SUPPLEMENTARY INFORMATION:

SI MATERIALS AND METHODS

Plant Material

Seeds were sterilized, stratified for 2 days at 4°C and sown onto Gamborg's B-5 Basal Medium (Sigma; G5893-10L) supplemented with 0.6% wt/vol agar. At 18 days seedlings were transferred to soil (Debco Seed Raising Mix supplemented with 1g/L Osmocote Extracted Mini patterned release fertilizer pellets) and grown at a light intensity of 120-190 $\mu mol.m^{-2}.s^{-1}$ using fluorescent tubes in 16 hour day cycle at 22°C/18°C (day/night). Preanthesis floral buds and 15 day old seedlings were collected, frozen in liquid nitrogen and stored at -80°C. All hybrids, backcrosses and outcrosses were generated by hand pollination. C24 *sde4* mutants were a gift from David Baulcombe (S1). The C24 *polIV* mutant was a gift from Drs Andy Eamens and Ming Bo Wang and contains a G to A conversion at nucleotide position 2126 of NRPD1a (*Pol IV*) resulting in a premature stop codon.

Genotyping backcrosses and outcrosses

At1g64790 was genotyped by digesting the PCR product with *BamH*I (Fermentas; bisulphite primer sequences are found in Table S2). The C24 allele contains a single *BamH*I site producing two products (43bp and ~500bp) while Ler contains no *BamH*I cut site. PCR machine settings were: 95°C/5 minutes, (95°C/1 minute, 55°C/2 minutes, 72°C/1.5 minutes) x 35, 72°C/6 minutes.

At3g43340 was genotyped by PCR using a forward primer specific to either C24 or Ler (Table S2). PCR machine settings were: 95°C/5 minutes, (95°C/30 seconds, 55°C/30 seconds, 72°C/40 seconds) x 30, 72°C/10 minutes.

Bisulphite PCR

Genomic DNA from preanthesis floral buds and 15 day old seedlings was extracted using the Plant DNeasy kit (Qiagen). 500ng-2µg of genomic DNA was bisulphite converted using the MethylEasy Xceed Kit (Human Genetic Signatures). Bisulphite PCR was carried out using unbiased bisulphite primers allowing amplification of both converted and unconverted

fragments (Table S2). Conversion rates were checked by amplifying a region of the chloroplast genome which showed a conversation rate >99% (Chloroplast region 15, ATPA2; Table S2). PCR machine settings for all bisulphite PCRs were: 95°C/5 minutes, (95°C/1 minutes, 55°C/2 minutes, 72°C/3 minutes) x 5, (95°C/1 minutes, 55°C/2 minutes, 72°C/1.5 minutes) x 35, 72°C/6 minutes, using Taq polymerase (NEB) or Kapa Robust 2G readymade mix (Kapa Biosystems). PCR products were gel extracted, ligated into pGEM-T Easy (Promega) and transformed into DH5α bacterial cells. To determine ^{m}C levels at least 12 clones were sequenced from parental lines and 24 clones sequenced from 1st , 2nd and 3rd generation plants. ^{m}C at a base were determined by dividing the number of ^{m}C in the clones by the total number of clones

McrBC-digest and Real-time PCR

25ng-50ng of genomic DNA was digested for 16 hours at 37°C with 1U of McrBC enzyme (NEB) followed by a heat inactivation step at 65°C for 15 minutes. For each digestion a control (undigested DNA) was also set up. Digested DNA was diluted into 200µl and 400µl, for 25ng and 50ng starting gDNA respectively. 5µl of the diluted DNA was used in each real-time PCR reaction. All results were obtained by digesting at least two biological replicates and two independent McrBC digests. For parents and F1 hybrids McrBC results are from replicates of pooled plants. In the case of plotted individuals, results shown represent the average of at least two McrBC digests.

Real-time PCR was carried out on a Corbett RG-6000 machine using the conditions: step 1: 94°C-10 min; step 2: 95°C-20 sec, 58°C-20 sec, 72°C-20 sec (45 cycles). McrBC digestion at At3g43340 was normalized to the reference gene *ACTIN* and then to the undigested control. Primer sequences are provided in Table S2. Digestion levels have been inverted to represent methylation levels.

Chromatin Immunoprecipitation (ChIP)

ChIP experiments were carried out as described (35) with the following alterations. 50mg of preanthesis floral buds were ground using liquid nitrogen in a mortar and pestle. The ground tissue was then added to 500µl of ChIP lysis buffer. Preanthesis floral bud chromatin was then digested with 15U of Micrococcal Nuclease (MNase; Thermo Scientific) for 14

minutes at 37°C. Digested material was then sonicated for 2 x 15sec (Amplitude = 30, cycle = 1) and centrifuged for 15minutes at 4°C. 5% of the supernatant was separated for total input control then the supernatant was separated into four aliquots (125 µl) and incubated with either no antibody (control), anti-H3K9me2 (pAB-060-050; Diagenode) or anti-H3K9ac (07-352; Upstate), along with Magna ChIP protein A magnetic beads (16-661; Millipore) for four hours at 4°C. Samples were then placed on a magnetic stand and washed four times with a low salt, high salt, LiCl and TE wash buffers for 5 minutes each at 4°C (33). DNA was then eluted 2 x 15 minutes at RT, digested with proteinase K for one hr at 45°C and DNA purified using a Qiagen PCR clean up kit.

ChIP-qRT-PCR

ChIP DNA was diluted to a 1/20 dilution and qRT-PCR carried out using the HT7900 and SDS 2.3 software (ABI) using 5 µl per reaction. Regions of interest were normalized to regions known to contain high levels of H3K9ac (*ACTIN*) and H3K9me2 (*TA3*) (S2) using the $2^{-\Delta\Delta CT}$ method (S3, Table S2). A two-tailed unequal variance T-test was used to determine significant differences in chromatin enrichment between samples.

Transcriptome data

Tissue for RNA extraction was harvested from aerial tissue from 15 DAS seedlings and preanthesis floral buds from C24/Ler F1 hybrids, C24, Ler and preanthesis floral buds from individual F2 C24/Ler progeny. Total RNA was extracted using QIAGEN RNeasy Plant Mini Kit™ with on column DNA digestion using the QIAGEN RNAse free DNAse set™. Libraries were prepared and sequenced by a service provider using the illumina True-seq kit and sequenced on an illumina Hi-Seq as mRNA-seq pair ended 100nt runs. Raw reads were mapped to the TAIR10 Arabidopsis reference genome using BioKanga (https://code.google.com/p/biokanga/) on default settings. Mapped reads were allocated to genomic features (including Transposable Elements) in the TAIR10 annotations using the BioKanga maploci function on default settings. Reads per genomic feature were standardised across samples using reads per million (mapped to a feature) per Kb (RPMKb) and these values used as indicators for mRNA levels (see Table S1). A two-tailed unequal variance T-test was used to determine significant differences in mRNA levels between samples.

SI REFERENCES:

1. Dalmay T, Hamilton A, Rudd S, Angell S, & Baulcombe DC (2000) An RNA-dependent rna polymerase gene in *Arabidopsis* is required for posttranscriptional gene silencing mediated by a transgene but not by a virus. *Cell* 101(5):543-553

2. Johnson LM, Cao XF, & Jacobsen SE (2002) Interplay between two epigenetic marks: DNA methylation and histone H3 lysine 9 methylation. *Current Biology* 12(16):1360-1367.

3. Livak KJ & Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(T)(-Delta Delta C) method. *Methods* 25(4):402-408.

SI FIGURES AND TABLES:

Figure S1: Experimental design to analyze the inheritance of TCM/TCdM-derived ^{m}C patterns observed in the F1 hybrid.

Figure S2: Ecotype variation at At1g64790 and At3g43340/50. ^{m}C levels of each individual ^{m}C are represented by blue (^{m}CG), red (^{m}CHG) and green (^{m}CHH) columns. For At1g64790 the regions which undergo TCdM (blue) and TCM (red) are highlighted in boxes. C24 and Ler graphs reproduced from (6).

Figure S3: The TCM event observed in C24/Ler hybrids also occurs in Col/Ler hybrids. The unmethylated Ler allele attains the same ^{m}C pattern as the methylated Col. SNPs were used to differentiate allelic ^{m}C levels. ^{m}C levels are represented by blue (^{m}CG), red (^{m}CHG) and green (^{m}CHH) columns. * denotes allele derived from F1.

Figure S4: Additional individuals analyzed for the inheritance of ^{m}C patterns at At3g43340/50. A) Four more backcross individuals with a Ler*/Ler genotype B) McrBC qRT-PCR of preanthesis floral buds from backcrossed individuals (Fig. 1B and S4A) C) Bisulphite PCR graphs of C24/Ler FI hybrid at 15 DAS seedlings. In A) & C) ^{m}C are represented by blue (^{m}CG), red (^{m}CHG) and green (^{m}CHH) columns. * denotes allele derived from F1 with individual plants labelled C to F. In B) column colours denote allelic combinations. Black dotted line represents MPV. Error bars = SEM.

Figure S5: Individuals analyzed for the inheritance of ^{m}C patterns at At3g43340/50 in outcrosses between C24/Ler and Cvi. ^{m}C are represented by blue (^{m}CG), red (^{m}CHG) and

green (^{m}CHH) columns. Boxes demonstrate how the individuals were derived. * denotes allele derived from F1 with individual plants labelled A to D.

Figure S6: Inheritance of ^{m}C patterns into the third generation. A) Experimental design used to examine the ^{m}C level in third generation plants derived from two backcrosses, or third generation plants derived from one backcross followed by one selfing. All third generation plants were derived from (Ler x F1) –A or –B. Red lines represent C24 allele, blue lines represent Ler allele and black dots represent ^{m}C B) McrBC qRT-PCR of parents, F1 hybrids and third generation plants at 15 DAS seedlings C) McrBC qRT-PCR of parents, F1 hybrids and individual third generation plants at 15das demonstrating the variability in ^{m}C between individuals with error bars representing the SEM of two independent McrBC digests. Column colours denote allelic combinations. Black dotted line represents MPV.

Figure S7: ^{m}C at the TCM region At1g64790 is lost in *polIV* mutants. Red box indicates region which normally undergoes TCM in the hybrid. ^{m}C levels are represented by blue (^{m}CG), red (^{m}CHG) and green (^{m}CHH) columns. C24 graph reproduced from (6).

Figure S8: Outcrosses, backcrosses and F2 individuals analyzed for the inheritance of ^{m}C patterns at At1g64790. 5 individuals were analyzed for both out/backcrosses and F2 plants. * denotes allele derived from F1 with individual plants labelled A to D. Boxes demonstrate how the individuals were derived. Regions which undergo TCdM (blue) and TCM (red) are highlighted in boxes. ^{m}C levels are represented by blue (^{m}CG), red (^{m}CHG) and green (^{m}CHH) columns. C24 and Ler graph reproduced from (6).

Figure S9: Histone acetylation and methylation levels at At1g64790. Bisulphite PCR graphs illustrate the regions analyzed by ChIP qRT-PCR (blue and green lines; Fig. 5). The blue and green graphs represent the results from primer set 1 (blue) and primer set 2 (green). H3K9ac levels at the TCM region were normalized against *ACTIN*, while H3K9me2 levels at the TCM region were normalized against TA3. For the ChIP graphs columns represent the relative fold change compared to C24. Error bars =SEM from three independent ChIP experiments. Bisulphite PCR graphs reproduced from (6). * indicates statistically significant differences ($P \leq 0.05$) in IP pull down between MPV and hybrids while * indicates statistically significant differences ($P \leq 0.05$) in IP pull down between the two parental accessions.

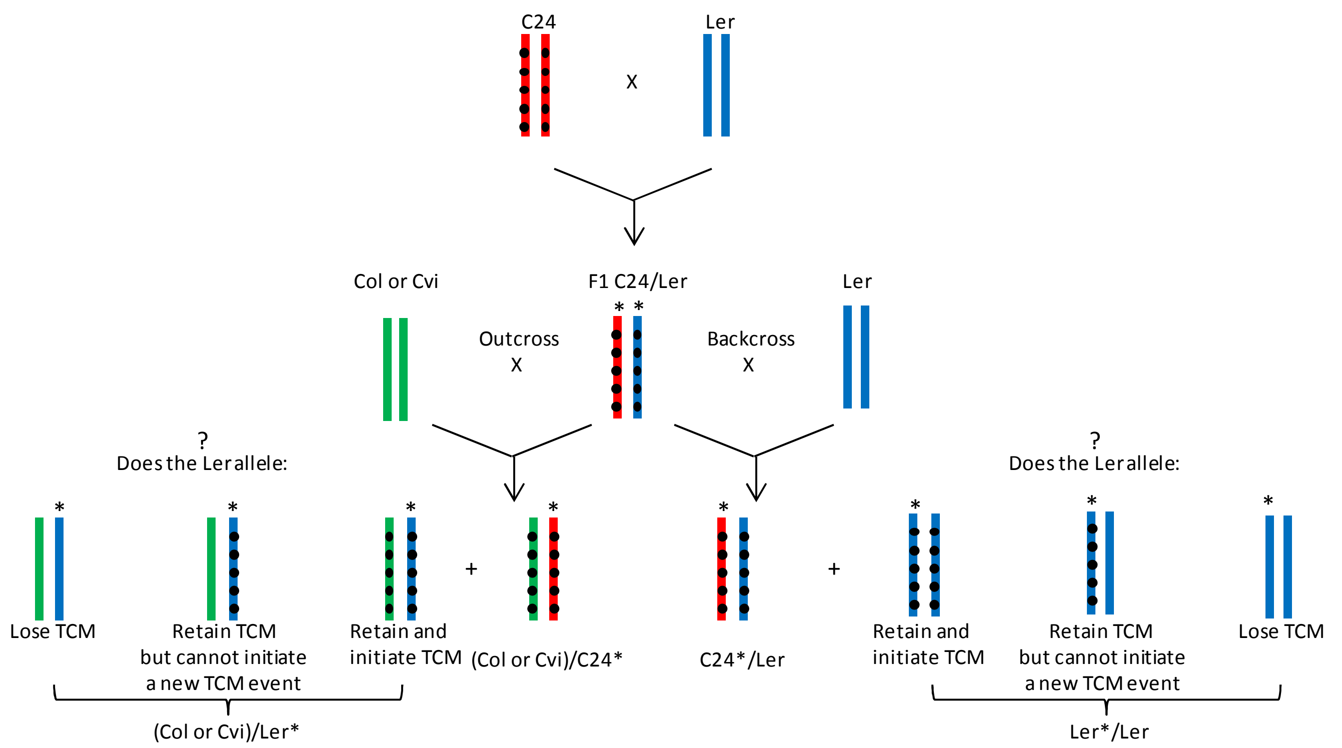
Table S1:

Deep sequencing data from 15 day old seedlings and preanthesis floral buds. Tables include number of reads mapped to genomic features (including TEs) along with raw read count numbers for At3g43340, At3g43350 and At1g64790. These numbers were used to calculate read per million per kilobase by the aligner software Biokanga (see SI material and methods). An unequal variance two-tailed Ttest was used to look for statistical differences between the average mRNA levels between the parents (red) and between the hybrids and MPV (green).

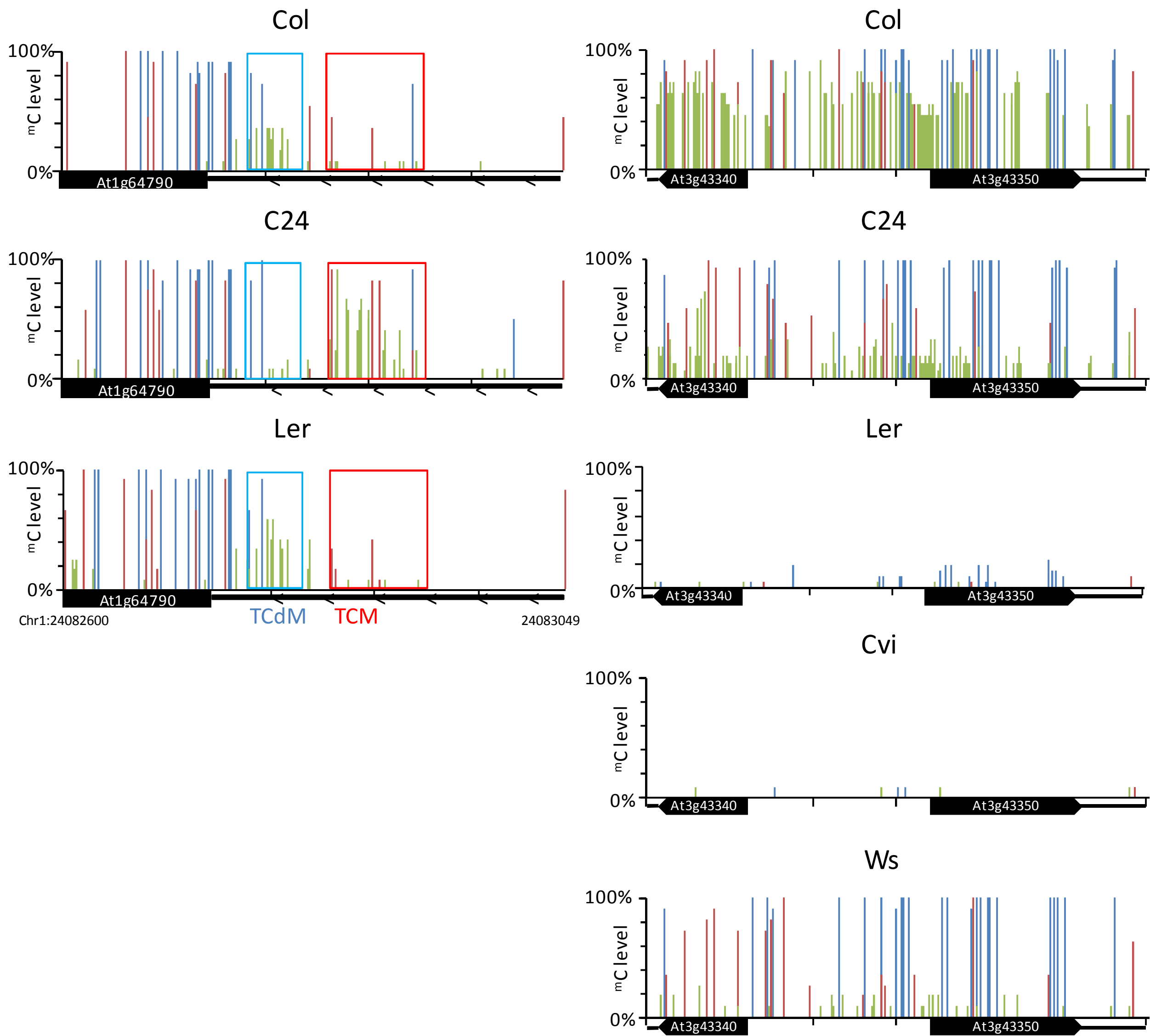
Table S2:

Primer sequences

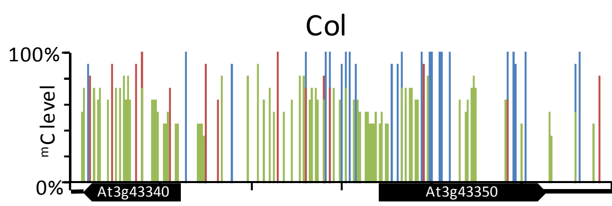
Supplementary figure 1

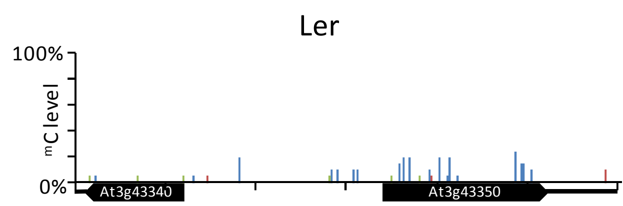


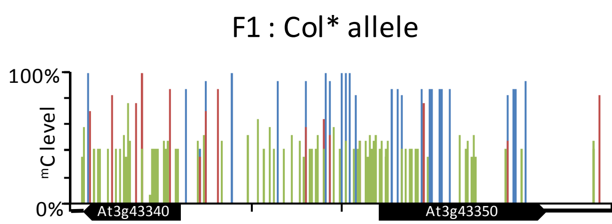
Supplementary figure 2

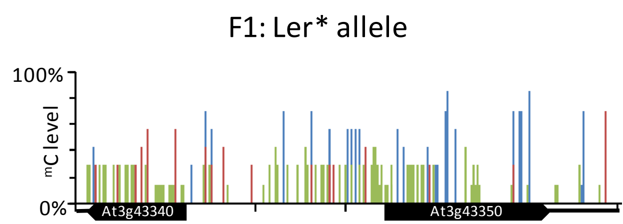


Supplementary figure 3









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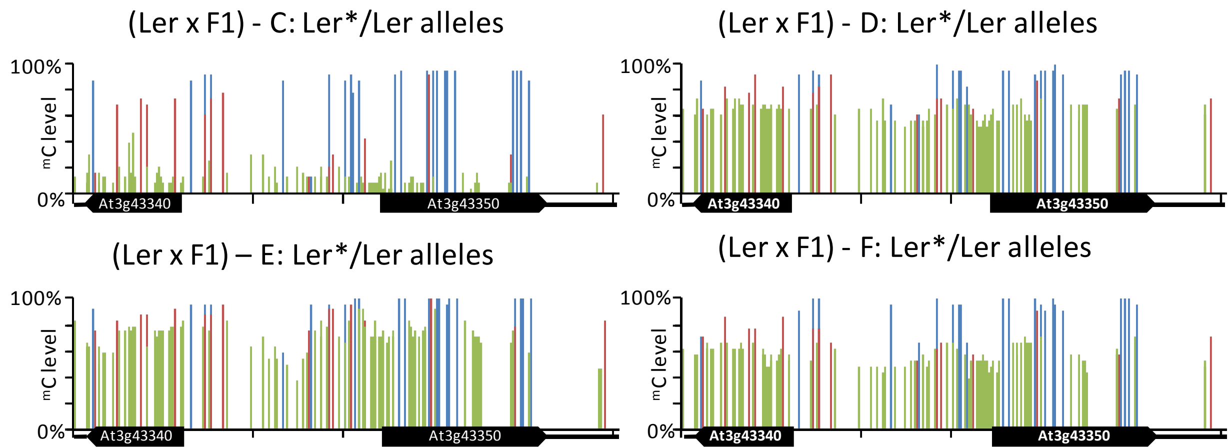
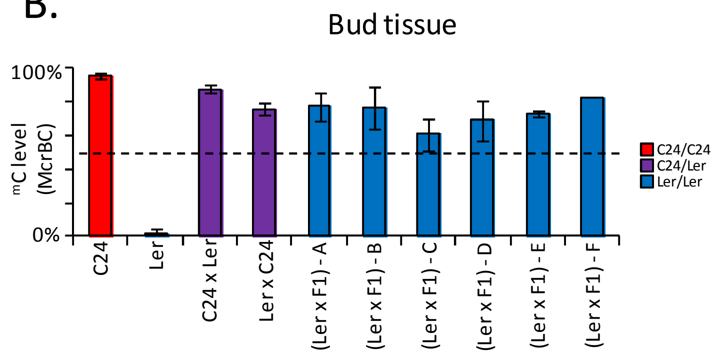
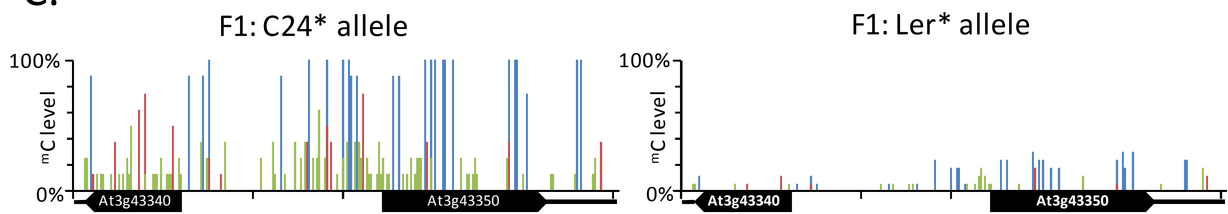
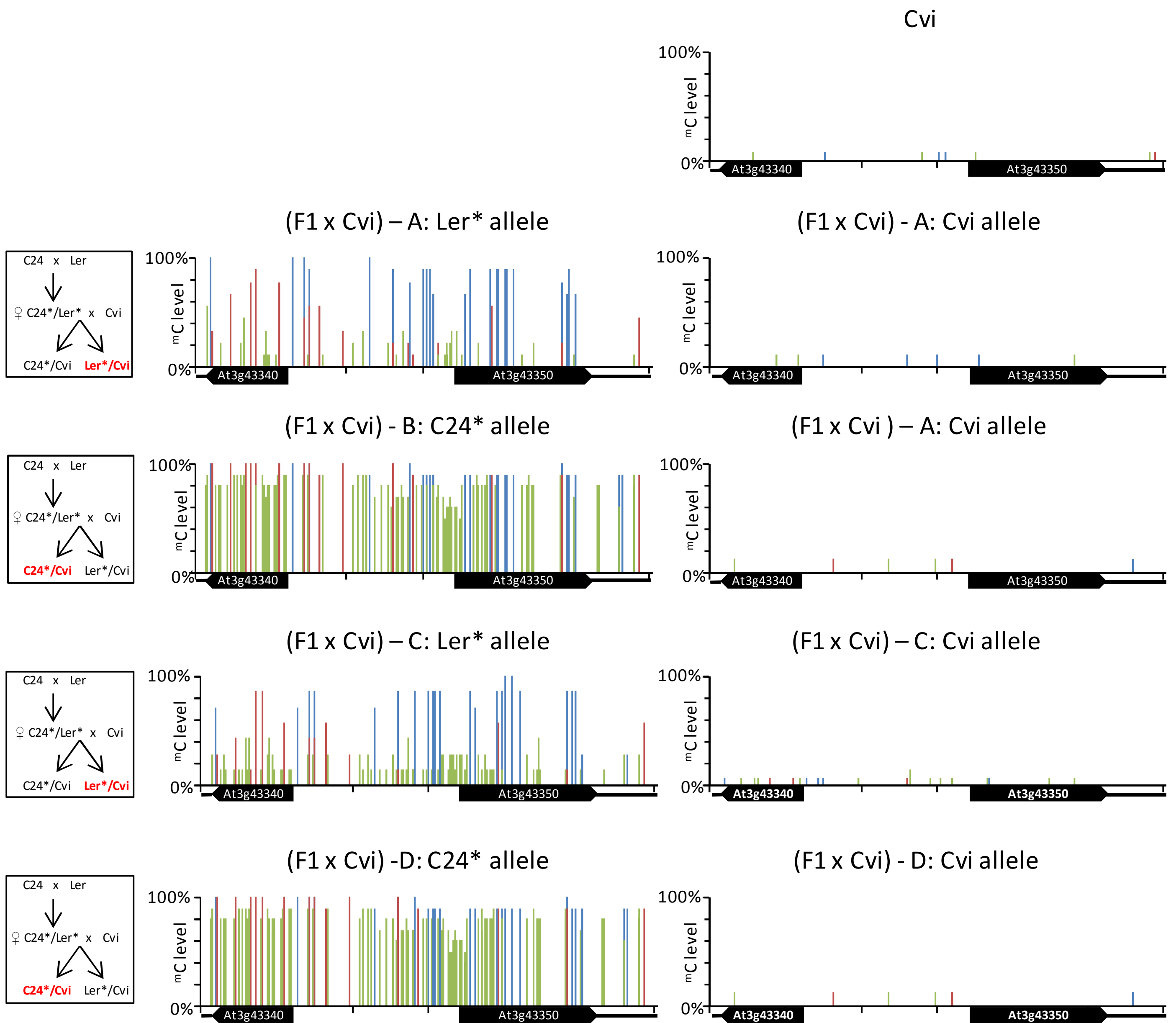


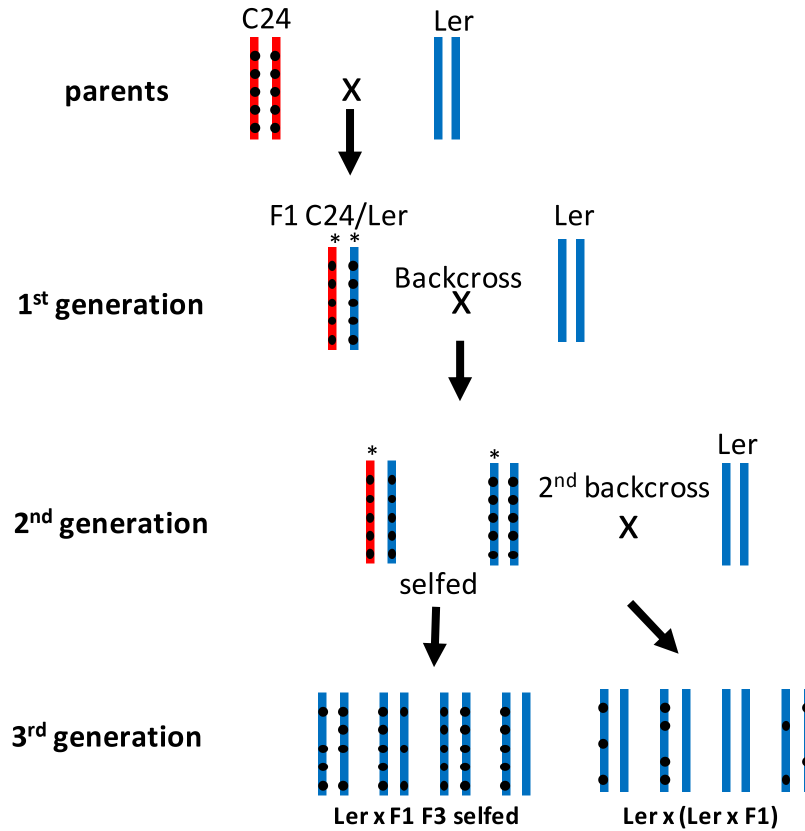


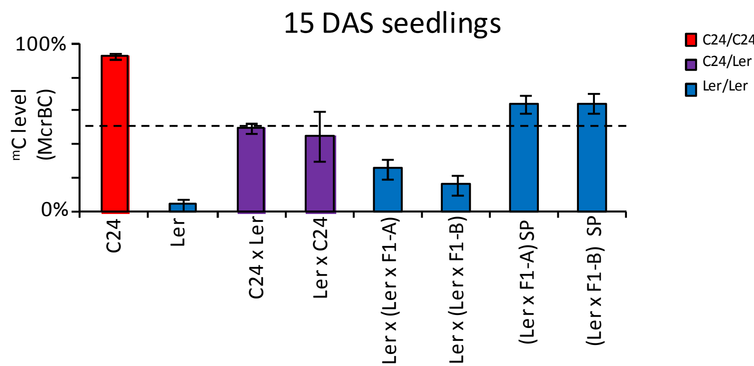


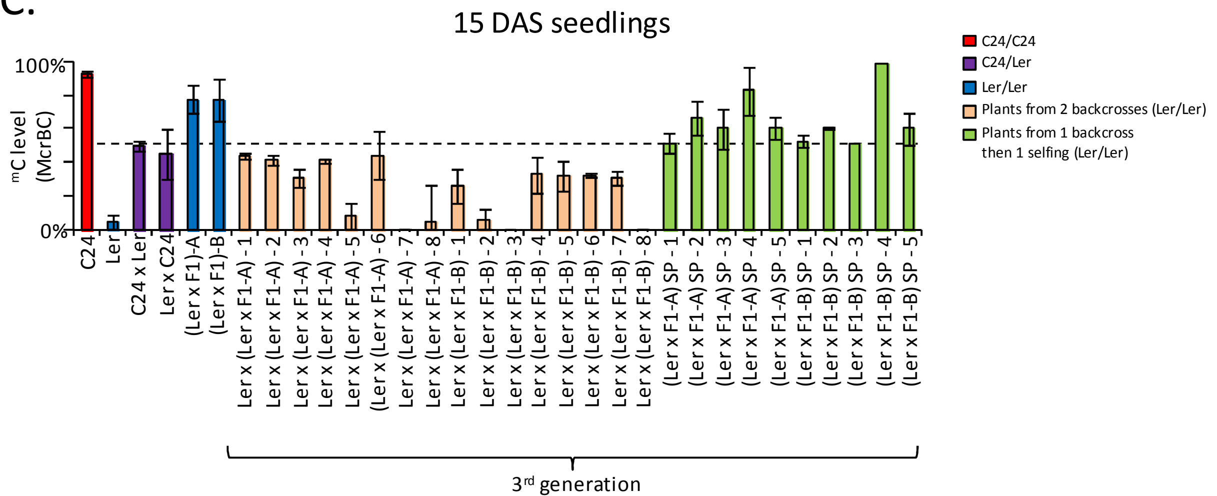
Supplementary figure 5



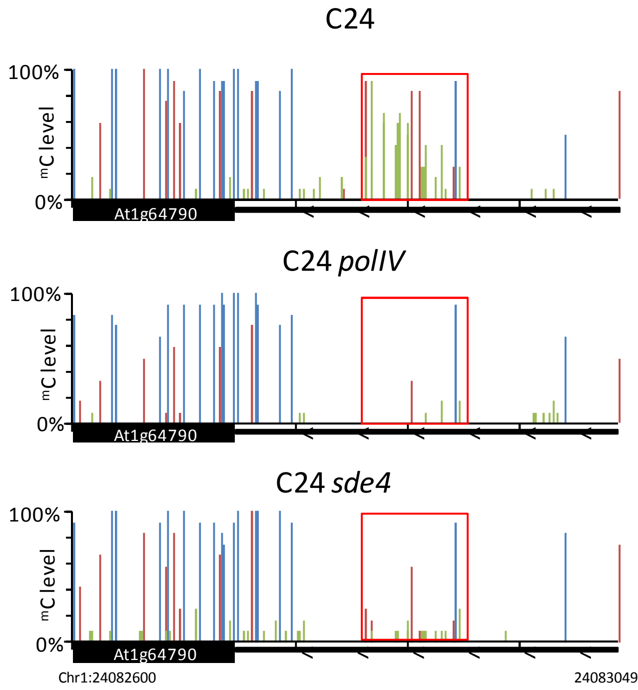
Supplementary figure 6



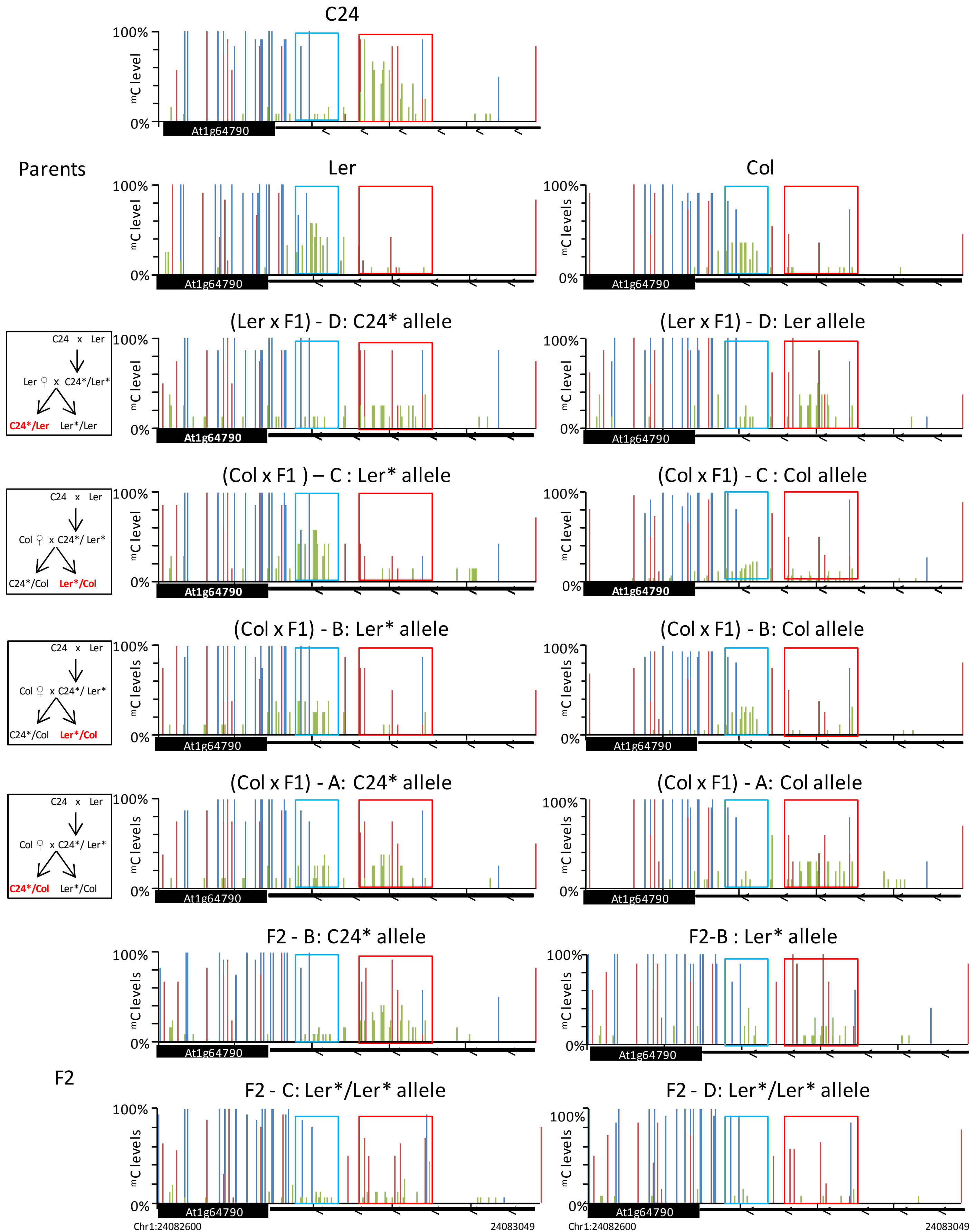




Supplementary figure 7



Supplementary figure 8



Supplementary figure 9

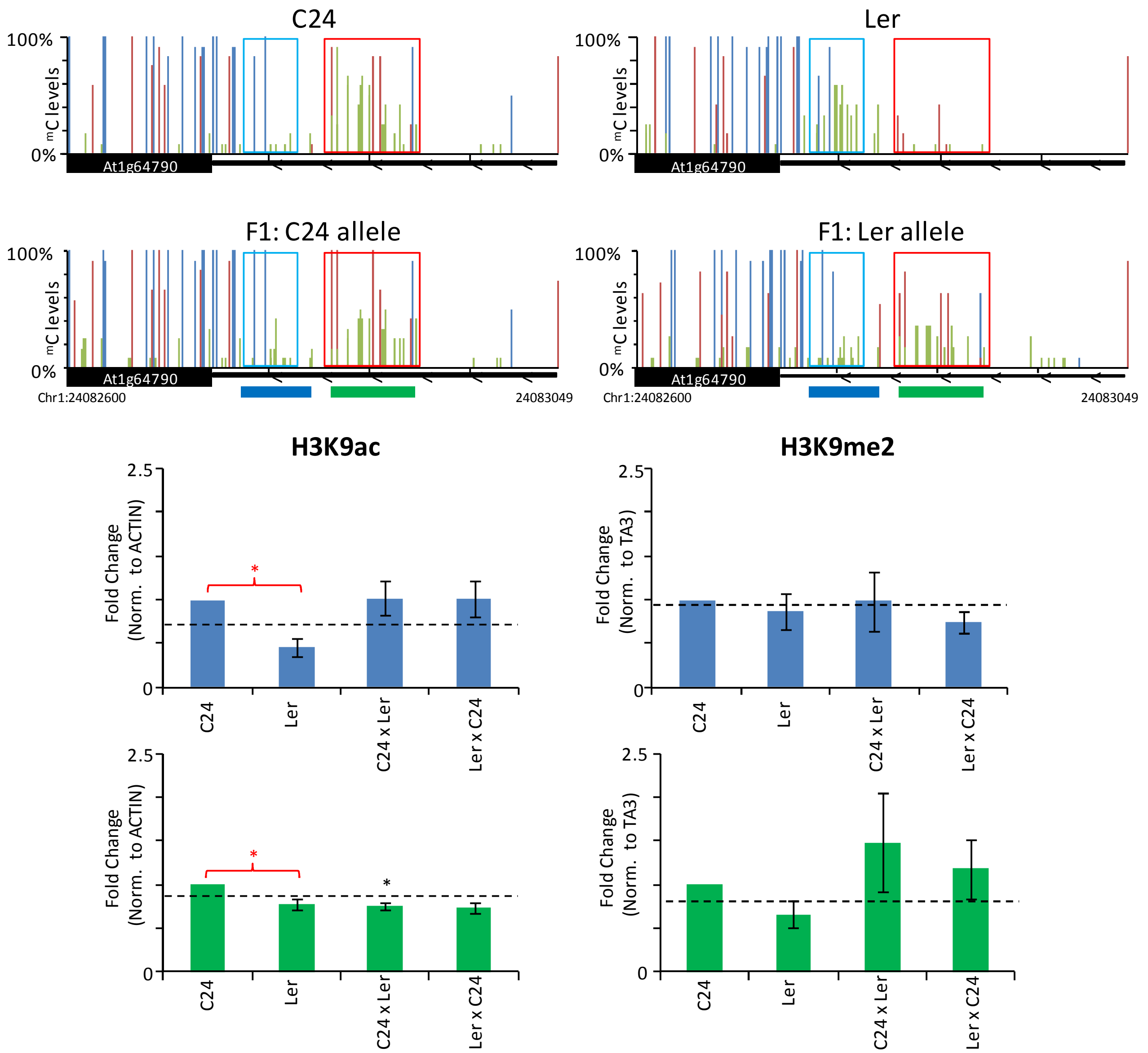


Table S1 : Deep sequencing data

Number of aligned reads mapping to TAIR 10 annotated features (used for RPMKb)

	15 DAS seedlings						pre-anthesis floral buds		
	RepA	RepB	RepC	RepD	RepE	RepF	A	B	C
C24	68544515	72742953	77700508	77250354	48915303	43269649	59526290	40813564	
Ler	75967316	71758991	78445675	74597367	82840226	34548979	63922311	21413779	
C24xLer	68105162	72247153	79289759	75791754			19319307	65771193	17370973
Ler x C24	74332606	74484998	60217489	41934086			36710190	41779241	19099460
F2-1							79743424		
F2-2							32506109		
F2-3							64444503		
F2-4							66747168		
F2-5							126638346		
F2-6							36164383		
F2-7							53834976		
F2-8							28065606		

Raw read counts At3g43340

	15 DAS seedlings						pre-anthesis floral buds		
	RepA	RepB	RepC	RepD	RepE	RepF	A	B	C
C24	14	24	9	19	10	7	8	4	
Ler	673	673	453	444	1314	477	676	186	
C24xLer	216	305	391	401			16	179	16
Ler x C24	343	452	273	217			21	124	34
F2-1							7		
F2-2							125		
F2-3							23		
F2-4							21		
F2-5							46		
F2-6							29		
F2-7							28		
F2-8							39		

Raw read counts At3g43350

	15 DAS seedlings						pre-anthesis floral buds		
	RepA	RepB	RepC	RepD	RepE	RepF	A	B	C
C24	83	96	112	100	82	73	83	47	
Ler	869	877	609	568	2307	825	872	266	
C24xLer	319	413	529	546			39	273	29
Ler x C24	471	597	458	349			93	237	102
F2-1							46		
F2-2							117		
F2-3							111		
F2-4							126		
F2-5							295		
F2-6							86		
F2-7							112		
F2-8							128		

Raw read counts At1g64790

	15 DAS seedlings						pre-anthesis floral buds		
	RepA	RepB	RepC	RepD	RepE	RepF	A	B	C
C24	9858	11550	14291	19714	8696	6650	11404	7533	
Ler	13352	11753	14468	16984	18440	6912	11265	3374	
C24xLer	11753	11204	17280	16686			3086	12274	2893
Ler x C24	10484	10424	10088	7877			6995	9078	4052
F2-1							15874		
F2-2							5305		
F2-3							12506		
F2-4							16385		
F2-5							27138		
F2-6							6565		
F2-7							11945		
F2-8							5564		

Standardized Reads (RPMKb) At3g43340

	15 DAS seedlings						pre-anthesis floral buds		
	RepA	RepB	RepC	RepD	RepE	RepF	A	B	C
C24	0.91	1.46	0.51	1.09	0.90	0.72	0.60	0.44	
Ler	39.37	41.68	25.67	26.45	70.50	61.36	47.00	38.60	
MPV	20.14	21.57	13.09	13.77	35.70	31.04	23.80	19.52	
C24xLer	14.10	18.76	21.92	23.51			3.68	12.10	4.09
Ler x C24	20.51	26.97	20.15	23.00			2.54	13.19	7.91
F2-1							2.17		
F2-2							17.09		
F2-3							1.59		
F2-4							1.40		
F2-5							1.61		
F2-6							3.56		
F2-7							2.31		
F2-8							1.11		

Standardized Reads (RPMKb) At3g43350

	15 DAS seedlings						pre-anthesis floral buds		
	RepA	RepB	RepC	RepD	RepE	RepF	A	B	C
C24	0.49	0.52	0.57	0.51	0.67	0.68	0.56	0.46	
Ler	4.60	4.90	3.11	3.05	11.17	9.58	5.17	4.98	
MPV	2.54	2.71	1.84	1.78	5.92	5.13	2.87	2.72	
C24xLer	1.88	2.29	2.67	2.89			0.81	1.66	0.67
Ler x C24	2.54	3.22	3.05	2.34			1.02	2.28	2.14
F2-1							0.64		
F2-2							1.44		
F2-3							0.69		
F2-4							0.76		
F2-5							0.93		
F2-6							0.95		
F2-7							0.83		
F2-8							0.66		

Standardized Reads (RPMKb) At1g64790

	15 DAS seedlings						pre-anthesis floral buds		
	RepA	RepB	RepC	RepD	RepE	RepF	A	B	C
C24	17.40	19.25	22.30	30.94	21.56	18.64	22.99	22.38	
Ler	21.32	19.86	22.36	27.61	26.99	24.26	21.37	19.11	
MPV	19.36	19.56	22.33	29.28	24.28	21.45	22.18	20.74	
C24xLer	20.92	18.80	26.43	26.70			19.17	22.63	19.98
Ler x C24	17.10	16.97	20.31	22.78			22.86	26.35	25.72
F2-1							24.14		
F2-2							19.58		
F2-3							23.29		
F2-4							29.77		
F2-5							25.98		
F2-6							22.01		
F2-7							26.62		
F2-8							24.04		

Average: Standardized Reads (RPMKb) At3g43340

	15 DAS seedlings			pre-anthesis floral buds		
	Average	SEM	Ttest (p≤0.05)	Average	SEM	Ttest (p≤0.05)
C24	0.931	0.133	0.002	0.516	0.081	0.063
Ler	44.172	7.471	0.002	42.803	4.199	0.063
C24xLer	19.573	2.074	0.507	6.623	2.739	0.023
Ler x C24	22.658	1.571	0.980	7.882	3.074	0.035
Combined F2's				3.856	1.910	0.009

Average: Standardized Reads (RPMKb) At3g43350

	15 DAS seedlings			pre-anthesis floral buds		
	Average	SEM	Ttest (p≤0.05)	Average	SEM	Ttest (p≤0.05)
C24	0.573	0.034	0.011	0.511	0.049	0.003
Ler	6.069	1.411	0.011	5.077	0.094	0.003
C24xLer	2.433	0.222	0.284	1.048	0.311	0.026
Ler x C24	2.788	0.208	0.504	1.811	0.399	0.129
Combined F2's				0.864	0.093	0.000

Average: Standardized Reads (RPMKb) At1g64790

	15 DAS seedlings			pre-anthesis floral buds		
	Average	SEM	Ttest (p≤0.05)	Average	SEM	Ttest (p≤0.05)
C24	21.682	1.997	0.410	22.68402	0.303644	0.259
Ler	23.733	1.273	0.410	20.23716	1.131772	0.259
C24xLer	23.213	1.984	0.846	20.59292	1.0447	0.543
Ler x C24	19.290	1.396	0.136	24.97858	1.072562	0.072
Combined F2's				24.42879	1.090502	0.062

Table S2: PCR primer sets

Bisulphite PCR primer sequences

Locus	Forward primer	Reverse Primer
C15	ATGATGTTGTTAGAATTTYATATAGG	CATCATTTARCTATCRCAATTCTTT
ATPA2	ATGGATTAGAAATTGGAYAAGTAAGAAAATTT	AATACCTTCTTTCAAAAARCTTTCTRCTTCA
AT1g64790	AGATGGTGATGTTYAGYGAATTT	TATTTTCTAATATCTAACTATACARACATA
AT3g43340	AGAAYTTTAAAAYTGAGAAAG	CTCAACAAAACARTARAAAAC

McRBC qRT-PCR primer sequences

Locus	Forward primer	Reverse Primer
ACTIN	CTAAGCTCTCAAGATCAAAGGC	AACATTGCAAAGAGTTTCAAGG
At3g43340	GGTCACGCTCACAGTTCTCTC	TCTTTTAGACTCCACCGCTCA

ChIP qRT-PCR primer sequences

Locus	Forward primer	Reverse Primer
At3g43340 primer set 1	GAAAGCTTTGGAAAAGTACCAC	CAAACGACACTTAATGGCAATC
At3g43340 primer set 2	CCGTGGTAATATATCTGCAATTTT	TCGTGAACGAGATGTTGAACC
At3g43340 primer set 3	GGTCACGCTCACAGTTCTCTC	TCTTTTAGACTCCACCGCTCA
At1g64790 primer set 1	CTTCAGAGCTGATCGGTGGT	TTGATTTAGGGTTGGACACG
At1g64790 primer set 2	CCTGGCCTGTTAATTTCCAT	TTTTGTCGGCCATTTTGATT
ACTIN 7	CGCTGTTGTTTCTCCTCCAT	GCGAACGGATCTAGACTCA
TA3	AGACAGCTCTGCGTGGAAGTC	TTATCAGTCTCAGCATTACACAG
FUS3	ACTTTTGCTACACTTGTTCACCA	CGCAACAAGATCTAATGCCACT

Allele specific primer sequences

Locus	Forward primer	Reverse Primer
At3g43340 C24 specific F	TTTAAAAAAAGAAAGATAATAAGAGC**AG**	CTCAACAACAAAACAGTAGAAAA
At3g43340 Ler specific F	TTTAAAAAAGAAAGATAATAAGAGC**TA**	