Supplemental Figure Legends

Supplemental Fig. 1. Genome-wide method for DNA methylation analysis using custom-design microarray in combination with methylated CpG island amplification (MCAM).

(A) Schematic of MCAM design. Agilent microarrays spotted with 244K oligonucleotide probes were designed to cover >80% of predicted *SmaI*/*XmaI* intervals up to 1.5 kb in size. These intervals (highlighted in orange) were annotated for their distances to known genes, CpG islands (CGIs) and repetitive sequences. Gene annotations were based on NCBI RefSeq (Jul 2008). CGIs were defined as at least 500bp long with GC content above 55% and CpG ratio above 0.65. Repetitive regions were masked in the genome downloaded from UCSC genome database using RepeatMasker (versions: RepBase Update 9.11). (B) Distributions of MCAM coverage across the gene body. Based on the genomic interval location relative to transcription start site (TSS) and transcription end site (TES), regions analyzed by MCAM were grouped into four categories: promoters; intragenic; 3' end and intergenic. Promoter refers to genomic interval located within 1kb upstream of TSS to 300bp downstream of TSS; 3' end is located within 1kb upstream to 300bp downstream of TES; intragenic is defined as within the gene transcripts, but neither promoter nor 3' end; all others are intergenic. Numbers of regions analyzed in each category are indicated. (C) Experiments to validate the MCAM performance. The experimental procedures have been described in detail previously (*1*). A female-female comparison of MCA products generated from peripheral blood lymphocytes (PBL) showed little difference (the correlation between these two individuals was 0.98, panel 1), indicating the arrays are reproducible. Using MCA products generated from normal male and female PBL, the genes on the X chromosome in female PBL were significantly hypermethylated compared with the same regions on male X chromosome (panel 2). Thus, MCA performs well, even in a situation where only 50%

methylation is expected. We then compared microarray results with quantitative bisulfite-pyrosequencing results for over 200 data points, and found a good correlation between these two independent methods (correlation was 0.87, panel 3).

Supplemental Fig. 2. Characterization of genomic regions undergoing methylation changes during differentiation of hESCs. (**A**) Genomic intervals are divided based on their association with CGIs as well as methylation changes during induced differentiation in hESCs. They are further subdivided based on their locations relative to genes. Within each bar, the relative proportions of regions enriched for H3K4me3/H3K27me3, H3K4me3 only, or H3K27me3 only are indicated. Relative to those in which methylation did not change, CGI-associated genomic intervals that gained methylation upon differentiation are enriched for the bivalent chromatin domain (H3K4me3/H3K27me3) (P<0.0001). (**B**) GO analysis of genes associated with CGIs that gained methylation during induced differentiation identifies a preponderance of developmental processes. For each significant GO term, the FDR adjusted P-value is indicated.

Supplemental Fig. 3. Methylation status of all CGIs located within the selected genes. DNA methylation was measured quantitatively at either 5' CGI (green) or 3' CGI (purple) for two classes of genes. Gene IDs are indicated on top of each graph. X-axis indicates differentiation status. Y-axis indicates methylation level. Values are given as means and standard deviations of results from more than two biological replicates.

Supplemental Fig. 4. Evolutionary conservation of *Hic1* gene locus. 6 kb of mouse and human *HIC1* sequences (from -670 bp, the start of *HIC1* transcription) were compared using

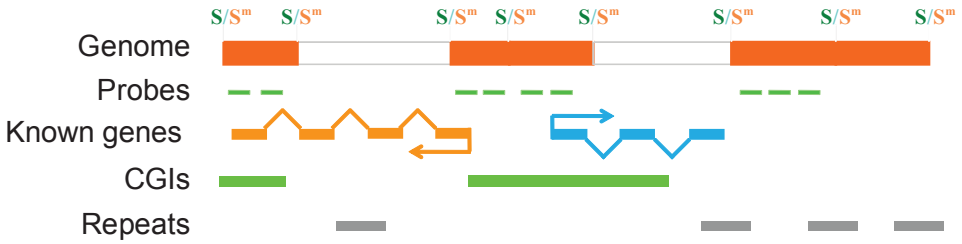
VISTA (http://www-gsd.lbl.gov/vista) (*2*) and a 100-bp window length. Human sequence is displayed on the x axis and percentage identity with mouse sequence is indicated on the y axis. Shaded areas represent regions with 75% or higher sequence identity.

Reference

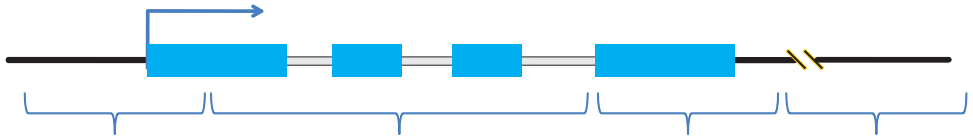
1. Shen, L., Kondo, Y., Guo, Y., Zhang, J., Zhang, L., Ahmed, S., Shu, J., Chen, X., Waterland, R. A., and Issa, J. P. (2007) Genome-wide profiling of DNA methylation reveals a class of normally methylated CpG island promoters, *PLoS Genet 3*, 2023-2036.
2. Mayor, C., Brudno, M., Schwartz, J. R., Poliakov, A., Rubin, E. M., Frazer, K. A., Pachter, L. S., and Dubchak, I. (2000) VISTA : visualizing global DNA sequence alignments of arbitrary length, *Bioinformatics 16*, 1046-1047.

Yu_Suppl Fig. 1

A DNA methylation custom array design



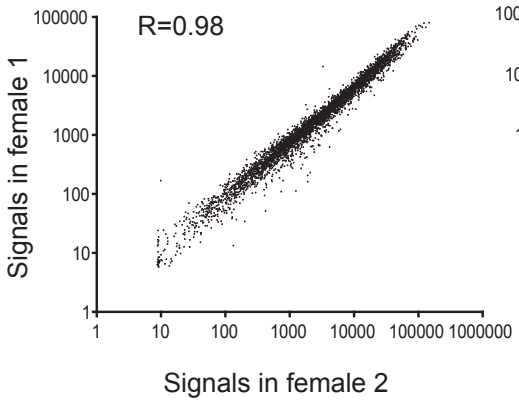
B Distribution of regions analyzed by MCAM



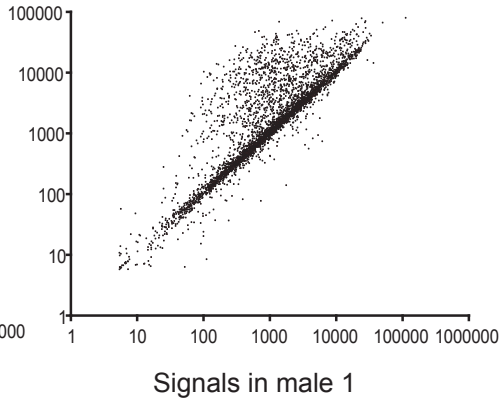
83927 total intervals				
CGI (32301)	14487	9843	1335	6636
non-CGI (51626)	1387	26721	1998	21520

C Validations of MCAM performance

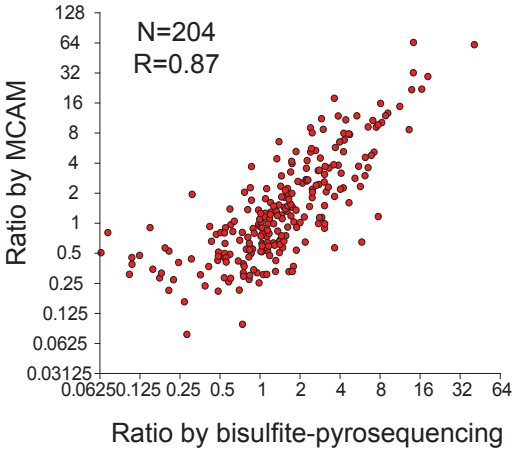
(1)

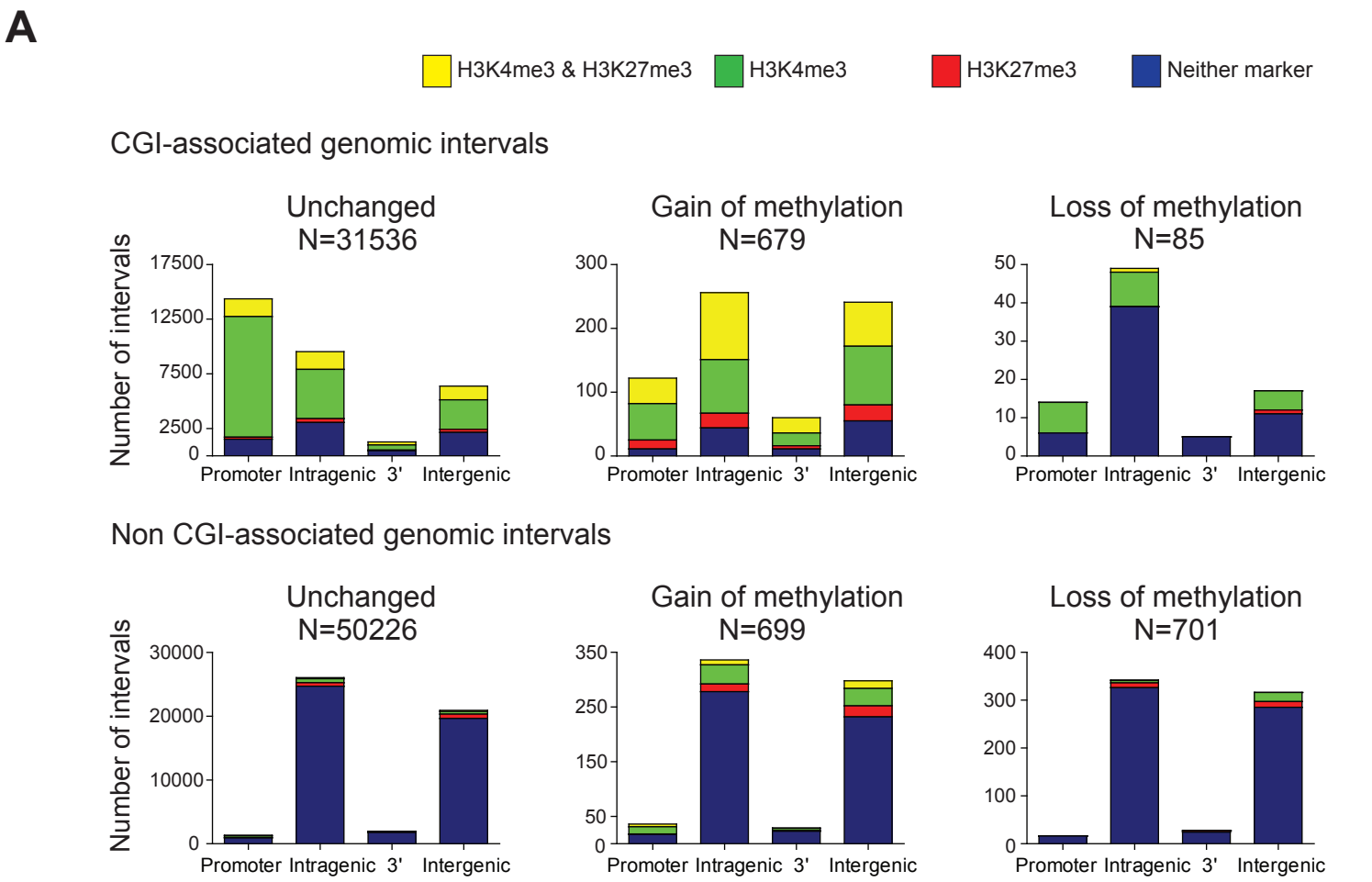


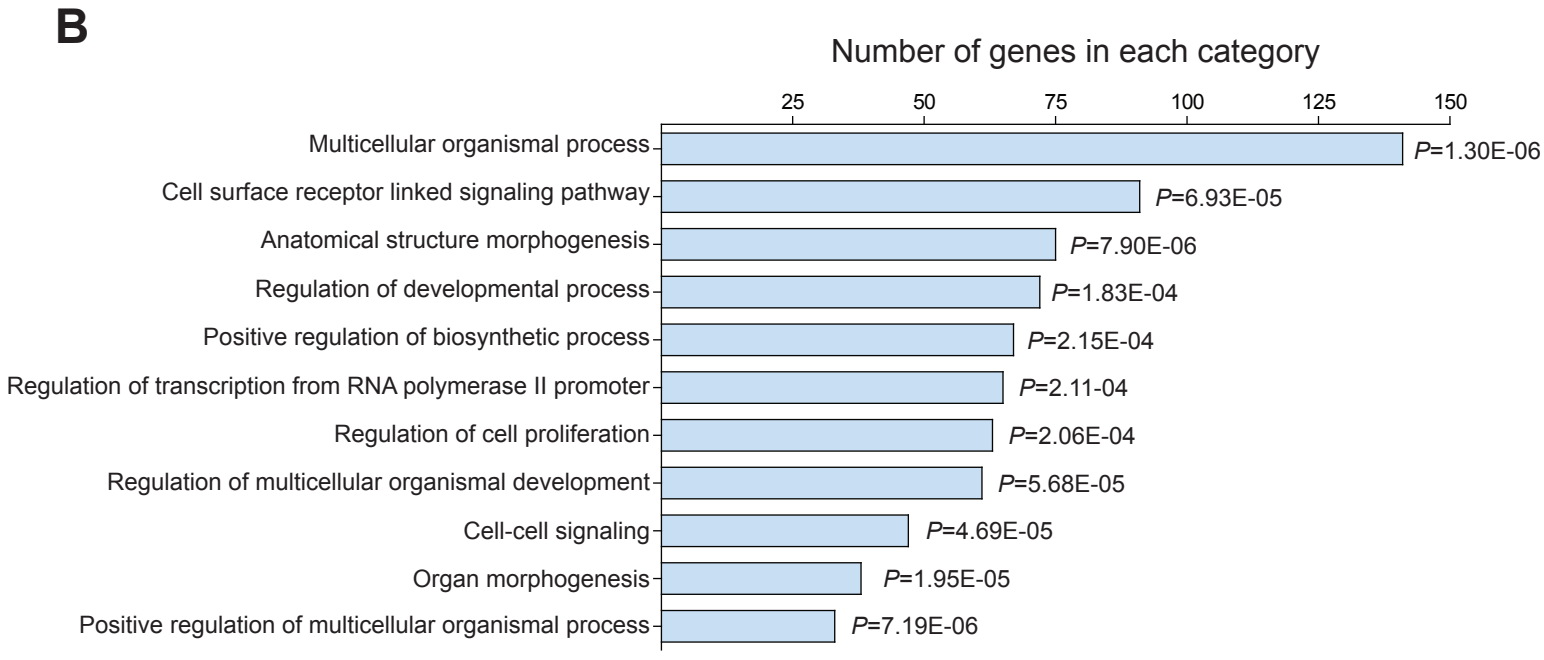
(2)



(3)

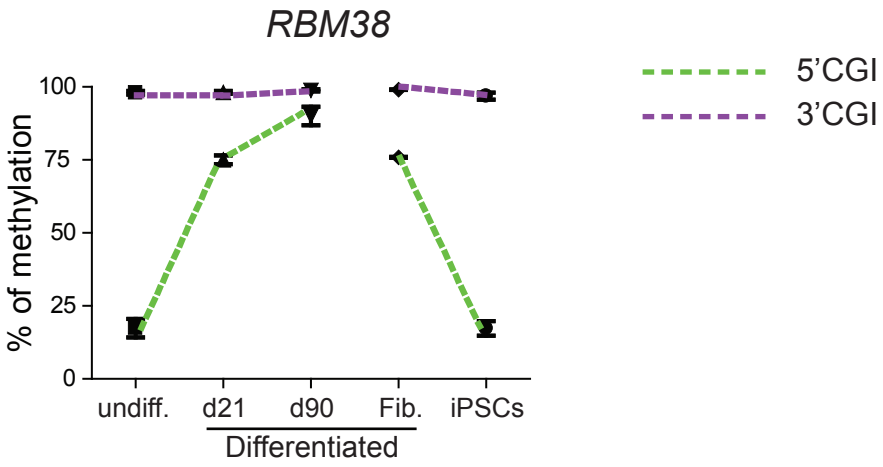



A
H3K4me3 & H3K27me3
H3K4me3
H3K27me3
Neither marker
CGI-associated genomic intervals
Unchanged
N=31536
Gain of methylation
N=679
Loss of methylation
N=85
Number of intervals
Promoter
Intragenic
3'
Intergenic
Non CGI-associated genomic intervals
Unchanged
N=50226
Gain of methylation
N=699
Loss of methylation
N=701

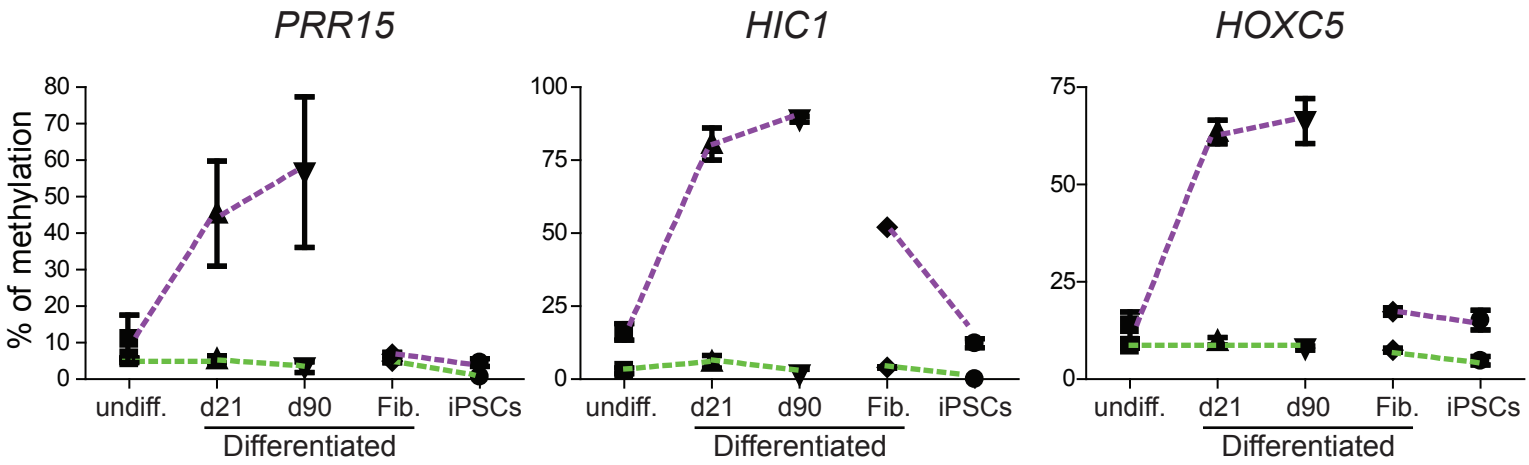

B
Number of genes in each category
Multicellular organismal process
Cell surface receptor linked signaling pathway
Anatomical structure morphogenesis
Regulation of developmental process
Positive regulation of biosynthetic process
Regulation of transcription from RNA polymerase II promoter
Regulation of cell proliferation
Regulation of multicellular organismal development
Cell-cell signaling
Organ morphogenesis
Positive regulation of multicellular organismal process
P=1.30E-06
P=6.93E-05
P=7.90E-06
P=1.83E-04
P=2.15E-04
P=2.11-04
P=2.06E-04
P=5.68E-05
P=4.69E-05
P=1.95E-05
P=7.19E-06

Yu_Suppl Fig. 3

A 5’ CGI associated gene



B 3’ CGI associated genes



Human Mar. 2006 chr17:1903440-1909443

Yu_Suppl Fig. 4

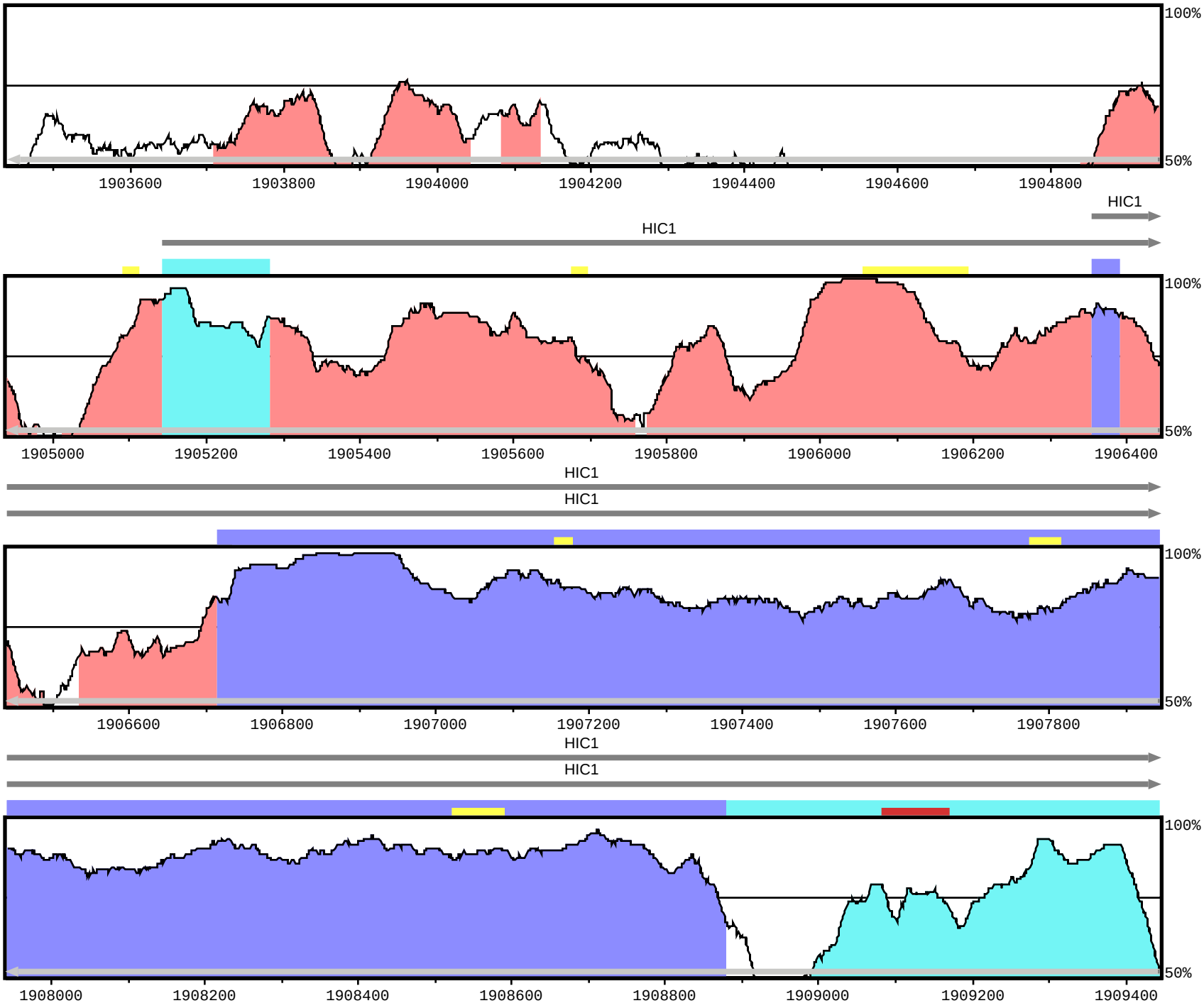
Alignment 1
sequence1
mhic1 (-)
90-6211
Criteria: 70%, 100 bp
Regions: 13

X-axis: Human Mar. 2006
Resolution: 1
Window size: 100 bp

contig
gene
exon
UTR
CNS
mRNA

Repeats:

LINE
LTR
SINE
RNA
DNA
Other



Supplemental Table 1. Primer sequences, PCR conditions, and assays for quantitative DNA methylation analysis by bisulfite-pyrosequencing

ID	Description	Accession No.	CpG island location	PCR conditions	
				Forward primer	Reverse primer
ALOX12	Arachidonate 12-lipoxygenase	NM_000697	Promoter	GGAGATTTYGGGAAGTGTTTTTATTTATG	CAAATCCCCRCCCCAAACTAACC
CMYA5	Cardiomyopathy associated 5	NM_153610	Promoter	GGTTAGAGTAGTYGGAGGGAGAATAT	CTAACTACCCTACAAACCCTAAAC
RBM38	RNA-binding region containing protein	NM_017495	Promoter	TTTAGAGGTGGAGGGTGTGATT	AAACCCCTTATTCAAAAACTAAAAATT
			3' end	GTGTAGTTTAGAGTGGTGATTTTAG	AACCCCCCTCACTACATCCTATCAAA
REC8L	Meiotic recombination protein REC8-like 1	NM_001048205	Promoter	GTTTTTTATTGGTTGTTAGGGTAA	CRATATCAAACCCCTAAACCTTAC
TCL1A	T-cell lymphoma-1	NM_001098725	Promoter	GTGGGTTTTGGTTTTGGGTATT	AAACCCRACCCCAACTAACC
MAP7D1	MAP7 domain containing 1	NM_018067	Intragenic	GTGTTTGYGTGGGTGTGTTGA	AAAACCCCCTCAATCCCC
PCDH8	Protocadherin 8 isoform 1 precursor	NM_002590	Intragenic	GGAGTTAAYGGGGAGTTGG	CCCRCCCCCTACTATTACC
TBX2	T-box 2	NM_005994	Intragenic	GGGTTGGTTTTTTGTTGAGTTAT	TAATCCAAAACCCCAAATTTACA
BCL11B	B-cell CLL/lymphoma 11B isoform 2	NM_022898	3' end	ATTAAGGTGGAGAAGGATTTGGA	AAACCCAAAAAAAAATCCTTCAT
HIC1	Hypermethylated in cancer 1	NM_006497	Promoter	GGGTAGGGTGGGTTTTTTT	CTTTCCCCACACTCTTATCTAAAAC
			3' end	TAGAGGGGGAGGGGTGTTA	TCCCCACTCCCCAACTCTA
HOXC5	Homeobox C5	NR_003084	Promoter	ATGGATAGGTTTTAGAGGTGTAGGTATTTA	TAATCTCCCCACTACTCTTAACTC
			3' end	GGTATTYGGGGTTTAGGGTAAGTT	CAAACTCTCRCCAACCTTTAACT
LASS1	Longevity assurance gene 1 isoform 1	NM_001492	3' end	AGGGGTTTAGGTTTTGGTTTATG	CCCRACCTACCCATAAAATCTC
NOS3	Nitric oxide synthase 3	NM_000603	3' end	GGTTGTGATTGGGAGGAGAGTTAT	CAAAAACCCTACACAACCCTAATT
PCDHGC5	Protocadherin gamma subfamily C	NM_032407	3' end	GGAYGGTAGTGATTTTATTTTTTTAAGAT	AAAATAAATACAAATTCAATCCCTTATAA
PRR15	Proline rich 15	NM_175887	Promoter	GGGAGGTGTTAGTAGGGAAGAG	CCCAACTAAATCAAATACTACTACTAATCT
			3' end	GGGAAGGGGTGGAGTGAA	CCCCCACTACCACTTCCTTACTTTT
SEMA6C	Semaphorin Y	NM_030913	3' end	GGGAGGTTTTTTTAGGTATT	CTCCCTTTAAAAATTAAAAC
TMEM190	Transmembrane protein 190	NM_139172	3' end	GTGAGGGTTTTTGGTTTTAGGT	CCAAAACAAACAAATACTACAACTCAA
CNTFR	Ciliary neurotrophic factor receptor	NM_147164	Intergenic	GGGGTAGGGGTAAGTTTTGTTTT	CCCACACAAAATATCCTCTCC
LY6E	Lymphocyte antigen 6 complex, locus E	NM_002346	Intergenic	GTGTTTTGGGTTTGGAGTAGAGA	ACRAAAAAAAAAAAACCCAATTC
ALX3	Aristaless-like homeobox 3	NM_006492	Intragenic	GGGTTAGGTAAAGGTTAGTGTG	TCCTTCCCAAATCCAACTACTT
GGN	Gametogenetin	NM_152657	Intragenic	AGTTGTGTTGGGGATATGGGTAAG	CRACTACAAATTCCCCATTTCTAA

*: Universal Primer: GGGACACCGCTGATCGTTTA (Ref 9)

Continued Supplemental Table 1.

	CpG island					Pyrosequencing Assay 1	
ID	**location**	**Biotin ***	**Anneal (°C)**	**w DMSO**	**Amplicon (bp)**	**Sequencing primer**	**Sequence to analyze**
ALOX12	Promoter	Regular	55	no	239	GGGGGTTTAGGTTTAT	TTYGATTTYGTTTTTTYGGGAGGTTTA
CMYA5	Promoter	Regular	60/57/54/51	no	276	GTAGTYGGAGGGAGAATATTAG	GYGYGGYGYGGGYGGTTTYGGTTT
RBM38	Promoter	Regular	55	no	148	GGGGTTTGTGTAGTTAT	AYGGTTTTTTTTTTYGTTTAGGAAAGTTYGGGG
	3' end	Regular	55	no	278	GTTGTTTTAGTTTTATATTGA	GTATAYGTYGGTTAGTTYGGTTTAYGTTTAGTATTTATYG
REC8L	Promoter	Regular	60/57/54/51	yes	162	YGATTTAGTTTTGTAGTAGG	YGGTTYGGGGTTATATYGYGGTYGTT
TCL1A	Promoter	Universal	55	no	234	TTTTAGTAGAGGTTTAGAGT	TTYGGTTYGGTAGTYGAGGGAAGYGGG
MAP7D1	Intragenic	Universal	60/57/54/51	yes	275	GATTTTTAGTAAATTAATGG	TYGGTATTATAGATYGAGAAGAAGTTATTYGGT
PCDH8	Intragenic	Universal	60/57/54/51	yes	236	GAGTTGTAGTAGTAGGA	GTYGYGYGAAGTTTTYGTTATYGGT
TBX2	Intragenic	Universal	55	no	253	GAAAGGTTAGGAAGGAAA	YGTYGGYGAGTGTTTGGGAT
BCL11B	3' end	Regular	60	no	125	GTAGTGGTTGGTGGG	TTAYGYGGYGTYGYGGTATTTTA
HIC1	Promoter	Regular	55	no	170	AGTATTTGTGAGTTTTTAGTTTAGG	TGYGYGGGAGGAAGGTAGYGGTTTGTGYGYGTAG
	3' end	Regular	60/57/54/51	yes	126	CAATAAAAATAAATATAATC	RCAAAAACCRAAAATAAAACTAAAAATACCCCRAC
HOXC5	Promoter	Regular	55	no	276	GGGTAGATATGGTTGTTAAT	TTTYGGGTTTATTTYGATYGTTTYGTTTGTAGYGT
	3' end	Universal	55	no	256	GAATTTTGGTTTGGGT	YGTATTTTYGGTTTTTA
LASS1	3' end	Universal	55	no	259	GTTTTATATTTTTTTTATAG	AYGGTGYGTTTYGAGTTTTAGYGTTTA
NOS3	3' end	Universal	50	no	216	GGGTATGGAATTTTGAG	TTYGAAGTYGYGTATTTTAGYGTAGTTT
PCDHGC5	3' end	Regular	55	no	206	AGTTAGGTGAGGGGT	TYGGYGTYGTTTYGGGYGATTTTTGGGGG
PRR15	Promoter	Regular	55	no	218	GGAAAGTAGTTTTGGATTTTTTAT	TYGGGTAGYGAGTTTGGYGGTTTTTYGTYGTYGTTT
	3' end	Regular	60/57/54/51	yes	186	TGTYGGGAGTTTTTAGGTTT	TTYGGTTATGGTYGATAGYGGYGAT
SEMA6C	3' end	Universal	55	no	213	GAAGTATTTTTTGTATTTGG	GTYGGTTYGAGGGTTATYGGGGT
TMEM190	3' end	Regular	60	yes	169	GTTTTAGGTTTTTGGGG	YGTGGGTTTG GYGGTYGGGAGTTTYGGGTT
CNTFR	Intergenic	Universal	60	no	165	GGTAYGGGAAGTAGGAGGT	YGGYGGTTTAGAGAGTTYGGGG
LY6E	Intergenic	Universal	55	no	224	GAGGGAGAGGAAGAGAGT	TTYGTTTYGTTTYGGGGTTTT
ALX3	Intragenic	Universal	55	no	164	AAAGGTTAGTGTGGGG	YGGGAGGGTGAATTATYGTTTTTYGGYGGYGGG
GGN	Intragenic	Universal	55	no	253	GGGGATATGGGTAAGAAG	GYGTGAGTGTYGAAGGTYGGGG

Continued Supplemental Table 1.

	Pyrosequencing Assay 2	
ID	**Sequencing primer**	**Sequence to analyze**
ALOX12	GGAGGTTTAGGAAGGT	TTYGTYGTATTTATTTYGTTGGTTTTATTTTGGTTYGGGT
CMYA5		
RBM38	GGTTATTTATGATTAGTATTTATA	YGTYGTTTYGTTTGTTAYGGTTGTTAGTTTYGTGGGT
REC8L	AGTYGTTATTTAATGAGGAG	YGAGGTGYGGTGTTTYGAAGYGTTYGTTTT
TCL1A	TTTTTTAGTAGTAGTAGAGG	GYGGYGGTYGGTGTYGTTGTTGGTYGGGGTTT
MAP7D1	GGTTTTTGGTTGAGAAG	YGGYGTTAGGTTYGGGAGTAG
PCDH8	GGGGGAGATATTGTTT	ATYGGYGATTTTTYGTAGGAGTTA
TBX2	GTTTGGGATGGGGTT	TTYGTTTYGGGATTTTTTTAYGAG
BCL11B	GGAGAAGGATTTGGAGTT	GTYGTTYGTYGYGTTTATTTYGTT
HIC1		
HOXC5	TGAATTTAGGGATGTATAGTTAGAA GAGTTAAGGTGGGTT	GGYGGTTYGTTYGGGYGTTGGAGGAGYGAGT YGGTTYGYGTTATAGGATTT
LASS1		
NOS3		
PCDHGC5	GTTTAGTAGTTTATAGTTTT	GGYGTTGGAGTTTGAYGTTATTYGGTTTYGTTTTAA
PRR15	AAGGGGTGGAGTGAA	YGGTYGGAGATTAYGTGGAGAAAGGGGTYGTT
SEMA6C	TTTTGAAAAGGGTGGA	YGTYGAGAAGTTTTAGTTGTTTTTGA
TMEM190	TTAATTTTGTTTTTTTTGTTTAGAA	AAYGTGYGGAGGAAGTATATGTGGGYGTTG
CNTFR	GTTTTGTTTTTTTTAAGATT	TYGTYGTAAYGTTTT
LY6E		
ALX3	AGGGAAGGTGTAGTGG	YGAYGGTTYGGAGTTTAG
GGN	TTTTGGAGAATAGGGG	YGGGGTYGGTGYGGAYGTATTAGATT

Supplemental Table 2. Primer sequences for bisulfite cloning and sequencing at *PRR15* gene locus

Primer Sets	Distance from TSS (bp)		Forward primer	Reverse primer
	CpG_start	CpG_end		
1	-359	-26	GGTAAATAGYGGATTTGTTAATTA	CCACCCTCCAAAAAAAAAAATTA
2	-214	64	ATGGGGTTAAGGGATAGTTGTTG	AACCAAAACCCTAAAACAACTAAA
3	149	381	GGAGTGAATAGTTAGGGTTTTATT	TAATCTCCTCTAACACCTCCAATT
4	358	621	AATTGGAGGTGTTAGAGGAGATT	ACCCCCRAAACCAAACTCTAT
5	622	914	TATTTTGGGGTGTTGTTTGGTAT	ATCCCATTCCCTCCTACTTTACAA
6	899	1125	TAGGAGGGAATGGGATGTTTGTA	CCRCCAAAAAAAAAAAATCTAATC
7	1120	1394	TGGYGGTTTYGGGAGGAGAATT	AAACTTCTAAAACCCCCCCTCTAC
8	1366	1643	GTTTTGTAGAGGGGGGGTTTTA	TTACTCCCCAAAACCCAATCTTAC
9	1834	2188	YGATGGGGGTAAATTTTGGAG	TTCCAAAACACCATCACCTAATC
10	2171	2318	GGTGATGGTGTTTTGGAAGATATT	CAATCCCCTTTCCTCCCTTATACT
11	2487	2690	TTGGGTGTYGGGAGTTTTTAGG	CCCRAAAACTACTAATCCAATCC
12	2469	2720	YGTTTTYGAGGAGATTGGGGTGTT	CCRCTTTAACCCTTCCATCTAAAT
13	2729	2900	TGTTTTYGTGGGGGTTATTAGGAA	CCCCCCAAACCAAACAAATT
14	3696	4122	AGGAAGGGAGAGAGGTATTAATT	AATCTCCTTCAAATACCCAAACT

Supplemental Table 3. Primer sequences for bisulfite pyrosequencing at mouse *Hic1* gene locus

Primer Sets	Distance from TSS (bp) CpG_start	CpG_end	Forward primer	Reverse primer
1	-674	-637	GGTGGATTTTATAATTTTTGAGTTTTAGG	AAACATCAAAAACACCCTTACTTA
2	-540	-490	GGTAGGGTTTTTAGTTGTATTAGTGAGA	CAAAAATACCAAAATACCTCTTCACTT
3	-359	-247	GGGGTTTAGAGTTATTATGGTAATTAATGA	CCCAAAACCTTAATATCTCACTAATACAA
4	-25	152	GAGTTTTTTATTGGAAATAGTTGGATAGAG	ATTTAAAAAAAACCCCCCCTATAAC
5	329	408	ATTATTTATTTGGATTTAGTGGAGAGAGA	ACAAACTAAAATCCCTCAAAACTACT
6	553	573	TGGAAGTAAGAGGATTAGGAGTTTAAG	ATCTCTCTCCACTAAATCCAAATAAATA
7	813	847	GAGGTTTGTAGATGAGGAGTTGAGTAGATT	ACCTTAAACTCCTAATCCTCTTACTT
8	1040	1074	AGGATTTATTTGGGGGGTATG	CTACTCAACTCCCCATCTACAAACCTCTT
9	1342	1366	GTGTTAGGGTAGAGTTTTTGTTAGTT	CATACCCCCCAAATAAATCCT
10	1548	1703	TGGTGGTAGTGGGGATTTATATTA	ACAAAAACCCTACCCTAACACAACTC
11	1797	1993	GGGGAGTTAAGTAGTTTTTAGGGG	CCACTACCACCAAACTACTAAAATT
12	2145	2168	ATAAGGAAGAAGGGAGTAAGATGTTTAT	CCCACCTACTTCCTACCT
13	2261	2284	TGGGAATTTTTAGGGAGAAATTTGT	CATCTTACTCCCCTCTTCCTTATCACTT
14	2730	2763	TTTTGGTGAGTTGATTGTTGAGTTGTA	TCAAACCCTAATACTCCATACCCCTAATC
15	2851	2881	GGTTTAGGTTTAGTAGGTTGTTATGTAT	CAACAATCAACCCACCAAAAACTTCTTA
16	2946	2957	GTAGGTAGTTGGTAGTAGTTAGTATAG	ACAACCCCTACCTCAAATCCCTAATAAT
17	3285	3319	TTGTTGGGTTAGGGTTTGAAGTATA	ACTAACTACTACCAACTACCTACA
18	3364	3502	GTTTTTTTTAGTTTTTGGAAGGGTAAAG	ACACCCACTACCCTAAACTATATACTTCAA
19	4394	4421	TTGATGAGGTTGAGTTGTTGAGTAAAT	AACCACTTAAACCTAAAACCCTTTAC
20	4525	4603	AGTTGTGAGGAGGGTTATAGTAAAGA	TCAACAACCCAACCTCATCAACCATATAAA
21	4821	4859	GTGTAGAAAGGTGGGGTTATTAT	AACCTCTACCCCCTAACTAAATTCACT
22	4919	4947	TGAGGTTAGGTGAGGTGTAGA	CCCCCCTCACAACTAAACAACTAAATCT
23	5171	5206	AGTAGGGAGATTTATAGTGAGAATG	CCTACCTCTCCCTATAACTCATT
24	5288	5327	AGGAAGAGGAGGGGATATTT	CACCCATTCTCACTATAAATCTCCCTACTA

Supplemental Table 4. Primer sequences for real-time CTCF ChIP assays

Region analyzed	Chr. Location	Forward primer	Reverse primer	Probe
PRR15_ 3' CpG island	chr7:29,606,201-29,606,259	CGCCGCAATTTGAAGATCTC	GCGCGCACTTTCCTCTTCT	CGGCCGCTTTAAG
HOXC5_3' CpG island	chr12:54,428,088-54,428,153	AGTTACACGCGCTACCAGACTCT	GCGAGTGAGGTAGCGGTTAAA	AACTCGAGAAAGAATTC
H19 DMR	chr11:2,021,040-2,021,101	GGGTCATCTGGGAATAGGACACT	GGATAATGCCCGACCTGAAG	ATGGGAGCCGCACCA
Ctr1_PRR15_promoter	chr7:29,603,474-29,603,528	TGCCCCAGGGTTCTGGTT	CTGCCCACCTGCTGGTTATT	CCCGAATCACTTGGC
Ctr2_HOXC5_outside of 3' CpG island	chr12:54,428,980-54,429,039	CACTGGACCCCAGCAAGTG	TTCGTGGCAGGGACTATGG	CTAGAGGCCCTTTGC

Supplemental Table 5. Primer sequences used to generate plasmids for luciferase reporter assays

Constructs	Primer sets	Primer seq	size(bp)	NCBI36/hg18 location
Promoter or Enhancer Assays				
PRR15-Pro: promoter assay positive control	Prr15-pro-F(NheI)	CTAGCTAGCATGCCCTGTGCCAATATC	1091	chr7:29568861-29569951
	Prr15-pro-R(XhoI)	CAACTCGAGAGGTGCAGGTAGGAACTT		
Pro-Luc: enhancer assay negative control	En-F(SacI)	ATAATAGAGCTCAGTTATTAATAGTAATCAATTACGG		
	En-R (NheI)	TATTATGCTAGCCAAAACAAACTCCCATTG		
CMV-Enh: enhancer assay positive control	CMV-PRO-F(XhoI)	ATAATACTCGAGGCACCAAAATCAACGG		
	CMV-PRO-R(HindIII)	TATTATAAGCTTCGCTAGCGGATCTGAC		
PRR15-3'CGI-forward	bl-920-F (NheI)	ATAATA GCTAGC GTCCTTCTCTCCTACTGG	920	chr7:29571718-29572637
	bl-920-R (XhoI)	TATTATCTCGAG GAGCTGCTGGTCCAGTC		
PRR15-3'CGI-reverse	bl-920R-F (XhoI)	ATAATA CTCGAG GTCCTTCTCTCCTACTGG	920	chr7:29605193-29606112
	bl-920R-R (SacI)	TATTAT GAGCTC GAGCTGCTGGTCCAGTC		
mHic1-3'CGI-forward	Hic1-F(SacI)	ATAATA GAGCTC AGCTACCTGCAGATCCCTGA	2155	chr11:74978530-74980684
	Hic1-R(XhoI)	TATTAT CTCGAG CACAGTGAGAATGGGTGTGG		
mHic1-3'CGI-reverse	Hic1R-2k-F(XhoI)	ATAATA CTCGAG AGCTACCTGCAGATCCCTGA	2155	chr11:74978530-74980684
	Hic1R-2k-R(SacI)	TATTAT GAGCTC CACAGTGAGAATGGGTGTGG		
Enhancer-Blocker Assays				
PRR15-3'CGI-forward	bl-920-F(BamHI)	ATAATA GGATCC GTCCTTCTCTCCTACTGGGATA	920	chr7:29571718-29572637
	bl-920-R(HindIII)	TATTAT AAGCTT GAGCTGCTGGTCCAGTC		
PRR15-3'CGI-reverse	bl-920R-F(HindIII)	TATTAT AAGCTT GTCCTTCTCTCCTACTGGGATA	920	chr7:29605193-29606112
	bl-920R-R(BamHI)	ATAATA GGATCC GAGCTGCTGGTCCAGTC		
mHic1-3'CGI-forward	bl-Hic1-F(BglII)	ATAATA AGATCT AGCTACCTGCAGATCCCTGA	2155	chr11:74978530-74980684
	bl-Hic1-R(HindIII)	TATTAT AAGCTT CACAGTGAGAATGGGTGTGG		
mHic1-3'CGI-reverse	bl-Hic1R-F(HindIII)	ATAATA AAGCTT AGCTACCTGCAGATCCCTGA	2155	chr11:74978530-74980684
	bl-Hic1R-R(BglII)	TATTAT AGATCT CACAGTGAGAATGGGTGTGG		

Supplemental Table 6. Differential methylation identified in hESCs after random differentiation

	Regions analyzed	Gain of methylation	Loss of methylation
Total	83927	2187 (2.6%)	1660 (2.0%)
CpG island associated	32301	1124 (3.5%)	201 (0.6%)
Non-CpG island associated	51626	1063 (2.1%)	1459 (2.8%)

Based on genome-wide methylation analysis in both H1 and H13 cell lines.
Significant methylation changes were identified in four biological replicates at either day 21 or day 90.