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Supplemental Data

Active and Repressive Chromatin Is Interspersed without Spreading in an Imprinted Gene Cluster in the Mammalian Genome

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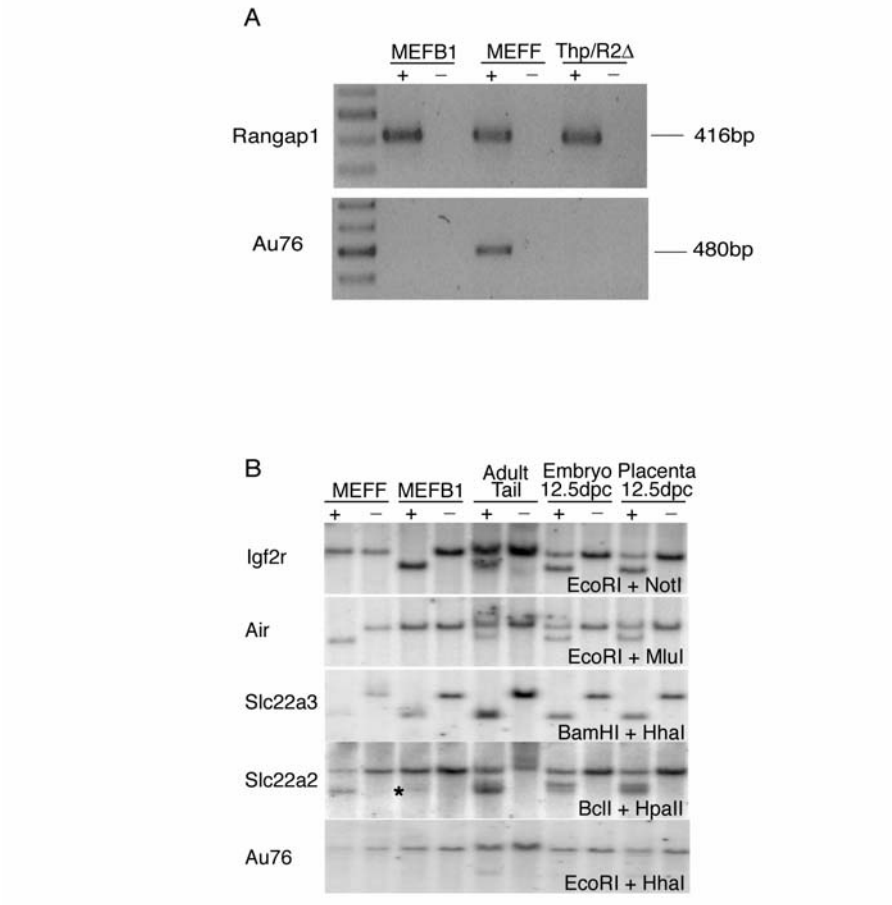

Figure S1. Lack Of *Au76*-psg Expression And DNA Methylation Status Of Genes In The *Igf2r* Cluster

(A) RT-PCR showing that the signal observed for the *Au76*-psg on both parental chromosomes in Fig.1B arises from cross-hybridization of the expressed ancestor *Rangap1*

gene located on chr.15. Sequence specific primers identify a 416bp band from the expressed *Rangap1* chr.15 gene sequence in all cell types and a 480bp band specific to *Au76* in MEFF cells carrying only the paternal chromosome. This latter signal arises from *Au76* sequences contained within the *Air* ncRNA as it is absent in T^{hp}/R2Δ cells that carry a paternal deletion of the *Air* promoter and lack *Air* expression (Wutz et al., 2001). (-), negative RT-PCR controls.

(**B)** DNA blots showing DNA methylation of promoter and pseudogene regions. Genomic DNA was digested either with a non-methyl sensitive plus a methyl sensitive enzyme (+ lanes, NotI, MluI, HhaI, HpaII) or with a single non-methyl sensitive enzyme (- lanes). The detection of the same sized band in (+) and (-) lanes indicates methylation. The *Igf2r* CpG island promoter (probe AJ249895 94104-99431) is methylated in MEFF cells that only contain a paternal chromosome, unmethylated in MEFB1 that only contain a maternal chromosome. Diploid adult and embryonic tissues contain both parental chromosomes and show a cut unmethylated and uncut methylated *Igf2r* fragment. The *Air* CpG island promoter (probe AJ249895 124992-126086) shows maternal-specific methylation at all stages. The *Slc22a3* CpG island promoter (probe UCSC Mouse Feb 2006 Chr.17: 12347702-12348110) is unmethylated (also at BssHII, data not shown). The *Slc22a2* promoter (probe UCSC Mouse Feb 2006 Chr.17: 12429198-12429606) lacks a CpG island and is partially methylated on both parental chromosomes. MEFF cells showed less methylation than MEFB1 cells (*faint band on original image). However, this is likely to be a cell variation as T^{hp}/+ and +/T^{hp} embryonic tissues lack parental-specific methylation (Zwart et al., 2001). The *Au76* fragment (probe AJ249895 bp43732-44140) is a CG poor region and is methylated on both parental chromosomes. Complete digestion by methyl-sensitive enzymes was confirmed by rehybridization of blots with the *Htf9* CpG island (Accession number X05830) that lacks DNA methylation (data not shown).

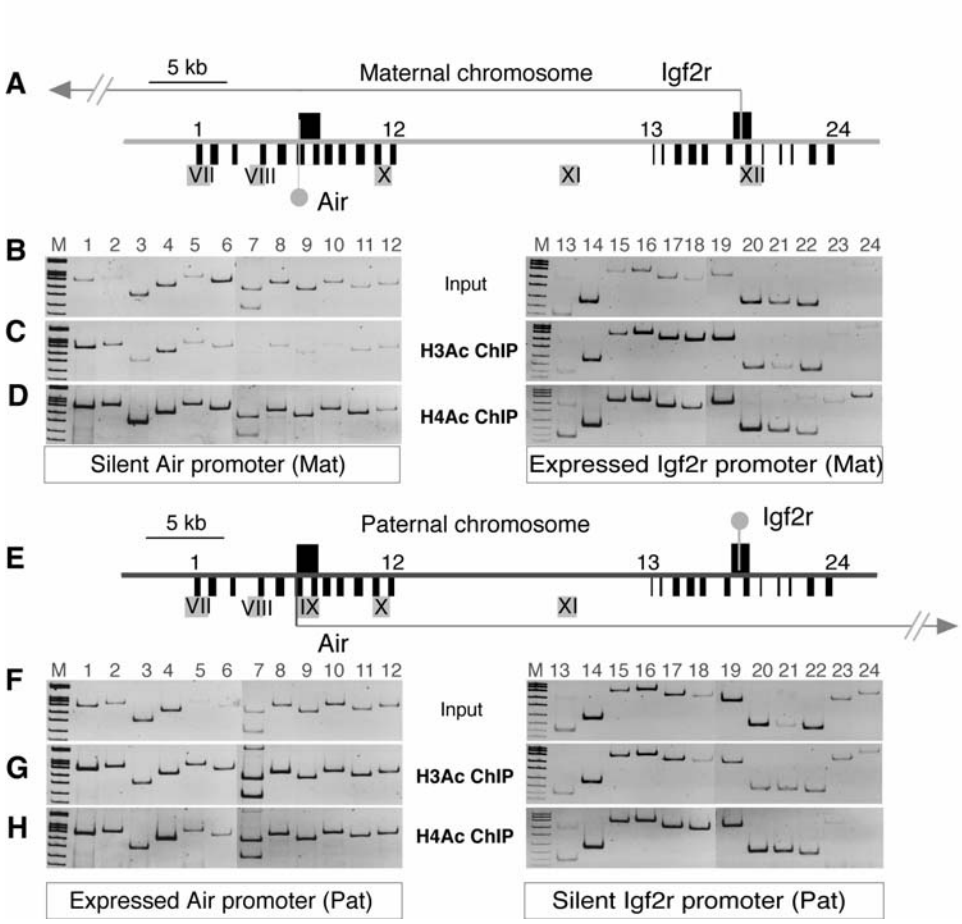

Figure S2. H3 And H4 Acetylation On The Expressed And Silent *Igf2r* And *Air* Promoters

(A) 40kb sequence based map showing the expression pattern of *Igf2r* and *Air* on the maternal chromosome (light grey line). Details as in Fig.2A.

(B-D) ChIP-PCR from MEFB1 cells that have only the maternal allele of the *Igf2r* cluster using multi-acetylation antibodies against H3 (K9+K14) acetylation (panels H3Ac) and H4 (K5+K8+K12+K16) acetylation (panels H4Ac).

(E) 40kb sequence based map showing the expression pattern of *Igf2r* and *Air* on the paternal chromosome (dark grey line).

(F-H) ChIP-PCR from MEFF cells that have only the paternal allele of the *Igf2r* cluster using multi-acetylation antibodies against H3 (K9+K14) acetylation (panels H3Ac) and H4 (K5+K8+K12+K16) acetylation (panels H4Ac).

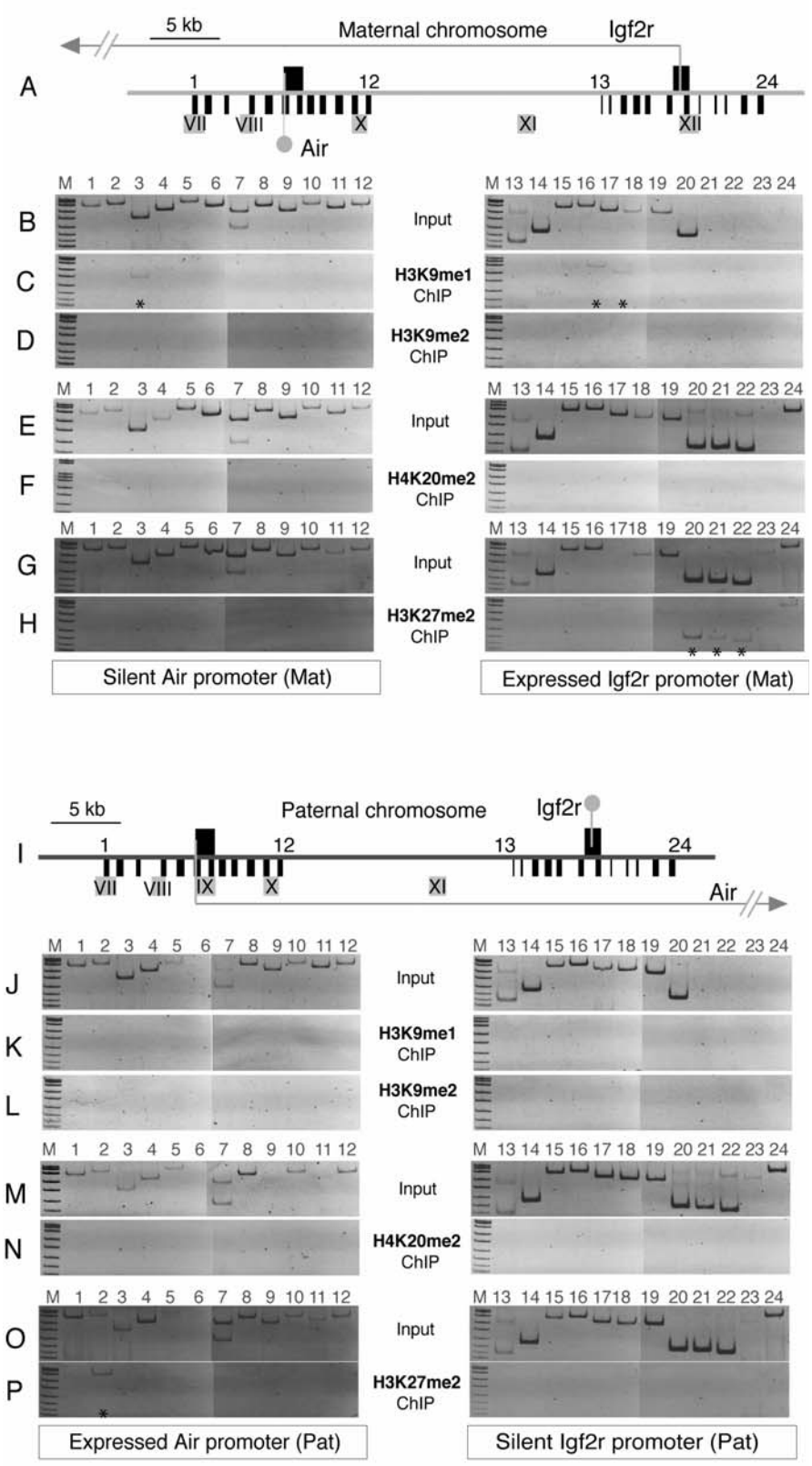

Figure S3. Mono And Di-Methyl Modifications Of H3K9, H3K27 And H4K20

(A) 40kb sequence based map showing the expression pattern of *Igf2r* and *Air* on the maternal chromosome (light grey line).

(B-H) ChIP-PCR from MEFB1 cells that have only the maternal allele of the *Igf2r* cluster using antibodies against H3K9me1, H3K9me2, H4K20me2 and H3K27me2. Primers 20-22 showed reproducible faint H3K27me2 signals.

(I) 40kb sequence based map showing the expression pattern of *Igf2r* and *Air* on the paternal chromosome (dark grey line).

(J-P) ChIP-PCR from MEFF cells having the paternal allele of the *Igf2r* cluster using antibodies against H3K9me1, H3K9me2, H4K20me2 and H3K27me2. A few primers variably produced weak H3K27me2 signals. *faint inconsistent signals.

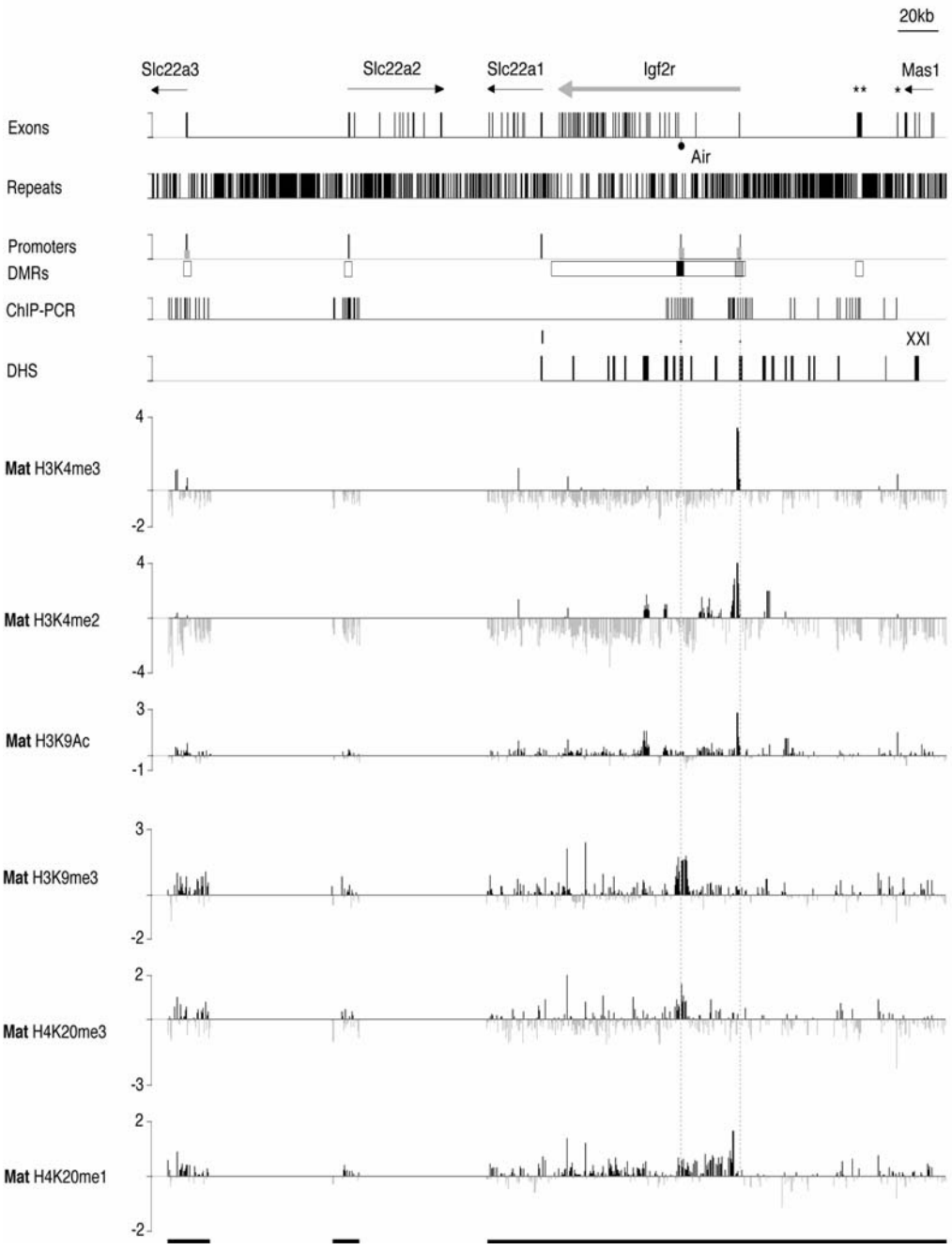

Figure S4. ChIP-Chip Assay Of Histone Marks On The Maternal Igf2r Imprinted Cluster

Biological replica of data in Fig.3 showing that active and repressive marks are interspersed and show limited spreading in the maternal *Igf2r* imprinted cluster. Details as Fig.3.

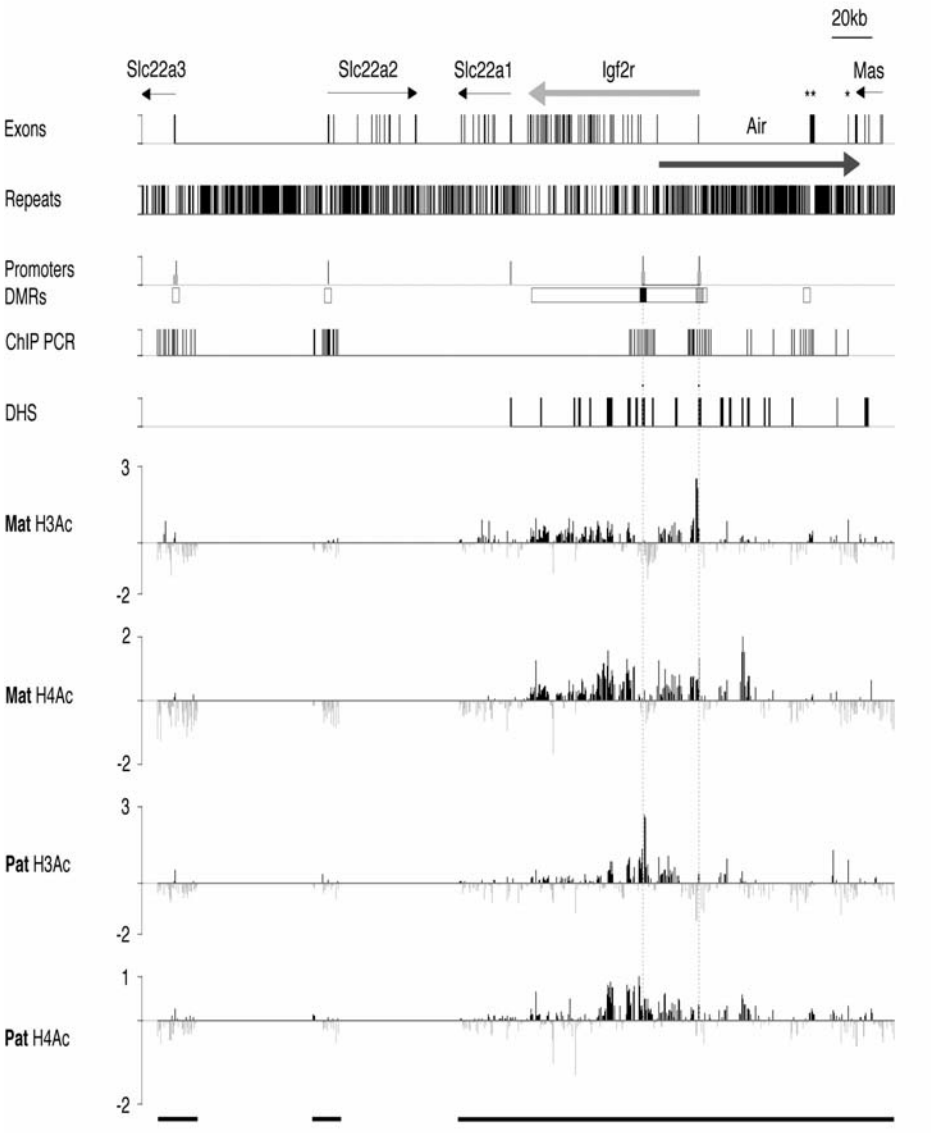

Figure S5. ChIP-Chip Assay Of General H3 And H4 Acetylation Patterns In The *Igf2r* Imprinted Gene Cluster

ChIP-DNA analyzed by PCR in Sup.Fig.2 was assayed by ChIP-Chip with a custom PCR tiling array as described in Fig.3. Mat; maternal chromosome analyzed in MEFB1 cells, Pat; paternal chromosome analyzed in MEFF cells.

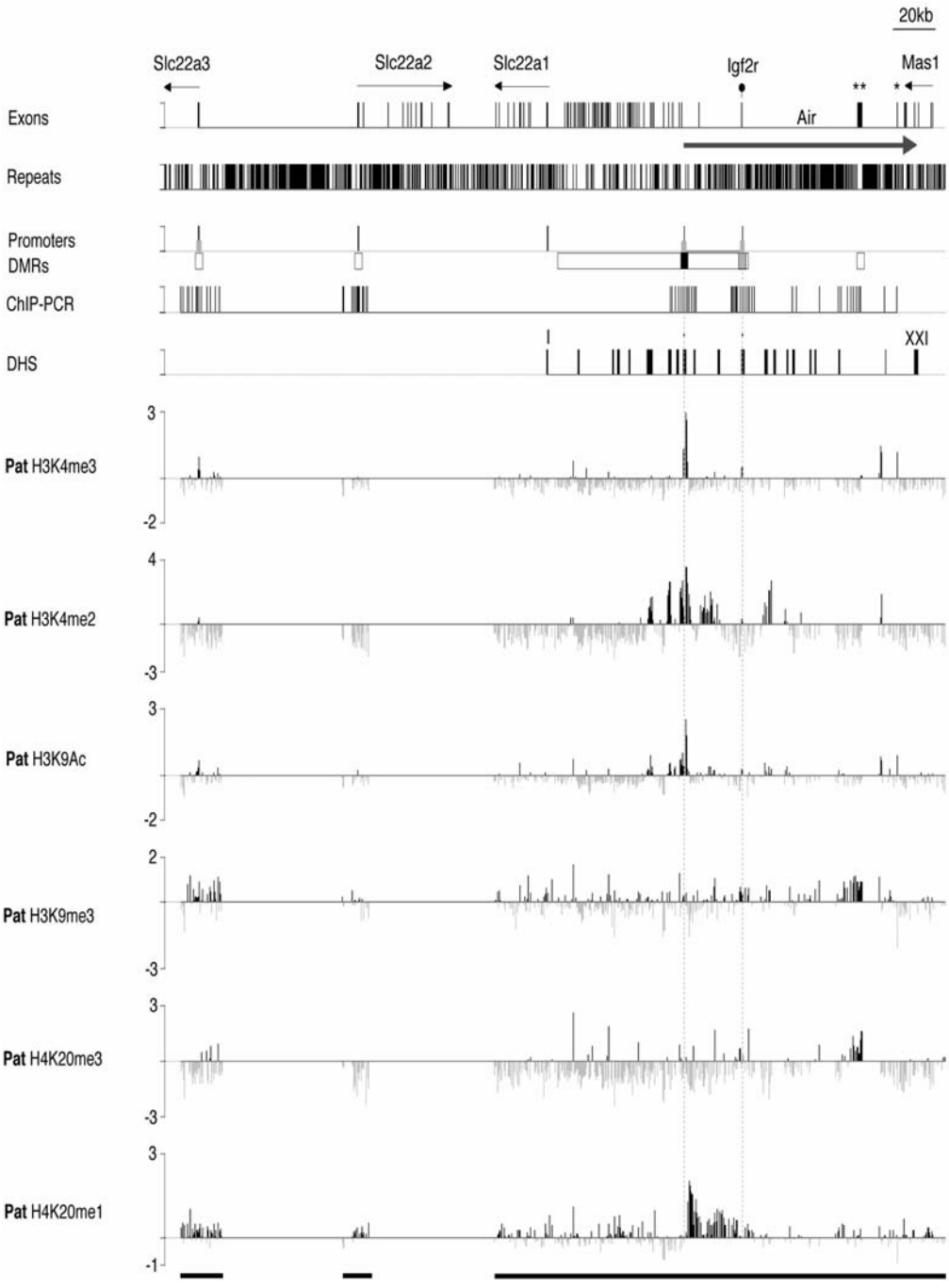

Figure S6. ChIP-Chip Assay Of Histone Marks On The Paternal Igf2r Imprinted Cluster

Biological replica of data in Fig.4 showing that active and repressive marks are interspersed and show limited spreading in the paternal *Igf2r* imprinted cluster. Details as Fig.4.

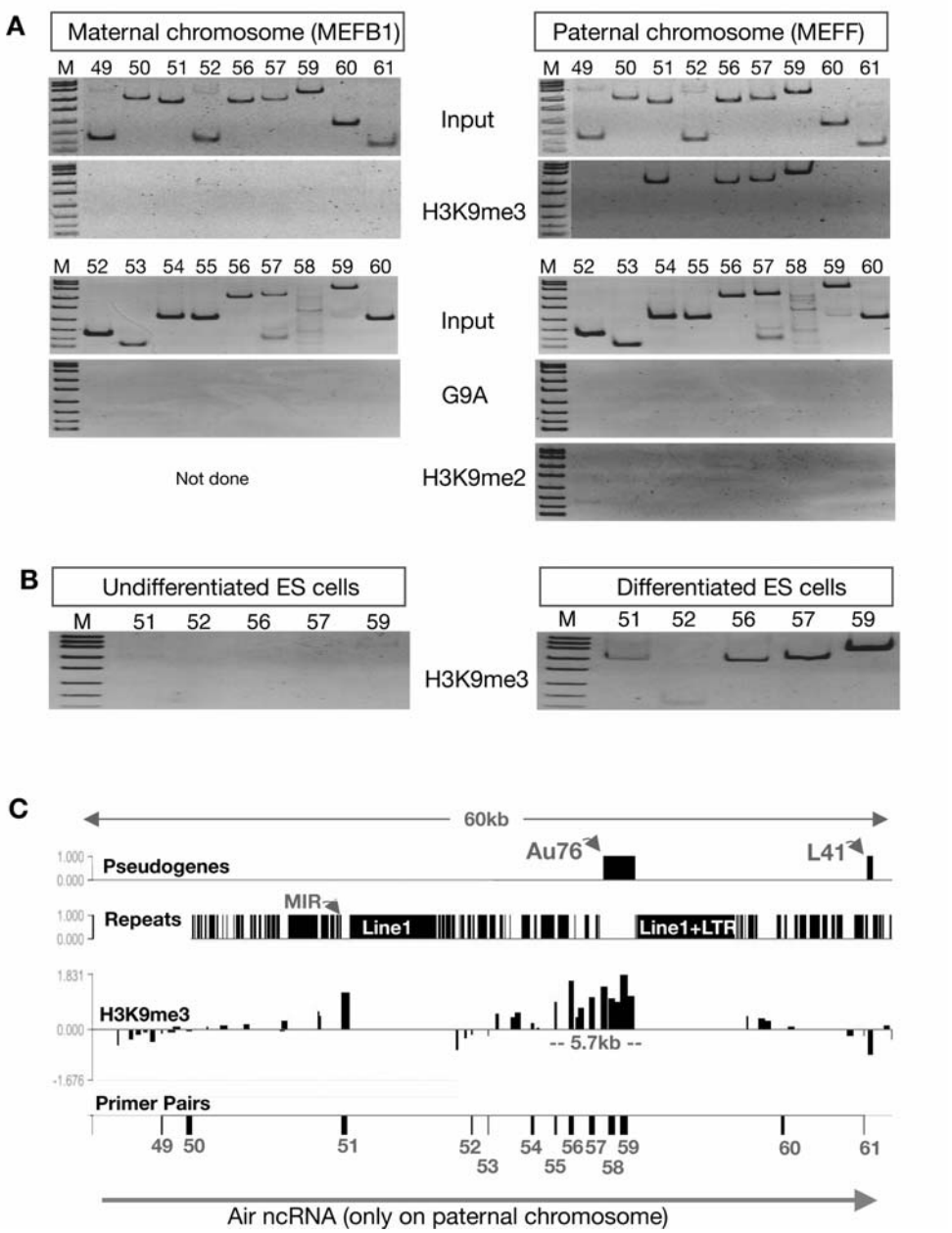

Figure S7. Paternal Specific Repressive Histone Modifications At The *Au76*-psg

(A) ChIP-PCR of H3K9me3, G9a and H3K9me2 modifications on the *Au76*-psg in MEFB1 (maternal chromosome) and MEFF (paternal chromosome) cells. H3K9me3 is also seen as a broad peak over the *Au76*-psg in the ChIP-Chip experiment only on the paternal chromosome (Fig.4) but H3K9me2 and G9a are not present on the paternal chromosome.

(B) ChIP-PCR of H3K9me3 in undifferentiated CCE ES cells and in differentiated CCE ES cells (treated in the absence of LIF with 5µM/ml Retinoic Acid for 5 days) shows that the H3K9me3 peak is not a germ line mark, but is acquired in ES cells during differentiation.

(C) An enlargement of a 60kb region from Fig.4 showing the relationship between the HC-peak on the *Au76*-psg (NCBI accession number AJ249895) and the interspersed repeat distribution. Primer pairs (N^o 49-61) used for ChIP-PCR are indicated below. Details as Fig.3. Note that repeats were not included in the custom PCR tiling array and that *Au76*-psg primers do not amplify the host *Rangap1* gene on chr.15 (Sup.Fig.1).

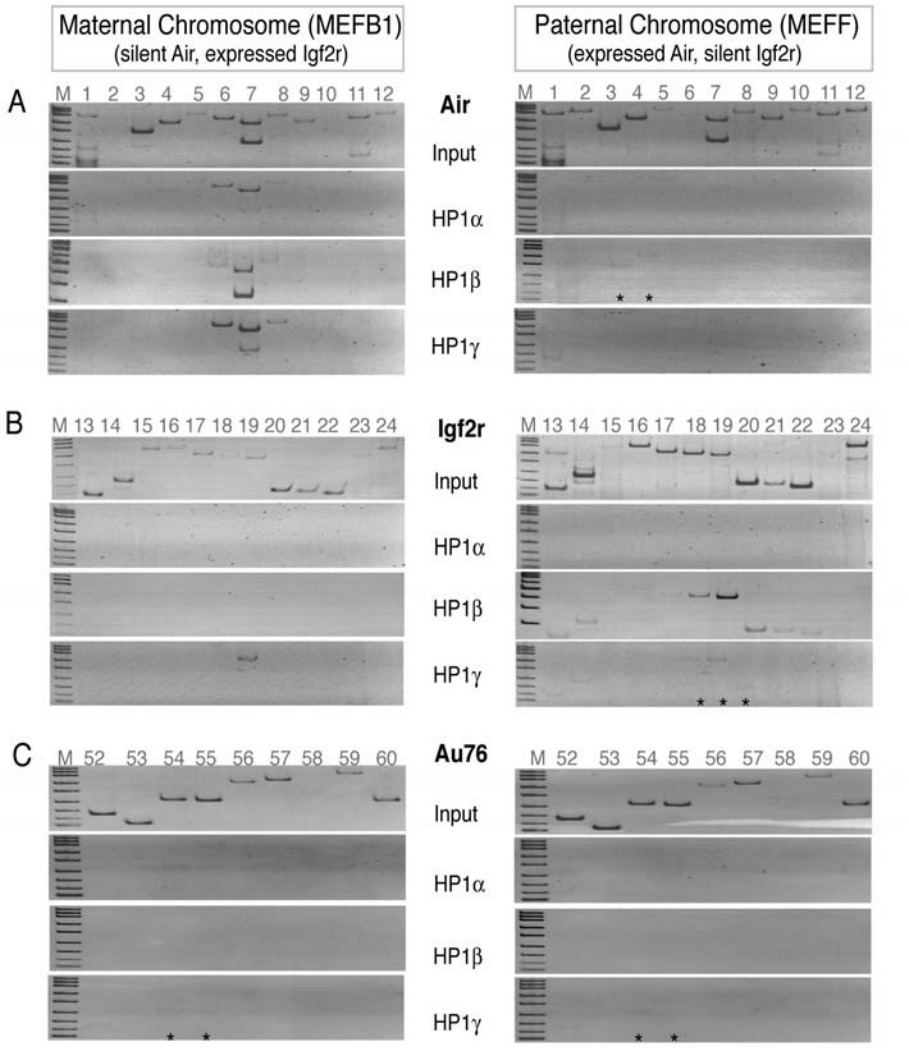

Figure S8. HP1 Isoforms On The Silent *Air* And *Igf2r* Promoters And The *Au76*-psg

ChIP-PCR analysis of the **(A)** *Air* promoter, **(B)** *Igf2r* promoter and **(C)** *Au76*-psg in MEFB1 cells containing only the maternal *Igf2r* cluster (left panels) and MEFF cells containing only the paternal *Igf2r* cluster (right panels), using antibodies for HP1α, HP1β and HP1γ. *faint reproducible signals.

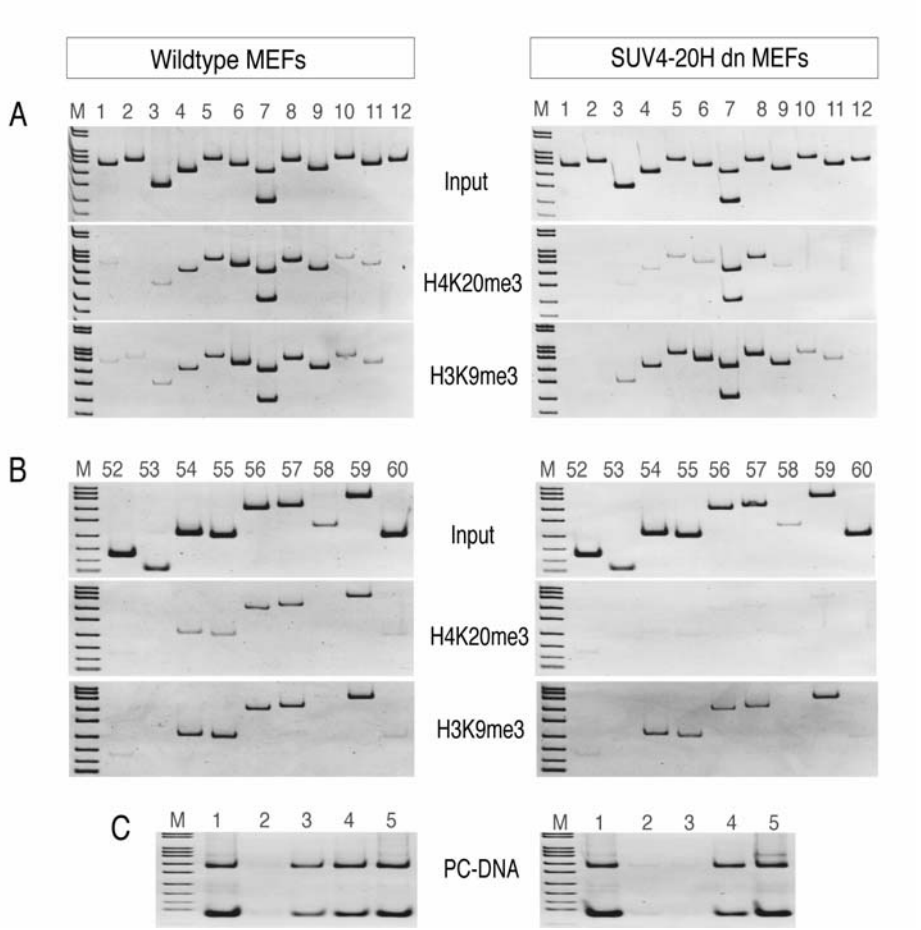

Figure S9. H3K9me3 Stability In Cells Lacking SUV4-20H Histone Tri-methylases

ChIP-PCR analysis of **(A)** *Air* promoter, **(B)** *Au76*-psg and **(C)** pericentric DNA in wildtype and mutant MEFs lacking SUV4-20H1,H2 (SUV4-20H dn) (Schotta et al., 2004) using antibodies for H3K9me3 and H4K20me3. Note that H4K20me3 is mostly lost from the *Au76*-psg in SUV4-20H null cells while it is reduced but still present at the silent *Air* promoter. In both cases the reduction in H4K20me3 does not change the H3K9me3 signal. Quantitative PCR showed that *Air* and *Igf2r* expression levels were similar in wildtype and mutant cells (data not shown). Lane numbers in **(A)**, **(B**) indicate primer pairs. Lane numbers in **(C)**: 1;Input, 2;Mock, 3;H4K20me3, 4;H3K9me3, 5;HP1β show that pericentric DNA loses H4K20me3 in SUV4-20H null cells as reported (Schotta et al., 2004).

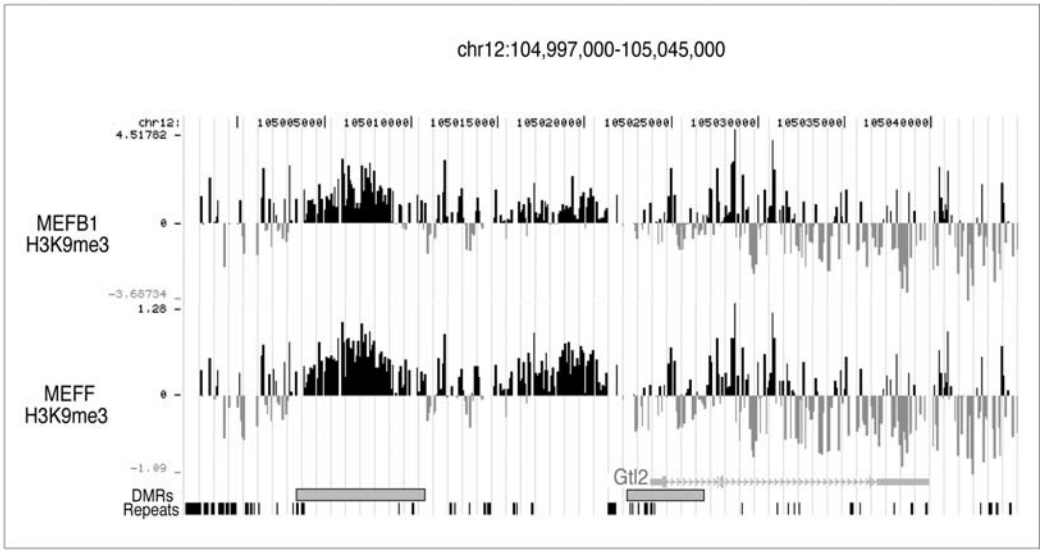

Figure S10. The Imprinted *Gtl2* ncRNA Is Marked Upstream By Focal Heterochromatin Peaks

ChIP-Chip showing the H3K9me3 profile over a 48kb region of mouse chromosome 12 (104,997,000-105,045,000) containing the imprinted *Gtl2* ncRNA indicated underneath. Preparation of the tiling array and hybridization were undertaken by Agilent Technologies, Inc. Two approximately 5kb long heterochromatin peaks can be seen, one is on the upstream DMR (known as the IG-DMR (Lin et al., 2003)), the other lies between this and the *Gtl2* transcription start. MEFB1 and MEFF cells are diploid for mouse chromosome 12 and provide biological replicates for this experiment. DMRs; regions showing parental-specific DNA methylation, Repeats; total repeats identified by RepeatMasker (www.repeatmasker.org).

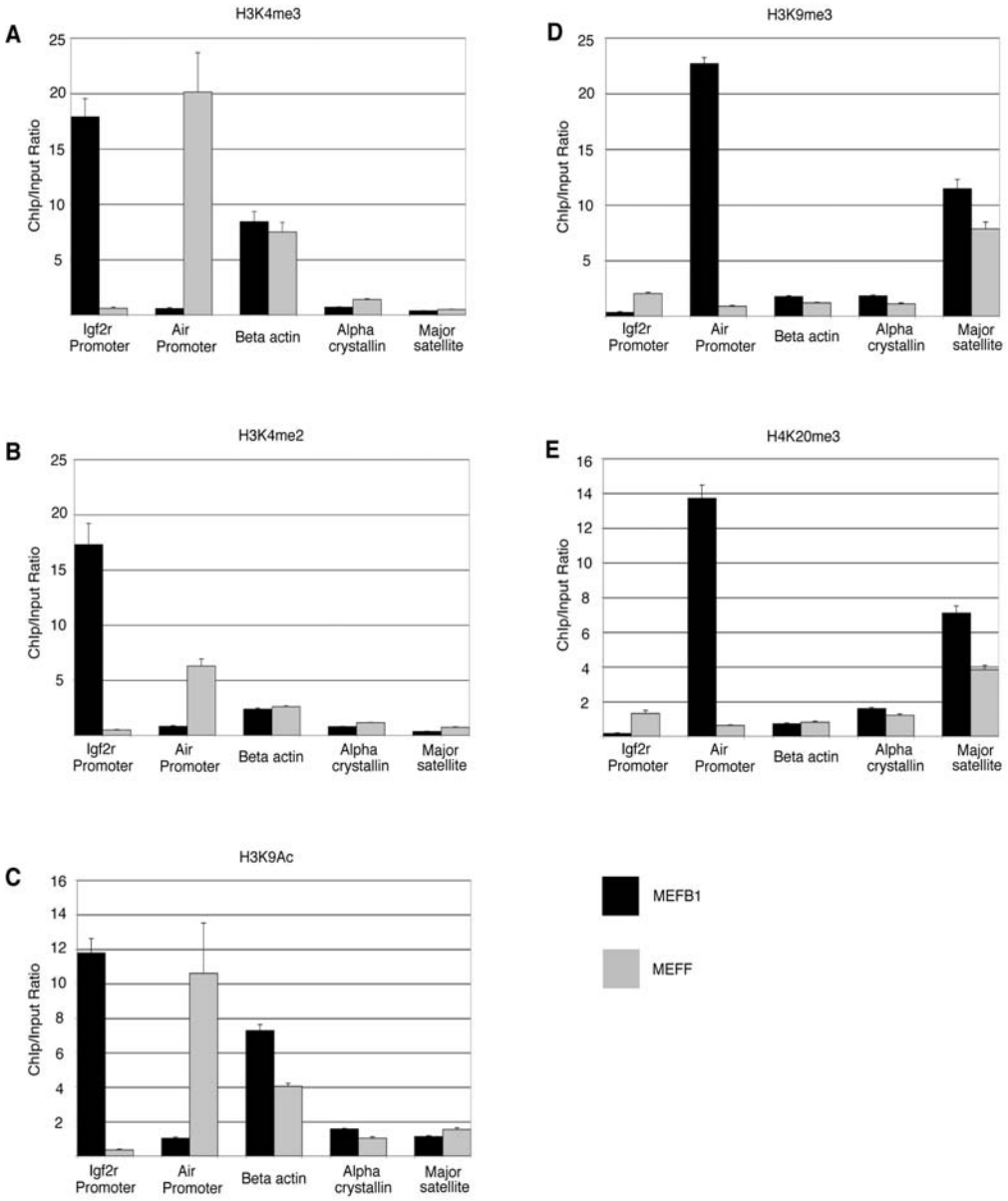

Figure S11. ChIP-Chip Control DNA Sequences

Controls for active **(A)** H3K4me3, **(B)** H3K4me2 **(C)** H3K9Ac and repressive **(D)** H3K9me3, **(E)** H4K20me3, histone modifications from the custom PCR genome tiling array. Each graph shows controls from one biological replica using data obtained from ChIP-Chip. The columns and error bars show, respectively, the average ChIP/Input ratio and standard deviation of the eight replicate spots on the array. This data shows that the antibodies used can distinguish active genes (beta actin) from silent genes (alpha crystallin) and heterochromatin (major satellites). This data also show that the MEFB1 and MEFF cells used here to identify

parental-specific modifications, are similarly modified on beta actin, alpha crystallin and the major satellites. Data for the *Igf2r*/*Air* promoters is shown for comparison. Columns represent the average ChIP/Input ratio of eight spots. Black columns; MEFB1, Grey columns; MEFF.

Table S1. Quantitative Real-Time Assays For ChIP-PCR Validation

Q-PCR assay	*Air*-132 TM assay	*Air*-128 TM assay	*Air*-UPSTREAM SG assay	*Air*-START SG assay	*Air*-126 TM assay	*Air*-125 TM assay
Position relative to primer pairs in Fig.2,3 Accession Nr:AJ249895	132542-132627 Primer-pair 1	128561-128642 Primer-pair 4	127006-127079 Between primer-pairs 5 & 6	126177-126243 Primer-pair 6a*	126212-126278 Primer-pair 6b*	125224-125297 Primer-pair 7
Primer sequence	**F:** GAACTGCCAGTGACAAGTGGTAAT **R**: GCACTGTTGCAGAAGTTTATAACCAA	**F**: GTTTGTTTCTGTGGATGTGAGGTT **R**: CAGAAGCTGGCCTAGACGTTTT	**F:** ACAGAGGTCACCAGGCTATTAACC **R:** AAGGGAGAGTAGATAACCTGTTTCCA	**F:** TTACCTCAGTTCCCCAGGATGT **R:** CTCTAGCGGGCAGCAAGAAG	**F**: GGCGGTGCTGTGCTTCTT **R**: TGCCGAGGCTTCAACATTATATC	**F**: CTGAGCTTTCCCTTCCCTTTC **R**: CGGAGCAATTCCGGTTGT
Taqman probe (TM assay)	TCAGGTTTCTCATGCAGGGTTACAGCAAC	CATTTGCACAGAGGTACCACGAGATTTAATTGTT	No probe (SG Assay)	No probe (SG Assay)	CTGCCCGCTAGAGCAAGGAGGGAT	ACCGCAACTCAGCACAACCAAGGATC
H3K4me3 1) ChIP/Input MEFB1 2) ChIP/Input MEFF 3) Enrichment Expressed^{F} / Silent^{B1}	1) 0.01531 2) 0.08209 3) **5.35891**	1) 0.02686 2) 0.02277 3) **0.84747**	1) 0.00630 2) 0.07139 3) **11.32509**	1) 0.01502 2) 12.27114 3) **816.83850**	1) 0.02083 2) 4.87977 3) **234.18800**	ND
H3K4me2 1) ChIP/Input MEFB1 2) ChIP/Input MEFF 3) Enrichment Expressed^{F} / Silent^{B1}	ND	1) 0.47628 2) 1.42550 3) **2.99300**	1) 0.13593 2) 7.40590 3) **54.48000**	1) 0.02285 2) 2.49985 3) **109.35000**	1) 0.05975 2) 3.56607 3) **59.67900**	1) 0.04049 2) 13.39350 3) **330.78200**
H3K9me3 1) ChIP/Input MEFB1 2) ChIP/Input MEFF 3) Enrichment Silent^{B1} / Expressed^{F}	ND	ND	1) 0.11458 2) 0.00968 3) **11.82759**	1) 0.25952 2) 0.01483 3) **17.49014**	ND	ND

Taqman probes and primers were designed by PrimerExpress and qPCR performed with the ABI PRISM 7000 using primers (900nM) and Taqman probe (200nM) under the following cycling conditions: 2 min 50^0C, 10 min 95^0C, 40 cycles of 15 s 95^0C and 1 min 60^0C. Quantification of Input and ChIP DNA amounts: DNA was extracted from MNase digested chromatin, equal amounts of MEFB1 and MEFF genomic DNAs were mixed and standard curves prepared for each real-time primer set. Relative quantification of Input and ChIP at different regions around *Air* promoter and statistics were performed as described in the manufacturer's protocol (Applied Biosystems). ChIP/Input ratio and the fold enrichment were calculated (expressed/silent or silent/expressed, B1=MEFB1, F=MEFF). *Note that the *Air*-Start SG assay overlaps the position of the *Air*-126 Taqman assay, yet produced 2–3 fold higher signals. The explanation for this difference is not clear since the SYBR green melting curves for this assay identified only one peak. TM Assay; Taqman Probe QPCR, SB Assay; SYBR GREEN QPCR.

Table S2. Distribution Of Histone Modifications On DNaseI Hypersensitivity Sites

DHS site	DHS present in MEFs	Single copy or Repetitive DHS	ECR non-ECR	Promoter expression in MEFs	Active histone modifications			Repressive histone modifications
					H3K4me3	H3K4me2	H3K9Ac	
I***	+	S/R	nd	*Slc22a1*-silent	- (P) - (M)	- (P) - (M)	- (P) - (M)	Tested: H3K9me1,2,3 H4K20me1,2,3 H3K27me1,2,3 general absence of repressive histone modifications with the exception of H3K27me1 and H4K20me1
II	-	S	-		- (P) - (M)	- (P) - (M)	- (P) - (M)	
III	+	R	-		- (P) - (M)	- (P) - (M)	- (P) - (M)	
IV	+	S/R	-		- (P) - (M)	- (P) - (M)	- (P) - (M)	
V	+	S	(+)		- (P) - (M)	- (P) - (M)	- (P) - (M)	
VI	+	S	+		- (P) - (M)	+ (P) + (M)	+ (P) + (M)	
VII	+	S	+		+ (P) - (M)	+ (P) + (M)	+ (P) + (M)	
VIII	+	R	(+)		- (P) - (M)	+ (P) - (M)	+ (P) - (M)	
IX**	+ (P)	S	-	*Air* Pat-exp	+ (P) - (M)	+ (P) - (M)	+ (P) - (M)	
X	+	S/R	-		- (P) - (M)	+ (P) + (M)	- (P) + (M)	
XI	+	R	-					
XII*	+ (M)	S	+	*Igf2r* Mat-exp	- (P) + (M)	- (P) + (M)	- (P) + (M)	
XIII	+	S/R	(+)		- (P) - (M)	+ (P) + (M)	- (P) - (M)	
XIV	+	R	-					
XV	+	S/R	-		- (P) - (M)	+ (P) - (M)	- (P) + (M)	
XVI	+	S	+		- (P) - (M)	+ (P) - (M)	- (P) + (M)	
XVII	-	S/R	-		- (P) - (M)	- (P) - (M)	- (P) - (M)	
XVIII	+	S/R	-		- (P) - (M)	- (P) - (M)	- (P) - (M)	
XIX	+	R	(+)		- (P) - (M)	- (P) - (M)	- (P) - (M)	
XX	+	S/R	(+)		- (P) - (M)	- (P) - (M)	- (P) - (M)	
XXI	-	S/R	-		- (P) - (M)	- (P) - (M)	- (P) - (M)	

***_Slc22a1_ promoter not expressed in MEF cells, ** _Air_ promoter paternally expressed, *_Igf2r_ promoter maternally expressed. Note that only DHS IX and XII that map to the transcription start respectively of _Air_ and _Igf2r_, show parental-specificity in MEF cells while the remaining DHS are present on both parental chromosomes. DHS sites not present in MEF cells were identified in mouse organs or ES cells (Pauler et al., 2005). + or -; positive or negative signal in 2 ChIP-Chip biological replicates (Fig.3, Sup.Fig.4). Signals on DHS VII-XII were also verified by PCR (Sup.Tab.1). M; maternal chromosome, P; paternal chromosome, ECR; evolutionary conserved sequence. Histone modifications linked to X+XI (spanning 13 kb) and to XIV-XV (spanning 5.2 kb) appeared between these two pairs of neighboring DHS. Bold type; DHS showing specific histone modifications. ECRs, (+) indicates an ECR within 2kb of the DHS.

Table S3. Characteristics of Heterochromatin peaks in the *Igf2r* imprinted gene cluster

	***Air* promoter HC peak**	***Igf2r* promoter HC peak**	***Au76*-psg HC peak**
H3K9me3	Maternal +++	Paternal ++	Paternal +++
HP1 α, β, γ	Maternal ++ (α, β, γ)	Paternal + (β), +/– (γ)	-
H4K20me3	Maternal +++	Paternal ++	Paternal +++
ESET/SETDB1	Maternal ++	Paternal +	Not detected
SUV39H null cells	Increased H3K9me3, HP1β and H4K20me3 signals	Increased H3K9me3, HP1β and H4K20me3 signals	No or minimal increased H3K9me3 and H4K20me3 signals
SUV4-20H null cells	Unchanged H3K9me3	not done	Unchanged H3K9me3
AirT allele	Present	Absent	Absent
Type of element	CpG island promoter 1.4kb	CpG island promoter 1.1kb	Pseudogene 2.3kb Host gene: chr.15 Rangap1
Expression in MEF cells	Paternally expressed	Maternally expressed	Not expressed
DNA methylation	Maternal (high density) (98 CpGs in 1.4kb)	Paternal (high density) (116 CpGs in 1.1kb kb)	Bi-parental (low density) (31 CpGs in 2.3 kb)
Silencing mechanism	DNA methylation	Air ncRNA expression	Promoter-less pseudogene
Transcription overlap	Overlapped by *Igf2r* mRNA	Overlapped by *Air* ncRNA	Overlapped by *Air* ncRNA

Table S4. Antibodies Used For Chromatin Immunoprecipitation

	Antibody	Source	Catalogue number	Lot numbers
1	H3K4me2	Upstate	07-030	24727
2	H3K4me3	Upstate	07-473	24503
3	H3K9Ac	Upstate	06-942	23997
4	H3Ac (K9+K14 Ac)	Upstate	06-599	25233
5	H4Ac (5,8,12,16 Ac)	Upstate	06-866	26393
6	H3K9me1	T. Jenuwein*	4858	6th bleed
7	H3K9me2	T. Jenuwein*	4677	6th bleed
8	H3K9me3	T. Jenuwein*	4861	6th bleed
9	H4K20me1	T. Jenuwein*	0077	3rd bleed
10	H4K20me2	T. Jenuwein*	0080	5th bleed
11	H4K20me3	T. Jenuwein*	0083	5th bleed
12	H3K27me1	T. Jenuwein*	8835	5th bleed
13	H3K27me2	T. Jenuwein*	8841	5th bleed
14	H3K27me3	T. Jenuwein*	6523	5th bleed
15	HP1α (clone 15.19s2)	Upstate	05-689	24257
16	HP1	Abcam	ab10478	57611
17	HP1γ (clone 42s2)	Upstate	05-690	24258
18	ESET	Upstate	07-378	24234, 31525.
19	**Anti-chicken IgG	Sigma	C2288	084K4752
20	***G9a /Eu-HMTase 2 (clone A8620A)	Perseus Proteomics	PP-A8620A-00	A1
21	G9a	Abcam	ab40542	248273
22	****SUV39H1	Abcam	ab12405	241399

* specificity described in Perez-Burgos et al., 2004, Methods Enzymol. 376, 234-254
**Mock antibody control included in all ChIP experiments and no fragments were amplified from this material
***Tested for IP in Tachibana et al., 2005, Genes Dev.19:815
****This antibody failed to ChIP pericentric chromatin (data not shown)

Table S5. Primer Pairs Used For ChIP-PCR

Primer Name	Forward Sequence	Reverse Sequence
Air Promoter		
1	AGTGGGTGGTAGGAATAGGG	GCCTCTGAGCAGTTTCCTG
2	GGGTGAAGGAAGTGTCTCAC	CCCACTCTCCAACTTAAAGC
3	CATGAAGGACTGACAGAACTAACC	TTACACCTGATCCTACTGTCCTG
4	AGTCAGGGTGTGGATTCTGC	GCAGAGAACGAATCATCCAAG
5	GGCTTGCCTAGTGCTTATCTG	TGCTCTGCAACATGACCTC
6	TCACCCTAGCGCTGAATCTC	TCCAGGACTGTAAGGCCATC
7*	CCCTGATCACAGAACCCTTC	AGGGTGAAAAGCTGCACAAG
Primer pair 7 spans direct repeats in the middle of the Air CpG island and consistently produced two bands. The sequence of both fragments showed they arose from the correct DNA fragment (data not shown)		
8	CAGAGGAGGTGGGAACAATG	GCATTTCGAGTGGATTCAGG
9	GGCCTGAGAGCCATTTAAACTTAG	CCTGAGATCCAAATAGGGTGTG
10	ACAGATGCTCCGCTTCCAG	GTCTGAGGGCTGCCAAATAAC
11	AAGCACCACAGTTCATGCTG	AGCCTCAGCCACTCAAGATG
12	AAGAGCTGAAGGCAGCCATA	TGAAGTCTAAAGTCACGGCACA
Igf2r Promoter		
13	TTTGACTTTTGACCCCTTGG	TCAGCAACAGAGAGGGAAGG
14	CTTCTGAGCTTGGCACAC	AGGCAGCTCTGCTTCTG
15	GAAGAGGCCAGGTAATGCAG	GTACACAAACGGAGGTGATCC
16	AGGCAGCTCCTTATGTGGTC	ACCGTGCTGTTCCAGTAACC
17	CACTTGTGCATGGTCATCTC	AACAGCTGCCTAGCTTGC
18	AGCCATTTGTCAGTGGTTGAAG	TCAGCCCCGTCTCATTCTG
19	TCCACCAGTCACCTAACTTGC	CCGGGTCACATGAGCATC
20	GTGCTCCATTTCCAAAGC	GGAGTTCATGTTTGTCCTCTC
21	AAAGCACAACATACCCATACAG	CGGGTGCCATAGATTAACCA
22	CTCTAGGGCAGTGTCTCACG	TGCTATGACTTATATTGGCATCC
23	TCATTTATCTCTGAAGGCAAGG	GAATTACTTAGTGACCTCCCTTCC
24	AGAAGGGGCAACAGATGAAC	GAAGGCGATTGAAAGTCACAG
Slc22a2 Promoter		
25	AAGGAAAGTGTGGGATGGAG	TGGCAGTGTGAAGGAAGC
26	TCCGAGGTCTTCTGCTCCTG	TCCAGCTTGGCCTTATATGCAG
27	TGAGGAACCCGATAGAAGATTG	GTCAAGGAGAGCATCTTGTGC
28	AAGACTCTGAACCCACTCAATG	TCTACCAGCTCAGTCCAACG
29	TGGGTGAAGAGGAAGGACAC	AGCGGCATTACAAACAATCC
30	TGCCTTCAGCAGCATTTG	GGTTCCAGTCCACCTCATACC
31	TCTCCTTCCTCAGCCAGTG	AGGGTTCTCCCTGACTACCTC
32	TGAGGTAGTCAGGGAGAACC	TGTCATGGTCTAACCTATAGCC
33	CCACACAGCATCACCTGAAC	CTGTCGGTGTGTTTGGATTG
34	CAATCCAAACACACCGACAG	ACCAGAGCTAGGAGGCACAG
35	GCTCTGTATAGATGGCCGTTAG	TGTTAGGGAGGCTTGAGAAAG
36	GCCCACAAGAACACACTGAC	CTCAAGGAGGAGGGAAACTG
Slc22a3 Promoter		
37	ATGCAGGATTTCTCCTCACC	GGTCTTCCCACCTAGACTGC
38	ACTCTGATTGGCTGCAACTG	CGATGCCCAGAACCTATCTC
39	TGACCATATCCCTTAAGACAGC	GGTCTAGTGAGCCCAAATCC
40	GTTCTCTCAACCTGTGATTCC	TTAAAGGGCAGATGTGGTG
41	CATGGTCAGCCTTAGTTCTGTG	CCGACTCAATGTCTGCACTC
42	ACCATCGTCAGCCAGGTAAG	TGGACACTGGAAAGAAGTTGC
43	GGTGCAGGTGCAGGTATGAA	TCCTCAGTGCCTGGTCGAA
44	ACATCACAAGCAGCCCAAAC	TCAGTGGCATTCCAGTACCC
45	CACATCATGGTCCACAGAGC	TGCTCCATTTCACAGTCAGG
46	CCTGGGCTGAACTCCAAAG	TGACCTCCCTGCTCAGCTAC
47	AAAGCAATGCAGATGAGCAG	AGGAAATGGTTCCCTGTTTG
48	ACTCCAGGAACTGGTATTGCTC	CCTCACAGGTGTTCCACAATC
Au76-psg		
49	TCTATAGGTCTGTTGTGGTGGAAC	AAGCCTGGTCAGCACTCTTC
50	AGCTCCAGGTGGGCACATTC	CCTCAAACAGTCTGTCCCTGTCAG
51	TGCAGGTCACATGAAGAAGG	ACGAGCAGGCTACCGAAAG
52	TCCCATTCCCTAACAGACCA	TAGAAGTTGCGGCTTGCATT
53	CTGATGCAGGGAGAGGTTATAC	ACGTCCTTTGTTAGGAGCAG
54	GAACCTGGTGTCAGGAGAGC	GGCAACAAGCAATACAAGTCC
55	CATCCAGCTTGGCCTTAGAG	CGGAAACCACCTTAGAAATGC
56	GAAGCAGGCATACCTTAGTATGAG	GACACAGGCAAACAACCCTAC
57	CAGCTGTATGCAAGCTCCAC	TGTTCAGGCACACACATACAAG
58	CAGGAAGTACATGCCCACAG	AAGGTGGCATCTGTGTTTCC
59	AGTCGGAGCTGAAGTGATGC	CCACCTCACCCTTCTCAATG
60	GCTGAATGAGCCTCTGAAAG	GGCCCAGATGCTGTTTTAC
61	GCAGCCATAAAGAGAAAAGG	GTATGCCTGGCCTCAACAG
Pericentric region – major satellites		
62	GACGACTTGAAAAATGACGAAATC	CATATTCCAGGTC52TCAGTGTGC
N.B., major satellites were analyzed with 12 PCR cycles (of 30 sec.@94^{0}C, 40 sec.@94^{0}C, 30 sec.@73^{0}C) (Peters et al., Mol.Cell 12, 1577-89)		

Supplemental References

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