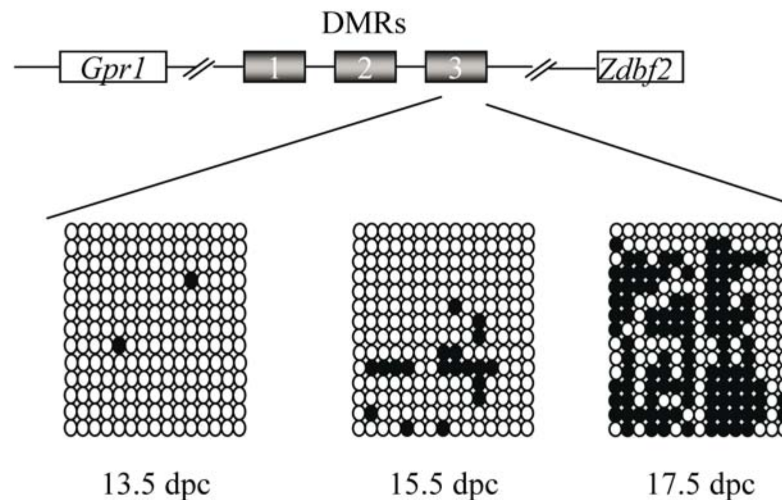
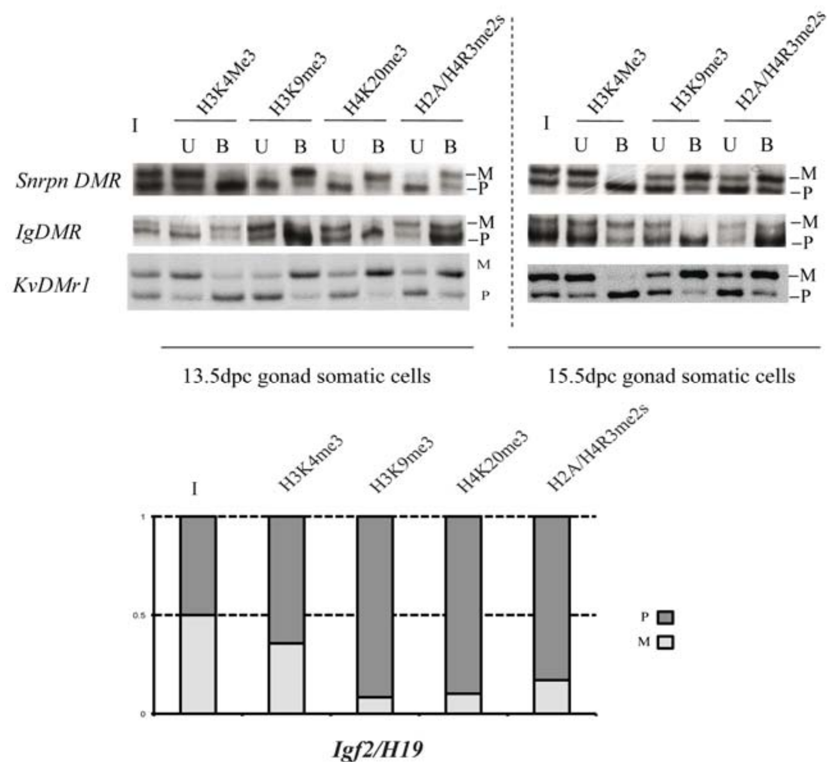
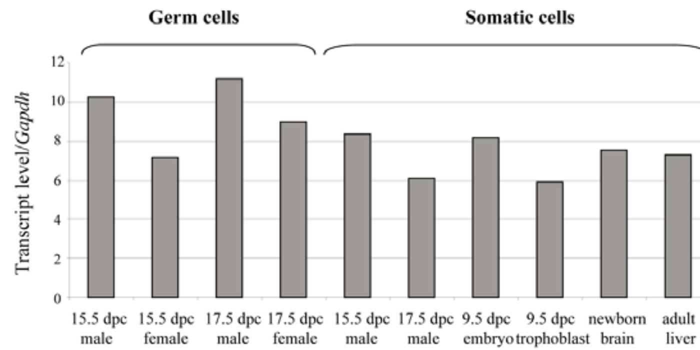


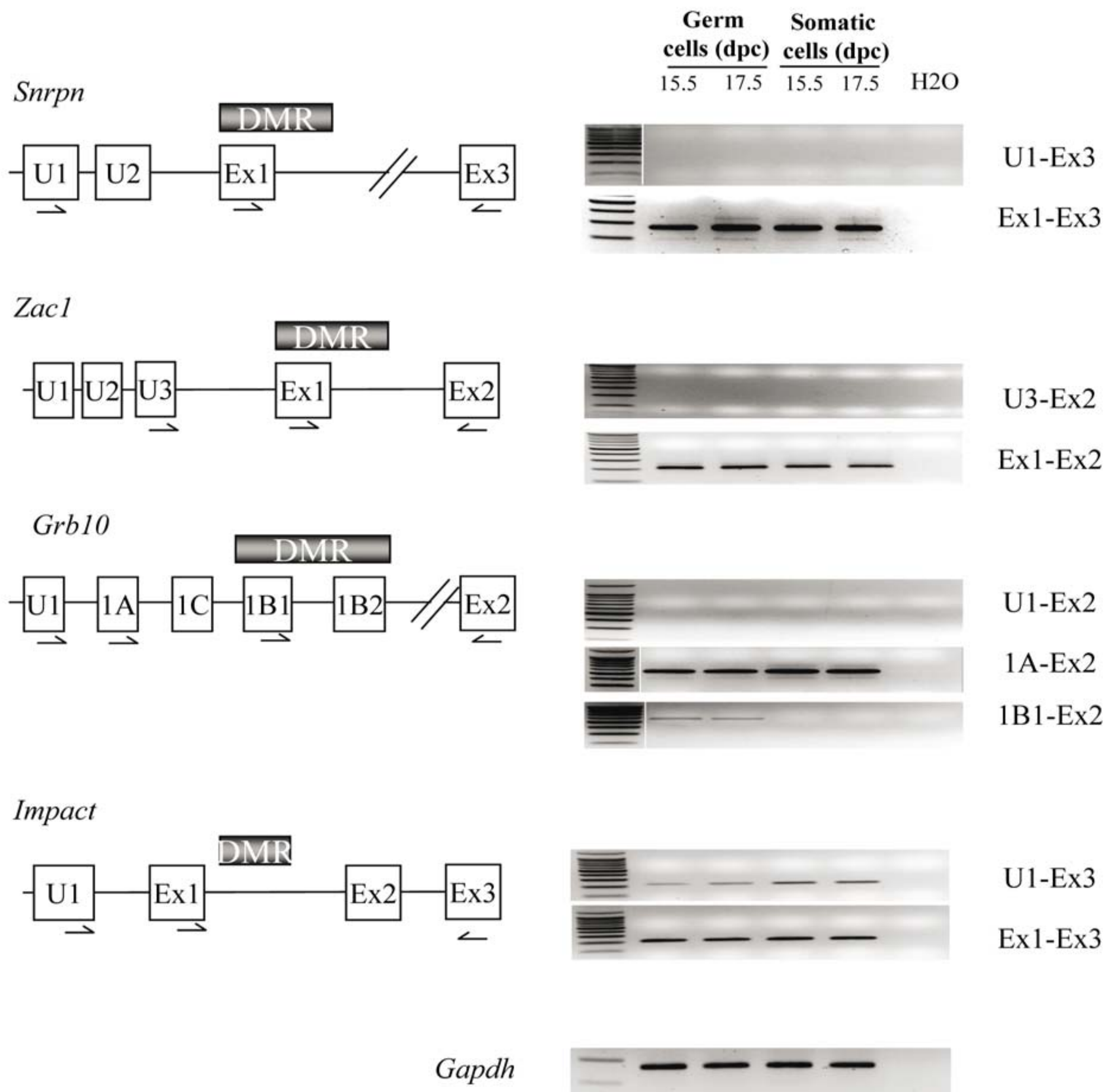
Supplementary Figure S1. DNA methylation acquisition at *Zdbf2* DMR3 in male germ cells. The *Gpr1-Zdbf2* imprinted locus has a tripartite DMR structure (*i.e*., DMR1, DMR2 and DMR3; Hiura et al, 2010 *Nucl Acids Res* **38**, 4929). Bisulphite sequencing data are shown for DMR3, on 13.5 dpc, 15.5 dpc and 17.5 dpc male germ cells. Each horizontal row of circles represents the CpGs on an individual chromosome. Methylated CpGs are depicted as solid circles, unmethylated CpGs as open circles. Maternal and paternal alleles were not distinguished in this assay.



Supplementary Figure S2. Allelic precipitation of histone modifications at ICRs in FACS-sorted somatic control cells. SSCP-based allelic discrimination following cChIP on (C57Bl/6J x JF1)F1 somatic cells (GFP-negative FACS-sorted cells) isolated from 13.5 dpc (left panel) and 15.5 dpc (right panel) male gonads. Representative results on the *Snrpn* DMR, Ig-DMR and KvDMR1 are shown. The lower panel shows results on the *H19* DMR (in 13.5 dpc somatic cells), obtained with an allelic real-time PCR-based discrimination assay. I, input chromatin; B, antibody-bound chromatin; U, unbound fraction; M, maternal allele; P, paternal allele. Note that the allelic assessment of H3K4me3 at the *H19* DMR is not informative since precipitation was at background levels only.

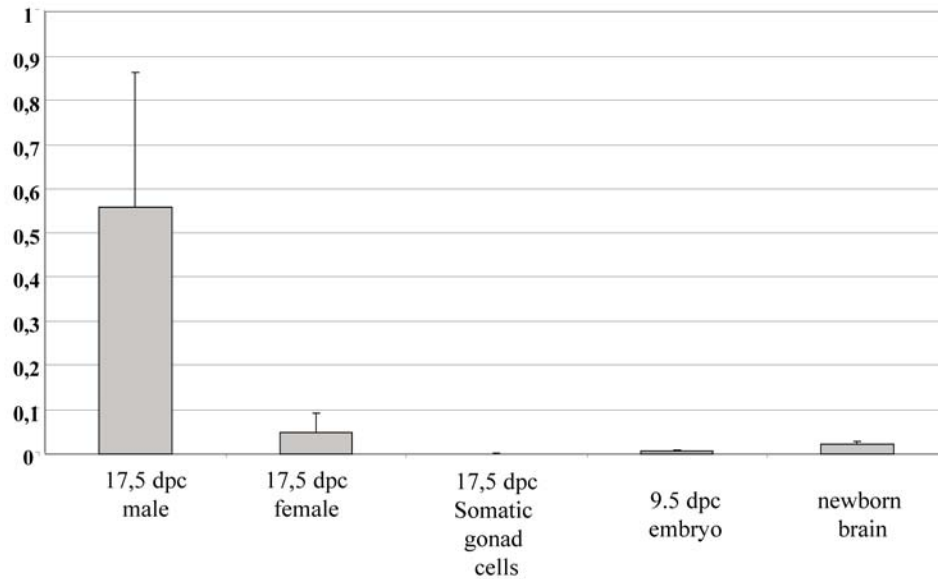


Supplementary Figure S3. *Rpl30* is highly expressed in male and female germ cells. Expression of *Rpl30* in germ cells (left panel), and somatic cells and tissues (right panel), analysed by quantitative RT-PCR. Graphs show the level of expression after normalization to the expression of *Gapdh*. The PCR primers used are described in Table SI.

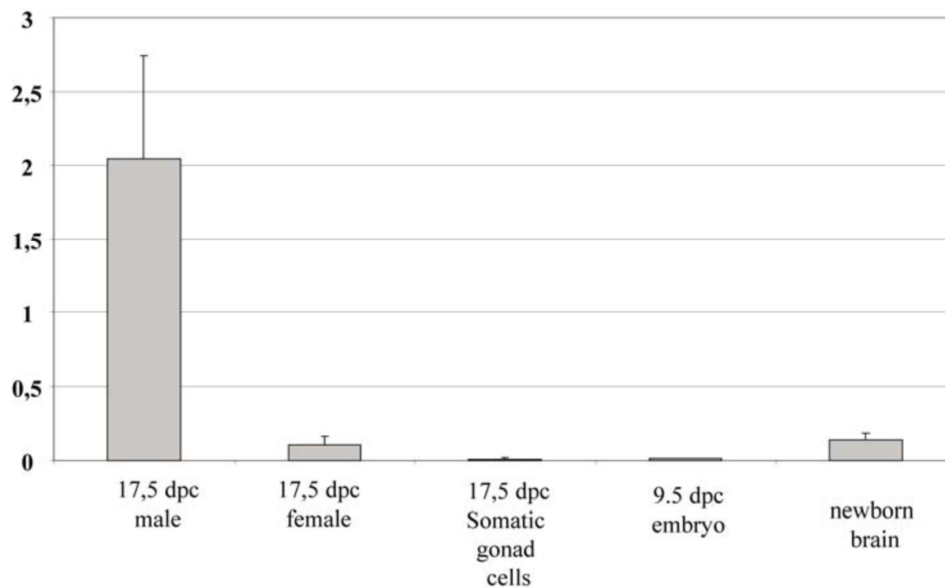


Supplementary Figure S4. Maternally-methylated ICRs are active promoters in foetal male germ cells. Promoter-specific RT-PCR analysis of male germ cells, and somatic control cells isolated from 15.5 and 17.5 dpc fetuses. Four loci known to be controlled by maternal ICRs were analysed by RT-PCR amplification (with 35 PCR cycles), followed by agarose gel electrophoresis, ethidium bromide staining of the gel, and gel imaging (Syngene scanning system). The four regions (DMRs) are all methylated on the maternal allele in somatic cells (Kobayashi H. *et al*, 2006; *Cytogenet Genome Res* 113, 130-137). At the *Snrpn* locus, no transcription is detected from upstream exon 1 (u1), but transcripts are readily amplified (from both parental alleles) between *Snrpn* exons 1 and 3 (Ex1-Ex3) indicating promoter activity of the DMR. Also at the *Zac1* locus, exon1-exon2 amplification was readily achieved (from both alleles), but not from upstream exons that are transcribed in somatic cells. In the embryo, the promoter 1B1 at the *Grb10* ICR is active in developing brain only (Sanz *et al,* 2008; *EMBO J* 27, 2523-2532). Transcription is detected in male germ cells as well. Additionally, the upstream 1A promoter is also active in male germ cells. At the *Impact* imprinted locus, transcription is detected at exon 1 within the DMR, and at the upstream exon U1.

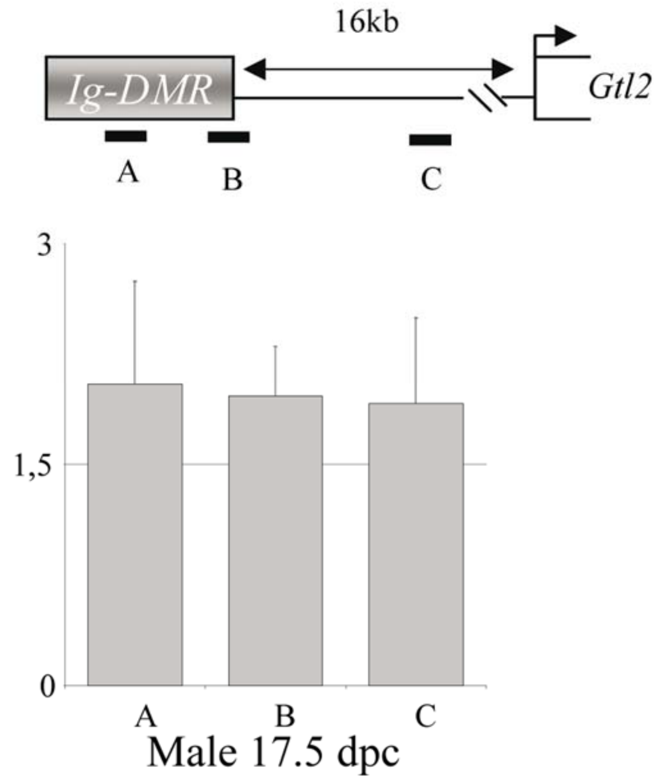
A) H19 DMR



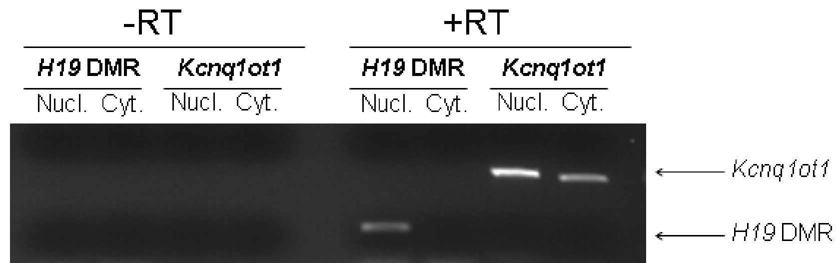
B) Ig-DMR



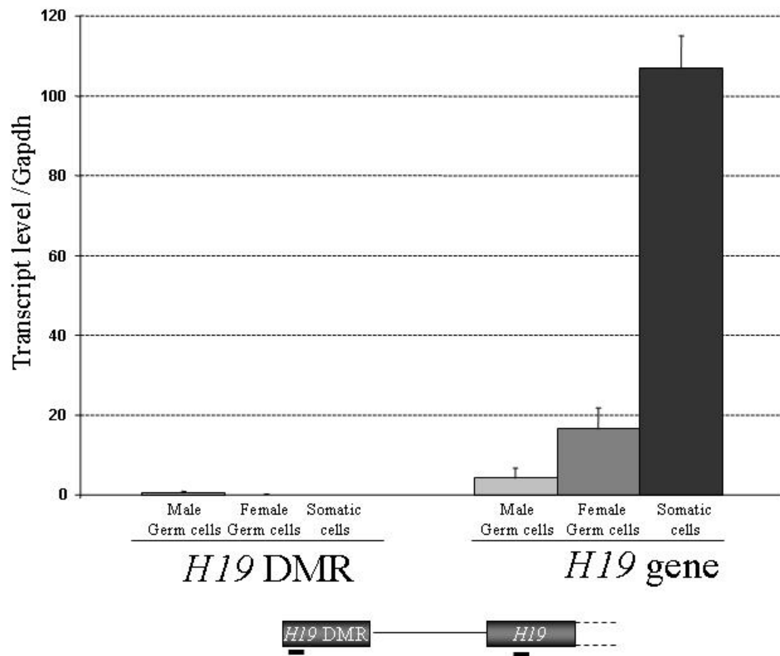
Supplementary Figure S5. Transcription across the *H19* DMR and Ig-DMR is predominant in male germ cells. Expression of *H19* DMR and *Ig-DMR* was analysed by quantitative RT-PCR in germ cells, whole 9.5 dpc embryos and newborn brain, as indicated. Graphs show the level of expression after normalization to that of *Gapdh*.



Supplementary Figure S6. Characterization of Ig-DMR non-coding RNAs in male germ cells. Quantitative RT-PCR analysis of regions located between the Ig-DMR and the *Gtl2* gene in male 17.5 dpc germ cells. Three regions were analysed (A, B, C; black bars). Measured levels of transcripts for each of the analysed regions are normalized to that of *Gapdh*.



Supplementary Figure S7. Predominantly nuclear localisation of the *H19* DMR transcript. Cytoplasmic (Cyt.) and nuclear (Nucl.) RNA fractions were extracted from male primordial germ cells, according to Jablonski and Caputi (2009) *J Virol* 83:981-992. Briefly, ~25,000 FACS-sorted male germ cells were resuspended in (10 mM NaCl, 2 mMMgCl, 10 mM Tris-HCl, pH 7.8, 5 mM dithiothreitol, 0.5% Igepal CA-630) and incubated on ice for 5 min. Nuclear pellet and cytoplasmic supernatant were collected by centrifugation at 8000 rpm for 5 min at 4°C. Total RNA was then isolated from the collected fractions using Trizol reagent (Invitrogen), and were treated with DNAseI (Roche). 100 ng of nuclear and cytoplasmic RNA was reverse transcribed using random hexamers and Superscript III (Invitrogen). 1µl of cDNA was used for PCR amplification using primers at the 5' side of the *H19* DMR (*H19* DMR) and at the Kcnq1ot1 nc-RNA, which is predominantly nuclear in localisation (Pandey *et al.* 2008; *Mol Cell* 32, 232-246). As a negative control, cDNAs were made without addition of reverse transcriptase (RT). PCR amplification products were migrated through an agarose gel, and then stained with ethidium bromide.



Supplementary Figure S8. Characterization of the level of expression of the *H19* DMR transcript and the *H19* gene transcript in male PGCs, female PGCs, and somatic control cells, at 15.5 dpc. Levels were determined by real-time PCR amplification, and are plotted relative to the expression of *Gapdh.*

SUPPLEMENTARY TABLE S1

Primers used for Bisulphite analysis

Region	Primer Sequences	Nucleotide position	Sequence ID	Allelic SNP (129Sv/B6 to JF1)	Reference
KvDMR1	F: GATGGTGTATTTTGGTTTAGTTA R: ATAACTAACTAATATAACCTCACC	1968-2181	AF119385		This study
Snrpn DMR	F : ATTGGTGAGTTAATTTTTTGGA R : ACAAAACTCCTACATCCTAAAA	68174- 68571	AF332579	G to A pos. 68314 A to G pos. 68479 in	Arnaud et al., 2006
H19 DMR	F :GTTGTGTAGATTTGGTTATAGT R :TTCTCCTAATCTCTAATCTAA	3303-3690	AF049091	T to Ø pos. 3476 C to A pos. 3534	Arnaud et al., 2006
Ig-DMR	F: TGTTGTGGTTTGTTATGGGTA R: TACAACCCTTCCCTCACTCCAA	81248-81574	AJ320506	G to A pos. 81275 C to A pos. 81421	This study
Zdbf2 DMR3	F: GAAAGGTTGTGGATATGTTA R: ATCAAAACATAACAAACCAAAATATCCAA	26492-26891	AL669947		Hiura et al., 2009

Primers used for SSCP analysis

Region	Primer Sequences	Nucleotide position	Sequence ID	Allelic SNP (129Sv/B6 to JF1)	Reference
KvDMR1	F: CGGATCACTTGAGCACTAC R: GCCAAGTGGATCGCGCCAAG	2229-2467	AF119385	T to C pos. 2392	Henckel et al., 2009
Snrpn DMR	F: AGGTTGTGACTGGGATCCTG R: TGCAGCGGCAACAGAACTTCT	68269- 68500	AF332579	G to A pos. 68314 A to G pos. 68479	Henckel et al., 2009
Ig-DMR	F:CTAAGGTACATCATGCTAGTGT R: AGCATAGCATAGCGGCTGCA	81248-81574	AJ320506	T to C pos.81242 G to A pos. 81275 C to A pos. 81421	This study

Primers used for quantitative allele-specific PCR analysis

Region	Primer Sequences	Nucleotide position	Sequence ID	Allelic SNP (129Sv/B6 to JF1)	Reference
H19 DMR	F-129sv/B6 specific: CTTACGGAATGGTCCCC**TT**C F-JF1 specific : CTTACGGAATTGGTCCCC**T**C R: TAGGTTACCTGGGACATTGC	3458-3643	AF049091	T to Ø pos. 3476	Henckel et al., 2009

Primers used for quantitative PCR analysis after ChIp

Region	Primer Sequences	Nucleotide position	Sequence ID	Reference
KvDMR1	F: CGGATCACTTGAGCACTAC R: GCCAAGTGGATCGCGCCAAG	2229-2467	AF119385	Henckel et al., 2009
Snrpn DMR	F: AGGTTGTGACTGGGATCCTG R: TGCAGCGGCAACAGAACTTCT	68269- 68500	AF332579.1	Henckel et al., 2009
H19 DMR	F: GATCAGGCATTTGTGCACTTAC R: TAGGTTACCTGGGACATTGC	3441-3643	AF049091	Henckel et al., 2009
Ig-DMR	F: GAAGACAAAGAGCAAGCCTGT R: TAGACAACGGTGAGCCAGGAT	82391-82587	AJ320506	Delaval et al., 2007
Rpl30 promoter	F: AGCACGCCCAAGACAACGTCA R: TGTGCGGTAGTTGGTTGCTA	25388787-25388559	NT039618.7	This study
IAPs	F: TATGCCGAGGGTGGTTCTCTA R: TGCGGCAAAACTTTATTGCTT	non applicable	non applicable	Delaval et al., 2007

Primers used for quantitative RT-PCR analysis

Region	Primer sequences	Nucleotide position	Sequence ID	Reference
KvDMR1	Upstream of *Kcnq1-ot1* transcription start site (product K1): F: GTCTCACACTTATTCAGAGTTA R: GACAGATGGTGAATAATGACT	1600-1817	AF119385	This study
	Downstream Kcnq1-ot1 transcription start site (product K2): F: CGGATCACTTGAGCACTAC R: GCCAAGTGGATCGCGCCAAG	2229-2467		Henckel et al., 2009
Snrpn	DMR, Upstream of Snrpn transcription start site (product S1): F: AT TTCCGCAGTA GGAATGCTCA R: TCCATTGCTTGCAAATCACT	67809-68053	AF332579.1	This study
	Exon 5 (product S2) : F: TGTGGGTAAGAGTAGCAAGA R: TGATCTTCCTGAACTCATCACA	515-664	NM_013670	This study
H19 DMR	Upstream part of DMR (product A): F: TCGGACTCCCAAATCAACAAGGTC R: CCTGGCCTCATGAAGCCCATC	1791-1976	AF049091	This study
	Downstream part of DMR (product B): F: GATCAGGCATTTGTGCACTTAC R: TAGGTTACCTGGGACATTGC	3441-3643		Henckel et al., 2009
	Between DMR and the *H19* gene (product C): F: GAAGTATAGGGGAGTATAGCA R: TGATCCTTTTCAGAGATCGGT	4159-4356		This study

	<u>Between DMR and the *H19* gene (product C’):</u> F: ACTAGGCTGAGGATCTGCCAAG R: CTCTGTCAACCAATCAGTACAT	4581-5452		This study
	<u>Between DMR and the *H19* gene (product D):</u> F: TCATACTCCGTGGGATAGTATG R: ATATAGCCCCGTAGCCTGCT	5501-5247		This study
	<u>5’ part of *H19* transcript (product E):</u> F: AGCAAAGGCATCGCAAAGGCT R: TTGCTAACTATCCTGCCTTTC	5673-6010		This study
Ig-DMR	<u>Upstream part of DMR:</u> F:CTAAGGTACATCATGCTAGTGT R: AGCATAGCATAGCGGCTGCA	81248-81574	AJ320506	This study
	<u>Downstream part of DMR:</u> F: GAAGACAAAGAGCAAGCCTGT R: TAGACAACGGTGAGCCAGGAT	82391-82587		Delaval et al., 2007
Gapdh transcript	F: ACAGTCCATGCCATCACTGCC R: GCCTGCTTCACCACCTTCTTG	574-798	NM_008084	This study
Rpl30 transcript	F:AGTCTCTGGAGTCGATCAACT R: AGCTGGACAGTTGTTGGCAAG	25388011-25388148	NT039618.7	This study

SUPPLEMENTARY TABLE S2

Details on antisera used in this study

Antibody	Company	N° cat	Lot
H3K4me3	Diagenode	PAb-030-050	001
H4K20me3	Millipore	07-463	31392
H3K9me3	Millipore	07-442	33453 JBC1361819
H2A/H4R3me2s	Abcam	ab5823	97454 520317
Anti-chicken IgY	Sigma	C-2288	083K4856