

Supplemental Figure 1. Whole mouse genome meDIP-on-chip and genome tiling array screen for DMRs using PG- and AG-derived stem cells and sperm.

A. Known imprinted DMRs in PG- and AG-derived cells were characterized by the COBRA method. The bisulphite-treated DNA amplified by PCR was digested with restriction enzymes (as representative results IG-DMR: *TaqI* and *Lit1*: *HpyCH4IV*) that cut only when the site in the genomic sample is methylated. The sizes of digested fragments are indicated on right (IG-DMR: 337 bp and *Lit1*: 185 bp). TS; DNA of TS cells, PG; DNA of PG-derived cells, AG; DNA of AG-derived cells, Sp; DNA of mature sperm.

B. Known imprinted DMRs and non-imprinted methylated regions estimated by meDIP quantitative real-time PCR method against DNA precipitated by the antibody against 5-methyl-cytosine. Black, gray and white bars represent cycle threshold (Ct) number subtracted Ct of meDIP DNA (meDIP) from that of whole genomic DNA (WG). Paternal DMRs: *H19*, IG-DMR and *Rasgrf1*. Maternal DMRs: *Nespas*, *Peg10*, *Peg3*, *Lit1*, *U2af1-rs1* and *Igf2r* (DMR2). Non-DMRs: *Nanog*, *Rest*, *Aicda*, *Tdrd12*, *Gdf3*, *Slc2a3*, *Aicda*, *Tdrd12* and *Utf1*.

Black, gray and white bars indicate data for sperm, AG- and PG-derived cells, respectively, which are different number of real-time PCR cycles of meDIP and amplified DNA, using Whole Genome Amplification kit.

Supplemental Figure 2. Three paternally methylated DMRs in the *Gpr1-Zdbf2* imprinted domain.

A. Bisulphite-PCR sequencing results for DMR1 (region 7) on genomic DNA prepared from E13.5 B6/JF1 fetus and placenta, and adult brain, liver, lung, heart and spleen. Each row represents a unique methylation profile within the pool of 20 clones sequenced. Closed and open circles represent methylated and unmethylated CpGs, respectively.

B. Bisulphite-PCR sequencing results for 16 regions on genomic DNA prepared from B6 sperm and the kidney from B6/JF1 adult mouse.

Supplemental Figure 3. Tissue specific-imprinted expression of the mouse *Gpr1*.

A. Direction expression analysis of mouse *Gpr1* gene. The 1st cDNA strands syntheses were performed using either the sense (S) or the antisense (AS) primer of the mouse *Gpr1* gene. Arrow indicates the cDNA product of *Gpr1* gene amplified by RT-PCR on right.

B. Analysis of mouse *Gpr1* gene. cDNA and genomic PCR products were amplified and sequenced directly from E13.5 embryos, placenta and yolk sac and adult materials obtained from B6/JF1 and JF1/B6 mice. Expression was biallelic in all tissues examined.

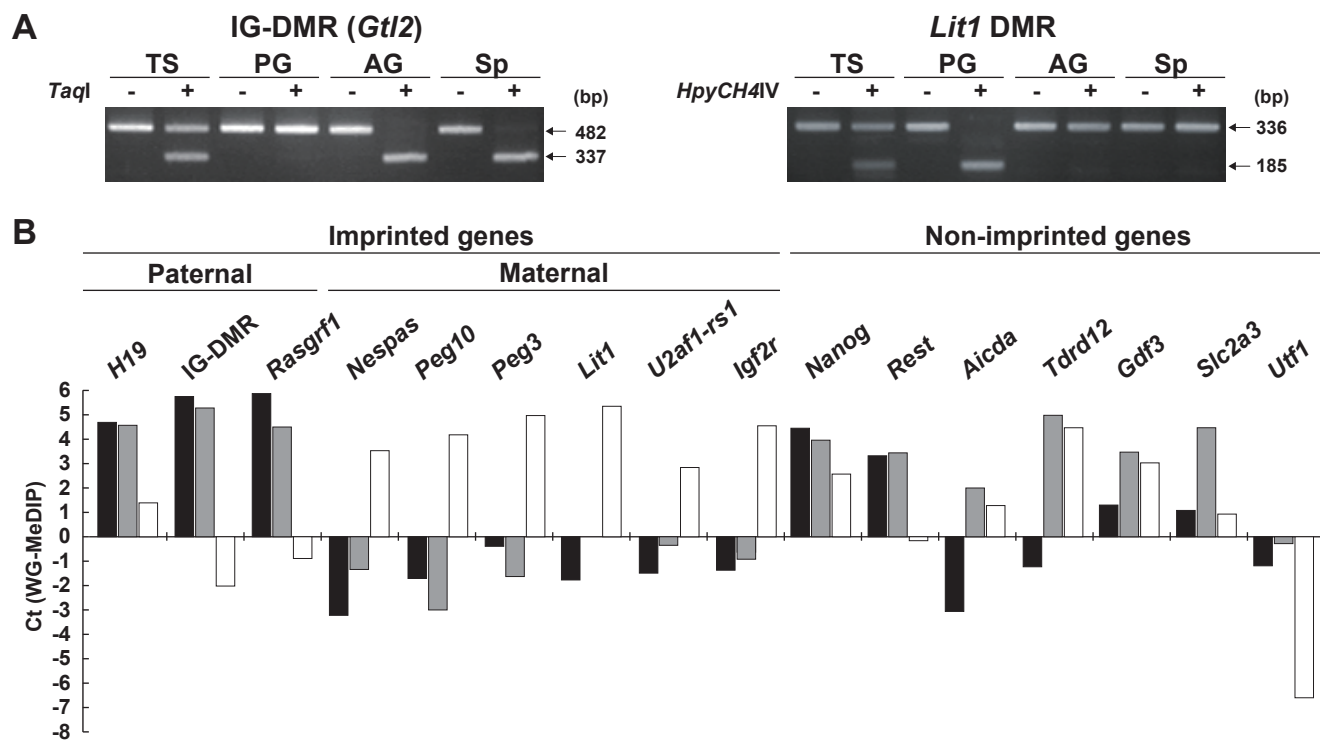
Supplemental Figure 4. Dot-plot comparison of the mouse DMR1 and human DMRh1.

Dots were placed at locations with identical nucleotides when more than 28 of 70 nucleotides were identical.

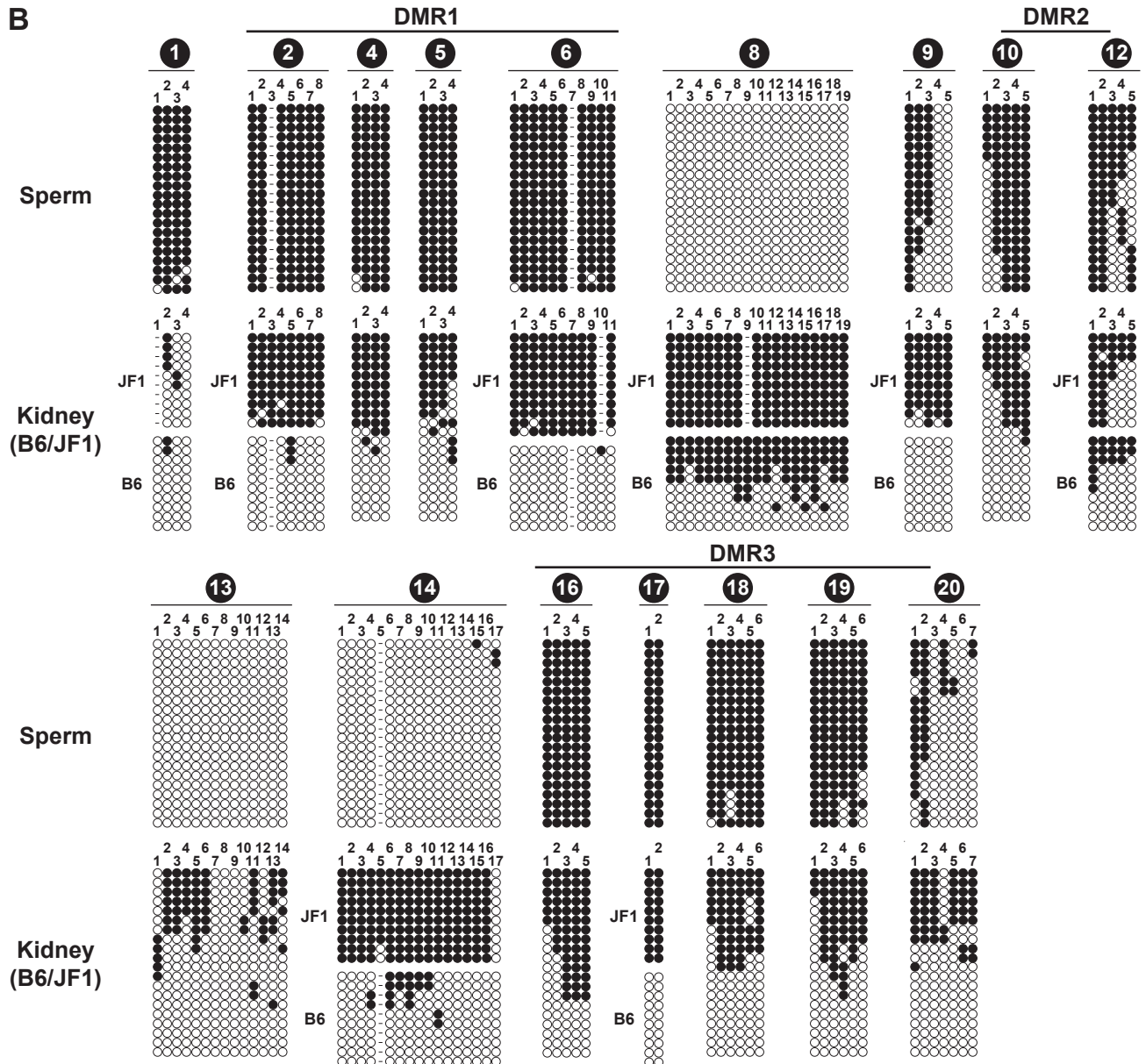
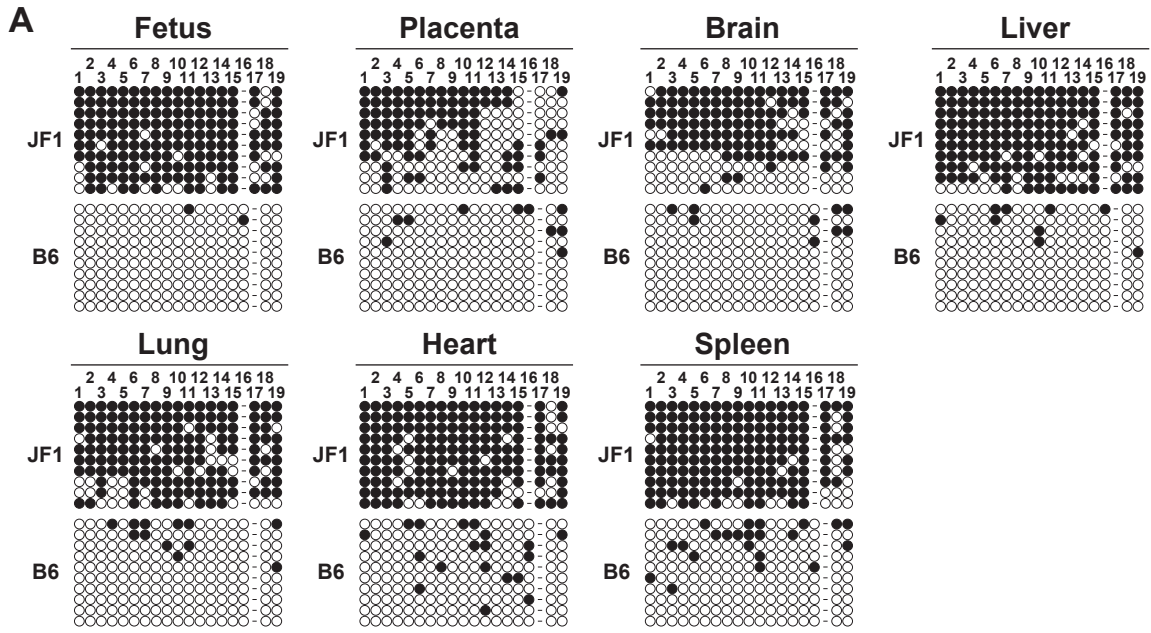
Supplemental Table S1. Primer sequences and PCR conditions.

Supplemental Table S2. DNA polymorphisms between B6 and JF1 strain and position of GenBank accession number AL669947.

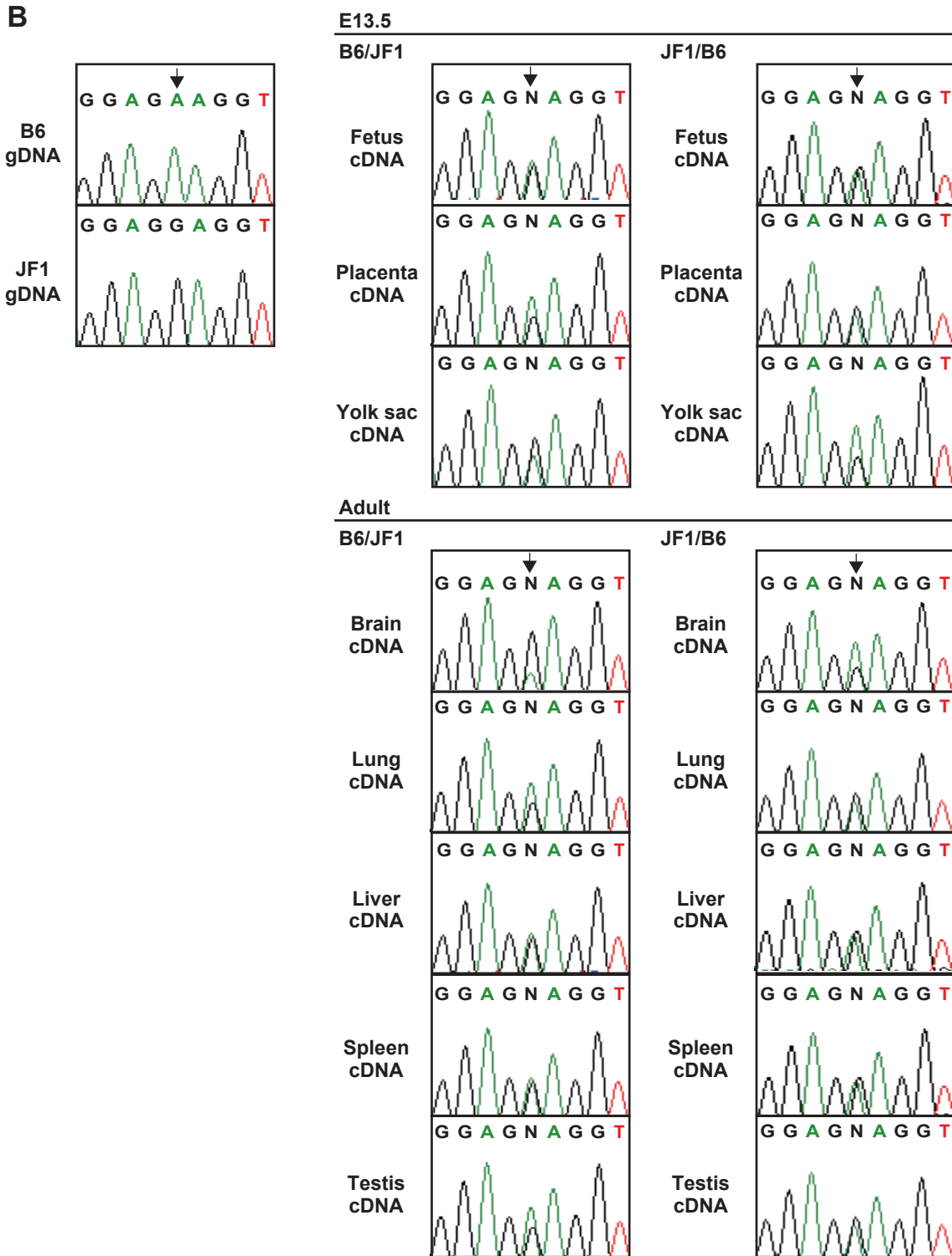
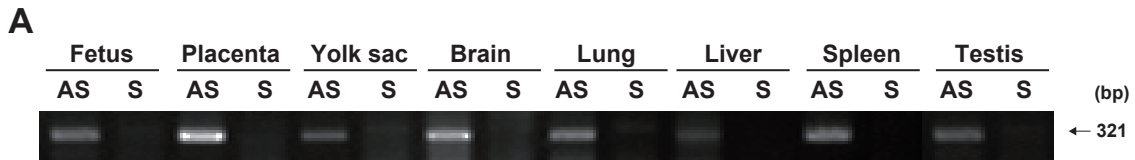
Supplemental Figure S1



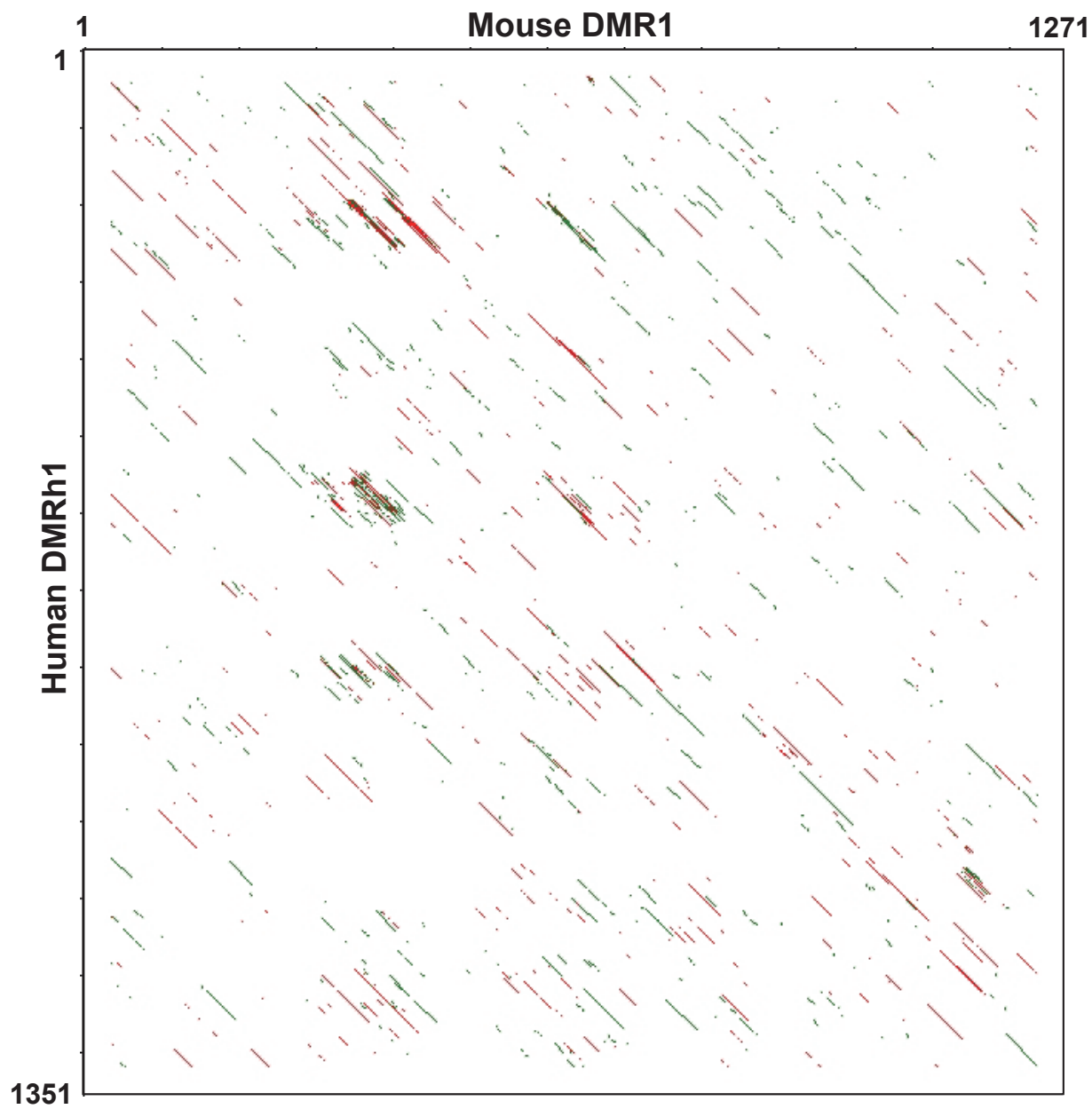
Supplemental Figure S2



Supplemental Figure S3



Supplemental Figure S4



Supplementary Table S1. Primer sequences and PCR conditions.

Locus	PCR	Primer sequence (5'-3')	Annealing temp	Cycles	Amplicon (bp)
Methylation assay					
Mouse					
IG-DMR (<i>Gtl2</i>)	1st	IG-DMR cBSF1 IG-DMR cBSR1	TTAAGGTATTTTTATTGATAAAAAATAGTAGT CCTACTCTATAATACCCATATAATTATACCA	55°C	30
	2nd	IG-DMR cBSF2 IG-DMR cBSR2	TTAGGAGTTAAGGAAAAGAAAAGAAATAGTAT/ TATACACAAAAATATATCTATAAACACCATA	55°C	482
<i>H19</i> DMR		H19 BisOF1 H19 BisOR2	TTGTGAGTGGAAGATTAAATGTTG ATCTTACCACCCTATAAATCCCT	57°C	423
<i>Rasgrf1</i> DMR	1st	<i>Rasgrf-1</i> BSF1 <i>Rasgrf-1</i> BSR1	ATGTGGTTTTGTAGTTGAA CAAAAACAACAATAAAGT	55°C	30
	2nd	<i>Rasgrf-1</i> BSF1 <i>Rasgrf-1</i> BSR2	ATGTGGTTTTGTAGTTGAA CAATAAAAAACAACAACAAC	55°C	467
<i>Nespas</i> DMR		<i>Nespas</i> BSF <i>Nespas</i> BSR	AGGGATGGTTTTATGGGGTTTTTGATT AACCAACTAAATCTCAACCACTAAC	57°C	337
<i>Gnas1A</i> DMR		<i>Gnas1A-C_F</i> <i>Gnas1A-C_R</i>	GGTATTATGTTGAAGATGGTTATGAAGTTAA AACATCATCTTCTTCATCAATATCACTATCC	60°C	519
<i>Peg10</i> DMR		<i>Peg10-E_F</i> <i>Peg10-E_R</i>	GTATTTAATTTGGAAAGTTGATAGGAGAG CTCCAACCACCAAAATCCCT	60°C	603
<i>Peg1</i> DMR	1st	<i>Peg1/Mest</i> BSF1 <i>Peg1/Mest</i> BSR1	GATTTGGGATATAAAGGTTAATGAG TCATTAATAAACACAAACCTCCTTTAC	55°C	30
	2nd	<i>Peg1/Mest</i> BSF2 <i>Peg1/Mest</i> BSR2	TTTTAGATTTTGAGGGTTTTAGGTTG AATCCCTTAAAAATCATCTTTCCACAC	55°C	563
<i>Peg3</i> DMR	1st	KH63 <i>Peg3</i> F1 KH64 <i>Peg3</i> R1	TTTTTGTAGTTTTGTGGGGTTTTTAATA AATCCCTATCACCTAAATACATCCCTACA	57°C	30
	2nd	KH65 <i>Peg3</i> F2 KH66 <i>Peg3</i> R2	TTGATAATAGTAGTTTGTAGTTGGTGGGTG ATCTACAACCTTATCAATCCCTTAAAAA	57°C	451
<i>Snrpn</i> DMR	1st	KH99 <i>Snrpn</i> F1 KH100 <i>Snrpn</i> R1	AAATTTGTGTAGTTTGTAAATTTGGG AAAATCCACAAACCACTAACCTTCC	57°C	30
	2nd	KH101 <i>Snrpn</i> F2 KH102 <i>Snrpn</i> R2	AATTATATTTATTTTAGATTGATAGTGAT TTTACAATCACTCCTCAAAACCAA	57°C	294
<i>Lit1</i> DMR	1st	<i>Lit-BS4</i> <i>Lit-BS2</i>	TAAGGTGAGTGGTTTGGATT AATCCCCACACCTAAATTC	57°C	30
	2nd	<i>Lit-BS4</i> <i>Lit-BS21</i>	TAAGGTGAGTGGTTTGGATT CCACTATAAACCCACACATA	57°C	336
<i>Zac1</i> DMR	1st	<i>Zac1</i> BSF1 <i>Zac1</i> BSR1	GGGTAGGTAAGTAGTGATAA CCTAAACACCAAAATAACA	54°C	30
	2nd	<i>Zac1</i> BSF2 <i>Zac1</i> BSR2	ATTTGGGTGTTTTAGTTGTA TACAAAACCAAAACCCCTTAC	54°C	383
<i>U2af1-rs1</i> DMR	1st	<i>U2af1-rs1</i> BSF1 <i>U2af1-rs1</i> BSR1	GGTTGAGTATTTTTATTGTA TACATAAACCTACCCATACA	55°C	30
	2nd	<i>U2af1-rs1</i> BSF3 <i>U2af1-rs1</i> BSR1	ATTGTAGATATTTGGATGAT TACATAAACCTACCCATACA	55°C	502
<i>Igf2r</i> DMR2	1st	<i>Igf2r</i> 13B-4 <i>Igf2r</i> 13B-2	TAGAGGATTTAGTATAATTTTAA CACTTTTAACTACCTCTCTTAC	55°C	30
	2nd	<i>Igf2r</i> 13B-5 <i>Igf2r</i> 13B-2	GAGGTTAAGGGTGAAGTTGAT CACTTTTAACTACCTCTCTTAC	57°C	490
<i>Impact</i> DMR		<i>Impact-C F</i> <i>Impact-C R</i>	TGTTTTGAAGGATGAGTATGAGAGTTTT CCCTACTAAA ACCTTACCTA CCTCTAAC	60°C	405
DMR region 1		R0-6467 BSF1 R0-6467 BSR1	TTTTTAATTAGTTGTTTTGTTTT AAAACCTAACCAATAAAATCTAATT	55°C	291
DMR region 2		R1-0208 BSF1 R1-0208 BSR1	GTTTTAGTGTAGTTTTGGGTTTTTTTT CCAAACAAAACAACAACCACT	58°C	505
DMR region 3		1700039101 Rik BSF 1700039101 Rik BSR	TTTGTGGTATGTGTAGAAGATTT ACAAATCATACTATCTACAAAAAAC	55°C	451
DMR region 4		R1-2831 BSF1 R1-2831 BSR1	GGAGTAGTATTTAGTTTTGTTTTTAT AAAAAATAAAAAAACACTAATAAT	57°C	241
DMR region 5		R1-3235 BSF1 R1-3235 BSR1	TAGGATTTTTGGAGTTGGAGTTAT TTAAACTCTCAACAATCATTACCAA	57°C	380
DMR region 6		R1-3948 BSF1 R1-3948 BSR1	TGTGATAGGTTTGTATGGTTTGT ATAATCAACTACYAAAAAACCCATA	55°C	365
DMR region 7		1P1 BSF2 1P1b BSR1	GATTTAGATTTAGTTGGTTAGTTTATAT CAAACCTAACCTACAAATACCTTATTACC	57°C	840
DMR region 8		R2-1331 BSF1 R2-1331 BSR1	TTATATTAGTTTGGGGGAGAGTT AAAACCTAAAACAAAAAATCTTAAC	55°C	406
DMR region 9		R2-3337 BSF1 R2-3337 BSR1	AAAGGGATATATGATTTTTATGTAAA ACTTTATTTCCAACAAATCCAAAC	55°C	414
DMR region 10		R2-3842 BSF1 R2-3842 BSR1	TATATTTTGTATGATTTGAAGTT AACAAAAAATCAAAATAAAAAACAT	55°C	503
DMR region 11		1P1down BSF1 1P1down BSR1	TAAATAAATAAAGGGTTGGTTAGTT AAATCAAACCTACAACTCCAAACTA	55°C	334
DMR region 12		R3-0105 BSF1 R3-0105 BSR1	GGTAGGAAGAGTAAATAAGGTTTT CTCAAAAATACAAAATACATAATAA	57°C	387
DMR region 13		R3-0619 BSF1 R3-0619 BSR1	TTATTGAGATGGAGTTATAGTGAAT TACTTAAACAATTCTAAAATCAATATAA	55°C	386

DMR region 14	R3-2035 BSF1 R3-2035 BSR1	TATTTTGTGGTTTTGAGGATT ACATACTACTAATAAATCCCATAAATATA	58°C	40	492
DMR region 15	CpG3com BSF1 CpG3com BSR1	AATTAAGATATTAGATTATGGATAGATTTTT ATAAACTCTCCAAAACCAAAAAA	57°C	40	339
DMR region 16	R3-7276 BSF1 R3-7276 BSR1	TATAATGTGAGTGGATTGTTAATTT AATACCAATATAAATACACAATAC	52°C	40	333
DMR region 17	R3-7980 BSF1 R3-7980 BSR1	TGAGTTTTGTGGTGTATATTTTA CATCCTAACCAACAATATTAATAAAC	55°C	40	185
DMR region 18	R4-0106 BSF1 R4-0106 BSR1	TGTGATTTATGTTTTGAGTTAGTTG ACTCCTACTTAAATCAAAAAATACC	55°C	40	383
DMR region 19	R4-0712 BSF1 R4-0712 BSR1	TATGGTATTTTGTAGTGGGTTT CAACTATTTAAACTATTCTTACTTCAAAC	55°C	40	411
DMR region 20	R4-1319 BSF1 R4-1319 BSR1	GTGTTTTAGTTGAAATATAGTTATGTTAG CCCTCTAAACTTAAATACAAA	55°C	40	301
Human					
<i>H19</i> DMR	H19 F2 H19 R1	TATATGGGTATTTTGGAGGTTTTT ATAAATATCCTATTCCCAATAACCCC	57°C	40	220
DMRh1	h2Pc4 BSF1 h2Pc4 BSR1	GTTTTGTAGTTAGATTGGAAAAATA AAAAATAAATACCTAAAAATAAAAAAC	55°C	40	210
DMRh2	h2Pc1 BSF1 h2Pc1 BSR1	TTTTATTTTTTGGTTGTGGAATA ACCTCTAAATAACTAAAACTATAATCATA	55°C	40	394
SNP analysis					
Human					
<i>GPR1</i>	GPR1 s3F2 GPR1 s3R0	ATCTTCACGTTCTTATCATTCACTG GGTGACTGTCTTCTCCACTTGAAC	60°C	35	324
Gene expression					
Mouse					
<i>Gpr1</i>	Gpr1 exF Gpr1 exR	CGGGGTGTCATTTCAAAAGT AGGGCATATAAGAACAGGGAGA	59°C	35	321
<i>Gapdh</i>	Gapdh exF Gapdh exR	GTGCTGGAGTCTACTGGTGC GAGCCCTCCACAATGCCAAA	60°C	30	241
Human					
<i>GPR1</i>	GPR1 exF2 GPR1 exR	GAAGGTACACCCAGGCATGACA TCCAGAACAAAAGCCAAACAA	57°C	40	236
<i>GAPDH</i>	GAPDH exF GAPDH exR	GTGCTGGAGTCCACTGGCGTC GAGTCTCCACAATACAAA	60°C	30	244
In situ hybridization probe					
Mouse					
<i>Zdbf2</i>	Zdbf2 H-F Zdbf2 H-R	CGGCAGCAGCAGCAGATCCT AAAAGACGCACCTTAGCCCTAA	60°C	40	593
<i>Gpr1</i>	Gpr1 exF2 Gpr1 exR2	CATTTCACTCTGGAGTTGGAAG TGGCAATGAAGGAATTAAC	60°C	35	453
Methylated-DNA immunoprecipitation					
Mouse					
<i>H19</i> DMR	ChIP-H19m3F1 ChIP-H19m3R1	AGGTTGGAACACTTGTGTTTCTGGAG TGGCCACGATATATAGGAGTATGCT	65°C	40	159
IG-DMR (Gtl2)	MeDIP IG-DMR F1 MeDIP IG-DMR R1	CGCTATGAACTACCGCTACG CGGCATTAGTACACGGCGAACCTCC	65°C	40	136
<i>Rasgrf1</i> DMR	MeDIP Rasgrf1 F1 MeDIP Rasgrf1 R1	CAGAGAGTATGTAAGCCAGAGC CAGCAATAGCGGTAGCCACGGATG	65°C	40	188
<i>Nespas</i> DMR	MeDIP Nespas F MeDIP Nespas R	GCCTGACCCAGCCCAATAGC GCCAACTACCCCTCCCAACATA	65°C	40	149
<i>Peg10</i> DMR	MeDIP Peg10 F2 MeDIP Peg10 R2	TGAGTCCCCAAATACGC GCAGTGCAATCCGTTCTGTA	65°C	40	74
<i>Peg3</i> DMR	ChIP Peg3 F1 ChIP Peg3 R1	ATGGGGTCTTGGATTGGTTAG TCTCCGCTTCTCCTCCGATGCT	65°C	40	136
<i>Lit1</i> DMR	ChIP Lit1 F2 ChIP Lit1 R2	TCGGTCGAGTCCCAAGGTGAGT ACAGCTACCACATAACAACAGC	65°C	40	184
<i>U2af1-rs1</i> DMR	MeDIP U2af1-rs1 F MeDIP U2af1-rs1 R	AGGGCTGAGCAITTTACTG ATCCCGCAGTATGTCTGATCTG	65°C	40	151
<i>Igf2r</i> DMR2	ChIP-Igf2r DMR2F ChIP-Igf2r DMR2R	GAGTTTTCTTGTAGCCAGAAATCTTCA TACCGAGGTGAGGGTTCCACTGAT	65°C	40	127
<i>Nanog</i>	Nanog-F5 Nanog-R5	TCAAGGCAACAGAGAAAAACCT GGGAAACCTGGGGAAATCT	60°C	40	147
<i>Rest</i>	REST-F REST-R	GCCGTGCTTTTATTTGTGC ACGAAGTGAGAGACCCAGTC	60°C	40	91
<i>Aicda</i>	Aicda-F Aicda-R	AGTCACGCTGGAGACCGATA GGCAAGCAGACAGGAAAT	60°C	40	133
<i>Tdrd12</i>	ECAT8-F ECAT8-R	GGTGCTGAAAGGTGAGGAGTG TAATCGGAACGCAAGCCTAC	60°C	40	109
<i>Gdf3</i>	Gdf3-F Gdf3-R	CGAGGCACAGGTTGGAA GCCAGACATCCGAGTTTCA	60°C	40	91
<i>Slc2a3</i>	Slc2a3-F Slc2a3-R	AGGACCCCAACAAGCAAAT TCTGTAGGACCCGAGGAACA	60°C	40	142
<i>Utf1</i>	UTF1-F1 UTF1-R1	AGAATAAGCAAGGCACAGG CCACACACGCTCTAAGGAC	60°C	40	127

Supplemental Table S2. DNA polymorphisms between B6 and JF1 strain and position of GenBank accession number AL669947.

Region	Nucleotide No. (AL669947)	C57BL/6	JF1
1	13907	G	A
2	14425	A	G
	14688-14689	-	TAATGTGTA
	14821	G	T
6	18187-18188	-	CGG
	18310-18346	GTTTGATCCAAACGGAAGATTTTCAGGGTCCTTCCCTTG	37 bp deletion
7	19155	G	A
	19243	G	C
8	19943	G	A
9	20780	G	A
11	23192	C	A
	23217-23218	GC	--
	23239-23240	-	TGTGTGTGTGTGTGTGTGTGTGTGTG
12	24484	A	G
	24540	A	G
14	25259	A	G
15	26681	C	T
	26819	C	T
17	29060	G	A