

## **Supplementary information, Figure S2**

**Fig. S2 Features of PRDM9-mediated H3K4me3. a** Stacked bar plot showing the genomic distribution of the newly generated and common H3K4me3 peaks on different

genomic features. **b** Profile of the average H3K4me3 tag density on common and newly generated H3K4me3 peaks in leptotene (left) and mid-zygotene (right) spermatocytes. H3K4me3 tag density was calculated using H3K4me3 reads coverage with 50-bp resolution. New: newly generated H3K4me3; Common: common H3K4me3. c UCSC genome browser view of the PRDM9-mediated H3K4me3 peaks, the strong peaks were highlighted with red shadings and the weak peaks were highlighted with blue shadings. Ctrl: control, mpL: mid-preleptotene spermatocytes, L: leptotene spermatocytes, mZ: mid-zygotene spermatocytes, e1P: early 1-pachytene spermatocytes, mP: midpachytene spermatocytes, D: diplotene spermatocytes. d Venn diagrams showing the overlap of defined SPO11-oligo DSB hotspots with the newly generated H3K4me3 peaks (left panel), and the common H3K4me3 peaks (right panel) in mid-zygotene. e Venn diagrams showing the overlap of DMC1-ssDNA defined hotspots with the newly generated H3K4me3 peaks (left panel), and the common H3K4me3 peaks (right panel) in mid-zygotene. f Profile of the average SPO11 oligos density on SPO11-oligo defined DSB hotspots with and without newly generated H3K4me3 peaks. SPO11 oligos density was calculated using SPO11 oligos coverage with 50-bp resolution. g The scatter plot showing the correlation between H3K4me3 tag density and SPO11-oligos tag density on the newly generated H3K4me3 peaks. SPO11-oligo tag density and H3K4me3 tag density were summed in the 1-kb around hotspot centers. h Profile of the average H3K4me3 tag density on DSB hotspots with and without newly generated H3K4me3 peaks in leptotene (left) and mid-zygotene (right) spermatocytes. Note that small peak of H3K4me3 was present even for the non-overlapping set of SPO11-oligo

hotspots. **i** Venn diagrams showing the overlap of  $Prdm9^{-/-}$  DSB hotspots with H3K4me3 in the  $Prdm9^{-/-}$  zygotene (left panel), common H3K4me3 peaks (middle panel) and newly generated H3K4me3 peaks (right panel) in the wild-type mid-zygotene.