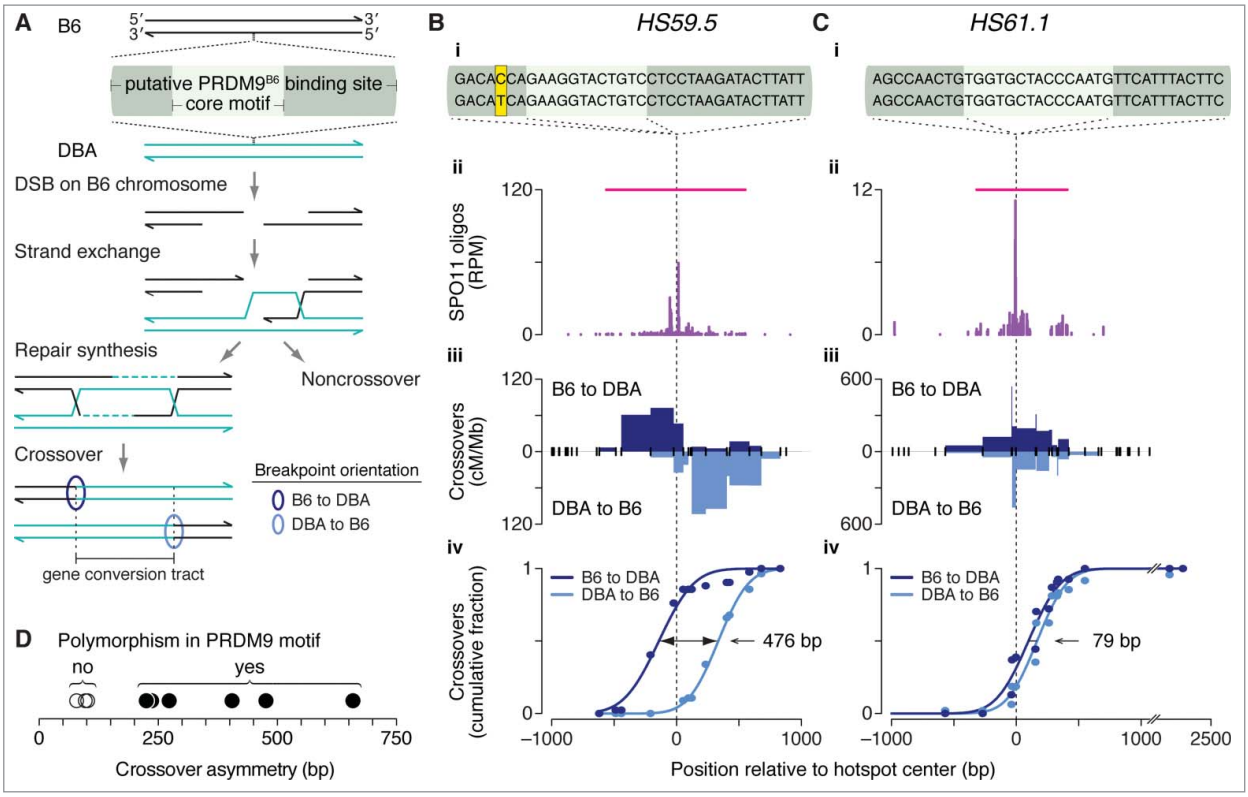

Figure 5. Crossover asymmetry in hotspots with polymorphisms in putative PRDM9 binding sites. (A) Model for crossover asymmetry. A sequence polymorphism in a PRDM9 binding site may affect relative DSB frequencies on the 2 hotspot alleles, manifested as asymmetry in the locations of crossover breakpoints. If DSBs are preferentially formed on the B6 chromosome of a B6 × DBA hybrid mouse, crossover breakpoints will tend to lie to the left of the hotspot center when recombinant products are assayed after PCR amplification in the B6-to-DBA orientation, and will tend to lie to the right when amplified in the DBA-to-B6 orientation. (B and C) Examples of crossover hotspots with (B) or without (C) crossover asymmetry. (i) B6 (top) and DBA (bottom) sequences of putative 36-bp binding sites for $PRDM9^{B6}$ at hotspot centers. The nucleotides shaded in yellow in *HS59.5* highlight a polymorphism between the B6 and DBA haplotypes. In *HS61.1*, the PRDM9 motif shown is on the Crick strand. (ii) SPO11-oligo maps. Red lines indicate SPO11-oligo hotspots. (iii) Crossover breakpoints (densities expressed as centiMorgans (cM) per Mb) mapped by allele-specific PCR on sperm DNA in the B6-to-DBA (top) and DBA-to-B6 (bottom) orientation.[102,103] Ticks represent tested polymorphisms. (iv) Cumulative distributions of crossover breakpoints with fitted Gaussian curves. The number indicates the distance between the 2 curves at the midpoint for each cumulative plot. Vertical dashed lines indicate hotspot centers. For hotspot *HS61.1*, zero values in both orientations at outlier position -1130 bp are not shown. (D) Crossover asymmetry is associated with presence of polymorphisms in putative PRDM9 binding sites at hotspots (Table S3). Crossover asymmetry was defined for each locus as the absolute difference between the midpoints of cumulative crossover breakpoint maps in the 2 orientations.