

# Supporting Information

Mirouze et al. 10.1073/pnas.1120841109

## SI Materials and Methods

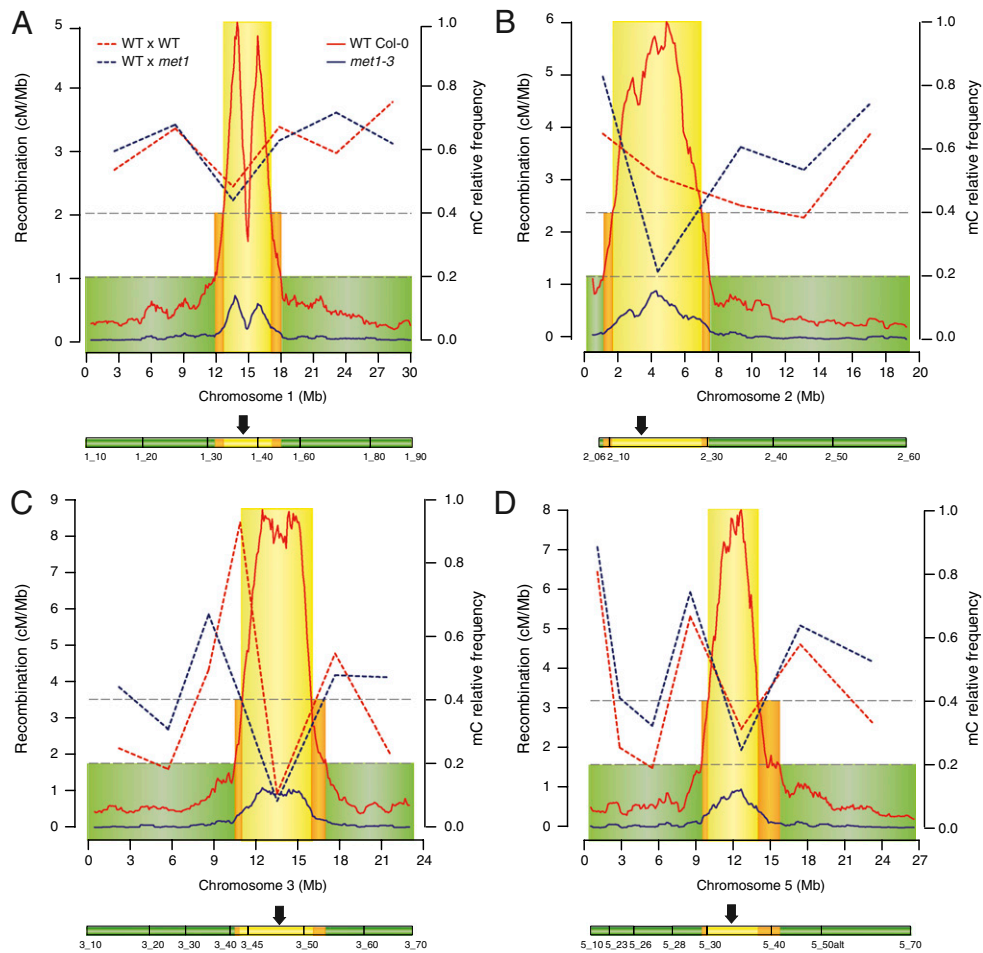
**INDEL Marker Analyses.** Plants from the four F2 populations were grown in vitro on 1/2 MS. Four-day-old individual seedlings were harvested and DNA extraction, PCR and genotyping were executed as described (1). See Table S5 for INDEL marker details.

**High-Throughput KASP Genotyping for SNPs.** For the genome-wide measurement of meiotic recombination, a set of 29 SNPs was selected across the *A. thaliana* genome according to the position of *met1*-like methylation blocks in the epiRILs epi01 and epi12 (2) and to chromatin properties (euchromatic vs. heterochromatic regions, see Fig. S1 and Table S1). Plants from the four F2 mapping populations were grown in vitro on 1/2MS. Individual seedlings were harvested and were either immediately frozen or lyophilized before DNA extraction 2 wk after germination. DNA extraction was carried out by using the NucleoSpin 96 Plant II kit (Macherey-Nagel), according to the manufacturer's instructions. Following the lysis step, the samples were loaded onto the Genesis RSP-150 robot (Tecan) for continuation of the extraction procedure. We used competitive allele-specific PCR genotyping technology to analyze single-nucleotide polymorphism (SNP) markers. For each SNP, KASP-genotyping primers were designed and validated. High-throughput genotyping was per-

formed on a 96 × 96 Dynamic Array IFC (Fluidigm), loading the 32 assays in triplicate. The 96 samples on each array were composed of 2–4 no-template controls, 2 samples of each known control (Columbia, Landsberg, or F1 heterozygote plant), and 86–88 F2 DNA samples. PCR cycling was performed on BioMark (Fluidigm) using the following conditions: 70 °C for 30 s (thermal mixing), 25 °C for 6 s, and 94 °C for 15 min (hot-start activation), followed by 10 cycles of touchdown PCR denaturing at 94 °C for 20 s and annealing/elongation at 65–57 °C for 1 min, dropping 0.8 °C per cycle. Amplification was proceeded for an additional 40 cycles of 94 °C for 15 s and 57 °C for 1 min. Endpoint fluorescence was measured at 37 °C on BioMark for the two allele-specific fluorescent dyes: the Columbia allele labeled by FAM (485 nm excitation, 520 nm emission) and the Landsberg allele labeled by VIC (534 nm excitation, 556 nm emission). ROX was used as an internal reference fluorescent dye (575 nm excitation, 610 nm emission). Usually, a recycling step was added for better clustering of data points. This step included 10 cycles of 95 °C for 15 s and 60 °C for 1 min. Data were analyzed by using the Fluidigm SNP Genotyping Analysis software. We estimated the error rate of our genotyping scoring to be 1%.

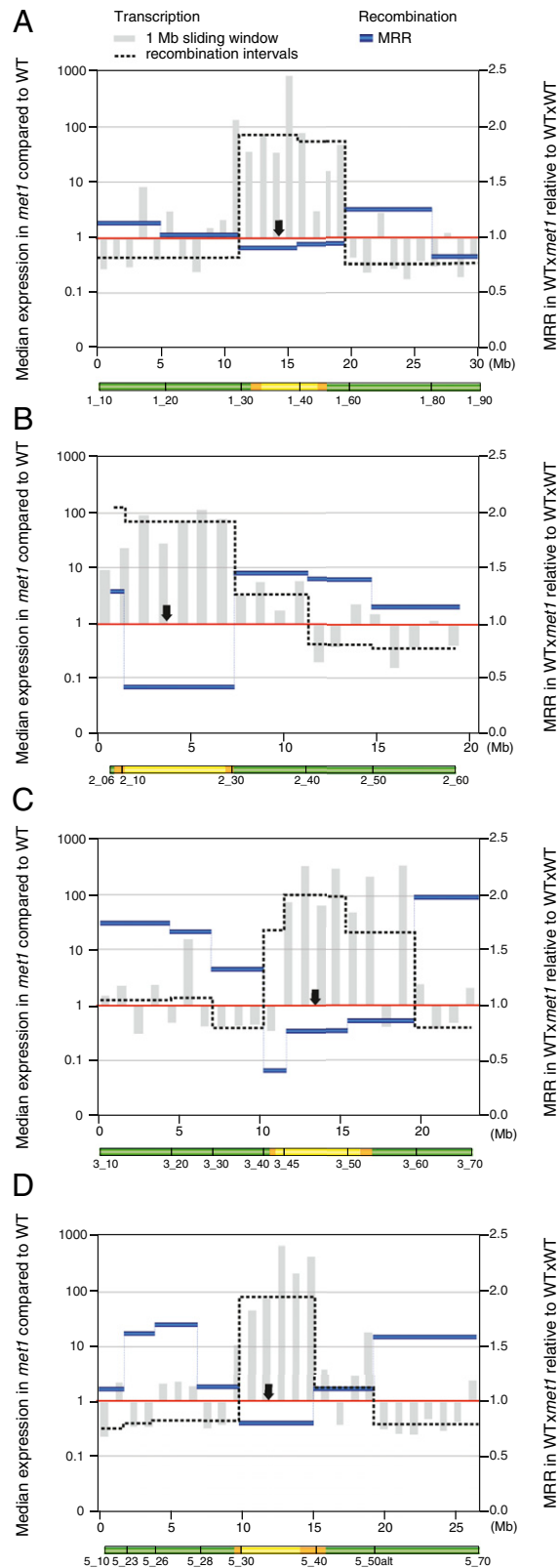
1. Edwards K, Johnstone C, Thompson C (1991) A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. *Nucleic Acids Res* 19:1349.

2. Reinders J, et al. (2009) Compromised stability of DNA methylation and transposon immobilization in mosaic *Arabidopsis* epigenomes. *Genes Dev* 23:939–950.



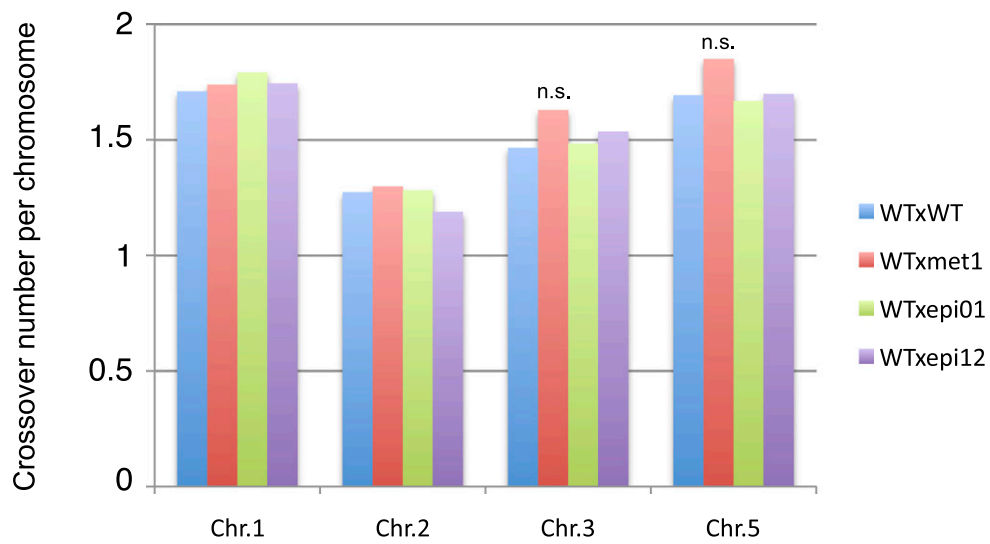
**Fig. S1.** Absolute recombination rates for WTxWT and WTxmet1 using KASP markers at intervals differing in their DNA methylation level. MRRs were calculated for each interval for WTxWT (red dotted curve) and WTxmet1 (blue dotted curve) populations and are represented along *Arabidopsis* chromosomes. The methylcytosine (mC) density plots were obtained from bisulfite-sequencing data (1, 2) using a 100,000-base sliding window with 10-base steps. The WT Col-0 (red curve) and met1-3 (blue curve) mC densities are relative to the highest methylation level detected in WT Col-0 plants. KASP intervals (green box, 0–20% mC; orange box, 20–40% mC, yellow box, >40% mC) analyzed in this study are depicted below the graphs. KASP marker details are given in Table S5. (A) Chromosome 1. Note that the drop at the centromere is due to the sequencing coverage (1). (B) Chromosome 2. (C) Chromosome 3. (D) Chromosome 5.

1. Lister R, et al. (2008) Highly integrated single-base resolution maps of the epigenome in *Arabidopsis*. *Cell* 133:523–536.
2. Cokus SJ, et al. (2008) Shotgun bisulphite sequencing of the *Arabidopsis* genome reveals DNA methylation patterning. *Nature* 452:215–219.



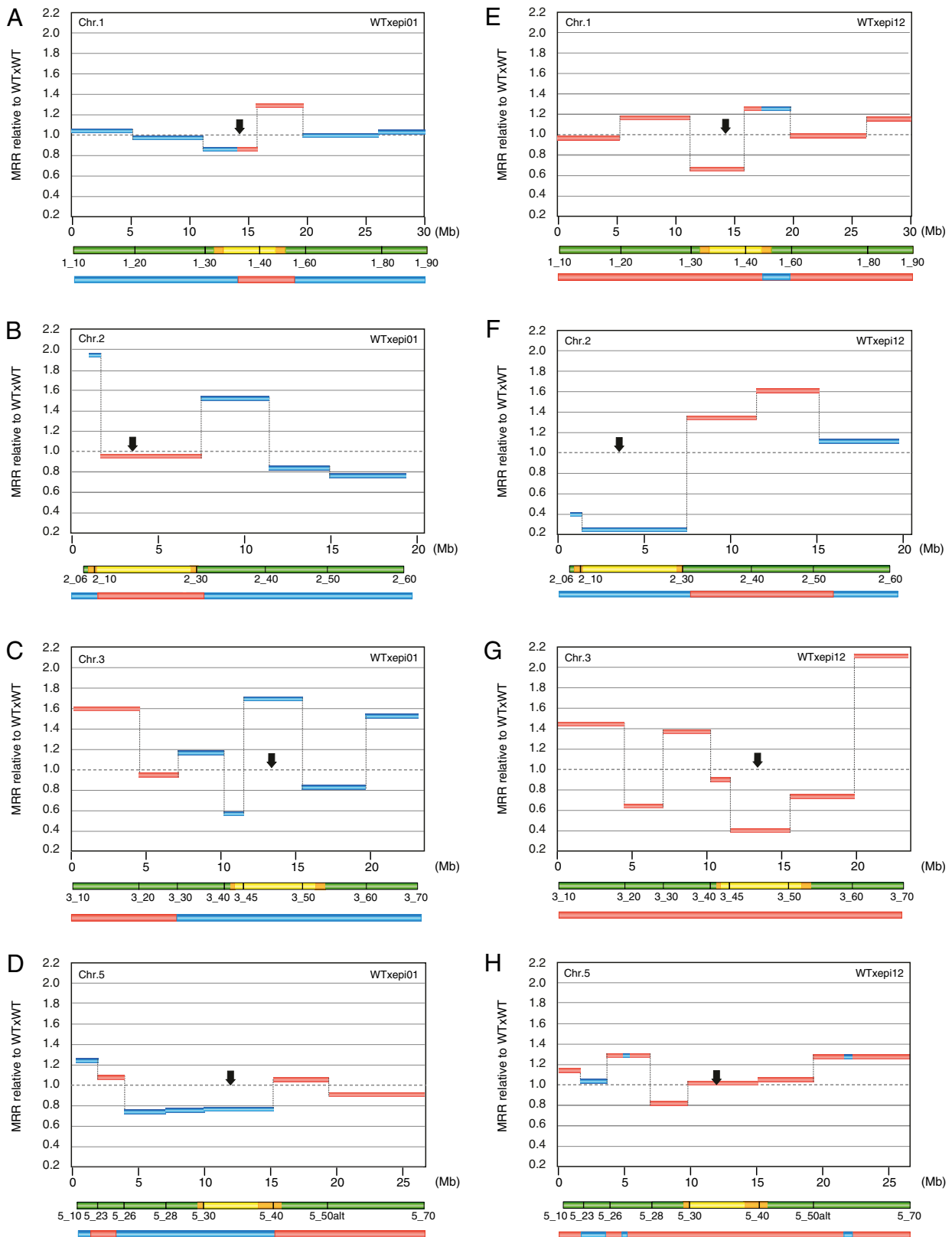
**Fig. S2.** Expression levels in *met1* compared with MRR in WT*xmet1*. Expression in *met1* mutant plants from Lister et al. (1) was analyzed by using a 1-Mb sliding window (gray bars), and the median level was calculated for each KASP interval (dotted black line). For comparison, relative MRR (WT*xmet1* relative to WTxWT, as in Fig. 2) is represented along the four chromosomes (black arrow, centromere localization; Mb, chromosome coordinates in Mb). The red lines mark both WT expression level and WTxWT recombination level set at 1. (A) Chromosome 1. (B) Chromosome 2. (C) Chromosome 3. (D) Chromosome 5.

1. Lister R, et al. (2008) Highly integrated single-base resolution maps of the epigenome in *Arabidopsis*. *Cell* 133:523–536.



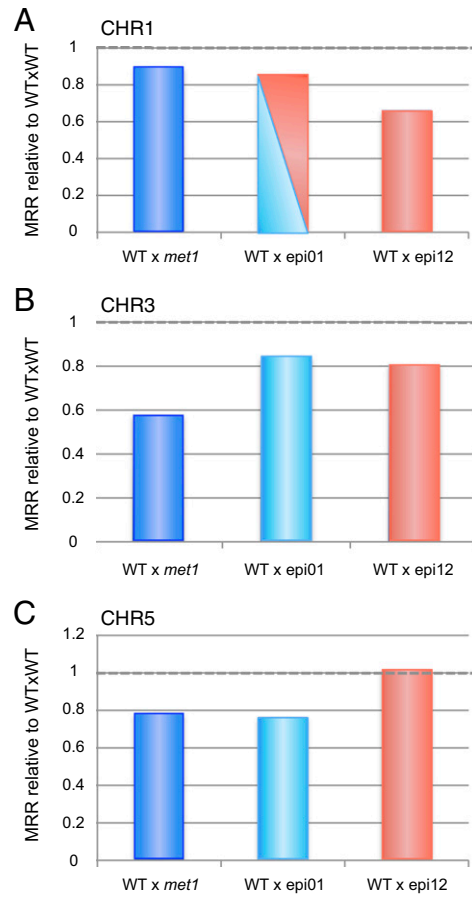
**Fig. S3.** CO number per chromosome per plant in the four F2 populations. CO numbers were calculated by using the CODA software (1). There was no significant (n.s.) difference ( $P > 0.01$ ,  $\chi^2$ ).

1. Gauthier F, Martin OC, Falque M (2011) CODA (crossover distribution analyzer): quantitative characterization of crossover position patterns along chromosomes. *BMC Bioinformatics* 12:27.



**Fig. 54.** Effect of the epi01 and epi12 parents on MRR along *Arabidopsis* chromosomes. F2 plants originating from the cross between WT and epi01 (WTxepi01 population) or WT and epi12 (WTxepi12 population) were genotyped by using KASP markers. Calculated MRRs were compared with the data obtained with the control cross (WTxWT). Legend is as in Fig. 4C. (A–D) WTxepi01 MRRs for chromosomes 1, 2, 3, 5, respectively. (E–H) WTxepi12 MRRs for chromosomes 1, 2, 3, 5, respectively. EpiRIL methylation map below each graph shows WT-like (light red boxes) and *met1*-like (light blue boxes) blocks inherited from WT and *met1* parents, respectively, as detected by genome-wide DNA methylation profiling (1). Black arrow, centromere location, Mb, chromosome coordinates in Mb.

1. Reinders J, et al. (2009) Compromised stability of DNA methylation and transposon immobilization in mosaic *Arabidopsis* epigenomes. *Genes Dev* 23:939–950.



**Fig. S5.** MRRs in heterochromatic intervals in  $WT \times met1$ ,  $WT \times epi01$ , and  $WT \times epi12$  relative to  $WT \times WT$ . F2 plants originating from the cross between WT and *epi01* ( $WT \times epi01$  population) and WT and *epi12* ( $WT \times epi12$  population) were genotyped by using KASP markers. Calculated MRRs were compared with the data obtained with the control cross ( $WT \times WT$ ). Legend is as in Fig. 4B. (A) Chromosome 1. (B) Chromosome 3. (C) Chromosome 5.

**Table S1. Meiotic recombination frequency calculations using KASP markers**

Left marker	Right marker	Interval size, Mb	Chromatin	DNA methylation pattern		Meiotic recombination frequencies, %						MRR, cM/Mb		MRR relative to WTxWT			
				epi01	epi12	WTxWT	WTxmet1	WTxepi01	WTxepi12	WTxWT	WTxmet1	WTxepi01	WTxepi12	WTxWT	WTxmet1	WTxepi01	WTxepi12
1_10	1_20	5.20	Euchromatin	met1-like	wt-like	13.86	15.58	14.50	13.54	2.66	2.99	2.79	2.60	1.00	1.12	1.05	0.98
1_20	1_30	6.03	Euchromatin	met1-like	wt-like	20.48	20.88	20.02	23.82	3.40	3.46	3.32	3.95	1.00	1.02	0.98	1.16
1_30	1_40	4.63	Heterochromatin	wt/met1-like	wt-like	10.95	9.84	9.41	7.27	2.37	2.13	2.03	1.57	1.00	0.90	0.86	0.66
1_40	1_60	3.93	Heterochromatin	wt-like	wt/met1-like	13.45	12.50	17.46	16.85	3.42	3.18	4.44	4.29	1.00	0.93	1.30	1.25
1_60	1_80	6.57	Euchromatin	met1-like	wt-like	19.45	24.14	19.40	19.28	2.96	3.68	2.95	2.94	1.00	1.24	1.00	0.99
1_80	1_90	3.81	Euchromatin	met1-like	wt-like	14.73	11.93	15.19	16.92	3.86	3.13	3.98	4.44	1.00	0.81	1.03	1.15
2_06	2_10	0.61	Heterochromatin	met1-like	met1-like	2.38	3.05	4.59	0.93	3.88	4.97	7.49	1.52	1.00	1.28	1.93	0.39
2_10	2_30	5.94	Heterochromatin	met1-like	met1-like	18.21	7.48	17.05	4.36	3.07	1.26	2.87	0.73	1.00	0.41	0.94	0.24
2_30	2_40	3.96	Euchromatin	met1-like	wt-like	9.95	14.37	14.97	13.32	2.51	3.63	3.78	3.36	1.00	1.44	1.50	1.34
2_40	2_50	3.58	Euchromatin	met1-like	wt-like	8.17	11.42	6.74	13.14	2.28	3.19	1.88	3.67	1.00	1.40	0.83	1.61
2_50	2_60	4.44	Euchromatin	met1-like	met1/wt-like	17.30	19.86	13.04	19.24	3.90	4.48	2.94	4.33	1.00	1.15	0.75	1.11
3_10	3_20	4.42	Euchromatin	wt-like	wt-like	9.71	17.09	15.48	14.01	2.20	3.86	3.50	3.17	1.00	1.76	1.59	1.44
3_20	3_30	2.60	Euchromatin	wt-like	wt-like	4.22	7.05	3.92	2.70	1.62	2.71	1.51	1.04	1.00	1.67	0.93	0.64
3_30	3_40	3.19	Euchromatin	met1-like	wt-like	13.83	18.67	15.88	18.89	4.34	5.86	4.98	5.93	1.00	1.35	1.15	1.37
3_40	3_45	1.30	Heterochromatin	met1-like	wt-like	10.92	4.69	6.08	9.78	8.38	3.60	4.67	7.51	1.00	0.43	0.56	0.90
3_45	3_50	3.98	Heterochromatin	met1-like	wt-like	3.79	2.97	6.39	1.51	0.95	0.75	1.61	0.38	1.00	0.78	1.69	0.40
3_50	3_60	4.31	Euchromatin	met1-like	wt-like	20.61	18.04	16.68	15.09	4.79	4.19	3.87	3.50	1.00	0.88	0.81	0.73
3_60	3_70	3.49	Euchromatin	met1-like	wt-like	7.20	14.43	10.91	15.18	2.06	4.13	3.12	4.35	1.00	2.00	1.51	2.11
5_10	5_23	1.55	Euchromatin	met1-like	wt-like	10.03	11.01	12.48	11.44	6.45	7.08	8.03	7.36	1.00	1.10	1.24	1.14
5_23	5_26	2.04	Euchromatin	wt-like	met1-like	4.12	6.65	4.48	4.23	2.02	3.27	2.20	2.08	1.00	1.61	1.09	1.03
5_26	5_28	3.19	Euchromatin	met1-like	wt-like	4.82	8.18	3.57	6.17	1.51	2.56	1.12	1.93	1.00	1.70	0.74	1.28
5_28	5_30	2.90	Euchromatin	met1-like	wt-like	15.43	17.22	11.67	12.61	5.33	5.94	4.03	4.35	1.00	1.12	0.76	0.82
5_30	5_40	5.30	Heterochromatin	met1-like	wt-like	13.20	10.41	10.09	13.43	2.49	1.96	1.91	2.54	1.00	0.79	0.76	1.02
5_40	5_50alt	4.18	Heterochromatin	wt-like	wt-like	19.30	21.28	20.37	20.29	4.62	5.10	4.88	4.86	1.00	1.10	1.06	1.05
5_50alt	5_70	7.37	Euchromatin	wt-like	wt-like	19.52	30.89	17.70	24.87	2.65	4.19	2.40	3.37	1.00	1.58	0.91	1.27

For each interval euchromatic or heterochromatic features (Fig. S1) and parental origin for DNA methylation [WT-like or met1-like, according to Reinders et al. (1); see Fig. S4] are indicated.

1. Reinders J, et al. (2009) Compromised stability of DNA methylation and transposon immobilization in mosaic *Arabidopsis* epigenomes. *Genes Dev* 23:939-950.

**Table S2. Expression of genes involved in meiotic recombination in epi12 compared with WT, as determined by genome-wide expression analysis**

Gene name	Gene ID	Epi vs. WT (fold change)	P value	Ref.
Early meiotic events				
AML1	AT5G61960	1.001	0.9696	1
AML2	AT2G42890	0.948	0.2835	1
AML3	AT4G18120	0.632	0.0002	1
AML4	At5G07290	0.988	0.8424	1
AML5	AT1G29400	0.943	0.3343	1
SWI1	AT5G51330	1.058	0.6687	1
MEI1	AT1G77320	0.912	0.3328	1
CDC45	AT3G25100	1.046	0.8001	1
SDS	AT1G14750	0.966	0.6426	1
TAM	AT1G77390	0.848	0.4489	1
MS5	AT4G20900	1.031	0.6945	1
MMD1	AT1G66170	0.993	0.9424	1
ASK1	AT1G75950	1.108	0.3699	1
CAP-E1	AT5G62410	1.065	0.5453	1
SC				
ASY1	AT1G67370	0.857	0.1100	1
ASY2	AT4G32200	0.956	0.4882	1
Cohesins and cohesion release				
SYN1	AT5G05490	1.056	0.7731	1
SCC3	AT2G47980	0.999	0.9873	1
AESP	AT4G22970	0.980	0.8596	1
DSB formation				
AtSPO11-1	AT3G13170	1.011	0.9296	2
AtSPO11-2	AT1G63990	0.838	0.1073	2
AtPRD1	AT4G14180	0.962	0.5693	2
AtPRD2	AT5G57880	ND	ND	2
AtPRD3	AT1G01690	1.132	0.3124	2
DSB processing				
AtMRE11	AT5G54260	0.936	0.3220	2
AtRAD50	AT2G31970	0.894	0.1994	2
AtNBS1	AT3G02680	0.915	0.3692	2
AtCOM1	AT3G52115	1.122	0.0780	2
DNA strand exchange				
AtRAD51	AT5G20850	1.151	0.5484	2
AtDMC1	AT3G22880	1.042	0.6255	2
AtRAD51C	AT2G45280	0.816	0.2128	2
AtXRCC3	AT5G57450	0.942	0.4132	2
AtRPA1a	AT2G06510	0.702	0.0023	2
AtBRCA2	AT5G01630	0.838	0.1499	2
AtMND1	AT4G29170	1.200	0.1395	2
Crossover formation				
RCK	AT3G27730	1.034	0.5856	2
AtMSH4	AT4G17380	0.920	0.5755	2
AtMSH5	AT3G20475	0.943	0.7556	2
AtZIP3	AT2G32270	1.074	0.4086	2
AtZIP4	AT1G10970	0.953	0.8139	2
AtPTD	AT1G12790	0.823	0.3952	2
AtSHOC1	AT5G52290	0.918	0.2183	2
AtZYP1a	AT1G22260	0.903	0.2385	2
AtZYP1b	AT1G22275	0.932	0.5938	2
AtMLH3	AT4G35520	0.869	0.0361	2
AtMUS81	AT4G30870	0.897	0.3579	2
Early recombination				
ATM	AT3G48190	0.981	0.8011	1
Late recombination				
MLH1	AT4G09140	1.014	0.9310	1

The list of genes involved in recombination was established from refs. 1 and 2. No gene was detected as being differentially up-regulated (>twofold) or down-regulated (>0.5-fold) in epi12 compared with WT. ND, not present on the array.

1. Mercier R, Grelon M (2008) Meiosis in plants: Ten years of gene discovery. *Cytogenet Genome Res* 120:281–290.
2. Osman K, Higgins JD, Sanchez-Moran E, Armstrong SJ, Franklin FCH (2011) Pathways to meiotic recombination in *Arabidopsis thaliana*. *New Phytol* 190:523–544.



**Table S3. INDEL marker details**

INDEL marker name	CHR	position (Mp)	Col allele (bp)	Forward Primer Sequence	Reverse Primer Sequence
T1O3	2	1.5	879	TGTGGTATTGGAATGATTTGAACTTCAGG	CCCTCTTCTCCTTTTCTCCTGTAACC
T26I20	2	6.3	762	CAAGAAATTGAAAAACAGAGACTGTGATCG	GATGTTACTCCGATCAAAGAGAGGAGG
T4C9	4	7.3	815	GACCAAGCTTCGTTATCGAAGATAACC	AAAGAGAACTCACCGCATACC
F13M23	4	12.8	800	TCTGAGATGAGAGAACGCAT	TGCAACTAACTTCAAAC

**Table S4. KASP marker details**

KASP marker	CHR	Position, bp	Col allele	Ler allele	SNP upstream sequence
1_10	1	32 209	T	C	GATCAACCTGAATGAGAGAAT
1_20	1	5 236 460	A	G	TTGACAGATTTGGTTCGTCT
1_30	1	11 267 086	T	G	AATCCATATTTCTGACCACGGAACGGAAAT
1_40	1	15 893 603	A	T	AAAGATTGTGTTAATGATTTTGATTT
1_60	1	19 821 699	T	C	CATGTTATAGTAGTAAACCGGA
1_80	1	26 388 796	G	A	TGAGAAATCCAGAAAGCCTGA
1_90	1	30 200 826	T	C	TGGGAAGTGGAGAGATCATT
2_06	2	876 519	A	G	GTAACACAAAGTATCACTTGGA
2_10	2	1 489 332	C	G	TCTCTATAAGGAATAGTATGTCAGGAGATAT
2_30	2	7 424 354	T	C	GATGATCTGAGCAATCAACAGAAAGAAG
2_40	2	11 385 252	C	T	CACCGTCCCATTGGTTGAT
2_50	2	14961718	C	T	AATTGAGTACCTGGAGC
2_60	2	19 400 043	G	T	TAGTAATGCTGAGCAACATC
3_10	3	68 195	C	T	TCACATCCCTGTTGATCATAACGATTCACTCA
3_20	3	4 491 688	C	T	TGTAAATTTGCAAAGAAATCAGAATA
3_30	3	7 092 629	C	T	AAGGAAAGGAACTAGTAATGATGAATT
3_40	3	10 278 811	T	G	AACTGTAAAACATGGGAAGG
3_45	3	11 581 079	G	T	GATCTATCACAGTATGAAGGACA
3_50	3	15 558 472	G	C	AAATTATGTGTAGATCATGTATC
3_60	3	19 864 963	A	T	TGCAGCAGCTTCCATGGACGCCGTCAC
3_70	3	23 358 425	G	A	GCAGAGGAGATGCTCAGAAGATCG
5_10	5	233 020	C	G	CTCTTATTGATGATTGATGATAT
5_23	5	1 787 276	A	G	TAACCGAAATGGATGTTCTT
5_26	5	3 823 313	G	A	AAGTTACAATTTGGATCTACG
5_28	5	7 016 783	T	G	TGTCACCGCCACTACAAGCT
5_30	5	9 913 838	A	G	TGTTTAAGGTTGGATGTGGCTAG
5_40	5	15 211 469	G	A	TTAATAGCACACGTGGCGAGATACATC
5_50alt	5	19 386 885	G	T	CTTCATCACATATCTGTTGATATC
5_70	5	26 757 636	G	T	ATCATAAAATCAAATG

**Table S5. Number of F2 plants analyzed for KASP genotyping in the four populations**

Marker (KASP)	WTxWT samples	WTx <i>met1</i> samples	WTxepi01 samples	WTxepi12 samples
1_10	148	179	172	171
1_20	149	184	171	170
1_30	146	181	172	169
1_40	144	179	172	167
1_60	151	180	172	167
1_80	150	171	169	170
1_90	148	182	170	170
2_06	150	183	169	166
2_10	150	183	170	167
2_30	150	181	166	167
2_40	149	176	170	164
2_50	149	180	170	164
2_60	149	179	170	163
3_10	149	178	171	170
3_20	149	178	169	171
3_30	148	181	172	170
3_40	146	178	170	169
3_45	149	181	171	169
3_50	150	174	170	169
3_60	147	175	169	168
3_70	148	183	171	169
5_10	148	169	171	167
5_23	149	183	172	171
5_26	151	181	171	169
5_28	150	182	172	169
5_30	149	181	169	169
5_40	150	181	171	172
5_50alt	149	173	86	165
5_70	148	179	170	172
Total	4,313	5,195	4,858	4,884