0, represent the five major and one intermediate (Löv-1) *FLC* haplotypes identified by ***Li et al., 2014***. The lab genotype Col *FRI* was also used in this study as a vernalization-requiring reference. (**B–D**) Temperature profiles experienced by plants at the three field sites, North Sweden – Ramsta (**B**), South Sweden – Ullstorp (**C**) and Norwich, UK (**D**) (***Source data 1***, as from ***Hepworth et al., 2018*** and ***Antoniou-Kourounioti et al., 2018***). Flowers above temperature profile indicate the median time of bolting of each of the natural accessions and of Col *FRI* (legend at bottom left corner). Black dots below temperature profile indicate the timepoints when plant material was collected for expression analysis. Black arrows below temperature profiles indicate time of transfer to greenhouse with long-day, warm conditions to assess degree of vernalization based on bolting time.

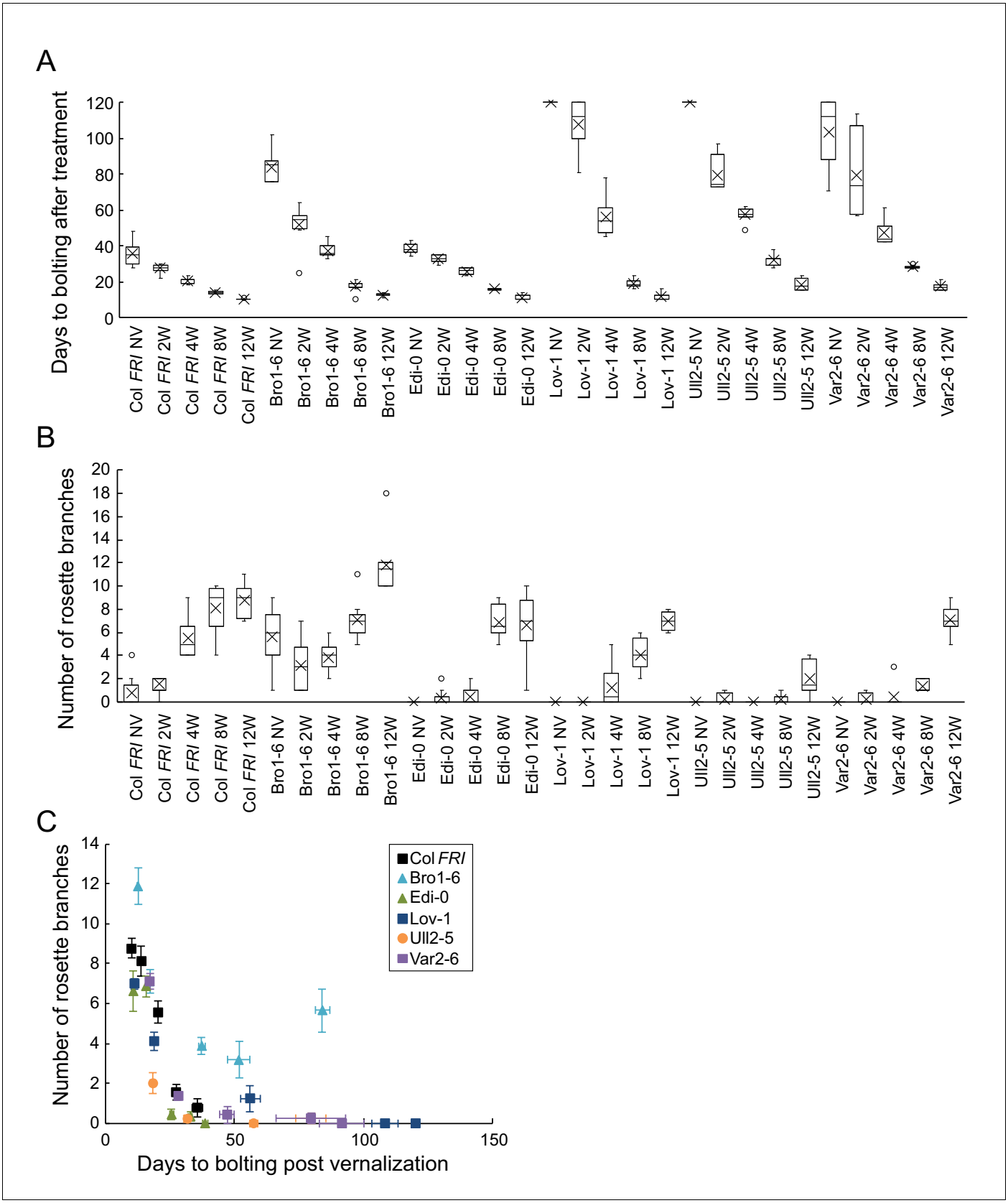

Figure 1—figure supplement 1. Increased vernalization reduces time to bolting, variability in bolting time, and increases rosette branch production in different accessions. (**A**) Time to bolting for accessions in a heated, lit greenhouse without vernalization (NV) or after weeks of vernalization at constant

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Figure 1—figure supplement 1 continued

5°C (*n*W). (**B**) Number of rosette branches for plants shown in A. Median (central line), mean (cross), interquartile range (box), range (whiskers) and outliers (circles, values more than 1.5 times the interquartile range outside of the interquartile range). Plants that did not flower within 120 days of transfer not shown, see ***Source data 7***. (**C**) Means per genotype and vernalization length treatment of rosette branch data presented in A and B, plotted against days to bolting. Error bars show s.e.m.

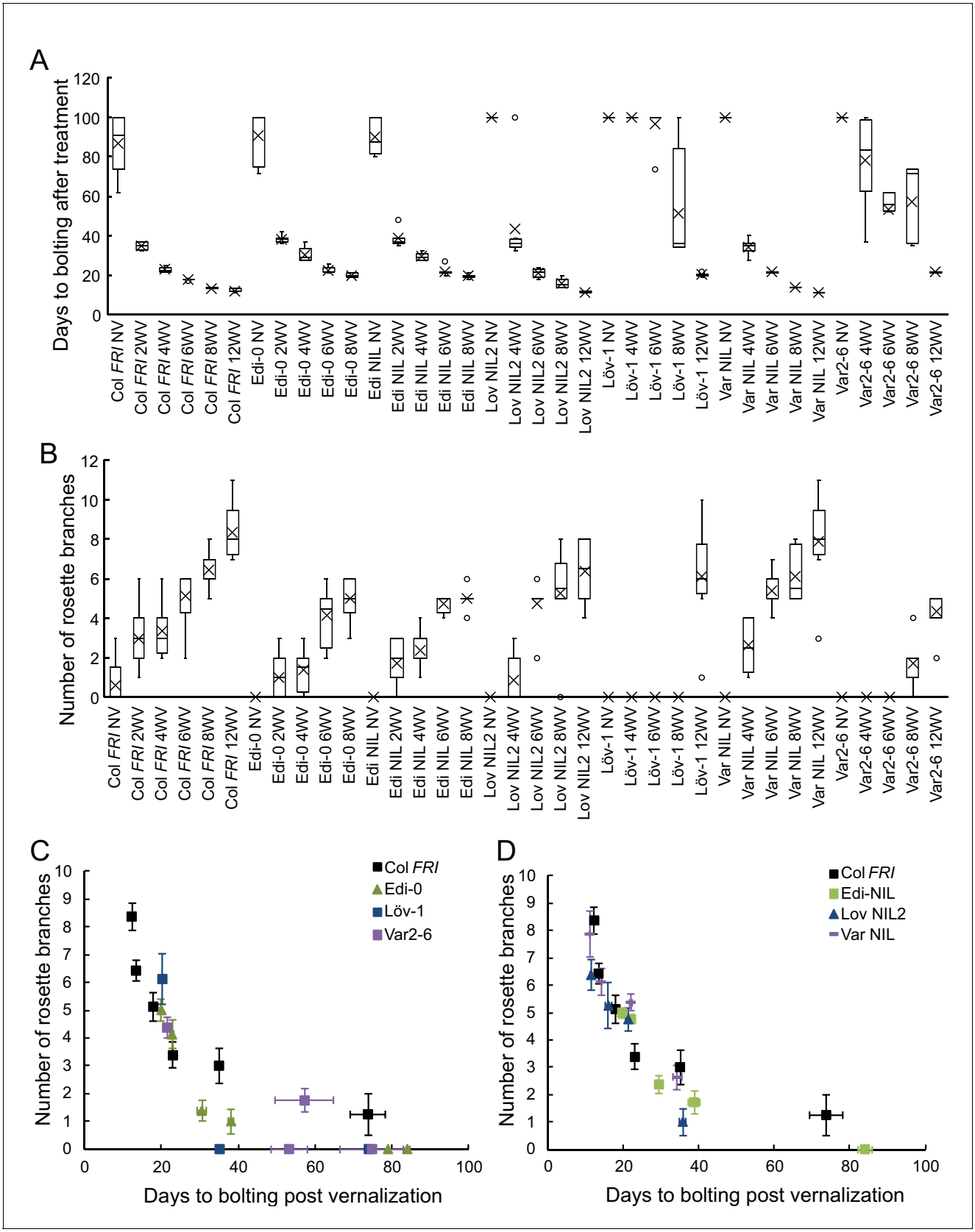

Figure 1—figure supplement 2. Increased vernalization reduces time to bolting and increased branch production with subtly different effects depending on *FLC* haplotype in the Col *FRI* background. (**A**) Time to bolting for selected accessions and NILs in a heated, lit greenhouse without

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Figure 1—figure supplement 2 continued

vernalization (NV) or after weeks of vernalization at constant 5°C (*n*W). (**B**) Number of rosette branches for plants shown in C. Median (central line), mean (cross), interquartile range (box), range (whiskers) and outliers (circles, values more than 1.5 times the interquartile range outside of the interquartile range). Plants that did not flower within 120 days of transfer not shown, see ***Source data 7***. (**C, D**) Means per genotype and vernalization length treatment of rosette branch data presented in A and B, plotted against days to bolting for; (**C**) accessions only and (**D**) NILs only. Error bars show s.e.m.

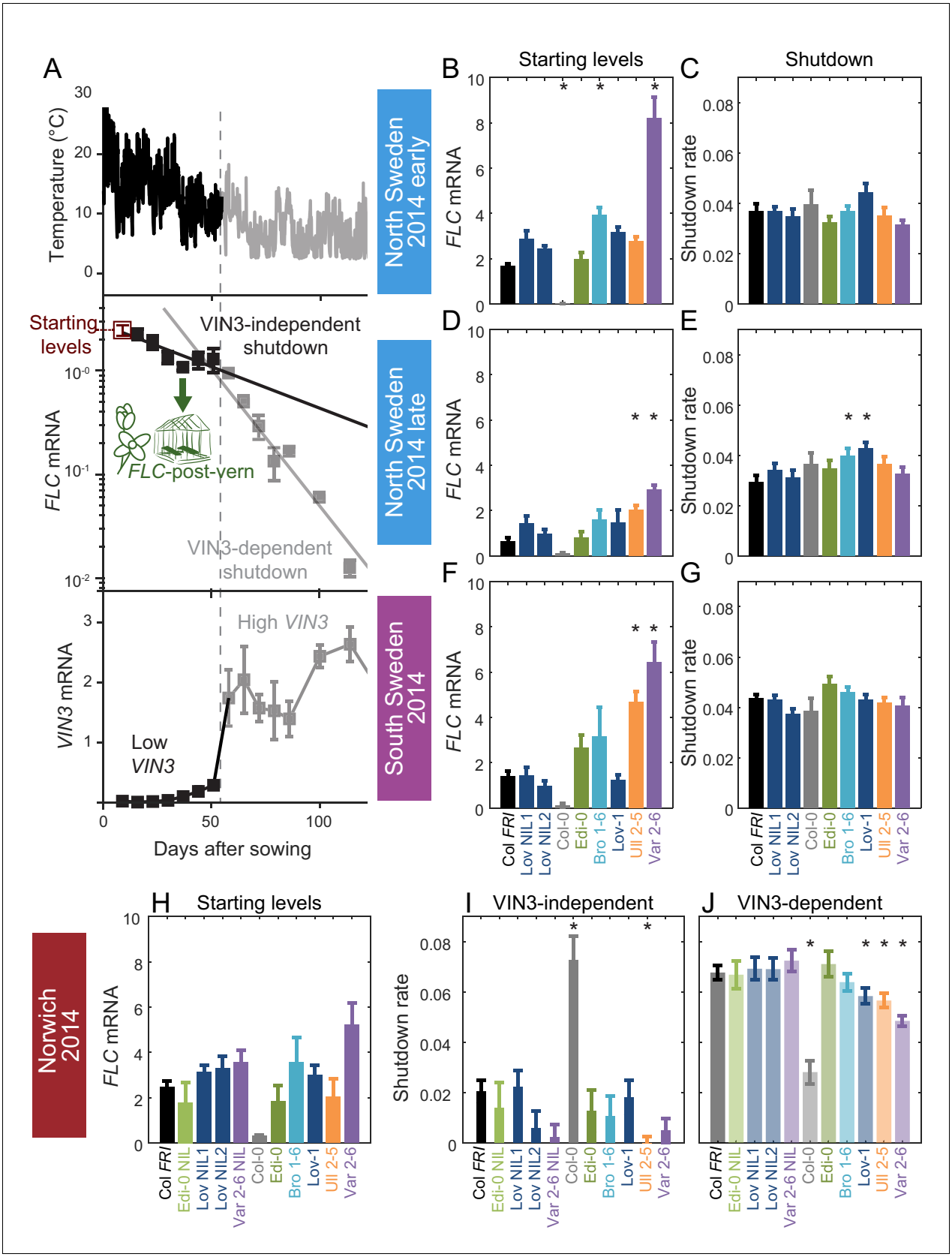

Figure 2. Downregulation in 2014–5 in Norwich, North Sweden (two plantings) and South Sweden for all NILs and accessions. (**A**) Experimental data for Col *FRI* in Norwich 2014–5, showing the temperature profile (top), *FLC* (middle) and *VIN3* (bottom) expression. Different shades indicate the separation

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of the VIN3-dependent (grey) and -independent (black) phases of *FLC* silencing (***Hepworth et al., 2018***) and equivalent times in *VIN3* and temperature profiles (as in ***Figure 1D***). Expression data were normalised to the control sample for 2014–5 (see Materials and methods). N = 6 except where samples were lost to death or degradation (see Materials and methods and ***Source data 2***). Error bars show standard error of the mean (s.e.m). The initial measurement in the field (Starting levels), the rate of downregulation before induction of *VIN3* expression (VIN3-independent, estimated from the slope of the fitted line) and the rate of downregulation after *VIN3* induction (VIN3-dependent) are the three features that were analysed and compared for each genotype and treatment in the next panels, based on the data of ***Figure 2—figure supplement 1***. A new feature is also shown, the FLC-post-vern, that is measured based on the flowering time from plants transferred to glasshouses with inductive conditions and how that relates to the *FLC* levels at the time of the transfer. (**B–J**) *FLC* downregulation analysed as level at first time point (Starting levels), and rate of downregulation (Shutdown – combining early and later timepoints for *FLC* data – see Materials and methods) for North (**B–E**) and South Sweden (**F–G**), or rate of downregulation before (VIN3-independent, dark bars) and after (VIN3-dependent, translucent bars) *VIN3* induction for Norwich (**H–J**). Features of genotypes that are significantly different to the reference line Col *FRI* are indicated by * (for Starting levels, ANOVA with Dunnett's post-hoc test, for Shutdown rates, Satterthwaite's t-tests on REML Linear mixed model). p-values for all comparisons are given in ***Supplementary file 1***. Rates of downregulation are given in units of 'a.u. per day', where the arbitrary units (a.u.) correspond to the normalised concentration of *FLC* mRNA, measured by qPCR. *VIN3* induction started at ~58 days in Norwich (***Figure 2—figure supplements 2–3***). Expression data were normalised to the control sample for 2014–5 (see Materials and methods). N = 6 except where samples were lost to death or degradation (see Materials and methods and ***Source data 2***). Error bars show s.e.

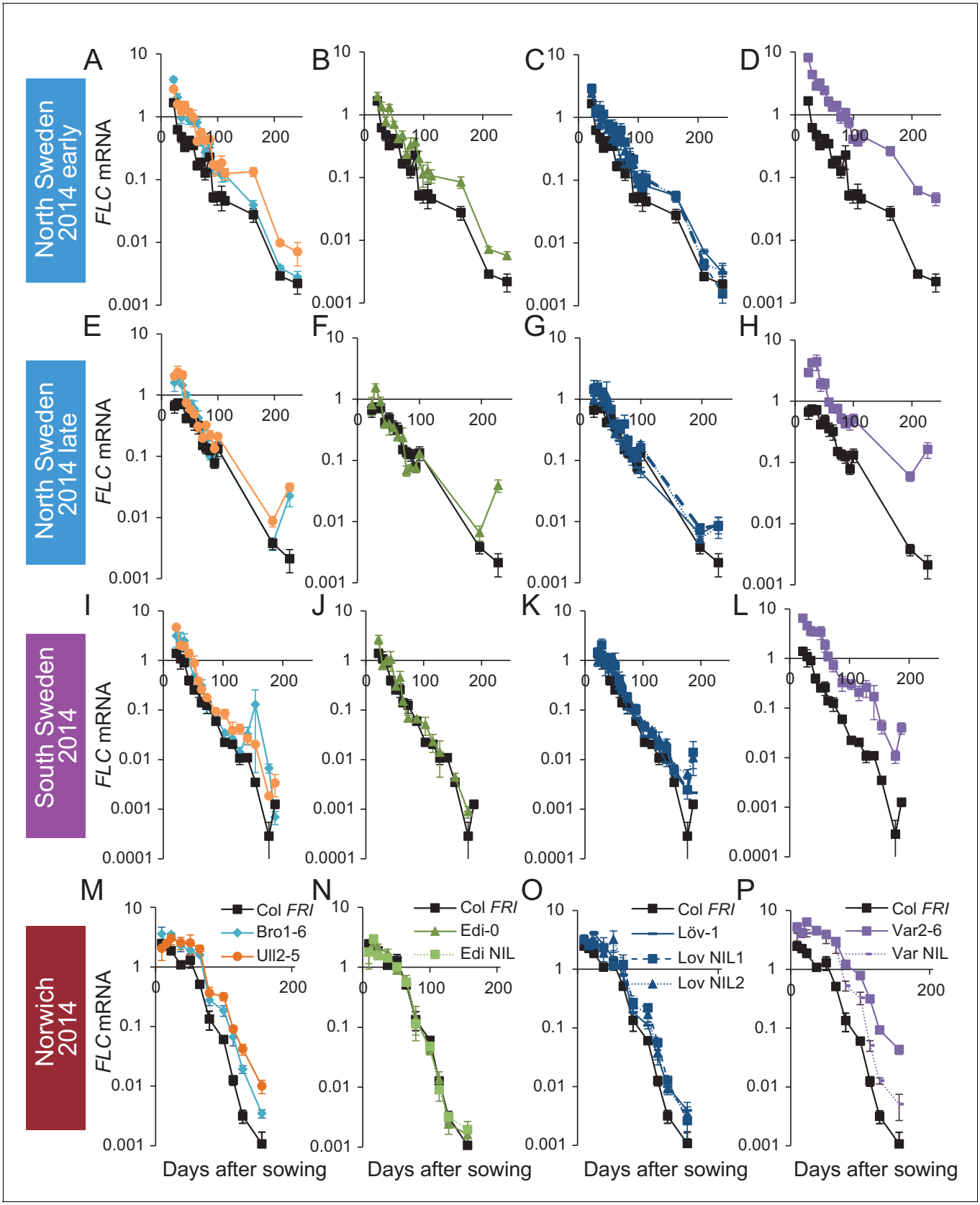

Figure 2—figure supplement 1. *FLC* downregulation in accessions and NILs in Norwich, North Sweden and South Sweden 2014–5. Expression normalised to control sample for 2014–5 (see Materials and methods). (**A–D**) Norwich, (**E–H**) South Sweden, (**I–L**) North Sweden first planting, (**M–P**)

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North Sweden second planting. N = 6 except where samples were lost to death or degradation (see Materials and methods and ***Source data 2***). Error bars show s.e.m.

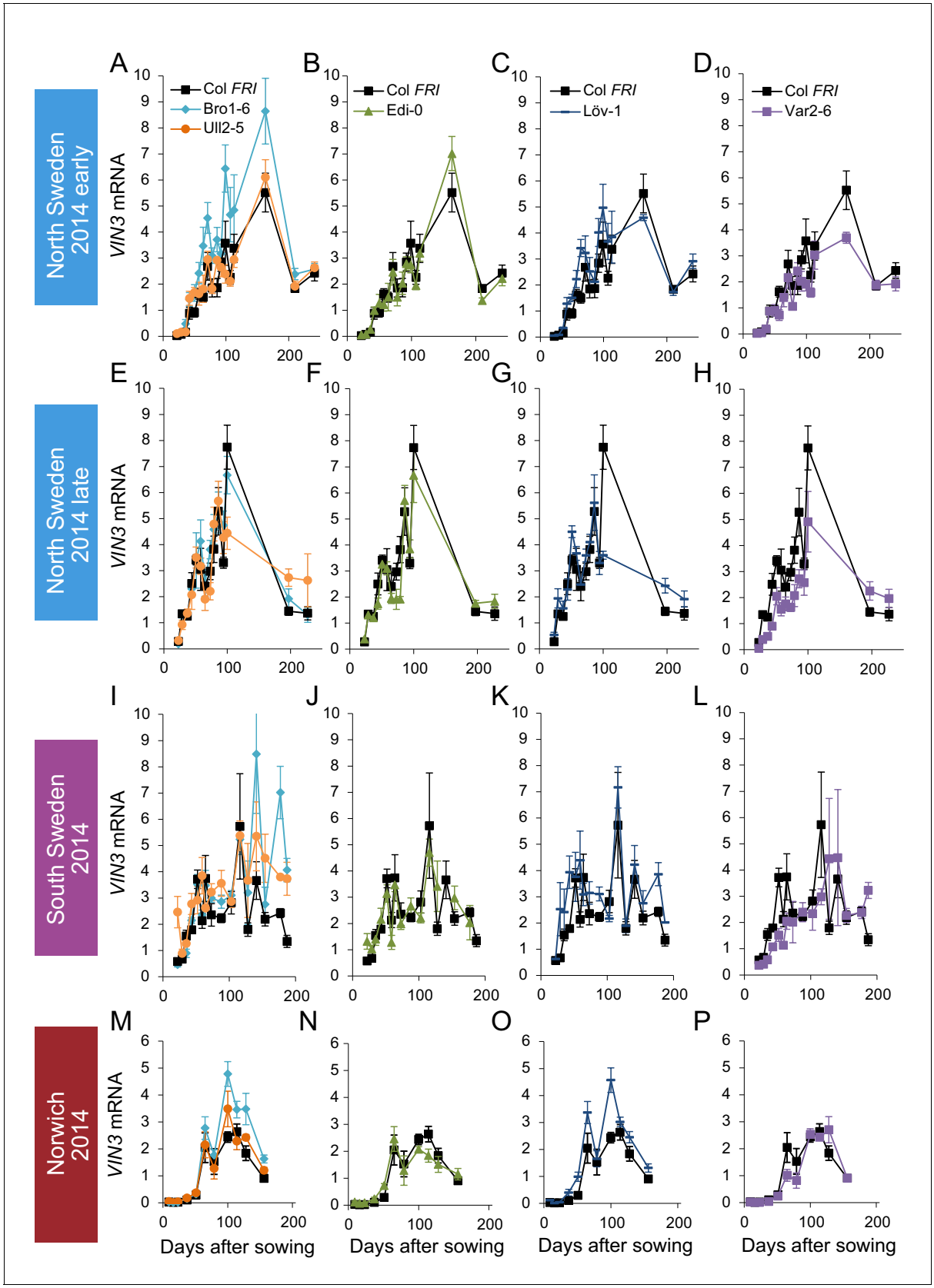
North Sweden 2014 early
North Sweden 2014 late
South Sweden 2014
Norwich 2014
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
VIN3 mRNA
Col FRI
Bro1-6
Ull2-5
Edi-0
Löv-1
Var2-6
Days after sowing

Figure 2—figure supplement 2. *VIN3* upregulation in accessions in Norwich, North Sweden and South Sweden 2014–5. *VIN3* expression normalised to control sample for 2014–5 (see Materials and methods). N = 6 except where samples were lost to death or degradation (see Materials and methods and ***Source data 2***). Error bars show standard error of the mean (s.e.m).

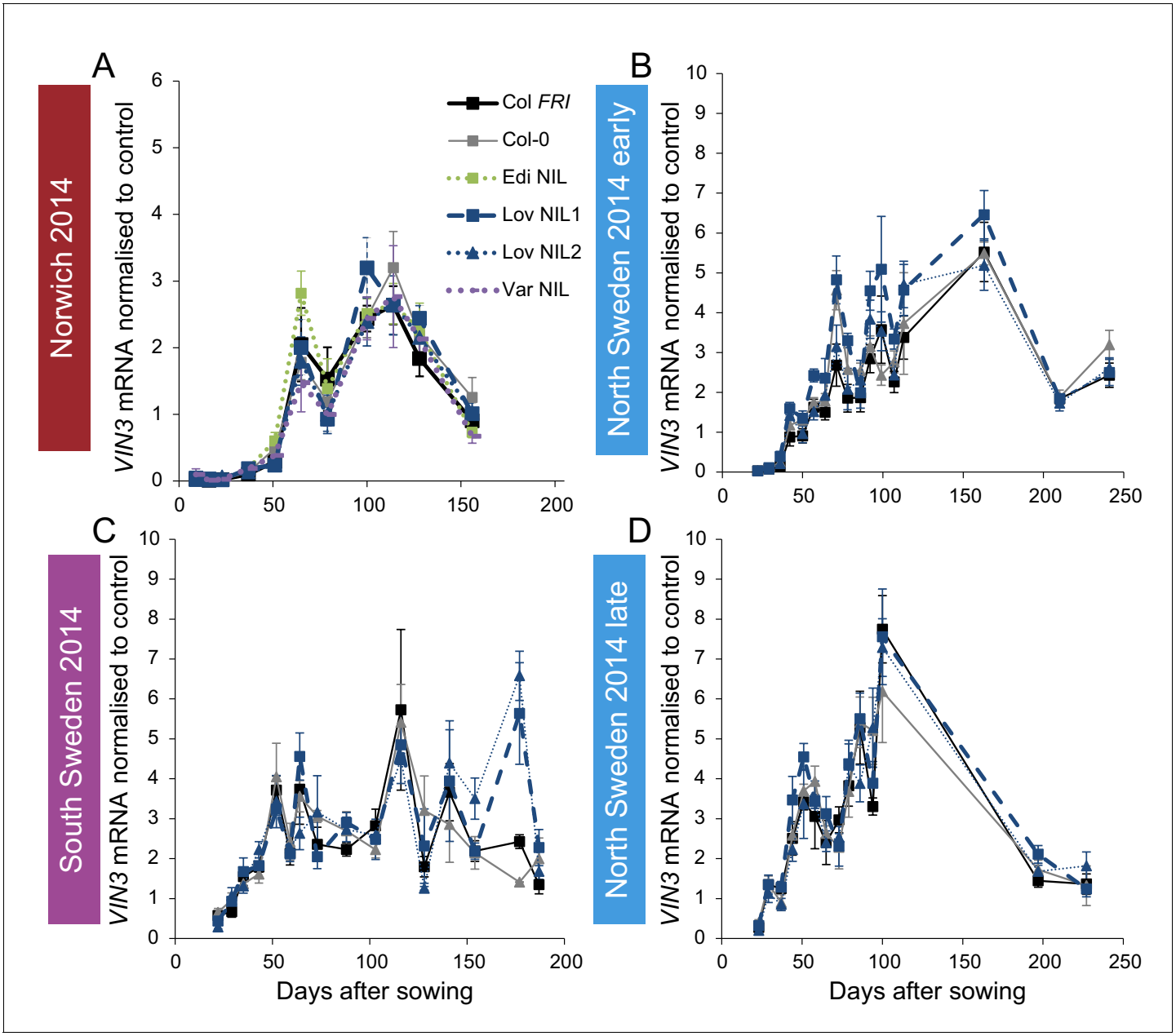

Figure 2—figure supplement 3. Expression of *VIN3* in NILs with the Col-0 *VIN3* allele in the field in 2014–2015. (**A**) Norwich, (**B**) North Sweden first planting, (**C**) South Sweden, (**D**) North Sweden second planting. N = 6 except where samples were lost to death or degradation (see Materials and methods and ***Source data 2***). Error bars show s.e.m.

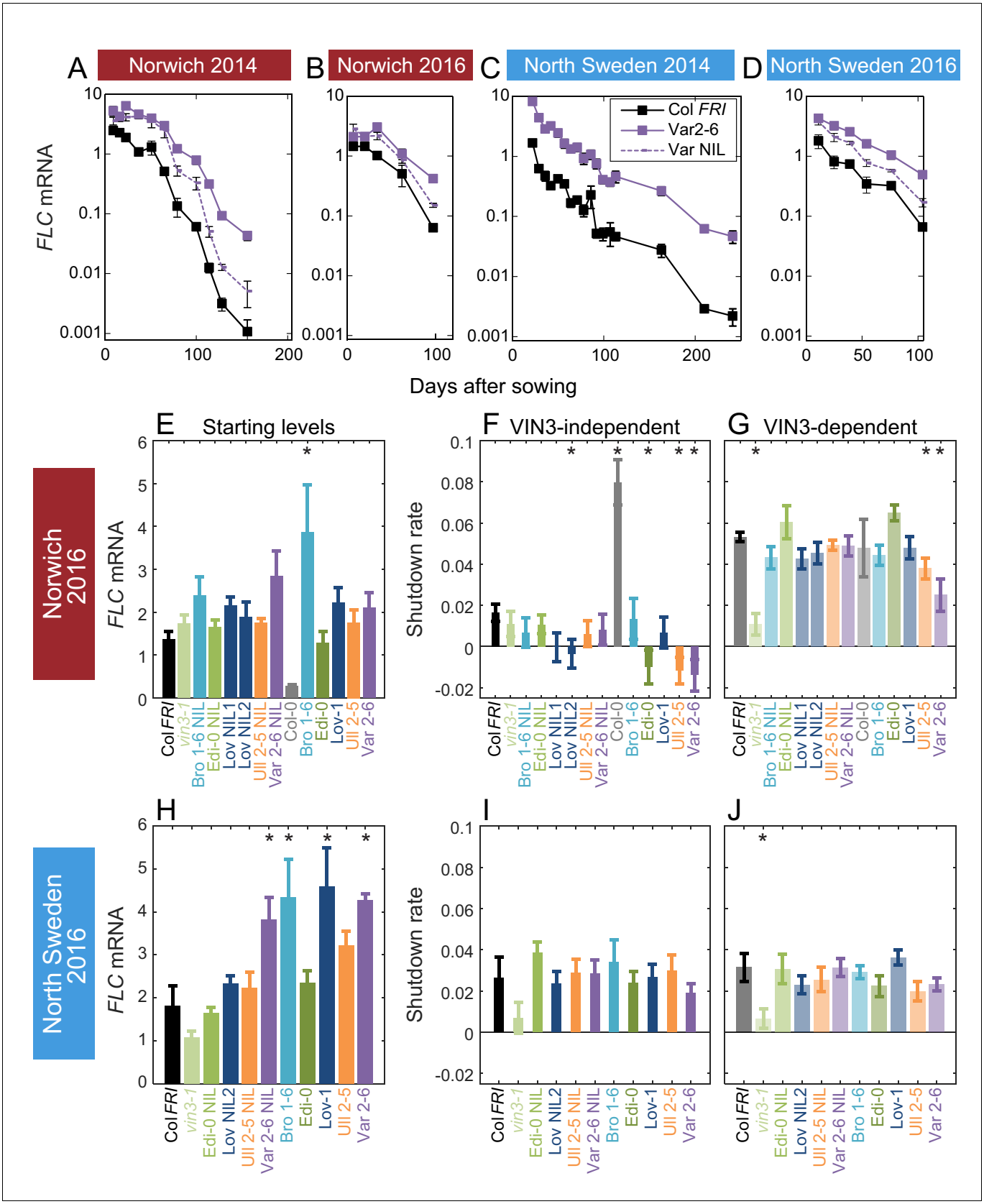

Figure 3. Downregulation in 2016 in Norwich and North Sweden for NILs and accessions show similar patterns of response to the first year. (**A–D**) *FLC* downregulation in Col *FRI*, Var2-6 and the Var NIL, as measured for Norwich and North Sweden in the winters of 2014–5 and 2016–7. (**E–J**) *FLC*

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Figure 3 continued

downregulation as Starting level and VIN3-independent and dependent rates. Features of genotypes that are significantly different to the reference line Col *FRI* are indicated by * (for Starting levels, ANOVA with Dunnett's post-hoc test, for Shutdown rates, Satterthwaite's t-tests on REML Linear mixed model). p-values for all comparisons are given in ***Supplementary file 1***. *VIN3* induction started at: Norwich 2016, ~48 days, North Sweden 2016,~46 days, see (***Figure 3—figure supplement 1***) Expression data were normalised to the corresponding control sample (2016–7, see Materials and methods). N = 6 except where samples were lost to death or degradation (see Materials and methods and ***Source data 3***). Rates of downregulation are given in units of 'a.u. per day', where the arbitrary units (a.u.) correspond to the normalised concentration of *FLC* mRNA. Error bars of bar plots show s.e., of line graphs show s.e.m.

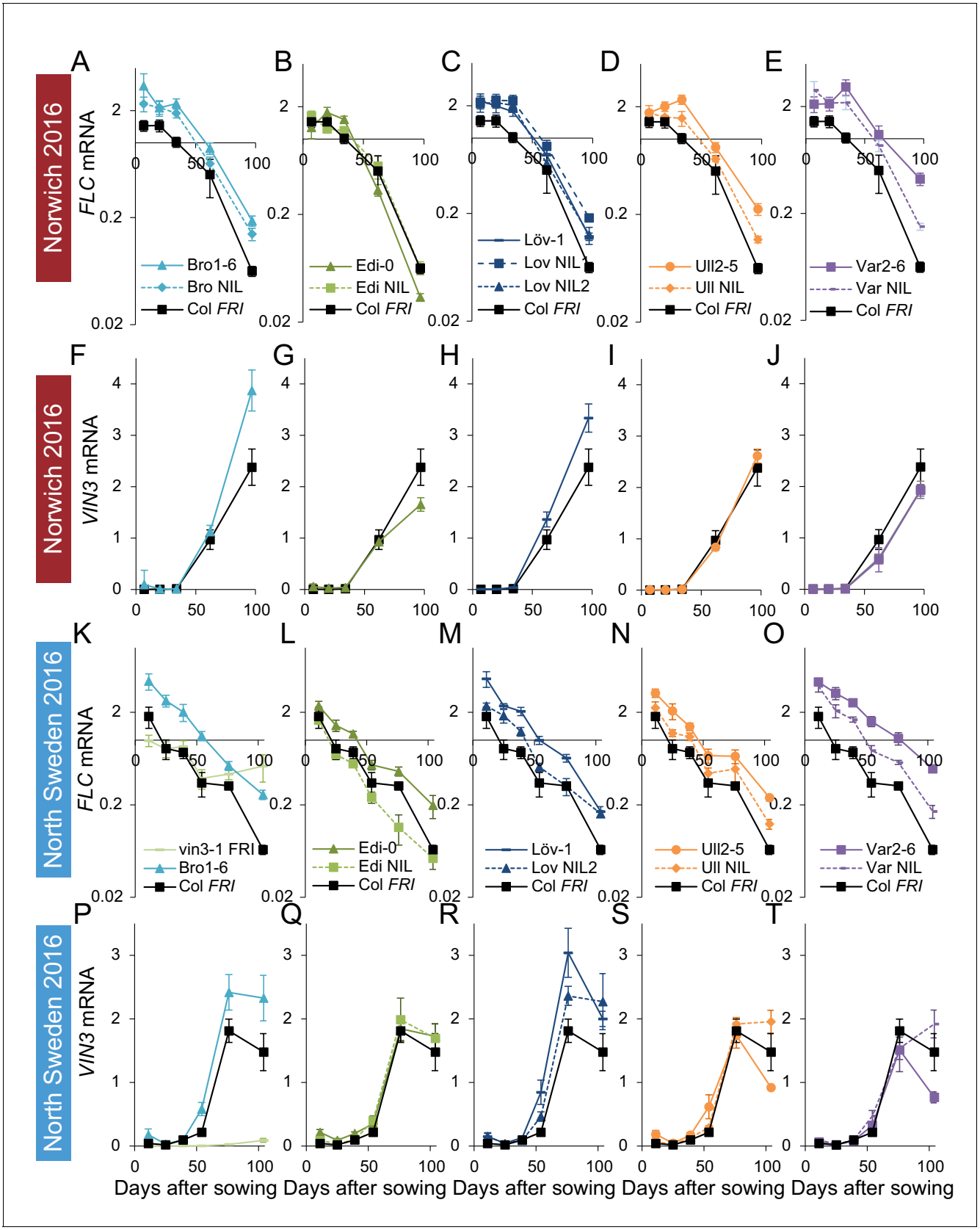

Figure 3—figure supplement 1. *FLC* downregulation and *VIN3* upregulation in accessions in Norwich and North Sweden in autumn/winter 2016. Expression normalised to control sample for 2016–7 (see Materials and methods). (**A–E**) Norwich *FLC* mRNA, (**F–J**) Norwich *VIN3* mRNA, (**K–O**) North

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Sweden *FLC* mRNA, (**P–T**) North Sweden *VIN3* mRNA. N = 6 except where samples were lost to death or degradation (see Materials and methods and ***Source data 3***). Error bars show s.e.m.

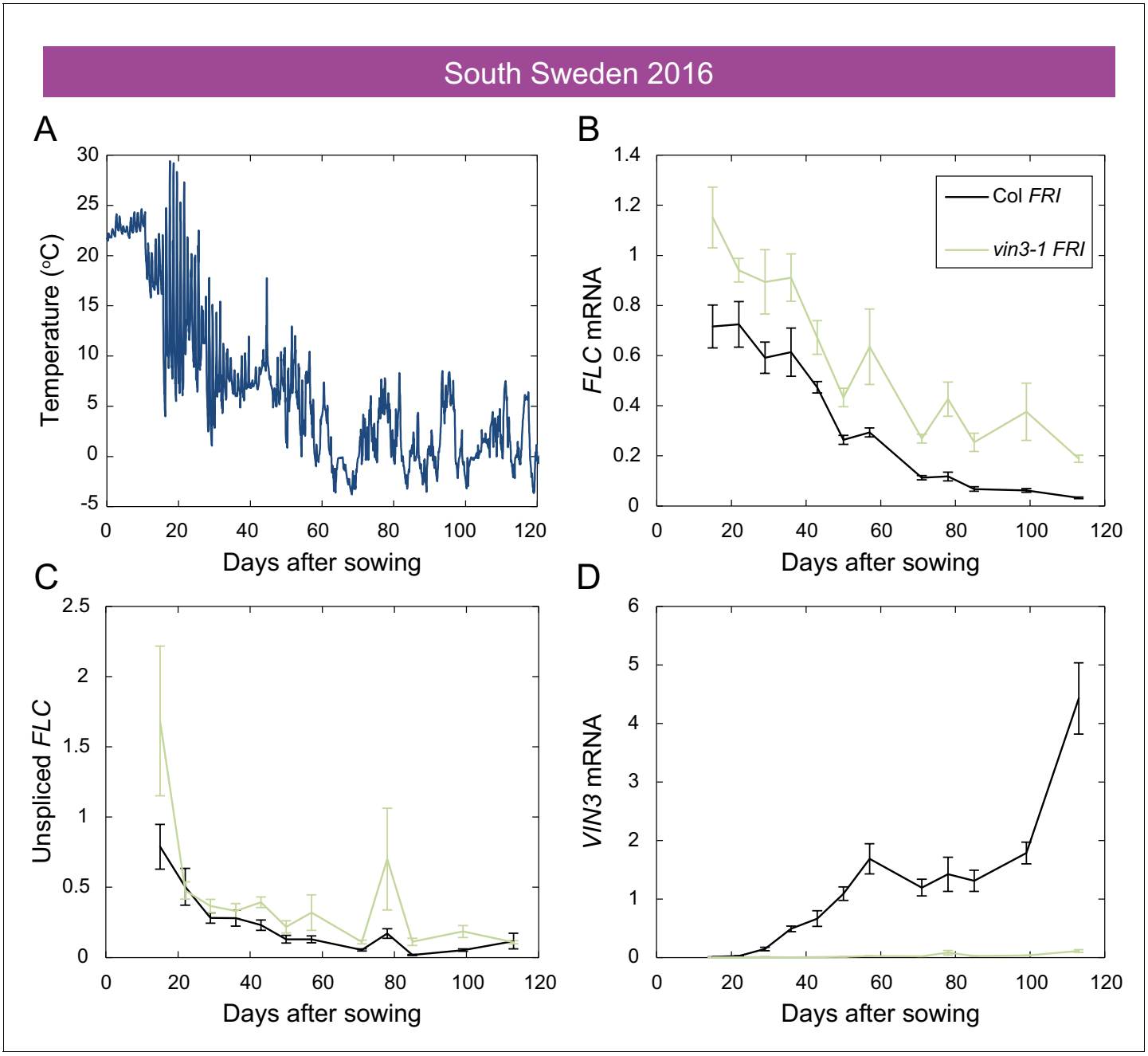

Figure 3—figure supplement 2. Downregulation of *FLC* and upregulation of *VIN3* in South Sweden in 2016. (**A**) Hourly temperature readings from plant level in South Sweden 2016. (**B**) *FLC* mRNA levels in the Col *FRI* and *vin3-1 FRI* accessions over autumn, with *vin3-1* showing less repression, especially later in the season. (**C**) Unspliced *FLC* levels. (**D**) *VIN3* mRNA levels, with *vin3-1 FRI* showing no induction. Expression normalised to control sample for 2016–7 (see Materials and methods). N = 6 except where samples were lost to death or degradation (see Materials and methods and ***Source data 3***). Error bars show s.e.m.

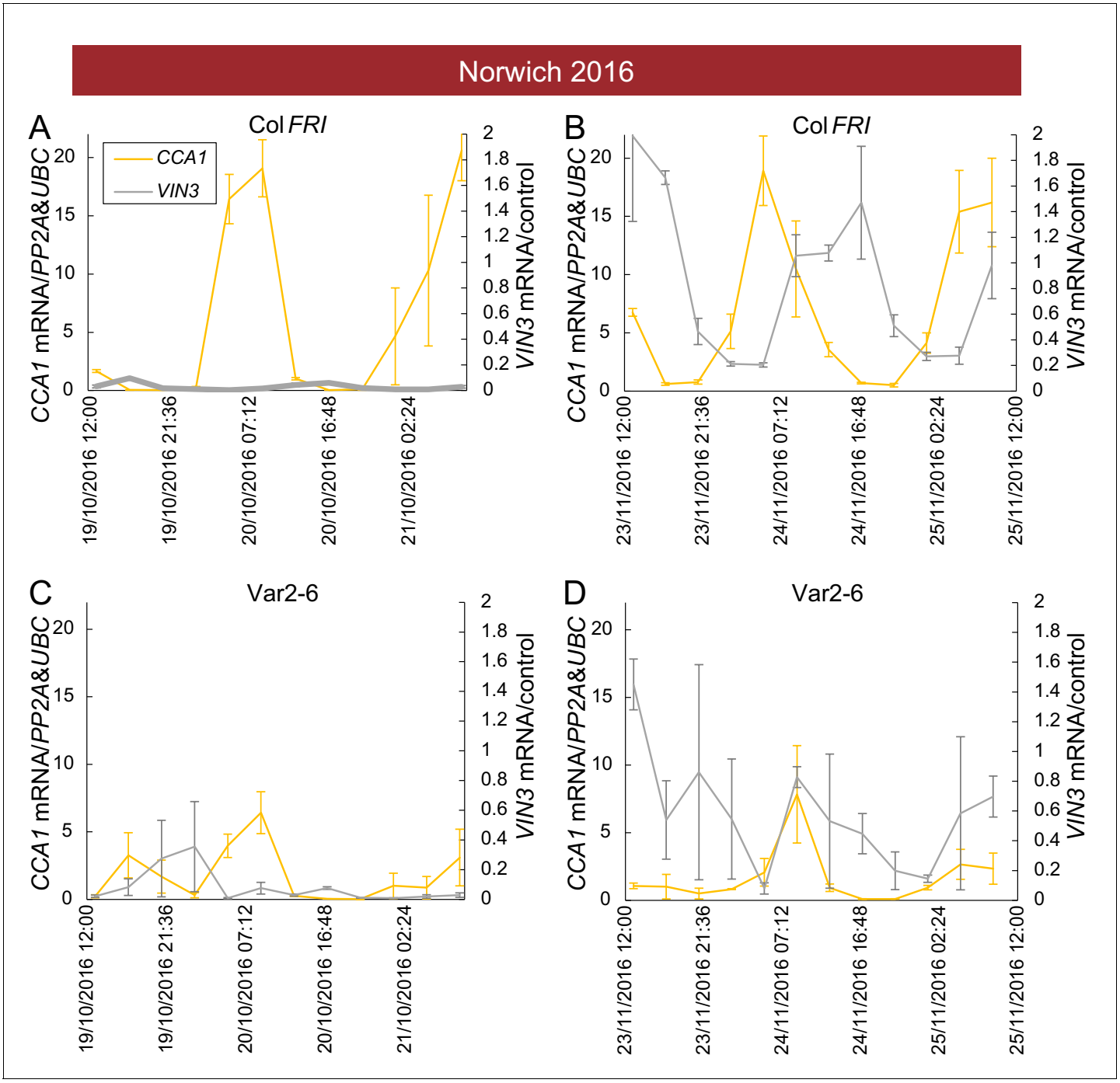

Figure 3—figure supplement 3. Low *VIN3* upregulation in Var2-6 is correlated with perturbation of the circadian clock. *VIN3* and *CCA1* expression measured over 48 hr in the field glasshouse in Norwich in 2016 in Col *FRI* and the Var2-6 accession. *CCA1* shows a circadian pattern throughout autumn in Col *FRI*, as does *VIN3* when it is upregulated later in the year. In Var2-6, *CCA1* expression is low, as is *VIN3* expression later in the year. (**A**) Expression in Col *FRI* in October. (**B**) Expression in Col *FRI* in November. (**C**) Expression in Var2-6 in October. (**D**) Expression in Var2-6 in November. N = 3, ***Source data 3***. Error bars show s.e.m.

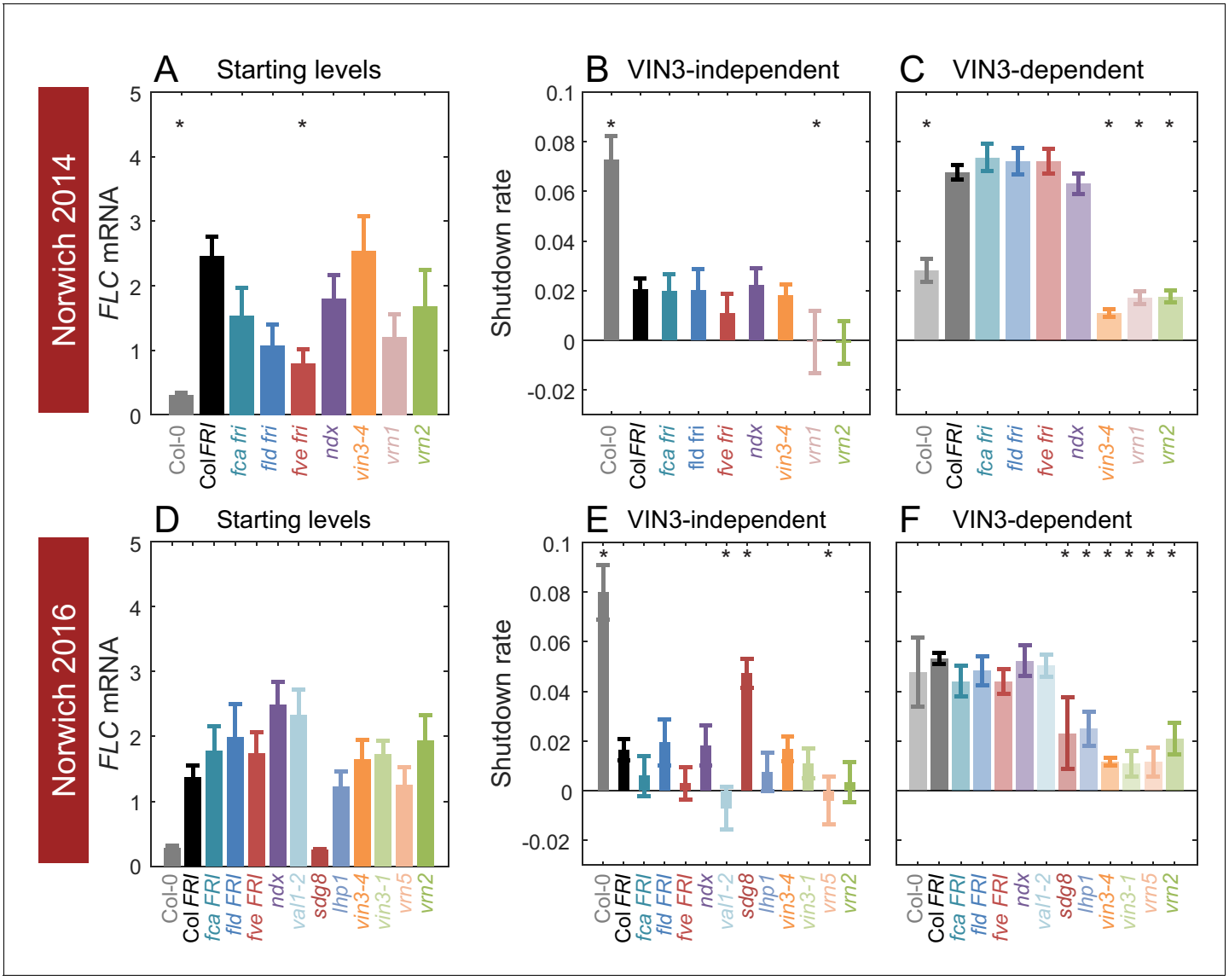

Figure 4. Starting levels and rates of downregulation of *FLC* in mutants and transgenics in field conditions in Norwich, UK. (**A–F**) *FLC* downregulation analysed as level at first time point (Starting levels, **A, D**), rate of downregulation before induction of *VIN3* expression (VIN3-independent, dark bars, **B, E**) and rate of downregulation after *VIN3* induction (VIN3-dependent, translucent bars, **C, F**). Features of genotypes that are significantly different to the reference line Col *FRI* are indicated by * (for Starting levels, ANOVA with Dunnett's post-hoc test, for Shutdown rates, Satterthwaite's t-tests on REML Linear mixed model). p-values for all comparisons are given in ***Supplementary file 1***. Rates of downregulation are given in units of 'a.u. per day', where the arbitrary units (a.u.) correspond to the normalised concentration of *FLC* mRNA. *VIN3* induction started at: Norwich 2014, ~58 days, see (***Figure 4—figure supplement 1***); Norwich 2016, ~48 days, see (***Figure 4—figure supplement 2***). All mutants are in the Col *FRI* background unless otherwise stated. Expression data were normalised to the corresponding control sample (for 2014–5 or 2016–7, see Materials and methods). N = 6 except where samples were lost to death or degradation (see Materials and methods and ***Source data 2*** and ***3***). Error bars show s.e.

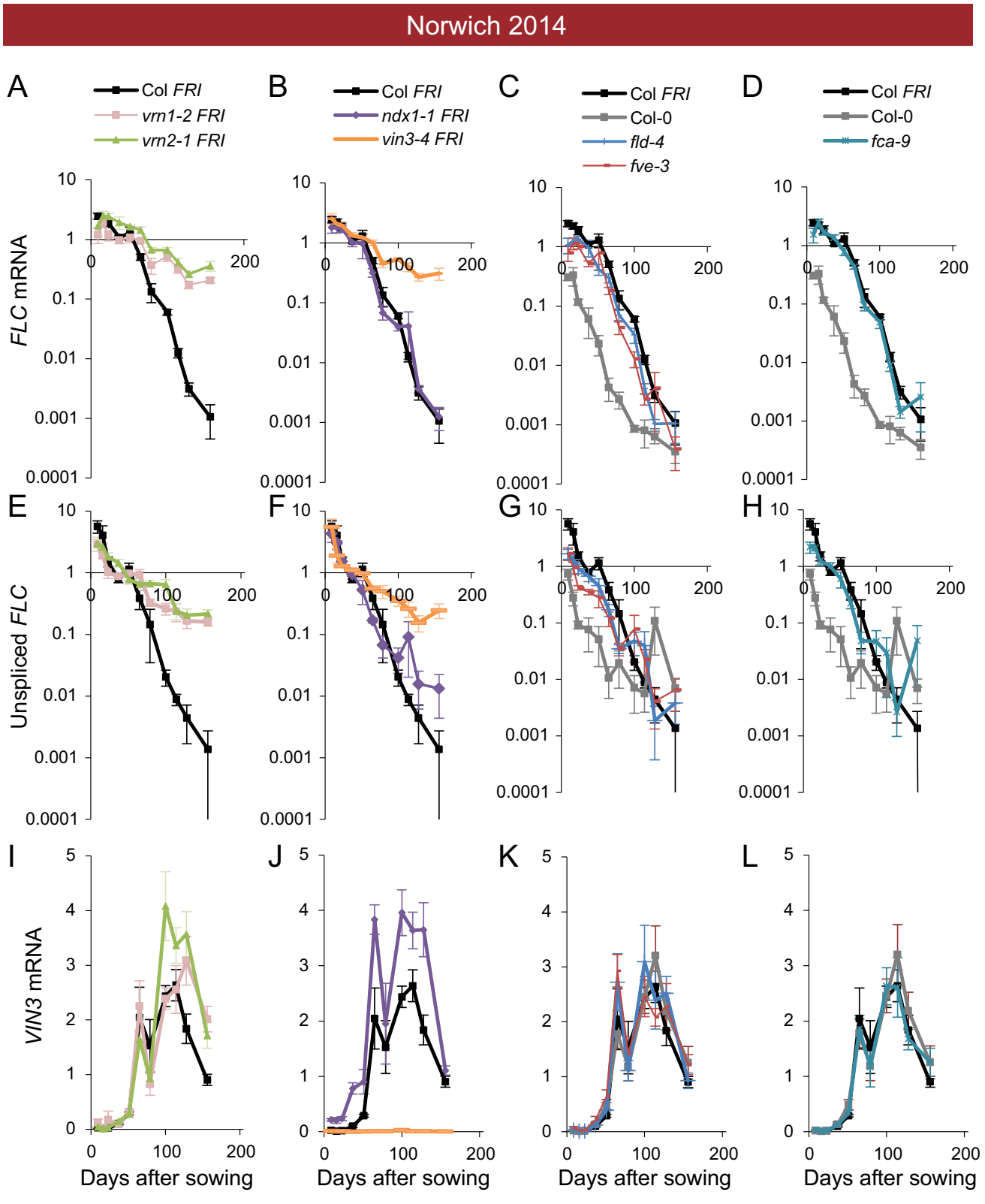
Norwich 2014
A
Col FRI
vrn1-2 FRI
vrn2-1 FRI
B
Col FRI
ndx1-1 FRI
vin3-4 FRI
C
Col FRI
Col-0
fld-4
fve-3
D
Col FRI
Col-0
fca-9
FLC mRNA
E
F
G
H
Unspliced FLC
I
J
K
L
VIN3 mRNA
Days after sowing

Figure 4—figure supplement 1. Expression of *FLC* and *VIN3* in all mutants in the field in Norwich 2014–2015. Expression normalised to control sample for 2014–5 (see Materials and methods). (**A–D**) *FLC* mRNA, (**E–H**) unspliced *FLC* transcript, (**I–L**) *VIN3* mRNA. N = 6 except where samples were lost to death or degradation (see Materials and methods and ***Source data 2***). Error bars show s.e.m.

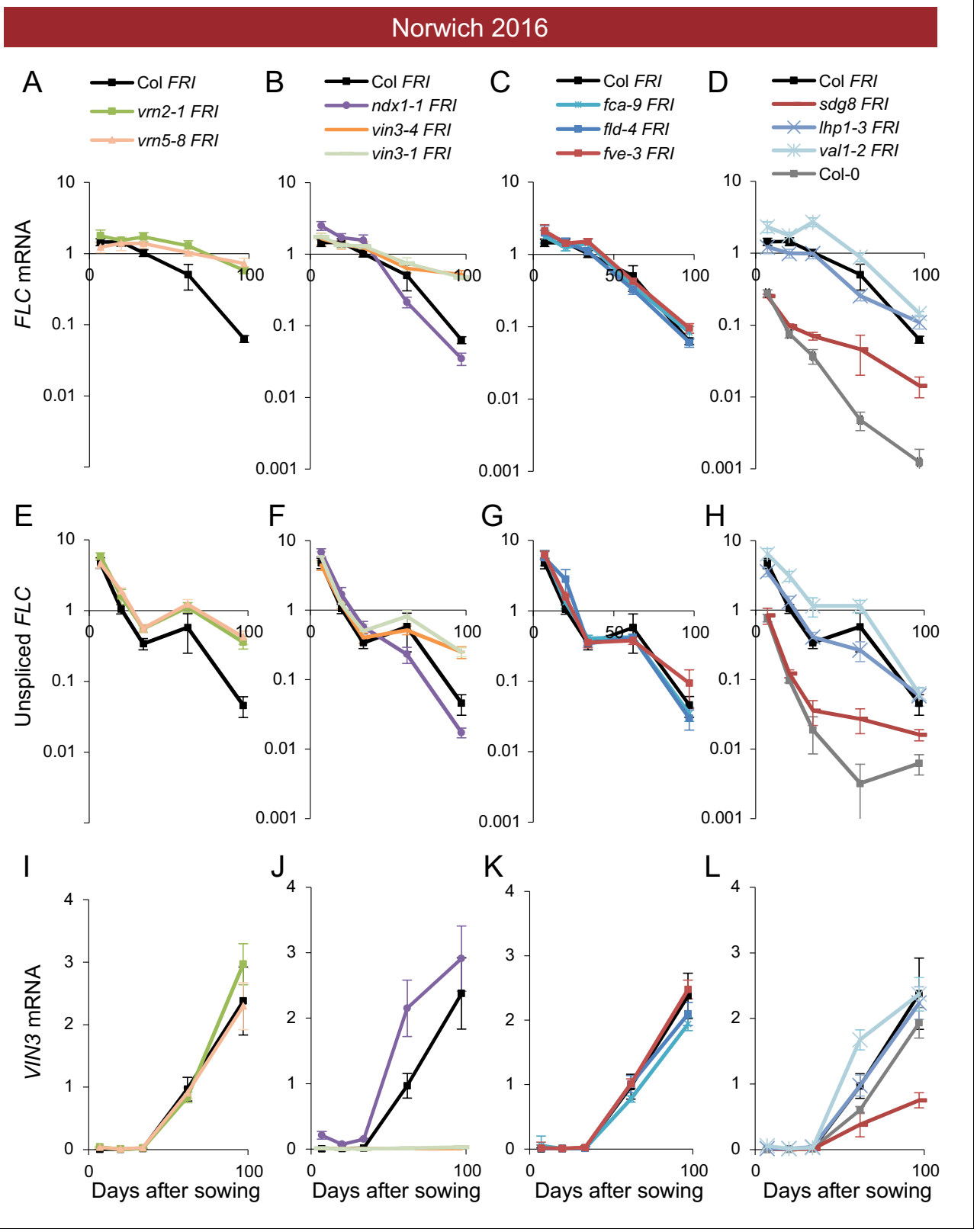
Norwich 2016
A
Col FRI
vrn2-1 FRI
vrn5-8 FRI
B
Col FRI
ndx1-1 FRI
vin3-4 FRI
vin3-1 FRI
C
Col FRI
fca-9 FRI
fld-4 FRI
fve-3 FRI
D
Col FRI
sdg8 FRI
lhp1-3 FRI
val1-2 FRI
Col-0
FLC mRNA
E
F
G
H
Unspliced FLC
I
J
K
L
VIN3 mRNA
Days after sowing
Days after sowing
Days after sowing
Days after sowing

Figure 4—figure supplement 2. Expression of *FLC* and *VIN3* in all mutants in the field in Norwich 2016–2017. Expression normalised to control sample for 2016–7 (see Materials and methods). (**A–D**) *FLC* mRNA, (**E–H**) unspliced *FLC* transcript, (**I–L**) *VIN3* mRNA. N = 6 except where samples were lost to death or degradation (see Materials and methods and ***Source data 2***). Error bars show s.e.m.

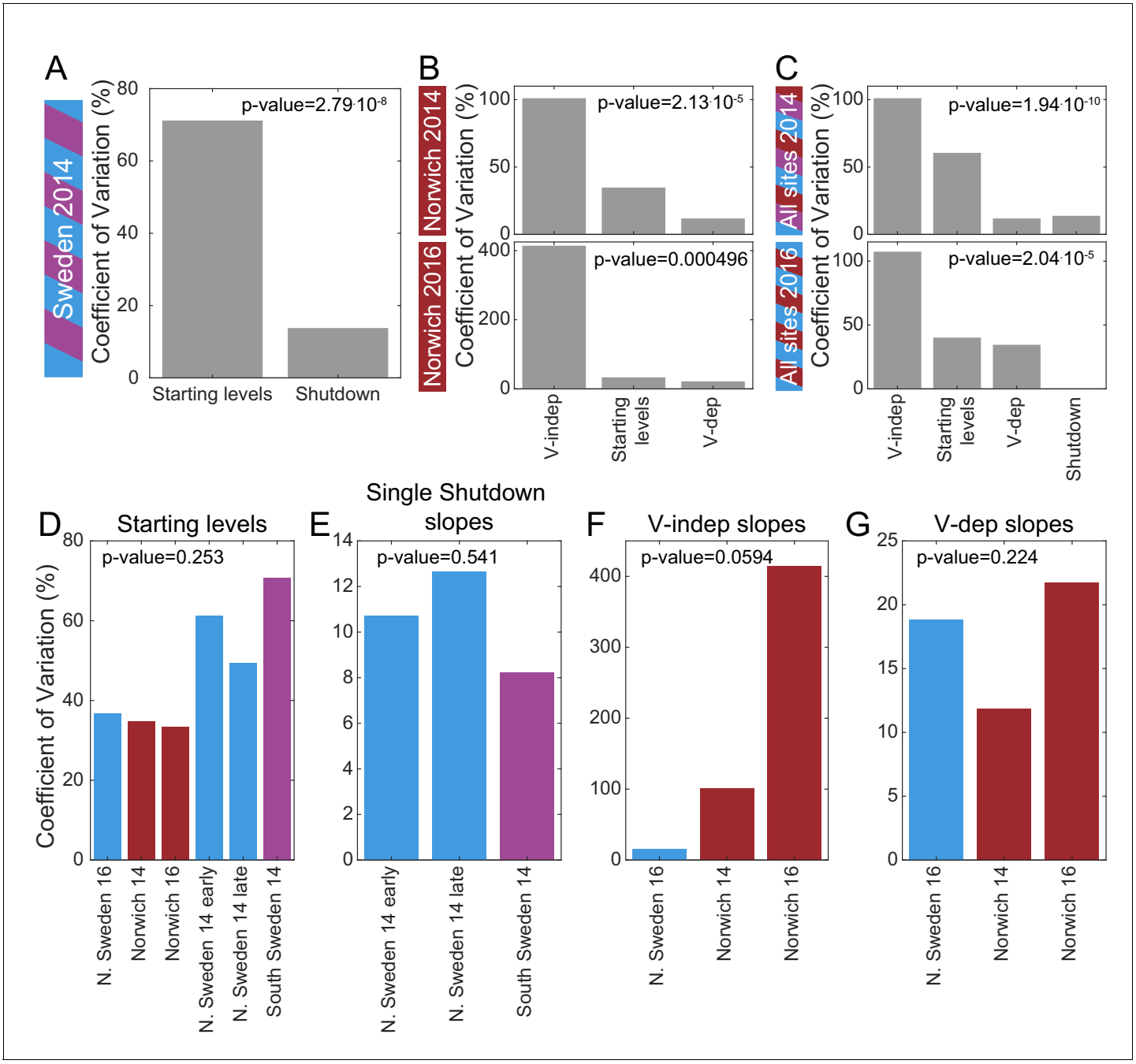

Figure 5. Mechanistic sources of natural variation in *FLC* levels across sites and years. (**A**) The coefficient of variation for the rates of shutdown and for the starting levels in all Sweden experiments in the first year. (**B**) Similarly in Norwich 2014 (top) and 2016 (bottom) but separately for the VIN3-independent (V-indep) and VIN3-dependent (V-dep) shutdown rates. (**C**) Comparison of the variability of the starting levels and the shutdown rates, separating V-dep and V-indep where appropriate, combining data from all sites in 2014 (top) and 2016 (bottom). 'Shutdown' refers to the combined V-dep/V-indep shutdown rate that was fitted in Sweden 2014, and so is not present in the 2016 results. (**D**) The coefficients of variation of the starting levels for each site/year. (**E**) The coefficients of variation of the single shutdown rates for the different plantings and sites in Sweden in 2014. (**F–G**) Similarly, for Sweden 2016 and Norwich in both years, separating the V-indep rates (**F**) and V-dep (**G**). Data from ***Source data 2*** and ***3***.

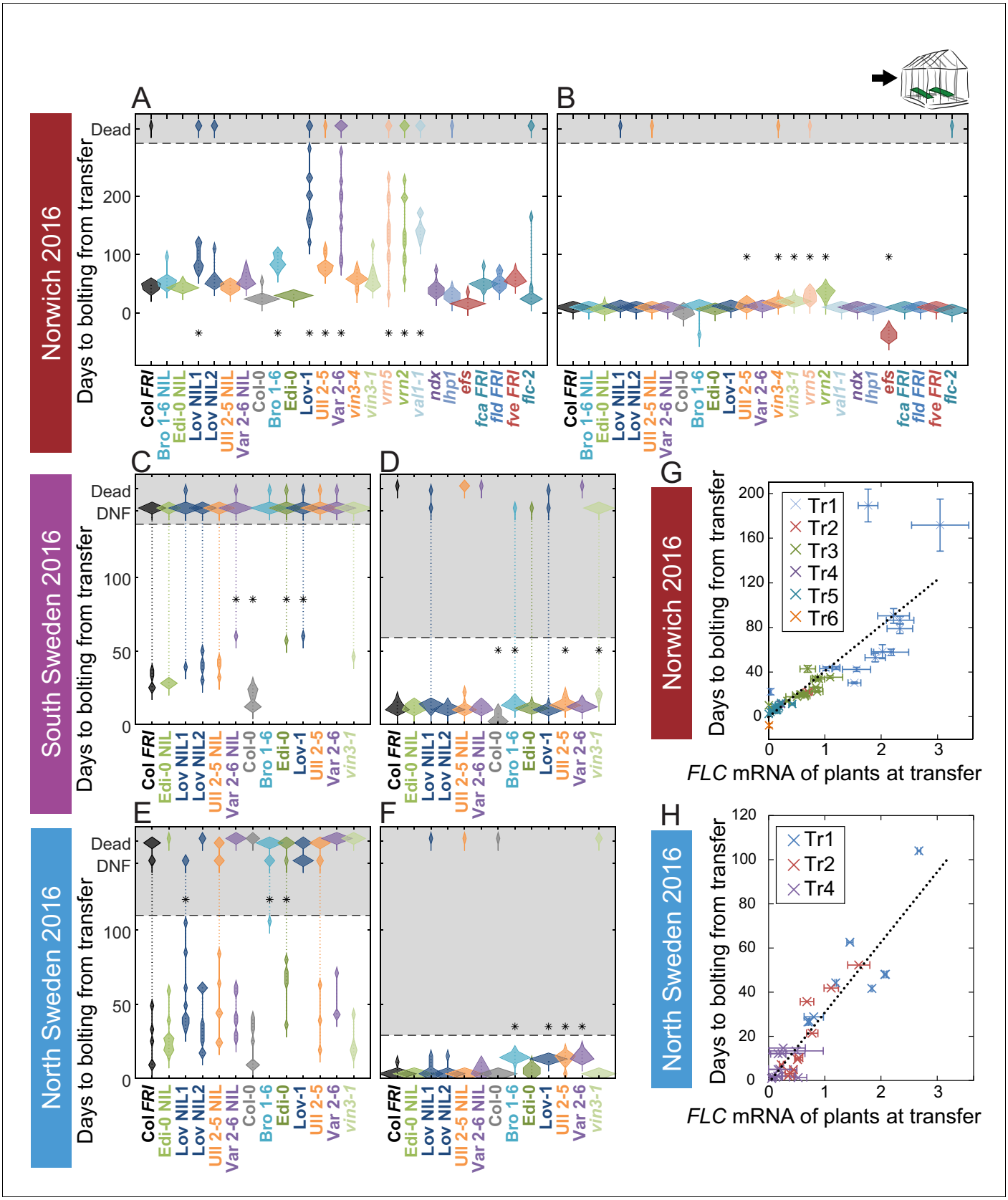

Figure 6. Vernalization requirement for *FLC* downregulation is saturated in natural winters. (**A–F**) Bolting time for accessions and NILs after transfer to floral-induction conditions from 'natural' winter 2016–7, in (**A**) Norwich 21/10/16 (**B**) Norwich 21/12/16 (**C**) South Sweden 01/10/2016 (**D**) South Sweden

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17/12/16 (**E**) North Sweden 06/09/2016 (**F**) North Sweden 24/11/2016. Plants that did not flower by 14/02/17 (**C, D**) or 23/12/16 (**E, F**) are shown as DNF and dead plants are indicated. Plots show the histogram of numbers of plants as the width of violin plots. A line connects the measurements to indicate the range. Flowering time of genotypes that are significantly different to the reference line Col *FRI* are indicated by * (ANOVA with Dunnett's post-hoc test). p-values for all comparisons are given in ***Supplementary file 3***. (**G–H**) North Sweden 2016 transfers for accessions and NILs, (**G**) mean time to bolting after transfer to floral-inductive conditions plotted against mean *FLC* expression per genotype at transfer, Norwich 2016–7, linear regression $R^2 = 0.68$, $p<0.001$. (**H**) Mean time to bolting after transfer to floral-inductive conditions plotted against mean *FLC* expression per genotype at transfer, North Sweden 2016–7. Genotypes that did not bolt within 205 days not shown, linear regression $R^2 = 0.85$, $p<0.001$. N = 12 plants except where plants died or (**E, H**) did not bolt within 205 days (***Source data 5***). Error bars for G and H show s.e.m.

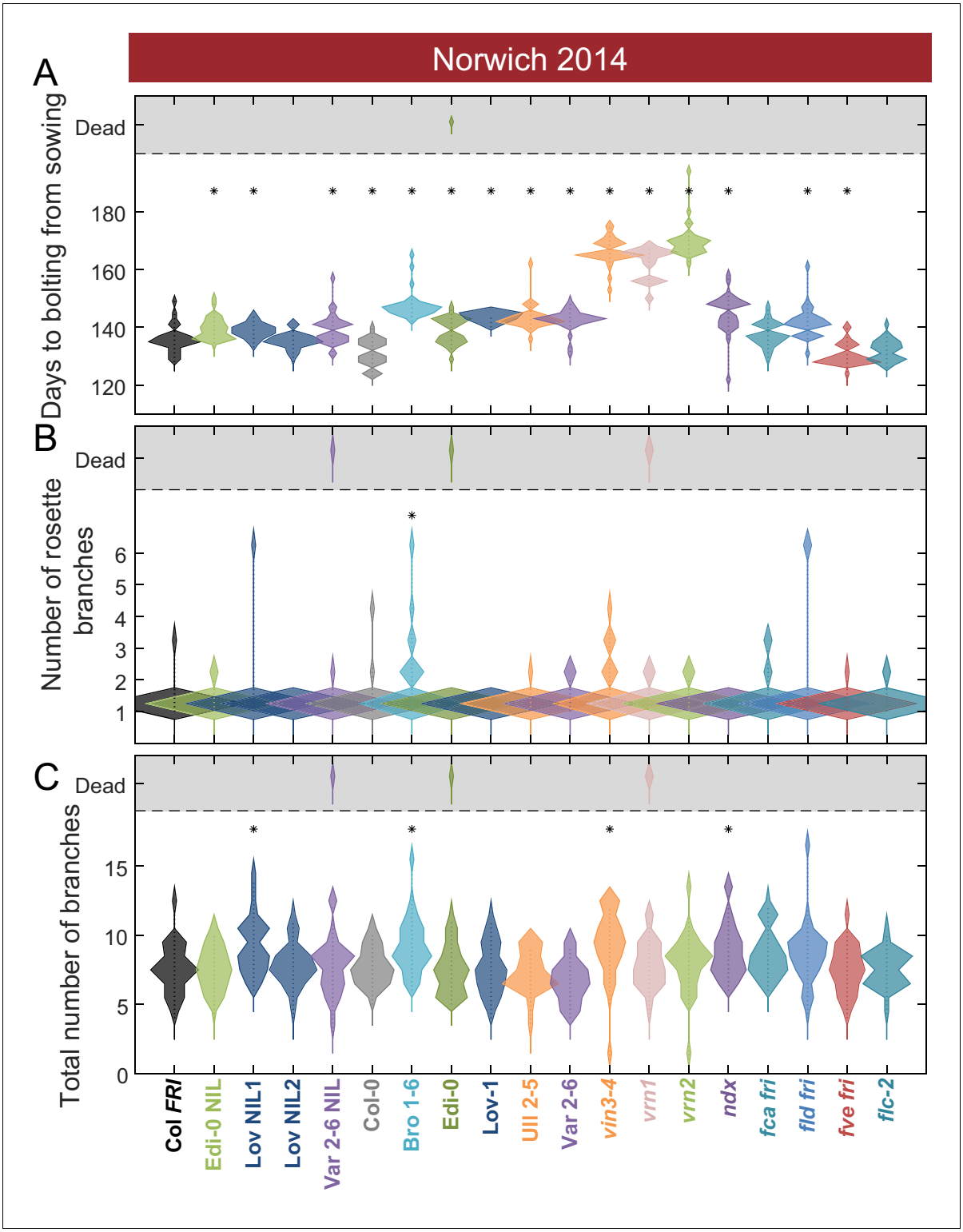

Figure 6—figure supplement 1. Flowering after winter in Norwich 2014–5 in the field was largely synchronous. (**A**) Time to bolting for each genotype in the 'field' glasshouse in Norwich 2014–5 experiment. (**B**) Number of rosette branches for plants shown in A. (**C**) Number of rosette and cauline branches for plants shown in A. Plots A-C show the histogram of numbers of plants as the width of violin plots. A line connects the measurements to indicate the range. N = 36, except where plants did not germinate, ***Source data 4***. Features of genotypes that are significantly different to the reference line Col *FRI* are indicated by * (ANOVA with Dunnett's post-hoc). p-values for all comparisons are given in ***Supplementary file 3***.

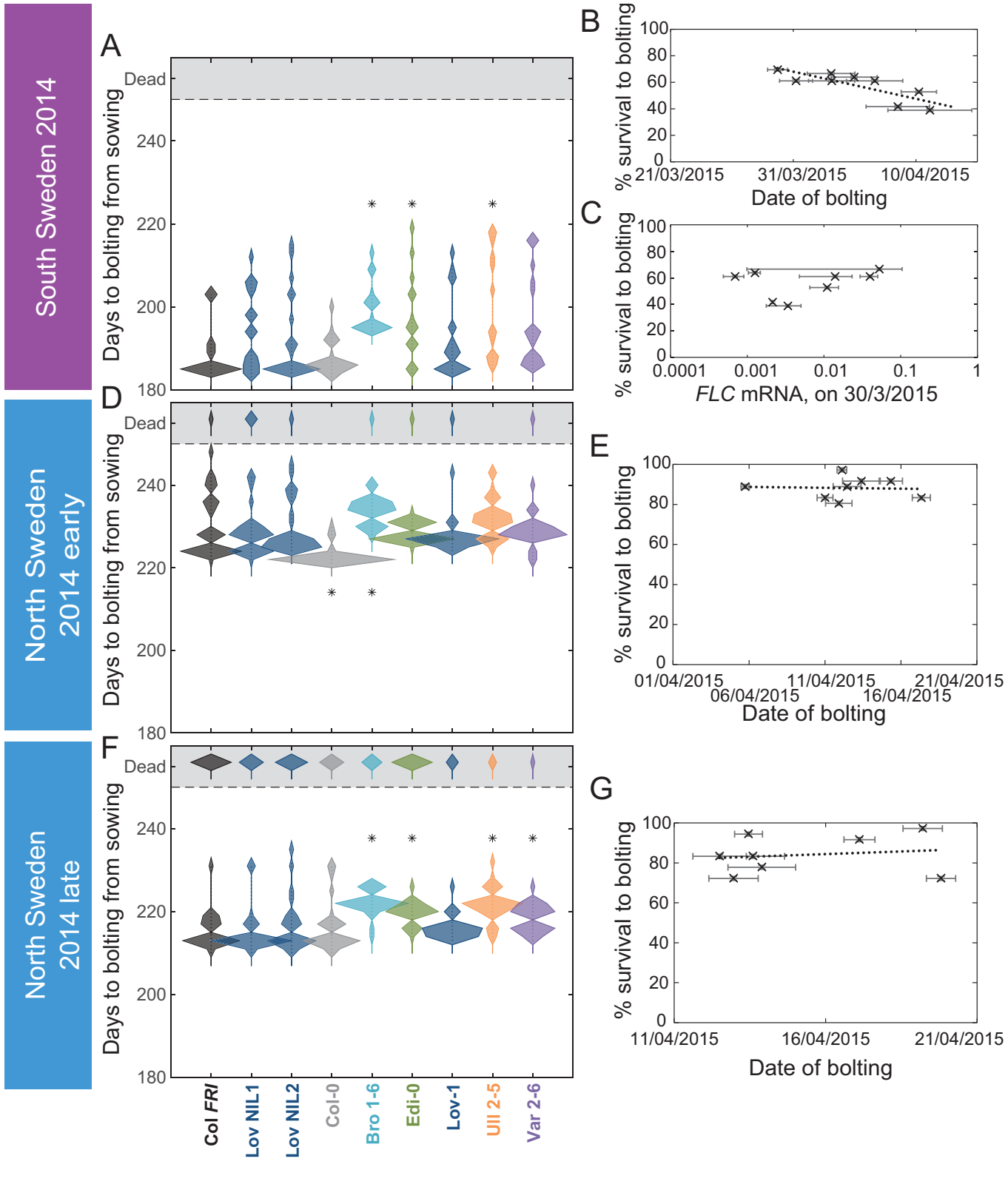

Figure 6—figure supplement 2. The transition to flowering after natural winters in South and North Sweden 2014–5 in the field was largely synchronous, while later bolting had a negative effect on survival only in South Sweden. (**A, D, F**) Time to bolting for each genotype, showing the

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histogram of numbers of plants as the width of violin plots. A line connects the measurements to indicate the range. Flowering time of genotypes that are significantly different to the reference line Col *FRI* are indicated by * (ANOVA with Dunnett's post-hoc). p-values for all comparisons are given in ***Supplementary file 3***. (**B, E, G**) Percentage of germinated plants of each genotype surviving to date of bolting, plotted against the mean date of bolting for that genotype. (**B**) South Sweden, Generalised Linear Models (GLMs) for binomial distribution, survival vs. date of bolting, p=0.0416. (**C**) South Sweden, percentage survival vs. mean *FLC* mRNA per genotype (normalised to control sample for 2014–5) on 30th March, GLM for binomial distribution, ns. (**E, G**) GLM for binomial distribution, ns. N = 36, except where plants died before flowering, ***Source data 4***. Error bars on scatter plots show s.e.m.

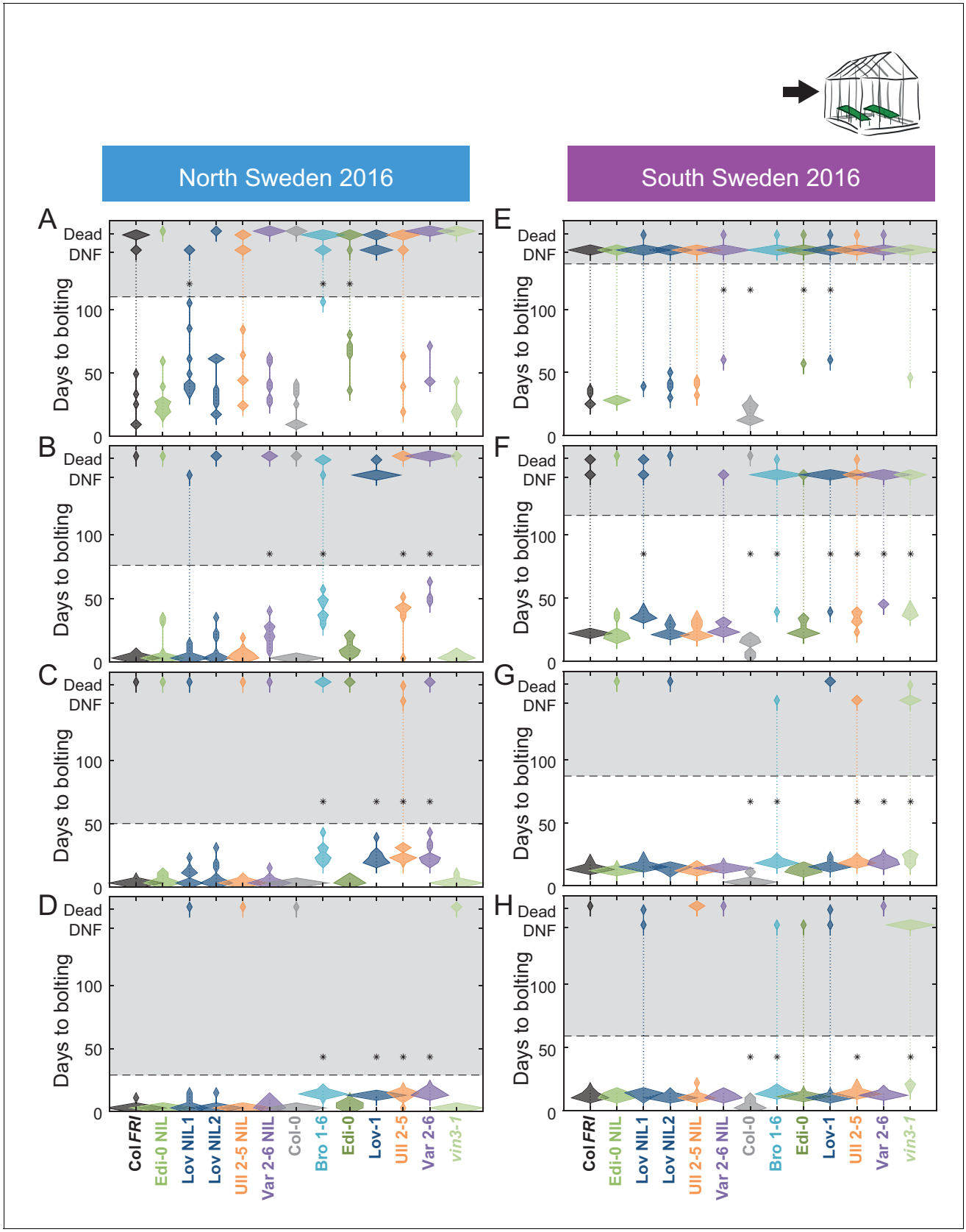

Figure 6—figure supplement 3. Bolting after transfer to warm, long-day conditions from winter in the field 2016–7 saturates at different rates in different genotypes in Sweden. Bolting time from sequential transfers to long-day warm conditions from the field, for each genotype and transfer. Plots

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show the histogram of numbers of plants as the width of violin plots. A line connects the measurements to indicate the range. Flowering time of genotypes that are significantly different to the reference line Col *FRI* are indicated by * (ANOVA with Dunnett's post-hoc). p-values for all comparisons are given in ***Supplementary file 3***. (**A–D**) North Sweden, transfer dates: 06/09/2016, 04/10/2016, 01/11/2016, 24/11/2016, experiment ended on 23/12/16. (**E–H**) South Sweden, transfer dates: 01/10/2016, 22/10/2016, 19/11/2016, 17/12/2016, experiment ended on 14/02/17. N = 12, except where plants died before flowering, ***Source data 5***.

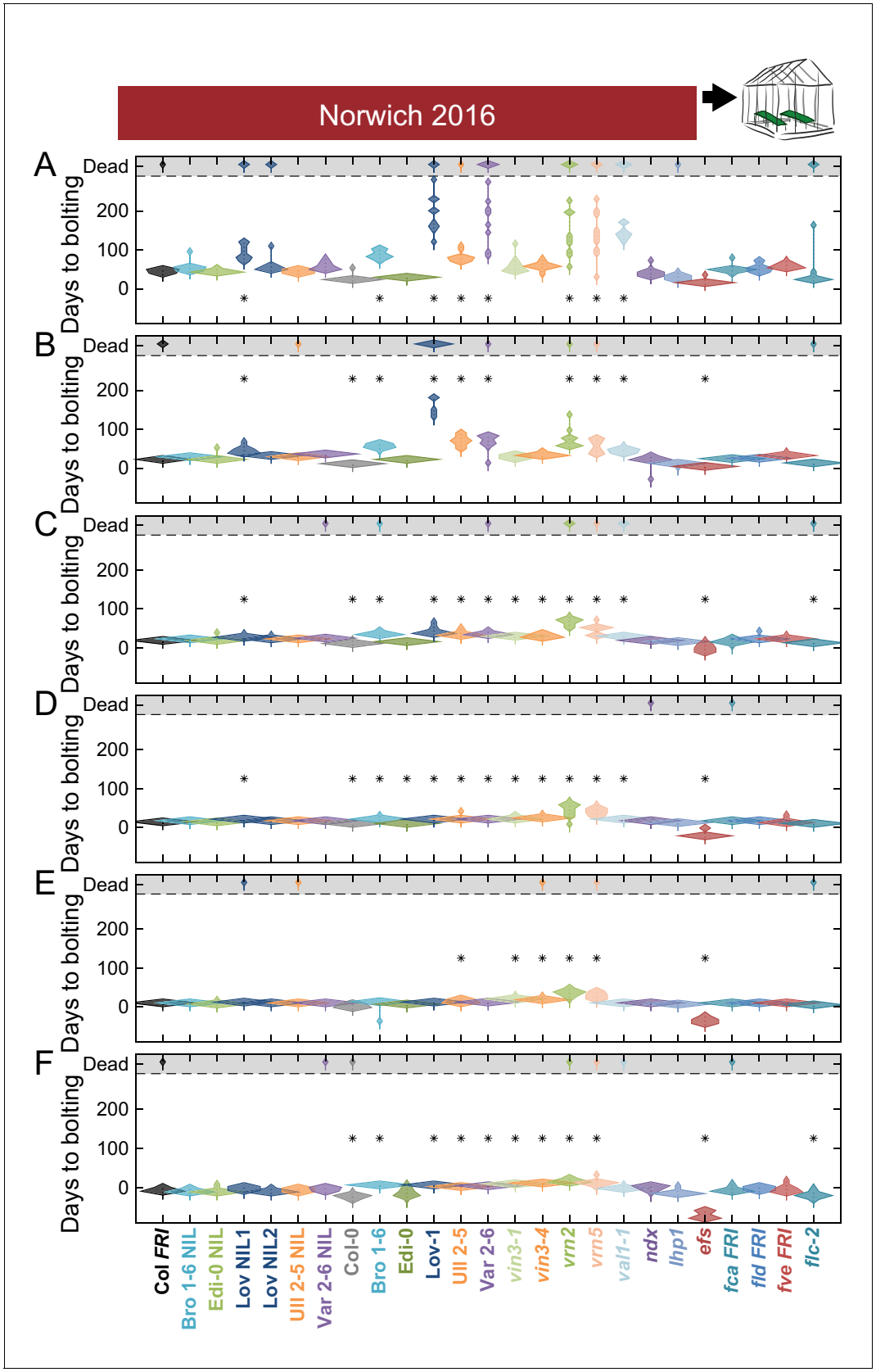

Figure 6—figure supplement 4. Bolting after transfer to warm, long-day conditions from winter in the field 2016–7 saturates at different rates in Norwich. Bolting from sequential transfers to long-day warm conditions from the field, for each genotype and transfer. The experiment was run until all

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Figure 6—figure supplement 4 continued

plants died. Plots show the histogram of numbers of plants as the width of violin plots. A line connects the measurements to indicate the range. Flowering time of genotypes that are significantly different to the reference line Col *FRI* are indicated by * (ANOVA with Dunnett's post-hoc). p-values for all comparisons are given in ***Supplementary file 3***. Transfers dates are (**A**) 21/10/2016, (**B**) 03/11/2016, (**C**) 17/11/2016, (**D**) 30/11/2016, (**E**) 21/12/2016, (**F**) 26/01/2017. N = 12, except where plants died before flowering, ***Source data 5***.

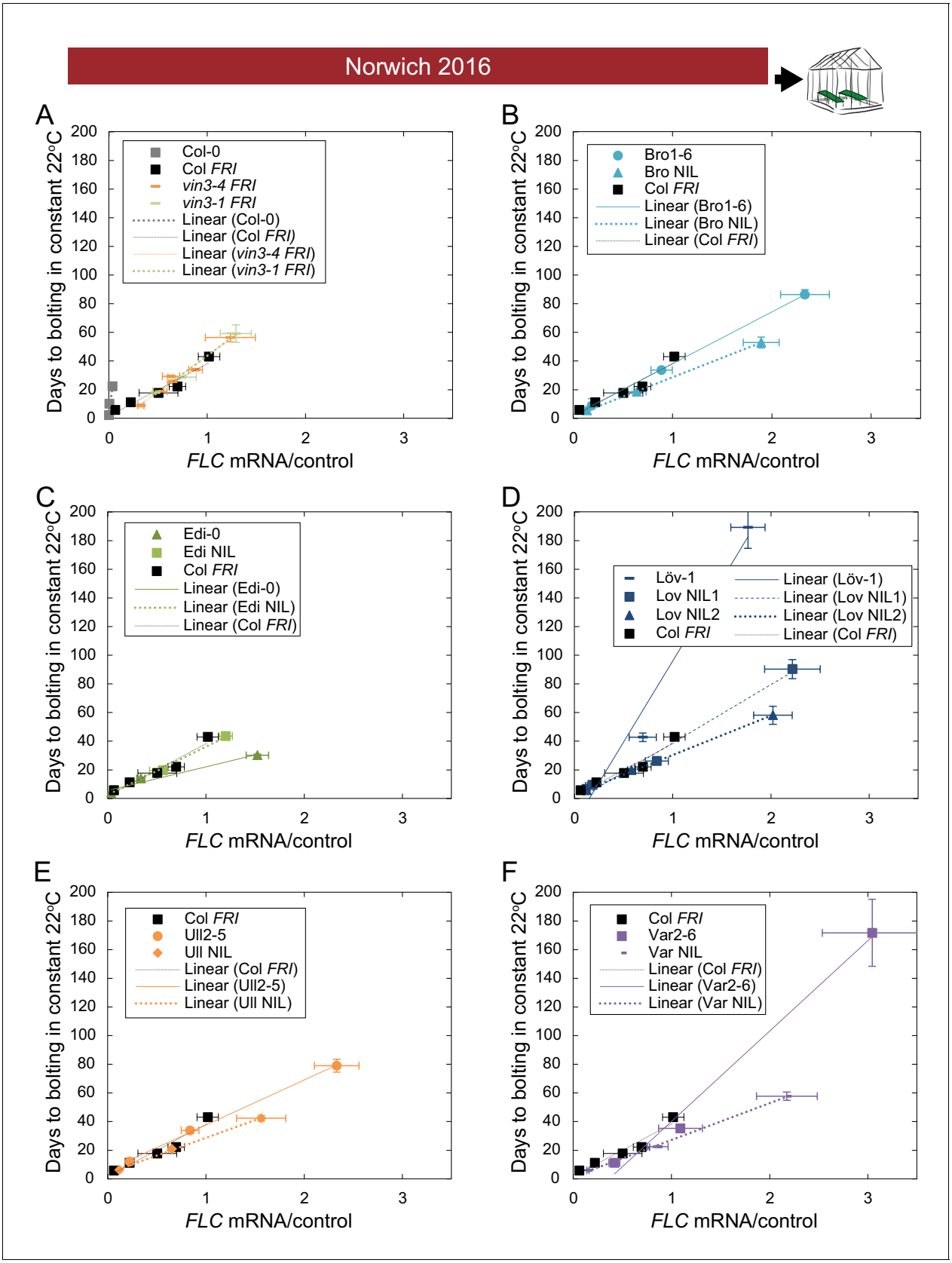

Figure 6—figure supplement 5. The relationship between time to floral transition and *FLC* expression at the end of cold (Norwich winter 2016–7) varies among accessions, both due to trans effects and due to the *FLC* alleles themselves. (**A–F**) Mean time to bolting of plants moved to a greenhouse

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Figure 6—figure supplement 5 continued

lit for 16 hr, and maintained at 22°C/18°C light/dark, plotted against the mean *FLC* mRNA expression from plants sampled in the Norwich field condition greenhouse on the day of transfer, with linear regression lines plotted for each genotype (the slope 'm' being the 'post-vern' value, with the y-intercept being the effect on days to bolt if the plants had no detectable *FLC*, see ***Table 1***). For all accessions and NILs over 3–6 transfers at different times during the winter, $R^2 = 0.68$ for linear regression, $p<0.001$. N = 6 for expression data, N = 12 for bolting data, except where plants died, ***Source data 3*** and ***5***. Error bars show s.e.m.

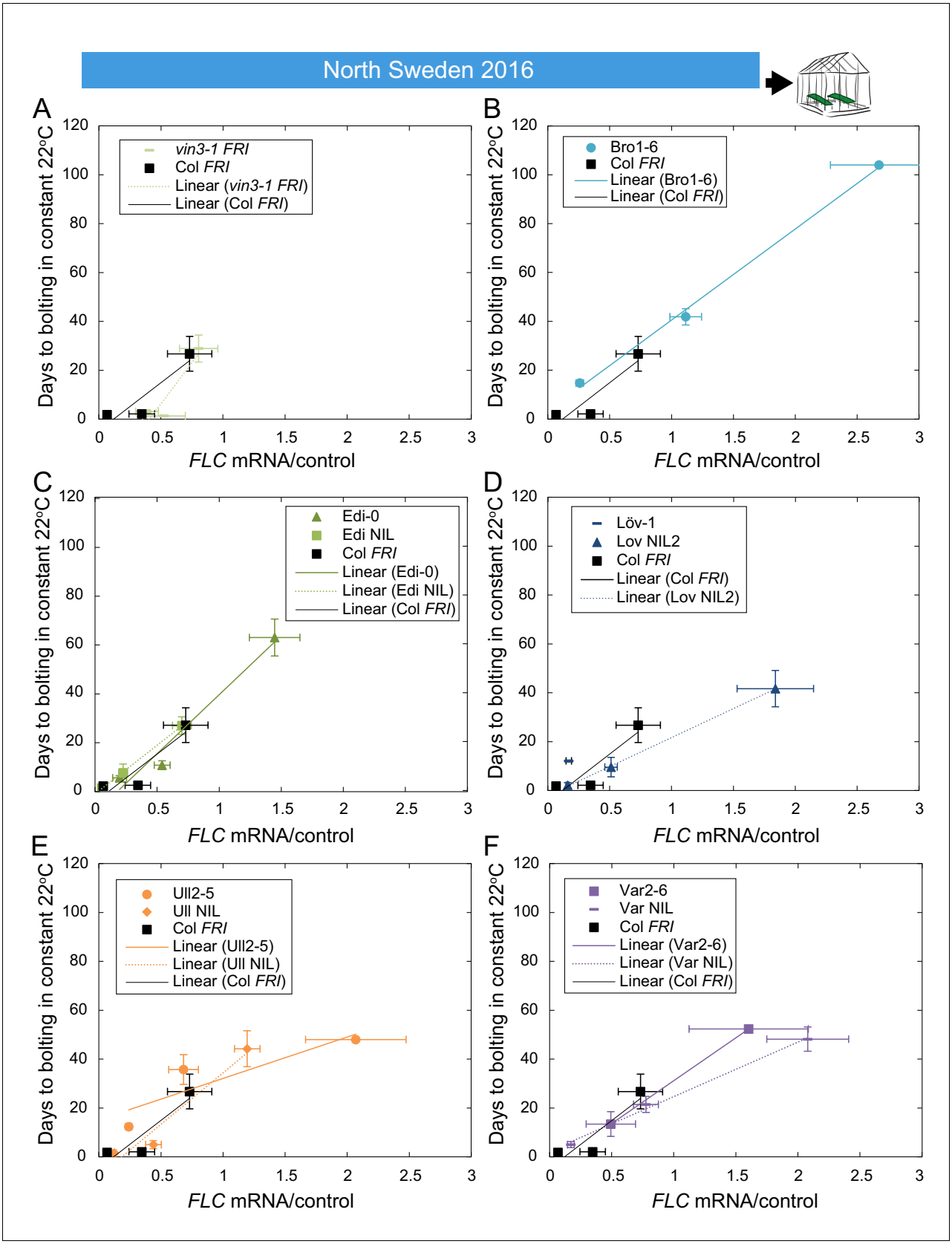

Figure 6—figure supplement 6. The relationship between time to floral transition and *FLC* expression at the end of cold in North Sweden winter 2016–7. Mean time to bolting of plants moved to a greenhouse lit for 16 hr, and maintained at 22°C, plotted against the mean *FLC* mRNA expression

Figure 6—figure supplement 6 continued on next page

Figure 6—figure supplement 6 continued

from plants sampled in the North Sweden field on or adjacent to the day of transfer, with linear regression lines plotted for each genotype (the slope 'm' being the 'post-vern' value, with the y-intercept being the effect on days to bolt if the plants had no detectable *FLC*, see ***Table 1***). (**A–F**) For all accessions and NILs over 3–6 transfers at different times during the winter, $R^2 = 0.68$ for linear regression, $p<0.001$. For D, there is no regression for Löv-1 as no Löv-1 plants from the first two transfers flowered within the 120 days of the experiment. N = 12 for bolting data, except where plants died, ***Source data 3*** and ***5***. Error bars show s.e.m.

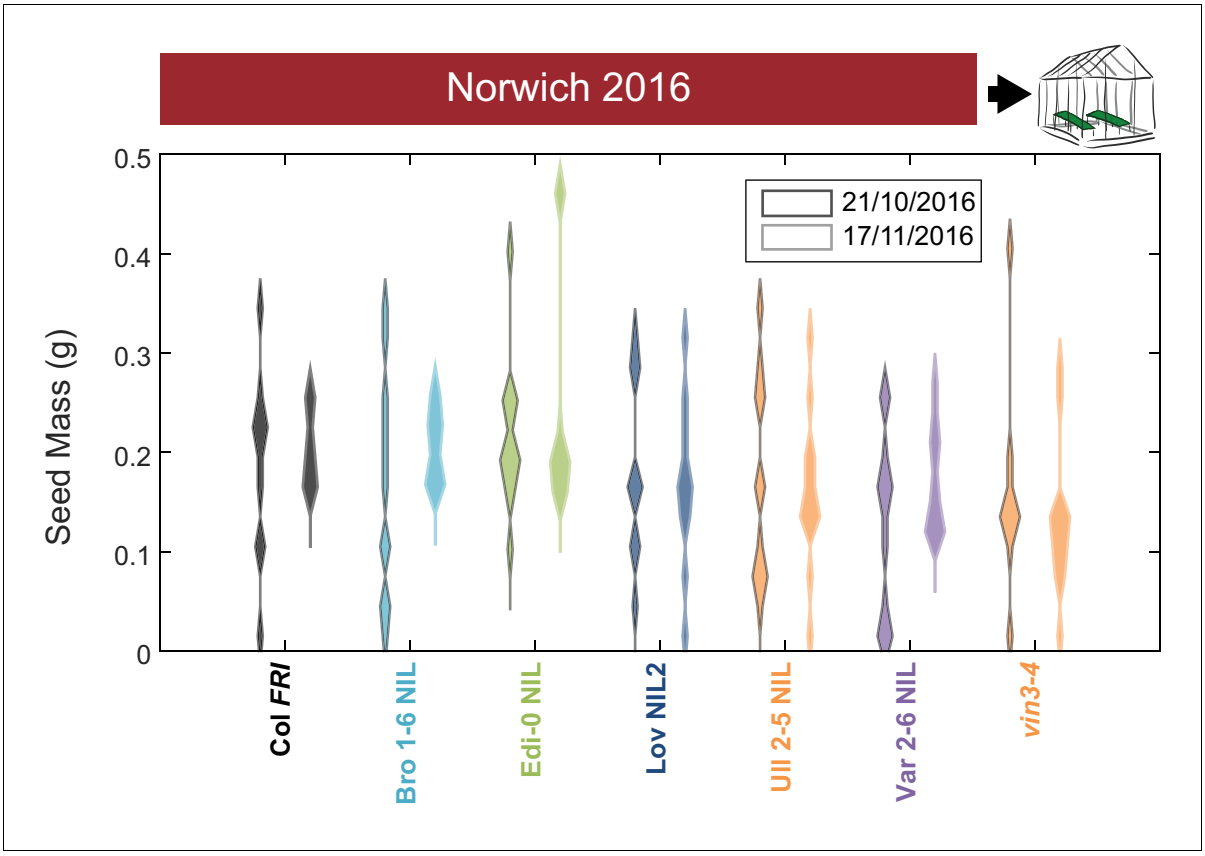

Figure 6—figure supplement 7. Increased vernalization increases the amount and reduces the variability of seed set. Total seed mass for Col *FRI*, NILs and the *vin3-4 FRI* mutant after transfer to floral-induction conditions from 'natural' winter in Norwich 2016–7 on 21/10/16 and 17/11/16 (flowering time in ***Figure 6—figure supplement 4A, C***). Plots show the histogram of numbers of plants as the width of violin plots. The time to bolt per plant negatively correlated with seed mass produced, $p<0.001$, Kenward-Roger's t-test on REML Linear mixed model with date of transfer as a random factor. N = 12 for transfer 21/10/2016, N = 8–12, mode = 12 for transfer 17/11/2015 due to losses, see ***Source data 5***.

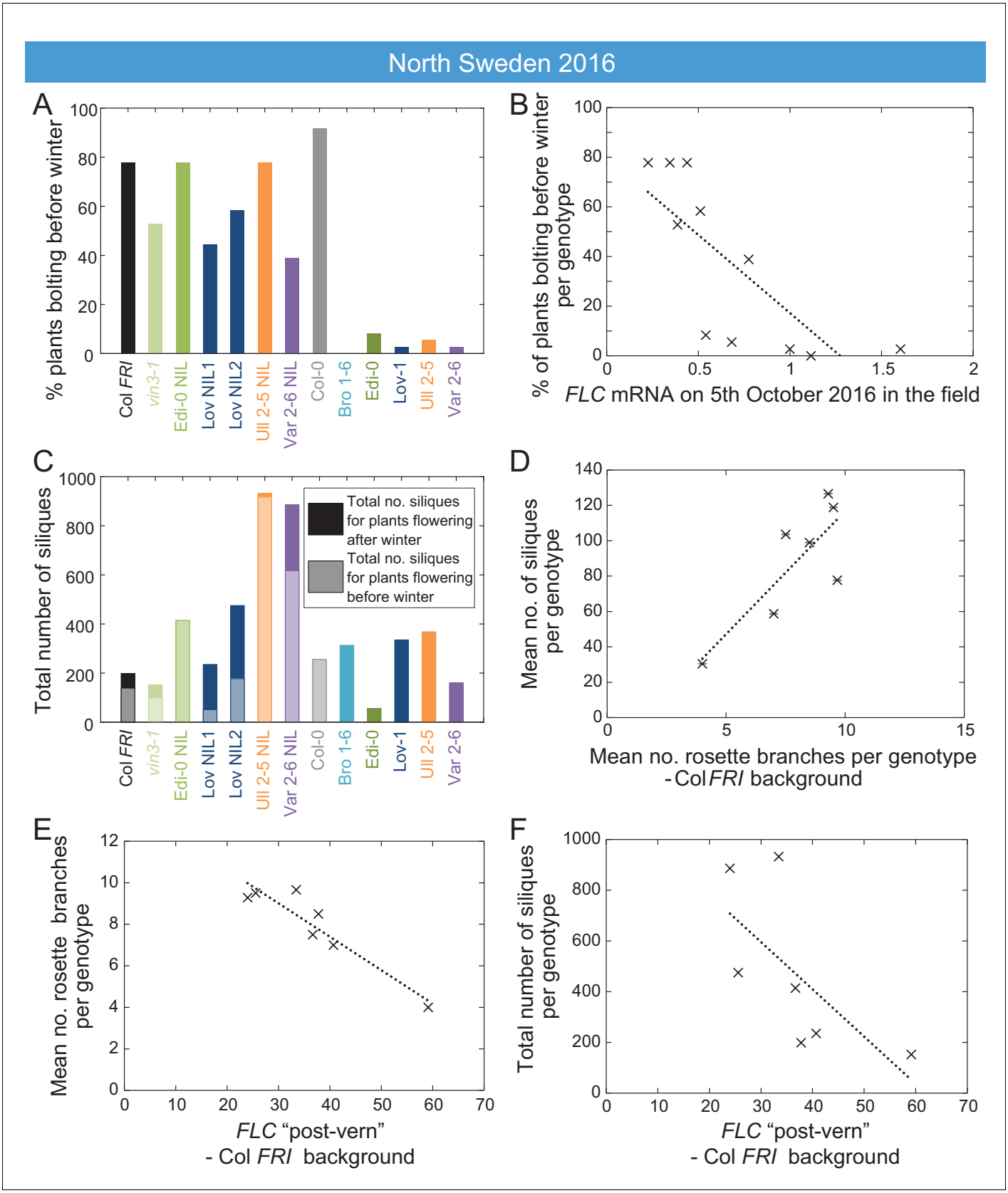

Figure 7. High FLC reduces precocious bolting in North Sweden in warm years. (**A**) Percentage of plants bolting before winter in the North Sweden 2016 experiment by genotype. Plants in the field were less likely to flower precociously before winter (18th November 2016) if they are accessions from

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more northerly latitudes or, to a lesser degree, if they are *FLC* introgression lines containing *FLC* haplotypes from SV accessions. (**B**) The percentage of plants transitioning to flowering before winter per genotype negatively correlated with *FLC* expression (normalised to control) on 5th October (R^2 = 0.59, p=0.0058). (**C**) Total number of siliques produced per genotype, showing contribution from plants that bolted before winter and plants that bolted after. Within the Col *FRI* genetic background there was no overall penalty in average silique number for surviving plants bolting before vs. after winter (92 and 77 per plant respectively, not significant in Mann-Whitney U test). (**D**) Mean silique production in plants surviving to set seed positively correlated to their mean rosette branch production for Col *FRI* genetic background genotypes (NILs and *vin3-4*; R^2 = 0.56, p-value=0.002). (**E**) Rosette branch production of Col *FRI* genotypes surviving to set seed is strongly negatively correlated with the *FLC* post-vern value for that genotype as from ***Table 1*** (R^2 = 0.86, p-value<0.002). (**F**) Total number of siliques produced by Col *FRI* background genotypes plotted against *FLC* post-vern, linear regression for post-vern effect alone, R^2 = 0.35, p-value=0.1. N = 36 plants sown (**A–C**), n for surviving plants (**D–F**) varies per genotype, see ***Source data 6***.

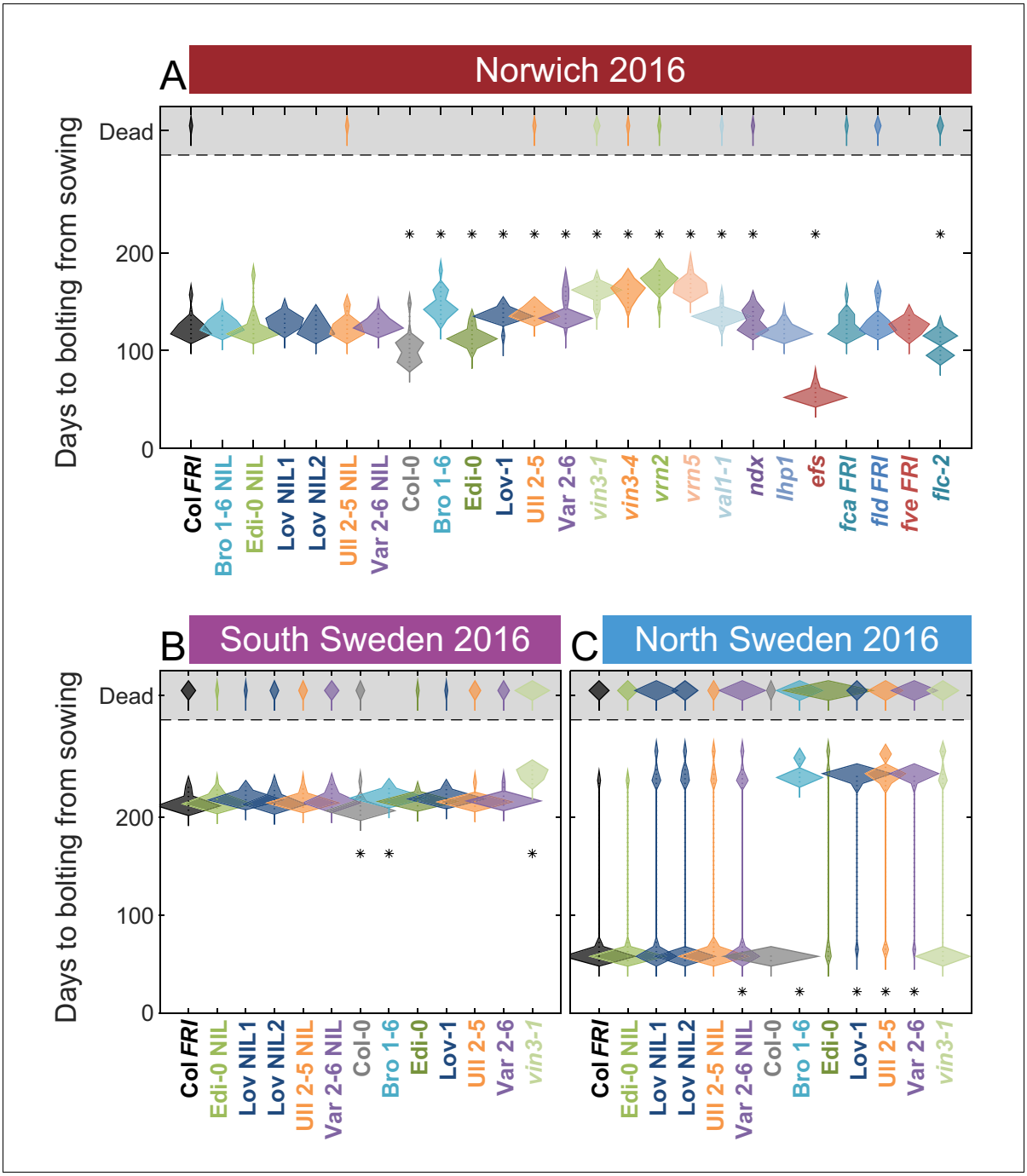

Figure 7—figure supplement 1. Flowering in the field across all sites in 2016–2017. Time to bolting for each genotype in (**A**) Norwich, (**B**) South Sweden and (**C**) North Sweden in 2016–7. Plots show the histogram of numbers of plants as the width of violin plots. A line connects the measurements to indicate the range. N = 24 for Norwich, N = 32 for South Sweden, N = 36 for North Sweden, ***Source data 6***. For Norwich 2016–7, nearby building works resulted in increased light pollution at night, possibly causing earlier flowering.

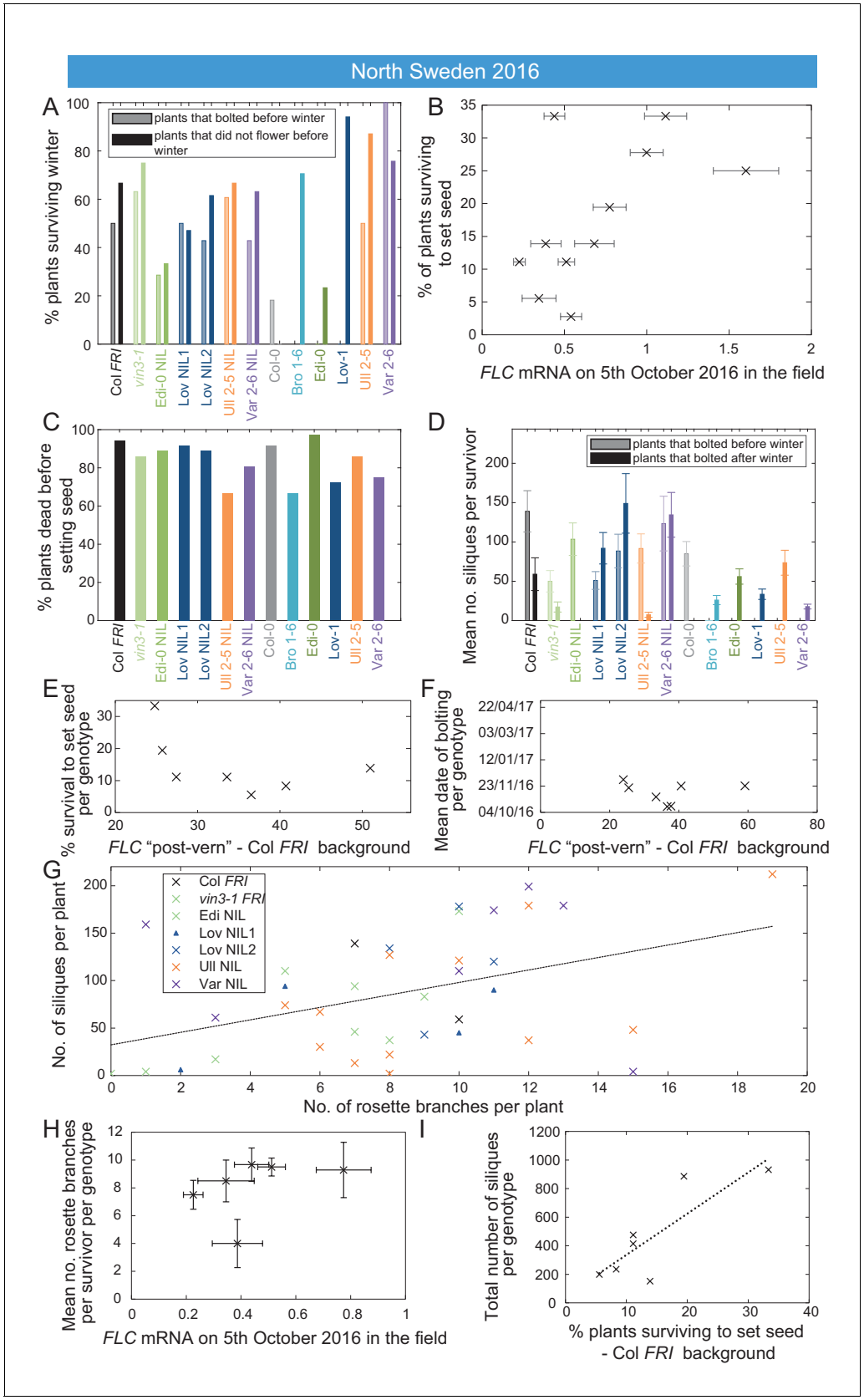

Figure 7—figure supplement 2. *FLC* affects fitness in North Sweden through bolting time and branching. Survival, branching and silique set in North Sweden are all correlated to aspects of *FLC* regulation. (**A**) Survival over winter of plants that bolted before winter in different genotypes vs. survival of

Figure 7—figure supplement 2 continued on next page

Figure 7—figure supplement 2 continued

plants that did not bolt before winter. (**B**) Survival to seed set plotted against *FLC* levels (normalised to control sample for 2016–7) in the field in North Sweden 2016 ($p<0.003$, Generalised Linear Models (GLMs) for binomial data). (**C**) Percentage mortality before setting seed was high for all genotypes. (**D**) Mean number of siliques for plants surviving to set seed that bolted before or after winter. (**E**) Survival in the field does not correlate with *FLC* post-vern for the Col *FRI* background (GLM with binomial distribution, p-value>0.1). (**F**) Date of bolting in the field does not correlate with *FLC* post-vern for the Col *FRI* background (linear regression, p-value>0.1). (**G**) Silique production by surviving Col *FRI* background plants correlates with number of rosette branches, though more weakly at the individual level than at the genotype average level (linear regression, $R^2 = 0.23$, p-value=0.004). (**H**) Rosette branching of surviving Col *FRI* background plants does not correlate with *FLC* mRNA as measured on 5th October in the field (linear regression, p-value>0.1). (**I**) Total number of siliques produced by Col *FRI* background genotypes plotted against percentage survival of that genotype to point of seed set, linear regression for survival effect alone, $R^2 = 0.64$, p-value=0.019. N = 36 plants sown (**A, B, C, E, I**) subsequent data based on survivors to seed set (**D, G, H**) and plants that survive to bolting (**F**), see ***Source data 6***. Error bars are s.e.m.