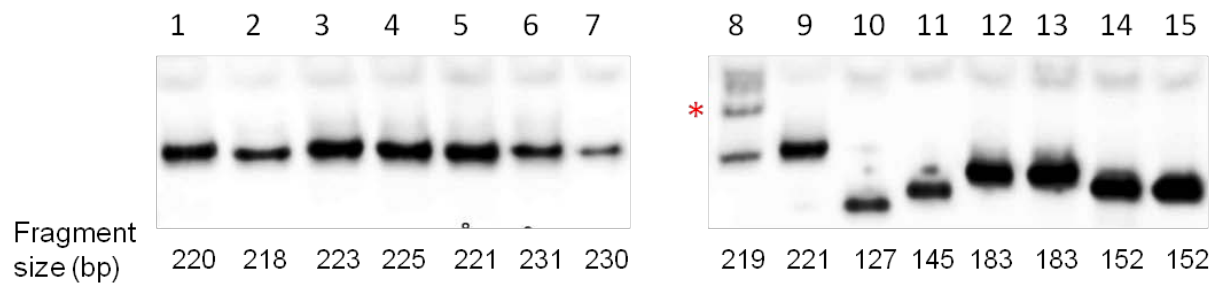


Additional file 4:

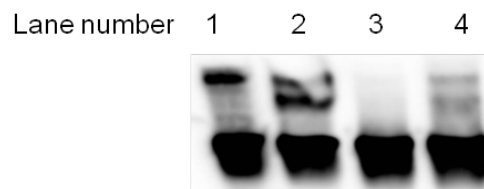
Figure S4. The PRDM9^{Cst} binding site of Esrrg-1.

(A) Binding of PCR-amplified fragments tiling Esrrg-1 to PRDM9^{Cst}.

Upper panel. Lanes 1–15: tiling-labeled fragments 1–15 incubated with PRDM9^{Cst}, as shown in Figure 3A. Additional bands present in all lanes are non-specific and probably caused by the presence of biotin-containing proteins in crude bacterial extract. Only the band in lane 8, indicated by an asterisk, was confirmed as specific by competition assay.



Lower panel. Competition assay for fragment 8. Lane 1, labeled fragment 8; lane 2, labeled fragment 8 + PRDM9^{Cst}; lane 3, labeled fragment 8 + PRDM9^{Cst} + excess of unlabeled fragment 8; lane 4, labeled fragment 8 + PRDM9^{Cst} + excess of unlabeled fragment 9; The second band in lane 1 is due to a misaligned fragment after denaturing; it disappears after adding bacterial extract. Unlabeled fragment 8 competes with the labeled fragment whereas fragment 9 does not.



(B) Sequence of Esrrg1 with the flanking SNPs in bold and the PRDM9^{Cst} binding site in bold, underlined. SNPs between C57BL/6J and CAST/EiJ are shown.

GTTTGGGAATCAGGAGGCATTGTCGAGACCTTTGACGGG[**T/C**]GTGGCAGGACTTTCACTAGTCTAAAGGCACCTT
CCCTTGTAAGTGAGATGGTATGGGAGGGAAGGCAAGACGAAGCAGAGAGACCTTTCTGGGCAGAGACGTGAGGCTCA
CTTCCTTCTCCAGGGATCTCAGGATCCCAGGCTCTTCCCTGGGACCTCCTTTCTGCTTGCTCTTCCTTCCTAACTCT
GGGAATTATTTATCATCATCTTAGCATATCCAGACTGAAGGTGTGAGCTGAAGGGTAGAACATAGATGAATGCTAGT
TTATTTTCTGTGCCTGGACTACCTTAATGTATTGCTTCTACTCCTAACCAGGGCTTGTTCTAATTTTATCCATGTT
CCTCAAGCCGGGTGAATGACGGCTACCCTGACCTCAGTATTGAGTTAGTATCAACTCATGAATAATTTGGAAGTATT
TGGAAGGAAACTGGATTGTTGGCTGATGCTTTGGCCTGTTTAAATATTTGTTCCCTGCTTCCCTCACAAATGCACCCC
A[**T/C**]TCTTTATAATATCTAGAAGCAGGCTGAAGTGTCTCCCTCCCAGGAACCTCTTTATAGCTGTCAACCCAATT
GATACATGTTTTGGGTCCATGTCATGACTTTTAGTCAGCATGGCTTCTATTGGCTTACTCATCCTTCCTGGTAGCTC
CGTCCCCTCAATTAATGAAGCATTAGGTTGGCATGAAAAGTGCATGTGACACACTGGGGAATGGTGGTTCTTCTGT
TTCTAGGTTCTTGGGAAGCTGAGGCCAAAGAGTGACTTGAGTGCAGAAATCTGAAGCCAGCCTTGGTAGCATACTGA
GACCCTACCACAAGAAATCTGCCAAATAGAGAAAGAAAGAAACACAGAAAGAGAAGGTGGACTGAGTTAGATGGCTC
TGTCCTCTCCGTACATGGTCCTTTAAGTATCACCATCAACTCTAGTTTGTGAAGTTTCTGTTCTGGAAAATGTGA
AGTTGTGGTAGAGATGGTGGCAGTGGCTAGCACTTGCCCTGAGGAAAGCCTGGGTGTCCTCTGACGGTCCCATCTCC
CATTTGTAGCCTTCAGTCTTCCACACTAGCACTGCTAGGTTTTTGTCTTGTGAAATATATATTTCTCAATGATCCTG
AACTTTGTATAGAACTCTGCAGAGTCATCAGGTAGTGTCTAAAGGGGGTAACATAGGGGGGAAAGTTATAACCTCAA
CTTCACTAACAGTGACAGAGTAAATGTGTTCTGTGTTATGT[G/A]TCTAAAGGAAGGAGGGTCTCCTCTTATCTC
CCAAATGTGTCTTTGACTTTGGTCTTTGCTTCAGCACTGAAATTCCTTCATAAGTAAAGAATTCTATTGCTACTTTA
ATGCCACTCAGAACTGGCCTTTTCTAATGTTCTAAGATTATGGCGCTCAGAATCCACCCCGCCCTC[T/G]CCAC
TCCCCTAGCTCGCAGTGACTGAGTTTAACTAGCAGCCCCGGTTAAGGTATTGATTTTAACTGAAGCCCAGAGGG
ACACAGGGCTGGAGTCAGCTGGACTCTTAAGCAGTCCTGGGAGACCAGA[G/A]TCATTTGCACATCGGAAAACAGC
TAATGGGCAGGGAATTAGGGCTTAGGTAGGCCTGTCCCTTCTGT[T/C]TTCCGTCCCTCACAGCTGCTATATGTCC
TGATAAAAAGGCAGGCAGACCCAAGACACCATATTTCAATTTCTTTCTCTCTCTCTCTCTTAAAAGTAAATTTTT
AAGAGTCTTTTATTTCTGATAAGGACACAGGTA[T/G]CTCAGATTGGCCTCAGACC[A/G]ACAAGAAAACATC
CCCCATCTCTGTCTCCAAGGGCTGGGAATACAGACATGTTGCTTTGTTTATGTGACCCCTAAGGGTCAACCCAG
AGCCTCATTTTTG[G/A]TTAGCGAACACTACTACTGAGCTGTACCCAAGGCTGAGTATTTTT[A/T]AAGGAAAC
TCAAGGAAGGGAAAATGATAGCTAGCCATTCTTTTTTACA[T/C]TGCAAAATTCTAGT[A/T]GTAGTGAA**ATACT**
TTGCAAATATCAAGGCTCTAATACAAATCCTACTGGCGACATACTTGGAAGAGTATCCTCTTGTGTGTCATTCTGTA
TGGAATAGTCTTAAGTTTTTGGGCTGCCAATTACTTGTAAG[G/C]TTCTTCTTTAAAATGT[T/C]CTATTAGCA
TGTATTCTCTATACATGAT[G/-]GGTGTGTGAAGTCTTCATGCATGGATGTGAGGTATTCTGATCACTGTTACCC
CGTCACCTTCTCTTCGCCCAGGCCCCGAGCTGCTCTTACCTCTCCCCTGCACAG[G/A][C/T]CTTGTAAGTCTCT
TCCTGTTTTGGACACCCAGCTGGGTTTCTGGCATATATCAGACGTGAGTATGAGCTGCTGTTCTGACCGGGGCAATC
CCATCCTTACCCAGTGTTTAGGACCCTAGTGGTTCCACCACACACCACCTTAAGCATCATCGTTTGTGTTTTGTTT
ACTTTGTTTGGCTCTCTGCAGTGAATGAGGGCAGTGTTACAGCAGGC
CTGGGTTGTCTTACATTGCTTTCTCTCCATGACTCGCTAGGGTCAGAGGTCCCCGATAATGCGCTGTGTGACTCCG
GCCTGAGCCATTTGTGTGCCCTGGAGATCTGAGGGTAACAGGCAGGGTCAGCATAACCTGCCAGATTCTCAGAGACC
CCTCTGTGAGTTGTGGGAGGACAGTAAAAAGCCTGTACGGACAGAGTAACCTTCCGTCCATGGGACTGCTTTTCA
TCAGATGGTGCTCACTTTGCCCAAGTGTAAGTATGAGGTCTACAGTCAGTGCTGCACATGCTAATGTGTTTCAATTTG
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CTTTCTCCCTAAAAGTTTATAGGTGTTTTGAGCTTCTGACAACACAGGCTTAAAGATGTGTGTAGCTTGGCTCCTGT
CAGTGCTTTTCTGGACTCCTTAAGTTATTCCTCAAAGCTGTTCTCTATTTAGGGAGGTAAAGTGATTTTCTGTGT
ATCCTGAAAAAAGAGGGATTTTATTTAATCCTTCTCTTCTCTTCTAGCCTATCTCC[-/T]TTTT