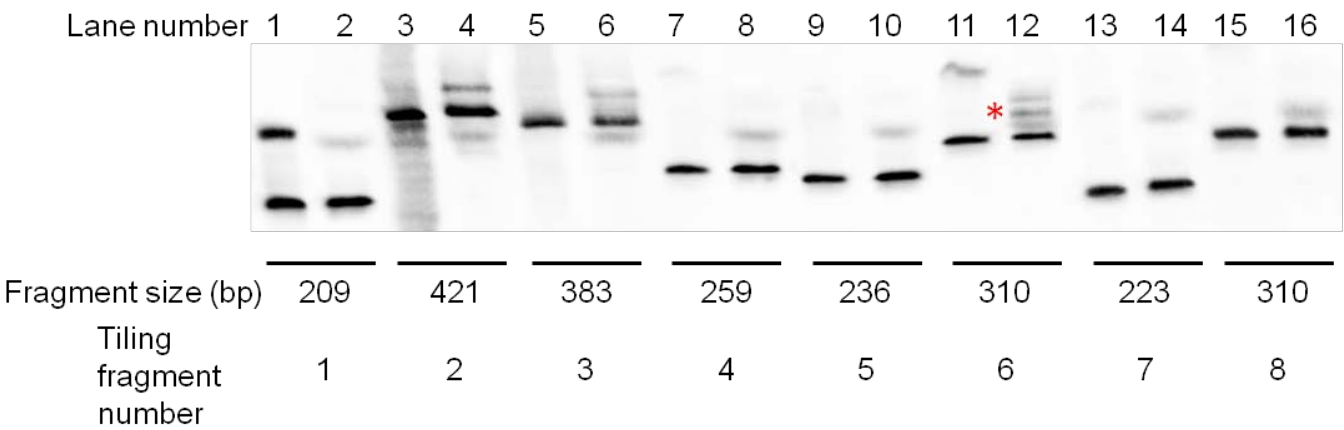


Additional file 3:

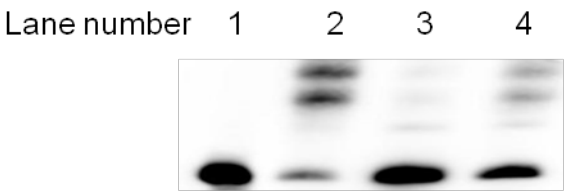
Figure S3. The PRDM9^{Dom2} binding site of Hlx1.

(A) Binding of PCR-amplified fragments tiling Hlx1 to PRDM9^{Cst}.

Upper panel. Odd numbered lanes (1, 3, 5, 7, 9, 11, 13, and 15) were loaded with tiling labeled fragments 1–8 alone, respectively. Even numbered lanes (2, 4, 6, 8, 10, 12, 14, and 16) were loaded with tiling fragments incubated with crude bacterial extract containing PRDM9^{Cst}. The substantial difference in fragment size is dictated by the presence of several repeated regions at the hotspot. Non-specific bands present in all lanes incubated with PRDM9^{Cst} are probably caused by the presence of biotin-containing proteins in crude bacterial extract or by non-specific binding of biotinylated PCR products to bacterial proteins. Only the band in lane 12 (fragment 6), indicated by the red asterisk, was confirmed as specific by competition assay (lower panel).



Lower panel. Competition assay for fragment 6. Lane 1, labeled fragment 6; lane 2, labeled fragment 6 + PRDM9^{Cst}; lane 3, labeled fragment 6 + PRDM9^{Cst} + excess of unlabeled fragment 6; lane 3, labeled fragment 6 + PRDM9^{Cst} + excess of unlabeled fragment 8; Unlabeled fragment 6 competes with the labeled fragment whereas fragment 8 does not.



(B) Sequence of Hlx1 with the flanking SNPs in bold and the PRDM9^{Cst} binding site in bold, underlined. SNPs between C57BL/6J and CAST/EiJ are shown.

AGAACTGACAGCAAT**[A/G]**GGGCCCAGGTTTGGCTCCAGGAAGCCTTCTGAATTG[C/A]TCTGCTTTC
TCTTAGCTTGCTGCTATCCCAGAACCA[T/G]CACAGACGCCACTCTGCCGGATACAGGCCACATGGAGC
TAAGGCAAGCCATCCCGAACCAGCTACGAGGGAGTCCAGGCAGAAGTGAGCCTAGCCAAGAACGGCGGAG
CCTGGCTCAGCTGACCTCAGCAGAACCGTTACGCGAGCACAGACTCTTGTT[C/A]CTCCACAACAGCTA
CCTACTAAAGCCGGGAGGCCAGTGGTCCTTGGCCTGTTTCAGGCTCC[G/A]TTTTCATATCTCACCTTA
AAATTCATTCCCTACAATGGAAGAC[-/A]AAAACTTAGTTATATTATAATTCATCCCTAGCAAATACT
CACTTCCCCTGTCTCT[-/TACTTCTAA]ACATTTTGTATGAACCATGCTTGGTTAAATTAATG[T/C]
TCGATGCTTGTGGGGCTGGAGAGATGACTCAGAGGCTAAGAGCTCACGATGCTCTTCCAGAGGACCCCGG
TTTCAGTTTCCAGAG[C/T]CCACACCAAGTGGCTCACAACCTGCCTGTAACCTCCAGCTCCAGAGAATTGC
TCTGACCGCCTCAG[G/A]CACCTGCATATACGTGGAACACCTAACATAGACAAAAATAAAATGCAAGT
AAGTATTTTT[T/A]AAAATACAGTGATTATACCTTGTTATATAAACGTCACTTATAGATGACTTAT[G
/A]TAATTT[C/A][A/T]AATTGTA[T/A]TTTTTAATTATTTCTTTTTTAATTATCATACAAAGTAAC
ATGTTTTCCTTGA[A/G]ATGTTTTCAAATAGCCTTTGTGTTGAAACCTTAAGTTCCTCTCCATCATCTC
CCTACTCCCTCTCCCAGCTTGTCCCCTCTACCTTCAGTATTTTCTTTCTTTTTTCATGTACATGTGAA
GGGACATAGGGAAGGGGAATTGTATTATGAAAGAGAGGCCACCAAGACTCAAGACCCCTGGAAGTACTTT
AAGACACAGAAGTCTTCAAGCAAGCTGTGACCTGAGACATTGGCTGCTCTTTAAACAGTGGAAGTCGTC
ATTGAAACACCATTTAGGTAATGGAGACACAAATAGAAACAACCTACCAAGAAGTTCCTATCAGCAGTGAA
AGGGTCACTTCTTTTTTTTTTTTTTTTTTTTTTTTGGTTAAATGCCACCCTGAACACCACCACTCCTT
TCCAGGCAGAATTCTGTGATAACCCATTCCAAGCAAGACTCTTGAACCTCCACAGCCCTAGGGTTTTCT
TTGGAGGGAAAGTATAAGGATGTTTTGTCTACCTCGGGTTGCTGGCTTCTTAAATTGATCCTTGCTACC
CTCCCCCTGCTCATTAGCCTTGATGTCCTGACCTACTCTCTTCTTTCTTTCTTTCTTTCTCTATTTCCC
CAAATCATTACCCTTACATACCTAAAACTGCACTAGT[T/C]CCTAGCCCTTCTTATCACAGCATACA
TTCCTTACTGTTTGGGTATAAGGTATCCAACCACCCAGATCATCTTGCTTGAAATACTAACTACATAGA
GCTGTAAGCTTCAACAGTATCAAAGCCTGACCCTCATTTGGCAGGGCTCCAGAGAAGCAGGGACACTGTC
AGGGAACTGGTTTGCACCTTACTTATTTCTTCTCTGCCCAGGCTATCTAGGTAC[G/A]TTGAGTTT
GGGCTTTTGTAGTTGTTATTGTATGTGTATGAGTGGTTGTG[A/C]GGTTATATGTGTATAATCATCT
GCATGTTGTGCCCTTGGTGACCAGAAGAGGGTGTCTATCCTCCAAAAGTGGAGGCAGAGTCAGTTGCAA
ACCACCATGTGGGTGCTGAGAGTTGAACCTGGGTCTTCTGCAAGATCAGTAAATGCTCTTAACCGACCAG
AGCCTGTACACTGATGTGGGAGGAGATGGTGGGTGAAT[-/A]

AGTGT[G/T]CAGACTTGGAC[C/T]CTGCCCTT[T/C]CTTT

ACGCATTCCACTAACCATGGGATGGAAGGTTCTGCCTCTCAAAACCTTCAGTTCTGCATCTGTGAAATCA
TATTA[G/A]CAACCTTGAGTCTAAGTGGTGGTGAGTATTAGACGTATATGCTC[G/A]GTTTATTA
ACTTCAGCTTCACGTCAG[A/G]GTAGGAGATGTATAAAGGGAAAAACAAAACAGAGCATAAGGTATAGT
AGCCTCGGGCAGCCATCTTCCAGTAACCTCCCCAAAATGATGAACACAAAGGGAAAGAGGAGAGGCACCTG
GTATATGTTCTCTAGGCCTTTTAGGAAACATGGCATTGTTCTTTGGCCACATACATGGGAATCTACAAG
AAGGGTGATATTGTGAACATCAAGGGAATGGGTACTGTTCAAAAAGGAATGCCCCATAAGTGCTACCACG
GCAAAACC[G/A]GAAGAGTCTACAATGTCACCCAGCATCCCGTAGGTATCATGGTAAACAAGCAAGTTA
AGGGCAAGATTCTGGCCAAGAGGACCAGTGTGTGGACTGAGCACATCAAGCACTCAAAGAGCAGAGACAG
CTTCTGAAGCGGGTGAAGGAGAACGATCAGAAGAAAAAGGAAGCCAAAGAGAAGGGCACCTGGGTGCAG
CTGAAGCGCCAGCCTGCGCCACCCAGAGAAGCACACTTTGTGAGGACTAACGA[T/A]AAAGAGCCTGAG
CTGTTGGAGCCATTCCATAC[G/A]AATTCATGGCCTAATGTACAAAAAT[G/A]AAATAAAGGACCAG
GACTGGAAAAA