



Figure 3. Spatial Relationships between PRDM9 Binding, H3K4me3, and DSBs

(A) SPO11 oligos map primarily between methylated nucleosomes. Data were locally normalized by dividing the signal at each base pair by the mean signal within each 2,001-bp window, then were averaged across hotspots. The SPO11-oligo profile was smoothed with a 51-bp Hann filter. See also Figure S3A.

(B) H3K4me3 is often highly asymmetric around hotspots. Heat maps (data in 5-bp bins) were ordered according to H3K4me3 asymmetry. Data were locally normalized, so color-coding reflects the local spatial pattern, not relative signal strength between hotspots.

(C) Similar SPO11-oligo patterns between hotspots with opposite H3K4me3 asymmetry. Each panel shows mean of locally normalized profiles (51-bp Hann filter for SPO11-oligo data) across the 20% of hotspots with the most asymmetric H3K4me3 patterns (left > right in top panel; right > left in bottom panel). See also Figure S3B.

(D) The hotspot-enriched 12-bp motif and its disposition within the larger PRDM9^{B6} binding site.

(E) Asymmetric average profile of SPO11 oligos (15-bp Hann filter) around motif midpoints (n = 9,060).

(F) SPO11-oligo spatial classes from k-means clustering.

(G) H3K4me3 patterns are similar despite different SPO11-oligo patterns (15-bp Hann filter) between the motif classes from (F).

(legend continued on next page)

(H and I) Similar SPO11-oligo counts (H) and H3K4me3 tag counts (I) for hotspots in each of the three PRDM9 motif classes. Counts are for 1,001-bp windows around hotspot centers. Boxplots are as defined in [Figure S2A](#) legend. In (I), a value of 1 was added to each hotspot to permit plotting of hotspots with no H3K4me3 tags.

(J) Schematic of modular PRDM9 DNA binding and histone methylation activities. ZnF, zinc-finger domain; MTase, methyltransferase domain.

(K) H3K4me3 is an imperfect predictor of DSB frequency. SPO11 oligos and H3K4me3 tag counts were summed in the 1,001-bp around hotspot centers. One H3K4me3 tag was added to each hotspot to permit plotting of hotspots with no H3K4me3 tags. Eight outliers ($H3K4me3 > 10^4$) are not shown.

See also [Figure S3](#).