Supplemental Table 1. Numbers of unique reads aligning to the maize genome sequence scaffold in S1-based mapping of DSB resection.

Sample	Number of unique mapping reads
S1-treated B73 - replicate 1	6804896
S1-treated B73 - replicate 2	11949874
B73 input DNA	14639531
Untreated B73 control	1788185
S1-treated B73 leaf (somatic cells)	6766
S1-treated *ameiotic1*	5168

Supplemental Table 2. Spearman correlation between samples in S1-based mapping of DSB resection.

	S1-treated B73 - replicate 1	S1-treated B73 - replicate 2	Untreated B73 control	B73 input DNA
S1-treated B73 - replicate 1	1	0.93	0.75	0.52
S1-treated B73 - replicate 2		1	0.77	0.54
Untreated B73 control			1	0.47
B73 input DNA				1

Supplemental Table 3. Numbers of unique reads mapping to the maize genome in MLH3 ChIP experiments.

Genotype	Stage	Antibody	Numbers of unique sequence reads (mln)
B73 x Mo17	zygotene	MLH3-C	29
B73 x Mo17	zygotene	MLH3-N	29
B73 x Mo17	pachytene	MLH3-C	64
B73 x Mo17	pachytene	MLH3-N	64
B73 x Mo17	diplotene	MLH3-C	27
B73 x Mo17	diplotene	MLH3-N	47
B73	zygotene	MLH3-C	73
B73	zygotene	MLH3-N	22
B73	pachytene	MLH3-C	10
B73	pachytene	MLH3-N	18
B73	diplotene	MLH3-C	18
B73	diplotene	MLH3-N	40

Supplemental Table 4. Numbers of MLH3 hotspots identified in the B73 x Mo17 hybrid colocalizing in experiments using antibodies targeting the C and N termini of MLH3. Window size = 2kb; q = 0.05.

Stage	Peak No. for MLH3-C	Peak No. for MLH3-N	No. of overlapping peaks	% of overlapping peaks
Zygotene	61	690	48	78.7
Pachytene	1115	1066	673	63.1
Diplotene	6945	63426	5503	79.2

Supplemental Table 5. Correlation between MLH3 patterns generated using antibodies targeting the C and N termini of MLH3 at different prophase I substages in the B73 x Mo17 hybrid. Table shows numbers of MLH3 peaks colocalizing in non-overlapping 2 kb windows distributed genome wide. q = 0.05.

| Pattern 1			*vs.*	Pattern 2			No. of overlapping peaks	% of overlapping peaks
Stage	Antibody	Peak No.		Stage	Antibody	Peak No.		
zygotene	MLH3-C	61		pachytene	MLH3-C	1115	9	14.8
zygotene	MLH3-C	61		diplotene	MLH3-C	6945	17	27.9
pachytene	MLH3-C	1115		diplotene	MLH3-C	6945	974	87.4
zygotene	MLH3-N	690		pachytene	MLH3-N	1066	14	2.0
zygotene	MLH3-N	690		diplotene	MLH3-N	63426	92	13.3
pachytene	MLH3-N	1066		diplotene	MLH3-N	63426	963	90.3
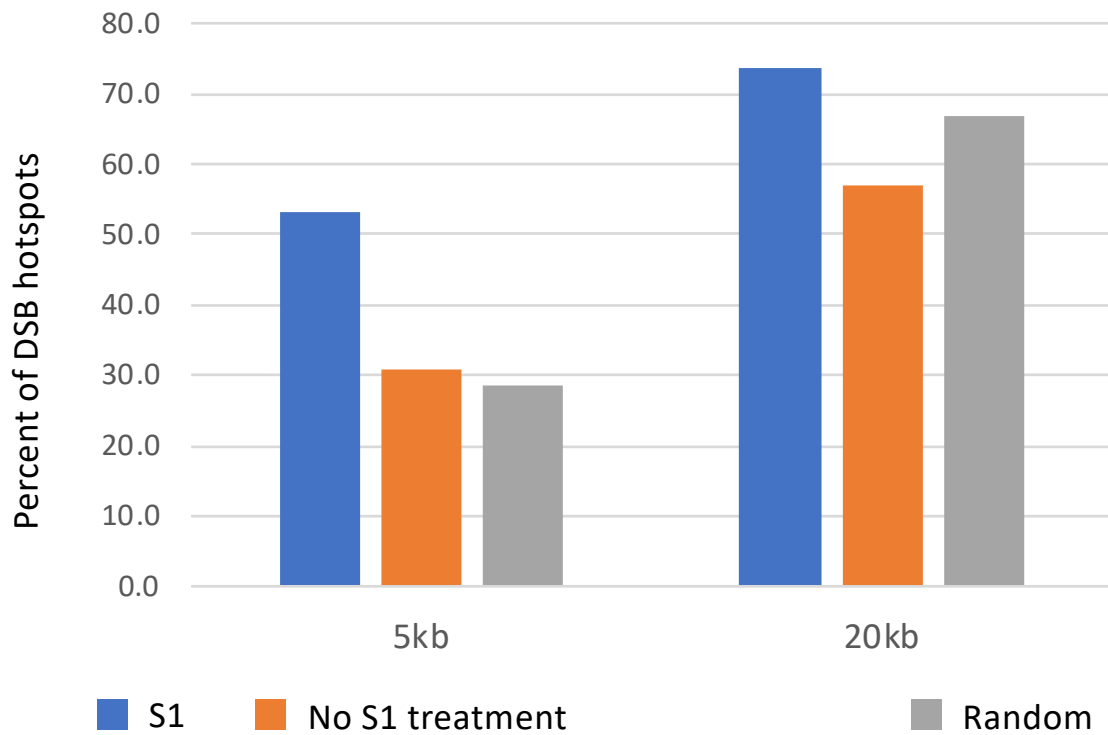
Supplemental Figure 1. Percent of DSB hotpots identified using RAD51 ChIP located within 5kb and 20kb from S1 resection sites 500bp to 2000bp in length.
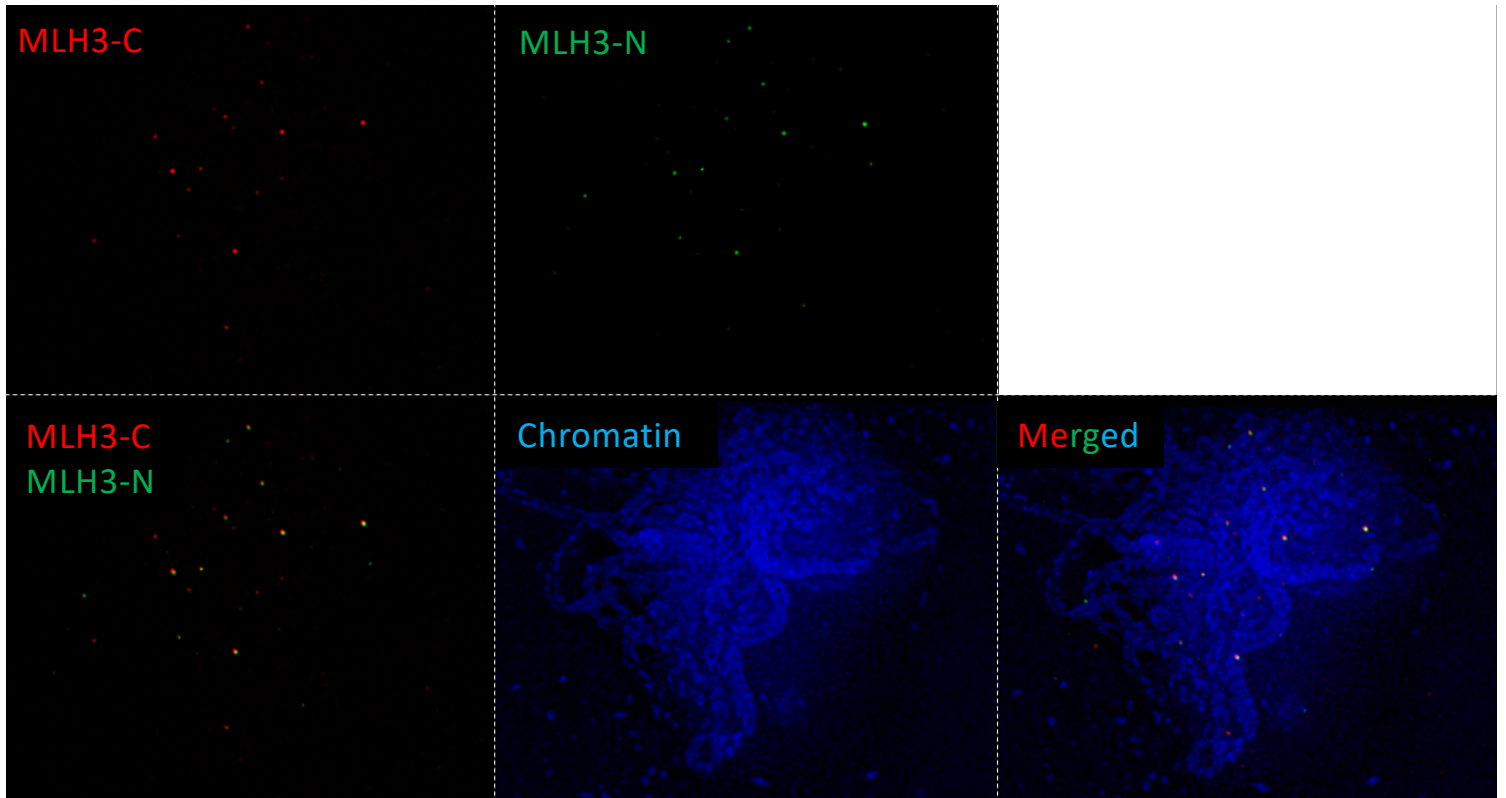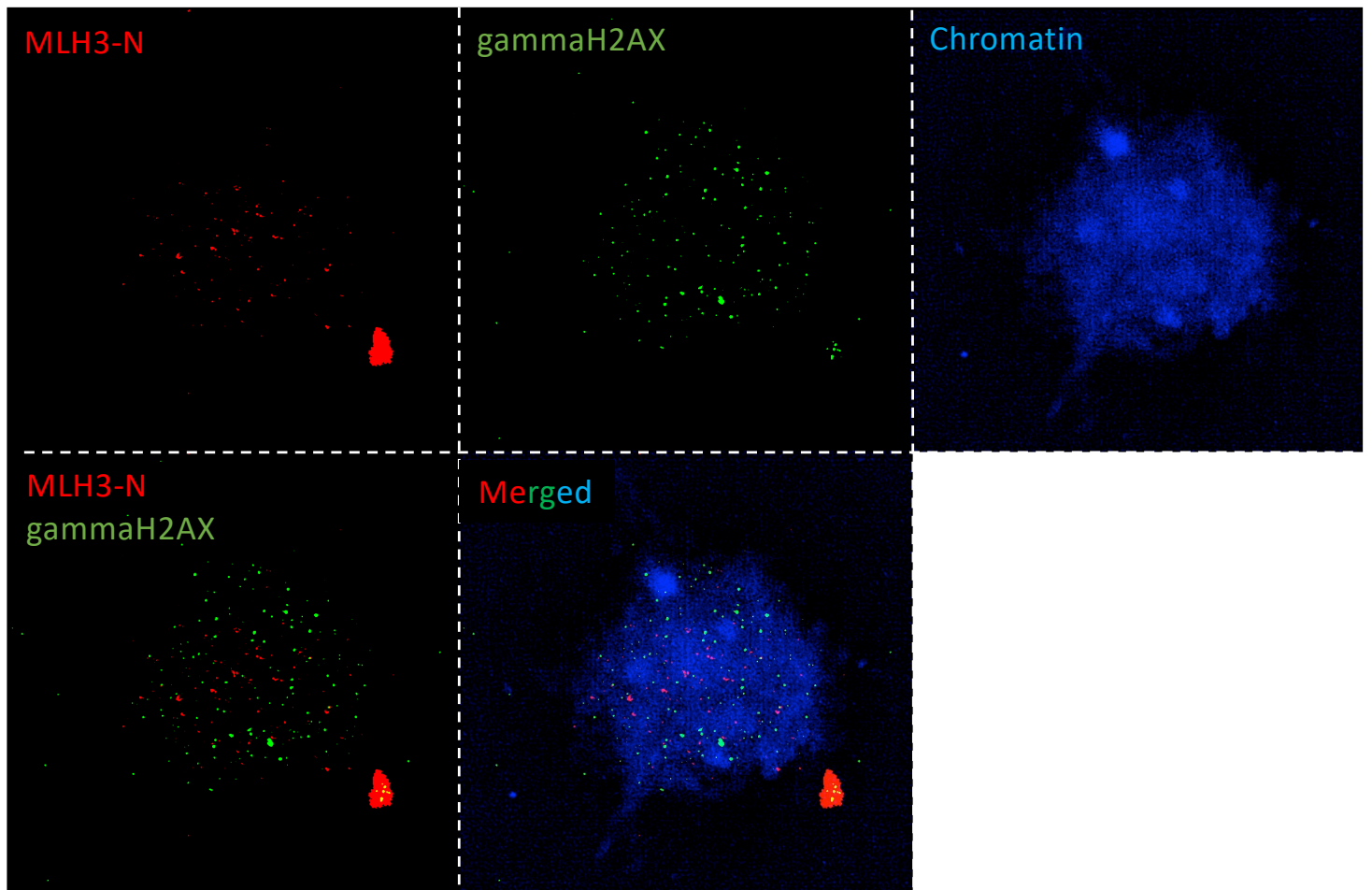
Supplementary Figure 2. Co-immunolabeling of MLH3-C and MLH3-N antibodies pachytene chromosomes in the B73 x Mo17 hybrid.
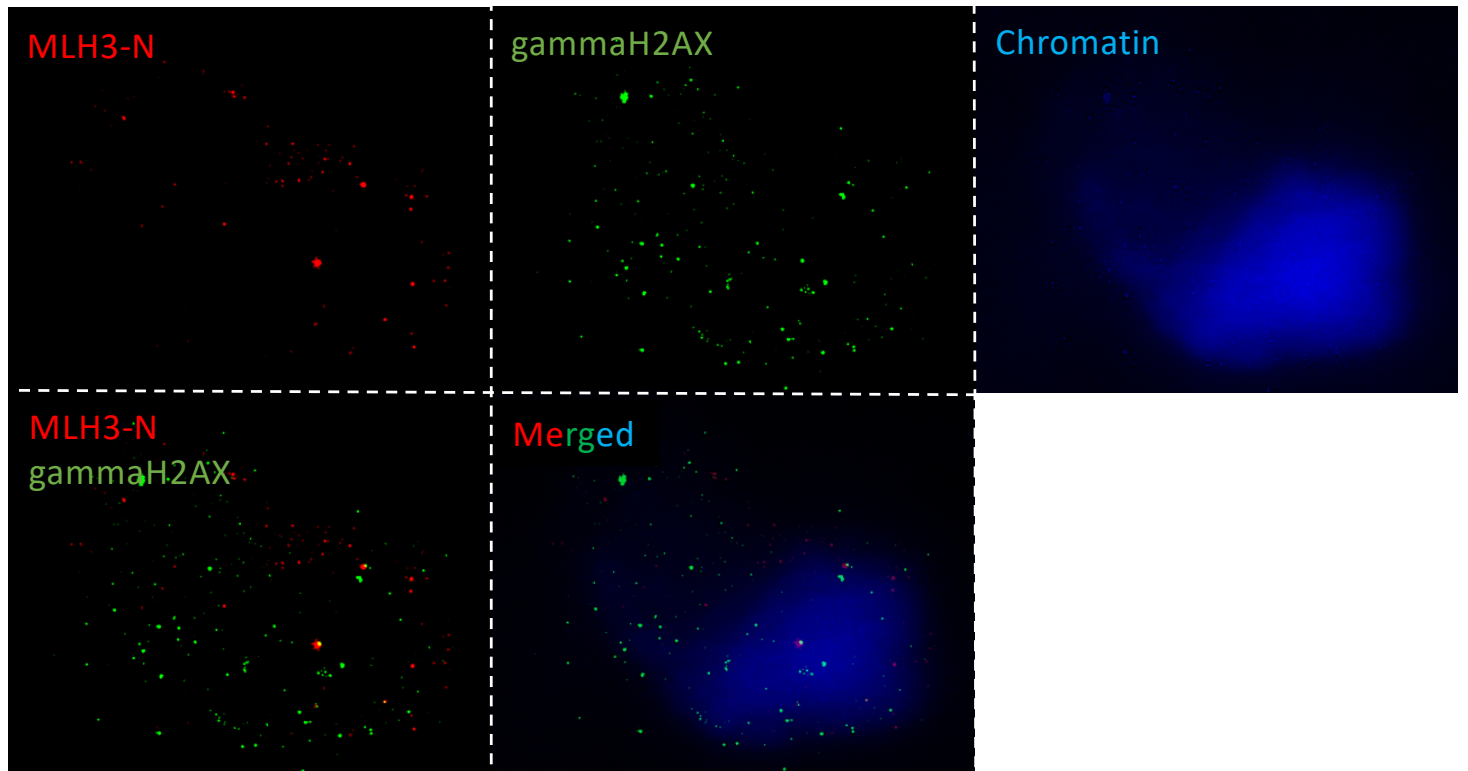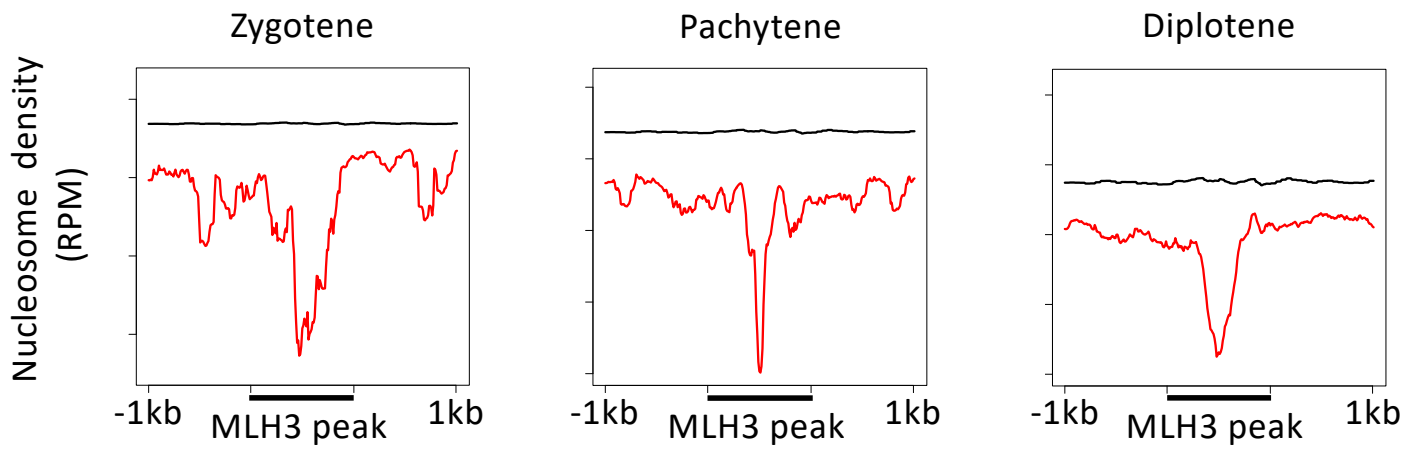
Supplementary Figure 3. Co-localization of MLH3-N foci with early recombination markers gammaH2AX (A) and RAD51 (B).

Supplementary Figure 4. Nucleosome occupancy measured by monococcal nuclease digestion at the sites of MLH3 hotspots detected at different substages of meiosis I in the maize inbred B73.