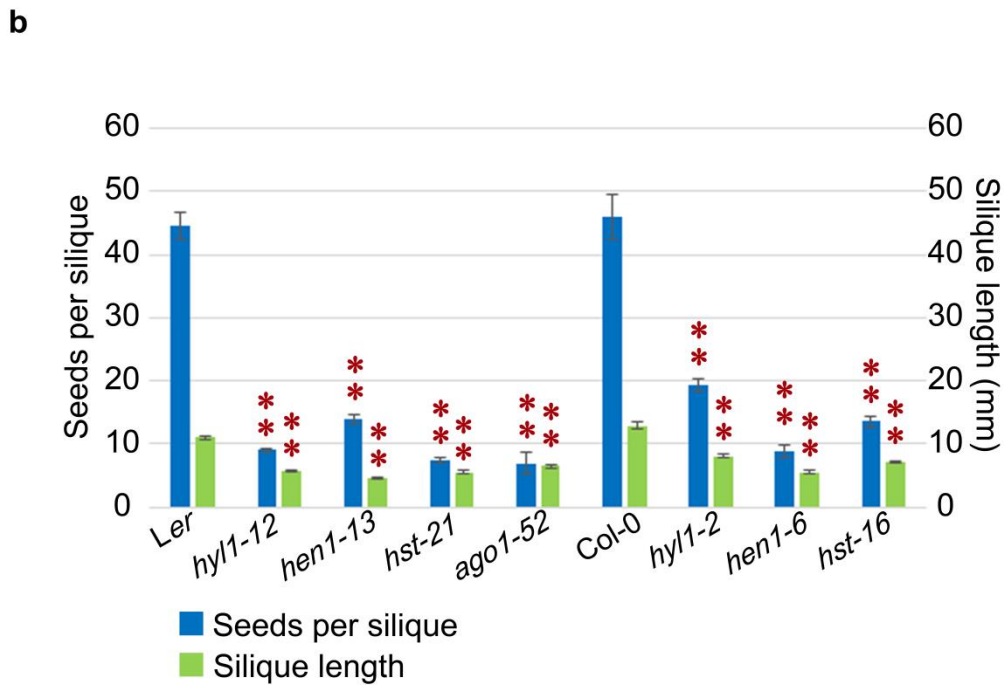
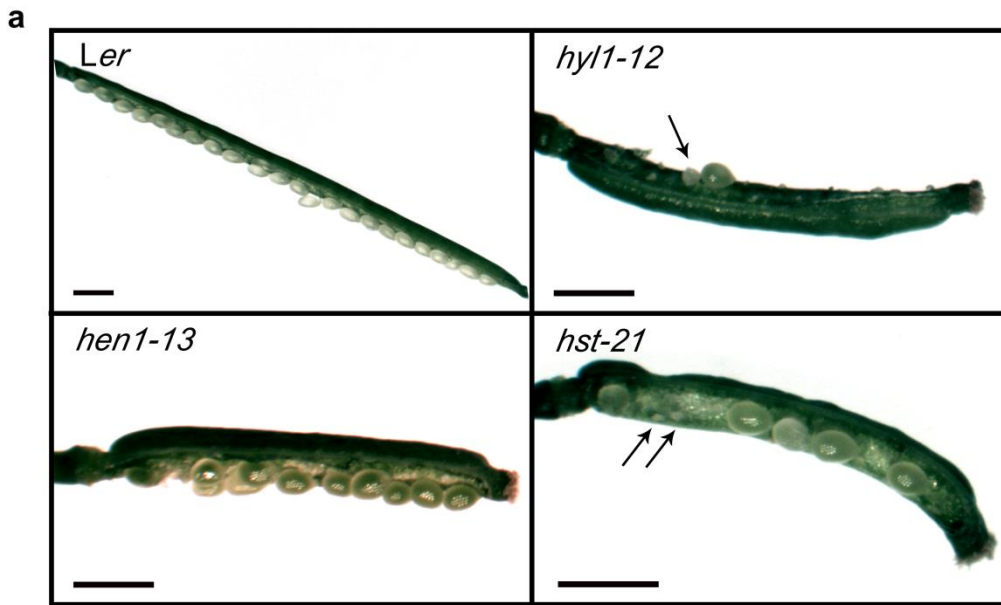


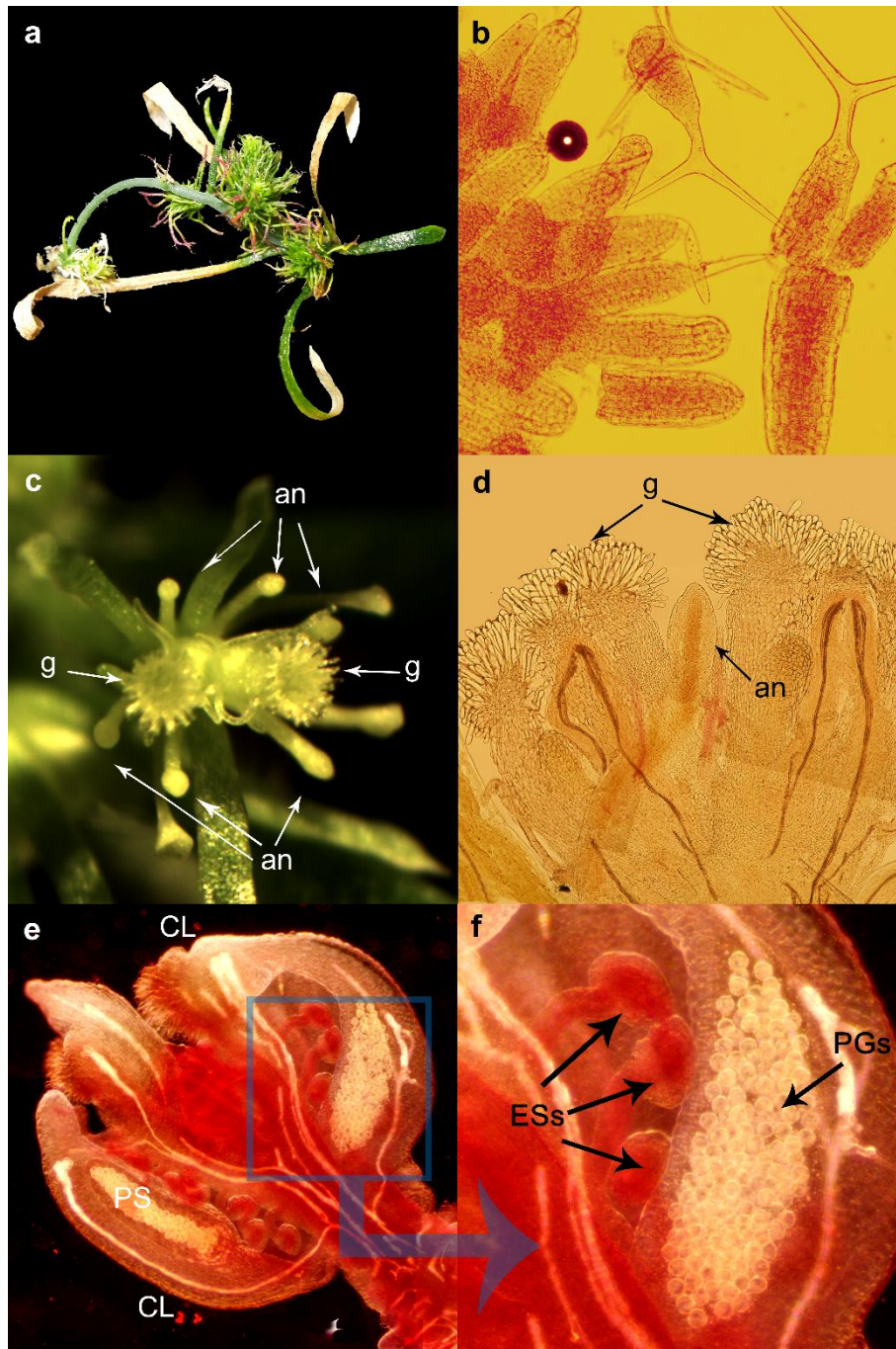
**Loss of function of Arabidopsis microRNA-machinery
genes impairs fertility, and has effects on homologous
recombination and meiotic chromatin dynamics**

Cecilia Oliver, Mónica Pradillo, Sara Jover-Gil, Nieves Cuñado,
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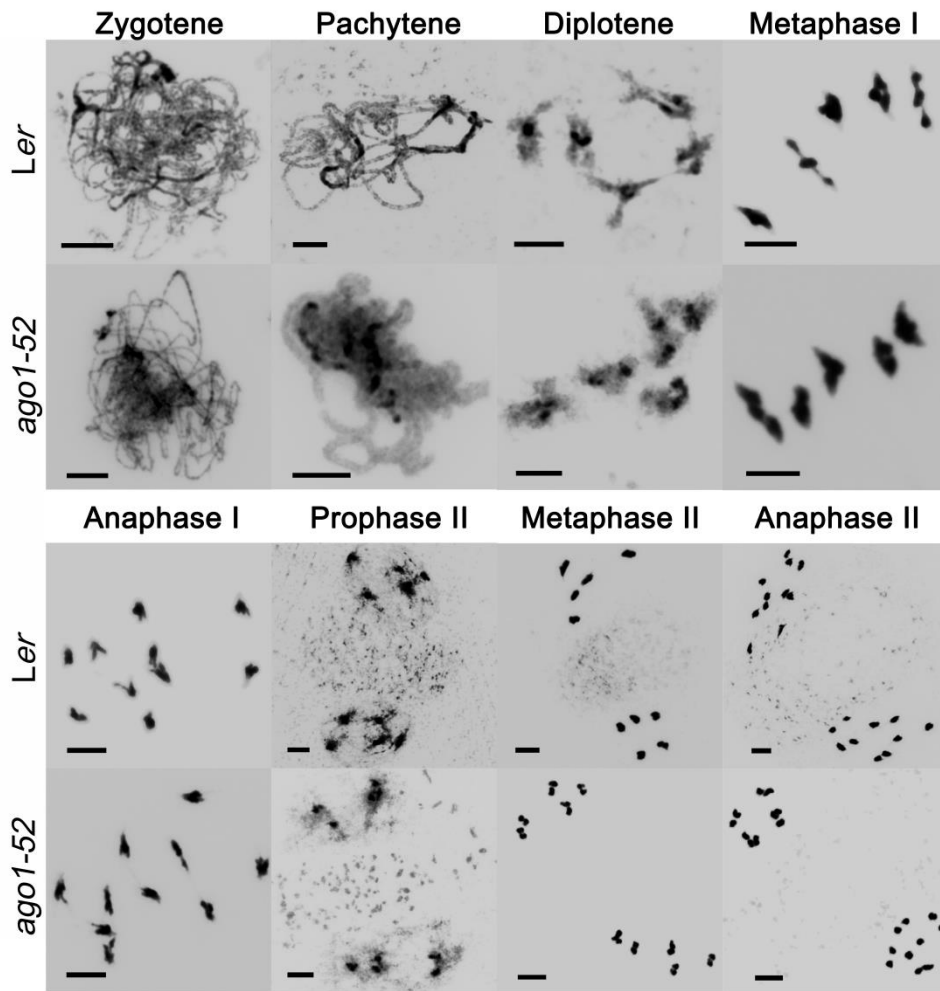
Supplementary Information



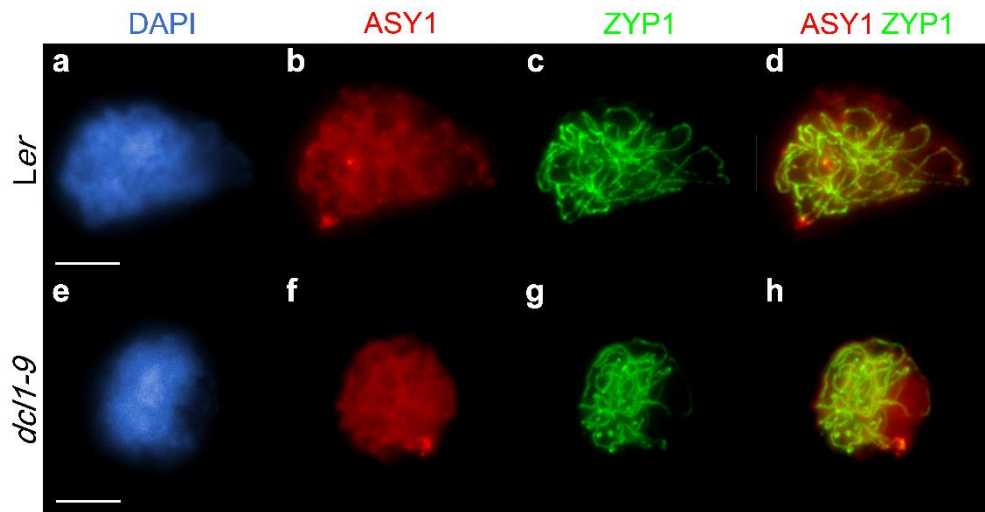
Supplementary Figure S1. Fertility in the *hyl1*, *hen1*, *hst* and *ago1* mutants. (a) Siliques from *Ler*, *hyl1-12*, *hen1-13* and *hst-21*. Arrows point to aborted seeds. Scale bars: 1 mm. **(b)** Seeds per silique and silique length. Error bars indicate standard error of the mean. Asterisks indicate a significant difference with the corresponding WT in a Mann-Whitney U test (**p < 0.01).



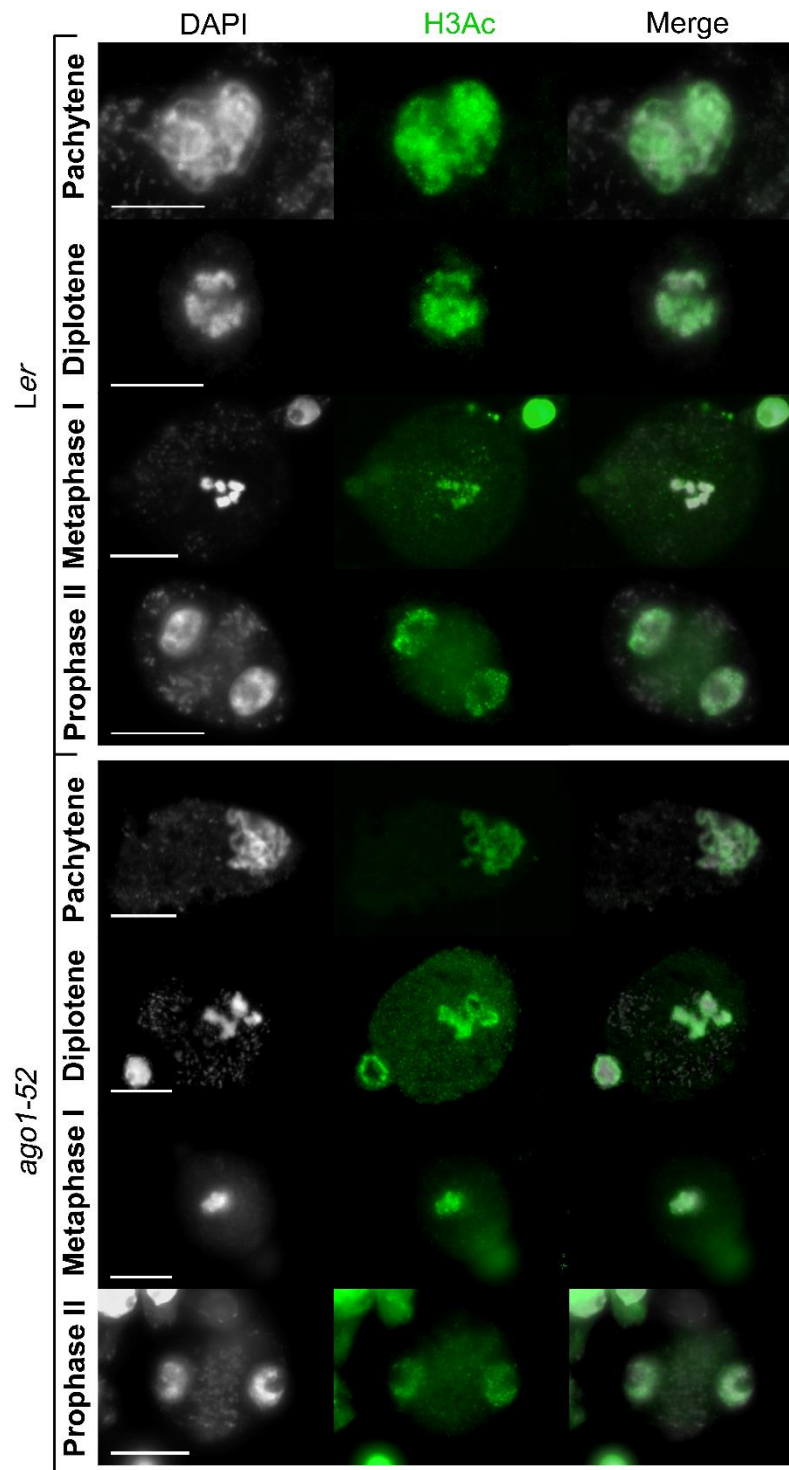
Supplementary Figure S2. Flower organ aberrations in *ago1-101* and *dcl1-9*. (a) *ago1-101* inflorescences formed by filamentous structures. (b) Detail of one of the filamentous structures shown in (a), stained with acetic carmine. (c) *ago1-101* flower displaying two gynoecia (g) and six anthers (an). (d) Acetic carmine staining of the flower shown in (b). (e) Acetic carmine staining of a *dcl1-9* gynoecium. (f) Magnification of (e), showing details of a pollen sac (PS) with pollen grains (PGs) within the carpel leaf (CL) and adjacent to the embryo sacs (ESs).



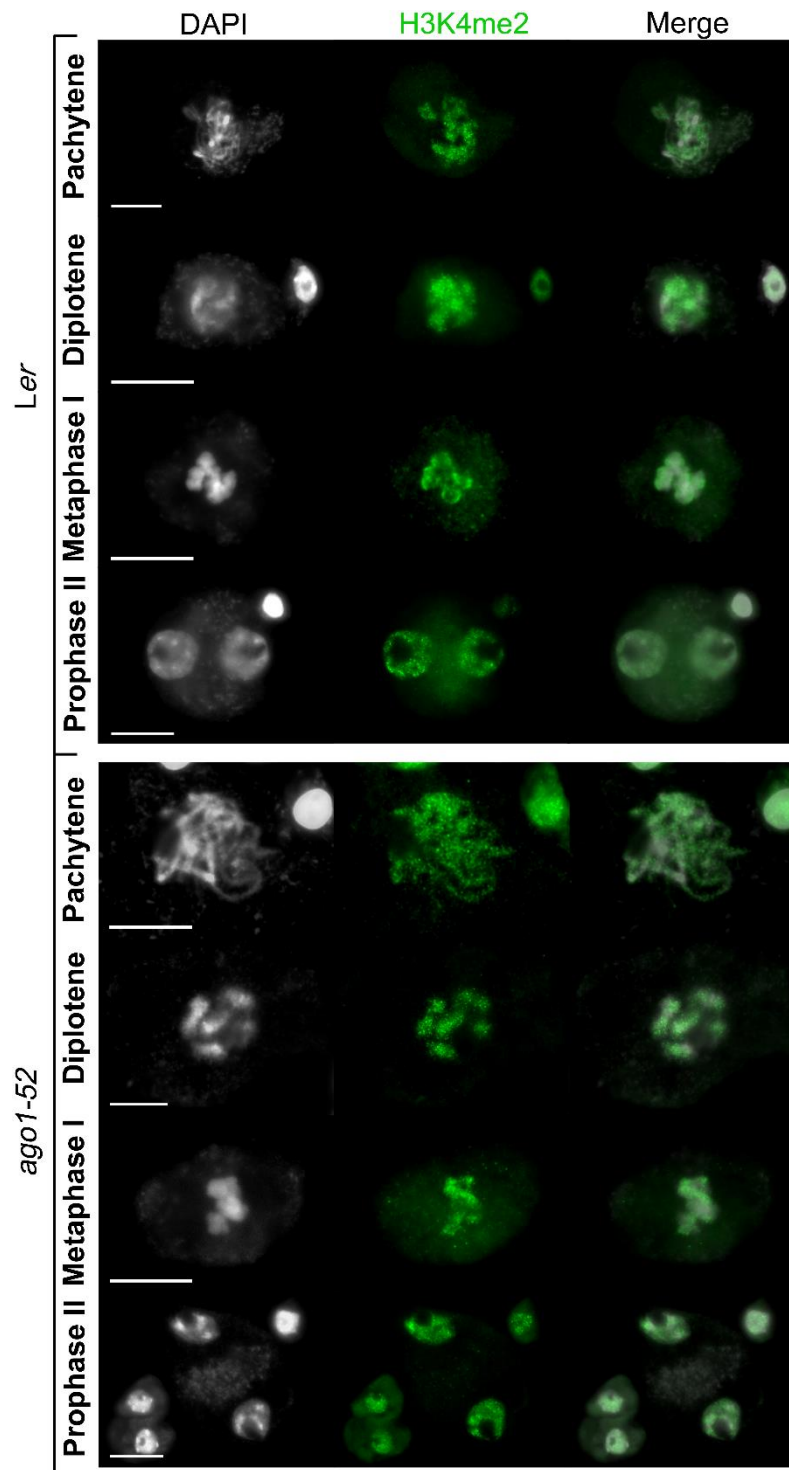
Supplementary Figure S3. Meiotic stages in PMCs from Ler and *ago1-52*. Bars = 5 μ m.



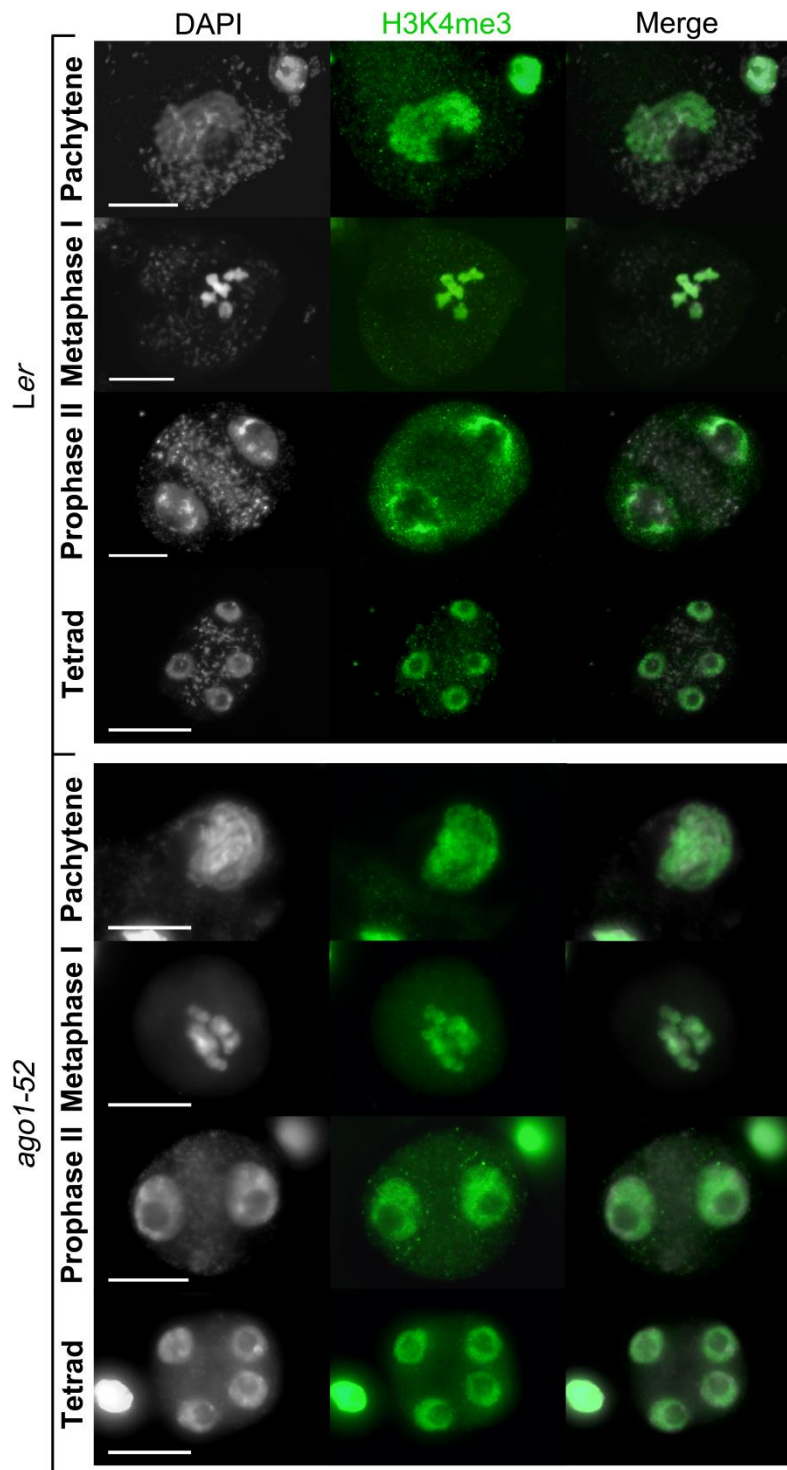
Supplementary Figure S4. ASY1 and ZYP1 immunolocalization in *Ler* and *dcl1-9* meiocytes. (a-d) *Ler* late pachytene. (e-h) *dcl1-9* late pachytene. Similar results were obtained with other mutants studied. Blue, red and green signals correspond to DAPI, ASY1 and ZYP1, respectively. Bars = 5 μm.



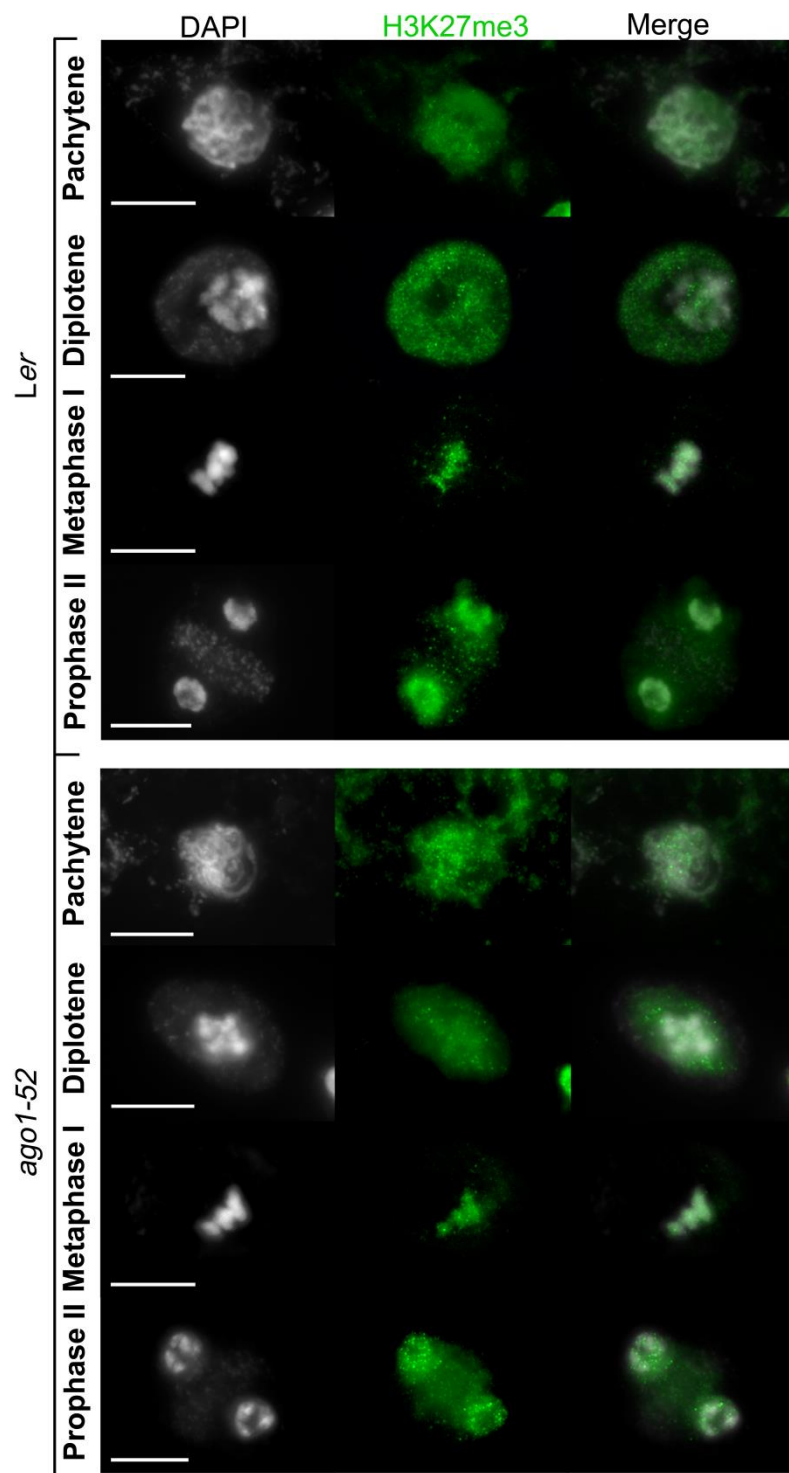
Supplementary Figure S5. Distribution pattern of H3Ac in PMCs from *Ler* and *ago1-52*. Representative images of H3Ac immunolocalization, which marks euchromatin regions. Similar results were obtained with other mutants studied. Bars = 5 μ m.



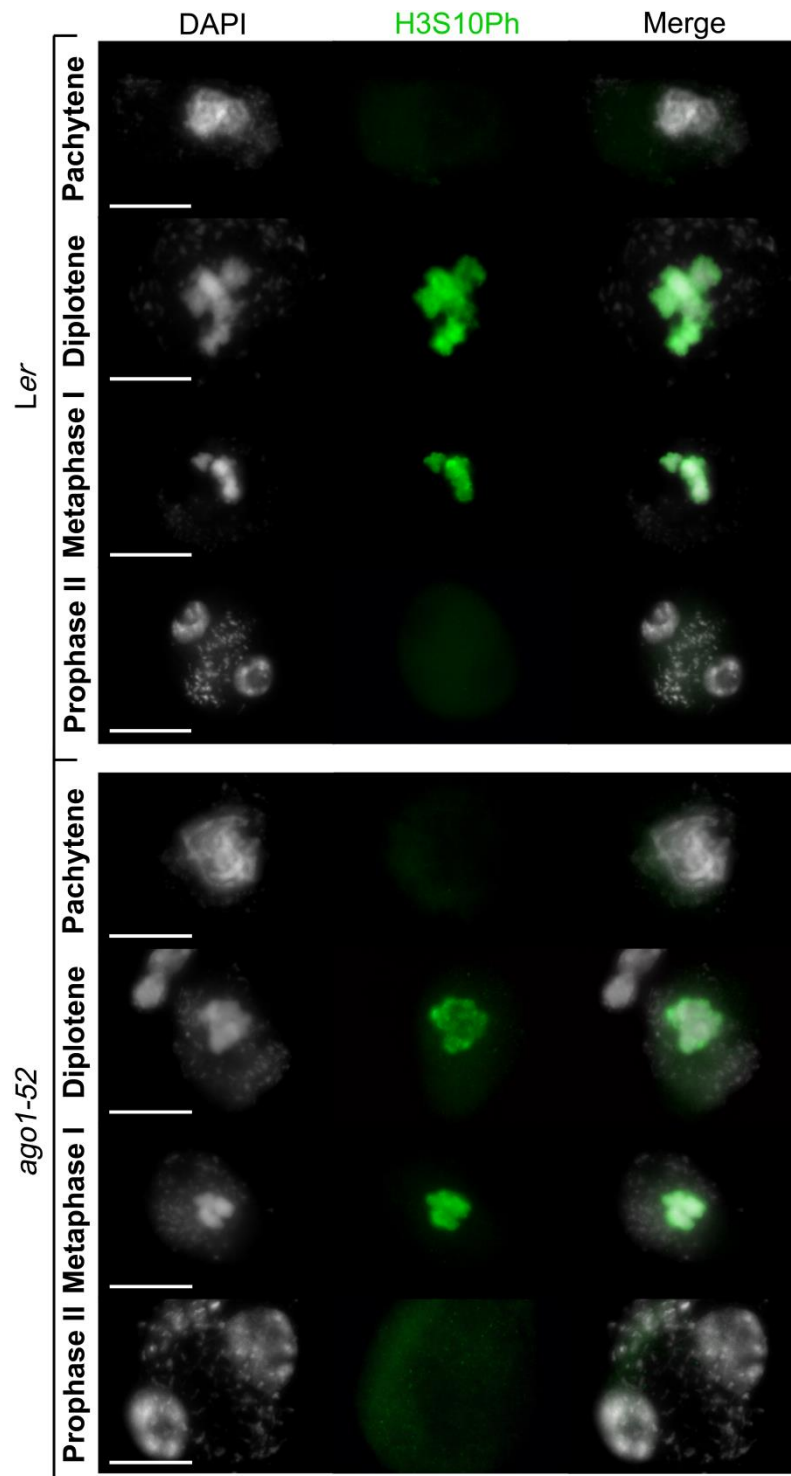
Supplementary Figure S6. Distribution pattern of H3K4me2 in PMCs from *Ler* and *ago1-52*. Representative images of H3K4me2 immunolocalization, which marks euchromatin regions. Similar results were obtained with other mutants studied. Bars = 5 μ m.



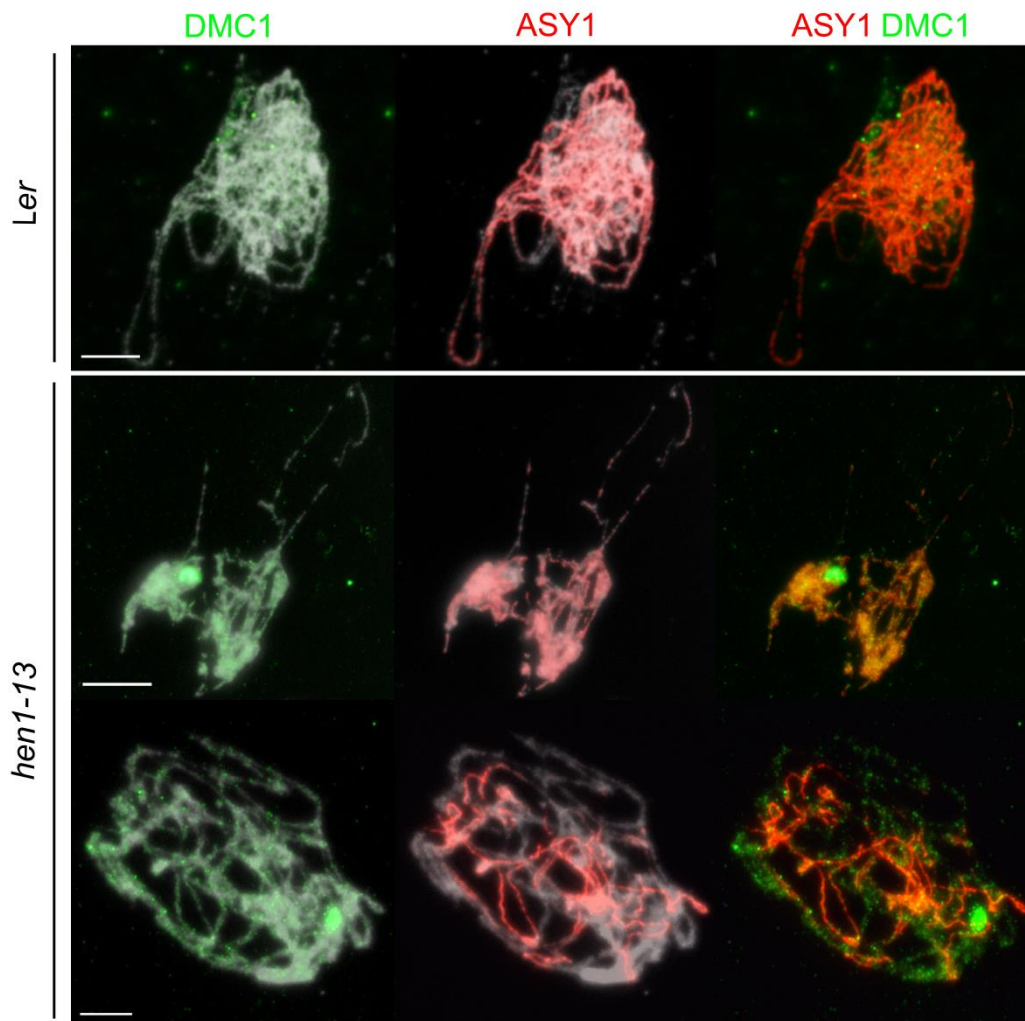
Supplementary Figure S7. Distribution pattern of H3K4me3 in PMCs from *Ler* and *ago1-52*. Representative images of H3K4me3 immunolocalization, which marks euchromatin regions. Similar results were obtained with other mutants studied. Bars = 5 μ m.



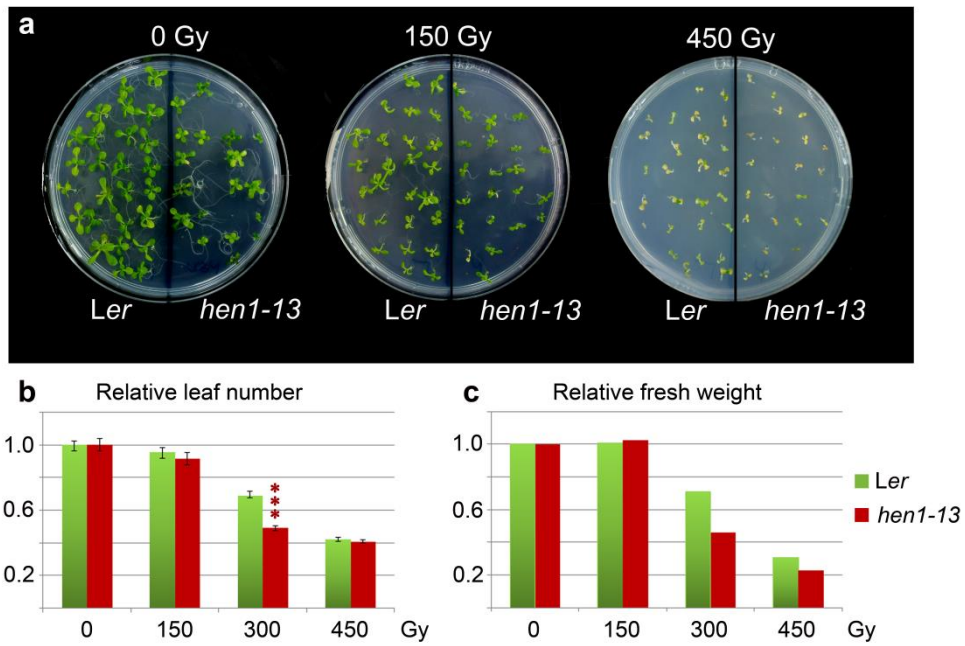
Supplementary Figure S8. Distribution pattern of H3K27me3 in PMCs from *Ler* and *ago1-52*. Representative images of H3K27me3 immunolocalization, which marks euchromatin regions. Similar results were obtained with other mutants studied. Bars = 5 μ m.



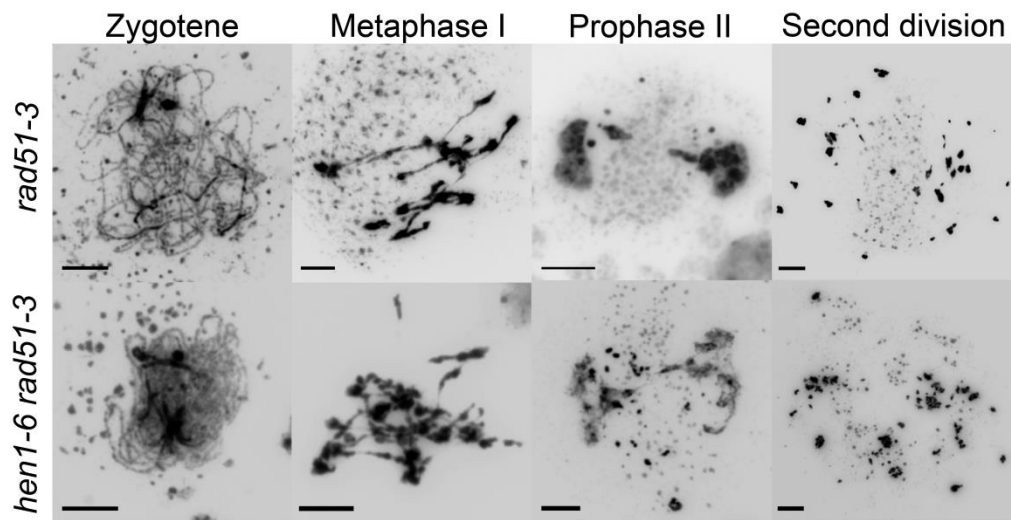
Supplementary Figure S9. Distribution pattern of H3S10Ph in PMCs from *Ler* and *ago1-52*. Representative images of H3S10Ph immunolocalization, which marks condensed chromosomes. Similar results were obtained with other mutants studied. Bars = 5 μ m.



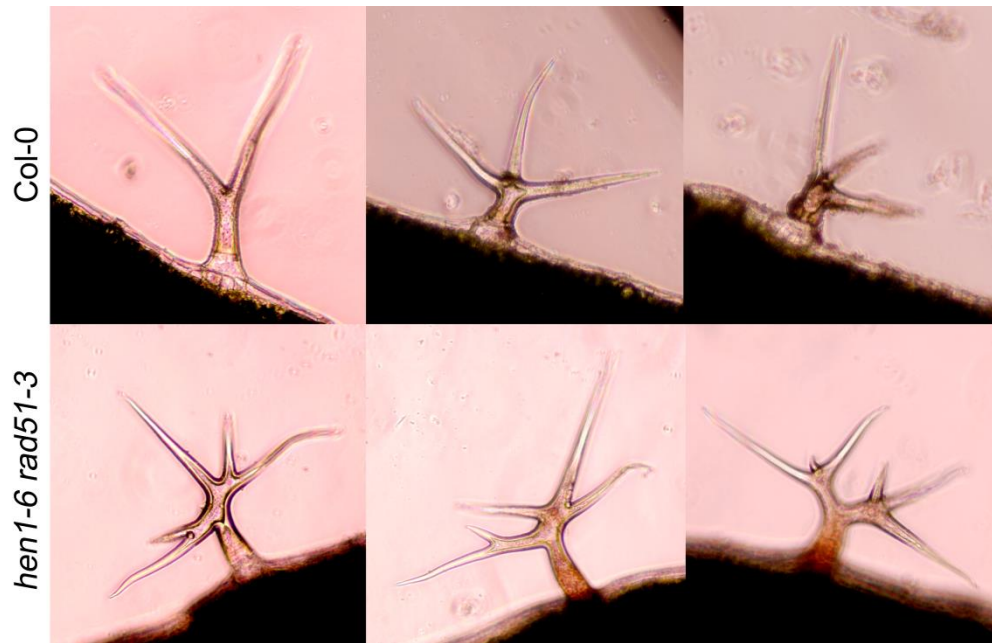
Supplementary Figure S10. DMC1 and ASY1 immunolocalization in *Ler* and *hen1-13* meiocytes. (a-c) *Ler*. (d-i) *hen1-13*. Green and red signals correspond to DMC1 and ASY1, respectively. Bars = 5 μ m.



Supplementary Figure S11. Sensitivity to gamma rays in *Ler* and *hen1-13*. (a) Representative images of plants exposed to 0, 150 and 450 Gy. (b) Relative leaf number after different doses of gamma irradiation. Leaf number was quantified in both genotypes and calculated in relation to the untreated control plants. Error bars indicate standard error of the mean. Asterisks indicate values significantly different from the corresponding wild type in a Mann-Whitney U test ($***p < 10^{-3}$). (c) Relative fresh weight after different doses of gamma irradiation. Fresh weight was measured in both genotypes and calculated in relation to the untreated control plants.



Supplementary Figure S12. PMCs from *rad51-3* and *hen1-6 rad51-3*. Bars = 5 μ m.



Supplementary Figure S13. Col-0 and *hen1-6 rad51-3* trichomes.

Supplementary Table S1: Mutants studied in this work.

Gene	Allele	Mutagen	Genetic background
At1g01040 (<i>DCL1</i>)	<i>dcl1-9</i> ¹	T-DNA	<i>Ler</i>
	<i>dcl1-16</i> ⁶	T-DNA	Col-0
At1g09700 (<i>HYL1</i>)	<i>hyl1-12</i> ²	Fast neutron	<i>Ler</i>
	<i>hyl1-2</i> ³	T-DNA	Col-0
At4g20910 (<i>HEN1</i>)	<i>hen1-13</i> ²	Fast neutron	<i>Ler</i>
	<i>hen1-6</i> ³	T-DNA	Col-0
At3g05040 (<i>HST</i>)	<i>hst-21</i> ⁴	EMS	<i>Ler</i>
	<i>hst-17</i> ⁶	T-DNA	Col-0
At1g48410 (<i>AGO1</i>)	<i>ago1-52</i> ²	EMS	<i>Ler</i>
	<i>ago1-101</i> ⁵	T-DNA	Col-0

¹⁻⁵Supplemental References. ⁶This work.

Supplementary Table S2: Percentage of decondensed nuclei at prophase I and metaphase I in PMCs from *ago1*, *dcl1*, *hen1*, *hst* and *hyl1* mutants.

Meiotic stage	Mutant	Decondensed nuclei (%)	Number of cells
Prophase I	<i>ago1-52</i>	37.74	53
	<i>dcl1-9</i>	40.00	20
	<i>hen1-13</i>	41.46	41
	<i>hst-21</i>	36.00	25
	<i>hyl1-12</i>	26.47	34
	<i>hen1-6</i>	34.29	35
	<i>hyl1-2</i>	40.00	30
Metaphase I	<i>hen1-6</i>	12.50	8
	<i>hst-17</i>	31.37	51
	<i>hyl1-2</i>	20.83	48

Supplementary Table S4: Oligonucleotide sets used for genotyping.

Gene	Line	Oligonucleotide sequences (5'→3')	
<i>DCL1</i>	<i>dcl1-16</i> (SALK_013118)	GACTTTTGGGATTCTCGAAGG	CCTCTCATCACTTTGCAAAGC
<i>HYL1</i>	<i>hyl1-2</i> (SALK_0064863)	AGTTCTCCCAGCGCTAATCTC	TTCTTGGAAATTGGATTGCAG
<i>HEN1</i>	<i>hen1-6</i> (SALK_090960)	GCATACTCCTACTCCAAAGGCGA	TGCCATCTTTCAATCCGAGCCT
<i>HST</i>	<i>hst-17</i> (SALK_033269)	GCAAAGCATCTCTTCTGATGGTGG	TGTCCATCACTGCTACATGCTGAG
<i>AGO1</i>	<i>ago1-101</i> (SALK_035319)	GGCAATCCAGCCTATACCTTC	TTCTCGATGAGTCACTTCAAC
<i>RAD51</i>	<i>rad51-3</i> (SAIL_873_C08)	TCTCAAGAACTTTGCAAGATGC	ATGCCAAGGTTGACAAGATTG

Supplementary Table S5: Antibodies used for immunolocalization.

Antibody	Dilution	Obtained in
anti-ASY1 ¹	1:1000	Rat
anti-ZYP1 ¹	1:250	Rabbit
anti-DMC1 ¹	1:300	Rabbit
anti-H3K9me2 ²	1:200	Rabbit
anti-H3K4me2 ³	1:200	Rabbit
anti-H3K4me3 ³	1:200	Rabbit
anti-H3K27me3 ²	1:200	Rabbit
anti-H3Ac ²	1:200	Rabbit
anti-H3S10Ph ⁴	1:200	Rabbit

¹Kindly donated by Prof. Chris Franklin, University of Birmingham, UK; ²Millipore; ³Abcam; ⁴Upstate.

Supplementary Table S6: Oligonucleotide sets used for RT-qPCR.

Gene		Oligonucleotide sequences (5'→3')		UPL	Amplification product (bp)
Symbol	AGI code	Forward primer	Reverse primer		
<i>SYN1</i>	At5g05490	TCAGCAGACTTTTATTTCCATGC	TCAGCTTGATGGAAGCTGCTG	129	93
<i>SMC1</i>	At3g54670	GCTCGAAGCTTGAAAAATTC	CCACGTCCTTTCTATTGGTCTC	63	96
<i>SMC6A</i>	At5g07660	TGCCTCAAGATGCAACAAAC	AAAGTCGAGAAAGACCGTTCC	150	76
<i>SMC6B</i>	At5g61460	TCGCACGAGAGGATAAAGAAA	TGACTCAAAGCCGAGGATG	68	106
<i>TOPII</i>	At3g23890	CCTATTCAGCAATGGACACAGA	TTCTTCTTTGCTCCTTGTCTACC	139	76
<i>SMC4A</i>	At5g48600	TGAAGGATAAACTGCAAACCAA	TGATCTCTGTATTGTTTTGTTCGAT	131	114
<i>CHR11</i>	At3g06400	AGCCAGACTTAAAGAGATGCAGA	CTCCCTTTTCCCTTATTGTTCA	67	108
<i>MET1</i>	At5g49160	CAACTGCAATGTGATTCTTAGGG	GCCTCTGTAGTAGAGACACAATCATC	39	75
<i>SWI1</i>	At5g51330	TTATTGGAGAAACCTTGCGTAAA	CCACTGTTTTCTTAAGCCATCC	69	60
<i>SPO11-1</i>	At3g13170	TTCCCAAACAGTGTCTTTTGC	TTCAAGTTCCAACCTCCATTG	143	113
<i>ATM</i>	At3g48190	AGGGTGGTGAGATGAGAAGC	TCTGTGTCAATTGCGTCTTGT	98	67
<i>ATR</i>	At5g40820	TTCAGCGCCCAAAGAAGA	GGCTTGCAAGGAATGGATA	3	67
<i>BRCA1</i>	At4g21070	CCAAGAAATTGGTCTTATCTTGC	AGTTCCGCAAATTCTGCAAT	100	73
<i>BRCA2B</i>	At5g01630	CACCTTAAAACCCGCAGTG	AGGTGATTTACAAGCACCGATT	140	117
<i>RAD50</i>	At2g31970	GCAGTGCAAGTCAAAGGTT	GGCCATCCAGGTTTGTAG	136	118
<i>RAD51C</i>	At2g45280	TCAACTAGCGCTTGCTTTAGG	AATACAGAATGACTCGGTTGGTG	54	65
<i>RAD51</i>	At5g20850	CATGCCACCACAACAAGG	ACATGGCGAGCTTATCACTTTAC	91	78
<i>DMC1</i>	At3g22880	TCAACGTTGCTGTCTACATGACT	GACCACCTGCTGGCTTTTT	31	90
<i>MSH4</i>	At4g17380	CAAGAATGGGGACAATGGAT	TGCATTATGAAAGCGGTCTCT	151	82
<i>MLH3</i>	At4g35520	GACTGAAGCAGACCTCACTTTG	GCCTTCAAATCGACAAGAGG	47	76
<i>MUS81</i>	At4g30870	GATATGTACCCAACGCTTTTGTG	CTTCTTGCGCCGAGACAT	29	73
<i>FANCM</i>	At1g35530	GCGGTAAAAAGCACATTAGAGG	GCTCCCGAAGAGACCTCAG	153	94

UPL: Universal Probe Library.

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