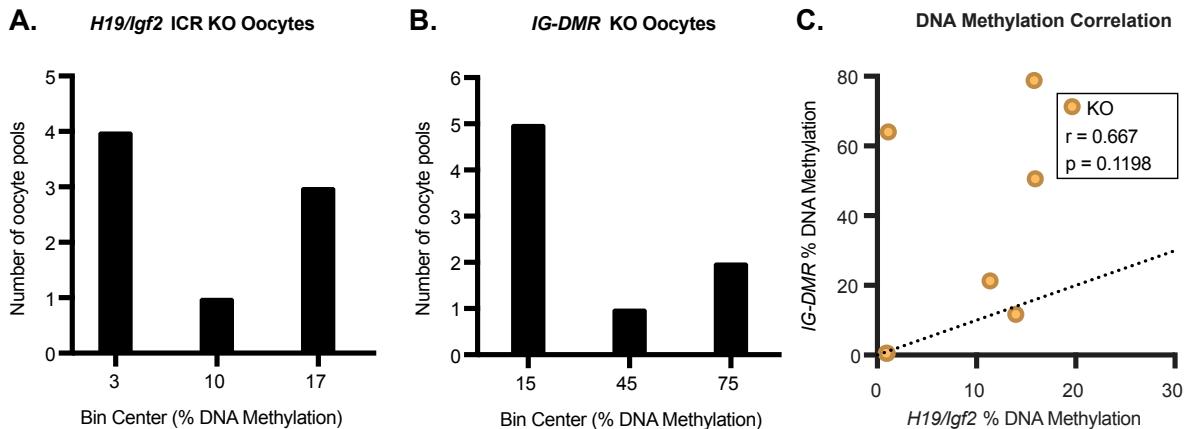
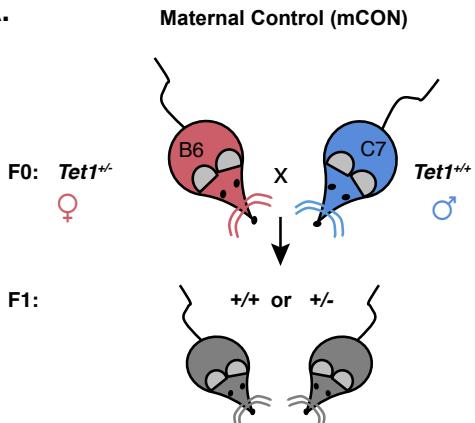
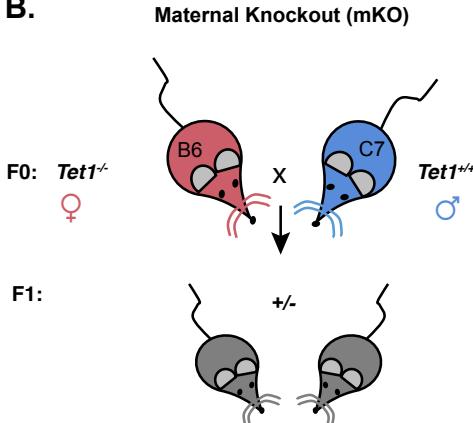
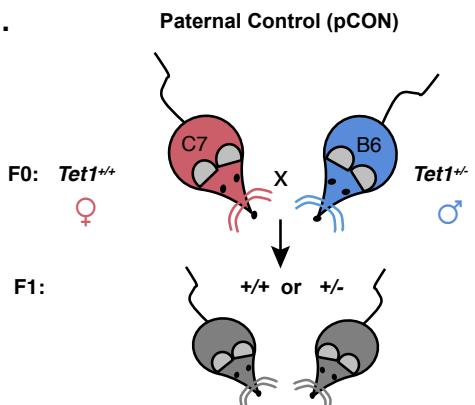
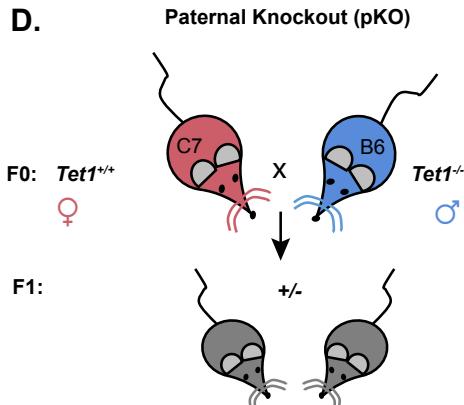
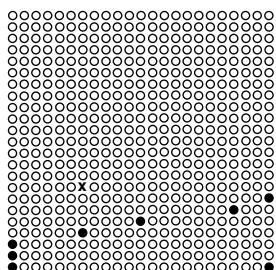
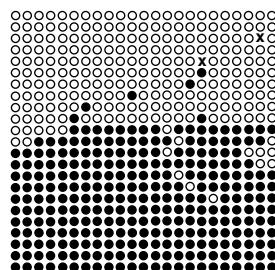
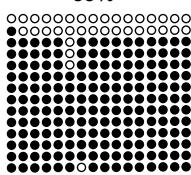
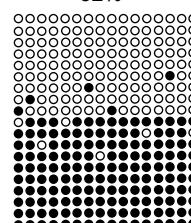


FIG. S1

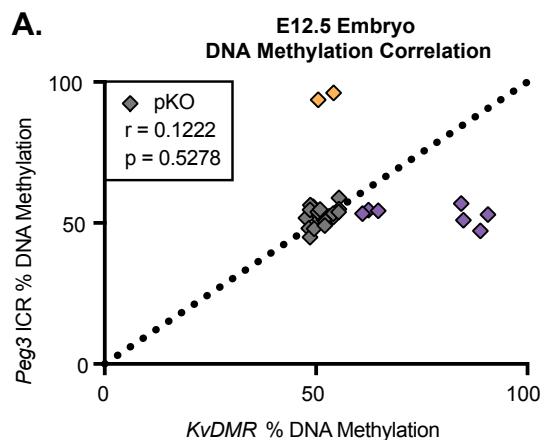
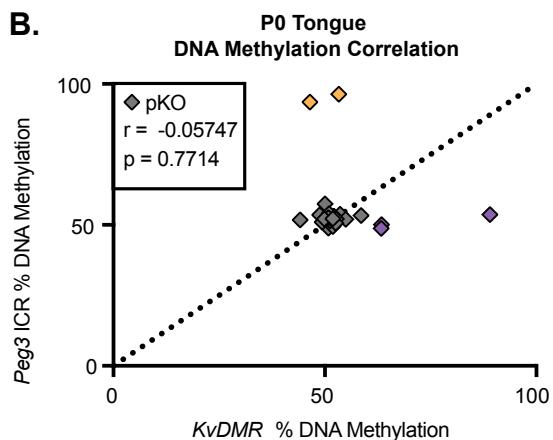
Supplemental Figure 1. Further characterization of oocyte DNA methylation. The DNA methylation data from Figure 1 (A) KO oocytes at the *H19/Igf2* ICR and (B) KO oocytes at the *IG-DMR* are plotted as histograms. (C) Correlation plot between KO oocyte pools at the *H19/Igf2* ICR versus the *IG-DMR*. r = Spearman correlation coefficient. Dashed line represents a hypothetical perfect correlation.

FIG. S2**A.****B.****C.****D.**

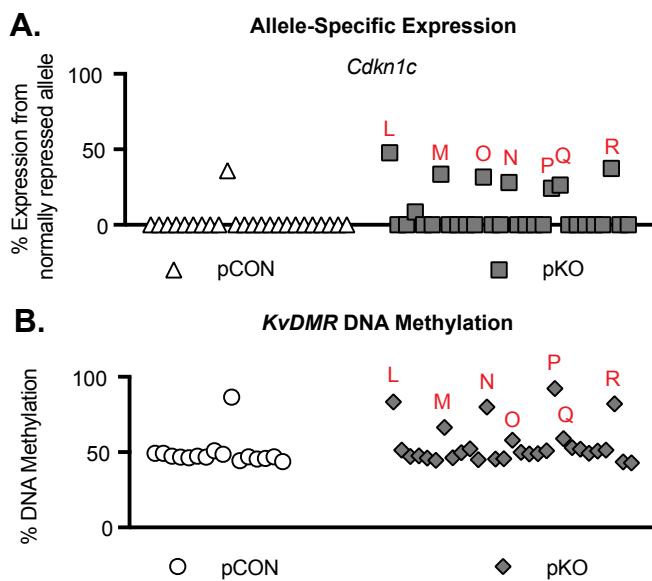
Supplemental Figure 2. F1 hybrid breeding schemes for analyzing allele-specific expression. (A) $Tet1^{-/-}$ female mice on the B6 background were mated to $Tet1^{+/-}$ mice on the C7 background to generate maternal control offspring (mCON) for analysis. (B) $Tet1^{-/-}$ female mice on the B6 background were mated to $Tet1^{+/-}$ mice on the C7 background to generate maternal KO offspring (mKO) for analysis. (C) $Tet1^{+/-}$ females on the C7 background were crossed to $Tet1^{-/-}$ males on the B6 background to generate paternal control offspring (pCON) for analysis. (D) $Tet1^{+/-}$ females on the C7 background were crossed to $Tet1^{-/-}$ males on the B6 background to generate paternal KO offspring (pKO) for analysis.

FIG. S3**A.*****Tet1* Sperm
Peg3 ICR**Het
2%KO
29%**B.*****Tet1* Brain (Somatic Control)
Peg3 ICR**Het
58%***H19/Igf2* ICR**Het
83%KO
98%***H19/Igf2* ICR**Het
52%

Supplemental Figure 3. Bisulfite sequencing validation of *Tet1* sperm DNA methylation. (A) Bisulfite sequencing of the *Peg3* ICR for both control heterozygous sperm and KO sperm. Bisulfite plots for the *H19/Igf2* ICR is shown as a control. (B) Bisulfite sequencing of a somatic control brain showing the expected methylation pattern at the *Peg3* ICR as a control. Each circle represents a CpG, white = unmethylated, black = methylated. Each row represents one cloned strand of DNA. Each bisulfite plot represents one animal and results from two technical PCR replicates.

FIG. S4**A.****B.**

Supplemental Figure 4. Hypermethylation at the *KvDMR* and *Peg3* ICRs is mutually exclusive in E12.5 pKO embryos and P0 newborns. (A) Correlation plot showing DNA methylation per E12.5 pKO embryo at the *KvDMR* and *Peg3* ICRs. (B) Correlation plot showing DNA methylation per P0 pup (tongue) at the *KvDMR* and *Peg3* ICRs. Gray diamonds show unaffected pKO pups. The orange diamonds highlight pups with *Peg3* hypermethylation. The purple diamonds highlight pups with *KvDMR* hypermethylation. r = Spearman correlation coefficient. Dotted line represents hypothetical perfect correlation.

FIG. S5

Supplemental Figure 5. Abnormal biallelic expression and DNA hypermethylation are consistent in the E12.5 placental tissues. (A) Allele-specific expression of *Cdkn1c* in the E12.5 placenta (corresponding to the embryos from Figure 6 in the main text). *Cdkn1c* (n) pCON = 24 placentas from 3 litters; pKO = 29 placentas from 7 litters. (B) Percent DNA methylation at the *KvDMR*. (n) pCON = 16 placentas from 2 litters; pKO = 29 placentas from 7 litters. Abnormal placentas are indicated with red letters and this lettering is consistent between all graphs in Figures 6B and 6C and supplemental Figures 5A and 5B. All placentas are in the same order from left to right in A and B.

Table S1: % DNA methylation at ICRs in Tet1 oocytes with inclusion data.

Mouse #	Genotype	ICR DNA Methylation (% Average)						<i>H19/Igf2</i> and/or <i>IG-DMR</i> ?	Number of Control ICRCs	TOP 2 avg >90%	Both Criteria Met?
		Paternally Methylated		Maternally Methylated (Control)							
5629A	WT	1.4	0.0	97.2		97.4		yes	2	97.3	yes
5826	WT		0.8	93.7	98.2	75.6		yes	3	95.9	yes
W956	WT	4.1	7.2	93.0	97.9	96.0		yes	3	96.9	yes
W1096	WT	3.1	15.1	84.9	92.6	94.5		yes	3	93.5	yes
W1542	WT	1.0	0.9	97.9		92.7		yes	2	95.3	yes
5442	Het	6.7	0.5	98.7	81.1	95.6		yes	3	97.1	yes
Het442	Het	2.0	0.8	89.0	97.7	81.9	64.9	yes	4	93.4	yes
H1095	Het	6.7	0.6	86.7	76.7	95.7		yes	3	91.2	yes
H1550	Het	0.7	5.5	91.4		94.6		yes	2	93.0	yes
LEP	KO	2.3		95.0		60.8	96.8	yes	3	95.9	yes
5619	KO	1.0	0.5	99.1	85.3	83.5		yes	3	92.2	yes
5828	KO	1.1	64.0	95.0	98.1	94.6		yes	3	96.5	yes
5834B	KO	0.9	0.5	98.0	98.0	94.7		yes	3	98.0	yes
Mut554A	KO	14.0	11.7	89.9	97.5	80.5	67.8	yes	4	97.8	yes
Mut445	KO	11.4	21.3	95.0	97.8	74.4		yes	3	96.4	yes
M1097	KO		0.8	96.1	98.3	91.9		yes	3	97.2	yes
M1027	KO	15.9	50.6	99.3	92.4	100.0		yes	3	99.6	yes
M1541	KO	15.8	78.8	92.1	89.5	100.0		yes	3	96.0	yes

Bold type indicates the top two most highly methylated maternally methylated ICRs

Table S3: Sex ratio information for both maternal and paternal Tet1 offspring.

Maternal E10.5 mCON				Maternal E10.5 mKO			
	Observed	Expected	TOTAL		Observed	Expected	TOTAL
Male	21	17	38	Male	18	18	36
Female	13	17	30	Female	18	18	36
TOTAL	34	34	68	TOTAL	36	36	72
Two-tailed Chi-Square: p = 0.170 Sex ratio is not significantly different				Two-tailed Chi-Square: 1.000 Sex ratio is not significantly different			
				Maternal E10.5 mKO			
					Affected	Unaffected	
				Male	3	16	
				Female	9	8	
				The two-tailed P value = 0.033 The association between rows and columns is significant.			
Maternal P0 mCON				Maternal P0 mKO			
	Observed	Expected	TOTAL		Observed	Expected	TOTAL
Male	8	8	16	Male	14	15	29
Female	8	8	16	Female	16	15	31
TOTAL	16	16	32	TOTAL	30	30	60
Two-tailed Chi-Square: p = 1.000 Sex ratio is not significantly different				Two-tailed Chi-Square: p = 0.715 Sex ratio is not significantly different			
				Maternal P0 mKO			
					Affected	Unaffected	
				Male	0	16	
				Female	3	13	Affected = J, K, and # (see Table S6)
				Two-tailed Fisher's Exact Test: p = 0.226 The association between rows and columns is not significant.			
Paternal E12.5 pCON				Paternal E12.5 pKO			
	Observed	Expected	TOTAL		Observed	Expected	TOTAL
Male	11	11.5	22.5	Male	10	14.5	24.5
Female	12	11.5	23.5	Female	19	14.5	33.5
TOTAL	23	23	46	TOTAL	29	29	58
Two-tailed Chi-Square: p = 0.835 Sex ratio is not significantly different				Two-tailed Chi-Square: p = 0.095 Sex ratio is not significantly different			
				Paternal E12.5 pKO			
					Affected	Unaffected	
				Male	3	7	
				Female	4	15	
				Two-tailed Fisher's Exact Test: p = 0.665 The association between rows and columns is not significant.			
Paternal P0 pCON				Paternal P0 pKO			
	Observed	Expected	TOTAL		Observed	Expected	TOTAL
Male	13	11.5	24.5	Male	17	14	31
Female	10	11.5	21.5	Female	11	14	25
TOTAL	23	23	46	TOTAL	28	28	56
Two-tailed Chi-Square: p = 0.532 Sex ratio is not significantly different				Two-tailed Chi-Square: p = 0.257 Sex ratio is not significantly different			
				Paternal P0 pKO			
					Affected	Unaffected	
				Male	0	17	
				Female	3	8	
				Two-tailed Fisher's Exact Test: p = 0.050 The association between rows and columns is not significant.			

Table S5: % DNA methylation at ICRs in Tet1 Sperm

		ICR DNA Methylation (% Average)					
		Paternally Methylated		Maternally Methylated			
Sample ID	Genotype	<i>H19/Igf2</i>	<i>IG-DMR</i>	<i>KvDMR</i>	<i>Peg3</i>	<i>Peg1</i>	<i>Snrpn</i>
W5062	WT	94.6		8.5	7.3		5.1
W5068	WT	95.7		2.5	1.6		2.3
WT1	WT	96.0	95.0	11.0	8.0	5.0	7.0
WT2	WT	96.0	95.0	12.0	4.0	5.0	3.0
WT3	WT	94.0	92.0	7.0	5.0	8.0	6.0
WT5183	WT	97.6	97.6	3.8	3.3	4.8	3.2
H4852	Het	95.4	96.9	4.0	3.4	5.8	4.2
H5059	Het	93.7	97.4	4.4	2.9	5.4	4.5
H5069	Het	97.7		9.6	5.3		13.8
H5103	Het	95.9	95.4	4.5	3.6	6.4	6.8
H5104	Het	94.0	97.3	4.8	6.0	6.9	4.6
H5105	Het	95.0	96.3	3.8	3.3	5.5	5.9
H5184	Het	94.1	97.0	4.4	6.0	6.1	2.3
Hep3	Het	93.9		6.7	5.0		5.3
Het5787	Het	97.4	96.9	5.3	5.3	8.2	5.0
Het5791	Het	97.1	97.2	4.4	3.4	6.6	3.9
M4848	KO	95.2		29.8	3.0		5.6
M4851	KO	93.7		33.6			18.5
M4859	KO	95.0		30.9	4.6		14.2
M5060	KO	94.8	95.1	33.9	10.6	20.4	5.1
M5064	KO	96.8	95.9	32.7	10.3	21.5	5.9
M5109	KO	91.6		39.0	9.8		28.8
M5311	KO	92.6	96.1	33.8	10.8	18.9	6.1
Mutep3	KO	93.0	96.4	34.3	11.6	23.5	7.0
Mutep4	KO	96.9	95.6	31.3	10.7	21.2	3.1
Mut5339	KO	91.7	88.4	36.5	19.9	26.0	10.1
Mut5781	KO	96.8	96.2	34.4	11.7	19.8	5.4
Mut5318	KO	95.2	89.4	37.9	11.9	21.4	5.5
Mut5333	KO	96.2	95.5	34.2	10.4	18.4	5.2
Mut5058	KO	96.5	95.1	35.8	11.2	21.4	5.2
Mut5728	KO	96.8	97.2	35.5	9.8	17.3	3.1
DKO 1	DKO	94.0	93.0	23.0	6.0	10.0	7.0
DKO 2	DKO	96.0	96.0	30.0	5.0	20.0	8.0
DKO 3	DKO	96.0	93.0	37.0	9.0	22.0	11.0
		<i>H19/Igf2</i>	<i>IG-DMR</i>	<i>KvDMR</i>	<i>Peg3</i>	<i>Peg1</i>	<i>Snrpn</i>
KO vs DKO, Mann-Whitney (p-value)		0.985	0.440	0.360	0.065	0.660	0.203

Table S8: List of primers used for each assay in this study.

Gene/Region	Primer	Primer Sequence	Assay	References
Tet1	13037	TCAGGGAGCTCATGGGAGACTA	Tet1 Genotyping	The Jackson Laboratory
Tet1	13038	TTAAAGCATGGGTGGAGTC	Tet1 Genotyping	(https://www2.jax.org/protocolsdb/f?p=116:5.0::NO:5:P5_MASTER_PROTOCOL_ID:P5_JRS_CODE:25442.017358)
Kdm5c/Kdm5d	Smc1	TGAAGCTTTCGGCTTGAC	Sex Genotyping	(Jay and Ciando, 2013)
Kdm5c/Kdm5d	Smc2	CCACTGCCAAATCTTGG	Sex Genotyping	
H19	H19 F	GCTCTGAAGAGCTGGACTG	qPCR	
H19	H19 R	ACTGCAGGACACATCCAC	qPCR	(Thorvaldsen et al., 2006)
Igf2	Igf2 F	CGCTTCAGTTTGCTGTTG	qPCR	
Igf2	Igf2 R	GCAGCACTTCCACGATG	qPCR	
Meg3/Gtl2	Meg3 F	TTCTGTGTCCTCAGGTT	qPCR	
Meg3/Gtl2	Meg3 R	ATCCGGGTCCTCAGTCCT	qPCR	
Dlk1	Dlk1 F	CGGGAAATTCTGGAAAATAG	qPCR	
Dlk1	Dlk1 R	TGTGCAGGAGCATTCGTA	qPCR	
Rplp0	Arpo F	TCCCCACTTACTGAAAAGTCAG	qPCR	
Rplp0	Arpo R	TCCGACTCTCTTTGCTTC	qPCR	
Rpl13a	Rpl13a F	ATCCCCTCACCCATTGACAA	qPCR	
Rpl13a	Rpl13a R	GCCCCAGGTAAAGCAAACCTT	qPCR	(Bougault et al., 2008)
Nono	Nono F	GCTCGTGAAGAACGGAGAT	qPCR	
Nono	Nono R	TTCTTGACGTCTCATCAAATCC	qPCR	(Plasschaert and Bartolomei, 2015)
H19	HE2 (F)	TGATGGAGGAGCACAGAGGG	Allele-Specific Expression	
H19	HE4 (R)	TTGATTCAACGAGACGGAC	Allele-Specific Expression	(Thorvaldsen et al., 2006)
Igf2	Igf2-18	ATCTGTGACCTCTTGGAGCAGG	Allele-Specific Expression	
Igf2	Igf2-20	GGGTTGTTAGAGCCAATCAA	Allele-Specific Expression	(Fortier et al., 2008)
Cdkn1c	p57-L	GCCAATGGGAAACGGTGG	Allele-Specific Expression	
Cdkn1c	p57-4	TACACCTTGGGACCAGCGTACTCC	Allele-Specific Expression	(Weaver et al., 2010)
Peg3	PG4	ATGCCACTCCCGTCAGCG	Allele-Specific Expression	
Peg3	PG7	GCTCATCTTGTGAACTTTG	Allele-Specific Expression	(Bhatnagar et al., 2014)
Kcnq1ot1	Lit1 F	ATTGGGAACCTGGGTGAGGC	Allele-Specific Expression	
Kcnq1ot1	Lit1 R	GGCACACGGTATGAGAAAATTG	Allele-Specific Expression	(Rivera et al., 2008)
Snrpn	Sn1 (F)	CTCCACAGGAATTAGAGGC	Allele-Specific Expression (Light Cycler)	
Snrpn	Sn3 (R)	GCAGTAAGGGGTCAAAAGC	Allele-Specific Expression (Light Cycler)	
Snrpn	SnMut (Snrpn sensor probe)	GAAGCATTTAGGGGAAGAGAA-fluorescein	Allele-Specific Expression (Light Cycler Probe)	(Szabo and Mann, 1995)
Snrpn	SnAnc (Snrpn anchor probe)	LC-Red640-GGCTGAGATTATCAACTGTATCTTAGGGTC-P	Allele-Specific Expression (Light Cycler Probe)	
H19/Igf2 ICR	H19/Igf2 ICR F	GGGTAGGATATGTTAGTTAGGTG	Pyrosequencing PCR	
H19/Igf2 ICR	H19/Igf2 ICR R-biotinylated	CTCATAAAACCCATAACTATAAAATCAT	Pyrosequencing PCR	
H19/Igf2 ICR	H19/Igf2 ICR Sequencing	TGTAAAGATTAGGGTTG	Pyrosequencing Sequencing Primer	
IG-DMR	IG-DMR F	GTGGTTGTTAGGGTAAGTTT	Pyrosequencing PCR	
IG-DMR	IG-DMR R-biotinylated	CCCTTCCCCTCAGCTAAACAAATTAA	Pyrosequencing PCR	
IG-DMR	IG-DMR sequencing	CTTATGGATTGGGTGTTAAG	Pyrosequencing Sequencing Primer	
Snrpn ICR	Snrpn F	GGTAGTTGTTTGTAGGATAT	Pyrosequencing PCR	
Snrpn ICR	Snrpn R- biotinylated	ACTAAACATCACAAACCCAACTAACCT	Pyrosequencing PCR	
Snrpn ICR	Snrpn Sequencing	GTGTTAGTTATTGTTGGGA	Pyrosequencing Sequencing Primer	
Peg3 ICR	Peg3 F	GGTTTTAAAGGTAATTGATAAGG	Pyrosequencing PCR	
Peg3 ICR	Peg3 R- biotinylated	CCCTATACACTAAACATCCCC	Pyrosequencing PCR	
Peg3 ICR	Peg3 Sequencing	AATTGATAAGGTTGAGATT	Pyrosequencing Sequencing Primer	
KvDMR	KvDMR F	TTTGTGTTTTTTGGAGACT	Pyrosequencing PCR	
KvDMR	KvDMR R-biotinylated	CCTCAAAACCCCTACT	Pyrosequencing PCR	
KvDMR	KvDMR Sequencing	CTAAAGTATTAAAGGTTAGAGTAA	Pyrosequencing Sequencing Primer	
Peg1/Mest ICR	Peg1/Mest ICR F	GGAGGTTTATATAAGTTGGTTTT	Pyrosequencing PCR	
Peg1/Mest ICR	Peg1/Mest ICR R-biotinylated	ACCACCAACTAACACTAAA	Pyrosequencing PCR	
Peg1/Mest ICR	Peg1/Mest Sequencing	GGTTTTATATAAGTATTGTTTTT	Pyrosequencing Sequencing Primer	
Peg3 ICR	Peg3A-BL (1st round)	TTTGATAAAGGGGTGTTT	Bisulfite Sequencing PCR	
Peg3 ICR	Peg3D-BL (1st round)	ACTCTAATATCCACTATAATAA	Bisulfite Sequencing PCR	
Peg3 ICR	Peg3B-BL (2nd round)	AGTGTGGGTATTAGATT	Bisulfite Sequencing PCR	
Peg3 ICR	Peg3C-BL (2nd round)	TAACAAAATCTACATCATC	Bisulfite Sequencing PCR	(Market-Velker et al., 2010)
H19/Igf2 ICR	BMsp21 (H19 A) (1st round)	GAGTATTAGGAGGTAAAGAATT	Bisulfite Sequencing PCR	
H19/Igf2 ICR	BHha113 (H19 D) (1st round)	ATCAAAAATCAATAACCCCT	Bisulfite Sequencing PCR	
H19/Igf2 ICR	Bmsp21c (H19 B) (2nd round)	GTAAGGAGATTATGTTTATTGTTGG	Bisulfite Sequencing PCR	
H19/Igf2 ICR	BHha114ct (H19 C) (second round)	CTAACCTCATAAAACCCATAACTAT	Bisulfite Sequencing PCR	(Ideraabdullah et al., 2014)

Table S9: List of PCR cycling conditions for each PCR used in this study.

Assay	Thermal Cycler	PCR conditions	Annealing Temperature (TA), °C	No. of cycles
Tet1	Thermo Electron Hybaid PCR Express Thermal Cycler	2 min denaturation at 94°C; number of cycles of [15 s at 94°C, 15 s at TA, and 40 s at 72°C]	60	35
Smc	BioRad C1000 Touch Thermal Cycler	5 min denaturation at 95°C; number of cycles of [15 s at 95°C, 1 min at TA, and 1 min at 72°C]; 7 min extension at 72°C	55	40
qPCR: All genes	7900HT Fast Real-Time PCR System	2 min hold at 50°C; 10 min hold at 95°C; number of cycles of [15 s at 95°C, 1 min at 60°C]; Melting Curve: 95°C for 15 s, 60°C for 15 s, 95°C for 15 s.	-	40
Allele-Specific <i>H19</i>	BioRad C1000 Touch Thermal Cycler	2 min denaturation at 95°C; number of cycles of [15 s at 95°C, 20 s at TA, and 20 s at 72°C]; 5 min extension at 72°C	58	21-31
Allele-Specific <i>Igf2</i>	BioRad C1000 Touch Thermal Cycler	2 min denaturation at 94°C; number of cycles of [20 s at 94°C, 20 s at TA, and 20 s at 72°C]; 5 min extension at 72°C	60	24-32
Allele-Specific <i>Cdkn1c</i>	BioRad C1000 Touch Thermal Cycler	2 min denaturation at 95°C; number of cycles of [15 s at 95°C, 20 s at TA, and 20 s at 72°C]; 5 min extension at 72°C	60	25-30
Allele-Specific <i>Peg3</i>	BioRad C1000 Touch Thermal Cycler	2 min denaturation at 94°C; number of cycles of [20 s at 95°C, 20 s at TA, and 20 s at 72°C]; 5 min extension at 72°C	60	29-34
Allele-Specific <i>Lit1/Kcnq1ot1</i>	BioRad C1000 Touch Thermal Cycler	2 min denaturation at 95°C; number of cycles of [20 s at 95°C, 20 s at TA, and 50 s at 72°C]; 5 min extension at 72°C	64	32-34
Allele-Specific <i>Snrpn</i>	Roche LightCycler 1.5	Amplification: 95°C 1 s (20°C/s), 50°C, 15 s (20°C/s), 72°C, 6 s (20°C/s); Melt: 95°C, 4 min (20°C/s), 35°C, 3 min (20°C/s), 40°C, 1 min (20°C/s), 45°C, 1 min (20°C/s), 85°C, 0 s, (0.5°C/s); Cooling: 40°C 30 s, (20°C/s)	-	Amplification: 45; Melt: 3; Cooling: 1
Pyrosequencing	BioRad C1000 Touch Thermal Cycler	(Hur et al., 2016)		
<i>Peg3</i> Bisulfite	BioRad C1000 Touch Thermal Cycler	First Round: 5 min denaturation at 94°C; number of cycles of [94°C for 30 s, TA for 30 s, 72°C for 1 min]; 10 min extension at 72°C	50	25
		Second Round: 5 min denaturation at 94°C; number of cycles of [94°C for 30 s, TA for 30 s, 72°C for 1 min]; 10 min extension at 72°C	53	35
<i>H19</i> Bisulfite	BioRad C1000 Touch Thermal Cycler	First Round: 5 min denaturation at 94°C; number of cycles of [94°C for 30 s, TA for 30 s, 72°C for 1 min]; 10 min extension at 72°C	50	25
		Second Round: 5 min denaturation at 94°C; number of cycles of [94°C for 30 s, TA for 30 s, 72°C for 1 min]; 10 min extension at 72°C	58	35

Supplemental References

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