

**Fig. S1**

H3K27me3, H3K9me2, and CHG methylation overlap on maternal alleles of PEGs in crosses *Ler* × *Col*.

**a** Overlap of genes marked by H3K27me3 and H3K9me2 on maternal alleles. Dark red inset marks genes that have H3K27me3 and H3K9me2 exclusively on their maternal alleles (excluding genes also marked on the paternal alleles).

**b** Genes marked by H3K27me3 and H3K9me2 have higher levels of H3K27me3. Box plots show mean values of z-scored H3K27me3 and H3K9me2 of genes where the maternal alleles are either marked by H3K27me3 alone or by a combination of H3K27me3 and H3K9me2. Genes containing maternal-specific H3K27me3 and H3K9me2 are particularly enriched for H3K27me3. Boxes show medians and the interquartile range, error bars show the full range excluding outliers. Notches show the 95% confidence interval for the median. Asterisks mark significance (Wilcoxon rank sum test,  $P$ -value <5.0E-3).

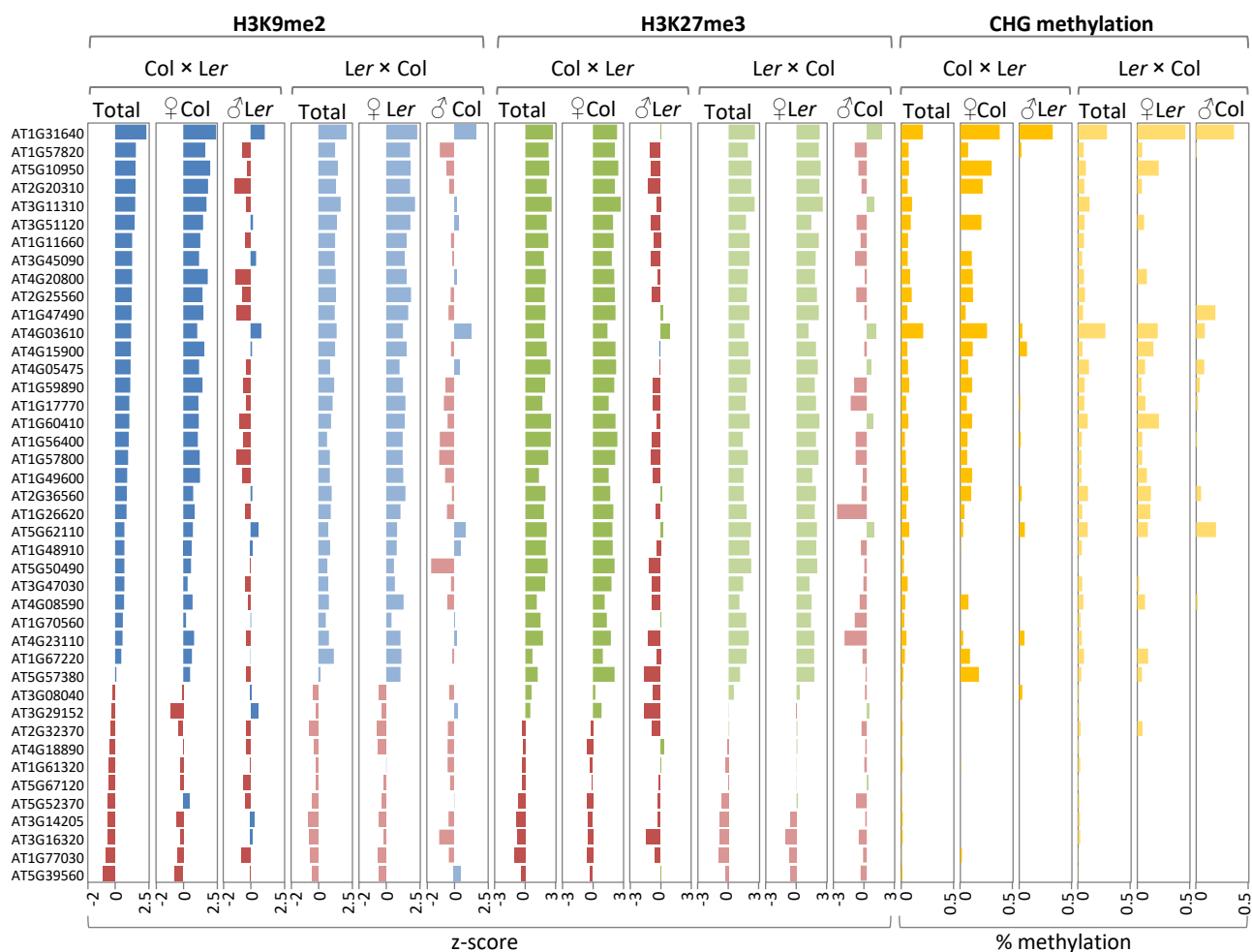
**c** The majority of PEGs are marked by H3K27me3 and H3K9me2 on their maternal alleles. Overlap of PEGs (Pignatta et al., 2014) with maternal and paternal H3K27me3 and H3K9me2.

**d** Levels of H3K27me3 and H3K9me2 on PEGs, MEGs and all genes. Box plots (as specified in panel b) represent mean values of z-scored H3K27me3 and, H3K9me2 Asterisks mark significance (Wilcoxon rank sum test,  $P$ -value <5.0E-3).

**e** Metagene plots showing average distribution of z-score normalized H3K27me3 and H3K9me2 levels on maternal and paternal alleles of PEGs (Pignatta et al., 2014).

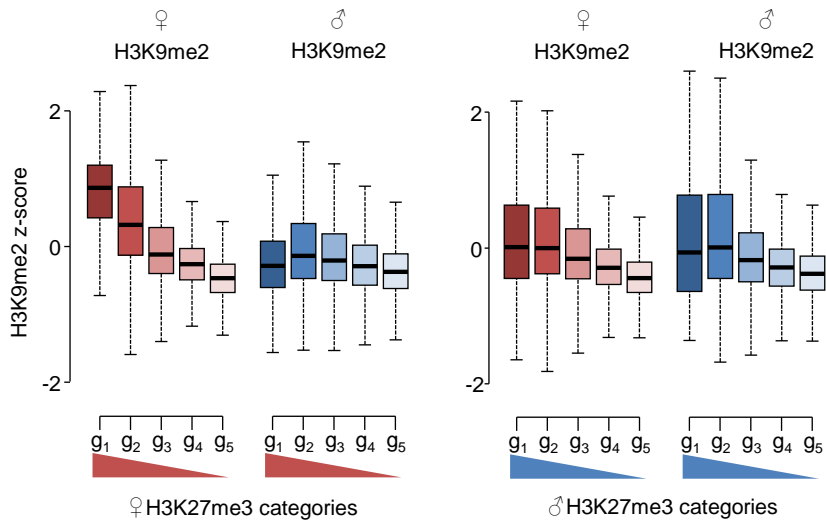
**f** Levels of DNA methylation in each sequence context (mCG, mCHG and mCHH) on PEGs, MEGs, and all genes. Box plots (as specified in panel b) show mean values of relative DNA methylation. Asterisks mark significance (Wilcoxon rank sum test,  $P$ -value <5.0E-2).

Data shown correspond to the cross *Ler* × *Col*, for reciprocal cross direction see Fig. 1.



**Fig.S2**

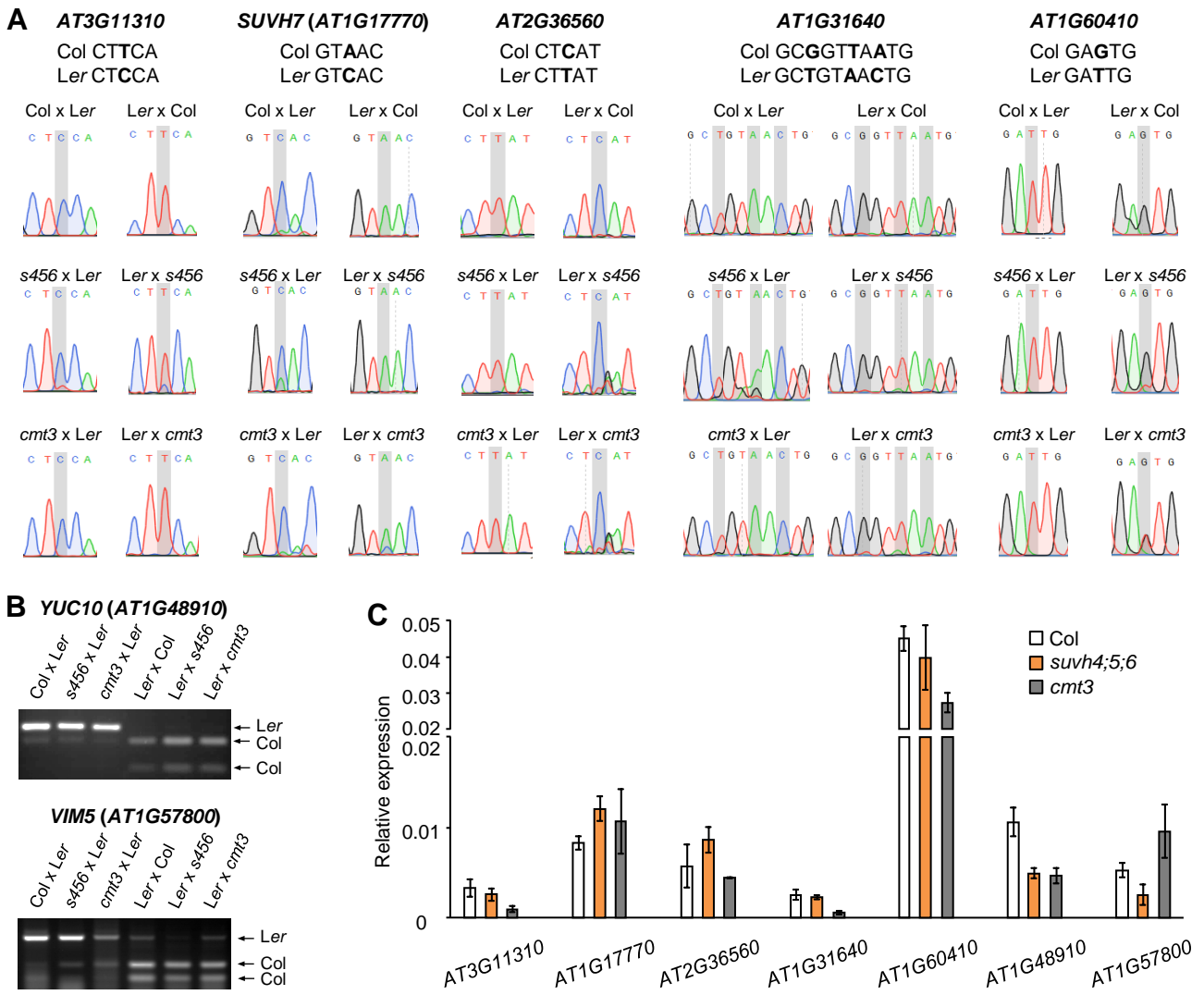
Total and parental-specific H3K27me3, H3K9me2, and CHG methylation levels in the endosperm of previously described PEGs [14].



**Fig. S3**

Increasing levels of H3K27me3 on maternal alleles correlates with increasing levels of H3K9me2.

Levels of maternal and paternal H3K9me2 on genes that have been grouped based on their level of H3K27me3 on maternal (left panels) and paternal alleles (right panels) from highest (g<sub>1</sub>) to lowest (g<sub>5</sub>) levels. For the z-score rank and number of genes in each category see Additional file 4: Table S5.



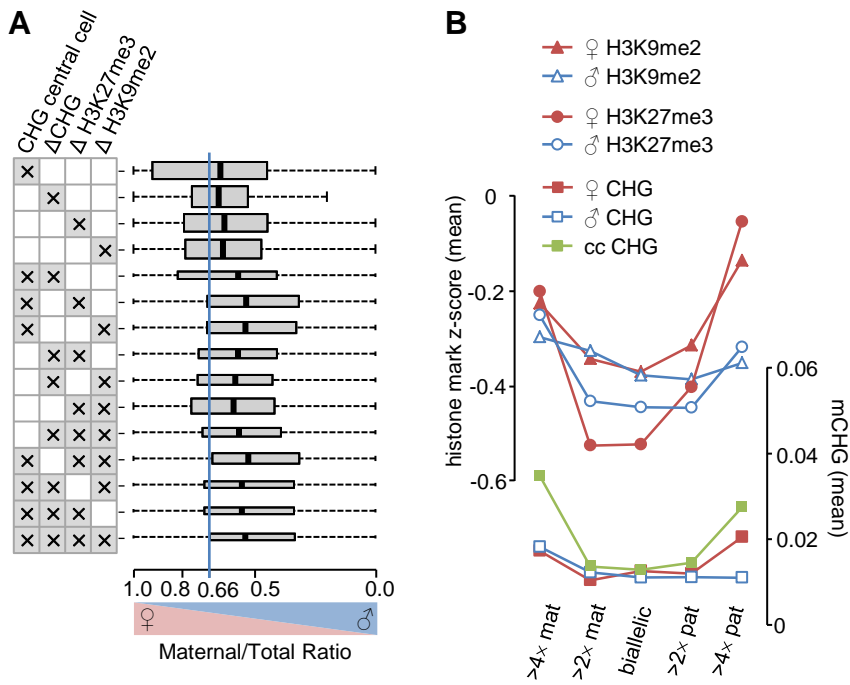
**Fig. S4**

Maternal alleles remain silenced in *svh4,5,6* and *cmt3* mutants.

**a** Imprinting assay of indicated PEGs in reciprocal crosses of Col and Ler and mutants for *svh4,5,6* (*s456*) and *cmt3* using Sanger -sequencing. Nucleotide polymorphisms allowing to distinguish parental alleles are shaded.

**b** Imprinting assay of indicated PEGs in reciprocal crosses of Col and Ler and mutants for *svh4,5,6* and *cmt3* using restriction-based analysis.

**c** qRT-PCR analysis of indicated PEGs in seeds of Col wild-type, *cmt3* and *svh4,5,6* mutants at 4 days after pollination. *PP2A* was used as a reference gene. Error bars show SEM. Expression changes in mutants are not significantly different compared to wild type (two-tailed t-test,  $P < 0.05$ ).



**Fig. S5**

Paternally-biased expression coincides with the combination of H3K27me3, H3K9me2, and CHG methylation in crosses *Ler* × *Col* (for reciprocal cross direction see Fig. 3).

**a** Box plots show mean values of maternal to total reads for genes marked by one or several of the following modifications: CHG methylation in the central cell, CHG methylation, H3K27me3, or H3K9me2 on the maternal alleles in the endosperm. For each modification only genes with top scores were included in the analysis (see Additional File 2: Table S1). Boxes show medians and the interquartile range, error bars show the full range excluding outliers. Widths of boxes are proportional to square-root of the number of genes in each category. Blue line indicates the expected ratio for biallelically expressed genes.

**b** Paternal expression bias correlates with high levels of CHG methylation in the central cell and H3K27me3 and H3K9me2 on maternal alleles in the endosperm. Plotted are mean values of CHG methylation, H3K27me3, and H3K9me2 on maternal and paternal alleles for genes with deviating parental expression levels from the expected two maternal to one paternal ratio.

**Table S3.**

GO terms enrichment of genes (P-value <0.01) in the highest score category (Score 12, see Additional File 2: Table S1).

<b>GO term</b>	<b>P-value</b>	<b>No. of genes</b>	<b>Description</b>
<b>biological process</b>			
GO:0006350	7.54E-05	28	transcription
GO:0045449	7.62E-05	27	regulation of transcription
GO:0019219	8.91E-05	27	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism
GO:0006351	1.36E-04	19	transcription, DNA-dependent
GO:0031323	1.47E-04	27	regulation of cellular metabolism
GO:0019222	1.89E-04	27	regulation of metabolism
GO:0006355	2.64E-04	18	regulation of transcription, DNA-dependent
GO:0051244	7.57E-04	27	regulation of cellular physiological process
GO:0050794	8.14E-04	27	regulation of cellular process
GO:0050791	1.12E-03	27	regulation of physiological process
GO:0050789	1.14E-03	29	regulation of biological process
GO:0016568	1.57E-03	4	chromatin modification
GO:0006139	2.30E-03	34	nucleobase, nucleoside, nucleotide and nucleic acid metabolism
<b>molecular function</b>			
GO:0030528	3.85E-04	28	transcription regulator activity
GO:0016782	7.63E-04	3	transferase activity, transferring sulfur-containing groups
GO:0003677	2.45E-03	29	DNA binding
GO:0003700	4.42E-03	23	transcription factor activity
GO:0003676	5.91E-03	35	nucleic acid binding
GO:0004722	6.20E-03	5	protein serine/threonine phosphatase activity
GO:0008415	8.24E-03	5	acyltransferase activity
GO:0016747	8.66E-03	5	transferase activity, transferring groups other than amino-acyl groups
<b>cellular component</b>			
GO:0005634	2.53E-03	30	nucleus

**Table S4.**

Fluorescence analysis of 4DAP seeds derived after indicated reciprocal crosses.

<b>Crosses</b>	<b>Line</b>	<b>No. of seeds observed</b>	<b>Expression in seed coat</b>	<b>Expression in endosperm</b>
<i>AT1G47530-GFP</i> × WT	L1	63	60	0
	L2	58	58	0
	L3	52	52	0
WT × <i>AT1G47530-GFP</i>	L1	62	0	0
	L2	52	0	0
	L3	56	0	0
<i>AT1G64660-GFP</i> × WT	L1	52	52	0
	L2	53	53	0
	L3	55	55	0
WT × <i>AT1G64660-GFP</i>	L1	62	0	53
	L2	53	0	45
	L3	57	No one	48

**Table S6.**

List of primers used in this study.

## Imprinting-by-sequencing

gene		Sequence 5' – 3'	product size (bp)
AT1G31640	FW	ACCAGTAGGGACATAGGGCTG	(Col/Ler) 334/334
	RV	TCTTAATCAGCTTAATGCCAGG	
AT1G60410	FW	TCGTTGGAGTAATCTCTGGTCG	(Col/Ler) 808/808
	RV	TCCCACCTTGGAGTAGTCATGG	
AT3G11310	FW	TGTAGAACCTGGAAAGACCGCTT	(Col/Ler) 385/385
	RV	CGGAGTAGGCTCATGATTGTCATTA	
AT1G17770	FW	GTGAAATGTGCTTAGTGGGGCTT	(Col/Ler) 271/271
	RV	CCTCTCACGACTCTAACGTCATTCC	
AT2G36560	FW	GAACGATGAGTCCAACGATGTGA	(Col/Ler) 363/365
	RV	GGTTAAACTCATCGTTCATCTCCT	

## Imprinting-by-restriction enzyme digestion

gene		Sequence 5' – 3'	product size (bp)	digestion fragments
AT1G57800 (VIM5)	FW	AAAGACCTTGGTTCCTAACAGG	(Col/Ler) 164/164	digestion BmsI (Col/Ler) 71+93/164
	RV	CTATGTTGACAATGAAGATCGTCC		
AT1G48910 (YUC10)	FW	GTTATCAATGGTGGGATTGGAA	(Col/Ler) 289/289	digestion HpaII (Col/Ler) 212+53+24/212+77
	RV	CCAAGATAGATCTGATGTCGTC		

## Expression analysis

gene		Sequence 5' – 3'
AT1G31640	FW	GACAACTCTCTGTGACATCAAAGCAT
	RV	CGAAATCACCTCTTGAACACCTT
AT1G60410	FW	CACATTTACGAACTGACCATCTC
	RV	AACGAAGCATCCAAGCCAAA
AT3G11310	FW	GGGAAAATACTCCAAGGAAAATAAGA
	RV	CTTTCCAGGTTCTACATGTCCTTTG
AT1G17770	FW	TGGGATGTATCTGGTTTCAAAGTTC
	RV	AGCAGGAGGTTGGTTTGGTTT
AT2G36560	FW	ACGATTCTACCTCACGGTTCAAG
	RV	CGCTCTCAGATATCGCCATTG
AT1G57800 (VIM5)	FW	GACGAGTAAAGGAAAGAAACCCATC
	RV	AGCATAAGAGCCAAAGCAAAGAGT
AT1G48910 (YUC10)	FW	AACCAACTCAATCCCAAACG
	RV	TGGCTAAGTGAAGTTTGAGACGAT



**(Table S6 continued)**

Primers used for generating reporter lines

<b>gene</b>		<b>Sequence 5' – 3'</b>
AT1G43580	FW	AACCTTCAGATGACTAAAGGTGGACTTGAATAGCC
	RV	CCCTTGCTCACCATTGATATCGTGCCGTCGCTTGCAAAGT
AT1G47530	FW	TTTTTTCACATGGGAAAGGATAAGACTTTGCCG
	RV	CCCTTGCTCACCATTGATATCAGCTCCTGCGCCGT
AT1G64660	FW	TTAACTACTATGGCTCATTTCCTCGAGACACAG
	RV	CCCTTGCTCACCATTGATATCAGCATTCTGAGGAATGCTTCTCG
AT2G30590	FW	GGTCTTAGATGGAGGAGATAGAAGGAACAAACAGAG
	RV	CCCTTGCTCACCATTGATATCAGAGTTGTTATAGCTTGAGATGGCAAT
AT4G15390	FW	GAGAAATCCATGGAGACGATGACGATGAAGGT
	RV	CCCTTGCTCACCATTGATATCAGAACCAAGACACTAGGGTTCATGG
AT5G53160	FW	AGGAAGAAGATGGAAGCTAACGGGATTGAGAAC
	RV	CCCTTGCTCACCATTGATATCAGGACTCTCGATTCTGTCGTGT
AT2G33620	FW	CCCTTGCTCACCATTGATATCCTCCAGGGCATGTTAATGGTGTATTG
	RV	GAGAAGAAAATGTCAGGATCTGAGACGGGT
<hr/>		
<b>Promoter</b>		<b>Sequence 5' – 3'</b>
AT1G43580	FW	AAGCTAAGCTCTAGTGATATCCCATATATGTTCAACCAAATCAGCTTTTAAGTGT
	RV	TTTAGTCATCTGAAGTTGATGGGTCGCC
AT1G47530	FW	AAGCTAAGCTCTAGTGATATCGTGGGAAATTTATTTTGAATATTTTAGGCGAAAAATA TTAATATTTCA
	RV	CTTTCCCATGTGAAAAAAGCTCTGTTTTAGTTTCTTCTTT
AT1G64660	FW	AAGCTAAGCTCTAGTGATATCAGAAATGTTGTTACAATCTTGATTTGTTGACTATATAT ATGAATGA
	RV	ATGAGCCATAGTAGTTAACTAAAGAAAAAGTAGAGCCTTTGTTAGGA
AT2G30590	FW	AAGCTAAGCTCTAGTGATATCTAGTATTGGAATCGCTGTGGTGTGTTGT
	RV	CTCCTCCATCTAAGAACCCTAATTTTTTCCCCCAGAAAAC
AT4G15390	FW	AAGCTAAGCTCTAGTGATATCAAAACATAAAGAAAGATATTGTACAATTGTGGTAGGT
	RV	CGTCTCCATGGATTTCTCTCCTCTTTAGACGAAGAGAAG
AT5G53160	FW	AAGCTAAGCTCTAGTGATATCGCTCTTCGAGTCAGGAAAAGTTAAGACT
	RV	AGCTTCCATCTTCTCCTCCGTCTTAAGCCACG
AT2G33620	FW	TCCTGACATTTTCTTCTCACTTTCCCAAACCAAAACAG
	RV	AAGCTAAGCTCTAGTGATATCATTAAAGCCGGTTTTTGCCTGAC

**Table S7.**

Quality of sequencing samples.

Table shows number of parental-specific reads in the samples analyzed in this study. Replicates are biological replicates.

<b>Replicates</b>	<b>Col reads</b>	<b>Ler reads</b>	<b>Observed</b>	<b>Expected</b>	<b>Contamination</b>
			<b>Ratio Col/Ler</b>	<b>Ratio Col/Ler</b>	
Col x Ler rep2	1 532 326	589 982	2,60	2,0	9%
Col x Ler rep3	3 527 140	1 155 593	3,05	2,0	14,9%
Ler x Col rep2	1 157 086	2 048 590	0,56	0,5	0%
Ler x Col rep3	1 566 319	3 177 167	0,49	0,5	0,30%