Figure S3. H3K4me3 and PRDM9 Binding, Related to Figure 3

(A) Mean locally normalized SPO11-oligo and H3K4me3 profiles. Data are the same as in Figure 3A, but zoomed to focus on the lower range of values for SPO11 oligos.

(B) Mean locally normalized SPO11-oligo and H3K4me3 profiles for subsets of hotspots. Top: 20% highest left-over-right (L > R) asymmetry; Bottom: 20% highest right-over-left (R > L) asymmetry (n = 2,792 for each group). Data are the same as in Figure 3C, but zoomed to focus on the lower range of values for SPO11 oligos. On average, the distribution of SPO11 oligos in secondary peaks to the left versus the right displays weak asymmetry in the same direction as H3K4me3 asymmetry. However, even at hotspots with strongly asymmetric H3K4me3, significant secondary peaks of SPO11 oligos can occur on the side that has low or no H3K4me3 signal.

(C) Enrichment of the 12-bp motif (see Figure 3D) coincides precisely with the central peak of SPO11 oligos. SPO11 oligos and motif density were each smoothed with a 51-bp Hann filter.

(D) The 12-bp motif enriched in SPO11-oligo hotspots is similar to motifs enriched in SSDS hotspots or in PRDM9[B6]-dependent H3K4me3 peaks and matches part of a larger 36-bp sequence to which PRDM9[B6] is predicted to bind (Brick et al., 2012; Baker et al., 2014, 2015; Davies et al., 2016). In the subset of hotspots lacking this "primary" 12-bp motif, a "secondary" 15-bp motif that largely overlaps the primary motif was enriched. A 15-bp motif largely overlapping the primary motif was also enriched in new hotspots arising in *Atm* null 1. See STAR Methods.

(E) Asymmetry in H3K4me3 signal is present in all three classes of PRDM9 binding sites. Shown for each of the three classes of PRDM9 motifs (from Figure 3F) are heat maps of H3K4me3 (left) and SPO11-oligo (right) signal around motif centers, ordered within each class according to asymmetry in local H3K4me3 pattern. Normalized SPO11-oligo values across each 1005-bp window were binned in 5-bp bins. k-means clustering of SPO11-oligo spatial patterns was carried out on SPO11-oligo profiles in 501-bp windows centered on motif occurrences and smoothed with a 15-bp Hann filter.

(F) The three PRDM9 motif classes have indistinguishable sequences. Sequence logos for 101-bp windows around motif midpoints are shown.