Supporting Information

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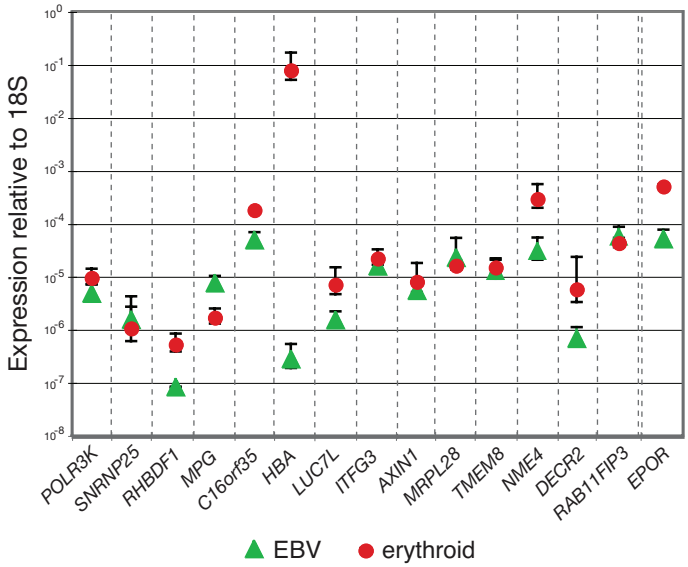

Fig. S1. Expression of genes contained within the terminal 500 kb of chromosome 16p, and an erythroid control gene *EPOR*, in EBV and erythroid cells. Expression was normalized to 18S. Values represent an average of three biological replicates ± 1 standard deviation. The *y* axis is a log scale. Although it appears that the expression of *LUC7L* increases in erythroid cells, comparison with hES cells (Fig. 1) suggests that expression actually decreases in EBV-transformed lymphocytes.

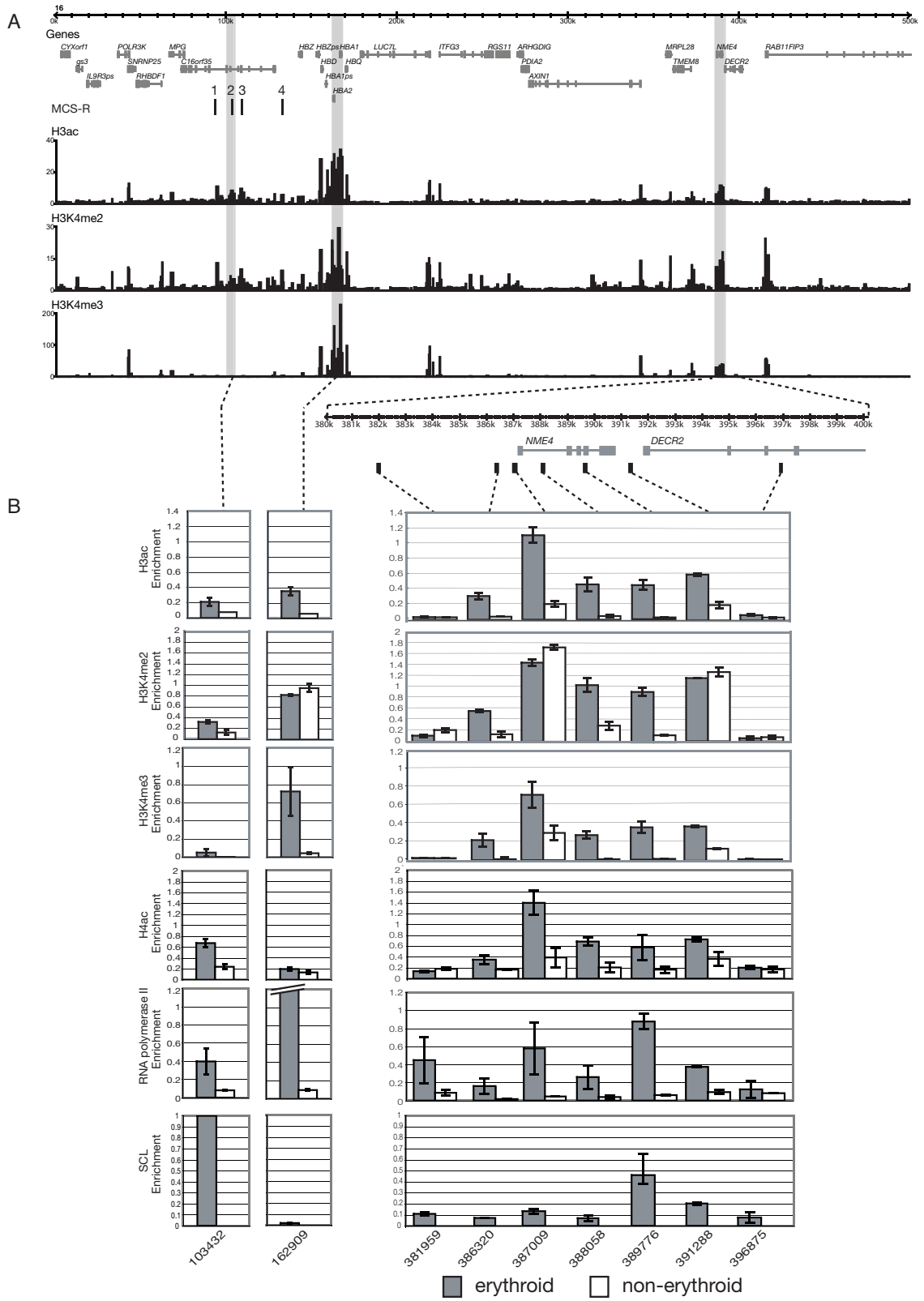

Fig. S2. Chromatin modifications, RNA polymerase II and SCL binding at *NME4*. (*A*) ChIP-chip analysis in erythroid cells of terminal 500 kb of chromosome 16p. (*B*) Real-time analysis of ChIP enrichment at MCS-R2 (HS-40), α-globin promoter, and *NME4* and surrounding area. Enrichment is relative to input and normalized to β-actin promoter, except SCL, which is normalized to amplicon 103432 (MCS-R2). Values represent the mean of three independent experiments $\pm$1 SD. Primer and probe information can be found in Table S6.

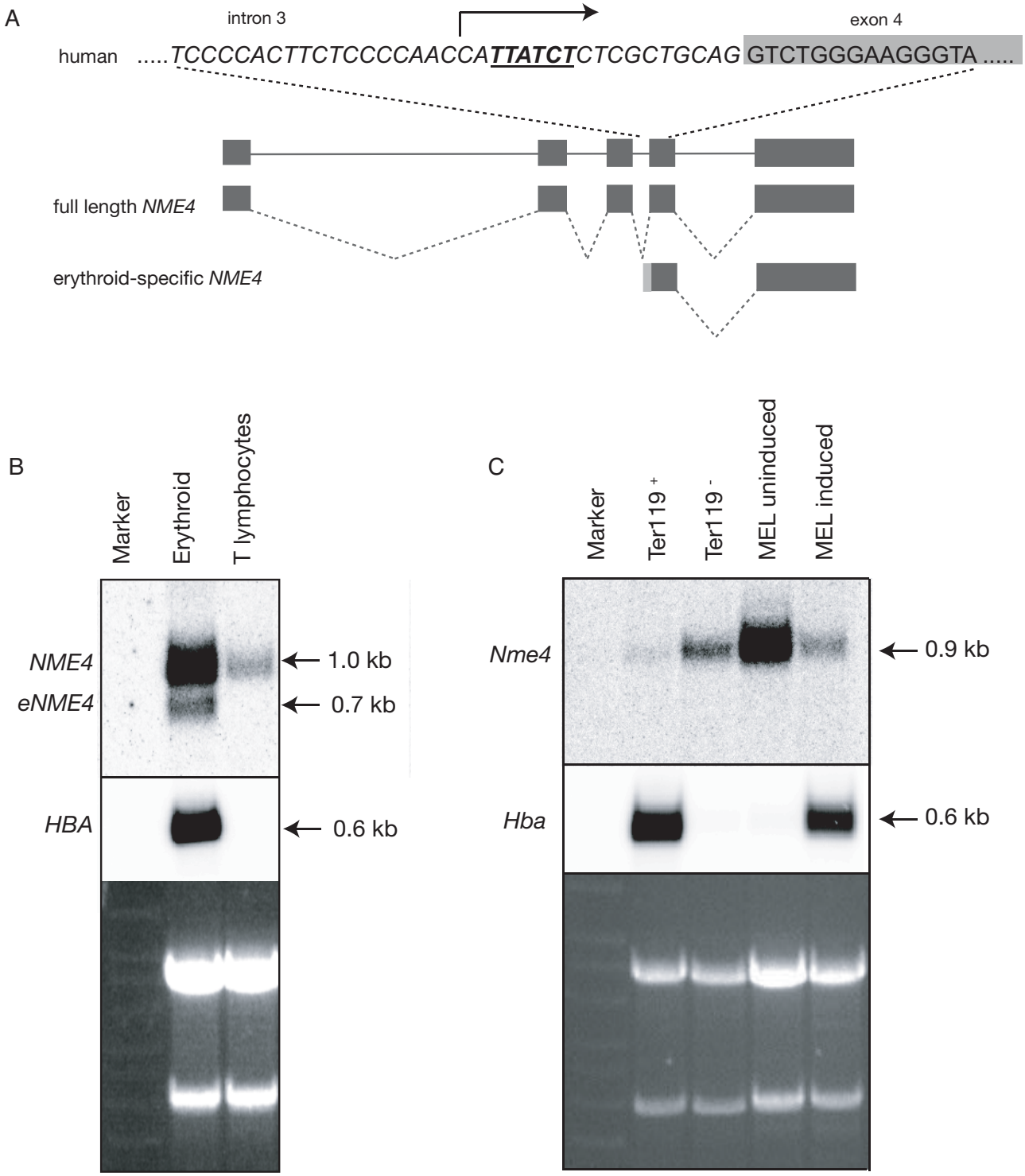

Fig. S3. Structure and expression of *NME4* and *eNME4* in erythroid and nonerythroid cells. (*A*) The GATA1 site contained within intron 3 of *NME4* is in bold and underlined. The arrow represents the start of transcription. (*Boxes*) exons; (*continuous lines*) introns; (*dashed lines*) splicing events. (*B*) Northern blot showing the up-regulation of *NME4* and the presence of *eNME4* compared to a nonerythroid control. (*C*) Northern blot showing *Nme4* expression in erythroid [Ter119$^+$ and MEL (mouse erythroleukemia) induced] and nonerythroid (Ter119$^-$) and MEL uninduced i.e. movement of the end bracket murine tissues. The blots were stripped and reprobed with corresponding α-globin probes. Probe information can be found in Table S3. Ethidium bromide stained gels (before blotting) are shown as loading controls.

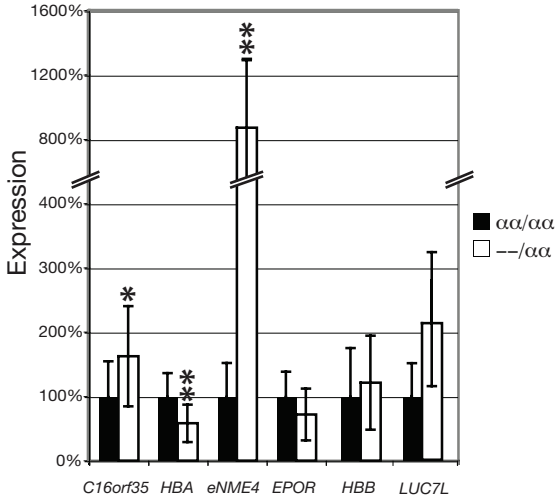

Fig. S4. Expression of *LUC7L* is not significantly different between controls and --/$\alpha\alpha$ samples. Data are as in Fig. 2*A*, with the addition of *LUC7L*. Student's *t* test $P = 0.071$.

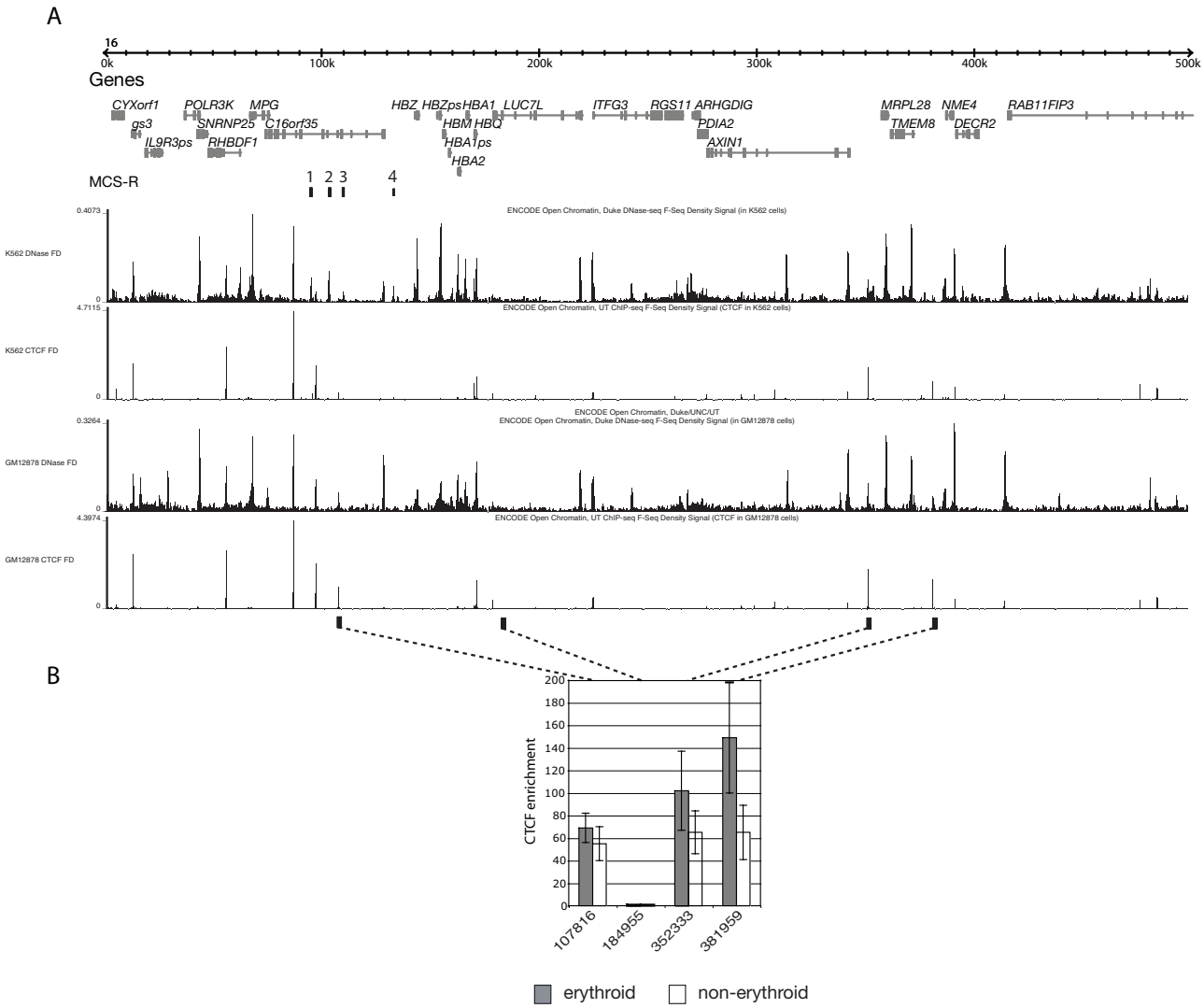

Fig. S5. DNaseI hypersensitive sites and CTCF binding sites across the terminal 500 kb of chromosome 16p. (*A*) Genome-wide DNase-seq and ChIP-seq in erythroid (K562; erythromyeloblastoid leukemia) and nonerythroid (GM12878; EBV-transformed B lymphocyte) cells. Data were generated by the Duke/UNC/UT-Austin/EBI ENCODE group and accessed via the University of California Santa Cruz genome browser (UCSC; www.genome.ucsc.edu). (*B*) Real-time analysis of CTCF-ChIP enrichment at selected amplicons between MCS-R2 and *NME4*. Enrichment is relative to 18S. Values represent the mean of three independent experiments ± 1 standard deviation. Primer and probe information can be found in Table S6.

Table S1. ABI expression assay IDs for expression analysis

Gene symbol	ABI assay-on-demand
EPOR	Hs00181092_m1
CD71	Hs00951094_m1
SNRNP25	Hs00430677_m1
RHBDF1	Hs00430530_m1
LUC7L	Hs00216077_m1
AXIN1	Hs00394718_m1
MRPL28	Hs00371771_m1
RAB11FIP3	Hs00206755_m1

Table S2. Primer sequences for expression analysis

Gene symbol	Forward primer	Reverse primer	Probe	5′ Label	3′ Label
POLR3K	CCCCTACGTGCACAACATCAC	CATCATCCACTTCTTTCAGTTTTGG	CCGCAAGGTAACAAA	6-FAM	MGB-NFQ
MPG	GCATCTATTTCTCAAGCCCAAAG	GGAGTTCTGTGCCATTAGGAAGTC	AGTTCTTCGACCAGCCGGCAGTCC	6-FAM	TAMRA
C16orf35	CAACGCCCTCAGCTTTGG	GCTGGTGAGGGTCATGTCATC	CCCCAACCAGCAGC	6-FAM	MGB-NFQ
HBA	GCCCTGGAGAGGATGTTCCT	CGTGGCTCAGGTCGAAGTG	CCTTCCCCACCACCAAGACCTACTTCC	6-FAM	TAMRA
ITFG3	GGCGGCCCGTTCAAG	GAGCCCACAAGAAGCACATCT	TCCCAGGGAACGCCGGTG	6-FAM	TAMRA
TMEM8	GCTGCAGTACTGCGACTTCTTG	TGAGCCGTGCCATGCA	CGGCCATCTGGGTCA	6-FAM	MGB-NFQ
NME4	CGTGATCCAGCGCTTTGAG	TGGTAGTGCTCGGCAAGGA	CTGCAGGCACCAGAGA	6-FAM	TAMRA
DECR2	GTTCCGGATTGCTGAGATTTTC	TCCTACTGGCAATCACCGTATG	ATGCGGCACGCT	6-FAM	MGB-NFQ
HBB	GTGCATCTGACTCCTGAGGAGAA	CCTCTGGGTCCAAGGGTAGAC	CACCAACTTCATCCACGTTCACCT	6-FAM	TAMRA
eNME4	TTATCTCTCGCTGCAGGTCTGG	CCACGGAGTCGCTGGCGTGG	CATGATTGGACACACCGACTCGGCT	6-FAM	TAMRA

Table S3. Northern probes for expression analysis

Northern probes	Start position	End position
NME4	Chr16:390341	Chr16:390554
Nme4	Chr17:26229074	Chr17:26228829
HBA	Chr16:163136, 166940	Chr16:163579, 167390
Hba	Chr11:32183936, 32196753	Chr11:32184462, 32197279

Table S4. Pyrosequencing primers

	Forward primer	Reverse primer	Sequence primer	5′ Label	3′ Label
NME4	*GTCTGGGAAGGGTACAATGTCGT	TGCTGATGTGGACGCTGAAGTC	GTCCAATCATGGCCC	*Biotin	—

Sequence to be analyzed (SNP shown in bold): Allele A (rev comp.) T**T**GAGGCGCG; Allele G (rev comp.) T**C**GAGGCGCG.
Dispensation order: E(nzyme)–S(ubstrate) T - G–A–C–G–C–G–C–A–G. Asterisk represents the presence of 5′ biotin on the forward primer.

Table S5. Pyrosequencing dispensation order and contribution of each allele to the peak height

Peak	Base	Allele contribution to signal
1	T	2×A+1×G
2	G	1×A
3	A	1×A
4	**C**	**1×G**
5	G	2×A+1×G
6	C	1×A
7	G	1×A
8	**C**	**1×A**
9	A	1×G
10	G	2×A+2×G

Calculation of allelic contribution: Proportion of G allele = peak 4 / (peak 4 + peak 8). Peaks used for calculations shown in bold.

Table S6. Primer sequences for ChIP analysis and 4C amplification

Amplicon	Forward primer	Reverse primer	Probe	5′ Label	3′ Label
ChIP					
103432	CAGGCTCCAGGCCCATATC	CCTCCTGCACTGTCCTTTGAC	TGCCCAAGAGCTCCTTCTGCAACC	6-FAM	TAMRA
107816	CTAATTTCTGTGCTCCCGGTTCT	CCTTGGAATGCTTCCCTTGA	CATCCAGCCAGCTTCACAAGGACCA	6-FAM	TAMRA
162909	GGGCCGGCACTCTTCTG	GGCCTTGACGTTGGTCTTGT	CCCACAGACTCAGAGAGAACCCACCATG	6-FAM	TAMRA
184955	CCACGATGGCATAAGGATAATCT	CATACTTTCCGTGCCCTTGTG	CGTGCTCTCATCCACACTGGGTATGG	6-FAM	TAMRA
352333	CGTCCCAAGAACGCCTCTT	GAGAAGCCTCGTCTCCAGACA	TCCAGGGCGCACCAGCATTG	6-FAM	TAMRA
381959	ACCAGATAGGCAGTCCATGCTT	GGCTGTACTGCCGCTCAGA	ATGGCAGTGAGACCCAGACGCAGTTC	6-FAM	TAMRA
386320	GAACAGGCTTTTTCAAGACTCATTAA	TGGGTAGATAGCACAGCATGCT	AAGTTCTAGCAGTGGGAGACCTAACCATTTCAAA	6-FAM	TAMRA
387009	CCGTGGGAAAGCGTTAAAAC	AACGCCAATTTCTGTTAAAAATCAG	TCGGTGTAAAGACGAATGCAATTCGAGAA	6-FAM	TAMRA
388058	CCTCCCGTGGGCTTCAG	AGACCCTGTATGTCCCACAGAATC	TAACTTTTCCTCCTGGCCTGGCGGA	6-FAM	TAMRA
389776	CCCCAACCATTATCTCTCGCT	CGGTGTGTCCAATCATGGC	AGGGTACAATGTCGTCCGCGCCTC	6-FAM	TAMRA
391288	TTGTAAGGAGTTGAACAGTAAAGAGGAA	ACCCCTGCCTGGCTCATAC	TGCACACCCAGTTCCATAACGTTGTTG	6-FAM	TAMRA
396875	GCCCCTCGCCCACACT	TCCTACTGGCAATCACCGTATG	TGCTTCTGGTTTTGCAGGCACGG	6-FAM	TAMRA
4C					
MCS-R2	CTGCTGATTACAACCTCTGGTGC	GAGCCTGGGGGAAAGGAGTAG			