Supporting Information

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SI Methods

DNA Isolation. DNA was isolated from the interphase/organic phase that remained after RNA isolation with TRIzol (Invitrogen). To facilitate the transfer of DNA into the aqueous phase, 0.5 mL of Back Extraction Buffer (4 M guanidine thiocyanate, 50 mM sodium citrate, and 1 M Tris) was added and samples were inverted several times. Samples were then spun at 12,000 relative centrifugal force (rcf) for 30 min at room temperature, the aqueous phase was transferred to a new tube, and DNA was precipitated by using isopropyl alcohol. After centrifugation at 12,000 rcf for 15 min at 4 °C, the supernatant was completely removed and the DNA pellet was washed in 75% ethanol and spun again at 12,000 rcf for 10 min at 4 °C. The supernatant was removed, and the samples were allowed to air dry for 10 min before being dissolved in 0.1× TE buffer.

Bisulfite Pyrosequencing. To further assess DNA methylation profiles of the *H19* and *Snrpn* DMRs, we used next-generation sequencing technology and performed custom pyrosequencing assays on the last six CpG sites for the *H19* DMR and seven CpG sites for the *Snrpn* DMR of the region previously analyzed by allele-specific bisulfite sequencing. In brief, 50 ng of bisulfite-treated DNA was amplified with the PyroMark PCR kit (Qiagen) by using the manufacturer's protocol in a 25-μL reaction. For the *H19* DMR, the primer sequences were as follows: forward primer 5′-GGGTAGGATATATGTATTTTTTAGGTTG-3′, biotinylated reverse primer 5′-CTCATAAAACCCATAACTATAAAATCAT-3′. For the *Snrpn* DMR, the primer sequences were as follows: forward primer 5′-GGTAGTTGTTTTTTGGTAGGATAT-3′, biotinylated reverse primer 5′-ACTAAAATCCACAAACCCAACTAACCT-3′. The reaction conditions were as follows: an initial denaturation step at 95 °C for 15 min, followed by 45 cycles at 95 °C for 30 s, 53 °C for 30 s, and 72 °C for 15 s, then a final extension and annealing step at 72 °C for 10 min. The biotinylated primer was used to facilitate isolation of single-stranded DNA templates by using streptavidin-Sepharose beads (Amersham) according to manufacturer's instructions. Ten microliters of the biotinlyated PCR product was used for pyrosequencing with either the *H19* DMR sequencing primer, 5′-TGTAAAGATTAGGGTTGT-3′, or the *Snrpn* DMR sequencing primer, 5′-GTGTAGTTATTGTTTGGGA-3′. Pyrosequencing was performed with the PyroMark Q96MD (Qiagen) system and the PyroMark Gold 96 reagents kit (Qiagen). The degree of methylation at each CpG site was quantified with Pyro Q-CpG software (Biotage) and determined by dividing the incorporation of cytosine by incorporation of cytosine plus thymine (%C = C/C+T) at each site. The percent methylation was calculated by averaging the degree of methylation at each of the CpG sites analyzed.

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Fig. S1. Allele-specific DNA methylation of the *H19*, *Snrpn,* and *Peg3* DMRs in somatic tissues of naturally and ICSI-derived female mice. (*A*) Methylation profile of the *H19* DMR in muscle tissue from naturally conceived and ICSI mice. (*B*) Methylation profile of the *Snrpn* DMR in brain tissue from naturally conceived and ICSI mice using standard bisulfite sequencing. (*C*) Methylation profile of the *Peg3* DMR in brain tissue from naturally conceived and ICSI mice using standard bisulfite sequencing. Other details are as described in Fig. 1.

Fig. S2. Bisulfite pyrosequencing analysis of the *H19* and *Snrpn* DMRs in different somatic tissues. (*A*) Global DNA methylation profile of the *H19* DMR in liver and brain tissues from naturally and ICSI-derived male mice. (*B*) Global DNA methylation profile of the *Snrpn* DMR in liver and muscle tissue from naturally conceived and ICSI-derived male mice. **P* < 0.005 compared with data from naturally conceived mice. Other details are as described in Fig. 1.

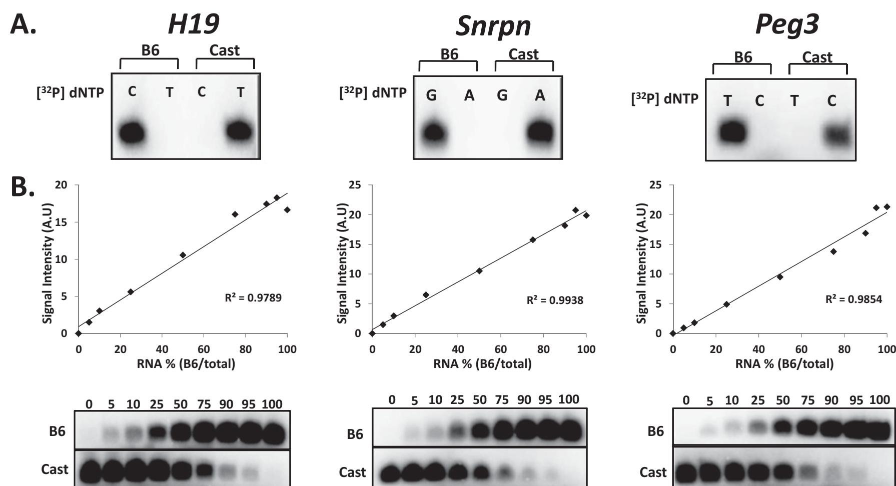


Fig. S3. Single nucleotide primer extension (SNuPE) assay for allele-specific expression of imprinted genes. RNA from C57BL/6 and B6(CAST7) mice was isolated from muscle for *H19* and brain for *Snrpn* and *Peg3*. RT-PCR was performed, and the amplified PCR products were gel-purified. The amplified DNA was then subjected to a primer extension reaction by using a primer that lies just 5′ to a SNP with [^{32}P] dNTPs that were specific to B6 and Cast alleles. (*A*) The SNuPE assay for *H19*, *Snrpn,* and *Peg3* showed allele-specific incorporation, whereas nonspecific incorporation was not detected. (*B*) Quantitative assessment of SNuPE assays. Different ratios of B6 or Cast RNA from muscle (*H19*) and brain (*Snrpn and Peg3*) were used to determine sensitivity and quantitation for each SNuPE assay. Each band was quantified by using data analysis software, and the signal intensity for 0% RNA was set to 0 and subtracted from all other values for background correction. The values obtained demonstrate that all three assays are relatively linear (R^2 > 0.97 for all three genes). B6, C57BL/6 mouse; Cast, B6 (CAST7) mouse.

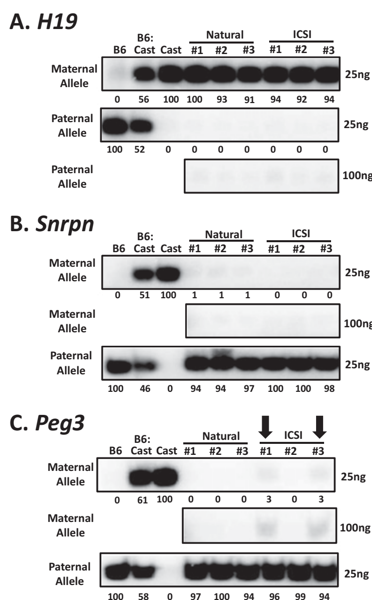


Fig. S4. Allele-specific expression of imprinted genes in the somatic tissues of naturally conceived and ICSI-derived female mice. (*A*) The SNuPE assay for *H19* was performed on RNA isolated from muscle tissue of natural and ICSI male mice. (*B*) The SNuPE assay for *Snrpn* was performed on RNA isolated from brain tissue of natural and ICSI female mice. (*C*) The SNuPE assay for *Peg3* was performed on RNA isolated from brain tissue of natural and ICSI female mice. The numbers below each lane indicate the percentage of expression compared with the internal controls. The amount of purified PCR product loaded onto the gel is indicated to the right of each gel. B6, 100% C57BL/6 RNA; Cast, 100% B6(CAST7) RNA; B6:Cast, 50% B6 RNA and 50% Cast RNA. Black arrows point to aberrant allele-specific gene expression patterns that constitute epimutations in ICSI-derived mice. Other details are as described in Fig. 2.

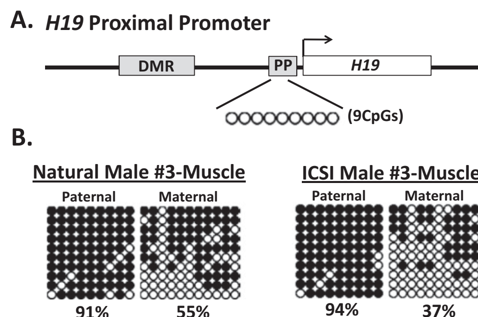
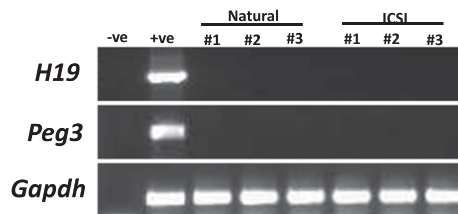


Fig. S5. Allele-specific DNA methylation of the *H19* proximal promoter in muscle tissue of naturally conceived and ICSI-derived male mice. (*A*) Schematic representation of the region analyzed by allele-specific bisulfite sequencing. The *H19* proximal promoter (PP) is a somatic DMR that is located in the promoter of the *H19* gene. The paternal (B6) allele has 9 CpG sites and the maternal (Cast) allele has 11 CpG sites. (*B*) Methylation profile of the *H19* PP in muscle tissue from naturally conceived male mouse no. 3 and ICSI-derived male mouse no. 3. Other details are as described in Fig. 1.



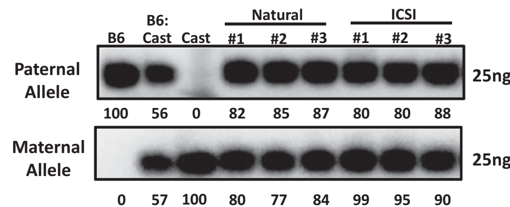


Fig. S6. Expression analysis of imprinted genes in advanced germ cells of naturally conceived and ICSI-derived male mice. (*A*) RT-PCR for *H19* and *Gapdh* was performed on RNA isolated from purified germ cells of natural and ICSI male mice. (*B*) The SNuPE assay for *Snrpn* was performed on RNA isolated from purified germ cells of natural and ICSI male mice. The numbers below each lane indicate the percentage of expression compared with the internal controls. The amount of purified PCR product loaded onto the gel is indicated to the right of each gel. -ve, no template; +ve, RNA from natural muscle tissue for *H19* and RNA from natural brain tissue for *Peg3* and *Gapdh*.

Table S1. Regions analyzed, primer sequences and PCR conditions for bisulfite assay

Gene	Accession	Position	Primer Type	Primer Sequence (5′-3′)	Annealing temperature	Reference
H19 DMR	U19619	1304-1726	OF	GAGTATTTAGGAGGTATAAGAATT	50°C	Trembaly et al., 1997
			OR	ATCAAAAACTAACATAAACCCTCT		
			IF	GTAAGGAGATTATGTTTATTTTTGG	55°C	
			IR	CCTCATTAATCCCATAACTAT		
H19 PP	U19619	4396-4780	OF	GTTGAGGATTTGTTAAGGTGTTATTGT	50°C	
			OR	AATAATAACTAATTTAAACACTCCTCACC		
			IF	GAGTGGTTATGATTGGTTAGTTTTTGAG	52°C	
			IR	AATAATAACTAATTTAAACACTCCTCACC		
Snrpn DMR	AF081460	2151-2570	OF	TATGTAATATGATATAGTTTAGAAATTAG	55°C	Lucifero et al., 2002
			OR	AATAAACCCAAATCTAAAATATTTTAATC		
			IF	AATTTGTGTGATGTTTGTAATTATTTGG	60°C	
			IR	ATAAAATACACTTTCACTACTAAAATCC		
Peg3 DMR	AF105262	2781-3384	OF	TTTTGATAAGGAGGTGTTT	50°C	Lucifero et al., 2004
			OR	ACTCTAATATCCACTATAATAA		
			IF	AGTGTGGGTGTATTAGATT	50°C	
			IR	TAACAAAACTTCTACATCATC		

OF, outside forward; OR, outside reverse; IF, inside forward; IR, inside reverse; DMR, differentially methylated region; PP proximal promoter

Table S2. Regions analyzed, primer sequences and PCR conditions for RT-PCR and SNuPE assay

Gene	Accession	Position	Primer Type	Primer Sequence (5′-3′)	Annealing temperature	Reference
H19	AK003142	147-788	F	CCTCAAGATGAAAGAAATGGT	55°C	Thorvaldsen et al. 2002; This study
			R	AACACTTTATGATGGAACTGC		
			S	TTGCCCTCAGACGGAGATGGA		
Snrpn	AK144386	921-1278	F	TGCTGCTGTTGCTGCTACTG	55°C	Szabo and Mann 1995
			R	GCAGTAAGAGGGGTCAAAAGC		
			S	CAATTTCACAAGAAGCATT		
Peg3	AF038939	3359-3596	F	AAGGCTCTGGTTGACAGTCGTG	55°C	Mann et al., 2004; This study
			R	TTCTCCTTGGTCTCACGGGC		
			S	CCCCACATTTTGGACATTCATA		

F, forward primer; R, reverse primer; S, SNuPE priemer