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Supplementary Information for

Regulation of interference-sensitive crossover distribution ensures crossover assurance in *Arabidopsis*

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This PDF file includes:
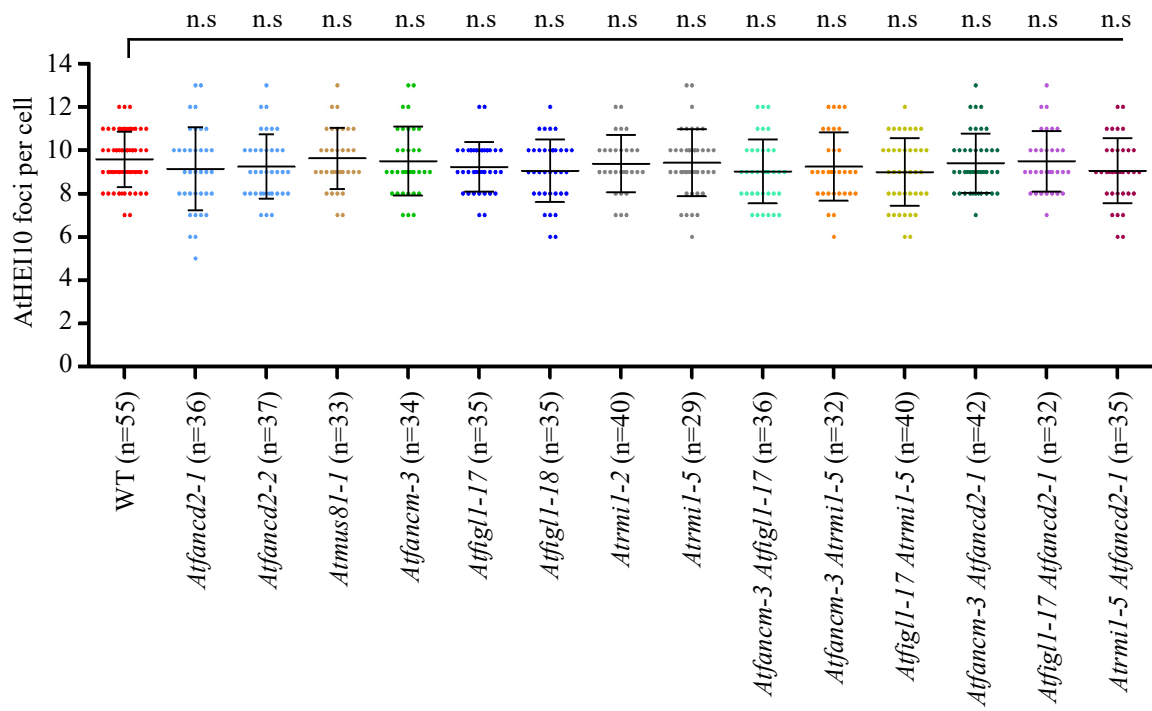
> Figures S1 to S4
> Tables S1 to S3

Fig. S1. Statistical analysis of the number of AtHEI10 foci per cell at diakinesis in the genotypes analyzed in this study. Error bars indicate SD. n.s, not significant, two-tailed Mann Whitney test. n, the number of cells analyzed.
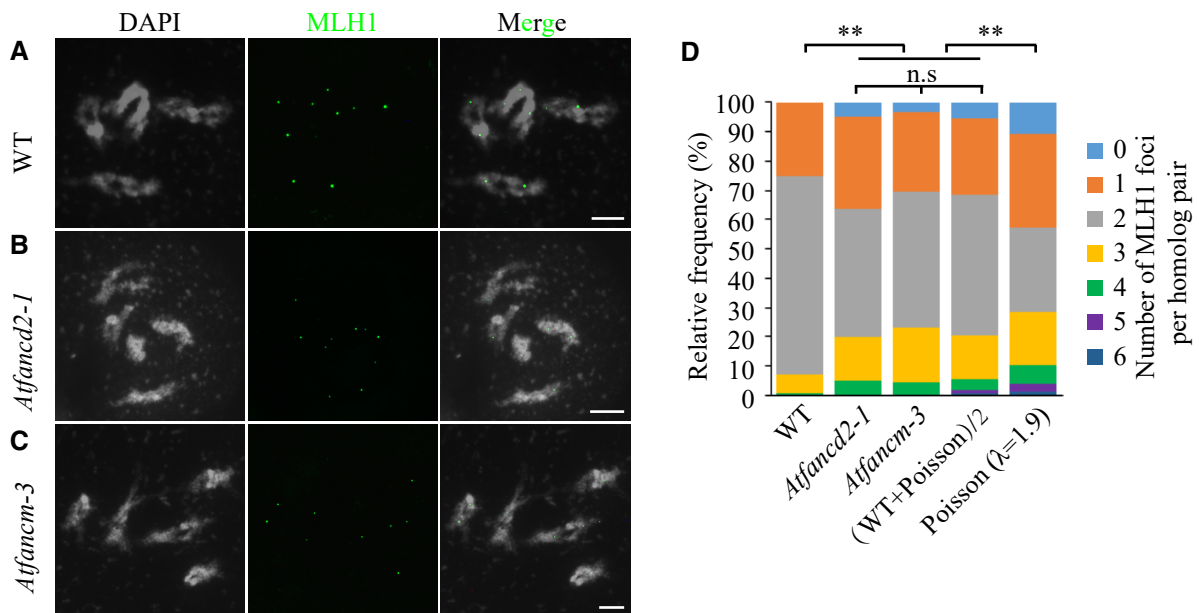
Fig. S2 The distribution pattern of AtMLH1 foci in WT, *Atfancd2-1* and *Atfancm-3*.
(A-C) Immunostaining of AtMLH1 at diakinesis in WT, *Atfancd2-1* and *Atfancm-3*. DAPI stained chromosomes are shown in white and AtMLH1 foci are shown in green. (D) Distributions of the number of AtMLH1 foci on pairs of homologous chromosome pairs (bivalents) in WT, *Atfancd2-1* and *Atfancm-3*. n.s not significant, ** $P < 0.01$ Chi-square test. Bars = 5 um.
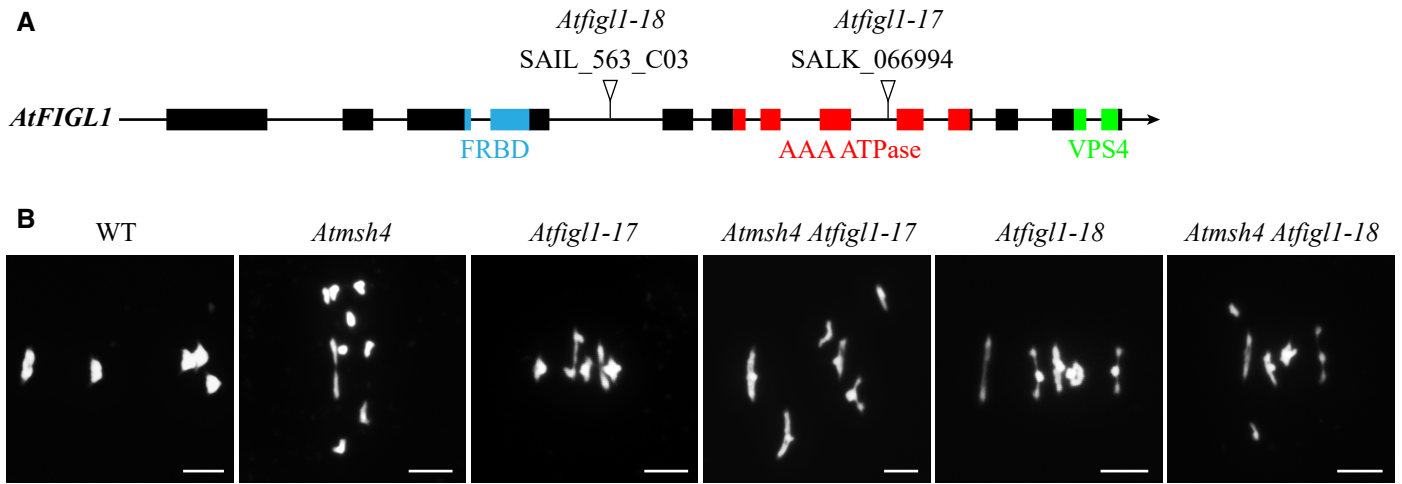
Fig. S3 Validation of two new *Atfigl1* alleles.
(A) Schematic positions of the T-DNA insertions in the *Atfigl1-17* and *Atfigl1-18* alleles. The blue, red and green rectangles indicate FRBD, AAA ATPase and VPS4 domains, respectively. The triangles indicate the positions of the T-DNA insertion.
(B) Chromosome morphologies of WT, *Atmsh4*, *Atfigl1-17*, *Atmsh4 Atfigl1-17*, *Atfigl1-18* and *Atmsh4 Atfigl1-18* at metaphase I. Bars = 5 um.
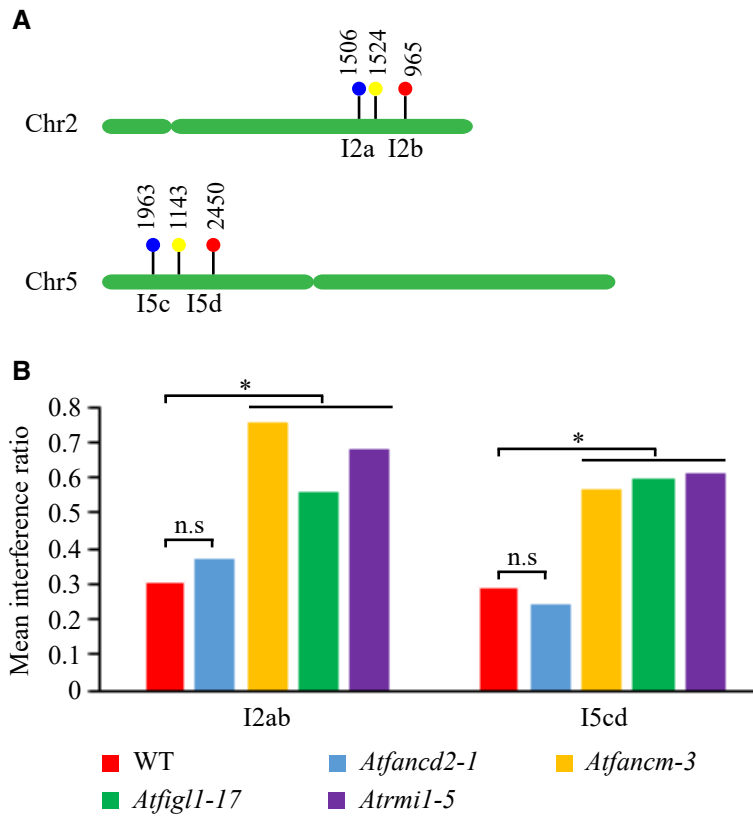
Fig. S4 CO interference analysis in WT, *Atfancd2-1*, *Atfancm-3*, *Atfigl1-17* and *Atrmi1-5*.
(A) Positions of intervals I2a, I2b, I5c and I5d in chromosomes marked by fluorescent proteins.
(B) Interference ratio (IR) was measured in two pairs of intervals I2ab and I5cd in WT, *Atfancd2-1*, *Atfancm-3*, *Atfigl1-17* and *Atrmi1-5*. The interference ratio is closer to 1, the interference is weaker. Using the raw data from table S2, calculations and statistical analyses have been performed according to Stahl Lab Online tools (https://elizabethhousworth.com/StahlLabOnlineTools/).
n.s not significant, * significant.

Table S1. P values for Chi-square test between the distribution of AtHEI10 foci among chromosomes in genotypes analyzed in this study.

	WT (n = 275)	Atmus81-1 (n = 165)	Atfancd2-1 (n = 180)	Atfancd2-2 (n = 185)	Atfancm-3 (n = 170)	Atfigl1-17 (n = 165)	Atfigl1-18 (n = 175)	Atrmi1-2 (n = 200)	Atrmi1-5 (n = 145)	Atfancm-3 Atfigl1-17 (n = 180)	Atfancm-3 Atrmi1-5 (n = 160)	Atfigl1-17 Atrmi1-5 (n = 195)	Atfancm-3 Atfancd2-1 (n = 210)	Atfigl1-17 Atfancd2-1 (n = 160)	Atrmi1-5 Atfancd2-1 (n = 175)	(WT + Poisson)/2
Atmus81-1	0.671															
Atfancd2-1	5.15E-05	0.002														
Atfancd2-2	1.81E-04	0.005	0.872													
Atfancm-3	2.72E-04	0.010	0.327	0.760												
Atfigl1-17	0.003	0.037	0.316	0.914	0.845											
Atfigl1-18	0.001	0.019	0.833	0.844	0.633	0.714										
Atrmi1-2	3.70E-05	0.003	0.497	0.130	0.603	0.205	0.586									
Atrmi1-5	0.001	0.011	0.817	0.987	0.796	0.934	0.856	0.152								
Atfancm-3 Atfigl1-17	0.003	0.029	0.651	0.860	0.664	0.874	0.975	0.858	0.640							
Atfancm-3 Atrmi1-5	0.002	0.040	0.373	0.701	0.947	0.848	0.821	0.764	0.513	0.806						
Atfigl1-17 Atrmi1-5	0.001	0.014	0.888	0.758	0.488	0.521	0.995	0.764	0.434	0.904	0.618					
Atfancm-3 Atfancd2-1	0.002	0.019	0.847	0.755	0.300	0.460	0.814	0.757	0.499	0.733	0.367	0.849				
Atfigl1-17 Atfancd2-1	1.31E-05	0.001	0.787	0.887	0.331	0.350	0.579	0.771	0.940	0.490	0.423	0.499	0.675			
Atrmi1-5 Atfancd2-1	4.35E-04	0.013	0.562	0.876	0.930	0.871	0.935	0.866	0.191	0.917	0.983	0.787	0.242	0.130		
(WT + Poisson)/2	0.004	0.035	0.523	0.920	0.706	0.971	0.735	0.975	0.124	0.841	0.729	0.607	0.618	0.492	0.784	
Poisson (λ =1.9)	2.22E-14	1.04E-10	0.001	0.002	0.003	0.001	0.001	0.009	0.001	3.90E-04	0.001	0.001	3.940E-04	0.003	0.003	0.004

Intervals	Genotypes	A	B	C	D	E	F	G	H	I	J	K	L	total
	Table S2. Raw data of FTL analyses in four intervals													
I2ab	WT	2085	205	141	1	1	1	1	0	0	0	0	0	2435
	Atfancd2-1	1105	105	60	2	0	0	0	0	0	0	0	0	1272
	Atfancm-3	1239	255	126	5	0	4	13	6	2	0	0	0	1650
	Atfancm-3 Atfancd2-1	1436	234	104	3	1	4	5	0	0	0	0	0	1787
	Atfigl1-17	1648	304	153	4	1	4	8	7	3	0	0	0	2132
	Atfigl1-17 Atfancd2-1	1437	330	156	5	2	3	9	8	3	0	0	0	1953
	Atrmi1-5	1540	232	125	4	3	2	5	5	1	0	0	0	1917
	Atrmi1-5 Atfancd2-1	1611	379	170	10	7	8	7	5	3	0	0	0	2200
I5cd	WT	869	139	117	2	1	2	0	0	1	0	0	0	1131
	Atfancd2-1	823	99	112	1	1	1	0	0	0	0	0	0	1037
	Atfancm-3	1193	379	289	15	16	12	17	16	4	0	0	0	1941
	Atfancm-3 Atfancd2-1	1238	260	213	13	11	8	12	10	3	0	0	0	1768
	Atfigl1-17	1003	280	187	8	7	10	12	11	5	0	0	0	1523
	Atfigl1-17 Atfancd2-1	1018	315	248	9	7	13	17	16	9	1	0	0	1653
	Atrmi1-5	1411	273	256	7	5	11	10	7	4	0	0	0	1984
	Atrmi1-5 Atfancd2-1	1308	391	324	8	9	13	18	14	8	1	0	0	2094

Table S3. The sequences of primers used in this study.	
Atfancd2-1 LP	AATTCACCGGAATGTCACAAC
Atfancd2-1 RP	AAAAGAATCACCACTTGCACG
Atfancd2-2 LP	ATTTAAGTTCAGGCCTCTTCTTCC
Atfancd2-2 RP	CCCAGTTTGGCATGTAGATTG
Atmus81-1 LP	TGGTGAAATCTAGCAACCCAG
Atmus81-1 RP	AATTTTCCACAAACCCTTTGG
Atmsh4 LP	CGGCTTCACTGCATCTATCTC
Atmsh4 RP	TGGAATGGATCAATGAGTTCC
Atfancm-3 LP	CACTCGAGCAATCTCCAAGTC
Atfancm-3 RP	TCTTGCCGGAATACATCTCAC
Atfigl1-17 LP	TTTCAAAGGTTGTCGTTCACC
Atfigl1-17 RP	CCTTCCGTTAGGTTGCATATG
Atfigl1-18 LP	TCAGGAAGAACGGCATGATAC
Atfigl1-18 RP	AATGGGCATGCATGTTATCTC
Atrmi1-2 LP	TCAAGGGGACAGAACAACAAC
Atrmi1-2 RP	TTTCAAGGGTTGAGCATATGC
Atrmi1-5 LP	ACTGTCAACCCTGAACCAGAG
Atrmi1-5 RP	TTTTGTACAACCTGCAGAGGG
LB3	TAGCATCTGAATTTCATAACCAATCTCGATACAC
LBb1.3	ATTTTGCCGATTTCGGAAC
GABItest1	CCCATTTGGACGTGAATGTAGACAC