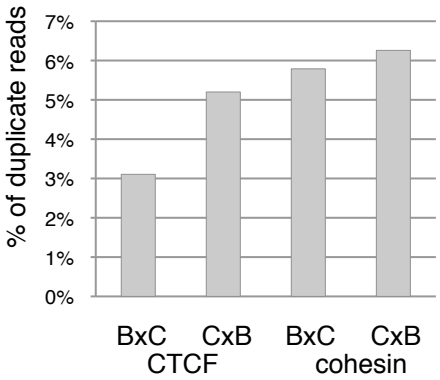


Figure S1: Read duplicates are shown for each ChIP-seq experiment. Clonal reads were removed prior to further analysis but overall these were a small proportion (<7%) of the total number of reads, indicative of the robustness of the ChIP-seq data.

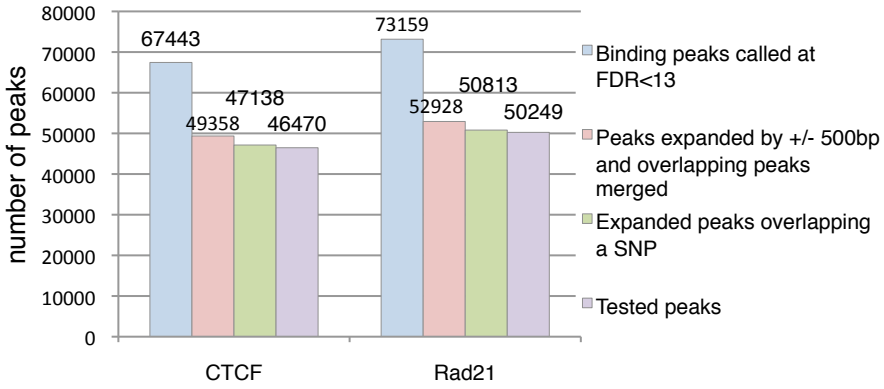


Figure S2: CTCF and cohesin binding regions were identified using Useq and resulted in the identification of 67,443 CTCF and 73,159 (Rad21) cohesin binding at an FDR < 13 (blue bars). To test mono-allelic binding CTCF and Rad21 binding regions were expanded by 500 bp and overlapping regions merged (red bars). Merged regions overlapping a SNP and thus where it is theoretically possible to test mono-allelic binding are shown (green bars). Where two or more reads with good enough quality overlapped the SNP a binomial test was conducted, and mono-allelic expression was assessed (purple bars).

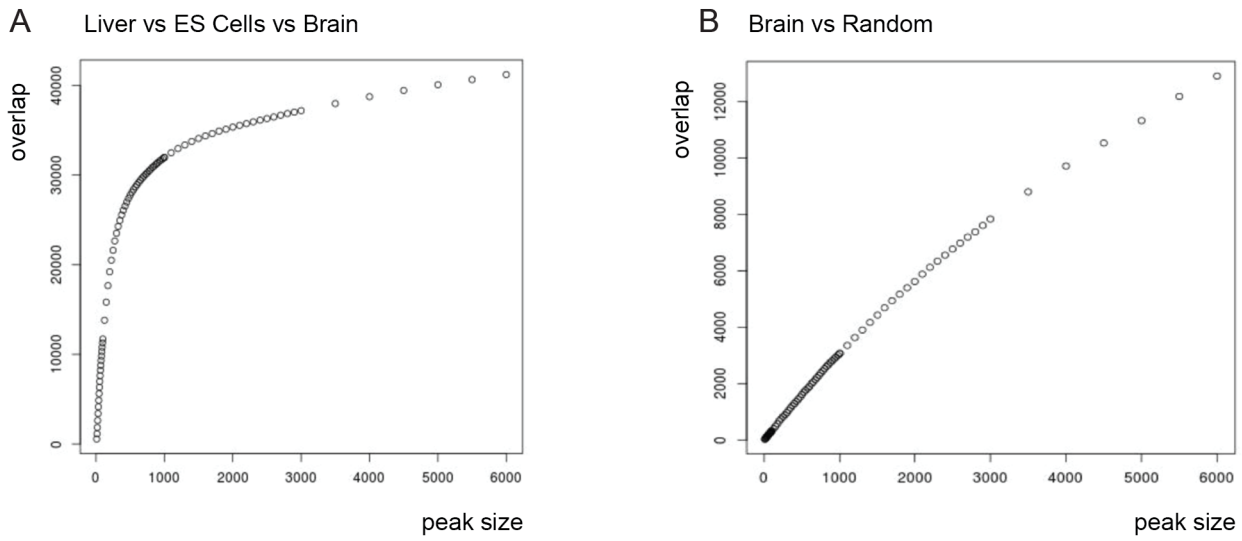


Figure S3: To identify the correct size of peak to make appropriate comparisons between datasets, the optimum size for the binding peak was determined by iterative comparison of overlaps in brain, liver (Schmidt et al. 2010) and ES cell (Chen et al. 2008) datasets: Graph (A) indicates that above a peak size of 1kb, any increase in overlap between the dataset is due to noise. Graph (B) is the control for (A) whereby a randomly created dataset is plotted against brain CTCF ChIP-seq data. Overlap of CTCF binding between tissues:

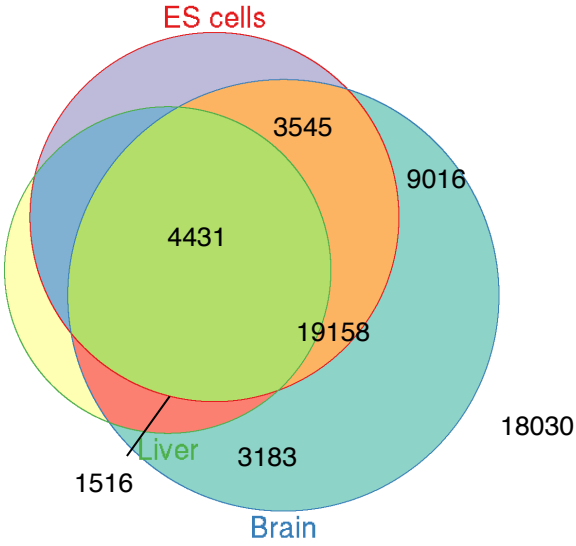


Figure S4: Overlap of CTCF binding between ES cells, liver and brain was repeated using CTCF ChIP-seq data from Shen et al (2012). CTCF binding for brain from Shen was not used due to the paucity of data available. Overlap of binding confirms the result seen in the original comparison indicating significant overlap of binding between all three tissues (Fig. 4A).

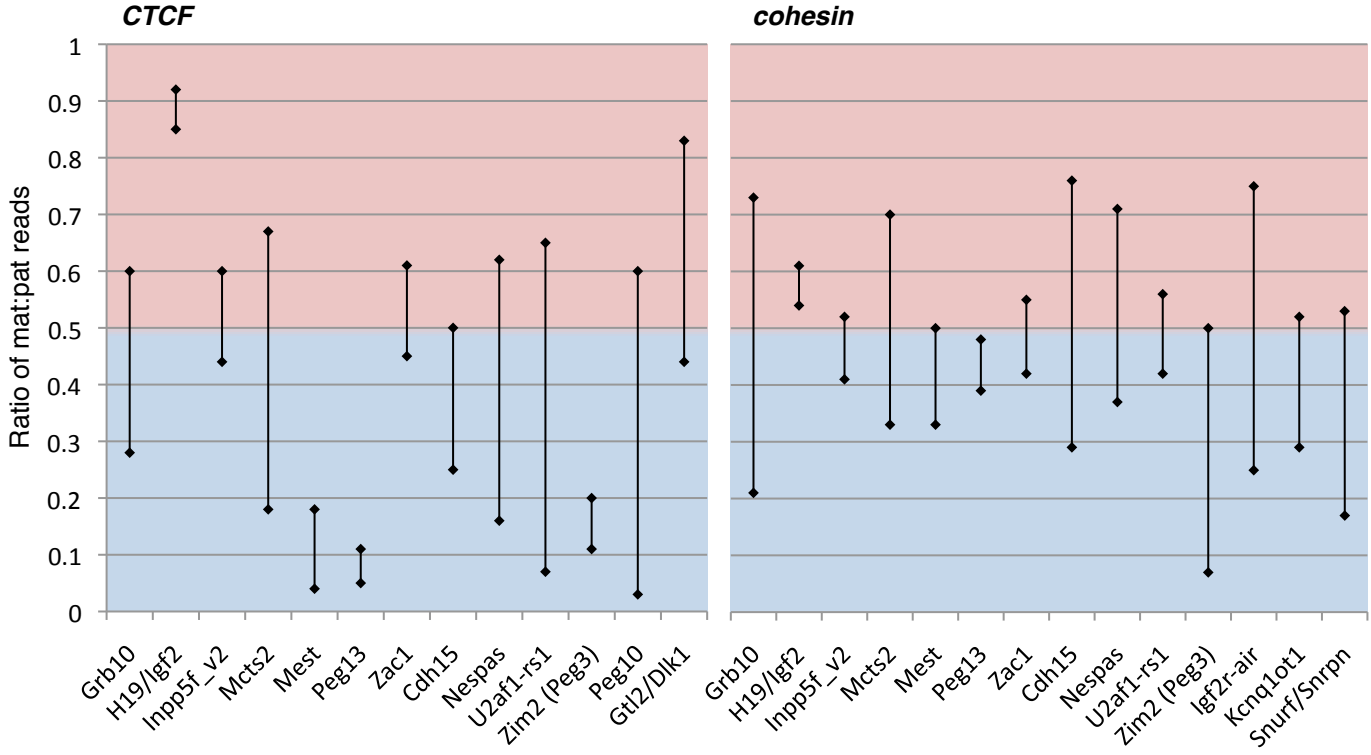
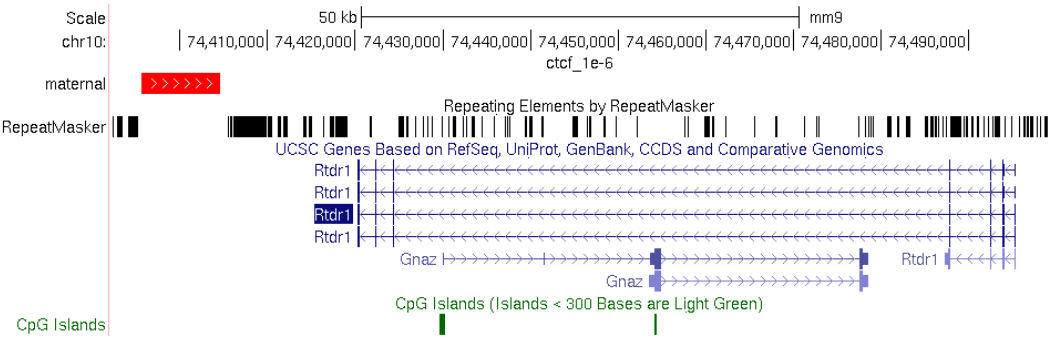
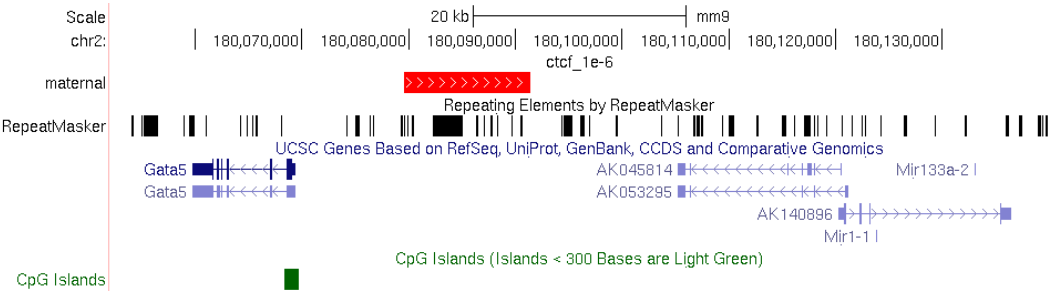


Figure S5: Plot of 95% confidence intervals for ratio of maternal:paternal reads of CTCF (left) and cohesin (right). These plots illustrate that the bias towards maternal or paternal binding is greater for CTCF than for cohesin. CTCF binding is suggestive of binding towards the unmethylated allele for all regions examined except for *Inpp5f_v2* and *Zac1,* where binding is bi-allelic. Regions where there was no SNP between Bl6 and cast could not be assessed and are not shown.

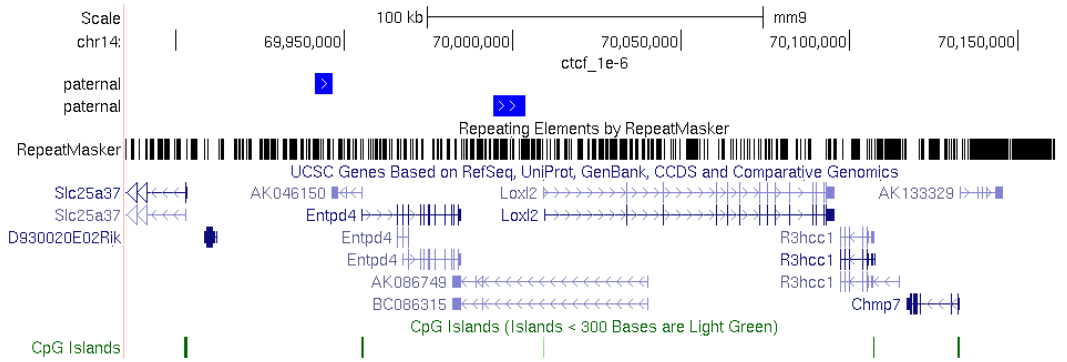
Rtdr/Gnaz - chr10:74,392,049-74,499,194



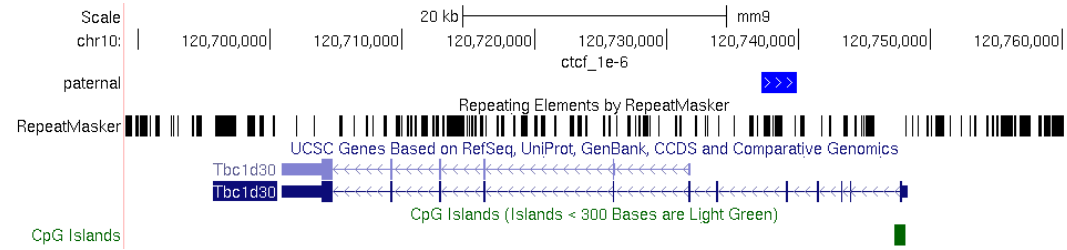
Gata5 - chr2:180,052,061-180,139,995



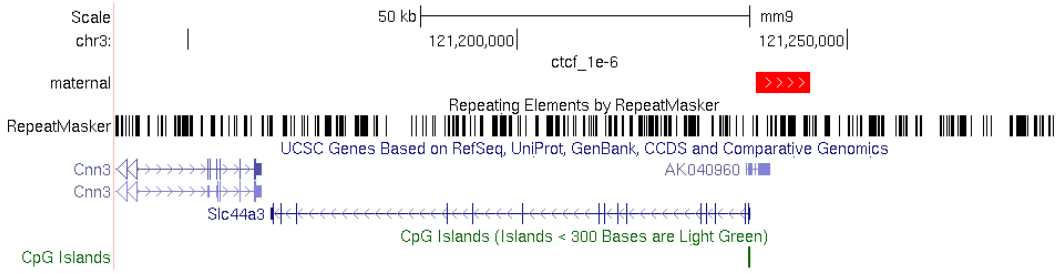
Entpd4/Loxl2 - chr14:69,884,977-70,163,630



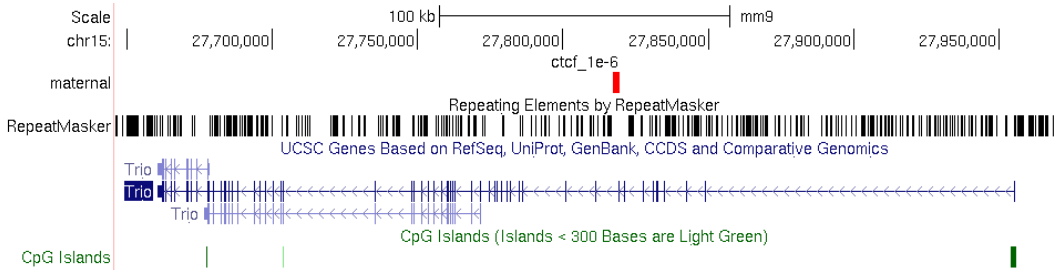
Tbc1d30 - chr10:120,689,034-120,760,088



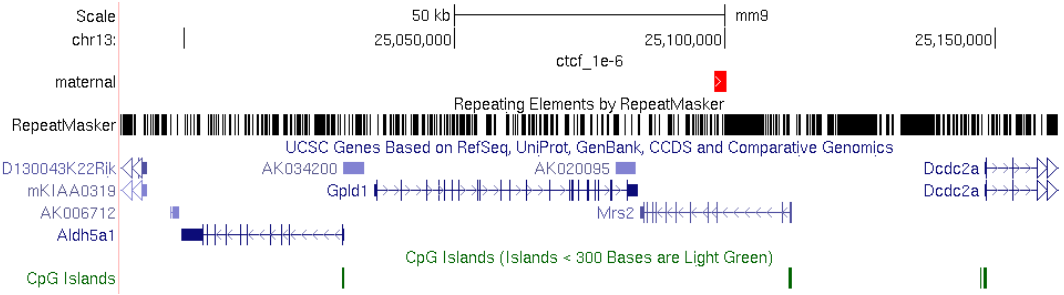
Slc44a3- chr3:121,138,949-121,281,439



Trio - chr15:27,646,061-27,968,903



Mrs2 - chr13:24,988,205-25,161,693



Snca - chr6:60,584,662-60,814,390

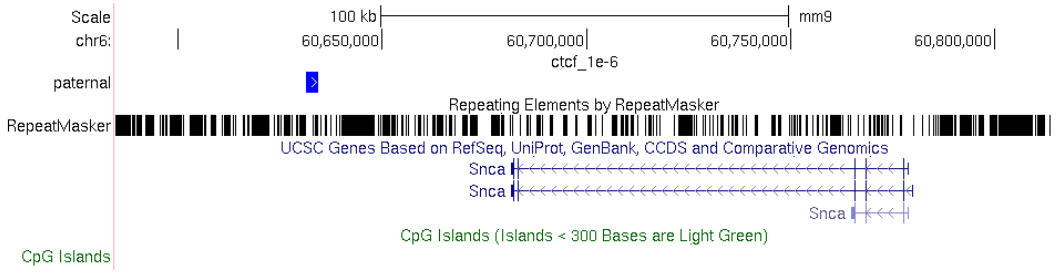


Figure S6: Regions of genomewide significant parent-of-origin specific CTCF binding displayed in UCSC genome browser for each genomewide significant region not associated with a known imprinted gene. Paternal binding is indicated as a blue box maternal binding as a red box.

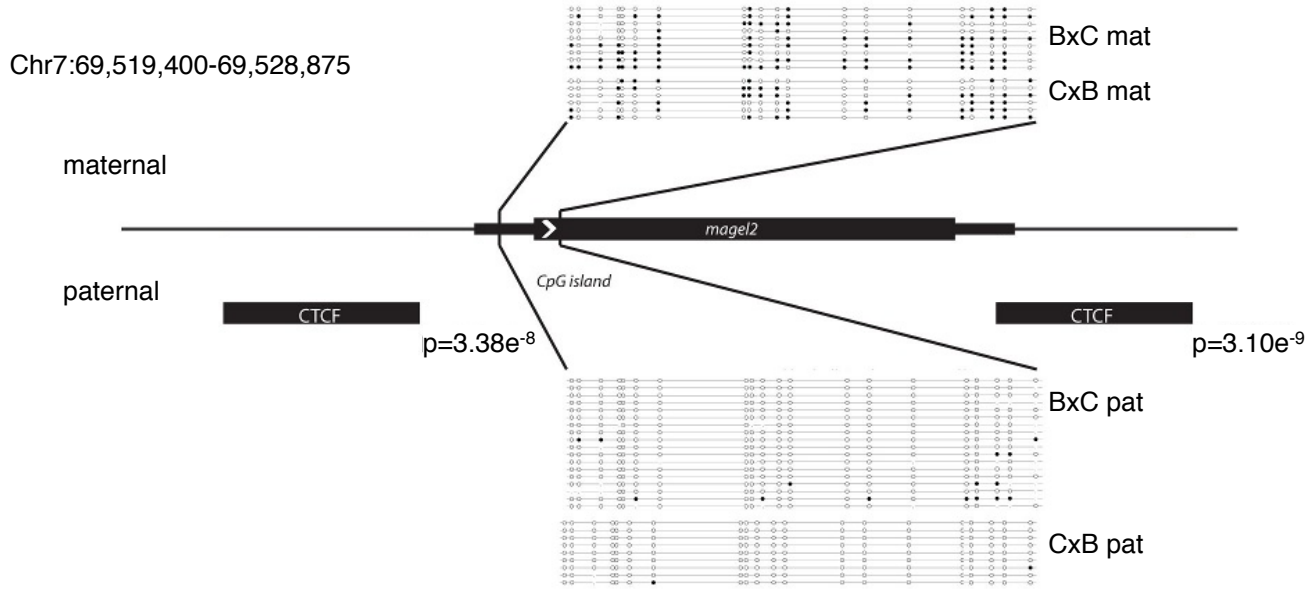


Figure S7: Methylation at the *Magel2* promoter. Due to the presence of multiple sites of paternal CTCF binding at the *Magel2/Peg13* locus and in particular two in close proximity to *Magel2*, the promoter CpG Island was tested for parent of origin specific methylation. Using locus specific bisulphite analysis in 3 week mouse brain a maternally methylated DMR was identified. Circles represent CpG dinucleotides, filled circles indicate methylated cytosine and white circles unmethylated cytosine residues. CTCF binding sites are shown.

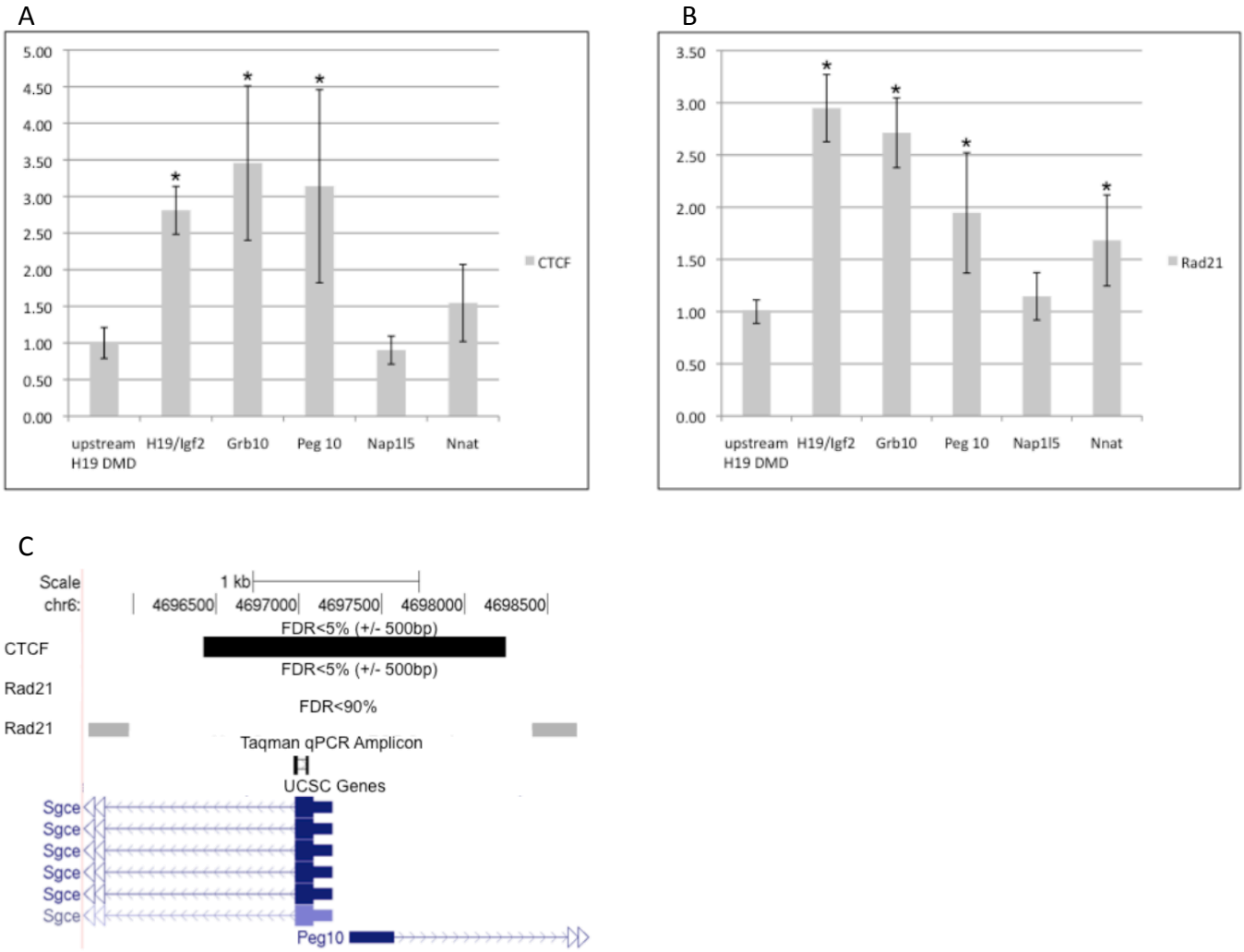


Figure S8: Validation of ChIP-seq binding. (A and B) Quantitative PCR analysis showing average CTCF (A) and cohesin (Rad21) (B) enrichment with standard deviations at five gDMRs. Regions that differ from the negative control upstream *H19* gDMR (DMD) with a $p < 0.05$ are denoted with an asterisk indicating that these regions show statistically significant levels of CTCF or cohesin binding above the level of the negative control. C. UCSC screenshot showing CTCF binding peak called at <5% FDR (black) and cohesin binding peaks called at <90% FDR (grey). The *Peg10* TaqMan™ qPCR location is indicated.

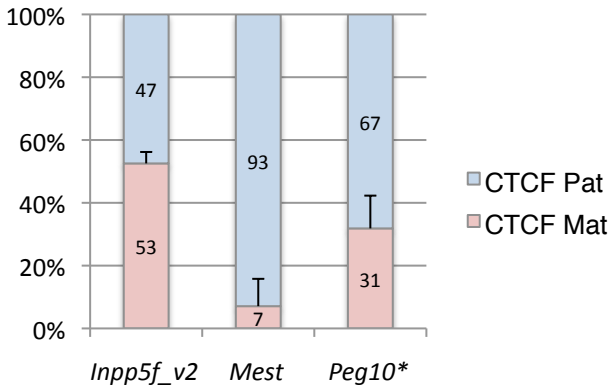


Figure S9: Validation of Table 1 using pyrosequencing to test allelic bias reveals results concordant with those seen using ChIP-seq. *Inpp5f_V2* shows approximately equal enrichment of maternal and paternal DNA indicative of bi-allelic binding, *Mest* shows a significant bias towards binding on the paternal allele and *Peg10* is suggestive of binding bias towards the paternal allele. * denotes 3 biological replicates, other samples were 4 biological replicates. Error bars represent the standard deviation of the percent maternal enrichment values.

	Binding peaks called at FDR<13	Peaks expanded by +/- 500bp and overlapping peaks merged	Expanded peaks overlapping a SNP	Tested peaks
CTCF	67443	49358	47138	46470
Rad21	73159	52928	50813	50249

Table S1: Number of peaks called by Useq with a statistical threshold of FDR < 13. To test for mono-allelic binding, peaks were expanded by 500 bp either side and overlapping peaks were merged. Peaks which contained a SNP between Bl6 and cast are shown and where two or more reads overlapped a SNP a binomial test was performed to test for mono-allelic binding resulting in 46,470 regions tested for CTCF and 50,249 for Rad21.

gDMR name	Methylated Allele	CTCF Binding	Mat Reads	Pat reads	95% CI	Cohesin Binding	Mat Reads	Pat reads	95% CI
CTCF and cohesin precisely colocalized									
Grb10	Maternal	Yes	2	7	0.28-0.60	Yes	7	8	0.21-0.73
H19/Igf2	Paternal	Yes	310	38	0.85-0.92	Yes	407	303	0.54-0.61
Inpp5f_v2	Maternal	Yes	85	78	0.44-0.60	Yes	150	173	0.41-0.52
Mcts2	Maternal	Yes	7	10	0.18-0.67	Yes	16	15	0.33-0.70
Mest	Maternal	Yes	8	75	0.04-0.18	Yes	57	82	0.33-0.50
Nnat	Maternal	Yes	*No SNP*			Yes	*No SNP*		
Peg13	Maternal	Yes	22	278	0.05-0.11	Yes	211	273	0.39-0.48
Zac1	Maternal	Yes	85	75	0.45-0.61	Yes	127	136	0.42-0.55
CTCF and cohesin not precisely colocalized									
Cdh15	Maternal	Yes	24	41	0.25-0.50	Yes	10	9	0.29-0.76
Nespas	Maternal	Yes	7	12	0.16-0.62	Yes	19	16	0.37-0.71
U2af1-rs1	Maternal	Yes	3	7	0.07-0.65	Yes	108	113	0.42-0.56
Zim2 (Peg3)	Maternal	Yes	39	215	0.11-0.20	Yes	4	13	0.07-0.50
CTCF binding only									
Peg10	Maternal	Yes	2	7	0.03-0.60	No			
Gtl2/Dlk1	Paternal	Yes	17	9	0.44-0.83	No			
IMPACT	Maternal	Yes	*No SNP*			No			
Cohesin binding only									
Igf2r-air	Maternal	No				Yes	8	8	0.25-0.75
Gnas-exon1A	Maternal	No				Yes	*No SNP*		
Kcnq1ot1	Maternal	No				Yes	30	43	0.29-0.52
Snurf/Snrpn	Maternal	No				Yes	10	20	0.17-0.53

Table S2: Shows the number of mapped maternal and paternal reads at each regions and the confidence intervals, which indicate the range of the ratio of maternal:paternal reads at a statistical threshold of 95%. Regions where the confidence intervals fell between 0.35-0.65 and spanned 0.5 were determined as bi-allelic binding sites, where CTCF or cohesin binds equally on both alleles.

	Called at p<0.001	Genomewide sig. ($p<1e^{-6}$)
CTCF	116	21
Rad21	38	0

Table S3: Number of peaks called as mono-allelic, regions are shown at statistically significant threshold of $p < 0.001$, and $p < 1e\text{-}6$ (which represents genomewide significance after correction for multiple testing) .

Gene	Chromosome	Primer	Expression in brain	Imprinted Expression	Notes
AK040960	**3**	**AK040960_1F**	No	n/a	
		AK040960_2R			
		AK040960_1F	No	n/a	
		AK040960_4R			
		AK040960_2F	No	n/a	
		AK040960_2R			
		AK040960_2F	No	n/a	
		AK040960_4R			
AK045814	**2**	**AK045814_1F**	Yes	No	
		AK045814_3R			
		AK045814_3F	Yes	No	Transcript size is different between C57BL/6 and *Mus m castaneus*
		AK045814_6R			
		AK814/295_F	Yes	No	No expression from the *Mus m castaneus* allele
		AK814/295_R			
		AK814/295_F2	No	n/a	
		AK814/295_R2			
AK046150	**14**	**AK046150_F**	Yes	No	
		AK046150_R			
AK086749	**14**	**AK086749_1F**	No	n/a	
		AK086749_4R			
		AK086749_2F	Yes	No	No expression from the C57BL/6 allele
		AK086749_4R			
Entpd4	**14**	**Entpd4_2F**	Yes	No	
		Entpd4_3R			
		Entpd4_9F	Yes	No	
		Entpd4_12R			
Gata5	**2**	**GATA5_4FA**	No	n/a	
		GATA5_6RA			
		GATA5_3F	No	n/a	
		GATA5_6RB			
		GATA5_4FB	No	n/a	
		GATA5_5R			
Gnaz	**10**	**GNAZ_3F**	Yes	No	
		GNAZ_4R			
		GNAZ_F	Yes	No	
		GNAZ_R			
		GNAZ_2F	Yes	No	
		GNAZ_3R			
Gpld1	**13**	**Gpld2_1F**	Yes	No	
		Gpld2_5R			
		Gpld2_5F	Yes	No	
		Gpld2_11R			
Loxl2	**14**	**Loxl2_1F**	No	n/a	
		Loxl2_2R			
		Loxl2_1F	No	n/a	
		Loxl2_3R			
		Loxl2_1aF	No	n/a	
		Loxl2_2R			
		Loxl2_1aF	No	n/a	
		Loxl2_3R			
Mrs2	**13**	**Mrs2_2F**	Yes	No	
		Mrs2_4R			
		Mrs2_5F	Yes	No	
		Mrs2_6R			
		Mrs2_2F	Yes	No	
		Mrs2_6R			
Rtdr1	**10**	**Rtdr1_4F**	Yes	No	
		Rtdr1_6RA			
		Rtdr1_5F	Yes	No	
		Rtdr1_6RB			
		Rtdr1_6F	No	n/a	
		Rtdr1_7R			
Slc44a3	**3**	**Slc44a3_3F**	Yes	No	
		Slc44a3_5R			
Snca	**6**	**SNCA_A1F**	No	n/a	
		SNCA_ST_2R			
		SNCA_1F	Yes	No	No expression from the C57BL/6 allele
		SNCA_ST_2R			
		SNCA_1F	Yes	No	No expression from the C57BL/6 allele
		SNCA_LT_6R			
		SNCA_A1F	Yes	No	No expression from the C57BL/6 allele
		SNCA_LT_6R			
Tbc1d30	**10**	**Tbc1d_2F**	Yes	No	
		Tbc1d_5R			
		Tbc1d_9F	Yes	No	
		Tbc1d_11R			
Trio	**15**	**Trio_F2**	Yes	No	
		Trio_R5			
		Trio_F22	Yes	No	
		Trio_R26			

Table S4: Allele-specific expression of candidate genes from Table 2. Primer sequences are found in Table S6.

Somatic & likely somatic DMR, or nearest gene/feature	DMR position (Xie et. al.)	Known not to be a gDMR	Methylated Allele	CTCF Binding?	Binding Allele	Allele-specific pval	Cohesin Binding?	Binding Allele	Allele-specific pval
Bind both CTCF and cohesin									
H19 promoter	chr7:149,763,483-149,765,230	Yes	P	Yes	Mat	$1.11e^{-074}$	Yes	Mat	$1.08e^{-004}$
Cdkn1c	chr7:150,645,240-150,647,381	Yes	P	Yes			Yes		
Gtl2	chr12:110,777,813-110,781,249	Yes	P	Yes	Mat	$6.10e^{-005}$	Yes		
Magel2	chr7:69,521,307-69,522,167	Yes	M	Yes	Pat	$3.38e^{-008}$	Yes	Pat	$3.00e^{-002}$
Casc1 intragenic*	chr6:145,136,212-145,136,335	ND	M	Yes			Yes		
Commd1 DMR2 (intragenic)*	chr11:22,880,151-22,880,300	ND	M	Yes			Yes		
Myo10 intragenic*	chr15:25,643,579-25,643,918	ND	M	Yes			Yes		
Eif2c2 diffuse DMR*	chr15:72,927,789-73,009,831	ND	P	Yes			Yes		
Gtl2-Mirg diffuse DMR*	chr12:110,783,188-110,989,333	ND	P	Yes	Mat	$6.10e^{-005}$	Yes		
Nhlrc1 downstream*	chr13:47,106,179-47,106,298	ND	M	Yes			Yes		
Bind CTCF only									
AK086712 promoter*	chr7:69,354,141-69,354,450	ND	M	Yes	Pat	$2.15e^{-002}$	No		
Snrpn U exon*	chr7:67,285,188-67,285,250	ND	M	Yes			No		
Neurog3 upstream*	chr10:61,590,706-61,590,749	ND	M	Yes	Pat	$2.00e^{-002}$	No		
Bind cohesin Only									
Nesp	chr2:174,109,010-174,113,395	Yes	P	No			Yes		
Igf2r	chr17:12,962,643-12,962,696	Yes	P	No			Yes		
H13 DMR2 (3' end)	chr2:152,533,500-152,533,900	Yes	M	No			Yes		
Pvt1 promoter*	chr15:61,868,725-61,868,885	ND	M	No			Yes		
Bind Neither CTCF and cohesin									
Ndn	chr7:69,493,100-69,493,181	Yes	M	No			No		
Mkrn3	chr7:69,564,012-69,565,740	Yes	M	No			No		
Dlk1	chr12:110,697,919-110,700,243	Yes	P	No			No		
Vwde promoter*	chr6:13,174,720-13,174,854	ND	P	No			No		
6330408a02Rik 3' end*	chr7:13,846,427-13,846,910	ND	M	No			No		
U80893 5' upstream*	chr7:68,084,346-68,085,190	ND	M	No			No		
*mir344b**	chr7:68,935,151-68,935,803	ND	M	No			No		
*mir344c**	chr7:68,982,367-68,982,367	ND	M	No			No		
*mir344**	chr7:69,022,442-69,022,857	ND	M	No			No		
*mir344-2**	chr7:69,084,650-69,084,878	ND	M	No			No		
*mir344g**	chr7:69,125,234-69,126,166	ND	M	No			No		
Magel2-Mrkn3 intergenic*	chr7:69,551,285-69,552,030	ND	M	No			No		
Grb10 DMR2 (intragenic)*	chr11:11,935,418-11,935,544	ND	M	No			No		
FR149454 promoter*	chr11:119,119,717-119,120,635	ND	M	No			No		
FR085584 promoter*	chr12:81,269,160-81,269,984	ND	M	No			No		

Table S5: Comparison of CTCF and cohesin binding at somatic DMRs identified by whole genome bisulphite sequencing (Xie et al, 2012). CTCF & cohesin, CTCF, cohesin and neither. The results for somatic DMRs are in close agreement with the results for gDMRs (Table 1). Allele-specific binding is defines at genomewide significance.

qPCR primers	Forward Primer	Reverse Primer	FAM Probe
Positive control *Rep1+2*	TCTTTAGGTTTGGCGCAATCGA	GACGTCTGCTGAATCAGTTGTG	TTTGCTGCCACCACGCG
Negative control *DMDup*	AGTGGTGTCTGTAATCTGGAGAGAA	TGAGCCTGCATCTTATTGAAGTGAT	CCATGTCCTATATAAGTAACACTC
Grb10	GCCGGCTAGCACAGACTT	CGGGAGCTGTCCACTGG	CCCGGTAGCGCGCACG
Nnat	GGACACCATGGGCTTGGT	GCAGCAAGATGTGAGACTAGATACA	ACCACACGGTGGCACAA
Peg10	GCCACTAACCTGTCAGTAAGAATGT	GGAGACCCTTGTGCTTGGA	CCCGACCCTTTCCCG
Nap1l5	GGGCAAGCTCTCCATAAAGTCTTTT	CGCGACCGGTGACAGT	CCCCAGGCCCCCGCG

Pyrosequencing primers	Forward Primer	5' Biotin labeled Reverse Primer	Sequencing primer
Inpp5f_v2	GCTACCCATGCCAGATTAGAAATT	AATGGCCAAGGATTAAAAGATACA	CATGCCAGATTAGAAATTAC
Mest	AGTATACCCTGGACCGCATTACGT	ATCTTCCCATGTTCGGGGAGT	GTCAGCTGGGTGGTC
Peg10	CTTCACACGCTACGAACTGATG	TTAAGCGCTCATTAGTACATCGTA	GCTTCGCTGTACTAATGG

Table S6: qPCR and Pyrosequencing primer sequences.

Primer name	Sequence
Rtdr1_4F	AAGCATGACATCATCCAAGC
Rtdr1_6R	TGGCCTTCACCTCCTCAT
Rtdr1_5F	GGAGAAGCTCCTTAGCCAAA
Rtdr1_6R	GATAGCATTGGCATCCAGAG
Rtdr1_6F	GTTGTCAGACACGGATGAGG
Rtdr1_7R	TCACTTTGATGGCTACTTCTGC
GNAZ_3F	GCAGCTCTTTGCTCTGACTG
GNAZ_4R	AAGAGGATGAGCGAGGTGTT
GNAZ_F	CAGTGGCTATGACCTGAAGC
GNAZ_R	CTCGAACTGACGTTGGATGT
GNAZ_2F	TTCGCAAGCCTTATGAAAAC
GNAZ_3R	GCTGCCTCTTTTTCCTCTG
AK045814_1F	AGAGCGCTGATGCAGAGTTA
AK045814_3R	TTCTGTCCATTTCACAGCAAG
AK814/295_F	AACCCGAGTTACCAGCAAAC
AK814/295_R	TTGTGGACCTCTGACTCTGC
AK045814_3F	CTGTGGGCTTGAGTCCAGT
AK045814_6R	AACAGTGTCAAAAGGGACCA
AK814/295_F2	TCTCTTGGCTGGTGCACTT
AK814/295_R2	TGAACTCCGGACCTTCAGA
GATA5_4F	AGAAAACGGAAGCCAAAGAA
GATA5_6R	GGCTGCTGACACAGGAACTA
GATA5_3F	CTGCTCCAACTGCCATACTG
GATA5_6R	AGGCAAAGTCTTCAGGTTCG
GATA5_4F	CACTTTGAAGGCAGAGTCCA
GATA5_5R	CAGCCTGCTTCTTTCTCCA
Ak046150_F	TTCTGGCTAGCCTGGTTGT
Ak046150_R	AGGGCACATTGTCAACATTT
AK086749_1F	AGCTTCCCTGAACCTGAAGT
AK086749_2F	ATCCGCAAGACAGACTATGG
AK086749_4R	AGGCTCTTCCTCTTCTGAGC
Loxl2_1F	TATAAAAGCCCCGGGAGA
Loxl2_1aF	GCTCGGAGCTTTTCTTCTG
Loxl2_2R	GGCCTCTACATAGCCCACTT
Loxl2_3R	TCAGTGTGCTTGCAGTCAGT
AK040960_1F	TCTCTGCAGAAACCTTCCAG
AK040960_2R	CCTACCTGCACAGACAGGAC
AK040960_2F	AATGGCGACACTCTGTCAA
AK040960_4R	TGTGCAAATGTCTACTTGAAGG
Slc44a3_3F	GAAGAACTCCCCAGTGGAAG
Slc44a3_5R	TGAACTCTGAGTGTAGTTGAAGGA
Mrs2_2F	ATGCCTCTCAAGCCACTTTA
Mrs2_4R	ACGTGCTGAAACCTCAAGTC
Mrs2_5F	TGAAGGCTGTGATAACTCCAG
Mrs2_6R	TGGAGTAGGACATGCAGCTT
Gpld2_1F	CTGGCATAGGTGGACAGTTT
Gpld2_5R	TGGAGTCCAGTGAGTCCTCT
Gpld2_5F	CTGGGAGAAGGACACAGAGA
Gpld2_11R	AGCATAAAGCTGGTCAAACG
SNCA_1F	TGCTTGGATTGGAAGAAGAG
SNCA_A1F	AGAGGCGCTGACAAATCA
SNCA_ST_2R	TCTATGATGCATGCAAGGAC
SNCA_LT_6R	TTCTTAGGCTTCAGGCTCATAG
Entpd4_2F	TTCTGTCATTGTGGATGACG
Entpd4_3R	GCCAGGATGCTAATGACAAC
Entpd4_9F	GCCAGCACAGTTCAGAAAA
Entpd4_12R	GTCTCTCAAGGGCAGGAAG
Tbc1d_2F	GGTCAAAATGGTTTTCAGCA
Tbc1d_5R	GGCCACAGTAGGAACTACAGC
Tbc1d_9F	GGCAGAGTTGAGGGAAAAGT
Tbc1d_11R	AGAACTATTTGCCTGCGATG
Trio_F2	GTTTTGCCAATTCTGAAGGA
Trio_R5	TCCAGCTTCAGTTTCCTCAC
Trio_F22	CCTGGGGATTTCTTCAGATT
Trio_R26	CAGGTGACGAAGCAGTGTC

Table S7: Primer sequences for allele specific expression testing of candidate genes.

Supplementary Methods 1

Chromatin immunoprecipitation

Banked P21 mouse brains were homogenized in 1ml of PBS pH8.0 with protease inhibitor (Roche:04693132001). Nuclei were centrifuged at 5000rpm for 5 mins. Subsequently centrifugation was at 5000rpm for 3 mins and resuspension in 1ml. Nuclei were washed 3X, cross-linked in 1ml of 5mM DTBP (Pierce:20665) on ice for 30 mins, washed twice in PBS, once in 0.1M Tris HCl pH8.0, 1.5M NaCl, and twice with PBS. Nuclei were cross-linked in 1% formaldehyde:PBS for 10 mins at 37°C and washed three times in PBS. Nuclei were lysed in 50mM Tris HCl pH8.0, 1% SDS, 10mM EDTA, with 0.1mM PMSF. DNA was quantified using a Nano-drop (Thermo Scientific).

Chromatin sonication

Chromatin was sonicated using a probe, amplitude 40 using nine 30 sec bursts, with 30 sec rests on ice. 15µl of sample was subjected to reverse crosslinking and electrophoresed on a 1% w/v agarose gel. Reverse cross-linking was achieved by adding 6µl 5M NaCl and 9µl water to the sample and incubating at 100˚C for 1 hour followed by 10µg RNAase A and 18µg Proteinase K treatment for 15 mins at RT.

Agarose bead chromatin immunoprecipitation.

5µg of chromatin was used for each sample plus 80µl ProteinA agarose fast flow beads (Millipore:16156), 1x complete protease inhibitor (Roche:04693132001) and buffer (16.7mM Tris HCl pH 8.0, 165mM NaCl, 20% Triton X-100, 1.2mM EDTA) to 600µl, rotated for two hours at 20rpm at 4˚C and beads separated by centrifugation.

8.5µl of CTCF (Millipore:07-729) or IgG (Abcam:ab17890) or 3µl of Rad21 antibody (Abcam:ab992) was added and diluted to 600µl, rotated overnight at 4˚C, transferred to Spin X columns (Costar:8160) with 60µl of agarose beads and rotated for 2 hours at 4˚C. Beads were washed in 800µl of buffer 1 (20mM Tris HCl pH 8.0, 150mM NaCl, 0.1% SDS, 1% Triton X 100, 2mM EDTA), rotated for 15 mins at 4˚C, repeated with buffer 2 (as buffer 1 but 500mM NaCl) and buffer 3 (10mM Tris HCl pH 8.0, 250mM NaCl, 1% Igepal, 5% sodium deoxycholate, 1mM EDTA). Beads were resuspended in 400µl water, 16µl 5M NaCl, incubated at 65˚C overnight and purified by phenol:chloroform extraction.

Quantitative PCR validation of CTCF and cohesin ChIP-seq

Applied Biosystems Custom TaqMan™ gene expression assays were designed (Supplementary Table S6 lists primers and FAM-conjugated probes) for each region. Positive and negative controls for CTCF and cohesin binding were chosen from the *H19/Igf2* differentially methylated domain region (Bell and Felsenfeld 2000; Hark et al. 2000; Kanduri et al. 2000). The negative control is upstream of the *H19/Igf2* gDMR and does not bind CTCF or cohesin (Stedman et al. 2008). Assays were designed to four other gDMRs, *Grb10, Peg10, Nap1l5* and *Nnat*. Of the regions we tested by qPCR, *Nnat* (Evans et al. 2005) and *Grb10* (Hikichi et al. 2003) were predicted to bind CTCF. TaqMan™ probes were designed to the centre of the CTCF and cohesin binding peaks except for *Nap1l5*, which did not exhibit enrichment, the probe was designed within the gDMR itself and constituted a negative control. Each region was assayed in duplicate and results were normalized to input and background.

CTCF and cohesin binding was assigned by performing a statistical comparison to the negative control region using a one-tailed Student's t-test. Regions with enrichment greater than the negative control with a $p<0.05$ were defined as CTCF or cohesin binding (Supplemental Fig. S8).